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COMMUNICATION

Apples Nutraceutic Properties Evaluation Through a Visible and Near-Infrared Portable System

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Abstract Non-destructive and rapid tools are required for predicting the optimum harvest window and for monitoring fruit quality during postharvest period. This study tested a portable, experimental visible/near-infrared (vis/NIR) spectrophotometer, more versatile and handy than traditional vis/NIR instruments, to measure phytonutrients active in human health and important in fruit storability. Parameters determining sensorial and quality properties of the fruit were also analyzed. The vis/NIR measurement was carried out in field using apples of "Golden Delicious" and "Stark Red Delicious" on tree. Calibration models were developed using PLS regression based on second derivative spectra. For "Golden Delicious" apple, the cross-validation R^2 for soluble solids content (SSC), chlorophyll, titratable acidity (TA), flesh firmness, total phenols, carotenoids, and ascorbic acid were 0.72, 0.86, 0.52, 0.44, 0.09, 0.77, and 0.50, respectively. The corresponding RMSECV were 0.78 °Brix, 0.50 nmol/cm^2, 0.59 g/L, 6.08 N, 0.10 mg/g, 0.08 nmol/cm^2, and 0.83 mg/100 g, respectively. For "Stark Red Delicious" similar calibration statistics were found for SSC, TA, flesh firmness, chlorophyll, and ascorbic acid content. A better calibration performance was achieved for total phenols, while for carotenoids it was less accurate. Cross-validation R^2 for "Stark Red Delicious" total anthocyanins, total flavonoids, and non-anthocyanic flavonoids were 0.67, 0.86, and 0.77, respectively. The corresponding RMSECV were 0.12, 0.14, and 0.15 mg/g, respectively. It was concluded that the portable vis/NIR instrument performed similarly to bench top or portable vis/NIR instruments reported in the literature.

Keywords Apple · Visible near-infrared · Nutraceutical compounds · Chemometrics · Quality evaluation

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Introduction

In recent years, developments in both chemometric and instrumentation have resulted in rapid methods capable of predicting the concentration of specific chemical constituents. In particular, visible/near-infrared (vis/NIR) and near-infrared spectroscopy (NIR) are rapid and non-destructive techniques, requiring minimal sample processing before analysis. Together with chemometric methods vis/NIR appears to be one of the most convenient and straightforward analytical tools for studying food products.

The NIR region of the electromagnetic spectrum lies between the visible and infrared regions and spans the wavelength range between 750 and 2,500 nm. This region contains information concerning the relative proportions of C–H, N–H, and O–H bounds which are the primary structural components of organic molecules (Williams and Norris 2002). Vis/NIR has been successfully used to measure a range of apple quality attributes such as soluble solids content (Quilitzsch and Hoberg 2003; Zude et al. 2006), titratable acidity (Peirs et al. 2002), starch index (Menesatti et al. 2009), chlorophyll content (Zude-Sasse et al. 2002), volatile compounds with the combined use of electronic nose (Di Natale et al. 2002),

phytonutrients (Merzlyak et al. 2003), firmness (Manley et al. 2007; Harker et al. 2002; Mehinagic et al. 2003), and mealiness (Moshou et al. 2003). This method, based on the evaluation of all pieces in the lot and not just one sample, has also been used to monitor ripening-associated changes of chlorophyll and carotenoid pigments (Solovchenko et al. 2005; Solovchenko et al. 2006), to determine harvest date (Peirs et al. 2005), storage duration (Camps et al. 2007), and the incidence of susceptibility to storage disorders (Nicolaï et al. 2006; Wulf et al. 2003; Clark et al. 2004; Clark et al. 2003).

Phytonutrients and antioxidant compounds not only determine the nutritional and nutraceutical features, but they are also related to the potential fruit storability (Veberic et al. 2005). If apples could be sorted in terms of storage performance, a suitable management of consignments would allow the optimization of product quality throughout a selling season.

Studies have shown a close relationship between the incidence of peel and flesh browning and low content of vitamin C in pome fruit (Veltman et al. 1999). The browning occurs when the content of ascorbic acid falls below a threshold value that depends on cultivar and harvest time and this value is experimentally detectable (Veltman et al. 2000; Eccher Zerbini et al. 2002).

The aim of this work was an innovative application of vis/NIR spectroscopy to study phytochemicals, present in some cases at low concentration in fruit, which not only have particular health functionality, but are also important for storability.

For this purpose, we used a portable system based on vis/NIR technology (Guidetti et al. 2010), a more simple and versatile device compared to bench top NIR instruments. Regarding phytochemicals, we analyzed ascorbic acid, total polyphenols, anthocyanins, flavonoids, and carotenoid content. In addition, the system was tested for the quick evaluation of consolidated parameters like acidity, firmness, soluble solid content, and chlorophyll content.

Materials and Methods

Portable Vis/NIR System

A portable system was tested in the vis/NIR range (450–980 nm). In this system, samples were hit by radiation produced by a lighting system, and the reflected component was measured by a spectrophotometer and registered through dedicated software. The system was composed of five elements: a lighting system, a fiber optic probe, a portable spectrophotometer, a PC for data acquisition control, and a battery.

The light source was a 50-W halogen lamp (Decostar Coolblue, Osram, Munich, Germany) with a color temperature of 4,500 K and maximum emission at 500 nm. The halogen spotlight was positioned at one end of a specially created metal holder, and the optical fiber transmitting radiation to the samples at the other end.

Light radiation was shone onto the fruit sample through a bidirectional fiber optic probe ("step index" type, model FCR-19IR200-2-ME-S1, Avantes, Eerbeek, The Netherlands; Fig. 1). The choice of this optical fiber was based on the need to acquire spectra in diffuse reflectance. The probe consisted of 19 fibers of 200 μm diameter, 17 fibers carry light to the samples and two fibers carry radiation back from the sample to the spectrophotometer. It allowed the light radiation produced by the halogen lamp to shine on the sample while simultaneously collecting the radiation coming from the sample and transferring it to the spectrophotometer. A supplementary plastic cap could be placed on the fiber optic probe to touch the sample perfectly while avoiding environmental light interference.

The fiber optic probe was connected to the portable spectrophotometer (AvaSpec-2048, Avantes, Eerbeek, The Netherlands). The spectrophotometer was equipped with a diffractive grating for acquisition in the spectral range 450–980 nm and a CCD sensor with a 2048 pixel matrix to

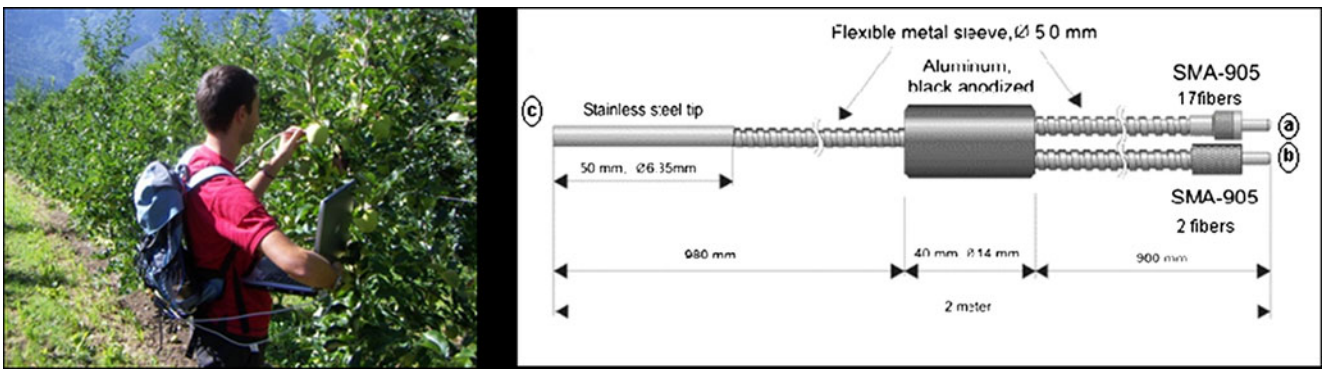

Fig. 1 Image of vis/NIR portable device at work and scheme of fiber optic probe: *c* the probe consists of 19 fibers of 200 μm diameter, *a* 17 fibers carry light to the samples, and *b* and two fibers carry radiation back from the sample to the spectrophotometer

record each wavelength signal intensity with a wavelength resolution of 0.3 nm.

The system was controlled by a portable PC with dedicated software for data processing and DAQ for automatic control of the halogen lamp. The device was powered by a 12-V battery that provided energy for the lamp during acquisitions of vis/NIR spectra. All components were housed in a backpack to allow transport in the field. The optical cable and PC remained outside the backpack.

Apple Samples

The research was carried out in Ponte in Valtellina (Sondrio, Northern Italy) on the two most representative local apple varieties: "Golden Delicious" and "Stark Red Delicious". Vis/NIR measurements were collected from apples on the trees just before fruit harvest. Samples were taken from orchards at two different altitudes (400 and 700 m a.s.l.) and with two different expositions (East and West), in order to obtain a representative data set of fruit grown under different conditions. Harvests were performed weekly during the end phase of the ripening of the fruit. Eight samplings were taken for "Golden Delicious" while six were taken for "Stark Red Delicious" from each orchard. A total of 160 samples for the "Golden Delicious" variety and 120 samples for the "Stark Red Delicious" variety were analyzed with the portable vis/NIR device. Vis/NIR spectra measurements were taken on each apple in two different points along the equator region. The two spectra were averaged in order to have a single averaged vis/NIR data for each fruit.

Portable vis/NIR devices are often used in controlled laboratory conditions (Antonucci et al. 2011; Camps and Christen 2009). Spectral analyses directly in field are more complicated because they are potentially sensitive to diurnal change of sunlight and temperature conditions that can influence instrument performances. To minimize these effects and standardize as much as possible lighting conditions, spectral acquisitions were done at the same time every morning. Tests were also done in field at different times of the day, underlining the fact that the intensity of artificial light produced by the lamp was always much greater than the sunlight near the measurement zone of the samples (contact zone between probe and fruit surface). There was also the action of the plastic cap on the end of the probe and the measurement on fruits not directly exposed to sunlight, which reduced the influence of sunlight to negligible values. Furthermore, carrying out measurements at the same time every morning reduced fluctuations of the signal due to temperature. In fact temperature during spectral acquisitions ranged from 15 to 25 °C. The sample temperature may be treated similarly to the variability of chemical components induced by differences between seasons, orchards, and cultivars (Peirs et al. 2003b). Spectral data set covers variations in our temperature range in a representative way and robust calibration models were developed (Peirs et al. 2003a). Moreover, the background noise was subtracted.

Total Soluble Solids, Titratable Acidity, and Firmness Determination

Analysis of fruit soluble solids content (SSC) (°Brix), firmness (N), and titratable acidity (TA) (g malic acid/L) was performed using the automatic "Pimprenelle" (Setop-Giraud Technologie, Cavaillon, France) system which gives a measurement for each single fruit of SSC and firmness and a mean value for titratable acidity for the batch (five fruits).

Determination of Total Phenols, Total Anthocyanins, Total Flavonoids, and Non-anthocyanic Flavonoids

For each sample preparation, five apples were peeled. Mesocarp and exocarp were separately immersed in liquid nitrogen and subsequently placed in a freezer at −80 °C. For the extraction of the polyphenols and of the anthocyanins, a solution of acidic ethanol (EtOH/HCl/H_2O 70:1:29, *v*/*v*) was used. Extraction of the phenols was performed on fruit of the two varieties while the anthocyanins and the flavonoids were tested only in the epicarp of "Stark Red Delicious". All the peels were weighed and blended to a homogeneous state. The supernatant was used for the analysis of total phenolic content according to the Folin-Ciocalteau method (Waterhouse 2005). One milliliter of Folin-Ciocalteau reagent, 0.5 mL of distilled water, and 2 mL of 20% Na_2CO_3 were added to 0.1 mL of extract. The solution was immediately diluted to a final volume of 20 mL with distilled water and then agitated. The optical density was measured after 90 min at 700 nm on a UV–vis spectrophotometer (Jasko model 7800, Tokyo, Japan). Results were reported as milligrams of catechin per liter of extract.

Total flavonoids and non-anthocyanic flavonoids were evaluated spectrophotometrically at 280 nm. A catechin standard curve was set and results were expressed as milligrams of catechin per liter of extract (Iriti et al. 2005).

Total anthocyanin content was estimated as malvidin 3-glucoside at 520 nm, using a molar absorptivity coefficient of 28,000 and expressed as milligrams per gram of fresh weight (Sinelli et al. 2008).

Determination of Chlorophyll and Carotenoids

Two disks of four different apples exocarp with a diameter of 1.2 cm were extracted in 95% ethanol and centrifuged at 10,000×*g* for 10 min. Pigments were immediately determined after extraction. Absorbance readings were taken at 665.2, 652.4, and 470 nm. Total chlorophyll and carotenoids were calculated by Lichtenthaler's formula (1987).

Ascorbate Determination

For ascorbate (ASA) analysis, 5 g of three fruit mesocarps were homogenized with 10 mL of cold 6% metaphosphoric acid in ice and centrifuged at 10,000×*g* for 30 min at 4 °C. The supernatant was transferred into a 25-mL volumetric flask at 4 °C. The pellet was washed with 8 mL of cold metaphosphoric acid solution and centrifuged. The supernatants were combined and diluted to the final volume with cold 6% metaphosphoric acid. After filtration through a 0.2-μm nylon filter, a 10-μL sample aliquot was injected onto an Inertsil ODS-3 (5 μm; 4.6×250 mm) GL Science column at 20 °C attached to a Series 200 LC pump (PerkinElmer, Norwalk, CT, USA) and eluted with 0.02 M orthophosphoric acid at a flow rate of 0.7 mL/min. Ascorbic acid was evaluated with an UV-975 intelligent UV/vis detector (Jasko, Tokyo, Japan) at 254 nm (Dalla Valle et al. 2007).

Data Processing

Chemometric analysis was performed using The Unscrambler software package (version 9.6, CAMO ASA, Oslo, Norway). Principal component analysis was performed on vis/NIR spectra to examine samples grouping and to identify outliers (Naes et al. 2000). Different treatments were applied to the vis/NIR spectra, namely scatter correction (multiplicative scatter correction) and derivatives, before building the calibration models. The first and second derivatives were performed using Savitzky-Golay transformation and smoothing (15 point and second-order filtering).

The vis/NIR spectra were correlated with ripening parameters (in accordance to indices including acidity, firmness, soluble solid, chlorophyll, and carotenoid contents) and with nutraceutic characteristics linked to the presence of bioactive compounds and antioxidants, including ascorbic acid, total polyphenols, anthocyanins, and flavonoids. Partial least square (PLS) regression algorithm was used. The PLS method performs particularly well when the various X-variables express common information, i.e., when there is a large amount of correlation, or even co-linearity, which is the case for spectral data of intact biological material (Nicolaï et al. 2007).

To evaluate the model accuracy the statistics used were the coefficient of determination in calibration (R_{cal}^2), the root mean standard error of calibration (RMSEC), the coefficient of determination in cross-validation (R_{cval}^2), and the root mean standard error of cross-validation (RMSECV) (Sinelli et al. 2008). Cross-validation is an internal validation method, usually used in the case of a small number of samples available for regression. With cross-validation some samples are kept out of the calibration and used for prediction. This is repeated until all samples have been kept out once. In this case full cross-validation was used, so only one sample at a time is kept out of the calibration.

The optimum calibrations were selected based on minimizing the RMSECV.

Percent errors (RMSEC% and RMSECV%) were also calculated as: RMSEC (%)=RMSEC/averaged reference values of each parameter. PLS models were calibrated for each apple variety separately.

Results and Discussion

Descriptive statistics for fruit qualitative and ripeness indices (SSC, titratable acidity, firmness, and total chlorophyll content) and for nutraceutical parameters (total anthocyanins, total phenols, total flavonoids and non-anthocyanic flavonoids, carotenoids, and ASA content) are shown in Tables 1 and 2 for "Golden Delicious" and "Stark Red Delicious", respectively. Wide variability in composition

Table 1 Descriptive statistics and statistics of the PLS models to predict maturity indices and nutraceutical properties of "Golden Delicious" apple

"Golden Delicious"	Number of samples (fruit)	Mean	SD	Calibration			Cross-validation		
				R^2	RMSEC	LV	R^2	RMSECV	RMSECV%
SSC (°Brix)	160	12.62	1.56	0.77	0.73	4	0.72	0.78	6.2
TA (g malic acid/L)	32 batches (160)	9.01	0.88	0.69	0.47	11	0.52	0.59	6.6
Firmness (*N*)	160	66.65	9.34	0.45	5.98	1	0.44	6.08	9.1
Chlorophyll (nmol/cm^2)	24 batches (96)	2.92	1.29	0.92	0.35	4	0.86	0.50	17.2
Ascorbic acid (mg/100 g)	34 batches (102)	5.36	2.12	0.88	0.40	3	0.50	0.83	15.5
Carotenoids (nmol/cm^2)	24 batches (96)	0.75	0.15	0.98	0.02	8	0.77	0.08	10.6
Total phenols (mg catechin/g)	32 batches (160)	0.50	0.11	0.12	0.10	2	0.09	0.10	20.0

For SSC and firmness that were measured on individual fruit, spectral data from two repeated measurements on the same fruit were averaged before PLS regression. For other attributes that were measured on a fruit batch basis, all spectral data from each fruit batch were averaged before PLS regression; 1 cm^2 of fruit exocarp weighs 0.068 g (±0.002 SD)

R^2 coefficient of determination, *RMSEC* root mean square error of calibration, *RMSECV* root mean square error of cross-validation, *LV* latent variables

Table 2 Descriptive statistics and statistics of the PLS models to predict maturity indices and nutraceutical properties of "Stark Red Delicious" apple

"Stark Red Delicious"	Number of samples (fruit)	Mean	SD	Calibration			Cross-validation		
				R^2	RMSEC	LV	R^2	RMSECV	RMSECV%
SSC (°Brix)	120	10.43	1.62	0.83	0.66	5	0.77	0.72	6.9
TA (g malic acid/L)	24 batches (120)	5.05	0.38	0.74	0.18	14	0.66	0.21	4.2
Firmness (*N*)	120	69.13	7.84	0.41	5.39	1	0.37	5.49	7.9
Chlorophyll (nmol/cm^2)	22 batches (88)	3.92	0.91	0.88	0.27	4	0.79	0.37	9.4
Ascorbic acid (mg/100 g)	30 batches (90)	0.38	0.59	0.46	0.07	2	0.41	0.07	18.4
Carotenoids (nmol/cm^2)	22 batches (88)	1.04	0.17	0.72	0.06	2	0.50	0.08	7.7
Total phenols (mg catechin/g)	24 batches (120)	3.34	0.43	0.62	0.06	5	0.56	0.06	1.8
Total anthocyanins (mg malvidin/g)	24 batches (120)	0.34	0.21	0.71	0.11	2	0.67	0.12	35.3
Total flavonoids (mg catechin/g)	24 batches (120)	2.77	0.54	0.90	0.11	2	0.86	0.14	5.0
Non-anthocyanic flavonoids (mg catechin/g)	24 batches (120)	2.37	0.39	0.96	0.07	7	0.77	0.15	6.3%

For SSC and firmness that were measured on individual fruit, spectral data from two repeated measurements on the same fruit were averaged before PLS regression. For other attributes that were measured on a fruit batch basis, all spectral data from each fruit batch were averaged before PLS regression; 1 cm^2 of fruit exocarp weighs 0.068 g (±0.002 SD)

R^2 coefficient of determination, *RMSEC* root mean square error of calibration, *RMSECV* root mean square error of cross-validation, *LV* latent variables

was observed as a result of different environmental and cultural conditions and ripening degrees (sampling times).

In Table 1 are also listed the statistics related to PLS models obtained by vis/NIR technology for SSC, firmness, chlorophyll, carotenoids, total polyphenols, and ASA in "Golden Delicious" apples. In Table 2 are mentioned the statistics related to PLS models obtained for "Stark Red Delicious". In addition to the parameters analyzed in "Golden Delicious", total anthocyanins, total flavonoids, and non-anthocyanic flavonoids were determined. Figures 2 and 3 show, as an example, the regression lines (cross-validation) of PLS models for SSC, chlorophyll, and carotenoids content in "Golden Delicious" and for SSC, chlorophyll, and flavonoids content in "Stark Red Delicious", respectively. Results regarding the nutraceutical parameters have shown different outcomes in the two cultivars.

The elaboration of a model for carotenoids displayed in both cultivar the presence of high correlation coefficients and low levels of standard error in cross-validation (RMSECV= 0.08 nmol/cm^2). Similar results were obtained by Merzlyak et al. (2003), showing for apples an estimation error in carotenoids determination of 0.08–0.10 nmol cm^{-2} with a vis/NIR spectrophotometer in the range 400–800 nm.

ASA data show high R^2 coefficient in calibration (0.88) in "Golden Delicious" and fair RMSECV values (0.83 mg/100 g). Results for ASA of "Stark Red Delicious" were less accurate (R^2=0.41 and RMSECV=0.07 in cross-validation). Only few reports are published in literature regarding ASA levels determined by NIR spectroscopy in fruits and vegetable tissue in general. Results of ASA prediction obtained by Xia et al. (2007) in Navel oranges and by Sinelli et al. (2008) in blueberry were similar to our findings.

The predicted results by PLS for total phenols were good in "Stark Red Delicious" ($R_{cv}{}^2$=0.56 and RMSECV= 0.06 mg catechin/g), while "Golden Delicious" were characterized by errors of prediction higher than those achieved for the other constituents. This problem of prediction could be justified by a very low concentration of this constituent in

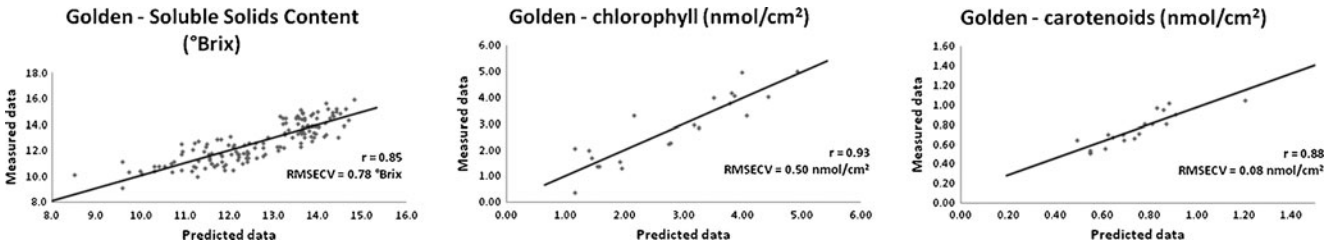

Fig. 2 Cross-validation line of PLS model built on "Golden Delicious" samples for soluble solids content (°Brix), chlorophyll, and carotenoids

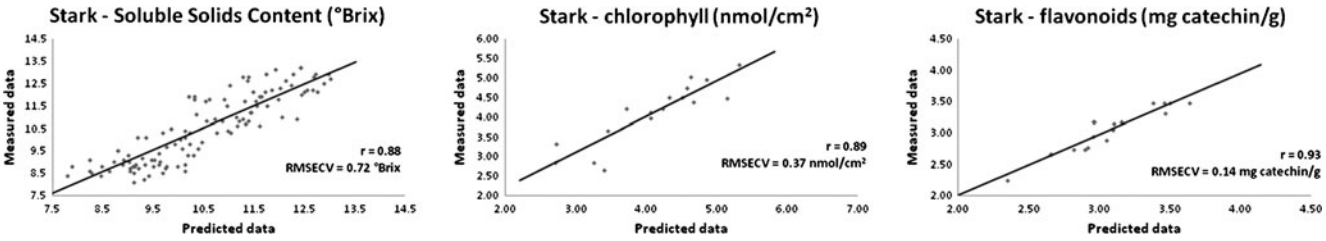

Fig. 3 Cross-validation line of PLS model built on "Stark Red Delicious" samples for soluble solids content (°Brix), chlorophyll, and flavonoids

this cultivar. On the other hand, RMSECV obtained for "Stark Red Delicious" was analogous to that achieved in olive oil (Mailer 2004).

Total anthocyanins in "Stark Red Delicious" were characterized by R_{cv}^2=0.67 and RMSECV of 0.12 mg malvidin/g. This value of RMSECV is probably caused by a very low level of this bioactive compound in this cultivar and a high data variability. Results are encouraging and slightly poorer compared to those obtained by Sinelli et al. in 2008, using an independent data set for validation (RMSEP=0.22 mg malvidin/g).

Good results were obtained for the prediction of "Stark Red Delicious" total flavonoids content (R^2=0.86, RMSECV=0.14 mg catechin/g). These are satisfactory results compared with literature: Zhang et al. (2008) showed models for flavonoids prediction on rice grain with r=0.66 and RMSECV=0.11 mg rutin equivalent/g. Lastly, very similar results were obtained for non-anthocyanic flavonoids non-destructive evaluation (R_{cv}^2=0.77, RMSECV=0.15 mg catechin/g).

Regarding the prediction of classical parameters like SSC, firmess, TA, and chlorophyll, good results were obtained in both the cultivar analyzed.

SSC estimation gave high R^2 values with a low standard error (RMSECV=0.8 °Brix) testifying a high level of prediction performance. In literature, results obtained on different fruits by different authors showed values of RMSECV about 0.6–1 °Brix (Nicolaï et al. 2007; Bobelyn et al. 2010; Yan-de and Yi-bin 2004). Results are also similar to those published for similar non-destructive applications on other fruits such as apricot (Camps and Christen 2009) and watermelon (Tian et al. 2007).

The models elaborated to estimate TA showed low values of RMSECV for both "Stark Red Delicious" and "Golden Delicious" (0.59 and 0.21 gmalic acid/L, respectively). Results are comparable to data published by other authors. Jha and Garg (2010) considered the potential of near-infrared spectroscopy in the wavelength range of 900–1,700 nm for determination of acidity for five cultivars of apple, including "Golden Delicious" and "Red Delicious". The lowest standard error of prediction with an independent validation set (SEP) was found to be 0.024%. Xiaoli and Yong (2006) investigated with vis/NIR spectroscopy the acidity (pH) of Chinese bayberry, achieving a SEP of 0.21.

The model elaborated for the evaluation of firmness showed encouraging results compared to those published by Fan et al. (2009), achieved by a more complicated application based on vis/NIR transmittance. Lu in 2007 used hyperspectral scattering imaging on "Golden Delicious" and "Red Delicious" apples, in the spectral region between 500 and 1,000 nm. Neural network models were built to predict fruit firmness obtaining, with an external validation set, a SEP of 6.2 N for "Golden Delicious" and 6.1 N for "Red Delicious". The application of NIR spectroscopy for the analysis of this parameter often encountered considerable difficulties, highlighted by some published studies (Nicolaï et al. 2008). Firmness decreases in parallel with a decrease in absorption in the 400–500 nm range, until reaching a minimum and then remains constant. This behavior of the firmness reflects the retention of carotenoids during ripening (Merzlyak et al. 1999). Finally, good correlation coefficients were achieved for chlorophyll prediction in both the cultivars (R_{cv}^2 about 0.9). The results obtained with this system are overall encouraging, similar to evaluation parameters for chemometric models that can be found in the literature (McGoverin et al. 2010; Zude et al. 2006).

Conclusion

Regarding the traditional indices of ripeness (SSC, TA, firmness, and chlorophyll content), similar results to the already existing data published in literature were obtained for both the varieties. In particular, compared to other portable vis/NIR instruments, the system tested in this work performed similarly in predicting SSC and TA, while slightly poorer in predicting firmness.

Good results, compared with those achieved with bench top vis/NIR instruments, were obtained in the estimation of carotenoids, total flavonoids, and non-anthocyanic flavonoids. Quite good results were also achieved for ASA, total phenols, and anthocyanins, compared to those published in literature for other produce.

Regarding calibration statistics for the two cultivars, only slight differences were recorded. In particular, different

results were obtained for total phenols, due probably to a very low concentration of this constituent in "Golden Delicious" with respect to "Stark Red Delicious".

The results are encouraging, although firmness and ASA need further investigation. Some modifications to the system should be taken into account, for example analyzing a wider spectral range for more accurate evaluation of all the parameters. The vis/NIR system could enable an effective quantitative and qualitative evaluation of the internal fruit quality and for large batches of produce, providing a better management of the processes of sorting, storing, and conservation of apples.

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