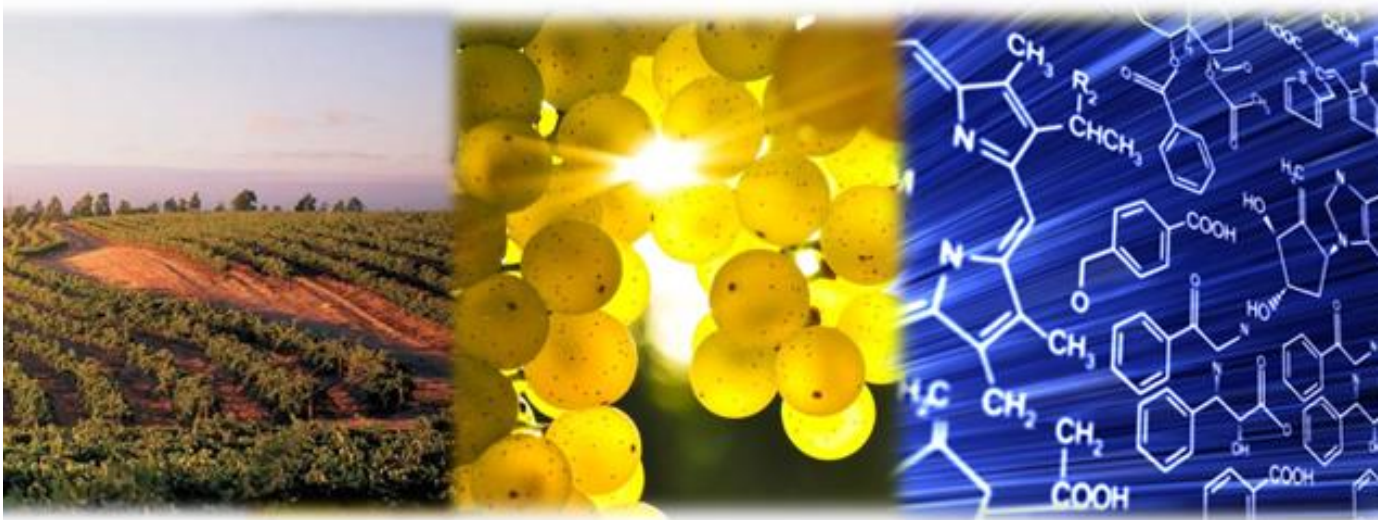
Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Objective measures of grape quality



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWR 1202**

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Research Organisation: **The Australian Wine Research Institute**

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1. Abstract

The aim of the project was to determine from existing and new objective chemical measures which ones can differentiate higher and lower value grape grades and whether any of these grape-derived compounds were also predictive of final wine style or grade for Australian Cabernet Sauvignon, Shiraz and Chardonnay grapes. The project identified objective chemical measures that can be used to define grape quality and are predictive of potential wine style in an Australian context. Targeted (individual compounds) and non-targeted (spectral fingerprints) analytical approaches were useful data types for grade prediction and specific chemical markers important for quality have been identified.

2. Executive Summary

2.1 Defining the need for objective measures in grape and wine production

The degree to which a wine achieves its intended style is related to compounds that arise from a variety of sources, such as from the grape berry either in free or precursor form, from fermentation of juices and musts, from oak storage or from ageing. The value of the grapes is currently assessed in a wide range of ways, for example, field-based assessments, including the condition of the vines, flavour of the fruit or the presence of disease, as well as certain quantitative chemical measures such as colour, Brix, pH or TA. Alternatively, value might be assigned based on the final quality of the wine that is achieved using those grapes. For the grower, this assessment determines how much they will be paid. For the winemaker, this assessment is critical to ensuring they have fruit that is appropriate to the value and style of the wine they intend to make as well as controlling raw material costs.

The type of assessments currently used are largely subjective. This subjectivity can result in uncertainty as to whether the maximum value possible for a parcel of grapes is achieved. As such, many growers and winemakers want to support the decision-making process by using objective chemical measures that are directly related to attributes that confer value such as key compounds responsible for taste, aroma, texture and appearance. This project is significant to growers because it relates to confidence and transparency in the realisation of maximum economic value for their fruit crop. For both growers and winemakers, objective chemical measures can provide specifications that allow the most value to be achieved from the starting material, the grapes. For winemakers it is significant because such measures offer the opportunity to stream fruit to maximise its value and minimise risk related to loss of value, for example by including higher value fruit with lower value fruit, and allow them to choose fruit of the value that relates best to a desired wine style.

2.2 Expected outcomes of the project

The initial aim of the project was to determine from a range of measurable chemical compounds in grapes, which of the measures, either independently or in combination, were able to differentiate between grape grades. During consultation with wine sector personnel, questions were raised that refined the project down to two more specific researchable questions. The first question was whether, regardless of the grade, chemical measurements could be used to ‘cluster’ fruit, that is to identify similar patches or highlight differences. This assumed no knowledge of grade and, for example, could be used to stream fruit. The second question was whether existing grading allocations could be predicted using previously identified, and some new, chemical measurements. This approach made the assumption that the existing current grading system was ‘accurate’ and sought to determine an objective basis for the process.

In addition to Wine Australia funding, the research was supported by Accolade Wines and FABAL. Accolade Wines uses a standardised approach for grading of all fruit and pays growers irrespective of the wine grade outcome. Grapes of three varieties (Cabernet Sauvignon, Shiraz and Chardonnay) from a range of quality grades were sourced by representative sampling of vineyards. A range of chemical analyses was performed to determine the concentration of key compounds known to affect wine style and key sensory properties. These included some traditional measures commonly used within the industry such as total soluble solids, pH, TA, anthocyanins and total phenolics. Other measures used less frequently such as malic acid and yeast assimilable nitrogen (YAN) were also included as well as new measurements: amino acid profile, dry matter, methoxypyrazines, C_6 compounds, phenol-free glycosyl glucose (GG) assay, tannin, β-damascenone, laccase and complete spectral fingerprints in the UV-vis, mid-infrared (MIR) and near-infrared (NIR) regions. Univariate or multivariate relationships using methodology such as partial least squares regression, principal component analysis and discriminant analysis were applied to the chemical measures and the grades to assess the extent to which they could be used to predict the grade. Multivariate data analysis was

also used to develop models that cluster fruit based on similar compositional variables to determine fruit parcels that are similar and which could guide streaming decisions.

The project was extended for a third year based on the recognition that it was important to establish whether any of the grape-derived compounds that can differentiate between grape grades were also predictive of final wine style or grade. It was noted that within a fruit grade, the potential for several wine styles might exist, driven by the presence of different grape-derived compounds. As such, there was consensus following discussion with Accolade Wines that the most valuable objective to address in this stage was to establish objective measures in grapes that can be used to support decision-making about what is the most appropriate style of wine that can be created from an individual batch of fruit. For this aspect of the work, grape batches were sourced from across four expected wine 'style' categories from two varieties (Shiraz and Chardonnay) and wine was prepared by a standardised procedure. Wines were then subjected to chemical analysis, quantitative descriptive sensory analysis and winemaker evaluation (grade, style).

The potential impacts of the project for the wine sector include the ability for grapegrowers to produce grapes to defined specifications, and for winemakers to select fruit with greater confidence that it will be appropriate for a targeted wine style. In addition, it is significant to grapegrowers because it relates to confidence and transparency in the realisation of maximum economic value for their grapes. For both growers and winemakers, objective chemical measures can provide specifications that allow the most value to be achieved from grapes. Developing an understanding of the synergistic relationships between available objective measures and well-established subjective systems has the potential to significantly reduce production costs and increase value by ensuring that fruit is used in the most appropriate production stream and that maximum value is returned from the end product. It may also lead to significant savings in the cost of monitoring crops through more effective application of resources and a clearer understanding of geographical and climatic drivers.

The second phase of the work (in 2015) could additionally afford winemakers the opportunity to stream fruit based on objective chemical measures to maximise its value and minimise risk related to loss of value, for example by avoiding batching higher value fruit with lower value fruit, and allow them to choose fruit of the value that relates best to a desired wine style. Furthermore, the management of vineyards to supply grapes of defined composition fit for a particular style of wine is a process that involves a wide range of inputs that use resources with varying impacts on maintaining a sustainable environment, for example chemicals for spraying, fuel for equipment, water and fertiliser. By better understanding the levels of chemical compounds in grapes that are required for each wine style, the resource inputs can be tailored and minimised, thus minimising the impact on the environment.

In conclusion, the maintenance of strong relationships along the value chain between key participants such as grapegrowers and winemakers is central to a sustainable Australian wine sector. Objective measures of quality may contribute significantly in some sections of the wine community to ensuring transparency, trust and the maximisation of value by providing an objective framework within which all parties understand what is expected, to achieve the highest value and most effective use of resources. Improving the profitability of vineyard enterprises and wine companies in rural Australia also provides social benefits for the regions.

2.3 Fruit grade prediction using targeted chemical analysis or spectral data

The question was asked: "Regardless of the grade, can chemical measurements be used to 'cluster' fruit to identify similar patches or highlight differences?". This assumed no pre-existing knowledge of the actual fruit grade and, if possible, could be used to stream fruit. In general, without using statistical modelling approaches, the ability to do this was limited.

Using principal component analysis (PCA), models could be developed to explain the variation and relationships between grape grades and grape chemical measures. It was found for the Cabernet

Sauvignon (CAS) and Shiraz (SHZ) samples studied, that discrimination of sample properties using PCA was indistinct, and in the case of the full chemical-analytical data set required multiple dimensions, that is, complex relationships existed amongst the samples. The primary separation of the samples based on their composition was found to be between those sourced from the warm Riverland region (also somewhat related to grade allocation) and samples obtained from other regions. Using this type of modelling, the analytical approaches used did not appear to be relevant as an aid to fruit streaming. Initial assessment of the Chardonnay (CHA) compositional data indicated that similar complexity and indistinct clustering of samples was found using PCA. However, using a reduced data set of only significant variables (high correlation loadings) which included ºBrix, pH, TA, malic acid, nitrogen (YAN, ammonia, alpha-amino nitrogen), malic acid, chloride, berry weight and the UV-visible spectrum between 280 and 370 nm (phenolics) a model could be developed that described 75% of the variability in the data in only two dimensions. Using this model, clear clustering of the samples was found, but this clustering was unrelated to region or grade, with the exception that Riverland (poor grade) samples were similar in composition. For CHA, therefore, some potential exists for unique parcels of fruit to be streamed for a winemaking end point based on their chemical similarity. Generally, however, the complexity of the relationships of chemical properties between fruit grades did not allow clear separation of the samples according to grade using PCA, suggesting that PCA-based clustering is not likely to be the most effective way of clustering fruit. The development of a model would therefore likely require some knowledge of commercial grade allocation (i.e. 'training') rather than just the raw data clustered by PCA. This was perhaps to be expected and indicates that a modelling approach that considers knowledge of grade is required to develop sound models capable of using targeted chemical analytes or spectral data capable of distinguishing grades.

The next question asked was "Can existing grading allocations be predicted using chemical measures?", for which the underlying assumption was that the current grading system is accurate. Using a modelling approach, the project team sought to establish an objective basis for the existing, predominantly subjective process. The results demonstrated that partial least squares regression (PLS) modelling and quadratic discriminant analysis (QDA) modelling could be used to develop predictive models for fruit grade. The relative strength of prediction varied depending on the type of analytical data used (e.g. spectral or targeted chemical analysis) and the type of modelling (PLS versus QDA) used.

Using targeted chemical analysis for the three varieties studied, values of R^2 (validation, R^2_{val}) achieved with PLS modelling were from 0.66 (66% of the variation in grade was explained by the linear relationship with the selected objective chemical measures) to 0.78. Somewhat better models were achieved using 'non-targeted' measurements (i.e. not measuring specific compounds, but rather overall 'fingerprints'), in particular the mid-infrared (MIR) spectrum of either grape homogenate or juice, with R^2_{val} between 0.78 and 0.86, indicating that grade could be more accurately predicted using this approach. The use of the PLS modelling approach for the prediction of grade was limited by the underlying assumption that grades are 'linearly' separated, i.e. by consistent, numerically defined increments between grades. This is in fact not the case, and grade could rather be said to be categorically defined. To overcome this, 'categorical' models (which consider grades as categories that are not necessarily consistently linearly separated) could potentially be applied using PLS-discriminant analysis (PLS-DA), but for the particular data sets defined in this study they could not be used successfully for grade prediction.

The use of QDA overcame the limitations of PLS-DA, and was found to be the most effective modelling technique to predict allocation grade, with prediction accuracies between 70 and 100%. Using QDA, certain analytical data sets better predicted grade by variety and season. CAS grade was best predicted from homogenate NIR spectra in 2013 with 90% accuracy and 87% accuracy in 2014. MIR spectra from grape homogenate performed maximally for CAS in 2014 with 100% accuracy. This was a significant outcome, as it showed that spectral data could be used for the development of highly predictive models for fruit grade classification. This is important, as some of the more complex

targeted analyses (i.e. of individual compounds) are not viable for many producers (due to cost and time delay) and by comparison, spectral methods are relatively low cost and rapid.

Similarly, SHZ allocation grade was predicted most effectively by QDA using homogenate NIR and the UV-visible spectrum, with 94% and 95% accuracy respectively. Using non-targeted juice MIR data, 93% prediction accuracy was the maximum achieved for CHA with QDA, which was similar to the accuracy achieved using targeted chemical data, at 95%. Interestingly, for CHA, no loss of prediction accuracy occurred when a limited suite of simple analytes was used: ºBrix, pH, TA, malic acid, nitrogen (YAN, ammonia, alpha-amino nitrogen), malic acid, chloride, berry weight and the UV-visible spectrum between 280 and 370 nm (phenolics). Many of these techniques are readily accessible to the wine industry and this was a significant result that showed grade could be easily predicted for CHA using a combination of these simple measurements. The results showed that QDA modelling provided a promising tool for grade prediction, in particular using non-targeted MIR and NIR spectra but also with simple chemical measures, and was a simple approach that could be highly valuable for the wine industry.

A limitation of the QDA modelling approach is that it cannot provide an indication of which individual grape compositional variables are important for predicting grade allocation. Although not as highly predictive as QDA, PLS modelling was useful in defining the important compounds that differentiate fruit grades from each other. Some traditional measures of quality were found to be important for grade prediction, for example titratable acidity (TA), pH, °Brix and berry weight, which, depending on grape variety, were either positively or negatively associated with grade. The use of the UV-visible spectrum of grape extracts, particularly the measurements at 280 nm (total phenolics), 370 nm (flavonols), 420 nm (yellow) and 520 nm (red varieties, colour), was shown to be important across vintages. For the red varieties CAS and SHZ, grape tannin was also important, and higher values for both tannin and UV-visible measures were associated with higher value fruit grades. This is in agreement with previous studies, which have indicated a relationship between fruit phenolics and quality. In CHA, higher levels of the 370 nm (flavonols) measure were associated with poorer quality fruit. This may reflect decisions made through visual assessments of the grapevine canopy (greater fruit exposure leads to increased flavonols) and is the first time a phenolics measure has been demonstrated as being of importance in defining quality in white grapes.

The relevance of grape juice nitrogen measures (as YAN, alpha-amino nitrogen, ammonia and amino acid profile) to quality were assessed for the first time in this study, and were significant for CAS, SHZ and CHA. These measures were found to be both positively and negatively associated with quality, driven by changes in specific amino acids, which varied seasonally. A significant finding was that the amino acids glutamic acid and proline were strongly related to fruit allocation grade across multiple varieties and seasons. In particular, glutamic acid was negatively associated with quality (i.e. higher amounts in lower value quality grades) in CAS and CHA, but positively in SHZ (i.e. higher amounts in higher value quality grades).

Grape-derived aroma compounds, either in the free volatile or precursor form were also explored as potential objective quality markers. For CAS in 2013, the free volatile compound β-damascenone and in 2014 phenolics-free glycosyl-glucose (which indirectly represents aroma potential) were found to be important predictors of higher value grape allocations respectively. For all the grape varieties studied, the C_6 volatiles E-2-hexenal, Z-3-hexanol and hexanol, which contribute to ‘grassy’, ‘green’ aromas, were significant, being either positively or negatively associated with allocation grade. For CHA in particular, the C_6 volatiles Z-3-hexanol and hexanol together with precursors to the volatile thiols 3-*S*-cysteinylhexan-1-ol (Cys-3-MH) and 3-*S*-glutathionylhexan-1-ol (Glut-3-MH), 3-*S*-cysteine-glycine-3MH (Cys-Gly-3MH) were elevated in higher value grapes. These observations are of importance in defining new grape objective measures.

Together these findings support some of the current measures used to define grape quality but have also highlighted the importance of compounds which have not previously been demonstrated as being

of importance to the objective measurement of grape quality in the Australian context. Both targeted (individual compounds) and non-targeted (spectral fingerprints) analytical approaches were shown to be useful for grade prediction, as well as the identification of specific chemical markers important for quality.

2.4 Wine grade and style prediction using grape objective measures

The project was extended for an additional season in 2015, to establish whether the compounds that were important for the definition of grape grade were of relevance to the final commercial wine grading and/or style category. This aspect of the study was performed over one vintage and region (Riverland), with a reduced sample set of only SHZ and CHA fruit from four grape allocation streams. Wines were made from the grape parcels in duplicate using a defined experimental procedure. The wines were given a commercial grading and style assignment by the producer, but were also subjected to quantitative descriptive sensory analysis to specifically define their aroma, flavour, texture and appearance attributes. It was evident that the producer assigned wines with diverse sensory profiles (i.e. multiple styles) to a single wine quality grade allocation. This limited the development of PLS or QDA models for wine style, but some comprehensive prediction of wine grade from grape composition was achievable. For CHA grapes, a PLS model with R^2_{cal} of 0.79 and R^2_{val} of 0.69 was obtained, with a QDA model 69% predictive of wine grade. For SHZ, PLS achieved R^2_{cal} of 0.81 and R^2_{val} of 0.58 in the prediction of grade, and QDA was not applicable in this instance due to wine category number limitations. Although the wine models were somewhat less robust than those developed for grape grade allocation, this was likely due to limitations in the sample numbers and the complexity of chemical transformations that occur to grape-derived compounds during the winemaking process. Considering this, the results obtained are reassuring and would likely be significantly improved by the addition of new data with a greater sample number per grade allocation.

The 2015 CHA 'grape chemistry-wine style' results showed some important overlap with the preliminary 2014 study. Grape compositional measures relevant to wine allocation grade in 2015 were similar to those identified as being important to grape allocation grade in 2014. As with the 2014 study, malic acid, C_6 volatiles Z-3-hexanol and hexanol together with precursors to the volatile thiols Cys-3-MH, Glut-3-MH and Cys-Gly-3MH were higher in grapes that produced wines of better grade (higher value). High value wines were strongly associated with increased berry weight, which had not previously been identified as being important in the 2014 results. Another variable that was expected to be relevant to grape quality, but was not significant in 2014 was laccase, which can be elevated in diseased fruit. In the 2015 CHA study, laccase was elevated in grapes that produced lower value wines, an important finding since this is potentially a new objective measure. As for the 2014 results, the 370 nm measure (flavonols) was significant in the prediction of wine grade. However, in the 2015 sample set which was limited to Riverland samples, 370 nm was found to be positively associated with higher value wines rather than the converse, which may indicate that within the warm Riverland region higher levels of canopy exposure may be important to the improvement of wine quality. Nevertheless, this result confirms that this subset of grape phenolics is important in defining final wine outcomes in CHA, and warrants further research.

In SHZ wines made in the 2015 study, it was again observed that some of the same compounds identified as being important in grape grade prediction in 2014 were significant in defining wine grades. Higher value wines were produced from grapes having higher ºBrix, glutamic acid, tannin, anthocyanin, chloride, laccase activity and E-2-hexenol. Similar to the 2014 study, grape Z-3-hexenol was lower in grapes which resulted in higher value wine. However, some differences were observed between the 2014 and 2015 studies, which may be due to factors that only come into play in the grape to wine transition. Glycosyl glucose was not found to contribute to the assessment of SHZ grape grade, yet in 2015, higher levels of this class of grape-derived compounds were correlated with higher value SHZ wine grades. This class of compounds forms free volatile compounds during the winemaking process, so the correlation with final wine properties is therefore to be expected. Such precursor compounds would not necessarily be assessed in a preliminary observational or sensory-

based grape assessment. However, this shows that a grape-based objective measure is potentially important in defining quality in wine.

In summary, the results of the 2013/2014 grape allocation grade study showed that targeted grape chemical analysis with data modelling gave a good indication that some traditionally measured compounds (pH, TA, °Brix, colour) could be useful as objective measures, as could some new chemical measures that had not previously been considered in the assessment of quality in the Australian context. Some of the new analytical measurements identified are already in use for quality assessment and grape streaming by international producers, and results from this work support their observations, providing a strong demonstration of their value to the Australian wine industry. A limitation of the analytes identified is that complex analyses may not be viable for some producers, due to cost and time delay for the analyses – however many of these analyses are available through third-party laboratories. Alternatively, it was important to note that for all the varieties studied, 'non-targeted' spectral fingerprint measurements, in particular MIR, could be used to achieve a high degree of grape grade prediction accuracy (≥90%) using QDA. Furthermore, for CHA, 93% prediction accuracy was achieved using simple grape chemical analyses that would be readily accessible to a winery laboratory. In terms of the 2015 'grape chemistry-wine style/grade' prediction study, there was a strong demonstration that grape composition can be used to support decisions about streaming fruit to particular wine styles or grades. Thus grape composition can support assessment of fruit potential for both wine quality grade (and style), with many of the objective measures identified in the 2014 'grape grade' study also being associated with 'wine style' outcomes. The results give a strong demonstration that with ongoing research, and greater sample numbers in stylistic categories, robust models could be developed to predict wine style or grade outcomes from objective grape-based analyses.

3 Background

The first point in the wine value chain is the vineyard and the grapes, therefore decisions made at this point are critical to achieving the desired final wine style. Grape-derived compounds responsible for appearance, aroma, texture and taste in wine are the primary contributors to the degree of fitness for purpose of wine grapes. Many of these compounds are known and are measurable, have meaning to the final sensory characteristics of the wine and can be manipulated through viticultural and/or winemaking practices. However, the application of objective chemical measures in Australia tends to be at a rudimentary level, with only one or two measures implemented by some companies.

The value of the grapes is currently assessed in a range of ways that are mainly subjective. The subjectivity of the assessment can result in uncertainty as to whether the maximum value possible for those grapes is achieved. As such, many growers and winemakers want to support this decision-making process by using objective chemical measures that directly relate to attributes that confer value such as key compounds responsible for taste, aroma, texture and appearance. The grower can be paid based on a field-based assessment of, for example the condition of the vines, flavour of the fruit, presence of disease, some quantitative chemical measures such as colour, Brix, pH, TA or they can be paid based on the final value of the wine that is achieved using those grapes. For the winemaker, this assessment is critical to ensuring they have fruit that is appropriate to the value and style of the wine they intend to make as well as controlling raw material costs.

The degree to which a wine achieves its intended style is related to compounds arising from a variety of sources, such as from the grape berry either in free or precursor form, from fermentation of juices and musts, from oak storage or from ageing. A range of these individual compounds is reported to have relationships with grades of grapes or wines and with sensory properties (Smith 2013). Colour has historically been used in Australia for grapes (Kassara and Kennedy, 2011) and colour and tannin relationships with wine grade are well established (Mercurio et al. 2010). Wine companies around the world are using varying degrees of these chemical measures for various applications, for example Gallo in the USA uses an index of multiple compounds primarily for fruit streaming (Cleary et al. 2013). Grower cooperatives in Germany are trialling measurements of released aroma from the glycosyl glucose assay (personal communication, Ulrich Fischer, 2014) and companies in USA, NZ, Italy, Portugal and South Africa use tannin and colour to support winemaking decisions.

By measuring a range of chemical compounds in multiple grape batches, the research described here aimed to determine how variable these were across a wide range of fruit grades. The primary aim of the project was to determine which of these chemical measurements in grapes, independently or in combination, were able to differentiate between grape grades. A further aim was to determine whether the fruit could be clustered (grouped) based on similarity of chemical composition. An expected output of the research was to assess the practical application of grape grading measurements and to support wine producers who intend to apply these measures in their systems. The pilot study was performed on a small scale in 2013 (CAS only), followed by a large-scale study in 2014 (CAS, SHZ and CHA).

An extension to the original 2013 and 2014 ‘fruit-focused’ project was a 2015 vintage trial that also included winemaking from different fruit grades for CHA and SHZ. After discussions with the industry partners, it was decided that the most valuable objective to address in the next stage of the research was to establish objective measures in grapes that could be used to support decision-making about the most appropriate style of wine that could be created from an individual batch of fruit. It was also important to establish whether compounds that can differentiate between grape grades were also predictive of final wine grade.

4 Project aims and performance targets

Year 1	Output	Target Date	Activities
a	Stage 1 of required data set compiled summarising analysis of key compounds and spectra of fresh Cabernet Sauvignon fruit and corresponding grades. A set of the same samples secured for frozen analyses required in stage 2 of 2013 data set.	30/06/2013	(i) Identify vineyards within the chosen fruit grading system, source representative fruit from them and determine the grade of the fruit (ii) Collect 20 bunch representative samples (minimum 10 per grade for each variety i.e. ~50-60 for Cabernet Sauvignon across the vineyards, collate grade associated with each fruit sample and deliver to AWRI. (iii) Identify and measure the most relevant compounds to measure in fresh Cabernet sauvignon berry samples. These are likely to include total soluble solids, pH/TA, malic acid, C_6 compounds and yeast assimilable nitrogen (YAN) (iv) Scan fresh Cabernet sauvignon grape homogenates by FT-MIR e.g. using Bruker alpha and/or other appropriate UV/VIS/NIR platforms. (v) compile preliminary data set summarising analysis of key compounds and spectra of fresh Cabernet Sauvignon fruit and corresponding grades.

Outputs and Activities 2013–14

Year 1	Output	Target Date	Activities
a	Stage 2 of required data set compiled summarising measured key compounds and spectra of frozen Cabernet Sauvignon fruit and corresponding grades. Recommendations provided to industry collaborators for sampling and measures in 2014.	31/12/2013	(i) Identify and measure the most relevant compounds to measure in frozen Cabernet Sauvignon fruit. These are likely to include anthocyanins/total phenolics/tannin, methoxypyrazines, phenol-free glycosylated glucose (GG), beta-damascenone, laccase, amino acid profiles and dry matter. (ii) Scan frozen Cabernet Sauvignon grape homogenates by FT-MIR e.g. using Bruker alpha and/or other appropriate UV/VIS/NIR platforms. Conduct preliminary correlation work to identify likely patterns. (iii) Review sampling and analysis methods used in 2013 and if necessary revise sampling and analysis methods to be used in 2014
b	Extension material outlining the project and measurements of key compounds	30/06/2014	(i) Identify vineyards within the chosen fruit grading system, source representative fruit from them and determine the grade of the fruit

	identified as indicating Cabernet fruit quality grading presented at an industry seminar (ASVO ideally) and at least one publication in an industry journal discussing vintage 2013 results.		(ii) Collect 20 bunch representative samples (minimum 10 per grade for each variety i.e. ~200) for Cabernet Sauvignon, Shiraz and Chardonnay across the vineyards, collate grade associated with each fruit sample and deliver to AWRI. (iii) Identify and measure the most relevant compounds to measure in fresh Cabernet Sauvignon, Shiraz and Chardonnay berry samples. These are likely to include total soluble solids, pH/TA, malic acid, C_6 compounds and yeast assimilable nitrogen (YAN) (iv) Scan fresh Cabernet Sauvignon, Shiraz and Chardonnay grape homogenates by FT-MIR e.g. using Bruker alpha and/or other appropriate UV/VIS/NIR platforms. (v) Prepare extension material summarising measurements and analysis of key compounds and spectra of fresh Cabernet Sauvignon fruit suitable for presentation to industry through at least two extension mechanisms

A variation was approved to add an additional vintage (2015) to the project, which included winemaking:

Outputs and Activities 2014–15

Year 2	Output	Target Date	Activities
a	Extension material including a 'practical guideline' containing information on at least (a) the variability of chemical measures (b) the relationships between the chemical measures and fruit grade and (c) a similarity model that clusters the fruit based on chemical composition provided to industry through AWRI e-News, posting on web sites, roadshow material and publication of outcomes in at least one industry journal and one peer-reviewed journal.	31/03/2015	(i) Measure the most relevant compounds to measure in frozen Cabernet Sauvignon, Shiraz and Chardonnay berry samples. These are likely to include anthocyanins/total phenolics/tannin, methoxypyrazines, phenol-free glycosylated glucose (GG), beta-damascenone, laccase, amino acid profiles and dry matter. (ii) Scan frozen Cabernet Sauvignon, Shiraz and Chardonnay grape homogenates by FT-MIR e.g. using Bruker alpha and/or other appropriate UV/VIS/NIR platforms. (iii) Perform statistical analyses to establish if relationships exist between targeted or spectral fruit composition measurements and fruit grade and if so, quantify what the relationships are. Prepare report and other extension material summarising opportunities to improve the streaming process of fruit on the basis of fruit composition or spectra (iv) Perform multivariate data analysis to group fruit into batches with similar fruit composition and to group fruit into batches with similar spectral fingerprints. (v) Prepare report, fact sheet, practical guidelines, AWRI roadshow presentation and other extension material summarising project outputs against stated objectives, including opportunities to improve

			streaming process of fruit on the basis of fruit composition or spectra and communicate to stakeholders through extension activities and/or trade journal publications and/or peer-reviewed publications as appropriate
b	Stage 1 of required 2015 data set compiled summarising analysis of key compounds and spectra of analytes from fresh Chardonnay and Shiraz fruit and corresponding grades and style categories. A set of the same samples secured for frozen analyses required in stage 2 of 2015 data set.	30/06/2015	(i) Identify four fruit batches for each of four style categories for each of Shiraz and Chardonnay. (ii) Collect 20 bunch representative samples across the vineyards, collate information (e.g. grade, yield etc.) associated with each fruit sample. (iii) Identify and measure compounds in fresh berry samples. (iv) Freeze grape samples for analysis in Stage 2 where required. (v) Scan grape homogenates by FT-MIR or UV/VIS/NIR.
c	Wine available from grapes fermented under consistent winemaking conditions.	30/06/2015	(i) Produce wine using standardised winemaking.

Outputs and Activities 2015–16

Year 3	Output	Target Date	Activities
c	Stage 2 of 2015 data set compiled summarising measured key compounds and spectra of fruit and corresponding grades and style categories. Recommendations provided to industry collaborators for sampling and measures in 2016.	31/12/2015	(i) Identify and measure the most relevant compounds to measure in fruit. (ii) Complete grape compositional analysis and collate data. (iii) Review analytes, sampling, analysis methods used in 2015 and if necessary revise for 2016.
d	Bottled 2015 wine available	31/12/2015	(i) Bottle V2015 wine.
e	Data available from Sensory descriptive	30/06/2016	(i) Perform sensory descriptive analysis of the

	analysis of 2015 wines		wines (ii) Provide wine to Industry partners for assessment of 'style' categories and sensory feedback.
f	Data available about relationships between targeted or spectral fruit composition measurements and wine 'style' category; and wine composition measurements and wine 'style' category	30/06/2016	(ii) Complete V2015 wine compositional analysis and collate data. (i) Perform data analyses to establish relationships between targeted or spectral fruit composition measurements and wine sensory attribute scores/'style' category; and wine composition measurements and wine sensory attribute scores/'style' category for vintage 2015.
g	Final Wine Australia report outlining the project and measurements of key compounds identified as having relationships with wine 'style'.	30/06/2016	(i) Prepare report from V2015 activities summarising opportunities to improve the streaming process of Chardonnay and Shiraz fruit on the basis of fruit composition or spectra.

A further variation was approved to extend delivery of the Final Report and Extension Activities, as below:

Outputs and Activities 2016–17

Year 4	Output	Target Date	Activities
a	Final Wine Australia report outlining the project and measurements of key compounds identified as having relationships with wine 'style'.	31/12/2016	(i) Prepare report from V2015 activities summarising opportunities to improve the streaming process of Chardonnay and Shiraz fruit on the basis of fruit composition or spectra.
b	Extension material including a 'practical guideline' containing information on at least (a) the variability of chemical measures (b) the relationships between the chemical measures and fruit grade (for years 1-3) and for	30/6/2017	(i) Prepare fact sheets/practical guidelines, for (a) and (b). (ii)Prepare an AWRI roadshow presentation and deliver in at least two regions. (iii)Prepare and deliver an AWITC Technical Conference Workshop on 'Objective Measures of Quality in Grapes and Wine. (iv) Offer a presentation to the ASVO Winemaking Seminar (November 2016).

	chemical measures, fruit grade and wine (year 3) provided to industry through AWRI eNews, posting on web sites, roadshow material and publication of outcomes in at least one industry journal and one peer-reviewed journal.		(v) Prepare and publish other extension material summarising project outputs against stated objective (including opportunities to improve streaming process of fruit on the basis of fruit composition or spectra) and communicate to stakeholders through trade journal publications (1xAWRI eNews, 1xTrade Journal article) and/or peer-reviewed publications as appropriate.

5 Method

5.1 Grape samples. The study was conducted over three grape production seasons (vintages), from 2013 to 2015. Grape regions and vineyards were selected by a commercial wine producer (Accolade Wines, Reynella, SA, Australia). In the first season (2013), a single grape variety was studied, Cabernet Sauvignon. A total of 46 samples and batches of fruit (~ 2 kg) were obtained. Fruit was selected across grades 2-7 (lower number = higher value/grade) across nine regions (Swan Valley (Swan), Western Australia (WA), Riverland, McLaren Vale (MCV), Langhorne Creek (LHC), Clare Valley (Clare), Padthaway (PTW), Coonawarra (COO) and Wrattonbully (WRA). In the second season (2014), ~ 2 kg of grapes were sampled for Cabernet Sauvignon, Shiraz and Chardonnay varieties across grades 2-9. Grapes were sourced from eight geographical areas for both vintages (Swan Valley, Riverland, McLaren Vale, Langhorne Creek, Clare Valley, Padthaway, Coonawarra and Wrattonbully) with additional samples obtained from Tasmania for Chardonnay only, in 2014. In 2015, the grape varieties studied were limited to Chardonnay and Shiraz only, from the warm Riverland region for a grape to wine study which required larger samples of fruit to be obtained (~ 100 kg). The allocation of grade was varied in 2015 from that used in 2013/14, following a regional streaming approach (Streams A - E, A = higher value, E = lower value). These streams were within the lower grades (6-9) studied in previous seasons, and were used to identify fruit batches representing four wine style categories by variety, with a total of 16 samples taken for each.

5.2 Commercial harvest. Grapes were harvested at commercial ripeness. Following harvesting, batches of fresh fruit were stored sealed, and immediately transported to the Australian Wine Research Institute. In 2015, a 20-bunch sub-sample of harvested fruit was taken for analysis from the ~ 110 kg harvested. Samples which required transport to South Australia from Western Australia or Tasmania were processed fresh and analysed for basic juice composition properties on site as described below. These were then frozen prior to transport. On arrival, fruit batches were stored at 4 °C for no more than six days, with the exception of the frozen samples which were kept at -20 °C. Grape batches were completely de-stemmed by hand, and thereafter, sub-samples were taken for analysis either fresh or frozen as described below.

5.3 Winemaking. In the 2015 season duplicate 50 kg fermentations were performed on Chardonnay and Shiraz grape samples. Fruit was chilled overnight and a dose of 100 ppm potassium metabisulfite was added at crushing. After crushing, juice pH was adjusted to 3.4 and 3.45 for Chardonnay and Shiraz respectively, and YAN was adjusted to 200 ppm (where required). After pressing, Chardonnay must was cold-settled with commercial pectinase for 48 h, and then inoculated with Lalvin QA23 (Lallemand Pty. Ltd Australia), according to the manufacturer's instructions and fermented to dryness at 15 ºC. Once dry, tanks were topped and lees stirred once a week, at 10 ºC for 12 weeks, then SO_2 adjusted to 80 ppm and bottled in 750 mL bottles (N_2 sparged) under screw-cap. Shiraz musts were inoculated with Zymaflore FX10 (Laffort, Australia) according to the manufacturer's instructions and fermented at 20 ºC, with plunging of the cap twice daily, until a Baume of 2 Be was reached. Thereafter, wine was pressed, and the free run and wine pressings were kept together. After fermentation to dryness, (<0.5 g/L sugar) wines were racked off lees and inoculated with Viniflora Oenos (Chr. Hansen, Australia) at 15 ºC. When malic acid was below 0.2 g/L, 60 ppm SO_2 was added and wines were bottled in 750 mL bottles (N_2 sparged) under screw-cap.

5.4 Commercial assignment of allocation grade. For commercial harvest, grapes were typically picked based on a berry sensory assessment once minimum/required sugar levels had been reached for a specific variety. Overall grading assessment included a combination of factors including historical vineyard performance, berry sensory assessment, disease/defect assessment and vineyard details such as vine age, canopy viability/health and forecast weather with other lesser considerations occasionally used such as harvester/freight availability and winery intake capacity. Fruit from lower grades (6-9) from the warm, Riverland region included additional assessments of

canopy exposure, berry size and for red varieties only, colour by NIR to preliminarily stream fruit with later assignment to a specific grade at the point of fruit intake (crush grade).

5.5 Quantitative Descriptive Sensory Analysis. A panel of 10 - 11 assessors with an average age of 54 years was convened for this study. Assessors attended three training sessions to determine appropriate descriptors for rating in the formal sessions. During these sessions, the assessors evaluated wines from the study which represented the full range of sensory properties. These wines were used by the assessors to generate and refine appropriate descriptive attributes and definitions through a consensus based approach. Wines were initially assessed by appearance, aroma and palate. In sessions two and three, standards for aroma attributes were presented and discussed and these standards were also available during the booth practice session and the formal assessment sessions. Following the three training sessions, tasters participated in a practice session in the sensory booths under the same conditions as those used for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms was determined. Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in isolated booths under daytime lighting, with randomised presentation order, except in the practice sessions where there was a constant presentation order. All samples were expectorated. In practice booth sessions the assessors were presented with four trays of four wines per tray. In formal booth sessions of two hours duration the assessors were presented with four trays of four wines per tray, and one tray with three wines. The assessors were forced to have a 60 second rest between samples and a ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. Samples were evaluated over three days of formal sessions. Wines evaluated during the study were presented to assessors twice in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.47B, Biosystemes, Couternon, France). The intensity of each attribute was rated using an unstructured 15 cm line scale from 0 to 10, with indented anchor points of ‘low’ and ‘high’ placed at 10% and 90% respectively. Data were acquired using Fizz sensory software (version 2.47B, Biosystemes, Couternon, France). Panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software, and included analysis of variance for the effect of judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples. All judges were found to be performing to an acceptable standard. Analysis of variance (ANOVA) was carried out using Minitab (Minitab Inc., Sydney, NSW). The effects of wine (W), judge (J) and replicate (R) were assessed. Following ANOVA, Tukey’s honestly significant difference (HSD) value was calculated (P=0.05). Principal component analysis (PCA) was conducted on the mean values averaged over panellists and replicates where appropriate, using the correlation matrix.

5.6 Commercial evaluation of wine style. Wines which had completed fermentation and bottling were sensorially assessed by a panel of winemakers from Accolade Wines. Certain wines were excluded from assessment due to faults (e.g. aldehydes) which prevented a complete stylistic assessment. Based on the preliminary sensory assessment, wines were allocated into style categories and grading, according to the standard commercial streaming process. For Chardonnay, the following styles and attributes were defined, with quality stream A-D in parenthesis: EE7 (A) lemon, nectarine, tight, lean, long; EH2 (B) peach, fleshy, juicy; EE6 (C) tropical peach, juicy; EHC (C) shaded, herbal, lean; EW8 (D) buttery, ripe (exposed). For Shiraz, the following styles and attributes were defined, with quality stream A-D in parenthesis: FR7 (A) ripe, dense, not jammy; FQ1 (B) bright red fruits; FK2 (C, top) good density, sweetness, light herbal notes; FV6 (C) bright, tropicality, sweet fruit; FA6 (D) bright, tropical, green, lacking sweetness; FA1 (D) simple green, herbal. For statistical analyses, both separation by style codes and grade were performed.

5.7 Grape processing and distribution for analysis. Multiple 200-berry samples were collected and transferred to plastic bags equipped with a re-sealable zipper. A fresh 200-berry sample was weighed for the assessment of average berry weight. For techniques which required grape

homogenisation, 200-berry grape samples were either processed fresh, or immediately frozen at -20 °C depending upon subsequent analyses required. Prior to homogenisation, frozen 200-berry samples were partially defrosted. Both fresh and defrosted 200-berry samples were homogenised cold (<10 ºC) and analysed on the day of homogenisation. Berry samples were homogenised in a Retsch Grindomix GM200 (Retsch GmbH & Co., Germany) at 8,000 rpm for 20 sec. The remainder of the destemmed berries following sub-sampling were manually crushed and strained and the resulting juice was analysed fresh, or aliquots (10 mL) were frozen at -20 °C for later analysis, as described below.

5.8 Basic juice compositional analysis. Fresh juice samples were centrifuged to remove solids and the clarified supernatant was analysed. Total soluble solids (as °Brix) was determined using an electronic refractometer. Juice pH was determined using a pH meter and combination electrode. Titratable acidity (TA) was determined by titrating with 0.33 M sodium hydroxide solution to a pH end point of 7 and 8.2 and expressed in g/L of tartaric acid equivalents. Ammonia concentration in fresh juices was determined using the Glutamate Dehydrogenase Enzymatic Bioanalysis UV-method test (Roche, Mannheim, Germany). Free α-amino acid nitrogen (AAN) in juice was determined by the *o*-phthaldialdehyde/N-acetyl-L-cysteine spectrophotometric assay procedure, which excludes proline (Dukes & Butzke, 1998). Juice yeast-assimilable nitrogen (YAN) was calculated by adding the nitrogen present as ammonium to the AAN concentration. Malic acid was determined enzymatically using a commercially available analytical kit (Roche, Mannheim, Germany).

5.9 Targeted compositional analysis of grape juices. Frozen juice samples were thawed and centrifuged prior to analysis. Chloride content was determined by Waite Analytical Services as described by Wheal and Palmer (2010). The amino acid profile of juices was performed by Metabolomics Australia, using the method of Boughton et al. (2011). The analysis of juice C_6 volatiles [(E)-2-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol] was done according to the procedure described in Capone et al (2012). A number of analyses were performed on Chardonnay juices to better mimic the expected extraction in fermentation, which generally excludes a significant period of skin contact. Chardonnay juice samples were scanned between 280 nm and 370 nm at 10 nm increments between measurements using a UV-visible spectrophotometer (Cary 300, Varian, Australia) to estimate the potential contribution of phenolics. Also, the analysis of volatile thiol precursors (3-*S*-cysteinylhexan-1-ol (Cys-3-MH) and 3-*S*-glutathionylhexan-1-ol (Glut-3-MH), 3-*S*-cysteine-glycine-3MH (Cys-Gly-3MH)) was performed according to a published method (Capone et al. 2010; Capone et al. 2011) on Chardonnay juice. Phenolic-free glycosyl glucose (GG) was analysed in Chardonnay juice, as described subsequently (5.13).

5.10 Near- and mid-infrared analysis of grape homogenates and juices. For the collection of mid-infrared (MIR) spectra, fresh grape homogenates and juices (fresh and frozen) were scanned using a platinum diamond attenuated total reflectance (ATR) single-reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany). The ATR–MIR spectra were recorded on OPUS software version 6.5 provided by Bruker Optics. The spectrum of each sample was obtained by taking the average of 64 scans at a resolution of 8 cm^{-1} and acquired between 4000 and 889 cm^{-1} with a background of 64 scans. The reference background spectra were recorded using deionised water. Water was also used to clean the ATR cell to avoid carry over between samples and dried using disposable lab wipes. For the collection of near-infrared (NIR) spectra, fresh grape homogenates were also scanned using an Antaris FT-NIR analyser (Thermo Scientific, Scoresby, Victoria) between 3,800 and 12,000 nm. Spectra were exported from Bacchus acquisition software in Grams format (Thermo Galactic).

5.11 Extraction of grape berry homogenates. For red varieties, Cabernet Sauvignon and Shiraz, extraction of homogenate was performed prior to analysis of tannin, anthocyanin (520 nm) and GG. Following homogenisation of previously frozen grape samples which had been stored for no

longer than three months at -20 ºC, a 1 g portion of homogenate was weighed into a centrifuge tube. Two separate extractions were performed in 10 mL of 50% v/v, aqueous ethanol, which was either non-acidified or at pH 2 for GG and phenolics analysis respectively. Tubes were capped and extractions carried out on a suspension mixer for 1 h at room temperature. The tube was then centrifuged at 1730 *g* for 10 min, and the supernatant analysed as described below.

5.12 Colour, tannin and UV-visible spectrum of grape berry homogenates. Tannin was analysed in grape homogenate extracts were analysed using a standard high-throughput method (Mercurio et al. 2007). Each analysis was performed in duplicate. Briefly, tannin concentration was analysed following precipitation of ethanolic homogenate extract by methyl cellulose (MCP) in 1.1 mL 96 well deep well plates. In 2015 only, grapes were also extracted using a wine-like (WL) protocol as reported previously (Bindon et al. 2014). A 25 μL sample of grape extract or wine was combined with 300 μL of 0.04% (w/v) methyl cellulose (Sigma Aldrich, St. Louis, MO, USA) solution, mixed on an automated plate shaker and left to stand for 3 min. A sample control was included where water was added in place of the methyl cellulose solution. A 200 μL aliquot of saturated ammonium sulfate (Sigma Aldrich, St. Louis, MO, USA) was added, followed by 475 μL water. Samples were mixed on an automated plate shaker and left to stand for 10 min. Centrifugation was performed for 5 min at maximum speed on a Hettich Universal 32 R centrifuge equipped with a Hettich 1645 rotor for 96 well plates (Adelab Scientific). Samples of supernatant (300 μL) from the methyl cellulose treated and control samples were transferred into a 370 μL 96 well UV plate and the 280 nm absorbance measured using a SpectraMax M2 Microplate Reader (Molecular Devices, Australia). Quantification was performed by analysing the difference in 280 nm absorbance between control and MCP-treated samples, using (-)-epicatechin (Sigma Aldrich, St. Louis, MO, USA) as the quantitative standard. To ensure standardisation of the tannin analysis across the staggered time points at which grape and wine sampling and extraction took place, a purified commercial seed extract (Tarac Technologies, Nuriootpa, Australia) was included for each 96 well plate assayed. Furthermore, the ethanolic extract of the grape homogenate (pH 2) was diluted 1 mL with 10 mL 1 M HCl and scanned across the UV-visible range using a spectrophotometer between 200 nm and 800 nm (Cary 300, Varian, Australia). Key wavelengths were selected (520 nm, 420 nm, 370 nm, 280 nm) for input into the targeted chemical compositional analysis models, as described below. The full UV-visible spectrum was also analysed using multivariate statistical methods independently of other grape analyses performed.

5.13 Phenolic-free glycosyl glucose analysis. The GG analysis was based on a modification of methods previously published (Zoecklein et al. 2000). Some aspects of the process were altered due to difficulties neutralising the samples following the acid hydrolysis which were observed to cause issues with the glucose analysis. Homogenate extracts of red grapes (as described above) were frozen prior to analysis. A set of quality controls were analysed alongside each batch of samples run to ensure consistent recoveries and results. A Shiraz homogenate extract and a Chardonnay juice were each spiked with n-octyl-β-D-glucopyranoside (Sigma, St Louis, MO, USA) at a set concentration range. Homogenate extract (4 mL) or Chardonnay juice (2 mL) were diluted ten-fold with glycine buffer (pH 12.5) and allowed to equilibrate for 30 min. Oasis HLB SPE cartridges (3cc, 60mg) were conditioned with methanol (1 mL) and Milli-Q water (3 mL), followed by glycine buffer (3 mL, pH 12.5). The samples were loaded on the cartridges, and washed with glycine buffer followed by Milli-Q water (4 x 3 mL). Full vacuum was briefly applied to the manifolds to remove excess water before eluting with methanol (1 mL) into 1.5 mL screw cap tubes. The methanol was removed from the samples under vacuum at 30 °C in a vacuum centrifuge (Labconco). The extracts were recovered in trifluoroacetic acid (2 M, 500 μL) and then heated for two hours at 100 °C. Due to issues with acid neutralisation, the trifluoroacetic acid was removed completely under vacuum overnight at 30°C using a vacuum centrifuge (Labconco). The hydrolysate was then recovered in 800 μL water and vortexed. A calibration curve of glucose was prepared in the range of 0-400 μM. The calibrants were prepared in 2 M trifluoroacetic acid and subjected to the same conditions as the samples, as outlined above. A glucose/fructose kit (Randox Laboratories Ltd.) was used to quantify the glucose content of the eluate using a UV/Vis

spectrometer microplate reader (SPECTRAmax M2, Molecular Devices). The concentration of the homogenate extract was related back to the original mass of homogenate, with the assumption that the 1 g of homogenate produced 0.5 mL of juice; a total volume of 10.5 mL for the extract, with final data expressed as µmol/kg.

5.14 Free β-damascenone in grape samples. The method of Perestrelo et. al. (2011) was adapted for the analysis of free β-damascenone. A 7 g of sample homogenate from previously frozen grapes (-20 ºC) was weighed into a 20 mL crimp-cap, headspace-SPME vial (Grace Davison). 100 µL of 20% sodium dodecyl sulfate solution was added and vortexed to mix. A 3 mL aliquot of saturated NaCl solution was added and vortexed to mix followed by 50 µL of 1.2 mg/L d_4-β-damascenone internal standard solution. Samples were prepared and analysed in duplicate. GCMS analysis was performed on an Agilent 7890 gas chromatograph equipped with a Gerstel MPS2 autosampler and coupled to an Agilent 5975C VL mass selective detector. Instrument control was performed with Agilent G1701EA Revision E.02.02 ChemStation software. The gas chromatograph was fitted with an Agilent VF-WAXms 30 m x 0.25 mm x 0.5 µm with a 0.5 m x 0.25 mm Restek Siltek retention gap. Helium (Ultra High Purity) was used as the carrier gas with linear velocity 36.2 cm/s, flow-rate 1.0 mL/min in constant flow mode. The oven temperature was started at 35 °C, held at this temperature for 1 min then increased to 195 °C at 10 °C/min and held at this temperature for 7 min. The vial and its contents were heated to 80 °C for 20 min in the heater/agitator with the agitator on for 5 sec and off for 2 sec at 500 rpm. A Supelco grey 2 cm SPME fibre was exposed to the sample during this heating time through the septum. The fibre was then injected into a split/splitless inlet in pulsed splitless mode. The analytes were desorbed into a Supelco 0.75mm ID sleeveless SPME liner for 10 min which was held at 200 °C. The purge flow to the split vent was 50mL/min at 0.6min with the septum purge flow turned off. The mass spectrometer quadrupole temperature was set at 150 °C, the source was set at 230 °C and the transfer line was held at 250 °C. EMV Mode was set to Gain Factor = 1.00 and spectra were recorded in SIM/Scan mode. The low mass threshold was 65 amu and the high mass threshold was 200 amu. The ions monitored in SIM mode were: *m/z* 73, 179 and 194 for d_4-β-damascenone and *m/z* 69, 175 and 190 for β-damascenone.

5.15 Grape methoxypyrazine analysis. This analysis was performed on homogenates from Cabernet Sauvignon grapes in only one of the two seasons studied (2013). The method was followed on grape homogenate as outlined previously (Bindon et al. 2013). Due to the absence of isobutyl methoxypyrazine in the majority of samples analysed in 2013 preventing any meaningful contribution to the assessment of quality grade, this measure was excluded in subsequent vintages and is not reported for the current study.

5.16 Determination of laccase activity. The substrate 2' 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), which is colourless in its reduced form but dark green in its oxidised form (Li et al. 2008), was used to determine laccase activity in the grape samples due to its increased sensitivity compared to other commonly used methods (Li et al. 2008). The spectrophotometric method described by Li et al. (2008) was used, only 0.1M sodium acetate buffer was used rather than 1 mM, and 1.0 mL additions of sample, buffer and ABTS solutions were made to give a final reaction mixture volume of 3.0 mL. Sample preparation was similar to that described by Grassin and Dubourdieu (1989), except that 0.5 g of PVPP was placed in a 10 mL syringe with glass wool plugs at the exit and entry points and eight mL of juice sample was used. Juice samples were allowed to percolate through the PVPP column in order to give more contact time and interaction between juice and PVPP before being pushed through. The first mL exiting the syringe was used for analysis. Laccase activity was expressed in terms of units per mL (Li et al. 2008).

5.17 Basic wine compositional analysis. Basic analysis of wine pH, TA and malic acid were conducted as described above. Residual sugar, as the sum of glucose and fructose, was determined enzymatically using a Randox kit (Randox Laboratories Ltd., Crumlin, Antrim, UK). Wine ethanol concentration was determined using a Foss WineScan FT 120 as described by the

manufacturer (Foss, Hillerød, Denmark). The UV-visible spectrum of Chardonnay wines was determined as described for juice, above.

5.18 Wine protein concentration and composition. The composition and concentration of haze-forming proteins in must and wines were assessed in Chardonnay wines using high performance liquid chromatography (HPLC) and on precipitated proteins with sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) (Marangon et al. 2012). HPLC analysis was performed as previously described (Van Sluyter et al. 2013) with some modifications. Separation was achieved using Agilent 1260 UHPLC with a Prozap Expedite C18 column (10 mm x 2.1 mm i.d., Grace technologies), a solvent system of 0.1% TFA/H_2O (Solvent A) and 0.1%TFA/ACN (Solvent B), at 0.75 mL/min and 35 °C column temperature. The mobile phase gradient was: 0-1 min 10-20% Solvent B, 1-4 min 20-40% B, 4-6 min 40-80% B, 6-7 min 80% B, 7.01 min 10% B, and 7-10 min 10% B. Detection of proteins was achieved by Diode Array monitoring at 210 nm. Identification of TLPs and chitinases was achieved by comparing the retention times of sample peaks with isolated standards (Van Sluyter et al. 2009) and quantitation was achieved by comparing the peak areas with those of a standard curve of thaumatin (15 mg/L to 500 mg/L) (Sigma, Australia).

5.19 Wine polysaccharide concentration and composition. Wine polysaccharides in Chardonnay and Shiraz were isolated following precipitation of 1 mL wine with five volumes of ethanol, at 4 ºC. Following centrifugation, retention of the precipitate and reconstitution in water, samples were dialysed against four changes of Milli-Q water over 3,500 Da molecular weight cut off. Samples were then freeze-dried, reconstituted in 2 M TFA and hydrolysed for 3 h at 100 ºC. Liquid was removed under vacuum at 30 ºC. Hydrolytically released monosaccharides were analysed according to a modification of Honda et al. (1989). The dry TFA-hydrolysates were resuspended in Milli-Q water containing 0.3 M of ribose and deoxy-glucose as internal standards (Sigma-Aldrich, St. Louis, MO, USA). The derivatising reagent was 0.5 M of methanolic 1-phenyl-3-methyl-5-pyrazolone (PMP) in 1 M NH4OH. For the derivatisation, 25 µL of sample was mixed with 96.2 µL of derivatising reagent and placed in a heating block at 70 ºC for 1 h. After this step, the samples were cooled on ice and neutralised with 25 µL of 10 M formic acid. Then, the samples were extracted twice with dibutyl ether (Sigma-Aldrich, St. Louis, MO, USA) and the upper layer was discarded. The excess of dibutyl ether was dried under vacuum at room temperature. PMP-monosaccharide derivatives were quantified by HPLC using a C18 column (Kinetex, 2.6 µm, 100 Å, 100 × 3.0 mm) protected with a guard cartridge (KrudKatcher Ultra HPLC in-line filter, 0.5 µm) (Phenomenex, Lane Cove, NSW, Australia). The mobile phases were solvent A, 10% (v/v) acetonitrile in 40 mM aqueous ammonium acetate, and solvent B, 70% (v/v) acetonitrile in water. The following linear gradient was used: for solvent A (with solvent B making up the remainder) 92% at 0 min; 84% at 12 min; to 0% at 12.5 min; 0% at 14 min, then returning to the starting conditions at 14.5−18.5 min, 92%. A flow rate of 0.6 mL/min was used with a column temperature of 30 °C. The PMP-monosaccharide derivatives were identified using commercial standards (Sigma-Aldrich, St. Louis, MO, USA).

5.20 Wine tannin and colour. Wine total tannin and colour properties in Shiraz wines were determined according to the methods of Mercurio et al. (2007). In addition, tannin compositional analysis and size were determined according to the methods of Kennedy and Jones (2001) and Kennedy and Taylor (2003) with the modifications outlined by Kassara and Kennedy (2011).

5.21 Wine volatiles. Fermentation products in wine samples from both grape varieties were analysed according to the method in Siebert et al. (2005). Low molecular weight sulfur-containing volatiles hydrogen sulfide (H2S), methanethiol (MeSH), ethanethiol (EtSH), methyl thioacetate (MeSAc), ethyl thioacetate (EtSAc), dimethyl sulfide (DMS), diethyl sulfide (DES), carbon disulfide (CS2), dimethyl disulfide (DMDS), diethyl disulfide (DEDS) were analysed according to Siebert et al. (2010) for both varieties. For Chardonnay wines only, the ‘tropical’ thiols 3-mercaptohexan-1-ol

(3-MH), 3-mercaptohexyl acetate (3-MHA), 4-mercapto-4-methylpentan-2-one (4-MMP) were also determined according to Capone et al. (2015). C_6 volatiles were analysed for all wines as described previously, using the method of Capone et al. (2012). Free β-damascenone in wines of both varieties was analysed as described above for grapes, with a 7 mL sample of wine pipetted into a 20 mL screw-cap, headspace-SPME vial (Agilent CrossLab) in place of 7 g of grape homogenate, with the remaining procedure constant. In addition, in 2015, the method of Kwasniewski et. al (2010) was adapted for the analysis of bound β-damascenone in addition to free, as follows. A 10 mL aliquot of wine was adjusted to pH 2 with 2 M hydrochloric acid, and 50 µL of 1.2 mg/L d_4-β-damascenone internal standard solution was then added. Samples were heated at 100 °C in a heating block for 60 min to facilitate hydrolysis. Immediately after heating, samples were placed in an ice bath to cool. Due to some precipitation during heating, samples were filtered through glass wool and Whatman #1 filter paper prior to SPE analysis. Samples were loaded onto 500 mg/6 mL LMS SPE cartridges (Agilent, Australia) that had been pre-conditioned with 5 mL DCM, 5 mL methanol and 10 mL Milli-Q water. After sample loading, samples were rinsed with 10 mL Milli-Q water and dried under vacuum. β-damascenone was eluted with 4 mL DCM. DCM was dried under a stream of nitrogen to a final volume of approximately 300 µL. Thereafter, analysis by GCMS was as described above.

5.22 Statistical analysis. Principal component analysis (PCA), partial least squares regression (PLS), linear (L)/quadratic (Q) discriminant analysis (DA), as well as combined PLS-DA were performed using the Unscrambler X 10.3 (CAMO Software, Oslo, Norway). PLS-DA was found to discriminate only extreme grades from the other samples, at best, and was excluded as a valid multivariate approach for the modelling of grape grade, wine grade or wine style. Prior to multivariate analyses, basic statistical comparisons were performed to confirm normality of sample distribution using the same software. Targeted chemical data were scaled as the inverse of the standard deviation for multivariate analyses. Where required, MIR and NIR data were normalised using the Savitzky–Golay algorithm, as indicated in the results. All PCA and PLS analyses were performed with cross validation.

6. Results and discussion

6.1 Preliminary study of grade prediction from grape analyses in 2013 Cabernet Sauvignon to provide a methodological basis for subsequent seasons studied (2014, 2015)

A preliminary investigation of the potential of targeted and non-targeted chemical analysis of Cabernet Sauvignon grape samples was performed in 2013, with the intention that this would inform the larger, multi-cultivar experiment for the second and third seasons of the study. For this section of the report, a more detailed discussion of the types of multivariate analyses performed, their relative success in predicting grade, and the way that this information was used to inform the subsequent years of study is provided. A summary of all the results from 2013 described below is shown in table format in Table 6.1, and will be similarly presented for the 2014 season.

Table 6.1 Results summary of all multivariate analyses performed in the 2013 season for Cabernet Sauvignon grape samples.

Analysis Type	PCA							PLS regression				Discriminant Analysis (Quadratic)	
		Calibration variance (%)			Validation variance (%)								
	PC no	PC_1	PC_2	PC_{max}	PC_1	PC_2	PC_{max}	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$	PC no	% accuracy
Chemistry all	7	26.73	47.00	79.40	18.29	37.02	62.02	2	0.67	0.49	0.79	5	84.78
UV-visible spectrum	2	93.52	98.87	98.87	92.92	98.73	98.73	2	0.53	0.51	0.81	5	86.96
MIR homogenate fresh, raw data	6	64.07	80.76	94.24	60.44	75.52	90.85	5	0.9	0.8	0.48	5	85.37
NIR homogenate fresh, raw data	3	71.11	91.68	99.51	69.56	90.52	99.41	6	0.63	0.42	0.79	5	90.24

For the discussion of later seasons studied, the presentation of multivariate results is summarised in table format rather than presented in detail, on the premise that this has been described in detail in the preliminary study.

6.1.1 Clustering of samples by grade or region according to various targeted and non-targeted analyses in the 2013 season

Besides the prediction of commercial grade (as defined by a set of subjective parameters) using targeted and non-targeted chemical analysis of grapes, an initial consideration in the study was to explore whether patterns existed within the data obtained which could enable clustering of fruit samples in terms of their chemical attributes, and to observe whether this reflected patterns in grade or region of origin. This type of data analysis could potentially enable the streaming of fruit to be performed based on the similarity (or dissimilarity) of grape chemical composition.

To this end, a PCA of standardised chemical data was performed for the 2013 Cabernet Sauvignon data set. A wide range of chemical data was collected from the grape samples. These included basic berry chemistry such as average berry weight (g), pH, TA 7 (g/L) and TA 8.2 (g/L), total soluble solids (as ºBrix), moisture (%), malic acid (g/L), α-amino nitrogen (mg/L), ammonia (mg/l), YAN (mg/L). Possible negative markers of quality included laccase activity (units/mL) and chloride (mg/kg). UV-visible spectral data included total phenolics A280 (AU), colour A520 (AU), A420 (AU), MCP tannin (mg/L epicatechin equivalents). Aroma compounds included C_6 (µg/L) compounds, methoxypyrazines (µg/L), phenolic-free glycosyl glucose (GG) in homogenate (µmol/kg), and free β-damascenone (µg/L). For a summary of the functions of these compounds in grapes and wines, refer to Smith (2013).

The first two principal components (PCs) accounted for 47% of the variation between samples (Table 6.1, Figure 6.1.1.1). Using only these first two PCs, clustering related to grade was less distinct than found for other types of non-targeted chemical analysis (discussed below). This means that the data were more complex, and hence required a greater number of PCs to discriminate grades, with the maximum variance explained using seven PCs only reaching 75% (Table 6.1). Instead, the regional clustering of samples based on their chemical attributes was stronger than observed for grade. Methoxypyrazine concentration in grapes was excluded as a variable in all multivariate analyses, being detected in less than 12% of the total grape samples.

The first PC was defined mainly by phenolics and colour in terms of positive loadings and by amino acids in terms of negative loadings (data not shown). The same chemical attributes also defined the second PC, and in this instance loadings for phenolics and colour were stronger (positive) for PC2, and amino acids more heterogeneous, being both positively and negatively loaded on PC2. From the results shown in Figure 6.1.1.1, grapes from the Riverland and Western Australia (WA) tended to separate from the other samples, as defined by PC2, being somewhat lower in phenolics/colour and certain amino acids, but had higher levels of other amino acids.

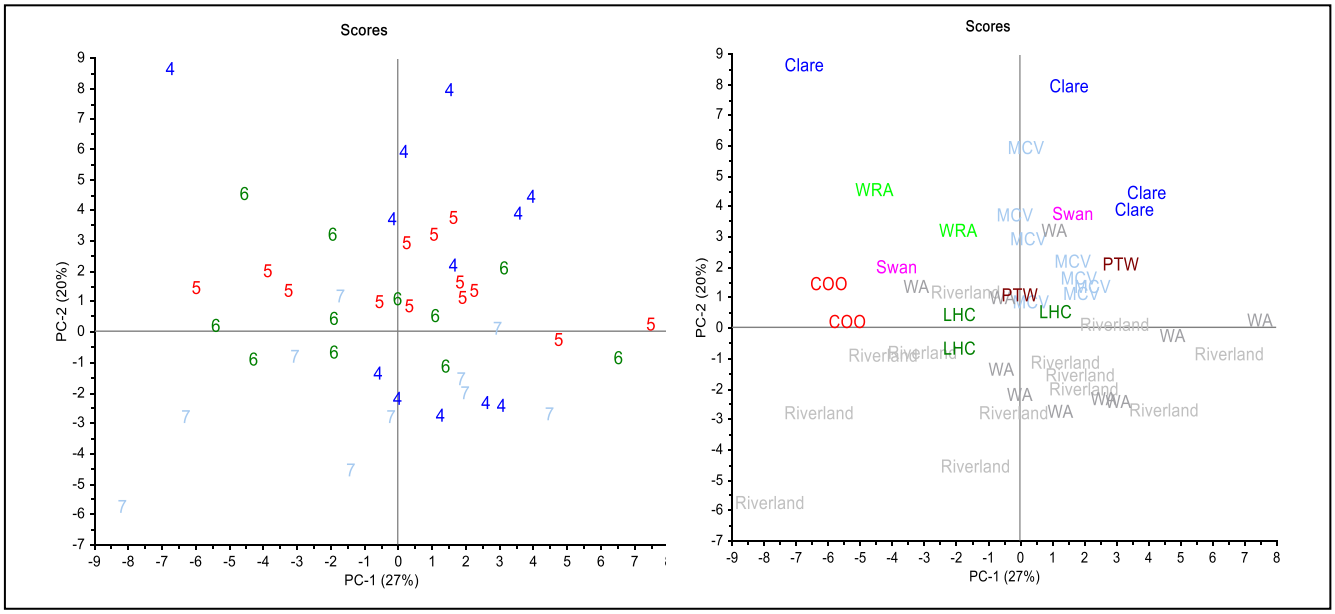


Figure 6.1.1.1. Principal component analysis scores plot for targeted chemical analytical data for 2013 Cabernet Sauvignon grapes, separated by grades 4-7 (left) and regions (right).

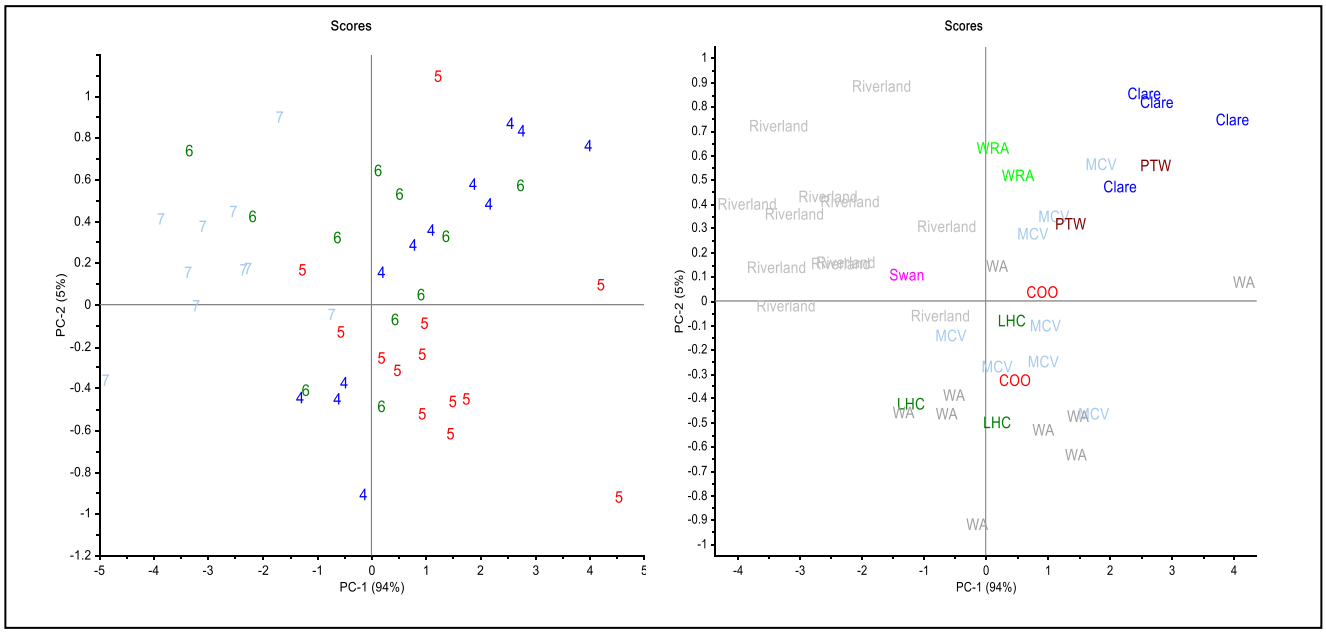


Figure 6.1.1.2. Principal component analysis scores plot for targeted UV-visible scan data for 2013 Cabernet Sauvignon grapes, separated by grades 4-7 (left) and regions (right)

A stronger clustering of samples was observed when the full UV-visible spectrum of grape homogenate extracts was analysed by PCA, in comparison with the full targeted chemical analysis (Figure 6.1.1.2); however this was primarily a separation of lower grades (7, primarily Riverland) from the other grades. The model was defined primarily by PC1, which explained 94% of the variability in the data set. This was also evident in the regional distribution of samples by PCA, with Riverland and Swan Valley clearly separated from the other regions. There was some clustering related to grade but also some signs of non-linearity. Overall, however, the UV-visible spectra showed only partial separation by grade or region, but could not be said to be distinct.

Using the MIR and NIR spectra for the 2013 Cabernet Sauvignon grape samples, (Figures 6.1.1.3 and 6.1.1.4) indistinct clustering was observed using PCA. While the first two PCs described 80% and 92% of the variation of the variation for MIR and NIR respectively, the spectra showed no distinct clustering by grade or region. The poor separation of the samples using NIR and MIR spectra indicates that the data structure (by region and grade) was more complex.

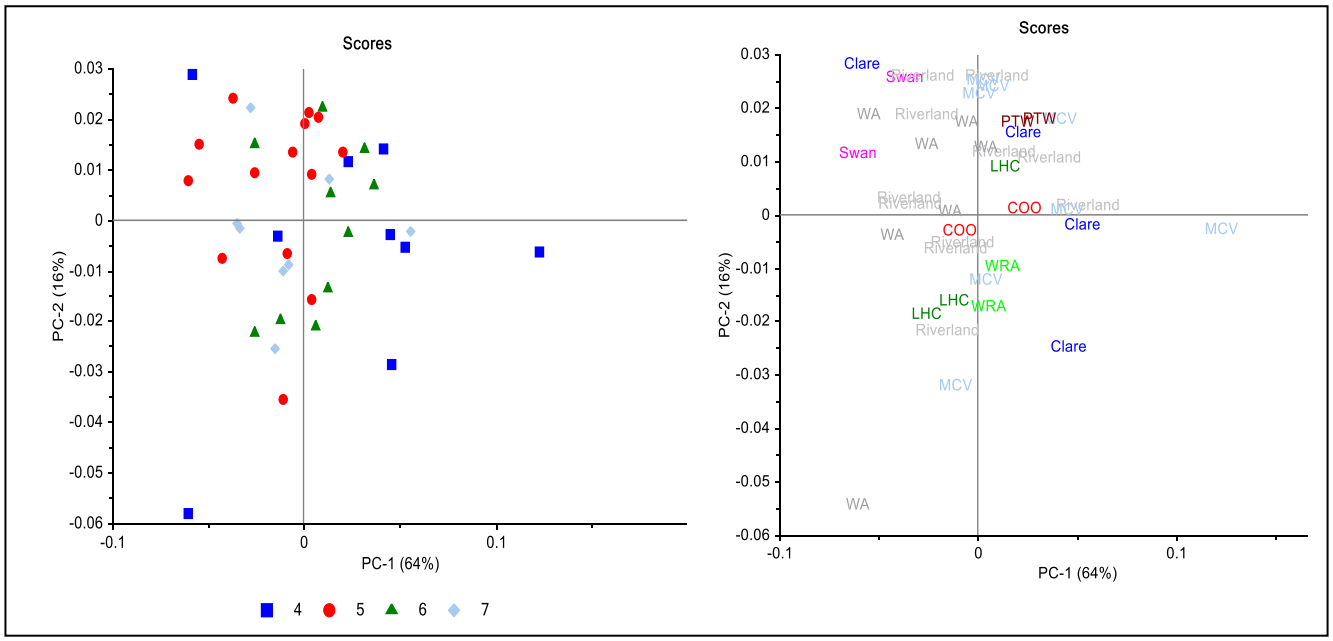


Figure 6.1.1.3. Principal component analysis scores plot for targeted homogenate MIR data for 2013 Cabernet Sauvignon grapes, separated by grades 4-7 (left) and regions (right).

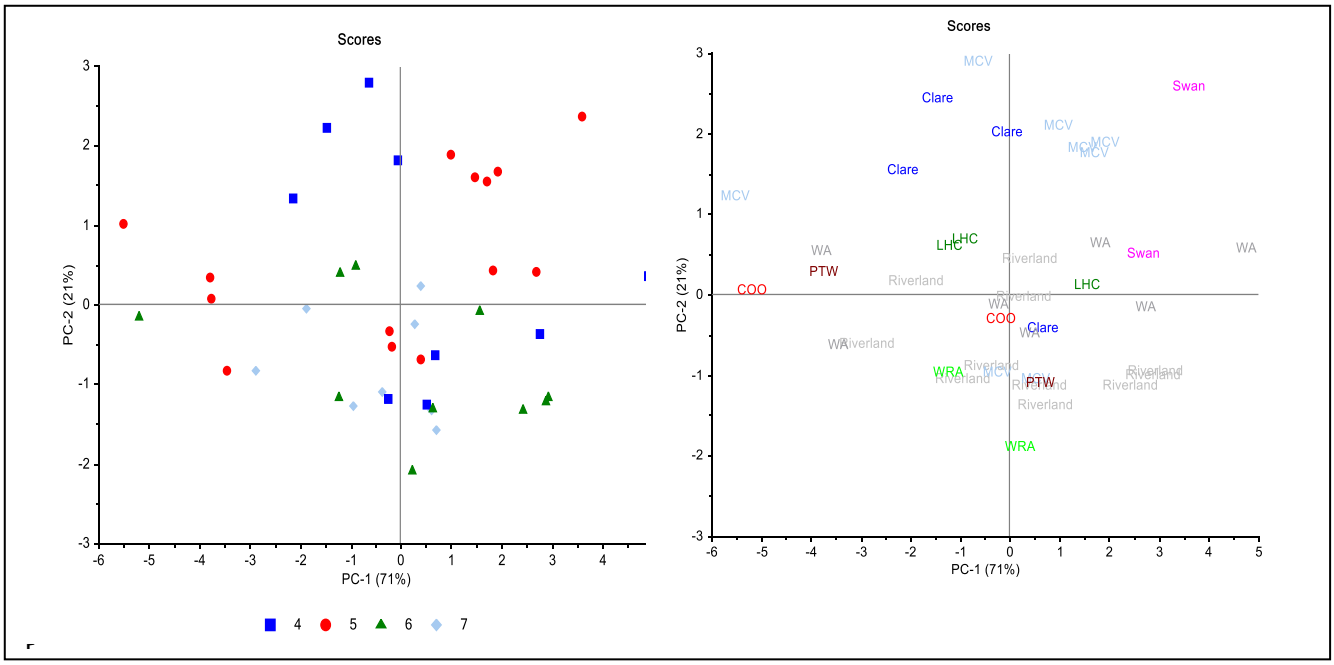


Figure 6.1.1.4. Principal component analysis scores plot for targeted homogenate NIR data for 2013 Cabernet Sauvignon grapes, separated by grades 4-7 (left) and regions (right).

6.1.2 CAS Grade prediction using partial least squares (PLS) in the 2013 season

Grade prediction was initially performed using PLS regression, where the grades were treated as numerical values. An advantage of this approach was that significant variables which define grading were identified, and cross-validation could be used to test the model. An uncertainty test was then used to identify the significant analytes contributing to the prediction of grade. Using PLS regression to describe the data set with targeted chemical analysis of the 2013 Cabernet Sauvignon grape samples, a model was obtained with only a moderate ability to predict grade (an R^2 of validation (R^2_{val}) of 0.49 and a root mean squared error of validation ($RMSE_{val}$) of 0.79) (Table 6.1, Figure 6.1.2.1). In terms of the $RMSE_{val}$ accuracy for grade prediction, given that the grades incorporated into the study were separated by a value of '1' apart from one another, an accurate $RMSE_{val}$ of prediction would be closer to 0.5.

Figure 6.1.2.2 shows the PLS model developed, but with only one factor (PC1) used for prediction, enabling the loadings for that factor to be examined directly to determine the variables significant to the prediction of grade. Coefficients were positive or negative depending on whether higher concentrations increased value (better grade) or decreased value (poorer grade) and only significant variables are shown. It was shown that total YAN, phenolics (A280), A420 (yellow) A520 (red colour) and free β-damascenone were positively associated (i.e. higher values) with higher value grades. Conversely, TA, cysteine, glutamic acid and glutathione were negatively associated (i.e. lower amounts) in higher value grades.

The PLS correlation with the UV-visible spectrum (240-700 nm) of the 50% ethanol extract of grape homogenates and grape grade resulted in an R^2_{val} of 0.51 and an $RMSE_{val}$ of 0.82 grade points. Using MIR spectra of grape homogenate an R^2_{val} of 0.80 was obtained in the prediction of grade which was a positive result, together with an $RMSE_{val}$ = 0.49 grade points (Table 6.1, Figure 6.1.2.1) which was (as discussed above) the best possible error considering that there

were no incremental scores in the reference data. The spectral data obtained for the NIR analysis resulted in an R^2_{val} of 0.42 and $RMSE_{val}$ of 0.80 grade points, similar to that derived from the UV-visible scans of the grape homogenate extract. In summary, PLS analysis of the targeted chemical analytical data set achieved R^2 and $RMSE_{val}$ results similar to that achievable using UV-visible spectra or NIR data. The best model for grade prediction using PLS was obtained with the MIR data.

6.1.3 CAS Grade prediction using discriminant analysis (DA) in the 2013 season

Using discriminant analysis (DA) with both spectral and chemical data using PCA scores, better prediction capacity was achieved for the 2013 Cabernet Sauvignon grape samples than for PLS. It should be noted that chemical data required standardisation to remove scaling effects prior to DA analysis, and since grading data are intrinsically categorical (rather than numerical) this type of analysis was appropriate. One drawback of the DA approach was that it was not possible to identify the drivers of the discrimination, and PLS therefore provided an indication of the main drivers of grape grade. Discriminant analysis applied to UV-visible spectra of grape extracts using the first five PCA factors (240-700 nm) allowed 40 out of 46 samples to be correctly predicted. The incorrect predictions were all in adjacent classes. Using the MIR spectra, 35 out of 41 samples were correctly predicted and five incorrect predictions were in adjacent classes, one was two classes away. Using the NIR spectra, 38 out of 41 samples were correctly predicted and all incorrect predictions were in adjacent classes.

Overall, the results suggest that mid-infrared spectral analysis of grape homogenates, with PLS regression modelling is a promising technique to assist in assessing/predicting grade. It should be noted that the ATR-MIR instrument used to collect the data provides a relatively low cost and rapid analytical method.

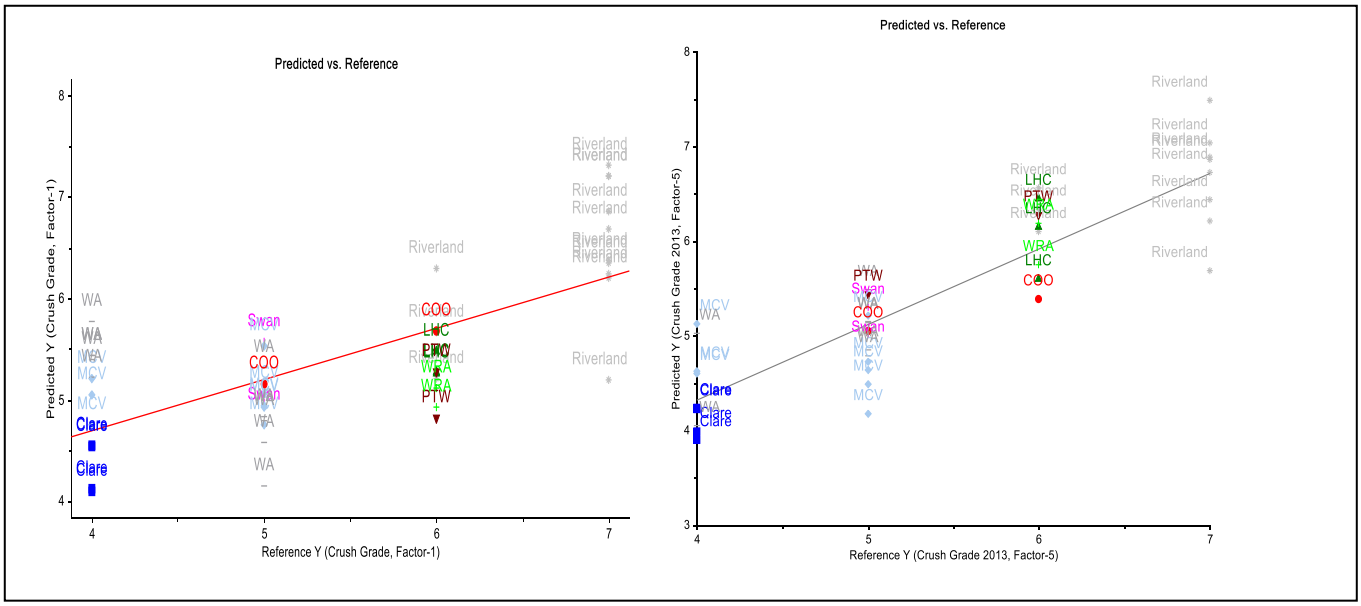


Figure: 6.1.2.1. Prediction of grade for 2013 Cabernet Sauvignon grape samples using PLS for targeted chemical analytical data (left) and MIR data (right)

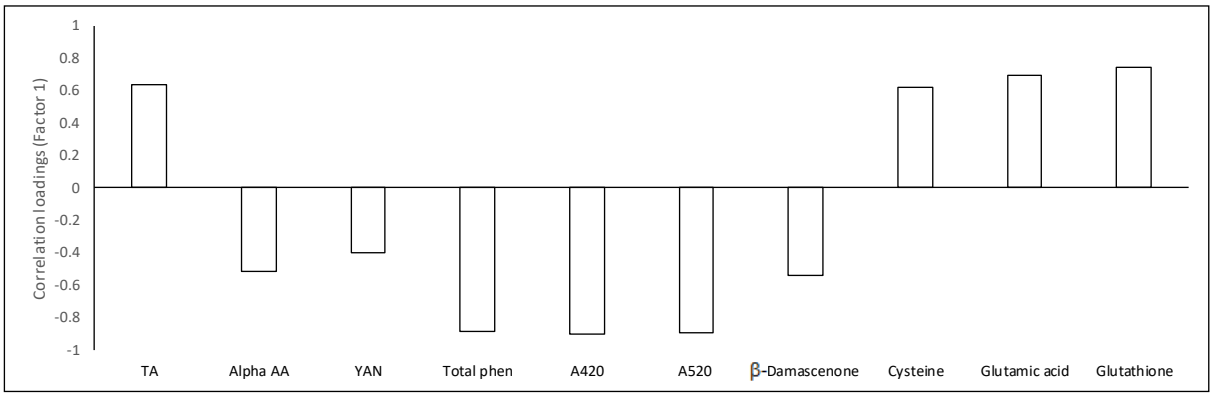


Figure 6.1.2.2 PLS loadings for the model developed for the prediction of Cabernet Sauvignon grape grade in 2013 from targeted chemical analytical data using one principal component (positive loadings associated with low value grades; negative loadings associated with high value grades).

6.2 Comprehensive study of grade prediction from analysis of three grape varieties in the 2014 season

In the 2014 season, the study was extended to include an additional two varieties, Chardonnay and Shiraz, and the work on Cabernet Sauvignon was repeated (with the exclusion of IBMP analysis based on the 2013 study). Additional to the 2013 study, MIR scans of juice were included in the non-targeted analysis suite in addition to homogenate. A summary of the results are shown in Tables 6.2.1 to 6.2.3.

6.2.1 Clustering of samples by grade or region according to various targeted and non-targeted analyses in the 2014 season using PCA

6.2.1.1 PCA of targeted chemical analysis and the UV-visible spectrum

Principal component analysis (PCA) of the grape samples was performed as for the 2013 season. For Cabernet Sauvignon, as observed in the first season of the study, the discrimination of samples using PCA was poor, requiring seven PCs to describe 80% of the variation (Table 6.2.1) and therefore indicating a complex data structure. The first two PCs of the PCA described 25% and 45% of the variation (calibration variance) respectively (Table 6.2.1) and the principal separation of the samples occurred on PC1 whereby the Riverland region (predominantly the lower grade fruit) was distinguished from the other regions studied (results not shown). The first PC of the PCA was defined by glycosyl glucose, phenolics (total phenolics or 280 nm, 320 nm, 370 nm, 420 nm and 520 nm) and MCP tannin; and these factors were negatively correlated to certain amino acids (glutamic acid), alpha-amino nitrogen and YAN (data not shown). However, in Cabernet Sauvignon, these measures did not clearly discriminate the remaining samples either by region or grade. This result, and the important metabolites defining the primary PCs, was similar to that observed in 2013.

Analysis of the Cabernet Sauvignon UV-vis spectral data gave a clearer PCA result, with 97% of the variation described in two PCs, 89% of which described PC1 (Table 6.2.1). This was similar to the result found in 2013, and similarly, indistinct separation of samples by either grade or region was found (data not shown). Clustering of Riverland samples (lower grade) was found, being low on PC1 which confirmed the observations using PCA for the complete analytical data set. For the remaining regions and grades studied, clustering of Great Southern samples was found with high

loadings on PC1 (high phenolics), but this was unrelated to grade. Overall, the observations with PCA were similar to those observed for 2013, and distinct sample clustering which might enable streaming using either targeted analysis or the UV-visible spectrum was not evident.

For the Shiraz samples analysed in 2014, PCA of the entire targeted analytical chemistry data set (Table 6.2.2) provided a better model of the variance within the data, with 65% described in two PCs. However, a maximum calibration variance of 86% was obtained using seven PCs, again showing that relationships within the data set were complex. As observed for Cabernet Sauvignon, the principal separation of samples was for the Riverland region (low score on PC1) as compared to other regions sampled, with a large distribution observed for the McLaren Vale region, and some clustering (high score on PC1) for the Great Southern, Clare and Padthaway regions (data not shown). PC1 was positively defined by amino acids (of which proline was most important), C_6 compounds, MCP tannin and phenolics (280, 320, 370, 420 and 520 nm). For grade as opposed to region, only grade 9 (low value) showed distinct clustering on PC1 (low scores), since this grading was largely defined by its origin in the Riverland region. As observed for Cabernet Sauvignon, the PCA model for only the UV-visible spectrum was well described in only two PCs, with 98% of the variance explained in PC1, confirming the importance of phenolics (280, 320, 370, 420 and 520 nm) in the initial PCA model. For the UV-visible spectrum PCA, the validation variance was also equivalent to the calibration variance. As for the initial chemical-analytical PCA model, clustering of the Riverland region, and quality grades 9 and 7, was observed with lower scores on PC1, being lower in phenolics overall. However, a lack of clear separation from the lower quality grades (4-6) from the McLaren Vale region was observed. As found for the Cabernet Sauvignon study, a clear clustering of samples which might facilitate streaming of 'like' batches of grapes based on their chemical similarity was not observed using PCA.

For the Chardonnay samples, using the full chemical-analytical data set a PCA model explaining 56% of the variation within the data in two PCs was developed (Table 6.2.3), which in five PCs could describe only 75% of the variation. This again demonstrated that relationships within the data were complex. However, for a subset of significant variables which included ºBrix, pH, TA, malic acid, nitrogen (YAN, ammonia, alpha-amino nitrogen), malic acid, chloride, berry weight and the UV-visible spectrum between 280 and 370 nm (phenolics) a PCA model explaining 99% of the variation in five PCs could be defined, with 76% described in two PCs. While no clear pattern of sample clustering could be observed with the whole chemical-analytical data set, with the subset of parameters, separation of Riverland samples, or grade 9 (high score on PC1) from the other regions and grades was achieved (data not shown). These samples were defined generally by higher levels of phenolics, higher pH, and lower acidity (TA, malic acid). PC2 (13% variation) was defined by nitrogen measures and no clustering of samples by grade or region was observed on this PC. This observation may indicate that the significant parameters (pH, acidity) are already used to determine grape quality grading, and are also defined on a regional basis. However, the observation that phenolics measures were important in determining allocation grade and were regionally defined was of interest, and may indicate the response of grape development to the hot, dry Riverland climate. Unrelated to the overarching factors of grade or region, sample groupings using the Chardonnay analytical subset could be observed according to their scores on PCs 1 and 2. This may highlight the possibility that fruit could be streamed according to its chemical similarity by the small number of parameters identified using PCA.

6.2.1.2 PCA of non-targeted mid- and near-infrared spectral analysis

In the 2014 season, MIR spectral data were collected for juice (fresh and frozen) and fresh homogenates. NIR scans were performed on fresh homogenates. For samples which had been transported frozen from other states within Australia, fresh MIR and NIR analysis was not performed, reducing the sample set slightly. Spectra could be analysed raw, and in some instances applying the Savitzky-Golay algorithm to the data improved the multivariate results.

Using either of these approaches, the PCA models developed could describe higher levels of variation within the data, using fewer PCs than for the chemical-analytical data set for all three varieties (Tables 6.2.1 to 6.2.3). The calibration and validation variance values were also similar or in some cases the same. This observation was similar to that observed using non-targeted analyses in the 2013 season. Also similar to the preliminary study, the spectra showed poor clustering of samples by grade or region (data not shown). The poor separation of the samples using NIR and MIR spectra indicate that the data structure was more complex.

Table 6.2.1 Summary of multivariate statistical analysis results for the assessment of Cabernet Sauvignon grape allocation grade based on chemical analytical data for the 2014 season.

Analysis Type	PLS													
	PCA						PLS regression				Discriminant analysis			
		Calibration variance (%)			Validation variance (%)						Linear		Quadratic	
	PC no	PC_1	PC_2	PC_{max}	PC_1	PC_2	PC_{max}	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$	PC no	% accuracy	PC no	% accuracy
Chemistry all	7	24.87	44.91	79.96	17.68	35.64	65.22	1	0.72	0.69	1.22	5	74.50	5	88.24
UV-visible spectrum	3	89.30	97.13	99.30	89.30	97.13	99.30	3	0.61	0.56	1.58	5	72.55	5	96.08
MIR juice fresh, raw data	3	89.44	95.32	98.70	89.44	94.08	98.70	2	0.79	0.76	1.09	5	84.62	3	94.87
MIR juice fresh, S. Golay	2	84.58	87.55	87.55	84.58	85.70	85.70	2	0.88	0.86	0.86	5	79.49	4	94.87
MIR juice frozen, raw data	2	96.69	98.52	98.52	96.69	98.52	98.52	4	0.81	0.77	1.15	5	68.63	5	88.24
MIR juice frozen, S. Golay	2	90.17	91.55	91.55	89.38	90.15	90.15	4	0.88	0.79	1.05	5	64.71	5	92.16
MIR homogenate fresh, raw data	3	85.99	93.01	97.06	85.99	91.59	97.06	5	0.89	0.84	0.94	5	79.49	5	100.0
MIR homogenate fresh, S. Golay	3	81.78	84.91	87.23	80.73	82.90	83.92	1	0.67	0.66	1.33	5	79.49	5	92.31
NIR homogenate fresh, raw data	2	76.38	98.52	98.52	74.98	98.52	98.52	5	0.74	0.65	1.32	5	69.23	5	76.92
NIR homogenate fresh, S. Golay	3	79.04	91.99	93.83	77.48	91.05	92.70	3	0.77	0.71	1.24	5	69.23	5	87.18

Table 6.2.2 Summary of multivariate statistical analysis results for the assessment of Shiraz grape allocation grade based on chemical analytical data for the 2014 season.

Analysis Type	PCA						PLS regression				Discriminant analysis			
		Calibration variance (%)			Validation variance (%)						Linear		Quadratic	
	PC no	PC_1	PC_2	PC_{max}	PC_1	PC_2	PC_{max}	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$	PC no	% accuracy	PC no	% accuracy
Chemistry all	7	47.79	64.59	85.68	44.43	58.75	74.15	3	0.77	0.66	1.38	5	58.62	5	85.00
UV-visible spectrum	2	98.34	99.60	99.60	98.34	99.60	99.60	6	0.80	0.75	1.18	5	62.07	5	94.83
MIR juice fresh, raw data	3	80.46	94.98	98.31	80.46	94.98	98.31	3	0.71	0.67	1.34	5	65.45	5	80.00
MIR juice fresh, S. Golay	3	86.63	89.90	91.80	86.63	88.98	90.69	2	0.72	0.72	1.33	5	54.55	5	80.00
MIR juice frozen, raw data	2	97.31	98.84	98.84	97.31	98.84	98.84	4	0.80	0.71	1.25	5	46.55	5	77.59
MIR juice frozen, S. Golay	2	90.97	94.25	94.25	90.97	94.25	94.25	2	0.64	0.61	1.44	5	48.28	5	82.76
MIR homogenate fresh, raw data	3	89.61	95.82	97.65	89.61	95.82	97.65	6	0.89	0.84	0.97	5	63.64	5	89.74
MIR homogenate fresh, S. Golay	3	86.31	88.79	92.38	86.31	88.79	90.78	3	0.79	0.69	1.33	4	56.36	4	70.91
NIR homogenate fresh, raw data	2	80.75	98.43	98.43	80.75	98.43	98.43	5	0.64	0.57	1.59	4	46.30	5	94.44
NIR homogenate fresh, S. Golay	4	74.08	92.70	95.13	74.08	92.70	95.53	6	0.91	0.61	1.49	5	62.90	5	87.04

Table 6.2.3 Summary of multivariate statistical analysis results for the assessment of Chardonnay grape allocation grade based on chemical analytical data for the 2014 season (na = not applicable).

Analysis Type	PCA						PLS regression				Discriminant analysis			
		Calibration variance (%)			Validation variance (%)						Linear		Quadratic	
	PC no	PC_1	PC_2	PC_{max}	PC_1	PC_2	PC_{max}	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$	PC no	% accuracy	PC no	% accuracy
Chemistry all	5	32.57	56.09	75.10	27.50	49.92	64.75	2	0.84	0.78	1.29	5	87.50	5	94.64
Chemistry sub-set*	7	62.37	76.30	98.81	59.90	71.19	97.42	3	0.74	0.68	1.54	5	85.71	5	94.64
MIR juice fresh, raw data	3	68.40	93.12	96.16	65.22	92.18	95.40	7	0.90	0.64	1.68	5	63.83	5	72.34
MIR juice fresh, S. Golay	2	63.92	70.72	70.72	61.64	64.36	64.36	3	0.78	0.66	1.64	5	68.09	5	92.98
MIR juice frozen, raw data	3	90.55	96.43	97.82	90.55	96.43	97.82	5	0.79	0.66	1.59	3	78.53	5	83.93
MIR juice frozen, S. Golay	2	81.29	88.90	88.90	81.29	88.21	88.21	6	0.88	0.72	1.46	4	63.33	5	83.93
MIR homogenate fresh, raw data	4	68.96	87.53	95.88	66.85	85.85	93.46	6	0.85	0.63	1.63	5	68.09	5	70.21
MIR homogenate fresh, S. Golay	4	65.65	72.71	80.85	64.32	69.91	75.48	5	0.91	0.78	1.29	5	59.57	5	78.72
NIR homogenate fresh, raw data	3	73.10	97.45	99.60	69.75	97.45	99.60	na	0.015	na	na	5	56.52	5	79.07
NIR homogenate fresh, S. Golay	3	88.58	94.37	96.15	88.58	94.37	96.15	na	0.019	na	na	5	53.19	5	85.71

*A sub set of basic berry analyses including pH, titratable acidity (pH 7 and 8.2), YAN, ammonia, Brix, alpha-amino nitrogen, malic acid, chloride, berry weight, UV-vis of juice at 280 - 370 nm (scan with 10 nm increments).

6.2.2 Grape allocation grade prediction using partial least squares (PLS) in the 2014 season

PLS regression models were developed for the prediction of allocation grade from the targeted and non-targeted analytical data sets for the three varieties studied in 2014, repeating the preliminary analysis of the first season. To identify significant variables, an uncertainty test was performed during the PLS analysis. For the PLS analysis, grades were either assigned numerical values or categories (PLS-DA). It was found that the PLS-DA approach was unsuccessful, and PLS models with poor regression values of calibration and validation were produced, with the exception that in some instances grade (as a category) 9 could be distinguished from the other samples. Due to the poor results using the PLS-DA approach, these data will not be presented. The results for the PLS analyses in 2014 are shown in Tables 6.2.1 to 6.2.3.

6.2.2.1 Grape grade prediction using PLS for chemical-analytical and UV-visible spectral data

For the 2014 Cabernet Sauvignon grape samples PLS regression analysis of the targeted chemical analysis data, a model was obtained with an ability to predict grade which was improved from that developed in 2013; with an R^2_{val} of 0.69 (PC1) and a slightly higher $RMSE_{val}$, of 1.22 (Table 6.2.1).

Figure 6.2.2.1 shows the PLS model developed with only one factor used for prediction to determine the significant variables. Similar to the 2013 results, phenolics (280, 320, 370, 420 and 520 nm) were important in the definition of higher value grades. Further to the results obtained from the preliminary study which highlighted multiple phenolics as being important to quality, MCP tannin was specifically highlighted as a quality marker in 2014. Unlike 2013, however, nitrogen measures (YAN, alpha-amino nitrogen) were negatively associated with higher value grades whereas the reverse was found in the previous year. The results for alpha-amino nitrogen values (which contributed to YAN) were driven mainly by increases in a particular amino acid, glutamic acid, which appeared to be a seasonal response. Glutamic acid was negatively associated with higher value grades, also observed in 2013. This indicates that this particular amino acid may be strongly influenced by season, but also was consistently associated with lower quality grading across two seasons, and warrants further research. Other important amino acids (isoleucine, proline) were positively correlated with higher value grades. In the second season of the study, neither TA nor free β-damascenone were found to be important in defining grade in Cabernet Sauvignon grapes, but rather C_6 compounds and glycosyl-glucose were negatively and positively associated with higher value grades, respectively. Chloride, and ºBrix were positively associated with higher quality grading.

As for the 2013 season, a PLS model for the UV-visible spectrum of the Cabernet Sauvignon grape data could be developed (Table 6.2.1), with similar R^2_{cal} and R^2_{val} values obtained, but a larger $RMSE_{val}$ indicating that differentiation between adjacent grades was less well modelled by the 2014 data than for the previous season. This potentially indicates that while phenolics were important in defining quality grade, as evidenced in the PLS model using all the whole chemical analytical data set, that other metabolites were also important, as discussed above. However, this observation also raises a potential limitation of the PLS modelling approach, in that it defines the grades as numerical values, whereas in reality this is not the case. To this end, the categorical approach defined by PLS-DA or DA would potentially be more effective. As discussed previously, however, the PLS-DA approach was found not to be effective in defining grape quality grade.

Taken together, the results indicate that for Cabernet Sauvignon, investigated over two seasons, certain key objective measures were important in predicting grade, and to some extent the relationships between these measures could be seasonally defined. Phenolics measures, as determined using the UV-visible spectrum seemed to make an important contribution to the prediction of grade inter-seasonally, and this observation may simply bear reference to the measures currently used by the producer to distinguish grape parcels, whether this is based on

current or historical data. The inter-seasonal importance of glutamic acid as a negative marker for quality appears to be of importance for ongoing study, since this compound has not previously been raised as being relevant to grape or wine production.

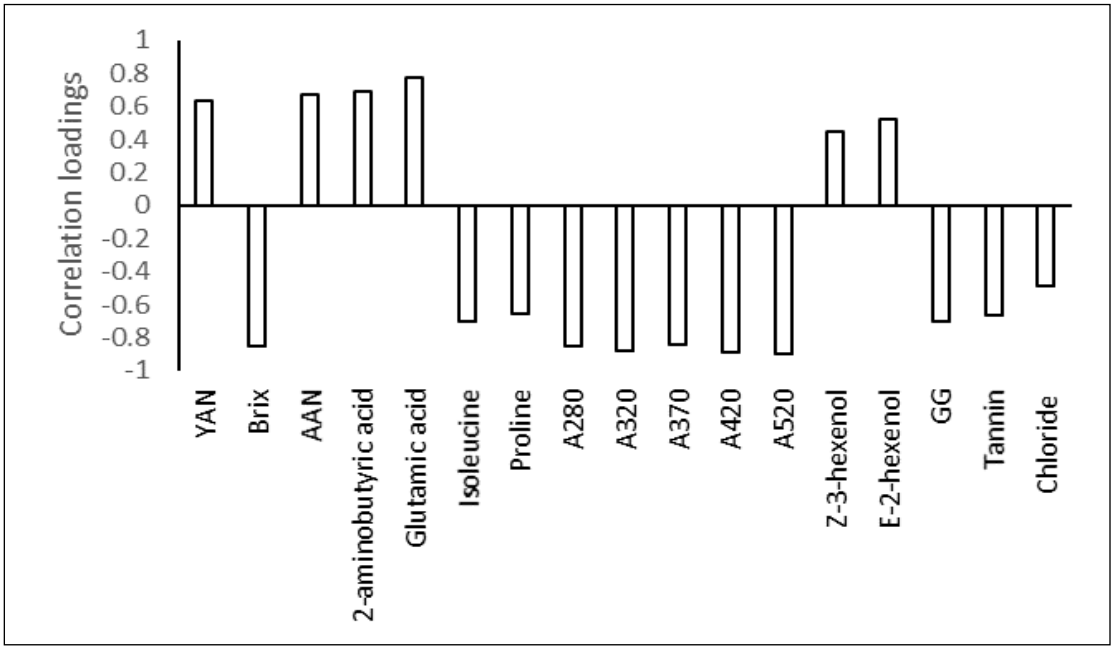


Figure 6.2.2.1 PLS loadings for the model developed for the prediction of Cabernet Sauvignon grape allocation grade in 2014 from targeted chemical analytical data using one principal component.

The prediction of allocation grade for Shiraz and Chardonnay grapes from chemical measures was performed for only one season, 2014. For Shiraz grapes, similar R^2_{cal}, R^2_{val} and $RMSE_{val}$ values were obtained to that observed for Cabernet Sauvignon, as described above (Table 6.2.2). For Shiraz, the key predictive measures identified (Figure 6.2.2.2) tended to be positively associated with quality grading, with only three measures being significantly negatively related: aspartic acid, Z-3-hexenol (a C_6 compound) and berry weight. Berry weight was the most significant negatively associated variable for quality grade prediction both in terms of correlation loadings and regression coefficients (data not shown, see Appendix 5) for the model. This has bearing on the observation that berry size has long been anecdotally used as a measure to indicate Shiraz quality, with smaller berries being preferred. This could also be an indicator that poorer grade fruit was obtained from more highly irrigated regions, such as the Riverland. The regional distinction of grade has been discussed previously in the PCA.

Many of the variables positively associated with quality grade were similar to those defined for Cabernet Sauvignon, with phenolics measures (280, 370, 420 and 520 nm) being important positive variables, as well as MCP tannin, ºBrix and chloride. Similar to the 2013 season Cabernet Sauvignon results, nitrogen measures such as YAN, alpha-amino nitrogen being positively associated with the prediction of higher quality grades. Interestingly, the amino acid (glutamic acid) associated negatively with quality was positively associated with higher quality in Shiraz. Certain other important amino acids, aspartic acid, isoleucine, serine and tyrosine were identified as positive quality markers. Contrary to what was observed for the 2014 Cabernet Sauvignon samples, one C_6 compound Z-2-hexenol was very strongly correlated with higher quality grade. A caveat in the results was that laccase activity, an important indicator of pathogen infection was also found to be positively associated with higher quality. This was expected to be a negative marker for quality at the outset of the project. The finding that a positive relationship with quality was observed was unexpected, and may have a bearing on the differences between the climatic (and pathogen prevalence) conditions between regions which produce fruits of different grades.

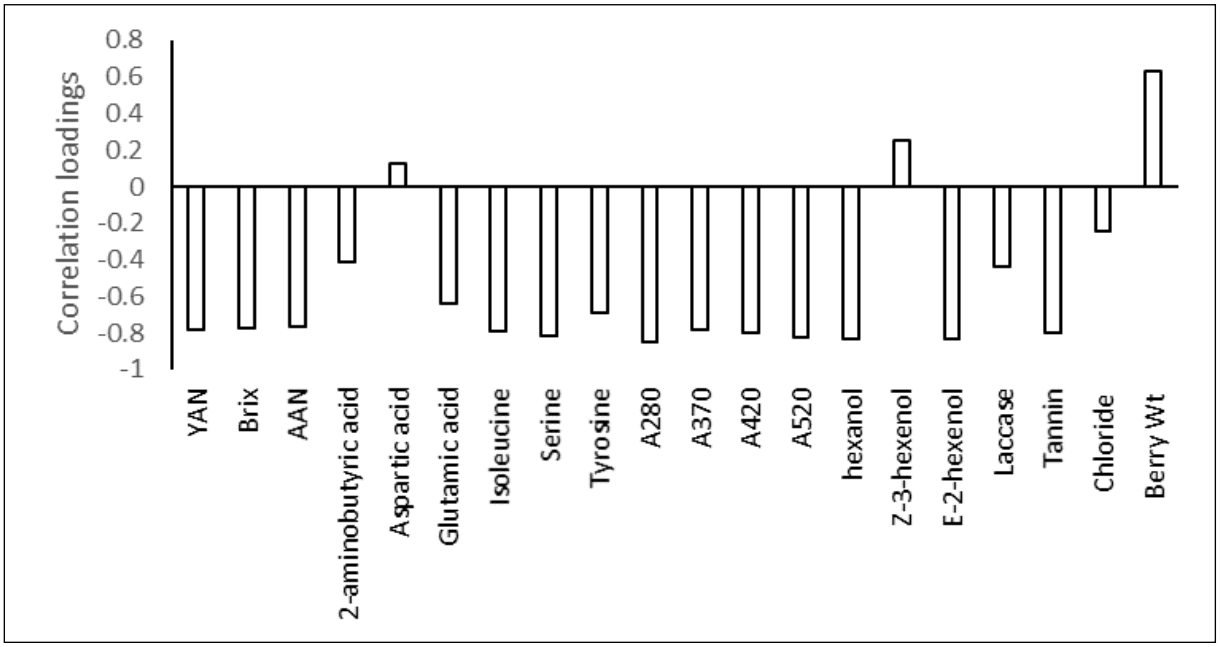


Figure 6.2.2.2 PLS loadings for the model developed for the prediction of Shiraz grape grade in 2014 from targeted chemical analytical data using one principal component.

The PLS analysis for grade allocation prediction from the Shiraz grape UV-visible spectrum gave far higher R^2_{cal} and R^2_{val} and values than observed for the Cabernet Sauvignon data sets (2013, 2014) at 0.8 and 0.75 respectively (Table 6.2.2). Further, the $RMSE_{val}$ value was lower for Shiraz, at 1.18, indicating that differentiation between grades as numerical increments was better than observed previously for Cabernet Sauvignon. This was a positive result due to the simplicity and accessibility of the UV-visible data. However, as discussed for the PCA, this result may indicate that UV-visible measures such as those use to identify phenolics (which were also important contributors to the PLS model developed for the whole targeted chemical data set) are currently, or were historically, in use for grade prediction by the producer. On the contrary, personal communication with the producer revealed that this was only in use in one of the regions analysed (Riverland) and that the technique used was an indirect measurement (NIR). Therefore, this result demonstrates that UV-visible data from Shiraz grapes can be used to distinguish grade, for which phenolics measures may be more important than other measures in defining grapes in terms of wine quality outcomes. A possible negative aspect of the result is that the PC number required to develop the PLS model was high, and indicates complex relationships within the data set. These will be discussed at a later stage in relation to the follow-up discriminant analysis approach also used to predict grade.

The earlier discussion of the PCA of Chardonnay grape compositional data showed that a subset of basic grape compositional data could be used successfully to model the sample set. Two PLS models were therefore developed for the prediction of grade from the Chardonnay chemical-analytical data, using all of the available measurements, and the subset discussed previously. From the initial PLS model developed to predict Chardonnay grade (Table 6.2.3), high values of R^2_{cal} and R^2_{val} were obtained at 0.84 and 0.78 respectively, which were better than those obtained for the red grape varieties studied. The $RMSE_{val}$ value for the PLS model was 1.29, similar to that observed for the other varieties, indicating that adjacent grades were not necessarily well separated. A positive aspect of the PLS model developed was that it was achieved within only two PCs, enabling a more successful identification of the important variables in grade assessment than previously for the red varieties.

The important variables defined for Chardonnay are shown in Figure 6.2.2.3, and both positive and negative attributes important to grade allocation could be identified. As also observed in the PCA, pH was higher in poor value grades and acidity as TA and malic acid were increased in higher value grade allocations. Interestingly, similar to the Cabernet Sauvignon results, glutamic acid was higher in poorer quality Chardonnay grapes. Higher levels of this amino acid were also correlated with proline, which may show that some environmental stress was present in the grapevines from poorer quality grades, since this amino acid tends to be produced under these conditions (Matthews and Anderson, 1988). Interestingly, proline was identified as a positive marker for higher value grades in the Shiraz PLS model which may indicate that the reverse was evident in the higher quality Shiraz vineyards. Associated with the notion that vineyard environmental conditions may be used to determine quality, the 370 nm measure in CHA was the only UV-visible wavelength that was important to quality, also being negatively associated (higher 370 nm in poorer quality grades). This UV-visible wavelength is associated with the class of phenolic compounds called flavonols, which generally increase under conditions of either water stress or sunlight exposure within the canopy (Kennedy et al. 2002). Lower chloride levels were also evident in higher quality grade allocations. Together, these observations point to the possibility that observations of either vineyard stress, canopy exposure, or both aspects, may be used to assess vineyards for quality. Personal communication with the producer revealed that canopy development and exposure are in part used for allocation decisions, making the 370 nm measure, and potentially proline and chloride, important toward identifying key objective measures for quality in Chardonnay.

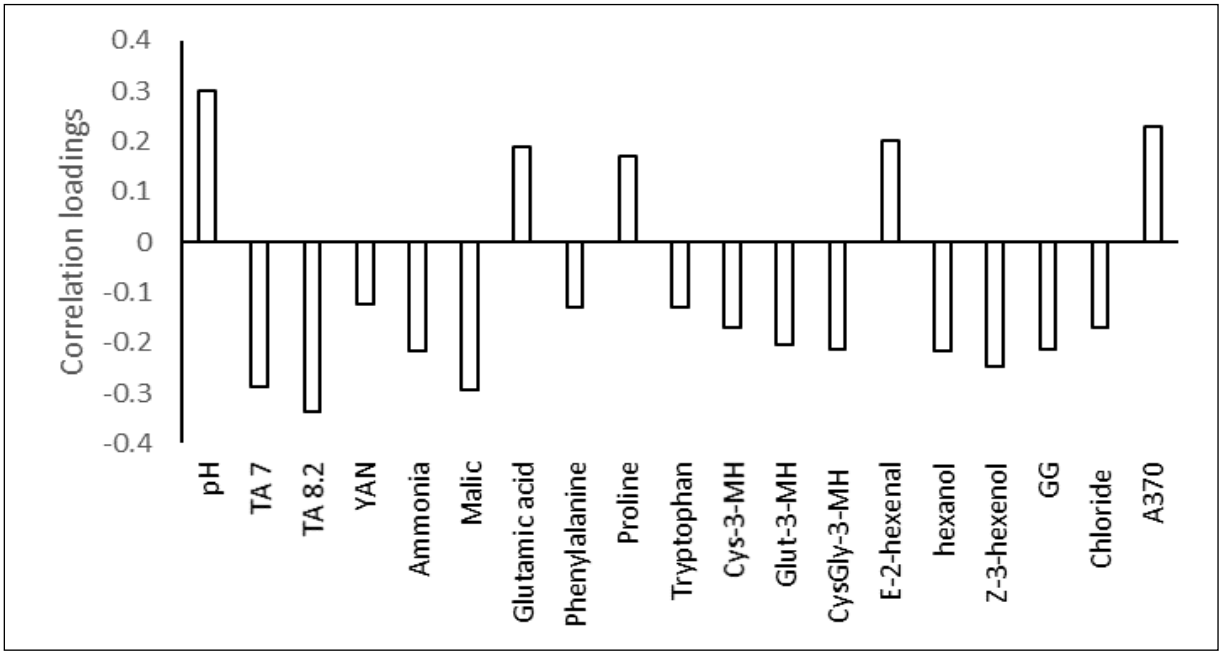


Figure 6.2.2.3 PLS loadings for the model developed for the prediction of Chardonnay grape grade in 2014 from targeted chemical analytical data using one principal component.

Similar to the 2013 Cabernet Sauvignon and the 2014 Shiraz results, Chardonnay nitrogen levels were important in the assessment of grade, however in the case of Chardonnay the high YAN levels observed in higher quality grades was due to increased ammonia and only two amino acids, phenylalanine and tryptophan. Important positive quality markers were aroma compounds and their precursors. The precursors to tropical thiol aromas: Cys-3-MH, CysGly-3-MH and Glut-3-MH were all elevated in higher value grades, as well as glycosyl glucose which would be expected to be associated with glycosylated aroma precursors. For the C_6 volatiles, E-2-hexenal was negatively associated, with hexanol and Z-3-hexanol positively associated with higher value allocation grades. The extent to which the free volatiles or aroma precursors identified might be detectable through berry tasting, or are environmentally regulated is unknown. It is therefore not

possible to speculate how these significant compounds (or classes or compounds) may be related to the current quality grading approach used by the producer. It is also possible that historical wine outcomes in terms of flavour and aroma may influence the allocation grading of the vineyard. Further research could aim to identify causative relationships between stress indicators such as proline, chloride and absorbance at 370 nm and the aroma volatiles and precursors discussed. Nevertheless, the identification of important classes of flavour/aroma compounds as being relevant in the objective prediction of grade was a promising result.

Further processing of the full chemical-analytical PLS model for Chardonnay with the uncertainty test, as well as observation of the initial PCA, enabled a subset of important variables to be identified. A follow-up PLS model for the data subset (Table 6.2.3) gave somewhat lower (but nonetheless significant) values of R^2_{cal} and R^2_{val} of 0.74 and 0.68 respectively. The $RMSE_{val}$ value for the PLS model was 1.54, being higher than that obtained for the full chemical-analytical data set. Nevertheless, the model developed indicated that only a few variables, easily accessible to commercial laboratories could be used to successfully predict grade. The selected variables for the reduced model were ºBrix, pH, TA, malic acid, nitrogen (YAN, ammonia, alpha-amino nitrogen), malic acid, chloride, berry weight and the UV-visible spectrum between 280 and 370 nm (phenolics). Overall, the chemical-analytical data set could more successfully be used for grade assessment in Chardonnay than the red grape varieties studied, and is a promising finding, suggesting that PLS modelling may be applied in the future for grape grade assessment using these objective chemical measures.

6.2.2.2 Grape grade prediction using PLS for non-targeted MIR and NIR spectral data

PLS models were developed for each of the three varieties studied in 2014 using MIR and NIR spectral information from homogenates and juice (MIR only). As discussed previously, these data were used successfully in the development of PCA models, with far more variation in the data sets being accounted for in a small number of PCs, although this approach had been less successful in separating (clustering) grape samples by grade. Tables 6.2.1, 6.2.2 and 6.2.3 show the MIR and NIR spectral PLS model results for the Cabernet Sauvignon, Shiraz and Chardonnay sample sets respectively. The MIR and NIR spectra were analysed either as raw data or following transformation with the Savitzky-Golay algorithm, as for the PCA described previously. The effect of transformation will not be compared between data sets, and only the better model will be described for the analyses performed.

Among the non-targeted spectral analyses collected for Cabernet Sauvignon, the MIR scans of either juice or homogenate could most successfully be used to model allocation grade, the same as was found in 2013 with the exception that juice was not analysed previously. The PLS models developed in 2014 had slightly lower values of R^2_{cal} and R^2_{val} in 2014 than in 2013. For juice MIR spectra, R^2_{cal} and R^2_{val} were 0.88 and 0.86 respectively, with an $RMSE_{val}$ value of 0.86. For homogenate MIR spectra, R^2_{cal} and R^2_{val} were 0.89 and 0.84 respectively, with an $RMSE_{val}$ value of 0.94. Although the $RMSE_{val}$ value of 0.46 found for the MIR homogenate spectra in 2013 was better (i.e. lower) than the 2014 result, the 2014 $RMSE_{val}$ values for the spectral models were nonetheless better than for all the models built using targeted analytical data. This indicates that better separation of adjacent grades and hence grade prediction could be achieved using this approach. Based on the 2013 and 2014 results for Cabernet Sauvignon, MIR spectral data either from juice or homogenate appears to be the most promising type of data for grade prediction using PLS modelling.

Similarly, PLS modelling of the Shiraz non-targeted MIR and NIR analyses revealed that MIR spectra from homogenate (and not juice) were most successful in the prediction of grade. Both higher values of R^2_{cal} and R^2_{val} at 0.89 and 0.84 respectively, and the lowest $RMSE_{val}$ value of 0.97 were achieved in the PLS model for MIR spectra from Shiraz homogenate. As observed for Cabernet Sauvignon, the PC number required for the PLS model was high, indicating that the

relationships within the spectral data set toward grade prediction were complex. It is nonetheless a valuable observation that for both red varieties studied that MIR spectra could be successfully used for PLS modelling of grade allocation, since this technology is readily accessible to commercial laboratories, easily applied and can be calibrated across multiple seasons.

A further positive finding was that for Chardonnay the MIR spectrum of homogenate also yielded the highest values of R^2_{cal} and R^2_{val} at 0.91 and 0.78 respectively in the PLS modelling of grade compared with the use of juice MIR or homogenate NIR spectra. Although the $RMSE_{val}$ value of 1.29 was lower than that obtained for Cabernet Sauvignon or Shiraz, this was the same as the lowest $RMSE_{val}$ value obtained using the Chardonnay chemical-analytical data set. This is of significance, since it indicates that complete separation of adjacent Chardonnay grades may not be realisable for Chardonnay, and broader grade categories may need to be defined if MIR/PLS modelling is used as an approach. The PLS model with MIR homogenates was also improved from the model developed with a subset of significant analytical variables for this variety, and hence has greater relevance as an application for grade prediction in grapes due to its simplicity and accessibility as a technique.

Although the PLS modelling of non-targeted spectra has revealed that MIR spectra from homogenates may be a useful tool for grade prediction across multiple varieties, a possible negative aspect of using the MIR approach is that important individual analytes cannot be identified (possibly resulting in less clarity for grape producers in terms of value assigned), and also that multiple years of calibration (by vintage and variety) would be required to increase its robustness. Nonetheless, the recognition that an objective tool can be used to predict a grading process subject to human subjectivity and error is a promising result.

6.2.3 Grape grade prediction using discriminant analysis (DA) in the 2014 season

In the preliminary study of Cabernet Sauvignon in 2013, the point was raised that the assignment of grades as numbers is not necessarily appropriate, and that the treatment of grades as categories in multivariate analysis is likely to improve the prediction process. As discussed previously, however, the use of PLS-DA analysis (i.e. assignment of grades as categories in PLS modelling) was unsuccessful using either the 2013 or 2014 data. Instead, quadratic DA (QDA) was found to provide better prediction than that obtained by PLS modelling. The use of QDA recognises that the gaps between grade categories are not necessarily linearly defined or consistent. There are some considerations that need to be taken into account when using QDA. Firstly, it can result in ‘over-fitting’ due to the assumption that a normal distribution exists within each category. To overcome this the analysis is performed using PCA scores, and not the native data. A further limitation is that QDA requires a minimum number of samples in each category. Thus, some grades with small sample numbers needed to be removed from the analysis, potentially excluding them from the grade prediction model. Increasing the robustness of the QDA approach would require repeated analysis of multiple vintages with greater numbers (or more even distribution) of samples per grade category. Tables 6.2.1, 6.2.2 and 6.2.3 show the results for the Cabernet Sauvignon, Shiraz and Chardonnay sample sets respectively. Generally, the QDA approach required five PCs to maximise the prediction accuracy, with a few exceptions, indicating the complex non-linear nature of the data.

The preliminary QDA of the 2013 Cabernet Sauvignon data had shown that NIR homogenate spectra most successfully predicted allocation grade, followed by the UV-visible spectra, and the least success was for the MIR spectra (Table 6.1) which differed from the results with PLS. However, for all the analytical data types used, prediction accuracy exceeded 85% using QDA. Higher values of prediction using QDA were obtained for the Cabernet Sauvignon analysis in 2014 than in 2013, with NIR spectra being the least effective. For the MIR homogenate data which also successfully modelled grade using PLS modelling in 2014, prediction was 100%.

Second to this, the UV-visible spectra were able to predict grade with 96% accuracy. In Shiraz, a similar prediction efficiency using QDA was obtained for the UV-visible spectra compared to Cabernet Sauvignon, at 95%. Shiraz MIR spectra were less successful for grade prediction. Interestingly, the Shiraz NIR spectra of homogenates were able to predict grade with 94% accuracy with QDA. Together, these results suggest that for the categorical, or DA approach for red varieties, the UV-visible spectral data were the most promising data type to distinguish grades, followed by non-targeted MIR/NIR measures.

For the 2014 Chardonnay data set, lower prediction capacity was observed using QDA for the non-targeted spectra than found for the red grape varieties studied, with the exception of fresh juice MIR spectra, for which QDA was able to predict grade allocation to 93% accuracy. Higher prediction accuracy was achieved using the targeted analytical data sets, and interestingly was 95% for both the complete and partial (significant subset from PCA/PLS discussed previously) data sets. Although a somewhat different conclusion from the PLS modelling approach where targeted analytical data less effectively predicted allocation grade for Chardonnay, this was nonetheless a positive finding. The linear DA results for the Chardonnay chemical-analytical data were only marginally lower than for QDA, indicating that some linearity exists within this model and it is unlikely to be subject to over-fitting with QDA. The similarity of the QDA of the Chardonnay compositional subset to that from the complete data set highlights that the important chemical variables for prediction could be identified, and this approach could provide grape suppliers with confidence in sample analysis and the assigned values. The suite of chemical measures identified in the Chardonnay subset are relatively simple and could easily be accessed by commercial laboratories and some are already in use (pH, TA, berry weight, ºBrix).

6.3 Relationships between grape composition (quality grade) and aspects of wine composition and style in the 2015 season

In the 2015 season, the study was limited to two varieties, Chardonnay and Shiraz, from a single growing region (Riverland) to explore the relationship between grape composition and wine style, as defined by both sensory attributes and the producer. This approach was limited to grape 'stylistic categories' as opposed to grade, but quality elements of the previous year's study were nonetheless applicable within the definition of style. However, a point of departure from the previous two years of the work was that a separate Riverland-based grading system was used to define quality/style rather than the broader producer-based system applied previously. Fruit was therefore graded A (high) to D (low) rather than on the point scale 1 (high) to 9 (low) used previously. The selections were also made to allocate wine 'style' categories as opposed to grade per se, therefore the distribution of samples by grade was not necessarily even. Due to this, the type of multivariate analyses used, as well as the results of the 2015 season could neither mimic nor be directly related to the 2014 season.

The premise of the proposed experiment was to determine whether objective measures could be identified from grapes to predict wine style, and also whether the final wine style and assigned wine grade had a bearing on grape grade. Within these objectives, other important outcomes could be identified. Much of grape and wine research has sought to understand the grape to wine relationship, and in turn the relationship between wine composition and sensory properties. Some rare studies have gone beyond this to explore the relationships of wine composition with style category, perceived quality, or both (Cadot et al. 2010). However, little is known of the relationship between grape composition and wine style, although this relationship is frequently assumed to exist at producer level. The proposed study therefore sought to apply chemical measurements to understand whether a relationship, if any, exists. To provide a conceptual framework for the type of interactions that can be explored within this data set, a schematic diagram is shown in Figure 6.3.1.

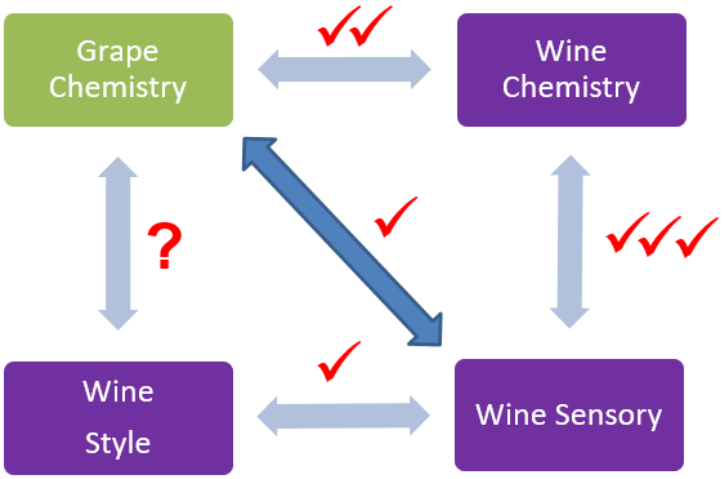


Figure 6.3.1. Schematic diagram indicating the relationships between grape or wine chemistry, wine sensory properties and style showing the degree to which these interactions have received attention in research, and for which data exists (amount of knowledge indicated by the number of ✓ symbols) and areas in which knowledge gaps exist (as indicated by ?).

6.3.1. Chardonnay and Shiraz fruit chemistry versus fruit grade in 2015

A repetition of the analysis done in 2013 and 2014 was performed on the 2015 grape compositional data for the 2015 Chardonnay and Shiraz grape samples. For Chardonnay, the sample set and grading range was narrower than the 2014 study, therefore PLS could not be used to further analyse the relationships between grape composition and grade. Instead, an exploratory PCA of Chardonnay (Figure 6.3.1.1) revealed a complex data set where the separation of samples according to either composition or grade occurred in multiple PCs (dimensions). Similar to what was observed for the 2014 Chardonnay PCA, only 60% of the variation within the data set could be described in the first two PCs, reaching 74% in three PCs. Grade clustering was not clear, and there was a large scatter among the samples on a compositional basis, as was also observed for the 2014 data. Some separation of grades A and B could be observed on PC1 and somewhat greater on PC2. Notably for the lower grades C and D, there was far greater scatter observed, with a lack of similarity in fruit sample composition. Interestingly, these C and D grade samples could be said to characterise the 'extremes' in grape composition observed for the current data set, which will be discussed in greater detail later.

Important grape compositional attributes that defined PC1 for the Chardonnay samples were amino acids, which were related to the broader nitrogen measures YAN and alpha-amino nitrogen. These were strongly related to increased laccase, volatile thiol precursors and the C_6 compounds hexanol, Z-3-hexenol and E-2-hexenol. These compounds appear in the left quadrant of the correlation loadings plot (Figure 6.3.1.1A). An inverse relationship of the three C_6 compounds discussed was found with another C_6 metabolite, E-2-hexenal. Significant variables defining PC2 were malic acid, pH, TA and dry matter (high loadings on PC2) and these showed an inverse relationship to UV-visible phenolics measures (importantly 280 nm, 320 nm and 370 nm), ºBrix and free α-ionone. Free β-ionone, β-damascenone and monoterpenes were not significant within PCs 1 and 2 for the PCA model developed. From the model for quality developed in 2014 for Chardonnay, some differences were found from those observed previously. For example, phenolics (370 nm) measures seemed to be somewhat higher in the Grade A fruit, and acidity lower. However, it is important to note that for the 2015 Chardonnay data set all samples were from one region, the Riverland (all grades 7-9 in the 2014 study), therefore some of the attributes which were significant in defining sample separation for the 2014 data set would be narrower in range in this instance, than observed across multiple regions and grades.

For the Shiraz grape samples for the 2015 season, PLS was possible due to a slightly wider grade range (A, B, D, E) and the results are shown in Figure 6.3.1.2. Using PLS, the fairly complex Shiraz compositional data set could be reduced to two PCs (dimensions) to predict grade, using significant variables R^2_{cal} (0.85), lower R^2_{val} (0.73), but the $RMSE_{val}$ was low (0.9) (data not shown). Some important distinguishing variables were similar to those found in 2014, but it was evident from the overlaid grade categories (Figure 6.3.1.2 A) that not all grades were well modelled by PLS regression, with Grade A in particular having certain samples separated from the others in the same category. In the PLS analysis, grape grades were primarily separated on PC1. Some additional compositional measures were introduced in the 2015 study, and for Shiraz this included an assay of 'wine-like' (WL) extractable phenolics (e.g. WL 280, WL 320 and WL tannin) as indicated in the right-hand quadrant of Figure 6.3.1.2 A. The WL assay has previously been used to determine variability in extractable tannin (primarily skin tannin) and anthocyanin in grapes and predicts tannin and colour levels in the wine made from this fruit (Bindon et al. 2014). In the current results for Riverland Shiraz fruit, the WL measure was well-correlated with the lower grades of fruit (D, E) and was also related to higher juice pH, and low titratable acidity in the fruit. Since higher levels of WL phenolics have generally been found to be associated with higher wine quality, specifically the concentration of finished tannin in the wine, this finding was unexpected. It is likely that under the warm conditions of the region that pH/TA were more important factors considered by the producer in terms of grade allocation, nevertheless a possible relationship between 'extractable phenolics' and these measures should be investigated further. Other important metabolites defining PC1 were ammonia and free β-ionone (not measured in 2014), which were both higher in Grade A fruit. For the PLS analysis of the 2015 Shiraz grape samples, PC2 was less important in separating grade than PC1. High correlation loadings on PC2 were defined by higher YAN levels, correlated with certain amino acids, and lower loadings for ºBrix, tannin (homogenate) and colour (520 nm, homogenate) which appeared to be important in distinguishing the samples, but not sample grade. These factors were important in defining grade in the 2014 season, and this observation again indicates that within the narrow regional source defined, they were less significant in the definition of grade under these conditions. However, as mentioned previously the 2014 and 2015 allocation grading systems are not directly comparable.

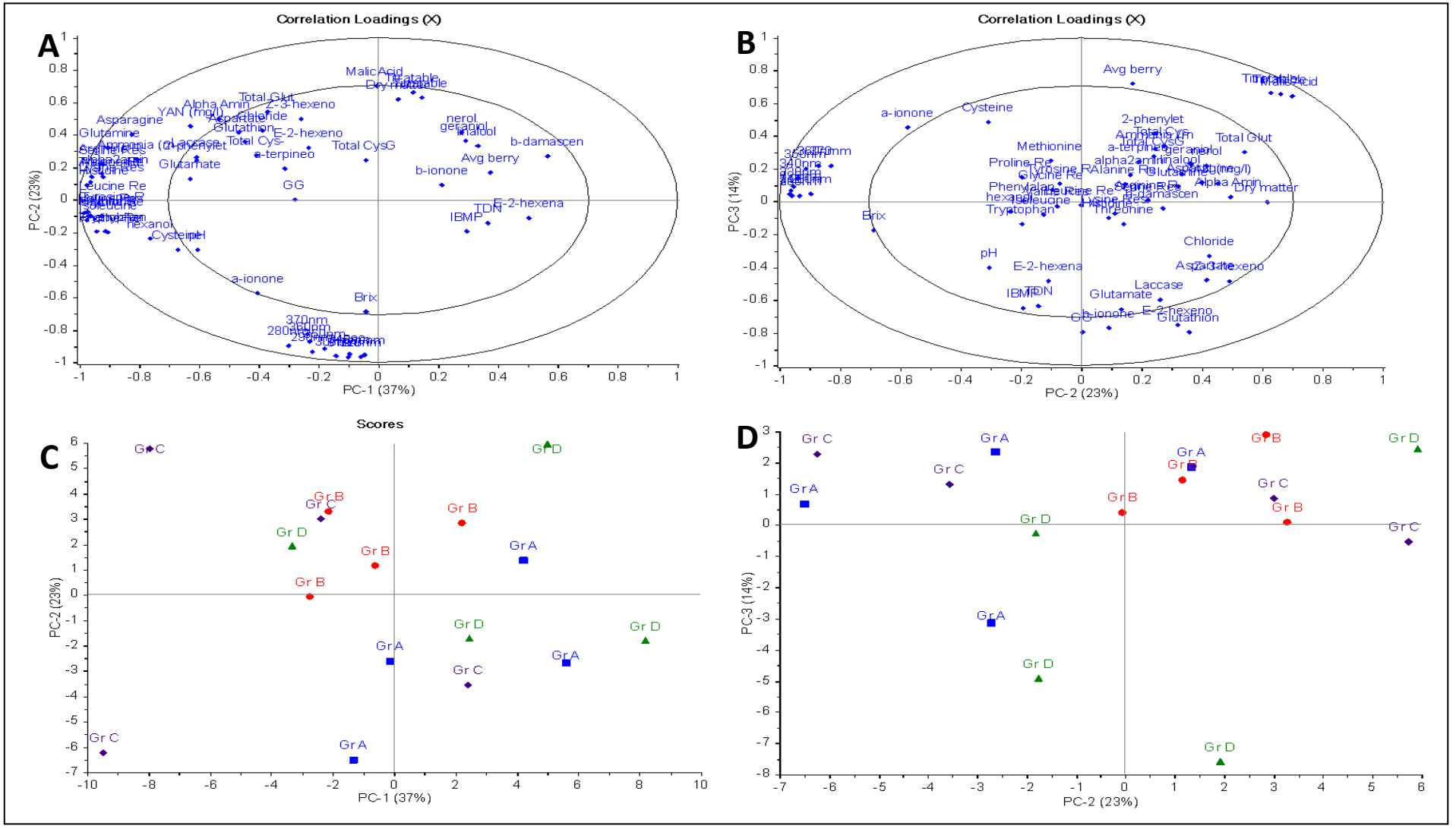


Figure 6.3.1.1. Principal component analysis of Chardonnay grape composition showing correlation loadings plots in three dimensions **A**. PC1 against PC2 grades and **B.** PC2 against PC3, with corresponding scores plots for the samples shown in **C**. and **D.** respectively. Grades are overlaid as coloured symbols (A=highest to D=lowest).

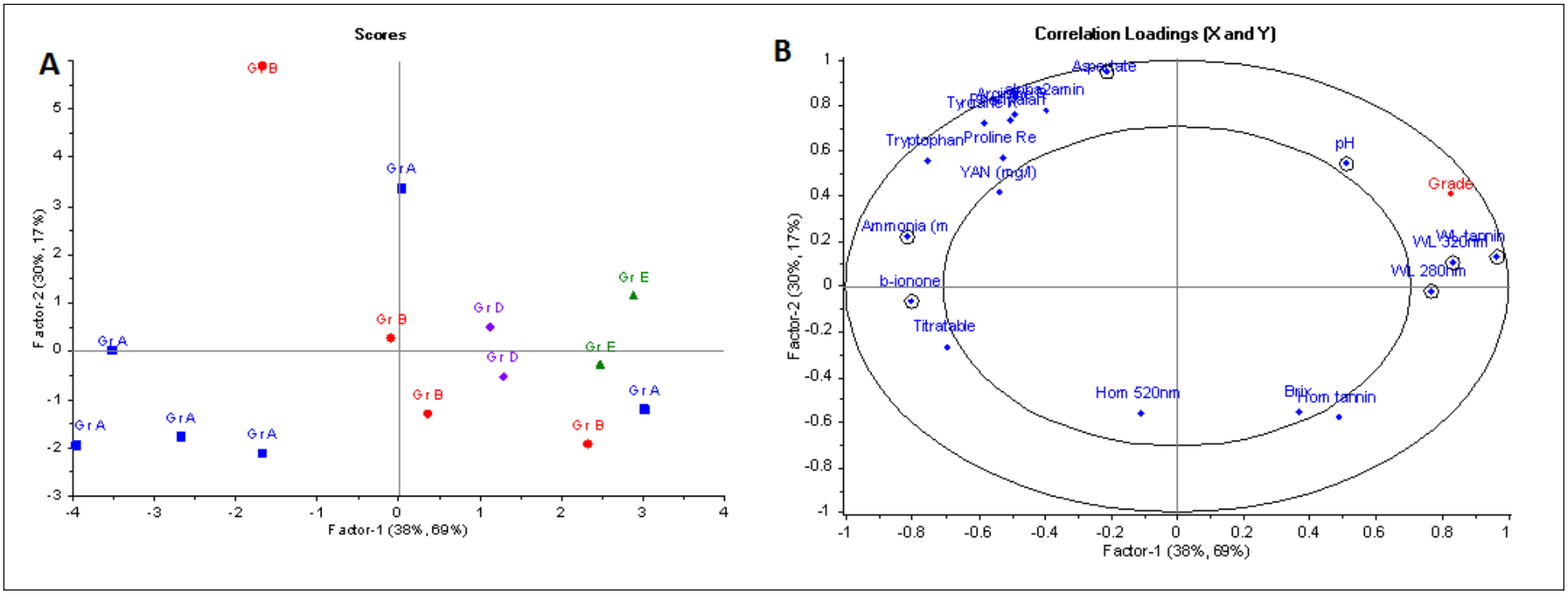


Figure 6.3.1.2. Principal component analysis of Shiraz grape composition showing **A**. correlation loadings plot in two dimensions and **B.** corresponding scores plots for the samples with grades overlaid as coloured symbols (A=highest to E=lowest). Significant variables based on the uncertainty test are circled.

6.3.2. Chardonnay and Shiraz wine sensory properties: an investigation of the implications for commercial fruit grade, wine grade and wine style allocation in 2015

Following sensory analysis, sensory differences between the Chardonnay and Shiraz wines produced from the various grade/style allocations were compared using ANOVA and PCA. The ANOVA results for the Chardonnay wines (Table 6.3.2.1) showed that all attributes identified differed significantly between wines ($p < 0.05$) except for 'green'/'grassy' aroma, 'stone fruit' flavour, 'green' flavour and 'astringency'. Astringency was found to be very close to significant ($p < 0.1$). Only 'spritz' was found to be significantly different by fermentation replicate within treatment, signifying that the winemaking replication was very consistent. For the Shiraz wines, the results of the ANOVA (Table 6.3.2.2) found that 23 of 31 attributes identified differed significantly by treatment ($p < 0.05$), with an additional two attributes (salt and acid) being very close to significant ($p < 0.1$).

For the PCA of the Chardonnay wine sensory attributes, the astringency, salt and acid attributes that were close to significant were included in the PCA. A clear visual separation of the main distinguishing properties of the wines can be seen using the PCA plot, which was performed only on significant sensory attributes found by the ANOVA for the mean values for each of the 16 wines. For this data set, the first three PCs were important in showing the variation in the data set. Figure 6.3.2.1 shows the PCA scores and loadings for PC1 and 2, which account for 61% of the variation in the data set. PC3 accounted for a further 10% of variation in the data set (data not shown). Sample variation was explored in terms of the allocated fruit grade, already discussed in terms of fruit composition, as well as the specific wine style and grading allocated to the wine sample set by the formal assessment of the producer, for which the result are shown in Table 6.3.2.3. The overlaid mapping of fruit grade allocation (Figure 6.3.2.1 A), wine style category (Figure 6.3.2.1B) and wine grade allocation (Figure 6.3.2.1 C) in the PCA plots showed some interesting distributions of the wines in terms of their commercial value. For fruit grading, Grade A fruit produced wines with lower 'box hedge'/'cooked vegetable'/'yellow' attributes, higher acidity and more 'balanced' or 'neutral' characteristics. Grade B overlapped closely with Grade A but some wines had higher 'passionfruit'/'tropical' notes. Fruit Grades C and D were not well separated from the others but a wider range (extremes) of sensory attributes was observed.

Table 6.3.2.1 ANOVA p-values for the Chardonnay wines.

Attribute	W	F(W)	W*J	J*F(W)	R*F(W)
Yellow Colour Intensity	<0.001***	0.090‡	0.002**	0.982	0.448
Overall Fruit Aroma	<0.001***	0.938	0.010*	0.331	0.175
Passionfruit	<0.001***	0.889	<0.001***	0.624	0.624
Pineapple	<0.001***	0.792	0.424	0.915	0.922
Stone fruit Aroma	<0.001***	0.331	0.570	0.936	0.757
Citrus Aroma	<0.001***	0.890	<0.001***	0.552	0.953
Confection/Floral	<0.001***	0.741	0.212	0.183	0.447
Green/Grassy Aroma	0.257	0.534	0.058‡	0.844	0.213
Box Hedge	<0.001***	0.538	<0.001***	0.636	0.481
Cooked Vegetable	<0.001***	0.388	<0.001***	0.727	0.609
Flint	<0.001***	0.392	<0.001***	0.409	0.284
Yeasty	0.001**	0.487	0.023*	0.103	0.438
Plastic/Chemical	<0.001***	0.933	<0.001***	0.595	0.504
Sweaty/Cheesy	<0.001***	0.770	<0.001***	0.697	0.603
Pungent	<0.001***	0.803	0.184	0.983	0.628
Overall Fruit Flavour	<0.001***	0.117	0.012*	0.956	0.744
Tropical Fruit	<0.001***	0.953	0.174	0.973	0.777
Stone fruit Flavour	0.375	0.505	0.005**	0.110	0.609
Citrus Flavour	<0.001***	0.485	0.635	0.552	0.689
Green Flavour	0.798	0.080‡	0.129	0.840	0.438
Yeasty Flavour	<0.001***	0.169	0.075‡	0.548	0.847
Viscosity	0.006**	0.557	0.221	0.996	0.858
Spritz	<0.001***	0.028*	0.032*	0.119	0.002**
Sweet	0.031*	0.223	0.280	0.853	0.174
Acid	0.001**	0.661	0.004**	0.951	0.843
Hotness	<0.001***	0.148	0.120	0.853	0.996
Astringency	0.057‡	0.693	0.003**	0.708	0.621
Bitter	<0.001***	0.189	0.032*	0.975	0.196
Fruit Aftertaste	<0.001***	0.713	0.097‡	0.999	0.509
df	15	32	150	160	16

†Significance levels are as follows: * p < 0.05; ** p < 0.01; *** p < 0.001; ‡ p < 0.10. J = Judge, W = Wine, R = Replicate, F = Ferment Replicate, df = degrees of freedom. All attributes were found to have a significant Judge effect (p<0.001)

Table 6.3.2.2. Shiraz ANOVA p-values for wine (W), judge (J), replicate (R) and their interactions

Attribute	Wine	Replicate	Wine*Judge	Wine*Replicate	Judge*Replicate
Opacity	<0.001***	0.97	<0.001***	0.001**	<0.001***
Purple Tinge	<0.001***	0.081‡	<0.001***	<0.001***	<0.001***
Overall Fruit Aroma	<0.001***	<0.001***	0.004**	0.45	0.001**
Red Fruit Aroma	0.001**	0.004**	0.344	0.721	0.044*
Dark Fruit Aroma	<0.001***	0.052	0.221	0.297	0.044*
Confection	<0.001***	0.117	0.179	0.022*	0.199
Floral	0.004**	0.002**	0.292	0.871	0.226
Green Aroma	<0.001***	0.572	0.008**	0.004**	0.797
Herbal	0.038*	0.043*	0.163	0.352	0.103
Cooked veg	<0.001***	0.031*	0.010*	0.036*	<0.001***
Sweet Spice	<0.001***	0.136	0.493	0.114	0.583
Vanilla Aroma	<0.001***	<0.001***	0.324	0.571	<0.001***
Pepper	0.040*	0.865	0.885	0.286	0.556
Earthy	0.031*	0.211	0.087‡	0.409	0.21
Chemical	0.279	0.024*	0.031*	0.089‡	0.361
Pungent	0.001**	0.641	0.134	0.196	0.738
Overall Fruit Flavour	<0.001***	0.635	0.044*	0.837	0.016*
Red Fruit Flavour	0.217	0.821	0.236	0.423	0.324
Dark Fruit Flavour	<0.001***	0.293	0.038*	0.736	0.87
Green Flavour	0.262	0.269	0.029*	0.732	0.059‡
Dried herb Flavour	0.001**	0.178	0.446	0.473	0.013*
Vanilla Flavour	0.478	0.9	0.082‡	0.367	0.065‡
Aniseed	0.73	0.844	0.992	0.549	0.215
Salt	0.065‡	0.040*	0.27	0.492	0.535
Sweet	0.007**	<0.001***	0.023*	0.364	0.006*
Viscosity	0.033*	0.551	0.971	0.346	0.018*
Acid	0.073‡	0.404	0.853	0.366	0.129
Hotness	0.361	0.621	0.98	0.049*	0.158
Astringency	0.001**	<0.001***	0.447	0.171	0.002**
Bitter	0.037*	0.028*	0.029*	0.794	0.043*
Fruit Aftertaste	<0.001***	0.283	0.202	0.314	0.376
df	22	1	176	22	8

†Significance levels are as follows: * p < 0.05; ** p < 0.01; *** p < 0.001; ‡ p < 0.10. df = degrees of freedom. All samples were significant by Judge p<0.001

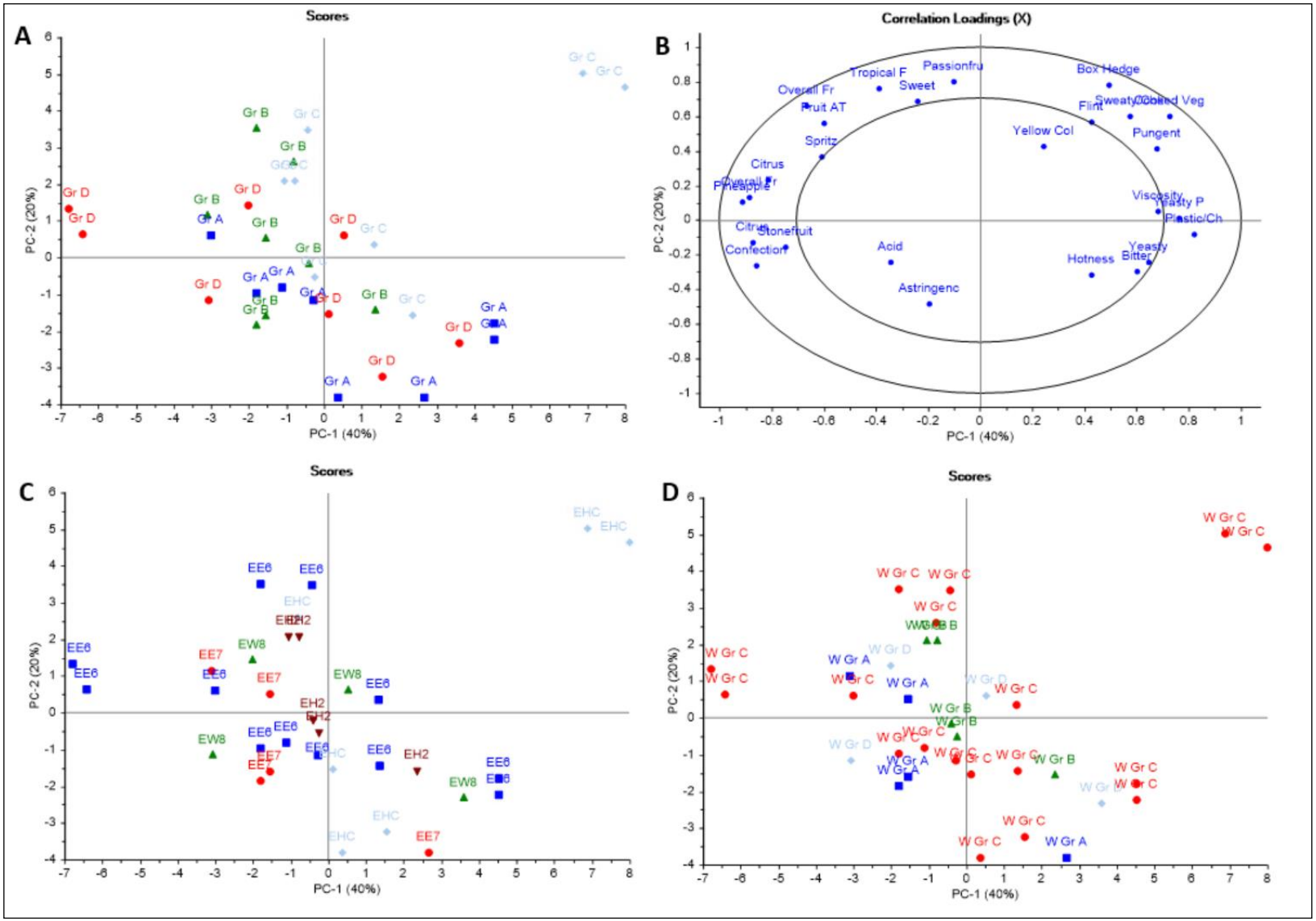


Figure 6.3.2.1 PCA of significant Chardonnay wine sensory properties in the 2015 season with **A.** Scores plot for samples overlaid by fruit grade **B.** Correlation loadings for sensory properties, **C.** Scores plot for samples overlaid by wine style allocation **D.** Scores plot for samples overlaid by wine grade allocation.

The distribution of the Chardonnay wine samples by wine style or wine grade within the PCA plot showed a similar pattern as that seen for fruit grade. Not all grade A fruit samples were streamed into Grade A wine, but it was interesting to note that no Grade D fruit samples were allocated above wine Grade C. Nevertheless, the separation between wine styles and grades according to their sensory properties was not distinct within the PCA plot. Wine grades A and B were close together with lower scores on PC2, and intermediate on PC1. Much scatter of wines grades C and D was observed, not clearly separated from A and B, yet greater extremes were observed. It could be said that the Grade A and B wines had a more narrowly distributed range of sensory attributes, and as discussed before, were generally more 'neutral' or 'balanced' and lower in the 'fruit', 'tropical flavour', 'sweetness', 'passionfruit', 'box hedge' and 'pungent' attributes.

Table 6.3.2.3. Commercial wine grade and style allocations for 2015 Chardonnay and Shiraz wines

Wine Grade	Wine Style	Description
Chardonnay wine allocation		
A	EE7	Lemon and nectarine, tight and lean, long
B	EH2	Peach, fleshy, juicy
C	EE6	Tropical peach, juicy
C	EHC	Shaded herbal and lean
D	EW8	Buttery, ripe (exposed)
Shiraz wine allocation		
A	FR7	Ripe and dense, without being jammy
B	FQ1	Bright red fruits
C	FK2	Good density and sweetness, light herbal notes
C	FV6	Bright, tropicaility, sweet fruit
D	FA6	Bright, tropical green lacking sweetness
D	FA1	Simple green, herbal

The same PCA approach was then performed on significant sensory attributes for the Shiraz wines found by the ANOVA for the mean values between each duplicate set of samples. For this data set, the first four PCs were important in showing the variation in the data set. Figure 1 shows the PCA scores and loadings for PC1 and 2, which account for 57% of the variation in the data set. Including PC3 and PC4 accounted for 76% of variation (not shown), in the data set, indicating that the relationships within the data were complex. Separation on PCs 1 and 2 were nonetheless useful in explaining the sensory differences among the samples and the distribution is shown in Figure 6.3.2.2. Not all Shiraz wines received a grading due to faults (aldehyde), and these appear in the PCA plot as numbers.

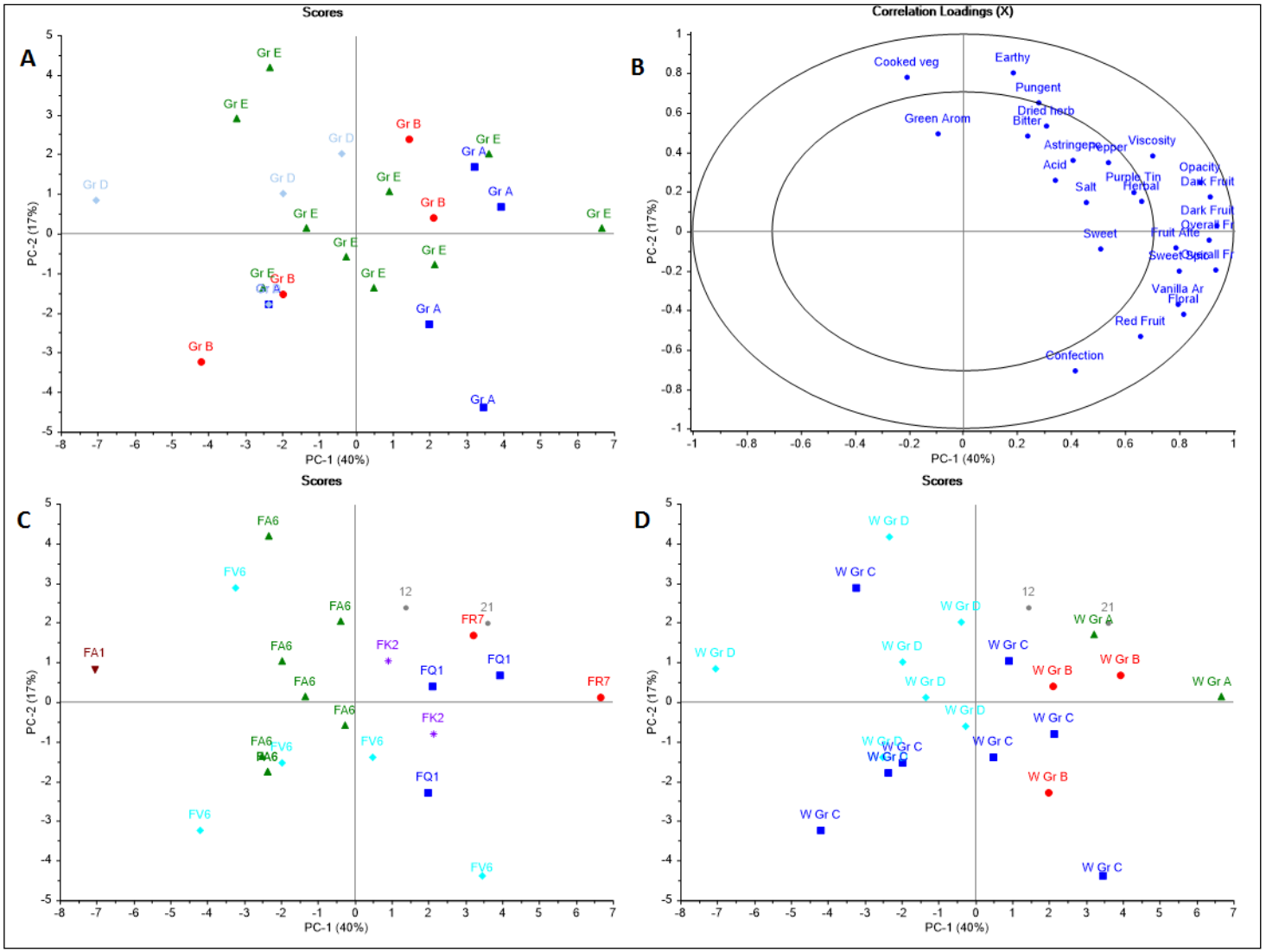


Figure 6.3.2.2 PCA of significant Shiraz wine sensory properties in the 2015 season with **A.** Scores plot for samples overlaid by fruit grade **B.** Correlation loadings for sensory properties, **C.** Scores plot for samples overlaid by wine style allocation **D.** Scores plot for samples overlaid by wine grade allocation.

The samples appeared to be broadly distributed within the PCA, with the significant sensory attributes nearly all contained in the two right hand quadrants (Figure 6.3.2.2 B). Generally the samples found to the left in the PCA were rated lower in intensity for most attributes and the samples to the right were rated higher. The distribution of the wines produced from fruit samples of different grades (Figure 6.3.2.2 A) showed that no clear pattern could be observed whereby fruit grades could be used to distinguish the final sensory outcomes of the wines. Samples within the PCA were also mapped according to the wine style and wine grade allocated by the producer (Table 6.3.2.3). It was interesting to observe that a large number of styles were allocated to the Shiraz wines (Figure 6.3.2.2 C), exceeding the expected allocation at the time of grape selection. The lower value wine grades C and D included wines from multiple styles, however wine grades A and B were individual styles. In terms of the fruit grade to wine allocation, fruit grade A did not necessarily produce wines of higher grade, or within a specific style, however most of the A grade fruit samples scored higher on PC1. It was important also to observe that many Grade E fruit samples produced wines of higher quality (A-C), and scoring higher on PC1. The distribution of the samples according to wine style and wine grade occurred mainly across PC1 (Figures 6.3.2.2 C and D), although the delineation between adjacent grades or styles was not precise. Attributes with the highest correlation loadings on PC1 were 'dark fruit' aroma and flavour, 'overall fruit' aroma and flavour, 'opacity', 'floral' aroma, 'sweet spice' aroma, 'vanilla' aroma, 'fruit' aftertaste and viscosity. Importantly, green aroma was not found to be an important discriminating variable (not significant in the correlation loadings plot).

6.3.3 Analysis of the ability of grape and wine compositional data sets to predict commercial wine style and wine grade using PLS and QDA, with reference to commercial grape grade

For both the Chardonnay and Shiraz wine samples for the 2015 season, the number of style categories assigned by the producer panel (Table 6.3.2.3) was too large to be modelled using PLS regression. Instead, a model using grouped style classes as wine 'grades' rather than specific style categories was possible. Some similarity existed between the specific grades assigned, for example in Shiraz, the lower value grades were all perceived to demonstrate more 'green' style than the wines of better grades, which were richer in fruit attributes and density.

Using the 2015 Chardonnay grape chemistry data to predict wine grade, a fairly strong R^2_{cal} of 0.82 could be obtained using PLS regression, although the R^2_{val} of 0.59 was low (Table 6.3.3.1) yet the $RMSE_{val}$ of 0.57 was also low. When a subset of significant grape chemistry analytes was used to define the PLS model (identified using the uncertainty test and analysis of correlation loadings), a similar model was obtained to that achieved using all of the targeted analytical data, although it was a complex model with variability described in 5 PCs.

Table 6.3.3.1. PLS model performance for Chardonnay and Shiraz grape and wine chemistry, wine sensory analysis and their respective combinations to predict commercial wine grade, nd = not determined due to poor performance of the data set in PLS.

Analysis Type	Chardonnay				Shiraz			
	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$	PC no	R^2_{cal}	R^2_{val}	$RMSE_{val}$
Grape chemistry, all data	4	0.82	0.59	0.57	1	0.68	0.35	0.80
Grape chemistry, significant variables	5	0.79	0.61	0.55	3	0.81	0.58	0.63
Wine chemistry, all data	6	0.90	0.52	0.64	2	0.82	0.64	0.58
Wine chemistry, significant variables					1	0.76	0.71	0.52
Wine sensory analysis					1	0.59	0.49	0.71
Wine aroma		nd			1	0.47	0.41	0.77
Wine palate and appearance					1	0.60	0.49	0.70
Wine chemistry and sensory analysis	5	0.91	0.46	0.67	6	0.99	0.76	0.51

6.3.3.1 Chardonnay wine grade prediction in 2015

Important variables that defined 2015 Chardonnay wine grade predicted from grape composition are shown in Figure 6.3.3.1. As discussed previously, the PLS analysis treats grades as numerical values, where variables important to higher grades (low value, 1) are negatively loaded, and variables associated with lower grades (high value, 4) are positively loaded. It was found for Chardonnay grape composition that higher berry weight was one of the most important variables in defining higher wine quality grades, as well as higher chloride and malic acid concentrations (Figure 6.3.3.1A). Interestingly, higher TA values were positively loaded i.e. higher in lower wine grades

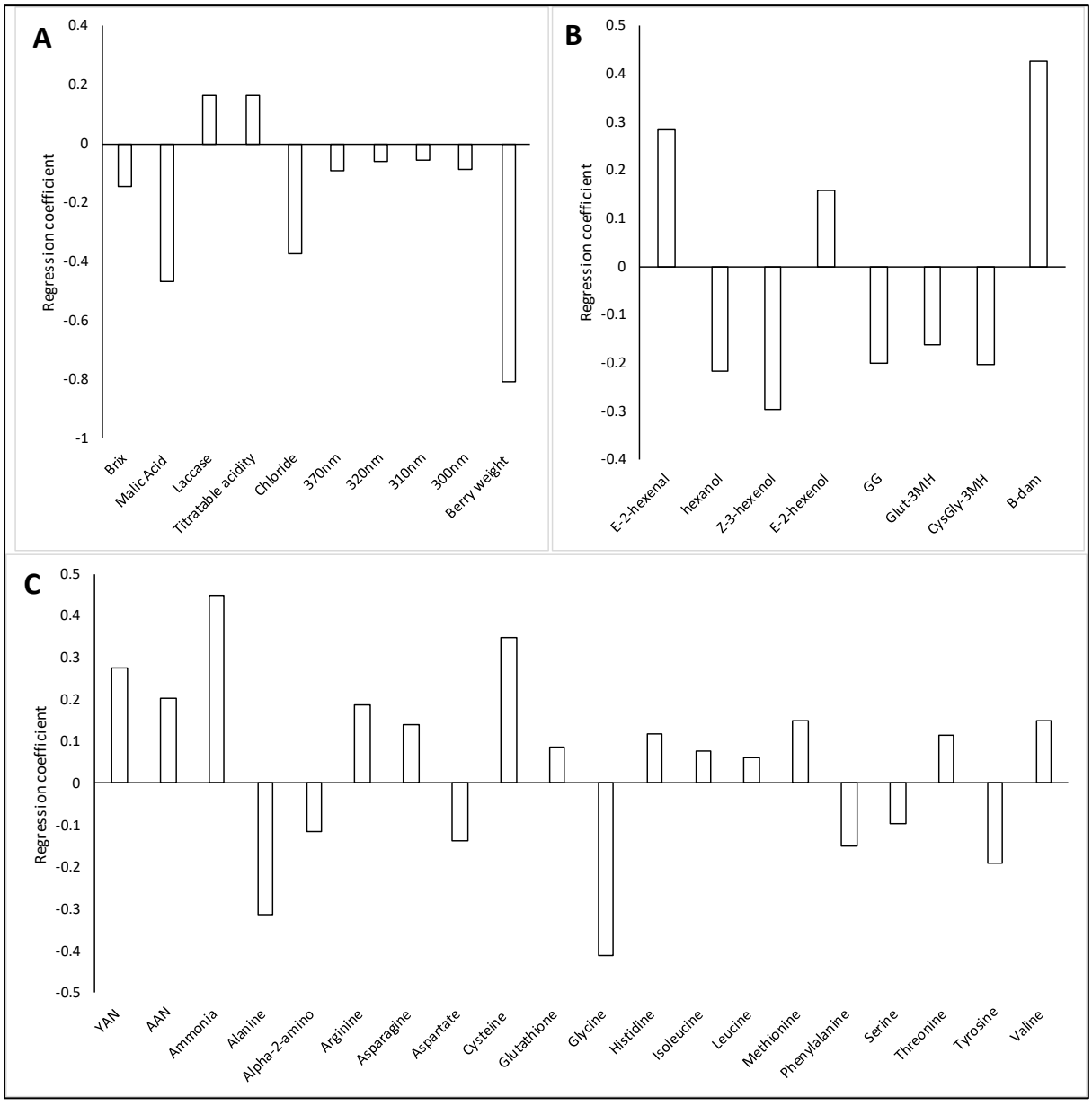


Figure 6.3.3.1 PLS regression coefficients for Chardonnay grape composition variables which were significant in predicting wine grade in the 2015 vintage showing **A.** basic grape measurements; **B.** volatile compound and precursors; **C.** nitrogen measures (including amino acids).

Also important in the grapes which produced higher quality wines in 2015 were elevated levels of phenolics (absorbance in the region 370 – 300 nm, flavonols). In the initial, broader 2014 study on grape allocation grade which incorporated regional differences, absorbance at 370 nm was lower in higher value grades, and was proposed to indicate canopy size or sun exposure differences since grape flavonols are positively correlated with sun exposure or water stress. In the narrower variability of values expected within the region of the Riverland, this result may indicate that canopy exposure may be an important variable in defining fruit and/or wine grading in vineyard assessment. A further important variable associated with lower value wines (positive coefficient) was higher laccase activity, which was not significant in the initial 2014 study. This was an important observation, since this might have a greater significance during the winemaking process and may not necessarily correlate with visual assessments conducted at vineyard level.

For the assessment of Chardonnay grape volatiles and volatile precursors (Figure 6.3.3.1B), C_6 compounds in grapes were important in defining final wine grade, but were both positively and negatively associated. Interestingly, higher hexanol and Z-3-hexenol were also associated with higher value grape grades in the 2014 study, as well as lower levels of E-2-hexenal which indicates that these may be important variables defining both grape and wine grading. As for the 2014 study on grape grade, higher concentrations of grape volatile thiol precursors and glycosyl glucose were associated with higher value wine grades. Of this class of compounds, the important aroma and flavour volatile β-damascenone was the variable with the highest positive coefficient, indicating that high concentrations associated with lower wine quality grade, an unexpected observation. However, as discussed previously, since higher wine quality gradings were associated with more 'neutral, balanced' sensory attributes, a lack of this compound may contribute to the neutrality of the wine, as will be discussed below.

In terms of grape nitrogen, these attributes were also positively and negatively associated with higher wine grades (Figure 6.3.3.1C). In the 2014 study, nitrogen (as YAN, alpha-amino nitrogen and ammonia) was positively associated with higher quality grapes, and in this instance was associated with poorer quality wines. However, as observed for the 2013 and 2014 Cabernet Sauvignon data sets, this class of compounds can demonstrate more seasonal variability.

For the assessment of the importance of wine chemistry and sensory attributes in the determination of wine grade (Table 6.3.3.1), wine sensory attributes alone were not able to produce a valid PLS model for wine grade. On the other hand wine chemical composition was able to more successfully predict wine grade, and when these data were included with certain significant wine sensory attributes, the model was not significantly altered. It should be noted, however, that the R^2_{val} values were not as high as the R^2_{cal} values for these models. Nevertheless, some useful information on important variables could be obtained from an assessment of the PLS models, and is shown in Figure 6.3.3.2.

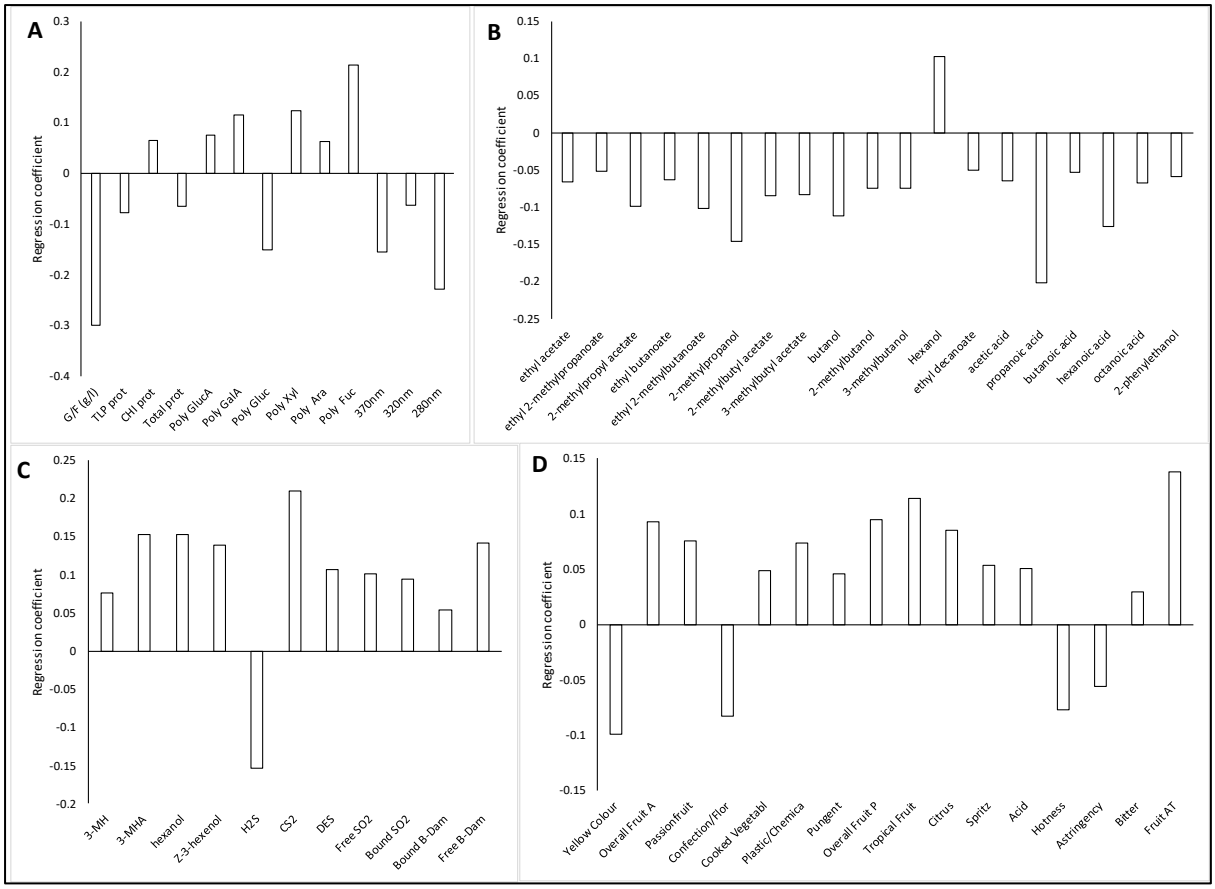


Figure 6.3.3.2 PLS regression coefficients for Chardonnay wine composition and sensory variables which were significant in predicting wine grade in the 2015 vintage showing **A.** wine non-volatiles including sugar, protein, polysaccharide (as component sugars) and phenolics; **B.** wine yeast-derived volatile esters, fatty acids and higher alcohols; **C.** wine volatile thiols, low molecular weight sulfur volatiles, C_6 compounds, and β-damascenone (free and bound); **D.** wine sensory attributes for aroma and palate.

One of the most strongly associated variables found in higher value grade wines was residual sugar (G/F) (Figure 6.3.3.2A). For the range of samples, the average residual sugar concentration was 1.18 g/L ± 0.5 standard deviation. Due to this, although a significant variable in the PLS, residual sugar was unlikely to exert a remarkable effect on the sensory properties of the wine. The individual wine proteins (TLP, CHI) which cause haze were found to be both positively and negatively associated with higher value quality grade, however higher levels of total wine protein were associated with higher grade wines. Conversely, higher levels of grape-derived polysaccharides (containing GlucA, GalA, Xyl, Ara, Fuc residues) were present in lower value grade wines, while polysaccharides rich in glucose (Gluc, possibly yeast-derived glucans) were strongly associated with increased wine value. As observed in the grape to wine grade relationship discussed previously, phenolics measures, in this case flavonols (370 nm, 320 nm) and total phenolics (280 nm), were increased in higher value wine grades.

In terms of Chardonnay volatile aroma compounds, yeast-derived esters, fatty acids and higher alcohols (Figure 6.3.3.2B) as well as grape-derived compounds (Figure 6.3.3.2C) were all increased in lower value wine grades, with the exception of propanoic acid and H_2S. The latter is well-known to be associated with ‘rotten egg’ odour and its identification as a positive attribute is therefore unexpected. The lowered contribution of most of the key aroma compounds in wine in higher value quality grades is in agreement with the observation that these wines tended to be more ‘balanced, neutral’ and lacked the extremes in style associated with the lower value grade categories.

Importantly, although hexanol, Z-3-hexenol and precursors to volatiles 3-MH and 3-MHA were elevated in grapes which produced higher value grade wines, the reverse was observed in terms of their profile in the wines. Higher value wines therefore had lower 3-MH, 3-MHA, hexanol and Z-3-hexenol. This may be due to the strong influence of other factors besides precursor level in grapes in determining their formation and release during winemaking (Capone et al. 2012). The observation in grapes that elevated levels of free β-damascenone were associated with increased wine quality grade was reflected in the wine profile as well, and included both free and bound forms. Although this compound is generally observed to be important in wine, its variability and influence in diverse wine styles is unknown. For the current data set, again highlighting the more neutral flavour and aroma profile associated with increased quality, reduced levels of this compound may contribute to this perception.

The Chardonnay sensory attributes that were significant for wine grade prediction within the PLS combined model developed for wine chemistry and sensory attributes are shown in Figure 6.3.3.2D. All 'fruity' attributes which might be deemed important to wine style were lowered as wine quality grade increased. Consistent with what was shown in Figure 6.3.3.1 for the PLS model of Chardonnay wine chemistry and sensory properties, far greater distribution in terms of sensory attributes and the associated wine chemistry were observed for wine grades other than A and B, which had a narrower distribution. Although unexpected, this was in keeping with the producer's allocation, as discussed previously. The only sensory attributes that were increased in wines of higher quality grade were yellow colour, a 'confection/floral' attribute, hotness and astringency.

The grape/wine chemistry and wine sensory data sets were then used to determine the ability of individual or combined attributes to predict wine style and grade, as well as grape grade (2015) (which was discussed in an earlier section of this report). For all data sets, QDA was used since LDA did not perform well as observed in the initial study on grape grade (Table 6.3.3.2). It was found that grape grade was best predicted by both the grape chemistry (100%), wine chemistry (100%) and the combined wine chemistry and sensory results (100%). Only slightly lower percentage accuracy was observed using Chardonnay wine sensory attributes alone, and this was lowest for wine aroma.

Table 6.3.3.2. Ability of data obtained for Chardonnay grape chemistry, wine chemistry and wine sensory attributes to predict either grape grade, wine style or wine grade using QDA (as % accuracy).

Dataset used to perform QDA	Grape grade	Commercial wine style (5)	Commercial wine grade (4)
All wine sensory attributes	97%	94%	91%
Wine aroma	85%	78%	78%
Wine palate	97%	94%	94%
Wine chemistry	100%	75%	94%
All wine chemistry and sensory	100%	91%	91%
Grape chemistry	100%	63%	69%

Similar patterns were observed between the prediction of wine style and wine grade, with wine palate attributes being most strongly predictive of both aspects. However for the prediction of wine grade, the wine chemistry and wine palate data sets performed similarly. In this instance, Chardonnay grape chemistry predicted wine style and wine grade less accurately using QDA than the wine data sets, achieving 63% and 69% accuracy reached respectively. In this instance, the PLS model for wine grade from grape chemistry (not possible for style) discussed previously provides more information on important grape attributes that can distinguish wine grades. From these results, it is evident that a predictive capacity exists between wine composition and wine style/grade, the development of an approach to more accurately (i.e. .69%) predict commercial outcomes from grape chemistry will require additional information. Some important leads for grape compositional markers have been

gained using the PLS modelling approach, but will need to be further refined across a more defined style category range than was achievable in the current study.

6.3.3.2 Shiraz wine grade prediction in 2015

For the 2015 Shiraz grape and wine data sets, most of the PLS models developed for the prediction of wine grade (Table 6.3.3.1) demonstrated a reduction in the validation (as %) as PC number increased, with the maximum attained using only one PC. Therefore, predictive models could largely be developed for one factor. As for the Chardonnay study, higher value or better grade wines received lower values (1, higher quality grade) and poorer-quality grades received higher values (4, lower quality grade). Using all of the grape chemistry data, a PLS model was developed for the prediction of wine grade, but with a low R^2_{val}. The R^2_{val} was increased when a refined model was produced using significant variables (defined using an uncertainty test and correlation loadings). The improved model used three PCs and had an R^2_{cal} of 0.81 and an R^2_{val} of 0.58. The $RMSE_{val}$ achieved was 0.63, indicating a better separation between adjacent wine grades than those found for the 2014 grape grade model discussed previously, albeit across a narrower range of wine grades.

Some key grape-based chemical attributes were useful in the prediction of final wine grade (Figure 6.3.3.3) although the number of significant variables was far smaller than observed for Chardonnay. Similar markers to those important for grape grade in 2014 were observed to be important for higher wine grades, notably elevated ºBrix, glutamic acid, tannin (including both measures of extractable/WL and homogenate tannin), anthocyanin/colour (extractable/WL and total homogenate), chloride, laccase activity and E-2-hexenol. Lower levels of Z-3-hexenol were also associated with grapes destined for higher wine grades, as for the 2014 grape grade analysis, although in this instance berry weight was not a significant variable. However, the observation that lower levels of dry matter were correlated with increased wine quality may be indirectly related to berry size. The 2015 study showed some interesting points of difference from the 2014 analysis which may reflect factors important in the grape to wine transition, but also seasonal variation. The free monoterpene geraniol was not measured in the earlier study, and was found to be a strong negative coefficient, present in higher quantities in grapes producing lower grade wines. Interestingly, in the 2014 study, glycosyl glucose was not found to contribute to the assessment of grape grade, yet in 2015, higher levels of this class of grape-derived compounds were correlated with higher quality wine grades.

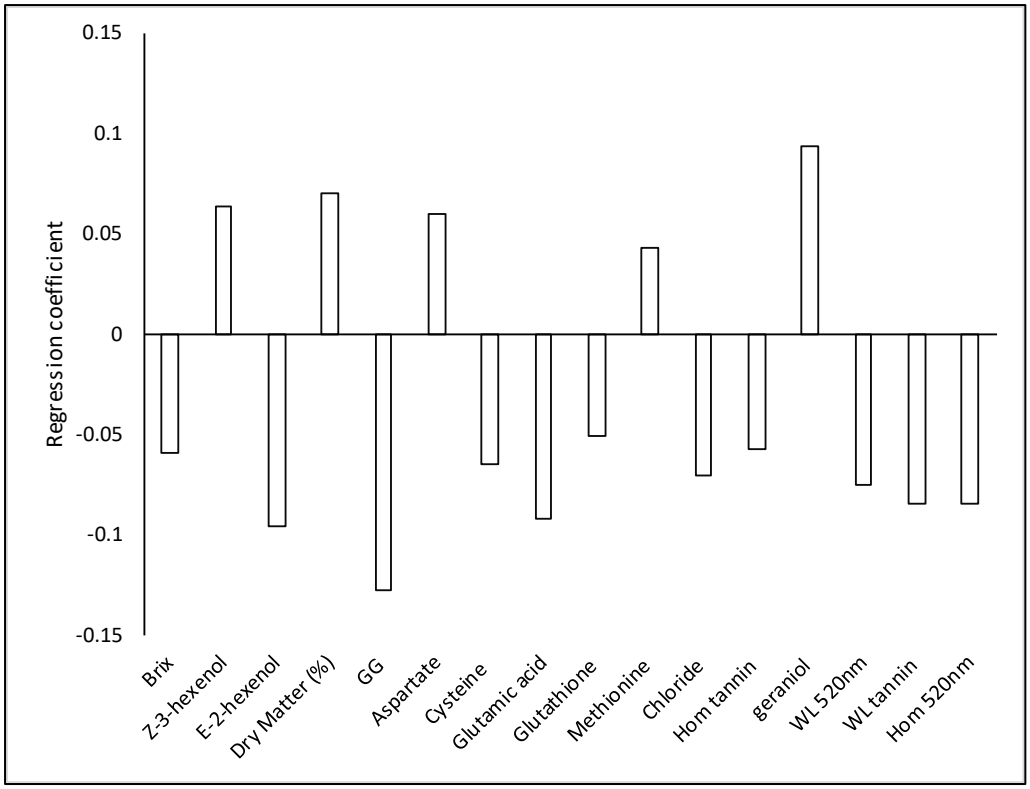


Figure 6.3.3.3. PLS regression coefficients for Shiraz grape composition variables which were significant in predicting wine grade in the 2015 vintage.

The PLS analysis of wine composition for the prediction of wine grade showed that the chemistry variables were more useful than the sensory attributes of the wine, which with two PCs and had an R^2_{cal} of 0.82 and an R^2_{val} of 0.64 and $RMSE_{val}$ achieved was 0.58 (Table 6.3.3.1). Using a reduced set of significant variables, R^2_{val} could be improved to 0.71 and $RMSE_{val}$ reduced to 0.52, indicating adjacent grades were well discriminated in the model. Sensory data did not provide useful modelling of wine grade, with palate and appearance data being somewhat better for modelling wine grade than the aroma data. A combination of sensory variables with the chemistry data yielded a strong PLS model with R^2_{cal} of 0.99 with a relatively low R^2_{val} of 0.76, with an $RMSE_{val}$ 0.51 using six PCs, and from this some information regarding important wine sensory variables could be obtained. However, the PLS model for the wine chemistry/wine grade analysis will be discussed independently of the sensory analysis. The significant variables for wine grade prediction from the analysis of Shiraz wine chemical composition using PLS are shown in Figure 6.3.3.4. These showed important similarity to the grape variables identified, which are known to be strongly related to wine compositional outcomes. Notably, phenolics measures in the wine which included total phenolics, tannin and its sub-qualities (trihydroxylation; %epigallocatechin or by inference proportion of skin tannin), total anthocyanin, non-bleachable pigment and wine colour density were all greater in higher quality wine grades. An interesting observation was the inverse relationship between tannin mass conversion (%MC) which refers to the extent to which tannin can be depolymerised to its component subunits (under heat and acid hydrolysis) and the 520/280 measure which refers to the proportion of colour incorporation within the tannin polymer. Some research has shown that incorporation of colour into tannin leads to a ‘softening’ of the astringency perception of tannins (Vidal et al. 2004), and is well known to decrease the extent to which these molecules may be cleaved during acid-catalysed thermolysis. Higher quality wine grades were associated with lowered tannin mass conversion, and higher colour incorporation. Astringency was not found to be important to the prediction of wine quality grade among the sensory attributes measures, yet the strong relationship of the tannin structure to wine quality may indicate that sub-qualities of mouth-feel perception, for example tannin ‘softness’ versus ‘drying’ may have been important discriminators by the producer. However, these attributes

were not detected by the sensory analysis panel used in this study, and the observation therefore remains speculative.

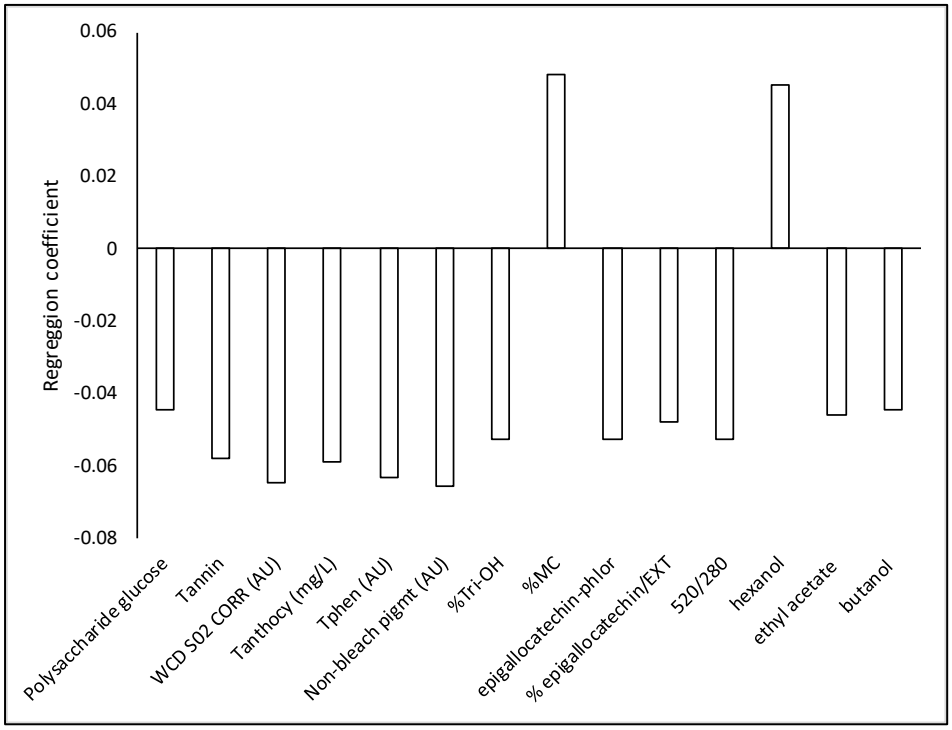


Figure 6.3.3.4. PLS regression coefficients for Shiraz wine compositional variables that were significant in predicting wine grade in the 2015 vintage.

Further to the phenolics data, polysaccharide-associated glucose (possibly yeast-derived glucans) was increased with better wine grades, as well as the yeast-derived volatiles ethyl acetate and butanol. An important negatively associated volatile was the C_6 compound hexanol, which was higher in poor-quality grade wines and which would be expected to be correlated with 'green', 'grassy' aromas (Bindon et al. 2013). However, as discussed previously, this attribute was not important in discriminating the Shiraz wines by PCA or PLS of their respective sensory attributes, nor was it relevant in the prediction of wine grade, but was an important factor used by the producer in the definition of style and grade (Table 6.3.2.3). It may therefore be an important variable to include in future studies that seek to understand the wine compositional factors which impact wine style as it pertains to 'green' attributes.

In the model combined with wine chemistry data, certain sensory attributes were found to be significant in the definition of wine grade using PLS (data not shown). However, the model required six PCs to adequately describe the variation in the data set, and the important sensory attributes discriminated grades differently on the PCs defined. Using the weighted regression coefficients, certain variables expected to be associated with improved quality such as 'dark fruit' flavour/aroma, 'purple' colour and 'viscosity' were found to be negative on PC1 (more in higher quality wines), but on PC6 were significant, and positively loaded (less in higher quality wines). This indicated that the relationships within the data set were extremely complex, and that multiple factors interacted in the definition of grade. The most important negative regression coefficients (data not shown) using six PCs were 'vanilla' and 'sweet spice' aromas, showing elevated levels in better quality wines.

As for the Chardonnay wine data set, QDA was also applied to the Shiraz wines to determine the variables that could be used to predict grape grade, and commercial wine style/grade (Table 6.3.3.3). Using this approach, the wine chemistry data set could successfully predict grape grade with 93% accuracy, and was improved to 100% when combined with the sensory data. On its own, the sensory attributes of the wines could predict grape grade with 87% accuracy. Grape chemical composition

needed to be modelled without one of the grape grades (B) due to a limited sample number, and the remaining grades could be predicted with 92% accuracy.

For the wine style and grade categories, problems arose due to the assignment of the number of categories defined, and the sample numbers in each. QDA requires a sufficient number of samples per category to be performed, but also the correct proportion of predictor variables to categories. Due to these issues, no QDA models for prediction could be developed for this variety.

Observing the complexity of the PLS model (combined for wine chemistry and sensory properties) and the inability to model the data using QDA due to the number of style categories defined, the point can be raised that the definition of wine style at the producer level is challenging. Although some important grape- and wine-based chemical variables were identified from the study, these appeared to be more important to broader definitions of grape or wine grade than to wine style. The fact that phenolics variables were the most significant discriminators of both grape and wine grade highlights that these may be the most important factors used by the producer to assess broad differences between quality streams. However, at the more advanced level, the subtle differences between flavour and aroma attributes (both grape and wine sensory attributes) were observed to interact in multiple dimensions (PCs) and the PCA and PLS models developed were complex, only partially explaining the variation in the data sets.

Table 6.3.3.3. Ability of data obtained for Shiraz grape chemistry and wine chemical and sensory attributes to predict either grape grade, wine style or wine grade using QDA (shown as % accuracy, nd = not determined).

Dataset used to perform QDA	Grape grade	Commercial wine style (6)	Commercial wine grade (4)
All wine sensory attributes	87%	nd	nd
Wine aroma	78%		
Wine palate	74%		
Wine chemistry	93%		
All wine chemistry and sensory	100%		
Grape chemistry	92% (no B)		

6.3.3.3 Usefulness of PLS and QDA modelling for the prediction of wine style from grape composition

To improve the PLS models developed, and increase the capacity of QDA modelling as a predictive approach, future research could begin at the point of wine style, seeking to accurately evaluate the sensory attributes that define stylistic categories. Working back from this point, wine and grape composition could be further explored, which given greater sample numbers could improve the robustness of the approach. Nevertheless, the study has shown some important grape and wine chemical markers that may point the way forward for research into this area, since these may be important for stylistic terms that were not captured as important attributes in the quantitative descriptive sensory analysis performed in this study. For example, broad categories that incorporate multiple terms such as those defined in Table 6.3.2.3 (‘lemon and nectarine, tight lean and long’ as opposed to ‘shaded, lean and long’ for Chardonnay or ‘bright red fruits’ versus ‘simple green and herbal’ for Shiraz) might be better used to characterise styles using napping (Holt et al. 2014). Following this, wine chemical markers of importance could be identified, and after that a progression to assess the relationship to grape composition.

7. Outcomes / conclusions

The planned outputs and performance targets for the project were achieved and the methodology/technology used was effective. In summary, the results of the 2013/2014 grape allocation grade study showed that targeted grape chemical analysis with data modelling gave a good indication that some traditionally measured compounds (pH, TA, °Brix, colour) could be useful as objective measures, as could some new chemical measures that had not previously considered in the assessment of quality in the Australian context. Some of the new analytical measurements identified are already in use for quality assessment and grape streaming by international producers, and the results of this project support their observations, providing a strong demonstration of their value to the Australian wine industry. A limitation of the analytes identified is that complex analyses may not be viable for some producers, due to cost and time delay for the analyses – however many of these analyses are available through third-party laboratories. Alternatively, it was important to note that for all the varieties studied, 'non-targeted' spectral fingerprint measurements, in particular MIR, could be used to achieve a high degree of grape grade prediction accuracy (≥90%) using QDA. Furthermore, for CHA, 93% prediction accuracy was achieved using simple grape chemical analyses that would be readily accessible to a winery laboratory.

In terms of the 2015 'grape chemistry-wine style/grade' prediction study, there was a strong demonstration that grape composition can be used to support decisions about streaming fruit for particular wine styles or grades. Thus grape composition can support assessment of fruit potential for both wine quality grade (and style), with many of the objective measures identified in the 2014 'grape grade' study also being associated with 'wine style' outcomes. The results give a strong demonstration that with ongoing research, and greater sample numbers in stylistic categories, robust models could be developed to predict wine style or grade outcomes from objective grape-based analyses.

A number of benefits from the project are apparent in terms of economic returns to grapegrowers or winemakers, improvements in the quality of grapes or wine, environmental benefits or benefits to the broader community. Potential impacts of this project for the wine sector include the ability for grapegrowers to more efficiently produce grapes to defined specifications, and for winemakers to select fruit with greater confidence that it will be appropriate for a targeted wine style. In addition, it is significant to grapegrowers because it relates to confidence and transparency in the realisation of maximum economic value for their grapes. For both growers and winemakers, objective chemical measures can provide specifications that allow the most value to be achieved from grapes. Developing an understanding of the synergistic relationships of available objective measures to well-established subjective systems has the potential to significantly reduce production costs and increase value by ensuring that fruit is used in the most efficient production stream and that maximum value is returned from the end product. It also may lead to significant savings in the costs of monitoring crops through more effective application of resources and clearer understandings of geographical and climatic drivers.

The second (2015) phase of the work could additionally afford winemakers the opportunity to stream fruit based on objective chemical measures to maximise its value and minimise risk related to loss of value, for example by avoiding batching higher value fruit with lower value fruit, and allow them to choose fruit of the value that relates best to a desired wine style. Furthermore, the management of vineyards to supply grapes of defined composition fit for a particular style of wine is a process that involves a wide range of inputs with varying impacts on the environment, for example, chemicals for spraying, fuel for equipment, water and fertiliser. By better understanding the levels of chemical compounds in grapes required for each wine style, the resource inputs can be tailored and minimised, thus minimising the impact on the environment.

In conclusion, the maintenance of strong relationships along the value chain between key participants such as grapegrowers and winemakers is central to a sustainable Australian wine sector. Objective measures of quality may contribute significantly in some sections of the wine community to ensuring transparency, trust and the maximisation of value by providing an objective framework within which all parties understand what is expected to achieve the highest value and most effective use of available resources. Improving the profitability of vineyard enterprises and wine companies in rural Australia also provides social benefits for the regions.

8. Recommendations

There are several future research and development directions arising from this research:

- The investigation of the relationships between grape composition and wine style needs to be continued with larger sets of grape samples and fewer, more defined, style categories. Such style categories would ideally be agreed upon by the wider industry, perhaps by an Industry Reference Group, as indicative of styles that are relevant to many producers. While acknowledging that a greater number of styles will be present across industry than can be catered for, there is clearly a broader set of styles that can be chosen for which relationships can be developed. It is possible that some of these may have regional typicity or 'terroir' factors associated with them.
- Further development efforts are required to provide decision support tools to help Australian grape and wine producers implement the types of data management and models that will allow businesses to capitalise on the objective measures identified in this report. Such systems are likely to be web-based and existing companies are already implementing these. The AWRI WineCloud could be one such platform. Nonetheless, currently there are laboratories that can provide the required analysis or spectroscopy equipment and consultants that can support the implementation of such systems for businesses.
- Continued extension activities are required to communicate the outcomes of the research, but perhaps more importantly a better understanding of the drivers for adoption of objective measures of quality would be valuable. International case studies of businesses that have implemented such systems and succeeded would be one such opportunity.

Appendix 1: Communication

The final extension activities are pending (June 2017) and as such, a summary of all communication activities and publications will be provided at the end of the project.

Appendix 2: Intellectual property

This project has generated knowledge, know-how and data on the subject of objective chemical measures of grape and wine quality which will be published and communicated as part of the project outcomes.

Appendix 3: References

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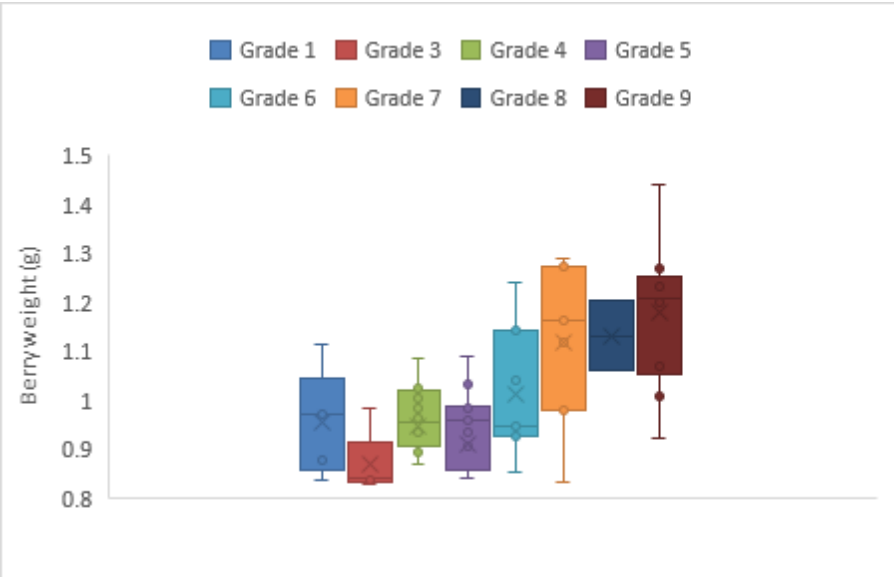
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Appendix 5: Supplementary data



Appendix 5.1 Box and whisker plot showing changes in Shiraz berry weight by grade allocation for the 2014 season.

Appendix 6: Budget reconciliation

A budget statement has been submitted online via Wine Australia's Clarity Investment Management System.