DEVELOPMENT OF QUALITY ASSESSMENT TOOLS FOR CHARDONNAY IN RELATION TO GRAPE, JUICE AND WINE COMPOSITION

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THESIS SUMMARY

Chardonnay is an important wine varietal and continues to be the main white wine variety grown in Australia, second only to Shiraz in total annual crush. The main gauges of white grape quality in the field, and the parameters used when making harvest decisions, are sugar and acid contents in the berry, despite the fact that one of the key components of the quality of a wine is its aroma. In the case of Chardonnay wines, aroma tends to be due to a complex mixture of esters, C_{13}-norisoprenoids, monoterpenoids, malolactic fermentation characters and oak volatiles; so this variety is impacted by more inputs than other white wine varieties. There is a need for objective tools to determine quality, both in grape and wine, and development of such tools implies understanding the underlying factors that modulate Chardonnay wine aroma and flavour. This includes aspects such as terroir and winemaking techniques, but also recognising compositional variables that can be measured in the berries to guide production of desired wine styles. This thesis comprises a number of studies that examine different factors that affect Chardonnay wine quality based on grape composition.

As a preliminary step to examine current trends in production that could affect final wine quality, a survey was distributed amongst Chardonnay wine producers in Australia. Over 150 respondents, or around 10% of all Chardonnay producers in Australia, participated in the survey. From the responses it was observed that production of Chardonnay wines was concentrated in the ultra- ($25-50) and super-premium ($15-24) categories rather than the lower priced segments. More importantly, the survey showed that although producers still relied mainly on total soluble solids (i.e. °Brix) and titrable acidity to determine grape maturity, berry tasting and grape flavour intensity were weighing as heavily in their decisions, especially when assigning a quality grade to the fruit. This emphasises the importance of the aroma-related compositional traits of the berries in the decision-making process, as well as that of understanding the chemical differences between berries from different quality grades. Other findings from the survey included trends in yeast selection, use of oak during fermentation and ageing according to price category, and changes in current Chardonnay wine styles.

Expert ratings and medals are currently one of the main ways by which the quality of wines is judged. A study was therefore undertaken to investigate the volatile molecules responsible for the differences between the quality ratings of commercial Chardonnay wines of different quality levels to pinpoint compounds that could be used to discriminate between higher and lower quality Chardonnay wines. The wine selection comprised oaked and non-oaked wines, ranging in price point, sales volume and wine writer quality score, sourced from Australia's main wine producing regions, which were assessed by a panel of eight industry experts. Correlation of sensory and chemical data indicated that nine volatile compounds were significantly and positively correlated to quality score and price, including oak and age-related volatiles (*Z*)- and (*E*)-oak lactones, furfural and diethyl succinate. Eleven volatiles were found to be negatively and significantly correlated to quality score, amongst them hexyl acetate, β-damascenone, 3-methyl butyl acetate, ethyl butanoate, ethyl hexanoate, hexanoic acid. Results also showed significant correlations between quality score and production method and vintage, where samples fermented in oak barrels and older than one vintage were preferred by the experts and thereby scored higher. In contrast, younger, more fruit-driven and simpler wines were all scored lower.

Quality of any wine depends on the quality and constitution of the grapes used in its production. Origin (or terroir) can have a large influence on the composition of the grapes through the effects of weather, topography and soil composition. As the main focus of the thesis, grapes sourced from different geographical regions in South Australia were studied for their effects on the composition of the ensuing wines. Berries collected during the 2014-2016 harvests from the Barossa Valley, Eden Valley, Clare Valley, the Adelaide Hills, Langhorne Creek, McLaren Vale and the Riverland were analysed and vinified to produce research-scale wines. Results from 2014 showed that it was possible for a trained panel to discriminate between wines from three distinct regions in South Australia (Adelaide Hills, Eden Valley and the Riverland) through sensory descriptive analysis, when the main attributes associated with each of these wines were generated. Both positive and negative sensory attributes were related to volatile compounds, which were used as markers of quality in small-scale fermentation wines. Discrimination between the different juices was possible based solely on their chemical composition (using compounds such as vitispirane, C_6-compounds, 5-methylfurfural, C_6-C_{10} acids and guaiacol, along with Zn, pH, TA and °Brix). Warmer weather in the Clare and Barossa Valleys related to higher quantities of hydrolytically-released β-ionone, vitispirane, 5-methylfurfural, guaiacol and 2,6-dimethoxyphenol and certain fatty acids but lower quantities of 1-hexanol, (*Z*)-linalool oxide and linalool. Chemical data from the other two vintages was assessed to validate and further refine the understanding of the regional effects, allowing models to be proposed that link Chardonnay grape composition and quality.

Industry requires rapid, low cost tools that can withstand the high throughput of vintage. To this effect, in addition to research based methodologies, work was started on the development of prediction models using partial least squares regression and mid-infrared (MIR) spectroscopy that could differentiate between grape juice from different origins and quality levels. Good separation was obtained in 2014 and 2016 between the tested regions (Adelaide Hills, Barossa Valley, Clare Valley, Eden Valley, Langhorne Creek and Riverland) using the fingerprint region (1500-800 cm^{-1}). Classification according to grade based solely on the fingerprint region proved more challenging although models were especially successful at classifying A-grade grapes both years. Future improvement and further work is required on these methodologies before they can be used routinely by the industry, but this initial work offers guidance and the promise of realising a rapid tool for discriminating grapes of different origins or quality levels.

The results of this thesis give insight into the different parameters affecting Chardonnay grape and wine quality, and contribute to the determination of objective indices that can be used by producers to grade their fruit, thereby potentially guiding their practices in the vineyard and winery. This knowledge can be used to further enhance understanding of the link between grape and wine composition and quality, and aid in the development of rapid assessment tools for the industry.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Joanna M. Gambetta M. Date

PUBLICATIONS

This thesis is a collection of manuscripts that were published in Journal of Agricultural Food Chemistry (JAFC), Food Analytical Methods, American Journal of Enology and Viticulture, Wine and Viticultural Journal and Wine and Viticultural Journal during candidature. The impact factor of JAFC according to Thomson Reuters Journal Citation Reports was of 2.912 in 2014 and the 5-year impact factor was 3.269. The impact factor of Food Analytical Methods in 2015 was 2.167 and the 5-year impact factor was 2.003. The impact factor of the American Journal of Enology and Viticulture was 1.579 and its 5-year impact factor 2.083.

The text and figures in Chapter 1 to 4 and 6 appear in different formats due to each journal's specific requirements. A statement of authorship, signed by all of the authors and listing individual contributions to the work, is included at the beginning of each chapter.

The thesis is based on the following publications (*indicates peer reviewed publications).

Chapter 1. Gambetta, J.M., Bastian, S.E.P., Cozzolino, C. & Jeffery, D. W. (2014). Factors Influencing the Aroma Composition of Chardonnay Wines. *J. Agric. Food Chem.*, 62, 6512-6534*

Chapter 2. Gambetta, J.M., Bastian, S.E.P., & Jeffery, D. W. (2016). Snapshot of Australian production practices for Chardonnay wine. *Wine and Viticultural Journal,* 5, 27-32

Chapter 3. Gambetta, J.M., Wang, J., Schmidtke, L., Bastian, S.E.P., Cozzolino, C. & Jeffery, D. W. (2017). Relating expert quality ratings of Australian Chardonnay wines to volatile composition and production method. *Am. J. Enol. Vitic.,* 68, 39-48*

Chapter 4. Gambetta, J.M., Bastian, S.E.P., Cozzolino, C. & Jeffery, D. W. (2016). Towards the Creation of a Wine Quality Prediction Index: Correlation of Chardonnay Juice and Wine Compositions from Different Regions and Quality Levels. *Food Anal. Methods*, 9, 2842-2855*

Chapter 5. Gambetta, J.M., Bastian, S.E.P., Cozzolino, C. & Jeffery, D. W. Exploring the effects of geographical origin on the chemical composition and quality grading of Vitis vinifera L. cv. Chardonnay grapes. *Molecules,* 22, 218*

An additional 2 publications co-authored by the candidate are given in the appendices.

CONFERENCES

University of Adelaide School of Agriculture, Food and Wine, Postgraduate symposium, 23rd September 2014, Adelaide

Presented a talk titled “Development of quality assessment tools for Chardonnay in relation to grape, juice and wine composition”

IVAS 2015 – 9th IN VINO Analytica Scientia, 14 to 17th July 2015, Trento.

Presented a talk titled “Creation of a wine quality prediction index; Correlation of aroma precursors developed in Chardonnay (*Vitis vinifera* L.) berries during ripening with final wine quality” and the poster titled “Prediction of phenolic composition of Shiraz wines using attenuated total reflectance mid-infrared (ATR-MIR) spectroscopy”.

16th Australian Wine Industry Technical Conference, 24 to 28th July 2016, Adelaide.

Presented a poster titled “Regionality effect in South Australian Chardonnay and its relationship to quality”

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CHAPTER 1

LITERATURE REVIEW

This literature review is covered in a review article on the factors influencing the aroma composition of Chardonnay wine aroma prepared during the first year of candidature. It covers the literature up to April 2014; the literature beyond this date has been included in the introductions of the publications covered in Chapters 2 to 4. A summary of the research aims is included at the end of this chapter.

Factors influencing the aroma composition of Chardonnay wines

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Overall percentage (%)	80%		
Certification:	This paper is a review on original research that was conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	4/11/2016

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Factors Influencing the Aroma Composition of Chardonnay Wines

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ABSTRACT: Chardonnay is one of the oldest and most widely distributed wine grape cultivars and is of commercial importance for the world's wine-producing nations. It is an extremely flexible variety that has adapted to different regions with varied weather and soil characteristics. Somewhat uniquely among white wines, Chardonnay lends itself to a wide variety of production styles, which can be tailored to the target market. Techniques such as skin maceration, barrel and stainless steel fermentation, use of selected or indigenous yeasts, malolactic fermentation, and aging in barrels with or without lees are all applicable and lead to different compositional outcomes. A number of research papers have been published with a view to understanding Chardonnay composition and quality as well as the impact of different enological techniques on the final product. This review summarizes current knowledge, explaining the influence of viticultural and production techniques on aroma composition, and poses directions for further research into Chardonnay wines.

KEYWORDS: *Chardonnay, grape, wine aroma, volatile compounds, winemaking, aging, viticulture*

INTRODUCTION

Chardonnay can be found in every wine-producing region of the world, from both the Casablanca and San Antonio valleys in Chile to the Barossa and Yarra valleys in Australia. It is the most widely planted variety in California,[1,2] the most important white wine grape in Australia (almost 25% of total Australian grape production),[3] and the main white variety and second most important grape cultivar in Chile (Table 1). In the United States, which is currently the world's main wine-consuming nation and export destination, Chardonnay accounts for 13.3% of all the wine consumed.[4]

Table 1. Hectares of Chardonnay Vines Planted and Percentage of Total Vineyard Area in Selected Wine-Producing Countries[1−3,5−11]

country	surface planted (ha)	% total vineyard area for country
France	47487	6.3
USA (California)	38475	19.9
Australia	25491	17.2
Chile	10970	8.7
South Africa	8278	6.5
Spain	6957	0.7
Argentina	6473	3.0
Germany	1388	1.4

Chardonnay first appeared in Burgundy, France, approximately 500 years ago, making it one of the oldest cultivars in the world.[12] This "ancient" variety originated from the crossing of Pinot and Gouais blanc.[13] Several clones of Chardonnay are available, some of which are favored by winemakers depending on the environmental conditions (e.g., temperature, rainfall, soil type) and the wine characteristics being sought.[12] However, according to Riaz et al.,[12] most of the variability observed among clones of the same grape variety is simply chimeric. These authors reported polymorphic markers in nine genotypes among Chardonnay clones, none of which were unique at more than one marker.[12]

Chardonnay can be considered a vigorous variety with a moderate grape yield; on average it produces 4−10 T/ha of grapes when cultivated on reasonably fertile soils. This is an early-ripening variety, better adapted to the Winkler climatic regions I (such as Burgundy, Tasmania, and Oregon; heat summation below 2500 degree days) and II (such as Bordeaux and the Yarra Valley; heat summation between 2501 and 3000 degree days).[14] Nonetheless, Chardonnay is very versatile and can adapt to different climates and soils, as evidenced by the varied sites and conditions where it is cultivated. When planted in cool regions, cropping levels should be monitored to ensure adequate ripening.[15] Disease-wise, Chardonnay is susceptible to powdery mildew, botrytis, and crown gall.[15]

This variety is genetically predisposed to a low percentage fruit set and millanderage (excessive numbers of small, ripe berries within a cluster), particularly when climatic conditions are adverse.[16] Budburst occurs relatively early in Chardonnay, around 3 days before the average onset of budburst for other varieties and 11 days before that for Semillon. Flowering also takes place early, as much as 9 days before the mean in hot regions such as the Barossa Valley in South Australia.[16] The lower buds of Chardonnay vines are sterile, so cane-pruning is more suitable for this variety than spur-pruning.[14,16] Additionally, this type of pruning is well suited for machine harvesting.[14]

Chardonnay berry clusters are small, cylindrical, and winged and can range from well-filled to compact. Berries are small, thin-skinned, round, and usually contain only one seed.[14,17] In most clones, seedless berries account for only 2% of the total bunch weight, although this percentage is higher for the Mendoza clone.[16] Chardonnay is an anisohydric variety, which

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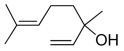
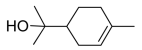
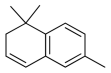
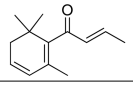
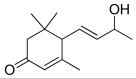
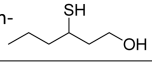
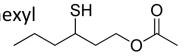
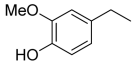
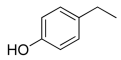
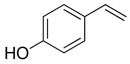
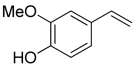
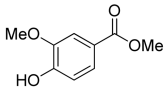
Table 2. Characteristics of Grape-Derived Odorants Important to Chardonnay Wine Typicity[a]

name and structure	aroma descriptor(s)	threshold (μg/L)	reported content in Chardonnay (μg/L)
linalool 	fruit, citrus[28,29]	25[30b]	2.0 – 142[31,32]
α-terpineol 	floral, musty, orange[33]	250[30 b]	0.3 – 181[31,34]
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) 	kerosene, petrol[35]	2[36c]	1 – 30[37]
β-damascenone 	stewed fruit, apple, peach[28,33]	0.05[38d]	66 –170[39]
3-oxo-α-ionol 	spicy[40]	N.A.[e]	19 – 2674[32,40]
3-sulfanylhexan-1-ol 	passionfruit, grapefruit[41]	0.06[41f]	0.010 – 0.148[42]
3-sulfanylhexyl acetate 	box tree, passion fruit[43]	0.004[43f]	0.006 – 0.100[42]
4-methyl-4-sulfanyl-pentan-2-one 	box tree[41]	0.0008[41f]	0.0007 – 0.023[42]
4-ethylguaiacol 	spice, phenolic[44]	33[30b]	0.2 – 50[40,45]
4-ethylphenol 	horse stable, medicinal, leather, phenolic[46,47]	440[48g]	<1 – 1194[49,50]
4-vinylphenol 	spicy, pharmaceutical[29]	180[51 g]	44 – 638[29]
4-vinylguaiacol 	smoke, phenolic[52]	40[38 d]	2.9 – 410.6[34,53]
methyl vanillate 	vanillin[46]	3,000[54f]	N.A.[e]

[a]Compounds are included in this table on the basis that they are present in grape berries or, more often, have precursors which are present in berries that are modified during fermentation or aging; that is, they are not produced de novo by winemaking microorganisms. Nonetheless, they may also be associated with other aspects of wine production, for example, storage in oak barrels. [b]11% aqueous ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid; pH adjusted to 3.4. [c]Calculated in a Chardonnay wine. [d]10% aqueous ethanol solution. [e]N.A., data not available. [f]10% aqueous ethanol solution; pH adjusted to 3.5. [g]Model aqueous alcohol solution.

means that the pathway for water to move back from the berry to the vine closes as cell vitality decreases, effectively stopping water backflow and late-ripening weight loss (shriveling).[16] Due to its anisohydric character, Chardonnay possesses a higher hydraulic conductance, which enables it to recover more rapidly from water stress after irrigation. Under water stress conditions, the decrease in stomatal conductance that Chardonnay undergoes is lower when compared to that of isohydric varieties, which in turn enables this variety to sustain a higher photosynthetic level and capacity.[18]

Chardonnay is undoubtedly a commercially important variety for the world's wine-producing nations, and its popularity among consumers is driven by the underlying chemical composition of the wines. In particular, aromas and flavors, resulting from volatile compounds derived from grapes, fermentation, and aging, are important to the quality of Chardonnay wines and, therefore, contribute to consumer liking.[19−22] The reported impacts of different viticultural and production practices that influence the aroma and flavor profiles and perceived quality of Chardonnay wines are summarized in this review. Future directions for research into factors that contribute to the differences in Chardonnay aroma composition are also outlined.

■ WINE AROMA AS AN INDICATOR OF QUALITY

Quality is a very subjective notion that, among other factors, drives the price a bottle of wine might fetch in the market. Wine quality depends not only on the physicochemical composition of the wine but also on what the consumer expects from it. Expectation will vary depending on the type of wine and from which standpoint quality is viewed from: young wine versus aged wine; producer versus consumer; low involvement versus high involvement level consumer.[23] Individual consumers will define this concept differently depending on their expectations and needs.[24] Various authors describe wine quality in terms of its fitness for purpose and the absence of faults,[19,23,25−27] but these terms are more related to production and establish only a very basic level of quality.

Journal of Agricultural and Food Chemistry Review

Table 3. Characteristics of Odorants Important to Chardonnay Wine Typicity Formed during Alcoholic and Malolactic Fermentation

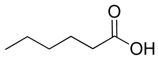
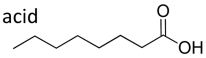
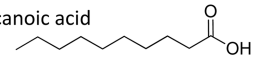
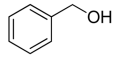
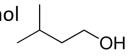
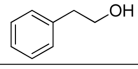
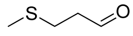
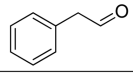
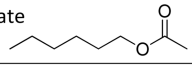
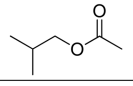
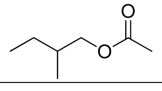
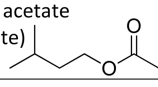
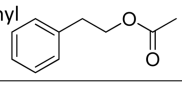
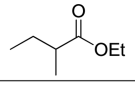
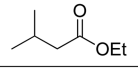
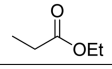
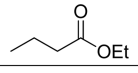
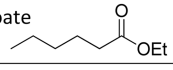
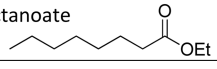
name and structure	aroma descriptor(s)	threshold (μg/L)	reported content in Chardonnay (μg/L)
acetic acid 	vinegar[68]	200000[38a]	6460 – 925000[40,62]
hexanoic acid 	rancid, pungent, green[28,52]	420[30b]	50 – 10430[67]
octanoic acid 	animal, spicy, cheese[29,44]	500[30b]	1150 – 50600[40,67]
decanoic acid 	vinegar, animal, fatty[29]	1000[30b]	40 – 14150[40,60]
benzyl alcohol 	fruity, floral[29]	10000[69c]	12 – 679[53,70]
1-propanol 	alcohol, ripe fruit[71]	306000[71d]	40 – 149000[40,67]
3-methyl-1-butanol (isoamyl alcohol) 	alcohol, harsh[72]	30000[38a]	50300 – 394900[59,67]
2-methylpropan-1-ol (isobutanol) 	green, fresh, fusel[73]	40000[38a]	160 – 45400[74,75]
2-phenylethanol 	rose[28,33]	14000[30b]	930 – 153800[40,70]
methional 	cooked vegetables[76]	0.5[76e]	N.A.[f]
phenylacet-aldehyde 	green, honey, floral, spicy[29,77]	1.0[78e]	4 – 28[29]
hexyl acetate 	apple[79]	1500[79a]	24 – 1590[63,80]
2-methylpropyl acetate 	strawberry[44]	1600[28b]	25 – 4600[59,62]
2-methylbutyl acetate 	banana, pear[81]	N.A.[f]	33 – 671[62,82]
3-methylbutyl acetate (isoamyl acetate) 	banana[29]	30[38a]	59 – 14900[63,67]
2-phenylethyl acetate 	floral, rose[73]	250[38a]	40 – 2000[67,82]
ethyl 2-methyl-butanoate 	strawberry, berry[44]	18[30b]	0.5 – 3.6[82]
ethyl 3-methyl-butanoate 	red fruit[33]	3[30b]	1.5 – 92[40,82]
ethyl propanoate 	sweet, ethereal, fruity[83]	1800[79a]	3 – 546[80,82]
ethyl butanoate 	fruity, strawberry[29,44]	20[38,84a]	60 – 1970[67]
ethyl hexanoate 	green apple, fruity, strawberry[29,33,44]	14[30b]	30 – 3960[63,70]
ethyl octanoate 	fruity, sweet[44]	5[30,38b]	30 – 3700[63,80]

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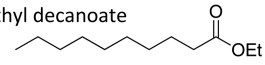
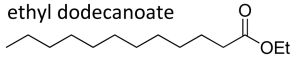
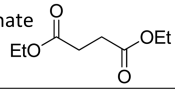
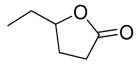
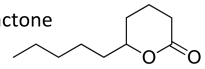
Table 3. continued

name and structure	aroma descriptor(s)	threshold (μg/L)	reported content in Chardonnay (μg/L)
ethyl decanoate 	oily, fruity, floral[83]	200[30b]	17 – 496[63,70]
ethyl dodecanoate 	oily, fruity, floral[83]	500[85g]	1 – 3100[62,63]
diethyl succinate 	caramel[29]	200000[79a]	90 – 4580[67]
γ-hexalactone 	coconut, fruit[86]	13000[79a]	N.A.[f]
δ-decalactone 	coconut, floral[29]	386[30b]	3 – 53[29]
diacetyl (2,3-butanedione) 	butter, cream[28,73]	100[38a]	173[87]
acetoin (3-hydroxy-2-butanone) 	fatty, wet, flowery, butter, cream[28,44,46,88]	150000[79a]	250 – 4800[40]
acetophenone 	flower, almond, fruity[33,89]	65[69h]	N.A.[f]

[a]10% aqueous ethanol solution. [b]11% aqueous ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid; pH adjusted to 3.4. [c]Hydroalcoholic solution. [d]10% hydroalcoholic solution, pH 3.5. [e]10% aqueous ethanol solution containing 5 g/L tartaric acid; pH adjusted to 3.2. [f]N.A., data not available. [g]14% aqueous ethanol solution; pH adjusted to 3.5 with tartaric acid. [h]Aqueous ethanol solution.

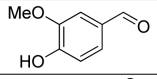
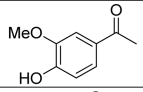
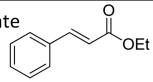
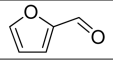
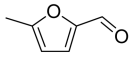
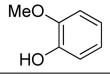
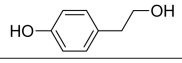
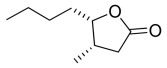
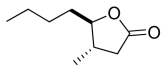
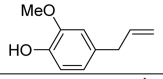
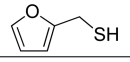
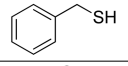
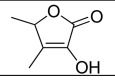
Consumers also rate quality according to how much pleasure it affords them[23] (which is too subjective to use as a parameter for production), ongoing trends, and wine flavor in particular.[23]

The flavor or "style" of a wine depends on its chemical composition; this relates to perceptions of astringency, bitterness, and acidity, among others, due to nonvolatile components, but relies particularly on the type and concentration of volatile molecules contributing to wine aroma. For Chardonnay wines, the most relevant aroma compounds are listed in Tables 2−4. A recent, but somewhat limited, study by Saliba et al.[21] of 21 commercial Australian Chardonnay wines identified at least five distinct styles (A−E), which are outlined in Figure 1. The presence of particular odorants such as diacetyl, thiols, and esters above their aroma thresholds allows the perception of attributes such as "butter", "tropical fruit", and "citrus" (Tables 2−4). These attributes in turn characterize the different styles described by Saliba et al.[21] and consequently have a major impact on Chardonnay quality and acceptance by consumers. However, the identification of strong links between Chardonnay grape and wine composition remains a holy grail. If wine producers are to fully exploit the capability of producing wines of a targeted style and quality for a specific market segment, a detailed understanding of Chardonnay impact odorants and the contribution of grape precursors and winemaking inputs is paramount in helping to address this need.

The advancement in extraction and analytical techniques has allowed scientists to delve further into the precursors and volatile molecules that contribute to the aroma of most wine varieties. However, given the wide array of existing styles and production techniques available to winemakers, the makeup of Chardonnay wine aroma and its relationship to quality have yet to be completely comprehended. Nonetheless, despite all of the possible combinations of enological techniques, studies by authors such as Jaffre et al.[55] and Ballester et al.[56] have demonstrated that a common olfactory representation (or prototype, as Ballester et al.[56] described it), independent of vintage and origin, does exist for Chardonnay wines among industry experts. It should be remembered, however, that it is the opinions and perceptions of consumers that matter in the marketplace, and these are underpinned by their notions of quality.

Ongoing research appears to be delving deeper into the understanding of Chardonnay wine quality and the different factors that affect it. The literature covers an array of fields, from studies on how quality is perceived by consumers[55,57,58] to the effect of winemaking techniques to incorporate more flavor, character, and uniqueness, such as the use of new yeast strains and sequential inoculation and thermotreatment of grapes, among others.[59−63] Particular interest exists among researchers in understanding "minerality", which is an essential (although somewhat poorly characterized) attribute of some of the most famous Chardonnay wines in the world such as those from Chablis and Burgundy.[64] Given the changes in global climate, studies are also required into the adaptation of viticultural practices and clone selection to continue producing high-quality wines, particularly as related to a drive to reduce alcohol levels. Even though anecdotal knowledge exists among producers as to the quality and style of fruit that can be obtained from each different Chardonnay clone available, there is a lack of scientific information as to the characteristics of these clones, and studies are needed on how they adapt to different terroirs and rootstocks.

Table 4. Odorants Derived from Oak Contact or Formed during Aging That Are Important to the Typicity of Chardonnay Wines

name and structure	aroma descriptor(s)	threshold (μg/L)	reported content in Chardonnay (μg/L)
vanillin 	vanilla[33]	200[38a]	3.9 – 1223[40,53]
acetovanillone 	floral, clove, vanilla[46]	1000[54b]	N.A.[c]
ethyl cinnamate 	cinnamon, sweet, floral, strawberry, plum[28,44,99]	1.1[30d]	1 – 33[40]
furfural 	almond[48]	14100[46d]	1 – 21190[40,45]
5-methyl-furfural 	warm, spicy[100]	20000[101]	trace – 37.5[45]
guaiacol 	phenolic, chemical, spice[28,33,44]	9.5[30d]	1.6 – 284[40,45]
tyrosol 	N.A.	N.A.[c]	600 – 17040[59,70]
cis-oak lactone 	coconut[77]	24[102e]	33 – 382[40]
trans-oak lactone 	coconut, oak[103]	172[102e]	10.2 – 1355 [40,45]
eugenol 	clove[48]	6[30d]	10 – 362[40,49]
2-methyltetrahydro-thiophen-3-one 	metallic, natural gas[104]	0.09[105a]	18000[106g]
2-furan-methanethiol 	roasted coffee[107]	0.0004[107f]	0.014[108]
benzene-methanethiol 	smoky, gunflint[109]	0.0003[109f]	0.03 – 0.04[109]
sotolon 	burnt, curry[28,33]	5[38a]	1.1 – 4.7[110]

[a]10% aqueous ethanol solution. [b]10% aqueous ethanol solution; pH adjusted to 3.5. [c]N.A., data not available. [d]11% aqueous ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid; pH adjusted to 3.4. [e]Neutral white wine. [f]12% aqueous ethanol solution containing 5 g/L tartaric acid; pH adjusted to 3.5. [g]As 1-octanol equivalents.

CHARDONNAY AROMA PROFILES

The headspace of Chardonnay wines has been tentatively determined to contain 243 volatiles detected through GC×GC-time-of-flight-mass spectrometry (TOF-MS),[65] which belong to a complex mixture of diverse chemical families including, but not restricted to, C_{13}-norisoprenoids, esters, alcohols, polyfunctional thiols, lactones, monoterpenoids, phenols, and acids.[39] Of these, the compounds derived from the grapes, winemaking, and aging listed in Tables 2–4 have been reported as having a positive relationship to the typicity of Chardonnay wines (i.e., the extent to which a wine is typical of the variety and is a good example of the Chardonnay wine concept), and some can be considered as character-relevant compounds for this variety.[29,55,56,66,67]

Not all of the compounds present in Tables 2–4 have an odor activity value (OAV) >1, which is normally taken as the value required for a compound to be a likely contributor to characteristic aroma. Their apparent importance to typical Chardonnay wine aroma highlights the fact that OAVs based on threshold values in a specific matrix (e.g., neutral white wine or 10% v/v aqueous ethanol) act merely as a guide when determining the importance of a compound to wine aroma, due to a significant effect of the matrix.[90–92]

When dealing with the typicity of a wine, with Sauvignon blanc being a good example, rather than search for impact compounds alone, researchers are also looking for those odorants that are essential for the wine to fit within a perceptual concept for that variety.[93] Similarly to quality, defining typicity requires a sensory analysis of samples; a number of sensory techniques are available for this purpose such as requiring the panelists to rate how well a wine belongs to a certain category and descriptive analysis or sorting of the samples, culminating with pairing and discrimination of the results based on the volatile composition of good and bad samples.[55,56,93] Other strategies to target relevant compounds include reconstitution analysis, which reveals the importance of certain volatiles to the overall aroma of the wine in question.[40] Consideration also needs to be given to the interactions among odorants, where differing concentrations of compounds in a mixture may have

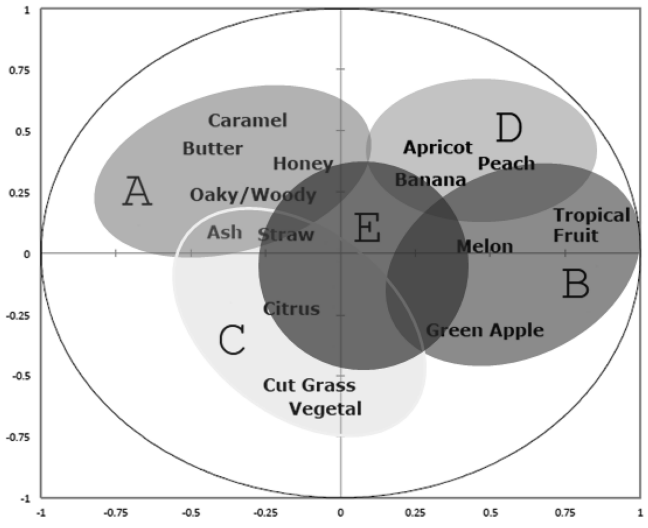


Figure 1. Representation of Chardonnay flavor profiles. A–E denote different commercial wine styles (based on data from Saliba et al.[21]).

synergistic or antagonistic effects and aromas may be enhanced or suppressed or odor quality altered as a result.[94−98]

Along the lines of seeking a perceptual concept for varietals, Louw et al.[67] analyzed 125 young unoaked Chardonnay wines, among other monovarietals from South Africa, using liquid−liquid extraction (LLE) and GC-flame ionization detection (FID), together with multivariate analysis and a "most used subset" of compounds to determine characteristic molecules that could be used to discriminate among cultivars. They showed that Chardonnay wines can be discriminated (with 74% correctly classified) by using the following eight volatile compounds: 2-phenylethanol, decanoic acid, diethyl succinate, ethyl hexanoate, ethyl decanoate, ethyl octanoate, hexyl acetate, and 1-propanol (Table 3). This prediction model, however, remains to be used to classify other sets of Chardonnay wines, particularly more developed ones, and the links between grape and wine composition for these eight specific compounds need clarification. They also observed that unlike most yeast-derived compounds (i.e., alcohols, acids, and esters such as those in Table 3), 2-phenylethanol, acetic acid, ethyl hexanoate, hexanoic acid, isoamyl alcohol, and 1-propanol were not affected by vintage, which could signify that their concentrations are characteristic of the Chardonnay cultivar. More work has to be done to confirm this, however.

Chardonnay juice does not possess any distinct aroma; however, certain precursors can be found in the must as well as the following volatile hydrolysis products: C_{13}-norisoprenoids, benzene derivatives, monoterpenoids, and aliphatic compounds as isolated by Sefton et al.[111] by reverse-phase chromatography followed by LLE and GC-MS. Although monoterpenoids (such as linalool and α-terpineol, Table 2) characterize the aroma of grape varieties such as Gewürztraminer and Muscats, Chardonnay is mostly deficient in these compounds, instead being dominated by C_{13}-norisoprenoids.[111] Studies by Lee and Noble,[40] Sefton et al.,[111] and Simpson et al.[39] suggest that the most relevant C_{13}-norisoprenoids to this variety's overall aroma are β-damascenone, 3-oxo-α-ionol, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and vitispirane.

C_{13}-Norisoprenoids such as β-damascenone and TDN are formed downstream from the oxidative cleavage of carotenoids (particularly lutein and β-carotene, with minor amounts of violaxanthin, neoxanthin, and several others), which occurs during grape berry ripening (e.g., Figure 2).[112−114] Although C_{13}-norisoprenoids can be found in their free form in the juice, they are usually present as glycoconjugates and easily degraded under acidic conditions.[115] β-Damascenone is a powerful odorant (odor threshold in model wine of 50 ng/L, Table 2) and aroma enhancer that, depending on concentration, can exhibit different odor qualities. At perithreshold concentrations, β-damascenone exhibits a "lemon balm" aroma, whereas at a concentration some 2 orders of magnitude higher it can be characterized as having "apple", "rose", and "honey" nuances.[116] Although β-damascenone is a ubiquitous wine component,[117] TDN, which possesses a "kerosene" aroma (Table 2), is typically associated with aged Riesling wines.[118] As determined by Lee and Noble[40] using GC−olfactometry (GC-O), 3-oxo-α-ionol possesses "spicy" notes at the concentrations found in Chardonnay wines (Table 2).

Polyfunctional thiols such as 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA), and 4-methyl-4-sulfanylpropan-2-one (4-MSP) are extremely potent odorants with very low aroma thresholds (60, 4.2, and 0.8 ng/L, respectively; Table 2) and aromas reminiscent of "box-tree", "grapefruit", and "passion fruit".[119,120] Both 3-SH and 4-MSP occur naturally in grapes as odorless glutathione and cysteine conjugates, which are released by yeast during alcoholic fermentation; 3-SHA is formed during alcoholic fermentation by esterification of 3-SH by alcohol acetyltransferase (AAT).[43,121,122] Although of particular significance to the typicity of Sauvignon blanc wines, these compounds are not as abundant in Chardonnay, yet still appear to be important.[42,119,123,124] The presence of other thiols such as benzenemethanethiol (BM) and 2-furanmethanethiol (FFT) at concentrations above their perception thresholds (0.3 ng/L[109] and 0.4 ng/L,[107] respectively) must also be noted (Table 4). BM has been described as having "flinty" and "smoky" (i.e., empyreumatic) notes, which may be related to Chardonnay wines' "mineral" character[64] and has an inconclusive formation

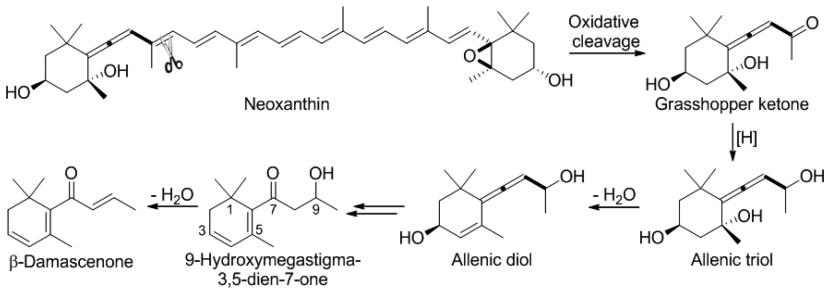


Figure 2. Formation of C_{13}-norisoprenoid β-damascenone from the carotenoid neoxanthin via oxidative cleavage, carbonyl reduction [H], and acid-catalyzed reactions. Although not depicted, glycosylated intermediates are also featured in this pathway.

pathway. On the other hand, FFT, with its strong "roast coffee" aroma, is formed from the furfural released by oak barrels and hydrogen sulfide formed during alcoholic fermentation.[125] Using a specific thiol extraction method (*p*-hydroxymercuribenzoate) together with GC-MS analysis, Tominaga et al.[109] determined that among wines from several appellation regions in France, Chardonnay possessed the highest concentrations of BM (30−32 ng/L), at 100 times above its detection threshold and 2−3 times more than all other assayed varieties. Mateo-Vivaracho et al.[42] have also confirmed the presence of suprathreshold concentrations of BM in Chardonnay (0.6−1.4 ng/L) through solid-phase extraction (SPE) and GC-MS analysis with negative chemical ionization (NCI). The different methodologies as well as the different origins of the wines employed by both research groups may account for the differences in quantities of BM found and in the hierarchy between Sauvignon blanc and Chardonnay in terms of these compounds. Comparison of Sauvignon blanc wines from different origins by Mateo-Vivaracho et al.[42] emphasized the importance that origin has on the content of all polyfunctional thiols (3-SH, 3-SHA, 4-MSP, BM, and FFT); BM concentration was highest in New Zealand wines, and FFT contents were found to be highest in French wines and lowest in those from Chile. However, these differences may be also due to viticultural and enological practices in each region. These two compounds have also been found at concentrations well above their perception thresholds in aged French Champagnes and have been shown to increase during aging.[126] Unfortunately, as with other polyfunctional thiols, analysis of BM and FFT is not a trivial undertaking (e.g., low abundance and reactive), and this may account for the fact that they have not been reported as characteristic of certain Chardonnay styles as yet.

Other important compounds are the esters (Table 3), which constitute the second most significant chemical group in the volatile fraction of Chardonnay wines. The esters are formed in excess during alcoholic fermentation by yeast metabolism and are responsible for "fruity" and "floral" aromas.[127,128] Wines contain two main types of odor-active esters: acetates of higher alcohols (formed by yeast AAT enzymes Atf1p and Atf2p, via condensation of their corresponding higher alcohols and acetyl CoA[129−131]) and ethyl esters of fatty acids (thought to be formed by esterification of the activated fatty acids (acyl CoA) during lipid biosynthesis mediated by acyl CoA:ethanol *O*-acyltransferase (EAT) enzymes Eeb1 and Eht1 together with other as yet unidentified enzymes[132]). Acetates are synthesized at higher concentrations than ethyl esters, and the ratio between both, as well as the concentration at which acetates are produced, is affected particularly by fermentation temperature, must nutrient content, and yeast strain rather than grape variety. Although both acetates and ethyl esters are liposoluble, excretion of ethyl esters through the yeast cell membrane becomes more difficult as chain length increases (only 8−17% of all ethyl decanoate produced by yeast is excreted into wine during alcoholic fermentation),[133] whereas 100% of all acetates produced are released.

The production of esters is modulated by the presence of cosubstrates and the activity of related synthesis and hydrolysis enzymes (esterases).[134] Formation of acetates depends on the concentration of unsaturated fatty acids (which inhibit AAT activity) available in the medium and carbon-to-nitrogen ratio.[134] A dynamic equilibrium exists between acetates and their corresponding acids and alcohols, which depends on the conditions of the medium.[135] Ethyl esters respond to the modification of most winemaking parameters in a similar way as acetates (albeit increases in nitrogen content or fermentation temperature have more pronounced effects on acetate formation). Higher concentrations of unsaturated fatty acids decreased ethyl ester production; however, inclusion of an additional carbon source to the medium increased only acetate production. Unlike acetates, synthesis of ethyl esters depends more on the availability of substrate (medium-chain fatty acids) rather than on enzyme expression level;[134] in turn, the amount and type of fatty acids available[136] depend on agricultural conditions and grape variety, making ethyl ester profiles more variety-dependent than that of acetates (except for hexyl acetate).[137] According to Smyth,[66] the most relevant esters for unwooded Chardonnay aroma appear to be ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexyl acetate, 2-methylbutyl acetate, and 3-methylbutyl acetate.

Moio,[138] Lorrain,[29] and Lee and Noble[40] all agree on the importance of volatile compounds such as guaiacol, *cis*- and *trans*-oak lactones, 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol, vanillin, methyl vanillate, and 5-methylfurfural (Tables 2 and 4) to the typicity of Chardonnay wines. These compounds derive mainly from aging in oak barrels, and some of them are even considered to be off-odors if they are present at concentrations above their perception threshold, as they are commonly associated with the metabolism of *Brettanomyces/Dekkera*.[47] However, some of these compounds, such as 4-vinylguaiacol, can also be found in wines that have had no contact with oak. 4-Vinylguaiacol is responsible for "smoke" and "phenolic" notes,[79] and although usually associated with aging in barrels, higher concentrations of this compound have been detected in some Chardonnay wines aged in stainless steel.[40] Spillman et al.[49] refer to 4-vinylguaiacol, 4-ethylguaiacol, 4-ethylphenol, and 4-vinylphenol as fermentation products rather than as compounds derived from oak contact. They are formed through decarboxylation of natural grape components ferulic and *p*-coumaric acids by *Saccharomyces cerevisiae* enzymatic activity[139] (hence their inclusion in Table 2). The concentration of these compounds appears to depend more on factors such as higher fermentation temperature and contact with lees than on the influence of oak wood.[140]

In their assessment of Australian Chardonnay wines, Saliba et al.[21] observed that five distinct styles resulted from the sensory analysis (Figure 1); among these, style A (a more "traditional/oaky" Chardonnay style, marked by "caramel", "butter", "honey", and "oak" attributes) and style B (a "crisp/fruity" style, with "tropical fruit", "melon", and "green apple" notes) seem to dominate the current market. From a volatile compound perspective, style A wines were marked by "woody", "smoky", "vanilla", "spicy", and "clove" odors, which are attributed to the presence of vanillin, guaiacol, 4-vinylguaiacol, furfural, and *cis*- and *trans*-oak lactones (Tables 2 and 4).[40,87] From the correlation matrix by Spillman et al.,[49] it can be inferred that the "smoky" notes present in Chardonnay wines aged in oak barriques are caused by the combined presence of guaiacol, 4-methylguaiacol, furfural, 5-methylfurfural, and, to a lesser extent, 4-ethylguaiacol and vanillin. Other oak-related aromas such as "cinnamon" and "coconut" were positively correlated to vanillin and *cis*-oak lactone (followed by guaiacol), respectively.

Unlike what might have been expected, the difference in resulting overall aroma between such A and B styles is not necessarily due to a different composition of characteristic

Table 5. Concentration (Micrograms per Liter) and Comparison of Volatile Compounds in Two Styles of Chardonnay Wines ("Fruity" and "Oaky") As Determined in Separate Studies

	Buettner et al.[87]			Lee and Noble[40]		
	fruity	oaky	factor of difference	fruity	oaky	factor of difference
ethyl isobutyrate	72.2	99.9	1.4	31		
ethyl butanoate	263	341.5	1.3	844	1040	1.2
ethyl isovalerate	9.2	19.9	2.2	42	41	1.0
3-methylbutyl acetate	943.7	163.5	0.2	519	349	0.7
ethyl hexanoate	757.2	737.5	1.0	843	600	0.7
linalool				50	31	0.6
2-phenylethanol	12415	24971	2.0	153840	116850	0.8
acetic acid	434232	489370	1.1	10000	11290	1.1
butanoic acid	1839	1611	0.9	1824	1505	0.8
trans-oak lactone	7.1	131.1	18.5	173	996	5.8
cis-oak lactone	17	214.8	12.6	33	382	11.6
2-methoxyphenol	2.7	9.9	3.7	25	284	11.4
ethyl cinnamate	1.5	3.1	2.1	3	6	2.0
eugenol	1.6	8.9	5.6	21	362	17.2
4-vinylguaiacol	50.5	49.3	1.0	1356	380	0.3
vanillin	48.5	241.6	5.0	107	1223	11.4

volatile molecules. A study by Buettner[87] of the potent odorants identified in two Chardonnay wines representative of a "traditional/oaky" and a "crisp/fruity" style (i.e., styles A and B according to Saliba et al.[21] in Figure 1) showed that they were both composed of the same volatiles, but each contained different amounts, leading to different time persistence levels for each attribute. Retronasal sensory assessment of both samples showed that the notes related to "oak" in style A were more persistent when judged by time−dilution analysis than the "fruity" ones in style B. Table 5 shows some of the results of this study, revealing that the concentration of *cis*-oak-lactone ("coconut" notes, Table 4) as measured by high-resolution (HR) GC-MS using stable isotope dilution analysis (SIDA) was almost 13 times higher in the A style wine than in the B style wine, and 3-methylbutyl acetate ("banana" notes, Table 3) was 6 times more abundant in the B style wine.

Lee and Noble[40] studied an array of California Chardonnay wines, two of which were characterized as belonging to the "crisp/fruity" and "traditional/oaky" styles (Figure 3). A comparison of their results to those obtained by Buettner[87] demonstrates similarities for certain compounds (Table 5). Both studies found concentrations of *cis*-oak lactone in the A styles that were approximately 13 times higher than in style B, as well as twice the concentration of ethyl cinnamate in the woodier styles than in the fruitier ones. The differences observed in the content of compounds related to fruity attributes were not as high as expected, and in some cases the difference found between both styles was not significant (i.e., ethyl hexanoate and ethyl butanoate). The use of aroma models and similarity tests by Lee and Noble[31] showed that even when trying to replicate wines with a high intensity of "fruity" aroma attributes, it was also necessary to incorporate "oak/spicy" volatiles (e.g., vanillin, furfural, 2-acetylfuran, *cis*- and *trans*-oak lactone, eugenol). The wines to which only "fruity" related aroma molecules (ethyl butanoate, 3-methylbutyl acetate, linalool, α-terpineol, and 2-phenylethyl acetate) were added were less representative and closer in profile to the base wine rather than the original "crisp/fruity" wine sample.

This highlights the potential importance of "oak" and "spicy" attributes to Chardonnay aroma even for "crisp/fruity" styles and explains the predominance of "woody" and "spicy" notes in

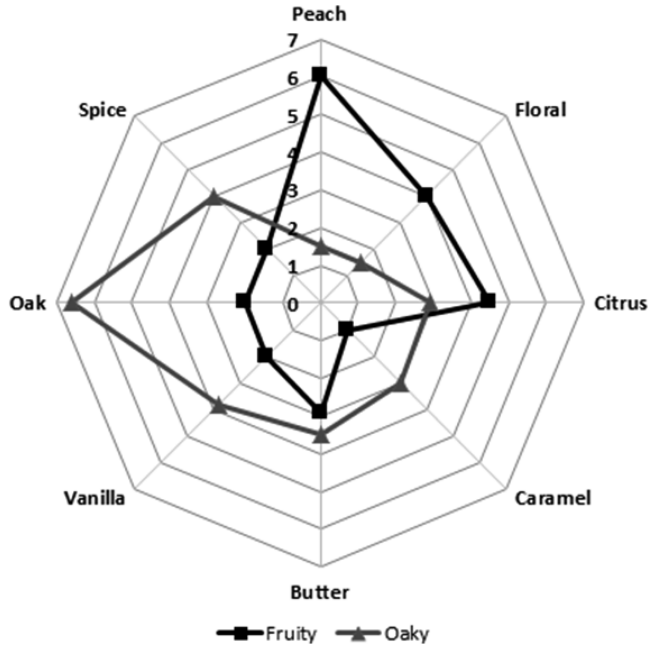


Figure 3. Aroma profiles of "fruity" and "oaky" Chardonnay wines (data from Lee and Noble[40]).

the "oaky" wine and lack thereof in the "fruity" style in the study by Buettner et al.[87] The only compounds not detected by tasters in the "fruity" sample were *cis*- and *trans*-oak lactone and eugenol; likewise, 3-methybutyl acetate was not detected by the tasters in the "oaky" wine and was, in fact, less abundant in the "oaky" style by a factor of 5.[87] The "fruity" style was also characterized by a higher detection frequency of linalool and α-terpineol,[40] which have been described as having "citrus" and "floral" notes.[40,79] Wines described as being "oaky" are seldom described as "fruity", and the work of Arrhenius et al.[37] showed a very significant negative correlation existed between the attributes "citrus" and "caramel/pumpkin" (this term is positively correlated to the presence of TDN). On the other hand, esters, which contribute "fruity" aromas, are often present in Chardonnay wines at concentrations well above their

detection thresholds; therefore, no significant differences have been observed in the detection frequency of most of these compounds between both wine styles.[40]

FACTORS AFFECTING AROMA DEVELOPMENT

A range of factors determine the aroma composition of a wine, including but not limited to grape maturity, vine nutrition, harvest method, alcoholic and malolactic fermentation, and aging. Among these, grape maturity and alcoholic fermentation are considered the most critical stages.[26] Compounds related to grape variety and typicity, such as monoterpenoids, C_{13}-norisoprenoids, pyrazines, and polyfunctional thiols, which derive from the berry itself, can vary in concentration and can be found either in free form or bound as glycosides or amino acid conjugates, depending on the grape variety.[141−143] During fermentation, *S. cerevisiae* metabolism produces many of the olfactory compounds identified as being important to wine, such as esters and fusel alcohols, from the nutrients, elements, and different compounds present in the juice or must.[141,144,145]

Juice Composition and Nutrient Content. A balanced content of nutrients in the juice (nitrogen, amino acids, lipids, vitamins, and metal cations), as well as optimum oxygen and pH levels before the start of fermentation,[62] are determinant of yeast biomass formation, yeast metabolism, and alcoholic fermentation rate. Chardonnay appears to be the variety with the most reported cases of difficult fermentations in Australia as per Schmidt et al.,[146] most probably due to low pH and, in some cases, low potassium (K) concentration. The pH of juice appears to affect yeast performance in one of three ways; either the strain is unaffected, or its performance is affected (negatively) but can be corrected by adding K to the medium, or it is affected and K cannot correct the problem. A low pH combined with a low K concentration alters the redox potential of the medium, which is then corrected by an excess production of acetic acid, which is itself inhibitory for yeast metabolism and thereby further compromises the progression of fermentation.[146]

Nutrient deficiencies increase the risk of incomplete fermentations, leading to the appearance of off-odors (e.g., H_2S and other sulfur compounds) and unstable wines prone to bacterial spoilage. By supplementing a juice deficient in yeast assimilable nitrogen (YAN), usually in the form of diammonium phosphate (DAP) as an ammonium source, lower amounts of higher alcohols (for example, those in Table 3) and branched-chained acids (isoacids) are produced, and a higher concentration of esters is synthesized, improving overall sensory quality.[75] However, an excessive addition of DAP will tend to produce wines with an undesirable "solvent" character and higher ethyl acetate and acetic acid contents.[82,147]

In terms of the type of nitrogen supplement used, Torrea et al.[82] demonstrated that the addition of inorganic ammonium nitrogen (as NH_4Cl) to Chardonnay juices resulted in an increased concentration of acetic acid in the corresponding wines, but lower quantities of higher alcohols than with other forms of nitrogen (i.e., amino acids and ammonium). When the amount of ammonium supplementation was increased, the final wine had low fruit-related aromas and high "acetic" and "nail polish remover" notes, due to acetic acid and ethyl acetate, respectively; of 16 aroma descriptors evaluated, 14 changed significantly when different types and quantities of nitrogen were supplied.[82] These results can be explained on the basis of yeast metabolism, in which amino acids may play a role in certain metabolic pathways or may act as precursors to esters.[148]

If nitrogen is supplied in the form of amino acids, greater amounts of esters will be produced and higher alcohol concentrations will decrease.[75,82] In general, higher amino acid content improves the capacity of yeast to adapt to and/or work under anaerobic conditions, activating the synthesis of fatty acids and increasing the amount of ethyl esters produced.[75] However, there appears to be no correlation between higher alcohol acetates and amino acid levels; the extent of utilization of a particular amino acid by yeast will depend on the nature of the amino acid. As an example, greater amounts of leucine (a precursor to 3-methyl-1-butanol) were utilized in fermentations in which it was supplemented, but the final concentration of 3-methylbutyl acetate was similar to that of control fermentations.[149]

It should be mentioned that not all yeasts are equally sensitive to every nitrogen source. Certain strains were revealed to be insensitive to specific sources, whereas strain 254D, which is a moderate ester producer, was very responsive to changes in nitrogen source. For example, the content of ethyl and 3-methylbutyl acetates produced by 254D was significantly higher when using DAP than when nitrogen was incorporated as amino acids. Furthermore, use of amino acids when working with 254D yielded the lowest maximum and final concentrations of acetate esters and fatty acid ethyl esters determined in the study when compared to DAP-supplemented and control fermentations.[149]

Nutrients other than nitrogen are important to fermentation performance and metabolite profile. Varela et al.[62] demonstrated that aroma composition can be affected through lipid and oxygen supplementation, thereby influencing ester and higher alcohol production in Chardonnay wines while limiting production of fatty acids, especially of acetic acid, as well as lowering acetaldehyde concentrations.[62] Furthermore, supplementation of musts with these nutrients not only stimulated yeast growth and metabolism but improved fermentation rates significantly and diminished the amount of residual sugar in the final product. Addition of either oxygen or lipids changed the proportions of acetate to ethyl esters and of branched-chain to medium-chain fatty acids while, unfortunately, also increasing, by approximately 2-fold, the amount of higher alcohols to levels oscillating between 320 and 400 mg/L. This means higher alcohols were above the value indicated by Rapp and Mandery[68] as contributing positively to wine complexity (300 mg/L). These authors also suggested that concentrations of higher alcohols in excess of 400 mg/L no longer contribute positively to the overall aroma of a wine but rather detract from it. Addition of lipids (as unsaturated fatty acids) and sterol yielded Chardonnay wines with more esters overall, whereas incorporation of oxygen (with and without addition of lipids) increased acetate esters but strongly diminished the total content of ethyl esters. In either case there was a reduction in hexanoic acid but an increase in 3-methylpropanoic acid and variable effects on the other volatile acids (excluding acetic acid, which was reduced as mentioned above). Addition of lipids can also improve the fermentation progress of overly clarified juices and avoid stuck fermentations and high acetic acid concentrations.[62,146,150]

Use of Enzymatic Preparations. Endogenous grape glycosidases have a low enzymatic activity at the pH and/or sugar content of most grape juices and are therefore capable of only a limited level of aroma release through glycoside

cleavage.[151,152] In addition, different enzymes are required to release bound volatiles such as monoterpenoids and C_{13}-norisoprenoids from their different glycosidic forms (i.e., β-D-glucosides and disaccharides).[153,154] Winemaking yeasts possess glycosidase activity[155] as highlighted by Chassagne et al.,[152] who revealed differences in the extent of glycoside hydrolysis during fermentation of a Chardonnay must. Model fermentations containing a Chardonnay glycosidic extract also showed that yeast glycosidases exhibited different extents of hydrolysis depending on the type of sugar involved (mostly glucose, arabinose, and rhamnose); by the end of fermentation 47% of glucose contained within the glycoside pool had been hydrolyzed, whereas glycosides containing rhamnose and arabinose had been almost completely metabolized.[61]

The use of enzymatic preparations with glycosidase activity has been demonstrated to increase the content of total monoterpenoids and C_{13}-norisoprenoids,[153,154] and enological preparations (of fungal origin) are available for use in winemaking to help improve aroma profiles.[155,156] Enzymes are typically added prior to fermentation during commercial winemaking (i.e., preparations used for juice extraction and clarification containing glycosidase side activities), but in a study of Chardonnay wines, glycosidases were added postfermentation, leading to increases of around 50% or more in total monoterpenoids and C_{13}-norisoprenoids.[32] Unlike grape glycosides, fungal glycosides are not inhibited under normal wine conditions[151] and have even been shown to enhance aroma characteristics when applied to dealcoholized wine containing grape aroma precursors.[157]

Winemaking Techniques. In response to current market trends and the popularity of certain wines, two marked tendencies exist now for Chardonnay aroma profiles: fruity and light styles are opposed by more flavored and complex (and possibly more evolved) styles. These disparate styles are targeted through choices made during winemaking, which have a strong influence on the overall aromas of Chardonnay wine, perhaps more so than any other white wine variety. Yeast produces esters during alcoholic fermentation, and given that they constitute one of the main groups of sensorially important compounds in Chardonnay, the choice of fermentation conditions and yeast strain will play a major role in overall wine aroma.[145] This explains why some non-Chardonnay wines, produced in the same style as a Chardonnay, have been confused by experts as belonging to the latter category during tasting.[55] In particular, Chardonnay aroma is marked by the presence of the ethyl esters of fatty acids (i.e., butanoate, hexanoate, octanoate, and decanoate) along with hexyl acetate (Table 3); the "fruity" nuances derived from variation in the concentrations of these compounds will be dependent on yeast strain, providing uniqueness to wine aroma.[158]

Chardonnay is one of the few white varieties that can endure a prolonged maceration.[159] This is fortuitous, because as shown with various grape varieties, a number of aroma compounds and precursors are located preferentially in grape skins; among them are monoterpenoids and their glycosides and precursors to β-damascenone and polyfunctional thiols.[53,160−163] Extended skin contact increases the total aroma content both in terms of concentrations of volatile molecules and as evaluated by sensory analysis.[34,164,165] Increased concentrations of linoleic and linolenic acids (precursors to C_6 compounds such as hexenals and, ultimately, 1-hexanol) have also been revealed through extended skin contact of Chardonnay musts,[166] and maceration with skins produced wines that were described as being more intense and "fruitier".[34,164] Macerated wines were also shown to be richer in C_6 compounds, particularly 1-hexanol.[34] According to Colagrande et al.,[167] the effect of C_6 alcohols and aldehydes depends on their concentration; when at low concentrations (<0.5 mg/L) their contribution is actually positive, adding to the typical aroma of Chardonnay wines, but they are also responsible for herbaceous flavors when their content is higher. The production of fruitier wines can be partly explained by the results of Dennis et al.,[137] which confirmed the role of certain C_6 compounds (1-hexanol, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol) as precursors to hexyl acetate after the respective reduction and/or acetylation. Hexyl acetate, with its pleasant "apple" aroma (Table 3), is typical of unwooded Chardonnay wines.[66]

It is worth noting that skin maceration will necessarily increase the extraction of grape phenolic compounds (i.e., nonvolatiles associated with taste, mouthfeel, and color (browning of white wine)), potentially having an adverse effect on white wine sensory properties. However, despite total phenolics being increased linearly with maceration time, no significant differences in bitterness or astringency of Chardonnay wines were found in studies employing different times for maceration treatments. After 16 or 24 h of maceration, the sensory effect of an increased level of phenolics was no different from the wines that did not undergo skin contact.[164,165] Temperature plays an important role, however, such that cooler temperatures minimize the extraction of phenols without overly affecting the extraction of aroma components.[168] Furthermore, maceration affected the pH, titrable acidity, total nitrogen and amino acid contents, and potassium, calcium, and magnesium levels.[164,165] Some of these parameters will influence fermentation performance and contribute to differences in aroma profiles.

Thermotreatment of grapes is usually reserved for red wines, where higher polyphenol and anthocyanin contents are sought for their effects on color and mouthfeel. Chardonnay juices or wines may also benefit from thermotreatments to improve the extraction of determinate compounds from grapes and accelerate the level of hydrolysis of glycosylated aroma precursors. A study by Francis et al.[61] showed that heat treatments of Chardonnay wines at around 45 °C for several weeks resulted in wines with sensory properties similar to those of wines that had been bottle-aged for several years. Thermal treatment reduced "floral", "pineapple", and "grassy" characters of the resulting wines and intensified their "oak", "honey", and "smoky" characteristics. On the contrary, heating of juices or wines for a short time (i.e., around 2−12 min) at 90 °C on a pilot scale (250 L) was shown not to produce any discernible sensory differences when compared to the unheated control wine. In either case, there were no oxidized, cooked, or maderized aromas. Thermal treatment of Chardonnay juice or wine using mild temperatures could be envisaged as a more rapid and economical alternative to ordinary aging of wines to obtain a more "evolved" profile.

A choice of fermentation vessels is available to winemakers when producing Chardonnay wines. Tanks constructed of steel, cement, or plastic as well as oak barrels and vats may be used, depending on the desired style, available infrastructure, and budget. Unlike stainless steel, barrels are not inert and interact with the juice during fermentation, resulting in higher concentrations of certain volatile compounds. According to González-Marco et al.[70] the total concentration of about 186 mg/L for higher alcohols in wines produced in new French oak

barrels was 42% greater than for stainless steel, but below the 400 mg/L total suggested as detrimental to wine aroma.[68] Ethyl acetate formed in oak barrels was approximately 32% higher, whereas other esters totaled almost 18 mg/L and were up to 4 times higher, particularly those of short- and medium-chain fatty acids, 3-methylbutyl acetate, and diethyl and monoethyl succinate, compared to stainless steel tanks. Hexanoic, octanoic, and decanoic acids were up to 6 times higher in oak barrel fermentations as well.

These outcomes can be rationalized by considering compositional differences between the fermentations in each type of vessel. Wood contains even-numbered saturated fatty acids (C2:0−C26:0), among a range of other volatile and nonvolatile compounds, which are extracted during fermentation, thus increasing the amount of esters and acids in the wine. The improved production of higher alcohols seemed to relate to a reduced consumption of amino acids in barrel-fermented wines, with the biggest differences being observed for 2-phenylethanol, benzyl alcohol, 1-hexanol, 1-propanol, 3-(methylthio)-1-propanol, methanol, tyrosol, and tryptophol.[70] Fermentation of wines in oak barrels also favors the extraction of typical oak-related volatiles such as oak lactones and furfurals, which undergo reduction to the corresponding furan alcohols because of a prolonged contact with lees.[140]

Yeast Influences. Yeasts affect the aroma composition and therefore quality of a wine in a number of ways. They synthesize odorant molecules de novo, such as esters and higher alcohols, release odorless precursors, and alter wine perception and flavor through the production of ethanol and the release of yeast constituents such as mannoproteins.[169] As well, the level of stress a yeast strain may tolerate will influence the amount of sulfur-containing malodorous compounds that will be formed in low-nitrogen situations. As outlined earlier, esters formed during fermentation comprise an important class of "fruity" aroma compounds in Chardonnay wine. Genetic variation between wine yeast strains and must composition leads to differences in expression of genes related to ester synthesis and hydrolysis.[149,169] Yeasts are also responsible for the cleavage of cysteinyl and glutathionyl conjugates and release of the corresponding polyfunctional thiols 3-SH, 3-SHA, and 4-MSP (see Table 2).[169] Typically, winemakers inoculate with selected strains of *Saccharomyces cerevisiae*, but a large diversity of yeast genera which can affect ester production exist as indigenous microflora.[170] A quick analysis of Australia's and America's major fine Chardonnay producers (based on wines with scores above 92 points as defined by James Halliday[171] and *Wine Spectator*) shows that many winemakers are choosing "natural" (i.e., wild or spontaneous) fermentations with indigenous yeasts (dominated initially by non-*Saccharomyces* yeast)[172] as a vehicle to incorporate more complexity and uniqueness into their wines. Wines from "natural" fermentations are not always scored better than inoculated wines, despite often possessing a higher aromatic intensity. This is because the use of non-*Saccharomyces* yeasts produces higher intensities of both positive and negative aromas, so scoring of the wine will depend on the balance between these compounds and the preference of the wine judge or consumer.[173]

Medina et al.[59] found that the use of commercial yeasts together with highly standardized procedures results in wines with uniform characteristics. Richter et al.[158] studied and characterized the metabolic footprint of 69 commercial yeast strains in Chardonnay wines. Despite the large number of strains studied, only four clusters, and therefore four wine aroma profiles, were identified through hierarchical clustering of their metabolites. Each cluster was identified by its particular ratio of acetate to ethyl esters, primarily due to a fluctuation in acetates. Each type of ester arises from discrete sets of precursors and enzymes, highlighting phenotypic differences among the clusters as a result of potential genetic variations. The final concentrations of esters in the wine depend on the maximum level attained during alcoholic fermentation, and the rate of formation, volatilization, and hydrolysis thereafter.[149] Esters are formed at different rates during fermentation, in particular, hexyl acetate and ethyl hexanoate, which derive from the modification of C_6 compounds by yeast. The earlier onset of production of these esters was suggested to depend on yeast cell growth, which relates to both the yeast strain and the medium conditions;[174] concentrations of hexyl acetate and ethyl hexanoate dropped significantly as fermentation progressed and ester degradation or volatilization overcame formation.[149]

New yeasts are still being selected and/or developed to provide winemakers with greater options to introduce complexity and increase diversity in aroma profiles. Strategies for the development of new strains include hybridization, mutagenesis, directed evolution, and genetic modification techniques such as the overexpression and introgression of genes related to the formation of specific aroma molecules or specific metabolic pathways.[169,175] Although genetically modified organisms are not currently permitted for use in the majority of wine-producing countries, and are anyhow met with high reticence by the public, they do constitute a very useful research tool. Gene deletions allow for metabolic pathways involved in the formation of key metabolites to be identified and comprehended, as has been the case in the elucidation of the mechanisms leading to the formation of polyfunctional thiols.[176] Even under the most favorable conditions (optimum fermentation temperature, extended skin maceration[177,178]), commercial yeast strains are able to release only 10% of all polyfunctional thiol precursors available. In response to this limitation, both Swiegers et al.[179] and Holt et al.[180] have developed yeast strains capable of releasing higher amounts of polyfunctional thiols due to enhanced carbon−sulfur (CS) lyase activity, through the insertion of the *Escherichia coli tna*A gene and by overexpressing the yeast gene *STR3*, respectively, in the commercial yeast VIN13 (considered to impart a high yield of thiols). Swiegers et al.[179] achieved a strain capable of releasing up to 25 times more 4-MSP and 3-SH than the original VIN13 strain, and Holt et al.[180] were able to confirm the CS β-lyase role of *STR3* gene product as well as design a yeast strain capable of releasing close to 10 times as much 3-SH as the original strain. Unfortunately, neither of these strains can be commercially applied.

Saberi et al.[63] isolated and studied two unique Burgundian strains individually and in cofermentations using Chardonnay juice, showing they were similar to each other and generally produced intermediate levels of higher alcohols, and ethyl and acetate esters, compared to the six commercial strains, which were more disparate to one another. The sensory profiles of the wines were estimated from descriptors and OAVs, showing the Burgundian strains produced higher alcohols (in particular, 1-butanol, 1-hexanol, and isobutanol) with OAVs of much less than 1 (minimizing fusel characters) and ethyl esters, responsible for pleasant "fruity" aromas, above their perception threshold (OAV > 1). Fermentations with mixed Burgundian strains tended to produce greater diversity in their profile of

volatile compounds, indicating some form of metabolic interaction.

Likewise, Orlic et al.[60] isolated seven indigenous strains of *Saccharomyces paradoxus* from the Zagreb region in Croatia. These strains have the capacity to produce higher levels of glycerol than *S. cerevisiae* and lower total amounts of higher alcohols. When used to ferment Chardonnay juice, *S. paradoxus* produced wines of similar or better organoleptic quality than those in which *S. cerevisiae* was used, with higher concentrations of 1-propanol and 1-hexanol and lower concentrations of isobutanol, capric acid, and ethyl acetate. However, they also produced lower amounts of 3-methylbutyl acetate and total volatile esters. Cell−cell communication and modification of gene expression through quorum sensing have been raised previously and may explain this phenomenon.[172] The results of Miller et al.[149] showed that no one yeast strain possesses a higher rate of accumulation for all of the desirable esters, so a choice has to be made according to the compounds that are to be maximized. Fermentations with mixed yeast populations therefore provide a potential means to better achieve the desired aroma outcome.

Unlike inoculated fermentations in which a single *S. cerevisiae* strain dominates throughout the whole process, spontaneous fermentations resemble more a relay course where different species and strains of yeast succeed one another as alcohol content increases and nutrients become scarcer. Sequential inoculations with chosen strains seek to imitate this progression while affording the winemaker more control over the process than in spontaneous fermentations; however, these schemes rarely incorporate more than two different yeasts. Medina et al.[59] used the apiculate yeast *Hanseniaspora vineae* (as a starter culture) together with *S. cerevisiae* to barrel ferment Chardonnay; this approach produced wines with more intense "fruity" characters and flavors than the wines fermented with only *S. cerevisiae*. Similarly, Soden et al.[181] produced Chardonnay wines with more intense desirable attributes such as "floral", "honey", and "apricot" when including *Candida stellata*. However, in both cases the concentration of ethyl acetate in the wine resulting from the sequential inoculation was almost double that of the control fermented with only *S. cerevisiae*, at levels above the perception threshold of this compound. When above its perception threshold, ethyl acetate possess a "solvent"/"varnish" aroma that is considered a fault and decreases wine quality. Contreras et al.[182] proved that sequential inoculation of Chardonnay musts with *Metschnikowia pulcherrima* and *S. cerevisiae* produced wines with lower ethanol contents as well as higher contents of esters (particularly the "pear" and "banana" smelling 2- and 3-methylbutyl acetate; see Table 3). Sequential inoculation with *M. pulcherrima* increased the concentration of higher alcohols, however, although these remained below their perception threshold, and augmented ethyl acetate 6.5-fold to a level above its perception threshold, at which it is negative to overall wine quality. As observed above, sequential inoculations can be used for a number of purposes, be it to incorporate more aroma complexity into the wine or to produce a wine with a certain desired characteristic, such as reduced ethanol content.

Unlike *S. cerevisiae*, where strains have been isolated, selected, and industrialized to minimize the production of off-odors and negative secondary products such as higher alcohols, acetic acid, and ethyl acetate, while ensuring the consumption of all fermentable sugars, similar work is still being undertaken for alternative yeasts. When using yeasts other than *S. cerevisiae*, a compromise is usually made by the winemaker in favor of the desired characteristic (e.g., trade-off with suboptimal fermentation performance). Parameters such as the optimum ratio between yeast strains and the ideal moment of inoculation with *S. cerevisiae*, depending on the desired style, have yet to be determined, and any possible synergistic effects among the different yeast species requires study. Ciani et al.[183] stated that using more than one yeast species may have unpredictable results, such as unexpected compounds or anomalous levels of components that can alter the final aroma of the wine; all of which must be tested before they can be commonly applied industrially.

White wine fermentations are typically conducted at cool temperatures (i.e., 10−18 °C), and bisulfite is a common winemaking additive used to eliminate indigenous yeasts on grapes prior to inoculation with *S. cerevisiae*. Fermentation temperature and sulfite addition are crucial when indigenous yeasts are used, however, as they are more sensitive to higher temperatures and the presence of sulfite than *S. cerevisiae*. At 21 °C, *S. cerevisiae* is 10 times more viable than any indigenous yeast, whereas an addition of 50 mg SO_2/L (as bisulfite) reduces any non-*Saccharomyces* population by a factor of 10.[173] Furthermore, for inoculated fermentations, ester formation will be affected by the level of inoculum and the sugar content (total soluble solids, TSS) of the must. When lower TSS are present, yeast should be inoculated at a higher level to maximize the ester formation potential. Due to a higher cell count and maximum percentage of viable cells, a high level of inoculation doubles the amount of esters produced in a low TSS situation compared to a low level of inoculation; however, this means that no differences can be detected from a per cell point of view. When TSS is higher and carbon energy sources sufficient, higher inoculation levels do not produce any significant effects on ester production.[127]

Despite the interesting aroma characteristics that may be obtained from using autochthonous yeasts, widespread preference for commercial options can be easily explained by the certainty they give the winemaker for the strain being inoculated and the favorable characteristics they will impart to the wine. Indigenous microflora can include a number of yeasts that produce undesired characteristics (i.e., elevated quantities of acetic acid and ethyl acetate, haziness, off-odors, etc.), that can induce wine spoilage such as *Kloeckera apiculata*, *Metschnikowia pulcherrima*, and *Brettanomyces* spp. For example, *Zygosaccharomyces bailli* produces elevated quantities of acetoin and *Saccharomyces prostoserdovii*, *Saccharomyces bayanus*, and *Zygosaccharomyces fermentati* generate flor-like films under aerobic conditions.[184]

Good hygiene and sound enological practices together with inoculation with selected commercial yeasts have basically eradicated problems with any of the aforementioned yeasts. However, given their resistance to high alcohol and SO_2 levels and low sugar content, *Brettanomyces* spp. pervade all winemaking regions. Contamination with *Brettanomyces* spp. results in higher concentrations of volatile phenols and isobutyric, isovaleric, and 2-methylbutyric acids (sweaty and cheesy aromas). Volatile phenols possess "smoky", "barnyard", "horse sweat", and "spicy" odors and arise from the decarboxylation of hydroxycinnamic acids into vinylphenols, which are then reduced into their corresponding ethylphenols.[47,184] Some strains such as *Brettanomyces intermedius* and *Brettanomyces lambicus* also have the ability to produce "mousy"-smelling 2-acetyltetrahydropyridine/pyrroline from

amino acid precursors;[185] others may produce high amounts of octanoic and decanoic acids (also known as "toxic fatty acids") that inhibit *S. cerevisiae* metabolism when present at the start of alcoholic fermentation.[184]

Bacterial Influences. Malolactic fermentation (MLF) of Chardonnay wines is a common practice in regions such as Burgundy and contributes the typical "buttery", "hazelnut", and "fresh bread" notes (and diminishes the intensity of "fresh" varietal odors such as "apple" and "grapefruit−orange")[186] associated with this variety from lactic acid bacteria (LAB) metabolites, particularly diacetyl and acetoin (Table 3). The contributions from MLF can differ due to grape variety[186] and, to some extent, the relative amounts of diacetyl and acetoin also relate to variety (thus, wine composition), with work by Flamini et al.[187] showing Chardonnay had a higher acetoin/diacetyl ratio than Cabernet Sauvignon. The desirability of this process depends on the style of wine being produced; winemakers have the choice of completely suppressing MLF, allowing it to occur only partially or on a portion of the wine, letting it take place naturally or encouraging it by adding LAB in the form of strains of *Oenococcus oeni*. In addition to deacidifying and helping stabilize wine by transforming malic acid into lactic acid, LAB also possess enzymatic activity, which further modifies wine aroma during MLF.[188] Grimaldi et al.,[189] D'Incecco et al.,[190] and Hernandez-Orte et al.[191] have confirmed the existence of glycosidase activity in *O. oeni* and its ability to release terpenes, C_{13}-norisoprenoids, and other glycoconjugates in model wine, even when MLF does not take place. This means that LAB not only contribute to the aroma of a wine through the production of diacetyl and acetoin but can also increase the amount of desirable compounds that are related to its typicity.[189]

As with yeast, development of indigenous bacteria can also have detrimental effects on the quality of the wine if production is not properly monitored and hygiene is not strictly observed. Several factors contribute to the spoilage of wines by bacteria such as high wine pH, elevated storage temperatures, insufficient SO_2, duration of MLF, strain of LAB present, and presence of residual sugar or O_2 during storage and aging.[184,192] Certain LAB, in particular *Pediococci* and *O. oeni*, possess enzymes that can decarboxylate amino acids into biogenic amines in wines with a high pH; these enzymes remain active even after the bacteria die.[193,194] These two strains are also responsible for the occurrence of "ropiness", in which high amounts of mucilaginous exopolysaccharides such as β-1,3-glucans are produced, conferring to the wine an oily appearance and a viscous texture, albeit no anomalous tastes or smells.[184,192] *Lactobacillus brevis* and *O. oeni* can cause what is known as "Tourne" disorder by fermenting tartaric acid into oxaloacetic acid, and subsequently succinic acid, or acetic acid and CO_2, causing a rise in pH, off-odors ("sauerkraut" and "mousy"), and a flat taste.[184] "Mousy" odors can also appear when 2-acetyltetrahydropyridine, 2-acetyl-1-pyrroline, and 2-ethyltetrahydropyridine are metabolized from ornithine and lysine.[195,196]

Of the eight known acetic acid bacteria (AAB) species, only *Acetobacter* sp. and *Gluconobacter* sp. are commonly found in grapes and wines.[184] These bacteria oxidize glucose and ethanol into acetic acid, acetaldehyde, and ethyl acetate.[192] Spoilage of musts occurs during pressing, when grapes are moldy and exhibit high levels of AAB.[184] Wines exposed to oxygen during aging (under *ullage* or with insufficient SO_2) are also susceptible to the development of AAB.

Contributions from Oak, Aging, and Lees. Chardonnay allows winemakers more stylistic choices than any other white wine variety; depending on the desired style, wines can be aged in oak and stored after bottling for long periods of time. Storage encompasses a number of variables (temperature, time, storage vessel, exposure to light, and closure) of which time and temperature are the most important ones,[74] because these promote and accelerate determinate chemical reactions among the different volatile and nonvolatile molecules in the wine. Unlike most other white wine varieties that are not aged, Chardonnay wines commonly spend a period of time, which may vary between several weeks and a year, in oak barrels. During barrel aging, furfural, 5-methylfurfural, furfuryl alcohol, guaiacol, 4-methylguaiacol, 4-propylguaiacol, eugenol, syringol, and oak lactone (both isomers) are extracted into wine from barrels (Table 4); the contents of 2-phenylethanol, 4-vinylguaiacol, and diethyl succinate also increase.[197]

Freshly cut oak does not contain the compounds that are desired in barrels and has too high a percentage of water; therefore, it must be seasoned and toasted before it can come into contact with wine.[198] Seasoning allows the wood to dry and prevents any shrinkage once the barrel has been formed. Compounds such as oak lactone and eugenol are formed during seasoning, and their concentration will depend on the wood's origin and where and for how long the wood is dried.[199,200] Furan aldehydes (furfural, 5-methylfurfural), vanillin, guaiacol, eugenol, and 4-methylguaiacol are degradation products formed by heat during wood cooperage from cellulose, hemicellulose, and lignin.[199] All of these oak compounds are responsible for the "smoky" and "spicy" aromas associated with barrel-aged Chardonnay wines.[140] Moio et al.[138] determined that guaiacol, derived from contact with oak, is one of Chardonnay's main impact odorants, denoting the relevance if this treatment for the typicity of certain styles of wine for this grape variety.

The amount of volatiles extracted from oak will vary according to the pH and ethanol content of the wine, as well as the barrel lot, its origin, and the number of years in use.[140] Although new French oak possesses on average 2−4 times more total volatile phenols than American oak, these compounds are formed during toasting and will depend on the degree of toasting rather than the origin of the wood. Certain compounds such as syringol, eugenol, and 4-methylguaiacol can decrease drastically between new and 2-year-old barrels to the point where no eugenol or 4-methylguaiacol may be found in wines aged in second-use barrels. Oak lactones are mainly formed from (3*S*,4*S*)-3-methyl-4-*O*-(6′-*O*-galloyl)-β-D-glucopyranosyloctanoic acid (galloylglucoside) and (3*S*,4*S*)-*cis*- and (3*S*,4*R*)-*trans*-3-methyl-4-*O*-β-D-glucopyranosyloctanoic acid during toasting and wine aging.[201] Oak lactones seem to be the exception to the general trend observed for most oak-derived compounds in wines, as the concentration of both isomers increased after 1 year of barrel use, with a more pronounced augmentation of the *cis*- rather than the *trans*-isomer. The ratio at which the *cis*- and *trans*-isomers are present will primarily depend on the origin of the wood, although seasoning can also play a role.[199] Among the oak lactones, the *cis*-isomer is more relevant to wine aroma and represents 57−90% of all oak lactones present,[202] and as highlighted by Maga,[203] only one enantiomer of each isomer can be found naturally in oak, that is, (4*S*,5*S*)-*cis*- and (4*S*,5*R*)-*trans*-oak lactones. *cis*-Oak lactone has been correlated with the intensity of "coconut" aroma in Chardonnay wine;[204] it is usually extracted at concentrations above its perception

Table 6. Concentration (Micrograms per Liter) and Standard Deviation (in Parentheses) of Odorants Relevant to Chardonnay Wine Typicity (Young, Bottle-Stored, and under Accelerated Aging Conditions)[a]

compound	young wine			stored wine (1 year; 18 °C)			accelerated aging		
hexyl acetate	72.7	(23)	a	5.6	(0.6)	b	14.1	(0.06)	c
ethyl butyrate	87.1	(7.9)		103	(11)		92.7	(1.7)	
ethyl hexanoate	674	(27)		871	(82)		830	(3.8)	
ethyl octanoate	1247	(199)		763	(48)		864	(2.7)	
ethyl decanoate	479	(35)	a	96.7	(16)	b	36.3	(1)	c
ethyl 4-oxopentanoate			a	4.52	(0.3)	b	3.40	(0.13)	a
ethyl dodecanoate	605	(47)	a	123	(10)	b	125	(14)	b
diethyl succinate	828	(47)	a	8930	(846)	b	4331	(135)	c
2-phenylethyl acetate	446	(117)	a	27.5	(9.1)	b	6.24	(3.4)	c
2-methyl-1-propanol	155	(11)	a	205	(38)	ab	286	(8.2)	b
1-butanol	15.2	(2.1)		18.4	(3.1)		22	(2.3)	
3-methyl-3-buten-1-ol	0.74	(0.2)							
1-hexanol	268	(88)		401	(98)		299	(0.9)	
(*E*)-3-hexen-1-ol	13.6	(2.7)		13.1	(4.7)		18.1	(0.4)	
(*Z*)-2-hexen-1-ol	1.56	(0.6)	a			a	4.13	(0.4)	b
linalool	5.93	(1.2)		3.35	(0.96)		2.16	(0.3)	
citronellol	4.33	(0.3)	a			b			b
geranic acid	67.1	(9.9)	a			b			b
benzaldehyde	11.5	(2.7)		4.91	(0.92)		12.9	(1.7)	
benzyl alcohol	112	(13)	a	169	(20)	b	108	(2.1)	ab
phenylethyl alcohol	5353	(13)	a	7876	(715)	b	6136	(250)	c
benzofuran			a	8.55	(1.34)	b	9.71	(0.25)	b
4-vinylguaiacol	126	(15)	a	226	(20)	b	212	(10)	b
γ-butyrolactone	19.9	(0.7)	a	18.3	(3)	a	0.06	(0.01)	b
γ-caprolactone			a	13.1	(1.49)	b	9.09	(0.42)	b
δ-decalactone	21	(2.8)	a	15.5	(2)	a	4.94	(1.2)	b
γ-undecalactone	226	(30)	a	436	(31)	b	552	(1.9)	b
furfural	5.52	(0.1)	a	69.2	(13)	b	92.8	(1.8)	b
5-methylfurfural			a	13.4	(1.8)	b	13.9	(3.0)	b
hexanoic acid	694	(8.1)	a	1019	(66)	b	827	(25)	c
octanoic acid	2545	(6.2)		2635	(7.8)		2718	(8.7)	
decanoic acid	558	(1.4)	a	381	(32)	b	400	(8.9)	b
dodecanoic acid	148	(0.7)	a	41.5	(13)	b	65.6	(2.4)	b
β-damascenone	13	(3.7)	a	14.4	(2.9)	a	45	(3.6)	b
3-oxo-α-ionol	154	(27)	a			b	99.5	(0.2)	a
3-hydroxy-β-damascenone	28.1	(5.4)	a			b	9.68	(0.28)	c
TDN			a	6.15	(0.86)	b	16.5	(0.8)	c

[a]Data from Cejudo-Bastante et al.[74] Where present, different letters across a row indicate significant differences ($p < 0.05$).

threshold (24 μg/L in white wine[102]) and at a low concentration is responsible for the "oaky" and "vanilla" aromas and of "coconut" notes when its concentration increases. The *trans*-isomer possesses a higher detection threshold of 172 μg/L (in white wine) and even when present at high levels does not seem to have an important influence on the overall aroma of the wine.[102,140,205]

As can be observed in Figure 1, Chardonnay wines can either have "smoky", "caramel", and "butter" attributes characteristic of a wine aged in oak or a more "fruity/crisp" character, dominated by "green apple" and "tropical fruit" notes. The Spearman rank-order correlation matrix by Spillman et al.[49] appears to support this claim. From the ranking of attributes the "green apple" attribute appears as very negatively correlated to guaiacol, 4-ethylguaiacol, furfural + furfuryl alcohol, and maltol and less significantly negatively correlated to 4-methylguaiacol, 5-methylfurfural, and furfuryl alcohol. "Green apple" was found to be highest in the control wine that had no contact with oak and exhibited a particularly significant negative correlation to "smoky"; the degree to which the "green apple" attribute was suppressed depended directly on the level of toasting of the barrel.

It is worth mentioning that oak barrel alternatives[45,206−208] (i.e., powders, chips, staves, plastic tanks lined with new or reclaimed oak staves) and different woods[209,210] (e.g., chestnut, beech, pine, cherry) have received attention from researchers. Although not specific to Chardonnay alone, as a whole, the use of oak barrel alternatives in particular offers an economical means of incorporating some of the desirable aroma compounds from oak without the time or expense associated with storing wine in oak barrels. The main factors to be considered when employing oak alternatives are the amount and surface area (shape and size) of the materials used. Chip-treated and barrel-aged wines differ in their contents of a range of volatiles, including vanillin, guaiacol, and furfural,[211] which translates accordingly into different aromas and flavors such as more pronounced "coconut" and "vanilla" together with more bitterness and astringency in white wines treated with chips.[45,212] Use of chips may also decrease the amount of perceived "fresh" and "unripe fruit" aromas and increase the

overall concentration of esters and wood-related compounds as well as the intensity of "oak" aromas in the treated wine.[213] In general, relative to unoaked wines, the use of oak chips improved the liking of wines by the assessors in a number of studies,[45,211,213] which makes it a useful and cost-effective technique for incorporation of oak into certain Chardonnay wines.

Unlike oak, glass is inert, and once a wine is bottled, it is deemed ready to be consumed. Nonetheless, bottle-aging encompasses a different set of changes from those taking place when wine is stored in barrels. For instance, from studies performed on Champagne wines, it is known that the concentration of FFT increases proportionally to the time spent in the bottle, whereas BM reaches a peak at 13−15 years, after which its concentration falls.[126] With regard to other aging phenomena, esters are particularly affected due to their acid−ester equilibria;[214,215] for a wine that had spent 1 year stored in a bottle at 18 °C, Cejudo-Bastante et al.[74] found a significant decrease in the concentration of acetates and certain short-chain fatty acid ethyl esters (C_8−C_{12}) responsible for "fruity" aromas of Chardonnay wines. As evidenced by those results (Table 6), time (storage period) and temperature (accelerated aging) introduced several of these changes in the aromatic profile of Chardonnay wines. Modifications included a decrease in acetates and short-chain fatty acid ethyl esters of octanoic, decanoic, and dodecanoic acid, and the disappearance of 3-methyl-3-buten-1-ol, citronellol, geranic acid, (*Z*)-2-hexen-1-ol, 3-hydroxy-*β*-damascenone, and 3-oxo-*α*-ionol (for the latter three the disappearance was true only in the case of storage time), thereby changing the aromatic profile of the wine. Storage time and accelerated aging induced the appearance of benzofuran, ethyl 4-oxopentanoate, 4-hydroxyhexanoic acid lactone (i.e., *γ*-caprolactone), 5-methyl-2-furancarboxaldehyde (i.e., 5-methylfurfural), and TDN. These compounds are responsible for some of the tertiary aromas of wine, and therefore wines containing high quantities of these volatiles are referred to as more "evolved".

Aging can take place in the presence or absence of yeast lees (autolyzed yeast cells). Aging on lees (often in barrel, but also in tank or bottle as is the case for Champagne) improves the organoleptic score of wines (including overall quality, persistence, color, and taste, as well as aroma) and attenuates the impact of wood on the wine's aroma.[106] During yeast autolysis, polysaccharides (mainly mannoproteins), amino acids, assimilable nitrogen, and other cell contents are released into the wine.[216,217] The presence of lees contributes to the formation of "floral" and "fruity" scented ketones and lactones and to an increase in monoterpenoid concentrations due to the release of *β*-glucosidase enzymes acting on precursors.[106] However, this technique also results in wines with a lower global ester content as yeast autolysis releases esterases, which hydrolyze some of the esters present, into the medium.

During aging, lees both adsorb positive compounds such as esters and glycosylated compounds and strip the wine of off-odors such as volatile phenols and malodorous sulfur compounds (e.g., methanethiol and ethanethiol; however, hydrogen sulfide, dimethyl sulfide, and thiophene were not absorbed).[218,219] In general, aging on lees creates a highly reductive environment that both protects the wine from oxidation and increases the possibility that these negative sulfur volatiles will be formed. This latter effect can be countered by frequent stirring to resuspend lees and create a limited oxidation, as well as by performing an early racking of the gross lees before any reduction odors appear. With time, lees do lose their ability to generate foul-smelling sulfur volatiles as sulfitoreductase activity progressively subsides.[220] Furthermore, both adsorption and hydrolysis reactions are enhanced when lees are stirred.[152,217] When volatile compounds are present at low concentrations, mannoproteins have been observed to enhance the perceived intensity of a number of aroma attributes in Chardonnay wines.[216] However, this effect on overall aroma and individual aroma attributes is not discernible when volatile compound concentrations are high.[106]

The presence of lees also affects the absorption of wood volatiles. Towey and Waterhouse[197] showed that during the last 60 days of a 150 day aging trial, the adsorption on lees of oak volatiles exceeded that of extraction by the wine. This implies that aging on lees decreased the overall oak volatile content in wine, which can be either positive or negative depending on the style of wine sought, the degree of toasting, and the age of the barrel.

Terroir: Regional and Environmental Effects. Terroir has been proven to be an important element in wine aroma profiles. Enological variables aside, each wine-producing region will impart certain aromatic characteristics to the wines produced through the unique combination of weather, soil composition, latitude, longitude, altitude, and viticulture practices.

Fruit set, ripening, and the accumulation and concentration of aroma precursors and free compounds in the berry are affected by environmental and agronomic practices such as canopy management, fertilization, and yield.[26,221−224] Berry development is very susceptible to changes in solar radiation, temperature, rainfall, irrigation, soil composition, and altitude.[225] In particular, sunlight seems to have the most impact on monoterpenoid and C_{13}-norisoprenoid concentrations among all cited parameters; an increase in sun exposure increases the amount of glycosidic aroma precursors present.[116,226] As mentioned previously, C_{13}-norisoprenoids arise from the enzymatic breakdown of carotenoids; if the berry receives a higher amount of sun exposure before veraison, the concentration of carotenoids will increase. If exposure to sunlight is higher after veraison, it will generally accelerate carotenoid breakdown and induce an increase in the amount of glycosylated C_{13}-norisoprenoids present.[227,228] Likewise, higher sunlight exposure increases monoterpenoid concentrations, with this effect being more pronounced in cooler years.[229] It must be noted, however, that the opposite seems to be true for the ubiquitous C_{13}-norisoprenoid *β*-damascenone, at least in the case of Cabernet Sauvignon. As Lee et al.[228] observed, concentrations of *β*-damascenone were highest and those of TDN, lowest, at lower sunlight exposure and temperature.

Arrhenius et al.[37] showed that within wines of a higher quality, sensory attributes were significantly correlated to the wine's origin. In turn, they found that aroma attributes were correlated only to secondary volatiles, such as 3-methylbutyl acetate with the term "green-apples/pears", TDN with "honey", and ethyl 2-hydroxypropanoate with "buttery". In fact, high-quality Chardonnay wines from the Carneros and Napa regions in California could be discriminated from other California wines on the basis of the concentration of 3-methylbutyl acetate, the level of which is unique to each of these regions. Environmental factors could be responsible for differentiating the regions; for example, the Carneros region has a different photoperiod from the Central Coast of California that may affect the rate of formation and accumulation of certain

compounds. This region could further be differentiated from the rest because of higher linalool content.[37] Williams et al.[230] and Marais et al.[231] observed that certain degradation products of carotenoids can be used as geographic markers for Chardonnay wines, because their concentrations vary depending on the temperature of the region; this is likely to be true of Australian Chardonnays, which seem to possess a distinct concentration of C_{13}-norisoprenoids that yield characteristic sensory attributes.[111,230]

Changes in environmental conditions between different vintages also affect the volatile composition of wines from a single vineyard (i.e., vintage effect). Louw et al.[67] showed that, with the exception of 2-phenylethanol, acetic acid, ethyl hexanoate, hexanoic acid, 3-methyl-1-butanol, and propanol, the level of 20 of 26 volatiles varied significantly during three consecutive South African vintages. That is, changes to fatty acids and their ethyl esters, along with higher alcohols and their acetates, were deemed to be caused by vintage effects. These compounds are associated with the volatiles derived from fermentation, implying an underlying change in grape composition (e.g., differences in amino acids and other nutrients) that was materialized upon fermentation.

Depending on intensity and timing, water deficit induces a reduction in Chardonnay wine quality, generating wines with high pH and low acidity and prone to atypical aging (i.e., wines with a lower intensity of varietal aromas and evident faults reminiscent of "acacia blossom", "furniture polish", "medicinal", and "naphthalene").[232,233] Atypical aging can be caused by suprathreshold levels of 2-aminocetophenone and other compounds such as sotolon that remain to be conclusively identified.[233−235] Research conducted by Reynolds et al.[236] showed that Chardonnay wines made from vines irrigated for different lengths of the season possessed higher intensities of "apple" and "citrus" compared to the nonirrigated controls and lower "earthy" aromas in three of four cases; however, consistent with the controls, wines showed greater atypical aging potential when the vines were subjected to irrigation up until lag phase or veraison. From their results, water stress could have interfered with the ability of grape berries to synthesize and accumulate monoterpenoids and ester precursors and may have promoted accumulation of compounds responsible for the atypical age character depending on the timing of water stress.

The characteristics of the soil where vines are planted is another environmental variable to consider when effects on wine composition are evaluated.[237] Soil properties can influence the depth that roots can penetrate, the availability of micronutrients, the retention of water, and therefore the level of hydric stress the plant is subjected to. All of these variables can affect the overall aroma of the subsequent wines. As Reynolds et al.[238] demonstrated, vineyards with high clay textures tended to yield Chardonnay wines with "vegetal", "earthy", and "citrus" characteristics, whereas wines made from grapes grown in sandy soils had "floral" and "melon" aromas. Although this study provides some guidance, at times there was a relationship between vine size and soil texture, and it was possible that vines in high-clay zones experienced some level of water stress. However, the results highlight the need to consider the impacts of the multiple facets of terroir on wine quality.

Weather can be partly countered through different agricultural techniques such as leaf removal and crop thinning. Leaf removal is particularly recommended in cold and cool climates to decrease the presence of "green" and "unripe" sensory attributes in the wine.[239] Under these conditions, leaf removal has been shown to increase the positive "floral" and "fruity" attributes. The effect of this treatment depends on when in the berry development cycle it is practiced; when applied during fruit set and veraison, the results are similar.[239] In areas with a high luminosity and potential for sunburn, a shaded bunch zone is preferable because a combination of high light intensity and temperature of the berry surface between 40 and 43 °C for only 5 min is enough to cause sunburn of mature Chardonnay berries. Sunburn damages the skin of the berry, decreasing quality and increasing concentrations of total phenolics, hydroxycinnamates, flavonoids, and tannins,[240−242] whereas under high light intensity and temperature conditions, shading can lead to crisper and more elegant wines that have more "citrus", "apple", and "quince" aromas.[16,224]

FUTURE DIRECTIONS

Given the popularity of Chardonnay wines and the global economic relevance of this variety, producers are always striving to produce better wines that cater to the current market trends. Understandably then, much work has been done to comprehend the volatile compounds present and the impact of each production step on the final aroma of Chardonnay wines. Over the years, quality has improved such that, for consumers, finding a good-quality wine is now extremely easy, but at the same time a certain homogeneity of flavor has appeared in the market, with two dominant styles arguably being responsible for most Chardonnays available in the market. In an industry where brand recognition goes only so far, uniqueness of the product is necessary. Determining whether this uniqueness is achieved at the vineyard level or via increased extraction of grape aroma compounds or through the use of different winemaking techniques requires more emphasis and understanding of the terroir dimension as well as a search for originality through the use of different yeast strains while preserving the typicity of Chardonnay.

Despite the definite existence of a typicity concept for Chardonnay, this has only started to be comprehended and requires deeper studies as this is a variety that can express itself in a multitude of profiles; Chardonnay is not as straightforward as other varieties (i.e., Sauvignon blanc or Muscats), which are dominated by distinct varietal compounds. Tackling its typicity requires multidisciplinary studies that analyze a number of variables responsible for the final product, rather than a one-directional focus.

Excellent advances in compositional knowledge have been detailed above, yet studies need to be carried out to confirm whether vintage-related changes between fatty acids and higher alcohols and their corresponding esters also occur in Chardonnay produced in different parts of the world and whether a trend can be determined. A better comprehension of the fate of aroma compound precursors extracted from the grapes is still required, as is the combined effect of the many components of terroir and how the most important ones are best manipulated and controlled. More work is therefore needed to correlate viticulture management with aroma/sensory profiles of the wines. In addition, very little is known of the potential of currently available Chardonnay clones, their interrelation with rootstocks, and any new clones that might be in development as related to other parameters such as yield, salt and drought tolerance, and vigor. Climate change will demand transformation and adaptation to new weather conditions with new choices that preserve the current quality standards.

Due to health concerns and taxation implications, as well as in response to the augmentation of alcoholic strength due to global warming and opting for riper fruit, low-alcohol wines and dealcoholization techniques have become a prime concern for the industry. Viable and cost-effective techniques that yield palatable and high-quality wines need to be formulated and tested on Chardonnay to determine the best alternative for producers. In a similar way, higher rates of individuals being susceptible to SO_2 and other industry additives, as well as dietary restrictions, pose new challenges for the wine industry, which will require the help of researchers to be solved.

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ABBREVIATIONS USED

T, tonnes; ha, hectares; TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene; GC, gas chromatography; GC-O, gas chromatography−olfactometry; TOF, time-of-flight; OAV, odor activity value; LLE, liquid−liquid extraction; SPE, solid phase extraction; NCI, negative chemical ionization; FID, flame ionization detector; HR, high resolution; SIDA, stable isotope dilution analysis; 3-SH, 3-sulfanylhexan-1-ol; 3-SHA, 3-sulfanylhexyl acetate; 4-MSP, 4-methyl-4-sulfanylpropan-2-one; BM, benzenemethanethiol; FFT, 2-furanmethanethiol; YAN, yeast assimilable nitrogen; DAP, diammonium phosphate; TSS, total soluble solids; MLF, malolactic fermentation; LAB, lactic acid bacteria; AAB, acetic acid bacteria

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■ NOTE ADDED AFTER ASAP PUBLICATION

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Summary of research aims

The main goals of this project were to develop objective measures to assess the quality of Chardonnay in relation to berries, juice and wine. Objective parameters and applicable tools are required in the vineyard, where the current grading system is highly subjective. The current approach does not encompass dimensions of grape quality such as aroma potential or amino acid composition that are relevant to wine production and are determinants of quality of the ensuing wine. In the winery, these tools are required as part of a decision making suite that can aid the winemaker to steer production of each wine to a particular consumer segment, or help to react to deviations in the fermentation process. To achieve this, the project had the following specific objectives:

1. Design and distribute a survey amongst winemakers to determine the current production practices related to Chardonnay wines in Australia.

2. Determine the main volatile compounds that influence Chardonnay wine quality and develop a predictive model for Chardonnay wine quality based on important volatiles.

3. Create a quality index for Chardonnay grapes that can be used in the vineyard to determine optimum harvest date according to desired wine profile and berry aroma composition.

4. Calibrate an IR spectrometer method capable of analysing Chardonnay grape and juice quality parameters in the field/cellar in a rapid, timely and affordable way.

<u>Objective 1: Survey of current winemaking practices for Chardonnay in Australia</u>

The quality of a wine is affected by all the operations included in its production such as harvest, fermentation vessel, yeast, etc. In order to determine which parameters were the most relevant to the overall study and which variables would be of most impact, a survey comprising 21 questions was distributed by email to wineries and vineyards producing Chardonnay in Australia. Responses showed trends in methods of assessment of grape maturity, criteria for allocation of fruit to quality level, nitrogen regime, choice of yeast and fermentation vessel, use of oak, and wine styles. Details of this study can be found in the publication presented in Chapter 2.

<u>Objective 2: Relating expert quality ratings of commercial Australian Chardonnay wines to volatile composition and production method</u>

Wine aroma is one component that is assessed when evaluating the quality of wine. Knowledge of the compositional markers that are driving the quality of a wine, and in particular the volatile molecules contributing to aroma, can contribute to the understanding of the processes that determine quality, and can assist in modulating practices that may have a negative impact. Commercial Chardonnay wines, comprising oaked and non-oaked wine selected from Australia's main winemaking regions, were assessed by an expert panel and analysed by HS-SPME-GC-MS. Thirty nine volatiles were studied in each wine and related to the quality score generated by the panel, as well as to price and production method. A prediction model was developed using partial least squares regression (PLS) in

order to classify wines into low, medium and high quality according to their volatile composition. Further details can be found in the publication presented in Chapter 3.

Objective 3: Creation of a quality index for Chardonnay grapes

The main criteria used to determine white grape maturity is pH, titrable acidity and soluble solids content. As valuable as these parameters are, studies have shown that they are not necessarily related to the accumulation of other metabolites that may impact quality, such as aroma precursors or free volatiles. Determining which variables relate to different regions and levels of grape quality will aid in the development of models to predict quality in the vineyard based on objective criteria such as grape chemical composition. Grapes were collected at commercial maturity from vineyards across South Australia (Adelaide Hills, Eden Valley, Clare Valley, Barossa Valley, Langhorne Creek, Riverland and McLaren Vale) during the 2014, 2015 and 2016 vintages. Berries were analysed for titrable acidity, soluble solids, pH, fatty acids, amino acids, elements, free volatile compounds, and glycosidic precursor content. Samples collected from different regions were also vinified on 500-mL and 5-L scales for chemical and sensory analysis. Wine samples were analysed by HS-SPME-GC-MS as in the previous study, and selected samples underwent sensory descriptive analysis. Results showed markers in the grapes and wines that could be used to discriminate between the different regions, and a prediction model was developed using partial least squares regression (PLS) and discriminant analysis (DA) to classify grapes into different quality levels. Details of these studies can be found in the publications presented in Chapter 4 and Chapter 5.

Objective 4: Infrared analysis of grapes and juice

Most of the techniques used in a research laboratory are incompatible with routine winery operation, particularly during vintage. Industry requires methods of analysis that do not necessitate complex sample preparation, and where results can be obtained rapidly and for low cost of analysis per sample. Calibration of mid- and near-infrared spectrometers (MIR and NIR) affords the possibility to discriminate samples with little to no sample preparation in a matter of seconds. Preliminary work was started using Chardonnay grapes and juice collected from three vintages (2014-16) to calibrate an MIR spectrometer, using the fingerprint region (1500-800 cm^{-1}). These results were correlated to other chemical parameters such as soluble solids (°Brix) and pH through PLS. The fingerprint region was also used to classify the samples into their regions of origin and quality grades through PLS-DA and DA. Details of the progress of this aspect of the project can be found in Chapter 6.

CHAPTER 2

Australian production practices for Chardonnay wine

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http://www.winetitles.com.au/

2.1 Introduction

Chapter two covers the results of a survey distributed amongst Australian Chardonnay grape and wine producers. The most salient results were highlighted and discussed. The aim of this survey was to determine the current production practices relating to Chardonnay wine and whether these changed according to bottle price, which was used as a proxy for quality. The survey distributed amongst producers is presented at the end of this chapter.

The sample population consisted of Australian Chardonnay grape and wine producers, estimated to number 1500 based on data published in the 2013 Australian and New Zealand Wine Industry Directory (Major, 2013). It was envisioned to collect at least 90 valid responses (6%), which would ensure at least a 95% confidence level in the analysis of the data.

A research questionnaire was developed to collect data of a predominantly qualitative nature. The survey included a total of 21 questions relating to Chardonnay wine production methods. Questions were constructed based on relevant demographic information from Wine Australia (2013) and knowledge of common practices used to produce Chardonnay wines. Experts in the field (including professors and winemakers) were consulted to validate the relevance of the proposed questions. The survey was constructed and collected using the internet survey facilitator Survey Monkey® (Palo Alto, Ca, USA). The survey was approved by the University of Adelaide Human Research Ethics committee (study No. H-2013-078) and the principles of the National Statement on Ethical Conduct in Human Research (NHMRC, 2007) were observed. Anonymity and confidentiality were assured. Contact information for further questions was made available for the researcher, the project coordinator and the Human Research Ethics Committee of the University of Adelaide. Consent was assumed on the basis of a returned and completed questionnaire.

Invitations to participate in the survey were distributed online via email on October 2013 and a reminder was sent two weeks later. This email included a weblink to direct participants to the survey and relevant information on the project. Participants were approached only after verifying that their winery produced Chardonnay wines as part of their global offer. Contact information for participants was obtained from the 2013 Australian and New Zealand Wine Industry Directory (Major, 2013) and internet.

The resulting data was analysed with Microsoft Excel 2013. The percentage represented by each answer within a question was calculated by summing alike answers and dividing the result by the total number of answers.

Major, M. (ed) (2013) The Australian and New Zealand Wine Industry Directory: 31st annual edition. 31st ed. Winetitles, Adelaide
National Health and Medical Research Council (NMHRC) (2015) National statement on ethical conduct in human research (2007)(Updated May 2015). Commonwealth of Australia, Canberra.
Wine Australia (2013) Market insights listing, accessed on 13 June 2013. https://www.wineaustralia.com/market-insights/search?category=GrapeAndWineProduction

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Principal Author

Name of Principal Author (Candidate)	Joanna M. Gambetta		
Contribution to the Paper	Designed the survey, contacted respondents, collected and analysed all data, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4/11/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Susan E.P. Bastian		
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Contribution to the Paper	Supervised the work, contributed to the research idea, and experimental design. Assisted in the preparation and editing of the manuscript.		
Signature		Date	4/11/2016

Snapshot of Australian production practices for Chardonnay wine

By Joanne Gambetta, Sue Bastian and David Jeffery, School of Agriculture, Food and Wine - Waite Campus, The University of Adelaide

How does your winery's handling of Chardonnay in the vineyard and winery compare with the rest of the Australian wine industry?

A PhD project titled 'Development of quality assessment tools for Chardonnay in relation to grape, juice and wine composition' began in 2013 with the main aim of determining objective parameters that could be measured in Chardonnay grapes and used to predict the final quality of the ensuing wine. As part of the initial scoping for the project we published a review on factors that influence Chardonnay wine aroma composition (Gambetta *et al.* 2014).

We chose to focus on Chardonnay due it being an important grape cultivar in Australia. According to the 2015 Vintage Report published by the Winemakers' Federation of Australia, Chardonnay continues to be the main white grape variety crushed. It accounts for 22.54% of the total of all varieties, or 376,339 tonnes; from 2014 to 2015 it saw an increase of 28,726 tonnes, and is second only to Shiraz (23.46% of total, 391,649 tonnes). In relation to the quality aspect of the project, there is impetus to build Australia's reputation as a fine wine producer (Gartry 2016), which necessitates the development of objective measures of wine quality and a better understanding of how quality can be managed, starting in the vineyard. As an initial activity of the PhD project, a survey of industry stakeholders was conducted to gain insight into current vineyard and winery practices associated with producing Chardonnay wines. The survey that was used may be viewed at https://www.surveymonkey.com/s/Chardonnay_wine_quality_survey

DISTRIBUTION OF SURVEY RESPONDENTS

Only wineries and vineyards producing Chardonnay were contacted, and more than 150 responses (around 10% of all Chardonnay producers (Major 2013)) were received from participants across Australia's wine-producing regions. ▸

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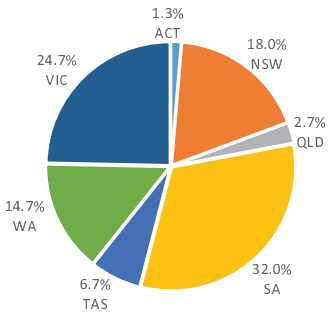

Figure 1. Distribution of survey respondents.

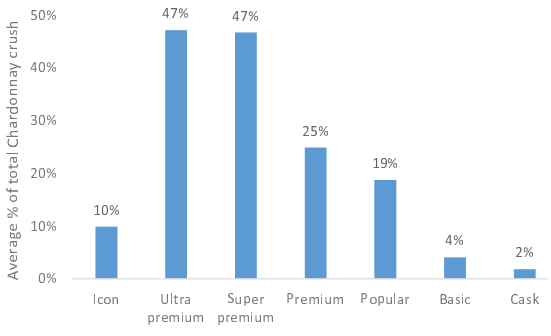

Figure 2. Proportion of Chardonnay quality levels.

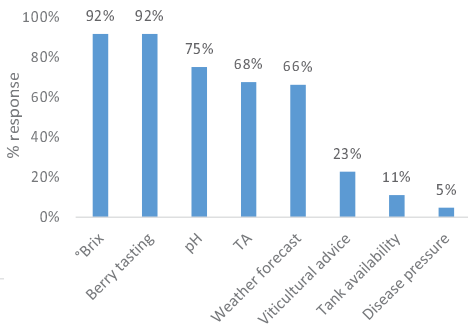

Figure 3. Harvesting criteria.

Approximately 57% of respondents were concentrated in South Australia (SA) and Victoria (VIC) and another 33% were located in New South Wales (NSW) and Western Australia (WA) (Figure 1). Our data roughly mirrors that published by the Australian Bureau of Statistics for the years 2014-15, which shows that 42% of all Chardonnay grapes grown in Australia were produced in SA followed by NSW (31%) and Victoria (24%). More specifically, 108,630 tonnes (32%) were produced in the Riverland (SA), 61,583 tonnes (18%) in Murray Darling (NSW/VIC) and 58,709 tonnes (17%) in the Riverina (NSW).

WINE QUALITY SEGMENTS

The ultimate quality of a wine encompasses a number of different dimensions – quality of raw materials, winemaking operations, sensory properties, packaging, etc that are often determined according to the market segment the wine is destined for. These different quality levels are usually related to price, where the Chardonnay wine allocation has been roughly segmented into icon (>$50), ultra-premium ($25-50), super premium ($15-24), premium ($10-14), popular ($5-9), basic (>$5) and cask wines. Any one producer will decide upon a mix of quality of their products which, in turn, will determine the types of operations carried out in the winery and sometimes also in the vineyard. Figure 2 shows that the Chardonnay offerings in Australia are dominated by the ultra and super premium range ($10-24), which accounted for 47% each. Cask (bag-in-box) only represented 2% of the respondents' total offerings, which indicates that Chardonnay production is already aimed at a higher quality configuration. This seems to correspond to both an adaptation in Chardonnay wine offerings, which had been experiencing a multi-year decline in domestic sales, and to profitability issues. According to Wine Australia's Annual Report (2012-13), the decline in sales (at least in the reported period) was centred in the lower end of the price spectrum, while the sales of Chardonnay wines priced over $20 were growing at double digit figures. Additionally, Keys (2010) explains that the production of low end Chardonnay wines is unsustainable due to production costs.

ASSESSMENT OF GRAPE MATURITY

One of the main drivers of wine quality is the condition of the fruit used for production. This, in turn, depends on critical decisions made in the vineyard, including the question of when to harvest. Measurement of sugar content continues to be one of the main criteria used in industry to determine Chardonnay berry maturity (92% of respondents, Figure 3). However, producers equally rely on berry tasting to determine when to harvest. Other criteria used include, in order of importance, pH (75%), titratable acidity (TA, 68%), weather forecast (66%), viticultural advice (23%), tank availability (11%) and the sanitary condition of the grapes and vineyard (5%).

Unlike red varieties, where strong correlations have been shown between the content of polyphenols and anthocyanins and final wine quality (Somers and Evans 1974), predicting the potential quality (at least in terms of harvest decision) of white wines remains a more complicated (and costly) task. The extent of berry tasting reveals that Chardonnay producers are looking at other characteristics, such as aroma profile, to complement their decision-making rather than relying on sugar content and other classic parameters (pH, TA) alone.

Berry tasting is carried out in the vineyard – however, with great variability in how this practice is conducted by each producer – and there is no way to quantify or compare results between different tasters. In the last decade, however, institutions like the Institut Cooperatif du Vin (ICV), Institut Français de la Vigne et du Vin (IFV), Institut National de la Recherche Agronomique (INRA), and the Ecole Superieure d'Agriculture d'Angers (ESA) have developed a more structured approach to this practice, which includes a scoresheet (Rousseau and Delteil 2000, Guyot and Dupraz 2004, Le Moigne *et al.* 2011) that enables the grape producer to qualify the degree of ripeness for each block of grapes assessed (Le Moigne *et al.* 2011). Correlations have been found between seed colour and berry ripeness (Olarte *et al.* 2012), and the ease of detaching the pedicel as well as skin thickness, amongst other variables, can be indicative of maturity (Rousseau and Delteil 2000). Different producers focus on different aspects of the berry and we were curious to know which of these were perceived as the most important. The responses show they are mostly concerned by the fruit's flavour intensity (98%), seed colour (60%), berry colour (56%), tannin ripeness (36%), pulp consistency (comprising the level of integrity of the pulp as well as its overall texture, which can go from firm to watery, 32%), skin thickness (28%), acid balance (9%) and flavour profile (8%) (Figure 4).

ALLOCATION OF FRUIT TO QUALITY LEVEL

Understanding how fruit is allocated to each quality bracket could perhaps allow us to appreciate the factors surrounding berry and juice composition that are most relevant to wine producers. Figure 5 shows that the predominating criterion used to allocate fruit is historical parcel records (56%), closely followed by the result of berry tasting (51%), and then wine style quotas (39%), acidity (26%) and sugar content (20%). However, a number

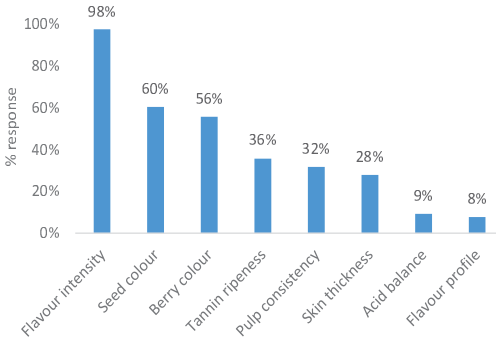

Figure 4. Important aspects of berry assessment.

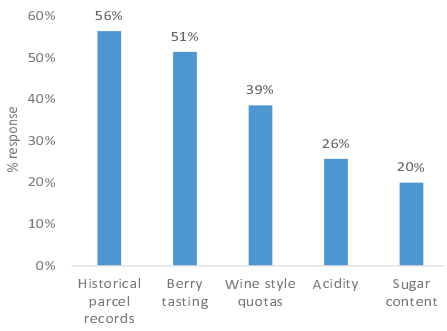

Figure 5. Fruit allocation criteria

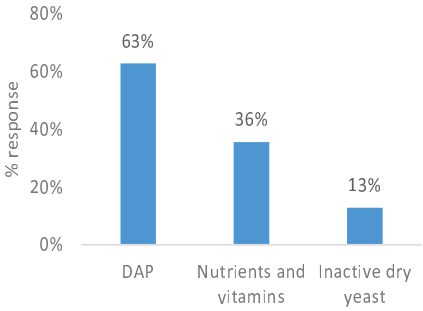

Figure 6. Nitrogen supplementation.

of respondents indicated that allocations were made only on the final wine, which means that no different considerations are made between parcels of fruit with regards to quality. Other criteria mentioned were market, vineyard location and management, wine style, and clone.

NITROGEN MANAGEMENT DURING FERMENTATION

Chardonnay has been cited as having frequent problems with sluggish and stuck fermentations, which can generate off-odours or microbiological instabilities that decrease the quality of the wine (Sommer *et al.* 2015, Ingledew and Kunkee 1985). This tendency is due to a combination of variety-related factors including higher amounts of octanoic and decanoic acids (toxic fatty acids), higher affinity for copper than magnesium, and a higher content of proline compared with other varieties that decreases the overall yeast assimilable nitrogen (YAN) available for yeast (Sommer *et al.* 2015). The simplest way to avoid sluggish or stuck fermentations seems to be ensuring an adequate nitrogen supply (140mg/L YAN when sugar content is moderate) prior to fermentation (Ingledew and Kunkee 1985). However, determination of nitrogen can be an onerous process, especially for smaller wineries that do not have high throughput instruments such as WineScan® during the hectic time of vintage. Over 60% of producers who participated in the survey do not determine nitrogen content, 15% determine YAN, 4% assess amino acid (AA) content and 17% determine both YAN and AAs. However, conscious of problems that can be caused by a lack of nitrogen, 73% of those producers who do no measurements still supplement YAN in their musts by adding either ammonium salts (diammonium phosphate, DAP), nutrients and vitamins, or inactive dry yeast. Amongst all participants, the preferred method of YAN supplementation is DAP addition (63%), whereas 36% use nutrients and vitamins, and 13% add inactive dry yeast (Figure 6). A number of alternative yeast supplements were mentioned by some producers, such as Nutristart®, GO-FERM®, and FermControl® – these combine ammonium salts, vitamins and inert yeasts, and are also commercialised under organic alternatives. Unlike DAP, use of vitamins, inactive dry yeast or alternative yeast supplements may have the additional benefit of contributing essential minerals such as magnesium and zinc to the juice as well as long chain fatty acids and sterols, which may help prevent the other problems associated specifically with Chardonnay fermentations (Sommer *et al.* 2015). From a quality perspective, it should be mentioned that the type and level of nitrogen supplementation has a complex effect on the final composition of the wine – too little nitrogen and few or no desirable esters will be formed, but too much nitrogen leads to increased ethyl acetate, acetic acid and higher alcohol concentrations. Ultimately, it is always better to correct musts where the nitrogen concentration is known (Torrea *et al.* 2011). These considerations also become important when selecting a yeast to work with as different strains have different YAN requirements and tolerances to high proline levels.

YEAST STRAINS AND WILD FERMENTS

The yeast selected will have a large impact on the final aroma profile (Gambetta *et al.* 2014) and, therefore, quality of the wine. Different yeasts exhibit diverse behaviour in relation to fermentation capabilities, ethanol formation, temperature tolerance, nutrient requirements, and other properties. Some yeasts are known to release more glycosides and/or thiols

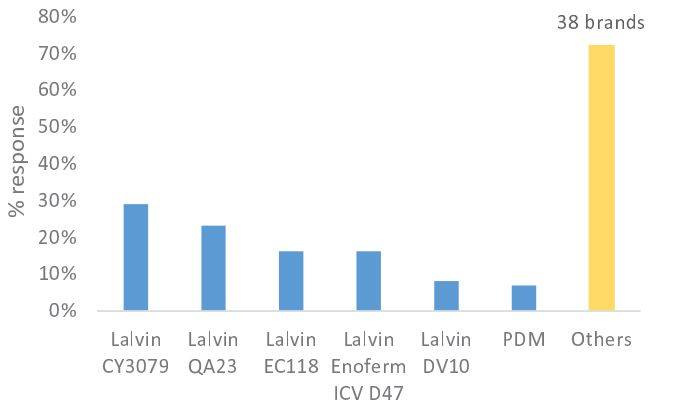

Figure 7. Commercial yeast choice.

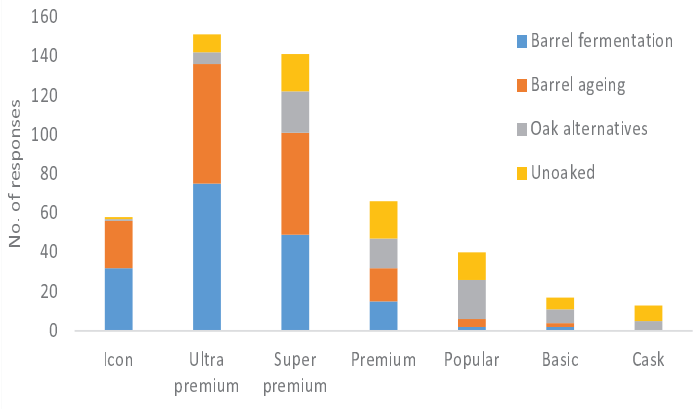

Figure 9. Method of oak incorporation.

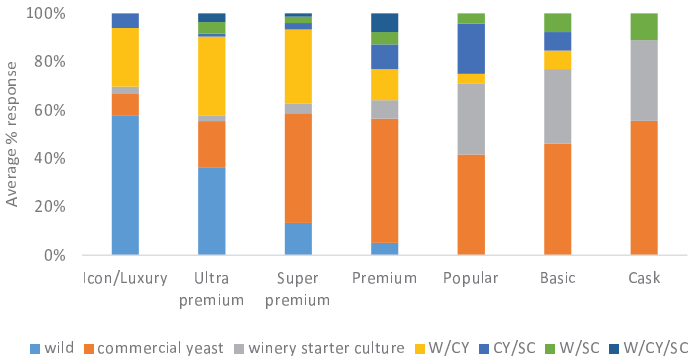

Figure 8. Choice of yeast according to product type.

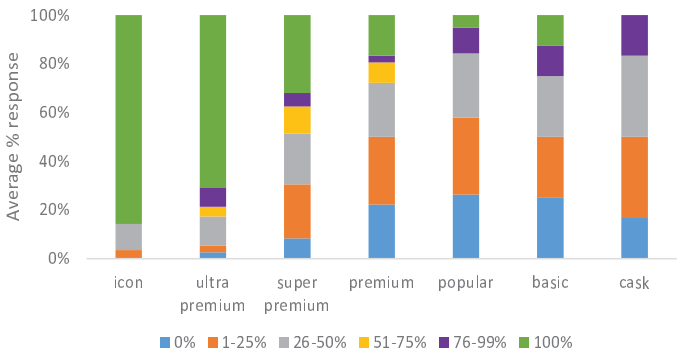

Figure 10. Proportion of oaked wine according to price bracket.

whereas others can enhance ester production or be considered relatively neutral. A particularly marked trend amongst respondents was the higher use of wild yeast (uninoculated fermentation) in the production of the most expensive brackets of wines. Figure 7 shows that 58% of all icon wines are produced exclusively with wild yeast (W), 24% with a combination of wild and commercial yeast (CY) and only 9% exclusively with commercial yeast. Unlike fermentations inoculated with commercial yeast, wild fermentations are conducted by a combination of non-*Saccharomyces* and indigenous *Saccharomyces* yeasts that work in relay as the fermentation progresses and alcohol content increases (Egli *et al.* 1998). In concert with the greater variety of species involved, a greater complexity of aromas (positive and negative) is obtained than with commercial yeast. However, the mix of strains is variable from year to year, can be affected by viticultural and winery factors including harvest and fermentation conditions, and usually leads to longer lag phases and fermentation times than with commercial yeast (Egli *et al.* 1998, Varela *et al.* 2009). On the other hand, according to respondents 56% of Chardonnay cask wines are produced solely with commercial yeasts and 33% with a winery starter culture (SC) (Figure 7), ensuring complete fermentations and consistent product profiles.

Based on frequency of citation, the preferred choices for commercial yeasts are CY3079, QA23, EC118 and Enoferm ICV D47 (Figure 8). In accordance with the varied types of Chardonnay wine styles available in the market (fresh and fruity, barrel-fermented and complex, etc), these yeasts have varied profiles (Table 1) and are adequate for very different styles of winemaking. CY3079 is better suited to barrel-fermented wines and aged sur lie wines, whereas QA23 is better for lighter and fruity Chardonnays (Table 1). Although producers are still predominantly using *S. cerevisiae* strains (73%), 18% of respondents are also using *S. bayanus* and some are even opting for non-traditional yeasts in a quest for more aroma diversity, using products such as Prelude and Zymaflore Alpha (*Torulaspora delbrueckii*) in a sequential inoculation with *S. cerevisiae*, or natural hybrids between *S. cerevisiae* and *S. kudriavzevii* (e.g. AWRI 1503) or *S. paradoxus* (e.g. Anchor Exotics).

MALOLACTIC FERMENTATION AND OAK USAGE

Icon and ultra-premium wines were the biggest groups to be barrel fermented (97% and 95%, respectively) and aged in barrels (73 and 77%, respectively) (Figure 9). Cask wines are fermented in stainless steel tanks and/or using oak alternatives according to the answers obtained and only 8% and 18% of the popular and basic wines, respectively, are barrel fermented. A very limited amount of the higher priced wines were made with oak alternatives (staves, chips, dust, etc) or without any oak contact at all.

Accordingly, a higher proportion of the final icon wines had contact with oak (Figure 10). As expected from the answers to the previous question, this proportion decreases markedly with price.

When it comes to Chardonnay wine, there is a clear preference for French oak (88% of respondents) over American oak, and 85% of respondents indicated that they prefer a medium toast irrespective of the origin, and only 2% choose a heavy toast.

Unlike other white wine varieties, Chardonnay wines often undergo malolactic fermentation (MLF), particularly when the wines are fermented and/or aged in oak barrels. MLF contributes aromas of caramel, honey and butter as well as a fuller

Table 1. Commercial yeast characteristics (Lallemand 2016).

Strain	CY3079 *S. cerevisiae*	QA23 *S. cerevisiae*	EC1118 *S. bayanus*	D47 *S. cerevisiae*
Killer factor	neutral	active	active	active
Lag phase	short-moderate	moderate	short	short
Fermentation vigour	moderate	high	high	moderate
Desirable T° limits	15-25	14-28	10-30	15-30
Nitrogen demand	moderate-high	low	low	moderate
Alc. %(v/v) tolerance*	14.5	16	18	15
MLF	unfriendly	-	neutral	friendly
Foam production	low	low	low	low
Other characteristics	Early onset of post fermentation autolysis	High in ß-glycosidase	-	High in ß-glycosidase, high polysaccharide producer
Recommended for	white wines, barrel fermentation, sur lie ageing	white wines	white, rose and red wines and secondary fermentation	barrel fermentation of white wines

*Depending on fermentation conditions

mouthfeel to the wine that are mostly desirable in styles that have undergone fermentation and/or aged with oak contact (Gambetta *et al.* 2014). Figure 11 shows that 34% of respondents do not allow their wines to undergo MLF, whereas a total of 36% conduct MLF (of which 9% indicated that it was reserved for higher quality wines), and 30% undertake partial MLF.

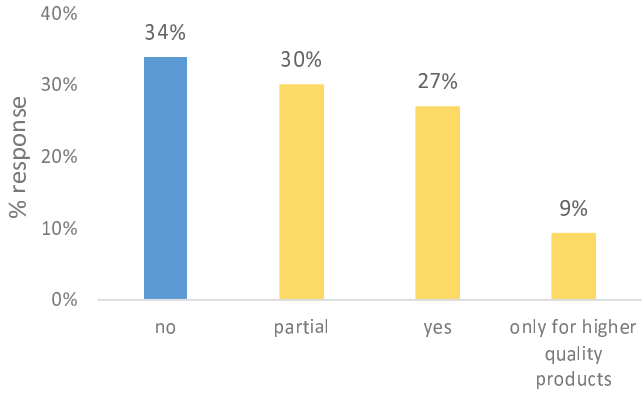

Figure 11. Use of malolactic fermentation.

CHARDONNAY WINE STYLES

Australia produces many conceivable Chardonnay styles. In their study of the positioning of Australian Chardonnay wines in the flavour map, Saliba *et al.* (2013) recognised wines that could match styles from every different Chardonnay producing region in the world. Wine style drives many of the choices described above - where to source fruit from, when to harvest, type of yeast, etc - and a word cloud was generated using the individual responses of the surveyed producers (Figure 12). Although wine style tends to change with target market and price bracket, a predominant number of producers described their wines as 'fruit-driven' followed by 'meant to age' or with ageing potential. Oak aromas, although still important to the overall Chardonnay wine concept, seem to have taken a back seat to fruit, adding complexity and subtle oak notes to the wine (Figure 12).

Figure 12. Chardonnay wine style word cloud derived from survey responses.

CONCLUSIONS

The quality of a wine is the result of both the operations used in its manufacture and the quality of the fruit. A look at the decisions made by winemakers during the production process gives us insight into the different considerations that weigh into this process and that ultimately determine the quality of a wine. Many of the decisions made in the winery, from the fruit that is purchased to the supplements used, will be decided depending on the market segment targetted. From the results of the survey, production of Chardonnay wines is now concentrated in the ultra ($25-50) and super-premium ($15-24) categories and potentially reflects a move away from the lower price points (due to lack of profitability), with only 1% of production destined for cask wine (bag-in-box). Harvest affects the quality of the fruit as it determines the final composition of the berries. The most important criteria used by respondents to determine grape maturity are °Brix and berry tasting, followed by pH. When tasting grapes, producers are primarily looking at the grapes' flavour intensity when determining when to harvest and grading the quality of the grapes. Once harvested, fruit is allocated into different quality levels in the winery based mainly on historical parcel records, but also on the results of berry tasting. Conscious of the importance of YAN management, 73% of producers who do not determine YAN or AA content still correct YAN levels. Overall, DAP addition is the preferred method to supplement YAN (63% of respondents) but some producers add vitamins and minerals or inactive dry yeast, or a combination of the three. Together with a higher interest in flavour intensity in the field, the use of wild yeast appears to have a common theme amongst wine producers to create a more unique aroma profile; evidently from our survey the use of wild yeast was predominantly amongst the higher priced wines (58% of icon wines are solely produced with wild yeast). Of those producers that are using commercial yeast, 18% use *S. bayanus* and some are even using sequential inoculations and hybrids. Together with wild fermentations, icon wines were also the category most likely to be barrel-fermented and aged

in barrels followed by ultra and super-premium wines. Super-premium wines also had a higher percentage of wines produced with oak alternatives or no oak compared with those other two categories. These results appear to reflect changes in drinking trends, where consumers are no longer seeking the traditional oaky, buttery Chardonnay, but are valuing more the expression of the fruit, whilst still seeking an oak backbone.

ACKNOWLEDGEMENTS

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WVJ

Chardonnay quality survey

Introduction

This survey is part of a GWRDC funded PhD research project of Joanna Gambetta from The University of Adelaide titled "Development of Quality Assesment Tools for Chardonnay in relation to Grape, Juice and Wine composition". Her PhD supervisors are Drs David Jeffery, Sue Bastian and Daniel Cozzolino. Joanna is a member of the Wine Science Group of the School of Agriculture, Food and Wine and may be contacted at joanna.gambetta@adelaide.edu.au.

The purpose of this survey is to find out what tools and criteria wine makers are using to define the quality and harvest date of their grapes in the vineyard and the style of wine they are destined for. The information generated in this survey, along with the other components of the project, will assist in determining quality parameters for Chardonnay wines, and how they correlate to grapes before harvest.

This survey will take approximately 15 minutes to complete. Consent to participate is implied by completing and returning this survey. Please provide your details, however they will remain confidential in a secure data base and will not be divulged to any other person. All data will be reported in aggregate and your anonymity is guaranteed. This survey has been reviewed and approved by the Human Research Ethics committee of The University of Adelaide.

If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the project co-ordinator or The University of Adelaide's Human Research Ethics Committee:

Project Co-ordinator: Dr David Jeffery
Telephone: (08) 8313 6649
email: david.jeffery@adelaide.edu.au

Human Research Ethics Committee
Telephone: (08)8313 6028
email: hrec@adelaide.edu.au

Chardonnay quality survey

Part 1. Questions related to vineyard activity

1. Please provide the following information. The information provided will only be read by the researchers incharge of the project

Name

Company

Position

2. What is the postcode where your winery is located?

ZIP/Postal Code:

Chardonnay quality survey

3. What are the sources of fruit that you use to make wine?

- ◯ Company vineyards only (go to question 4 and skip question 5)
- ◯ Contract growers only (skip question 4 and go to question 5)
- ◯ Combination of both (go to question 4 and then to question 5)

4. What is the area of your own vineyard/s that you source your fruit from?

- ☐ Small ≤10 Hectares
- ☐ Medium 10-30 Hectares
- ☐ Large 30-100 Hectares
- ☐ Very large > 100 Hectares

5. What is the area of the contract vineyard/s that you source your fruit from?

- ☐ Small ≤10 Hectares
- ☐ Medium 10-30 Hectares
- ☐ Large 30-100 Hectares
- ☐ Very large > 100 Hectares

6. Approximately, what is your annual total Chardonnay crush? What percentage of your global crush does it represent?

Tonnes of Chardonnay berries crushed

%

7. How do you determine Chardonnay grape maturity and harvest date? (Check all that apply)

- ☐ pH
- ☐ Titrable acidity
- ☐ Brix/Baume degrees
- ☐ Berry tasting
- ☐ Weather forecast
- ☐ Tank availability
- ☐ Viticultural advice
- ☐ Other (please specify)

8. Only if you perform berry tasting, what attributes do you look for while sampling berries? (Check all that apply)

- ☐ Berry colour
- ☐ Flavour intensity
- ☐ Pulp consistency
- ☐ Tannin ripeness
- ☐ Skin thickness
- ☐ Seed colour
- ☐ Other (please specify)

Chardonnay quality survey

Part 2. Questions related to winery activity

9. What size is your winery?

- ◯ Less than 20 tonnes per year
- ◯ 20 to 49 tonnes per year
- ◯ 50 to 99 tonnes per year
- ◯ 100 to 249 tonnes per year
- ◯ 250 to 499 tonnes per year
- ◯ 500 to 999 tonnes per year
- ◯ 1000 to 2499 tonnes per year
- ◯ 2500 to 4999 tonnes per year
- ◯ 5000 to 9999 tonnes per year
- ◯ 10000 to 19999 tonnes per year
- ◯ 20000 or more tonnes per year

*All questions from this point onwards relate only to Chardonnay wines.

10. What percentage of total Chardonnay crush is represented by each of the following quality levels? Please allocate the quantity produced yearly over the different quality levels so that it totals 100% (using 0 where a level is not produced).

Icons/Luxury ($50+/bottle)	
Ultra premium ($25 - $50/bottle)	
Super premium ($15 - $25/bottle)	
Premium ($10 - $15/bottle)	
Popular ($5 - $10/bottle)	
Basic (less than $5/bottle)	
Cask	

11. What criteria do you use to allocate fruit to each quality level? Please specify

- ☐ Historical parcel records
- ☐ Wine style quotas
- ☐ Acidity
- ☐ Other (please specify)
- ☐ Sugar content
- ☐ Berry tasting

Chardonnay quality survey

All of the following questions relate to cellar operations such as DAP additions and inoculation in order to try to find out how these parameters vary according to Chardonnay wine quality.

12. Do you determine the nitrogen content of your musts?

- ◯ Yes – total nitrogen content
- ◯ Yes – amino acid composition
- ◯ Yes - both
- ◯ No

13. How do you correct the YAN of your musts? (Check all that apply)

- ☐ DAP
- ☐ Nutrients and vitamins
- ☐ Other (please specify)
- ☐ Inactive dry yeast
- ☐ Not applicable

14. Where applicable for each quality level, please indicate which method is used for conducting alcoholic fermentation.

	Iconic/Luxury	Ultra premium	Super premium	Premium	Popular	Basic	Cask
Wild yeast	☐	☐	☐	☐	☐	☐	☐
Active dry yeast	☐	☐	☐	☐	☐	☐	☐
Winery starter culture	☐	☐	☐	☐	☐	☐	☐

Other (please specify)

15. If you use commercial yeast, please specify the strain(s) you use for producing Chardonnay wines, and whether you use different yeasts for different fruit parcels, in particular with regard to different quality levels.

16. Do you encourage malolactic fermentation of your wines? If so, would you please elaborate?

- ☐ No
- ☐ Spontaneous
- ☐ Inoculation with malolactic bacteria
- ☐ Only partial
- ☐ Only for higher quality wines

Other (please specify)

Chardonnay quality survey

17. Where appropriate, please specify the method of any oak incorporation by checking the relevant boxes.

	Barrel fermentation	Ageing in barrels	Oak alternatives (i.e. chips,staves,dust)	Unoaked
Icons/Luxury	☐	☐	☐	☐
Ultra premium	☐	☐	☐	☐
Super Premium	☐	☐	☐	☐
Premium	☐	☐	☐	☐
Popular	☐	☐	☐	☐
Basic	☐	☐	☐	☐
Cask	☐	☐	☐	☐

Other (please specify)

18. Which type of oak and toast level do you favour?

	Light toast	Medium toast	Heavy toast
French	◯	◯	◯
American	◯	◯	◯

Other (please specify)

19. What percentage of the wines used in the blend of each of your different quality level is oaked? (0-100%)

Icons/Luxury	
Ultra premium	
Super Premium	
Premium	
Popular	
Basic	
Cask	

20. How would you describe the style and profile of your wines (i.e. fruit-driven, oak-driven, meant to be aged, etc.)? Do you have different profiles for your different wine qualities?

21. Do you have any thoughts on how to improve berry quality determination? (i.e. berry sensory training; a quality index related to berry and wine composition; rapid measurement of key parameters; texture analysis/skin hardness, etc.)

Chardonnay quality survey

Thank you!

Thank you for your participation. A summary of the findings from this survey will be sent to you in the future. We reiterate that your anonymity is guaranteed.

CHAPTER 3

Relating expert quality ratings of Australian Chardonnay wines to volatile composition and production method

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Name of Principal Author (Candidate)	Joanne M. Gambetta
Contribution to the Paper	Designed experiments, performed experimental work, conducted sensory trials with expert tasters, analysed and interpreted data, drafted and constructed the manuscript.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 4/11/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Susan E.P. Bastian
Contribution to the Paper	Contributed to the research idea, sensory analysis design, interpretation of the data and editing of the manuscript and acted as corresponding author.
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Name of Co-Author	Leigh M. Schmidtke
Contribution to the Paper	Performed curve resolution analysis of all chromatograms and edited the manuscript.
Signature	Date 4/11/2016

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Contribution to the Paper	Assisted with volatiles determination and manuscript editing
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Contribution to the Paper	Supervised the work, contributed to the research idea and experimental design. Assisted in the preparation and editing of the manuscript.
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Relating Expert Quality Ratings of Australian Chardonnay Wines to Volatile Composition and Production Method

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Abstract: Chardonnay is a neutral grape variety offering a diversity of wine styles that are popular among consumers. The links between wine production methods and Chardonnay wine volatile composition, as determinants of quality, require further elucidation. Over 80 commercial Australian Chardonnay wines were assessed by expert panelists who were asked to define four distinct levels of quality in a blind tasting. Wine aroma volatiles in each wine were analyzed by solid-phase microextraction-gas chromatography-mass spectrometry, and multivariate statistical techniques were used to examine the relationship between volatile composition and quality as defined by the experts. Of 39 aroma compounds quantified, nine volatiles (including *cis*- and *trans*-oak lactones, furfural, and diethyl succinate) correlated significantly and positively with Chardonnay wine quality, while 11 volatiles (including fruity esters and monoterpenoids) correlated negatively. Compounds associated with oak contact and malolactic fermentation were present at highest concentrations in higher-quality wines as perceived by wine experts. Lower scores were assigned to younger but less complex wines, which were richer in fruity esters and other grape-derived compounds. A model was developed using partial least squares regression based on these results, which permitted classification of the Chardonnay wines into high-, medium-, and low-quality brackets depending on their relative concentrations of *cis*- and *trans*-oak lactone, ethyl lactate, and 2-methyl-1-propanol (positive) and of 1-propanol and 1-hexanol (negative). There was a significant and positive correlation ($r = 0.469$, $p < 0.0001$) between retail price and quality score, underlying the usefulness of price as an indicator of quality, although it failed to entirely explain quality (as judged by experts) and should therefore be used in conjunction with other quality cues.

Key words: Chardonnay, gas chromatography, principal component analysis, oak lactones, sensory, wine quality

For most goods, value is determined by quality, often measured as nutritional value for food or level of craftsmanship for material objects such as clothes or furniture. For non-commodity hedonic goods such as wine, quality becomes more difficult to define because consumption is not related to nutrition and the steps used in the winemaking process (i.e., craftsmanship) cannot be truly or easily appreciated by the consumer (Schiefer and Fischer 2008). Quality is officially defined by the International Standardization Organization (2008) as "the ability of a set of inherent characteristics of a product, system, or process to fulfil requirements of customers and other interested parties". However, it is often unclear what these requirements are, particularly for products such as wine, where quality cannot be determined solely by chemical analysis, but instead depends on a range of organoleptic properties (e.g., color, taste, aroma) and the amount of pleasure it affords the consumer (Charters and Pettigrew 2007). Therefore, the quality of a wine cannot be assessed without having tasted the product first, and more often than not, consumers are unable to taste a wine before buying it. Consumers have to rely on a series of quality cues such as brand, price, medals, advertising, packaging, reputation, and the advice and/or judgment of experts, to make a decision (Charters and Pettigrew 2007, Gawel and Godden 2008, Schiefer and Fischer 2008, Lockshin et al. 2009, Sáenz-Navajas et al. 2016).

Sensory judges can be divided into consumers, trained assessors, and experts, according to their level of exposure to the product and sensory training. Although not necessarily formally trained as sensory panelists, wine experts are individuals who, through repeated contact with wine, have honed the ability to focus on individual attributes, identify wine defects, and recognize volatile compounds (Gawel and Godden 2008). In many cases, their experience allows them to recognize wine variety, region, and style, to judge how well a sample complies with these categories, and to produce repeatable and consistent judgments on wine quality (Gawel and Godden 2008).

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Expert tasters score the quality of a wine based on the absence of faults and the presence of desirable aromas, among other attributes such as color, texture, taste, balance, and complexity. All aromas perceived in wine depend on the concentrations of multiple volatile compounds which are interpreted and labeled by the brain after olfaction, if and when they occur at concentrations above their odor detection threshold (Rapp and Mandery 1986). A series of studies have confirmed that wine quality depends on physicochemical characteristics such as aroma composition, and these studies have tried to establish correlations with specific volatile components (San Juan et al. 2012, Hopfer et al. 2015). Taking this a step further and determining which compounds correlate to a specific quality level can help develop objective and rapid ways to screen for wine quality. Additionally, such a correlation could enable quality monitoring during production so winemakers could adjust procedures to improve or better target a specific quality level.

Previous research has either concentrated on relating the impact of specific procedures to overall sensory quality or particular marker compounds, or on recognizing which volatiles define the typicity of a variety. For instance, red wines with higher quality levels present higher concentrations of aroma compounds with "pleasant notes" such as ethyl esters, C_{13}-norisoprenoids, and oak-derived components, and lower concentrations of detrimental odorants such as 4-ethylphenol, phenylacetaldehyde, and methional (San Juan et al. 2012). The same remains to be done for white wines such as Chardonnay.

Chardonnay is one of the most widely planted varietals in the world and is grown in most winemaking regions (Gambetta et al. 2014). It is a very flexible variety, with fruit-driven characteristics that lends itself to a number of winemaking techniques, such as barrel fermentation and aging in oak, without these winemaking attributes necessarily becoming the dominant feature. Over 240 different volatile components have been identified in Chardonnay wines, including an assortment of esters, alcohols, acids, lactones, and ketones arising from fermentation or oak storage. Among these compounds, not all impact the overall aroma of the wine; some volatiles have no associated aroma or are present at infrathreshold levels (Welke et al. 2014).

Of particular importance to Chardonnay wines are esters (both straight-chain fatty acid ethyl esters and branched-chain acetate esters), C_{13}-norisoprenoids (β-damascenone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)), and oak volatiles (oak lactones, guaiacol), which, depending on concentration and precise composition, will impact the level of typicity and therefore quality, of the wine (Gambetta et al. 2014). C_{13}-Norisoprenoids are formed during berry ripening from carotenoids in the grapes. Esters are synthesized in wine during vinification through yeast metabolism and oak volatiles come from contact with toasted oak wood during fermentation and/or maturation. Together with duration of oak contact, variables such as grape sunlight exposure, irrigation, yeast strain, vinification technique, and aging affect the concentrations of these important compounds (Gambetta et al. 2014).

Given the importance of aroma to wine quality and the usefulness of expert opinions to determine quality, this study aimed to improve understanding of the link between compositional differences in aroma volatiles measured by solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) and quality as determined by an industry expert tasting panel. Samples consisted of commercial Australian Chardonnay wines encompassing a wide range of quality and price. Chromatographic data were aligned and integrated using multivariate curve resolution techniques, and relationships between quality and volatile composition were investigated using chemometrics and network analysis.

Materials and Methods

Samples. Eighty-three commercial Chardonnay wines (three bottles each) from vintages spanning 2010 to 2013 were donated by producers from New South Wales, South Australia, Tasmania, Victoria, and Western Australia. Samples were chosen for having a rating >90 points by James Halliday (Halliday 2013) or for having a sales ranking in the top 10% at one of Australia's main wine retail chains (G. Hindson, personal communication, 2013). Details of the wine samples, tasting scores, and basic chemical data are reported (Supplemental Table 1). Wineries kindly provided additional proprietary information regarding winemaking and maturation techniques. Wines were stored at 15°C for about two months prior to use.

Reagents, standards, and materials. Reference compounds (purity ≥97%) consisting of ethyl butanoate, ethyl 2-methylbutanoate, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl furoate, ethyl 2-phenylacetate, diethyl succinate, hexyl acetate, 3-methylbutyl acetate, 2-phenylethyl acetate, 3-methylbutyl octanoate, ethyl lactate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, 1-hexanol, 1-octanol, 2-phenylethanol, linalool, α-terpineol, limonene, β-damascenone, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, benzaldehyde, nonanal, oak lactone (mixture of isomers), and furfural were purchased from either Sigma-Aldrich or Alfa Aesar. Deuterated internal standards of d_4-3-methyl-1-butanol, d_3-hexyl acetate, d_{13}-1-hexanol, d_5-2-phenylethanol, and d_{19}-decanoic acid were supplied by CDN Isotopes, and d_5-ethyl nonanoate was synthesized as described previously (Boss et al. 2015). Absolute ethanol (Merck) and sodium chloride (JT Baker) were analytical grade and water was obtained from a Milli-Q purification system (Millipore).

Wine sensory assessment. Sensory evaluation of samples was conducted during a single day in November 2013 by eight industry professionals (winemakers, professors, and retailers with experience in white wines) who met the criteria defining them as wine experts (Parr et al. 2004). Wine samples (30 mL) were served at room temperature (~20°C) in clear INAO (ISO standard) 215 mL glasses covered with a transparent plastic lid. All wines were coded (three-digit code, Supplemental Table 1) and presented in a randomized order. To cope with fatigue, quality assessment was broken down into three sessions with 1-hr enforced breaks. Within a session, samples were presented in flights of five wines with 10-min breaks between flights. Panelists were provided with water and plain crackers to cleanse their palate, evaluation

sheets, and a bucket in which to expectorate the samples. Each panelist was seated at a separate table. Samples were scored using the Australian Wine Show system on a 20-point scale (Iland et al. 2009) and a four-level quality score (A to D) was determined and agreed upon by all members of the panel prior to tasting. Before commencing assessments of samples, the panelists discussed and defined the criteria corresponding to each quality category (Table 1) and tasted and sorted four wines considered to be representative of each bracket (this was an iterative process; wines were provided according to their price and characteristics by the panel leader, where price was used as a proxy quality indicator). This was done to calibrate the panel members with each other and ascertain that they had reached a consensus on the different quality categories. Experts were asked to declass and not score any wine presenting a serious fault (e.g., oxidation or cork taint).

Basic wine composition. The pH and titrable acidity (TA, reported as g/L tartaric acid equivalents) of the wines were measured using a combined pH meter and autotitrator (Crison, CompacTitrator, Crison Instruments), and percent alcohol was determined using an alcoholizer (Alcolyzer Wine ME+DMA 4500M, Anton Paar). All measurements were conducted in duplicate within six months of the sensory evaluation.

Volatile analysis by HS-SPME-GC-MS. Wines were analyzed immediately after sensory analysis from the same bottles as described (Wang et al. 2016a) with modification. Wine (0.5 mL) was aliquoted into a 20 mL SPME vial (Supelco), diluted with water (4.5 mL), and 2 g sodium chloride was added. The vial was spiked with an internal standard solution (10 µL) consisting of a mixture of deuterated standards in absolute ethanol (d_4-3-methyl-1-butanol [2380 mg/L], d_3-hexyl acetate [25 mg/L], d_{13}-1-hexanol [50 mg/L], d_5-2-phenylethanol [500 mg/L], d_{19}-decanoic acid [50 mg/L], and d_5-ethyl nonanoate [1.2 mg/L]), sealed tightly with a PTFE-lined cap (Supelco), and the contents were homogenized with a vortex mixer.

Table 1 Criteria for Chardonnay wine quality categories defined by the expert panel.

Category/ score (pts)	Description
A/18-20	Barrel fermented or aged (although not necessarily aged)
	Balanced
	Texture
	Good acid support and length
	Richness on palate
	Fruit on palate (stone fruit, white peach, nectarine, grapefruit, and pith notes)
	Exhibit flintiness and nuttiness (described as notes of cashew and hazelnut)
B/16-17.9	All of the above but less intense
	Lesser length and balance than A
	Maybe more oaked
	Lacking in finesse
C/14-15.9	Good commercial wines without faults
	Fresher than A and B and more fruit-driven
	No phenolics
	Lacking acid support
	Striving to be B but overdone
D/<14	Simple wines
	Clean
	Fruity, leaner than other categories
	Lack length
	More tropical and vegetal aromas
	Can be dominated by oak- or malolactic-related aromas

Multivariate curve resolution analysis of GC-MS data. Data processing and treatment was performed using MATLAB (version R2012a 7.14.0.739, The Mathworks) after exporting the GC-MS files in netCDF format from Agilent Chemstation (E.02.02.1431). Extracted ion chromatograms of all samples were overlaid, aligned, and integrated using an approach modified from one previously described (Schmidtke et al. 2013). Elution time windows for each analyte of interest, including the internal standards, were chosen by visual inspection of extracted ion chromatograms. Chromatograms were aligned using the icoshift algorithm (Savorani et al. 2010), and peak areas were extracted from the aligned elution profiles for all samples using a trapezoid integration.

Statistical analysis. Data was processed using XLSTAT ver. 2014.05.03 (Addinsoft), Gephi ver. 0.9.1 (Bastian et al. 2009), and The Unscrambler X (CAMO AS, version 10.3). Comparison and correlation of scores, prices, vintage, and analyte concentrations were executed by one-way ANOVA and Pearson's correlation analysis. ANOVA of compositional variables was accomplished using vintage, quality category, and fermentation vessel as explanatory variables for the differences among samples. Principal component analysis (PCA) was performed on the normalized concentrations of significantly different analytes (using quality category as the explanatory variable) using variables with scores ≥0.7 in the Pearson correlation matrix. Network analysis was carried out on significantly different variables (using score as the explanatory variable) with strong positive ($r \geq 0.6$) or negative ($r \leq -0.6$) correlations among each other, as described (Wang et al. 2016b). Score (*y*-variable) was related to all wine compositional data (*x*-variables) using partial least squares regression (PLS) analysis. The overall set of samples was randomly split into calibration (~2/3 of the samples) and validation (~1/3) sets using the Kennard-Stone algorithm. The prediction ability of the model was tested on the validation set using the root-mean-square error of prediction (RMSEP), the residual predictive deviation (RPD), the correlation coefficient (CC), the slope of the regression curve for the predicted *y*-variable (*m*), and the percentage of variance explained by the model (%EV). All variables were normalized before analysis.

Results and Discussion

Sensory analysis by expert tasters. Consensus among expert panelists resulted in descriptions of four different wine quality categories prior to a blind tasting of 83 commercial Chardonnay wines (Table 1). Wines included in category A had a score of 18 points or more, those in category B had 16 to 17.9 points, category C had 14 to 15.9 points, and category D had below 14 points.

Scores for all samples ranged between 14.2 and 18.1 points: only one sample was categorized as A. ANOVA of scores resulted in significant differences ($p < 0.0001$) among vintages. The highest scores were given to 2010 wines and the lowest to those produced in 2013 (Supplemental Table 1). Given the description determined by the experts for each category (Table 1), this outcome was somewhat expected, as higher scores were given to wines that had been aged and thereby contained more "evolved" aromas. Significant differences ($p < 0.01$) were observed between scores of wines that underwent barrel fermentation in oak wood and those fermented in stainless steel tanks (Supplemental Table 1), which accords with expert panelists considering that wines fermented in the presence of oak were of higher quality. This agrees with the higher liking reported by panelists for Chardonnay wines fermented and/or aged in the presence of oak over those fermented in stainless steel (Liberatore et al. 2010). The incorporation of oak in the form of barrels, staves, chips, or other alternatives (Gambetta et al. 2014) during alcoholic fermentation not only imparted "smoky" and "woody" characters that were rated favorably by our expert panel, but also decreased the impact of "unripe fruit" and "fresh fruit" aromas and flavors that are typical of young white wines (Pérez-Coello et al. 2000). All younger wines assessed in our study (2013 vintage) corresponded to a fruitier, fresher, usually unoaked style. The panelists in this study classified these wines in category C because they lacked the higher complexity, balance, texture, and aroma profile sought for higher quality categories (Supplemental Table 1).

Volatile composition, typicity, and quality score. Samples were chosen to represent the current available offering of Chardonnay wines in the Australian market (Supplemental Table 1). They originated in the main Australian wine-producing regions from grapes grown in a variety of climates, and encompassed varied winemaking styles (no oak, barrel-fermented, uninoculated fermentation, etc.), were from different vintages, and were sold at a variety of prices. Volatiles analysis was undertaken using SPME-GC-MS and data handling was greatly simplified by employing multivariate curve resolution techniques (see Materials and Methods for details). The relative concentrations of 39 aroma volatiles determined for the 83 Chardonnay wines revealed considerable variability for some compounds (Table 2). Seventeen analytes were significantly different among the different quality categories ($p < 0.05$): ethyl butanoate; 3-methylbutyl acetate; hexyl acetate; ethyl hexanoate; diethyl succinate; ethyl lactate; 1-propanol; 2-methyl-1-propanol; 2-phenylethanol; linalool; α-terpineol; hexanoic, octanoic, and dodecanoic acids; furfural; and *cis*- and *trans*-oak lactones. These aroma volatiles are formed during berry ripening, alcoholic fermentation, and aging (Gambetta et al. 2014), and their variation illustrates the large intravarietal differences among Chardonnay wines resulting from geographic origin, vintage, and viticultural and enological practices. Of these compounds, ethyl butanoate, ethyl hexanoate, 3-methylbutyl acetate, linalool, diethyl succinate, and octanoic acid have correlated positively with the typicity of Chardonnay wines (Gambetta et al. 2014). Conversely, the majority of these compounds, together with several other esters and β-damascenone, correlated negatively with the overall quality score assigned by our experts (Figure 1). Most studies on the typicity of Chardonnay wine have only been conducted on unoaked samples, but oak-derived characteristics were favored by the current study's tasting panel, which could account for our findings.

PCA analysis was conducted with significantly different variables using quality score as the explanatory variable and production details, vintage, quality score, and price as supplementary variables (Figure 1). Together, PC1 and PC2 explained 76.2% of the variability in the data and showed a segregation of samples according to quality score and fermentation vessel along the F1 axis, where higher scoring and barrel fermented (BF) samples were located to the left of the PCA plot, and lower-scoring samples to the right, were fermented in stainless steel vessels (SSF) or produced with SSF/BF/oak alternatives. Samples in the upper left quadrant had more *cis*- and *trans*-oak lactones and, as explained by the supplementary variables, these samples tended to be older (more than one year) and fermented and/or aged in oak barrels. From a sensory perspective, the oak lactones are the most important compounds released by oak into wine and their presence in wine is affected by the age, origin, and volume of the barrel (Pérez-Prieto et al. 2002). These two molecules were recognized as part of a set of 15 key odor-active compounds necessary to reconstitute the aroma of California Chardonnay wines, which are traditionally oaked (Lee and Noble 2006). Lower-scoring samples split into two groups along the F2 axis: located in the lower right quadrant were samples mainly from the 2013 vintage, which contained higher concentrations of hexyl acetate, 3-methylbutyl acetate, and β-damascenone, and in the upper right quadrant was a more heterogeneous group of samples, predominantly produced with SSF/staves or SSF/BF, characterized by higher amounts of hexanoic acid, ethyl hexanoate, and ethyl butanoate. Hexanoic acid, ethyl hexanoate, ethyl butanoate, hexyl acetate, 3-methylbutyl acetate, and β-damascenone either directly impart or enhance "fruity" and "vegetal" (or "green") aromas (San Juan et al. 2011, Gambetta et al. 2014) that were associated with the lower quality brackets by the experts (Table 1).

As a useful visualization tool (Wang et al. 2016b), network analysis was used to reveal 17 strong positive ($r \geq 0.6$) and two strong negative ($r \leq -0.6$) correlations or "edges" arising between significantly different aroma volatiles, basic chemical parameters, and quality score (Figure 2, positive correlations only). Positive correlations resulted in three distinct modules: on the far right, a module consisting of hexanoic acid and the fruity esters ethyl butanoate and ethyl hexanoate; in the middle, another module with the fruity acetates 3-methylbutyl, 2-phenylethyl, and hexyl acetate and the varietal compounds linalool, α-terpineol, and β-damascenone; and on the far left, a module with the oak-derived volatiles *cis*- and *trans*-oak lactone and furfural, which correlated strongly with quality score and price. As expected, the fatty acid hexanoic acid correlated very strongly with its corresponding ethyl ester

Table 2 Relative concentrations[a] of volatile compounds analyzed in 83 commercial Australian Chardonnay wines of vintages from 2010 to 2013.

		All Samples			2010-12 vintages	2013 vintage	SSF[e]	BF[f]	BA[g]
Compound	**Ion[b]**	**Min**	**Max**	**Sig[c]**	**mean (n = 12)[d]**	**mean (n = 71)**	**mean (n = 13)**	**mean (n = 43)**	**mean (n = 61)**
Ethyl esters									
Ethyl butanoate[h]	71	24	92	***	49 (29%)	61 (22%)	63 (18%)	42 (26%)	47 (26%)
Ethyl 2-methylbutanoate[i]	102	0.042	0.70	ns	0.22 (53%)	0.095 (51%)	0.12 (47%)	0.23 (51%)	0.23 (51%)
Ethyl hexanoate[i]	88	49	140	***	85 (21%)	104 (15%)	104 (13%)	77 (19%)	83 (19%)
Ethyl lactate[h]	45	14	614	***	205 (75%)	42 (73%)	44 (82%)	252 (66%)	215 (66%)
Ethyl octanoate[i]	88	153	411	ns	267 (20%)	270 (25%)	305 (22%)	251 (21%)	256 (21%)
Ethyl 2-furoate[j]	112	0.061	31	ns	9.6 (49%)	6.2 (32%)	8.5 (49%)	10 (43%)	9.5 (43%)
Ethyl decanoate[i]	88	35	222	ns	107 (33%)	107 (42%)	118 (35%)	107 (33%)	102 (33%)
Diethyl succinate[j]	101	113	6838	***	2347 (58%)	500 (48%)	694 (81%)	2679 (51%)	2412 (51%)
Ethyl 2-phenylacetate[j]	91	0.088	78	ns	15 (82%)	7.6 (57%)	9.0 (69%)	16 (88%)	15.2 (88%)
Ethyl dodecanoate[k]	183	130	1180	ns	433 (47%)	372 (28%)	397 (33%)	442 (51%)	432 (51%)
Acetate esters									
Ethyl acetate[l]	61	1130	8031	ns	2290 (42%)	1835 (17%)	1833 (17%)	2463 (26%)	2381 (26%)
3-Methylbutyl acetate[i]	70	3.2	121	***	19 (63%)	73 (37%)	58 (57%)	17 (78%)	19 (78%)
Hexyl acetate[i]	56	29	56	***	32 (9%)	43 (13%)	40 (19%)	31 (10%)	32 (10%)
Other esters									
3-Methylbutyl hexanoate[h]	70	274	914	ns	570 (23%)	572 (29%)	633 (24%)	538 (24%)	550 (24%)
3-Methylbutyl octanoate[h]	127	2.6	18	ns	7.8 (42%)	7.0 (43%)	8.3 (38%)	7.4 (44%)	7.2 (44%)
2-Phenylethyl acetate[j]	104	52	3227	ns	395 (108%)	1009 (33%)	746 (57%)	348 (134%)	400 (134%)
Alcohols									
1-Propanol[h]	59	2.3	9.0	**	4.4 (35%)	5.0 (30%)	5.6 (23%)	4.1 (38%)	4.2 (38%)
2-Methyl-1-propanol[l]	31	59	293	ns	178 (28%)	120 (21%)	111 (27%)	193 (25%)	183 (25%)
3-Methyl-1-butanol[l]	55	9724	19655	ns	13975 (15%)	11824 (16%)	12286 (14%)	13974 (16%)	14016 (16%)
1-Hexanol[h]	56	51	290	ns	138 (34%)	121 (17%)	145 (36%)	125 (38%)	133 (38%)
2-Ethyl-1-hexanol[h]	57	26	69	ns	39 (26%)	36 (27%)	40 (23%)	39 (27%)	38 (27%)
1-Octanol[h]	56	5.6	19	ns	11 (28%)	9.5 (28%)	10 (29%)	11 (29%)	11 (29%)
2-Phenylethanol[j]	91	882	10297	*	2511 (71%)	1797 (56%)	1478 (24%)	2443 (76%)	2520 (76%)
Isoprenoids									
Limonene[i]	68	0.041	0.32	ns	0.14 (34%)	0.17 (33%)	0.18 (30%)	0.13 (39%)	0.14 (39%)
Linalool[h]	80	0.18	3.9	*	0.77 (65%)	1.4 (60%)	1.4 (63%)	0.6 (58%)	0.7 (58%)
α-Terpineol[j]	93	3.3	14	*	6.4 (34%)	7.1 (40%)	8.7 (30%)	5.8 (32%)	6.1 (32%)
Citronellol[h]	69	1.9	15	ns	4.5 (51%)	5.3 (22%)	5.1 (31%)	4.1 (56%)	4.3 (56%)
β-Damascenone[j]	121	1.7	25	ns	4.2 (41%)	14 (39%)	10 (66%)	4.0 (49%)	4.3 (49%)
Acids									
Hexanoic acid[h]	60	161	400	*	265 (22%)	299 (15%)	311 (14%)	242 (20%)	259 (20%)
Octanoic acid[m]	60	344	659	*	473 (15%)	486 (13%)	502 (13%)	451 (13%)	468 (13%)
Nonanoic acid[m]	60	1.3	119	ns	23 (122%)	11 (92%)	25 (134%)	23 (115%)	21 (115%)
Decanoic acid[m]	60	119	307	ns	217 (13%)	219 (19%)	233 (17%)	214 (12%)	213 (12%)
Dodecanoic acid[m]	73	2.9	7.6	*	5.4 (20%)	4.8 (20%)	5.3 (17%)	5.4 (22%)	5.3 (22%)
Carbonyls									
Furfural[j]	96	8.2	331	*	104 (80%)	26 (79%)	27 (68%)	133 (62%)	113 (62%)
Benzaldehyde[j]	106	1.9	18	ns	5.0 (49%)	3.5 (44%)	4.5 (91%)	4.8 (38%)	4.9 (38%)
Methional[j]	48	1.7	24	ns	12 (43%)	11 (32%)	10 (43%)	12 (45%)	12 (45%)
Oak-related									
cis-Oak lactone[j]	99	2.1	80	***	32 (62%)	11 (52%)	6 (47%)	42 (39%)	36 (39%)
trans-Oak lactone[j]	99	0.91	85	***	28 (65%)	8.2 (84%)	9.6 (79%)	36 (44%)	30 (44%)
4-Ethylguaiacol[j]	152	0.12	20	ns	1.1 (276%)	0.39 (57%)	0.48 (48%)	0.86 (263%)	0.83 (263%)

[a]Expressed as µg of internal standard/L.
[b]Ion used for integration by multivariate curve resolution.
[c]Significant differences when using quality category as explanatory variable: *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$; ns, not significant.
[d]Percent relative standard deviation is shown in parenthesis next to mean values.
[e]With alcoholic fermentation in stainless steel tanks only.
[f]With alcoholic fermentation in oak barrels only.
[g]With partial or complete aging in oak barrels.
[h]d_{13}-1-Hexanol was used as an internal standard.
[i]d_3-Hexyl acetate was used as an internal standard.
[j]d_5-2-Phenylethanol was used as an internal standard.
[k]d_5-Ethyl nonanoate was used as an internal standard.
[l]d_4-3-Methyl-1-butanol was used as an internal standard.
[m]d_{19}-Decanoic acid was used as an internal standard.

(r = 0.88) and to ethyl butanoate (r = 0.72), as a result of their common biosynthetic pathways (Nykänen 1986). The association between the compounds in the middle reflects their higher concentrations in the younger 2013 wines and the common biosynthetic pathways of 3-methylbutyl and hexyl acetates (r = 0.85) and linalool and α-terpineol (r = 0.64; Nykänen 1986). Negative edges were also observed (data not shown) between 2-methyl-1-propanol and ethyl hexanoate and between diethyl succinate and hexyl acetate. Both 2-methyl-1-propanol and diethyl succinate were more abundant in samples older than one year, while concentrations of ethyl hexanoate and hexyl acetate were lower. Fermentation in barrels both suppresses formation of hexyl acetate and promotes that of diethyl succinate (Liberatore et al. 2010).

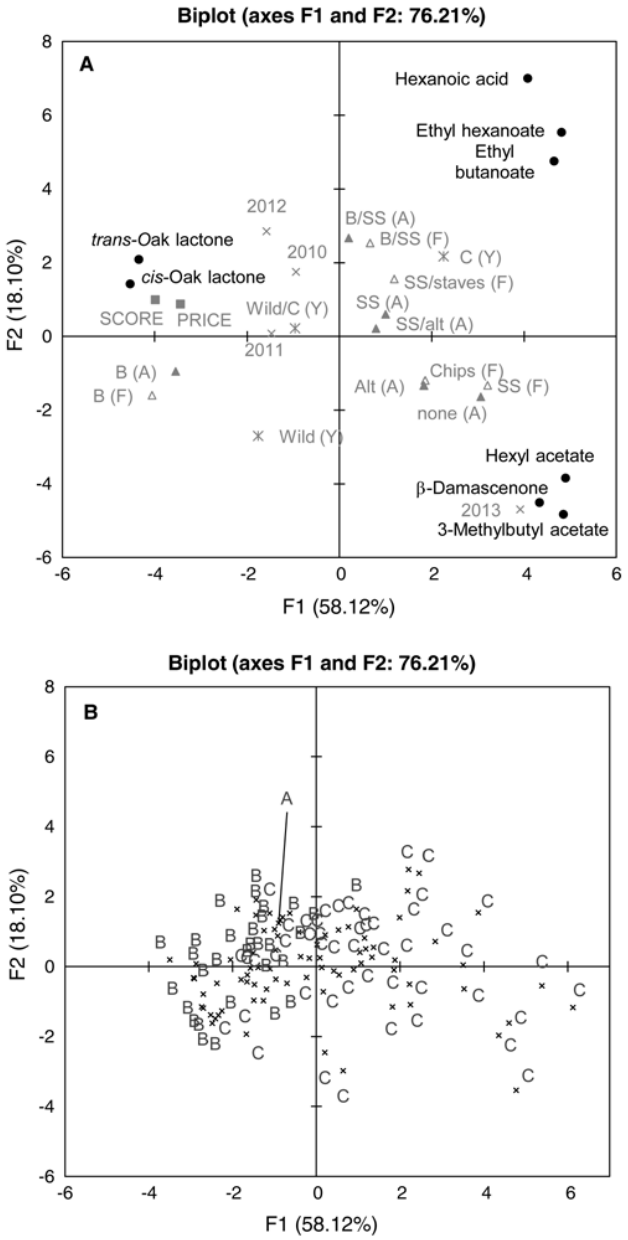

Figure 1 Principal component analysis showing (**A**) loadings and (**B**) scores of significantly different variables ($p < 0.05$) with score as explanatory variable for all Chardonnay wine samples. Active variables are shown in black and supplementary variables and quality score in gray. (Y), Yeast; (C), commercially available yeast; (F), alcoholic fermentation; (A), aging; (B), barrel; (SS), stainless steel tanks; (Alt), alternative oak sources (chips, staves).

Effect of wine age. Significantly different variables ($p < 0.05$) using year of vintage as the explanatory variable (Figure 3) revealed trends consistent with a previous study of red wines from different price points (San Juan et al. 2012), despite the difference in wine type and grape variety studied. In general, varietal compounds such as β-damascenone, linalool, and limonene declined with wine age and were most abundant in the youngest and lowest-scored wines (Table 2 and Figure 3). The average concentration of β-damascenone declined sharply in samples older than one vintage. Under low-pH conditions, β-damascenone is lost due to acid-catalyzed cyclization or nucleophilic attack, particularly in the presence of the nucleophile SO_2 (Daniel et al. 2004). Likewise, the concentrations of 3-methylbutyl acetate, hexyl acetate, 2-phenylethyl acetate, ethyl butanoate, and ethyl hexanoate were greatest in the youngest wines. Such changes in the volatile profile of a wine as a function of age depend on the duration and conditions of storage (temperature, oxygen concentration, and exposure to light) (Cejudo-Bastante et al. 2011). In general, "young wine", "fruity", and "floral" characters decrease rapidly in white wine during aging, mostly due to loss of acetate esters and ethyl esters of short-chain fatty acids, which undergo acid hydrolysis over time (Guchu et al. 2006, Cejudo-Bastante et al. 2011), and to acid-catalyzed rearrangement of monoterpenoids (e.g., linalool, geraniol) into forms with less-intense aromas such as α-terpineol (Marais 1983). Any increases in temperature

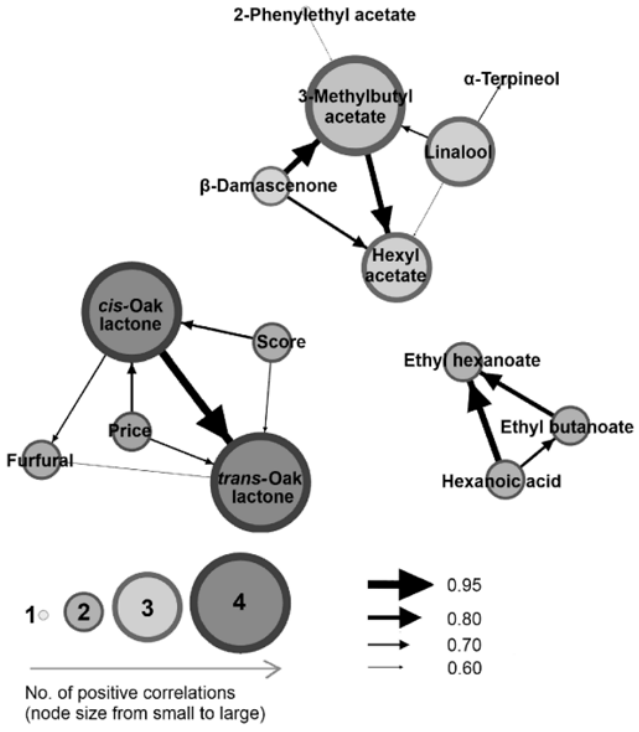

Figure 2 Network analysis of significant variables ($p < 0.05$) that have strong positive relationships ($r \geq 0.6$) with each other.

or light exposure will accelerate these reactions (Guchu et al. 2006, Cejudo-Bastante et al. 2011).

In contrast, ethyl acetate, diethyl succinate, ethyl lactate, ethyl 2-furoate, ethyl 2-phenylacetate, and ethyl 2-methylbutanoate were more abundant in the older wines (Table 2). Although difficult, a distinction should be made between the effects of bottle and barrel aging, as barrel aging encompasses a series of other phenomena such as extraction of oak volatiles, contact with lees, and processes associated with the "low oxidation" conditions of barrel storage (see section "Winemaking techniques"; Garde-Cerdán and Ancín-Azpilicueta 2006). The presence of ethyl 2-furoate is due largely to oak contact, but also to aging itself (Makhotkina and Kilmartin 2012) and formation of ethyl lactate increases during malolactic fermentation (MLF). Aging time allows esterification of acids such as succinic acid into the corresponding diethyl succinate (Ancín-Azpilicueta et al. 2009) and of branched-chain fatty acids, leading to esters such as ethyl 2-methylbutanoate and ethyl 2-furoate (Makhotkina and Kilmartin 2012). As a general rule, most "young wines" (2013 vintage) were aged in barrels for shorter periods than the older vintages, if at all (Supplemental Table 1), and were on the market in the same year as harvest. Consequently, they also spent less time in bottle, allowing less opportunity for most aging-related changes to take place compared to older wines. *cis*- and *trans*-oak lactone concentrations correlated very strongly with score (r = 0.70 and r = 0.64, respectively, $p < 0.05$) and price (r = 0.70 and r = 0.67, respectively, $p < 0.05$; Figure 2), and were significantly different ($p < 0.05$) between the 2013 samples and all others, again mostly because of a decrease or lack of time in contact with oak wood. On the other hand, hexanoic acid, hexyl acetate, and ethyl hexanoate, with their predominantly "green" and "apple" notes (Gambetta et al. 2014), not only correlated negatively to both price (from r = -0.31 to -0.41, $p < 0.05$) and score (from r = -0.39 to -0.52, $p < 0.05$), but were also more abundant in the 2013 samples (Table 2). These compounds have been cited as important to unoaked Chardonnay wine typicity (Smyth 2005), so it was of interest that such compounds were associated negatively with price and quality by experts.

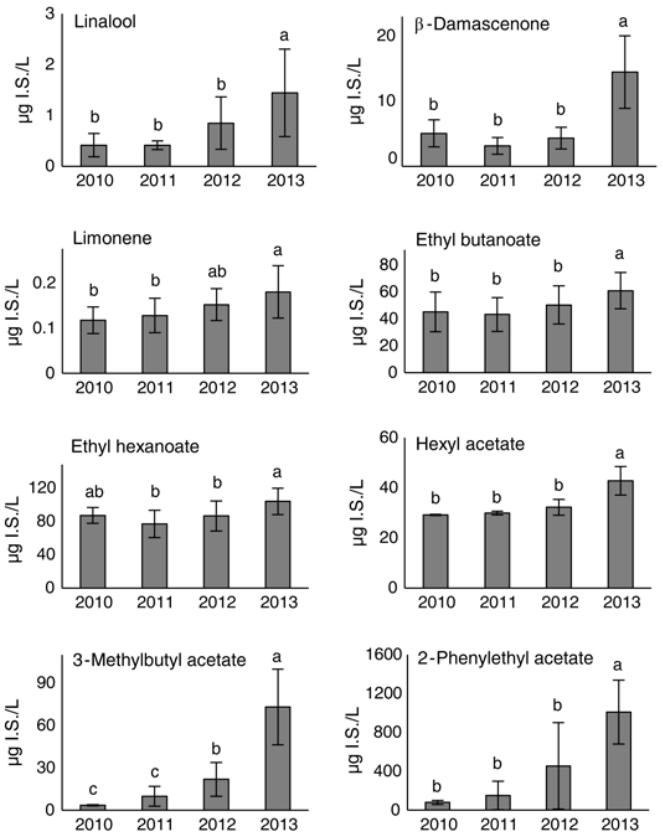

Figure 3 Mean relative concentrations (µg of internal standard/L) of linalool, β-damascenone, 2-phenylethyl acetate, limonene, ethyl butanoate, ethyl hexanoate, 3-methylbutyl acetate, and hexyl acetate, which were significantly different ($p < 0.05$) among all Chardonnay wine samples with year of vintage as the explanatory variable. Different letters in the figures (a, b, c) indicate significant differences between vintages according to Tukey's HSD with $\alpha = 0.05$.

Winemaking techniques. Oak volatiles are incorporated into wine either by fermenting and/or aging in barrels or through the presence of oak barrel alternatives such as chips or staves (Gambetta et al. 2014). Roughly 50% of all samples analyzed were completely fermented in oak barrels rather than in stainless steel tanks, and barrel fermentation was the method of choice for the most expensive wines (Supplemental Table 1). Tasting scores revealed a clear association with samples fermented in barrels (Supplemental Table 1), with a predominance of oak-related volatiles associated with the aroma of the highest-rated samples (left hand side of F1-axis, Figure 1). Independently of wine vintage, wine quality scores correlated very strongly and positively with the presence of *cis*- and *trans*-oak lactone in the wine, as mentioned above. Oak lactones are among the most important volatile compounds released into Chardonnay wine during contact with oak and contribute a "coconut" and "oaky" aroma when present at concentrations above their detection threshold (Spillman et al. 2004). The concentrations of *cis*- and *trans*-oak lactones were five and six times greater, respectively, in 2010 Chardonnay wines than in 2013 samples. However, it should be noted that only one sample was assigned to category A and this sample did not have the highest amounts of these lactones. Further inspection of the data indicated that extremely high quantities of *cis*- and *trans*-oak lactone do not contribute further to improving the quality score of a sample. Furfural, which is formed in oak during coopering (Spillman et al. 2004) and extracted during fermentation and aging in barrels, contributed positively to the overall quality score of the wines (r = 0.48, $p = 0.05$), and was related to the oak lactones (in the same module as score and price) through network analysis (Figure 2).

Unlike stainless steel, oak wood is porous and allows microoxygenation of wine. In addition, oak is not inert: it adsorbs as well as contributes aroma compounds, and alters the production of fermentation volatiles by yeast (González-Marco et al. 2008, Liberatore et al. 2010). For example, barrel fermentation depresses nitrogen consumption and increases production of fermentation volatiles such as 2-phenylethanol

and other higher alcohols (González-Marco et al. 2008). Significantly higher average quantities of 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl 2-methyl butanoate, and diethyl succinate were found in barrel-fermented samples than in those fermented in stainless steel (Table 1). Concentrations of most esters, particularly esters of higher alcohols, were appreciably lower in wines fermented and/or aged in barrels (Figure 1A and Table 1), consistent with other published findings (Ancín-Azpilicueta et al. 2009, Liberatore et al. 2010).

A higher proportion of the more expensive wines were produced, exclusively or in part, by fermenting with autochthonous (wild) yeast (Supplemental Table 1). Data provided for these commercial wines revealed a wide range of yeast choices: 49 samples were fermented exclusively with a commercial yeast strain, of which five were priced at or above AU$40, 19 wines were fermented exclusively with autochthonous yeast, of which eight were priced at or above AU$40, and the remaining 15 used a combination of both, of which three were priced at or above AU$40. According to the PCA (Figure 1), samples produced strictly with a commercial yeast strain (and mostly fermented in stainless steel with no barrel aging) scored significantly lower than the rest and could be observed mainly to the right of the F1-axis. However, this association could well be a coincidence of production costs and reflect the more expensive techniques reserved for production of higher-quality, more expensive wines.

Quality models. Quality ratings provided by the panel and the chemical composition of the Chardonnay wines were assessed using PLS (Figure 4). The model explained 67% of the variance in the volatile composition data (*x*-variable) and 66% of the variance in score (*y*-variable). RMSEP was 0.53, the CC was 0.84, and the RPD was 1.79. RPD values between 1.5 and 3.0 imply that the model can be used for classifying wines as of low, medium, or high quality (Williams 2001), which was sufficient for the purposes of this study. This low RPD value stems from the small range in quality scores (standard deviation), which ranged from 14.2 to 18.1 despite the large number of samples. Consistent with observations throughout the study, the oak-derived *cis*- and *trans*-oak lactones were the two components with the strongest positive effect on the model, followed by ethyl lactate (an MLF metabolite) and 2-methyl-1-propanol (a potential marker of barrel fermentation; Figure 4). The negative contributions of 1-hexanol and 1-propanol were expected based on the details presented above. Although not detracting from our findings, the scope of this work was limited to the effect of aroma compounds on the overall quality of Chardonnay wines. The inclusion of texture and taste attributes would more completely model the full dimensions of Chardonnay wine quality, as they impact important parameters such as mouthfeel, complexity, and balance.

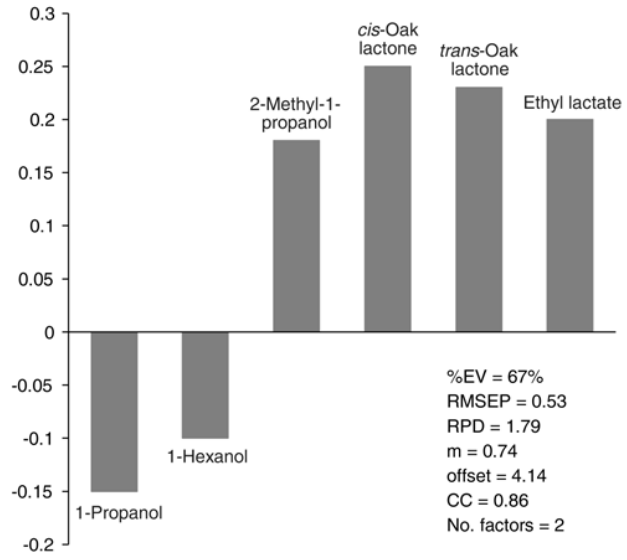

Figure 4 Standardized regression coefficients of the volatile compounds included in the PLS model that linked quality with aroma chemical composition, including model quality parameters. %EV: percentage of variance explained by the model; RMSEP: root-mean-square prediction error; RPD: residual predictive deviation; m: slope of the regression curve between real and predicted *y*-variables; and CC: correlation coefficient between real and predicted *y*-variables.

Relationship between expert quality rating and price, wine critic score, and sales. Price is regarded by many consumers as an indicator of quality, which leads to an assumption that more expensive wines are better (Lockshin et al. 2009). Pearson correlation analysis of our results revealed this was partly true: there was significant positive correlation ($r = 0.45$, $p < 0.0001$) between retail price and score, with the highest average prices also belonging to the oldest wines. However, although the price of a wine partly explains its quality, other variables such as method of production, aging, vintage, etc., need to be taken into account to completely explain the score given to each wine. Likewise, higher prices do not necessarily equate to better wines: the highest-scored wine in our study set retailed for ~AU$40.00, and prices for wines in the C category ranged between AU$7.00 and AU$85.00 (Supplemental Table 1). Wine prices tend not to reflect quality and depend more on other factors such as winery reputation (25% of price variability) and marketing costs (Combris et al. 1997).

Several specialty magazines in the wine market routinely rank wines and serve as a purchase guide for consumers. Comparison of published scores (Halliday 2013) with those awarded by the expert panel exhibited only a moderate positive correlation ($r = 0.44$, $p = 0.0095$), which was expected given the multiple factors that affect judging scores such as setting, order of wine presentation, and number of panelists (Lawless and Heymann 2010). In addition, based on sales volume and ranking obtained from a major Australian liquor retail chain (G. Hindson, personal communication, 2013), consumer purchase behavior is largely explained by retail price ($r = 0.73$, $p = 0.000$), as observed elsewhere (Batt and Dean 2000, Cronley et al. 2005, Lockshin et al. 2006, Veale and Quester 2009), followed by wine guide score ($r = 0.332$, $p = 0.0443$). These correlations might vary if data from different retail sources was included, such as boutique stores that tend to be frequented by more involved consumers (Batt and Dean 2000, Lockshin et al. 2006).

Conclusion

Expert tasters evaluated 83 commercial Chardonnay wines spanning a range of prices, origins, production methods, and vintages, with the samples being representative of the Australian Chardonnay wines available at the time. Significant differences were found between the chemical compositions of all score brackets. Expert tasters scored more highly those samples fermented in oak barrels, which had higher concentrations of oak-related volatiles, particularly *cis*- and *trans*-oak lactones, and lower concentrations of esters and isoprenoids. Younger wines, which also tended to be unoaked, clustered together due to their higher concentrations of β-damascenone and esters such as hexyl acetate, ethyl hexanoate, and ethyl butanoate, consistent with the Chardonnay typicity concept. However, these samples were scored lower by the expert tasters. A model was constructed using PLS to relate expert quality ratings to the chemical composition of the wines. The *cis*- and *trans*-oak lactones, 2-methyl-1-propanol, and ethyl lactate had positive effects on quality score, whereas 1-hexanol and 1-propanol were associated with lower quality wines. However, wine quality also depends on mouthfeel and taste properties, which are determined by polysaccharide, tannin, and residual sugar concentrations, among other factors. Future studies should include these nonvolatile constituents to offer a more complete picture. Furthermore, the experts considered that only one wine could be assigned as top quality (category A). The highest-scored group of wines was category B, which were described as more oaked than the A-scored wine. This could explain the high impact of oak lactones on quality scores. As demonstrated with our sample set, wine quality is determined not only by grape composition, but also by a range of decisions made by the winemaker. The use of oak and type of fermentation vessel, yeast employed, the quality of the fruit streamed into each wine quality tier, and the attention given to the operations thereafter, all have an impact on the final quality of the product. Linking compositional differences to Chardonnay wine quality judged by experts has identified a range of targets that may help winemakers tune the quality of their wines by adjusting winemaking protocols.

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Supplemental Data for:

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Code	Vintage	Region[a]	Fermentation[b]	Aging[c]	Yeast[d]	Price	% Alc	pH	TA (g/L)	Closure[e]	Mean tasting score	Quality category
359	2012	TAS	BF	BA (9)	C	$40.00	13.4	3.19	6.27	1	18.1	A
105	2012	Mix	BF	BA/SSA (11)	wild/C	$61.00	13.6	3.39	7.62	1	17.6	B
155	2012	VIC	BF	BA (9)	C	$30.00	13.2	3.26	6.46	1	17.4	B
780	2011	WA	BF	BA (11)	wild	$105.00	13.3	3.13	7.91	1	17.3	B
795	2012	VIC	BF	BA (7)	wild/C	$25.00	12.7	3.23	6.74	1	17.2	B
715	2010	SA	BF	BA (9)	C/wild	$83.00	13.2	3.24	5.62	1	17.1	B
893	2012	SA	BF	BA (10)	wild/C	$28.00	13.6	3.21	6.08	1	17.1	B
928	2011	WA	SSF/BF	BA/SSA (9)	C/wild	$23.50	13.8	3.24	6.75	1	17.1	B
935	2012	WA	BF	BA (9)	C	$39.00	12.8	3.31	6.10	1	17.1	B
236	2012	VIC	BF	BA (9)	C	$55.00	13.3	3.45	5.20	1	17.0	B
733	2012	WA	BF	BA/SSA (10)	wild	$71.00	13.6	3.39	6.50	1	17.0	B
812	2012	SA	BF	BA (3)	C/wild	$12.50	13.2	3.18	6.70	5	17.0	B
689	2012	VIC	BF	BA (9)	C/wild	$27.00	12.6	3.20	6.26	1	16.9	B
94	2012	NSW	BF	BA (8)	wild/C	$19.00	12.3	3.32	7.63	1	16.8	B
491	2012	Mix	BF	BA/SSA (10)	wild/C	$29.00	13.5	3.29	7.26	1	16.8	B
660	2012	SA	BF	BA (15)	wild	$18.00	14.6	3.35	6.00	1	16.8	B
634	2011	VIC	BF	BA (9)	wild	$47.00	12.1	3.42	6.03	1	16.7	B
224	2012	TAS	BF	BA/SSA (11)	wild	$40.00	13.6	3.54	6.65	1	16.5	B
414	2011	VIC	BF	BA (9)	wild	$25.00	11.8	3.38	7.24	1	16.5	B
549	2012	VIC	BF	BA (9)	wild	$52.00	13.4	3.29	7.22	1	16.5	B
40	2012	SA	BF	BA (8)	wild	$19.00	12.7	3.21	6.38	1	16.4	B
524	2012	SA	SSF/BF	BA (6)	C	$15.00	13.4	3.27	6.20	1	16.4	B
614	2012	WA	SSF/BF	BA (12)	C	$27.00	13.2	3.18	7.00	1	16.4	B
838	2012	NSW	BF	BA (9)	C	$34.00	12.4	3.13	6.16	1	16.4	B
874	2012	WA	BF	BA (9)	wild	$25.00	12.5	3.18	6.73	1	16.4	B
173	2010	SA	BF	BA (9)	C	$125.00	13.5	3.19	5.89	1	16.3	B
502	2012	WA	BF	BA/SSA (10)	wild	$38.00	14.1	3.44	6.67	1	16.3	B
531	2012	SA	SSF/BF	BA (10)	C	$35.00	13.5	3.28	6.25	1	16.3	B
141	2012	SA	BF	BA (9)	C	$40.00	13.9	3.11	7.26	1	16.2	B
948	2012	NSW	SSF/BF	BA/SSA (8)	C	$30.00	12.9	3.34	5.16	1	16.2	B
579	2012	VIC	SSF/BF	Staves (9)	C	$13.00	13.2	3.32	5.93	1	16.1	B
913	2012	SA	BF	BA (9)	wild	$31.50	13.0	3.27	7.10	1	16.1	B
466	2012	SA	BF	BA (5)	C	$20.00	13.5	3.42	5.82	1	16.0	B
697	2012	SA	SSF/BF	BA/SSA (8)	C	$19.00	12.7	3.33	7.51	1	16.0	B
370	2010	WA	BF	BA (9)	wild/C	$50.00	13.3	3.27	7.00	1	15.9	C
809	2013	SA	BF	BA (12)	SSL	$25.00	13.0	3.31	6.42	1	15.9	C
58	2012	VIC	SSF/BF	BA/SSA (6)	C	$18.00	12.7	3.37	6.43	1	15.8	C
86	2011	WA	BF	BA (12)	C	$85.00	13.9	3.36	5.70	1	15.8	C
126	2012	SA	BF	BA (9)	C	$20.00	13.8	3.42	5.31	1	15.8	C
825	2012	VIC	BF	BA (9)	C	$28.50	13.1	3.11	7.25	1	15.8	C
863	2012	SA	BF	BA (9)	wild	$42.00	12.8	3.20	6.51	1	15.8	C
957	2011	VIC	BF	BA (18)	wild	$120.00	13.3	3.39	6.87	3	15.8	C
585	2012	TAS	BF	BA (8)	wild/C	$37.00	13.1	3.24	6.84	1	15.6	C
762	2012	WA	SSF	SSA (5)	C	$12.00	13.5	3.30	7.32	1	15.6	C
592	2012	SA	BF	BA (7)	wild/C	$18.00	14.1	3.15	6.90	1	15.5	C
45	2012	SA	SSF/Staves	BA (7)	C	$15.00	13.4	3.29	5.27	1	15.4	C
346	2012	VIC	SSF/BF	BA/SSA (12)	C	$14.00	12.5	3.36	6.50	1	15.4	C
384	2013	SA	SSF/BF	BA (9)	wild/C	$18.00	13.2	3.21	5.73	1	15.4	C
977	2012	SA	BF	Staves (3)	C	$17.00	13.9	3.33	5.80	1	15.4	C

Supplemental Data for:

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Code	Vintage	Region[a]	Fermentation[b]	Aging[c]	Yeast[d]	Price	% Alc	pH	TA (g/L)	Closure[e]	Mean tasting score	Quality category
206	2012	SA	BF	BA (14)	C	$35.00	12.6	3.48	5.28	1	15.3	C
482	2012	VIC	SSF	SSA (3)	C	$7.50	12.9	3.36	6.75	1	15.3	C
537	2011	SA	BF	BA (7)	C	$30.00	12.7	3.02	8.74	1	15.3	C
249	2012	SA	SSF/Staves	SSA/Staves (6)	C	$17.00	13.3	3.15	6.28	1	15.1	C
291	2012	SA	SSF/Staves	BA (9)	C	$15.00	13.3	3.30	5.80	1	15.1	C
304	2013	SA	SSF	Staves (6)	C	$11.00	12.9	3.35	5.66	1	15.1	C
401	2012	SA	BF	BA (10)	C	$22.21	14.2	3.35	7.55	1	15.1	C
757	2012	SA	SSF/Staves	Staves (6)	C	$13.00	13.5	3.34	5.30	1	15.1	C
278	*2012*	SEA	SSF/BF	BA/SSA (12)	C	$12.90	13.0	3.34	6.11	1	15.0	C
195	2012	SEA	SSF/BF	SSA (6)	C	$13.00	13.5	3.60	6.22	1	14.9	C
623	2013	NSW	SSF	none	C	$7.00	11.8	3.33	6.59	2	14.9	C
647	2013	Mix	Chips	Chips (9)	C	$7.00	13.8	3.45	6.41	1	14.9	C
679	2013	SA	SSF	none	C	$15.00	13.4	3.33	5.72	1	14.9	C
880	2012	SA	SSF/Staves	BA/SSA (10)	C	$17.00	13.6	3.11	6.11	1	14.9	C
71	2012	VIC	BF	BA (9)	C	$20.00	13.5	3.24	5.31	1	14.8	C
336	2012	SA	SSF/BF	BA (9)	C	$23.00	13.8	3.24	5.73	1	14.8	C
990	2012	SA	SSF	none	C	$9.90	13.0	3.35	6.40	1	14.8	C
168	2013	SA	Chips	chips (9)	C	$10.00	13.5	3.48	5.80	2	14.7	C
181	2013	NSW	Chips	chips (4)	C	$9.00	13.7	3.38	7.50	2	14.7	C
728	2012	SEA	SSF	SSA/BF (8)	C	$7.00	12.9	3.32	6.50	1	14.7	C
744	2011	VIC	BF	BA (9)	wild	$25.00	12.2	3.40	6.20	1	14.7	C
816	2013	SA	SSF	none	wild	$15.00	12.6	3.18	6.70	1	14.7	C
253	2012	SA	SSF	none	wild	$18.00	14.2	3.28	6.35	1	14.6	C
456	2013	SA	SSF/Staves	SSA/Staves (4)	C	$12.00	12.9	3.38	5.70	2	14.5	C
469	2012	SA	SSF	SSA/chips (3)	wild	$9.00	13.8	3.52	6.60	1	14.5	C
702	2013	VIC	BF	BA (9)	wild	$22.00	12.6	3.41	6.97	1	14.5	C
217	2012	SA	BF	BA (10)	C	$19.00	13.5	3.21	5.92	1	14.4	C
318	2011	SA	SSF	none	C	$13.00	11.6	3.29	5.98	1	14.4	C
437	2013	Mix	SSF	none	wild/C	$9.99	13.3	3.49	6.29	2	14.4	C
511	2012	Mix	SSF/BF	BA/SSA (12)	C	$15.00	14.0	3.34	6.38	4	14.4	C
21	2011	SA	SSF/BF	BA (6)	wild/C	$14.00	11.72	3.25	6.20	1	14.3	C
289	2012	WA	BF	BA (8)	wild	$45.00	12.6	3.43	6.43	1	14.3	C
555	2012	SA	SSF/BF	BA/SSA (9)	C	$18.00	13.0	3.26	5.29	1	14.3	C
845	2012	SA	SSF	SSA (3)	C	$7.99	13.5	3.38	5.62	1	14.2	C

[a]NSW: New South Wales; SA: South Australia; SEA: South Eastern Australia; TAS: Tasmania; VIC: Victoria; WA: Western Australia; Mix: origin of grapes not specified.
[b]AF: alcoholic fermentation; BF: barrel-fermented; SSF: fermented in stainless steel tanks.
[c]BA: aged in oak barrels; SSA: aged in stainless steel tanks; time of aging in months is given in parentheses.
[d]C: commercial yeast strain; wild: autochthonous yeast strain.
[e]1: screw cap lined with tin saran; 2: screw cap lined with saranex; 3: natural cork; 4: agglomerated cork; 5: agglomerated "twin top" cork with natural cork ends.

CHAPTER 4

Towards the creation of a wine quality prediction index: Correlation of Chardonnay juice and wine compositions from different regions and quality levels

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Name of Principal Author (Candidate)	Joanna M. Gambetta		
Contribution to the Paper	Designed experiments, performed experimental work and sensory trials, analysed and interpreted data, drafted and constructed the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4/11/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Susan E.P. Bastian		
Contribution to the Paper	Contributed to the research idea and sensory analysis design, and editing of the manuscript.		
Signature		Date	4/11/2016

Name of Co-Author	Daniel Cozzolino		
Contribution to the Paper	Supervised the work, contributed to the research idea and design of analysis by mid-infrared spectroscopy, assisted with data analysis and editing of the manuscript.		
Signature		Date	4/11/2016

Name of Co-Author	David W. Jeffery		
Contribution to the Paper	Supervised the work, contributed to the research idea and experimental design. Assisted in the preparation and editing of the manuscript and acted as the corresponding author.		
Signature		Date	4/11/2016

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Towards the Creation of a Wine Quality Prediction Index: Correlation of Chardonnay Juice and Wine Compositions from Different Regions and Quality Levels

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Abstract Wine quality depends upon the composition of the grapes used in its production, which in turn depends on the weather and soil of the growing region together with viticultural practices. Region is used by many winemakers as a proxy for quality but objective quality measures are lacking. This study examined the compositional aspects of Chardonnay wines produced with berries from different regions. Through descriptive analysis, distinct sensory profiles were recognised for three diverse regions in South Australia (Adelaide Hills, Eden Valley, Riverland), which helped to pinpoint compounds relating to higher- and lower-quality Chardonnay wines. Correlations between the content of elements, fatty acids, free volatiles and conjugated glycosides in berries from different quality levels, and the composition of their corresponding wines, were investigated. Higher berry concentrations of linalool, (*E*)-linalool oxide, (*Z*)-3-hexen-1-ol, decanoic acid, vitispirane, Cu, Zn, and behenic acid, and lower °Brix and pH levels were related to higher quality wines.

Keywords Chardonnay · Sensory descriptive analysis · Principal component analysis · Isoprenoids · Partial least squares regression · Objective measures of quality

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Introduction

The quality of any wine is intrinsically dependent on the quality and composition of the grapes used to produce it. In traditional winemaking countries such as France and Germany, wine quality is determined by geographic origin or the *terroir* of the wine (Seguin 1986). Terroir describes the relationship between an agricultural product and its geographical origin and considers that the region of production might influence the product's characteristics (Seguin 1986). In the case of wine, terroir involves the interactions of grapevine, vineyard, climate and human factors such as viticultural and oenological practices. More specifically, mesoclimatic variability has to be taken into account, as well as altitude, inclination, orientation and composition of the soil (Seguin 1986). Together, the various aspects of terroir affect the development and composition of the grapes, which in turn influences the characters of the wine, so terroir can be seen as a proxy for wine quality. As such, certain viticultural regions are regarded as producing better quality fruit, which is often reflected in the different retail prices of Chardonnay wines originating from various regions (Schamel and Anderson 2003).

Linked to terroir, grape maturity is also an important driver of grape composition and affects wine style and quality. Indices such as the Winkler Thermal Index and the Heliothermal Index of Huglin help to classify different regions into distinct climatic zones, according to the amount of degree-days and daily temperatures, and relate them to potential berry sugar content (Tonietto and Carboneau 2004). Every grape variety possesses a maturity index calculated as the amount of degree-days it requires to fully mature, making varieties better suited to some growing regions rather than others. Tonietto and Carboneau (2004) proposed that other criteria be taken into account, such as night temperature and rainfall. Whichever index is used, differences in precipitation,

wind, temperature and levels of sun exposure affect berry development and the accumulation of acids, sugars and aroma compounds (Jones and Davis 2000; Rocha et al. 2010), such that the overall combination of a given grape variety and characteristics of the vineyard location will result in better quality fruit from certain regions compared to others.

In terms of measuring quality, most indices consider berry sugar accumulation as an indicator of grape maturity; it is only through empirical knowledge that any classification pertaining to wine quality is taken into account (Forde et al. 2011). The same holds true for harvest date determination, which is mainly calculated by measuring sugar, pH and acid content in the berry. However, aroma compounds are of paramount importance to wine quality, and some measure (or marker) of aroma potential should also be taken into account when determining optimum harvest date and grading/allocating fruit.

Chardonnay grape and wine quality is of particular interest given the importance of this variety, both in Australia and internationally. C_{13}-norisoprenoids, such as β-damascenone and the monoterpenoids linalool and α-terpineol, usually bound as glycosides, are considered to be amongst the main grape-derived aroma compounds contributing to Chardonnay wine quality and typicity (Gambetta et al. 2014). C_{13}-norisoprenoids are derived from the enzymatic and photochemical degradation of carotenoids (Lee et al. 2007); carotenoids (tetraterpenoids, C_{40}) and monoterpenoids (C_{10}), are formed via the terpenoid biosynthetic pathway. Higher sunlight exposure, without incurring overexposure, leads to higher quantities of both C_{13}-norisoprenoids and monoterpenoids (Lee et al. 2007). This is managed through viticultural practices such as leaf removal, which increases the amounts of these aroma compounds by decreasing the extent of berry shading (Duchêne and Schneider 2005; Lee et al. 2007). On the contrary, overexposure of berries to sunlight leads to a loss of monoterpenoids through transformation or degradation reactions.

Beyond the effects of sunlight, temperature constitutes another important factor affecting the development of secondary metabolites in the grape and Duchêne and Schneider (2005) have suggested that cool conditions are better for the development of aromas. At equal sugar concentrations, higher temperatures have been suggested to decrease the concentration of monoterpenoids (Belancic et al. 1997) and lower the overall concentration of C_{13}-norisoprenoids (Marais et al. 1999) whilst increasing the presence of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and its carotenoid precursors (Marais et al. 1992). Whereas most C_{13}-norisoprenoids and monoterpenoids contribute desirable wine aroma attributes, TDN can contribute to the aroma of bottle-aged white wine (particularly Riesling) when present at low concentrations but imparts an undesirable "kerosene" aroma at concentrations above its perception threshold (Marais et al. 1992).

Other than these direct grape contributors to wine aroma, an array of volatile components arise during fermentation and ageing that can be linked to grape composition to an extent. Thus, aroma compounds such as isoamyl acetate, octanoic and hexanoic acids, diethyl succinate, 2-phenylethyl acetate, ethyl hexanoate and ethyl butanoate also contribute to the typicity and overall quality of Chardonnay wines (Jaffré et al. 2011).

During ripening, a number of phenomena take place such as the accumulation of sugars and decrease in acidity, with these attributes being routinely measured to guide decision-making. On the other hand, there is also accumulation and degradation of a range of components that have direct relevance to wine quality, but these factors are not easily included as part of the decision-making package. This study begins to address this gap, by identifying compositional features that can be tentatively used as potential markers to predict Chardonnay wine quality in the vineyard in relation to different geographical origins of the grapes.

Materials and Methods

Chemicals

Aroma reference compounds were obtained from either Sigma-Aldrich (Castle Hill, NSW, Australia) or Alfa Aesar (Ward Hill, MA, USA) with a purity of ≥ 97 %. Deuterium-labelled internal standards consisting of d_4-3-methyl-1-butanol, d_3-hexyl acetate, d_{13}-1-hexanol, d_5-phenylethanol and d_{19}-decanoic acid were supplied by CDN Isotopes (Point-Claire, Quebec, CN), and d_5-ethyl dodecanoate was synthesised previously (Boss et al. 2015). Additional details regarding materials and preparation of solutions appear in Supplementary Information.

Grapes

V. vinifera var. Chardonnay berries were collected at commercial maturity from commercial vineyards in Barossa ($n = 5$), Eden ($n = 9$), and Clare Valleys ($n = 9$), Adelaide Hills ($n = 8$), and Riverland ($n = 2$), South Australia during the 2014 vintage. A 4-kg sample was collected from each vineyard for general analysis and small-lot vinifications, whereas 20 kg was collected for vinification from the vineyards used to prepare wines for sensory analysis. Grapes were carefully stored at −20 °C until required and destemmed as necessary whilst frozen.

Vinification

Wines for Sensory Analysis

Wines required for sensory analysis were vinified on a 5-L scale in triplicate according to Ristic et al. (2013). More specific details of the winemaking appear in Supplementary Information.

Small-Scale Ferments for Chemical Analysis

Wines required for chemical analysis were fermented on a 500-mL scale in triplicate. Destemmed berries were defrosted at room temperature 2 h prior to crushing, and SO_2 (50 mg/L as a solution of potassium metabisulfite) was added to each sample. Berries were crushed manually and centrifuged for 5 min at 4250 r.c.f. and 10 °C in a Beckman J21 centrifuge. The clarified juices were decanted into 500-mL Schott bottles, and pH was corrected to 3.4 with tartaric acid. Additions of DAP (1 mL, 472 mg/L solution) and PVPP (1 mL, 130 mg/L suspension) were added to each sample followed by 5 mL of yeast starter culture (PDM, Maurivin®, 1 mL/100 mL juice). After the end of fermentation (residual sugars < 2 g/L, verified using Clinitest tablets), wines were racked, adjusted with PMS solution to give 57 mg/L of free SO_2, corrected again to pH 3.4 where necessary and cold stabilised at 4 °C for 1 week. Wines were then filtered using a 0.22-μm S-Pak ® membrane filter (Merck), bottled and cellared at 15 °C until required.

Sensory Analysis

Two wines were produced for descriptive analysis (DA) (Stone and Sidel 2004) from each of the three regions (Riverland, Eden Valley and Adelaide Hills) spanning the most distinct grape quality levels. The panel was composed of 12 assessors (6 male, 6 female, ranging in age from 25 to 50) with previous DA experience, who undertook a series of training and formal evaluation sessions. Additional details related to training, formal assessments and sensory attributes appear in Supplementary Information.

Wine and Juice Basic Chemical Analysis

Wine ethanol content (% *v*/*v*) was evaluated using an Alcolyzer Wine ME/DMA 4500 M (Anton Paar, Austria). Titrable acidity (TA, expressed as g/L of tartaric acid) and pH were measured using a combined pH meter and autotitrator (CompacTitrator, Crison Instruments, S.A., Allela, Spain) (Iland et al. 2004). Residual sugar (glucose + fructose) was determined using an enzymatic test kit (Megazyme, Wicklow, Ireland). All measurements were performed in duplicate.

Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry

HS-SPME-GC-MS analysis of all samples was carried out in duplicate in 20-cm SPME vials (Supelco) containing 2-g sodium chloride. After addition of samples and internal standards, vials were tightly sealed with a PTFE-lined cap and homogenised with a vortex mixer. HS-SPME was carried out using a Gerstel autosampler (Lasersan Australasia Pty Ltd., Robina, QLD, Australia) fitted with a DVB/CAR/PDMS SPME fibre (50/30 μm, 1 cm, 23 gauge). GC-MS analysis was performed with an Agilent 7890A gas chromatograph coupled to a 5975C mass selective detector (Agilent, Forest Hill, VIC, Australia). A deactivated SPME inlet liner (0.75 mm i.d., Supelco) was used and the GC column was a DB-WAX (60 m, 0.25 mm, 0.25 μm, Agilent J&W, Folsom, CA, USA). Specific incubation and oven programme conditions for each type of sample are outlined below. The transfer line was set at 230 °C, and positive ion electron impact spectra at 70 eV were recorded in the range m/z 35–350 for scan runs. Instrument control and data analysis were performed with Agilent ChemStation software (E.02.02.1431). Analyte identity was determined by comparison with authentic reference compounds. When these were unavailable, identification was carried out through mass spectral library matches (NBS 75K), and comparison of retention indices and mass spectrometric data with those reported in the literature.

Screening of Free Volatiles from Grapes

Grape juice (5 mL), prepared by manually crushing 200 g of previously destemmed and defrosted grapes with 200 μL of a 100 mg/mL PMS solution, was added to a 20-cm SPME vial (Supelco) containing 2 g of sodium chloride. A 25 μL aliquot of internal standard solution, consisting of a mixture of deuterated standards in ethanol (d_4-3-methyl-1-butanol, 1.2 μg/L; d_3-hexyl acetate, 0.05 μg/L; d_{13}-1-hexanol, 0.04 μg/L; d_5-2-phenylethanol, 0.5 μg/L; d_{19}-decanoic acid, 0.24 μg/L and d_5-ethyl dodecanoate, 0.21 μg/L), was added. Samples were incubated for 10 min at 35 °C and extracted for 20 min at 35 °C with an agitation speed of 250 rpm. Desorption into the inlet was performed at 200 °C for 6 min in splitless mode, and the splitter was opened after 3 min. Ultrapure helium was used as carrier gas at a constant flow of 1.15 mL min^{-1}. The oven temperature programme started at 40 °C for 4 min and increased to 220 °C at 5 °C/min, before being held at this temperature for 10 min.

Analysis of Wine Volatiles

Wine (0.5 mL) was added to a 20-mL SPME vial containing 2 g NaCl, together with 10 μL of ethanolic internal standard mixture (d_4-3-methyl-1-butanol, 2.4 μg/L; d_3-hexyl acetate, 0.025 μg/L; d_{13}-1-hexanol, 0.05 μg/L; d_5-2-phenylethanol, 0.5 μg/L; d_{19}-decanoic acid, 0.05 μg/L and d_5-ethyl dodecanoate, 0.001 μg/L) and 4.5 mL of Milli-Q water as described by Wang et al. (2016). Vials were tightly sealed with a PTFE-lined cap, homogenised with a vortex mixer and subjected to GC-MS analysis as described above, with the following modifications. Extraction temperature was set at 50 °C,

inlet temperature was 240 °C and the oven programme was held at 40 °C for 1 min, increased to 130 at 2 °C min^{-1}, then to 212 at 5 °C min^{-1}, and finally to 250 at 15 °C min^{-1}, which was held for 10 min.

GC-MS Analysis of Hydrolysed Grape Glycosides

Volatile compounds present as glycosidic precursors in grapes were determined according to the method of Hernandez-Orte et al. (2015), using the GC-MS instrumentation described above configured for liquid injection.

Element Analysis by Inductively Couple Plasma-Optical Emission Spectroscopy (ICP-OES)

Elements comprising Fe, Mn, B, Cu, Zn, Ca, Mg, Na, K, P, S, Al, Ti, Cr, Cd, Pb, As, Se, Mo, Co and Ni were analysed by ICP-OES at Waite Analytical Services laboratory according to the procedure of Wheal et al. (2011).

Fatty Acid Analysis by GC-Flame Ionisation Detection (GC-FID)

Analysis of saturated and unsaturated fatty acids was performed by Waite Analytical Services laboratory using a modified Bligh and Dyer extraction technique and GC-FID as described by Makrides et al. (1996).

Mid-Infrared (MIR) Analysis of Grape Juice

The MIR spectrum of each juice sample was acquired with a Bruker Alpha spectrometer (Bruker Optics GmbH, Ettlingen, Germany) coupled with a platinum diamond attenuated total reflectance (ATR) single reflection module cell. Spectra resulted from an average of 64 scans (resolution of 4 cm^{-1}) acquired between 4000 and 375 cm^{-1}. The scanner velocity was 7.5 kHz with a background of 64 scans. Air was used as reference background. MIR spectra were recorded using OPUS v.6.5 software (Bruker Optics GmbH).

Statistical Analysis

Basic chemical data were processed with Microsoft Excel 2010. Data are presented as mean values with standard deviation from replicate determinations. Two-way analysis of variance (ANOVA) was performed on all the attributes assessed by the sensory panel together with a Tukey's HSD ($p<0.05$) using SenPaq, version 5.01 (Qi Statistics, Reading, UK) to determine the effects of treatment, fermentation replicate nested within treatment, judge and replicate. Canonical variate analysis (CVA, SenPaq) was employed to analyse all significantly different attributes; the number of dimensions was chosen based on the value of the corresponding eigenvalue. One-way ANOVA (region) was performed on all chemical data using XLSTAT (version 2014.05.03, Addinsoft, Paris, France) to determine significantly different variables which were later used for all other analysis. The means of significantly different attributes and chemical data were subjected to principal component analysis (PCA) using XLSTAT. Data for PCA were normalised prior to analysis and aroma volatiles were grouped if highly correlated ($r>0.95$), and the number of principal components (PC) used was determined from scree plots. The Pearson correlation matrix was calculated and inspected for attributes that were significantly correlated at $\alpha=0.05$. Partial least squares regression analysis (PLS) was carried out using The Unscrambler X (CAMO AS, version 10.3, Oslo, Norway) to relate wine chemical data (y variables) with juice compositional data (x variables) using PLS2, with all data standardised prior to analysis. Wine variables for which a significant correlation could be found ($r\geq0.6$) were further analysed using PLS1. PLS models were developed using cross validation (leave one out). MIR spectral data were processed with The Unscrambler X software using the second derivative (20 smoothing points and second polynomial order) of the fingerprint region (1800–800 cm^{-1}) in order to remove and correct for baseline effects (Savitsky and Golay 1964).

Results and Discussion

Sensory and Compositional Analysis of Regional Chardonnay Wines

Using a consistent winemaking approach, we explored the sensory and volatile profiles of wines produced with fruit from two different vineyards in three distinct quality regions in South Australia—the Riverland (RVL), Eden Valley (EV) and Adelaide Hills (ADL).

As observed by Saliba et al. (2013), a wide range of attributes can be used to describe Chardonnay depending on origin and style such as *lemon*, *lime*, *confectionary*, *tropical fruit*, *melon*, *peach*, *apricot*, *green apple and honey*. All of these typical aroma notes seemed to be represented in varying degrees in the different samples, with attributes such as *tropical fruit*, *melon* and *green apple* appearing to be more intense in ADL samples, followed by EV (which was rated as more intense for *honey* notes than the others) and to a lesser extent in those from RVL (Fig. 1b). The ANOVA showed significant differences ($p<0.05$) for all attributes with the exception of *green apple*, *doughy*, *citrus* (palate), *stonefruit/melon* (palate) and *tropical* (palate) between the different origins (Supplementary Information, Table S2). Canonical variate analysis (CVA) was conducted on the significant DA attributes in order to classify samples by region. The CVA scores and loadings plots revealed that 89.2 % of the variance was explained by the first two canonical variates, with the majority

of that (80.2 %) accounted for by CV1 (Fig. 1a, b). Clear separation between the wines from each origin is evident, with the wines from ADL located to the far right side of the score plot (Fig. 1a). These wines had the highest ratings of *tropical*, *citrus*, *floral*, *stonefruit/melon* and *herbaceous* aromas as well as *green apple* and *herbaceous* flavours (Fig. 1b). The Adelaide Hills has been catalogued as an "ultra-premium" region (Schamel and Anderson 2003), and given the results from the DA, was therefore used as the reference for higher quality wines. Chardonnay wines produced from RVL fruit were located on the opposite side of the biplot and were described by the panel as possessing more intense *beer, mushroom, yeasty* and *caramel* aromas, *honey* flavour and having more body (Fig. 1a, b). They were also perceived as hotter, more astringent and bitter, as well as longer in *afterflavour* than the rest. Wines from EV were located in the middle of the plot (Fig. 1a) and were judged to have characteristics that were intermediate between the RVL and ADL samples for most statistically significant attributes. Interestingly, apart from wines made from RVL fruit, the other two regions were seen as slightly divergent in their sensory profiles.

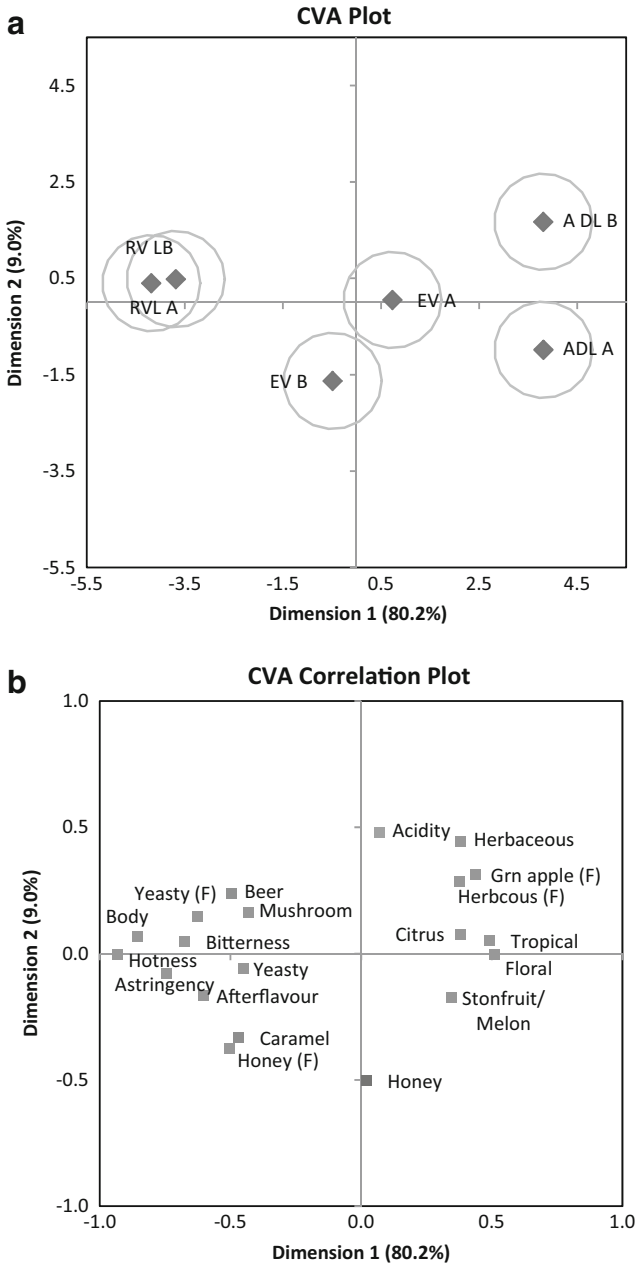

Fig. 1 CVA plot generated from DA mean intensity rating data for Chardonnay wines made with fruit originating from Riverland (RVL), Eden Valley (EV) and Adelaide Hills (ADL) showing **a** scores with 95 % confidence ellipses and **b** loadings. (F) designates flavour attributes

HS-SPME-GC-MS was used to quantify 28 compounds in the six wines that underwent DA (Supplementary Information, Table S3). All compounds were detected at quantities well within the ranges previously reported for Chardonnay wines in the literature (Gambetta et al. 2014) with the following compounds present at levels above their perception threshold in all samples: 3-methyl-1-butanol, 2-phenylethanol (except ADL B), 1-octanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, β-damascenone, and hexanoic, octanoic and decanoic acid. The ANOVA showed significant differences ($p < 0.05$) between samples for all compounds with the exception of β-damascenone, limonene, hexanoic acid, dodecanoic acid, 2-methyl-1-propanol, 1-octanol, and 2-ethyl-1-hexanol. Linalool, isoamyl acetate, hexyl acetate, octanoic acid, decanoic acid and 1-hexanol were found in higher concentrations in the samples from ADL, followed by those from EV. All of these, with the exception of 1-hexanol, contribute positively to the typicity of Chardonnay wines (Gambetta et al. 2014). Ethyl acetate, ethyl 2-methyl butanoate, 3-methyl-1-butanol, acetic acid and diethyl succinate were higher in the samples produced from RVL grapes, and α-terpineol was found in greater concentrations in EV samples.

PCA of SPME-GC-MS and sensory data for the samples revealed some relationships between sensory attributes and volatile compounds, which in turn may be related to quality. The first two PCs shown in the PCA biplot (Fig. 2) explained 78.7 % of the total variation in the samples, with the majority of the variance (63.7 %) explained by PC1 and an additional 15 % explained by PC2. Samples from different origins were clearly differentiated along PC1 based on their content of 1-hexanol, acetates (hexyl and isoamyl), linalool and acids (octanoic and decanoic) on the left and higher concentrations of acetic acid, 3-methyl-1-butanol, ethyl-2-methylbutanoate and diethyl succinate on the right. Separation of wines along PC2 was based mainly on their concentration of ethyl decanoate and α-terpineol, which in turn appear to contribute more to the differentiation between samples within the same region than between different regions.

Samples were located in different quadrants of the biplot and characterised by different volatile profiles (Fig. 2).

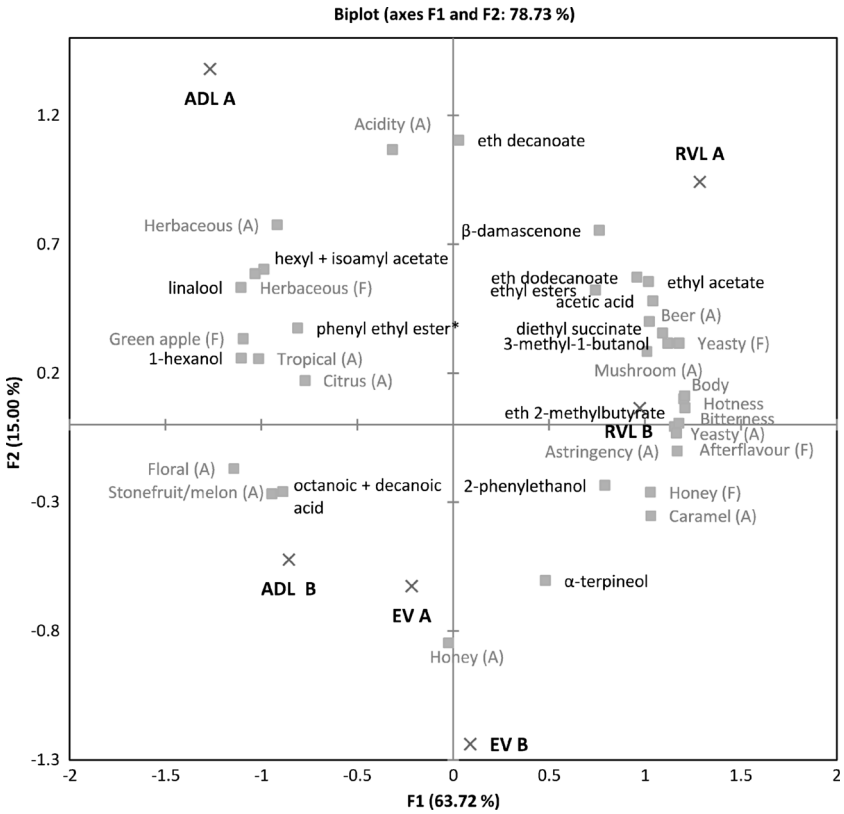

Fig. 2 PCA biplot showing scores and loadings of the standardised means for significant ($p < 0.05$) sensory attributes from DA and volatile compounds from GC-MS analysis determined for Chardonnay wines made with fruit originating from the Riverland (RVL), Eden Valley (EV) and Adelaide Hills (ADL). (A) designates an aroma attribute and (F) designates a flavour attribute. *phenylethyl ester designates the grouping of 2-phenylethyl acetate and ethyl phenylacetate

Chardonnay wines produced from ADL fruit contained higher amounts of 1-hexanol (Supplementary Information, Table S3; ADL A 2602 μg/L, ADL B 1547 μg/L) than the other wines, which relates well with the sensory results (Fig. 1). 1-Hexanol is normally described as possessing *herbaceous and grassy* notes (Wang et al. 2016) and was very highly correlated ($r = 0.82$) to higher intensities of *herbaceous* aroma and palate attributes perceived for the ADL wines, in particular sample ADL A, as well as to *green apple* flavour ($r = 0.88$) and *floral* aroma ($r = 0.83$). These samples also had higher concentrations of linalool (21.5 and 15 μg/L), which was very significantly correlated to *tropical* ($r = 0.85$) and *herbaceous* ($r = 0.94$) aroma notes and to *green apple* ($r = 0.91$) and *herbaceous* ($r = 0.95$) flavours. Linalool, described as having *floral, citrus, lemon* and *herbaceous* notes, has been deemed to be important to Chardonnay wine typicity (Lorrain et al. 2006) and may be acting additively with other compounds such as 1-hexanol to impart these aromas and flavours to the wine. ADL A in particular was characterised by a greater concentration of acetates (hexyl and isoamyl, Supplementary Information, Table S3), which were very highly correlated to the *herbaceous* aroma and flavour attributes ($r = 0.90$) and *tropical* aroma of the samples ($r = 0.84$). Hexyl and isoamyl acetates, characterised by having *apple* and *banana* notes, respectively, have not only been mentioned as being amongst the most relevant esters for unwooded Chardonnay aroma, but it has also been proposed that their levels (together with those of other compounds) could be used to discriminate Chardonnay wines from those made from other cultivars (Gambetta et al. 2014). Decanoic and octanoic acids, described with terms such as *cheesy* and *fatty* (Gambetta et al. 2014), were also higher in the ADL samples (especially wine B) and seemed to contribute positively to the perception of *citrus* ($r = 0.87$), *stonefruit/melon* ($r = 0.92$), *tropical* ($r = 0.85$) and *floral* ($r = 0.90$) aromas, but negatively to that of the *yeasty* ($r = -0.83$) *mushroom* ($r = -0.96$) and *beer* ($r = -0.95$) aromas. Smyth (2005) also reported strong correlations between fatty acids (decanoic, hexanoic and octanoic) and the perception of *citrus* in a study of unwooded Chardonnays. In contrast, RVL samples, which were located to the right of the biplot, possessed the lowest amounts of 1-hexanol, linalool and hexyl and isoamyl acetates (Supplementary Information, Table S3) amongst the three sets of samples and the highest ratings of the attributes *yeasty*, *caramel*, *beer* and *mushroom*, explaining the negative correlations observed between these attributes and these compounds. 3-Methyl-1-butanol, a higher alcohol normally described as having *alcohol* and *harsh* notes, was observed in greater concentrations in samples from RVL (RVL A, 200 mg/L and RVL B, 196 mg/L) and seemed to contribute to the overall perception of bitterness ($r = 0.96$), hotness ($r = 0.96$) and astringency ($r = 0.92$) in these samples, as well as to their higher intensity of the *beer* aroma ($r = 0.85$) and *yeasty* flavour ($r = 0.93$) and lower perception of *floral* aroma ($r = -0.83$). Likewise, the higher concentrations of 2-phenylethanol appeared to contribute to

the overall sensation of bitterness ($r = 0.83$). Ethyl 2-methylbutanoate, although normally characterised as possessing *berry* notes, appeared to be contributing strongly to the higher perceptions of *yeasty* aroma and flavour ($r = 0.85$ and $r = 0.82$) as well as to *honey* flavour ($r = 0.82$) in the RVL samples, particularly RVL A. As mentioned before, EV samples had the highest concentrations of α-terpineol (EV A, 16 μg/L and EV B, 17 μg/L) which led to the highest intensities in *honey* aroma ($r = 0.62$).

Temperature influences the rate of accumulation of sugars and catabolism of acids, as well as the development of aroma molecules during ripening (Coombe 1987). A perusal of the heat degree day (HDD) values for each region (Supplementary Information, Table S4) explains the differences observed in aroma descriptors. According to Winkler's Thermal Index, Chardonnay is a region I variety (Amerine and Winkler 1944), which means that it requires at least 850 HDD to mature, and that it will adequately ripen in the South Australian locations being studied (Jones et al. 2010). ADL has the coolest weather and highest altitude of all three regions (Supplementary Information, Table S4) and is considered a cool climate (Saliba et al. 2013); as a result, grapes grown in this region had the highest TA and lowest °Brix (Supplementary Information, Table S5) and a predominance of herbaceous aromas and corresponding higher concentrations of 1-hexanol in wines (Figs. 1 and 2). The cooler weather also seemed to promote the production or preservation of linalool, which was reflected by *tropical* and *floral* wine aromas. Not unlike what we observed, cool climate Chardonnay wines such as these are often characterised by *grassy* and *citrus* notes (Saliba et al. 2013). On the other hand, RVL is a much warmer region, with temperatures quite often surpassing 40 °C during the ripening season. Whilst RVL is normally classified as belonging to region IV according to the Winkler's Thermal Index, a year like 2014 meant it actually classified as region V (HDD 2378), the warmest possible region for viticulture (Jones et al. 2010). As a consequence of these higher temperatures and riper fruit (Supplementary Information, Table S5), the wines produced from RVL grapes had a greater % of alcohol by volume (% *v*/*v*) (RVL A, 14.4 % *v*/*v*; RVL B, 14 % *v*/*v*, Supplementary Information, Table S5) than the samples from EV (average of 12 % *v*/*v*) and ADL (average 9.2 % *v*/*v*), which explains why the panel perceived the RVL wines as hotter and more intensely bitter. Jones et al. (2008) observed that ethanol affects the perception of bitterness in model wines, with higher ethanol contents enhancing bitterness as well as increasing the sensation of *dryness* and *roughness*. The higher temperatures of the RVL region (Supplementary Information, Table S4) also explain the lower amounts of *herbaceous* aromas but higher intensity of *caramel, honey* and *beer* attributes in the samples.

Small-Scale Chardonnay Ferments

Composition of Juice from Different Regions

Chardonnay berry samples encompassing a range of quality levels were collected from 31 vineyard blocks from different vineyards in the Adelaide Hills (ADL, $n = 8$), Eden Valley (EV, $n = 9$), Clare Valley (CV, $n = 9$) and Barossa Valley (BV, $n = 5$) in order to undertake compositional analysis and compare the characteristics of each different region. Quality levels were allocated by the producers based on region and historical data for each vineyard, as well as on the result of informal berry tastings in the field by the winemakers (data not shown). The DA analysis discussed above resulted in a set of compounds in the wine that could be related to different regions and qualities. Thus, a larger set of samples with a more varied array of qualities was used to confirm whether these markers were pertinent to other regions and quality levels and to determine how they relate to the composition of the berries. In this way, we could begin to pinpoint compounds which can be later on used to estimate potential wine quality from the vineyard.

Harvest timing was based on commercial maturity considerations and spanned from early February until early March, as a result of the climate in the different regions. Values for °Brix, pH and titratable acidity (TA) at harvest (i.e., the usual measures of grape maturity and indicative of wine style and quality) showed significant differences between the regions ($p < 0.0001$), with ADL being the most different and less mature than the others (Supplementary Information, Table S6).

Element composition of the grapes was determined (Supplementary Information, Table S7) because this has previously been shown to enable association of geographical regions with the provenance of grapes or wines, and certain metals can influence wine quality (Hopfer et al. 2015). There were significant differences amongst the sites for all elements ($p < 0.05$), with the exception of Fe, and the trace elements Cr, Cd, Pb, As, Se, Mo, Co, Ni, which were below the detection limit. Most notable are the higher concentrations of sodium (32 mg/L), potassium (1850 mg/L) and phosphorus (232.6 mg/L) in the samples from BV and the higher content of magnesium (132.1 mg/L), calcium (114.7 mg/L), zinc (0.87 mg/L) and titanium (0.09 mg/L) in the ADL samples. Samples from EV had less calcium (75 mg/L) and sodium (10.3 mg/L) than the rest, and together with those from the ADL, the most copper (2.10 and 2.14 mg/L, respectively). Sodium content in all samples was below the sensory threshold of 220 mg/L in Chardonnay grape juice (de Loryn et al. 2014). When compared to the element composition of 96 commercial Chardonnay juices, concentrations of Ca, Na, Mg, P, S, Mn, B, Cu, Zn in our samples were within the range detected by Schmidt et al. (2011), whilst Fe, Al and K exceeded their maximum observed values in all of our

samples (with the exception of K in ADL). As shown by Boulton (1980), high concentrations of potassium (and in some cases sodium) in the berries are undesirable since this cation decreases the amount of free tartaric acid and increases the pH of the juice, as can be verified by the higher pH values of the BV samples. Zn, on the other hand, is an essential mineral for yeast metabolism during alcoholic fermentation. It acts as an activator of the terminal alcohologenic Zn-metalloenzyme ethanol dehydrogenase and yeast requires at least 0.1 mg/L (may vary according to yeast strain) to avoid sluggish fermentations (Walker 2004). All samples contained amounts of Zn above this threshold.

Lipid composition of grapes is affected by growing conditions and has important implications for fermentation performance and the formation of wine aroma compounds (Ugliano and Henschke 2009). A number of grape fatty acids were therefore determined (Supplementary Information, Table S8), to assess their importance in discriminating wines from the different regions and quality levels. Gallander and Peng (1980) mentioned palmitic (16:0), stearic (18:0), arachidic (20:0) and behenic (22:0) acids as being the major saturated fatty acids in all grape varieties sampled in their study. This was also true for the Chardonnay samples we assayed, with the inclusion of lignoceric acid (24:0). The concentrations of all saturated fatty acids reported by those authors were above the amounts found in ADL, BV, CV and EV, with the exception of lignoceric acid, which was lower in DeChaunac and Seyval grapes studied by Gallander and Peng (1980). This might be due to differences in the grape varieties but also in origins and growing conditions. In general, intravineyard variability of fatty acid content was extremely high, making it difficult to observe any significant differences between locations.

Although Chardonnay juice does not possess any distinct aroma, it does contain a number of volatile compounds, either free or conjugated as glycosides that after fermentation will determine the volatile composition and quality of the resulting wine (Gambetta et al. 2014). Table 1 shows the mean concentrations of free volatiles detected by SPME-GC-MS, and Table 2 shows the mean concentrations of the aroma compounds released from their glycosidic precursors in the juices from each location. In the same way as observed by Kalua and Boss (2009), the free volatile fraction was dominated by C_6 compounds formed in the berry during ripening through the lipoxygenase pathway. 2-Phenylethanol, which has been described as being related to later berry development, was also present in all samples. Significant differences were observed between samples ($p<0.05$) for 3-methyl-1-butanol, ethyl hexanoate, hexanoic acid, 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, (*E*)-linalool oxide, linalool, diethyl succinate, β-damascenone and 2-phenylethanol. Most alcohols (with the exception of 3-methyl-1-butanol which was higher in BV), β-damascenone and (*E*)-linalool oxide were higher in ADL samples, whilst linalool was higher in EV. In contrast, levels of C_{13}-norisoprenoids (vitispirane, β-damascenone, TDN and β-ionone) released by hydrolysis (Table 2) were higher in BV followed by CV and lowest in ADL and EV. Hydrolysis of glycosides also revealed higher contents of (*Z*)- and (*E*)-linalool oxide in ADL and of α-terpineol in EV.

PCA was performed using significantly different juice compositional variables (Tables 1 and 2, Supplementary Tables S6–S8). Figure 3 shows the first two PCs, which accounted for 66.02 % of the variability in the data. Despite the relatively close geographic proximity of all regions, some segregation of the juices was possible based on their origin, especially when differentiating ADL (extreme left) and BV (extreme right) from EV and CV (middle of the plot) along PC1 (which accounted for 41.63 % of the variation), mainly driven by the concentration of hexanal, 2-hexenal, 1-octanol, vitispirane, 5-methylfurfural (5-MF), phenylacetaldehyde, diethyl succinate, acids (hexanoic, octanoic, decanoic, (*E*)-2-hexenoic and *n*-hexadecanoic), guaiacol, 2,6-dimethoxyphenol (2,6-DMP) and °Brix. PC2 (24.39 %) helped to separate ADL and BV samples from CV and EV based on their differences in pH, TA, Zn and 1-hexanol levels. The biplot also showed a clear discrimination between juices from ADL (top left quadrant) compared to the other regions. This difference is strongly driven by the higher titrable acidities in these grapes, lower °Brix, lower pH and higher contents of Zn, Cu and Ca in ADL juices. Significantly higher quantities of the C_6 compounds 1-hexanol and (*E*)-2-hexen-1-ol, and of linalool oxide (Table 1), also contributed to the discrimination of these ADL samples. The warmer climates (BV, CV) were characterised by higher quantities of the C_{13}-norisoprenoids β-ionone and vitispirane, and 5-MF, as well as of guaiacol, 2,6-DMP and a number of fatty acids for BV juices in particular. As expected, weather as represented by HDD and total rainfall (in the critical months of January and February) had a significant impact on the degree of maturity and volatile profile of the grapes. Higher HDD values led to higher °Brix ($r=0.80$) and lower TA ($r=-0.67$) values, as seen in the higher maturity of BV and CV grapes. The higher HDD levels had significant negative effects on the final concentrations of 1-hexanol ($r=-0.37$), (*Z*)-linalool oxide ($r=-0.55$) and linalool ($r=-0.69$) and stimulated the production of vitispirane ($r=0.62$), β-ionone ($r=0.66$) and 5MF ($r=0.81$). Higher maturity levels lead to higher concentrations of C_{13}-norisoprenoids, and Chardonnay in Australia is prone to sunburn in the warmer regions (Greer et al. 2006), which causes degradation of carotenoids and likely contributes to the elevated concentrations of C_{13}-norisoprenoids. On the contrary, rain had different effects depending on when it occurred. Higher January rainfall appeared to have delayed the maturation process and exhibited strong significant negative correlations with °Brix ($r=-0.70$), pH ($r=-0.36$), 1-octanol ($r=-0.60$), 2,6-DMP ($r=-0.46$), guaiacol ($r=-0.51$), all aldehydes and C_{13}-

Table 1 Mean concentrations of free volatile compounds detected in Chardonnay juices

	ADL	BV	CV	EV
Ethyl esters				
Ethyl acetate*	49 ± 57	76 ± 11	26 ± 18	18 ± 8.4
Ethyl hexanoate*	2.8 ± 1.2b	3.2 ± 1.4a	5.0 ± 2.0b	3.0 ± 1.1b
Ethyl octanoate*	0.85 ± 0.55	1.2 ± 0.38	1.5 ± 1.5	0.88 ± 0.79
Acetate esters				
Isoamyl acetate*	8.1 ± 12b	5.5 ± 1.6	2.2 ± 0.80	2.1 ± 1.8
Hexyl acetate*	1.6 ± 0.92	3.0 ± 0.59	5.3 ± 8.5	4.8 ± 9.1
Alcohols				
3-Methyl-1-butanol*	18 ± 32	178 ± 152	32 ± 74	6.8 ± 2.6
1-Hexanol*	290 ± 88a	242 ± 70ab	105 ± 27b	123 ± 64b
(*E*)-3-Hexen-1-ol*	3.3 ± 4.7	1.2 ± 0.79	0.61 ± 0.23	0.59 ± 0.29
(*Z*)-3-Hexen-1-ol*	16 ± 7.7a	7.5 ± 2.4b	4.4 ± 0.7b	6.3 ± 1.4b
(*E*)-2-Hexen-1-ol*	106 ± 46a	98 ± 53ab	35 ± 14b	51 ± 46ab
(*Z*)-2-Hexen-1-ol*	13 ± 24	45 ± 75	2.1 ± 3.4	1.82.7
1-Octen-3-ol*	41 ± 21	23 ± 24	23 ± 10	41 ± 17
2-Ethyl-1-hexanol*	15 ± 15	2.4 ± 1.4	6.7 ± 6.9	10 ± 17
1-Octanol*	3.1 ± 2.7	2.7 ± 2.3	2.8 ± 1.8	1.6 ± 0.5
2-Phenylethanol**	166 ± 41a	98 ± 63b	113 ± 12b	121 ± 20b
Isoprenoid				
Eucalyptol*	0.42 ± 0.19	3.4 ± 5.2	0.39 ± 0.18	1.7 ± 1.8
(*Z*)-Linalool oxide*	4.3 ± 4.1a	0.18 ± 0.07b	0.47 ± 0.25b	2.1 ± 1.3ab
Linalool*	6.9 ± 4.5a	0.52 ± 0.11b	0.82 ± 0.39b	9.5 ± 3.8a
β-Damascenone**	124 ± 47	22 ± 10	59 ± 50	67 ± 44
Acids				
Hexanoic acid*	50 ± 24a	90 ± 19a	63 ± 19ab	37 ± 13b
Carbonyl				
Hexanal*	138 ± 126	79 ± 21	134 ± 86	201 ± 140
(*E*)-2-Hexenal*	120 ± 117	278 ± 159	109 ± 76	115 ± 87
Nonanal*	16 ± 33	3.1 ± 0.6	5.7 ± 2.9	5.9 ± 2.7
Benzaldehyde**	121 ± 53	80 ± 11	199 ± 131	136 ± 55
Diethyl succinate**	0.20 ± 0.14	0.29 ± 0.08	0.31 ± 0.07	0.12 ± 0.05

For each region, means ± SD (duplicate measurements for each sample) with different letters between columns (a, b, c) are significantly different ($p < 0.05$) according to Tukey (HSD) pairwise comparison. Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$) and Eden Valley (EV, $n = 9$). Values expressed as μg/L equivalents of *d_{13}-1-hexanol or **d_5-2-phenylethanol

norisoprenoids, although there was a positive relationship with linalool ($r = 0.72$). Higher rainfall in February, however, had no effect on the final °Brix level of the grapes but a negative impact on TA ($r = -0.67$). February rain had very little impact on most volatile compounds, with the exception of linalool ($r = 0.37$), 2,6-DMP ($r = -0.43$) and guaiacol ($r = -0.39$).

MIR Analysis of Juices Unlike most of the methodologies employed above, MIR is a rapid analysis technique known for its ease of operation and minimal to no sample preparation, which makes it ideal for use in wineries, particularly during very busy periods such as harvest. It has been successfully used to measure juice compositional data, verify wine authenticity and classify different wine styles and cultivars (Bevin et al. 2008) and therefore could potentially discriminate between different grape origins and qualities. PCA analysis of the second derivative of the fingerprint region of the MIR spectra of juices from the four growing regions (Supplementary Information, Fig. S1) showed that it was possible to obtain a separation of samples from ADL, BV and EV using this analytical tool. However, as with the other analyses, there was some overlap between the samples from CV, BV and EV. This was somewhat expected for the samples from BV and CV, given their climatic similarity and geographic proximity. The first two principal components accounted for 96 % of the total variance of the spectra, and the highest loadings for PC1 were found at 1069, 1030, 1003 and 1127 cm^{-1} (data not

Table 2 Mean concentrations of volatiles detected after hydrolysis of glycosides extracted from Chardonnay juices (expressed as mg/L of d_{13}-1-hexanol equivalents)

	ADL	BV	CV	EV
Alcohols				
1-Octanol	0.31 ± 0.19b	1.0 ± 0.60a	0.23 ± 0.08b	0.23 ± 0.15b
Benzyl alcohol	0.075 ± 0.022	0.075 ± 0.013	0.063 ± 0.012	0.067 ± 0.01
Isoprenoids				
(*Z*)-Linalool oxide	0.17 ± 0.13	0.13 ± 0.061	0.068 ± 0.028	0.11 ± 0.038
(*E*)-Linalool oxide	0.11 ± 0.079	0.085 ± 0.034	0.068 ± 0.028	0.068 ± 0.022
Vitispirane *(sum of isomers)*	0.042 ± 0.024bc	0.11 ± 0.001a	0.083 ± 0.035ab	0.042 ± 0.038c
α-Terpineol	0.071 ± 0.057	0.098 ± 0.006	0.081 ± 0.04	0.14 ± 0.12
β-Damascenone	0.15 ± 0.048	0.19 ± 0.002	0.14 ± 0.04	0.14 ± 0.05
TDN	0.064 ± 0.052	0.15 ± 0.001	0.15 ± 0.09	0.11 ± 0.11
β-Ionone	0.44 ± 0.15b	0.90 ± 0.075a	0.89 ± 0.23a	0.67 ± 0.27ab
2,6-Dimethyl-7-octene-2,6-diol	0.10 ± 0.017b	0.12 ± 0.010ab	0.12 ± 0.02a	0.11 ± 0.02ab
Carbonyls				
Hexanal	0.091 ± 0.032b	0.24 ± 0.16a	0.087 ± 0.026b	0.082 ± 0.030b
2-Hexenal	0.12 ± 0.05b	0.24 ± 0.10a	0.070 ± 0.028b	0.087 ± 0.012b
5-Methylfurfural	0.043 ± 0.030c	0.20 ± 0.020a	0.12 ± 0.031b	0.06 ± 0.017c
Phenylacetaldehyde	0.038 ± 0.022b	0.12 ± 0.072a	0.077 ± 0.018ab	0.056 ± 0.034b
Diethyl succinate	0.061 ± 0.10b	0.22 ± 0.20a	0.021 ± 0.006b	0.020 ± 0.012b
Acids				
Hexanoic acid	0.87 ± 1.1b	2.4 ± 2.0a	0.44 ± 0.14b	0.47 ± 0.19b
(*E*)-2-Hexenoic acid	0.49 ± 0.66b	1.3 ± 1.2a	0.14 ± 0.07b	0.21 ± 0.13b
Octanoic Acid	17.3 ± 22.1b	53 ± 44a	11 ± 2.2b	9.4 ± 2.6b
Nonanoic acid	24.4 ± 6.6ab	32 ± 11a	25 ± 5.8ab	23 ± 3.7b
Decanoic acid	3.9 ± 4.5ab	7.9 ± 5.9a	2.0 ± 0.82b	2.8 ± 1.9b
n-Hexadecanoic acid	44 ± 30ab	77 ± 50a	27 ± 11b	43 ± 31ab
Octadecanoic acid	30 ± 23	31 ± 7.0	23 ± 9.6	29 ± 25
Volatile phenols				
Guaiacol	0.019 ± 0.015ab	0.032 ± 0.006a	0.023 ± 0.011ab	0.010 ± 0.009b
4-Vinylguaiacol	1.4 ± 0.89	2.2 ± 0.17	1.9 ± 1.1	0.90 ± 0.90
2,6-Dimethoxyphenol	0.072 ± 0.062ab	0.12 ± 0.033a	0.078 ± 0.053ab	0.028 ± 0.027b

For each region, means ± SD (duplicate measurements for each sample) with different letters between columns (a, b, c) are significantly different ($p < 0.05$) according to ANOVA and Tukey (HSD) pairwise comparison. Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$) and Eden Valley (EV, $n = 9$)

shown). As indicated by Bevin et al. (2008), the region spanning between 1080 and 1045 cm^{-1} relates to the C-OH of sugars (C-O stretch for glucose at 1030 cm^{-1} and fructose at 1060 cm^{-1}), which contribute to the separation between regions. This was confirmed through PLS analysis, which yielded the coefficient of determination (R^2) of 0.72 between the sugar content of the samples and MIR spectral data (residual predictive deviation, RPD = 1.7, SECV = 1.9). Although no absorptions corresponding to carboxylic acid were found in the loadings, TA is an important parameter affecting berry and wine quality so the correlation between TA and MIR spectral data was also tested; this resulted in a strong R^2 of 0.75 (RPD = 1.9, SECV = 2.0). These are useful parameters for assessing grape ripeness and MIR could be envisaged as an important component of a quality measure, especially if additional parameters can be predicted.

Composition of Corresponding Small-Scale Wines

Small lot Chardonnay wines were produced from the 31 different assayed vineyards in order to evaluate and compare their compositions and relate that back to the grapes. Major volatiles were analysed by HS-SPME-GC-MS (Supplementary Information, Table S9) and PCA of the significantly different volatile compounds, together with wine ethanol content, yielded similar trends to the analysis of juice composition. As in the samples for sensory analysis, 3-methyl-1-butanol, 1-octanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, β-damascenone, and hexanoic, octanoic and decanoic acids were above their respective perception thresholds in all samples. Unlike the samples for sensory analysis, however, 2-phenylethanol was found at levels above its detection threshold of 14,000 μg/L

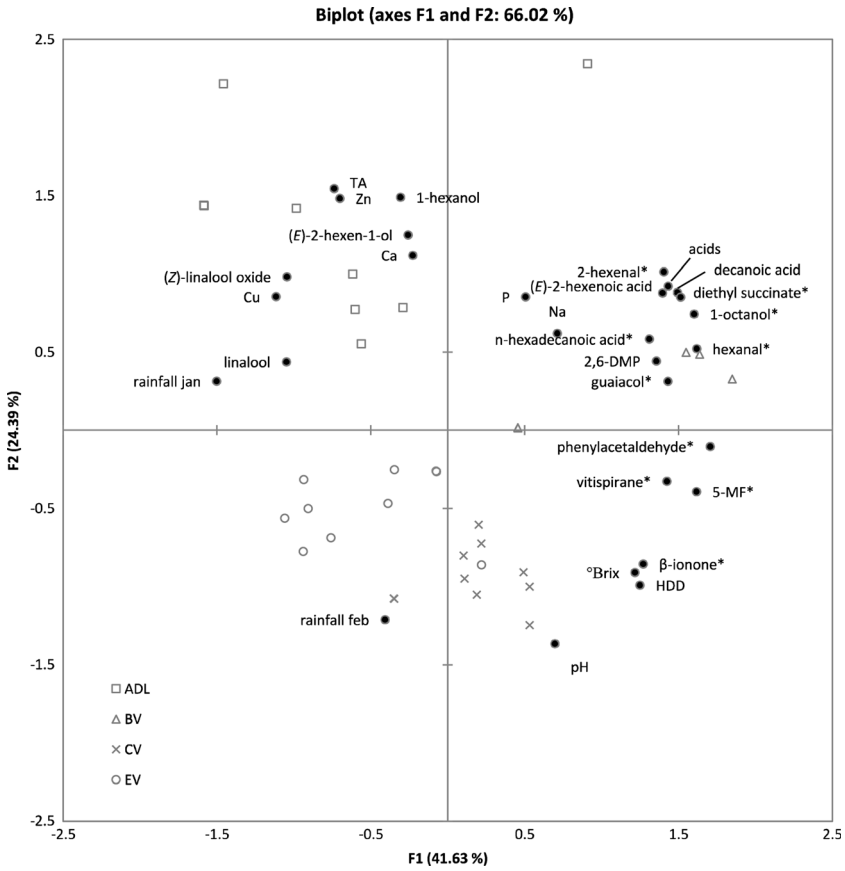

Fig. 3 PCA biplot of the standardised means of the significant ($p < 0.05$) volatile compounds, elements and basic chemical parameters for juice samples from Eden Valley (EV), Barossa Valley (BV), Clare Valley (CV) and Adelaide Hills (ADL). * denotes volatiles detected after hydrolysis of glycosides extracted from juice. 5-MF, 5-methylfurfural; 2,6-DMP, 2,6-dimethoxyphenol

(Gambetta et al. 2014) only in samples from CV (15,880 μg/L). The mean concentration of 2-phenylethyl acetate in EV samples (268 μg/L) was above its threshold of 250 μg/L, as were the concentrations of ethyl decanoate (threshold of 200 μg/L) and linalool (threshold of 25 μg/L) (Gambetta et al. 2014) in ADL, CV and EV samples. The first two PCs (Fig. 4) accounted for 54.65 % of the variability and showed a clear separation of ADL samples from the rest, as well as a good separation of samples from EV and BV. Separation of the ADL samples occurred mainly along PC2, due to their lower average concentrations of ethanol, 3-methyl-1-butanol, 2-phenylethanol, 1-propanol, 2-methyl-1-propanol and ethyl phenylacetate and a higher concentration of hexyl acetate, compared to CV samples which were opposite in volatile profile. Samples from EV were found to the right side of PC1 with a higher concentration of the esters, including ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate and isoamyl acetate and greater amounts of the monoterpenoids linalool, α-terpineol and limonene, whilst samples from BV were located to the left hand side and having lower concentrations of these compounds. PC3 (data not shown) explained an additional 8.4 % of the variability and corroborated the trends observed in the first two PCs, as well as providing extra information on the separation between different blocks from the same region, mainly driven by ethyl phenylacetate, α-terpineol and 2-phenylethanol.

In general, it could be observed that samples from the cooler regions (ADL, EV) contained higher amounts of desirable esters and monoterpenoids (fruity and floral aromas), whereas those from the warmer regions (BV, CV) had greater levels of the higher alcohols. All of these compounds have been cited as being typical of Chardonnay wine aroma (Gambetta et al. 2014), but linalool, isoamyl acetate and ethyl hexanoate in particular have a positive effect on the typicity of Chardonnay, together with diethyl succinate, ethyl butanoate, hexyl acetate and C6-C10 acids (Jaffré et al. 2011; Smyth 2005). The higher ethanol concentration was to be expected in the BV and CV samples as a result of the higher initial sugar concentration in the juices (Supplementary Information, Table S6). Given that all samples were supplemented with DAP at the beginning of fermentation to avoid any shortage of assimilable nitrogen, the greater production of higher alcohols, especially in the CV wines (179 mg/L in total), may be attributed to the higher sugar concentration of the initial juice and the processes involved in sugar catabolism (Nisbet et al. 2014).

Linking Juice and Wine Compositions to Quality

PLS regression of juice and wine components was used to determine strong correlations in the multivariate space

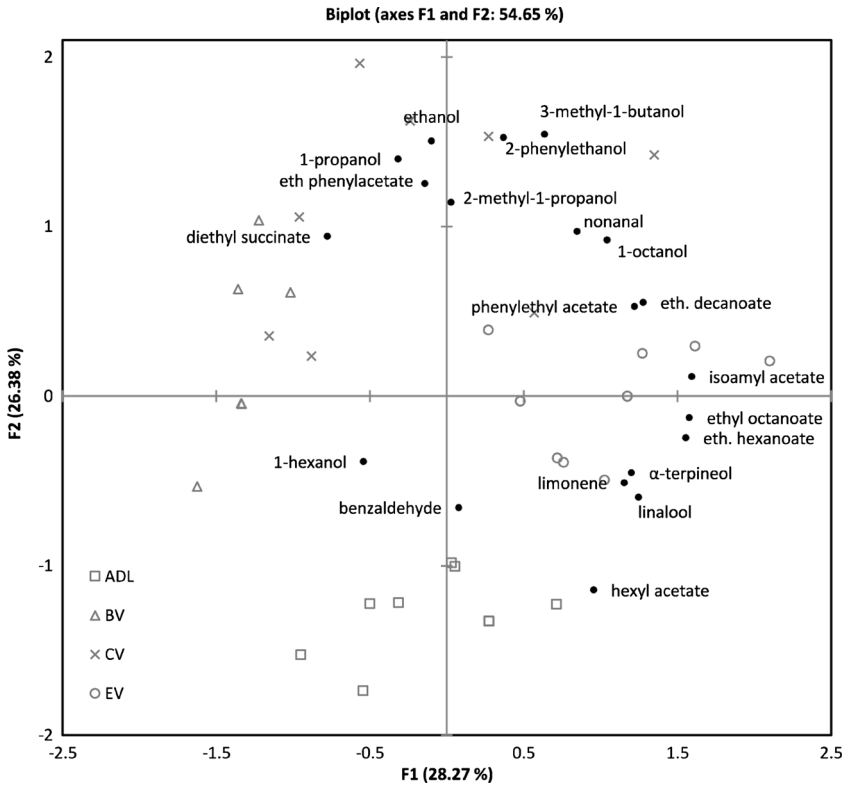

Fig. 4 PCA biplot of standardised means of the significant ($p < 0.05$) volatile compounds in samples arising from small-scale vinifications using fruit obtained from Eden Valley (EV), Barossa Valley (BV), Clare Valley (CV) and Adelaide Hills (ADL)

(Supplementary Information, Fig. S2 a–e), using variables that differed significantly amongst the different regions. Although these correlations do not necessarily indicate cause and effect, they do indicate relationships that could be exploited to predict quality in the vineyard. The analysis yielded five strong positive relationships (R^2 predicted vs. measured, validation) for the wine attributes linalool ($R^2 = 0.79$, SECV = 0.022 μg/L), hexyl acetate ($R^2 = 0.73$, SECV = 54 μg/L), 2-phenylethyl acetate ($R^2 = 0.64$, SECV = 46 μg/L), 2-phenylethanol ($R^2 = 0.72$, SECV = 2113 μg/L) and 3-methyl-1-butanol ($R^2 = 0.60$, SECV = 12,835 μg/L) as a function of grape compositional parameters. Although the RPD values were low (i.e., ≤ 2, Supplementary Information, Table S10), they were suitable for the purpose of classification, since the aim was not to accurately quantify the wine analytes using these variables.

Based on the published information for Chardonnay wine composition (Jaffré et al. 2011; Lorrain et al. 2006), the results of the sensory DA ("Sensory and compositional analysis of regional Chardonnay wines" section) and empirical knowledge about the quality of Chardonnay wines arising from each region, higher quantities of wine volatiles linalool, hexyl acetate and 2-phenylethyl acetate were deemed desirable and markers of higher quality wines, whilst increased levels of the higher alcohols 3-methyl-1-butanol and 2-phenylethanol were regarded as markers of lower quality wines. In terms of grape components, higher levels of Cu, Zn, linalool oxide, (*Z*)-3-hexen-1-ol, 1-hexanol, behenic acid (22:0), decanoic acid, linalool and vitispirane were regarded as desirable. In general, higher maturity levels represented by higher sugar contents and higher pH/ lower TA values were detrimental to the presence of positive compounds and favoured the production of higher alcohols. The enhanced levels of higher alcohols in wines would be predictable based on the °Brix, TA and (*Z*)-3-hexen-1-ol content, together with the concentration of stearic acid (for 3-methyl-1-butanol) or Cu and Zn (for 2-phenylethanol). From the correlations, it appears that a higher level of Cu and Zn stimulated the formation of hexyl acetate whilst depressing the synthesis of 2-phenylethanol. This not only relates to the fact that these elements are most abundant in the samples from ADL but also to observations by Walker (2004) who noted that higher concentrations of Zn, and in particular Cu, increased yeast biomass and growth, thereby stimulating alcoholic fermentation (and the enzymatic reactions that ensue).

Conclusions

The composition of Chardonnay juices and wines originating in different regions of South Australia was strongly modulated by climatic factors. This not only affected ripening dates but also the concentrations of a range of compounds, such as 1-hexanol and the monoterpenoids linalool and α-terpineol present in the juice, and the levels of higher alcohols formed in the corresponding wines. Additional to weather factors, soil

composition seemed to play a major role in the behaviour of fermentations, as it affected the levels of Zn, Mg and K in the juice and therefore impacted the concentration of certain analytes in the wines. Key molecules related to each different region were pinpointed and can be further monitored in future studies to confirm their adequacy as predictors of wine quality; in particular, desirable esters and monoterpenoids were associated with cooler regions whereas wines derived from the warmer regions had greater levels of the higher alcohols. Strong positive relationships were obtained for the wine volatiles linalool, hexyl acetate, 2-phenylethyl acetate, 2-phenylethanol and 3-methyl-1-butanol as a function of grape compositional parameters, which provided the basis for objective measures that can predict wine quality in the vineyard. MIR proved to be capable of distinguishing amongst grapes from different origins and could therefore be envisaged as a rapid tool for classification in the vineyard or winery. It was also shown that a panel of trained assessors can discriminate between wines produced from juices originating from different regions, providing verification of the important influence that provenance and climate have on final sensory characteristics of a wine.

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Compliance with Ethical Standards This sensory study was approved by the Human Research Ethics Committee of The University of Adelaide (Project No. 2013–078).

Conflict of Interest Joanna M. Gambetta declares that she has no conflict of interest. Daniel Cozzolino, declares that he has no conflict of interest. Susan E.P. Bastiana declares that she has no conflict of interest. David W. Jeffery declares that he has no conflict of interest.

Informed Consent Informed consent is not applicable in this study.

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Supplemental Data.

2. Materials and Methods

2.1 Chemicals

LiChrolut EN resin and ethanol, methanol and dichloromethane were purchased from Merck (Kilsyth, Victoria, Australia) and L-ascorbic acid from Sigma-Aldrich. Ethyl acetate was provided by Chem-Supply (Gillman, SA, Australia), citric acid monohydrate and sodium fluoride were purchased from VWR International (Leuven, Belgium) and sodium chloride was obtained from JT Baker (Phillipsburg, NJ, USA). Potassium metabisulfite (PMS), diammonium phosphate (DAP) and polyvinylpolypyrrolidone (PVPP) were supplied by Winequip (Newton, SA, Australia). Stock solutions of standards were prepared volumetrically in absolute ethanol and stored at –20 °C and working solutions were stored at 4 °C until required. All solvents and chemicals were analytical reagent grade unless otherwise stated. Water was obtained from a Milli-Q purification system (Millipore,North Ryde, NSW, Australia). Divinylbenzene-carboxen-poly(dimethylsiloxane (DVB/CAR/PDMS) SPME fibres were obtained from Supelco (Bellefonte, PA).

2.3 Vinification

2.3.1 Wines for sensory analysis

Grapes were defrosted overnight at 4 °C and crushed using a small water bag press (800-1000 kPa) in the presence of dry ice. Individual lots of juice were treated with SO_2 (50 mg/L, using a solution of PMS) and pectolytic enzyme (Novozymes VinoClear®, Novozymes Australia, North Rocks, NSW, Australia; 0.03 g/L) and left to clarify overnight at 4 °C. After clarification the musts were decanted and the pH was adjusted to 3.4 with tartaric acid. Each juice was supplemented with DAP (150 mg/L), inoculated with PDM yeast (Maurivin®; AB MAURI, Toowoomba, QLD, Australia) rehydrated and inoculated according to manufacturer's instructions, and transferred to 5 L glass fermenters equipped with fermentation locks. Juices were fermented to dryness (<2 g/L, determined using Clinitest tablets supplied by Enoltech, Victoria, Australia) at 15 °C, then racked from gross lees and cold stabilised at 0 °C for 3-4 weeks. Residual sugar was tested using an enzymatic test kit (Megazyme, Wicklow, Ireland) to verify that wines were dry (<2 g/L of reducing sugars). Adjustments were made to yield wines with a pH of 3.4 and free SO_2 of 30 mg/L prior to bottling in 375 mL bottles under screw cap closures. Bottles were cellared at 15 °C until required (at least 8 weeks before conducting sensory analysis).

2.4 Sensory analysis

Descriptive terms were generated by the panel during 2 h training sessions over 4 weeks and panellists familiarised themselves with the sample set and scoring system. Two formal evaluation sessions of 2 h duration were carried out in individual booths under fluorescent light over two weeks. Wine samples (30 mL) were presented to each assessor in black INAO (ISO standard) 215 mL tasting glasses covered with a petri dish. The panel rated the intensity of 12 aroma and 13 palate attributes (Table S1) on a 15-cm unstructured line scale anchored by "low", "medium" and "high" prompts at 10%, 50% and 90% of the scale, respectively. In order to alleviate palate fatigue, breaks were enforced during the tasting (1 min after each sample and 5 min every 5 samples) and assessors were provided with pectin solution (1 g/L) and crackers. Data was collected using Fizz software, version 2.47b (Biosystèmes, Couternon, France). Each vinification replicate was evaluated in triplicate.

Table S1. Aroma and flavour attributes generated from sensory descriptive analysis.

Attribute	Aroma (A)/Palate (P)	Description
Citrus	A, P	Aroma/flavour of lemon, lime and/or orange
Green apple	A, P	Aroma/flavour of granny smith apple
Stonefruit/melon	A, P	Aroma/flavour of apricot, peach, yellow nectarine, melon
Tropical	A, P	Aroma/flavour of pineapple, pawpaw, lychee, passionfruit
Floral	A	Aroma of orange blossom and rose water
Herbaceous	A, P	Aroma/flavour of fresh cut grass
Mushroom/earthy	A	Aroma/flavour of mushroom and earth
Honey	A, P	Aroma/ flavour of honey
Caramel	A, P	Aroma/ flavour of butterscotch candy
Yeasty	A, P	Aroma/flavour of yeast
Doughy	A, P	Aroma/ flavour of pizza dough and crackers
Beer	A	Smell of hops and oxidation
Acidity	P	Overall intensity of sour/acid taste
Bitterness	P	Intensity of bitter taste
Astringency	P	Overall Intensity of astringency sensation
Hotness	P	Overall intensity of alcohol or burning sensation
Body	P	Mouthfeel perception on palate
Afterflavour	P	Time that any flavour perception persists after expectoration of wine

Table S2. Results of sensory descriptive analysis (DA) for six Chardonnay wines.[a]

Attribute	Minimum	Maximum	Median	Mean	SD[b]	Significance[c]
Citrus	0.00	13.50	3.30	4.11	3.13	*
Stonefruit/Melon	0.00	13.50	4.58	5.03	3.33	*
Tropical	0.00	13.50	3.75	4.86	3.64	**
Floral	0.00	13.50	2.10	3.03	2.80	*
Caramel	0.00	12.00	2.55	3.39	2.91	*
Mushroom	0.00	13.20	1.65	3.04	3.24	*
Honey	0.00	13.50	3.75	4.20	3.09	*
Yeasty	0.00	14.25	3.15	3.80	3.13	*
Beer	0.00	14.55	4.05	4.48	3.09	**
Green apple	0.00	13.50	2.63	3.62	3.34	ns
Doughy	0.00	13.05	1.80	2.84	2.91	ns
Herbaceous	0.00	13.35	1.50	2.65	3.00	*
Acidity	0.90	15.00	11.25	10.27	3.18	*
Bitterness	0.00	14.70	5.55	5.79	3.48	***
Hotness	0.00	14.70	7.05	7.23	3.72	***
Astringency	0.00	14.40	4.80	5.60	3.35	***
Body	0.30	13.50	5.70	5.73	3.03	***
Citrus (F)[d]	0.00	13.50	6.60	6.55	3.36	ns
Stone Fruit (F)	0.00	13.50	4.20	4.80	3.19	ns
Tropical (F)	0.00	14.25	3.75	4.57	3.49	ns
Green apple (F)	0.00	14.85	6.15	6.07	3.88	*
Herbaceous (F)	0.00	13.50	2.10	3.19	3.17	*
Honey (F)	0.00	12.30	2.55	3.37	3.06	**
Yeasty (F)	0.00	13.50	1.95	3.07	3.18	***
Afterflavour	0.15	15.00	7.50	7.15	3.89	**

[a] Evaluated on a 0-15 scale. [b] SD, standard deviation. [c] Significant differences among wines: *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$; ns, not significant. [d] (F) denotes flavour attributes

Table S3. Mean concentrations of volatile compounds (µg/L) for six Chardonnay wines produced for descriptive analysis.[a]

	ADL A	ADL B	EV A	EV B	RVL A	RVL B
Ethyl esters						
Ethyl butanoate	462±70 bc	360±17 c	527±20 b	492±25 bc	710±61 a	492±61 bc
Ethyl 2-methylbutanoate	4.8±0.60 c	5.3±0.50 c	14±0.58 b	15±2.1b	28±3.2 a	19±3.2 b
Ethyl hexanoate	898±167 bc	664±27 c	1121±36 ab	984±48 b	1357±85 a	863±85 bc
Ethyl octanoate	566±167 b	398±11 b	678±65 ab	560±21 b	902±48 a	509±48 b
Ethyl decanoate	170±66	117±21	127±31	112±11	199±4.3	115±4.3
Diethyl succinate	238±27 c	349±59 c	1117±114 bc	1185±51 bc	4222±107 a	1830±107 b
Ethyl phenylacetate	2.0±0.11 a	1.1±0.06 cd	1.6±0.07 b	1.4±0.22 bc	1.0±0.07 d	1.3±0.07 bcd
Ethyl dodecanoate	5.1±2.1 b	27±20 b	23±7.5 ab	24±11 b	25±2.6 a	26±2.6 ab
Acetate esters						
Ethyl acetate	54105±21652 b	48642±16704 b	60388±6386 b	45004±25481 b	137571±3785 a	84037±3785 ab
Isoamyl acetate	3875±1157 a	2304±523 ab	1165±229 b	1817±635 b	1264±107 b	929±107 b
Hexyl acetate	607±123 a	217±14 b	97±3.3 bc	93±14 c	23±3.9 c	35±3.9 c
2-Phenylethyl acetate	132±8.1 a	71±2.9 cd	103±4.0 b	89±17 bc	63±4.8 d	83±4.8 bcd
Alcohols						
2-Methyl-1-propanol	18882±5389	16764±3385	15165±3630	14244±1190	20860±855	21276±855
3-Methyl-1-butanol	136250±7375 bc	106856±1894 c	157865±8383 b	156965±844 b	200554±7395 a	195805±7395 a
1-Hexanol	2602±161 a	1547±28 b	1396±66 b	1561±149 b	633±33 d	973±33 c
2-Ethyl-1-hexanol	20.6±3.0	18±0.65	20±0.78	20±2.1	21±0.69	20±0.70
1-Octanol	3.0±1.1	2.7±0.07	2.1±0.09	2.1±0.16	2.7±0.03	2.5±0.03
2-Phenylethanol	16933±371 bc	9281±655 c	36548±1318 a	26703±11377 ab	26700±482 ab	35009±482 a
Isoprenoids						
Limonene	1.5±0.12	1.4±0.59	1.2±0.03	1.6±0.20	1.07±0.56	2.03±0.56
Linalool	21.5±4.9 a	15±5.7 ab	13±5.6 ab	9.0±2.4 b	7.6±0.59 b	7.0±0.60 b
α-Terpineol	9.4±1.2 b	9.0±1.0 b	16±4.4 ab	17±4.1 a	15±0.45 ab	10±0.45 ab
β-Damascenone	6.5±1.2	5.4±0.31	5.5±0.32	6.7±0.20	8.5±0.10	6.9±0.10
Carbonyls						
Benzaldehyde	1.2±0.87	1.49±0.4	0.36±0.20	0.80±0.13	0.58±0.15	0.52±0.15
Acids						
Acetic acid	48277±2114 b	53750±6286 b	77535±22912 b	68410±12537 b	244063±30429 a	113311±30429 b
Hexanoic acid	3229±353	2616±122	3140±330	2924±158	3326±157	2393±157

Octanoic acid	30724±80 a	30341±1186 a	31040±5425 a	28585±2686 a	23494±1249 ab	20392±1249 b
Decanoic acid	5725±51 a	5900±150 a	5735±302 a	5623±234 a	5145±125 ab	4301±125 b
Dodecanoic acid	115±7	111±13	116±25	119±22	142±7.4	125±7.3

[a] For each region, means ± SD (three winemaking replicates each measured in duplicate) with different letters (a, b, c, d) are significantly different ($p<0.05$) according to Tukey's (HSD) pairwise comparison.

Table S4. Climatic data for the different regions (2014).[a]

	Mean T (°C)		Highest T (°C)		Rainfall (mm)		Elevation (m)[b]	HDD[b]	WI[c]
	Jan min/max	*Feb min/max*	*Jan*	*Feb*	*Jan*	*Feb*			
Adelaide Hills	13.7/ 29.5	13.3/ 26.9	42.8	43.0	16.6	93.6	400-500	1270	I
Eden Valley	15.0/ 29.9	14.5/ 27.0	41.1	41.6	16.8	114.6	380-550	1390	II
Barossa Valley	16.0/ 33.4	16.2/ 30.2	42.5	42.1	7.6	98.0	250-370	1710	III
Clare Valley	15.8/ 32.1	15.3 /25.5	41.9	42.3	11.6	102.4	400-500	1770	III
Riverland	16.8/ 35.5	16.5 /32.8	45.4	44.5	8.4	93.8	20	2084	IV

[a] Australian Government Bureau of Meteorology (2015) [b] Wine Atlas of Australia (Halliday, 2014). [c] WI, Winkler Index.

Table S5. pH, °Brix and TA mean values of grapes used to produce wines for sensory analysis, and % alcohol values of corresponding wines.[a]

	pH	TA (g/L)	°Brix	% abv
A Hills A	3.57	9.4	16.7	9.2
A Hills B	3.55	10.5	17.0	9.2
Eden A	3.74	6.1	20.0	11.9
Eden B	3.92	6.1	20.8	12.0
Riverland A	4.10	5.0	24.8	14.4
Riverland B	4.08	5.8	24.0	14.0

[a] TA, titratable acidity; % abv, % alcohol by volume (% v/v).

Table S6. pH, °Brix and TA mean values for each region used to produce small-scale wines, with standard deviations within a region[a]

	pH	TA (g/L)	°Brix
Adelaide Hills	3.52±0.10 b	9.66±0.8 a	18.1±0.8 c
Barossa	3.97±0.16 a	6.68±0.5 b	22.4±1.0 a
Clare Valley	3.96±0.12 a	6.09±0.3 b	21.6±0.7 ab
Eden Valley	4.00±0.15 a	6.37±0.6 b	20.5±1.0 b

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a column (a, b, c) are significantly different ($p<0.05$) according to Tukey's (HSD) pairwise comparison. Adelaide Hills (ADL, n= 8), Barossa Valley (BV, n=5), Clare Valley (CV, n=9) and Eden Valley (EV, n=9).

Table S7. Mean element concentrations (mg/L) with standard deviations within region for Chardonnay berries used to produce small-scale wines.[a]

	ADL	BV	CV	EV
Fe	2.8±1.3 ab	2.6±1.1 ab	3.4±1.2 a	2.1±0.4 b
Mn	0.74±0.17 a	0.48±0.11 b	0.65±0.19 ab	0.75±0.11 a
B	6.2±0.63 ab	5.5±1.3 b	7.5±1.4 b	5.8±0.4 4a
Cu	2.14±0.44 a	1.0±0.10 b	0.71±0.08 b	2.1±0.39 a
Zn	0.87±0.098 a	0.50±0.11 bc	0.43±0.08 c	0.58±0.12 b
Ca	115±26 a	97±8.4 ab	95±9.6 ab	75±7.6 b
Mg	132±14 a	119±22 ab	118±12 ab	111±4.6 b
Na	21±7.6 ab	32±11 a	19±7.7 bc	10±1.7 c
K	1317±96 ab	1850±349 a	1513±242 ab	1523±197 b
P	205±46 ab	236±38 a	180±18 b	176±10 b
S	91±8.1	91±9.8	79±5.6	79±6.7
Al	1.6±1.1 ab	1.1±1.1 ab	2.2±1.0 a	0.65±0.27 b
Ti	0.09±0.05 a	0.03±0.02 b	0.05±0.02 b	0.04±0.02 ab

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a column (a, b, c) are significantly different ($p<0.05$) according to Tukey's (HSD) pairwise comparison. Adelaide Hills (ADL, n= 8), Barossa Valley (BV, n=5), Clare Valley (CV, n=9) and Eden Valley (EV, n=9).

Table S8. Mean fatty acid concentrations (mg/100g of sample) with standard deviations within a region for Chardonnay berries used to produce small-scale wines.[a]

	14:0	16:0	18:0	20:0	22:0	24:0	18:1n-9	18:1n-7	18:3n-3	18:2n-6
ADL	0.04±0.02	1.07±0.56	0.47±0.27	0.16±0.07	0.24±0.08	0.57±0.19	0.45±0.28	0.06±0.03	0.40±0.18	1.75±1.0
BV	0.03±0.006	0.86±0.23	0.36±0.10	0.12±0.01	0.14±0.02	0.35±0.06	0.68±0.01	0.06±0.01	0.29±0.08	2.28±0.58
CV	0.04±0.03	1.15±0.83	0.54±0.43	0.18±0.11	0.22±0.12	0.51±0.31	0.48±0.04	0.05±0.04	0.39±0.20	1.81±1.4
EV	0.03±0.007	0.74±0.16	0.30±0.09	0.15±0.04	0.25±0.05	0.47±0.11	0.34±0.01	0.04±0.01	0.31±0.06	1.33±0.53

[a] For each region, means ± SD (duplicate measurements for each sample) are given. Adelaide Hills (ADL, n= 8), Barossa Valley (BV, n=5), Clare Valley (CV, n=9) and Eden Valley (EV, n=9).

Table S9. Mean concentrations of volatile compounds with standard deviations within a region for small-scale Chardonnay wines.[a]

	ADL	BV	CV	EV
Ethanol (% v/v)	10.3±0.71 c	13.0±0.55 a	12.4±0.56 ab	12.0±0.52 b
Ethyl esters				
Ethyl butanoate	373±94	361±154	409±175	547±146
Ethyl 2-methylbutanoate	1.60±0.41	2.63±2.0	1.88±1.7	1.60±0.48
Ethyl hexanoate	794±157 ab	625±82 b	746±171 b	974±172 a
Ethyl octanoate	579±59 a	411±122 b	566±106 ab	687±98 a
Ethyl decanoate	224±49 ab	157±42 b	336±156 ab	348±95 a
Diethyl succinate	49±24 b	352±219 a	220±198 ab	75±54 b
Ethyl phenylacetate	0.05±0.04 b	0.08±0.03 ab	0.09±0.05 a	0.06±0.01 ab
Ethyl dodecanoate	48±19	51±8.9	75±62	66±21
Acetate esters				
Ethyl acetate	74920±14971	66060±13847	73939±35451	95316±35688
Isoamyl acetate	5421±1861 ab	2753±995 b	5623±3548 ab	8401±3178 a
Hexyl acetate	254±86 a	73±24 b	95±76 b	187±60 ab
2-Phenylethyl acetate	171±46 b	162±35 b	220±78 ab	268±51 a
Alcohols				
1-Propanol	76±11b	106±39 ab	117±18 a	93±16 ab
2-Methyl-1-propanol	26851±6709 ab	25588±4313 ab	38622±12598 a	24762±4795 b
3-Methyl-1-butanol	93424±108556 b	121620±15198 a	123524±17588 a	122982±8801 a
1-Hexanol	744±217 ab	898±165 a	493±115 b	599±185 b
2-Ethyl-1-hexanol	10±6.0	10±1.7	12±2.1	10±2.1
1-Octanol	2.8±0.64 b	1.9±0.66 b	4.5±1.3 a	4.2±1.0 a
2-Phenylethanol	7258±1353 c	12963±684 b	15880±2173 a	13787±1836 ab
Isoprenoids				
Limonene	2.4±0.28 ab	2.2±0.57 b	1.8±0.16 b	2.9±0.43 a
Linalool	73±31 b	19±4.0 c	25±9.2 c	117±28 a
α-Terpineol	8.2±1.4 ab	3.4±1.8 c	4.3±2.4 bc	10.4±3.6 a
β-Damascenone	17±3.6	17±6.5	20±5.4	19±3.2
Carbonyl				
Nonanal	10±2.6 b	10±1.9 b	14±2.0 a	13±2.8 a
Benzaldehyde	0.70±0.27 a	0.43±0.09 ab	0.49±0.13 ab	0.39±0.09 b
Acids				
Acetic acid	19218±4164	25963±7995	22265±7213	25245±7841
Hexanoic acid	4410±531	3447±825	4500±893	4762±929
Octanoic acid	32000±2871	24810±5577	29901±5481	31930±5636
Decanoic acid	4792±247	3926±802	4311±543	4825±615
Dodecanoic acid	75±42	75±25	162±115	82±33

[a] Concentrations in µg/L unless indicated otherwise. For each region, means with different letters within a column (a, b, c, d) are significantly different ($p<0.05$) according to Tukey's (HSD) pairwise comparison. Adelaide Hills (ADL, n= 8), Barossa Valley (BV, n=5), Clare Valley (CV, n=9) and Eden Valley (EV, n=9).

Table S10: PLS prediction parameters for aroma volatiles in small-scale Chardonnay wines using juice compositional variables as predictors. [a]

	Mean (µg/L)	SD	R^2	SECV (µg/L)	RPD
Hexyl acetate	159	100	0.73	54	1.8
Linalool	0.063	0.047	0.79	0.022	2.1
2-Phenylethyl acetate	12577	72	0.64	46	1.6
3-Methyl-1-butanol	115292	18927	0.60	12627	1.5
2-Phenylethanol	12577	3778	0.72	2113	1.8

[a] SD, standard deviation; SECV, standard error of cross-validation; RPD, residual predictive deviation.

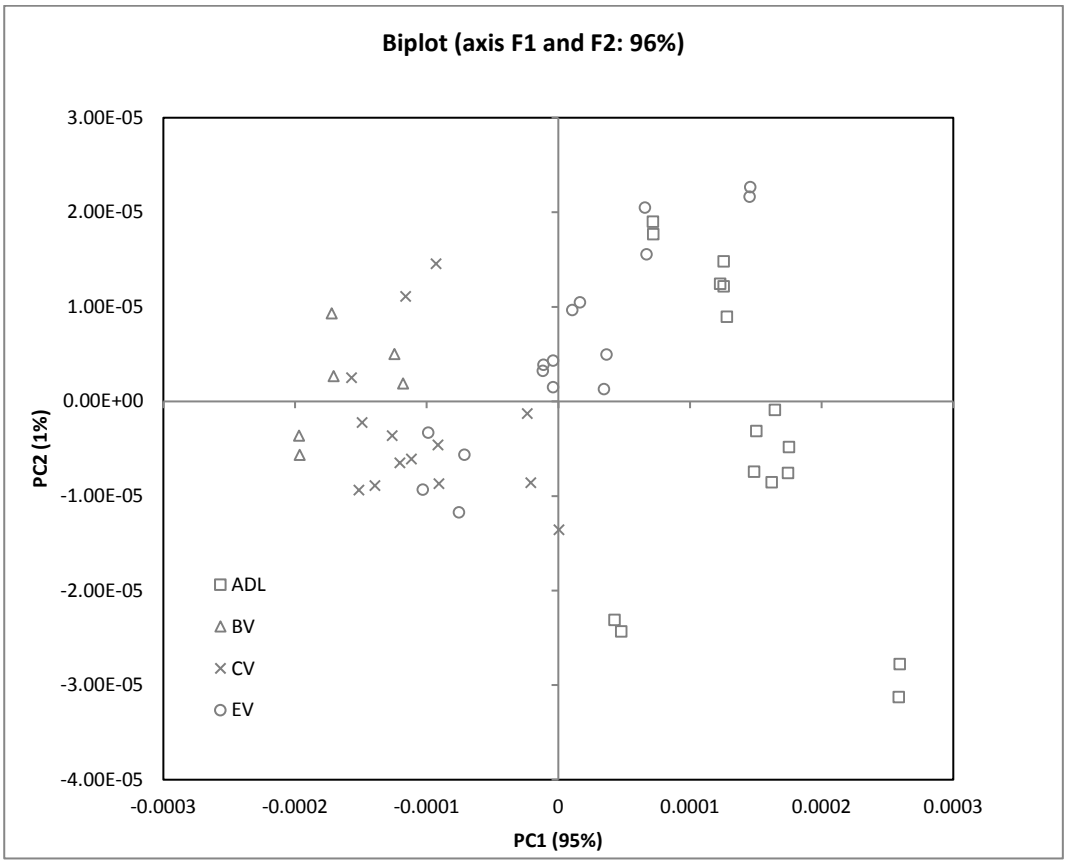

Fig. S1: PCA biplot of the second derivative of juice samples (fingerprint region) analysed in duplicate by mid-infrared spectroscopy.

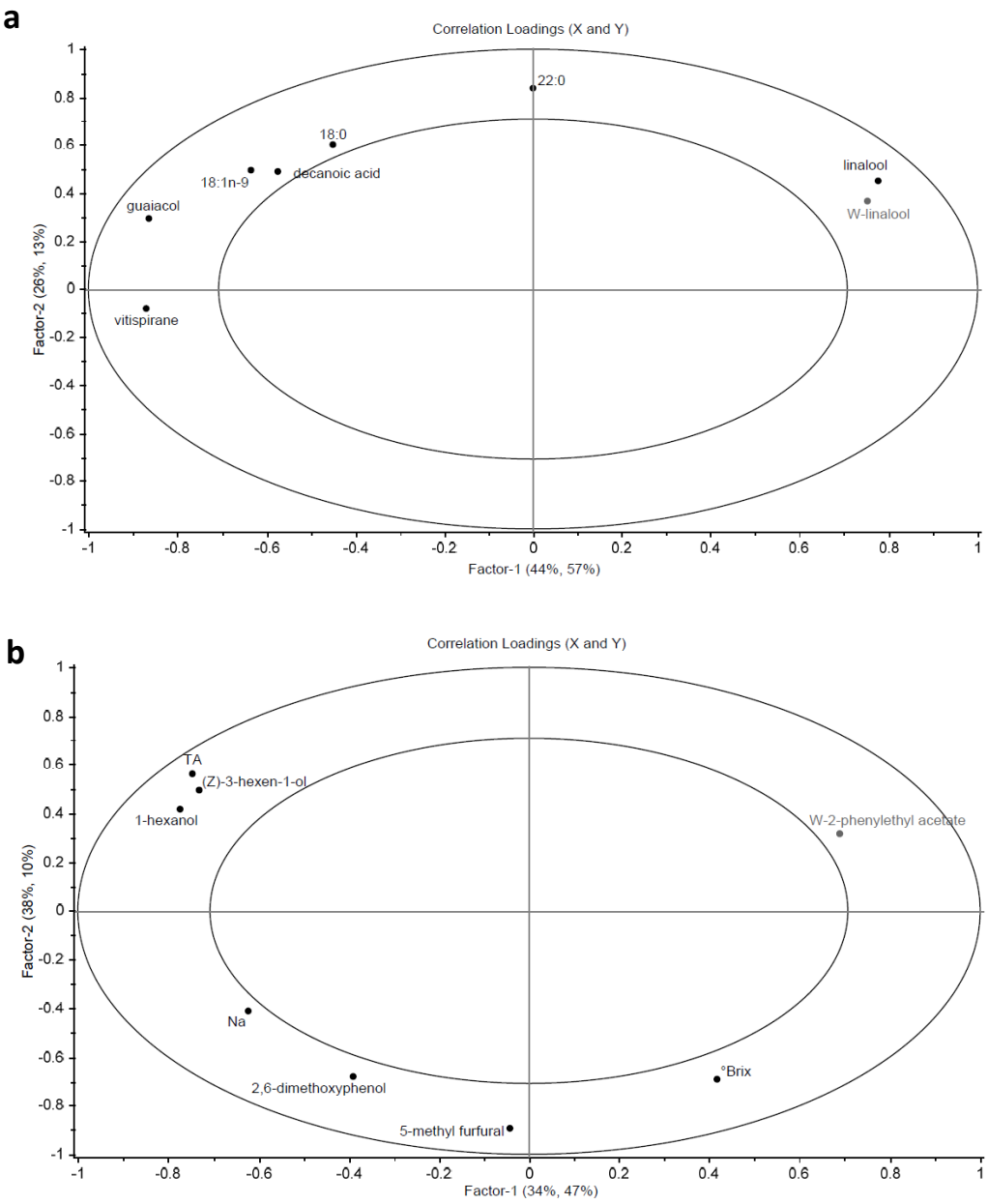

Figure S2: Scores plot and *X* and *Y* loadings plot from PLS regression for the wine compounds (**a**) linalool, and (**b**) phenylethyl acetate using juice compositional data. *X* loadings (juice compositional data) are shown in black, *Y* loadings (wine compounds) are shown in grey. The letter W in front of a compound indicates it is a wine compound.

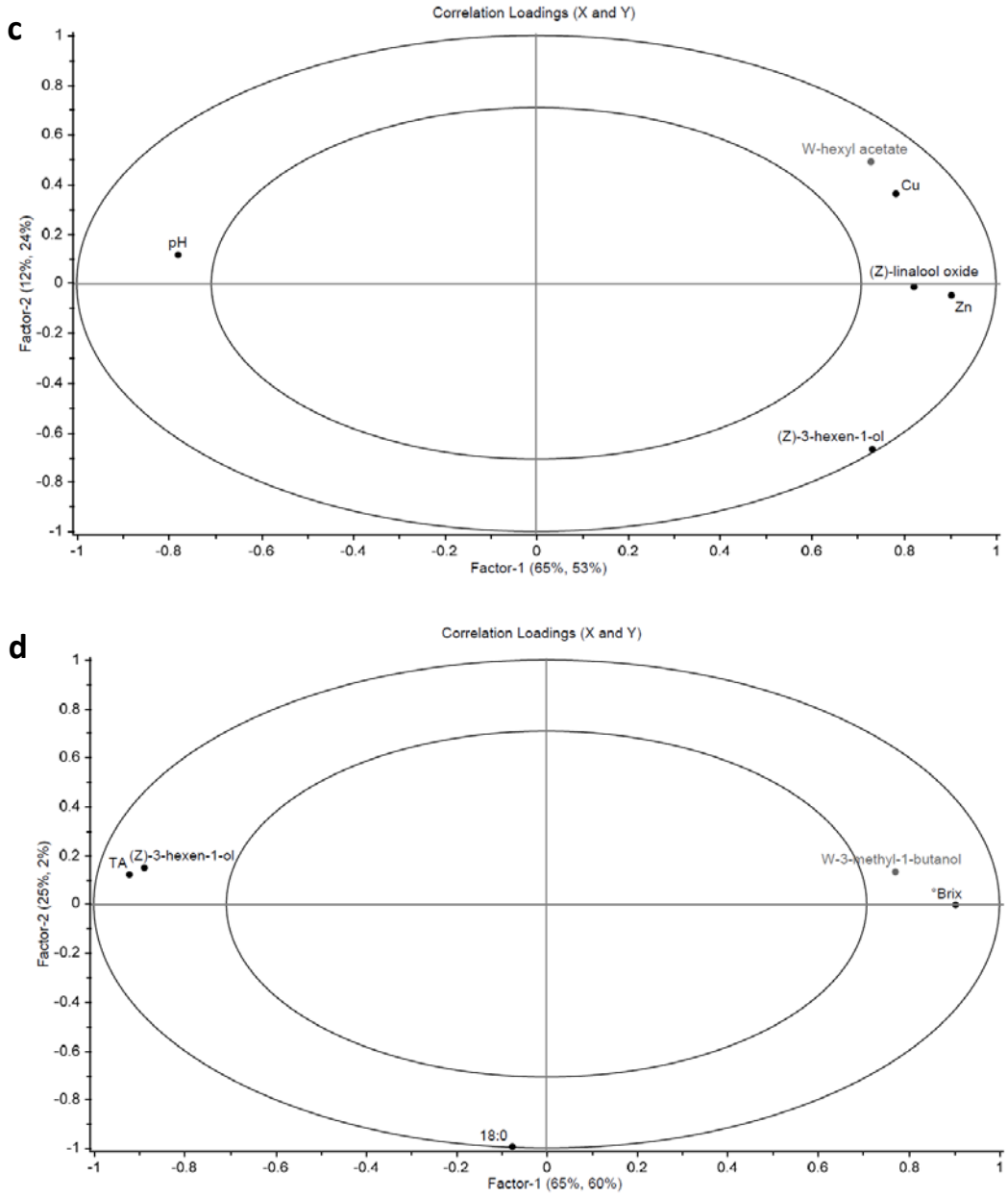

Figure S2 contd.: Scores plot and *X* and *Y* loadings plot from PLS regression for the wine compounds (**c**) hexyl acetate, and (**d**) 3-methyl-1-butanol using juice compositional data. *X* loadings (juice compositional data) are shown in black, *Y* loadings (wine compounds) are shown in grey. The letter W in front of a compound indicates it is a wine compound.

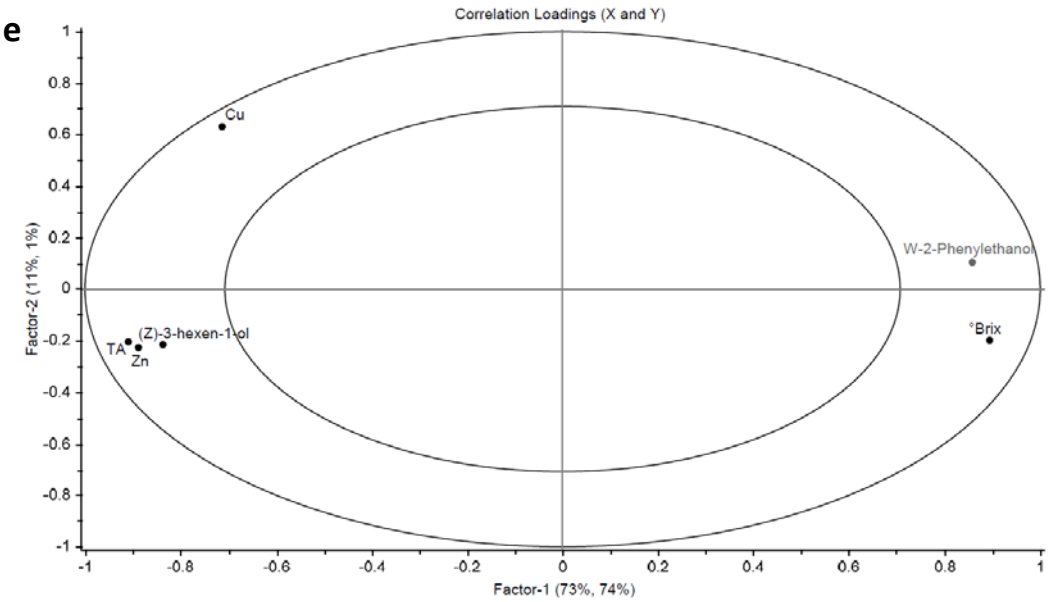

Figure S2 contd.: Scores plot and *X* and *Y* loadings plot from PLS regression for the wine compound (**e**) 2-phenylethanol using juice compositional data. *X* loadings (juice compositional data) are shown in black, *Y* loadings (wine compounds) are shown in grey. The letter W in front of a compound indicates it is a wine compound.

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CHAPTER 5

Exploring the effects of geographical origin on the chemical composition and quality grading of *Vitis vinifera* L. cv. Chardonnay grapes

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Name of Principal Author (Candidate)	Joanna M. Gambetta		
Contribution to the Paper	Designed experiments, performed experimental work, analysed and interpreted data, drafted and constructed the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	06/03/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Supervised the work, contributed to the research idea, experimental design and interpretation of the data. Assisted in the preparation and editing of the manuscript and acted as the corresponding author.		
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Exploring the Effects of Geographical Origin on the Chemical Composition and Quality Grading of *Vitis vinifera* L. cv. Chardonnay Grapes

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Abstract: The relationship between berry chemical composition, region of origin and quality grade was investigated for Chardonnay grapes sourced from vineyards located in seven South Australian Geographical Indications (GI). Measurements of basic chemical parameters, amino acids, elements, and free and bound volatiles were conducted for grapes collected during 2015 and 2016. Multiple factor analysis (MFA) was used to determine the sets of data that best discriminated each GI and quality grade. Important components for the discrimination of grapes based on GI were 2-phenylethanol, benzyl alcohol and C_6 compounds, as well as Cu, Zn, and Mg, titratable acidity (TA), total soluble solids (TSS), and pH. Discriminant analysis (DA) based on MFA results correctly classified 100% of the samples into GI in 2015 and 2016. Classification according to grade was achieved based on the results for elements such as Cu, Na, Fe, volatiles including C_6 and aryl alcohols, hydrolytically-released volatiles such as (*Z*)-linalool oxide and vitispirane, pH, TSS, alanine and proline. Correct classification through DA according to grade was 100% for both vintages. Significant correlations were observed between climate, GI, grade, and berry composition. Climate influenced the synthesis of free and bound volatiles as well as amino acids, sugars, and acids, as a result of higher temperatures and precipitation.

Keywords: Chardonnay; geographical indication; volatile composition; elements; multiple factor analysis

1. Introduction

Place of origin has an important influence on the style, quality and prestige of regional produce such as wine. Protected "Geographical Indications" (GI) have thus arisen as a way to identify goods that originate from a particular region "where a given quality, reputation or other characteristic of the product is essentially attributable to its geographical origin" [1]. GI are used in recognition of the influence of local factors on the characteristics of products that can help differentiate them in the global market [2]. Wine provenance and quality begins with the grapevines that are grown in a multitude of regions around the world, some of which are better suited to certain varieties than others. Climate and vineyard characters such as geology and soil affect vine phenology, and vine water and mineral status, modifying grape chemical composition [3] and enabling discrimination of fruit from different regions [4–7]. Knowledge of the specific composition of the grapes originating from different GI would allow the characterisation, authentication and valorisation of each GI.

The presence of major and trace minerals in the berry directly impacts yeast behaviour and modifies wine sensory profile and quality [8]. Concentrations in the berry depend on the availability of elements such as Fe, Zn and Mg in the soil, which are modulated by agricultural practices, particularly irrigation and fertilisation [9]. A number of studies have demonstrated that wine mineral profile is highly correlated to region and soil type, leading to the use of mineral composition to discriminate between wines from different origins [4]. However, few studies have been performed on grapes. Cugnetto et al. [10] were able to discriminate between grapes from the Alpine and Langhe regions based on their Ba, Mn, Si, Sr, and Ti concentrations, and Protano and Rossi [11] classified grapes according to the composition of the soil of origin using Ba, Rb, and Sr as markers. However, both of these studies dealt with viticultural areas with distinctly different soil profiles. In fact, Cugnetto et al. [10] were unable to discriminate between regions that were closer together based solely on berry element composition.

Conceptual representations exist for wines originating from different geographical origins [12]; Cabernet Sauvignon wines from California and Australia, Riesling wines from Germany, and Sauvignon Blanc wines from New Zealand, amongst others, have been shown to possess distinct and typical sensory characteristics that allow them to be recognised and discriminated from wines produced in other regions [13–17]. These typical sensory representations correspond to the biochemical profiles, and particularly the volatile aroma compositions of each set of wines, where clear correlations exist between the presence of certain aroma notes, for example floral, citrus and lime, and compounds such as monoterpenoids [18]. The responsible secondary metabolites are produced in the berry through a series of biosynthetic pathways which are modulated by viticultural practices, and particularly, by climatic phenomena [19,20]. This knowledge has led to investigations of the link between volatile composition and GI in order to classify samples according to their origin [13,21–24]. Aroma compounds are found in free and bound forms in the berry. Free forms include alcohols, aldehydes, acetates and isoprenoids [25], and bound forms (in terms of being hydrolytically-releasable) involve aglycones such as C_{13}-norisoprenoids, aliphatic and aromatic alcohols, shikimic acid metabolites, and monoterpenoids linked to sugars [26].

The successful classification of grapes and wines according to their origin is based on data sets that are intrinsically multivariate, and often combines results from various analytical techniques [4]. Chemometric tools are needed to determine the most important factors that need to be measured to predict origin, as well as to recognise patterns in the data and develop classification models [27], and techniques such as principal component analysis (PCA), multiple factor analysis (MFA), cluster analysis (CA), discriminant analysis (DA), and partial least squares (PLS) regression have commonly been used. PCA is an exploratory technique used to reduce the dimensionality of a data set [27]. MFA is similarly exploratory and allows the simultaneous analysis of multiple data sets, structured in groups, in which each group of variables is weighted [28]. DA is capable of classifying samples into pre-established categories and PLS regression is mainly used to relate blocks of variables measured on sets of objects [29–31].

In this study we used chemometric approaches to explore the effects of geographical origin on the chemical composition of Chardonnay grapes obtained from seven GI in South Australia during two vintages, and thereby determined the variables capable of discriminating between the regions of origin. Grape allocation grade was then used to try to pinpoint the chemical variables driving the grades assigned by winemakers in order to investigate objective measures of Chardonnay grape quality.

2. Results and Discussion

2.1. Understanding Regional Effects on Grape Composition

Grape samples were collected at commercial maturity during the 2015 and 2016 vintages from different GI across South Australia spanning the Adelaide Hills (ADL), Barossa Valley (BV), Clare Valley (CV), Eden Valley (EV), McLaren Vale (MV), Langhorne Creek (LC) and the Riverland

(RVL). Measurements of basic chemical parameters (TSS, TA and pH), 12 elements, 28 free and 29 conjugated volatile compounds and 19 amino acids were performed on the various grape samples (Supplementary Tables S1–S5). Examination of these compositional variables by two-way ANOVA (using region and vintage as the explaining variables) showed statistically significant interactions ($p < 0.05$) between both variables for most of the evaluated parameters. Significant differences were found for most compounds measured between both years with the exception of: Na, S, Fe, B, Zn, P, serine, histidine, threonine, leucine, phenylalanine, arginine, lysine, isoleucine, the free volatiles isoamyl acetate, hexanoic acid, (*E*)-3-hexen-1-ol, 2-ethyl-1-hexanol, linalool, (*Z*)-linalool oxide, hexanal and the conjugated volatiles 2,6-dimethyl-7-octene-2,6-diol, 2,6-dimethoxyphenol and decanoic acid. Additionally, PCA (results not shown) of the combined 2015 and 2016 analytical data revealed a clear clustering of samples according to vintage, so it was decided to treat both vintages separately. Vintage effects are common given the large but variable influences that seasonal changes have on berry metabolite composition [32,33], as climate has been shown to have a greater effect on fruit composition than soil and cultivar [34].

2.1.1. Classification According to Origin Using Multiple Factor Analysis of Analytical Variables

To elucidate the relationship between grape chemical composition and the region of origin, MFA was applied to significantly different compositional variables (using GI as the explaining variable). MFA is a *k*-table methodology that allows the simultaneous analysis of multiple data sets acquired on the same group of samples, and unlike other methodologies, it ensures that no single set dominates the common solution. Only data sets with RV coefficients superior to 0.6 were retained, and MFA was recalculated for 2015 using the data sets corresponding to elements, free volatiles, basic chemistry and bound volatiles (Figure 1) and for 2016 using elements, free volatiles, and basic chemistry (Figure 2).

2.1.2. Differentiating Variables

The first three dimensions of the MFA plot corresponding to the 2015 vintage accounted for 49% of the total variance (Figure 1). Four groups of samples were observable in the F1/F2 plot (Figure 1A). Group 1, comprising ADL and EV samples, was located in the lower right quadrant of F1/F2, group 2 containing BV, CV, and RVL samples was located in the lower left quadrant of F1/F2, and two groups consisting separately of LC and MV samples, were located on the positive side of F2. Inclusion of F3 (Figure 1B) allowed the separation of CV samples from the rest of group 2 along the F3-axis (lower left quadrant of F1/F3, Figure 1B). Examination of F1 loadings (Figure 1C) revealed that the separation between group 1 and 2 was due to a higher pH and higher concentrations of Cu, Zn, free 2-phenylethanol and benzyl alcohol, and lower concentrations of free (*Z*)-linalool oxide and linalool in the ADL and EV samples (Supplementary Tables S1, S2, and S3, Supplementary Figure S1) to the right along F1. Notably, all samples on the left side of F1 (Figure 1A) originated from regions with high night time temperatures during the berry ripening period in January and February (Supplementary Table S6), which correlated with lower concentrations of 2-phenylethanol and benzyl alcohol ($r = -0.49$ and $r = -0.64$ respectively, $p < 0.001$) and higher contents of linalool (only January, $r = 0.69$, $p < 0.0001$). Benzyl derivatives are derived from L-phenylalanine and are formed through a coupled decarboxylation and oxidation reaction (2-phenylethanol) or through deamination of phenylalanine into (*E*)-cinnamic acid and subsequent oxidation (benzyl alcohol) [25]. These compounds have been reported to be present at higher concentrations in Glera grapes at véraison, decreasing thereafter as maturity progresses [35]. As observed by Alessandrini et al. [35], benzyl derivatives were also the most abundant in our study for the two sites with the highest altitudes, ADL and EV.

The presence of certain trace elements has been used in several studies to determine the geographical origin of wines and grapes, as they reflect the geochemistry of the soil the vines were grown in [4]. Cu and Zn were most abundant in ADL and EV (Figure 1A,C, Supplementary Table S2, Supplementary Figure S1), which contributed to their separation from the rest of the sites. Separation of

LC and MV from group 1 and 2 along F2 (Figure 1A) was driven by higher contents of the free volatiles isoamyl acetate and 3-methyl-1-butanol, and bound 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and 2-phenylethanol (Figure 1C, Supplementary Tables S3 and S5) and lower concentrations of bound hexanoic acid. MV had both the highest growing degree day (GDD) values and night time temperatures in 2015 (Supplementary Table S6), contributing to the formation of free 3-methyl-1-butanol and isoamyl acetate, and bound TDN and 2-phenylethanol, along with the degradation of hexanoic acid (bound). Similarly, higher formation of 3-methyl-1-butanol has been reported in Glera berries in warm sites compared to cool sites in the Conegliano-Valdobbiadene appellation [35]. A lower concentration of the C_6 compounds 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)- and (*E*)-hexen-2-ol (Supplementary Table S3) and lower TA in CV samples (Supplementary Figure S1 and Table S1), together with higher quantities of bound 3-oxo-α-ionol, explained the separation of CV from group 2 along F3 (Figure 1B,D). Mean concentrations of 3-oxo-α-ionol, arising in the berry from the corresponding carotenoid as a function of the amount of light received after véraison [36], were significantly higher in CV and lowest in ADL, LC and MV. Concentrations were correlated to the average amount of light received by these regions (r = 0.50, $p < 0.001$), where CV had the highest level of irradiation (25.1 MJ/m^2, Supplementary Table S6) in February (i.e., commercial harvest month for all regions except RVL).

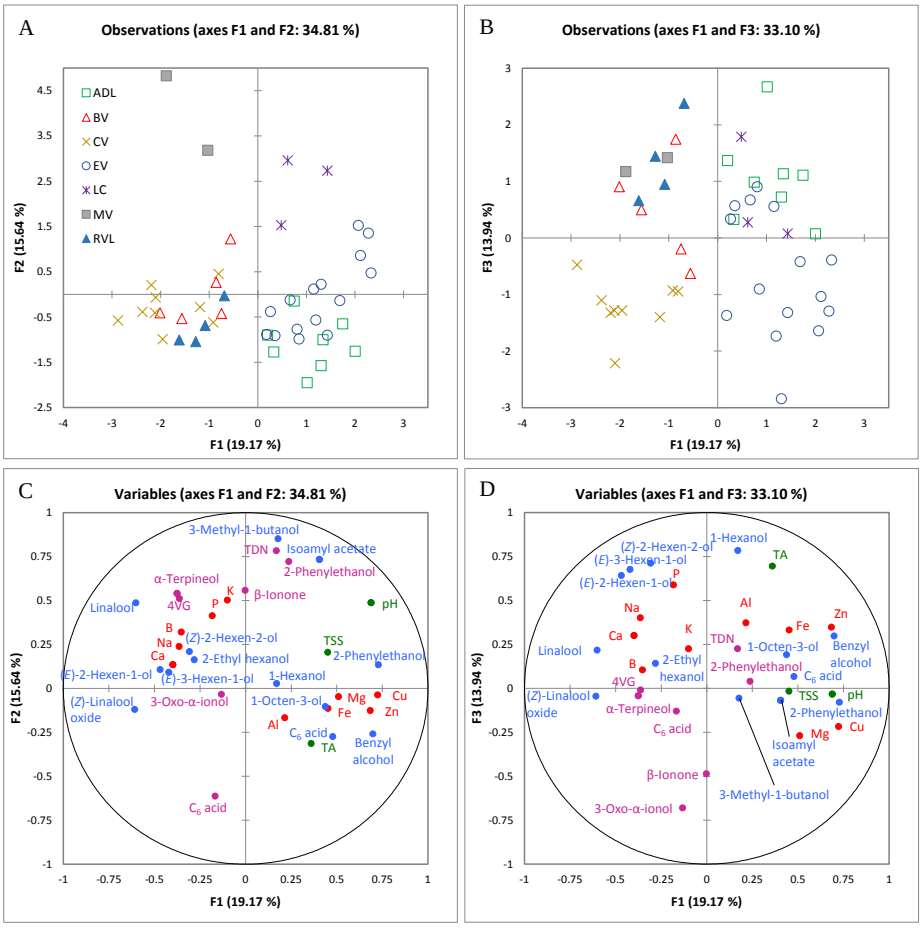

Figure 1. Multiple factor analysis (MFA) of elements, free and bound volatiles and basic chemistry data for all seven Geographical Indications (GI) in 2015, showing the scores projected on F1 and F2 (**A**) and F1 and F3 (**B**) for each GI, and loadings of variables used in the analysis on F1 and F2 (**C**) and F1 and F3 (**D**) (basic chemistry in green, elements in red, free volatiles in blue and bound volatiles in violet). ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; MV, McLaren Vale; RVL, Riverland. Abbreviations (e.g., TSS, 4VG, TDN) are the same as specified in the text.

Although not observable in the MFA plots, it was possible to discriminate RVL samples from all others in 2015 based solely on the composition of elements. RVL had the lowest Mg content of all samples (Supplementary Table S2, 73 mg/L) and the highest concentrations of Al (2.0 mg/L) and P (175 mg/L).

Examination of the GDD values, minimum and maximum temperatures, and days above 25 °C and 30 °C (Supplementary Table S6) for both vintages and for each region revealed that 2016 was a warmer year than 2015. However, due to rain events during early February 2016 (i.e., harvest period, Supplementary Table S6), the harvest date for all sites except RVL was not more advanced in relation to the previous year (Supplementary Table S1). In 2016, the first three dimensions of the MFA plot (Figure 2) accounted for 56% of the total variance. Discrimination along F1 was again driven in the positive direction by Cu, Zn, benzyl alcohol, and 2-phenylethanol concentrations. Separation along F2 was related to TA in the positive direction and total soluble solids (TSS, as °Brix) in the negative (Figure 2C), and F3 was mainly driven in the positive direction by the presence of higher amounts of (*Z*)-3-hexen-1-ol and to a lesser extent Mg, and in the negative direction by TSS (Figure 2D).

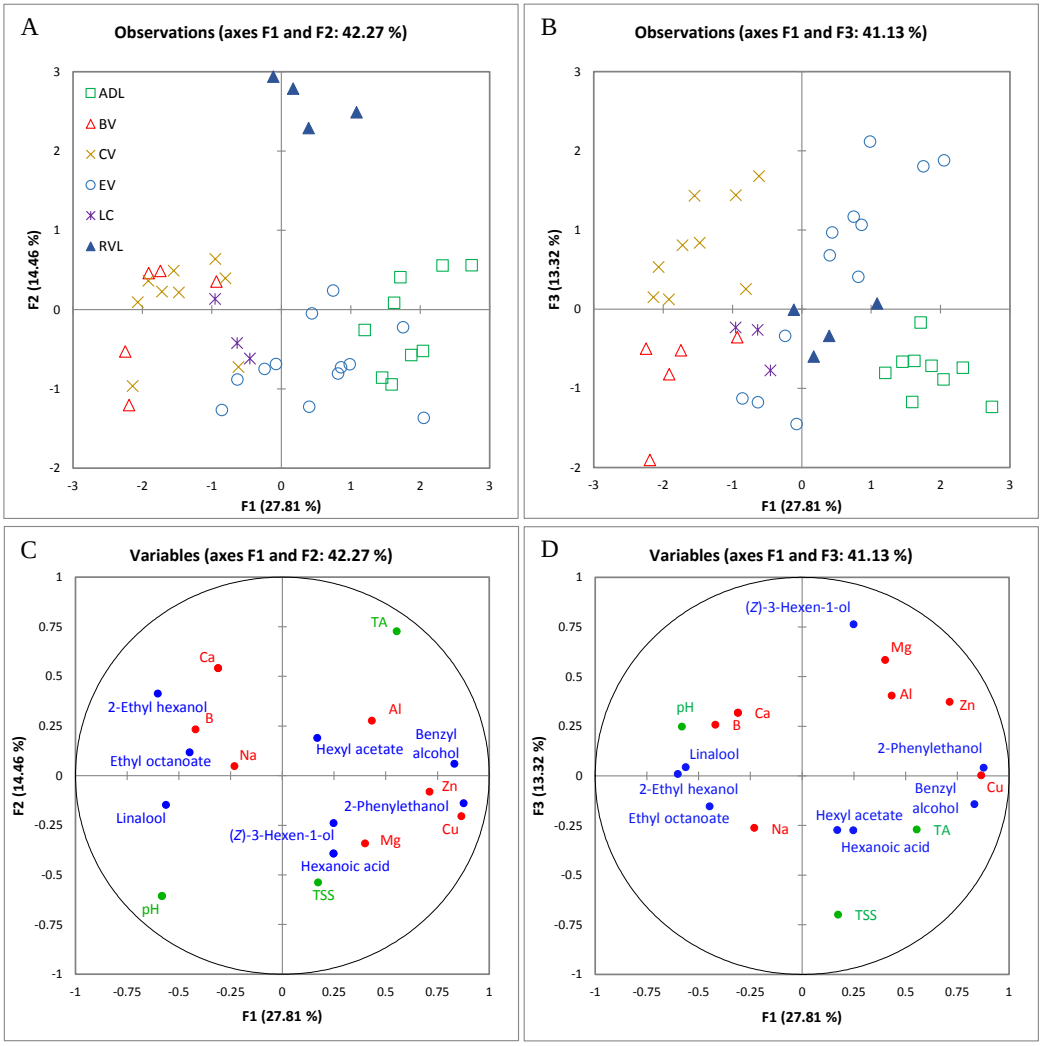

Figure 2. MFA of elements, free volatiles and basic chemistry data for all six GI in 2016, showing the scores projected on F1 and F2 (**A**) and F1 and F3 (**B**) for each GI, and loadings of variables used in the analysis on F1 and F2 (**C**) and F1 and F3 (**D**) (basic chemistry in green, elements in red and free volatiles in blue). ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; RVL, Riverland. Abbreviations (e.g., TSS, TA) are the same as specified in the text.

Discrimination of ADL was observed mainly along F1 (Figure 2B, F1/F3, lower right quadrant) and driven by its higher concentrations of free 2-phenylethanol and benzyl alcohol (Supplementary Table S3, Supplementary Figure S1), Cu and Zn content (Supplementary Table S2), and lower 2-ethyl-1-hexanol concentration. This confirms the trends observed during 2015, and matches with the previous results for this region [37]. Given that 2016 was a warmer year, the importance of cooler nights to the decrease in concentration of 2-phenylethanol and benzyl alcohol seems to have been accentuated. Strong negative correlations were found for the 2016 vintage between night time temperature and the respective concentrations of these alcohols ($r = -0.78$ and $r = -0.71$, $p < 0.0001$). Unlike 2015, RVL samples in 2016 could be easily distinguished from all other regions in the MFA multivariate space along F2, mainly because of their higher concentrations of TA (Figure 2C and Supplementary Table S1, 9.5 g/L as tartaric acid) and Ca (65 mg/L), and lower pH.

Pearson correlation analysis showed that for both years, the presence of 2-ethyl-1-hexanol was favoured by higher GDD values ($r_{2015} = 0.53$ and $r_{2016} = 0.64$, $p < 0.0005$). BV and CV, which had the highest mean January and February maximum temperatures after RVL (Supplementary Table S6), were observed to the extreme left of F1 (Figure 2A) opposite ADL, due to their lower concentrations of 2-phenylethanol and benzyl alcohol, as well as higher amounts of linalool, 2-ethyl-1-hexanol, ethyl octanoate, Ca, and B (Figure 2C). CV could be discriminated from BV along F3 (Figure 2B) mainly due to a slightly higher concentration of (*Z*)-3-hexen-1-ol and lower TSS (Figure 2D). LC and EV exhibited an intermediate profile in 2016 between the warmer and cooler regions, probably due to warmer nights compared to 2015.

2.1.3. Major and Trace Elements

Although most elements did not show any marked trends between regions across both vintages, some patterns could be observed for certain elements such as Zn and P. For both vintages, the mean concentrations of Zn in ADL were significantly different and higher than those of all other regions according to Tukey's HSD post hoc test, followed by EV, and those from BV were significantly lower than those from all other regions (Supplementary Table S2). This can be partly attributed to the application of seaweed extract (*Ascophyllum nodosum*) to vines in the ADL vineyards, which has been shown to significantly increase vine Zn content [38]. Perusal of the Australian Soil Resource Information System (ASRIS) [39] also showed a higher concentration of this element in the root active zone in the soils of the ADL region than in all other regions (with the exception of RVL for which such information was not available). Zn plays an essential structural and functional role in yeast cells and is required as an essential cofactor for enzymatic activity where it binds to catalytic active sites and acts as an activator of the terminal alcohologenic Zn-metalloenzyme ethanol dehydrogenase during fermentation. Zn deficiencies lead to slow or incomplete fermentations [40]. Comparison of means showed that P concentrations were significantly different and higher in the RVL during both vintages (and MV in 2015), and lowest in ADL and EV. The corresponding ASRIS data sheets [39] indicated that P contents were marginal in the selected ADL and EV sites. Additionally, unlike RVL, both the ADL and EV sites had soils with an acidic pH, which lowers the availability to the plant of any P present due to fixation by aluminium or iron [41]. Higher levels of Cu were measured in ADL and EV samples, with the lowest mean contents being found in CV (Supplementary Table S2, Supplementary Figure S1), which reflect the composition of these regions' soils. The mean relative concentrations of Fe were significantly different and higher for both years in ADL than in all other sites (Supplementary Figure S1). Fe and Cu are needed at low concentrations as co-factors in cell metabolism, however, they can be toxic to yeast development when present at around 6 mg/L or higher [42]. Fe and Cu concentrations were well below this value for all samples in both years. Berry concentrations of Mg changed from year to year for most regions, but EV had the highest mean relative concentrations of this element in both vintages. Mg is an essential mineral for good fermentation performance–it is involved in every phosphate-transferring enzymatic process–and is crucial to metabolic activities

including glycolysis and alcoholic fermentation [43]. According to Sommer et al. [44], *S. cerevisiae* preferentially utilises Mg^{2+} cations during fermentation and biomass formation.

2.1.4. Amino Acids

Overall, the amino acid results showed no discernible pattern contributing to the separation of samples by region in either year: any regional effects that may have existed are seemingly overshadowed by other factors. Amino acid concentrations can be greatly impacted by the degree of berry maturity and water stress, level of fertilisation, and other viticultural parameters [45], and considerable variation between vintages has been reported previously [46]. Amino acids are important contributors to quality as they act as precursors to many key compounds including, but not restricted to, higher alcohols, aldehydes, and esters [47]. The Ehrlich pathway gives rise to higher alcohols through the degradation of the corresponding amino acid [48]. Higher alcohols can have both positive and negative effects on wine quality depending on their concentrations [47,48] and can be transformed during fermentation into their corresponding fruity esters. Esters are crucial to Chardonnay wine quality, as they impart desirable aromas and constitute one of the main odorant classes of this grape variety [49]. Amino acids also affect yeast metabolism, regulating the formation of compounds that are detrimental to wine quality such as the volatile sulfur compounds (e.g. hydrogen sulfide, methyl mercaptan) [47]. Looking at the data more closely, RVL samples contained higher levels of aspartic acid in 2016 (Supplementary Table S4, 84 mg/L) that made them distinct from all other regions. Likewise, ADL could be described by higher contents of alanine (249 mg/L), serine (127 mg/L), glutamic acid (183 mg/L) and glutamine + glycine (GLN + GLY, 246 mg/L) than all other regions for that vintage. EV had the second highest levels of serine (120 mg/L) and glutamic acid (161 mg/L) in 2016. Relative contents of phenylalanine were lowest in ADL, BV, and RVL in both years.

Consistent with the work of Stines et al. [50], proline and arginine were the two predominant amino acids in the grapes (in both vintages) and the content of proline was in line with the average of 742 mg/L published by Amerine and Ough [51]. Unlike other grape varieties, Chardonnay favours proline accumulation from véraison onwards, which can be potentially problematic. Proline is non-assimilable by yeast and higher amounts relative to forms of yeast assimilable nitrogen (YAN) can lead to challenging fermentations due to the lower total amount of YAN available. As a whole, higher amounts of proline were observed during 2015 than 2016. During both years, lysine, isoleucine, leucine, tyrosine and β-alanine were found at the lowest concentrations. Their combined concentrations amounted to less than 100 mg/L or 5% of the total amino acid content. Isoleucine, leucine, and lysine have also been cited by Hernández-Orte et al. [46] as being minor amino acids in Tempranillo grapes.

2.1.5. Glycosides

Similarly to amino acids, free and bound volatiles did not differ specifically according to the geographical origin of the grapes. The profile of bound volatiles in the form of glycosidic aroma precursors is dependent on maturity level and weather as well as grapevine canopy [19]. Consequently, the results obtained for bound volatiles for each year and region varied considerably. For 2015 samples, in contrast to what was observed for the corresponding free alcohols with respect to night time temperature, hydrolytically-released 2-phenylethanol and benzyl alcohol concentrations were highest in MV and LC samples (Figure 1, Supplementary Table S5), with these being two of the regions with the highest night time temperatures. MV also had significantly higher levels of TDN, α-terpineol, and 4-vinylguaiacol (4VG) than all other regions. Formation of C_{13}-norisoprenoids, including TDN, is affected by sunlight and heat. TDN is a carotenoid degradation product, and the concentrations of its carotenoid precursors (lutein and β-carotenoid) have been shown to increase in grapes from hot regions [52,53]. Likewise, the formation of glycosidically-bound monoterpenoids such as α-terpineol is influenced by berry microclimate, where levels are higher in fruit with greater sunlight exposure (and higher bunch temperature) [54]. ADL together with CV presented the highest levels of 2,6-dimethyl-7-octene-2,6-diol and MV had the lowest.

In 2016, there were no significant differences between the concentrations of hydrolytically-released 2-phenylethanol and benzyl alcohol in samples from the different regions (Supplementary Table S5). This seems to be partly explained by the overall higher temperatures in 2016. BV and CV had the highest mean concentrations of vitispirane, 2,6-dimethyl-7-octene-2,6-diol, 5-methylfurfural (5-MF), β-damascenone, α-terpineol, and β-ionone. Higher levels of 5-MF, vitispirane, β-ionone and β-damascenone accorded with preceding results for BV and CV, which were compared to ADL and EV in that study [37]. As observed previously, the presence of these four compounds in the berries exhibited significant correlations to GDD (r = 0.53, r = 0.56, r = 0.56, and r = 0.43 for 5-MF, vitispirane, β-ionone, and β-damascenone, respectively, $p < 0.001$), as well as to February maximum temperatures and number of days over 25 °C during January and February (Supplementary Table S6). As stated previously, carotenoid degradation increases in hotter climates with the corresponding augmentation of C_{13}-norisoprenoid concentrations in the berry [52,53]. Additionally, heat stress in berries during ripening results in a partial anaerobic metabolism and the production of ethanol, CO_2, and fermentation by-products including 5-MF [55–57]. Correspondingly, the mean concentrations of α-terpineol and 5-MF were lowest in EV and LC. These two regions had less days over 25 °C during January and February in 2016 than all other sites, which appears to also have had an impact on the production of 3-methyl-1-butanol, (*E*)- and (*Z*)-linalool oxide, linalool, (*E*)-2-hexenal, and 4VG, which were lower in these samples. 3-Oxo-α-ionol was again found in the highest concentrations in CV, which had the highest solar exposure of all regions harvested in February (24.8 MJ/m^2, Supplementary Table S6). Despite RVL being the hottest region from which samples were sourced, most of the abovementioned compounds were found at the lowest concentrations in RVL samples, likely as a result of the earlier harvest dates in 2016. Grapes from this region were less ripe than the remainder (Supplementary Table S1, TSS = 18.9 °Brix, TA = 9.5 g/L) and therefore most C_{13}-norisoprenoids and monoterpenoids, which are synthesised during ripening [36], were found in lower concentrations. Methyl vanillate, 2,6-dimethoxyphenol, α-terpinene, hexanoic acid, octanoic acid, 4VG, guaiacol, 3-methyl-1-butanol, (*E*)-2-hexenal, and (*E*)- and (*Z*)-linalool oxides were higher in ADL than all other regions. As a whole, due to the effect of rain during ripening in 2016, lower amounts of bound monoterpenoids and C_{13}-norisoprenoids were observed compared to 2015 [58,59].

2.2. Prediction of Geographical Indication Based on Composition Variables

A number of studies have been conducted to discriminate wines according to geographical origin but very few have done so on grapes [4]. Amongst the grape studies, successful results have been obtained when using mineral elements for clearly distinct geological regions [10,11]. However, these studies have failed to distinguish regions that are closer in proximity based exclusively on a single class of berry constituents such as elements. Unlike PCA and MFA, discriminant analysis (DA) is a supervised classification technique that requires prior knowledge of class membership. DA was carried out using backward stepwise selection of variables and full cross-validation to predict the membership of a sample to a particular GI (Figure 3). Overall classification rates for the years 2015 (n = 50) and 2016 (n = 45) were 100%. Based on the results of MFA, the variables used by the model to discriminate between regions in 2015 were Ca, K, Mg, Na, Fe, B, Cu, P, Al, the free volatiles isoamyl acetate, ethyl hexanoate, hexanoic acid, 1-hexanol, (*E*)-3-hexen-1-ol, (*E*)- and (*Z*)-2-hexen-1-ol, (*Z*)-linalool oxide, 1-octen-3-ol, linalool, 2-phenylethanol, benzyl alcohol, pH, and TA, and the bound volatiles α-terpineol, hexanoic acid, TDN, and benzyl alcohol (Figure 3C). In 2016, classification relied on the presence of Ca, Mg, Na, B, Cu, the free volatiles hexyl acetate, (*E*)-3-hexen-1-ol, ethyl octanoate, 2-ethyl-1-hexanol, linalool, benzyl alcohol, and pH and TSS (Figure 3D). These results indicate the potential for the classification of grapes according to origin but should be considered as exploratory; a larger set of samples from each GI is required to confirm these outcomes.

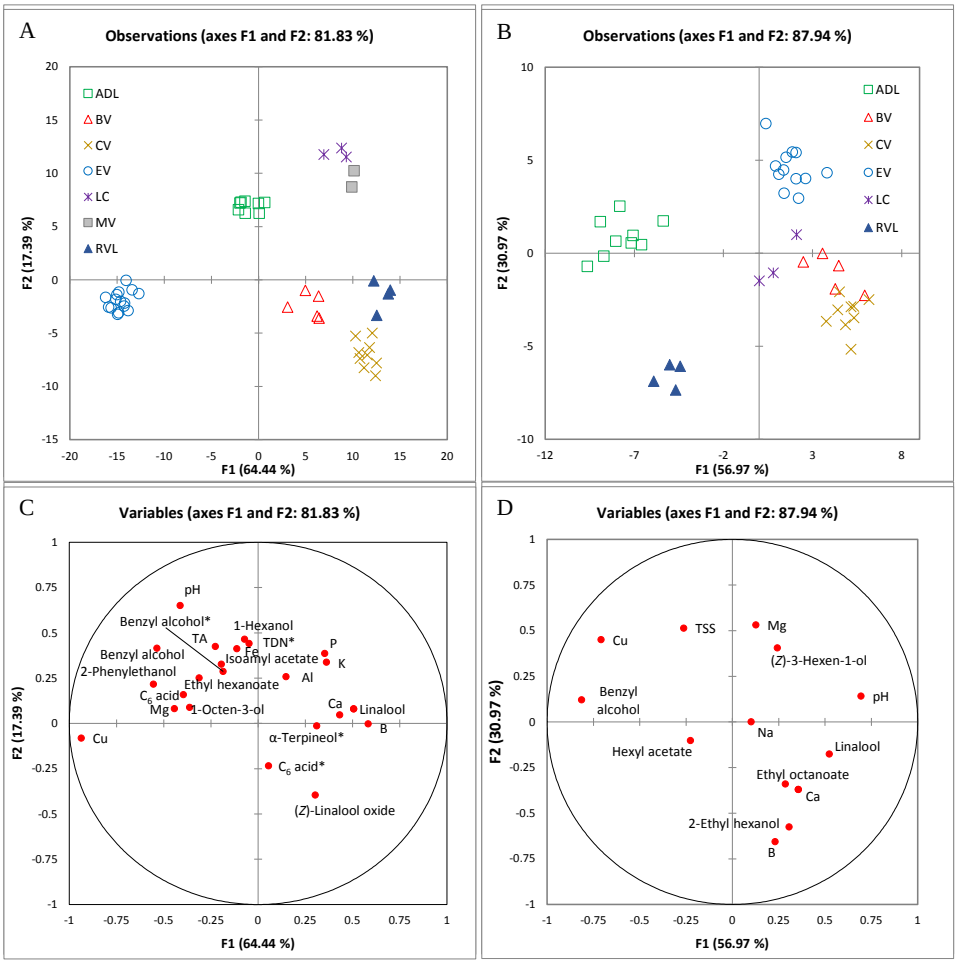

Figure 3

Figure 3. Distribution of grape samples in the two coordinate system defined by discriminant analysis showing the two canonical variables with the highest discrimination power. Scores projected onto F1 and F2 for 2015 (**A**) and 2016 (**B**) show samples grouped according to GI where ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; MV, McLaren Vale; RVL, Riverland. Loadings of variables used in the analysis are shown for 2015 (**C**) and for 2016 (**D**) where * denotes volatiles detected after hydrolysis of glycosides extracted from juice. Abbreviations (e.g., TSS, TDN) are the same as specified in the text.

2.3. Correlating Grape Grading and Grape Composition

Grades are normally allocated to Chardonnay grapes by winemakers in Australia (and likely elsewhere) based on the location and previous knowledge of the vineyard, basic chemical parameters such as pH, TA and TSS, and flavour profile as judged during berry tasting in the vineyard [60]. However, the tasting process does not necessarily take into account the true flavour composition and aroma potential of the berries. Key volatile compounds such as the monoterpenoids and C_{13}-norisoprenoids are synthesised through biological pathways that are independent and, depending on climatic and viticultural conditions, often asynchronous to sugar accumulation [61]. Conscious that technological maturity (based on basic chemical parameters to determine harvest timing) and aroma maturity do not always coincide, companies are looking to develop indices which include volatile compounds to better stream grapes into the different desired fruit categories [62]. MFA of significantly different attributes (according to allocated grade) was carried out on berry composition for 2015 and 2016, and followed up with DA with full cross-validation to identify the parameters that most influenced the allocated grades (Figure 4). In 2015 and 2016, 100% of the samples were correctly classified as A-, C- or D-grade. These correct classification rates exceed the grade prediction

results for Chardonnay grapes mentioned by Smith [63]. However, as a result of the large effect that vintage year has on the composition of the berries, the different grading systems used by winemakers in each study, and the different measurements taken into account, some variances could be observed between the results of the prediction models from our work versus those reported by Smith. Classification in 2015 was based on the concentration of Na, Fe, Cu, Al, C_6 alcohols (1-hexanol, (*E*)-3-hexen-1-ol, 2-ethyl-1-hexanol), pH, TA, and hydrolytically-released vitispirane and 5-MF. Samples corresponding to A-grade fruit had higher TA, higher concentrations of Al and Fe, and lower levels of bound 5-MF. C-grade samples had overall lower concentrations of 1-hexanol and Al, and higher concentrations of Cu and vitispirane, whereas D-grade samples were characterised by higher levels of (*E*)-3-hexen-1-ol, 2-ethyl-1-hexanol, Na, and bound 5-MF, and lower pH and lower concentrations of Cu and vitispirane. Classification in 2016 depended on the levels of Cu, benzyl alcohol, alanine, proline, and hydrolytically-released (*Z*)-linalool oxide, hexanoic acid, 3-oxo-α-ionol, and 2,6-dimethoxyphenol (2,6-DMP). A-grade samples were correctly classified based mainly on their higher mean concentrations of benzyl alcohol, Cu, proline, alanine, and bound hexanoic acid and 2,6-DMP. C-grade samples had higher levels of bound 3-oxo-α-ionol, and D-grade samples had the lowest levels of hydrolytically-released 3-oxo-α-ionol and (*Z*)-linalool oxide.

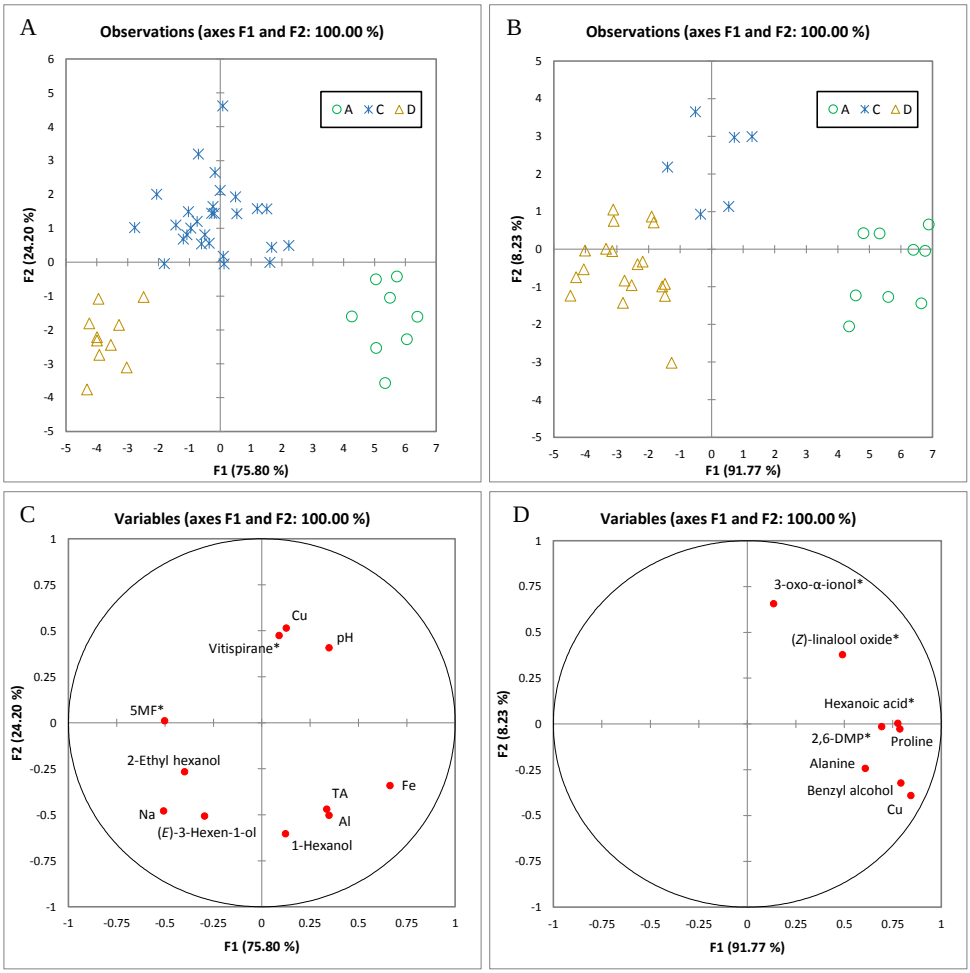

Figure 4. Distribution of grape samples in the two coordinate system defined by discriminant analysis showing the two canonical variables with the highest discrimination power. Scores projected onto F1 and F2 for 2015 (**A**) and 2016 (**B**) shows samples grouped according to quality grade (A, C and D). Loadings of variables used in the analysis are shown for 2015 (**C**) and for 2016 (**D**) where * denotes volatiles detected after hydrolysis of glycosides extracted from juice. Abbreviations (e.g., TA, 5-MF, 2,6-DMP) are the same as specified in the text.

Impact of Climate on Berry Quality Grades

Climate has been shown to have a significant impact on the final composition and quality of both grapes and wines [3]. Pearson correlation analysis revealed a significant negative correlation between allocated Chardonnay grape quality grade and GDD ($r_{2015} = -0.80$ and $r_{2016} = -0.60$, $p < 0.0005$). RVL had the highest GDD for both years (2370 and 2670, respectively, Supplementary Table S6), and the corresponding grapes were graded as D, in line with the correlation analysis results. However, whereas RVL grapes constituted the lowest grade assigned in 2015, grapes from certain areas in CV and EV, and all BV vineyards, were classified as D in 2016, making this relationship with GDD less clear. Temperature has a large effect on grape composition by impacting the rate of photosynthesis and the formation and degradation of important metabolites during maturation, and thus affects quality [3]. Higher temperatures decrease the concentration of organic acids, namely malate [64], and increase the amount of shrivelled and sunburnt berries [65]. This is especially relevant to Chardonnay grapes, which are very susceptible to sunburn under high temperature conditions. Sunburn increases the concentration of secondary phenolic compounds, and as shown for Sauvignon blanc grapes, shifts wine aroma profiles from fresh and fruity to phenolic and neutral, with a consequent loss of quality [66,67]. Closer examination of our results revealed that the effect of GDD on allocated grade was partly driven by the negative influence of higher night time temperatures (measured as minimum average temperature), mainly in January ($r_{2015} = -0.78$ and $r_{2016} = -0.85$, $p < 0.005$) but also in February ($r_{2015} = -0.70$, $p < 0.001$), and maximum average temperatures in February ($r_{2015} = -0.78$ and $r_{2016} = -0.59$, $p < 0.001$). A significant negative effect of temperatures above 30 °C was observed on the quality of the grapes, with $r_{2015} = -0.87$ and $r_{2016} = -0.70$ ($p < 0.0001$). This means that higher temperatures, and particularly higher night time temperatures, negatively affected the development of positive characteristics in Chardonnay grapes. Higher temperatures cause volatilisation of aroma compounds such as the monoterpenes and enhance their biotransformation and degradation, and decrease benzenoid concentrations [68]. According to temperature sensitivity models, rises in temperature result in significant losses of quality for Chardonnay grapes (measured as the price paid per tonne of grapes) [69]. Significant correlations between the allocated grade and precipitation that occurred during January and February were found only for the 2016 vintage ($r_{Jan} = 0.53$ and $r_{Feb} = 0.34$, $p < 0.05$). Precipitation during both months was moderate, and distributed over a period of several days, which gave viticulturists enough time to apply fungicides (when possible) and adjust irrigation regimes to avoid the potential for berry burst and increased disease pressure. The moderate levels of rain in 2016 seem to have delayed maturity slightly, giving berries the possibility to ripen more slowly despite the warmer weather.

3. Materials and Methods

3.1. Chemicals

All chemicals were of analytical reagent grade unless otherwise stated. Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). All aroma reference compounds were obtained from either Sigma-Aldrich (Castle-Hill, NSW, Australia), Alfa-Aesar (Ward Hill, MA, USA), Riedel-de Haën (Seelze, Germany), or Hopkin and Williams (London, UK). Deuterium-labelled internal standards consisting of d_4-3-methyl-1-butanol, d_3-hexyl acetate, d_{13}-1-hexanol, d_5-phenyl ethanol, and d_{19}-decanoic acid were supplied by CDN Isotopes (Point-Claire, Quebec, CN, Canada), and d_5-ethyl dodecanoate was synthesised previously [70].

3.2. Grapes

V. vinifera L. cv. Chardonnay berry bunch samples (4 kg) were randomly harvested from commercial vineyards in the Adelaide Hills ($n = 8$), Eden Valley ($n_{2015} = 15$, $n_{2016} = 12$), Clare Valley ($n = 9$), Barossa Valley ($n = 5$), Langhorne Creek ($n = 3$), McLaren Vale (only 2015, $n = 2$), and the Riverland ($n = 4$), South Australia, at commercial maturity (~21.6 °Brix) during the 2015 and 2016

vintages. The number of samples collected was the same for each site for both vintages unless stated otherwise. Regions were located around the following GPS coordinates: Adelaide Hills, S: 34°56′15 and E: 138°52′36; Eden Valley, S: 34°37′03 and E: 139°02′27; Clare Valley, S: 33°57′16 and E: 138°39′12; Barossa Valley, S: 34°38′26 and E: 138°53′41; Langhorne Creek, S: 35°19′42 and E: 138°58′35; McLaren Vale, S: 35°11′12 and E: 138°33′24; Riverland, S: 34°04′28 and E: 139°52′03. Samples were transported on ice and were carefully stored at −20 °C until required for analysis and were destemmed as necessary while frozen. Quality grades (scale of A–E, where A was the highest grade) provided by the wine companies were allocated to fruit from each vineyard based on wine sensory characteristics after the wines had been finalised.

3.3. Juice Basic Chemical Analysis

Titrable acidity (TA, expressed as g/L of tartaric acid at pH 8.2) and pH were measured using a combined pH meter and autotitrator (CompacTitrator, Crison Instruments, S.A., Allela, Spain) [71]. Total soluble solids of grapes (TSS, expressed as °Brix) was determined using a digital refractometer (Atago pocket, Atago Co., Ltd., Tokyo, Japan).

3.4. Headspace-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) of Free Volatiles from Grapes

A total of 28 compounds were semi-quantified in Chardonnay juice as previously described [37]. All analyses were performed in duplicate.

3.5. Analysis of Hydrolysed Grape Glycosides by Gas Chromatography-Mass Spectrometry (GC-MS)

Glycosidic precursors in grapes were measured using the method described by Hernandez-Orte et al. [19] and the GC-MS instrumentation described previously [37].

3.6. Element Analysis by Inductively Couple Plasma-Optical Emission Spectroscopy (ICP-OES)

Juice samples were analysed for aluminium (Al), arsenic (As), boron (B), cadmium (Cd), calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), phosphorus (P), selenium (Se), sodium (Na), sulfur (S), and zinc (Zn) by ICP-OES after acid digestion. Analyses were performed by the CSIRO Analytical Services Unit according to Wheal et al. [72].

3.7. Amino Acid Analysis by HPLC with Diode Array Detection

Amino acids in grape samples were quantified according to Boss et al. [70] using an AccQ Fluor reagent kit (Waters Corporation, Milford, MA, USA) and solid-phase extraction clean-up step. The glycine peak co-eluted with glutamine and was therefore reported together as "GLN + GLY".

3.8. Climatic and Soil Data

Weather data (maximum and minimum daily temperatures (°C), daily rainfall (mm), and solar exposure (MJ/m^2)) were obtained from the Australian Bureau of Meteorology [73]. Growing degree days (GDD base 10 °C) were calculated for the active vegetation period (1 October–30 April). Soil profiles were obtained from the Australian Soil Resource Information System based on proximity and GPS coordinates [39].

3.9. Data Analysis

XLSTAT (version 2014.05.03, Addinsoft, Paris, France) was used to conduct two-way analysis of variance (ANOVA) on all instrumental measurements to test the effect of vintage and region. Principal component analysis (PCA) was conducted on the means of all significantly different parameters (region as the explaining variable) after normalisation (1/standard deviation) to elucidate the differences

between grapes according to the vintage. The number of principal components (PC) was selected based on their eigenvalues and scree plots [74]. Multiple factor analysis (MFA) was conducted using XLSTAT on significantly different compositional variables (according to region and quality grade). RV cut-off values for inclusion were set at 0.6 [75]. Discriminant analysis (DA) was then performed to classify samples into their respective regions of origin and quality grade. The analysis was performed with XLSTAT on the variables selected by MFA analysis and using stepwise selection of variables according to their discriminating power, as measured by an *F* statistic. Performance of all classifications was evaluated by leave-one out full-cross validation.

4. Conclusions

In accord with other reports, the year of vintage exerted a strong influence on the composition of Chardonnay grapes originating from seven different GI in South Australia, as a result of changes in the weather between 2015 and 2016. Measurement of elements (minerals), amino acids, basic chemical composition, and free and bound volatile compounds, in conjunction with chemometric treatment of the data sets, allowed the discrimination of the different regions using both MFA and DA. Two of the main compounds driving the separation in both years were 2-phenylethanol and benzyl alcohol, the concentrations of which were higher in the cooler sites. These results indicated that preservation of 2-phenylethanol and benzyl alcohol is highly correlated to cooler nights rather than cooler overall mean temperatures.

Mineral composition proved to be a useful parameter to discriminate between grapes from different origins, which agrees well with other studies. Concentrations of Zn, Cu, and Mg contributed to the separation of ADL and EV from other GI in both vintages, indicating possible markers related to origin that can be used consistently, and independent of weather and vintage. ADL berries also contained high levels of Zn, Fe, and Al that were characteristic of the soil in the ADL region. Relationships between soil and berry mineral composition could be further corroborated with renewed soil analysis of each particular site, as soil composition can be modified through viticultural practices.

With respect to bound volatiles, the concentration of 3-oxo-α-ionol was highest in CV during both vintages, consistent with this region having one of the highest levels of solar radiation. Based on the first three factors of the MFA, successful classification models for GI were built using DA. All samples were correctly classified according to their region of origin in 2015 and 2016. Successful classification models were also built according to the allocated grape quality grade. Correct grade classification of 100% of the samples in 2015 was based on the concentrations of Cu, Na, Fe, Al, 1-hexanol, (*E*)-3-hexen-1-ol, 2-ethyl-1-hexanol, pH, TA and hydrolytically-released vitispirane, and 5-MF. In 2016, 100% of the samples were correctly classified based on the levels of Cu, benzyl alcohol, alanine, proline, and hydrolytically-released (*Z*)-linalool oxide, 3-oxo-α-ionol, 2,6-DMP, 5-MF, and hexanoic acid. However, these results need to be confirmed with further studies encompassing a larger number of samples, especially for some regions.

Pearson correlation revealed the effect of climate on the Chardonnay berry quality grades, with significant negative correlations found between GDD and quality grade for both years, and a particularly large negative influence of higher night time temperatures on allocated grape quality. These results provide some insight into the factors to target in order to manipulate fruit quality in warm regions, particularly with respect to diurnal temperature differences. Thus, methods of bunch-zone cooling could be investigated, especially during heatwave events, or more optimal vineyard locations chosen in view of changes to the climate that are envisaged to occur over the coming decades.

Supplementary Materials: The following are available online at http://www.mdpi.com/1420-3049/22/2/218/s1, Figure S1. Boxplots of main compositional variables used to discriminate between Chardonnay grape samples from seven GI in South Australia and quality grades in 2015 and 2016: (A) Fe 2015, (B) Fe 2016, (C) Cu 2015, (D) Cu 2016, (E) Zn 2015, (F) Zn 2016, (G) Al 2015, (H) Al 2016, (I) 2-Phenylethanol 2015, (J) 2-Phenylethanol 2016, (K) Benzyl alcohol 2015, (L) Benzyl alcohol 2016, (M) Total soluble solids 2015, (N) Total soluble solids 2016, (O) Titratable acidity 2015, (P) Titratable acidity 2016, (Q) β-Ionone* 2015, (R) β-Ionone* 2016, (S) 5-MF* 2015,

(T) 5-MF* 2016; Table S1. Harvest date and mean values of pH, total soluble solids (°Brix), and titratable acidity (TA) for Chardonnay grapes harvested from seven GI in South Australia in 2015 and 2016; Table S2. Mean element concentrations (mg/L) in harvest samples of Chardonnay berries collected from seven GI within South Australia in 2015 and 2016; Table S3. Mean concentrations (expressed as mg/L of deuterated internal standard) of free volatile compounds determined in harvest samples of Chardonnay berries collected from seven GI within South Australia in 2015 and 2016; Table S4. Mean content (mg/L) of amino acids in Chardonnay berries at harvest from seven GI in South Australia in 2015 and 2016; Table S5. Mean concentrations of hydrolytically-released volatile compounds determined in harvest samples of Chardonnay berries collected from seven GI within South Australia in 2015 and 2016; Table S6. Weather data for all regions sampled, including mean, minimum, maximum, and highest temperature for the months of January and February, number of days when the temperature exceeded 25 and 30 °C during the January-February period, GDD values, and total rainfall and solar exposure for the months of January and February.

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Conflicts of Interest: The authors declare no competing financial interest.

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Sample Availability: Samples of the compounds are not available.

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Supplementary Information: Exploring the Effects of Geographical Origin on the Chemical Composition and Quality Grading of *Vitis vinifera* L. cv. Chardonnay Grapes

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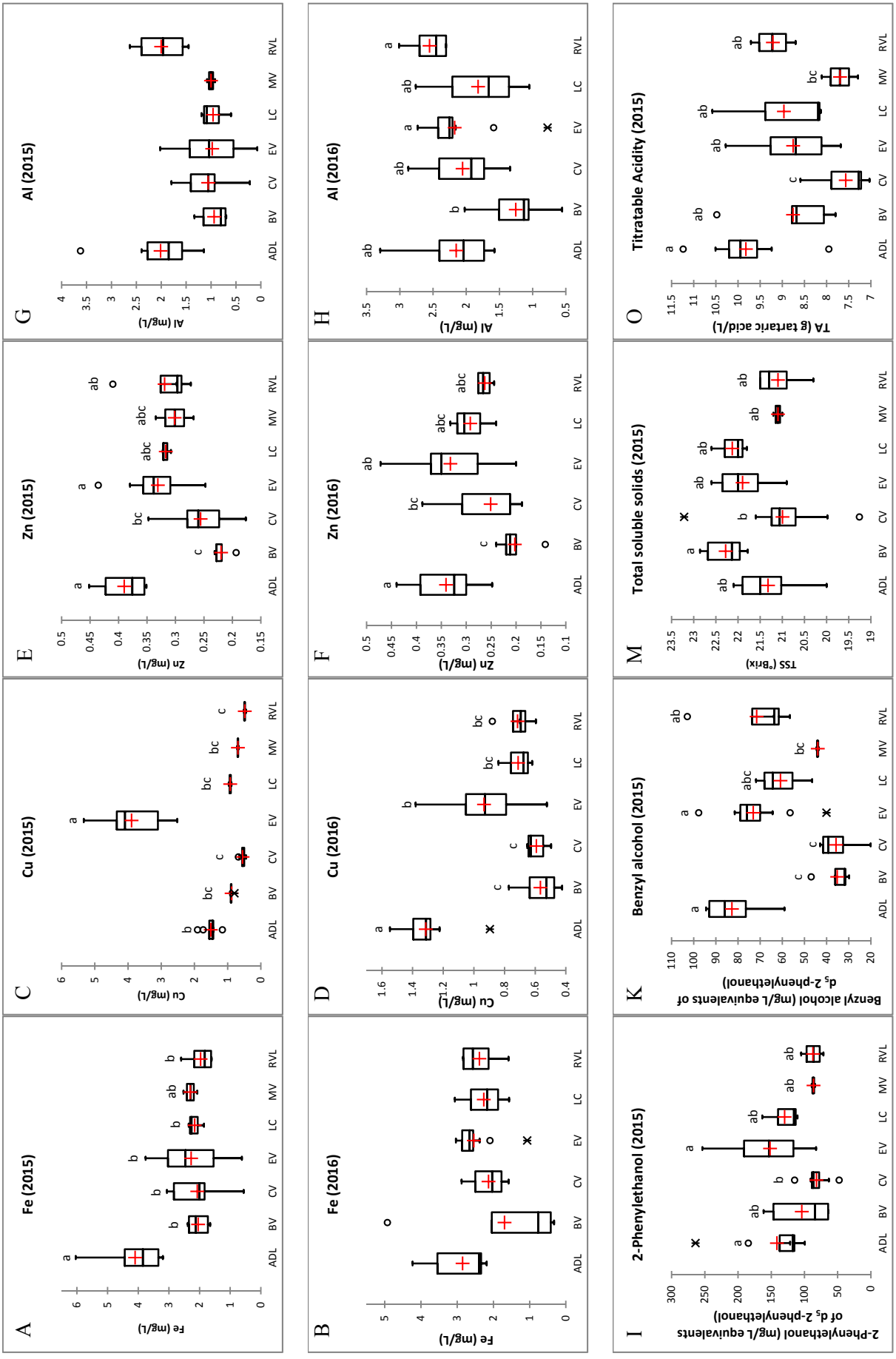

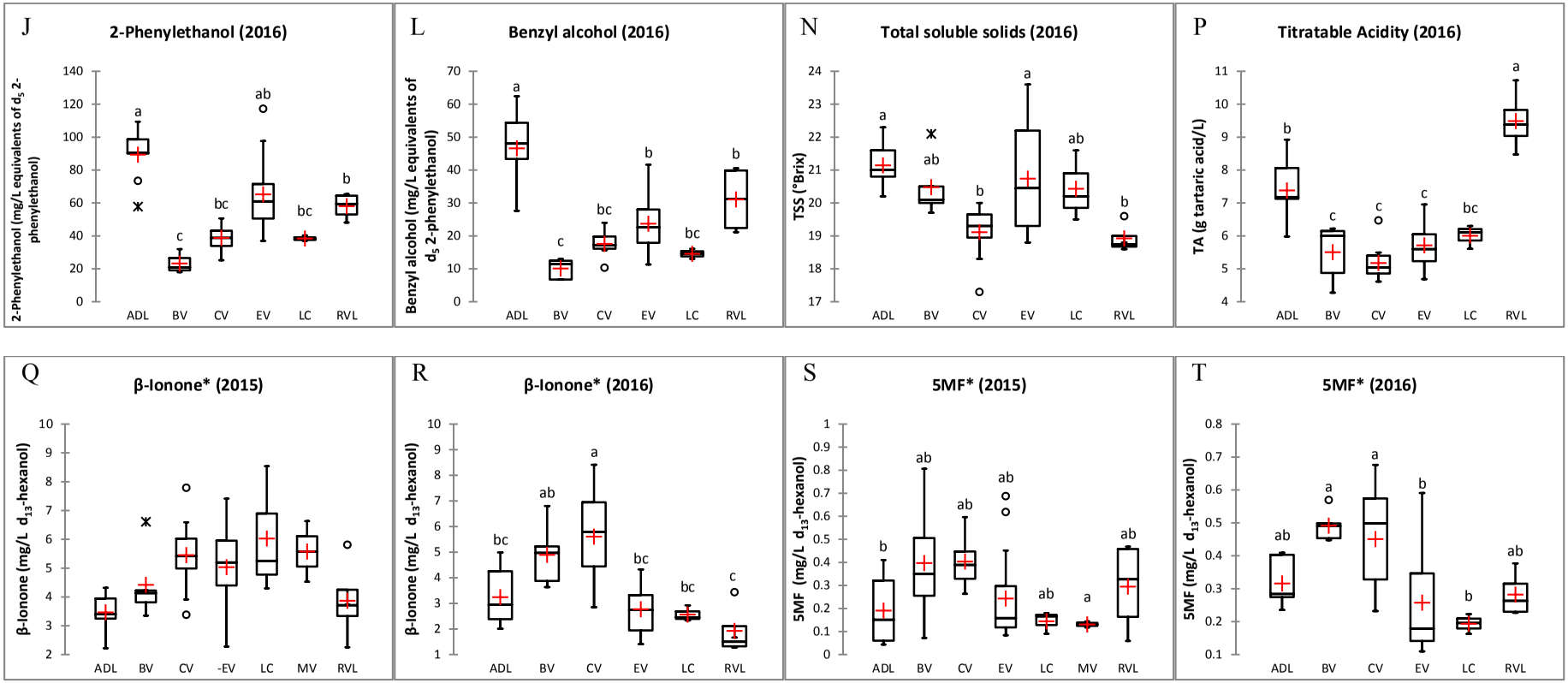

Figure S1. Boxplots of main compositional variables used to discriminate between Chardonnay grape samples from seven GI in South Australia and quality grades in 2015 and 2016: (**A**) Fe 2015, (**B**) Fe 2016, (**C**) Cu 2015, (**D**) Cu 2016, (**E**) Zn 2015, (**F**) Zn 2016, (**G**) Al 2015, (**H**) Al 2016, (**I**) 2-Phenylethanol 2015, (**J**) 2-Phenylethanol 2016, (**K**) Benzyl alcohol 2015, (**L**) Benzyl alcohol 2016, (**M**) Total soluble solids 2015, (**N**) Total soluble solids 2016, (**O**) Titratable acidity 2015, (**P**) Titratable acidity 2016, (**Q**) β-Ionone* 2015, (**R**) β-Ionone* 2016, (**S**) 5MF* 2015, (**T**) 5MF* 2016. ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; MV, McLaren Vale (only 2015); RVL, Riverland. * denotes volatiles detected after hydrolysis of glycosides extracted from juice. 5MF, 5-methylfurfural. [a] For each compound, regions with different letters (a, b, c) above the box have significantly different ($p < 0.05$) mean values according to Tukey's (HSD) pairwise comparison

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Table S1. Harvest date and mean values of pH, total soluble solids (TSS) and titratable acidity (TA) for Chardonnay grapes harvested from seven Geographical Indications in South Australia in 2015 and 2016. [a]

	Harvest		pH				TA(g Tartaric Acid/L)				TSS (°Brix)			
	2015	2016	2015		2016		2015		2016		2015		2016	
ADL [b]	16/2	16/2	3.5	(0.1)b	3.2	(0.1)b	9.8	(1.0)a	7.4	(0.9)b	21.3	(0.8)ab	21.1	(0.7)a
BV	3/2	2/2	3.2	(0.0)c	3.4	(0.1)a	8.8	(0.9)ab	5.5	(0.8)c	22.3	(0.4)a	20.5	(0.8)ab
CV	30/1	4/2	3.3	(0.1)c	3.4	(0.1)a	7.6	(0.5)c	5.2	(0.5)c	21.0	(1.0)b	19.1	(0.8)b
EV	17/2	18/2	3.5	(0.1)b	3.4	(0.1)a	8.8	(0.8)ab	5.7	(0.6)c	21.9	(0.5)ab	20.7	(1.6)a
LC	10/2	15/2	3.8	(0.1)a	3.4	(0.0)a	9.0	(1.4)ab	6.0	(0.3)bc	22.1	(0.4)ab	20.4	(0.9)ab
MV	6/2	- [c]	3.6	(0.1)ab	-	-	7.7	(0.6)bc	-	-	21.1	(0.1)ab	-	-
RVL	23/1	14/1	3.3	(0.0)c	3.1	(0.0)b	9.2	(0.4)ab	9.5	(0.8)a	21.1	(0.5)ab	18.9	(0.4)b

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a column (a, b, c) are significantly different ($p < 0.05$) according to Tukey's (HSD) pairwise comparison. [b] Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$), Eden Valley (EV, $n_{2015} = 15$ and $n_{2016} = 12$), Langhorne Creek (LC, $n = 3$), Riverland (RVL, $n = 4$), McLaren Vale (MV, $n = 2$). [c] -, not sampled.

Table S2. Mean element concentrations (mg/L) in harvest samples of Chardonnay berries collected from seven Geographical Indications within South Australia in 2015 and 2016. [a]

2015	**ADL** [b]		**BV**		**CV**		**EV**		**LC**		**RVL**		**MV**	
Calcium	66	(18)	66	(5.7)	74	(9.8)	55	(12)	74	(12)	63	(12)	77	(1.5)
Potassium	1176	(86)ab	1436	(119)a	1168	(239)ab	1010	(230)b	1503	(60)a	947	(57)b	1525	(50)a
Magnesium	97	(10)a	89	(3.7)ab	91	(12)a	96	(6.5)a	92	(3.1)a	73	(1.9)b	85	(2.8)ab
Sodium	16	(3.5)b	40	(14)a	17	(5.4)b	13	(3.4)b	45	(14)a	56	(32)a	41	(9)a
Sulfur	67	(6.6)a	58	(8.9)ab	49	(7.4)b	59	(6.0)ab	53	(2.5)b	60	(4.2)ab	59	(1.2)ab
Iron	4.1	(1.0)a	2.0	(0.3)b	2.1	(0.9)b	2.3	(0.9)b	2.2	(0.3)b	2.0	(0.5)b	2.3	(0.3)ab
Manganese	0.43	(0.08)	0.37	(0.10)	0.52	(0.24)	0.44	(0.17)	0.33	(0.04)	0.32	(0.07)	0.42	(0.09)
Boron	3.8	(0.51)abc	3.4	(0.4)c	5.2	(1.4)ab	3.6	(0.5)bc	5.3	(0.4)a	5.4	(0.8)a	5.3	(0.4)ab
Copper	1.5	(0.2)b	0.88	(0.05)bc	0.55	(0.07)c	3.9	(0.85)a	0.92	(0.04)bc	0.48	(0.02)c	0.69	(0.03)bc
Zinc	0.39	(0.04)a	0.22	(0.02)c	0.26	(0.05)bc	0.33	(0.05)a	0.32	(0.01)abc	0.32	(0.06)ab	0.30	(0.05)abc
Phosphorus	100	(16)c	116	(14)bc	91	(25)c	101	(26)c	162	(29)ab	175	(7.9)a	176	(10)a
Aluminium	2.0	(0.78)	0.94	(0.29)	1.1	(0.5)	0.98	(0.61)	0.96	(0.32)	2.0	(0.6)	1.0	(0.1)
2016														
Calcium	39	(13)b	55	(20)ab	61	(14)a	51	(7.0)ab	43	(16)ab	65	(5.2)a	- [c]	
Potassium	1298	(166)	1457	(256)	1512	(141)	1367	(220)	1548	(167)	1414	(239)	-	
Magnesium	80	(3.9)ab	80	(6.3)ab	78	(16)b	100	(24)a	65	(9.6)b	70	(9.3)b	-	
Sodium	16	(4.0)ab	52	(53)a	8.8	(4.2)b	15	(19)ab	24	(5.4)ab	14	(7.2)ab	-	
Sulfur	55	(4.9)	56	(7.0)	54	(8.1)	58	(13)	50	(2.5)	52	(9.7)	-	
Iron	2.9	(0.8)	1.7	(1.9)	2.1	(0.5)	2.5	(0.5)	2.3	(0.8)	2.4	(0.6)	-	
Manganese	0.24	(0.07)	0.29	(0.14)	0.31	(0.07)	0.41	(0.21)	0.21	(0.07)	0.32	(0.05)	-	
Boron	3.7	(0.4)bc	4.3	(0.8)abc	5.3	(1.3)a	3.3	(0.9)c	5.1	(0.5)ab	5.0	(1.0)ab	-	
Copper	1.3	(0.2)a	0.57	(0.14)c	0.59	(0.06)c	0.93	(0.26)b	0.71	(0.11)bc	0.72	(0.12)bc	-	
Zinc	0.34	(0.07)a	0.20	(0.04)c	0.25	(0.07)bc	0.33	(0.07)ab	0.29	(0.05)abc	0.26	(0.05)abc	-	
Phosphorus	91	(16)b	129	(22)ab	98	(23)b	128	(27)ab	137	(40)ab	145	(36)a	-	
Aluminium	2.2	(0.6)ab	1.3	(0.5)b	2.1	(0.5)ab	2.2	(0.5)a	1.8	(0.9)ab	2.6	(0.3)a	-	

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a row (a, b, c) are significantly different ($p < 0.05$) according to Tukey's (HSD) pairwise comparison. [b] Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$), Eden Valley (EV, $n_{2015} = 15$ and $n_{2016} = 12$), Langhorne Creek (LC, $n = 3$), Riverland (RVL, $n = 4$), McLaren Vale (MV, $n = 2$). [c] -, not sampled.

Table S3. Mean concentrations (expressed as mg/L of deuterated internal standard) of free volatile compounds determined in harvest samples of Chardonnay berries collected from seven Geographical Indications within South Australia in 2015 and 2016. [a]

2015	ADL [b]		BV		CV		EV		LC		RVL		MV	
Ethyl esters														
Ethyl pentanoate* [c]	15	(7.1)b	16	(6.5)b	13	(2.9)b	18	(9)b	63	(54)a	15	(12)b	85	(42)a
Ethyl hexanoate*	3.9	(1.5)	3.3	(1.5)	3.6	(1.0)	7.2	(4.4)	8.7	(4.6)	2.7	(0.6)	11	(4)
Ethyl octanoate*	1.0	(1.3)	0.68	(0.72)	0.32	(0.15)	4.19	(11)	0.90	(0.08)	0.56	(0.51)	1.89	(1.6)
Diethyl succinate**	0.24	(0.27)ab	0.34	(0.29)ab	0.08	(0.03)b	0.43	(0.42)a	0.22	(0.15)ab	0.13	(0.08)ab	0.20	(0.08)ab
Acetate esters														
Isoamyl acetate*	2.5	(2.2)ab	3.7	(4.5)ab	1.3	(0.4)b	6.2	(6.6)ab	12	(6.1)a	2.1	(1.6)ab	12	(0.0)ab
Hexyl acetate*	3.0	(2.3)	3.0	(1.5)	1.3	(0.5)	2.9	(2.3)	2.8	(1.3)	2.1	(1.4)	3.9	(1.9)
Alcohols														
3-Methyl-1-butanol*	7.5	(2.9)c	6.9	(4.1)c	7.7	(1.9)c	13	(7.4)bc	24	(17)ab	5.5	(0.4)c	43	(23)a
1-Hexanol*	219	(49)a	207	(16)ab	132	(14)b	177	(34)ab	181	(8)ab	206	(81)ab	220	(25)a
(*E*)-3-Hexen-1-ol*	0.68	(0.21)ab	1.0	(0.24)a	0.63	(0.10)b	0.59	(0.16)b	0.70	(0.20)ab	0.77	(0.29)ab	1.0	(0.06)a
(*Z*)-3-Hexen-1-ol*	11	(3.1)a	7.0	(0.7)b	8.8	(2.7)ab	8.0	(1.2)ab	7.3	(0.8)ab	7.2	(2.1)ab	5.5	(0.4)b
(*E*)-2-Hexen-1-ol*	66	(30)abc	103	(15)ab	60	(14)abc	44	(20)c	57	(7)bc	83	(44)abc	108	(2)a
(*Z*)-2-Hexen-2-ol*	1.8	(0.6)ab	2.0	(0.4)ab	1.5	(0.3)ab	1.3	(0.8)b	2.3	(0.7)ab	1.9	(0.3)ab	2.8	(0.0)a
1-Octen-3-ol*	16	(3)	14	(6)	9.4	(1)	17	(7)	11	(3)	18	(10)	12	(2)
2-Ethyl-1-hexanol*	2.0	(0.4)b	3.6	(1.1)ab	3.2	(0.4)ab	2.3	(0.5)b	3.6	(1.9)ab	7.0	(7.0)a	4.7	(0.1)ab
1-Octanol*	1.6	(0.75)a	1.0	(0.47)ab	0.92	(0.25)b	1.3	(0.26)ab	1.5	(0.51)ab	1.7	(1.4)a	1.7	(0.5)a
2-Phenylethanol**	142	(56)a	104	(47)ab	82	(19)b	152	(49)a	130	(29)ab	87	(15)ab	87	(4)ab
Benzyl alcohol**	83	(13)a	35	(7)c	36	(8)c	73	(13)a	61	(13)abc	72	(21)ab	44	(1)bc
Isoprenoid														
Eucalyptol*	0.56	(0.60)	0.19	(0.04)	0.13	(0.07)	0.44	(0.35)	0.19	(0.18)	0.13	(0.11)	0.88	(0.98)
(*Z*)-Linalool oxide*	0.21	(0.19)ab	0.47	(0.45)ab	0.77	(0.41)a	0.28	(0.30)ab	0.10	(0.17)b	0.26	(0.2)ab	0.58	(0.02)ab
Linalool*	1.1	(0.56)c	3.5	(1.7)b	3.1	(2.1)b	0.95	(0.75)c	1.4	(0.08)bc	1.6	(0.96)bc	11	(2.8)a
β-Damascenone**	42	(55)	38	(32)	60	(44)	38	(37)	8.3	(10)	61	(35)	10	(11)
Acids														
Hexanoic acid*	56	(21)a	51	(6)ab	34	(9)b	52	(12)a	38	(12)ab	42	(21)ab	36	(10)ab

Table S3. *Cont.*

2015	ADL [b]	BV	CV	EV	LC	RVL	MV	2015	ADL [b]	BV	CV	EV	LC	RVL
Carbonyls														
Hexanal*	60	(52)	47	(23)	33	(18)	51	(27)	41	(6.3)	62	(74)	36	(13)
(*E*)-2-Hexenal*	97	(43)ab	130	(106)	71	(19)bc	69	(44)bc	44	(5)bc	36	(16)c	57	(5)bc
2-Octanone*	0.41	(0.24)	0.34	(0.10)	0.34	(0.04)	0.48	(0.19)	0.38	(0.16)	0.41	(0.18)	0.52	(0.14)
Nonanal*	3.1	(1.9)a	2.4	(1.2)ab	1.8	(0.5)b	3.3	(1.3)a	2.0	(0.5)ab	2.3	(1.8)ab	2.7	(0.5)ab
Isophorone**	3.3	(1.3)	3.1	(0.9)	2.7	(0.4)	3.2	(0.8)	3.2	(0.1)	2.7	(1.0)	3.5	(0.1)
Benzaldehyde**	4.4	(1.3)ab	3.2	(1.7)ab	2.8	(0.6)b	4.7	(1.6)a	5.9	(2.1)a	3.3	(0.8)ab	5.0	(1.1)a
2016														
Ethyl esters														
Ethyl pentanoate*	5.6	(1.7)	7.8	(2.0)	7.9	(1.5)	7.9	(2.6)	5.8	(2.0)	7.4	(2.4)	- [d]	
Ethyl hexanoate*	1.0	(0.3)	1.9	(0.9)	1.6	(1.0)	1.2	(0.4)	1.6	(1.2)	2.0	(1.5)	-	
Ethyl octanoate*	0.46	(0.23)b	1.1	(0.52)a	0.67	(0.26)ab	0.46	(0.12)b	0.47	(0.18)b	0.68	(0.22)ab	-	
Diethyl succinate**	0.036	(0.016)	0.055	(0.032)	0.050	(0.016)	0.032	(0.010)	0.030	(0.011)	0.050	(0.012)	-	
Acetate esters														
Isoamyl acetate*	3.6	(0.4)	3.6	(1.8)	3.0	(0.7)	3.3	(0.5)	2.9	(0.8)	4.5	(0.8)	-	
Hexyl acetate*	1.5	(0.7)ab	1.9	(0.2)a	1.0	(0.4)b	1.1	(0.4)ab	1.4	(0.5)ab	1.6	(0.7)ab	-	
Alcohols														
3-Methyl-1-butanol*	5.4	(1.6)	1.9	(0.6)	5.9	(4.7)	5.9	(2.7)	4.8	(2.7)	2.3	(1.4)	-	
1-Hexanol*	236	(32)	213	(53)	180	(28)	239	(79)	240	(44)	225	(34)	-	
(*E*)-3-Hexen-1-ol*	0.68	(0.17)	0.93	(0.29)	0.68	(0.15)	0.82	(0.32)	0.68	(0.25)	0.59	(0.13)	-	
(*Z*)-3-Hexen-1-ol*	14	(3)ab	12	(3)ab	18	(6)ab	25	(12)a	10	(2)b	12	(2)ab	-	
(*E*)-2-Hexen-1-ol*	107	(21)	169	(45)	132	(22)	167	(76)	119	(35)	145	(33)	-	
(*Z*)-2-Hexen-2-ol*	1.8	(0.6)a	2.5	(0.7)a	1.9	(0.4)a	2.5	(1.4)a	1.5	(0.4)a	1.6	(0.6)a	-	
1-Octen-3-ol*	10	(3.2)	7.5	(3.0)	7.9	(3.1)	9.2	(3.8)	6.5	(1.8)	7.0	(2.1)	-	
2-Ethyl-1-hexanol*	2.4	(0.4)b	4.9	(1.6)a	4.5	(1.6)a	2.9	(0.4)b	2.8	(0.4)b	5.4	(2.0)b	-	
1-Octanol*	0.98	(0.36)	1.0	(0.2)	1.0	(0.2)	1.0	(0.3)	0.66	(0.20)	0.91	(0.16)	-	
2-Phenylethanol**	89	(15)a	23	(6)c	39	(7)bc	65	(24)ab	38	(2)bc	58	(8)b	-	
Benzyl alcohol**	47	(11)a	10	(3)c	18	(4)bc	24	(9)b	15	(2)bc	31	(10)b	-	

Table S3. *Cont.*

2016	ADL [b]	BV	CV	EV	LC	RVL	MV	2015	ADL [b]	BV	CV	EV	LC	RVL
Isoprenoid														
Eucalyptol*	0.065	(0.026)	0.49	(0.9)	0.19	(0.2)	0.095	(0.034)	0.041	(0.034)	0.19	(0.08)		-
(*Z*)-Linalool oxide*	0.26	(0.10)ab	0.33	(0.34)ab	0.43	(0.17)a	0.21	(0.13)b	0.06	(0.06)b	0.15	(0.02)b		-
Linalool*	0.91	(0.37)b	1.8	(1.5)ab	3.0	(1.1)a	1.4	(1.2)b	0.88	(0.4)b	0.65	(0.14)b		-
β-Damascenone**	22	(24)	18	(28)	25	(25)	26	(20)	3	(3)	26	(21)		-
Acids														
Hexanoic acid*	62	(14)a	56	(13)ab	43	(17)ab	48	(15)ab	52	(10)ab	35	(6)b		-
Carbonyls														
Hexanal*	68	(51)	14	(6)	65	(64)	68	(32)	68	(64)	29	(13)		-
(*E*)-2-Hexenal*	149	(34)	136	(60)	186	(91)	195	(62)	159	(39)	110	(30)		-
2-Octanone*	0.46	(0.16)ab	0.70	(0.05)a	0.57	(0.16)ab	0.41	(0.09)b	0.45	(0.23)ab	0.67	(0.10)a		-
Nonanal*	1.6	(0.5)b	3.3	(0.7)a	2.3	(0.8)ab	2.1	(0.9)b	1.4	(0.4)b	1.4	(0.2)b		-
Isophorone**	2.2	(0.4)	2.2	(0.3)	2.8	(0.9)	3.3	(1.0)	2.2	(0.6)	2.2	(0.4)		-
Benzaldehyde**	2.5	(0.5)	2.2	(0.2)	2.3	(0.4)	2.0	(0.4)	2.0	(0.2)	2.2	(0.1)		-

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a row (a, b, c) are significantly different ($p < 0.05$) according to Tukey's (HSD) pairwise comparison. [b] Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$), Eden Valley (EV, $n_{2015} = 15$ and $n_{2016} = 12$), Langhorne Creek (LC, $n = 3$), Riverland (RVL, $n = 4$), McLaren Vale (MV, $n = 2$). [c] Values expressed as mg/L equivalents of *d_{13}-1-hexanol or **d_5-2-phenylethanol. [d] -, not sampled.

Table S4. Mean content (mg/L) of amino acids in Chardonnay berries at harvest from seven Geographical Indications in South Australia in 2015 and 2016. [a]

2015	**ADL** [b]		**BV**		**CV**		**EV**		**LC**		**RVL**		**MV**	
Aspartic acid	26	(8.5)a	29	(14)a	12	(3.9)b	25	(6.2)a	19	(10)ab	25	(6.7)ab	9.6	(2.8)b
Asparagine	26	(11)	35	(23)	16	(9.2)	29	(6.8)	41	(24)	28	(14)	20	(8.9)
Serine	113	(23)ab	115	(46)ab	72	(15)c	133	(23)a	84	(16)bc	88	(4.7)bc	51	(9.2)c
Glutamic acid	59	(19)a	60	(27)a	26	(9.4)b	59	(13)a	52	(10)ab	59	(17)a	36	(7.6)ab
Histidine	60	(20)ab	62	(29)ab	35	(11)b	72	(17)a	56	(5.6)ab	62	(22)ab	33	(10)b
GLN + GLY	267	(80)a	265	(213)a	99	(55)b	335	(66)a	161	(104)ab	265	(49)ab	53	(3.4)b
Arginine	370	(111)ab	457	(169)ab	301	(136)b	485	(61)a	454	(52)ab	456	(66)ab	225	(88)b
Threonine	87	(21)ab	100	(30)ab	78	(15)b	118	(26)a	96	(3.4)ab	81	(3.8)b	64	(19)b
β-Alanine	22	(2.1)bc	24	(2.7)ab	20	(2.5)c	24	(2.5)b	29	(0.8)a	25	(1.0)ab	24	(2.3)ab
Alanine	264	(29)ab	227	(72)b	148	(55)c	307	(28)a	201	(27)bc	253	(24)ab	103	(10)c
Proline	631	(157)b	749	(154)ab	454	(164)b	749	(127)ab	959	(111)a	794	(130)ab	803	(209)ab
γ-Aminobutyric acid	217	(30)ab	223	(41)ab	168	(31)b	239	(40)a	256	(23)a	226	(33)ab	181	(64)ab
Tyrosine	7.7	(2.6)ab	9.8	(4.1)a	4.1	(1.1)b	6.4	(2.7)ab	6.3	(1.2)ab	5.8	(1.2)ab	5.0	(1.0)ab
Valine	38	(16)ab	32	(11)ab	25	(6.6)b	47	(13)a	39	(4.3)ab	32	(0.7)ab	23	(7.3)ab
Methionine	6.6	(3.6)	5.2	(2.2)	3.4	(0.6)	7.1	(4.0)	3.7	(0.5)	4.3	(0.8)	4.2	(0.3)
Lysine	8.3	(2.9)	10	(3.1)	8.2	(3.0)	9.5	(2.4)	10	(2.1)	11	(1.5)	6.4	(2.7)
Isoleucine	17	(12)	15	(5.6)	14	(4.3)	28	(19)	20	(3.2)	13	(0.5)	12	(4.9)
Leucine	24	(12)	26	(10)	19	(4.5)	35	(19)	34	(5.4)	22	(0.9)	19	(6.5)
Phenylalanine	29	(13)	25	(8.1)	20	(6.2)	36	(18)	33	(4.6)	25	(3.1)	23	(9.1)
2016														
Aspartic acid	65	(13)ab	28	(16)c	29	(9.4)c	40	(13)c	42	(10)bc	84	(14)a	- [c]	
Asparagine	15	(3.6)	11	(2.8)	17	(16)	18	(11)	27	(6.6)	15	(6.4)	-	
Serine	127	(15)a	105	(41)ab	84	(26)b	120	(41)ab	80	(5.4)b	88	(9.3)ab	-	
Glutamic acid	183	(54)a	141	(34)ab	122	(12)b	161	(37)ab	151	(20)ab	156	(11)ab	-	
Histidine	63	(13)	70	(29)	65	(25)	74	(35)	47	(6.3)	51	(12)	-	
GLN + GLY	246	(107)a	99	(51)b	117	(77)b	158	(93)ab	98	(28)b	218	(105)ab	-	
Arginine	510	(130)	412	(186)	369	(183)	468	(225)	384	(51)	344	(150)	-	
Threonine	116	(8.6)	105	(27)	95	(30)	118	(36)	90	(2.3)	75	(6.3)	-	
β-Alanine	20	(1.9)	18	(1.1)	19	(1.6)	20	(1.6)	20	(1.7)	18	(1.6)	-	
Alanine	249	(48)a	120	(65)c	130	(68)c	150	(51)bc	136	(31)bc	231	(34)ab	-	
Proline	784	(130)a	445	(161)b	364	(160)b	512	(203)b	657	(119)ab	330	(82)b	-	
γ-Aminobutyric acid	109	(62)	104	(38)	102	(38)	93	(24)	96	(11)	58	(12)	-	
Tyrosine	17	(1.8)	18	(6.4)	16	(6.0)	21	(8.1)	13	(1.5)	14	(2.5)	-	
Valine	31	(2.7)	25	(11)	28	(16)	32	(10)	25	(1.1)	22	(1.6)	-	

Table S4. *Cont.*

2016	ADL [b]	BV	CV	EV	LC	RVL	MV	2015	ADL [b]	BV	CV	EV	LC	RVL
Methionine	9.4	(3.9)b	18	(4.1)a	13	(6.6)ab	8.2	(3.0)b	7.0	(4.6)b	20	(1.8)a		-
Lysine	8.0	(1.1)	9.1	(3.5)	8.1	(2.7)	9.0	(3.1)	8.3	(1.1)	6.6	(1.6)		-
Isoleucine	15	(1.9)	14	(5.9)	18	(13)	20	(8.6)	14	(0.7)	12	(1.3)		-
Leucine	23	(2.8)	20	(7.7)	22	(13)	25	(8.6)	21	(0.8)	13	(1.9)		-
Phenylalanine	24	(4.0)	23	(6.0)	24	(11)	32	(10)	27	(4.6)	18	(1.6)		-

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a row (a, b, c) are significantly different ($p < 0.05$) according to Tukey's (HSD) pairwise comparison. [b] Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$), Eden Valley (EV, $n_{2015} = 15$ and $n_{2016} = 12$), Langhorne Creek (LC, $n = 3$), Riverland (RVL, $n = 4$), McLaren Vale (MV, $n = 2$). GLN + GLY, Glutamine and glycine. [c] -, not sampled.

Table S5. Mean concentrations (expressed as mg/L of deuterated internal standard) of hydrolytically-released volatile compounds determined in harvest samples of Chardonnay berries collected from seven Geographical Indications within South Australia in 2015 and 2016. [a]

2015	ADL [b]		BV		CV		EV		LC		RVL		MV	
Alcohols														
3-Methyl-1-butanol	0.52	(0.13)	0.48	(0.23)	0.53	(0.11)	0.62	(0.26)	0.74	(0.21)	0.46	(0.21)	0.68	(0.16)
Benzyl Alcohol** [c]	1.8	(0.44)ab	2.6	(1.9)ab	1.3	(0.36)b	2.9	(1.9)ab	4.0	(1.8)ab	2.1	(1.1)ab	4.7	(0.66)a
2-Phenylethanol**	2.0	(0.5)c	3.0	(2.1)bc	1.5	(0.4)c	3.1	(2.0)bc	5.4	(2.3)ab	1.3	(0.2)c	6.9	(1.6)a
Isoprenoids														
(*E*)-Linalool oxide*	1.4	(0.35)	1.5	(0.87)	1.7	(0.37)	1.8	(0.88)	2.1	(0.68)	0.96	(0.66)	2.5	(1.2)
(*Z*)-Linalool oxide*	0.91	(0.26)	0.92	(0.58)	1.0	(0.26)	1.3	(0.59)	1.4	(0.42)	0.67	(0.42)	1.6	(0.79)
α-Terpinene*	0.062	(0.020)	0.020	(0.020)	0.042	(0.017)	0.047	(0.029)	0.047	(0.038)	0.041	(0.047)	0.052	(0.013)
Vitispirane (sum of isomers)*	0.20	(0.12)	0.089	(0.09)	0.31	(0.16)	0.27	(0.17)	0.43	(0.29)	0.13	(0.20)	0.40	(0.03)
Linalool*	0.066	(0.031)	0.042	(0.004)	0.068	(0.025)	0.066	(0.030)	0.057	(0.010)	0.031	(0.021)	0.055	(0.004)
α-Terpineol*	0.48	(0.14)b	0.49	(0.29)b	0.83	(0.26)ab	0.52	(0.18)b	0.61	(0.04)b	0.47	(0.37)b	1.4	(0.64)a
β-Damascenone*	0.81	(0.38)	0.54	(0.42)	0.95	(0.45)	1.2	(0.72)	1.6	(0.36)	0.64	(0.65)	1.8	(0.81)
TDN*	0.36	(0.22)c	0.43	(0.40)bc	0.21	(0.13)c	0.65	(0.53)bc	1.4	(0.89)ab	0.35	(0.51)c	2.2	(1.1)a
β-Ionone*	3.5	(0.67)	4.4	(1.3)	5.5	(1.3)	5.0	(1.5)	6.0	(2.2)	3.9	(1.5)	5.6	(1.5)
2,6-Dimethyl-7-octene-2,6-diol*	0.51	(0.17)ab	0.40	(0.11)b	0.81	(0.36)a	0.44	(0.17)b	0.41	(0.22)b	0.57	(0.20)ab	0.46	(0.02)ab
3-Oxo-α-ionol*	7.8	(1.1)b	11	(3.6)ab	12	(2.5)a	10	(2.3)ab	7.2	(1.2)b	8.9	(0.7)ab	6.6	(1.2)b

Table S5. *Cont.*

2015	ADL [b]	BV	CV	EV	LC	RVL	MV	2015	ADL [b]	BV	CV	EV	LC	RVL
Carbonyls														
Hexanal*	0.83	(0.31)	0.74	(0.57)	0.61	(0.30)	1.3	(0.83)	1.4	(0.55)	0.56	(0.20)	0.75	(0.14)
(*E*)-2-Hexenal*	1.0	(0.62)	0.70	(0.70)	0.72	(0.28)	0.98	(0.43)	1.3	(0.44)	0.61	(0.44)	0.80	(0.06)
5-Methyl furfural*	0.28	(0.08)b	0.58	(0.30)ab	0.40	(0.10)ab	0.46	(0.16)ab	0.58	(0.15)ab	0.46	(0.18)ab	0.63	(0.21)a
Phenylacetaldehyde**	0.64	(0.33)	0.40	(0.41)	0.37	(0.11)	0.86	(0.52)	0.90	(0.24)	0.26	(0.15)	0.48	(0.06)
Benzaldehyde*	0.84	(0.43)	0.89	(0.61)	0.52	(0.14)	1.1	(0.70)	1.5	(0.57)	0.66	(0.44)	1.4	(0.0)
Acetovanillone*	0.85	(0.20)	1.1	(0.64)	1.2	(0.43)	1.3	(0.54)	1.2	(0.35)	0.82	(0.14)	1.4	(0.56)
Acids														
Hexanoic acid*	24	(9.1)	15	(2.6)	24	(6.0)	18	(6.6)	12	(1.8)	21	(3.3)	9	(4.9)
Octanoic Acid*	1.2	(0.47)	0.98	(0.58)	1.0	(0.20)	1.7	(1.0)	1.5	(0.59)	1.0	(0.46)	1.4	(0.44)
Hexadecanoic acid*	4.4	(2.7)	4.9	(3.6)	3.3	(1.2)	5.6	(4.5)	5.4	(2.2)	3.1	(1.7)	6.1	(2.1)
Volatile phenols														
Guaiacol*	0.078	(0.026)	0.064	(0.018)	0.066	(0.016)	0.088	(0.053)	0.13	(0.026)	0.058	(0.016)	0.14	(0.086)
4-Vinylguaiacol*	7.8	(2.6)b	11	(5.5)ab	11	(4.9)ab	9.3	(3.9)b	11	(4.2)ab	10	(1.0)ab	20	(8.0)a
4-Allyl-2,6-dimethoxyphenol**	0.56	(0.19)	0.73	(0.27)	0.48	(0.21)	0.75	(0.39)	0.72	(0.16)	0.65	(0.17)	0.62	(0.10)
Vanillin**	0.44	(0.15)	0.56	(0.26)	0.49	(0.10)	0.54	(0.23)	0.58	(0.15)	0.33	(0.07)	0.63	(0.04)
Methyl vanillate**	0.87	(0.14)ab	0.82	(0.52)ab	0.48	(0.17)b	1.1	(0.49)a	0.95	(0.25)ab	0.77	(0.14)ab	0.50	(0.19)ab
2,6-Dimethoxyphenol*	0.12	(0.05)	0.13	(0.07)	0.093	(0.030)	0.14	(0.07)	0.13	(0.02)	0.11	(0.02)	0.17	(0.08)
2016														
Alcohols														
3-Methyl-1-butanol	0.16	(0.01)a	0.15	(0.03)ab	0.14	(0.04)ab	0.12	(0.03)b	0.11	(0.01)b	0.13	(0.04)ab		- [d]
Benzyl Alcohol**	2.1	(1.9)	1.6	(0.07)	2.3	(1.8)	1.4	(0.46)	1.5	(0.04)	1.5	(0.26)		-
2-Phenylethanol**	1.4	(0.82)	1.1	(0.18)	1.3	(0.81)	0.94	(0.44)	1.1	(0.07)	0.86	(0.17)		-
Isoprenoids														
(*E*)-Linalool oxide*	0.72	(0.27)a	0.52	(0.11)ab	0.56	(0.17)ab	0.37	(0.20)b	0.30	(0.02)b	0.39	(0.33)ab		-
(*Z*)-Linalool oxide*	0.51	(0.21)a	0.40	(0.07)ab	0.47	(0.12)a	0.26	(0.14)b	0.23	(0.03)b	0.31	(0.21)ab		-
α-Terpinene*	0.0071	(0.0026)a	0.0044	(0.0019)ab	0.0046	(0.0022)ab	0.0037	(0.0013)b	0.0056	(0.0058)ab	0.0039	(0.0020)ab		-
Vitispirane (sum of isomers)*	0.033	(0.011)b	0.058	(0.016)a	0.045	(0.019)ab	0.030	(0.009)b	0.019	(0.005)b	0.024	(0.017)b		-
Linalool*	0.015	(0.005)ab	0.021	(0.009)a	0.014	(0.008)ab	0.0086	(0.0052)b	0.0060	(0.0007)b	0.012	(0.008)ab		-
α-Terpineol*	0.26	(0.06)ab	0.38	(0.13)a	0.29	(0.09)ab	0.18	(0.10)b	0.16	(0.05)b	0.25	(0.17)ab		-
β-Damascenone*	0.24	(0.04)ab	0.27	(0.07)a	0.25	(0.08)a	0.16	(0.06)b	0.21	(0.12)ab	0.15	(0.03)b		-

Table S5. *Cont.*

2016	ADL[b]	BV	CV	EV	LC	RVL	MV	2015	ADL[b]	BV	CV	EV	LC	RVL
Isoprenoids														
TDN*	0.23	(0.15)	0.35	(0.21)	0.22	(0.13)	0.15	(0.19)	0.059	(0.011)	0.15	(0.13)		-
β-Ionone*	3.2	(1.2)bc	4.9	(1.3)ab	5.6	(2.0)a	2.8	(1.0)bc	2.6	(0.3)bc	1.9	(1.0)c		-
2,6-Dimethyl-7-octene-2,6-diol*	0.51	(0.10)ab	0.70	(0.19)a	0.53	(0.29)ab	0.29	(0.14)b	0.19	(0.03)b	0.42	(0.26)ab		-
3-Oxo-α-ionol*	8.0	(5.3)ab	6.2	(0.66)ab	12	(6.8)a	5.7	(1.3)b	7.4	(0.33)ab	5.0	(0.97)b		-
Carbonyls														
Hexanal*	0.51	(0.21)a	0.31	(0.08)ab	0.39	(0.27)ab	0.25	(0.14)b	0.31	(0.07)ab	0.34	(0.10)ab		-
(*E*)-2-Hexenal*	0.49	(0.17)a	0.43	(0.13)ab	0.38	(0.24)b	0.26	(0.11)b	0.20	(0.02)b	0.38	(0.21)ab		-
5-Methyl furfural*	0.32	(0.07)ab	0.49	(0.05)a	0.45	(0.16)a	0.26	(0.17)b	0.19	(0.03)b	0.28	(0.07)ab		-
Phenylacetaldehyde**	0.16	(0.03)	0.16	(0.07)	0.16	(0.09)	0.12	(0.07)	0.07	(0.01)	0.12	(0.11)		-
Benzaldehyde*	0.20	(0.04)	0.25	(0.08)	0.19	(0.06)	0.16	(0.05)	0.14	(0.08)	0.17	(0.05)		-
Acetovanillone*	0.76	(0.48)	0.50	(0.09)	0.87	(0.37)	0.58	(0.13)	0.68	(0.08)	0.50	(0.04)		-
Acids														
Hexanoic acid*	4.9	(1.7)a	2.1	(0.4)b	2.4	(1.1)b	1.9	(1.1)b	1.5	(0.2)b	2.2	(2.2)b		-
Octanoic Acid*	0.54	(0.16)a	0.32	(0.08)ab	0.29	(0.14)b	0.23	(0.14)b	0.10	(0.01)b	0.36	(0.20)ab		-
Hexadecanoic acid*	3.3	(2.1)	1.6	(0.4)	2.3	(1.6)	1.7	(0.8)	1.4	(0.7)	1.9	(0.4)		-
Volatile phenols														
Guaiacol*	0.11	(0.04)a	0.067	(0.010)ab	0.086	(0.040)ab	0.055	(0.028)b	0.054	(0.005)b	0.076	(0.034)ab		-
4-Vinylguaiacol*	25	(9.8)a	14	(2.2)ab	16	(11)ab	10	(9.3)b	6.2	(0.50)b	14	(9.0)ab		-
4-Allyl-2,6-dimethoxyphenol**	0.29	(0.07)ab	0.24	(0.05)ab	0.39	(0.31)a	0.15	(0.10)b	0.23	(0.04)ab	0.24	(0.07)ab		-
Vanillin**	0.21	(0.06)	0.21	(0.03)	0.21	(0.09)	0.11	(0.05)	0.22	(0.06)	0.18	(0.06)		-
Methyl vanillate**	0.50	(0.21)a	0.14	(0.02)b	0.29	(0.19)b	0.18	(0.10)b	0.35	(0.01)ab	0.28	(0.10)b		-
2,6-Dimethoxyphenol*	0.31	(0.15)a	0.10	(0.02)b	0.15	(0.09)b	0.10	(0.08)b	0.066	(0.005)b	0.17	(0.11)ab		-

[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a row (a, b, c) are significantly different ($p < 0.05$) according to Tukey's (HSD) pairwise comparison. [b] Adelaide Hills (ADL, $n = 8$), Barossa Valley (BV, $n = 5$), Clare Valley (CV, $n = 9$) and Eden Valley (EV, $n_{2015} = 15$ and $n_{2016} = 12$), Langhorne Creek (LC, $n = 3$), Riverland (RVL, $n = 4$), McLaren Vale (MV, $n = 2$). [c] Values expressed as mg/L equivalents of *d_{13}-1-hexanol or **d_5-2-phenylethanol. [d] -, not sampled.

Table S6. Weather data for all regions sampled, including mean, minimum, maximum, and highest temperature for the months of January and February, number of days when temperature exceeded 25 and 30 °C during the January-February period, GDD [a] values, and total rainfall and solar exposure for the months of January and February.

	Mean T (°C)		Highest T (°C)		No. of Days (°C)		GDD [a]	Rainfall (mm)		Solar Exposure (MJ/m²)	
	min/max	min/max									
	Jan	Feb	Jan	Feb	>25	>30		Jan	Feb	Jan	Feb
2015											
ADL [b]	12.5/25.8	13.7/29.8	41.2	39.6	37	20	1639	52.8	0.0	23.7	23.7
BV	15.9/28.7	16.2/33.4	43.6	41.2	50	28	2074	55.4	3.4	24.4	24.2
CV	14.7/27.4	15.3/32.3	40.8	39.5	45	26	1884	62.8	0.0	23.7	25.1
EV	13.2/25.0	14.7/29.6	40.0	37.2	35	23	1576	89.2	1.0	23.6	24.2
LC	14.9/26.5	15.0/29.2	43.5	39.4	32	18	1839	66.2	0.8	23.5	22.3
MV	17.9/27.0	17.7/30.4	42.3	40.0	39	22	2086	33.8	0.2	24.0	23.9
RVL	15.7/31.4	15.6/35.2	45.1	44.1	57	40	2307	31.6	0.0	25.5	25.4
2016											
ADL	13.9/29.1	13.3/26.6	38.7	36.1	41	18	1998	35.5	37.6	25.6	23.5
BV	16.3/31.8	13.1/29.6	39.9	37.2	53	34	2406	30.8	5.4	25.5	24.6
CV	15.7/30.2	13.8/29.5	38.1	38.2	47	29	2227	35.8	18.0	24.6	24.8
EV	15.2/28.0	13.2/26.8	36.6	36.4	39	22	1985	30.8	27.8	25.7	24.6
LC	15.3/28.2	14.6/26.0	37.2	37.0	34	16	2101	17.0	19.6	25.1	22.0
MV	17.9/28.9	16.3/26.6	37.2	37.5	35	19	2388	29.2	44.6	26.3	22.9
RVL	16.4/33.9	14.7/33.6	43.3	44.6	56	43	2670	21.0	0.0	25.8	25.4

[a] Growing degree days base 10 °C. [b] ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; RVL, Riverland; MV, McLaren Vale.

CHAPTER 6

Classification of Chardonnay grapes according to geographic indication and quality grade and assessment of maturity through mid-infrared spectroscopy

6.1 Introduction

The final quality of a wine depends to a large extent on the quality of the grapes used to produce it. Quality is linked to grape chemical composition, which influences the final sensory profile of the ensuing wine through differences in the concentrations of free and bound volatiles, amino acids, total soluble solids (TSS), pH, titratable acidity (TA), essential yeast nutrients, phenolics, and more. (Cadot, Caillé, Samson, Barbeau, & Cheynier, 2012; Dennis, Keyzers, Kalua, Maffei, Nicholson, & Boss, 2012; Gambetta, Bastian, Cozzolino, & Jeffery, 2014; Garde-Cerdán & Ancín-Azpilicueta, 2008; Hernández-Orte, Cacho, & Ferreira, 2002; Keyzers & Boss, 2010). As such, objective criteria to assess the quality of grapes is required to: improve management of viticultural practices in the vineyard in regards to desired profile; optimise decisions regarding harvest timing; more effectively stream fruit to the desired wine style; tailor winemaking operations to the chemical composition of the grapes. However, the development of objective criteria and tools to assess the quality of grapes continues to pose a challenge to the industry.

So far, the most frequently used criteria to determine the quality of Chardonnay berries in Australia are historical parcel records, berry tasting, basic chemical measurements (TSS, TA and pH) and yield in tonnes/ha (Gambetta, Bastian, & Jeffery, 2016; Longbottom, Simon, Krstic, & Johnson, 2013). Historical parcel records contain useful information about the performance and past quality of the block or vineyard over time but do not take into account the particular conditions of the vintage in question, and can only be revised once the fruit has been processed. Furthermore, using records is not applicable to new vineyards, new varieties or new contract growers. Other quality indicators are necessary, such as berry tasting, which is a useful tool to determine grape quality that has become widely adopted in the Chardonnay vineyard (Gambetta et al., 2016; Olarte Mantilla, Collins, Iland, Johnson, & Bastian, 2012). This practise usually focuses on describing the level of technological maturity of the berry, intensity of pulp aroma, and skin and seed maturity (Olarte Mantilla et al., 2012). Disadvantageously, it can be extremely time-consuming and subjective, with the results varying depending on who is performing the tasting and how it is being conducted.

TSS, TA and pH are valuable parameters that are easy to measure and have traditionally correlated well with grape maturity. As berries ripen, sugar accumulates and TSS rises, which may coincide with desirable increases in flavour potential, however, these phenomena are not mutually dependent (Robinson, Boss, Solomon, Trengove, Heymann, & Ebeler, 2014). Another criteria used frequently to determine quality, is crop yield. Lower yields have been linked to higher quality wines as they modify the balance of the vine and therefore affect accumulation of grape metabolites. However, the level of yield that is suited to each variety/wine style/climate is poorly understood, therefore no threshold, or "ideal" yield, exists. Relationships between sensory attributes and different treatments to control yield have led to mixed results, leading to the conclusion that the method used to control yield might be more important than yield itself, making it an ill-suited criteria to evaluate grape and wine quality, at least on its own (Matthews & Nuzzo, 2007).

Environmental factors such as soil type, climate, solar exposure, and altitude, together, and amongst other factors, known as "*terroir*", have been shown to modulate grape composition and grape

quality (Alessandrini, Gaiotti, Belfiore, Matarese, D'onofrio, & Tomasi, 2016; Pereira, Gaudillere, van Leeuwen, Hilbert, Maucourt, Deborde, et al., 2006). Climate and soil affect grape composition through changes in berry temperature, shading, vine phenology and water status, mineral status, etc. These in turn impact enzyme activity and gene expression within the fruit (Robinson et al., 2014). Dal Santo, Tornielli, Zenoni, Fasoli, Farina, Anesi, et al. (2013) demonstrated that 18% of the genes in grapevines are impacted by weather, which affects the formation of aroma compounds and polyphenols, accumulation of sugar, and catabolism of acids during ripening, and thereby influences grape quality. Consequently, certain vineyard locations are recognised as being better suited for a range of wine grape varieties. Indeed, geographical origin is commonly used as a proxy for quality both by producers and consumers, who pay more per ton of grapes or bottle of wine from selected regions (Anderson, 2016; Lockshin, Jarvis, d'Hauteville, & Perrouty, 2006).

Vintage is a hectic period when a large number of samples need to be processed in a small window of time. Determination of grape composition is crucial to make decisions that range from when to harvest to choices about additives (DAP, tartaric acid, SO_2, enzymes, etc.) to employ during winemaking. Additionally, given the size and available resources of most wineries, any analytical measurements undertaken need to be quick, require little to no sample processing and have a low cost per sample (Cozzolino, Cynkar, Shah, & Smith, 2011; Cozzolino, Parker, Dambergs, Herderich, & Gishen, 2006). In this respect, infrared spectroscopy techniques have emerged as an attractive alternative, with increasingly portable devices available at more affordable prices (Cozzolino et al., 2011).

Since its first use in the wine realm by Kaffka and Norris (1976), many researchers have worked to develop infrared applications for grape and wine analysis. The most widespread applications to date include the determination of alcohol content in wine using near infrared (NIR)-based analysers, and the routine analysis of volatile acidity, pH, malic acid, tartaric acid, lactic acid, glucose and fructose, acetic acid and polyphenols in wine and juice by fourier transform mid-infrared (FT-MIR) spectroscopy (International Organisation of Vine and Wine (OIV), 2010). The coupling of infrared spectral analysis to multivariate techniques affords the possibility to not only replace routine analysis of single wine components, but to perform qualitative analysis of samples, allowing their discrimination and/or classification and the creation of characteristic fingerprints (Cozzolino, Holdstock, Dambergs, Cynkar, & Smith, 2009). More recently, both NIR and MIR have been used to discriminate between wines made with different cultivars (Arana, Jarén, & Arazuri, 2005; Bevin, Dambergs, Fergusson, & Cozzolino, 2008; Cozzolino, Cynkar, Shah, & Smith, 2012; Edelmann, Diewok, Schuster, & Lendl, 2001) and origins (Liu, Cozzolino, Cynkar, Gishen, & Colby, 2006; Riovanto, Cynkar, Berzaghi, & Cozzolino, 2011).

The aim of this study was to conduct a preliminary evaluation of the capacity of ATR-MIR, in combination with chemometric techniques, to discriminate between juices from different regions of South Australia and to predict the quality grade of the fruit obtained from each region. The potential of ATR-MIR to measure compositional parameters of grape juice that are currently used by the wine producers, such as TSS and TA, was also evaluated.

6.2 Materials and Methods

6.2.1 Samples

Grape bunches were manually collected at commercial maturity from commercial South Australian vineyards located in the Geographical Indications (GI) of Adelaide Hills, Barossa Valley, Clare Valley, Eden Valley, Langhorne Creek and Riverland (Wine Australia, 2015) during the 2014 and 2016 vintages. Table 1 shows the regions and numbers of samples collected each year. Samples were stored at -20 °C until required and destemmed while frozen.

Table 1. List of samples collected during the 2014 and 2016 vintages for MIR analysis from different GI across South Australia

GI	2014	2016
Adelaide Hills (ADL)	n = 8	n = 9
Barossa Valley (BV)	n = 3	n = 5
Clare Valley (CV)	n = 9	n = 9
Eden Valley (EV)	n = 9	n = 12
Langhorne Creek (LC)	-[a]	n = 3
Riverland (RVL)	-	n = 4

[a] No samples collected.

6.2.2 Juice basic chemical analysis

Titrable acidity (TA, expressed as g/L of tartaric acid at pH 8.2) and pH were measured using a combined pH meter and autotitrator (CompacTitrator, Crison Instruments, S.A., Allela, Spain) (Iland, Bruer, Edwards, Weeks, & Wilkes, 2004). Total soluble solids (TSS, expressed as °Brix) were determined using a digital refractometer (Atago pocket, Atago Co., Ltd, Tokyo, Japan). All measurements were performed in duplicate.

6.2.3 Mid-Infrared (MIR) Analysis of Grape Juice

Grape juice was prepared by manually crushing 200 g of previously destemmed and defrosted grapes in small plastic bags with 200 µL of a 100 mg/mL potassium metabisulfite solution. The MIR spectrum of each juice sample was acquired in duplicate with a Bruker Alpha spectrometer (Bruker Optics GmbH, Ettlingen, Germany) coupled with a platinum diamond attenuated total reflectance (ATR) single reflection module cell. Spectra resulted from an average of 64 scans (resolution of 4 cm^{-1}) acquired between 4000 and 375 cm^{-1}. The scanner velocity was 7.5 kHz with a background of 64 scans. Air was used as reference background. MIR spectra were recorded using OPUS v.6.5 software (Bruker Optics GmbH).

6.2.4 Statistical Analysis

MIR spectral data were processed and analysed with The Unscrambler X software (CAMO AS, version 10.3, Oslo, Norway) using the second derivative (20 smoothing points and second polynomial

order) of the fingerprint region (1500–800 cm^{-1}) in order to remove and correct for baseline effects (Savitsky & Golay, 1964). Principal component analysis (PCA) was performed on the transformed data to examine any segmentation in the data according to sample origin. The number of principal components (PC) used was determined from scree plots. Partial least squares regression (PLS) models were constructed in order to predict TSS and TA content in the samples using the MIR spectra. The statistics calculated for the calibration included the standard error of prediction (SECV), the residual predictive deviation (RPD), and the coefficient of determination (R^2). All chemical variables were normalised before analysis (1/standard deviation). Discriminant models were developed using linear discriminant analysis (LDA) and discriminant partial least squares (PLS-DA) as described by Naes, Isaksson, Fearn, and Davies (2002), Matthias (1999) and Cozzolino et al. (2009). LDA was performed on the first three PCA scores, which gave the highest levels of separation in the models developed. In PLS-DA, a dummy matrix was constructed where each sample was assigned a value of either 0 or 1 depending on whether it belonged to the category (region, grade) or not. As such, a four-dimensional (4D) matrix was developed for 2014 and a six-dimensional (6D) one for 2016. Samples were then allocated to the category with the highest predicted value (Bevilacqua, Bucci, Magrì, Magrì, Nescatelli, & Marini, 2013). All PCA, PLS, LDA and PLS-DA models were developed using full cross validation.

6.3 Results and Discussion

Chardonnay berry samples from different GI in South Australia were collected during 2014 and 2016 and analysed using ATR-MIR. A limited number of samples from each region meant that the results should be considered as exploratory, but they were still sufficient to enable a preliminary assessment of the potential application of ATR-MIR to classify Chardonnay grape quality.

6.3.1 PCA of results according to region

Results obtained from the second derivative of ATR-MIR spectra of grape juices were analysed using Principal Component Analysis (PCA) to visualise any groupings in the data according to GI. Figures 1 and 2 show the score plot of the first and second principal components (PC) for the 2014 and 2016 vintages.

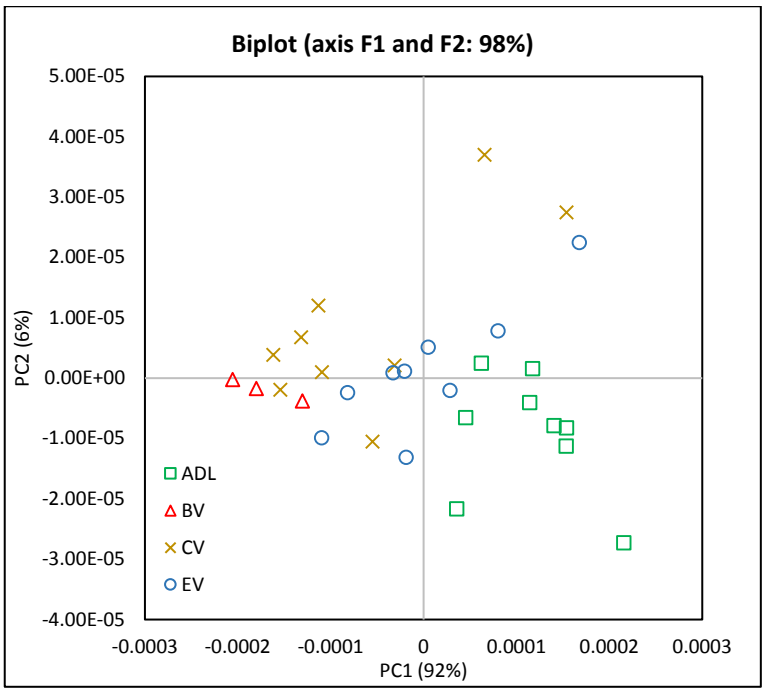

Figure 1. Score plot of the first two principal components of Chardonnay grapes collected during 2014 and analysed using ATR-MIR spectroscopy.

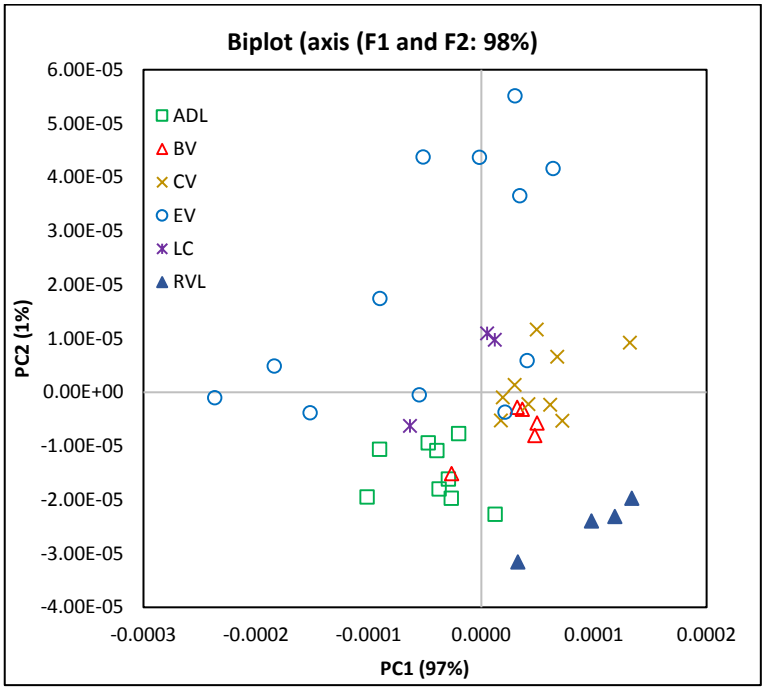

Figure 2. Score plot of the first two principal components of Chardonnay grapes collected during 2016 and analysed using MIR spectroscopy.

Some separation between the different regions can be observed for both vintages. The differences between the results for each vintage were anticipated due to environmental effects altering grape composition each year, as well as the inclusion of extra samples in 2016. In both cases, the first PCs accounted for 98% of the total variation in the ATR-MIR spectra: for 2014, PC1 explained

92% of the variation and PC2 explained 6%; for 2016, PC1 accounted for 97% and PC2 for 1%. As observed by Cozzolino et al. (2012), most of the variation in the spectra was concentrated in the 1200-900 cm^{-1} range, where bands characteristic of sugars and organic acids are located. Inspection of the loadings showed that the predominant peaks contributing to the variability in the data were located, in order of importance, at 1070, 1030, 1002, 1026 and 1167 cm^{-1} (Figure 3).

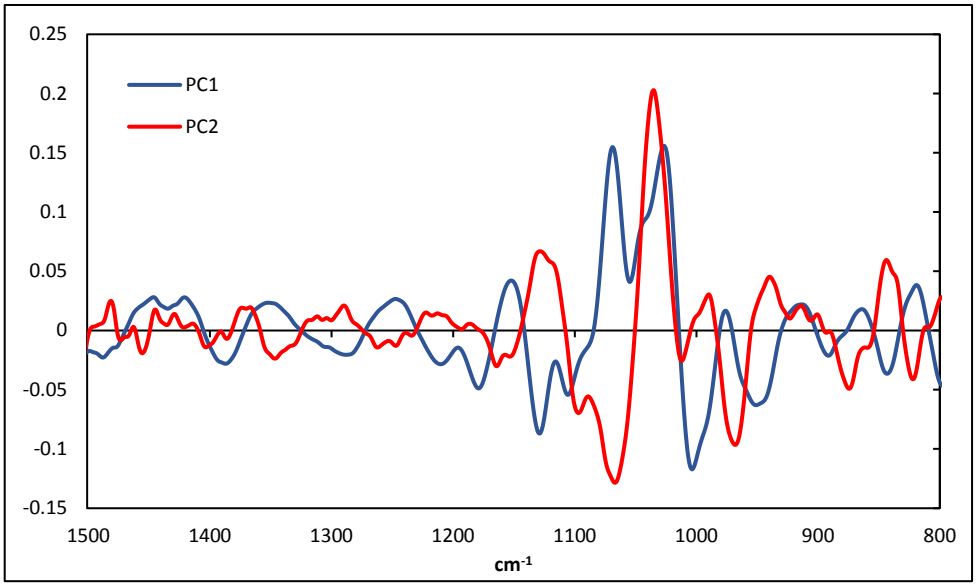

Figure 3. Loadings of the first two principal components using the second derivative of MIR spectra for 2016 grape samples.

These absorptions are mainly due to the presence of polysaccharides in the samples, which according to Stuart (1996) and Shah (2010) are related to the CH-OH and alkyl frequencies of sugars located in the region between 1000 and 1600 cm^{-1}. The two main bands driving the differences between the samples were located at 1030 and 1070 cm^{-1} and can be attributed to glucose and fructose, respectively (Bevin et al., 2008). This was confirmed for both years through PLS analysis of the TSS content of the samples in conjunction with their MIR spectra (fingerprint region), which yielded coefficients of determination (R^2) of 0.62 and 0.81 (Table 3). The inclusion of a bigger set of samples with a wider range of values in 2016 strengthened the prediction capabilities of the model, as can be seen by the improved R^2 and RPD. However, due to the modest RPD values obtained, these models should be considered as qualitative, and be used to classify samples as having low, medium or high levels of TSS. To achieve higher RPD values, a wide range of reference values is required or a low error in the prediction (SECV) (Shah, Cynkar, Smith, & Cozzolino, 2010).

Table 3. PLS calibration statistics for TSS and TA in Chardonnay grape juice samples analysed by ATR-MIR spectroscopy for the 2014 and 2016 vintages.

		Mean	SD[a]	R^2 [b]	SECV [c]	RPD[d]	Factors
2014	TSS	20.3	1.8	0.62	1.15	1.6	2
	TA	7.14	1.54	0.76	1.02	2.0	2
2016	TSS	20.2	1.4	0.80	0.59	2.4	4
	TA	6.31	1.49	0.87	0.55	2.7	2

[a] SD, Standard deviation. [b] R^2, coefficient of determination. [C] SECV, standard error of prediction. [d] RPD, residual predictive deviation (RPD= SD/SECV)

Bands corresponding to the vibration of organic acids (as drivers of TA) have also been shown to influence the spectra in the region between 1500 and 900 cm^{-1} (Schindler, Vonach, Lendl, & Kellner, 1998). Correlations were found between TA and the fingerprint region through PLS (Table 3), although it was necessary to expand the selection up to 1800 cm^{-1} to better model this parameter. Inspection of the loadings led to identification of regions located at 1733-1716 and 1760-1756 that were key to modelling TA. This accords with C=O stretching frequencies of organic acids at around 1700 cm^{-1} (Shah et al., 2010).

Table 4. Chemical composition of Chardonnay berries collected during the 2014 and 2016 vintages

GI[a]	Vintage	pH	TSS (°Brix)	TA (g/L)
2014				
ADL	2014	3.5 (0.1)b[b]	18.1 (0.9)c	9.7 (0.9)a
BV	2014	4.0 (0.2)a	22.4 (1.2)a	6.7 (0.6)b
CV	2014	4.0 (0.1)a	21.6 (0.7)ab	6.1 (0.3)b
EV	2014	4.0 (0.2)a	20.5 (1.0)b	6.4 (0.5)b
2016				
ADL	2016	3.2 (0.1)b	21.1 (0.7)a	7.4 (0.5)b
BV	2016	3.4 (0.1)a	20.5 (0.9)ab	5.5 (0.8)c
CV	2016	3.4 (0.1)a	19.1 (0.8)b	5.2 (0.4)c
EV	2016	3.4 (0.1)a	20.7 (1.7)a	5.8 (0.6)c
LC	2016	3.4 (0.0)a	20.4 (1.1)ab	6.0 (0.4)bc
RVL	2016	3.1 (0.1)b	18.9 (0.5)b	9.5 (0.2)a

[a] ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; RVL, Riverland.[a] For each region, means ± SD (duplicate measurements for each sample) with different letters within a column (a, b, c) are significantly different ($p<0.05$) according to Tukey's (HSD) pairwise comparison.

Figures 1 and 2 show a clear discrimination between samples from the Adelaide Hills (ADL) and Clare Valley (CV) along PC2 for both vintages, and ADL and Barossa Valley (BV) in 2014. As elucidated from the loadings, part of this discrimination was being driven by TSS and TA, which as some of the more abundant components in grapes after water, had a major influence on the ATR-MIR spectra. ADL samples had the highest TA and lowest TSS values in 2014 (Table 4) which led to their grouping and separation from all other regions at the lower right quadrant from all other regions. Similarly, samples

from the Riverland (RVL), located at the lower right corner of the 2016 plot (Fig. 2), clearly segregated from those from all other regions due to its lower TSS and higher TA that vintage.

Discrimination between samples picked from BV and CV was difficult for both years. This was not surprising given their similar climates (Table 5). Both regions were the warmest ones sampled in 2014, and other than RVL (which was picked 15 days earlier), were the warmest again in 2016. Individual examination of BV samples in 2016 showed also that the one sample not clustering with the rest from this region had a higher sugar content (22.1 °Brix), more akin to the mean value obtained for ADL (21.1 °Brix, Table 4).

Table 5. Weather for all regions sampled, including mean, minimum, maximum and highest temperature for January and February, total precipitation and GDD for the 2014 and 2016 vintages

GI	Vintage	Mean T (°C)		Highest T (°C)		Rainfall (mm)		GDD[a]
		Jan min/max	*Feb min/max*	*Jan*	*Feb*	*Jan*	*Feb*	
2014								
ADL	2014	13.7/ 29.5	13.3/ 26.9	42.8	43.0	16.6	93.6	1592
BV	2014	16.0/ 33.4	16.2/ 30.2	42.5	42.1	7.6	98.0	2086
CV	2014	15.8/ 32.1	15.3 /25.5	41.9	42.3	11.6	102.4	1896
EV	2014	15.0/ 29.9	14.5/ 27.0	41.1	41.6	16.8	114.6	1623
2016								
ADL	2016	13.9/ 29.1	13.3/ 26.6	38.7	36.1	35.5	37.6	1998
BV	2016	16.3/ 31.8	13.1/ 29.6	39.9	37.2	30.8	5.4	2406
CV	2016	15.7/ 30.2	13.8/ 29.5	38.1	38.2	35.8	18.0	2227
EV	2016	15.2/ 28.0	13.2/ 26.8	36.6	36.4	30.8	27.8	1985
LC	2016	15.3/ 28.2	14.6/ 26.0	37.2	37.0	17.0	19.6	2101
RVL	2016	16.4/ 33.9	14.7/ 33.6	43.3	44.6	21.0	0.0	2670

[a] GDD, Growing degree days base 10 °C. ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; EV, Eden Valley; LC, Langhorne Creek; RVL, Riverland.

6.3.2 Classification according to region

Although successful classification of wines according to origin has been attempted by a few authors (Cozzolino, Smyth, & Gishen, 2003; Liu et al., 2006; Riovanto et al., 2011), only Arana et al. (2005) have done this for grapes, using the varieties Viura and Chardonnay for two locations in Spain. Using Chardonnay grapes from different South Australian GI, a classification model developed with PLS-DA using the fingerprint region of the MIR spectra for the 2014 and 2016 vintages had an overall success of 83 and 81%, respectively (Table 6). The inclusion of more samples in 2016 seems to have improved the performance of the model for most regions with respect to 2014, particularly with respect to the classification of samples from BV and EV. Based on the results for each individual region (data not shown), only one ADL sample was misclassified in 2016, but inspection of its TA and TSS values revealed it to be within the range of those obtained for the rest of the group. Classification of BV samples in 2014 proved challenging, stemming in part from their compositional similarities to CV. This led to a low classification success of 33%. This class seemed to have been better modelled in 2016

through the inclusion of more samples. As could have been expected based on the 2016 PCA plot (Fig. 2), a BV sample was misclassified as ADL in 2016 (due to a higher TSS value), decreasing the success rate of classification for BV in this year. Although PLS works to maximise among-groups and within-groups variability, it was impossible to discriminate LC samples (2016) from those from other regions based solely on ATR-MIR spectra (Table 6).

Table 6. PLS-DA classification rates of Chardonnay juice samples according to region of origin for six GI in South Australia.

	2014		2016	
ADLa	100%	(9/9)[b]	89%	(8/9)
BV	33%	(1/3)	80%	(4/5)
CV	100%	(9/9)	89%	(8/9)
EV	67%	(6/9)	83%	(10/12)
LC			0%	(0/3)
RVL			100%	(4/4)
Overall	80%		83%	

[a] ADL, Adelaide Hills; BV, Barossa Valley; CV, Clare Valley; LC, Langhorne Creek; RVL, Riverland. [b] Samples correctly classified and number of samples. [b] -, not sampled.

6.3.3 Classification according to grade

Linear discriminant analysis (LDA) and PLS-DA regression were used to develop models capable of predicting the quality grades allocated to parcels of fruit originating from different vineyards, and the results were compared. Fruit is graded by companies on a rating scale such as A-E (with A being the highest grade), based mainly on the organoleptic properties of the fruit and the production requirements of the company. This approach means that this criteria can change from company to company, and from winemaker to winemaker. Additionally, these grades are usually not final until the wine has been produced and tasted, which underlines the difficulty in correctly grading fruit.

LDA was applied to the first three principal component scores to sort the samples according to their quality grade. LDA is a supervised classification technique, designed to maximise between-group variability. In this situation, PCA was used to reduce the dimensionality of the data and derive the first three components in order to be able to perform LDA (Cozzolino, Smyth, Cynkar, Dambergs, & Gishen, 2005). PLS-DA is a variant of PLS, used when the Y-variable is categorical. It can be thought of as a penalised canonical correlation analysis with a PCA in the *X*-space and a PCA in the *Y*-space providing the penalties (Barker & Rayens, 2003). In a comparison, based on data in Table 7 there was a slightly better overall prediction capability for PLS-DA (83% in 2014 and 81% in 2016) than LDA (73% in 2014 and 65% in 2016). This is probably due to the fact that unlike PLS-DA, the initial PCA that LDA was based on is not capable of distinguishing “among-groups” and “within-groups” variability. When the “within-groups” variability dominates, PLS has been reported to outperform PCA (Barker & Rayens, 2003).

Table 7 shows the individual classification by each technique of grape juices into each individual quality category. As observed, A-grade was the best modelled class for both techniques, with

classification rates of (or close to) 100% for both vintages. In 2014, PLS-DA showed better results at classifying C-grade samples than D-grade, a situation that reversed in 2016. This is probably due to the fact that there were too few D-grade samples in 2014 and C-grade samples in 2016 to train the model properly with respect to the other two categories. LDA, however, had a more consistent behaviour across the vintages for C-grade, albeit its success rate dropped in relation to D-grade from 2014 to 2016. As for sample misclassification, this was due to the proximity of the C-grade and D-grade classes in the multivariate space. There were very few cases where C- and D-grade samples were misclassified as A-grade. This only occurred for two cases in 2016 when using PLS-DA, and was explained by these samples having higher TSS that made them more similar to the A-grade samples from that vintage. No samples were misclassified as belonging to the A-grade in 2014 when using PLS-DA. A larger set of samples encompassing all quality levels, and the introduction of other potentially crucial variables, such as elements or polyphenols, are required to improve the prediction capabilities of the model. Since sugars and acids constitute the major compounds in juice they tend to mask other critical components, and a preliminary cleanup to eliminate sugars and acids has been used by other authors in order to better observe the effect of minor compounds such as polyphenols on the overall spectra (Edelmann et al., 2001; Fragoso, Aceña, Guasch, Busto, & Mestres, 2011). However, this sort of additional step might move away from the realms of being rapid and cheap, which is the advantage of MIR, although further aspects could be explored.

Table 7. PLS-DA and LDA results for the classification of Chardonnay grape samples from 2014 and 2016 vintages into different quality categories (A, C, D).

	PLS-DA			LDA		
	A	C	D	A	C	D
2014						
Correct	9	16	0	8	10	4
Total	9	16	5	9	16	5
%	100%	100%	0%	89%	63%	80%
2016						
Correct	9	0	18	9	4	9
Total	9	6	19	9	6	19
%	100%	0%	95%	100%	67%	47%

6.4 Conclusion

This preliminary study verified the possibility to discriminate Chardonnay berries from different GI in South Australia and of different quality grades using ATR-MIR. The ATR-MIR spectra were used to develop predictive models to quantify TSS and TA, which are the two main parameters used to determine optimum harvest maturity in Chardonnay grapes. Due to their low RPD values, these models can only be used for qualitative purposes, but they do verify the existence of a relationship between the selected region of the spectra and these analytes. The results from this study also showed through the combination of chemometric analysis and spectroscopic methods, that grapes belonging to different regions can be classified correctly with an overall success rate across GI of up to 83%.

Advances were also made into the creation of models capable of discriminating Chardonnay grape samples from different quality grades. Using PLS-DA, successful classifications of 83% and 79% overall were achieved across grade categories for 2014 and 2016, respectively. Models were especially successful in modelling the A-grade grapes throughout both vintages, demonstrating that this class was well defined. However, this model still requires a larger input of samples from different grades and perhaps, extra chemical data to strengthen its prediction capabilities.

Despite the limited number of samples, this study showed the capabilities of ATR-MIR as a potential tool to help winemakers grade fruit from different producers and origins. Furthermore, given the advances in miniaturisation and portability being achieved in the field, it is foreseeable for in-field applications to be developed in the near future. Portable applications have already been successfully tested to assess soil, milk, and cereal, amongst other matrices. Furthermore, non-destructive applications that permit the analysis of intact berries need to be evaluated, as this would simplify testing of samples, particularly while in the field.

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CHAPTER 7

Concluding remarks and future perspectives

7. Concluding remarks and future perspectives

Quality of hedonic goods such as wine has always been a difficult concept to define as it is highly subjective. To some extent, it can be described as the degree to which a grape or wine satisfies the wine style it was intended for, whether this is fruity, fresh, or meant for ageing, etc. In this respect, different levels of fruit quality could be understood depending on the price range and style of the wine it will be used to produce. Price range drives the types of operations performed in the vineyard (machine vs. manual harvesting, cost of inputs, irrigation, bunch thinning, etc.) and at the winery (fermentation in stainless steel tanks vs. oak barrels, use of oak chips and staves, choice of yeast, ageing, etc.). In the absence of an objective grading system, many producers have traditionally used region of origin together with basic chemical parameters such as titratable acidity (TA), pH and total soluble solids (TSS), and berry tasting to distinguish between higher and lower quality fruit. Understanding the factors that contribute to grape quality, beyond the traditional parameters of TA, pH and TSS, is a growing concern amongst producers and winemakers. An objective grading system would provide them with a clear scale by which to benchmark fruit, a better understanding of the impact of certain viticultural practices on the final quality of grapes, and a better gauge to monitor ripening and determine optimum harvest timing. Thereby allowing the production of fruit that is better suited for each wine style.

Chardonnay is the main white winegrape variety in Australia, accounting for 22.54% of the total of all varieties, and is second only to Shiraz in its share of overall production. Unlike red varieties, where strong correlations have been shown between grape colour and anthocyanin profile and wine quality, no such marker has been developed for white berries. Therefore, the research discussed in this thesis was undertaken to provide a better understanding between the chemical composition of Chardonnay grapes and their region of origin and quality grade, as well as between Chardonnay wine aroma composition and the grades allocated by expert tasters.

7.1 Conclusions

7.1.1 Snapshot of Australian production practices for Chardonnay wine

An online based survey was designed and distributed to determine current practices in Australian Chardonnay winemaking, fulfilling Objective 1 of the project. More than 150 winemakers (approximately 10% of all Australian Chardonnay wine producers listed in the 2013 wine industry directory data base) answered the 21-question survey. The results showed that Chardonnay wine production was primarily concentrated in the ultra- and super-premium ranges (AU$ 15-50), with only a small fraction of respondents producing any bag-in-box wine. This appeared important, because the market segment being targeted by the producer will determine many of the decisions made concerning production, from the quality of fruit to the operations in the winery.

Although most of the producers who answered the survey still relied heavily on TSS to determine when to harvest, just as many based their decisions on the results of berry tasting, which indicated their concern with flavour profile and aroma potential. When asked about the main attribute they were looking at during berry tasting, most indicated flavour intensity. The criteria used to stream fruit into the different quality categories was also of interest. Respondents indicated that fruit

streaming was based on historical parcel records but also on the results of berry tasting. When asked about the profile of their wines, most producers answered “fruit-driven”, underscoring the importance of the flavour profile of the grapes originally used to produce these wines. The outcome of the survey demonstrated winemakers’ concerns with the aroma profile of the berries and their wines.

7.1.2 Relating expert quality ratings of commercial Australian Chardonnay wines to volatile composition and production method

Objective 2 of this project was to determine which volatile molecules could be used to explain the quality ratings of commercial Chardonnay wines. Wines (n = 83) were selected from Australia’s main winemaking regions, spanning different price ranges (AU$7-125) and vintages (2010-2013), and comprising oaked and unoaked samples, being representative of the Australian Chardonnay wines available at the time. Scores were judged by eight expert tasters, all industry professionals (winemakers, professors and retailers with experience in white wine) using the Australian Wine Show system on a 20-point scale, and a four-level quality score (A-D) determined and agreed upon by all the members of the panel prior to tasting. Basic wine chemical parameters (alcohol, TA and pH) and 39 volatile compounds were determined in the wines and related to the quality score generated by the panel, as well as to price and production method.

Overall, panellists scored more highly the wines fermented and/or aged in oak barrels, which contained higher quantities of oak related volatiles, especially *cis*- and *trans*-oak lactone, and lower concentrations of esters and isoprenoids. Conversely, younger-release wines fermented exclusively in stainless steel tanks were rated lower in quality. These wines had higher concentrations of β-damascenone and the esters hexyl acetate, ethyl hexanoate and ethyl butanoate. However, only one sample was considered by the experts as worthy of an A-grade, 40% of the samples were considered as B-grade and 59% as C-grade. Both B- and C-grades were described by the experts as being “more oaked” than A-grade, which would at least partly explain the high impact encountered in this study for the oak-related volatiles.

Network analysis was used to illustrate correlations between the chemical compounds, and also between chemical compounds, quality score and price. Both score and price were shown to be significantly correlated to the presence of *cis*- and *trans*-oak lactones and furfural, all of which correlated with each other. These molecules were more abundant in the older samples, which had been fermented and aged in oak barrels. As determined in Chapter 2 and established from the product descriptions, fermentation and ageing in barrels are techniques reserved for the more expensive wines. A second node was made up by the esters ethyl hexanoate, ethyl butanoate and hexanoic acid, all of which share common biosynthetic pathways. A third node corresponded to 3-methylbutyl acetate, hexyl acetate, linalool, α-terpineol, 2-phenylethylacetate and β-damascenone, all of which were more abundant in the 2013 samples that had had no contact with oak. A prediction model was developed using partial least squares regression (PLS) in order to classify wines into low, medium and high quality according to their volatile composition. This model confirmed the positive influences of *cis*- and *trans*-oak lactone on the overall quality as assessed by the expert panel, as well as that of 2-methyl-1-propanol and the negative impact of higher concentrations of 1-hexanol and 1-propanol.

7.1.3 Towards the creation of a wine quality prediction index: Correlation of Chardonnay juice and wine compositions from different regions and quality levels

In traditional winemaking countries wine quality is determined by geographic origin or *terroir*. The concept of t*erroir* encompasses the soil, climate and human factors such as viticultural practices that modulate the composition of the berries and ensuing wine. This study addressed Objective 3 by evaluating the compositional aspect of grapes, sourced from different Geographical Indications (GI) in South Australia, and their corresponding wines. Firstly, sensory descriptive analysis was undertaken on wines produced with fruit from three distinct regions in South Australia, Adelaide Hills (ADL), Eden Valley (EV) and Riverland (RVL), in order to determine whether wines made with fruit from different regions could be discriminated by a trained sensory panel. The regions were chosen because they represented high, middle and low quality fruit according to producers and price payed per tonne of fruit. The panel was composed of 12 assessors (6 male and 6 female) who generated 12 aroma and 13 palate attributes. Clear separation of all three regions was achieved through canonical variate analysis (CVA) of the significantly different sensory attributes. ADL was characterised by more intense *tropical, citrus, floral*, *stonefruit/melon, herbaceous* and *green apple* aromas and flavours, whereas RVL displayed more intense *yeasty, caramel, beer* and *mushroom* aromas and *honey* flavour as well as more body, astringency and bitterness. EV was judged as intermediate between both profiles.

HS-SPME-GC-MS was used to quantify 28 compounds in these wines, which were then correlated to the sensory data in order to pinpoint compounds relating to each region which could thereafter be used as proxies for quality. Of particular interest were 1-hexanol, linalool, hexyl and isoamyl acetate, octanoic and decanoic acid, 3-methyl-1-butanol, 2-phenylethanol, and ethyl-2-methylbutanoate.

Berry samples were collected from 31 blocks from four different GI - ADL, EV, Barossa Valley (BV) and Clare Valley (CV) - in order to undertake compositional analyses and compare the chemical characteristics of each region. Analysis included free and bound volatile compounds, basic chemistry, fatty acids, and determination of trace and major elements. Significant differences were observed between the four sites for all elements with the exception of Fe. Higher concentrations of Na, K and P were observed in BV, higher levels of Mg, Ca, Zn and Ti were determined in ADL samples, and both ADL and EV had the highest levels of Cu amongst all regions. According to principal component analysis (PCA), samples were separated along PC1 based on their concentrations of hexanal, 2-hexenal, 1-octanol, vitispirane, 5-methylfurfural (5-MF), phenylacetaldehyde, diethyl succinate, acids (hexanoic, octanoic, decanoic, (*E*)-2-hexenoic and hexadecanoic), guaiacol, 2,6-dimethoxyphenol (2,6-DMP) and TSS. PC2 was driven mainly by pH, TA, Zn and 1-hexanol.

Climatic factors were seen to have a strong influence on the composition of Chardonnay juices from the different regions. Weather affected ripening dates and the concentration of 1-hexanol, linalool, (*Z*)-linalool oxide, vitispirane, β-ionone and 5-MF in the juice as well as the levels of desirable esters, monoterpenoids and higher alcohols in the corresponding wines.

PLS was used to determine associations among wine volatiles and grape compositional parameters. Strong correlations in the multivariate space were obtained for the wine compounds linalool, hexyl acetate, 2-phenylethyl acetate, 2-phenylethanol and 3-methyl-1-butanol. It would be of

interest to pursue this relationships in the future with model fermentations to validate the effect of the grape compositional variables pinpointed.

Finally, mid-infrared (MIR) spectroscopy was investigated as a rapid analysis technique to discriminate between juices from different origins and qualities. PCA of the 2nd derivative of the fingerprint region of the MIR spectra showed that it was possible to separate ADL, BV and EV samples, albeit with some overlap between samples from CV, BV and EV. Strong correlations were also found between the MIR spectra and TSS (R^2 = 0.72) and TA (R^2 = 0.75) of the samples.

7.1.4 Exploring the effects of geographical origin on the chemical composition and quality grading of Vitis Vinifera L. cv.of Chardonnay grapes

This study expanded on the results obtained in Chapter 4 through the evaluation of berries collected from several GI throughout South Australian during the 2015 and 2016 vintages. Samples were collected from Adelaide Hills (ADL), Barossa Valley (BV), Clare Valley (CV), Eden Valley (EV), Langhorne Creek (LC), McLaren Vale (MV, 2015 only) and Riverland (RVL). The composition of amino acids, elements, basic chemistry and free and bound volatiles was evaluated and compared. PCA analysis of overall results demonstrated a strong influence of vintage on the composition of berries from all the regions sampled.

Discrimination of samples according to GI was possible through the use of multiple factor analysis (MFA) and discriminant analysis (DA). In 2015, separation of the different regions was based on the concentrations of Cu, Zn, free 2-phenylethanol, benzyl alcohol, (*Z*)-linalool oxide, linalool, isoamyl acetate, 3-methyl-1-butanol, 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)- and (*E*)-hexen-2-ol, glycosidically-released TDN, 2-phenylethanol, 3-oxo-α-ionol and hexanoic acid, and TA. In 2016, discrimination depended on Cu, Zn, Mg, Ca, B, Na, 2-phenylethanol, benzyl alcohol, (*Z*)-3-hexen-1-ol, 2-ethyl-1-hexanol, linalool, ethyl octanoate, TA and TSS in the berries. Pearson correlation values indicated that preservation of 2-phenylethanol and benzyl alcohol was highly correlated to lower night temperatures rather than only lower overall mean temperatures. These two compounds were more abundant for both vintages in fruit from ADL and EV. ADL and EV also contained higher amounts of Zn, Cu and Mg. The confirmation of these results during both vintages indicated the presence of potential regional markers for both GI. Higher concentrations of 3-oxo-α-ionol detected in CV both years were linked to the higher levels of irradiation in this GI.

DA using backward stepwise selection of variables was performed on the results of the MFA analyses and correctly classified 100% of samples in 2015 and 2016 according to region of origin. The variables used by the model to discriminate between regions in 2015 were Ca, K, Mg, Na, Fe, B, Cu, P, Al, the free volatiles isoamyl acetate, ethyl hexanoate, hexanoic acid, 1-hexanol, (*E*)-3-hexen-1-ol, (*E*)- and (*Z*)-2-hexen-1-ol, (*Z*)-linalool oxide, 1-octen-3-ol, linalool, 2-phenylethanol, benzyl alcohol, pH, and TA, and the bound volatiles α-terpineol, hexanoic acid, TDN and benzyl alcohol. In 2016, classification relied on the presence of Ca, Mg, Na, B, Cu, the free volatiles hexyl acetate, (*E*)-3-hexen-1-ol, ethyl octanoate, 2-ethyl-1-hexanol, linalool, benzyl alcohol, and pH and TSS. Successful classifications using DA were also achieved according to quality grade (100% correct in both vintages). Discrimination in 2015 depended on the levels of Na, Fe**,** Cu, Al**,** 1-hexanol, (*E*)-3-hexen-1-ol, 2-ethyl-1-hexanol, pH, TA, and hydrolytically-released vitispirane, 5-methylfurfural (5MF). In 2016, discrimination was driven by

Cu, 2-phenylethanol, benzyl alcohol, pH, TSS, alanine, proline, and hydrolytically-released (*E*)- and (*Z*)-linalool oxide, 3-oxo-α-ionol, β-ionone, 5MF and 4-vinylguaiacol. Climate affected the concentrations of free and bound volatiles and some amino acids present in the berries, and as such, had a significant effect on berry quality grade. Significant negative correlations were observed between GDD and quality grade, and night time temperatures and quality grade.

7.1.5 Classification of Chardonnay grapes according to geographic indication and quality grade and assessment of maturity through mid-infrared spectroscopy

The last objective of the project (Objective 4) was to explore the capability of rapid, non-destructive techniques such as mid-infrared (MIR) spectroscopy to classify berry samples according to origin and quality grade. Samples were collected during 2014 and 2016 from Adelaide Hills (ADL), Barossa Valley (BV), Clare Valley (CV), Eden Valley (EV), Langhorne Creek (LC, 2016 only) and Riverland (RVL, 2016 only). PCA demonstrated that it was possible to discriminate to a certain extent between samples from different origins based solely on the fingerprint region (1500-800 cm^{-1}) of the MIR spectra. Overlap occurred mainly between BV and CV samples, as was noted in Chapters 4 and 5 with other methodologies, due to the geographical proximity and climatic similarity of these two areas. As observed for LC in Chapter 5, it was difficult to completely segregate the samples from this GI in 2016 solely using the MIR spectra. This indicated that some extra compositional information, such as elements or polyphenol content, may be required to successfully separate all GI completely. Inspection of the loadings indicated that the predominant peaks were located at 1167, 1070, 1030, 1026 and 1002 cm^{-1}, which are mainly due to the presence of sugars, in particular glucose and fructose. This was confirmed through PLS analysis of TSS and MIR spectra of all samples for each year. A prediction model was also developed for TA using PLS. Although good coefficients of determination were observed, RPD values were too low to consider using any of the models to predict the value of these compounds in the berries. As demonstrated by the improvement in coefficient of determination and RPD values between 2014 and 2016, the use of a larger set of samples is needed to strengthen the model, as well as a wider range of values.

PLS-DA and linear discriminant analysis (LDA) were used to classify samples into their corresponding regions of origin and quality grades. In 2014, 83% of samples were classified correctly according to GI using PLS-DA, and 81% in 2016. As for quality grade, LDA assigned 73% of samples correctly in 2014 and 65% in 2016, whereas PLS-DA yielded 83% correctly classified in 2014 and 79% in 2016. Amongst all classes, A-grade was modelled the best, with 100% successful prediction by PLS-DA for both vintages, pointing to a clear separation of this class from the rest. Although this study had an exploratory character, the results outlined in this chapter point to the potential value of MIR as part of a suite of routine analytical tools in the winery. This is particularly interesting for bigger operations that need to process numerous samples each day. Work on this subject should be further explored, in particular with regards to portable equipment and measurements in the field.

7.2 Future directions

a) Based on the results of Chapter 3 and summarised under heading 7.1.2, it would be appropriate to conduct sensory descriptive analysis (DA) of wines from each quality grade in order to understand

the aroma profiles each category represents. Consumer testing of selected samples could then be undertaken to evaluate how well expert opinions actually represents consumer preferences, and to evaluate the acceptability of more evolved samples vs. younger, fruitier ones. As pointed out in the manuscript, Chardonnay wine quality also depends on mouthfeel properties, which were not part of the aims of the study but are important when trying to fully understand the drivers of Chardonnay wine quality. The effects of mouthfeel traits on perceptions of quality remain to be comprehended in the context of a holistic approach to describing Chardonnay wines.

b) Small-lot ferments were produced during the 2014 and 2015 vintages with the addition of diammonium phosphate (DAP) and tartaric acid according to standard industry practice, to correct any deficiencies in the juice and prevent problems with stuck fermentations. However, TA and yeast assimilable nitrogen levels are characteristics intrinsically related to each GI. Their supplementation might have diminished otherwise noticeable differences in aroma profile that could have aided in the discrimination and characterisation of the different regions, and helped to elucidate links to quality. The effect of regionality without these corrections should be explored to observe the effect of juice compositional differences on the volatile profile of the corresponding wines.
c) Chemometric models described in Chapters 4, 5 and 6 were mostly of an exploratory nature due to the limited number of samples. Results and prediction models should be expanded with a larger set of samples in order to confirm the trends observed. Moreover, given the overwhelming effect of vintage on the results, additional years of data would be advisable to confirm the observed trends.
d) Although some interesting correlations between soil composition and juice and wine elements have been pointed out throughout this study, these require confirmation with updated soil analyses of the specific sampled sites at the time of harvest.
e) Certain important berry compositional markers of region and quality have been determined, however there is a lack of information about when and under which situations these compounds are formed in white berries. Studies into the evolution of these compounds, and how different viticultural practices affect them, are required to be able to eventually adapt viticultural practices according to the desired wine style. The use of other complimentary analytical techniques such as LC-MS and ^{1}H NMR could also help better elucidate formation pathways. The use of alternative statistical techniques to handle the data and generate prediction models should also be investigated.

In summary, this project has provided insight into aspects driving Australian Chardonnay grape and wine quality. Through the responses gathered in the online survey, the main ongoing practices for production of Chardonnay wine were identified, as well as the main criteria used to determine optimum harvest and streaming of fruit. This project has also contributed knowledge on the main volatile compounds impacting Chardonnay wine quality as judged by experts, and on their relationship to production practices. This is important as it sheds light on objective drivers of quality that can be measured and modulated by winemakers in order to obtain desired wine styles. On this note, the impact of different chemical variables (elements, amino acids, fatty acids, free and bound volatiles, TA, TSS and pH) on the quality of Chardonnay grapes sourced from different Australian GI was evaluated. Advances were made into the characterisation of each of these GI in terms of berry composition, and chemometric analysis was used to discriminate berries according to origin. Links were found between

fruit components and their quality grade as judged by winemakers, identifying markers that may be modulated through appropriate viticultural techniques. These markers may assist in the creation of objective grading criteria for Chardonnay grapes that can be used by winemakers to communicate with grapegrowers. Finally, it was determined that ATR-MIR shows good promise as a rapid analytical tool to discriminate between berries of different origins and quality grades with minimal sample preparation.

List of abbreviations

%EV	percentage of variance explained by the model
2,6-DMP	2,6-dimethoxyphenol
3-SH	3-sulfanylhexan-1-ol
3-SHA	3- sulfanylhexyl acetate
4-MSP	4-methyl-4-sulfanylpropan-2- one
5-MF	5-methylfurfural
AA	amino acids
AAB	acetic acid bacteria
AAT	alcohol acetyltransferase
ADL	Adelaide Hills
ANOVA	analysis of variance
ATR	attenuated total reflectance
BF	barrel fermented
BM	benzenemethanethiol
BV	Barossa Valley
CC	correlation coefficient
CV	Claire Valley
CVA	canonical variate analysis
CY	commercial yeast
DA	descriptive analysis
DA	discriminant analysis
DAP	diammonium phosphate
ESA	École Supérieure d'Agriculture d'Angers
EV	Eden Valley
FFT	2-furanmethanethiol
FID	flame ionization detector
GC	gas chromatography
GC-O	gas chromatography–olfactometry
GDD	growing degree day
ha	hectares
HDD	heat degree day
HR	high resolution
HS	headspace
ICP-OES	inductively coupled plasma optical emission spectrometry
ICV	Institut Coopératif du Vin
IFV	Institut Français de la Vigne et du Vin
INRA	Institut National de la Recherche Agronomique
LAB	lactic acid bacteria
LC	Langhorne Creek
LLE	liquid–liquid extraction
LOD	limit of detection

LOQ	limit of quantitation
MIR	mid-infrared
MLF	malolactic fermentation
MV	McLaren Vale
NCI	negative chemical ionization
ND	not detected
NIR	near-infrared
NIST	National Institute of Standards and Technology
NSW	New South Wales
OAV	odour activity value
PC	principal components
PCA	principal component analysis
PLS	partial least squares regression analysis
PMS	potassium metabisulfite
PVPP	polyvinylpolypyrrolidone
R^2	coefficient of determination
RMSEP	root-mean-square error of prediction
RPD	residual predictive deviation
RT	retention time
RVL	Riverland
SA	South Australia
SC	starter culture
SD	standard deviation
SECV	square error of cross validation
SIDA	stable isotope dilution analysis
SO_2	sulfur dioxide
SPE	solid phase extraction
SPME	solid-phase microextraction
SSF	stainless steel fermented
T	tonnes
TA	titratable acidity
TDN	1,1,6-trimethyl-1,2-dihydronaphthalene
TOF	time-of-flight
TOF-MS	time-of-flight-mass spectrometry
TSS	total soluble solids
VIC	Victoria
W	wild yeast
WA	Western Australia
YAN	yeast assimilable nitrogen

Appendices

These are additional additional peer reviewed papers generated during candidature, which I have co-authored.

Appendix 1

Comprehensive study of volatile compounds in two Australian rosé wines: Aroma Extract Dilution Analysis (AEDA) of extracts prepared using Solvent-Assisted Flavor Evaporation (SAFE) or Headspace Solid-Phase Extraction (HS-SPE)

Jiaming Wang, Joanna M. Gambetta and David W. Jeffery

J. Agric. Food Chem., **2016**, 64, 3838-3848

Appendix 2

Predicting phenolic composition of Shiraz wines using Attenuated Total Reflectance Mid-Infrared (ATR-MIR) spectroscopy

Renata Ristic, Daniel Cozzolino, David W. Jeffery, Joanna M. Gambetta and Susan E. P. Bastian

Am. J. Enol. Vitic., **2016**, 67, 460-465

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Comprehensive Study of Volatile Compounds in Two Australian Rosé Wines: Aroma Extract Dilution Analysis (AEDA) of Extracts Prepared Using Solvent-Assisted Flavor Evaporation (SAFE) or Headspace Solid-Phase Extraction (HS-SPE)

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Supporting Information

ABSTRACT: Two rosé wines, representing a tropical and a fruity/floral style, were chosen from a previous study for further exploration by aroma extract dilution analysis (AEDA) and quantitative analysis. Volatiles were extracted using either liquid−liquid extraction (LLE) followed by solvent-assisted flavor evaporation (SAFE) or a recently developed dynamic headspace (HS) sampling method utilizing solid-phase extraction (SPE) cartridges. AEDA was conducted using gas chromatography−mass spectrometry/olfactometry (GC-MS/O) and a total of 51 aroma compounds with a flavor dilution (FD) factor ≥3 were detected. Quantitative analysis of 92 volatiles was undertaken in both wines for calculation of odor activity values. The fruity and floral wine style was mostly driven by 2-phenylethanol, β-damascenone, and a range of esters, whereas 3-SHA and several volatile acids were seen as essential for the tropical style. When extraction methods were compared, HS-SPE was as efficient as SAFE for extracting most esters and higher alcohols, which were associated with fruity and floral characters, but it was difficult to capture volatiles with greater polarity or higher boiling point that may still be important to perceived wine aroma.

KEYWORDS: *rosé wine, volatile composition, liquid−liquid extraction, headspace sampling, GC-O, flavor dilution factor, odor activity value*

INTRODUCTION

Aroma is one of the most important sensory components that contributes to wine quality, varietal characters, and consumer acceptance, but the study of wine aroma is not a straightforward undertaking. Aside from the inherent genetic differences in individuals that can influence perception of aromas, consideration needs to be given to the biological and chemical origins of wine aroma volatiles, the concentration ranges spanning many orders of magnitude, and the influences of grape variety and matrix interactions. Fortunately, continuous advances in analytical technology, such as in gas chromatography instrumentation and sample preparation techniques, and decades of research in flavor chemistry have contributed to current methodologies and understanding.

One very useful technology that has arisen is gas chromatography−olfactometry (GC-O), often performed in conjunction with detection by mass spectrometry (MS). Quite uniquely for an analytical instrument, GC-O utilizes human olfaction (sense of smell) in combination with a conventional instrument detector to simultaneously evaluate odor characteristics and chemical identity (at least in the case of MS) for chromatographically separated volatile components. GC-O can be conducted in a number of ways to evaluate the potential sensory importance of odorants,[1] with one of the most common, aroma extract dilution analysis (AEDA), being based on threshold concentrations in air. This relatively simple (albeit time-consuming) approach provides quantitative information on odorants (intensity) and is used to assess their relative importance to wine aroma. This is achieved by calculating a flavor dilution (FD) factor for each odorant, being the highest dilution level at which an odor is still detected, which can be plotted against retention index (RI) to produce an aromagram (olfactogram). An aroma model can be proposed upon the identification and quantification of significant odorants and the calculation of odor activity values (OAV) from threshold data.[2] Evidently, a GC-O strategy does not model the enhancing or suppressive effects of odorant mixtures, which could occur in a real matrix,[3] and reconstitution/omission sensory studies are often undertaken to account for any perceptual interactions and verify an aroma model.[1,2,4] In general, however, odorants with high OAVs and/or with aromas that are readily distinguishable are likely to have an impact on wine aroma.[1,5]

Besides different GC-O strategies to assess the importance of various odorants to overall wine aroma, preparation of a representative sample of the original wine is always a fundamental issue.[1,5,6] Different methodologies have been developed to obtain samples for study by GC-O more generally and can be applied to wine, but none offer a universal approach to extracting relevant odorants. Liquid−liquid extraction (LLE) using various organic solvents provides for a simple and exhaustive extraction but without selectivity; virtually all volatiles and some nonvolatiles are recovered from the

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wine.[5,7] LLE is accompanied by concentration of the solvent by distillation or with a stream of nitrogen in a sample concentrator. Distillation can be rapidly and conveniently carried out under high vacuum using solvent-assisted flavor evaporation (SAFE), with a SAFE apparatus allowing careful isolation of volatile compounds from a solvent extract.[7] Solid-phase extraction (SPE) is extensively used for isolation of volatiles and gives a result similar to that of LLE,[8] albeit with more selectivity and efficiency and using lower quantities of organic solvent.

Extracts obtained by LLE followed by SAFE or from SPE are unlikely to reflect the profile of volatiles released from the original matrix that end up being perceived during olfactory evaluation.[9] Furthermore, the concentration step to remove solvent can lead to loss of highly volatile components, and injected solvent can mask the detection of compounds during GC analysis.[2] Headspace (HS) methods fill a gap in the extraction technique repertoire, as they can more closely approximate the volatile aroma fraction of a wine. In particular, dynamic HS extraction using a purge and trap system seems to be more representative of the original sample and has been successfully applied in wine research.[6,10] Recent efforts toward obtaining a representative HS extract (from model wine) have involved the development and refinement of a purge and trap system utilizing a specific flask and commercial SPE cartridge containing polymeric sorbent.[6,9] An alternative HS method involves solid-phase microextraction (SPME), which is much less time-consuming than other methods because it allows for direct AEDA of a wine sample, either following successive dilution[5] or by altering the split ratio of the GC injector port.[11] In terms of being able to obtain a representative SPME method, however, there are many parameters requiring careful optimization (e.g., different fiber coating, fiber length, extraction time and temperature, volume of the sample), making HS-SPME approaches challenging to optimize[1,5] and not widely adopted for wine aroma characterization using AEDA compared to the other techniques.

Characterization of rosé wine aroma is of interest due to the somewhat peculiar nature of the production technique, which uses red grape varieties such as Pinot noir, Grenache, and Cabernet Sauvignon, among others, in conjunction with white winemaking practices. Although there is some extraction of grape skin components such as red pigments, unlike red winemaking the grape juice does not macerate with the grape solids during fermentation to produce rosé wine. Despite the limited skin contact, different grape varietals can still play a role in the aroma profile of the corresponding wines. Researchers have studied aroma compounds of rosé wines from Turkey, Spain, and France.[12−14] More recently, different styles of commercial Australian rosé wine have been proposed, with sensory analysis of a range of wines revealing characters such as oaky/spicy, tropical/citrus, fruit-driven, and floral.[15] Fruity aroma attributes have often been found to be important to rosé wine aroma and can be associated with a number of ethyl esters and higher alcohol acetates and also with grape-derived aroma compounds such as polyfunctional thiols including 3-sulfanyl-1-hexanol (3-SH) and its corresponding ester 3-sulfanylhexyl acetate (3-SHA),[10,12−17] and the C_{13}-norisoprenoid β-damascenone.

A previous study on rosé wine from Australia identified, among other sensory characters, fruity and confectionery notes in a number of wines and tropical and citrus attributes in others.[15] On the basis of these differences, two rosé wines representing fruity/floral versus tropical were selected for GC-MS/O analysis to evaluate the volatile compounds driving the particular sensory styles. A recent headspace sampling method,[6] refined by Escudero et al.[9] but so far used only for synthetic wine spiked with a selection of volatiles, was modified and compared with LLE and SAFE to evaluate the differences in the AEDA results for the two wines. Quantitative analysis of a wide range of volatiles was also undertaken to calculate OAVs. This study provides understanding of the important aroma compounds in two different Australian rosé wines and also offers guidance in obtaining a representative volatile extract for wine aroma research.

MATERIALS AND METHODS

Chemicals. Volatile compounds (≥97% purity) used in quantitative analysis and as reference standards during GC-O (ethyl 2-methylpropanoate, 2,3-butanedione, 2-methyl-2-butanol, ethyl 3-methylbutanoate, butyl acetate, 2-methyl-1-propanol, 3-methylbutyl acetate, 1-butanol, 3-methyl-1-butanol, ethyl hexanoate, hexyl acetate, 3-hydroxybutan-2-one, 3-methyl-1-pentanol, ethyl lactate, 1-hexanol, (*Z*)-3-hexenol, ethyl octanoate, acetic acid, 3-methylbutyl hexanoate, furfural, octyl acetate, 3-isobutyl-2-methoxypyrazine (IBMP), 2,3-butanediol, linalool, 2-methylpropanoic acid, butanoic acid, ethyl decanoate, γ-butyrolactone, furfuryl alcohol, 3-methylbutanoic acid, diethyl succinate, methionol, 3-sulfanylhexyl acetate (3-SHA), benzyl acetate, δ-valerolactone, 2-phenylethyl acetate, β-damascenone, hexanoic acid, guaiacol, benzyl alcohol, 2-phenylethanol, anisaldehyde, octanoic acid, γ-decalactone, decanoic acid, diethyl tartrate, 2-furoic acid, benzoic acid, dodecanoic acid) were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia), except for ethyl butanoate and ethyl 2-methylbutanoate, which were supplied by Alfa Aesar (Ward Hill, MA, USA). Sodium chloride was supplied by J. T. Baker (Phillipsburg, NJ, USA), and analytical grade solvents were obtained from Merck (Kilsyth, VIC, Australia). GC grade solvents were supplied by VWR International (Tingalpa, QLD, Australia). Deuterium-labeled compounds were supplied by CDN Isotopes (Pointe-Claire, QC, Canada). Stock solutions of standards were prepared volumetrically in absolute ethanol and stored at −20 °C, and working solutions were stored at 4 °C until required. All chemicals were of analytical reagent grade unless otherwise stated, and water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia).

Wine Samples. One fruity and floral (Cabernet Sauvignon) and one tropical (Shiraz) rosé wine (Supporting Information, Table S1) were selected on the basis of the results of a previous study[15] and following an informal tasting of a selection of rosé wines conducted with wine researchers at The University of Adelaide (UA). To accomplish this, two candidates for each style were chosen, and 10 experienced assessors were asked to select the most representative sample for each style. The wines were bottled under screw cap and donated by local wineries. A 2014 commercial rosé wine (bag-in-box, 12.5% v/v ethanol, pH 3.40, titratable acidity (TA) = 6.8 g/L, SO_2 (free) = 29 mg/L, SO_2 (total) = 134 mg/L) was used as a base wine for calibration of the quantitation methods and also for training purposes during the GC-O study to allow sniffers to become familiar with the GC-O process.

Basic Wine Composition. Alcohol, TA, pH, and residual sugar (glucose + fructose) were measured as previously described.[15] Free and total SO_2 were determined by the aspiration method. All measurements were performed in duplicate (Supporting Information, Table S1).

Isolation of Volatiles for AEDA. Both samples were extracted in duplicate, but no differences were detected between replicates by GC-MS analysis. Only one extract from each replicate was chosen to conduct AEDA.

LLE-SAFE Extract. Wine (100 mL) was extracted with CH_2Cl_2 (3 × 50 mL) using a separating funnel and vigorous shaking for 10 min. The combined organic phases were spiked with a 2-octanol solution (0.75 mL of 500 mg/L in ethanol) as internal standard and washed

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with a saturated sodium chloride solution (150 mL) and dried over anhydrous sodium sulfate. After filtration and concentration to approximately 100 mL by distillation on a water bath at 40 °C with a Vigreux column (50 × 1 cm), the volatiles were isolated by means of SAFE. The apparatus was thermostated at 40 °C and kept under high vacuum (10^{-3} Pa), and the sample was added dropwise into the evaporation flask over 30 min. After an additional 5 min, the vacuum was released and the distillate was thawed at room temperature. The SAFE extract was concentrated to 100 μL by distillation, again using a Vigreux column as described above, and aliquots of extract were stored at −20 °C until required for GC-O analysis. These samples arising from LLE and SAFE were designated T-SAFE and F-SAFE for tropical and fruity/floral wines, respectively.

HS-SPE Extract. The procedure was adopted from a previous study[9] with modifications. Different extraction conditions including different cartridge size (1, 3, and 20 mL), mass of sorbent (300, 400, and 500 mg), and length of extraction (60, 100, 180, 360, and 720 min) were evaluated by GC-MS to obtain the optimal parameters using the bag-in-box rosé wine. On the basis of the total peak area and peak heights, the combination of 500 mg of resin in a 20 mL cartridge with 360 min extraction time was chosen. Wine (100 mL) was added to a customized flask (Supporting Information, Figure S1) and purged without agitation at room temperature. Volatiles were trapped with 500 mg of LiChrolut EN sorbent (Merck, Kilsyth, VIC, Australia) packed into 20 mL polypropylene SPE tubes (fitted with PTFE frits), which had been previously washed with CH_2Cl_2 (25 mL) and dried. A controlled stream of nitrogen (500 mL/min), which did not disturb the liquid surface, was applied to the headspace of the wine for 6 h. The cartridge was removed and dried using a stream of nitrogen (0.6 bar, 10 min). Analytes were subsequently recovered with CH_2Cl_2/MeOH (95:5 v/v, 4 mL) using a dropwise elution rate. The extract was spiked with 2-octanol solution (0.02 mL of 500 mg/L in ethanol, to keep the same concentration as LLE) as internal standard and concentrated under a stream of nitrogen (0.6 bar, 10 min) to a final volume of 100 μL. Extracts were stored as described above until required. These samples arising from HS extraction were designated T-HS and F-HS for tropical and fruity/floral wines, respectively.

Gas Chromatography−Mass Spectrometry/Olfactometry (GC-MS/O). *GC-MS Conditions.* Analyses were performed using an Agilent 7890 GC equipped with a Gerstel MPS autosampler (Lasersan Australasia Pty Ltd., Robina, QLD, Australia) and coupled to a 5897 mass selective detector (Agilent, Palo Alto, CA, USA). The GC was also fitted with a Gerstel olfactory detection port (ODP series 1). A DB-Wax column (60 m × 0.25 mm, 0.25 μm film thickness Agilent J&W, Folsom, CA, USA) was used with helium as carrier gas (Coregas, Cavan, SA, Australia) in constant pressure mode (263.9 kPa, nominal initial flow = 2.6 mL/min). The oven was held at 40 °C for 5 min and then heated at 3 °C/min to 240 °C and held at this temperature for 5 min. Splitless injection mode was used for liquid injections (2 μL), and the split vent was opened after 3 min. A single taper, ultrainert liner with glass wool was used (splitless, deactivated, 4 mm i.d., 900 μL, Agilent). The MS transfer line was set at 250 °C, and electron impact spectra at 70 eV were recorded in the range m/z 35−350. The MS quadrupole was set at 150 °C, and the source was set at 230 °C. The transfer line to the ODP and the humidifier mixing chamber were set at 250 and 200 °C, respectively. The humidified gas and makeup gas in the ODP system were nitrogen (Coregas) with preset rates at 12 and 50 mL/min, respectively. The capillary column lengths from splitter to ODP and MS were set using an Agilent pressure flow calculator to achieve a 2:1 split ratio. Simultaneous detection of MS signal and odorant by olfaction was verified by injecting a CH_2Cl_2 solution containing several volatiles that have distinguishing odors. Instrument control and data analysis were performed with Agilent ChemStation software (E.02.02.1431), and the Gerstel autosampler was controlled with Maestro software integrated version 1.3.3.51/3.3.

Identification of Volatiles. Compound identity was verified by comparing the following: mass spectra with library matches (NBS 75K) and authentic reference compounds; calculated RI (using C7−C40 alkanes, Sigma-Aldrich) with those obtained from AromaOffice 1D (version 2.01.00 (2012/03/09, Gerstel K.K., Tokyo, Japan) for a DB-Wax column; odor quality with those of reference compounds or literature reports.

Aroma Extract Dilution Analysis (AEDA). GC-O analysis was conducted on volatile extracts prepared by SAFE and HS-SPE. Five sniffers (four males, one female, average age of 32 years) were involved for the AEDA study. Four sniffers had previous GC-O experience and participated in an earlier rosé wine sensory descriptive analysis (DA) panel.[15] The total sniff time for each assessor was up to 50 min (consisting of two separate 25 min sessions), and assessors conducted up to five runs per day with at least a 25 min break between sessions. Assessors evaluated the undiluted base wine extracts three times for training purposes before the formal analysis, to eliminate potential gaps in detecting odor active regions and to ensure consistency of detection. A recorder system (ODP-Recorder, Gerstel GmbH & Co. KG, version 3.0.2.2) was used, and the comments of panelists were saved and simultaneously recognized by the software while the button was being held down on the recorder. The sensory vocabulary for the recorder system was developed from attributes generated in the previous rosé wine DA panel[15] and from terms used during GC-O test runs. Extracts were stepwise diluted with CH_2Cl_2 (1:2 (v/v) to yield dilutions of 3, 9, 27, 81, etc., and up to 177147 relative to the original extracts (i.e., 3^n where n = 1, 2, 3, etc.)). After analysis by GC-O, FD factors of each odor-active compound in the four samples (T-SAFE, T-HS, F-SAFE, and F-HS) were determined. FD was defined as the maximum dilution at which three of five sniffers could still perceive the odorant. Compounds that had FD factors ≥3 in at least one sample were studied further.

Quantitation of Volatiles. *HS-SPME-GC-MS Analysis.* A total of 28 compounds (one of which was tentatively identified) were quantified using a previous method for rosé wine.[15] Standards at three concentrations (covering the concentration range and evenly spaced) in base wine were analyzed in duplicate to develop calibration functions for quantitation.

HS-SPME-GC-MS Analysis with Selected Ion Monitoring (SIM). A SIM method was established to quantify a further 34 compounds (10 of which were tentatively identified) using the SPME parameters and sample preparation as reported in a previous study.[15] Samples were analyzed on an Agilent 6890 GC equipped with a 5973N MS. A deactivated SPME inlet liner (0.75 mm i.d., Supelco) and DB-Wax column (60 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W) were used with a constant flow rate of 1.5 mL/min and an average velocity of 31 cm/s. The inlet temperature was set to 240 °C, with the pressure set at 157.8 kPa, and splitless injection mode was used. The split vent was opened after 3 min. The oven was held at 40 °C for 5 min, then heated at 2 °C/min to 240 °C, and held at this temperature for 10 min. The MS transfer line was set to 240 °C, the MS source was 230 °C, the quadrupole was 150 °C, and electron impact spectra were recorded at 70 eV. Ultrapure helium (Coregas) was used as the carrier gas. Authentic compounds were first analyzed in scan mode to select the SIM ions for each analyte. On the basis of retention time, 15 SIM groups with dwell times ranging from 20 to 100 ms were established. Instrument control and data analysis were performed with Agilent ChemStation software (E.02.02.1431). Calibration, validation, and calculation of the limit of detection (LOD) and limit of quantitation (LOQ) for each analyte were undertaken as described previously.[15] The retention index (RI), SIM ions, regression coefficient (R^2), and calibrated concentration range for each compound are given in the Supporting Information (Table S2).

Analysis for Other Volatiles. C_6 compounds were quantified by HS-SPME-GC-MS,[18] and polyfunctional thiols (3-sulfanyl-1-hexanol (3-SH), 3-sulfanylhexyl acetate (3-SHA), 4-methyl-4-sulfanylpentan-2-one (4-MSP), furfurylthiol (FT), and benzenemethanethiol (BMT)) were determined by HPLC-MS/MS after derivatization.[19] Analyses of methoxypyrazines,[20] oak volatiles,[21] and oxidation volatiles[22] were performed by the Australian Wine Research Institute (AWRI) Commercial Services Laboratory (Adelaide, Australia) using published methods. A further 11 tentatively identified compounds that were important according to AEDA were semiquantified on the basis of

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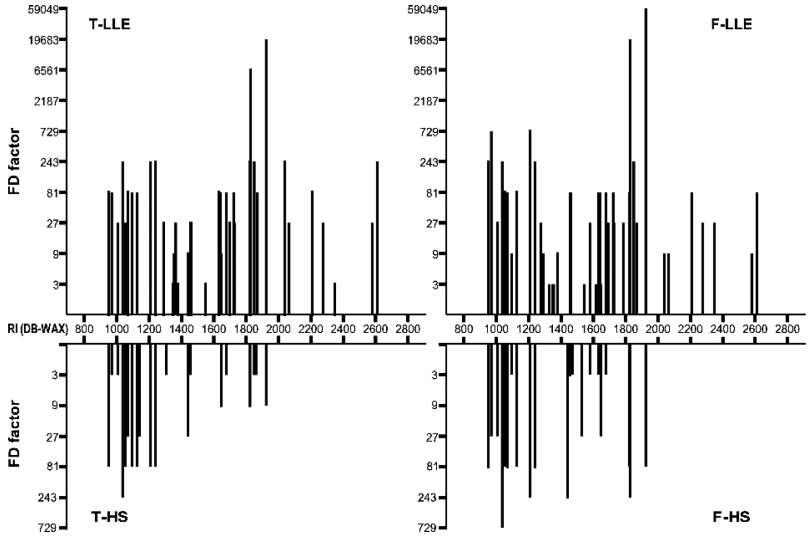

Figure 1. Flavor dilution (FD) aromagrams of volatile fractions (FD ≥ 3) isolated from two representatives rosé wines (tropical, T; and fruity/floral, F) with two extraction techniques (LLE-SAFE and HS).

their equivalence to other calibrated compounds. In total, 92 volatile compounds were quantified.

Data Analysis. Quantitative chemical data are presented as mean values with standard deviation from replicate determinations (Microsoft Excel 2010). Flavor dilution (FD) aromagrams were created in SPSS-20 (SPSS Inc., Chicago, IL, USA) and refined in Adobe Illustrator CS6 (Adobe Systems, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

GC-O and AEDA. Extracts from two commercial rosé wines, with one being tropical in style and the other fruity and floral, were obtained using LLE followed by SAFE, as well as with HS-SPE. Using AEDA, a total of 51 odorants were determined with an FD factor ≥3; the highest FD factors obtained were 59049 (i.e., 3^{10}) for 2-phenylethanol and 729 (i.e., 3^6) for ethyl butanoate in SAFE and HS samples, respectively (Figure 1 and Table 1). According to the AEDA results, the most important aroma compounds in these two wines were a number of fermentation compounds, especially 3-methyl-1-butanol, ethyl 2-methylpropanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate, hexanoic acid, and 2-phenylethanol, along with β-damascenone, a grape-derived compound, which was similar to the AEDA results reported for Çalkarası[12] and Grenache[13] rosé wines.

Comparison of Extraction Methods and Identity of Important Odorants. It was not surprising that a greater number of odor-active compounds were detected in samples obtained by SAFE and, especially, those compounds with larger RIs (Figure 1). Heavier volatiles and those with greater polarity are not as easily extracted with the HS-SPE process,[6,9] whereas SAFE is an exhaustive extraction procedure. In contrast, light volatiles may be lost during extraction using SAFE, and a dynamic HS method might better represent the aroma compounds that are perceived during olfactory assessment of wine.[9] Whereas the HS-SPE purge and trap technique might come closest to being an ideal extraction method, as pointed out earlier, there is no universal approach to chemically assessing wine aroma. Highly volatile compounds such as dimethyl sulfide (DMS) would be better captured with static HS sampling,[23] so for the most complete assessment of wine aroma a combination of extraction techniques would be necessary to prepare samples for GC-O.

The FD factors for both SAFE and HS samples were similar and relatively high at the beginning of the aromagrams (Figure 1), which indicated not only the sensory importance of the more volatile compounds (such as fermentation esters and alcohols) but also that the SAFE and HS-SPE techniques performed similarly in the extraction of such compounds. As the volatility of compounds decreased and their polarity increased, FD factors for HS samples gradually tapered off compared with SAFE samples until odorants were barely perceptible around the middle part of a GC-O run (RI ≈ 1900). On the contrary, SAFE samples contained odorants with the highest FD factors at around this time and odorants could still be detected for some time afterward.

The highest FD factor for SAFE samples was determined for 2-phenylethanol (59049 and 19683 for F-SAFE and T-SAFE, respectively, Table 1), a compound responsible for rose aroma that has been identified in previous rosé wine studies.[12,13] The second highest FD was observed for β-damascenone (19683 and 6561 for F-SAFE and T-SAFE, respectively), a ubiquitous odorant with fruity-floral aroma[33] found to be a key aroma compound related to fruity aroma in Provencal[14] and Australian[15] rosé wines. Guaiacol (smoky and bacon) and δ-decalactone (caramel and coconut), which both eluted relatively late, were principally detected in SAFE samples. Also, FD factors of volatile acids and isoprenoids were higher in SAFE samples, which suggested that they were not easily volatilized to be trapped in the HS extraction process. For HS samples, ethyl butanoate had the highest FD factor for both tropical (FD = 243) and fruity (FD = 729) samples; this fatty acid ethyl ester was described as having red fruit and confectionery notes by the sniffers (Table 1) and contributes strawberry notes in red wines.[34] Ethyl octanoate, a related ester with fruity characters, which has been reported as an important odorant in Grenache rosé wine,[13] had an FD factor that was higher in both HS samples compared with SAFE, particularly for the fruity/floral sample (Table 1). For most other early-eluting volatiles, such as branched-chain ethyl esters, fusel alcohols, and 3-methylbutyl (isoamyl) acetate (Table 1), the FD factors were around the same as for the SAFE samples, which suggested that the HS-SPE extraction method could be as efficient as SAFE for the majority of the more highly volatile

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Table 1. Details of Odorants Detected by AEDA (with FD ≥ 3) of Rosé Wine Extracts Prepared by Two Different Extraction Techniques

				FD factor					OAV	
RI[a]	odorant description[b]	identity[c] determined by	identity[d]	T-SAFE	T-HS	F-SAFE	F-HS	threshold[e]	tropical	fruit/floral
952	fruity, tropical fruit	MS, O, RI	ethyl 2-methylpropanoate	81	81	243	81	15[24]	2.7	3.0
969	butter, yogurt	MS, O, RI	2,3-butanedione	81	3	729	27	100[25]	6.3	13.0
1009	plastic, solvent, fly spray	MS, O, RI	2-methyl-2-butanol	27	3	27	27	n/a[f]	–[g]	–
1036	red fruit, confectionery	MS, O, RI	ethyl butanoate	243	243	243	729	20[24]	20.5	22.4
1052	strawberry, bubble gum	MS, O, RI	ethyl 2-methylbutanoate	27	81	81	81	18[24]	<0.1	<0.1
1060	caramel, yogurt	MS, *O*, RI	2,3-pentanedione	1	0	9	3	900[26]	–	–
1070	citrus, tropical fruit, artificial fruit	MS, O, RI	ethyl 3-methylbutanoate	81	27	81	81	3[24]	3.7	7.0
1094	fusel, amyl alcohol	MS, O, RI	2-methyl-1-propanol	81	81	9	3	40000[24]	0.2	0.3
1126	banana	MS, O, RI	3-methylbutyl acetate	81	81	81	81	30[24]	108	87
1142	fruity, alcoholic	MS, O, RI	1-butanol	0	27	0	0	150000[27]	<0.1	<0.1
1210	solvent, sweaty feet	MS, O, RI	3-methyl-1-butanol	243	81	729	243	30000[25]	4.6	5.6
1239	confectionery, strawberry, green apple, Chinese white spirit	MS, O, RI	ethyl hexanoate	243	81	243	81	14[24]	139	50
1276	confectionery, fruity	MS, O, RI	hexyl acetate	1	1	27	1	1500[27]	0.2	0.1
1289	wet, sweaty	MS, O, RI	3-hydroxybutan-2-one	27	1	9	1	150000[27]	<0.1	<0.1
1305	solvent	MS, *O*, RI	1-hydroxy-2-propanone	0	3	1	1	50000[28]	–	–
1327	green, solvent	MS, O, RI	3-methyl-1-pentanol	1	1	3	1	830[29]	0.1	0.1
1348	yeasty, creamy	MS, O, RI	ethyl lactate	3	1	3	0	154000[27]	0.1	0.06
1355	spicy, green	MS, O, RI	1-hexanol	9	1	3	1	8000[24]	0.3	0.3
1362	burning, alcohol	MS, *O*, RI	2-hydroxy-3-pentanone	27	1	1	0	n/a	–	–
1365	green	MS, O, RI	(*Z*)-3-hexenol	27	0	0	0	400[24]	0.5	0.2
1378	solvent, earthy	MS, *O*, RI	3-ethoxy-1-propanol	3	0	9	0	100[26]	–	–
1439	green, fruity	MS, O, RI	ethyl octanoate	9	27	1	243	20[27]	279	145
1456	vinegar	MS, O, RI	acetic acid	27	3	81	3	200000[25]	0.9	0.5
1461	caramel, yeasty	MS, O, RI	3-methylbutyl hexanoate	27	0	81	0	900[28]	<0.01	<0.01
1472	floral, candy, fruity	MS, O, RI	furfural	1	0	1	3	14100[24]	0.01	<0.01
1530	capsicum	MS, O, RI	IBMP	1	0	0	27	0.002[27]	–	–
1542	floral, creamy	MS, O, RI	2,3-butanediol	1	1	3	0	100000[29]	0.3	0.2
1549	floral	MS, O, RI	linalool	3	0	0	1	25[24]	0.7	0.2
1616	moldy	MS, *O*, RI	dehydrolinalool	0	0	3	0	n/a	–	–
1632	sweaty, cheesy	MS, O, RI	butanoic acid	81	1	81	3	173[24]	10.3	4.9
1641	burnt, floral	MS, O, RI	ethyl decanoate	81	1	81	3	200[24]	17.0	6.0
1647	sweaty	MS, O, RI	γ-butyrolactone	9	9	3	27	35000[30]	0.5	0.3
1677	cheesy, sweaty	MS, O, RI	3-methylbutanoic acid	81	3	81	3	33[24]	2.8	3.5
1679	onion	*MS, O*, RI	2-methyl-3-(methyldithio)furan	27	0	0	1	0.3[31]	–	–
1681	creamy	MS, O, RI	diethyl succinate	0	1	27	0	200000[27]	<0.01	<0.01
1693	fruity	MS, *O*, RI	ethyl 9-decenoate	0	0	27	0	n/a	–	–
1722	caramel, yeasty, burnt milk, bready	MS, O, RI	methionol	81	1	81	0	1000[24]	0.3	0.4
1727	passionfruit, tropical, thiols	MS, O, RI	3-SHA	27	0	27	1	0.004[32]	5.1	3.1
1784	green, woody	MS, *O*, RI	diethyl glutarate	1	0	27	0	5000[28]	–	–
1824	honey, floral	MS, O, RI	2-phenylethyl acetate	243	9	81	81	250[25]	0.6	0.2
1828	fruity, tobacco, woody, floral	MS, O, RI	β-damascenone	6561	0	19683	243	0.05[25]	62	74
1850	sweaty, acid	MS, O, RI	hexanoic acid	243	3	243	1	420[24]	10.1	4.2
1864	smoky, burnt plastic	MS, *O*, RI	*N*-(3-methylbutyl) acetamide	0	3	1	0	n/a	–	–
1870	smoky, bacon	MS, O, RI	guaiacol	81	1	27	1	9.5[24]	0.6	0.2
1923	roses, perfume	MS, O, RI	2-phenylethanol	19683	9	59049	81	10000[25]	1.3	1.3
2039	aniseed, caramel, popcorn	MS, O, RI	anisaldehyde	243	1	9	1	20[28]	3.6	1.5
2066	leesy, acidic	MS, O, RI	octanoic acid	27	0	9	0	500[24]	14.8	6.0
2207	caramel, coconut	MS, *O*, RI	δ-decalactone	81	0	81	1	386[24]	–	–
2277	sweaty	MS, O, RI	decanoic acid	27	1	27	1	1000[24]	3.3	2.1
2347	hospital, cheesy	MS, O, RI	diethyl tartrate	3	0	27	0	n/a	–	–
2578	pungent, coconut, acidic, sweet	MS, *O*, RI	phenylacetic acid	27	0	9	0	2500[13]	–	–

[a]RI, retention index calculated using a series of alkanes (C7−C40). [b]Summarized based on the comments from sniffers. [c]MS, mass spectrum matches with authentic compound and/or library; *MS* (italicized), mass spectrum matches with literature; O, odor matches with authentic compound; *O* (italicized), odor matches with literature; RI, retention index matches with literature and/or authentic compound. [d]Underlined

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Table 1. continued

compounds were tentatively identified. [e]Concentration in μg/L and literature reference as superscript number. In refs 13 and 31 the matrix was 10% water/ethanol solution at pH 3.2; in ref 24 the matrix was an 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH; in ref 25 the matrix was 10% water/ethanol solution; in ref 26 the matrix was 10% water/ethanol solution, adjusted to pH 3.5 with tartaric acid; in ref 27 thresholds were calculated using a 12% water/ethanol mixture; in ref 28 the thresholds of 1-hydroxy-2-propanone and 3-methylbutyl hexanoate were determined in beer, and the threshold of diethyl glutarate was determined in 18% water/alcohol solution with 100g/L sugar at pH 3.5 and the threshold of anisaldehyde was determined in water; in ref 29 as specified in Fenaroli's Handbook; in refs 30 and 32 the matrix was water/ethanol solution. [f]Not available. [g]"–" indicates the OAV of the compound was not determined either because its threshold was not available, its concentration was under the LOQ, or it was semiquantified.

compounds in wine. The FD factors of 2-phenylethanol and β-damascenone in the F-HS sample, in particular, showed them to be important odorants, but the values were much lower than the corresponding SAFE sample values due to the decrease in volatility of these odorants. Around the time these compounds eluted marked the point where odorants were no longer detectable in HS samples, and it may also indicate the stage at which odorants start to become overemphasized (in terms of sensory importance) in the SAFE samples.

Odorants in Fruity and Tropical Wine Styles. With regard to the different volatile compounds in the two rosé wines, there were several more odorants with an FD factor ≥3 in the fruity/floral sample compared to the tropical one (Table 1), such as 2,3-pentanedione and hexyl acetate. Ethyl and acetate esters related to fruity characters in rosé wines,[10,12−17] such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl butanoate, ethyl hexanoate, and 3-methylbutyl acetate, also had FD factors that were quite often higher in the fruity/floral sample. Whereas 3-methylbutyl acetate has been determined as an impact odorant in its own right if the concentration is high enough, individual ethyl esters arising during fermentation via the same pathways (i.e., esterification of branched versus straight chain fatty acids) do not have such a role, but can be considered as impact families.[1] Perceptual interaction of esters in the wine matrix can lead to additive and enhancing effects that modulate fruity notes, particularly the berry aromas of red wines.[30] Although not captured with the two extraction techniques employed here, the common sulfur volatile DMS can also interact with esters to enhance the perception of fruity aromas.[35] Esters such as ethyl 2-methylpropanoate and ethyl hexanoate have been deemed to be important in Pinot noir wine[36,37] and red wines made from either Merlot, Cabernet Sauvignon, or Grenache.[38] Ethyl 2-methylbutanoate was suggested as a key aroma compound in Dornfelder red wine[39] and was one of the volatiles with the highest FD factor in red wine made from Merlot or Cabernet Sauvignon.[38] For acetate esters, 3-methylbutyl acetate, responsible for confectionery and fruity notes in wine[25] and one of the key odorants in a Çalkarası rosé wine[12] and a selection of Australian rosé wines,[15] had an FD factor of 81 across the four samples, which was similar to the observation in Pinot noir wines.[36] 2-Phenylethyl acetate (honey, floral) also had high FD factors and was important in Çalkarası rosé wine.[12] Hexyl acetate, which has fruity characters,[36,40] was not very strong in samples except for F-SAFE, with a moderate FD factor at 27. Despite these esters being ubiquitous fermentation volatiles, grape variety and composition may play a role in their formation,[41] and given that rosé wines are made from different red grape varieties, it is interesting to observe parallels in the importance of these esters. The scarcely reported ester 3-methylbutyl hexanoate, which was detected only in SAFE samples with FD = 27 and 81 for tropical and fruity/floral samples, respectively, had caramel and yeast aromas. Diethyl esters of succinic, glutaric and tartaric acids, which arise during aging, were detected by sniffers with an FD of 27 in the F-SAFE sample. Diethyl succinate can be negatively associated with fruity aromas,[17] as can diethyl tartrate, although it has been suggested there was no direct effect on the fruitiness of wines.[42] Beside these compounds, ethyl lactate (yeasty) and ethyl 9-decenoate (fruity) were detected mainly with low FD factors in SAFE samples, indicating little contribution to aroma profile of these wines.

A group of higher alcohols were perceived and identified in extracts of the two wines. 2-Phenylethanol had the highest FD factor of all odorants in both SAFE samples, with a higher FD factor in fruity/floral extracts compared to tropical ones for both SAFE and HS extraction methods. These results parallel those for 2-phenylethanol having the highest FD factor in Pinot noir wine from New Zealand[36] and the United States,[37] Dornfelder red wine from Germany,[39] and aged red wine from Spain.[43] 3-Methyl-1-butanol, a commonly abundant higher alcohol, was one of the most powerful odorants (solvent and sweaty) in this study and received higher FDs in the fruity/floral sample, which in concert with other higher alcohols can induce perceptual changes to fruity aromas in wine.[44] 2-Methyl-1-propanol, another common higher alcohol, was observed with moderate FD factors and was more perceivable in tropical extracts (81 in T-SAFE and T-HS, 9 in F-SAFE, and 3 in F-HS). (*Z*)-3-hexenol, a grape-derived alcohol with green and grassy notes,[34] was detected only in the tropical SAFE sample with an FD value of 27. Methionol (meaty, bready, and yeasty), related to methionine content in grapes[45] and responsible for negative aromas potentially affecting red wine quality,[34] had FD = 81 in both SAFE samples. 2,3-Butanediol (floral, vanilla), which is a malolactic fermentation (MLF) product, apparently contributed more to the fruity/floral sample with FD = 3 for the extract obtained by SAFE. Aside from these higher alcohols, 2-methyl-2-butanol (plastic, solvent), 1-butanol (fruity, alcoholic), 3-methyl-1-pentanol (green, solvent), 1-hexanol (spicy, green), and 3-ethoxy-1-propanol (solvent, earthy) were all identified by AEDA, but none had an FD factor >27.

Volatile acids such as 3-methylbutanoic acid, acetic acid, and other even-numbered medium-chain fatty acids (C_4−C_{10}) and phenylacetic acid were detected by AEDA. Acetic acid (vinegar), the main volatile acid in wine,[4] had FD = 3 in both HS samples, but its FD for the fruity/floral sample was larger than the tropical sample using SAFE; this was reasonable given acetic acid may have a positive impact on the overall fruity aroma expression.[46] 3-Methylbutanoic acid, which had cheesy and sweaty aromas, had the same FD factors in both samples (81 for SAFE and 3 for HS) and was not differentiated according to the aroma styles of the two wines. Butanoic acid, which had similar sweaty notes in wine,[39] had the same FD factors as 3-methylbutanoic acid in both samples obtained by SAFE, and only slightly higher FD in fruity/floral sample with HS sampling. Hexanoic acid, with FD = 243 in both tropical and fruity/floral SAFE samples, was found to be a key odorant

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Table 2. Volatile Compound Concentrations (μg/L) Determined for Two Rosé Wines[a]

compound	tropical	fruity/floral	compound	tropical	fruity/floral
ethyl esters			**acids**		
ethyl butanoate	410.3 ± 35.6[b]	448.4 ± 34.4	acetic acid	189115 ± 2033	95969 ± 4035
ethyl hexanoate	1940 ± 93	699 ± 13	butanoic acid	1780 ± 57	850.6 ± 34.8
ethyl octanoate	5587 ± 404	2903 ± 163	hexanoic acid	4236 ± 62	1777 ± 103
ethyl decanoate	3407 ± 22	1194 ± 10	octanoic acid	7412 ± 70	3001 ± 43
ethyl dodecanoate	61.9 ± 0.6	46.4 ± 0.4	decanoic acid	3372 ± 166	2099 ± 83
ethyl lactate	15685 ± 234	9750 ± 582	dodecanoic acid	6546 ± 453	3189 ± 58
ethyl furoate	22.5 ± 0.5	28.3 ± 0.5	2-methylpropanoic acid	516.9 ± 24.2	437.3 ± 30.8
ethyl 2-methylbutanoate	0.66 ± 0.01	1.39 ± 0.12	3-methylbutanoic acid	93.7 ± 0.9	114.2 ± 6.5
ethyl 3-methylbutanoate	11.1 ± 0.2	21.1 ± 1.1	benzoic acid	1283 ± 70	1166 ± 8
ethyl 2-methylpropanoate	39.8 ± 3.9	45.6 ± 2.1	2-furoic acid	253.7 ± 14.4	121.7 ± 2.6
ethyl 9-decenoate[c]	2.01 ± 0.07	39.50 ± 0.70	phenylacetic acid[h]	1538 ± 36	4536 ± 74
diethyl succinate	1324 ± 38	1571 ± 122	**phenols**		
diethyl tartrate	641.6 ± 36	462 ± 31	guaiacol	6.00 ± 1.00	2.00 ± 1.00
diethyl glutarate[d]	68.4 ± 1.8	117.4 ± 6.6	4-ethylguaiacol	<10.0	<10.0
acetate esters			4-ethylphenol	<10.0	<10.0
butyl acetate	2.46 ± 0.13	1.31 ± 0.07	4-methylguaiacol	<1.00	<1.00
hexyl acetate	291.6 ± 0.2	108.0 ± 8.4	eugenol	<10.0	<10.0
octyl acetate	3.04 ± 0.05	7.55 ± 0.29	**aldehydes**		
2-phenylethyl acetate	143.8 ± 0.7	53.8 ± 0.8	hexanal	10.0 ± 0.5	11.0 ± 0.6
3-methylbutyl acetate	3235 ± 263	2623 ± 148	(*E*)-2-hexenal	0.50 ± 0.025	0.55 ± 0.028
benzyl acetate	7.77 ± 0.06	2.52	(*E*)-2-heptenal	<0.01	<0.01
other esters			(*E*)-2-octenal	0.02 ± 0.001	0.03 ± 0.002
3-methylbutyl hexanoate	5.93 ± 0.58	6.39 ± 0.43	(*E*)-2-nonenal	0.08 ± 0.004	0.53 ± 0.027
alcohols			2-methylpropanal	12.0 ± 0.6	16.0 ± 0.8
1-butanol	619 ± 55	531 ± 12	3-methylbutanal	5.8 ± 0.3	11.0 ± 0.6
1-hexanol	2754 ± 33	2191 ± 22	furfural	200.0 ± 20.0	105.0 ± 10.5
2-methyl-2-butanol	132.1 ± 11.0	130.7 ± 10.3	5-methylfurfural	<10.0	<10.0
3-methyl-1-butanol	136977 ± 949	168369 ± 2882	benzaldehyde	105.0 ± 5.3	81.0 ± 4.1
2-methyl-1-propanol	6800 ± 169	11540 ± 763	anisaldehyde	71.22 ± 3.37	29.25 ± 0.83
3-methyl-1-pentanol	85.9 ± 0.5	117.4 ± 2.3	phenylacetaldehyde	10.00 ± 0.50	8.40 ± 0.42
2-ethyl-1-hexanol	26.5 ± 0.3	31.9 ± 1.6	vanillin	<10	<10
3-ethoxy-1-propanol[e]	123.0 ± 5.0	57.6 ± 0.9	methional	<0.01	0.43 ± 0.02
benzyl alcohol	128.0 ± 2.1	91.2 ± 6.3	**ketones**		
2-phenylethanol	12653 ± 214	12520 ± 38	1-hydroxy-2-propanone[i]	240.0 ± 7.6	150.1 ± 39.2
(*Z*)-3-hexen-1-ol	193.5 ± 0.7	66.5 ± 0.7	2-hydroxy-3-pentanone[i]	166.5 ± 1.6	67.3 ± 7.8
(*E*)-2-hexenol	≤0.20[f]	≤0.20	3-hydroxybutan-2-one	646.0 ± 42.9	322.1 ± 1.3
methionol	311 ± 16	424 ± 21	2,3-butanedione	626.4 ± 27.5	1297 ± 31
furfuryl alcohol	123.4 ± 4.1	108.3 ± 7.3	2,3-pentanedione[j]	279.6 ± 6.7	363.0 ± 4.3
2,3-butanediol	31630 ± 1471	19457 ± 1535	**lactones**		
isoprenoids			γ-butyrolactone	17004 ± 1659	12075 ± 874
linalool	17.1 ± 1.4	5.9 ± 0.1	δ-valerolactone	42.5 ± 0.5	36.2 ± 1.2
α-terpineol	21.5 ± 0.1	31.9 ± 0.3	γ-decalactone	2.61 ± 0.01	2.07 ± 0.09
β-ionone	7.25 ± 0.03	7.23 ± 0.01	δ-decalactone[k]	1.03 ± 0.08	2.06 ± 0.21
β-damascenone	3.12 ± 0.16	3.70 ± 0.25	*cis*-oak lactone	<10	<10
dehydrolinalool[g]	0.99 ± 0.01	1.90 ± 0.34	*trans*-oak lactone	<10	<10
thiols			sotolon	0.75 ± 0.004	<0.01
4-MSP (ng/L)	≤1.1	≤1.1	**others**		
3-SH (ng/L)	532 ± 2	539 ± 1	mesityl oxide	≤0.5	≤0.5
3-SHA (ng/L)	20.2 ± 0.3	12.5 ± 0.1	2-methyl-3-(methyldithio)furan[i]	31.9 ± 0.2	23.9 ± 1.6
FT (ng/L)	≤1	≤1	*N*-(3-methylbutyl)acetamide[l]	8.8 ± 0.7	23.0 ± 1.6
BMT (ng/L)	1.90 ± 3.72	10.50 ± 0.07	homofuraneol	312.0 ± 15.6	132.0 ± 6.6

[a]Concentrations in μg/L unless specified otherwise. The aroma descriptors, thresholds, and OAVs of compounds can be found in Table 1 and in the Supporting Information (Table S3). [b]Values are shown as the mean ± SD (standard deviation) of duplicate analyses. [c]Equivalent to ethyl decanoate. [d]Equivalent to diethyl tartrate. [e]Equivalent to 1-butanol. [f]"≤" or "<" indicates the content was below the limit of quantitation (LOQ). [g]Equivalent to linalool. [h]Equivalent to dodecanoic acid. [i]Equivalent to 3-hydroxybutan-2-one. [j]Equivalent to 2,3-butanedione. [k]Equivalent to γ-decalactone. [l]Equivalent to 3-methylbutyl acetate.

in a Pinot noir study[37] and had a sweaty and acid smell. Decanoic acid (sweaty) and phenylacetic acid (pungent, acidic) had the same FD factors in both samples under each extraction technique and did not seemingly contribute to differences in tropical and fruity/floral wines. Contrarily, octanoic acid (leesy, acidic) had a larger FD factor in the tropical SAFE sample (FD

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= 27) compared with the fruity/floral extract (FD = 9), indicating that it might contribute more to the aroma characteristics of the tropical wine style.

Isoprenoids contributed markedly according to the AEDA results. Altogether, linalool, β-damascenone, and dehydrolinalool were detected with FD factors ≥3 in at least one sample. Most notably, β-damascenone had higher FD factors irrespective of extraction technique in fruity/floral extracts (F-SAFE = 19683 and F-HS = 6561) compared to tropical extracts (T-SAFE = 6561, T-HS = 0), which was in agreement with its fruity and floral aroma nuances. β-Damascenone has also been reported with the highest FD factor in Pinot noir wines[36] and aged red wine,[43] and may play a role in enhancing fruity characters in rosé wines.[10,13] Besides β-damascenone, the FD factors of the other isoprenoids were not above 27, indicating their limited contribution to overall wine aroma profiles.

Several ketones were identified by AEDA, with three of them being related to MLF. The first was 2,3-butanedione (butter, yogurt), also known as diacetyl, which had larger FD factors in fruity/floral samples (FD = 729/27) compared to the tropical samples (FD = 81/3) for each extraction method. This appears to be consistent with a GC-O study of premium Spanish red wines, in which it was described as having a strawberry aroma (as well as the usual lactic descriptor).[34] 2,3-Butanedione was generally higher in FD factor when the current results were compared with different white wines[47] (which do not necessarily go through MLF), but similar to that reported for a Barossa Valley Shiraz wine that had been through MLF.[40] The other two MLF products, 3-hydroxybutan-2-one (wet, sweaty) and 2,3-pentanedione (caramel, yogurt), behaved differently; 3-hydroxybutan-2-one was more important in the T-SAFE sample with FD = 27 compared to F-SAFE with FD = 9, whereas 2,3-pentanedione was higher in fruity/floral samples (FD = 9/3) with either extraction technique. Additionally, 1-hydroxy-2-propanone and 2-hydroxy-3-pentanone were detected, and both were associated with solvent aromas. 2-Hydroxy-3-pentanone seemed to be more important in the tropical sample with FD = 27 in T-SAFE, whereas 1-hydroxy-2-propanone had less contribution, with lower FD factors that did not seem to relate to the wine styles. Apart from the (hydroxy)ketones, two aldehydes were detected during AEDA. Anisaldehyde, which has been detected in sherry musts[48] and oak wood,[49] had aniseed, caramel, and popcorn nuances and was determined to have a large FD factor of 243 in the T-SAFE sample. Furfural (floral, candy, fruity), a Maillard-related volatile that can be released by contact with toasted oak,[33] contributed little to either sample with FD ≤ 3 in accord with its general unimportance as an aroma volatile, although indirect effects on aroma (increased oak intensity, decreased fruit intensity) have been noted previously.[50]

Two varietal impact compounds were identified by AEDA, namely, the polyfunctional thiol 3-SHA and the methoxypyrazine 3-isobutyl-2-methoxypyrazine (IBMP). 3-SHA, synonymous with Sauvignon blanc aroma and closely associated with tropical aroma in wines,[32] was detected with the same FD factor of 27 in both SAFE samples. On this basis it was not distinctly responsible for the tropical nuances of the Shiraz wine that was designated a tropical style. Unlike the somewhat special case of Sauvignon blanc wines,[20] the tropical style of this rosé wine was not solely driven by varietal thiols acting as character impact compounds, although they have been found in other studies to be important odorants in rosé wines.[12,13,15,17] On the other hand, IBMP is often found to be an impact compound in Sauvignon varietal wines, where it can be responsible for green bell pepper characters at very low levels (several ng/L).[51] Only the F-HS sample (obtained from the Cabernet Sauvignon wine) had FD = 27 for IBMP, and despite not being detected during quantitative analysis, it was clearly perceived by the sniffers.

Other compounds identified in this study were of mixed (including ill-defined) origins and included γ-butyrolactone (sweaty), δ-decalactone (caramel, coconut), and guaiacol (smoky, bacon), which were all determined in rosé wines previously.[12,13] 2-Methyl-3-(methyldithio)furan, which had a distinguishing onion aroma, was detected only in the T-SAFE sample with FD = 27. It has previously been found in red wines and could potentially arise via Maillard reaction of ribose and cysteine, followed by mixed disulfide formation with methanethiol.[31] *N*-(3-Methylbutyl)acetamide, which had smoky and burnt plastic characters, has formerly been determined in Amarone[52] and fortified wines,[53] and its concentration was found to increase with a longer skin-contact time.[54] This latter observation suggested that it could be a marker of the extent of maceration on skins during rosé wine production.

Volatile Compound Quantitation and Calculation of OAVs. In one of the most comprehensive assessments of volatiles accomplished on rosé wine to date, a total of 92 compounds were quantified for two different wine styles (Table 2). Among these, esters, alcohols, and volatile acids, mainly arising during fermentation, together accounted for more than half of the total, with the remaining compounds detected and quantified comprising phenols, carbonyls, lactones, isoprenoids, thiols, and furans.

Along with using an existing SPME scan method[15] to quantify 27 volatiles and several other published methods[18−22] for a number of specific compounds, an SPME-GC-MS SIM method was developed to quantify a further 34 compounds. The R^2 values of each calibrated analyte were ≥0.99, and calibrations were linear across the concentration range (Supporting Information, Table S2). Precision at low and high concentrations ranged from 1 to 17%, and recoveries varied between 91 and 118%. LOQ values were below the reported aroma detection thresholds for the analytes.

Most compounds were detected and quantified in both tropical and fruity/floral wine samples at concentrations that were consistent with other rosé wine studies.[10,12−15] The largest OAVs (Table 1) were obtained for ethyl octanoate (green, fruity) in both tropical (OAV = 279) and fruity/floral (OAV = 145) samples, which suggested it had a large contribution to both aroma styles, but perhaps more so for the tropical style wine. Ethyl octanoate was similarly found with a large mean OAV (135) in a study of 26 Australian rosé wines[15] and had the fourth largest OAV in Grenache rosé.[13] Ethyl hexanoate (confectionery and strawberry) had the second largest OAV in the tropical style wine (OAV = 139) as did 3-methylbutyl acetate (banana) in the fruity/floral wine (OAV = 87), with these high OAV results being consistent with previous studies on rosé wines;[12,15] both esters had substantially higher OAVs in the tropical wine in contrast to the fruity/floral one. The C_{13}-norisoprenoid β-damascenone had OAVs ≥ 50 for both samples, which was entirely consistent with the large FD factor obtained for this compound by AEDA. These results accord well with other research, in which ethyl hexanoate, 3-methylbutyl acetate, and β-damascenone were determined as the top three odorants with the largest OAVs in Çalkarası rosé[12] and were among the top five most important odorants in

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Grenache rosé[13] and a set of Australian rosé wines.[15] Other esters, such as ethyl decanoate, along with butanoic, hexanoic, and octanoic acids, had larger OAVs (at least double) in the tropical wine compared to the fruity/floral one. Tropical odorant 3-SHA had a 1.6-fold larger OAV in the tropical wine (OAV = 5.1), which could potentially help to further differentiate the two styles being studied. In contrast, 2,3-butanedione, ethyl butanoate, ethyl 3-methylbutanoate, and 3-methyl-1-butanol had larger OAVs in the fruity/floral sample, with the two esters in particular likely to be driving the fruity characters. On the other hand, 3-methyl-1-butanol can attenuate fruity characters in model solution[44] but has also been described as intensifying berry notes when added to dearomatized red wine,[14] which implies a variable effect depending on the matrix. It was interesting to note that although 2-phenylethanol was suggested as an important volatile from the AEDA results, on the basis of calculated OAVs it was seemingly not the case and only just reached values above its threshold (OAV = 1.3).

Compounds not detected by AEDA were quantified at levels (Table 2) mostly below or around their corresponding aroma detection thresholds (i.e., OAV < 1, Supporting Information, Table S3), except for four compounds, β-ionone, 3-SH, BMT, and phenylacetaldehyde. β-Ionone, which has descriptors such as violet and fruity aroma,[36] was determined with an OAV of around 80 in both wines. This is a substantially greater value than previously found for other rosé wines, in which β-ionone had an OAV of 3−6.[10,12] 3-SH contributes grapefruit aroma to wine[32] and had OAV around 9 for both samples, which accords well with other rosé wine studies[10,12] but contrasts with 3-SH having the largest OAV among odorants in Grenache rosé (OAV = 67).[13] BMT, which seems not to have been reported previously in AEDA studies on wine, was determined to have OAV = 35 in the fruity/floral sample, with this magnitude being consistent with previous rosé wine studies.[10,12] Phenylacetaldehyde, which induces honey and floral notes,[22] had OAV = 10 in the tropical wine and OAV = 8.4 in the fruity and floral one. Despite the relatively large OAVs, the lack of any sizable difference between the two rosé wines for these particular compounds, aside from BMT, indicated that in isolation they did not differentiate the different sensory styles.

To summarize the outcomes of this work, when the AEDA results for the two isolation methods are compared, HS was as good as SAFE for odorants eluting at the beginning of a GC run with a DB-Wax column. These compounds were primarily esters and higher alcohols associated with floral and fruity characters. As the RI of analytes increased, the exhaustive extraction ability of LLE became more evident; the compounds with higher boiling points and lower K_{aw} (air−water partition coefficient) were difficult to sample by the dynamic HS-SPE technique. However, for wine that was mostly fruity and floral driven, HS was sufficient to extract aroma compounds that were reflective of the sensory characters of the wine. From another perspective, HS was environmentally friendly compared with SAFE as the former was almost solvent free. Despite this advantage, light volatiles such as DMS were not captured by either extraction method, and it seems that AEDA information from at least two sample preparation strategies (i.e., static and dynamic HS methods or static HS and SAFE) is needed to determine a representative aroma model for olfactory analysis.

From the AEDA study and quantitative analysis, 2-phenylethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl 2-methylpropanoate, 3-methyl-1-butanol, 2-phenylethanol, hexanoic acid, and one grape-derived compound, β-damascenone, were deemed to have an important impact on sensory profiles in both samples. In particular, compounds such as 2-phenylethanol, β-damascenone, and a range of esters more associated with fruity and floral characters were apparently important to the fruity/floral rosé wine, whereas 3-SHA and several volatile acids were more related to the tropical style wine. The study was also explained in terms of OAVs calculated from quantitative results and published thresholds; ethyl octanoate, 3-methylbutyl acetate, ethyl hexanoate, β-damascenone, and β-ionone all had relatively large OAVs, along with BMT to a lesser extent. Within these compounds, the three esters were more associated with the tropical wine, whereas β-damascenone and BMT apparently contributed more to the fruity/floral wine. The concentration of β-ionone was not different between the two samples.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b01030.

Wine basic chemical composition; method characteristics for volatile compounds determined by HS-SPME-GC-MS SIM; aroma properties for volatile compounds quantified; customized flask for conducting HS-SPE analysis (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; LLE, liquid−liquid extraction; SAFE, solvent-assisted flavor evaporation; HS-SPE, headspace-solid phase extraction; FD, flavor dilution; 3-SHA, 3-sulfanylhexyl acetate; GC-O, gas chromatography−olfactometry; GC-MS, gas chromatography−mass spectrometry; RI,

retention index; OAV, odor activity value; HS-SPME, headspace-solid phase microextraction; 3-SH, 3-sulfanyl-1-hexanol; TA, titratable acidity; ODP, olfactory detection port; DA, descriptive analysis; SIM, selected ion monitoring; LOD, limit of detection; LOQ, limit of quantitation; 4-MSP, 4-methyl-4-sulfanylpentan-2-one; FT, furfurylthiol; BMT, benzenemethanethiol; IBMP, 3-isobutyl-2-methoxypyrazine; DMS, dimethyl sulfide; K_{aw}, air−water partition coefficient

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Research Note

Prediction of Phenolic Composition of Shiraz Wines Using Attenuated Total Reflectance Mid-Infrared (ATR-MIR) Spectroscopy

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Abstract: Phenolic compounds play a critical role in determining red wine color, taste, flavor, and mouthfeel sensory attributes. Additionally, they contribute to wine aging and provide wine stability. This study evaluated the use of an attenuated total reflection (ATR) mid-infrared (MIR) spectroscopy to measure phenolic compounds in Shiraz wine samples of different quality levels obtained from 24 Australian wine geographical indications. Partial least squares (PLS) regression using the second derivative of the whole MIR spectrum produced the coefficient of determination (R^2) in calibration and standard error in cross-validation (SECV) for different attributes. In particular, SO_2-resistant pigments had R^2 = 0.58 (SECV=0.58 au), total anthocyanins had R^2 = 0.61 (SECV=32 mg/L), wine color density had R^2 = 0.51 (SECV=0.56 au), and total phenolics had R^2 = 0.60 (SECV=5.7 au). These results demonstrated the potential use of ATR-MIR spectroscopy with PLS regression as a rapid method to measure important parameters related to red wine phenolic composition and wine quality.

Key words: anthocyanins, chemical composition, phenolics, quality, SO_2-resistant pigments

Phenolic compounds from red wine grapes play a major role in red wine color, flavor, and mouthfeel sensory attributes (Kennedy et al. 2006). Additionally, they influence wine aging and provide several health benefits due to a range of bioactivities (Friedman 2014). Wine phenolic compounds mainly originate from grapes, but they also arise after microbial modifications and through winemaking additions (e.g., oak and enological tannins). As such, their concentrations depend on the grape variety, harvest date, viticultural management, and applied winemaking techniques (Downey et al. 2006, Kennedy et al. 2006). Red wine phenolic composition is crucial to wine quality, and several studies have reported strong correlations between wine color density (WCD), anthocyanins, tannins and total phenolics, and wine quality score/grade and/or retail price (Mercurio et al. 2010, Ristic et al. 2010, Kassara and Kennedy 2011, Caceres et al. 2012).

Wine color intensity plays an important role in consumer preference (Parr et al. 2002) and perceived wine quality (Parpinello et al. 2009). Wine consumers preferred moderate levels of tannins and astringency in Shiraz wines (Bastian et al. 2010), whereas winemakers and highly knowledgeable wine consumers acknowledge that higher amounts of tannins balanced with other wine components are required to achieve greater wine complexity, structure, and stability. Given the inter-relationship between winemaking practices and manipulation of red wine quality to meet consumer preferences, there is still general interest in developing rapid (and potentially real-time) methodologies to analyze wine color and phenolic composition.

Multiple analytical approaches, including spectrophotometric and chromatographic methods, can be employed to determine the phenolic composition of wines. Wine color and total phenolic composition have been analyzed routinely using simple but timely UV-vis spectral measurements developed by Somers (Iland et al. 2004, Mercurio et al. 2007). Although HPLC applications with diode array or mass spectrometric detection are precise, they are time-consuming and expensive to use for routine analysis, requiring several pre-processing steps (e.g., sample extraction and dilution) and specific operational requirements (Lorrain et al. 2013). In contrast, methods and techniques based on infrared (IR) spectroscopy are rapid, can be non-destructive, require minimal preparation of samples, and provide significant time and cost savings. Several

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studies have applied mid-infrared (MIR) spectroscopy using short path-length cells (transmission) for the evaluation of wine characteristics, including origin and variety (Bevin et al. 2008, Cozzolino et al. 2011a, Riovanto et al. 2011), sparkling wine style and quality (Culbert et al. 2015), red wine tannins (Mercurio et al. 2010, Cozzolino 2015, Ricci et al. 2015), and smoke taint in wines (Fudge et al. 2012). However, few studies have employed attenuated total reflectance (ATR) in conjunction with MIR for wine analysis (Cozzolino 2015).

ATR is a sampling technique combined with IR spectroscopy that allows direct examination of samples in the solid or liquid state (Milosevic 2012, Ricci et al. 2013). Previous studies demonstrated the application of Fourier transform MIR (FT-MIR) spectroscopy to measure sugars and other compositional parameters in various beverages, including fruit juices, milk, beer, olive oil, and wine samples using a transmission cell as the sampling attachment (Bevin et al. 2008, Cozzolino et al. 2011b, Fudge et al. 2012). Recent developments of ATR cells have simplified sample handling and minimized common measurement issues associated with transmission cells, which has considerably improved the routine analysis of liquid samples using IR spectroscopy (Milosevic 2012).

This study evaluated the use of an ATR cell combined with MIR spectroscopy and chemometrics to measure phenolic compounds in Shiraz wine samples of different quality levels obtained from 24 Australian wine geographical indications (GIs).

Materials and Methods

Wine samples. One hundred Australian Shiraz wines were sourced from a subset of 24 wine GIs (http://www.wineaustralia.com/en/Production and Exporting/Register of Protected GIs and Other Terms/Geographical Indications.aspx) across Australia as follows: Barossa Valley (24), McLaren Vale (16), Margaret River (8), Yarra Valley (7), Adelaide Hills (6), Coonawarra (5), Heathcote (5), Riverina (4), Clare Valley (4), Great Southern (3), Hunter Valley (3), Grampians (2), Geographe (2), Canberra (1), Central Ranges (1), Orange (1), Beechworth (1), Murray Darling (1), Bendigo (1), Great Western (1), Pyrenees (1), Rutherglen (1), King Valley (1), and Swan Valley (1). The vintages dated from 2009 to 2013, and the retail prices ranged from AU$3 to AU$185. Selection of the wines was based on a market report of the highest selling Shiraz wines in different price categories, wine show results, popularity among wine consumers, and recommendations from wine experts.

Wine sensory assessment. An expert panel of eight wine industry personnel was convened, with the panelists meeting the definition of expert according to Parr et al. (2002). There were two female and six male judges between 25 and 65 years old. Under blind conditions, judges were asked to rate all wines on a scale from 1 to 20, following the conditions of the Australian 20-point wine show scoring system (Rankine 1986, Dunphy and Lockshin 1998), and place them consensually into one of four quality categories based on the Australian wine show system of gold (G), silver (S), or bronze (B) medal, or no medal (NM) but of sound quality.

Spectral analysis of wines. All 100 wines were analyzed in triplicate using modified Somers measurements (Mercurio et al. 2007) and ATR-MIR spectroscopy as detailed in previous reports (Riovanto et al. 2011, Culbert et al. 2015).

Statistical analysis. ATR-MIR spectra were exported in GRAMS format (*.spc) from the OPUS software into The Unscrambler X software (version 10.1, CAMO ASA) for preprocessing and chemometric analysis. Spectral data were processed using the second derivative of Savitzky-Golay (40 smoothing points and second polynomial order) to remove and correct for baseline variation, followed by chemometric analysis (Savitzky and Golay 1964, Naes et al. 2002).

Initial principal component analysis (PCA) was performed to determine relevant and interpretable structure in the data and detect sample outliers. The PLS regression (PLS1 algorithm) models were developed using Hotelling's T^2 statistic with 95% confidence ellipse included in the score plot to reveal potential outliers lying outside the ellipse (Naes et al. 2002). Samples were divided into calibration (n = 70) and validation (n = 30) sets at a ratio of ~3:1 as previously proposed, taking into consideration that the range of the actual values in the calibration set covered the values in the validation set (Brereton 2000). Calibration models were developed using PLS regression with full cross-validation (Naes et al. 2002). The PLS models were evaluated using the coefficient of determination in cross-validation (R^2) and standard error of cross-validation (SECV), as well as residual predictive deviation (RPD = SD/SECV) (Williams 2001, Naes et al. 2002). Correlation coefficients for wine quality, retail price, and phenolic composition were obtained using the statistical package XLSTAT version 4.07, 2014 (Addinsoft SARL).

Results and Discussion

Relationship of WCD and phenolic composition with wine quality score of Shiraz wines. One hundred commercial Shiraz wines from 24 Australian wine GIs were assigned by an expert panel into four quality levels (gold, silver, and bronze medals, or no medal) based on intrinsic wine attributes such as color, aroma, flavor, and mouthfeel properties. Group G (gold medal, highest quality) contained 10 wines originating from Barossa Valley (5), Clare Valley (2), Great Western (1), and McLaren Vale (2), with retail prices ranging from AU$8 to $120. Group S (silver medal) included 19 wines sourced from Barossa Valley (8), McLaren Vale (3), Adelaide Hills (2), Margaret River (1), Grampians (1), Hunter Valley (1), Geographe (1), Yarra Valley (1), and Heathcote (1) with prices ranging from AU$9 to $155. The third quality group (B, bronze medal), had 39 wines from 17 wine regions, including Barossa Valley (6), McLaren Vale (6), Adelaide Hills (4), Coonawarra (4), Yarra Valley (4), Margaret River (3), Great Southern (2), Central Ranges (1), Clare Valley (1), Geographe (1), Heathcote (1), Hunter Valley (1), King Valley (1), Pyrenees (1), Riverina (1), Rutherglen (1), and Swan Valley (1) (AU$7 to $185). The remaining 32 wines from 16 wine regions were placed in the fourth group (NM, no medal) and priced at AU$3 to $115.

WCD and phenolic composition of the 100 Shiraz wines were determined to assess their relationship with wine quality level assigned by experts and wine retail price. WCD (sum of pigments absorbing at 420 and 520 nm), wine hue (ratio of absorbance at 520 and 420 nm), SO_2-resistant pigments (a measure of stable [non-bleachable] red wine color), and total phenolics (based on absorbance at 280 nm) showed significant correlations with wine quality level and retail price (Table 1). The wines in group G were characterized by higher WCD (10.5 to 16.4), wine hue (0.74 to 0.94), content of total anthocyanins (70.9 to 259.5 mg/L), SO_2-resistant pigments (2.9 to 5.7 au), and total phenolics (39.8 to 65.9 au). The group S wines had wider ranges of WCD (9.3 to 16.1), wine hue (0.72 to 0.93), total anthocyanins (21.6 to 203.6 mg/L), SO_2-resistant pigments (2.8 to 6.0 au), and total phenolics (35.2 to 64.5 au), which reflected larger differences in wine age (Supplementary Table 1). For the third and fourth quality groups, the ranges were as follows: WCD, 6.8 to 18.3 (B) and 4.3 to 14.0 (NM); wine hue, 0.71 to 0.94 (B) and 0.73 to 1.00 (NM); total anthocyanins, 29.2 to 218.2 mg/L (B) and 27.4 to 206.9 mg/L (NM); SO_2-resistant pigments, 2.2 to 7.1 au (B) and 1.8 to 5.6 au (NM); and total phenolics, 32.5 to 65.9 au (B) and 30.1 to 70.7 au (NM). WCD and phenolic composition have been shown to exhibit positive relationships with wine quality score or grade (Mercurio et al. 2010, Ristic et al. 2010, Kassara and Kennedy 2011). Phenolic compounds, such as monomeric anthocyanins, stable wine pigments, and tannins (Figure 1), contribute to wine color and in general, wines with deeper color (i.e., higher WCD) had a greater concentration of phenolic compounds as well as higher flavor intensity and body according to sensory assessment, and consequently received higher quality scores. Unsurprisingly, these types of wines are usually sold at higher prices (Mercurio et al. 2010, Kassara and Kennedy 2011, Fanzone et al. 2012).

Table 1 Value ranges for phenolic parameters and pairwise correlations (and significance) of wine color and phenolic measurements with quality category and retail price.

Phenolic parameters	Range	Quality category		Retail price	
		r[a]	*p*-value	r[a]	*p*-value
Wine color density	4.3–16.4	0.50	<0.001	0.44	<0.001
Wine hue	0.73–1.00	-0.16	0.01	0.24	<0.001
Anthocyanins (mg/L)	19.4–259.6	0.10	ns[b]	-0.31	<0.001
SO_2-resistant pigments	1.8–7.1	0.38	<0.001	0.37	<0.001
Total phenolics (au)	30.0–65.9	0.36	<0.001	0.40	<0.001

[a]r, correlation coefficient.
[b]ns, not significant.

In the current study, significant positive correlations between wine quality level and i) wine retail prices ($p < 0.001$), and ii) wine geographical indications ($p < 0.05$), have been established, but not between GIs and wine retail price. Wine price is generally assumed to be an indicator of wine quality, although a price increase is not necessarily paralleled by an increase in quality in the Australian wine market. The high quality wines may be bought at relatively lower price points, and very often, the wine region and winery reputation are better indicators of wine quality and retail price (Horowitz

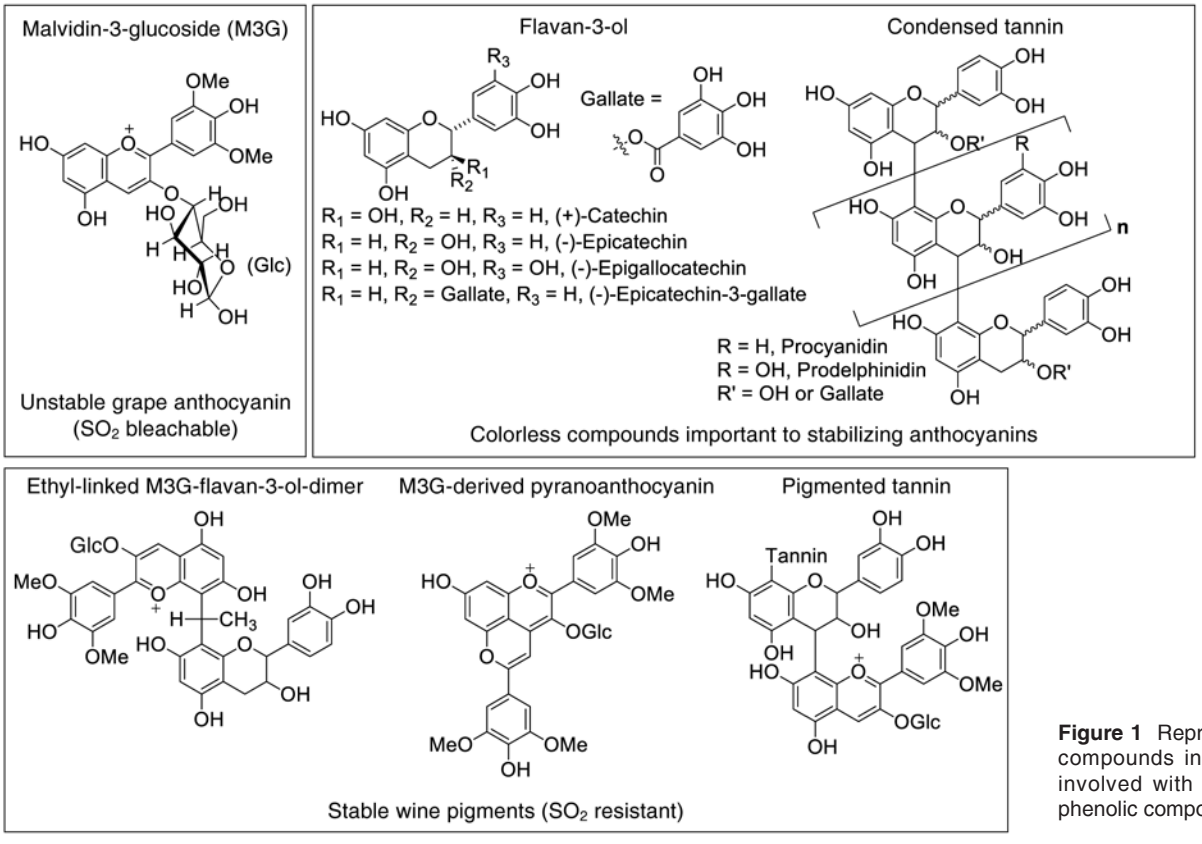

Figure 1 Representative compounds in red wine involved with color and phenolic composition.

and Lockshin 2002, San Juan et al. 2012). However, it should be noted that selection of Australian commercial Shiraz wines for this study was driven by the need to include a range of wine qualities, and distribution of wines was uneven across wine regions.

ATR-MIR spectroscopy of Shiraz wines. A comparison of the ATR-MIR spectra showed that most of the variation occurred in the region between 1700 and 800/cm. However, the loadings indicated that the different compounds in the region between 1350 and 900/cm best contribute to the calibrations for the measured parameters, thus, this study focused on this "fingerprint" region. The deconvoluted ATR-MIR mean spectrum (second derivative) of wine samples is shown in Figure 2. Strong and characteristic absorbance peaks attributed to sugars (i.e., glucose, fructose), polysaccharides, and other carbohydrates are observed between 1500 and 900/cm (Subramanian and Rodrigez-Saona 2009, Shah et al. 2010), whereas the peaks arising between 1600 and 1000/cm are associated with the CH-OH and alkyl functionalities of sugars. Furthermore, peaks in the spectral region between 1500 and 1200/cm were attributed to deformations of C–C–H, CH_2, and H–C–O groups, whereas stretching modes of C–C and C–O bonds appear between 1200 and 950/cm (Subramanian and Rodrigez-Saona 2009, Shah et al. 2010). Other absorption bands were observed in the carbohydrate region between 1200 and 900/cm (C–O–C), indicating the presence of carbohydrate-like components (Subramanian and Rodrigez-Saona 2009, Shah et al. 2010). The amide I and II groups, proteins, and water are associated with absorption between 1635 and 1550/cm (Subramanian and Rodrigez-Saona 2009, Shah et al. 2010). The region between 1100 and 1000/cm can be assigned to aromatic groups, such as C-C and C-C-O functional groups, that are associated with different phenolic compounds present in red wine.

Calibration statistics and interpretation. Figure 3 shows the score plot of the first two principal components (PCs) obtained from the ATR-MIR spectra in the calibration set. The first two PCs explain 89% of the variation (PC1 66.8% and PC2 22.2%) in the ATR-MIR spectra of the analyzed Shiraz samples. From the PCA, some separation was observed between samples according to quality level (particularly with gold medal wines), indicating that the MIR spectra contains information associated with specific characteristics (e.g., sugars and phenolics) of the wines that relate to their phenolic composition and quality.

The eigenvectors derived from the PCA (not shown) indicated patterns in the spectra similar to those described below. Consistent with this result, the types of compounds that contribute to red wine color and phenolic composition (Figure 1) have absorptions in the specific MIR regions identified by PLS loadings because of the functional groups associated with anthocyanins and polyphenols. The mean and standard deviation (SD) for the content of phenolic components measured in the set of Shiraz wines are shown in Table 2. Although the samples showed wide variability in composition,

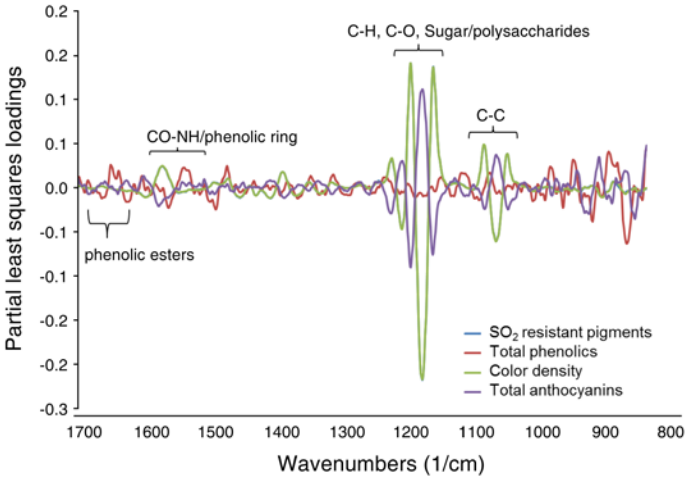

Figure 2 Partial least squares loadings derived from the calibration models (second derivative or mid-infrared spectra in the fingerprint region, showing tentative functional group assignments).

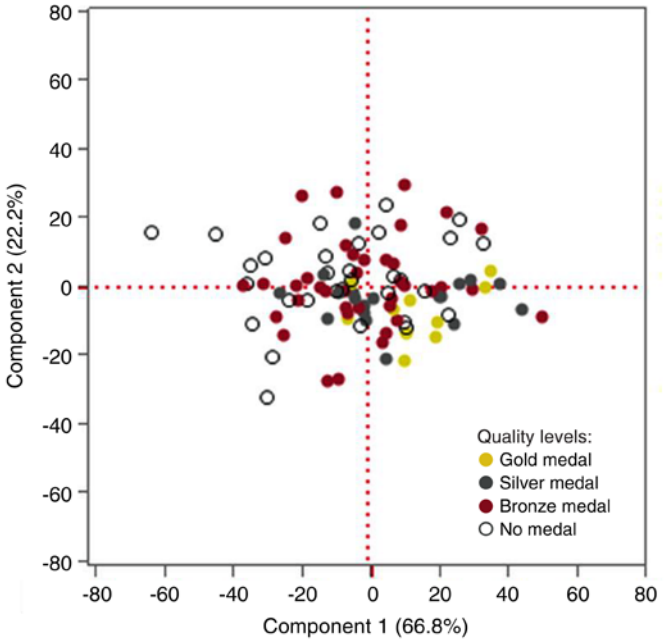

Figure 3 Principal component score plot of 100 Australian Shiraz wines analyzed using attenuated total reflectance mid-infrared fingerprint region (1700 to 800/cm). Quality levels were assigned by the expert panel based on the Australian 20-point wine show scoring system.

Table 2 Descriptive (mean and SD) and partial least squares cross validation statistics for the wine phenolic parameters predicted using attenuated total reflectance mid-infrared spectroscopy.

	Mean	SD[a]	R^2	SECV[a]	RPD[a]	PLS[b]
Wine color density	10.7	2.7	0.51	0.56	4.8	4
SO_2-resistant pigments (au)	3.5	1.1	0.58	0.58	1.8	3
Total anthocyanins (mg/L)	128.1	55.4	0.61	32	1.8	3
Total phenolics (au)	43.8	8.2	0.60	5.7	1.5	3

[a]SD: standard deviation, SECV: standard error of cross validation, RPD: SD/SECV.
[b]Number of partial least squares (PLS) loadings used to develop the calibration models.

the analytical values observed in this set of Shiraz wines were in accordance with those reported by other authors (Mercurio et al. 2010, Kassara and Kennedy 2011, McRae et al. 2012).

After obtaining the second derivative of the whole MIR spectrum, PLS regression provided the coefficient of determination in calibration (R^2) and SECV for the different wine composition attributes (Table 2). From the calibration models, SO_2-resistant pigments had $R^2 = 0.58$ (SECV = 0.58 au), total anthocyanins had $R^2 = 0.61$ (SECV = 32 mg/L), WCD had $R^2 = 0.51$ (SECV = 0.56 au), and total phenolics had $R^2 = 0.60$ (SECV = 5.7 au). These promising results highlighted the potential of using ATR-MIR spectroscopy with PLS regression to rapidly measure important parameters related to the phenolic composition of wines.

Further evaluation of the statistics using RPD values, which varied between 1.5 and 2.5 for most of the wine parameters, implied that the PLS calibrations are best utilized for qualitative measurements. In contrast, WCD had an RPD value of 4.8, indicating that the calibration can be readily used to predict this parameter in wine. Previous reports indicate that a large SECV compared with the range of compositions (based on SD) leads to a comparatively small RPD value, meaning that the resulting PLS calibration model may not be sufficiently robust for routine analysis (Williams 2001). On the contrary, a higher RPD value (>3) increases the ability of the model to accurately predict the measured variable in new samples. Lower values for RPD may occur when reference values fall within a narrow range (small SD) or when SECV is large relative to the variability of reference values (Williams 2001). Despite the positive results, especially for WCD, analysis of more samples is necessary to extend the range of phenolic compositions covered to obtain more robust calibrations.

The loadings were used to define the variables (i.e., wavenumbers) that are key to describing variation in the data set, as well as to identify unusual variables and to determine the characteristic dimensionality of the data (Karoui et al. 2010). The PLS loadings indicated that regions in the MIR spectra (1100 to 1000/cm) related to aromatic, C-C, and C-C-O functional groups were most important for explaining the calibration models for the color and phenolic parameters analyzed (Figure 2). This is a sensible result considering the specific IR-active functional groups present in wine phenolic compounds.

Conclusion

An expert panel assessed wine quality for 100 Shiraz wines at different price points and from different regions, and WCD, SO_2-resistant pigments, and total phenolics were measured for each wine. Relationships between wine quality level and i) retail price ($p < 0.001$), and ii) wine region ($p < 0.05$), were established, and typical red wine phenolic measures had strong positive correlations with wine quality level and retail price. Wines with higher WCD had a greater concentration of phenolic compounds, and consequently received higher quality scores. In conjunction, ATR-MIR spectroscopy combined with PCA and PLS regression was evaluated as a rapid analytical technique to predict red wine phenolic parameters. This study demonstrated that the second derivative of the MIR spectra predicted WCD and aspects of phenolic composition of Shiraz wines measured by standard techniques. In addition, the MIR spectra contained relevant functional group information related to the wine color parameters and enabled some separation of wine quality by PCA. The results were very promising, particularly for WCD, although analysis of more samples is necessary to extend the range of phenolic compositions covered to improve the robustness, specificity, and accuracy of MIR calibration. Further studies should also include flavor profile and sensory assessments of wines.

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