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Grape quality parameters that influence wine flavour and aroma: identification, confirmation and application to industry.



FINAL REPORT to

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Grape quality parameters that influence wine flavour and aroma: identification, confirmation and application to industry (CSP1201).

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2 Abstract

Cabernet Sauvignon and Chardonnay grape sensory attributes and chemical compositions were mined to predict relationships with wine sensory characteristics. In parallel, winemaker panels defined sensory characteristics associated with quality Cabernet Sauvignon or Chardonnay wines. Relating the sensory characteristics of Cabernet Sauvignon or Chardonnay grapes to wines was challenging, as was modelling the entire wine sensory space using grape measures. However, modelling individual wine characteristics successfully linked blocks of grape measures to wine attributes. Knowledge generated from this project will form the basis for future development of measures of grape flavour potential and strategies to produce fit for purpose fruit.

3 Executive Summary

Considerable research into the chemical basis for sensory attributes in wine has been undertaken but there has been less focus on understanding the links of grape composition to wine chemistry and wine sensory properties. In limited cases, a wine sensory attribute can be assigned directly to a specific grape metabolite; for example, pepper due to rotundone, green capsicum resulting from 3-isobutyl-2-methoxypyrazine, and floral characters from monoterpenes. Beyond that, sugars, amino acids, lipids, micronutrients, and other grape constituents will contribute to the suite of compounds produced during winemaking that are important to wine sensory properties. An increasing body of evidence emphasises the importance of grape composition on the potential of a wine to have certain sensory characteristics. Nonetheless, the previously identified gap in the literature still remains, and there is a lack of knowledge that explains how parcels of grapes from the same variety, and possibly same vineyard, can result in very different wine sensory outcomes. In addition, there is little information tracing vineyard management practices and effects of the environment on production of grape metabolites that subsequently influence wine chemistry and sensory. With regards to "quality" measures, some success has been achieved with red grape colour and this project aimed to develop other measures that predict wine sensory outcomes.

The project methodology consisted of three main parts. 1) In each of the first three vintages of the project (2013-15), 25 Cabernet Sauvignon grape parcels were obtained from our industry partners from both warm and cool growing regions and vinified using an identical small-scale fermentation protocol. A subsample of each grape parcel was analysed using multiple methods to quantify various classes of compounds including: amino acids, volatile compounds, bound volatile compounds, anthocyanins, flavonols, tannins, fatty acids and total phenolics. Other measures such as CIELab colour, normal harvest parameters and activities from lipoxygenase pathway enzymes were also conducted, as well as berry sensory analysis (BSA). The corresponding wines were analysed by a trained descriptive analysis (DA) sensory panel and rated for quality by a winemaker panel. The multiple datasets were then analysed for predictive relationships. 2) A similar experimental setup was applied to Chardonnay for two vintages (2015-16). Again, 25 grape parcels were obtained each year from our industry partners in vineyards spread across South Australia. These were vinified using a controlled protocol, and the wines profiled by DA and assessed for quality by a winemaker panel. BSA was conducted on the grape parcels and measures deemed to be relevant to Chardonnay fruit composition were applied to the fruit. Predictive relationships among the data were explored in a similar way to Cabernet Sauvignon. 3) Biochemical methods were used to study the pathways of two classes of secondary metabolites during Cabernet Sauvignon berry development, to explore whether genetic and biochemical markers could indicate changes in berry metabolism.

The study of Cabernet Sauvignon over three vintages showed that the major driver of differences in the sensory attributes of the wines was the region of origin. In general, the wines from the Riverland had lower sensory scores for dark fruit flavour and aroma, body, overall flavour and aroma and astringency, compared to the wines from other regions. These same wine sensory characteristics were identified by the winemaker sensory panel as indicators of higher quality, whereas lower quality wines were described as green, simple and poor in colour. Interestingly, some Riverland Cabernet Sauvignon wines that were graded higher than others, possessed "balance", which is a holistic sensory percept that the detailed sensory profiling did not capture. To achieve a more complete understanding of wine quality, the drivers for concepts such as balance (and complexity), and not merely a list of specific wine sensory attributes, clearly require further investigations.

Because the study involved such a large number of data sets collected during an extensive metabolomics analysis of the grapes (12 data blocks for Cabernet Sauvignon, 9 for Chardonnay), a novel data analysis method called sequential and orthogonalised partial least squares (SO-PLS) was used to select data blocks that were predictive of wine sensory attributes. It also highlighted the data blocks that were least frequently used to model sensory perception and can likely be removed from future studies. The current study confirmed previous findings that some grape volatile measures are important for modelling sensory

attributes in both Cabernet Sauvignon and Chardonnay wines. However, many other data blocks, arising from the quantification of groups of grape metabolites beyond grape volatiles, were better at modelling a range of wine sensory characteristics, and novel correlations between particular sensory attributes and blocks of grape metabolites were demonstrated. The grape target compounds may not necessarily be precursors to wine aroma volatiles, but may act as markers that indicate altered berry metabolism and composition. These grape biochemical markers of wine sensory outcomes would be useful in streaming or grading fruit once suitable protocols for their measurement can be developed and verified.

Winemaker quality assessment proved successful with the Cabernet Sauvignon wines but did not significantly discriminate the Chardonnay wines. When vinified with a simple, identical protocol, sensory differences across Chardonnay wines were detectable albeit subtle, and these results suggest that quality drivers of commercial Chardonnay wines are most likely not derived solely from grapes. Sensory differences were not consistent across the vintages and often did not relate to regionality. All nine blocks of grape measures were used in the models developed to predict wine sensory attributes so there appear to be many potential indicators of Chardonnay wine flavour. Our results suggest that vinification factors likely contribute more to the variation in sensory characteristics of commercial Chardonnay wines than the grapes, and perhaps grape measures of quality would be more suited to red varieties or other white varieties with distinct varietal characteristics.

Principal component analysis (PCA) of the data blocks enabled observation of the year to year variation in grape measures, yielding information about their stability across regions and vintages. This enables the identification of aspects of grape composition that can potentially be manipulated in the vineyard and those that may be more prone to variability due to unknown or uncontrollable environmental factors. Amino acid composition of the Cabernet Sauvignon grape samples was similar in samples taken from the same vineyard across the vintages, and was not primarily driven by region. This suggests that something intrinsic to the vineyard influences grape amino acid composition but exploration is required to determine whether this can be managed. Other variables, such as anthocyanin or tannin composition, appeared to be driven mainly by region, suggesting that broad climatic or regional management differences may be important determinants. Bound volatile compound and fatty acid compositions were somewhat related to region but they also varied from year to year. Their concentrations may be altered by environmental variables and could be managed if conditions in the bunch zone could be altered to mimic environmental changes.

Studies of the expression of genes from the lipoxygenase pathway and those responsible for the breakdown of carotenoids showed that the enzyme activity in the fruit was often the result of multiple genes and that maximal gene expression was often separated temporally from the peak in enzyme activity. This would make it difficult to use gene expression assays to predict pathway flux at harvest. Nevertheless, understanding where and when these pathways are active in the fruit is important for the development of strategies to manage the production of important aroma precursor compounds in the vineyard. This has important implications given the outcome that carotenoid content in Cabernet Sauvignon may predict the concentration of β-damascenone, a compound implicated in red wine quality.

The range of techniques and different intellectual approaches from analytical and separations chemists, plant physiologists, biochemists, oenologists and sensory scientists gave this project a genuinely multidisciplinary approach. The research team's insights into grape chemistry have produced some novel and exciting results and have established a foundation of basic research that will eventually lead to changes in the way grapes are assessed and ultimately grown.

This study has brought together the complementary skills of three research teams at the University of Adelaide and CSIRO and we have identified a number of grape data blocks that are associated with specific Cabernet Sauvignon and Chardonnay wine sensory attributes. This is a significant step towards developing objective means of predicting wine sensory properties from grape measures.

4 Background

The future competitiveness of the Australian wine industry will depend on its ability to reliably export a high quality product at reasonable cost and to produce new wine styles suited to changing market needs. Expansion of the industry into new markets will require the generation of a product suited to the new consumers. A major determinant of consumer preference is the flavour and aroma of the wine. However, little is understood of how compounds in the grape berries contribute to the final flavour and aroma characteristics of the wine and how the biophysical environment, within-vineyard variability and management influences flavour development.

This lack of understanding about the role of grape composition in determining wine sensory profiles also means that there is no technology for the objective measurement of grape flavour and aroma attributes that growers and wineries can easily use to assess their product. The Australian wine industry has a voluntary code of conduct that has been developed to ensure "a common Australian winegrape supply contract framework" and to "provide a dispute resolution system to manage disagreements which exist over price or quality assessments" (Anon. 2014). The code of conduct refers to a document that provides a summary of best practice for assessing quality of fruit (Allan 2003). To directly quote from this document: "flavour and character requirements, such as tannin structure, are determined by wineries according to their product requirements and their winemaking style. These are often difficult to quantify, both as a specification and as an assignment". To develop technologies to measure compounds contributing to consumer appeal we need to examine the links between grape composition and wine sensory characteristics so we can identify berry-derived compounds that are either indicators of or contributing to specific wine flavour and aroma attributes.

Volatile and semi-volatile compounds in wine are predominantly responsible for the flavour and aroma perceived by consumers (Ebeler and Thorngate 2009, Robinson et al. 2014a, b). While these compounds have multiple origins during winemaking, the major raw material in this process is the grapes. There is a growing body of evidence that grape composition has a significant impact on wine sensory attributes. The most distinctive example of this is demonstrated by the ability of different grape varieties to produce wines with different sensory characteristics. This is due to genetic differences that determine the biochemical composition of fruit at harvest. However, there are also effects of vintage, region, vineyard variation and vineyard management that influence wine flavour and aroma (e.g. Bramley et al. 2011, Forde et al. 2011, Robinson et al. 2011b, Robinson et al. 2012). This suggests that environmental factors and vineyard management can alter berry composition, presumably through changes in gene expression, enzyme activity or the rate of chemical reactions in the berries but virtually nothing is known about the influence of these practices on specific flavour-active compounds or their non-volatile precursors. Equally important is the determination of optimal harvest time and the question of the apparent temporal difference between the accumulation of sugars and flavour compounds, which has led to a gradual increase in the alcohol content of Australian red wines as attempts are made to increase the flavour components.

The complexity of wine chemistry and how this relates to the sensory properties of wine restricts studies into the impact of vineyard variables on wine style. Therefore, the determination of a compound or group of compounds that contribute to a certain flavour or aroma character is a goal for scientists trying to develop a better understanding of how wine style is constructed chemically. Some compounds that contribute to varietal sensory characteristics have been identified, such as the methoxypyrazines, which contribute vegetal or earthy aromas to certain varieties (e.g. Cabernet Sauvignon and Sauvignon Blanc) and varietal thiols, which are important contributors to Sauvignon Blanc flavour and aroma (for review see Robinson et al. 2014a). However, such compounds are not solely responsible for the sensory attributes of the respective varietal wines; changes in the total volatile profile will alter the sensory perception of the wines and ultimately determine consumer preference. Furthermore, wine volatile composition depends greatly on the composition of the grapes used to produce that wine. The profound influence that grape composition has on wine style not only necessitates effective grading and streaming of fruit, but also provides the potential to manage wine flavour and aroma more effectively in the vineyard. Objective measures of fruit flavour

potential and a means of predicting wine sensory attributes from grape composition will improve our ability to efficiently grow grapes to suit desired wine styles. Therefore, the identification of grape-derived precursors contributing to wine flavour and aroma is a key research outcome that is essential for growing grapes fit for purpose. This will allow for refined decisions about harvest timing as well as batching and streaming of fruit to consistently produce desired wine styles. More importantly, it will provide tools to optimise grape flavour potential and assess quality in the vineyard, and deliver the means of producing grapes with a desired chemical profile that can be used to make wines of a specified flavour profile.

In summary, this project aimed to confirm grape volatile composition to wine sensory relationships observed in a previous project, and to identify new molecules that contribute to or may act as indicators of certain flavour and aroma characteristics of wine. This will eventually enable the development of technologies to measure the abundance of these molecules in grapes and monitor the product for its ability to match certain consumer appeal. Adoption of such technologies could aid the grape and wine industry in streaming fruit for batches of wine to be marketed to specific groups of consumers. There are also no scientifically-validated methods of flavour management in the vineyard that provide producers the ability to better manage the flavour potential of their grapes. This work is made more difficult by the fact that markers of specific wine styles are not available to guide decision making in the vineyard. Therefore, robust grape measures that are known to predict wine sensory outcomes will streamline research into the relationships between location, viticultural practices, environmental variables and grape quality. Such research will also lead to better advice to industry on how to manage vineyard variability to optimise returns, and eventually, to a more directed approach to grape-growing to achieve certain flavours, as our understanding of the viticultural and environmental effects on the production of grape secondary metabolites improves.

5 Project Aims and Performance Targets

This project had four main objectives aimed at developing knowledge and tools to provide objective measurements of grape flavour and the potential to manipulate wine flavour and aroma in the vineyard to achieve specific wine sensory outcomes.

1. To validate links between grape compounds and physical attributes with wine sensory properties that have been identified as drivers of consumer preference.
2. To further investigate a number of candidate grape compounds, genes and physical characteristics (as identified in previous GWRDC funded projects) to determine whether measurements of these grape parameters can be applied as a robust objective measure of grape flavour potential in red varieties such as Cabernet Sauvignon.
3. To adapt this approach to Chardonnay by testing correlative measures in a large set of matching grape and wine samples. This approach will also involve investigations into non-volatile components of the fruit that affect the wine matrix.
4. Develop tools to evaluate the flux of grape metabolites through pathways in the fruit that lead to the production of important wine flavour and aroma compounds and to understand the timing of their production in fruit development.

The planned outputs as described in the original grant application are listed below.

Year 1 (half year 2013):

Output	Target Date	Activities
1. Project Industry Reference Group established	05/13	Establish an Industry Reference Group for the project to facilitate strong collaboration with industry and help guide the application of the project to practical delivery.
2. Accurate method for measurement of targeted grape compounds and improved method for non-targeted analysis of volatile compounds for metabolomic profiling of grape and wine samples developed.	06/13	Develop an accurate GC/MS method to quantify 10 target compounds identified in previous project (CSP05/04) as predicting wine sensory attributes. Refine non-targeted GC/MS method of wine volatile analysis to include multiple deuterated standards.
3. A preliminary list of Cabernet Sauvignon grape compounds confirmed as markers of sensory and hence quality attributes (part I)	06/13	Hire new Postdoctoral Fellow (University of Adelaide). Engage industry partners from throughout South Australia, harvest at least 20 samples from independent sites, perform sensory analysis of selected grape samples, store tissue for further analyses, undertake small scale winemaking on at least 20 samples.

NB: There was some delay to the completion of output 1 as Wine Australia decided to coordinate the establishment of an industry reference group to go across multiple projects. Output 3 was also delayed due to the late appointment of the postdoctoral fellow as the subcontract was not signed. A variation of the end date of output three to 06/14 was agreed to.

Year 2 (2013/2014):

Output	Target Date	Activities
1. A preliminary list of Cabernet Sauvignon grape compounds or physical measures confirmed as markers of sensory characters and hence quality attributes (part II).	06/14	Recruitment and training of sensory descriptive analysis panelists. Determination of scores for at least 15 wine sensory attributes from at least 20 small scale Cabernet Sauvignon wines. Analyse grape and wine samples using appropriate techniques. Obtain concentrations of at least 15 target metabolites in grape samples and at least 50 compounds from wines. Statistical analysis of sensory scores and predictive berry measures from 2013 vintage Cabernet Sauvignon wines to identify interrelationships. Present findings to IRG to review and determine any preliminary dissemination options
2. New measures of additional grape parameters that correlate with wine sensory characteristics identified.	06/14	Literature searches and personal communications with other researchers worldwide conducted to capture information already developed. Potential targets or methods identified and required consumables and materials obtained. Trial at least two new methods of measuring potential grape indicators of wine sensory outcomes including one based on berry viscosity and another measuring non-volatile grape compounds. Test against at least 5 Cabernet Sauvignon samples obtained in the 2013 vintage. Synthesise standards when required.
3. A refined list of Cabernet Sauvignon grape compounds or physical measures confirmed as markers of sensory characters and hence quality attributes (part I).	06/14	Engage industry partners from throughout South Australia, harvest at least 20 samples from independent sites, perform sensory analysis of selected grape samples, store tissue for further analyses, undertake small scale winemaking on at least 20 samples.
4. Communication material describing the preliminary results and future prospects of this project for dissemination to industry through the GWRDC Innovators Network and other extension mechanisms.	06/14	Produce material (facts sheets, presentations, articles etc) for the GWRDC website.

Year 3 (2014/2015):

Output	Target Date	Activities
1. A refined list of Cabernet Sauvignon grape compounds or physical measures confirmed as markers of sensory characters and hence quality attributes (part II).	06/15	Recruitment and training of sensory descriptive analysis panelists. Determination of scores for at least 15 wine sensory attributes from at least 20 small scale Cabernet Sauvignon wines. Analyse grape and wine samples using appropriate techniques. Obtain concentrations of at least 15 target metabolites in grape samples and at least 50 compounds from wines. Statistical analysis of sensory scores and predictive berry measures from 2013 and 2014 vintage Cabernet Sauvignon wines to identify and confirm interrelationships. Present findings to IRG to review and determine any preliminary dissemination options
2. Extended list of grape compounds or other measures predictive of wine sensory attributes for Cabernet Sauvignon developed, and preliminary list of measures relevant to Chardonnay quality developed. (part I)	06/15	Engage industry partners from throughout South Australia, harvest at least 20 samples from independent sites for each variety, perform sensory analysis of selected grape samples, store tissue for further analyses, undertake small scale winemaking on at least 20 samples of each variety.
3. Analytical techniques for profiling metabolites in Chardonnay grapes/juice to enable comparison with sensory attributes developed and optimised.	06/15	Trial targeted and non-targeted GC/MS analytical methods used with Cabernet Sauvignon on Chardonnay samples. Test measures of other grape parameters developed in the earlier stages of the project with Chardonnay samples. Optimise methods and synthesise standards if necessary.
4. Genetic or biochemical assays developed for testing flux through important grape secondary metabolite pathways to determine the timing of compound production.	06/15	Design and test molecular methods to quantify expression of genes from two secondary metabolite pathways found to be important for precursor production in grapes.
5. Communication of project results	06/15	Prepare paper for publication in industry journal to disseminate project outcomes to interested parties.

6. Progress report including a review of the project	06/15	In consultation with GWRDC, conduct a review of the project and make recommendations for future work. (Stop/go review point)

NB: A variation request was agreed to that changed the end date of output 6 in year three of the project to 12/15.

Year 4 (2015/16):

Output	Target Date	Activities
1. Extended lists developed of grape compounds or other measures predictive of wine sensory attributes for Cabernet Sauvignon and preliminary list of measures relevant to Chardonnay quality (part II).	06/16	Recruitment and training of sensory descriptive analysis panelists. Determination of scores for at least 15 wine sensory attributes from at least 20 small scale Cabernet Sauvignon and Chardonnay wines. Analyse grape and wine samples using appropriate techniques. Obtain concentrations of at least 15 target metabolites in grape samples and at least 50 non-targeted compounds from wines. Statistical analysis of sensory scores and predictive berry measures from 2015 vintage Chardonnay wines to identify interrelationships. Statistical analysis of sensory scores and predictive berry measures from 2013, 2014 and 2015 vintage Cabernet Sauvignon wines to identify interrelationships. Analyse and compare data sets from experiments over three seasons. Make recommendations for parameters to use as potential measures of quality. Present findings to IRG to review and determine any preliminary dissemination options
2. A refined list of Chardonnay grape compounds or physical measures confirmed as markers of sensory characters and hence quality attributes (part I).	06/16	Engage industry partners from throughout South Australia, harvest at least 20 samples from independent sites, store tissue for further analyses, undertake small scale winemaking on at least 20 samples.
3. Understanding of the timing of secondary metabolite production for two target pathways during fruit development to determine the optimum timing of assays for quality prediction.	06/16	Analyse flux through secondary metabolite pathways (e.g. lipoxygenase and carotenoid pathways) in grape berries using appropriate molecular or biochemical techniques.

NB: Output 1 was delayed as data collection from the 2015 grape samples was not completed. This was completed soon into the next financial year which allowed the analyses of the three years to be completed soon after.

Year 5 (2016/17):

Output	Target Date	Activities
1. Communication of research results to industry.	07/16	Present results of work at AWITC conference as oral and/or poster presentation.
2. Understanding of the varietal differences between Cabernet Sauvignon and Chardonnay in two target pathways to determine ability to apply flux assays to multiple cultivars and publish results.	06/17	Analyse flux through secondary metabolite pathways (e.g. lipoxygenase and carotenoid pathways) in grape berries from Chardonnay and Cabernet Sauvignon using appropriate molecular or biochemical techniques. Publication of results in research journal.
3. A refined list of Chardonnay grape compounds or physical measures confirmed as markers of sensory characters and hence quality attributes (part II). Final lists of grape compounds or other measures predictive of wine sensory attributes for both cultivars prepared.	06/17	Recruitment and training of sensory descriptive analysis panelists. Determination of scores for at least 15 wine sensory attributes from at least 20 small scale Cabernet Sauvignon wines. Analyse grape and wine samples using appropriate techniques. Obtain concentrations of at least 15 target metabolites in grape samples and at least 50 non-targeted compounds from wines. Statistical analysis of sensory scores and predictive berry measures from all vintages and both varieties to identify interrelationships. Make recommendations for parameters to use as potential measures of quality.
4. Knowledge concerning how grape precursor pathways are affected by region and vintage as a means to identify those that can be targeted for viticultural control.	06/17	Statistical analysis to compare analytical chemistry results from multiple seasons to determine the plasticity of production of grape secondary metabolites. Make recommendations for future research concerning growing grapes for specific wine outcomes.
5. Communication of project results and recommendations to interested parties	06/17	Prepare final report, prepare papers for publication to industry and peer reviewed journals, disseminate final project outcomes to interested parties. Make recommendations for the further development of methods quantifying gene expression or enzyme activity as means of measuring fruit flavor potential.

NB: The end dates for outputs 2-5 were changed to 12/17 after a variation request was agreed to.

6 Method

6.1 Overview

In developing this project, our hypothesis was that grape composition has a strong influence on wine volatile composition and, as a result, wine sensory attributes. Therefore, it should be possible to develop measures of grape composition that can predict wine sensory characteristics. In order to achieve this, chemical (e.g. GC-MS), sensory (descriptive analysis) and physical (e.g. calliper) measures of grapes that may predict wine sensory attributes were applied to a large set of Cabernet Sauvignon grape samples, obtained from our industry partners from both warm and cool growing regions. Corresponding small-scale fermentations were conducted under controlled conditions to eliminate winemaking variables and descriptive sensory analyses were conducted on the wines. Advanced statistical analysis of these grape compositional measures and the corresponding sensory data were conducted to develop models to predict wine sensory attributes from grape composition. Winemaking quality assessment was also conducted on the wines to provide information on the sensory attribute associated with high and low quality wines.

The methods we developed using Cabernet Sauvignon were also applied to Chardonnay samples from various regions provided by industry partners. Overall, the aims were to provide information on the applicability of measures developed in Cabernet Sauvignon to be used in other varieties, to increase our array of quality assays to include non-volatile compounds and identify potential grape quality measures in Chardonnay.

Molecular and biochemical methods (e.g. enzyme and gene expression assays) were also used to improve our knowledge of how the biochemical pathways of secondary metabolite accumulation are controlled during berry development and to investigate the use of genetic and biochemical markers to indicate changes in metabolism and therefore changes in fruit characteristics

6.2 Grape Sampling

Our approach required that the samples used had a broad range of differences in fruit composition and wine sensory attributes. We therefore sampled grapes from throughout South Australia, across eight regions for Cabernet Sauvignon and 12 regions for Chardonnay. Where possible, we also obtained grapes from vineyards that had been consistently graded at different levels within each region. At each site, ~60 kg of grapes were randomly sampled from throughout the vineyard block from all areas of the canopy. The grapes were transported back to the laboratory where a 500 g subsample was taken for berry sensory analysis. Five 100 g subsamples of individual berries were also randomly taken from each parcel and immediately frozen in liquid nitrogen and stored at -80 °C pending further analysis. In some cases samples were deseeded once frozen and, when required, samples were homogenised using an IKA A11 basic analytical mill (IKA, Staufen, Germany) in preparation for various analyses. The rest of the grapes were delivered to the Waite Campus winery where they were fermented according to published vinification procedures (Niimi et al. 2017).

6.2.1 CABERNET SAUVIGNON SAMPLES

Cabernet Sauvignon samples were harvested in the 2013, 2014 and 2015 vintages. In each year, 25 samples were obtained from grape growing regions around South Australia, namely: Barossa Valley (BV) (2), Clare Valley (CV) (2), Coonawarra (CWA) (4), Eden Valley (EV) (2), McLaren Vale (McV) (2), Langhorne Creek (LC) (2), Riverland (RVL) (9) and Wrattonbully (WBY) (2). The same sites were used across all three vintages for the Cabernet Sauvignon parcels from BV, CV, EV, McV, LC and WBY, but only 2 of the 4 CWA sites were the same for all three vintages and only 4 of the 9 were sampled every year from RVL (Table 1).

Table 1. Codes and harvest dates for Cabernet Sauvignon samples.

CODE	GROWING REGION	HARVEST DATES
BV1	Barossa Valley	25/02/2013; 13/03/2014; 19/02/2015
BV2	Barossa Valley	01/03/2013; 16/03/2014; 19/02/2015
CV1	Clare Valley	27/02/2013; 06/03/2014; 19/02/2015
CV2	Clare Valley	27/02/2013; 06/03/2014; 19/02/2015
CWA1	Coonawarra	15/03/2013; 16/04/2014
CWA2	Coonawarra	15/03/2013; 16/04/2014; 20/03/2015
CWA3	Coonawarra	20/03/2013; 09/04/2014; 26/03/2015
CWA4	Coonawarra	9/04/2013
CWA5	Coonawarra	16/04/2014; 20/03/2015
CWA6	Coonawarra	26/03/2015
EV1	Eden Valley	25/02/2013; 01/04/2014; 26/02/2015
EV2	Eden Valley	01/03/2013; 06/04/2014; 26/02/2015
LC1	Langhorne Creek	13/03/2013; 14/03/2014; 23/02/2015
LC2	Langhorne Creek	13/03/2013; 14/03/2014; 23/02/2015
McV1	McLaren Vale	22/02/2013; 12/03/2014; 20/02/2015
McV2	McLaren Vale	22/02/2013; 12/03/2014; 20/02/2015
RVL1	Riverland	21/02/2013
RVL2	Riverland	21/02/2013
RVL3	Riverland	18/02/2013; 04/03/2014; 25/02/2015
RVL4	Riverland	18/02/2013; 06/02/2014; 12/02/2015
RVL5	Riverland	21/02/2013
RVL6	Riverland	19/02/2013; 11/03/2014; 25/02/2015
RVL7	Riverland	15/02/2013; 11/03/2014; 12/02/2015
RVL8	Riverland	19/02/2013
RVL9	Riverland	19/02/2013
RVL10	Riverland	07/03/2014; 25/02/2015
RVL11	Riverland	04/03/2014; 18/02/2015
RVL12	Riverland	04/03/2014; 18/02/2015
RVL13	Riverland	07/03/2014; 18/02/2015
RVL14	Riverland	06/02/2014; 12/02/2015

CODE	GROWING REGION	HARVEST DATES
WBY1	Wrattonbully	15/03/2013; 09/04/2014; 20/03/2015
WBY2	Wrattonbully	20/03/2013; 09/04/2014; 26/03/2015

6.2.2 CHARDONNAY SAMPLES

Chardonnay samples were obtained in the 2015 and 2016 vintages. Like the Cabernet Sauvignon samples, the aim was to have as broad a range of sensory characteristics in the wines as possible, so the fruit was sourced from throughout South Australia. In 2015, 25 fruit parcels were obtained from: Adelaide Hills (ADEH) (2), BV (2), CV (3), CWA (1), EV (3), LC (1), McV (2), Robe (1), RVL (9), WBY (1). Nineteen of these sites were used again in 2016 with the final 25 samples coming from: ADEH (2), Bordertown (BTN) (1), BV (1), CV (3), CWA (1), EV (4), LC (1), Robe (1), RVL (9), Padthaway (PWY) (1), WBY (1).

Table 2. Codes and harvest dates for Chardonnay samples.

CODE	GROWING REGION	HARVEST DATES
ADEH1	Adelaide Hills	19/02/2015; 18/02/2016
ADEH2	Adelaide Hills	26/02/2015; 18/02/2016
BTN1	Bordertown	02/02/2016
BV1	Barossa Valley	03/02/2015; 01/02/2016
BV2	Barossa Valley	13/02/2015
CV1	Clare Valley	02/02/2015; 04/02/2016
CV2	Clare Valley	02/02/2015; 01/02/2016
CV3	Clare Valley	02/02/2015; 04/02/2016
CWA1	Coonawarra	15/02/2015; 17/02/2016
EV1	Eden Valley	16/02/2015; 11/02/2016
EV2	Eden Valley	13/02/2015
EV3	Eden Valley	16/02/2015; 11/02/2016
EV4	Eden Valley	11/02/2014
EV5	Eden Valley	11/02/2016
LC1	Langhorne Creek	10/02/2015; 15/02/2016
McV1	McLaren Vale	06/02/2015
McV2	McLaren Vale	06/02/2015
PWY	Padthaway	02/02/2016
Robe	Robe	24/02/2015; 17/02/2016
RVL1	Riverland	23/01/2015

RVL2	Riverland	23/01/2015
RVL3	Riverland	04/02/2015; 28/01/2016
RVL4	Riverland	11/02/2015; 28/01/2016
RVL5	Riverland	04/02/2015; 29/01/2016
RVL6	Riverland	04/02/2015; 28/01/2016
RVL7	Riverland	11/02/2015; 29/01/2016
RVL8	Riverland	04/02/2015; 29/01/2016
RVL9	Riverland	12/02/2015; 29/01/2016
RVL10	Riverland	28/01/2016
RVL11	Riverland	29/01/2016
WBY1	Wrattonbully	15/02/2015; 17/02/2016

6.3 Grape Measurements

6.3.1 HARVEST MEASURES

Typical fruit harvest measurements were made when the parcels were returned to the laboratory for subsampling. Bunch weights were obtained for 25 randomly sampled bunches, and berry weight was determined for 30 berries. Total soluble solids (°Brix) were measured in the same thirty berries using an RFM710 digital refractometer (Bellingham Stanley, Tunbridge Wells, UK). Other measures such as pH, titratable acidity (TA) and yeast assimiliable nitrogen (YAN) were also obtained. To measure the pH and TA, 100 berries of each sample were selected randomly, squeezed to obtain juice which was centrifuged at 1890 × *g* for 5 min at room temperature. The supernatant was recovered and the pH and TA were measured in triplicate using a combined pH meter and autotitrator (Crison, CompacT Titrator, Crison Instruments, South Australia). YAN measurements were obtained in triplicate using an OenoFOSS analyzer (FOSS, Hilleroed, Denmark).

6.3.2 PHENYLPROPANOIDS

Phenylpropanoids comprise a large group of compounds found in plants. The ones most important for grape and wine are the anthocyanins, the flavonols and the tannins.

6.3.2.1 Anthocyanins

These are the compounds responsible for the colour of the fruit and wine. They were measured using high performance liquid chromatography (HPLC) which allows us to also determine the abundance of each of the 15 main anthocyanin types that are in the fruit skins. The method used was identical to that published in Downey and Rochfort (2008).

6.3.2.2 Flavonols

Flavonols are another class of phenylpropanoid compounds found in grapes and wine that are produced via the sample biosynthesis pathway as the anthocyanins. These compounds are thought to help protect the plant from UV-damage. In a wine context, the flavonols positively affect wine colour by co-pigmentation

with anthocyanins, and are anti-oxidants and anti-inflammatory compounds. The method of Downey and Rochfort (2008) was also used to quantify flavonols in the grape samples.

6.3.2.3 Tannins

Tannins are polymeric phenylpropanoid compounds responsible for aspects of wine mouthfeel, astringency, bitterness and colour. A published HPLC method (Hanlin and Downey 2009), was used to profile the berry skin tannin composition with the various flavan-3-ol terminal and extension subunits quantified along with an estimate of the degree of polymerisation.

6.3.3 AMINO ACIDS

Analysis of the 20 proteinogenic amino acids as well as β-alanine and γ-aminobutyric acid was conducted on ground whole berry samples using the method described in Boss et al. (2015).

6.3.4 TARGETED GRAPE VOLATILE COMPOUNDS

A previous study had found that the concentration of some Cabernet Sauvignon grape volatile compounds were associated with certain wine sensory attributes (Forde et al. 2011; Table 3). A new solid phase microextraction (SPME), gas chromatography-mass spectrometry (GC-MS) method was developed to accurately quantify these compounds in grape samples.

Whole grapes were ground to a powder using an IKA A11 analytical mill. In a 20 mL crimp-capped amber vial, 2.5 g frozen grape powder, 2.5 mL 4 mM ZnSO4 and 2.5 g NaCl were mixed. To this mixture 50 µL of a standard solution was added, which consisted of d_3-ethyl acetate (197 µg/L, C/D/N Isotopes Inc., Pointe-Claire, Canada); d_{16}-octanal (164 µg/L, C/D/N Isotopes Inc.); 2n-hexyl furan (270 µg/L, Sigma-Aldrich, Sydney, Australia); (Z)-3-hepten-1-ol (3.4 mg/L, Sigma-Aldrich); d_{17}-2-ethyl-1-hexanol (133 µg/L, C/D/N Isotopes Inc.); d_{11}-hexanoic acid (372 µg/L, C/D/N Isotopes Inc.); d_7-benzyl alcohol (104 µg/L, C/D/N Isotopes Inc.). Because hexanal was present in high concentrations, a second vial with only 1 g of frozen grape powder was added to 4 mL of 4mM ZnSO4 and 2.5 g NaCl before the addition of 20 µL of an 89 mg/L d_{12}-hexanal standard solution (C/D/N Isotopes Inc.).

The method utilised a 7890A gas chromatograph (Agilent) equipped with a MP2 auto-sampler (Gerstal) and a 5975C mass spectrometer (Agilent). The samples were equilibrated in a shaking sample incubator at 40 °C for 5 min at 250 rpm. Volatile compounds were then extracted at 40 °C with agitation at 250 rpm for 30 min with a divinylbenzene-carboxen-polydimethylsiloxane fibre (2 cm, 23-Gauge, 50/30 µm DVB-CAR-PDMS, Supelco). The fibre was then inserted into the inlet for 1 min at 220 °C to desorb the volatile compounds. A ZB-Wax column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm; Phenomenex) was used for separation of the compounds using helium as a carrier gas at a constant flow of 1.5 mL/min. The chromatography was performed with an initial hold at 35 °C for 1.5 min, then a temperature gradient of 7 °C/min from 35 °C to 245 °C and finally the oven was held isothermally for 3.5 min. Mass spectrometry was performed with the transfer line temperature at 250 °C, a source temperature of 230 °C, the quadrupole temperature at 150 °C and an ionising potential of 70 eV. Selected ions were monitored and the mass channels used for each compound are listed in Table 3. 3-Isobutyl-2-methoxypyraine (IBMP) was analysed in the Cabernet Sauvignon grape samples using the method published in Dunlevy et al. (2010). The grape analyses were performed in biological triplicate and quantification of compounds was achieved by calculating the ratio of the target ion peak area and that of the relevant internal standard and comparing this to a calibration curve.

Table 3. A list of the compounds quantified using a targeted SPME GC-MS method and the quantifier and qualifier ions used. Internal standards are underlined above the compounds they were used for quantification. Attributes in italics were negatively associated with the compound and (a) = aroma, (f) = flavour, (v) = visual, and (t) = aftertaste.

COMPOUND	ASSOCIATED SENSORY ATTRIBUTE(S) (FORDE ET AL. 2011)[1]	QUANTIFIER IONS	QUALIFIER IONS
d_8-Ethyl acetate		66	46, 76
Ethyl acetate	pepper (a)	61	73, 88
d_{16}-Octanal		96	112, 124
Heptanal	spicy (a)	70	81, 86
Octanal	*berry (f)*	84	100, 110
(*E,E*)-2,4-Heptadienal	berry (f)	110	81
Decanal	*viscosity (v), pungent (a), astringency (t)*	112	95, 128
2-Hexylfuran		152	108, 123
2-Pentylfuran	aroma impact (a)	138	109, 123
(*Z*)-3-Hepten-1-ol		81	67, 96
(*Z*)-3-Hexen-1-ol	*colour intensity (v), flavour impact (f), warming (t)*	67	55, 82
d_{17}-2-Ethyl-1-hexanol		94	80, 112
2-Ethyl-1-hexanol	green (f)	83	70, 98
d_{11}-Hexanoic acid		63	77, 93
Hexanoic acid	*colour intensity (v)*	60	87
d_7-Benzyl alcohol		115	85, 98
Benzyl alcohol	*viscosity (v)*	108	77, 91
d_{12}-Hexanal		80	92
Hexanal	*pepper (f)*	72	82
d_3-IBMP		127	95, 154
IBMP	woody/tobacco (a,f)	124	94, 151

6.3.5 NON-TARGETED GRAPE VOLATILE COMPOUNDS

The analysis of non-targeted grape volatile compounds was conducted using the method outlined in Kalua and Boss (2009) but with a different sample preparation and a greater number of internal standards. In short, the sample preparation was altered by the addition of 2.5 mL 4mM $ZnSO_4$ to the vial to inhibit lipoxygenase

activity to 2.5 g of powdered grape tissue and 2.5g NaCl. The internal standards added were the same ones used for the targeted grape volatile analysis method described above in section 6.3.3. The internal standard used for each compound was designated by using the one with the most similar functional group. The spectra were recorded in scan mode (range: m/z 35-350, scan rate: 4.45 scans/s).

Table 4 below lists the non-targeted grape volatile compounds that we quantified relative to an internal standard across the samples.

Table 4. Compounds for which relative quantities were obtained in the headspace of crushed grape samples.

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA (CH ONLY)
2-Hexyl methyl ether (Ch)	882	C	1
2-Butanone (Ch)	888	B	2
3-Methyltetrahydrofuran (Ch)	892	C	3
2-Methylbutanal	946	B	4
3-Methylbutanal	949	A	5
Hexyl methyl ether (Ch)	951	B	6
2-Ethylfuran (Ch)	952	B	7
2,3-Dimethyloctane (Ch)	959	C	8
3-Methylnonane (Ch)	968	B	9
Pentanal (Ch)	970	A	10
Methyl butanoate (Ch)	980	B	11
Decane (Ch)	1000	A	12
α-Pinene (Ch)	1014	A	13
1-Penten-3-one (Ch)	1015	A	14
4-Heptanone (Ch)	1020	B	15
2-Butenal (Ch)	1036	B	16
3-Pentanone (CS)	1054	B	
2-Butylfuran (Ch)	1109	B	17
(*E*)-2-Pentenal	1110	A	18
4-Methyl-3-penten-2-one (Ch)	1115	B	19
(*Z*)-3-Hexenal (Ch)	1133	B	20
Monoterpene 1 (Ch)	1137	C	21
1-Butanol	1143	A	22

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA (CH ONLY)
1-Penten-3-ol (Ch)	1155	A	23
1,1-Dimethoxyhexane (Ch)	1158	A	24
2-Heptanone (CS)	1168	A	
Methyl hexanoate (Ch)	1174	B	25
Cyclohexane oxide (Ch)	1198	A	26
(*E*)-2-Hexenal (Ch)	1215	A	27
1-Pentanol	1255	A	28
3-Octanone (CS)	1262	A	
Unknown 1 (Ch)	1287	C	29
(*Z*)-3-Hexenyl acetate (Ch)	1293	A	30
2,2,6-Trimethylcyclohexanone (CS)	1302	B	
(*Z*)-2-Penten-1-ol	1322	B	31
(*Z*)-2-Heptenal	1323	B	32
3-Ethoxypentane (Ch)	1329	C	33
6-Methyl-5-hepten-2-one	1333	A	34
1-Hexanol (Ch)	1334	A	35
(*E*)-3-Hexen-1-ol (Ch)	1343	A	36
Nonanal (CS)	1375	A	
2,4-Hexadienal	1378	A	37
(*E*)-2-Hexen-1-ol (Ch)	1401	A	38
(*E*)-2-Octenal (CS)	1416	A	
Ethyl octanoate (CS)	1420	A	
1-Octen-3-ol	1452	A	39
1-Heptanol (CS)	1462	A	
(*E*,*Z*)-2,4-Heptadienal (CS)	1465	A	
Benzaldehyde	1532	A	40
Linalool (Ch)	1545	A	41
1-Octanol (CS)	1560	A	

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA (CH ONLY)
Unknown 2	1594	C	42
(*E*)-2-Octen-1-ol (CS)	1615	A	
β-Cyclocitral (CS)	1624	B	
2-Phenylethanal	1633	A	43
5-Ethyl-2(5H)-furanone	1748	B	44
β-Damascenone (CS)	1830	A	
2-Phenylethanol (CS)	1901	A	
β–Ionone (CS)	1948	A	

[1] RI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}). [2]A, identity confirmed by matching mass spectra and RI with that of authentic standards; B, tentative assignment based upon comparison with mass spectral libraries and published RI. C, compound not identified or named based on mass spectral match only. (Ch) = quantified in Chardonnay only. (CS) = quantified in Cabernet Sauvignon only.

6.3.6 GRAPE 'BOUND' VOLATILE COMPOUNDS

The extraction of "bound" volatiles from the grape samples was achieved using a method adapted from that of Martin et al. (2012). Two g of powdered grape was mixed with 8 mL of water, mixed every 2 min for 10 min and centrifuged at 3500 × *g* for 15 min at 4 °C. The supernatant was then passed through a Strata-X SPE column (60 mg/3 ml, Phenomenex) conditioned previously with 3 ml methanol and 3 ml water. The column was washed with 6ml water and 6 ml pentane before the "bound" volatiles were eluted with 6ml methanol. The extracts were then dried using a vacuum evaporator without heat (Savant SpeedVac, ThermoFisher, MA, USA).

Hydrolysis of the "bound" volatiles was conducted using citric acid and heat, and compounds released were extracted using diethyl ether and hexane (1:1), as outlined in Kotseridis et al. (1999). A 10 µl aliquot of 45 mg/L ethyl-4-acetyl benzoate in 50% ethanol was added as an internal standard. The extracted volatile compounds were analysed by GCMS (Agilent 6890N gas chromatograph and 5973 mass spectrometer). A 1 µL aliquot of each hydrolysate was injected into an inlet in pulsed splitless mode at a temperature of 220 °C and separated on a ZB-Wax column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm; Phenomenex). The pressure pulse was 25.0 psi for 0.6 min at which time purging at 30 ml/min began. Helium was used as the carrier gas at a constant flow of 1.2 ml/min. The temperature program of the oven was as follows: 40 °C, held for 1 min; ramped at 10 °C/min up to 90 °C; ramped at 4 °C/min to 190 °C; ramped at 10 °C/min to 240 °C and held for 10 min. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range: m/z 35-350, scan rate: 4.45 scans/s).

A laboratory generated library (377 compounds) as well as the US National Institute of Standards and Technology-11 (NIST-11) and the Wiley Registry 9th Edition mass spectral libraries was used to identity volatile compounds. Many of the compounds could only be tentatively identified or remain unknown (Table 5).

Table 5. Compounds for which relative quantities were obtained in the bound volatile fractions of the grape samples.

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
(*E*)-2-Hexenal	1205	A	1

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl) furan	1226	B	2
Megastigmatrienone 1	1291	B	3
Unknown 1	1293	C	4
Nonanal	1379	A	5
(*Z*)-Linalool oxide	1425	A	6
α-Ionene	1431	B	7
(*E*)-Linalool oxide	1451	A	8
2-Ethyl-1-hexanol	1468	A	9
Benzaldehyde	1506	A	10
Vitispirane 1 and 2	1507	B	11
Megastigmatrienone 2	1553	B	12
1-Terpinenol	1554	B	13
Unknown 2	1555	C	14
Menthol	1621	B	15
2-Methylbenzaldehyde	1632	B	16
3-Carene	1636	B	17
Unknown 3	1660	C	18
4-Oxoisophorone	1673	A	19
α-Terpineol	1677	A	20
3,3,5,6-Tetramethyl-1-indanone	1715	B	21
1,2-Dihydro-1,1,6-trimethylnaphthalene (TDN)	1722	B	22
4-Ethylbenzaldehyde	1792	A	23
β-Damascenone	1801	A	24
1(2,3,6-Trimethylphenyl)but-1,3-diene (TPB)	1807	B	25
p-Cymen-8-ol	1832	B	26
Guaiacol	1885	A	27
Hexanoic Acid	1845	A	28

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Benzyl Alcohol	1864	A	29
α-Calacorene	1891	B	30
Unknown 4	1895	C	31
Unknown 5	1905	C	32
Unknown 6	1919	C	33
2-Ethyl hexanoic Acid	1944	A	34
Heptanoic Acid	1952	A	35
2,5,8-Trimethyl-1,2-dihydro-naphthalene	1971	B	36
Dihydro-β-ionone	1978	B	37
Unknown 7	1987	C	38
Octanoic Acid	2057	A	39
1-(2,3,6-Trimethylphenyl)but-3-en-2-one	2090	B	40
Nonanoic acid	2161	A	41
4-Vinylguaiacol	2180	A	42
2,5,8-Trimethyl-1,4-dihydro-naphthalene	2182	B	43
6-(3-Hydroxybut-1-enyl)-2,2,6-trimethyl cyclohex-4-en-1-one	2193	B	44
Unknown 8	2244	C	45
2,6-Dimethoxyphenol	2256	A	46
Decanoic acid	2274	A	47
4-(2,3,6-Trimethylphenyl)-but-3-en-2-one	2285	B	48
1-(2,3,6-Trimethylphenyl)-3-butanone	2376	B	49
4-Vinylphenol	2385	B	50
1-(2,3,6-Trimethylphenyl)-2-butanone	2443	B	51
9-Hydroxymegastigm-7-en-3-one	2453	B	52

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Benzoic acid	2471	B	53
Unknown 9	2506	C	54
3-Hydroxy-β-damascone	2528	B	55
Vanillin	2559	A	56
Methyl vanillate	2592	B	57
3-Oxo-α-ionol	2600+	B	58
Unknown 10	2600+	C	59
Unknown 11	2600+	C	60
Vanillyl acetone	2600+	B	61
Methyl syringate	2600+	B	62

[1] RI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}). [2]A, identity confirmed by matching mass spectra and RI with that of authentic standards; B, tentative assignment based upon comparison with mass spectral libraries and/or published RI; C, compound not identified.

6.3.7 COLOUR MEASUREMENTS

Homogenised berry extracts were prepared in triplicate using the preparation method for total phenolics (Iland et al. 2013). These extracts were analysed using a Cintra 4040 UV-Visible Spectrometer (GBC Scientific Equipment Pty Ltd, Braeside, VIC, Australia) for their CIELab colour tristimulus. The parameters measured were: lightness (L*); redness – greenness (a*); and yellowness – blueness (b*). This was achieved by measuring transmittance from 380 to 780 nm at 1 nm intervals with the samples in 1 mm quartz cuvettes. Values of a* and b* were used to calculate the chroma (colour strength: C*) and hue angle of the homogenate extracts (hab).

6.3.8 TOTAL PHENOLICS AND TANNINS

Total phenolics and tannins were analysed using the methods published in Iland et al. (2013) and Sarneckis et al. (2006).

6.3.9 FATTY ACIDS

Fatty acids were extracted from ground, de-seeded berry samples using the method of Han and Yuan (2009). One g of berry tissue and 1 mL of isopropanol were mixed and incubated at 75 °C for 15 min and then cooled to room temperature. The internal standard (50 µl of 1.2 mM d_{33}-heptadecanoic acid (C/D/N Isotopes Inc.)) was added, as was 1 ml of chloroform and 2 ml of methanol and the sample was mixed for 1 h on a flatbed stirrer. Separation of the fatty acids and their methylation was conducted according to Yunoki et al. (2004) with some modification. First, to partition the phases, 1 mL of both chloroform and water were added, the solutions mixed and then centrifuged at 200 × *g* for 2 min. The lower chloroform layer was collected, re-extracted with 3 mL of chloroform:methanol:water (3:48:47) twice then concentrated under N_2. To methylate the fatty acids, 2 ml methanol in 5% HCl was added and the samples incubated for 2 h at 95 °C. The samples were then cooled to room temperature and 1 mL water and 2 mL n-hexane added. After shaking the upper layer was collected and the bottom layer extracted twice more with 2 mL n-hexane. The combined

n-hexane extracts were concentrated under N_2. The extract was resuspended in 100 µL dichloromethane for injection onto the GCMS. A 1:10 dilution was also made for quantification of the more abundant fatty acids.

Chromatographic separation of the fatty acids was conducted with an Agilent 6890 gas chromatograph and 5973 mass spectrometer. The inlet was in split mode (10:1) at a temperature of 230 °C, and 1 µL of each sample (undiluted and diluted) was injected onto a ZB-5 column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm; Phenomenex). Helium was the carrier gas and this was maintained at a constant flow of 1.5mL/min. The oven temperature program was as follows: initial 40 °C, then ramped at 12 °C/min up to 200 °C, then increasing at 0.5 °C/min to 230 °C, followed by a ramp at 3 °C/min until a final temperature of 250 °C was reached and held for 15 min. The mass spectrometer was set with a transfer line temperature of 250 °C, source temperature of 240 °C, quadrupole temperature of 150 °C and ionising potential at 70 eV. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range: m/z 40-500, scan rate: 3.15 scans/s).

Table 6 lists the extracted ions used to quantify the fatty acid methyl esters relative to the internal standard (methyl ester of D_{33}-heptadecanoic acid). The identities of the fatty acid methyl esters were determined by comparing mass spectra with those of authentic standards and spectral libraries.

Table 6. List of methyl esters quantified in the grape fatty acid extracts, Kovats indices (LRI) and the ions used for quantification.

COMPOUND	LRI	QUANTIFIER IONS
Methyl dodecanoate (12:0)	1503	66
Methyl tridecanoate (13:0)	1610	74
Methyl tetradecanoate (14:0)	1710	74
Methyl 4-pentadecenoate (15:1 n-11)	1768	74
Methyl (*Z*)-10-pentadecenoate (15:1 n-5)	1773	55
Methyl pentadecanoate (15:0)	1791	74
(*E*)-2,4-Phytadiene	1848	82
Methyl (*E*,*E*) 7,10-hexadecadienoate (tr16:2 n-6)	1861	67
Methyl (*Z*)-9-hexadecenoate (16:1 n-7)	1868	55
Methyl (*Z*,*Z*)-9,12-heptadecadienoate (17:2 n-5)	1959	67
Methyl (*Z*)-10-heptadecenoate (17:1 n-7)	1966	55
Methyl (*Z*)-9-heptadecenoate (17:1 n-8)	1970	55
Methyl heptadecanoate (17:0)	1990	74
Methyl 2-hydroxyhexadecanoate (16:0 OH)	2015	97
Methyl elaidate (tr18:1 n-9)	2090	264
Methyl nonadecanoate (19:0)	2205	74

COMPOUND	LRI	QUANTIFIER IONS
Methyl (*Z*,*Z*)-11,14-eicosadienoate (20:2 n-6)	2278	81
Methyl (*Z*)-13-eicosanoate (20:1 n-7)	2283	55
Methyl (*Z*,*Z*,*Z*)-11,14,17-eicosatrienoate (20:3 n-3)	2285	79
Methyl eicosanoate (20:0)	2311	74
Methyl heneicosanoate (21:0)	2406	74
Methyl docosanoate (22:0)	2502	74
Methyl tricosanoate (23:0)	2607	74
Methyl tetracosanoate (24:0)	2713	74
Methyl pentacosanoate (25:0)	2804	74
Methyl hexacosanoate (26:0)	2910	74
Methyl heptacosanoate (27:0)	3001	74
Quantified in 1:10 dilution		
Methyl hexadecanoate (16:0)	1906	74
Methyl linoleate (18:2 n-6)	2049	67
Methyl linolenate (18:3 n-3)	2057	79
Methyl octadecanoate (18:0)	2108	74

6.3.10 LIPOXYGENASE PATHWAY ENZYMES

Enzymes from the lipoxygenase pathway were assayed using methods adapted from the literature as outlined below.

6.3.10.1 Lipoxygenase (LOX)

The lipoxygenase assay was based on the methods published by Bonnet and Crouzet (1977) and Axelrod et al. (1981). To 1 g frozen ground grape material, 1 mL cold extraction buffer (0.5M Tris-HCl pH 8.0, 1% (w/v) ascorbic acid, 1% (w/v) EDTA, 1% (w/v) PVP) was added and mixed thoroughly. The mixture was then centrifuged at 3250 x *g* for 15 min at 4 °C and 1 mL of the supernatant collected. To the supernatant, 20 µL cold 1M $CaCl_2$ was added and then the extract was allowed to sit for 2 h at 4 °C. Following this the mixture was centrifuged for 5 min at 13000 x *g* at 4 °C and the supernatant represents the crude enzyme extract. Enzyme activity was then measure spectrophotometrically. To a quartz cuvette, 2.96 mL 0.2 M potassium phosphate buffer (pH 6.5) and 30 µL 10mM linoleic acid were added. The cuvette was capped, inverted 3 times to mix and placed in a temperature controlled cuvette holder at 25 °C. The rise in absorbance at 234 nm was measured at 10 sec intervals over 2 min to correct for non-enzymatic oxidation of linoleic acid. Then 10 µL of the crude enzyme extract was added to the cuvette which was inverted a further 3 times to mix. Again the rise in absorbance at 234 nm was measured at 10 sec intervals over 3 min at 25 °C to quantify the formation of conjugated diene hydroperoxide.

6.3.10.2 Hydroperoxide Lyase (HPL)

Hydroperoxide lyase was assayed using a method adapted from those of Vick (1991) and Anthon and Barrett (2003). To 100 mg of ground grape tissue was added 300ul of cold extraction buffer (1M Tris-HCl pH 8.0, 5mM EDTA, 10% (v/v) glycerol, 1% (w/v) PVP, 1% (v/v) TritonX-100, 10 mM β-mercaptoethanol, 1mM PMSF) in a 1.5 mL microfuge tube and flicked to mix. The mixture was centrifuged for 5 min at 13000 x *g* at 4 °C, and the supernatant collected (the crude preparation can be stored at -80 °C at this point). The spectrophotometer was zeroed against a semi-micro polymethyl methacrylate (PMMA) cuvette containing water. To another cuvette, 960 µL 50mM sodium phosphate (pH 6.5), 5 µL 14mM NADH, 10 µL of yeast alcohol dehydrogenase (5U/µL), and 5 µl 10mM 13-hydroperoxylinolenic acid in ethanol were added. The mixture was inverted 3 times to mix and placed in a temperature controlled cuvette holder at 25 °C. The background oxidation rate of NADH in the reaction mixture was measured at 340 nm for 2 min then 50 µL of the enzyme extract was added to give a final volume of 1.3 mL. Again, the cuvette was inverted 3 times to mix and the decrease in absorbance at 340 nm measured for an additional 2 min at 25 °C. To account for the background oxidation rate of NADH in the sample, another cuvette was prepared as above, but the 13-hydroperoxylinolenic acid was replaced with 5 µL of ethanol and the absorbance at 340 nM measured again. After subtraction of the background oxidation rate of NADH in the reaction mixture and sample, the net enzyme rate was expressed as the number of moles of NADH oxidised per min per gram of grape tissue.

6.3.10.3 Alcohol dehydrogenase (ADH)

The methods of Molina et al. (1986), Tesniere & Verries (2000) and Tesniere et al. (2004), were adapted to measure alcohol dehydrogenase activity in the grape tissue. The preparation of the crude enzyme extract was identical to that described in section 6.3.9.2. A macro PMMA cuvette containing water was used as the blank. In another cuvette, 50 µL of the crude enzyme extract and 2.85 mL 50mM sodium phosphate buffer pH 5.8 were mixed at room temperature. Then 50 µL 14 mM NADH was added and the cuvette inverted 3 times to mix. This was then placed in the temperature controlled cuvette holder at 25 °C for 2 min to equilibrate. The enzyme assay was initiated by the addition of 50 µL 0.3M acetaldehyde and the inversion of the cuvette 3 times to mix. The decrease in absorbance was measured at 340nm at 10 second intervals for 3 min without stirring. ADH activity is expressed as µM of NADH consumed per min per g fresh weight, as acetaldehyde is reduced.

6.3.11 GENE EXPRESSION STUDIES

6.3.11.1 Plant tissue

The grapes used for the developmental studies were harvested during a trial conducted in the 2008-9 vintage from a vineyard in the Riverland. The site and the sampling regime are outlined in Kalua and Boss (2010). In brief, whole bunches were harvested fortnightly from 2 weeks postflowering (wpf) until commercial harvest and transported back to the Waite Campus on ice. Individual berries were then removed from bunches and snap frozen in liquid nitrogen pending further analysis.

6.3.11.2 Gene identification, qRT-PCR primer design and analysis.

Members of gene families putatively encoding enzymes from the lipoxygenase pathway and enzymes responsible for the degradation of carotenoids were identified using existing literature and database searches. For the lipoxygenase pathway, the identity of grapevine lipoxygenase (LOX) family members were obtained from the paper of Podolyan et al. (2010). Initial identification of hydroperoxide lyase (HPL) family members was obtained from Zhu et al. (2012) and further homologues were identified after a BLAST search of the NCBI database (Altschul et al. 1990). Alcohol dehydrogenase (ADH) family members were obtained from Tesniere & Verries (2000). Carotenoid cleavage dioxygenase (CCD) genes have also been cloned previously from grapevine (Matthieu et al. 2005; Guillaumie et al. 2011).

Primers in the 3' ends of the genes, and preferably in the 3' untranslated region, were designed using Geneious version 9.1.2 (http://www.geneious.com, Kearse et al., 2012). Genomic DNA was purified from Cabernet Sauvignon leaves using a method previously described (Thomas et al. 1993) and sequences

corresponding to the 3' end regions were amplified following a published protocol (D'Onofrio et al. 2009). qRT-PCR was conducted in triplicate on cDNA purified from grape berries throughout development using the methods described in Dunlevy et al. (2010).

6.3.12 GRAPE SENSORY ANALYSIS

6.3.12.1 Cabernet Sauvignon

Grape berry sensory analysis was conducted as described in Niimi et al. (2017). In brief, assessors were trained over five 2 h sessions involving: taste and mouthfeel discrimination; the berry sensory assessment protocol; attribute generation; reference standard development and; practise evaluations. The finalised list of 30 attributes covered four categories, physical, pulp, skins, and seeds (Table 7).

All samples (4 berries per sample) were evaluated at commercial harvest (approx. 23-25 °Brix) in the 2014 and 2015 vintages. In the 2014 vintage two to four samples were presented in triplicate across ten sessions. In the 2015 vintage, berry ripening was faster than 2014, which meant that in each session nine to 15 berry samples were presented. A total of six sessions were required to evaluate all samples in triplicate in the 2015 vintage.

All attributes were rated on unstructured 15 cm line scales consisting of either two anchors at 10% and 90%, or three anchors at 10%, 50%, and 90%. The assessors were given one minute breaks between each sample and a 5 minute break after every fourth sample. Filtered water, crackers, and pectin solution (1 g/L) were used as palate cleansers. Samples were evaluated in sensory booths at ambient temperature and under natural white light. Data was acquired using Fizz acquisition Ver. 2.47b (biosystèms, Courternon, France).

Table 7. Attribute list of berry sensory analysis with definitions and reference standards.

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Appearance and physical	
Berry Colour	Colour of ripe Cabernet Sauvignon berry from pink, purple, to black (Photo colour chart of berries)
Firmness	Resistance felt when gently squeezed (Marshmallows [Pascall, Ringwood, VIC] and Coles raspberry gummies)
Pedicel removal	Ease with which peduncle detaches from berry
Pulp	
Juiciness	Relative amount of juice released from berry (Cinsaut grapes, Vaillard Blanc grapes[4])
Detachability	The ease at which pulp separates from skin
Gelatinous	How jelly like the flesh is (Chambourcin Q106-3SB 8218 grapes, Chardonnay grapes[4])
Sweetness[1]	Perception of sweetness (Grape juice [Berri, Docklands, VIC] [13 °Brix] to added Shiraz grape concentrate [25 °Brix] [Pfeiffer Enterprises, Loxton, SA, Australia])
Acidity[1]	Perception of sourness (0.5g/L and 1.5g/L tartaric acid [Tarac Technologies, Nuriootpa, SA] in grape juice)
Dark berry flavour	Combined flavour of plums, blue berries, blackcurrants (Crushed fresh blueberries, blackcurrants, and red plum)
Red confectionary flavour	Combined flavour of artificial raspberry, cherries, and bubble gum (Coles raspberry gummies)

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Dried fruit flavour	Combined flavour of dried prunes figs, and raisins (Diced dried figs [Fruit for life, Girraween, NSW] and dried prunes [Woolworths home brand, Bella vista, NSW])
Tropical/melon flavour	Combined flavour of watermelon, honey dew rockmelon, passionfruit (Diced rockmelon, honey dew, watermelon, and passionfruit)
Green flavour[2]	Combination of grass with hints of capsicum and snow peas (Capsicum, grass, snow peas)
Jam flavour[2]	Flavour of cooked red fruit and sugar (St Dalfours four fruit jam, Marmande, France)
Skin characteristics	
Acidity[1]	Perception of sourness of the skin (As above)
Dark berry flavour	Combined flavour of plums, blue berries, blackcurrants (As above)
Dried fruit flavour	Combined flavour of dried prunes figs, and raisins (As above)
Vegetal green	Combined flavour of lettuce, green beans, snow peas, hay, and straw (Fresh red oak lettuce)
Bitterness[1]	Perception of bitterness in the skin (Quinine sulfate 10mg/L and 40mg/L [Sigma Aldrich, St Louis, MO, USA])
Astringency[1]	The mouth-feel of dryness when skins are chewed (Tannin 0.5g/L and 3 g/L ([Tarac Technologies, Nuriootpa, SA])
Disintegration	Resulting particle size after chewing skins
Tannic intensity	Level of surface resistance between tongue and roof of mouth when skins are chewed (Satin fabric and high grade sandpaper P400)
Re-salivation	Length of time in seconds required to re-salivate
Seed characteristics	
Colour	The colour of the backside of seed ranging from green to brown (Colour chart of seeds [Winter et al. 2004])
Astringency[1,3] (uncrushed)	The mouth-feel of dryness when seeds are placed in mouth (As above)
Brittleness	The degree at which seeds shatter to fragments (Passionfruit and toasted sunflower seeds)
Toasted flavor	Perceived level of bread crust, wood, dusty, and earthy apparent in taste (Toasted white bread)
Roasted nut flavor	Perceived level of roasted peanuts and hazelnuts apparent in taste (Peanuts [Nobby's beer nuts, Chatswood, NSW] and Hazelnuts [Woolworths select, Bella Vista, NSW])
Herb green flavor	Perceived level of fresh parsley, green tea, grass, thyme (Grass, green tea bag [Dilma, Brandon Park, VIC])
Astringency (crushed)	The mouth-feel of dryness when seeds are chewed (As above)
Bitterness[1]	Perception of bitterness when seeds are chewed (As above)

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Tannic intensity (seed)	Level of surface resistance between tongue and roof of mouth when seeds are chewed (As above)

[1]Standards prepared in grape juice (Berri Pty Ltd. Docklands, VIC, Australia). [2]Specific to 2014 vintage. [3]Specific to 2015 vintage. [4]Grapes obtained from Coombe vineyard, Waite Campus, University of Adelaide.

6.3.12.2 Chardonnay

Grape berry sensory analysis was conducted by adapting the method from section 6.3.11.1. Assessors were trained in a similar manner as above and attributes generated were customised to Chardonnay grapes (Table 8). All samples were evaluated at commercial harvest (approx. 21-24 °Brix) in both 2015 and 2016 vintages. As ripening was comparatively faster than Cabernet Sauvignon, between 6 and 18 samples (including replicates) were evaluated depending on the number of vineyard sites that were commercially ripe. The berries were presented in paper cupcake holders, labelled with random three digit codes and randomly allocated into compartments of a plastic storage box for each assessor. The evaluation procedure was identical to that described above. All samples from 2015 and 2016 vintages were evaluated in triplicate across the eight and seven sessions, respectively.

Table 8. Attribute list of berry sensory analysis with definitions and reference standards for the assessment of Chardonnay.

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Appearance and physical	
Berry Color	Color of ripe berry (photo color chart of berries)
Firmness	Resistance felt when gently squeezed (Pascall Marshmallows [Cadbury, Ringwood, VIC, Australia] and raspberry confectionary [Nestlé, Rhodes, NSW, Australia])
Pedicel removal	Resistance felt when removing pedicel
Pedicel pulp	Quantity of pulp on pedicel (photo chart)
Pulp	
Juiciness	Relative amount of juice released from berry (Vaillard Blanc grapes from Coombe vineyard)
Detachability	The ease at which pulp separates from skin (Muscat from Coombe vineyard)
Gelatinous	How jelly like the flesh is (Chardonnay grapes in 2015 and Muscat grapes in 2016 from Coombe Vineyard)
Sweetness	Perception of sweetness (30 g/L and 70 g/L sucrose in water [Woolworths Homebrand, Bella Vista, NSW, Australia])
Acidity	Perception of sourness (1 g/L and 3 g/L tartaric acid in water [Tarac Technologies, Nuriootpa, SA, Australia])
Green apple	Flavor of fresh green apples (sliced Granny Smith apple)
Apple juice	Flavor of ripe apple juice (Ashton Valley Crush apple juice [Ashton Valley Fresh Pty Ltd, Ashton, SA, Australia])

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Tropical	Flavor of tropical fruits (watermelon, rockmelon, and canned pineapple [Golden Circle, Northgate, QLD, Australia])
Citrus	Flavor of lemon, orange, grapefruit, and zest (Valencia orange, ruby grapefruit, and lemon)
Skin	
Acidity	Perception of sourness of the skin (as above)
Green apple	Flavor of fresh green apples (sliced Granny Smith apple)
Green	Flavor of leaf and grass (fresh grass cuttings)
Thickness	Sensation of thickness when chewing skins
Bitterness	Perception of bitterness in the skin (quinine sulfate 10 mg/L and 30 mg/L [Sigma Aldrich, St Louis, MO, USA])
Astringency	The mouth-feel of dryness when skins are chewed (grape seed extract 0.5 g/L and 2 g/L [Tarac Technologies, Nuriootpa, SA, Australia])
Disintegration	Resulting particle size after chewing skins
Tannic intensity	Level of surface resistance between tongue and roof of mouth when skins are chewed (satin fabric and high grade sandpaper P400)
Re-salivation	Length of time in seconds required to re-salivate
Seed	
Color	The color of the backside of seed ranging from green to brown (color chart of seeds [Winter et al. 2004])
Astringency (uncrushed)	The mouth-feel of dryness when seeds are placed in mouth (as above)
Brittleness	The degree at which seeds shatter to fragments (passionfruit and toasted sunflower seeds)
Nutty	Flavor of hazelnuts (hazelnuts [Woolworths select, Bella Vista, NSW, Australia])
Wood	Flavor of cedar wood/pencil shavings (pencil shavings)
Astringency (crushed)	The mouth-feel of dryness when seeds are chewed (as above)
Bitterness	Perception of bitterness when seeds are chewed (as above)
Tannic intensity (seed)	Level of surface resistance between tongue and roof of mouth when seeds are chewed (as above)

6.4 Wine Measurements

6.4.1 WINE CHEMICAL MEASURES

Basic chemical measures of the wine were made including pH, TA, ethanol, VA, malic acid, residual sugar. Additional measures were made which were colour measures using the Somers assay and direct CIELab measures on the wines, and total tannin measures using the MCP method.

6.4.2 WINE VOLATILE COMPOUND ANALYSIS

Wine volatile compounds were analysed using the method described in Boss et al. (2014), except the standard mix was changed slightly by the replacement of methyl nonanoate with d_5-ethyl nonanoate. So 10 µL of the mix added equated to: d_{13}-hexanol (9.2 µg for 1:2 dilution, 0.92 µg for 1:100 dilution; C/D/N Isotopes Inc.); d_{11}-hexanoic acid (9.3 µg for 1:2 dilution, 0.93 µg for 1:100 dilution; C/D/N Isotopes Inc.); d_{16}-octanal (0.821 µg for 1:2 dilution, 82.1 ng for 1:100 dilution; C/D/N Isotopes Inc.); d_5-ethyl nonanoate (61.2 ng for 1:2 dilution, 6.12 ng for 1:100 dilution, Boss et al. 2015); d_3-linalool (17.3 ng for 1:2 dilution and 1.7 ng for 1:100 dilution, C/D/N Isotopes Inc.). Sixty eight compounds were quantified in the Cabernet Sauvignon wine and 159 in the Chardonnay wines and these are listed below (Table 9).

Table 9. Compounds for which relative quantities were obtained in the volatile fractions of the wine samples.

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Ethyl acetate	888	A	1
Ethyl propanoate	953	A	2
Ethyl isobutyrate	962	A	3
Propyl acetate (Ch)	967	A	4
Methyl butanoate (Ch)	983	B	5
Decane (Ch)	1000	A	6
Isobutyl acetate	1009	A	7
α-Pinene	1020	A	8
Ethyl butanoate	1033	A	9
1-Propanol (Ch)	1039	A	10
Ethyl 2-methylbutanoate	1050	A	11
Ethyl isovalerate	1067	A	12
Isobutanol	1107	A	13
Isoamyl acetate	1121	A	14
Ethyl valerate	1125	A	15
1-Butanol (Ch)	1149	A	16

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Ethyl crotonate (Ch)	1153	A	17
α-Terpinene (CS)	1154	A	18
Pentyl acetate (Ch)	1160	A	19
Unknown1 (Ch)	1163	C	20
1,4-Dimethylbenzene (Ch)	1164	B	21
2-Heptanone (Ch)	1169	A	22
Methyl hexanoate (Ch)	1170	A	23
Isoamyl propanoate	1173	A	24
Ethyl 4-methylpentanoate (Ch)	1200	B	25
Isoamyl alcohol	1201	A	26
Unknown 2 (Ch)	1216	C	27
Ethyl hexanoate	1235	A	28
1-Pentanol (Ch)	1239	A	29
Styrene (Ch)	1239	A	30
Isoamyl butanoate (Ch)	1248	A	31
Hexyl acetate	1256	A	32
Ethyl (*E*)-3-hexenoate	1273	B	33
Isoamyl isovalerate (Ch)	1280	B	34
Ethyl (*Z*)-3-hexenoate	1283	A	35
(*E*)-3-Hexenyl acetate (Ch)	1287	B	36
4-Methyl-1-pentanol	1288	A	37
(*E*)-3-Hexenyl acetate (Ch)	1298	A	38
Propyl hexanoate (Ch)	1303	A	39
2-Heptanol (CS)	1304	A	40
3-Methyl-1-pentanol	1310	A	41
Monoterpene 1 (Ch)	1315	C	42
Ethyl heptanoate	1319	A	43
Ethyl lactate	1329	A	44
Ethyl (*E*)-2-hexenoate	1329	A	45

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
1-Hexanol	1338	A	46
Unknown 3 (Ch)	1349	C	47
Isobutyl hexanoate (Ch)	1340	B	48
(*E*)-3-Hexen-1-ol	1347	A	49
3-Ethoxy-1-propanol	1360	A	50
Heptyl acetate (Ch)	1361	A	51
(*Z*)-3-Hexen-1-ol	1367	A	52
2-Nonanone (Ch)	1373	A	53
Methyl octanoate (Ch)	1375	A	54
Unknown 4 (Ch)	1394	C	55
Ethyl octanoate	1428	A	56
1-Octen-3-ol (CS)	1432	A	57
1-Heptanol (CS)	1437	A	58
p-Menth-1-en-8-ol (Ch)	1440	C	59
Furfural (Ch)	1446	A	60
Isoamyl hexanoate	1447	A	61
Acetic acid (Ch)	1448	A	62
Octyl acetate (Ch)	1460	A	63
Linalyl acetate (Ch)	1467	C	64
m-Mentha-1,8-diene	1473	C	65
Dihydro-2-3H-thiophenone (Ch)	1481	C	66
2-Ethyl-1-hexanol	1483	A	67
p-Mentha-1,4(8)-diene	1493	C	68
2-Nonanol	1501	A	69
Dihydro-2-methyl-3(2H)-thiophenone (Ch)	1504	A	70
Propyl octanoate	1505	A	71
Benzaldehyde	1506	A	72
Vitispirane 1 and 2	1507	B	73

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Ethyl nonanoate	1517	A	74
Ethyl 2-hydroxy-4-methylpentanoate (Ch)	1520	B	75
Linalool	1526	A	76
1-Octanol	1535	A	77
Unknown 5 (Ch)	1536	C	78
Isobutyl octanoate	1538	B	79
Ethyl 3-(methylthio)propionate (Ch)	1550	A	80
Dihydro-3,5-dimethyl-2(3H)-furanone	1553	C	81
Methyl decanoate (Ch)	1580	A	82
2-Undecanone (Ch)	1583	A	83
Geranylgeraniol (Ch)	1587	C	84
Hotrienol (Ch)	1593	B	85
β-Cyclocitral (Ch)	1600	A	86
Ethyl 2-furoate	1606	A	87
4-Hydroxybutanoic acid (CS)	1607	C	88
Butyrolactone (Ch)	1607	A	89
3-(Methylthio)propyl acetate (Ch)	1607	A	90
Riesling acetal (Ch)	1608	B	91
Ethyl methyl succinate (Ch)	1622	B	92
Butanoic acid (CS)	1624	A	93
Ethyl decanoate (1/100)	1626	A	94
1-Nonanol	1642	A	95
Citronellyl acetate (Ch)	1645	A	96
Isoamyl octanoate	1646	A	97
Ethyl benzoate (CS)	1649	A	98
β-Farnesene (CS)	1649	A	99
Unknown 6 (Ch)	1653	C	100
Diethyl succinate (Ch)	1660	A	101
Isovaleric acid (base)	1673	A	102

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Unknown 7 (Ch)	1675	C	103
p-(1-Hydroxy-1-methylethyl)acetophenone (Ch)	1692	C	104
Ethyl 9-decenoate (Ch)	1685	B	105
3-(Methylthio)-1-propanol	1700	A	106
Norisoprenoid 1	1703	C	107
Unknown 8 (Ch)	1710	C	108
Monoterpene 3 (Ch)	1714	C	109
Propyl decanoate (Ch)	1720	A	110
Sesquiterpene 1 (Ch)	1723	C	111
Unknown 9 (Ch)	1724	C	112
Unknown 10 (Ch)	1725	C	113
Unknown 11 (Ch)	1728	C	114
Unknown 12 (Ch)	1730	C	115
TDN	1731	B	116
Geranyl acetate (Ch)	1736	A	117
Unknown 13 (Ch)	1741	C	118
1-Decanol	1744	A	119
β-Citronellol	1748	A	120
Unknown 14 (Ch)	1750	C	121
α-Curcumene (Ch)	1759	B	122
Ethyl phenylacetate	1768	A	123
Ethyl 4-hydroxybutanoate (Ch)	1775	B	124
1,2-Octanediol (Ch)	1783	B	125
2-(4-Methoxyphenyl)thiophene (Ch)	1789	C	126
Methyl dodecanoate (Ch)	1793	B	127
2-Phenylethyl acetate	1797	A	128
β-Damascenone	1802	A	129
Norisoprenoid 2 (Ch)	1815	C	130

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Ethyl dodecanoate	1828	A	131
Hexanoic acid	1837	A	132
Benzyl alcohol (CS)	1865	A	133
Norisoprenoid 3 (Ch)	1878	C	134
3-Hydroxy-2,2,4-trimethylpentyl 2-methylpropanoate (Ch)	1884	B	135
Isoamyl decanoate (Ch)	1883	B	136
Ethyl dihydrocinnamate (Ch)	1884	B	137
Ethyl 3-hydroxydodecanoate (Ch)	1890	B	138
2-Phenylethyl alcohol (1/100)	1895	A	139
Unknown 15 (Ch)	1896	C	140
Unknown 16 (Ch)	1897	C	141
Unknown 17 (Ch)	1905	C	141
3-(2,6,6,-trimethyl-1-cyclohexen-1-yl)-2-propenal (Ch)	1919	C	142
2-Ethylhexanoic acid (Ch)	1930	B	143
2-Phenylethyl butyrate (Ch)	1945	A	144
1-Dodecanol (Ch)	1949	A	145
Monoterpene 4 (Ch)	1963	C	146
Unknown 18 (Ch)	1975	C	147
Norisoprenoid 4 (Ch)	1987	C	148
α-Cadinol (Ch)	1999	C	149
(*E*)-Nerolidol (Ch)	2025	A	150
Unknown 19 (Ch)	2032	C	151
Ethyl tetradecanoate	2045	A	152
Octanoic acid	2052	A	153
Ethyl 3-hydroxytridecanoate (Ch)	2088	C	154
Ethyl cinnamate (Ch)	2095	A	155
Sesquiterpene 1 (Ch)	2151	C	156
2-Phenylethyl hexanoate (Ch)	2155	A	157

COMPOUND	RETENTION INDICES[1]	IDENTIFICATION[2]	REFERENCE NUMBER IN PCA
Nonanoic acid (Ch)	2163	A	158
Unknown 20 (Ch)	2215	C	159
4-Vinylguaiacol	2235	A	160
Sesquiterpene 2 (Ch)	2241	C	161
Unknown 21 (Ch)	2250	C	162
7-Methoxy-1-tetralone	2253	C	163
Decanoic acid	2269	A	164
9-Decenoic acid (Ch)	2343	A	165
4-Vinylphenol	2355	B	166

[1] RI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}). [2]A, identity confirmed by matching mass spectra and RI with that of authentic standards; B, tentative assignment based upon comparison with mass spectral libraries and published RI; C, unknown compound, possible identity listed if match with mass spectral library is strong. (Ch) = quantified in Chardonnay only. (CS) = quantified in Cabernet Sauvignon only.

6.4.3 WINE DESCRIPTIVE ANALYSIS

6.4.3.1 Cabernet Sauvignon

Descriptive sensory analysis (DA) is a method that determines the differences among samples based on the intensities of attributes that are perceived by the senses; e.g. appearance, smell, taste, texture, and hearing. For the DA of the Cabernet Sauvignon wine samples from the three vintages, a panel of assessors were recruited (n=10) based on their interest, availability and previous experience in DA panels. Five training sessions were held to develop a list of attributes covering five categories including colour, aroma, taste/mouthfeel, flavour, and aftertaste (Table 10). Training spanned between five and ten sessions depending on the vintage. All attributes were rated on unstructured 15 cm line scales.

Table 10. List of attributes, definitions, and reference standards used for DA of Cabernet Sauvignon wines.

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)	SCALE ANCHORS
Color		
Hue	Hue color of the wine (Color chart (Wine folly, Woodinville, WA))	Ruby - Purple
Depth of color	The wine body color intensity	Pale - Deep
Aroma		
Overall aroma	Overall aroma intensity	Low, Medium, High
Dark fruit	Perception of ripe blackberry and blackcurrant (Four fruit jam [St Dalfours, Marmande, France]) *(30 µL Blackberry natural flavour , 1 tsp Ribena)*	Low, Medium, High
Red fruit	Perception of fresh raspberry and strawberry (Fresh strawberry and raspberries)	Low, Medium, High
Dried fruit[£]	Perception of dried prune, raisins, and dry fig aroma (1 dry prune cut in pieces [Woolworths home brand, Bella vista, NSW, Australia], ½ dry fig cut in pieces	Low, Medium, High

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)	SCALE ANCHORS
	[Fruit for Life, Girraween, NSW, Australia], 6 raisins cut in pieces [Sunbeam, Irymple, VIC])	
Jam	Perception of jam or cooked fruit (Plum jam [Granny's secret, Foodland, Adelaide, SA])	Low, Medium, High
Confectionery	Perception of red confectionery (Strawberries and cream [Allen's, Rhodes, NSW], raspberry confectionery, raspberry cordial [Cottee's, Tullamarine, VIC])	Low, Medium, High
Pepper	Perception of pepper (white, green, black) (Ground black and white pepper [Black & Gold, Macquarie Park, NSW])	Low, Medium, High
Spice	Perception of sweet spice (Mixed spice and extra cinnamon [Masterfoods, Wyong, NSW])	Low, Medium, High
Alcohol	The aroma perception of ethanol (Ethanol, 30% [Tarac Technologies, Nuriootpa, SA])	Low, Medium, High
Savory	Perception of black olives and smoked bacon (Black olive [Kalamata sliced, Always Fresh, Scoresby, VIC], smoked bacon [Schulz Butchers smokehouse bacon, Angaston, Barossa Valley, SA])	Low, Medium, High
Green	Perception of capsicum, tomato leaf, eucalyptus (Fresh green capsicum, tomato peduncle, eucalyptus leaf, hedge stalk)	Low, Medium, High
Tobacco/leather	Perception of dried cigar tobacco and leather (Leather, cigar [Jose Piedra])	Low, Medium, High
Rubber	Perception of rubber and Band-Aid (Rubber band and Leukoplast, BSN Medical GmbH, Hamburg, Germany)	Low, Medium, High
Earthy	Perception of earth and mushrooms (Fresh mushroom, soil)	Low, Medium, High
Earthy/leather[£]	Perception of earth, mushrooms, and leather (3 pieces of leather [4 cm each, cut in pieces], ½ fresh mushroom cut in pieces, 1 tsp soil [Waite campus soil])	Low, Medium, High
Flavor		
Overall flavor	Overall flavor intensity	Low, Medium, High
Dark fruit	Perception of fresh blackberry and blackcurrant (Frozen blueberries and blackberries)	Low, Medium, High
Red fruit	Perception of fresh raspberry and strawberry (As above)	Low, Medium, High
Jam	Perception of jam or cooked fruit (Plum jam (As above)	Low, Medium, High
Confectionery	Perception of red confectionery (As above)	Low, Medium, High
Pepper	Perception of pepper (white, green, black) (As above)	Low, Medium, High
Spice	Perception of sweet spice (As above)	Low, Medium, High
Savoury	Perception of black olives and smoked bacon (As above)	Low, Medium, High
Green	Perception of capsicum, tomato leaf, eucalyptus (As above)	Low, Medium, High
Rubber	Perception of rubber and Band-Aid (As above)	Low, Medium, High
Taste		

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)	SCALE ANCHORS
Acid	Acidic perception of wine on palate	Low, Moderate, Very acidic
Fruit sweetness	Perception of sweetness coming from fruit	Low, Medium, High
Bitterness	Perception of bitterness	Low, Medium, High
Mouthfeel		
Body	Mouth filling sensation of wine in palate	Light, Medium
Astringency	Amount of drying sensation of tannins after tasting wine	Low, Medium, High
Alcohol intensity	Amount of warming sensation caused by alcohol content	Mild, Warm, Hot
Tannin quality	Sensation of tannin coarseness	Fine, velvet, coarse sand paper
Aftertaste		
Length	Duration of any wine attributes perceived after expectorating	<10 sec, 15 sec, <20 sec
Bitter[£]	Perception of bitterness after expectorating wine	Low, medium, high
Fruit length[£]	Time that wine fruit flavour remains on palate after spitting	>2sec, 5 sec, 10 sec, >20 sec
Phenolic Length[£]	Time that phenolic length remains on palate after spitting	>2sec, 5 sec, 10 sec, >20 sec

[£] Attributes specific to 2013

6.4.3.2 Chardonnay

For the DA of the 25 Chardonnay wines from two vintages, a panel of assessors were recruited (n=9-11) who were screened using International Standards Organisation guidelines for the recruitment of a sensory panel (ISO 13300-2:2006). Ten training sessions were held to develop a list of attributes covering five categories including colour, aroma, taste/mouthfeel, flavour, and aftertaste (Table 11). All attributes were rated on unstructured 15 cm line scales. Wines were assessed for in mouth properties in Black INAO tasting glasses and colour was separately assessed in clear tasting glasses.

Table 11. List of attributes, definitions, and reference standards used for DA of Chardonnay wines.

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Aroma	
Tropical fruits	Tropical fruits (canned passionfruit [John West, Mentone, VIC, Australia], fresh pineapple, rock melon, banana)
Citrus	Lemon and limes (fresh lemons and limes)
Green apple	Green apples (fresh granny smith apples)
Peach	Peaches (canned peaches [Goulburn Valley, Shepparton, VIC, Australia])
Honey	Honey (honey (Capilano, Richlands, QLD, Australia))
Medicinal	Chemicals (4-ethyl phenol (400 µg/L) [Sigma Aldrich, Castle Hill, NSW, Australia] and surgical tape [Leukoplast GMBH, Hamburg, Germany])
Green[£]	Fresh cut plants (grass and hedge clippings)
Earthy[£]	Dampness (mushrooms and damp dirt)

ATTRIBUTE	DEFINITION (REFERENCE STANDARD)
Taste	
Sourness	Acids (citric acid (1 g/L) [McKenzie's, Altona, VIC, Australia])
Sweetness	Sugars (sucrose (10 g/L) [Woolworths Home brand, Bella Vista, NSW, Australia])
Bitterness	Bitterness (quinine (50 mg/L) [Sigma Aldrich, St Louis, MO, USA])
Flavor	
Citrus	Lemons and limes (as above)
Tropical fruits	Tropical fruits (as above)
Green apple	Green apples (as above)
Peach	Peaches (as above)
Mouth-feel	
Astringency	Sensation of mouth puckering (Grape seed extract 2 g/L in water [Tarac Technologies, Nuriootpa, SA, Australia])
Heat	Burning sensation in oral cavity (Ethanol in water 15% [Tarac Technologies])
Viscosity[£]	Sensation of fullness (Xanthan gum 0.5 g in water [The Melbourne Food Ingredient Depot, East Brunswick, VIC, Australia])
After-taste	
Length	Duration of mouth sensation
Bitterness	The aftertaste of bitterness
Sourness	The aftertaste of acids
Heat	The persistence of burning sensation in oral cavity
Astringency	The persistence of the sensation of mouth puckering

[£] Attributes specific to 2015

6.4.4 WINEMAKER SORTING TASK

As one objective of the project was to determine measures that could relate to wine quality, the wines from each vintage were graded by winemakers. We determined the perceived quality ratings of the wines as they may provide opportunities to find relationships to the perceived quality of the grapes.

Winemakers were recruited for the evaluation from within South Australia. The experience of these winemakers in the wine industry ranged from 6 to more than 26 years. All of the winemakers taste wine as part of their job description, with most also having experience in judging wine and were included using previously proposed criteria (Parr et al. 2002). Prior to starting the sorting task, the winemakers were introduced to the task and were notified that the wines had completed both primary and secondary fermentation for Cabernet Sauvignon or only primary fermentation for Chardonnay and had not been aged in oak. A discussion was held with the group to define four different quality levels of wines; premium, high, moderate, and low qualities (in certain vintages an extra quality level was added which was "faulty"; Table 12). These definitions were formed generically and broadly so as to provide a framework for the winemakers to categorise the wines into quality groups whilst allowing freedom for their individual opinions to be incorporated. The winemakers sorted the wines into different quality grades as if the wine were being allocated in a commercial winemaking setting in triplicate. The definitions of quality that spanned from premium quality to low quality were set prior to the sorting.

6.4.5 DATA ANALYSIS

6.4.5.1 Univariate statistical analyses

Descriptive statistics and the distribution of the data was first visualised by plotting box plots to identify outliers, for which the raw data was then checked to confirm the values. The significance of any differences

between samples was tested by ANOVA and, if significant (p<0.05), was followed by Fisher's least significance difference test (LSD). All univariate statistical analyses were conducted using IBM SPSS Statistics ver. 20 (IBM Australia, Sydney, NSW, Australia).

6.4.5.2 Principal components analysis

The simplest way to visualise the relationships among a set of samples upon which multiple measurements have been made is to use a technique called Principal Component Analysis (PCA). PCA seeks to emphasise the variation among the samples and shows us the strongest patterns in a set of data. Often the first two principal components (PCs) are plotted and the percentage of the variation represented by each PC is quoted, with the largest percentage plotted across the horizontal axis (PC1) and the next largest on the vertical axis (PC2). The plots in this report often have the "scores" and "loadings" plotted together in a biplot. The scores show how the various samples relate to each other, but also how they correspond to the measures, which are called the loadings. So samples in the biplot that are found far apart with regards to the horizontal, vertical or both axes are less similar compared to those located close to one another. Measures in the biplot are collocated with certain samples because those samples have higher values for those measures compared to samples found on the opposite side of the plot (this may be horizontally, vertically or diagonally across the plot). In other cases the score and loadings plots are presented separately because the large number of variables makes the biplot cluttered and difficult to read. These are interpreted in the same way as a biplot.

PCA in this study were conducted using The Unscrambler X 10.1 (Camo, Oslo, Norway). Data were standardized using the 1/SDev option for variable weighting, with cross validation and use of the non-linear iterative partial least squares (NIPALS) algorithm with no rotation.

6.4.5.3 Multidimensional scaling (MDS)

The data from the winemaking sorting tasks were analysed using multidimensional scaling (MDS) using IBM SPSS Statistics. The procedure used for data preparation and analysis with MDS was the same as that previously published (Niimi et al. 2016).

6.4.5.4 Partial Least Squares (PLS) modelling

To determine the relationships between the sensory attributes and basic chemical measures of grapes and the sensory attributes of wine in the two varieties Cabernet Sauvignon and Chardonnay, data were analysed using partial least squares (PLS) regression using The Unscrambler X. The procedures used to undertake PLS along with pre-processing of data were the same as those previously published (Niimi et al. 2017, Niimi et al. 2018).

6.4.5.5 Sequential and orthogonalised-partial least squares (SO-PLS) modelling

To determine the relationships between grape measures and the wine sensory profiles and attributes, a series of PLS models were used. As there were multiple data sets (blocks) generated from measures of grape composition in both the Cabernet Sauvignon and Chardonnay (12 and 9 blocks of measures, respectively), a data analysis technique that would systematically analyse the contribution of each individual block for modelling and thus their level of importance for the models, was necessary. Sequential and orthogonalised-PLS (SO-PLS) was used as the method manages multiple data blocks for modelling by means of orthogonalisation (Menichelli et al. 2014). Data blocks were added orthogonally to each model (entire sensory profile, and for single sensory attributes of Cabernet Sauvignon and Chardonnay) until there was no further improvement as indicated by the root mean squared error of validation, the number of components used for each block, and the percentage of improvement obtained in the validated explained variance. All analyses for SO-PLS modelling was performed using Python programming language (Python ver. 3.5) using the packages numpy, IPython, Pandas, and statsmodels (Oliphant 2007, Peréz and Granger 2007, McKinney 2010, Seabold and Perktold 2010). The SO-PLS procedure was coded in-house.

Table 12. List of definitions formed by the winemakers prior to sorting of Cabernet Sauvignon and Chardonnay wines.

Quality grade	Cabernet			Chardonnay	
	2013	**2014**	**2015**	**2015**	**2016**
A: Premium	*Wines that are complex with good structure and tannins, expression of varietal and regional characteristics, and is balanced and has good length.*	*Intense, dense with varietal characteristics, excellent structure and balanced tannin, complexity, length and texture.*	*Dark fruits, herbs and spice character. Balanced flavour and acid, weight, length, vibrant colour and flavours. Concentrated, has varietal definition, and complex*	*Intense varietal character, complexity, robustness, balanced flavours (no overripe/under ripe characters), full palate, good texture, fresh and vibrant, long finish. Handled with appropriate wine making technique. Has aging potential. These wines express their origins.*	*Wine with balance, persistence, acidity (structure), complexity, creamy, with stone fruit and zesty/citrus flavours, phenolics and tannins, good fruit and flavour intensity, vibrancy, freshness with fruit purity, minerality (flinty) flavour and good sulphites.*
B: High	*Wines with some varietal expression, flavour intensity that is above average, with tannin and body.*	*As explained in A grade, but to a lower intensity and complexity*	*Wines that lack the finesse of grade A. It still has dark fruit, herb and spice characters, balanced flavours, weight, length, vibrancy, some varietal definition and complexity*	*Good intensity in varietal character, some complexity and balanced flavours. Good texture, full palate characters. Some level of purity to the overall wine. It is fresh and vibrant, handled appropriately, has aging potential.*	*Balanced wines with freshness and vibrancy. Missing some key aspects from grade A. Good complexity, some persistence and intensity, acid structure, less complete but sound wine.*
C: Moderate	*Wines that are simple, soft and low to moderate flavour intensity and varietal expression.*	*Simple wine that is soft and has moderate varietal characteristics. It has less body and structure.*	*Wines that are green, fruit driven and has confectionary character. They lack varietal definition, length and complexity. They are simple and low in flavour intensity, soft acid/tannins, hollow and can be sweet*	*Moderate intensity, some varietal character, round structure, low complexity. Clean, fresh, balanced acidity/fruit, and some depth. Low evidence of origin, maybe unbalanced overall, lacks finesse, low aging potential and moderate length.*	*Overripe or under ripe tropical fruits. Less intense than grade B attributes. Higher amounts of bitterness/phenolics, simple with soft acidity, neutral flavour, less balanced, definition, and citrus flavours than grade B. Commercially acceptable.*
D: Low	*Wines that either have no varietal expression, lack of balance, or contain faults.*	*Under/overripe (green to jammy) with low flavour intensity, unbalanced structure and lacks varietal characteristics and colour.*	*Wines that are unbalanced and low in complexity. They are thin/dilute, lacks varietal definition and are dry red wines (non-distinct). It can have too much/little acid, ripeness of flavours, tannins and can be faulty*	*Overripe/under ripe, lacks varietal definition, unbalanced. Typically has too low/high acidity, unclean, and short. It can also be faulty.*	*Low intensity, diluted, soft phenolics, Overripe, high phenolics, bitter. The wine is out of balance. Can have green flavours, simple, short, too sweet, lacks fruit flavour.*
E: Faulty	-	*Wine that has faults.*	-	-	*Faulty wines, commercially unacceptable.*

7 Results/Discussion

7.1 Cabernet Sauvignon Grape Measures

A broad range of Cabernet Sauvignon grape samples from hot, warm and cool growing regions of South Australia was obtained from our industry partners in the 2013, 2014 and 2015 vintages. Small-scale fermentations (50 kg) were carried out on the parcels of fruit, including malolactic fermentation. Chemical and sensory measures were made on the Cabernet Sauvignon fruit and wine from the three vintages and then the data mined for significant relationships between fruit composition and sensory attributes. The results from this work is outlined below.

7.1.1 GRAPE HARVEST MEASURES

7.1.1.1 °Brix

In 2013, harvest dates ranged from 15/02/2013 in the Riverland (RVL) to 9/04/2013 in Coonawarra (CWA; Table 1). The target total soluble solids (TSS) range was 23-25 °Brix, although this was dependent upon the desired °Brix/°Baume sought by the companies supplying the fruit. Nevertheless, in most cases the samples were close to this range with many having a mean TSS only slightly higher than 25 °Brix (Table 13).

In 2014, grape parcels fell between 22.2 and 26.2 °Brix (Table 13) and were harvested between 06/02/2014 in the RVL to 16/04/2015 in CWA (Table 1). Four of the nine RVL samples had TSS levels slightly lower than the target range.

The 2015 harvest was complicated by an overlap with the Chardonnay samples collected in the same year. However, TSS ranged from 22.6 to 26.7 °Brix (Table 13) and there was a tendency for them to be higher in this vintage compared to the other years. The Cabernet Sauvignon samples were harvested between 12/02/15 and 26/03/15.

Table 13 °Brix means (n=30) for each of the Cabernet Sauvignon grape parcels from the three vintages. The Least Significant Difference (LSD) at the bottom of the table is the minimum difference between two means within each year necessary for a significant difference at $p < 0.05$.

VINEYARD	°BRIX		
	2013	2014	2015
BV1	26.0	23.8	24.9
BV2	23.6	24.2	25.4
CV1	24.8	24.0	26.7
CV2	25.3	24.5	25.9
CWA1	25.1	24.3	
CWA2	24.7	24.5	24.0
CWA3	25.5	24.3	25.2
CWA4	24.2		

VINEYARD	°BRIX		
	2013	2014	2015
CWA5		22.2	23.8
CWA6			24.8
EV1	22.7	25.5	23.9
EV2	22.0	24.6	22.6
LC1	25.0	23.3	25.1
LC2	26.0	23.8	23.4
McV1	26.7	25.8	25.6
McV2	25.9	26.2	26.5
RVL1	25.4		
RVL2	24.2		
RVL3	25.5	22.6	24.1
RVL4	26.0	24.0	25.7
RVL5	24.8		
RVL6	25.1	24.4	24.2
RVL7	23.5	23.2	26.5
RVL8	25.4		
RVL9	26.8		
RVL10		22.6	23.4
RVL11		22.9	22.6
RVL12		24.9	24.2
RVL13		22.6	26.7
RVL14		24.2	26.3
WBY1	26.3	25.4	25.6
WBY2	25.8	25.3	24.1
LSD (p<0.05)	*0.7*	*0.6*	*0.8*

7.1.1.2 Berry Weight

In 2013, the average berry size ranged from 0.73 g in a Clare Valley (CV) sample to 1.21 g in a parcel from Wrattonbully (WBY; Figure 1). The RVL samples were generally larger than the overall average (0.92 g), ranging from 0.76 g (RVL9) and 1.14 g (RVL5). While there has been a lot of discussion over the years about berry size and skin/flesh ratios in relation to fruit quality, recent work suggests that skin thickness needs to be taken into account as this also influences the percentage skin mass per berry (Matthews and Kriedemann 2006).

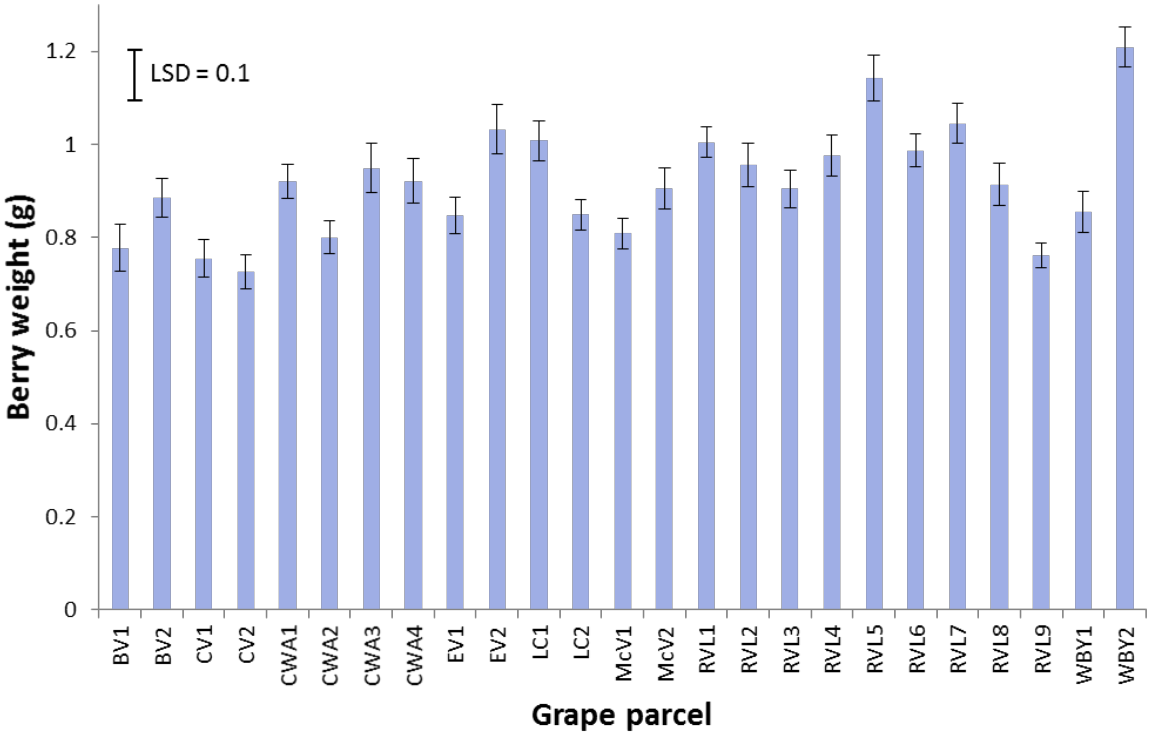


Figure 1. Average berry weight of 30 berries randomly sampled from each site in 2013. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

The mean berry size in 2014 (0.86 g) was slightly less than that in 2013. The variability in berry size across the vineyards ranged from 0.57 g in the McLaren Vale sample McV1 to 1.20 g in one of the RVL samples, RVL12 (Figure 2). Some similarity was seen across the first two vintages, with the CV and McV1 fruit generally smaller than those from other sites.

Berries were larger in the 2015 vintage with the mean berry size being 1.10 g. The WBY2 fruit was the largest, as it was in 2013 (Figure 3) and other sites which consistently had relatively large berries were RVL12, RVL10 and EV2. The berries from the two CV sites were again amongst the smallest, although RVL7 provided small fruit sizes in the 2014 and 2105 vintages.

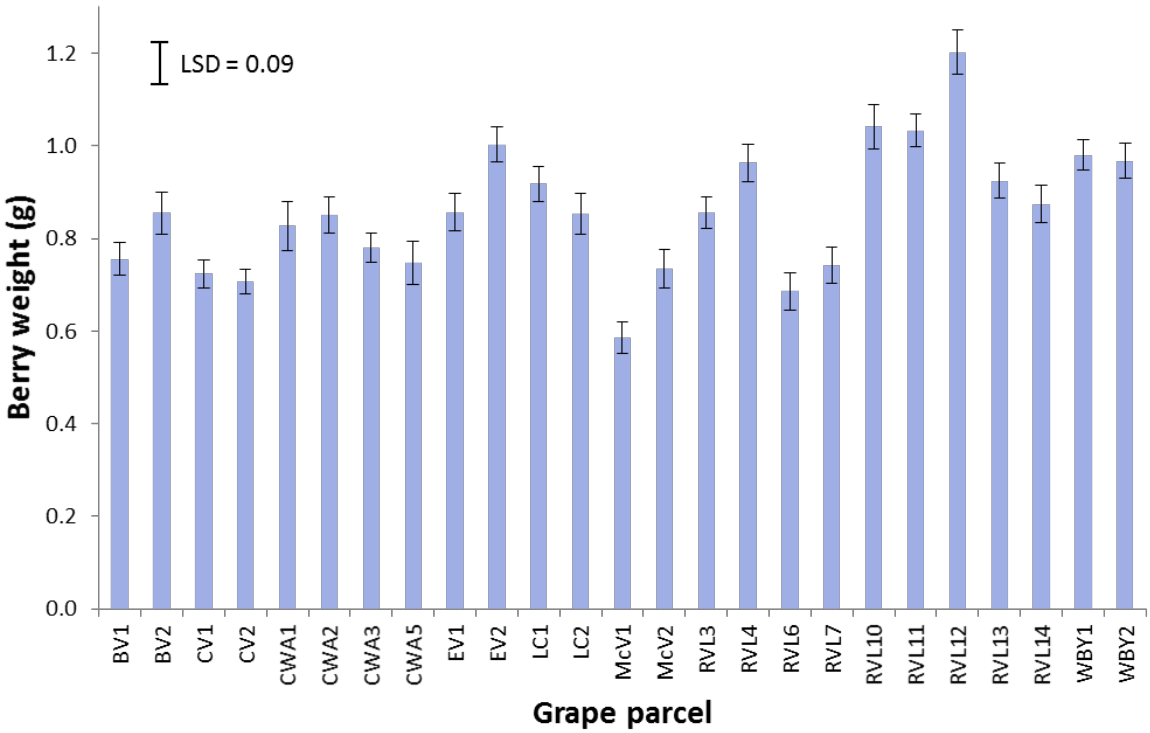


Figure 2. Average berry weight of 30 berries randomly sampled from each site in 2014. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

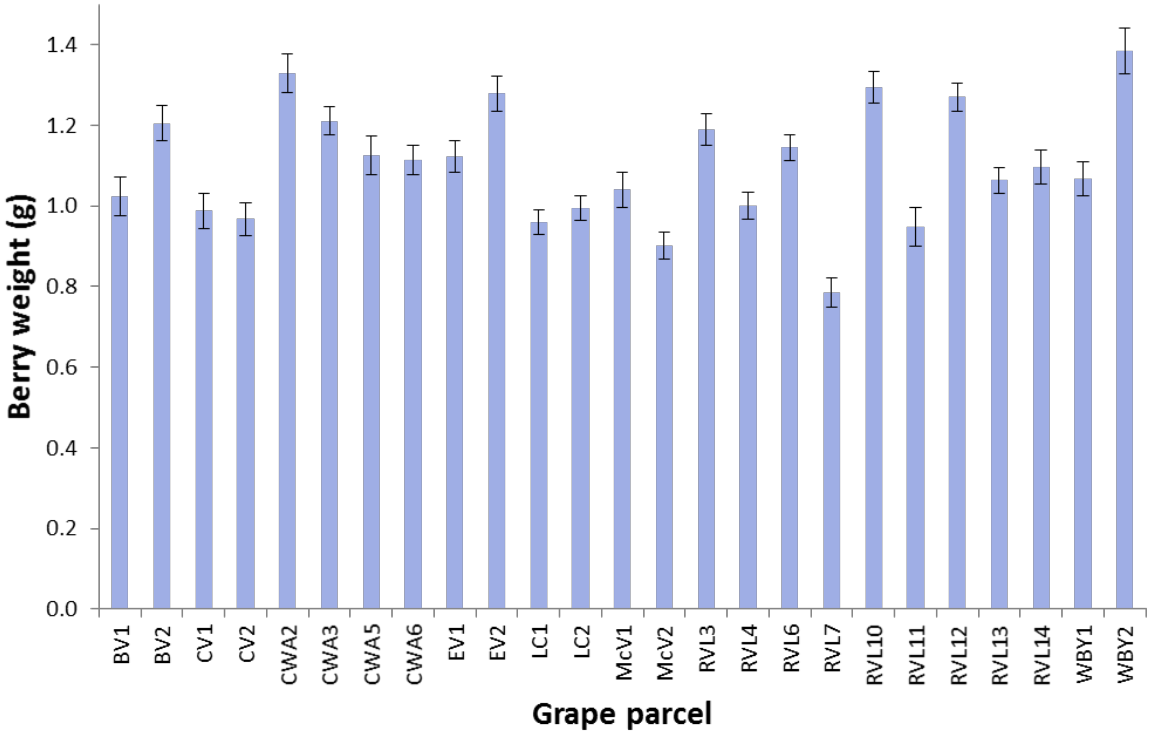


Figure 3. Average berry weight of 30 berries randomly sampled from each site in 2015. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

7.1.1.3 Bunch Weight

Bunch weights did not appear to be driven wholly by berry size in 2013, which is evident when a comparison of Figure 1 and Figure 4 below is made. The RVL samples were spread across the range of bunch weights with one (RVL2) very close to the total average bunch weight (116 g) and four higher and four lower than this. The largest bunch weights were measured in the samples from WBY (205 g and 170 g), conversely a parcel each from Eden Valley (EV) and Barossa Valley (BV) had average bunch weighs of about 75 g and were the lowest recorded.

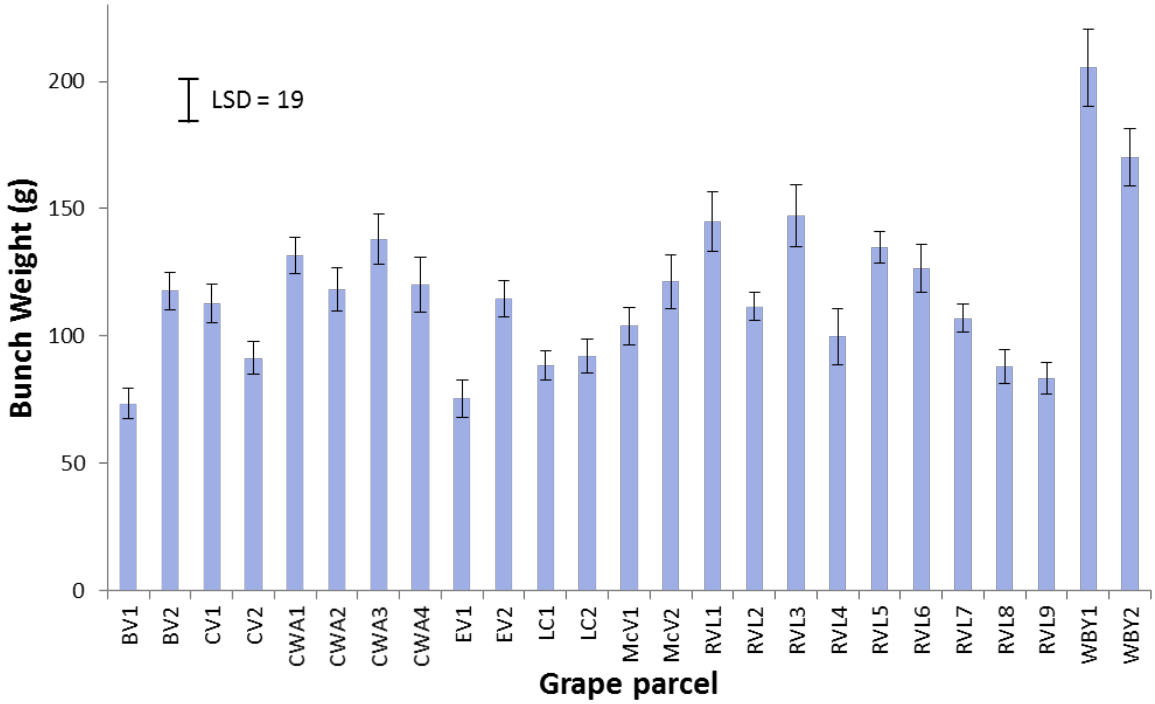


Figure 4. Average bunch weight of 25 bunches randomly sampled from each site in 2013. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

Bunch weights in 2014 were again variable, but comparing Figure 2 and Figure 5 suggests that berry size may have had more of an influence on bunch weights in 2014 compared to 2013. Three RVL vineyards (RVL10, RVL12 & RVL13) had the highest bunch weights of 145, 140 and 142 g respectively. The lowest bunch weights were recorded in the parcels harvested from CWA5 being on average 40 g per bunch. For those sites that were sampled in both 2013 and 2014, the bunch weights were generally smaller in 2014. Mean bunch weight in 2014 was 87 g compared to 117 g in 2013.

In 2015, the Cabernet Sauvignon bunch weights were again highly variable among the sites (Figure 6). There may have been a moderate influence of berry weight on bunch weights in this vintage (cf Figure 3 and Figure 6). Highest bunch weights were observed in RVL10 again (176 g) and the lowest were recorded in the parcels harvested from Langhorne Creek (LC; 65 and 51 g). Mean bunch weight was similar to that seen in 2013, being 113 g in 2015.

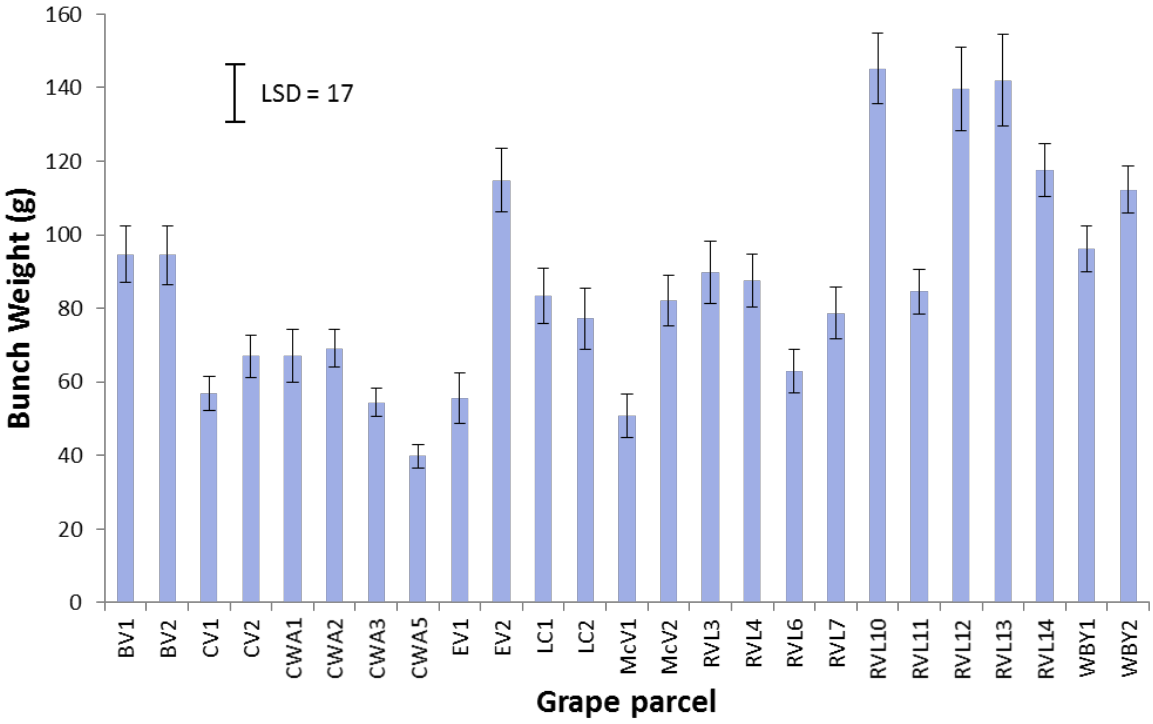


Figure 5. Average bunch weight of 25 bunches randomly sampled from each site in 2014. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

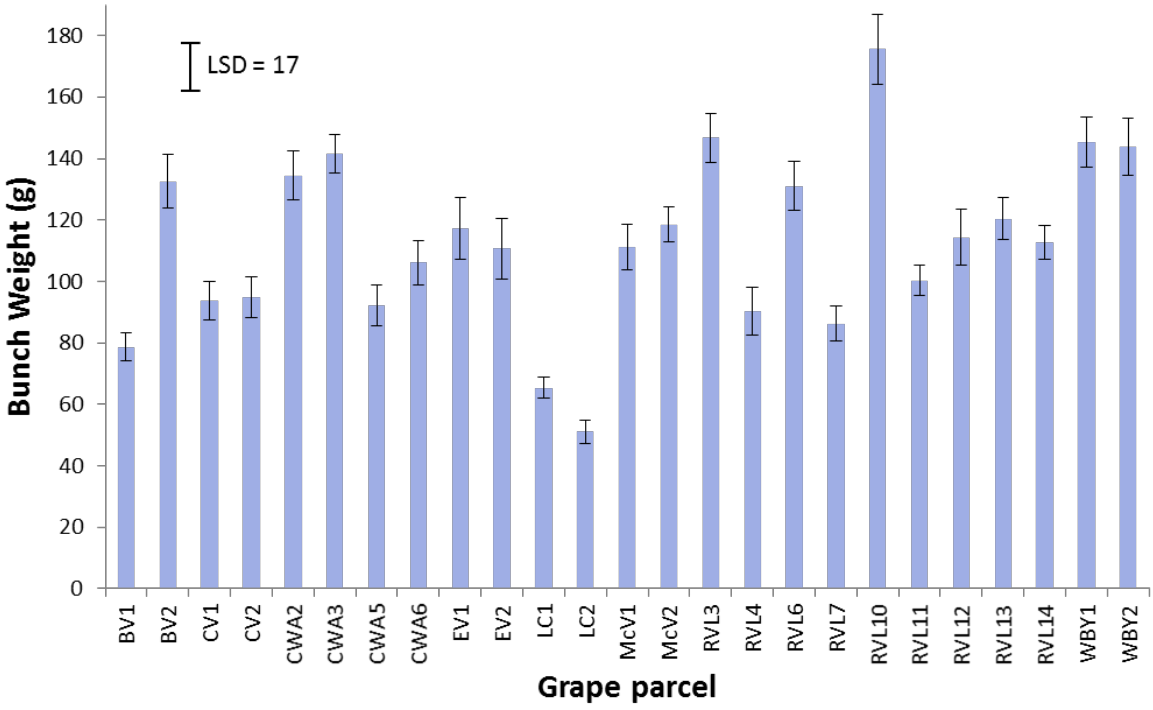


Figure 6. Average bunch weight of 25 bunches randomly sampled from each site in 2015. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

7.1.1.4 Colour, Total Phenolics and CIELAB

Measures of colour and total phenolics in grapes are routinely conducted to assess aspects of quality based on the impact these factors will have on wine colour and, potentially, mouthfeel. To compliment the colour and phenolic assays, CIELAB measurements of the grapes were also conducted. This is a quick means to get an assessment of colour in the fruit and is used for many horticultural crops (Pathare et al. 2013).

The CIELAB measures were analysed using PCA alongside the colour and phenolics measures as this is a good way to visualise the relationship among the measurements and the samples. TSS measures were also included to gauge the influence of fruit maturity on the colour and phenolic measures. In 2013, 83% of the variation was explained by the separation on the horizontal axis (Figure 7). This separates all of the RVL samples, except RVL9, from most of the other grape parcels. The measures driving this separation were lightness (L*) and blue-yellow (b*) on the left of the plot which were associated with the RVL samples. Total colour was collocated with chroma (C*) and red-green (a*) and these were driving horizontal separation with samples on the right having higher values for these measures. Total phenolics were also located on the right of the plot. Separation on the vertical axis was driven mainly by Brix, with more mature fruit located near the top of the plot. It is interesting that the least coloured non-RVL samples (EV1 & 2, BV2) were those with the lowest Brix, indicating the effect of maturity on colour (Figure 7).

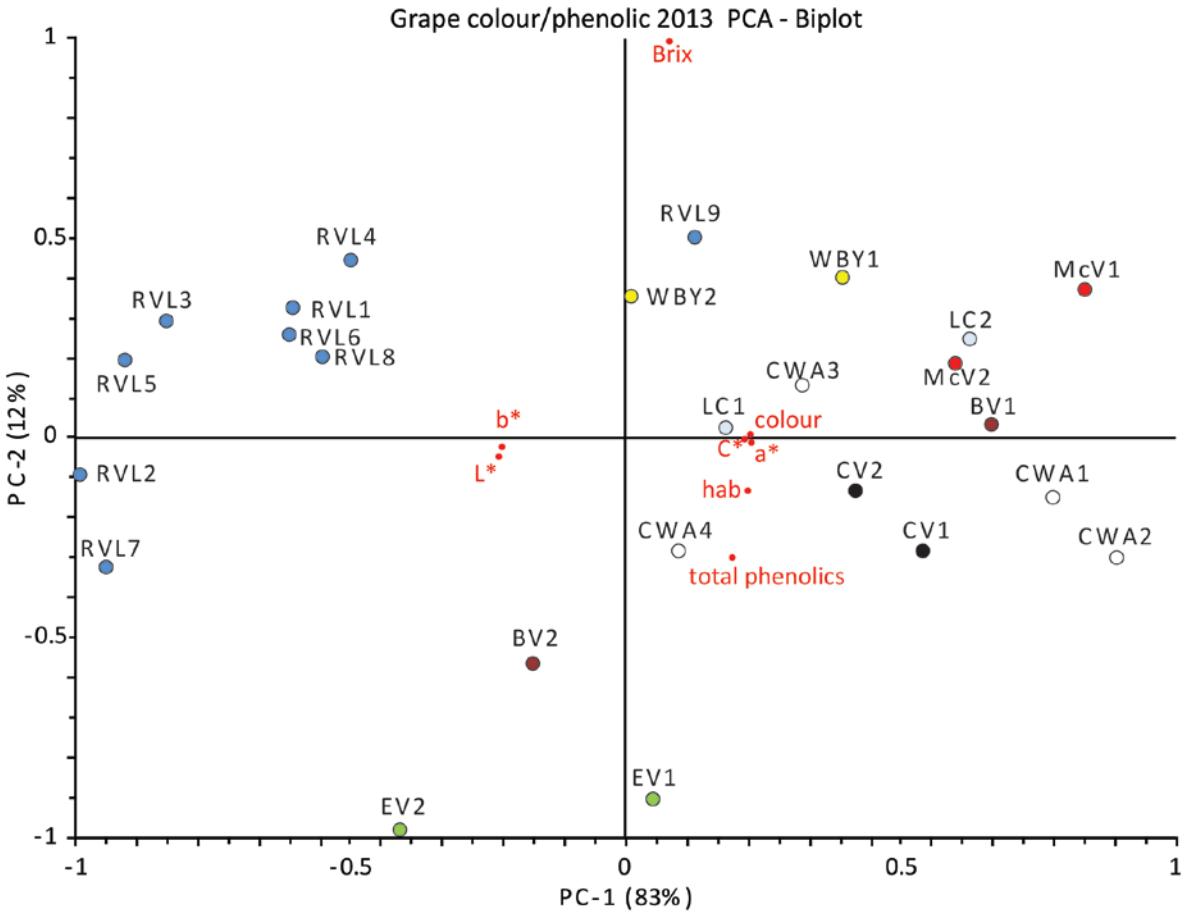


Figure 7. PCA biplot showing the scores (coloured circles and black font) of the 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the associated colour, phenolic, CIELAB and Brix loadings (red circles and red font).

A very similar pattern was seen for the scores and loadings in the PCA plot of the 2014 samples. The RVL sites were located on the left of the plot, corresponding to those samples with higher L* and b* scores (Figure 8). The samples with greater colour were located on the right of the plot and 82% of the total variation was described by this first PC. Again, colour measured spectrophotometrically collocated with the C* and a* measures from CIELAB, and total phenolics was also found in a similar region of the plot to the colour measures. Separation on the vertical axis was again driven solely by Brix, but only represented 10% of the total variation among the samples. One difference noted in 2014 was that the non-RVL sites with the lowest colour measures (EV2 & BV2) were not necessarily those that had the lowest TSS (Figure 8).

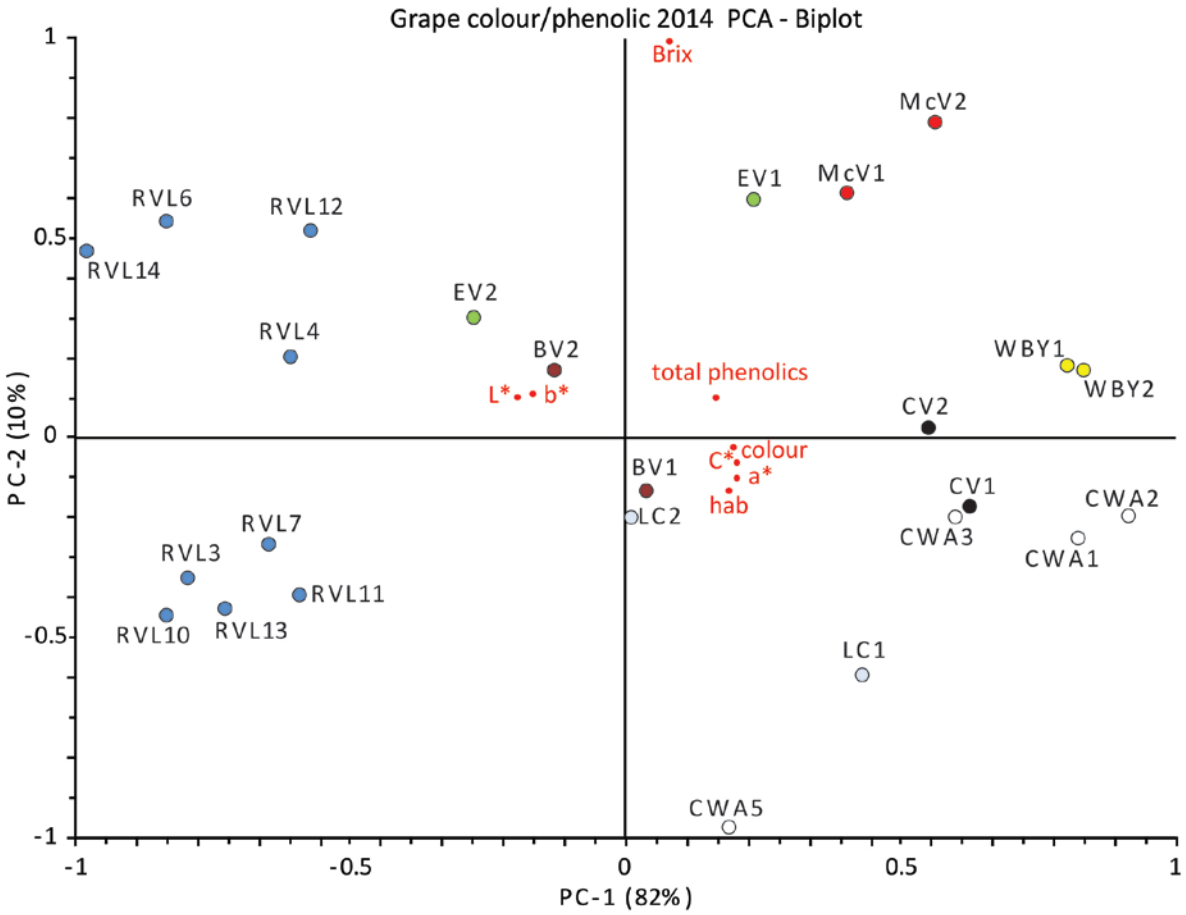


Figure 8. PCA biplot showing the scores (coloured circles and black font) of the 25 different 2014 Cabernet Sauvignon grape samples from eight regions across South Australia and the associated colour, phenolic, CIELAB and Brix loadings (red circles and red font).

In 2015, the PCA confirmed the findings from the previous two vintages. The RVL samples were located on the left of the plot due to higher L* and b* scores (Figure 9). Driving separation on the right side of the horizontal axis were total colour, C* and a*, and non-RVL had higher scores for these measures. PC1 described 76% of the total variation and the vertical axis 14%, which was again discriminating samples based mainly on Brix, although total phenolics were also important. Like 2013, it was observed that some of the non-RVL sites with the lowest Brix were also those that had lower colour and total phenolics (Figure 9).

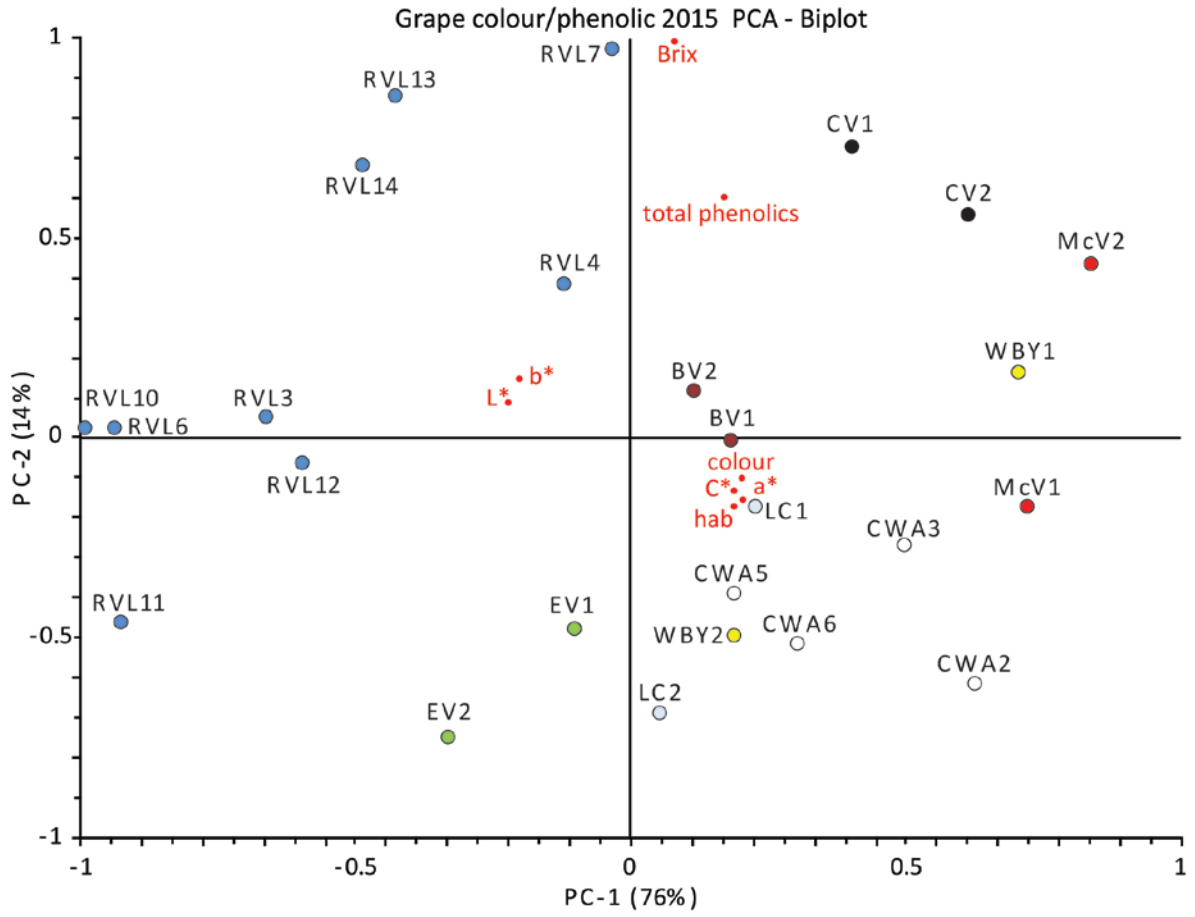


Figure 9. PCA biplot showing the scores (coloured circles and black font) of the 25 different 2015 Cabernet Sauvignon grape samples from eight regions across South Australia and the associated colour, phenolic, CIELAB and Brix loadings (red circles and red font).

Colour and phenolic measures across the three vintages gave consistent patterns. In general, the RVL samples had lower colour and phenolics, whereas samples from CV, CWA and McV contained the more of these compounds. In two of the three years, lower colour and phenolics appeared to be related to earlier harvests and lower Brix measures. The strong co-localisation of the CIELAB measures of C*and a* with the traditional colour measures suggests either technology is applicable so CIELAB should be considered as an alternative if it is cheaper and/or quicker than current protocols.

7.1.1.5 pH, Titrable Acidity (TA) and Yeast Assimilable Nitrogen (YAN)

Analyses of pH, TA and YAN were conducted on the fruit at receival. The results for 2013 are listed in the table below (Table 14). pH values for the fruit parcels ranged from 3.4 to 4.26. The pH of the fruit from CWA tended to be relatively low and those from LC were above 4. TA was lowest in the BV1 and CV2 samples (2.7) and was highest in the RVL5 sample at 5.7. YAN was highly variable, but all wines were supplemented to avoid stuck ferments. Nevertheless, the YAN in the fruit itself ranged from 168 to 393 mg/L. The RVL parcels, in general, had lower amounts of YAN than those harvested from other regions.

Table 14. Measures of pH, TA and YAN in the Cabernet Sauvignon grape parcels in 2013. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

PARCEL	pH	TA (g/L)	YAN (mg/L)
BV1	4.03	2.7	298
BV2	3.71	3.4	393
CV1	3.75	3.1	280
CV2	3.84	2.7	289
CWA1	3.53	3.8	257
CWA2	3.52	4.0	269
CWA3	3.51	5.1	260
CWA4	3.51	4.7	236
EV1	4.00	2.8	382
EV2	3.40	4.7	282
LC1	4.18	3.0	352
LC2	4.26	3.1	311
McV1	3.60	3.7	236
McV2	3.45	4.1	251
RVL1	3.52	4.6	175
RVL2	3.70	4.3	210
RVL3	3.91	3.1	258
RVL4	3.81	4.3	176
RVL5	3.48	5.7	168
RVL6	3.83	3.5	230
RVL7	3.59	4.4	256
RVL8	3.88	3.9	279
RVL9	3.73	3.9	194
WBY1	3.75	3.4	271
WBY2	3.69	4.0	202
LSD	*0.22*	*0.9*	*37*

In 2014, the pH values for the grape parcels ranged from 3.48 to 3.95 (Table 15) and were generally lower than those measured in 2013. There was no major trend across the regions with regards to pH in 2014. TA

was lowest in the WBY1 sample (4.9) and was highest at 7.1 in BV2, RVL3 and RVL10. YAN was again highly variable ranging from 134 mg/L (RVL11) to 401 mg/L (BV1). The RVL sites again had relatively lower YAN, but some other sites were also low in YAN including CWA1 and LC2 (Table 15).

Table 15. Measures of pH, TA and YAN in the grape parcels in 2014. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

PARCEL	pH	TA (g/L)	YAN (g/L)
BV1	3.84	6.1	401
BV2	3.65	7.1	377
CV1	3.69	6.0	301
CV2	3.76	5.8	247
CWA1	3.46	6.3	139
CWA2	3.59	6.6	257
CWA3	3.64	6.2	333
CWA5	3.48	7.0	292
EV1	3.73	6.7	395
EV2	3.72	6.4	367
LC1	3.73	6.3	273
LC2	3.74	6.3	215
McV1	3.87	6.2	251
McV2	3.71	6.3	235
RVL3	3.95	7.1	194
RVL4	3.78	5.2	139
RVL6	3.86	6.4	197
RVL7	3.77	6.4	197
RVL10	3.62	7.1	239
RVL11	3.88	5.2	134
RVL12	3.78	6.4	255
RVL13	3.79	6.4	275
RVL14	3.71	6.2	179
WBY1	3.94	4.9	326
WBY2	3.72	6.3	235
LSD	*0.09*	*0.8*	*59*

The grape parcels harvested in 2015 had pH values lower than those seen in 2013 and were similar to those from 2014. The pH ranged from 3.39 to 3.88 (Table 16; Table 15) and did not seem to been affected by the region the fruit was grown in. TA ranged from 4.1 in the McV1 parcel to 7.0 in EV2 (Table 16) and again it was difficult to see any effect of region on these values. YAN was highest in most samples in this vintage compared with 2013 and 2014, with the values ranging from 221 mg/L (RVL11) to 501 mg/L (BV2). It was not so obvious that the RVL grapes were lower in YAN than the other sites in 2015, with samples from WBY and McV having similar YAN concentrations to the RVL samples (Table 16).

Table 16. Measures of pH, TA and YAN in the grape parcels in 2015.

PARCEL	pH	TA (g/L)	YAN (g/L)
BV1	3.66	5.5	450
BV2	3.66	6.4	501
CV1	3.63	4.7	259
CV2	3.58	5.5	266
CWA2	3.67	5.5	285
CWA3	3.42	5.5	329
CWA5	3.42	6.7	285
CWA6	3.39	6.9	317
EV1	3.69	5.4	428
EV2	3.58	7.0	437
LC1	3.80	5.0	391
LC2	3.71	5.2	333
McV1	3.71	4.1	301
McV2	3.49	4.5	272
RVL3	3.84	5.9	321
RVL4	3.67	4.9	238
RVL6	3.72	6.0	336
RVL7	3.66	4.6	364
RVL10	3.58	5.6	282
RVL11	3.55	5.1	221
RVL12	3.60	6.0	384
RVL13	3.68	5.0	346

PARCEL	pH	TA (g/L)	YAN (g/L)
RVL14	3.75	5.4	243
WBY1	3.88	4.3	269
WBY2	3.47	6.4	229
LSD	*0.11*	*0.8*	*25*

7.1.2 GRAPE CHEMICAL MEASURES

In this study we have measured a range of compounds in the fruit in a bid to both confirm associations we saw between these and wine sensory properties in a previous study (Forde *et al*. 2011) and to investigate other berry parameters as potential indicators of wine sensory outcomes. The results for these measurements are presented below. They are divided into sections based on the type of compound and methodology used to quantify them. We have also provided a short discussion on the results of these measures as, individually, these measurements are interesting indicators of fruit composition. This has the potential to tell us something about how fruit composition is affected by growing region and vintage. It also allows us to identify those compounds that are most sensitive to vineyard variation and differences in conditions during different vintages.

The true value in making these measurements will be the use of these Cabernet Sauvignon measures from the three vintages to compare fruit composition with the data obtained from the descriptive sensory analysis of the wines. Using specially developed chemometric data analyses, we then sought to determine if these compounds and parameters are good indicators of wine sensory attributes and hence may be used to assess the wine style potential and quality of the fruit. This work is presented in later sections of this report.

7.1.2.1 Anthocyanins

These are the compounds responsible for the colour of the fruit and wine. They were measured using HPLC, which allows the quantification of each of the 15 main anthocyanin types that are present in the berry skins. As a result, the relative make-up of the anthocyanin pool in the fruit can be determined as this may affect hue or colour longevity.

The total concentration of anthocyanins in the fruit samples from 2013 are shown in Figure 10. The overall mean was 0.78 mg anthocyanins per g de-seeded berry fresh weight but there was significant variation in the anthocyanin content of the fruit, ranging from 0.42 – 1.25 mg/g. The anthocyanin content of the RVL samples (mean of 0.53 mg/g) was generally lower than the rest of the samples (mean of 0.93 mg/g), and there did not appear to be a region that consistently produced fruit with higher anthocyanin concentrations than the rest of the regions from where the grapes were harvested.

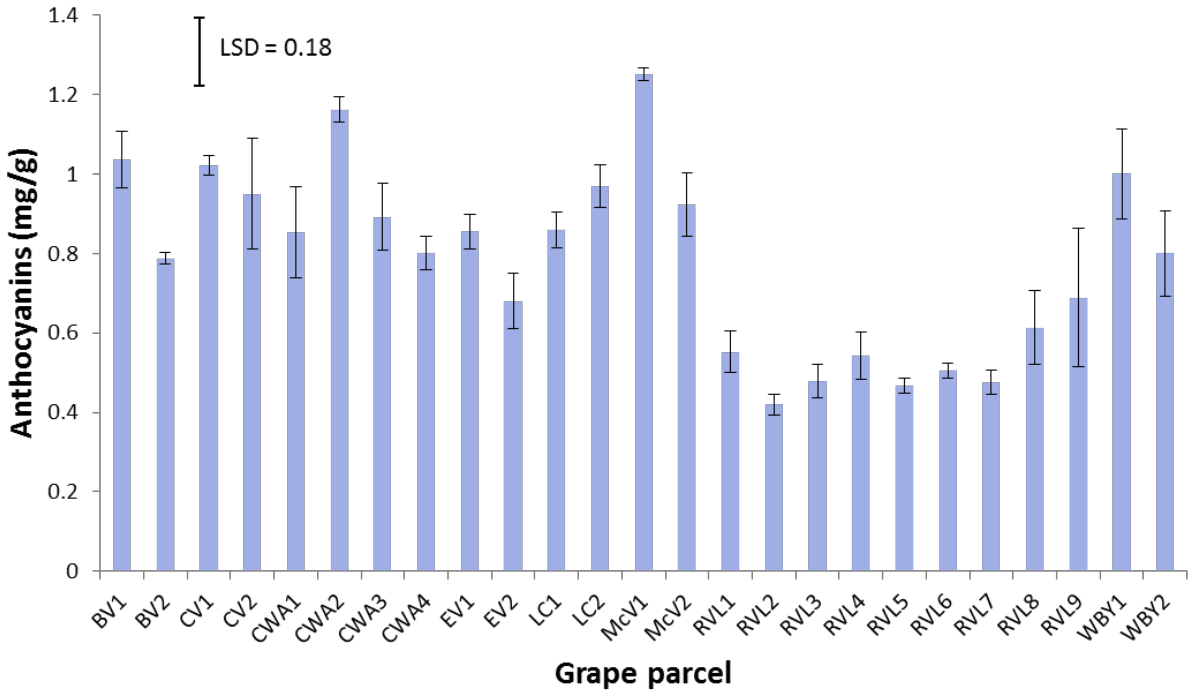


Figure 10. Total anthocyanins extracted from whole berries in 2013 presented as mg per g fresh weight. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

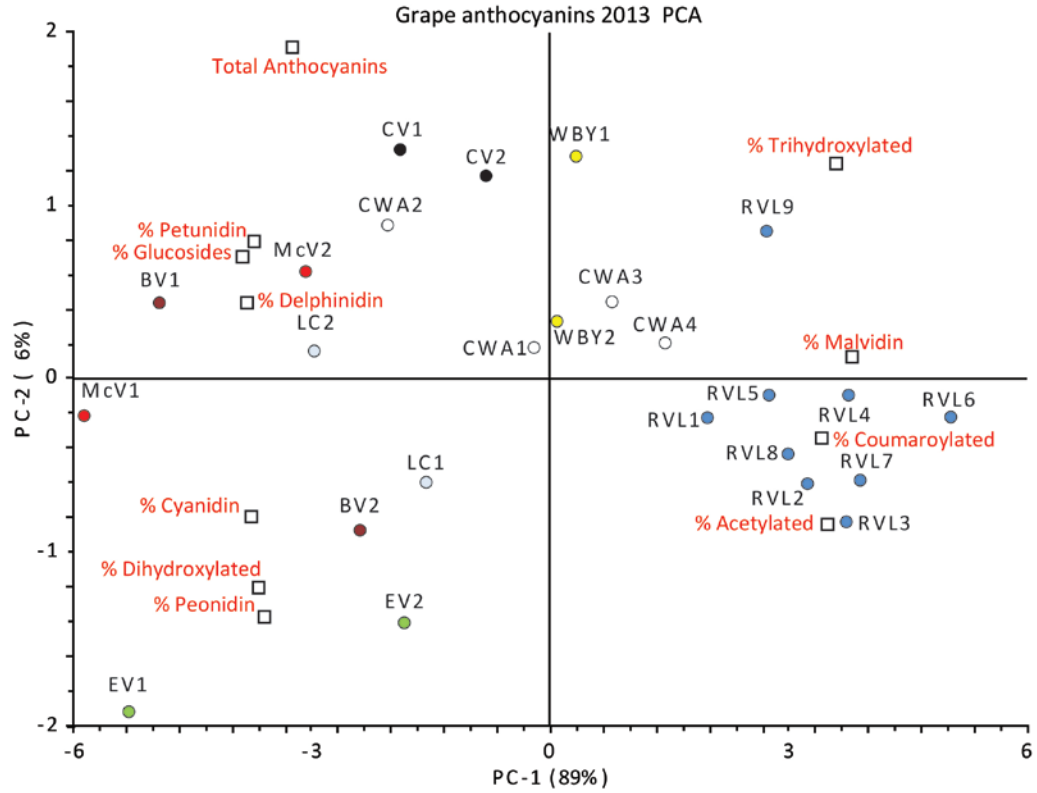


Figure 11. PCA plot showing the scores (coloured circles and black font) of the 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the associated anthocyanin profile parameter loadings (open squares and red font).

The PCA biplot for the anthocyanin profiles of the 2013 Cabernet Sauvignon grape samples is presented in Figure 11. Most of the variation in the profiles (89%) was described by the separation of the samples on the horizontal axis which represents PC1. The separation on PC2 only represented 6% of the variation, which was probably marginally above the error associated with the anthocyanin measurements. The samples from RVL in 2013 were located together on the right hand side of the plot. This region of the PCA plot corresponded to samples that had a higher percentage of malvidin-containing, trihydroxylated, acetylated or coumaroylated anthocyanin species. However, the samples with higher anthocyanin concentrations were found on the left hand side of the plot. This corresponded to those samples that had a higher percentage of glycosylated or dihydroxylated anthocyanins as well as the other four anthocyanidin types found in grape berries (Figure 11).

In 2014, the mean total anthocyanins across the sites was remarkably similar to 2013, being 0.73 mg anthocyanins per gram of de-seeded berry fresh weight. The range went from 0.34 mg/g (RVL10) to 1.21 mg/g in the CWA2 and CWA3 samples. Again, the RVL samples (0.48 mg/g) tended to have less anthocyanin than those from the other regions (0.87 mg/g). Three of the CWA samples stood out as they had higher anthocyanin concentrations than the rest of the samples (Figure 12).

The PCA biplot of the scores and loadings from the compositional analyses in 2014 showed a similar overall pattern to that seen in 2013 (Figure 13). The right side of the plot contained the RVL sites and these co-localised with scores for malvidin-based anthocyanidins as well as anthocyanins that are trihydroxylated and acylated with acetyl or coumaroyl groups. It is noteworthy that in 2014 the samples from WBY and CWA clustered on the far left of the plot, whereas in 2013 these samples were located closer to the RVL samples. It doesn't appear that this was solely driven by total anthocyanin concentration (cf Figure 12) and was perhaps related to the anthocyanin composition.

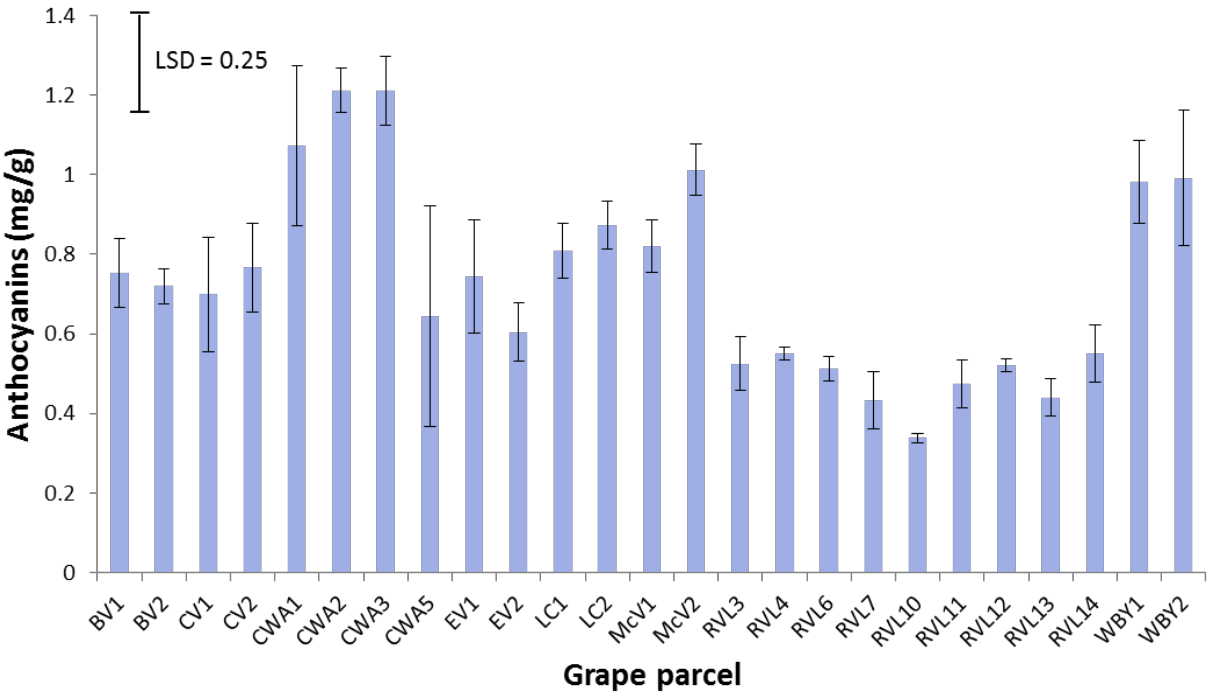


Figure 12. Total anthocyanins extracted from whole berries in 2014 presented as mg per g fresh weight. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

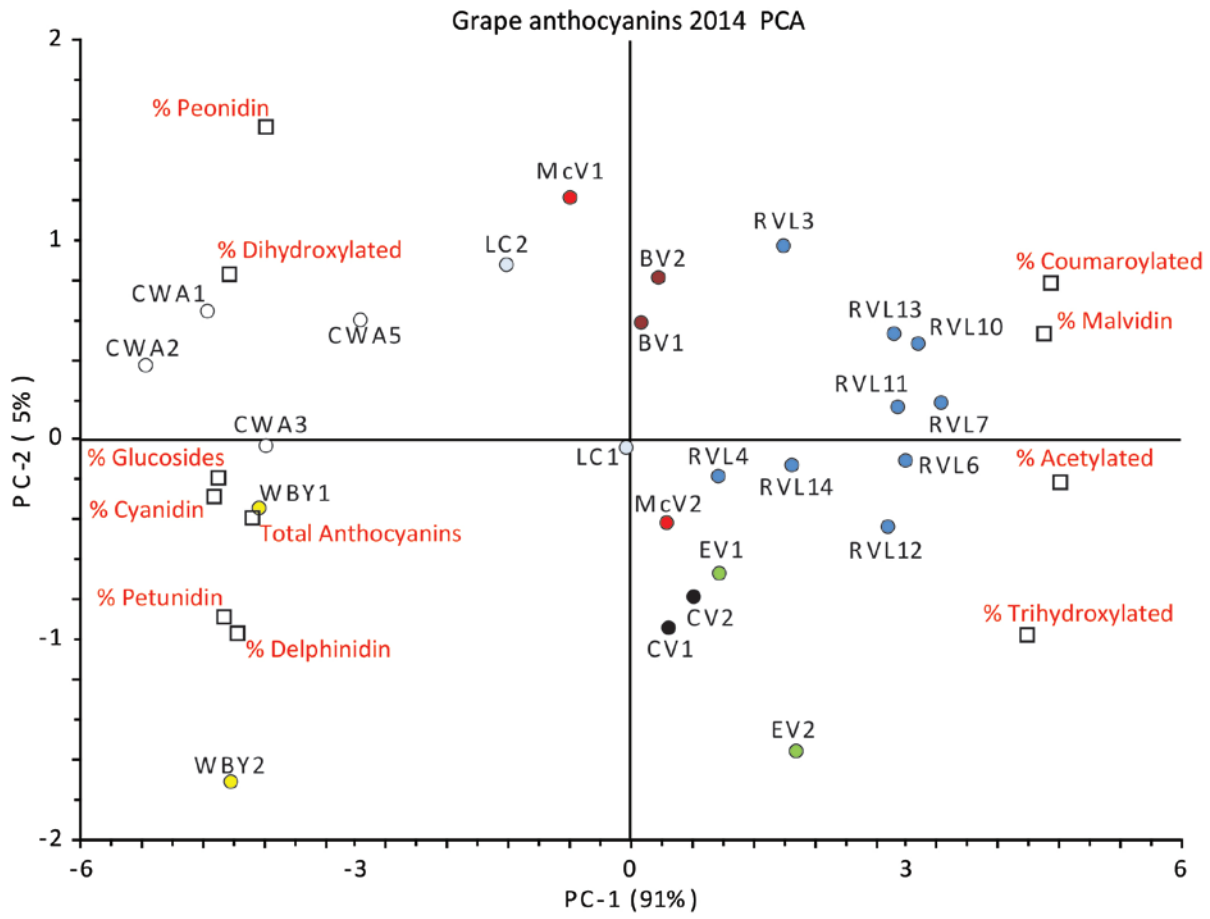


Figure 13. PCA plot showing the scores (A) of the 25 different 2014 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and (B) the associated anthocyanin profile parameter loadings.

The total anthocyanins across the sites were the highest in 2015 with a mean of 0.86 mg anthocyanins per gram of de-seeded berry fresh weight. This was 0.13 mg/g fresh weight more anthocyanins compared to 2014, and this increase appeared to be consistent across the sites. For example, the range in 2015 was 0.46 mg/g fresh weight (RVL6) to 1.32 mg/g fresh weight in the CWA2 sample (cf 0.34-1.21 mg/g in 2014). The RVL sites had a lower mean (0.62 mg/g) than the samples from the other regions (0.99 mg/g). CWA2 and CWA3 were again the two sites that had the highest concentration of anthocyanins in the fruit (Figure 14).

PCA showed that most of the variation was described on PC-1 (83%), which was a lower percentage than the other two vintages (Figure 15). The measures that were separated on the horizontal axis were the same as seen in the other years, with the RVL samples grouped on the right-hand side of the plot collocated with higher percentage of malvidin-based anthocyanins, trihydroxylated anthocyanins or those acylated with acetyl or coumaroyl groups. Interestingly, the samples from BV or EV were grouped in the lower left of the biplot, which appeared to indicate that those samples had a higher proportion of dihydroxylated anthocyanins compared with the other grape samples (Figure 15).

If we assume that the fruit at the RVL sites were exposed to the highest temperatures during berry development across the three vintages, these findings support those of Mori et al. (2007), who found that the malvidin anthocyanin species and the acylated anthocyanins were less effected by high temperature than the other anthocyanin types found in Cabernet Sauvignon berries. This could have implications for the hue of the wine, as the different anthocyanins will absorb at different wave lengths.

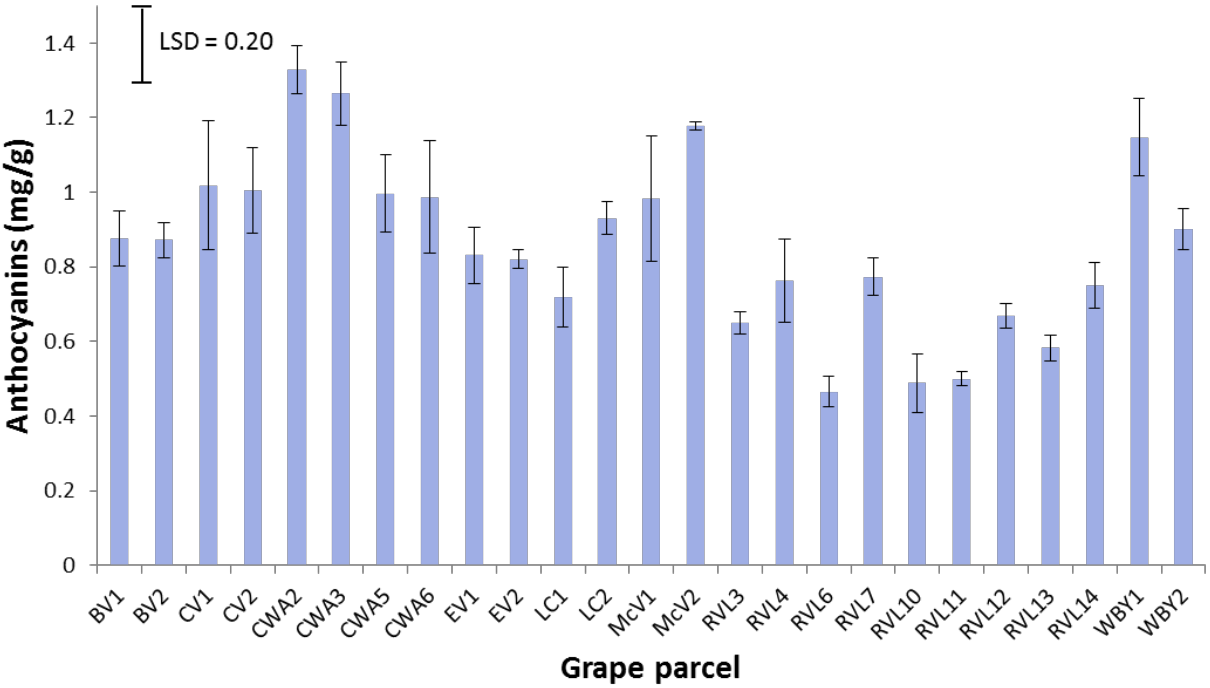


Figure 14. Total anthocyanins extracted from whole berries in 2015 presented as mg per g fresh weight. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

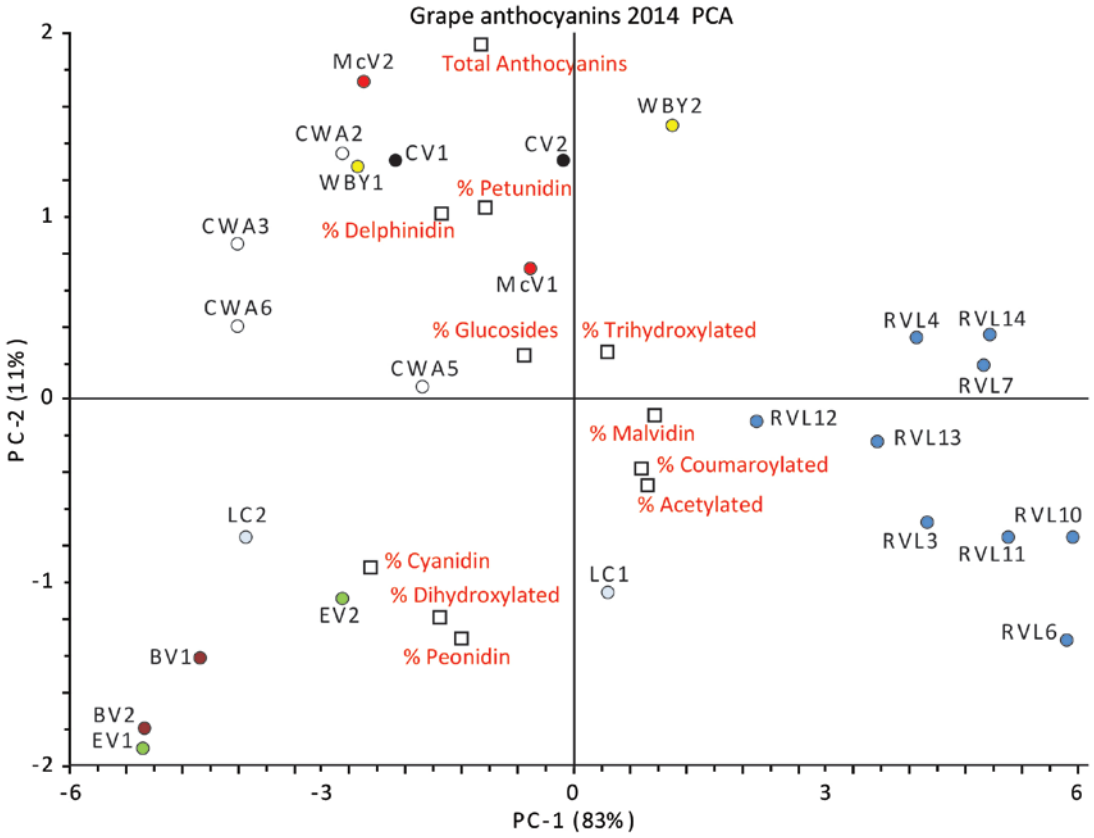


Figure 15. PCA plot showing the scores (A) of the 2015 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and (B) the associated anthocyanin profile parameter loadings.

7.1.2.2. Flavonols

Flavonols are produced via the same biosynthetic pathway as grape anthocyanins. These compounds are thought to help protect the plant from UV-damage. In a wine context, flavonols positively affect wine colour by co-pigmentation with anthocyanins, and are anti-oxidant compounds. The levels of flavonols in the grapes were determined by HPLC in the same analyses as those used for the anthocyanins.

Total flavonols (mg/g of deseeded berry) in the 2013 Cabernet Sauvignon fruit are shown in Figure 16. The mean was 0.122 mg/g berry but there was significant variation between the samples with a range of 0.04 – 0.27 mg / g berry and the mean of the RVL samples (0.07) was below the mean of the other samples (0.15). However, the RVL8 sample was comparable with some of the samples obtained from other regions and the WBY2 grapes had a total flavonol concentration that was similar to those grown in the RVL. There was a correlation (R^2 = 0.671) between the levels of anthocyanins and flavonols in grapes (data not shown), suggesting the whole pathway leading to these two classes of compounds is regulated in a similar manner by the environmental conditions experienced by the fruit in the various vineyards.

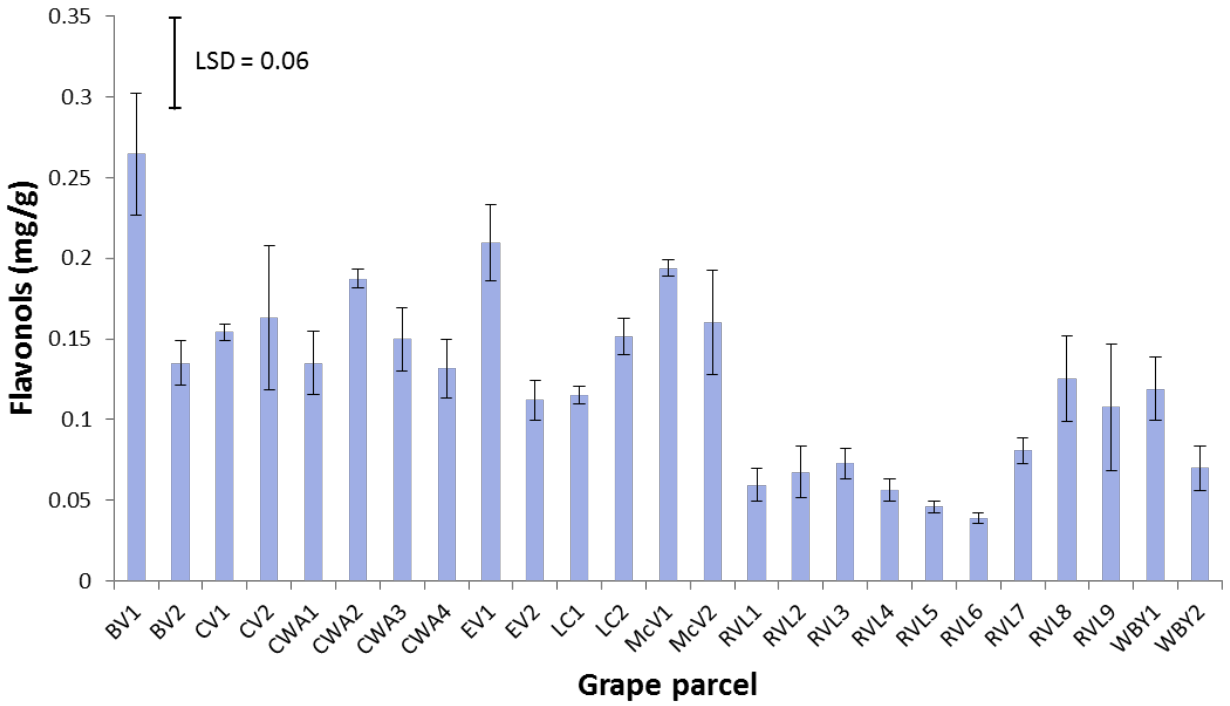


Figure 16. Total flavonols extracted from whole berries in 2013 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

PCA of the flavonol profiles showed that the total flavonol measurement was associated with higher amounts of kaempferol-3-*O*-glucuronide, the loadings of which were collocated in the top right quadrant of the biplot (Figure 17). Most of the variation (78%) was described in the first PC and in general the samples with high total flavonols are on the right hand side of the PCA and those with less flavonols, on the left side of the plot, tended to have a greater proportion of kaempferol-3-*O*-glucoside and laricitin-3-*O*-galactoside.

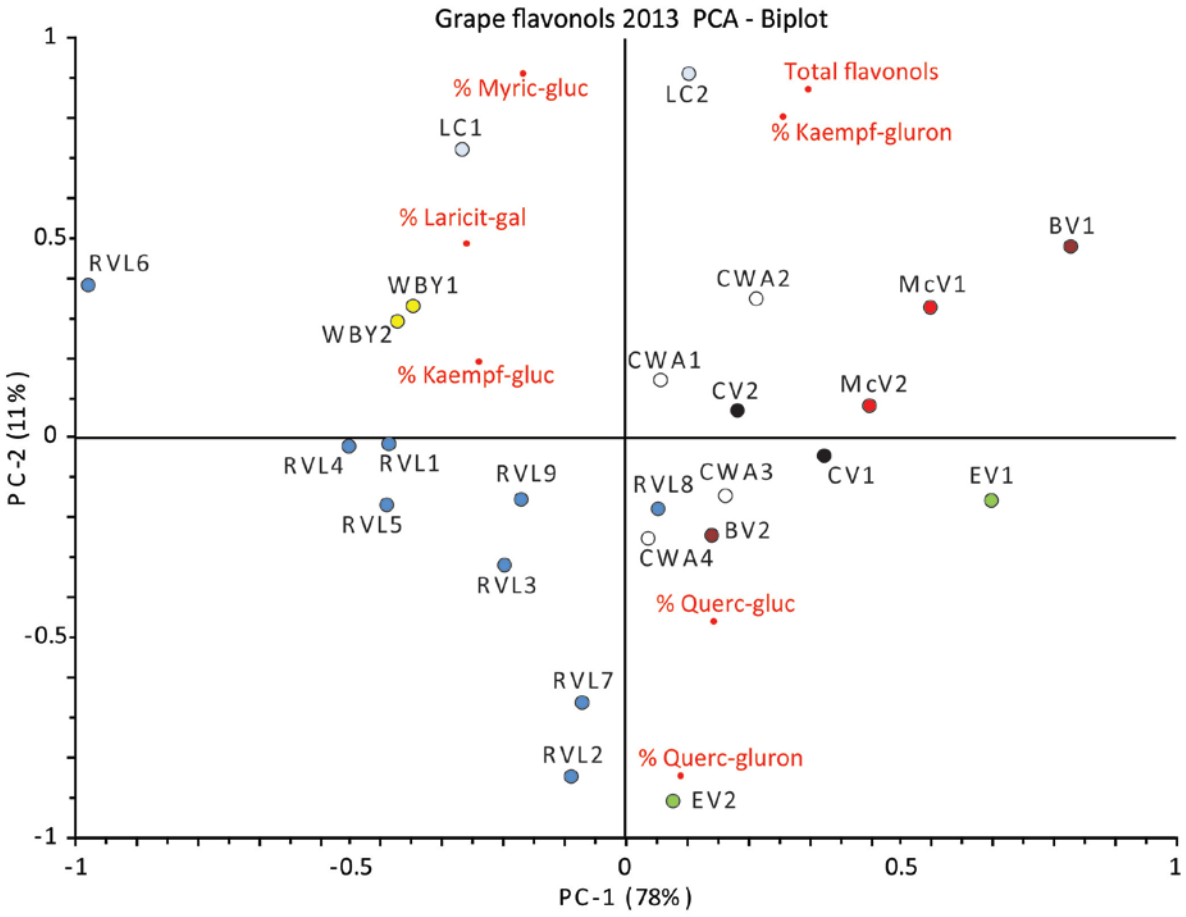


Figure 17. PCA biplot showing the scores of the 25 different 2013 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated flavonoid profile parameter loadings in red. Kaempf-gluc = Kaempferol-3-*O*-glucoside, Kaempf-gluron = Kaempferol-3-*O*-glucuronide, Laricit-gal = Laricitrin-3-*O*-galactoside, Myric-gluc = Myricetin-3-*O*-glucoside, Querc-gluc = Quercetin-3-*O*-glucoside, Querc-gluron = Quercetin-3-*O*-glucuronide.

Similar to the anthocyanin comparisons between the 2013 and 2014 vintages, the mean total flavonols amongst the samples in 2014 was 0.125 mg/g having been 0.122 mg/g in 2013. The total flavonol concentrations ranged from 0.05 – 0.21 mg/g in 2014 (Figure 18). The highest concentrations were recorded in the CWA1-3 and McV samples and, in this vintage, the flavonol concentrations in the EV2 and WBY2 samples were comparable to those grown in the RVL (Figure 18).

The PCA of the flavonol data from 2014 (Figure 19) showed similar trends to those seen in 2013 (Figure 17; Figure 19). The majority of the variation among the samples was described by PC1 which accounted for 71% of the total variation. The samples most abundant in total flavonols were located on the right side of the plot which also often coincided with the samples with the most kaempferol-3-glucuronide, although these parameters were separated on the second principal component. The grapes with less total flavonols were associated with a higher proportion of kaempferol-3-*O*-glucoside and laricitin-3-*O*-galactoside as was also seen in 2013. An exception is the CWA5 sample that had a relatively low overall flavonol concentration, but was high in the percentage of quercetin-3-*O*-glucoside and, as such, it was found on the right hand side of the biplot (Figure 19).

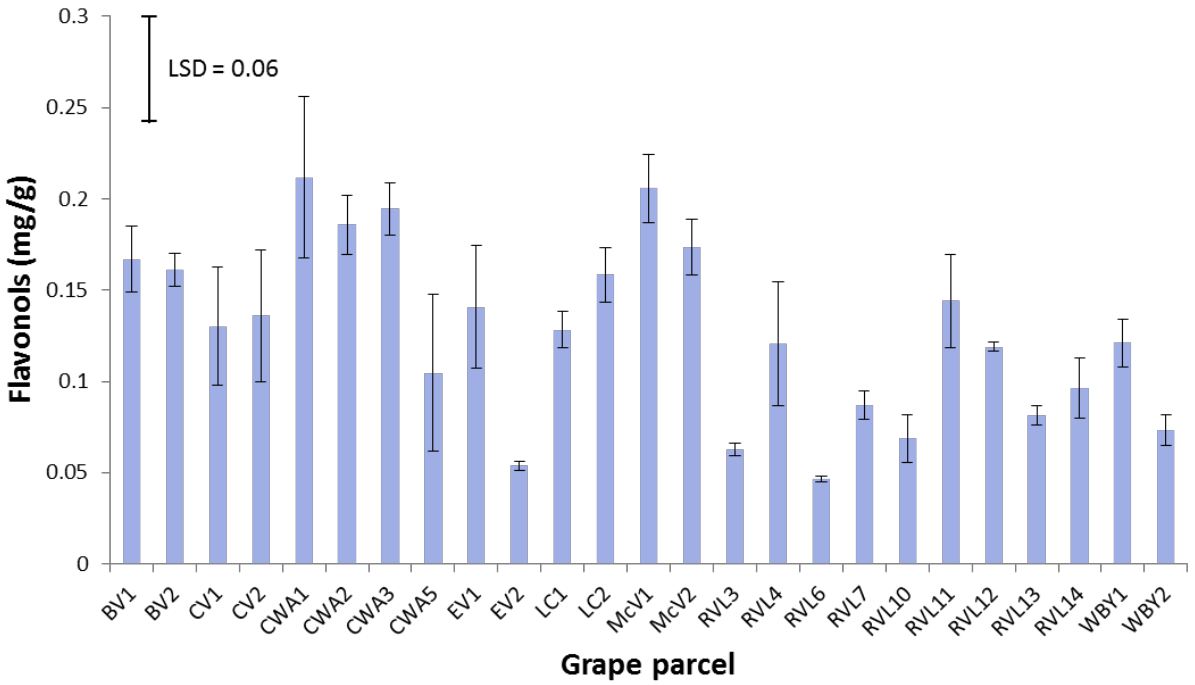


Figure 18. Total flavonols extracted from whole berries in 2014 presented as mg per g fresh weight of de-seeded berries. Error bar is ± standard error. LSD = minimum difference for a significant difference at $p < 0.05$.

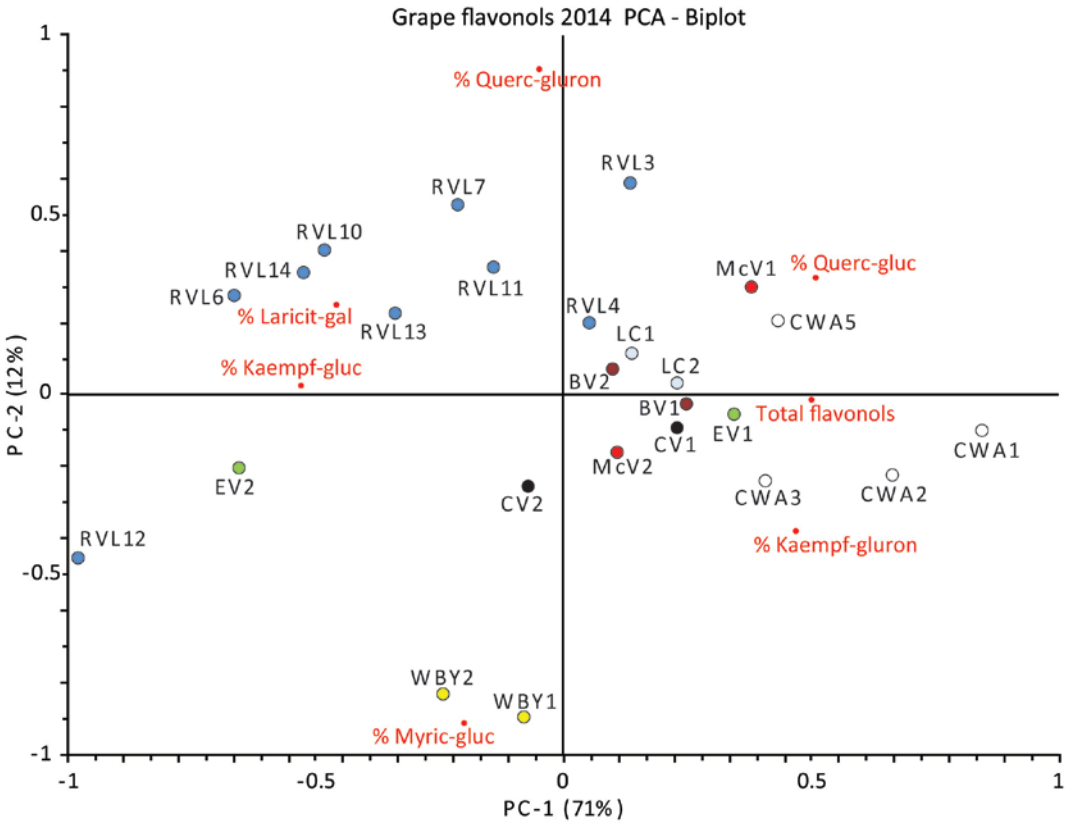


Figure 19. PCA biplot showing the scores of the 25 different 2014 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated flavonoid profile parameter loadings in red. Kaempf-gluc = Kaempferol-3-*O*-glucoside, Kaempf-gluron = Kaempferol-3-*O*-glucuronide, Laricit-gal = Laricitrin-3-O-galactoside, Myric-gluc = Myricetin-3-O-glucoside, Querc-gluc = Quercetin-3-O-glucoside, Querc-gluron = Quercetin-3-*O*-glucuronide.

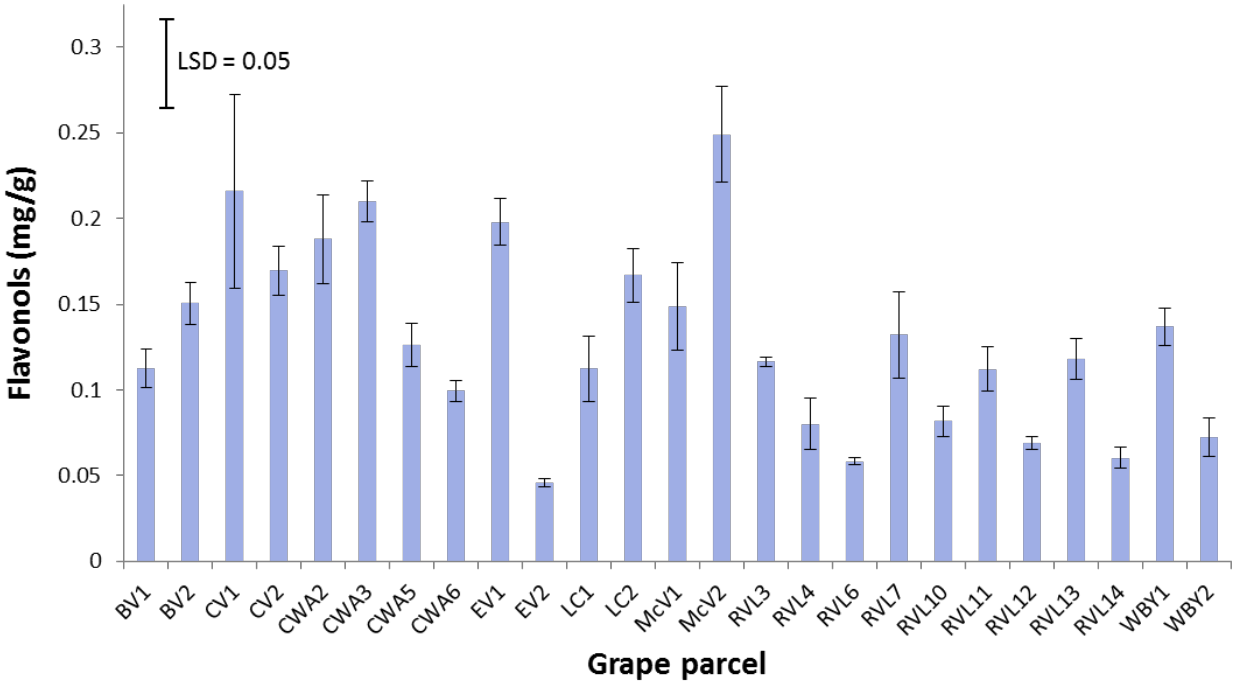


Figure 20. Total flavonols extracted from whole berries in 2015 presented as mg per g fresh weight of de-seeded berries. Error bar is ± standard error. LSD = minimum difference for a significant difference at $p < 0.05$.

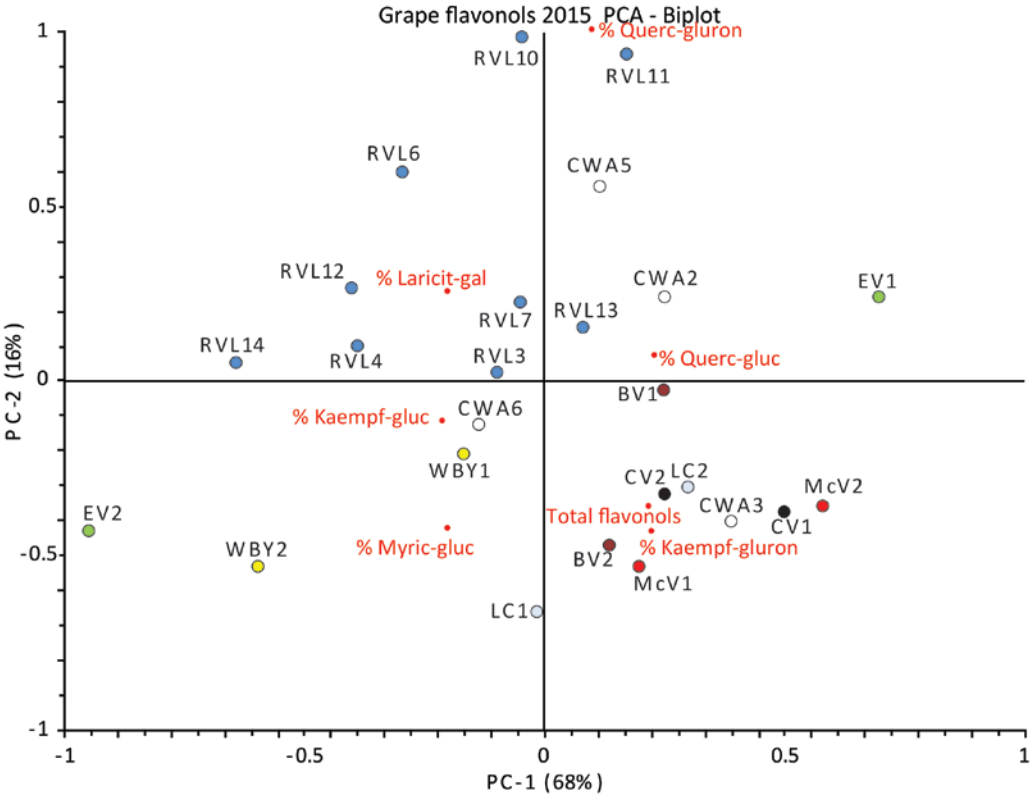


Figure 21. PCA biplot showing the scores of the 25 different 2015 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated flavonoid profile parameter loadings in red. Kaempf-gluc = Kaempferol-3-*O*-glucoside, Kaempf-gluron = Kaempferol-3-*O*-glucuronide, Laricit-gal = Laricitrin-3-O-galactoside, Myric-gluc = Myricetin-3-O-glucoside, Querc-gluc = Quercetin-3-O-glucoside, Querc-gluron = Quercetin-3-*O*-glucuronide.

The mean total flavonols amongst the samples in 2015 was 0.129 mg/g, very similar to the means observed in the previous two vintages, and the concentrations ranged from 0.04 – 0.25 mg/g (Figure 20). Again the highest concentrations were recorded in the CWA2-3 and McV2 samples, although the flavonol concentrations in CV1 and EV1 were also high in 2015. In this vintage, the lowest flavonol concentrations were measured in the EV2 sample and the WBY samples had concentrations similar to those grown in the RVL (Figure 20).

In the PCA biplot, the samples most abundant in total flavonols were located on the right side of the plot which was also where the samples that had the highest percentage of total flavonols as kaempferol-3-glucuronide were located (Figure 21). The separation on the horizontal axis represented 68% of the total variation with the vertical axis describing only 16% of the variation. Most of the RVL samples were associated with higher a percentage of and laricitin-3-*O*-galactoside and were located in the top left hand side of the biplot. The WBY samples were found on the lower left hand side of the plot indicating that they had a higher percentage of myricetin-3-O-glucoside than the other samples (Figure 21).

7.1.2.3. Tannins

Tannins in red wine (specifically condensed tannins) are important for wine astringency and mouthfeel properties, and long term colour stability. As these small scale wines did not have any oak contact, the tannin composition of the wine was totally dependent on the tannins extracted from the grapes. Total tannins in homogenised whole berries was measured using the MCP tannin method (Sarneckis et al. 2006). The concentration of total tannin in the skin of the grape samples was determined following chemical extraction, hydrolysis of the tannin polymers and analysis by HPLC.

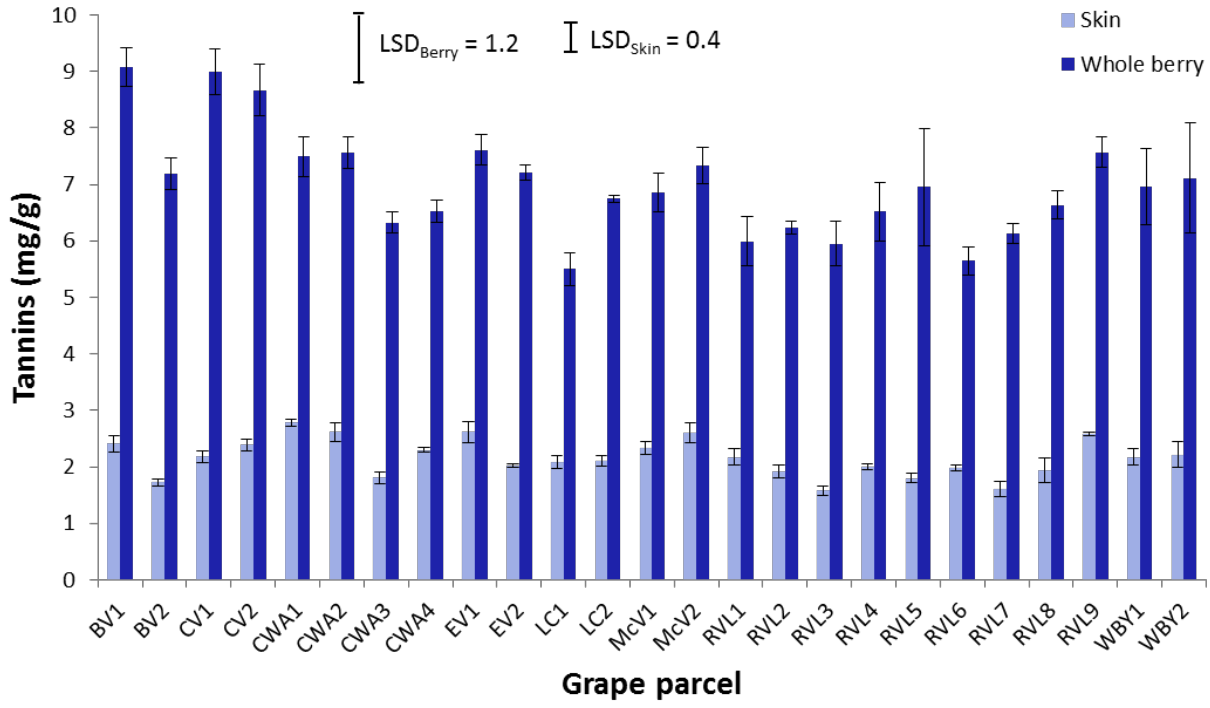


Figure 22. Total tannins extracted from either whole (Whole berry) or de-seeded berries (Skin) in 2013 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

The range of tannin concentrations across the 2013 samples (whole berry: 5.5 – 9.1 mg/g, skin: 1.6 – 2.8 mg/g) was not as large as it was for anthocyanins or flavonols (Figure 22). There was a weak correlation (R^2 = 0.358) between the levels of anthocyanins and skin tannins in the grape samples (data not shown). Also, fruit from the warmer regions was not necessarily always lower in total tannins. However, it should be noted

that these represent only the total tannins and that the length (extent of polymerisation) and structure (sub-unit and linkage composition) of the tannins may be more important than the total amount, especially from a sensory perspective.

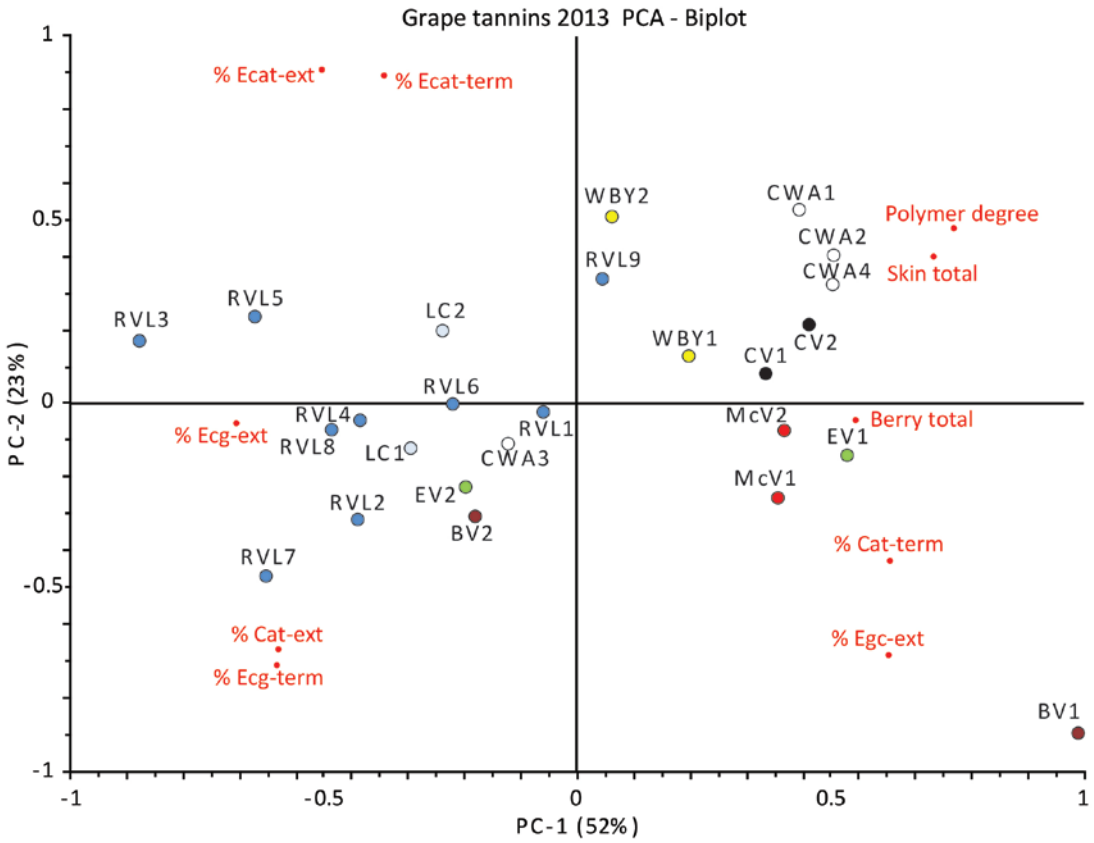


Figure 23. PCA biplot showing the scores of the 25 different 2013 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated tannin profile parameter loadings in red. Berry total = total whole berry tannin, Cat-ext = catechin extension subunits, Cat-term = catechin terminal subunits, Ecat-ext = epicatechin extension subunits, Ecat-term = epicatechin terminal subunits, Ecg-ext = epicatechin gallate extension subunits, Ecg-term = epicatechin gallate terminal subunits, Egc-ext = epigallocatechin extension subunits, Polymer degree = degree of polymerisation, Skin total = total skin tannin.

PCA of the tannin subunit data showed that there did seem to be a relationship between the amount of skin tannins and the degree of polymerisation - the samples that had more tannin also had a greater degree of polymerisation (Figure 23). These and total whole berry tannin were the main positive drivers (right hand side) of PC1 which described 52% of the total variation. In contrast, a higher percentage of epicatechin gallate extension subunits was associated with samples on the left of the biplot. The majority of the samples in the left-hand side of the plot were those from the RVL, whereas three out of four of the CWA samples were associated with higher skin and whole berry tannin, as were the CV, McV and WBY samples. The EV and BV samples were split with one on the left and the other on the right of the plot and the LC samples were collocated with the RVL samples. The BV1 sample appeared to be an outlier and this was driven by a higher percentage of epigallocatechin extension subunits and less epicatechin subunits than the other samples. A higher percentage of catechin terminal and epigallocatechin subunits was associated with the samples that had more skin tannin. Some of the variation was described on PC2, which mainly separated those samples with a greater percentage of epicatechin subunits from those with more catechin subunits (Figure 23).

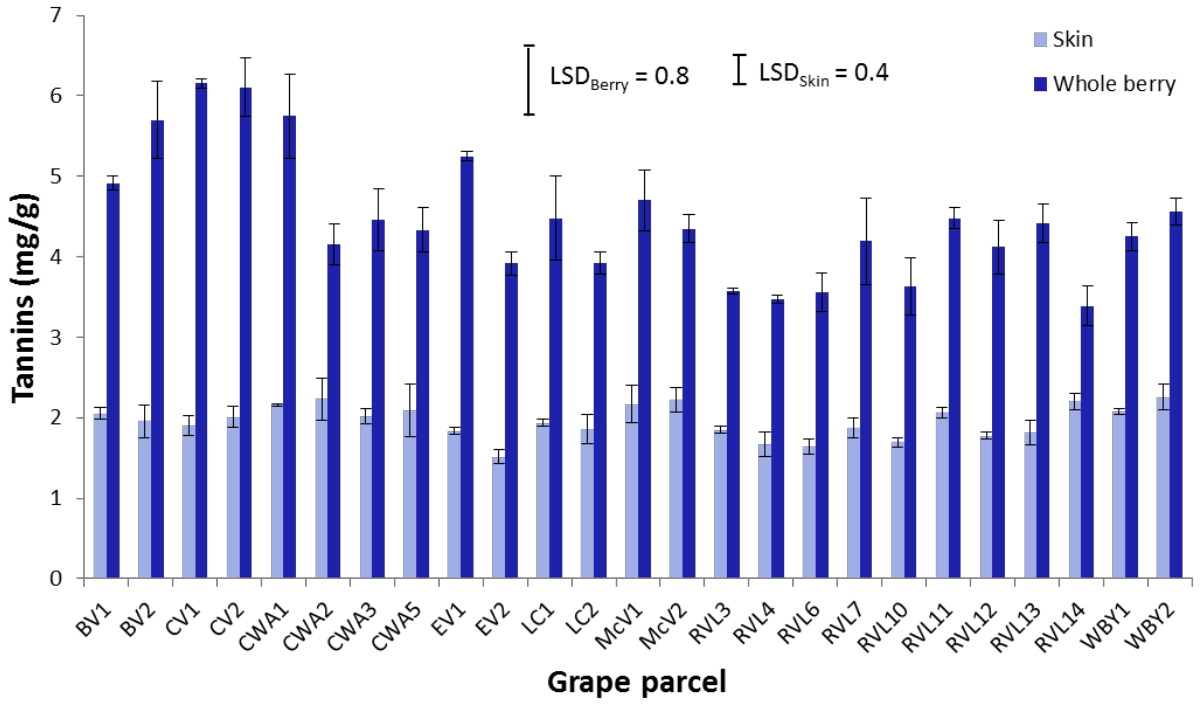


Figure 24. Total tannins extracted from either whole (Whole berry) or de-seeded berries (Skin) in 2014. Error bar is ± standard error. LSD = minimum difference for a significant difference at $p < 0.05$.

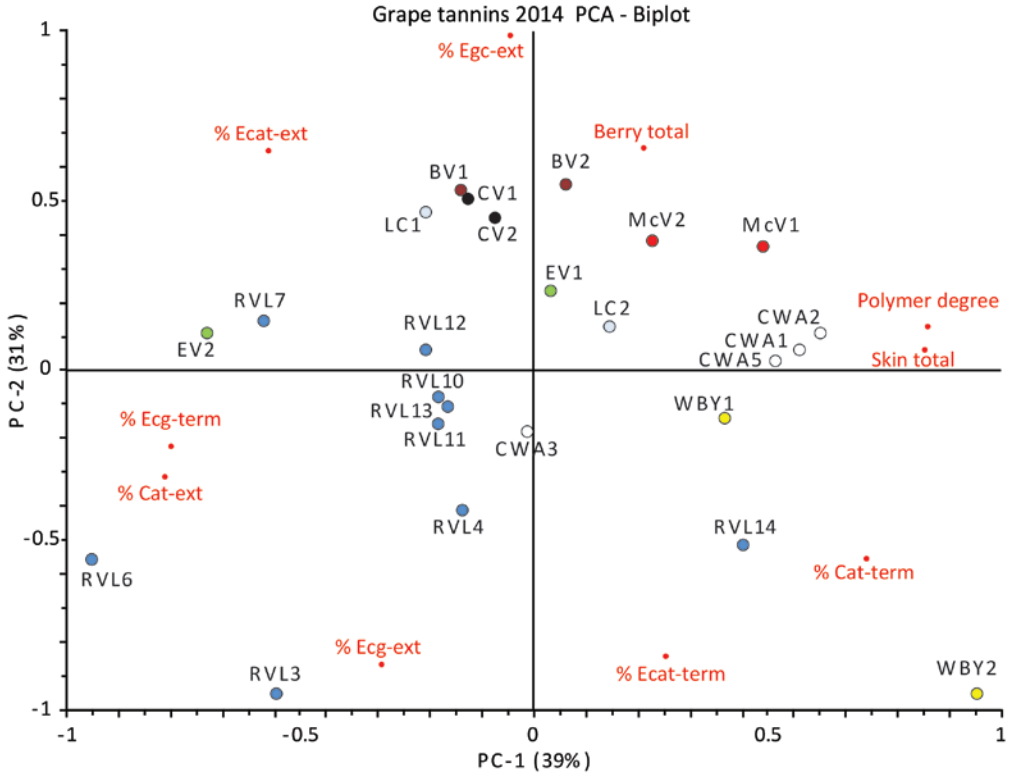


Figure 25. PCA biplot showing the scores of the 25 different 2014 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated tannin profile parameter loadings in red. Berry total = total whole berry tannin, Cat-ext = catechin extension subunits, Cat-term = catechin terminal subunits, Ecat-ext = epicatechin extension subunits, Ecat-term = epicatechin terminal subunits, Ecg-ext = epicatechin gallate extension subunits, Ecg-term = epicatechin gallate terminal subunits, Egc-ext = epigallocatechin extension subunits, Polymer degree = degree of polymerisation, Skin total = total skin tannin.

Total tannins in the whole berries were lower in 2014 (4.5 mg/g) compared to 2013 (7.0 mg/g) and this was also reflected in the total skin tannins which averaged 1.95 mg/g in 2014 compared to 2.16 mg/g in 2013. The range of the total berry tannins in the 2014 grape samples was again relatively narrow, extending from 3.4 mg/g to 6.2 mg/g (Figure 24). The samples from CV had the highest tannin concentrations, as they did in 2013, with the BV2 and CWA1 also amongst the parcels with the most tannin in 2014.

PCA of the tannin profiles obtained from the 2014 samples showed a slightly different relationship amongst the variables compared to 2013. The main positive driver on PC1 was skin tannin rather than total berry tannin and the degree of polymerisation was again associated with total skin tannin (Figure 25). The variation in total berry tannin was described more by PC2 than PC1 as was seen in 2013, and on the opposite side of the biplot was the percentage of epicatechin gallate extension subunits which was observed in 2013 (Figure 23 & Figure 25). In 2014, the percentage of epicatechin terminal subunits was associated with the percentage of catechin terminal subunits, which was not seen in 2013. The BV1 sample, which was an outlier in 2013, was again associated with a high percentage of epigallocatechin extension subunits and WBY2 was associated with a high percentage of epicatechin terminal subunits (Figure 23 & Figure 25). In both years, the RVL3 sample collocated with the percentage of epicatechin gallate extension subunits on the biplot.

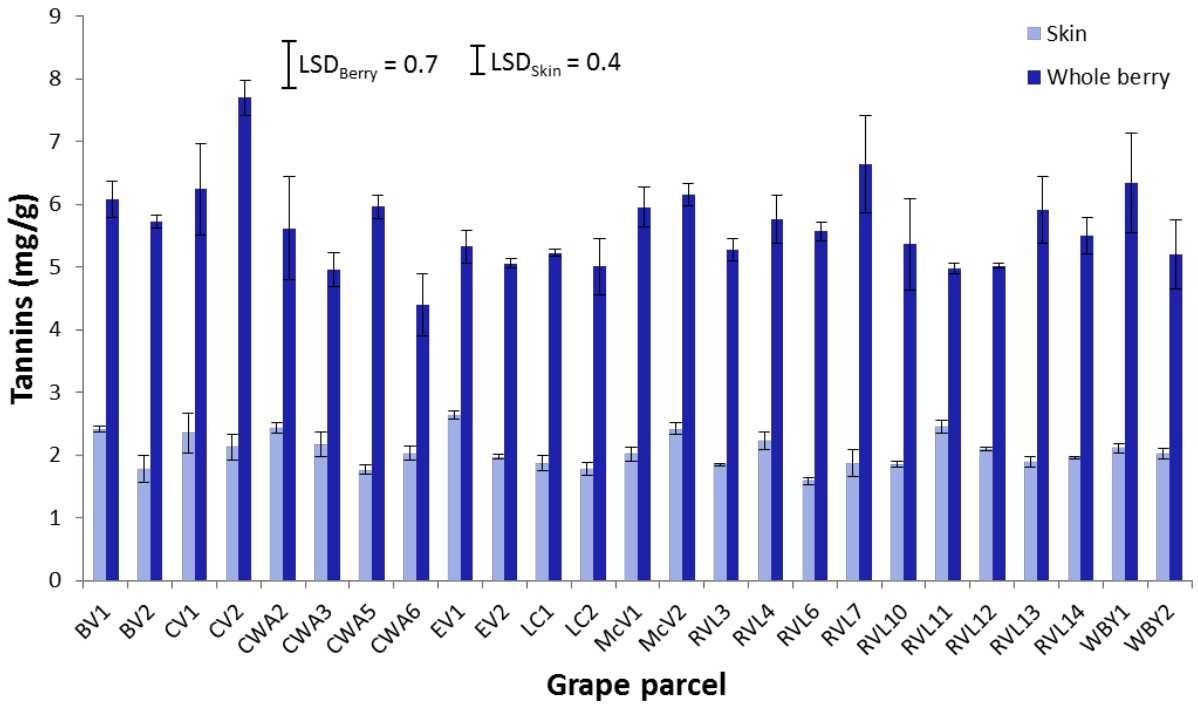


Figure 26. Total tannins extracted from either whole (Whole berry) or de-seeded berries (Skin) in 2015 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

The mean total tannins measured in whole berries of the 2015 Cabernet Sauvignon samples (5.6 mg/g fresh weight) was lower than 2013 (7 mg/g), but higher than 2014 (4.5 mg/g). Skin tannins were also intermediate, having a mean of 2.07 mg/g in 2015. The CV samples were again among those with the highest whole berry tannin concentrations, and surprisingly the RVL7 sample had the second highest concentration of tannin measured in 2015 (Figure 26). The skin tannin concentrations did not correlate well with the total berry tannins illustrating the effect of seed tannins on the whole berry measures. Concentrations in 2015 ranged from 4.4 mg/g to 7.7 mg/g for whole berries and 1.6 mg/g to 2.6 mg/g for skin tannins.

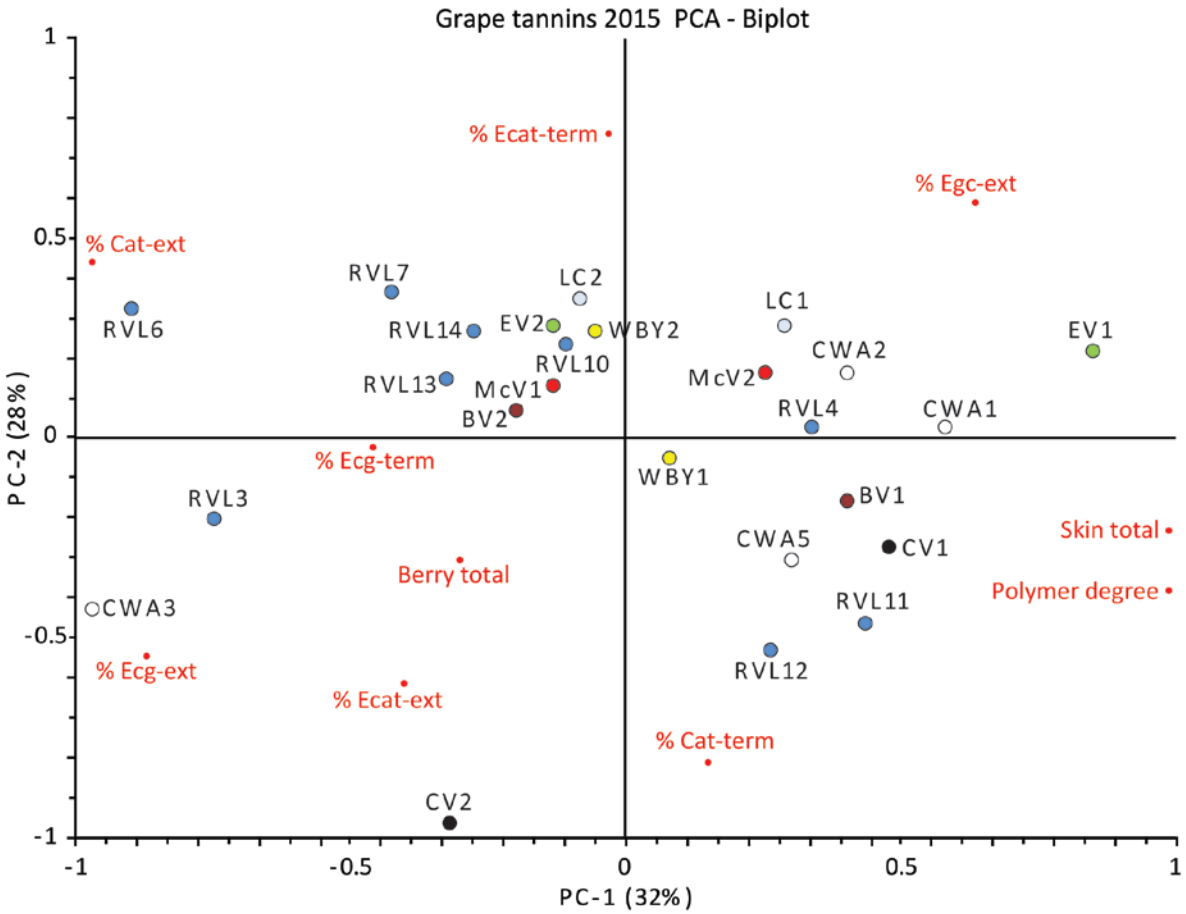


Figure 27. PCA biplot showing the scores of the 25 different 2015 Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia and the associated tannin profile parameter loadings in red. Berry total = total whole berry tannin, Cat-ext = catechin extension subunits, Cat-term = catechin terminal subunits, Ecat-ext = epicatechin extension subunits, Ecat-term = epicatechin terminal subunits, Ecg-ext = epicatechin gallate extension subunits, Ecg-term = epicatechin gallate terminal subunits, Egc-ext = epigallocatechin extension subunits, Polymer degree = degree of polymerisation, Skin total = total skin tannin.

The 2015 tannin profiles of the Cabernet Sauvingon grape parcels were subjected to PCA (Figure 27). The first two principal components described 60% of the total variation, but neither were driven to any great extent by whole berry tannins. PC1 discriminated those samples with higher skin tannin and a greater degree of polymerisation on the right of the plot from the samples that had a higher percentage of catechin and epicatechin gallate extension subunits. PC2 separated the grape samples with a high percentage of catechin terminal subunits compared to those with more epicatechin terminal subunits. The CV2 sample was an outlier due to it having relatively high percentages of epicatechin extension and catechin terminal subunits. The location of RVL3 & 6 and CWA3 on the right of the plot was driven by high percentages of catechin and epicatechin gallate extension subunits.

7.1.2.4. Targeted Grape Volatile Compounds

In a previously funded Wine Australia project it was found that the concentration of some Cabernet Sauvignon grape volatile compounds were associated with certain wine sensory attributes (Forde et al. 2011; Table 3). It should be noted that some of the volatile compounds were negatively associated with some attributes. That is, as the amount of some compounds increased in the berry, the scores for the associated wine attribute decreased and these are *italicised* in Table 3. A new solid phase microextraction (SPME), gas

chromatography-mass spectrometry (GC-MS) method was developed to accurately quantify 12 of these compounds in the grape samples. Some of the compounds showing grape-wine sensory links from the Forde et al. (2011) paper were not measured in this study. For example, 2-heptanol was not discriminating the wine flavour attributes "sweet", "acidic" and "pepper", which made its use questionable and so it was not measured. Two other compounds, nonanoic acid and geraniol, could not be detected using the new solid phase microextraction (SPME) methods employed (the previous study used a "twister" stir-bar sorptive microextraction method). In the case of "aroma impact" and "spicy" flavour, we had other associated compounds we could use, although geraniol was the only grape compound that showed close association with "overall aftertaste".

The easiest way to visualise how these measures discriminate the different grape parcels is to plot them using PCA as discussed above. In the 2013 samples, the separation on the horizontal axis was driven by differences in the concentration of a number of volatile aldehydes in the grapes and benzyl alcohol (Figure 28). This separation also seems to be influenced by °Brix which co-localised with benzyl alcohol. In general, samples from the same region are not well separated on PC1, with the exception of the CWA samples and RVL7. The second PC began to discriminate some of the samples obtained from the same region. The compounds that were driving the discrimination on PC2 were ethyl acetate, 2-pentyl furan and hexanoic acid concentrations (top of the plot) and the concentration of (*Z*)-3-hexen-1-ol produced in the grapes. IBMP is not discriminating the samples and this is probably due to the fact that the 2013 vintage was one in which IBMP concentrations were not high in grape samples from all regions (Figure 29).

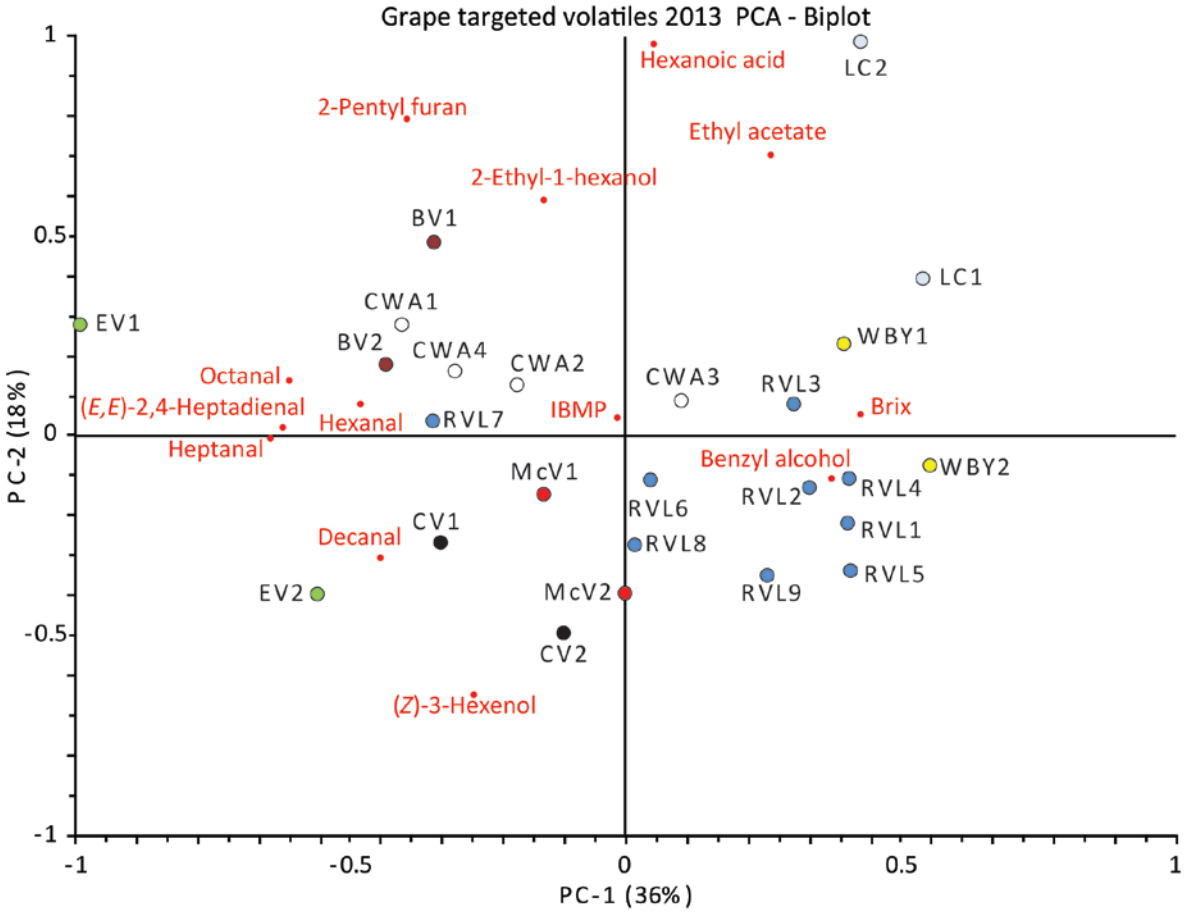


Figure 28. PCA biplot of 25 different Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia in 2013 and examples of some of the associated grape parameters measured in the fruit.

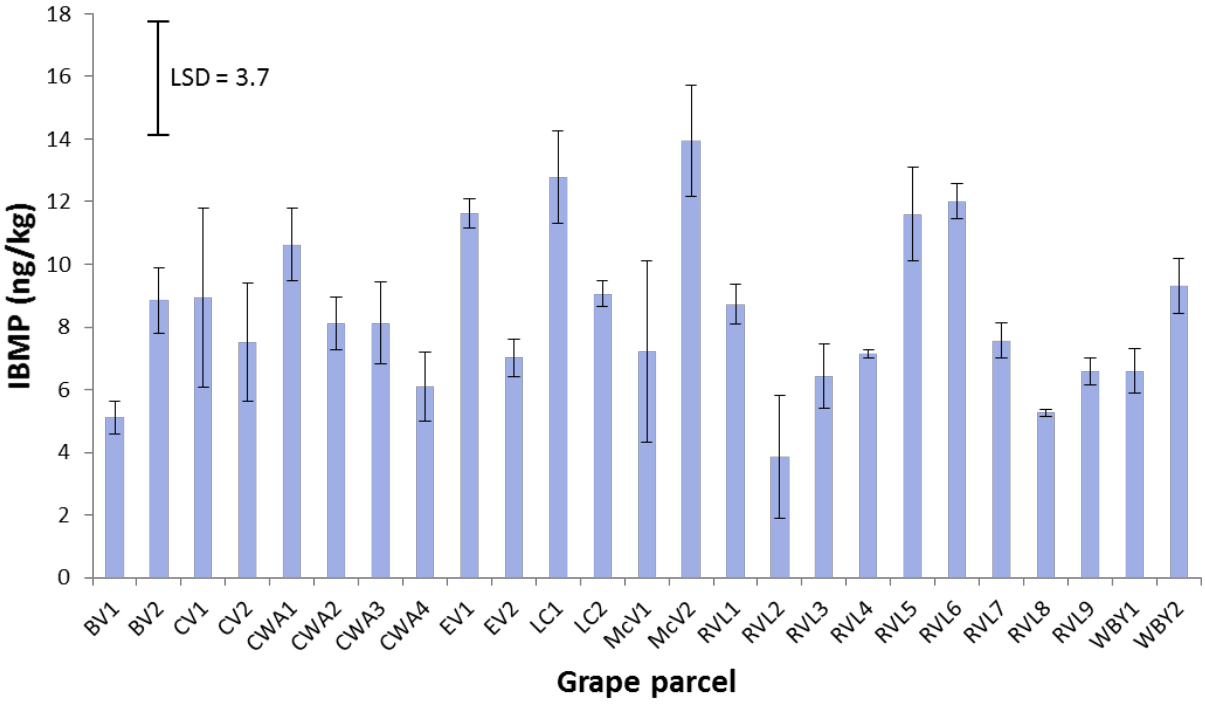


Figure 29. 3-Isobutyl-2-methoxypyrazine (IBMP) extracted from whole berries in 2013 presented as ng per kg fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

In the 2014 vintage, hexanoic acid was not detected in the samples and so was eliminated from the PCA. The first two PCs explained 63% of the total variation with 39% explained by separation on the horizontal axis (Figure 30). The relationship among some of the compounds measured was similar to that seen in 2013. The volatile aldehydes octanal, heptanal, decanal and (*E,E*)-2,4-heptadienal were again located together and on the opposite side of the biplot as benzyl alcohol (Figure 30). Ethyl acetate and 2-pentyl furan were again separated from (*Z*)-3-hexenol on PC2. However, IBMP was strongly driving the separation on PC2 and it co-localised with (Z)-3-hexenol and hexanal. All three compounds have "green" type sensory attributes. Variation within the samples from the RVL, CWA and EV regions was described more by PC1 than PC2, but the BV, CV, LC, McV and WBY samples were not very well discriminated by this analysis (Figure 30). The most notable aspect of the IBMP concentrations in the berries was the relatively high amounts found in the WBY samples compared to those from other regions (Figure 30).

The PCA of the targeted volatile compound measurements of Cabernet Sauvignon samples in 2015 showed some differences to the previous two years (Figure 32). While the volatile aldehydes again were collocated and drove the variation described on PC1, benzyl alcohol was not found at the opposite side of PC1 as it was in 2013 and 2014 (Figure 28 & Figure 30). PC2 was driven by differences in ethyl acetate and (*Z*)-3-hexenol similar to the previous two years, but Brix was also important for separation on this PC and was associated with higher ethyl acetate concentrations (Figure 32). Again the power of the measures to discriminate samples from the same region was mixed. For example, LC samples were discriminated on both PC1 and PC2, whereas the CWA samples were mainly separated on PC1 and the RVL samples more on PC2 than PC1. Others, like the BV and WBY samples, were found closely together in the PCA suggesting the targeted volatile measures do not vary much in these samples (Figure 32). IBMP did not discriminate the samples on either PC1 or PC2, but drove separation on PC3, along with benzyl alcohol (data not shown). IBMP concentrations were very high in some samples in 2015 (Figure 33). The EV2 sample had 68 ng/kg IBMP which was about three times more than any other sample in 2013 and 2014. Both BV samples and WBY2 had concentrations of IBMP that would likely have an impact on the sensory attributes of wine made from those grapes.

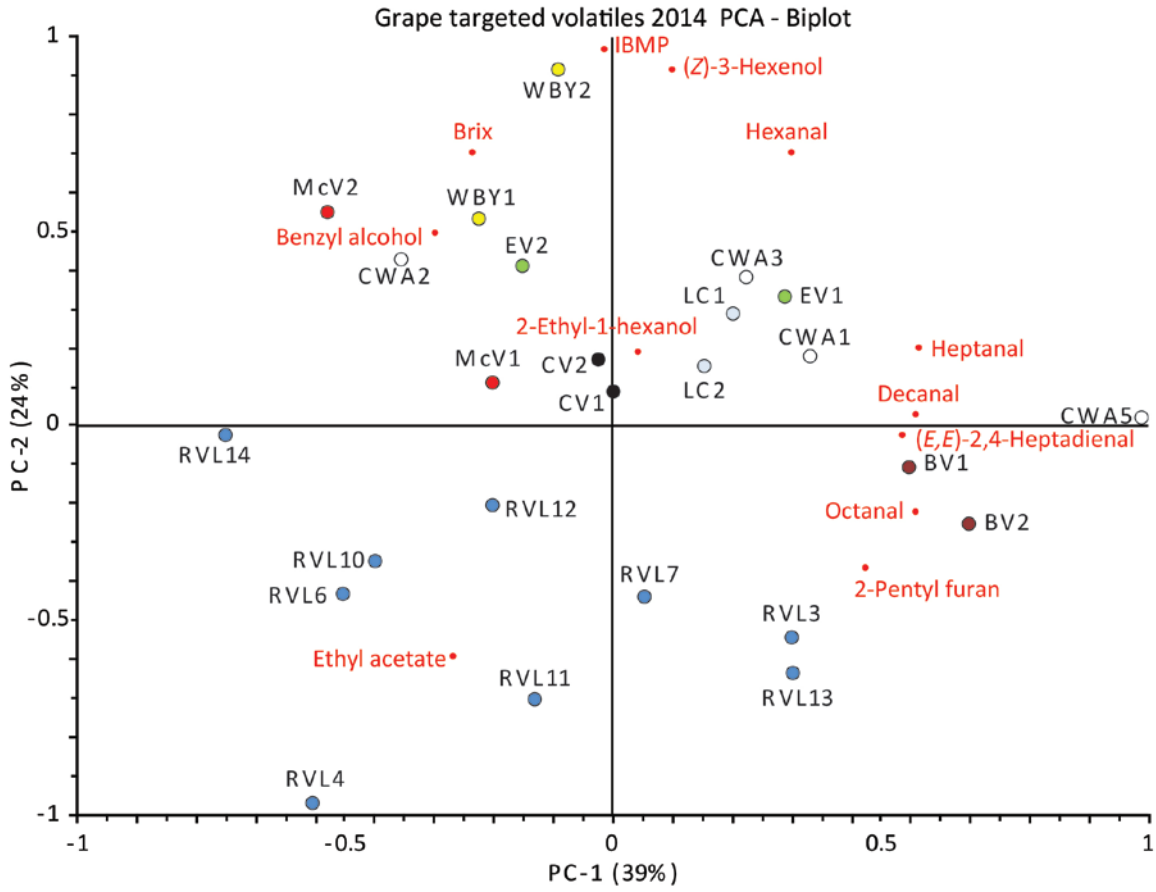


Figure 30. PCA biplot of 25 different Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia in 2014 and examples of some of the associated grape parameters measured in the fruit.

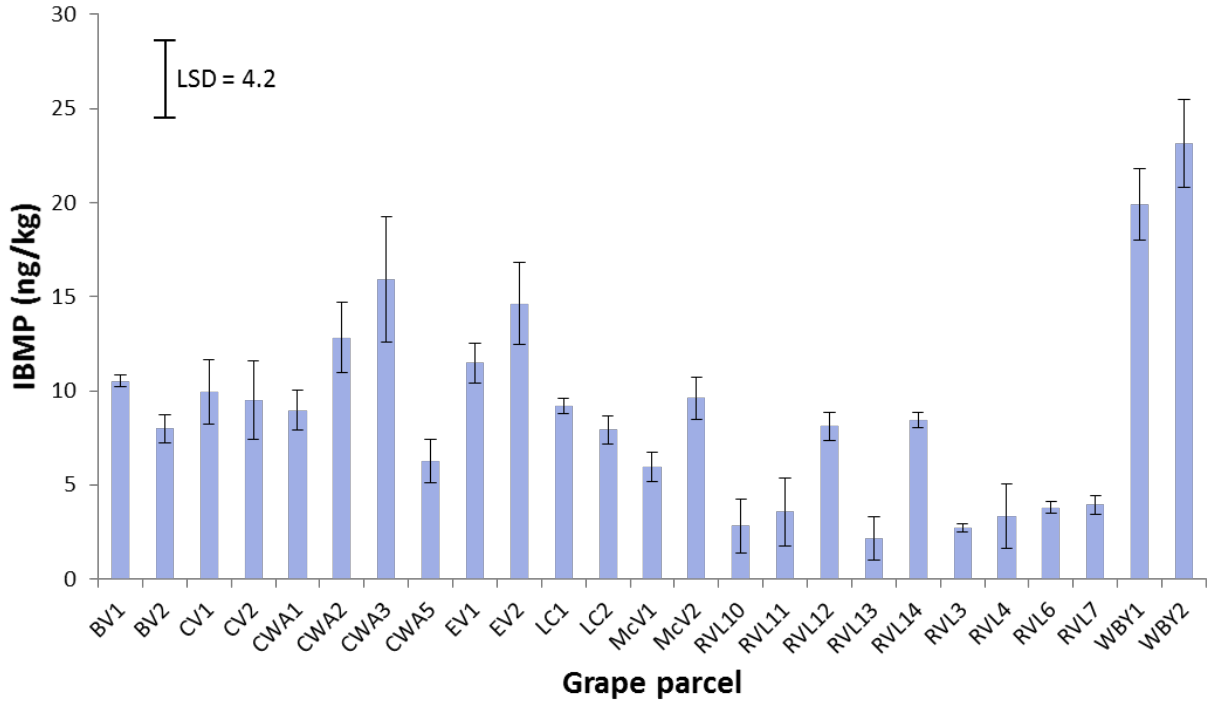


Figure 31. 3-Isobutyl-2-methoxypyrazine (IBMP) extracted from whole berries in 2014 presented as ng per kg fresh weight. Error bar is ± standard error. LSD = minimum difference for a significant difference at $p < 0.05$.

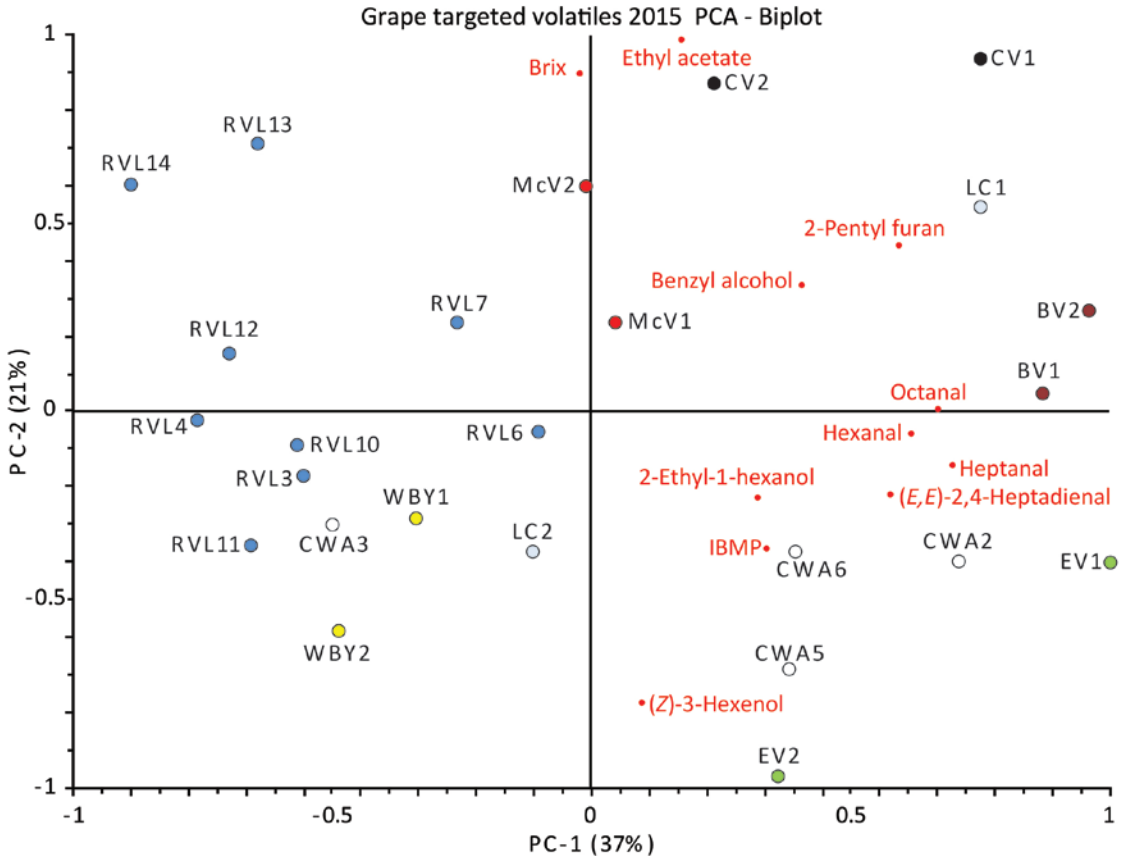


Figure 32. PCA biplot of 25 different Cabernet Sauvignon grape samples from eight regions (coloured) across South Australia in 2015 and examples of some of the associated grape parameters measured in the fruit.

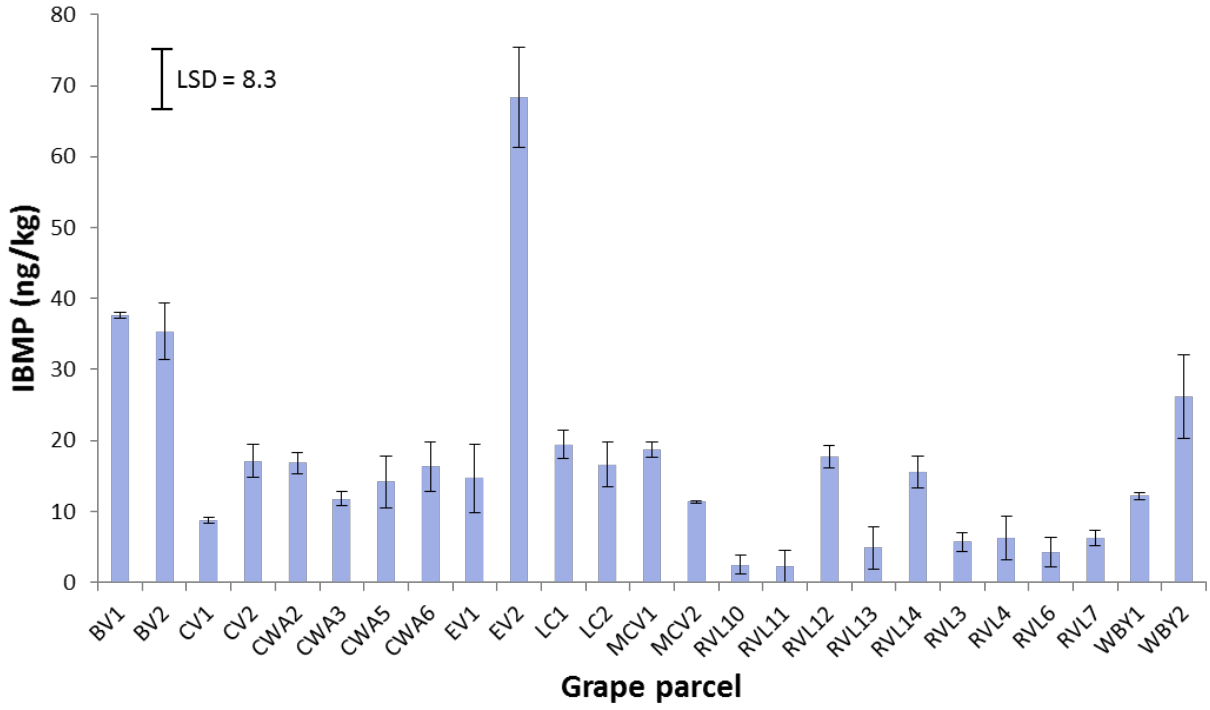


Figure 33. 3-Isobutyl-2-methoxypyrazine (IBMP) extracted from whole berries in 2015 presented as ng per kg fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.1.2.5. Non-targeted Grape Volatile Compounds

Other major volatile compounds extracted from the headspace above crushed berries were also measured in the grape samples (see Table 4). In general, a profile of the volatile compounds from crushed grapes is much simpler than the profile of volatiles from a wine and this is due to the complexity added by the yeast during fermentation. Nevertheless, the volatile composition of the fruit should reflect the changes in metabolism in the berries as influenced by factors such as climate, soil properties, management, harvest timing and rootstock. This in turn will influence wine style by altering the source of nutrients or precursors of the yeast or by altering yeast metabolism.

While the raw data is of interest, in the context of this report it is easier to visualise the overall relationship between the concentration of the compounds and the samples by conducting a PCA of the data. Because of the number of compounds and samples used, we have split the biplot into scores for the samples and loadings for the compounds to make it easy to read the labels. The interpretation is still the same as for those plots where scores and loadings are overlaid, in that the compounds and samples found in the same spatial region on the plots indicate a close association.

The PCA of the non-targeted grape volatile compounds in 2013 (Figure 34) gave us a different pattern of separation amongst the samples than was seen when the targeted volatile compounds were analysed (Figure 28). However, the samples from the same region were generally located in close proximity and so regionality would appear to be driving most of the differences in fruit composition. The RVL, LC and WBY samples clustered on the left of the plot and were characterised by higher concentrations of benzaldehyde and 3-pentanone, and lower amounts of the aldehydes and alcohols that were found on the right side of the loading plot. Most of these aldehydes and alcohols are the products of lipid degradation. There was some discrimination on this principal component between the LC samples. The separation on PC2 was interesting as it distinguished those samples with high norisoprenoids (compounds such as β-damascenone resulting from the degradation of carotenoids) from those with high concentrations of aromatic compounds such as 2-phenylethanal and 2-phenylethanol. The norisoprenoids are important grape-derived aroma impact compounds in wine, with β-damascenone shown to enhance the perception of fruit sensory characteristics and β-ionone contributing to floral/violet characters. The ketones 3-octanone and 2-heptanone were also important variables for the discrimination of the samples by PC2 and were located near the norisoprenoids on the loadings plot (Figure 34). There is also some evidence of ethyl octanoate production in some of the fruit samples which was presumably from fungal infection or yeast activity, as grapes do not usually produce this ester by their own metabolism.

In 2014, the RVL samples were no longer clustered by the PCA, but were spread across PC1 (Figure 35). PC1 also separated the EV, McV, LC and CWA samples. The samples on the right of the plot were characterised by having higher concentrations of benzaldehyde, as was seen in 2013. Those on the right were associated with greater abundance of many alcohols and aldehydes. PC2 separated the RVL samples from the CWA grape parcels. The separation on PC2 again involved norisoprenoids, with both β-damascenone and β-cyclocitral (β-ionone was not detected in the samples in 2014) found in the upper section of the loadings plot. However, there were a number of unsaturated aldehydes that were also positively influencing PC2. In contrast to 2013, 2-heptanone and 3-octanone were negatively driving PC2 and were not associated with the CWA samples. The samples within the BV, CV and WBY regions were not discriminated on the first 2 PCs (Figure 35).

The volatile composition of the grape parcels in 2015 could not discriminate the BV or WBY samples, and the McV samples were also located close to one another on the score plot (Figure 36). The RVL samples were separated on both PC1 and PC2 in 2015 with the RVL10 sample grouping with three CWA samples. PC1 provided good separation of the EV and LC samples, and the CWA3 grape parcel was located on the left of the plot away from the other CWA samples. Separation on the right side of PC1 was mainly driven by the concentration of 2-heptanone. The samples on the left side of the plot were characterised by having higher concentrations of a number of alcohols and aldehydes. PC2 discriminated several of the RVL samples and also separated the CV samples from each other. The norisoprenoids were again important for driving PC2,

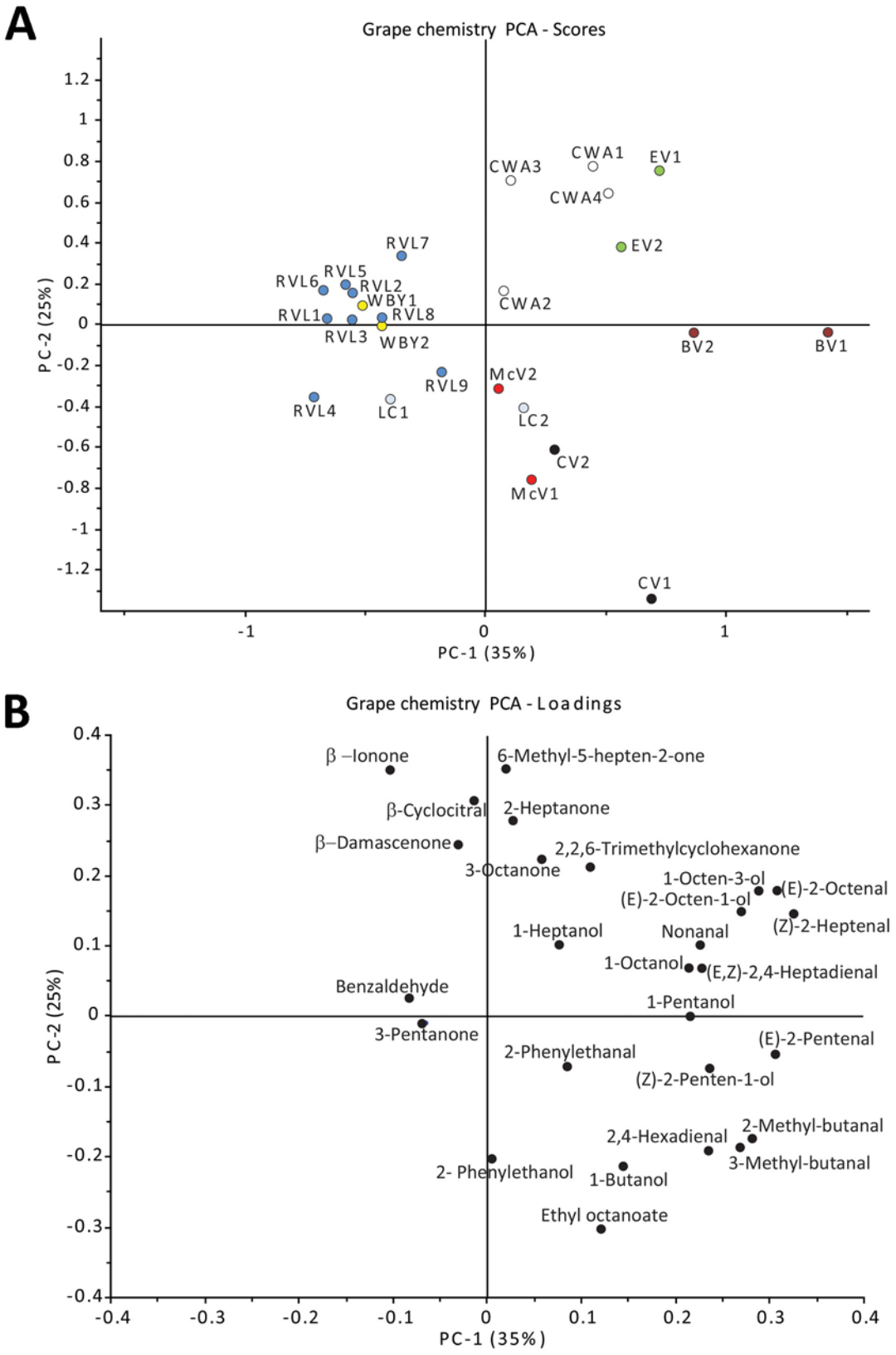


Figure 34. PCA scores (A) and loadings (B) plots of 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the volatile compounds quantified in the headspace of the crushed grapes.

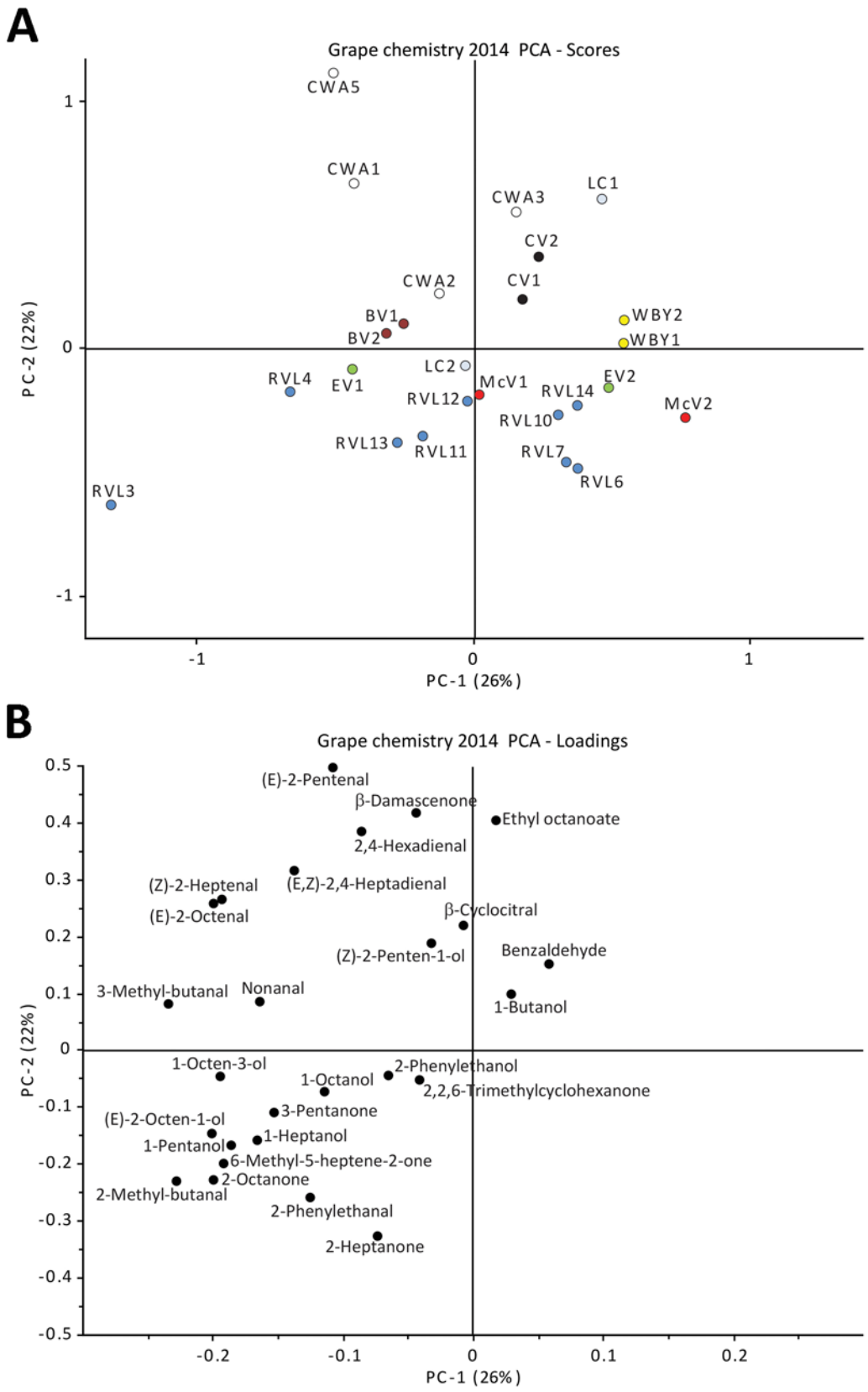


Figure 35. PCA scores (A) and loadings (B) plots of 25 different 2014 Cabernet Sauvignon grape samples from eight regions across South Australia and the volatile compounds quantified in the headspace of the crushed grapes.

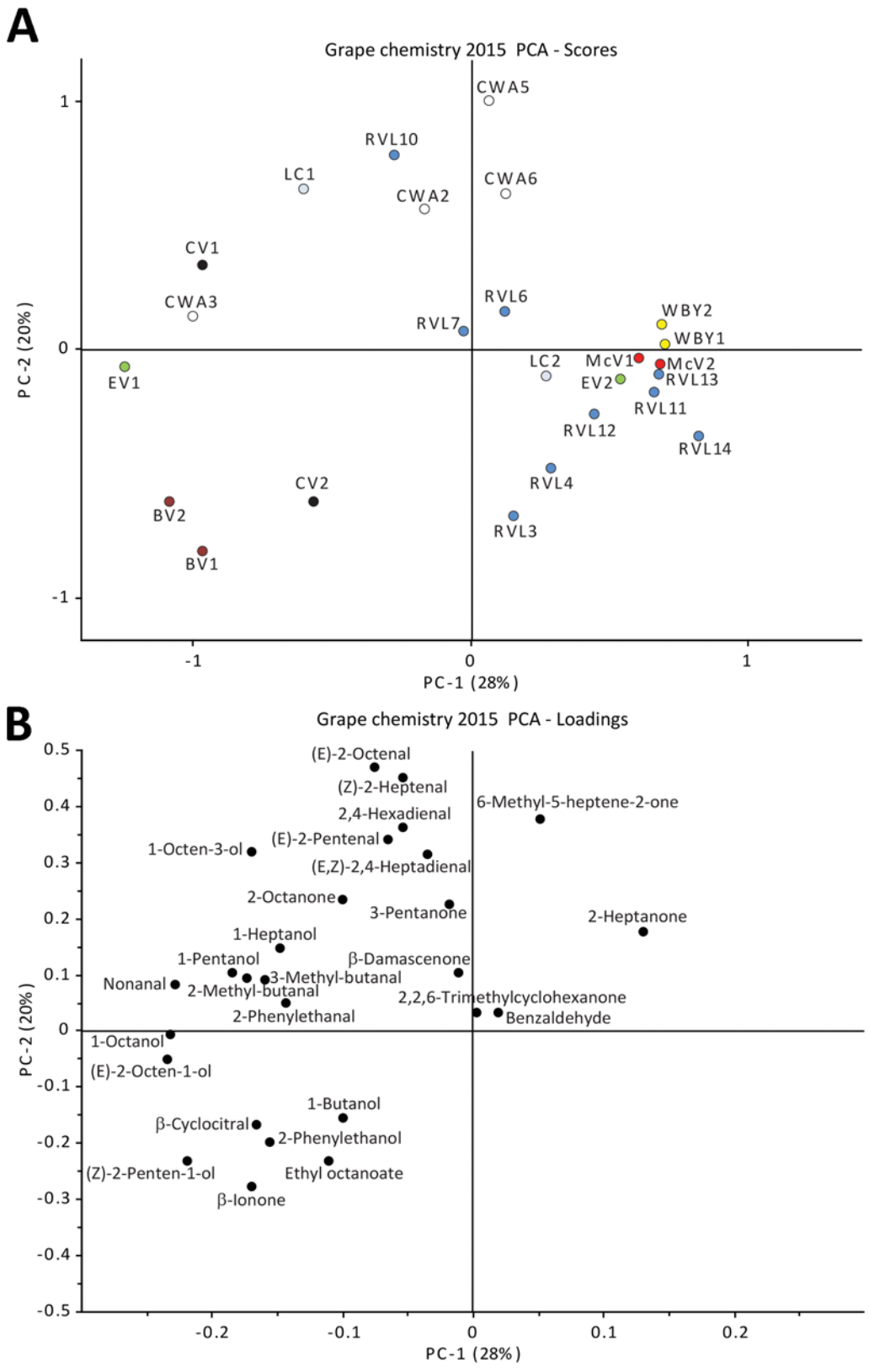


Figure 36. PCA scores (A) and loadings (B) plots of 25 different 2015 Cabernet Sauvignon grape samples from eight regions across South Australia and the volatile compounds quantified in the headspace of the crushed grapes.

but not β-damascenone. β-Ionone was detected in 2015 and was found in higher concentrations in the samples located in the lower part of the plot. Compounds with a positive influence on PC2 included a number of unsaturated aldehydes (Figure 36).

In general, the non-targeted volatiles separated samples according to region of origin, although some separation of parcels taken from within a region was observed. The pattern of the sample separation seen in the score plots and the compounds driving these separations was variable across vintages.

7.1.2.6. Amino Acids

Amino acids are implicated in wine volatile compound production in two main ways. One is that they contribute to the amount of yeast assimilable nitrogen (YAN) that can influence the fermentation process. While it is claimed that YAN increases ester production during fermentation, there is conflicting evidence in the literature. For example, in previous experiments where more than three levels of YAN have been used to examine effects on volatile production, and thus allowing trends to be observed, ester production has not shown a strong positive relationship to YAN, especially at high YAN levels (Carrau et al. 2008; Villanova et al. 2007). The variation in effects observed in different experiments reported in the literature suggests that the composition of grape juice determines how the effect of changing YAN levels will be manifested in the production of wine esters (Garde-Cerdan et al. 2008; Hernandez-Orte et al. 2006; Miller et al. 2007; Ugliano et al. 2008). Furthermore, increasing YAN to high levels may increase the production of ethyl carbamate, a known carcinogen, during fermentation. The other is that amino acids are also precursors of certain wine volatile components. The fusel acids and alcohols are formed mainly from catabolism of amino acids and these compounds can in turn be esterified. These precursors may come from the fruit or be produced by the yeast themselves and so the impact of both sources should be considered.

The concentrations of amino acids in the fruit samples were determined to investigate if they are predictive of any sensory attributes in the wine. The method utilised the derivatisation of the amino acids and their detection by fluorescence using HPLC. It allowed the quantification of all proteogenic amino acids in the grape samples, except tryptophan, which was below the detection threshold of the method. Two non-proteogenic amino acids, β-alanine and γ-aminobutyric acid (GABA) were also quantified. β-Alanine is important for the production of Co-A and may influence ester production during fermentation (Boss et al. 2015). GABA is a neurotransmitter in animals, and seems to play a role in stress responses in plants (Bouché & Fromm 2004). Once more, it is best to view the data by a PCA plot as this again gives us an overall idea of the relationships amongst the amino acid profiles and the grape samples.

For the 2013 amino acid profiles, separation on the first PC (65%) was driven by the total amino acid concentration on the right of the plot (Figure 37). Glutamic acid was located on the left of the plot on PC1, but its loading was 4-8 fold less than the amino acids on the right of the plot meaning it was not driving the separation on PC1 very strongly. Some discrimination of samples within regions on the first PC was observed (e.g. WBY, RVL and EV), whereas samples from other regions still grouped together. The analysis also showed that the samples from BV and LC tended to have higher concentrations of many of the amino acids measured than those from the other regions. The second PC discriminated the BV samples and to some extent the McV samples (Figure 37) and was driven by differences in the concentration of aspartic acid (top of the plot) and glycine, methionine and γ-aminobutyric acid (bottom of the plot). The LC samples were distinguished from those harvested from other regions as they had higher concentrations of glycine, methionine and γ-aminobutyric acid.

It is interesting to note that the PCA plot of the amino acid concentrations in the 2014 Cabernet Sauvignon samples (Figure 38) had a similar overall pattern to that seen in 2013. PC1 described slightly less of the overall variance among the samples (56%) compared to 2013, but again there was discrimination of the WBY or RVL samples on this PC. The CWA samples were also discriminated from each other on PC1. The main driver of this separation was the total amount of amino acids which means that most of the amino acids cluster on the right side of the loadings plot (Figure 38). Again the BV and LC samples were associated with higher concentrations of amino acids, but both EV samples were located in this side of the plot, whereas only EV1 was collocated with the loading for total amino acids in 2013. PC2 only described 15% of the overall variation

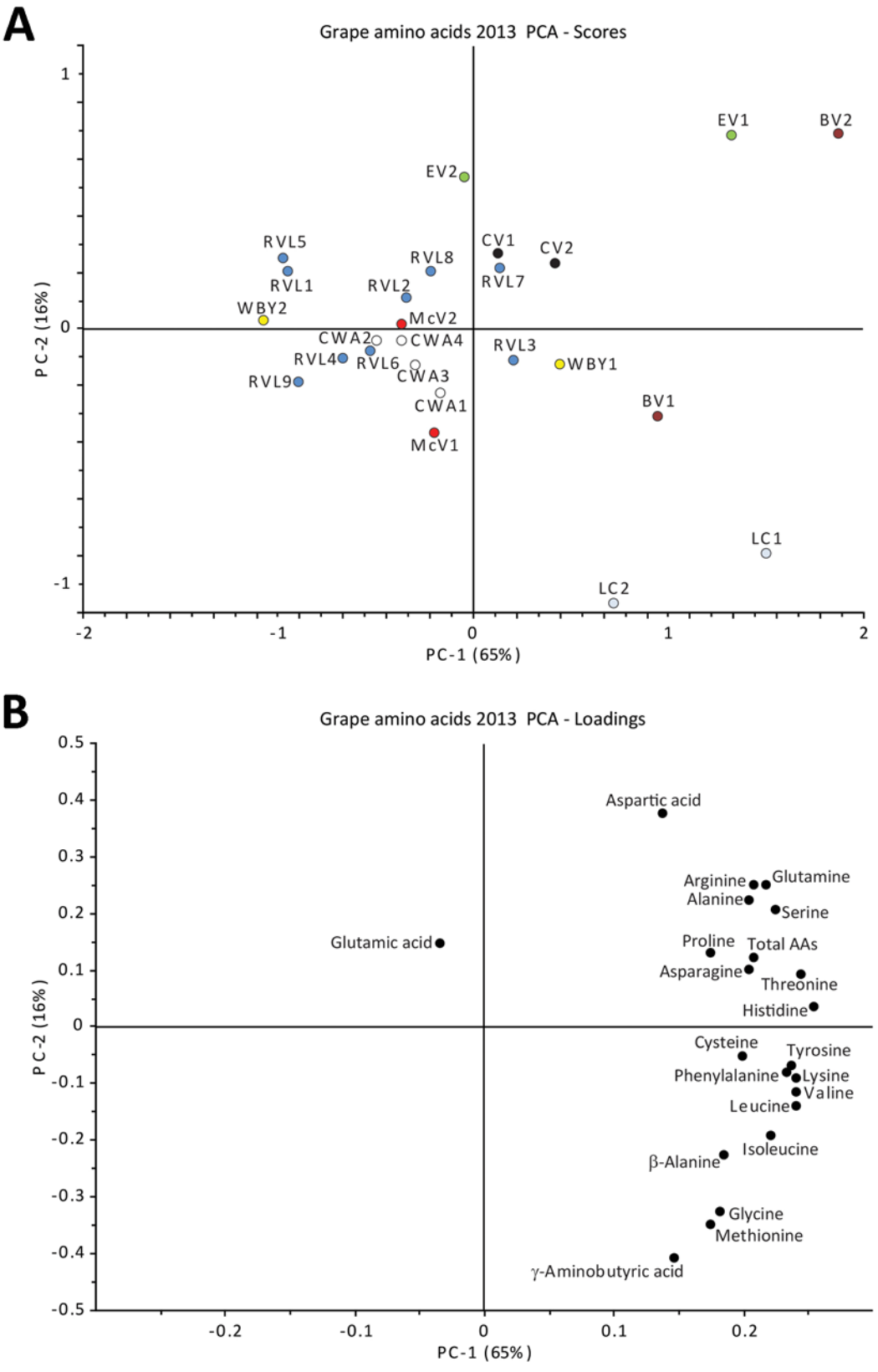


Figure 37. PCA scores (A) and loadings (B) plots of 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the amino acids quantified in the grape juice.

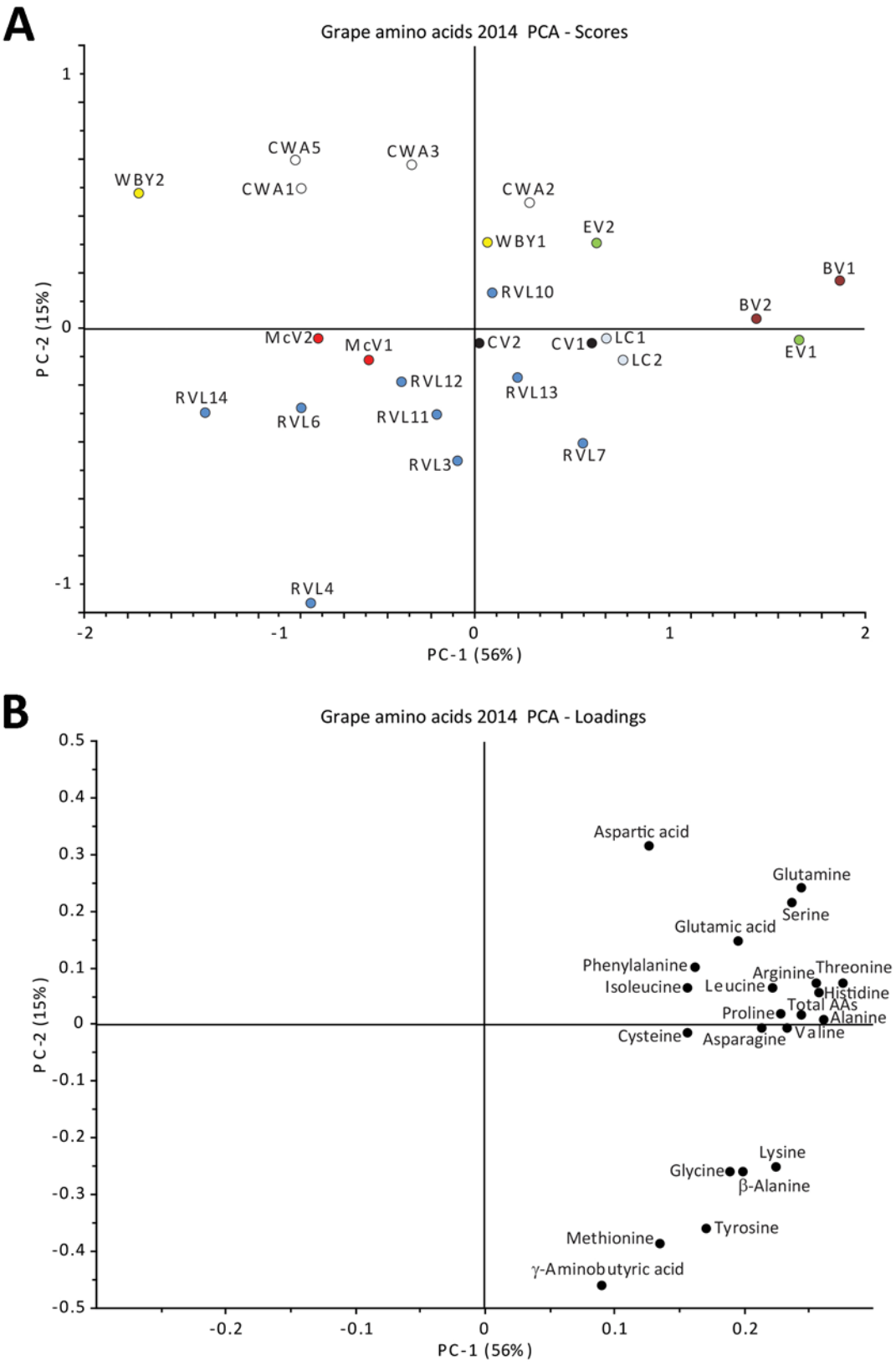


Figure 38. PCA scores (A) and loadings (B) plots of 25 different 2014 Cabernet Sauvignon grape samples from eight regions across South Australia and the amino acids quantified in the grape juice.

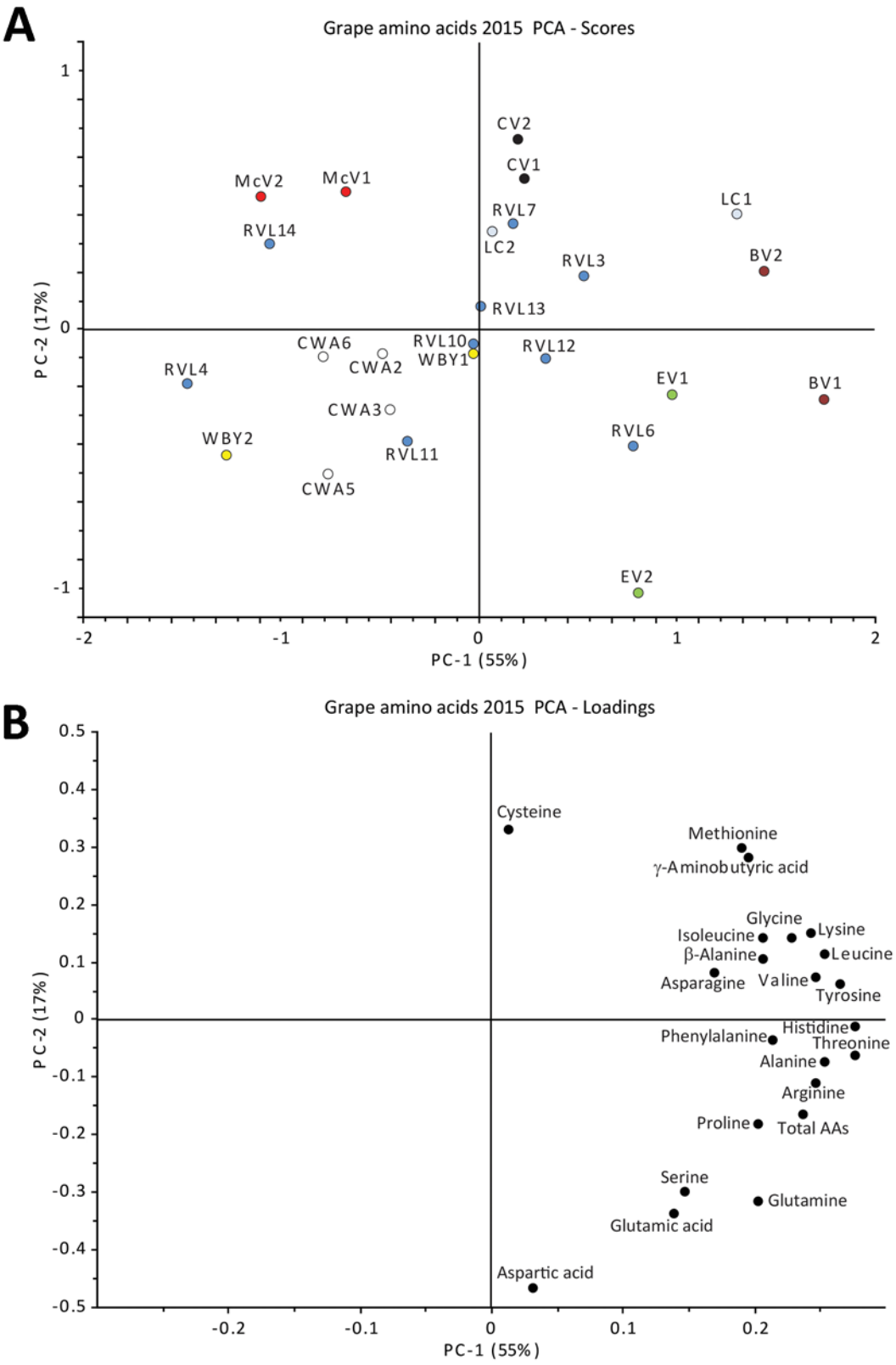


Figure 39. PCA scores (A) and loadings (B) plots of 25 different 2015 Cabernet Sauvignon grape samples from eight regions across South Australia and the amino acids quantified in the grape juice.

and separated samples with higher aspartic acid concentrations in the upper half of the plot with those that have higher glycine, tyrosine, methionine and γ-aminobutyric acid concentrations at the bottom of the plot

(Figure 38). This was again in close agreement with what was observed in 2013, although the LC samples did not appear to have higher concentrations of glycine, methionine and γ-aminobutyric acid as they did in 2013.

Analysis of the amino acids from the grapes harvested during the 2015 vintage showed a remarkably similar trend to the first two years of the study (Figure 39). PC1 explained 55% of the total variance and again discriminated the RVL samples and accounted for some variation in the WBY and LC samples. This was driven by total amino acids on the right side of the plot, where yet again the majority of the amino acids were found in the loadings plot (Figure 39). The BV and EV samples were located on this side of the plot, along with LC1. The amino acids driving separation on PC2 (17%) were aspartic acid in the lower half of the plot with methionine and γ-aminobutyric located in the upper half (Figure 39). One difference in the 2015 data was that cysteine was an important variable in the discrimination on PC2, whereas it was not driving this in the other two vintages.

The similarity between the PCA analyses of the amino acid concentrations in the samples across the three vintages suggests that the differential production of these important grape metabolites is related to the region/vineyard more than to environmental variables. This is important as it means that vineyard interventions designed to alter the amino acid profiles may be robust across vintages as was generally seen in this study. The next challenge is to determine the variables responsible for these differences and to test the efficacy of vineyard interventions in manipulating these amino acid profiles.

7.1.2.7. Bound Precursors of β-Damascenone and Other Bound Volatile Compounds

β-Damascenone in wine is thought to be derived from non-volatile precursors (Puglisi et al. 2001, 2005) produced in berries from the enzymatic degradation of carotenoids (Mathieu et al. 2005). Several authors suggest that β-damascenone has a high odour activity value in red wine and so has a direct impact on red wine aroma (e.g. Aznar et al. 2001; Ferreira et al. 2000, 2002). In contrast, recent work by Pineau et al. (2007) illustrates that β-damascenone concentrations in wine are below threshold in that matrix. Nevertheless, the presence of low amounts of β-damascenone in wine appeared to enhance the odour impact of other flavour impact compounds related to fruity characters (Pineau et al. 2007). Our previous study, discussed above (Forde et al. 2011), supported the findings of Pineau et al. (2007) as a correlation was seen between β-damascenone concentration in wine and overall odour impact.

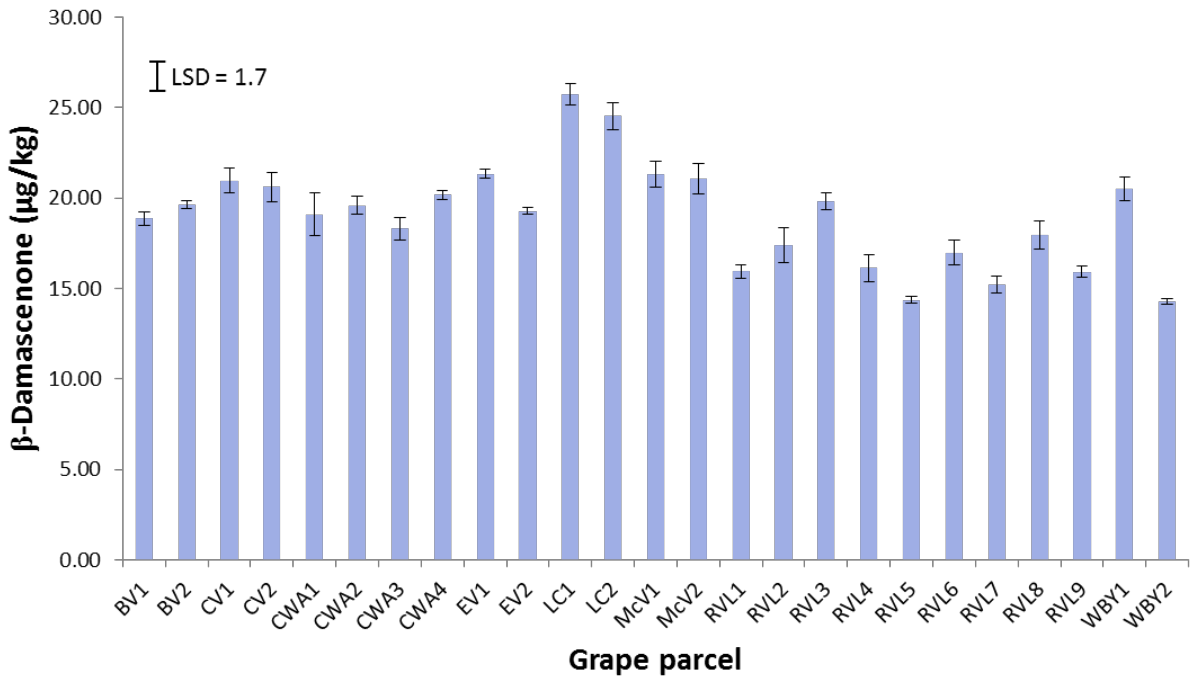


Figure 40. Bound β-damascenone concentrations in grape berries harvested from various South Australia vineyards in 2013. Error bar is ± standard error. LSD = minimum difference for a significant difference at $p < 0.05$.

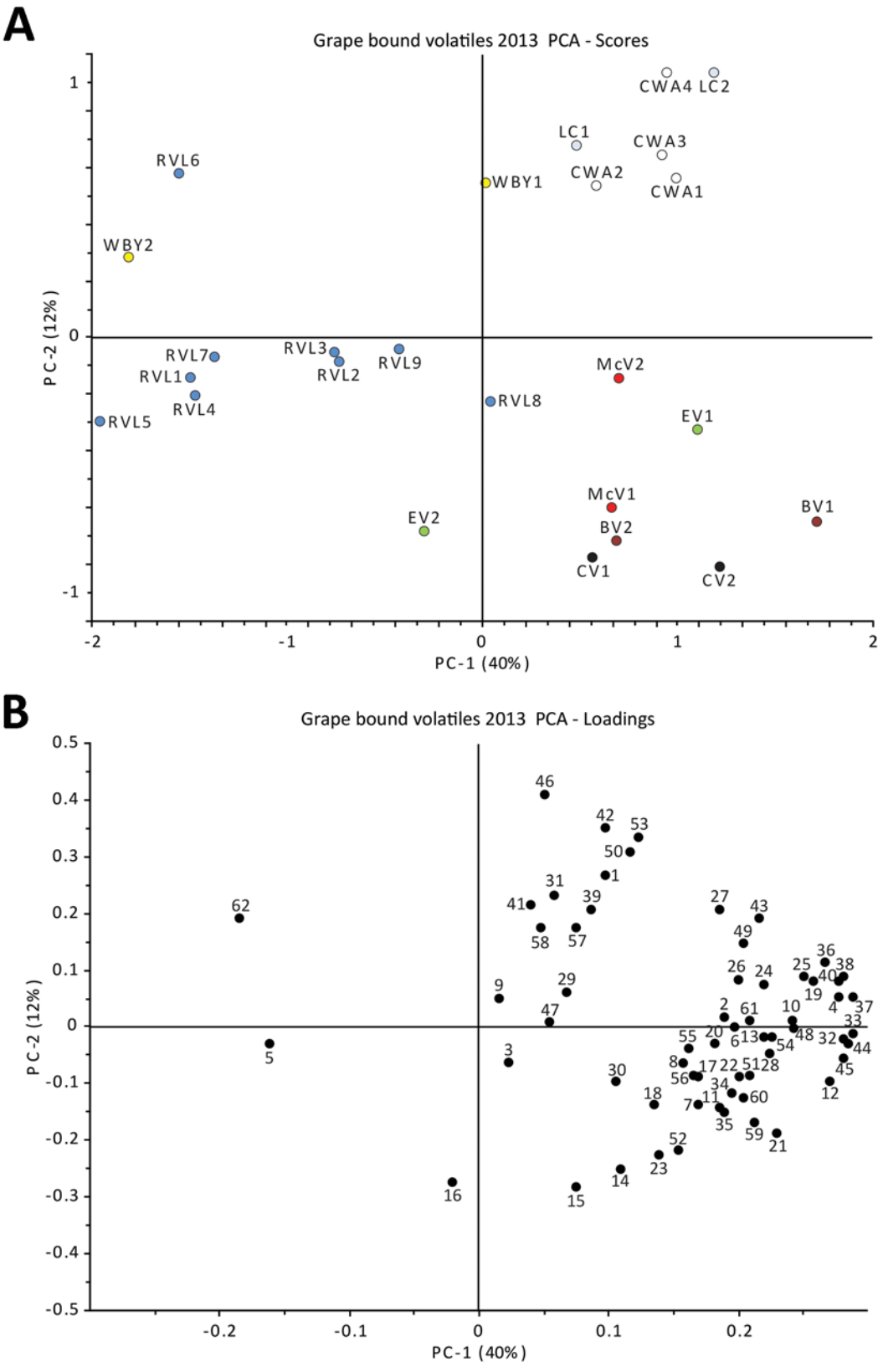


Figure 41. PCA scores (A) and loadings (B) plots of 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the bound volatiles quantified in the grapes. The numbers represent the individual compounds as listed in Table 5.

The amount of free β-damascenone in the headspace above the crushed grapes had already been determined (section 7.1.2.5), but as the bound forms of this compound are also thought to be important, these were quantified as well. A method was developed using solid phase extraction and acid hydrolysis of the bound forms and a liquid extraction before GC-MS analysis (section 6.3.6). Because the method allowed the detection of other volatile compounds released from the bound volatile fraction, a further 60 compounds were quantified in these samples, although some were not detected in certain vintages. The data are presented as PCA plots, except β-damascenone concentrations because of the perceived importance of this compound.

The concentration of bound β-damascenone in the various grape samples was surprisingly similar in the 2013 vintage (Figure 40). There was a trend for the RVL sites to have lower concentrations than those grapes harvested from other regions. Also the two samples from LC had the highest concentrations of bound β-damascenone measured. PCA of the bound volatile profiles revealed some interesting groupings among the samples, which was generally based on region (Figure 41). Most of the RVL samples were located on the left side of the score plot, although WBY2 was also found in the same area. The other WBY sample was collocated with the LC and CWA samples on the top right hand quarter of the plot, and the lower left hand side was where the BV, CV, McV and one of the EV samples were grouped (Figure 41). Forty percent of the variation was described by the horizontal axis, which separated most of the RVL samples and WBY1 from the others. The separation of the two groups of samples on the right hand side of the plot on PC2 was only accounting for 12% of the total variation. There were many compounds driving separation on the horizontal axis, with the norisoprenoids dihydro-β-ionone, megastigmatrienone2 and 1-(2,3,6-trimethylphenyl)but-3-en-2-one along with several unknowns being associated with samples on the right of the plot, and higher concentrations of methyl syringate and nonanal being found in the samples on the left side. The separation on the vertical axis was due to higher concentrations of the aromatic compounds 4-vinylguaiacol, 2,6-dimethoxyphenol, 4-vinylphenol and benzoic acid in samples at the top of the plot and more 2-methylbenzaldehyde, 4-ethylbenzaldehyde and menthol in samples near the bottom (Figure 41).

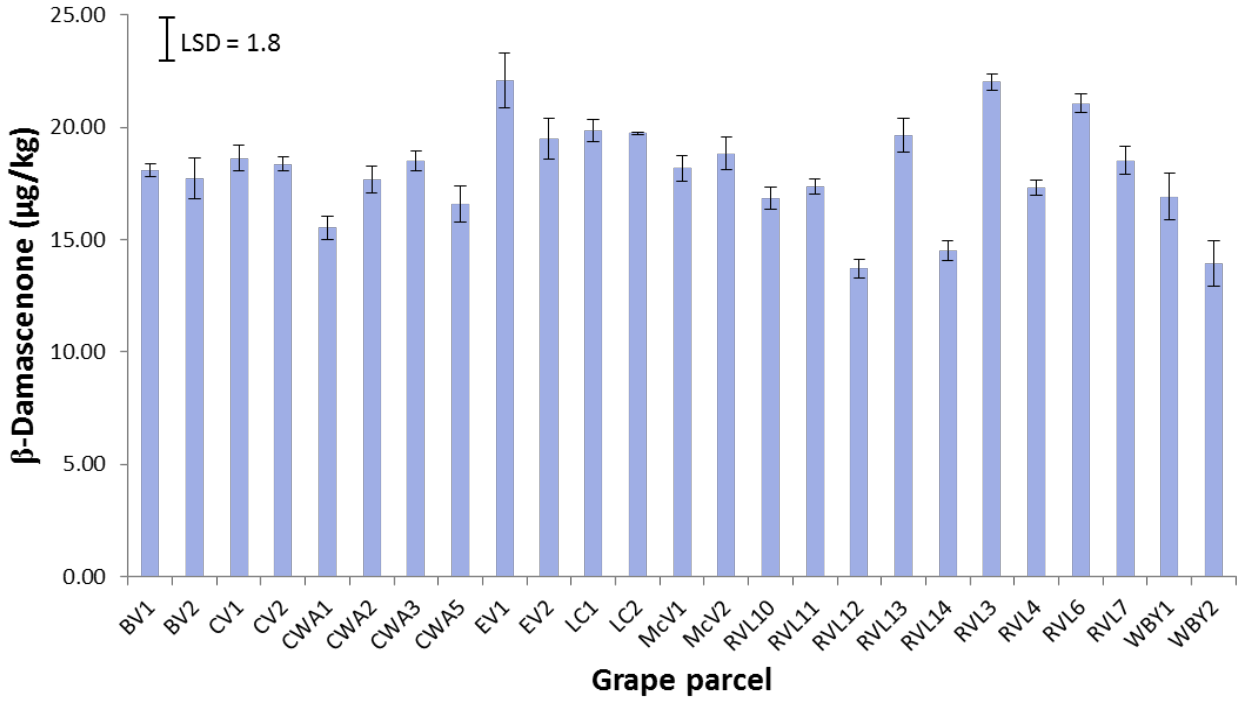


Figure 42. Bound β-damascenone concentrations in grape berries harvested from various South Australia vineyards in 2014. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

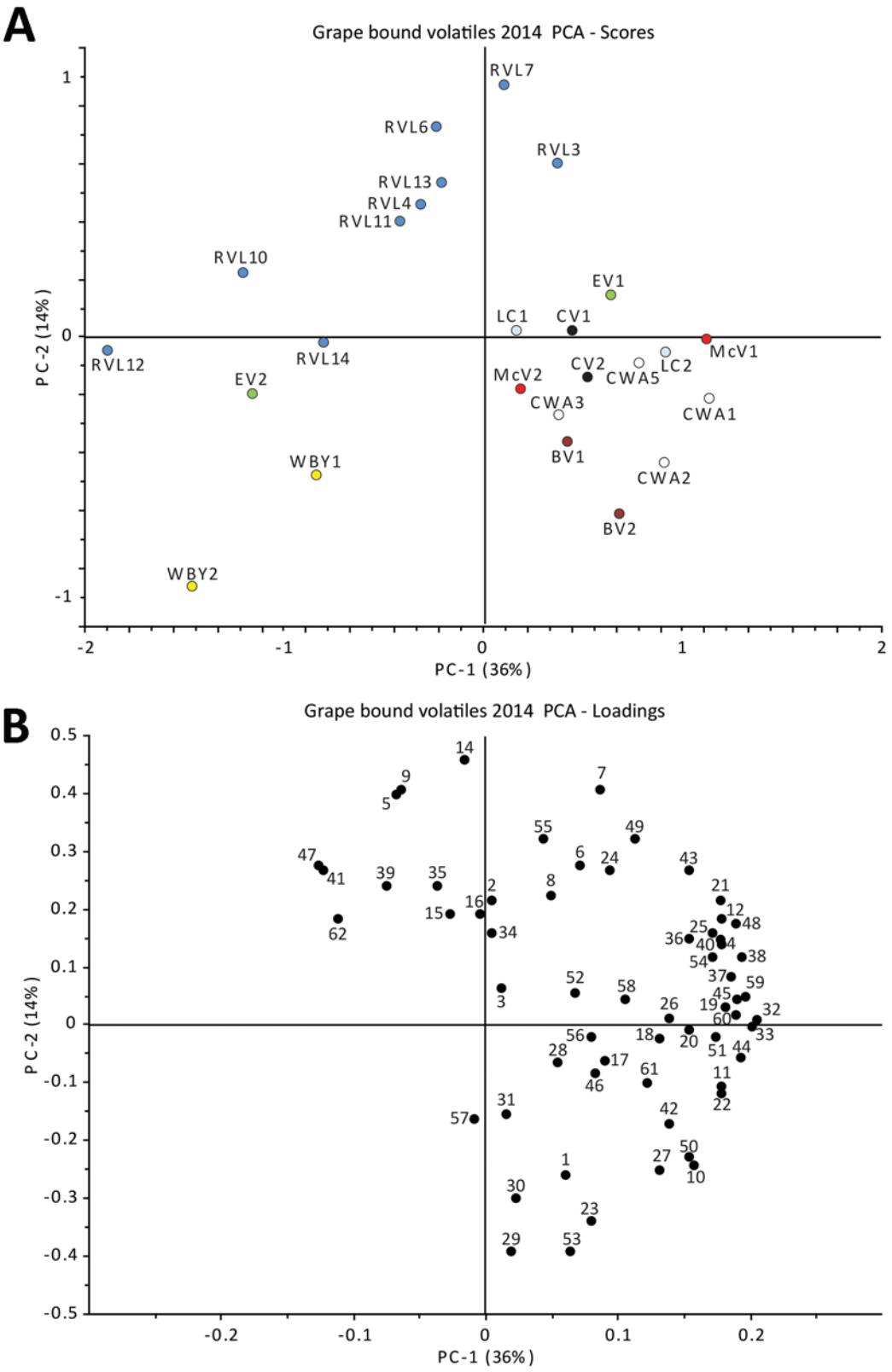


Figure 43. PCA scores (A) and loadings (B) plots of 25 different 2014 Cabernet Sauvignon grape samples from eight regions across South Australia and the bound volatiles quantified in the grapes. The numbers represent the individual compounds as listed in Table 5.

The bound β-damascenone concentrations in the grape samples from the 2014 vintage were similar to those observed in 2013 and again did not vary much across the samples ranging from 13.7 to 22.1 µg/kg (Figure 42). There did not seem to be a relationship between region and bound β-damascenone concentration in the 2014 samples. When the relationship among the bound volatile profiles of the grape samples was visualised using PCA, there was some grouping based on the region of origin (Figure 43), but the pattern was different to that seen in 2013 (Figure 41). The RVL samples grouped loosely in the top right quarter of the plot, but there was some separation of these samples on both axes. The BV, CV, CWA, McV and LC samples were all found in the right of the plot, with most in the lower right quarter, whereas the WBY samples were located in the lower left of the plot. The EV samples were separated on PC1 with EV1 on the right of the plot and EV2 on the left. The compounds driving this separation on the horizontal axis were similar to those in 2013, with dihydro-β-ionone and the same unknown compounds associated with the samples on the left of the plot and methyl syringate, decanoic acid and nonanoic acid concentrations being higher in the samples on the right. PC1 described 36% of the variation in the samples, whereas PC2 only accounted for 14%. The samples near the top of the vertical axis were associated with higher concentrations of nonanal, α-ionene and 2-ethyl-1-hexanol, whereas the samples in the lower part of the plot had higher concentrations of 4-ethylbenzaldehyde, benzyl alcohol and benzoic acid (Figure 43). There were some similarities between the compounds associated with certain regions in the 2013 and 2014 vintages. For example, nonanal and methyl syringate collocated with the RVL samples in both vintages. The CWA samples were associated with the compounds benzoic acid, 4-vinylphenol, guaiacol and 4-vinylguaiacol in both vintages (Figure 41, Figure 43).

The grapes from the 2015 vintage had the broadest range of bound β-damascenone concentrations seen in the three years of the study. The lowest concentration of 12.2 µg/kg was seen in the RVL12 sample and 37.4 µg/kg of bound β-damascenone was measured in the LC1 sample (Figure 44). Again there was no major trend with regards to the regional effect on bound β-damascenone concentrations. The PCA of the bound volatile profiles measured in the fruit revealed a very different picture compared with the previous two vintages (Figure 45).

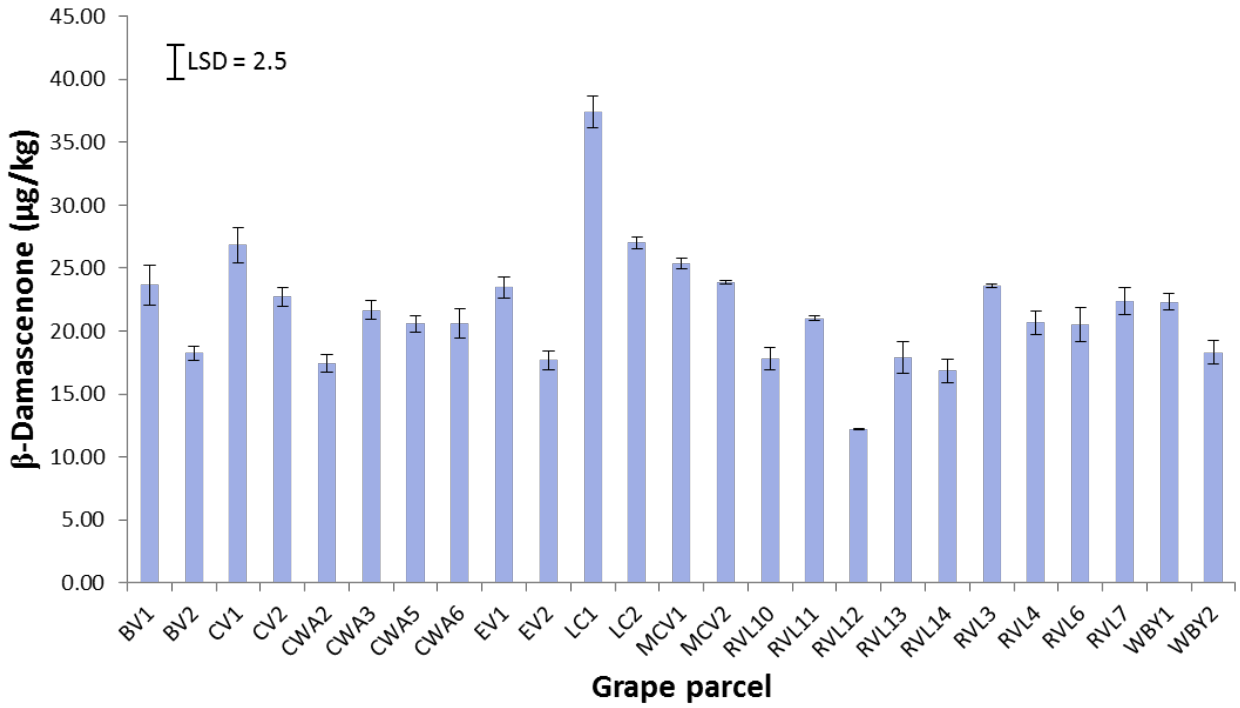


Figure 44. Bound β-damascenone concentrations in grape berries harvested from various South Australia vineyards in 2015. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

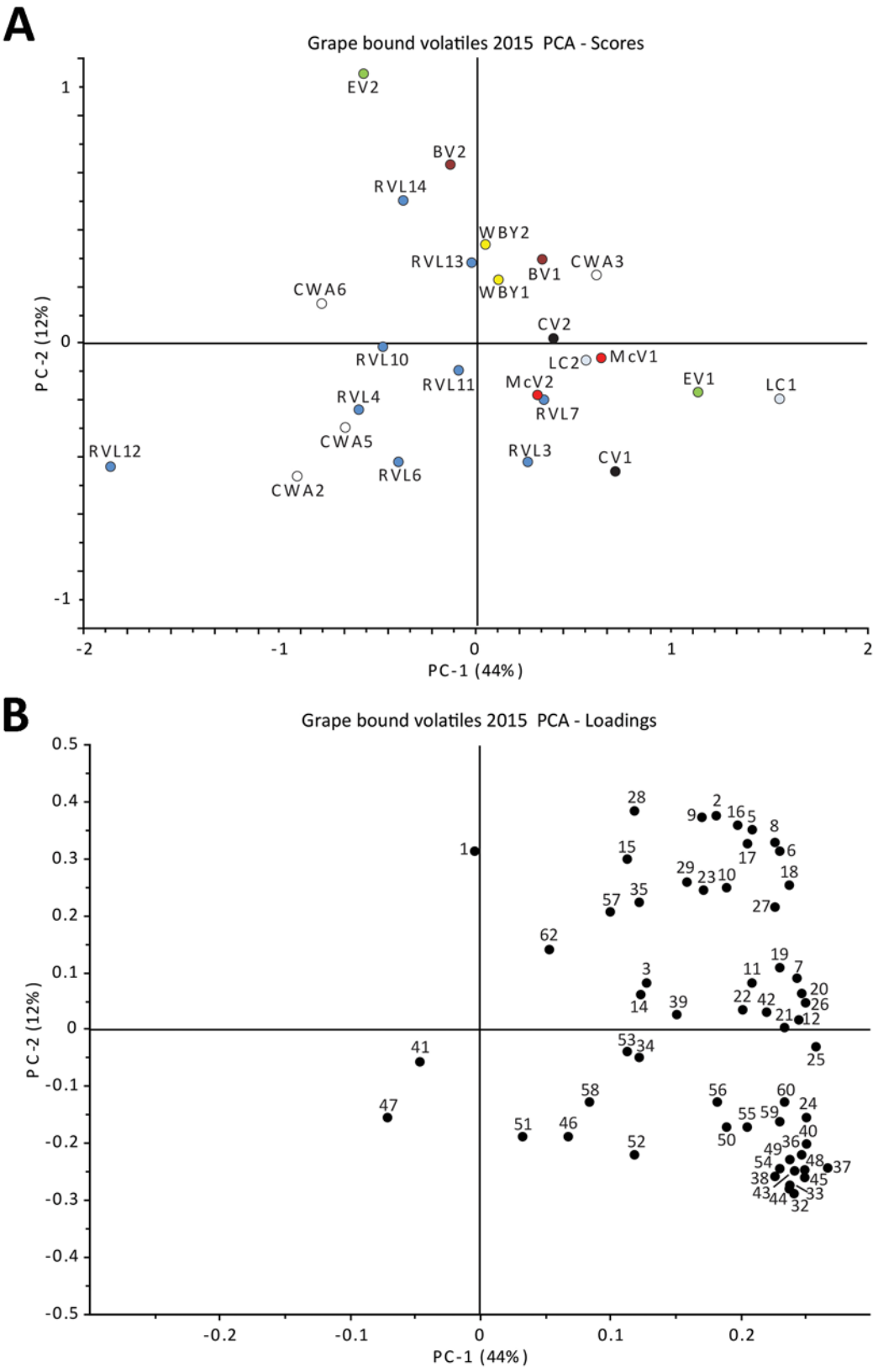


Figure 45. PCA scores (A) and loadings (B) plots of 25 different 2015 Cabernet Sauvignon grape samples from eight regions across South Australia and the bound volatiles quantified in the grapes. The numbers represent the individual compounds as listed in Table 5.

The first PCA described 44% of the variation and discriminated most of the RVL sites, three CWA samples and EV2 from the other grape parcels (Figure 45). PC2 discriminated the BV and CV samples as well as CWA grape parcels and some of the RVL sites. Plotting PC1 versus PC3 (data not shown) did group the RVL samples in the top left quarter of the plot – where they collocate with CWA2, 5 and 6. There were many compounds driving the separation on the horizontal axis, with norisoprenoids such as 1(2,3,6-trimethylphenyl)but-1,3-diene and dihydro-β-ionone being associated with compounds on the right side, and a lower concentration of a lot of bound volatiles distinguishing those parcels on the left of the plot (Figure 45). The separation by PC2 was driven by higher concentrations of tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)-furan, 2-ethyl-1-hexanol, 3-carene and hexanoic acid at the top of the plot and more 6-(3-hydroxybut-1-enyl)-2,2,6-trimethyl cyclohex-4-en-1-one at the bottom of the plot.

While there were some similarities in the bound volatile profiles of the grape parcels in the 2013 and 2014 vintages, the same analysis of the fruit harvested in 2015 did not show much in common with the previous vintages. The concentration of bound β-damascenone did not vary greatly in the first two vintages with the highest and lowest concentrations being less than 2-fold different. This suggests that the variation in the concentration of this compound across vineyards would not be great enough to be used to distinguish a series of grape parcels into multiple grades.

7.1.2.8. Fatty Acids

From studies on the volatile composition grapes it was observed that the main source of headspace volatile compounds was the degradation of fatty acids (Kalua and Boss 2009, 2010). This can occur in several ways and there are distinct sets of compounds produced from the different degradation pathways (Van Dyck 2007). For example, the enzymatic breakdown of linoleic acid via the lipoxygenase pathway should result in the production of hexanal and (*E*)-2-nonenal and the corresponding alcohols hexanol and (*E*)-2-nonenol (Dunlevy *et al*. 2009). Whereas, singlet oxygen will produce 2-heptenal, 2,4-heptadienal, 2-butenal and 2-pentylfuran and triplet oxygen will degrade the lipid to release hexanal, nonenal and 2-octenal (Van Dyck 2007). Total lipids are of interest as they may all be targets for singlet and triplet oxygen, and free lipids, which will be available for enzymatic degradation by the lipoxygenase pathway are also important. The method used to quantify the individual fatty acid moieties of the lipids incorporates a simple solvent extraction of all the lipids in de-seeded berries followed by methylation to enable detection of the methyl esters by GS-MS. Thirty one fatty acids were quantified using this method and the data was summarised using PCA.

In 2013, the fatty acid profiles were able to discriminate samples collected within some of the growing regions, but not others (Figure 46). For example, some of the RVL samples were separated on the horizontal axis, as were the samples from CWA, EV, LC and WBY. The second PC only described 15% of the total variation compared to PC1, which represented 33%, and PC2 discriminated some of the regions from each other (e.g. CV compared to McV). The samples on the right of the plot were associated with higher concentrations of many of the fatty acids measured (Figure 46). Those fatty acids driving the separation on the right side of the plot were the saturated even-numbered fatty acids ranging from tetradecanoic acid (14:0) to hexacosanoic acid (26:0), along with heptadecanoic (17:0), heneicosanoic (21:0) and tricosanoic (23:0) acids. The left side of the plot was associated with lower amounts of almost all of the fatty acids. Separation on the vertical axis was driven by heptacosanoic (27:0), dodecanoic (12:0) and 2-hydroxy-hexadecanoic (16:0 OH) acids towards the top of the plot, while the lower section was associated with greater abundance of saturated and unsaturated even numbered fatty acids with lengths between 20 and 24 carbon atoms (Figure 46).

The PCA plot of the fatty acid profiles obtained from the 2014 vintage grapes, at first glance, appeared to be quite different from 2013 (Figure 46 and Figure 47). However, closer inspection showed that the first two PCs appeared to be swapped, which meant that, in 2014, PC1 (31%) is driven by the fatty acid composition, whereas PC2 reflected more the overall concentration of fatty acids in the samples (Figure 47). PC1 discriminated samples based on region, with BV, McV and RVL samples located on the left of the plot and the CV, CWA and WBY samples found on the right. PC2 gave some discrimination of the grapes from the RVL, with parcels from BV, CWA, EV and LC also found on either side of the horizontal axis (Figure 47). The drivers

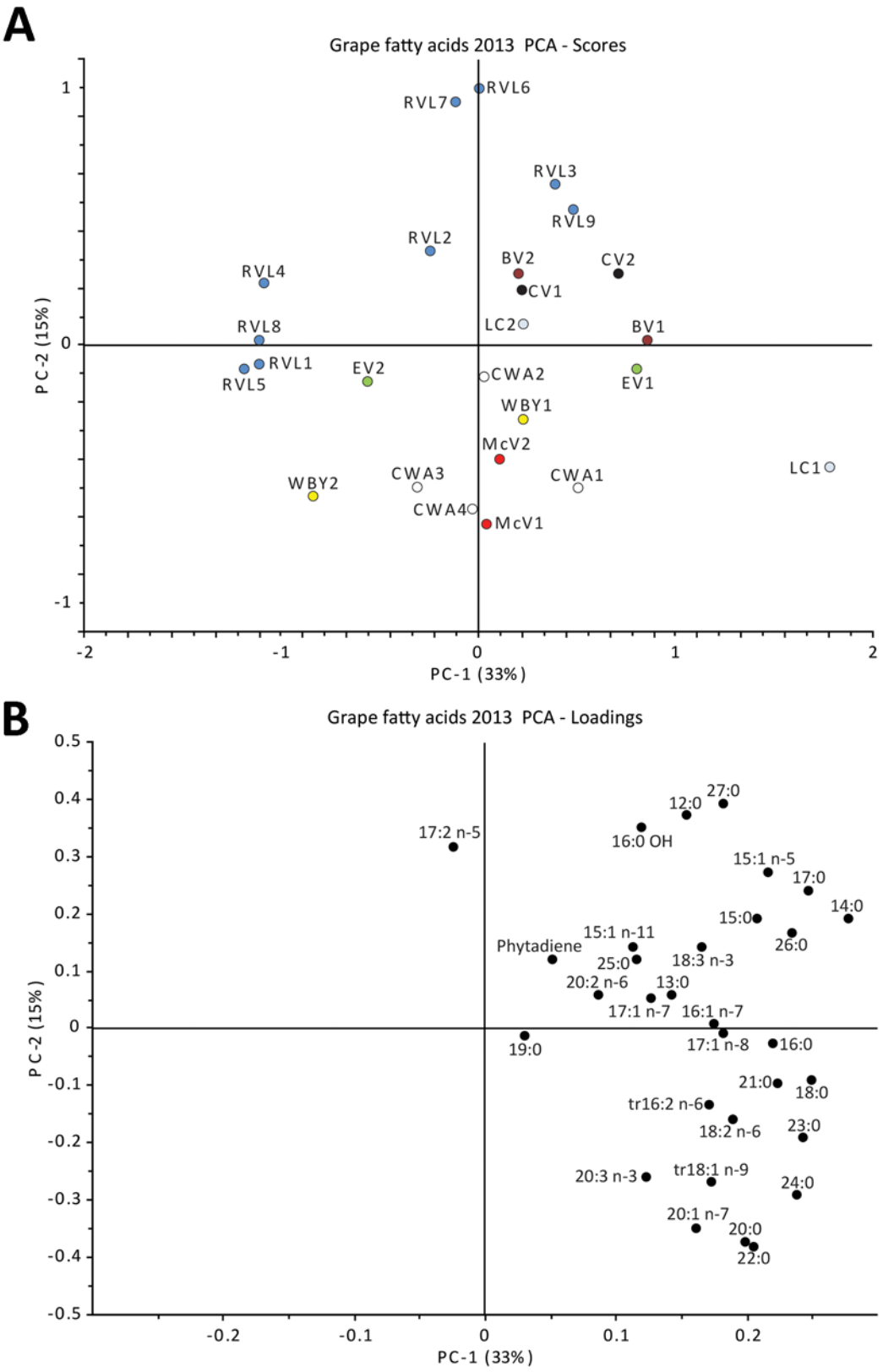


Figure 46. PCA scores (A) and loadings (B) plots of 25 different 2013 Cabernet Sauvignon grape samples from eight regions across South Australia and the fatty acids quantified in the grapes. The denotations of the various fatty acids are listed in Table 6.

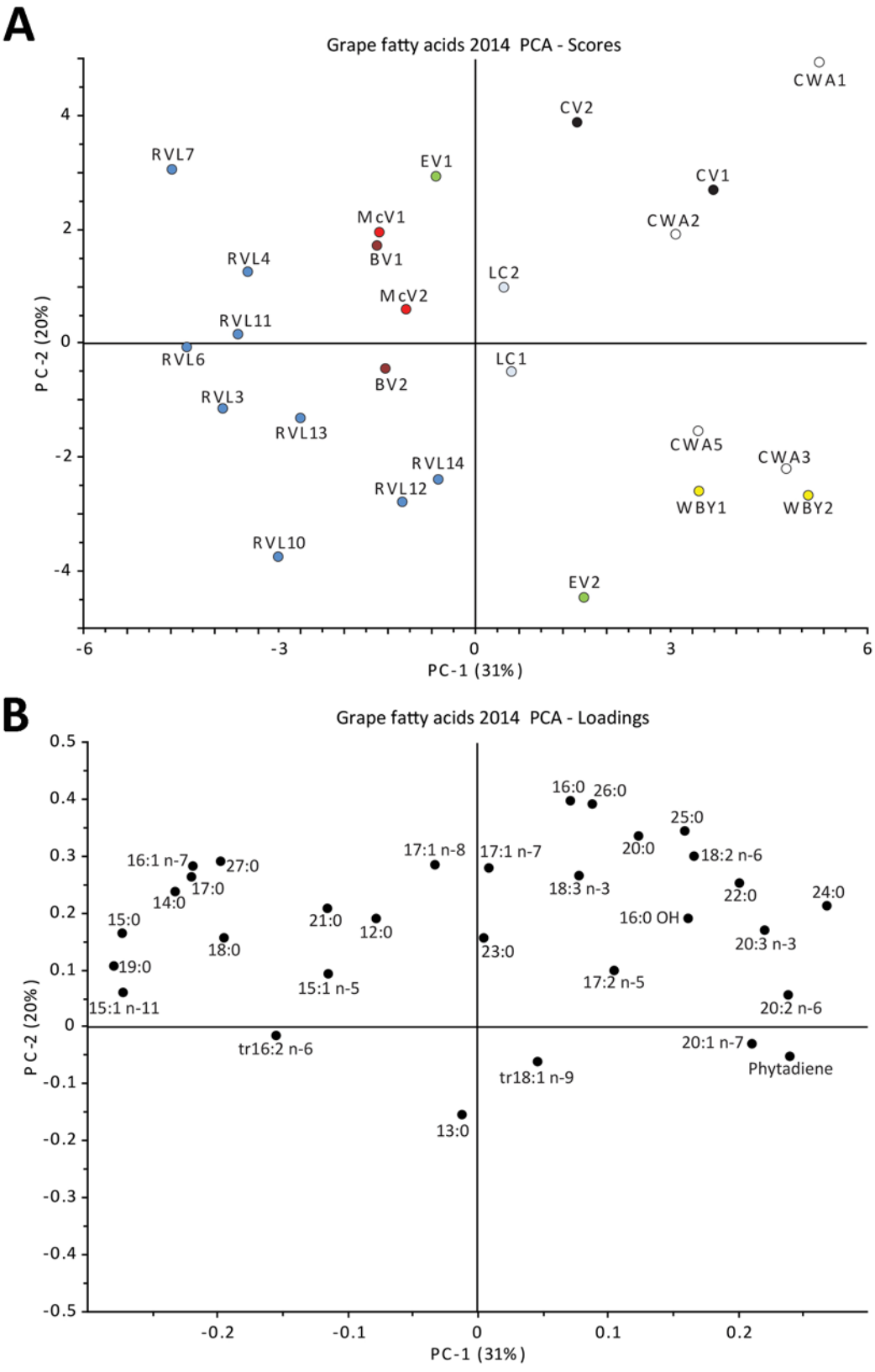


Figure 47. PCA scores (A) and loadings (B) plots of 25 different 2014 Cabernet Sauvignon grape samples from eight regions across South Australia based on the fatty acids quantified in the grapes. The denotations of the various fatty acids are listed in Table 6.

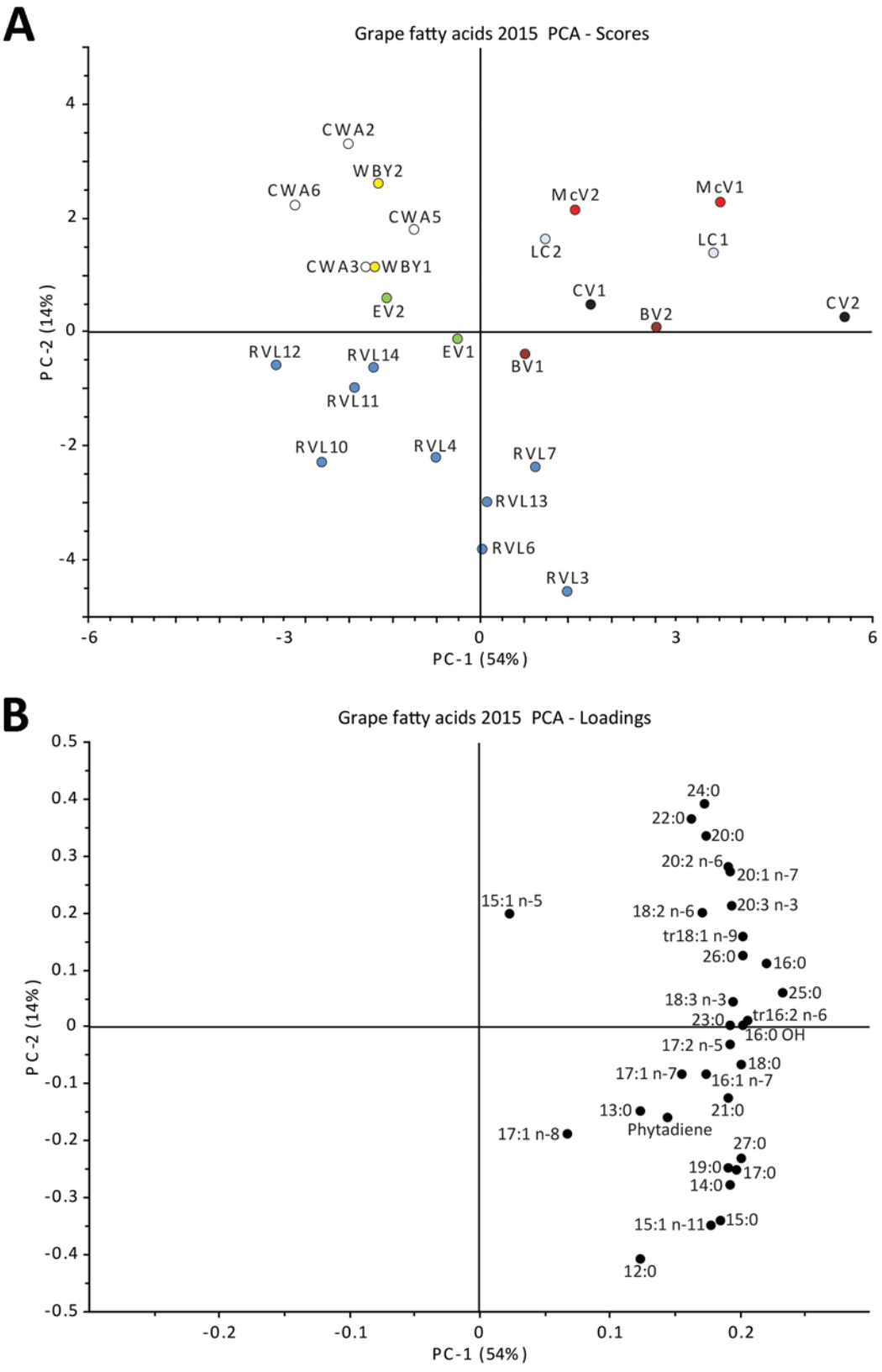


Figure 48. PCA scores (A) and loadings (B) plots of 25 different 2015 Cabernet Sauvignon grape samples from eight regions across South Australia based on the fatty acids quantified in the grapes. The denotations of the various fatty acids are listed in Table 6.

of the discrimination of the samples on the right of the plot on PC1 were unsaturated 20 carbon fatty acids as well as docosanoic (22:0), tetrasanoic (24:0) acids and (*E*)-2,4-phytadiene. On the left of the plot, the samples were associated with higher concentrations of the odd numbered fatty acids pentadecanoic (15:0),

(*Z*)-10-pentadecanoic (15:1 n-5) and nonadecanoic (19:0) acids. PC2 separated the samples with generally higher concentrations of many different fatty acids at the top of the plot from those samples that were not distinguished by high concentrations of any of the fatty acids measured (Figure 47).

The PCA of the 2015 fatty acid profiles of the Cabernet Sauvignon grapes (Figure 48) showed a similar pattern to the 2013 samples (Figure 46). PC1 explained 54% of the total variation and discriminated CWA, EV and WBY parcels from BV, EV, LC and McV fruit. It also allowed discrimination among the RVL samples. This PC was driven by the higher concentration of most of the fatty acids measured on the right side of the plot, associated with the BV, CV, LC and McV samples, and the lower concentration of most of these fatty acids on the left side (Figure 48). PC2 was predominantly describing the separation of the RVL samples from those obtained from the other sites. The RVL samples were associated with higher concentrations of shorter even-numbered fatty acids (e.g. dodecanoic and tetradecanoic acids) and odd-numbered fatty acids, whereas the grapes from the other sites had a greater abundance of longer chain even-numbered fatty acids (Figure 48).

Some similarities were observed in the fatty acid profiles across the three vintages. The RVL samples were mainly associated with lower concentrations of fatty acids and a higher concentration of odd-numbered and relatively short, even-numbered fatty acids. The CWA and WBY parcels tended to have a higher concentration of long even-numbered fatty acids compared to the other parcels. No consistent pattern across the vintages was seen for the other sites suggesting that environmental effects have a strong influence on grape fatty acid composition in Cabernet Sauvignon grapes.

7.1.3 ACTIVITIES OF ENZYMES FROM THE LIPOXYGENASE PATHWAY

The lipoxygenase pathway (Figure 49) is one of the most important sources of volatile compounds in grapes. It catalyses the release of six carbon (C_6) volatile compounds from unsaturated fatty acids when most plant tissues are damaged and is responsible for the distinctive odour of freshly mown grass. As such, most of the C_6 compounds are described as having a “green” of “cut grass” aroma, which are often seen as being undesirable in red wines.

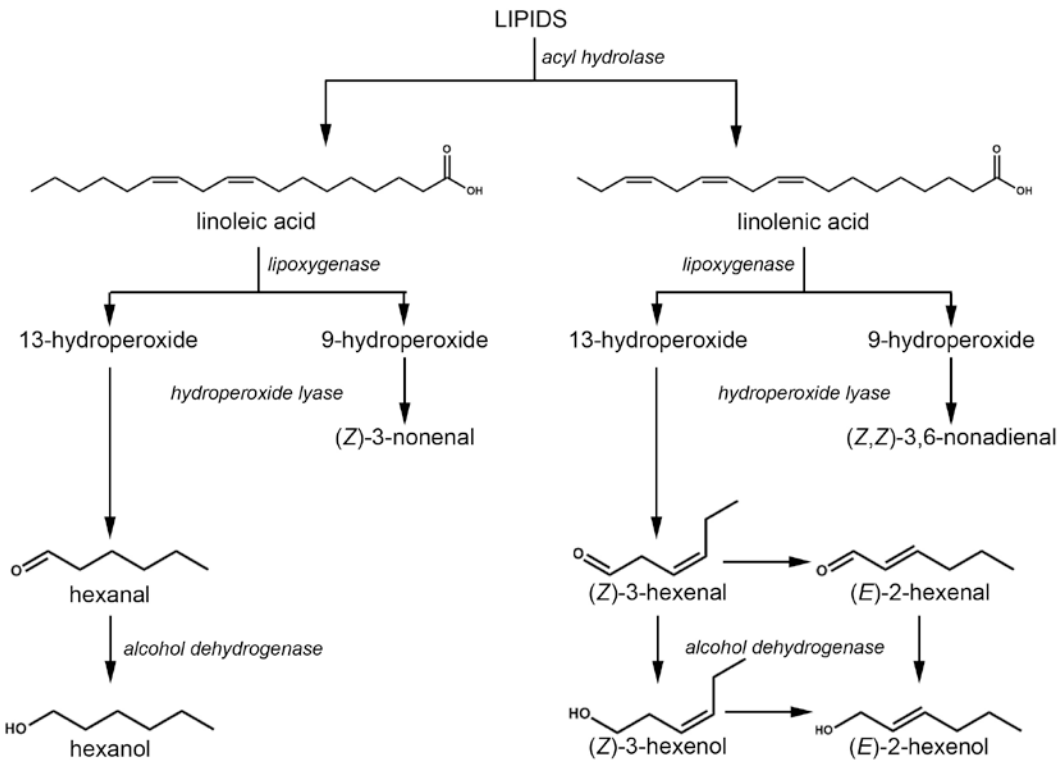


Figure 49. The lipoxygenase pathway

Given the prevalence of these C_6 compounds in crushed grapes, assays to determine the activity of the three major lipoxygenase pathway enzymes in the harvested fruit were developed. The hypothesis was that the activity of lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH) would influence the total amount of C_6 compounds produced after crushing as well as the relative proportions of alcohols and aldehydes, which could have implications for the production of related compounds during winemaking (Dennis et al. 2012).

7.1.3.1 Lipoxygenase (LOX)

Lipoxygenase is the first committed step of the pathway and catalyses the production of a lipid hydroperoxide at the double bond at C-9 or C-13 of linoleic or linolenic acid (Figure 49). Lipoxygenases generally have specificity for either site and in ripe grapes the predominant activity is towards C-13. The C-13 lipoxygenase activity was assayed in the harvested fruit, and it was found that there was only a small amount of variation measured in the different grape parcels (Figure 50). In 2013, the LOX activity varied from 0.49 µmol/min/g in EV2 to 0.7 µmol/min/g in EV1. No obvious trend with regard to any regional effect on LOX activity was observed. The 2014, the grapes had a greater range of LOX activities (Figure 51), with the maximum activity seen in the CWA2 and CWA3 samples (0.84 µmol/min/g) and the lowest measured in the RVL3 fruit (0.47 µmol/min/g). This still represents less than a 2-fold difference between the extremes. The mean LOX activity in 2014 was 0.68 µmol/min/g, which was greater than that observed in 2013 (0.57 µmol/min/g). There was a slight trend for the CWA samples to have higher LOX activities than those sampled from other regions, but the effect was not marked. Finally, in 2015, the extent of LOX activities was again quite narrow ranging from 0.48 µmol/min/g in two samples (RVL10 and WBY1) to 0.79 µmol/min/g in the BV2 sample (Figure 52). The mean LOX activity fell between those observed in 2013 and 2014, being 0.60 µmol/min/g in 2015. The BV and CV grapes had relatively higher LOX activity than the other sites in 2015, but the difference was not great. There was little consistency in the LOX activities of the samples across the three vintages, which may reflect that: differences in harvest timing may influence LOX activity in fruit; environmental effects that differ among the vintages influence LOX activity more than any site or regional effect; the LOX activity in the fruit is stable across region and vintage and the subtle differences observed may be due to sampling or experimental error.

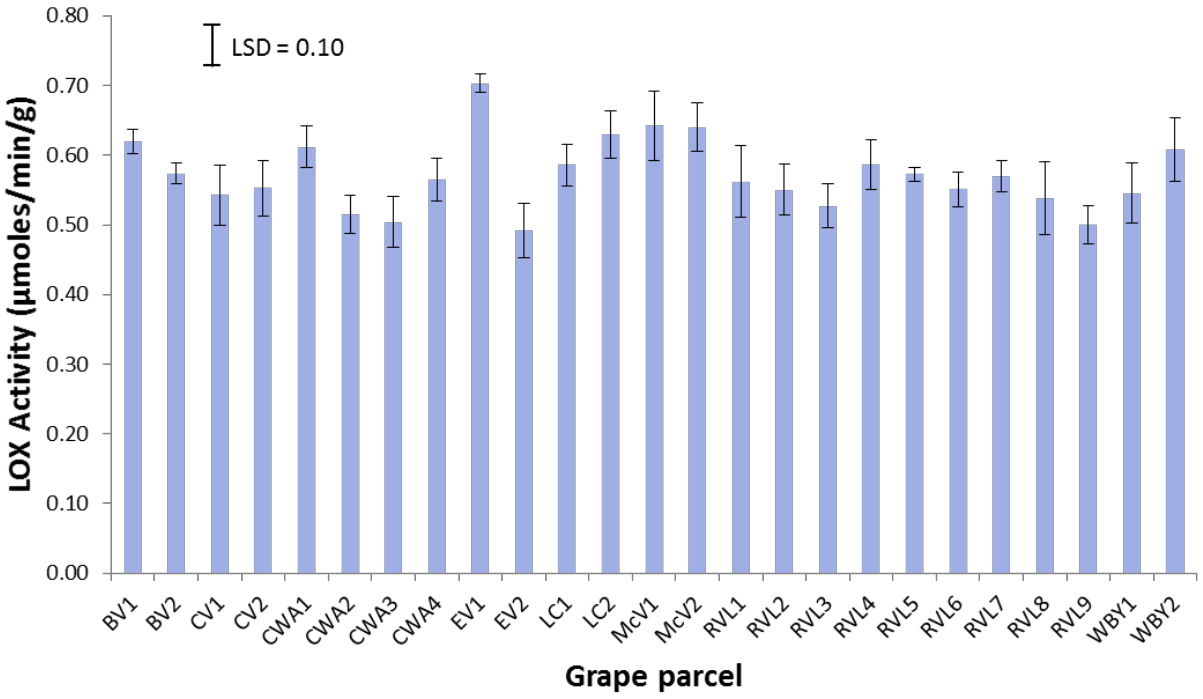


Figure 50. Lipoxygenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2013. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

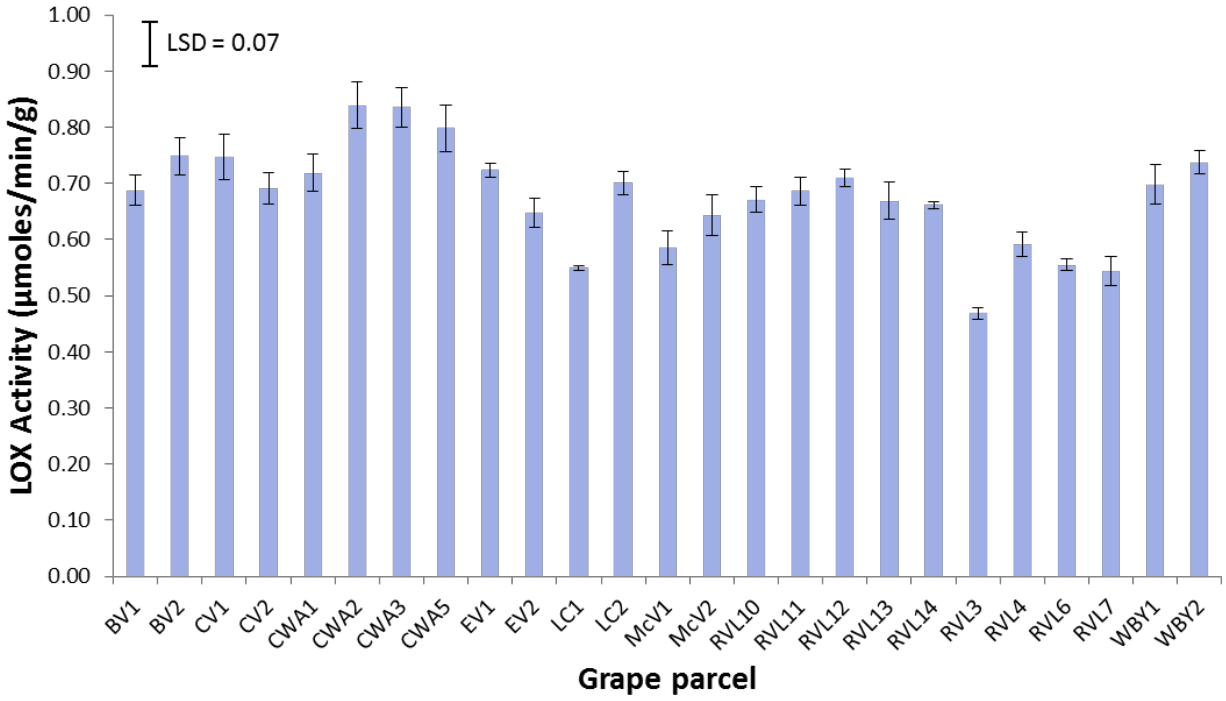


Figure 51. Lipoxygenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2014. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

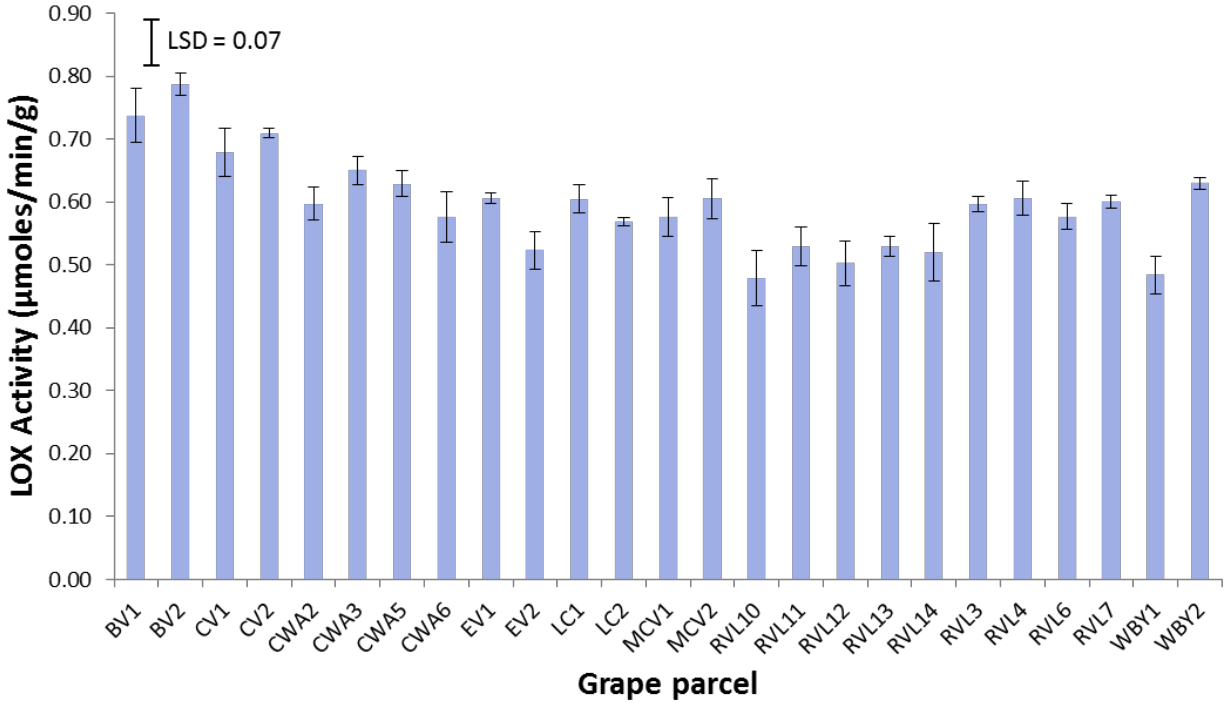


Figure 52. Lipoxygenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.1.3.2 Hydroperoxide lyase (HPL)

Hydroperoxide lyase catalyses the cleavage of the hydroperoxy fatty acids, formed by the action of LOX, into omega-oxo acids and volatile C_6- and C_9-aldehydes (Figure 49). However, it has also been shown that spontaneous degradation of the 13-hydroperoxide formed from linoleic acid can occur (Duan et al. 2005). Nevertheless, the presence of HPL enzyme activity would increase the flux down this pathway to the production of C_6 aldehydes and alcohols.

Similar to LOX, the range of HPL activities measured in the fruit harvested in 2013 was not great (Figure 53), the lowest being 0.81 µmol/min/g (WBY2) and the highest 1.15 µmol/min/g from the BV2 sample. No major trends were observed across the sites. This was also true for the 2014 samples, although most of the lower HPL activities were measured in RVL samples (Figure 54). The mean HPL activity was lower in the 2014 samples (0.75 µmol/min/g) than in the 2013 grapes (1.00 µmol/min/g). Again the range of activities was not great with a less than 2-fold difference between the lowest activity, 0.62 µmol/min/g in the RVL10 sample, and the highest measured activity, which was 0.89 µmol/min/g in the BV1 grapes (Figure 54). The 2015 vintage followed the pattern observed in the previous two vintages. That is, the variation among the HPL activities in the grape parcels was low, with the range in activities being from 0.64 µmol/min/g in CWA6 to 0.98 µmol/min/g in BV1 (Figure 55). Samples from CWA, RVL and WBY had slightly lower HPL activities than those from other regions. The mean activity in 2015 was 0.83 µmol/min/g, intermediate to the mean activities measured in the other two vintages.

Looking across the three vintages, the same conclusions that were made about the LOX activities are appropriate for the HPL activities measured. There appears to be no general trend across the sites and vintages. Again, the lack of variation in these enzyme activities may reflect the fact that the HPL activity is stable across the regions and vintages.

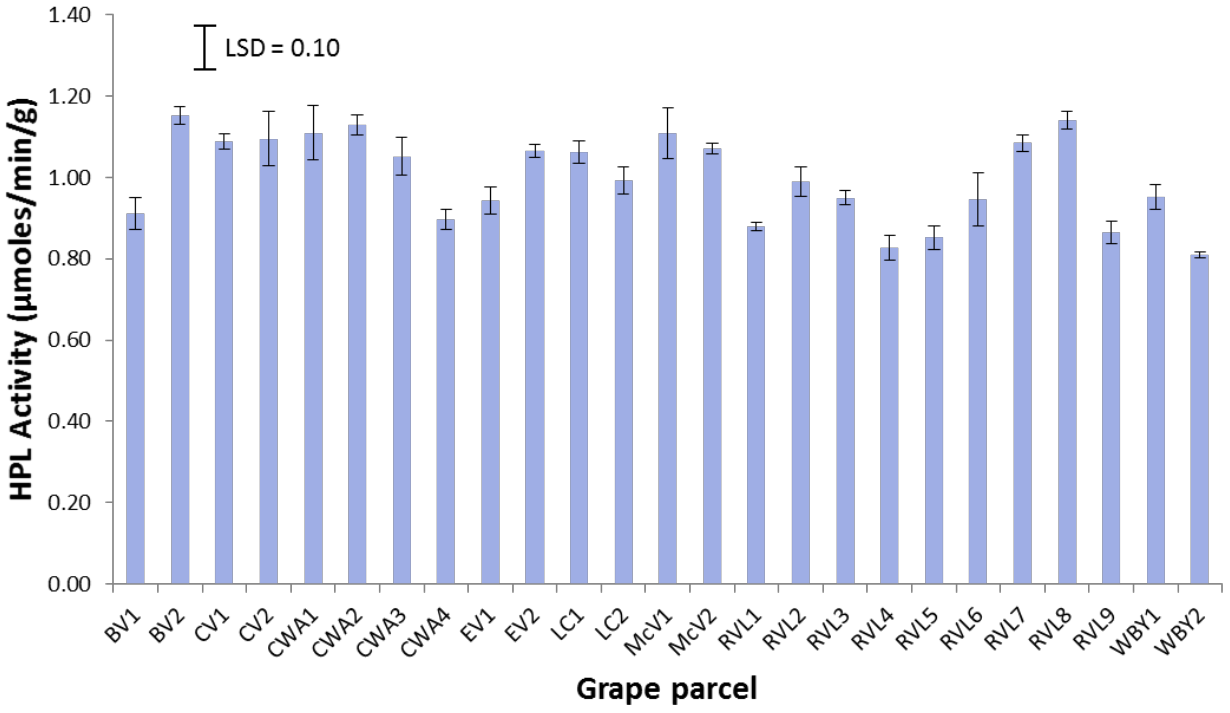


Figure 53. Hydroperoxide lyase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2013. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

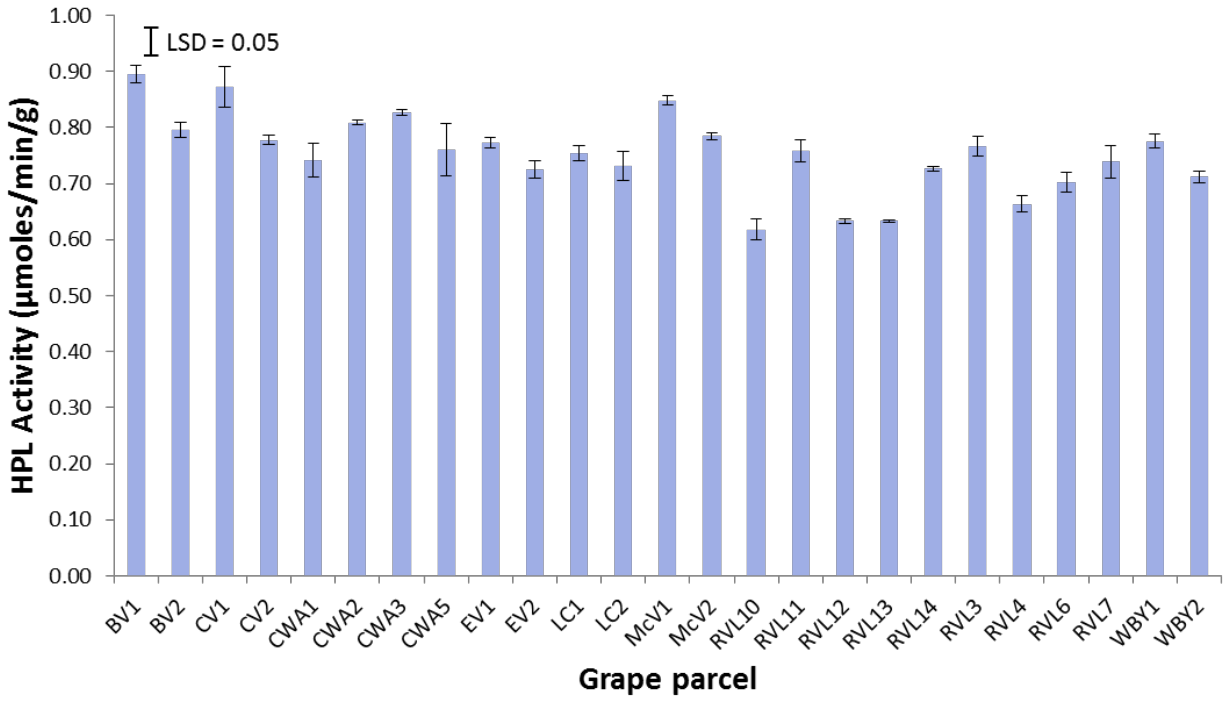


Figure 54. Hydroperoxide lyase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2014. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

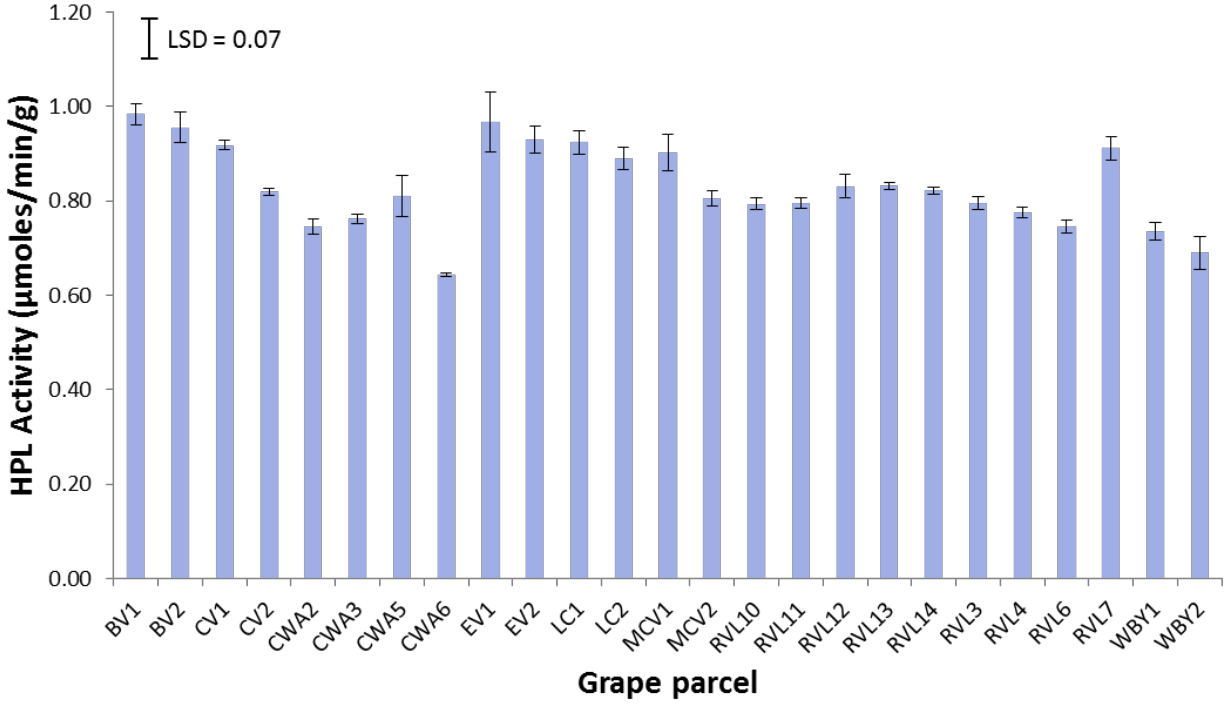


Figure 55. Hydroperoxide lyase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.1.3.3 Alcohol dehydrogenase (ADH)

Alcohol dehydrogenase is the last enzyme in the lipoxygenase pathway that, despite its name, actually catalyses the reduction of C_6 aldehydes to alcohols (Figure 49). Altering ADH activity in tomatoes has been shown to have an effect on the volatile composition and sensory attributes of the fruit by affecting the C_6 aldehyde/alcohol ratios (Speirs et al. 1998). It is assumed differences in activities in the parcels of fruit would have the same effect in grapes, although how this might influence wine composition is complicated by the fact that these compounds may be metabolised during fermentation (Dennis et al. 2012).

Of the three enzymes assayed in the grapes, ADH showed the most variation in activities across the parcels. In 2013, the ADH activities ranged from 1.26 µmol/min/g in the McV2 sample to 2.95 µmol/min/g in the RVL8 grapes (Figure 56). The samples from BV, CV, EV and McV had lower ADH activities than those from other regions, and the grapes from the LC sites had relatively high ADH activities. The mean of the ADH activities measured in the grapes from the 2014 vintage was identical to the 2013 mean of 1.96 µmol/min/g. However, in 2014, the CWA samples had some of the lowest ADH activities and other sites that had low ADH activity in 2013 had relatively high ADH activities in 2014 (Figure 57). The range of ADH activities in the 2014 vintage went from 1.10 µmol/min/g (CWA5 and RVL14) to 2.71 µmol/min/g (EV1). The CWA samples were again among those with the lowest ADH activities in the 2015 vintage, and the LC grapes had high ADH activities (Figure 58). While the range of ADH activities in 2015 was the broadest, the lowest being 0.46 µmol/min/g in the CWA5 sample and the highest was 2.95 µmol/min/g in the LC1 grapes, the mean was the lowest of the three vintages (1.72 µmol/min/g).

The ADH activity measures were the most promising of the three LOX pathway enzymes assayed in the fruit because of the greater range of values measured within each vintage, which could lead to greater discrimination in the multivariate analyses. The trends in relative activities observed across the three vintages were variable, but often samples from the same region had similar activities in a single vintage. This could mean that environmental factors are influencing ADH activity in the fruit from year to year, but those samples within a region are experiencing the same conditions and are, hence, relatively similar.

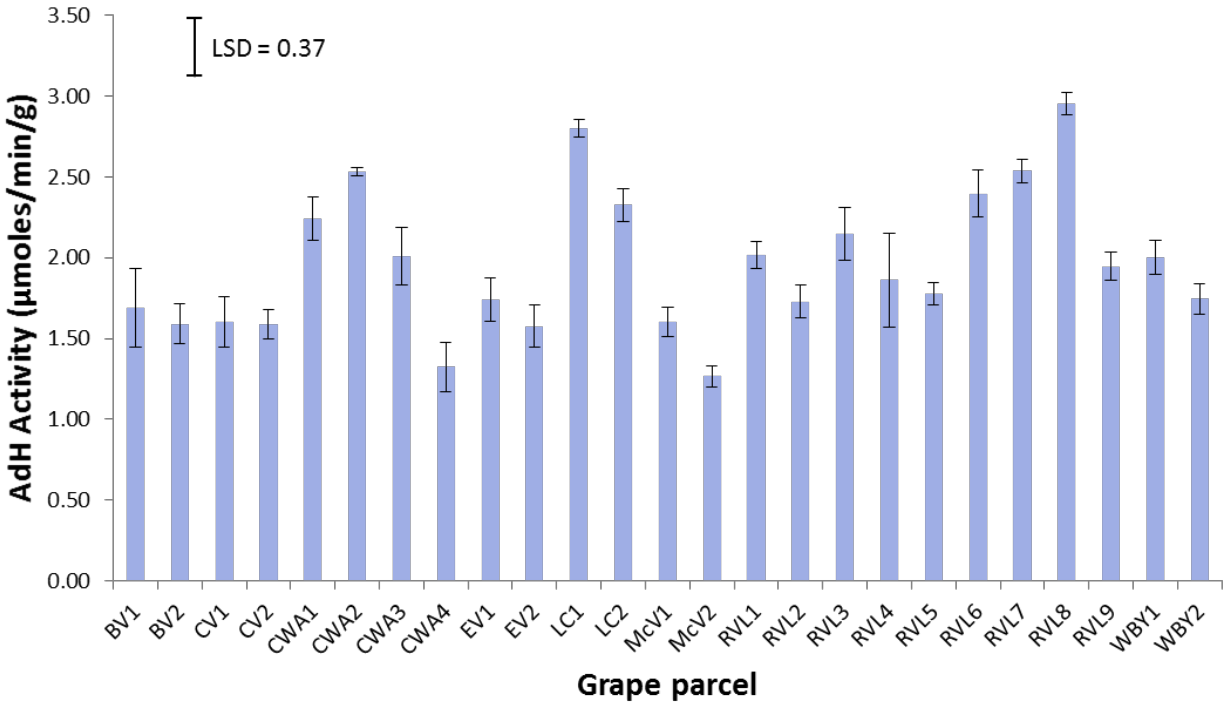


Figure 56. Alcohol dehydrogenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2013. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

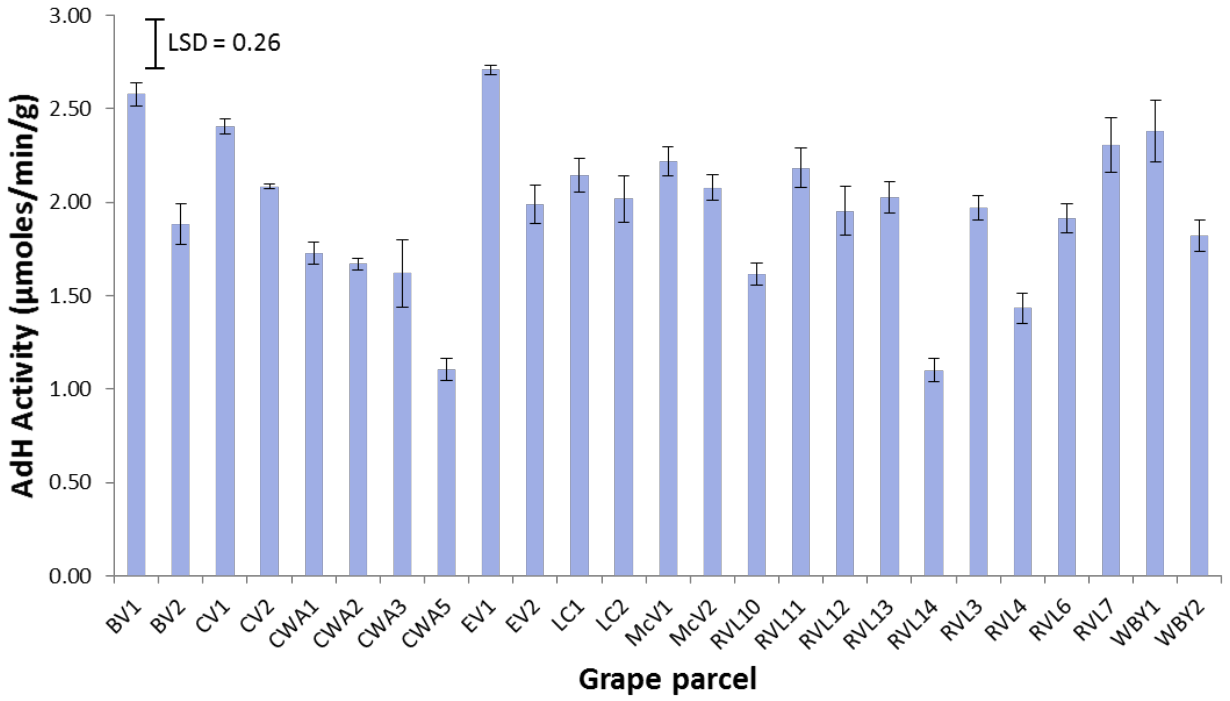


Figure 57. Alcohol dehydrogenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2014. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

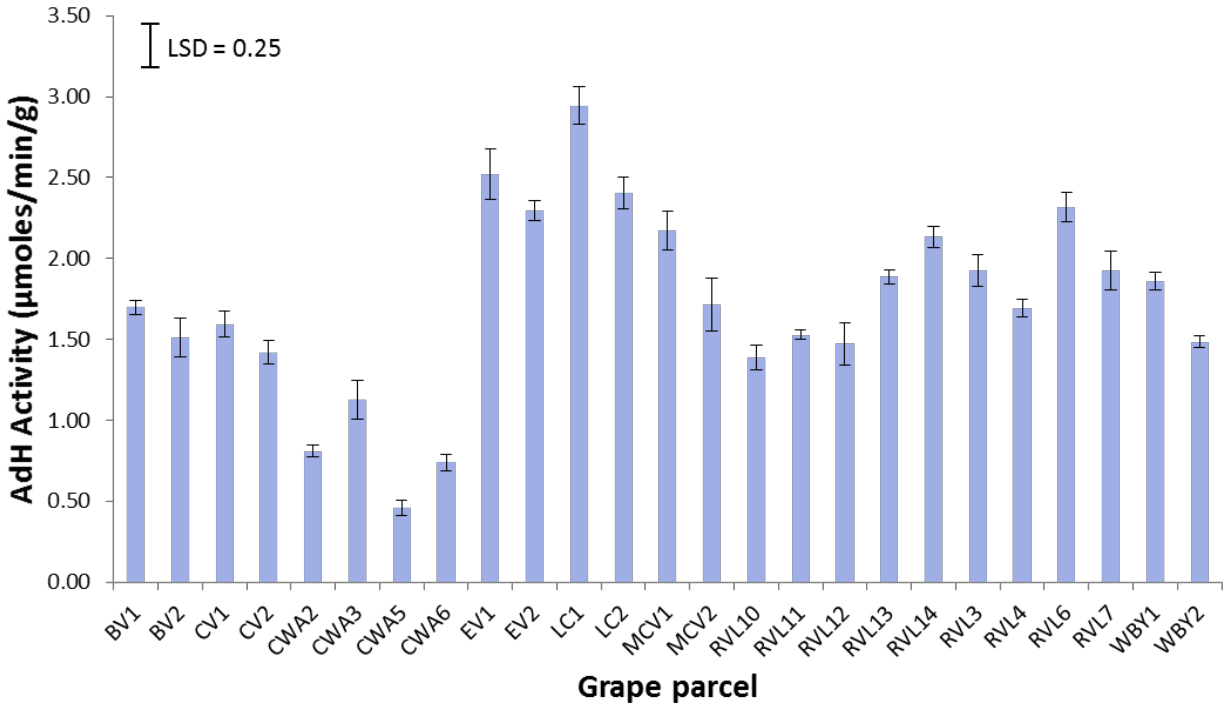


Figure 58. Alcohol dehydrogenase activity in crude protein extracts from homogenates of whole Cabernet Sauvignon berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.1.4 BERRY SENSORY ASSESSMENT (BSA)

Sensory assessments of berries were conducted in this project as it is common in the grape and wine industry to taste the grapes in the vineyard to assist in decision making processes (Olarte Mantilla et al. 2012, Gambetta et al. 2016). The results of berry tastings are, however, very subjective as it is mostly conducted to the discretion of the industry members and very little to no information is available pertaining to differences in the sensory perception of grapes within a variety and across so-called qualities. The BSA of Cabernet Sauvignon were completed by the sensory panel in the 2014 and 2015 vintages. Overall, the taste of grapes appeared to be discriminable by regions, however, the sensory properties of the grapes differed by vintage.

The BSA of 2014 Cabernet Sauvignon samples showed a clear discrimination on PC1 between RVL samples and those from LC, CV, CWA, and WBY (Figure 59). The main variables contributing to the discrimination was deformability, total phenolics (Tot' phen'), crushed seed astringency (SD_Astrin' crushed), seed tannic intensity (SD_Tannic intensity), seed bitterness (SD Bitterness), anthocyanins, tropical pulp flavor (P_Tropical), vegetal green skin flavor (SK_Veg' green), chroma, TA, as well as skin disintegration (SK_Disint'). The second PC separated RVL14, McV2, BV2, CWA2, and WBY from the RVL10, RVL7, CV1 samples. The following variables contributed to the discrimination of the samples; pedicel removability (Ph_Ped' rem'), detachment of pulp from skin (P_Detach'), berry firmness (Ph_Firm), juiciness (P_Juiciness), dried fruit flavor (P_Dried Fruit), berry color (Ph_Color), and Brix. Least discriminating samples were RVL12, McV1, BV1, EV2, LC1, and LC2.



Figure 59. PCA biplot of scores (black) and loadings (red) of 2014 Cabernet Sauvignon BSA results. Supplementary variables are plotted in addition (blue).



Figure 60. PCA biplot of scores (black) and loadings (red) of 2015 Cabernet Sauvignon BSA results. Supplementary variables are plotted in addition (blue).

The BSA of the 2015 Cabernet Sauvignon samples allowed them to be discriminated based largely on region, similar to 2014 vintage. The CV and McV samples separated from CWA, BV, and EV along PC 1 (Figure 60). Attributes that resulted in sample separation were juiciness (P-Juiciness), skin disintegration (SK_Disint'), TA, skin dark berry and dried fruit flavors (SK_Dark Berry, SK_Dried fruit, P_Dried Fruit) and Brix. Further regional discrimination was detected on PC2 for the RVL and to a lesser extent the WBY samples. Attributes that led to the discrimination of these samples were pedicel removal (Ph_Ped' rem'), berry firmness (Ph_Firm), Chroma, and Anthocyanins. Eight attributes belonging to pulp, skins, and seeds discriminated specifically CWA2 (Figure 60).

In all cases, the supplementary variables of basic chemistry measures matched with the sensory attributes. The high TA values correlated well with pulp acidity (as did a negative correlation with pH), sweetness of pulp with TSS, and deformability correlated negatively with berry firmness when measured in the Cabernet Sauvignon samples.

7.2 Cabernet Sauvignon Wine Analyses

7.2.1 BASIC WINE CHEMISTRY

The wines were produced from the 50 kg parcels of grapes by the WIC Winemaking Service on the Waite Campus in Adelaide. A controlled methodology was used to reduce the variables in the study to those

concerning grape composition and not wine-making practices. Each wine was allowed to undergo malo-lactic fermentation to assist the sensory studies. The usual array of wine chemistry measurements were made on the wines. Glucose/fructose and acetic acid were analysed by enzymatic method, alcohol analysis was performed by NIR, and pH and TA were obtained by auto-titrator. All of these measures were completed by AWRI Commercial Services.

In 2013, the pH of the wines ranged from 3.44 to 3.72 and these were adjusted before fermentation and after bottling (Table 17). TA was lowest in the EV2 sample at 4.6 g/L and highest in CWA3 (5.8 g/L). The targeted range of alcohols was 13-15%, and most fell into this range. The lowest percent alcohols were observed in the EV wines, whereas the RVL9 wines was 15.7% alcohol. The fermentations all continued to dryness as indicated by the low glucose and fructose measures, and malo-lactic fermentation was successfully completed in all of the 2013 samples (Table 17). Acetic acid measures were all below 500 mg/L, except RVL9 which contained 630 mg/L.

Table 17. Wine chemistry at bottling of 2013 Cabernet Sauvignon. TA= titratable acidity, ALC=alcohol, GLU + FRU= glucose and fructose residual sugar.

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
BV1	3.62	5.1	14.4	0.6	<0.05	0.28
BV2	3.66	4.8	12.9	0.5	<0.05	<0.1
CV1	3.63	4.9	13.5	0.6	<0.05	0.28
CV2	3.52	5.2	13.7	0.6	<0.05	0.2
CWA1	3.66	5.0	14.4	0.7	<0.05	0.37
CWA2	3.68	4.9	14.6	0.7	<0.05	0.38
CWA3	3.66	5.8	14.7	0.4	<0.05	0.43
CWA4	3.59	5.1	13.7	0.5	<0.05	0.17
EV1	3.59	4.8	12.7	< 0.3	0.06	0.23
EV2	3.66	4.6	12.0	0.4	0.06	0.19
LC1	3.63	5.3	14.2	0.2	<0.05	0.16
LC2	3.67	5.3	15.3	0.7	<0.05	0.3
McV1	3.60	5.2	14.9	0.5	<0.05	0.45
McV2	3.68	5.0	15.0	0.5	<0.05	0.44
RVL1	3.66	5.5	14.7	0.32	<0.05	0.2
RVL2	3.59	5.5	13.9	0.28	<0.05	< 0.1
RVL3	3.59	5.0	14.3	0.5	<0.05	0.19
RVL4	3.65	5.3	14.9	0.3	0.05	0.5
RVL5	3.88	5.5	14.0	0.3	<0.05	< 0.1
RVL6	3.68	5.0	14.6	0.3	0.08	0.31

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
RVL7	3.62	4.7	13.2	0.8	0.05	<0.1
RVL8	3.65	5.4	14.3	0.6	0.06	0.21
RVL9	3.69	5.6	15.7	0.3	<0.05	0.63
WBY1	3.58	4.9	14.5	0.8	<0.05	< 0.1
WBY2	3.64	4.9	14.6	0.5	<0.05	0.34

The pH values of the 2014 Cabernet Sauvignon wines were generally lower than those in 2013 and they varied from 3.13 (RVL11) to 3.56 (CWA1). TA's ranged from 5.9 g/L in CWA1, CWA5 and RVL13 to 8.1 g/L in RVL3 (Table 18). Again most of the wines fell into the targeted range (13-15%), with the lowest being 12.0% (CWA5) and highest 14.9% (RVL12). The wines were all dry and had successfully completed malo-lactic fermentation (Table 18). The maximum acetic acid concentration was measured in the McV2 sample and at 420 mg/L it should have little impact on wine sensory attributes.

Table 18. Wine chemistry of 2014 Cabernet Sauvignon. TA= titratable acidity, ALC=alcohol, GLU + FRU= glucose and fructose residual sugar.

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
BV1	3.27	6.7	13.2	0.5	<0.05	< 0.16
BV2	3.41	6.0	13.3	0.4	<0.05	< 0.16
CV1	3.33	6.1	13.4	< 0.3	<0.05	< 0.16
CV2	3.39	6.1	13.7	< 0.3	<0.05	0.23
CWA1	3.56	5.9	13.6	0.1	<0.05	0.28
CWA2	3.46	6.2	13.2	< 0.1	<0.05	0.24
CWA3	3.40	6.5	13.1	< 0.1	<0.05	0.24
CWA5	3.38	5.9	12.0	<0.1	<0.05	0.25
EV1	3.40	6.4	14.6	0.5	<0.05	< 0.16
EV2	3.41	6.5	13.9	0.1	<0.05	0.26
LC1	3.35	6.3	12.8	<0.1	<0.05	0.19
LC2	3.52	6.0	13.1	< 0.1	<0.05	0.20
McV1	3.45	7.0	14.3	0.3	<0.05	0.38
McV2	3.54	6.7	14.6	<0.3	<0.05	0.42
RVL3	3.17	8.1	13.9	0.5	<0.05	0.39
RVL4	3.46	6.3	14.0	0.2	<0.05	0.24
RVL6	3.34	6.2	13.2	< 0.1	<0.05	0.27

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
RVL7	3.45	6.7	14.4	<0.3	<0.05	0.3
RVL10	3.44	6.0	13.5	< 0.1	<0.05	0.27
RVL11	3.13	7.1	13.8	0.4	<0.05	0.41
RVL12	3.55	6.3	14.9	0.3	<0.05	0.39
RVL13	3.43	5.9	13.4	<0.1	<0.05	0.24
RVL14	3.34	6.8	14.2	0.5	<0.05	0.37
WBY1	3.43	6.6	14.3	0.1	<0.05	0.25
WBY2	3.53	6.5	14.1	<0.1	<0.05	0.35

The pH values of the 2015 Cabernet Sauvignon wines were the highest of the three vintages. The CWA5 sample had the lowest pH of 3.41 and RVL14 had the highest pH at 4.20 (Table 19). Not surprisingly, TA's were also lower in 2015 compared to 2014, ranging from 4.3 g/L in RVL11 and RVL12 to 6.0 g/L in CV1. While most of the wines fell into the targeted alcohol range (13-15%), some were higher with the CV wines being over 16% and the McV wines above 15.5%. Again the wines were all dry and had successfully completed malo-lactic fermentation (Table 19). Acetic acid measures were all relatively low, with the maximum amount of 410 mg/L measured in CWA2, RVL4 and RVL12.

Table 19. Wine chemistry of 2015 Cabernet Sauvignon. TA= titratable acidity, ALC=alcohol, GLU + FRU= glucose and fructose residual sugar.

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
BV1	4.01	4.4	14.1	0.1	<0.05	0.21
BV2	3.87	4.9	14.7	0.6	<0.05	<0.25
CV1	3.62	6.0	16.3	0.4	<0.05	0.28
CV2	3.89	5.0	16.4	0.4	<0.05	0.27
CWA2	3.75	4.9	14.1	0.6	<0.05	0.41
CWA3	3.65	4.8	14.8	0.5	<0.05	0.31
CWA5	3.48	5.2	13.6	0.7	<0.05	<0.25
CWA6	3.64	5.0	14.5	0.7	<0.05	0.26
EV1	3.61	5.3	14.2	0.8	<0.05	<0.25
EV2	3.55	5.3	14.2	0.7	<0.05	<0.25
LC1	3.64	5.4	14.3	0.7	<0.05	<0.25
LC2	3.63	5.2	13.6	0.4	<0.05	0.25
McV1	3.88	5.7	15.8	0.2	<0.05	0.31

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
McV2	3.80	5.7	15.6	0.2	<0.05	0.35
RVL3	3.58	5.7	14.1	0.6	<0.05	<0.25
RVL4	3.94	5.1	13.9	<0.3	<0.05	0.41
RVL6	3.53	5.6	13.7	0.6	<0.05	<0.25
RVL7	3.81	5.2	14.4	0.6	<0.05	<0.25
RVL10	3.56	5.4	13.2	0.5	<0.05	<0.25
RVL11	4.11	4.3	12.8	<0.3	<0.05	<0.25
RVL12	4.18	4.3	13.7	0.1	<0.05	0.41
RVL13	4.09	4.7	15.2	0.1	<0.05	0.28
RVL14	4.20	5.1	15.5	0.1	<0.05	0.36
WBY1	3.68	5.1	15.1	0.6	<0.05	0.31
WBY2	3.71	4.9	14.5	0.4	<0.05	0.38

7.2.2 WINE COLOUR AND PHENOLIC MEASURES

A range of assays were performed for the measurement of colour and phenolics of Cabernet Sauvignon wines. These were supplementary to the similar measures made in the grapes and were not part of the main aspects of the project. Nevertheless, CIELab measurements, methyl cellulose precipitable tannin assays, and Somers assays were performed using methods described above and those from literature (Somers and Evans 1974, 1977). Results per vintage can be visualised as a summary with PCA plots (Figure 61, Figure 62, Figure 63). Consistent trends were seen across vintages, where RVL samples were comparatively higher in L* values (lightness), hue angles and hue values from the Somers assay, and b* values (yellowness). In contrast, the remaining samples were consistently higher in total phenolics, chroma and a* (depth of colour and redness), total tannins, colour densities, and SO_2 resistant pigments. The exact projections of samples towards these measures varied by year, indicating that their subtleties are influenced by season.

7.2.3 WINE VOLATILE PROFILES

Many flavour and aroma attributes in wine are sensed in the olfactory bulb of the consumer as volatile compounds interact with the collection of receptors at the top of the nasal cavity. As such, a measure of the volatile composition can give some insights into potential sensory characteristics of a wine. However, the interpretation of such data is confounded by the fact that different compounds have different odour thresholds and so those present in the highest amount aren't necessarily the ones that have the most influence on the flavour and aroma of the wine. There are also synergistic and antagonistic (masking) interactions among the compounds that have been observed, but these remain poorly understood. While the main focus of this study was to relate grape measures to wine sensory profiles, we also collected wine volatile data at the same time as the sensory analyses were conducted. It was thought that these data sets

may help in deciphering any relationships observed between grape composition and specific wine sensory attributes.

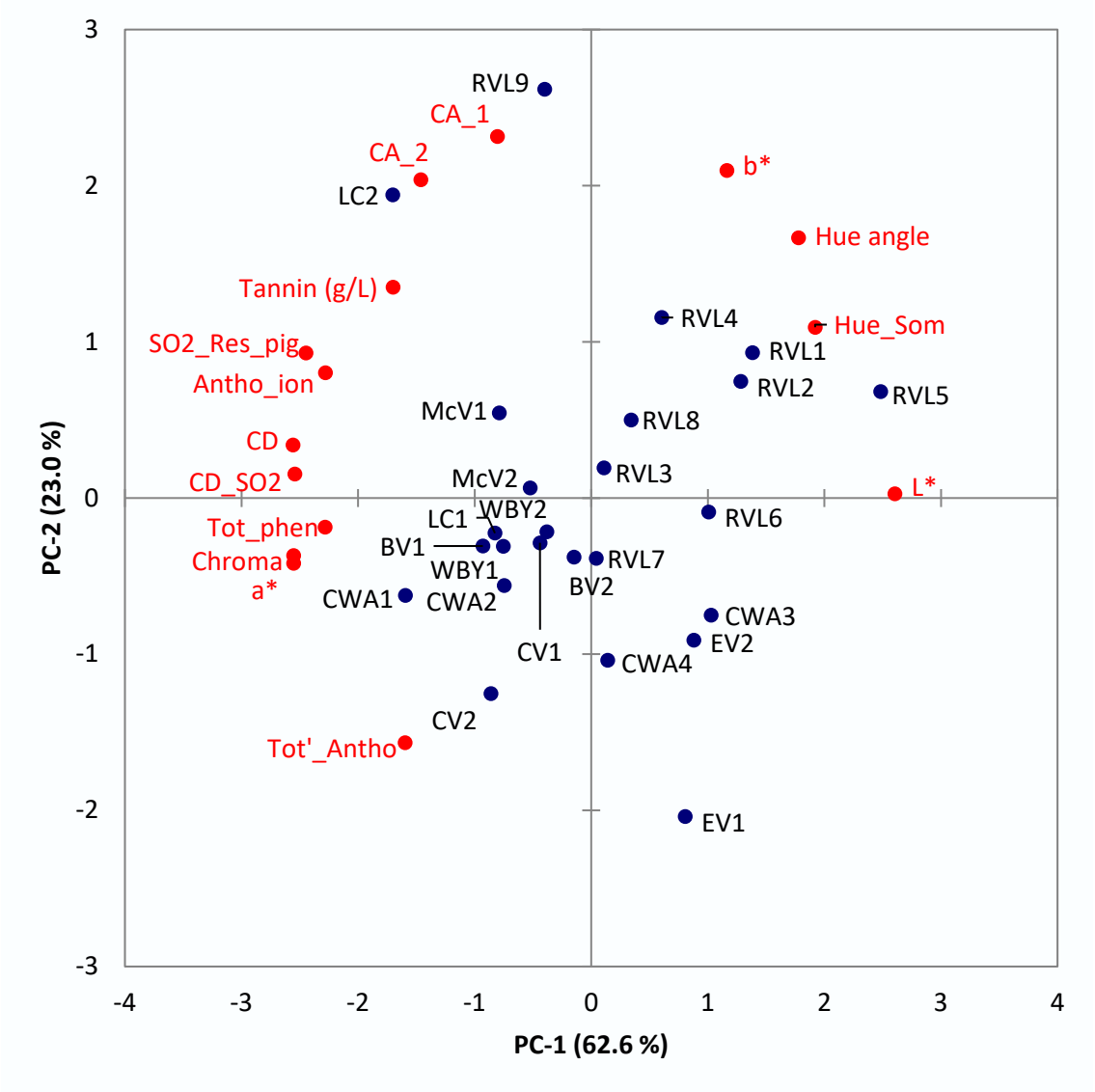


Figure 61. PCA biplot of scores (black) and loadings (red) of Cabernet Sauvignon wines from 2013 measured for CIELab colour, total tannins, and Somers assay. CA = chemical age, CD = colour density.

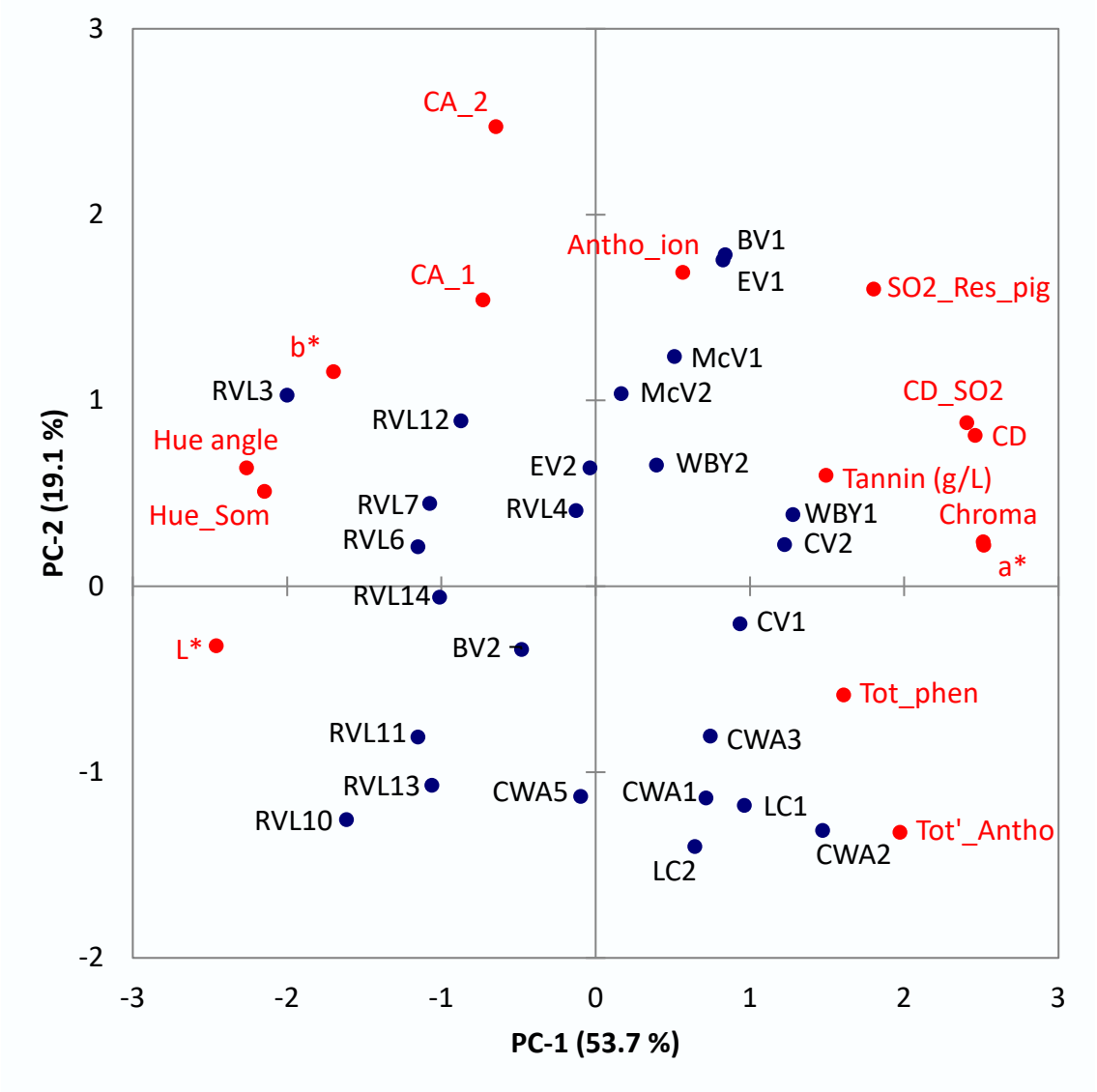


Figure 62. PCA biplot of scores (black) and loadings (red) of Cabernet Sauvignon wines from 2014 measured for CIELab colour, total tannins, and Somers assay. CA = chemical age, CD = colour density.

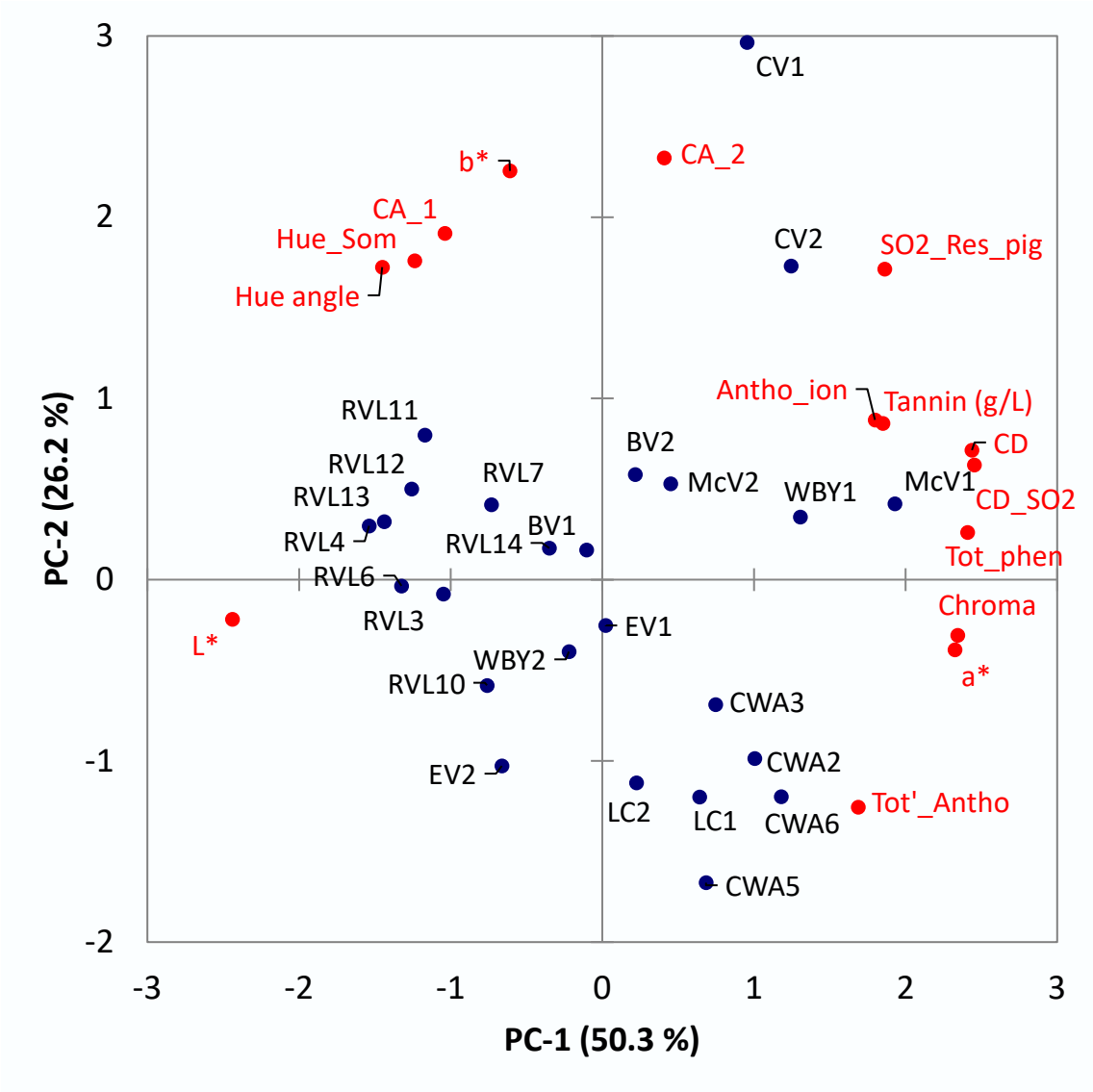


Figure 63. PCA biplot of scores (black) and loadings (red) of Cabernet Sauvignon wines from 2014 measured for CIELab colour, total tannins, and Somers assay. CA = chemical age, CD = colour density.

The wine volatile profile data is best visualised using PCA to understand how wines relate to one another with regards to their volatile composition and which compounds are most associated with different wine samples.

The 2013 wines were not modelled very well by PCA. The first two PCs only account for 43% of the variation in the data, with PC1 representing 27% and PC2, 16% (Figure 64). On the right side of PC1 most of the RVL samples, as well as those from CWA and McV were grouped and the BV, CV, EV and LC samples were located on the left of the plot. RVL7 was the only RVL sample on the left of the plot and the WBY samples were found on either side. The compounds driving the separation on the right side of the plot were 4-hydroxybutanoic acid (88), 2-phenylethyl alcohol (139), isoamyl alcohol (26) and ethyl 2-methyl butanoate (11). The esters isoamyl octanoate (97), isobutyl octanoate (79), isoamyl acetate (14), 2-phenylethyl acetate (128) and hexyl acetate (32) were collocated on the left of the plot along with hexanoic acid (132). It is interesting that isoamyl alcohol and phenylethyl alcohol were found on opposite sides of the PCA plot from their corresponding acetate esters, suggesting that the samples on the left had a greater capacity for acetate ester production from these alcohols than those on the right. The vertical separation was driven by β-citronellol (120) and 1-hexanol (46) towards the bottom of the plot and ethyl acetate (1), ethyl isobutyrate (3) and ethyl isovalerate (12) near the top (Figure 64).

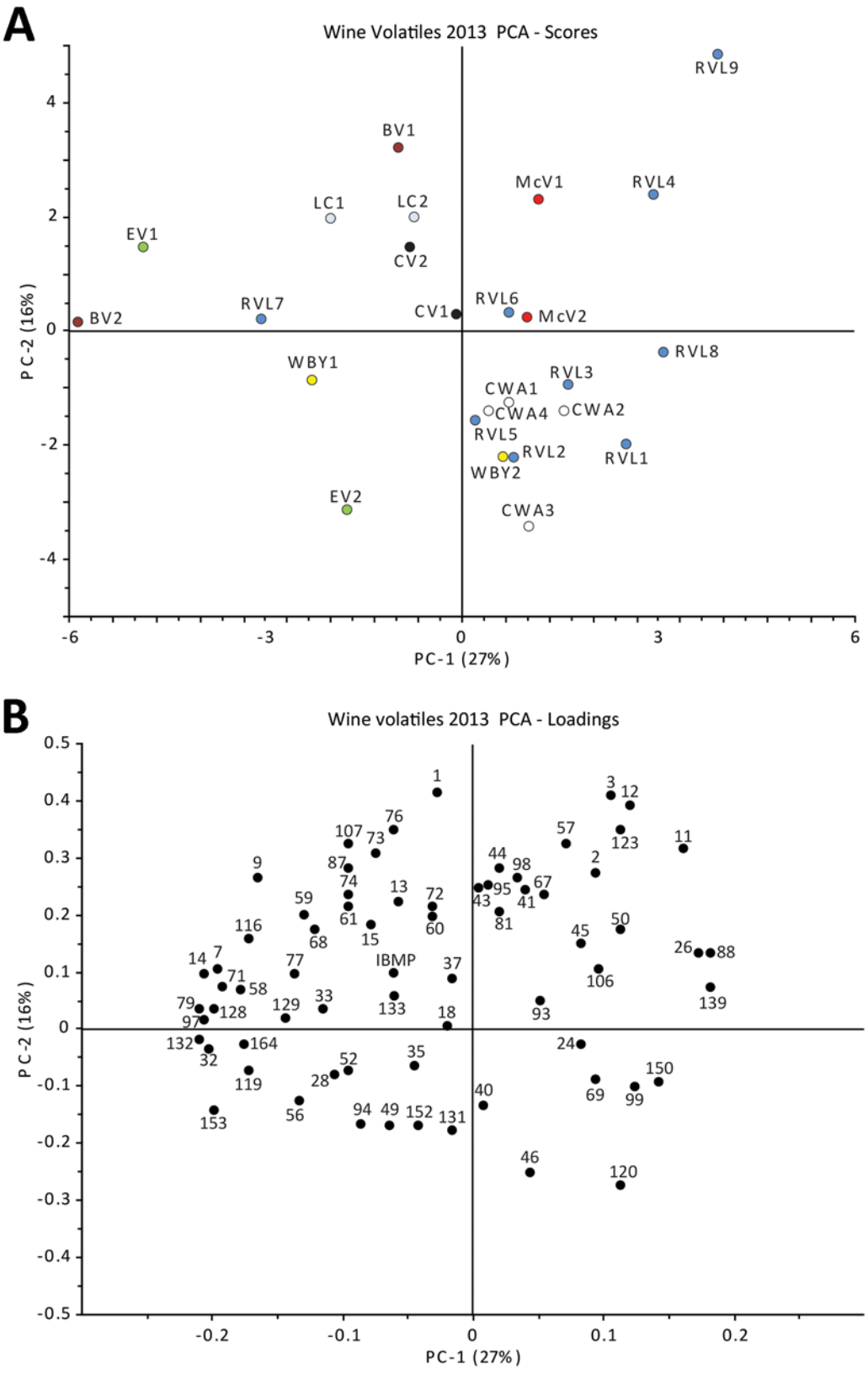


Figure 64. PCA scores (A) and loadings (B) plots of 25 different 2013 Cabernet Sauvignon wines from eight regions across South Australia based on the volatile profiles. The denotations of the wine volatile compounds are listed in Table 9.

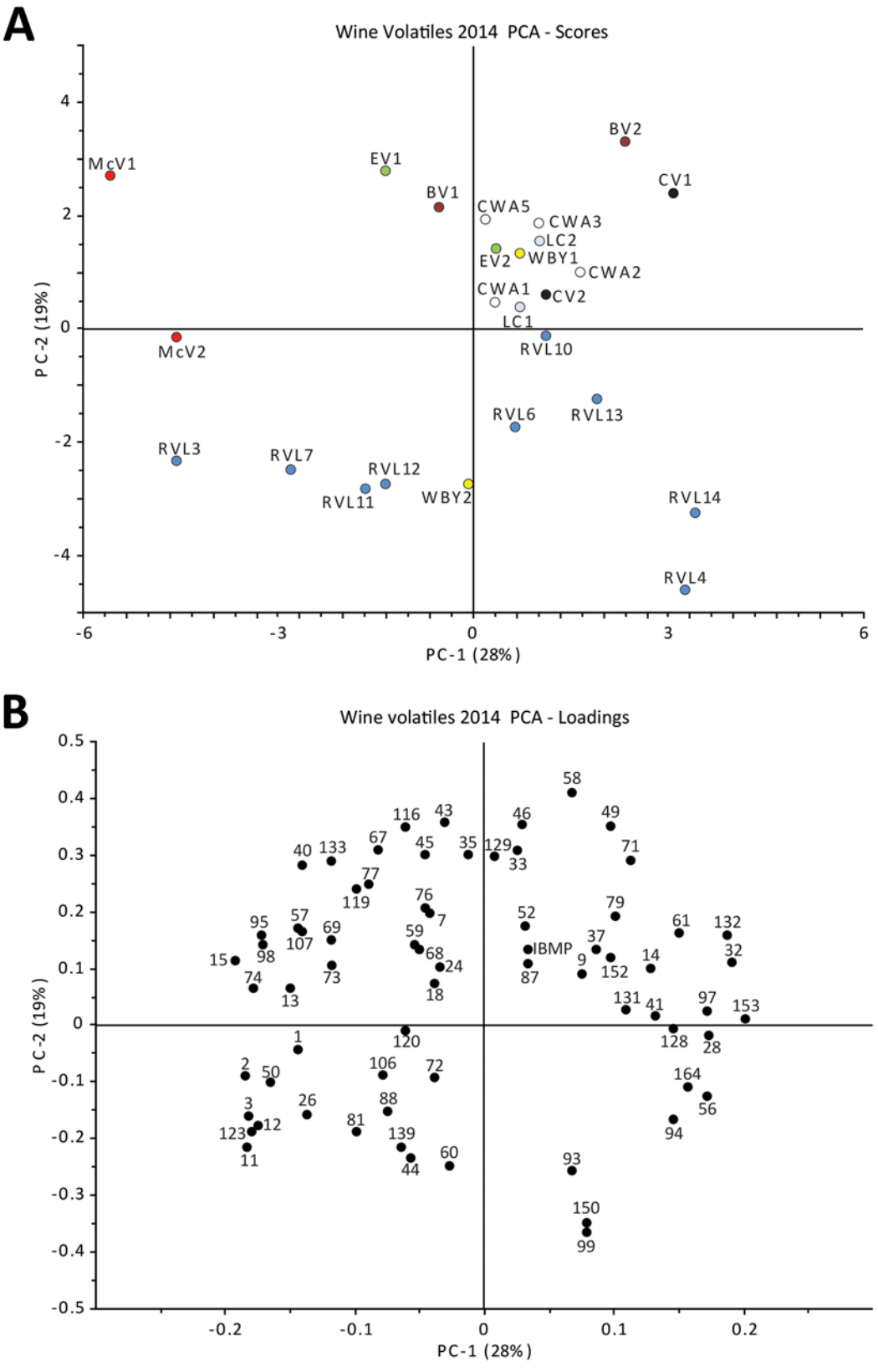


Figure 65. PCA scores (A) and loadings (B) plots of 25 different 2014 Cabernet Sauvignon wines from eight regions across South Australia based on the volatile profiles. The denotations of the wine volatile compounds are listed in Table 9.

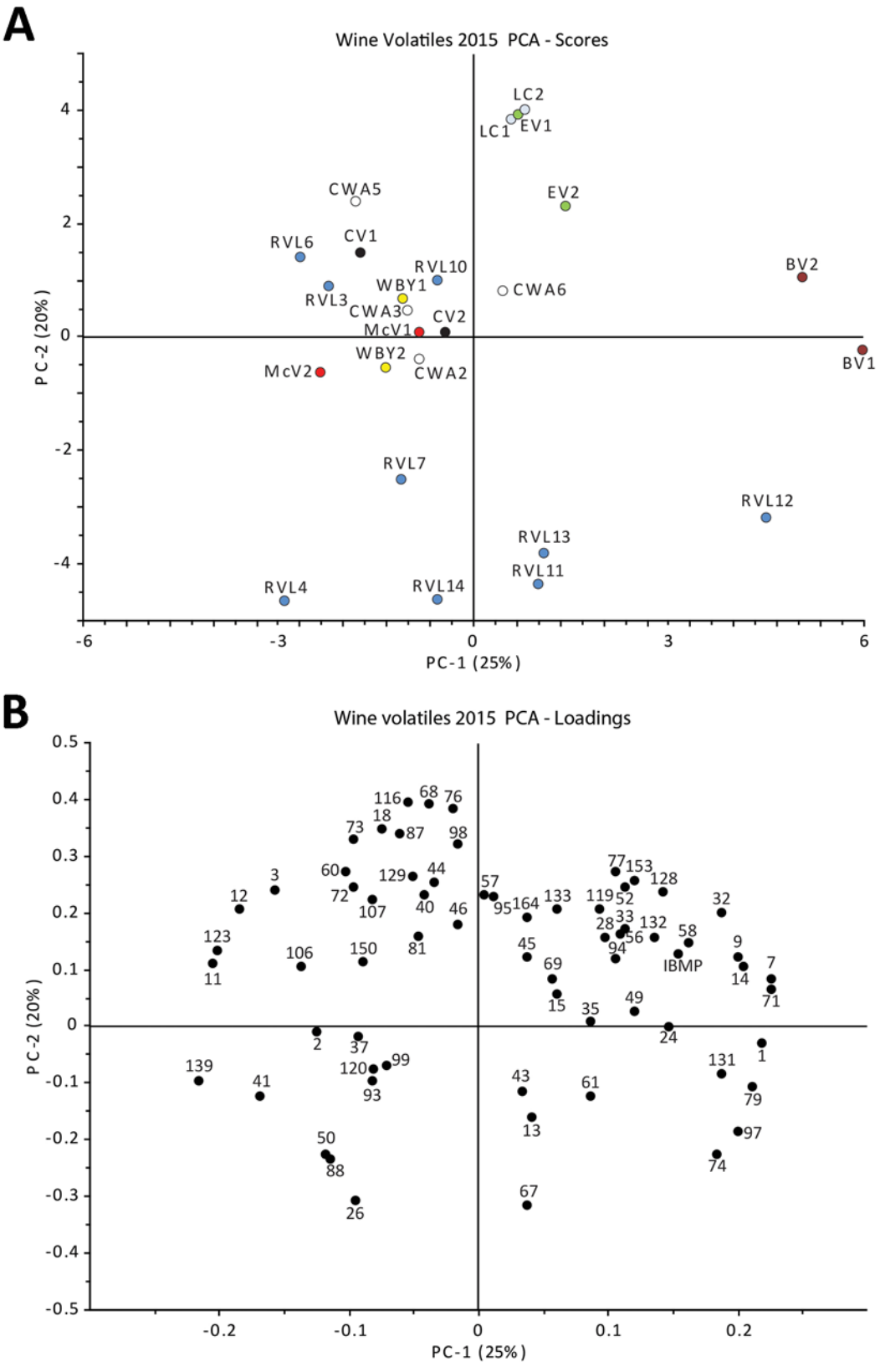


Figure 66. PCA scores (A) and loadings (B) plots of 25 different 2015 Cabernet Sauvignon wines from eight regions across South Australia based on the volatile profiles. The denotations of the wine volatile compounds are listed in Table 9.

The wine volatile profiles were again not modelled well by the PCA in 2014. The first PC described 28% of the variation and PC2 described 19% (Figure 65). The grouping of the samples was quite different to that seen in 2013. The McV samples, a subset of the RVL wines as well as BV1, EV1 and WBY2 were located on the left of the plot and the rest were found on the right side (Figure 65). The wines on the right of the plot were associated with greater concentrations of hexanoic (132) and octanoic acid (153), ethyl hexanoate (28), ethyl octanoate (56) ethyl decanote (94), hexyl acetate (32), 2-phenylethyl acetate (128), isoamyl hexanoate (61) and isoamyl octanoate (97). Those on the left had higher amounts of a number of ethyl esters namely: ethyl propanoate (2), ethyl isobutyrate (3), ethyl 2-methylbutanoate (11), ethyl isovalerate (12), ethyl valerate (15), ethyl nonanoate (74) and ethyl phenylacetate (123). PC2 discriminated the RVL samples, as well as WBY2, from the wines produced from grapes harvested from the other sites (Figure 65). The non-RVL wines at the top of the plot collocated with ethyl heptanoate (43), 1-hexanol (46), (*E*)-3-hexen-1-ol (49), 1-heptanol (58) and TDN (116), whereas those on the bottom of the plot had more β-farnesene (99) and (*E*)-nerolidol (150).

The pattern of separation of the 2015 samples was different from the other two vintages, although, again, the PCA model was describing less than 50% of the variation in the first two PCs (Figure 66). PC1 described 25% of the total variation and discriminated the BV, EV and LC wines as well as CWA6 and three RVL wines from the other sites. PC2 separated RVL4, 7, 11-14 and to some extent BV1, CWA2, McV2 and WBY2 from the wines produced from other parcels of grapes (Figure 66). Separation on the horizontal axis was driven by a number of esters on the right, namely: ethyl acetate (1), isobutyl acetate (7), ethyl butanoate (9), isoamyl acetate (14), propyl octanoate (71) and isobutyl octanoate (79), and, on the left, the samples had higher concentrations of ethyl 2-methylbutanoate (11), ethyl isovalerate (12), ethyl phenylacetate (123) and 2-phenylethyl alcohol (139). It is interesting that three of the compounds driving separation on the vertical axis were monoterpenes, with α-tepinene (18), *p*-mentha-1,4(8)-diene (68) and linalool (76) being found at the top of the plot alongside TDN (116). The samples found towards the bottom of the plot were associated with higher amounts of isoamyl alcohol (26), 3-ethoxy-1-propanol (50), 2-ethyl-1-hexanol (67), ethyl nonanoate (74) and 4-hydroxybutanoic acid (88).

Looking at the three vintages, the relationships among the volatile profiles of the wines from the different sites were quite different year to year. For example, the RVL samples clustered with those from CWA and McV in 2013 (Figure 64), whereas in 2014 they grouped by themselves at the bottom of the plot (Figure 65) and in 2015 they were split into two groups (Figure 66). What may be of more interest is the re-occurrence of certain volatiles as important drivers of the separation on either PC1 or PC2. Ethyl 2-methylbutanoate (11) and ethyl isovalerate (12) appeared to be important in all three vintages, while ethyl acetate (1), ethyl isobutyrate (3), isoamyl acetate (14), isoamyl alcohol (26), hexyl acetate (32), 1-hexanol (46), ethyl nonanoate (74, isobutyl octanoate (79), 4-hydroxybutanoic acid (88), isoamyl octanoate (97), TDN (116), ethyl phenylacetate (123), 2-phenyl acetate (128), hexanoic acid (132) and 2-phenylethyl alcohol (139) were important drivers in two vintages. The predominance of esters in this list suggests that the differences in grape composition among the parcels can have an influence on the production of these yeast–derived compounds during the fermentation process.

7.2.4 WINE DESCRIPTIVE SENSORY ANALYSIS

Descriptive sensory analysis (DA) is a method that determines the differences among samples based on the intensities of attributes that are perceived by the senses; e.g. appearance, smell, taste and texture. For the DA of the 25 wine samples from each of the 2013-15 vintages, a panel of assessors were recruited (n=10) based on their interest, availability and previous experience in DA panels. All wines were assessed within a year of bottling using the protocol outlined in section 6.4.3.

In 2013, 16 of the 28 attributes were significantly different across the wine samples ($p < 0.05$). Significant attributes detected were from the colour (2), aroma (4), taste (1), mouthfeel (4), flavour (4), and aftertaste (1) sensory categories. These attributes were analysed using PCA to visualise the entire data set for associations among attributes and wine samples, and the first two dimensions accounted for almost 74% of the explained variance (Figure 67). The observation that is most apparent is that the samples tend to

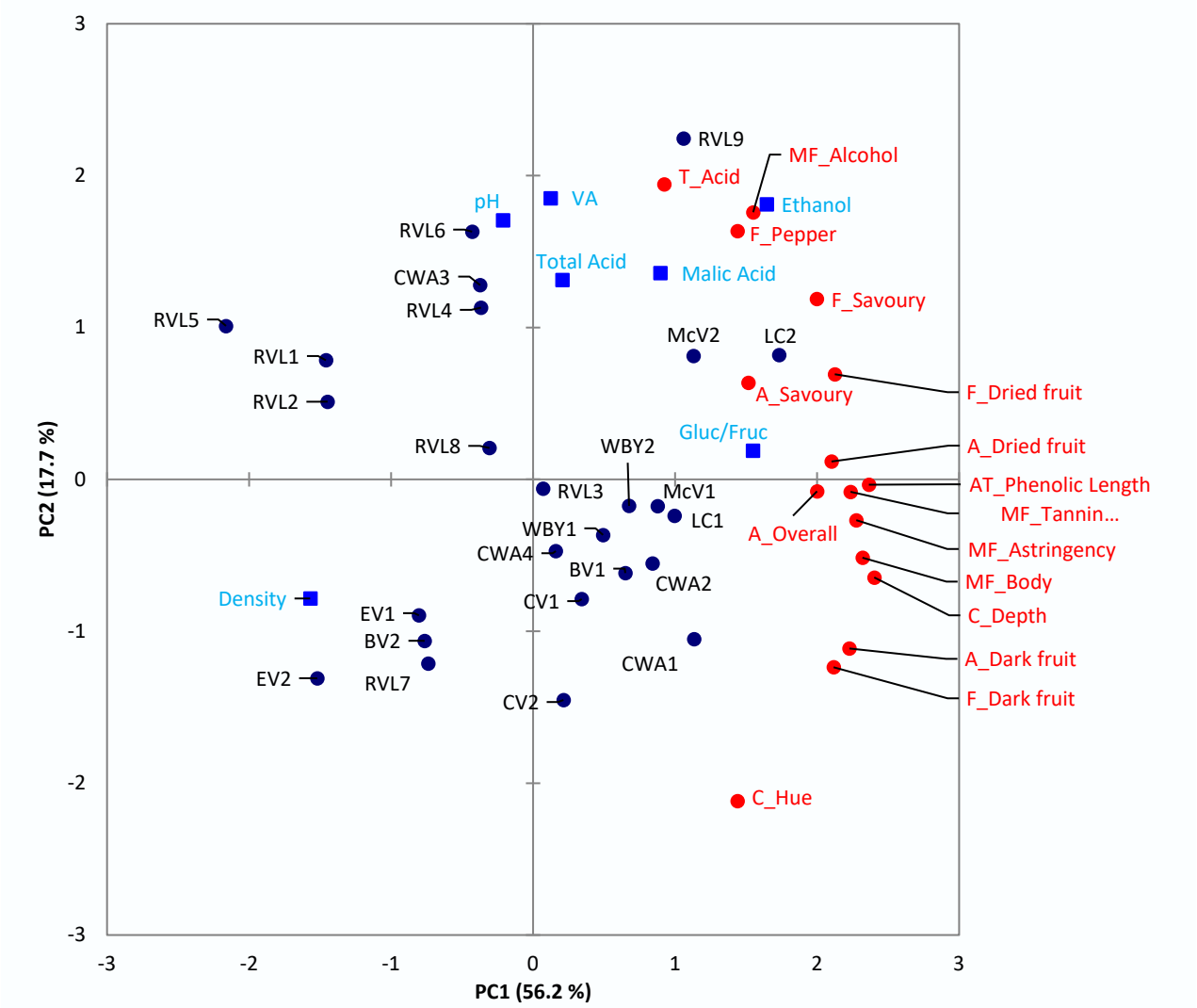


Figure 67. PCA biplot of the scores (circles) and loadings (red squares) on PC 1 vs 2 using significantly different sensory attributes of 2013 Cabernet Sauvignon wines. Supplementary chemical variables are included as blue squares.

be grouped based on the region of origin. This is another demonstration of the importance of region in determining Cabernet Sauvignon wine sensory properties. All of the attributes were highly loaded on the first PC. Differences in samples by separation on the horizontal axis explained just over half of the variation seen amongst the sensory attributes of the wine (Figure 67). This separation was driven mainly by the mouthfeel attributes of astringency, tannin quality and wine body, the aroma and palate attributes dark fruit and dried fruit, and phenolic aftertaste. Samples on the right hand side of the plot (i.e. from CWA, LC, McV and WBY) were more associated with these attributes. There was some separation of samples from the same region on this axis (e.g. BV and RVL), but in general the samples from the same region were not discriminated. In particular McV2 and LC2 were associated with savoury flavour and aroma and dried fruit, while CWA1 and 2 were associated with dark fruit aroma and flavour, colours of hue and depth. The second principal component explained approximately 17% of the variation (Figure 67). This PC allowed some discrimination amongst the RVL samples and one of the CWA samples from the others taken from that region. But, in general, those wines from the same region were found close together on the plot. The major sensory characters driving the separation on the vertical axis were alcohol intensity and acid taste, at the top of the plot, and hue colour towards the bottom of the plot (Figure 67).

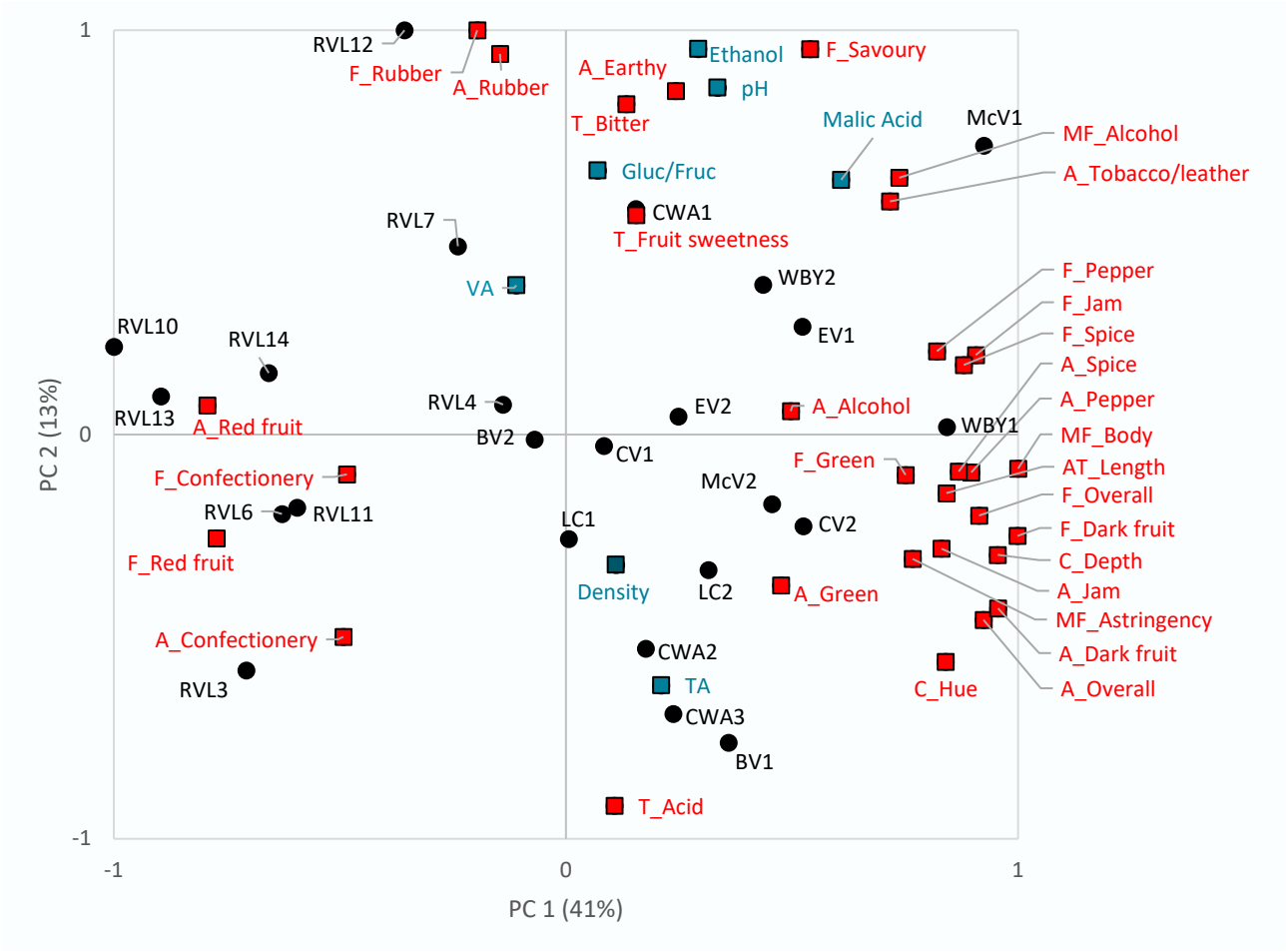


Figure 68. PCA biplot of the scores (circles) and loadings (red squares) on PC 1 vs 2 using significantly different attributes of 2014 Cabernet Sauvignon wines. Supplementary chemical variables are included as blue squares.

Thirty one of the 32 sensory attributes assessed by DA were found to be significantly different ($p < 0.05$) among the samples in 2014. These included attributes from the colour (2), aroma (12), taste (3), mouthfeel (3), flavour (10), and aftertaste (1) categories. The CWA5 sample was perceived to have a fault and was eliminated from the analysis. Again the significantly different attributes were analysed using PCA and the first two principle components account for 54% of the explained variance (Figure 68). The samples from the same region tended to be located together on the biplot. Separation on the horizontal axis explained 41% of the variation seen amongst the sensory attributes of the wine. Similar to the 2013 wines, the separation on the right side of the plot was driven mainly by many sensory attributes relating to dark fruit characteristics, colour, body and astringency, but also included spicy and pepper characteristics as well as overall flavour and aroma (Figure 68). Samples on the right hand side of the plot (i.e. from CWA, CV, EV, LC, McV and WBY) tended to be more associated with these attributes. Unlike the 2013 wines, those 2014 wines located on the left of the plot were associated with certain sensory characteristics, namely, red fruit and confectionery flavour and aroma. The RVL wines were grouped in this part of the PCA plot (Figure 68). The second principal component explained 13% of the variation and allowed some discrimination amongst the RVL samples, one of the CWA samples from the others taken from that region and separation of the McV samples. The major sensory characters driving the separation on the vertical axis were rubber flavour and aroma, savoury flavour, earthy aroma and bitter taste at the top of the plot, and acid taste and colour hue at the bottom of the plot (Figure 68). The McV1 sample was associated with higher scores for alcohol mouthfeel and tobacco/leather aroma. Interestingly, the green aroma and flavour attributes co-localised with dark and jammy fruit characteristics in this PCA, when they are often found on opposite sides of sensory profile PCAs of Cabernet Sauvignon wines (e.g. Hjelmeland et al. 2013, Robinson et al. 2011). This may reflect the distinction between "dark" fruit and "red" fruit that was made by the panel in this study which may not have been analysed in

previous sensory studies. Green and red fruit characters were found on opposite sides of the PCA in the current study (Figure 68).

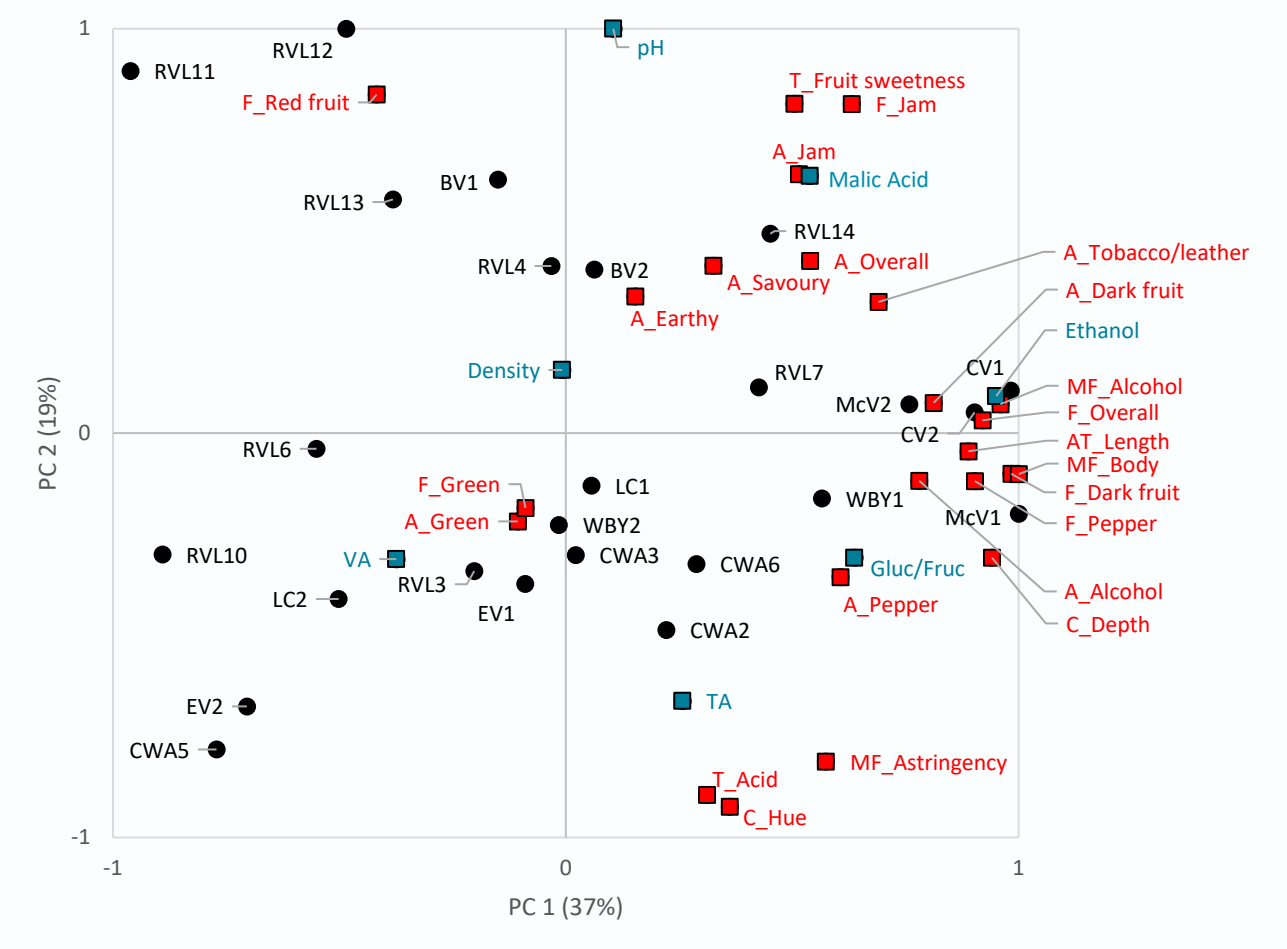


Figure 69. PCA biplot of the scores (circles) and loadings (red squares) on PC 1 vs 2 using significantly different attributes of 2015 Cabernet Sauvignon wines. Supplementary chemical variables are included as blue squares

In 2015, 23 of the 28 sensory attributes assessed by DA were found to be significantly different ($p < 0.05$) among the samples. These included attributes from the colour (2), aroma (9), taste (2), mouthfeel (3), flavour (6), and aftertaste (1) categories. The first two principle components accounted for 56% of the explained variance (Figure 69). Again the samples from the same region tended to be located together on the biplot, but the groupings in 2015 were the least obvious of the three vintages. Separation on the horizontal axis explained 37% of the variation and, like the other two vintages, the separation on the right side of the plot was driven mainly by many sensory attributes relating to dark fruit characteristics, colour, body and astringency (Figure 69). Samples on the right hand side of the plot (i.e. from CWA, CV and McV) tended to be more associated with these attributes. Red fruit flavour was again driving separation on the left of the plot, but was also influencing separation on PC2. Seven of the RVL wines were located on the left of the PCA plot, but so were samples from CWA, EV, BV and LC (Figure 69). The second principal component explained 19% of the variation and allowed some discrimination amongst the RVL samples. The major sensory characters driving the separation on the vertical axis were jam flavour and aroma, red fruit flavour and fruit sweetness at the top of the plot, and acid taste, astringency and colour hue at the bottom of the plot. The RVL14 sample was associated with higher scores for jam flavour and aroma and fruit sweetness. The green aroma and flavour attributes were not modelled well in the first 2 PCs (Figure 69).

From the Cabernet Sauvignon wine descriptive analyses, the observation that is most apparent is that the samples tended to be grouped based on the region of origin and this was consistent throughout the three vintages (Figure 67, Figure 68, Figure 69). This is another demonstration of the importance of region in determining Cabernet Sauvignon wine sensory properties. The obvious sample discrimination that was observed was the separation of RVL samples from the remaining samples. All of the attributes were highly loaded on the first principal component (PC), with the exception of red fruit and confectionery for 2014 and 2015 vintages. Conversely, samples from McV and WBY1 appeared to consistently group together and projected in the opposite direction from the RVL samples. The attributes that discriminated the McV and WBY1 samples were mainly those related to body, mouthfeel and dark fruits, but the attributes varied by vintage, which demonstrated their dependency on vintage variation.

7.2.5 WINE QUALITY ASSESSMENT USING A WINEMAKER PANEL

As one of the objectives of the project was to determine grape measures that could predict wine quality, the Cabernet Sauvignon wines from the three vintages were graded by winemakers. This was done in order to determine the perceived quality of the wines as this may provide an opportunity to find relationships with the perceived quality of the grapes. For the quality assessment winemakers (n = 6 – 9) were recruited for evaluation from within South Australia.

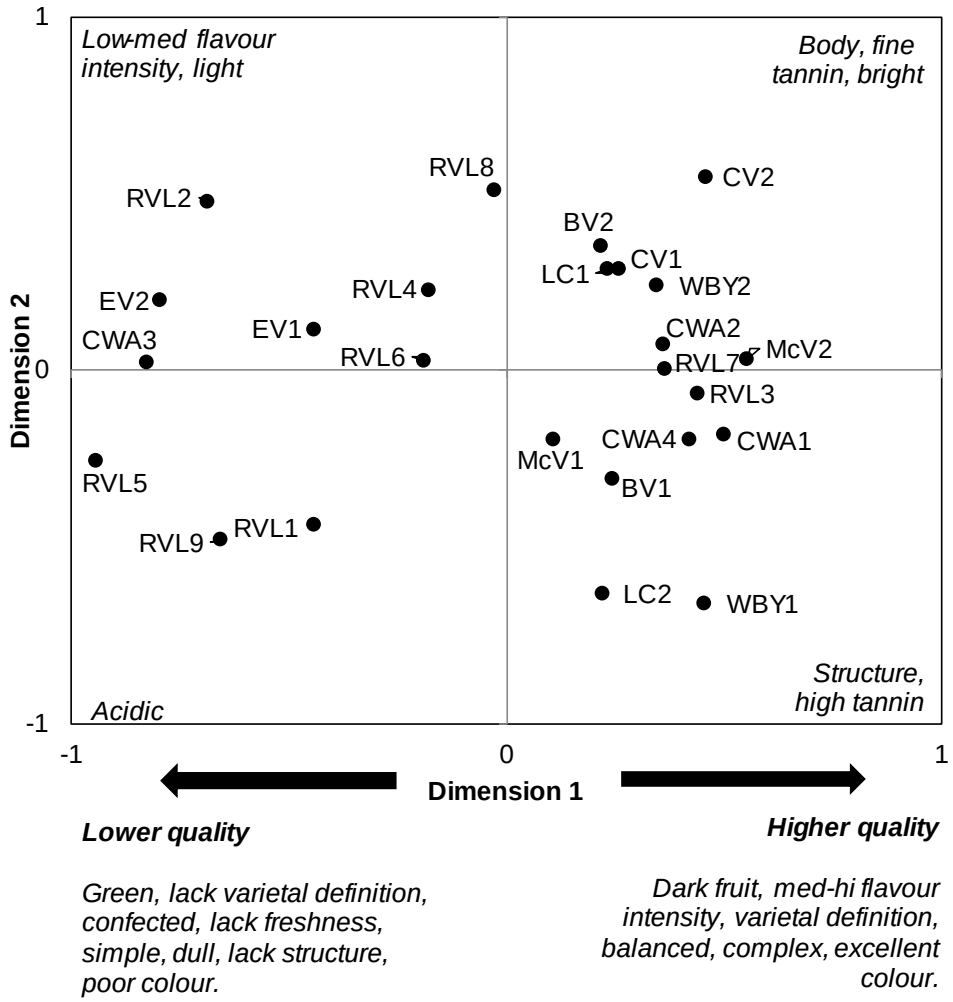


Figure 70. MDS plot of Cabernet Sauvignon from 2013. Descriptions under the plot denote for generic characters of wines associated with different quality as depicted by the arrows. Descriptions in *italic* within the plot denote for characters specific to the samples projected in the different areas of the matrix.

The data obtained from the quality assessment of the wines were analysed using multidimensional scaling (MDS). The interpretation of the data is similar to the PCA plots described earlier, only the plot shows similarity in samples based on inter-sample distances, i.e. samples close together have been frequently sorted together and are similar to each other and vice versa. In 2013, the characters that were considered to be indicative of higher quality Cabernet Sauvignon wine, according to the winemakers, were dark fruit characters, medium to high overall flavour intensity, expression of varietal definition, balance, complexity, good colour, and good structure. Samples that possessed these characters were those from BV, CV, CWA, LC, McV and WBY (Figure 70). These samples also were considered to be more associated with the DA attributes that included aromas of savoury, dried and dark fruits, flavours of dried and dark fruits, pepper, savoury, as well as hue, depth, body, astringency, coarser tannins, and long phenolic length (Figure 67). These attributes are consistent with quality characters such as the overall flavour intensity, expression of varietal definition, colour, and structure. Most of the RVL samples and both EV samples were considered by the winemakers to be of lower quality and were green, lacked varietal expression, were confected, simple, lacked structure, and had poor colour (Figure 70). While these characters were not significantly different according to the descriptive analysis, these samples were associated with lower scores for colour and body, and the dark and dried fruit sensory attributes (Figure 67).

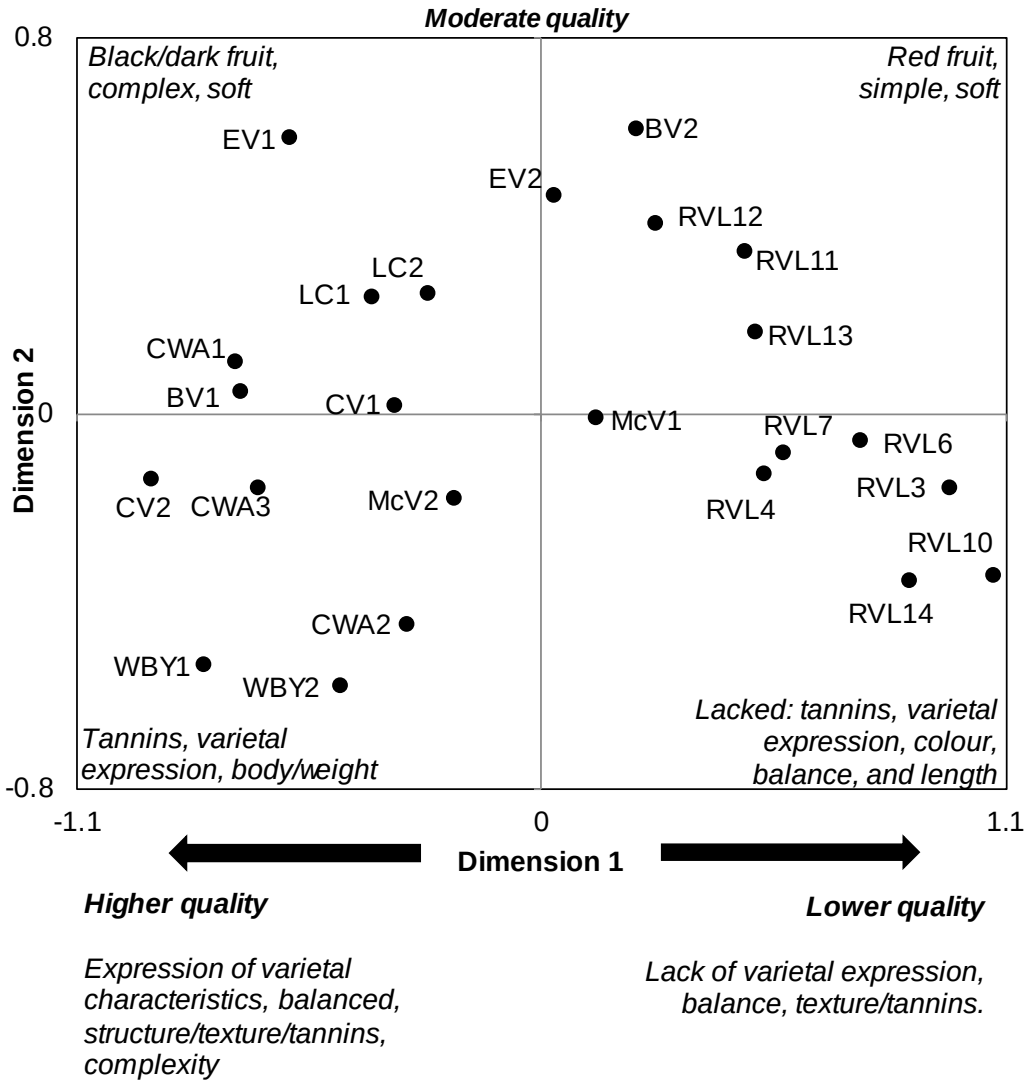


Figure 71. MDS plot of Cabernet Sauvignon from 2014. Descriptions under the plot denote for generic characters of wines associated with different quality as depicted by the arrows. Descriptions in *italic* within the plot denote for characters specific to the samples projected in the different areas of the matrix.

In 2014, the winemakers considered higher quality Cabernet Sauvignon wines to have good expression of varietal definition and balance with good structure, texture, tannins and complexity. These characters were found in the wines from CV, CWA, LC and WBY and one of the wines from BV, EV and McV (Figure 71). The DA of these wines found them to have higher scores for overall flavour and aroma, body, astringency and colour as well as dark fruit, jam and spice sensory attributes (Figure 68). Again these attributes aligned with the winemaker definitions. All of the RVL samples and the BV2, EV2 and McV1 samples were considered by the winemakers to be of lower quality as they lacked varietal expression, and had poor balance, texture and tannins (Figure 71). Most of these wines were scored higher for red fruit and confection characters in the descriptive analysis (Figure 68), which match some of the descriptions used for some of the wines regarded as being of lower quality. The McV1 wine was considered to have high alcohol mouthfeel and a tobacco/leather aroma (Figure 68) which may have affected the balance of the wine, hence it was rated as a lower quality wine by the winemakers (Figure 71).

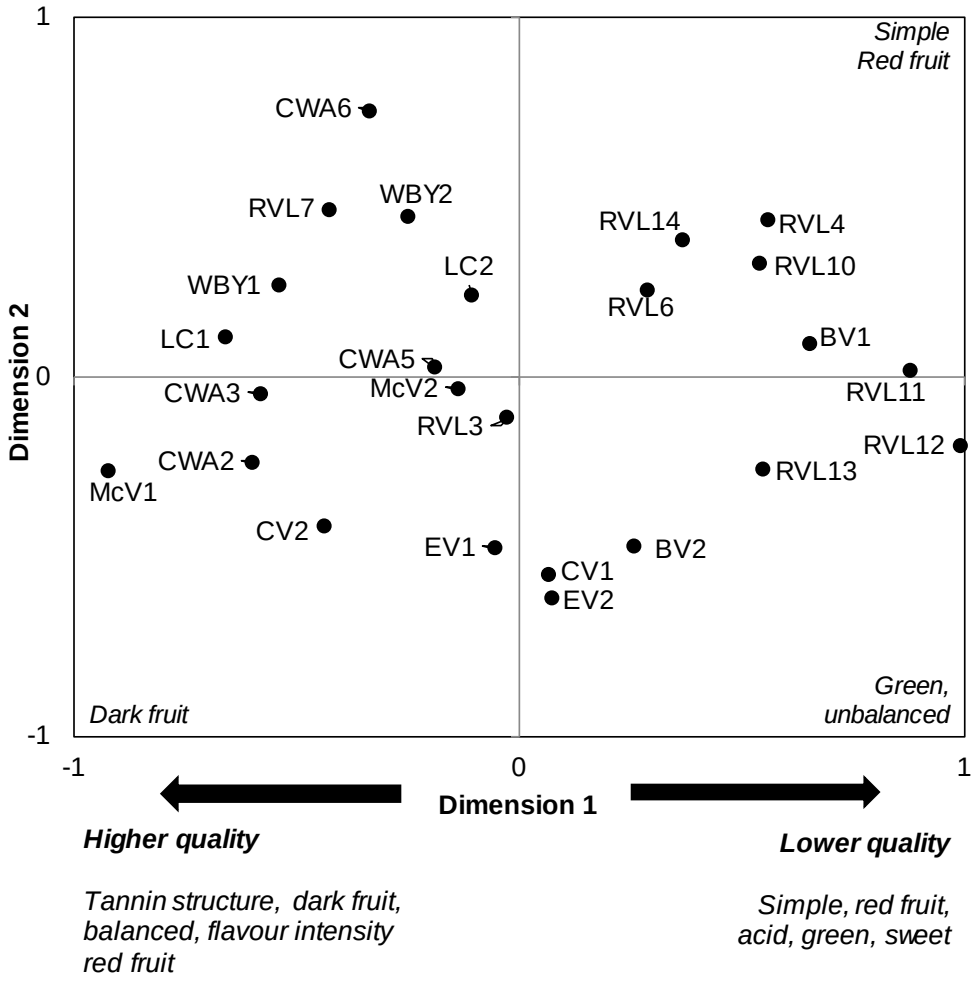


Figure 72. MDS plot of Cabernet Sauvignon from 2015. Descriptions under the plot denote for generic characters of wines associated with different quality as depicted by the arrows. Descriptions in *italic* within the plot denote for characters specific to the samples projected in the different areas of the matrix.

Similar terms were used by the winemakers in 2015 to describe higher quality wines that had been used in the previous two vintages, including balanced, good tannin structure, dark fruit characters, and high flavour intensity. These characters were seen in the 2015 wines from CWA, LC, McV and WBY and one of the CV and

EV wines (Figure 72). This was the only vintage where the Cabernet Sauvignon descriptive analysis didn't closely match the grading by the winemakers. While the McV and three of the CWA wines had high scores for colour, body and dark fruit characters, both CV wines, and only one of the WBY wines were associated with these sensory attributes (Figure 69). Strangely, red fruit was included as an indicator of both high and low quality wines (Figure 72), although wines that were green, simple, acid or sweet were also regarded by the winemakers as being of lower quality. All of the RVL wines except RVL3 and 7 were graded as lower quality as were both BV wines and CV1 and EV2. Generally, this matches the descriptive analysis of the wines except for CV1, which was collocated with some of the high quality wines, and RVL14, although this wine was somewhat of an outlier having high fruit sweetness which may have been seen to be unbalanced (Figure 69).

Overall, there were advantages in determining the qualities and analysing the data in such a way, as it showed the reasons for the qualities of wine samples. Generally, there was good discrimination in quality with clear reasoning based on sensory descriptions for the Cabernet Sauvignon wines. This stemmed from the wide range of qualities determined among the wines. High quality Cabernet Sauvignon wines were consistently described to have good tannin structure, varietal characteristics, balance, complexity, and body. In the Cabernet Sauvignon wines, there was consistent discrimination of wine quality where, at a broad level, RVL samples were always comparatively lower quality than the remaining samples. The defining characteristics for their lower quality were due to poor texture, colour, lack of varietal definition, the wines being simple, and possessing red fruit or green characters. Interestingly, RVL3 and 7 were higher in quality relative to the remaining RVL samples in the 2013 and 2015 vintages. This may have been due to these wines being seen as balanced, which is an attribute that is not easy to measure by descriptive analysis. It is interesting to note that these wines are located relatively close to the origin in the 2013 (Figure 67) and 2015 PCAs (Figure 69), which suggests they are not distinguished by having high or low sensory scores in any of the attributes tested.

7.3 Relating Cabernet Sauvignon Grape Measures to Wine Sensory Perception

7.3.1 RELATING BERRY SENSORY ANALYSIS (BSA) TO WINE DESCRIPTIVE ANALYSIS.

The BSA method was proposed as a tool to predict wine style (Winter et al. 2004), but this has not been experimentally confirmed. The only work that has tested this was in Shiraz, which found seed bitterness to be a negative contributor to savoury spice aroma in wine (Olarte Mantilla et al. 2015). The utility of BSA to predict wine sensory attributes was tested in Cabernet Sauvignon for the 2014 and 2015 vintages (Niimi et al. 2017). Data from BSA were related to wine descriptive analysis using the multivariate statistical method partial least squares (PLS). For the Cabernet Sauvignon samples, seven wine sensory attributes were found to have moderate to high correlations with BSA attributes (maximum of 79.4% validated explained variance;

Table 20; Niimi et al. 2017). Four of these attributes were consistent across vintages, namely, depth of colour, dark fruit aroma and flavour, and body mouthfeel. The predictor variables (berry sensory attributes) that modelled these wine sensory attributes, however, differed by vintage and it was difficult to conclude with certainty that he BSA attributes predicted wine sensory characters. An in depth narrative about these analyses can be found in the manuscript Niimi et al. (2017).

Table 20. PLS1 model parameters of for 2014 and 2015 vintages showing explained variances for X and Y variables for the first two components, R^2 and RMSE of both calibration and validation models (Y-variables with R^2_{Val} > 0.6 have been included). C = colour, A = aroma, F = flavour, and MF = mouthfeel.

Y-VARIABLE ATTRIBUTE	NO. OF COMPONENTS	R^2_{CAL}	R^2_{VAL}	$RMSE_{CAL}$	$RMSE_{VAL}$
		2014			
C_Hue	1	0.728	0.704	1.812	1.971
C_Depth	1	0.807	0.788	1.483	1.623
A_Overall	2	0.757	0.675	0.327	0.395
A_Dark fruit	2	0.837	0.792	0.580	0.685
F_Overall	2	0.628	0.525	0.375	0.442
F_Dark fruit	2	0.802	0.752	0.627	0.732
MF_Body	2	0.786	0.734	0.885	1.029
		2015			
C_Hue	1	0.631	0.559	0.956	1.089
C_Depth	1	0.823	0.794	0.961	1.089
A_Dark fruit	1	0.636	0.557	0.430	0.494
F_Dark fruit	1	0.671	0.632	0.482	0.531
MF_Body	1	0.715	0.678	0.347	0.384
MF_Alcohol	1	0.715	0.659	0.289	0.329
Ethanol	1	0.833	0.813	0.397	0.439

7.3.2 MODELLING OF GRAPE BERRY MEASURES TO CABERNET SAUVIGNON WINE SENSORY PROFILES

An objective of this project was to find grape measures that were potentially important in their contribution in determining wine style and subsequently quality, in both Cabernet Sauvignon and Chardonnay varieties. With so many chemical measures made in the grapes (12 different measurement types), a structured approach that would appropriately handle many data blocks was necessary. Simply joining all the data sets together to make one large table for analysis and modelling as one large data set could potentially miss important measures that may explain the differences in the wine sensory profiles of the samples. Also, it was found that linear models of sensory attributes using the grape measures in a single block of data were not robust across the vintages (data not shown). For these reasons, the data was analysed using the sequential and orthogonalised-partial least squares (SO-PLS) modelling method. This method models multiple X predictor blocks to predict a response data set (Y) by sequentially orthogonalising the X blocks with respect to each other (Naes et al. 2011). By orthgonalisation, the integrity of each X data set is maintained and the analyst can determine the relative contribution of each orthogonalised block in explaining the prediction of the Y data block. A feasibility study was conducted on one vintage (2013 Cabernet Sauvignon) and it was

found that modelling single Y variables (single sensory attributes) produced models with enhanced explained variance compared to modelling the entire sensory profile at once (Niimi et al. 2018, submitted). Hence, the analyses of the multiple vintage data sets were performed on single sensory attributes. The models were built with sequential orthogonalisation of X-blocks until addition of blocks no longer improved the explained variance of models. The nature of each data block is shown in Table 21.

Table 21. Identities of blocks and their analysis method, use for the data analysis of Cabernet Sauvignon grapes. X blocks are predictor data and Y are the response to be predicted.

DATA BLOCK	MEASUREMENT	ANALYSIS METHOD
X_{01}	Harvest measures	Weight, TSS, pH, TA
X_{02}	Amino acids	HPLC
X_{03}	Targeted volatile compounds	GC-MS
X_{04}	Non-targeted volatile compounds	GC-MS
X_{05}	Bound volatile compounds	GC-MS
X_{06}	Colour	CIELab tristimulus
X_{07}	Total tannin and phenolics	UV spectrophotometry
X_{08}	Anthocyanins	HPLC
X_{09}	Tannins	HPLC
X_{10}	Flavonols	HPLC
X_{11}	Fatty acids	GC-MS
X_{12}	Lipoxygenase pathway enzyme activities	Spectrophotometric
Y	Sensory profiles	Descriptive analysis

Variation of data by vintage overrode the variation by samples. Therefore vintage effect was removed from the data in order to model based on underlying sample differences that were primarily driven by regional variation. Predictive models for Cabernet Sauvignon were determined for each sensory attribute based on 74 samples across 3 vintages (one sample, 2014 CWA5, was removed as it was an outlier as the wine contained a fault). A total of 19 attributes were modelled using either PLS1 or SO-PLS1 (Table 22). From the 19 attributes, 8 attributes were best modelled with 1 data block and any further orthogonal addition of X blocks did not improve the validated explained variance. Eleven attributes were modelled with SO-PLS1 using two data blocks and any further addition of a third block did not improve the validated explained variance. Depth of colour showed the highest explained variance by total tannin and phenolics measures (71.9%). The poorest model in contrast was confectionary aroma, which was modelled using flavonols only (17.7%). Dark

fruit character in both aroma and flavour were most explained by total tannin and phenolics as the major predictor data block.

Table 22. Validated explained variances, RMSECV, and associated number of components used for each X block to model individual sensory attributes of Cabernet Sauvignon wines spanning 3 vintages 2013 – 2015. C_ = colour, A_ = aroma, F_ = flavour, T_ = taste, and MF_ = mouthfeel.

Y VARIABLES	BLOCK		RMSECV[A]	COMPONENTS[B]	EXPLAINED VARIANCE	
	1ST	2ND			CALIBRATED	VALIDATED
C_Hue	X06	X03	0.672	1_1	59.8	54.7
C_Depth	X07	-	0.530	2	73.2	71.9
A_Overall	X10	X01	0.700	2_1	57.0	49.6
A_Dark fruit	X07	X08	0.619	2_1	64.5	60.9
A_Red fruit	X10	-	0.835	2	32.8	25.3
A_Confectionery	X10	-	0.881	2	24.7	17.3
A_Savoury	X01	-	0.868	3	38.0	24.5
A_Green	X03	X02	0.718	2_1	57.9	41.0
A_Pepper	X06	-	0.848	1	41.7	24.7
F_Dark fruit	X07	X01	0.634	2_1	64.4	57.8
F_Red fruit	X11	-	0.870	1	33.1	21.6
F_Confectionery	X06	-	0.875	1	13.8	10.9
F_Green	X03	-	0.846	1	35.1	20.3
F_Pepper	X01	X03	0.731	2_2	47.4	41.5
F_Savoury	X01	X06	0.816	3_1	38.4	33.4
T_Bitterness	X02	-	0.875	2	30.8	21.3
MF_Body	X06	X01	0.648	1_1	62.1	57.1
MF_Astringency	X06	-	0.703	1	51.1	49.7
MF_Alcohol	X01	-	0.647	2	60.5	53.7

[A]Root mean square error of cross validation

[B]Components with more than one block are arranged as those for the first block_second block

The appropriateness of the data analysis was confirmed by the modelling of select attributes with X blocks that were expected to explain their perception, For example, total tannin and phenolics as well as CIELab measures modelled colour attributes, targeted volatiles (which includes IBMP) modelled green attributes, and harvest measures (which includes TSS) were predictors of alcohol mouthfeel (Table 22).

The data analyses showed that nine data blocks (grape chemical measures) contributed to modelling single sensory attributes. Thus three blocks were not used at all for modelling any sensory attribute and these blocks were X04 (non-targeted grape volatiles), X06 (bound volatiles), and X07 (enzymes). This suggests that these measures are not important blocks of data for the development of objective measures of quality of Cabernet Sauvignon grapes.

7.4 Chardonnay Grape Measures

To accompany the analysis of grape and wine data sets in the red wine variety Cabernet Sauvignon, the last two years of the project included parallel analyses of Chardonnay. Again the aim of this part of the project was to explore relationships between grape measures and berry sensory analysis, and sensory profiles of wines made using a controlled winemaking protocol.

7.4.1 GRAPE HARVEST MEASURES

7.4.1.1 °Brix

In 2015, Chardonnay harvest dates ranged from 23/01/2015 in the RVL to 26/02/2015 in the Adelaide Hills (ADEH; Table 2). The target total soluble solids (TSS) range was 20 -24 °Brix, although this was dependent upon the desired °Brix/°Baume sought by the companies supplying the fruit. Nevertheless, in most cases samples were close to this range with some having a mean TSS only slightly higher than 24 °Brix. The RVL7 sample was somewhat of an outlier with a °Brix of 25.8 (Table 23).

In 2016, the TSS of the grape parcels were very much lower than those harvested the year before and they fell between 19.0 and 24.7 °Brix with the majority below 22 °Brix (Table 23). The grapes were harvested between 28/01/2016 in the RVL to 18/02/2016 in the ADEH (Table 2).

Table 23. °Brix means (n=30) for each of the Chardonnay grape parcels from the two vintages. The Least Significant Difference (LSD) at the bottom of the table is the minimum difference between two means within each year necessary for a significant difference at $p < 0.05$.

VINEYARD	°BRIX	
	2015	2016
ChADEH1	22.7	20.6
ChADEH2	22.3	19.5
ChBTN1		19.2
ChBV1	21.6	20.3
ChBV2	24.4	
ChCV1	22.0	20.9
ChCV2	21.1	20.3
ChCV3	21.5	19.2
ChCWA1	20.9	21.6
ChEV1	22.3	19.1
ChEV2	20.9	
ChEV3	22.5	22.7
ChEV4		21.2
ChEV5		20.2

VINEYARD	°BRIX	
	2015	2016
ChLC1	22.6	20.7
ChMcV1	22.7	
ChMcV2	22.0	
ChPWY1		21.0
ChROBE 1	22.1	21.6
ChRVL1	21.3	
ChRVL2	21.8	
ChRVL3	24.4	20.9
ChRVL4	23.9	20.1
ChRVL5	22.4	22.1
ChRVL6	23.6	22.7
ChRVL7	25.8	19.0
ChRVL8	23.9	21.1
ChRVL9	24.8	21.3
ChRVL10		24.7
ChRVL11		21.1
ChWBY1	23.9	21.9
LSD (p<0.05)	*0.8*	*0.9*

7.4.1.2 Berry weights

In 2015, the average berry size ranged from 1.72 g in the EV1 sample to 0.94 g in the parcel from WBY (Figure 73). There were no main trends evident across the regions with regard to Chardonnay berry weights in 2015.

Berries were generally smaller in the 2016 vintage with the mean berry size being 1.08 g compared to 1.36 g in the 2015 vintage. The RVL samples tended to be bigger than those from the other regions in 2016 (Figure 74). The RVL6 and 8 fruit were the largest with mean berry weights of 1.51 g, and the smallest berries were those sampled from EV1 (0.52 g). EV1 was the vineyard with the largest fruit in 2015 and the drastic differences in berry size between the vintages probably reflects rainfall differences during the growing season as the EV1 vines were not irrigated.

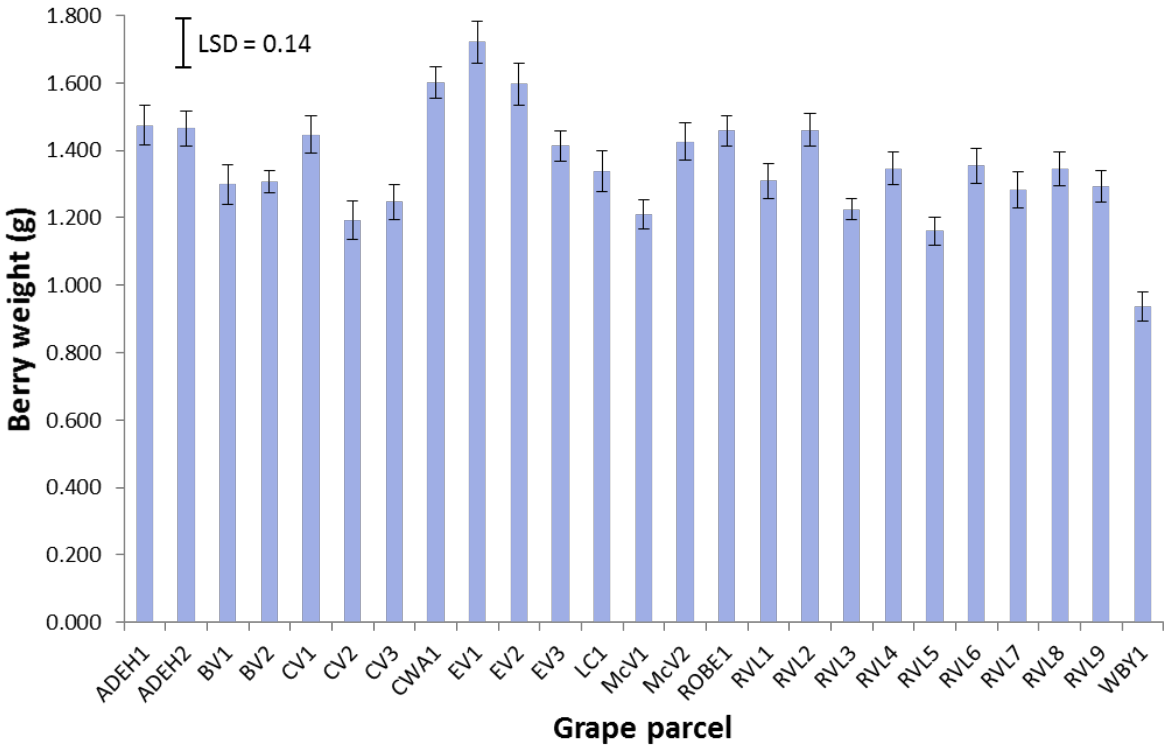


Figure 73. Average berry weight of 30 Chardonnay berries randomly sampled from each site in 2015. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

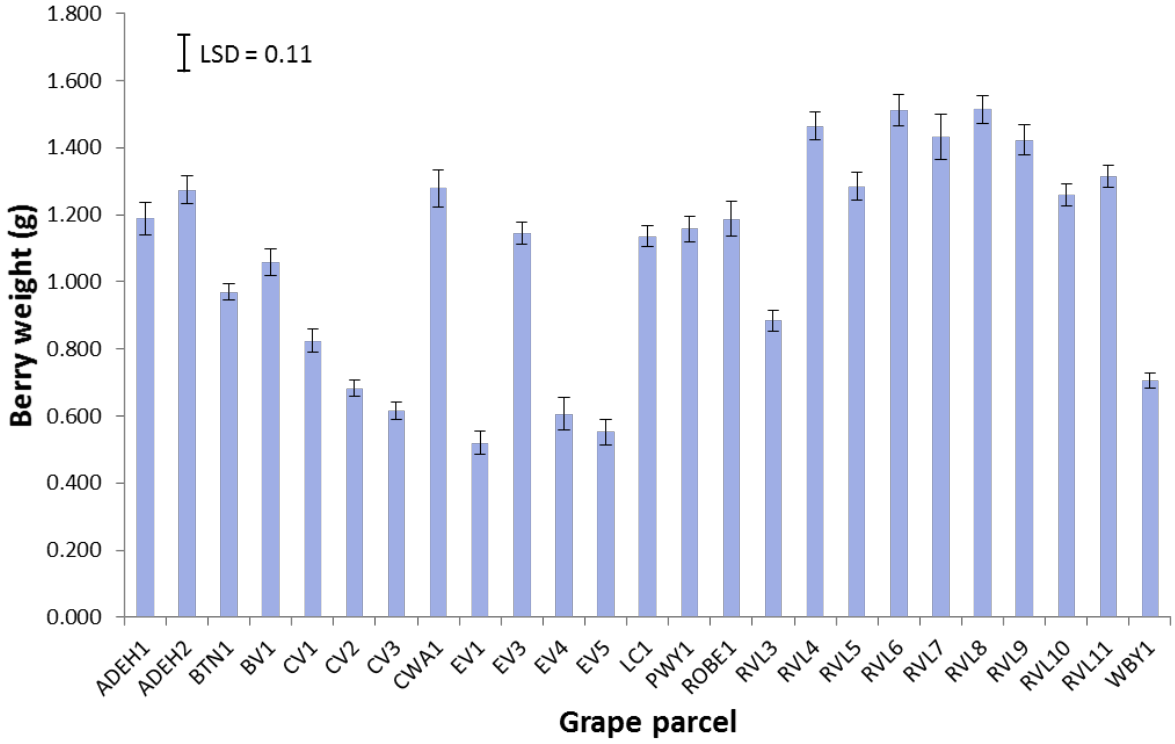


Figure 74. Average berry weight of 30 Chardonnay berries randomly sampled from each site in 2016. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

7.4.1.3 Bunch Weight

Bunch weights did not appear to be driven strongly by berry size in 2015 (cf Figure 73 and Figure 75). The bunch weights were also more variable than the berry weights in 2015. The smallest bunches were harvested from the CV3 and EV3 vineyards, with mean bunch weights of 81 g (Figure 75). The largest bunch weights were measured in the samples from RVL2 and ADEH2 (176 g and 168 g), which were well above the overall mean for 2015 of 121 g per bunch.

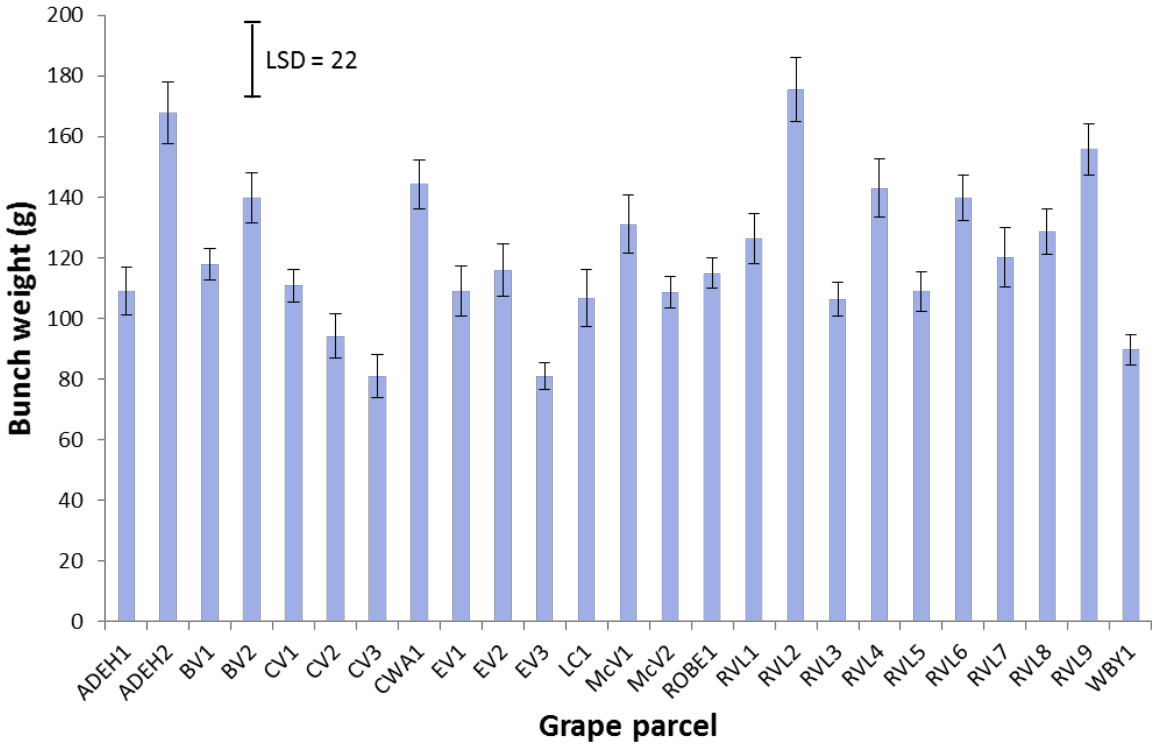


Figure 75. Average bunch weight of 25 Chardonnay bunches randomly sampled from each site in 2015. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

Bunch weights in 2016 were relatively more variable than those in 2015, and a comparison of Figure 74 and Figure 76Figure 5 suggests that berry size may have had more of an influence on bunch weights in 2015 compared to 2016. The highest bunch weights were measured in the ADEH2 and CWA1 samples, being 168 g and 163 g respectively. The lowest bunch weights were recorded in the parcels harvested from CV2 & 3 and EV1, 4 & 5, ranging from 65 g to 75 g (Figure 76). The overall mean bunch weight was remarkably similar to that in 2015, being 117 g in 2016.

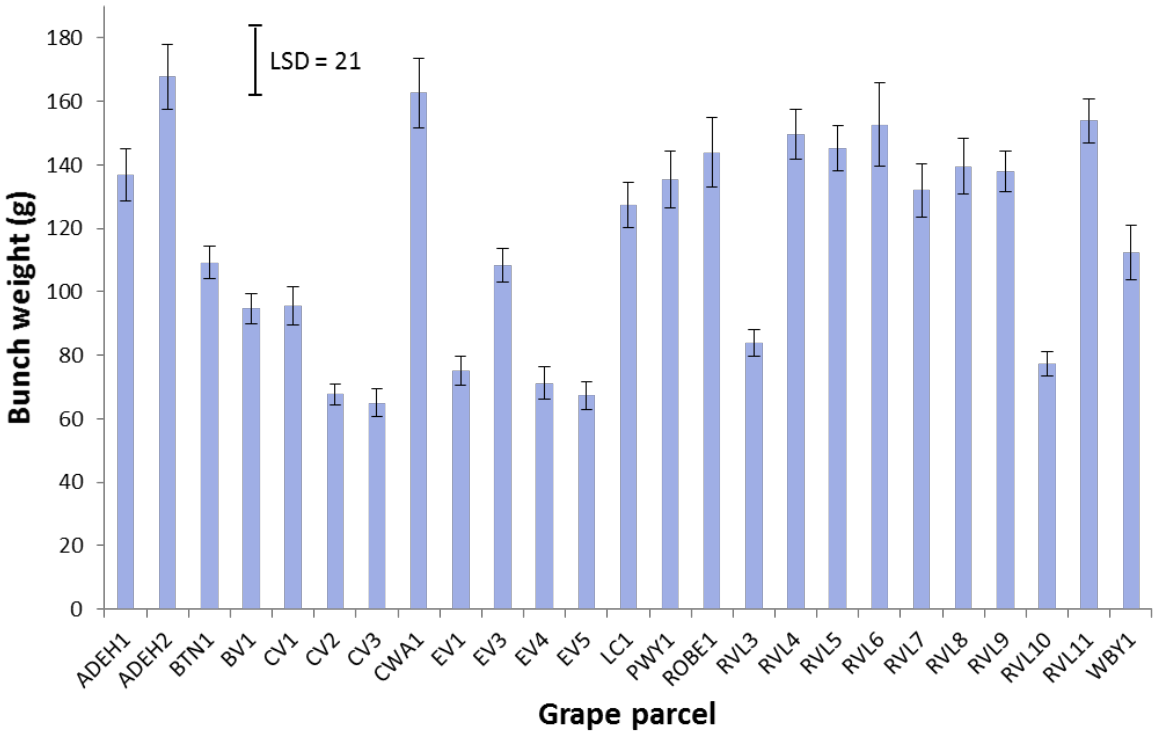


Figure 76. Average bunch weight of 25 Chardonnay bunches randomly sampled from each site in 2016. Error bar is ± standard error. The LSD represents the minimum difference between two means necessary for a significant difference at $p < 0.05$.

7.4.1.4 pH, Titrable Acidity (TA) and Yeast Assimilable Nitrogen (YAN)

Analyses of pH, TA and YAN were conducted on the fruit at receival. The results for the 2015 Chardonnay parcels are listed in the table below (Table 24). pH values for the fruit parcels ranged from 3.12 to 3.7. The pH of the fruit from RVL tended to be relatively higher in pH than those from the other regions. TA was lowest in the RVL5 sample (6.7 g/L) and was highest in the RVL1 sample at 13 g/L. YAN was variable, but all wines were supplemented to avoid stuck ferments. Nevertheless, the YAN in the fruit itself ranged from 132 to 378 mg/L. No clear trends were observed in YAN values across the sites.

Table 24. Measures of pH, TA and YAN in the Chardonnay grape parcels in 2015.

PARCEL	pH	TA (g/L)	YAN (g/L)
ChADEH1	3.47	9.9	176
ChADEH2	3.23	9.3	162
ChBV1	3.28	10.4	185
ChBV2	3.52	8.6	230
ChCV1	3.37	8.7	155
ChCV2	3.43	8.8	146
ChCV3	3.41	9.2	217
ChCWA1	3.12	12.5	206

PARCEL	pH	TA (g/L)	YAN (g/L)
ChEV1	3.34	9.5	290
ChEV2	3.17	9.2	215
ChEV3	3.22	10.0	215
ChLC1	3.42	10.9	183
ChMcV1	3.33	10.8	140
ChMcV2	3.41	9.7	132
ChROBE1	3.22	8.6	257
ChRVL1	3.31	13.0	195
ChRVL2	3.32	11.6	218
ChRVL3	3.58	8.4	249
ChRVL4	3.55	7.0	197
ChRVL5	3.55	6.7	175
ChRVL6	3.50	7.9	217
ChRVL7	3.70	8.5	378
ChRVL8	3.48	7.8	205
ChRVL9	3.61	7.4	225
ChWBY1	3.38	9.6	339

The grape parcels harvested in 2016 had pH values generally lower than those observed in 2015, which probably reflects the earlier harvest and lower TSS values for these grapes. The pH ranged from 3.08 to 3.65 (Table 25) and was higher in the RVL parcels. TA ranged from 4.8 g/L in the RVL3 parcel to 8.1 in EV1 (Table 25) and it was difficult to see any effect of region on these values. YAN was very variable in this vintage with the values ranging from 46 mg/L (RVL10) to 326 mg/L (EV5). Again no clear trends were observed among the regions for YAN, and the extremely low RVL10 sample was very much an outlier (Table 25).

Table 25. Measures of pH, TA and YAN in the Chardonnay grape parcels in 2016.

PARCEL	pH	TA (g/L)	YAN (g/L)
ChADEH1	3.11	6.4	105
ChADEH2	3.21	6.2	128
ChBTN1	3.08	7.8	126
ChBV1	3.30	6.4	175
ChCV1	3.25	6.5	150
ChCV2	3.25	6.4	190

PARCEL	pH	TA (g/L)	YAN (g/L)
ChCV3	3.29	6.5	215
ChCWA1	3.20	6.9	198
ChEV1	3.23	8.1	295
ChEV3	3.30	6.0	149
ChEV4	3.23	7.7	314
ChEV5	3.26	6.9	326
ChLC1	3.33	6.2	124
ChPWY1	3.27	6.3	187
ChROBE1	3.21	5.7	225
ChRVL3	3.62	4.8	277
ChRVL4	3.39	6.8	210
ChRVL5	3.65	5.4	251
ChRVL6	3.59	5.7	201
ChRVL7	3.44	6.7	327
ChRVL8	3.53	5.9	210
ChRVL9	3.53	6.4	241
ChRVL10	3.46	6.0	46
ChRVL11	3.47	6.2	207
ChWBY1	3.31	6.3	231

7.4.2 GRAPE CHEMICAL MEASURES

As for the Cabernet Sauvignon grapes, a range of compounds were measured in the Chardonnay fruit to investigate whether berry parameters are potential indicators of wine sensory outcomes in this white wine variety. The results for these measurements are presented below in sections based on the type of compound and methodology used to quantify them. Some of the measures conducted on the Cabernet Sauvignon grapes were not relevant in the Chardonnay fruit and so there are less blocks of data in this section. Again short discussions have been written about the results of these measures as, individually, these measurements are interesting indicators of fruit composition. This also allows for the identification of compounds that are most sensitive to vineyard variation and differences in conditions during different vintages.

Ultimately, these Chardonnay fruit measurements were compared with the data obtained from the descriptive sensory analysis of the wines. The SO-PLS chemometric data analyses were again used to determine which blocks of data were good indicators of wine sensory attributes and hence may be used to assess the wine style potential and quality of the fruit. This work is presented in later sections of this report.

7.4.2.1 Flavonols

Total flavonols were measured in deseeded berries. For the 2015 Chardonnay samples the mean flavonol concentration was 0.076 mg/g but there was significant variation between the samples with a range of 0.02 – 0.16 mg/g (Figure 77). These values were much lower than the flavonol concentrations measures in the Cabernet Sauvignon samples from the same vintage (Figure 20). The mean flavonol concentration of the RVL samples (0.04 mg/g) was below the mean of the other samples (0.10 mg/g), although the ADEH1 sample had a concentration of flavonols similar to the RVL parcels. The low amounts in the RVL sites may reflect the management of the vineyards where larger canopies may be maintained to reduce sun-burn and it is known that light exposure is a major factor in inducing flavonol production in grapes (e.g. Price et al. 1995, Haselgrove et al. 2000)

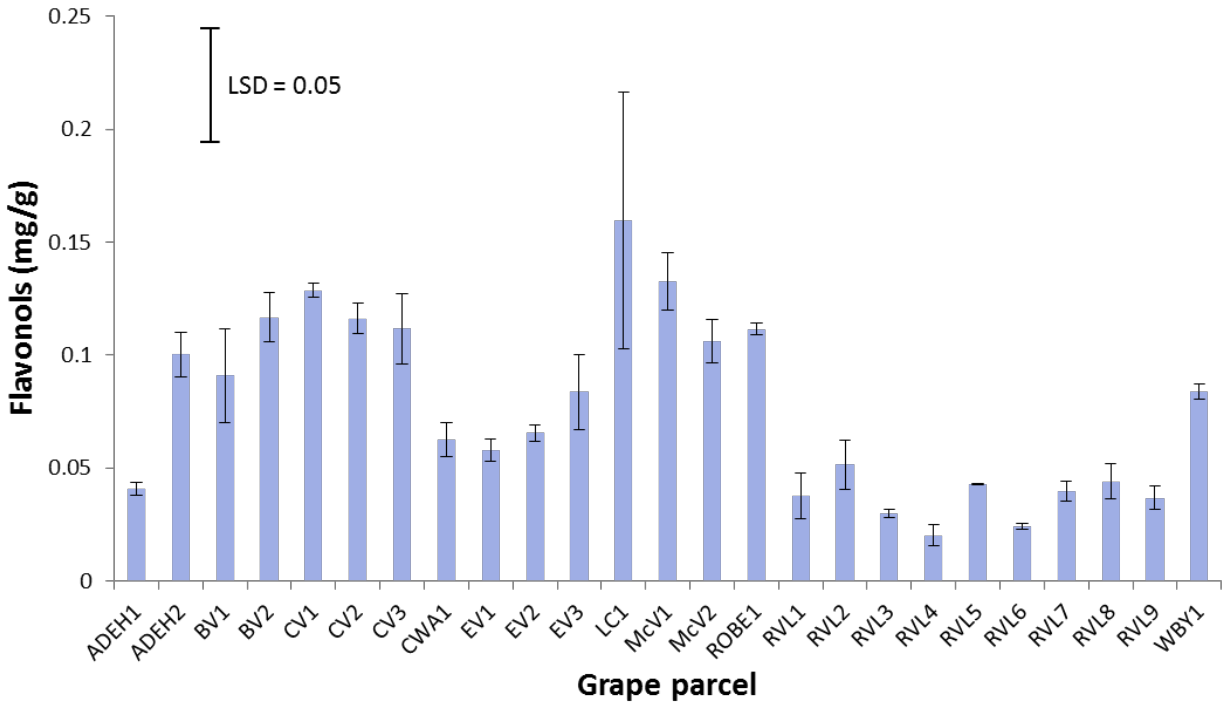


Figure 77. Total flavonols extracted from whole Chardonnay berries in 2015 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

PCA of the 2015 Chardonnay flavonol profiles showed that the total flavonol measurement was associated with higher amounts of kaempferol-3-*O*-caffeoylate and kaempferol-3-*O*-glucoside, the loadings of which were collocated on the right side of the biplot (Figure 78). Most of the variation (59%) was described in the first PC and, in general, the samples with high total flavonols were on the right hand side of the PCA and those with less flavonols were on the left side of the plot. It was interesting that the samples with lower concentrations of flavonols were associated with higher concentrations kaempferol-3-*O*-glucuronide (Figure 78), which is in direct contrast to the Cabernet Sauvignon samples where this compound collocated with the total flavonol content of the sample (e.g. Figure 16). There was some separation of the samples on the second PC, and this was driven by higher percentages of quercetin-3-*O*-glucoside in the samples located towards the top of the PCA biplot (Figure 78).

The total flavonols measured in the 2016 Chardonnay samples had similar trends to those seen in 2015 (Figure 79). Again the RVL samples had the lowest flavonol concentrations, although fruit from the RVL10 site were an outlier in that they possessed flavonol concentrations comparable to those seen in parcels of fruit from other regions. There appeared to be three groups of samples based on total flavonol concentrations: the low RVL samples, the Bordertown (BTN), BV, CWA, EV1, LC1, Padthaway (PWY) and WBY

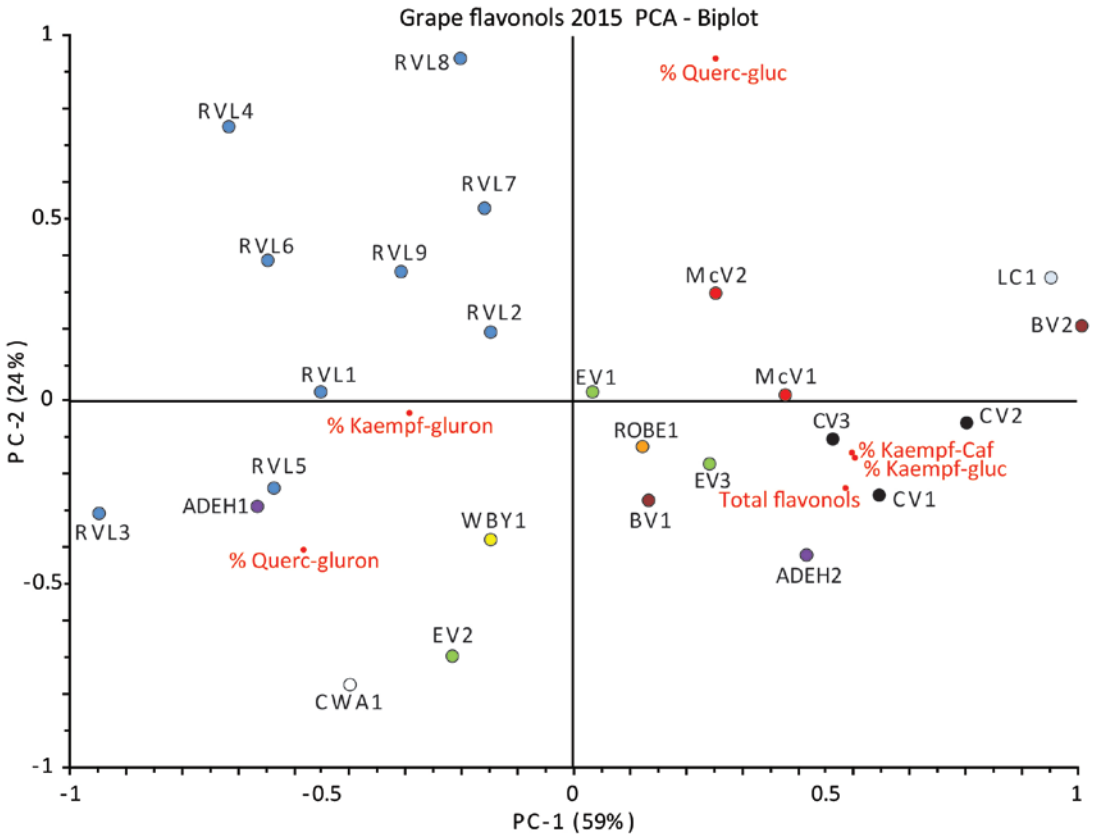


Figure 78. PCA biplot showing the scores of the 25 different 2015 Chardonnay grape samples from South Australia and the associated flavonoid profile parameter loadings in red. Kaempf-gluc = Kaempferol-3-*O*-glucoside, Kaempf-gluron = Kaempferol-3-*O*-glucuronide, Kaempf-Caf = Kaempferol-3-*O*-caffeoylate, Querc-gluc = Quercetin-3-*O*-glucoside, Querc-gluron = Quercetin-3-*O*-glucuronide.

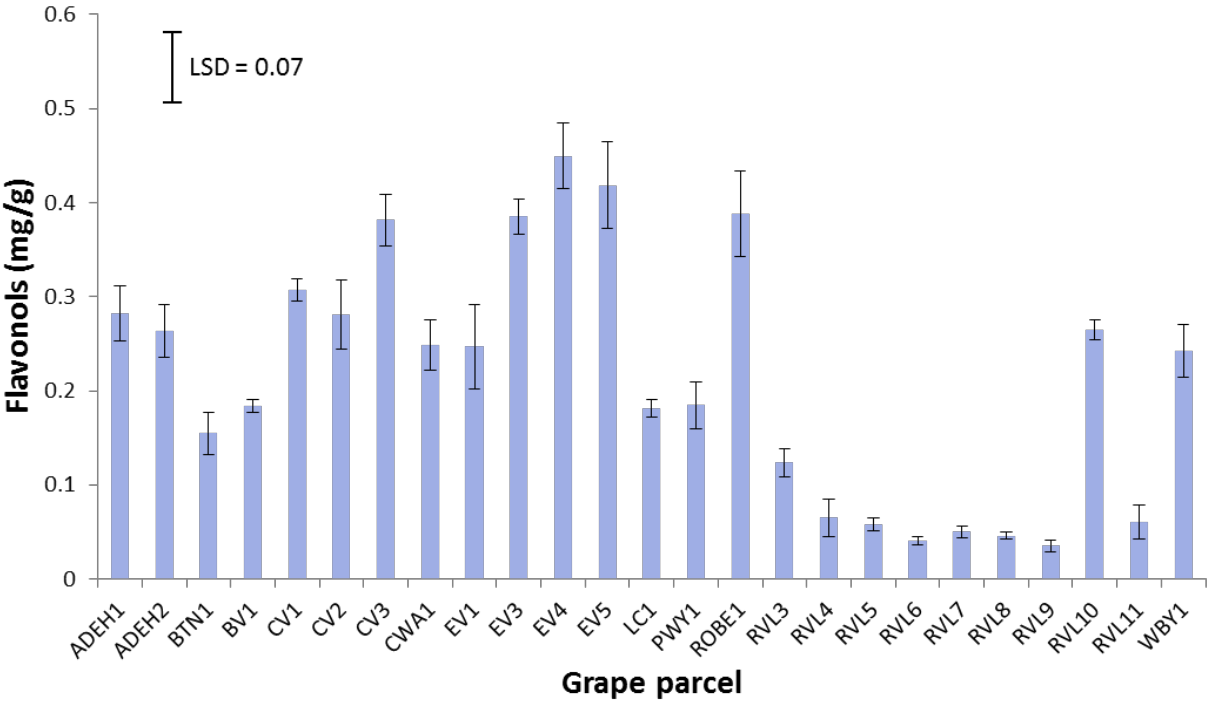


Figure 79. Total flavonols extracted from whole Chardonnay berries in 2016 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

samples with intermediate concentrations of flavonols, and the ADEH, CV, EV3-5 and ROBE sites with high flavonol concentrations (Figure 79). The mean flavonol concentration for the Chardonnay samples in 2016 was 0.21 mg/g, which was more than double the mean in 2015, and concentrations ranged from 0.04 mg/g to 0.45 mg/g.

The PCA of the flavonol profiles of the 2016 Chardonnay samples was similar to that seen in 2015. The samples on the right of the plot were those with higher concentrations of flavonols and these tended to have a higher percentage of kaempferol-3-*O*-caffeoylate and kaempferol-3-*O*-glucoside (Figure 80). Again, on the opposite side of the plot to the total flavonols was found the percentage of kaempferol-3-*O*-glucuronide. Separation on the second PC was driven positively by kaempferol-3-*O*-glucuronide and quercitin-3-*O*-glucoside and negatively by quercitin-3-*O*-glucuronide (Figure 80). Samples from the same region tended to group together, especially on the horizontal axis.

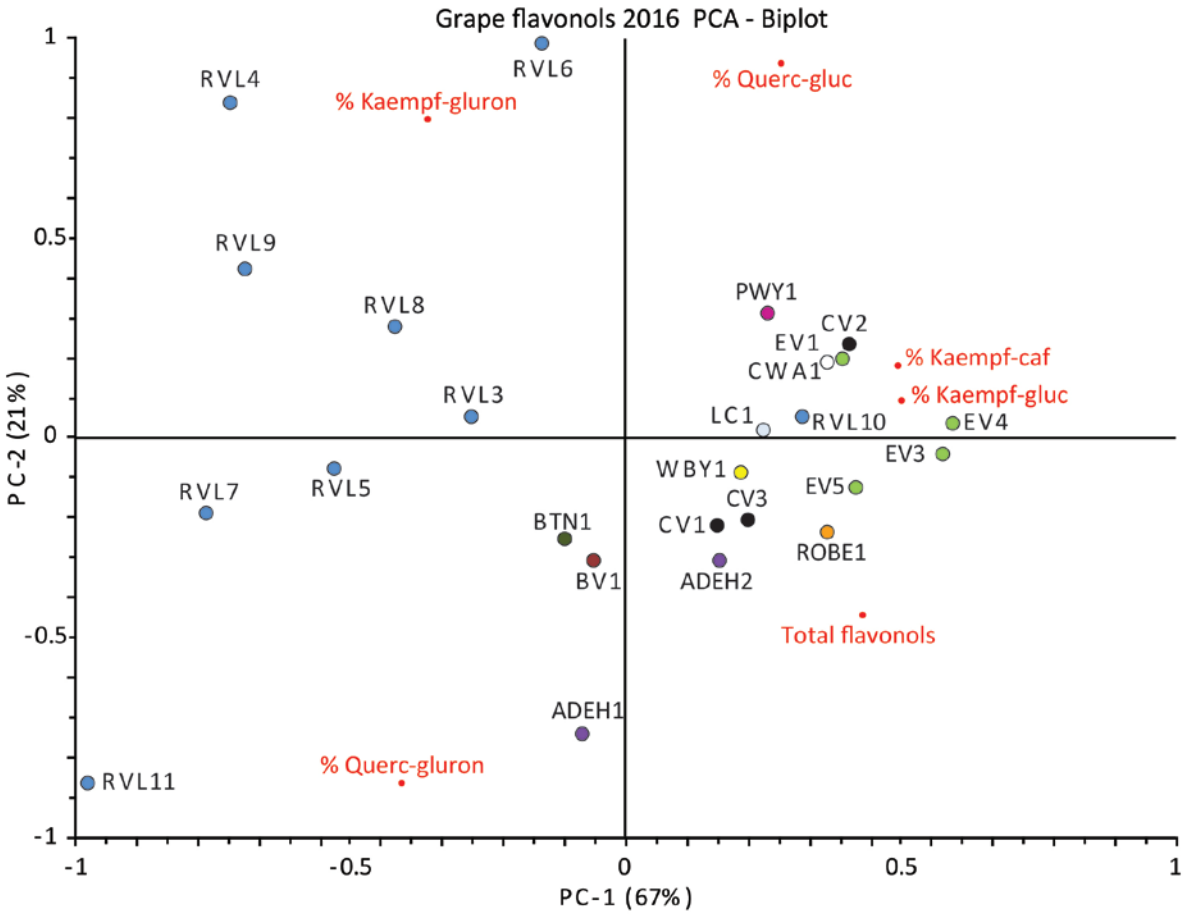


Figure 80. PCA biplot showing the scores of the 25 different 2016 Chardonnay grape samples from South Australia and the associated flavonoid profile parameter loadings in red. Kaempf-gluc = Kaempferol-3-*O*-glucoside, Kaempf-gluron = Kaempferol-3-*O*-glucuronide, Kaempf-Caf = Kaempferol-3-*O*-caffeoylate, Querc-gluc = Quercetin-3-*O*-glucoside, Querc-gluron = Quercetin-3-*O*-glucuronide.

7.4.2.2 Tannins

Tannins in white wine grapes are less important for the sensory properties of the wines than in red wines as there are lower concentrations present due to winemaking differences. However, they still can be responsible for bitter taste (Singleton 1992) and may interact with proteins to promote the production of wine haze (Waters et al. 1995). The differences in concentrations in grape samples may be caused by

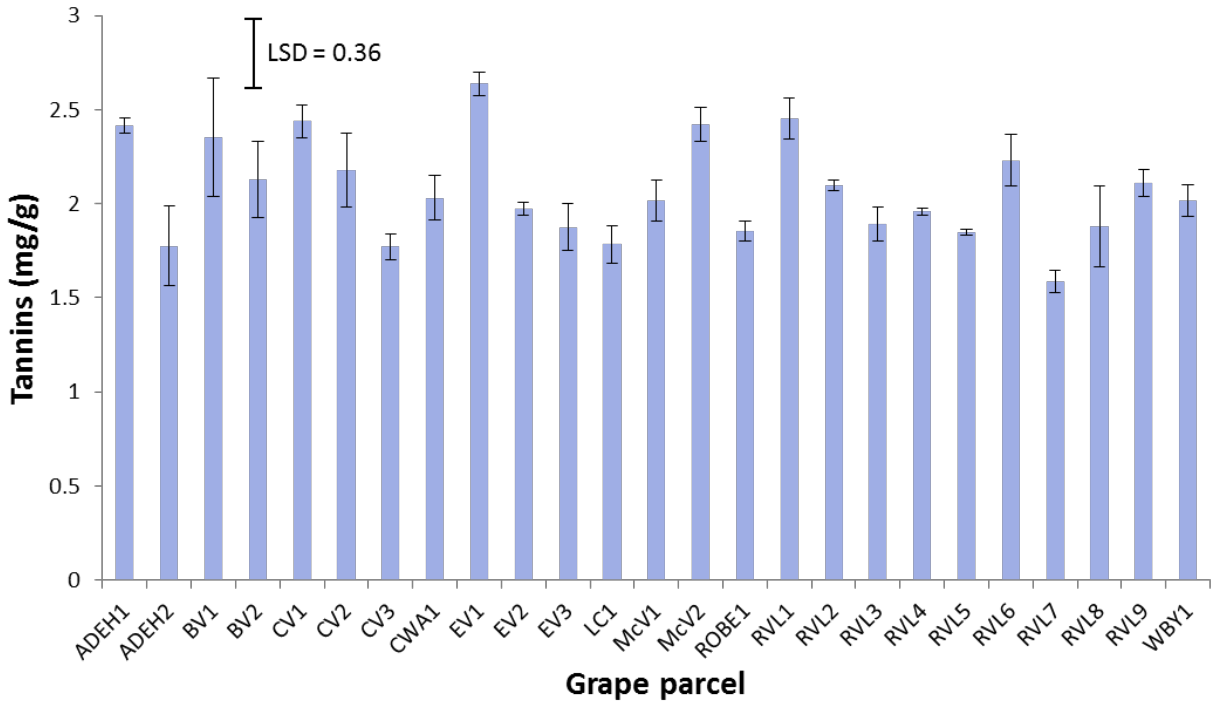


Figure 81. Total tannins extracted from de-seeded Chardonnay berries in 2015 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

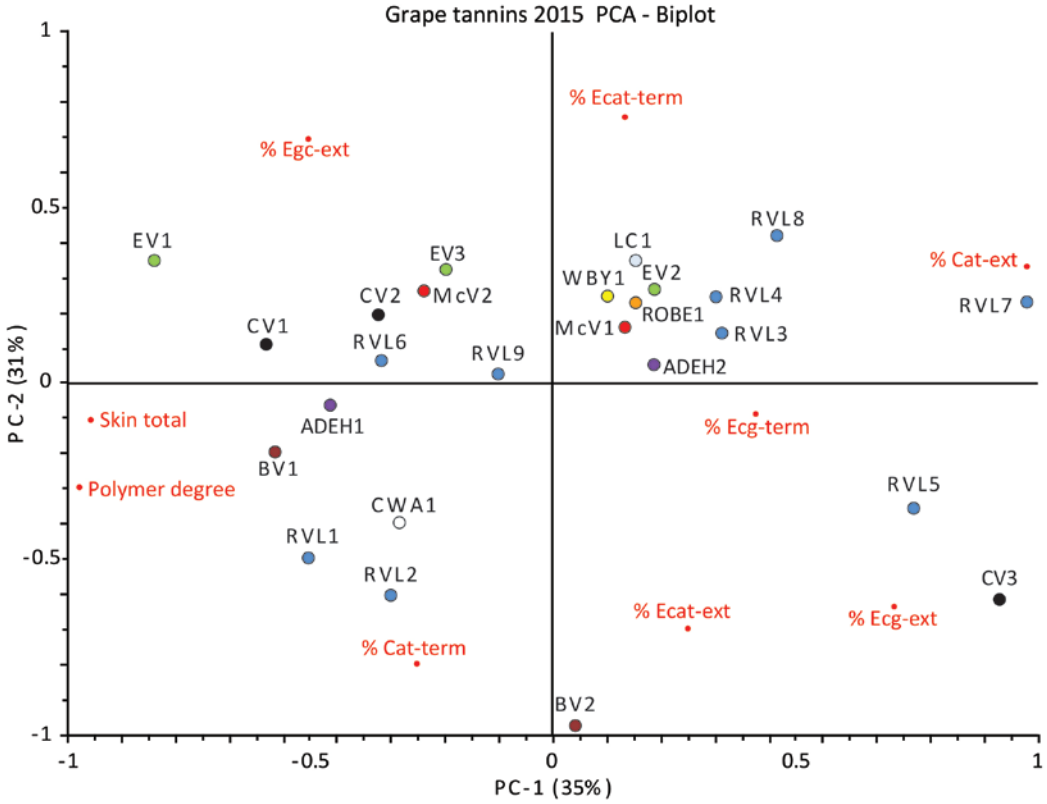


Figure 82. PCA biplot showing the scores of the 25 different 2015 Chardonnay grape samples and the associated tannin profile parameter loadings in red. Cat-ext = catechin extension subunits, Cat-term = catechin terminal subunits, Ecat-ext = epicatechin extension subunits, Ecat-term = epicatechin terminal subunits, Ecg-ext = epicatechin gallate extension subunits, Ecg-term = epicatechin gallate terminal subunits, Egc-ext = epigallocatechin extension subunits, Polymer degree = degree of polymerisation, Skin total = total skin tannin.

management or environmental variables and as such may be indicators of certain wine styles. As these small scale wines were not exposed to oak, the tannin composition of the wine was totally dependent on the tannins extracted from the grapes during the initial production of free-run juice. The concentration of total tannin in the skin of the grape samples was determined following chemical extraction, hydrolysis of the tannin polymers and analysis by HPLC.

The range of tannin concentrations across the 2015 Chardonnay samples went from 1.59 mg/g in the RVL7 sample to 2.64 mg/g in EV1 (Figure 81). This range was less than that seen for flavonols. The grapes from the warmer regions were not necessarily always lower in total tannins. However, it should be noted that these represent only the total tannins and that the length (extent of polymerisation) and structure (sub-unit and linkage composition) of the tannins may be more important than the total amount, especially from a sensory perspective.

PCA of the tannin subunit data confirmed what was seen with the Cabernet Sauvignon data in that there seemed to be a relationship between the amount of skin tannins and the degree of polymerisation (Figure 82). In 2015, these were also the main positive drivers of PC1 which described 35% of the total variation, and, in contrast, a higher percentage of catechin and epicatechin gallate extension subunits was associated with samples on the right of the biplot. It is interesting that there was no regional bias in the grouping of the samples in the biplot, with RVL samples found on either side of the plot and also samples from CV and EV discriminated on PC1 (Figure 82). This is similar to what was seen in the Cabernet Sauvignon samples in 2015 (Figure 27), but contrasts with the 2013 and 2014 vintages where samples from the same regions tended to group together (Figure 23 & Figure 25). Just under a third of the total variation was described on PC2, which mainly separated those samples with a greater percentage of epicatechin terminal and epigallocatechin extension subunits from those with more catechin terminal and epicatechin and epicatechin gallate extension subunits (Figure 82).

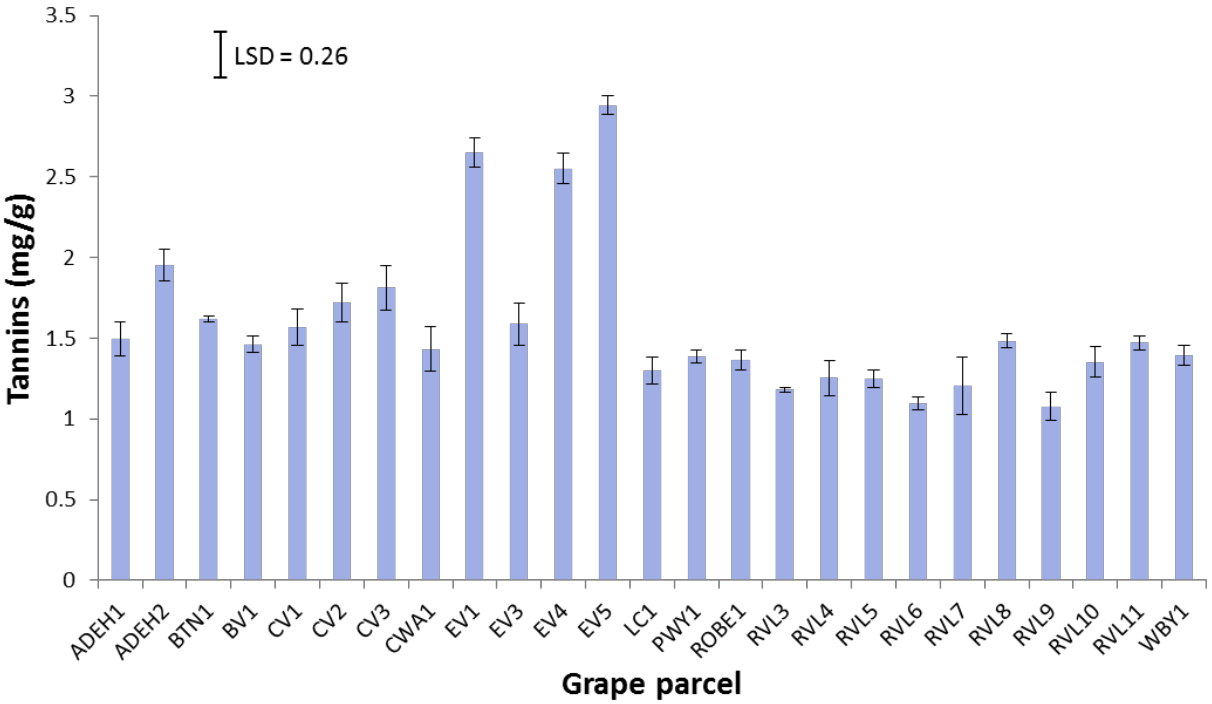


Figure 83. Total tannins extracted from de-seeded Chardonnay berries in 2015 presented as mg per g fresh weight. Error bar is ± standard error. LSD = minimum difference between two means for a significant difference at $p < 0.05$.

The 2016 vintage saw the widest range of skin tannins observed of any of the four years of sampling. High concentrations of tannins were seen in three of the EV samples with EV5 having the highest at 2.94 mg/g and the lowest concentration was seen in the RVL9 sample with 1.08 mg/g (Figure 83). The mean tannin concentration in 2016 was 1.58 mg/g, which was about three-quarters of the mean in 2015 (2.07 mg/g). The

RVL samples were noticeably lower in tannins compared to other rgions in 2016, something that was not observed in 2015.

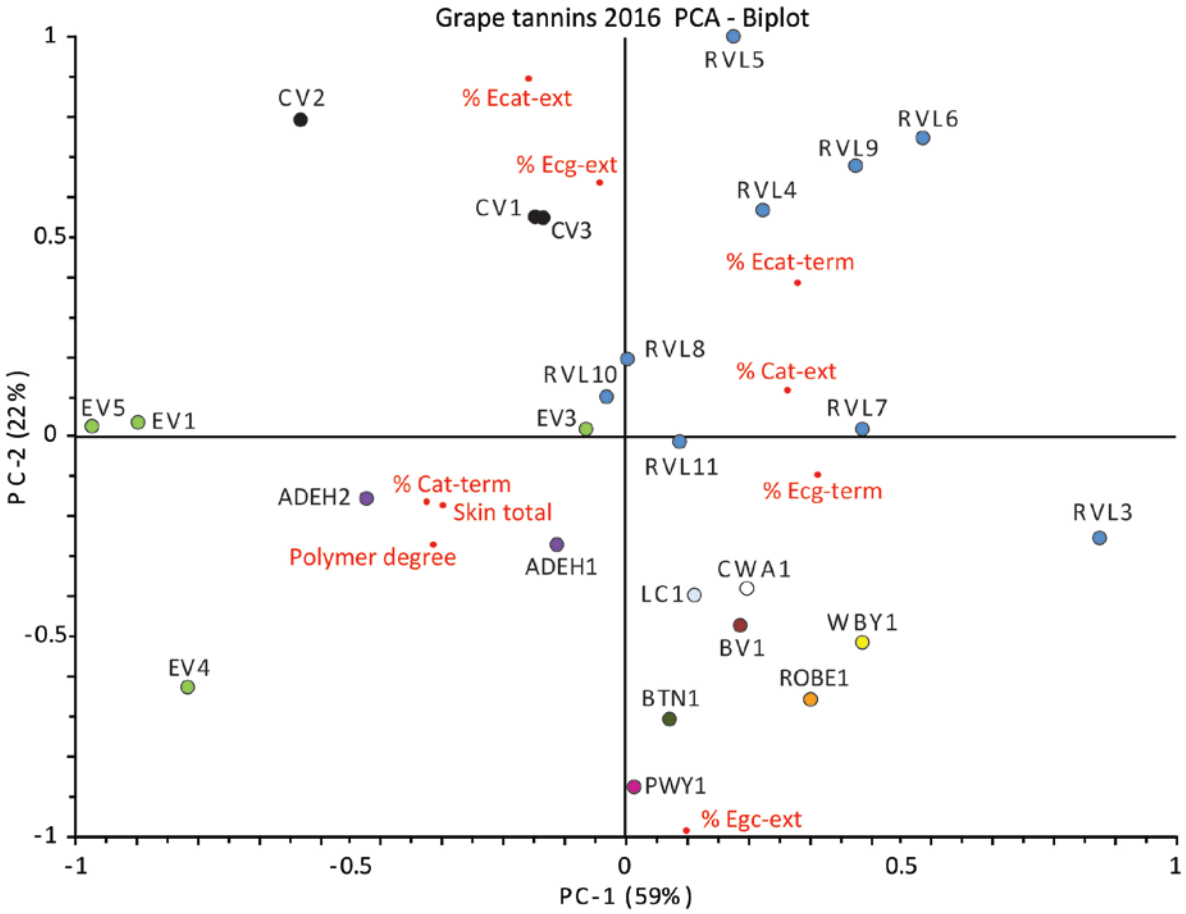


Figure 84. PCA biplot showing the scores of the 25 different 2015 Chardonnay grape samples and the associated tannin profile parameter loadings in red. Cat-ext = catechin extension subunits, Cat-term = catechin terminal subunits, Ecat-ext = epicatechin extension subunits, Ecat-term = epicatechin terminal subunits, Ecg-ext = epicatechin gallate extension subunits, Ecg-term = epicatechin gallate terminal subunits, Egc-ext = epigallocatechin extension subunits, Polymer degree = degree of polymerisation, Skin total = total skin tannin.

Principal component analysis of the tannin profiles obtained from the 2016 samples showed a slightly different relationship amongst the variables compared to 2015. The main driver on PC1 was again skin tannin and the degree of polymerisation, but the percentage of catechin terminal subunits was also closely associated with total skin tannin (Figure 84). The ADEH, CV and EV samples are found on the left of the plot associated with more skin tannin. On the opposite side of the biplot lay the percentage of catechin extension subunits, which was similar to that observed in 2015, but epicatechin and epicatechin gallate terminal subunits also drive separation on the horizontal axis. The RVL samples were found on the right of the plot along with many of the single samples taken from various regions. PC1 described more of the variation in 2016 (59%) compared to 2015 (35%). Epicatechin and epicatechin gallate extension subunit percentage were driving separation on PC2 towards the top of the plot and higher epigallocatechin subunit percentages are associated with samples near the bottom of the plot (Figure 84). The main contrast with the 2015 Chardonnay tannin profiles is that, in 2016, the samples were grouped on the PCA biplot according to region. The contrasting results from the different vintages, which was also seen in the Cabernet Sauvignon samples, suggests that environmental effects may have a strong influence on skin tannin composition.

7.4.2.3 Targeted Grape Volatile Compounds

A new solid phase microextraction (SPME), gas chromatography-mass spectrometry (GC-MS) method was developed to accurately quantify a number of compounds that had shown grape-wine sensory associations in previous work using Cabernet Sauvignon (Forde et al. 2011) and these were also measured in the Chardonnay grapes. While they may not necessarily be linked to similar sensory attributes in both varieties given the differences in wine production, they may be indicators of certain environmental conditions that the fruit has experienced during development and hence may still act as markers of certain Chardonnay wine styles.

Only nine of the 12 target compounds could be detected in the 2015 Chardonnay grapes. PCA is the easiest way to visualise how these measures discriminate the different grape parcels as discussed above, but the first two PCs only described 47% of the total variation (Figure 85). The separation on the horizontal axis was driven by differences in the concentration of (*E*,*E*)-2,4-heptadienal, 2-ethyl-1-hexanol and (*Z*)-3-hexen-1-ol on the left of the plot and °Brix on the right (Figure 85). PC1 did separate samples according to region, with the RVL parcels located on the right and the BV, EV, CWA and WBY samples found on the left. Some discrimination among the ADEH, CV and McV samples was seen on PC1. The second principal component began to discriminate some of the RVL samples. The compounds that are driving this are ethyl acetate, three aliphatic aldehydes and benzyl alcohol (top of the plot) and the concentration of (*Z*)-3-hexen-1-ol is associated with samples towards the bottom of the biplot (Figure 85).

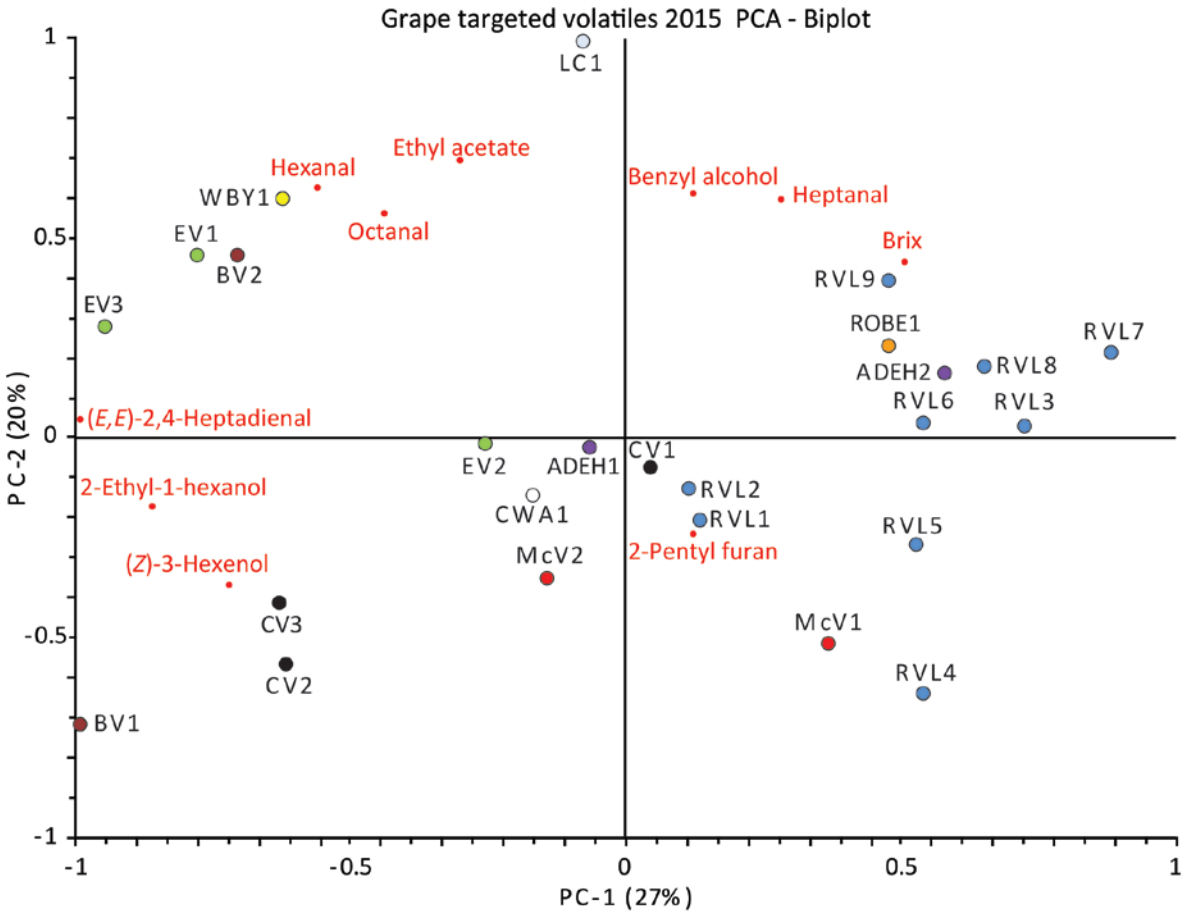


Figure 85. PCA biplot of 25 different Chardonnay grape samples from across South Australia in 2015 and associated loadings for the grape parameters measured in the fruit.

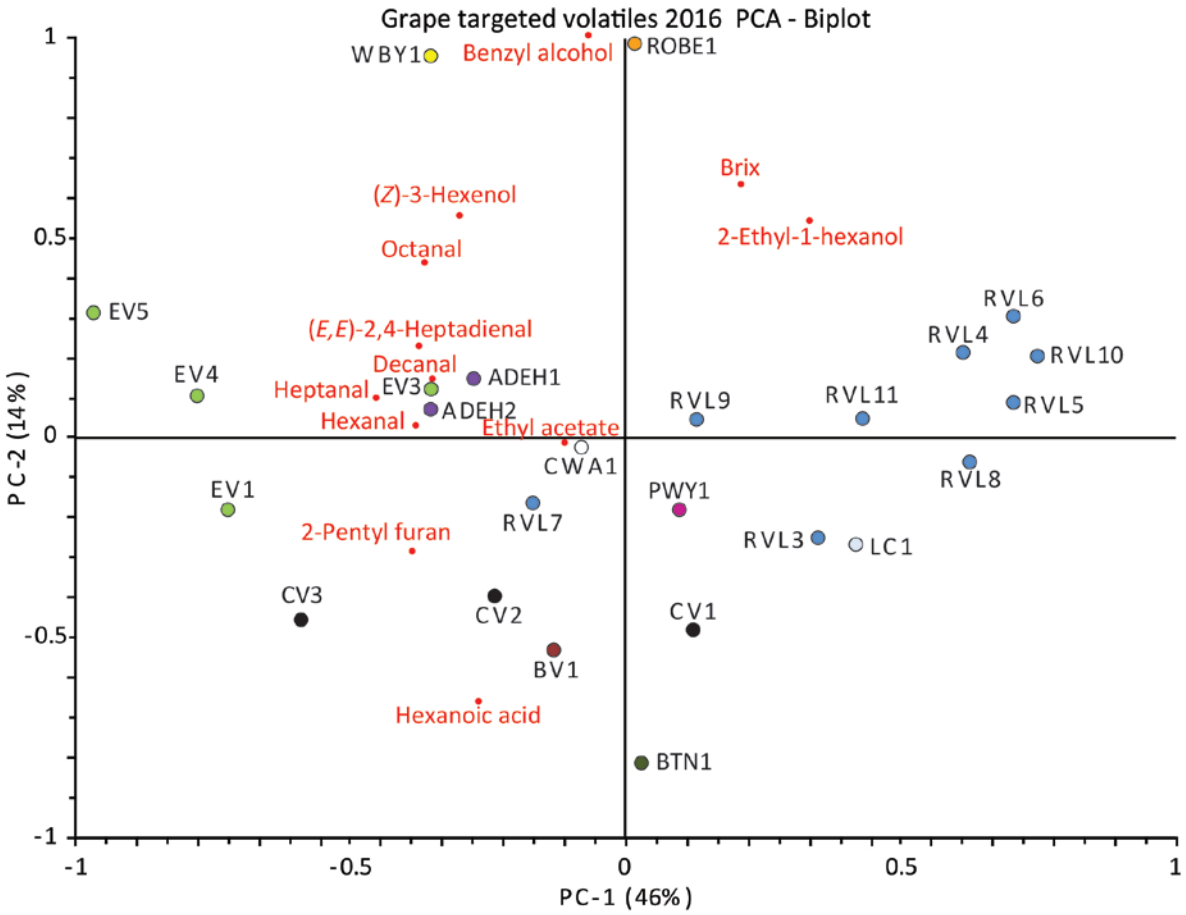


Figure 86. PCA biplot of 25 different Chardonnay grape samples from across South Australia in 2016 and associated loadings for the grape parameters measured in the fruit.

In the 2016 vintage, hexanoic acid and decanal were detected in the samples and so were included in the PCA. The first two PCs explained 60% of the total variation with 46% being explained by separation on the horizontal axis (Figure 86). The volatile aldehydes hexanal, octanal, heptanal, decanal and (*E,E*)-2,4-heptadienal were located together with the EV samples but on the opposite side of the biplot to 2-ethyl-1-hexanol, which was associated with the RVL samples in 2016 (Figure 86). Benzyl alcohol concentration was strongly driving discrimination on PC2 with hexanoic acid located on the bottom of the plot. Variation within the samples from the RVL and CV regions was described more by PC1 than PC2, but the ADEH and EV samples were not well discriminated by this analysis (Figure 86). PC2 mainly described the difference between the ROBE and WBY samples, which were high in benzyl alcohol, and the BTN and BV samples which were associated with high hexanoic acid concentrations.

Some consistencies were observed across the vintages in the targeted grape volatile analyses. With regards to the samples, the PCA analyses placed the EV and RVL sites at opposite ends of PC1 and the EV samples collocated with (*E,E*)-2,4-heptadienal in both vintages. The WBY sample was associated with high benzyl alcohol concentrations in both years. The CV2 and 3 samples were also located on the left of the plot in both years. The movement of 2-ethyl-1-hexanol from one side of PC1 to the other, and hence becoming associated with completely different samples suggests this may not be a very stable marker of grape composition.

7.4.2.4 Non-targeted Grape Volatile Compounds

Other major volatile compounds extracted from the headspace above crushed Chardonnay berries were also measured and the profiles visualised by PCA (see Table 4). Because of the number of compounds and samples used, we have split the PCA biplot into scores for the samples and loadings for the compounds to make it easy to interpret. Codes for the loadings plot are included in Table 4.

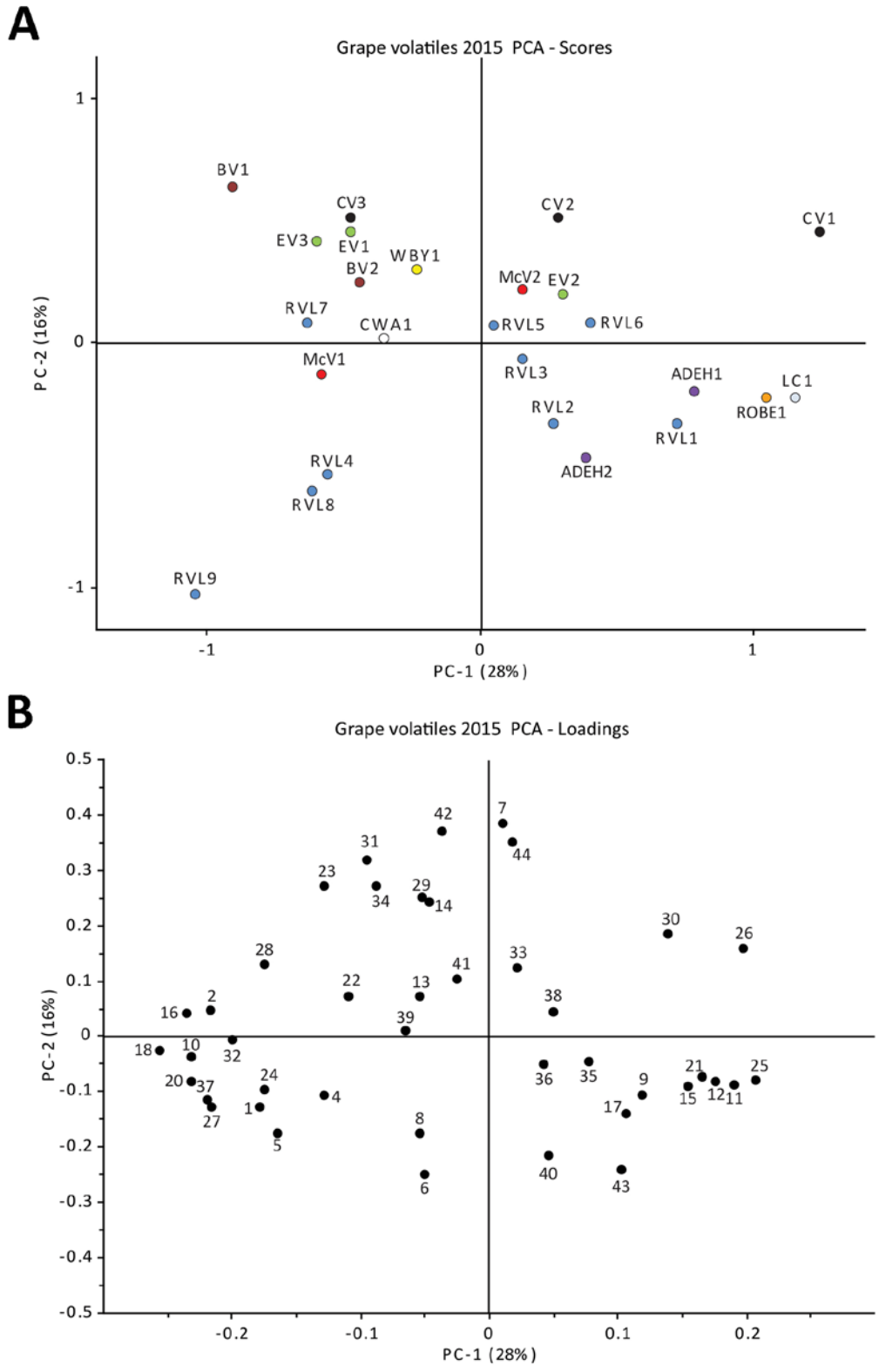


Figure 87. PCA scores (A) and loadings (B) plots of 25 different 2015 Chardonnay grape samples from across South Australia and the volatile compounds quantified in the headspace of the crushed grapes.

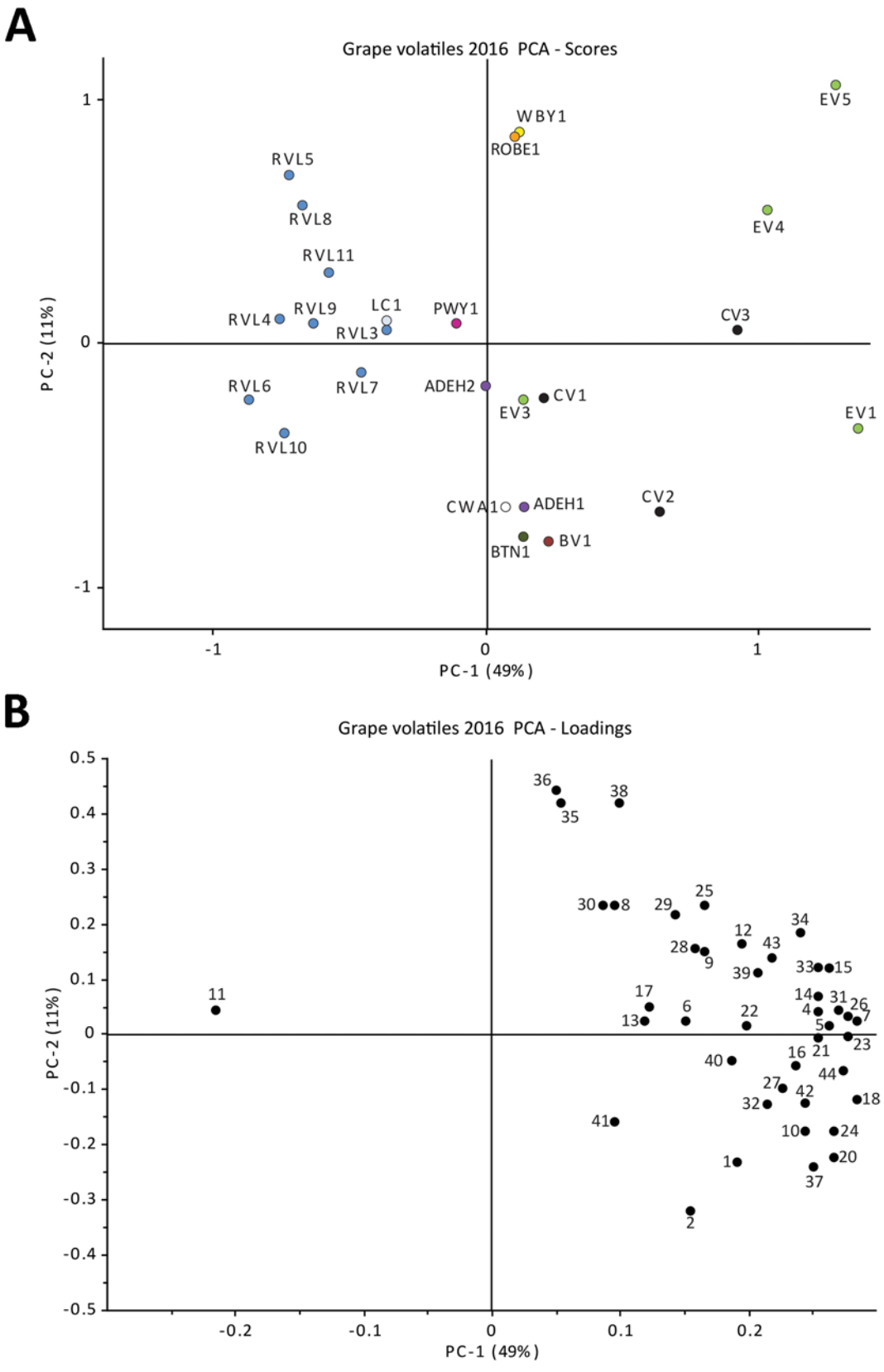


Figure 88. PCA scores (A) and loadings (B) plots of 25 different 2016 Chardonnay grape samples from across South Australia and the volatile compounds quantified in the headspace of the crushed grapes.

The PCA of the non-targeted grape volatile compounds in 2015 (Figure 87) showed a different pattern of separation among the samples than was seen when the targeted volatile compounds were analysed (Figure 85). The grouping according to region, seen in the targeted analysis, was lost in the non-targeted volatile

profiling (Figure 87). PC1 accounted for 28% of the total variation and some samples from the same region (i.e. CV, EV, McV and RVL) were discriminated by this PC. The ADEH, ROBE and LC samples were located on the right of the plot and were characterised by higher concentrations of the esters methyl butanoate and hexanoate, as well as decane and cyclohexane oxide. The BV, CWA and WBY samples were on the left of the plot and were collocated on the PCA plot with the aldehydes pentanal, 2-butenal, (*E*)-2-pentenal and (*Z*)-3-hexenal (Figure 87). The separation on PC2 distinguished six of the RVL samples as well as the ADEH, ROBE, LC and McV1 at the bottom of the plot from the rest of the samples. The compounds associated with the samples at the top of the plot were 2-ethyl furan, (*E*)-2-penten-1-ol and 5-ethyl-2(5H)-furanone and those at the bottom had higher concentrations of benzaldehyde, 2-phenylethanal and hexyl methyl ether (Figure 87).

For the 2016 volatile compound profiles, the results of the PCA looked very different to those in 2015. First, PC1 described a lot more of the variation (49%), and separated many of the samples by region (Figure 88). The RVL samples were found on the left of the plot, along with the samples from PWY and LC, and the rest were on the right. The loadings plot also showed a very different pattern from that seen in 2015, with most of the volatile compounds grouped on the right of the plot and only one (methyl butanoate) on the left (Figure 88). The compounds driving the separation on the right of plot included the five-carbon compounds (E)-2-pentenal, 1-penten-3-ol and (Z)-2-penten-1-ol as well as 2-ethyl furan, cyclohexane oxide and 5-ethyl-2(5H)-furanone. Separation on PC2 only represented 11% of the total variation. It allowed discrimination of some of the CV, EV and RVL samples from each other as well as the ROBE and WBY1 samples at the top of the plot from those harvested in ADEH, BTN, BV and CWA (Figure 88). The compounds driving this separation were the C_6 compounds 1-hexanol, (*E*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol at the top of the plot and 2-butanone at the bottom.

The difference between the results of the PCA on the 2015 and 2016 volatile profiles of the Chardonnay grapes suggests that the volatile composition of the fruit is altered significantly by environmental conditions. Interestingly, the PCA of the 2015 Cabernet Sauvignon non-volatile profiles also failed to group the samples from the same region as much as was observed in previous vintages (Figure 34-36). It would be interesting to compare climatic conditions for this vintage to see if any unusual observations were made in this growing season.

7.4.2.5 Amino Acids

As mentioned previously, amino acids are implicated in wine volatile compound production in two main ways: i) they contribute to the amount of yeast assimilable nitrogen (YAN) that can influence the fermentation process and ii) amino acids are also precursors of certain wine volatile components. The concentration of amino acids in the fruit samples were profiled to investigate if they are predictive of any sensory attributes in the wine. It is best to view the data by a PCA plot as this again gives us an overall picture of the relationships amongst the amino acid profiles and the grape samples.

In the 2015 Chardonnay samples, separation on the first PC (66%) was driven by the total amino acid concentration on the right of the plot, which was also where all the amino acid loadings were located (Figure 89). Some discrimination of the RVL samples was seen on the first PC, whereas samples from other regions (e.g. ADEH, CV and McV) still grouped together. The analysis also showed that the RVL7 and WBY samples had the highest concentrations of many of the amino acids measured, while the ADEH, CV and McV grapes had some of the lowest amino acid concentrations. The second PC (12%) discriminated the RVL4 and 9 samples from the others harvested in the RVL, as well as the BV, LC and McV samples from those obtained from ADEH, CWA and WBY (Figure 89). The samples at the top of the plot had more aspartic acid compared to the others, and higher concentrations of isoleucine, lysine phenylalanine and β-alanine were associated with the samples at the bottom of the plot.

The PCA plot of the amino acid concentrations in the 2016 Chardonnay samples (Figure 90) had similar overall pattern to that of the 2015 samples. PC1 again described about two-thirds of the overall variance among the samples (68%). The location of the samples on the horizontal axis was also similar in the two years. Most of the EV samples, as well as the CWA and WBY grapes, were located on the right of the plot with the ADEH and CV samples on the left (Figure 90). The RVL3, 7and 9 samples were on the left of the plot as they were in the 2015 amino acid analysis (Figure 89). The samples on the right were again associated with having higher concentrations of all of the amino acids than those on the left. PC2 discriminated the RVL samples

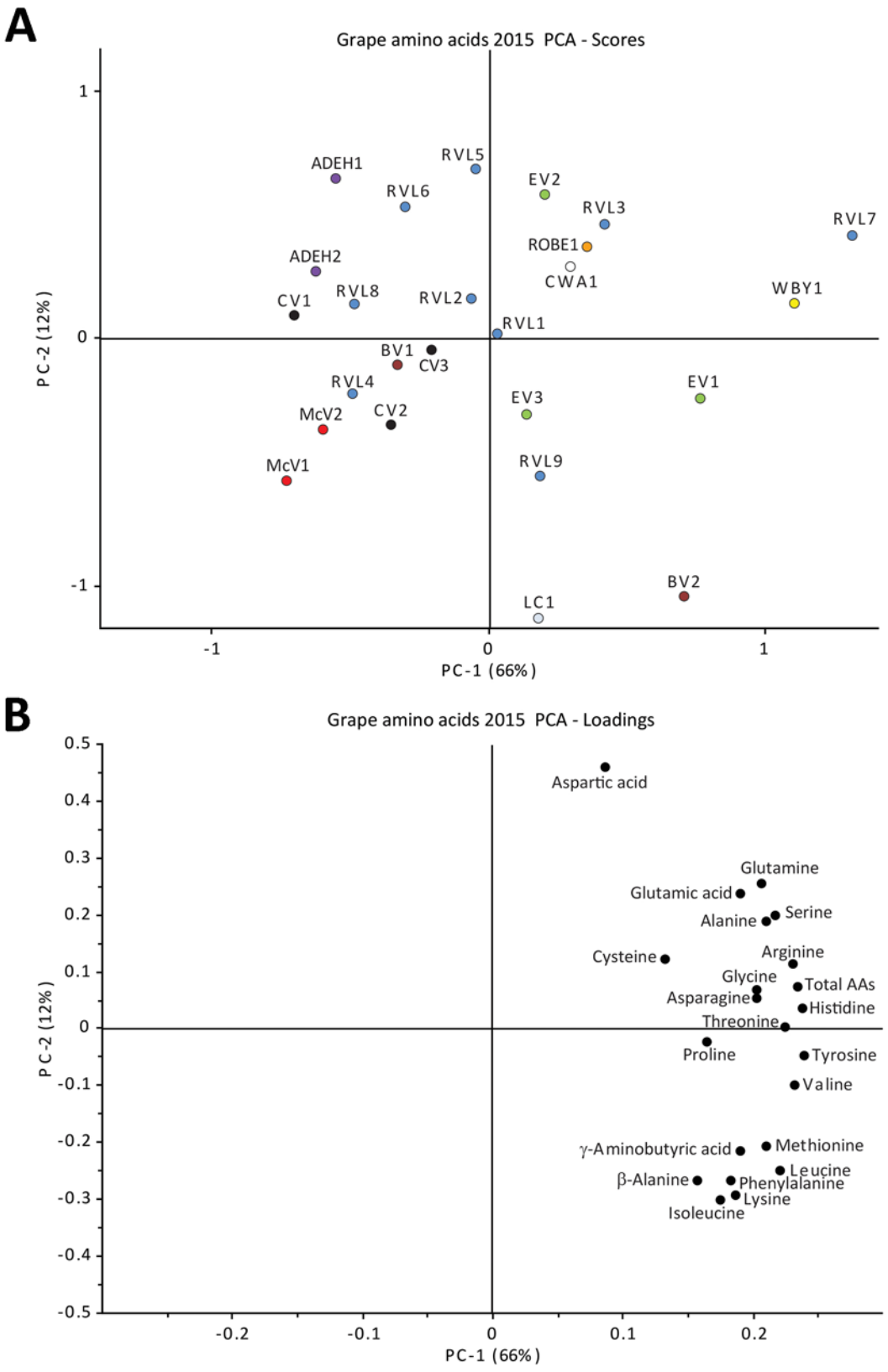


Figure 89. PCA scores (A) and loadings (B) plots of 25 different Chardonnay grape samples from across South Australia and the amino acids quantified in the grape juice.

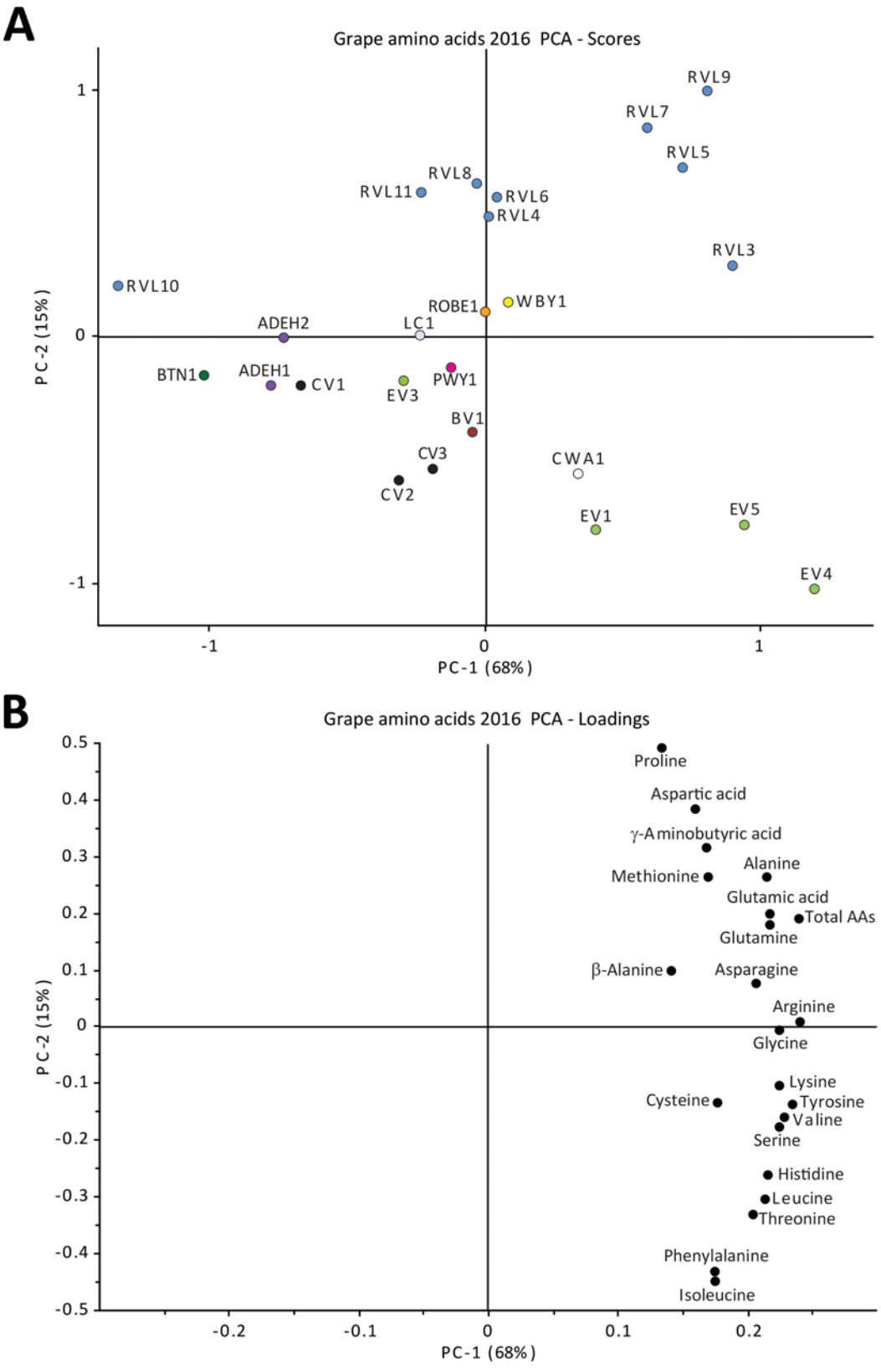


Figure 90. PCA scores (A) and loadings (B) plots of 25 different Chardonnay grape samples from across South Australia and the amino acids quantified in the grape juice.

from most of the other samples (Figure 90). The amino acids driving the discrimination on PC2 in 2016 were similar to those in 2015. Aspartic acid was a driver of separation towards the top of the plot, although proline was more important than it was in 2015. Towards the bottom of the plot, the samples were associated with

higher concentrations of isoleucine and phenylalanine, both of which were also driving separation in this direction in 2015.

It is interesting that the amino acid profiles across the vintages in Chardonnay were relatively stable as this was also seen in the Cabernet Sauvignon samples. The main driver in each sample set was the presence of higher amounts of total amino acids in some samples, and this tended to describe about two thirds of the total variation. It is highly likely that this reflects nitrogen availability of the vines, which may be due to variables such as fertiliser management in the vineyard or rootstock differences (Bell and Henschke 2005). The stability suggests that this is a trait that can be managed in the vineyard to alter wine composition based on total amino acid availability.

7.4.2.6 Bound Precursors of Volatile Compounds

Bound volatile compounds were also examined in the Chardonnay grapes as they have the potential to be released during winemaking and influence wine volatile profiles in this variety (Sefton et al. 1993). PCA of the 2015 bound volatile profiles revealed groupings amongst the samples that was generally based on region (Figure 91). The RVL samples were located on the left side of the score plot, along with the ADEH and CWA samples and two from EV. The other EV sample was found on the right of the plot along with the samples from BV, CV, LC, McV, ROBE and WBY (Figure 91). This separation on the horizontal axis accounted for 36% of the total variation among the samples. There were many compounds driving separation on the horizontal axis, especially towards the right side of the plot with several norisoprenoids, such as dihydro-β-ionone and 4-(2,3,6-trimethylphenyl)but-3-en-2-one, found on this side of the loadings plot (Figure 91). PC2 only explained 12% of the total variation and discriminated the ADEH, CV1, LC1 and RVL1, 2, 4 & 9 samples at the bottom of the plot from the others. The separation on the vertical axis was due to higher concentrations of the aromatic compound benzoic acid, as well as heptanoic and octanoic acids in samples at the top of the plot and more 2-ethyl-1-hexanol, 2-methyl-benzaldehyde, decanoic acid and α-calacorene in samples located near the bottom (Figure 91).

When the relationship among the bound volatile profiles of the 2016 Chardonnay grape samples was visualised using PCA, there was again clustering of samples based on the region of origin (Figure 92), but the pattern was different to that seen in 2015 (Figure 91). Most of the RVL samples were again found in the left of the plot along with the ADEH, CWA, EV3, LC, PWY and ROBE samples. The exceptions were the RVL3 and 10 samples which were located on the right of the plot along with the BTN, BV, CV, WBY and other EV samples. The loadings plot showed that there were a lot of compounds driving separation on the right of the horizontal axis (Figure 92). The cluster of compounds in the top right corner was composed mainly of norisoprenoids, similar to those that drove this separation in the previous vintage. PC1 described 37% of the variation in the samples, whereas PC2 accounted for 24%. The samples found towards the top of the vertical axis were associated with higher concentrations of benzoic and nonanoic acid, whereas the samples in the lower part of the plot had higher concentrations of nonanal, α-ionene, 2-ethyl-1-hexanol and 2-methylbenzaldehyde (Figure 92).

There were some similarities between the bound volatile profiles of the Chardonnay grapes from the 2015 and 2016 vintages. For example, in both vintages most of the RVL samples were located on the left of the PCA plot and these generally had lower concentrations of most of the bound volatile compounds which clustered on the right of the plot (Figure 91 and Figure 92). Conversely, the CV, BV and WBY samples were consistently found to be associated with higher amounts of these bound volatiles. Most of the compounds on the far right of the loadings plot were norisoprenoids which are derived from the enzymatic breakdown of carotenoids (Mathieu et al. 2005). Carotenoids are compounds that protect the photosynthetic apparatus (Choudhury & Behera 2001) in plants and hence their synthesis is induced by light (Pizarro & Stange 2009). The larger canopies used in the RVL region to prevent damage to the fruit by the sun could be influencing the bound volatile profiles by reducing the amount of carotenoids and their norisoprenoid breakdown products.

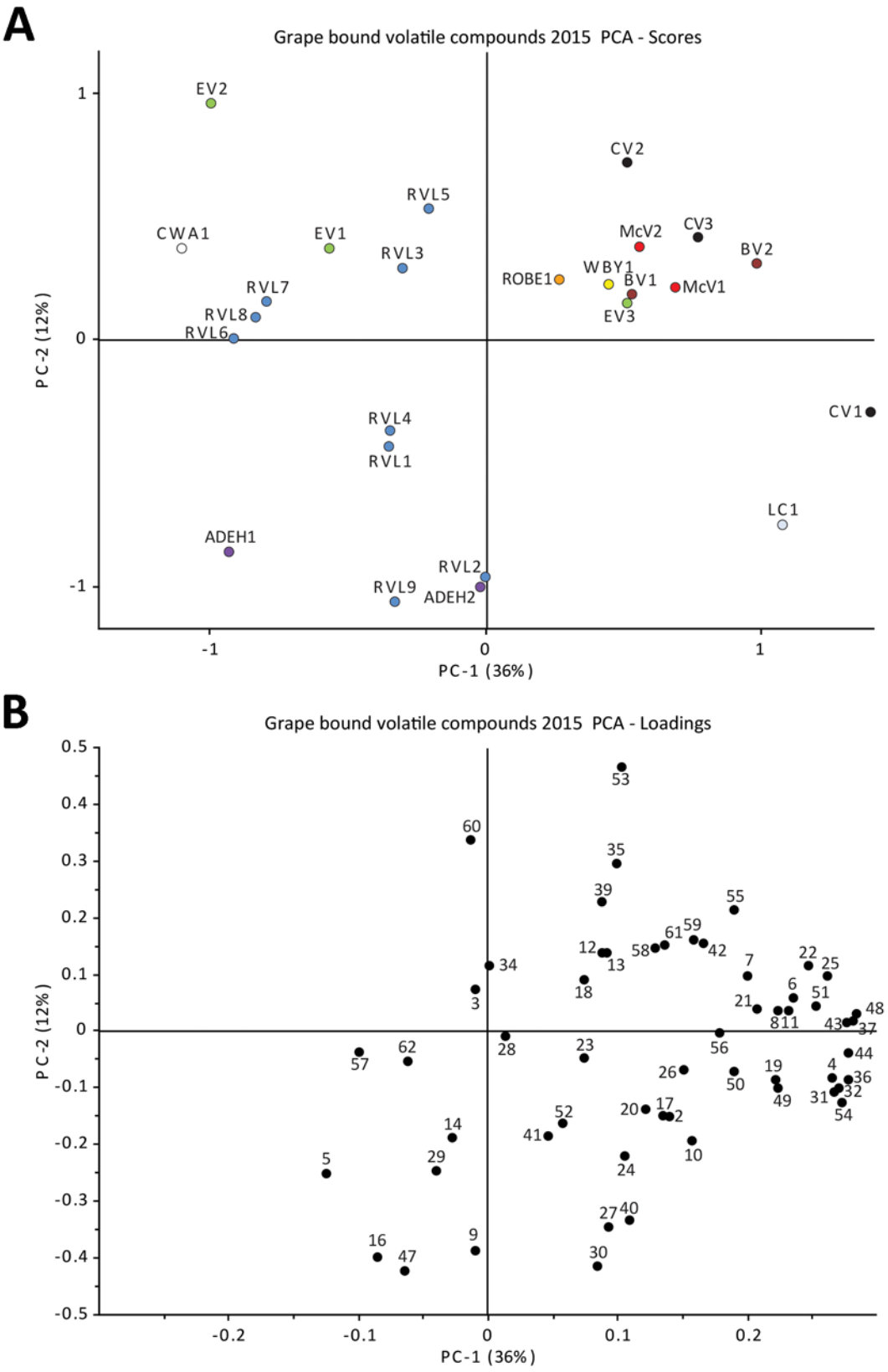


Figure 91. PCA scores (A) and loadings (B) plots of 25 different 2015 Chardonnay grape samples from across South Australia and the bound volatiles quantified in the grapes. The numbers represent the individual compounds as listed in Table 5.

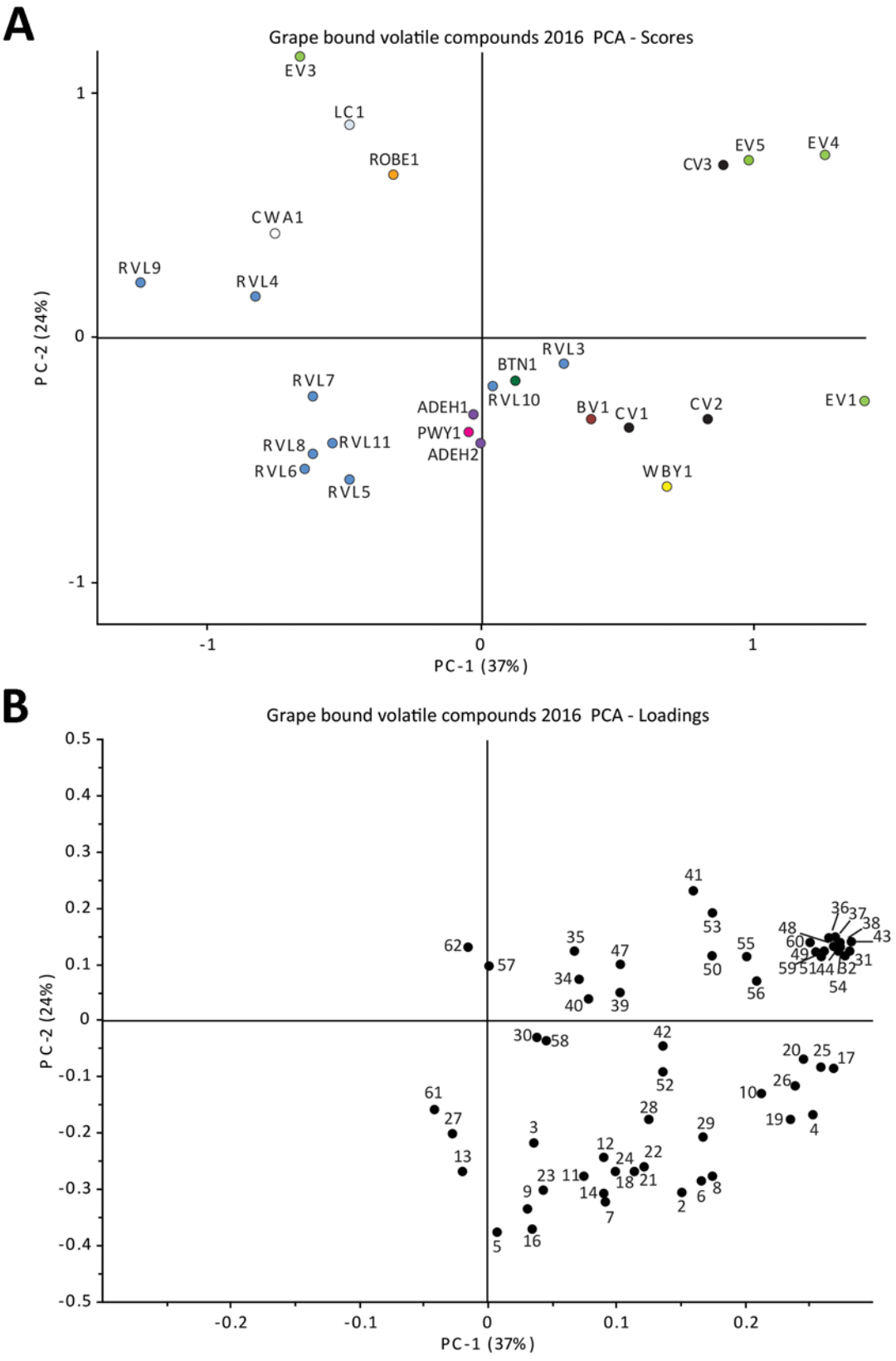


Figure 92. PCA scores (A) and loadings (B) plots of 25 different 2016 Chardonnay grape samples from across South Australia and the bound volatiles quantified in the grapes. The numbers represent the individual compounds as listed in Table 5.

7.4.2.7 Fatty Acids

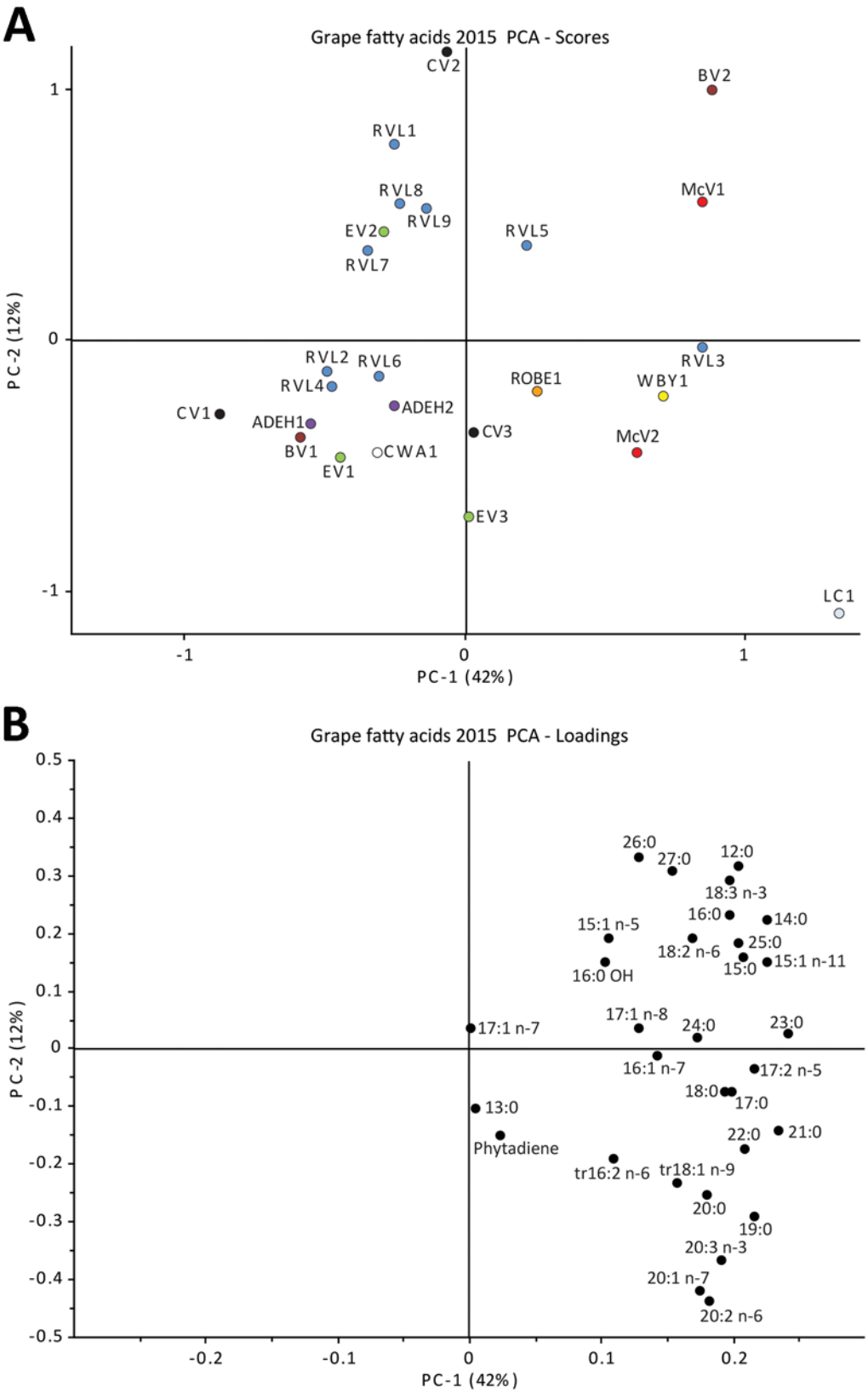


Figure 93. PCA scores (A) and loadings (B) plots of 25 different 2015 Chardonnay grape samples from South Australia based on the fatty acids quantified in the grapes. The denotations of the various fatty acids are listed in Table 6.

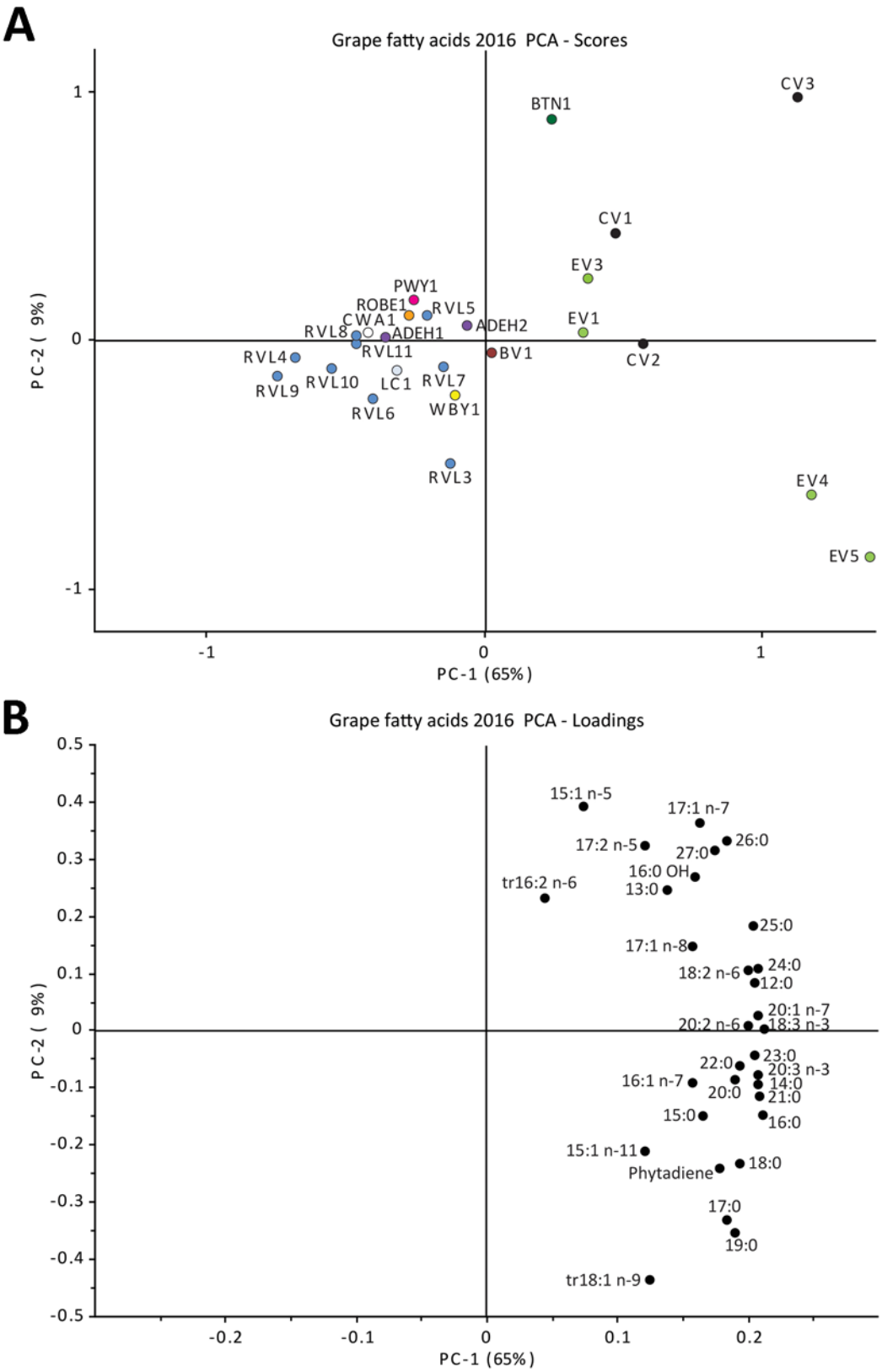


Figure 94. PCA scores (A) and loadings (B) plots of 25 different 2016 Chardonnay grape samples from South Australia based on the fatty acids quantified in the grapes. The denotations of the various fatty acids are listed in Table 6.

As outlined above, many of the volatile compounds in crushed grapes are formed from the degradation of fatty acids (Kalua and Boss 2009, 2010). As such, the fatty acids have the potential to be the precursors of

many wine volatile compounds (Dennis et al. 2012) and also have the potential to alter yeast metabolism and therefore the relative concentration of yeast-derived components of wine (Duan et al. 2015). Fatty acid abundances were measured in deseeded Chardonnay grapes in both vintages.

In 2015, the PCA of the fatty acid profiles was able to discriminate samples from within growing regions, with the ADEH samples being the exception (Figure 93). For example, the RVL3 sample, and to a lesser extent RVL5, were separated from the other RVL samples on the horizontal axis, as were the samples from BV and, marginally, CV and EV. The second PC only described 12% of the total variation compared to PC1, which represented 42%, and PC2 also discriminated some of the samples from the same region (e.g. BV, CV, EV, McV, and RVL). The samples on the left of the plot were associated with higher concentrations of many of the fatty acids measured (Figure 93). Those fatty acids driving the separation on the right side of the plot were the saturated odd-numbered fatty acids ranging from pentadecanoic (15:0) to pentacosanoic acid (25:0), along with tetradecanoic (14:0) and docosanoic (22:0) acids, and the unsaturated 4-pentadecenoic (15:1 n-11) and (*Z,Z*)-9,12-heptadecadienoic (17:2 n-5) acids. The samples on the left side of the plot were associated with lower amounts of almost all of the fatty acids. Separation on the vertical axis was driven by hexacosanoic (26:0), heptacosanoic (27:0), dodecanoic (12:0) and linolenic (18:3 n-3) acids at the top of the plot, while the lower section was associated with greater abundance of saturated and unsaturated 20 carbon fatty acids and nonadecanoic acid (Figure 93).

The PCA of the fatty acid profiles obtained from the 2016 vintage Chardonnay grapes showed a quite different pattern from that seen in 2015 (Figure 93 and Figure 94). PC1 described 65% of the total variation and was again driven by the overall concentration of fatty acids in the samples. PC1 discriminated samples based on region, with the BTN, CV and EV samples located on the left of the plot with the rest found on the right. PC2 only represented 9% of the variation in the fatty acid profiles of the grape samples. It gave poor discrimination of the samples on the left of the plot, but some of the BTN, CV and EV samples were separated well on the right of the plot (Figure 94). The drivers of the discrimination of the samples on the right of the plot on PC1 were almost all of the fatty acids measured including odd- and even-numbered unsaturated fatty acids and some 18 and 20 carbon unsaturated fatty acids (Figure 94). Samples on the left of the plot were associated with lower concentrations of all the fatty acids measured. PC2 separated the samples with higher concentrations of some odd-numbered unsaturated fatty acids as well as hexacosanoic (26:0) and heptaconsanoic (27:0) acids at the top of the plot with samples that had more oleic (18:1 n-9), nonadecanoic (19:0) and heptadecanoic (17:0) acids at the bottom (Figure 94).

Some similarities were observed in the fatty acid profiles across the two vintages. The ADEH, CWA and RVL samples were generally associated with lower concentrations of fatty acids. However, the fruit from other regions were variable across the two vintages with the LC, ROBE and WBY samples associated with higher fatty acids in 2015 and lower in 2016, whereas the opposite was true for the CV and EV samples (Figure 93 and Figure 94). There was no trend with regards to the samples associated with more specific types of fatty acids, as discriminated on PC2, across both vintages. It could be that the data need to be mined deeper and that relative composition could be more important. For example, perhaps there are differences in ratios of saturated to unsaturated or odd- to even-numbered fatty acids that are more important than the overall concentrations. It may also be important to have an indication of the water content of the fruit, as this could be what is influencing the total fatty acid concentrations more than any change in biosynthesis, although any difference in the grapes would also continue through to the wine. Total fatty acid differences may also reflect differences in the size of the cells in the berries, something that does not appear to have been investigated with regards to wine style and quality.

7.4.3 ACTIVITIES OF ENZYMES FROM THE LIPOXYGENASE PATHWAY

The lipoxygenase pathway is the main enzymatic means by which unsaturated fatty acids are broken down in grape berries. The activity of the pathway is important in determining the concentration of C_6 volatile aldehydes and alcohols present in crushed grapes and these are the compounds that dominate volatile profiles of grapes. The activities of the three main enzymes of this pathway were assayed in the harvested

berries to see if these activities were predictive of any wine sensory attributes, given their importance in determining grape volatile composition.

7.4.3.1 Lipoxygenase (LOX)

Lipoxygenase is the first committed step of the pathway and catalyses the production of a lipid hydroperoxide at the double bond at C-9 or C-13 of linoleic or linolenic acid (Figure 49). Lipoxygenases generally have specificity for either site and in ripe grapes the predominant activity is towards C-13. C-13 lipoxygenase activity was assayed in the harvested fruit, and there was more variation among the Chardonnay grape parcels than was seen in the Cabernet Sauvignon samples (see Section 7.1.3.1). In 2015, the LOX activity varied from 0.31 µmol/min/g in CWA1 to 0.66 µmol/min/g in ROBE1. No obvious trend with regard to a regional effect on LOX activity was observed and most of the activities were very similar, with the CWA1 and EV2 samples standing out as the LOX activity in these samples was lower than that observed in the rest of the samples (Figure 95).

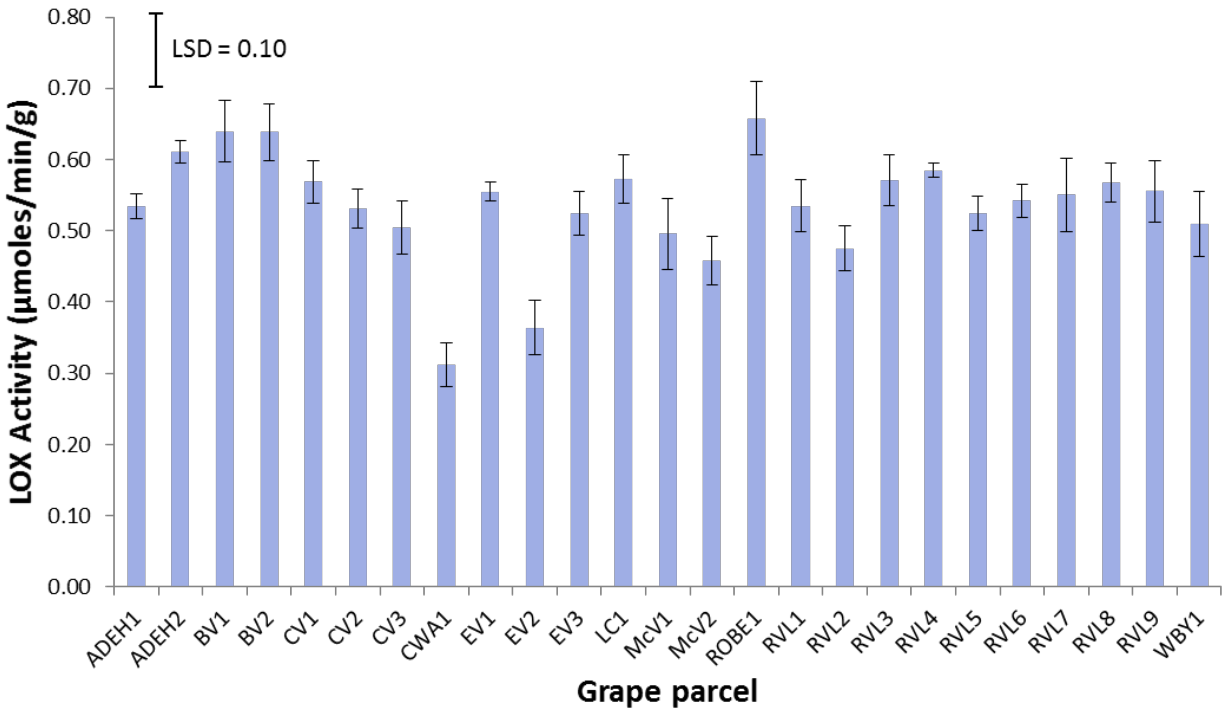


Figure 95. Lipoxygenase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

The 2014 Chardonnay grapes had a slightly greater range of LOX activities (Figure 96), with the maximum activity seen in the ADEH2 sample (0.78 µmol/min/g) and the lowest measured in the RVL11 fruit (0.34 µmol/min/g), representing slightly more than a 2-fold difference between extremes. The mean LOX activity in 2016 was 0.61 µmol/min/g, slightly greater than that observed in 2015 which was 0.54 µmol/min/g. There was a trend for the RVL samples to have lower LOX activities than those sampled from other regions in 2016 and the WBY sample also had relatively low LOX activity in 2016.

There seemed to be little consistency in the relative LOX activities of the samples across the two vintages which was similar to that observed in the Cabernet Sauvignon samples. This lack of consistency may reflect differences in harvest timing that may influence LOX activity in the fruit or could be due to environmental effects that differ among the vintages which influence LOX activity more than any site or regional effect.

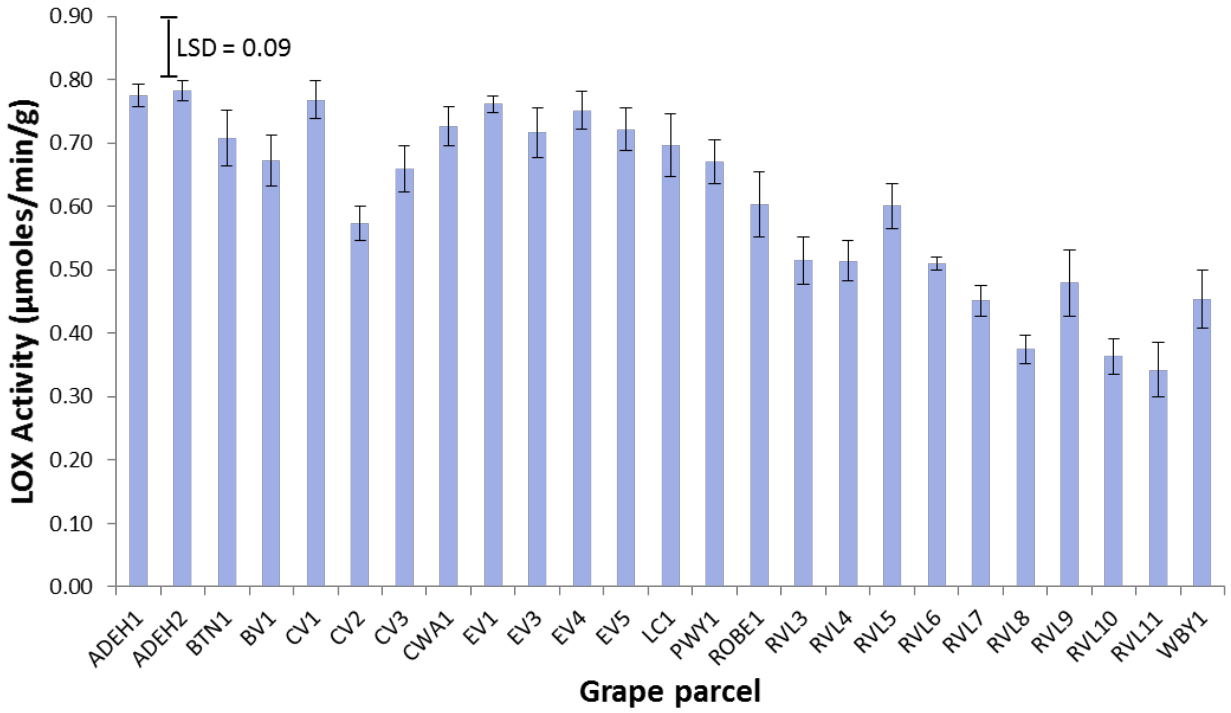


Figure 96. Lipoxygenase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2016. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.4.3.2 Hydroperoxide lyase (HPL)

Hydroperoxide lyase catalyses the cleavage of the hydroperoxy fatty acids, formed by the action of LOX, into omega-oxo acids and volatile C6- and C9-aldehydes (Figure 49). Although spontaneous degradation of the 13-hydroperoxide formed from linoleic acid can occur (Duan et al. 2005), the presence of HPL enzyme activity would increase the flux down this pathway to the production of C_6 aldehydes and alcohols.

The range of HPL activities measured in the Chardonnay fruit harvested in 2015 was not great (Figure 97), the lowest being 0.87 µmol/min/g (RVL9) and the highest 1.02 µmol/min/g in the ROBE sample. The HPL activities in Chardonnay were more uniform than those measured in the Cabernet Sauvignon samples in each vintage (section 7.1.3.2). However, there was more variation in the HPL activities in the Chardonnay samples from 2016 and, like LOX, most of the lower HPL activities were measured in RVL samples (Figure 98). The mean HPL activity was higher in the 2016 samples (1.10 µmol/min/g) than in the 2015 grapes (0.93 µmol/min/g). The difference of HPL activities was slightly greater than 2-fold between the lowest activity, 0.70 µmol/min/g in the RVL10 sample, and the highest measured activity, which was 1.45 µmol/min/g in the EV1 grapes (Figure 98).

Looking across the two vintages, the same conclusions that were made about the LOX activities are appropriate for the HPL activities measured as well. There appears to be no general trend across the sites and vintages. Again, the lack of any great variation in these enzyme activities may reflect the fact that the HPL activity is relatively stable across the region and vintages.

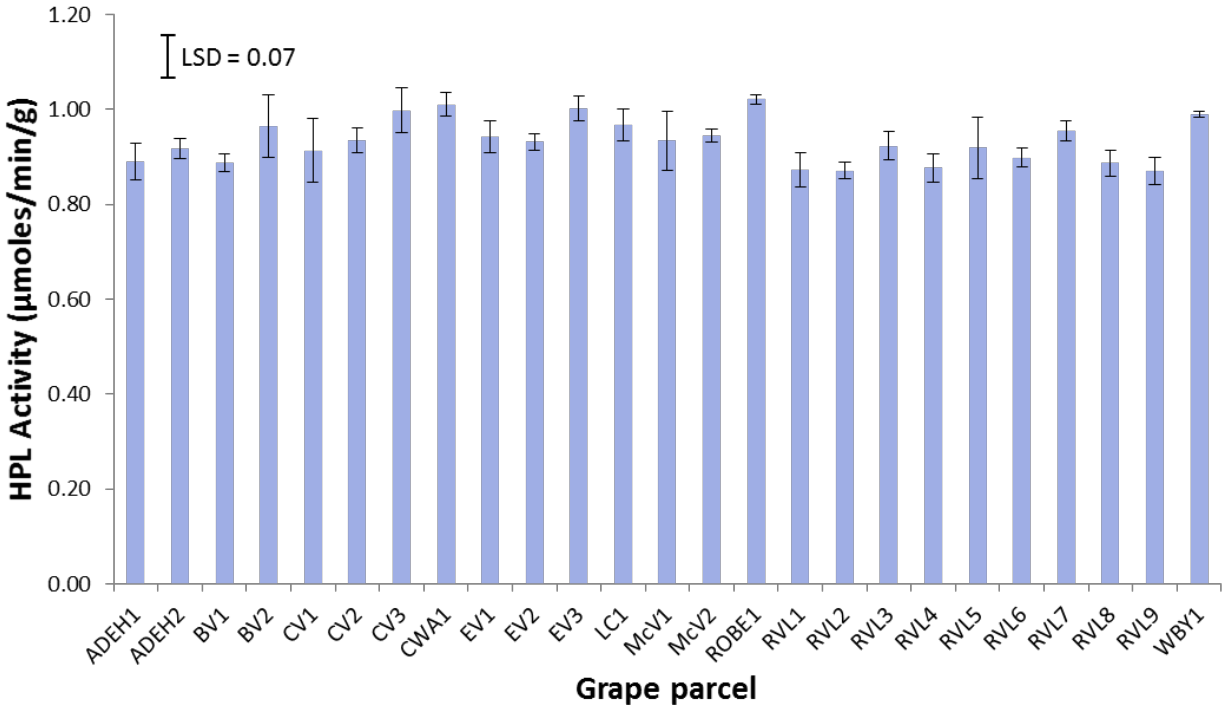


Figure 97. Hydroperoxide lyase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

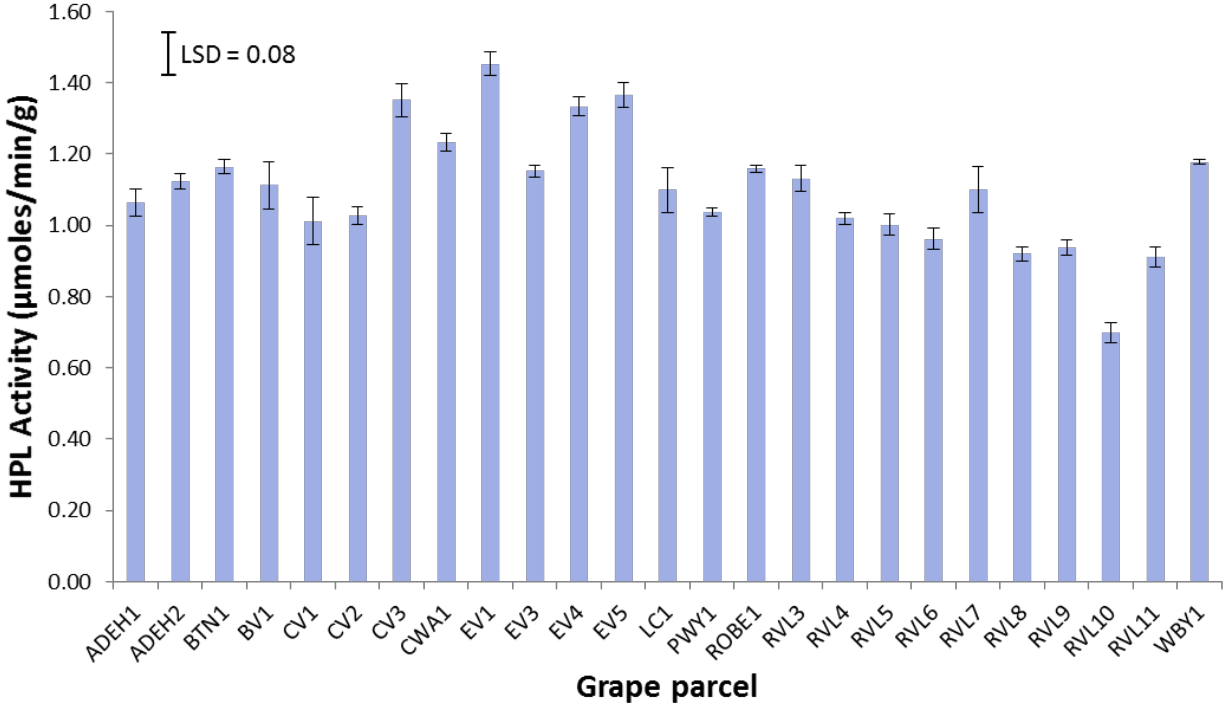


Figure 98. Hydroperoxide lyase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2016. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.4.3.3 Alcohol dehydrogenase (ADH)

Alcohol dehydrogenase is the last enzyme in the lipoxygenase pathway that catalyses the reduction of C_6 aldehydes to alcohols (Figure 49). Differences in ADH activities in the parcels of fruit may alter the ratio between aldehydes and alcohols in the must, although how this might influence wine composition is complicated by the fact that these compounds may be metabolised during fermentation (Dennis et al. 2012). ADH activity was measure in the Chardonnay grape parcels in both years.

Of the three enzymes assayed in the grapes, ADH showed the most variation in activities across the Chardonnay samples similar to what was seen in Cabernet Sauvignon (section 7.1.3.3). In 2015, the ADH activities ranged from 0.38 µmol/min/g in the BV1 sample to 1.63 µmol/min/g in the RVL7 grapes (Figure 99). There were no major trends in ADH activities among the regions and much variation was seen within the RVL samples.

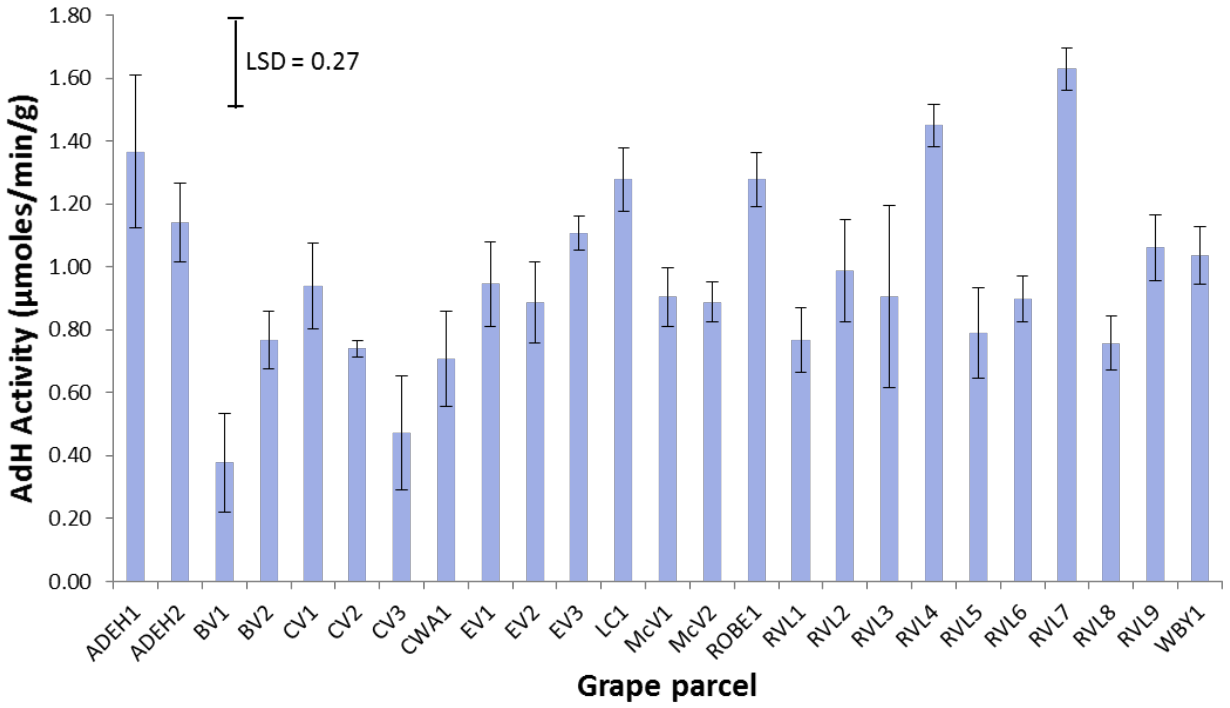


Figure 99. Alcohol dehydrogenase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2015. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

The mean of the ADH activities measured in the grapes from the 2016 vintage (1.13 µmol/min/g) was slightly higher than the 2015 mean of 0.96 µmol/min/g. The ADH activities in the 2016 samples were quite different to those observed in 2015. For example, the BV1 grapes had the lowest ADH activity in 2015, but had one of the highest ADH activities in 2016 (Figure 100). The ADH activities in the 2016 Chardonnay berries ranged from 0.64 µmol/min/g (BTN1) to 1.57 µmol/min/g (RVL3).

Similar to what was observed in the Cabernet Sauvignon samples, the ADH activity measures were the most promising of the three LOX pathway enzymes assayed in the fruit because of the greater range of values measured within each vintage which could lead to greater discrimination in multivariate analyses. The activities in Chardonnay were lower than those observed in Cabernet Sauvignon, but this may be related to differences in harvest timing between these cultivars.

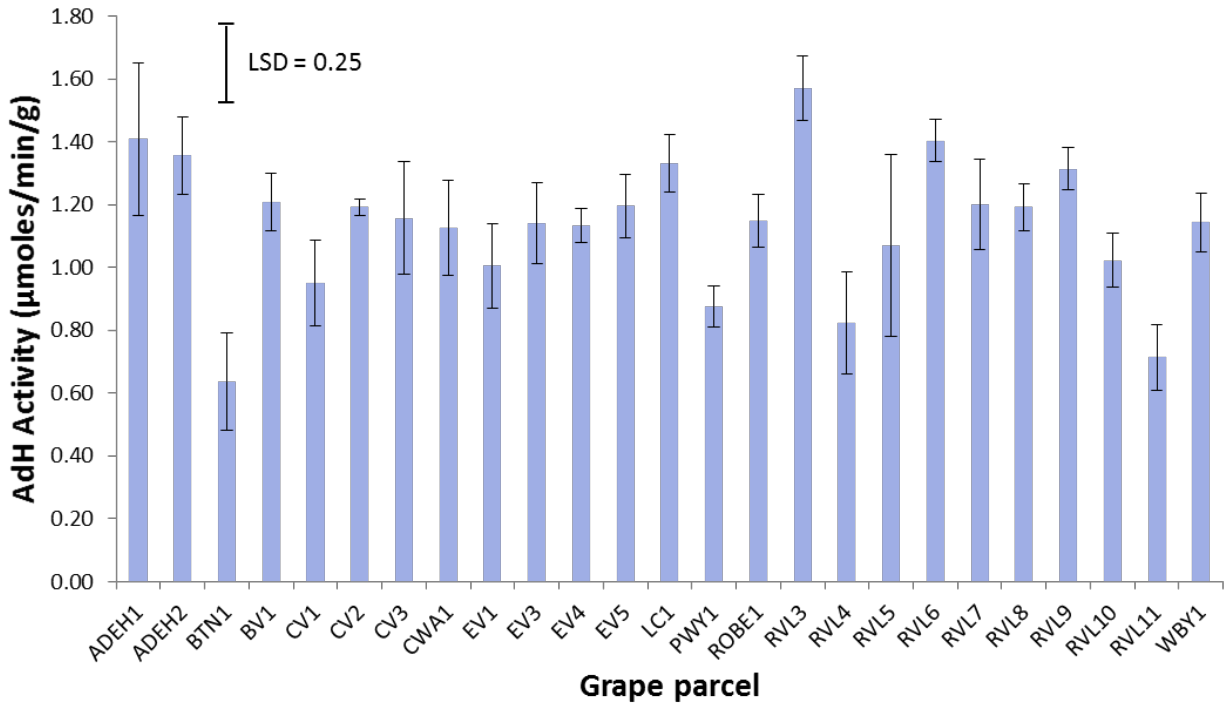


Figure 100. Alcohol dehydrogenase activity in crude protein extracts from homogenates of whole Chardonnay berries harvested in 2016. Error bars represent ± standard error (n=3). LSD = minimum difference between two means for a significant difference at $p < 0.05$.

7.4.4 BERRY SENSORY ASSESSMENT (BSA)

The BSA of 2015 Chardonnay samples allowed them to be discriminated by 21 berry attributes. Samples were grouped by region, such as EV, ADEH, McV, and almost all RVL samples on the first two PCs which together described 65% of the total variation (Figure 101). PC1 discriminated the RVL and McV samples from those harvested from ADEH, EV and WBY, whereas PC2 allowed discrimination between some of the RVL samples. Looking at the sensory attributes significantly different among the grapes, the EV and CWA samples had more acidic pulp and citrus flavor, more resistance in pedicel removal, thicker skins and firmer berries than the other samples. The ADEH and WBY samples were associated with having a gelatinous pulp and the McV samples had sweet pulp with tropical and apple juice flavors. The majority of the 2015 Chardonnay RVL samples were darker in seed color than the other parcels (Figure 101). Also the samples that were located towards the top of the biplot were associated with more astringent and bitter seeds than the other samples. The correlation between some of the sensory attributes was interesting with green apple and citrus tasting pulp being associated with skin thickness, but negatively correlated with tropical and apple juice pulp flavor as well as sweetness (Figure 101).

The 2016 vintage Chardonnay samples were discriminated by 19 berry attributes. The ADEH samples and most of EV samples formed distinct groups that were separate from the remaining samples, many of which clustered around the origin suggesting they weren't discriminated well by the first 2 PCs (Figure 102). The ADEH samples had more pulp on pedicel upon removal, higher skin disintegration and thickness in comparison to the remaining samples. The EV1, 4 and 5 samples had a higher skin tannic intensity, astringency, and bitterness, and high resistance of pedicels upon removal from the berries. Five RVL samples (RVL5, 6, 8, 9 and 10) had pulp that were gelatinous and a yellow berry color. Berries of RVL7 and 11 were firm. Four samples (CWA1, EV3, LC1 and ROBE1) possessed juicy, sweet pulp that was more intense in apple juice flavor, and thick skins.

As was observed with the Cabernet Sauvignon samples, the supplementary variables of basic grape chemistry matched with the sensory attributes. The high TA values correlated with pulp acidity (as did a negative

correlation with pH) and the sweetness of pulp collocated with TSS. There were some common relationships between samples and sensory attributes across the vintages. For example, the EV samples were associated with high pulp acidity and high resistance of pedicels upon removal from the berries in both years. The ADEH samples had greater skin thickness than many of the other samples in both vintages. However, the relation of some of the berry sensory attributes to one another were inconsistent across the two vintages. For example, skin thickness was negatively correlated to apple juice pulp flavor and sweetness in 2015, but positively correlated in 2016 (Figure 102).

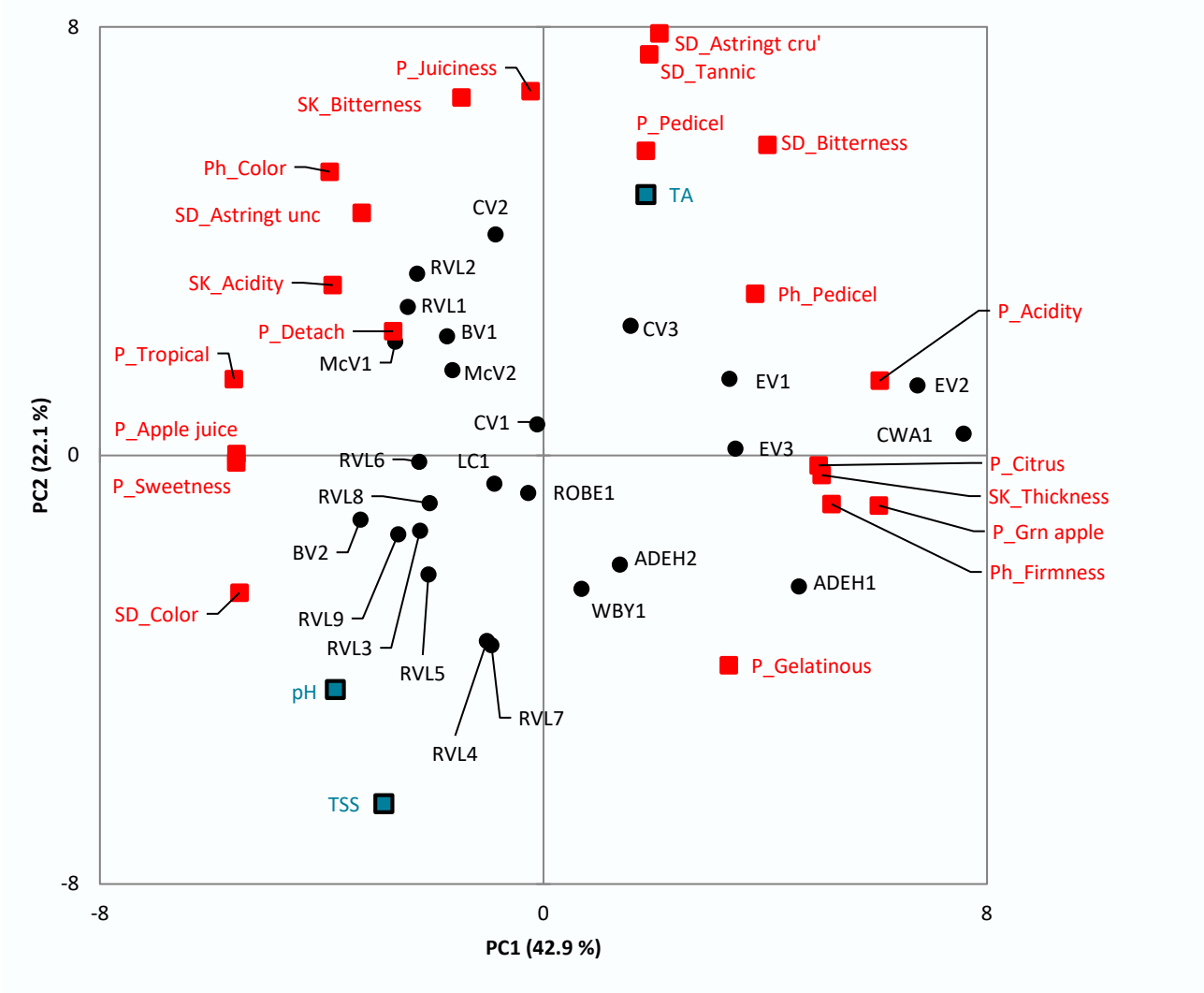


Figure 101. PCA biplot of scores (black) and loadings (red) of 2015 Chardonnay BSA results. Supplementary variables are plotted in addition (blue).

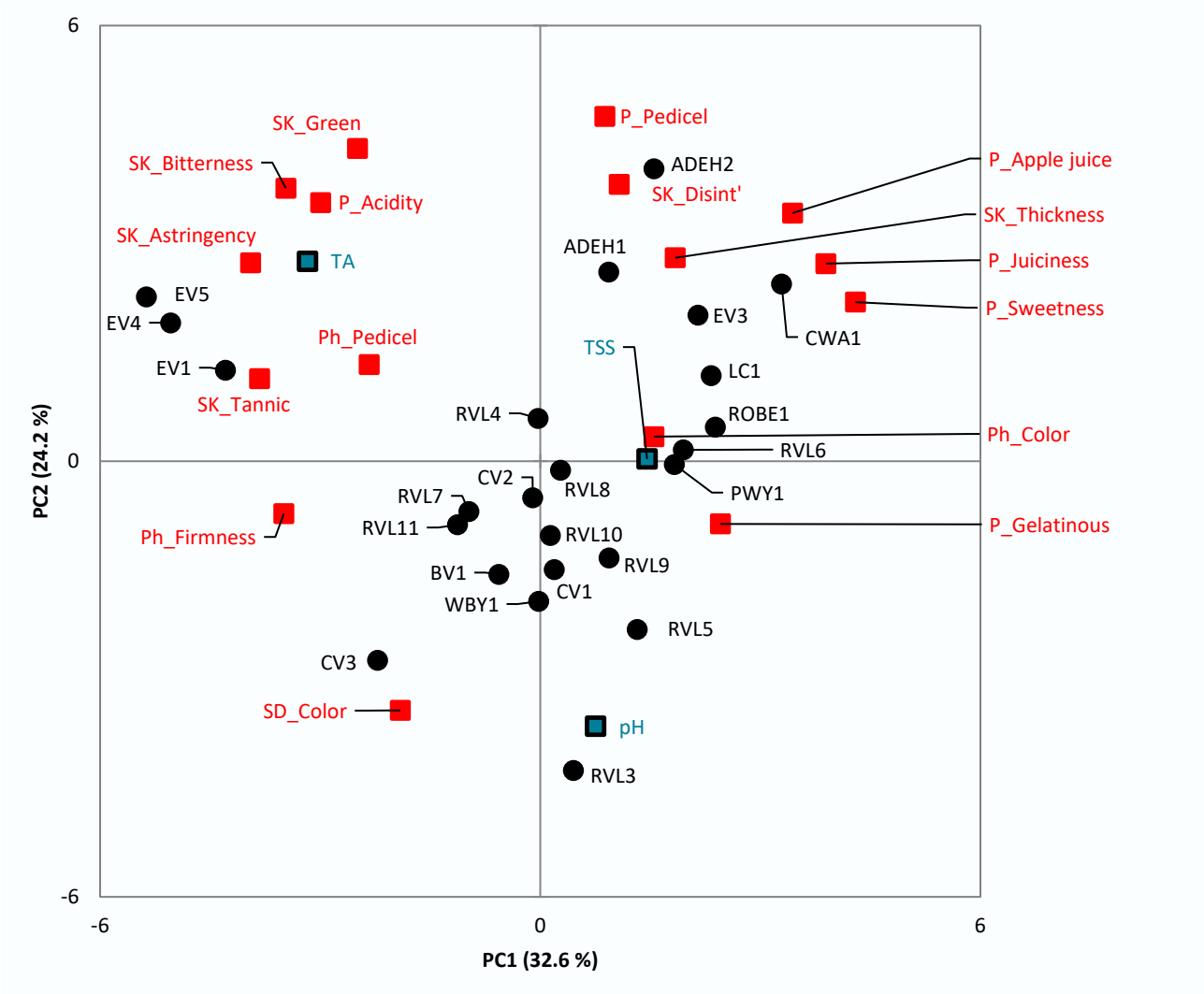


Figure 102. PCA biplot of scores (black) and loadings (red) of 2016 Chardonnay BSA results. Supplementary variables are plotted in addition (blue).

7.5 Chardonnay Wine Analyses

7.5.1 BASIC WINE CHEMISTRY

The wines were produced from the 50 kg parcels of Chardonnay grapes by the WIC Winemaking Service on the Waite Campus in Adelaide. A controlled methodology was used to reduce the variables in the study to those concerning grape composition and not wine-making practices. Unlike the Cabernet Sauvignon wine making protocol it was decided not to put the Chardonnay wines through malo-lactic fermentation. The usual array of wine chemistry measurements were made on the wines. Glucose/fructose and acetic acid were analysed by enzymatic methods, alcohol analysis was performed by NIR, and pH and TA were obtained by auto-titrator. All of these measures were completed by AWRI Commercial Services.

In 2015, the pH of the wines ranged from 2.96 to 3.54 and these were not adjusted before fermentation or after bottling (Table 26). TA was lowest in the EV2 sample at 5.69 g/L and highest in CWA1 (11.71 g/L). The targeted range of alcohols was 12-14%, and most fell into this range. The lowest % alcohols was observed in the CWA1 wine (11.8%), whereas four of the RVL wines had over 14% alcohol. The fermentations all continued to dryness as indicated by the low glucose and fructose measures, and malic acids were high indicating that none of the wines had spontaneously completed malo-lactic fermentation (Table 26). Acetic acid measures were all below 350 mg/L.

Table 26. Chemical measures of 2015 Chardonnay wines. TA= titratable acidity, ALC=alcohol, GLU + FRU= glucose and fructose residual sugar.

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALATE (g/L)	ACETIC ACID (g/L)
ChADEH1	3.08	7.73	13.6	1.0	2.80	0.15
ChADEH2	3.06	7.54	13.6	1.9	2.33	0.17
ChBV1	3.12	9.01	13.0	1.6	2.70	0.21
ChBV2	3.54	5.69	14.7	1.3	3.17	0.23
ChCV1	3.17	8.50	13.2	2.9	2.47	0.21
ChCV2	3.15	11.46	12.8	2.6	2.53	0.23
ChCV3	3.10	10.74	12.4	1.4	2.90	0.21
ChCWA1	2.96	11.71	11.8	0.8	4.57	0.30
ChEV1	3.23	8.13	13.4	0.6	3.13	0.20
ChEV2	3.06	9.56	12.4	0.5	3.60	0.23
ChEV3	3.01	9.20	13.6	1.3	2.90	0.24
ChLC1	3.30	7.97	14.0	1.5	2.63	0.18
ChMcV1	3.22	8.20	13.7	1.2	2.63	0.25
ChMcV2	3.15	8.61	13.6	1.1	2.33	0.23
ChROBE1	3.02	7.70	13.7	1.0	2.00	0.20
ChRVL1	3.44	8.92	12.2	1.6	3.07	0.19
ChRVL2	3.26	8.81	12.2	2.1	3.20	0.17
ChRVL3	3.16	8.93	14.0	0.7	2.67	0.32
ChRVL4	3.47	8.72	14.6	1.9	3.33	0.19
ChRVL5	3.37	7.35	13.0	1.1	2.67	0.22
ChRVL6	3.51	7.41	13.6	1.9	3.17	0.20
ChRVL7	3.54	8.94	14.3	0.1	3.77	0.25
ChRVL8	3.50	7.97	13.7	1.4	3.27	0.26
ChRVL9	3.47	8.46	14.6	1.8	3.37	0.19
ChWBY1	3.14	8.34	13.7	0.9	2.87	0.22

The pH values of the 2016 Chardonnay wines were slightly higher than those in 2015 with means of 3.36 and 3.24 respectively. In 2016 pH varied from 3.16 (BTN1) to 3.89 (RVL5). TAs ranged from 4.49 g/L in RVL5 to 7.72 g/L in CWA1 (Table 27). Many of the wines in 2016 were slightly lower than the targeted alcohol percentage range (12-14%) with nine have less than 12% alcohol and one of these less than 10% alcohol

(CV3). In contrast RVL10 contained 14.1% alcohol (Table 27). The wines were all dry, but unfortunately the BTN1 sample completed malo-lactic fermentation and was therefore eliminated from the wine sensory analyses (Table 27). The maximum acetic acid measure was made in the ROBE1 sample, and at 420 mg/L it should have minimal impact on wine sensory attributes.

Table 27. Chemical measures of 2016 Chardonnay wines. TA= titratable acidity, ALC=alcohol, GLU + FRU= glucose and fructose residual sugar.

PARCEL	pH	TA (g/L)	% ALC (v/v)	GLU + FRU (g/L)	MALIC (g/L)	ACETIC ACID (g/L)
ChADEH1	3.21	7.5	12.3	< 0.3	1.92	0.39
ChADEH2	3.24	7.3	11.8	< 0.3	2.19	0.39
ChBTN1	3.16	4.9	11.1	< 0.3	0.06	0.27
ChBV1	3.47	5	12.2	0.6	1.94	0.25
ChCV1	3.46	4.9	12.1	0.6	1.44	< 0.25
ChCV2	3.39	5.2	11.6	0.3	1.35	< 0.25
ChCV3	3.40	5.1	10.8	< 0.3	1.35	< 0.25
ChCWA1	3.19	7.6	12.3	< 0.3	2.13	0.41
ChEV1	3.24	6	11.2	0.3	1.44	< 0.25
ChEV3	3.38	5.8	12.2	0.5	1.65	0.29
ChEV4	3.26	5.9	12.4	0.5	1.35	< 0.25
ChEV5	3.36	5.5	11.6	0.5	1.29	< 0.25
ChLC1	3.40	6.7	12.4	< 0.3	2.31	0.38
ChPWY1	3.35	5.4	12.1	0.9	1.77	< 0.25
ChROBE1	3.27	7.1	12.9	< 0.3	1.83	0.42
ChRVL3	3.31	5.8	12.3	0.7	1.46	0.28
ChRVL4	3.34	6.3	11.9	0.8	3.26	0.26
ChRVL5	3.89	4.5	12.9	0.7	2.76	0.25
ChRVL6	3.32	6.1	13.3	1.3	2.31	< 0.25
ChRVL7	3.40	6	11	0.3	2.94	0.28
ChRVL8	3.39	5.6	12.1	1.3	2.34	< 0.25
ChRVL9	3.39	5.9	12.5	1.2	2.79	0.26
ChRVL10	3.27	6.7	14.1	< 0.3	2.16	0.31
ChRVL11	3.39	5.7	11.6	0.8	2.42	0.28
ChWBY1	3.40	6.7	12.5	< 0.3	2.16	0.39

7.5.2 WINE VOLATILE PROFILES

As outlined above, a measure of the volatile composition of a wine can give some insights into the potential sensory characteristics that it can possess, although the complex inter-relationships among compounds in the wine make prediction difficult. Nevertheless, it was thought that these data sets may help in deciphering any relationships observed between grape composition and specific wine sensory attributes. For example, they may tell us whether the grape composition to wine sensory relationship is casual via a related change in wine volatile composition or perhaps the grape measure is more a marker of how the fruit has been grown that has, in turn, influenced wine style.

The Chardonnay wine volatile profiles were more complex than the Cabernet Sauvignon ones as 159 compounds were quantified. The wine volatile profile data are again best visualised using PCA to understand how wines relate to one another with regards to their overall volatile composition and which compounds are most associated with different wine samples.

The volatile profiles of the 2015 Chardonnay wines were not modelled very well by PCA. The first two PCs only accounted for 42% of the variation in the data, with PC1 representing 24% and PC2, 18% (Figure 103). There was grouping of samples based on region, with the ADEH, CV and McV samples located in similar positions along PC1, and the RVL sites were loosely grouped at the bottom of the plot. PC1 discriminated two subsets of the RVL samples and separated the BV samples from each other (Figure 103). PC2 separated the RVL samples from most of the wines produced using grapes harvested from other regions. The compounds driving the separation on the right side of the plot, and associated with the McV samples, were ethyl phenylacetate (123), 2-phenylethyl alcohol (139), ethyl 4-methylpentanoate (25), ethyl 2-methyl butanoate (11) and ethyl isovalerate (12). The esters isoamyl acetate (14), (*E*)-3-hexenyl acetate (38) propyl octanoate (71) and propyl decanoate (110) were collocated on the left of the plot along with an unknown compound (47). The vertical separation was driven by several norisoprenoids and terpenoids towards the top of the plot including vitispirane (73), β-cyclocitral (86) and α-cadinol (149), which, in the case of the increased norisoprenoid content, may indicate greater sun exposure of the grapes. However, β-damascenone (129) was one of the compounds that was associated with the RVL Chardonnay samples at the bottom of the plot which suggests this generalisation can't be made for all of the norisoprenoids (Figure 103). Other compounds located at the bottom of the loadings plot were ethyl 4-hydroxybutanoate (124), 4-vinylguiacol (16) and methyl dodecanoate (127).

The pattern of separation of the 2016 samples was different from that observed in 2015, although, again, the PCA model is describing less than 50% of the variation in the first two PCs (Figure 104). PC1 described 33% of the total variation and discriminated most of the RVL, CV and EV wines from those made from grapes harvested from other regions. PC2 separated seven of the RVL wines and the ADEH, BV and LC wines from those produced from other parcels of grapes (Figure 104). Separation on the horizontal axis was driven by a number of esters on the right, including ethyl 2-methylbutanoate (11), ethyl isovalerate (12), isoamyl isovalerate (34), ethyl 3-(methylthio)propionate (80) and ethyl phenylacetate (123) along with isovaleric acid (102). On the left of the plot, the samples had higher concentrations of the esters ethyl crotonate (17), hexyl acetate (32), isobutyl hexanoate (48), ethyl octanoate (56) and octyl acetate (63), and both hexanoic (132) and decanoic acids (164). Similar to 2015, many of the compounds driving separation on the vertical axis were norisoprenoids such as vitispirane (73), Riesling acetal (91) and TDN (116). The samples found toward the bottom of the plot were associated with higher amounts of 2-heptanone (22), 2-nonanone (53), benzaldehyde (72) and ethyl 4-hydroxybutanoate (124) (Figure 104).

Of interest in the wine volatile analysis is the fact that similar compounds were driving the separation on the first two PCs in both years. For example, ethyl 2-methylbutanoate, ethyl isovalerate and ethyl phenylacetate were important variables for PC1 in both vintages and separation on PC2 was driven by the concentration of norisoprenoids. In both vintages, the RVL samples were mainly located in the bottom half of the plot, whereas the norisoprenoids on the loadings plot were located towards the top of the plot. In contrast, the wine samples associated with the three esters consistently found on the right of the loadings plot were different in both vintages. Nevertheless, it suggests that the production of these yeast-derived compounds is strongly driven by fruit composition and hence they are important for the discrimination of wines made from different regions.

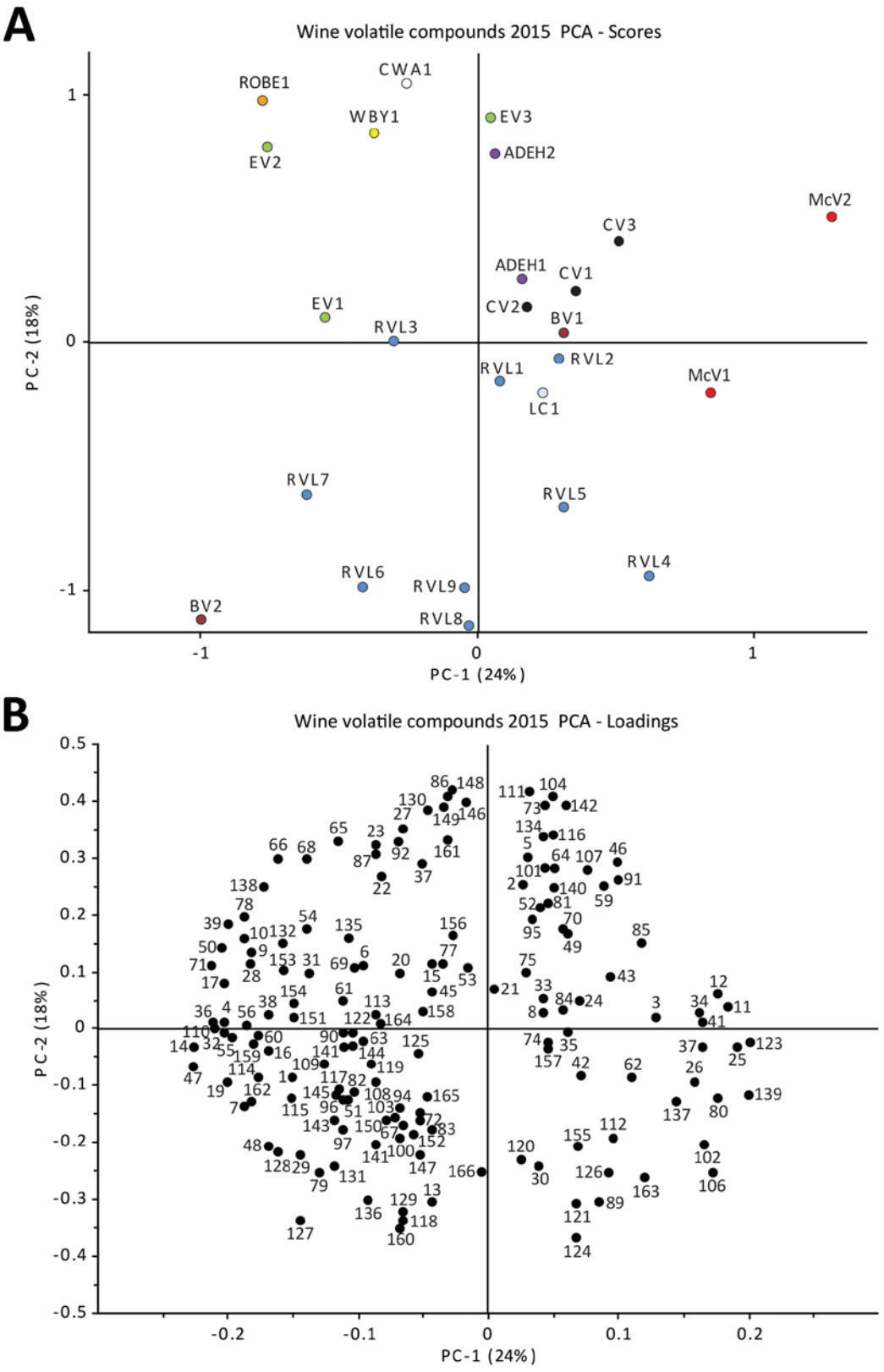


Figure 103. PCA scores (A) and loadings (B) plots of 25 different 2015 Chardonnay wines from South Australia based on their volatile profiles. The denotations of the wine volatile compounds are listed in Table 9.

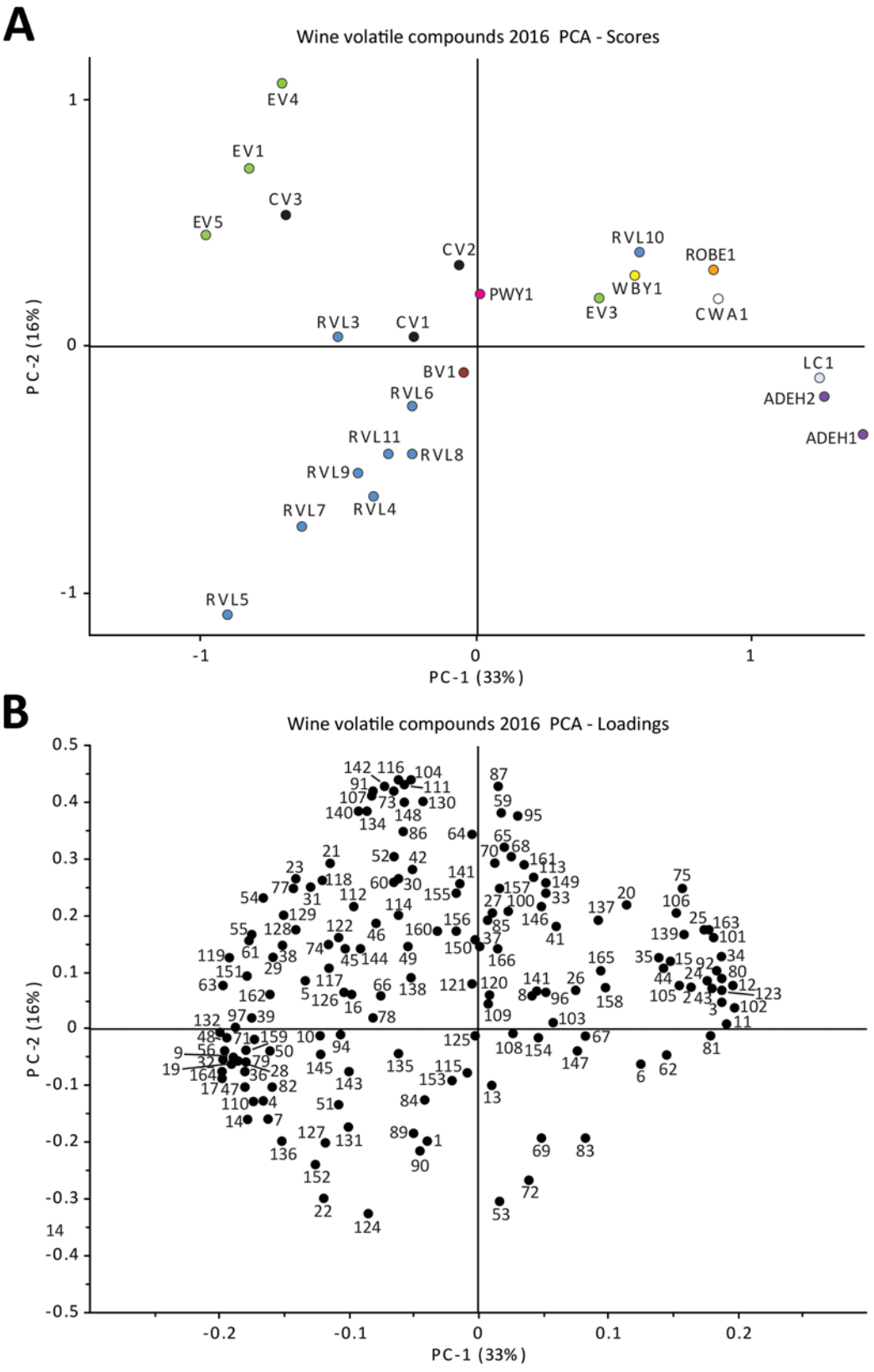


Figure 104. PCA scores (A) and loadings (B) plots of 25 different 2016 Chardonnay wines from South Australia based on their volatile profiles. The denotations of the wine volatile compounds are listed in Table 9.

7.5.3 WINE DESCRIPTIVE SENSORY ANALYSIS

Descriptive sensory analysis was also conducted on the Chardonnay wines from both vintages using a trained sensory panel. For the Chardonnay wines, 23 sensory attributes were assessed by the trained panel, including aroma, taste, flavour, mouthfeel and aftertaste descriptors (Table 11). All wines were assessed within a year of bottling using the protocol outlines in section 6.4.3.

In 2015, 20 attributes were significantly different across samples ($p < 0.05$). Significant attributes included those from the aroma (6), taste (3), mouthfeel (3), flavour (3), and aftertaste (5) categories. These attributes were analysed using PCA to visualise the entire data set for associations among attributes and wine samples, and the first two dimensions account for just over 61% of the explained variance (Figure 105). Samples were discriminated most by sour taste and aftertaste on PC1, as well as astringent aftertaste and mouthfeel, bitterness, heat, and viscosity, and further by tropical fruits and peach flavours and aromas, and earthy and medicinal aromas on PC 2 (Figure 105). The Chardonnay wines made from the CWA and EV fruit tended to be more sour and astringent with citrus flavour while those on the left of the plot, especially the RVL6 and BV2 wines, were bitter, hot and viscous. The CV and McV wines, and to a lesser extent those made from EV grape parcels, grouped together, but these groups were not discriminated by any particular loadings.

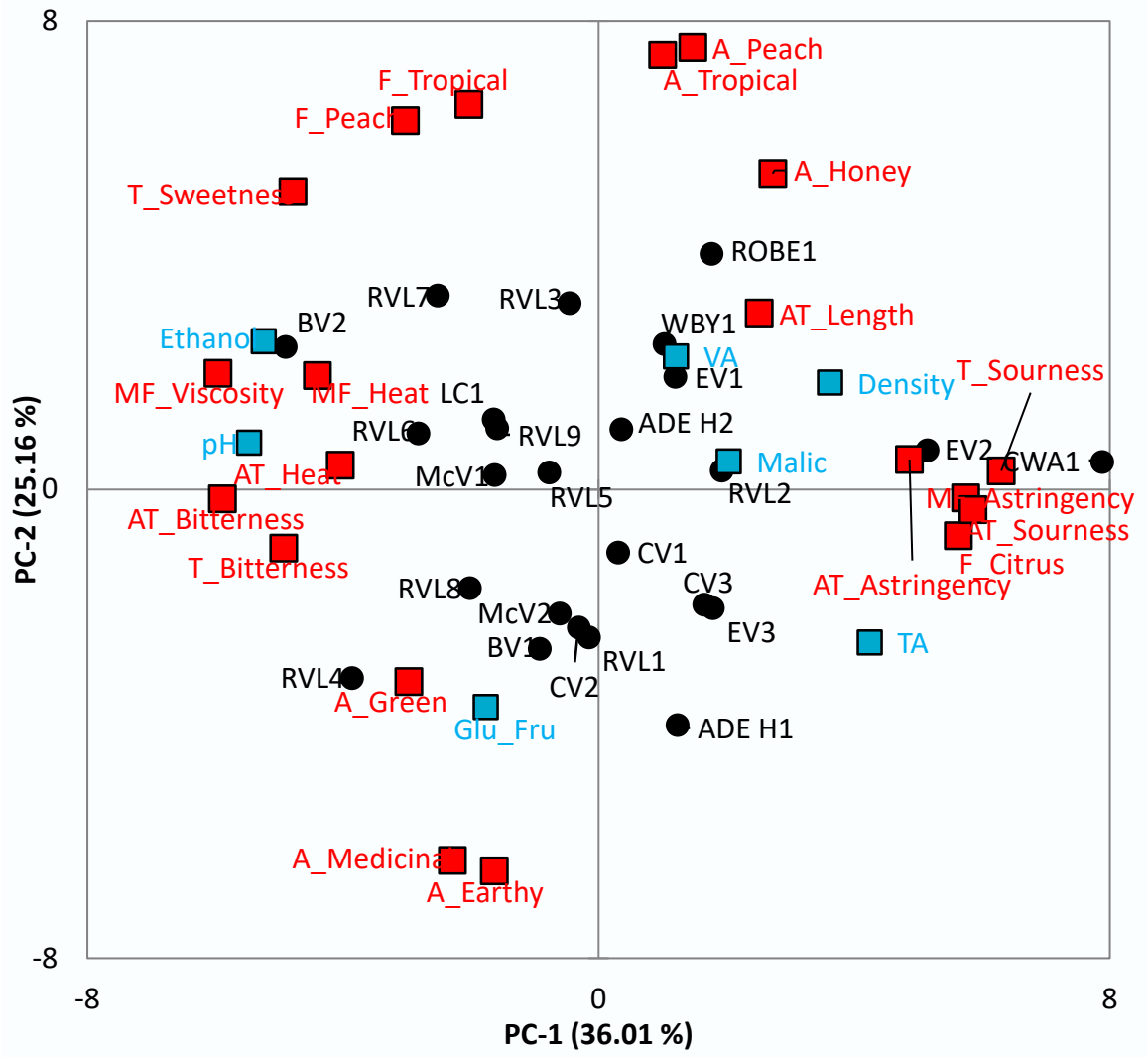


Figure 105. PCA biplot of the scores and loadings on PC 1 vs 2 using significantly different attributes of 2015 Chardonnay wines. Sample names are ADE H = Adelaide Hills, BV = Barossa Valley, CV = Clare Valley, CWA = Coonawarra, EV = Eden Valley, LC = Langhorne Creek, McV = McLaren Vale, RVL = Riverland, ROBE = Robe, WBY = Wrattonbully.

In 2016, only 24 of the 25 wines were assessed by the trained panel as the BTN1 wine was eliminated from the study as it had completed malo-lactic fermentation unlike the rest of the wines. Fourteen of the 20 sensory attributes assessed by descriptive analysis were found to be significantly different ($p < 0.05$) among the 2016 Chardonnay wine samples. These included attributes from the aroma (3), taste (2), mouthfeel (2), flavour (4), and aftertaste (3) categories. Again the significantly different attributes were analysed using PCA and the first two principle components accounted for 68% of the explained variance (Figure 106). The first thing to note is that there were eight samples that were not discriminated very well by the sensory analysis and thus they clustered in the centre of the biplot. The sensory attributes associated with the EV5, BV1, CV1, RVL3 and RVL5 wines on the right of the plot were peach and tropical flavours, sweetness, and tropical aroma. The wines on the opposite side of the plot (i.e. ROBE1, RVL10 and WBY1) had more medicinal and astringent characters (Figure 106). Two other clusters of wine samples were characterised by having higher scores for honey aroma (CV3, RVL7 and RVL4) or citrus flavour and sourness (ADEH1, ADEH2 and CWA1)

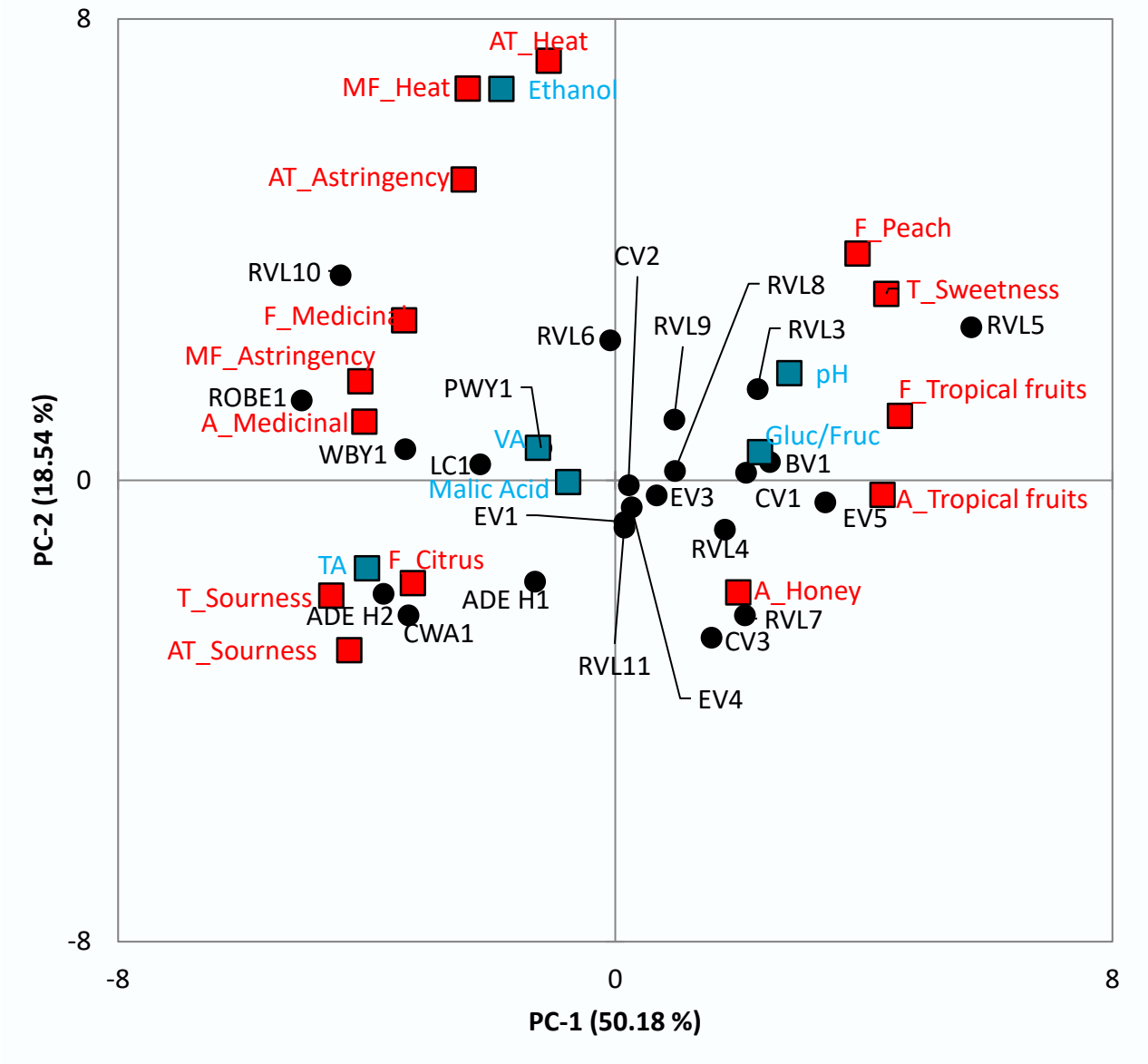


Figure 106. PCA biplot of the scores and loadings on PC 1 vs 2 using significantly different attributes of 2016 Chardonnay wines. Sample names are ADE H = Adelaide Hills, BV = Barossa Valley, CV = Clare Valley, CWA = Coonawarra, EV = Eden Valley, LC = Langhorne Creek, McV = McLaren Vale, PWY = Padthaway, RVL = Riverland, ROBE = Robe, WBY = Wrattonbully.

For both vintages, chemical measures agreed with related sensory attributes including heat with ethanol and sourness with TA and pH (negative). In the 2016 vintage, residual sugar was correlated with sweetness. Similar to the BSA results, scores did not consistently project with the same loadings across vintages, indicating that regional differences were not among the primary drivers of consistent sample discrimination across vintages.

Interestingly, while significantly different sensory attributes were detected in both the 2015 and 2016 Chardonnay wines, their effect sizes were small, which indicated that the actual perceptual differences across the samples were subtle. From a perceptual perspective, it is likely that the scores that were most extremely projected on the PCA biplots, opposite to each other, were the samples that were perceivably different while the remaining wines in between were not.

7.5.4 WINE QUALITY ASSESSMENT USING A WINEMAKER PANEL

In an attempt to correlate specific wine sensory attributes to the perceived quality of the wines, the Chardonnay wines from the 2 vintages were graded by winemakers. It was thought that indications of the perceived quality of the wines may also provide an opportunity to find relationships with the perceived quality of the grapes. Wine makers (n = 6 – 9) were recruited for evaluation from within South Australia and assessed the wines as outlined in section 6.4.4.

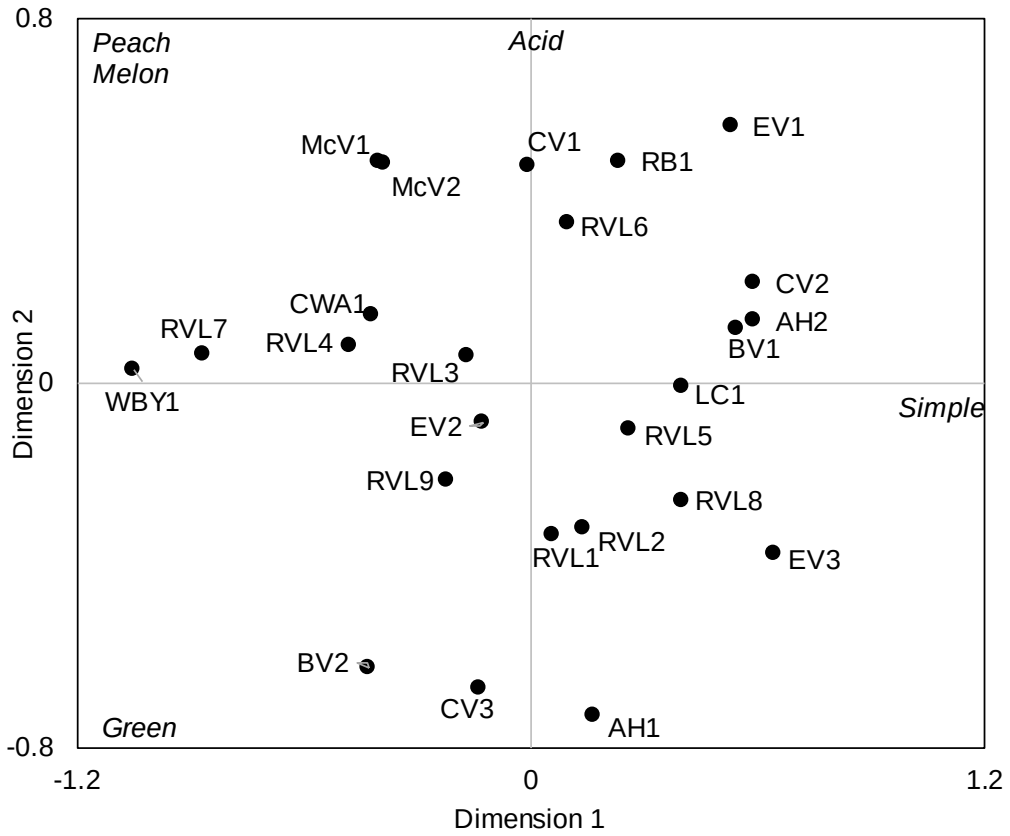


Figure 107. MDS plot of Chardonnay wines from 2015. Descriptions under the plot denote for generic characters of wines associated with different quality as depicted by the arrows. Descriptions in *italic* within the plot denote for characters specific to the samples projected in the different areas of the matrix. (Note: AH = Adelaide Hills and RB1 = Robe in this figure)

The Chardonnay wines, in contrast to what was observed with the Cabernet Sauvignon wines, were not well discriminated by quality scores. The samples were also not discriminated by region in either year (Figure 107 and Figure 108). Further, the differences in wine qualities were much narrower than that seen for the

Cabernet Sauvignon wines, ranging from low to moderate qualities in both the 2015 and 2016 vintages. This lack of discrimination in quality was reflected on the MDS plots where sample discrimination was based only on sensory descriptions (Figure 107 and Figure 108). The relatively poor discrimination in wine quality suggests that Chardonnay wine quality is not strongly driven by differences in the fruit when the wines are all vinified with an identical protocol. It is possible that the winemakers' perceptions of Chardonnay wine quality were biased by them being informed that the wines were vinified without any oak contact; oak is considered a major driver of Chardonnay quality (Gambetta et al. 2016). The results of the winemaker quality assessment suggest that Chardonnay quality is less driven by the raw material and perhaps more driven by vinification parameters. A finding that warrants further investigation is the tropical flavour character that have emerged in some of the Chardonnay wines (Figure 105 and Figure 106). It is possible that this character was due to the presence of varietal thiols, which are normally associated with Sauvignon blanc, but have recently been linked to Chardonnay wines (Capone et al. 2017). Indeed, analysis of the varietal thiols in the 2015 Chardonnay wines found high concentrations in the RVL7, WBY1 and McV1 samples, which correlated with tropical and peach flavour in those wines (Sanders 2016). Interestingly, informal discussions with the winemakers revealed that they considered that tropical characters indicated the fruit was overripe, which is a characteristics of low quality Chardonnay wines. It is difficult to come to this conclusion with certainty in the current study, especially given that there was a lack of quality discrimination among the Chardonnay wines, and this requires further study.

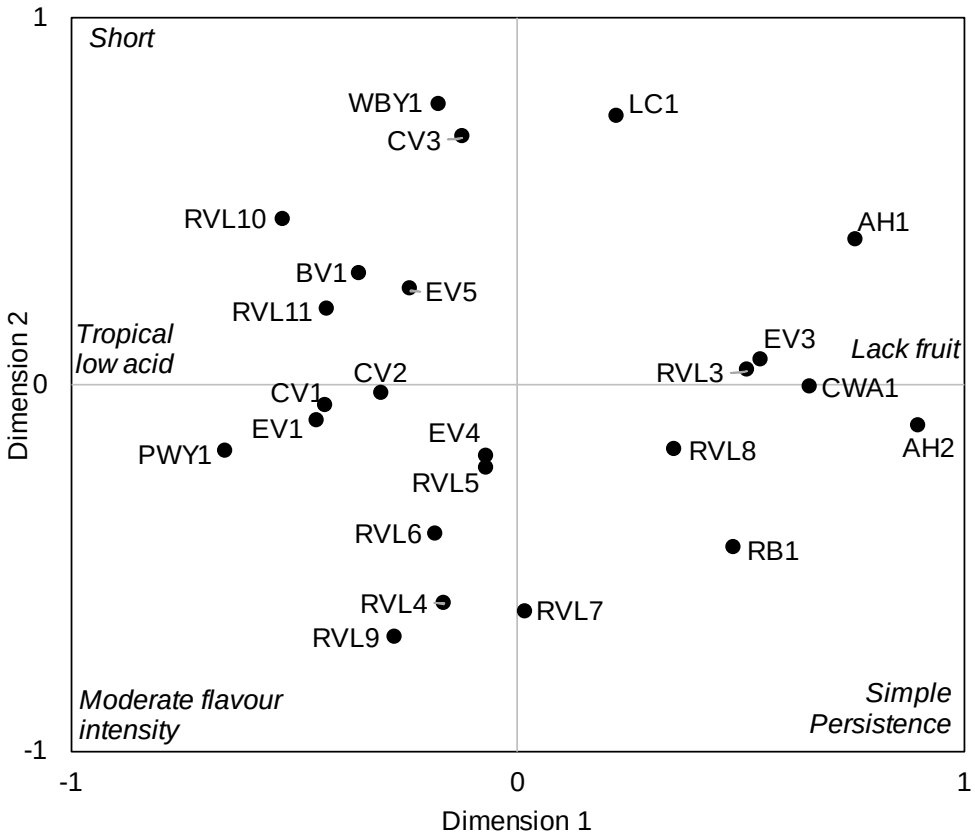


Figure 108. MDS plot of Chardonnay wines from 2016. Descriptions under the plot denote for generic characters of wines associated with different quality as depicted by the arrows. Descriptions in *italic* within the plot denote for characters specific to the samples projected in the different areas of the matrix. (Note: AH = Adelaide Hills and RB1 = Robe in this figure)

7.6 Relating Chardonnay Grape Measures to Wine Sensory Perception

7.6.1 RELATING BERRY SENSORY ANALYSIS (BSA) TO WINE DESCRIPTIVE ANALYSIS

Berry sensory assessment is used to predict wine style and hence grape quality, but there are limited robust studies testing these relationships. Work in Shiraz (Olarte Mantilla et al. 2015) and Cabernet Sauvignon (Niimi et al. 2017) have found some correlations, but these are either limited to a few wine sensory attributes or inconsistent across vintages. Scores from the BSA were compared with those from wine sensory perception in the Chardonnay samples from both vintages and it was found that Chardonnay did not produce consistent models between 2015 and 2016 vintages (Table 28). Wine sour taste and sour aftertaste were modelled best (0.66% and 0.62% validated explained variance, respectively in 2015 vintage). Ethanol content and pH were also modelled satisfactorily with BSA and grape chemical measures. On the other hand, BSA of the 2016 Chardonnay wines only produced poor models and all attributes that were modelled had an R^2 of validated explained variance below 0.5. A likely reason for this is because the Chardonnay grapes did not possess as much textural and visual sensory dimensions as the Cabernet Sauvignon grapes. Therefore the relative difficulty in discriminating the sensory characteristics of Chardonnay wines was also reflected in the poor models. Using BSA for to indicate potential wine style for Chardonnay does not appear to be possible because of this. For more details of this work see Niimi et al. (2018).

Table 28. PLS1 models determined for wine sensory attributes for 2015 and 2016 vintages using BSA attributes and grape chemistry variables. T = taste, MF = mouth-feel, and AT = aftertaste.

VINTAGE	Y-VARIABLE	NO. COMPONENTS	R^2_{CAL}	R^2_{VAL}	$RMSE_{CAL}$	$RMSE_{VAL}$
2015	T_Sourness	1	0.716	0.666	4.3	4.9
	T_Sweetness	1	0.668	0.602	3.1	3.5
	MF_Astringency	1	0.616	0.533	3.5	4.0
	MF_Heat	1	0.613	0.526	3.5	4.0
	MF_Viscosity	1	0.663	0.586	2.7	3.1
	AT_Length	1	0.650	0.589	3.4	3.9
	AT_Bitterness	1	0.612	0.566	4.2	4.6
	AT_Sourness	1	0.671	0.618	4.3	4.9
	Ethanol	2	0.892	0.854	0.25	0.31
	pH	1	0.835	0.808	0.07	0.08
2016	T_Sourness	1	0.309	0.227	5.9	6.5
	T_Sweetness	1	0.320	0.233	5.6	6.2
	AT_Sourness	1	0.397	0.350	5.7	6.2
	Ethanol	1	0.469	0.340	0.57	0.66

7.6.2 MODELLING OF GRAPE BERRY MEASURES TO CHARDONNAY WINE SENSORY PROFILES

Extending the applicability of the grape metabolomics measures and their prediction of sensory attributes in wine for the Chardonnay variety was also an objective of the current project. While less groups of chemical measures were made in the Chardonnay grapes compared to Cabernet Sauvignon (9 versus 12 different measurement types), this still required a structured approach that would appropriately handle these different data blocks. Therefore, the data was again analysed using sequential and orthogonalised-partial least squares (SO-PLS) modelling method (Naes et al. 2011).

The nine grape chemical measures were used as predictor blocks to relate to wine sensory measures and, as explained above in section 7.3.2, the same processes of using PLS and SO-PLS data analyses were applied. The summary of the data blocks used for the modelling process is shown in Table 29.

Table 29. Identities of blocks and their analysis method, use for the data analysis of Chardonnay grapes. X blocks are predictor data and Y are the response to be predicted.

DATA BLOCK*	MEASUREMENT	ANALYSIS METHOD
X_{01}	Harvest measures	Weight, TSS‡, pH, TA#
X_{02}	Targeted volatile compounds	GC-MS
X_{03}	Non-targeted volatile compounds	GC-MS
X_{04}	Bound volatile compounds	GC-MS
X_{05}	Amino acids	HPLC
X_{06}	Lipoxygenase pathway enzyme activities	Spectrophotometric
X_{07}	Tannins	HPLC
X_{08}	Flavonols	HPLC
X_{09}	Fatty acids	GC-MS
Y	Sensory profiles	Descriptive analysis

Again variation by vintage was greater than the variation among the samples and so vintage effect was removed before modelling. A total of 16 wine sensory attributes were modelled using the nine Chardonnay grape chemical measures (Table 30). Five attributes were modelled using PLS1 where optimum models were obtained with only one X block. These were: honey and medicinal aromas, sweet taste, green apple flavour, and length of aftertaste. Length of aftertaste was modelled the poorest, where two components were used to obtained a validated explained variance marginally above 10%, the lower limit set for the analyses (Table 30). This attribute was modelled with targeted volatile compounds. The remaining eleven attributes were modelled with SO-PLS1, with bitter aftertaste being modelled with the highest explained variance of 56.7%.

This attribute was modelled with harvest measures followed by amino acids. However, this differed from bitter taste, which was modelled with non-targeted volatiles and flavonols (Table 30).

Table 30. Validated explained variances, RMSECV, and associated number of components used for each X block to model individual sensory attributes of Chardonnay wines spanning the 2015 and 2016 vintages. C_ = colour, A_ = aroma, F_ = flavour, T_ = taste, and MF_ = mouthfeel.

Y VARIABLES	BLOCK		RMSECV[A]	COMPONENTS[B]	EXPLAINED VARIANCE	
	1ST	2ND			CALIBRATED	VALIDATED
A_Tropical fruits	X05	X08	0.777	1_3	50.3	39.6
A_Honey	X02		0.855	2_1	46.3	26.8
A_Medicinal	X05		0.933	1_1	17.0	12.9
T_Sourness	X09	X06	0.751	2_1	61.0	42.7
T_Sweetness	X09		0.820	2	49.2	32.7
T_Bitterness	X03	X08	0.828	2_1	61.5	31.4
F_Citrus	X06	X03	0.789	1_2	65.2	37.7
F_Tropical fruits	X05	X07	0.709	1_1	59.4	49.7
F_Green apple	X01		0.900	1	33.1	18.8
F_Peach	X01	X05	0.833	1_1	45.1	30.5
MF_Heat	X01	X09	0.706	1_2	71.8	50.2
AT_Length	X02		0.939	2	36.7	11.7
AT_Bitterness	X01	X05	0.658	1_2	68.5	56.7
AT_Sourness	X09	X04	0.760	2_1	61.6	42.2
AT_Heat	X01	X04	0.741	1_2	71.0	45.0
AT_Astringency	X09	X02	0.824	2_1	59.2	32.0

[A]Root mean square error of cross validation. [B]Components with more than one block are arranged as those for the first block_second block.

Some of the attributes confirmed expectations of how they might be modelled. For example, heat in both mouthfeel and aftertaste were modelled with harvest measures and samples with high TSS were often found to have high scores for heat intensity in both mouthfeel and aftertaste. An interesting finding was that tropical fruits aroma and flavour were both modelled with amino acids as the first block. These could show the links of tropical fruit flavours to amino acids such as cysteine, where a portion of thiols are cysteinylated in must and later released into wine (Thibon et al. 2008). These precursors are known to be in Chardonnay juice (Capone et al. 2017) and results from our study further demonstrate that this sensory characteristic can be present in Chardonnay wines. Some other attributes also showed possibly associative correlations with X data blocks. Examples were the green apple and peach flavour attributes, where the two attributes were modelled with harvest measures (X01). Within the harvest measures block, green apple flavour was correlated with titratable acidity, while peach flavour was correlated with high pH and °Brix values.

Unlike the Cabernet Sauvignon modelling, each of the nine data blocks (grape measures) were used to model at least one of the Chardonnay wine sensory attributes (Table 30). Harvest measures and amino acids were used in five of the models, whereas measures of tannins were used only once, and as the second block in the model predicting tropical flavour (Table 30). The non-targeted volatile compound block, which was not used for modelling in Cabernet Sauvignon, modelled bitter taste and citrus flavour in the Chardonnay samples.

Also, measures of bound volatiles and lipoxygenase enzymes in the Chardonnay grapes were included in the models for hot and sour aftertaste and citrus flavour and sour taste of the Chardonnay wines respectively. Some of the X data blocks used in the models may be more correlative than causative. For example, it was found that measures of fatty acids (X09) contributed to the models of the taste attributes of sourness and sweetness, and sourness and astringent aftertastes. Fatty acids above 10 carbons in length are not known to elicit sour, sweet, or astringent perceptions although some have been described as bitter and pungent (Schiffman and Dackis 1974).

7.7 Grape Secondary Metabolite Pathways

One of the aims of the project was to study pathways involved in the production of grape secondary metabolites that were involved in the production of compounds that have the potential to influence wine composition and hence sensory attributes. Two classes of grape compounds that seem to be common to most varieties are the C_6 aldehydes and alcohols, which are produced as a result of the lipoxygenase pathway, and the C_{13}-norisoprenoids which result from the degradation of carotenoids (Dunlevy et al. 2009). Therefore, we studied aspects of these pathways during grape berry development or in some of the samples collected in this study to investigate the regulation of flux through these pathways and to explore the possibility of using gene expression data to gauge berry quality.

7.7.1 GRAPE DEVELOPMENTAL SAMPLES

The Cabernet Sauvignon grapes used to study changes in gene expression or enzyme activities during berry development were sampled from a vineyard in the Riverland. Grape bunches were collected fortnightly, starting at two weeks post-flowering (2wpf), and transported to the laboratory on ice for berry weight and total soluble solids (°Brix) measurements (Figure 109). Veraison occurred between 8 and 10 wpf, and increases in berry weight followed the normal developmental patterns (Figure 109).

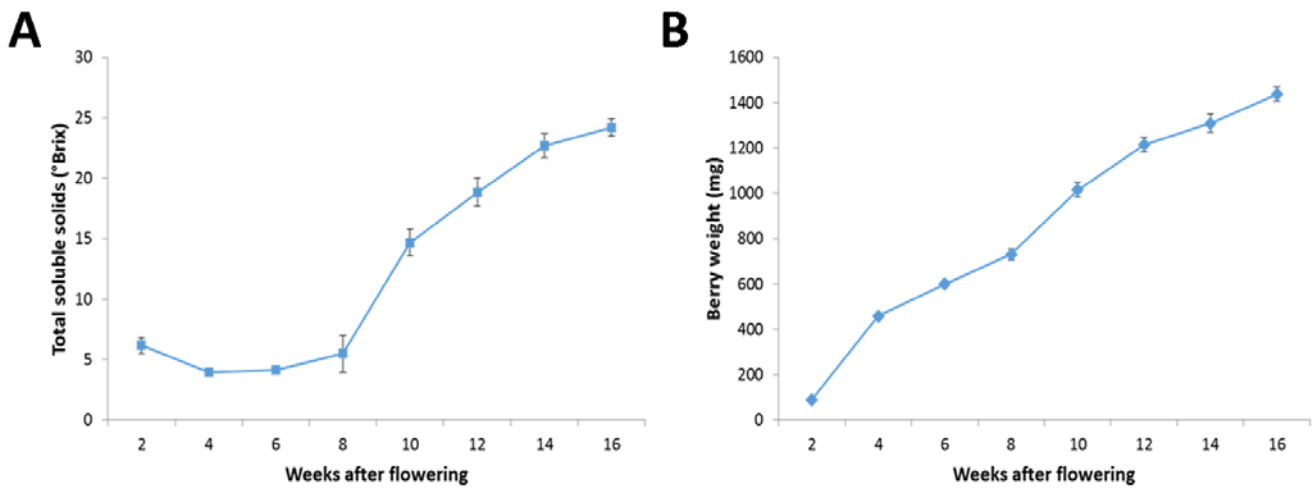


Figure 109. Total soluble solids and berry weight throughout Cabernet Sauvignon berry development.

7.7.2 LIPOXYGENASE PATHWAY

As outlined in section 7.1.3, the lipoxygenase pathway (Figure 49) is highly active in grape berries and results in the production of C_6 alcohols and aldehydes from the unsaturated fatty acids linoleic and linolenic acid. There are three main enzymes in the pathway: lipoxygenase, hydroperoxide lyase and alcohol dehydrogenase. All three enzymes are encoded by multigene families in grapes and a number of these genes have been cloned and characterised in previous studies (Tesniere & Verries 2000, Podolyan et al. 2010, Zhu et al. 2012). qRT-PCR assays for a number of these genes were developed to study them all in one developmental series of grapes. The availability of enzyme assays for each of these three enzyme classes also allowed these activities to be followed during berry development and related to changes in the expression of the corresponding genes. The amount of volatile C_6 compounds was also determined in the samples.

7.7.2.1 Total Lipoxygenase-derived Volatile Compounds

The total flux through the pathway can be studied by looking at the final products, which are the C_6 alcohols and aldehydes and their corresponding esters. The most abundant volatile compounds produced as a result of lipoxygenase pathway activity were quantified in the grapes throughout development. The compounds quantified included: hexanol, hexanal, hexyl acetate, (*E*)-2-hexenal, (*E*)-2-hexenol, (*E*)-3-hexenol, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate. The total abundances of these compounds are shown in Figure 110. There appeared to be two phases of LOX-derived compound production during berry development. The first occurred very early in berry development and may be a carry-over from the metabolism of the flower. There was then a second peak in production of these compounds immediately after veraison (Figure 110). The final stages of berry development around harvest were characterised by a general decrease in the concentration of LOX-derived volatile compounds.

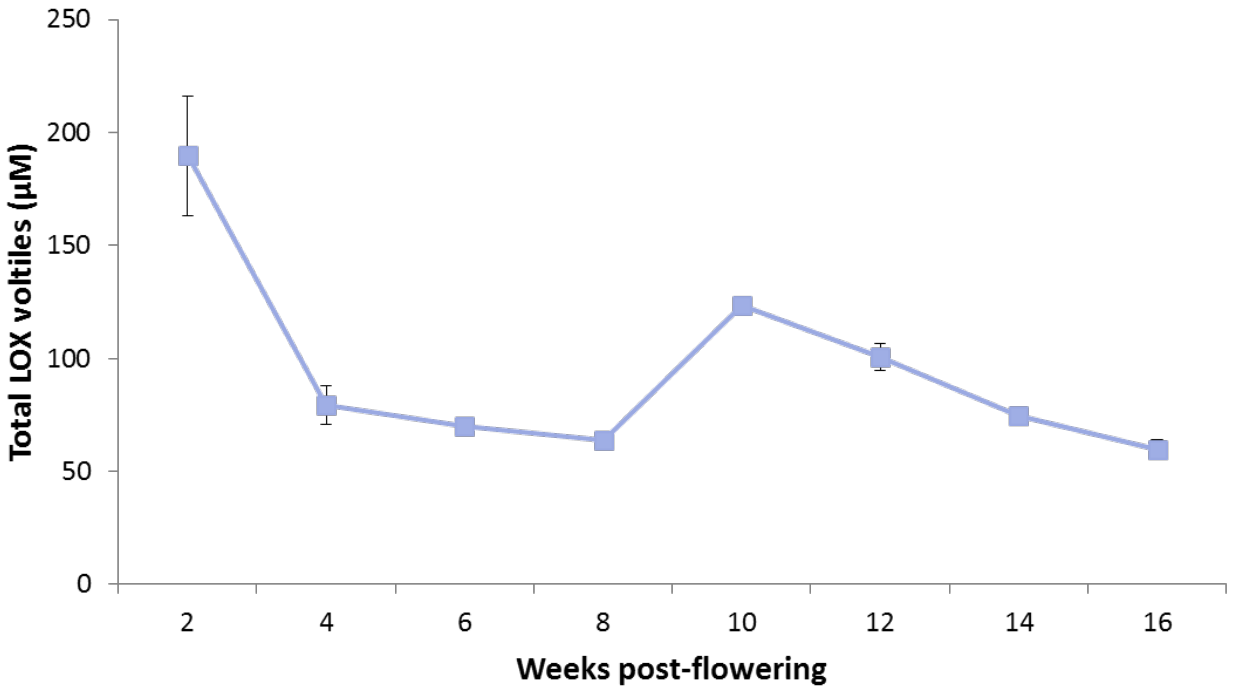


Figure 110. Total LOX-derived volatile compounds quantified in Cabernet Sauvignon berries sampled throughout berry development. Error bars are ± SE, n = 3.

7.7.2.2 Lipoxygenase Enzyme Activity and Gene Expression

The total lipoxygenase activity was determined in berries at each of the developmental stages using the method outlined in section 6.3.10.1. It was found that LOX activity was highest in the grapes early in development, between 2 and 4 wpf (Figure 111). There was then a dramatic decrease in LOX activity which remained low until 8 wpf. After verasion there was then a steady increase in the LOX activity in the Cabernet Sauvignon berries through to harvest (Figure 111).

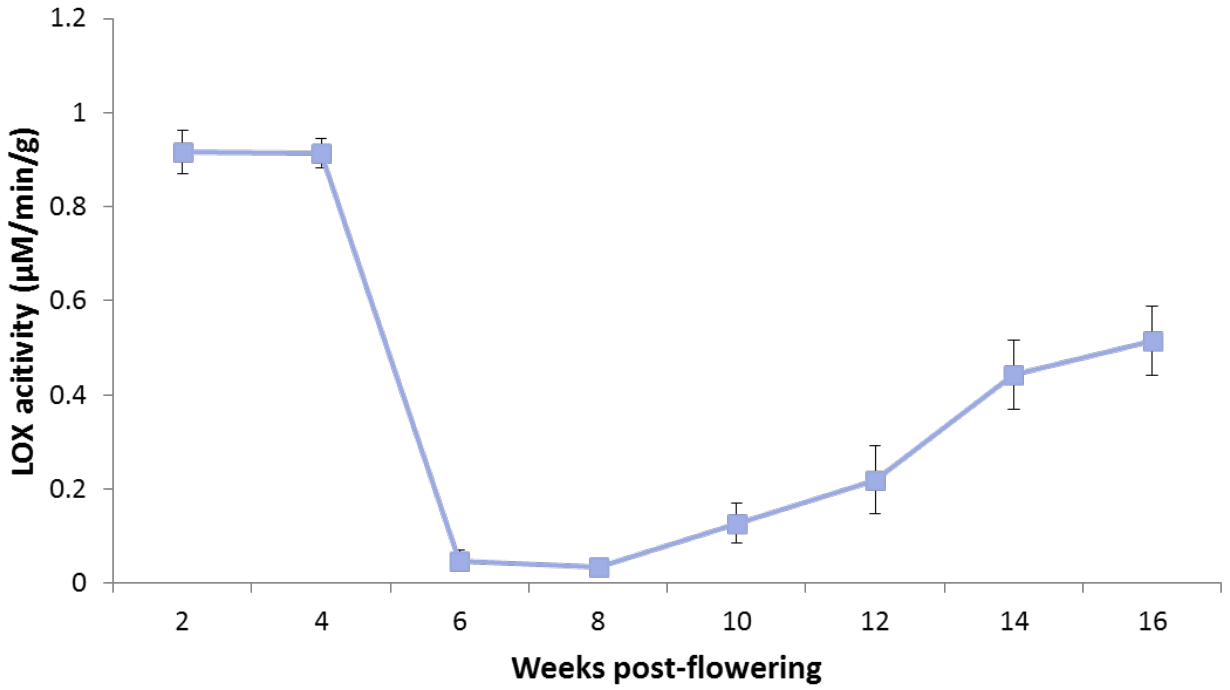


Figure 111. LOX activity quantified in Cabernet Sauvignon berries sampled throughout berry development. Error bars are ± SE, n = 3.

The expression of LOX genes was also studied in this Cabernet Sauvignon berry developmental series. We focused on the putative 13-LOX genes as these are the enzymes that lead to the production of C_6 compounds. There are also grape genes that encode 9-LOX enzymes, which are involved in the production of nine-carbon volatile compounds. The LOX genes in the grape genome that encode the 13-LOX genes were identified by Podolyan et al. (2010) and are listed in Table 31.

Table 31. Grape 13-LOX genes examined in this study.

GENE ID	LOCUS NUMBER	CHROMOSOME
VvLOXA	LOC100243312	Chr 6
VvLOXE	LOC100241684	Chr 6
VvLOXG	LOC100262173	Chr 6
VvLOXH	LOC100255333	Chr 6

GENE ID	LOCUS NUMBER	CHROMOSOME
VvLOXJ	LOC100257867	Chr 13
VvLOXO	LOC100241150	Chr 9
VvLOXP	LOC100244202	Chr 1
VvLOXS	LOC100267183	not-mapped to any Chr

Primers were designed within or overlapping with the 3'-untranslated regions of these genes and expression was quantified in the berries throughout development. No expression was detected for the *VvLOXG* and *VvLOXS* genes in any of the samples.

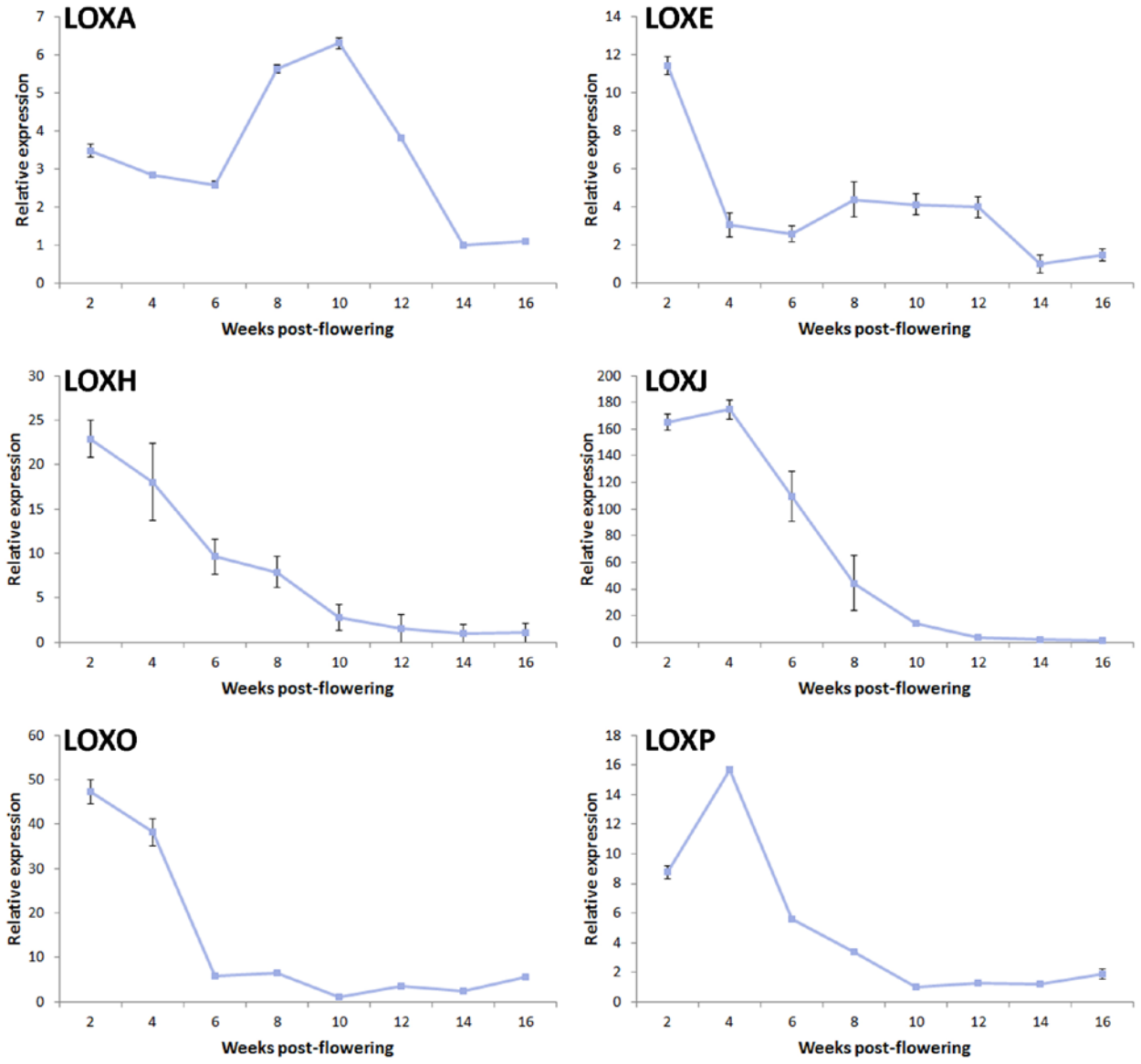


Figure 112. Relative expression of six 13-LOX genes throughout Cabernet Sauvignon berry development.

Five of the six 13-LOX genes that were expressed in the berries showed a similar pattern of expression during development (Figure 112). *VvLOXE*, *H*, *J*, *O* and *P* were expressed at the highest amount in the 2 and/or 4 wpf samples, after which expression decreased and remained relatively low through until harvest. LOXA peaked in expression at 10 wpf after which expression decreased and remained low in the last two sample points (Figure 112). It is interesting to note that none of the 13-LOX genes had an expression pattern that matched the total 13-LOX activities measured in the same fruit samples (Figure 111). There could be several reasons for this. First, it is likely that multiple 13-LOX genes were contributing to the overall enzyme activity in the fruit. The LOXA and LOXO genes have been shown to be expressed in different grape berry tissues (Podolyan et al. 2010), but the assays in this study were conducted using total berries and so the relative contributions of each gene cannot be discriminated. Second, it is possible that the stability of the enzyme in the berry changes over time. For example, early in development the enzyme may be rapidly turned over due to the presence of specific proteases, but after veraison these may be absent which allows the slow accumulation of 13-LOX activity over time from relatively low 13-LOX gene expression. Third, it is possible that some of the 9-LOX genes that we did not examine for gene expression, actually encode enzymes with dual-positional-specificity and have both 9- and 13-LOX activity. This has been observed in other plant species (e.g. Liu and Han 2010) and so it is possible that these types of genes may contribute to the activity seen in the berries. Nevertheless, it appears that multiple genes are contributing to 13-LOX activity in the grapes which makes the use gene expression as an assay for LOX pathway flux difficult.

7.7.2.3 Hydroperoxide Lyase Enzyme Activity and Gene Expression

Hydroperoxide lyase activity was also determined in berries at each developmental stage using the method outlined in section 6.3.10.2. In contrast to LOX, HPL activity was highest in the grapes sampled after veraison (Figure 113). HPL activity was lowest in the 2 wpf samples then steadily increased until 10 wpf, after which HPL activity slightly declined through to harvest (Figure 113).

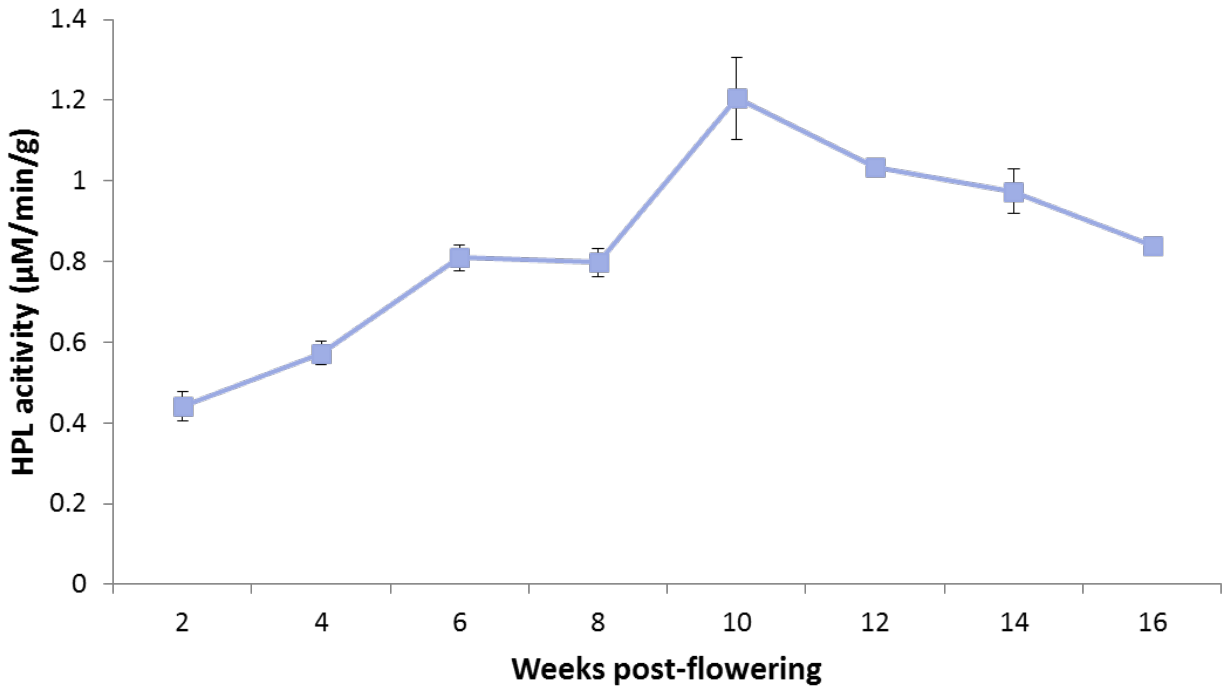


Figure 113. HPL activity quantified in Cabernet Sauvignon berries sampled throughout berry development. Error bars are ± SE, n = 3.

Zhu et al. (2012) characterised two *HPL* genes in grapevine. These were called *VvHPL1* and *VvHPL2* and correspond to the locus numbers LOC100251750 and LOC100526774 respectively. Both were expressed in the Cabernet Sauvignon developmental fruit samples. The expression of *VvHPL1* was low until veraison when

it increased ~2-fold, followed by a decline in expression through to harvest. The expression of *VvHPL2* was variable in the biological replicates, but was generally higher in the 8 week period from 6 to 12 wpf compared to the other samples (Figure 114).

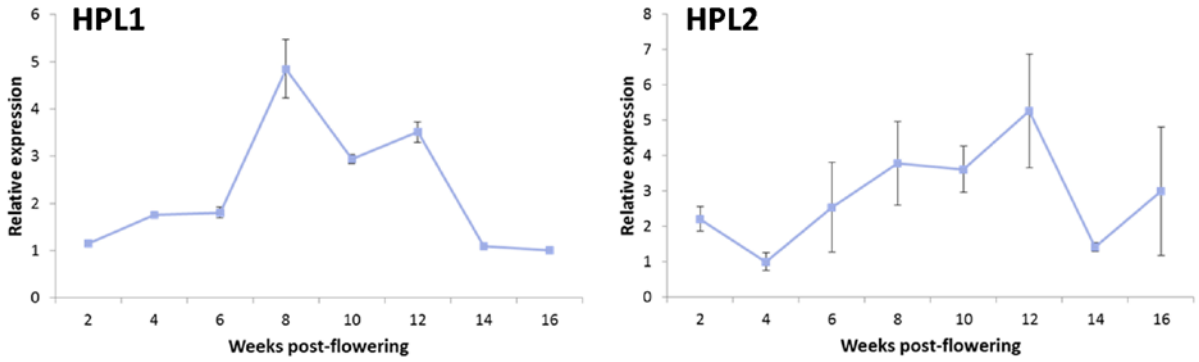


Figure 114. Relative expression of two HPL genes throughout Cabernet Sauvignon berry development.

The expression of neither gene matched the change in HPL enzyme activity in the samples (Figure 113). We would expect gene expression to slightly precede enzyme activity as it may take time for the protein to accumulate after mRNA transcription and subsequent translation of the protein. It is likely that both genes were contributing to the total enzyme activity which again would make developing assays from the gene expression difficult.

With regards to the relationship between enzyme activity and the flux through the pathway, to determine the overall amount of lipoxygenase pathway products (Figure 110), it appears that neither LOX nor HPL activity was limiting C_6 compound production during development. In fact, it appears that the high amount of lipoxygenase pathway products early in development is driven by high LOX activity (and gene expression) and that the subsequent increase after veraison may have more to do with HPL activity and gene expression. This putative biphasic control of the pathway again makes using activity or gene expression measures to determine flux through the pathway in different grape parcels difficult.

7.7.2.4 Alcohol Dehydrogenase Enzyme Activity and Gene Expression

When studying the effect of ADH activity on products of the lipoxygenase pathway, it is probably more important to look at the ratio of aldehyde to alcohol C_6 products rather than the total LOX pathway products. So the ratios between the main C_6 aldehydes and their respective alcohols were calculated at each stage of development (Figure 115). The first thing to note is that the calculated ratios were very low as the amount of aldehyde greatly exceeded that of the corresponding alcohol. For the hexanol:hexanal ratio, this showed a constant rise from the 2wpf sample up to 14 wpf, after which the ratio decreased slightly (Figure 115). The ratio between the unsaturated C_6 pair of (*E*)-2-hexenol:(*E*)-2-hexenal peaked at 4wpf followed by a decrease until after 8wpf when the ratio increased again (Figure 115).

Alcohol dehydrogenase activity was also determined in the developmental series of Cabernet Sauvignon grape samples. ADH activity was generally low in the fruit until 12 wpf, when expression began to increase and the highest activity was measured in the harvest sample at 16 wpf (Figure 116). The change in activity of the ADH enzyme across development does not match the changes in the alcohol:aldehyde ratio (Figure 115). It is likely responsible for the increase in the alcohol:aldehyde ratios observed later in fruit development, but the pre-veraison increase in the ratios cannot be explained by higher ADH activity in the fruit. It is possible that the relative stability of either C_6 compound had an effect. There was a large decrease in LOX activity (Figure 111) and LOX-derived volatile compounds (Figure 110) early in fruit development, and so if (*E*)-2-hexenol was more stable than (*E*)-2-hexenal in the fruit this may change the ratio in an environment when the total flux through the pathway was greatly reduced. It is also possible that the aldehyde was being converted to a product other than the alcohol at these stages of development, perhaps into the

corresponding carboxylic acid through oxidation. Likewise the alcohol may be formed from other precursors, perhaps by esterase activity on a relevant ester. Either way it would appear that measurements of ADH activity may not be good indicators of the relative amounts of C_6 alcohols and aldehydes during berry development.

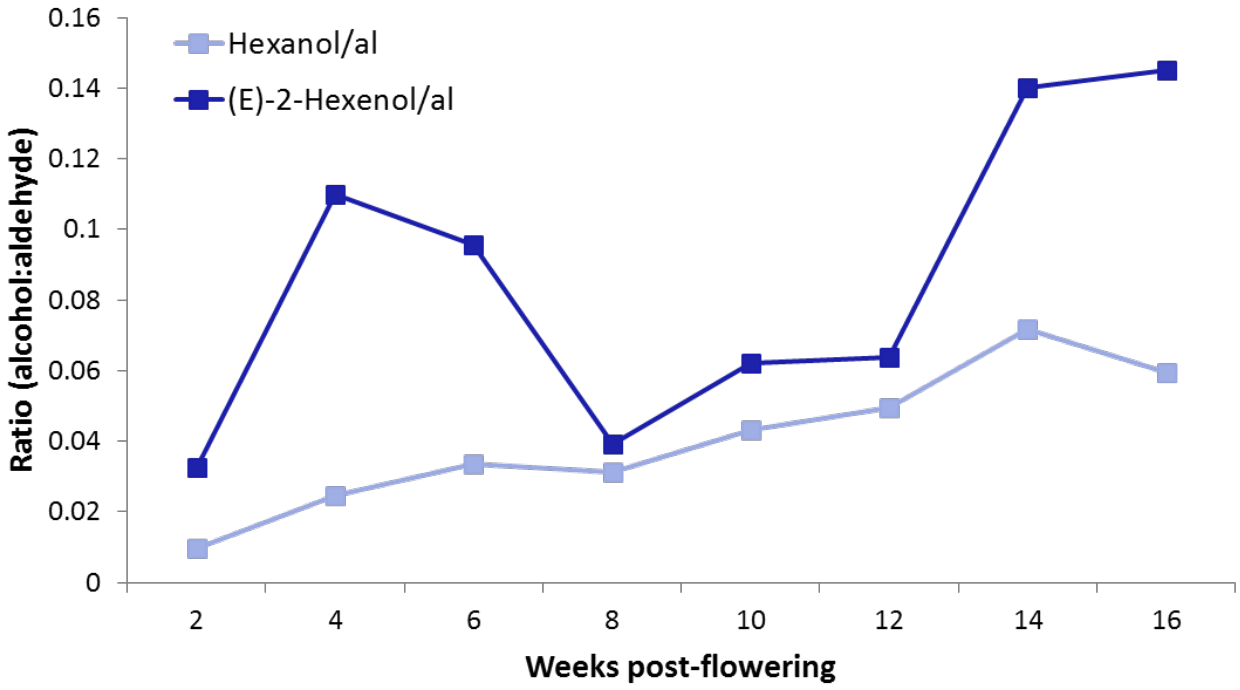


Figure 115. Ratio of alcohol to aldehyde for the two main C_6 compound classes found in grape berries.

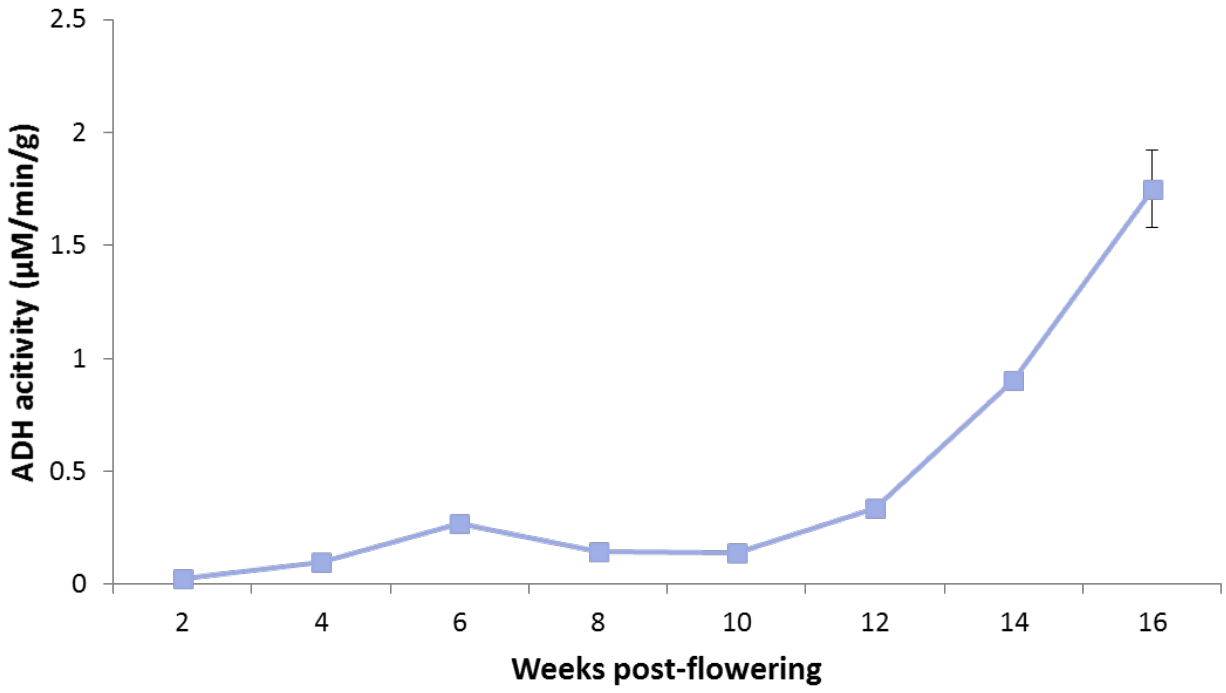


Figure 116. ADH activity quantified in Cabernet Sauvignon berries sampled throughout berry development. Error bars are ± SE, n = 3.

Tesniere and Verries (2000) identified three ADH genes in the grape genome, namely *VvADH1* (LOC100232853), *VvADH2* (LOC100232854) and *VvADH3* (LOC100233102) and their expression was determined in the Cabernet Sauvignon berry developmental series (Figure 117). Of the three genes examined, it appeared that *VvADH2* may have had the biggest impact on ADH enzyme activity in the fruit. Its expression began to increase at veraison, preceding the increase in ADH activity (Figure 116). However, expression tended to level off after 12 wpf, while ADH activity continued to rise, presumably as the amount of enzyme accumulated due to the continued high level of ADH gene expression. This suggests that assaying the expression of *VvADH2* may not be a good predictor of ADH activity or the amount of C_6 alcohols in the fruit.

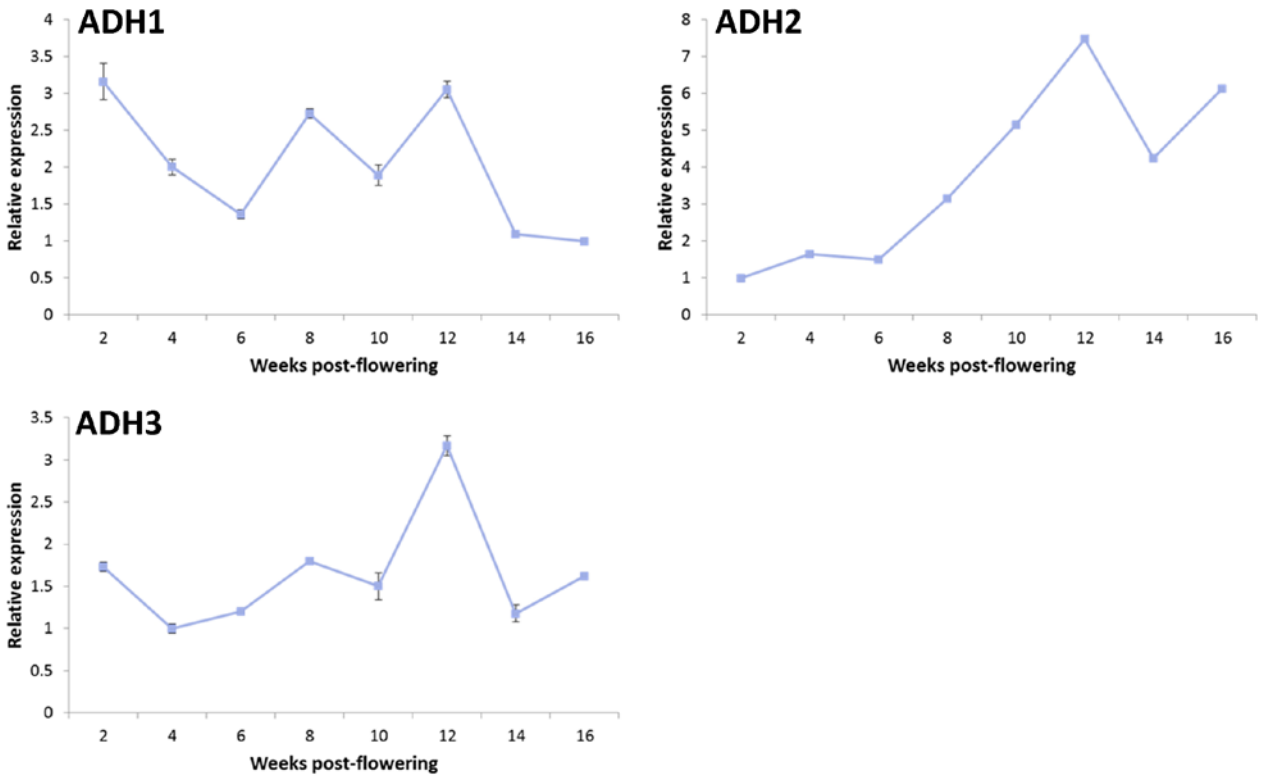


Figure 117. Relative expression of three ADH genes throughout Cabernet Sauvignon berry development.

7.7.3 CAROTENOID DEGRADATION (NORISOPRENOID PRODUCTION)

Norisoprenoids that are important for wine flavour, such as β-damascenone and β-ionone, are formed by the enzymatic degradation of carotenoids (for review see Dunlevy et al. 2009). It if thought that these 13-carbon norisoprenoids are produced predominantly by the activity of enzymes known as carotenoid cleavage dioxygenases (CCDs; Mathieu et al. 2005).

7.7.3.1 Norisoprenoids in Wines from Throughout Development

Because the norisoprenoids are present as both free and bound forms, the quantification of these compounds in the fruit is difficult. The samples that were used in the developmental experiments outlined in this report had also been vinified and the wine volatile compounds studied in a previous project (Boss et al. 2018). This allowed us to observe the potential for the grapes to contribute to the norisoprenoid pool in corresponding wines throughout development.

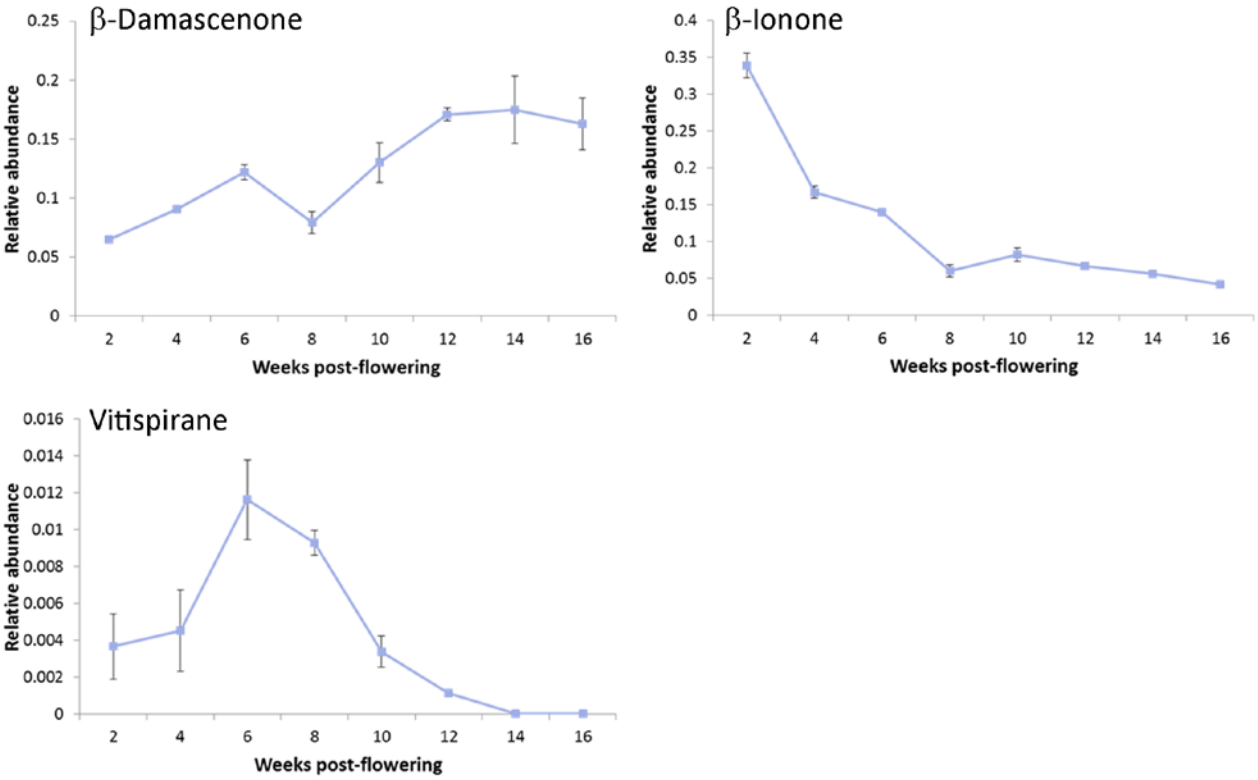


Figure 118. Norisoprenoids quantified in Cabernet Sauvignon wines produced from berries sampled throughout berry development. Quantified relative to an internal standard. Error bars are ± SE, n = 3.

All three of the main C_{13}-norisoprenoids measured had different accumulation patterns in the wines (Figure 118; Boss et al. 2018). β-Damascenone had two phases of increasing abundance separated by a slight drop in concentration at veraison, whereas β-ionone was present in maximal amounts in the first sample taken 2 wpf, and concentration subsequently decreased until levelling off at veraison (Figure 118). Vitispirane accumulation was different again, peaking at 6wpf and then decreasing through to harvest (Figure 118).

7.7.3.2 Expression of Carotenoid Cleavage Dioxygenases (CCDs) Throughout Development

The expression of the *CCD* genes did not seem to match any of the accumulation patterns of the norisoprenoids very well (Figure 119). All three of the genes increased in expression after veraison, which implicates them in the production of β-damascenone. However, all three genes were not expressed very highly in the early samples when β-ionone and vitispirane were present in the wines in the highest amounts. It is possible that there are other *CCD-like* genes in grapevine that might catalyse the production of these norisoprenoids given that the *VvCCD4* genes do not seem to have the ability to make β-ionone (Lashbrooke et al. 2013). It is also possible that the carotenoids were being degraded into norisoprenoids by the action of lipoxygenases. It is assumed that co-oxidation of carotenoids can occur when LOX generates a peroxyl radical by enzymic oxidation of an unsaturated lipid (Wu et al. 1999). While the action of the CCD enzymes should be more specific and generate a C_{13}-norisoprenoid, co-oxidation via lipoxygenase activity should happen at any double bond on the carotenoid and so should result in norisoprenoids of other lengths. Such compounds, such as β-cyclocitral and 6-methyl-5-hepten-2-one are sometimes seen in grape and wine samples. The potential for there to be multiple origins of norisoprenoids again suggests that measuring CCD gene expression may not be a good indicator of the potential of a parcel of grapes to produce some of these important flavour and aroma compounds.

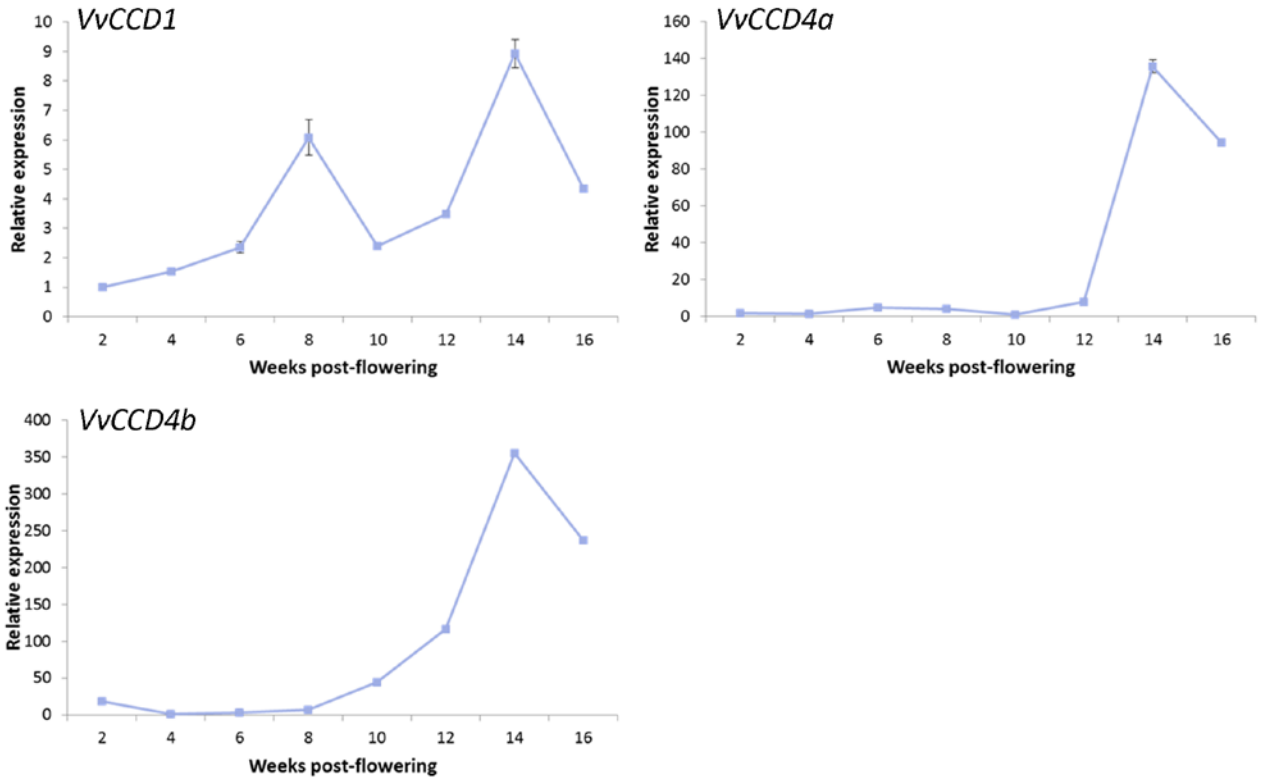


Figure 119. Relative expression of three CCD genes throughout Cabernet Sauvignon berry development.

7.7.3.3 Carotenoids in Ripe Grapes and Correlations with Grape and Wine Norisoprenoids

As carotenoids are the precursors of the norisoprenoids, an alternative approach to predicting norisoprenoid content in the wine might involve measurement of carotenoids. If CCD activities in the grapes are relatively similar or not limiting, the availability of substrate may determine the concentration of norisoprenoids in the grapes and wines. With this in mind, a method to quantify individual carotenoids was established in the laboratory and these compounds measured in the 2015 Cabernet Sauvignon and Chardonnay samples. The identity of five carotenoids was confirmed in the extracts: β-carotene, lutein, zeaxanthin, neoxanthin and violaxanthin. PCA of the carotenoid concentrations along with relevant norisoprenoids quantified in the grapes and wine enabled inter-relationships to be visualised.

For the Cabernet Sauvignon samples, the BV, CV and EV sites produced fruit with the most β-carotene, lutein, zeaxanthin and neoxanthin (Figure 120). These four carotenoids clustered together in the PCA biplot suggesting that there are growing conditions that encourage the accumulation of almost all of the carotenoids. The concentration of free grape β-ionone also collocated with these four carotenoids, which is not surprising given it probably originates from the degradation of β-carotene (Dunlevy et al. 2009). Violaxanthin did not group with the other carotenoids and was one of the main drivers of separation on PC2 (Figure 120). The concentrations of bound vitispirane and β-damascenone in the grapes and also the free aglycones present in the headspace of the wines were collocated with violaxanthin at the top of the PCA. This was somewhat surprising given that it is predicted that the precursors of β-damascenone originate from neoxanthin or 9'-*cis*-neoxanthin degradation (Bexman et al. 2005, Isoe et al. 1973). The positioning of violaxanthin somewhat to the opposite side of PC1 as zeaxanthin makes sense if the xanthophyll cycle is active in the ripening fruit (Demmig-Adams & Adams 1996). It is possible that the larger canopies in the RVL vineyards were inducing the epoxidation of zeaxanthin and increasing violaxanthin concentrations. However, other variables must have been driving violaxanthin concentrations given the fact that it was a main factor on PC2, and it is known that nitrogen and temperature can also influence the ratio of zeaxanthin to violaxanthin in plants (Demmig-Adams & Adams 1996).

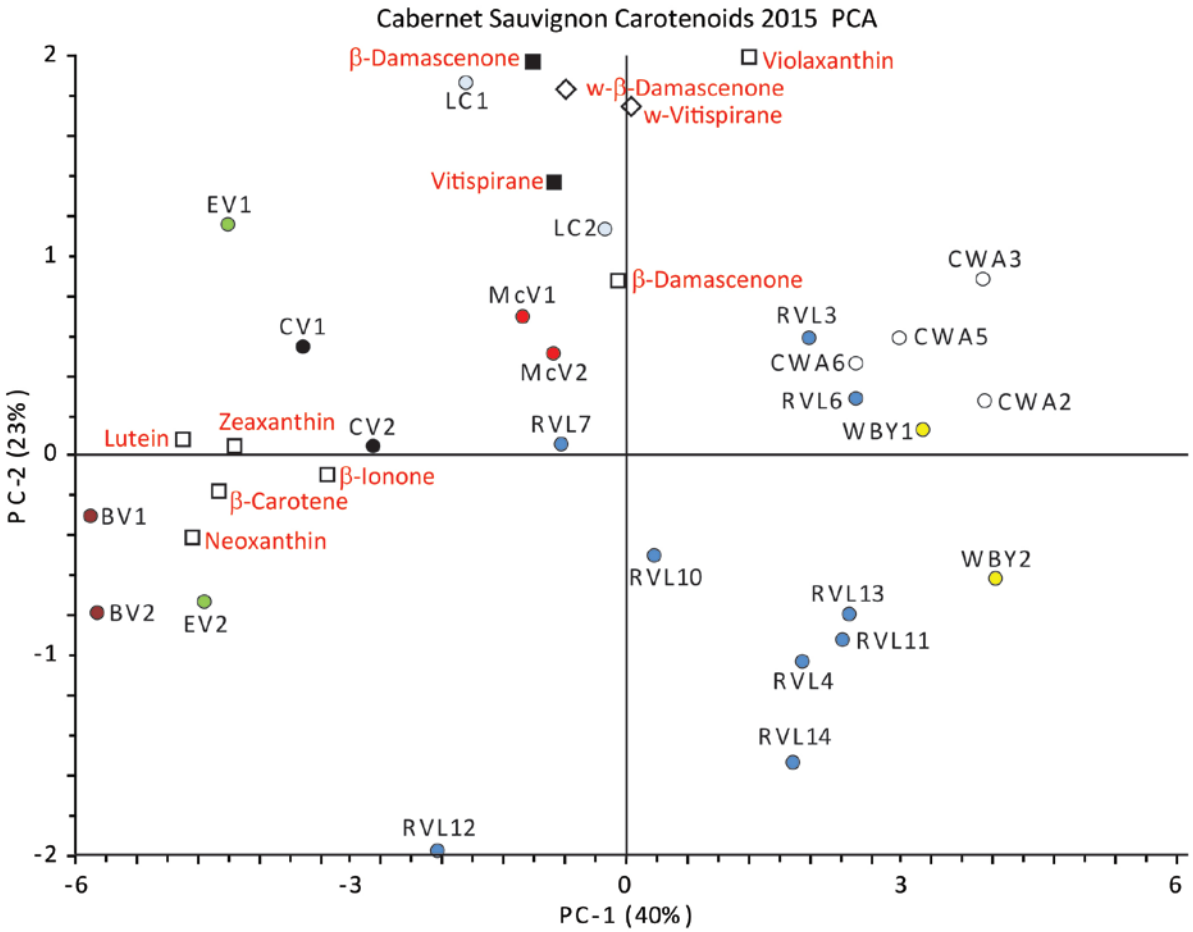


Figure 120. PCA plot showing the scores (coloured circles and black font) of the 25 different 2015 Cabernet Sauvignon grape samples and the carotenoid or free β-damascenone loadings (open squares and red font), bound norisoprenoid loadings (closed squares and red font) or wine norisoprenoid loadings (open diamonds and red font).

The carotenoids in the Chardonnay grapes did not show the same inter-relationships as seen in Cabernet Sauvignon. First, there was a cluster of four carotenoids again driving separation on PC1, but they were β-carotene, lutein, violaxanthin and neoxanthin. In Chardonnay, zeaxanthin was located on its own, although this time on the opposite side of PC1 (Figure 121). The grouping of the RVL samples on the right of the plot, the same side as violaxanthin, would appear to make sense given the high canopy/low light environment these grapes would encounter in the vineyard. The RVL Chardonnay wines also appeared to be those with some of the highest concentrations of β-damascenone. There was little correlation between the amount of bound β-damascenone and free wine β-damascenone in Chardonnay (Figure 121) compared to the close relationship seen between these variables in Cabernet Sauvignon (Figure 120). This divergence is presumably because of the difference in winemaking techniques between the varieties.

Overall, there seems to be potential in using carotenoid measures, especially violaxanthin, as a way of predicting wine β-damascenone concentrations in Cabernet Sauvignon wines. While measuring bound β-damascenone was also a good indicator, this method may not be available to as many laboratories given it requires a gas chromatograph. It would be interesting to look into this relationship more thoroughly and to explore variables in the vineyard that could alter violaxanthin and overall carotenoid concentrations. The role of the xanthophyll cycle in the plastids of ripening berries in determining the carotenoid composition of the fruit would also be an area of interest.

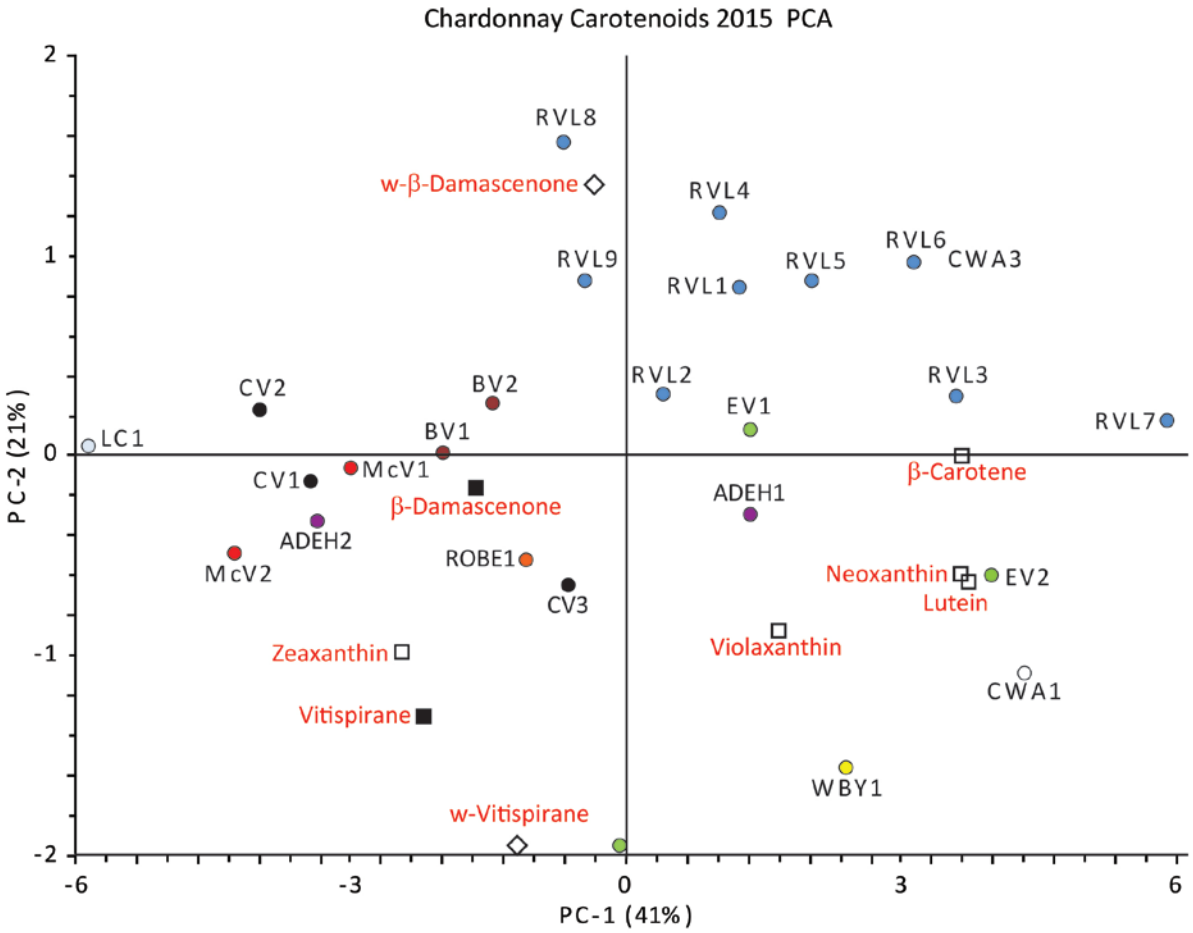


Figure 121. PCA plot showing the scores (coloured circles and black font) of the 25 different 2015 Chardonnay grape samples and the carotenoid loadings (open squares and red font), bound norisoprenoid loadings (closed squares and red font) or wine norisoprenoid loadings (open diamonds and red font).

8 Outcomes/Conclusion

Researchers around the world continue to increase our knowledge of how compounds contribute to the sensory attributes of wine. However, there is a large gap in our understanding of how compounds in the grape berries contribute to the final aroma and flavour characteristics of the wine. Furthermore, there is no technology for the objective measurement of the potential for a parcel of grapes to generate a particular wine style that growers and wineries can easily use to assess their product. This lack of understanding of the links between grape composition and wine sensory characteristics also means that there are no scientifically-validated methods that provide producers with the ability to better manage the flavour potential of their grapes in the vineyard. The project described in this report tested our ability to predict wine sensory outcomes from grape composition and has made considerable progress towards achieving this goal. The outputs listed below have the potential to greatly benefit the Australian wine industry, provide a solid basis for future work to determine the link between berry and wine composition, and indicate areas where future research will be beneficial.

First, we have identified a number of grape data blocks that are associated with specific Cabernet Sauvignon and Chardonnay wine sensory attributes. This project was designed to build upon a previous Wine Australia project (CSP 05/04) where it was found that certain grape volatile compounds correlated with Cabernet Sauvignon wine sensory attributes. In the current study, some of the same grape volatile measures were found to be important for modelling sensory attributes in both Cabernet Sauvignon and Chardonnay wines. However, many of the other data blocks, arising from the quantification of other groups of grape metabolites, were better at modelling a range of wine sensory characteristics. Initially, this expanded our potential targets for designing objective measures of quality from volatile compounds to ten other blocks of data. Eight of the blocks of data measured in Cabernet Sauvignon grapes and all nine blocks measured in the Chardonnay grapes were used in the models. Some of the associations detected in this study concur with data from previous studies, whereas others demonstrate novel correlations between particular sensory attributes and blocks of grape metabolites. The grape target compounds may not necessarily be precursors to wine volatile compounds, but may act as markers that indicate altered berry metabolism and therefore ultimate wine composition. This would arise from the up- or down-regulation of certain biochemical pathways within the grape in response to growth conditions. These grape biochemical markers of wine sensory outcomes would be useful in streaming or grading fruit once suitable protocols for their measurement are developed and verified. Such objective measures of berry flavour potential would be of much benefit to the industry.

Second, a novel data analysis method was shown to be useful for determining the most important grape chemical measures that best predict wine sensory profiles and single attributes. Because the study involved such a large number of data sets collected during an extensive metabolomics analysis of the grapes, a data analysis method that handles multiple blocks of data was required. SO-PLS assisted in a robust selection of grape chemical measures, which will assist in the selection of measures for future research endeavours in the development of objective measures of quality. It also highlighted the data blocks that were least frequently used to model sensory perception. In Cabernet Sauvignon, eight of the twelve data blocks were used for the models and thus the remaining four grape chemical measures (non-targeted and bound volatiles, enzyme activity, and tannin measures) could be removed from the list in future studies. This already is a significant step towards focusing in on grape measures that correlate more to wine sensory perception.

Third, the use of a sorting task with winemakers to assess quality of research wines was shown to be a useful technique for Cabernet Sauvignon. Consistent variations in quality were seen with the Cabernet Sauvignon samples and with the method employed it was clear to see exactly why some samples were higher in quality than others. The winemakers provided similar high quality descriptors each vintage including dark fruit, high flavour intensity, good tannin structure and balance. Such descriptors are good targets for quality measures. An interesting finding was the fact that two samples from the RVL region were considered to have moderate quality in two vintages, on par with samples from some of the other regions more associated with a premium product. The sensory drivers that clearly distinguished these samples were more of a holistic nature, such as

‘balance’ and ‘complexity’ that detailed sensory profiling in this instance did not capture. The drivers of concepts such as balance and complexity clearly require further investigation in order to enhance our understanding of wine quality beyond relying on profiles of wine sensory attribute intensity perception.

Fourth, the same quality assessment that proved successful with the Cabernet Sauvignon wines did not significantly discriminate the Chardonnay wines. When vinified with a simple identical protocol, it was found that there were only relatively subtle sensory differences across the Chardonnay wines, which is especially interesting given the remarkably wide variation in price per tonne across Chardonnay growing regions. These results indicate that quality drivers of commercial Chardonnay wines are most likely not derived predominantly from the grapes and it appears likely that winemaking factors would contribute more to the variation in sensory characteristics. Further research into other white varieties with distinct varietal characters, for example Riesling, Sauvignon blanc, and Gewürtztraminer, would probably present a different story.

Fifth, analysis of the year to year variation among the different blocks of grape measures revealed information about their stability across regions and vintages. This is important as it identifies which aspects of grape composition can potentially be manipulated in the vineyard, and those that may be more prone to variability in environmental factors beyond our control. For example, the amino acid composition of the Cabernet Sauvignon grape samples was similar in the samples across the vintages, and was not primarily driven by region. This suggests that something intrinsic to the vineyard influences grape amino acid composition and further exploration is needed to determine whether this can be managed. Other variables, such as anthocyanin or tannin composition, clustered the samples mainly by region, suggesting that coarse climatic differences may be important determinants of the fruit composition of these metabolites, so management may be difficult. Bound volatile compound and fatty acid compositions were somewhat related to region, but they also varied from year to year, meaning their production is more flexible. So these compounds might be managed if means to alter conditions in the bunch zone that mimic these environmental changes can be determined.

Sixth, it was found that the biochemical markers designed to measure the activity in specific biosynthesis pathways may not be practical or useful in predicting wine sensory outcomes. However, they do provide important insights into the timing of the production of metabolites in grapes and can help inform strategies for manipulation of fruit composition in the vineyard. Measures of the enzyme activities of the three major lipoxygenase pathway enzymes in Cabernet Sauvignon grapes were not useful in modelling any of the wine sensory attributes significantly different among the samples. In Chardonnay the lipoxygenase pathway measures helped model sour taste and citrus aroma. Studies of the expression of genes involved in the lipoxygenase pathway or the breakdown of carotenoids showed that the enzyme activity in the fruit is often the result of multiple genes and the maximal gene expression is often separated temporally from the peak in enzyme activity. This would make it difficult to use gene expression assays to predict pathway flux at harvest. However, from this work it was found that carotenoid content in Cabernet Sauvignon may predict wine β-damascenone concentration, which has implications for red wine quality.

Seventh, berry sensory analysis appeared to have limited utility in predicting specific wine sensory outcomes. Cabernet Sauvignon berry samples were consistently discriminated by sensory properties within and across vintages, and five sensory attributes of wine were consistently modelled with moderate to high regressions using berry sensory attributes and simple berry chemical measures. However, finding berry sensory attributes that consistently related to wine style and profile was challenging and it was found that basic chemical measures including TSS, anthocyanins, and chroma of grape homogenates were more reliable predictors of wine sensory attributes. Relating the sensory characteristics of Chardonnay grapes to the wines was also challenging and perhaps indicative that variation in style of these varietal wines are is not greatly dependent on the raw grape material.

Finally, this project has enhanced the Australian wine industry’s capability to conduct research into grape and wine flavour and aroma. Our approach has been grape-centric and adds to the existing research into wine flavour and aroma. This has resulted in the development of several new methodologies that will improve our ability to discover and measure grape metabolites responsible for wine style and to develop means to alter the levels of these compounds in the field. The grape and wine samples collected during this project are available to be used for further research for the next few years and the data sets will also be

mined further in the years to come. This work will also be built on in projects at the ARC Training Centre for Innovative Wine Production where studies into Cabernet Sauvignon will continue. We have trained postdoctoral fellows, students and research assistants in the field of flavour and aroma research and both CSIRO and the University of Adelaide now have established research teams with the knowledge required to undertake such work. Furthermore, the CSIRO and University of Adelaide laboratories on the Waite Campus collectively have a suite of analytical instruments for grape and wine metabolite studies and staff highly skilled in their use and maintenance. Collaborations developed with Australian and international researchers continue to allow the latest knowledge and techniques to be rapidly applied to issues facing the Australian wine industry to improve grape growing practices and improve flavour and aroma outcomes.

9 Recommendations

1. Continue scientific research

Our current research has identified a number of blocks of grape measures that are potential markers for Cabernet Sauvignon and Chardonnay wine sensory attributes. There are a number of potential future research streams that could build on this work to provide practical outcomes for the Australian wine industry. Some will be pursued in the ARC Training Centre for Innovative Wine Production.

Objective measures of quality development

- Further mine the data collected to see if specific compounds within a block are driving the correlations thus reducing the number of metabolites that need to be measured.
- Look for predictive relationships between grape composition and wine volatile profiles to help explain the wine sensory results.
- Meta-analysis of all Wine Australia projects that have examined aspects of grape composition, wine composition and wine sensory to discover robust relationships.
- Use the sensory data from this study to guide the identification of grape metabolites from the stored samples which have abundances that correlate with specific sensory attributes.
- Demonstrate grape composition to wine sensory links in a controlled system.
- Explore the application of the potential quality measures to other grape varieties.

Sensory and winemaking methodologies

- Develop methods to quantify holistic descriptors of wine such as balance and complexity.
- Explore the use of novel sensory methodologies such as rate-all-that-apply (RATA) to analyse both the quality and sensory attributes of experimental wines.
- Determine if dark fruit characters linked to higher quality are influenced by wine colour.
- Develop a new controlled winemaking protocol including oak treatment for use with Chardonnay experiments.
- Explore how much oak treatment influences winemakers' perceptions of quality in Chardonnay and test on wines made with grapes harvested from a range of regions throughout South Australia.

Grape metabolite pathways

- Identify important genes or enzymes from the pathways that lead to the production of the grape metabolites in the predictive blocks of grape measures.
- Examine their expression during fruit development to determine where and when expressed to help inform strategies to alter grape composition to drive wine style.
- Explore how gene expression and enzyme activity can be alter by practical management techniques in the vineyard.
- Study the Influence of machine harvesting on grape metabolite pathways and determine the potential to modify flavour postharvest.

2. Explore practical applications

The profound influence that grape composition has on Cabernet Sauvignon wine style requires effective grading and streaming of fruit, but also provides the potential to manage wine flavour and aroma more effectively in the vineyard. Objective measures of fruit flavour potential and a means of predicting wine

sensory attributes from grape composition could provide a step change in improving our ability to efficiently grow grapes to suit desired wine styles. It will help to promote improved decisions about harvest timing as well as batching and streaming of fruit to consistently produce desired wine styles. More importantly, it will provide tools to optimise grape flavour potential in the vineyard and deliver the means of producing grapes with a desired chemical profile that can be used to make wines of a specified flavour profile. While this project has identified target blocks of compounds, further practical trials are needed to extend these results to enable generic recommendations. We also need to begin identifying means of developing simple, fast, accurate and cheap assays that can be conducted in the vineyard or at the weigh station under the hectic conditions at vintage to aid decision making at various points of the grape growing process.

3. Communication with industry

Australian grape growers, viticulturists and winemakers are the target audiences for the ultimate outcomes of this research. The knowledge and techniques developed should be of value to both cool and hot climate producers. The outcomes from this project that will impact on the industry concern the understanding of the role that grape composition has on wine chemistry and how this information can be used to inform vineyard management decisions to manipulate wine style, predict harvest timing and improve the streaming of grape parcels. The research outcomes will continue to be disseminated through scientific journals, industry journals, conferences (for example the Australian Wine Industry Technical Conference), industry organised meetings and if suitable through the public media via factsheets and news releases. As some of the research was conducted in commercial vineyards, this has already aided in the progression of further trialling and uptake, which will have to be undertaken with industry support and input. It is expected that this will result in downstream application and evaluation in the commercial world with rapid feedback expected from industry collaborators on the results and application of the research.

10 Appendix 1: Communication

Journal articles, conference proceedings, book chapters

Bastian SEP (2014) "Regions, Wine Quality and Sensory Measures in Cabernet Sauvignon". In: Searching for the Sweet Spot – the quest for optimal yield and quality" at ASVO seminar Mildura, 24th-25th July 2014.

Niimi J, Boss PK, Jeffery DW, Bastian SEP (2017) Linking the sensory and composition of *Vitis vinifera* cv. Cabernet Sauvignon grape berries to wine. American Journal of Enology and Viticulture 68: 357-368.

Niimi J, Boss PK, Jeffery DW, Bastian SEP (2018) Linking the sensory properties of grape berries with wines for *Vitis vinifera* cv. Chardonnay. American Journal of Enology and Viticulture (In press).

Niimi J, Boss PK, Bastian SEP (2018) Sensory profiles and quality of research Cabernet Sauvignon wines are discriminated better than research Chardonnay wines. Food Research International (In press).

Niimi J, Tomic O, Næs T, Jeffery DW, Bastian SEP, Boss PK (2018) Application of sequential and orthogonalized-partial least squares (SO-PLS) regression to predict sensory properties of Cabernet Sauvignon wines from grape chemical composition. Food Chemistry (submitted).

Industry communications and extension activities

Boss PK (2014) Who's the big boss man then? Australian and New Zealand Grapegrower and Winemaker 601: 29.

Boss PK, Bastian SEP, Jeffery DW (2014) Linking grape measurement to wine sensory outcomes. Australian and New Zealand Grapegrower and Winemaker, 605: 49-52.

Bastian SEP. Regions, Wine Quality and Sensory Measures in Cabernet Sauvignon. ASVO seminar Mildura, 24th-25th July 2014. Oral presentation.

Niimi J, Boss PK, Cozzolino D, Jeffery DW, Lemba C, Johnson TE, Olarte-Mantilla S, Bastian SEP. Sensory attributes differences in Cabernet Sauvignon wines of varied provenance can be discriminated with mid infrared spectroscopy. Macrowine. Stellenbosch, South Africa, 7th-10th September 2014. Oral presentation.

Niimi J, Boss PK, Cozzolino D, Jeffery DW, Lemba C, Johnson TE, Olarte-Mantilla S, Bastian SEP. Sensory attributes differences in Cabernet Sauvignon wines of varied provenance can be discriminated with mid infrared spectroscopy. Crush. Adelaide. Australia, 25th-26th November 2014. Oral presentation.

Niimi J, Jeffery DW, Boss PK, Bastian SEP. Quality evaluation and sensory characteristics of research wine. Crush. Adelaide, Australia, 20 November, 2015. Oral presentation.

Boss PK, Robinson SP, Bastian SEP, Jeffery DW and Niimi J. The search for grape chemical markers of wine quality. Australia-Italy Grape and Wheat Symposium. Canberra, Australia, 3-4 December 2015. Oral presentation.

Boss PK. Flavour/aroma development in the berry: concepts and practical approaches. Oregon Wine Symposium. Portland, Oregon, USA, 22-23 February 2016. Oral presentation.

Boss PK, Robinson SP, Bastian SEP, Jeffery DW and Niimi J. Grape quality parameters that influence wine flavour and aroma. Treasury Technical Group. Adelaide, Australia, 3 May 2016. Oral presentation.

Boss PK. Flavour/aroma development in the berry. X International Symposium on Grapevine Physiology and Biotechnology. Verona, Italy, 13-18 June 2016. Oral presentation.

Niimi J, Jeffery DW, Boss PK, Bastian SEP. Linking sensory properties of Cabernet Sauvignon berries and wines. 16th AWITC Adelaide, Australia, 25-28 July 2016. Poster presentation.

Niimi J. Objective measures of quality. 16th AWITC Adelaide, Australia, 25-28 July 2016. Workshop participation.

Boss PK. Grapes for style: the impact of berry ripening on wine quality. 16th AWITC Adelaide, Australia, 25-28 July 2016. Workshop participation.

Niimi J, Tomic O, Jeffery DW, Bastian SEP, Næs T, Boss PK. Analysis of multiple grape measurements to relate to wine sensory characteristics using a multi-block SO-PLS method Eurosense, Dijon, France 2016. Oral presentation.

Niimi J, Tomic O, Jeffery DW, Bastian SEP Næs T, Boss PK. Key grape measures that predict sensory profiles of Cabernet Sauvignon wines. Crush. Adelaide, Australia, November, 2017. Oral presentation.

Publications arising from collaborations associated with this project

Boss PK, Böttcher C, Davies C (2014) Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. American Journal of Enology and Viticulture 65: 341-353.

Davies C, Nicholson EL, Böttcher C, Burbidge CA, Bastian SEP, Harvey K, Huang A, Taylor D, Boss PK (2015) Shiraz wines made from grape berries (Vitis vinifera) delayed in ripening by plant growth regulator treatment have elevated rotundone levels and 'pepper' flavor and aroma. Journal of Agricultural and Food Chemistry 63: 2137-2144.

Ward SC, Petrie PR, Johnson TE, Boss PK, Bastian SEP (2015) Unripe berries and petioles in *Vitis vinifera* "Cabernet Sauvignon" fermentations impacts sensory and chemical profiles. American Journal of Enology and Viticulture. 66: 435-443.

Boss PK, Pearce AD, Zhao Y, Nicholson EL, Dennis EG and Jeffery DW (2015) Potential grape-derived contributions to volatile ester concentrations in wine. Molecules 20: 7845-7873.

Ristic R, Boss PK, Wilkinson K (2015) Influence of fruit maturity at harvest on the intensity of smoke taint in wine. Molecules 20: 8913-8927.

Gambetta JM, Cozzolino D, Bastian SEP, Jeffery DW (2016) Towards the creation of a wine quality prediction index: correlation of Chardonnay juice and wine compositions from different regions and quality levels. Food Analytical Methods 9: 2842-2855.

Jiang W, Niimi J, Ristic R, Bastian SEP (2016) The effects of immersive context and wine flavor on consumer wine flavor perception and emotions elicited. American Journal of Enology and Viticulture. 68:1-10.

Böttcher C, Boss PK, Harvey KE, Burbidge CA, Davies C (2017) Peduncle-girdling of Shiraz (*Vitis vinifera* L.) bunches alters amino acid and hormone content of grapes and affects wine volatile compounds depending on berry sugar levels. Australian Journal of Grape and Wine Research (In press).

Olarte-Mantilla S, Collins C, Iland P, Kidman K, Ristic R, Boss PK, Jordans C, Bastian SEP (2017) Shiraz (*Vitis vinifera* L.) berry and wine sensory profiles and composition are modulated by rootstocks American Journal of Enology and Viticulture (In press).

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Hranilovic A, Li S, Ristic R, Boss PK, Bindon K, Grbin P, van der Westhuizen T, Jiranek V (2017) Chemical and sensory profiling of Shiraz wines co-fermented with commercial non-*Saccharomyces* inocula. Australian Journal of Grape and Wine Research (In press).

Boss PK, Kalua CM, Nicholson EL, Maffei SM, Böttcher C, Davies C (2017) Fermentation of grapes throughout development identifies stages critical to the development of wine volatile composition. Australian Journal of Grape and Wine Research (In press).

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Niimi J, Liu M, Bastian SEP (2017) Flavour-tactile cross-modal sensory interactions: The case for astringency. Food Quality and Preference 62:106-10.

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Gambetta JM, Schmidtke LM, Wang J, Cozzolino D, Bastian, SEP, Jeffery DW (2017) Relating expert quality ratings of Australian Chardonnay wines to volatile composition and production method. American Journal of Enology and Viticulture 68: 39-48.

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11 Appendix 2: Intellectual property

Intellectual property arising from this project has been communicated by various means to both the industry and scientific communities through various channels. This is outlined in Appendix 1. The knowledge gained regarding the important blocks of grape measurements that may predict wine sensory attributes will be useful to the industry. This will assist in decision making and catalyse discussion and innovation to further modify vineyard practices to achieve positive changes in grape composition. Dissemination of knowledge has been through various media including; publications in peer-reviewed grape and wine scientific journals, industry journals, oral and poster presentations to growers through national and regional meetings and presentations to individual companies.

The identification of potential blocks of grape measures of wine sensory outcomes that was achieved in this project would need to be confirmed in a broader industry study to assess robustness across regions, varieties and vintages. Proof of strong correlation between grape metabolites and wine sensory attributes would then provide the impetus to develop rapid and cheap measures of these target compounds to assist in fruit streaming and decision making in the vineyard.

12 Appendix 3: References

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13 Appendix 4: Staff

All staff below spent periods of time on the project:

Dr Paul K. Boss

Associate Professor Susan E. P. Bastian

Associate Professor David W. Jeffery

Dr Jun Niimi

Suzanne M. Maffei

Emily L. Nicholson

Dr Simon P. Robinson

Dr Claudia Schüürmann

14 Appendix 5: Other relevant material

Collaborating researchers

Researchers involved in this project interact with a number of other researchers in CSIRO, Australia and the rest of the world. The benefit of such interactions to the Australian wine industry is felt through the wealth of knowledge and expertise that can be brought to bear on issues of industry concern. A list of our major collaborators on this project is included below:

1. International

Dr Oliver Tomic (Faculty of Science and Technology, Norwegian University of Life Sciences)

Professor Tormod Næs (Norwegian Institute of Food, Fishery and Aquaculture)

2. Australian academic and scientific

Dr Roberta De Bei (University of Adelaide)

Associate Professor Cassandra Collins (University of Adelaide)

Anthony Pearce (University of Adelaide)

Yanjia Zhao (University of Adelaide)

Associate Professor Daniel Cozzolino (Central Queensland University)

Dr Joanna Gambetta (University of Adelaide/ Charles Sturt University)

Ross Sanders (University of Adelaide)

3. Australian wine industry

Treasury Wine Estates (Paul Petrie, Amy Richards, Jana Shepherd, Catherine Kidman, Catherine Wotton, Angus Davidson)

CCW Ltd (Andrew Weekes, Nicole Pitman, Ian Macrae)

Yalumba (Brooke Howell, Louisa Rose, Dan Newson)

Chalk Hill Wines (Jock and John Harvey)

WIC winemaking service (Michael Coode, John Gledhill)

4. CSIRO

Dr Christopher Davies (Agriculture and Food)

Dr Christine Böttcher (Agriculture and Food)

15 Appendix 6: Budget reconciliation

To be sent by Shane Wynn in a separate document.

FOR FURTHER INFORMATION

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