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Non-destructive Measurement of Lycopene Content in High Soluble Solids Stored Tomato (*Solanum Lycopersicum* Mill. cv Rinka 409)

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***Abstract*— High-quality fruits can be produced by controlling the environmental factors, i.e. temperature, relative humidity, carbon dioxide, and light intensity. The tomato *(Solanum lycopersicum)* variety *'Rinka 409'*, was grown hydroponically with a high-wire system in high technology greenhouse, Ehime University, Japan. The water stress treatment using visual monitoring system was used to produce the high soluble solids tomato fruits. Moreover, a high lycopene content of tomato can be produced by controlling the storage condition. Non-destructive measurement has been known to provide a fast and accurate quality measurement of fruits and vegetables. The objectives of this study were to develop a precise position for the non-destructive measurement of the lycopene content in high soluble solids stored tomato using Vis/NIR spectroscopy and evaluate its performance. In this study, the high soluble solids of tomato fruits were grown under water stress treatment and stored after harvesting at 25°C in a cool incubator for 7 days. The tomato spectra were measured using Vis/NIR spectroscopy with the wavelength 500-1010 nm. The estimation of the lycopene content was based on the statistical model using multivariate analysis. From this study, it is concluded that the measurement of lycopene content in high soluble solids of tomato fruits after storage by using the top part of the tomato, was better than if it is compared to the side part. It was best determined using PLS analysis with Visible/Near-infrared Spectroscopy non-destructively, with the R 0.98 and RMSE 0.80.**

***Keywords*— high soluble solids tomato; lycopene; non-destructive measurement; stored tomato; visible/near-infrared spectroscopy.**

I. INTRODUCTION

In high technology greenhouse, the environmental factors can be controlled for optimal plant growth. Quality is a high priority for greenhouse crops, requiring much care in pest and disease management, not only to secure yields but also to obtain a high-value product. Greenhouse technology uses solar irradiance to support the production of plant and to protect the plant against external environment [1].

In the high technology greenhouse, the speaking plant has been studied for years, especially at Ehime University, Japan. The speaking plant is the idea of using observed crop processes as the variables to be controlled. The use of internet technology for environmental control in the greenhouse has been used for years [2] as an online monitoring of crop responses as early warning systems to detect and prevent the unintended condition by giving substantial resources needed [3]. Online monitoring system can be used to control the greenhouse environment, such as temperature, humidity, light intensity, and other environmental information using WiFi and it provides a stable, feasible, and low-cost solution for greenhouse production management [4]. The artificial lighting in a greenhouse can be used not only for replacing or supplementing the sunlight but also for boosting growth and is designed by the crop's photoperiodicity, natural day length, average hours of sunlight, solar radiation angle and intensity, the amount of structure-induced shading.

By using high technology, the high soluble solids of fruits can be produced in the greenhouse. The high soluble solids tomato fruits can be produced using water stress treatment with an early detection technique based on the projected plant area calculated from digital color images captured by digital camera [5]. The water stress is known can give positive effect to soluble solids content (SSC), with more than 10% increase compared to controls [6], [7].

Tomato (*Solanum Lycopersicum* Mill.) is one of the world's most popular vegetables. Tomato can be grown in the home garden and commercially. Tomato is widely used

as a vegetable, cooking ingredient, and drank as a juice. It is one of the most popular salad vegetables in the raw state and can be made into soups, conserves, pickles, ketchup, and other products. It can be served raw, baked, stewed, fried, and as a sauce with various other foods.

As the population is getting higher and people tend to live healthily, the demand for tomatoes increases, especially from 2015 to 2016. In Indonesia, according to Statistics Indonesia 2016 on The National Socioeconomic Survey [8], the national consumption of tomato around the year 2016 was 1149.16 million kg, increased from the year before, which was 1065.42 million kg. When choosing the fresh tomato, consumers have considerations before purchase it. Consumer preference plays an important role in their purchasing decision. As the people's living condition getting improve as well as the development of social economy, consumer's preference is also changing from quantity to quality gradually. People are paying attention to the quality, not only the appearance, color, firmness, flavor, but also other compounds of nutritional interest and storage characteristics [9]. The intrinsic quality attributes of those products available at harvest can also reach consumers intact and consequently affect consumer preference. One of the consumer dissatisfactions with tomato products is a lack of fresh tomato flavor [9]. Therefore, it is essential to produce a high-quality tomato to meet the consumer's expectation.

Tomato is rich in vitamin A and vitamin C, which are good for human health. 100 g of fresh tomato fruit contain on average 94.2 g of water, 0.95 g of proteins, 2.6 g of carbohydrates, 0.21 g of fat, 0.61 g of minerals, 19 mg of vitamin C, and 592 µg of carotenoids [11]. Tomato fruit is widely known for its good nutrients and has a high lycopene content. Tomato contains a complex mixture of bioactive components, plays a role as a dietary source of nutrients, such as a mixture of carotenoids, including lycopene, β-carotene, and lutein. The carotenoids in tomato fruits are accumulated during the ripening process, which also related to the ethylene, and the increase of lycopene [12]. Carotenoids which take part in a wide range of physiological processes are lipid-soluble molecules that contribute to color in many fruits and vegetables and some have been associated with several health benefits. Consumption of carotenoids has been associated with reduced risk of cardiac and heart disease, prostate cancer, and age-related macular degeneration [13].

Lycopene is one of the carotenoids substances. Lycopene is the bright red carotenoid phytochemical that gives tomatoes and other red fruits their color. Color is one of the most important quality factors that affect tomato appearance and is determined by skin and flesh pigmentation. When the tomato fruit undergoes the developmental stage, the chlorophyll experience a degradation, which is marked green-to-red color change [14]. Tomatoes are climacteric fruits, thus ripening processes, including lycopene synthesis and accumulation, are dependent on ethylene production [15]. Lycopene serves as a potent antioxidant in humans and is said to lower the risk of a variety of cancers [16]. Lycopene is a carotenoid that protects against many diseases by alleviating oxidative stress. Lycopene is also beneficial for controlling epileptic seizures and a potential functional food alternative in epilepsy treatment [17].

Since lycopene content in tomato fruit is increased under optimum temperature, it is essential to control the storage environment for providing valuable tomato fruit. Based on the previous study [18], the optimum temperature to store the tomato for getting a higher lycopene content was 25°C. Therefore, in this study, the tomato fruits will be stored at 25°C for 7 days.

Non-destructive measurements of quality parameters have been conducted in many agricultural products and have proved to be rapid and accurate in estimating the quality factors involved [19]. NIR spectra acquired 2-position spectra of the fruit was acceptable and could provide precise and stable calibration equations [20],. Vis/NIR spectroscopy offers the possibility to classify and predict the internal quality of fruits and vegetables. In the previous study [21], the lycopene content of tomato fruit was measured using Vis/NIR spectroscopy with the wavelength range between 500 nm and 1010 nm.

The objectives of this study were to develop a precise position for the non-destructive measurement of the lycopene content in high soluble solids stored tomato using Vis/NIR spectroscopy and evaluate its performance. This study was expected to provide useful information by giving feedback to the farmer for enhancing the quality of high soluble solids tomato with high lycopene content by modifying the storage condition.

II. Materials and Method

A. Plant Material

Tomato plants (*Solanum Lycopersicum* Mill., cv Rinka 409) were grown hydroponically with the high-wire system in high technology greenhouse (1.3 ha) at Ehime University (33°50' N, 132°47' E). The greenhouse had a full controlling system (temperature, relative humidity, carbon dioxide, light intensity) to support the seasonal change and will be adjusted to the real-time condition. The sowing was done on July 22, 2018, and the tomato seedling was transplanted to Rockwool slabs (0.3 x 0.25 x 0.91 m) (Grotop expert, Grodan, Roermond, Netherlands) on September 6, 2018.

The tomato plants were grown with water stress treatment. The visual monitoring system was applied in the intelligent greenhouse to detect the water stress condition of the tomato plant. Water stress condition will affect physiological and photosynthetic activities in plant [22], [23],, and the tomato fruits will increase their bioactive compounds [24].

A total of 96 ripe tomatoes were harvested from the high-technology greenhouse at Ehime University. The experiment was conducted from January 15, 2019, to January 28, 2019. The fruits were harvested manually and brought to the laboratory at Ehime University directly to be analyzed.

B. Storage Equipment

Tomatoes were stored in cool incubators (A 1201-2V, (Ltd.) Ikuta Sangyo Co., Ltd., Ueda, Japan) for seven days. The incubator had 2 doors with the outer dimension of the incubator was 0.45 [L] x 0.32 [W] x 0.63 [H] m and the inner dimension was 0.3 [L] x 0.2 [W] x 0.25 [H] m and it was divided into two parts using a partition. The incubators were set at 25°C in temperature and monitored using a Thermo Recorder (TR-72wf, (Ltd.) T & D) data logger. The

average for the temperature for both periods recorded was 25 ± 3 °C and for relative humidity was 51.4 %. The incubator used to store the tomatoes, and the data logger used were shown in Fig. 1 (a) and (b) respectively.

(a) (b)

Fig. 1 Incubators Used for Storing the Tomato Fruits (a) Thermo Recorder Used as Data Logger (b)

C. Fruit Quality Measurement

Tomato fruit quality for the diameter, fresh weight, sweetness, and lycopene content was measured before storage (day 0^{th}), and after storage (day 2^{nd}, 4^{th}, and 7^{th}). The measurements conducted at the Research Center of Department of Bio-mechanical System, Faculty of Agriculture, Ehime University, Japan.

Non-destructive methods were used for measuring the diameter, fresh weight, and spectrum from the fruits. The diameter was measured using a Digital Caliper Ruler (BLD-100, Niigata Seiki co., Ltd), and the fresh weight was measured using a Digital Weighing Scale (EK-300i, AND Company, Ltd). The destructive method was used for measuring the sweetness using Digital Handheld "Pocket" Refractometer PAL-1 (ATAGO U.S.A., Inc) and for measuring the lycopene content using Spectrophotometer (U-1900, Hitachi High-Technologies Co., Tokyo, Japan). The data of the lycopene content measured destructively was used to make the model in the statistical analysis.

The lycopene content measured destructively as a puree and determined based on the conventional method [25]. The lycopene content was extracted two times with acetone. The first one was using 35 mL, and the second one was using 15 ml of acetone. Tomato fruits were crushed using a blender to get the puree. About 1.5 g of the puree was put in 35 mL of the brown centrifuge tubes. Lycopene extraction solution (35 mL) consisting of acetone was added to the tubes and shaken for 10 minutes with 1.000 rpm using a shaker (MS-300, As One Co., Osaka, Japan). Then, the supernatant solution was moved to a brown measuring flask of 50 mL, and the residual samples left was shaken again with 15 mL of acetone for 5 minutes with 1.500 rpm. Then, the supernatant solution was moved to the brown measuring flask, and acetone was added until the solution reached 50 mL. The supernatant solution was filtered using a 0.45 µ disposable filter (ADVAN-TEC, Tokyo Roshi Kaisha, Ltd., Tokyo, Japan). The absorbance of the supernatant solution then was measured at 505 nm using a spectrophotometer (U-1900, Hitachi High-Technologies Co., Tokyo, Japan). The lycopene content was calculated using the equation (Equation 1) with the absorption coefficient of 3.150 %-^{1}cm $^{-1}$ [26], [27].

$$Lycopene\ content\ (mg/100g) = \frac{absorbance\ at\ 505\ nm}{0.315 \times sample\ (g)} \times 10 \quad (1)$$

D. Spectra Acquisition

Using the KUBOTA Fruit Selector instrument (K-BA100R, KUBOTA corporation), the spectra were obtained. The fruit was placed on the black holder and covered by the black cloth to avoid any scattered sunlight during the measurement. Each sample was measured two times, the top part, and the side part. The fiber optics directed the tungsten light from the halogen lamp to the fruit. The spectra were obtained in two positions. The first one was using a top part of the tomato against the black holder, and the second one was using a side part of the tomato against the black holder (Fig. 2).

(a) Top (b) Side

Fig. 2 Spectra Measurement

By using the wavelength range from 500 nm to 1010 nm (interval between wavelength was 2 nm), measurement storage time of 40 ms, average measurement frequency of 5, measurement dummy frequency of 2, and V10 filter for the wavelength calibration for repeatability, the spectra were obtained. The fruit selector spectroscopy scheme is shown in Fig. 3.

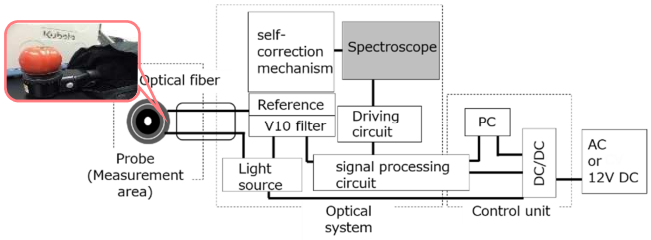


Fig. 3. Fruit Selector Spectroscopy Scheme

Kubota Fruit Selector was used as a Visible/Near-infrared spectroscopy to measure the spectra of lycopene. The instrument consisted of a probe and the main unit. It measured the spectra when the tomato fruit skin touched the probe. The probe itself consisted of ring light arranged in a concentric pattern. The light emitted from the ring light went through the sample and spread inside the sample. Then the data was passed to the main unit. The spectra data were obtained before the samples were subjected to destructive experiments. The spectral data obtained then be analyzed using Unscrambler 10.3 (CAMO-software, Norway).

E. Statistical Analysis

The spectra were analyzed using the Unscrambler 10.3 software. Since many spectra data obtained contains background information besides sample information, such as noise, uncertainties, and others, it is necessary to do pre-processing data before the modelling. The pre-processing should be done to get a reliable, accurate, and stable calibration models. The analysis was done according to Fig. 4.

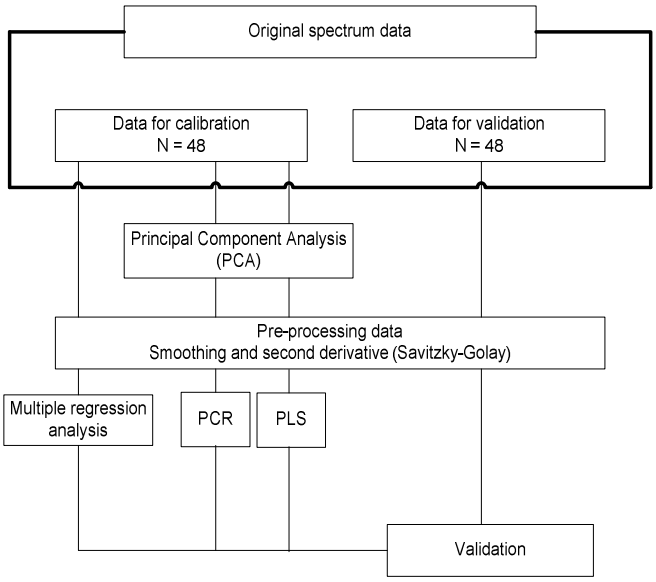
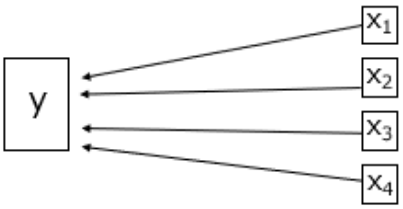


Fig. 4 Analytical Diagram Using Visible/Near-infrared Spectroscopy

The spectral data were used to develop calibration and validation models. The method used was principal component analysis (PCA). PCA searched for directions of maximum variability in sample grouping and used them as new axes called principle components which could be used as new variables instead of the original data, in following calculations. The prediction performance was evaluated by the correlation coefficient (r), and root mean square error of cross-validation (RMSEV). The ideal model should have a higher R-value and lower RMSEV values.

The original spectra data were divided into two parts, for calibration, and for validation. Pre-processing data was used to reduce noise. Then the analyses were done using MLR, PCR, and PLS. Multivariate analysis was performed using the Unscrambler software version 10.3.

In Multiple linear analysis (MLR), the Y is estimated using the multiple explanatory variables (Fig.5).



y : objective variable

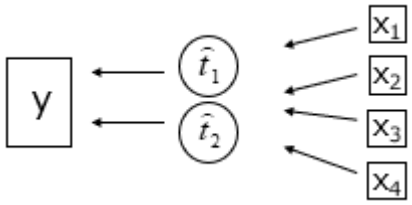
x_i : explanatory variable

Fig. 5 Multiple Linear Regression Analysis

$$y = b_0 + b_1x_1 + b_2x_2 \quad (2)$$

Y is the lycopene content (estimation), while the b_0 is the intercept, b_1, b_2 is the regression coefficient, the x_1 is the absorbance at 560 nm, and x_2 is the absorbance at 674 nm.

In PCR (Principal component regression), the Y is estimated by replacing the multiple explanatory variables with principal components (Fig.6).

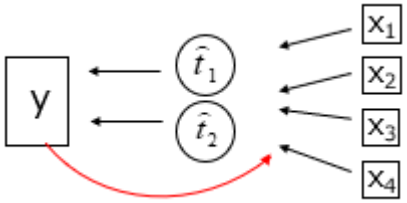


t_i : principal component

x_i : explanatory variable

Fig. 6 Principal Component Regression Analysis

In PLS (partial least square), the Y is estimated by replacing the multiple explanatory variables and y with principal components (Fig.7).



t_i : principal component

x_i : explanatory variable

Fig. 7 Partial Least Square Analysis

The accuracy of the models was expressed as the root mean square error of cross-validation (RMSECV). The cross-validation was performed to avoid over-fitting of the model. The RMSECV was calculated as follows [28]:

$$RMSECV = \sqrt{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2/N} \quad (3)$$

Where ŷi and yi are the model-estimated value and the reference value for sample i, respectively, and N is the number of samples.

III. Results and Discussions

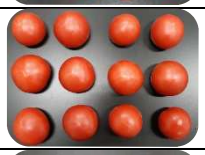
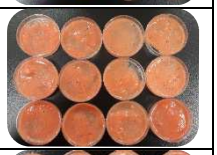
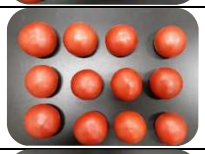
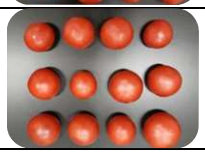
A. Tomato Fruit Quality After Storage

The tomato fruit qualities were measured before storage (at day 0^{th}) and after storage (at day 2^{nd}, 4^{th}, and 7^{th}). The tomatoes were harvested at the ripe stage and developed a light red color. After harvesting, the tomatoes were put in the cool incubator directly to avoid any light that could

affect the quality of the fruits [29]. The lycopene synthesis was inhibited when the intense solar radiation exposed it. Twelve tomatoes of each experiment were then taken for the quality measurement.

The tomatoes color was getting darker as the storage day longer. The red color and quality of tomatoes could be attributed mainly to the presence of the major carotenoid, lycopene, representing a key factor for their commercial value. In plants, carotenoids are localized in cellular plastids. The plastids have differences in their capacity to synthesize and sequester carotenoids and have a role in carotenogenic activity, carotenoid stability, and pigment diversity and [30].

TABLE I
TOMATO APPEARANCE BEFORE AND AFTER STORAGE

Storage Day	Tomato Appearance	Tomato Puree
0		
2		
4		
7		

Lycopene in tomato fruits is known to increase when stored under optimum temperature. The result shown in Table 2 showed that the lycopene content was increasing as the storage day longer when stored at 25°C. Sufficiently high temperature (22-25°C) along with dense foliage to protect fruits from direct exposure to the sun is the most appropriate conditions to enhance the lycopene synthesis [29],. At the previous study [18], the lycopene content stored at 25°C for 7 days increased almost 4 times. While in this study, the lycopene content at Day 7 increased almost 3 times compared to Day 0.

The sweetness of the water stress tomato had an average of 8 Brix % (Table 2). Water stress would affect in the reduction of transpiration but increased in fruit nutrient uptake [31], which result in the improvement of organoleptic and functional quality [32], such as the increase of soluble solids (enhance sweetness and flavor). As tomatoes ripen, there was a significant increase in their fructose and glucose contents. These sugars were the most significant contributor to the soluble solids content, and the correlation between the soluble solids and the sugars in the tomatoes is high [33]. The changes in the constituents of the soluble solids might result from a change in the glucose/fructose ratio and the organic acids in the tomatoes after harvest [34]. Soluble solids in tomato fruits are considered important, especially for tomatoes grown for processing. The average of soluble solids content in industrial tomatoes must be at least 5 Brix % [35],. The soluble solids content is an important parameter to determine the maturity and quality of the tomato fruit. The composition of soluble solids is commonly determining the flavor of the fruit [36].

Their diameter and fresh weight usually denote the size of the tomato fruits. In general, water stress affected the diameter and weight of the fruits. According to this study, the average weight of the water stress tomato was 67.5 g. The low weight of the fruit was due to the soil was dry for a long time, which affects the low fruit water content. This prolonged dryness decreased the accumulation of moisture in the fruit [35]. The calcium deficiency in plants and particularly in the developing fruits was causing a decrease in fresh weight fruit grown under water stress condition [31].

TABLE II
TOMATO FRUIT QUALITY AFTER STORAGE

	Storage Day	Diameter (mm)	Fresh weight (g)	Sweetness (brix %)	Lycopene (mg/100g)
Period 1	Day 0	55.15	71.18	7.76	4.81
	Day 2	54.45	67.37	8.10	8.78
	Day 4	70.79	54.21	7.51	12.10
	Day 7	54.49	69.61	8.02	13.96
Period 2	Day 0	55.30	71.40	8.47	4.50
	Day 2	54.39	70.64	7.92	9.14
	Day 4	53.57	66.75	8.69	11.65
	Day 7	54.00	70.80	8.21	13.03

As the tomato ripened and developed red color, the chlorophyll content decreased, and the organoleptic properties of the fruit changed. Under thermal processing, the natural structural barrier, such as plant cell walls and plastid are destroyed, thereby releasing lycopene [37]. It also describes that the degradation of lycopene will run continuously as the product temperature increased [38].

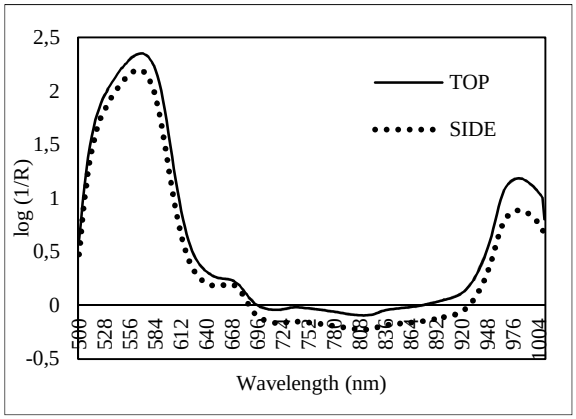
Carotenoids were largely accumulated in tomato during fruit ripening by the disappearance of chlorophylls and transformation of chloroplast into chromoplast during the lag phase that precedes maturation. This results in a change in tomato fruit during ripening and lycopene were synthesized rapidly over the entire ripening period [29].

B. Spectra Analysis

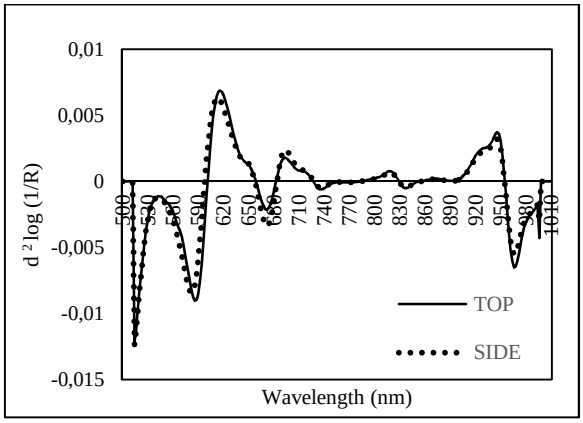
The wavelength used in the multiple regression analysis was based on reference [21]. The spectrum of the red tomatoes was compared with the spectrum of pink and green tomatoes. The result of pre-processing of the data, the difference in absorbance was seen greatly at 560 nm and 674 nm [21]. Therefore, the explanatory variable used in the multiple regression analysis was absorbance 560 nm and 674 nm. The measurement of spectra in this study was done using the top and side of tomatoes. The spectra of both top and side part of the tomato were presented in Fig. 8.

The spectral shape and trend of the fruit at both parts of the tomato were similar (Fig.8). The absorptions of the top part of the tomato were slightly higher than the side part were probably attributed to the pericarp that contained

lycopene. The pre-processing data for the top and side part of tomato used Savitzky Golay second derivative (Fig.5b). The spectra difference between them was the ratio of the maximum absorption, and it was closely related to the composition of carotenoid extracts. The peak and depression spectra for the second derivative spectral data showed the strong and weak absorbance characteristics of tomato, which might be due to the presence of lycopene content in the tomato.



(a)



(b)

Fig. 8 Original Spectra (a) and Second Derivative Spectra (b) for Top and Side Tomato

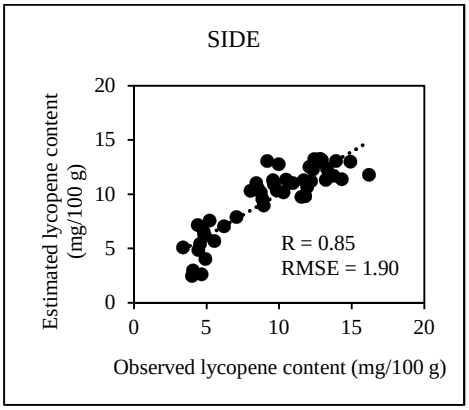
C. Statistical Analysis

The lycopene content in tomato fruits was estimated using MLR, PCR, and PLS. The estimation using the MLR used absorbance of 560 nm and 674 nm, which was obtained from preliminary experiments [21] as the explanatory variables. After pre-processing data, the data were divided into two parts, which was for calibration and for validation. Table 3 showed the equation obtained for the lycopene estimation using MLR. The estimated and observed lycopene content for the top and side part of tomato using MLR, PCR, and PLS were shown in Fig. 9 and were summarized in Table 4.

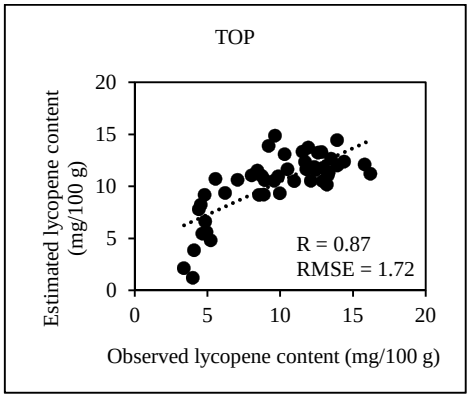
TABLE III
MULTIPLE LINEAR REGRESSION FOR LYCOPENE ESTIMATION

TOP	$Y = 1438.28\ x_1 + 380.3111\ x_2 + 14.0516$
SIDE	$Y = 1398.034\ x_1 + 27.9551\ x_2 + 13.9695$

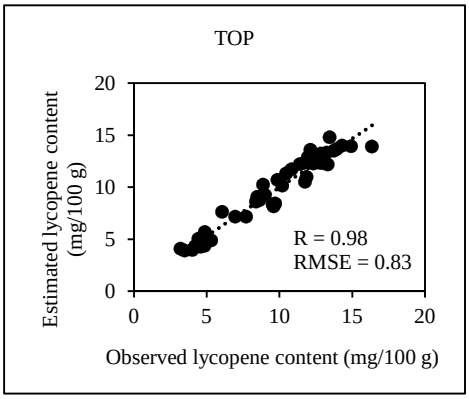
x_1: absorbance at 560 nm
x_2: absorbance at 674 nm



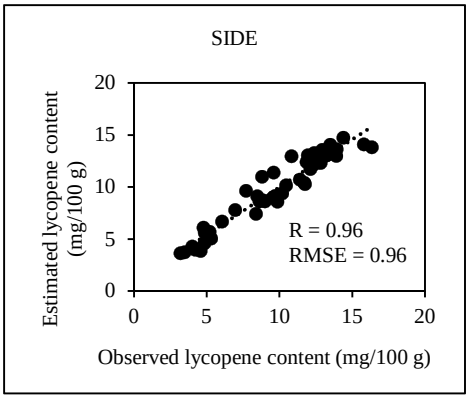
(a)



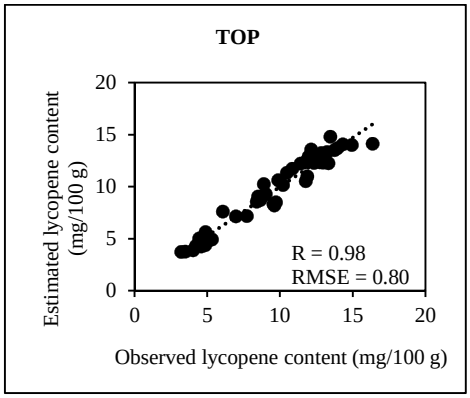
(b)



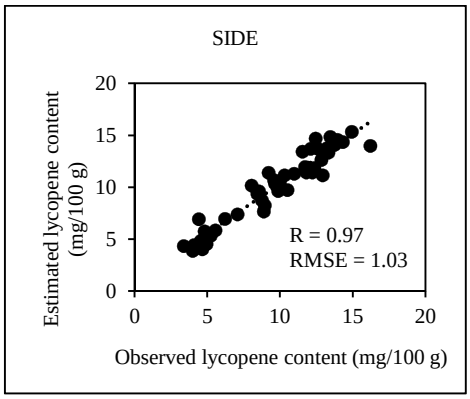
(c)



(d)



(e)



(f)

Fig. 9 Estimated and Observed Lycopene Content for Top and Side Part of Tomato Using MLR (a) and (b), PCR (c) and (d), PLS (e) and (f)

TABLE IV
SUMMARY OF STATISTICAL ANALYSIS

Tomato Part		MLR (Estimation)	PCR	PLS (Estimation)
TOP	R	0.85	0.98	**0.98**
	RMSE	1.90	0.83	**0.80**
SIDE	R	0.87	0.96	0.97
	RMSE	1.72	0.96	1.03

Using 48 samples data for the calibration, and 48 data for the validation, the lycopene content was estimated using Multiple Linear Regression. For the top part of the tomato, the R was 0.85 and the RMSE obtained was 1.90, while for the side part, the R was 0.87 and the RMSE obtained was 1.72. The lycopene content was also estimated using PCR and PLS. From PCR top part, the R was 0.98 and the RMSE obtained was 0.83, while for the side part, the R was 0.96 and the RMSE obtained was 0.96. From PLS top part, the R was 0.98 and the RMSE obtained was 0.80, while for the side part, the R was 0.97 and the RMSE obtained was 1.03.

According to Table 4, it was shown that PLS gave optimum results compared to other method since the RMSE value was the smallest. Generally, the larger the correlation coefficient, the smaller the root mean square error, and it indicates that the overall performance of the model is better [39]. It could be said that the lycopene content in high soluble solids of tomato fruits (grown under water stress treatment) after storage was best determined using PLS analysis with Visible/Near-infrared Spectroscopy non-destructively. Besides, the estimation of lycopene using the top part of the tomato was better compared to the side part. That was due to the distribution of lycopene in tomato fruit. Lycopene was found predominantly in the chromoplasts of plant tissues which is predominate in the outer part of the pericarp [29]. In pericarp, lycopene exists as small globules, suspended in the tomato pulp throughout the fruit. After tomatoes reach the mature stage, elongated crystals, or crystalloid of lycopene form in association with extended thylakoids and appear in the chromoplasts.

From the top part of the tomato, the light pass the outer pericarp and columella. It is located in the middle of the tomato. At the side of tomato, the light past many parts of tomato, such as the outer pericarp, locular cavity, seeds, and columella. It explained that the top part of tomato was more accurate to estimate the lycopene content in tomato fruit than the side part.

IV. CONCLUSION

From this study, it could be concluded that the measurement of lycopene content in high soluble solids stored tomato. It using the top part of the tomato was better compared to the side part, and it was best determined using PLS analysis with Visible/Near-infrared Spectroscopy non-destructively, with the R 0.98 and RMSE 0.80.

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