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NON-DESTRUCTIVE QUANTIFICATION OF CAROTENOIDS IN INTACT WATERMELON (*Citrullus lanatus*) USING ON-LINE NEAR INFRARED SPECTROSCOPY

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ABSTRACT

The major interest of carotenoids is related to their antioxidant action in the organism. A great interest has recently been focused on lycopene and β-carotene. Red flesh watermelon is, together with tomato skin, the main food sources of lycopene as the most abundant carotenoid. The use of NIRS in the post harvesting stage has permitted to rapidly quantify lycopene, β-carotene content and total soluble solids (TSS) of single intact fruits. 135 samples were picked up in the period of 2013-2015. Fruits were submitted to NIR radiation while transporting along the conveyor belt system in stationary and in movement, and at different positions on the belt.
800 spectra from 100 samples were collected as calibration set in the 900-1700 nm interval using an on-line diode array NIR instrument (NIROnLine, Buchi, Switzerland. Calibration models were performed using PLS regression on pretreated spectra (derivatives and SNV) in the ranges 2.65-151.75 mg/kg (lycopene) and 0.19-9.39 mg/kg (β-carotene) and 5.3-13.7% (soluble solids). External validation were carried out with 35 samples and on 35 spectra. The PLS models for intact watermelon could predict lycopene concentration with R^2 of 0.877 and SECV of 15.68 mg/kg, β-carotene concentration with R^2 of 0.822 and SECV of 0.81 mg/kg and TSS with R^2 of 0.836 and SECV of 0.8%. External validation have confirmed predictive ability with R2 of 0.805 and RMSEP of 16.19 mg/kg for lycopene, with R^2 of 0.737 and RMSEP of 0.96 mg/kg for β-carotene and with R^2 of 0.707 and RMSEP of 1.4%.

KEYWORDS: watermelon, lycopene, β-carotene, carotenoids, NIR spectroscopy

INTRODUCTION

Fruit consumption is no longer merely a result of taste and personal preference, but is being a concern of health due to the fruit nutrients content[1]. Fruits and fruit-based products are increasingly becoming a significant component of the human diet worldwide and are associated with reduced risks of some types of cancer and cardiovascular diseases[2]. These beneficial properties appears to be related to antioxidants, mainly carotenoids. Even though human body does not synthetize carotenoids, they are ubiquitous and contribute to a number of biochemical processes balancing the oxidative stress of cells[3]. Chemically, they are a group of pigments, varying from yellow to red in colour, characterized by a C40 carbon chain highly unsaturated[4]. Lycopene exhibit the highest antioxidant activity and singlet oxygen quenching ability of all dietary carotenoids[5]. Alongside of tomatoes, which has been extensively studied as source of lycopene[6,7], watermelon has moved up to the front of the line in recent research studies because the mean lycopene concentration of watermelon (37-122 mg/kg, depending on ripening stage and temperature during crop growth) is about 40% higher than the year-round mean for raw tomato (25-90 mg/kg). Moreover, in watermelon, lycopene is the most abundant (84-97%) carotenoid[8].

Due to the increasing popularity of lycopene as one of the important nutraceuticals for use in food and nutritional supplements, there is a great interest in developing lycopene rich products and using lycopene from watermelon. It requires adequate measurement methods. Available assays are based on chromatography or UV-Vis spectroscopy, both starting with extraction of lycopene from samples in hazardous organic solvents, and are time consuming, tedious and destructive[9]. The application of analytical techniques rapid, solvent-free and easily usable in the field and in food processing industries would present an enormous advantage over the conventional wet chemistry-based approaches. NIR spectroscopy allied to multivariate calibration techniques fulfills all the above

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requirements[10]. NIR spectroscopy has already been applied to non-destructive determination of lycopene in tomato pulp and skin[11]. Despite difficulties due to the optical thickness of rind and high water content (typically 90% w/w), NIR diffuse reflectance method has been also successfully used to predict total sugar content in watermelon, as total soluble solids (TSS)[12]. However, to our knowledge, there is no published data on the quantitative determination of lycopene and β-carotene based on NIR spectroscopy in intact watermelon. Therefore, the main objective of this study was to assess the application of NIR spectroscopy for predicting the content of lycopene and β-carotene, simultaneously estimating also the total soluble solids (TTS). Based on the results obtained, NIR was used on-line for watermelons selection while passing on the conveyor belt before packing. The final aim was to provide an important issue for agri-food product valorization using NIR technology.

MATERIALS AND METHODS

Watermelon fruit samples

The watermelon (*Citrullus lanatus*) cultivar MINIROSSA®, selected as having high carotenoids content, has been used for all the experimental work. MINIROSSA® has characterized by small size (diameter, 100-150 mm) and very thin and striped green rind (<0.5 mm). All fruits were grown in open field in the same area (North-East of Italy) under a Mediterranean climate during 3 campaigns from 2013 to 2015. Fruits were harvested on multiple harvest dates from late June to mid Septemper of every year at dates according to the maturity stage for each one. No standardized index for watermelon ripening is available, so field ripeness was usually judged by thumping the rind, giving an unavoidable margin of error. During 2013, 40 samples were picked up corresponding to 100% grade of ripening, whilst in 2014 60 samples were samples, 12 for each of the 5 ripening stage: 50% (completely unripe), 80% (almost ripe) 100% (ripe), 110% (just overripe) and 130% (completely overripe). In 2015, other 35 fruits were sampled at randomly different grades of ripening from the field.

A total of 135 samples were used for development and external validation of regression models for each parameter od interest (lycopene, β-carotene e TSS). 100 samples were used for the calibration set, 35 for external validation set. Lycopene concentration varied from 2.65 and 151.75 mg/kg of fresh pulp, β-carotene concentration from 0.19 to 9.39 mg/kg of fresh pulp and TSS concentration from 5.3 to 13.7%. In the other new 35 samples for external validation, concentration intervals of 7.0- 141.2 mg/kg, 2.00-11.6 mg/kg and 8.8-13.2% for lycopene, β-carotene and TTS respectively, was found.

Spectral measurement

The reflectance spectra of intact watermelon were collected with a NIR On-Line® X-One (Buchi, Switzerland), equipped with diode array detector and tungsten-halogen dual lamp. The selected wavelength range used was from 900 to 1700 nm, with a measuring time of 10 milliseconds and a reading every 10 nm, for a total of 80 readings per sampled area. Whole watermelons were placed on the belt in such a way that the distance between the light window and the fruit rind was 40 mm. In 2013 sample campaign, each fruit was submitted to light beam at two different positions on the stationary belt, obtaining 80 spectra. In 2014 each fruit was sampled on moving belt at different speed rate (3-4-5 fruits/sec) and at 4 different positions, in order to simulate when fruits randomly fall down on the belt from the collecting containers, obtaining 720 spectra. Finally, in 2015, for each of 35 fruits while passing on the belt at random position one spectrum was collected.

Data analysis

Partial least squares (PLS) regression were computed on the calibration set of 800 spectra using SX-Plus software (Buchi, Switzerland). Spectra were previously pre-treated with 1st derivative and SNV. Model performance was determined using the blockwise cross-validation approach making a leave-out representing 20% of the sample, and evaluated by means of coefficient of determination (R^2), standard error of calibration (SEC) and standard error of cross- validation (SECV). Outliers' analysis was performed by the software using Mahalanobis distance criterion. External validation was performed comparing NIR predicted data and reference data with Microsoft Excel® 2010 and calculating coefficient of determination (R^2) and the root mean square error of prediction (RMSEP) as reported by Martens, 2001[13].

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Reference assays

Carotenoids concentration in watermelon fresh pulp was determined after extraction in chloroform by means of reversed phase HPLC (Jasco, Japan) method[14]. Total soluble solid content was obtained as refractometric Brix Degree measurement.

RESULTS AND DISCUSSION

Original spectra are shown in Figure 1(A) and 1(B). Baseline offset of the spectra in 2014 could be due to the effect of movement at the different speed rate, whilst in 2013 spectra were collected on stationary belt. The score plot (Figure 1(C)) has evidenced the presence of two clusters based on year of sampling. It's worthwhile noting that inside the 2014 group, a separated sub-cluster corresponding to overripe samples (130%) can be clearly recognized. Spectra were surely influenced by the completely different physical-chemical characteristics of over-ripe watermelons in comparison with the other ripening stages.

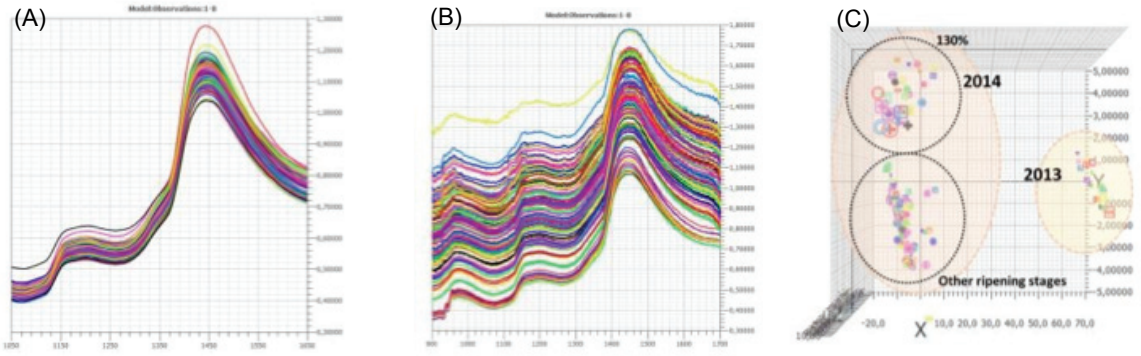

Figure 1. Original spectra of intact watermelon collected in 2013 (A) and 2014 (B) and calibration set scores plot (C).

Since spectra were collected on fresh fruit, they are dominated by a large absorption of water at around 1460 nm, owing to the first O-H overtone and O-H combination band, respectively (15). Typically, spectra of fresh materials do not allow an easy visual interpretation, because of the presence of several interferences. Nevertheless, wavelengths regions of the spectra that evidenced the highest correlation are in the ranges 900-1050 and 1400-1500 nm corresponding to 2nd and 1st overtone of O-H bonds of sugars and water, and in the ranges 1100-1250, 1300-1350 and 1650-1700 nm, corresponding to 3rd overtone, combo bands and 1st overtone respectively, of C-H bonds of carotenoids. PLS models for calibration of the three parameters of interest are shown in Figure 2.

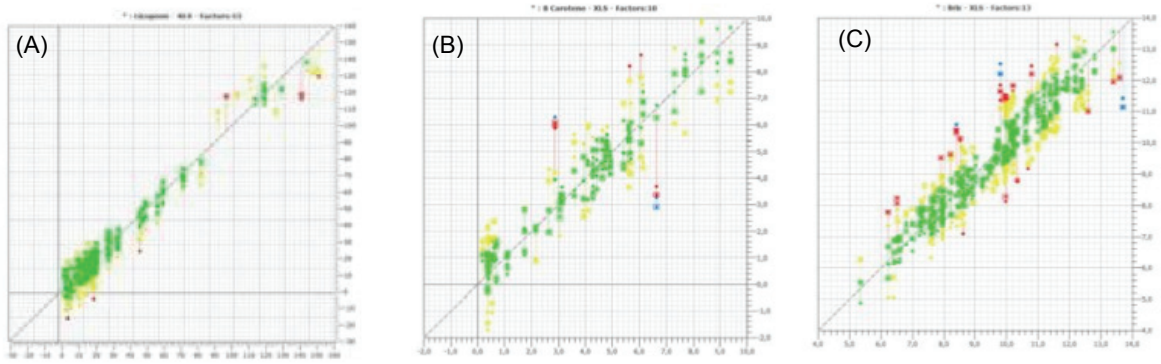

Figure 2. Calibration and cross validation curves for lycopene (A), b-carotene (B) and TSS (C). Red and blue samples are outliers. They are visualized but excluded from the models.

For lycopene and β-carotene, data from 2013 and 2014 are almost completely separated in 2 different parts of the curves, in particular samples harvested in 2013 had meanly lower concentration values than samples harvested in 2014, due to the different climate condition between the two years. In fact, carotenoids concentration in watermelon is strongly dependent on rainfall frequency and external temperature[16]. 2013 was characterized by particularly warm and dry weather, contrary to 2014, which will be mentioned in Italy as one of the colder and humid summer for decades. Differently from carotenoids TSS data are completely mixed up. TSS, which could be approximated to sugars content, depends on ripening stage rather than on climate conditions.

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External validation plots were depicted in Figure 3.

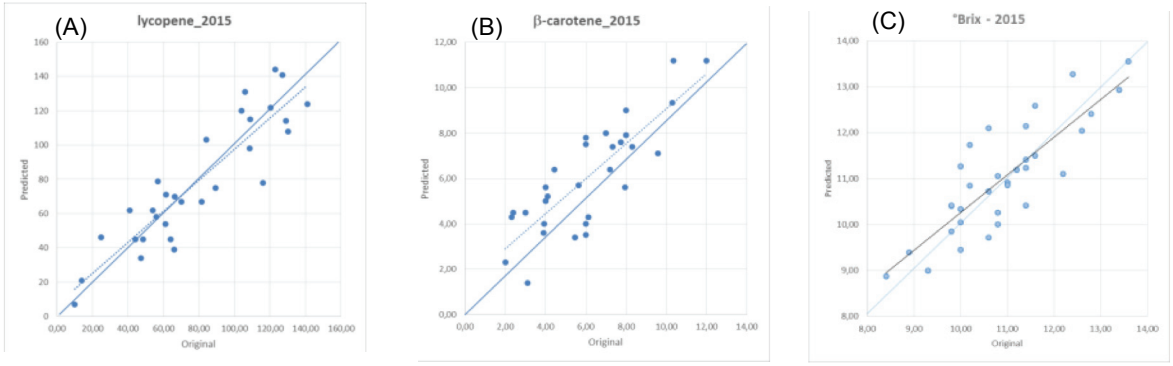

Figure 3. External validation plot for lycopene (A), β-carotene (B) and TSS (C).

Performance was evaluated plotting NIR predicted data and reference analysis for lycopene, β-carotene and TSS of 35 randomly harvested samples and based on single spectrum acquired when fruits pass on the convey-or belt. RMSEP values could be considered quite satisfactory, even though improvable, because in all the three cases it corresponds to errors about or below 20%. A summary of statistical results of calibration, cross-validation and external validation are reported in Table 1.

Table 1. Statistics of calibration, cross-validation and external validation results for intact watermelons.

	Lycopene (mg/kg)		**β-carotene (mg/kg)**		**TTS (%)**	
	CAL	EXT VAL	CAL	EXT VAL	CAL	EXT VAL
#sample	100	35	100	35	100	35
Range	2.65-151.75	7.00-141.23	0.19-9.39	2.00-11.68	5.3-13.7	8.8-13.2
#spectra	759	35	735	35	754	35
R^2	0.877	-	0.822	-	0.836	-
SEC	14.8	-	0.75	-	0.7	-
R^2CV	0.756	-	0.810	-	0.820	-
SECV	15.7	-	0.81	-	0.8	-
R^2	-	0.805	-	0.737	-	0.707
RMSEP	-	16.2	-	0.98	-	1.4

CONCLUSION

Application of NIR on-line in the postharvest watermelon processing represents a significant step forward in improving quality of products. Characterizing carotenoids content and TSS as sugars index for each intact fruit could permit to farmers to valorize the nutritional value of fruits and so increasing consumer's awareness on beneficial effects on health deriving from fresh products consumption in human diet.

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