Effects of preprocessing techniques in developing a calibration model for soluble solid and acidity in 'Gedong Gincu' mango using NIR spectroscopy

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Abstract

Near infrared spectroscopic (NIRS) techniques have shown promise as rapid and non-destructive tools to evaluate the internal quality attributes of fruits. The objective of this work were to develop a calibration model for prediction soluble solids content (SSC) and acidity in 'Gedong Gincu' mango using NIRS and to analyze the effects of different preprocessing techniques on the acuracy of the calibration model. The prediction models were developed by partial least square (PLS) regression. NIR reflectance spectra were measured at wavelength of 1000-2500 nm using NIRFlex N-500 fiber optic solid. The results show that for SSC, the best preprocessing method was smoothng 3 points (sa3) with PLS factor=15, r=0.82, SEC=0.5ºBrix, SEP=1.28ºBrix, CVc 5.8% and RPD=1.52. For acidity, the best preprocessing method was first derivative Savitzky-Golay-9 ponts (dg1) with PLS factor=3, r=0.74, SEC=0.01%, SEP=0.12%, CVc=38.1% and RPD=1.33. This findings showed the important role of preprocessing method in developing accurate models for the prediction of mango internal quality characteristics.

Keyword-NIR spectroscopy, preprocessing, soluble solid content, acidity, 'Gedong Gincu' mango

I. INTRODUCTION

Recently, the quality evaluation of fruits is an important issue. Fruits classification into their tastes are commonly judged by aroma and experiences. It is becoming increasingly difficult due to consummer demands on a prime quality guarantee. Recent trend in agribusiness is declining reliance on subjective assessment of quality and increasing adoption of objective, quantitative and non-destructive techniques of quality assessment. Non-destructive instrument-based methods are preferred to destructive techniques because they allow the measurement and analysis of individual fruit, reduce waste and permit repeated measures on the same item over time. Near infra red spectroscopy (NIRS) is a fast, easy-to-use and non-destructive analytical technique (Day and Fearn, 1982). One of the main advantages of NIRS is that allow several constituents to be evaluated at the same time. NIRS has been used to assess the internal quality of fruits such as apple (Fan et al., 2009; He et al., 2006; Lu et al., 2000), pear (Fu et al., 2007; Nicolaı̈ et al., 2008; Sirisomboon et al., 2007), kiwifruit (Moghimi et al., 2010), peach (Golic and Walsh, 2006), avocado (Clark et al., 2003), mango (Saranwong et al., 2004; Purwanto et al., 2013a; Purwanto et al; 2013b) and papaya (Purwanto et al., 2015).

NIRS instrumentation has developed in response to the need for speed in analyses and flexibility in adapting to different sample states. However, modern NIRS provides a large amount of spectral data that contains noise and is influenced by a number of physical, chemical, and structural variables. In addition, it is difficult to distinguish subtle spectral differences between samples (Blanco and Villarroya, 2002). Because of these reasons, NIRS requires chemometrics to extract information from noisy data. There is no substitute for optimal data collection, but, after proper data collection, preprocessing of spectral data is the most important step.

Preprocessing of NIRS spectral data has become an integral part of chemometrics modeling. The objective of the preprocessing is to remove physical phenomena in the spectra in order to improve the subsequent multivariate regression, classification model or exploratory analysis (Rinnan et al., 2009). The standard methodology when comparing various preprocessing techniques and selecting different wavelengths is to compare prediction statistics computed with an independent set of data not used to make the actual calibration model (Skibsted et al., 2004). Researchers have studied different integral parts of chemometrics such as different preprocessing techniques, variable reduction methods, multivariate calibration methods, and different

NIRS modes in order to construct accurate and reliable models (Moghimi et al., 2010). The objective of this work were to develop a calibration model for prediction soluble solids content (SSC) and acidity in 'Gedong Gincu' mango by using NIRS and to examine the effects of different preprocessing techniques on the acuracy of the calibration model.

II. METHODOLOGY

A. Sample preparation

'Gedong Gincu' mango fruits were harvested from farmer's orchard and were hand harvested at different picking date. After being harvested at the same day, samples of mango fruits were then delivered to the Laboratory within 8 hours at ambient temperature. The fruits was cleaned, sorted and randomly separated into two groups of 66 fruits. Each group of fruits were then stored at cold storage with temperature set of 8 and 13 ^{o}C (65-75% RH). After acquisition of spectra, SSC measurement was made with a digital refractometer (*Atago* PR-201) and titratable acidity was measured by the titrimetric method (AOAC, 2000). All experiments, including spectra acquisition and quality analysis were carried out every 2 days during period storage of 22 days.

B. Spectra acquisition

Spectra of mango fruits were collected from sample of fruits in the range of 1000–2500 nm with an increment of 5 nm using NIRFlex N-500 (Büchi Labortechnik AG, Flawil, Switzerland) at room temperature of 27ºC. Spectra data were collected by measuring the diffuse reflectance of the samples in triplicate. Data collection of NIR spectra were conducted by using NIRWare 1.2 software (Büchi Labortechnik AG, Flawil, Switzerland).

C. Chemometrics

Water, which is the most important chemical constituent of most fruit, strongly absorbs near infrared radiation (Nicolaı¨ et al., 2007). The near infrared spectrum is essentially composed of a large set of overtones and combination bands. In addition, complex chemical composition of a typical fruit causes the near infrared spectrum to be highly convoluted. The spectrum may further be complicated by wavelength dependent scattering effects, tissue heterogeneities, instrumental noise, ambient effects and other sources of variability (Nicolaı¨ et al., 2007). Consequently, NIRS requires chemometrics to extract as much relevant information as possible from the analytical data (Blanco and Villarroya, 2002). Chemometrics has been used to extend and improve the potential application of NIRS technique in food industry (Cen and He, 2007). Chemometrics is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods. Chemometrics in NIRS analysis includes three facets: 1) spectral data preprocessing; 2) building calibration models for quantitative and qualitative analysis; 3) model transfer (Cen and He, 2007). This work has focused on spectral data preprocessing and the calibration models for quantitative and qualitative analysis.

D. Preprocessing

A large amount of spectral data is usually obtained from NIR instruments and yields useful analytical information (Blanco and Villarroya, 2002; Osborne et al., 1993). However, the data acquired from NIR spectrometer contains background information and noise besides sample information. In order to obtain reliable, accurate and stable calibration models, it is very necessary to preprocess spectral data before modelling (Cen and He, 2007). Spectral preprocessing techniques are required to remove any irrelevant information including noise, uncertainties, variability, interactions and unrecognised features. In this study several preprocessing methods were used such as smoothing 3 points (sa3), normalization (n01), first derivative Savitzky-Golay 9 points (dg1), combination of normalization and first derivative Savitzky-Golay 9 points (n01, dg1), and the multiplicative scatter correction (MSC). Preprocessing NIR spectral was conducted by using NIRCal 5.2 software (Büchi Labortechnik AG, Flawil, Switzerland).

E. Calibration methods

The data were randomly split into two groups by NIRCal 5.2 software, a calibration set and a validation set, in a ratio of 2:1. The multivariate-regression methods most frequently used in NIR spectroscopy are principal component regression (PCR) and partial least square (PLS) regression. PLS models are slightly better than the PCR because they do not include latent variables that are less important to describe the variance of the quality parameter (Jong, 1993). In this work, the PLS method was used to develop calibration models to predict SSC and acidity in 'Gedong Gincu' mango fruits. PLS found the directions of greatest variability by considering both spectral and measured property information, with the new axes, called PLS factors (Blanco and Villarroya, 2002). The performance of the PLS regression model was evaluated to avoid over-fitting. The error was evaluated in terms of coefficient of correlation (r) between the reference value and the predicted value. Statistical parameters used to evaluate the developed NIR calibration model were:

1) Bias, i.e., the average deviation between the reference value (x_n) and the predicted value (y_n) of validation set (V-Set). It is recommended that Bias should be equal to zero (Williams and Norris, 1990).

$$Bias = \frac{1}{N}\sum(x_n - y_n)$$

2) The standard error of calibration set (SEC), i.e., the standard deviation of the differences between the reference value (x_n) and the predicted value (y_n) of calibration set (C-Set).

$$SEC = \sqrt{\frac{1}{N-1}\sum(x_n - y_n - Bias)^2}$$

3) The standard error of validation set (SEP), i.e., the standard deviation of the differences between the reference value (x_n) and the predicted value (y_n) of V-Set.

$$SEP = \sqrt{\frac{1}{N-1}\sum(x_n - y_n - Bias)^2}$$

4) Coefficient of correlation (r) between the reference value (x_n) and the predicted value (y_n).

$$r = \frac{\sum(x_n - \bar{x}_n)(y_n - \bar{y}_n)}{\sqrt{\sum(x_n - \bar{x}_n)^2 \sum(y_n - \bar{y}_n)^2}}$$

The model is considered more useful when r value approaches 1, whereby r value is larger than 0.90, considered as high correlation (Williams and Norris, 1990).

5) Coefficient of variation (CV) in C-set and V-set:

$$CVc = \frac{SEC}{\bar{x}}\ x\ 100$$

$$CVv = \frac{SEP}{\bar{x}}\ x\ 100$$

6) Ratio of prediction to deviation (RPD).

$$RPD = \frac{\mathrm{SD}}{SEP}$$

A very reliable calibration could be achieved when the value of CVc in C-Set was lower than 5% and the value of CVv in V-Set was lower than 10% (Mlček et al., 2006). N = number of sample; x_n = reference value; y_n = predicted value by NIR. RPD explaining by which factor the prediction accuracy has been increased compared to using the mean composition for all samples (Saeys et al., 2005).

III. Result and Discussion

Table 1 shows the summary statistics of SSC and acidity in 'Gedong Gincu' mango fruits for calibration set and validation set for all samples selected in each data set. Figure 1 shows original spectra in 'Gedong Gincu' mango fruits. The original ones show that there is a parallel shift of spectra. It occurred because spectra data of NIR did not only contain sample information, but also background information as well as noises. Therefore, preprocessing was needed before modelling to get reliable, accurate and stable calibration model (Cen and H, 2007). Spectra data resulted from diffuse reflectance measurement at solid sample would be followed by scattering noise as a result of particle size difference (Chen et al., 2013). This case was supported by Blanco and Villarroya (2002) who acknowledged that physical properties of solid samples influence spectra of solid samples. Spectra pattern of NIR reflectance indicated that wavelength of 1215-1395 nm was CH2, 1450 nm and 1940 nm were water, 1765 nm was CH2 and cellulose, and 2252-2400 was carbohydrate.

Table 1. Statistical properties of SSC and acidity in 'Gedong Gincu' mango fruit

Propertis	Sample set	Number of spectra	Minimum	Maximum	Average	Standard deviation
SSC (^{o}Brix)	Calibration set	281	13.73	23.67	18.06	1.82
	Validation set	138	16.03	23.27	18.63	1.68
Acidity (%)	Calibration set	275	0.06	0.68	0.25	0.14
	Validation set	133	0.06	0.58	0.3	0.16

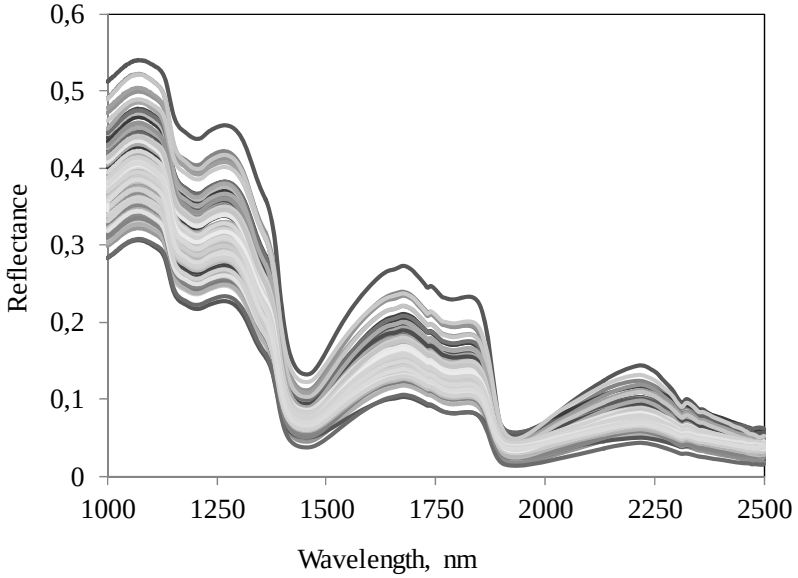

Figure 1. Original spectra NIR reflectance in 'Gedong Gincu' mango fruits

A. Effects of different preprocessing

Various calibration models were developed by using different preprocessing techniques on the spectral data. Each calibration model was used to predict SSC and acidity of prediction data set in order to verify the improved ability of models based on different preprocessing techniques. A proper model was evaluated based on the low SEP and SEC, a high coefficient of correlation between the predicted and measured value of each property and RPD higher than 1.5 (Schmilovitch dkk, 2000). RPD value obtained was highly dependent on the diversity of data. The greater the diversity the greater RPD values obtained (Mouazen et al., 2005). Models have been developed using different number of PLS factors and different preprocessing techniques. PLS factors are an effective method to reduce dimension in NIRS analysis. The spectra information of sample component is showed by the latent variables. Furthermore, the selection of number of PLS factors is essential to reduce noise and use the whole of spectra information (Chen et al., 2013). The model will depend on the data set and provide poor prediction if too much number of PLS factors is used. On contrary, the model will not be large enough to explain the data variability if too few number of PLS factors were used (Cozzolono et al., 2009). All paragraphs must be indented. All paragraphs must be justified, i.e. both left-justified and right-justified.

Performance of parameters of the model using different preprocessing methods to predict SSC and acidity in 'Gedong Gincu' mango fruits is shown in Table 2. For SSC and acidity spectra preprocessing of sa3 and dg1 produced the best calibration model compared to other spectra preprocessing. Those preprocessing techniques increased coefficient of correlation between measured and predicted parameter attributes from 0.8 to 0.82ºBrix for SSC, 0.66 to 0.74% for acidity and whilst SEP for both parameters attributes value decreased. PLS factors of calibration set for SSC and acidity were 15 and 3. The model with application of preprocessing methods of sa3 for SSC and dg1 for acidity showed higher accuracy and precision than the original model. This indicated that preprocessing was important prior to develop a model as it would improve the accuracy and precision of calibration model (Udelhoven et al., 2003; Ouyang et al., 2012).

B. Prediction of internal quality attributes

For validation set of SSC, it was obtained SEP and r were 1.30 and 0.68. Based on RPD values obtained, the resulting model showed an excellent model in predicting SSC in 'Gedong Gincu" mango fruit. This is because the RPD values obtained was higher than 1.5 (Mouazen et al., 2005). For acidity, the validation set of SEP and r were resulted 0.16 and 0.69 with RPD 1.33. PLS prediction results for soluble solids content and acidity are presented in the scatter plots Fig. 2 and 3. In both figures, the ordinate and abscissa represent the predicted and measured fitted values of the appropriate parameters, respectively. The prediction performances of these models were excellent with high correlation coefficient and low in both SEP and SEC for each characteristic. The best model for the prediction of SSC was developed when sa3 was used as preprocessing and a 15 factor PLS model was found to be sufficient for determining the soluble solids content of intact 'Gedong Gincu' mango fruit. The coefficient of correlation between the measured and the predicted SSC for best model was as high as 0.82 (Fig. 2). RPD was higher than 1.5 which was indicated that the developed model could be used to predict SSC

accurately. Lu (2001) reported for the cherries (Samvar.) with r=0.89 and SEP=0.65. Lu, 2001 also obtained better results in cherries (Hedel- finger var.) with r=0.97 and SEP=0.71.

The best model for the prediction of acidity was achieved when dg1 was used as preprocessing and a 5 factor PLS calibration model was used for determining the acidity of intact 'Gedongf Gincu' mango fruit. The coefficient of correlation between the measured and the predicted acidity for best model was 0.74. It was found that RPD was 1.33 or lower than 1.5. The acid content in 'Gedong Gincu' mango fruit is quite lower which was implied in the accuracy of the developed model. The accuracy of the acidity model is better than the result of Schmilovitch dkk, (2000) for 'Tommy Atkins' mango fruit with R^2 =0.39. The accuracy of the developed model based on the CV parameters, except CVv for SSC, all the values of CVc and CVv were higher than 5% and 10% as reported by (Mlček et al., 2006).

Table 2. The prediction results of SSC and acidity with different preprocessing techniques

Quality attributes	Pre-processing	Calibration set			Validation set		
		Number of PLS factor F	SEC	r	SEP	r	RPD
SSC	Original data	14	1.09	0.80	1.20	0.68	1.52
	sa3	15	1.04	0.82	1.30	0.68	1.52
	n01	13	1.08	0.81	1.30	0.66	1.40
	dg1	6	1.15	0.78	1.40	0.62	1.30
	n01, dg1	6	1.12	0.79	1.40	0.64	1.30
	MSC	3	1.10	0.8	1.30	0.67	1.40
Acidity	Original data	8	0.11	0.66	0.13	0.61	1.23
	sa3	6	0.13	0.48	0.17	0.30	0.94
	n01	6	0.12	0.54	0.14	0.46	1.14
	dg1	3	0.01	0.74	0.12	0.69	1.33
	n01, dg1	2	0.10	0.69	0.12	0.65	1.33
	MSC	6	0.12	0.56	0.15	0.45	1.10

IV. CONCLUSION

It has been demonstrated that NIR spectroscopy is an appropriate tool for prediction of internal quality parameters such as SSC and acidity in 'Gedong Gincu' mango fruit. Moreover, it was found that NIRS in combination with chemometrics methods could lead to proper models. It was crucial to choose an appropriate preprocessing method and calibration technique. The effects of different preprocessing techniques in developing calibration model were investigated. The sa3 indicated the best results with high correlation coefficient (r=0.81), SEP = 1.30ºBix, RPD=1.52 were achieved with 15 PLS factors; in the dg1 of acidity model was found the correlation of coefficient r=0.69, SEP=0.12 and RPD 1.33 with 5 PLS factors. These findings showed that preprocessing methods could affect the accuracy of the NIR calibration models.

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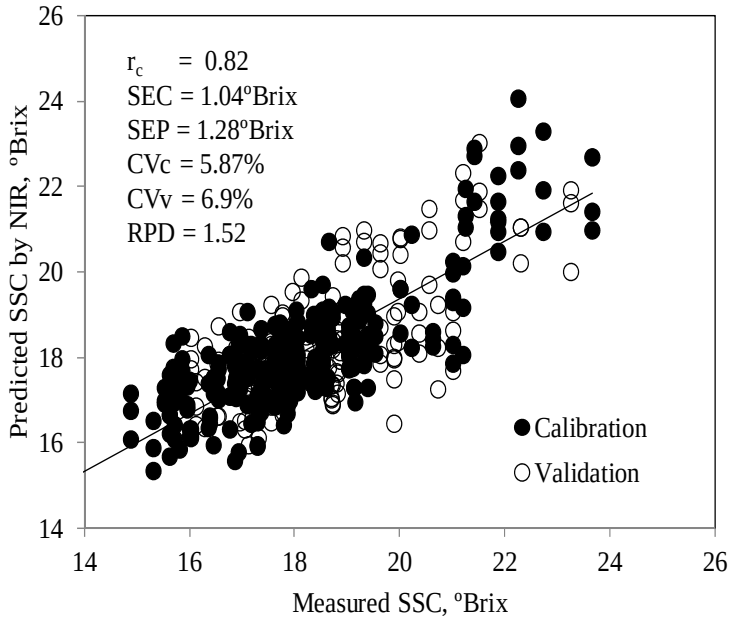

Figure 2. Calibration and validation or prediction of SSC with preprocessing of sa3

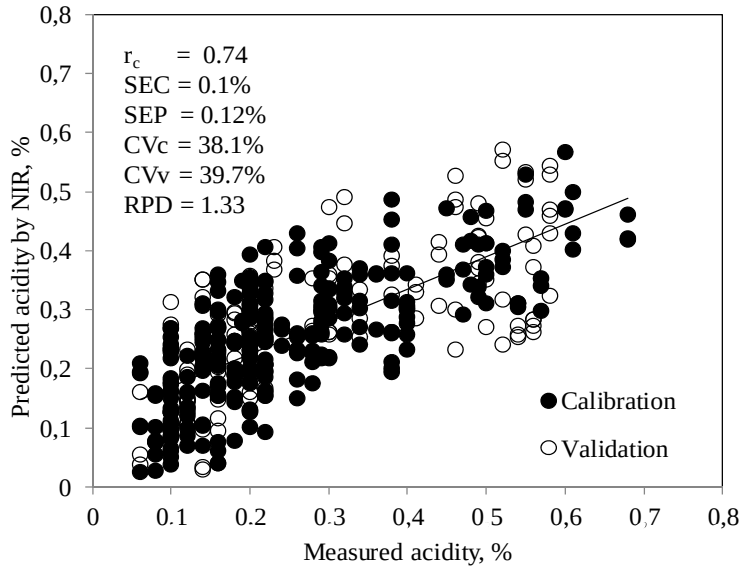

Figure 3. Calibration and validation for prediction of acidity with preprocessing of dg1

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