# Assessment of relationships between grape chemical composition and grape allocation grade

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## Introduction

Because the first point in the value chain is the vineyard and the grapes, decisions made at this point are critical to achieving the desired wine style. Compounds derived from grapes responsible for appearance, aroma, texture and taste in wine are the primary contributors to the degree of fitness of purpose of winegrapes. Many of these compounds are known and are measurable, have meaning to the final sensory state of the wine and are able to be manipulated through viticultural and/or winemaking practices. However, the application of objective chemical measures in Australia tends to be at a fairly rudimentary level and lags behind overseas competitors, with only one or two measures implemented by some companies. The subjectivity of the existing assessments can result in uncertainty relating to whether the maximum value possible for those grapes is achieved.

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

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

By measuring a range of chemical compounds in multiple grape batches of different grades, the research described herein aims to determine how variable the chemical measures are across a wide range of fruit grades, whether there is a relationship between the chemical measures and the fruit grade and whether the fruit can be clustered based on similarity of chemical composition. A further aim of the research is to assess the practical application of grape grading measurements and to support wine producers who intend to apply these measures in their systems.

Potential impacts for the wine sector include the ability for grapegrowers to more efficiently produce grapes to defined specifications, and for winemakers to select fruit with greater confidence that it will be appropriate for a targeted wine style. In addition, it is significant to grapegrowers because it relates to confidence and transparency in the realization of maximum economic value for their grapes. For both growers and winemakers, objective chemical measures can provide specifications that allow the most value to be achieved from grapes. For winemakers such measures offer the opportunity to stream fruit to maximise its value and minimise risk related to loss of value, for example by including higher value fruit with lower value fruit, and allow to choose fruit of the value that relates best to a desired wine style.

## Results and discussion

The initial aim of the project was to determine from a range of measurable chemical compounds in grapes which of the measures, independently or in combination, are able to differentiate between grape grades. During consultation with wine sector personnel the questions that were raised refined the project down to two, more specific researchable questions. Firstly, regardless of the grade, can chemical measurements be used to 'cluster' fruit to identify similar patches or highlight differences? This assumes no knowledge of grade and for e.g. could be used to stream fruit. Secondly, can existing grading allocations be predicted using some of the previously identified (and some new) chemical measurements? This assumes the current grading system is 'accurate' and seeks to objectify the process.

Grapes from a range of quality grades were sourced by representative sampling of vineyards and a range of chemical analyses were performed to determine the concentration of key compounds known to affect wine style and key sensory properties. These included: total soluble solids, pH, TA, malic acid, yeast assimilable nitrogen (YAN), amino acid profile, dry matter, methoxypyrazines, C6 compounds, phenol-free glycosyl glucose (GG) assay, anthocyanins, total phenolics, tannin, β-damascenone, laccase and spectral fingerprints in the UV-Vis, mid infrared (MIR) and near infrared (NIR) regions. The grades were sourced from the grower or winery contracted to make wine from those grapes. Univariate or multivariate relationships using methodology such as partial least squares regression, principal component analysis, as well as multiple linear regression were applied to the chemical measures and the grades to assess the extent to which these measures were able to be used to predict the grade. Multivariate data analysis was also used to develop models that cluster fruit based on similar compositional variables to determine fruit parcels which are similar and which could guide streaming decisions.

The research was undertaken with the support of Accolade Wines and FABAL as partners. Accolade Wines uses one standardised

approach for grading of all fruit and pays growers independent of the wine grade outcome. Cabernet Sauvignon grapes only were studied in 2013 and 46 samples were selected across grades 2–7 (2 = higher value, 7 = lower value) across 9 regions (Swan Valley, Western Australia (SW), Riverland, McLaren Vale, Langhorne Creek, Clare Valley, Padthaway, Coonawarra and Wrattonbully).

### Principal Component Analysis: Spectroscopy

UV-Vis, MIR and NIR Spectra were all collected for grape samples. UV-Vis scans were performed on 50% ethanol grape homogenate extract, while MIR and NIR were performed on grape homogenate. Principal Component Analysis (PCA) of UV-Vis spectra (240–700 nm) showed that the first two PC's accounted for 99% of the variation between samples. There was some clustering related to grade but also some signs of non-linearity. There was also clustering related to region, with Riverland and Swan Hill very distinct from other regions. PCA using the MIR and NIR spectra showed no distinct clustering by grade or region. In summary, PCA with UV-Vis spectra of grape homogenate extracts shows some separation by grade or region, but not distinct. NIR and MIR showed very little separation with the first PC's so the data structure is more complex.

### Spectroscopy data: Grade prediction using discriminant Analysis or PLS regression.

Grade prediction can be performed using discriminant analysis (DA) with both spectral and chemical data using PCA scores. It should be noted that chemical data must be standardised to remove scaling effects and grading data is categorical so this type of analysis is quite appropriate. One drawback is that it can be harder to identify the drivers of the discrimination e.g. wavelength x, y, z or compound a, b, c.

Discriminant analysis applied to UV-Vis spectra of grape extracts using the first 5 PCA factors (240–700 nm) allowed 40 out of 46 samples to be correctly predicted. The incorrect predictions were all in adjacent classes. Using the MIR spectra, 35 out of 41 samples were correctly predicted and 5 incorrect predictions were in adjacent classes, 1 was 2 classes away. Using the NIR spectra, 38 out of 41 samples were correctly predicted and all incorrect predictions were in adjacent classes.

Grade prediction can also be performed using PLS regression, where the grades are treated as numerical values and predicted using PLS regression. An advantage is that significant variables will be identified and cross-validation can be used to test the model. Again, chemical data must be standardised. The PLS correlation between UV-Vis (240–700 nm) spectra of 50% ethanol grape homogenate extract and grape grade resulted in $R^2$= 0.51 and a standard error of cross validation (SECV)= 0.82 grade points (perfect prediction would be 0.5 as there is no fractional grading in reference data). Using MIR spectra of grape homogenate gave an $R^2$= 0.80 and a SECV= 0.49 grade points i.e. the best possible error considering there are no incremental scores in reference data. NIR data resulted in an $R^2$= 0.42 and a SECV= 0.80 grade points, similar to that derived from the UV-Vis scans of 50% grape homogenate extract.

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

### Principal Component Analysis: Chemical data

A wide range of chemical data was collected from the grape samples. These included basic berry chemistry such as average berry weight (g), pH, TA7 (g/L) and TA8.2 (g/L), Brix, moisture (%), malic acid (g/L), α-amino nitrogen (mg/L), ammonia(mg/L), YAN (mg/L). Possible negative markers of quality included laccase activity (units/mL), chloride (mg/kg). UV-Vis Spectral data included Total phenolics A280 (AU), Colour A520 (AU), A420 (AU), MCP tannin (mg/L epicat. eq.). Aroma compounds included C-6 (µg/L) compounds, methoxypyrazines (µg/L), GG in homogenate (µMol/kg), free β-damascenone (µg/L). For a summary of the functions of these compounds in grapes and wines, please refer to (Smith 2013).

PCA using the standardised chemical data resulted in a model in which the first two PC's accounted for 47% of the variation between samples. Using only the first two PCs, the clustering related to grade is less distinct than when using UV-Vis spectral data (i.e. more PC's needed to discriminate grades). Regional clustering was stronger than that by grade when compared with UV-Vis spectral data, and there was stronger overlap of other regions with Riverland.

### Chemical data: Grade prediction using discriminant Analysis or PLS regression

Discriminant analysis yielded a model capable of correctly predicting the grade of 39 out of 46 samples. Almost half of incorrect predictions were 2 classes away. Using PLS regression on the chemical data yielded a model with moderate ability to predict grade; $R^2$= 0.49 and SECV= 0.79 grade points i.e. similar in accuracy to that using UV-Vis scan data or NIR data, but not as good as that using MIR data. An uncertainty test was then used to identify the significant analytes contributing to the prediction of grade.

In Figure 1 only 1 factor used for prediction so loadings for that factor can be examined directly. Coefficients can be positive or negative depending on whether higher concentrations increase value (better grade) or decrease value (poorer grade) and only significant variables are shown.

### A new objective measure: 'Wine extractable' tannin

In the production of red wines, it is crucial to optimise the extraction of grape-derived phenolic compounds, primarily anthocyanin and condensed tannin (proanthocyanidin) in order to ensure the development of stable wine color and desirable textural properties. Tannins extracted during winemaking potentially originate from grape skins, seeds and pulp (Bindon et al. 2010) while in most commercially-produced grape varieties, anthocyanins occur only in the grape skins. The extraction of phenolics from these respective grape components during fermentation differs significantly, with anthocyanin and skin tannin being extracted early (Cerpa-Calderon et al. 2008; Peyrot des Gachons and Kennedy 2003) and seed tannin being extracted later.

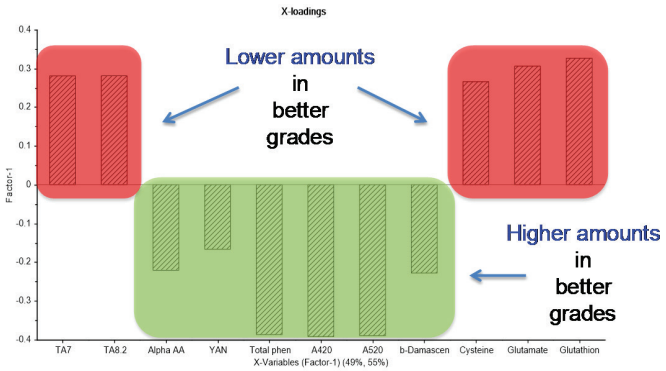


**Figure 1.** PLS model predicting grade with loadings for significant chemical measures. Figure 1 allows inference of some 'lead' targets capable of differentiating grape grades. YAN, Total Phenolics, A420 and A520 and β-damascenone are positively associated (i.e. there are higher amounts) in higher value grades. TA, cysteine, glutamate and glutathione are negatively associated (i.e. there are lower amounts) in higher value grades.

While a significant amount of tannin is present in grape pulp, this appears to remain nonextractable during fermentation, potentially due to a strong association with pulp cell wall constituents (Bindon et al. 2010).

Color, as well as anthocyanin and tannin concentrations in wine, have been shown to be positively associated with market value grade, and they are therefore useful as objective markers for wine quality (Mercurio et al. 2010; Kassara and Kennedy 2011). Most analytical phenolic methods aim to achieve as close to an exhaustive extraction as possible, and this is evident in techniques applied for grape tannin and anthocyanin. Wine, on the other hand, represents a partial extraction from the grape solids. As a result, using an exhaustive extraction method might not capture information regarding the intrinsic variability in the kinetics of tannin and anthocyanin extraction during fermentation, as well as potential back-binding events to cell wall constituents. In light of this, a desirable, commercially relevant outcome for wine industry producers would be a procedure for the prediction of anthocyanin and tannin concentrations achievable during fermentation. Yet, despite this clear indication that the strategic management of wine tannin and anthocyanin is important, until now (Bindon et al. 2014) a rapid analytical method to predict wine phenolic profile from grapes had not been developed.

Cabernet Sauvignon and Shiraz grapes were sourced from different regions within Australia, and microvinified with a) with a contact period of six days. Grape samples were extracted using two protocols: a 15% v/v ethanol, 10 g/L tartaric acid extract of gently crushed berries (wine-like) and a 50% v/v ethanol, pH 2 extract of grape berry homogenate (Figure 2).

It was found that in wine-like extracts, both grape tannin (Figure 3) and anthocyanin concentrations were strongly correlated to wine tannin ($R^2$=0.91), anthocyanin($R^2$=0.94) and color density achieved during the skin contact period. Also, both Cabernet sauvignon and Shiraz fitted the same relationship, indicating that a variety-specific model is not required, at least for these two major Australian varieties. Thus an indication of the likely extractable tannin from grapes can be determined by using this wine-like extraction procedure.

A poor relationship ($R^2$=0.46) was observed between grape tannin concentration in homogenate extracts and final wine tannin concentration, but a strong relationship ($R^2$=0.92) was found for anthocyanin concentration (Figure 4). When the data obtained from 50% ethanol extraction of homogenate was treated separately by individual grape variety, a stronger relationship between grape tannin and wine tannin concentration was observed for Shiraz ($R^2$=0.91) and Cabernet sauvignon ($R^2$=0.75). Thus the 50% ethanol grape homogenate extracts which are commonly prepared in Australia to measure grape colour can be used to give an indication of extractable grape tannin, although for this dataset Shiraz is predicted with higher accuracy than Cabernet Sauvignon.

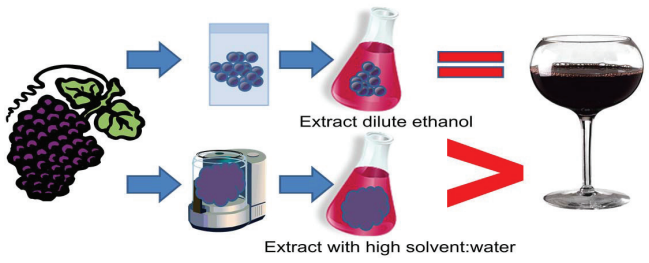


**Figure 2.** Whole grape extracts were prepared either by using hand crushed berries in acidified 15% ethanol or more complete grape extractions using 50% ethanol following homogenisation. One kg fermentations were then performed and the tannin concentration in each type of grape extract was compared with the final wine tannin concentration.


## Summary

Principal Component Analysis (PCA) with UV-Vis spectra of grape homogenate extracts shows some separation by grade or region, but not distinct. NIR and MIR show very little separation with the first PC's so data structure is more complex. Overall, the results suggest that mid infrared (MIR) spectra of grape homogenate, with PLS regression modelling is a promising technique to assist in assessing/predicting grade. Using chemical data and PLS regression gives moderate ability to predict grade and has identified some 'lead' targets to monitor for the 2014 sample set.

In addition, we propose a new grape objective measure of quality, 'wine extractable tannin' to complement the existing 'total grape tannin' measure. In a commercial context, decision-making regarding the optimal winemaking approach for particular grape batches could be enhanced by knowledge of the tannin and anthocyanin extractable during fermentation. For example, for high-tannin grapes, adequate extraction of phenolics may be obtained during a short skin contact period, whereas for low-tannin grapes extending maceration time may be considered. The benchmarking of grape samples for their predicted wine tannin, anthocyanin, and color could facilitate com-


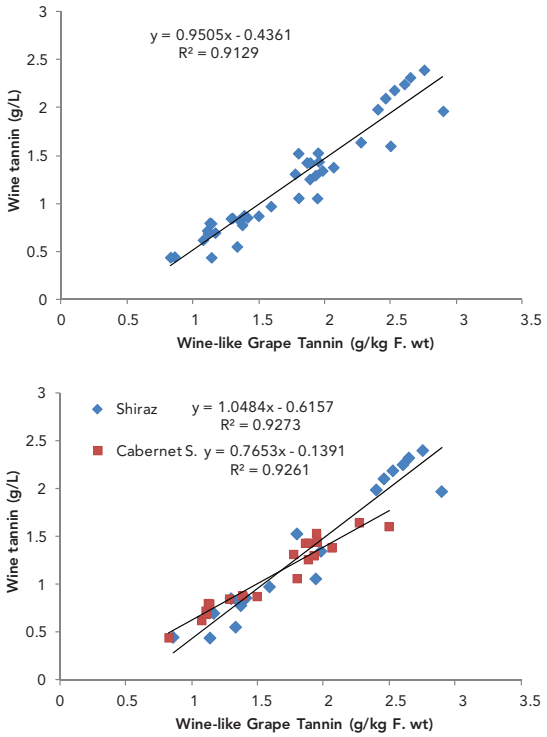


**Figure 3.** Strong correlations were observed between wine-like grape extracts in 15% ethanol and the final tannin concentration in the wine for Australian Cabernet Sauvignon and Shiraz.

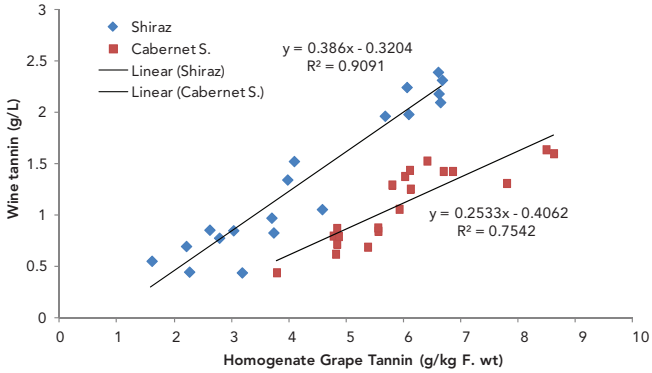


**Figure 4.** When the data obtained from 50% ethanol extraction of homogenate was treated separately by individual grape variety, a stronger relationship between grape tannin and wine tannin concentration was observed for Shiraz ($R^2$=0.91) and Cabernet Sauvignon ($R^2$=0.75).

parisons by season, region, vineyard, grape variety or management practices, and would be of great advantage in winemaking.

Developing an understanding of the synergistic relationships of available objective measures to well-established subjective systems has the potential to significantly reduce production costs and increase value by ensuring that fruit is used in the most efficient production stream and that maximum value is returned from the end product. It also may lead to significant savings in the costs of monitoring crops through more effective application of resources and clearer understandings of geographical and climatic drivers. The maintenance of strong relationships along the value chain between key participants such as grapegrowers and winemakers is central to a sustainable Australian wine sector. Objective measures of quality may contribute significantly in some sections of the wine community to ensuring transparency, trust and the maximisation of value is achieved by providing an objective framework within which all parties understand what is expected to achieve the highest value and most effective use of available resources. Improving the profitability of vineyard enterprises and wine companies in rural Australia has social advantage to the regions.

## Acknowledgements

The Australian Wine Research Institute, a member of the Wine Innovation Cluster in Adelaide, is supported by Australian grapegrowers and winemakers through their investment body, the Australian Grape and Wine Authority with matching funds from the Australian Government.

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