PRELIMINARY STUDIES ON POLYPHENOL ASSESSMENT BY FOURIER TRANSFORM-NEAR INFRARED SPECTROSCOPY (FT-NIR) IN GRAPE BERRIES•

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Keywords: *Vitis vinifera* cv 'Barbera', berries, anthocyanins

1. INTRODUCTION

Grape quality evaluation is now a pressing need for the wine industry. Routine assessment of grape quality involves the evaluation of total soluble solids (TSS), titratable acidity (TA) and must pH; but it is long-time known that measurements of chemical compounds tied to secondary metabolism, such as polyphenols, are more important for properly defining berry quality. As polyphenols determination is very expensive and time-consuming, alternative techniques, such as NIR spectroscopy, have been tested in viticulture. NIR-based methods have been applied to measure grape TSS, pH, TA (Fernandez-Novales *et al*., 2009; Cao *et al*., 2010) and anthocyanins (Dambergs *et al*., 2006). However, they have widely been used for determining quality of grape homogenates and wines (Janik *et al*., 2007; Smyth *et al*., 2008), rather than of single or pooled berries, with the only exception of a few studies (Cozzolino *et al*., 2004; Gonzáles-Caballero *et al*., 2010; Guidetti *et al*., 2010). In this work, we applied NIR spectroscopy to evaluate ripening parameters and anthocyanin contents in intact berries of *Vitis vinifera* L. cv 'Barbera'. In particular, NIR predictive capacity was tested using the High Pressure Liquid Chromatography (HPLC) as reference method, in order to develop a database of calibration models for each analysis of interest.

2. MATERIALS AND METHODS

2.1. Sampling

The trial was carried out on 11 diverse vineyards of *Vitis vinifera* cv 'Barbera', located in the Southern Piedmont area (North-West Italy). In each vineyard, samples of about 100 berries were taken at harvest from three randomised plots (15 vines each). At the lab, 30 berries were randomly chosen among samples from each vineyard and divided into three groups of 10 berries each. Triplicates were thus submitted to NIR and HPLC analyses, whereas the remaining berries were manually crushed and on the obtained must total soluble solids (TSS, °Brix), pH and titratable acidity (TA, g L^{-1} tartaric acid) were measured.

• QUAD. VITIC. ENOL. UNIV. TORINO, 31, 2009-2010

2.2. Spectral acquisition and chemical analysis

Samples of ten berries each were scanned to acquire NIR absorption spectra, working with a FT-NIR spectrometer (NIRFlex N500; Büchi) in reflectance mode, in the 40-100 m^{-1} range at 80 mm^{-1} resolution. Later, skins were obtained from each ten berries sample and extracted in a pH 3.2 ethanol buffer (120 mL L^{-1} ethanol, 5 g L^{-1} tartaric acid, 2 g L^{-1} $Na_2S_2O_5$, 22 mL L^{-1} NaOH 1 N) at 30 °C for 72 h. Anthocyanin content was detected by HPLC/DAD analysis, using a Perkin Elmer series 200-L, in accordance with a previously published method (Ferrandino, Guidoni, 2010).

2.3. Multivariate data analysis

Both spectra acquisition and chemometric analyses were carried out using the suited software NIRCal v.5.2 (Büchi). Mathematical pre-treatments were applied on raw absorption spectra and average spectra were calculated every ten NIR acquired spectra, to properly correlate NIR predicted values with each HPLC analysis. The partial least squares regression (PLS) coupled with the C-set/V-set validation method was employed to run calibrations. Three fourths of the dataset were used as calibration set (C-set) and the remaining fourth, termed validation set (V-set), was used to test the predictive capacity of the calibration model developed, according to other publications (Cozzolino *et al*., 2008; Gonzáles-Caballero *et al*., 2010).

3. RESULTS AND DISCUSSION

3.1. Assessment of TSS, pH and TA

The best results in terms of calibration and predictive capacity for TSS, pH and TA (tab. 1) highlight differences between the performances of the fibre optic probe and the autosampler NIR applications. Good results were obtained for TSS assessment in terms of both calibration (R^2 C-set = 0.89; SEC = ± 0.90 °Brix) and predictive ability (R^2 V-set = 0.83; SEC = ± 0.93 °Brix), in accordance with results previously described by other authors (Cao *et al*., 2010; Gonzáles-Caballero *et al*., 2010). We successfully elaborated calibrations also for pH and TA, as demonstrated by R^2 V-set values, standard errors of prediction (SEP) and Q values (tab. 1).

Tab. 1 - Statistical parameters obtained for routine quality indexes, assessed by either the NIR fibre optic probe or the NIR autosampler.

Calibrated Property	Application Used	R^2 C-set	R^2 V-set	SEC	SEP	Q value
TSS	fibre optic probe	0.89	0.83	± 0.90	± 0.93	0.67
	autosampler	0.93	0.82	± 0.78	± 0.81	0.66
pH	fibre optic probe	0.97	0.91	± 0.02	± 0.03	0.84
	autosampler	0.93	0.82	± 0.03	± 0.04	0.84
TA	fibre optic probe	0.96	0.94	± 1.15	± 1.18	0.72
	autosampler	0.89	0.87	± 1.78	± 1.78	0.62

3.2. Assessment of total anthocyanins

Anthocyanin quantitative calibrations can often be affected by the features of the sample matrix. This phenomenon causes a non-linearity condition, often observed in calibrations elaborated with samples from different growing regions, different seasons and/or different grape cultivars (Dambergs *et al*., 2006; Janik *et al*., 2007). This problem is particularly evident in intensively coloured fruit, as berries of the 'Barbera', where anthocyanin concentrations can reach values of even 2.5 g kg^{-1} of grape at harvest. In order to get over this interference, we worked on selected sample sets coming from vineyards of the same cultivar and collected from only one vintage. Calibrations to predict anthocyanin contents at the berry level were obtained (tab. 2), and the efficiency of measurements done by using the fibre optic probe or the autosampler applications is also compared.

Tab. 2 - PLS regression statistics obtained for total anthocyanins. Data are expressed in three different units (mg kg^{-1} berries; mg $berry^{-1}$ and mg g^{-1} berry skin).

Calibrated Property	Application Used	R^2 C-set	R^2 V-set	SEC	SEP	Q value	SE HPLC
Total anthocyanins (mg kg^{-1} berries)	fibre optic probe	0.93	0.95	± 92.00	± 94.00	0.34	± 96.00
	autosampler	0.92	0.91	± 97.00	± 97.00	0.40	
Total anthocyanins (mg $berry^{-1}$)	fibre optic probe	0.87	0.83	± 0.30	± 0.38	0.71	± 0.29
	autosampler	0.85	0.84	± 0.30	± 0.35	0.72	
Total anthocyanins (mg g^{-1} berry skin)	fibre optic probe	0.83	0.76	± 2.00	± 2.10	0.50	± 1.60
	autosampler	0.87	0.77	± 2.00	± 2.20	0.55	

Abstract

NIR spectroscopy has widely been tested in viticulture as powerful alternative to traditional analytical methods in the field of quality evaluation. NIR instruments have been used for assessing must and wine quality features in several works, but little information regarding their application on whole berries for polyphenol determination is available. In this study, we applied NIR technique to assess anthocyanin content in *Vitis vinifera* L. cv 'Barbera' intact berries, by collecting samples from different vineyards of Southern Piedmont (Italy). NIR analyses were performed using a FT-NIR spectrometer, whereas anthocyanins were detected according to a previously published method. Calibrations, elaborated to test NIR predictive capacity against HPLC measurements, showed a good correlation for the parameter total anthocyanins mg kg^{-1} ($R^2 = 0.93$; SEP = ± 94 mg kg^{-1}).

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