Note

Nondestructive Evaluation of Anthocyanin Concentration and Soluble Solid Content at the Vine and Blossom Ends of Green Mature Mangoes during Storage by Hyperspectral Spectroscopy

Yoshio MAKINO^{1*}, Aiko ISAMI¹, Takehiro SUHARA¹, Kenjiro GOTO¹, Seiichi OSHITA¹, Yoshinori KAWAGOE², Shinichiro KUROI³, Yohanes A. PURWANTO⁴, Usman AHMAD⁴ and SUTRISNO⁴

¹ Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

² Nihon University, Kameino 1866, Fujisawa, Kanagawa 252-0880, Japan

³ Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe 657-8501, Japan

⁴ Bogor Agricultural University, Gedung Fateta Lt 2, Kampus IPB Darmaga, Bogor, Indonesia

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Internal and external qualities of mangoes during storage were evaluated by hyperspectral spectroscopy. Green mature mangoes were harvested in Okinawa Prefecture, Japan, and stored for 14 d at 27°C [relative humidity 90%]. Spectral reflectance (380 – 1000 nm), anthocyanin concentration, chlorophyll concentration in skin, and soluble solid content (SSC) in flesh were measured during storage. Anthocyanin concentration and SSC were nondestructively determined with correlation coefficients of 0.88 [root mean square error of cross validation (RMSECV) 2.96 mg·100 g f.w.⁻¹] and 0.73 (RMSECV 0.98%), respectively, using partial least squares regression (PLSR) models. The proposed nondestructive method may be effective for evaluating internal and external qualities of mangoes simultaneously.

Keywords: *Mangifera indica* L., pigment, postharvest, quality, statistical analysis, tropical fruit

Introduction

Mango is a major fruit worldwide, with the total production of mangoes, mangosteens, and guavas reaching 42 million tons (FAOSTAT, 2012)ⁱ. In Japan, mangoes are produced mainly in the Okinawa and Miyazaki prefectures (3,300 tons in 2011), and production is increasing yearly (Statistical report by the Ministry of Agriculture, Forestry and Fisheries, 2011)ⁱⁱ. Kikuchi *et al.* (2011) reported that mangoes are graded based on the percentage of red color on the surface of the fruit; mangoes are subjectively graded with the naked eye. Jha *et al.* (2007) modeled color values of “Dashehari” mangoes, measured using a HunterLab colorimeter, by multiple linear regression (MLR), principal components regression (PCR), and partial least squares regression (PLSR) analyses. Anthocyanin is responsible for the red color of this fruit. (Berardini *et al.*, 2005). However, no reports until now have

described the objective evaluation of redness on the basis of anthocyanin concentration. That is, scientifically based prediction methods to evaluate skin redness as anthocyanin concentration are desired.

In contrast, there have been several studies on the nondestructive evaluation of internal qualities of intact mango fruit by near-infrared spectroscopy (NIRS). Tanabe *et al.* (1995, 1996) measured the soluble solid content (SSC) of “Irwin” mangoes. Guthrie and Walsh (1997) measured the dry matter (DM) of “Kensington” mangoes. Schmilovitch *et al.* (2000) determined the SSC, acidity, firmness, and storage period of “Tommy Atkins” mangoes. Hasbullah *et al.* (2002) predicted the internal qualities of heat-treated “Irwin” mangoes. Saranwong *et al.* (2004) predicted the SSC, DM, and starch concentrations of “Mahajanaka” mangoes. Subedi *et al.* (2007)

*To whom correspondence should be addressed.

E-mail: amakino@mail.ecc.u-tokyo.ac.jp

and Subedi and Walsh (2011) predicted the DM and SSC of four varieties of mangoes. Kader (2002) reported that sweetness is one of the most important qualities for fruits in general. Accordingly, SSC should be considered as a criterion for fruit grading along with the red color. Thus, a nondestructive method for evaluating fruit contents should be developed. A hyperspectral camera was chosen in this study because it has been applied to the measurement of spectral reflectance/absorbance with spatial information (Sun, 2010).

In this study, an objective analysis of the surface redness of mangoes was attempted using a hyperspectral camera. The effectiveness of this method was investigated by evaluating differences in skin redness depending on the sampling point. Skin redness differs between the vine and blossom ends (Kikuchi *et al.*, 2011). Furthermore, nondestructive prediction of SSC, one of the most important fruit qualities, was attempted with the hyperspectral camera. These contents change during storage owing to enzymatic activities (Medlicott *et al.*, 1986; Saranwong *et al.*, 2004). Therefore, we aimed to nondestructively monitor these contents post-harvest using the hyperspectral camera.

Materials and Methods

Samples and storage method Green mature mangoes (*Mangifera indica* L., cv. Irwin) were harvested on July 5, 2012 at a farmland in Yaese (Okinawa Prefecture, Japan). After harvest, the samples were transported to the laboratory within 24 h at ambient temperatures. This maturity level was selected because pigments and SSC changes are greater at this stage than at complete maturity during postharvest ripening (Makino *et al.*, 2011). Samples consisting of 23 fruits were stored at constant temperature and humidity (27°C, 90% R.H.) for 14 d for subsequent experiments. Three fruits were selected at 2 d intervals and used for subsequent experiments. Samples with a wide range of SSC and skin color were prepared during storage.

Spectral reflectance measurement with a hyperspectral camera system The spectral reflectance of mangoes was measured at a range of 380–1000 nm using a hyperspectral camera system (JFE

Techno-Research Co., Tokyo, Japan) (Fig. 1, Table 1), with a wavelength resolution of 5 nm and a stage driving velocity of 0.41 mm s⁻¹. (Siripatrawan *et al.*, 2011). The light intensity reflected from the sample was converted by adjusting the intensity of the dark current to 0 and that of the white standard to 1. A SpectrumAnalyzer[®] ver. 1.8.6 (JFE Techno-Research Co.) was used for storing and processing the “hypercube data” (Sun, 2010) obtained with the hyperspectral camera system. The spectral reflectance of each sample was measured at 2 d intervals during storage.

Destructive and nondestructive analysis of SSC in flesh, anthocyanin and chlorophyll concentrations in skin The relationships between reflectance and anthocyanin concentration, chlorophyll concentration, and SSC were determined according to the method of Nicotra *et al.* (2003). The spectral reflectance of a mango was measured before and after sampling. A cuboidal piece of flesh (15 × 20 × 15 mm) was sampled from the vine and blossom ends. The mean spectral reflectance was calculated at the sampling site using the SpectrumAnalyzer[®].

Sampled skin was halved to measure anthocyanin and

Fig. 1. Overview of hyperspectral camera system (JFE Techno-Research, Co., Tokyo, Japan)

Table 1. Components of the hyperspectral camera system assembled by JFE Techno-Research Corporation (Tokyo, Japan)

Part	Component	Model	Maker
A	Sample	Mango	—
B	Sample stage	SGSP26-200	Sigma Koki Co., Ltd. (Tokyo, Japan)
C	16 mm lens	C1614A	HOYA Corporation (Tokyo, Japan)
D	Prism/grating/prism assembly	V10C ImSpector	Spectral Imaging Ltd. (Oulu, Finland)
E	12 bit CCD camera	MC1002PF	Texas Instruments Inc. (Dallas, USA)
F	150 W Xe lamp	Super Bright 152S	SAN-EI Electric Co., Ltd. (Osaka, Japan)
G	150 W tungsten halogen lamp	ColdSpot PCS-UHX	Nippon P•I Co., Ltd. (Tokyo, Japan)
H	250 mm illumination rod	PDL-T-250F8	Nippon P•I Co., Ltd. (Tokyo, Japan)
I	17 inch monitor	E178FPc	Dell Co., Ltd. (Kawasaki, Japan)
J	Desktop computer ^a	Vostro 220	Dell Co., Ltd. (Kawasaki, Japan)
K	40 mm × 220 mm white reference	Spectralon	Labsphere Inc. (North Sutton, USA)

^a Implemented with SpectrumAnalyzer ver. 1.8.6 (JFE Techno-Research Co., Tokyo, Japan)

chlorophyll after denaturation of chlorophyllase by heating at 100°C for 3 min and homogenization. Anthocyanin concentration in the sampled skin was measured according to the method of Nagata *et al.* (2006). Formic acid (5%) was used as the solvent for anthocyanin extraction. The absorbance at 530 nm of the extract was converted to the mass of delphinidin (molar extinction coefficient: $2.154 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$), a kind of anthocyanin. Chlorophyll *a* and *b* concentration was measured according to the method of Porra *et al.* (1989). Acetone (80%) was used for chlorophyll extraction and absorbance was measured at 645 and 663 nm. A nyal[®] nylon screen (pore size: Ø68 µm; Sefar AS, Heiden, Switzerland) was used for squeezing the sampled flesh. The SSC of the sampled flesh preparation was measured using a PR-201 digital refractometer (Atago Co. Ltd., Tokyo, Japan).

Prediction of anthocyanin concentration and SSC by statistical models Second derivatives of 46 reflectance spectra (Savitzky and Golay, 1964) were calculated as the independent variables. PLSR was conducted to predict SSC and anthocyanin as dependent variables using Unscrambler[®] ver. 10.3 (CAMO Software AS., Oslo, Norway). The prediction models were validated by leave-one-out cross-validation. Then, the smoothing points to calculate second derivatives of spectra were selected as the number in which the root mean square error of the cross validation (RMSECV) values was minimal. The number of latent variables (LVs) selected was based on the maximal total variance of the dependent variables. Effects of storage period and the sampling site on anthocyanin concentration and SSC were estimated by two-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test using JMP[®] Pro ver. 10.02 (SAS Inst., Cary, USA).

Results and Discussion

Changes in anthocyanin concentration and SSC at the vine and blossom ends measured by destructive methods According to the results of two-way ANOVA, differences in sampling site and storage period did not significantly affect SSC measurement. Krishnamurthy and Subramanyam (1973) reported that SSC of mangoes increased during storage due to postharvest ripening. Hasbullah *et al.* (2002) compared the SSC of heat-treated "Irwin" mangoes between the vine and blossom ends by NIRS and reported that SSC at the vine end was greater than that at the blossom end. The results of SSC in the present study differed from the previous reports. The variation of measured data observed in the current study was possibly caused by individual variability, given that each datum was taken from a different sample.

In contrast, differences in the sampling site significantly affected anthocyanin concentration measurement. This compound is the main pigment responsible for the red color of the skin (Barardini *et al.*, 2005). As redness at the vine end is normally greater than that at the blossom end (Kikuchi *et al.*, 2011), anthocyanin concentration may be affected by differences in the

sampling site. However, the storage period did not affect the variability of the measured data. Medlicott *et al.* (1986) reported a decrease in anthocyanin during storage. The results of the current study differ from the previous report. However, these data for anthocyanin concentration and SSC can be used for calibration of spectral data for nondestructive prediction.

Prediction model for anthocyanin concentration and SSC Results of PLSR are shown in Fig. 2. According to Williams and Norris (2001), the correlation coefficients revealed that prediction models for anthocyanin and SSC were effective for screening and rough screening, respectively. In the 1990s, MLR was frequently used for predicting mango quality by NIRS (Tanabe *et al.*, 1995, 1996; Guthrie and Walsh, 1997). More recently, PCR and PLSR have been commonly used for prediction (Schmilovitch *et al.*, 2000; Hasbullah *et al.*, 2002; Saranwong *et al.*, 2004; Subedi *et al.*, 2007; Subedi and Walsh, 2011). In previous studies, internal quality was predicted with these linear models constructed from spectral reflectance/absorbance. The correlation coefficient for SSC

Fig. 2. Relationship between measured and predicted values of (A) anthocyanin concentration in the skin and (B) soluble solid content in the flesh of mangoes modeled by partial least squares regression analysis

The open circles denote actual values and the straight lines denote regression lines. Correlation coefficients were (A) 0.88 and (B) 0.73; root mean square errors for cross validation were (A) 2.96 mg·100 g f.w.⁻¹ and (B) 0.98%; the numbers of latent variables were (A) 7 and (B) 3.

Fig. 3. Score plots of latent variables, LV 1 and LV 2 calculated by partial least squares regression analysis grouped on the basis of (A) anthocyanin concentration in the skin (threshold, $6 \text{ mg} \cdot 100 \text{ g f.w.}^{-1}$; closed circles, high; open circles, low) and of (B) chlorophyll *a* concentration in the skin (threshold, $2.5 \text{ mg} \cdot 100 \text{ g f.w.}^{-1}$; closed circles, high; open circles, low); (C) loading plot (solid line, LV 1; dashed line, LV 2); and (D) spectral reflectance (black lines, vine end; gray lines, blossom end); the number of data points for Savitzky–Golay smoothing was 11

in the current study was lower than those in the previous studies (0.80–0.96). This finding suggests that SSC prediction using hyperspectral spectroscopy is more difficult than that by point measurement. NIRS is effective for the internal prediction of SSC due to its high permeability. This indicates that this kind of electromagnetic ray significantly diffuses in the internal tissues. Therefore, it may be more difficult to evaluate differences in the concentration of internal substances between sites in a fruit than between fruits as previously reported using this kind of ray. This is the first study to report the nondestructive prediction of anthocyanin concentration in mango skin.

Score plots, loading plots and second derivatives of spectral reflectance around light absorption caused by anthocyanin are shown in Fig. 3. According to Fig. 3(A), LV 2 explained anthocyanin concentration. Nagata *et al.* (2006), using hyperspectral imaging, found that anthocyanin in purple-fleshed sweet potatoes absorbed visible light at around 525 nm. In the present study, in contrast, anthocyanin in mango skin absorbed light at around 570 nm, as shown in Fig. 3(D). When light is absorbed by a molecular species, a positive-valued peak appears as

the second derivative of the spectral reflectance. Thus, loading around 570 nm [Fig. 3(C)] may explain the light absorption by anthocyanin. A value of the second derivative of spectral reflectance can be separated by PLSR into an LV score and a loading. As LV 2 scores increase with anthocyanin concentration, positive values of loading are considered to contribute to the light absorption observed in the second derivative of reflectance. The peak at 600 nm in Fig. 3(C) may not be associated with anthocyanin, judging from the result in Fig. 3(D). According to Fig. 3(B), LV 1 explained the chlorophyll *a* concentration in the skin. Chlorophyll *a* and *b* in terrestrial plants (Tanaka, 2009) absorb visible light at 663 and 645 nm, respectively (Porra *et al.*, 1989), and the concentration of chlorophyll *a* is much higher than that of chlorophyll *b* (Nicotra *et al.*, 2002). Changes in concentrations of chlorophyll *a* and *b* at each end over time are shown in Fig. 4. This result differed from the trends observed for anthocyanin and SSC. However, Medlicott *et al.* (1986) reported that chlorophyll decreases during postharvest ripening. Therefore, the trends shown in Fig. 4 agreed with the result in the previous report. According to the results of two-way ANOVA (Table 2),

Fig. 4. Changes over time in (A) chlorophyll *a* and (B) chlorophyll *b* concentrations in mango skin analyzed destructively; (C) spectral reflectance (black lines, vine end; gray lines, blossom end); the number of data points for Savitzky–Golay smoothing was 11. Closed and open circles denote the means of three measured values $\pm$ standard error at the vine and blossom ends, respectively. Within the same figure, symbols followed by the same letter are not significantly different ($P < 0.05$; Tukey's honestly significant difference test).

storage period and sampling site significantly affected the variance of the measured data. The concentrations of chlorophyll *a* and *b* at 0 d were significantly higher than those after 0 d. The score of LV 1 at the blossom end was lower than that at the vine end, indicating that the negative loading for LV 1 at around 680 nm was influenced by chlorophyll *a*, as shown in Fig. 3(C). Thus, loading around 680 nm [Fig. 3(C)] may explain the light absorption by chlorophyll

a. That is, LV 1 may indicate that sampling sites with high concentrations of chlorophyll *a* contain low concentrations of anthocyanin. Understanding this relationship may contribute to the accuracy of the prediction model for anthocyanin concentration.

Score plots, loading plots and second derivatives of spectral reflectance around light absorption caused by free sugar are shown in Fig. 5. According to the score plot, LV 2 explained SSC [Fig. 5(A)]. Tanabe *et al.* (1995) confirmed that sucrose, fructose and glucose absorbed visible light at 750, 840, and 850 nm, respectively, using pure chemical solutions. However, light around 745 and 835 nm may be influenced by free sugars, as shown in Fig. 5(D). Accordingly, the loading at 755 and 835 nm [Fig. 5(C)] may correspond to light absorption by free sugars. As shown in Fig. 5(B), LV 1 may indicate the concentration of chlorophyll *a* in a similar manner to anthocyanin concentration. In Fig. 5(C), the loading at the wavelength absorbed by chlorophyll *a* shows a large negative value. LV 1 may indicate that a sample with a high concentration of chlorophyll *a* contains a low concentration of SSC. During postharvest ripening, SSC increases (Krishnamurthy and Subramanyam, 1973) and chlorophyll decreases (Medlicott *et al.*, 1986) over time, suggesting that changes in SSC may be associated with changes in chlorophyll concentration.

In the present study, we described a method to nondestructively predict anthocyanin concentration in the skin and SSC in the flesh using a hyperspectral camera combined with PLSR. Evaluation of anthocyanin distribution affects the grading of fruits, and in turn the market price. When threshold values have been determined for grading, based on the distribution of anthocyanin in fruit, the proposed method may be applied to the market grading of mangoes by determining the number of pixels, which reflects skin anthocyanin content, that exceed the threshold values.

Chlorophyll *a* concentration contributed to the accuracy of the prediction model. This observation suggests that the models we have proposed are valid for evaluating mangoes including chlorophyll *a* harvested at the green mature stage. Models applicable to fully mature fruits using a hyperspectral camera should be considered in future.

Conclusions

Hyperspectral camera measurements in combination with PLSR were used for nondestructively measuring anthocyanin concentration in the skin and SSC in the flesh of green mature “Irwin” mangoes during storage and showed the correlation coefficients of 0.88 (RMSECV 2.96 mg·100 g f.w.⁻¹) and 0.73 (RMSECV 0.98%), respectively.

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Table 2. Two-way analysis of variance of the effect of storage period and sampling site of mangoes on chlorophyll *a* and *b* concentrations in skin measured by destructive analysis

Content	Effect	Degree of freedom	Sum of square	F	p
Chlorophyll <i>a</i>	Storage period	7	7490	19.3	<0.0001***
	Sampled site	1	424	7.64	0.0097**
	Interaction	7	477	1.23	0.318
	Error	30	1660		
	Total	45	10100		
Chlorophyll <i>b</i>	Storage period	7	294	16.4	<0.0001***
	Sampled site	1	11.7	4.57	0.0411*
	Interaction	7	19.5	1.09	0.397
	Error	30	74.2		
	Total	45	402		

Significant at *95, **99 and ***99.9% levels, respectively.

Fig. 5. Score plots of (A) LV 1 and LV 2 calculated by partial least squares regression analysis grouped on the basis of SSC in the flesh (threshold, 11%; closed circles, high; open circles, low) and (B) chlorophyll *a* concentration in the skin (threshold, 2.5 mg·100 g f.w.⁻¹; closed circles, high; open circles, low); (C) loading plot (solid line, LV 1; dashed line, LV 2); and (D) spectral reflectance (black lines, vine end; gray lines, blossom end); the number of data points for Savitzky–Golay smoothing was 7

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