Rapid and non-destructive prediction of total soluble solids of guava fruits at various storage periods using handheld near-infrared instrument

Kusumiyati1*, Y Hadiwijaya2, I E Putri2, S Mubarok1 and J S Hamdani1
1Department of Agronomy, Agriculture Faculty, Universitas Padjadjaran, Indonesia
2Graduate Student in Agronomy, Agriculture Faculty, Universitas Padjadjaran, Indonesia

*E-mail: kusumiyati@unpad.ac.id

Abstract. Guava fruit is one of the most popular horticulture products because it has various health benefits. Once fruit is harvested, the fruit is still carrying out the respiration process during storage which results in changes in physical and chemical properties. One of chemical properties that is concerned by consumers is the total soluble solids, which explain the rough sugar content. The study examined the use of handheld near-infrared instruments to predict the total soluble solids of guava fruit at various storage periods rapidly and non-destructively. The research method used in this study was multivariate data analysis. Spectra pre-treatments were applied to correct the spectra and increase the accuracy of prediction. Calibration model was done by partial least squares regression (PLSR) and principal component regression (PCR). The results showed that the use of handheld near-infrared instrument was able to predict the total soluble solids of guava fruit with high accuracy. The best calibration model was produced by PLSR calibration method integrated with orthogonal signal correction (OSC) spectra preprocessing technique.

1. Introduction
Total dissolved solids (TSS) are an important parameter of fruit quality. TSS states the amounts of soluble solids in liquid. TSS value affects the taste of the fruit, because it can indicate the level of sweetness of the fruit. TSS is dominated by total sugar content and a small portion of soluble proteins, amino acids and other organic materials [1]. Determination of total soluble solids is generally carried out by destructive method, which requires laboratory testing, besides this method damages the fruit. The destructive method of TSS measurement is usually performed by refractometer. The measurement is done by dripping the liquid fruit extract on the detector. TSS value is expressed by %Brix, the value displayed is based on ratio of the speed of light in vacuum and speed of light through the sample. The more concentrated the solution shows the more TSS in the solution, it causes the slower speed of light penetrates the solution. However, fruit quality measurement by destructive method leads the fruit to be damaged and result in the fruit being unable to be marketed. Therefore, it is needed a method of measurement that is fast, and non-destructive. A method that can be used is near-infrared spectroscopy (NIRS).

NIRS has been introduced as an alternative method of fruit quality measurement. This method has different types based on its wavelength. Near-infrared radiation ranged between 700 nm-2500 nm. The application of NIRS has been widely used to measure fruit quality, including TSS of passion fruit [2], sapodilla [3], banana [4], and black mulberry [5]. In addition, this method was able to differentiate
fruit species [6]. NIRS is a method of detecting fruit quality by utilizing light interactions with matter. The light radiated from the spectrometer can be reflected, absorbed, transmitted or scattered by the fruit samples. The measurement of spectra data usually produces noise, pre-processing of spectra is needed before developing the calibration model.

Spectra data that contain noise and overlap can be corrected using spectra pre-processing technique. There are various techniques of spectra pre-processing, such as orthogonal signal correction (OSC) and second derivative savitzky-golay (d2a). OSC is a technique to reduce the variation of predictor variables that are not correlated with variations of response variables [6]. This technique takes an approach based on response variables, hence it is expected to improve the results of regression analysis. Saad et al. [7] have proven that the use of OSC in TSS measurement of tomato using Vis-NIRS produces the best coefficient of determination value of 0.99. d2a is a technique for overlapping the spectrum and clarifying the peaks and valleys of the spectrum. Guava is classified as non-climacteric fruit due to the low respiration rate in the harvested fruit. Low respiration rate results in changes in fruit quality that are not as fast as those of climacteric fruits. The research conducted by Dewi et al. [8] concluded that the storage of guava at 0, 4, and 8 days had an effect on vitamin c levels. NIRS analysis requires chemometrics to extract the information from the spectra data. Chemometrics includes spectra pre-processing, calibration, and validation. Calibration methods that have been widely used are partial least squares regression (PLSR) and principal component regression (PCR). PLS maximizes the covariance between dependent and independent variables, with the result that latent variables are correlated with the dependent variables. PCR is a series of analyzes by reducing data based on principal component analysis (PCA) and then performing a regression using the new components and reference data. Validation is needed to check the accuracy of calibration model. The study aimed to non-destructively predict the TSS of guava fruits at various storage periods using a handheld near-infrared instrument, and also to compare various spectra pre-processing techniques and calibration methods to obtain the best calibration model.

2. Materials and Methods

2.1. Samples preparation
The samples used in this study were guava fruits cv. Crystal harvested from the plantation in Tomo, Sumedang, West Java. Fruits were harvested at the same maturity level. The numbers of samples used were 120 samples. Samples were numbered, then the whole samples were divided into 3 groups. 40 samples were stored for 0 day, 40 samples were stored for 4 days, and the others 40 samples were stored for 8 days. The purpose of the storage was to obtain samples that have different degrees of maturity. Storage was carried out by using fruit baskets at room temperature around 27ºC. Afterwards, 2/3 of the total samples were used for the calibration stage, while for the validation stage were 1/3 of the total samples.

2.2. Spectra data acquisition
NIRS spectra data were measured at six different points, divided into 3 points on the front side and 3 points on the back side of the fruit. Handheld near-infrared instrument with wavelengths of 702 nm-1065 nm was used to obtain NIRS spectra. Spectra acquisition was run at room temperature (27ºC). The principle of spectra measurement is to emit photons from halogen lamps to the fruit samples, reflected light detected by sensors, then digitized to acquire spectra data. Spectra measurement of intact fruit is based on reflectance spectra. The reflectance spectra were then transformed into absorbance spectra data (log 1/R).

2.3. Destructive measurement
Refractometer was used to measured total soluble solids of guava fruits. The guava samples were shredded, then the extracted liquids were measured using refractometer, TSS value is described in %Brix. TSS is the rough sugar content. Destructive measurement was made to obtain reference data for regression analysis.
2.4. NIRS spectra pre-processing
NIRS spectra data obtained from spectra measurements often contain other information besides information from samples such as background information and noise, thus causing calibration models to be less accurate. In order to build an accurate calibration model, it is necessary to perform spectra pre-processing to reduce noise and improve the calibration model. The pre-processing of spectra used in this study were the d2a and OSC techniques. Spectra pre-processing of d2a clarifies the peaks and valleys of NIRS spectra, while the OSC method corrects the NIRS spectra based on reference data (the results of destructive analysis).

3. Results and Discussion
3.1. Effects of NIRS spectra pre-processing
When light is illuminated to the fruit, some of the light is absorbed. The amount of light absorbed varies on various types of fruit. This is due to the different physical and chemical composition of each type of fruit. Pre-processing of spectra data used to develop the calibration model depends on the type of material and the chemical content of the material to be predicted. The pre-processing techniques of NIRS data used in this study were d2a and OSC.

Original absorbance spectra obtained at the spectra acquisition stage were transformed into d2a and OSC spectra. The purpose of the d2a spectra pre-treatment is to predict the chemical properties in the fruit which have a small percentage. The spectra pre-treatment of d2a can eliminate overlapping spectra and decompose hidden spectra. [9] informed that the use of d2a pre-treated spectra was able to predict sapodilla water content well. The OSC technique reduces variance in the original spectra that does not correlate with the results of destructive analysis. The use of OSC pre-processing techniques is effective to increase the value of $R^2$ in predicting the SSC of mangoes. Figure 1 shows original absorbance spectra, d2a, and OSC.

3.2. TSS of guava at various storage periods
Table 1 presented the mean of guava fruits TSS at various storage periods. The mean value of TSS was increased during storage. The is caused by the process of breaking down carbohydrates into more liquid compounds such as glucose, sucrose, and fructose. By storing the samples at various periods, the TSS value of the guava would be more varied. Accordingly, the calibration model to be developed would have wider range of TSS prediction.

| Days of Storage | Average | Standard Deviation |
|-----------------|---------|--------------------|
| 0               | 7.82    | 0.59               |
| 4               | 8.59    | 0.82               |
| 8               | 8.80    | 1.13               |

3.3. Calibration and validation model
Development of TSS calibration model was carried out using the PLSR and PCR methods combined with spectra pre-processing. Based on the results of the study, the use of various calibration methods and spectra pre-processing techniques gave different effects on the accuracy of the calibration model. The development of calibration and validation model of guava fruit TSS using PLSR and PCR methods integrated with spectra pre-processing techniques are described in Table 2.
Figure 1. Original absorbance spectra (a), d2a pre-treated spectra (b), OSC pre-treated spectra (c).

Table 2. Calibration and validation model of prediction of guava fruit TSS using PLSR and PCR methods combined with various spectra pre-processing techniques.

| Calibration Method | Spectra pre-processing | Factor | Calibration $R^2$ | RMSE | Validation $R^2$ | RMSEP | RPD | Consistency (%) |
|--------------------|------------------------|--------|------------------|------|------------------|-------|-----|-----------------|
|                   | Original               | 10     | 0.85             | 0.38 | 0.73             | 0.47  | 1.87| 80              |
|                   | d2a                    | 8      | 0.86             | 0.37 | 0.74             | 0.46  | 1.91| 80              |
|                   | OSC                    | 2      | **0.85**         | **0.39** | **0.72**         | **0.48** | **1.83** | 81             |
|                   | Original               | 9      | 0.72             | 0.52 | 0.60             | 0.62  | 1.41| 83              |
|                   | d2a                    | 14     | 0.68             | 0.56 | 0.49             | 0.66  | 1.33| 85              |
|                   | OSC                    | 3      | **0.79**         | **0.45** | **0.70**         | **0.50** | **1.75** | 90             |

The value of consistency in the entire models showed values above 80%, which mean the overall models were sufficient. Spectra pre-processing with OSC technique displayed the highest consistency values in both calibration methods. These were indicated by the consistency value of 81% in the PLSR calibration method and the value of 90% in the PCR calibration method. The excellent calibration model provides consistency value ranged from 80-110%. Spectra pre-processing with the OSC technique indicated the lowest factor compared to the d2a technique and original absorbance spectra in both calibration methods. This is in line with the findings stated by Zulfahrizal [10] that the use OSC spectra correction gave lower factor than original spectra. Factor value obtained by OSC and PLSR calibration techniques was 2, while PCR calibration obtained factor value of 3. Low factor value indicates low disturbance in the analyzed spectra. Pre-processing technique with the d2a and PLSR calibration obtained factor value that was smaller than the original absorbance spectra. On the contrary, in PCR calibration, the value of the factor obtained was greater than the original absorbance spectra. It revealed that the pre-processing of d2a technique is not suitable to be integrated with PCR calibration because it causes the factor value greater than the original spectra and low accuracy of the
model. This is affirmed by low values of $R^2$ calibration and validation values of 0.68 and 0.49, respectively. RMSEC (root mean squares error of calibration) and RMSEP (root mean squares error of prediction) obtained were also higher than other spectra, which were 0.52 and 0.62, respectively. In addition, the value of RPD was recorded as the lowest, which amounted to 1.33. RPD (ratio of performance to deviation) is calculated to assess the goodness of fit of the model. RPD value above 1.5 denotes that the model can distinguish variations in the data [11]. In the PLSR calibration, all three types of spectra obtained RPD value above 1.5, but in the PCR calibration only the OSC spectra acquired RPD value above 1.5. Moreover, the RMSEC and RMSEP in the PLSR calibration achieved lower value than the PCR calibration, whereas the PLSR calibration displayed higher $R^2$ than the PCR method in all analyzed spectra. It concluded that the PLSR calibration was better than PCR. The best spectra pre-processing technique was shown by OSC spectra, besides showing high calibration and validation accuracy, the lowest factor compared to other techniques, and also the most important was the highest consistency values in both calibration methods. The best model for predicting TSS of guava was represented by the PLSR calibration method combined with OSC pre-treated spectra with calibration, validation, RMSEC, RMSEP, RPD, and consistency values of 0.85, 0.72, 0.39, 0.48, 1.83, and 81%, respectively.

| Value   | Calibration | Validation |
|---------|-------------|------------|
|         | Measured    | NIRS Prediction | Measured | NIRS Prediction |
| Minimum | 5.53        | 6.28       | 6.35     | 6.57           |
| Maximum | 11.16       | 11.09      | 10.53    | 10.23          |
| Mean    | 8.36        | 8.36       | 8.37     | 8.48           |

Table 3 presented the comparison between NIRS prediction and measured TSS using refractometer. Calibration set of measured TSS ranged from 5.53-11.16, while the measured of validation set ranged from 6.35-10.35. It is necessary to provide high range of calibration set since the model is expected to predict unknown (independent) samples. The results showed that NIRS prediction closed to the measured value. It proved that NIRS method displayed the high accuracy and was reliable to predict TSS content of guava fruits.

4. Conclusion
TSS of guava fruits at various storage periods can be predicted by using a handheld near-infrared instrument. Various spectra pre-processing techniques and calibration methods affected the model accuracy. The best calibration method was produced by the PLSR method, while the best spectra pre-processing technique was performed by the OSC technique. The best calibration model was obtained by PLSR calibration method combined with OSC spectra pre-processing technique.

References
[1] Bexiga F, Rodrigues D, Guerra R, Brázio A, Bulegas T, Cavaco A M, Antunes M D and de Oliveira J V 2017 Postharvest Biol. Technol. 132 23-30.
[2] Maniwara P, Nakano K, Boonyakiat D, Ohashi S, Hiroi M and Tohyama T 2014 J. Food Eng. 143 33-43.
[3] Kusumi K, Mubarok S, Hamdani J S, Farida F, Sutari W, Hadiwijaya Y, Putri I E, Mutiarawati T and Hadiwijaya Y 2018 J. Food Agric. Environ. 16 49-53.
[4] Zude M 2003 Fruits 58 135-42.
[5] Soltanikazemi M, Abdanan Mehdizadeh S and Heydari M 2017 Int. J. Food Prop. 20 2437-47.
[6] Kusumiyati K, Hadiwijaya Y and Putri I E 2019 J. Biodjati 4 89-95.
[7] Saad A G, Jaiswal P and Jha S N 2014 Int. J. 2 632-9.
[8] Dewi Y U, Sumantri S and Utami P I 2007 Pharmacy: J. Farm. Indonesia 5 100-5.
[9] Kusumiyati K, Hadiwijaya Y and Putri I E 2018 IOP Conf. Series: Earth Environ. Sci. 207 p 012047.
[10] Zulfahrizal Z 2014 The development of non-destructive measurement method to determine the quality and fermentation of intact cacao beans using nir spectroscopy Master Thesis (Bogor, Indonesia: Institut Pertanian Bogor).
[11] Saeys W, Mouazen A M and Ramon H 2005 Biosys. Eng. 91 393-402.