Determination of several phenolic compounds in red wine by near infrared transmittance spectroscopy

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Introduction

Phenolic compounds are some of the main constituents responsible for colour and flavour in red wines[1]. These compounds undergo complex reactions, including condensation and polymerisation resulting in changes in the colour and organoleptic properties of wine[1]. Wine phenolic profiles depend on several parameters such as grape variety, the vinification technique and the numerous reactions that take place during processing and storage[2, 3]. The influence of these alterations on the quality and economic value of wine is undeniable, hence the considerable interest in gaining an understanding of the processes involved.

Recently developed methods for phenolic compound analysis involve time-consuming, laborious, costly procedures and the use of chemical reagents and complicated and expensive techniques including high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy[4]. During recent years, new developments in rapid instrumentation and chemometric techniques have resulted in the development of rapid methods relating multivariate spectral data to the concentration of specific chemical constituents[5]. Near infrared (NIR) spectroscopy is a physical and non-destructive technique[6] that uses the region of the electromagnetic spectrum with wavelengths between 750 and 2500 nm. NIR spectra generally appear smooth since they consist of many overlapped bands[6]. The NIR region contains information concerning relative proportions of C-H, N-H and O-H bonds, hence spectral information from this region can be used to analyse the organic constituents of agricultural products[6].

Recently, reports describing the application of NIR spectroscopy as an alternative to HPLC for the prediction of phenolic compounds in tea and plant extracts have been published[7, 8]. The present study evaluated the use of near infrared transmittance (NIT) spectroscopy as an alternative rapid method to HPLC for predicting phenolic compounds, such as cyanidin-3-glucoside (C3G), delphinidin-3-glucoside (D3G), malvidin-3-glucoside (M3G), petunidin-3-glucoside (P3G), peonidin-3-glucoside (Peo3G), tannins (T), pigmented polymers (PP), quercetin (Q) and vitisin A, in red wine fermentations.

Materials and methods

Grapes of the Cabernet Sauvignon variety were sourced from a single vineyard in the Padthaway region of South Australia. The grapes were machine harvested at optimum maturity (24°Brixon 8th April 2002 and transported to the Roseworthy Hickinbotham Wine Science Laboratory at the Waite Campus of the University of Adelaide. The bins of grapes were divided at random to make up 400 kg lots which were then de-stemmed and crushed and pumped into twelve small-scale temperature

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controlled fermenter tanks, six of each being of two different types, *viz.* rotary (*Vinomatic*, 1100 L) and static (*Potter*, 900 L). The fermenters were then inoculated with yeast cultures. For each type of fermenter, one of two different strains of yeast were used, namely *Saccharomyces cerevisae* (AWR838) and *Saccharomyces bayanus* (AWR1375) from the Australian Wine Research Institute culture collection, that had been cultured aerobically at 20°C in sterile grape juice. The alcoholic fermentation was carried out with each combination of fermenter type and yeast strain controlled at two different temperatures (20 and 28°C). Samples were taken from each tank twice daily (at 8 am and 6 pm) from day zero up to day six. From day six onwards, samples were taken at 8 am only. A total of 166 samples were collected.

Samples from the fermentations were clarified by centrifugation (4 000 rpm for 5 min) prior to scanning in transmission mode (400–2500 nm at an interval of 2 nm) in a rectangular cuvette having a 1 mm path length using a *FOSS NIRSystems6500* scanning monochromator instrument (FOSS NIRSystems, Silver Spring, Maryland USA). Samples were heated to 33°C for 3 min in the instrument before scanning. Spectral collection and spectral data processing were manipulated using *Vision* software (version 1.0, FOSS NIRSystems, Silver Spring, Maryland USA). Data were stored as absorbance calculated as the logarithm of the reciprocal of the transmittance (log 1/T). Reference analysis was conducted using an HPLC method.

Near infrared calibrations were developed using the *WinISI II* software (version 1.5, Infrasoft International, LLC, USA) Equations were developed using modified partial least squares (MPLS) regression with internal cross-validation[9]. Scatter correction and smoothing were performed using standard normal variate and detrend transformations (SNVD)[10]. Mathematical treatments applied to the spectra were (1,4,4,1) and (2,10,10,2). The first number indicates the order of derivative (one is the first derivative of log 1/T), the second number is the gap in data points over which the derivative is calculated; the third and fourth number refers to the number of data points over which the first and second smoothing is applied[9].

Calibration statistics calculated included the standard error of calibration (SEC), the coefficient of determination in calibration (R^2_{cal}), the standard error of cross validation (SECV) and the coefficient of determination in cross validation (R^2_{val}). The optimum calibrations were selected based on minimising the SECV with the highest R^2_{cal}.[9]

Results and discussion

Figure 1 shows the mean spectrum of samples from day zero to day six. Major absorption bands were found for day zero at 1450 nm, and at 1950 nm, related to OH stretch second overtone and OH combination bands of water, respectively[11]. A minor peak near 2270 nm is most likely to be related to glucose and fructose[12] present in high concentrations before fermentation.

After day zero, absorption peaks corresponding to anthocyanin pigments appeared, most noticeably that at 538 nm, related to the ionised forms of anthocyanin moieties[12]. During the course of fermentation, this peak appeared to shift to slightly lower wavelengths. There was also an increase in intensity of the 2270 nm peak, and the appearance and increase in intensity of a peak at 2304 nm. The 2270 nm peak relates to the CH combination band from the CH_3 group of ethanol and the 2304 nm peak relates to the CH combination band of the CH_2 group of ethanol[11, 13].

Anthocyanins have strong, distinct absorbance in the visible wavelength region. In the NIR region, vibrations of CH in aromatic compounds may allow discrimination of phenolic compounds from the matrix (mainly sugar/acid/ethanol/water) of fermenting grape juice. Unfortunately there is a lot of overlap of aromatic CH peaks with other CH peaks resulting from the more abundant matrix

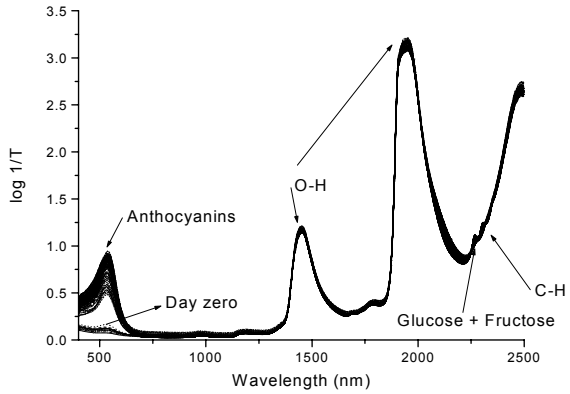

Figure 1. Vis and NIR spectra of red wine during fermentation.

components. However, the aromatic CH combination band at approximately 2150 nm[14] stands clear in this sample matrix: progressive changes in absorbance can be seen in this region during the course of fermentation.

The optimum calibrations were found to be where the first derivative pre-treatment of spectra was used and the calibration and cross validation statistics for the phenolic compounds are shown in Table 1. The coefficients of determination in calibration and cross validation were considered satisfactory and most were higher than 0.90. Further work must be performed to determine the specificity of these calibrations as there are significant cross-correlations among the various phenolic compounds measured and they all increase in synchrony with ethanol during fermentation.

Table 1. Statistics for the sample set and near infrared calibrations for prediction of phenolic compounds in red wine fermentations (first derivative and SNVD).

Constituent	Mean	SD	SEC	R^2_{Cal}	SECV	R^2_{Val}
D3G [1]	6217	2082	485.0	0.95	556.7	0.92
C3G [1]	640	360	81.2	0.95	107.1	0.91
P3G [1]	5000	1659	360.4	0.95	414.2	0.93
Peo3G [1]	3743	797	234.8	0.91	318.9	0.83
M3G [2]	267.3	72.8	9.9	0.98	12.3	0.97
Vitisin A	425	275	42.9	0.96	67.8	0.94
PP [2]	8.9	6.1	1.4	0.98	1.6	0.97
Tannins [3]	102.5	53.7	7.9	0.97	11.0	0.95
Quercetin [1]	1012	584	92.5	0.97	109.8	0.96

Notes: 1. HPLC peak area; 2. mg/L as M3G; 3. mg/L as catechin. Mean is the average concentration of the samples set as determined by the reference method; SD is the standard deviation of the concentration as determined by the reference method; SEC is the standard error in calibration; R^2_{Cal} is the coefficient of determination in calibration; SECV is the standard error in cross validation; R^2_{Val} is the coefficient of determination in cross validation.

Conclusions

We suggest that NIT spectroscopy may be used to predict phenolic compounds in ferments and red wine samples. It offers great potential in research applications as a complementary and alternative technique to HPLC by allowing larger numbers of samples to be measured quickly. The technique also has potential to be used as a tool for on-line fermentation monitoring during commercial winemaking. Further work will be carried out to determine the specificity of the calibrations over different varieties and vintages.

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