The Measurement of Soluble Solids Content in Snake Fruit (*Salacca Edulis Reinw*) cv. Pondoh Using A Portable Spectrometer

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Abstract: In this research we reported the use of near infrared spectroscopy for nondestructive soluble solids content (SSC) measurement in intact snake fruit (*Salacca edulis Reinw*) cv. Pondoh. The spectra of 100 samples were acquired in the range of 300-1040 nm using an available, low cost portable spectrometer (VISNIR USB4000). The spectrometer used an array of linear CCD as a detector. The LS-1 Tungsten Halogen lamp is used as light source. This system is also equipped with a fiber optic. Snake fruits were placed on the sample holder and the spectra were measured using 100 ms of integration time and 50 scans for averaging. The spectra were acquired in two different positions in the middle of fruit. The total scanning time was 10 s for each fruit. The SSC of snake fruits were measured destructively following the spectra measurement using a digital refractometer. A portion of snake fruit flesh with associated with the point of spectra measurement was cut and juiced. Then the juice of snake fruit was placed on a digital refractometer. The SSC was quantified in Brix value. The trial free version of The Unscrambler V.9.1 was used as chemometrics tools to extract the useful information from the spectra. The calibration model was developed using Partial Least Squares Regression 1 (PLSR1) for three types of spectra, original, smoothing and second derivative spectra. The calibration model was evaluated using some parameters such as coefficient of determination (R^2), standard error of prediction (SEP), bias between actual and predicted SSC value and ratio prediction to deviation (RPD) parameter. The possibility of using near infrared spectroscopy to measure SSC of intact snake fruit nondestructively was successfully demonstrated. The best calibration model with 3.32 of RPD value could be obtained.

Keywords: snake fruit, near infrared spectroscopy, intact fruit, soluble solids content, nondestructive method, ratio prediction to deviation

1. INTRODUCTION

There is an increasing in the consumption of tropical fruits as ingredients of diets in the world such as Europe and North America (Gorinstein et al. 2009). Indonesia, which has many exotic tropical fruits such as mangosteen, pineapple and snake fruit, can be main exporter of tropical fruit to provide tropical fruits which high quality. For this purpose, a non-destructive quality evaluation of the tropical fruits to meet the export market criteria is important to be considered.

The use of near infrared spectroscopy as non-destructive quality evaluation seems to be a good choice since that this technique has become popular and ready to use. Many researchers have reported the successful application this technique in measuring internal quality of several fruits. It has been demonstrated that near infrared spectroscopy, especially in the short wavelength are highly correlated to the SSC of apples, mangos, oranges, pears, tomatoes, kiwi fruits, and etc. (Subedi et al. 2007; McGlone et al. 2002). A complete review of using near infrared spectroscopy technique for internal quality inspection in fruits is well described by Nicolai et al. (2007). In the review, it is clearly that many researchers use this technique with various kind of near infrared wavelength range and sample presentation. Some of them use visible and short wavelength 700-1100 nm (Ventura et al. 1998; McGlone et al. 2002; Temma et al. 2002; Saranwong et al. 2003; Walsh et al. 2004) and others use short and long wavelength 700-2500 nm (Guthrie and Walsh 1997; Ying et al. 2005). Reflectance and interactance (also similar to absorbance) are widely used for sample presentation. The near infrared instrumentation was developed rapidly and now there is an increasing of using a low cost and portable NIR instrumentation for measuring internal quality of fruits and vegetables, especially for soluble solids content (SSC) determination (Temma et al. 2002; Saranwong et al. 2003). As mentioned by Gorinstein et al. (2009) snake fruit has similarity in characteristic with kiwi fruit such as polyphenols content and antioxidant value. Both fruits are recommended for good diet ingredients. For kiwi fruit, the

use of near infrared spectroscopy has already conducted with promising result (r = 0.99 and SEC = 0.72%Brix for SSC determination) (Slaughter and Crisosto, 1998). Recent work also confirmed the successful result of using FT-NIR diffuse reflectance spectroscopy in measuring the firmness of kiwi fruit (Fu et al. 2007). However, the use of a portable near infrared spectroscopy instrumentation for non-destructive measurement of SSC in intact snake fruit has not yet been reported

For snake fruit especially `pondoh` cultivars, the quality of product mainly determined by its sweetness. However, it is very common that the degree of sweetness vary due to different place of cultivation. To export snake fruit, it is important to sort the product based on the homogeneity in degree of sweetness. In this research, a potential use of a low cost and portable NIR spectrometer to measure SSC of snake fruit will be proposed. Then the calibration model for predicting the SSC of snake fruit will be developed and validated.

2. MATERIALS AND METHODS

2.1. Materials

A number of 100 snake fruits (*Salacca Edulis Reinw*) cv. Pondoh were used. This snake fruit is originated from Indonesia. The fruits were harvested from the same orchard at Lampung province, Indonesia. To obtain a wide range of SSC measurement, two groups of sample were selected. The first group was samples with high SSC; most of them are big in size and dark in skin appearance. The second one was samples with relatively low SSC; most of them are small in size and clear in skin appearance. The expert farmer did this selection. The spectral acquisitions were done in the same day of harvest time.

2.2. Spectra measurement method

The spectra of snake fruits were acquired in absorbance mode using a low cost and portable spectrometer (VIS-NIR USB4000, The Ocean Optics, USA). This spectrometer has wavelength range 300-1040 nm. The spectrometer used an array of linear silicon CCD as a detector. The LS-1 Tungsten Halogen lamp is used as light source. This system is also equipped with a fibre optic (2 m in length and 400 μm of its probe diameter). Snake fruits were placed on the sample holder and the spectra were measured using 100 ms of integration time and 50 scans for averaging. A diffuse reflectance standard reference (model WS-1, The Ocean Optics, USA) measurement was made every time prior to a sample spectra acquisition. The spectra were acquired in two different positions in the middle of fruit as shown in the Fig.1. Therefore, the total scanning time was 10 s for each fruit. As the temperature has a considerable effect on the near infrared spectrum of chemical component, it needs to avoid the fluctuating of temperature of sample (Maeda et al. 1995). For this purpose, the sample temperature was maintained at 25°C using a water bath prior to the spectra measurement (Peirs et al. 2003).

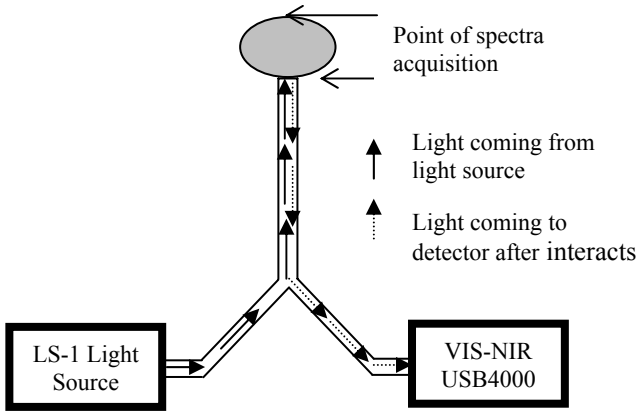

Fig. 1. The spectra acquisition of intact snake fruit in absorbance mode in two different positions.

2.3. The reference method

The SSC of snake fruits were immediately measured destructively following the spectra measurement using a digital refractometer (model PR-201α (Brix 0.0-60.0%), ATAGO, Co., Tokyo, Japan). This refractometer has measuring accuracy ±0.1% and measurement temperature 10-40°C (automatic temperature compensation). The temperature of the samples for SSC measurement was not measured. However, since that the SSC measurement was conducted at the room temperature, the influence of the temperature to the SSC measurement using this refractometer could be negligible. A portion of snake fruit flesh with associated with the point of spectra measurement was cut and juiced. Then the juice of snake fruit was placed on a digital refractometer. The SSC was quantified in Brix value. Then the average of two SSC measurements was used as a reference value. In order to predict the reference parameter (SSC), calibration and validation sample sets were performed. Table 1 showed the descriptive statistics of calibration and validation samples used for developing calibration model and performing validation test.

Table 1. Descriptive statistic of samples used for developing calibration and validation in intact snake fruit.

Item	Calibration sample set	Validation sample set
Number of samples	50	50
Minimum value	12.10	12.00
Maximum value	20.15	20.10
Mean	16.13	16.27
Standard deviation	2.95	3.06
Unit	%Brix	%Brix

2.4. Spectra analysis

In this research, a short near infrared wavelength range of 700-1040 nm will be used for further analysis. Relative absorbance spectra were calculated by using (1) (Suhandy 2009):

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right) \quad (1)$$

Where:

S_λ = Intensity of sample at wavelength λ

D_λ = Intensity of dark at wavelength λ

R_λ = Intensity of reference at wavelength λ

The "relative absorbance spectra" are called hereafter "spectra". In this research, original spectra refer to the spectra that having without any treatment. Smoothing spectra are spectra, which are calculated by moving average algorithm with 5 segment of averaging. Second derivative spectra are calculated from smoothing spectra using Savitky-Golay algorithm (number of gap right and left: 10 nm, total gap is 20 nm). The averaging technique is used to reduce the number of wavelengths or to smooth the spectrum of snake fruit. The derivation of spectra is used to remove the shift baseline and superposed peak. Calculation of derivation spectra based on Savitky-Golay in order of 2 is very popular and improve the calibration model results. The trial free version of The Unscrambler V.9.1 (CAMO AS, Trondheim, Norway) was used as chemometrics tools to calculate these spectra treatment and to extract the useful information from the spectra by developing the calibration model and performing validation test. The calibration model was developed using Partial Least Squares Regression 1 (PLSR1) for three types of spectra, original, smoothing and second derivative spectra. Performance of the calibration model was evaluated using following statistical parameters such as coefficient of determination between predicted and measured SSC (R^2), standard error of prediction (SEP), bias between actual and predicted SSC value and ratio prediction to deviation (RPD) parameter. The use of root mean square error of prediction (RMSEP) was also important parameter to be considered in evaluating quality of calibration model. Many researchers also used these evaluation parameters to evaluate the performance of calibration models for SSC determination using near infrared spectroscopy (McGlone and Kawano 1998; Flores et al. 2009). A good calibration model should have high of R^2, low both SEC and SEP but also a small difference between SEC and SEP. The value of RMSEP should be as lower as possible to get high prediction accuracy. The calibration model should have as greater as possible of RPD value. An RPD between 1.5 and 2 means that the calibration model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above correspond to good and excellent prediction accuracy, respectively. The external validation method known as test set was conducted for each calibration model based on a sample set which has not been used in developing calibration models. This sample set was known as validation sample set that is described in Table 1. The results of this process were bias and standard error of prediction (SEP).

The following control limits are assumed to evaluate the accuracy of the best developed calibration model (Shenk and Westerhaus 1996):

Limit control SEP = 1.30 × SEC (standard error of calibration)
Limit control bias = ±0.60 × SEC (standard error of calibration)

3. RESULT AND DISCUSSIONS

3.1. Spectra of snake fruit

Fig.2 demonstrated the original spectra of snake fruit having low, middle and high SSC value. Though there is a slight different spectrum among them, we cannot notice a peak in the spectra due to different in SSC value. Fig. 3 was the second derivative spectra of Fig. 2. From these spectra, we can easily identify the peak of the spectra due to different value of SSC. The peak was mostly identified at water band absorption near 970 nm and 990 nm. It is similar to many other researcher findings (Osborne et al. 1993; Lammertyn et al. 2000; Williams 2001). The domination of water band absorption is addressed to fact that water is the most important chemical constituent of ripen snake fruit.

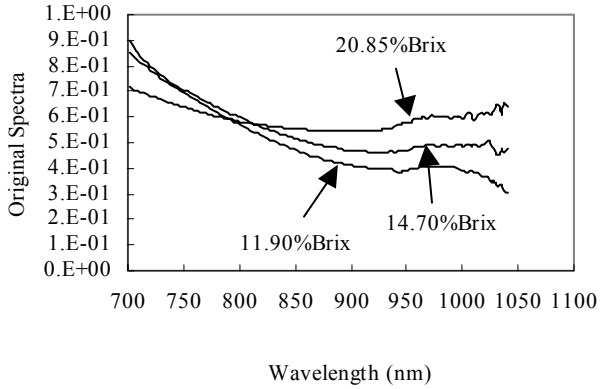

Fig. 2. Original spectra of snake fruit with low, middle and high SSC value.

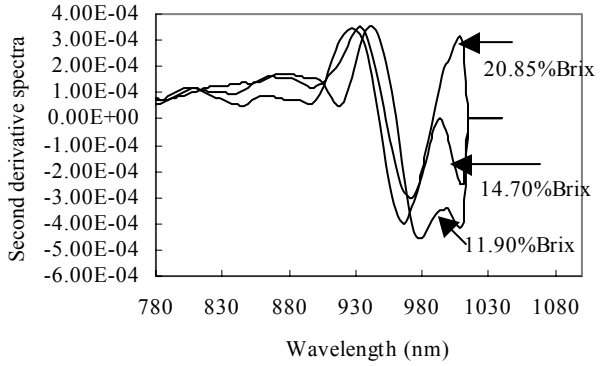

Fig. 3. Second derivative spectra of snake fruit with low, middle and high SSC value.

3.2. Developing a calibration model

For all types of spectra, the calibration and validation results were very promising. Table 2 showed the calibration results for SSC determination in intact snake fruit. The calibration has range of coefficient determination (R^2) =0.85 ~ 0.94 in

original spectra. The range of R^2 were 0.81 ~ 0.94 and 0.86 ~ 0.90 for smoothing and second derivative spectra, respectively. The entire calibration model also had low standard error of calibration (SEC). The SEC varied from 0.67 to 1.17 for original spectra, 0.75 ~ 1.29 for smoothing spectra, and 0.88 ~ 1.11 for second derivative spectra.

From the table it is also clear that using whole spectrum of short near infrared spectroscopy (700-1040 nm) of second derivative spectra gave the best calibration model. It has lowest of SEP = 0.92% Brix and highest of RPD = 3.32. It has also relatively small of difference between SEC and SEP (0.04). For bias = 0.08 and SEP = 0.92%Brix, the best calibration model had 0.924 of RMSEP value. The RMSEP is calculated by using (2) (Golic and Walsh 2006):

$$RMSEP^2 = SEP^2 + bias^2 \quad (2)$$

This result was comparable with that recorded by other authors using short wavelength (300-1100 nm). Reported values include: RMSEP = 0.615, R^2= 0.861 noted by Liu and Ying (2005) using 812-1100 nm of FT NIR spectroscopy; SEP = 0.73, bias = 0.17, RPD = 2.35 and R^2 = 0.83 noted by Khuriyati and Matsuoka (2004) using 305-1150 nm of NIR transmittance method. It is also comparable with work reported by Shao and He (2007) of measuring SSC in bayberry (R^2 = 0.90 SEP = 0.40, RMSEP = 0.42 and bias = -0.14); RMSEP = 0.51 and R^2 = 0.91 reported by Cayuela (2008) of predicting SSC in intact oranges; R^2 = 0.90 and RMSEP = 0.39%Brix demonstrated by McGlone and Kawano (1998) for SSC assessment in kiwifruit using 800-1000 nm. However, our work has slight lower performance than that reported by McGlone et al. (2002) using NIR methods for SSC measurement in kiwifruit (R^2 = 0.94, RMSEP = 0.32).

The best calibration model using wavelength range of 700-1040 nm of second derivative spectra seems to be robust model since only three factors (F) or latent variables were used in the calibration model. Hu et al. (2005) reported similar result in detecting internal quality of tomato fruit using Vis/NIR spectroscopy technique. The calibration model was developed using three factors.

Table 2.Calibration and validation results for determination of SSC in intact snake fruit using original, smoothing and second derivative spectra.

λ (nm)	F	R^2	SEC	SEP	Bias	RPD
Original						
700-900	5	0.94	0.76	1.18	-0.09	2.59
700-920	5	0.94	0.74	1.11	-0.01	2.76
700-940	6	0.94	0.75	1.02	0.14	3.01
700-960	6	0.92	0.82	1.04	-0.20	2.95
700-980	6	0.92	0.80	1.02	-0.18	3.00
700-1040	5	0.92	0.81	0.98	-0.08	3.13
Smoothing						
700-900	5	0.90	0.95	1.11	-0.13	2.76
700-920	5	0.90	0.96	1.06	-0.10	2.88
700-940	6	0.90	0.88	1.03	0.00	2.96
700-960	6	0.90	0.88	1.05	-0.18	2.91
700-980	7	0.94	0.75	1.02	0.03	3.00
700-1040	5	0.90	0.92	0.96	-0.12	3.19
Second Derivative						
700-900	2	0.86	1.11	0.99	-0.01	3.09
700-920	2	0.88	0.99	0.98	0.12	3.11
700-940	2	0.88	0.97	0.95	0.14	3.21
700-960	3	0.90	0.90	0.93	0.08	3.28
700-980	3	0.90	0.90	0.93	0.08	3.28
700-1040	**3**	**0.90**	**0.88**	**0.92**	**0.08**	**3.32**

3.3. Regression coefficient versus wavelength

The importance wavelength can be clearly identified by plotting the regression coefficient against the wavelength. Fig.4 presented the plot of regression coefficient and wavelength. There were two major positive peaks that associated to water absorption band at 768 nm and 990 nm (Williams 2001). The major negative peaks can be observed at 879 nm, which is associated with carbohydrate absorption band, and 950 nm, which is associated with sugar absorption band (Williams 2001). Recent work also confirmed the importance of wavelength 990 nm for predicting the SSC in fruits (Shao and He 2007). Shao and He (2007) also invented the importance of sugar absorption band at 950 nm in determining the SSC of bayberry fruit. The wavelength of 950 nm was correspondence with the absorption of 3rd overtone stretch of CH and 2nd and 3rd overtone of OH (Rodriguez-Saona et al. 2001; Slobodan and Yukihiro 2001).

The importance of wavelength 879 nm in our research was close to other authors. Saranwong et al. (2003) described the importance of wavelength 878 nm for SSC determination in intact mango. Dull et al. (1992) also used optimum wavelengths at 884 nm and 913 nm for SSC determination in melons. Khuriyati and Matsuoka (2004) confirmed the importance of wavelength at 884 nm in developing calibration model for SSC determination in intact tomato.

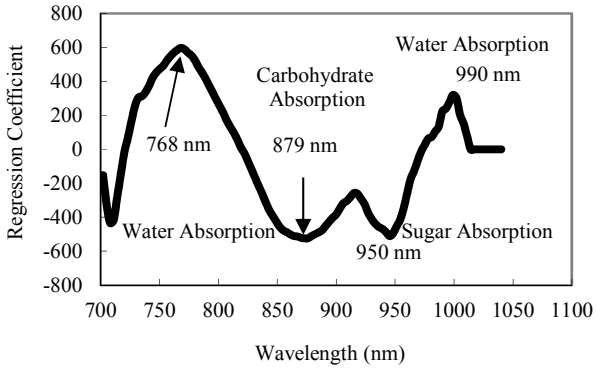

Fig. 4. The regression coefficient versus wavelength for calibration model of 700-1040 nm of second derivative spectra.

3.4. Validation of the calibration model

In this research, we applied test set method of validation. In this validation method, all calibration models were validated using different samples used for developing calibration model. The validation results showed that for all developed calibration

models the bias was low. The SEP resulted from this study was relatively high compared to other authors (Khuriyati and Matsuoka 2004; Shao and He 2007). The SEP varied from 0.92 to 1.18. However, the all prediction also resulted in high value of ratio prediction to deviation (RPD). The best calibration model with SEP = 0.92 gave the value of RPD = 3.32.

The use of RPD value was more reliable than SEP value in measuring the performance of validation. It is clear that the SEP value is relative and its meaning dependant on the value of its standard deviation (SD). In this study, the SD is 3.06 (Table 1). It is greater than SD = 1.72 in Khuriyati and Matsuoka (2004) which has SEP = 0.73 and RPD = 2.35. Our RPD was also better than that reported by McGlone and Kawano (1998) (RPD = 3.1) and could be excellent calibration mode since that the RPD was higher than 3.

The best calibration model with SEC = 0.88 gave the value of limit control as follow:

Limit control of SEP = 1.14
Limit control of bias = ±0.53

Since that the obtained value of SEP and bias (SEP = 0.92, bias = 0.08) are lower than that limit control, showing that the best calibration model ensure accurate predictions.

Scatter plot between measured and predicted values of SSC is shown in Fig. 5. By a 95% confidence pair *t-test*, there were no significant differences between the SSC of snake fruit measured using refractometry method and that predicted by near infrared spectroscopy method.

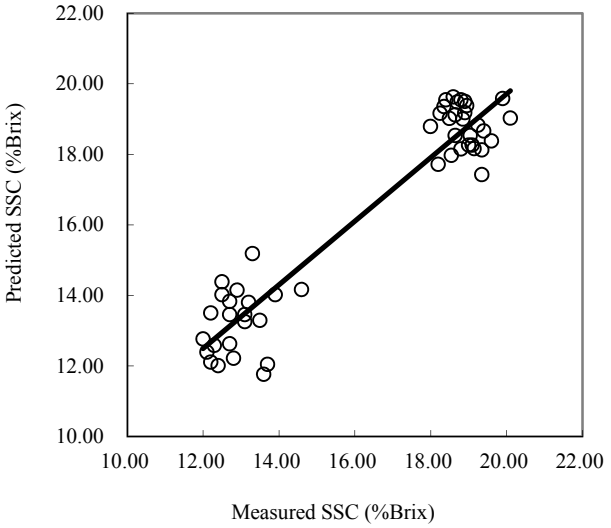

Fig. 5. Scatter plot between actual and predicted SSC value in wavelength range of 700-1040 nm of second derivative spectra.

4. CONCLUSIONS

In this research, the potentiality of using a portable near infrared spectrometer for non-destructive SSC measurement in intact snake fruit was successfully demonstrated instead of the traditional method of refractometry. The SSC of snake fruits can be predicted well using the best calibration model resulted from this study with SEP of 0.92% Brix. This study will open the possibility to perform an automatic sorting system for snake fruits based on the SSC value. In this study, the total scanning time was 10 s for each fruit. To obtain a fast measurement, for example in the real grading system, however 10 s of scanning time was relatively too long. We can reduce this scanning time by acquiring the spectra in one position in the middle for each fruit. By this measurement, the total scanning time will be 5 s for each fruit.

5. ACKNOWLEDGEMENTS

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