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Araştırma Makalesi/*Research Article (Original Paper)*

Non-destructive determination of vitamin C and lycopene contents of intact cv. Newton tomatoes using NIR spectroscopy

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Abstract: Lycopene and vitamin C are two vital compositions of tomatoes. Analytical quantification of these components using common destructive methods is expensive and time consuming. In this study, the feasibility of using near-infrared (NIR) spectroscopy at the range of 930-1650nm was assessed to measure the lycopene and vitamin C contents of intact cv. Newton tomatoes. To develop prediction models based on spectral data and analytical measurements achieved using common destructive methods, partial least squares (PLS) regression was utilized. Before modeling, some pre-processing techniques were also used to reduce the irrelevant data from the spectra. The prediction accuracy of the models was evaluated using root mean square error of cross validation (RMSECV), correlation coefficient (r_{cv}) and residual predictive deviation (RPD). The best prediction models had RMSECV of 2.256 µg g^{-1} and 1.087 mg $100g^{-1}$, r_{cv} of 0.840 and 0.818, and RPD of 1.835 and 1.701 for lycopene and vitamin C contents, respectively. Results indicated that NIR spectroscopy can predict the lycopene and vitamin C contents of tomato non-destructively with good and fair accuracy, respectively.

Keywords: Lycopene, NIR spectroscopy, Tomato, Vitamin C

NIR Spektroskopisi Kullanılarak Bütün Haldeki Newton Domates Çeşidinde C Vitamini ve Likopen İçeriğinin Tahribatsız Tespiti

Öz: Likopen ve C vitamini, domateste iki hayati bileşendir. Bu bileşenlerin yaygın parçalama yöntemleri ile analitik olarak ölçülmesi pahalı ve zaman alıcıdır. Bu çalışmada, 930-1650nm aralığında yakın kızılötesi (NIR) spektroskopi kullanımı, bütün haldeki Newton çeşidi domateslerinin likopen ve C vitamini içeriğini ölçmek için değerlendirilmiştir. Spektral verilere dayanan ve yaygın parçalama yöntemleri ile elde edilen analitik ölçümlere dayanan tahmin modelleri geliştirmek için, kısmi en küçük kareler (PLS) regresyonu kullanılmıştır. Modellemeden önce, spektrumdaki alakasız verileri azaltmak için bazı ön işleme teknikleri de kullanılmıştır. Modellerin tahmin doğruluğu, çapraz doğrulama karekök kare hatası (RMSECV), korelasyon katsayısı (r_{cv}) ve rezidüel tahmin sapması (RPD) kullanılarak değerlendirilmiştir. Likopen ve C vitamini içeriği için en iyi tahmin modelleri, sırasıyla RMSECV değeri 2.256 µg/g ve 1.087 mg/100g; 0.8v ve 0.818 r_{cv} ve 1.835 ve 1.701 RPD olarak bulunmuştur. Sonuçlar, NIR spektroskopisinin, bütün haldeki domatesin sırasıyla likopen ve C vitamini içeriklerini, iyi ve adil bir doğrulukla tahmin edebileceğini göstermektedir.

Anahtar kelimeler: Likopen, NIR spektroskopisi, Domates, C Vitamini

Introduction

Tomato (*Solanum lycopersicum* L.) is a popular plant in the world (Abushita et al. 1997; Song et al. 2015). It is eaten directly as raw vegetable or consumed as processed products like ketch-up, sauce, juice, diced, soup, paste and puree (Bodunde et al. 1993). Tomato fruit is a good source in terms of vitamins and minerals (Sigmund and Gustav 1991; Van Eck et al. 2006; Yekbun and Turgay 2017).

One of the most vital compositions of tomato is lycopene, a kind of carotenoid. It is found in many vegetables and fruits with red color such as tomatoes and watermelon (Pohar et al. 2003; Özlem 2016). Human cannot synthesize lycopene and obtain it exclusively from diets (Tapiero 2004). Lycopene reduces oxidative damage to cells by scavenging of the free radicals and may have protective effects against carcinogens in the liver, brain, colon, breast, cervix and prostate, therefore consumption of lycopene is associated with reduced incidence of certain types of cancers. It can improve the morphology and mobility of sperm cells and may improve male fertility. In addition, lycopene has preventive effects against coronary heart disease (Rao and Agarwal 2000; Bramley, 2000; Manson et al., 1993; Giovannucci et al. 1995; Narisaw et al. 1996; Martinez-Ferrer et al. 2006). Heat processing of tomato makes the availability of lycopene more than that in the raw product (Gartner et al. 1997; Agarwal and Rao 2000).

Vitamin C (ascorbic acid) is an antioxidant which neutralizes free radicals (Gorton and Javis 1999). Therefore, recommendations have been made to increase intake of plants rich in vitamin C to lower risk of cardiovascular diseases and cancers (Steinmetz and Plotter 1991). Vitamin C improves the human immune system and prevents high blood cholesterol levels. Among fruits and vegetables, the highest level of vitamin C is found in tomato, broccoli, citrus, mango and watermelon (Wintergerst et al. 2006).

Lycopene and vitamin C contents of fruits and vegetables are commonly measured using destructive methods which are both expensive and time consuming. Hence, it is important to develop a technique for rapid, low-cost and reliable measurement of these compounds, non-destructively.

Near-infrared (NIR) spectroscopy as an applicable non-destructive technique, has been utilized to assess the quality of many horticultural products. Researches have been confirmed the good ability of this technique for measuring soluble solids contents (SSC) in various fruits such as apple (Bobelyn et al. 2010; Zude et al. 2006), apricot (Bureau et al. 2009; Camps and Christen 2009), plum (Golic and Walsh 2006), grape (Cao et al. 2010; Guidetti et al. 2010), peach (Golic and Walsh 2006), strawberry (Sanchez et al. 2011), tomato (Flores et al. 2009; He et al. 2005; Pedro and Ferreira2007; Shao et al. 2007), banana (Jaiswal et al. 2012) and orange (Jamshdi et al. 2014). However, other attributes with low concentration in fruits and vegetables such as lycopene and vitamin C which are more difficult to measure have been less studied using NIR spectroscopy (Nicolai et al. 2014). While little publications have been assessed the feasibility of using this technique for measuring lycopene contents in tomatoes (Clement et al. 2008; Pedro and Ferreira 2005; Saad et al. 2014; Szuvandzsiev et al. 2014), no reports found about determination of vitamin C contents in tomatoes using this technique.

This research aims: 1- to assess the ability of using NIR spectroscopy for predicting both lycopene and vitamin C contents in intact Newton tomatoes, non-destructively, 2- to develop the reliable calibration models for prediction of both lycopene and vitamin C contents of the Newton tomatoes. 3- to introduce the best pre-processing methods for developing the calibration models.

Materials and methods

Data acquisition

Measurements were carried out on total 120 Newton tomatoes prepared from local markets. Before NIR spectroscopy, length and diameter of the samples were measured and they were stored at room temperature (about 25 °C) for about 12 hours to minimize the temperature effect on the spectral data. In this research, an EPP 2000 NIR spectrometer (Stellarnet, Inc. USA) was utilized to collect the spectra of tomatoes in interactance mode. The detector of the spectrometer was indium gallium arsenide (InGaAs). The range of spectrometer operation was 900-1700 nm. A 20W dc tungsten halogen light (StellarNet, Inc. USA) was used as light source. One bifurcated optical fiber placed underneath the tomato to guide light radiation towards the sample and to bring back the internal reflectance to the spectrometer. For each tomato, spectra at three points around equatorial locations and five scans in each point were saved using SpectraWiz software (StellarNet, Inc. USA). The mean spectrum of each tomato was obtained from 15 scans. All reflectance spectra of samples were transformed to absorbance spectra to provide linear correlations between the spectral data and lycopene and vitamin C contents measured using reference methods (Nicolai et al. 2007).

Data pre-processing

In all the absorbance spectra, the first 30 wavelength data and the last 50 wavelength data were ignored to eliminate noises at two edges of each spectrum. Then, the spectral range of 930 to 1650 nm was considered for model developing. The data obtained from the spectrometer may contain unwanted information such as scattering effects, instrumental noises and sample size effects which would affect the accuracy of calibration models. Many pre-processing methods are used to reduce such unwanted information and develop more reliable and accurate models (Cen and He 2007; Nicolai et al. 2007).

In this research, two normalization methods of multiplicative scatter correction (MSC) and standard normal variate transformation (SNV) were performed individually and in combination with first derivative (D_1) for reducing the irreverent information and improving the models' accuracy. MSC compensates the physical effects of additive (offset shift) and/or multiplicative (tilt) in the NIR spectra such as the non-uniform scattering throughout the NIR spectrum as the degree of scattering is dependent on the wavelength, the sample size, distance variation of the sample and detector and the refractive index (Nicolai et al. 2007; Lu 2001). SNV also compensates the effects of additive and multiplicative light, sample size, variation of distance between the sample and detector (Lu 2001). First derivation is often used for removing the constant background and enhancing the resolution of the spectra. (Condolfi et al. 1999; Lammertyn et al. 1998).

Reference measurements

To measure lycopene content of the tomatoes, method of Markovic et al. (2006) was used. Sample of tomato (approximately 5g) was homogenized using a blender and placed in 200ml flask wrapped with aluminum foil to protect it from light. A 100ml mixture of hexane–acetone–ethanol (2/1/1, v/v/v) was added to the flask and agitated continuously for 10 minutes on a magnetic stirrer plate. Thereafter, 15ml of water was added to flask, followed by agitation for another 5 minutes. The solution was separated into distinct layers of polar and non-polar. The solution of hexane containing lycopene was filtered using filter paper through 0.2mm filter paper and the filtrate was then diluted with a mixture of hexane–acetone–ethanol (2/1/1, v/v/v). Colorless residue on the filter paper indicated that extraction of lycopene was rapid and complete. Absorbance of the hexane solution containing lycopene at 472 nm on a spectrophotometer was measured and lycopene content was estimated.

To determine vitamin C content of the samples, titration method was used (AOAC 1990). To this end, vitamin C was extracted from tomato samples with metaphosphoric acid. The filtered extract was then titrated with 2,6-dichlorophenolindophenol (DCIP) which was reduced by the vitamin C, changing it from a blue color to colorless.

Model development and evaluation

Before developing the models, 9 samples were removed because of the mistake in reference measurements. One outlier which was the sample containing interferences having a negative influence on modeling was detected using principal component analysis (PCA) and removed (Jamshidi et al. 2015; Viscarra Rossel 2008). After removing the 10 outlier samples, partial least squares (PLS) regression was performed to build the calibration models based on reference measurements for lycopene and vitamin C contents and the spectral information of the samples. To validate the models, full cross validation was performed. The maximum number of latent variables (LVs) investigated for calibration models was 15 to avoid over-fitting and noise modeling.

Calibration models were evaluated using the root mean square of cross validation (RMSECV), correlation coefficient of cross validation (r_{cv}) and the residual prediction deviation (RPD) which are defined as follows (Nicolai et al., 2007):

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}{n}} \quad (1)$$

$$r_{cv} = \sqrt{\frac{\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}{\sum_{i=1}^{n}(y_m-\hat{y}_i)^2}} \quad (2)$$

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{RMSECV}} \quad (3)$$

Where, n is the number of samples; y_i is the measured value of the i_{th} sample using the reference method, $\hat{y}_i$ is predicted value of the ith sample when model is developed without i_{th} sample; y_m is the average of observed value of the desired attribute (lycopene or vitamin C) and SD is standard deviation of desired attribute.

A good model should have a low RMSECV and high r_{cv} and RPD (Viscarra Rossel et al 2008). RPD values were assessed based on the classification reported by Mouazen et al. (2010) as follows: an RPD < 1.0 shows very poor prediction by model and use of the model is not recommended; 1 < RPD < 1.4 shows poor prediction and model can distinguish only high values of attribute from low values; 1.4 < RPD < 1.8 shows fair predictions which can be used for correlation and assessment; 1.8 < RPD < 2.0 shows good predictions which can be used for quantitative prediction and an RPD value between 2.0 and 2.5 or above indicates very good and excellent prediction accuracies for the model, respectively. All pre-processing methods, PLS regression and models' validation were done by means of ParLeS software version 3.1 (Viscarra Rossel 2008).

Results and Discussion

Statistics of the samples

Descriptive statistics such as minimum (Min), maximum (Max), mean and standard deviation (SD) for size properties (length and diameter), lycopene content and vitamin C content of 110 tomato samples are shown in Table 1. The length and the diameter of the tomatoes were in the range of 4.19-5.96 and 5.41-7.78 cm. The ranges of lycopene and vitamin C content in the samples were from 17.35 to 41.59 µg/g and 22.78 to 29.87 mg/100g, respectively. The variation of lycopene content was more than the variation of vitamin C content in the samples.

Table 1. Descriptive statistic for size properties, lycopene and vitamin C content of the samples

Attribute	Min	Max	Mean	SD
Length (cm)	4.19	5.96	5.22	0.36
Diameter (cm)	5.41	7.78	6.72	0.47
Lycopene content (µg/g)	17.35	41.59	25.04	4.14
Vitamin C content (mg/100 g)	22.78	29.87	26.01	1.85

NIR spectra and the effect of pre-processing techniques

Fig. 1 shows the absorbance spectra of 50 typical samples and the effect of pre-processing techniques (MSC, SNV, MSC+D_1 and SNV + D_1) on them. The spectra had some peaks in specific wavelengths due to stretching vibration of O–H or C–H overtones relative to the concentration of some internal characteristics of tomatoes with these functional groups. Normalizing methods of MSC and SNV did not affect on the shape of the spectra and the position of the peaks. However, these pre-processing methods led spectra to be close together and their peaks to be clearer. There was a broad peak around 980 nm due to the second overtone of O-H, and two distinctive peaks around 1200 and 1400 nm related to the second overtones of C-H, CH_2 or CH_3 and the first overtone of O-H, respectively. Considering the spectra processed using MSC and SNV indicates that the spectra had a small peak around 1600 nm related to the first overtones of C-H, CH_2 or CH_3 (Cen and He 2007). First derivative of the absorbance spectra after MSC and SNV normalizing confirmed and highlighted the mentioned peaks. Peak around 980 nm has been confirmed by Flores et al. (2009); Saad et al. (2014) and Szuvandzsiev et al. (2014) and the peak around 1200 nm was similar to the peak in the spectra reported by Pedro and Ferreira (2005) and Flores et al. (2009). The spectra reported by He et al. (2005) and Flores et al. (2009) conformed the peak around 1400 nm.

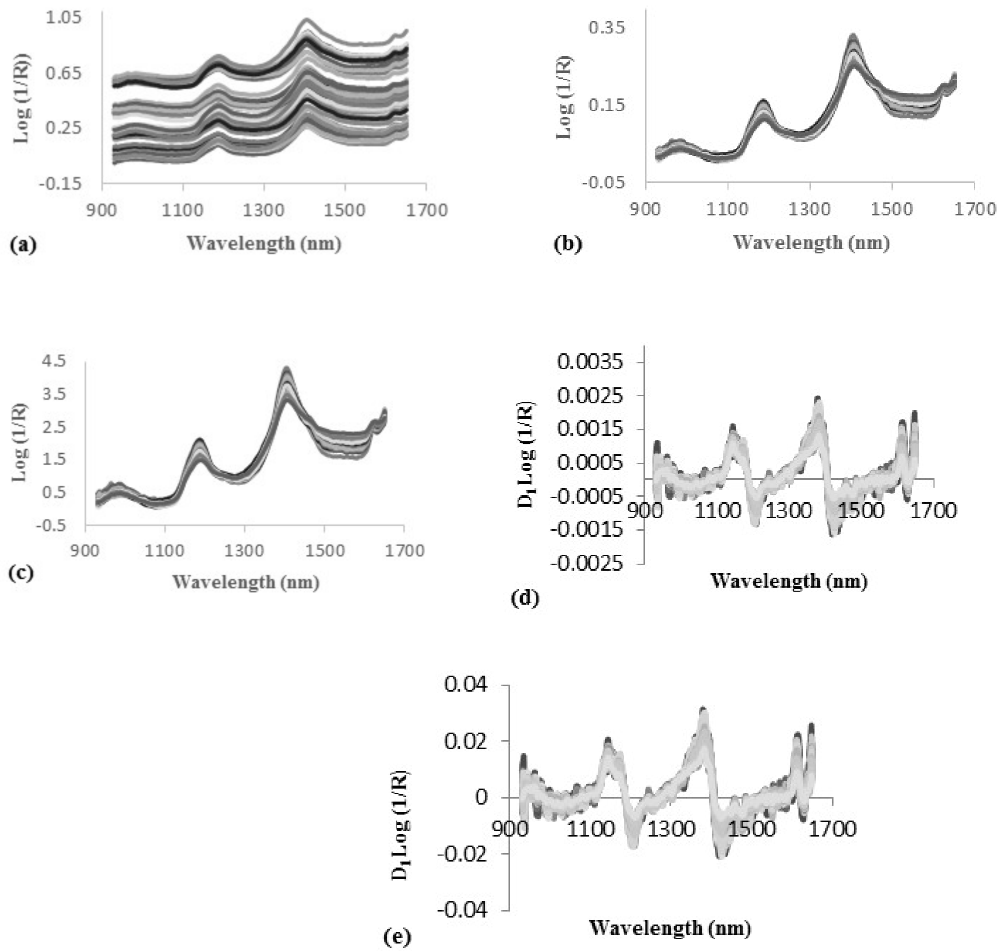

Figure 1. 50 typical NIR absorbance spectra of Newton tomatoes in 930-1650 nm without pre-processing (a), and pre-processed using MSC (b), SNV (c), MSC + D_1 (d) and SNV + D_1 (e)

Prediction of lycopene and vitamin C contents

Table 2 indicates the results of the developed models based on different pre-processing techniques for lycopene and vitamin C content prediction.

Table 2. Results of the developed models based on different pre-processing techniques for lycopene and vitamin C content prediction

Inner property	Pre-processing method	Optimum LV	RMSECV	r_{cv}	RPD
Lycopene content (µg/g)	-	10	2.623	0.778	1.578
	MSC	9	2.280	0.835	1.816
	SNV	9	2.426	0.812	1.707
	MSC+D_1	5	2.256	0.840	1.835
	SNV+D_1	5	2.329	0.828	1.776
Vitamin C content (mg/100 g)	-	12	1.305	0.734	1.418
	MSC	10	1.173	0.777	1.577
	SNV	10	1.194	0.768	1.549
	MSC+D_1	12	1.087	0.818	1.701
	SNV+D_1	10	1.098	0.810	1.685

*Bold values show the best model prediction for lycopene content and vitamin c content.

According to the table, all developed models had ability to predict both lycopene and vitamin C content of the tomatoes (RPD >1.4). The pre-processing methods of MSC and SNV individually and in combination with D_1 reduced RMSECV and increased r_{cv} and RPD of the models for both lycopene and vitamin C content in comparison with the model developed without any pre-processing. These normalization methods especially MSC compensated the physical effects in the spectra related to size variation of the tomatoes and increased the accuracy of the models. Using D_1 after normalizing the spectra obtained better results for prediction of both lycopene and vitamin C content of the samples in comparison with using MSC or SNV individually.

For lycopene prediction, it was noted that using D_1 after MSC or SNV reduces the number of LVs (from 9 to 5) and leads to simplification of the model. The prediction results based on MSC and MSC+D_1 were close together but the model developed using MSC + D_1 could predict the lycopene content with fewer LVs. In addition, using the absorbance spectra with no pre-processing and using the pre-processing technique of SNV and SNV + D_1 led to fair predictions for the calibration models of lycopene content (1.4 <RPD< 1.8). Therefore, the best model was obtained using MSC + D_1 with LV = 5 which could predict the lycopene content of the samples with good accuracy (RMSECV = 2.256, r_{cv} = 0.840 and RPD = 1.835).

Fig. 2a shows the variation of RMSECV against LVs using the best model and how to choose optimal LV. At first, RMSECV decreases with increasing of LVs to reach optimum LVs (LVs = 5) and then increases. If the LVs is less than or more than 5, the model will be under-fitted or over-fitted, respectively. The correlation between the predicted and measured values of lycopene content in the samples for the best model is presented in Fig. 3a.

The accuracy of the best model for lycopene prediction was close to the accuracy reported by Szuvandzsiev et al. (2014) in 530-980 nm (r^2 =0.75 and RMSECV = 1.99). The accuracy of the best model reported by Saad et al. (2014) in 350-1050 nm (r^2 = 0.99 and RMSECV = 0.91), Pedro and Ferreira (2005) in 4500 – 9500 cm^{-1} (r_{val} =0.999 and RMSEP = 21.578), Baranska et al. (2006) in 100 – 4000cm^{-1} (r^2 = 0.85) and Clement et al. (2008) in 400 -1500 nm (r^2 =0.98 and RMSECV =3.15) were better than the accuracy of the best model in this research. However, comparison of the results of different NIR spectroscopy for lycopene content measurement is not recommended because of different wavelength range, different instrument and different concentration of lycopene in different varieties.

As it can be seen in Table 2, all the prediction models had fair predictions for the vitamin C content of the samples (1.4 < RPD< 1.8). The best model for predicting the vitamin C content was also obtained when MSC + D_1 was used to pre-process the spectra (RMSECV = 1.087, r_{cv} = 0.818 and RPD = 1.701). However, the number of LVs was not less (LV = 12).

Considering variation RMSECV against LVs for the best prediction model of vitamin C contents (Fig. 2b) shows increasing LVs to reach LV = 12 (LVs with minimum RMSECV) increases the model accuracy. Number of LVs more than 12 complicates the model and causes the model over-fitting. Measured values of vitamin C against predicted values using the best developed model are shown in Fig. 3b.

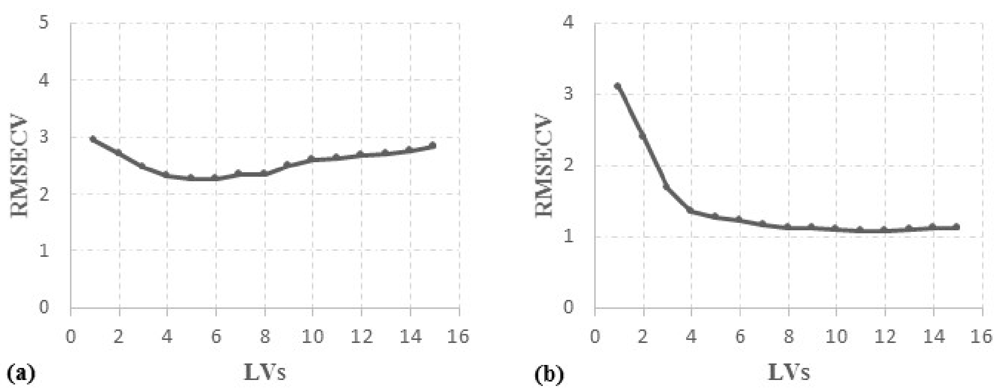

Figure 2. Variation of RMSECV against LVs using the best model for predicting lycopene content (a) and vitamin C content (b).

While measurement of vitamin C content using NIR spectroscopy method have been investigated in apple by Pissard et al. (2012) (r_p = 0.71 to r_p = 0.81 and RPD =1.11 to RPD=1.57), kiwifruit by Fu et al. (2005) (r_p = 0.93), and chilly by Wang et al. (2011) (r_p = 0.803), no reports found on tomato.

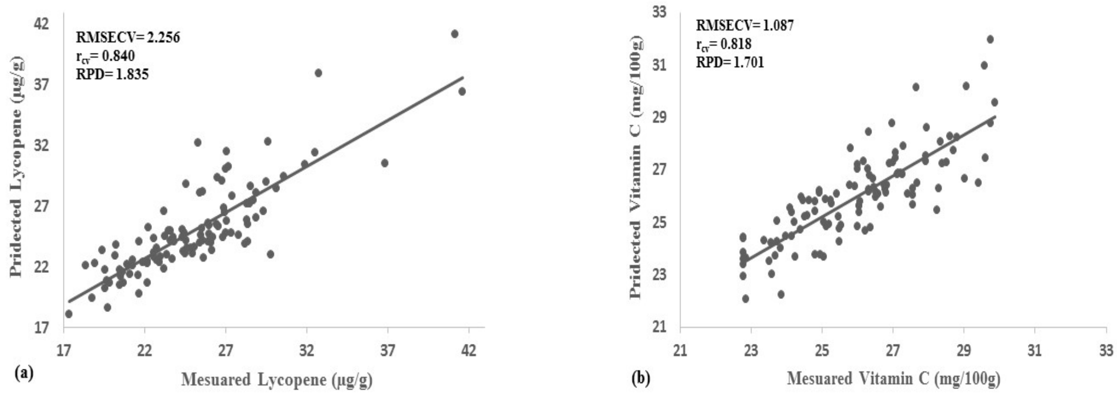

Figure 3. NIR spectroscopy predicted results versus reference measured contents of the lycopene (a) and vitamin C (b) for the best PLS model.

Conclusion

In this research, the potential of NIR spectroscopy in the region of 930 – 1650 nm was assessed for rapid prediction of the lycopene and vitamin C contents of Newton tomatoes, non-destructively. In conclusion, following results were achieved:

- NIR spectroscopy combined with PLS models developed based on the pre-processing method of MSC + D_1 can predict the lycopene and vitamin C contents of the tomatoes.
- The best developed model had good accuracy (RMSECV =2.256 µg/g, r_{cv} = 0.840 and RPD = 1.835) for prediction of lycopene contents of the samples.
- The best developed model had fair accuracy (RMSECV = 1.087 mg/g, r_{cv} = 0.818 and RPD = 1.701) for prediction of vitamin C contents of the samples.
- It was noted that NIR spectroscopy can be utilized for online tomato sorting in terms of lycopene and vitamin C content when the high accuracy is not desired.

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