Detection of Hardening Pericarp Disorder and Determination of Firmness at Hardening Area in Mangosteen by Visible-Near Infrared Reflectance Spectroscopy

S. Workhwa and S. Teerachaichayut*

Department of Food Science, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand

**Corresponding author, Email: ktsontis@kmitl.ac.th*

Abstract

Mangosteen (*Garcinia mangostona* L.) is an economically important fruit grown commercially in Thailand for domestic consumption and export. The fruit has a thick and hard pericarp. However, hardening pericarp disorder can easily occur as a result of compression or impact during harvest and transport. Classification and prediction of hardening pericarp disorder in mangosteen was investigated using visible-near infrared spectroscopy (Vis/NIRS). Reflectance spectra were acquired on each of 1100 mangosteen samples. The number of samples for training and test set was 733 and 367 samples, respectively. Partial least squares-discriminant analysis (PLS-DA) was used for quantitative analysis. The results of discriminant analysis of normal and hardening pericarp samples using leave-one-out cross-validation achieved an average total accuracy of 92.92%. A further goal was quantitative analysis of firmness of mangosteens with hardening pericarp using Vis/NIR measurements. The optimum calibration model was pretreated using standard normal variate transformation (SNV) pretreatment and was developed using partial least squares regression (PLSR). The model was proven useful for prediction of the degree of pericarp hardening of mangosteen. The coefficients of correlation (R) and root mean square error of cross validation (RMSECV) were 0.89 and 2.67N respectively. This technique has potential use for nondestructive and rapid classification of quality for mangosteen.

Keywords: mangosteen, nondestructive, hardening pericarp, Visible-NIR reflectance spectroscopy

Introduction

Mangosteen (*Garcinia mangosteen* L.) is often referred as the "Queen of the tropical fruit" because it is considered to be an outstanding delicious fruit (Morton, 1987). The origin of mangosteen is believed to be in Southeast Asia. Today, it is mainly found in India, Myanmar, Sri Lanka and Thailand (Jung et al., 2006). An internal mangosteen defect called hardening pericarp occurs due to compression or impact from mechanical force. Such forces are present in almost every step of fruit processing. This defect is marked by a rapid increase in pericarp firmness (Tongdee and Suwanagul, 1989). Hardened pericarp cannot be sorted by visual inspection. The only way to detect it is by cutting, a destructive detection method. At present, there is no practical non-destructive method to detect hardened pericarp.

In recent years, nondestructive methods have been proposed for assessing fruit firmness. This include vibration based measurement techniques (Langenakens et al., 1997), X-ray imaging (Schatzki et al., 1997), laser vibrometers (Terasaki et al., 2001) and acoustic measurements (Schotte et al., 1999).

Vis/NIRS has been commercially used in agricultural industries (Donghai et al., 2006). It is a nondestructive technique frequently used to quantitatively and qualitatively evaluate the quality of agricultural products NIR reflectance spectroscopy has been used to quantitatively assess

firmness of a wide number of fruits including peaches (Slaughter, 1995), grapes (Herrera et al., 2003), kiwifruits (Clark et al., 2004), apples (Zude et al., 2006), watermelons (Tian et al., 2007), tomatoes (Shao et al., 2007), pears (Nicolai et al., 2008) and oranges (Cayuela, 2008). Valero et al. (2004) found clear correlations between measured firmness values and predicted values of a calibration model for apple tissues. However, nondestructive discrimination was applied to use for qualitative measurement.

In previous studies, NIR spectroscopy was used for internal quality determination such as gamboges disorder and translucent flesh disorder (Teerachaichayut et al., 2008, Teerachaichayut et al., 2010b, Teerachaichayut et al., 2010a, Terdwongworakul et al., 2012). Reflectance NIR spectroscopy was used for determination of moisture content and peels hardness of mangosteen (Ahmad et al., 2014). Short wavelength near infrared spectroscopy was used to detect total soluble solids content of mangosteen (Teerachaichayut et al., 2009a). Models for total soluble solids content were used to identify translucent flesh disorder in mangosteen (Teerachaichayut et al., 2009b). Peerapattana et al., (2013) established a model for estimating the concentration of alphamangostin (aM) in mangosteen pericarp powder. Teerachaichayut et al., (2011) showed good results of qualitative analysis of hardened mangosteen's pericarp using SW-NIR transmittance spectroscopy in the wavelength range of 660-960 nm. In the transmittance mode, spectral information of whole fruit including pericarp, flesh and seed was acquired. It may be better to use reflectance mode instead of transmittance mode because only spectral information around the surface is needed for determination of pericarp quality. Hypothetically, reflectance mode may be more suitable for detecting hardened pericarp. Hence, the goal of this study was to investigate the use of reflectance Vis/NIR spectroscopy to predict and classify hardened pericarp of mangosteen.

Materials and Methods

Fruit

Mangosteens (*Garcinia mangostana* L.) of uniform color and size (54-62 g) were harvested at commercial ripeness (Capirig et al., 2001) in Nakornnayok Province, Thailand. The fruits were harvested carefully in order to minimize physical damage. After harvesting, the fruits were transported to our laboratory within 6 h in ventilated corrugated fiberboard boxes. The fruits were cleaned by being softly wiped with dried cloth and then stored for 24h in an air-conditioned room at 25^{o}C and 80% relative humidity before being tested on the following day.

Spectral Acquisition

Each sample was measured at an equatorial position of the fruit body as shown in Figure 1. Individual measurements were made using a Vis/NIR spectrometer in reflectance mode (FOSS, Model XDS-RCA Analyzer, Denmark) over the wavelength range of 400-2500 nm. The light source was a 50W tungsten-halogen lamp. The position of illumination was perpendicular to the fruit surface during measurement. Reflected light diffusely scattered through a detector positioned at a 45^{o} angle from the measuring point and light source.

Preparation of Hardening Pericarp Mangosteen and Firmness Measurement

All samples (N=1,100) were divided into two groups: 550 samples of mangosteens with normal pericarp and 550 samples of mangosteens with to be hardened pericarp. Samples from the to-be-hardened pericarp group were dropped from a height of 100 cm onto a concrete floor in order to develop hardened pericarp (Ketsa and Koolpluksee 1993). The resulting impact area on the surface of each fruit was marked. Then samples were stored for at least 3 hours at 25^{o}C and 80% RH before Vis/NIR measurement. Each sample was scanned 4 times at several quarter-equatorial points of the fruit. Only for the hardened pericarp samples, scans started at the impact area and then to the other points at every 90^{o} rotation. An average spectrum of each fruit was used for analysis. At the same point of the Vis/NIR scan in each sample, firmness was measured in using texture analyzer (Model: TA.XT Plus, UK) with a flat-tipped 2 mm diameter stainless-steel cylindrical probe at a speed of 10 mm s^{-1} and 5 mm penetration. The texture analyzer showed the unit value of firmness in Newton (N). After firmness measurements, all mangosteens

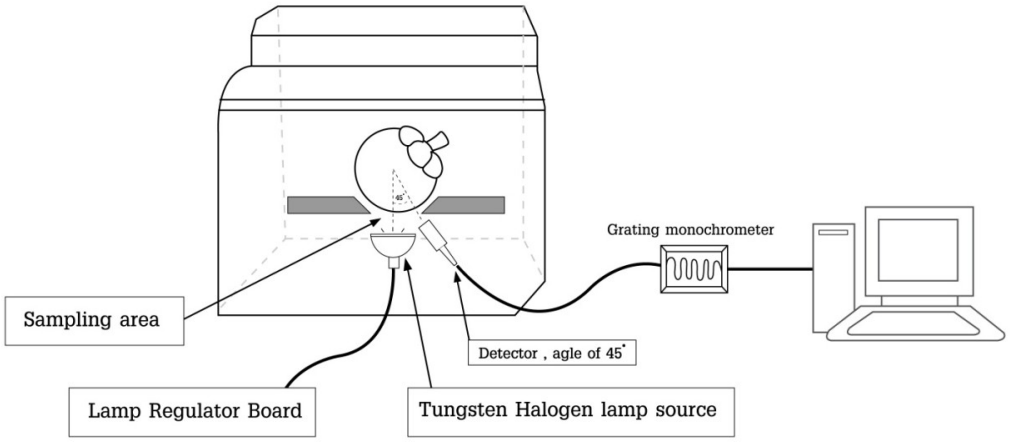

Figure 1 Schematic diagram of Vis/NIR spectrometer and sample presentation for all measurements.

were cut open with a sharp knife perpendicular to the stem-calyx axis and their pericarp appearance was carefully inspected visually to confirm that they were in the right group.

Preparation of Mangosteen pericarp for Scanning Electron Microscopy (SEM)

To investigate cell structure of pericarp, both normal and hardening pericarp were cut into 5 mm cubes in a dish of 0.1 M phosphate buffer pH 7.3 for SEM. Subsequently, pericarp pieces were transferred immediately into a primary fixative solution containing 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4°C and stored overnight as described by Bozzola and Russell (1999) with slight modification for anatomical study. Tissues were washed in the same buffer vehicle used in the glutaraldehyde fixation step. Washing was extremely important because it eliminates any free unreacted glutaraldehyde remaining in the tissues. Next, the specimens were post-fixed in 1% osmium tetroxide in the same buffer for 2 hours. Then, the specimens were dehydrated by stepwise exposure to ethanol-buffer mixtures (20, 40, 60, 80 and 100%) allowing 15 minutes in each. Critical point drying in liquid CO_2 was then done. For SEM, the dried specimen samples were mounted on specimen studs and sputter coated with gold. Coated samples were stored in a desiccator for one day before examination by SEM at 10 kV (JEOL Model JDX 3530).

Data Analysis

For qualitative analysis, the total 1100 samples of mangosteen were classified as normal samples (n = 550) and the hardening pericarp samples (n = 550). Partial least squares discriminant analysis (PLS-DA) was used to evaluate the efficiency classification of normal and hardened pericarp mangosteens. The normal pericarp samples were assigned to a value of 0 and hardening pericarp samples were assigned to a value of 1. All samples were combined and divided to the calibration set (n = 733) and prediction set (n = 367) with almost the same distribution of normal and hardening pericarp in each set. Spectra pretreatments were considered to obtain optimal results. All calibration models were analyzed using Unscrambler 9.7 software (CAMO Oslo, Norway).

The Vis/NIR reflectance absorbance spectra of hardening pericarp mangosteen were used for quantitative evaluation. The spectral pretreatments (original, smoothing (median filter), standard normal variate transformation (SNV), differentiation (second derivative) and combined spectral pretreatments) and wavelength range (400-1100 nm, 1100-1800 nm, 1100-2500, 1800-2500 and 400-2500 nm) of the calibration set were investigated in order to provide the best calibration model for firmness.

For quantitative analysis, a total of 433 hardening pericarp samples were divided into a calibration set (288 samples) and a prediction set (145 samples). The calibration model for firmness was constructed using partial least squares regression (PLSR) and evaluated by a full cross validation method. The statistic of R, RMSECV, root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used to judge the success and accuracy of the models.

Results and Discussion

Visible-Near Infrared Spectral Characteristic of Mangosteen

Figure 2A shows the average Vis/NIR spectra of normal and hardening pericarp mangosteens in the range of 400-2500 nm. Both show dominant fifth bands at 530, 970, 1170, 1450 and 1940 nm. The peak at 530 nm is in the visible region and is relate to the color of xanthophylls pigment (Penuelas and Filella, 1998). The spectra were dominated by the water spectrum with overtone bands of the OH at 970, 1170 and 1450 nm (Polesello and Giangiacomo, 1981) and 1940 nm assigned to C-H and O-H. Moreover, it also had a small peak at 1676 nm assigned to C-H in aromatic skeletal structure lignin (Terdwongworakul et al., 2005). The combination bands overlapped and were difficult to assign they have much chemical information (Schwanninger et al., 2011). Therefore, the second derivative treatments were applied for resolving the overlap spectral region as shown in Figure 2B. In the visible region of 600-750 nm, spectra are in the inset for clearer observation. It is seen that the absorbance of the hardening pericarp was higher than of normal pericarp since they were of different color. In the area of hardening pericarp, a dark brown color was observed as a result of accelerated oxidation of phenolic substances. The oxidative products resulted in pericarp xanthophylls (Amiot et al., 1977; Vance et al., 1980; Bunsiri et al., 2003). Therefore, their different absorbance is perhaps influenced by brown pigment at 680 nm (Penuelas and Filella., 1998); this is the red end of visible spectrum.

The Detection of Hardening Pericarp Disorder

All samples of normal and hardened pericarp mangosteens were grouped before classification analysis. They had to be in the correct group. In the experiment, after samples were cut open. Some samples could not be judged for certain from their appearance whether they had normal or hardened pericarp; therefore, these samples were not used for classification analysis. The Vis/NIR spectra of normal mangosteen (identifier = 0) and hardening pericarp mangosteen (identifier = 1) were used for qualitative evaluation by PLS-DA. A predicted value of 0.5 was used as a cut off value for discrimination. If predicted value was equal or less than 0.5, it defined as normal mangosteen. If predicted value was higher than 0.5, it indicated hardening pericarp mangosteen. The spectral pretreatments of the calibration set in the visible and Vis/NIR wavelength range were determined and compared as shown in Table 1. Although the model built from the visible wavelength range gave good discrimination results, but the model built from the whole wavelength range gave the best results. Therefore, Vis/NIRS is more suitable for discrimination.

The combination of smoothing (median filter) and second derivative differentiation spectral pretreatments resulted in an optimal accuracy of 97.14 % (712 from 733 samples) for classification by cross validation. Then it was used for establishment of the calibration model. The classification results of the prediction set showed 92.92% accuracy (341 of 367 samples) as shown in Table 2.

Figure 3A shows performance of the calibration model by cross validation in the calibration set. The accuracy was 97.81% for prediction of normal mangosteen and 96.46% for predicting hardening pericarp. For classification in the prediction set, the accuracy was 96.20% for prediction of normal mangosteen and 89.62% for hardening pericarp mangosteen as shown in (Figure 3B).

These results showed that our classification of normal and hardened pericarp mangosteen using reflectance Vis/NIRS obtained a better accuracy when compared to that in a previous study by Teerachaichayut et al. (2011), which transmittance NIRS was used. It is possible that reflectance Vis/NIRS obtains information only from pericarp.

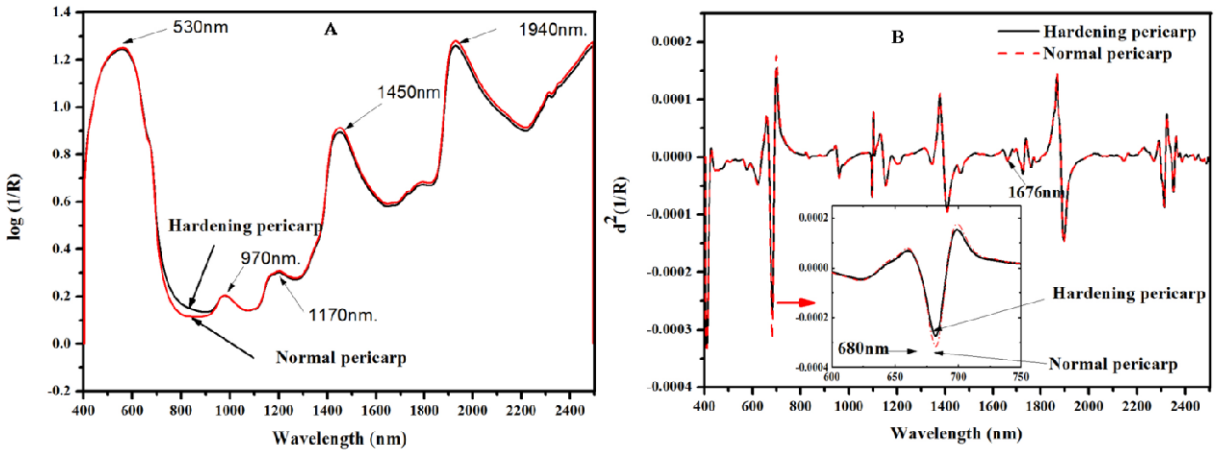

Figure 2 Averaged original reflectance spectra range 400-2500 nm (A), Averaged second derivative spectra range 400-2500 nm (B)

Table 1 PLS-DA results for the classification of samples in the training set with various pretreatment models.

Spectral pretreatment	Wavelength Region (nm)	LV	n	Normal pericarp (0)		Hardening pericarp (1)		%Total Accuracy
				Corrected	Uncorrected	Corrected	Uncorrected	
Original	400-700	12	733	334/366	32/366	331/367	36/367	90.72
	400-2500	11	733	356/366	10/366	334/367	33/367	94.13
Smoothing	400-700	15	733	341/366	25/366	335/367	32/367	92.20
	400-2500	5	733	348/366	18/366	334/367	33.367	93.04
2 nd derivative	400-700	6	733	302/366	64/366	315/367	52/367	84.18
	400-2500	12	733	346/366	20/366	331/367	36/367	92.36
SNV	400-700	17	733	340/366	26/366	326/367	41/367	90.86
	400-2500	11	733	356/366	10/366	333/367	34/367	93.99
Smoothing+2nd derivative	400-700	14	733	345/366	21/366	338/367	29/367	93.18
	400-2500	13	733	358/366	8/366	354/367	13/367	97.14
Smoothing+SNV	400-700	16	733	344/366	21/366	327/367	40/367	91.54
	400-2500	17	733	354/366	12/366	337/367	30/367	94.27

LV: Number of latent variable, n: Number of sample

while transmittance NIRS obtains its information from pericarp and from flesh and seeds as well. The penetration depth of NIR light is up to 4 mm in the 700-900 nm range and about 2-3 mm in the 900-1900 nm range in apple fruit (Lammertyn et al., 2000). In the current study, Vis/NIR light from the reflectance mode yielded enough information about mangosteen pericarp in the short wavelength range

The Determination of Firmness

The firmness values of 433 hardening pericarp mangosteens were investigated using a texture analyzer. It was seen that the firmness in the test set were in the range of the training set and had a similar deviation. This ensured that the prediction results were accurate, as shown in Table 3.

Preprocessing was performed on the average spectrum of the calibration set spectra. The calibration and cross-validation statistical parameters of the PLSR model for pericarp firmness prediction are presented in Table 4. The best result from cross validation of the calibration set was selected by observing the lowest value of RMSECV and the lowest number of latent variable (LV). It was seen that the SNV pretreatment in the

Table 2 Results of PLS-DA classification for normal pericarp and hardening pericarp mangosteens of samples in test set using the definitive model.

Spectral pretreatment	Wavelength Region (nm)	LV	n	Normal pericarp (0)		Hardening pericarp (1)		%Total accuracy
				Corrected	Uncorrected	Corrected	Uncorrected	
Smoothing+2nd derivative	400-700	14	367	(177/184) 96.20%	(7/184) 3.80%	(163/183) 91.25	(20/183) 8.74%	92.26
	400-2500	13	367	(177/184) 96.20%	(7/184) 3.80%	(164/183) 89.62%	(19/183) 10.38%	92.92

LV: Number of latent variable, n: Number of sample

Table 3 Descriptive statistics of the firmness value in the calibration and prediction sets of mangosteen.

Parameter	Data set	n	Range (N)	Mean	SD
Firmness (N)	Total sample	433	3.86-35.97	12.71	6.05
	Training set	288	3.86-35.97	12.72	6.06
	Test set	145	4.05-26.31	12.70	6.03

n: Number of sample, N: Newton, SD: Standard deviation

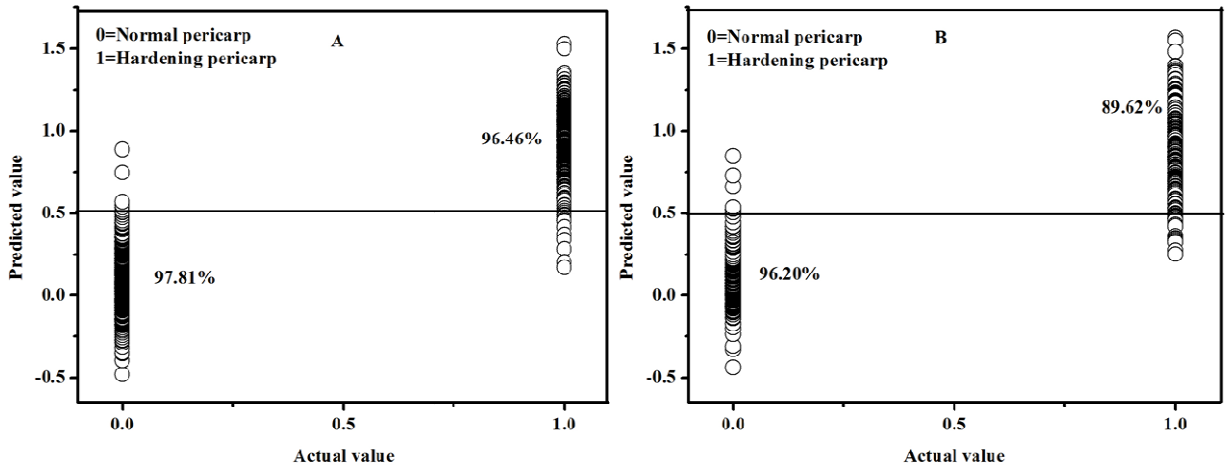

Figure 3 Classification results of training set (A) and test set (B) for normal and hardening pericarp mangosteens

wavelength range of 400-1100 nm gave optimal results with R of 0.89, RMSECV of 2.83 N and LV of 8.

The calibration model was developed for firmness prediction using the SNV pretreatments in wavelength range of 400-1100 nm and provided the best result by PLSR. The results obtained R of 0.89 and RMSEC of 2.67N for the calibration set and R of 0.86, RMSEC of 3.13N for the prediction set. Scatter plots of measured firmness and predicted firmness for mangosteen are shown in Figure 4.

Scanning Electron Microscope Study of Mangosteen Pericarp Hardening

Regarding physiological changes (Tissue hardening disorder) related to the appearance of agricultural products, mechanical damage is one of the main causes (Kays, 1999). Mechanical property of agricultural products is affected by mechanical damage (Kays, 1991). Lignin biosynthesis is a plant's defensive response to wounds. Lignin deposition in plant cell walls is one of the mechanisms that toughen plant vascular tissues

Table 4 The PLSR results of spectral pretreatment and wavelength in the calibration set

Spectral pretreatment	Wavelength Region (nm)	LV	Calibration (Training set)	
			R	RMSECV
SNV	400-2500	6	0.89	2.79
	400-1100	8	0.89	2.83
	1100-1800	14	0.78	3.73
	1100-2500	6	0.77	3.83
	1800-2500	15	0.74	4.04

LV: Number of latent variable, R: Correlation coefficient; RMSECV: Root mean square error crosses validation

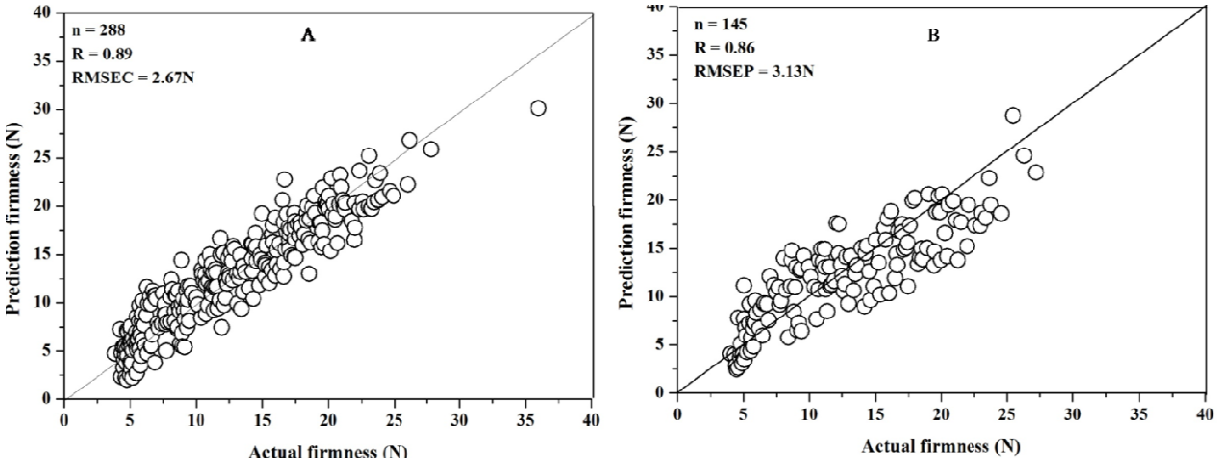

Figure 4 The scatter plots of calibration (n=288) (A) and prediction (n=145) (B) for normal and hardening pericarp mangosteens

(Vance et al., 1980). In addition, turgor pressure has a major influence on tissue strength and macroscopic fruit firmness. It is lost when fruits are deprived of water (Oey et al., 2007). In Figure 5, two pictures of scanning electron microscopy (SEM) of hardened pericarp shows rigidly structured mangosteen pericarp cells in a single layer of interior tissue. Tightly-packed turgid cells were observed in normal pericarp tissue (Figure 5A) while discontinuous cuticle covering the pericarp and big space between flaccid cells were observed in hardened pericarp tissue (Figure 5B). This phenomenon probably occurred because cells in the hardened pericarp tissue were broken from impact and cytoplasmic water in the cells was removed; the cells lost their turgor pressure after impact, and hence became wilted. The color of the tissue that changed from pink to dark brown may indicate the presence of enzymatic browning reaction due to peroxidase activity in the lignification process (Ketsa et al., 1998; Valentines et al., 2005). SEM showed that the hardened pericarp tissue was not continuous due to damage at the impact area. The wounded tissue might be associated with intensification of lignin deposition. Synthesis of lignin is the main reason for the increased firmness of wounded tissue. Firmness of damaged mangosteen pericarp increases rapidly after impact (Ketsa and Atantee, 1998). Furthermore, rapid moisture loss from mangosteen fruit after impact may be related to water vapor movement to the outside atmosphere through lenticels, injured areas or directly through the cuticle. Mangosteen fruit rapidly lost moisture once harvested. The majority of initial moisture loss resulting from hardening pericarp would accelerate the oxidation of phenolic substances. The oxidative products resulted in dark brown color of the inner mangosteen pericarps. This explains the increase of absorbance in the wavelength range of 600-800 nm

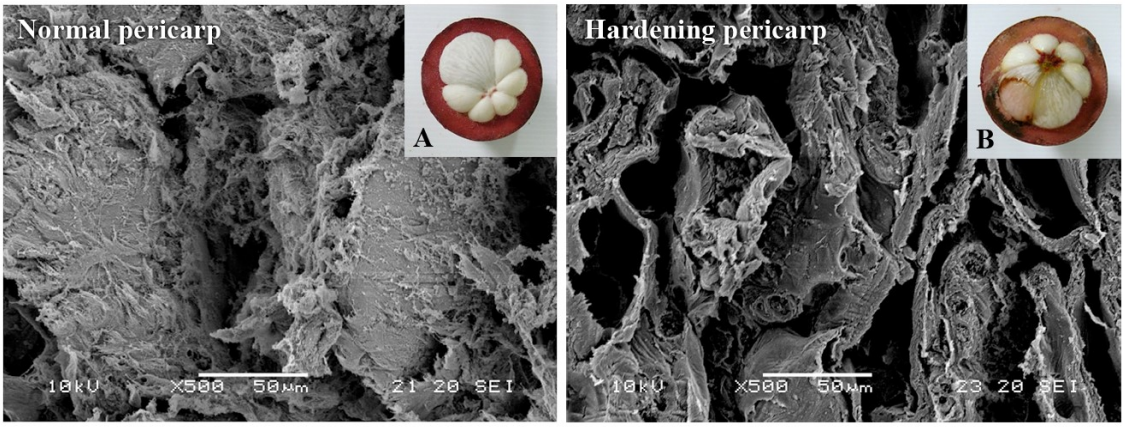

Figure 5 SEM micrographs of the normal (A) and hardening pericarp mangosteen (B)

as seen in the Vis/NIR region. The difference between normal and hardened pericarp in color, water and lignin content resulted in different absorbance values in the absorption bands of color (530 nm), water (970, 1170 and 1450 nm) and lignin (1676 nm).

Conclusions

Color, water and lignin are attributes that can be used for discrimination between normal and hardened pericarp. By comparing the statistical results, it was found that the model using the Vis/NIR wavelength region (400-2500 nm) was more efficient than the model using the visible wavelength region (400- 700 nm). The results of this study indicate that it is possible to develop a nondestructive technique using Vis/NIR reflectance spectroscopy for measuring the internal quality of intact mangosteen fruit. PLSR has good potential to estimate hardening pericarp disorder in mangosteen from their infrared spectra. The Vis/NIR calibration equations of reflectance spectra were sufficiently accurate to determine the internal quality firmness of mangosteen fruits. For firmness, the PLS model with standard normal variate transformation (SNV) pretreatments in wavelength range of 400-1100 nm gave the best results. In order to classify the normal and hardening pericarp mangosteens, the PLS-DA model was developed that could be used for prediction this defect. Good classification was obtained, with an accuracy of 92.92%. This shows that Vis/NIR reflectance spectroscopy has the potential for use in nondestructive discrimination of normal and hardening pericarp mangosteens. Although discrimination with the wavelengths in the visible region was good, but overall, the best discrimination results were obtained by using the whole region of wavelengths. Also found that, SEM displayed the tissue of hard rind mangosteen consisting of broken cells while that of intact fruit consisting of normal cells. It can be a feasible detector for an on-line sorting system.

Acknowledgments

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