

Determination of Total Carotene and Vitamin C in Chili Powder (*Capsicum annuum* L.) Non-destructively Using Near-Infrared Spectroscopy

Nafis Khuriyati^{a,*}, Anggoro Cahyo Sukartiko^a, Moh Affan Fajar Falah^a, Ririn Nur Alfiani^a

^a Department of Agroindustrial Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Corresponding author: *nafis.khuriyati@ugm.ac.id

Abstract— Chili (*Capsicum annuum* L.) is an important source of total carotene and vitamin C. Both substances are widely used in food processing materials, supporting a healthy immune system and medicine. However, destructive testing often obtains information about the substances, which damages the tested material and requires a relatively long analysis. Therefore, this research aims to develop calibration models of total carotene and vitamin C in chili powder for non-destructive testing using near-infrared spectroscopy. The samples consist of four groups of color, i.e., light green, dark green, red tinge, and red, with a total of 84 samples. Seventy percent of the sample was used for calibration, while the rest of the sample was used for validation. Spectra were measured using the NIRFlex N-500 instrument at a wavelength of 1000 nm to 2500 nm and analyzed with the partial least square (PLS) method using three spectral pre-treatments, which are multiplicative scatter correction (MSC), first derivative savitzky-golay, and de-trending. The accuracy and model reliability was determined by the coefficient of determination (R^2) and the residual predictive deviation (RPD). The best calibration models were successfully obtained when the spectrum was processed using the first derivative savitzky-golay pre-treatment with 6 and 5 PLS factors for vitamin C and total carotene, respectively. Both models were accurate and can be potentially used for determining the total carotene and vitamin C in chili powder samples non-destructively.

Keywords—Chili powder; near-infrared spectroscopy; total carotene; vitamin C.

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I. INTRODUCTION

Chili (*Capsicum annuum* L.) contains a pigment called carotene, which can be transformed into vitamin A (Pro-Vitamin A) in the human body. The green color of chili pepper is originally caused by the chlorophyll pigment [1], while the orange and yellow color is caused by the carotenoid pigment [2], [3]. The process of color transformation in a chili occurs during the transition of a substance in fruit from the chloroplast to the chromoplast during the ripening process. Since the chromo-plastic organelles are connected to the capacity of the fruit tissue, this consequently leads to an excess of carotenoid pigment. This high carotenoid concentration in the chromoplast tissue is responsible for fruit's red color [4], [5].

Chemically, carotenoid is a class of hydrocarbon (pure carotene hydrocarbon, containing no oxygen atom) and xanthophyll (carotene-carrying oxygen atom) [6]. The carotenoid acts as provitamin A, protecting body tissue from sunlight and free radicals [7]. Furthermore, this compound

cannot be produced in the human body but is only reported in plants and some animals, and carotenoid is responsible for the red, orange, or yellow color in various fruits and vegetables, including chili.

Chili also contains more vitamin C compared to other plants. A plant can only produce vitamin C because it contains the microsomal enzyme L-gulonolactone oxidase, which catalyzes the production of ascorbic acid. Furthermore, vitamin C or ascorbic acid is a derivative of hexose, which is classified as a carbohydrate and is widely associated with monosaccharides [8].

In the last few decades, many non-destructive methods have been developed to measure substances in food materials, and the most commonly used are imaging and spectroscopy, which are mainly the two forms of optical measurement. Unlike mechanical technology, which directly measures the features of particular food material, optical technology only provides a non-direct measurement and relies on the calibration model to combine the optical properties or features with the attributes of the texture [9]. One of the spectroscopy

methods to measure substances in food materials is the Near Infrared (NIR) method.

NIR is an analytical method that has advantages as a non-destructive method because it does not cause environmental pollution, does not require chemical reagents, and enables faster analysis [10], [11]. This method has already been applied in many fields of research, particularly in food quality assessment. For instance, rapid measurement of special-grade flat green tea [12], prediction of bioactive compounds in barley [10], detection of heavy metals in vegetable oil [13], also prediction of antioxidant activity and water content in chili powder [14]. As a result, this method was done well to predict chemical properties non-destructively combined with chemometric analysis.

In contrast, NIR also has a disadvantage in that the resulting data with the spectra distribution is quite complicated. The chemical information of most food material samples tested with the NIR is blurred by a change in the spectrum caused by a physical characteristic, for example, the size of the material particle. Therefore, the NIR is a secondary method that needs calibration against the reference method to measure certain substances [15]. A secondary analysis method means a method in need of calibration from the reference method.

The process of building a calibration model was performed using Partial Least Square (PLS) with data processing. This analysis was used to build a linear regression model with predicted variables and observable variables in a new space, with the category variable in a separate column [16]. The parameters used to show the reliability of the regression model are coefficient of correlation value (R^2), Residual Predictive Deviation (RPD), and model consistency [17].

The multivariate calibration technique is often used to gain essential information from NIR spectra analysis [18]. The NIR calibration is a prerequisite for the estimation of many characteristics in the material since an exact characteristic curve analysis with a reliable method is required. Calibration always requires an input task in the beginning, but because it is well-calibrated, the NIR system can be more efficient, quicker, and safer [19]. Therefore, a calibration model was developed to determine or predict substances in chili powder based on NIR Spectra. This study aims to develop calibration models for deciding chili powder's total carotene and vitamin C. The non-destructive testing with NIR spectroscopy is a way to reduce measurement time and is carried out without damaging the tested samples.

II. MATERIALS AND METHOD

A. Conceptual Research

The conceptual outline of this research can be seen in Fig.1. In the food industry, chili powder was used as a condiment for ingredients such as making chili paste, sauces, and seasoning [20]. Meanwhile, in the pharmaceutical industry, chili peppers were used as a mixed ingredient for the manufacture of balsam, inhalers, ginger beer drinks, and others. Usually, to find out the chemical content in the agricultural product, destructive measurements are taken. Unfortunately, this method has disadvantages such as the material cannot be reused, needing a large sample preparation, using chemical substances, and being time-consuming [21].

Currently, several instrumentation techniques have been developed to determine chemical content rapidly. One of the techniques is non-destructive measurement using *Near Infrared Spectra*.

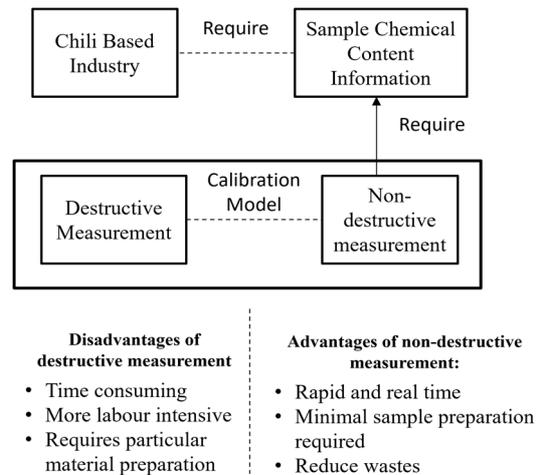


Fig. 1 Conceptual outline of research

Near-infrared (NIR) has been widely used to analyze characteristics with fast and *low-cost* analysis capabilities. NIR has also been applied in food technology to analyze and control food ingredients, quality, pesticide residues, and so forth [22]. NIR technology was developed as one of the non-destructive methods that can analyze at high speed, does not cause pollution, use simple sample preparations, and does not require chemicals [21], [23], [24]. NIR is increasingly used in process and environmental, food, agriculture, pharmaceutical, and polymer analyses.

In order to develop the non-destructive measurement, we need to build a calibration model between destructive and non-destructive measurement. Therefore, findings from this study may provide a rapid model for the determination of total carotene and vitamin C in chili powder using near-infrared spectroscopy and can be applied in industry as well.

B. Sample and Preparation

Fig. 2 shows the flow chart of the research method. The fresh chilies were obtained from farmers in Special Region of Yogyakarta, Indonesia. The fresh chilies consisted of four color groups: light green, dark green, red tinge, and red chilies. These four colors were intentionally selected so that the samples could represent a wider color range of chilies.

The chili powder was prepared following the Standard Operational Procedure issued by Agricultural Products Processing Directory, which started with sorting, then washing, draining, drying, and milling. The fresh chilies were sorted according to the classified color. They were then washed with water to remove the dirt stuck on the chilies and dried by a cabinet dryer at a temperature of 60°C for 15 hours to reduce the water moisture. In addition, the dried chilies were milled by a dry mill into powder. A 60-mesh sifter then sifted this powder to be the same size as chili powder and packaged it into a clip-seal plastic bag of 0.5 g weight, totaling 84 samples. The fresh chili sample is shown in Fig.3.

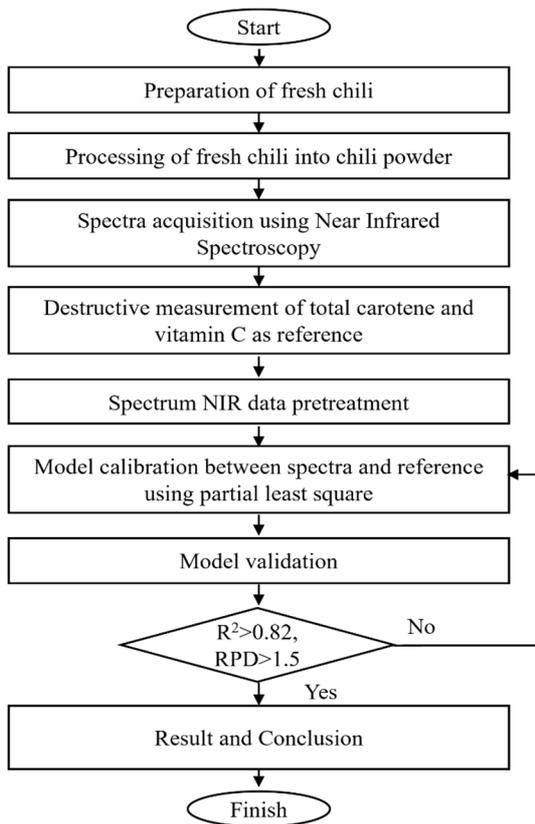


Fig. 2 Flow chart of research method



Fig. 3 Fresh Chilies in various color

C. NIR Spectra Acquisition

The NIRflex N-500 Fiber Optic Solids Cell (Büchi Labortechnik AG, Flawil, Switzerland) instrument used to acquire the spectrum pattern of chili powder is shown in Fig. 4.



Fig. 4 Near-infrared spectroscopy

Furthermore, 0.5-gram chili powder was placed into a vial tube. The sample was then closely radiated by infrared at a

wavelength of 1000-2500 nm. The tube filled with samples was then rotated 12° times from the starting point each time the wave interval was changed. This acquisition was performed for 84 samples.

D. Total Carotene Measurement

The total carotene substance was measured using adsorption column chromatography modified [25]. Chili powder of 0.5 g was poured into a reaction tube with a screw cap, ± 2 ml of petroleum ether, and acetone in a ratio of 1:1 was added and then introduced into a vortex until the liquid became homogeneous. The residue was set aside until sedimentation. Then it was taken by pipet and placed into a screw cap testing tube. Next, the acquired filtrate was placed into a separatory funnel, added with aquadest, and shaken. The liquid was set aside until there was a separation between the ether phase and acetone. The acetone in the bottom layer was therefore streamed outside of the separatory funnel and discarded. The washing process was carried out twice. The ether phase or Petroleum ether fraction on the top was placed into the volumetric flask and diluted up to 10 ml. After that, the resulting absorbance was measured at a wavelength of 450 nm. This was performed using Spectrophotometry Ultraviolet-Visible Model 721N instrument (Hangzhou West Tune Trading Co., Limited, Zhejiang, China).

E. Vitamin C Measurement

Vitamin C tests were performed using the iodine titration method. The sample, which had been previously crushed, was weighed and taken by 5 grams and then dissolved in a 100 ml flask. This liquid was then filtered, and the filtrate was pipetted into 25 ml. Then, a few drops of indicator strength were added and quickly titrated with 0.01 N iodine solution until it turned blue. The vitamin C compound can be calculated using the formula below:

$$\text{Vit C (mg/100g)} = \frac{V_{I2} \times 0.99 \times F_p \times 100}{W_s} \quad (1)$$

V_{I2} = Volume of Iodine (mL)

F_p = Factor of Dilution

W_s = Weight of Sample

F. Data Analysis

The graphics produced by raw spectra still have much noise. Therefore, pre-treatment was required. Pre-treatment is a process of changing the value of a data variable that has so as to make it better to analyze. The use of pre-treatment is to improve the distribution of skewed or unsymmetrical variables using algorithms and eliminate disruptive noise in the spectrum by smoothing curves. Data that is changed through pre-treatment is absorbance data at each NIR wavelength, while chemical data is not changed because it is used as a reference. Three pre-treatments are used: multiplicative scatter correction (MSC), first derivative savitzky-golay, and de-trending.

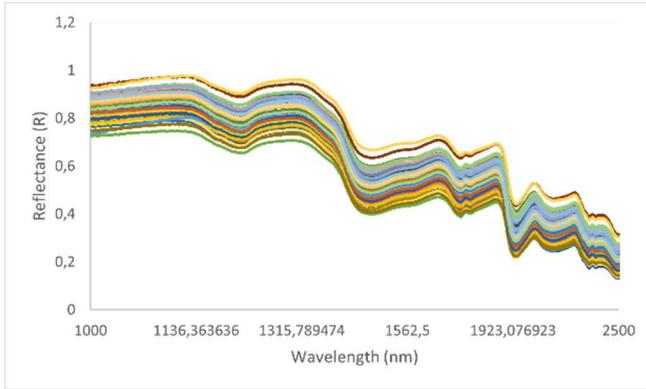
The data analysis was conducted using the Unscrambler X version 11.0. The calibration models were developed using PLS regression. In addition, PLS is a non-parametric, multivariable approach for predicting the calibration model that assigns the response variable y to an x -predictor group [26], [27]. Based on research conducted by Anggraeni [28] and Hayati [29] on the comparison of PLS methods with

principal component regression (PCR), it was shown that the PLS method provides optimum results. The reference and spectra data were used to develop calibration models for determining the total carotene and vitamin C.

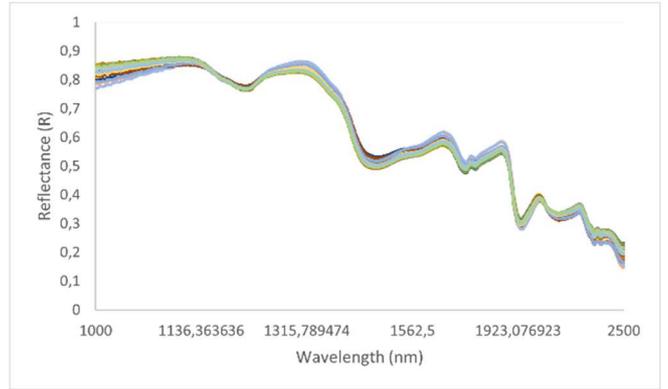
The optimization of the PLS regression calibration model was taken from the cross-validation procedure [30], in which all chili powder samples (84) were divided into two data groups. The first group is called calibration data, used to develop the model, while the second group is validation data, which is used to evaluate and validate the model's reliability [18]. This grouping of calibration and validation data has a proportion of seventy and thirty percent of total samples, respectively. The calibration data consist of 59 samples, and the rest 25 data are categorized into validation data.

The parameters used to evaluate model reliability were determined by the coefficient of correlation value (R^2), RPD, and model consistency [17]. A model has good performance if its value of $R^2 \geq 80\%$ [31] and RPD is greater than 2, which shows the model's accuracy, and the consistency varies between 70-110%. Model consistency was gained from SEC/SEP ratio.

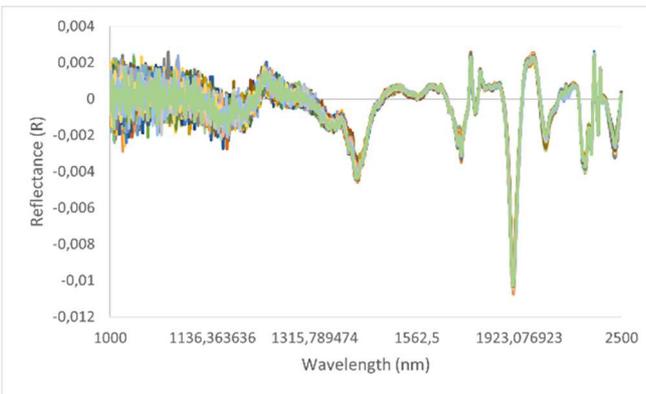
$$R^2 = \sqrt{1 - \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (2)$$



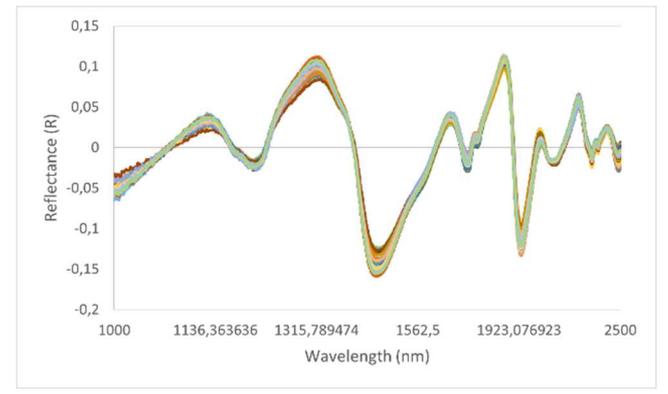
(a)



(b)



(c)



(d)

Fig. 5 Spectrum result (a) raw spectra (b) multiplicative scatter correction (c) first derivative savitzky-golay (d) de-trending

The NIR spectra data not only show sample information but also contains background information or noise. Pre-processing or pre-treatment is a step to improve deficient spectra due to light refraction, while data acquisition, noise, outer circle disturbance, low reproducibility measurement

$$SEC = \sqrt{\frac{\sum_{i=1}^n (y_i - y_{c_i})^2}{n - p - 1}} \quad (3)$$

$$SEP = \sqrt{\frac{\sum_{i=1}^n [(y_i - y_{v_i}) - \text{bias}]^2}{n - 1}} \quad (4)$$

III. RESULTS AND DISCUSSION

A. Spectra Analysis

The spectra acquisition of eighty-four samples was recorded between 1000 nm and 2500 nm. There were peaks in each spectrum indicating the presence of a chemical bond. The spectra plot in Fig. 5 shows the valley (inverted peak) as a sign of a substance in the chili powder. The valley of the wave can be seen at a wavelength ranging from 1190-1240 nm, 1422-1430 nm, 1700-1730 nm, 1950-1960 nm, 2060-2080 nm, and 2420-2500 nm. Total carotene as a substance consisting of a CH_3 bond can be detected at a wavelength of 1190-1240 nm and 1700-1730 nm, combinations while wavebands between 1680 and 1754 nm could be allocated to the first overtones of the C-H stretching and combinations modes [32]. Meanwhile, 1000-1052 nm mainly reflects the information of orange skin or vitamin C [33].

a relatively consistent baseline [22], while the first derivative savitzky-golay to remove spectral variation caused by the scattering process and the particle size.

Fig. 5 shows the difference between raw spectra and three pre-treatment spectra. Multiplicative scatters correction reduced basic variations and eliminated the scattering effect. In addition, it was also able to reduce the number of latent variables, compared to non-pretreatment methods. The results of the MSC pre-treatment are shown in Fig. 5 (b), which can improve the spectrum results, making it smoother and denser than the raw spectra.

The first derivative savitzky-golay is a method for reducing noise without removing important information from the data, reducing baseline effects, and increasing smaller absorption peaks, and producing an ordinate linear offset. Savitzky-golay's algorithm matches polynomials for each successive curve segment to replace the original value with more regular variations. Fig. 5 (c) shows that the pre-treatment spectra significantly differ from the raw ones.

The pre-treatment of de-trending uses to remove nonlinear trends in spectra. The pre-treatment also calculated the baseline function as the least squares fit of a polynomial, for the spectrum data. The de-trending was further applied to the individual spectrum. De-trending spectra correction was taken into account because it was stated in several studies that this spectra correction method was fit and needed to be applied when dealing with bulk samples [29]. As the polynomial order de-trending increases, additional baseline effects are removed. Zero order to correct the offset, first order to correct the offset and tilt, as well as the second order to correct the offset, tilt, and curvature. This research used the second-order de-trending correction method to improve the PLS prediction accuracy for total carotene and vitamin C content. These results have very significant differences from the raw spectra. There was a change between the valley shape and peaks produced from the spectrum of chili powder. The peak and depression spectra for the de-trending spectral data showed the chili powder's strong and weak absorbance characteristics, which may be due to the chemical content in the chili powder. Fig. 5 (d) presents the spectrum results of de-trending.

B. Total Carotene and Vitamin C Analysis

Table 1 shows that the total carotene and vitamin C of 84 samples is varied and has a wide range because they were obtained from various maturity levels of chili, from green to red.

TABLE I
TOTAL CAROTENE AND VITAMIN C OF SAMPLE

Color of Chili Powder	Number of samples	Average of total carotene (%)	Average of vitamin C(mg/100g)
Light Green	21	60.41 ^a	383.62 ^a
Dark Green	21	119.41 ^b	473.95 ^b
Red Tinge	22	276.05 ^c	597.12 ^c
Red	20	408.45 ^d	714.45 ^d

Note: In the same column, different letters show significant differences at $p \leq 0.05$.

The average of total carotene was around 60.41% to 408.45%, meanwhile the average of vitamin C around 383.62

mg/100g to 714.45 mg/100 g. Other than that, it showed a significant difference between light, dark green, red tinge, and red chili powder samples ($p \leq 0.05$) with the amount of the chili powder.

C. Calibration of Models

In this research, 84 samples were divided into two subsets. One was called a calibration set in which all samples were used for calibrating the model. The other was called the validation set, where all samples were used to test the model's performance. To achieve a robust model, the selection of the sample for the calibration of the model was important and carried out as follows. First, all samples were sorted according to their reference value (total carotene and vitamin C measured using the destructive method), then divided into two for calibration and validation sets. The division was done in such a way that the reference values of the validation set were within the range of reference values of calibration one. Moreover, the average of reference value for the two sample sets was almost the same. The calibration set contains 59 samples, and the validation set contains 25 samples as shown in Table 2.

Calibrating model using a regression tool was used to establish a model between reference measurement and NIR spectra in the calibration set. The optimizing model was carried out by validation. The total carotene and vitamin C of samples measured using destructive testing were used as a reference for developing a calibration model that would determine the success of using NIR to estimate parameters of the quality of the sample. The detailed descriptive statistics of the reference value in the calibration and validation sets are shown in Table 2.

TABLE II
DESCRIPTIVE STATISTICS OF REFERENCE VALUE IN THE CALIBRATION AND VALIDATION SETS

Parameter	Subsets	Number of samples	Average	SD	Max.	Min.
Total carotene	Calibration set	59	212.4	139.3	472.2	56.6
	Validation set	25	208.9	139.5	463.6	58.2
Vitamin C	Calibration set	59	538.4	139.4	805.0	292.5
	Validation set	25	546.8	137.2	794.7	332.7

D. Regression Model for Predicting Total Carotene

Fig. 6 can be seen that the value of R² of the calibration and validation model, from raw spectra and three pre-treatments, achieves a value higher than 80%. Therefore, all models can be classified as good performance [31]. The next evaluation parameter is the RPD or ratio between the standard deviation of the data reference and SEP. Based on Fig. 6, the RPD value of model using raw spectra, MSC pre-treatment, first derivative savitzky-golay pre-treatment, and de-trending pre-treatment has a number greater than 2. It is therefore concluded that the models have good accuracy. Similar effects can be overcome by successfully using MSC. Savitzky-Golay smoothing was able to eliminate the noises like baseline drift, tilt, and reverse. [35]. Meanwhile, de-trending reduced noise wave interference so that the spectra became smoother than before.

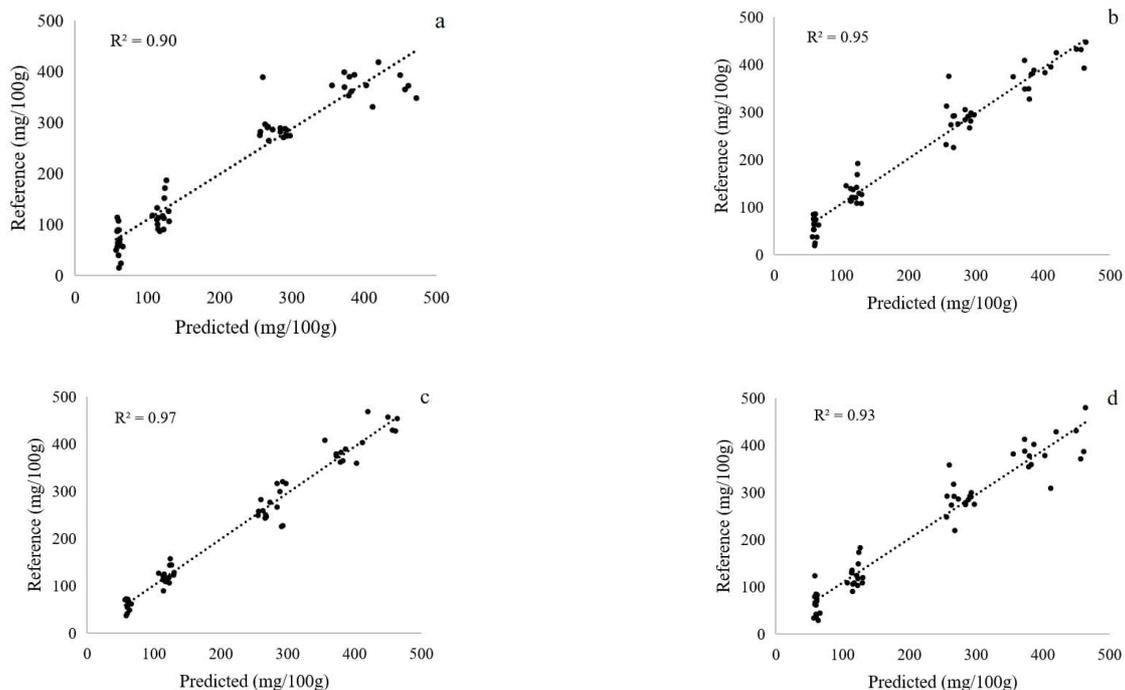


Fig. 6 Reference and predicted total carotene using (a) raw spectra (b) multiplicative scatter correction (c) first derivative savitzky-golay (d) de-trending.

Tiaprasit and Sangpithukwong [17] suggested that a good model should consider SEC/SEP ratio or model consistency. If the value of SEC is small and SEP is big, it can lead to overfitting. Meanwhile, as the SEP value is small and, conversely SEC value is big, this leads to underfitting. If the value of model consistency is greater than 98%, therefore, no model has overfitting data. However, a model resulting from raw spectra or no pre-treatment leads to an underfitting data prediction. Underfitting is a condition in which a model performs poorly in its calibration data. This happens simply because the model is too simple and therefore cannot describe

the predictive value well. Nonetheless, the model from spectra with three pre-treatments allows the consistency value ranging from 98.5-112.8%, which can be considered to have a good performance.

In addition, it can be concluded the model evaluation with the aforementioned evaluation parameter leads to three calibration models, which are good and reliable. The best pre-treatment to predict total carotene was the first derivative savitzky-golay with a determination coefficient value (R^2) of 0.97 and using 5 PLS factor.

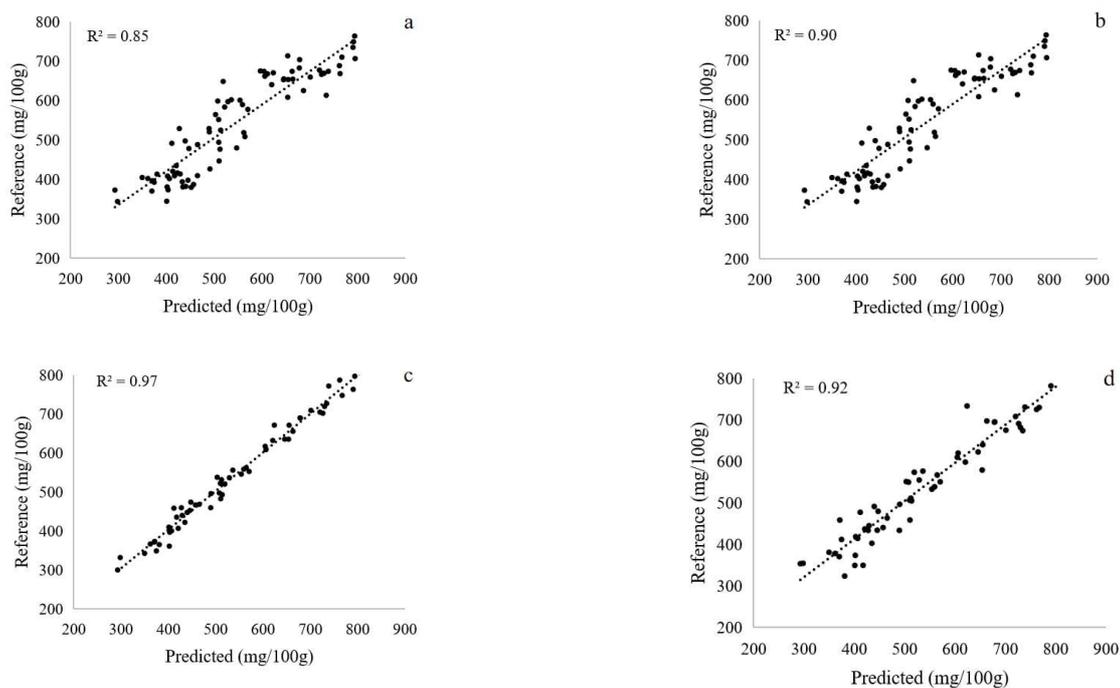


Fig. 7 Reference and predicted vitamin C using (a) raw spectra (b) multiplicative scatter correction (c) first derivative savitzky-golay (d) de-trending.

E. Regression Model for Predicting Vitamin C

Partial least square is one of the most classical multivariate regression tools. The number of PLS factors and the spectra pre-processing are critical factors in calibrating the model [36]. Furthermore, the number of PLS factors and the spectral pre-processing were optimized by cross-validation and determined according to the first local minimum of root mean square error of cross-validation, plotted concerning the number of PLS factors. Both spectral pre-treatment and number of PLS factors significantly affect the PLS model's performance.

The calibration model is the correlation between the NIR absorbance and vitamin C. The best calibration model was determined from the R^2 and the RPD values [15], [36]. In predicting vitamin C, all built models have achieved acceptable results, with the R^2 values of all models higher than 0.80 [37]. The regression model for predicting vitamin C is shown in Fig. 7.

The model from spectra with three pre-treatments allows the consistency value ranging from 92.5-105.4%, which can be considered good performance. The statistical analysis result using PLS for each pre-treatment can be seen in Table 3.

The best pre-treatment to predict vitamin C was the first derivative savitzky-golay with 6 PLS factor. This method can reduce noise without removing important information from the data, decreasing base effects and increasing smaller absorption peaks, producing an ordinate linear offset. Savitzky-golay algorithm adjusts the polynomials for each successive curve segment in such a way that it can replace the original value with more regular variations. By analyzing the first derivative, changes can be seen when compared with the original data. This method is able to predict total carotene and vitamin C non-destructively.

TABLE III
SUMMARY OF STATISTICAL ANALYSIS USING PARTIAL LEAST SQUARE

Parameter	Pre-treatment	R^2	RPD	Consistency value (%)
Total Carotene	Non-pretreatment	0.90	2.8	112.8
	MSC	0.95	2.7	98.5
	1 st der.	0.97	3.5	102.4
	Savitzky golay			
Vitamin C	De-trending	0.93	2.9	99.5
	Non-pretreatment	0.85	2.0	101.2
	MSC	0.90	2.0	105.4
	1 st der.	0.97	2.8	102.8
	Savitzky golay			
	De-trending	0.92	2.3	92.5

IV. CONCLUSION

From this study, it can be concluded that the calibration models can be developed to determine the substance of total carotene and vitamin C in chili powder based on NIR spectra. A good performance calibration model can be managed by spectral pre-treatment using multiplicative scatter correction, first derivative savitzky-golay, and de-trending. The three spectral pre-treatments for the calibration model of total carotene with coefficient of determination (R^2) value ranges between 0.90 and 0.97 and therefore RPD value between 2.70-3.50 can be stated to have high accuracy. Meanwhile, the three spectral pre-treatments for vitamin C resulting

calibration model with determination coefficient value (R^2) ranges between 0.85-0.97, and therefore the RPD value between 2.00-2.80. The best pretreatment of both model total carotene and vitamin C was first derivative savitzky-golay, with 6 and 5 PLS factors for vitamin C and total carotene, respectively. The consistency value ranges between 92-105%, which can be categorized as a model of good performance.

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