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Prediction of lycopene and β-carotene in tomatoes by portable chroma-meter and VIS/NIR spectra

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ABSTRACT

The aim of this study was to examine the possibility of non-destructive estimation of lycopene and β-carotene content in intact tomato fruit. Representative tomato fruits were harvested at different maturity stages and measurement of visible/near infrared (VIS/NIR) spectra and color variables were followed by reference analysis of lycopene and β-carotene. Models predicting lycopene and β-carotene from color variables and VIS/NIR spectra of intact tomato were developed. Regression coefficient between color variables (a*, a*/b*, and (a*/b*)^2) and reference values of lycopene and β-carotene content were (0.90, 0.98 and 0.52) and (0.75, 0.81 and 0.58), respectively for the prediction set. Meanwhile, after observing predictive *p*-values in multiple regression, best equations were developed to predict the contents of lycopene and β-carotene from color values with regression coefficient of 0.97 and 0.85 for lycopene and β-carotene, respectively. On the other hand, the selected partial least square (PLS) model of VIS/NIR spectra had good predictive power for lycopene and β-carotene showing high correlation coefficient of 0.85 and 0.77, respectively, between measured and predicted samples. This study revealed that, estimation of the lycopene and β-carotene content in tomatoes could be achieved by a portable chroma meter and VIS/NIR spectroscopy, with a possible application at field and agricultural processing centers, respectively.

1. Introduction

Carotenoids are a group of pigments occurring naturally in the chromoplasts and chloroplasts of plants. They are divided into two classes: xanthophylls (which contain oxygen) such as lutein, and carotenes (which are purely hydrocarbons, and without oxygen) such as β-carotene and lycopene (Giorio et al., 2007; Pandurangaiah et al., 2016). Carotenoids serve to absorb light energy for use in photosynthesis, and they protect chlorophylls and other elements of the photosynthetic apparatus from photodamage (Ramel et al., 2012). Although carotenoids are essential nutrients, they cannot be synthesized by animals and humans, and thus have to be consumed through the diet (Latowsk et al., 2014).

The characteristic pigmentation of tomato fruit is the result of synthesis of carotenoids, which is associated with the change in fruit color from green to red as chloroplasts are transformed to chromoplasts (Pék et al., 2010). Ripe tomato fruits accumulate large amounts of red linear carotene (lycopene) and small amount of its orange cyclisation pro vitamin A product (β-carotene) (Rosati et al., 2000; Carrillo-López and Yahia, 2014). Carotenoids have capacity to quench singlet oxygen as well as triplet chlorophylls through a physical mechanism involving transfer of excitation energy followed by thermal deactivation (Ramel et al., 2012). They are efficient free radical scavengers, and modulate the immune system (Rao and Agarwal, 1999). Regular intake of an adequate amount of fresh tomatoes or tomato products prevents the development of various types of cancers; strong evidence has been found for cancers of the lung, stomach, and prostate gland, and suggestive data were reported on their beneficial effects in cancers of the cervix, breast, oral cavity, pancreas, colorectum, and esophagus as well as cardiovascular diseases (Giovannucci et al., 2002; Hadley et al., 2003; Tilahun et al., 2017).

Different techniques have been used for lycopene and β-carotene content measurement; most of the methods currently used for tomato lycopene and β-carotene content measurement are destructive; HPLC analysis allows accurate quantification but it is laborious, requires proper skill to produce consistent results and uses toxic solvents (Kimura and Rodriguez-Amaya, 1999; Hyman et al., 2004). Spectrophotometric measurement of either fruit extract (Rao and Agarwal,

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1998) or puree (Davis et al., 2003; Hyman et al., 2004) is also common but this evaluation method is also costly, time consuming, requires sample preparation and needs precautions to avoid pigment oxidation during extraction. This calls for the development of a rapid, low-cost, reliable and reproducible analytical method. Pasquini (2003) reported that NIR range spectra produce qualitative and quantitative information derived from the interaction between NIR spectra and organic compounds that forming the substance. Both chromaticity values (Arias et al., 2000; Hyman et al., 2004) and VIS/NIR spectra (Clément et al., 2008; Baranska et al., 2006) have been reported as rapid, low-cost and reliable methods. However, to the best of our knowledge, prediction of both lycopene and β-carotene at the same time from color variables and VIS/NIRS spectra of tomato fruit has not yet been reported. The aim of this study was therefore, to develop models suitable to predict lycopene and β-carotene content of tomato fruit based on VIS/NIR spectra and color values from Minolta chroma meter.

2. Materials and methods

2.1. Chemicals

The chemicals used (hexane, ethanol and acetone) were of analytical grade and obtained from Dae-Jung Chemicals, Korea; standard β-carotene was purchased from Sigma-Aldrich, Korea

2.2. Plant materials

Fruit of “244” tomato cultivar which is commonly grown by the surrounding farmers at the Kangwon province of South Korea were harvested at breaker, pink and red maturity stages in four consecutive harvests at 7 days interval using tomato color chart (USDA, 1997) from greenhouses in spring 2016. The three maturity stages (breaker, pink and red) were represented equally when the spectra readings, color readings and sampling were done. Color readings were taken immediately after taking the VIS/NIR spectroscopy readings and samples for reference analysis (lycopene and β-carotene) were frozen by liquid nitrogen and stored in deep freezer (–80 °C) until analysis.

2.3. Color measurement and analysis

Fruits were selected based on their uniformity and freedom from defects and blemishes. Hunter a^* (redness), b^* (yellowness), and L^* (lightness) values (McGuire, 1992) were determined using a CR – 400 chroma meter (Minolta, Tokyo, Japan). Color variables were measured three times from the equator region of each tomato fruit samples and the average determined.

Samples were divided into calibration and prediction sets to compare the performance of regression models developed by using color variables. Fruit samples were harvested during 4 consecutive harvesting periods. 60 fruit samples (breaker, pink and red stages; 20 each) were used during each harvesting period. The fruit samples (180 fruit) from the first three consecutive harvests were used for calibration set and fruit samples from the fourth harvest (60 fruit) were used for prediction set. A total of 240 fruit samples were used for the experiment. Color values were measured 3 times from the equator region of each tomato.

2.4. VIS/NIR spectra measurement and analysis

The transmittance spectra of tomatoes were acquired from intact tomato fruit using VIS/NIR spectrometer (Life & Tech, CO, Ltd, Yongin, Korea) (Fig. 1A) in spectral region of 500–1100 nm with three (12 V/100 W) halogen lamp as a source of VIS/NIR light and fruit holder to keep the fruit right above the detector (Fig. 1B), the integration time was set to 300 m s and the measurement was done 6 times (different fruit directions) per 1 fruit to reduce noise from being included. A total of 3500 data were saved for each measurement at 0.2 nm spectrum resolution. NIR spectrometer was connected to computer for data transmission.

Samples were divided into calibration and prediction sets to compare the performance of the regression models developed with PLS model. A total of 1440 spectra readings representing breaker, pink and red stages were obtained from fruits harvested at four different harvesting period. Outliers were excluded and a total of 1160 spectra were used for analysis. The calibration samples were randomly selected and used for model development using the leave one out cross validation procedure. Half of the samples (580 spectra readings) were used for calibration models, and the remaining half (580 spectra readings) were used for cross validation. The original spectra were transformed by Hanning window, Standard Normal Variate (SNV), Multiplicative Scattering Correction (MSC) and first derivatives to reduce systematic noise and remove unwanted information. To select the optimal number of latent variables in PLS model, cross validation was performed based on lowest predicted residual error sum of squares (PRESS) value.

To establish a linear relationship between spectral data and measured references, partial least square (PLS) regression analysis was performed with MATLAB R2012b (Version 8.0.0.783, The Math Works, Inc., Natick, MA, USA).

The performance of the developed PLS model was evaluated in terms of RMSECV (root mean square of standard error in cross validation), RMSEP (root mean square of standard error in prediction), coefficient of determination for cross validation (R^2) and correlation coefficient for prediction(r). A predictive model with fewer bias value, lower RMSECV and lower RMSEP is considered to be good prediction model.

2.5. Reference analysis: lycopene and β-Carotene

Lycopene content of triplicate tomato fruit samples were determined according to the method of Fish et al. (2002), with some modifications. Homogenized frozen tomato samples (1 g of each) were placed into vials, to which was added 5 mL acetone, 5 mL of ethanol, and 10.0 mL of hexane. Vials were then centrifuged (5,871 × g for 15 min). Afterwards, 3 mL of deionized water was added to each vial, and the samples were shaken for another 5 min. Vials were left at room temperature for 5 min without agitation to allow phase separation. The absorbance of the hexane (upper) layer was measured with a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 503 nm versus a blank of hexane solvent. The lycopene content of the samples was then expressed as mg kg^{-1} of fresh weight according to the method reported by Fish et al. (2002).

β-Carotene content of triplicate tomato fruit samples were determined according to the method of Park and Kim (2002). The same procedures followed for lycopene content determination were used and absorbance of the hexane layer was measured with a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 448 nm versus a blank of hexane solvent. β-Carotene content was quantified by comparing the sample readings with the standard curve and expressed as mg kg^{-1} fresh weight of the sample.

3. Results and discussion

3.1. Color variables vs. reference analysis

Mean and ranges for reference lycopene, lycopene estimated by using the measured color variables in the calibration data set and lycopene predicted from the measured color variables in the prediction data set are shown in Table 1. The variability of the results is also shown in Table 1, represented as standard deviation (SD). R^2, RMSECV and RPD values of the calibration data set ranged between 0.42–0.94, 1.06–3.25 and 0.76–2.80, respectively. In the prediction data set, the corresponding values were 0.50–0.98, 1.11–4.90 and 1.07–4.43, respectively for r, RMSEP and RPD (Table 1). Hunter's L values and (a^*/

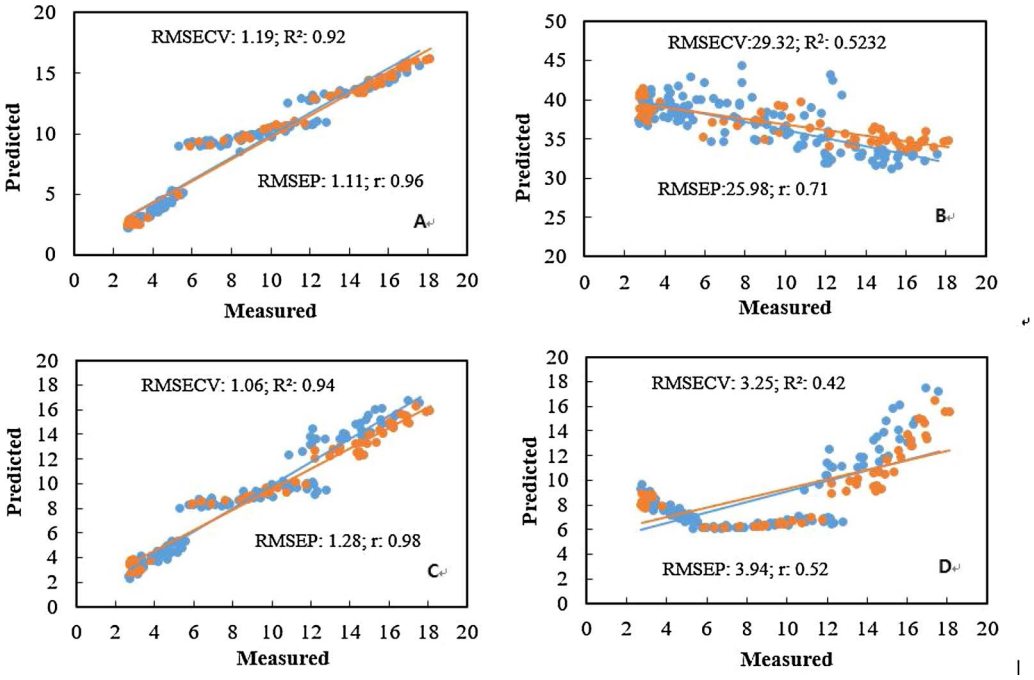

Fig. 1. Reference vs. Predicted scores of lycopene in the calibration (blue) and prediction (red) sets with PLS models using: A. Hunter's a* values; B. Hunter's b* values; C. a*/b* values; and D. $(a^*/b^*)^2$ values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$b^*)^2$ values had lower R^2 (0.49 and 0.42, respectively) as compared to a^*(0.92) and a^*/b^*(0.94) (Table 1 and Fig. 1). The RMSECV values for Hunter's L^*, a^*, b^*, a^*/b^* and $(a^*/b^*)^2$ were 3.05, 1.19, 2.95, 1.06 and 3.25, respectively (Table 1, and Fig. 1). For the prediction data set, the PLS model for a^*/b^* had the highest coefficient of correlation (0.98), followed by a^*(0.90) and b^*(0.71). The lowest RMSEP value (1.11) was observed in a^* values, followed by a^*/b^*(1.28) while the highest value (4.90) was observed in L^*, followed by b^*(4.05) (Table 1 and Fig. 1). Similar to the present study, Arias et al. (2000) reported linear regressions of the lycopene content and a^*/b^* with high coefficient of correlation (0.96) for Laura tomatoes. Hyman et al. (2004) also evaluated 24 tomato genotypes in two harvests and reported higher correlation (0.74) of $(a^*/b^*)^{2.5}$ for whole fruit and (0.94) of a^{*4} for puree values with actual lycopene measured by HPLC. Davis et al. (2003) evaluated puree absorbance method for four different cultivars and tomato products, they observed linear correlation coefficients with lycopene content determined by hexane extraction/spectrophotometry of R^2 (0.97) for fresh tomato, and (0.88) for tomato products. In the present study, Hunter's L values and $(a^*/b^*)^2$ values had lower R^2 (0.50 and 0.52, respectively) (Table 1 and Fig. 2). Unlike the present finding, D'Souza et al. (1992) reported the best R^2 (0.75) of $(a^*/b^*)^2$ and lycopene for three tomato cultivars.

Table 2 shows mean and ranges for reference β-carotene, β-carotene estimated by using the measured color variables in the calibration data set and β-carotene predicted from the measured color variables in the prediction data set. The variability of the results, represented as standard deviation (SD), is also shown in Table 2. R^2, RMSECV and RPD values of the calibration data set ranged between 0.48–0.74, 0.95–1.34 and 0.68–1.04, respectively. In the prediction data set, the corresponding values were 0.58–0.81, 0.84–1.44 and 0.65–2.08, respectively for r, RMSEP and RPD (Table 2). Hunter's L^* values and $(a^*/b^*)^2$ values had lower R^2 (0.63 and 0.58, respectively) as compared to a^*(0.75) and a^*/b^*(0.81) (Table 2 and Fig. 2). The RMSECV values for Hunter's L^*, a^*, b^*, a^*/b^* and $(a^*/b^*)^2$ were 1.34, 1.10, 1.21, 0.95 and 1.25, respectively (Table 2, and Fig. 2). For the prediction data set, the PLS model for a^*/b^* had the highest coefficient of correlation (0.81), followed by a^*(0.75) and b^*(0.69). $(a^*/b^*)^2$ on the other hand, had the lowest coefficient of correlation (0.58) (Table 2 and Fig. 2). Hyman

Table 1
Statistics for calibration and prediction of lycopene using tomato color variables.

Set	Parameters	Sample number	Mean	Range	SD	R^2	RMSECV	RPD
Calibration	Reference lycopene	180	8.45	2.72–17.59	4.29			
	Hunter's L*		8.45	1.93–13.50	3.01	0.49	3.05	2.45
	Hunter's a*		8.45	2.14–15.57	4.12	0.92	1.19	0.94
	Hunter's b*		8.44	1.16–14.22	3.11	0.52	2.95	2.33
	a*/b*		8.45	2.23–16.67	4.16	0.94	1.06	0.76
	$(a^*/b^*)^2$		8.45	6.04–17.46	2.79	0.42	3.25	2.80
Set	Parameters	Sample number	Mean	Range	SD	r	RMSEP	RPD
Prediction	Reference lycopene	60	10.63	2.75–18.13	5.32			
	Hunter's L*		7.62	0.27–13.62	2.76	0.50	4.90	4.43
	Hunter's a*		10.29	2.41–16.19	4.88	0.90	1.11	1.07
	Hunter's b*		8.78	3.99–11.86	2.26	0.71	4.05	3.60
	a*/b*		10.04	2.75–16.23	4.46	0.98	1.28	1.07
	$(a^*/b^*)^2$		9.55	6.10–16.40	2.87	0.52	3.94	3.68

RMSECV: Root mean square error of cross validation; RMSEP: root mean square error of prediction; RPD: Residual Predictive Deviation; R^2: coefficient of determination in cross validation and r: coefficient of correlation in prediction data set.

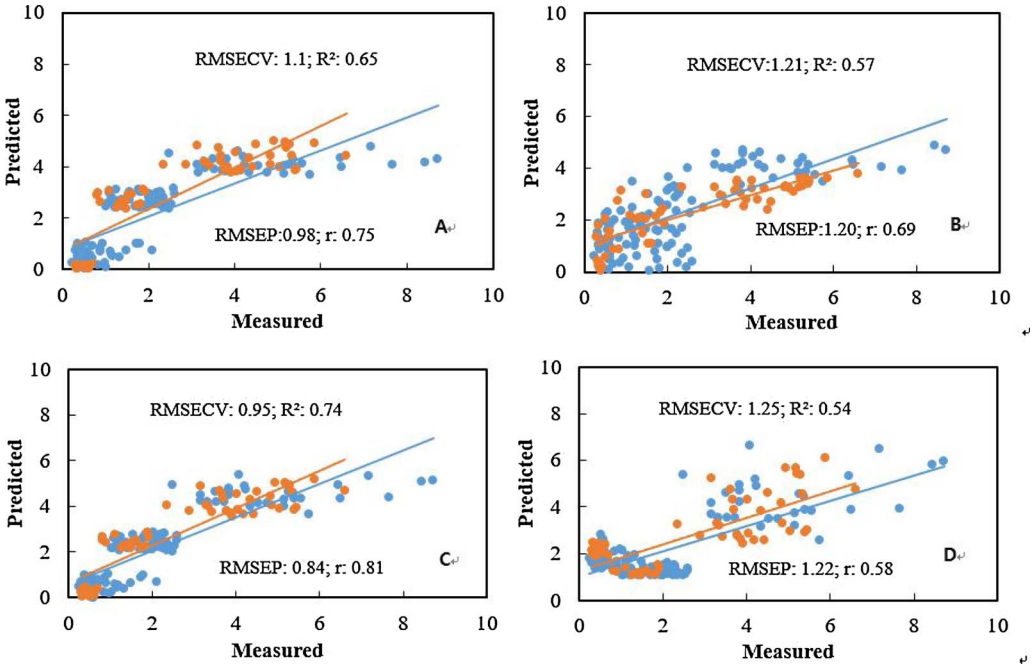

Fig. 2. Reference vs. Predicted scores of β-carotene in the calibration (blue) and prediction (red) sets with PLS models using: A. Hunter's a* values; B. Hunter's b* values; C. a*/b* values; and D. $(a^*/b^*)^2$ values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Statistics for calibration and prediction of β-Carotene using tomato color variables.

Set	Parameters	Sample number	Mean	Range	SD	R^2	RMSECV	RPD
Calibration	Reference β-carotene	180	2.20	0.24–8.72	1.86			
	Hunter's L*		2.23	0.05–4.33	1.22	0.48	1.34	0.93
	Hunter's a*		2.20	0.02–4.77	1.48	0.65	1.10	0.78
	Hunter's b*		2.21	0.04–4.89	1.39	0.57	1.21	0.96
	a*/b*		2.20	0.00–5.35	1.59	0.74	0.95	0.68
	$(a^*/b^*)^2$		2.23	1.05–6.62	1.36	0.54	1.25	1.04
Set	Parameters	Sample number	Mean	Range	SD	r	RMSEP	RPD
Prediction	Reference β-carotene	60	2.64	0.30–6.60	1.90			
	Hunter's L*		2.01	0.06–6.90	1.18	0.63	1.44	2.08
	Hunter's a*		2.87	0.01–5.00	1.77	0.75	0.98	0.79
	Hunter's b*		2.32	0.03–3.79	1.08	0.69	1.20	0.96
	a*/b*		2.81	0.02–5.18	1.71	0.81	0.84	0.65
	$(a^*/b^*)^2$		2.76	1.08–6.11	1.40	0.58	1.22	0.99

RMSECV: Root mean square error of cross validation; RMSEP: root mean square error of prediction; RPD: Residual Predictive Deviation; R^2: coefficient of determination in cross validation and r: coefficient of correlation in prediction data set.

et al. (2004) reported low results for a^*/b^* (0.43) and a^*(0.01) but similar result b^*(0.55) for selected regression models relating color values taken on whole fruit with β-carotene content. The lowest RMSEP value (0.84) was observed in a^*/b^* values, followed by a^*(0.98) while the highest value (1.44) was observed in L^*, followed by $(a^*/b^*)^2$ (1.22) (Table 2 and Fig. 2).

Different PLS models were developed on the basis of color variables of tomato fruit to predict lycopene and β-carotene content. Pertinent results were obtained for both dependent variables (lycopene and β-carotene content). The PLS models of a^*/b^* and a^* had, higher R^2/r and lower RMSECV/P values compared to the other color variables for both lycopene and β-carotene. The higher r and the lower RMSEP for both dependent variables showed the promising predictive capabilities of the developed models (Figs. 1 and 2). On the other hand, L^* and $(a^*/b^*)^2$ had lower R^2/r and higher RMSECV/P values compared to the other color variables for both lycopene and β-carotene. This indicates that the model had less power to predict both lycopene and β-carotene. Arias et al. (2000) reported best regressions of the lycopene content measured by HPLC with the lycopene predicted from a^*, a^*/b^*, and $(a^*/b^*)^2$ color factors; and they recommend to use a^*/b^* for predicting the lycopene content in Laura tomatoes. In addition, a positive correlation (r = 0.99) was reported by Periago et al. (2004) between lycopene content and color parameter $(a^*/b^*)^2$ values. Hyman et al. (2004) reported R^2 (0.54) for puree and (0.55) for whole fruit after regressing β-carotene measured by HPLC on the a^{*2} and b^* values, respectively. Similar to the present study, they observed higher correlation coefficients between color values and lycopene content than between color values and β-carotene content.

After observing predictive analysis in multiple regression, a* and a*/b* in lycopene prediction and (a^*/b^*) and $(a^*/b^*)^2$ in β-carotene prediction were found to have very high predictive *p*-values; the following predictive equations were then found to be the best equations to predict lycopene and β-carotene from color values

$$\text{Lycopene content (mg kg}^{-1}) = 7.58 + 0.174\,(a^*) + 6.8\,(a^*/b^*)$$

$$\text{Lycopene content (mg kg}^{-1}) = 7.58 + 0.174\,(a^*) + 6.8\,(a^*/b^*)$$

$$\text{β-carotene (mg kg}^{-1}) = 1.417 + 2.722\,(a^*/b^*) + 2.241(a^*/b^*)^2$$

$$\text{β-carotene (mg kg}^{-1}) = 1.417 + 2.722\,(a^*/b^*) + 2.241(a^*/b^*)^2$$

Reference vs. predicted scores of lycopene and β-carotene in the

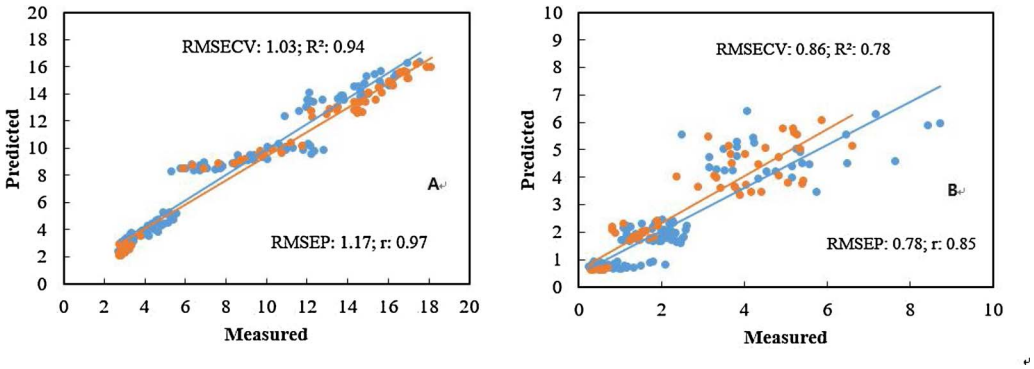

Fig. 3. Reference vs. predicted scores of lycopene (A) and β-carotene (B) in the calibration (blue) and prediction (red) sets with multivariate PLS models. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

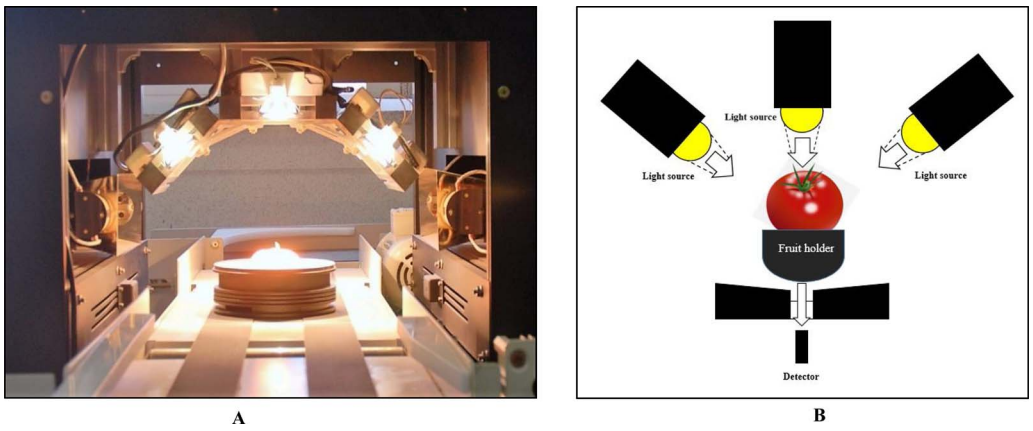

Fig. 4. Transmittance measuring machine (A) and transmittance measurement system (B) during VIS/NIR spectra measurement of tomato fruit.

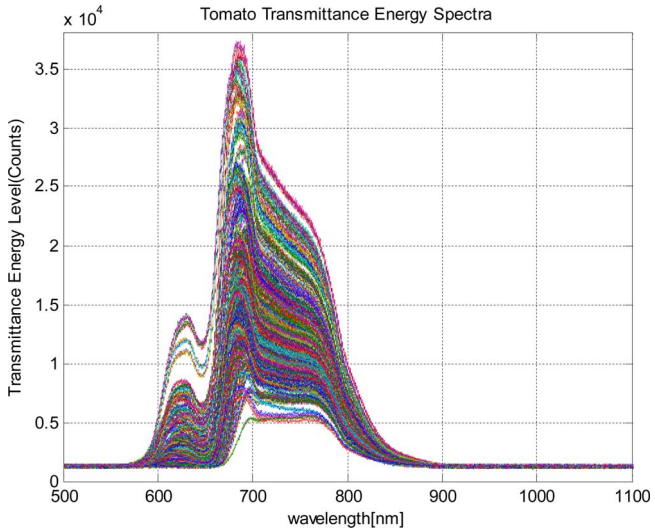

Fig. 5. Tomato transmittance energy spectra curves obtained using VIS/NIRS.

calibration and prediction sets with multivariate PLS models had shown a promising result to use the model. For the prediction data set, multivariate PLS model had the highest coefficient of correlation (0.97) for lycopene and (0.85) for β-carotene (Fig. 3).

3.2. VIS/NIR spectra vs. reference analysis

Near-infrared spectroscopy (NIRS) has been used for food quality evaluation and gained broad acceptance (Rodriguez-Saona and Allendorf, 2011). Clément et al. (2008) suggested the use of visible and short-wave NIR region (400–1000 nm) for the measurement of lycopene content and color variables (Fig. 4). In the present study, intact tomato transmittance energy spectra were recorded in the wavelength range of 500–1100 nm as shown in Fig. 5. PLS models were also developed on the basis of VIS/NIR spectra of intact tomato to predict lycopene and β-carotene content, and promising results were obtained for both dependent variables. R^2 and RMSEC for reference vs. VIS/NIR values of lycopene in calibration set were 0.89 and 1.56, respectively (Fig. 6A). Meanwhile, RMSEP for reference vs. VIS/NIR values of lycopene in prediction set were 0.85 and 1.79, respectively (Fig. 6B). On the other hand, R^2 and RMSEC for reference vs. VIS/NIR values of β-carotene in calibration set were 0.88 and 0.63, respectively (Fig. 7A), while RMSEP for reference vs. VIS/NIR values of β-carotene in prediction set were 0.77 and 1.00, respectively (Fig. 7B). Numerous efforts have been made to estimate the physico-chemical properties of tomato fruits according to their infrared spectra. Radzevičius et al. (2016) reported that NIR spectra can be used to estimate dry matter, soluble solids content, fruit skin and flesh firmness in tomato fruit with a regression coefficient of 0.91, 0.81, 0.91 and 0.96, respectively. Clément et al. (2008) conducted an experiment on tomato cultivars obtained from retailers and greenhouse to simultaneously measure quality parameters in non-destructive manner using VIS/NIR reflectance spectroscopy in 400–1500 nm range and chemometrics; they reported an accurate lycopene content prediction (r^2 = 0.98) along with color variables such as Hunter's a^* (r^2 = 0.98), L^*, and b^* (r^2 = 0.92). In the present study, lycopene was predicted more precisely than β-carotene unlike Baranska et al. (2006) who reported precise prediction of β-carotene (r^2 = 91.19) than lycopene (r^2 = 0.85), using NIR spectroscopy.

In conclusion, the current study indicated the possibility of using color values and VIS/NIR spectra to evaluate carotenoids in intact tomato fruit. Prediction of carotenoids from intact tomato fruit is faster and cheaper than from destructive analysis. It could also allow the use of portable Chroma meter in the field since the equipment can be easily carried and suitable to use. The results also revealed the possibility of using VIS/NIR spectroscopy for non-destructive carotenoids estimation

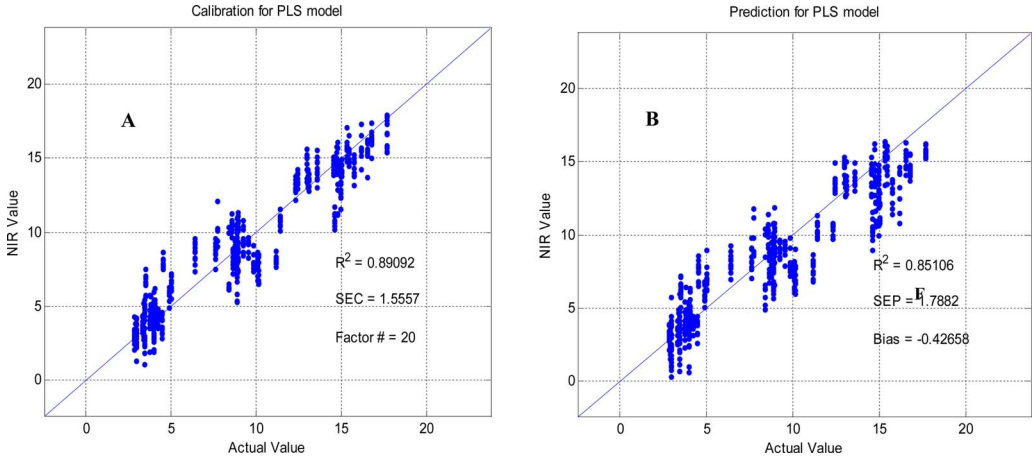

Fig. 6. Reference vs. VIS/NIR values of lycopene in the calibration (A) and prediction (B) sets with PLS models.

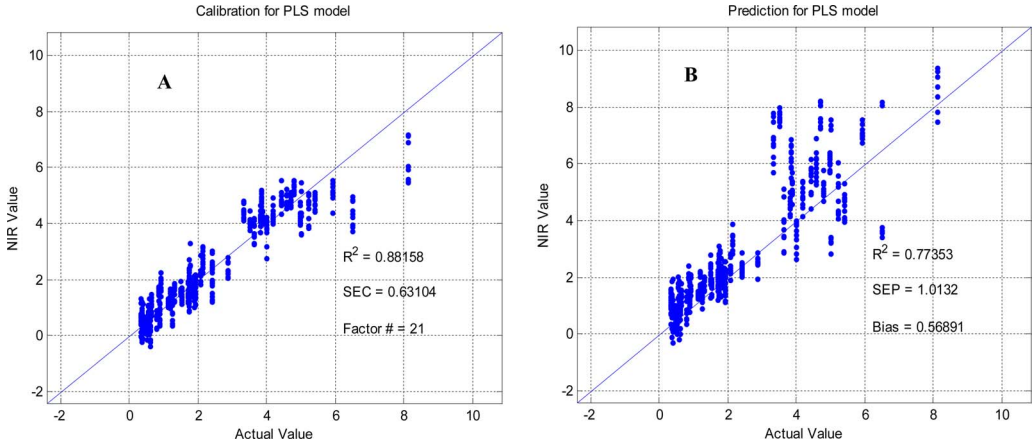

Fig. 7. Reference vs. VIS/NIR values of β-carotene in the calibration (A) and prediction (B) sets with PLS models.

in the agricultural processing centers to sort tomato fruit on a conveyor belt. Further investigation on additional varieties using color values and VIS/NIR spectra to evaluate carotenoids in intact tomato fruit could help to develop more robust models.

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