Use of Attenuated Total Reflectance Midinfrared for Rapid and Real-Time Analysis of Compositional Parameters in Commercial White Grape Juice

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A simple and fast midinfrared (MIR) spectroscopy method was developed for simultaneously determining total soluble solids (TSS, °Brix), pH, total phenolics, ammonia, free amino nitrogen (FAN), and yeast assimilable nitrogen (YAN) contents in grape juice samples using attenuated total reflectance (ATR). Results from this study demonstrated the capability of ATR-MIR coupled with partial least-squares regression to measure TSS and pH and to monitor FAN, ammonia, and YAN in a wide range of grape juice samples. The standard error in cross-validation and the residual predictive deviation obtained were 0.20 °Brix and 9 for TSS, 0.07 and 3.3 for pH, 14.8 mg/L and 2 for ammonia, 28.3 mg/L and 2 for FAN, and 36.9 mg/L and 2 for YAN, respectively. Both the time of analysis and the volume of sample required were considerably reduced as compared to the transmission MIR measurements currently used by the wine industry.

KEYWORDS: Attenuated total reflectance; midinfrared; phenolics; total soluble solids; pH; grape juice

INTRODUCTION

The increasing interest in sustainable and high-quality production in the agriculture and food industry has prompted the need to develop more automated and precise monitoring analytical systems (*1*). Optimized process control is essential to address safety rules and maintain the commercial viability and sustainable wine industry. This implies, among other aspects, a rapid assessment of the chemical and physical properties of raw materials (e.g., grape and juice), process streams (e.g., ferments), and end products (e.g., wine).

Currently, many sensors are used in the agro-food industries to assess, monitor, and control composition. These sensors can be divided into physical sensors to monitor temperature, pressure, flow rate, and humidity or chemical sensors that are commonly used to assess conductivity, pH, and sugars (*1*). Developing a rapid objective measure of the properties of grapes and wine will allow winemakers to use more precise methods to grow grapes with desirable qualities and will allow wine producers to test hundreds of grape samples in a few hours and plan their farming techniques accordingly (*1*, *2*).

The development of sampling accessories attached to a wide range of infrared (IR) spectrophotometers, such as attenuated total reflectance (ATR) cells, has led to major improvements in routine analysis, by simplifying sample handling and avoiding measurement problems often found using transmission cells (*3*). The ATR sample presentation relies on a sample absorbing the incoming IR radiation by internal reflection from the surface of a high-refractive index transparent medium (*3*). The ATR absorption mechanism is due to two phenomena: a rapid change in sample refractive index across an IR absorption peak and the molar absorption coefficient attributed to the vibrational mode. The change in refractive index can cause severe peak distortions in the midinfrared (MIR) spectrum, particularly if the incident angle is near the critical angle, but such distortions are usually small at the higher angles near 45° used in most ATR accessories (*3*).

Several reports have illustrated the application of Fourier-transformed (FT) MIR spectroscopy using transmission cells to quantify sugars and other compositional parameters in several fruit juices, olive pulp, milk, and wine samples (*4−10*). In commercial FT-MIR spectrophotometers commonly used by the wine industry for routine analysis, grape juice and wine samples are analyzed through a short path length transmission cell. The advantage of the transmission cell is that it provides very accurate and reproducible spectroscopic measurements and is easily temperature controlled. However, the use of transmission cells in FT-MIR spectroscopy has several drawbacks such as filling and cleaning the cell, variation of sample path length due to window wear, and turbidity of the sample, among others (*4*). The aim of this study is to evaluate the potential of ATR-MIR spectroscopy to measure several compositional parameters of grape juice samples currently used by the wine industry.

MATERIALS AND METHODS

Samples and Reference Data. Juice samples were sourced from commercial wineries in South Australia, during 2007 to 2009 vintages. A total of 108 clarified juice samples comprising Chardonnay (CH), Riesling (RH), Viognier (VI), Semillon (SEM), Pinot Gris (PG), and Sauvignon Blanc (SB) were used in this study. Clarified juices were sampled from

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winery tanks before yeast inoculation. Juice samples (c. 500 mL) were immediately frozen after sampling, delivered frozen to AWRI, and kept stored frozen at −18 °C before analysis. Prior to analysis, juice samples were thawed overnight at 4 °C and carefully decanted into clean containers. Total soluble solids (TSS, °Brix) were measured in the juice using a digital portable refractometer (Atago Co., Ltd., Japan). The pH of juices was measured using an Orion Portable Meter (Thermo Scientific, Beverly, MA), equipped with an Orion ROSS epoxy body combination pH electrode (Thermo Scientific) by directly immersing the pH electrode in the juice at room temperature (c. 22 °C). For total phenolics (TP) analysis, 0.3 mL of juice was acidified with 1 M HCl (5.7 mL), and the absorbance was measured at 280 nm, using a Varian Cary 300 (Varian, Mulgrave, Australia) spectrophotometer. TP was calculated according to the method of Iland et al. (*11*) and expressed as absorbance units (au).

A second set of samples was sourced from one winery during 2009 vintage (*n* = 350). Ammonia, free amino nitrogen (FAN), and yeast assimilable nitrogen (YAN) were measured in the juice and ferment samples. In this experiment, fresh nonprocessed juice samples comprising CH, RH, VI, SEM, and PG were obtained and analyzed on the same day of arrival at the winery. Ammonia and FAN were measured using an enzymatic method (Roche Applied Science, Mannheim, Germany), while YAN content was calculated as FAN + (0.824 × ammonia) (*11*).

ATR-MIR Instrument. Samples were scanned using a platinum diamond ATR single reflection sampling module cell mounted in a Bruker Alpha instrument (Bruker Optics GmbH, Ettlingen, Germany). The 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 4000 and 375 cm^{-1}, with a scanner velocity of 7.5 kHz and a background of 64 scans. The reference background spectra were recorded using deionized water. Water was used to clean the ATR cell to avoid carry over between samples and was dried using disposable lab wipes. The negative areas observed in the MIR spectra due to backgrounding were omitted when the calibration and validation models were developed.

Data Analysis. Spectra were exported from the OPUS software in GRAMS format (*.spc) into The Unscrambler software (CAMO ASA, Oslo, Norway) for chemometric analysis. Principal component analysis (PCA) was performed before partial least-squares regression (PLS1) models were developed to determine any relevant and interpretable structure in the data and to detect outliers (*12, 13*). Calibration models between chemical compositional values and MIR spectra were developed using the PLS1 regression method with full cross-validation. The optimum number of terms in the PLS1 calibration models was indicated by the lowest number of factors that gave the minimum value of the prediction residual error sum of squares in cross-validation to avoid overfitting in the models (*12, 14*). The ATR-MIR spectral data were transformed with standard normal variate (SNV) to correct multiplicative interferences and variations in baseline shifts (*15*).

Statistics calculated for the calibrations included the coefficient of determination in cross-validation (R^2), the standard error of cross-validation (SECV), bias, and slope. The prediction accuracy of the TSS, TP, and pH models was tested by full cross-validation using the SECV and the residual predictive deviation (RPD = SD/SECV) (*12, 14*).

To test the prediction accuracy of the calibration models developed for ammonia, FAN, and YAN, the data set was split randomly into training (*N* = 175) and validation (*N* = 175), respectively. Selection of both training and validation sets was performed using the algorithms available in the WinISI (v. 1.50) software package. Samples used for the NIR analysis were selected to represent the whole spectral and chemical variability in the target population in the calibration and validation groups, respectively. The Mahalanobis distance (*H*) was used as a criterion for selecting those samples in the population that were more variable on the basis of spectra features. The ISI algorithm CENTER was used to establish population boundaries with a maximum standardized *H* distance of 3.0. Then, the ISI algorithm SELECT was used for efficient selection, by choosing samples with a minimum standardized *H* distance of 0.6 from their nearest neighbors. Statistics calculated included the coefficient of correlation (*R*), the standard error of prediction (SEP), slope, and bias.
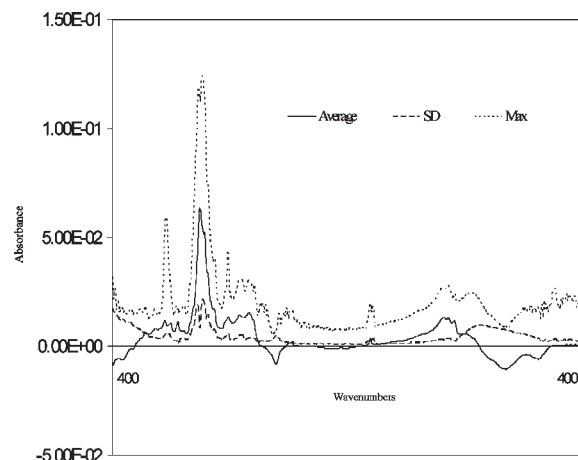
Figure 1. Mean, standard deviation, and 95% confidence interval of ATR-MIR spectra of grape juice samples.

RESULTS AND DISCUSSION

Determination of TSS, pH, and TP. The ATR-MIR spectra of grape juice samples were dominated by intense peaks around 780, 1070, and 1256 cm^{-1} due to water, sugars, and phenolic compounds (**Figure 1**). These peaks are related to the CH−OH and alkyl frequencies for sugars (e.g., glucose and fructose) between 1000 and 1600 cm^{-1} (*16*). Most of the spectroscopic variation was observed around 1100−1550 and 2850−2950 cm^{-1} and was mainly related to different chemical compositions (e.g., sugars and phenolic compounds) of the different varieties of grape juice analyzed. The spectroscopic absorbances of the strongest ATR peaks were far lower than the almost complete absorbance obtained for transmittance spectra reported elsewhere (*2*). Intense and characteristic bands in the region between 1500 and 900 cm^{-1} for sugars (e.g., sucrose and fructose) and organic acids (e.g., malic acid) were observed, as reported by other authors when fruit juice samples were analyzed using ATR-MIR (*17*). Bands between 1500 and 1200 cm^{-1} are assigned to deformations of −CH$_2$ and deformations of C−C−H and H−C−O, respectively (*17−20*), whereas peaks between 1200 and 950 cm^{-1} are explained by stretching modes of C−C and C−O (*17, 18*).

Figure 2 shows the score plot of the first two principal components obtained from the ATR-MIR spectra. The first two principal components explain 90% of the variation in the ATR-MIR spectra related to the different grape juice varieties analyzed. A separation between CH and R grape juices was observed, although two CH samples were mixed with the RH. No spectroscopic outlier samples were observed. The analysis of the first three eigenvectors (data not shown) derived from the PCA analysis indicated that sugars and phenolics are the main chemical compounds responsible for the separation between these varieties.

Statistics for the PLS1 calibrations developed using the first set of grape juice samples analyzed are shown in **Table 1**. The R^2 and the SECV were 0.98 and 0.20 for TSS (°Brix), 0.86 and 0.07 for pH, and 0.53 and 1.01 for TP (au). The RPD values obtained were 9, 3.3, and 2 for TSS, pH, and TP, respectively. Additionally, calibrations were developed for TP using single varieties; however, no improvements in the statistics were observed.

The regression coefficients for the optimal PLS1 calibrations present strong, sharp, and well-defined peaks at 1031, 1061, 1361, 1429, 2889, and 2941 cm^{-1} mostly related to sugars and water (not shown). The peak around 1060 cm^{-1} is related to glucose and fructose as reported by other authors in several juice samples (*4, 16−19*). In the case of TSS, strong CH−OH frequencies due to
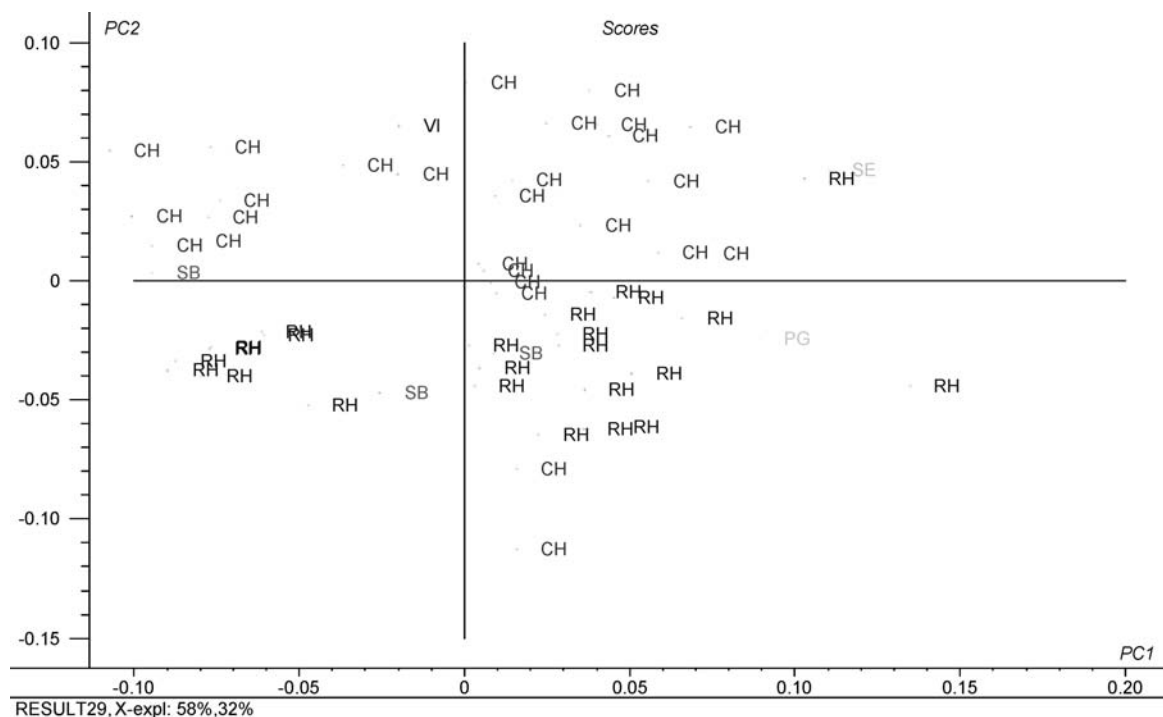
Figure 2. Score plot of the first two principal components of grape juice samples analyzed using ATR-MIR spectra.

Table 1. Cross-Validation Statistics for TSS, pH, and TP in Grape Juice Samples Analyzed by ATR-MIR Spectroscopy Using SNV Transformation[a]

	mean	SD	SEL	R^2	SECV	slope	PLS terms	RPD
TSS (°Brix)	20.83	1.68	0.2	0.98	0.20	0.98	5	9
pH	3.11	0.23	0.05	0.86	0.07	0.86	11	3.3
TP all samples (au)	9.8	1.80		0.53	1.01	0.18	7	2
TP CH (au) ($n = 32$)	9.2	1.67		0.40	2.0	0.17	4	1
TP RH (au) ($n = 24$)	10.15	1.89		0.77	1.01	0.55	10	2

[a] SEL, standard error lab method; SD, standard deviation.

Table 2. Cross-Validation and Prediction Statistics for FAN, Ammonia, and YAN in Grape Juice Samples Analyzed by ATR-MIR Spectroscopy Using SNV Transformation[a]

	ammonia (mg/L)	FAN (mg/L)	YAN (mg/L)
	calibration		
mean	34.3	71.2	99.4
SEL	0.5	0.5	0.5
SD	30.5	59.1	76.9
R^2	0.92	0.79	0.80
SECV	14.8	28.3	36.9
bias	0.08	0.73	0.15
slope	0.72	0.74	0.75
PLS terms	11	10	8
RPD	2	2	2.1
	validation		
mean	32.1	75.8	101.7
SD	29.3	57.2	72.4
R	0.82	0.75	0.79
SEP	17.2	36.7	42.4
bias	−0.23	4.1	2.9

[a] SEL, standard error lab method; SD, standard deviation.

sugar at 1033 and 1061 cm^{-1} and CH$_2$ at 1361, 1429, 2890, and 2990 cm^{-1} were observed (*21−23*). This confirms that there is a strong correlation between the sugar concentration and the calibrations for TSS (°Brix) and pH. The regression coefficient for TP showed small and sharp peaks related to aromatic ring C−C and aromatic−OH at 1321 and 1601 cm^{-1}. The regression coefficients observed for pH were heavily influenced by strong negative peaks due to COOH group frequency at 1241, 1393, and 1729 cm^{-1} and due to C=O stretching of organic acids around 1700 cm^{-1} (*16, 21*).

Determination of YAN, FAN, and Ammonia. Table 2 shows the calibration and prediction statistics for ammonia, YAN, and FAN of grape juices and ferments analyzed using ATR-MIR. Analytical values of juice ammonia, YAN, and FAN ranged from 0 to 150, 0 to 300, and 3 to 384 mg/L, respectively. The R^2 and SECV were 0.79 and 28.3 mg/L for FAN, 0.92 and 14.8 mg/L for ammonia, and 0.80 and 36.9 mg/L for YAN. The R and SEP were 0.75 and 36.7 mg/L for FAN, 0.82 and 17.2 mg/L for ammonia, and 0.79 and 42.4 mg/L for YAN. The RPD values obtained for ammonia, FAN, and YAN were equal or higher than 2, indicating that these calibrations can be used for qualitative determination of nitrogen fractions in grape juice and ferments (low, medium, and high). It is well-known that if the SECV is large as compared with the range of composition (as SD), a relatively small RPD value results, and the PLS calibration model is considered not robust (*24*). The higher the value of the RPD is,

the greater the probability of the model to predict the analyte accurately in samples outside the calibration set (*24*). An RPD value greater than three is considered fair and recommended for screening purposes, while a RPD value greater than five is considered good for quality control. Lower RPD values can result from a narrow range of the reference values (small SD) or from a large error in the prediction (SECV) as compared with the variability of the reference values (*24, 25*). However, for the purpose of qualitative interpretations using calibration models, RPD values lower than 1.5 are considered insufficient for most applications, whereas RPD values equal or greater than 2 are considered acceptable (*25*). In this study, the RPD values obtained for ammonia, FAN, and YAN were considered
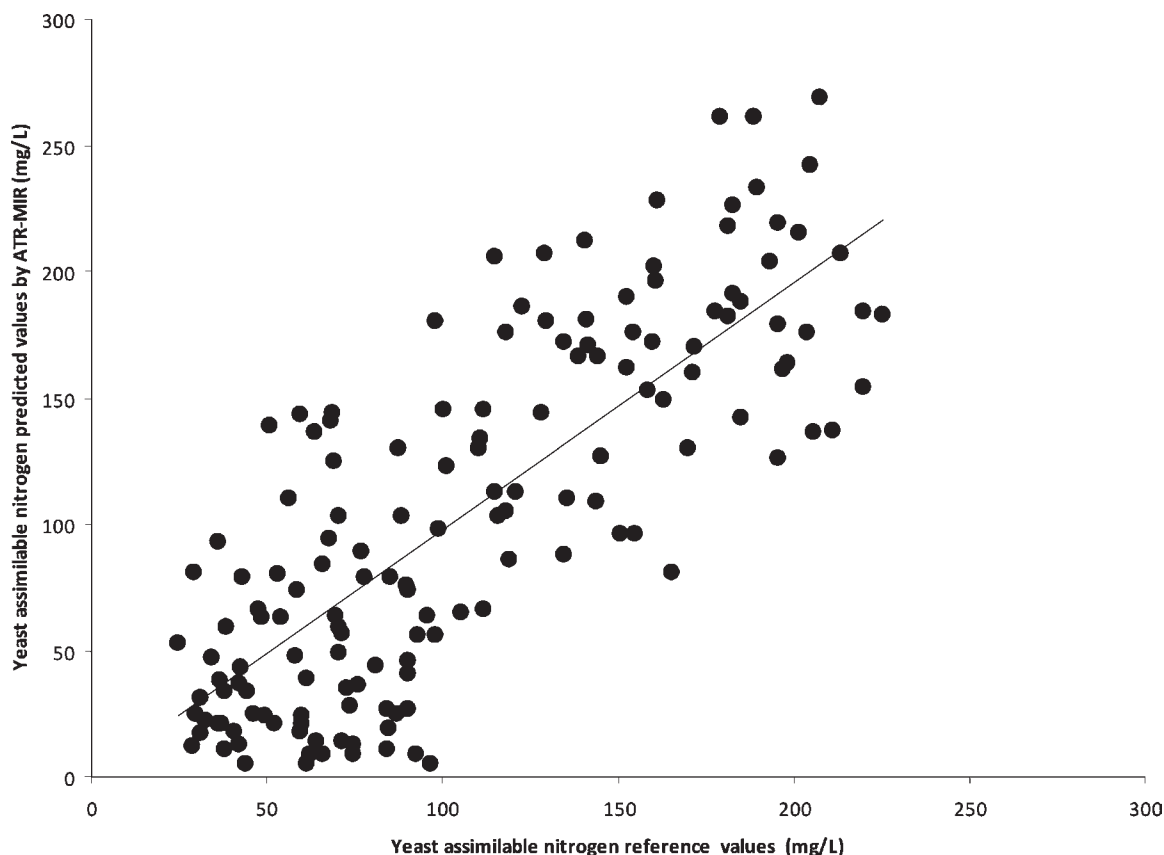
Figure 3. ATR-MIR predicted YAN values versus reference values for grape juice in validation.

adequate for monitoring these analytes in grape juice and wine ferments in a winery.

Regression coefficients for the optimal PLS1 calibrations for ammonia, FAN, and YAN showed strong peaks around 1650 and 1589 cm^{-1} related with N−H bending and N=O stretching and around 1570−1450 and 1300−1370 cm^{-1} related with N−H stretching (*16*). **Figure 3** shows the relationship between the reference method values and the predicted values for YAN using ATR-MIR.

The results from this study showed that ATR-MIR spectroscopy is capable of both measuring and monitoring grape juice parameters in an industrial situation. Therefore, it offers considerable advantages as compared to using traditional transmission cells in MIR, as the sample presentation is simple, requiring only 100 μL applied onto the ATR crystal. Unlike transmission, the ATR does not require a short path length cell, with inherent problems such as interference fringes and cell cleaning.

The practical implications of this study showed that ATR-MIR spectroscopy confers valuable benefits for the wine industry. Examination of the ATR-MIR calibration regression coefficients or PLS1 loadings might provide insights into aspects of juice chemistry and composition that are related to characteristics of interest sought after by winemakers. It also provides a rapid tool to monitor the fermentation process. These methods also offer the possibility to develop relationships between spectra and reference methods to measure several parameters simultaneously reducing the time of analysis, requiring minimal sample preparation.

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