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Application News

No.A463

Spectrophotometric Analysis

Reflectance Measurements of an Apple and Pear, and Prediction of Elapsed Days by Multivariate Analysis

Fruit gradually undergoes change with the passage of time after harvesting. Examination of how the physical properties of fruit change over time is important, but if testing requires cutting out a portion of the fruit sample, the number of samples tested would most likely be greatly reduced. Non-destructive fruit properties inspection testing is commonly conducted using a spectrophotometer, and testing of sweetness and acidity by this means is well known.

This type of testing is valuable not only for understanding the physical properties, but for quality management as well. Ripeness, in particular, can be judged based on the number of days that have elapsed from the time of harvesting from the tree. Fruit ripeness is known to be associated with the change in chlorophyll content.[1), 2)] Here, we conducted a simulation experiment in which we investigated the time-course changes in purchased fruit samples over a period of days, focusing on changes in the absorption peak of chlorophyll. The results of measurement of apple and pear samples using a UV-VIS-NIR spectrophotometer revealed a correlation between the reflectance spectra of the samples and the number of days elapsed from the time they were obtained. Applying multivariate analysis, we used a prediction formula to calculate and predict the number of elapsed days from the time the samples were obtained. Good results were obtained with the apple sample, as reported in this paper.

Measurement Method

We measured the total light reflectance in an apple and a pear using the SolidSpec-3700DUV UV-VIS-NIR spectrophotometer equipped with an integrating sphere. The purchased apple was of the "Fuji" variety, and the pear was the "Nansui" variety. Reflectance measurements were conducted using fluoro-resin Spectralon® standard target plates from Labsphere Inc. (USA). Three measurements were taken for each of the 2 samples at each measurement session, and the samples were positioned at nearly the same position, respectively, for each measurement session over the course of the experiment. After purchasing the samples, time-course measurements were taken at day 0 (purchase date), day 7, 14, 21, 28, and day 35, all the while keeping the samples at room temperature. The experiment was conducted during the autumn, from October to November.

Fig. 1 and Fig. 2 show the apple and pear, respectively, mounted in the integrating sphere, and Table 1 shows the measurement conditions. The SolidSpec-3700DUV has a large sample chamber, and is equipped with an integrating sphere as standard, permitting measurement of large samples like apples and pears. Below, the results of analysis of the apple are presented first, and the results obtained with the pear are discussed later.

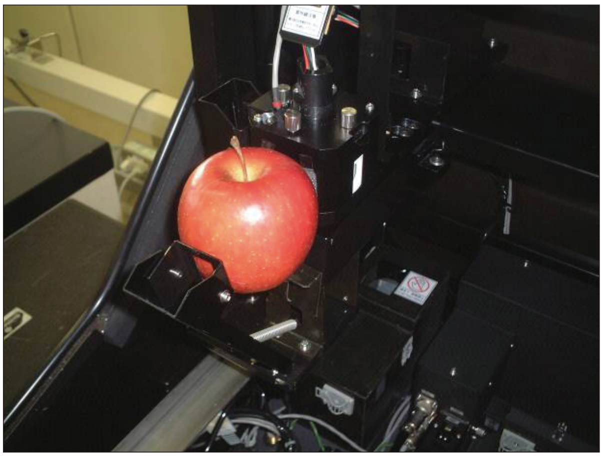

Fig. 1 Apple Mounted in Integrating Sphere

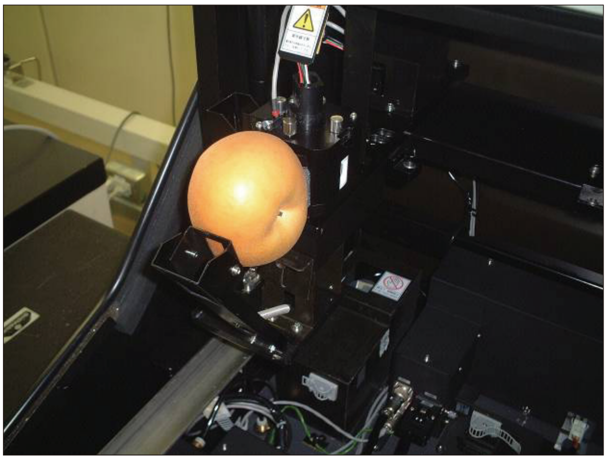

Fig. 2 Pear Mounted in Integrating Sphere

Table 1 Analytical Conditions

Instrument	: SolidSpec-3700DUV UV-VIS-NIR spectrophotometer
Measurement wavelength range	: 200 nm - 2700 nm
Scan speed	: Medium
Sampling pitch	: 1.0 nm
Photometric value	: Reflectance
Slit width	: (20) nm
Detector switching wavelengths	: 870 nm, 1650 nm

Measurement Results for the Apple and Prediction of Elapsed Days by Multivariate Analysis

The results for the apple are shown in Fig. 3. It is clear from this figure that there is a large spectral change in the visible region, but almost no change in the infrared region. Fig. 4 presents an expanded view of the visible region in which there is a large spectral change. The downward peak in the vicinity of 680 nm is due to the absorption of chlorophyll.[1), 2)] This peak clearly became smaller as the number of elapsed days increased.

Using the data in the region of this peak, it is possible to make predictions about the number of elapsed days the apple was left standing. Therefore, we tried calculating this number of elapsed days using a prediction formula based on quantitative methods of multivariate analysis. We used two types of multivariate analysis, the PLS (Partial Least Squares) method and the multiple linear regression method, and then compared the prediction accuracy between the two. For the PLS method, we utilized data centering.

Of the data obtained from the three repeat measurements, we used those of the first and second analyses to generate a calibration model. The third data points were used as verification data for evaluating the prediction accuracy of the calibration model. Using the PLS method, we created a calibration model using all of the spectral data within the range of 450 nm to 750 nm, and using the multiple linear regression method, we created a calibration model using the spectral data associated with three wavelengths, 540 nm, 664 nm and 676 nm. Fig. 5 shows the data within the selected wavelength range using the PLS method as a blue line, and the wavelength positions of the data using multiple linear regression as red arrow marks.

Each of the generated calibration models was used to predict the elapsed number of days associated with the verification data (data obtained from the third measurement). The results are shown in Table 2. Good results were obtained using both methods, with the data matching within ±3 days of the actual elapsed number of days. The calculations were conducted using the Unscrambler® multivariate analysis software, a product of CAMO. Regarding this analysis, principal component analysis was conducted using mean-centering. Multiple regression calculations were conducted using the "regression analysis" function of the Microsoft Excel® spreadsheet software.

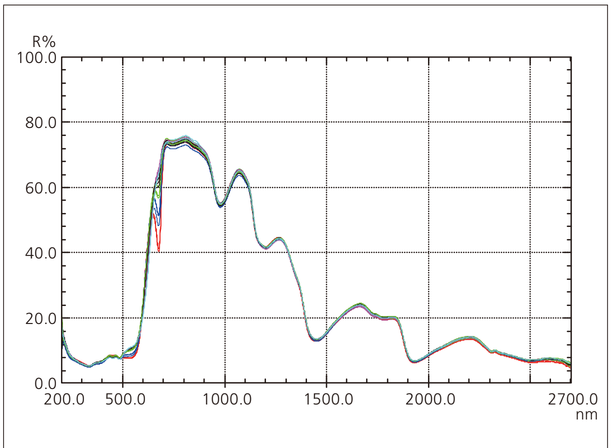

Fig. 3 Reflectance Spectra of an Apple
(Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

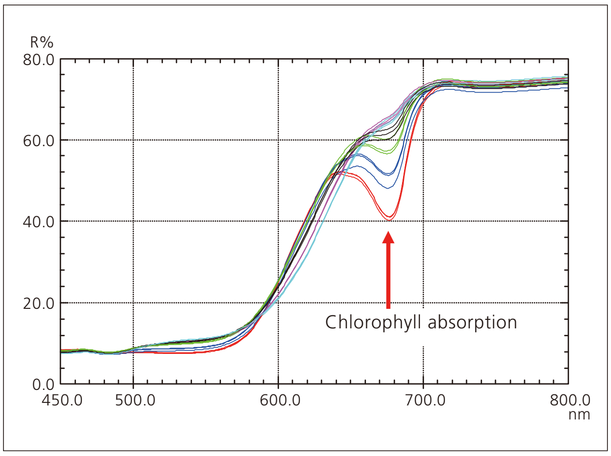

Fig. 4 Expanded Spectra of Fig. 3 (450 nm - 800 nm)
(Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

Table 2 Prediction Results for Verification Data

Actual Elapsed Days	Prediction Results with PLS Method Verification Data	Prediction Results with Multiple Linear Regression Verification Data
0	-0.66	2.56
7	7.33	6.10
14	15.26	12.77
21	21.72	21.02
28	30.32	27.54
35	35.19	33.92

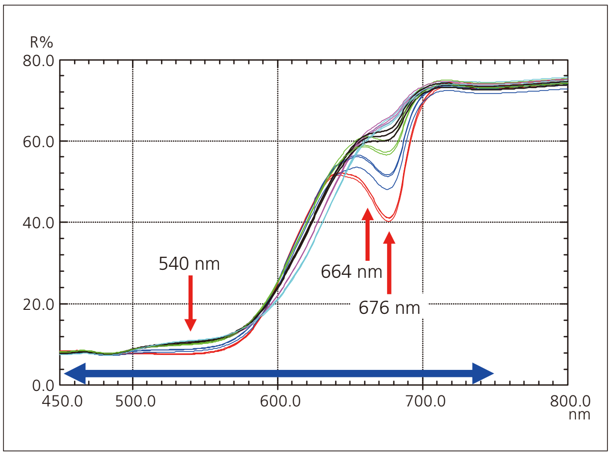

Fig. 5 Data Used for Partial Least Squares (PLS) and Multiple Linear Regression
(Blue Line Range: PLS, Red Arrow: Multiple Linear Regression)
(Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

Measurement Results for the Pear and Prediction of Elapsed Days by Multivariate Analysis

We conducted measurements for the pear just as we did for the apple. The measurement results for the pear are shown in Fig. 6. The regions from 500 nm to 1000 nm and 1000 nm to 1800 nm are expanded in Fig. 7 and Fig. 8, respectively. The weak absorption peak of chlorophyll can be seen in the vicinity of 680 nm in Fig. 7. Looking at Fig. 8, a big difference is noticeable between the spectrum at Day 0 and those of all the other elapsed days, but this is presumed to be due to a change in the hardness of the pear.[2)]

Fig. 9 shows the expanded region of the chlorophyll absorption peak of Fig. 6, and Fig. 10 shows only the first measured spectrum of those in Fig. 9. From Fig. 9 and Fig. 10, it is clear that while the absorption peak of chlorophyll is extremely weak compared to that of the apple, the spectrum changes in a way that is correlated with the elapsed number of days. The greater the elapse of time, the smaller becomes the spectral "constriction" corresponding to absorption.

Using the data in the vicinity of this chlorophyll absorption region, as in the case of the apple, we created calibration models by the PLS and multiple linear regression methods, respectively. Data associated with the 640 - 740 nm wavelength range was used for the PLS method, and those associated with 655 nm, 676 nm and 700 nm were used for the multiple linear regression method. Fig. 11 shows the data within the wavelength range using the PLS method as a blue line, and the wavelength positions of the data using multiple linear regression as red arrow markings.

Using each of the generated calibration models, we predicted the number of elapsed days for the verification sample (data associated with the third sample measurement). Those results are shown in Table 3. The calculated prediction of 35 days differed significantly from the actual number of days in both the multiple linear regression method and the PLS method. Thus, the good result obtained with the apple was not obtained with the pear. It is believed that this might be due to the low absorption of chlorophyll in the nansui pear used here, possibly introducing a degree of error.

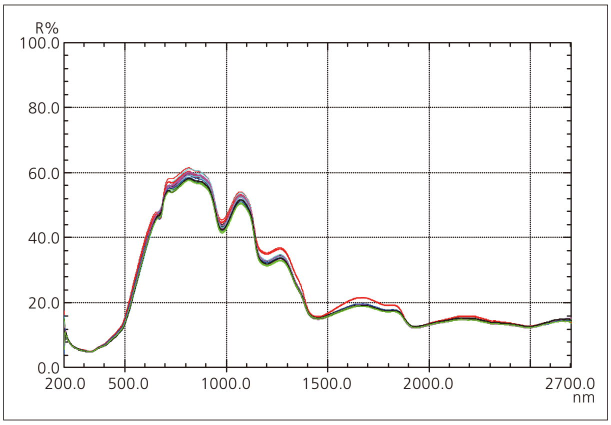

Fig. 6 Reflectance Spectra of a Pear (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

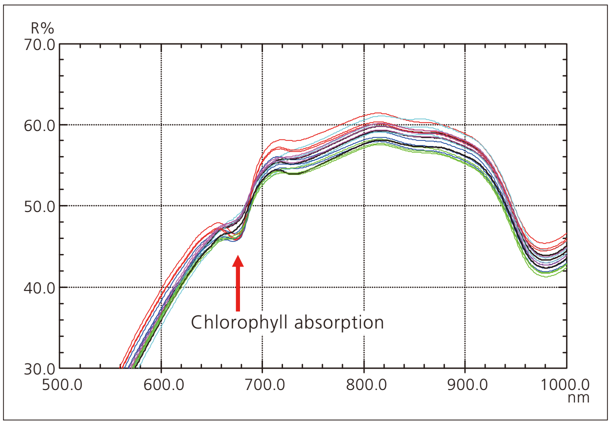

Fig. 7 Expanded Spectra of Fig. 6 (500 nm - 1000 nm) (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

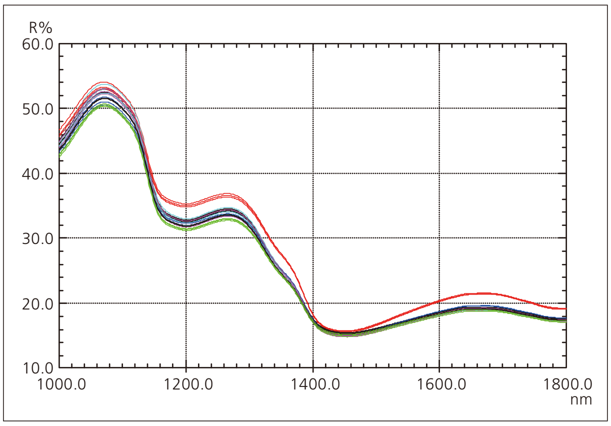

Fig. 8 Expanded Spectra of Fig. 6 (1000 nm - 1800 nm) (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

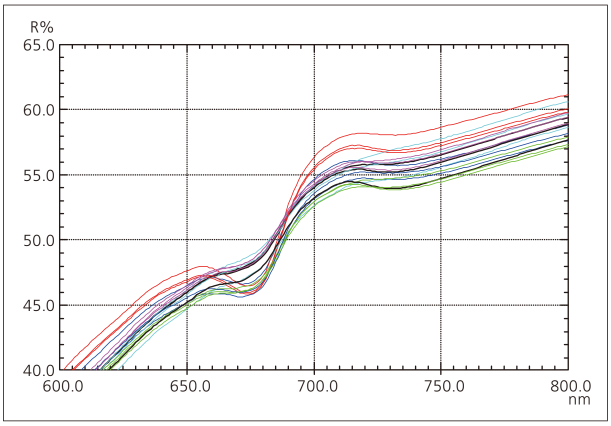

Fig. 9 Expanded Spectra of Fig. 6 (600 nm - 800 nm) (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

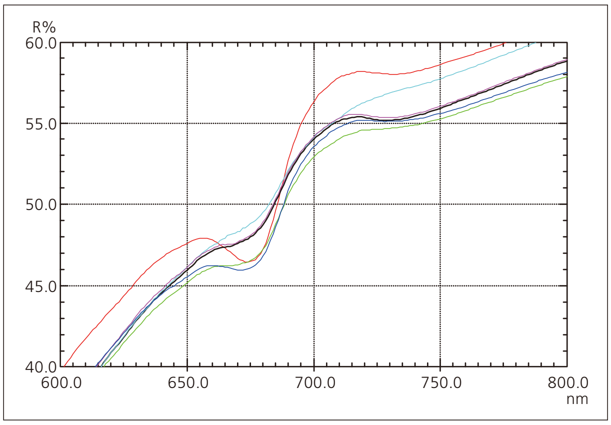

Fig. 10 First Spectra Measured for Each Elapsed Day in Fig. 9 (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

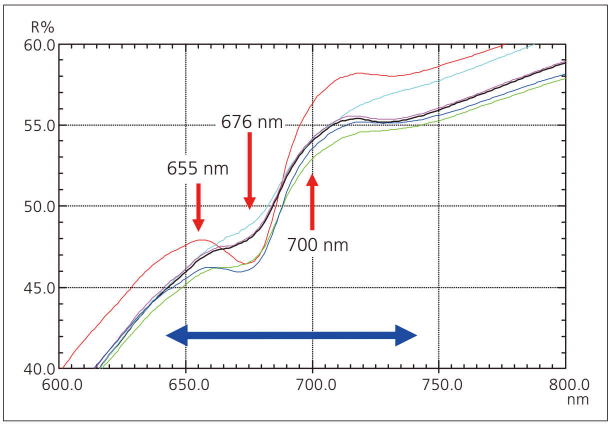

Fig. 11 Data used for Partial Least Squares (PLS) and Multiple Linear Regression (Red: 0 Days, Blue: 7 Days, Green: 14 Days, Black: 21 Days, Purple: 28 Days, Light Blue: 35 Days)

Table 3 Prediction Results for Verification Data

Actual Elapsed Days	Prediction Results with PLS Method Verification Data	Prediction Results with Multiple Linear Regression Verification Data
0	-1.49	-1.54
7	5.55	6.17
14	12.65	13.51
21	21.07	21.54
28	28.99	28.16
35	23.42	25.15

Conclusion

By applying multivariate analysis to the reflectance data of samples measured at 7 day intervals, we were able to predict the number of elapsed days within ±3 days during the course of 35 days. In the case of the pear, however, the prediction accuracy was poor compared to that for the apple, and this was thought to be due to the low absorption of chlorophyll. These results may also be subject to variation due to such factors as sample type, number of measurement samples, as well as the season when measurement is conducted. It is also necessary to consider that the results will depend on when the sample is harvested from the tree. However, because there is a definite correlation between the number of days that elapsed from the date of purchase and the absorption of chlorophyll, the present study suggests that this method can be applied to predict the number elapsed days from the date of harvest. It is believed that the combination of spectroscopy and multivariate analysis is effective for studying the maturity and degradation of chlorophyll-containing fruit.

[References]
1) Takefumi Kudo, Hiroshi Murotani, Masahiro Hazima, Souta Suzuki, Moriaki Wakaki: "Dependence of Maturity on Surface Color of Pear," Proceedings of the Faculty of Engineering of Tokai University, Vol. 46 No.2 p.29-34 (2006)
2) Yasuyuki Sagara: "Food Preference Measurement, Evaluation and Advanced Technologies - Food Sensitivity Engineering Proposal," Journal of Food Science and Technology Vol. 41, Supplement No. 6

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