Application eBook

Food & Beverage Science

JASCO

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In order to detect the food frauds and to get the information about species, quality and production area of foods, it is mandatory to analyze the components in food. Since the several components are included in food, the proper information needs to be extracted, and the various methods (chromatography, mass spectrometer, etc) are used for separating/analyzing the components. However, these methods has the disadvantages that analytical instruments are expensive, and much time and labor is required. In addition, sample pretreatments and skilled analytical techniques are also required.

Spectroscopy analysis, which can evaluate rapidly and easily, is expected to be effective tool, and the analytical method by Excitation-Emission Matrix (EEM) attracts attention recently. Focusing on the components with fluorophore, it is possible to perform the component estimation and quantitative analysis by extracting each EEM from the mixed EEM of food product. Its extraction can be done by PARAFAC (Parallel Factor Analysis), which is one of multivariate analysis.

As a measurement example, this article shows the fluorescence measurement result of olive oils (6 kinds of extra virgin olive oil, 2 kinds of pure olive oil), and also shows the classification result by PARAFAC.

Identification of components in olive oil

EEM after processing (spectral correction and Raman rejection of solvent) are shown in Figure 1.The peak locations in Figure 1 and literature* were used to estimate the components of the samples and are the following: oxidation product (Excitation: 300 to 400 nm, Emission: 320 to 500 nm) and chlorophyll (Excitation: 300 to 700 nm, Emission: 650 to 800 nm).

*Kongbonga YGM, Ghalila H, Onana MB, Majdi Y, Lakhdar ZB, Mezlini H and Ghalila SS, *Food and Nutrition Sciences*, **2**, 692-699, (2011).

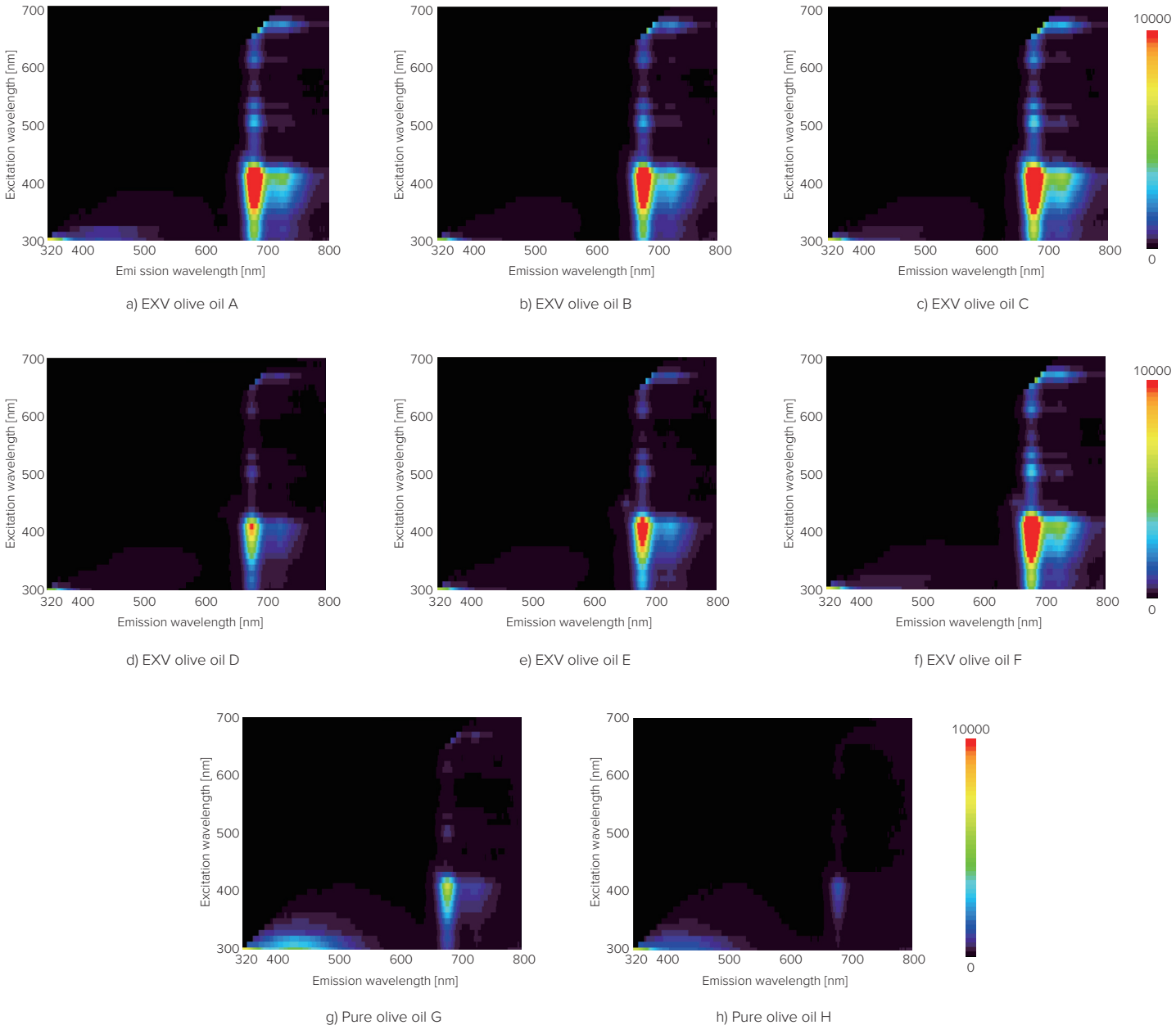


Figure 1. EEM of olive oil samples

PARAFAC was performed on the EEM and the number of component spectra was set to 2. Figure 2 shows the component EEM calculated by PARAFAC. The first component EEM is chlorophyll and the second is an oxidation product.

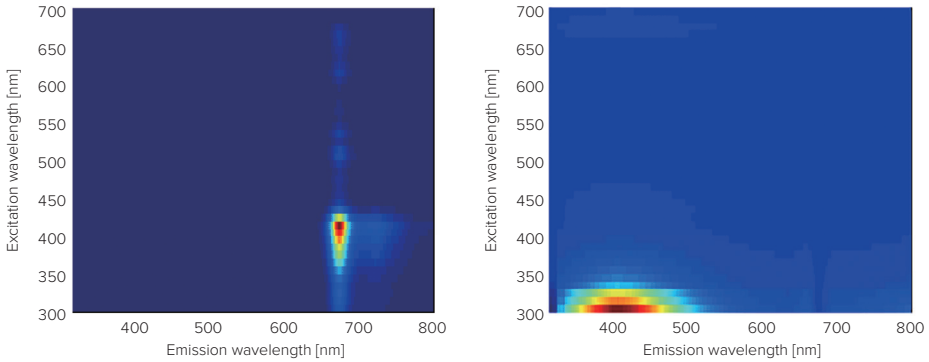


Figure 2. First (left) and second (right) component EEM calculated by PARAFAC.

Classification of olive oil

Figure 3 shows the score plot for the first component (chlorophyll) and the second component (an oxidation product). Regarding the distribution in the extra virgin olive oil, the score for the first component is large while the second is small. However, the distribution in the pure olive oil shows the score of first component is small while the second is large. This trend is related to the production process of the olive oil. Extra virgin olive oil is produced by squeezing and filtering the fruit of the olive without any chemical treatment process, and the acidity of the olive oil is less than 0.8%. On the other hand, pure olive oil is a blend of virgin olive oil and refined olive oil, so the acidity of pure olive oil is less than 1%. In addition, the chlorophyll content of pure olive oil is small because it is reduced during the production process for refining the olive oil.

PARAFAC analysis of EEM can perform the discrimination of food product as well as the identification of component in food product by verifying the EEM of each component. This technique may also be applied for the food ingredient identification, the quality control monitoring and the food fraud detection.

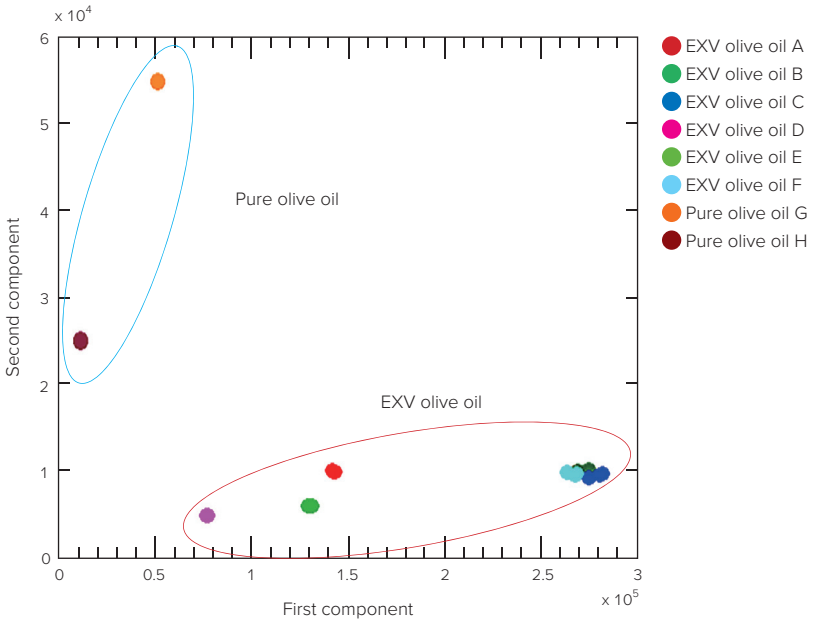


Figure 3. Score plot of the first and second components analyzed by PARAFAC

Quantitative analysis of free fatty acids in vegetable oil (UHPLC-ADAM derivatization)

HPLC is widely used for quantitative analysis of fatty acids in oil, and their components are generally detected by UV detector, refractive index detector and evaporative light scattering detector (ELSD). In the case that the amount of target component is very small, the derivatization method is preferred because it offers much higher sensitivity. Among the various derivatization methods, 9- Anthryldiazomethane (ADAM) is most commonly used because it reacts with fatty acids easily at room temperature and enables to highly sensitive and selective analysis.

This article shows the analysis result of free fatty acids in vegetable oil using ADAM derivatization by Ultra High-performance Liquid Chromatography (UHPLC).

Pre-column derivatization with ADAM reagent

Figure 1 shows the procedure of ADAM derivatization, and Figure 2 shows the reaction mechanism of ADAM reagent with fatty acid. In this time, the fluorophore was attached to fatty acids by pre-column derivatization, which is the method that the sample is derivatized before injecting it to the column.

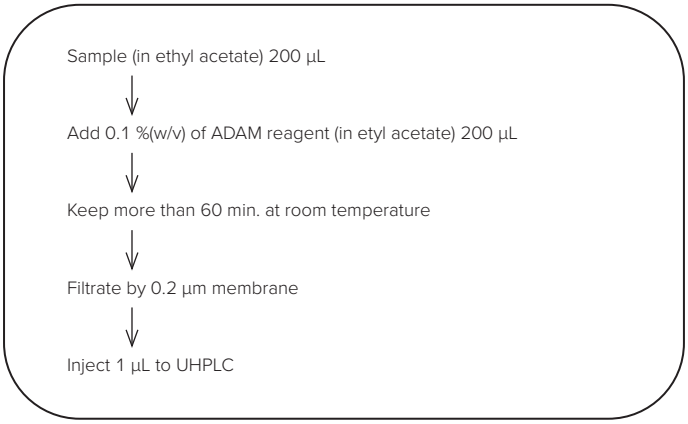


Figure 1. Procedure of ADAM derivatization

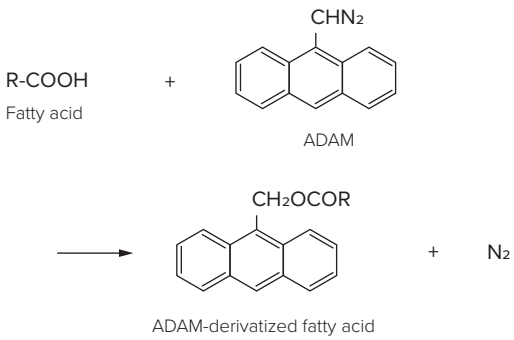
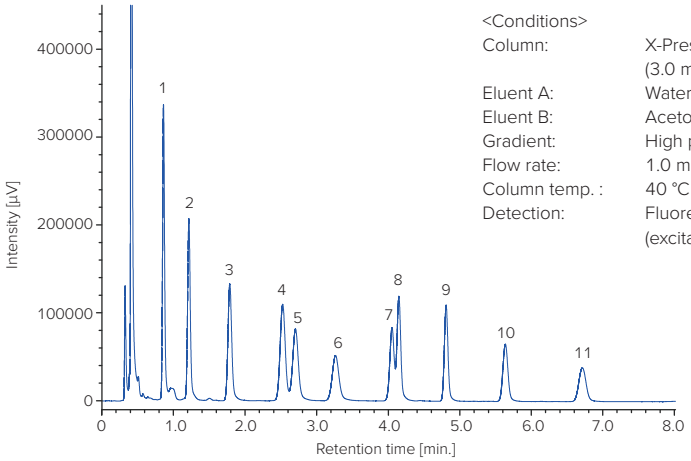


Figure 2. Reaction mechanism of ADAM reagent with fatty acid

Analysis of standard fatty acids

Figure 3 shows the chromatograph of the standard fatty acids derivatized with ADAM. 11 different fatty acids were separated within 7 minutes.



<Conditions>

Column:	X-PressPak V-C18-W (3.0 mmI.D. × 50 mmL, 2.0 μm)
Eluent A:	Water/Methanol (5/95)
Eluent B:	Acetonitrile/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp. :	40 °C
Detection:	Fluorescence detector (excitation: 365 nm, emission: 412 nm)

Figure 3. Chromatogram of standard fatty acids derivatized with ADAM

1: Caprylic acid (C8), 2: Capric acid (C10), 3: Lauric acid (C12), 4: Linolenic acid (C18:3), 5: Myristic acid(C14), 6: Linoleic acid (C18:2), 7: Palmitic acid (C16), 8: Oleic acid (C18:1), 9: Stearic acid (C18),10: Arachidic acid (C20), 11: Behenic acid (C22)

Analysis of fatty acids in vegetable oils

Figure 4 shows the chromatograms of fatty acids in rice oil and coconut oil derivatized with ADAM, respectively. Table 1 shows quantitative values of fatty acids in rice oil and coconut oil.

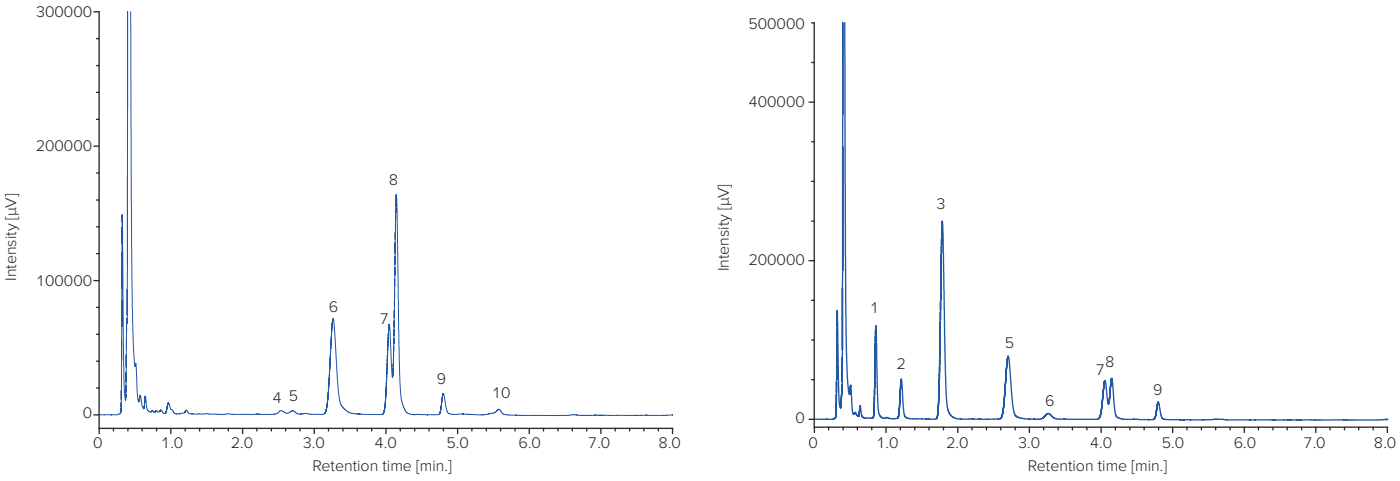


Figure 4. Chromatograms of ADAM derivatives in rice oil (left) and coconut oil (right)

Peak numbers and chromatographic conditions are the same as in Figure 3.

Preparation: 1.0 g of oil was dissolved in ethyl acetate, and then its volume was made up to 10 mL. After that, procedure of ADAM derivatization was followed as in Figure 1.

Table 1. Quantitative values (mg/g) of rice oil and coconut oil

Fatty oil	C8	C10	C12	C14	C16	C18	C18:1	C18:2	C18:3	C20	C22
rice oil	-	-	-	0.010	0.23	0.044	0.41	0.38	0.007	0.020	-
coconut oil	0.017	0.012	0.85	0.45	0.26	0.089	0.20	0.055	-	-	-

Component analysis of dairy product (FTIR)

It is important to know the components (or ingredients) in foods to solve the problems on the manufacturing process and quality control, and IR spectroscopy is effective tool to get such information. However, since many ingredients are included in food, IR spectrum which these information is added in is detected, and it is hard to extract the information which the user needs. In order to get the proper data from measurement data, chemometrics is used frequently. Examples of chemometrics technique are the principal component analysis (PCA) and the partial least squares (PLS) regression.

PCA is the technique that can perform the dimensionality reduction of the data during minimizing the information loss of high-dimensional data (such as spectra). Its technique enables to find the characteristics (latent variables) from multi-data (spectra), which can perform the identification and classification of data.

Although PLS regression can also perform the dimentionality reduction like PCA, it is the regression analysis utilizing the latent variables, which are found so that the relation (covariance) between the latent variable and the response variable is maximized. Its technique can create the robuster model than the general multiple regression analysis, and it is strong against the noise.

This article shows the component analysis result of dairy product by PCA, and also shows the quantitative analysis result of protein in food product by PLS. Measurement data were acquired by IR spectroscopy for these analyses.

Component analysis of milk by PCA

IR spectra were acquired by using ATR. Figure 1 shows the IR difference spectra between milk and water. Milk consists mainly of 4 components (water, protein, sugar and fat), and approx. 90 % of milk is water. Subtracting water spectrum from milk spectrum could observe the specific functional group bands of each main component.

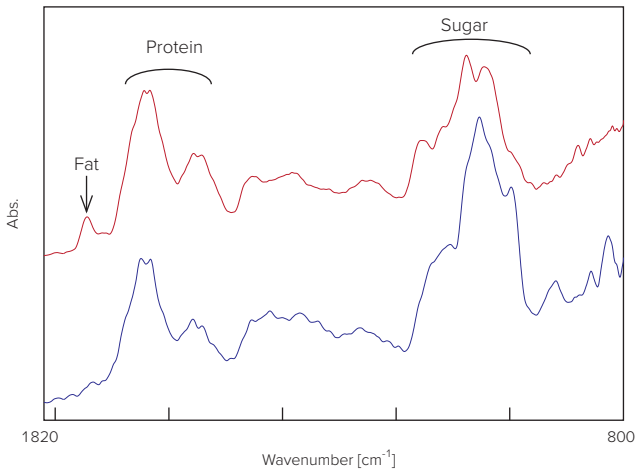


Figure 1. IR difference spectra between milk and water
(red: milk, blue: coffee with milk)

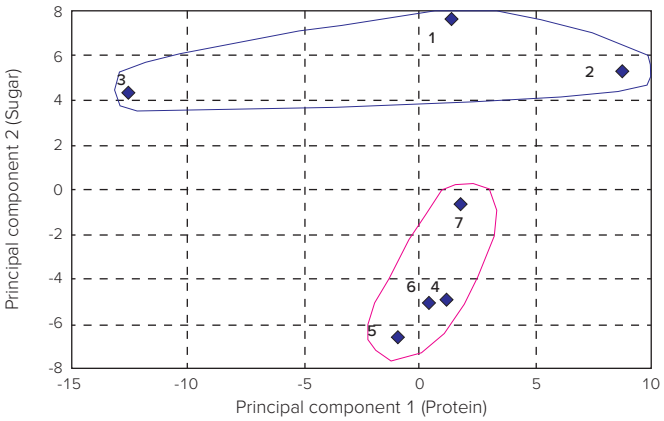


Figure 2. Principal component scores
1. Coffee with milk (A), 2. Coffee with milk (B), 3. Coffee with milk (C), 4. Milk (D), 5. Milk (E), 6. Milk (F), 7. Milk (G)

Next, 7 kinds of commercial milk were measured, and classification of them were performed by using PCA (Figure 2). Figure 2 indicates that the component ratios of milk samples (No. 4 to 7) are almost the same. Regarding the coffee with milk (No.1 to 3), it indicates that the sugar ratios of them are almost the same, and they are largely different in the other component ratio.

Quantitative anaylysis of protein in food products

The several food samples including the protein (table 1) were measured by IR spectroscopy. Figure 3 shows the near-IR spectra of them. Although these spectra are similar, there are slight difference among them. Next, PLS calibration model were created by using the measured data and the protein concentration value which is described on the label of each food sample (table 1). Figure 4 shows the correlation between the spectrum and protein concentration. Its correlation coefficient was 0.994, which means that it is good calibration model.

Table 1. Food samples and their protein concentration (on label)

Food sample	Protein concentration
Gouda cheese	18.0 %
Natural cheese	28.0 %
Worcester sauce	0.9 %
Yogurt	4.2 %
Soybean milk yogurt	4.0 %
Miso (salted bean-paste)	10.0 %

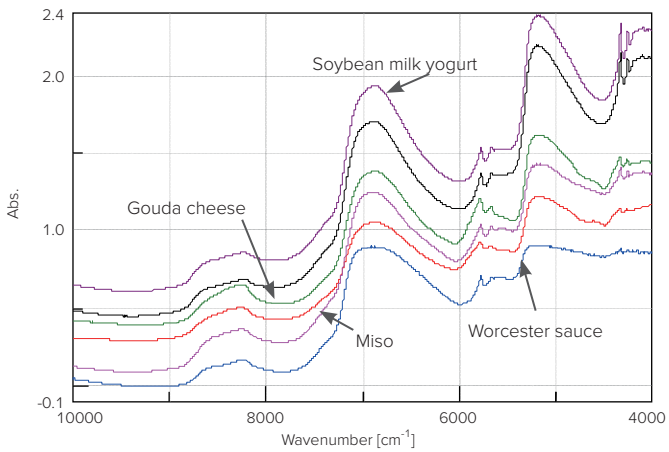


Figure 3. Near-IR spectra of food samples

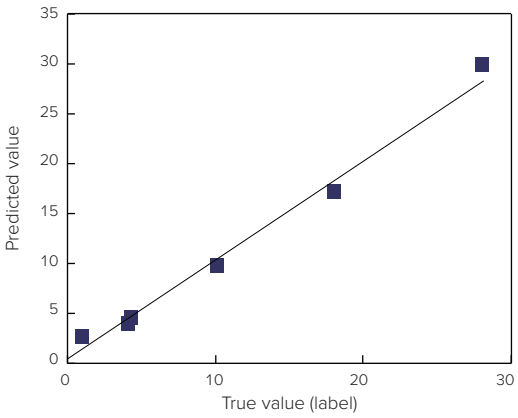


Figure 4. PLS calibration model of protein

Simultaneous quantitative analysis of multi-component in food product

Near-IR spectroscopy is widely used for quantitative analysis of components in food product. Its method has the feature that it is possible to perform non-destructive measurement without any special sample preparation. Therefore, not depending on the status of sample (such as liquid, paste, solid or particle), just putting it on the sampling plate or cell in the analytical instrument can perform the measurement easily. In addition, since the multivariate analysis can provide the quantitative information of mult-component from one spectrum simultaneously without splitting the spectrum into several spectra, applying its technique enables to get a lot of information rapidly and easliy.

This article shows the simultaneous quantitative analysis result of each component in commercial food product by a series of near-IR measurement.

Simultaneous quantitative analysis of multi-component in the condensed soup

15 kinds of condensed soup, whose concentration of each component is already known, were prepared, and diffuse transmission measurements of them were performed by using UV/Vis-NIR spectrophotometer with integrating sphere unit. Figure 1 shows the spectra of each condensed soup. Although these spectra are similar, there are slight difference among them. Next, based on the measured data, the calibration model of salt was created by PLS (Figure 2). As a result, the calibration model with high correlation coefficient could be obtained. It means that the salt can be quantified by using the phenomenon that the peak of water is shifted depending on the salt concenctration even though the salt does not have the absorption in near-IR region.

Calibration models of other components (water and protein) were also created by using the same method. Table 1 shows the correlation coefficients of each calibration model. It indicates that the calibration models were created with high correlation coefficient, and means that this technique can quantify the multi-components simultaneously.

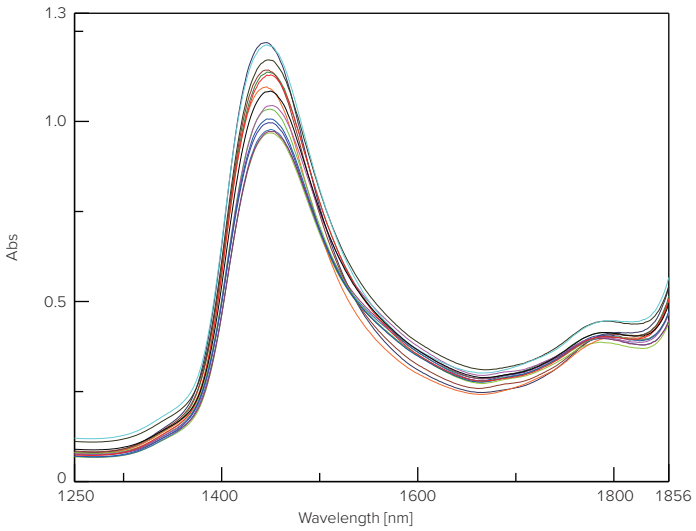


Figure 1. Spectra of each condensed soup

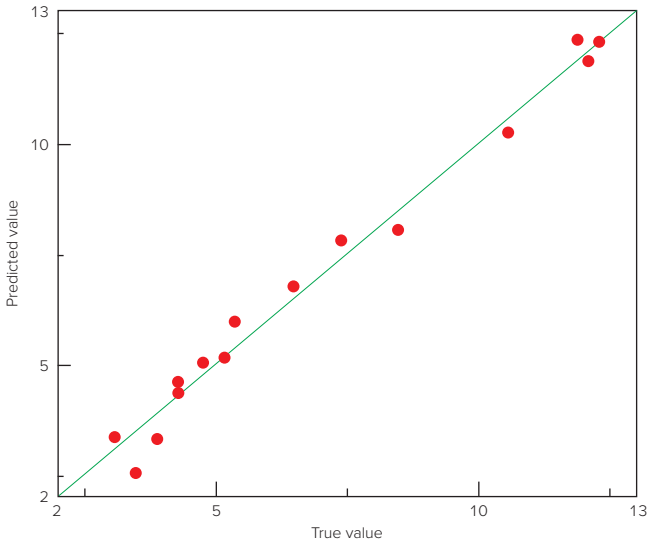


Figure 2. PLS calibration model of salt

Table 1. Calibration model of each component and their correlation coefficient

Component	Number of samples	Range (%)	Correlation coefficient	Error of prediction
Water	15	55.7 to 77.4	0.961	2.04
Protein	15	1.0 to 4.7	0.955	0.470
Salt	15	3.1 to 12.3	0.992	0.415

Simultaneous quantitative analysis of multi-component in soybean

Soybeans were measured without destroying them by using Fourier-Transform near-IR spectrometer (FT-NIR). Figure 3 shows the near-IR spectra of each soybean. Although these spectra are similar, there are slight difference among them.

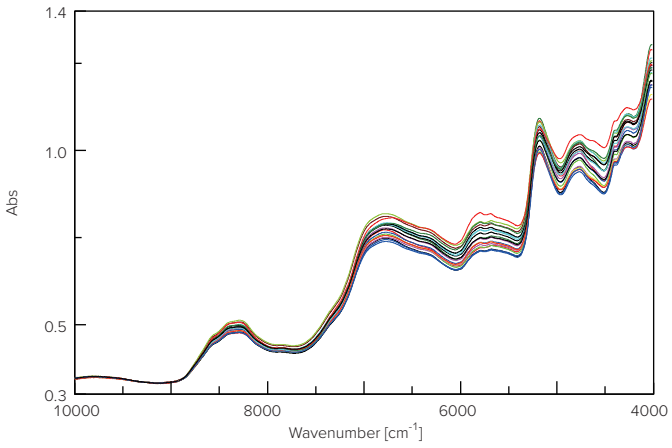


Figure 3. Near-IR spectra of each soybean

Next, PLS calibration models of each component were created by using measured near-IR spectra. Quantitative information of each component which is measured by wet chemistry method is used for the true value of PLS calibration model. Figure 4 shows the calibration models which were created simultaneously by using the measured value of other components (water, carbohydrate and fat) as well as protein. These models of each component were created with high correlation between the true value and the predicted value, and it means that this technique can quantify the multi-components simultaneously.

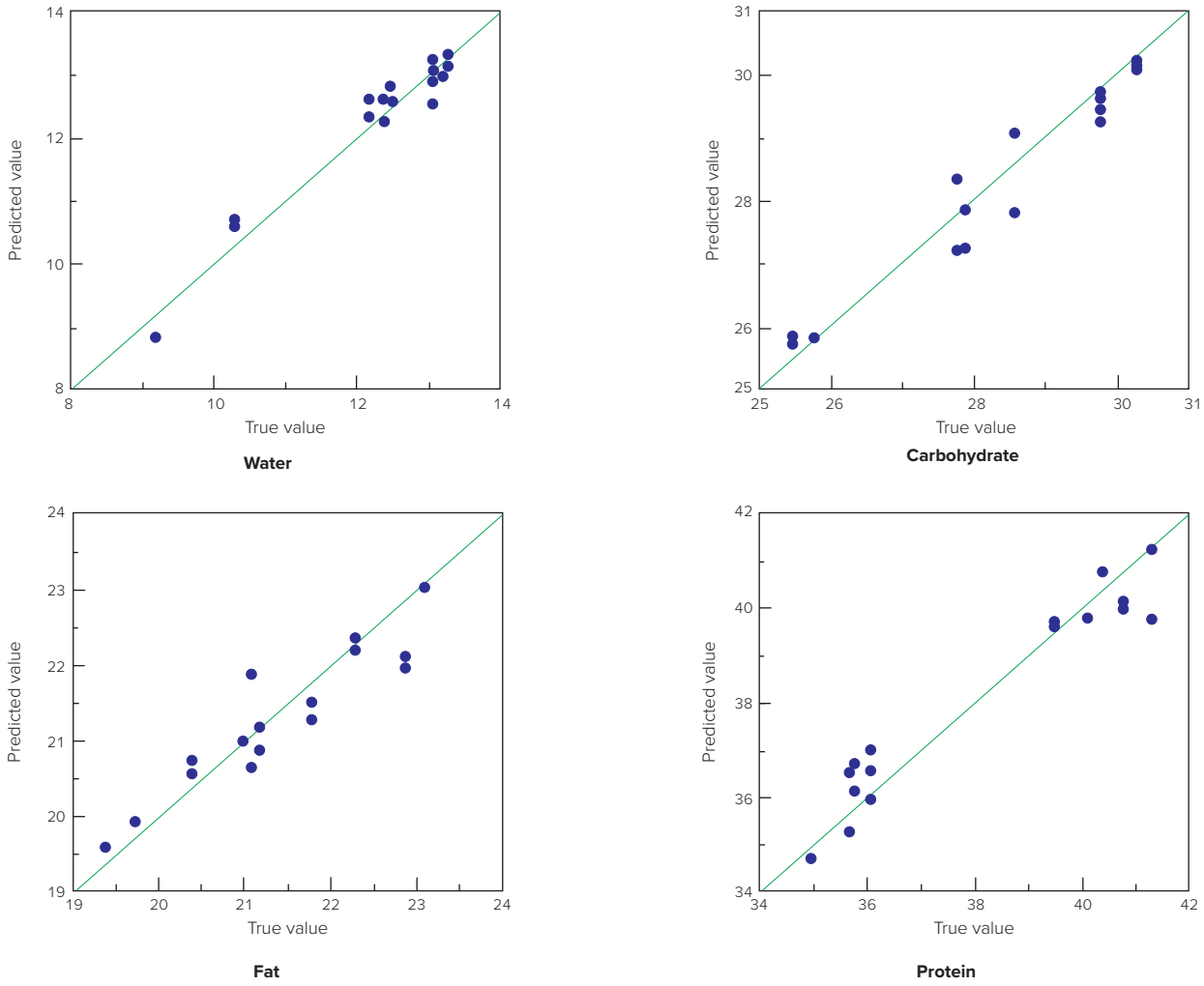


Figure 4. PLS calibration models

FT-NIR system has the following advantage comparing to the other spectroscopy system, and can be used for quantifying the multi-components in food product simultaneously.

- Excellent wavenumber accuracy
- Bright and high throughput
- Continuous measurement and monitoring available
- Simultaneous measurement in entire region

JASCO can suggest the suitable solution in accordance to the user's demands.

Quantitative analysis of amino acids in functional beverage (HPLC-OPA derivatization)

Among the various methods for analyzing amino acids, HPLC is widely used as easy and rapid analytical method. Since almost amino acid does not have the absorption and fluorescence in the region from UV to visible light, these targets need to be derivatized by using reagent for detecting with high sensitivity.

As an example of derivatization methods, the derivatization of *o*-phthalaldehyde (OPA) is well-known, and makes it possible to attach the fluorophore (chromophore) so that HPLC can analyze them. Its method enables highly sensitive and selective analysis of each amino acid, and makes it possible to do by general optical detector (UV detector and fluorescence detector).

This article shows the analysis results of OPA-derivatized amino acids by using HPLC with UV detector or fluorescence detector.

Derivatization with OPA

Figure 1 shows the reaction mechanism of OPA reagent with amino acids. Its reagent has the features that it reacts with amino acids rapidly even at room temperature. Since the reaction rate depends on the amino acid and the derivatives are begun to decompose after reaction, it operation needs to be done carefully.

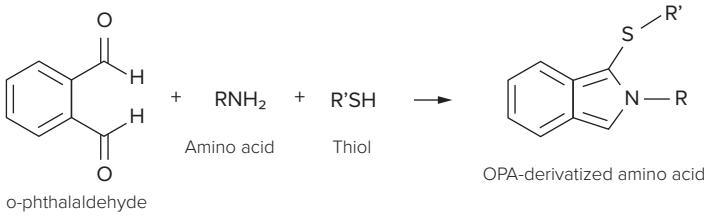


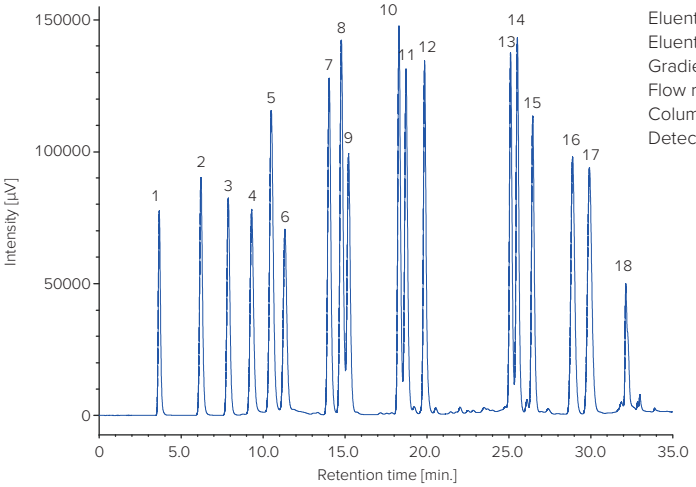
Figure 1. Reaction mechanism of OPA reagent with amino acid

Since OPA-derivatized amino acid has the fluorophore, it is possible to perform high sensitive detection (on the order of fmol) by selecting the proper detection wavelength (excitation: 340 to 350 nm, emission: 450 to 460 nm).

In this time, amino acids were analyzed using OPA pre-column derivatization, which is the method that the sample is derivatized before injecting it to the column. The automated pre-column derivatiation function of autosampler was used because it can do the derivatization operation reproducibly. v

Amino acid analysis using fluorescence detection

Setting the excitation wavelength and emission wavelength to 345 nm and 455 nm respectively, the amino acids were analyzed. Figure 2 shows the chromatogram of standard amino acids, and Figure 3 shows the chromatogram of functional beverage. Both could be acquired with good separation.



<Conditions>

Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent A:	Sodium acetate buffer/methanol/THF (89/10/1)
Eluent B:	Methanol/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	20 °C
Detection:	Fluorescence detector (excitation: 345 nm, emission: 455 nm)

Figure 2. Chromatogram of standard amino acids

1: Aspartic acid, 2: Glutamic acid, 3: Asparagine, 4: Histidine, 5: Serine, 6: Glutamine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Taurine, 11: Alanine, 12: Tyrosine, 13: Methionine, 14: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Lysine

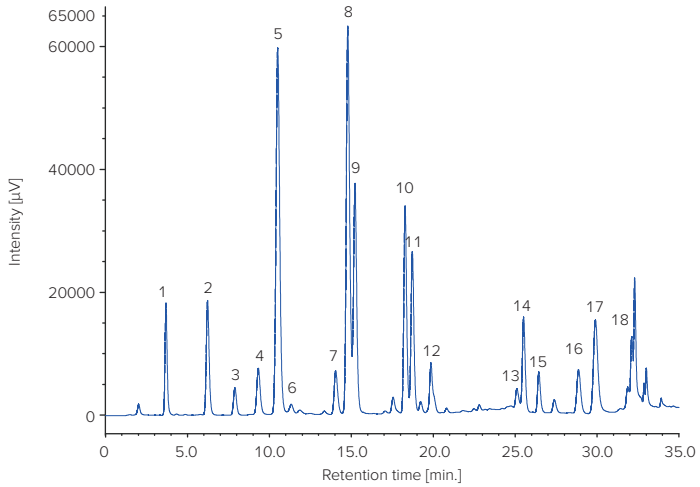


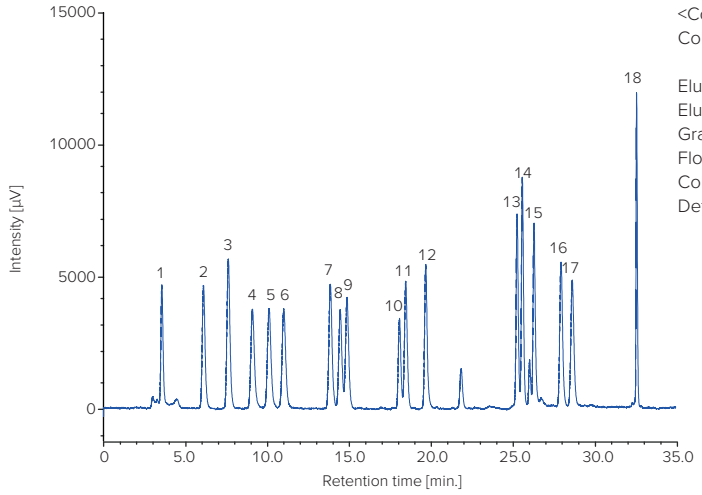
Figure 3. Chromatogram of functional beverage

Peak numbers and chromatographic conditions are the same as in Figure 2.

Preparation: Functional beverage was diluted 100 times with 0.01 M HCl, and then it was filtrated with 0.2 µm membrane. After that, 10 µL was injected to HPLC.

Amino acid analysis using UV absorption detection

OPA-derivatized amino acids can be detected even by UV detector. Setting the absorption wavelength to 338 nm, amino acids were analyzed. Figure 4 shows the chromatogram of standard amino acids, and Figure 5 shows the chromatogram of functional beverage. Both could be acquired with good separation even by UV detector.



<Conditions>

Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 μm)
Eluent A:	Sodium acetate buffer/methanol/THF (89/10/1)
Eluent B:	Methanol/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	20 °C
Detection:	UV detector (absorption wavelength: 338 nm)

Figure 4. Chromatogram of standard amino acids

1: Aspartic acid, 2: Glutamic acid, 3: Asparagine, 4: Histidine, 5: Serine, 6: Glutamine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Taurine, 11: Alanine, 12: Tyrosine, 13: Methionine, 14: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Lysine

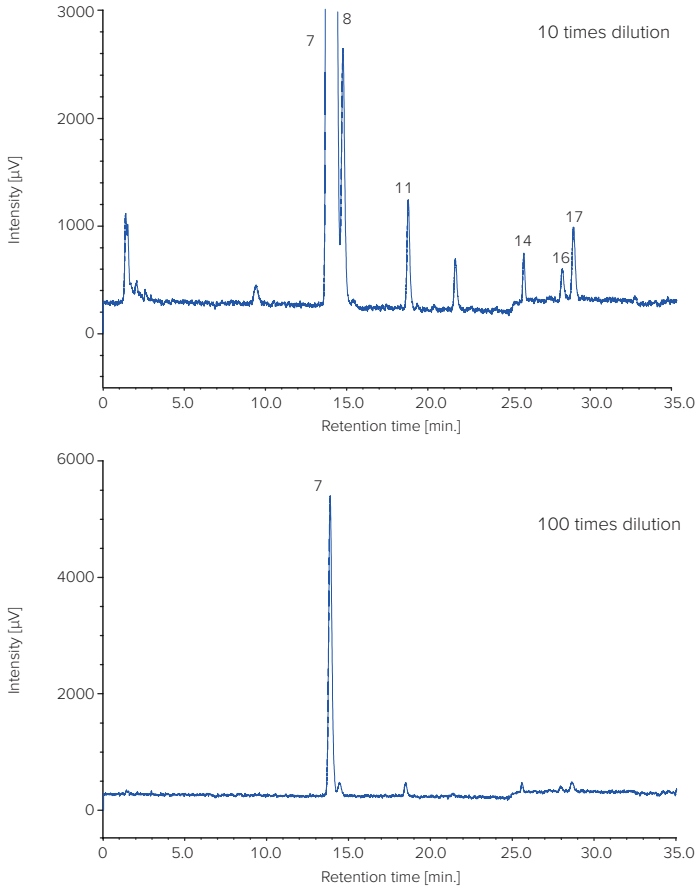


Figure 5. Chromatogram of functional beverage

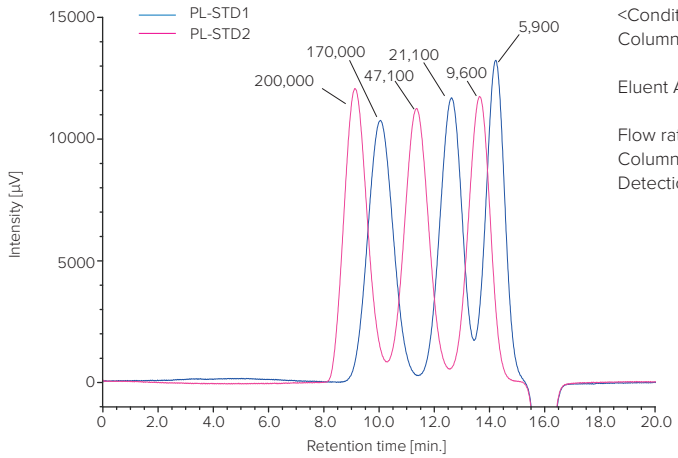
Peak numbers and chromatographic conditions are the same as in Figure 4.

Preparation: Functional beverage was diluted 10 times and 100 times with 0.01 M HCl respectively. After that, 5 μL was injected to HPLC.

Molecular weight distribution analysis of condroitin sulfate sodium salt (HPLC-SEC column)

Chondroitin sulfate (MW 20,000 to 50,000), which is one of mucopolysaccharide, has several types (A to E),and are found in animal connective tissue (cartilage, skin, etc.). Chondroitin sulfate is widely used in the manufacture of health foods and medicines. In this article, chondroitin sulfate (sodium salt) was analyzed using a SEC (size exclusion chromatography) column with an exclusion limit of 300,000 molecular weight (pullulan) and detection using refractive index (RI detector). A pullulan (PL) calibration curve was created from a standard mixture, and the resulting molecular weights calculated from the standard PL are described below.

Figure 1 shows the chromatogram of standard PL mixture for molecular weight calibration, and Figure 2 shows the one of chondroitin sulfate sodium salt.



<Conditions>

Column:	Shodex Asahipak GF-510 HQ (7.5 mmI.D. × 300 mmL, 5 μm)
Eluent A:	0.04 M Sodium dihydrogen phosphate anhydrous-0.06 M Disodium hydrogen phosphate anhydrous
Flow rate:	0.6 mL/min.
Column temp.:	30 °C
Detection:	RI detector

Figure 1. Chromatogram of standard PL mixture for molecular weight caligration

(Each value on the chromatogram is M_p (peak top molecular weight).)

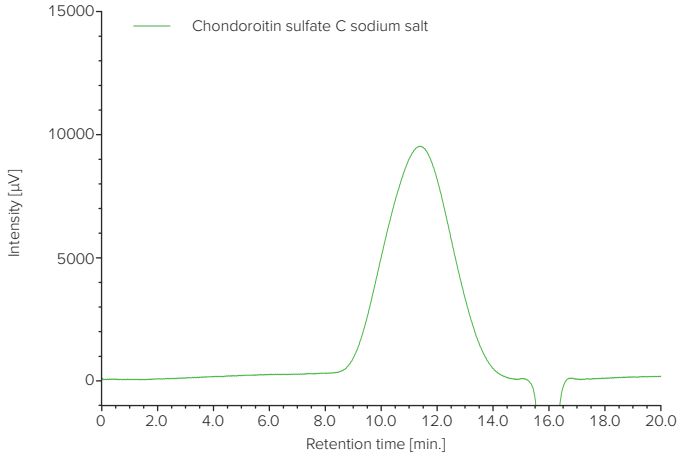


Figure 2. Chromatogram of condroitin sulfate sodium salt

Chromatographic conditions are the same as in Figure 1.

Figure 3. shows the molecular weight calibration curve created by retention volume (elution time) and peak top molecular weight of standard PL.

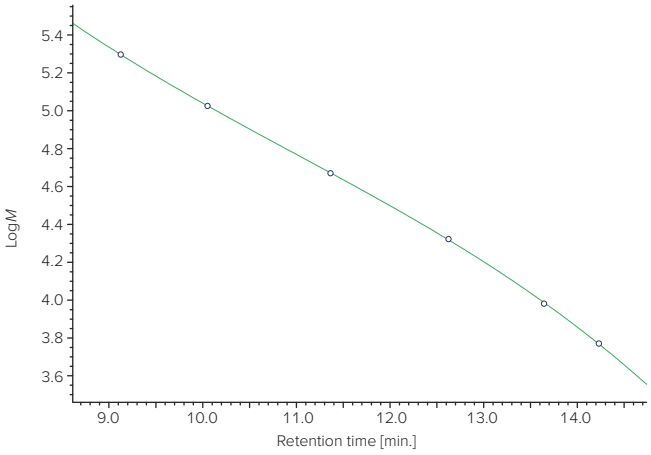


Figure 3. Molecuar weight calibration curve created with standard PL mixture

Figure 4 shows chromatogram of chondroitin sulfate sodium salt and molecular weight calibration curve, and Figure 5 shows the molecular weight distribution caluculation curve. The integral molecular weight distribution curve shows the relationship between molecular weight (logarithmic value) and the percentage of a specific molecular weight in the total of molecules. The differential molecular weight distribution curve is used to plot the molecular weight (logarithmic value) on the x-axis against the weight fraction differentiated from a logarithmic value of molecular weight (d*w*/d(log*M*)) on the y-axis. When normalized, the use of this curve makes it possible to compare a chromatogram and a molecular weight distribution derived using different columns and measurement conditions.

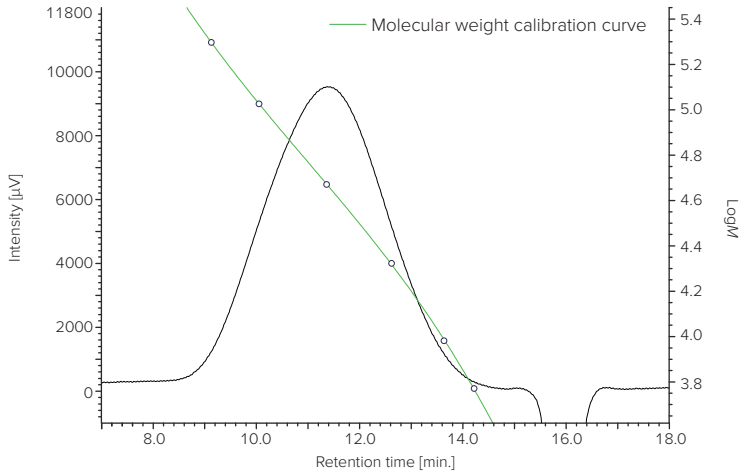


Figure 4. Chromatogram of chondroitin sulfate sodium salt and molecular weight calibration curve of standard PL

(The figure in blue represents M_p calculated using standard PL)

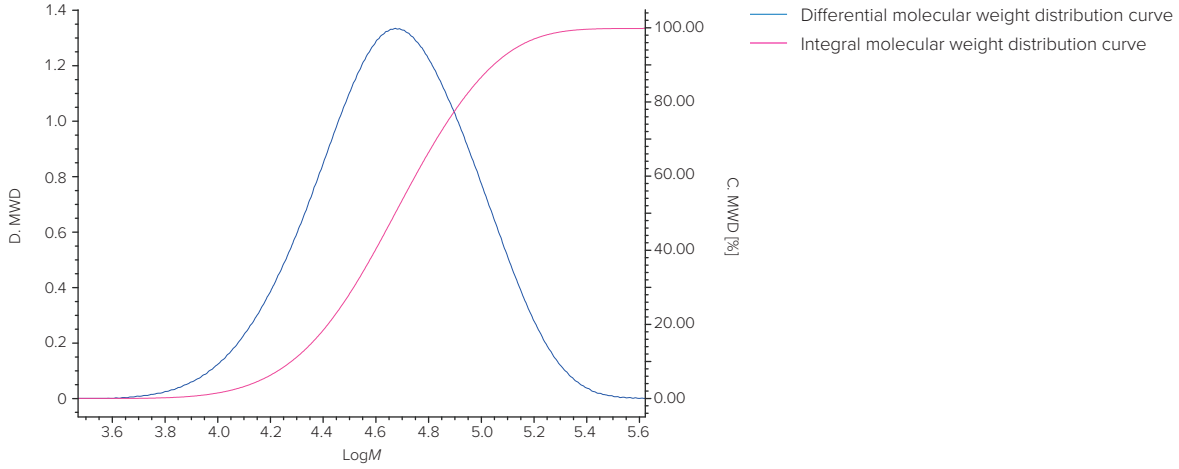


Figure 5. Molecular weight distribution curves of chondroitin sulfate sodium salt

Table 1 shows the molecular weight caluculation result by standard PL. The molecular weight averages of polymer materials obtained by size exclusion chromatography (SEC) includes the number-average (M_n), weight average (M_w), z-average (M_z), and viscosity-average (M_v) molecular weights. The distribution of these molecular weight averages bears in general a relationship such as $M_n \leq M_v \leq M_w \leq M_z$. In the case where $M_n = M_v = M_w = M_z$, there is no molecular weight distribution (mono-dispersed).

Table 1. Molecular Weight of Chondroitin Sulfate Sodium Salt

M_p	M_n	M_w	M_z	M_v	M_w/M_n	M_z/M_w
46812	36668	57914	85947	57914	1.58	1.48

Quantitative analysis of sugars (HPLC)

HPLC is widely used for quantitative analysis and quality evaluation of sugars in food. Depending on the type of sugar which is separated, various chromatography such as hydrophilic interaction chromatography (HILIC), size exclustion chromatography (SEC), ion-exchange chromatography and ligand-exchange chromatography are used. Since the sugar does not have the absorption in the UV region, refractive index detector (RI detector) or evaporative light scattering detector (ELSD) are used generally. Or, the sugars can also be detected by derivatization for attaching fluorophore or chromophore. This article shows the direct detection example of sugars by RI detector, and also shows the fluorescence detection example by post-column derivatization.

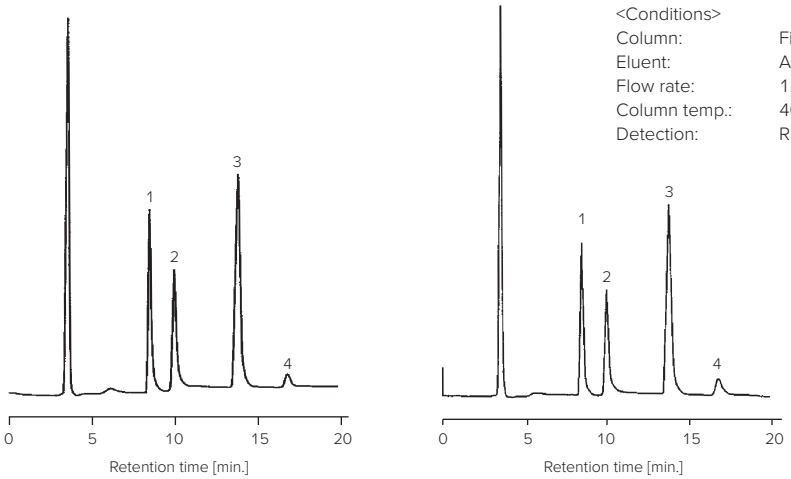
Direct detection of sugars by RI detector

RI detector is the versatile detector utilizing the difference between the refractive indices of eluent and sample, and can detect the sugar even though it does not have UV absorption. In this case, HILIC mode column is generally used.

JASCO RI detector has the following features, and enables to perform stable measurement because it is affected by the external environment hardly.

- Baseline stability by cell temperature control
- Expandability to semi-micro scale and preparative scale

Figure 1 shows the chromatograms of strawberry jam and orange marmalade, and Table 1 shows the quantitative analysis result. Monosaccharides (fructose and glucose) and disaccharides (sucrose and maltose) could be separated and quantified very well.



<Conditions>

Column:	Finepak SIL NH_2
Eluent:	Acetonitrile/Water (75/25)
Flow rate:	1.0 mL/min.
Column temp.:	40 °C
Detection:	RI detector

Figure 1. Chromatograms of strawberry jam (left) and orange marmalade (right)

1: Fructose, 2: Glucose, 3: Sucrose, 4: Maltose

Preparation: 1.0 g of sample was weighed, and then 5 mL of water was added. After mixing it, it was filtrated with 0.45 µm membrane. After that, 5 µL was injected to HPLC.

Table 1. Quantitative analysis result

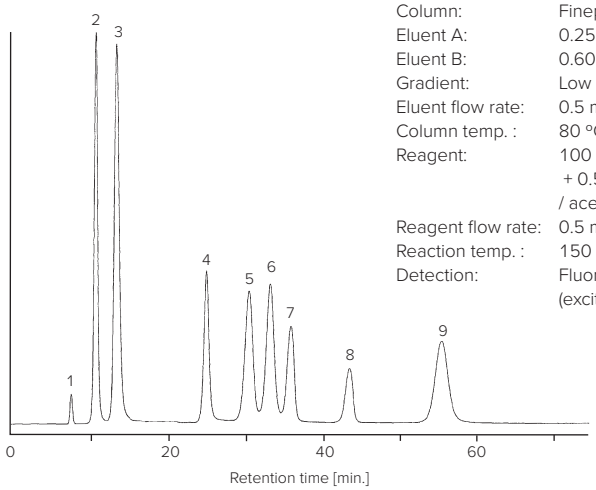
	Strawberry jam	Orange marmalade
Fructose	1.618	1.322
Glucose	2.804	2.609
Sucrose	2.565	2.251
Maltose	0.583	0.873

Fluorescence detection by post-column derivatization

In the case that the sample includes the contaminants during the HPLC analysis of sugars, proper qualitative and quantitative analysis cannot be performed because RI detector measures the contaminants as well as the target components. In addition, even when the amount of target component is very small, it cannot be performed because the sensitivity of its detector is not enough.

Although sugars do not own the chromophore, it can be turned to the derivative with fluorophore by reaction of guanidine and metaperiodic acid. Applying its properties to HPLC eables to perform highly sensitive and selective detection. This article shows the fluorescence detection result of sugars by post-column derivatization (the method that the sample is derivatized after injecting it to the column), whose reproducibility is good. The anion exchange column was used because its column can separate more sugars.

Figure 2 shows the chromatogram of standard sugars, and Figure 3 and 4 show the chromatograms of extract of vegetable stew and the condensed noodle soup, respectively. Each component could be separated with good resolution.



<Conditions>

Column:	Finepak GEL SA-121 (6.0 mmI.D. × 100 mmL)
Eluent A:	0.25 M borate buffer (pH 8.0)
Eluent B:	0.60 M borate buffer (pH 9.0)
Gradient:	Low pressure gradient elution
Eluent flow rate:	0.5 mL/min.
Column temp. :	80 °C
Reagent:	100 mM borate + 50 mM guanidine hydrochloride + 0.5 mM sodium metaperiodate (pH 8.0, adjust with NaOH) / acetonitrile (80/20)
Reagent flow rate:	0.5 mL/min
Reaction temp. :	150 °C
Detection:	Fluorescence detector (excitation: 310 nm, emission: 415 nm)

Figure 2. Chromatogram of standard sugars

1: Sucrose, 2: Maltose, 3: Lactose, 4: Mannose, 5: Fructose, 6: Galactose, 7: Sorbitol, 8: Mannitol, 9: Glucose

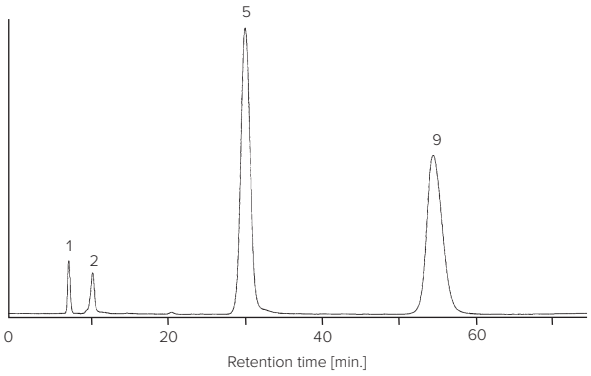


Figure 3. Chromatogram of extract of vegetable stew

Peak numbers and chromatographic conditions are the same as in Figure 2.

Preparation: Extract was filtrated with 0.45 µm membrane, an then it was diluted 5 times with water. After that, 20 µL was injected to HPLC.

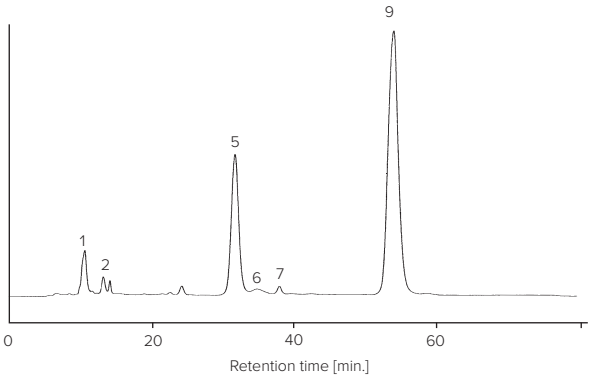


Figure 4. Chromatogram of the condensed noodle soup

Peak numbers and chromatographic conditions are the same as in Figure 2.

Preparation: The condensed noodle soup was added to hot water (60 °C), and it was ultrasonicated for 1 minutes. After it was warmed in hot bath (65 °C) for 5 minutes, it was left for 30 minutes at room temperature. And then, the supernatant (10 mL) was collected, and it was applied to centrifugal separation (4000 rpm, 10 minutes). 6 % perchloric acid (1 mL) and chloroform (1 mL) was added to the obtained supernatant (1 mL), and it was stirred. After that, the obtained supernatant was filtrated with 0.45 µm membrane, and 20 µL was injected to HPLC.

Rapid separation of orgainic acids in beer (HPLC-ODS column)

Organic acids in fermented foods are produced as the byproduct during fermentation process of lactic acid bacteria, and are the compounds that produce tastes such as sourness and deliciousness. In addition, they are also expected to have the oxidation resistant and antibacterial activity, and their functional features attract the attention.

Generally, the ion exclusion column is used for separating the organic acids, and adding the pH indicator (BTB: bromothymol blue) after column enhances the selectivity between target and contamitant compounds (Figure 1). Ion exclusion column is used for applying to the separation of various organic acids (high/low hydrophobicity). If only organic acids with low hydrophobicity is evaluated, reversed-phase column is good option of high speed and high sensitivity analysis . This article shows the separation example of organic acid by using ODS column.

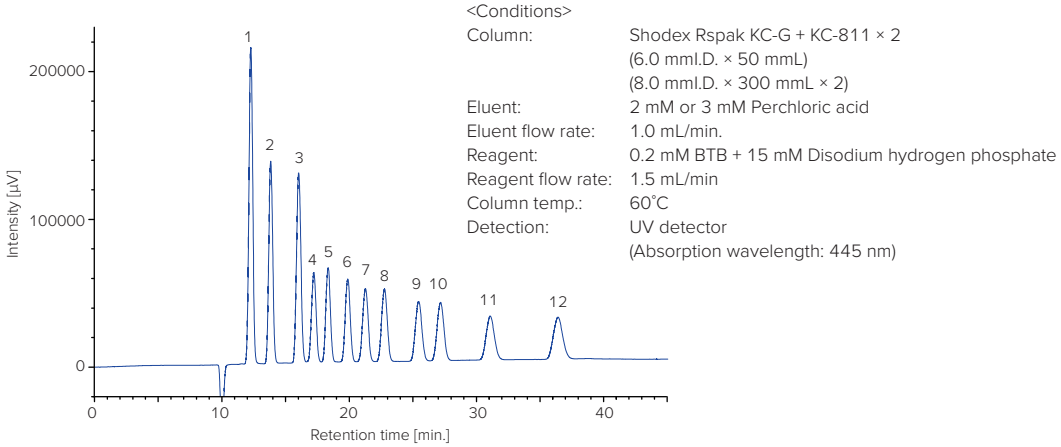
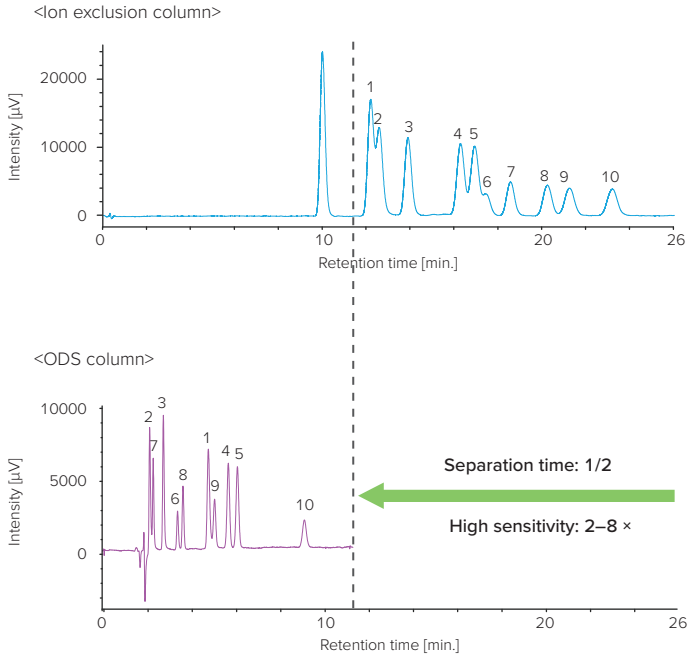


Figure 1. Chromatogram of standard organic acids (by ion exclusion column)

1: Citric acid, 2: Malic acid, 3: Succinic acid, 4: Lactic acid, 5: Formic acid, 6: Acetic acid, 7: Pyroglutamic acid, 8: Propionic acid, 9: Isobutyric acid, 10: n-Butyric acid, 11: Isovaleric acid, 12: n-Valeric acid (5.0 mmol/L each)

Analysis of standard organic acids

Figure 2 shows the chromatograms of standard organic acids by ODS column and ion exclusion column. Separation method by ODS column could separate 10 organic acids in half the measurement time by ion exclusion column (12 min.), and also improved their separation. In addition, it could also enhance the sensitivity (2 to 8 times).



<Conditions (ion exclusion column)>

Column:	Shodex Rspak KC-G + KC-811 × 2 (6.0 mmI.D. × 50 mmL) (8.0 mmI.D. × 300 mmL × 2)
Eluent:	2 mM or 3 mM Perchloric acid
Eluent flow rate:	1.0 mL/min.
Reagent:	0.2 mM BTB + 15 mM Disodium hydrogen phosphate
Reagent flow rate:	1.5 mL/min.
Column temp.:	60°C
Detection:	UV detector (absorption wavelength: 445 nm)

<Conditions (ODS column)>

Column:	InertSustain AQ-C18 (4.0 mmI.D. × 10 mmL, 5 μm) (4.6 mmI.D. × 150 mmL, 5 μm)
Eluent:	5 mM Perchloric acid
Eluent flow rate:	1.0 mL/min.
Reagent:	0.2 mM BTB + 15 mM Disodium hydrogen phosphate
Reagent flow rate:	1.0 mL/min.
Column temp.:	40°C
Detection:	UV detector (absorption wavelength: 445 nm)

Figure 2. Chromatograms of standard organic acids (by ion exclusion column and ODS column)

1: Citric acid, 2: Tartaric acid, 3: Malic acid, 4: Succinic acid, 5: Fumaric acid, 6: Lactic acid, 7: Formic acid, 8: Acetic acid, 9: Pyroglutamic acid, 10: Propionic acid, 0.5 mM each

Comparison of quantitative values of organic acids in beer

Figure 3 shows the chromatogram of beer, and Table 1 shows the quantitative values of organic acids in beer. Both methods could provide the same quantitative values.

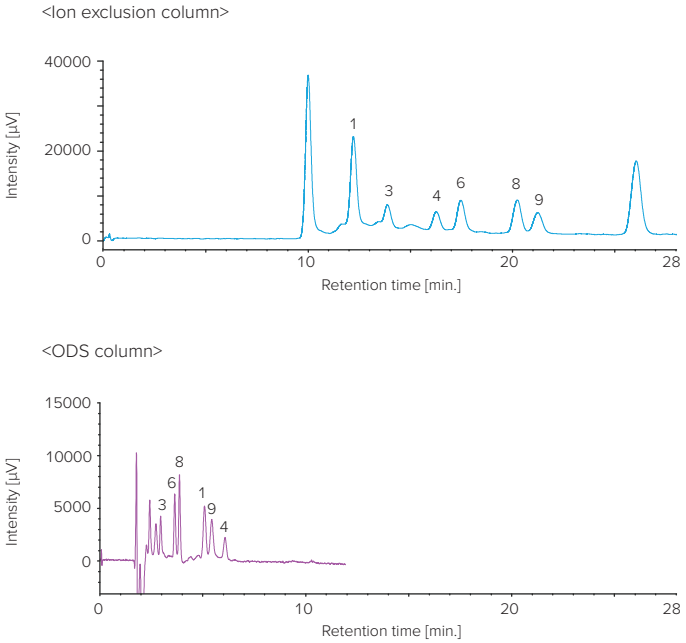


Table 1. Quantitative values of organic acids in beer (mM)

	Ion exclusion column	ODS column
Malic acid	0.19	0.17
Lactic acid	0.97	0.98
Acetic acid	0.83	0.95
Citric acid	0.58	0.49
Pyroglutamic acid	0.58	0.69
Succinic acid	0.17	0.16

Figure 3. Chromatograms of beer (by ion exclusion column and ODS column)

Peak numbers and chromatographic conditions are the same as in Figure 1.

Preparation: Beer was diluted twice with 1.5 mM perchloric acid, and then it was filtrated with 0.45 μm membrane.

Rapid separation of nonvolatile amines in wine (RHPLC-OPA derivatization)

Nonvolatile amines are produced during the microbe decay process of proteins and amino acids. Since several nonvolatile amines including histamine causes the food poisoning, their quantitative evaluation should be performed for quality control.

HPLC method using dansyl chloride (DNS-Cl) derivatization is generally used for analyzing the nonvolatile amines. However, its method needs many labors to pretreat the sample, and needs long time to measure the sample (approx. 40 minutes). Pre-column derivatization by *o*-phthalaldehyde (OPA) is expected to reduce the complicated pretreatment because it reacts the sample rapidly and easily and enables to perform the flourescence detection. This article shows the separation example of nonvolatile amines by using RHPLC (Rapid Separation LC) with OPA derivatization.

Comparison of derivatization methods (DNS-Cl reagent and OPA reagent)

Figure 1 and 2 show the reaction mechanisms of DNS-Cl derivatization and OPA derivatiation, respectively.

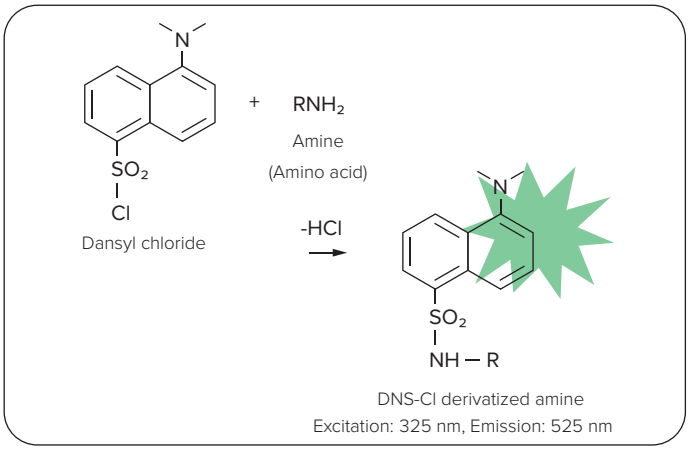


Figure 1. Reaction mechanism of DNS-Cl derivatization

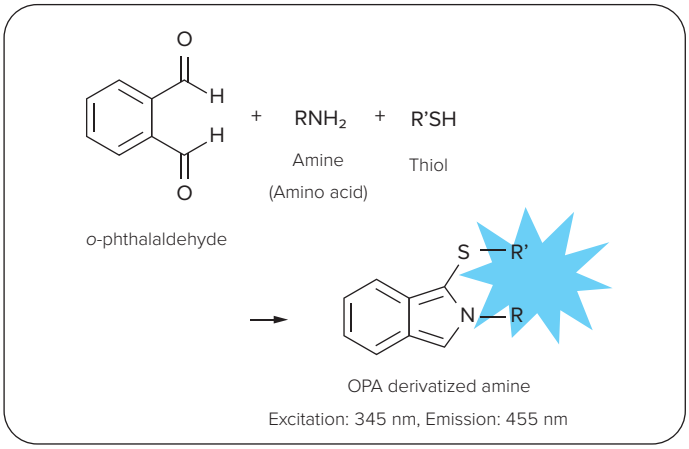
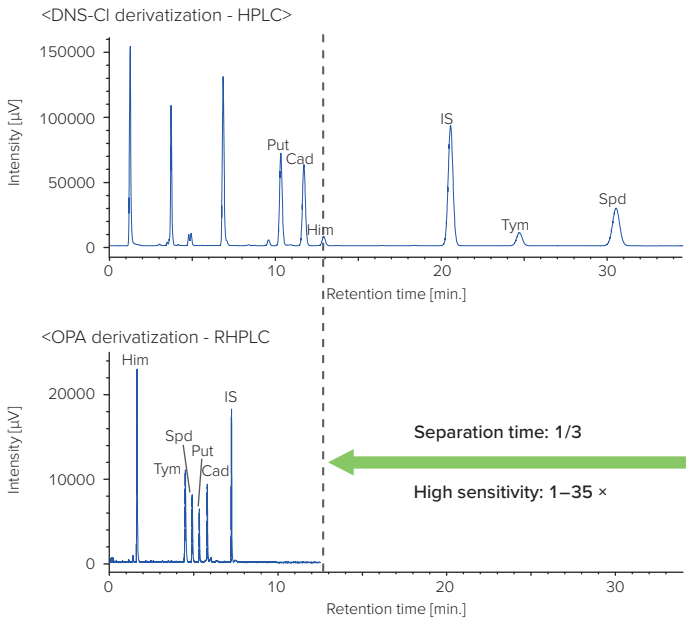


Figure 2. Reaction mechanism of OPA derivatization

There are many process to perform DNS-Cl derivatization (sample preparation, reaction, extraction, concentration and re-dissolution), and it takes long.time to pretreat it. In the case of OPA derivatization, only setting the autosampler after preparing the reagent, it runs automatically for reaction process. OPA derivatization is done in one-fourth the process time of DNS-Cl derivatization.

Analysis of standard nonvolatile amines

Figure 3 shows the chromatograms of standard nonvolatile amines by HPLC method and RHPLC method, respectively. RHPLC method could separate 6 nonvolatile amines in one-third the measurement time of conventional HPLC method (13 min.), and also improved their separation. In addition, it could also enhance the sensitivity (1 to 35 times).



<Conditions (HPLC)>

Column:	Inertsil ODS-SP (4.6 mmI.D. × 250 mmL, 5 μm)
Eluent:	Water/Acetonitrile (35/65), Isocratic
Flow rate:	1.2 mL/min.
Column temp.:	40 °C
Detection:	Fluorescence detector (exciation: 325 nm, emission: 525 nm)

<Conditions (RHPLC)>

Column:	Sunshell C18 (2.1 mmI.D. × 50 mmL, 2.6 μm)
Eluent A:	1M Citrate buffer 3.5 mL in Water
Eluent B:	1M Citrate buffer 3.5 mL in water/acetonitrile/ethanol (5/4/1)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	40 °C
Detection:	Fluorescence detector (excitation: 345 nm, emission: 455 nm)

Figure 3. Chromatograms of nonvolatile amines (by HPLC and RHPLC)

Put: Putrescine, Cad: Cadaverine, Him: Histamine, IS: 1,8-diaminooctane, Tym: Tyramine, Spd: Spermidine

Comparison of quantitative values of nonvolatile amines in wine

Figure 4 shows the chromatogram of wine, and Table 1 shows the quantitative values of nonvolatile amines in wine. Both methods could provide the same quantitative values.

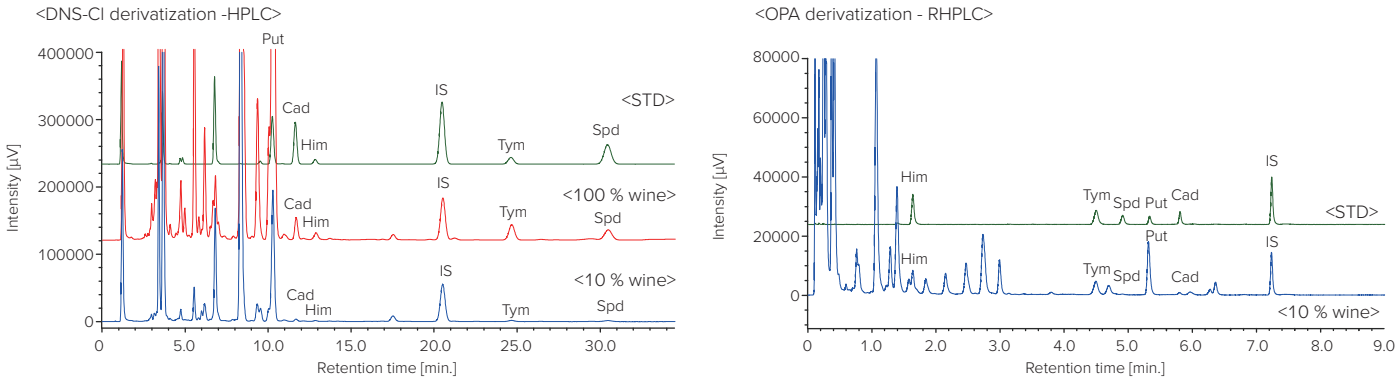


Figure 4. Chromatograms of wine (by HPLC and RHPLC)

Peak numbers and chromatographic conditions are the same as in Figure 3.

Preparation: Wine was diluted 10 times with water, and then it was filtrated with 0.45 μm membrane.

Table 1. Quantitative values of nonvolatile amines in wine (μM)

	HPLC	UHPLC
Him	25.2	24.9
Tym	32.0	28.1
Cad	3.95	5.07
Put	226.4	203.1
Spd	4.13	4.11

Identification test of food additives (FTIR)

Manufacturing or using the food additives, each country has settled the reguration for their identification, purity and quality, and controls them strictly so that the additives which does not conform to the regulation are not sold/used. Food/additive manufacturers need to verify whether their products conform to the regulation, and also need to confirm if they conform to the regulation the import country has settled when exporting them.

Test methods are described clearly in the regulation, and indicates that several additives should be identified by "Infrared spectrometry". Although comparing the measurement spectrum of sample with the reference spectrum or the measurement spectrum of the reference standard confirms its identification, the skilled technique is required to analyze the spetrum data. As the one of solutions, using the comparison algorithm enables to evaluate the identification objectively. The examples of comparison algorithms are shown as below.

- Euclidean distance

  It evaluates the summation of the square of absorbance difference at each wavenumber. Its algorithm is used for comparing the spectra including the broad bands.
- Euclidean distance after differentiation

  It evaluates the summation of the square of absorbance difference at each wavenumber after differentiation. Its algorithm is used for comparing the spectra including the baseline drift, or for comparing the spectra including the sharp peaks.
- Correlation coefficient

  It evaluates the correlation between spectra. Its algorithm is used for comparing the spectra including much noise.

This article shows the evaluation examples of *p*-methylacetophenone (used as flavor) and aspartame (used as sweetener) in accordance with the test methods that Japanese authorities specify (Japan's Specifications and Standards for Food Additives).

Identification test of *p*-methylacetophenone

The liquid sample was held between two KBr windows, and was measured by FTIR instrument (Liquid Film Method). Figure 1 shows the measurement result.

The regulation says that the both spectra should exhibit similar intensities of absorption at the same wavenumbers when comparing with the reference spectrum. Selecting the comparison algorithm depending on the situation, quantifying the similarity of database enables to distinguish the slight difference between spectra easily even if they look very similar.

"Euclidean distance after differentiation" was used as the comparison algorithm, whose score is 0 when the similarity is high. Since two spectra were compared after differentiation to offset the slope of baseline, they could be compared accurately. In order to clarify the criteria, several samples were measured and the euclidean distances between sample and reference were calculated beforehand. As a result, its criteria was set to 60.

The score of measurement result shown in Figure 1 is 37.29, which could be judged as "PASS" objectively because its score is lower than the criteria.

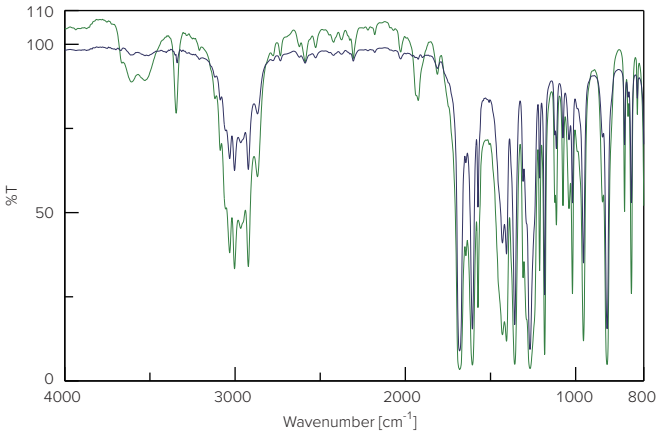


Figure 1. IR spectra of *p*-methylacetophenone
(green: sample, blue: reference standard)

Identification test of aspartame

The paste sample was produced by mixing the triturated sample with liquid paraffin. Its paste was held between two KBr windows, and was measured by FTIR instrument (Paste Method). Figure 2 shows the measurement result.

The regulation says that the spectrum should exhibit absorption bands at specific wavenumbers. Its test can be performed objectively by setting the wavenumbers and tolerances at software.

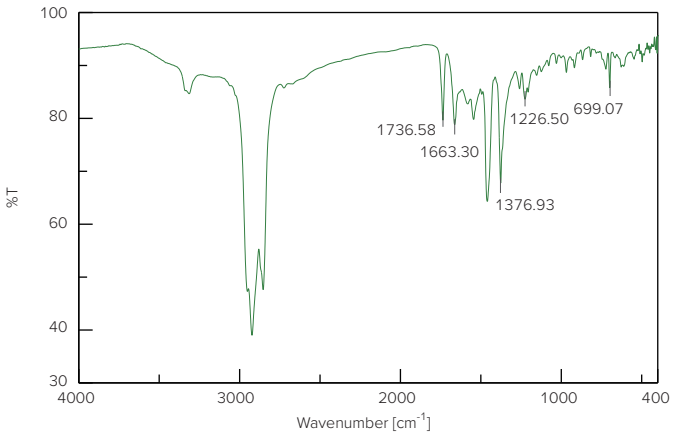


Figure 2. IR spectrum of aspartame

Table 1. Test result

Peak wavenumber (tolerance)	Measurement value	Pass/Fail
1737.00 cm^{-1} (5.00 cm^{-1})	1736.58 cm^{-1}	Pass
1666.00 cm^{-1} (5.00 cm^{-1})	1663.30 cm^{-1}	Pass
1379.00 cm^{-1} (5.00 cm^{-1})	1376.93 cm^{-1}	Pass
1227.00 cm^{-1} (5.00 cm^{-1})	1226.50 cm^{-1}	Pass
699.00 cm^{-1} (5.00 cm^{-1})	699.07 cm^{-1}	Pass

JASCO can provide the FTIR system to evaluate the products objectively, which does not need the skilled technique to analyze IR spectra, This system has the following functions, which enables to perform the product evaluation easily and routinely.

- Database registration of IR spectrum of reference standards
- Quantification of spectrum comparison between IR spectra of reference and sample
- 7 kinds of comparison algorithm in accordance with the comparison purpose
- Objective evaluation by pass/fail judgement function
- CFR Part 11 available

Analysis of preservatives and sweeteners (HPLC)

Food additives serve useful functions to enrich the consumer's food life, and are essential ingredients during food processing. There are various additives: 1) sweeteners/color additives/spices for improving the flavor, appearance and colorness of food, 2)preservatives for preventing the food spoilage and the changes in color, flavor or texture, and for maintaining the freshness, 3)nutrients for fortification.

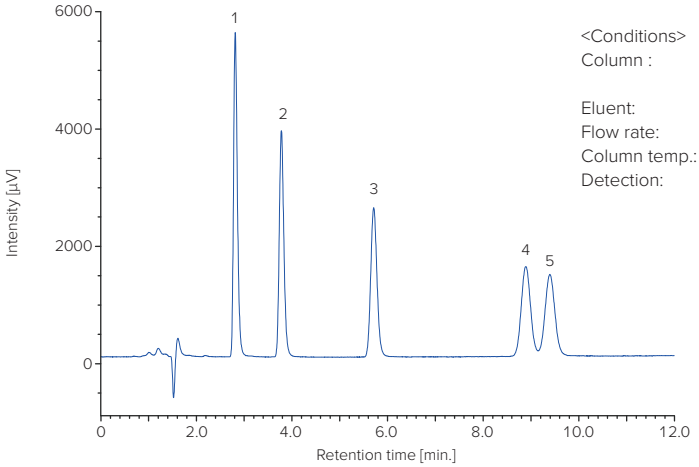
However, since almost food additives may have adverse health effects depending on the amount to be used, the authorities in each country strictly study, regulate and monitor them for safe use. In the case of FDA, when evaluating the safty of a substance and whether it should be approved, FDA considers: 1) the composition and properties of the substance, 2) the amount that would typically be consumed, 3) immediate and long-term health effects, 4) various safety factors. The evaluation determines an appropriate level of use that includes a built-in safety margin - a factor that allows for uncertainty about the levels of consumption that are expected to be harmless. Food manufacturers need to verify whether their products conform to the regulation, and also need to confirm if they conform to the regulation the import country has settled when exporting them.

Japan also regulates the manufacture, use and sale of the food additives, and publishes the specifications and standards for food, food additives, etc. (Food Sanitation Act). In addition, the analysis methods for each food additive are also published for their compliance check, and HPLC method is specified in many case of analysis of food additive. This article show the HPLC measurement examples of preservatives and sweetener according to the analysis method that Japanese authorities publish.

*U.S. FOOD & DRUG ADMINISTRATION (2010, April), *Overview of Food Ingredients, Additives & Colors*, https://www.fda.gov/food/food-ingredients-packaging/overview-food-ingredients-additives-colors

Analysis of *p*-hydroxybenzoate esters

p-hydroxybenzoate esters are added to foods as preservatives because it has the antibacterial activity against various bacteria. Figure 1 shows the chromatogram of standard p-hydroxybenzoate esters. 5 components were eluted with good resolution within 10 minutes.



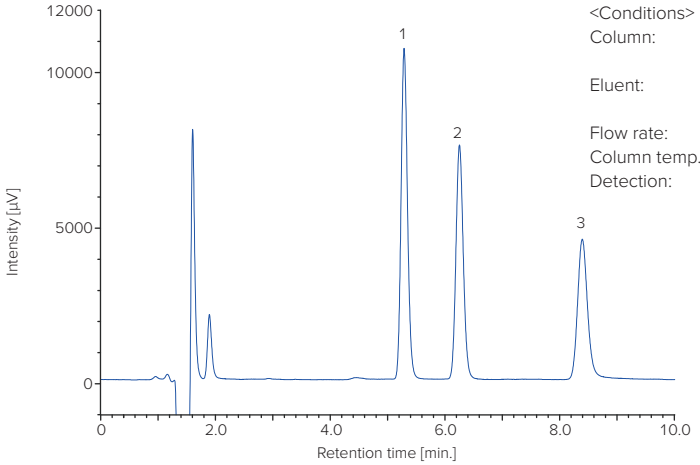
<Conditions>
Column : CrestPak C18S
(4.6 mmI.D. × 150 mmL, 5 µm)
Eluent: Acetonitrile/5 mmol/L Citrate buffer (pH 4.1) (60/40)
Flow rate: 1.0 mL/min.
Column temp.: 40 °C
Detection: UV detector
(absorption wavelength: 270 nm)

Figure 1. Chromatogram of standard *p*-hydroxybenzoate esters

1: Methyl *p*-hydroxybenzoate, 2: Ethyl *p*-hydroxybenzoate, 3: Propyl *p*-hydroxybenzoate,
4: Isobutyl *p*-hydroxybenzoate, 5: Butyl *p*-hydroxybenzoate

Analysis of bensoic acid, sorbic acid and dehydroacetic acid

Benzoic acid, sorbic acid and dehydroacetic acid are also added to foods as preservatives. Figure 2 shows the chromatogram of standard mixture. 3 components were eluted with good resolution within 9 minutes.



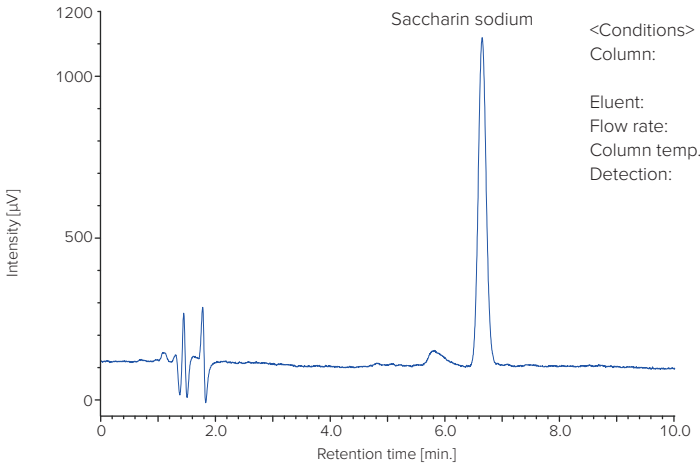
<Conditions>
Column: CrestPak C18S
(4.6 mmI.D. × 150 mmL, 5 µm)
Eluent: 5 mmol/L Citrate buffer (pH 4.1) / Acetonitrile / Methanol (70/20/10)
Flow rate: 1.0 mL/min.
Column temp.: 40 °C
Detection: UV detector
(absorption wavelength: 230 nm)

Figure 2. Chromatogram of standard mixture (benzoic acid, sorbic acid and dehydroacetic acid)

1: Benzoic acid, 2: Sorbic acid, 3: Dehydroacetic acid

Analysis of saccharin sodium

Saccharin sodium is used as sweetreener. Figure 3 shows the chromatogram of standard saccharin sodium.



<Conditions>
Column: CrestPak C18S
(4.6 mmI.D. × 150 mmL, 5 µm)
Eluent: 0.05 mol/L Phosphate buffer (pH 6.9)/Methanol (90/10)
Flow rate: 1.0 mL/min
Column temp.: 40 °C
Detection: UV detector
(absorption wavelength: 230 nm)

Figure 3. Chromatogram of saccharin sodium

Rapid separation of food additives in functional beverage (UHPLC)

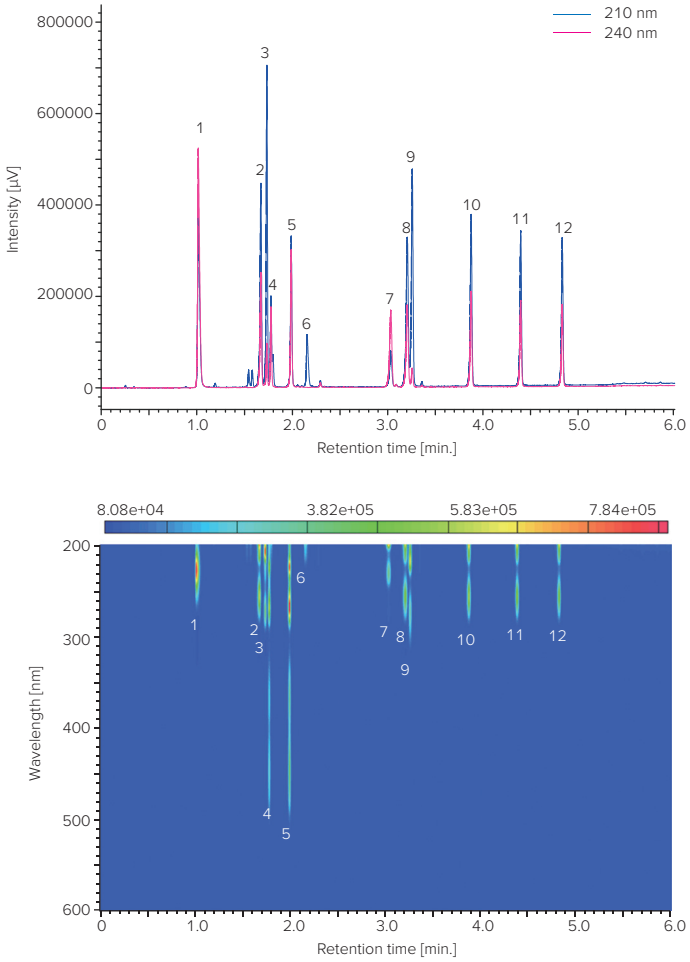
HPLC is widely used for component evaluation of the additives in food because it is easy to operate. Increasing the number of sample, the demands that a lot of samples want to be measured in shorter time than the conventional method have increased. In addition, since the absorption wavelength is different depending on the components, it may be difficult to monitor all components at one absorption wavelength if there are a lot of components.

Using the PDA detector for Ultra High-performance Liquid Chromatography (UHPLC), which can perform high-speed data acquisition (100 spectra/sec.), enables to measure the sample in shorter time than the conventional method. The retention time is not varied even at any absorption wavelength, and it is possible to perform the proper qualification analysis by comparing the UV spectra of unknown sample acquired from PDA detector with the one of standard sample.

This article shows the UHPLC analysis result of food additives in functional beverage.

Analysis of standard food additives

Figure 1 shows the chromatogram and contour plot of standard food additives. 12 components were clearly separated within 5 minutes.



<Conditions>

Column:	ZORBAX Eclipse Plus C18 (3 mmI.D. x 50 mmL, 1.8 µm)
Eluent A:	0.3% Phosphoric acid solution
Eluent B:	Acetonitrile
Gradient:	High pressure gradient elution
Flow rate:	0.8 mL/min.
Column temp.:	40 °C
Detection:	PDA detector (absorption wavelength: 200 to 650 nm)

Figure 1. Chromatogram of standard food additives

1: Acesulfame K (0.2mg/mL), 2: *p*-Hydroxybenzoic acid (0.05mg/mL), 3: Caffeine(0.05mg/mL), 4: Vitamin B_2 sodium phosphate(Riboflavin sodium phosphate) (0.1mg/mL), 5: Vitamin B_2(Riboflavin) (0.1mg/mL), 6: Aspartame (0.1mg/mL), 7: Benzoic acid (0.05mg/mL), 8: Methyl p-hydroxybenzoate (0.05mg/mL), 9: Propyl gallate (0.05mg/mL), 10: Ethyl *p*-hydroxybenzoate (0.05mg/mL), 11: Propyl *p*-hydroxybenzoate (0.05mg/mL), 12: Butyl *p*-hydroxybenzoate (0.05mg/mL)

Analysis of food additives in functional beverage

Figure 2 and 3 shows the chromatogram and the contour plot of functional beverage, respectively. Figure 4 shows the chromatograms at 4 different wavelengths.

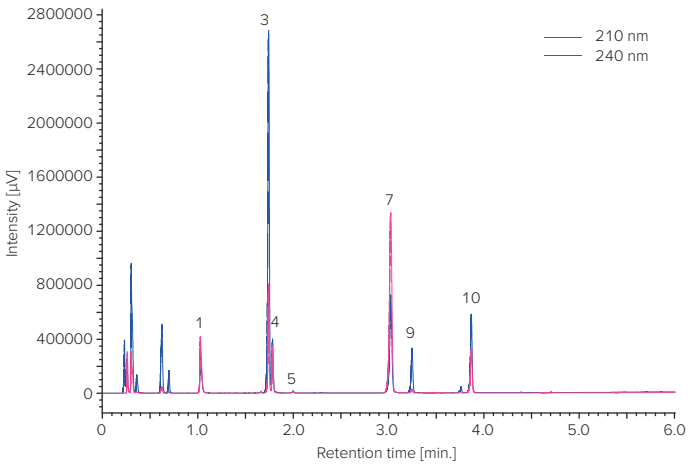


Figure 2. Chromatogram of functional beverage

Peak numbers and chromatographic conditions are the same as in Figure 1.

Preparation: Functional beverage was filtrated with 0.2 µm membrane.

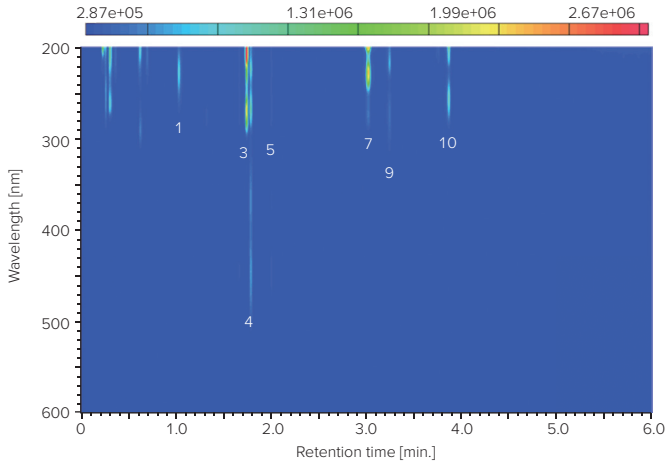


Figure 3. Chromatogram of functional beverage (contour plot)

Peak numbers and chromatographic conditions are the same as in Figure 1.

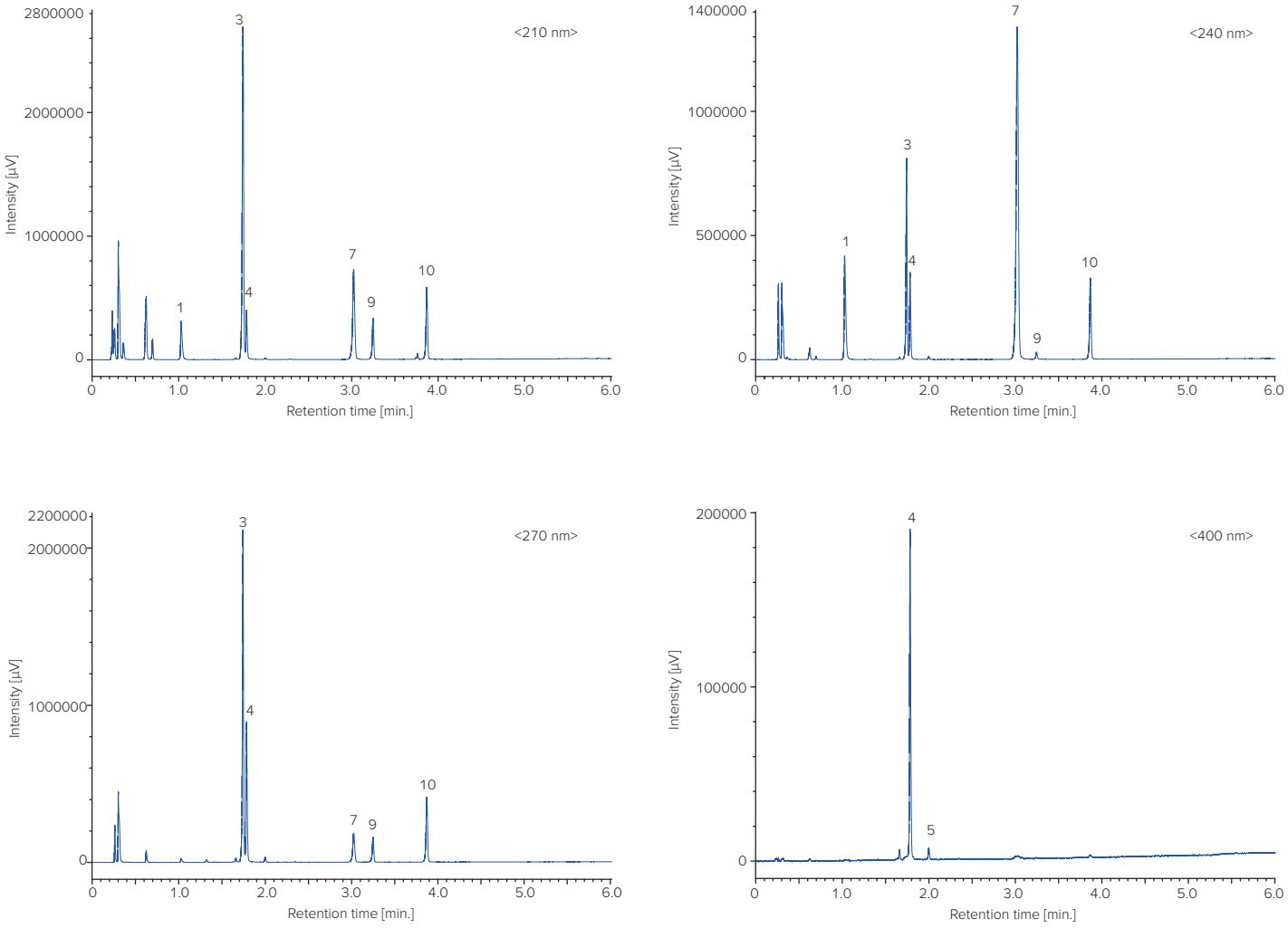


Figure 4. Chromatograms of functional beverage at 4 different wavelengths

Peak numbers and chromatographic conditions are the same as in Figure 1.

Conbined analysis of food package films (micro IR/micro Raman)

Package films have the roles to protect the food quality and to extend the time limitation of food freshness/consumption. On the other hand, the defects of package films lead to food deterioration, which may cause the accidents that it injures humans and things. Therefore, its R&D are being progressed constantly, and quality control in manufacturing process need to be performed based on the thought of HACCP (Hazard Analysis and Critical Control Point).

There are a lot of materials for packaging (metal, glass, paper etc.), and the plastic material is widely used for food packaging due to high safety and hygiene. IR microscope and Raman microscope are powerful tool to evaluate the impurities and the conformation of packaging film. Two instrument have mutual complementary relationship, and obtaining both spectra can provide the various information. In addition, analyzing the spectrum data by chemometrics can extract the component information properly. Visualizing the component distribution, it enables to provide detailed information of contamination, and also to provide the effective breakthroughs for R&D.

This article shows the evaluation example of packaging film.

Combined analysis of multi-layer film

Cross-section piece of multi-layer film was created, and its same position was measured by IR microscope and Raman microscope. Figure 1 shows the observation image of cross-section of multi-layer film, and Figure 2 and 3 show the principal component spectra calculated by MCR (Multivariate Curve Resolution) and chemical images calculated by the score of the principal component spectra. As a result of both principal component spectra and chemical images, the distribution status of polypropylene (PP), polyethylene (PE) and polyethylene terephthalate (PET) could be visualized. Cellulose with large infrared activity was specifically detected by IR microscope, and titanium oxide (TiO_2) was specifically detected as a very thin layer (several μm) by Raman microscope. Since cellulose is not used in this film, it indicates that there is the high possibility that cellulose is mixed as foreign matter,. By using both systems, the complementary information was acquired.

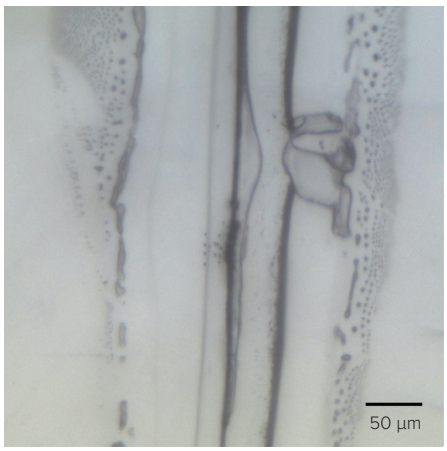


Figure 1. Observation view

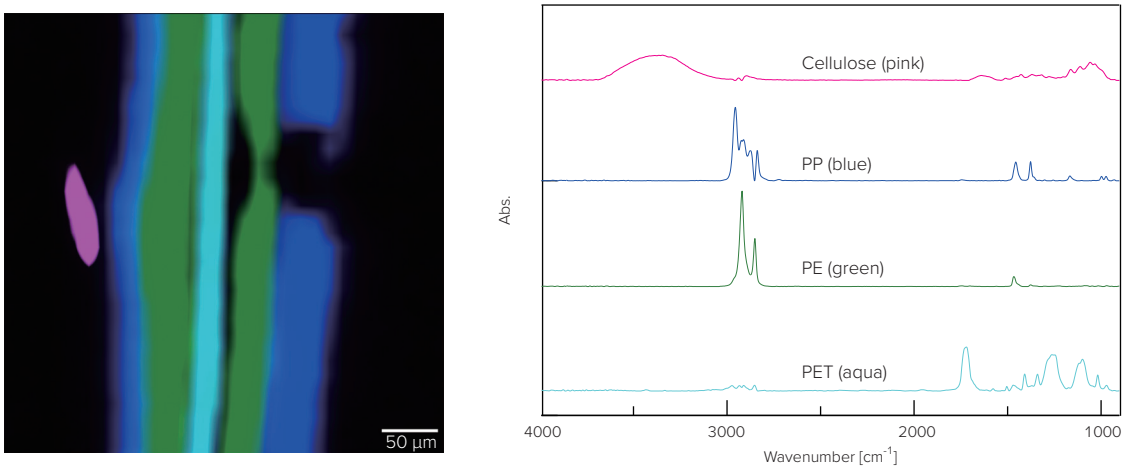


Figure 2. Principal component spectra (right) and chemical image (left) (IR microscope)

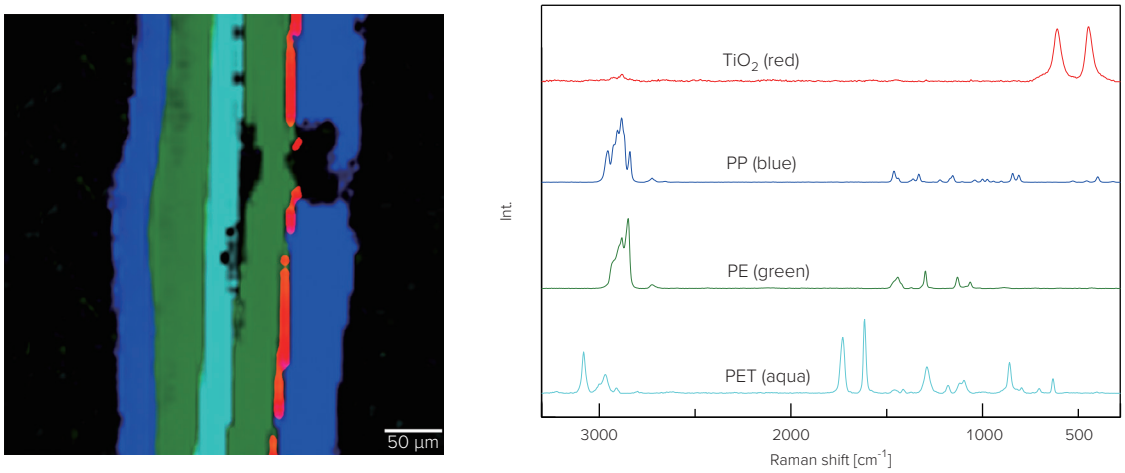


Figure 3. Principal component spectra (right) and chemical image (left) (Raman microscope)

JASCO can provide innovative accessory, IQ frame, which enables to obtain Infrared and Raman information in the same area quickly and conveniently. Its accessory is useful for microscopic analysis between different facilities and re-measurement of samples that are removed once from the stage as well as the combined analysis of IR spectroscopy and Raman spectroscopy.

Combined analysis of impurities in food (micro IR/micro Raman)

Recalls by the contaminant problems in food product occurs all around the world, and some authorities disclose the details of them. There are various reasons why the food products have been recalled, which include the contaminations of rubber and resin by deteriorating the parts in product line equipment or by human error. Therefore, it is important to specify the reason of contamination to manufacture the food products safely.

Recently, some countries obligates the manufacturers to have the hygiene control by HACCP (Hazard Analysis and Critical Control Point), and they are addressing it with the strict quality control so that the recall does not occur.

IR microscope and Raman microscope are powerful tool to evaluate the impurities and the conformation of packaging film. Two instrument have mutual complementary relationship, and obtaining both spectra can provide the various information. In addition, analyzing the spectrum data by chemometrics can extract the component information properly, which can be the evidence to find out the causes of product defect.

This article shows the evaluation example of the plastic particles by using IR microscope and Raman microscope.

Analysis of plastic particles by IR microscope

The sample sprayed with plastic particles was measured by IR microscope. Figure 1 shows the observation image, and Figure 2 shows the principal component spectra calculated by MCR (Multivariate Curve Resolution) and chemical image calculated by the score of the principal component spectra. As a result, protein was found in addition to four plastics: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS).

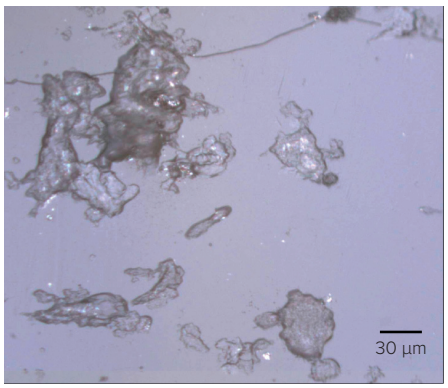


Figure 1. Observation view

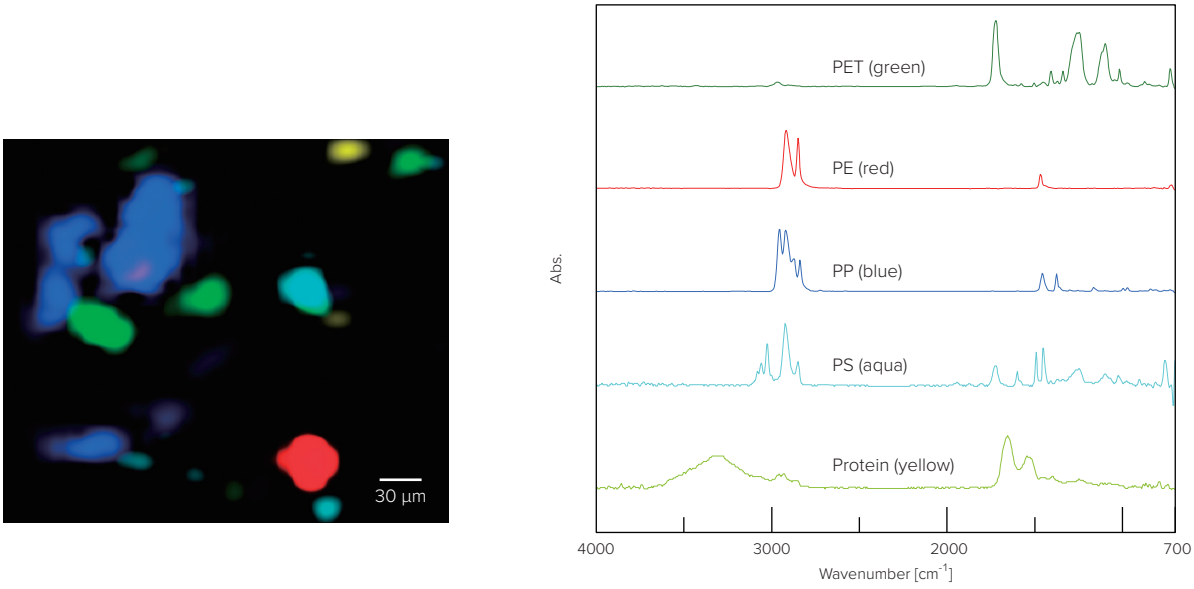


Figure 2. Principal component spectra (right) and chemical image (left) (IR microscope)

Analysis of plastic particles by Raman microscope

Utilizing IQ frame, Raman measurement was performed at the same position measured by IR microscope. Figure 3 shows the principal component spectra calculated by MCR (Multivariate Curve Resolution) and chemical image by the peak height of the key band. As a result, carbon was found in addition to the four plastics previously identified by IR: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS). In analysis by IR microscope, protein was detected in addition to the four plastics, whereas in analysis by Raman microscope, carbon was detected without protein detection. These results are obtained because Raman spectroscopy has low detection sensitivity for protein, but it is good for carbon analysis.

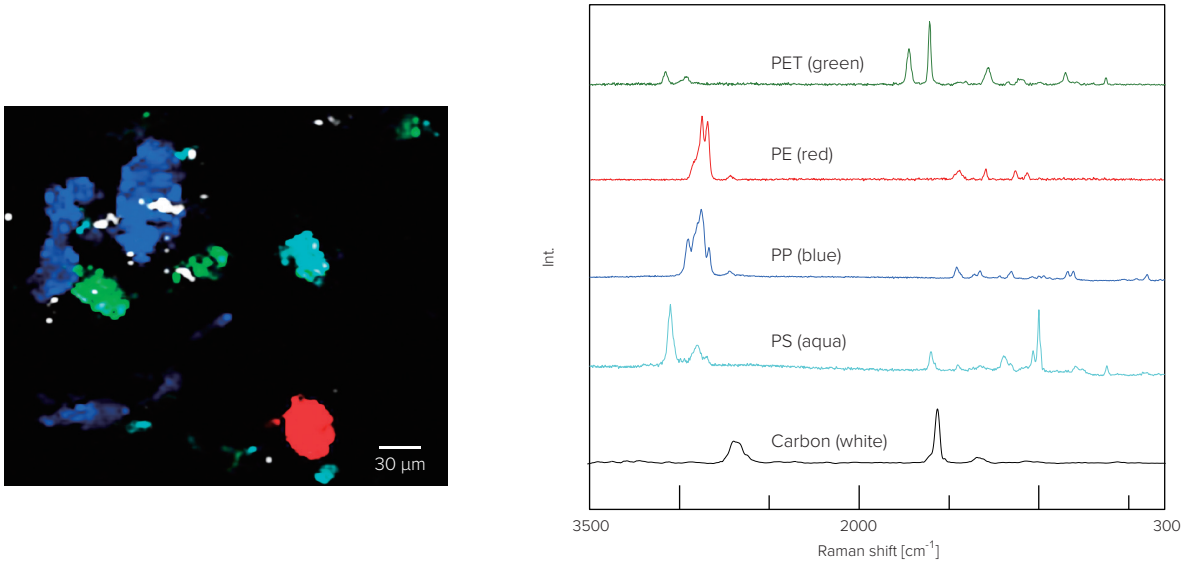


Figure 3. Principal component spectra (right) and chemical image (left) (Raman microscope)

Overlay of imaging results (IR and Raman)

As a result of the each sample imaging shown in Figure 2 and 3, only five components could be detected in the plastic particle sample by either IR or Raman spectroscopy separately. However, the results of combined analysis indicated that six different components could be detected and imaged.

In order to visualize the complete analytical information, both sets of imaging data were overlaid (Figure 4), this showed the protein detected by IR analysis together with the five other components, polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS) and carbon detected by Raman analysis.

These results indicate that using the strong points of both IR and Raman analysis can be combined to be more useful than a single analytical technique alone.

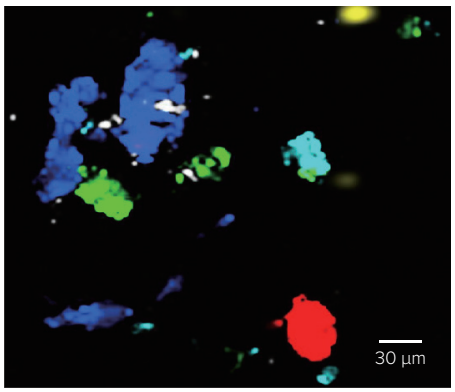


Figure 4. Overlaid image of IR and Raman

Time estimation of pest contamination (Circular dichroism)

Pest contamination occurs anytime (from farm to table). When the problem occurs, the manufacturers need to estimate the point where the pests have been added to, and need to improve the product processing as soon as possible.

Monitoring the protein denaturation of pest can be one of clues to estimate the time of contamination. Utilizing the behavior that the high order structure of protein varies irreversibly during heating it, it is feasible that the time of pest contamination about the heat treated food is estimated by checking whether the denaturation occurs or not (Figure 1).

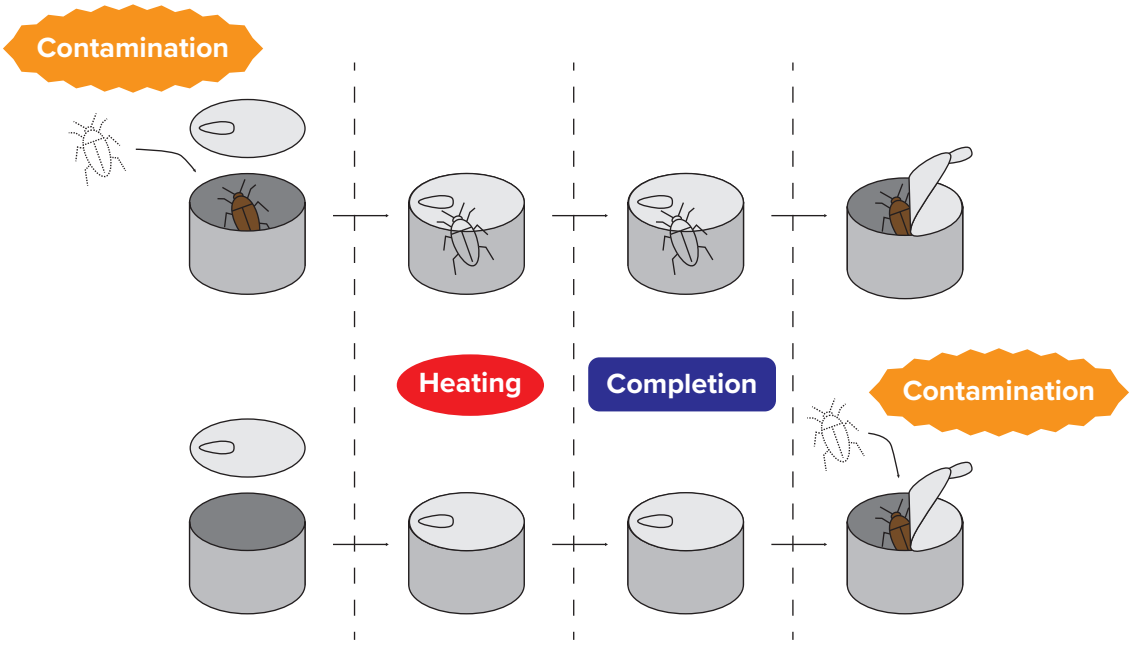


Figure 1. Time estimation of pest contamination

(Top: contamination during the food processing, Bottom: contamination after food processing)

Since CD spectrometer can detect the change of high order structure of protein, it is expected as the effective tool to estimate the time of pest contamination.

This article evaluated whether CD spectrometer can provide the clues of pest contamination by using protein extracted from pest.

CD spectrum changes of pest femur extract

Figure 2 shows the CD spectra of the pest femur extract and commercial myosin before/after heating. Both spectra are similar very well, and it indicates that there are many myosins in pest femur extract. Figure 3 shows the result of secondary structure estimation analysis by CD spectrum of myosin, which indicates that the ratio of secondary structure was varied during heating.

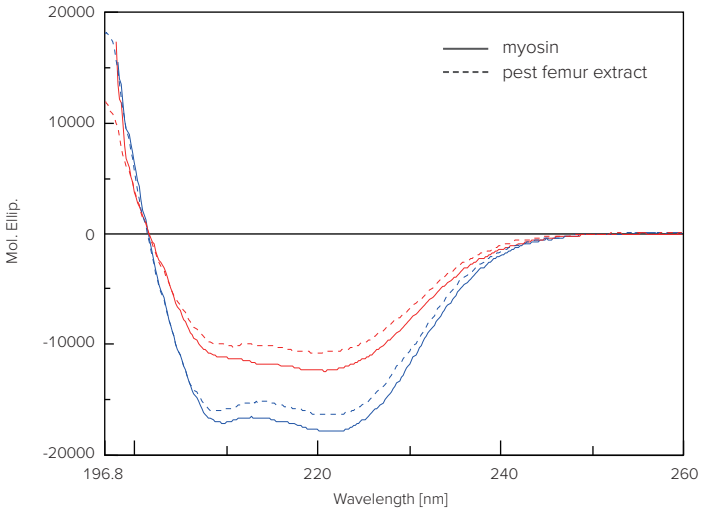


Figure 2. CD spectrum changes(blue: non-heating, red: heating)

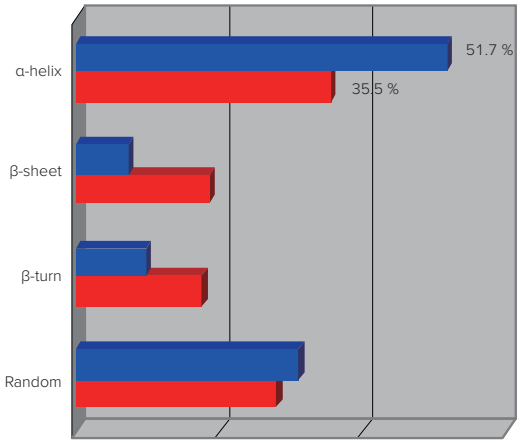


Figure 3. Secondary structure analysis (blue: non-heating, red: heating)

Denaturation temperature of myosin

Figure 4 shows the temperature monitoring result of myosin. As a result, the denaturation temperature of myosin was 46.2 °C, and it indicates that it is possible to estimate the time of pest contamination if the heating treatment temperature is higher than 46.2 °C. If the denaturation is not confirmed, it is estimated that the pest has been added to the product after completing it.

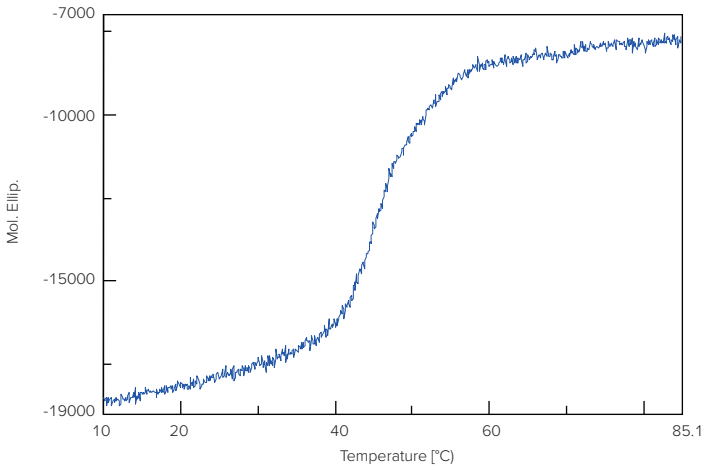


Figure 4. Temperature monitoring of myosin

Since the structure of myosin is almost same as the one in many species, it is applicable to many pests. In addition, since the denaturation temperature is 46.2 °C, it also applicable to the manufacturing process with wide sterilizing temperature. It is expected that its estimation by CD measurement is applied for the heat treated food (such as canned food, retort pouch).

JASCO CORPORATION
2967-5, Ishikawa-machi, Hachioji-shi, Tokyo 192-8537 Japan
Tel: +81-42-649-5177 Fax:+81-42-646-4515 Web: www.jasco.co.jp
Japan

JASCO INTERNATIONAL CO., LTD.
11-10, Myojin-cho 1-chome, Hachioji-shi, Tokyo 192-0046, Japan
Tel: +81-42-649-3247 Fax: +81-42-649-3518 Web: www.jascoint.co.jp/english/
Australia, Hong Kong, India, Indonesia, Korea, Malaysia, New Zealand, Pakistan,
Philippines, Russia and CIS countries, Singapore, Taiwan, Thailand, Vietnam

JASCO INCORPORATED
28600 Mary' s Court, Easton, Maryland 21601, U.S.A.
Tel: +1-410-822-1220 Fax: +1-410-822-7526 Web: www.jascoinc.com
Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Guatemala, Mexico,
Paraguay, Peru, Puerto Rico, United States of America, Uruguay, Venezuela

JASCO EUROPE S.R.L.
Via Luigi Cadorna 1, 23894 Cremella (LC), Italy
Tel: +39-039-9215811 Fax: +39-039-9215835 Web: www.jasco-europe.com
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JASCO CHINA (SHANGHAI) CO., LTD.
Room No.D, 10F, World Plaza, 855 Pudong South Road, Pudong New Area, Shanghai, China
Tel: +86-21-6888-7871 Fax: +86-21-6888-7879 Web: www.jasco-global.com
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E6402-2103