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Determination of quality parameters in Cavendish banana during ripening by NIR spectroscopy

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Abstract: Studies have been carried out to determine the chemical (soluble solid content) and physical (firmness) parameters of locally grown Cavendish banana by near infrared (NIR) spectroscopy. NIR spectra in the wavelength region of 680-2500 nm were obtained from a total of 408 Cavendish bananas of different ripeness indices. Chemometrics using multiple linear regression (MLR) was applied to develop calibration models for prediction of firmness and soluble solid content (SSC) of Cavendish banana. Results showed that NIR spectroscopy had the feasibility for non-destructive determination of the quality of Cavendish banana. The coefficient of determination (R^2) for firmness and SSC calibration models at different ripeness indices ranged from 0.78 to 0.86 and 0.75 to 0.96, respectively. The calibration models were validated using independent sets of data and prediction models developed with the root mean square error of prediction (RMSEP) ranged from 0.01 to 0.26 kgf and 0.039 to 0.788 Brix for firmness and SSC, respectively. The multi-index models showed considerable robustness but higher prediction error with RMSEP of 0.336 kgf for firmness and 0.937% Brix for SSC compared to index specific model.

Keywords: Cavendish, NIR, multiple linear regression, firmness, soluble solid content

Introduction

The Cavendish banana is the most widely grown banana cultivar worldwide. It is a triploid AAA cultivar of *Musa acuminata* or scientifically known as *Musa acuminate* Colla *cv*. 'Dwarf Cavendish'. In Malaysia, banana is the second largest cultivated fruits, covering about 16% of the total fruit production areas with Cavendish variety mainly cultivated and commercialized for export. Cavendish bananas are harvested at mature green stage (ripeness index 2) and could be stored in chilled condition for up to about 40 days while in transit. Appropriate temperature, humidity, time, air circulation, maturity and ethylene gas are all required for ripening of bananas. During banana ripening, the peel color changes from green to yellow as flavor develops and pulp softens (Meng *et al*., 1997). As banana are typical fruits having a climacteric rise, the ripening treatment of banana could be artificially made by using controlled ethylene gas (Soltani *et al*., 2010). The commercial ripening facilities for banana fruits involved automation and programmatic ethylene gas control by trained laborers. However, this method is lack of monitoring system to detect the ripening quality of banana fruits that may have uneven ripening. This makes determining and monitoring of quality parameters in the orchard, pack house and delivery points important in producing acceptable fresh bananas for the market. It is therefore crucial to adopt a non-destructive detection method in an easy operating application to predict the quality of bananas during ripening, thus ensuring consistent supply of high quality bananas for export.

Firmness is considered a useful criterion for ascertaining the eating quality and ripeness of banana. Flesh firmness decreases down to a relatively narrow optimal eating range of 0.7-0.4 kgf beyond which the fruit becomes senescent. Softening or loss of pulp firmness during ripening has been attributed to the solubilization of peptic substances in the cell wall and middle lamella (Dadzie, 1998). The instrument most commonly used for determination of fruit firmness is the hand-held penetrometer (Subedi and Walsh, 2009). This instrument measures the peak force required to plunge a cylinder of known diameter to a given distance into the fruit pulp (Abbott *et al*., 1997). The use of penetrometer for measurement of firmness is time consuming and destructive.

Another characteristic of banana ripening is the increase in sugars as starch is converted to soluble solids, of which sucrose comprised more than 70% of the total sugars in fully ripe banana (Marriott *et al*., 2006). As with many other fruits, sugar content is usually regarded as evaluation of fruit quality for banana. The SSC of banana is often believed to be linked to consumer taste preference. Generally, fruits above 12 % Brix are considered more acceptable to consumers (McGlone and Kawano, 1998).

Most of the current methods of determining the quality parameters use destructive measures that involve crushing to determine firmness and juicing to measure the SSC of fruits. As it is impossible

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to destructively test every unit of fruit, a subset of a batch were usually taken as representative of the entire batch. Consequently, the quality of the entire batch of fruit could not be accurately assessed because of large variability that may exist between individual fruit as a result of pre and postharvest factors (Louw and Theron, 2010). The banana industry can therefore benefit from non-destructive technology that rapidly predicts the quality parameters of individual fruits. Current technologies that applied non-destructive quality measurement include Magnetic Resonance Imaging (MRI) (Andaur *et al*., 2004), Fourier Transform Infrared (FTIR) (Bellincontrol *et al*., 2009), Laser-induced Fluorescence Spectroscopy (LIFS) (Wulf *et al.,* 2005), Time-Resolved Reflectance Spectroscopy (Zerbini *et al.,* 2005), Proton Transfer Reaction Mass Spectrometry (PTR-MS) (Barbon *et al.,* 2005), and Near Infrared Spectroscopy (NIRS) (Dolores *et al*., 2009). Several attempts have been carried out to predict the banana firmness non-destructively. These include using sonic technique (Finney *et al*., 1967), delayed light emission (Chuma *et al*., 1980), optical chlorophyll sensing system (Meng *et al*., 1997), sound velocity (Subedi and Wash, 2009) and by capacitance technique (Soltani *et al*., 2010). However, these are only fundamental approaches that were far from reaching a practical application. Measurements of quality parameters based on NIRS are most promising with a wide range of application. In NIRS, the fruit is irradiated with light in the near infrared spectral region (600-2500 nm). The spectra in this region contain abundant information reflecting the structure of molecules as well as attributes of fruits such as firmness, total soluble solids, etc. NIR absorption bands are produced when NIR radiation at specific frequencies resonates at the same frequency as the molecular bond in the test sample. This allows association of a specific wavelength with a specific chemical bond vibration generating a specific spectrum that in turn is related to concentration of a specific component. The reflected or transmitted radiation by test samples is then mathematically compared with the spectra of reference samples that have been assayed previously by standardized wet chemistry or non-NIR methods. A specialized computer software (chemometrics) then uses the mathematical relationship to combine the NIR spectra and accompanying chemistry analysis as reference to generate a NIR predictive model used to predict composition of the test samples (Sapienza *et al*., 2008).

NIR has been used as a rapid and nondestructive technique for measuring the SSC and firmness of several commodities. Among the commodities are apple (Ying and Lin, 2004), kiwifruit (Slaughter and Crisosto, 1998), plums (Louw and Theron, 2010), peach and mango (Subedi and Walsh, 2009), nectarine (Golic and Walsh, 2006) and citrus (Zude *et al*., 2008). Subedi and Walsh (2009) used visible-short wave near infrared (400-1100 nm) interactance spectroscopy to measure the firmness of banana. Determination of individual sugar content in banana was performed by Tarkosova and Copikova (2000) using high performance liquid chromatography as the reference method to NIR. While the use of near infrared spectroscopy for measurement of fruit firmness and SSC have been reported, the utility of the technique on banana has not been proven. This study aims to evaluate the use of NIR spectroscopy as a rapid and nondestructive alternative for the accurate prediction of quality parameters that corresponds to the different stages of ripeness in Cavendish banana.

Materials and Methods

Sample preparation

Bananas (*Musa Cavendish* Colla) of an even maturity (Index 2 – firm and green) (CSIRO, 1972) were harvested weekly from a local banana farm at Landeh, Padawan, Sarawak for a period of four months (Nov – Feb). The bananas were transported to the laboratory within 24 hour of harvest and kept at ~17^{o}C prior to ripening treatment within 72 hour. Ripening of fruits was initiated at 17^{o}C at 95% relative humidity in a controlled atmosphere containing ethylene gas at a concentration of 100 ppm (0.01% by volume in air). After 24 hour, bananas were assessed or left to ripen at ambient temperature and subsequently withdrawn for assessment when they reached their respective ripening stages (Index 2-7). During the experiment period, a total of 408 bananas were collected and analyzed.

Firmness and SSC assessment

Banana pulp firmness measurements were conducted with a penetrometer mounted on a test stand using a 7 mm diameter cylindrical probe (Wagner, USA). Firmness was defined as maximum force (kgf) required until tissue failure (Soltani *et al*., 2010) and measurements were taken at the middle part from stem end and distal end of the banana. To determine the SSC of the bananas, fruit were juiced individually using a commercial fruit blender. Fifty grams of flesh sample from the banana was diluted in 50 ml of distilled water and blended for 1 min until homogenized and turned juicy. The juice was further centrifuged at 6000 rpm for 6 min (Hettich, Germany). A drop of supernatant from each sample was placed onto a refractometer (Atago, Japan) to measure the soluble solid levels in % Brix. This value was corrected for sample dilution to give the final SSC measurement.

Spectral acquisition

*S*pectra were collected over the wavelength range of 680-2500 nm using a NIR reflectance spectrometer equipped with tungsten halogen lamp as light source (Unity Scientific, NSW). Wavelength accuracy was based on scanning diffraction grating monochromatic of nominal band width of 10 nm and 1 nm steps. The NIR spectra were taken from the middle part of the fruit, equal distant from proximal and distal ends by averaging 15 scans. Periodic reference scan was performed every 30 min. A trial and error process was used to determine the portion of the spectral region scanned which would provide the best prediction of firmness and SSC using Multiple Linear Regression (MLR) calibration technique.

Data analysis and modeling

The chemometrics software package, CalStar version 2.0 was used for calibration model development using MLR technique. Data processing procedure was conducted to optimize the data set by sample set compression, spectral data compression and data smoothing by omitting outliers. The MLR method was optimized at 5 wavelengths combination for all ripeness index and quality parameter. Only the informative wavelength region was retained from the initial wavelength interval of 680-2500 nm and used in further calculations. Calibration model performance was assessed in terms of coefficient of determination (R^2) and standard error of estimation (SEE). The accuracy and robustness of model were validated in terms of R^2, standard error of prediction (SEP) and root mean square error of prediction (RMSEP) of samples not related to the calibration population, slope and bias (Miller and Miller, 2005).

For all NIR calculations, samples were manually separated into calibration and validation sets. The sample size for MLR calibration recommended by industry standard (ASTM E1655-97 NIR Std) is 40-50 samples with minimum of 10 samples should be used to determine the reproducibility of the analysis. In this study, validation of the calibration model was set at minimum of 10 independent samples from a different population in order to acquire the best prediction model possible.

Data from 6 ripening stages were pooled to create reference data for the multi-Index model. The mean and standard deviation (SD) values of each ripeness index were also determined and Duncan Multiple Range Test (DMRT) was performed. Model performance was described by the following statistical terms (Calstar™, 2008):

R^2: R^2 measures the agreement between reference values and predicted values. The maximum value is 1.0, which indicates a perfect prediction. As a rule of thumb value above 0.75 normally indicates a useable model.

SEE: Standard error of estimate indicates how well the calibration equation fits the data. The smaller the value indicates the lower prediction errors of the calibration.

SEP: Standard error of prediction indicates the imprecision (quality) of the validation model. The smaller the value the better the model.

Bias: Bias is a parallel shift adjusted in the calibration curve, the average of differences between actual value and NIR predicted value.

RMSEP: Root mean square error of prediction indicates the prediction error or validation variance. RMSEP equals to SEP when assuming that bias is close to 0.

Results

Quality measurements and spectral description

The changes of firmness and SSC of Cavendish banana during ripening were shown in Table 1. Pulp firmness declined from 3.23 kgf to 0.52 kgf as ripening progressed from Index 2 (day 0) to Index 7 (day 7). The firmness of bananas at Index 2, 3 and 4 were significantly different ($p<0.05$) while the firmness at index 5, 6 and 7 did not differ significantly. The mean values of SSC increased from 4.68% Brix at Index 2 to 19.9% Brix at Index 7 in a quadratic pattern.

The characteristics of the data sets used for MLR model development were shown in Table 2. Larger data sets were split into calibration and validation sets that are comparable to each other. The SSC and firmness for bananas of the combined ripeness stages ranged from 1.80-22.80% Brix and 0.34-4.04 kgf respectively.

The typical NIR spectra for six ripening indices are presented in Figure 1. The spectrum for the respective ripening stages were similar and all show 5 broad absorption peaks around the 970, 1190, 1450, 1790 and 1940 nm regions.

Table 1. Quality parameters of Cavendish banana during ripening

Day	Ripening Index	Description of ripening stage	N	Firmness ± SD (kgf)	SSC ± SD (% Brix)
0	2	Green. 75% ripeness. Fruit is suitable for distance market	54	3.23[a] ± 1.01	4.68[a] ± 2.39
2	3	More green than yellow. 85% ripeness	48	1.33[b] ± 0.71	11.52[b] ± 4.63
3	4	More yellow than green. Fruit is almost ripe and not suitable for distance market	88	0.88[c] ± 0.36	15.09[c] ± 3.68
4	5	Yellow with some green at the end of the fruit and only suitable for local market	69	0.66[d] ± 0.13	17.49[d] ± 2.83
5	6	Yellow overall. Ripe fruit and at the best stage for eating fresh	66	0.60[d] ± 0.11	18.84[e] ± 1.64
7	7	Yellow overall with brown speckles. The fruit is too ripe and have a short lifespan	83	0.52[d] ± 0.09	19.19[e] ± 1.87

Different letters in superscript indicate means are significantly different ($p<0.05$).
Index 1 - Dark green and not ripe for harvest.
N number of sample

Table 2. Characteristic of data sets used for calibration and validation of Cavendish banana at different ripening stages and combination of all stages

Ripeness Index		SSC (% Brix)				Firmness (Kgf)			
		Mean	SD (±)	Range	N	Mean	SD (±)	Range	N
2	Cal	4.73	2.34	2.00-6.00	47	3.55	0.75	2.18-4.92	52
	Val	3.72	0.51	2.60-4.50	10	3.45	0.58	2.60-4.70	22
3	Cal	11.47	4.65	6.00-20.00	36	1.47	0.68	0.68-3.20	52
	Val	12.20	3.66	8.00-17.00	10	1.47	0.45	0.68-2.00	13
4	Cal	14.95	3.38	5.40-21.40	51	0.88	0.29	0.54-1.72	58
	Val	12.75	3.10	8.40-16.50	18	1.00	0.18	0.74-1.35	11
5	Cal	17.42	2.82	13.20-21.60	70	0.66	0.11	0.46-0.92	66
	Val	17.49	1.52	15.00-20.00	10	0.66	0.10	0.50-0.90	20
6	Cal	18.82	1.24	17.00-22.80	33	0.62	0.07	0.48-0.74	35
	Val	18.83	0.61	17.80-20.00	15	0.63	0.03	0.60-0.70	13
7	Cal	19.72	1.21	16.30-23.00	44	0.53	0.08	0.35-0.70	44
	Val	19.95	1.71	17.60-22.00	11	0.55	0.10	0.36-0.65	11
All	Cal	13.93	6.08	1.80-22.80	346	1.40	1.22	0.34-4.04	330
	Val	14.03	6.69	2.80-21.60	15	1.32	1.09	0.36-3.98	26

SD Standard deviation
Cal Calibration set
Va Validation set

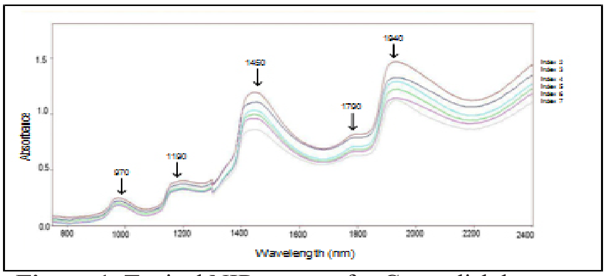

Figure 1. Typical NIR spectra for Cavendish bananas taken at different ripening stages

NIRS assessment of SSC

MLR calibration and prediction results for SSC and firmness are presented in Table 3. The index specific calibration models for all bananas ripeness index indicated a high correlation between NIR and the measured SSC values with R^2 ranging from 0.745 to 0.955. Model complexity determined using external validation (independent dataset from calibration) is also presented in Table 3. The high model precision indicated that the majority of the variance was reproduced in the prediction model using limited number of samples (N range from 10-18). The R^2 of SSC in the validation model ranged from 0.865 to 0.997, while RMSEP ranged from 0.039 to 0.788% Brix. When all the samples from 6 ripeness indices were pooled and a multi-index SSC model was developed, the R^2 increased to 0.914 and 0.981 in both calibration and validation model, respectively. However, a higher prediction error was observed with SEE and SEP increased to 2.438%Brix and 0.959% Brix, respectively. The narrow wavelength range of 700-1000 nm was shown to produce higher R^2 in a multi-index model compared to the index specific models.

The actual versus predicted values of SSC for the multi-index calibration and validation model are shown in Figure 2(a) and 2(c). The calibration dataset showed that the distribution of samples were more concentrated at higher SSC values. Nevertheless, the samples used for the calibration had included the complete range of SSC values to adequately describe the variation in the range. The accuracies of the developed calibration for SSC were successfully validated using random external samples, which are shown in the score plot of the MLR validation model.

NIRS assessment of firmness

The firmness calibration models for index specific bananas performed well with R^2 ranged from 0.789 to 0.843 and SEE ranged from 0.05 to 0.379 kgf. The multi-index model gave a similar result of R^2 values of 0.855 and SEE equals to 0.418 kgf. When validated using independent samples, the predictability increased in both the multi-index (R^2=0.905) and index specific model (R^2=0.849-0.979) except for bananas ripeness of index 3 (R^2=0.779). However, the decrease in R^2 shown in index 3 did not increase

Table 3. Performance of calibration and prediction model for sugar content and firmness of Cavendish banana

Parameter	Index	Calibration			Validation			
		R^2	SEE	Wavelength (nm)	R^2	SEP	Bias	RMSEP
SSC (%Brix)	2	0.783	0.696	1878-2440	0.997	0.034	0.022	0.039
	3	0.878	1.562	680-2474	0.972	0.649	-0.491	0.788
	4	0.955	0.973	743-2465	0.993	0.274	0.161	0.311
	5	0.745	1.429	717-2434	0.865	0.669	-0.266	0.688
	6	0.855	0.825	680-2467	0.937	0.246	0.146	0.279
	7	0.853	0.585	1026-2166	0.987	0.202	-0.021	0.194
	ALL	0.914	2.438	705-992	0.981	0.959	0.144	0.937
Firmness (Kgf)	2	0.837	0.379	713-2415	0.860	0.226	0.131	0.256
	3	0.843	0.345	741-1120	0.779	0.206	-0.038	0.199
	4	0.841	0.170	725-1394	0.979	0.028	0.026	0.038
	5	0.785	0.071	727-1696	0.971	0.017	0.010	0.019
	6	0.780	0.050	793-2438	0.849	0.013	0.006	0.014
	7	0.815	0.050	713-1294	0.940	0.010	0.001	0.010
	ALL	0.855	0.418	688-957	0.905	0.343	-0.018	0.336

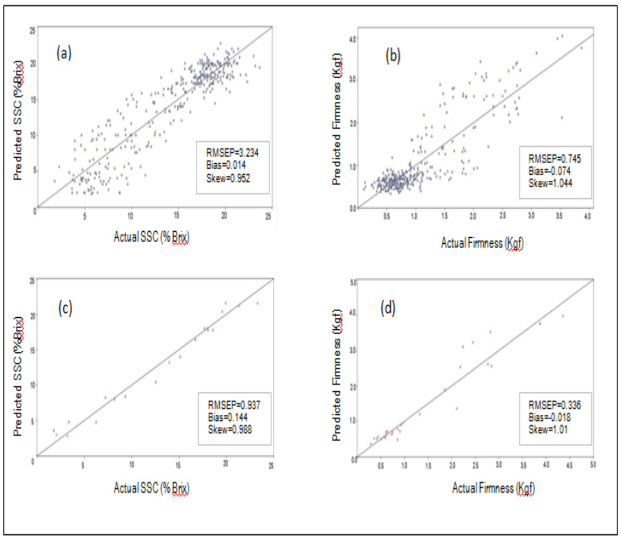

Figure 2. Non destructive NIRS prediction for (a) SSC and (b) firmness in calibration model; and (c) SSC and (d) firmness in validation model plotted against actual reference data for all ripening indices combined

the prediction error (RMSEP= 0.199). When all the samples from 6 ripeness indices were pooled and a multi-index firmness model was developed, an increase in R^2 values was also shown. Nevertheless, a higher prediction error was observed (SEE=0.418% Brix; SEP=0.343%Brix), similar to the SSC multi-index model.

The actual versus predicted values of firmness for the multi-index calibration and validation model are shown in Figure 2(b) and 2(d). The calibration dataset with 330 samples indicated a higher distribution of the samples at lower firmness reading. This was in accordance to the descriptive statistic result on the overall firmness measurement that bananas of index 5, 6 and 7 were not significantly different from each other. The firmness for bananas of index 5, 6 and 7 shared the similar firmness reading in a narrow range of 0.52 to 0.66 kgf (Table1). When firmness regression model was used to predict the data for 26 independent bananas samples, prediction result of the validation model were acceptable with bias of 0.018%Brix as shown in Figure (2d).

Discussion

The changes of SSC in bananas of index 2 to index 7 during ripening were in a quadratic pattern that is similar to the increase reported by Marriot *et al.* (2006) and Soltani *et al.* (2010). There was a significant decrease in firmness from day 2 to day 7, similar to those reported by Morita *et al.,* (1992). The decrease in firmness was mainly due to the softening of banana fruit that was associated with the breakdown of pectin substances and movement of water from the rind of the banana to pulp during ripening.

A strong peak centered at ~970 nm was observed from the NIR spectra of Cavendish bananas. This is in accordance to the report by William and Norris (1987), indicating a strong water absorption band exists at 958 nm. Peaks at 1190 and 1790 nm also agree with the second and third C-H overtone regions associated with sugar solutions (Osborne *et al.*, 1993). The slight peaks shown beyond 2300 nm may falls within the combinations region associated with the C-H and C-H combinations grouping. The absorbance pattern shown can be loosely related to the functional groups associated with water and sugar as banana consists of 70% water and showed a rising sugar content throughout ripening. The influence of wavelength regions was studied by progressively reducing spectral across the range. In general, a reduced spectral range was essential for developing good models.

MLR, partial least square (PLS) or other multivariate techniques are typically employed in the non destructive assessment of fruit quality through correlation with the NIR spectra. Many variables like temperature, geographic region, harvesting time, cultivar, data pre-treatment and model algorithm could affect the performance of a predictive model (Golic and Walsh, 2006). In this study, the bananas used were cultivar and index specific, harvested from a specific location over a period of four months. The calibration that cover the complete range of quality values were used in order to provide sufficient variability in the dataset on which the model is to be based. Including more variability in the calibration set is expected to increase the prediction accuracy, but on the other hand, more data would also result in increased chance of adding atypical data (Lin and Ying, 2009). Thus, it is important and reasonable to control the amount of spectral variability by using the MLR calibration model because the ripening stage is already a known priori for determination of SSC and firmness in banana. By using MLR, precise calibration equations were developed in this study, similar to those reported by Lin and Ying (2009) for other fruits attributes. However, prediction model over fitting was shown and the model might be less robust if other pretreatment was not employed.

The NIRS techniques could model the SSC and firmness directly or indirectly through measurement of correlated attributes because it is commonly known that banana skin colors changes as fruit starts ripening. However, this change can be upset by certain storage temperatures, harvesting timing and ethylene treatment. The skin color of banana may failed to yellow while conversion of starch to sugar and change in firmness proceeds. This is a challenge as inability to interpret the secondary relationships will lower the confidence in the robustness of the model. For example, the firmness model may not necessarily has a casual origin in direct firmness changes at all. Other features of the fruits are changing with ripening and the SSC and firmness model might be influenced by simultaneous changes in these features. Thus it is suggested that index specific model should be developed in addition to a multi-index model as all the index specific models have smaller prediction error. Nevertheless, when calibration models are low in robustness the inclusion of more than one ripening index with increased range can increase the predictability of the models.

It was reported that the instruments and methods used to measure the reference data and the size of the wavelength interval could influence the quality of the prediction results (Sohn and Cho, 2000). Subedi and Walsh (2009) reported a model for banana firmness with acceptable predictability of R^2 equals to 0.76 using 140 calibration samples and 20 validation samples measured at a short wavelength near infrared (SWNIR) of 730-935 nm. In this study, the predictability for the multi-index model showed R^2 value of 0.90 using 330 calibration samples and 26 validation samples measured at the range of 688-957 nm. On the other hand, the prediction result for SSC validation model with R^2 equals to 0.98 showed similar result with those obtained by Fan *et al.* (2009), Louw and Theron (2010) in apple and plum, respectively. To date, there was little information on the use of NIR to measure the SSC of banana except those reported by Tarkosova and Copikova (2010) with R^2 of 0.98 for total sugar content.

The performance of the calibration model for firmness was fair, though not better than SSC. This is in accordance with the literature that the accuracy of calibration models for firmness is usually worse than for SSC (Penchaiya *et al*., 2009). This is because the firmness is a physical parameter that has limitation due to the changes in pectin and water absorbance bands compared to chemical parameter of SSC. It is likely that better prediction for firmness can be obtained by separating the contributions of scattering and absorption based on time or spatially resolved techniques (Nicolai *et al*., 2008). A negligible bias was observed in both firmness and SSC when validating the model based on an external validation set which indicates considerable robustness of the multi-index calibration model.

Conclusions

From this study, it can be concluded that NIR can be used to develop prediction models for internal quality of Cavendish banana such as firmness and SSC. The quality of the model performance was cultivar specific with special emphasis on the different ripeness indices. Although the multi-index models outperformed the single index models in terms of R^2 values, it is suggested that the index specific model be developed as it had smaller prediction errors. MLR can be used to estimate component concentrations, chemical and physical properties from their NIR reflectance spectra. These findings can be utilized by the banana industry to improve the efficiency of ethylene treatment and in developing a grading system that is based on the rapid prediction of quality parameter of the fruit. Further research is needed to improve the precision by using different coefficient for data pre-processing such as second derivative transformation.

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