Prediction of colour and pH in grapes using a diode array spectrophotometer (400 - 1100 nm)

Cozzolino, D.; Esler, M.B.[a]; Gishen, M., Dambergs, R.G.; Cynkar, W.U.; Boehm, D.R.; Francis, I.L.; Høj, P.B.

The Australian Wine Research Institute. Waite Road, Urrbrae. PO Box 197, Glen Osmond, South Australia 5064, Australia. Fax: + 61 (8) 8303 6601. Email: Daniel.Cozzolino@awri.com.au

[a] *Present address: Chemistry Section, Bureau International des Poids et Mesures, Pavillon de Breteuil, F-92312 Sevres Cedex, France.*

Introduction

During recent years, the combination of novel rapid instrumentation and chemometric techniques has resulted in the development of rapid methods relating multivariate data, such as near infrared (NIR) spectra of samples, to the concentration of specific chemical constituents[1,2,3]. Consequently, the demand for traditional analysis using chemical reagents is reduced. Near infrared (NIR) reflectance spectroscopy is one such instrumental technique that has been applied commercially in Japan for the non-invasive estimation of total soluble sugars of fruit as an indicator of eating quality[4]. Published reports of such applications have largely involved either the use of research grade NIR instrumentation, suited for packing shed or field use, or the use of purpose-built spectrophotometers, unsuited for general applications[5,6].

During the 1990s, several low cost diode array spectrophotometers capable of operation up to 1050 nm became commercially available and have been used for applications in different foods, however, no reports were found in relation to grape analysis[6,7,8]. The high-speed operation of diode array spectrophotometers offers the possibility to acquire spectral information from relatively large surface areas of sample in a short time, and this in turn offers the possibility of their application for on-line analysis. NIR is a physical and non-destructive technique using the region of the electromagnetic spectrum that lies between the visible and infrared region (400 – 2500) nm[2,3,10].

A NIR spectrum of any sample usually consists of many overlapping bands and could be considered as the summation of the individual spectra of its major chemical components[3,9,10]. The NIR region contains information concerning relative proportions of C-H; N-H and O-H bonds of the organic molecules which constitute the matrix of food/agricultural products[3]. The prediction of constituent concentrations in a matrix by NIR requires a calibration to be developed that relates NIR spectral measurements to the desired constituent or property being determined[2,10]. Calibration is the key to successful use of the NIR technique and there are a number of essential steps required to develop a calibration including sample selection, acquisition of spectral and reference data, pre-treatment of spectral data, derivation of the regression model and validation of the model.

The objective of this study was to evaluate the usefulness of a diode array spectrophotometer to predict colour and pH in red grapes.

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Materials and methods

Samples used for scanning were whole grape berry samples of Merlot (n= 24), Shiraz (n= 41) and Cabernet Sauvignon (n= 42) grape varieties, sourced from the Riverland region of South Australia. A further set of samples of Shiraz (n= 13) were collected from the Langhorne Creek region of South Australia. Samples were collected during the vintage of 2001 and were kept frozen for two months at -18° C before scanning.

Homogenates were prepared at room temperature using an *Ultra-Turrax T25* high-speed homogeniser with an *F25N* dispersing head (Janke and Kunkel GmbH Co. Germany). Berry samples of approximately 50 g were homogenised in 120 mL plastic containers at 24,000 rpm for 60 seconds.

The spectrophotometer used for this work was a Zeiss *CORONA* (model: *CORONA 45VISNIR*, Carl Zeiss, Germany) equipped with a 10 W halogen lamp as the light source and a silicon diode array detector able to collect spectra from the visible (VIS) and near infrared (NIR) regions of the spectrum (400 to 1100 nm at 3.2 nm wavelength resolution). Samples of red grape homogenate were placed in a glass beaker (diameter approximately 80 mm) while whole berry samples were placed in a glass Petri dish (diameter 120 mm) for scanning. Both whole grapes and homogenate samples were placed on the sensor head and illuminated from below as recommended by the instrument supplier. Reference spectra were obtained by measuring a white ceramic tile while the dark reference spectra were recorded with a hood over the detector module.

All laboratory analyses were conducted in duplicate and the mean result used as the reference data for calibration development. Duplicate samples (approximately 1g each) from thoroughly mixed homogenates were scooped into pre-tared centrifuge tubes and their masses recorded. Aqueous ethanol (10 mL, 50% v/v) was added to extract the anthocyanins from grape tissue, the tubes capped and the content mixed for one hour using a Chiltern rotating wheel. After this period, the tubes were centrifuged (*Universal 32R Hettich*, Tuttlingen, Germany) at 4000 rpm for 10 min. A 200 μL aliquot of the supernatant was taken and acidified with 3.8 mL of 1M HCl and the concentration of total anthocyanins (expressed as equivalent malvidin-3-glucoside) determined spectrophotometrically at 520 nm using a UV/Visible spectrophotometer (*Cary 300*, Varian, Australia).[11,12,13]

The pH of the grapes was measured on homogenates using an *Orion Advanced* portable pH meter (model *250A*, Thermo Orion, USA) equipped with an *Orion ROSS* epoxy body combination electrode (model *815600*, Thermo Orion, USA).

The *Aspect Plus* software (Carl Zeiss, Germany) was used to acquire the NIR spectra. Spectral and associated laboratory reference data were exported to *The Unscrambler* software (version 7.5 CAMO, Norway) for chemometric analysis and calibration development. Partial least squares (PLS) regression with full random cross validation was used as the method for development of calibrations. The cross validation was performed using six segments, with 19 samples for each segment. No pre-treatments or smoothing were applied to the spectra prior to calibration regression. Calibrations were developed using the visible (VIS: 400–700 nm), NIR (700–1100 nm), and VIS+NIR (400–1100 nm) wavelength regions. The coefficient of correlation (R^2_{cal}) and the standard errors in calibration (SEC) and cross validation (SECV) were calculated.

Results and discussion

The absorption band found around 540 nm (Fig. 1) is assumed to be related principally to anthocyanin pigments which are the major source of colour in red grapes[12,13]. Ionised anthocyanins absorb at this wavelength and have a red colour, but there are a number of other forms that can be

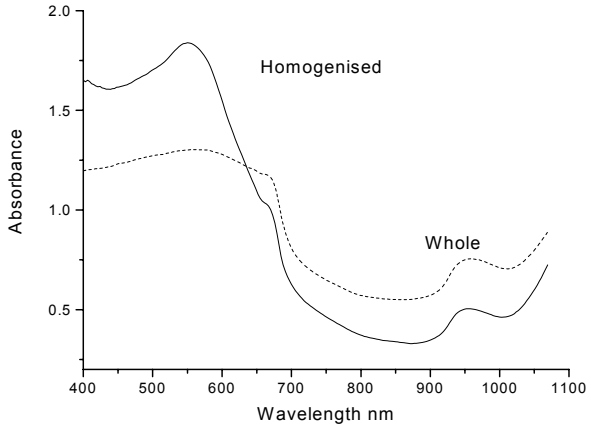

Figure 1. Vis and NIR mean spectrum of homogenised and whole grape samples.

colourless, blue or yellow[12,13]. However, the high absorbance observed in the visible region between 500 to 700 nm can also be indicative of an abundance of chlorophyll as well as other red pigments (carotenoids as well as anthocyanins) in fruits and vegetables[5,12,14]. In the region 400 to 600 nm, the absorbance spectra for whole grapes were generally lower than those of homogenised grapes.

The absorption band at 970 nm (O-H second overtone) is principally associated with the water content of the grape berries (ripe grape berries typically contain 75 to 80 % water by weight)[15]. However, there may be some overlap with absorption related to the CH stretch third overtone, associated with carbohydrates[15,16].

Statistics for PLS calibrations developed for homogenised grape samples using the visible (VIS: 400–700 nm), NIR (700–1100 nm), and VIS+NIR (400–1100 nm) regions are shown in Table 1.

Table 1. Calibrations for colour and pH on homogenised red grapes.

Constituent	Wavelength range (nm)	n	R^2_{cal}	SEC	SECV	RPD
Colour (mg g^{-1})	400 – 1100	118	0.96	0.05	0.06	4.2
	400 – 700	118	0.92	0.06	0.07	3.6
	700 – 1100	118	0.92	0.07	0.08	4.2
pH	400 – 1100	116	0.81	0.05	0.05	2.2
	400 – 700	116	0.90	0.03	0.04	2.8
	700 – 1100	116	0.64	0.07	0.08	1.4

n, number of samples in calibration after outlier eliminated; R^2_{cal}, coefficient of determination of calibration; SEC, standard error of calibration; SECV, standard error of cross validation; and RPD = SD/SECV.

The coefficients of determination in calibration, $R^2_{cal,}$ for colour were greater than 0.90 for all regions, but were lower than 0.90 for pH. Calibrations for whole grape berries were less successful as shown in Table 2.

Table 2. Calibrations for colour and pH on whole red grapes.

Constituent	Wavelength range (nm)	n	R^2_{cal}	SEC	SECV	RPD
Colour (mg g^{-1})	400 – 1100	118	0.48	0.13	0.14	1.8
	400 – 700	118	0.46	0.13	0.14	1.8
	700 – 1100	118	0.62	0.12	0.13	1.9
pH	400 – 1100	116	0.70	0.07	0.08	1.4
	400 – 700	116	0.62	0.07	0.08	1.4
	700 – 1100	116	0.61	0.07	0.08	1.4

n, number of samples in calibration set; R^2_{cal}, coefficient of determination of calibration; SEC, standard error of calibration; SECV, standard error of cross validation; and RPD = SD/SECV.

For colour the R^2_{cal} were less than 0.70 for all wavelength regions, and for pH, the best calibration was obtained using the full VIS+NIR region (R^2_{cal} = 0.70; SECV = 0.07).

In both presentation modes examined here, the calibration loadings are strongly influenced by the visible region of the spectra (400 to 700 nm) (data not presented). The optimal VIS/NIR calibration model for colour in homogenate samples required the full range (400 to 1100 nm) which contains the absorbance of pigments as well as the water region (980 nm). This may be due to the fact that some of the anthocyanin pigmented forms have absorption peaks that tail into the NIR region, and some forms of anthocyanin are not pigmented. Alternatively, the inclusion of the water peak (980 nm) may be related to cross-correlation of anthocyanin levels with the concentration of total soluble solids. For whole grapes the best VIS/NIR model for colour was obtained using the NIR region alone: this may be related to the fact that spectra of whole grapes appeared to be relatively indistinct in the visible region in comparison with homogenates (Fig.1).

The Ratio of standard error of Performance to standard Deviation (RPD), defined as the ratio between the standard deviation of the population (SD) and the standard error in cross validation (SECV) for the NIRS calibrations, is a useful statistic that is often used to evaluate how well a calibration model can predict chemical data[17]. If the error in estimation for a constituent (SECV) is large compared with the spread in composition of that sample in the population (as SD), and therefore having a relatively small RPD, the NIR calibration models are considered not robust. The higher the value of the RPD the greater the power of the model to predict the chemical composition. An RPD greater than three is considered very good for prediction purposes. In the present work, the RPD was less than three for pH calibrations in both presentations, higher than three for colour in the homogenised presentation, and less than two for colour calibrations on intact presentation.

The relatively poor calibration models obtained for whole grape berries might be explained by several factors such as surface properties of the sample, unevenness of the sample surface and scattering. In addition, the natural variation in degree of ripeness both between and around the surface of intact berries and the resultant variations in skin colour could also contribute to variations in spectral response. In this case a relatively small sample of whole berries was scanned in only one orientation- scanning a larger sample area may result in improved accuracy. It has been reported that

when a piece of fruit or vegetable is exposed to white light, about four per cent of the incident light is reflected at the outer surface, causing specular reflectance[5]. The remaining 96 per cent of incident energy is transmitted through the surface into the cellular structure of the product where it is either scattered by the small interfaces within the tissue or absorbed by cellular constituents[5]. The complex physical structure of the grape tissues creates an optically dense product that is difficult to penetrate and alters the path length travelled by the light so that the amount of tissue analysed is not known with certainty in whole grapes.

Conclusions

The calibration models obtained for homogenised red grape samples suggest that the diode array spectrophotometers have the potential for rapid measurement of colour and pH for that mode of sample presentation. The use of diode array instruments could potentially increase the speed and frequency of analysis of grape composition in the wine industry due to their inherent speed. Intact presentation of red grape berries to the spectrophotometer has been investigated since it may offer advantages in further increasing the speed of analysis and provide potential for on-line or on-farm applications. Diode array spectrophotometers with a limited range of wavelengths are less expensive and more "rugged" than traditional scanning monochromator instruments capable of scanning the full visible and near infrared wavelength range and may be more suited to field work. More research must be done into the spectral variability observed in relation to intact presentation of red grape berries to the instrument to realise the potential benefits of this application in the grape and wine industry.

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