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ASSESSMENT OF INTERNAL QUALITY OF JAPANESE PUMPKIN (*CUCURBITA MAXIMA*) USING NEAR INFRARED SPECTROSCOPY

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ABSTRACT

Near infrared spectroscopy (NIRS) is a non-destructive technique for fruit quality assessment. This study applied NIRS to assess internal quality of Japanese pumpkin fruit during maturation. Japanese pumpkin fruit (*Cucurbita maxima*) were harvested at three stages of fruit maturation, namely, immature, mature and over-mature, i.e., 40, 50 and 60 days after fruit set (DAFS). It was found that there was a possibility to use short wavelength region of near infrared radiation (700-1100 nm) to determine quality of Japanese pumpkin in terms of total soluble solids (TSS). The spectra were measured by NIRSystem 6500. Partial least squares regression (PLSR) was introduced to develop prediction models for the aforementioned parameters. The best PLS model for quality assessment of Japanese pumpkin fruit was found in TSS, resulting the coefficient of determination (R^2) of 0.77. The prediction model also provided low values of SEC, SEP and bias which were 0.62%, 0.96% and 0.13%, respectively.

Keywords: Japanese pumpkin, near infrared, internal quality

INTRODUCTION

Kabocha or Japanese pumpkin (*Cucurbita maxima)* is a Japanese variety of winter squash. It belongs to the genus *Cucurbita* and the family *Curcurbitaceae*, It is one of best-selling vegetables of the Royal Project Foundation, Thailand, considered to be a health-promoting vegetable due to its high contents of multivitamins and antioxidative-pigmented compounds such as carotenoids and phenolic compounds (Hurst *et al.,* 1995). It is commonly called Japanese pumpkin. In Thailand, it is called "Japanese Fak Thong" or "golden squash". It is rich in beta-carotene, iron, vitamin C, potassium and smaller traces of calcium, folic acid and multivitamin B. It is typically small (about 1-2.5kg), mature fruit of Japanese pumpkin has dark green skin and dark orange flesh. Japanese pumpkin maturity is more difficult to determine. The internal quality is the most important index of Japanese pumpkin fruit maturity. Nowadays, most of Japanese pumpkin fruits are rejected because it is frequently found that immature fruits are mixed. As a harvesting time cannot evaluate maturity of Japanese pumpkin fruits and mostly is assessed by human experience, they are evaluated by day counting after planting and checking stem dryness. Although these methods can assess maturity of Japanese pumpkin fruit and provide reliable results but still there are losses arising from the mix of immature fruits and low efficiency after long-time working. Near infrared technology is recommended for agricultural produce for maturity evaluation and internal quality assessment because it is a fast, no chemical use and non-destructive technology. Many researchers used NIR spectroscopy as a non-destructive method for maturity evaluation and internal quality assessment of different kinds of agricultural products such as pumpkin fruit (Murakami *et al*., 1992; Saridnirun *et al.*, 2011), Suphap *et al.*, 2011), summer squash (*Cucurbita pepo L.*) (Blanco-Díaz *et al*., 2014; Martínez-Valdivieso *et al*., 2014), mangoes (Saranwong *et al*., 2001, 2003, 2004; Theanjumpol *et al.*, 2010), apples (Liu and Ying, 2005; Liu *et al*., 2007), strawberry (Noimanee *et al.*, 2012), sweetcorn (Noimanee *et al*., 2012) and kiwifruits (Clark *et al*., 2004). Besides, NIRs is applied to assess fruit quality by means of its chemical and physical properties, for example, total soluble solids, starch, chlorophyll, carotenoid and others. Thus, the objective of the present study is designed to study the possibility of near infrared spectroscopy to evaluate internal quality of Japanese pumpkin fruit.

MATERIALS AND METHODS

Samples preparation: Japanese pumpkin fruits from "Mone Khoa", the Royal Project Foundation Development Center, Mae Tang District, Chiang Mai, Thailand (Highland area and low temperature) were harvested at 3 stages, immature (40days after fruit set; DAFS), mature (50 DAFS) and over-mature (60 DAFS). At each stage, 90 fruits were harvested. The samples were separated into two samples set; 50 fruits for calibration sample set and 40 fruits for validation sample set. Then the sample fruits were transported to our laboratory of Postharvest Technology Research Institute, Chiang Mai University and stored samples at 25˚C during experiment. The samples were measured for morphological characteristics including thickness, diameter, height and weight before spectrum acquisition. The thickness, diameter and height of Japanese pumpkin were measured by digital vernier caliper (Mituyo, Japan). The fruit weight was measured by digital fine balance 2 digits increment (Mettler Toledo, Switzerland).

Spectrum acquisition data: NIR spectrum of Japanese pumpkin fruits was measured in short wavelength region from 700-1100 nm (14,285-9,090 cm^{-1}) at harvest date with NIR system 6500 spectrophotometer (Foss NIRSystem, Silver Spring, USA). The NIRsystems 6500, all spectra were acquired raw spectrum using interactance mode with the fiber optic probe showed in Figure 1. To control the temperature of the samples at 25°C, a water bath (EYELA, Japan) covered with thin polyethylene film. It was used for control the samples temperature by dipping in 10 minutes before acquisition and during experiment controlled room temperature at 25°C showed in Figure 2. Both sides of the fruit were measured for NIR spectrum by averaging 50 scans. A reference measurement of Teflon cylinder was performed every 5 samples.

Figure-1: NIRsystem 6500 with the fiber optic "interactance probe" was used to measure the spectrum of Japanese pumpkin fruit.

Figure-2: Japanese pumpkin fruit with the temperature ontrolled at 25°C by dipping in a low-temperature bath.

Chemical analysis: Within thirty minutes after spectra acquisition, a portion of each fruit side irradiated by NIR was cut about 3*3*3 cm (length*width*depth) used for total soluble solids (TSS), dry matter (DM) and starch determination. The total soluble solids (TSS) content was measured by a digital refractometer (PAL-1, Atago, Japan). Each fruits were used to determine TSS content by using the juice squeezed using high speed blender from the cut fraction. The measurement was taken, and TSS content of each fruit (%) was displayed (Hai *et al*., 2015). Dry matter (DM) was determined by chopping the sample fruit into small pieces about 10 grams. Then the sample were weight by digital balance 2 digits (Mettler Toledo, Switzer-land) and then put in hot air oven (Air oven Venticell 111, MMM Medcenter Einrichtungen GmbH, Germany) 70ºC for 48 hours (AOAC, 2000) to get dry sample weight and calculated by comparing the weight deference before and after drying. Dry matter was reported as percent dry matter (%DM) calculated from Eq. (1).

$$\%\ Dry\ matter = \left(\frac{(dry weight\ (g))}{(fresh\ weight\ (g))}\right) \times 100 \quad (1)$$

Starch content was measured with "Total Starch Assay Kit" (Megazyme, Catalog No. K-TSTA, Wicklow, Ireland) That is, the dried sample to pass a 0.5 mm screened mill "Cyclotec 1093" (Foss Tecator AB, Hoganas, Sweden) for starch analysis. Add flour sample (about 100 mg, weighed accurately) to centrifuge tube and then add 5 mL of aqueous ethanol (80% v/v), and incubate the tube at 80ºC for 5 minute. Mix the content and add 5 mL of 80% aqueous ethanol. Then centrifuge at 3,000 rpm for 10 minute and discard supernatant. Immediately add 3 mL of thermostable α-amylase in MOPS buffer (50 mM, pH 7.0) and incubate in a hot water bath at 100°C for 6 minutes. Place the tube in a water bath at 50ºC; then add sodium acetate buffer (4 mL, 200 mM, pH 4.5), followed by amylo-glucosidase (0.1 mL, 20U) and mixed by vortex for 30 minutes. Transfer the solution to 100 mL volumetric flask and then adjust with distilled water. Mixed and centrifuged solution at 3,000 rpm for 10 minutes. Transfer sample solution to test tube add 3.0 mL of GOPOD reagent, and incubate the tube at 50ºC for 15 minutes. Then, the absorbance at 510 nm for sample and glucose control is read against the reagent blank with spectrophotometer UV/VIS model specord40 (Analytik jena, Germany). and calculated for the amount of starch contents was calculated as 90% of measured glucose (McCleary *et al.,* 1997) as follows Eq. (2).

$$Starch\ (\%) = \Delta E \times F \times \left(\frac{1}{W}\right) \times 90 \qquad (2)$$

where: ΔE =Absorbance (reaction) read against the reagent blank.,

F = 100 (μg of glucose) / abs. of 100 μg of glucose.

W = The weight in milligrams of the flour analysed.

Data preprocessing and calibration equation development: In this study, all NIR spectra were explained in term of the absorbance (log(1/R)). The spectrum data were transformed with the second derivative was performed with Savitzky-Golay method (10 nm averaging for left and right side) and smoothing (SM) and multiplicative scatter correlation (MSC) used for develop the model. the samples including spectral data, TSS value, DM value and starch value, were separated into two sets. Calibration set was used for model development and validation set was used to test the model. Then, the partial least squares regression (PLSR) was used to develop the model in conjunction with the Unscrambler® program Version 9.8 (CAMO, Oslo, Norway) to evaluate Japanese pumpkin fruit quality. It was used in order to build predictive model of TSS, DM and starch. The calibration model efficiency was illustrated by correlation coefficient between prediction value and actual value. The effective models were defined by correlation coefficient of 0.71 or higher as reported by Williams (2007). In this study, the calibration and prediction models efficiency were determine by the following statistic values in terms of correlation coefficient (R^2), standard error of prediction (SEP), standard error of calibration (SEC), residual predictive deviation (RPD) value and bias value. The calculations as following equations:

$$SEP = \sqrt{\frac{\sum_{i=1}^{n_p}(x_i - y_i - Bias)^2}{n_p - 1}} \qquad (3)$$

$$SEC = \left(\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N-1}\right)^{\frac{1}{2}} \qquad (4)$$

$$RPD = \frac{S.D._{pre}}{SEP} \qquad (5)$$

$$Bias = \frac{\sum_{i=1}^{n_p}(x_i - y_i)}{n_p} \qquad (6)$$

where x_i is the predicted value of TSS, DM and starch; y_i is the measured value; n_p is the number of observations used in validation set; N is total number of samples; and $S.D._{pre}$ is the standard deviation of measured values in validation set.

RESULTS AND DISCUSSION

Morphological characteristic: The maximum, minimum, mean and standard deviation (S.D.) values of Japanese pumpkin fruit in term of fruit weight, fruit diameter, fruit height and pulp thickness of samples were used in calibration and validation sets shown in Table 1. The diameter, height, weight and thickness of Japanese pumpkin fruit were showed that the average of diameter and height of samples at 40 DAFS were 9.22 and 9.02 mm, respectively. The averages of diameter of Japanese pumpkin fruit at 50 and 60 DAFS were 14.69 and 15.25 mm, respectively. Moreover, the mean of height at 50 and 60 DAFS were 9.92 and 10.48 mm, respectively. Fruit weight of Japanese pumpkin fruit greatly increased during maturity stage and fruit weight of them were 1110.95, 1185.13 and 1321.92 g, at 40, 50 and 60 DAFS, respectively. However, thickness of edible part of Japanese pumpkin fruit used in the experiment varied between 2.3-3.7 cm depend on maturity of the samples. For typical NIRs analysis, diameter, shape, size, thickness, rough skin and opaque property of sample fruit directly affects light absorption and scattering.

Table-1: Morphological characteristics of Japanese pumpkin used in this study

Parameter	Min.	Max.	Mean	S.E.	S.D.
Height (cm)	8.3	11.5	9.9	0.07	0.62
Diameter (cm)	11.5	16	13.75	0.20	1.89
Weight (g)	776.7	1484.9	1164.55	16.04	152.2
Thickness(cm)	2.3	3.7	3	0.09	0.9

Table shows the value of mean Standard error (SE.)
Table shows the value of mean Standard deviation (SD.)

Chemical properties of Japanese pumpkin fruit distributions: The range, mean and S.D. of total soluble solid, dry matter and starch of calibration and validation sets of each stage are shown in Table 2. A typical Japanese pumpkin fruit contained 9-11% of soluble solids. Generally levels of TSS in pumpkin fruit was 11% or greater are considered necessary for good eating quality in pumpkin (Loy, 2004). On the other hand, Sombun *et al.,* 2012 reported that soluble solid content in pumpkin was cultivated in Thailand contained with 11-15% of soluble solids. It depend on maturity, area of cultivation and varieties. In this study, Japanese pumpkin fruit samples contained total soluble solids (TSS) in a standard range. As for dry matter (DM), typically, a fully mature fruit contain 20-30% (Harvey *et al.,* 1997), however, DM value in this study were below 20%. The low value of DM might be explained by cultivation varieties, production area and production. Percentages of starch at each maturity stage were 19.27, 21.45 and 20.94, respectively. They also changed after fruit development, TSS and DM increased during fruit maturity while percent starch was slightly increased during mature stage and decreased to 20.94% after 50 DAFS.

Table-2: Distribution of chemical properties of Japanese pumpkin in calibration and validation sets.

Parameter	Data set	No. fruit	Statistical parameter					
			Max.	Min.	Mean	S.D.	S.E.	Replication (Time/fruit)
TSS (%)	Calibration	50	14.1	7.0	10.44	1.65	0.23	2
	Validation	40	14.1	7.2	10.17	1.68	0.24	2
DM (%)	Calibration	50	20.7	10.61	14.57	3.13	0.44	2
	Validation	40	21.0	10.78	14.29	2.83	0.40	2
Starch (%)	Calibration	50	22.4	16.8	19.66	1.89	0.27	2
	Validation	40	22.4	16.8	19.62	2.01	0.28	2

Table shows the value of Standard error of the mean (SE.)
Table shows the value of mean Standard deviation (SD.)

Feasibility of near infrared application: Figure-3A exhibits the mean original spectra of Japanese pumpkin fruit from interactance mode which shows a clear peak at 975 nm which is related to the second overtone of water. Normally, peak of water molecules shows a clear peak at 980 nm (Osborne, 1986), 964 nm (Kawano *et al*., 1995) and 976 nm (Jannok *et al*., 2014). The absorbance of water molecules in Japanese pumpkin fruit decreased when maturity increased. The spectra were shifted upward or downward, depending on the scattering conditions of fruit flesh (Osborne, 1993). The 2nd derivative technique was used to transform the spectral data to reduce the effect of baseline shift, overlapping peak and noise. The result of this present study showed that the peak of water molecules of original spectra Japanese pumpkin fruits changed from 975 to 960 nm after changed to second derivative (Figure-3B). The NIR could be used to predict the internal quality of Japanese pumpkin fruit in short wavelength range of 710-1090 nm because it contributed with the organic molecules, and contain bonds of C-H, O-H, C-O and C-C (Osborne *et al.,* 1993). Thus, it is possible to use NIR for determination internal quality of Japanese pumpkin fruit. In this study, NIR is sensitive to the concentration of organic materials but unfortunately, the original spectrum of Japanese pumpkin showed only clear peak at 975 nm. Their peak overlapped the peak of water molecule since the wavelength range 950-980 nm is the effective wavelengths of water, because the strong water absorbance bands are present around 960 nm and 760 nm for the second and third harmonics of the fundamental O-H stretching vibration (Liu *et al*., 2010).

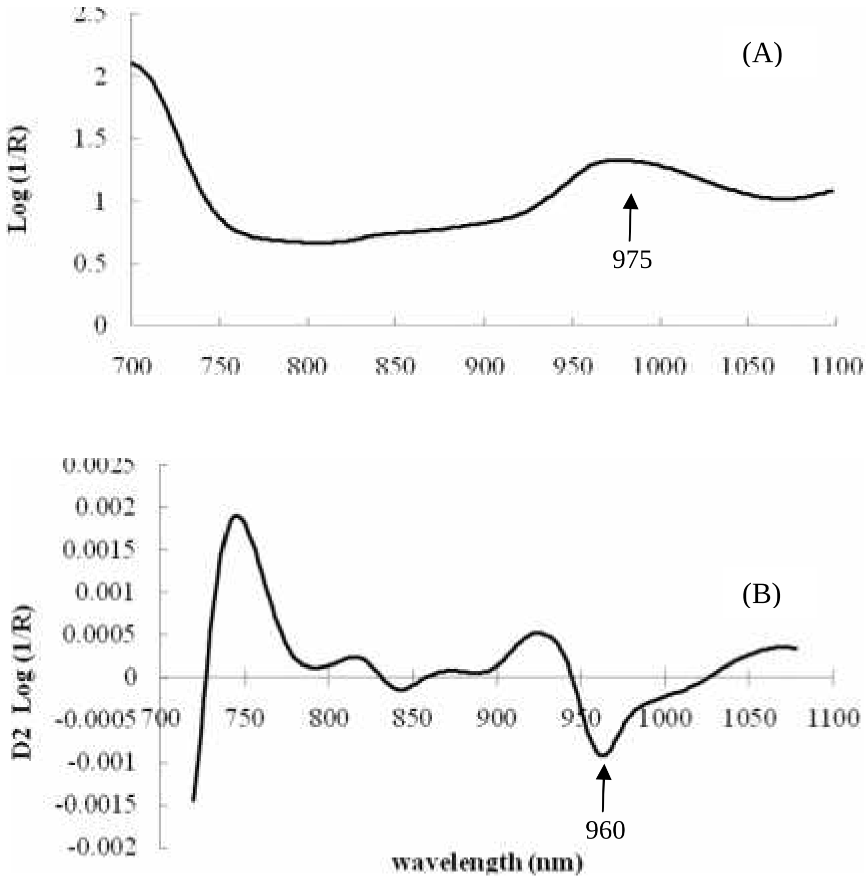
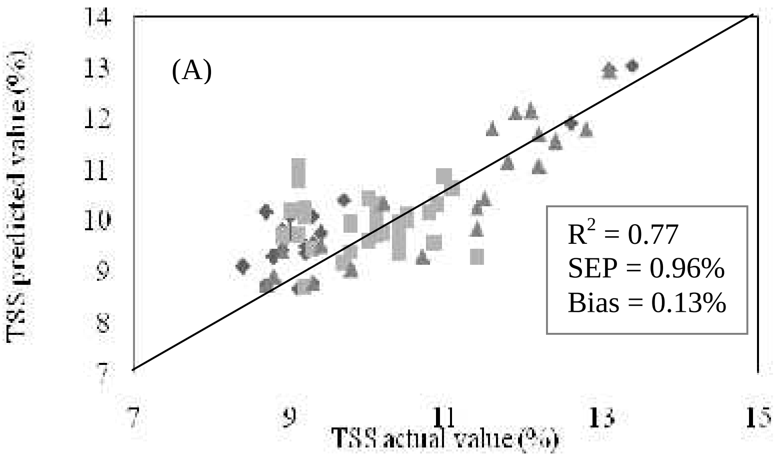


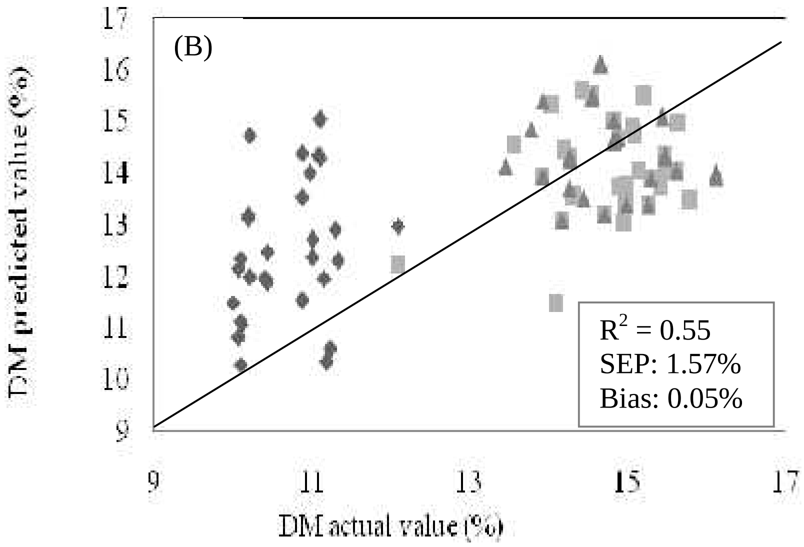
Figure 3. Original and second-derivative spectra of Japanese pumpkin fruit at each stage.

NIR spectrophotometer was applied to assess internal fruit quality by average of chemical and physical properties itself, for examples, the potential of interactance mode of NIRs with fiber optics was studied to estimate the soluble solid content. The spectra from 680 nm to 1235 nm were obtained by NIR spectroscopy (Kawano *et al*., 1992 and 1995). NIRs can be used to assess physiological characteristics such as apple firmness (Cho *et al.*, 1992). The measurement of tiratable acidity (TA) of satsumas showed that error of NIR determination was about 20% (Miyamoto *et al.*, 1998). Likewise, Saranwong *et al*., (2004) and Theanjumpol (2012) successively developed the NIR calibration equations for harvest quality prediction of unripe mango (hard-green) fruit by means of its dry matter (DM) and starch content. In addition, Murakami *et al*., (1992) reported the moisture, reducing sugar and total sugar contents of pumpkin fruit which were measured by NIR spectroscopy without distraction. As a result of the experiments, SEP of the moisture, reducing sugars and total sugar were 3.94, 1.20 and 0.84%, respectively.

The results of calibration and prediction for TSS: The results of PLS regression performed for TSS were developed using the treated spectral data with MSC and second derivative (10 nm average for left and right sides) in the spectral range 710-1090 nm wavelength range. Many research were suggested that the range of 850-1088 nm is the effective wavelengths for sugar prediction because the sugar absorbance bands are present around 908 nm (Kawano *et al*., 1992; William and Norris, 2001). The regression coefficient plot exhibited the highest value at various wavelengths which affected the PLS model. Theanjumpol (2014) reported that the results of TSS and DM gave high value at the similar wavelength: 816, 904, 944 and 976 nm whereas TA had high value at wavelength 778, 888 956 and 986 nm. To reduce effect of base line shift, overlapping peaks of water, light scattering, peaks shift, effect of sample opaque, sample shape and particle size, the spectral data were treated with pretreatment mathematic methods before they were used to develop the PLS models (Osbone, 1993; Williams and Norris, 2001; Theanjumpol, 2014). In this study, the best PLS model of TSS validations by second derivative and MSC pretreatment showed R^2, SEC, SEP and the average of the difference between actual and prediction value (bias) and RPD were 0.77, 0.62%, 0.96%, 0.13% and 1.75 respectively showed in Table 3. Williams and Norris (2001) explained about RPD value which related the SEP to the SD and simplifies the interpretation of SEP. The value of RPD indicated efficient NIR predictions and RPD values were not lower than 6.72, RPD values ranging between 6.5-8.0 indicate a very good performance of the model.

The scattered plot of TSS analysis data of Japanese pumpkin fruit in validation sets showed in (Figure-4A). They showed a high correlation between TSS actual value (conventional method) and TSS predicted value (NIR predicted value) but the prediction results were not excellent because R^2 not higher than 0.71. It occur from influenced of Japanese pumpkin morphological effect, internal space, thickness and opaque property affecting absorbance spectra. Meanwhile, Nicolaï *et al.*, (2007) reported that a good model for prediction should provide a high R^2 and low SEC and SEP, but both of them should not high difference from each other.





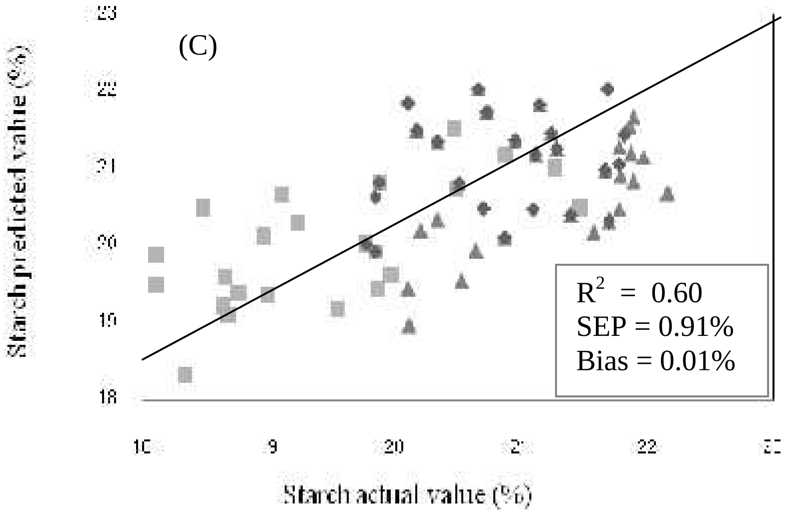


Figure-4: Scattered plot of TSS, DM and starch prediction results of Japanese pumpkin fruit

The results of calibration and prediction for DM: The PLS model of DM are shown in Table-3. They were developed PLS model following TSS model step, the spectra of Japanese pumpkin fruit were treated using the mathematical methods. The best results PLS model of DM was developed using the treated spectral data with only 2nd derivative (10 nm average for left and right sides) in 710-1090 nm wavelength range. The PLS model validations showed R^2, SEC, SEP, bias and RPD of DM were, 0.55, 1.40%, 1.57%, 0.05% and 1.80, respectively. Figure 4B shows the scattered plot of DM analysis data in whole stage of Japanese pumpkin fruit. They show a low correlation between DM actual value and predicted value (NIR predicted value) meanwhile, the regression coefficient plot exhibited the lower value at various wavelengths which affected the PLSR model. In our study, the prediction of DM results were not excellent because influenced of the Japanese pumpkin fruit itself. As to morphological of Japanese pumpkin fruit effect, thickness and opaque of pumpkin fruit might interrupt the light path, affecting absorbance spectra, the ability of light to pass through objects and diminishing model accuracy. Especially, effect of uniformity of internal quality of fruit might also decrease model accuracy. Thus, it necessary to keep data of the spectral acquisition at many position of sample fruits and take many number of repeats (Maniwara *et al.*, 2014; Nicolaï et al., 2007).

The results of calibration and prediction for starch: The result of starch prediction was showed in Table 3. In term of prediction, the highest R^2 value were obtained with the full range of acquisition spectra. The best result of starch was developed PLS model by using the original spectral data in 700-1100 nm wavelength range. The results were an R^2 of 0.60, SEC of 0.75%, SEP of 0.91%, bias of 0.01% and RPD of 2.20, respectively. The scattered plot of starch value between actual value and NIR predicted value of Japanese pumpkin fruit in both calibration and validation sets are showed in Figure 4C. The result showed that their scattered plot had low correlation. Meanwhile, the regression coefficient plot exhibited the lower value at various wavelengths, which affected the PLSR model similar to scattered plot of dry matter.

Table-3: Result for calibration and validation of Japanese pumpkin fruit quality.

Quality parameter	Pre-treatment	Wavelength (nm)	F	R^2	SEC	SEP	Bias	RPD
TSS	Original	700-1100	6	0.69	0.73	1.10	0.06	1.52
	MSC	700-1100	6	0.70	0.72	1.19	0.03	1.41
	2nd	710-1090	6	0.46	0.97	1.03	0.07	1.63
	2nd+MSC	**710-1090**	**6**	**0.77**	**0.62**	**0.96**	**0.13**	**1.75**
DM	Original	700-1100	6	0.54	1.41	1.35	0.24	2.09
	MSC	700-1100	6	0.54	1.40	1.36	0.12	2.08
	2nd+MSC	690-1090	**6**	0.43	1.57	1.60	0.29	1.76
	2nd	**710-1090**	6	**0.55**	**1.40**	**1.57**	**0.05**	**1.80**
Starch	MSC	750-1100	6	0.51	0.83	0.97	0.01	2.07
	2nd	690-1090	6	0.53	0.81	1.00	0.1	2.01
	2nd+MSC	690-1090	6	0.35	0.96	1.01	0.06	2.01
	SM+MSC	690-1090	6	0.56	0.79	0.89	0.06	2.25
	Original	**700-1100**	**6**	**0.60**	**0.75**	**0.91**	**0.01**	**2.20**

Note: TSS: total soluble solid (%); DM: dry matter (%w/w); MSC: multiplicative scatter correction; SM: smoothing; 2nd: second derivative; F: number of factors used in the calibration equation; R^2: correlation of determination; SEC : standard error of calibration; SEP: standard error of prediction and Bias: average of difference between actual value and NIR predicted value

Structure of the common calibration model: The results of regression coefficient plot showed that total soluble solids (TSS), dry matter (DM) and starch (ST) gave high values of regression coefficient at respective wavelength, which related to internal quality of Japanese pumpkin fruit. In this study, the results showed that TSS gave high regression coefficient values at wavelength 798, 820, 880 and 955 nm whereas DM had high value at 798, 825, 880, 920 and 955 nm and starch gave high values at wavelength 798, 832 and 944 nm showed in Figure 5. In addition, Slaughter and Thomson (2003) identified NIR absorbance bands for water at 960 nm while Williams and Norris (1987) identified water absorbance peak at 834, 938, 958, 978, 986, 994, 1010 and 1099 nm. Sugar and total soluble solid content were showed absorbance peak at 838, 888, 913, 978 and 1005 nm. Starch showed peak at 878, 901 918 979, 1030 and 1053 nm. Katayama *et al*. (1996) reported that effective wavelength of starch exhibited positive wavelength at 874 and 948 nm and negative wavelength at 765, 910, 997 and 1161 nm. For moisture content, positive wavelength exists at 910 and 999 nm and negative at 881 and 948 nm. For sugar, correlation coefficients were significant and positive wavelength at 732, 833 and 950 nm and negative at 914 and 1030 whereas Murakami *et al.* (1992) selected effective wavelength of sugar at 833, 888 and 912 nm. Moreovre, the effective wavelength of day matter was showed absorbance peak at 908, 944 and 974 nm, their starch were 884, 928, 958 and 992 nm. (Theanjumpol, 2012 and Sarawong, 2003).

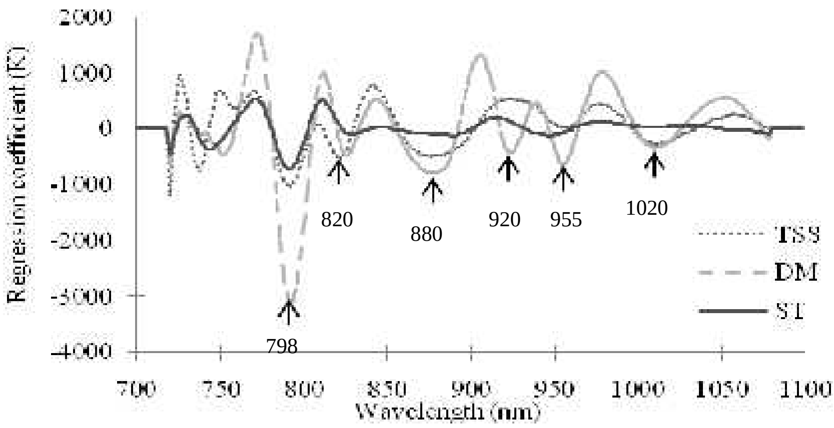


Figure-5: Regression coefficient plot of PLS calibration models of TSS, DM and starch of Japanese pumpkin fruit at whole maturity stages.

CONCLUSIONS: In this study, the NIRs can be applied to check the internal quality of Japanese pumpkin fruit, in terms of TSS. It can be possible to predict the quality of Japanese pumpkin fruit but in terms of DM and starch cannot be predicted. The best result of internal quality prediction except for TSS parameter was showed in the correlation coefficient of determination; R^2 was 0.77, SEC and SEP were 0.62% and 0.96%, respectively. the combination was PLS model with MSC and 2^{nd} derivative spectral pretreatment for internal assessment of Japanese pumpkin quality using only TSS model. These techniques are applicable for samples with low light scattering and low optical density (Slaughter *et al.,* 2003). The main sources of error were the shape of sample fruit, ununiformed fruit quality, Japanese pumpkin fruit translucence and methodology of measurement. These errors occurred because of the rough surface of the pumpkin epicarp, NIR rays cannot pass through deep enough of the sample fruit and the difference in circumference. These influenced the reflected NIR ray. Almost all of the NIR rays of the pumpkin were reflected from the surface and just a small portion of the beam of NIR ray penetrated and returned to the detector of the sensor. Therefore, the irregular shape of the surface influences the absorbance.

However the prediction value should be done the bias correction when they were used for the routine analysis**.** If a higher accuracy of PLS model is required, preparation of the sample and methodology of measurement are necessary to be improved, for example, the fiber optic probe could be designed for Japanese pumpkin to reduce the influence of the round surface of pumpkin and fruit opaque.

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