JEOL

Applications Note
Food Analysis Solutions

Solutions for Innovation

Foodnote

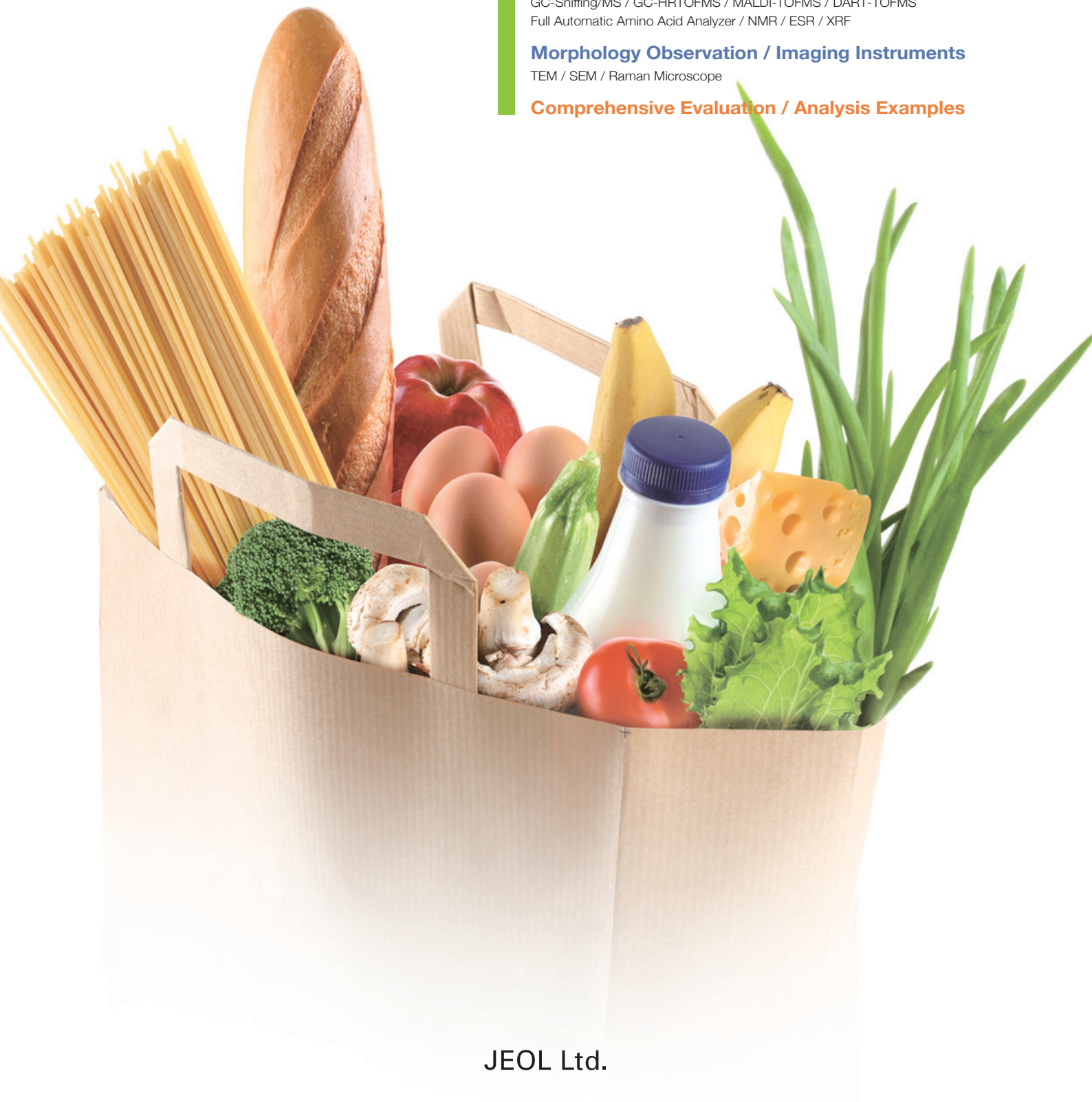
Chemical Analysis Instruments

GC-Sniffing/MS / GC-HRTOFMS / MALDI-TOFMS / DART-TOFMS
Full Automatic Amino Acid Analyzer / NMR / ESR / XRF

Morphology Observation / Imaging Instruments

TEM / SEM / Raman Microscope

Comprehensive Evaluation / Analysis Examples



JEOL Ltd.

Food Analysis Solutions

Foodnote

Introduction

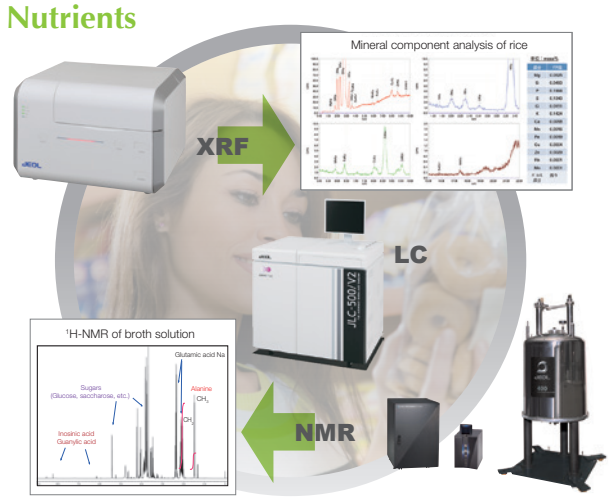
Four demands have developed in the food product field for highly accurate, objective evaluation technologies to analyze aspects such as nutritional components, flavors and tastes, texture, and antioxidation functions. JEOL offers numerous analytical tools to support both "food safety & security" as well as various evaluations of primary, secondary and tertiary functions of foodstuffs which are useful for a wide range of users associated with this field.

This Foodnote introduces the features of each of the instruments and actual analysis examples, and is designed for researchers and engineers who are considering purchases of instruments. This brochure also presents comprehensive evaluations and analysis solutions that can be achieved with combinations of multiple instruments.

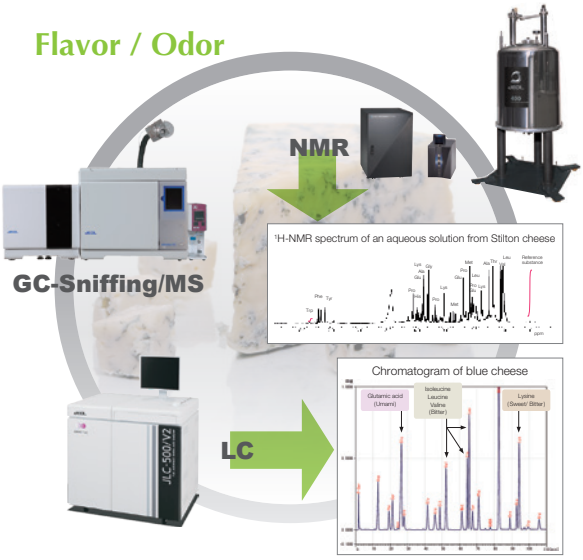
INDEX

Recommended JEOL instrument line-up depending on analytical purposes

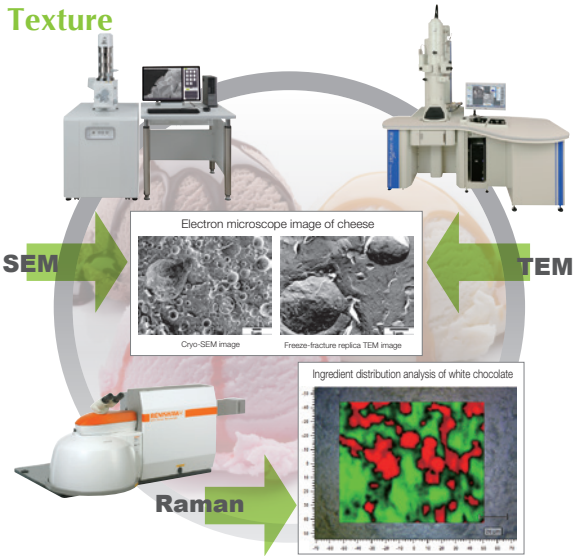
Nutrients



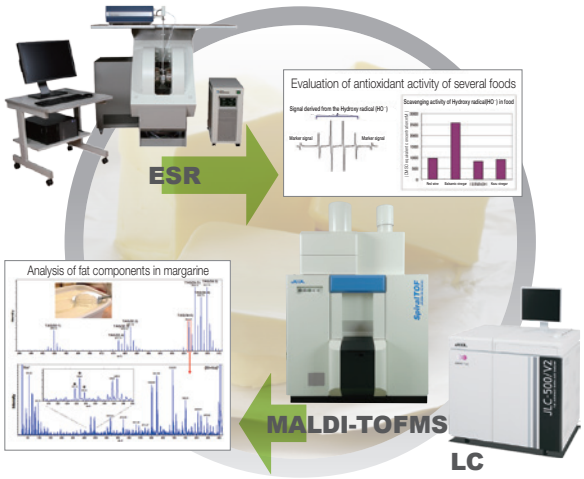
Flavor / Odor



Texture



Functionality

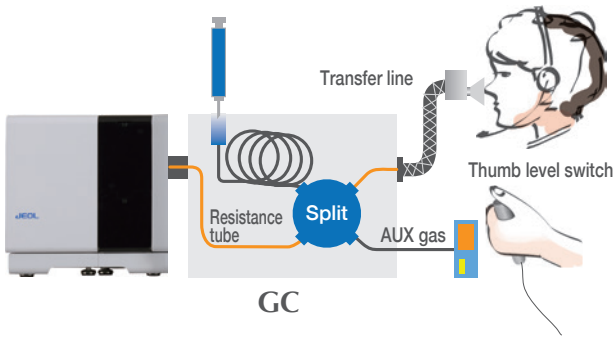


Quality assurance

Package

1-1 GC-Sniffing/MS

The GC-Sniffing / MS is a combined analysis system that is composed of a kind of "Sniffing" analysis method and a mass spectrometer (MS) for detecting components that have been separated by using gas chromatography (GC). By simultaneously analyzing the sensory index called "odor" and the underlying chemical components, it is possible to estimate the chemical substances that are the sources of specific odors by using library searches of the mass spectra. The MS system uses a quadrupole mass spectrometer and the system can support a variety of applications such as environmental analysis and material packaging analysis.



GC-Sniffing/MS
(Quadrupole mass spectrometer JMS-Q1500GC)

Odor analyses can be easily performed by any user!

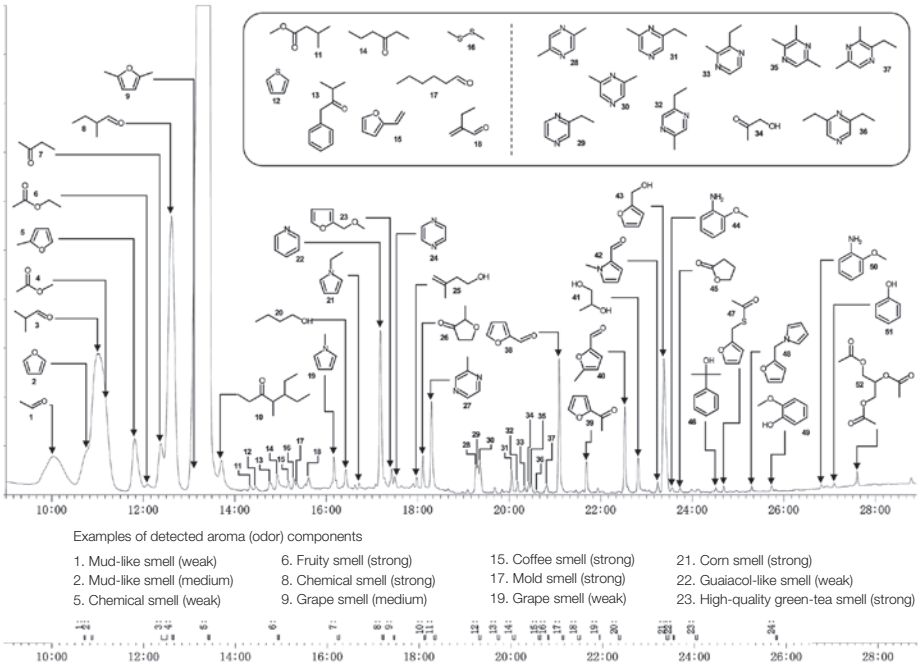
Features

- MS provides a highly-sensitive analysis, thus allowing a greater branching ratio to the sniffing line.
- The branching ratios can be easily calculated by using the advanced calculation software.
- The "Notification function" in the software informs you when a peak comes out on the MS, thus supporting the timing for the sniffing operation.
- The use of the headspace auto sampler enables the highly-sensitive measurement of volatile components in both solid and liquid samples, without any pre-treatment.

Case study

Analysis of aroma components in coffee beverage

Combining the headspace auto sampler with the HS-GC-MS measurements makes it possible to detect volatile components that are found in many odors. By simultaneously performing sniffing, the sensory evaluation combined with measurements is useful for identifying flavor components for product development as well as isolating off-flavor (off-odors) in the case of complaints. As an example of an HS-GC-sniffing / MS application, the results for measurements of the trace aroma components in a commercial canned coffee beverage are shown below.



Examples of detected aroma (odor) components

1. Mud-like smell (weak)
2. Mud-like smell (medium)
5. Chemical smell (weak)
6. Fruity smell (strong)
8. Chemical smell (strong)
9. Grape smell (medium)
15. Coffee smell (strong)
17. Mold smell (strong)
19. Grape smell (weak)
21. Corn smell (strong)
22. Guaiacol-like smell (weak)
23. High-quality green-tea smell (strong)

1-2 Gas Chromatograph – High Resolution Time-Of-Flight Mass Spectrometer (GC-HRTOFMS)

The Gas Chromatograph – High Resolution Time-Of-Flight Mass Spectrometer (GC-HRTOFMS) provides an ability to acquire high-sensitivity, high-speed mass spectra while retaining high mass resolution and accuracy for the substance components separated by GC. An ionization method can be selected from the usual electron ionization (EI) as well as methods like chemical ionization (CI) and field ionization (FI), enabling analysis of a wide range of volatile substances.

Gas Chromatograph – High Resolution Time-Of-Flight Mass Spectrometer JMS-T200GC "AccuTOF™ GCx"

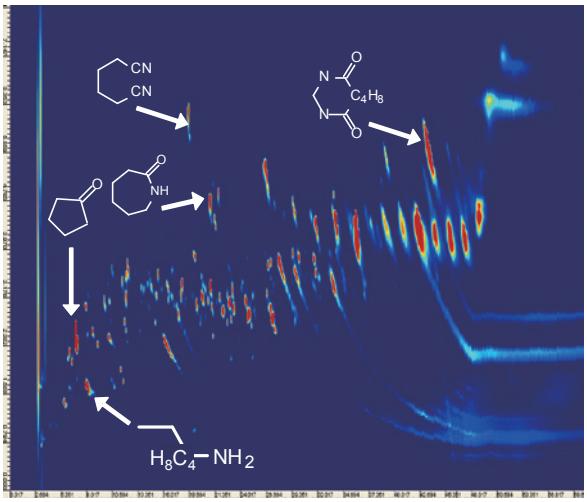
Routine execution of high-end research analysis, such as identification of unknowns!

Features

- Allows routine accurate mass analysis and is exceptional for structural analysis of unknown substances.
- The high mass resolution supports highly-selective quantification.
- Measurements can be made by GC and direct insertion probe.
- Since you can select the ionization method (from EI, CI, FI, FD), analysis is possible for a wide range of materials.
- Even more detailed analysis is possible by incorporating comprehensive two-dimensional gas chromatography (GC x GC), even for specimens that are not adequately separated into their components with ordinary GC/MS.

Case study Analysis of macro-molecular material using Pyrolysis GC x GC-HRTOFMS

During the analysis of macromolecular materials, especially for measurements that combine a pyrolysis device and GC-MS, there are cases in which very large numbers of chemical components are generated by pyrolysis. As a result, the separation from conventional (one-dimensional) GC does not provide sufficient chromatographic separation, so that the structural information about the macromolecular material cannot be obtained.
By combining pyrolysis with GC x GC-HRTOFMS, it is possible to perform even more detailed analysis of macromolecular materials by taking advantage of the high-separation capability of GC x GC and the exceptional mass resolution of HRTOFMS. Furthermore, by using the soft ionization method (FI) in addition to the hard ionization method (EI), it is also possible to confirm the molecular weight of unknown components, enabling an even more precise analysis.



Analysis results for 6, 6 nylon using Pyrolysis GC x GC-HRTOFMS

1-3 Matrix Assisted Laser Desorption Ionization – Time-Of-Flight Mass Spectrometer (MALDI-TOFMS)

Matrix Assisted Laser Desorption Ionization – Time-Of-Flight Mass Spectrometer (MALDI-TOFMS) is suitable for the analysis of a wide range of substances, from low molecular weight compounds like amino acids to high molecular weight compounds like synthetic polymers and proteins. Furthermore, the MS/MS spectra obtained from MS/MS measurements using high precursor ion selectivity and high-energy collision-induced dissociation (HE-CID) provide even more detailed structural information from the analytes, particularly positional information about the carbon-carbon double bonds. It is also possible to generate images using the mass spectra (MS imaging) by acquiring a sequence of mass spectra while continuously shifting the exposure position of the laser. This allows you to visually capture the distribution of a target compound within a sample.

MALDI-TOFMS
JMS-S3000
"SpiralTOF™"

Capable of handling a wide range of substances, from low to high molecular weights!

Features

- The adoption of SpiralTOF techniques enables high-resolution mass separation.
- Detection with high mass accuracy is possible even for measurements of low molecular weight molecules, like amino acids.
- Detailed structural information can be obtained from single-ion MS/MS with HE-CID.
- Using imaging, it is possible to visually display the distribution of the target substances.

Case study Analysis of fat components in margarin

Triacyglycerol (TAG) is contained in edible oils such as olive oil and margarine, and consists of one molecule of glycerol and three molecules of fatty acids. They build the structure of ester bond and each fatty acid has a different number of carbon atoms and double bonds and also a different position for the double bonds. Therefore until recently, it has been extremely difficult to identify each TAG, individually form and clarifying the structures. The results of an analysis for a commercially-available margarine using the MALDI-TOFMS JMS-S3000 "SpiralTOF™" are shown in the figure on the left. In the upper mass spectrum, there are multiple peaks attributable to the detected TAGs. From the accurate mass of these peaks, it is possible to confirm the total number of carbons and double bonds in the 3 fatty acids that constituted the TAG. The results of the MS/MS measurements using HE-CID for each peak (shown in the lower image of Figure 1 for TAG(54:6)) identified the double bond positions and elemental composition of each fatty acids, as well as the bonding position.

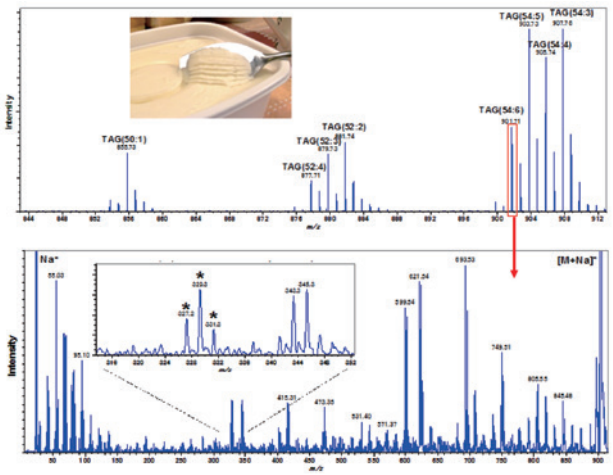


Fig. 1 Mass spectra of margarine
Upper: Enlarged spectrum of the TAG mass region
Lower: TAG (54:6) (*m/z* 901.71) product ion spectrum

Since the MALDI-TOFMS JMS-S3000 "SpiralTOF™" is capable of high mass resolution, high precursor selectivity, in addition to HE-CID, and only requires an extremely simple sample preparation, the JMS-S3000 is highly regarded as an ideal tool for the structural analysis of TAG in edible oils, including margarine.

m/z	Number of acyl group carbons and double bonds	Composition of each fatty acid			
855.7	50 : 1	(16:0,16:0,18:1)			
877.7	52 : 4	(16:0,18:2,18:2)	(16:0,18:3,18:1)		
879.7	52 : 3	(16:0,18:1,18:2)	(16:0,18:2,18:1)		
881.7	52 : 2	(16:0,18:1,18:1)			
901.7	54 : 6	(18:2,18:1,18:3)	(18:1,18:2,18:3)	(18:2,18:2、18:2)	(18:1,18:3,18:2)
903.7	54 : 5	(18:1,18:2,18:2)	(18:1,18:3,18:1)		
905.7	54 : 4	(18:1,18:1,18:2)	(18:1,18:2,18:1)		
907.7	54 : 3	(18:1,18:1,18:1)			

Table 1. TAG *m/z* value detected in margarine and fatty acid composition

1-4 DART-Time-Of-Flight Mass Spectrometer (DART-TOFMS)

DART (Direct Analysis in Real Time) is an ambient ionization method that allows non-contact ionization under atmospheric pressure.
By using a mass spectrometer equipped with a DART™ ion source, the analysis of volatile substances in samples of any form, including gas, liquid, solid, or solid surface, can be performed, through detection by mass spectrometry without pretreatment. In addition, by combining DART with time-of-flight mass spectrometry, it is possible to identify or estimate the composition based on the accurate mass and isotope ratio data for the detected components.

Liquid Chromatography
Time-Of-Flight Mass Spectrometer
JMS-T100LP
"AccuTOF™ LC-plus 4G"+ DART™

Easy analysis without sample pretreatment!

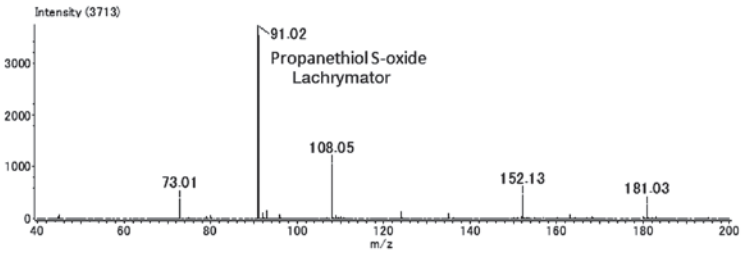
Features

- The sample ionization occurs in open atmosphere, so a wide variety of samples can be instantly analyzed.
- No pretreatment is required.
- The non-contact sample ionization allows direct measurement, even for high-concentration samples.
- The short analysis time delivers high throughput.
- A wide range of materials can be measured, from nonpolar to polar compounds.

Case study

Detection of lachrymators released from onions

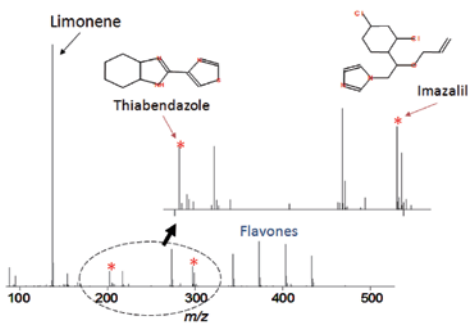
It is widely known that “Slicing onions makes you cry.” This is caused by a tear-inducing substance (lachrymator) that is contained in onions. When the onion is cut, the lachrymator vaporizes, causing irritation when it comes into contact with human eyes. This lachrymator is a relatively unstable substance, however, so it has been difficult to analyze with the conventional detection methods used for mass spectrometry. Using the JMS-T100LP "AccuTOF™ LC-plus 4G", which incorporates DART – an ionization method that does not require any pretreatment, it is possible to easily detect the Propanethiol S-oxide that is the lachrymator by performing DART measurements on a freshly-cut slice of onion.



Case study

Detection of antifungal agents on the surface of an orange

Since DART can measure organic compounds adhered to the surface of a substance without any pretreatment, it can be used to easily detect residual agricultural chemical on the surfaces of fruits, for example.



1-5 Full Automatic Amino Acid Analyzer

The Full Automatic Amino Acid Analyzer JLC-500/V2 is a system dedicated to amino acid analysis using the post-label ninhydrin method.
Amino acids separated by the cation exchange resin are derivatized with the ninhydrin reagent and detected with a visible light detector.
These systems are actively used for a variety of applications, including development and quality control of functional foods, evaluation of flavor components, (glutamate, etc.) calculation of amino acid scores in food, and determination of mixture ratios for livestock feeds.

Full Automatic Amino Acid Analyzer JLC-500/V2

Superior quantification with a dedicated amino acid analyzer supports your product development and quality control!

Features

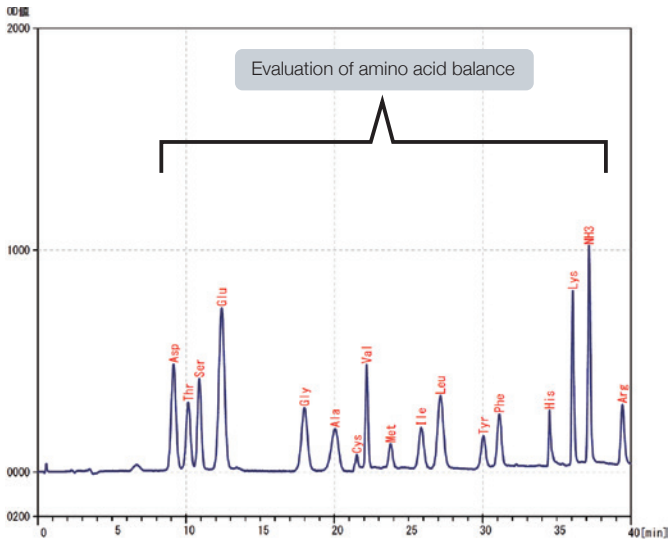
- Capable of simultaneously analyzing more than 41 types of amino acids.
- The construction of a system specialized for amino acid analysis achieves superior quantification, reproducibility and durability.
- All reagents are provided in kits, with no need for troublesome preparation.
- Any user can perform analysis with the dedicated, intuitive-operation software.
- A full-range of user support is provided after installation, from creation of analysis methods to consultations about pre-treatment methods.

Case study Evaluation of amino acid balance in livestock feed

Analysis of amino acid composition in livestock feed and subsequent adjustment of the amino-acid component balance, make it possible for the amino acid analyzer to reduce the total nitrogen content in excrement.
Feeds that satisfy official standard are certified as “Environmental Load Reduction Feeds”.
The Full Automatic Amino Acid Analyzer is effective for the development and quality control of “Environmental Load Reduction Feeds”. It is necessary to perform some preprocessing (Hydrolysis of proteins) for the analysis.

[Pretreatment procedure]

Pig feed
↓
Hydrolysis by 6N-HCl
(110 °C, 24h)
↓
Neutralization
↓
Filtration
↓
Dilution
↓
Analysis



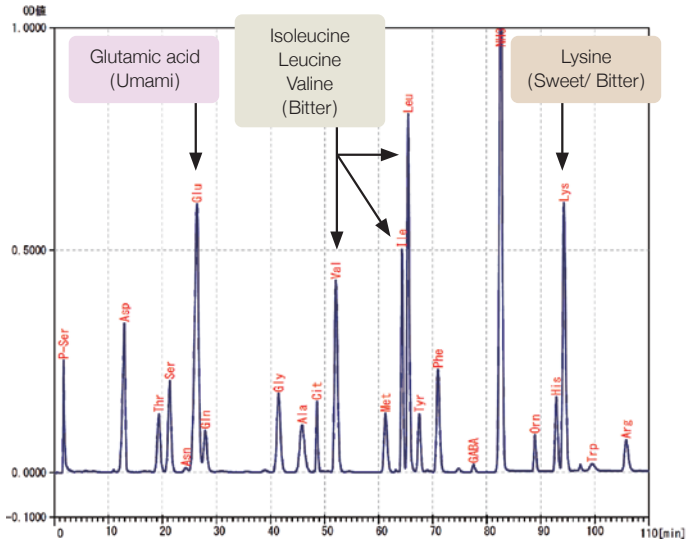
Chromatogram of pig feed

Case study Evaluation of flavor in blue cheese

It is known that the flavors of cheese are characterized by the fermentation products of various amino acids. From the analysis of blue cheese, the amino acids that characterize the flavor are found to include Glu, Val, Leu, and Lys. Evaluation of the relation between the amino acids and the flavor can be applied to desired flavor profile.

[Pretreatment procedure]

Blue cheese
↓
Amino acid extraction
(Homogenization in pure water)
↓
Elimination of solid part
(Centrifuge separation)
↓
Deproteination
↓
Filtration
↓
Analysis



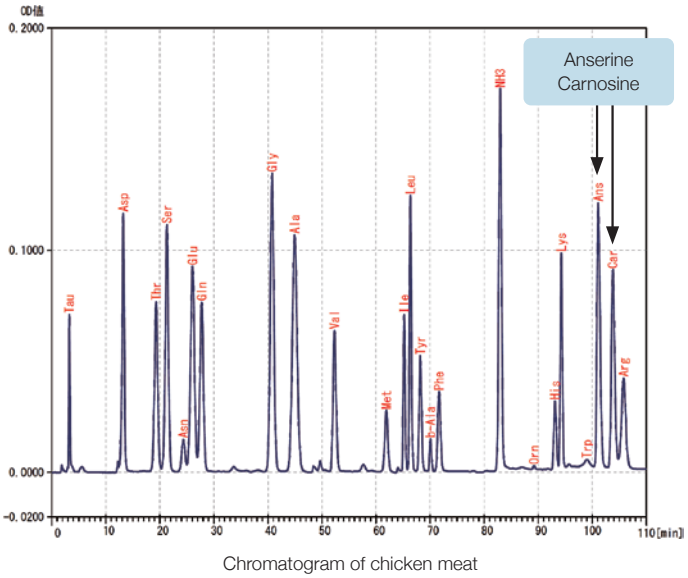
Chromatogram of blue cheese

Case study Evaluation of antioxidants in chicken meat

Chicken meat has rich antioxidants effect compounds such as anserine and carnosine. Evaluating the relation between the breeding condition and amino acid (dipeptides) is useful for developing higher added-value food products.

[Pretreatment procedure]

Chicken meat
↓
Amino acid extraction
(Homogenization in pure water)
↓
Elimination of solid part
(Centrifuge separation)
↓
Deproteination
↓
Filtration
↓
Analysis



Chromatogram of chicken meat

1-6 Nuclear Magnetic Resonance System (NMR)

NMR is an analytical method that focuses on specific nuclei (^{1}H, ^{13}C ··) in molecules, and provides information about the environment around the observed nucleus and/or surrounding structure. Qualitative and/or quantitative results can be determined by measurement of NMR spectra.

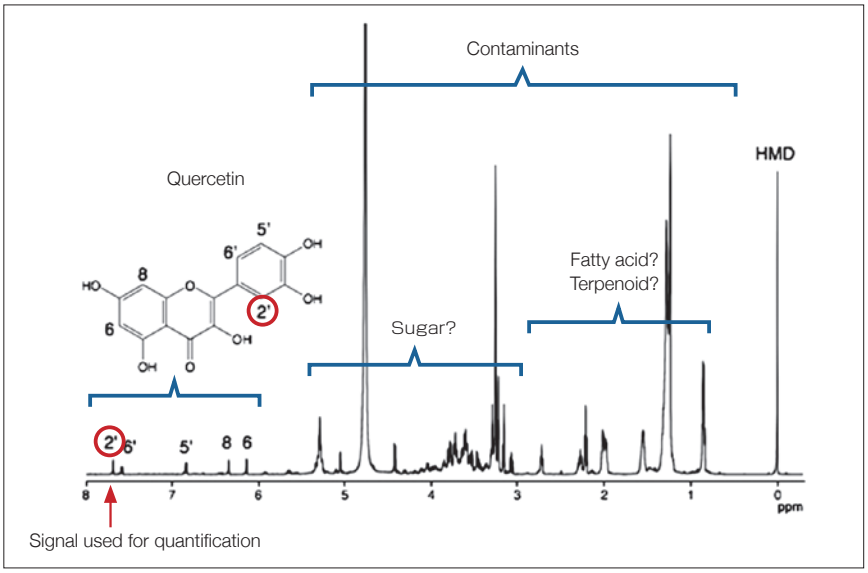
NMR System JNM-ECZ400S

Features

- Samples may be analyzed either in the solid or liquid state.
- No complicated sample preparation required.
- Accurate quantification without the reference material which is same as the analyte.
- No calibration curves needed for quantification.
- Efficient sample screening is possible, even for multi-component mixtures, i.e., foods.
- By combination with multivariate analysis, there is no need for analysis of individual spectra .

Case study Quantification of quercetin in tartary buckwheat

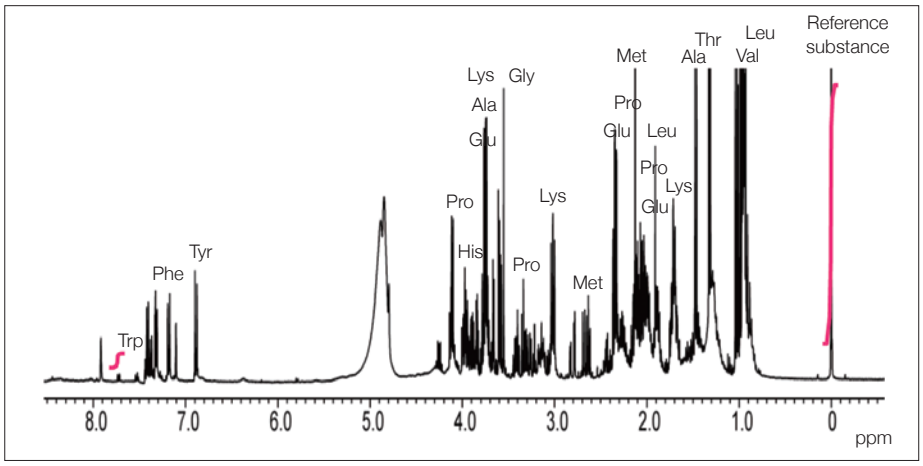
Tartary buckwheat contains a lot of Rutin as a functional flavonoid. Quercetin, a flavonoid, is mainly detected as a degradation product of rutin in samples, as there are rutin degrading enzymes present in Tartary buckwheat noodle. Quantification of quercetin in Tartary buckwheat noodle was carried out using qNMR with HMD (Hexamethyl disilane) as an internal reference standard. The content of quercetin was determined 1.58 ± 0.14 mg per gram of Tartary buckwheat. NMR allows for accurate rapid quantification of analytes derived from natural sources when it is difficult to obtain reference material for quantification.



^{1}H NMR spectrum of methanol extract of tartary buckwheat noodles

Case study Screening of flavor components in blue cheese

NMR resonances corresponding to amino acid residues were observed from a water extract from blue cheese. The molar concentration of tryptophan was determined to be 2.77 mM by comparing the integrals of the tryptophan resonance to that of the internal reference. Sample preparation time is minimal followed immediately by data collection which takes approximately 10 minutes. As a rapid screening procedure NMR is a very valuable technique to analyze water soluble samples.



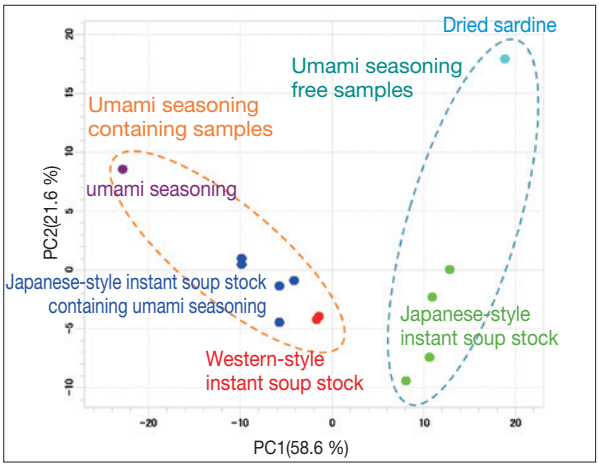
^{1}H-NMR spectrum of aqueous extraction of blue cheese

Case study Classification of broth

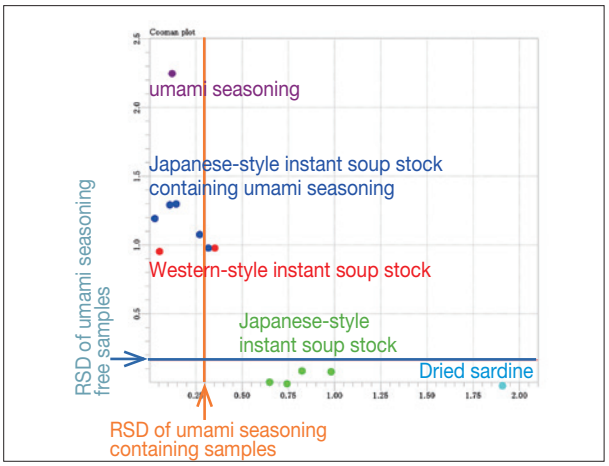
A variety of broths were measured by NMR. All acquired data were batch processed and the chemometric technique of multivariate analysis was performed on the resultant spectra. From the results of principal component analysis (PCA: left figure) it can be shown that the broth samples can be classified into 5 groups. There is also a distinct separation into two larger groups, depending on whether the broth contained umami seasoning or not.

In addition, it is possible to estimate an unknown analytical sample by using soft independent modeling of class analogy (SIMCA: right figure). In the figure on the right, the group that contains the umami seasoning is on the left side of the orange line. The group that has no added umami seasoning is below the blue line. By plotting the data of unknown sample in this model, it is possible to see the distance of the unknown sample from two lines. This makes it very easy to determine whether the unknown sample belongs to either group.

Multivariate analysis in conjunction with batch processing makes NMR a versatile method to classify samples into groups without complicated sample preparation. This method is highly suitable for effective primary screening of multicomponent samples, like foods, providing a powerful tool for quality control.



Grouping by principal component analysis of broth ^{1}H-NMR data



Classification of broth ^{1}H-NMR data using soft independent modeling of class analogy

1-7 Electron Spin Resonance System (ESR)

The ESR system selectively detects radicals within a sample. The radicals are highly reactive due to instability that is the result of molecules having only 1 electron in a bond that should normally have 2 electrons.
Therefore, the generation of radicals in food leads to deterioration of the quality of food. ESR can measure such radicals and also enables you to evaluate the antioxidant properties of food.
The ESR system consists of a microwave oscillator, a pair of electromagnets, and a spectrometer. Samples are placed into a dedicated cell and set into a holder section between the electromagnets. A spectrum acquired by sweeping a magnetic field while irradiating the sample with a fixed-frequency microwave.

ESR system JES-X320

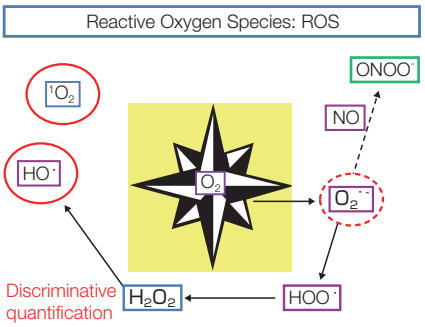
Simple pretreatment for selective radical detection!

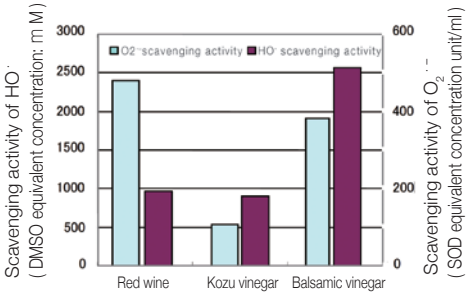
Features

- Selective detection of only the radicals within the specimen.
- High sensitivity.
- Identification of radicals using the spectrum patterns and g value.
- Non-destructive analysis.
- Suitable for any sample state.

Case study Evaluation of antioxidant activity of foods (Reactive oxygen scavenging)

The superoxide ($O_2^{\cdot -}$), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$) shown in the left portion of the figure are generated within living tissue and are thought to be a cause of many diseases. These kinds of reactive oxygen species can be measured with ESR, making it possible to evaluate antioxidant activity separately for each of the reactive oxygen species.





Difference in antioxidant activity for $HO^{\cdot}$ and $O_2^{\cdot -}$ depending on the sample type
Even for the same types of food, the antioxidant activity differs depending on raw materials and manufacturing methods.

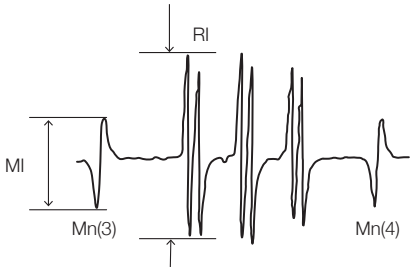
Case study Beer flavor test

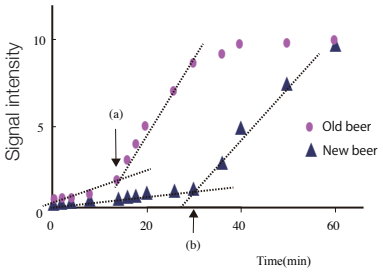
The fresh taste of beer is thought to be lost over time due to oxidation by reactive oxygen generated in the beer itself. During accelerated deterioration tests at 60°C, the fresh beer, containing a large amount of antioxidants, suppresses the generation of the radicals for a long time, while the radicals arise quickly in the old beer.
This trend correlates well to the sensory taste testing, indicating that ESR can be used to forecast the shelf life by evaluating the radical suppression period of the beer.

$$\text{Signal intensity} = \frac{\text{RI}}{\text{MI}}$$

RI: Radical intensity
MI: Mn marker intensity

Radical intensity is normalized with intensity of the Mn marker associated with the instrument





1-8 X-Ray Fluorescence Spectrometer (XRF)

The XRF is an instrument capable of identifying and measuring the types and the concentrations of the elements contained in a sample by measuring fluorescent X-rays emitted from the sample when it is irradiated with X-rays. With Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES), the sample preparation such as liquid solution is necessary. X-ray Fluorescence analysis allows samples to be measured non-destructively without a complicated preparation, so this method has the advantage of being able to handle solid, powder or liquid samples.
In the field of food products, it is possible to measure the mineral components in the various nutritional ingredients that are included in a raw material or a product. XRF is an ideal tool for screening from high concentration of minerals (Ca, P, Mg, K, S, Na, Cl) to trace minerals (Mn, Cu, Zn, Se, Mo, Co...)
In addition, it is useful for analyzing contamination during the manufacturing process as well as for quality control of packaging materials.

Energy Dispersive X-ray Fluorescence Spectrometer
JSX-1000S (Element Eye™)

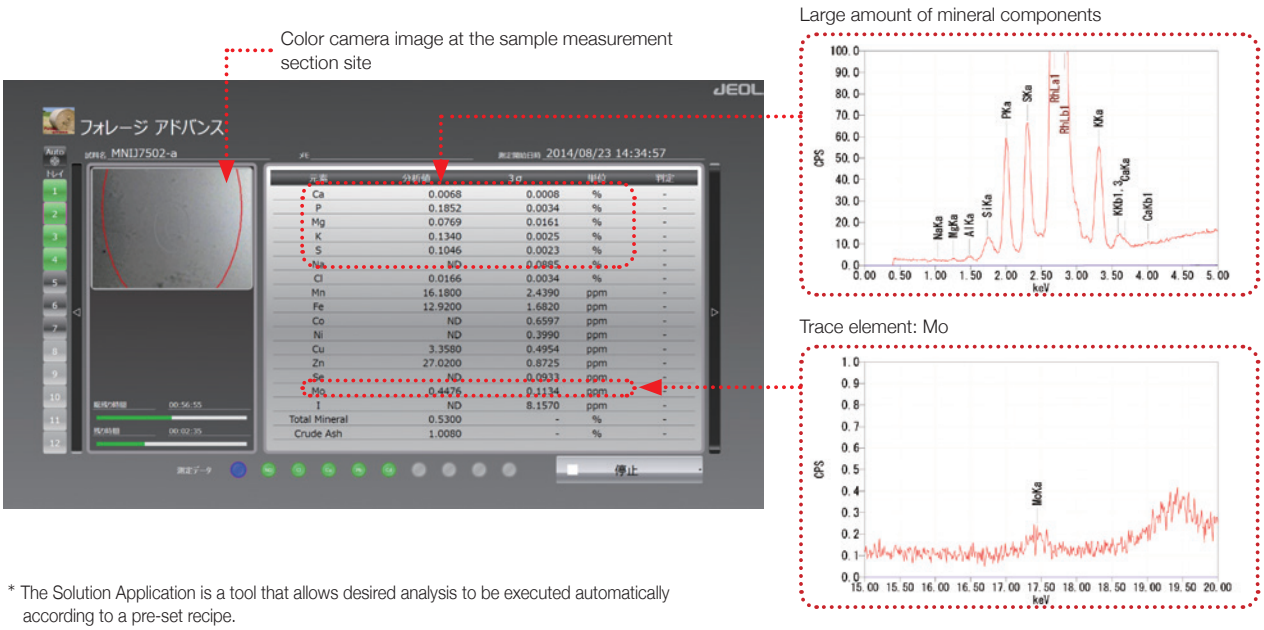
Ideal for quick analysis of both a large amount of minerals and trace minerals!

Features

- Simultaneous analysis of elements from Na to U.
- Analysis for solid, powder and liquid samples.
- Non-destructive analysis.
  (Measurement with sample state preserved, any pretreatments not needed)
- Wide dynamic range. (Quantification from sub-ppm to 100%)
- Standard-less quantification.
  (Preparation of standard samples or creation of calibration curves not needed)
- Rapid analysis.
  (Only a few minutes from sample preparation to acquisition of results)

Case study Mineral component analysis of white rice

This is a measurement example of the mineral components in white rice using the forage solution application developed for screening the mineral components in grass used for grazing purposes. It was possible to perform measurement of 7 minerals in large quantities as well as 9 trace minerals contained in the white rice by just a single click. The results are displayed in just a few minutes. It is also possible to estimate the ash of the non-mineral component.
The ability to determine quickly the minerals of various foodstuffs is very useful for the evaluation of food prototypes and for designing the mineral component composition in food product development.



* The Solution Application is a tool that allows desired analysis to be executed automatically according to a pre-set recipe.

2-1 Transmission Electron Microscope (TEM)

Replica method (TEM)

The freeze-fracture method and the freeze-etching method are both replica methods. For the former method, the specimen is frozen (physically fixed), then split (fractured), and a replica film is made on a fractured surface. The specimen is then dissolved to extract only the replica film for TEM observation. In the latter method, the frozen (physically-fixed) specimen is freeze-fractured and the ice is sublimated; then, the same procedure is used to fabricate the replica film. The specimen is then dissolved to extract only the replica film for TEM observation.

Main specifications (High-contrast spec.)
Resolution: 0.2 nm (lattice image)
Acceleration voltage: 40 to 120 kV
Magnification
MAG mode: X 200 to 1,200,000
LOW MAG mode: X 10 to 1,000

Fig. 1 JEM-1400Plus appearance and specifications

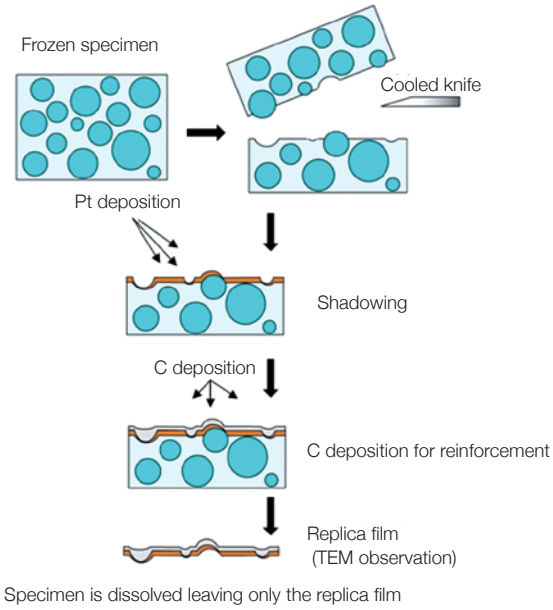


Fig. 2 Freeze-fracture method procedure

After performing SEM observation of a wide field of view, TEM observation is useful for acquiring information about more detailed portions. The replica method is one of specimen preparation techniques for TEM. The high-contrast obtained with the JEM-1400Plus is ideal for TEM observations of this kind of soft material.

Observation example Processed cheese

Since the film replicates the irregularities of the fractured surface, it is possible to observe three-dimensional structures as on an SEM image. This resolution is higher than that of an SEM image, making it possible to observe even finer structures, such as the casein protein. In addition, since the specimen created using the replica film method can be stored, it can be observed repeatedly.

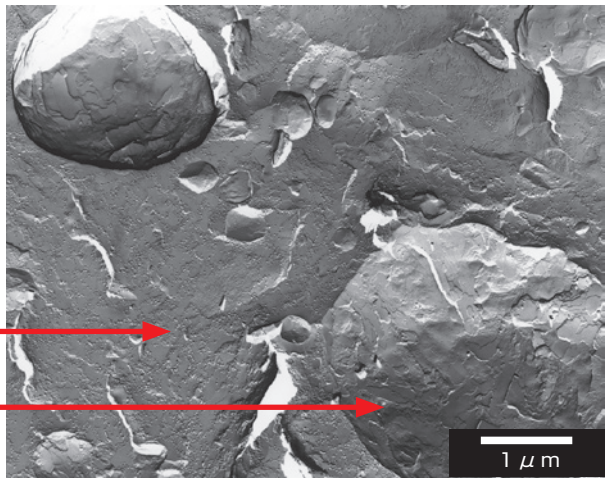


Fig. 3 TEM image of a replica film of processed cheese (Black/white inverted)

Observation example Ice cream

The ice and fine structures of the fat globules on the surface can be observed.

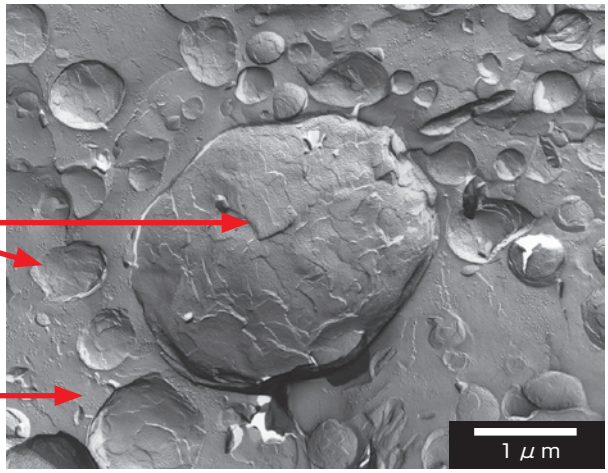


Fig. 4 TEM image of a replica film of ice cream (Black/white inverted)

Observation example Coffee creamer

Observation of fat globules in a specimen of rapidly frozen coffee creamer.
Since the freezing is performed rapidly, the distribution of the fat globules in the creamer is preserved. It is also possible to observe the particle size distribution, surface and cross-sectional structures in greater detail.

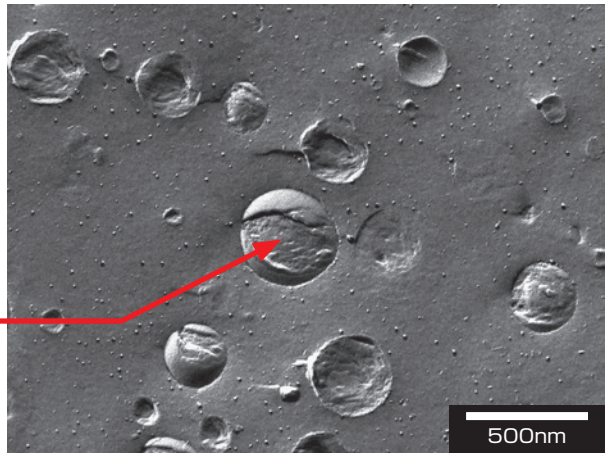


Fig. 5 TEM image of a replica film of coffee creamer (Black/white inverted)

2-2 Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) is a surface-observation instrument that scans a focused electron beam (probe) over the specimen surface. Then, the SEM detects secondary or backscattered electrons emitted from the surface, amplifies the output signals from the detector for transferring them to the display unit, and subsequently displays these signals on a monitor screen on the display unit in synchronism with the scan of the electron probe. In addition, attaching an energy-dispersive X-ray spectrometer (EDS) to the SEM enables you to identify and pinpoint constituent elements in any location from the area of interest in an acquired image.
In the food product fields, the SEM is suitable for a wide range of applications, including observation of surface structures of packaging materials, analysis of the relationships between food morphology and texture, and element analysis of any foreign particles mixed into food products or packaging.

Scanning Electron Microscope JSM-IT500

Constituent elements are located from an area with morphology imaged!

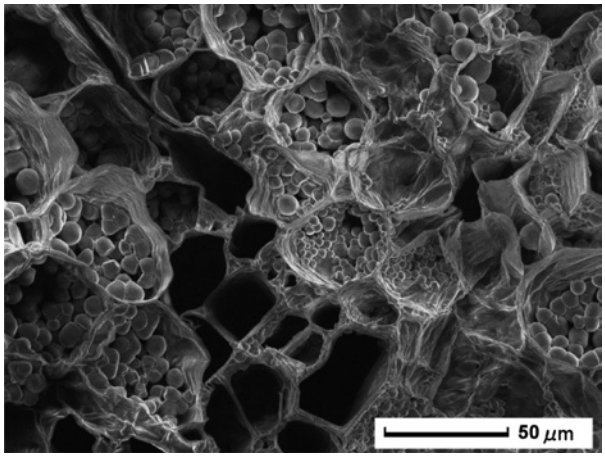
Features

- Surface structures are imaged at magnifications up to several hundreds of thousands (up to several millions depending on the SEM type).
- Observation of differences in chemical composition.
- Element analysis of the entire observation field.
- Element analysis of a specific point on the image.
- Element distribution mapping.

Observation example

Cross section of sweet potato

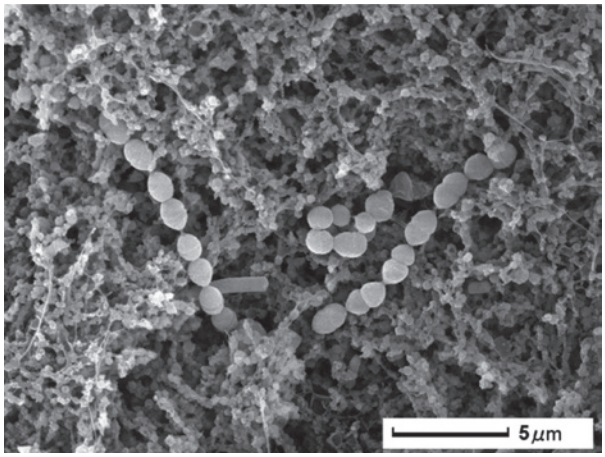
The image to the right shows a cross section of a sweet potato.
Topographic structures of multiple solidified starch particles can be observed.



Specimen: Cross section of sweet potato
Accelerating voltage: 5 kV
Magnification: ×500

Yogurt

The image to the right shows the surface structure of yogurt. After finishing chemical fixation of a specimen of yogurt, performing dehydration, drying and coating of the specimen enables you to observe both the morphology of yogurt and the structure of lactic acid bacteria.

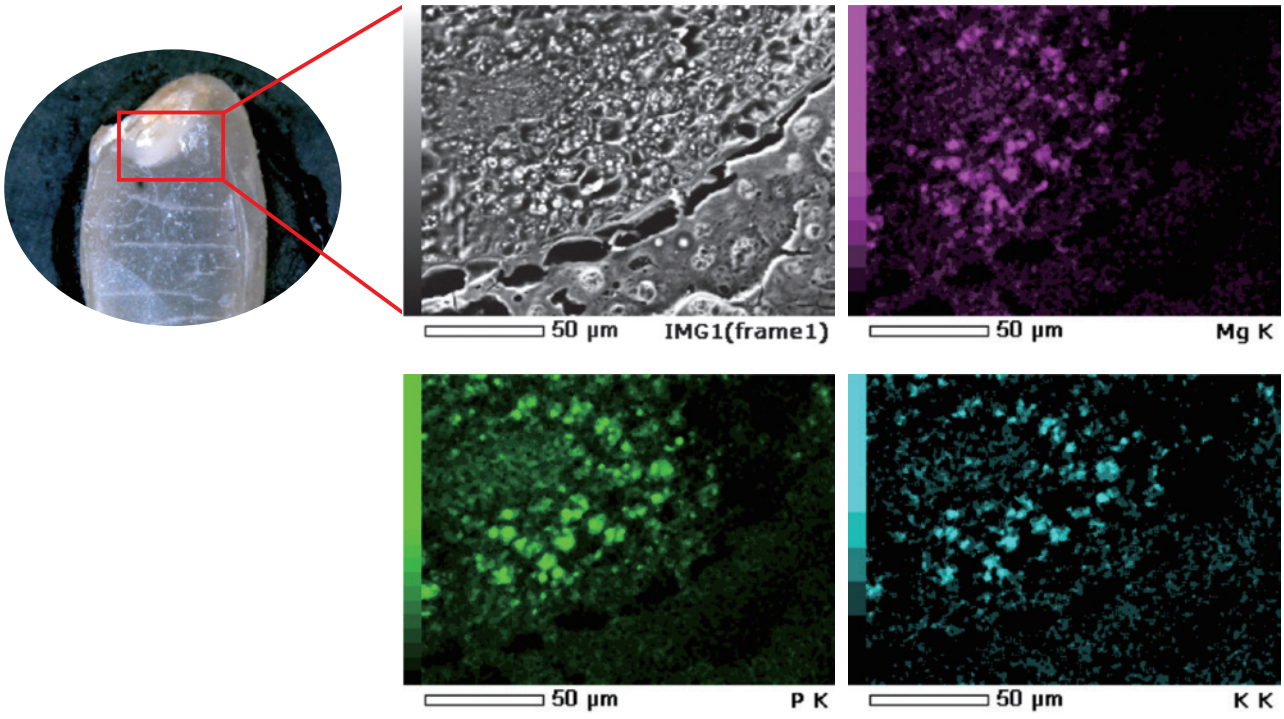


Specimen: Yogurt
Accelerating voltage: 15 kV
Magnification: ×5000

Analysis of distribution of main constituent elements in brown rice

Images show element maps of a cross section of a brown rice. When a backscattered electron image (compositional image) is taken from an area of a brown rice grain, whiter particles (indicating larger average atomic number) are easily viewed. Analyzing these particles by EDS reveals that the brown rice contains mineral components such as Mg, P, and K.

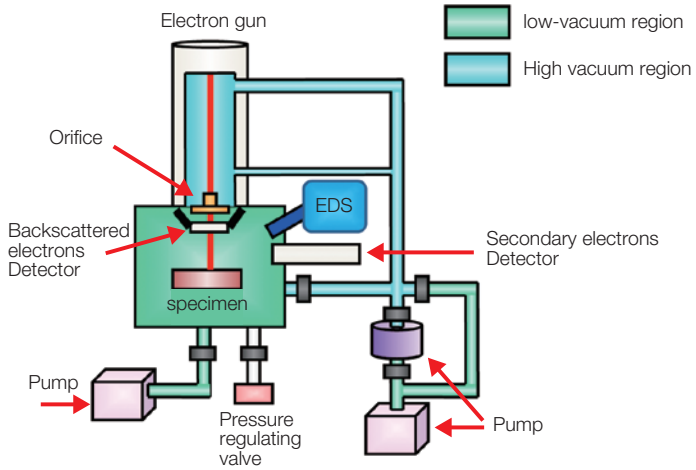
Furthermore, the mapping function of EDS enables you to visually confirm the areas where many relevant elements are contained.
This function is effectively used to check where harmful substances accumulate in foods.
Topographic structures of multiple solidified starch particles can be observed.



Specimen: Brown rice
Accelerating voltage: 15 kV
Magnification: ×700

Low-Vacuum Mode

The inside of the SEM microscope column needs to be maintained at high vacuum. For specimens that contain water or oil (in foods), it is difficult to preserve its morphology. Thus, such specimens are generally subject to pretreatment, including fixation, dehydration, drying and coating. But there have been increasing demands for observing these specimens in their native states without the pretreatment. The low-vacuum (LV) SEM is designed to observe those specimens with no pretreatment by raising the pressure inside the specimen chamber. The LV SEM enables you to observe non-conductive specimens while dramatically reducing the charging.
The LV SEM can increase the pressure by isolating the vacuum system of the specimen chamber from the other sections of the SEM.



Low-vacuum SEM schematic

Specimen observation in native state without pretreatment!

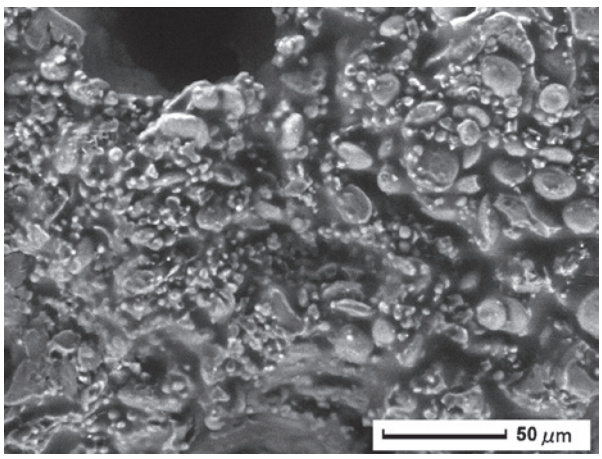
Features

- Non-conductive specimens are imaged and subject to element analysis without coating.
- Observation and element analysis of specimens containing water or oil.
- Observation and element analysis of out-gassing specimens.
- Dynamic observation of non-conductive specimens by heating or stretching them.
- Observation of special specimens that cannot be subject to artificial treatment, including cultural assets and commercial products to be inspected.

Observation example

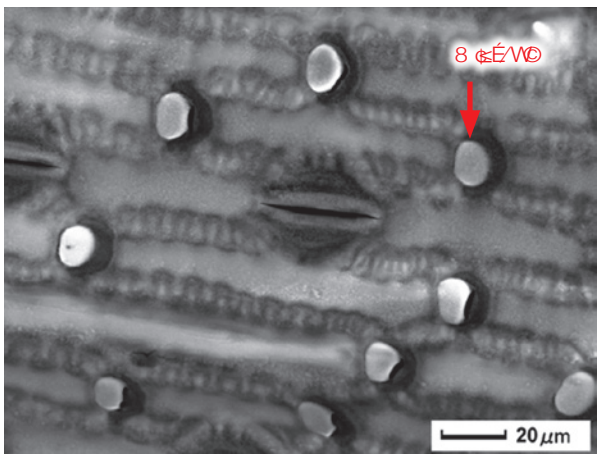
Observation of food products using the LV SEM

This image shows a chocolate coolie in its native state (with no pretreatment). The structures of starch particles and lipids are revealed. A black area at the upper-left in the image shows a void.



Specimen: Chocolate coolie
Accelerating voltage: 15 kV
Magnification: ×500

This image shows dried timothy, an ingredient in pet food. The structures of stomas and distribution of minerals are visible.



Specimen: Timothy
Accelerating voltage: 15 kV
Magnification: ×700

Observation techniques for water-containing specimens

An electron microscope is an effective tool to visualize fine structures that affect textures of food products. Various techniques that capture native morphologies of food products containing oil, water and bubbles are available (Fig. 1). Each technique is selected depending on observation requirements or analytical purpose.

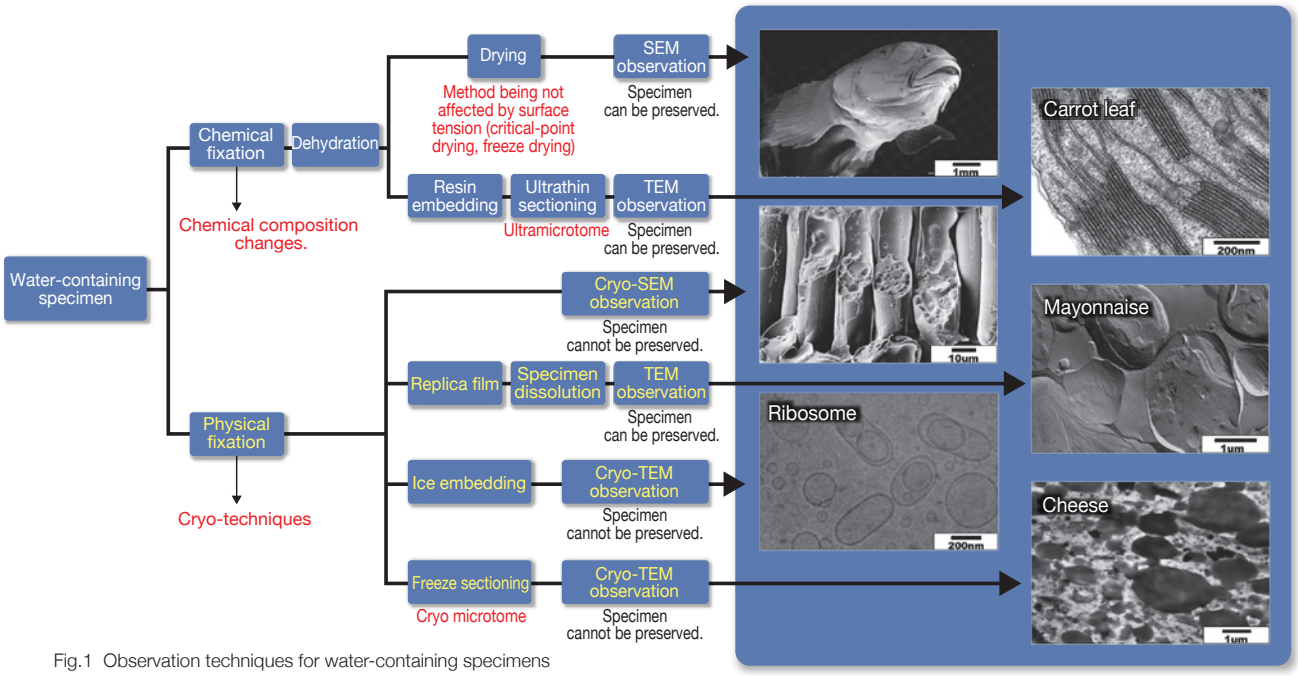


Fig.1 Observation techniques for water-containing specimens

Observation and analysis of cheese (process cheese)

Comparison of chemical fixation and physical fixation (freezing) using process cheese is presented below.

Chemical fixation

Analysis examples of cross sections prepared by chemical fixation are shown below (Fig. 2). When chemical fixation with glutaraldehyde (GA) is applied, fat globules flow and they are observed as voids. Applying double fixation of GA and osmium tetroxide (OsO_4) to a cheese fixes the flat globules, enabling you to observe ball-like structures. This result clarifies that a change in chemical composition arises due to resin substitution.

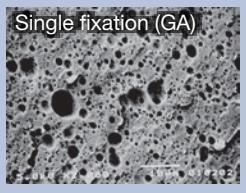
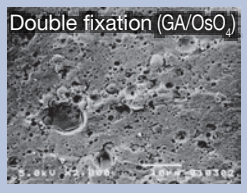
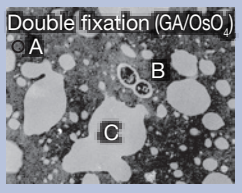


Fig. 2-1 SEM observation of cheese using chemical fixation.
A cheese to which chemical fixation was applied was subject to critical-point drying for SEM observation.





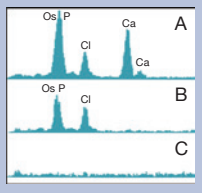


Fig. 2-2 TEM image of ultrathin section of cheese using chemical fixation and the result of EDS analysis for the sectioned cheese.
A cheese to which double fixation was applied was subject to epon embedding and ultrathin sectioning. Then, the prepared section was subject to TEM observation and EDS analysis. Osmium used for double fixation and chlorine in a resin-embedded region were detected.

Physical fixation (freezing)

Analysis examples of cross sections prepared by Cryo-techniques are shown below (Fig. 3). Fine structures of fat globules and casein proteins are clearly visualized, enabling you to observe the specimen structure in its native state and also to perform EDS analysis for this specimen.

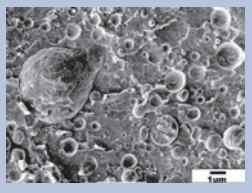


Fig. 3-1 Cryo-SEM image
Fat globules are clearly observed.

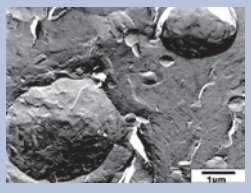
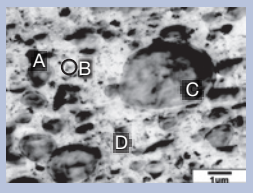


Fig. 3-2 TEM image of freeze-fractured replica.
Distribution of casein proteins is observed.



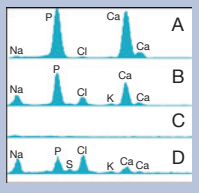


Fig. 3-3 Cryo-TEM image of frozen section and the result of EDS analysis for the section.
Since the elements originating only from the specimen are detected, accurate EDS analysis is enabled.

Cryo-SEM (Observation of water-containing specimens)

Construction of Cryo-SEM

Cryo-techniques are required to perform SEM or TEM observation of a frozen, water-containing specimen. For SEM observation of such a specimen, the Cryo-SEM is effective. Figure 1-1 shows the schematic diagram of the Cryo-SEM. The Cryo-SEM consists of the cryo-preparation chamber to perform freeze fracturing and coating for a cooled specimen and the cooling stage for SEM observation. Liquid nitrogen is used to cool both the specimen treatment stage for the cryo-preparation chamber and the cooling stage for SEM observation. Figure 1-2 is an example of this chamber (Gatan ALTO2500). One unit of this chamber is a cryo knife to fracture a rapidly-frozen specimen in the air, under vacuum. In addition, the chamber is fitted with a sputtering device to coat the specimen with Pt or other metals. The temperature of the specimen stage is controllable, enabling fracturing, etching and coating at an appropriate specimen temperature.

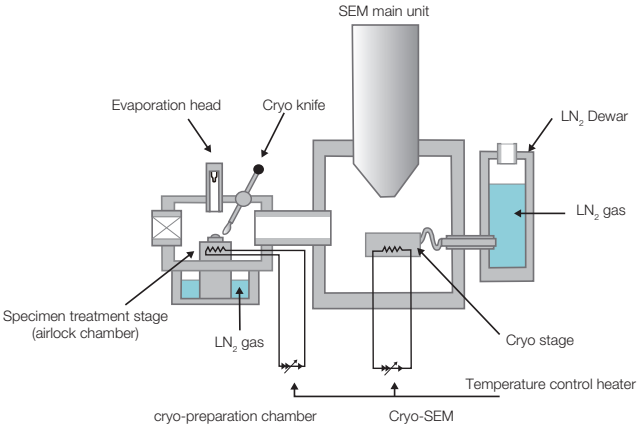


Fig. 1-1 Cryo-SEM basic diagram

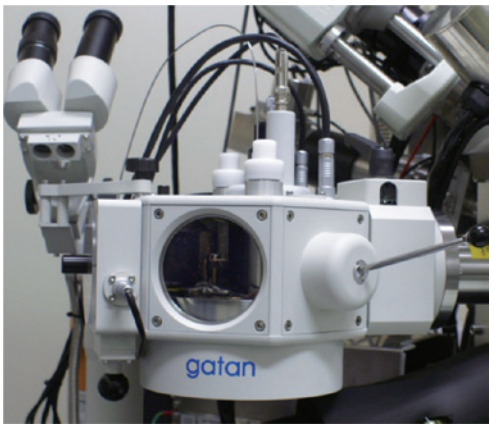


Fig. 1-2 Cryo-preparation chamber (ALTO2500)

Observation example

Basic capability (etching)

The temperature of the specimen stage for the Cryo-SEM is controllable, thus enabling ice on a freeze-fractured specimen to be sublimated. This capability gives information on water distribution in the specimen.

Comparison images of freeze-fractured O/W emulsion (whipped cream) before and after etching are shown in Figures 2-1 and 2-2. Figure 2-2 reveals that after etching is applied to the specimen, ice sublimates and only oily parts remain.

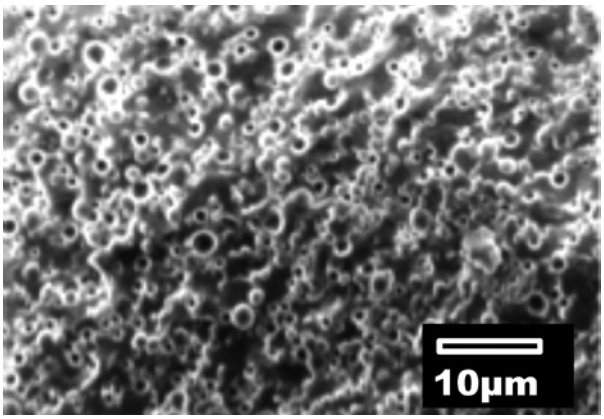


Fig. 2-1 O/W emulsion (Before etching)

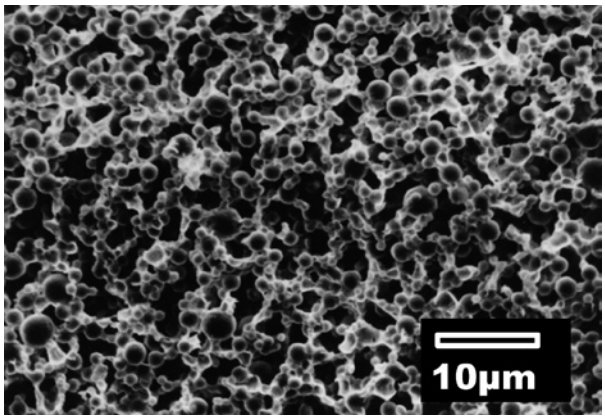


Fig. 2-2 O/W emulsion (After etching)

Application to processed cheese

Figure 2 shows a Cryo-SEM image of a freeze-fractured processed cheese. The grain size distribution of constituent materials (fat globules, etc.) and surface structures are observed. In addition, combined use with the low-vacuum mode allows element analysis without conductive coating. Cryo-SEM based evaluation plays a suitable role for providing information that integrates surface structures with textures.

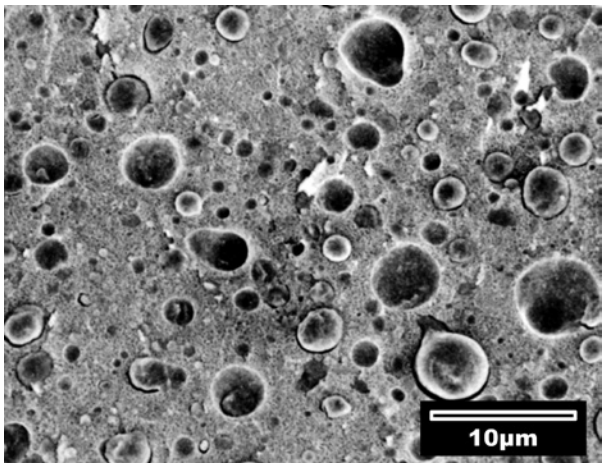


Fig. 2 Cryo-SEM image of freeze-fractured surface of cheese (Pt evaporated).

Application to mayonnaise

Figure 3 shows a Cryo-SEM image of a freeze-fractured mayonnaise specimen. The distribution of ball-shape oils and surface/cross-sectional structures are observed. Thus, Cryo-SEM is suited to observe fine structures of various emulsions.

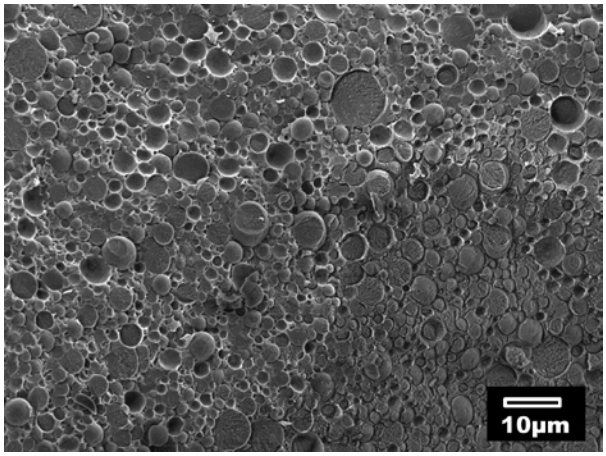


Fig. 3 Cryo-SEM image of freeze-fractured surface of mayonnaise (Pt evaporated).

Application to whipped cream

Figure 4 shows a Cryo-SEM image of a freeze-fractured whipped cream specimen. This image reveals that fat globules are uniformly distributed before whipping, but after whipping, surface structures drastically change because gas bubbles are formed in the cream.

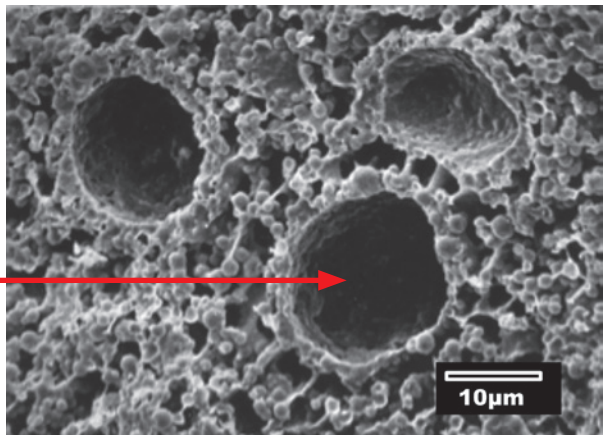


Fig.4 Cryo-SEM image of freeze-fractured surface of whipped cream (Pt evaporated).

Application to grape skin

Figure 5 shows a Cryo-SEM image of a freeze-fractured grape-skin specimen. Textures in grape cells are clearly observed.
The use of Cryo-SEM enables you to observe surface structures on plant textures and analyze constituent elements.

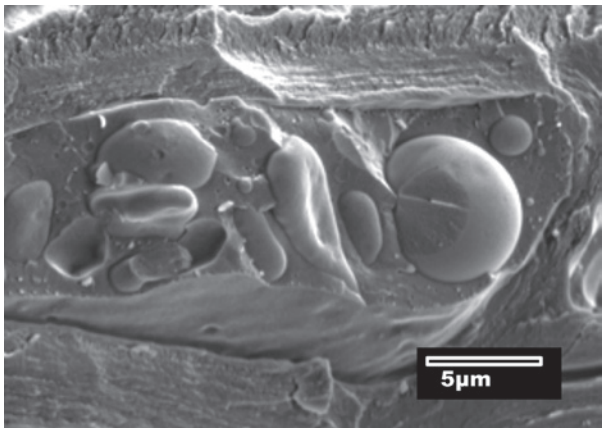


Fig. 5 Cryo-SEM image of freeze-fractured surface of grape skin (Pt evaporated).

2-3 Micro Raman Spectrometer "Raman Microscope"

A micro Raman spectrometer "Raman Microscope" is an instrument for identifying compounds or analyzing molecular and crystal structures by irradiating a sample with a focused laser beam and measuring a spectrum of inelastically scattered "Raman scattered light". The Raman Microscope enables non-destructive, non-contact analysis, therefore pretreatment of the sample is not required. This is a big advantage of this instrument.
In the field of food products, the Raman Microscope is used for a variety of applications, such as analysis of chemical components in raw materials, identification of trace-amount intermingled foreign substances, component distribution analysis of processed food products at the micro meter level, and structure analysis and functional evaluation of packaging materials.

inVia Reflex Raman Microscope by Renishaw

Good at chemical imaging using high-speed mapping function!

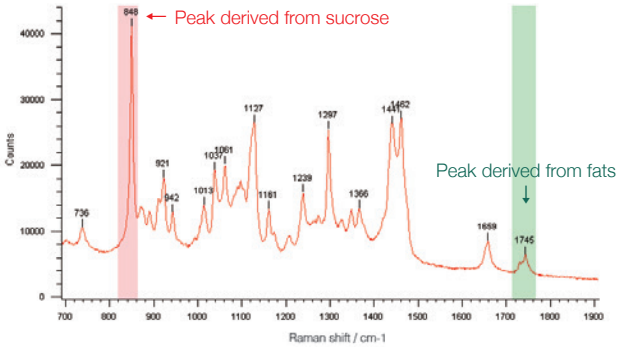
Features

- Non-destructive, non-contact analysis.
- Analysis under atmospheric pressure. (no need for vacuum or atmosphere control)
- Direct acquisition of information about the chemical bonds.
- Capable of spatial resolution up to a sub-μm level.
- Supports chemical imaging to visualize the distribution of each compound or group of atoms.
- Non-destructive depth analysis of transparent specimens.
- Supports optional measurements with specimen heating / cooling.

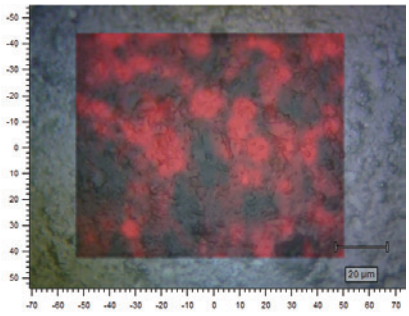
Case study

Principal component-distribution analysis of white chocolate

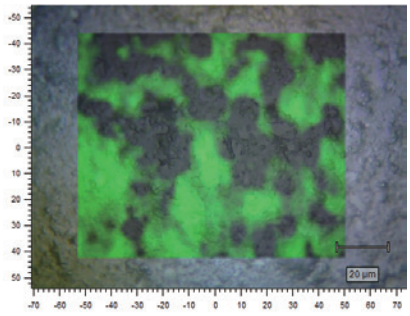
This is an example of mapping performed for the surface of white chocolate in a native state, without pretreatment. By constructing images based on various Raman signal intensities for the sucrose and fats from all the observed spectra, the distribution of both components can be visualized. From this kind of component distribution image, it is possible to see the mixing state (emulsified state) of materials that influence the flavor and texture. This obtained result provides information useful for understanding state change caused by the storage environment, as well as for product development and the optimization of production processes.



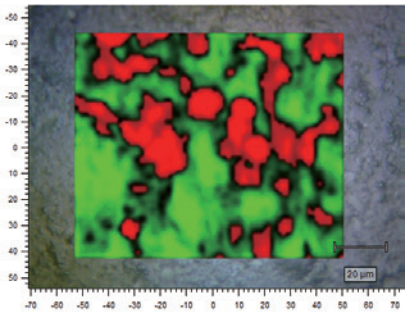
Average Raman spectrum of white chocolate (785 nm laser excitation)



Sucrose distribution image



Fat distribution image



Composition image of both distributions

3-1 Comprehensive Evaluation of Foods using Scientific Instruments
- Analysis of Broth : Using XRF, Full Automatic Amino Acid Analyzer, NMR, GC-MS -

1. Introduction

Broth “Dashi” is a seasoning that contains the umami ingredients. It is a liquid food preparation that is used in cooking and is made by extracting the umami from ingredients like meats, vegetables, mushroom, seaweeds, etc. (From Wikipedia) In recent years, powdered broth granules have become common due to the ease of use.
We have used several kinds of commercially available broth granules as the test samples. The comprehensive evaluation was made using various scientific instruments, including mineral and amino acid content analysis and identification of odorous components.




Fluorescent X-ray : Mineral analysis
Full Automatic Amino Acid Analyzer : Identification / Quantification of amino acids
Nuclear Magnetic Resonance system: Screening of components in samples
GC-MS : Analysis of volatile components

The evaluation results from using the various instruments are shown below.

Table 1 Tested powdered broth samples

	Main ingredient	Additives		
		Chemical seasoning	Table salt	Sugar
Broth 1	Dried kelp	○	○	○
Broth 2	Anchovy	○	○	○
Broth 3	Dried kelp	×	×	○
Broth 4	Shitake mushroom, Kelp, Bonito	×	×	○
Broth 5	Bonito	○	○	○
Broth 6	Bonito	○	○	○
Broth 7	Anchovy	×	×	×
Chemical seasoning	—	—	—	—

○ · · · Added
× · · · Not added

2. Component Analysis: Mineral analysis using XRF

The X-Ray Fluorescence Spectrometer (XRF) is an instrument for investigating the types and the concentrations of the elements contained in the samples using fluorescent X-rays. There is no sample preparation required, and the FP (Fundamental Parameter) method enables quick and simple composition analysis without preparing standard samples, which makes easy quantification possible.
Figure 1 shows the spectra of a chemical seasoning and a bonito broth. The quantification results of the main elements are shown in Table 2. It is clear that the bonito broth contains a greater variety of minerals than the chemical seasoning. Since a concentration of a little less than 3% was detected for chlorine, it is estimated that some sodium chloride is contained.
Figure 2 is radar charts made based on the quantification results which were detected in the 7 broth granule samples in Table 1. The potassium content is high in samples 1, 3, and 4, which use kelp as a raw material. The bonito and anchovy-based samples, 2, 5, and 6 show high calcium levels. The samples with no added salt, 3, 4, and 7, can be seen to have lower amounts of chlorine.
The chart of sample 7 indicates that it contains a good balance of a variety of minerals.

Energy Dispersive X-Ray Fluorescence Spectrometer
JSX-1000S (Element Eye™)

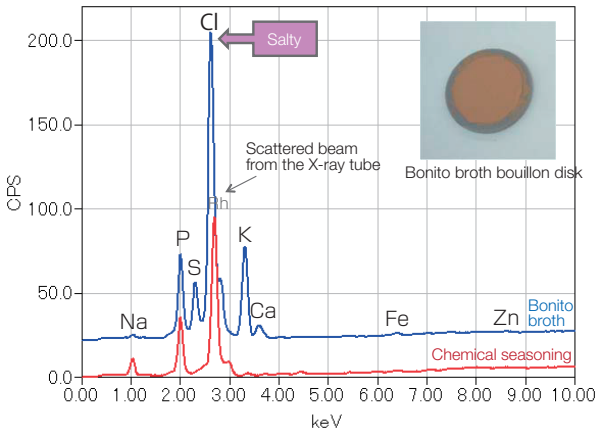


Fig. 1 XRF spectrum of chemical seasoning and bonito broth

Component	Chemical seasoning	Bonito broth
Na	12.10	2.30
P	0.75	0.92
S		0.34
Cl		2.70
K		1.20
Ca		0.04
Fe		0.005
Zn		0.001
(Organic substance)	87.80	92.50

Unit : %

Table 2 Element composition analysis results

* Light elements, C, H, O, N derived from organic materials, which are not detected with XRF, are specified as the balance (residual) components, and quantified by the FP method.

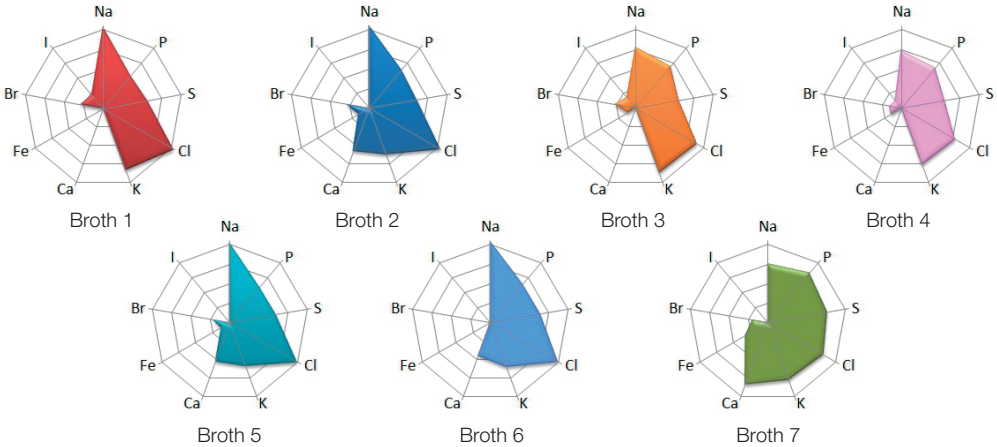


Fig. 2 XRF analysis of various powdered broths

3. Component Analysis: Amino acid analysis

Figure 3 shows the amino acid content in the broth granules. The diagram shows the content of Glutamic acid and the other amino acids. In the broths of chemical seasoning added, the most of the amino acid content is Glutamic acid, it can be inferred that the great deal of Glutamic acid is added. The other hand, the broth with no chemical seasoning shows lower Glutamic acid content.
Figure 4 shows the amount of amino acids in the broth other than Glutamic acid. In the broths that do not have chemical seasoning additives, the characteristic profiles of the main ingredient are shown, such as the Asparagine contained in the kelp, and the Histidine and Taurine contained in the anchovy.
In the broth with added chemical seasoning, most did not contain much of any amino acids other than Glutamic acid, although broth sample 5 was confirmed to contain a lot of sweet-tasting Alanine. Since only the Alanine content is high, it seems likely that it was added to increase the sweetness. This kind of amino-acid analysis enables you to understand the characteristics of a material. This understanding can be applied to help design flavor.

Full Automatic Amino Acid Analyzer (JLC-500/V2)

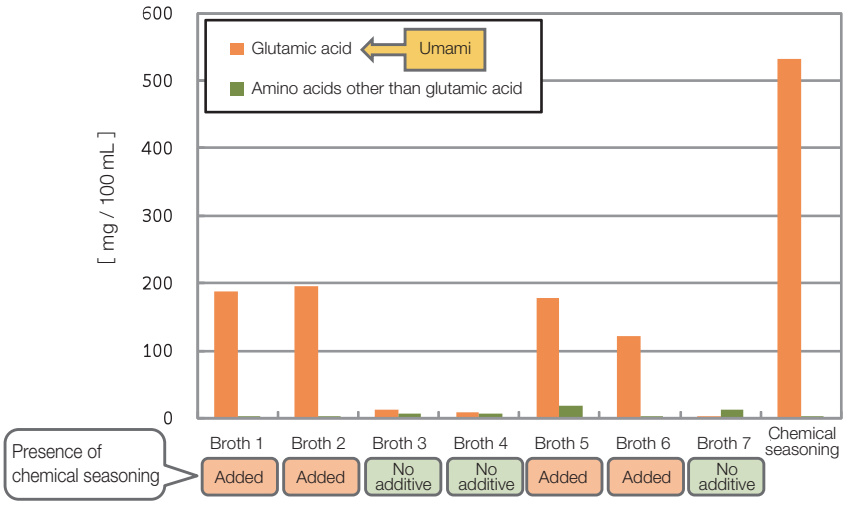


Fig. 3 Amino acid content in various broth granules (glutamic acid)

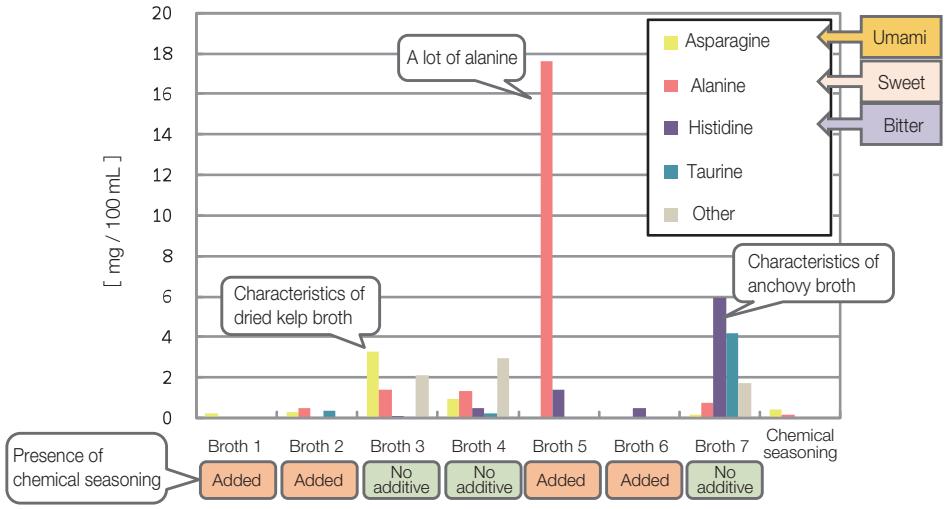


Fig. 4 Amino acid content in various broth granules (other than glutamic acid)

4. Component Analysis: NMR

NMR is used for qualitative and quantitative analysis because of its ability to observe nuclei in molecules directly. Since the analysis can be performed non-destructively, with the analyte in an original state, without any preparation like component separation, it can be used for comprehensive analysis as well as so-called screening assay. Figure 5 shows the results for a bonito broth. The only sample preparation was making an aqueous solution.
From the spectrum pattern, it is possible to confirm the presence of amino acids, sugars, inosinic acid and guanylic acid all at once. Since the signal intensity on the NMR spectrum is proportional to the number of moles, by using a comparison of the integrated values it was found that the ratio of alanine and glutamic acid was 1:6.
The measurement time for an acquisition of NMR spectra is a few minutes for an automated measurement. Therefore, it is possible to perform a screening assay in analytical sample in a short amount of time.

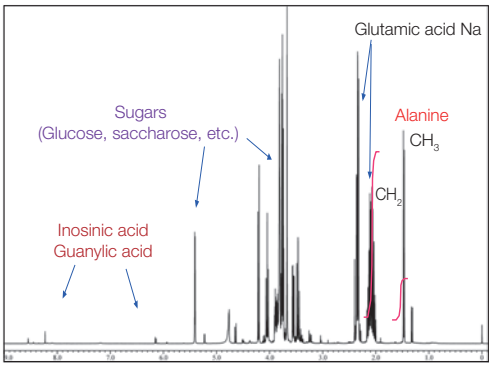
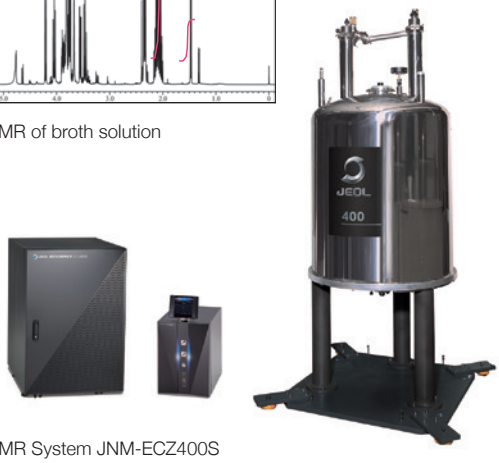


Fig. 5 ^{1}H-NMR of broth solution



NMR System JNM-ECZ400S

5. Components Analysis: Odor analysis using GC-MS System

Total Ion Current Chromatograms (TICC) of 3 types Broths are shown in Figure 6.
The upper chromatogram is for Broth 6, the middle is the TICC for Broth 4, and the bottom is for Broth 7.
As seen in Figure 6, Broth 7, which has only dried sardine as the raw material, was found to contain more odor components than the other samples. Specifically, compared to the other broths, Broth 7 contained much more Cresol which is well known as fumigation odor.
The bonito flavored Broth 6 contained a high level of Guaiacol. These results showed clearly that each of the broths has distinct odor characteristics. For the products containing added sugar, the results of the Maltol content ratios confirmed that there was almost no difference.

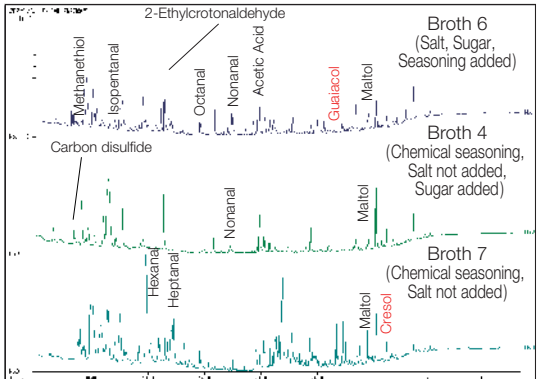


Fig. 6 Aroma analysis results of seasonings

HS-GC-MS System (JMS-Q1500GC)

6. Summary

Recently there has been a variety of research on food tastes and flavors, with a focus not only on the sensory aspects, but also on numerical quantification using scientific instruments. Here, we used commercially-available broth granules as the test samples, and evaluated the nutritional components, flavor and taste from a chemistry perspective to make qualitative and quantitative evaluation using multiple scientific instruments. This is equivalent to the evaluation of the primary and secondary functions of the foods.

3-2 Comprehensive Evaluation of Foods using Scientific Instruments
- Analysis of Cheese: Using SEM, Full Automatic Amino Acid Analyzer, GC-MS and NMR -

1. Introduction

Cheese is produced by fermenting the milk of a cow or sheep using a microorganism, such as a mold. These are dairy products with unique flavors and tastes.
Among the various types, the blue cheeses are well known for extremely distinctive flavors and smells.
In this example, 3 representative types of blue cheese, Gorgonzola, Stilton, and Roquefort, were used as the test samples. Comprehensive evaluation was performed using various scientific instruments, including simple surface observations, quantification of amino acid content, and analysis of the characteristic odor components.

Benchtop Scanning Electron Microscope :	Surface observation
Nuclear Magnetic Resonance System :	Screening of solutions extracted from samples
Full Automatic Amino Acid Analyzer :	Qualitative and Quantitative analysis of amino acids
Head space (HS) – Sniffing – GC-MS system :	Analysis of volatile components

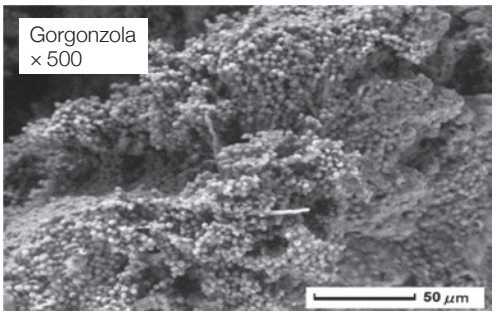
The evaluation results from using the various instruments are shown below.

	Cheese name	Region of origin	Raw material	Blue mold	Production method	Taste (JEOL testing)
Blue cheese (Aged cheese)	Gorgonzola (picante) 	Italy	Cow milk	P.galaucum	Blue mold mixed into the raw milk	Some bitterness Seems a good pairing with alcohol
	Stilton 	England	Cow milk (low fat milk)	P.roqueforti	The whey is removed from the solidified milk, then the curd is diced on a support called a Stilton sink. Salt is added and then it is solidified.	Seems a good pairing with alcohol Strong salt flavor Seemed most full-bodied
	Roquefort 	France	Sheep milk	P.roqueforti	Sheep milk is heated, lactobacillus and enzymes are added to harden. Whey is removed and the curd is molded and aged. The blue mold added into the cheese propagates.	Milder than other blue cheeses

Table 1 World's 3 major blue cheese varieties and human taste sense evaluation

2. Observation of blue mold in blue cheeses

The JCM-6000 behchtop SEM was used to observe blue mold in each cheese. Acquired SEM images are shown below. With the naked eye, three kinds of cheese are found to have different shapes. But in SEM observation, the surface structures of Stilton and Roquefort are similar to each other because the kinds of blue mold used for ferment are the same. On the other hand, Gorgonzola that contains blue mold different from the two former cheeses was found to exhibit different surface structures.



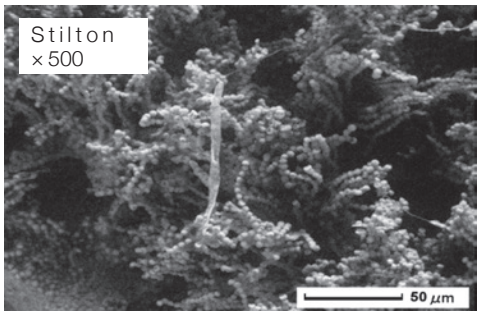
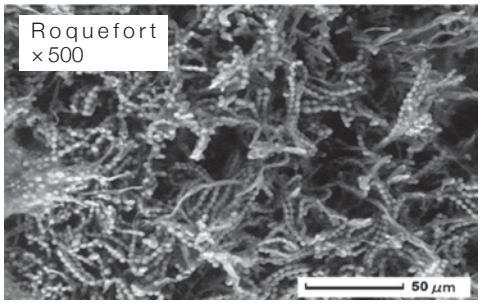


Fig. 1 SEM images

Benchtop scanning electron microscope
JCM-6000



3. Component Analysis: NMR

NMR is used for qualitative and quantitative analysis because of its ability to observe nuclei in molecules directly. Since analysis can be performed non-destructively, with the analyte in an original state, without any preparation like component separation, it can be used for comprehensive analysis as well as a so-called screening assay.
Figure 2 shows the results for an aqueous solution of Stilton cheese. There are signals for which it is not possible to make a complete assignment but NMR signals corresponding to amino acids were observed on the NMR spectrum. From intensity of the signals derived from tryptophan (Trp), confirmed at around 7.8 ppm, a quantification analysis can be performed, and the integral value ratio with the reference substance indicates an amount of about 2.8 mM.
From the NMR analysis of the aqueous solution of the Stilton cheese, it was found that the main components were amino acids. The measurement time was approximately 10 minutes. Use of NMR as a screening technique is rapid as it does not require complicated sample preparation.

NMR System JNM-ECZ400S

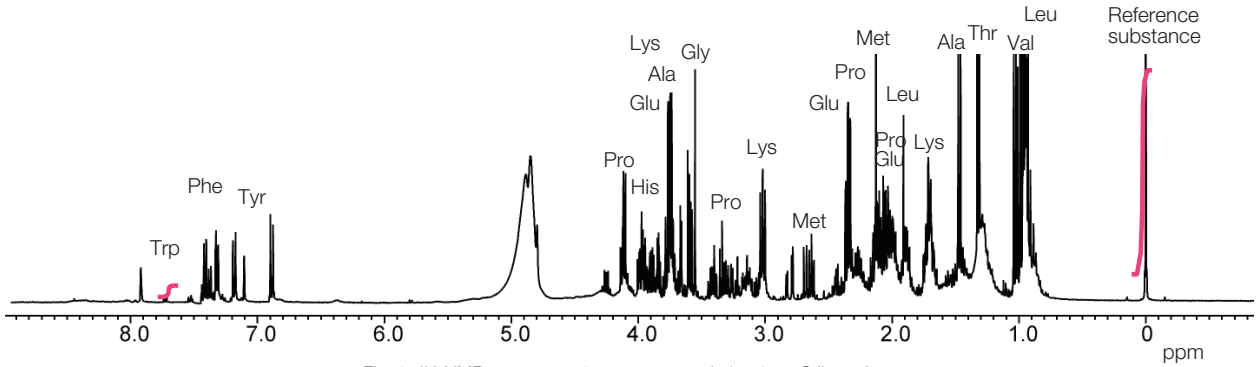


Fig. 2 ^{1}H-NMR spectrum of an aqueous solution from Stilton cheese

4. Component Analysis: Amino acid analysis

Figure 3 shows the radar charts of the amino acid analysis results for the various cheeses. The solid red line in each chart indicates the content of each amino acid. The green dotted line indicated the stimulation threshold level required for humans to detect the taste derived from each amino acid.
In these analysis results, the amino acids of Glu (umami), Leu, Met, Val, His, Phe, Ile (bitter) and Lys (sweet) were detected at levels exceeding the stimulation threshold for recognition of the flavor by humans.
Especially, Stilton had a large amount of amino acids. In addition, it was found that Glu (umami) and Leu tend to exhibit larger amounts of bitterness in comparison to the other 2 types of blue cheese. The taste assessment by human testers shown in Table 1 is consistent with the present result. It is suggested that there is a close relation between the amino acid contents evaluated in this study and the tastes of 3 types of cheese.

Full Automatic Amino Acid Analyzer (JLC-500/V2)

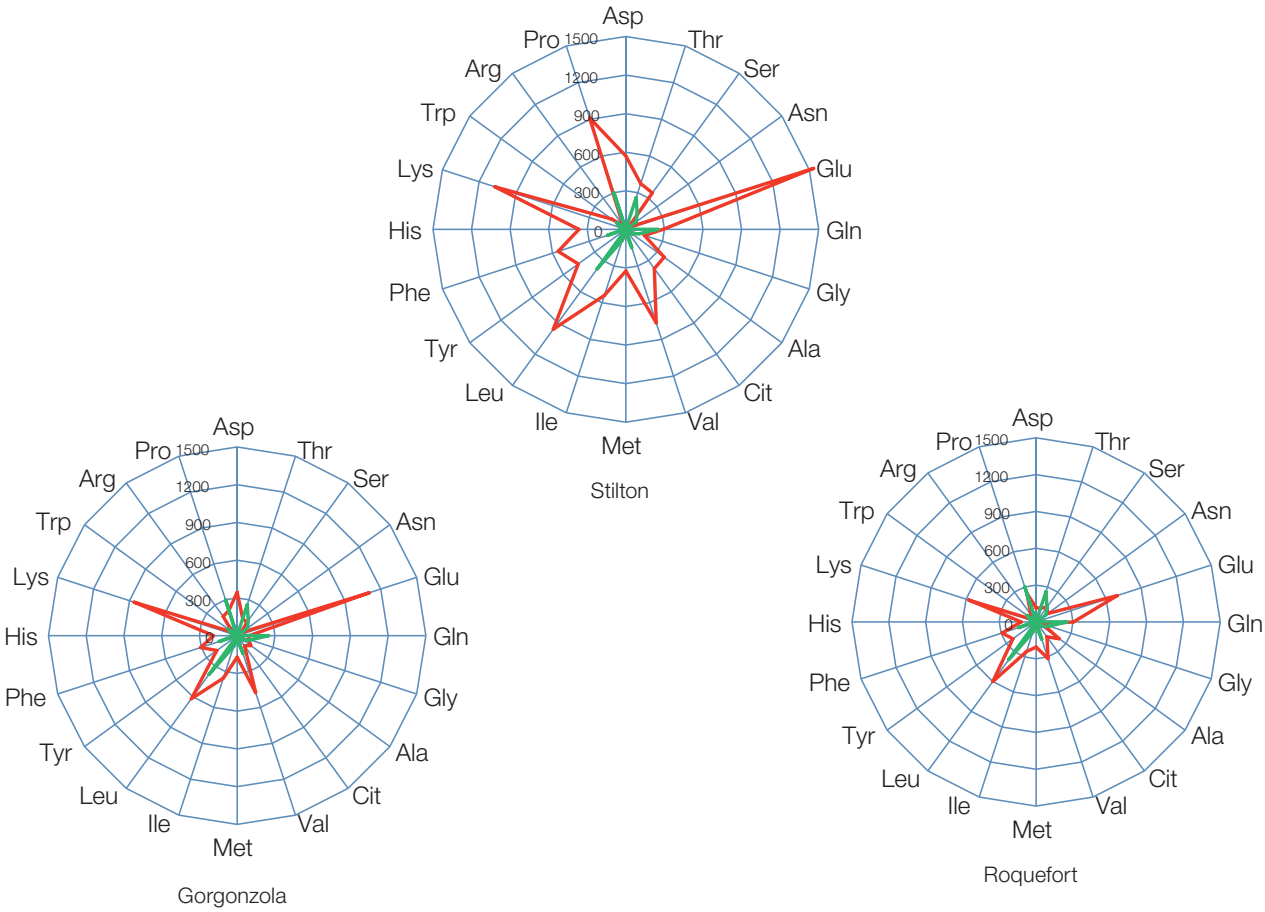


Fig. 3 Amino acid content in blue cheese

5. Component Analysis: HS-Sniffing-GC-MS system

Figure 4 shows the results for the measurements of 3 types of blue cheese made with the HS-Sniffing / GC-MS system. The upper portion is the Total Ion Current Chromatogram (TICC) acquired using GC-MS. The middle portion shows the odors sensed by a human tester during the sniffing analysis. The bottom portion displays the names of the chemical substances that are the first candidates from the library search results for the spectra acquired from the peaks in the TICC. As shown in these results, an HS-GC-MS system with a sniffing system makes it possible to evaluate the odor component of a sample while comparing and correlating the chemical analysis results from the GC-MS with the sensory results from sniffing.

In this study, the analysis results from the sniffing tests of the 3 types of blue cheese are similar, and it was clarified that the characteristic odor for each cheese is influenced by the odors of the chemical substances found in trace amounts, as well as the content ratio of these trace chemical substances.

The sniffing results indicate that the fishy odors and odors derived from fermentation are sensed in the short retention time region, and that the characteristic blue cheese odors and medicinal odors are detected later as the retention time increases.

Gorgonzola Stilton Roquefort

HS-Sniffing- GC/MS System (JMS-Q1500GC)

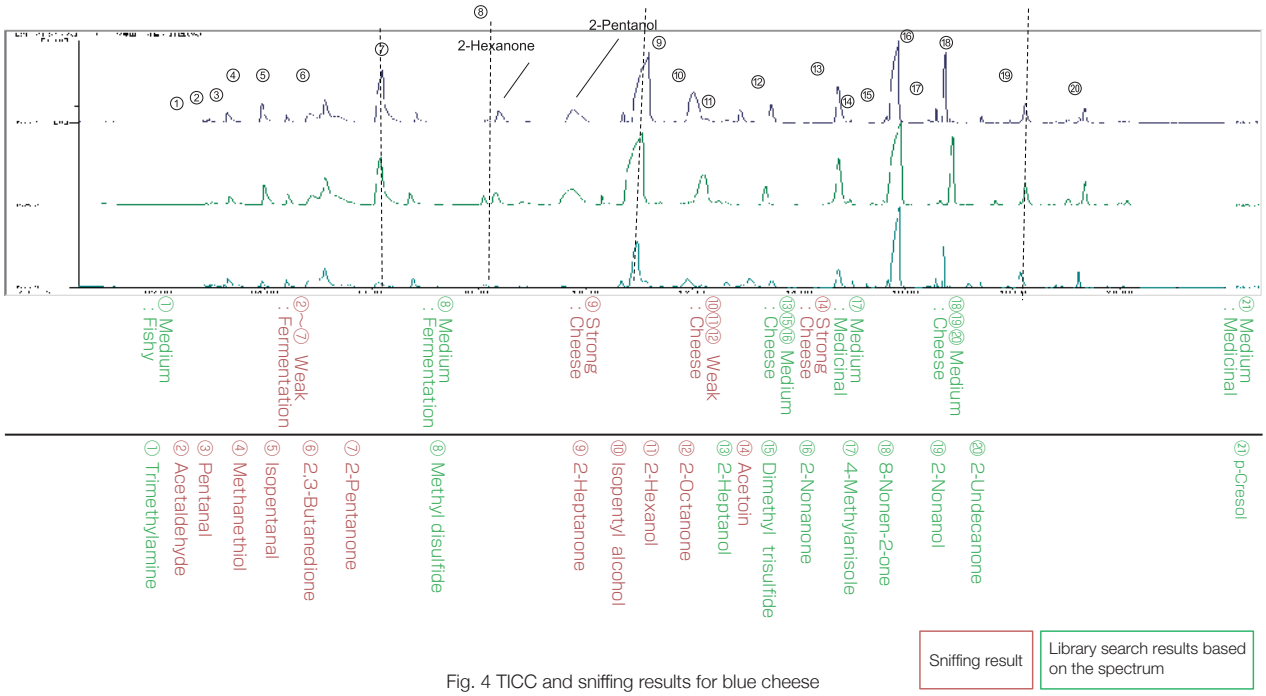


Fig. 4 TICC and sniffing results for blue cheese

6. Summary

Recently there has been a variety of research on flavors and fragrances, with a focus not only on the sensory aspects, but also on numerical quantification using scientific instruments.

Here, 3 representative types of blue cheese were used as the test samples, and multiple scientific instruments were used to observe the morphology, and make qualitative and quantitative evaluation from a chemistry perspective of the flavors and tastes.

It was demonstrated that the amino acids and volatile components are important factors in determining the characteristic tastes and smells of cheeses. Thus, by using multiple scientific instruments it is possible to make an effective evaluation with 1 sample.

3-3 Comprehensive Evaluation of Foods using Scientific Instruments
~Analysis of Potato Snacks : Using Full Automatic Amino Acid Analyzer, Micro Raman Spectrometer, ESR, GC/MS ~

1. Introduction

There are many snack foods available that can be easily enjoyed anytime and anywhere. This is partly because manufacturers are careful to manage the expiration dates on the products. For this test, a potato snack food (fried snack) was chosen as the sample, and measured with four kinds of instruments to make comparisons in order to clarify the changes that occur over time for a given food.

Prior to the measurements, sensory tests were conducted. Even for a sample within the recommended expiration date, when compared to a newer sample there was a clear difference noted in both the taste and flavor. Samples of products both within and past the recommended expiration date set by the manufacturer were prepared as shown in Figure 1. Scientific analysis of the differences was made using the analysis instruments described below.

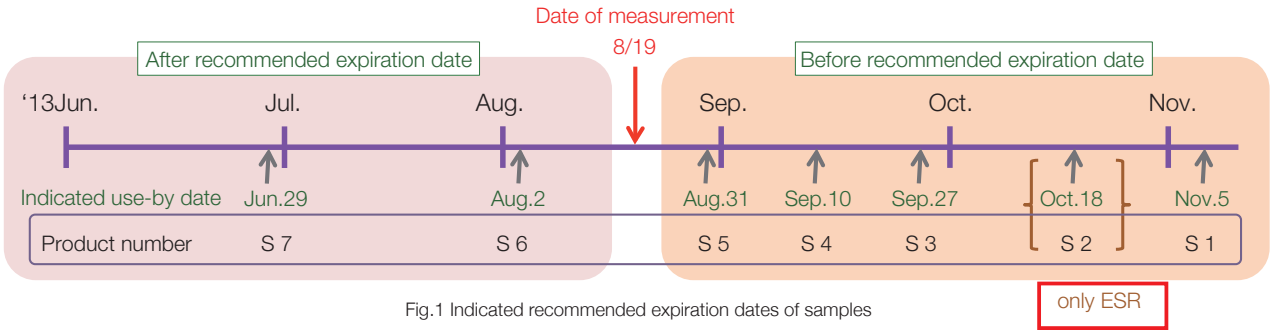


Fig.1 Indicated recommended expiration dates of samples

Full Automatic Amino Acid Analyzer : Search for indicators of taste change
Raman Microscope : Component distribution analysis and deterioration assessment of lipids, etc.
Electron Spin Resonance System : Search for components related to lipid oxidation
GC-MS : Analysis of volatile components

The evaluation results from using the various instruments are shown below.

2. Component Analysis: Amino Acid Analysis

Full Automatic Amino Acid Analyzers are actively utilized in a wide range of fields in the food industry, including development of highly-palatable foods (like fermented foods), development of functional foods, and for quality assurance. It is well known that the different amino acids give rise to different food flavors. For this study, we investigated the relationship between the amino acids and the changes in flavor that accompany deterioration of a potato snack food. Figure 2 is a summary of the changes over time of each amino acid. For all amino acids, there was no significant change in the amount, and it was confirmed that the content remained nearly constant. This suggests that the changes in flavor that were recognized in the sensory tests were mainly caused by something other than the amino acids.

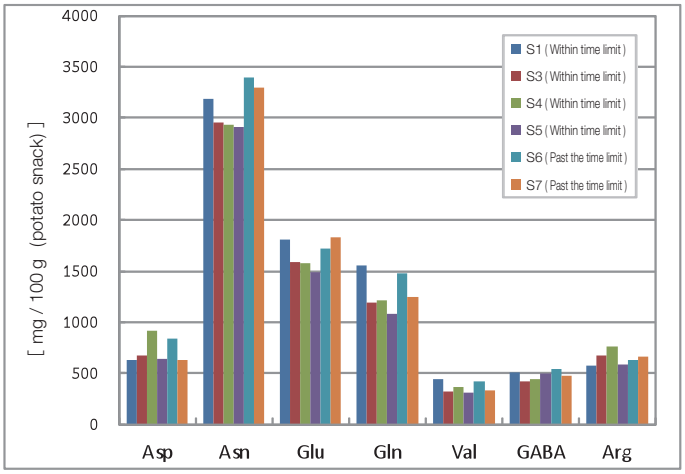


Fig. 2 Amino acid analysis results for a potato snack food

Full Automatic Amino Acid Analyzer (JLC-500/V2)

3. Component Analysis: Lipid analysis with Raman Microscope

Raman spectrometry is a method for analyzing crystal and molecular structures and for identifying compounds using the scattered light that is generated when a sample is irradiated with a focused laser beam.
To evaluate the deterioration of lipids in the potato snack, the edible oils contained in the samples were measured. The spectra of the S1 (freshest) and S7 (past the recommended expiration date) oils, as well as the spectrum for the S1 oil after thermal degradation are shown in Figure 3-1. When oil degrades there should be a decrease in the peak intensity of the series of peaks derived from the C=C bonds in the triacylglycerol molecules, but there was almost no intensity difference observed for S1 and S7. Figure 3-2 is a distribution image of the ester C=O bond (from edible oil) obtained by mapping the actual surface of S1 without any sample pretreatment. Using this kind of Raman imaging analysis, it is possible to visualize the distribution at the micro level of an indicator compound for the component deterioration.

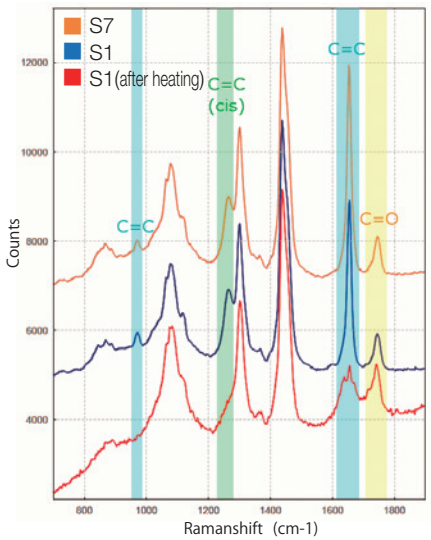


Fig. 3-1 Raman spectrum of edible oils (TAG) in a potato snack food

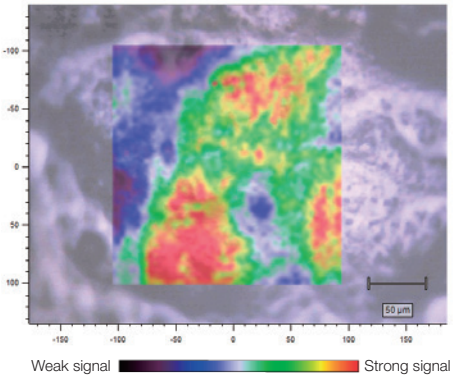


Fig. 3-2 Mapping analysis of a potato snack food

Color map image by intensity of the peaks derived from the ester structure C=O bonds of the edible oils.

inVia Reflex Raman Microscope by Renishaw

4. Component Analysis: Antioxidant evaluation using ESR

ESR detects the radicals in a sample. Signals are identified from the position at which they appear and the waveform, and quantification is possible from the signal intensity. Because of the high selectivity, pretreatment is not required, allowing direct measurement of the samples. For a fat-processed potato snack, we expected to detect lipid radicals generated by oxidation, and the spectra obtained are shown in Figure 4-1. Since there is no split in any case, and the g-value indicating (g = 2.0056) at ambient temperature as well as they were stable, it is considered likely that this is derived from the ascorbic acid radical ($AA^{\cdot}$).

For each sample the total amount of the obtained radicals was determined, and the values normalized by the collected amount are shown in Figure 4-2. With the horizontal axis taken as the remaining shelf life, we can see a bimodal pattern for the changes in the radical amount. For the sample with a remaining shelf life of 2 months, there is a peak in the radical amount. This is thought to be the result of the production of $AA^{\cdot}$ resulting from the antioxidation activity of the antioxidant AA. Since $AA^{\cdot}$ is relatively stable, it accumulates. In products with shorter remaining shelf live, it is thought that the AA is depleted and the $AA^{\cdot}$ decreases. However, this kind of food product contains vitamin E (V.E.) There is data from a separate experiment indicating that the radical quantity is suppressed by V.E. at the stage after the AA is depleted.

Later, the radical amount increases, but this is thought to be because the V.E. has been depleted. It is interesting that the timing of this effect is consistent with the indicated recommended expiration date.

In this way it is possible to use ESR to observe the antioxidant species radicals in fat-processed food to gain the information on the stages of oxidation degradation.

Electron Spin Resonance system (JES-X320)

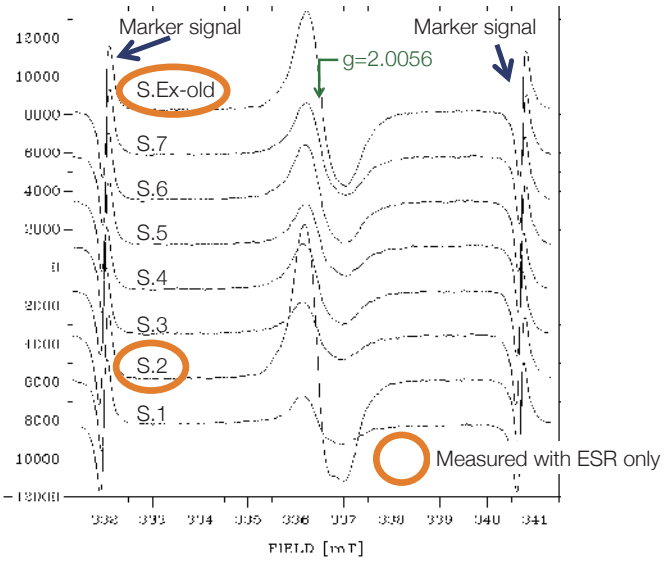


Fig. 4-1 ESR spectra of a potato snack food

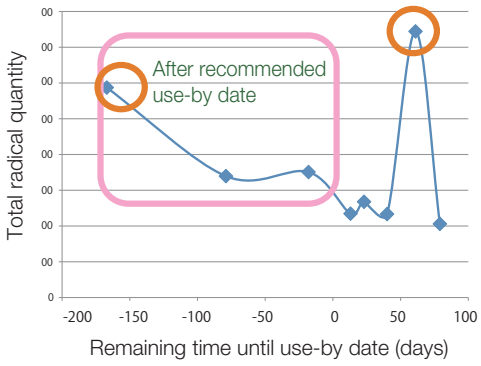


Fig. 4-2 Comparison for total radical amount in various samples

5. Component Analysis: Odor analysis using the Sniffing GC-MS

Figure 5-1 shows the results for the measurements of potato snacks using the Sniffing – GC-MS system. The upper portion is the Total Ion Current Chromatogram (TICC) acquired using GC-MS. The middle portion shows the odors sensed by a human tester during the sniffing analysis. The bottom portion displays the names of the chemical substances that are the first candidates from the library search results for the spectra acquired from the peaks observed on the TICC. As shown in these results, an HS-GC-MS system with a sniffing system makes it possible to evaluate the odor component of a sample while comparing and correlating the chemical analysis results from the GC-MS with the sensory results from sniffing.

For the potato snack odors in this study, it was possible to recognize oily and potato odors in the sensory test and to tentatively identify the corresponding compounds using a library search. The area of the peaks in the extracted ion chromatogram (EIC) from the characteristic *m/z* of the estimated compounds is compared with the ratio of the other peak areas by taking the peak area for the sample with the most-recent manufacture date as 1. With regard to the components associated with the sensation of an oily odor, it was confirmed that there are a number of compounds that show an increasing concentrations as the product ages. For the compound associated with the sensation of a potato odor, there were no differences in the date of manufacture.

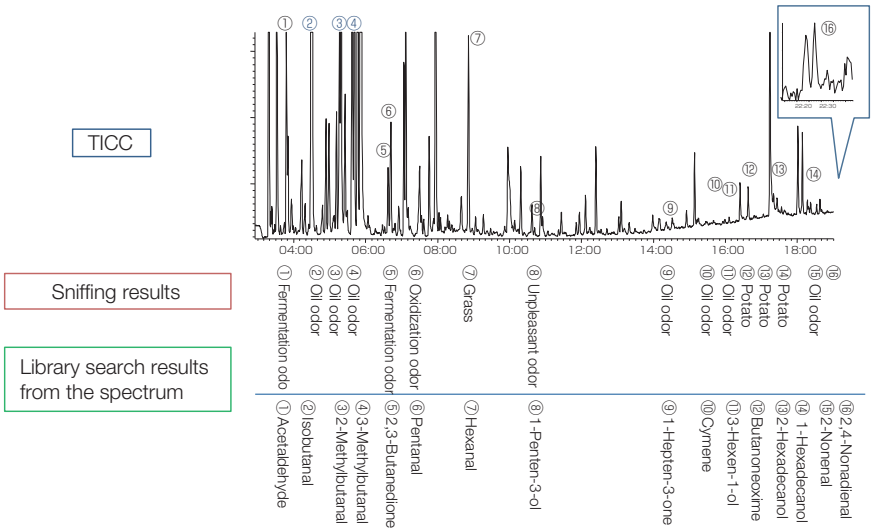


Fig. 5-1 TICC and sniffing results for potato snack food

HS-Sniffing- GC/MS system (JMS-Q1500GC)

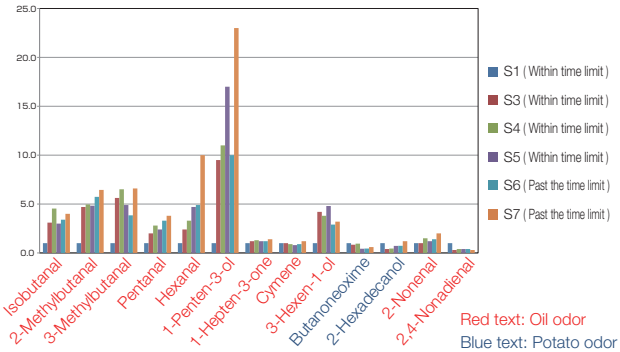


Fig. 5-2Comparison of specimen compounds for which oily and potato odors were sensed

6. Summary

It has been said that the taste of foods may depend more on the flavor than on factors like the amino acids. Using the Full Automatic Amino Acid Analyzer and the Raman Microscope, there were no significant changes observed in the amino acid or lipids composition. This may be regarded to be the result of the quality control efforts of the food manufacturers. Nevertheless, the observation of the radicals derived from the antioxidants using ESR and the changes in the taste components observed using GC-MS suggest that changes are occurring slightly for lipids. As illustrated by these examples, measuring the same sample with a variety of scientific instruments makes it possible to obtain multifaceted, complementary analytical data. The group of analytical instruments recommended by JEOL can be used for a wide range of applications, including quality control for processed foods.

Table of Food Analysis / Evaluation Instruments

		Mass Spectrometer	Full Automatic Amino Acid Analyzer	Nuclear Magnetic Resonance System	Electron Spin Resonance System	Energy Dispersive X-Ray Fluorescence Spectrometer	Transmission Electron Microscope	Scanning Electron Microscope	Raman Microscope
Nutrition	Fat analysis / evaluation / observation	●		●			●	●	●
	Protein analysis / evaluation / observation	●	●				●		●
	Sugars analysis	●		●					●
	Vitamin analysis	●		●					●
	Inorganic substance analysis					●		●	●
Aroma / Flavor	Amino acid analysis	●	●	●					●
	Organic component analysis	●		●					●
	Aroma / Fragrance analysis	●							
	Taste component analysis	●	●	●		●			
Texture	Viscosity evaluation						●	●	●
	Mouthfeel evaluation						●	●	●
	Chewiness evaluation						●	●	
Functionality	Evaluation of antioxidant function of soluble components				●				
	Evaluation of susceptibility to fat oxidation				●				
	Functional component analysis	●	●						
Quality & Safety management	Heavy metal analysis					●	●	●	
	Residual pesticide analysis	●		●					
	Toxic component analysis	●		●		●			
	Bacteria observation						●	●	●
	Source / Variety identification			●		●			
	Food inclusion / contaminant analysis / observation	●		●		●	●	●	●
	Food additive analysis	●		●		●	●	●	●
	Nutritional component identification	●	●	●		●	●	●	●
	Particle size analysis						●	●	●
	Pigment analysis	●		●		●			●
	Inorganic component analysis					●	●	●	●
Packaging materials	Packaging material research and development	●		●		●	●	●	●
	Packaging film analysis / observation	●				●	●	●	●
	Desiccant / Product quality preserver evaluation					●			●
	Analysis of deterioration of packaging resins	●			●				●
	Analysis of foreign particles adhered to / embedded in packaging materials					●		●	●

*Appearance or specifications subject to change without notice.

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