Non-destructive determination of free acid and malic acid content in apples using near infrared spectroscopy

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Introduction

In Korea, the acidity of apple fruits is considered to be as important a factor in determining the quality and grading of fruit as sweetness. Consumers require the right balance between a sweet and a sour taste. The acid content of Fuji apple fruit is generally 0.3–0.5% and it decreases rapidly during storage in comparison with brix. Therefore, it is necessary to develop a non-destructive measuring technique for acidity. We have already reported on the possibility of using near infrared (NIR) reflectance spectroscopy for determining the sweetness of apples.[1] In this study we investigated the feasibility of using NIR reflectance spectroscopy for the nondestructive measurement of acidity in apple fruit and to improve the accuracy of the measurement.

Materials and methods

Apple fruit (Fuji variety) of the cultivar and growing district of Kyung-Pook in Korea were collected from 1996 to 1998. Approximately 2,000 fruit were used in this study. The combined apple samples of apple fruits collected were stored for between 1 and 5 months at 2°C. The temperature of the apple fruit for spectral measurement was maintained at about 15°C.

The free acid content was measured by 0.005 N and 0.001 N NaOH. Intact, browned and heat-treated, squeezed apple juice was used for titration. The malic acid content was measured by HPLC. Analytical conditions were as follows: eluent, 0.1% phosphoric acid; column, Ionpak KC-811; column temperature, 40°C; detector, Shimadzu RID-10 A.

The NIR reflectance spectrometer used was an InfraAlyzer 500C (Bran+Luebbe Co., Germany) and the wavelength region used for the NIR analysis was 1100–2500 nm with 2 nm intervals. The equator surface of intact apple fruit was scanned using the sample holder and the apple juice sample was presented in an aluminium cell with a quartz surface. Spectral attributes were correlated against chemical data of the intact fruit. Chemometrics models were constructed using a calibration set and an independent validation set to evaluate the predictive ability of the models. Data treatment was performed using IDAS software (Bran+Luebbe Co., Germany). Analysis involved stepwise multiple linear regression (MLR) of the original and derivative spectral data. Model performance was reported as the correlation coefficient (*R*) and the standard error of prediction (*SEP*).

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Table 1. Results of MLR analysis for free acid content in apple fruit at harvesting season.

Calibration and prediction sample sets	Range %	Mean %	*R*	*SEC* %	*SEP* %
Apple with same harvest time (8 Nov.)	0.24 – 0.38	0.31	0.54	0.029	0.032
Apple with different harvest time (30 Oct. + 8 Nov. + 16 Nov.)	0.20 – 0.41	0.31	0.77	0.028	0.030

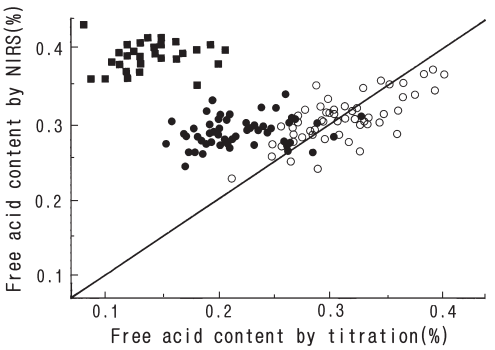

Figure 1. Results for free acid content in stored apple fruit using the calibration equation developed for the harvesting season (❍ : before storage, ● : 2 month storage, ■ : 4 month storage).

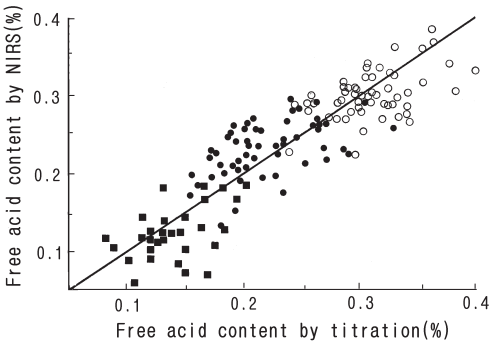

Figure 2. Plot of NIR predicted v. measured free acid content of stored apple fruit. (❍ : before storage, ● : 2 month storage, ■ : 4 month storage).

Results and discussions

Free acid

The effect of the colour of squeezed apple juice during titration on prediction accuracy was confirmed. When brown apple juice was used, the accuracy was lower than intact or heat treated juice (data not shown). The NaOH concentration did not affect prediction accuracy. The effect of sample collection in the calibration model for apple fruit at harvest season is presented in Table 1. When apples collected at the same harvest time were used, *R* was 0.54 and *SEP* was 0.032%. However, when samples collected at different harvest times were used, the prediction accuracy was greater; *R* was 0.77 and *SEP* was 0.03%. Fig-

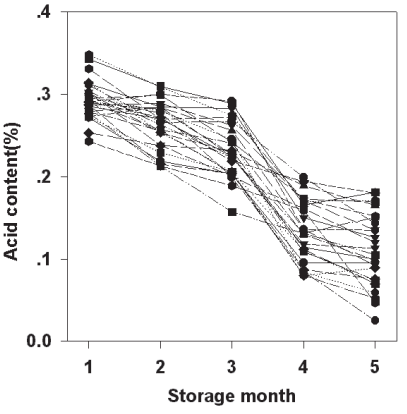

Figure 3. NIR monitoring of free acid content in apple fruits during storage.

Table 2. Results of MLR analysis for malic acid content in stored apple fruit using raw original and derivative spectra.

Mathematic treatment	*n*	Range (kg)	Mean (kg)	R	*SEC* %	*SEP* %
Raw	Cal(134),pre(90)	0.07–0.40	0.27	0.83	0.040	0.039
1st derivative	Cal(134),pre(90)	0.07–0.40	0.27	0.86	0.036	0.037
2nd derivative	Cal(134),pre(90)	0.07–0.40	0.27	0.82	0.041	0.037

ure 1 shows the result for stored apple fruit in this calibration. The application was nearly impossible. This was caused by the range of free acid in the prediction set not agreeing with the calibration set. A new calibration model was developed for the stored apple fruit, using 224 combined samples (calibration set: 134 and prediction set: 140). The result of the prediction was acceptable (Figure 2); *R* and *SEP* were 0.90 and 0.04%, respectively. The wavelengths used were 1180, 2160, 2140 and 2200 nm. Figure 3 shows the monitoring of free acid content of apple fruit stored for 5 months at 2°C. It was possible to determine the non-destructive measurement of the free acid content in apple fruit and to monitor the decreasing acidity in the storehouse to *c*. 9% error rate. Miyamoto *et al*.[2] have reported that the classification of high acid content of mandarins at *c*. 20% error rate is possible by using a PLS model.

Malic acid

Most of the organic acid in apple fruit, over 80%, is malic acid. The predicted results of the amount of malic acid in combined apple fruit was acceptably accurate; *R* was 0.83 and *SEP* was 0.039%. The prediction accuracy for free acid content was similar whether MLR analysis was performed on derivative data or on the raw spectral data (Table 2). The wavelengths used were 1744 nm, 1766 nm, 2296 nm and 2244 nm. A plot of NIR predicted v. measured malic acid content in apple fruit is presented in Figure 4(a). There was a high correlation between measured malic acid content and the predicted value of the apple juice [Figure 4(b)]. The results of the MLR analysis were *R*, 0.922 and *SEP,* 0.035%. Wavelengths used were 2288 nm and 1748 nm. The accuracy was higher than for intact apple fruit. This calibration model could be applied to commercial fruit juice. Figure 5 shows the changes in the difference spectra by adding malic acid in water. The second derivative absorption value in the

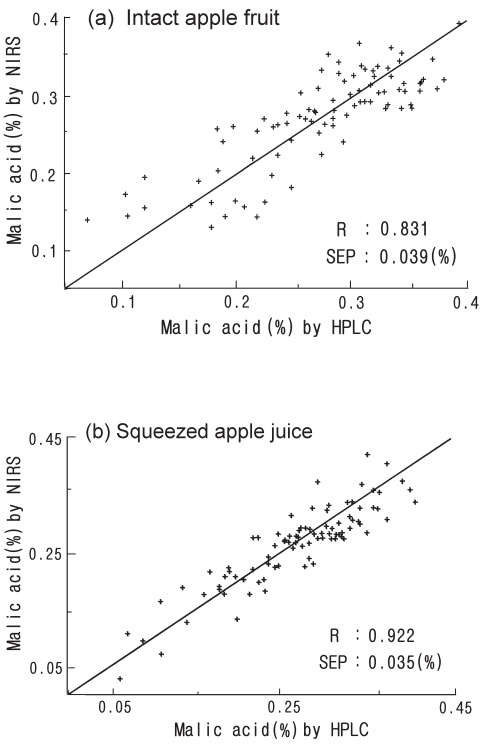

Figure 4. Plot of NIR predicted v. measured malic acid content of (a) intact apple fruit and (b) squeezed apple juice.

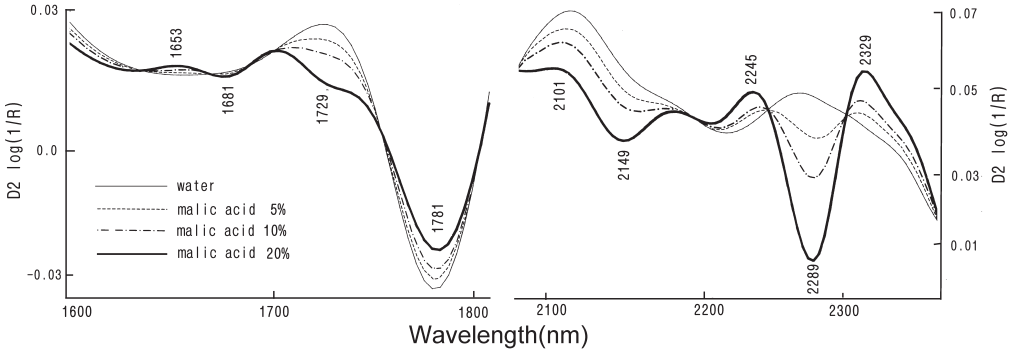

Figure 5. Second derivative NIR spectra of solutions with different malic content.

1700–1750 nm region, 2100–2200 nm region and 2250–2310 nm region made positive contributions to the regression equation for malic acid content. The wavelengths in this region were used to develop the calibration model for apple acidity. A similar phenomenon was also reported by Fujiwara *et al*.,[3] for the measurement of citric acid content in satsuma mandarin juice using wavelengths of 1718 nm and 2290 nm.

References

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