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Application News

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Spectrophotometric Analysis

Quantitative Analysis of Fat in Mayonnaise by Reflectance Spectroscopy and Multivariate Analysis

Mayonnaise is one of various seasonings that are used on a daily basis. Reflecting the health consciousness that is prevalent in recent years, fat content-adjusted mayonnaise products have been sold in great quantities. Accordingly, it is important that the fat content listed on the label reflect the fat content determined through accurate measurement. Fat content in mayonnaise is typically determined using the Soxhlet extraction method. However, the process involving the repeated evaporation of solvent required to extract the target substance is quite time consuming.
We therefore investigated a simple method based on reflectance measurement for this fat quantitation. Combining reflectance measurement using a screw-cap vial with multivariate analysis, we demonstrated the ease with which fat quantitation can be accomplished. Multivariate analysis was conducted using both multiple regression and the PLS method, and the quantitative accuracy was compared between the two methods. Both methods were found to provide good results. Furthermore, good quantitative accuracy using both the multiple regression and the PLS method were obtained even when the original mayonnaise containers were used "as is." Here, we introduce the results of this investigation.

Total Luminous Reflectance Measurement of Mayonnaise

The measurement samples included eight types of mayonnaise containing different levels of fat content. Table 1 shows the fat content of each sample as indicated on labels affixed to mayonnaise containers. The samples are indicated using the letters A – H, based on descending order of the indicated fat content.
For this investigation, measurements were conducted after a jig for securing all the samples in the same position was attached to the integrating sphere. After transferring a sample into a glass screw-cap vial, and setting the vial in the integrating sphere as shown in Fig. 1, each sample type was measured twice, replacing the vial for the same sample between the two measurements. Disposable screw-top vials were used for measurement. Thus, a total of 16 data points (8×2=16) were obtained. In addition, we used as a reference plate for reflectance measurement a Spectralon® fluorine-based resin white plate obtained from Labsphere Inc. (United States).
The measurement conditions and measurement results are shown in Table 2 and Fig. 2, respectively. The vertical axis of Fig. 2 expresses the absorbance-related $\log_{10}(R_0/R)$ value. Here, R_0 is reflection intensity of the standard white plate, and R is the reflection intensity of the sample. The "Abs." of Fig. 2 represents $\log_{10}(R_0/R)$. As is evident from Fig. 2, the results from each set of two measurements are practically overlapped, indicating that switching of the screw-top vials had little effect on the results.
Fig. 3 shows an expanded view of Fig. 2 in the range of 1000 nm – 1500 nm. The region in which fat absorption occurs (in the vicinity of 1210 nm) is circled in red. Focusing on this absorption region, it is clear that the greater the fat content of the sample, the larger the absorption peak.

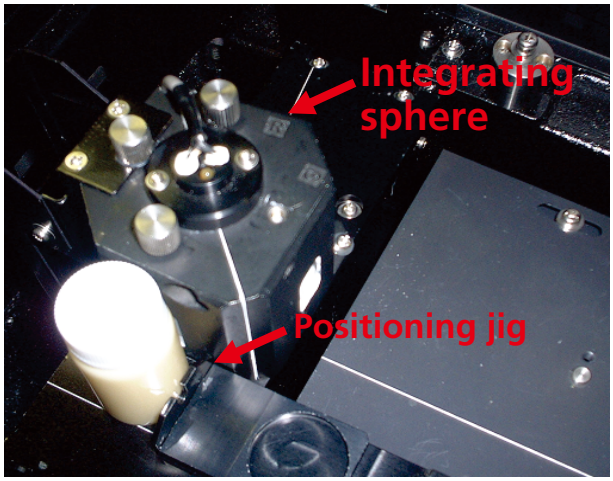

Fig. 1 Sample Set in an Integrating Sphere

Table 1 Measured Mayonnaise Types (8 Kinds)

Sample	Fat Content Indicated on Label (g/15 g)
A	11.8
B	11.2
C	9.8
D	8.6
E	5.2
F	5.1
G	3.6
H	2.2

Table 2 Analytical Conditions

Instrument	: UV-3600 Ultraviolet-Visible-Near Infrared Spectrophotometer, MPC-3100 Large-Sample Compartment (with built-in integrating sphere)
Measurement Wavelength Range	: 200 nm – 2300 nm
Scan Speed	: Medium
Sampling Pitch	: 2.0 nm
Photometric Value	: Reflectance
Slit Width	: (20) nm

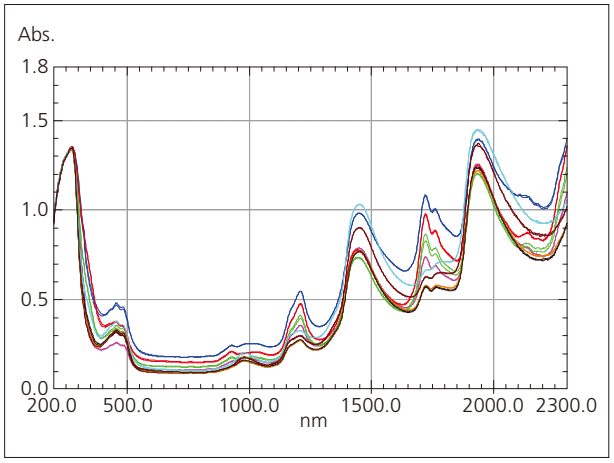

Fig. 2 Total Luminous Measurement Spectra
Red: A, Green: B, Blue: C, Purple: D, Black: E, Orange: F, Brown: G, Light Blue: H

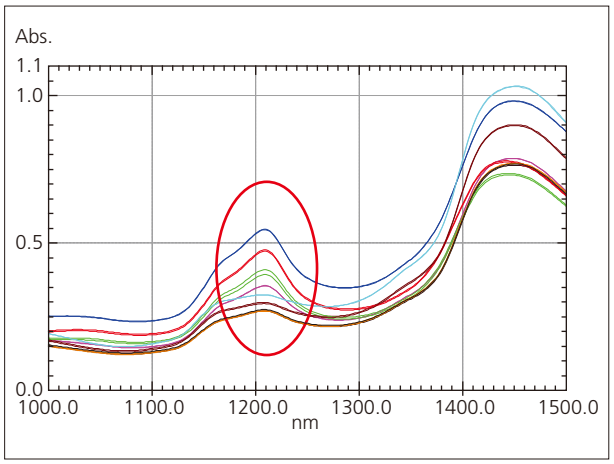

Fig. 3 Expanded Spectra of Fig. 2
Red: A, Green: B, Blue: C, Purple: D, Black: E, Orange: F, Brown: G, Light Blue: H

Results of Quantitative Analysis

Quantitation of the fat content was conducted by applying the multiple regression method and the PLS method of multivariate analysis on the obtained data. We created a calibration model for each of the methods based on the standard samples A, C, E, G and H of Table 1. We used the fat content values listed on the mayonnaise product labels as the true fat content values of the standard samples.
Samples B, D and F of Table 1 were used as verification samples for predicting the accuracy of the calibration model. Table 3 shows the results predicted for the fat content of the verification samples using each of the calibration models.
Comparing the results, there were no large differences in quantitation accuracy between the two methods, indicating good results. Here, the root mean square error of prediction (RMSEP) of Table 3 is an index that represents the mean difference between the predicted and actual results based on the values defined in Fig. 4.
Calculations associated with the PLS method were conducted using The Unscrambler®1) multivariate analysis software of CAMO Software company. In addition, regression analysis calculations for the multiple regression method were conducted using the "Regression Analysis" feature of the Microsoft Excel®2) spreadsheet software.

Note: Four wavelengths, 1150 nm, 1210 nm, 1240 nm, and 1280 nm were used for the calculations by the multiple regression method. As for the PLS method, calculations were conducted by the center averaging process with respect to the total data between 1150 nm – 1280 nm.

Table 3 Prediction Results of Fat Calculated by Each Calibration Model for Validation Samples Measured Using Screw-Cap Vials

Sample	Fat Content Indicated on Label (g/15 g)	Predicted Results According to Multiple Regression Method	Predicted Results According to PLS Method
B (first)	11.2	10.17	10.02
B (second)	11.2	10.72	10.50
D (first)	8.6	8.05	8.23
D (second)	8.6	7.87	8.20
F (first)	5.1	4.90	4.89
F (second)	5.1	4.99	5.00
RMSEP		0.603	0.610

*RMSEP：Root Mean Square Error of Prediction

$$RMSEP=\sqrt{\frac{\sum_{i=1}^{n}(y'_i-y_i)^2}{N}}$$

Where:
y'i : expected value
yi : actual value
N : number of evaluation samples

Fig. 4 Definition Formula for RMSEP

Measurement of Mayonnaise with Container

The container materials of the mayonnaise samples measured here all consisted of the polyethylene (PE) and ethylene-vinyl alcohol copolymer resin (EVOH). Focusing on that point, we then attempted measurement of the mayonnaise samples "as is" in their respective containers.
The mayonnaise container thickness varied depending on the sample. In this case, therefore, the influence of transmittance due to variation in container thicknesses should also be reflected in the data. This, in terms of multivariate analysis, means that an additional factor(s) may be needed to account for the noise added by the additional substance. Multivariate analysis permits correction for variation due to other components. Therefore, in this case, it means that variation due to thickness can be used for correction of the mayonnaise data.
A photograph of a sample set in the integrating sphere is shown in Fig. 5, and the measurement results for all the samples, A – H, are shown in Fig. 6. The measurement conditions are the same as those shown in Table 2. Each sample was measured twice, while repositioning it in the integrating sphere between measurements.
Fig. 7 shows an expanded view of the region of Fig. 6, and the region in which fat absorption occurs (in the vicinity of 1210 nm) is circled in red. Here, as well, it is clear that the greater the fat content in a sample, the larger the absorption peak in this region. Table 1 lists the fat content for each of the samples.

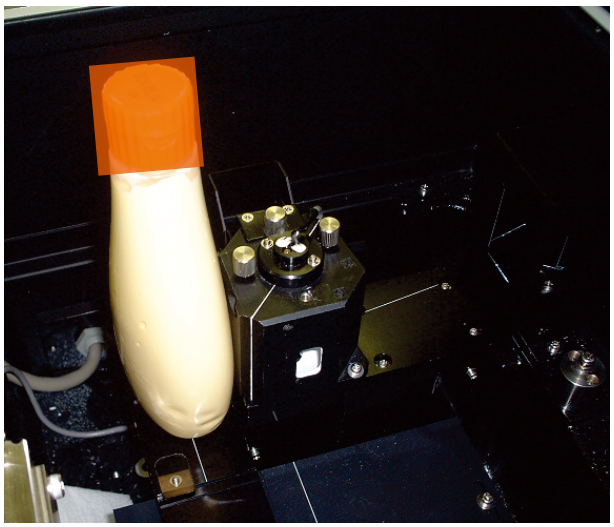

Fig. 5 Sample Set in an Integrating Sphere

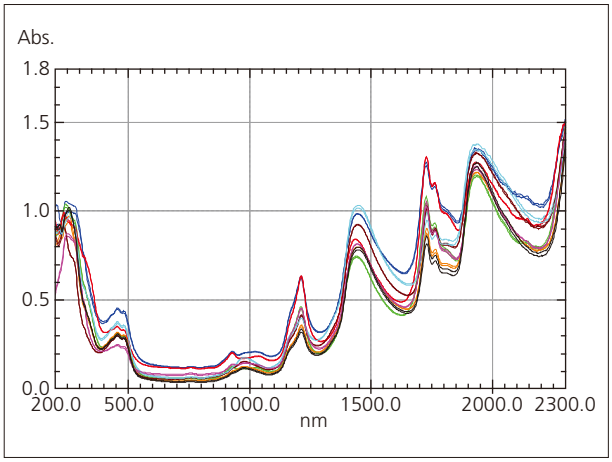

Fig. 6 Total Luminous Measurement Spectra
Red: A, Green: B, Blue: C, Purple: D, Black: E, Orange: F, Brown: G, Light Blue: H

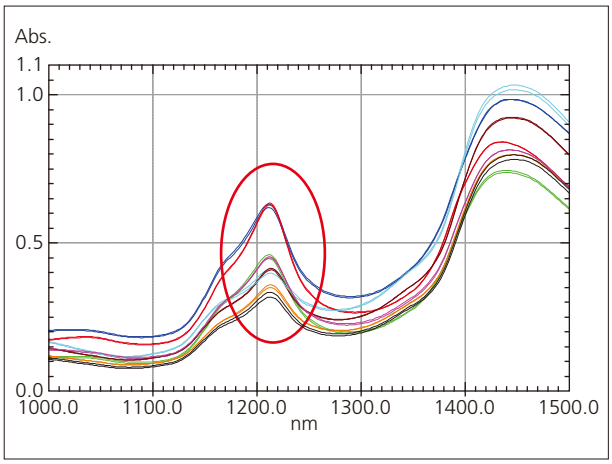

Fig. 7 Expanded Spectra in Fig. 6
Red: A, Green: B, Blue: C, Purple: D, Black: E, Orange: F, Brown: G, Light Blue: H

Results of Quantitative Analysis of Mayonnaise Measured "As Is" in its Container

Quantitation of fat content was conducted in the same way as that for the mayonnaise in screw-cap vials. The predicted results for each of the calibration models corresponding to validation sample are shown in Table 4. The data indicate that good results were obtained using both the multiple regression and PLS method.

Note: Data at 4 wavelengths, 1180 nm, 1210 nm, 1240 nm and 1300 nm, were used for calculation by the multiple regression method. With the PLS method, calculation was conducted by center averaging with respect to all data between 1100 nm – 1300 nm.

Table 4 Prediction Results for Fat Calculated by Each Calibration Model for Validation Samples Measured "As Is" in their Containers

Sample	Fat Content Indicated on Label (g/15 g)	Predicted Results According to Multiple Regression Method	Predicted Results According to PLS Method
B (first)	11.2	11.10	11.28
B (second)	11.2	10.96	11.25
D (first)	8.6	8.02	7.98
D (second)	8.6	8.17	8.18
F (first)	5.1	4.86	4.76
F(second)	5.1	4.95	4.83
RMSEP		0.334	0.355

Conclusion

We conducted measurement of mayonnaise with the samples transferred into screw-cap vials for measurement, in addition to measurement of the mayonnaises samples in their original containers. The measurement data were then subjected to multivariate analysis using both multiple regression and PLS analysis for determination of fat content. We also compared the quantitative accuracy obtained by these two methods. Good results were obtained using multivariate analysis and PLS analysis with respect to measurement using both the vials and the original containers.
Conventionally, highly viscous cream-state samples have been measured by the transmittance method with the samples coated on glass plates, etc., but measurement with this method has generally been difficult due to uneven coating, etc. Using the method presented here, however, it was found that measurement can be conducted easily and with good accuracy.
The results obtained here suggest that this technique is effective for quantitative analysis of creamy samples such as mayonnaise.

1) The Unscrambler is a registered trademark or trademark of CAMO.
2) Excel is a registered trademark or trademark of Microsoft Corporation.

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