GRAPE AND WINE ANALYSIS – ENHANCING THE POWER OF SPECTROSCOPY WITH CHEMOMETRICS. A REVIEW OF SOME APPLICATIONS IN THE AUSTRALIAN WINE INDUSTRY

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Abstract

Spectroscopic techniques offer the potential to simplify and reduce analytical times for a range of grape and wine analytes. It is this aspect, together with the ability to simultaneously measure several analytes, which was the impetus for developing spectroscopic methods. The Australian Wine Research Institute (AWRI) has long used spectroscopic analysis of wines in the ultraviolet (UV) and visible (Vis) wavelengths, and since 1998 has been investigating applications of spectroscopic techniques in the near infrared (NIR) and mid-infrared (MIR) wavelength regions of the electromagnetic spectrum for the rapid analysis and quality control of both grapes and wine by the Australian wine industry.

This paper reviews the use of several spectroscopic techniques, including NIR, MIR, and Vis, combined with chemometrics, to assess grape and wine composition in the Australian wine industry. The achievements, current research, and proposed further applications of different spectroscopic techniques are discussed in studies into the assessments of red grape composition and of fungal diseases in grapes, monitoring phenolic compounds during red wine fermentations, quality grading of red, white and fortified wine styles, monitoring wine distillation processes, and yeast strain classification.

Introduction

The Australian Wine Research Institute (AWRI) has been investigating the application of rapid analytical techniques such as visible (Vis), near infrared (NIR) and mid-infrared (MIR) spectroscopy since 1998. The project has had a primary focus on furthering the development of NIR methods for the rapid analysis of red grapes, including total anthocyanins, total soluble solids (TSS, °Brix) and pH. This is because the measurement of red grape colour (expressed as total anthocyanins) has shown great promise as an indicator of red wine quality, especially in the low to medium colour range (Kennedy 2002). It was demonstrated that for a set of wines made under carefully controlled conditions with grapes from a specific region, red grape colour was correlated with both wine quality score and wine flavour intensity (Francis et al. 1999, 2005, Gishen et al. 2002, Gishen and Dambergs 2002a,b, Kennedy 2002). However, since the laboratory techniques for the determination of red grape colour are time consuming and laborious, NIR spectroscopy offers the capability of rapid analysis (Gishen and Dambergs 1998).

The objectives of this review paper are to discuss the advances made with several spectroscopic techniques used in combination with chemometrics, to assess grape and wine composition in the Australian wine industry.

Spectroscopy and chemometrics

No single technique can solve all analytical problems but spectroscopic techniques in the Vis, NIR, and MIR wavelength regions of the electromagnetic spectrum have many advantages over chemical, physical and other instrumental methods of food and beverage analysis (Osborne et al. 1993, Shenk and Westerhaus 1993).

Among these techniques NIR spectroscopy has come of age through the development of computers for the application of multivariate statistical methods to easily acquired reflectance and transmission spectra. Herschel discovered infrared energy in 1800; however at that time it was not known that there was a continuous spectrum of electromagnetic energy (Batten 1998, Deaville and Flinn 2000, McClure 2003).

The near infrared region of the electromagnetic spectrum lies between the visible and infrared region and is usually defined by the wavelength range between 700 to 2500 nanometres (nm) (Osborne et al. 1993, Deaville and Flinn 2000). However, most analytical applications of NIR use the wavelength range between 1100 and 2500 nm (McClure 2003). This region is characterised by absorption bands caused by stretching vibrations of hydrogen (H) covalently bonded to either carbon (C), oxygen (O) or nitrogen (N) atoms (Osborne et al. 1993, Batten 1998). The absorption bands seen in the near infrared region are the weaker overtones (between 1000 and 1900 nm) and combinations (between 1900 and 2500 nm) of the much stronger fundamental vibrations that occur in the MIR region (Osborne et al. 1993, Deaville and Flinn 2000).

Traditionally, much of the research into wine composition has been conducted in a manner that can be described as 'univariate' in nature, since it only examined the effect (response) of a single variable on the overall matrix (Munck et al. 1998, Jaumot et al. 2004). At the time that many statistical methods were developed, i.e. around the 1920s (Bendell et al. 1999), samples were considered cheap and measurements expensive. Since that time, the nature of technology has changed, so that now, relatively speaking, samples are expensive and measurements cheap. Analysing the effect of one variable at a time by analysis of variance (ANOVA) techniques can give useful descriptive information, but this will not give specific information about relationships among variables and other important relationships in the entire matrix (Wold 1995, Martens and Naes 1996). Multivariate analysis (chemometrics) was developed in the late 1960s, and introduced by a number of research groups in chemistry, mainly in the fields of analytical, physical and organic chemistry due to (1) the introduction of instrumentation giving multivariate responses for each sample analysed, and (2) the availability of computers (Wold 1995, Munck et al. 1998, Otto 1999, Siebert 2001).

With modern chemical measurements we are often confronted with so much data that the essential information may not be readily evident. Certainly that can be the case with chromatographic or spectral data for which many different observations (peaks or wavelengths) have been collected. Each individual measurement can be thought of as a different dimension. Traditionally as analysts, we strive to eliminate matrix interference in our methods by isolating or extracting the analyte we wish to measure, thus making the measurement apparently simple and certain. However, this ignores the possible effects of chemical and physical interactions between the large amounts of constituents present in the sample – this will be especially evident for such complex materials as grapes and wine. Univariate models do not consider the contributions of more than one variable source and can result in models that could be an oversimplification. Therefore, we need to look at the sample in its entirety, and not just at a single component, if we wish to untangle all the complicated interactions between the constituents and understand their combined effects on the whole matrix. The secret to understanding lies in the matrix itself. Multivariate methods provide the means to move beyond the one-dimensional (univariate) world. In many cases, multivariate analysis can reveal constituents that are important through the various interferences and inter actions (Wold 1995, Geladi 2003).

Today, many food quality measurement techniques are multivariate and based on indirect measurements of the chemical and physical properties (Martens and Naes 1996, Geladi 2003). A typical characteristic of many of the most useful of these instrumental techniques is that, paradoxically, the measurement variable might not have a direct relationship with the property of interest, for instance the concentration of a particular chemical in the sample – that is, the technique is a correlative method. The explanation for this is often found in chemical and physical interferences. Spectroscopic techniques provide the possibility of obtaining more information from a single measurement because they can record responses at many wavelengths simultaneously, and it then becomes essential to use multivariate analysis in order to extract the information. Specific details of the numerous algorithms, formulas and procedures used in multivariate analysis can be found in more specialised literature (Wold 1995, Martens and Naes 1996, Munck et al. 1998, Otto 1999, Siebert 2001, Geladi 2003).

Application of spectroscopy in grape and wine analysis

Red grape composition
The Australian wine industry continues to search for the means to objectively assess the composition of grapes and wines for quality control and payment purposes (Gishen et al. 2000, 2002, Gishen and Dambergs 2002a,b, Francis et al. 2005). Conventional laboratory techniques for the determination of different quality characteristics of grapes and wine are tedious and time consuming. For example, the conventional method for total anthocyanins involves extraction, centrifugation, dilution then equilibration prior to measurement (Cynkar et al. 2004a,b). These time-consuming and technically demanding procedures are a barrier to the widespread uptake and use of quality descriptors by the grape and wine industry (Gishen et al. 2000). NIR spectroscopy with its inherent speed and relative cost effectiveness compared to traditional laboratory techniques, has been subjected to increasing attention as a technique for the rapid determination of several grape and wine quality parameters.

The most common use of NIR spectroscopy in winery laboratories has been for the determination of the alcohol content of wine (Osborne et al. 1993, Medrano et al. 1995, Dambergs et al. 2004). This technology has now been put into practice by several large wine companies for determination of the concentration of total anthocyanins (colour) in red grapes (Gishen et al. 2002, Kennedy 2002). The AWRI initially concentrated efforts on development of an NIR method for determining colour (total anthocyanins), total soluble solids (TSS) and pH in red grapes that would be suitable for practical industry use (Dambergs et al. 2000, 2003, Esler et al. 2002, Gishen et al. 2002).

The prediction of quality parameters in red grapes using NIR began with the scanning of homogenised grape samples using a research grade laboratory NIR spectrophotometer and the development of calibration models between reference values and NIR data, and the test or validation using unknown samples (Dambergs et al. 2003). With NIR methods for total anthocyanins in red grapes, it has been observed that with large data sets incorporating many vintages, regions and grape varieties, partial least squares (PLS) calibrations show pronounced non-linearity (Dambergs et al. 2003) (Figure 1a). Non-linearity can be corrected with the LOCAL algorithm (local weighted regression) (Figure 1b) due to its property of 'local' spectral matching of unknowns with calibration samples (Shenk and Wester haus 1993, Dambergs et al. 2003).
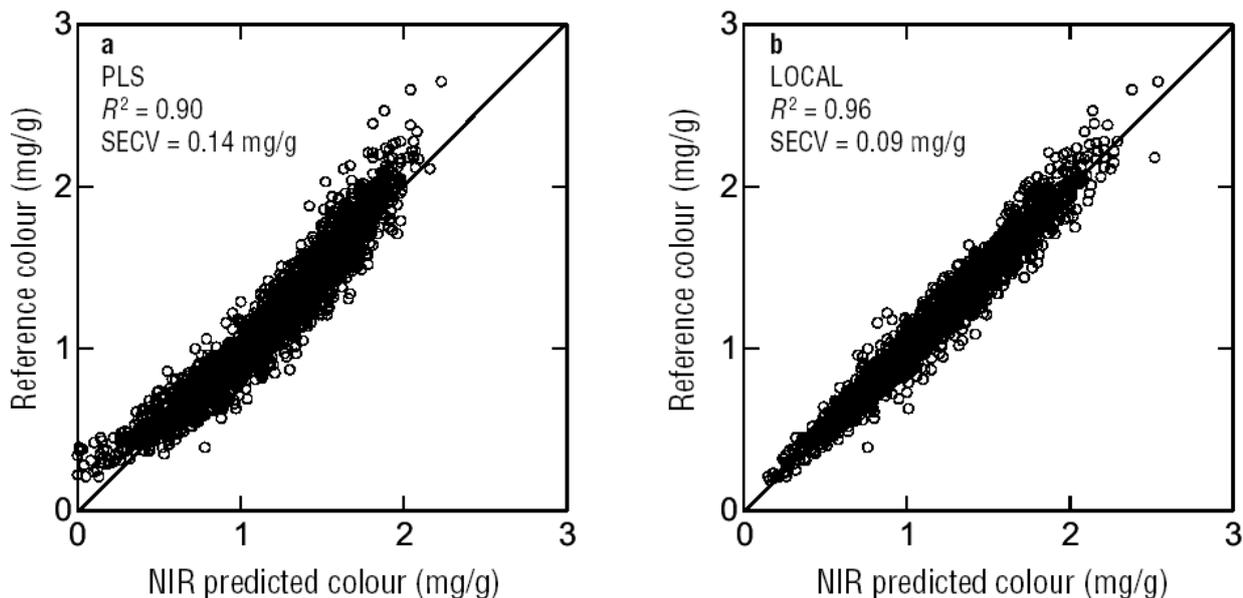

Figure 1. A comparison of red grape colour prediction performance using (a) partial least squares (PLS, The Unscrambler) or (b) LOCAL (WinISI) algorithms. Data are for approximately 2300 A comparison of red grape colour prediction performance using (a) partial least squares (PLS, The Unscrambler) or (b) LOCAL (WinISI) algorithms. Data are for approximately 2300 samples from 3 vintages, 10 regions and 10 grape varieties

As an alternative for grape colour calibrations, a simple way to reduce error is to split samples into analyte ranges, irrespective of other classifying parameters such as region, vintage and variety (Dambergs et al. 2003) (Table 1).

Table 1. Partial least squares (PLS) calibration statistics for NIR measurement of red grape colour (mg/g) in samples from 3 vintages, 10 regions and 10 varieties separated by colour range (mid-range: 0.5–1.5 mg/g; extreme-range: >-1.5, <-0.5 mg/g).

Calibration set	n	Validation set	SECV	SEP	R^2
All samples, 1999–2001	2287	–	0.13	–	0.92
Mid-range colour	1618	–	0.09	–	0.89
Mid-range colour	1618	Extreme-range colour	–	0.16	0.92
20% Subset of mid-range	324		0.09	–	0.91
20% Subset of mid-range	324	80% Subset of mid-range	–	0.10	0.87

Where n is the number of samples, SECV is the standard error of cross validation, SEP is the standard error of prediction, and R2 is the coefficient of determination.

However, to predict the whole range encountered pre-supposes that sufficient calibration samples are available at all levels across the range. The recent availability of faster Vis-NIR spectrophoto meters with alternative optical configurations has provided the possibility of presenting the grape samples to the instrument without the need for homogenisation (Cozzolino et al. 2004a, 2005c). The possibility of simplifying the sample presentation for NIR prediction of colour, TSS and pH could dramatically increase sample throughput. This mode of presentation offers the possibility of scanning whole berry samples, or even single berries. Preliminary investigations for whole grape berry presentation using a diode array spectrophotometer are promising, indicating that NIR may have potential for use at the weighbridge or for in-field analysis (Cozzolino et al. 2004a). Further details on data acquisition, calibration development and validation can be found elsewhere (Esler et al. 2002, Dambergs et al. 2003).

Fungal diseases in grapes
In measuring grape quality, there is also a need for objective measures of negative quality parameters such as the degree of mould contamination, particularly with mechanically harvested grapes, where visual assessment can be difficult. We have explored the use of NIR spectroscopy for the detection of powdery mildew, caused by the fungus *Erysiphe necator* Schwein. var. necator (formally *Uncinula necator),* in wine grapes (Dambergs et al. 2005a).

Samples of Chardonnay grapes with varying degrees of powdery mildew infection (classified visually) were homogenised then scanned in reflectance mode over a 400–2500 nm wavelength range. The homogenates were also analysed for powdery mildew DNA content (Stummer et al. 2002). The powdery mildew DNA content correlated with the visual infection classification and strong spectral correlations with infection level were also observed, including spectral changes not related to pH and TSS differences. There were no systematic inter-correlations of infection level with pH and TSS, precluding the possibility that the infection level calibrations could be based on these parameters.

The spectral data were reduced by principal component analysis (PCA) and the first four PCA scores were used with a quadratic discriminant analysis algorithm to classify infection level: 100% classification was achieved in calibration mode and 92% classification using cross-validation. The PLS analysis was used for prediction of the powdery mildew DNA content using the spectral data and the degree of accuracy was sufficient to clearly discriminate the lowest infection level (1–10%) from the uninfected samples. Only two PLS factors were used, indicating the strength of the spectral correlations, but it must be noted that this was a small data set and the results must be confirmed with larger, more diverse sample sets. The implication of this work is that it might be possible to discriminate

infected fruit at the weighbridge to provide a 'go/no-go' test to identify suspect fruit for further detailed analysis to determine suitability for winemaking.

Monitoring phenolic compounds during red wine fermentation

Investigations of process scale red wine fermentation trials conducted at the Hickinbotham Roseworthy Wine Science Laboratory at the University of Adelaide in three vintages were carried out with the objective of examining the ability of NIR spectroscopy to predict the concentration, and monitor the extraction and evolution, of phenolic compounds during red wine fermentation. If successful, NIR spectroscopy techniques offer advantages as a rapid, low cost and non-invasive tool for monitoring the fermentation process.

Samples were sourced from fermentation trials conducted during the 2001, 2002 and 2003 vintage harvests using two different types of fermenters, and inoculated with different yeast. Details of the experimental design have been presented elsewhere (Cozzolino et al. 2004b). Preliminary results showed that NIR spectroscopy could predict the concentration of major anthocyanins such as malvidin-3-glucoside (R^2 = 0.91 and SECV = 28.0 mg/L), pigmented polymers (R^2 = 0.87 and SECV = 5.9 mg/L), and tannins (R^2 = 0.83 and SECV = 131.1 mg/L), in Cabernet Sauvignon and Shiraz wines during fermentation (Cozzolino et al. 2004b). However the specificity of the calibrations developed must be confirmed, as there are many simultaneous changes occurring during fermentation.

Principal component analysis (PCA) was performed in order to identify the dominant patterns in the spectra of the sample set. The PCA revealed that the first four principal components (PCs) explained almost 99% of the spectral variation of the samples. Figure 2 shows the first three PCs for the Cabernet Sauvignon fermentations.
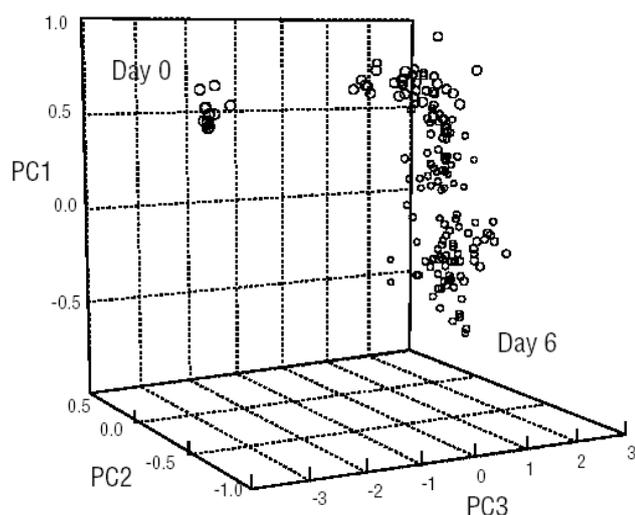

Figure 2. The relationship between the first three principal component scores of the spectra of samples taken during the time course of Cabernet Sauvignon fermentations

It is of note that both varieties showed the same trend or pattern in PC scores during the time course of both the fermentation process itself and maturation of the wine after pressing. This suggests that it might be possible to qualitatively monitor fermentations directly using NIR spectroscopy without the need for any compositional analysis (traditionally the fermentation is monitored through the analysis of sugars, temperature, glycerol, yeast count, etc.).

The highest loading on the first PC, which explained almost 80% of the variation, was around 540 nm (Figure 3). Absorption in this spectral region is dominated by colour pigments, principally anthocyanins, and is characteristic for red wines. PC2 explained another 15% of the variation and the loadings plot showed a high and inverse correlation with the visible region around 540 nm, and high positive correlation with the region around 2200– 2300 nm.
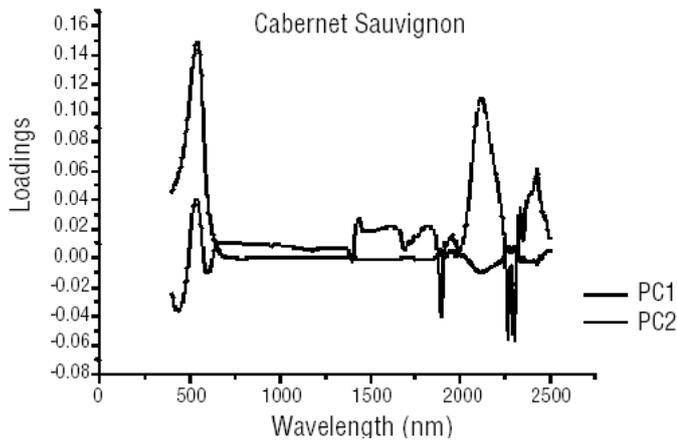
Figure 3. Loadings for the first two principal components of the spectra of samples taken during the time course of Cabernet Sauvignon fermentations

Other researchers have assigned the latter region as relating to complex reactions involving tannins and phenolic compounds (Soukupová et al. 2002). The various components of the wine matrix change during fermentation, in complex yet inter-related ways – spectroscopy and chemometrics offer simplified methods for monitoring this process, allowing better control to achieve a desired outcome.

Wine quality grading

Red wines

The ability to accurately assess wine quality is an important part of the winemaking process, particularly when allocating batches of wines to styles determined by consumer requirements. Grape pricing is often determined by the quality category of the resulting wine – so called 'end use' payment.

Wine quality, in terms of sensory characteristics, is normally a subjective measure, performed by experienced winemakers, wine competition judges or wine tasting panellists. By nature, such assessments can be biased by individual preferences and may be subject to day-to-day variation. An objective quality grading method would therefore be of great assistance in the wine industry. Flavour compounds are often present in concentrations below the detection limit of NIR spectroscopy, but the more abundant organic compounds offer possibilities for objective quality grading by this technique. It has also been demonstrated that wine quality rankings (as the score or allocation assigned to wines by sensory panels) for red and fortified wines could be discriminated by Vis-NIR spectroscopy (Dambergs et al. 2002a). Furthermore, it has been demonstrated that Vis-NIR spectroscopy can predict wine quality as judged by both commercial wine quality rankings and wine show scores (Dambergs et al. 2000, 2002a).
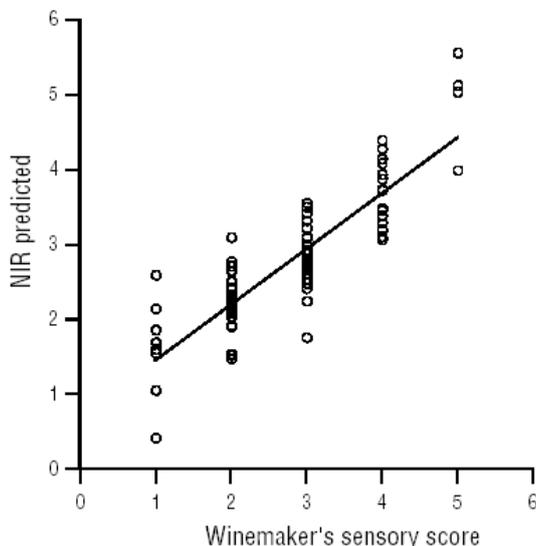

Figure 4. Relationship between actual and predicted values for Cabernet Sauvignon wine grade using a calibration prepared from Vis–NIR spectra of commercial wine

Figure 4 shows a correlation plot of wine end-use quality grading versus NIR predicted quality values for Cabernet Sauvignon dry red wines. The reference quality scores were segmented by category (on a 1–5 scale, with 1 being lowest quality category), whereas the NIR values were on a continuous scale with fractions of a grade predicted – this would have reduced the R2 (0.76) value for the prediction of quality. Due to the segmented nature of the reference data the theoretical minimum error when attempting to predict fractions of scores was a grade score of 0.5 – the SECV of the NIR predicted values was 0.6 grade points. The Cabernet Sauvignon dry red calibration used PLS factor loadings in the wavelengths related to wine pigments (anthocyanins and polymerised variants thereof) (Somers 1998), ethanol and possibly phenolic compounds (Osborne et al. 1993, Cozzolino et al. 2004b), (Figure 5). Only a small number of factors were required and the strongest loadings were in the anthocyanin / polymerised pigment spectral region (400–700 nm).
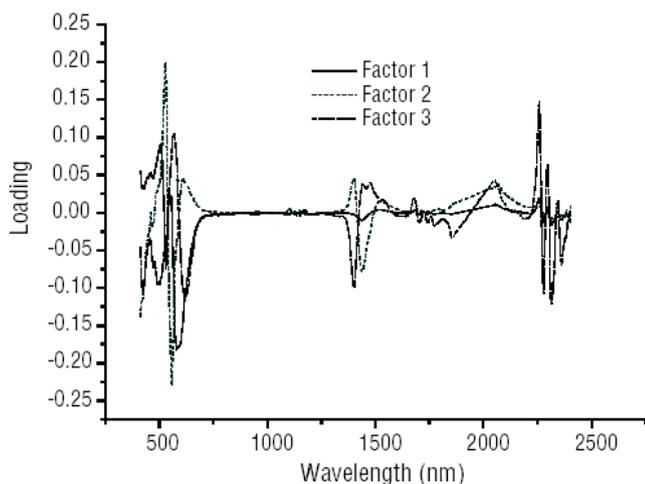

Figure 5. Wavelength loadings for the three partial least squares regression factors used in a quality grading calibration of Cabernet Sauvignon wine

Restricting the wavelengths to 400–700 nm produced calibrations of similar accuracy to those using the full available wavelength range (400–2500 nm), highlighting the importance of the visible region (Dambergs et al. 2002a). This application could provide a rapid assessment or pre-screening tool to add to the range of analyses available to winemakers. It could allow preliminary blend allocation of large numbers of batches of wines prior to sensory assessment. Winemakers may be able to develop 'profiles' for their blends as in-house NIR calibrations.

NIR calibrations based on sensory scores will tend to be difficult to obtain due to variation between individual wine tasters, and may not pick up compounds that are present at low concentrations yet have potent sensory properties. Nevertheless, interpretation of spectral data may provide valuable insight into the more abundant parameters affecting wine quality and highlight the interactions that occur within the complex wine matrix in governing sensory properties (Dambergs et al. 2002a, Francis et al. 1999, 2005, Gishen et al. 2000).

The correlations between NIR spectra and sensory data obtained using wine show samples were less significant in general, in comparison with the commercial grading data shown, for example, in Figure 4 (Dambergs et al. 2002a). The commercial samples were all from one major producer, from one growing area and were graded immediately ex-vintage, with minimal oak treatment. With most dry red classes in wine shows the samples may span vintages, growing areas and winemaking styles, even though they may be made from only one grape variety.

For dry red wines, the best calibrations were obtained with a class of Pinot Noir – a variety that tends to be produced in limited areas in Australia and would represent the least matrix variation. Similar to the winemakers allocation trial, the highest loadings were observed predominantly in the Vis region (wine pigments).

Fortified wines

With wine show data, the strongest correlations between spectral and reference data were obtained with a tawny port class and the SECV represented a 6% error, relative to the mean score (Dambergs et al. 2002a). During the maturation process of sweet fortified wines, Maillard browning compounds are formed and water is lost through the barrels in preference to ethanol, producing 'concentrated', darkly coloured wines with high alcohol content.

The tawny port quality calibrations indicated strongest loadings in the water regions of the spectrum, suggesting that 'concentration' of the wines was important, whilst the visible and alcohol regions of the spectrum also featured as important factors. It has previously been demonstrated that NIR can predict alcohol and total dry extract in this type of wine (Medrano et al. 1995) – the wine show calibrations suggested that these are both important quality parameters.

White wines

Commercially available bottles of Australian Riesling wines were sourced from a broader wine flavour study (Cozzolino et al. 2003, Smyth et al. 2003). The wines showed a diversity of sensory characteristics and ranged in vintage from 1993 to 2002. The wines were from several viticultural regions within the states of South Australia (60%), Victoria (15%), Western Australia (10%), New South Wales (5%), and Tasmania (5%), with the remainder being non-regional blends (5%). Analyses were made of the PLS calibration models developed at different wavelength regions for honey, estery, lemon, caramel, toasty, perfumed floral and passionfruit aroma properties, and overall flavour and sweetness palate properties in white wines as assessed by a trained sensory panel.

The results showed good correlations between spectral and sensory properties (R > 0.70) for estery, honey, toasty, caramel, perfumed floral and lemon, while poor correlations (R < about 0.55) were found in most cases for passionfruit, sweetness and overall flavour, respectively. Overall, the best calibration statistics were obtained when the combination of Vis-NIR regions was used.

The results show that honey, estery and lemon aroma characters were well predicted using the Vis-NIR regions. It was also clear that the volatile chemical composition of the sample alone did not account for the correlations that were observed (Smyth et al. 2003), and that other perhaps less specific methods such as sensory, which are a manifestation of the complex interaction occurring within the whole of the matrix, have much to contribute. However, in developing such models, it is known that the more properties (variables) used for classification, the more objects (samples) are needed to get a robust model. In the study described here, only a limited number of wines were used, and therefore, caution must be exercised in extending the applicability of the technique until further validation work is completed (Cozzolino et al. 2005a).

Grape spirit

Grape spirit is produced by distillation of wine or wine- and grape-derived process waste, and is used in the production of fortified wines. Methanol concentrations in grape marc, one of the major sources of distillation raw materials, can be high due to the action of mould and bacteria in the raw product (Dambergs et al. 2002b). The methanol concentration in the final product must be minimised to comply with food regulations, and operating continuous stills can be difficult without rapid feedback of methanol analytical data to allow fine tuning of the stills in a timely manner.

Opportunities for monitoring of the wine distillation process by NIR spectroscopy have been demonstrated (Dambergs et al. 2002b). In comparison to wine, the distillation process streams represent relatively simple matrices consisting of predominantly ethanol, water and minor quantities of other volatile organic compounds. Two key analytes that are routinely monitored during the distillation process are ethanol and methanol, which have characteristic NIR spectra based on differences in relative concentrations of CH_3 groups, wavelength shifts for OH groups and a CH_2 group unique to ethanol (Figure 6). NIR calibrations have been developed for both compounds using PLS and multiple linear regression (MLR) methods with transmission spectra of wine fortifying spirit using gas chromatography as the reference method. The PLS calibrations approached the accuracy of the

reference methods, with an R2 of 0.99 and a SECV of 0.06 g/L for methanol and an R2 of 0.96 and SECV of 0.08 % v/v for ethanol.
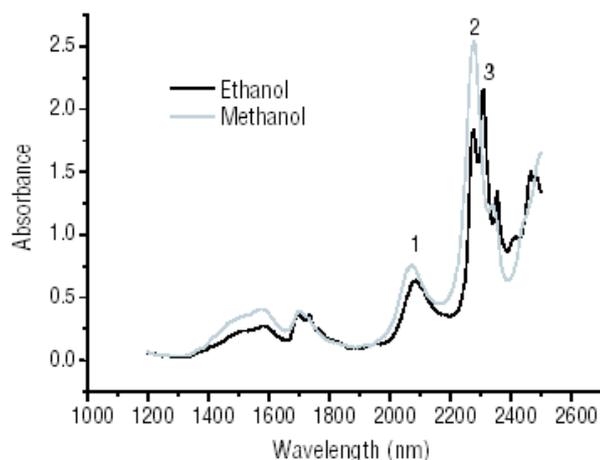

Figure 6. Near infrared spectra of ethanol and methanol (transmission, 1 mm pathlength).
Region 1 relates to OH vibration combinations, region 2 relates to CH combinations from the CH3 group and region 3 relates to CH combinations from the CH2 group.

The calibrations were very robust as indicated by high values for the ratio of the standard deviation of the reference data to the standard error of prediction of the calibration. The ratios for calibrations for various distillation fractions were in the range of 10 to 20, implying they were robust and suitable for routine analytical use (Fearn 2002). MLR calibrations were less accurate, but nevertheless robust across vintages (Dambergs et al. 2002b).

Although the distillate matrices are relatively simple, the concentration of the analytes can vary in the order of a hundred-fold between various distillation fractions (process streams), necessitating the use of separate calibrations for different fractions to attain best accuracy. Nonlinearities and wavelength shifts are observed in mixtures of alcohols as a result of hydrogen bonding changes and polymerisation. Nevertheless, with suitably sized spectral databases, locally weighted regression or artificial neural network (ANN) calibrations may offer improved accuracy by ameliorating these effects, allowing the use of a single calibration model for all distillation fractions (Li et al. 1999, Dambergs et al. 2002b).

Yeast strain classification by near infrared spectroscopy and soft independent modelling of class analogies (SIMCA)

In the last few years, both microbial and plant metabolite analysis has shifted from specific assays toward methods offering both high accuracy and sensitivity in highly complex mixtures of compounds. Large-scale metabolome analysis is based on the use of gas chromatography mass spectrometry (GC/MS) and liquid chromatography-MS (LC/MS) (Sweetlove et al. 2004). Both NIR and Fourier Transform NIR (FTNIR) spectroscopy have been examined to assess their suitability as tools for yeast identification (Halasz et al. 1997), yeast protein measurement (Majara et al. 1998), and the detection and identification of bacterial strains (Kansiz et al. 1999, Rodriguez-Saona et al. 2001, Irudayaraj et al. 2002). One of the advantages of NIR spectroscopy is not only the ability of this technology to assess chemical structures through the analysis of the molecular bonds in the near infrared spectrum (O-H, N-H, C-H), but also its capacity to build a spectrum characteristic of the sample, which behaves as a 'fingerprint' of the sample (Downey 1994, 1996). Accordingly, the application of NIR spectroscopy and multivariate analysis as a rapid screening technique to discriminate different yeast strains with particular metabolic profiles was studied.

Samples used were fermentation supernatants of different Saccharomyces cerevisiae deletion strains provided by the Molecular Biology group at AWRI, scanned over the Vis-NIR range (400 to 2500 nm) in transmittance mode with a 1 mm pathlength (Cozzolino et al. 2005b). Multivariate classification models were developed using the spectra of the yeast strains. PCA models for each of the yeast deletion strains were performed and then used to develop soft independent models of class analogies (SIMCA)

classification models (Geladi 2003). The SIMCA models for the yeast strains were based on the wildtype laboratory strain and are presented in Figure 7.
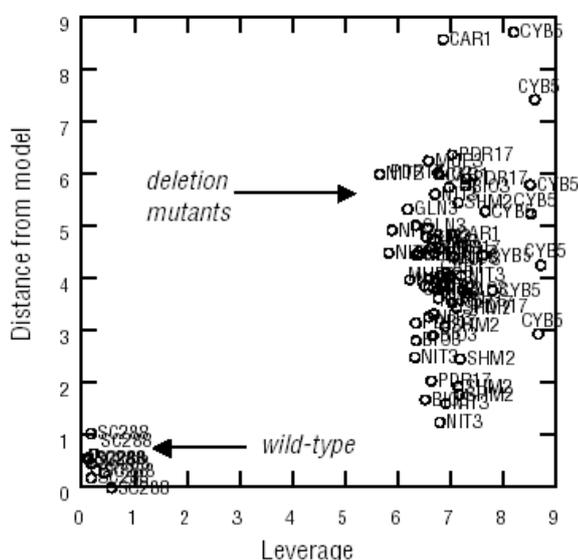

Figure 7. Cooman's plot of a discrimination model applied to classification of yeast strains based on the wild-type laboratory strain SC288 using spectra of fermentation media supernatants

The models showed that the deletion strains were correctly classified as different from the wild-type laboratory strain. This demonstrated the power of combining NIR spectroscopy and multivariate techniques to enable the rapid selection of yeast strains with specific abilities from a large population, and to classify samples that have similar characteristics.

The combination of different analytical techniques with multivariate methods could be used as a tool for fingerprinting yeast strains on a large scale. However, it must be recognised that the chemical basis of this separation is not addressed using this methodology and other analytical techniques (i.e. GC/MS) need to be used in order to reveal the fundamental causes of the separation.

Comparison of NIR and Mid-infrared spectroscopic techniques

Mid-infrared (MIR) has also been investigated as an alternative spectroscopic method to NIR for the rapid prediction of wine and grape properties. The MIR range uses spectral information arising from the fundamental, and very intense, molecular vibrations leading to the overtone vibrations observed in the NIR for C-H, N-H and O-H molecular groups, but is also sensitive to some C-O, C-N and C-S groups not detectable in the NIR range (McClure 2003). It was therefore expected that MIR might offer advantages in enhancing sensitivity for the prediction of some wine and grape properties, although it is known to be insensitive to the colour information observed in the visible spectral region. The better-defined and understood spectral features characteristic of MIR may assist in the interpretation of some of the underlying grape chemistry.

Commercially available instruments using this technology have been available to the wine industry for some time as the AWRI has previously reported (Gishen and Holdstock 2000). Such instruments can be supplied with built-in calibrations for more than 15 analytes in various types of wine or juice, and have found increasing acceptance in the Australian wine industry in recent times (Chris Bevin, pers. comm.).

Studies conducted at AWRI with the MIR technique have investigated the rapid and simple sample presentation method of attenuated total reflectance (ATR) as described several decades ago (Flournoy and Schaffers 1966). With this technique, the grape homogenate sample is simply smeared onto the ATR crystal and scanned, thereby eliminating many of the problems associated with the use of sample cuvettes, which would otherwise require a very small path length in the MIR spectral range.

From a comparison of NIR and MIR-ATR in the analysis of grape homogenates, calibration statistics for the two techniques suggested that MIR may have superior predictive capability for pH and TSS, but is less accurate than NIR for colour (Janik et al. 2005). However, the best prediction for colour was achieved using a combination of MIR and NIR (data not presented).

Another application where MIR outperforms NIR is in the prediction of amino-nitrogen, ammonia and yeast assimilable nitrogen (Table 2) (Dambergs et al. 2005b) in grape juice.

Table 2. Calibration statistics for the prediction of ammonia, amino-nitrogen (amino-N) and yeast assimilable nitrogen (YAN), using near infrared (NIR) and mid-infrared (MIR) spectra

	Near Infrared					Mid-Infrared				
	R^2	F	SECV (mg/L)	SECV (as % of mean value)	SD/SECV	R^2	F	SECV (mg/L)	SECV (as % of mean value)	SD/SECV
Ammonia	0.52	5	15.5	21.5	1.4	0.87	5	8.0	11.1	2.8
Amino N	0.81	8	24.9	9.7	2.3	0.94	8	15.0	5.9	3.8
YAN	0.77	8	34.2	10.9	2.1	0.94	6	18.0	5.7	4.0

Where R2 is the coefficient of determination for the predicted value versus the value measured by the reference method, F is the number of partial least squares (PLS) factors used in the calibration, SECV is the standard error of cross-validation, SD/SECV is the standard error of the reference data over the SECV for the calibration. When the latter value is greater than 3 the calibration is more likely to be robust and suitable for routine use.

Conclusion

Spectroscopy combined with chemometric methods has been shown to be a powerful tool for quality assessment of both grapes and wines. As with many other primary produce-based industries, payment for quality of the raw material is a major issue and will become more important as supply and demand come into balance in the Australian wine industry.

Recent advances in chemometrics software and computing power have greatly enhanced the development of rapid analytical methods based on spectroscopic data and their subsequent application in a wide range of agricultural industries. Although the instrumentation may require a large capital outlay and can be reasonably complex to calibrate and maintain, with more development it is anticipated that more cost-effective, simple instruments will be available for general use by the wine industry.

Further, the technology offers the exciting future prospect of small, cheap, portable hand-held instruments, which would be of great benefit to the whole supply chain of the industry. Spectroscopy offers the ability of simple, rapid testing of grapes, with little or no sample preparation.

Winemakers and grape growers could benefit if the compositional quality of grapes could be rapidly and non-destructively assessed using spectroscopy at the weighbridge or even whilst still on the vine.

Visible (Vis), near infrared (NIR) and mid-infrared (MIR) spectroscopic analytical techniques are beginning to gain acceptance in the wine industry. As the technology of spectroscopic instrumentation and chemometrics advances, the resulting spin-offs may further assist the industry in its quest to define and objectively measure grape and wine quality.

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