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DETERMINATION OF CAROTENOIDS IN TOMATO PRODUCTS USING VIS/NIR SPECTROSCOPY

Abdel Gawad Saad[*1], *Zoltán Pék*[2], *Péter Szuvandzsiev*[2], *Daood Hussein Gehad*[3], , *Lajos Helyes*[2]

Address(es):
[1] Biological Engineering Dept., Agricultural Engineering Research Institute (AEnRI), Agricultural Research Center (ARC), Egypt.
[2] Institute of Horticulture, Szent István University, Gödöllő, Hungary.
[3] Regional University Knowledge Centre, Szent István University, Gödöllő, Hungary.

*Corresponding author: en_gawad2000@yahoo.com

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ABSTRACT

Carotenoids analysis is complicated by their tendency to react with radical species, resulting in oxidative breakdown and isomerization during extraction. Hence, analysis methods should be rapid and avoid unnecessary exposure to high temperature, acids, and so on. The aim of this work to estimate carotenoid contents of processed tomato products non-destructively. The mean values obtained by visible and near-infrared Vis/NIR spectroscopy and by high performance liquid chromatography (HPLC) for eight carotenoid contents (ß-carotene, 5-*cis* lycopene, 13-*cis* lycopene, 9-*cis* lycopene, all-*trans* lycopene, zeaxanthin, lycoxanthin and total carotenoids) in four processed tomato products from five different brands were compared. The carotenoid contents were measured using HPLC, and these results were then used to develop partial least squares regression (PLSR) models to predict carotenoid components from Vis/NIR spectra of the same samples. A good correlation was found between HPLC measurements and the Vis/NIRs (590-790 nm) predictions for ß-carotene (R_P^2= 0.88), 9-*cis* lycopene (R_P^2= 0.86), total carotenoids (R_P^2= 0.84), 13-*cis* lycopene (R_P^2= 0.83), 5-*cis* lycopene (R_P^2=0.80), zeaxanthin (R_P^2= 0.80) to passable for all-*trans* lycopene (R_P^2= 0.70), but there was only a poor correlation (R_P^2= 0.20) for the lycoxanthin component. The overall results indicated that Vis/NIRs could be applied to assess carotenoid contents of different processed tomato products.

Keywords: Vis/NIR Spectroscopy; Non-destructive; Carotenoid assessment; Processed tomato

INTRODUCTION

Tomato (*Lycopersicumesculentum*) is one of the most consumed vegetable in the world, either as a fresh or as a processed product. With regards to the economic importance and consumption in the whole world after potatoes, tomatoes are in second place, since they are used in the food industry as raw material for the production of several products such as juices, sauces, purees, pastes, and canned tomatoes. Recently, the consumption of tomatoes has been associated with the prevention of several diseases, like some cancers and cardiovascular diseases (**Basu and Imrhan, 2007; Tan *et al.*, 2010**), mainly due to their content of antioxidants, including carotenes (lycopene as well as ß-carotene), tocopherol, ascorbic acid, and phenolic compounds (**Jacob *et al.*, 2010**).
Carotenoids are mostly found intracellularly in the chromoplast and chloroplast membranes in plants, they have been structurally classified as carotenoids, including β-carotene, α-carotene, and xanthophylls such as β-cryptoxanthin, zeaxanthin, lutein, violaxanthin, fucoxanthin and neoxanthin (**Gómez-García and Ochoa-Alejo, 2013**). Approximately 750 natural carotenoids had been identified and extracted from different plant sources (**Maoka, 2009**). Lycopene is one of the most important carotenoids in tomato (**Nasir *et al.*, 2015**). In five processing tomato cultivars, **García-Valverde and co-workers (2013)** found 83.2 to 97.6 mg kg^{-1}fw. of total lycopene and 3.0 to 6.1 mg kg^{-1}fw. of ß-carotene. All-*trans* lycopene represents a 79–91% and *cis* lycopene isomers a 9–21% of total lycopene in tomatoes, tomato soup and tomato paste (**Markovic *et al.*, 2006**).
Bioavailable lycopene concentrations in processed tomato products such as paste, puree, ketchup, juice, soup, and sauce is higher than in fresh tomato (**Agarwal *et al.*, 2001**). For this reason, tomato sauce is a preferable source as opposed to raw tomatoes (**Alda *et al.*, 2009**). Due to the increasing popularity of lycopene as one of the important nutraceuticals for use in food and nutritional supplements, tomato manufacturers are interested in developing carotenoids rich products and ingredients during processing of tomatoes, and in the increased bioavailability of lycopene (**Alda *et al.*, 2009**). Thus the assessment of lycopene is a very important procedure of the final products (**Mert, 2012**). Present methods of carotenoids assay require its extraction from samples by hazardous organic solvents, are time-consuming, require extensive sample preparation, tedious, hazardous and destructive (**Rivera and Canela, 2012**).
Partial least square regression (PLSR) has been widely used for non-destructive food quality evaluation of agricultural products by NIR spectroscopy (**Huang *et al.*, 2014; Wang *et al.*, 2015**). PLSR offers an edge over Principal Components Regression (PCR) because the resulting spectral vectors are directly related to the constituents of interest. On the other hand, PCR takes the vectors merely representing the most common spectral variations in the spectral data, totally ignoring their relation to the components of interest until the final regression step (**Scholz *et al.*, 2005; Pu *et al.*, 2015**).
Nowadays, It has become urgently required to search for rapid methods to estimate tomato product components. The aim of this work was to use Vis/NIRs as a rapid and easy method to estimate the carotenoids in some processed tomato products.

MATERIAL AND METHODS

Processed tomato products

A 100 samples of tomato paste (5), puree (5), ketchup (5) and juice (5) were considered for build a model. Five different commercial brands of each product were bought from local grocery stores. Besides that, twenty new samples were used for tested the PLSR models. All of these samples were taken from different commercial batches produced on different days within a two-month period to represent a wide span of variation in the characteristics of processed tomato products. All samples were tested in four replications each.

Spectral acquisition

Reflectance spectra of processed tomato samples were acquired using a portable spectro-radiometer (FieldSpecHandHeld 2™, Analytical Spectral Devices (ASD), Inc., Boulder, USA) with wavelength ranges of 325–1075 nm. The sample was placed in a black sample cup (diameter 75 mm) at 25 ±1mm height, and instrument positioned at 20 mm above the samples when collecting the spectra. All of the spectral data were stored in a computer and processed using

the RS3 software for Windows (Analytical Spectral Devices, Inc., Boulder, USA), designed with a graphical user interface. The reflectance spectra were transformed into ASCII format by using the ASD ViewSpecPro software. Then, the average of four spectra for each sample was taken.

Analysis of carotenoid components

Extraction

Carotenoids extraction has been done from processed tomato products according to the method of **Daood *et al.*, (2013)**. Five gram samples of processed tomato have been taken in triplicate followed by disintegrated in a crucible mortar in the presence of quartz sand. The water was then removed by adding 25 ml of methanol along with the repeat disintegration of the aggregating bulk. After the addition of 70 ml of a 6:1 dichloroethan-methanol solution, the mixture was transferred quantitatively into 100 ml conical flask. Moreover, the mixture was shaken up to 15 min by a mechanical shaker. When there is not a clear separation from the polar phase (water + methanol) of dichloroethane phase, few drops of double distilled water were added. Separation of two phases has been carried out with a separatory funnel and the lower layer consisting lipids in dichloroethane was dried over anhydrous sodium sulphate. The organic solvent was evaporated under vacuum by rotary evaporator (IKA® RV10, Sigma-Aldrich Ltd., Budapest, Hungary) at maximum 40 °C and the residues were re-dissolved again in 5ml of HPLC acetone.

HPLC equipments and conditions

For the analyse of carotenoid compounds, a Chromaster liquid chromatographic instrument (Hitachi, Japan) consisting of a Model 5110 Gradient pump, a Model 5210 auto-sampler and a Model 5430 photodiode array detector was used. Operation and data processing were performed by EZ Chroma Elite software. The separation of carotenoids was done on Accucore C-30, 150 x 4.6 mm, 2.6 µm column using gradient elution starting with 100 % methanol (A), changing to 40 % ter-butyl-methyl-ether in methanol (B) in 35min and returning to 100% A in 5 min with a flow rate of 0.7 ml/min according to **Daood *et al.*, (2013)**. The column affluent was detected at their maximum absorption wavelength for identification and quantification. The retention properties and spectral characteristics of the detected peaks were compared with some available standard materials like lycopene, *β*-carotene and zeaxanthin (Sigma-Aldrich Ltd., Budapest, Hungary). In case of absence of standards the tentative identification was done on basis of comparison of retention times and spectral characteristics with literature data. Additionally, the compounds were quantified as either lycopene- or *β*-carotene-equivalent based on their spectral characteristics (figure 1).

Data analysis

Statistical analysis

Statistical analyses of processed tomato physico-chemical properties were carried out using statistical software (IBM SPSS Statistics 22.0). Data were analysed by (ANOVA) at LSD p= 0.05. The coefficient of variation (CV), defined as the ratio of the standard deviation of the mean was calculated, multiplied by 100 (**Magwaza *et al.*, 2014**).

Chemometrics, calibration, validation and prediction procedures

The spectrometry data were analysed using Unscrambler software (The Unscrambler X version 10.2, CAMO Software AS, Oslo, Norway). PLSR was carried out to develop linear prediction models between reflectance spectral data and different carotenoid composition of different processed tomato products at the wavelength region of 590 to 790 nm. By applying the random selection procedure, the samples were split into two groups, a group of calibration (100 samples) and a group of prediction set (20 samples). Cross validation procedures were used for calibration and validation. The validation set was used to test the predictability of the PLSR models. Prediction ability of PLSR models was evaluated based on the coefficient of determination value (R_P^2), root mean square error of prediction (RMSEP), standard error of calibration (SEC), standard error of prediction (SEP) and bias from the validation data set. Generally, RMSEP is used to evaluate calibrations on an independent set and RMSEC plays only a minor instructive role.

RESULTS AND DISCUSSION

Four different processed tomato products (paste, puree, ketchup and juice) were used in this study. All samples were chosen based on their type of processing to cover a wide range of caroenoid contents as possible. Geometrical isomerisation of all-trans lycopene is significant (**Khoo et al., 2011**), especially the effect of heat transfer resulted in higher proportion of *cis* isomers (**Meléndez-Martínez *et al.*, 2014; Honda *et al.*, 2015**). An overview of the mean values, maximum, minimum, standard deviation (SD), and coefficient of variation (CV) for ß-carotene, 5-*cis* lycopene, 13-*cis* lycopene, 9-*cis* lycopene, all-*trans* lycopene, zeaxanthin, lycoxanthin and total carotenoids (calculated as the sum of the concentrations of the individual carotenoid type) in the samples used in the calibration validation sets are presented in Table 1. In the samples set, there was a vast variation in carotenoid composition, and the samples covered most of the variability of different processed tomato products.

Table 1 A summary of different carotenoid components of samples used in calibration and prediction sets.

Parameters	Calibration set (100 samples)					Prediction set (20 samples)				
	Mean	Min.	Max.	SD	CV	Mean	Min.	Max.	SD	CV
Total carotenoids	970.30	254.88	1763.00	452.42	46.63	964.04	267.39	1730.00	436.11	45.24
ß-carotene	34.24	14.58	74.06	17.91	52.32	33.68	14.44	70.50	17.43	51.77
All-*trans* lycopene	784.71	246.47	1399.00	333.63	42.52	789.36	265.40	1376.00	321.69	40.75
5-*cis* lycopene	7.68	1.03	17.92	5.52	71.89	7.72	1.35	17.94	5.62	72.80
9-*cis* lycopene	13.59	1.52	33.74	8.40	61.81	13.96	1.42	35.90	8.86	63.52
13-*cis* lycopene	51.01	9.06	113.22	33.71	66.10	50.95	10.58	113.74	33.48	65.70
Lycoxanthin	16.88	3.75	32.79	8.69	51.44	16.55	4.20	29.51	8.46	51.10
Zeaxanthin	8.25	1.67	17.55	5.22	63.33	8.15	2.35	16.57	5.00	61.30

In figure 2 some typical Vis/NIR spectra obtained for different processed tomato products are shown. These include the spectra from the different processed tomato products with the highest to lowest measured carotenoid concentrations (paste, puree, juice and ketchup), respectively. The shape of the original spectra was quite homogeneous, and no outliers were distinguished a priori by visual inspection. Consistent baselines offset and bias were present. These are quite common features in the NIR spectra acquired by diffuse reflectance techniques. Obviously, a significant variation in the spectra at wavelength range (500–1000 nm), may be due to the difference in colour and cooked process reactions between processed tomato products (**Eichner *et al.*, 1996**).

In Table 2, the statistics of the calibrations, full cross validations and prediction sets for the different carotenoid components, including RMSEP and R_P^2 values for the equations of best fit obtained for each of carotenoid components at wavelength range (590–790 nm) were presented. Actually, this range has been used to measure pigments, ß-carotene and lycopene in tomato, as in a previous study of tomato puree (**Szuvandzsiev *et al.*, 2014**). However, most of the analysed samples contain, besides zeaxanthin, and other carotenoid components that can be evaluated at this wavelength range. The R_P^2 values of the cross-validation and RMSEP are shown also in the same table.

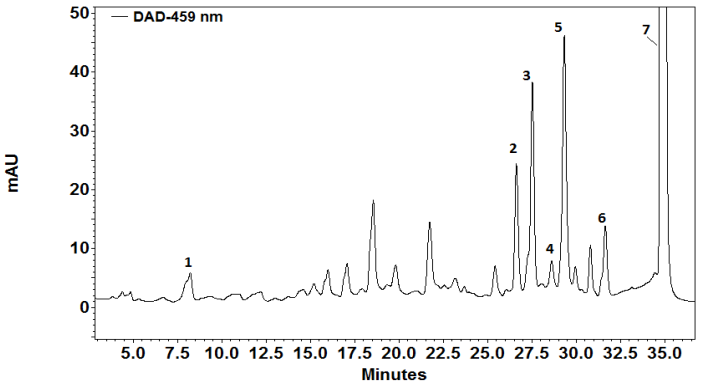


Figure 1 HPLC profile of tomato carotenoids separated on C30 column, core, 150×4.6 mm column with gradient elution of TBME in Methanol. Peak identification as: **1.** Zeaxanthin, **2.** Lycoxanthin, **3.** *β*-carotene, **4.** 5-*cis* lycopene, **5.***13-cis* lycopene, **6.** 9-*cis* lycopene and **7.** All-*trans* lycopene.

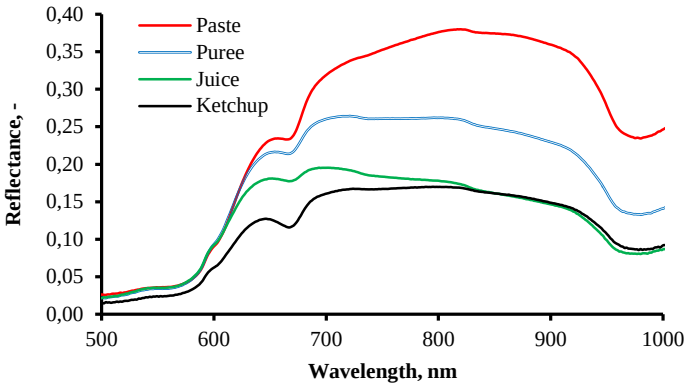


Figure 2 Mean reflectance spectra of paste, puree, juice and ketchup samples.

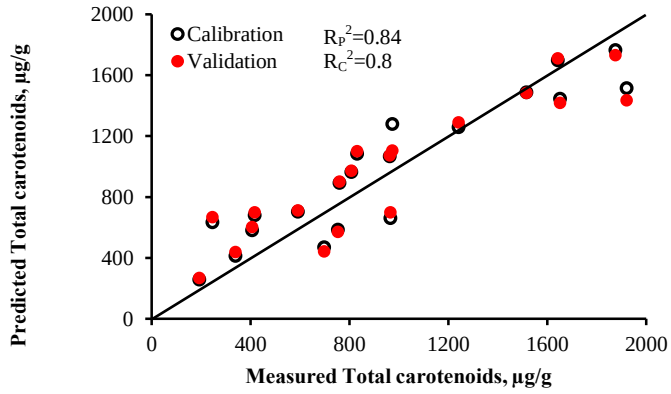
Table 2 Results of PLSR models built by using Vis/NIR (590–790 nm) for the different carotenoids in processed tomato products.

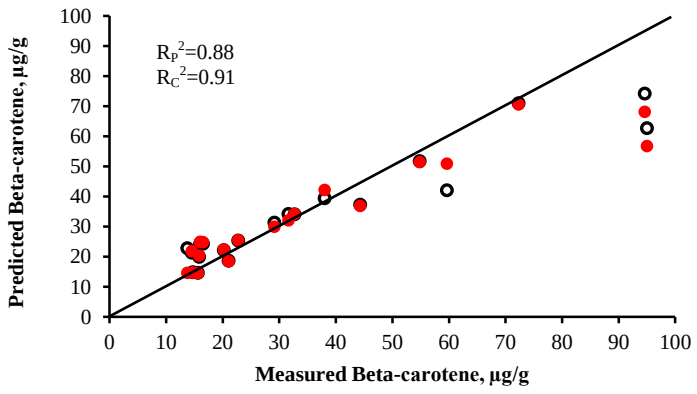
Parameters	Calibration set					Full-cross validation					Prediction set				
	RMSEC	SEC	R_C^2	Bias	Slope	RMSEV	SEV	R_V^2	Bias	Slope	RMSEP	SEP	R_P^2	Bias	Slope
Total carotenoids	284.94	292.3	0.85	1.29e-5	0.78	296.1	301.2	0.84	0.008	0.75	315.83	323.97	0.84	-6.28	0.74
ß-carotene	15.47	15.88	0.91	3.34e-7	0.66	17.05	17.74	0.89	-0.02	0.64	17.69	18.15	0.88	-0.36	0.63
All-*trans* Lycopene	255.31	261.9	0.72	1.2e-5	0.66	266.91	273.5	0.71	0.0004	0.64	282.17	289.5	0.70	-5.34	0.62
5-*cis* lycopene	2.66	2.73	0.82	-8.9e-8	0.79	2.93	3.02	0.81	-0.001	0.80	3.098	3.18	0.80	-0.06	0.80
9-*cis* lycopene	5.12	5.26	0.88	-5.9e-8	0.83	5.74	5.92	0.87	-0.003	0.85	5.97	6.13	0.86	-0.03	0.86
13-*cis* lycopene	13.10	13.44	0.88	7.15e-7	0.85	14.86	15.02	0.85	0.0007	0.84	15.06	15.45	0.83	-0.26	0.83
Lycoxanthin	11.53	11.83	0.35	3.4e-7	0.35	12.08	18.71	0.22	0.002	0.29	12.93	13.26	0.20	-0.33	0.26
Zeaxanthin	2.87	2.95	0.83	2.74e-7	0.79	3.09	3.18	0.81	0.001	0.77	3.28	3.36	0.80	-0.11	0.75

The PLSR model's prediction performance applied to the test sets for each carotenoid compound generated R_P^2 values ranging from 0.20 for lycoxanthin to 0.88 for ß-carotene contents. As shown in Table 2, a good correlation was found between HPLC measurements and the Vis/NIRs (590–790 nm) predictions for ß-carotene (R_P^2= 0.88), 9-*cis* lycopene (R_P^2= 0.86), total carotenoids (R_P^2= 0.84), 13-*cis* lycopene (R_P^2= 0.83), and fairly acceptable 5-*cis* lycopene (R_P^2= 0.80), zeaxanthin (R_P^2= 0.80) to passable for all-*trans* lycopene (R_P^2= 0.70), but there was only a poor correlation (R_P^2= 0.20) for the lycoxanthin component.There is no report, which tried to create a predictive model for estimation of lycopene isomers from Vis/NIRs. Although, lycopene content of tomato is well defined by non-destructively measured colour values of fruits in CIELab colour system (**Brandt *et al.*, 2006; Saad *et al.*, 2016a**), or in Vis/NIRs (**Szuvandzsiev *et al.*, 2014; Saad *et al.*, 2014; Saad *et al.*, 2016b**).

The RMSEP values ranging from 3.18 µg/g for 5-*cis* lycopene to 323.97 µg/g for total carotenoids. Although low RMSEP values are desirable, the low RMSEP values for zeaxanthin,5-*cis* lycopene and 9-*cis* lycopene have to be interpreted in light of the low actual concentrations and low % proportions of these components in samples (Table 2). Because of these low concentrations, they have quite low coefficients of determination, due to the proportionally greater technical errors associated with the HPLC and Vis/NIRS measurements of these compounds (**Mireei *et al.*, 2014**).

Initially, PLSR models were developed in the whole range of spectra (325–1075 nm) for predicting various carotenoid contents of processed tomato products non-destructively. PLSR is a usable multivariate regression method when there are few samples and several variables, and the data are multicollinear, especially in tomato puree (**Szuvandzsiev *et al.*, 2014**). Thereafter the numbers of wavelengths were sequentially minimized to select best performing group of wavelength so as to reduce the cost of the instrument. The linear regression between reflectance data and different carotenoid contents are presented in figure 3, at the range of spectra (590–790 nm). A high correlation was found for ß-carotene R_P^2= 0.88, RMSEP= 17.69 µg/g and bias= -0.36.





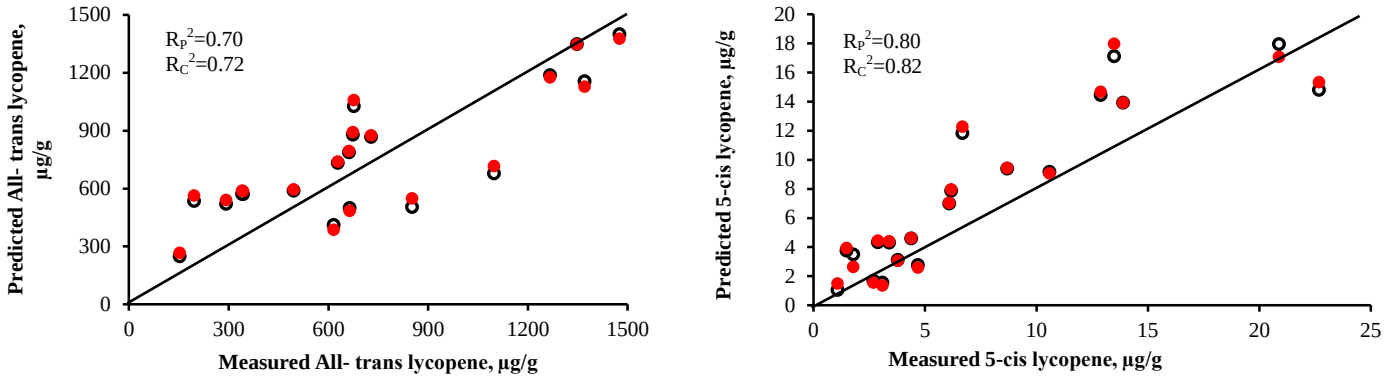


Figure 3 The scatter plots of measured and predicted total carotenoids, *β*-carotene, *all-trans* lycopene and *5-cis* lycopene for the optimal PLSR models.

The PLSR model was used for measuring the model's ability in 5-*cis* lycopene, 13-*cis* lycopene, 9-*cis* lycopene and all-*trans* lycopene prediction for half of the samples (prediction set). The developed models were found to be more suitable for prediction. Scatter plots of the models developed based on data in the wavelength of 590-790 nm has been shown in figures. 3, 4. It showed to be highly adequate in the correlation between the measured values of processed tomato along with the prediction. The PLSR model curve was indicated R_P^2=0.86 RMSEP= 5.97 µg/g and Bias= -0.03 for prediction set samples were used in 9-*cis* lycopene prediction. For 13-*cis* lycopene prediction the R_P^2= 0.83, RMSEP= 15.06 µg/g and bias= -0.26. In addition to the 5-*cis* lycopene and all-*trans* lycopene prediction R_P^2= 0.80, 0.70; RMSEP= 3.1 µg/g, 282.17 µg/g and bias= -0.06 and -5.43, respectively. The results closely resemble those in the study of lycopene (**Pék *et al.*, 2014; Szuvandzsiev *et al.*, 2014**). This study results regarding the determination of lycopene in fresh tomato are in agreement with the findings of previous studies (**Clement *et al.*, 2008; Saad *et al.*, 2014; Deák *et al.*, 2015**).

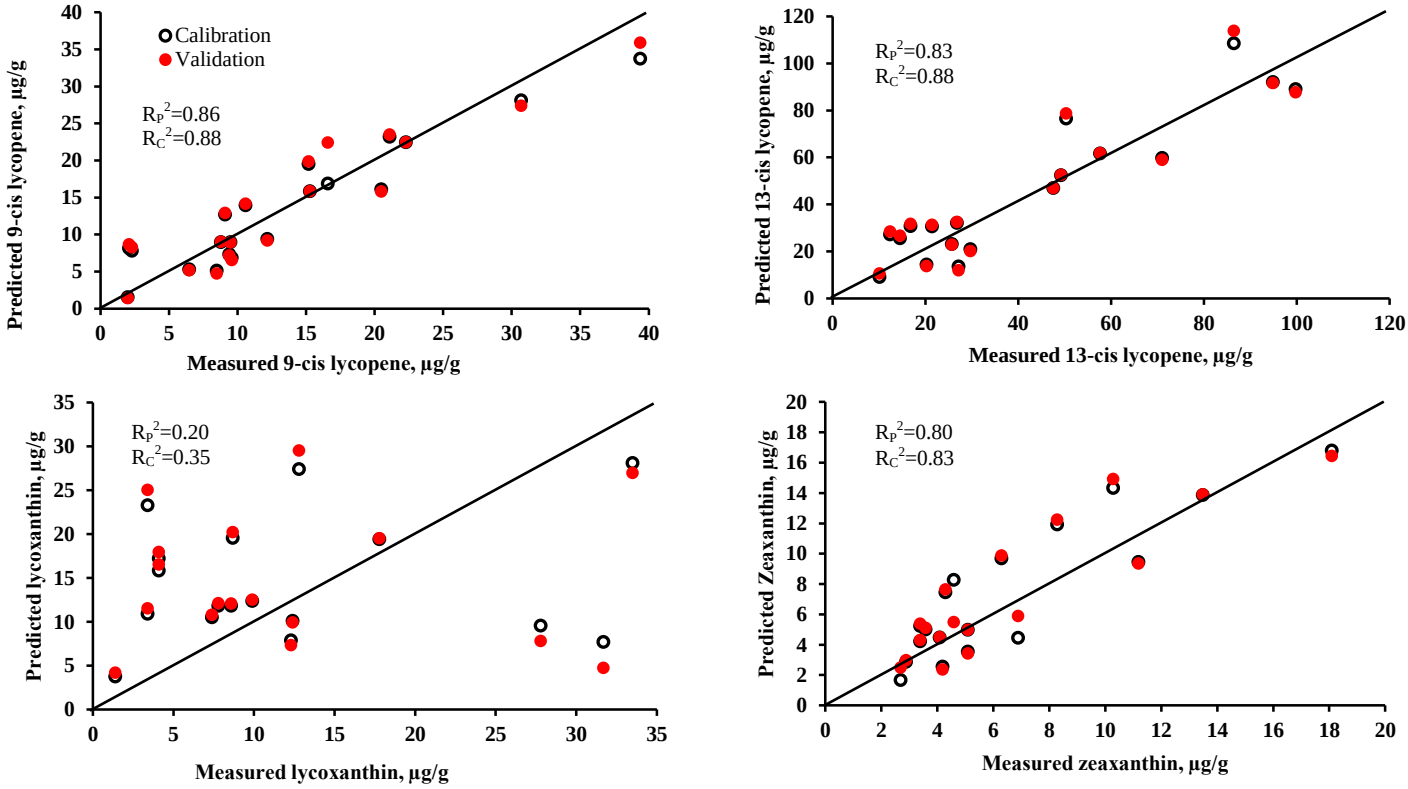


Figure 4 The scatter plots of measured and predicted 9-*cis* lycopene, 13-*cis* lycopene, lycoxanthin and zeaxanthin for the optimal PLSR models.

CONCLUSION

The overall results indicated that Vis/NIRs, as a rapid and non-destructive method, could be applied to estimate carotenoid contents of processed tomato products. A good correlation was found between HPLC measurements and Vis/NIRS predictions for ß-carotene, 9-*cis* lycopene, total carotenoids, 13-*cis* lycopene, 5-*cis* lycopene and zeaxanthin. There are no many previous research results of discrimination methods for different lycopene isomers using Vis/NIRs. It must be highlighted that results obtained from the analysis of processed tomato products, without any preliminary sample preparation, could be applied in combination with Vis/NIR technology for online control during tomato processing.

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