Monitoring electric conductivity and macronutrient elements in *Vitis vinifera* grapes by near infrared reflectance spectroscopy

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Introduction

Elements such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) are macro and micronutrients that contribute to the growth, yield and quality of grapes. The distribution of soil elements (i.e. minerals) can vary considerably with growing region, soil type, age of the vineyard, and rootstock (among others factors).[1] Grape berry growth is supported by imports of carbohydrates, water and mineral nutrients. The description of these imports provides some information about the compounds required to support berry development. Additionally, grape berry mineral composition plays an important role in fruit development, disease resistance and wine chemical composition.[1] Near infrared (NIR) reflectance spectroscopy has been used to determine macro and micronutrients in a wide range of agricultural products and foods.[2,3,4] However, the determination of inorganic compounds is more challenging compared to organic compounds, owing to the indirect relationship between the element involved and the spectra obtained.[3] NIR reflectance spectroscopy has previously been used to determine the mineral concentration of plant tissues, due to the association between minerals and organic functional groups in the food matrix, and the subsequent effect on O-H bonding.[3] The estimation of mineral elements by NIR reflectance spectroscopy generally depends on the presence of those elements in mixtures of organic or hydrated compounds and salts (cations and anions).[4] Despite the reports of many satisfactory calibrations for mineral elements for a particular material, it is difficult to apply the results from one food to another. The characteristic bonds between of organic molecules and mineral compounds can vary with season, as well as by species, food type and processing method. Such variation may lead to unstable calibrations with inconsistent results, and be difficult to expand beyond a well characterised population. Therefore, the development of universal calibrations for minerals is difficult. The aim of this study was to evaluate the potential of NIR reflectance spectroscopy as a method to quantitatively measure macronutrient minerals (Mg, K, P and S) and electric conductivity (EC) in red grape homogenates.

Materials and Methods

Samples

Wine grape (*Vitis vinifera* L.) samples (n = 209) of Cabernet Sauvignon, Shiraz, Merlot, Pinot Noir and Chardonnay cultivars were collected during two consecutive vintages (2009 and 2010) at harvest (20–24°Brix), as whole berries and stored frozen (-18ºC) for up to six months before analysis. Samples (approximately 100 g) were homogenised using a commercially available homogeniser (8000 rpm for 20 seconds on a Retsch Grindomix GM200; Retsch GmbH & Co, Haan, Germany). Frozen samples were always thawed overnight at 4ºC prior to homogenisation. Samples were homogenised cold (<10ºC) and analysed on the day of homogenisation at room temperature (ca. 24–25ºC).

Reference analysis

The samples were analysed for potassium (K), magnesium (Mg), phosphorus (P), and sulphur (S) after acid digestion using inductively coupled plasma optical emission spectrometry (ICPOES), performed by Waite Analytical Services, School of Agriculture and Wine, University of Adelaide. Samples were digested with nitric acid and hydrochloric acid (http://www.adelaide.edu.au/was/). All analyses were done in duplicate and expressed as mg.kg⁻¹. Electric conductivity (EC) was measured using an Orion 250A portable pH-meter (Thermo-Orion, Beverley, MA, USA). Measurements were taken in duplicate by directly immersing the pH electrode in the homogenate at room temperature (ca. 22°C).

Spectral and chemometric analysis

Grape homogenate samples were scanned in reflectance mode (400–2500 nm) using a scanning monochromator FOSS NIRSystems6500 (FOSS NIRSystems, Silver Springs MD, USA). Spectral data were collected using Vision software (version 1.0, FOSS NIRSystems, Silver Spring MD, USA). Samples were scanned in a rectangular cuvette with a 10 mm path-length. The spectrum of each sample was the average of 32 successive scans (1050 data points per scan). Calibration models (n = 120) for the elemental concentration and EC measurements were performed using partial least squares regression (PLS) (The Unscrambler, version 9.7, CAMO, ASA, Oslo, Norway) using samples collected during the 2009 vintage (1100–2500 nm wavelength range). Calibration models were developed using full cross validation and the number of terms in the PLS calibration models were determined by the PRESS (prediction residual error sum of squares) function in order to avoid overfitting. The PRESS value is a statistical parameter that helps to know how well a calibration model can predict the level of analyte omitted during cross-validation. It was calculated from the prediction errors in cross-validation and plotted as a function of the number of factors used in the PLS calibration. The PLS method is a soft-modelling method for constructing predictive models when the factors are many and highly collinear, and allows a model to be calculated that was tested on external samples observing its prediction ability. The predictive ability of the NIR calibration models was demonstrated using an independent validation set. Validation of the PLS calibration models was carried out using samples from the 2010 vintage (n = 89). Before PLS models were developed, multiplicative scatter correction (MSC) was used as a transformation method in order to compensate for additive and/or multiplicative effects in spectral data (The Unscrambler software, version 9.7, CAMO, ASA, Oslo, Norway).[5] The predictive ability of calibrations was assessed from the r^2, the standard error of prediction (SEP) and the ratio of standard error of prediction to standard deviation, RPD value (SD/SEP).[6,8]

Results and Discussion

The NIR calibration statistics for the macronutrient elemental concentrations and EC measurements in red grape homogenates are shown in Table 1. The validation statistics (SEP, r^2, slope and bias) for the elements and EC measured in grape berries showed that good predictions were obtained for EC (SEP: 13.4 mS) and S (SEP: 34.5 mg.kg^{-1}) while intermediate predictions were obtained for Mg. The results showed that P and S might be measured by NIR reflectance spectroscopy in red grape homogenates. The RPD values obtained for the calibration models were lower than 3 indicating that the PLS calibrations could be used only as indicative of the mineral content in grape homogenates.[6] It must be taken into consideration, however, that it is often difficult to assign wavelengths to specific molecular absorptions in the NIR region. This is especially true in the case of metal ions in solution, where the NIR absorption spectra are most likely affected by changes in the hydrogen bonding around the metal cations and associated organic compounds.[7,8] If the element being measured is bonded to organic compounds then a distortion of the spectrum may be detectable at certain wavelengths, suggesting that NIR reflectance spectroscopy might predict some elements.

Table 1. Calibration statistics for minerals and electric conductivity of grape homogenates measured by near infrared spectroscopy (n = 120).

	R^2	SECV	Slope	RPD	LV
Mg (mg.kg^{-1})	0.84	12.93	0.74	2.2	8
K (mg.kg^{-1})	0.78	285.34	0.65	1.7	6
P (mg.kg^{-1})	0.70	40.19	0.56	1.7	6
S (mg.kg^{-1})	0.88	14.45	0.82	2.4	7
EC (mS)	0.87	7.66	0.78	2.0	4

R^2: coefficient of determination in cross validation, SECV: standard error in cross validation, RPD: residual predictive deviation (SD/SECV), LV: latent variables; Ca: calcium; Mg: magnesium; K: ptassium; P: phosphorus; S: sulphur; EC: electric conductivity.

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Conclusion

This study has shown that NIR spectra can be used to predict the concentration of Mg, S and EC in grape berries. However, these NIR calibrations are not suitable for quantitative routine use in an analytical laboratory, and further development with larger data sets (e.g. varieties, regions and vintages) will be required for the calibrations to become stable.

Acknowledgements

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