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Detection of spongy pulp in guava using light properties and near infrared spectroscopy

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Abstract

Spongy pulp defect in guava is recognised by a dry-looking pulp with a brown colour, and not acceptable to consumers which causes substantial loss in value. Current detection of spongy pulp uses visual assessment of the flesh, which is half cut from the sample. The present work aimed to develop a classifying model based on non-destructive technique for the detection of spongy guava. Guava samples harvested at full maturity were determined for visible light properties, visible light reflectance, and near infrared reflectance. The light properties and light reflectance of guava peel were used to derive a classification model which was then compared with a near infrared reflectance model, which in turn provided absorbance of the flesh and peel using stepwise discriminant analysis. The models were used to classify the guavas into normal and spongy flesh groups, which were assigned with reference to the visual assessment on half cut samples. The classification accuracy for the model using gloss and light reflectance at 650 nm (chlorophyll *b*) was 90.4%. However, the model developed from the near infrared absorbance provided better accuracy (92.7%). It appeared that the largest wavenumber at 4,721 cm^{-1} contributed to the total sugar content, which implied that spongy and normal guavas had different total sugar contents in the flesh. The present work demonstrated the potential of near infrared spectroscopy to discriminate spongy from normal guavas. However, the accuracy of the classification could be further improved by analysing more samples from the next season.

Introduction

Guava is considered as one of the important fruits in Thailand as it is highly nutritious and has good flavour. Its richness in vitamin C, dietary fibre, and lycopene ranks it among the healthier fruits (Sidhu, 2006). In particular, 'Kimju' guava is preferred by the majority of consumers due to its thin peel, crispy flesh, and good flavour (Poubol *et al.,* 2018). Harvesting guava at the right maturity gives good flavour and texture. However, properly harvested guava does not guarantee overall good quality as the harvested fruit may have internal defects such as spongy pulp. The spongy pulp is recognised by a dry-looking pulp with a brown colour (Janave and Sharma, 2008). This defect is not acceptable to consumers, and causes substantial loss to the value of the fruit. Furthermore, this defect cannot be detected from the external appearance. Therefore, a non-destructive technique is required to detect the occurrence of spongy pulp inside the fruit. One non-destructive technique has been reported for identifying spongy tissue in 'Alphonso' mangoes, which is the most popular Indian cultivar (Thomas *et al.,* 1993). In that research, X-ray images and photographs of fully grown, green, unripe, and ripe mangoes developing spongy tissue revealed dark grey patches associated with internal cavities, which contrasted with the light grey areas of healthy flesh.

Near infrared spectroscopy (NIRS) has several advantages over other techniques in terms of the evaluation of fruit internal quality because it is non-destructive, easily applied, and allows for rapid measurement and minimal sample preparation (Pasquini, 2018). NIRS has been applied for the non-destructive assessment of the internal quality of many kinds of fruits including apple (Fan *et al.,* 2019), pomelo (Puangsombut *et al.,* 2012), mangosteen (Teerachaichayut *et al.,* 2007), black mulberry (Soltanikazemi *et al.,* 2017), and apricot (Buyukcan and Kavdir, 2017). There have been a number of investigations applying NIRS for fruit classification. Discrimination among apple varieties into Copefrut Royal Gala, Red Delicious, and Fuji was achieved with 100% accuracy (He *et al.,* 2007). Visible/NIR spectroscopy was applied with a

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recognition rate of 100% for the non-destructive classification of orange samples in accordance with their geographical areas and growing conditions (Shao *et al.,* 2009).

Many applications of NIRS have been reported with respect to the detection of internal defect. One of them involved the assessment of astringency caused by soluble tannins in persimmons (Noypitak *et al.,* 2015). Normal persimmons could be differentiated from astringent ones with an accuracy of 97.1% based on NIR spectra of the stem-end flesh. Another investigation applied transmission visible-NIR spectroscopy to categorise the 'Yali' Chinese pear into pears with brown cores, and those of acceptable quality, providing a correct score of 95.4% (Han *et al.,* 2006). Furthermore, brown heart in pear could also be detected by visible-NIR spectroscopy (Fu *et al.,* 2007). The detection of translucency in mangosteen was also accomplished using short wavelength NIRS with the classification between normal and translucent mangosteens, achieving 92.0% accuracy (Teerachaichayut *et al.,* 2007).

The light reflectance properties of fruit were also studied as a sorting index for fruit quality. Jha *et al.* (2005) applied multiple linear regression to create models for the prediction of total soluble solids as a sweetness indicator of intact mangoes using the visible spectrum in the range 440 - 480 nm. The calibrated values of total soluble solids highly correlated with the predicted values. Hoa *et al.* (2006) assessed the flesh and skin colour of dragon fruit using the hue angle system. Thus far, however, there has been no known report on the application of the visual spectrum to detect spongy pulp in guava.

Regarding guava, visible and NIRS have been applied to predict the soluble solid content with good performance providing a multiple correlation coefficient of 0.947, and a standard error of prediction of 0.721 Brix, respectively (Hsieh and Lee, 2005). However, there has been no report on the application of NIRS and light properties for the detection of spongy pulp in guava. Therefore, the objective of the present work was to study the use of NIRS and light properties to classify spongy flesh guavas from normal guavas.

Materials and methods

Sampling

Samples of 'Kimju' guava were harvested at 120 d after anthesis, when the fruits were fully mature, from the orchards located at the central region of Thailand in May 2019. All 188 guava samples were kept at 25°C overnight for acclimatisation before performing successive measurements on the following day.

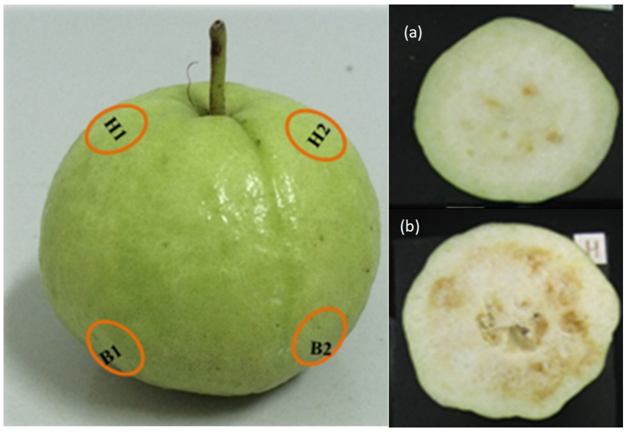

Figure 1. Four positions on the guava fruit where optical reflectance was measured, and the images of (a) normal guava, and (b) spongy guava.

Light property measurement

Each guava sample was measured for optical reflectance in the visible region (400 - 700 nm) using a spectrophotometer (Spectro-guide, Sphere gloss BYK-Gardner, USA) at four positions, two of which were adjacent to the stem, while the other two were close to the fruit end (Figure 1). Spongy pulp was reported by the farmers to be found mostly in these four locations. In addition, the Hunter L^*, a^*, and b^* colour scale and gloss value were recorded to indicate the fruit colour and visual appearance. The measurement at each position was done in duplicate (Figure 2a). These four measurements were averaged and used for further analyses.

NIR spectral measurement

The NIR spectra of each guava sample were attained using a spectrophotometer (MPA FT-NIR, Bruker Optics, Ettlingen, Germany) in the range 4,000 - 12,500 cm^{-1} at 32 cm^{-1} resolutions (Figure 2b). The average of 32 scans was calculated to denote the spectrum of each measurement. The measurement locations on the sample were the same as those used for the visible measurements. Each location was scanned twice to obtain duplication of the spectra.

Spongy flesh assessment

Visual inspection of half-cut guava sample was used to determine whether the flesh sample was spongy. The spongy flesh was characterised by a brown colour and a sponge-like corky texture. Following the inspection, 168 samples were

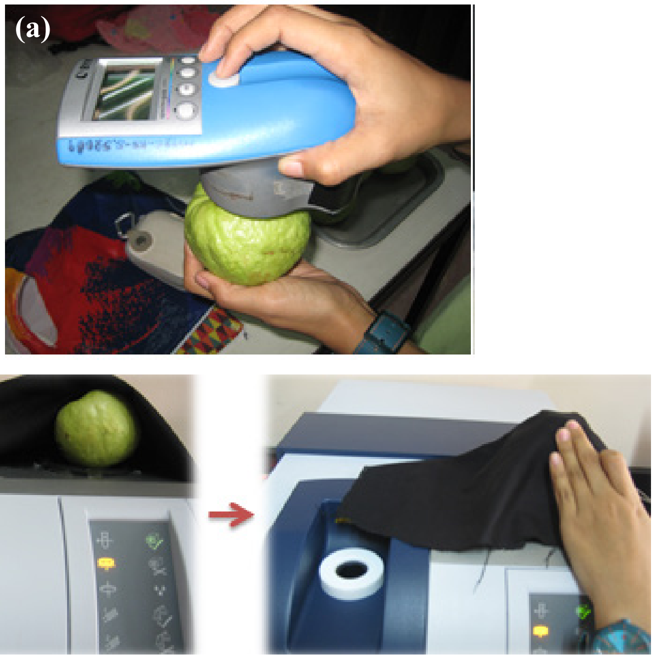

Figure 2. The measurement of (a) the optical reflectance, and (b) near infrared absorbance showing the placement of the guava sample covered with black cloth to prevent surrounding light from entering the scanning window.

identified with normal flesh, and 20 samples had spongy flesh (Figure 1).

Soluble solid content measurement

The flesh adjacent to the measured location was removed, and its juice separated using a manual fruit squeezer. The extracted juice sample was centrifuged to remove the dispersed solid particles from the liquid using a centrifuge at a speed of 8,500 rpm (PLC-012, Gemmy Industrial Co., Ltd., Taiwan). The clear juice from each guava sample was then determined for the soluble solid content (SSC) using a digital refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan). Each juice sample was measured twice for replication. All measurements were averaged to represent the SSC of the whole guava, and used for further analyses.

Statistical analysis

For the first part of the analyses, statistical testing was performed to determine any significant differences between values of L^*, a^*, b^*, gloss, and SSC of the normal and spongy guavas. Student's *t*-test was performed to analyse difference at 95% confidence level.

In the second part of the analyses, the classifying models were constructed by performing discriminant analysis (SPSS version 9.0, Chicago, IL, USA) to separate the guava into spongy and normal groups. Discriminant analysis is a multivariate technique implemented to develop linear functions of multiple variables that allow the maximum difference between two or more groups to be obtained while minimising the deviation within each group (Tabachnick and Fidell, 1996). The model performance of classification pertaining to the percentage of correct classification was subsequently determined using leave-one-out cross validation by which each sample was classified using the classifying models developed from all samples other than that sample.

Initially, the first classifying model was developed using L^*, a^*, b^*, gloss, and light reflectance at each wavelength. Then, for the purpose of comparison, the second classifying model was also created based on NIR spectra. A stepwise procedure was applied in the process of model development to reduce the number of classifying variables, selecting only optimal variables. The procedure of variable selection in the stepwise method was based on maximising an F ratio which was calculated from the Mahalanobis distance between groups. The stepwise method began with a classifying model that did not contain any of the predictors. At each step, the classificatory variable with maximum F ratio that exceeded the entry criteria was added to the model (Tabachnick and Fidell, 1996).

In the case of the model based on the NIR spectra, to minimise the scattering effect and enhance the classification performance, commonly used pre-treatments (Huang *et al.,* 2010) such as multiplicative scatter correction (MSC), standard normal variate (SNV), and second derivative (2D) were applied to the NIR spectra prior to the analyses using the Unscrambler software (version 9.8, Camo, Oslo, Norway). The MSC is used to remove additive and multiplicative effects on the spectra. The SNV is more useful when path length and baseline variation occurred between samples. The 2D resolves overlapping peaks in the spectrum, and removes the constant and baseline shift between the samples (Huang *et al.,* 2010).

For result interpretation, each structural coefficient of each classification variable was investigated for its contribution to the classification model. Each structural coefficient represents the correlation between a given classification variable and discriminant function (Tabachnick and Fidell, 1996) with a higher absolute value indicating that the variable contributes better at discriminating between groups.

Results and discussion

Characteristics of light reflectance and near infrared absorbance

The average light reflectance of the normal guavas was lower than that of spongy guavas from a wavelength range of 450 - 700 nm (Figure 3). A strong peak of light reflectance appeared at around 670 nm, which was similar to the light reflectance of young blueberry (Yang *et al.,* 2015) and immature dragon fruit (Wanitchang *et al.,* 2010). The reflectance was treated using 2D to resolve the peaks (Figure 4). Visual inspection of the 2D-treated reflectance in Figure 4 revealed that there was a big difference in absorbance at a wavelength of 650 nm, where the normal guavas absorbed more light than the spongy guavas. Absorbance at 650 nm corresponds with chlorophyll *b* (Pålsson *et al.,* 1994).

In the NIR region, the spectra were treated using the SNV to reduce the effect of scattering caused by surface differences of the guavas (Figure 5). Two strong peaks near 6,888 cm^{-1} (1,452 nm)

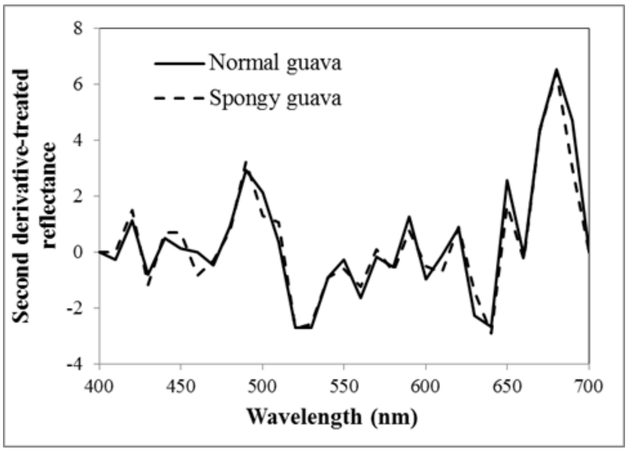

Figure 4. Average second derivative-treated reflectance of normal and spongy guavas.

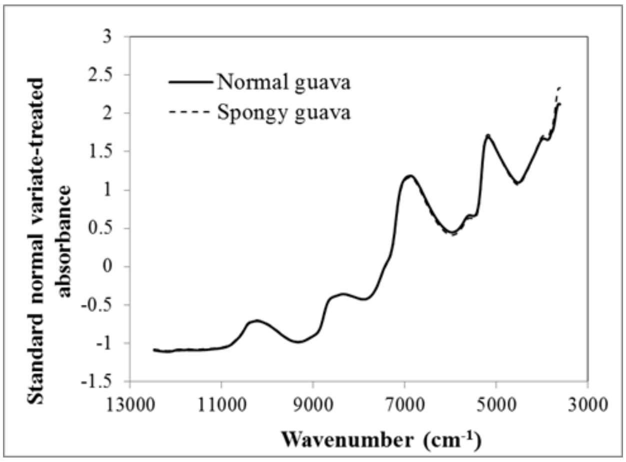

Figure 5. Average near infrared spectra pre-treated with standard normal variate between normal and spongy guavas.

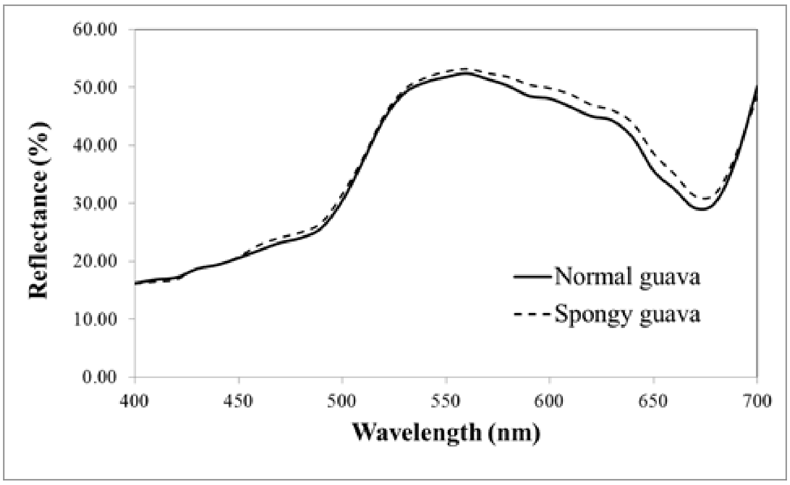

Figure 3. Average visible spectra of normal and spongy guavas.

and 5,160 cm^{-1} (1,938 nm) were associated with the first overtone of O–H stretching of H_2O and a combination of vibration as a result of the O–H stretching + O–H deformation of H_2O, respectively (Osborne and Fearn, 1986). In general, the normal guavas absorbed more light at longer wavenumbers between 7,000 and 5,500 cm^{-1} than did the spongy guavas.

Comparison of light properties between normal and spongy guavas

Table 1 shows a comparison of the light properties between normal and spongy guavas. The spongy guavas had a significantly (p-value < 0.05) higher value of L^* than normal guavas, which resulted in the spongy fruit surface appearing brighter than that of normal fruit. Greenness, as indicated by the negative value of a^*, was higher for the surface of spongy guavas than for the normal guavas. The a^* value was significantly (p-value < 0.05) different between both groups of guavas. Additionally, the surface of the normal guavas appeared significantly (p-value < 0.05) glossier than that of the spongy guavas.

Performance of classifying model based on light reflectance and light properties

A classification model was developed based on the light reflectance and other light properties using discriminant analysis with a stepwise procedure for the selection of the optimum properties. The classificatory variables that were selected by the stepwise method included gloss value and light reflectance at both 650 and 700 nm. The discriminant analysis produced two classification equations:

Normal guava = -223.8 - 3.7 (Gloss) - 1.7 (LR at 650 nm) + 10.4 (LR at 700 nm) (Eq. 1)

Spongy guava = -212.3 - 4.2 (Gloss) - 0.9 (LR at 650 nm) + 9.7 (LR at 700 nm) (Eq. 2)

where, LR = light reflectance.

These two equations were used for classification by substituting the gloss value, and LR at 650 and 700 nm measured from the sample. The resultant values calculated from the two equations were compared, and the equation with higher value was the class to which the sample belonged. For example, if the calculated value of the normal guava equation was higher, the sample was classified into the normal guava class.

The classification performance of the model based on the light reflectance and other light properties are shown in Table 2. Cross validation resulted in the model showing good classification with an accuracy of 90.4%. The normal guavas were predicted with better accuracy (95.8%) than the spongy ones (45.0%). Considering the structural coefficients which related light reflectance and light properties to the discriminant factors (Tabachnick and Fidell, 1996), the gloss property contributed the most to the classification with a coefficient of 0.604. This gloss property was in agreement with the Student's t-test results in Table 1 which indicated a significant difference between the normal and spongy guavas. The second highest contributing parameter to the classification based on the structural coefficient value was the reflectance at 650 nm (coefficient of -0.467), which confirmed the difference in the 2D of reflectance shown in Figure 4, as the largest difference occurred at 650 nm. This implied that the surfaces of normal and spongy guavas contained different chlorophyll b levels which corresponded to reflectance at 650 nm.

Performance of classifying model based on near infrared absorbance

Table 1. Light properties and soluble solid contents of normal and spongy guavas. The p-values were obtained by the Student's t-test.

Parameter	Normal guava	Spongy guava	p-value
L^*	72.63 ± 1.54	73.37 ± 1.41	0.0418
a^*	-6.52 ± 0.94	-5.74 ± 0.95	0.0006
b^*	33.47 ± 1.10	33.98 ± 1.35	0.0566
Gloss	4.57 ± 1.37	2.98 ± 0.69	0.0000
Soluble solid content	10.65 ± 1.36	9.17 ± 1.40	0.0300

Table 2. Discriminant analysis results showing performance of classification between normal and spongy guavas based on light reflectance and near infrared absorbance.

Parameter	Actual group	Correctly classified guava (%)	Predicted group	
			Normal guava	Spongy guava
Light reflectance at wavelengths 650 and 700 nm and gloss value	Normal guava	95.8	161	7
	Spongy guava	45.0	11	9
	Total**	90.4		
SNV*-treated NIR absorbance at wavenumbers 3,718, 3,950, 4,721, 5,300, 5,801, and 6,958 cm^{-1}	Normal guava	96.9	154	5
	Spongy guava	57..9	8	11
	Total**	92.7		

*SNV = standard normal variate; **Total = percentage of total number of correctly predicted samples into both groups divided by the total number of all samples.

The NIR spectra of all guava samples were examined visually, and 10 spectra showed abnormal peaks, and thus were removed as outliers from the analysis. The optimum classifying model was developed using discriminant analysis with the SNV-treated absorbance at optimum wavenumbers being selected using the stepwise technique. Cross validation was performed on the developed model, and the resultant classification performance is shown in Table 2. Similar to the performance of the light-classification model, the normal guavas were predicted with greater accuracy than the spongy guavas. However, in terms of overall accuracy, the NIR model performed better with an accuracy of 92.7% as compared to the light model. Furthermore, both normal and spongy guavas were predicted with better accuracy using the NIR model than the light model. As a result of the stepwise technique, the optimal wavenumbers contributing to the classification were 3,718, 3,950, 4,721, 5,300, 5,801, and 6,958 cm^{-1}. Considering the structural coefficients of the optimal wavenumbers, the absorbance at 4,721 cm^{-1} contributed the most to the classification, as absorbance at 4,721 cm^{-1} (2,118 nm) was a characteristic wavenumber of the total sugar content (Robert *et al.,* 1993). This indicated that the normal and spongy guavas contained different total sugar contents. This could be partially confirmed by a comparison with spongy tissue in mango, where it has been reported that the spongy tissue in affected pulp had low sugar content because the flesh remained unripe due to the biochemical disturbances (Sivakumar *et al.,* 2011). The total soluble solids content was determined, and the normal guavas had a significantly higher content (10.65 Brix) than the spongy guavas (9.17 °Brix).

Conclusion

The classification into normal and spongy guavas was achieved based on light properties and light reflectance, and was compared with NIR reflectance. The classification accuracy using the model based on the gloss and light reflectance at 650 nm was 90.4%. However, the accuracy was improved to 92.7% when the model was based on NIR reflectance at wavenumbers of 3718, 3950, 4721, 5300, 5801, and 6958 cm^{-1}. This suggested that the difference between the spongy and normal guavas could be attributed to chlorophyll *b* (reflectance at 650 nm), glossiness of the surface, and total sugar content (absorbance at 4721 cm^{-1}). However, further investigation is needed to make the model more robust by including more samples withspongy flesh, and performing the analysis using other techniques such as K-Nearest Neighbor, support vector machine, or artificial neural networks.

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