

Spectra evolution over on-vine holding of *Italia* table grapes: prediction of maturity and discrimination for harvest times using a Vis-NIR hyperspectral device

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Abstract

Measurement of certain grape quality parameters (sugars, acidity, and pH-value) is essential for the determination of the optimum harvest time. Non-destructive analytical techniques, including near infrared (NIR) spectroscopy, can be valid alternatives to traditional analytical methods for the determination of maturity indexes, enabling the possibility of on-field applications. This work aims to study the reliability to monitor spectra changes related with ripening of table grapes and to select optimal wavelengths for the discrimination of bunches from different harvests, in addition to the prediction of total soluble solids, pH, titratable acidity, phenols and antioxidant activity of table grapes. Grapes were harvested four times from the same plants at day 0 (I HT), and after 11 (II HT), 27 (III HT) and 48 (IV HT) days. Spectra were acquired from the images obtained using a spectral scanner Vis-NIR (ver 1.4.; DV Srl, Padova, Italy), with a detector in the region between 400-1000 nm principal component analysis was used to remove outliers followed by spectra pre-treatment. The best prediction model was achieved for soluble solids with the regression coefficient values of 0.91 for calibration and 0.88 for validation followed by titratable acidity (0.71 and 0.78) and antioxidant activity (0.68 and 0.62). In addition an excellent correlation was observed between spectra and days before harvest (R^2 of 0.98 for calibration and prediction models) indicating that is possible to relate spectra changes with ripening, leading also to the effective discrimination of the fruits from the different harvest times. The results showed that this technique may be a valid sup-

port to select the optimal harvest time also based on the prediction of the maturity related constituents.

Introduction

According to a report of the Food and Agriculture Organisation (FAO) the table grapes are among the most widely consumed fruits in the world with 27% of the total grape production being used as fresh fruits (FAO, 2009). Many factors contribute towards consumer acceptability of table grapes including size, shape, colour, skin thickness, crispiness, flesh firmness, brightness, colour uniformity, berry size, flavour, nutritional content, maturity stage and harvest time (Cliff *et al.*, 1996; Crisosto *et al.*, 2003; Piva *et al.*, 2006). The factors of cardinal significance in this regard are total soluble solids (TSS), titratable acidity (TA), and TSS/TA ratio, representing the major contributing factors towards acceptable ripeness (Guelfat-Reich and Safran, 1971; Peppi *et al.*, 2006; Jayasena and Cameron, 2008). Most of the researchers use total soluble TSS, TA, colour and volatile compounds as commercial harvest ripening indices (Sonogo *et al.*, 2002; Wei *et al.*, 2002).

The measurement of these quality parameters using the destructive instrumental and analytical practices require expert labour, the use of chemical agents, and costly equipments. Despite being time consuming and expensive, these analytical techniques provide analysis for a limited number of samples, therefore, present researchers in the field of postharvest are oriented towards non-destructive food techniques which are fast, and allow to analyse a higher number of samples and repetitions of the same sample in real time (Costa *et al.*, 2009). Many studies have successfully implemented non-destructive analytical techniques for the prediction of the quality attributes of various fruits and vegetables (Slaughter *et al.*, 1996; Pedro and Ferreira, 2007; Chia *et al.*, 2012; Ignat *et al.*, 2012).

In case of grapes, TSS, pH, TA, phenolic content, sugar content and antioxidant activity lied in the sphere of interest of many researches due to the significant relation of these analytes with the grape and wine quality, in fact most of them have been studied for wine grapes whereas literature on table grapes is scarce.

González-Caballero *et al.* (2010), achieved high R^2 values of 0.89 and 0.87 for the TSS and reducing sugar content with the standard error of cross validation (SECV) of 1.41°Brix and 17.13 g/L, respectively. Reducing sugar content study during the stages of grape ripening, wine making and ageing was conducted by Fernández-Novales *et al.* (2009). González-Caballero *et al.* (2011) also studied the changes in the internal quality attributes of wine grapes and excellent precision was obtained for TSS and reducing sugars with an R^2 as high as 0.94 and good precision was obtained for pH, TA, malic acid and tartaric acid with R^2 values ranging from 0.73 to 0.87.

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Damberg *et al.* (2006) found that the predictions of the pH and anthocyanins were based on the visible range and those for the TSS were based on the near infrared (NIR) range. The sugar content of the grapes using portable Vis-NIR devices have also been measured, as in a study conducted by Wu *et al.* (2008); the reflectance spectra of the grape berries were collected in the Vis-NIR range obtaining an R^2 of prediction of 0.908 with a root mean square error of prediction (RMSEP) of 0.112 g/L.

Nogales-Bueno *et al.* (2014) used the NIR in the range of 900-1700 nm for non-destructive prediction of phenolic content of grape skin, TSS, TA and pH developing both individual models and global models. The values of R^2 for the individual models of these parameters were very high (more than 0.90) for red grapes. Encouraging values were achieved for the global model, with $R^2=0.77$ and SEP=1.97 mg g⁻¹ for phenolic content in grape skin; 0.97 and 1.61 °Brix for sugars; 0.96 and 3.89 g L⁻¹ for TA, and 0.92 and 0.18, for pH. One other study evaluated the feasibility of using hyperspectral imaging in the Vis-NIR range for the prediction of anthocyanins, polyphenols, sugars and density (González-Caballero *et al.*, 2012). Other works also predicted phenolic content including flavonols (Ferrer-Gallego *et al.*, 2011) and anthocyanins (Fernandes *et al.*, 2011) of wine grapes. Moreover fructose and glucose concentration, pH value, TA and glycerol, gluconic acid and acetic acid were also predicted with on-line near Vis-NIR spectrometer upon grape reception at wineries (Porep *et al.*, 2015). As for table grapes, few works are available, and only predicting maturity indexes; Baiano *et al.* (2012) applied hyperspectral imaging for the determination of TA and TSS for table grapes. Satisfactory R^2 were found for white and red grapes being 0.95 and 0.82 in case of TA, 0.94 and 0.93 for TSS and 0.80 and 0.90 for pH.

In addition, Piazzolla *et al.* (2013) evaluated the feasibility of using spectral Vis-NIR images to discriminate table grapes from different harvest times and reported excellent potential of this technique, being able to correctly classify almost all samples (non error rate of 99%).

Since most of the work refers to wine grapes, the objective of this work was to evaluate the feasibility of using spectral information to characterise table grape quality (including relevant nutritional parameters) also in relation to different harvest times in terms of internal components.

Particularly this work aimed to use spectral information in the Vis-NIR range, obtained with an innovative hyperspectral scanner, for a comprehensive study of ripening changes of table grapes over on-vine holding by exploring the feasibility of: i) predicting soluble solids content, pH, titratable acidity, phenols and antioxidant activity; ii) monitoring the correlation of spectra changes with the time of on vine holding; iii) selecting the most relevant wavelengths for the discrimination of fruit from different harvest times.

Materials and methods

Experimental design

First harvest of table grapes of the variety cv. *Italia*, grown in location Cellamare (Province of Bari, Italy), was done on the 8th of October, 2010 (I HT) followed by subsequent harvests after 11 (II HT), 27 (III HT) and 48 (IV HT) days. The first harvest time corresponded to the commercial maturity, according to grower decision, whereas the following were aimed to monitor quality of on-vine held grapes, since in Southern Italy is a very common practice postponing the harvest up to Christmas. Grapes were cultivated

with the Apulia canopy grape system and covered with net and low-density polyethylene plastic film with 170 µm thickness. At the first harvest 15 plants were marked in a row of the field, according to grower indications, and at each harvest time, 2 bunches per plant were harvested (for a total of 30 bunches) and transported to the Postharvest Laboratory of the University of Foggia.

Fifteen berries from each bunch were randomly selected, and used for chemical analysis after the acquisition of hyperspectral images. The berries were then squeezed obtaining one sample juice for each bunch, simulating the commercial procedure applied to decide the moment to harvest based on the juice of some bunches. Correspondingly, the spectra were averaged for a total of 120 spectra (2 bunches × 15 plants × 4 harvest times), one for each sample juice.

Quality determination

After each harvest the maturity stage and nutritional content of the grapes were determined by recording the values of TSS, TA, pH, antioxidant activity and total phenol content by destructive analysis of the grape juice obtained from each bunch. Moreover colour and berry firmness were also monitored, to characterise differences at harvest. A digital refractometer (Atago PR32-Palette; ATAGO CO., Ltd., Tokyo, Japan) was used to measure the TSS; measurements of pH and TA, expressed in percentage of tartaric acid, were carried out with an automatic titrator (TitroMatic CRISON 1S; Crison Instrument, Barcelona, Spain). For measuring phenol content and antioxidant activity, 5 g of berries were homogenised with an Ultraturrax (IKA T18 basic; IKA®-Werke GmbH & Co. KG, Staufen, Germany) after the addition of 3 × 10³ mg kg⁻¹ of methanol plus 3% formic acid. The extracts were then centrifuged at 5°C and 9000 rpm for 10 min. Total phenols were determined according to the method of Singleton and Rossi (1965). Each extract (100 µL) was mixed with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L⁻¹). After 2 h standing in the dark, the absorbance of the solution was read at 725 nm against a blank using a spectrophotometer (UV-1700; Shimadzu Corp., Jiangsu, China). The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as grams of gallic acid per kilogram of fresh weight (g GA kg⁻¹). Antioxidant assay was performed following the procedure described by Brand-Williams *et al.* (1995) with minor modifications. The diluted sample (50 µL) was pipetted into 0.95 mL of diphenylpicrylhydrazyl solution to initiate the reaction. The absorbance was read after 24 h at 515 nm. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was used as a standard and the antioxidant activity was reported in milligrams of Trolox equivalents per kilogram of fresh weight (g TE kg⁻¹). Firmness was determined on 15 berries for each cluster and defined as the force (N) required to compress each berry for 3 mm between two parallel plates using an Instron Universal Testing Machine (model 3343; Instron Inc., Norwood, MA, USA), at a speed of 50 mm/min. Colour information were extracted as described in the following paragraph.

A one-way analysis of variance was performed on quality attributes at harvest and mean values were separated with Tukey's test ($P<0.05$). Data were analysed with the StatGraphics Centurion software (v. 16.1.11; StatPoint Technologies, Inc., Warrenton, VA, USA).

Vis-NIR spectral acquisition

Hyperspectral imaging (v. 1.4.5; DV Srl, Padova, Italy) system consisted of a charge-coupled device (CCD), a 12-bit camera con-

nected to a V10 type spectrograph (400-1000 nm, 25 μm slit, resolution 5 nm; ImSpector V10, Specim Ltd., Haarlem, The Netherlands) coupled with a standard C-mount f16 mm lens. The optics of this imaging system helped to study the fruit properties associated to the spectral range of 400-1000 nm of reflectance with 5 nm of resolution. The target was placed at a distance of 360 mm from the camera. The light source consisted of a 150W halogen lamp (EKE 21 V 150 W, Tokyo, Japan) mounted at an angle of 45° to the horizontal plane, and of an optic fibre that transfers the radiation to a linear light diffuser. The camera spectrograph assembly was supplied with a stepper motor to move the unit through the field of view of the camera and carry out a line-by-line scan of the sample. The spectral images were collected in a dark room where the halogen light was the only light source. One scan per sample, for 15 randomly selected berries per bunch was done with an acquisition speed of 3 mm s⁻¹ in the Vis-NIR range. The analysis was performed on fruits conditioned at room temperature (approx. 20°C).

The hyperspectral images were first corrected with a white and a dark reference. The dark reference was used to remove the effect of dark current of the CCD detectors that are thermally sensitive. All the spectra were extracted using the company software, SS scanner v. 1.3.4.6. (DV Srl). A region of interest corresponding to the maximum inscribed rectangle was manually selected on each berry and then the spectra of the 15 berries corresponding to a single bunch were averaged. The software also allowed to automatic calculate L*, a*, b* colour values and the Hue Angle was then calculated, as following:

$$H = \arctg \frac{b^*}{a^*} \quad (1)$$

Spectra processing and modelling

The acquired spectral data were analysed using the Unscrambler packing software version 9.1 (CAMO ASA, Oslo, Norway). All the reflectance measurements were first transformed to absorbance values using $\log(1/R)$ according to the law of Lambert-Beer. The spectra were analysed with a principal component analysis central model to identify and eliminate defective spectral outliers.

Spectra were then pre-treated by different mathematical methods. Pre-treatment methods [smoothing; multiple scatter correction (MSC); Savitzky-Golay derivative; baseline; standard normal variate] tested individually and in combination on the whole dataset.

A partial least squares regression (PLS) algorithm was applied after each transformation to select the best transformation for the prediction TSS, pH, TA, phenols and antioxidant activity, using all 121 wavelengths.

Particularly, PLS model seeks to correlate spectral variations (X) with defined value (Y), and each component is obtained by maximising the covariance between Y and all possible linear functions of X. This leads to components, which are more directly related to variability in Y than are original variables.

After selection of the best transformation, calibration and prediction models were developed. From the total of 120 spectra, 80 samples were randomly selected for the calibration data set and 40 for the prediction data set. The performance of the PLS regression, was evaluated by comparing values of coefficient of correlation (R) for calibration, the root mean square error of calibration (RMSEC), the coefficient of correlation for cross validation (RSECv), the root mean square error of cross validation (RMSECv), the standard error of prevision (SEP) and RMSEP.

In addition, a linear discriminant analysis (LDA) was tested with the aim of discriminating bunches from the 4 harvest times, using a forward stepwise model. This model first reviews all the

Table 1. Quality parameters of *Italia* table grapes at different harvest times.

Parameter		Harvest times			
		I HT	II HT	III HT	IV HT
TSS (°Brix)	Mean	18.25 ^a	17.18 ^a	17.60 ^a	18.24 ^a
	Range	15.75-19.78	14.55-18.38	13.03-19.83	13.80-20.18
	SD	1.43	1.26	1.8	1.77
pH value	Mean	3.95 ^b	4.01 ^b	4.06 ^b	4.2 ^{4a}
	Range	3.88-4.26	3.75-4.23	3.78-4.25	3.83-4.59
	SD	0.15	0.12	0.14	0.16
Titratable acidity (tartaric ac. g/100 g)	Mean	0.39 ^a	0.33 ^b	0.32 ^b	0.32 ^b
	Range	0.27-0.48	0.28-0.40	0.27-0.38	0.30-0.38
	SD	0.04	0.04	0.03	0.02
Phenols (gallic ac. mg/100 g)	Mean	55.37 ^a	33.38 ^b	38.39 ^b	42.53 ^b
	Range	34.91-87.39	22.67-42.88	25.00-60.20	21.46-72.03
	SD	17.28	0.25	9.88	15.29
Antioxidant activity (Trolox mg/100 g)	Mean	279.82 ^a	179.15 ^c	189.44 ^c	230.91 ^b
	Range	228.13-343.49	144.92-211.92	148.52-265.09	179.82-315.15
	SD	42.67	24.32	42.43	34.74
Firmness (N)	Mean	7.35 ^a	6.62 ^b	6.70 ^b	6.89 ^b
	Range	5.50-12.32	4.23-10.45	5.14-9.96	4.49-10.75
	SD	1.72	1.51	1.43	1.6
Hue angle (°)	Mean	110.59 ^a	108.33 ^{ab}	106.47 ^b	100.43 ^c
	Range	107.59-113.13	104.50-111.86	102.63-109.03	94.75-104.97
	SD	0.53	1.99	2.15	3.54

HT, harvest time; TSS, total soluble solids; SD, standard deviation. ^{a-c}Within each column different letters indicate mean values significantly different (P<0.05).

variables and includes, step by steps, the ones that have the biggest weight for the discrimination between groups. In this way 14 wavelengths were selected over 121. Using only these 14 wavelengths was also possible to find correlation between absorbance spectra and days of grapes holding on the vine, starting from the first harvest (0 days) up to the last harvest (48 days).

Results and discussion

Grape composition

Table 1 shows the evolution of quality parameters as harvesting proceeded over time. Harvest time influenced all quality attributes except TSS. Titratable acidity decreased during ripening, with grapes of I HT showing a higher TA (0.39% tartaric acid) than grapes from the other harvest times (about 0.32%); accordingly pH increased with the harvest date from 3.95 to 4.24, with the last harvest being significantly higher than the previous 3. Furthermore, it can be also observed that also the phenols decreased during ripen-

ing, and particularly after the I HT (55.37 gallic ac. mg/100 g). Antioxidant activity did not follow a linear trend, with the proceeding of the ripening presenting highest values at I HT (279 mg/100 g), then decreasing at II HT (about 179.1 mg/100 g), and then increasing again at III HT (about 189.4 mg/100 g) and at IV HT (230 mg/100 g). Firmness decreased during the ripening reporting the highest value at I HT (7.35). Also the hue angle decreased significantly during the ripening, starting from 110.50 up to 100.43 at the last harvest; this changes indicated a loss of the green component which can be associated to the chlorophyll degradation and a consequent increase of the yellow component.

Calibration and external prediction developments

Principal component analysis did not reveal the presence of spectra outliers, according to the values of the Mahalanobis distance (H), using a threshold of 3.0, therefore, the number of analysed samples was 120. In Figure 1 are shown the raw 120 spectra. The whole dataset, was then used to select the best pre-treatment for each quality parameter. The selected results of the model obtained with the best transformations for each quality attribute, are shown in Table 2.

Table 2. Calibration and cross validation partial least squares regression models for total soluble solids, pH, titratable acidity, total phenols and antioxidant activity of *Italia* table grapes (n=120) using different mathematical pre-treatments.

Parameter	Pre-treatment	SD	R_{cal}	RMSEC	SEC	R_{CV}	RMSECV	SECV	PLS factors
TSS (°Brix)	No transformation	1.56	0.06	0.04	0.04	0.43	0.08	0.08	11
	Smoothing S. Golay	1.47	0.05	0.06	0.06	0.04	0.08	0.08	11
	MSC	1.56	0.06	0.05	0.04	0.04	0.08	0.08	11
	1° Der. S. Golay	1.71	0.01	0.06	0.06	0.05	0.08	0.05	13
	2° Der. S. Golay	1.33	0.00	0.07	0.09	0.03	0.10	0.10	5
	Baseline	1.64	0.06	0.07	0.07	0.00	0.05	0.05	13
	SNV	1.64	0.06	0.07	0.07	0.04	0.08	0.08	13
	pH	No transformation	0.12	0.55	0.16	0.16	0.60	0.15	0.15
Smoothing S. Golay		0.11	0.60	0.15	0.15	0.55	0.16	0.16	3
MSC		0.11	0.55	0.16	0.16	0.50	0.15	0.17	2
1° Der. S. Golay		0.12	0.62	0.15	0.15	0.53	0.16	0.16	3
2° Der. S. Golay		0.11	0.57	0.16	0.16	0.50	0.17	0.17	2
Baseline		0.11	0.58	0.15	0.16	0.52	0.16	0.16	3
SNV		0.11	0.56	0.16	0.16	0.52	0.16	0.16	2
Titratable acidity (tartaric ac. g/100 g)		No transformation	0.03	0.71	0.04	0.04	0.62	0.04	0.04
	Smoothing S. Golay	0.02	0.69	0.04	0.04	0.60	0.04	0.04	7
	MSC	0.03	0.73	0.03	0.03	0.62	0.04	0.04	7
	1° Der. S. Golay	0.02	0.64	0.04	0.04	0.56	0.04	0.04	3
	2° Der. S. Golay	0.02	0.64	0.04	0.04	0.53	0.04	0.04	3
	Baseline	0.03	0.71	0.04	0.04	0.60	0.04	0.04	8
	SNV	0.03	0.72	0.04	0.04	0.60	0.04	0.04	7
	Phenols (gallic ac. mg/100 g)	No transformation	4.09	0.14	17.95	18.02	0.23	17.59	17.66
Smoothing S. Golay		4.08	0.23	17.59	16.66	0.12	18.01	18.09	1
MSC		6.78	0.37	16.74	16.81	0.27	17.47	17.55	3
1° Der. S. Golay		6.63	0.37	16.80	16.87	0.27	17.51	17.59	2
2° Der. S. Golay		7.17	0.40	16.58	16.65	0.27	17.53	17.60	2
Baseline		6.73	0.37	16.76	16.83	0.27	17.53	17.60	3
SNV		6.08	0.34	17.00	17.08	0.21	17.79	17.87	2
Antioxidant activity (Trolox mg/100 g)		No transformation	41.88	0.67	46.52	46.71	0.56	52.32	52.54
	Smoothing S. Golay	40.96	0.65	47.32	47.52	0.55	53.00	53.19	5
	MSC	41.34	0.66	47.00	47.19	0.58	51.38	51.59	5
	1° Der. S. Golay	41.88	0.67	46.52	46.71	0.57	51.54	51.75	3
	2° Der. S. Golay	40.07	0.64	48.08	48.28	0.54	52.81	53.04	3
	Baseline	42.07	0.67	46.35	46.54	0.57	52.06	52.27	6
	SNV	39.04	0.62	48.90	49.11	0.55	52.39	52.60	4

SD, standard deviation; R_{cal} , R value for calibration set; RMSEC, root mean square error of calibration; SEC, square error of calibration; R_{CV} , R values for cross-validation set; RMSECV, root mean square error of cross validation; SECV, coefficient of correlation for cross validation; PLS, partial least squares regression; TSS, total soluble solids; MSC, multiplicative scatter correction; SNV, standard normal variate.

Based on the highest R and the lowest $RMSEC$, the first Savitzky-Golay derivative was the optimal transformation for TSS and pH, with R values of 0.90 and 0.62 respectively in calibration, and 0.67 and 0.53, in cross validation. Figure 2 shows the plot between the regression coefficients and the wavelengths for the calibration of total soluble solids; it can be observed that throughout the length of the spectral range there are many wavelengths relevant for the final prediction. As for pH highest regression coefficients were detected at 695, 870 and 905 (data not shown). For TA and antioxidant activity, MSC was found to be the optimal pre-treatment giving an R value of 0.73 and 0.66 in calibration and 0.62 and 0.58, respectively in cross validation.

For phenols, the best mathematical transformation was the second Savitzky-Golay derivative, with R value of 0.40 in calibration and 0.27 in cross-validation. The maximum peaks contributing to the model performance were observed at 420, 445 and 720 nm (data not shown).

Table 3 shows the statistics for the calibration and for the prediction model of TSS, pH, TA, total phenols, and antioxidant activity when using external samples not included in the calibration, while in the Figure 3 are shown the performance of the models of prediction for each quality attributes by optimal pre-treatment. Model for predicting soluble solids presented a very satisfactory performance, with a value of R_{cal} of 0.91, $RMSEC$ of 0.77°Brix, while the value of R_{pred} was found to be 0.88, with $RMSEP$ of 0.95°Brix. As for residual prediction deviation, the obtained value close to 2 (1.92) indicates that that coarse quantitative predictions are possible by using the model (Nicolai *et al.*, 2007). These results were similar to those reported in the studies of Cao *et al.* (2010) in which the TSS of grape berries belonging to three different varieties were measured by using 2 different model development techniques *i.e.*, PLS and genetic algorithm coupled with least square support vector machine (GA-LS-SVM). The obtained values of R of prediction were approximately equal to the values obtained in the present study being 0.91 for both PLS and GA-LS-SVM with $RMSEP$ of 0.93 and 0.96°Brix. The results of the present study were also comparable to those of another study conducted by Baiano *et al.* (2012) using the same device. These authors predicted the TSS of red and white grape berries with a value of R^2 for prediction to be 0.93 and 0.94, respectively. The error rates for this parameter are lower than those reported by other authors using a different instrument in the NIR wavelength range from 900-1700 nm (Nogales-Bueno *et al.*, 2014). Hence, a good capacity of correlation was achieved in numerous other works on prediction of TSS for wine and table grapes (González-Caballero *et al.*, 2011; Parpinello *et al.*, 2013).

The best prediction obtained in this study for pH was not as encouraging as those found in other studies. In the present study

the highest value of R_{cal} obtained was 0.58 with a $RMSEC$ of 0.15, which is much lower as compared to the results of Baiano *et al.* (2012) in which R value for white grapes was 0.80. Similarly, in a study conducted by Cao *et al.* (2010) the models resulted in an R value of 0.97 for wine grapes. González-Caballero *et al.* (2010) on single berries of wine-grapes with coefficient of determination (R^2) of 0.64 in prediction using Vis-NIR range. The main reason for this difference on the results may be the method for setting the Y vector during the reference development. In all these studies the berries were individually juiced but in case of the present study a single value of pH was taken for a cluster of 15 berries. In case of TA, R_{cal} of 0.71 with $RMSEC$ of 0.03% tartaric acid, and R_{pred} of 0.78 with $RMSEP$ of 0.04% tartaric acid was yielded. These results presented a better performance as compared to González-

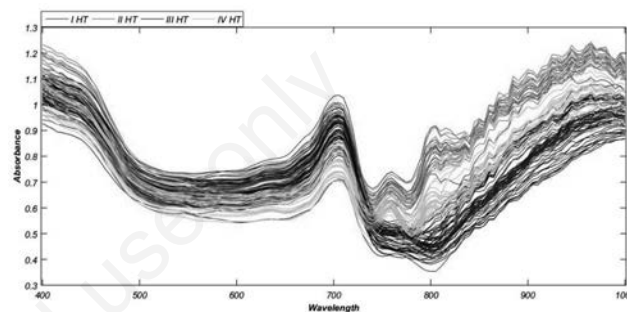


Figure 1. Row spectra of individual *Italia* table grape berries. HT, harvest time.

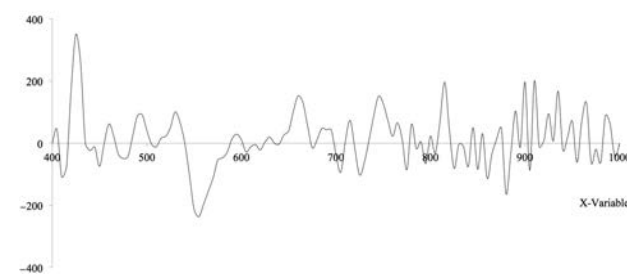


Figure 2. Regression coefficients of the partial least squares regression model for the calibration of total soluble solids on *Italia* table grapes.

Table 3. Performance of model of prediction for each quality attributes by optimal pre-treatment.

Parameter	Pre-treatment	SD	Calibration (80 samples)			Prediction (40 samples)			RPD	PLS factors
			R_{cal}	RMSEC	SEC	R_{pred}	RMSEP	SEP		
TSS (°Brix)	1° Der. S. Golay	1.85	0.91	0.77	0.78	0.88	0.95	0.97	1.92	13
pH	1° Der. S. Golay	0.18	0.58	0.15	0.15	0.70	0.16	0.16	1.13	3
Titrateable acidity (tartaric ac. g/100 g)	MSC	0.03	0.71	0.03	0.03	0.78	0.04	0.03	0.86	7
Phenols (gallic ac. mg/100 g)	2° Der. S. Golay	17.83	0.41	16.18	16.28	0.36	17.37	17.59	1.01	2
Antioxidant activity (Trolox mg/100 g)	MSC	62.90	0.68	45.98	46.25	0.62	48.98	49.54	1.27	5

SD, standard deviation; R_{cal} , R value for calibration set; RMSEC, root mean square error of calibration; SEC, square error of calibration; R_{pred} , R value for prediction set; RMSEP, root mean square error of prediction; SEP, standard error of prevision; RPD, residual prediction deviation; PLS, partial least squares regression; TSS, total soluble solids; MSC, multiplicative scatter correction.

Caballero *et al.* (2010) work in which TA for the berries were measured over a wavelength range of 380-1650 nm giving an R^2 of 0.33 in cross validation. The R values for TA in the present study are lower as compared to the study conducted by Baiano *et al.* (2012) in which the prediction values for TA were as high as 0.95 and 0.82 for white and red grapes, respectively, but similar to those obtained by Nogales-Bueno *et al.* (2014). The better results obtained in prediction for TA and pH compared to calibration, may be explained by the fact that models were not enough robust and may be by having selected by chance external data set which better described the correlation between spectra and analytic measure, compared to sample used for the calibration model.

Finally some consideration should be drawn on prediction performances of TSS, TA and PH models in relation to laboratory error. In fact, despite the lower R^2 of TA and pH models, compared to TSS, prediction errors were very reasonable, if compared to laboratory errors, respectively, 0.59 for TSS, 0.10 for pH and 0.02 for TA. As general rule, SEP values should be as closer as possible to laboratory error, being considered excellent if not higher than 1.5 and good if around 2-3 time the laboratory error. In this case it can be observed as for all these 3 parameters the SEP was not higher than 1.6 times the laboratory error (for TSS), being even lower for TA and pH. These findings suggest that the lower performances of the models may be attributed to the high variance of the modelled parameter in the juice, which may be expected since it is made from 15 berries, and to the error of the reference method. For antioxidant activity, the best statistics obtained were $R_{cal}=0.68$, $RMSEC=45.98$ mg Trolox/100 g in calibration and $R_{pred}=0.62$, $RMSEP=48.98$ mg Trolox/100 g in prediction, and no published studies reported the application of Vis-NIR spectroscopy on determination of antioxidant activity of grapes yet. In case of phenol content, lowest performances were observed in prediction, in particular a value of 0.41 for R in calibration and 0.36 in prediction, the same results were published by Kemps *et al.* (2010), in which the value of correlation (R) in calibration ranged between 0.36 to 0.60 for various grape varieties. González-Neves *et al.* (2010) reported a value of (R^2) of 0.98 for total phenols in validation model using red grapes *Graciano*. Similar results were generated in a study conducted by Nogales-Bueno *et al.* (2014) in which the results of R^2 values of 0.89 for red grapes, 0.80 for white grapes, and 0.77 for global models, were reported. Results demonstrated that, while good performances were observed for TSS, followed by TA, pH and antioxidant activity, in agreement with the results of the other authors with research on grapes (Cao *et al.*, 2010; González-Caballero *et al.*, 2010, 2011; Baiano *et al.*, 2012; Nogales-Bueno *et al.*, 2014), a limited potential was seen in the performance of the PLS model in case of phenol content. In case of antioxidant activity, no comparison was possible since it has not been previously reported in any paper.

Harvest times discrimination

Results of the LDA allowed discriminating the 4 classes only by using 14 variables, showing a significant discrimination (Wilks' Lambda 0.000243313, $P < 0.0000$) between the 4 classes. The variables selected, were 420, 580, 585, 630, 745, 760, 770, 780, 800, 805, 865, 870, 925 and 970 nm as showed in the discriminant Equation 1:

$$0.360959*420 + 0.295888*580 + 0.557965*585 - 0.379512*630 - 0.633662*745 - 0.585279*760 + 0.519507*770 + 0.961174*780 + 0.595383*800 + 1.32246*805 + 0.592633*865 + 0.29153*870 - 0.083511*925 + 0.537856*970 \quad (2)$$

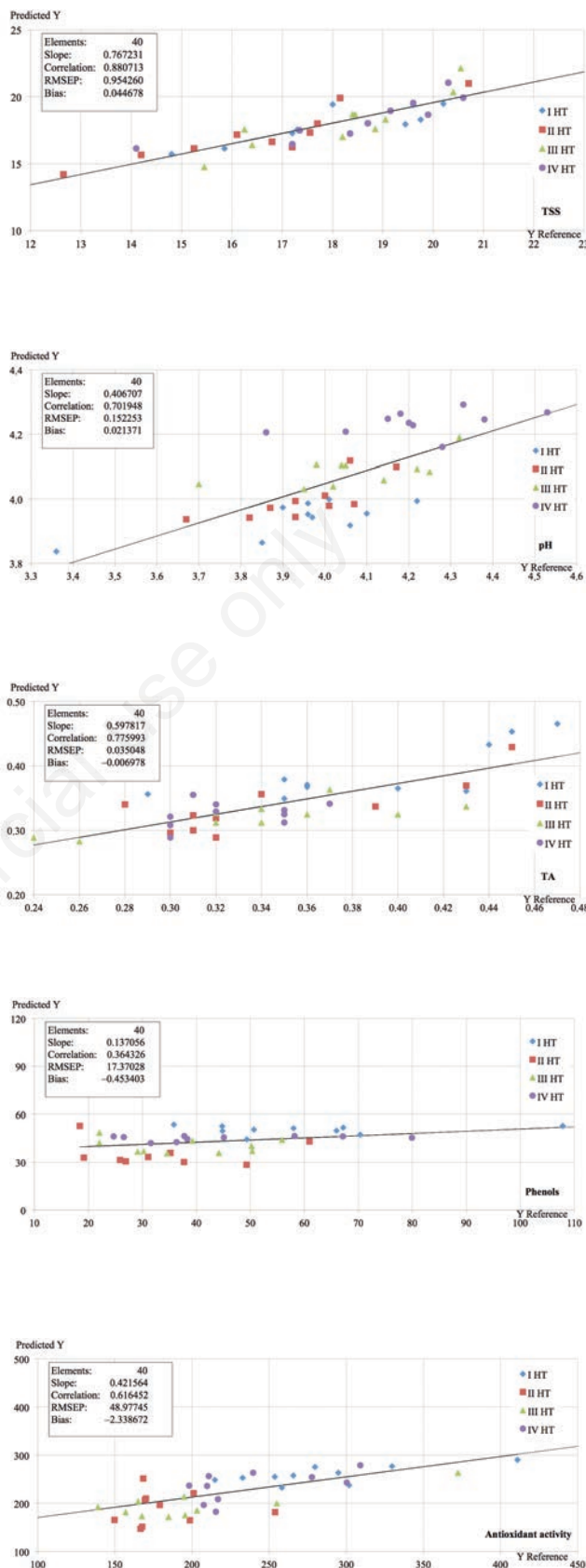


Figure 3. Performance of model of prediction for each quality attributes by optimal pre-treatment. TSS, of total soluble solids; TA, titratable acidity.

The results of classification using the derived discriminant functions are shown in Table 4. Amongst the 120 observations used to fit the model, 119 (or 99.1667%) were correctly classified, with only one sample belonging to II HT, being classified in I HT. These results confirmed the excellent capability of hyperspectral imaging to discriminate among grapes from different harvest time as reported by Piazzolla *et al.* (2013), but were optimised in term of number of wavelengths used for the model. Moreover this information were also used for further monitoring on quality changes over time on the plant. In fact, by using only the information contained in 14 wavelengths it was possible to predict the number of days for those grapes held on the plant after the first harvest. Models built to predict the days on the vine since the first harvest (0 day for HT1, 11 days for HT 2, 27 days for HT3 and 48 days for HT4) gave excellent for both calibration and external data set. In particular value of 0.98 for R, RMSEC of 3.29 days and SEC of 3.31 days were found in calibration, and comparable performances were obtained in prediction (R of 0.98, and SEP of 3.95 days). Relation of the spectra with time on the plants can be also observed in Figure 4 showing the predicted days on the plants for the external data set. These results confirmed that it was possible to discriminate grapes from different harvest times with a percentage of correct classification of 99.2% as shown in Table 4, but also suggested a possible way to monitor ripening on the plant by studying the spectra changes over time.

Conclusions

The results of this study confirm the suitability of using Vis-NIR hyperspectral scanner for reliable prediction of various analytes in table grapes, as already reported for wine grapes. Good prediction models were achieved for TSS, TA and pH, and promising results were also obtained for antioxidant activity, which may be further improved. In addition to this, results of this study showed that

Table 4. Classification results by linear discriminant analysis at different harvest time for *Italia* grapes.

Qualitative groups	I HT	II HT	III HT	IV HT
I HT	30	1	-	-
II HT	-	29	-	-
III HT	-	-	30	-
IV HT	-	-	-	30
Percentage correctly classified	100%	96.67%	100%	100%

HT, harvest time.

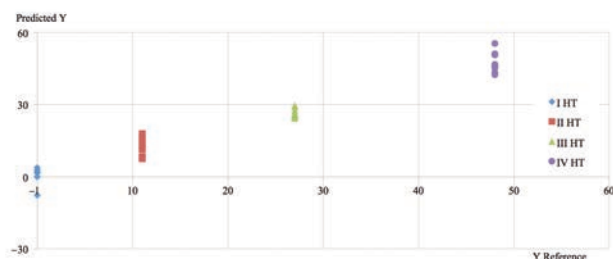


Figure 4. Performance of the linear discriminant analysis model to predict the numbers of days for grapes held on the plant after the first harvest.

analysing spectra changes over time during on-vine holding of table grapes was possible to monitor ripening and to correctly classify grapes by harvest time, using only 14 wavelengths. These findings encourage further implementation of this method to monitor ripening of table grapes in the vineyard and better define most suitable harvest time also taking into account quality attributes more significant in terms of nutritional value of the product.

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