Featured Articles

Fluorescence Pattern Analysis to Assist Food Safety

—Food Analysis Technology Driven by Fluorescence Fingerprints—

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OVERVIEW: Fluorescence fingerprint analysis uses the large quantity of information contained in fluorescence patterns generated by organic components in samples to identify quality or place of origin, or to detect harmful substances. It is a simple and low-cost method that is gaining attention for its food industry applications. The Fluorescence Spectrophotometer F-7100 made by Hitachi High-Tech Science incorporates several technologies that enable high-throughput fluorescence fingerprint measurement. It is designed to have a full range of specialized functions for fluorescence fingerprint measurement, such as a new automatic filter accessory device that simply and rapidly removes the effect of spectrometer-specific higher-order light. This article presents its features and some example applications.

INTRODUCTION

FLUORESCENCE fingerprint analysis measures the fluorescence pattern (fluorescence fingerprint) emitted by a sample and performs statistical multivariate analysis on a massive quantity of numerical data representing parameters such as fluorescence wavelength and intensity. Recent advances in information processing technology have enabled analysis of large-volume data, making fluorescence fingerprint analysis practical and expanding its scope of application. The level of attention being paid to food industry applications has increased dramatically over the past few years, with expectations for applications such as the identification of sample types and places of origin[(1), (2)], the calculation of sample mixture ratios[(3)], the detection of harmful substances such as mycotoxins[(4) - (7)], and the quantification of functional components.

This article describes the fluorescence fingerprint analysis method and its features, and presents some of the latest example applications.

FLUORESCENCE FINGERPRINT ANALYSIS

Fluorescence Fingerprints

When light (excitation light) is shone on a sample, light of various wavelengths (fluorescent light) is emitted from the organic substances contained in the sample. Fluorescent light contains three items of information: the wavelength of the excitation light (the excitation wavelength), the wavelength of the fluorescent light from the sample (the fluorescence wavelength), and the intensity of the fluorescent light from the sample (the fluorescence intensity). A fluorescence fingerprint is obtained by plotting these items of information in three dimensions as an aerial view or contour map.

As an example, Fig. 1 shows the fluorescence fingerprint of a sample of pineapple juice. The excitation wavelength is shown on the vertical axis, the fluorescence wavelength is shown on the horizontal axis, and the fluorescence intensity is represented by contour lines. The plot resembles a human fingerprint, and is therefore called a fluorescence fingerprint. Since fluorescent light is observed at longer wavelengths than excitation wavelengths (Stokes' law), the fluorescence fingerprint appears at the bottom-right of the diagonal. The fluorescence intensity is plotted in the form of contour lines since changing the excitation wavelength will not result in a change in fluorescence wavelength for the same component.

Peaks (a) to (e) were detected in this fluorescence fingerprint. These peaks come from the fluorescent components contained in the sample. From previously reported examples[(8)], it can be inferred that they include (a) L-tyrosine from aromatic amino acid, (b) L-tryptophan, (c) ferulic acid and lignins from cell walls, (d) vitamin B_2 (riboflavin), and (e) chlorophylls. Different places of origin or growth conditions will cause changes in the quantities of these components,

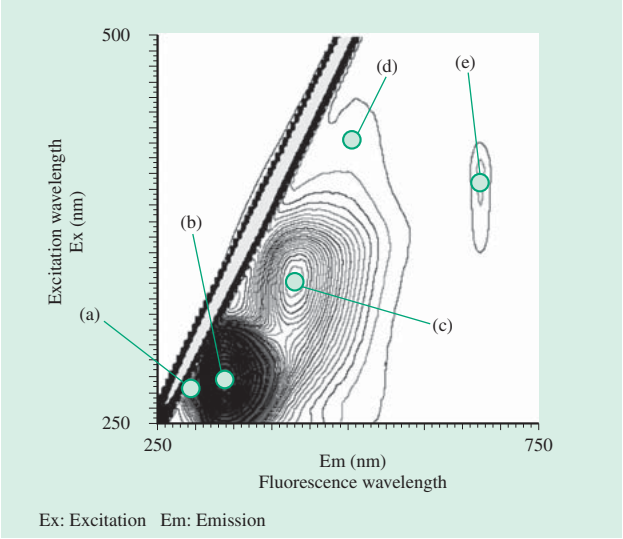

Fig. 1—Fluorescence Fingerprint Example (Pineapple Juice). The vertical axis represents the excitation wavelength, the horizontal axis represents the fluorescence wavelength, and the contour lines represent the fluorescence intensity. Peaks were detected for fluorescent components (a) to (e) contained in the sample. From previously reported examples, it can be inferred that they include (a) L-tyrosine from aromatic amino acid, (b) L-tryptophan, (c) ferulic acid and lignins from cell walls, (d) vitamin B_2 (riboflavin), and (e) chlorophylls.

enabling sample-specific fluorescence fingerprints to be acquired that enable identification of information such as place of origin.

Fluorescence Fingerprint Analysis Method and Identification Applications

Fluorescence fingerprints are a measurement method that has been known for about 30 years. But with a single measurement requiring anywhere from tens of minutes to hours to complete, it was initially an inconvenient method, and required time for data analysis. As a result, only a single wavelength of an excitation light/fluorescent light peak for a component of interest was previously used to identify or quantify the component. Recent improvements in measuring equipment performance and advances in large-volume data analysis technology have enabled the use of statistical methods for comprehensive analysis of fluorescent light and excitation light spectra (large-volume data consisting of information for many wavelengths and intensities), greatly expanding the potential for identification applications.

Fig. 2 is a conceptual diagram illustrating the fluorescence fingerprint analysis method. Multiple samples with characteristics (such as place of origin or quality) that are known beforehand are obtained (these samples are called known samples). The fluorescence fingerprint data of each sample is measured. This data is subjected to multivariate analysis to create an 'identification model' from the known samples. The plotted points in the identification model correspond to each known sample. The fluorescence fingerprint data of the unknown sample is then measured and plotted in the identification model to determine which known sample the unknown sample most closely resembles, enabling identification of its place of origin and quality.

Fluorescence fingerprint data is an aggregate of anywhere from thousands to tens of thousands of data points. To enable use of this large volume of data, multivariate analysis is applied to condense it into a small number of variables that express the data's features. For example, principal component analysis is a method that lowers the dimensions of large-volume data by condensing it into variables (feature values) called principal component scores. By using principal component scores 1 and 2 on the horizontal and vertical axes, the identification model of Fig. 2 displays large-volume fluorescence fingerprint data at a single coordinate using two principal component scores. This plot indicates which group the unknown sample belongs to, enabling easy identification.

Features of Fluorescence Fingerprint Analysis

Fluorescence fingerprint analysis has the following three major features:

(1) Ability to perform identification analysis from a massive quantity of information

The wavelengths of excitation light and fluorescent light are generally measured in a range extending from ultraviolet to visible light (about 200 to 800 nm). The intensity information acquired consists of tens of thousands of data points. This massive quantity of numerical data is subjected to multivariate analysis to condense it into several variables for use in identification analysis.

(2) Simplicity of analysis

Equipment-based analysis of food or farm products generally requires preprocessing involving several procedures. However, fluorescence fingerprint analysis measures the fluorescence possessed by the sample itself (auto-fluorescence), enabling measurement with a minimal amount of preprocessing (such as pulverization, dissolving, or filtration), and direct analysis of samples such as fruit fragments or raw meat. Analysis is also possible on samples in either a liquid or solid state (such as in bar or powder form).

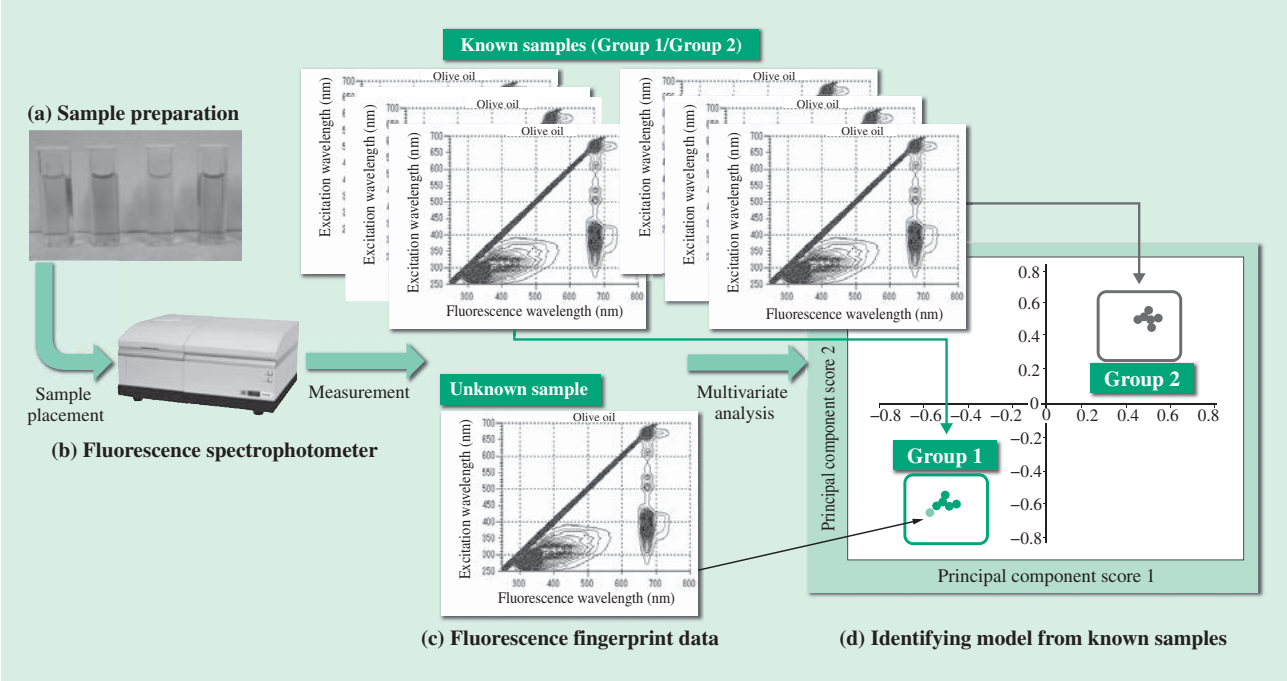

Fig. 2—Concept of Fluorescence Fingerprint Analysis Method.
This method obtains multiple samples with characteristics that are known beforehand (known samples) and measures the fluorescence fingerprint data of each one. This data is subjected to multivariate analysis to create an identifying model from the known samples. The fluorescence fingerprint data of the unknown sample is then measured and subjected to multivariate analysis. The result is plotted over the identifying model, enabling the group that the unknown sample most closely resembles to be inferred.

(3) Lower analysis cost

Fluorescence fingerprint analysis has lower startup and running costs than other methods using analysis equipment. In relation to mycotoxin analysis for example, Fujita et al.[(4) - (7)] reported that fluorescence fingerprint analysis can be expected to lower the cost of analysis when used for sample selection (screening analysis), a preprocess for procedures such as high-cost mass analysis.

FLUORESCENCE FINGERPRINT MEASUREMENT SYSTEMS

Fluorescence fingerprint analysis previously had limited applications due to the time required for measurement. However, advances in the sensitivity and speed of the fluorescence spectrophotometers used for fluorescence measurement that began about ten years ago have improved throughput to the point where a single measurement can be completed in a matter of minutes, enabling a large quantity of fluorescence fingerprint measurements. Fluorescence fingerprint measurement requires scanning of the excitation light from the ultraviolet range to the visible range, and continuous measurement of the wavelength and intensity of the fluorescent light (fluorescent spectrum). Fluorescence spectrophotometers therefore require high-speed/high-precision measurement performance.

Hitachi High-Tech Science Corporation's Fluorescence Spectrophotometer F-7100 has a three-dimensional fluorescent spectrum measurement mode for fluorescence fingerprint measurement. It also incorporates several technologies designed to increase the throughput of fluorescence fingerprint measurement, such as one of the fastest scan speeds in its class (60,000 nm per minute), automatic setting of response processes for high-speed scanning, and rapid excitation wavelength switching (see Fig. 3).

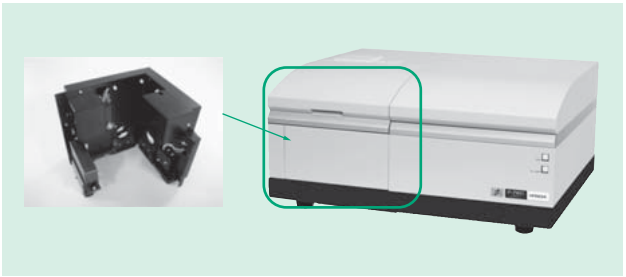

Fig. 3—Appearance of the F-7100 and Automatic Filter Accessory.
Mounting the automatic filter accessory device in the F-7100 enables simple and rapid removal of the effects of higher-order light that appear as ghost peaks.

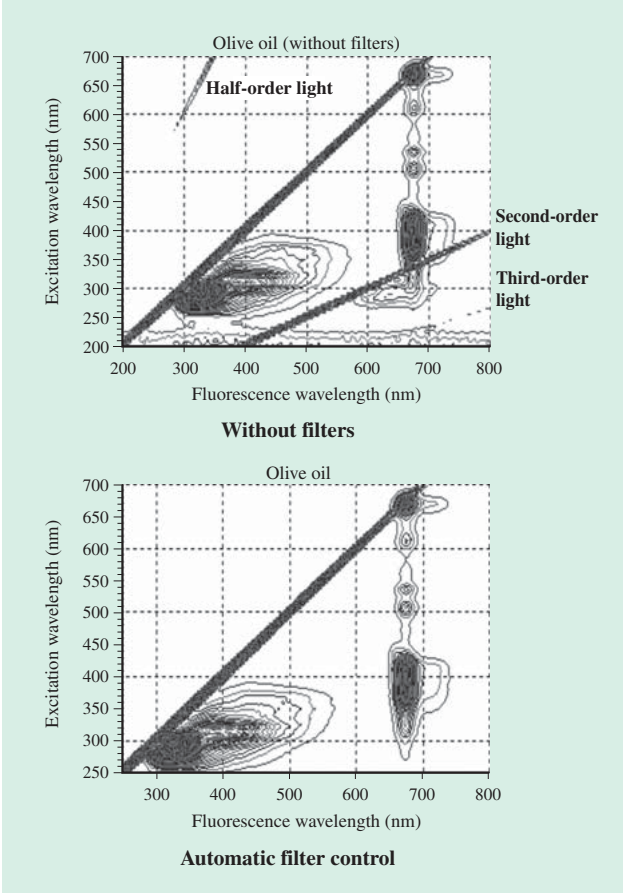

Fig. 4—Comparison of Fluorescence Fingerprint Data With and Without Filters.
The graphs show fluorescence fingerprints for olive oil. Without filters, unneeded spectrometer-specific higher-order light (such as second- and third-order light) is superimposed on the fluorescence fingerprint. The automatic filter accessory automatically inserts a filter corresponding to the measurement conditions, enabling the effects of higher-order light to be eliminated.

One of the challenges of fluorescence fingerprint measurement is removing the effects of spectrometer-specific higher-order light (such as second- or third-order light) that appears as ghost peaks. A new automatic filter accessory was developed to solve this problem. It automatically inserts filters corresponding to the measurement conditions, enabling simple and rapid removal of the effects of higher-order light (see Fig. 4). To improve data analysis precision, the number of effective data points that can be used in fluorescence fingerprint analysis has been increased from about 4,500 to about 10,000 for the standard wavelength range. The data processing unit has also been given a full range of specialized functions for fluorescence fingerprint measurement, such as a fluorescence fingerprint peak display function, and a function for transferring data to multivariate analysis software.

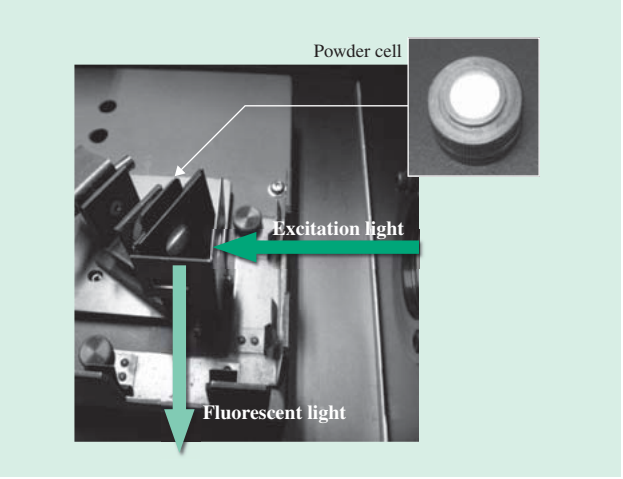

Fig. 5—Sample Placement.
To measure the fluorescence fingerprint of starch, about 0.7 mL of the sample was sealed in a powder cell, which was placed in a solid sample holder enabling measurement of the fluorescent light from the sample's surface.

EXAMPLE APPLICATIONS

Identifying Starches by Their Raw Materials

Using samples of starch from corn, potatoes, and wheat, Hitachi High-Tech Science attempted to identify each type of starch from the results of measuring its fluorescence fingerprint. About 0.7 mL of each sample was sealed in a powder cell, which was placed in a solid sample holder enabling measurement of the fluorescent light from the sample's surface (see Fig. 5). Fig. 6 shows the fluorescence fingerprints obtained. Hitachi High-Tech Science found several peaks for each sample. The peaks observed near the 300-nm fluorescence wavelength are hypothesized to come from amino acid components of proteins. The aerial views of the fluorescence fingerprints make it difficult to identify the differences in the samples. However, while the samples had no major differences in peak wavelengths, differences were found in their peak intensities. Hitachi High-Tech Science therefore extracted the fluorescence intensity of typical wavelengths corresponding to intensity peaks and valleys, and performed multivariate analysis (principal component analysis).

Fig. 7 shows the results. As the figure shows, the plot for each raw material is grouped differently. Furthermore, the mixed sample has a different plot position from the pure samples and is hypothesized to be a mixture made from corn and potato starches. So, by applying principal component analysis to fluorescence fingerprint data as described, it should be possible to identify what raw material an unknown

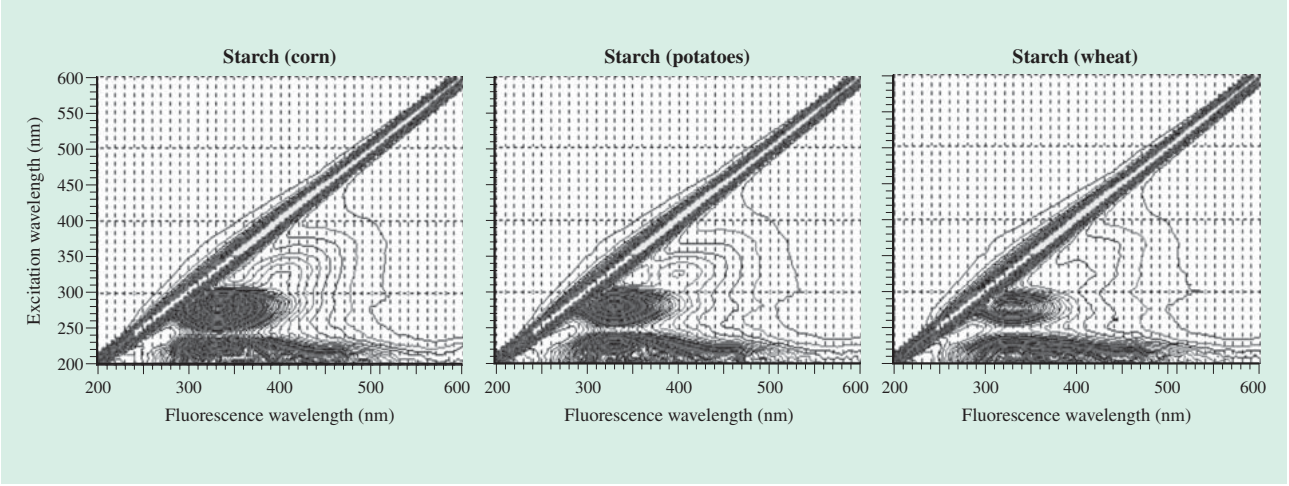

Fig. 6—Fluorescence Fingerprints of Starch from Different Raw Materials.
The graphs show the fluorescence fingerprint of starch from corn (left), potatoes (middle), and wheat (right). The peaks observed near the 300-nm fluorescence wavelength are hypothesized to come from amino acid components of proteins. No major differences were found in the peak wavelengths that appeared for each sample, making it difficult to identify differences in the fluorescence fingerprints from the aerial views.

sample is made from, for applications in quality control and defective product analysis.

Analysis Identifying Olive Oil

Hitachi High-Tech Science measured the fluorescence fingerprint of samples of commercially available, regular olive oil and virgin olive oil. Fig. 8 shows the measurement results. The samples were placed in cells made of polymethyl methacrylate (PMMA) without dilution or other preprocessing, and the fluorescent light from the surfaces of the samples was measured. For all of the olive oil samples, Hitachi High-Tech Science found fluorescence peaks in two regions: Region 1 (with an excitation wavelength of 250 to 450 nm and fluorescence wavelength of 300 to 600 nm), and Region 2 (with an excitation wavelength of 300 to 700 nm and fluorescence wavelength of 650 to 750 nm). Region 1 suggests fluorescence corresponding to an oxidation product, and Region 2 suggests fluorescence corresponding to chlorophyll[8]. The fluorescence intensity of fluorescence peak (i) (excitation wavelength of 320 nm, fluorescence wavelength of 400 nm) was extracted from Region 1, and the fluorescence intensity of fluorescence peak (ii) (excitation wavelength of 415 nm, fluorescence wavelength of 675 nm) was extracted from Region 2. Hitachi High-Tech Science calculated the fluorescence intensity ratio [(ii)/(i)]. As shown by the numbers in the graphs in Fig. 8, a comparison of fluorescence intensity ratios indicates that the values are higher for virgin olive oil than for regular olive oil.

Virgin olive oil is extracted from the olive fruit and is not refined or processed, which results in a high level of the chlorophyll contained in the olive fruit being detected [fluorescence peak (ii)]. On the other hand, the chlorophyll content of regular olive oil may be reduced through refining and processing,

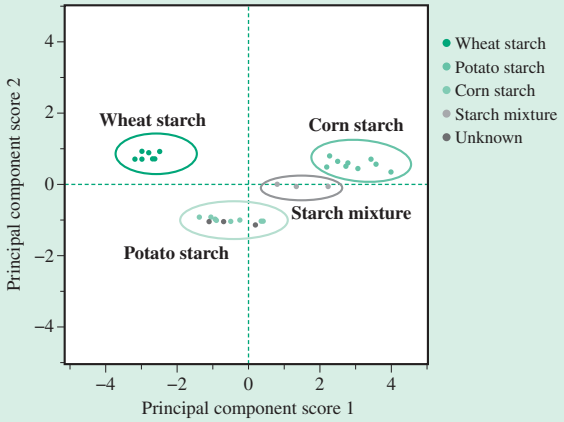

Fig. 7—Principal Component Analysis Results for Starch Fluorescence Fingerprints.
Principal component analysis lowers the dimensions of large-volume data by condensing it into principal component scores, which are variables that represent data features. The plot for each raw material falls into a distinct grouping, making it easy to identify the differences among the samples. The mixed sample is hypothesized to be a mixture of corn starch and potato starch. The unknown sample is hypothesized to be potato starch.

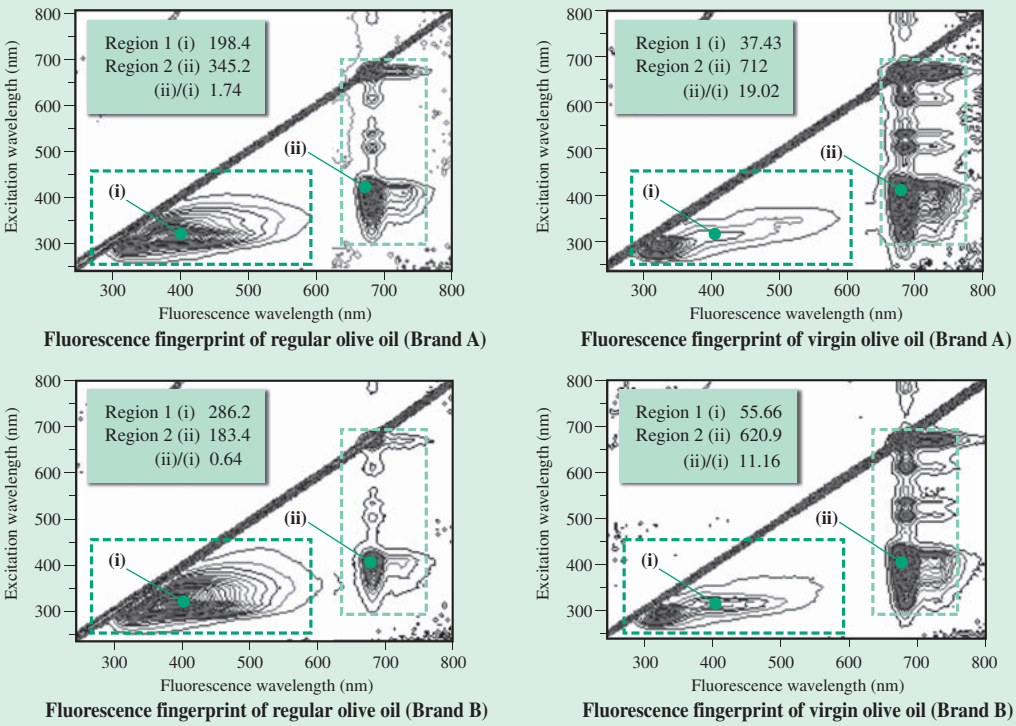

Fig. 8—Olive Oil Fluorescence Fingerprints.
Fluorescence peaks were found in two regions: Region 1 (excitation wavelength of 250 to 450 nm, fluorescence wavelength of 300 to 600 nm), and Region 2 (excitation wavelength of 300 to 700 nm, fluorescence wavelength of 650 to 750 nm). The fluorescence intensity ratio [(ii)/(i)] was calculated to identify the differences between regular and virgin olive oils.

which results in a low level of chlorophyll being detected. Similarly, the fluorescence corresponding to an oxidation product [fluorescence peak (i)] is lower in virgin olive oil. In practice, the quality of olive oil is determined by criteria such as its acidity, aroma, and flavor. So, while this method cannot determine olive oil quality, fluorescence fingerprint data can be used to enable simple evaluation of counterfeit vegetable oil products, a topic that has been in the spotlight recently.

CONCLUSIONS

A wide variety of samples exist in the food industry, with assessment criteria that also vary greatly according to the objective. Research has therefore been done on expanding applications, such as creating analytical methods that are tailored to samples and objectives[6]. Many sources have reported research results about attempts to apply fluorescence fingerprint analysis to the food industry, and awareness of the method has been increasing dramatically over the past few years. Fluorescence fingerprint analysis was created as a measurement method that is simpler and faster than conventional methods. Hitachi High-Tech Science will continue working on developing applications and improving equipment function/performance to help this technology contribute to society.

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