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ANALYSIS OF PUMPKIN QUALITY BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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Introduction

Nondestructive analysis of quality of fruits and vegetables, for eating and processing, has become very important and numerous methods have been developed and investigated. Mechanical, electrical and optical methods are some of the nondestructive methods for determination of quality. In the mechanical method, the quality is determined by measuring the sound of samples struck or sound vibration^{1,2,3)}. In the electric method, fresh and damaged samples are separated by measuring their impedance⁴⁾. Near-Infrared (NIR) Reflectance spectroscopy is one of the optical methods.

According to quantum physics, the oscillators of molecules absorb or emit energy by a quantum of energy equal in value to $h\nu$, where h is Planck's constant and ν is the frequency of the oscillator⁵⁾. In the NIR region, the absorbed frequency that occurs is an overtone or combination bands of ν . Therefore, if the molecular frequencies of components and the NIR absorbance by these components are known, the component concentration can be determined.

The purpose of this study was to determine the quality of fruits and vegetables by NIR Spectroscopy. In this research, pumpkins of the cultivar 'Ebisu', produced in Hokkaido, were used. Pumpkin quality was evaluated by measuring the moisture, reducing sugar and total sugar contents. The accuracy of the NIR spectroscopy for measuring the contents of pumpkin was determined.

Materials and Methods

1. Samples and instruments

The pumpkins used are of the 'Ebisu' variety, produced in Mori-township, Hokkaido, and harvested on 8 September, 1991.

The measuring system used in this study is shown in Figure 1, with the NIR instrument NIRS 6500 manufactured by NIRSystems, Inc. This instrument was a grating monochromator type that could measure the absorbance from 400 to

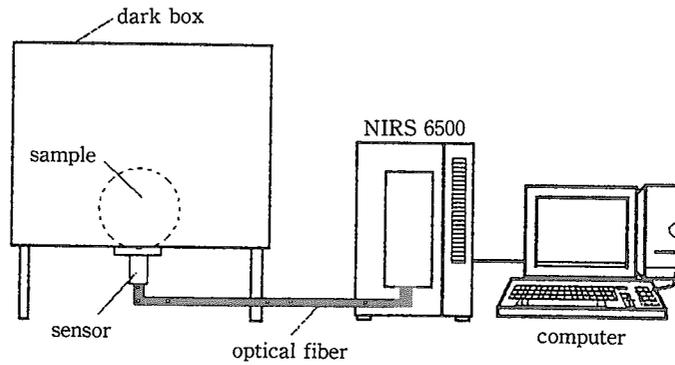


Fig. 1. Measuring system.

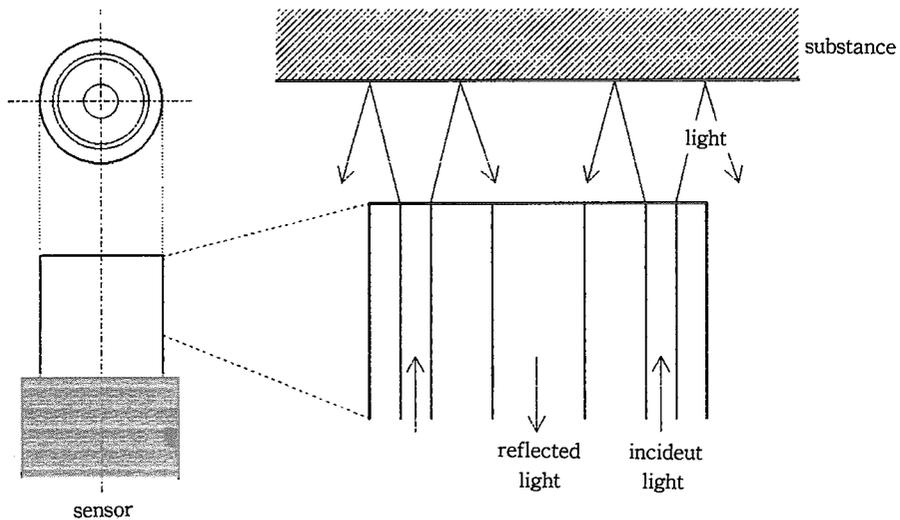


Fig. 2. Structure of the sensor.

2500 nm for each 2 nm in the near-infrared region. To measure the spectrum of samples in a nondestructive mode, a dark box was made in which light was completely shut out. The box and the NIR instrument were connected with an optical-fiber. A sensor of the optical-fiber was placed at the bottom of the dark box. This sensor and optical fiber are called 'Transmission Detector Module'. The useful measuring range was from 800 to 1600 nm. The sensor is shown schematically in Figure 2. An incident beam was radiated from outside of the sensor and the reflected beam was received at the inside of the sensor.

2. NIR analysis

One pumpkin was divided into four pieces and each piece was treated as one sample. Each sample was placed close to the sensor in the dark box (Figure 3)

to obtain the beam reflected from the inside. The spectrum of the sample was measured at 2 different points on the epicarp located in the middle part between the bottom and top. The mean spectrum was to be the spectrum of pumpkin. The second derivative of this spectrum was used to calculate the component concentration. In the experiment, 16 pumpkins or 64 samples were used. All samples were kept overnight at 20.0 to 26.0°C to obtain a uniform temperature for measurement of the spectrum.

From a preliminary experiment, it was known that NIR ray penetrated through the pumpkin epicarp up to about 10 mm thickness and returned to the sensor. So the reflected ray sent the information of the pumpkin within this part. Then the correlation in some components between this part and the whole pumpkin was measured. As a result, the correlation coefficients (r) obtained for moisture, reducing sugar and total sugar contents were 0.93, 0.93 and 0.93 respectively, thus validating by NIR spectroscopy method.

3. Laboratory analysis method

To estimate the predicted value with NIR spectroscopy method, some appropriate chemical analyses are required. In this experiment, the following methods were used.

The moisture content of pumpkins was determined by oven-drying at 70°C for 24 hr⁶⁾.

The reducing sugar (the sum of glucose and fructose) and the total sugar contents (the sum of the reducing sugar and sucrose) of the pumpkin were determined by the Somogyi-Nelson method^{7,8)}.

4. Statistics

The standard deviation of the difference (SDD) was calculated by the following equation ;

$$SDD = \sqrt{\frac{\sum_{i=1}^n d_i^2}{2n}}$$

where

d_i : difference between measured values

n : number of samples

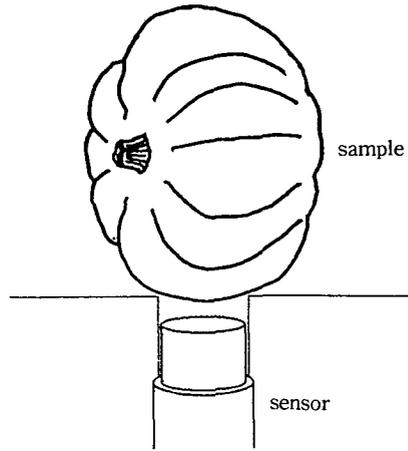


Fig. 3. Measuring the pumpkin.

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

$$\text{SEC} = \sqrt{\frac{\sum_{i=1}^n (X_i - Y_i)^2}{n - p - 1}}$$

where

X_i : value of laboratory analysis

Y_i : predicted value of NIR

p : number of wavelengths for calibration equation

The bias was calculated by

$$\text{bias} = \frac{1}{n} \sum X_i - \frac{1}{n} \sum Y_i = \bar{X} - \bar{Y}$$

where

\bar{X} : mean value of X_i

\bar{Y} : mean value of Y_i

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

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^n \{(X_i - Y_i) - (\bar{X} - \bar{Y})\}^2}{n - 1}}$$

Results

1. Accuracy of laboratory analysis

SDD of laboratory analysis was evaluated. Each sample was measured at 3 points for moisture content and 4 points for reducing and total sugar contents. The results are shown in Table 1. It was found that each of the constituents was measured with adequate accuracy.

Table 1. Standard error of laboratory analysis.

Items	n	Standard Error(%)
moisture	74	0.2
reducing sugars	99	0.1
total sugars	102	0.1

2. Measuring the compositions of pumpkin

For calibration, 32 samples were selected arbitrarily from the 64 samples and used to calibrate each constituent. The moisture content in pumpkin fluctuated

Table 2. Estimation of calibration

	n	max	min	mean	r ²	SEC
moisture						
lab. analysis	32	84.1%	64.2%	74.5%		
calibration	32	83.5	62.6	74.8	0.843***	2.69
reducing sugars						
lab. analysis	32	5.3	0.7	3.2		
calibration	32	5.1	0.2	3.3	0.770***	0.77
total sugars						
lab. analysis	32	11.0	7.0	9.4		
calibration	32	11.1	8.1	9.3	0.634***	0.71

*** 0.1% significant

Table 3. Estimation of the NIR prediction

	n	max	min	mean	r ²	Bias	SEP
moisture							
lab. analysis	32	84.3%	63.0%	74.2%			
NIR prediction	32	86.2	57.1	75.3	0.690***	-1.1	3.94
reducing sugars							
lab. analysis	32	5.5	0.6	3.2			
NIR prediction	32	5.9	- 0.1	3.3	0.450***	-0.1	1.20
total sugars							
lab. analysis	32	10.7	7.0	9.3			
NIR prediction	32	11.4	7.9	9.3	0.287**	0.0	0.84

** 1% significant

*** 0.1% significant

from 64.2 to 84.1 per cent, the reducing sugars content, from 0.7 to 5.3 per cent and the total sugar content, from 7.0 to 11.0 per cent. The following method was used to determine the calibration variables : Firstly, the correlation coefficient between values of laboratory analysis and the second derivative of raw spectrums of pumpkin were calculated. Secondly, the first variable of multiple regression was determined as the wavelength that showed a negative correlation coefficient shown in the identification of wavelength of the near-infrared absorber table⁹⁾. The estimation of calibration and NIR prediction are shown in Table 2 and 3, respectively.

(1) Moisture

Selected wavelengths were 834, 938, 958 and 978 nm. These wavelengths were the first variable and the other variables were calculated by the all-possible subsets method. As a result of the t-distribution test for calculated multiple regression, the coefficient of variables could be rejected at 1 per cent up to 3 variables. Calibration was evaluated with bias, SEP and the correlation coefficient. The calibration in which bias and SEP were near 0 and r was near 1 is well estimated.

$$\text{moisture}(\%) = 43.26 - 567.7X_{840} - 216.3X_{880} - 46.24X_{958}$$

where

X_{840} : absorbance at 840 nm

X_{880} : absorbance at 880 nm

X_{958} : absorbance at 958 nm

The correlation between the laboratory analysis value and the predicted value of NIR is shown in Figure 4. According to the statistical analysis, SEP was about 4 per cent. In general, the main constituent of pumpkin was moisture, over 70 per cent and absorbance of moisture content was very strong. Therefore, it would be easy to measure the moisture content of pumpkin. In fact, however, these errors occurred because of the rough surface of the pumpkin epicarp and the difference in circumference. These influenced the reflected NIR ray. Almost all of the NIR rays of the pumpkin was 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 influenced the absorbance.

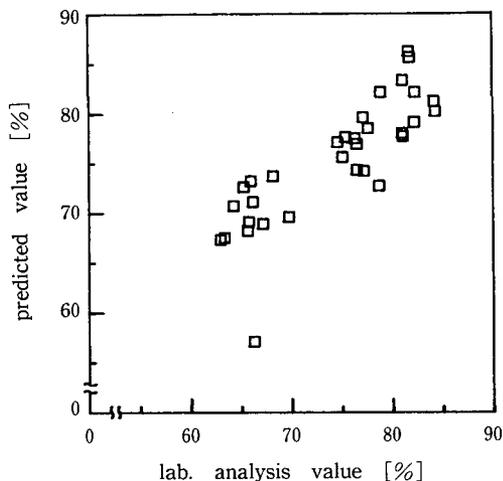


Fig. 4. Correlation between laboratory analysis and the NIR predicted values for moisture content.

(2) Reducing sugars

Selected wavelengths were 838 and 888 nm. The t-distribution test for the multiple regression was used, in the same way as during determinations of moisture content. As a result of this test, the coefficient of variables could be rejected at 1 per cent up to 3 variables. The following equation was determined as the calibration for the reducing sugar content.

$$\text{reducing sugars}(\%) = 14.24 - 160.6X_{838} - 87.81X_{880} - 97.21X_{1018}$$

where

X_{838} : absorbance at 838 nm

X_{880} : absorbance at 880 nm

X_{1018} : absorbance at 1018 nm

The correlation between the value of laboratory analysis and the predicted value of the NIR is shown in Figure 5. As a result of the statistical analysis, SEP

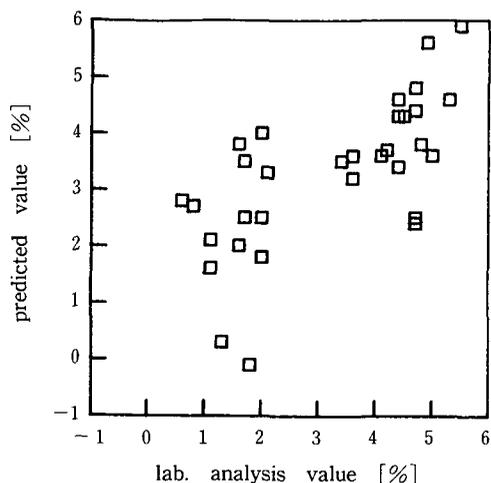


Fig. 5. Correlation between laboratory analysis and the NIR predicted values for reducing sugar content.

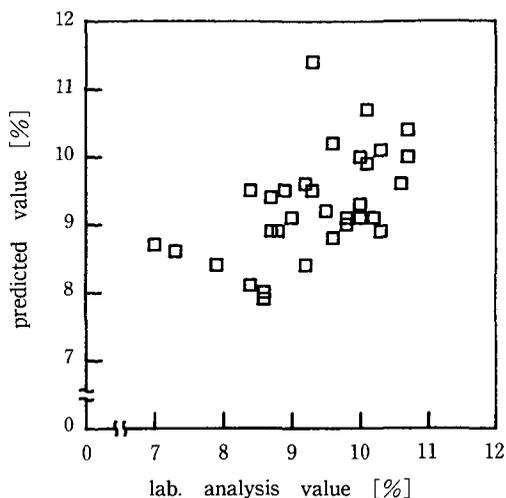


Fig. 6. Correlation between laboratory analysis and the NIR predicted values for total sugar content.

was about 1.2 per cent which represented over 30 per cent of the reducing sugar content of pumpkin. This value had no adequate accuracy. The following causes were considered : 1) During first determinations, there was low correlation coefficient value determined between the laboratory analysis and the predicted value of NIR. Hence, the strong absorbance of reducing sugars was not in the NIR region from 800 to 1100 nm. 2) Since the amount of reducing sugar was smaller than the amount of other constituents of the sample, it was thought that difference in absorbance between the reducing sugars and other constituents would be difficult to measure.

(3) Total sugars

Selected wavelength was only 912 nm. As a result of the t-distribution test, the coefficient of variables could be rejected at 1 per cent up to 3 variables. The following equation was determined as the calibration for the total sugar content.

$$\text{total sugars(\%)} = 13.78 + 62.50X_{840} + 33.78X_{882} - 15.34X_{912}$$

where

X_{840} : absorbance at 840 nm

X_{882} : absorbance at 882 nm

X_{912} : absorbance at 912 nm

The correlation between values of the laboratory analysis and predicted values of NIR is shown in Figure 6. It was expected that the error occurred because of the same cause as in the determination of reducing sugars.

Conclusion

The moisture, reducing sugar and total sugar contents 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 per cent, respectively. If a higher accuracy is required, preparation of the sample is necessary. For example, the sample could be cut prior to spectrum measurement to reduce the influence of the rough surface of pumpkin.

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