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Objective measures of Shiraz grape and wine quality in premium Australian vineyards



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1. Abstract:

The identification and use of objective measures for the accurate determination of red grape quality is of great interest for many Australian grape and wine producers. To achieve this objective, collaboration between wine producers and researchers is essential to identify practical grape quality measures that are associated with specific wine quality grades and styles. Building on previous work on grape and wine objective methods across multiple Shiraz grades, the study presented in this report aimed to understand the compositional differences between a narrow range of premium Barossa Shiraz categories. In both seasons of the study, the specified quality grades (1 and 2) could not be defined by targeted grape objective measures alone. However, certain premium vineyards did not produce wines in the expected premium wine category within a season, and could be designated as 'marginal' vineyards. Using two different approaches over two seasons of the study, it was found that very high levels of grape amino acids and ammonia were associated with vineyards categorised as 'marginal' and could be associated with losses in wine quality. The results suggest that grape nitrogen should be carefully managed in both the vineyard and at the winery as a first step to optimise wine quality.

2. Executive summary

An earlier Wine Australia-funded project (AWR1202) had aimed to identify the key objective measures in grapes which might be used to define commercial quality grading across a wide range of grades from the poorest quality through to premium for a single wine producer. This was particularly successful for Shiraz grapes, where the most important variables for defining quality grade were phenolics (colour and tannin) and nitrogen (amino acids and ammonia). Based on this successful proof-of-principle study, there was interest from another producer (Pernod Ricard Winemakers) to use the same objective tools to understand the difference between premium and ultra-premium Barossa Shiraz. This led to the initiation in 2016 of a new project on premium Shiraz quality, which is presented in this report. This project looked more specifically at grape phenolics using new techniques such as extractable tannin and colour, as well as non-targeted phenolic profiling, which enables multiple unknown compounds to be identified. Since total must nitrogen and individual amino acids are also known to influence the fermentation-derived volatile profile of wines, these measures were also included in the new project on premium Shiraz. Other techniques investigated were the use of mid-infrared (MIR), near-infrared (NIR) and UV-visible spectra of juices or homogenates (or their extracts).

In the 2017 and 2018 seasons, the producer identified 20 and 10 Barossa Valley Shiraz vineyards that were within the premium 'intended use' classifications 1 and 2 respectively. In the first season, five samples were taken across each vineyard, and in the second season ten samples were taken to gain greater understanding of within-vineyard variability. Grapes were analysed for multiple targeted and non-targeted objective measures. In the first season, the grape batches were also put through a micro-fermentation process. Finally, for commercial winemaking, each vineyard was harvested and kept separate in the winery for fermentation and barrel ageing in old oak over six to eight months. Commercial wines were then evaluated by a panel of winemakers to assign an 'intended use' classification and analysed for their chemical and sensory properties at the AWRI. The assigned 'intended use' grades spanned four quality categories: 1 (highest quality), 1.5, 2 and 3. Data generated from the study were analysed using multivariate statistical techniques in order to predict the respective grape and wine grades.

In the 2017 season, using the results of grape analysis alone, it was not possible to accurately predict the 'intended use' grade of more than 46% of the grape samples using multivariate statistics. Nevertheless, some grape-based measurements could be identified as more significant than others in distinguishing grape grades 1 and 2. Prediction models were also built using data from the micro-ferment wines associated with each grape batch. The wines were assessed immediately at the completion of fermentation, and were therefore not aged, and reflected only the basic extraction and conversion of compounds during fermentation. The inclusion of the wine data gave stronger models for grape grade prediction using multivariate statistics, at 70% accuracy. When data from non-targeted grape and wine phenolic analysis were added to the prediction models, their accuracy was improved up to 79%, and certain grape and wine compounds were further identified by mass spectrometry. The results of the grape grade study showed that although some grape-based measures could be identified

as relevant in the prediction of grape grade, strong models of prediction could not be developed without the inclusion of wine data. This indicated that the conversion of grape metabolites during fermentation is related to, but not strictly correlated with, their concentration or extractability from the grape source. Further research into the identification of important wine metabolites from the non-targeted phenolic analysis may potentially shed light on other grape precursors which were not detected in the grape-based assays but which may be relevant to the definition of quality grade in the premium category.

Although the vineyards selected for the study were only from premium grape grades 1 and 2, it was evident after the commercial winemaking was completed that the vineyard grading system did not closely reflect the final wine grade assigned by the winemaker panel. In light of this finding, it was deemed relevant to the goals of the project to determine whether grape or wine chemical measures might be useful in predicting the 'intended use' wine grade, since this was of commercial importance. The same grape and wine chemical data used in the prediction of grape grade were therefore applied in models to predict the final wine grade. From this process, it was found that wine grades 1, 1.5 and 2 could not be distinguished by the targeted chemical data, but the models could strongly predict wine grade 3, at between 71 and 75% accuracy depending on the type of data used. From the grape data, it was found that grape batches that produced grade 3 wines had higher levels of grape nitrogen, including ammonia and total YAN and multiple amino acids. It was also observed that while total grape colour was relevant to the model, the anthocyanin to tannin ratio was more important as a predictor of grade 3 wines, and was generally lower than in grapes which resulted in wines graded to categories 1, 1.5 and 2. The sensory properties of the commercial wines from the different grade classes were also compared. It was found that large increases in the negative aroma attribute 'tinned vegetable' was associated with the grade 3 category relative to the higher wine quality grades, with losses in 'dark fruit' aroma and flavour also seen for grade 3 wines, accompanied by increases in 'red fruit' aroma and flavour. Grade 3 wines also had a browner colour, and had less opacity and purple colour than wines from the better quality grades. The relationship of grape nitrogen to the development of negative attributes or losses of positive attributes will require further study.

Based on observations from the 2017 season which showed that some vineyards had a large degree of variability in certain grape objective measures, the second year of the study aimed to look more closely at within-vineyard variation. From the analysis of the grape data in 2018/19, it was clear that significant within- and between-vineyard variability existed in the vineyards studied. The inclusion of variability data into the study was an important step forward, since it was found that high variability in phenolics, and high absolute levels of nitrogen (not necessarily nitrogen variability) were associated with 'marginal' vineyards (grade 2 vineyards not consistently performing to the expected wine quality outcome). This finding was not completely validated through the multivariate modelling approach and will require further research. Nevertheless, although grape nitrogen was generally highest in the 'marginal' vineyards, it was also found to be one of the grape-based measures which was generally more variable across all vineyards.

An important conclusion was that due to the importance of grape nitrogen to quality found in both seasons of the study, managing total vineyard nitrogen levels, as well as potentially reducing within-vineyard nitrogen variability, may be a factor of importance to improve quality across the board. Nitrogen management is also potentially important for quality control since DAP addition is a critical element of winemaking. Nitrogen fertiliser application in the vineyard and DAP addition in the winery should therefore be carefully considered in combination. To this end, growers and winemakers could apply different management strategies to heterogeneous vineyards to reduce variation and achieve quality goals. For example, a vineyard could be managed to reduce variability, or sub-sections of a vineyard could be harvested separately and subjected to different DAP addition protocols.

A final important result was that when using non-targeted grape-based measures in the 2018 season, prediction of both grape grades 1 and 2, as well as 'marginal' (grade 2/3) vineyards was possible with homogenate MIR spectra. The strongest prediction allowed the separation of 'marginal' vineyards from the other grape grade categories ($R2 = 0.8$, for 2/3 'marginal' vineyards). Although not realised in the first season of the study, this was a promising result, with a more reliable and stronger model being produced using a readily available and easy-to-use method. Using MIR appears at this stage a most promising tool for detection of lower quality grapes early and quickly in the production chain. Future directions for the Australian wine industry would be to support the increased use of MIR for scanning grape homogenates early in the production process, not only for the prediction of grade but also for the

development of rapid objective measures of grape composition for which calibrations due not yet exist (e.g. extractable phenolics) or which require further validation (e.g. YAN). A further key step would be to increase the skills base within the industry to use and apply non-targeted approaches such as MIR for the ongoing validation and prediction of vineyard and wine grade, and targeted improvements to management practices, according to individual producer needs.

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3. Background:

For Shiraz, only one grape compound (rotundone) has been identified that links directly with varietal wine characters, and it is notably absent from wines produced in warmer climates, where many of Australia's premium wine regions are located. Results from the earlier AWR1202 project showed that non-volatile compounds such as phenolics, overall nitrogen status (yeast assimilable nitrogen, YAN) and individual amino acids were more significant drivers of quality differences in Shiraz grapes than volatile compounds and their precursors. This was not unexpected, since the importance of tannin concentration and wine colour to Shiraz wine quality is well known (Kassara and Kennedy 2011). Total must nitrogen and individual amino acids are also known to influence fatty acid and ester formation in wines (Vilanova et al. 2007), which are important contributors to fermentation-derived flavour and aroma, but which in excess may exert a negative influence on wine quality (Ugliano et al. 2010). New techniques have been developed to determine grape phenolic composition such as tannin extractability (Bindon et al. 2014) and non-targeted phenolic profiling (Lloyd et al. 2015) which to date have been applied to multiple cultivars and regions. Shiraz grapes have been shown to have considerable variation in phenolics measures between regions, which may contribute to regional differences in wine style or quality.

A significant knowledge gap and constraint for uptake by industry of grape objective measures for quality determination is the largely unknown seasonal, regional and within-vineyard variability. Historically, colour as a quality measure was confounded by both significant seasonal variation and a lack of consistency in sampling and analysis techniques. Both the wine industry and the research community recognise the need for grape objective measures to be used effectively in order to appropriately reward growers, and for wineries to stream fruit to specification. In the context of Australian production, certain producers such as Pernod Ricard Winemakers (PRW) routinely analyse grapes destined for winemaking for phenolics and YAN, measures defined as important for Shiraz in project AWR1202. Hence the opportunity was obvious to extend earlier work to incorporate these and other new analytical tools, to obtain meaningful data on the relationship between grape variability (vineyard, grade), wine composition and final wine quality.

Pernod Ricard Winemakers is a significant player in the domestic and international market for Shiraz wine, of which 28% qualifies in the premium category. Internal grading is used within PRW to establish quality grades Q1 (highest quality) through to Q5, with Barossa Valley fruit being graded more narrowly from Q1 to Q3. PRW has a strong chemical analytical capability in-house, by which both grape and wine composition can be monitored. However, the consistent production of premium grapes is hindered by the inability to predict variability between and within vineyards, on both a regional and seasonal basis. In order to accurately grade grapes destined for winemaking within the premium category, a greater understanding of the relationship between grape composition and wine style is needed. The AWR1202 project had identified a suite of analytical parameters for Shiraz grapes that were found to be useful for predicting commercial grade, showing that non-volatile compounds (phenolics, amino

acids) were the most important factors for Shiraz. An opportunity was identified to apply this suite of analytical tools to a narrower, premium quality grade range, and explore additional non-targeted metabolomics approaches for comprehensive profiling of grape composition. From each vineyard, wines were produced to enable an evaluation of grape composition/grade on wine quality and style. Ultimately, the study aimed to determine whether there are significant compositional differences between grades and better understand their implications for wine quality outcomes.

4. Project aims and performance targets:

The objectives defined for the project were as follows:

- Determine whether premium Shiraz fruit can be defined by within- or between-vineyard variability for selected objective measures (revision point in project based on outcomes in year 1, see note 1 in budget information).
- Define whether PRW grape quality grading can be complemented by grape objective measures using selected statistical methods and rank their importance, if any.
- Determine the relationship between grape composition (pooled by vineyard) and wine composition based on selected objective measures.
- Observe whether stylistic and/or quality categories can be defined for wines and assign sensory attributes which most strongly define them.
- Assess the relationships between wine sensory attributes, wine quality/style category, grape and wine chemistry using multivariate modelling.
- Develop practical guidelines for industry on the relevance of grape objective measures to defining premium fruit grade or wine style/quality.
- In consultation with viticultural and winery staff from PRW, identify possible management strategies to add value or minimise costs of premium Shiraz production.
- Prepare and conduct appropriate extension activities to communicate the research results to Australian stakeholders.

Differences from the original project application

Referring to Tables 4.1, 4.2 and 4.3, some changes to the original project outputs were made during the course of the two-year project. A key change was introduced due to the decision by PRW to investigate Q1 and Q2 quality grades, instead of Q3. A decision was also taken at the project review point to have a deeper focus on within-vineyard variability in the second year of the project, which maintained the grape sample numbers but explored a smaller number of vineyards with increased sampling points. Other minor changes were made to improve the experimental design, as indicated below.

[a]Indicates a change from the original project application. At the start of the 2017 season, the producer made the decision to investigate differences between grades Q1 and Q2. This decision was maintained in the second year of the study, with the exception that two vineyards which inconsistently performed at Q2 quality were included (defined as Q3).

[b]Indicates a change from the original project outline which stated that only grape samples and commercial wines were to be analysed. In order to overcome concerns that differences in commercial winemaking such as tannin addition or enzyme use would confound the grape to wine relationship, triplicate small-scale (250 g) micro-ferments were also produced from each grape sample in year one. In the second year micro-ferments were made from a pooled grape sample from each vineyard, in triplicate. This was done addition to the commercial winemaking.

[c]Indicates that napping was not performed due to a greater number of wine samples being required for this technique. Instead, Quantitative Descriptive Analysis (QDA) was performed on all available commercial samples which were deemed not to be outliers (faulty or over-oaked) during the AWRI bench test, rather than a sub-set of samples.

[d]Indicates a change based on the one-year review point. Within-vineyard variability was selected as a key objective for further study in year 2 of the project. Based on this decision, 10 vineyards with 10 sampling points were chosen rather than one vineyard with 100 points, consistent with the producer's preferences. From these samples, 30 experimental wines and 10 commercial wines were expected to be produced.

Table 4.1. FY1 Outputs and Activities

Output	Target Date	Activities
List of 20 Shiraz vineyards identified for use in the project	31 December 2016	• Review historical PRW data and consult with a reference group of PRW viticulturists and winemakers to identify vineyards that consistently perform to Q2 and Q3[a] grades.
Samples of grapes representing within-vineyard heterogeneity	30 June 2017	• Collect five samples from each of the identified vineyards at commercial harvest to capture within-vineyard heterogeneity. • Organise for processed grape samples to be delivered to AWRI.
Wines from grape quality grades Q2 and Q3[a]	30 June 2017	• Source grapes at commercial harvest from each vineyard. • Produce 20 wines[b] from grape quality grades Q2 and Q3[a] according to commercial procedure, retaining the grape parcels intact and applying standard oak treatment.

Table 4.2. FY2 Outputs and Activities

Output	Target Date	Activities
Data from grape compositional analysis and knowledge of relationships between grape composition and quality grade	31 December 2017	• Conduct analysis of total soluble solids, pH, TA, malic acid, yeast assimilable nitrogen (YAN), alpha-amino nitrogen in grape juice • Conduct analysis of grape amino acid profile • Conduct analysis of grape anthocyanins, total phenolics, total and extractable grape tannin, • Conduct spectral fingerprinting (UV/Vis scan, NIR, MIR). • Perform basic statistics to determine regional, within-vineyard and quality-based differences in grape compositional attributes. Conduct multivariate modelling of grape composition and quality attributes to define primary drivers of differences in grade, if any.
Project review and agreed priorities for second year of research.	31 December 2017	• Hold meeting with project team and Wine Australia to discuss year 1 results.

		• Identify the strongest drivers of variability in grapes and decide whether vineyard mapping should be conducted in year 2. • Prepare variation request if required.
Data from wine compositional analysis and non-targeted phenolics analysis in grapes and wines Data from wine napping[c] and descriptive sensory analysis	31 December 2017	• Conduct analysis of wine[b] pH, TA, residual sugar and alcohol completed • Complete wine proline[b] analysis • Complete wine colour and tannin[b] analysis • Complete phenolic profile of 100 grape samples • Complete phenolic analysis of 20[b] wine samples • Carry out wine sensory napping[c] at PRW with a reference panel of winemakers • Conduct quantitative descriptive analysis of a sub-set of wine style categories defined by napping • Undertake project review to determine whether additional analysis of wine fermentation products and/or damascenone is required.
Knowledge of grape and wine composition, and the relationship to wine style and quality grade	30 June 2018	• Perform multivariate modelling on grape composition in relation to wine composition. • Determine relationships between compositional attributes and wine style and/or quality grade.
Subject to the review point following Year 1, this Year 2 output will be: Grape samples from 20 Shiraz vineyards[d] Or Grape samples from different sections within one vineyard[d]	30 June 2018	• Identify vineyard(s) of premium quality grade from which 10 grape batches of two quality grades, Q2 and Q3[a], will be sourced. • Collect 100 grape samples from: a. the 20 identified vineyards at commercial harvest (5 per vineyard)[d]. b. or, from within one premium vineyard known to have high variability[d]. • Arrange for processed grape samples to be delivered to the AWRI.
Wines from heterogenous grape parcels, as identified by compositional analysis	30 June 2018	• Produce 20 wines[b,d] according to commercial procedure, and apply standard oak treatment.

Table 4.3. FY3 Outputs and Activities

Output	Target Date	Activities
Data from general grape compositional analysis	30 September 2018	• Conduct analysis of total soluble solids, pH, TA, malic acid, yeast assimilable nitrogen (YAN), alpha-amino nitrogen in grape juice • Conduct analysis of grape amino acid profile • Conduct analysis of grape anthocyanins, total phenolics, total and extractable grape tannin, • Conduct spectral fingerprinting (UV/Vis scan, NIR, MIR).
Knowledge of relationships between grape composition and quality grade	31 December 2018	• Perform basic statistics and multivariate modelling to assess differences in grape compositional attributes. • Define primary drivers of differences in grape composition, if any.
Data from wine[b] compositional analysis and wine napping and sensory analysis	31 December 2018	• Conduct analysis of wine[b] pH, TA, residual sugar and alcohol completed • Complete wine proline[b] analysis • Complete wine colour and tannin[b] analysis • Carry out wine napping[c] at PRW with a reference panel of winemakers at PRW • Conduct quantitative descriptive analysis of a sub-set of wine style categories defined by napping[c] • Undertake a review as to whether additional analysis of wine fermentation products and/or damascenone is required.
Data from non-targeted phenolics analysis in grapes and wines	31 December 2018	• Complete phenolic profile of 100 grape samples • Complete phenolic analysis of 20 wine samples[b,d]
Knowledge of relationships between grape and wine composition, and the relationship to wine style/quality	30 June 2019	• Perform multivariate modelling on grape composition in relation to wine composition. • Determine relationships between compositional attributes and wine style and/or quality/vineyard parameters.
Extension materials and final report	30 June 2019	• Prepare extension materials from research outputs summarising opportunities to improve the streaming process of Shiraz fruit on the basis of fruit composition or other identified parameters for presentation to industry through at least two extension mechanisms. • Prepare and submit final report to Wine Australia

5. Methods:

5.1 Grape samples. The study was conducted in two grape production seasons (vintages), from February to March of 2017 and 2018 respectively. In the 2017 season, Shiraz grapes were sourced from 20 premium Barossa Valley vineyards (designated S1 to S20) from a commercial producer, Pernod Ricard Winemakers (PRW). In In the 2018 season, 10 vineyards were selected (including a sub-set carried over from the previous season). In the second season, vineyards were assessed for yield, cordon length and canopy properties (VitiCanopy app, Lobet et al. 2013) by the producer. Allocation gradings of 1 (highest quality) and 2 were assigned according to the producer's 'intended use' specifications, based on within-season vineyard and grape assessments, as well as historical performance (grape assessment at harvest and final commercial wine allocation, see section 5.8). For both seasons, all sub-regions in the Barossa sampled were identified as warm climate, and elevations ranged from 231 to 339 m above sea level. Vineyards were of different specifications in terms of clone, rootstock (most on own roots), planting date, soil type and management practices, and specific data were not available for all sites. Approximately 2 kg of grapes were collected per sampling point, a minimum sample of 30 bunches. In the 2017 season, 15 of the PRW vineyards, multiple locations (4-5) within a vineyard site were sampled in order to obtain data on vineyard heterogeneity. In the 2018 season, 10 samples were collected at widely-distributed points within each vineyard for the same purpose. Samples were collected at, or within one week of commercial harvest for a specific vineyard. Following harvesting, batches of fresh fruit were sealed in clear plastic bags, and stored at 4°C Where possible, five samples per vineyard were obtained from distinct locations to obtain an indication of within-vineyard variation. Samples were stored as whole bunches, sealed, at 4 °C for no more than 5 days before being processed. A total of 82 grape samples were processed for the 2017 experiment. A total of 100 grape samples were processed for the 2018 experiment.

5.2 Grape processing and distribution for analysis. For both the 2017 and 2018 experiments, grape batches were completely de-stemmed by hand, and thereafter, sub-samples of berries were taken for analysis either fresh or frozen as described below. A fresh 200-berry sample was weighed for the assessment of average berry weight and transferred to a plastic bag equipped with a re-sealable zipper (snap-lock). The 200 berry sample was gently crushed, by hand, in the snap-lock bag and strained and the juice was collected in a measuring cylinder for an assessment of juice volume. Juice was centrifuged at 1730 *g* for 5 minutes and 50 mL was recovered and analysed fresh for pH, TA, °Brix and YAN as described below. Aliquots (10 mL) were frozen at -20°C for later analysis of the MIR spectrum, as described below. Duplicate 250 g grape samples were prepared in snap-lock bags and stored at 4°C for no more than 4 hours, and later prepared for small-scale fermentation as described below. Triplicate 50 g samples of fresh grapes were prepared in small snap-lock bags and the weight recorded for analysis of extractable phenolics, as described below. For techniques that required grape homogenisation, a 200 g grape sample was immediately frozen at -20°C. Prior to homogenisation, the frozen 200 g berry samples were partially defrosted. Berry samples were homogenised in a Retsch Grindomix GM200 (Retsch GmbH & Co., Germany) at 8,000 rpm for 20 seconds. Homogenates were kept at less than 4°C in 70 mL screw-cap plastic containers until analysed, on the same day as homogenisation was performed.

5.3 **Basic juice compositional analysis**. Fresh juice samples were centrifuged to remove solids as described above 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 pH end-points 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*-phthaldehyde/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).

5.4 **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 4,000 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 the cell was 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).

5.5 **Wine-like grape extraction**. To estimate extractable grape tannin and measure colour (520 nm), phenolics (280 nm) and absorbance in the UV-visible spectrum, grapes were extracted according to a published protocol (Bindon et al. 2014). Fresh triplicate 50 g berry samples in snap-lock bags were crushed gently by hand. The musts (solids and juice) obtained were transferred into 70 mL plastic screw-cap containers, using a spatula to ensure all berry contents were removed from each bag. Aqueous ethanol (15 mL of 40% adjusted to pH 3.4 with a 10 g/L aqueous tartaric acid solution) was added and the capped containers were gently shaken on their side at 60 rpm on a medium orbital shaker (EOM5, Ratek) for 40 h at room temperature (22 °C). Afterwards, the extracts were pressed through a 1 mm^2 sieve, and the extract volume recorded. The extracts were transferred into 50 mL tubes, centrifuged at 1730 *g* for 5 min, and the supernatants were analysed for tannin, colour and absorbance in the UV-visible spectrum as described below.

5.6 **Extraction of grape berry homogenates.** Following homogenisation of previously frozen 200 g grape samples which had been stored for no longer than three months at -20ºC, a 1 g portion of homogenate was weighed into a 10 mL screw-cap centrifuge tube. Extractions were performed in 10 mL of 50% v/v, aqueous ethanol. Tubes were capped and extractions carried out on a suspension mixer for 2 hours at room temperature (22 °C). The tube was then centrifuged at 1730 *g* for 10 minutes, and the supernatant analysed as described below.

5.7 **Micro-scale winemaking.** Triplicate 250 g fermentations were performed on fresh grape samples in the 2017 season. Grapes were gently crushed by hand in a snap-lock plastic bag and transferred to a 500 mL plastic container equipped with a screw-cap lid, fitted with a rubber seal and airlock. To the crushed grapes, 12.5 mg (as a 250 μL aliquot in water) of potassium metabisulfite (PMS), 100 mg (as a 1 mL aliquot in water) of hydrated *S. cerevisiae* PDM (Maurivin, Sydney, Australia) and 75 mg (as a 200 μL aliquot in water) diammonium phosphate (DAP) were added, and mixed well using a metal spatula. A plastic cylinder covered with fine plastic mesh was inserted into each container, designed specifically to keep the cap submerged during fermentation. Ferments were transferred to a temperature-controlled room and fermented at 27ºC for six days and stirred once daily. Thereafter ferments were pressed through fine plastic mesh, and the residual glucose was tested using Keto Diabur 5000 test strips (Roche Diagnostics, NSW, Australia). If the result was > 2% glucose, fermentation was continued at room temperature in a Schott bottle fitted with a rubber seal and airlock. Once dry for glucose, 26 mg of PMS was added to a 130 mL aliquot of wine, which was then decanted into a 120 mL container with no ullage and settled at 4°C for a minimum of 48 hours. Wines were racked off gross lees, pH adjusted to <3.5 using tartaric acid in water (50% w/w), and bottled in 100 mL glass bottles with no ullage, and stored at 4°C until analysed (within one month)

5.8 **Grading of vineyard blocks, commercial winemaking and wine grade assessment**. Blocks were assessed for quality by winemakers. At least one row was selected that was representative of the block, and this could not be an outside row. The number of rows selected per block depended upon the vineyard area, intended use, yield, geography, within-vineyard variability and row length. For each row, winemakers walked at least three panels into the row before starting to assess vine and grape berry attributes. A least four vines per row were assessed for vine and grape berry attributes, and the vines were expected to be randomly spaced along both sides of the row. The assessment only included fresh grapes that were representative of the block, and did not include damaged berries, unless this was otherwise not possible. Suitably trained viticultural staff independently assessed the block for pest and disease status. Assessments included leaf condition, bunch exposure, berry size, berry shrivel, and berry sensory attributes (sugar/acid balance, skin chewiness/thickness and tannin intensity). Vineyards were harvested at commercial ripeness and fermented as separate units in the winery. However, winemaker decisions regarding the use of enzymes, tannin addition and the length of maceration time were not controlled for experimental purposes. After fermentation, wines were barrel-aged in old oak to prevent the introduction of excessive oak character to the wines. After fermentation, wines were assessed blind by the winemaking team following a familiarisation tasting of available styles, and classified to a style and quality grade category. Winemaker assessment took place in June in both 2017 and 2018.

5.9 **Basic wine compositional analysis**. Micro-scale wines were clarified by centrifugation and scanned in a Thermo-Fisher Microdom instrument, which combines two spectrophotometers in series via flow cells, to collect spectra over the UV-Vis and MIR wavelength ranges. Data on wine pH, TA, alcohol, residual sugar and volatile acidity (VA) were obtained using the instrument calibration. Commercial wines were analysed for their basic composition using a Foss WineScan FT 120 as described by the manufacturer (Foss, Hillerød, Denmark).

5.10 **Colour, tannin and UV-visible spectrum of extracts and wines.** Tannin was analysed in grape extracts and wines using a standard high-throughput method (Mercurio et al. 2007). Each analysis was performed in a minimum of duplicates. Briefly, tannin concentration was analysed following precipitation of ethanolic homogenate extract by methyl cellulose (MCP) in 1.1 mL 96 well deep well plates. 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 minutes. 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 minutes. Centrifugation was performed for five minutes at maximum speed on a Hettich Universal 32 R centrifuge equipped with a Hettich 1645 rotor for 96 well plates (Adelab Scientific). A 300 μL sample of supernatant from each of the methyl-cellulose-treated and control samples was 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, grape extracts were diluted with 1 M HCl and scanned across the UV-visible range using a spectrophotometer between 200 nm and 800 nm (Cary 300, Varian, Australia). Wine colour properties were also determined according to the high-throughput modified Somers assay described by Mercurio et al. (2007).

5.11 **Analysis of commercial wine.** Aside from the general measures described, commercial wines were also analysed for tannin composition and polysaccharides. Tannin was isolated using solid

phase extraction on Oasis HLB (3 mL, 60 mg, 30 µm) cartridges (Waters, Rydalmere, NSW, Australia) as modified for the isolation of total tannin (Kassara & Kennedy 2011). Tannin isolates were reconstituted in methanol, and analysed using phloroglucinolysis (Kennedy and Jones 2001) to determine tannin subunit composition and mean degree of polymerisation (mDP) using the HPLC method described previously (Kennedy and Taylor 2003). Samples were also analysed by gel permeation chromatography (GPC) to determine tannin size distribution. The GPC approach was adapted from the original method (Kennedy and Taylor 2003) to allow for increased size distribution resolution of high molecular mass material, as described previously (Bindon and Kennedy 2011). For polysaccharide analysis, a 1 mL aliquot of wine was added to 5 mL of absolute ethanol and allowed to precipitate at 4 C for 18 hours. Samples were centrifuged at 8000 *g* for 5 minutes and the supernatant was discarded. The pellet was washed with 5 mL ice-cold 80% v/v ethanol, re-centrifuged, and the recovered pellet allowed to air dry briefly to remove excess ethanol. The pellets were reconstituted in Milli-Q water, frozen at −20°C and lyophilised. Lyophilised samples were reconstituted in 2 M TFA and hydrolysed at 100 C for 3 hours. Hydrolysates were cooled on ice, concentrated under vacuum at 30 C in a Heto vacuum centrifuge (Heto-Holten A/S, Allerod, Denmark) and resuspended in Milli-Q water, dialysed, reconstituted and hydrolysed according to the protocol described by Bindon et al. (2019). Monosaccharides released from polysaccharides following hydrolysis were quantified using an adaptation of a published method (Honda et al. 1989), and analysed by HPLC according to Bindon et al. 2019). In the second season of the study, a sub-set of the commercial wines were also analysed for low molecular weight sulfur compounds (Siebert et al. 2010), polyfunctional thiols (Capone et al. 2015), C6 compounds (Capone et al. 2012), norisoprenoids and monoterpenes (Pedersen et al. 2003, Ugliano et al. 2008).

5.12 **Non-targeted phenolic profiling of grapes and wines**. For each grape sample, 25 berries were frozen using liquid nitrogen, roughly crushed with a pestle and subsequently milled in a cryo-mill to obtain a homogenous powder. The powder was transferred to 50 mL tubes and kept frozen. Each sample (2 g) was weighed in a 10 mL yellow cap tube and stored at -80℃ before extraction. The powder was extracted with water (1 mL), methanol (2 mL) and chloroform (2 mL). The sample was vortexed for 1 minute and centrifuged at 3,750 rpm at 4℃ for 15 minutes. The aqueous phase was transferred to a 10 mL tube and the sample re-extracted using a water-methanol solution (1:2). The sample was vortexed and centrifuged. The two aqueous phase fractions were combined and centrifuged for 10 minutes. For micro-scale wines, a clean-up procedure was applied using Strata-X reversed phase SPE cartridges. After conditioning the cartridge (1mL MeOH and 1 mL water), 2 mL of each sample was diluted with 8 mL of water and loaded to the cartridge. The eluted fraction was discarded. The retained fraction was recovered in sequential elution with 1 mL aqueous MeOH (2%) followed by 1 mL MeOH. Between elution steps the cartridge was dried at full vacuum for five minutes. The eluted fractions were collected in test tubes and methanol was evaporated. The methanolic grape and wine extracts were pooled and evaporated to dryness under a stream of nitrogen at 35℃. Samples were reconstituted using LCMS solvents, 25 µL of solvent A (0.5% MeOH in MilliQ water) and then 75 µL of solvent B (40% acetonitrile in MeOH) and analysed using LC-QTOFMS. Separation was performed on an Agilent 1200SL HPLC coupled to a Bruker microTOFQ-II. For HPLC, 1 µL injection volume was injected to a Phenomenex Kinetex PFP (150 mm x 2.1 mm) column, at an initial flow rate of 0.4 mL/min with 100% solvent A (2% formic acid, 0.5% methanol in Milli-Q water). Solvent B, which contained 2% formic acid, 2% Milli-Q water, 40% acetonitrile in methanol, was varied throughout the 42-minute run as described in Table 5.12, together with the flow rate. The HPLC oven temperature was set at 30°C and the DAD acquisition range was 200-500 nm. For MS, samples were acquired in MS negative mode. The MS was operated at a source temperature of 200°C, with a capillary voltage of, 3500 V, and end plate offset voltage of -500 V, nebuliser pressure of 2.0 Bar and a dry gas flow rate of 8.0 $L.min^{-1}$. The mass range was 50-1650 m/z and the acquisition rate was 0.5 Hz. A calibration solution of sodium formate (5 mM sodium hydroxide in 50 % (v/v) 2-propanol) was introduced during LCMS analysis via an inline post-column switching valve and sample loop. Using Bruker's DataAnalysis (v4.0 SP4) software, mass spectra (line) were calibrated in the range 90-1000 m/z from the sodium formate

clusters using an enhanced quadratic algorithm. The final number of molecular features detected was 105. The term 'molecular feature' describes a two-dimensional bounded signal: a chromatographic peak (retention time) and a mass spectral peak (m/z). Analysis was performed by the AWRI's Metabolomics Australia node.

Table 5.12. Flow rate and solvent gradient for the non-targeted profiling of phenolics by LC-MS.

Time (min)	% Solvent A	% Solvent B	Flow Rate
0	0	0	0.4 ml/min
6.25	99	1	
20.00	92.5	7.5	
30.00	40	60	
33.00	10	90	
38.00	10	90	
38.20	100	0	
38.40	10	90	
38.60	100	0	
38.80	10	90	
39	100	0	
42	100	0	

5.13 **Amino acid analysis of grape juices and wines.** The amino acid profile of juices and wines was performed by Metabolomics Australia, using the method of Boughton et al. (2011).

5.14 **Wine volatiles.** Yeast volatile fermentation products were analysed using HS-SPME–GCMS, with SIDA as described previously (Siebert et al. 2005). Twenty-seven compounds, including ethyl and acetate esters, higher alcohols and volatile acids were quantified.

5.15 **Sensory analysis**. For both seasons of the study, sensory analysis of the wines was conducted by quantitative descriptive analysis (QDA). The original project description indicated that napping would be done, followed by QDA on a sub-set of wines with key sensory indicators which defined specific styles (or grades). However, due to the limited number of wines available for sensory assessment, it was decided to proceed with QDA on all wines. For the 2017 season, of the 20 vineyards studied, five commercial wines could not be made available for sensory analysis from the producer. For the remaining 15 commercial wines, a preliminary sensory evaluation by a panel of expert technical wine assessors resulted in exclusion of one wine due to excessive oak flavour influence. Fourteen wines were evaluated using QDA, and were across four commercial quality designations. For grade 1 ,which represented the highest quality, there were four wines. For the remaining grade categories there were four wines in grade 1.5, three wines in grade 2, and three wines in grade 3. For the 2018 season, two of the ten wines produced for the study were not available for sensory analysis. QDA was performed on eight wines, none of which were excluded from sensory analysis. The wines evaluated were across the same four quality categories defined in the first season, albeit skewed toward a greater proportion in quality grades 1 and 1.5.

5.16 **Statistical analysis**. For the assessment of grape and wine compositional variability, and the prediction of grade categories, principal component analysis (PCA), partial least squares (PLS), PLS regression (PLSR) or PLS combined with discriminant analysis (PLS-DA) were used with the Unscrambler X 10.3 (CAMO Software, Oslo, Norway) software. Prior to multivariate analyses, basic statistical comparisons were performed to confirm normality of sample distribution using the same software. PCA was performed to observe variability within the datasets and to determine sample outliers. PLS-DA was used for grade prediction. Targeted chemical data and non-targeted non-

volatile data were scaled as the inverse of the standard deviation for multivariate analyses. Where required, MIR and NIR data were transformed using the Standard Normal Variate (SNV) and/or the Savitzky–Golay derivatives, and were not scaled for statistical analysis. All PCA and PLS analyses were performed with full cross validation.

For the processing of wine sensory results, panel performance was assessed using Fizz software and R with the SensomineR and FactomineR packages. The performance assessment included analysis of variance for the effect of judge, wine, presentation replicate and their interactions, degree of agreement with the panel mean, degree of discrimination across samples and the residual standard deviation of each judge by attribute. All subsequent data analysis was carried out using XLSTAT (XLSTAT 2017, Paris, France). Using analysis of variance (ANOVA) the effects of wine, judge, presentation replicate, judge by wine, judge by presentation replicate and wine by presentation replicate effects were assessed, treating judge as a random effect. Following ANOVA, Tukey's honest significant difference (HSD) value was calculated (P=0.05) for the wine effect. PCA was conducted for the mean data of the significant (P<0.05) and close to significant (P<0.10) attributes of each of the wines using the correlation matrix.

6. Results and discussion

6.1 Results of the 2017 season

6.1.1 Prediction of vineyard grade through targeted grape and wine analysis in 2017

Using the results of grape analysis alone, it was not possible to accurately predict the 'intended use' grade of more than 46% of the grape samples using multivariate statistics (Table 6.1.1). In all cases an uncertainty test was used to select a sub-set of the most significant predictive variables and in some cases improved the prediction of the validated model (R^2_{val}). Prediction models were also built using data from the micro-ferment wines associated with each grape batch. The wines were assessed immediately at the completion of fermentation, and were therefore not aged, and reflect only the basic extraction and conversion of compounds during fermentation. The inclusion of the wine data gave stronger models for grape grade prediction using multivariate statistics, at 70% accuracy (Table 6.1.1).This enabled the identification of both grape and wine variables that were relevant in defining the two fruit grades (1 and 2). The better performance of the prediction model with both data types indicated that the conversion of grape-derived metabolites during fermentation may have been relevant to the historical performance of the respective vineyards in terms of commercial wine quality outcomes, and informed the current grape grade.

The PLS-DA model(s) developed using targeted grape and wine analysis were complex and required multiple principal components (PCs). The initial PCA results (not shown) did not show separation of the grade categories on the first two PCs which describe the greatest quantitative variance between samples. Rather, more information about grade separation by PCA was found in PCs 3, 4, and 5. Due to this, it is therefore important to note variables identified as significant in the prediction of grade by PLS-DA are more likely to reflect relative, and not absolute differences in the target compounds. Therefore, to better visualise the results for the significant predictors of grade, these are presented as the weighted regression coefficients from 7 PCs (Figure 6.1.1).

For Grade 1 grapes, positive predictors were extractable (wine-like) grape anthocyanin, total wine anthocyanin and wine colour density. Grade 1 grapes were predicted by lower extractable and total grape tannin relative to the higher extractable colour. It was found that wine tannin was correlated with grape extractable tannin, polymeric pigment and wine colour density in the initial PCA results (not shown), but was not found to be a significant predictor of quality grade in the final PLS-DA model. As such, it was relevant to observe that there was wide variability in the absolute quantities of grape and wine phenolics across the two grade categories, but Grade 1 had proportionally higher extractable anthocyanin to extractable tannin (ratio). Due to this finding, it was considered that the ratio of extractable or total anthocyanin to tannin (A/T ratio) might be a relevant objective measure. It was found following PCA of the targeted analytical data including the A/T ratio, that it was inversely correlated with

extractable and total tannin, as well as the wine chemical age 2 (result not shown). However, adding the A/T ratio to the PLS-DA model did not improve the accuracy of grape grade prediction. Rather, it can be seen from Figure 6.1.1 that chemical age 2 was an important predictor of grade, and was negatively associated with Grade 1 fruit. Since the chemical age 2 measure provides an indication of the proportion of polymeric pigment to total red pigment in the wine (Somers and Evans, 1977) it was not surprising that this measure was negatively correlated with the grape A/T ratio in the PCA, since it indicated that proportionally more anthocyanin had reacted with tannin to form polymeric pigment in the wines made from Grade 2 grapes during the ferment. On the other hand, wines made from Grade 1 grapes retained more monomeric anthocyanin. In this case, the chemical age 2 value provided the same information as the A/T ratio. Since the micro-ferments used to build the prediction models were not aged, the question of how the colour composition of the respective commercial wines made from the grapes of different grades might respond to ageing was considered to be important, and will be discussed later in this report.

Grape nitrogen measures and wine fermentation products were also assessed for their importance in defining grape quality grade (Figure 6.1.1). As discussed previously, the predictors do not indicate absolute levels of the specified analytes which were associated with the respective grades, given that the initial PCA analysis showed greater separation of the samples by grade on the higher PC levels. It was found from the PLS-DA prediction model that a number of fermentation esters, including ethyl acetate in wines, were negative predictors of Grade 1, as were the amino acids glutamate, tryptophan and alanine in grapes. On the other hand, ammonia and the amino acids tyrosine, valine and serine in grapes were positively associated with Grade 1 grapes. In micro-ferment wines, the ester ethyl octanoate was also positively associated with Grade 1 grapes.

The results of the grape grade study showed that although some grape-based measures could be identified as relevant in the prediction of premium grape grades, stronger prediction models could not be developed without the inclusion of wine data. This indicates that the conversion of grape metabolites during fermentation is related to, but not strictly correlated with their concentration in, or extractability from the grape source. It was therefore considered that important wine metabolites from the non-targeted phenolic analysis could potentially shed light on other grape precursors not detected in the grape-based assays which may have been relevant to the definition of quality grade in the premium category. This study will be discussed in the following section, together with the work conducted using spectral analysis of the grapes.

Table 6.1.1. PLS regression model outputs for the prediction of grape grades 1 and 2 from targeted and non-targeted analytical data showing the optimal number of principal components (PC no) required, the R^2 coefficients of calibration (R^2_{cal}) and validation (R^2_{val}), and the root mean squared error of calibration ($RSME_{cal}$) and validation ($RSME_{val}$).

Analysis Type[a]	Data selected	PLS-DA model parameters				
		PC no	R^2_{cal}	R^2_{val}	$RMSE_{cal}$	$RMSE_{val}$
Targeted grape analysis	all data	7	0.68	0.30	0.27	0.41
	sig var[b]	6	0.63	0.46	0.30	0.36
Targeted grape and wine analysis	all data	7	0.81	0.52	0.21	0.34
	sig var	7	0.83	0.70	0.20	0.27
Non-targeted grape non-volatiles	all data	5	0.76	0.39	0.24	0.38
	sig var	2	0.53	0.47	0.33	0.36
Non-targeted wine non-volatiles	all data	4	0.77	0.52	0.23	0.34
	sig var	3	0.77	0.71	0.23	0.26
Non-targeted grape and wine non-volatiles	all data	5	0.88	0.54	0.17	0.33
	sig var	4	0.86	0.79	0.18	0.23
All targeted and non-targeted analysis of grape and wine, significant variables only	all data	7	0.92	0.77	0.13	0.24
	sig var	3	0.56	0.46	0.32	0.36
UV-vis scan from grape homogenate extract	all data	7	0.56	0.40	0.32	0.38
	sig var	7	0.53	0.39	0.33	0.39

[a] Model results from mid-infrared data of juice and homogenate, as well as from near-infrared data of homogenate are not shown; [b] Sig var indicates a sub set of significant variables selected using an uncertainty test and high correlation loadings

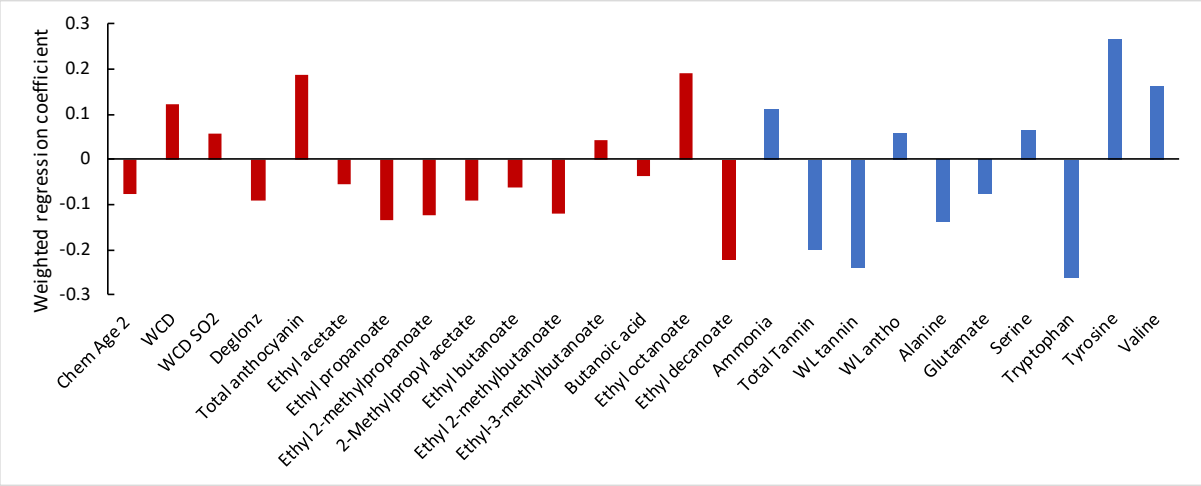


Figure 6.1.1. Weighted regression coefficients for the PLS-DA model to predict grape grades 1 and 2 using a combination of targeted analysis from both micro-ferments (red) and grapes (blue) showing significant predictors (weighted regression coefficient >0.03); Chem Age, chemical age; WCD, wine colour density; Degionz, degree of anthocyanin ionisation; WL, wine-like/extractable grape tannin or colour; antho, anthocyanin.

6.1.2 Non-targeted analysis of grapes and wines in 2017

Despite the finding that tannin and colour were important in the prediction of grape grade, using the UV-visible spectrum of grape homogenate extracts did not produce successful prediction models (Table 6.1.1), and nor did the use of spectra of juice and/or homogenate obtained in the MIR and NIR ranges (results not shown). This finding was contrary to the results of a previous study (Bindon et al. 2017a, Bindon et al. 2017b) which found that these types of spectral analysis most successfully predicted a wide range of Shiraz grape quality grades, and performed better than targeted analysis. As an alternative analytical approach, non-targeted metabolomics was used for comprehensive profiling of grape composition, including phenolic compounds. This provided data known as 'molecular features', which describe a two-dimensional bounded signal: a chromatographic peak (retention time) and a mass spectral peak (m/z) which could be compared to the variable, grape grade. For the grape samples, the final number of molecular features detected was 105, and for the associated wines the number detected was 456. PLS-DA models built for grape grade prediction using only the 105 molecular features from grapes did not perform better than that achieved with targeted grape analysis, at 47% accuracy (Table 6.1.1). For the 456 molecular features identified in micro-ferment wines, the accuracy increased to 77%, and when grape molecular features were included, improved to 79%. When this data was combined with important predictor compounds identified from targeted analysis, the prediction accuracy did not improve further and remained at 77%. The results for the PLS-DA model are shown in Figure 6.1.2, showing that, unlike the targeted grape analysis, a simpler separation of samples from quality grades 1 and 2 occurred on one PC, which described 68% of the variation in grade (Y). A further 10% of the variation in Y was defined by the second PC. This indicates that should key molecular features be identified by mass spectrometry, simpler discriminative modelling of grade might be achievable. Using this approach, a number of molecular features from grapes and micro-scale wines which were shown to be most significant in the prediction of the two grape quality grades were further investigated and tentatively identified.

Of the significant molecular features identified as predictors of grape grade using PLS-DA, only four from either the grape- or wine-based measures respectively could be tentatively identified using mass spectrometry and comparison to reference data (Table 6.1.2). For the positively identified wine features, ethyl gallate had the highest regression coefficient that was positively associated with grade 1 in the PLS-DA model. Procyanidin B2 in wine, positively identified by mass spectrometry, also had a high (positive for grade 1) regression coefficient, ranked 7th after ethyl gallate. For the grape-based molecular features included in the PLS-DA model, features G8 and G80 had the most strongly weighted positive and negative regression coefficients respectively, but could not be identified using mass spectrometry. Two isomers of coutaric acid (Table 6.1.2) were found to be negatively associated with better grape grade, and had the most strongly weighted regression coefficients in the PLS-DA after the two non-identified grape compounds. Tryptophan, although an amino acid, was also detected and positively identified by the non-targeted phenolic profiling method, and was shown to be negatively associated with grape grade, as observed for the targeted amino acid results. Interestingly, a compound labelled as gossypin (gossypetin-8-glucoside) was putatively identified in grapes for the first time, to the best knowledge of the researchers at the time of reporting. Gossypin is abundant in *Hibiscus vitifolius*, flowers, as well as other plant species, and has been reported to exert anti-diabetic, antioxidant, anti-inflammatory, anticancer and anti-convulsive effects (Fathima et al. 2015, Saravanamuttu and Sudarsanam 2012). This compound was found to be positively associated with grade 1 grapes relative to grade 2, but did not have a strongly weighted regression coefficient in the model relative to coutaric acid and other non-identified grape compounds.

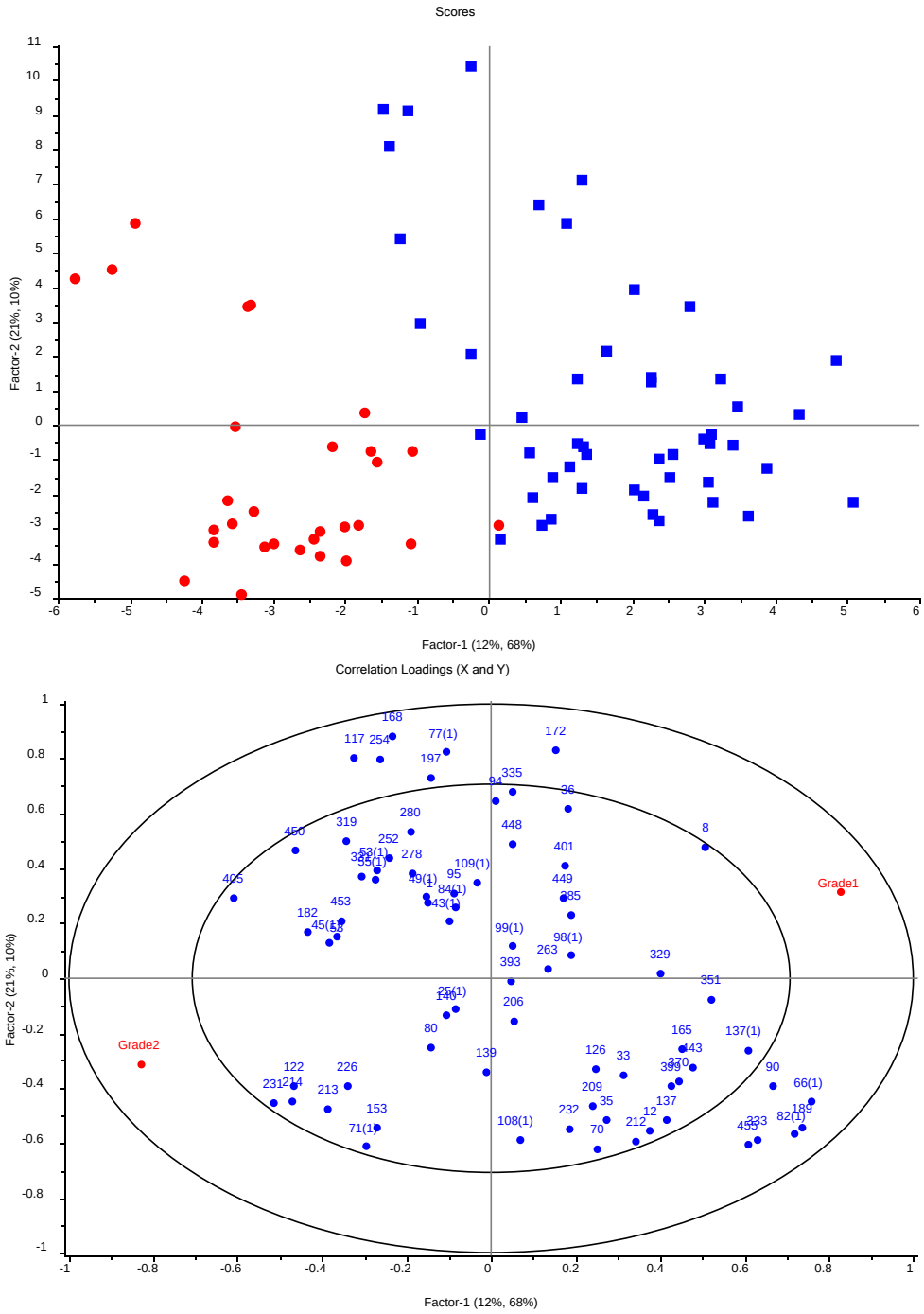


Figure 6.1.2. Prediction of grape grade by partial least squares-discriminant analysis using significant molecular features from grapes and micro-scale wines (shown as their feature ID numbers) identified by an uncertainty test showing A. scores plot and B. correlation loadings of the first two principal components (brackets indicate duplicate molecular features identified in grapes (number only) and wines (followed by the number 1 in brackets).

Table 6.1.2. Tentative identification by mass spectrometry (MS) of grape and wine molecular features shown to be significant in the prediction of grape grade, showing the ID number for each feature, the exact mass (*m/z)* derived from the MS/MS data, match factor and putative identification together with a proposed molecular formula.

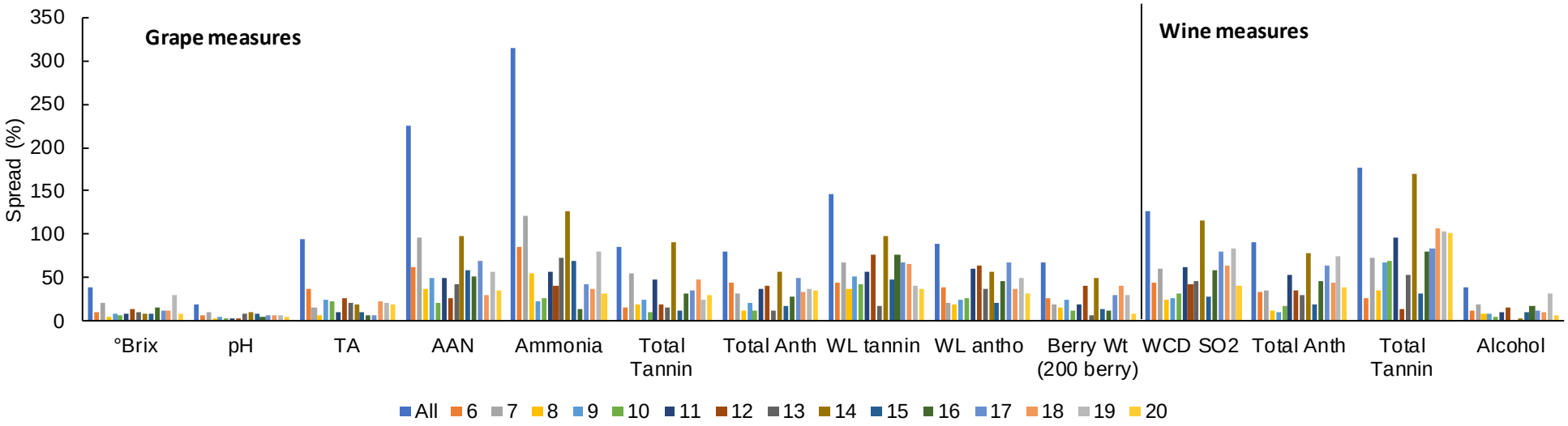
Feature ID	m/z	Retention time	Library match	Match Factor	Proposed Formula[a]
Grape measurement					
G139	295.0459	5.23	Coutaric acid (isomer 1)	99	C13H12O8
G140	295.0442	6.86	Coutaric acid (isomer2)	97	C13H12O8
G1	203.0807	7.98	Tryptophan	97.9	C11H12N2O2
G35	481.0975	23.78	Gossypetin-8-C-glucoside	97.56	C21H22O13
Wine measurement					
W71	157.0492	5.35	3-hydroxysebacic acid[b]	54.6	C7H10O4
W172	197.046	12.41	Ethyl gallate	97.9	C9H10O5
W263	577.1318	13.47	Procyanidin B2	82.62	C30H26O12
W99	509.2192	25.33	Cyanidin-3-glucoside[b]	57.68	C22H38O13

[a] Measured masses were detected as a negative charged adduct [M-H]-, while the proposed formulas refer to the neutral, uncharged, molecule [M]); [b] A partial match indicates a library match where the observed fragmentation pattern was present, but the molecular weight of the compound was different

6.1.3 Variability in grape and wine composition (targeted) for the 2017 samples

Multiple samples were collected from a number of vineyards in the 2017 study which allowed an investigation of within-vineyard variability relative to between-vineyard variability. Using the same PCA discussed previously of the targeted grape and wine analysis (results not shown), the scores plot for the samples (results not shown) was then used to identify vineyards that were relatively homogeneous or more variable (more heterogeneous) in their fruit composition and fermentation outcomes. Another, simpler way to describe this variation is to determine the 'spread' value as [100x(max-min)]/median] for each variable, both between and within vineyards. These results for a sub-set of variables are shown in Figure 6.1.3. It can be seen that certain grape and wine variables had greater variation than others, with nitrogen measures (ammonia, alpha-amino nitrogen, YAN), tannin and anthocyanin varying widely, while °Brix, pH and wine alcohol varied little. The greatest variation was observed for nitrogen measures, and although certain vineyards had greater spread in these measures than others, the variation between vineyards was greater than the within-vineyard variation. For phenolic measures such as grape and wine tannin, as well as wine colour density, within-vineyard variation was as high as that observed between vineyards. This leads to an important conclusion, that the variability within vineyards may potentially be so great that no correlation between grape-based measurements and commercial wine composition can be observed, despite some quality measures in grape and wine being important predictors of grape grade (Figure 6.1.1). The relevance of these observations will be discussed further below, in the context of implications of the project outcomes for commercial wine quality and composition.

Figure 6.1.3. Plot of the calculated spread (%) in the variation among all vineyards (all) and within individual vineyards (6-20) of selected grape and wine parameters (TA, titratable acidity; AAN, alpha-amino nitrogen; Anth, anthocyanin; WL, extractable tannin or colour; WCD SO2, bisulfite-corrected wine colour density).



6.1.4 Quantitative descriptive sensory analysis of the 2017 commercial wines

For the 2017 season, of the 20 vineyards studied, five commercial wines could not be made available for sensory analysis from the producer. For the remaining 15 commercial wines, a preliminary sensory evaluation by a panel of expert technical wine assessors resulted in exclusion of one wine due to excessive oak flavour influence. Fourteen wines were evaluated using QDA, across four commercial quality designations. For grade 1 which represented the highest quality, there were four wines. For the remaining grade categories there were four wines in grade 1.5, three wines in grade 2, and three wines in grade 3.

From the ANOVA to determine whether important differences were found between the wines, 22 attributes rated by the panel differed significantly ($P<0.05$) among the wines (Table 12.1, Appendix 5). These attributes included: 'opacity', 'purple colour', 'brown colour', 'red fruit aroma', 'dark fruit aroma', 'blackcurrant aroma', 'vanilla/chocolate aroma', 'tinned vegetable aroma', 'drain (reduction) aroma', 'tobacco aroma', 'woody aroma', 'stalky aroma', 'pungent aroma', 'red fruit flavour', 'dark fruit flavour', 'blackcurrant flavour', 'woody flavour', 'tobacco flavour', 'viscosity (body)', 'astringency', 'hotness', 'bitterness' and 'fruit aftertaste'. Four attributes: 'coconut aroma', 'pepper aroma', 'sweet spice aroma' and 'vanilla/chocolate' flavour were close to being significant ($P<0.10$). There were significant judge by wine interaction effects for 16 attributes, reflecting some inconsistent use of these terms by judges (see Table 12.1 in Appendix 12).

The PCA for the wines (Figure 6.1.4) was created using the 26 attributes that were significantly different ($P<0.05$) and close to significant ($P<0.10$) across the 14 Shiraz wines. PCs 1, 2 and 3 (not shown) explained 47.0% 18.0% and 9.4% of the variance in the data, respectively. Wines plotted to the far right of the Figure were rated highly in those attributes highly positively loaded on PC1, notably 'brown colour', 'tinned veg aroma', 'drain (reductive) aroma', 'stalky aroma' and 'red fruit flavour', and these wines were all in the quality category 3. Conversely, wines plotted to the left of the figure were rated lower in these attributes, and highly in the attributes highly negatively loaded on PC1, notably 'opacity', 'purple colour', 'dark fruit aroma', 'vanilla/chocolate aroma', 'woody aroma', 'dark fruit flavour', 'vanilla/chocolate flavour', 'woody flavour' and 'viscosity'. The grade 1 wines were all plotted to the left of Figure 6.1.4, with a more dispersed distribution for grades 1.5 and 2. Wines plotted higher on the vertical axis (PC2) were rated highly in 'blackcurrant' aroma and flavour, opposed to wines plotted low on the figure which were rated highly for 'tobacco' aroma and flavour and 'drain' aroma. A third PC describing a further 9.3% of the variance was also relevant in separating the wines on the basis of the attributes 'coconut aroma' and 'fruit aftertaste' (positive) or by 'pungent aroma', 'pepper aroma' and 'hotness' (negative), but did not contribute to bring a clear discrimination of the wines according to their grades (results not shown).

Based on this assessment, it was interesting to observe that although the vineyards selected for the study were only from premium grape grades 1 and 2, it was evident after the commercial winemaking process was completed that the vineyard grading system did not closely reflect the wine grade assigned by the winemaker panel. In light of this finding, it was also deemed relevant to the goals of the project to determine whether grape or wine chemical measures might be useful in predicting the final wine grade. However, because there was insufficient separation of the quality grades based on wine sensory attributes, it was considered more likely that a model could be developed to predict which batches of grapes graded 1 and 2 would not result in premium quality wine (i.e. would end up at wine grade 3) rather than to predict each wine grade category.

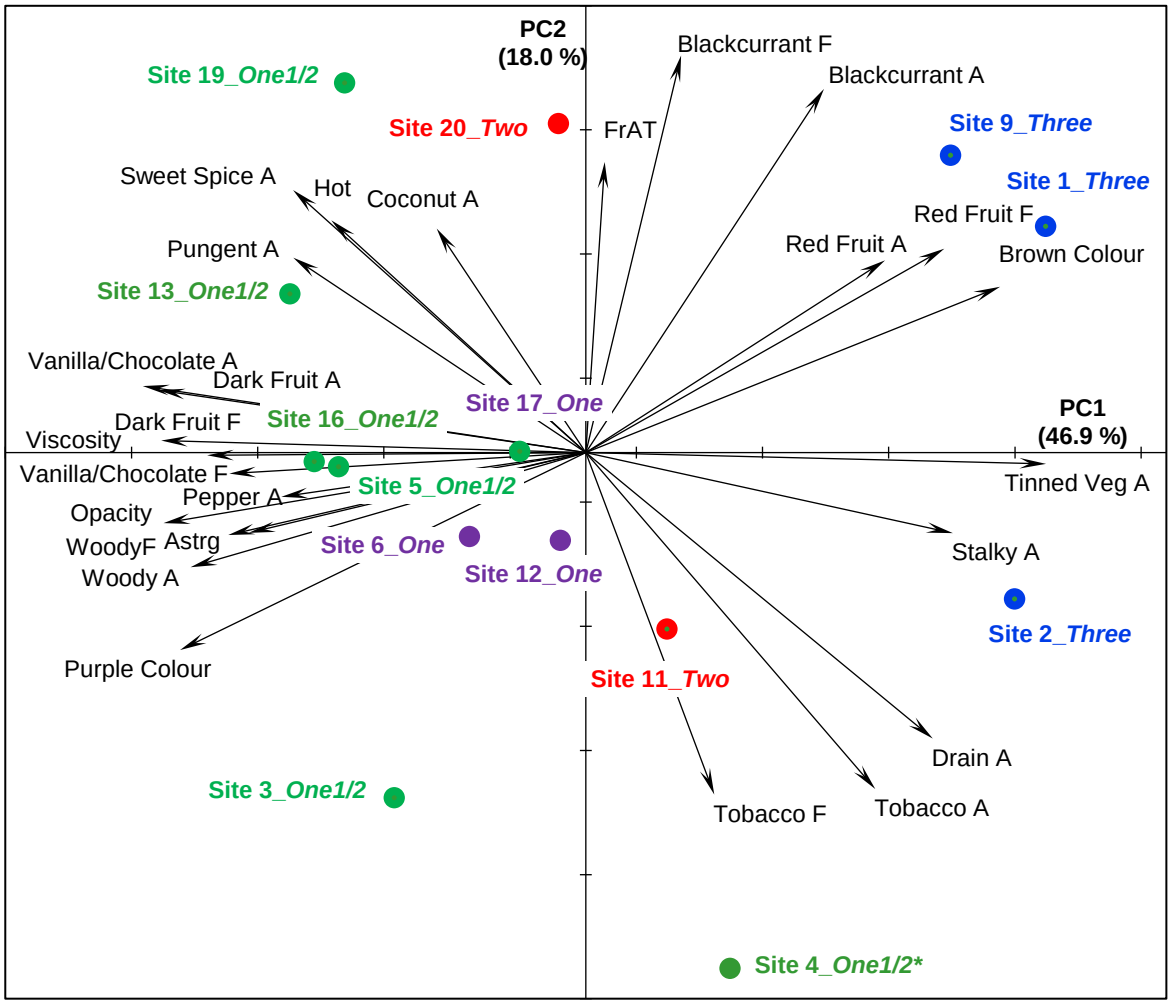


Figure 6.1.4. Principal component biplot of significant (P<0.05) and close to significant sensory attributes (P<0.10) based on principal components 1 and 2 for the Shiraz wines. Different colours represent the industry partner's commercial wine quality category designation, with purple representing grade 1, green representing grade 1.5, red representing grade 2 and blue representing grade 3. (A: Aroma, F: Flavour, *Site 4 was later classified as grade 3 for blending and bottling).

6.1.5 Prediction of wine sensory properties (2017) from the compositional analysis of commercial wines.

Using the analytical data generated for the commercial wines, PLSR models were developed to predict the sensory properties described in 6.1.4. Of the 26 sensory attributes which were different among the wines, only eight variables were well-modelled by the analytical data generated for the wines, where the R^2 of validation was greater than 0.58. Of these variables, a sub-set of the analytical data was identified using an uncertainty test which could be used to successfully predict the sensory results, either improving or maintaining the R^2 of calibration and validation from the original model. These R^2 results are shown in Table 6.1.5 and exclude only one sensory variable, 'red fruit' flavour, which was defined by variables other than those identified by the uncertainty test and will be discussed independently. The weighted regression coefficients for each significant attribute are shown in Figure 6.1.5.1.

Of the seven sensory variables predicted by the sub-set of analytical data, the appearance attributes 'opacity', 'purple colour' and 'brown colour' were best modelled by PLSR. The attributes 'opacity' and 'purple colour' tended to correlate in the PLSR model, as found in the PCA (Figure 6.1.4), and were

defined similarly by the wine compositional measures. The highest positive regression coefficients for 'opacity' and 'purple colour' were the degree of anthocyanin ionisation, tannin concentration, the proportion of epicatechin-gallate in the tannin (as both extension and terminal unit), and the tannin molecular mass by GPC (Figure 6.1.5.1). Generally, these wine sensory attributes were associated with a lower wine hue, which was not unexpected. Interestingly, and contradictory to what is observed in the literature, wine colour density was not highly weighted in the PLSR model. In terms of the significant wine fermentation products identified by the PLSR model, 'opacity' and 'purple colour' were variably predicted by these, and had lower regression coefficients than some of the important aroma and flavour variables, and were therefore considered not to be of importance (Figure 6.1.5.1 B). It was found that the 'brown colour' attribute tended to correlate negatively with those of 'opacity' and 'purple colour', as shown by the PCA (Figure 6.1.5.1 A, Figure 6.1.4). It was therefore not surprising that 'brown colour' was strongly predicted by the same variables defined for 'opacity' and 'purple colour', but with negatively weighted regression coefficients.

Table 6.1.5. R-squared of calibration and validation determined for the prediction of wine sensory attributes by PLS from a sub-set of significant wine (commercial) and grape analytical variables identified by an uncertainty test (A, aroma; F, flavour; four principal components for wine model; two principal components for grape model).

	Commercial wine		Grape	
Sensory attribute	R^2_{cal}	R^2_{val}	R^2_{cal}	R^2_{val}
Opacity	0.92	0.85	0.86	0.79
Purple Colour	0.82	0.74	0.79	0.64
Brown Colour	0.81	0.72	0.79	0.62
Dark Fruit A	0.82	0.71	0.64	0.40
Tinned Vegetable A	0.79	0.58	0.73	0.50
Dark Fruit F	0.84	0.65	0.76	0.61
Astringency	0.70	0.65	-	-

The 'astringency' attribute was reasonably well-modelled by PLSR, yielding high positive regression coefficients for total phenolics, tannin concentration and tannin molecular mass by gel permeation chromatography. Interestingly, the 'astringency' attribute had higher positive regression coefficients for wine colour density than were observed for the 'opacity' attribute. Similarly to 'opacity' and 'purple colour', the astringency attribute was defined by higher contribution of epicatechin-gallate as part of the tannin compositional subunits, both as a terminal and an extension unit. This may possibly indicate a greater contribution of seed tannin in more astringent wines, but this could also be due to oenotannin addition as a commercial practice. Some association of fermentation products and 'astringency' was found by PLSR (Figure 6.1.5.1 B), but did not exhibit high regression coefficients relative to the other flavour and aroma attributes defined as significant by the model.

Three flavour and aroma attributes were also reasonably well-modelled by the PLSR, demonstrating high regression coefficients for both phenolics measures and fermentation products (Figures 6.1.5.1 A and 6.1.5.1 B).Generally, 'dark fruit' flavour and aroma were co-correlated in the model, as found for the PCA, and were defined by similar wine compositional attributes. 'Tinned vegetable' aroma was negatively related to the 'dark fruit' attributes, and was predicted by the same wine compositional factors, albeit oppositely loaded in the model. 'Dark fruit' attributes were positively defined by higher contributions of 2-methylbutanol and negatively by hexyl acetate and 3-methylbutyl acetate. Although ethyl propanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate have been previously implicated in 'dark fruit' aroma by reconstitution studies (Pineau et al 2009), only ethyl 2-methylbutanoate was positively associated with 'dark fruit' aroma.

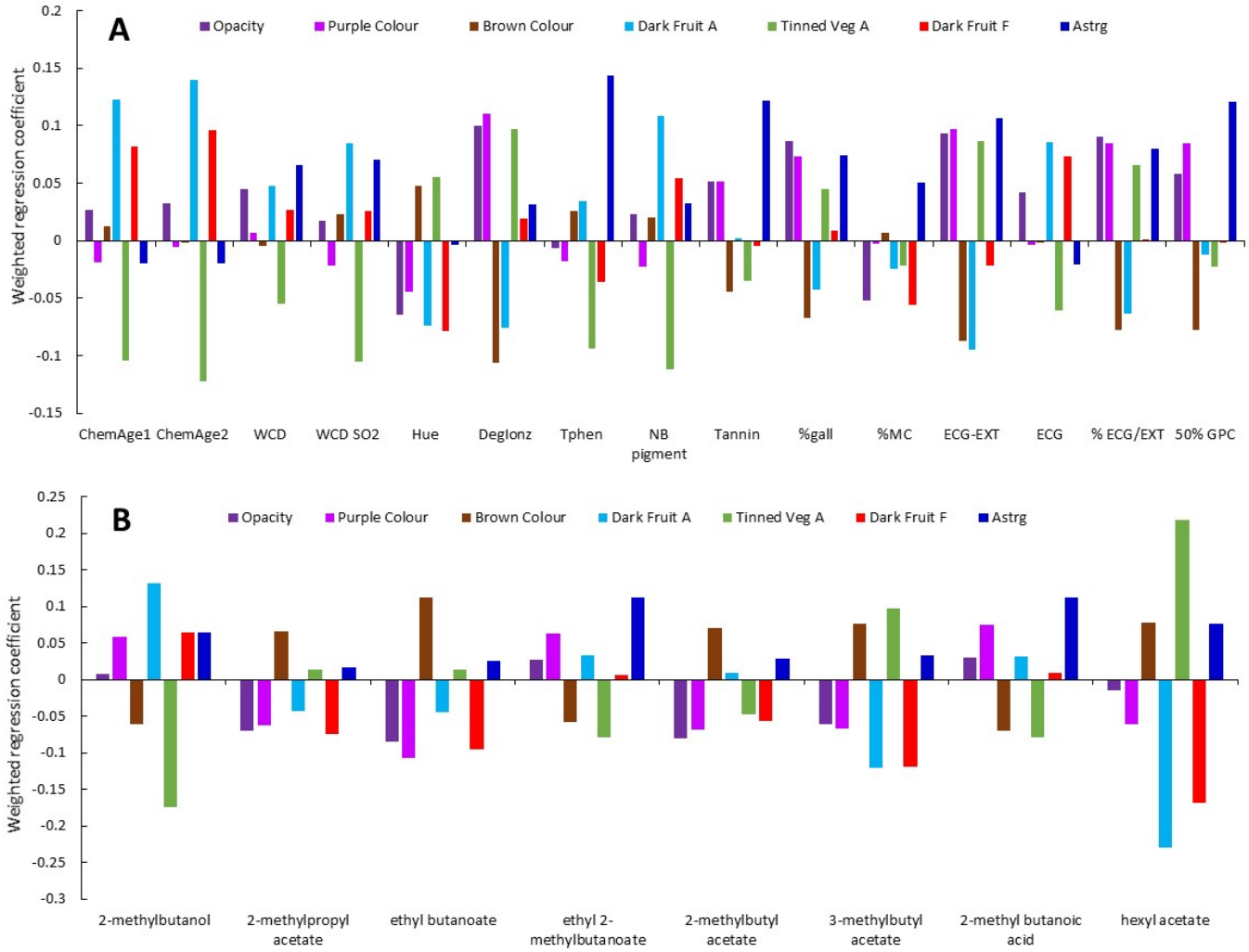


Figure 6.1.5.1. Weighted regression coefficients for the prediction of sensory attributes from targeted analysis of commercial wines showing A. phenolic composition, B. fermentation products (A, aroma; F, flavour; Astrg, astringency; ChemAge, chemical age; WCD, wine colour density; WD SO2, bisulfite-resistant wine colour density; Degionz, degree of anthocyanin ionisation; Tphen, total phenolics; %gall, % tannin galloylation; %MC, % conversion of tannin to subunits by phloroglucinolysis; ECG, epicatechin-gallate; EXT, extension unit; 50% GPC, molecular mass average by gel permeation chromatography).

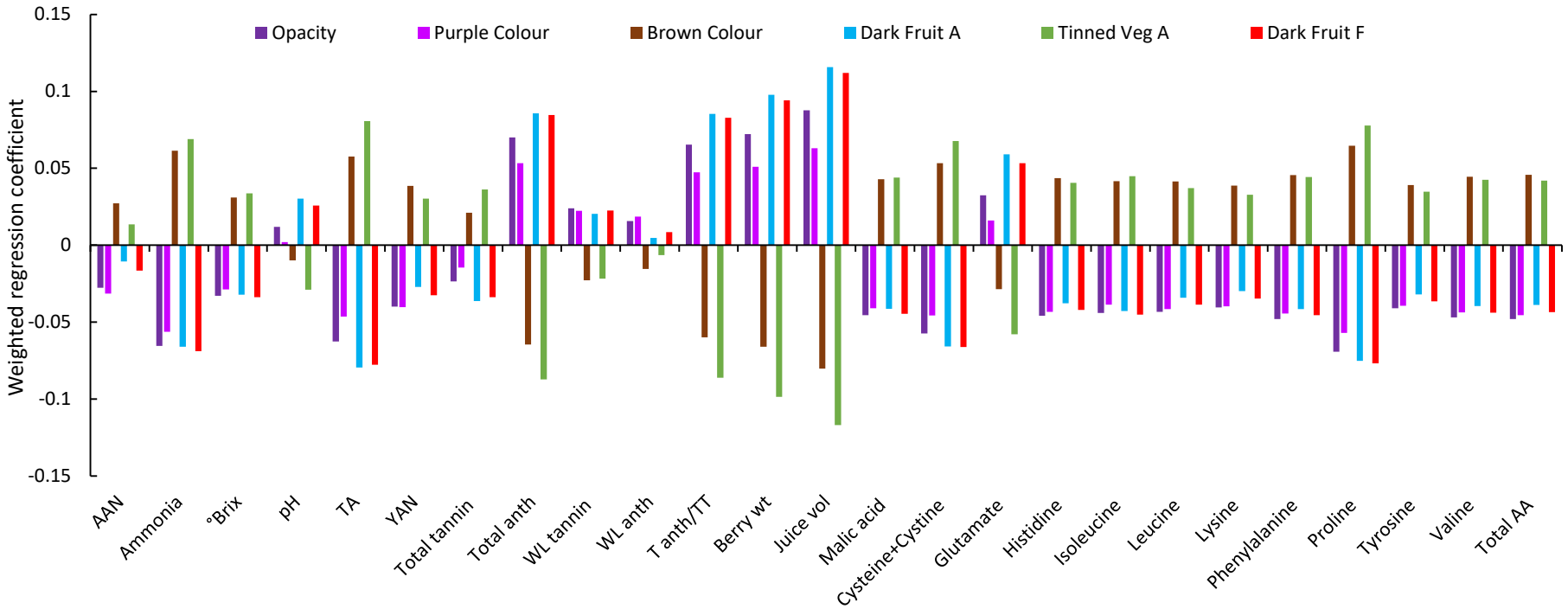


Figure 6.1.5.2. Weighted regression coefficients for the prediction of sensory attributes from targeted analysis of grapes pooled across vineyards, variables with weaker regression coefficients were included in the analysis but removed from the figure (A, aroma; F, flavour; TA, titratable acidity; Anth, anthocyanin; WL, extractable tannin or colour; T anth/TT, total anthocyanin to total tannin ratio; A, amino acids).

Conversely, hexyl acetate had the highest regression coefficient in the PLSR model. Hexyl acetate has been previously described as contributing to 'red berry' aromas in wine (Forde et al. 2011) but its corresponding alcohol hexanol contributes to attributes described as 'green, fruity, sweet, fatty, fresh, apple, pear'. The 'red fruit' aroma attribute was not well modelled by the wine chemical data, but the 'red fruit' flavour attribute could be predicted using wine compounds additional to the model presented here (R^2 validation = 0.65, data not shown), including ethyl acetate and butanoic acid, which both had high positive regression coefficients. However, hexyl acetate also had a high positive regression coefficient for the prediction of 'red fruit' flavour, and was negatively associated with 'dark fruit' aroma and flavour as also shown in the PCA (Figure 6.1.4). Interestingly, the 'tinned vegetable' aroma attribute which was positively correlated with 'red fruit' attributes in the PCA was predicted by PLSR with positive regression coefficients for hexyl acetate, and was negatively associated with 2-methylbutanol (Figure 6.1.5 B).

For the prediction of sensory attributes from the targeted grape chemical analysis, a poorer model with weaker R-squares of predictions was obtained than observed for the model developed using the commercial wine data (Table 6.1.5). However, similar to what was found for the PLSR model from wine data, the sensory properties 'opacity', 'purple colour' and 'brown colour' were better predicted using the grape data. The regression coefficients for the prediction of sensory attributes from grape data are shown in Figure 6.1.5.2.

It was evident that the berry size variables (berry weight and juice volume) had the strongest regression coefficients in the PLSR model, being positively associated with 'opacity', 'purple colour', 'dark fruit' attributes, and negatively associated with 'brown colour' and 'tinned vegetable' aroma. This was surprising, considering that smaller berry size is commonly perceived to be positively associated with wine quality within the wine industry at large, although this notion is not necessarily supported by the literature (Walker et al. 2005). Beyond berry size measures, total grape colour and a calculated grape anthocyanin to total tannin ratio were positively associated with 'opacity', 'purple colour' and 'dark fruit' attributes, while showing a negative relationship negative with 'tinned vegetable' aroma and 'brown colour'. Total grape tannin had the opposite association with these sensory properties, but had a weak negative regression coefficient in the PLSR model, as did the extractable measures of tannin and anthocyanin.

To relate this observation back to the results described above from the PLSR model developed from the commercial wine analysis, 'tinned vegetable' aroma and 'brown colour' attributes (Figure 6.1.5 A) were associated with wines with lower total wine tannin. The observation that 'astringency' was not well predicted by the PLSR model for grape attributes (Table 6.1.5) as well as was from the commercial wine analysis (Figure 6.1.5A) was likely due to addition of oenological tannins by the producer to all of the commercial wines. This is further corroborated by results from micro-ferments which demonstrated that extractable grape tannin and wine tannin from micro-ferments were correlated (R^2=0.72 pooled across vineyard samples), but were only weakly related to wine tannin concentration in the commercial wines. This effectively rendered the measurement of tannin in grapes or micro-ferments of less relevance when relating these values to the finished commercial product. From the current results, it would appear that a higher grape anthocyanin to tannin ratio may result in meaningful differences in the visual appearance of the wines, independent of oenological tannin additions, which might have greater implications for astringency.

A further important observation from the PLSR model for grape analysis and wine sensory properties was that a number of nitrogen measures, namely ammonia, total amino acids, YAN and multiple amino acids including proline were positively associated with 'tinned vegetable' aroma and 'brown colour' in the commercial wines. On the other hand, these same measures were negatively associated with 'opacity', 'purple colour' and 'dark fruit' attributes. This would suggest that excess fermentable nitrogen in grapes, in particular ammonia and certain amino acids, may have contributed to the development of

'tinned vegetable aroma' and 'brown colour' in the corresponding wines. However, a direct association of nitrogen measures with browning remains unclear and might warrant further research.

6.1.6 Prediction of commercial wine grade from targeted grape and wine analysis in 2017.

As outlined above, it was evident that a number of grape parcels did not produce wines with the expected specifications for the premium 'intended use' category after the completion of commercial winemaking. A number of PLS-DA models were therefore developed to predict the final commercial wine grade using the different datasets generated. Using these various datasets, it was found that none of the models could effectively discriminate wine grades 1, 1.5 and 2 from one another, but they could strongly predict wine grade 3, with up to 75% accuracy depending on the type of data used (Table 6.1.6).

Using the targeted grape data generated from the grape grade study, the wine grade 3 category could be predicted with an accuracy of 73%. It was found that grape batches which were otherwise of 'intended use' grape grades 1 and 2 but were downgraded to category 3 based on wine assessments had higher levels of grape nitrogen, including ammonia, total amino acids, total YAN and proline (Figure 6.1.6 A). Multiple amino acids, and also their total concentration including with proline, had positive regression coefficients in the prediction of wine grade 3. Interestingly, grape total soluble solids (°Brix), TA and malic acid (highest regression coefficient in the PLS-DA), also had positive regression coefficients for the prediction of grade 3 wines. Sugar and acidity are basic compositional measures already used in the wine industry; this finding indicates that they are nonetheless relevant to the definition of grade.

Table 6.1.6. PLS regression model outputs for the prediction of wine grade 3 from targeted and non-targeted analytical data of grapes, micro-ferments and commercial wines, showing the optimal number of principal components (PC no) required for the model, the R^2 coefficients of calibration (R^2_{cal}) and validation (R^2_{val}), and the root mean squared error of calibration ($RSME_{cal}$) and validation ($RSME_{val}$).

Analysis Type[a]	Data selected	PLS-DA model parameters				
		PC no	R^2_{cal}	R^2_{val}	$RMSE_{cal}$	$RMSE_{val}$
Targeted grape analysis	all data	4	0.75	0.72	0.18	0.19
	sig var[b]	2	0.73	0.73	0.19	0.19
Targeted grape and wine[c] analysis	all data	3	0.73	0.67	0.19	0.21
	sig var	3	0.74	0.71	0.18	0.20
Non-targeted grape non-volatiles	all data	3	0.56	0.49	0.24	0.26
	sig var	3	0.59	0.54	0.23	0.25
Non-targeted wine[c] non-volatiles	all data	5	0.77	0.47	0.26	0.31
	sig var	2	0.74	0.71	0.19	0.20
Non-targeted grape and wine[c] non-volatiles	all data	7	0.84	0.72	0.15	0.20
	sig var	2	0.78	0.75	0.17	0.19
Targeted and non-targeted analysis of grape and wine[c], sig var	all data	2	0.78	0.74	0.17	0.18
	sig var	2	0.78	0.74	0.17	0.18
Commercial wine[d] targeted analysis	all data	1	0.70	0.54	0.24	0.26
	sig var	1	0.59	0.49	0.23	0.30
Commercial wine[d] targeted analysis and sensory	all data	1	0.74	0.63	0.21	0.27
	sig var	1	0.68	0.62	0.23	0.27
Commercial wine[d] sensory analysis only	all data	1	0.78	0.71	0.19	0.24
	sig var	1	0.72	0.68	0.21	0.25
Grape juice mid-infrared[e] data	all data	3	0.69	0.59	0.19	0.22
	sig var	2	0.62	0.58	0.21	0.23
Homogenate mid-infrared data	all data	6	0.66	0.35	0.19	0.27
	sig var	-	-	-	-	-
UV-vis scan from grape homogenate extract	all data	5	0.48	0.39	0.26	0.29
	sig var	-	-	-	-	-

[a] Model results from near-infrared data of homogenate are not shown; [b] Sig var indicates a sub set of significant variables selected using an uncertainty test and high correlation loadings; [c] Micro-ferment wine data; [d] Commercial wine data; [e] Savitzky-Golay transformed

Grape phenolics measures were also found to be significant in the prediction of the grade 3 wine category. Grapes that produced grade 3 wines had higher total and extractable tannin than those that produced better wine quality grades, but had a lower anthocyanin to tannin ratio (Figure 6.1.6 A). In interpreting this result, it is important to note that there was a wide distribution of total and extractable tannin (and associated wine tannin) among the grape samples, being both high and low in grades 1, 1.5 and 2. As a result, the model indicated that while extractable and total tannin were always high in grapes that produced wine grade 3, there was a lower relative contribution of grape-derived anthocyanin. It would therefore appear that the lack of sufficient anthocyanin, in the presence of high grape-derived tannin, may have affected the final wine quality outcome, as previously discussed for the sensory results.

From the PLS-DA models developed for the prediction of wine grade 3, the inclusion of micro-ferment data did not significantly improve the R^2 of calibration and validation (Table 6.1.6), contrary to what was previously observed for the grape grade study. These models will therefore not be discussed further in this report. Furthermore, the targeted analysis of the commercial wines also had less predictive capacity for wine grade 3 than the other data types, which was surprising. The commercial wine sensory data performed better in the predictive model than did the targeted data, but not as well as the targeted grape analysis. The PLS-DA results for the wine sensory data alone are shown in Figure 6.1.6 B, and showed that wines from grade 3 were characterised by a higher 'brown colour', 'tinned vegetable aroma', and 'red fruit' attributes than grades 1-2. The lower grade wines also had a lower contribution of 'opacity', 'purple colour', 'dark fruit' attributes, 'woody flavour' and 'astringency'.

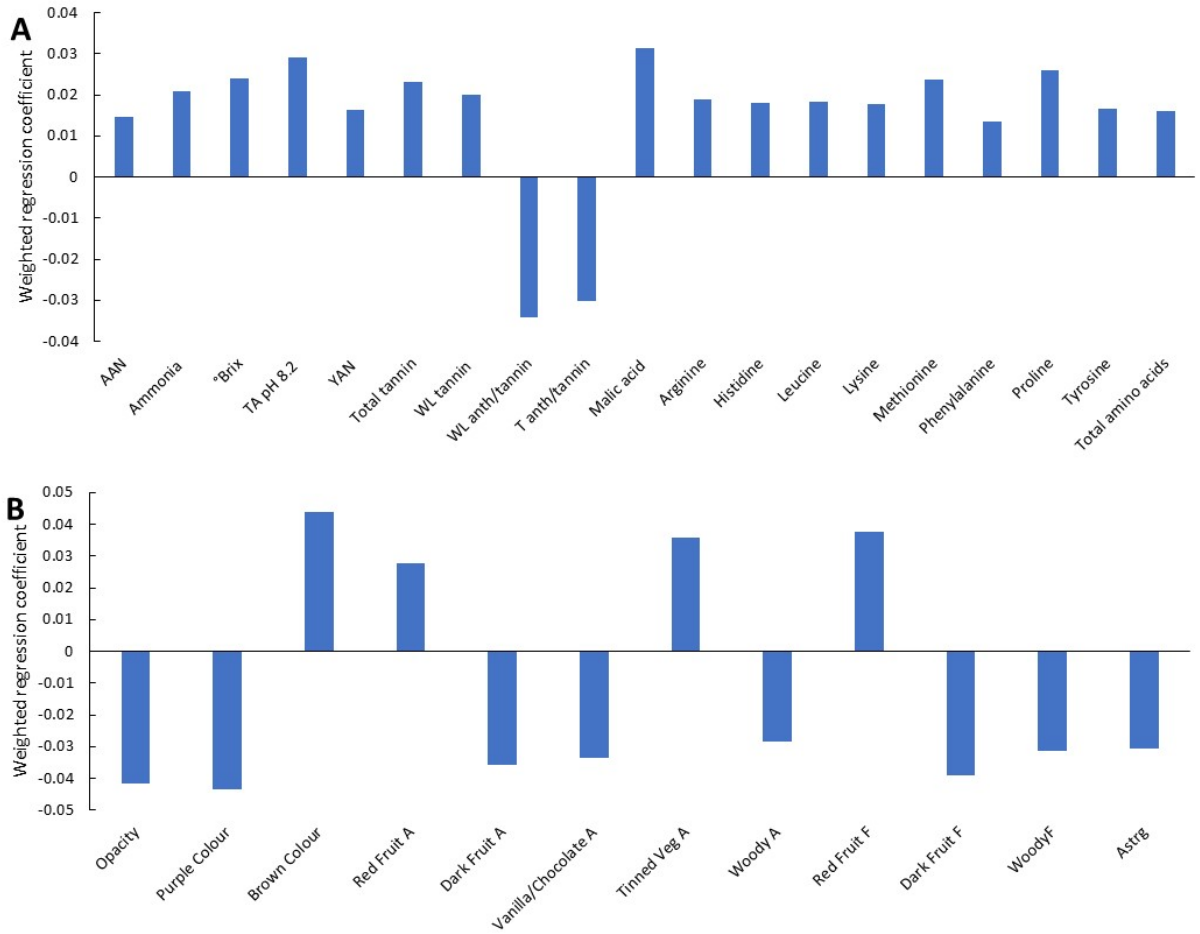


Figure 6.1.6. Weighted regression coefficients for the PLS-DA model to predict wine grade 3 using A. targeted grape analysis, B. commercial wine sensory analysis, showing significant predictors identified using an uncertainty test or high correlation loadings (A, aroma; F, flavour; AAN, alpha amino nitrogen; TA, titratable acidity at pH 8.2 end-point; YAN, yeast assimilable nitrogen; anth, anthocyanin, WL, extractable grape tannin or colour and associated ratio; T anth/tannin, ratio of total grape anthocyanin to tannin; Astrg, astringency).

Considering that there was no clear definition of the wine categories 1, 1.5 and 2 based on the preliminary PCA analysis, it was not surprising that only grade 3 wines could be successfully predicted by the PLS-DA model. The PLS-DA results were in strong agreement with the sensory study, but also pinpointed some associated grape-based variables which may contribute to losses of quality during the commercial winemaking process, leading in particular to 'brown colour' and 'cooked vegetable' attributes, with important losses of 'dark fruit' aroma and flavour. These will be further discussed in section 6.1.8 as well as in the conclusions section of this report.

6.1.7 Prediction of commercial wine grade from non-targeted analysis in 2017.

As for the grape grade study, non-targeted measures of grape homogenate and juice using MIR, a UV-visible scan of homogenate extracts (Table 6.1.6) and NIR scans of homogenate (not shown) could not produce prediction models with R^2 of greater than 0.59. This was a surprising result considering that these techniques were previously found to be successful in the prediction of Shiraz grade, albeit from a wider range of quality categories than studied in the current project (Bindon et al. 2017a, Bindon et al. 2017b). On the other hand, the non-targeted LC-MS profiling approach was able to reproduce the prediction capacity of the PLS-DA using targeted grape analysis, when a combination of significant grape and micro-ferment variables were used (R^2 = 0.75). This observation points towards a potential contribution of grape compounds which are not detected or not well detected by spectral scans. The model based on non-targeted LC-MS profiling was not improved further when targeted grape and wine analysis was included in the PLS-DA and will therefore be discussed separately. The maximal variance of the PLS-DA model was explained by the first two PCs. From the model it was evident that wine grade 3 samples were primarily defined by PC1, with a greater distribution of grades 1, 1.5 and 2 observed over PC2. Using the key molecular features detected by LC-MS and the PLS-DA, further structural elucidation was performed using mass spectrometry.

Table 6.1.7. Putative identification of grape and wine molecular features shown to be significant in the prediction of wine grade 3 by high-resolution mass spectrometry (MS) showing the ID number for each feature, the m/z intensity values derived from the MS/MS fragmentation pattern, derived match factor and putative identification with a proposed molecular formula.

Feature ID	mz	Retention time	Library match	Match Factor	Proposed Formula[a]
Grape measurement					
G128	331.0669	2.88	Gallic acid[b]	54.6	C13H16O10
G20	451.1258	12.31	Epicatechin[b]	80.5	C21H24O11
G30	289.0971	18.34	Epicatechin	95.9	C15H14O6
Wine measurement					
W128	169.0142	1.81	Gallic Acid	96.1	C7H6O5
W201	577.1318	14.97	Procyanidin B1	82.57	C30H26O12
W289	289.0729	18.95	Epicatechin	96.6	C15H14O6

[a] Measured masses were detected as a negative charged adduct [M-H]-, while the proposed formulas refer to the neutral, uncharged, molecule [M]); [b] A partial match indicates a library match where the observed fragmentation pattern was present, but the molecular weight of the compound was different

Of the four grape and 40 wine (micro-ferment) molecular features identified by PLS-DA as having a significant predictive capacity for grade 3 wines, 6 could be tentatively identified by mass spectrometry and are shown in Table 6.1.7, all of which had high positive regression coefficients for grade 3 prediction. For both grapes and wines, epicatechin was putatively identified, while gallic acid was found to be important for grape samples, and procyanidin B1 for wines. All of the identified phenolics are commonly associated with grapes and wines, and can be analysed by HPLC with diode array detection in a research laboratory setting. However, their use as quality markers for grapes and wines is currently limited to relatively few laboratories focusing on wine, with limited information about their role as potential quality markers in the current literature. Inclusion of the measurement of these common

phenolics in future studies on quality, and their incorporation into a standard phenolics analysis repertoire will shed further light on their relevance as grape and wine quality markers.

6.1.8 General summary of the 2017 results and project plan for the second season

The results of the first (2017) season of the study highlighted some important new knowledge related to both grape and wine quality. The grape grade study showed that predictive models could be built for the 'intended use' vineyard quality categories. However, these required micro-scale ferment data as well as a number of non-targeted phenolic variables (many of which were not identified by mass spectroscopy) to be effective. It was therefore considered unlikely based on the first season of the results that meaningful models could be developed for use in industry by this approach. Moreover, the observation that a number of fruit parcels in the 'intended use' grape grades 1 and 2 did not produce commercial wines in the expected wine grade category highlighted that more effective models could be based on the final wine outcome. From the models developed in 2017 for the prediction of wine grade, it was evident that simple grape-based measures could potentially be identified to predict whether a batch of fruit would meet the requirements for the premium quality designation (grades 1, 1.5 and 2) or should be more appropriately assigned to producing a lower wine grade category (grade 3). The objective measures identified as being of relevance were primarily the grape anthocyanin/tannin ratio and grape nitrogen measures.

An important observation in the first season of the study was that large within-vineyard variability was found for the measurement of grape nitrogen (ammonia and amino acids) and phenolics (extractable and total tannin and colour). For phenolics, the within-vineyard variability in some cases exceeded the variability found between vineyards. Since both classes of compounds were relevant to the prediction of wine grade, it was considered that significant within-vineyard variation might contribute to final wine quality outcomes, in particular when a vineyard is harvested as a whole. In other words, heterogeneous vineyards with a wider distribution of grape quality within the vineyard could be expected to offer less predictive certainty for commercial wine quality outcomes. As such, there was a clear need to better understand within-vineyard variability and its relationship to wine quality outcomes in the second year of the study.

6.2 Results of the 2018 season

6.2.1 Understanding within- and between-vineyard variability in the 2018 season

The original research plan stipulated a review point in the second season with a decision pending as to whether to repeat the first year of the study, or to study variability within one single vineyard. To gain an understanding of wine quality outcomes across multiple vineyard blocks it was therefore decided in the second season of the study to reduce the number of vineyards studied from 20 to 10, but to increase the sample number per vineyard from 5 to 10. Some of the vineyards were retained between the two seasons of the study, but certain vineyards identified as being of poorer quality were discontinued. In addition, two of the vineyards had been initially classified as being of 'intended use' grade 2, but historically performed variably over many seasons and the associated wines had been frequently downgraded in terms of final wine quality category. An assessment of vineyard metrics such as yield, cordon length and canopy density indicated that these two sites had generally higher vigour than the others included in the study. These vineyards were designated 'grade 3' for the purposes of the experiment, while the remaining eight vineyards were distributed evenly between grades 1 and 2.

To obtain an overview of the within-vineyard variability for the targeted grape objective measures, the spread was determined as a percentage value (maximum-minimum)/median for each vineyard block and is shown in Table 6.2.1. In addition, the quartile (25^{th}-75^{th} quartile average/median) data was used to remove the effect of outliers on the measure of variation. From Table 6.2.1 it can be seen that the greatest variability for all vineyards was found in nitrogen measures, as both ammonia and alpha-amino nitrogen (summed as YAN). Looking at the spread values, the grapes from Block 3 presented the highest variability in alpha ammonia nitrogen concentration; however, this was found to be due to the contribution of one outlier (data not shown). Once this outlier was removed from the calculation, Block 3 was actually more uniform than Block 2, and this was confirmed by the quartile result, which was lower for Block 3 than Block 2. Block 8 had the smallest spread value, which meant that the grapes

were more uniform in alpha amino nitrogen concentration than the other nine vineyards. Block 6 also had one outlier sample which increased the spread result, whereas it was otherwise quite uniform.

Based on the ANOVA, there were significant differences in grape alpha amino nitrogen concentration among the 10 vineyards, especially between blocks 1 and 9. The average alpha amino nitrogen level in Block 9 was significantly higher than in the other vineyards, while Block 1 was mostly lower. The ammonia concentration followed a similar trend in terms of spread, and also between blocks, as seen for alpha amino nitrogen. As a result, YAN showed a similar distribution for both of its components (data not shown). According to Table 6.2.1, Block 10 was the most variable vineyard in grape ammonia concentration with the highest spread value, and Block 8 had the least spread. There appeared to be less influence of outliers in the spread values for ammonia, with the exception of Block 5, which expanded the spread value. With the outlier removed, Block 5 would in fact have the most uniform grape ammonia concentration of the ten vineyards. Furthermore, significant differences among vineyards in ammonia concentration were found using ANOVA and, as discussed before, the trend was analogous to that shown for alpha amino nitrogen.

After nitrogen measures, the analysis of extractable (wine-like, WL) colour and tannin showed the greatest variability within individual blocks. Blocks 2 and 4 (colour only) had single-sample outliers that increased the spread, but had lower values when the ratio of the quartile to the median was compared. However, for Blocks 8 and 9, a similar spread was found for WL colour and tannin, but the measure was not influenced by outliers, hence having higher % quartile values than Blocks 2 and 4. In this case, the spread reflects a true heterogeneity within these vineyards. Most of the vineyard blocks with the exception of 8 and 9 were relatively homogenous for extractable tannin and colour measures. From the ANOVA results, Block 6 had a higher average WL tannin concentration than the other vineyards overall, while Block 10 had the lowest. Although Block 9 had locations with some of the highest extractable grape tannin concentrations found, the large variability of this vineyard meant that it was equivalent to Blocks 1, 5 and 6. Grape WL anthocyanin concentration had a positive relationship with homogenate (total) grape anthocyanin concentration (result not shown), with an $R^2 = 0.72$. Other studies (Bindon et al. 2014) have shown stronger relationships between extractable and total anthocyanin in grapes but it was interesting to note that total anthocyanin was very high, at approximately 2 mg/g for all blocks, possibly reflecting the premium quality designation of these vineyards. The variability in total anthocyanin between blocks was therefore low. However, Blocks 1 and 8 had significantly different grape anthocyanin concentrations while Block 9 had the greatest variability in WL anthocyanin concentration. Block 6 was the most uniform among the vineyards in terms of total anthocyanin. Comparatively, the rest of the vineyards were relatively homogenous for total anthocyanin, as discussed. Total tannin (homogenate extract) in grapes was not well correlated with extractable tannin, contrary to other studies (Bindon et al. 2014). As for total anthocyanin, total tannin in the samples studied was far higher than observed previously from a range of locations, at ≥ 6 mg/g. The concentrations of both anthocyanin and tannin in these samples were therefore at the upper limit of what has been previously observed (Bindon et al. 2014). This could be due to seasonal differences, but could also be because of the narrow quality grade studied and the location (Barossa). According to the spread values in Table 6.2.1, Block 3 grapes had the most variable grape homogenate tannin concentration, and Block 6 was the most homogenous for this measure. Notwithstanding, Block 1, 2 and 5 had relatively high spread values, but this was due to outliers and these were otherwise homogenous according to the quartile values. Block 1 generally had higher total grape tannin concentration than the other blocks while Block 5 had the lowest, again following a different trend to that observed for extractable tannin.

Table 6.2.1. Targeted objective measures of grape composition taken for 10 samples across 10 blocks in the 2018 season, showing significant differences for the means (ANOVA; P<0.0001, post-hoc T-test) as different letters, with italicised indices of variation shown as the coefficient of variation, spread and quartile (25-75%)/median as percentages.

Grape compositional measure	Block	1	2	3	4	5	6	7	8	9	10
°Brix	Mean	24.9cd	24.7d	23.1e	24.3d	26.0b	27.1a	25.7bc	24.9cd	26.5ab	25.9b
	Coeff. of variation (%)	*4*	*3*	*4*	*3*	*1*	*1*	*7*	*6*	*4*	*3*
	Spread (%)	*10*	*15*	*9*	*22*	*7*	*11*	*12*	*17*	*11*	*17*
	Quartile/median (%)	*4*	*3*	*4*	*3*	*1*	*1*	*7*	*6*	*4*	*3*
pH	Mean	3.92f	4.03e	4.08de	3.90f	4.16bcd	4.34a	4.26ab	4.07de	4.21bc	4.12cde
	Coeff. of variation (%)	*3*	*3*	*1*	*3*	*3*	*2*	*2*	*5*	*5*	*3*
	Spread (%)	*5*	*8*	*6*	*14*	*4*	*8*	*5*	*9*	*14*	*12*
	Quartile/median (%)	*3*	*3*	*1*	*3*	*3*	*2*	*2*	*5*	*5*	*3*
Berry wt (g)	Mean	1.09	1.14	1.14	1.20	1.11	1.19	1.17	1.20	1.10	1.23
	Coeff. of variation (%)	*21*	*7*	*15*	*16*	*9*	*3*	*18*	*9*	*25*	*11*
	Spread (%)	*46*	*63*	*39*	*54*	*21*	*22*	*39*	*19*	*58*	*28*
	Quartile/median (%)	*21*	*7*	*15*	*16*	*9*	*3*	*18*	*9*	*25*	*11*
Juice volume (mL/berry)	Mean	0.52d	0.61c	0.61c	0.67abc	0.64bc	0.72ab	0.70abc	0.72ab	0.65abc	0.73a
	Coeff. of variation (%)	*20*	*5*	*7*	*12*	*17*	*9*	*28*	*15*	*31*	*16*
	Spread (%)	*26*	*56*	*59*	*58*	*32*	*25*	*57*	*31*	*50*	*58*
	Quartile/median (%)	*20*	*5*	*7*	*12*	*17*	*9*	*28*	*15*	*31*	*16*
Titratable Acidity (g/L)	Mean	3.00a	3.29b	3.03c	2.97c	2.44cd	2.66cd	2.78de	2.91ef	3.54fg	2.56g
	Coeff. of variation (%)	*6*	*5*	*14*	*6*	*7*	*4*	*13*	*13*	*19*	*3*
	Spread (%)	*10*	*22*	*20*	*41*	*16*	*11*	*33*	*17*	*32*	*24*
	Quartile/median (%)	*6*	*5*	*14*	*6*	*7*	*4*	*13*	*13*	*19*	*3*
Yeas assimilable nitrogen (mg/L)	Mean	113.9f	216cde	231cd	171e	180de	208cde	299b	235c	427a	195cde
	Coeff. of variation (%)	*33*	*52*	*44*	*30*	*17*	*15*	*40*	*31*	*34*	*54*
	Spread (%)	*65*	*101*	*139*	*65*	*61*	*59*	*85*	*45*	*54*	*103*
	Quartile/median (%)	*33*	*52*	*44*	*30*	*17*	*15*	*40*	*31*	*34*	*54*
Alpha amino nitrogen (mg/L)	Mean	95.6e	173.4c	185.5c	137.7cd	150.7de	174.8cd	236.0b	190.5c	328.5a	153.9cd
	Coeff. of variation (%)	*34*	*34*	*57*	*38*	*17*	*19*	*43*	*33*	*32*	*79*
	Spread (%)	*118*	*108*	*158*	*105*	*73*	*70*	*112*	*60*	*76*	*172*
	Quartile/median (%)	*34*	*34*	*57*	*38*	*17*	*19*	*43*	*33*	*32*	*79*
Ammonia (mg/L)	Mean	22.3e	51.8c	56.0c	40.3cd	34.7de	40.0cd	75.8b	54.4c	119.2a	50.0cd
	Coeff. of variation (%)	*28*	*56*	*39*	*30*	*17*	*15*	*35*	*28*	*30*	*52*
	Spread (%)	*63*	*98*	*135*	*55*	*59*	*61*	*76*	*48*	*56*	*95*
	Quartile/median (%)	*28*	*56*	*39*	*30*	*17*	*15*	*35*	*28*	*30*	*52*
WL anthocyanin concentration (mg/g)	Mean	2.2a	1.96bc	1.87cd	1.94bcd	1.92bcd	2.12ab	1.95bc	1.72d	2.12ab	1.78cd
	Coeff. of variation (%)	*8*	*12*	*10*	*15*	*10*	*11*	*19*	*42*	*26*	*18*
	Spread (%)	*26*	*51*	*32*	*61*	*26*	*20*	*37*	*60*	*61*	*40*
	Quartile/median (%)	*8*	*12*	*10*	*15*	*10*	*11*	*19*	*42*	*26*	*18*
WL tannin concentration (mg/g)	Mean	2.04a	1.68bc	1.66bc	1.67bc	1.92ab	2.08a	1.75bc	1.60c	2.06a	1.61c
	Coeff. of variation (%)	*6*	*10*	*17*	*17*	*13*	*10*	*19*	*39*	*43*	*22*
	Spread (%)	*29*	*57*	*42*	*59*	*38*	*26*	*55*	*70*	*60*	*69*
	Quartile/median (%)	*6*	*10*	*17*	*17*	*13*	*10*	*19*	*39*	*43*	*22*
Anthocyanin concentration (mg/g)	Mean	2.39a	2.25ab	2.09bcd	2.20ab	1.92de	2.16bc	2.14bc	1.85e	2.18ab	1.95cde
	Coeff. of variation (%)	*12*	*7*	*10*	*8*	*5*	*7*	*21*	*24*	*22*	*9*
	Spread (%)	*31*	*46*	*28*	*36*	*19*	*18*	*31*	*41*	*54*	*43*
	Quartile/median (%)	*12*	*7*	*10*	*8*	*5*	*7*	*21*	*24*	*22*	*9*
Tannin concentration (mg/g)	Mean	8.82a	7.80bc	6.72de	6.91d	6.03e	8.59ab	7.01cd	6.52de	7.86bc	7.18cd
	Coeff. of variation (%)	*12*	*8*	*29*	*12*	*10*	*4*	*21*	*16*	*26*	*9*
	Spread (%)	*56*	*55*	*62*	*29*	*42*	*13*	*39*	*45*	*40*	*39*
	Quartile/median (%)	*12*	*8*	*29*	*12*	*10*	*4*	*21*	*16*	*26*	*9*

From the spread values in Table 6.2.1, vineyards were relatively uniform for °Brix and pH compared with the other objective measures assessed, and these measures will not be discussed further. Larger within-vineyard differences were found for berry weight, berry volume and TA. According to spread numbers, Block 6 was the most uniform in berry juice volume with the lowest spread value and Block 7 was the most variable vineyard. Based on the ANOVA, there was no significant difference among the 10 vineyards in grape berry weight. Block 8 had the most homogenous berry weight while Blocks 5 and 9 were more variable. As found for other studies (Walker et al. 2005), although berry weight has been thought in the past to be important for grape phenolic concentration, no correlation between berry weight, juice volume and phenolic concentration (extractable or total) was found. For TA, Block 9 was the most variable vineyard, and also had the highest observed TA levels. Excluding vineyards for which outliers influenced spread values, most were fairly uniform for TA. This uniformity likely allowed for significant differences in the average TA between blocks to be calculated, since absolute differences between blocks were very small.

The results of the variability study showed that vineyard heterogeneity or homogeneity can be important considerations when evaluating a vineyard's response in terms of basic grape objective measures. Firstly, certain attributes such as alpha amino nitrogen, ammonia and in turn, YAN, were found to vary quite significantly within a single block. Nitrogen inputs during winemaking are a critical point in wine production and where/when a sample is taken from a vineyard, harvest bin or crusher can eventually influence the estimate of required DAP additions in the winery. It was also clear from these results that some vineyards showed true heterogeneity, whereas others were more uniform, with the exception of a single sample which was markedly different from the others (outlier). Depending on the compound analysed, the influence of the outlier on the average or spread (%) of a given vineyard could be more or less important and the quartile data therefore helped to compensate for the effects of outliers. In the next section of the study, vineyard heterogeneity or homogeneity was considered as an additional parameter in the evaluation of quality grade. To achieve this, the values from Table 6.2.1 were included in the multivariate analysis.

6.2.2 Prediction of vineyard and wine grade from vineyard variability data and targeted grape analysis in 2018

In the quality prediction section of the 2018 results, grape and wine quality evaluation and prediction are presented side by side. As for the 2017 season, it was evident that the final wine quality categories spanned four classes: 1, 1.5, 2 and 3, while the 10 vineyards had been assigned to 'intended use' quality grades 1 and 2 (Table 6.2.2). Unlike the first season of the study, only one wine was assigned to quality category 2. Based on the initial assessment that vineyards 7 and 9 were 'marginal' blocks that had been observed by the producer to respond variably by season (either attaining quality grade 2, or being downgraded to 3) it was found that only site 9 was assigned grade 3 during the final wine grade assessment. Site 7, on the other hand, was assigned to quality grade 1.5. Two other vineyards of 'intended use' grade 2 (sites 5 and 8) were associated with a final wine quality designation of grade 3. None of the sites of 'intended use' grade 1 were graded lower than 1.5 in the final wine quality category, which was of interest given that the first season of the study showed more variability by quality category.

Using targeted grape analysis, neither grape grades 1 and 2, nor the wine grade categories, could be predicted using PLS-DA. On the other hand, for vineyards which were classified by the producer as 'grade 3', a model could be developed using PLS-DA which gave a satisfactory R^2 of calibration of 0.66, and an R^2 of validation of 0.55. Although this was not a strong model of prediction, it could nonetheless provide some guidelines as to the fruit compositional characteristics that caused these vineyards to be deemed 'marginal' in quality.

As discussed previously (Table 6.2.1) both the 'marginal' quality vineyards (Sites 7 and 9) had the highest YAN, ammonia and alpha amino nitrogen, but did not necessarily present the greatest within-vineyard variability in these measures in comparison with the other vineyards studied. According to the PLS-DA results shown in Figure 6.2.2.1, grade 3 was driven primarily by Factor-1, with a higher juice pH and higher concentrations of nitrogen measures overall, including multiple amino acids, together with glutamate. Interestingly, higher concentrations of grape-derived glutamate had been found to be positive predictors of quality in a previous study of Shiraz (Bindon et al. 2017a, Bindon et al. 2017b). Although not separated by the PLS-DA model, some separation of grades 1 and 2 was evident on Factor-2, although incomplete. Factor-2 was defined primarily by berry weight (higher loadings) and acidity (lower loadings), with grape grades 1 and 2 being higher and lower on this factor respectively.

Table 6.2.2. Block number, grape grade and commercial wine grade assigned (vineyards designated 2/3 were grade category 2, with variable outcomes having been observed over different years and therefore downgraded to 3).

Site	Grape Grade	Wine Grade
1	2	2
2	2	1.5
3	1	1.5
4	1	1
5	2	3
6	1	1
7	2/3	1.5
8	2	3
9	2/3	3
10	1	1

As discussed previously, a further goal of the 2018 study was to explore the importance of within-vineyard variation (as opposed to between-vineyard variation) in defining vineyard quality and final wine quality. Following the initial PCA of the entire dataset of 100 grape samples (result not shown), a further PCA was performed that included the combined averages of data from the samples for each vineyard. This made it possible to study the wine chemical results as well as the % variability within each vineyard by the PCA. Based on the results from Table 6.2.1, the % quartile data were a more appropriate measure of within-vineyard variability since this measure excluded extremes introduced by single outliers, and were therefore used in the final PCA. For the assessment of variability data by PCA (Figure 6.2.2.2 A) the first two PCs described 55% of the variability in the dataset. Significant variables for the first PC1 were total nitrogen and variability measures for extractable and total phenolics (tannin and anthocyanin) as positive correlation loadings. PC2 was defined positively by absolute measures of extractable tannin and colour, °Brix, and pH, and negatively by measures of nitrogen variability. Amino acids were excluded from this analysis since they tracked strongly with YAN and alpha amino nitrogen, and did not contribute further to the model. Based on the distribution of the 10 vineyard sites according to the scores plots, it was evident that ‘marginal’ vineyards (sites 7 and 9, grade 3) were characterised by higher variability in phenolics, lower levels of extractable phenolics and higher absolute grape nitrogen (Figure 6.2.2.2 B). From the plot of the final wine grades (Figure 6.2.2.2 C), it was evident that one of the vineyards classified as ‘intended use’ grade 2 was downgraded to 3 in the final wine assessment and was grouped with sites 7 and 9 as being variable in phenolics and high in nitrogen. Another site downgraded to wine grade 3 (site 5) was grouped with better quality wine grades by PCA. From this assessment it appeared that total vineyard nitrogen and within-vineyard variation in phenolics had a greater impact on final wine quality outcomes than other measures. However, the classification of site 5 as final wine quality grade 3 was not clearly defined by the PCA results.

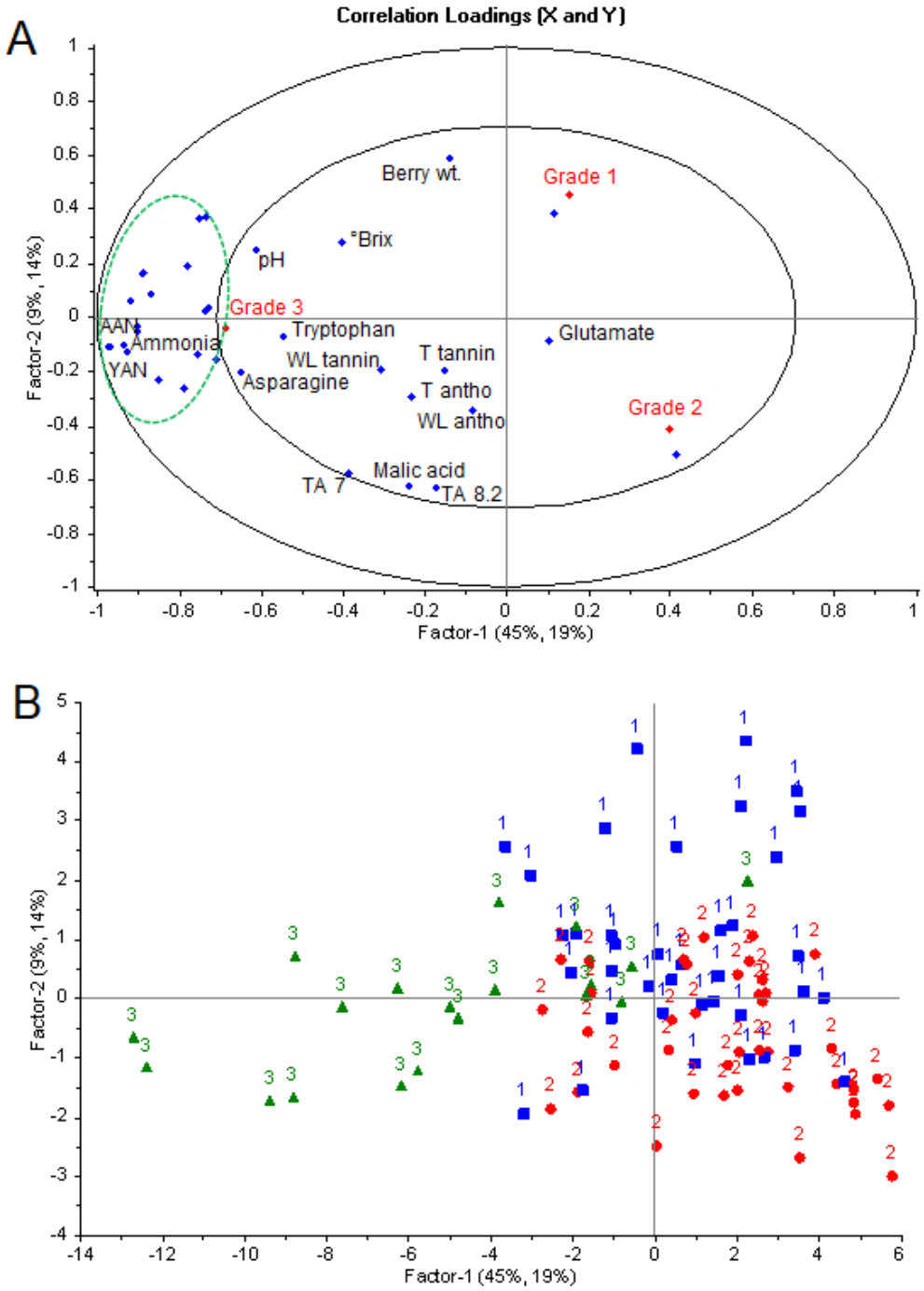


Figure 6.2.2.1. Partial least squares-discriminant analysis of basic grape objective measures (including amino acids) showing A. correlation loadings, B. scores plot for vineyard quality grades where 3 represents 'marginal' blocks of quality grade 2 (green broken line represents multiple amino acids, other than those labelled in the plot; WL, extractable anthocyanin or tannin under wine-like conditions; anth, anthocyanin; T, total; TA, titratable acidity to pH end points 7 and 8.2; AAN, alpha amino nitrogen; YAN, yeast assimilable nitrogen).

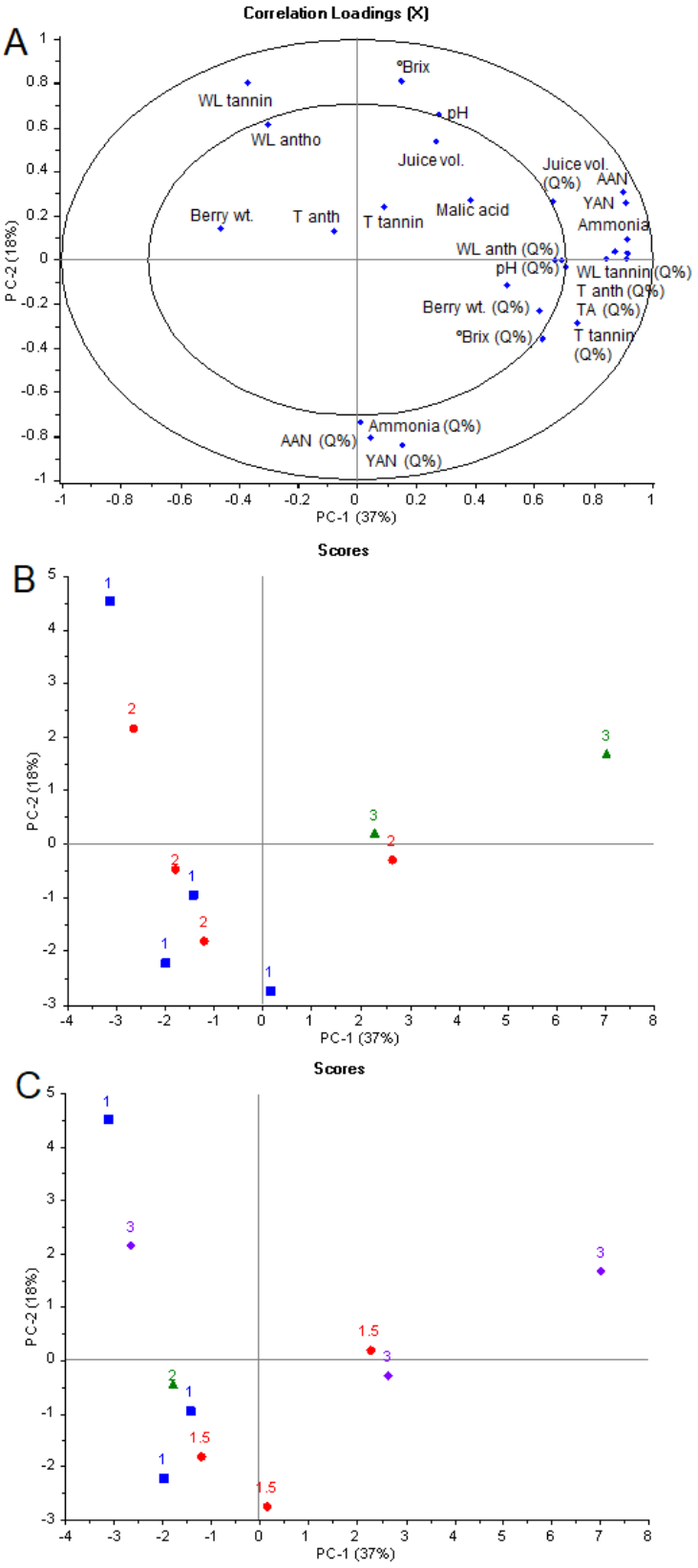


Figure 6.2.2.2. Principal component analysis of basic grape objective measures (excluding amino acids) showing A. correlation loadings, B. scores plot for vineyard quality grades where 3 represents 'marginal' blocks of quality grade 2 and C. scores plot for final wine grade (WL, extractable anthocyanin or tannin; anth, anthocyanin; T, total; TA, titratable acidity; AAN, alpha amino nitrogen; YAN, yeast assimilable nitrogen; Q%, variation across 10 grape samples within a vineyard as the 25-75th quartile average/mean (as %).

6.2.3 Partial least squares-discriminant analysis to predict vineyard and wine grade from non-targeted grape analysis in 2018

In the 2018 season, the same non-targeted measures were applied to grapes as for the first year of the study, namely: MIR analysis of juice and homogenate, NIR of homogenate, a UV-visible scan of homogenate extract and non-targeted profiling of grape extracts by LC-MS. These approaches performed variably in the PLS-DA models depending upon whether grape or wine grade were predicted. As for the first season of the study, successful models could not be built using the NIR (homogenate), MIR (juice) and UV-visible spectra, and this aspect will not be discussed further. The data generated from the non-targeted profiling of the grape samples could not be used to successfully predict grape grades 1 and 2, or the final wine grades. However, the designation of 'marginal' vineyards as grade 3 enabled a better PLS-DA models to be built based on the non-targeted LC-MS data, but prediction accuracy did not exceed 62%. As a result, no further work was done to attempt to identify key molecular features in 2018 study.

The use of MIR spectra from grape homogenate achieved better prediction models for grape grade in the second season of the study, improving on those built using targeted grape analysis. Grape grades 1 and 2 could be predicted with PLS-DA of homogenate MIR spectra (note: but not from MIR spectra of grape juice) with R^2 of calibration 0.75, and R^2 of validation 0.68 with a sub-set of spectra defined using an uncertainty test (6 PCs, RSME of validation = 0.28). When the 'marginal' grade 3 vineyards (sites 7 and 9) were designated as a separate category in the PLS-DA model, grade 3 vineyards could be predicted from a sub-set of homogenate MIR spectra with an R^2 of calibration 0.85, and R^2 of validation 0.80 with a sub-set of spectra defined using an uncertainty test (7 PCs, RSME of validation = 0.18). Using this model, some separation of the grade 3 samples from grades 1 and 2 could be demonstrated via scores plots (Figure 6.2.3). However, it is important to note that the model required 7 PCs to best explain the Y variance.

Prediction of wine grade using MIR homogenate spectra was not as successful as for grape grade, with only wine grade 1 being separated from the other wine grades at 52% accuracy, despite previous reports that homogenate MIR data can be used successfully for the prediction of Shiraz grape grade (Bindon et al. 2017a, Bindon et al. 2017b). The current models developed for grape grade prediction are based on a limited sample number and would need to be further validated by the addition of more samples and possibly multiple grades (including those of poorer quality). Yet overall the use of grape homogenate MIR spectra appears to be a promising approach for grape grade prediction.

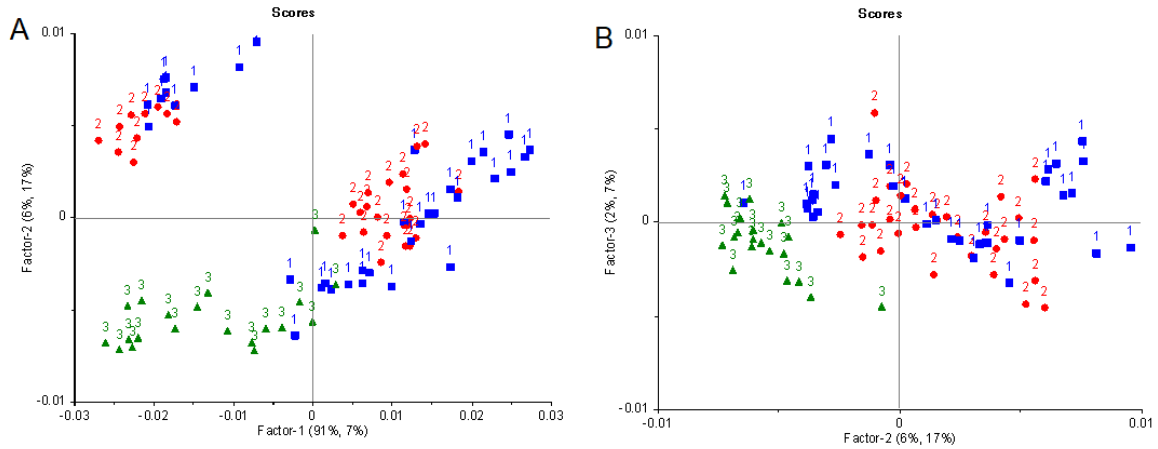


Figure 6.2.3. Scores plots from partial least squares-discriminant analysis of mid-infrared spectra of homogenate showing the separation of vineyard quality grades (where 3 represents 'marginal' blocks of quality grade 2) A. Using principal components 1 and 2, and B. using principal components 2 and 3.

6.2.4 Relationship between grape and wine composition and wine sensory properties in the 2018 season

The 2018 study aimed to evaluate the differences in the sensory properties of the 2018 Shiraz wines made from grapes from multiple vineyards in the Barossa Valley, each classified according to the PRW quality designation. It was intended to assess all 10 wines from the experiment by sensory analysis; however, wines from sites 5 and 9, both in quality grade 3, had already been processed further by the producer and only the remaining eight commercial wines could be examined in this study. All wines were evaluated using quantitative sensory descriptive analysis. For the study, three wines were allocated to each of grades 1 or 1.5 by the producer, and one wine was allocated to each of grades 2 and 3 respectively. ANOVA revealed 20 attributes rated by the sensory panel differed significantly (P<0.05) between the Shiraz wines: 'opacity', 'purple colour', 'dark fruit aroma', 'red fruit aroma', 'earthy aroma', 'vanilla/chocolate aroma', 'blueberry aroma', 'stone fruit aroma', 'drain (reductive) aroma', 'leather aroma', 'pungency', 'astringency', 'bitterness', 'hotness', 'sweetness', 'dark fruit flavour', 'red fruit flavour',' earthy flavour', 'spices flavour' and 'fruit aftertaste'. There were significant judge by wine interaction effects for 18 attributes, reflecting some inconsistent use of these terms by judges and these are shown in Appendix 12 (Table 12.2). The PCA for the wines (Figure 6.2.4.1) was created using the 20 attributes that were significantly different (P<0.05) across the eight Shiraz wines. PCs 1, 2 and 3 explained 66.4%, 15.4% and 9.6% (PC3 not shown) of the variance in the data, respectively. Overall, the separation of the wines by PCA was small. Wine from site 1 was plotted to the far right of the figure as rated highly in those attributes highly positively loaded on PC1, notably 'earthy flavour', 'earthy aroma', 'leather aroma' and 'drain (reductive) aroma', and this wine was in the quality category two. Conversely, wines plotted to the left of the figure were rated lower in these attributes, and higher in the attributes highly negatively loaded on PC1, notably 'blueberry aroma', 'red fruit flavour', 'vanilla/chocolate aroma', 'dark fruit aroma', 'dark fruit flavour' and 'fruit aftertaste'. The wines from quality categories 1 and 1.5 were mostly plotted to the centre of Figure 6.2.4.1, and were slightly higher on PC2 than the wines from categories 2 and 3. The wine from site 8 was plotted lower on the vertical axis (PC2) and rated highly in 'stone fruit aroma' and 'sweetness', opposed to wines plotted high on the figure which were rated higher for 'opacity', 'purple colour' and 'astringency'.

The unbalanced distribution of the wines by quality category meant that a clear prediction by PLS-DA was not possible for this dataset. Instead, PLS-DA was done using combined wine analytical and sensory data to build a model to predict the original grape grades 1 and 2, as well as wine grade 1 and 1.5 (excluding wine categories 2 and 3 from the model). As such, a fairly strong model of prediction could be built for wine grades 1 and 1.5, at 79% accuracy. A reasonable PLS-DA model could also be developed for the original grape grade, at 62% accuracy. The results for the important variables which defined these categories are shown in Figure 6.2.4.2.

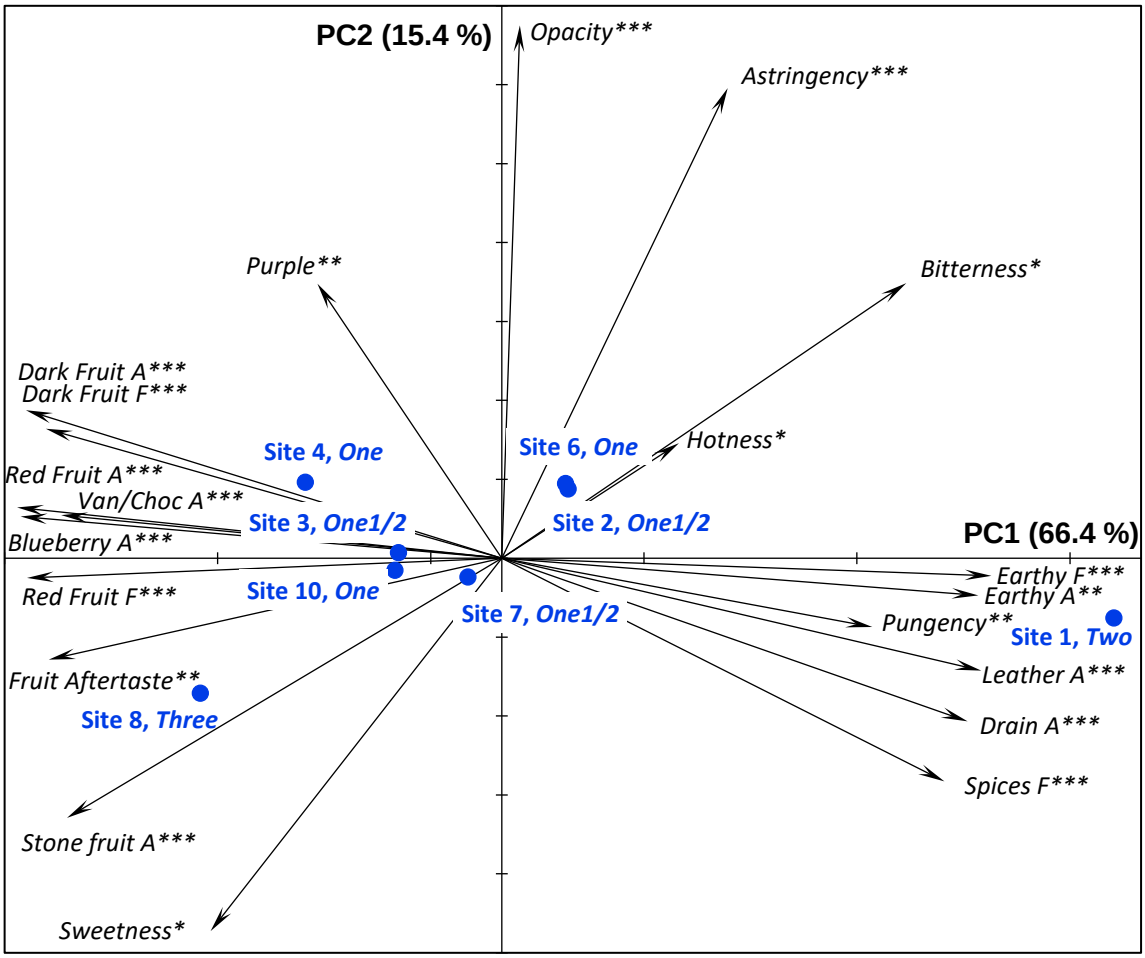


Figure 6.2.4.1. Principal component biplot of significant (*P*<0.05) and close to significant sensory attributes (*P*<0.10) on principal components 1 and 2 for the 2018 Shiraz wines, where italicised text indicates the quality category assigned by the producer. Significantly different sensory properties by ANOVA are shown as follows: *P<0.05; ** P<0.01; *** P<0.001; A: Aroma, F: Flavour.

Wines produced from both the original grape grade 1, and wine grade 1 were characterised by a greater contribution of 'opacity' and 'purple colour' (Figure 6.2.4.2), which was related to elevated wine colour density, tannin molecular mass and mean degree of polymerisation, as well as the proportion of epigallocatechin as a skin tannin proxy. Grade 1 wines were also characterised by a slightly lower tannin mass conversion, which relates to the extent to which tannin can be cleaved to subunits following acid-catalysed depolymerisation. This is interesting, because it is currently thought that tannin with a lower mass conversion might be less astringent. Different volatiles defined wines produced from either grape grade 1 or wine grade 1, but both were characterised by lower β-ionone. Wines produced from grape grade 1 tended to have lower hydrogen sulfide, mesityl oxide, hexanol and Z-3-hexenol. On the other hand, grade 1 wines had higher 2-methyl propyl acetate and ethyl acetate, and lower cis- and trans-rose oxide. There were no clear aroma or flavour descriptors which defined the different wines by PLS-DA, and hence further rationalisation for the observed differences in wine volatiles was not attempted. Further modelling was conducted for this sub-set of wines using the average grape compositional values from the targeted analysis, including the measures of within-vineyard variation, but satisfactory PLS-DA models could not be developed. This was not surprising given that grades 1 and 1.5 could not be distinguished using the targeted analysis described in the earlier sections. Given that grape phenolics (total and extractable tannin and anthocyanin) were very high in the sample set studied (Table 6.2.1), likely due to the fact that all were from the producer's premium quality category, it was evident that factors other than absolute concentration or relative extractability may have driven the subtle differences in wine phenolics and appearance that were important to the final wine classification. Further work will be needed to understand the factors that affect the development of wine colour properties in the premium category.

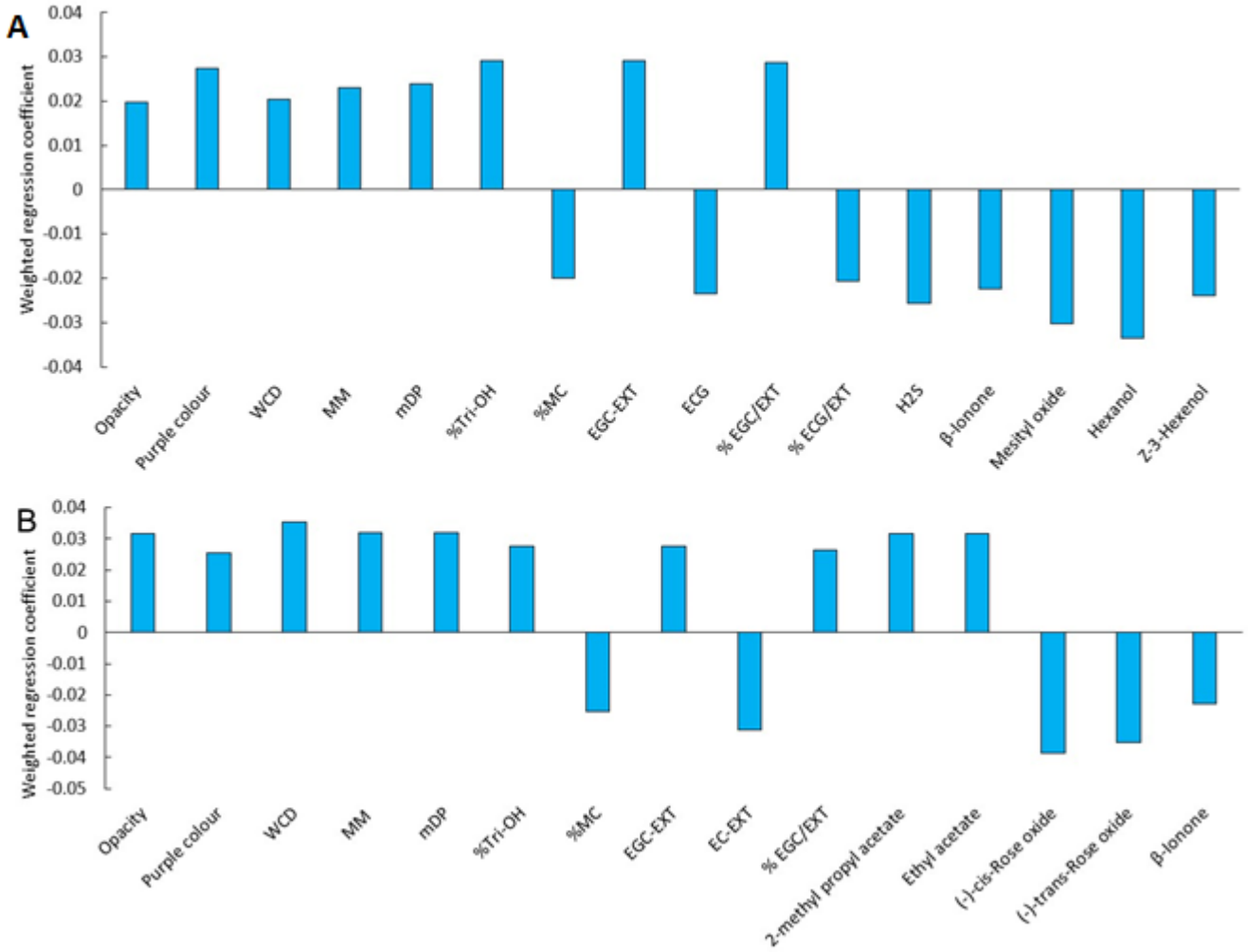


Figure 6.2.4.2. Weighted regression coefficients for the prediction of A. grape grades 1 and 2 (R^2calibration = 0.84, R^2validation = 0.62, RSMEvalidation = 0.35, 1 PC) and B. wine grades 1 and 1.5 (R^2calibration = 0.99, R^2validation = 0.79, RSMEvalidation = 0.28, 2 PCs) from commercial wine sensory and compositional data by partial least squares discriminant analysis (positive regression coefficients correspond to grape and wine grade 1); WCD, wine colour density; MM, tannin molecular mass by phloroglucinolysis; mDP, mean degree of tannin polymerisation; %Tri-OH, contribution of epigallocatechin; %MC, tannin conversion to subunits as a function of concentration; EGC, tannin epigallocatechin; EC, tannin epicatechin; EXT, tannin extension subunit.

7. Outcomes and conclusions:

Since wine colour and the associated grape compositional properties which lead to high wine colour are well known to be related to wine quality, it is not surprising that this was again confirmed for both seasons of the project and this validates the overall approach chosen for this study. Surprisingly, the relevance of grape and vineyard nitrogen as grape quality parameters as demonstrated in this study has until now been largely overlooked , and nitrogen measures are not routinely used as a standard parameter of 'fitness for purpose' in processing grapes at the winery. Data from both seasons of the study indicated that vineyards otherwise identified as being of premium grade, but with high grape nitrogen, could lose their assigned quality during the winemaking process, possibly due to the formation of off-odours or reduced levels of key positive aroma compounds. In the second season of the study this was definitively shown for only one vineyard (site 9) which had extremely high levels of grape nitrogen. The measurement of nitrogen in the vineyard through petiole or juice analysis is currently performed by only 40% or less of Australia's producers (Nordestgaard 2019). Approximately 65% of wineries that crush more than 10,000 tonnes adjust diammonium phosphate (DAP) additions to must based on YAN measurements, but this drops to 30% or less for smaller producers that crush 1,000 tonnes or under (Nordestgaard, 2019). In light of this, the measurement of grape juice YAN prior to DAP additions in the winery is relatively simple and accessible for many wineries, and can be used to prevent excess addition of nitrogen to must. In consideration of the results presented in this study, increasing the use of nitrogen measurements by both small- and larger-scale producers within Australia would be an important element to maintain and further improve Shiraz wine quality.

A further important observation from both seasons of the study was that a high degree of within-vineyard variability was found for certain sites, in some cases exceeding the variation among different sites. Measures traditionally used to determine grape quality or uniformity such as °Brix, pH and berry weight tended to be more uniform within sites, while nitrogen and phenolics measures varied widely. In both seasons of the study, the values for extractable tannin and colour could vary as much within a single vineyard as between regions within South Australia (Bindon et al. 2014). The outcomes of the modelling studies did not definitively show that within-vineyard variability could lead to a decrease in final wine quality. However, it was nonetheless of interest to note that the two vineyards designated 'marginal' quality in 2018 (which both also had both high vineyard nitrogen and vigour) and one vineyard which was grape grade 2 and downgraded to quality category 3 at commercial wine assessment were all highly variable in phenolics. It would be advisable for producers to monitor vineyards for variability in phenolics, in particular to determine whether sections of a vineyard should be harvested as separate parcels (streaming) for commercial winemaking based on potential quality outcomes. Considering that the analysis of multiple sampling locations within a vineyard would be time-consuming and laborious, a future research direction could be to make better use of rapid, hand-held or tractor-mounted devices which can be used for direct measurements in the vineyard (Power et al. 2019).

Regarding the initial objective of the study, to provide objective tools to determine differences between premium grade categories, the results of the current project were less clear. In the first season, which included micro-scale fermentations on every grape sample, it was found that non-targeted LC-MS analysis of both grapes and wines could provide a reasonable prediction of the two grape grade categories. However, given the complexity of this analysis, and the observation that only a few target compounds could be identified by their mass spectra using existing databases, it is unlikely that this process would directly lead to a commercially relevant outcome as an objective measure of quality. Nonetheless, from the first season of the project some key compounds already known in grapes and wines could be identified as possible quality markers for validation in future studies.

Despite the finding that mid-infrared analysis was not successfully applied in the first season of the study, the positive outcome in the second vintage enabled both the prediction of grape grade and 'marginal' blocks from homogenate MIR spectra. This highlighted that mid-infrared spectroscopy should not be overlooked as an objective tool by the wine industry, and also that choosing an appropriate sample type (grape homogenates and not grape juice) can be equally important for successful application. MIR spectroscopy can be rapidly deployed on grape samples, and does not require a high degree of technical competence by laboratory staff at the winery. However, it is important to note that there is a need within the wine industry to develop a broader competency to make better use of the spectra generated, not only as a tool to determine existing grade categories, but also to develop and

validate predictive calibrations for non-targeted objective measures which are not as yet commonplace, such as YAN or extractable and total phenolics.

8. Recommendations:

The results of both the former AWRI1202 project and the current study have highlighted that there is great potential for the use of refined grape objective measures within the wine industry. The first project provided a strong 'proof-of-principle' outcome across a range of grape varieties, regions and grades, giving a clear message to industry that the technology is available and useful, but requires ongoing commitment by producers at the operational level. The current study presented a similar message to a single producer, PRW, and has resulted in the company's commitment to incorporate objective measures as part of its future internal R and D program (see extension in 9.3 below).

For future directions for grape objective measures research, the following main points need to be considered:

a. The current grading systems tend to be defined on a producer by producer basis, and are tailored to the available fruit source (and therefore often regionally defined), scale of production and wine style categories which go to market. It is therefore unlikely that a universal standard of grape objective measures can be established in the future.
b. Future research might explore the potential of new/alternative/faster/cheaper analytical approaches or technologies; for example, data generation technologies that can be integrated at scale with vineyard and winery decision-making processes.
c. Future research might also explore opportunities beyond streaming or grading of grapes, and include the application of grape objective measures to achieve and quantify targeted improvements to viticultural management practices in the context of achieving sustainability and/or productivity enhancements.

A further observation based on the project presented in this report is that there are benefits if future projects in this area are initiated by industry partner(s) as research collaborations, and therefore the industry partner is invested in the project outcomes from the outset. This project has also identified a critical need for investment in professionals with skills in multivariate modelling and validation of models, and for resourcing over multiple vintages.

9. Appendix 1: Communication:

9.1 Conference presentations:

Bindon, K., Kassara, S., Macintyre, J., Zhang, W., Do, Z., Nicolotti, L., Chen, F., Solomon, M., Ford, C. Zoning in on Shiraz grape quality. Crush Symposium, Charles Hawker Centre, Urrbrae, 26 September 2018.

9.2 Publications:

Bindon, K., Kassara, S., Espinase Nandorfy, D., Nicolotti, L., Do, Z., Johnson, D. 2020. Identifying objective measures for Barossa Valley Shiraz grapes. *Wine Vitic. J.* Summer edition, *in press*.

9.3 Extension:

Two feedback sessions were held with PRW research, technical management, viticulture and winemaking staff during the course of the study. The first session was held on 18 October 2017 and aimed to provide outcomes from the first season of the study, and to inform the following year's research plan. Based on this session, further discussion was held to focus more on within-vineyard variability in the second season. After the second season was completed, a further half-day session was held on 13 June 2018 with local and international PRW staff, which involved presentations and a 'round table' discussion to guide PRW's global R&D Strategy. Based on this session, PRW was advised strongly to pursue objective measures research, particularly to control vineyard and winery nitrogen additions but also to increase the in-house focus on grape measurements, including MIR spectroscopy. PRW was also advised to broaden the quality categories being investigated. A project was initiated in collaboration with AWRI Commercial Services in 2019 to look more broadly at PRW's grape quality categories, with a PRW employee assigned to data collection and analysis and to be trained in multivariate modelling.

A further extension activity took place at the AWRI on 11 October 2018 for a group of 14 wine producers. A session on 'Objective measures of grape quality' was presented as part of an all-day workshop for the 'First Families of Wine'. The talk included results from the study presented in this report and generated interest and discussion regarding the implementation of objective measures by these producers.

Future extension activities are planned for AWRI's roadshow program. The results of the study will be partitioned into two presentations, one on 'Managing compositional variability in the vineyard and winery', and one on 'Objective measures of grape quality'. The latter presentation will be merged with the results of the former AWRI1202 project to build on the AWRI's ongoing narrative to the greater wine industry regarding the importance of objective measures.

10. Appendix 2: Intellectual Property

Nothing to report.

11. Appendix 3: References

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12.Appendix 4: Staff

12.1 AWRI staff:

- Dr Keren Bindon, project management, data analysis and interpretation, writing and communication of results.
- Dr Luca Nicolotti, non-targeted phenolics analysis, identification of unknown target compounds by mass-spectrometry.
- Dr Zung Do, multivariate analysis of 2017 data by principal component analysis.
- Dr Vilma Hysenaj, analysis of grape and wine amino acids.
- Mr Damian Espinase Nandorfy, sensory analysis of the wines, data analysis and interpretation.
- Mrs Stella Kassara, targeted non-volatile analysis of grapes and wines.
- Mr Luke Qi, sample preparation, analysis of grape homogenates and juices by MIR and NIR.
- Mr Mark Solomon, analysis of wine fermentation products (2017).
- Mr Flynn Watson, analysis of wine volatiles (2018).
- Ms Allie Kulcsar, analysis of volatile sulfur compounds (2018).

12.2 Collaborators

- Dr Jean Macintyre, PRW, industry project leadership and management.
- Mr Philip Deverell, PRW, identification of vineyards, measurement of grapevine GPS coordinates, yield and canopy indices, coordination of grape sample collection.
- Mr Andrew Johns, PRW, grape sample analysis and coordination of deliveries (2017).
- Prof Chris Ford, University of Adelaide, supervisor to MSc students (2017 and 2018).
- Ms Fangzhou Chen, University of Adelaide, MSc student (2017), sample processing, performing small-scale fermentations, sample analysis and data compilation.
- Ms Wenjing Zhang, University of Adelaide, MSc student (2018), sample processing, performing small-scale fermentations, sample analysis, data compilation, interpretation and writing.

12. Appendix 5: Supplementary data

Table 12.1. F-ratios, significance, degrees of freedom and mean square error from the analysis of variance of the sensory properties of 14 commercial Shiraz wines in the 2017 season.

Attribute[a]	Wine	Rep	J*Wine	J*Rep	Rep*Wine	MSE
Opacity	28.07***	1.90	1.68**	1.27	1.61	0.29
Purple Colour	11.96***	0.07	4.92***	1.91	0.98	0.58
Brown Colour	14.62***	1.33	2.77***	0.69	0.66	0.50
Overall Fruit A	1.55	0.07	2.11***	1.97*	1.05	0.51
Red Fruit A	2.27*	0.08	1.04	0.78	0.62	2.41
Dark Fruit A	1.87*	0.62	1.90***	1.71	0.70	1.14
Blackcurrant A	1.88*	0.00	2.21***	2.52*	1.32	2.50
Floral A	1.07	0.08	1.13	4.18***	1.31	1.12
Jammy A	0.60	0.98	1.52*	1.68	1.73	1.64
Coconut A	1.59[b]	2.23	1.51*	3.31***	1.28	0.80
Vanilla/Chocolate A	2.87***	0.73	1.42	4.68***	0.82	1.69
Tinned Veg A	3.56***	0.62	1.37	1.07	0.62	2.75
Pepper A	1.61[b]	1.11	1.34	0.97	0.90	1.49
Drain A	4.16***	1.46	1.61**	2.69**	0.57	2.60
Tobacco A	2.07*	4.01	1.93***	0.73	1.14	1.84
Woody A	5.29***	0.09	1.68**	1.19	0.45	1.73
Sweet Spice A	1.72[b]	0.17	1.35	6.58***	2.71**	1.39
Eucalyptus A	1.46	0.01	1.38*	2.14*	1.16	1.62
Stalky A	3.80***	0.13	1.12	2.13*	0.59	1.56
Medicinal A	1.56	0.06	1.13	2.28*	0.94	2.30
Pungent A	2.49**	0.19	1.08	2.58**	0.84	0.60
Overall Fruit F	1.41	0.39	1.97***	2.77**	0.85	0.40
Red Fruit F	2.20*	0.00	1.53*	0.85	0.61	1.75
Dark Fruit F	2.27**	0.13	1.76***	1.71	0.86	1.14
Blackcurrant F	2.07*	0.50	2.19***	4.62***	0.80	1.84
Jammy F	0.88	2.82	1.15	1.82	1.03	1.68
Sweet Spice F	1.27	0.95	1.12	3.20**	1.44	1.62
Stalky F	1.37	0.38	1.16	1.94	0.52	1.50
Vanilla/Chocolate F	1.69[b]	1.38	1.58**	9.27***	1.80*	1.13
Eucalyptus F	1.47	0.01	1.07	1.66	1.47	1.41
Woody F	2.81**	0.12	1.16	1.25	0.74	1.73
Tobacco F	2.98***	0.52	0.92	2.53*	0.22	1.83
Viscosity	2.56**	3.91	0.97	0.74	1.17	1.00
Acidity	1.23	0.03	1.03	0.85	0.72	0.58
Astringency	8.95***	0.09	1.95***	3.32***	1.95*	0.67
Hotness	2.32**	3.53	1.57**	0.80	1.64	0.79
Bitterness	1.37	0.03	1.42*	4.48***	0.50	0.58
Fruit AT	2.23*	5.79*	1.29	1.55	0.41	0.60
df	13	1	117	9	13	117

[a]Significance levels are as follows: *P<0.05; ** P<0.01; *** P<0.001; [b]P<0.10. df = degrees of freedom. Judge effect was significant for all attributes (P<0.05) except attributes; *sweet spice* and *medicinal aromas*, which had P-values of 0.077 and 0.086, respectively. Rep = presentation replicate, J = Judge, MSE = mean square error. A: aroma, F: Flavour, AT: Aftertaste.

Table 12.2. F-ratios, significance, degrees of freedom and mean square error from the analysis of variance of Shiraz wine sensory attributes in the 2018 season.

Attribute[a]	**Wine**	**Rep**	**J*Wine**	**J*Rep**	**Rep*Wine**	**MSE**
Opacity	9.84***	1.89	1.6**	2.82***	1.63[b]	0.409
Purple	3.19**	1.88	1.54**	2.89***	2.22**	0.560
Dark Fruit A	5.92***	0.22	2.17***	1.45[b]	0.96	1.137
Red Fruit A	9.38***	0.85	1.45*	0.93	1.18	2.370
Earthy A	3.97**	0.13	1.87***	0.91	0.86	1.550
Vanilla/Chocolate A	10.08***	0.03	1.78**	0.92	1.17	1.360
Blueberry A	6.11***	0.18	1.53*	0.7	0.88	2.001
Pepper A	0.64	1.34	1.1	1.46[b]	1.23	0.800
Stone fruit A	5.08***	1.17	1.7**	0.66	1.17	1.458
Spices A	1.76	1.07	2.7***	1.54[b]	1.78*	1.282
Herbal A	0.24	0.82	2.17***	1.9*	1.22	1.523
Drain A	26.69***	0.56	2.18***	1.77*	1.55[b]	1.935
Leather A	6.96***	2.46	2.21***	1.16	0.88	1.669
Pungency	2.98**	2.6[b]	0.75	0.65	0.52	0.877
Acid	1.62	0.21	1.34[b]	0.94	0.72	0.748
Astringency	15.45***	0.06	1.09	1.07	1.58[b]	0.829
Bitterness	2.83*	0.05	1.2	1.5[b]	0.76	0.992
Viscosity	0.63	0.49	0.96	2.6***	0.85	0.629
Hotness	2.42*	1.39	0.67	0.87	1.1	1.132
Sweetness	2.42*	0.75	1.6**	1.43[b]	1.16	1.354
Dark Fruit F	5.94***	0.1	2.83***	1.4	0.87	0.711
Red Fruit F	6.11***	0.44	2.13***	1.33	1.61[b]	1.774
Earthy F	4.53***	0.74	1.58**	0.84	1.5	1.394
Spices F	5.11***	0.22	2.81***	1.49[b]	1.19	0.894
Fruit Aftertaste	4.09**	0.06	2.87***	1.23	1.49	0.867
df	*7*	*2*	*84*	*24*	*14*	*168*

[a]Significance levels are as follows: **P*<0.05; ** *P*<0.01; *** *P*<0.001; [b]*P*<0.10. df = degrees of freedom. Judge effect was significant for all attributes (*P*<0.05) except attributes; *sweet spice* and *medicinal aromas*, which had P-values of 0.077 and 0.086, respectively. Rep = presentation replicate, J = Judge, MSE = mean square error. A: aroma, F: Flavour, AT: Aftertaste.

14.Appendix 6: Budget reconciliation

Not applicable.