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ANALYSIS OF APPLE QUALITY BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

— Measurement of moisture, sugar and apple acid content
in different varieties using a single calibration equation —

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Introduction

Differences in the components - both qualitative and quantitative - of fruits and vegetables observable in different species and varieties affect the spectra obtained with NIR spectroscopy. Hence, when measuring them, in general, one calibration equation has been used for each combination of component and variety. But establishing a calibration equation for each component and variety is time-consuming and requires a lot of samples. A single calibration equation method would be preferable. In this paper, component concentrations in different varieties of apples were predicted using a single calibration equation and the accuracy of this method was assessed.

Materials and Methods

In this experiment, the varieties "Tsugaru", "Redgold" and "Fuji", produced in the Yoichi Orchard of Hokkaido University, were used as samples. In order to get a wide range of component concentrations, fruits of a variety were harvested at 3 times : a week before the full ripening, just at the full ripening and a week after the full ripening. In 1992, the full ripening of "Tsugaru", "Redgold" and "Fuji" occurred on 19 September, 15 October and 29 October, respectively.

Picked samples were washed in water and then stored in an electric refrigerator for 1 to 6 days. Prior to measuring the spectra, samples were kept overnight in an incubator at 25°C to obtain a uniform temperature ; this is because temperature influences the spectrum of a sample. After measuring the spectra, the samples were stored in an electric refrigerator overnight and the next morning the components were measured by laboratory analysis methods. The measuring

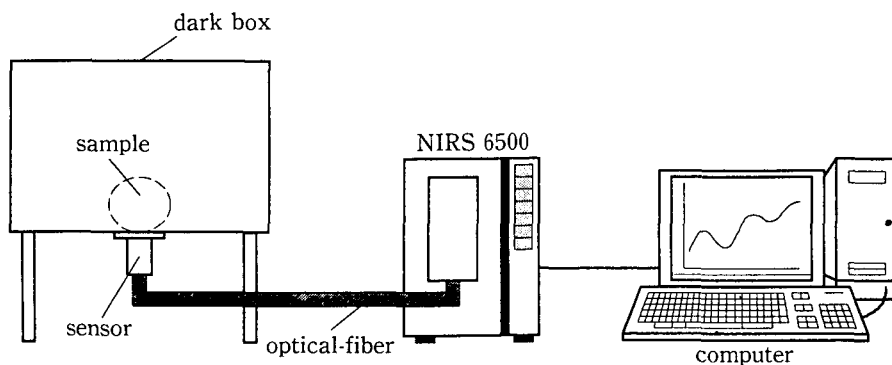


Figure 1. Measuring system.

system used in this study is shown in Figure 1. The spectrophotometer employed was the NIRS 6500, produced by NIRSystems Inc. To measure the spectra of samples non-destructively, a dark box was constructed so as to allow no light to enter. The box and the NIR instrument were connected with optical-fiber, with the sensor placed at the bottom of the dark box. The system consisting of the sensor and optical-fiber is called a 'Transmission Detector Module', and it has a dual structure in the sense that an incident beam is radiated from the outside of the sensor and the reflected one is received at the inside of the sensor. Each sample was placed close to the optical-fiber in the dark box.

The spectrum of the sample was measured at 8 different points in the epicarp located half way between the bottom and top of the sample. The mean spectrum was deemed to be the spectrum of the apple. The second derivative of this spectrum was used to calculate the component concentration. Segment and gap for calculating the second derivative were 20nm and 10nm, respectively.

Polystyrene board was used as a reference. When measuring the reference, it was placed above the optical-fiber, allowing a 3mm gap.

The measuring range for spectra and reference was from 800 to 1100nm and absorbance was measured at each 2nm. Scanning was made 120 times.

Laboratory analysis method

(1) Moisture content

An apple was cut into slices each 5mm thick and one of them was measured for the moisture content. The method used was oven-drying at 70°C for 24 hours¹⁾. The moisture content value was taken from the mean of 3 points in each slice. However, slices in which the maximum difference among the points was over 1% were excluded. The dryer used in this procedure was Dryer PS-11, produced by Tabai Co.

(2) Sugar content

An apple was cut into slices each 5mm thick and one of them was measured for the sugar content. The slice was mixed together with distilled water in a blender. The reducing sugar and total sugar contained in the solution were evaluated by the Somogyi-Nelson method^{2,3}.

(3) Apple acid

Acids in apples are mainly compounds of apple acid, so that this component was measured. The solution used to measure the sugar content was also used to measure the apple acid. The method was 0.01N-NaOH titration⁴.

Calibration

To establish the calibration equation, 10 samples were drawn from each variety, thus a total of 30 samples was examined. In the same way, for a validation set, another 30 samples were selected. The wavelengths used to obtain the calibration equation were selected by the following method. The wavelength at which absorption by some component of the apple occurred was taken as the first wavelength, and multiple regressions were produced by the use of the first wavelength and each of the other wavelengths. Then, the correlation coefficients of these equations were calculated. The wavelength at which the correlation coefficient had the highest value was employed as the second wavelength. In the same way, the other wavelengths were selected.

Statistics

The standard error of calibration (SEC) was calculated by the following equation :

$$SEC = \sqrt{\frac{\sum_{i=1}^n (x_i - y_i)^2}{n - p - 1}}$$

where

x_i : value by laboratory analysis

y_i : value predicted by NIR

p : number of wavelengths in calibration equation

n : number of samples

The bias was calculated by :

$$\text{Bias} = \frac{1}{n} \sum_{i=1}^n x_i - \frac{1}{n} \sum_{i=1}^n y_i = \bar{x} - \bar{y}$$

where

\bar{x} : mean value of x

\bar{y} : mean value of y

The standard error of prediction (SEP) was the standard deviation of the prediction error and was calculated by :

$$SEP = \sqrt{\frac{\sum_{i=1}^n \{(x_i - y_i) - (\bar{x} - \bar{y})\}^2}{n-1}}$$

Results

Component ranges of the calibration and validation sets are shown in Table 1.

Table 1. Component range of calibration and validation sets.

item	calibration			validation		
	maximum	minimum	mean	maximum	minimum	mean
moisture (%)	86.9	83.5	85.5	86.7	84.0	85.6
reducing sugar (%)	8.8	5.1	6.5	7.7	5.3	6.8
total sugar (%)	13.9	8.4	10.9	13.4	8.5	10.6
apple acid (%)	0.45	0.23	0.34	0.45	0.27	0.35

(1) Moisture content

The wavelengths that resulted in the highest correlation coefficients for the laboratory analysis value and the second derivative value of the absorbance at each wavelength were 952, 950 and 946nm for "Tsugaru", "Redgold" and "Fuji", respectively, thus being very close to each other. In raw spectra, an absorption that can be associated with occurred at 970nm⁵⁾. Therefore, it is likely that the absorption at these wavelengths was caused by the moisture content in the samples. The results obtained here (Figure 2) for the absorption wavelengths associated with moisture content show a shift to the left when compared with the results obtained by B. G. Osborne. This shift might be related to the influence of some other components in the samples. When calculating correlation coefficients for laboratory analysis values and second derivative values of absorbance in the calibration sample set, the highest correlation was at 948nm. Calibration produced with 948nm as the first wavelength gave the following equation.

$$\text{moisture} = 106.3 + 396.9_{x948} - 28.90_{x1016} - 12.63_{x838} + 1442_{x1048} + 721.2_{x990}$$

where

x_{948} : $d^2\log(1/R)$ at 948nm

x_{1016} : $d^2\log(1/R)$ at 1016nm

x_{838} : $d^2\log(1/R)$ at 838nm

x_{1048} : $d^2\log(1/R)$ at 1048nm

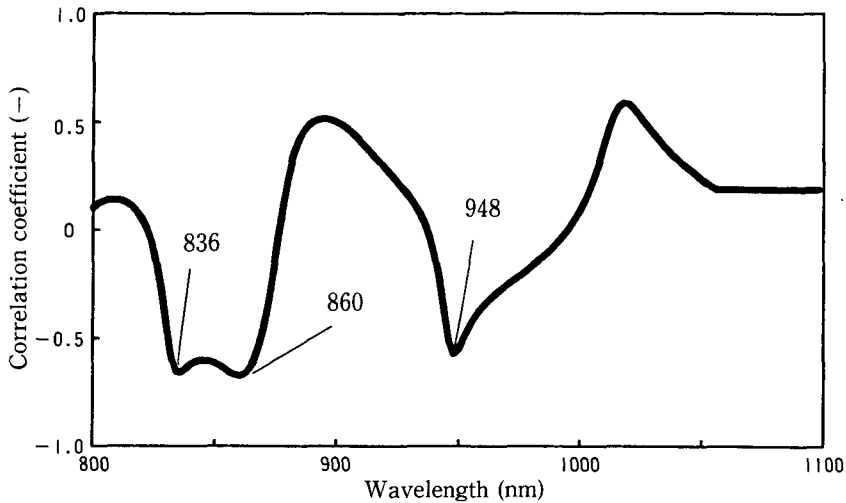


Figure 2. The relationship between wavelengths and correlation coefficients in moisture content.

x_{990} : $d^2\log(1/R)$ at 990nm

The validation of this calibration is shown in Figure 3. Since SEP was 0.4%, this calibration had sufficient precision for the prediction of the moisture content of apples. In general, as the main constituent was moisture (over 75%), absorbance by moisture content in the NIR region was very strong. This indicates that measuring the moisture content of apples in this way should not be difficult.

(2) Reducing sugar

The reducing sugar content of the samples was about 6%, very low when compared with moisture content. Therefore, there was the possibility that weak absorbance by reducing sugar would be hidden by the strong absorbance by moisture.

The relationship between wavelengths and correlation coefficients is shown in Figure 4. High correlation coefficients occurred at 890nm. The calibration obtained is expressed in the following equation :

$$\text{reducing sugar} = 26.30 - 560.1x_{890} - 417.5x_{980} - 598.0x_{932} - 167.9x_{842}$$

where

x_{890} : $d^2\log(1/R)$ at 890nm

x_{980} : $d^2\log(1/R)$ at 980nm

x_{932} : $d^2\log(1/R)$ at 932nm

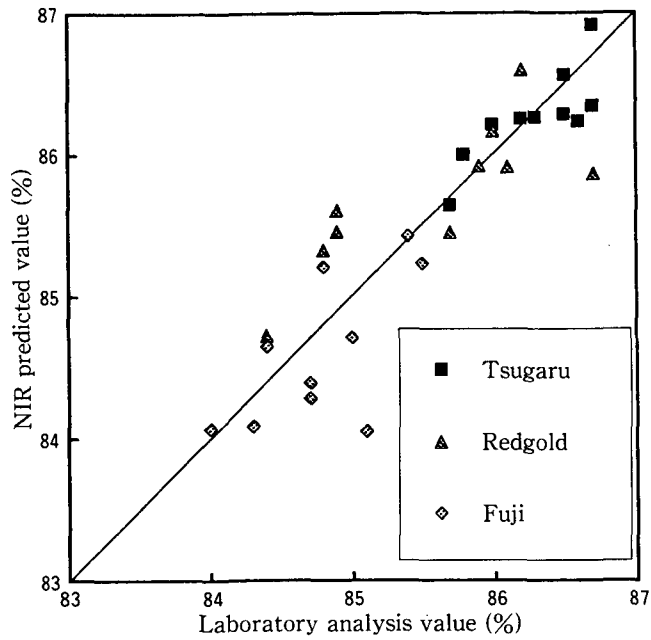


Figure 3. Validation of the calibration equation of moisture content.

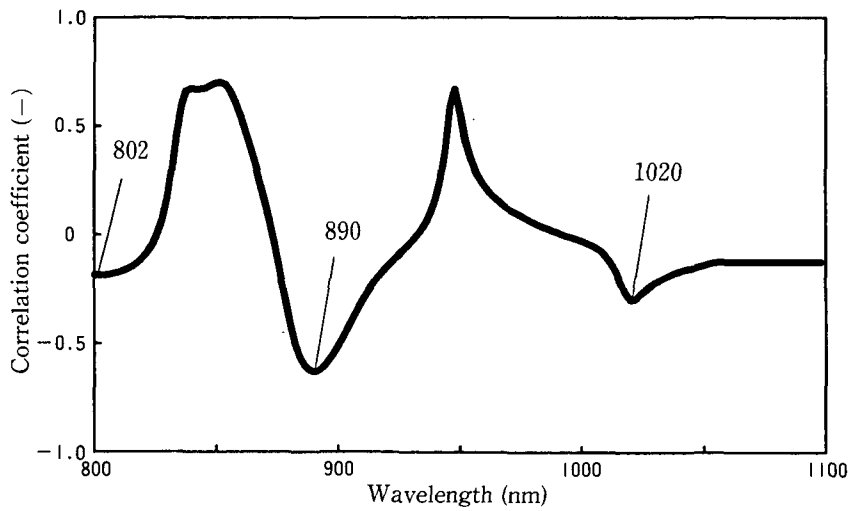


Figure 4. The relationship between wavelengths and correlation coefficients in reducing sugar content.

x_{842} : $d^2\log(1/R)$ at 842nm

In Figure 5, the result of validation of this calibration is shown. SEP was 0.7%. As Figure 5 suggests, this low SEP might be the influence of the spread values corresponding to the prediction for "Redgold".

The reducing sugar of "Redgold" used to produce the calibration set ranged from 5.1 to 6.0%. On the other hand, the range used in the validation set was from 5.3 to 7.0%. Thus, the concentration in samples used for the estimate was in over-range, and it is thought that this caused the high SEP.

(3) Total sugar

Correlation coefficients for absorbance at each wavelength and laboratory analysis values are shown in Figure 6. High correlation coefficients occurred at 902nm. It was thought that absorbance at this wavelength was by sucrose. Therefore, calibration of total sugar was produced with a first wavelength of 902nm.

$$\text{total sugar} = 138.0 - 768.4x_{902} + 1124x_{880} - 400.2x_{818} - 238.2x_{956} - 300.4x_{858}$$

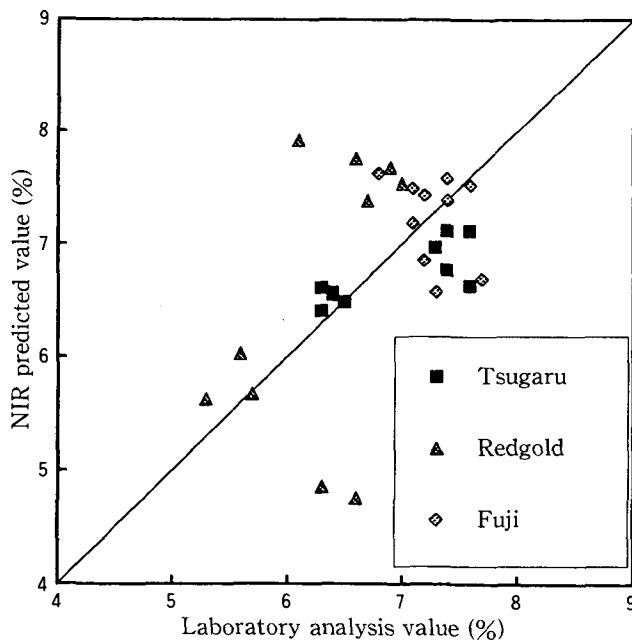


Figure 5. Validation of the calibration equation of reducing sugar content.

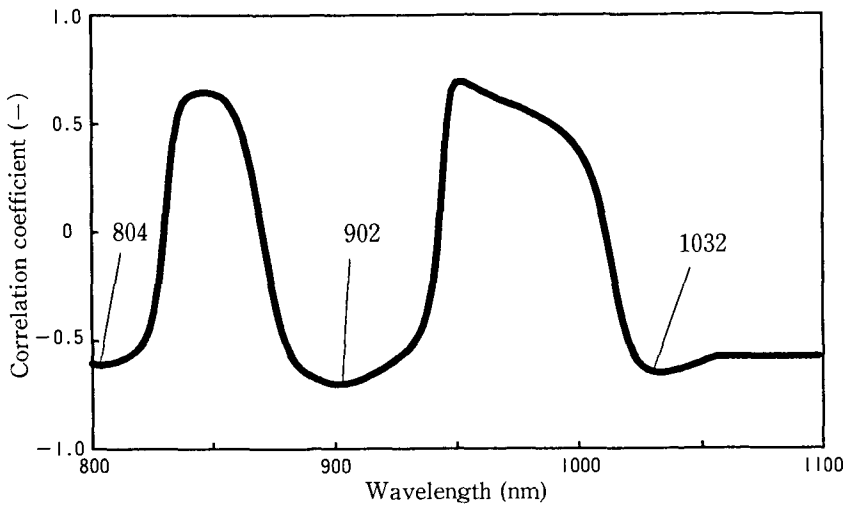


Figure 6. The relationship between wavelengths and correlation coefficients in total sugar content.

where

x_{902} : $d^2\log(1/R)$ at 902nm

x_{880} : $d^2\log(1/R)$ at 880nm

x_{818} : $d^2\log(1/R)$ at 818nm

x_{956} : $d^2\log(1/R)$ at 956nm

x_{858} : $d^2\log(1/R)$ at 858nm

The result of validation is shown in Figure 7. SEP was 0.7%. In this case, the predicted values of the concentration of total sugar in the calibration were distributed uniformly. Thus, the accuracy was enough to predict the component concentration.

(4) Apple acid

In the same way, the correlation coefficients of this component is shown in Figure 8. High correlation coefficient occurred at 1012nm.

$$\text{Apple acid} = 1.529 - 6.913x_{1012} - 2.740x_{946} + 20.37x_{872} - 1.505x_{1056}$$

where

x_{1012} : $d^2\log(1/R)$ at 1012nm

x_{946} : $d^2\log(1/R)$ at 946nm

x_{872} : $d^2\log(1/R)$ at 872nm

x_{1056} : $d^2\log(1/R)$ at 1056nm

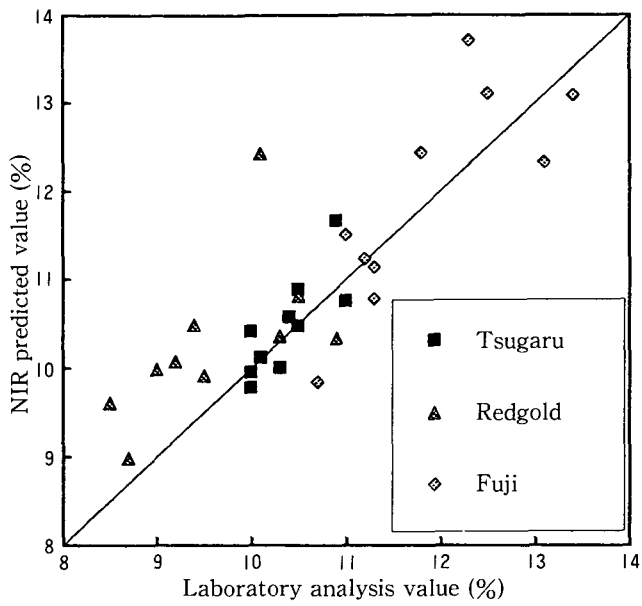


Figure 7. Validation of the calibration equation of total sugar content.

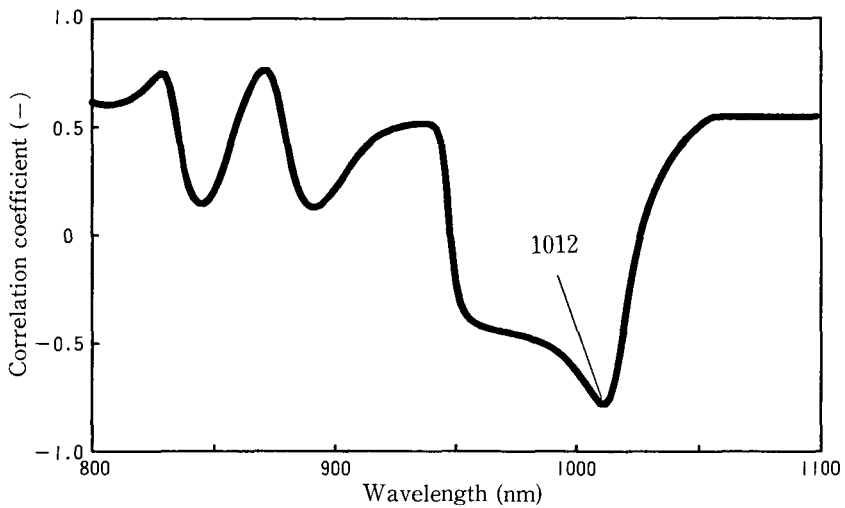


Figure 8. The relationship between wavelengths and correlation coefficients in apple acid content.

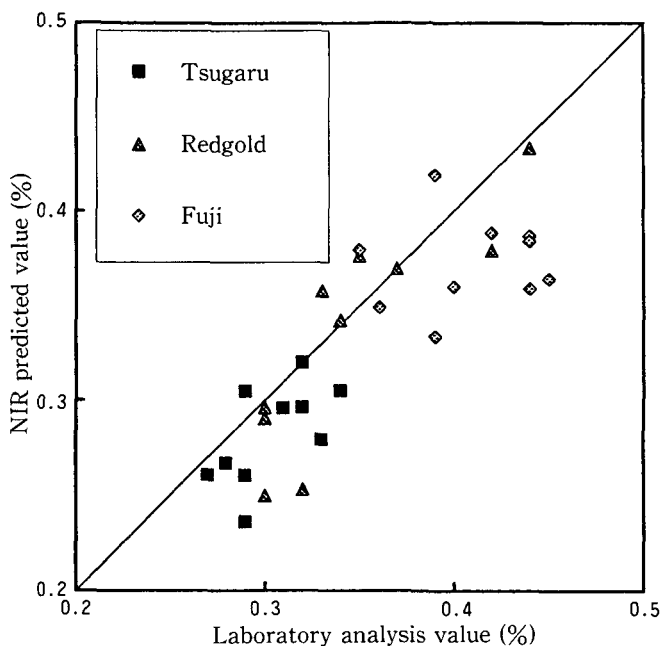


Figure 9. Validation of the calibration equation of apple acid content.

The results of validation are shown in Figure 9. SEP was 0.03% and there was no bias. Again, there were enough precision to predict the apple acid content.

Discussion

The SEP's of the calibration equations established with each variety, and with the mixture of the varieties, are shown in Table 2. NIR rays radiated to the apples were reflected to the surface of the sensor after diffusing inside the apples. From preliminary experiments, it was known that NIR rays penetrate through the **Table 2.** The SEP's of the calibration equations established both with each variety and with the mixture of the varieties.

item	moisture (%)	reducing sugar (%)	total sugar (%)	apple acid (%)
Tsugaru	0.3	0.7	0.8	0.04
Redgold	0.4	0.5	0.6	0.03
Fuji	0.6	0.4	0.6	0.04
Mixture	0.4	0.7	0.7	0.03

apple's epicarp to a depth of up to 15mm. Thus the reflected rays carry information about that part of the interior of the apple. Correlations between components in that part of the apple and those components in the whole were determined for the variety "Fuji". The correlation coefficients obtained for moisture, reducing sugar, total sugar and apple acid content were 0.81, 0.85, 0.81 and 0.88, respectively, and standard deviations were 0.7, 0.6, 0.8 and 0.02%, respectively. Since there were some deviations of components, a certain error was unavoidable. But it was possible to predict the component concentration by a single calibration equation, because there was little difference between the SEP's of each calibration equations. But, in the case of reducing sugar, variation in the component concentration of the sample used for the calibration set affected the accuracy of prediction greatly. Accordingly, if a higher accuracy is required, a more uniform deviation of component concentration is necessary.

Summary

Moisture, reducing sugar, total sugar and apple acid in 3 varieties of apples were measured using a single calibration equation. The varieties used in this experiment were "Tsugaru", "Redgold" and "Fuji", produced in the Yoichi Orchard, Hokkaido University. Ten samples were selected arbitrarily from each variety and a total of 30 samples was used for the calibration set. Another 30 samples were selected for the validation set. The second derivative of this spectrum was used to calculate the component concentration. Segment and gap for calculating the second derivative were 20nm and 10nm, respectively. The range of a spectrum was from 800 to 1100nm and the absorbance was measured at each 2nm. Scanning was made 120 times.

As a result of these experiments, the SEP's of moisture, reducing sugar, total sugar and apple acid were 0.4, 0.7, 0.7 and 0.03%, respectively.

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