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Application News

No.A524

Spectrophotometric Analysis

Differentiating Olive Oils Using UV-VIS Spectrophotometer and Spectrofluorophotometer

Introduction

There is growing interest in food safety and organic foods, with an increasing number of shops specializing in a variety of food products and increasing circulation of high price import goods. One of these foods is olive oil, which is supposed to have both health and aesthetic benefits. The most expensive form of olive oil is extra virgin olive oil, which is regulated by the International Olive Council. Only olive oil that is chemically unprocessed, produced by squeezing and filtering olive fruit, and with an acidity of no more than 0.8 % qualifies as extra virgin olive oil. Another olive oil called pure olive oil is created by purification and high-temperature treatment. Differentiating between extra virgin olive oil and pure olive oil based on appearance alone is difficult. This article describes an attempt to differentiate between these two olive oil types by spectrum measurement using Shimadzu UV-2700 UV-VIS spectrophotometer and RF-6000 spectrofluorophotometer, then performing multivariate analysis.

Absorbance Measurement of Olive Oils

Fig. 1 shows Shimadzu UV-2700 that was used to measure absorbance. Fig. 2 shows some of the olive oils tested. They each differ in terms of color, odor, and place of origin. Ten different extra virgin olive oils were prepared from a total of 6 producers. Samples were named in the format "○ × E", where "○" was replaced by letters A through F to refer to each producer, and "×" was replaced by each producer's consecutive numbers in order of increasing olive oil price. Pure olive oil was also prepared from producers A and B. These samples were named in the format "○ × P". Each olive oil was placed in a quartz cell, then its absorption spectrum was measured. Measurement conditions are shown in Table 1, and three spectra representative of the spectra obtained are shown in Fig. 3. Absorption peak wavelengths are almost identical between spectra, though with obvious differences in their degree of absorption. Results confirmed the extra virgin olive oils tended to exhibit higher absorbance than the pure olive oils.

Table 1 UV-2700 Measurement Conditions

Spectrum Type	: Absorption spectrum
Measurement Wavelength Range	: 330 nm to 800 nm
Scanning Speed	: Intermediate
Sampling Pitch	: 0.5 nm
Light Source Switching Wavelength	: 323 nm

Fig. 1 UV-2700 UV-VIS Spectrophotometer

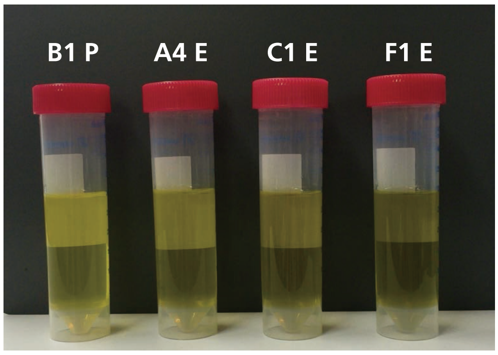

Fig. 2 Various Olive Oils

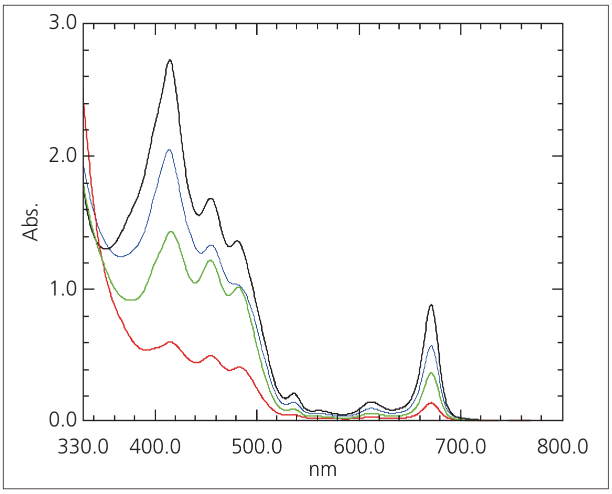

Fig. 3 Olive Oil Absorption Spectra
Red: B1 P, Green: A4 E, Blue: C1 E, Black: F1 E

Multivariate Analysis Using Absorption Spectra

We attempted to differentiate between pure olive oil and extra virgin olive oil by performing a multivariate analysis of the results obtained by absorbance measurement. The Unscrambler®X[1)] multivariate analysis software was used to perform difference analysis on absorbance at 7 peak wavelengths.

Principal component analysis (PCA) and cluster analysis were used to differentiate between olive oil types. With PCA, scores are calculated to allow visual differentiation by the analyst (score plot). A loading plot is also used to determine characteristic factors in each grouping that have a strong influence on the score plot. Cluster analysis differentiates samples based on a tree diagram. The shorter the horizontal line that connects each sample, the more similar those samples.

The score plot obtained by PCA is shown in Fig. 4. Pure olive oils are clustered in the negative direction along the dominant PC-1 axis, while extra virgin olive oils are clustered in the positive direction along the dominant PC-1 axis. This shows successful differentiation between olive oil types. The loading plot in Fig. 5 shows a characteristic of the extra virgin olive oils tested is strong signals at short wavelengths, such as 415 nm and 454.5 nm.

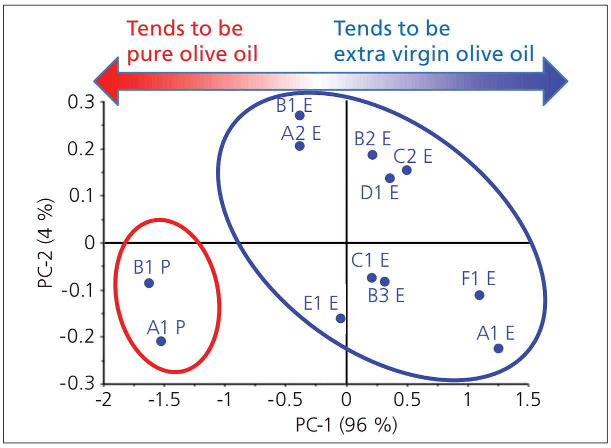

Fig. 4 Score Plot Based on Olive Oil Absorbance

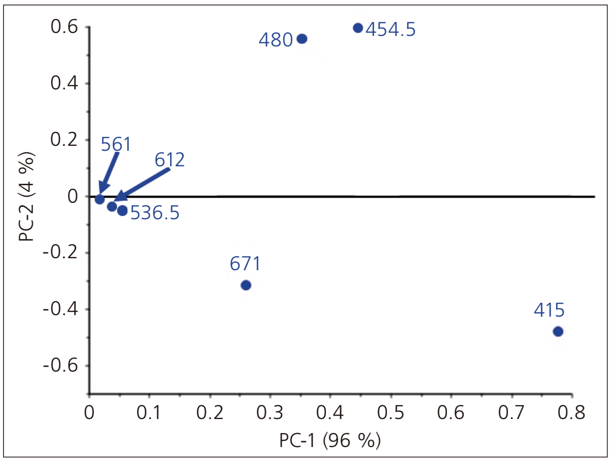

Fig. 5 Loading Plot Based on Olive Oil Absorbance

Cluster analysis results are shown in Fig. 6. At a glance, the tree diagram shows the olive oils separated into 2 groups and the degree of similarity between the samples. This result shows we successfully differentiated between pure olive oils and extra virgin olive oils.

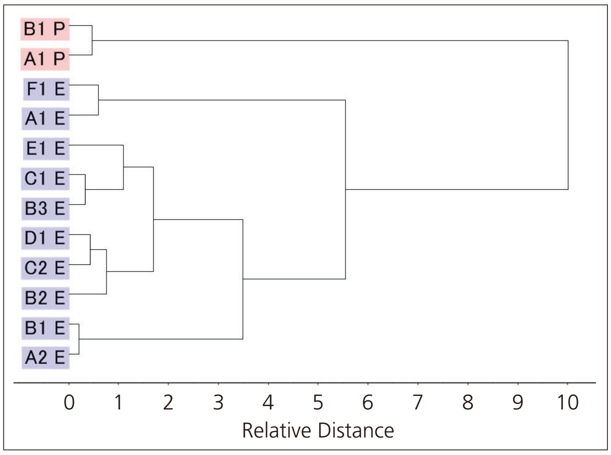

Fig. 6 Cluster Analysis Result Based on Olive Oil Absorbance

Three-Dimensional Spectra Measurements of Olive Oils

The three-dimensional emission spectra of olive oils were measured using Shimadzu RF-6000 spectrofluorophotometer. Fig. 7 shows the instrument used. Due to the high absorbance exhibited by the samples, a solid sample holder (Fig. 8) was used to compensate for self-absorption effects. Self-absorption is the phenomenon of light emitted by the sample being absorbed by the sample itself. When the absorption spectrum of a highly absorbing sample is measured in a normal cell holder, the amount of emission light that enters the detector can be reduced due to emission light being absorbed by the sample itself. A solid sample holder was used to direct excitation light towards the corner of the quartz cell as shown in Fig. 9. This reduces the amount of sample through which emission light travels, and so reduces the effects of self-absorption.

Fig. 7 RF-6000 Spectrofluorophotometer

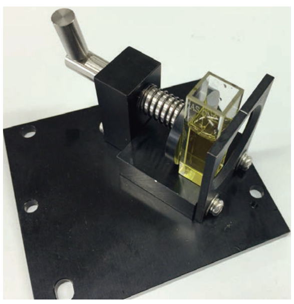

Fig. 8 Solid Sample Holder

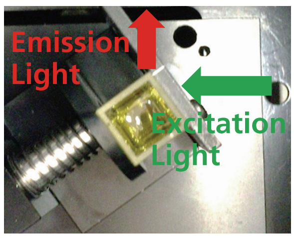

Fig. 9 Measurement Method

Table 2 shows the measurement conditions used. A filter (IHU310) that blocks light below 310 nm was placed in the path of emission light to prevent high-order excitation light reaching the detector. Fig. 10 shows two of the three-dimensional spectral diagrams obtained. Emission light predicted to be derived from chlorophyll was confirmed at Em 680 nm in both samples. Emission light at this wavelength was detected more strongly from extra virgin olive oil samples compared to pure olive oil samples. Also, strong emission light in the vicinity of Em 400 nm was mainly detected from pure olive oil samples. This 400 nm emission light is predicted to be derived from vitamins. Fig. 11 compares a pure olive oil and an extra virgin olive oil showing emission spectra obtained at an excitation wavelength of 300 nm. The excitation light region shown in Fig. 11 is indicated by the white dotted lines in Fig. 10. The emission spectra show different peak strengths and peak tail shapes between 300 nm and 500 nm.

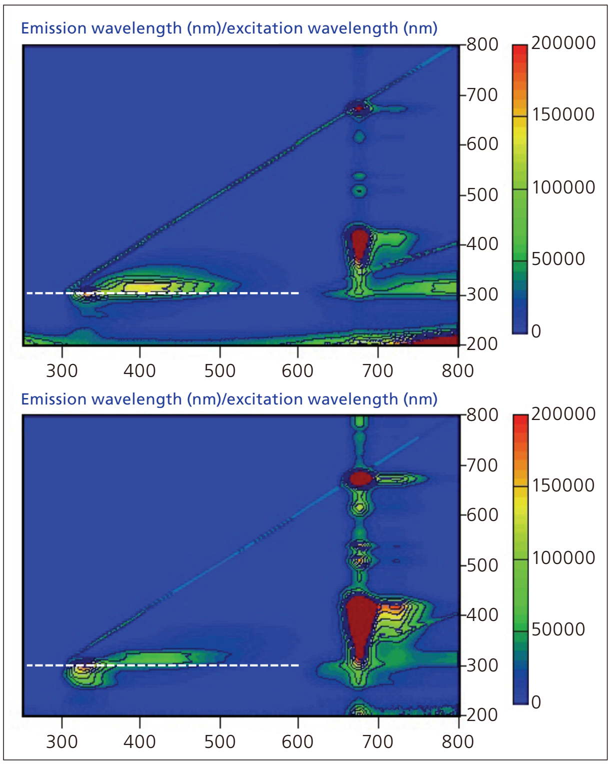

Fig. 10 Three-Dimensional Spectra of Olive Oils
Top: A1 P, Bottom: F1 E

Multivariate Analysis Based on Three-Dimensional Spectra

Similar to absorbance analysis, a multivariate analysis was performed on the three-dimensional spectra to differentiate between pure olive oils and extra virgin olive oils. Emission light intensity at the 10 points shown in Fig. 12 (A through J) was used for this analysis.

Table 2 RF-6000 Measurement Conditions

Optional Accessory	: Solid sample holder, IHU310
Spectrum Type	: 3D spectrum
Measurement Wavelength Range	: Ex 200 nm to 800 nm Em 250 nm to 800 nm
Scanning Speed	: 6000 nm/min
Wavelength Interval	: Ex 5.0 nm, Em 1.0 nm
Bandwidth	: Ex 5.0 nm, Em 5.0 nm
Sensitivity	: Low

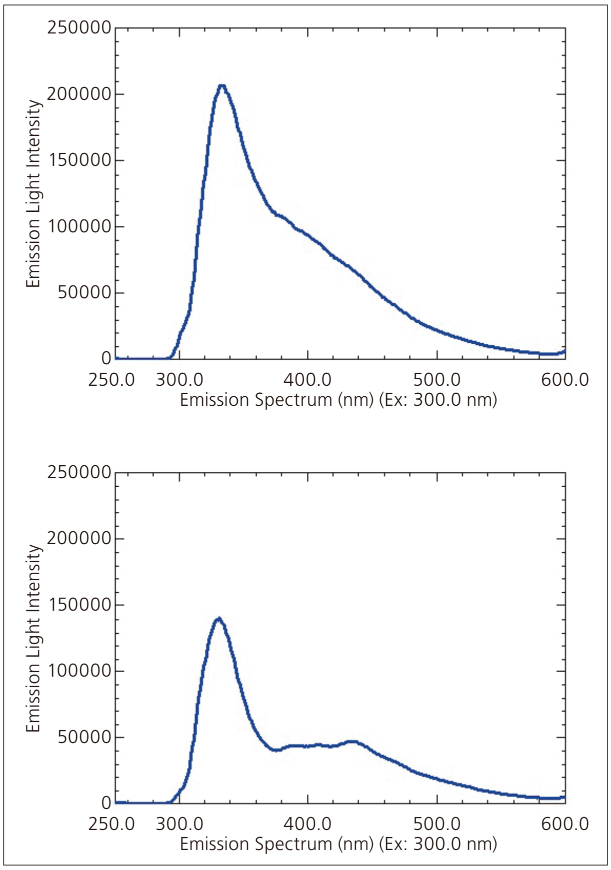

Fig. 11 Emission Spectrum of Olive Oils Excited at 300 nm
Top: A1 P, Bottom: F1 E

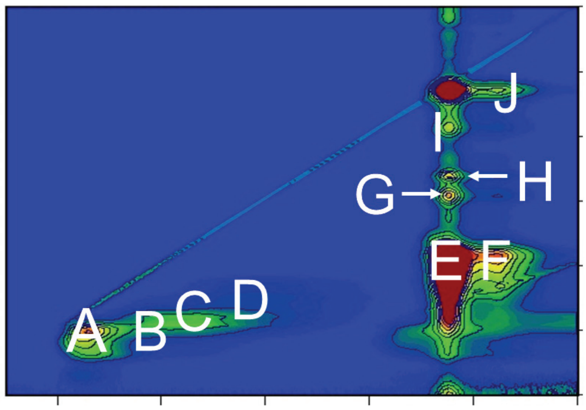

Fig. 12 Analysis Points for Multivariate Analysis

The score plot obtained by PCA is shown in Fig. 13. Pure olive oils are clustered in the positive direction along the dominant PC-1 axis and extra virgin olive oils are clustered in the negative direction along the dominant PC-1 axis. This shows the olive oil types have been separated.
Compared to the score plot created based on absorbance, the A2 E and B1 E samples are positioned closer to the pure olive oil samples. While A2 E and B1 E are both extra virgin olive oils, we can predict they are similar to pure olive oils. The loading plot is shown in Fig. 14. It shows a characteristic of the pure olive oils is for strong signals at emission light analysis points A through D, which are points predicted to be derived from vitamins.

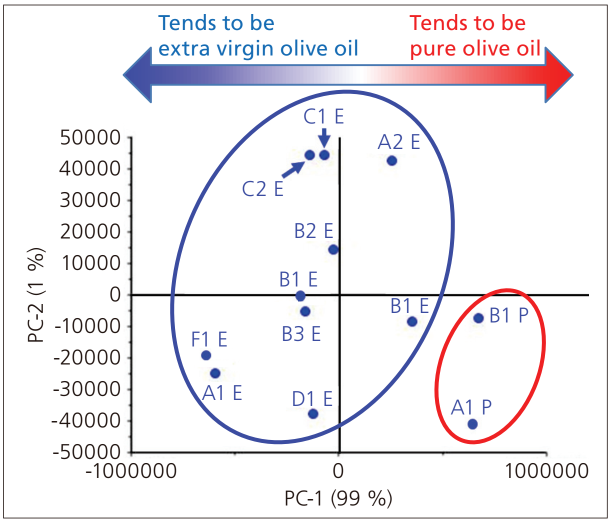

Fig. 13 Score Plot Based on Olive Oil Absorbance

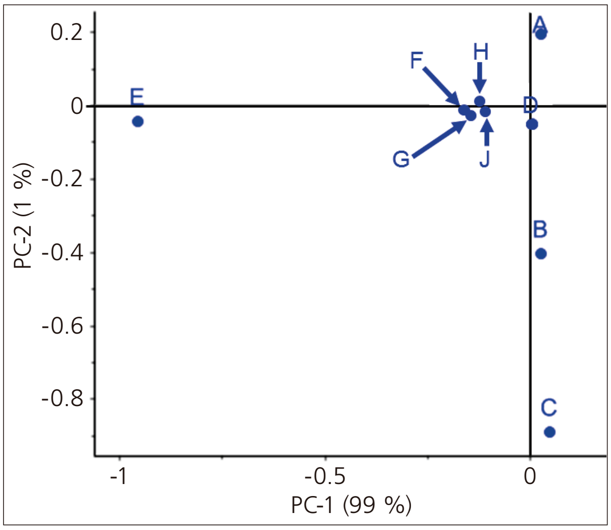

Fig. 14 Loading Plot Based on Olive Oil Absorbance

Results of the cluster analysis are shown in Fig. 15. Compared to the results obtained based on absorbance, the A2 E and B1 E samples are included in the pure olive oil group. This result also shows that while these samples are extra virgin olive oils, they tend to be closer to pure olive oils. Using emission light spectra, we could predict which extra virgin olive oils are likely to contain more vitamins.

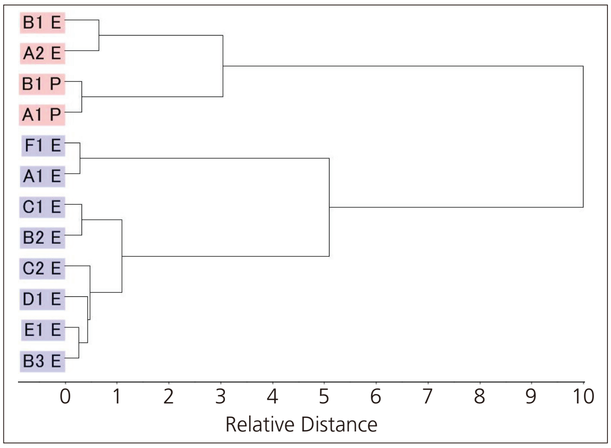

Fig. 15 Cluster Analysis Result Based on Olive Oil Absorbance

Conclusion

Shimadzu UV-2700 UV-VIS spectrophotometer was used to measure absorption spectra. Results showed the extra virgin olive oils have a higher absorbance than the pure olive oils. Next, the three-dimensional emission spectra of olive oils were measured using Shimadzu RF-6000 spectrofluorophotometer. Differences in emission light intensity and peak width were confirmed in the three-dimensional emission spectra results. Multivariate analysis was then performed based on each spectrum. This allowed successful differentiation between the extra virgin olive oils and pure olive oils, while results obtained from the RF-6000 allowed identification of extra virgin olive oils particularly similar to pure olive oils.

<Acknowledgments>
We are grateful to Kazumi Hashimoto of the Polymer Photonics Laboratory, Master's Program of Innovative Materials, Graduate School of Science and Technology, Kyoto Institute of Technology for providing assistance in performing these measurements.

1) The Unscrambler®X is a trademark or registered trademark of CAMO Software.

First Edition: Dec. 2016