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Application News

No.A508

Spectrophotometric Analysis

Variation Analysis of Food – Three Dimensional Fluorescence Spectra of Black Tea Leaves with Different Places of Origin –

There are concerns in the food industry about food safety and security, including contamination and foods that disguise their true place of origin. Confirming the place of origin of branded goods and imported goods is sometimes impossible based on visual analysis and simple testing alone, and with the introduction of the Trans-Pacific Partnership (TPP) the number of these goods is going to increase.
Three dimensional (3D) fluorescence analysis represents the fluorescent characteristics of a substance as a spectral map. 3D fluorescence spectra and analysis of these spectra promises to be able to distinguish between different material types and places of origin.
We describe using the RF-6000 spectrofluorophotometer to obtain 3D spectra for black tea leaves from different places of origin, and attempting to distinguish between different places of origin by performing multivariate analysis of these results.

Three-Dimensional Spectra of Black Tea Leaves

Fig. 1 shows the RF-6000 system used. Fluorescence maps were obtained using the 3D spectrum mode in LabSolutions RF, which is the RF-6000 control software. Fig. 2 shows commercially available black tea leaves of different places of origin. Each sample of tea leaves had its own particular smell, and the shape of the leaves was different in each sample.
As shown in Fig. 3, samples were placed into a solid sample dish, which was placed in a solid sample holder. Analytical conditions are shown in Table 1, and measurements were repeated six times for each sample, refilling the solid sample dish for each measurement. Preliminary measurements showed fluorescence occurred at wavelengths longer than 500 nm, so a Y50 filter was placed on the emission side during measurements. When taking the excitation wavelength range and emission wavelength range into consideration, filters can be used to remove higher-order light from excitation light. Refer to UV Talk Letter Vol. 17 for information on a method of avoiding the detection of higher-order light by a spectrofluorophotometer.

Table 1 Analytical Conditions

Instrument	: RF-6000 Solid sample holder
Spectrum type	: 3D spectrum
Measured wavelength range	: Ex 300 nm to 750 nm, Em 550 nm to 850 nm
Scanning speed	: 6000 nm/min
Wavelength interval	: Ex 5.0 mm, Em 1.0 nm
Bandwidth	: Ex 5.0 mm, Em 3.0 nm
Sensitivity	: Low
Emission filter	: Y50

Fig. 1 RF-6000 Spectrofluorophotometer

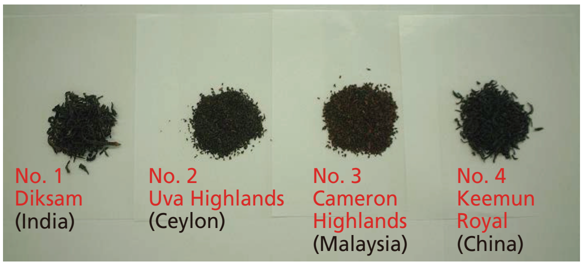

Fig. 2 Black Tea Leaves of Different Places of Origin

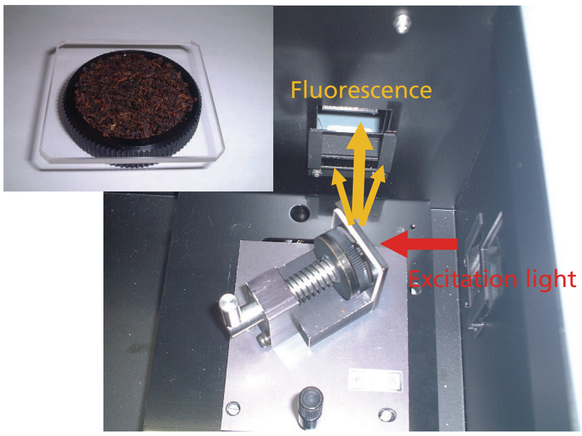

Fig. 3 Sample Dish and Sample Chamber

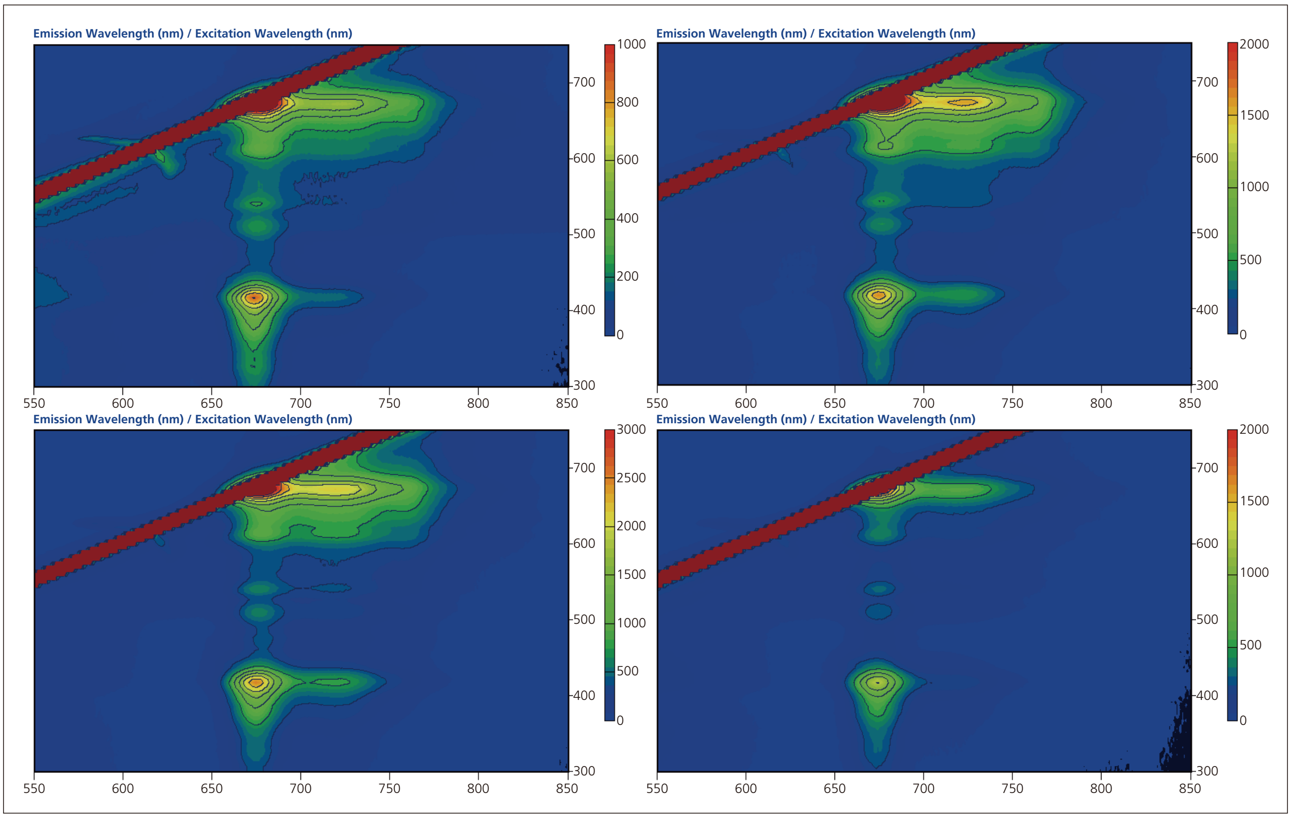

Fig. 4 3D Fluorescence Spectra of Black Tea Leaves
Upper Left No. 1 India, Upper Right No. 2 Ceylon, Lower Left No. 3 Malaysia, Lower Right No. 4 China

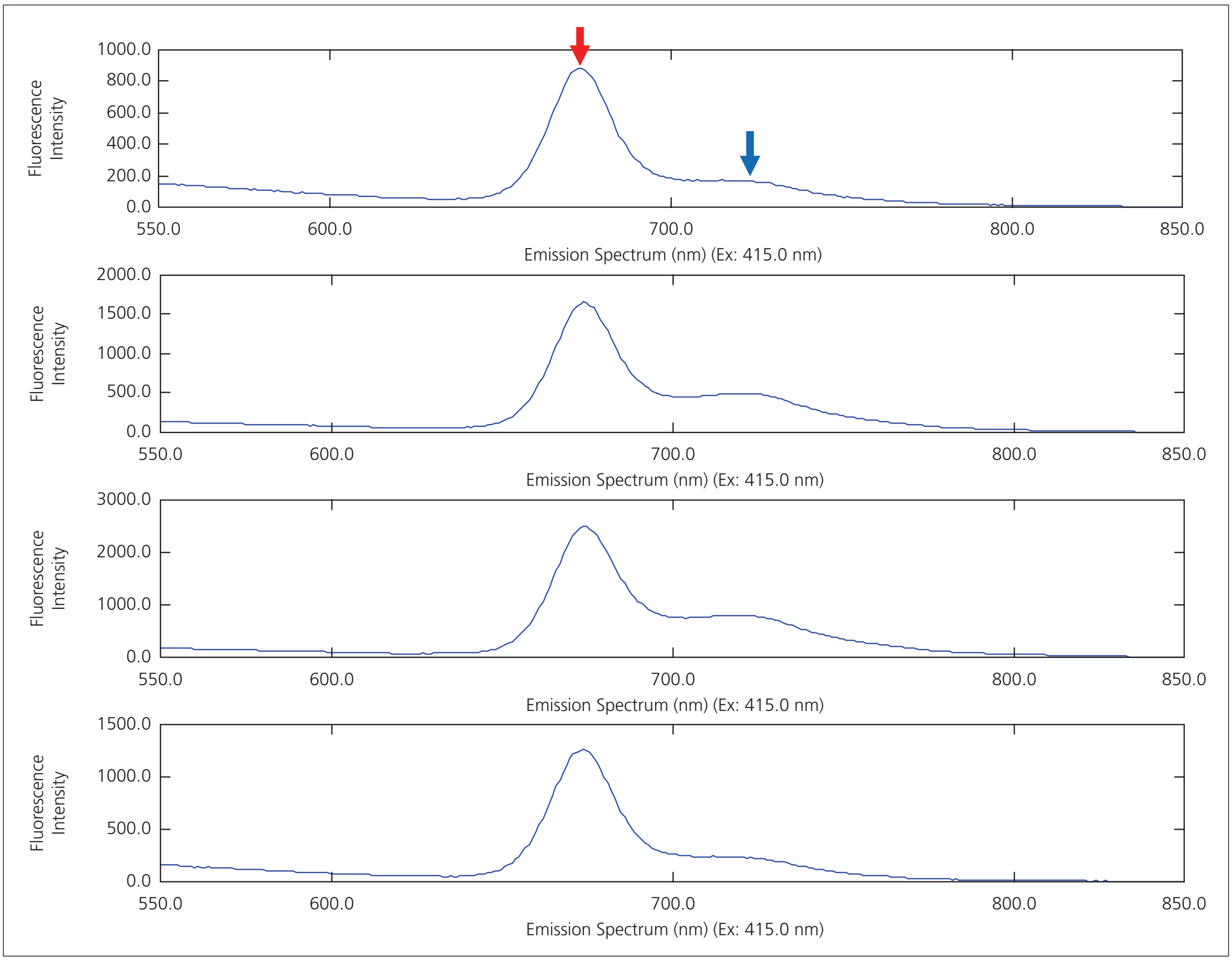

Fig. 5 Fluorescence Spectra of Black Tea Leaves Excited at 415 nm
In Order from the Top: No. 1 India, No. 2 Ceylon, No. 3 Malaysia, No. 4 China

Fig. 4 shows the 3D spectra obtained from each sample. Excitation wavelength (Ex) is shown on the vertical axis of the 3D spectra, emission wavelength (Em) on the horizontal axis, and fluorescence intensity is represented according to color. Cursory examination of the 3D spectra allows us to evaluate at which excitation wavelengths fluorescence occurs. For these samples, we observed fluorescence in the region of Em 680 nm at multiple excitation wavelengths. Fluorescence was also confirmed in the region of Em 720 nm.

Fig. 5 shows the emission spectra obtained at an excitation wavelength of Ex 415 nm. Each sample has a different ratio between the emission peaks at 680 nm and 720 nm and different emission peak shapes. Differences that are difficult to distinguish in the 3D spectra can be determined by evaluating separate emission spectra.

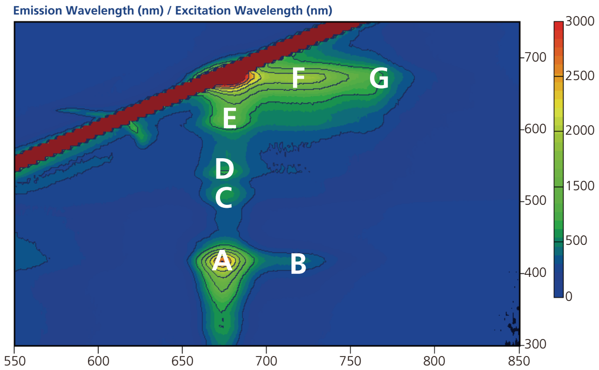

Fig. 6 3D Spectra and Analysis Points (No. 3 Malaysia)

Multivariate Analysis Using 3D Spectra

We used the 3D spectra of black tea leaves to perform multivariate analysis and investigate the differences between each sample. We performed central area averaging for fluorescence intensity at the seven points shown in Fig. 6, then performed variance analysis on these points using The Unscrambler® Note 1 multivariate analysis software. A score plot of results obtained by principal component analysis (PCA) is shown in Fig. 7, and a loading plot is shown in Fig. 8. The score plot can be used to visualize and group the data. The horizontal axis on the score plot is the first principal component score, the vertical axis the second principal component score, and the score of each component determines the position of each measurement. Because there is a matching and relative positional relationship between the score plot and loading plot, the loading plot can be used to show which components have a strong effect in each group. In this case, the black tea leaves from Ceylon and Malaysia are relatively close to each other on the score plot, and the black tea leaves from India and China are also relatively close to each other. Examining the loading plot, the emission peaks of A and F in Fig. 6 are relatively large for data on the right side of the score plot, and the emission peaks of B, C, D, E, and G in Fig. 6 are relatively large for data on the left side of the score plot. Based on these results, it was estimated the A and F components of black tea leaves from Ceylon and Malaysia have relatively high emission intensities, and the B, C, D, E, and G components of black tea leaves from India and China have relatively high emission intensities.

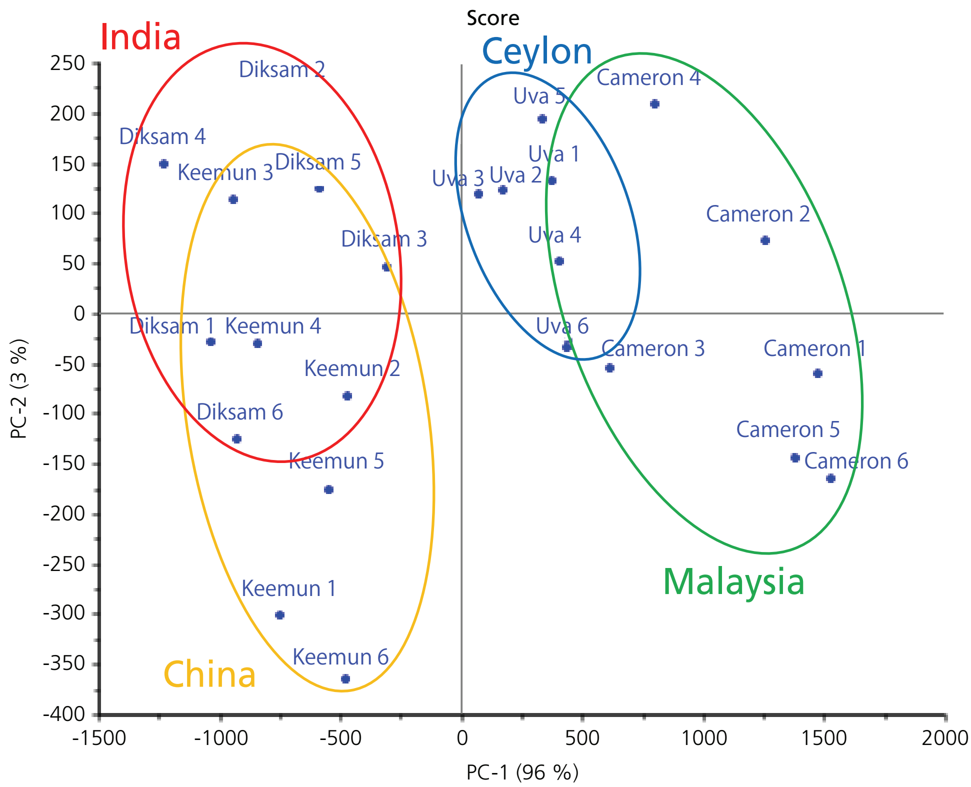

Fig. 7 Score Plot for Black Tea Leaves

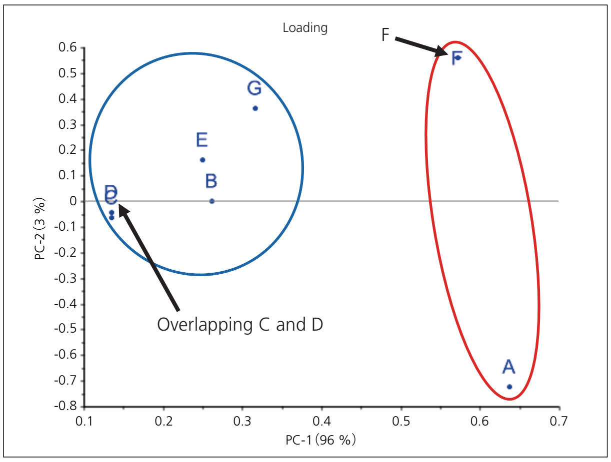

Fig. 8 Loading Plot for Black Tea Leaves

Fig. 9 shows the results of cluster analysis. Cluster analysis creates a sample order based on similarity, and is a technique used to group samples. In Fig. 9, a shorter connecting distance along the horizontal axis denotes a closer relationship. Finally, a tree diagram (dendrogram) can be produced. In our case, broad grouping puts Uva Highlands (Ceylon) and Cameron Highlands (Malaysia) in the same cluster, while Diksam (India) and Keemun (China) form another cluster.
We performed multivariate analysis on 3D spectra to classify black tea leaves based on place of origin characteristics.

Conclusion

We used the RF-6000 spectrofluorophotometer to obtain 3D spectra for black tea leaves from different places of origin and performed multivariate analysis on these spectral results. We mapped the fluorescence of black tea leaves based on their 3D spectra, then performed multivariate analysis to characterize the black tea leaves based on their differing places of origin.

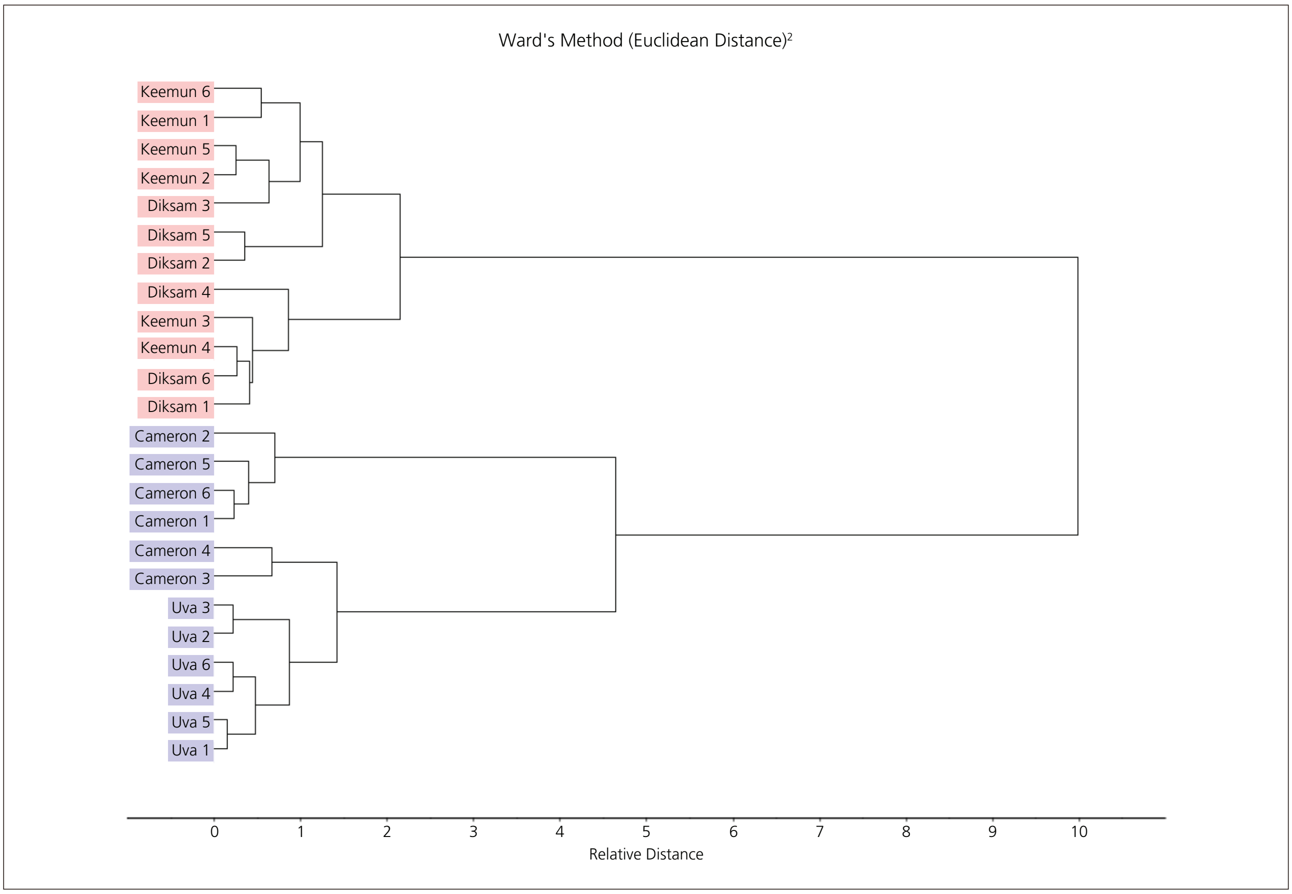

Fig. 9 Cluster Analysis of Black Tea Leaves

Note 1: The Unscrambler® is a trademark or registered trademark of CAMO Software AS.

First Edition: Aug. 2016