C10G-E090

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Evaluation of the Deliciousness of Artificial Meat

Introduction

According to a June 2019 report by the United Nations, the world population will increase from 7.7 billion to 9.7 billion in 2050. It states that due to rapid population growth, countermeasures against food problems such as starvation and malnutrition are urgently needed. Global meat consumption has increased 5-fold over the past 50 years and shows no sign of decreasing. It has also been reported that livestock products account for approximately 20 % of global greenhouse gas emissions, and that livestock products cause soil and water pollution.

In recent years, "artificial meat" that artificially reproduces the texture, flavor, and appearance of meat has been developed. There are two types of artificial meat: "plant-based meat (PBM)," which is made mainly from vegetable proteins such as soybeans, peas and mushrooms, and "cultured meat," which is made by culturing stem cells from animals such as cattle. Both are attracting growing interest in food markets around the world.

This application book summarizes methods for evaluating the deliciousness of artificial meat.

Contents

Comparison of the Flavors of Plant-Based Meat and Organic Beef (GC-MS)

Raw meat on its own has little aroma; therefore, almost all aromas associated with "meatiness" are created during the cooking process by the Maillard Reaction between amino acids and reducing sugars. That reaction determines which non-volatile precursors release volatile aroma compounds. Plant-based meat (PBM), products created to resemble animal meat in both look and taste, are growing in popularity. A plant protein such as soy protein concentrate, along with colors, stabilizers, and oils, is used to successfully mimic meat flavor and texture. And, just like in animal meat, the amino acids of that protein undergo the Maillard Reaction.

Solid phase microextraction (SPME) is a solvent-less extraction technique which makes use of a sorbent fiber to ad/absorb compounds from a headspace or liquid sample (Fig. 1). Headspace SPME improves selectivity and sensitivity for volatile compounds and reduces matrix effects. The new SPME Arrow contains a greater quantity of sorbent phase and larger surface area than a traditional fiber, allowing for greater analyte extraction in less time.

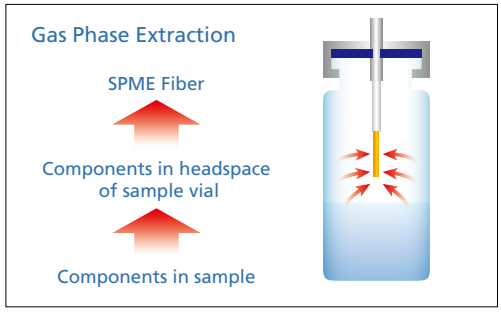


Fig. 1 The Principle of SPME

This study compared the aroma of cooked meat substitutes with that of regular cooked meat by using the SPME-GC/MS method, which is suitable for qualitative analysis of the aroma.

Samples of PBM were run with the SPME Arrow (Fig. 2), and the volatile profile was compared against that of the organic beef. Similar compounds, such as fatty acids and Maillard browning reaction products, were found in both types of meat (Table 1).

The differences can be explained by the different and wide variety of precursors present in PBM since it contains amino acids and sugars from various sources as opposed to regular meat.

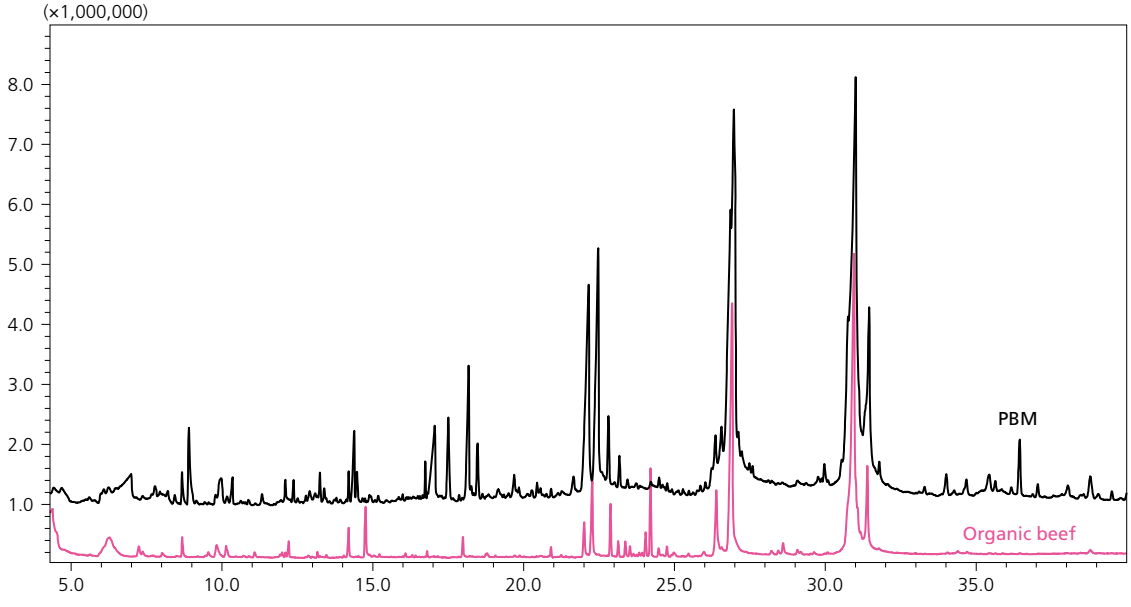


Fig. 2 Overlaid Representative Chromatograms for PBM (black) and Organic Beef (pink) at 10 min Extractions with the SPME Arrow

Table 1 Compounds Detected in PBM and Organic Beef Samples with the SPME Arrow

PBM	Organic Beef
1,3-Propanediol	Propanoic acid, 2-hydroxy-, methyl ester, (.+/–.)-
Pentaethylene glycol	Dimethyl sulfone
2(5*H*)-Furanone	Glycerin
Glycerin	3-Pentanone, 2,4-dimethyl-
Furaneol	Hexyl *n*-valerate
3,5-Octadien-2-one,(*E*,*E*)-	Nonanal
Nonanal	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
Maltol	2(3*H*)-Furanone, dihydro-4-hydroxy-
4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Octanoic acid
2(3*H*)-Furanone, dihydro-4-hydroxy-	Thiophene, 2,3-dihydro-
Octanoic acid	Piperidine, 1-nitroso-
Caprolactam	Nonanoic acid
2-Decenal, (*E*)-	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
Nonanoic acid	*n*-Decanoic acid
2-*n*-Octylfuran	Niacinamide
2,4-Decadienal, (*E*,*E*)-	6,10-Dodecadien-1-ol, 3,7,11-trimethyl-
cis-4-Decenal	2-Tridecanone
n-Decanoic acid	Dodecanoic acid
2-Tridecanone	Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester
Tetradecane	Eicosane
Thiazole, 4,5-dimethyl-	1-Hexadecanol
n-Nonylcyclohexane	Methanone, (1-hydroxycyclohexyl)phenyl-
Dodecanoic acid	Hexadecenoic acid, *Z*-11-
1-Pentadecyne	Tetradecanoic acid
8-Heptadecene	1-Dodecanol, 3,7,11-trimethyl-
2-Dodecanone	Octadecane
Tetradecanoic acid	Hexadecane, 2,6,10,14-tetramethyl-
Tetradecanoic acid, ethyl ester	Tetradecanal
Erucic acid	Pentadecanal
n-Hexadecanoic acid	Pentadecanoic acid
Oleic Acid	2-Heptadecanone
Octadecanoic acid	δ-Dodecalactone
Hexadecanamide	Erucic acid
	n-Hexadecanoic acid
	Heptadecanoic acid
	2(3*H*)-Furanone, 5-dodecyldihydro-
	Oleic Acid
	Octadecanoic acid
	8,11,14-Eicosatrienoic acid, (*Z*,*Z*,*Z*)-
	Squalene

Classification of Meat Through Flavor Analysis by Machine Learning (GC-MS)

Foods are comprised of numerous components and the quality of foods may not be consistent even for the same food products. Differences in quality can be caused by slight differences in the components that comprise food products. For this reason, methods are being investigated to estimate and identify the subjective properties of foods, such as taste, smell, and deterioration, based on their components. One such method that is expected to be effective revolves around learning the relationship between components and subjective properties of a known sample and then utilizing those results for an unknown sample.

In this study, data on the volatile components of properly refrigerated and decomposed meat was used as training data, and data from unknown samples was used to validate the identification method.

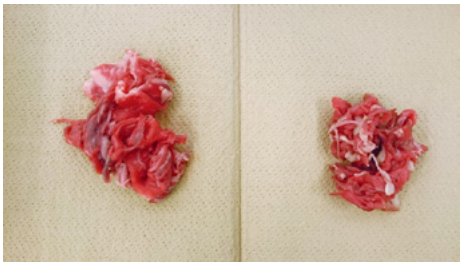


Fig. 3 Left: Properly Refrigerated Sample (4 °C sample)
Right: Sample Exposed to a 40 °C Environment for 3 Hours (40 °C sample)

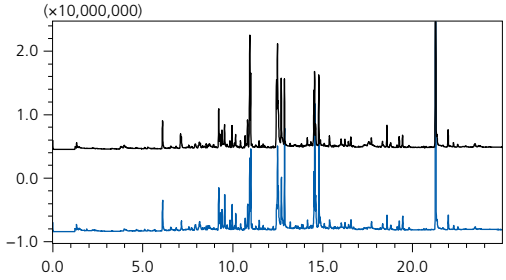


Fig. 4 Example Total Ion Chromatograms
Black: 4 °C sample, Blue: 40 °C sample

The vials were heated at 200 °C for 15 minutes and the resulting vapor was collected by SPME and analyzed in scan mode. As there were many samples, we used the AOC-6000 Plus for injection since it is capable of collection, adsorption, and desorption automatically by SPME. Comparison of the total ion chromatograms did not reveal any peaks characteristic to each sample (Fig. 4).

A quality discrimination method was developed based on data obtained by analysis using a support vector machine (SVM) as the classifier. Of 116 samples of collected data, 92 were used for learning and 24 for validation (Fig. 5).

Utilizing the hyperparameters optimized with the training set, we classified the 24 samples of the test set. Of the 24 samples, 23 samples were classified correctly. Table 3 lists the results, indicating the refrigerated samples as “Positive” and the deteriorated samples as “Negative”.

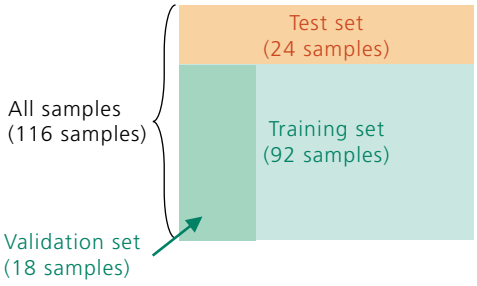


Fig. 5 Division of Datasets

Table 2 Classification Results of the 24 Samples of the Test Set

	True	False
Positive	12	0
Negative	11	1
Precision	95.8 %	

AOC-6000 Plus + GCMS System

Comparison of Amino Acids of Soy Meat and Chicken (LC)

There are five basic tastes, including deliciousness, which are perceived by people. The amount and kind of amino acids contribute to taste components. Of all the amino acids, glutamic acid is widely known as a component of the delicious taste. Further, the types and component ratios of amino acids largely control the flavor of food products. For example, glycine and alanine are associated with sweetness, valine and leucine with bitterness, and aspartic acid and glutamic acid with deliciousness.

The Shimadzu post-column method employs an automatic analysis system that utilizes fluorescence derivatization by *o*-phthalaldehyde (OPA) in detection after separation of the amino acids by gradient elution using a cation exchange column (Fig. 6). Although simultaneous analysis of amino acids is conducted using the optimized time programs, highly accurate amino acid analysis is possible without labor-intensive preparation using a dedicated amino acid mobile phase kit and reaction reagent kit.

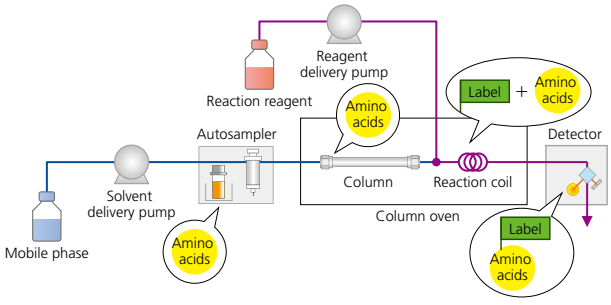
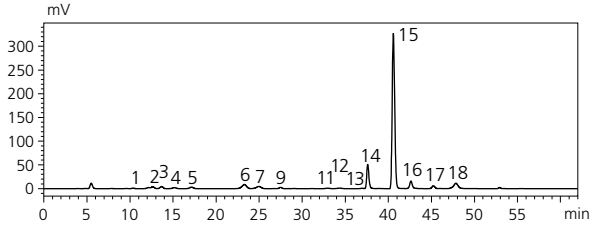


Fig. 6 Flow Diagram of Amino Acids Analysis System

Nexera Post-Column Amino Acids Analysis System

Fig. 7 and Fig. 9 show examples of analysis of 18 free amino acids obtained by extraction from dried soy meat and chopped chicken breast meat samples, respectively. Fig. 8 and Fig. 10 describe the pretreatment protocol for each analysis. Comparing dried soybean and chopped chicken breast chromatograms shows the differences in taste characteristics.



1. Aspartic Acid
2. Threonine
3. Serine
4. Glutamic Acid
5. Proline
6. Glycine
7. Alanine
9. Valine
11. Isoleucine
12. Leucine
13. Tyrosine
14. Phenylalanine
15. Histidine
16. Lysine
17. Ammonia
18. Arginine

Fig. 7 Analysis of Chopped Chicken Breast Meat

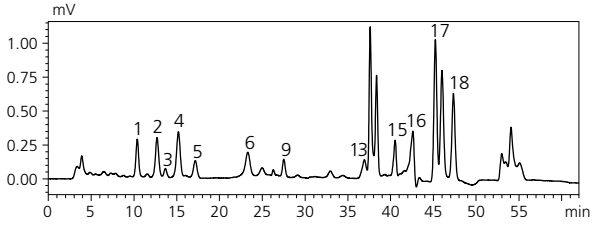
Chopped chicken breast meat 1.668 g
- 0.2 mol/L sodium citrate buffer (pH 2.2) (10 mL)
  Homogenize (1 minute)
  Centrifuge (5000 rpm × 5 minutes)

Supernatant
- Ultrafiltration filter (10K)
  Centrifuge (10000 rpm × 10 minutes)

Filtrate 100 µL
- 0.2 mol/L sodium citrate buffer (pH 2.2) (900 µL)

HPLC

Fig. 8 Pretreatment Protocol of Chopped Chicken Breast Meat



1. Aspartic Acid
2. Threonine
3. Serine
4. Glutamic Acid
5. Proline
6. Glycine
9. Valine
13. Tyrosine
15. Histidine
16. Lysine
17. Ammonia
18. Arginine

Fig. 9 Analysis of Dried Soy Meat

Dry soy meat 109 mg
- 0.2 mol/L sodium citrate buffer (pH 2.2) (20 mL)
  Stand at room temperature, 2 hours
  Homogenize (1 minute)

Supernatant
- Ultrafiltration filter (10K)
  Centrifuge (10000 rpm × 10 minutes)

Filtrate 100 µL
- 0.2 mol/L sodium citrate buffer (pH 2.2) (400 µL)

HPLC

Fig. 10 Pretreatment Protocol of Dried Soy Meat

Comparison of the Primary Metabolites in Ground Beef and Plant-Based Meat (LC-MS)

With heightening interest in sustainable and healthy diet lifestyles, plant-based meat (PBM) is getting more and more attention. As demand increases for developing new products of PBM, there is a corresponding focus on the flavor quality of the products. However, it is sometimes difficult to precisely evaluate flavor that includes taste and aroma because highly complex combinations of numerous chemical compounds may define these factors. Widely-targeted analysis of the primary metabolites, such as amino acids, organic acids and nucleosides, is one great idea to accomplish this evaluation.

This study shows the results of analyzing four different PBM products and ground beef with the primary metabolites evaluation approach.

Four different commercially available PBM products (Product 1 to 4) and ground beef were prepared for this analysis.100 mg of meat samples were cooked in a 200 °C oven for 20 min. 0.75 mL of methanol was put into the sample for deproteinization and delipidation. After centrifugation, supernatant was taken, and filtration was performed with a 0.45 mm pore size filter. The filtered solution was stored at –20 °C as an original stock sample solution. The sample solution for metabolites analysis was prepared by diluting the stock sample solution to 5000-fold with water to minimize the matrix effect.

It was confirmed that most compounds showed good recovery in the range of 80 to 120 % in diluted sample solvents, and all 55 compound peak area values were considered as relative concentrations in each sample. The peak shapes of glutamic acid and inosine, representative deliciousness components, are shown in Fig. 11. Data including metabolite analysis for principal component analysis are summarized. The score plots obtained from principal component analysis are shown in Fig. 12. The score plot can be interpreted as the "total similarity" of each sample for taste. The more similar the pattern of data, the closer the sample dots are to each other. This allows the similarity of each sample to be reviewed without subjective factors.

The score plot shows that ground beef differs from the four types of PBM. The results from analyzing the primary metabolites that contribute to taste suggest that there is a difference in taste between ground beef and PBM.

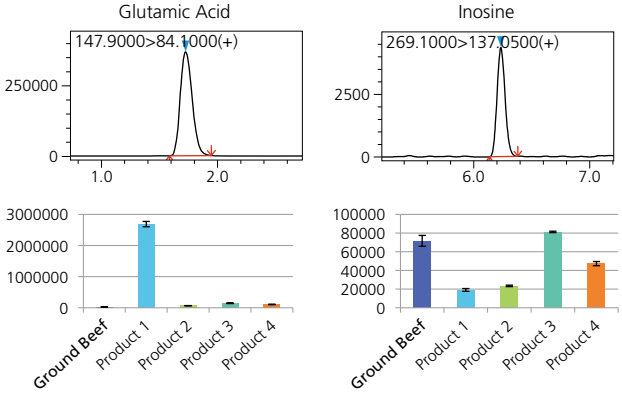


Fig. 11 Peak Shape of Glutamic Acid and Inosine in Product 1 (upper) and Comparison Between Samples of Glutamic Acid and Inosine (lower)

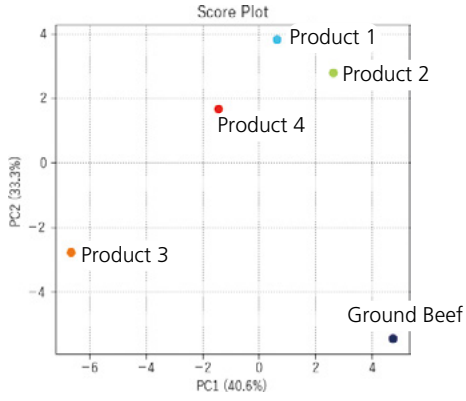
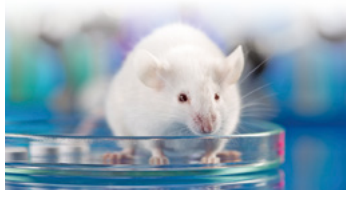


Fig.12 Score Plot of the Result from Principal Component Analysis

LC/MS/MS Method Package for Primary Metabolites Ver. 3

LC/MS is the most widely used technique for metabolomics because many metabolites, such as sugars, amino acids, and organic acids, are hydrophilic compounds and can be measured with simple sample preparation. Combining Shimadzu's highly sensitive and ultra-fast LC-MS/MS with a method package for primary metabolites enables rapid and efficient quantitative metabolite analysis.



Target Metabolic Pathway	Method	Number of Registered Compounds
Glycolysis, pentose phosphate pathway, coenzyme, etc.	Ion pair method	55
Methylation cycle, urea cycle, TCA cycle, etc.	Non-ion pair method	97

This package includes two methods, permitting selection of the method that suits the target compounds and instrument environment. It should be noted that the PFPP column is used with the non-ion pair method.

Comparison of Meatball Texture Between Plant-Based Meat and Chicken

(Texture Analyzer)

The texture of food, including the sense of crispness, springiness, firmness, and the feeling on the tongue, is an important element that together with taste has an impact on the deliciousness of food.

Food texture is normally evaluated using sensory tests. However, sensory tests are often difficult to reproduce, due to individual differences in people's sensations and physical condition. Shimadzu's texture analyzer supplements sensory tests by obtaining objective results in the form of numerical values for use in the field of food development. Here, the principle of the evaluation of texture characteristics and a comparison of the texture of plant-based meat (PBM) and chicken meatballs are introduced.

The texture you feel when you eat food is closely related to the physical properties of food. Fig. 13 shows the stress-strain curve obtained from a fracture test, and Table 3 shows the characteristic values obtained from the results.

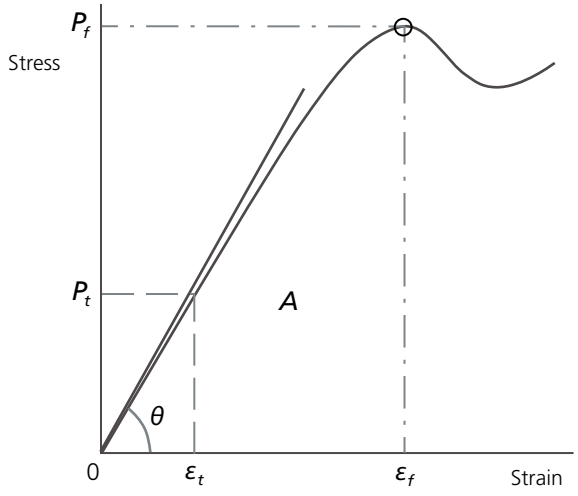


Fig. 13 Stress-Strain Curve

Table 3 Fracture Mechanic Properties

Fracture Strength	Force needed to break an object (P_f)
Fracture Energy	The amount of energy consumed to generate cracks per unit area (A)
Fracture Strain	Strain under load of fracture strength (ε_f)
Initial Elastic Modulus	The amount of deformation that is caused by a small stress. Calculated from the slope of the Stress-Strain curve within a certain range of linearity ($tan\theta$)

The principle of a texture test is shown in Fig. 14. Place the food on the base plate and compress and pull the top-mounted plate twice in a constant velocity linear motion. Fig. 15 shows the curve obtained as a result of the test. From this result, it is possible to calculate various texture parameters. Table 4 describes definitions of textural characteristics for food and shows the formula to obtain the texture characteristic.

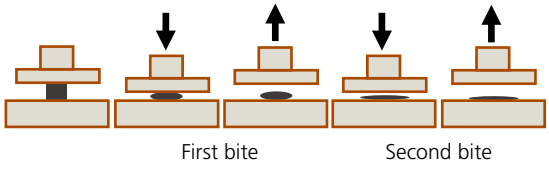


Fig. 14 Schematic Diagram of Texture Test

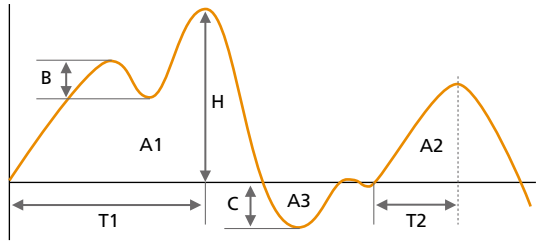


Fig. 15 Example of a Method for Evaluating Texture Characteristics

Table 4 Definitions of Textural Characteristics for Food

Properties	Primary Properties	Secondary Properties	Common Terms	Description of Properties
Mechanical Properties	Hardness		Soft – Firm/Hard	Force required for a given deformation volume. Internal cohesive forces that give the food its shape (**H**)
	Cohesiveness (**A2/A1**)	Fracturability	Crumbly – Crunchy – Brittle	Force required to crush food. Related to hardness and cohesiveness. (**B**)
		Chewiness	Soft – Tough	Energy required to chew solid food until it can be swallowed. Related to hardness, cohesiveness, and springiness. (**H×A2/A1×T2/T1**)
		Gumminess	Crumbly – Powdery – Pasty – Rubbery	Energy required to chew semi-solid food until it can be swallowed. Related to hardness and cohesiveness. (**H×A2/A1**)
	Springiness		Plastic/Ductile – Elastic	Proportion of a deflection caused by an external force that returns to the original position after the force is removed. (**T2/T1**)
	Adhesiveness		Gooey – Sticky – Slimy	Force required to overcome the attractive force between the surface of a food product and other things (such as the tongue, teeth, and palate). (**A3**)

Note: Alina Surmacka Szczesniak Texture-related terminology was arranged and systematized for the first time internationally in 1963.

We conducted a comparative test on the texture of commercially available plant-based meatballs and chicken meatballs. In this study, the texture of meatballs made of two different meats was confirmed by a shear fracture test that simulates biting movements using a jig with a sharp tip and a compression texture test that simulates chewing movements. Prior to instrumental analysis, a sensory evaluation of chicken and plant-based meatballs was performed. When comparing chicken meatballs and plant-based meatballs in terms of texture, the latter had a harder texture. As for elasticity, chicken meatballs had stronger elasticity.

Fig. 16 shows the test force-displacement curves of two samples and Table 6 shows the result of the shear fracture test. The plant-based meatball has a higher fracture strength, which indicates hardness, than chicken meatballs. Fig. 17 shows the profile of the compression texture test. Compared to chicken-derived products, plant-based meatballs had a higher force under loading conditions with less elasticity, which is the property to restore deformation. It is consistent with the result of the sensory test.

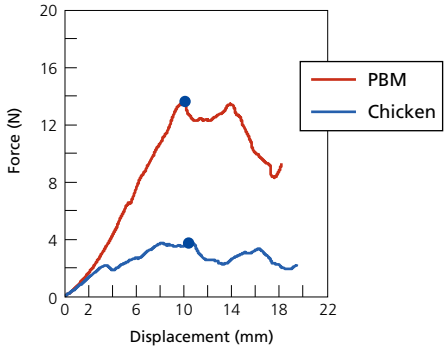


Fig. 16 Force-Displacement Curve for Two Types of Meatballs

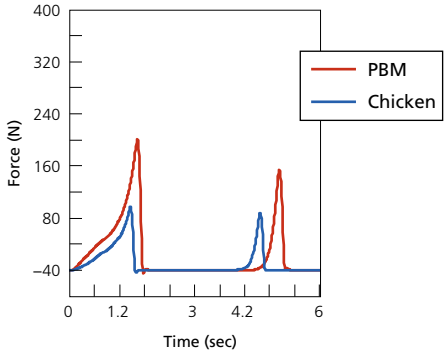


Fig. 17 Profile of Compression Texture Test for Two Types of Meatballs

Table 5 Result of Shear Fracture Test

	PBM	Chicken
Fracture Strength (N)	13.6	3.7

Table 6 Result of Compression Texture Test

	PBM	Chicken
Hardness (N)	202	97
Elasticity	0.41	0.53

Protein Denaturation and Texture Analysis of Chicken

(DSC & Texture Analyzer)

The deliciousness of food is greatly influenced by taste due to chemical interactions with the tongue, in addition to the physical sensations of hardness, softness, and juiciness sensed by the teeth. This study shows an overall evaluation of texture in fried chicken heated over different time periods. DSC was to measure protein denaturation, and a precision universal testing machine was used to measure the hardness.

Chicken breast seasoned with spices was placed in an aluminum sealed cell and heated at a rate of 10 °C/min (Fig. 18). Endothermic peaks were seen at 57 °C, 64 °C and 78 °C. It is presumed that the endothermic peaks correspond to denaturation of myocin at 57 °C, connective tissue at 64 °C, and actin at 78 °C.

After confirming that the temperature at the center of fried chicken breasts was 70 °C, the chicken breasts were kept warm for 0 min, 1 hour, 2 hours, and 4 hours, respectively, at which times the chicken breasts were immediately frozen by shock freezing. Samples were heated at a rate of 10 °C/min up to 100 °C (Fig. 19). An endothermic peak due to denaturation of the protein actin is seen within one hour of heat retention. This means that un-denatured protein still remains. On the other hand, when heat retention continues beyond two hours, no endothermic peaks are seen and the softness is lost. Thus, this correlates to the experience that the longer heat retention continues, the dryer the texture becomes.

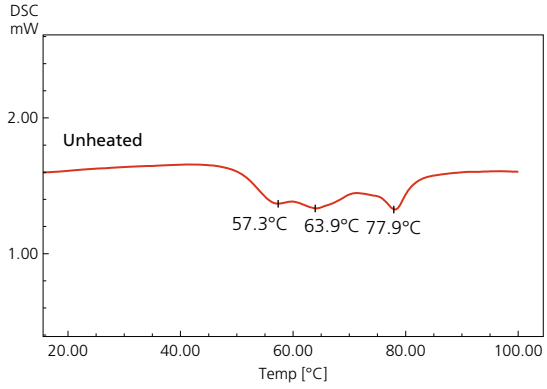


Fig. 18 DSC Curve of Chicken

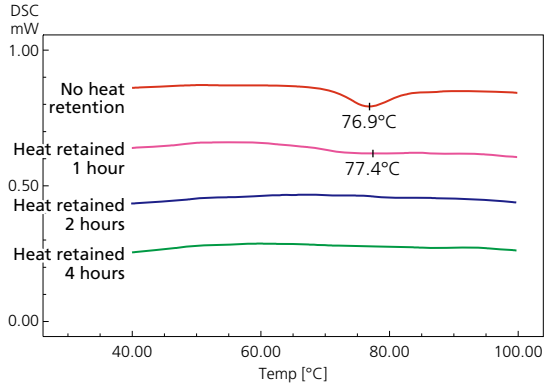


Fig. 19 DSC Curves of Fried Chicken Subjected to Different Heat Retention Times

Next, the chicken breast fiber was evaluated for hardness by orthogonal shearing. Fig. 20 shows the force and time graph and Table 7 shows the hardness and elasticity calculated based on these test results. It is clear that while the hardness rose marginally up to a heat retention time of one hour, the specimen suddenly became harder after heat retention exceeded two hours. This also correlates to the DSC measurement result in which protein denaturation is seen within one hour.

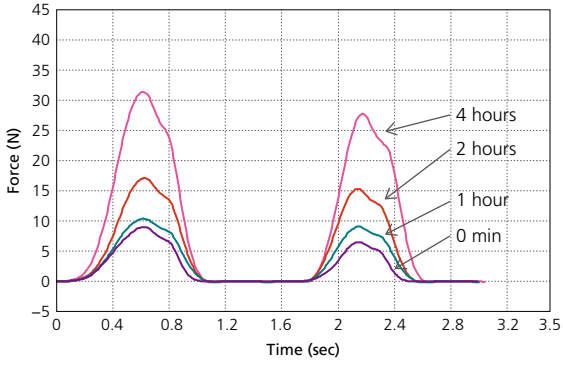


Fig. 20 Profile of Compression Texture Test for Fried Chicken

Table 7 Results of Compression Texture Test

Heat Retention Time	Hardness (N)
0 min	8.87
1 hour	10.15
2 hours	16.83
4 hours	32.77

Simultaneous Analysis of Culture Supernatant of Mammalian Cells (LC-MS)

Cell culture technology, which has been mainly used in the medical field, has been applied and researched all over the world for more efficient production of livestock products such as meat, chicken eggs, and milk. The challenge with developing cultured meats is to reduce manufacturing costs in order to trade at prices comparable to traditional meats. For this reason, development and control of an effective culture process are required.

Optimization and control of the cultural process requires routine monitoring of medium conditions such as pH, dissolved gas, carbon source (glucose), and nitrogen source (glutamine). Culture media also consist of various other biologically important compounds such as vitamins, nucleic acids, and other primary metabolites, which would lead to a more detailed understanding of the bioprocess if monitored together. To meet the demand for comprehensive analysis of a medium component, we optimized the analytical conditions and developed this "Method Package for Cell Culture Profiling" that can monitor relative abundance of the 95 compounds listed in Table 8. Using this Method Package, we demonstrated the change in abundance of culture medium components associated with hybridoma growth over a period of five days.

Table 8 List of Target Compounds (Method Package for Cell Culture Profiling)

No.	Compound Name	Class.
1	2-Isopropylmalic acid	IS
2	Gluconic acid	Carbohydrate
3	Glucosamine	Carbohydrate
4	Hexose (Glucose)	Carbohydrate
5	Sucrose	Carbohydrate
6	Threonic acid	Carbohydrate
7	2-Aminoadipic acid	Amino acid
8	4-Aminobutyric acid	Amino acid
9	4-Hydroxyproline	Amino acid
10	5-Glutamylcysteine	Amino acid
11	5-Oxoproline	Amino acid
12	Alanine	Amino acid
13	Alanyl-glutamine	Amino acid
14	Arginine	Amino acid
15	Asparagine	Amino acid
16	Aspartic acid	Amino acid
17	Citrulline	Amino acid
18	Cystathionine	Amino acid
19	Cysteine	Amino acid
20	Cystine	Amino acid
21	Glutamic acid	Amino acid
22	Glutamine	Amino acid
23	Glutathione	Amino acid
24	Glycine	Amino acid
25	Glycyl-glutamine	Amino acid
26	Histidine	Amino acid
27	Isoleucine	Amino acid
28	Kynurenine	Amino acid
29	Leucine	Amino acid
30	Lysine	Amino acid
31	Methionine	Amino acid
32	Methionine sulfoxide	Amino acid
33	*N*-Acetylaspartic acid	Amino acid
34	*N*-Acetylcysteine	Amino acid
35	Ornithine	Amino acid
36	Oxidized glutathione	Amino acid
37	Phenylalanine	Amino acid
38	Pipecolic acid	Amino acid
39	Proline	Amino acid
40	Serine	Amino acid
41	Threonine	Amino acid
42	Tryptophan	Amino acid
43	Tyrosine	Amino acid
44	Valine	Amino acid
45	4-Aminobenzoic acid	Vitamin
46	Ascorbic acid	Vitamin
47	Ascorbic acid 2-phosphate	Vitamin
48	Biotin	Vitamin
49	Choline	Vitamin
50	Cyanocobalamin	Vitamin
51	Ergocalciferol	Vitamin
52	Folic acid	Vitamin
53	Folinic acid	Vitamin
54	Lipoic acid	Vitamin
55	Niacinamide	Vitamin
56	Nicotinic acid	Vitamin
57	Pantothenic acid	Vitamin
58	Pyridoxal	Vitamin
59	Pyridoxine	Vitamin
60	Riboflavin	Vitamin
61	Tocopherol acetate	Vitamin
62	Adenine	Nucleic acid
63	Adenosine	Nucleic acid
64	Adenosine monophosphate	Nucleic acid
65	Cytidine	Nucleic acid
66	Cytidine monophosphate	Nucleic acid
67	Deoxycytidine	Nucleic acid
68	Guanine	Nucleic acid
69	Guanosine	Nucleic acid
70	Guanosine monophosphate	Nucleic acid
71	Hypoxanthine	Nucleic acid
72	Inosine	Nucleic acid
73	Thymidine	Nucleic acid
74	Thymine	Nucleic acid
75	Uracil	Nucleic acid
76	Uric acid	Nucleic acid
77	Uridine	Nucleic acid
78	Xanthine	Nucleic acid
79	Xanthosine	Nucleic acid
80	Penicillin G	Antibiotics
81	2-Aminoethanol	Other
82	2-Ketoisovaleric acid	Other
83	3-Methyl-2-oxovaleric acid	Other
84	4-Hydroxyphenyllactic acid	Other
85	Citric acid	Other
86	Ethylenediamine	Other
87	Fumaric acid	Other
88	Glyceric acid	Other
89	Histamine	Other
90	Isocitric acid	Other
91	Lactic acid	Other
92	Malic acid	Other
93	*O*-Phosphoethanolamine	Other
94	Putrescine	Other
95	Pyruvic acid	Other
96	Succinic acid	Other

A murine hybridoma cell line was cultured in DMEM (see Table 9 for conditions) and its culture supernatant was sampled every 24 hours for five days after inoculation. A sample was prepared by adding an internal standard to the sample and then removing proteins by taking supernatant after mixing with acetonitrile, which was further diluted with ultrapure water prior to injection. 1 µL was injected into the LC-MS for simultaneous MRM quantitation of all 96 compounds. Fig. 21 shows a growth curve and viability plot of the cell line, and Fig. 22 shows the quantitative value (ratio of peak area with respect to internal standard) of representative compounds over five days.

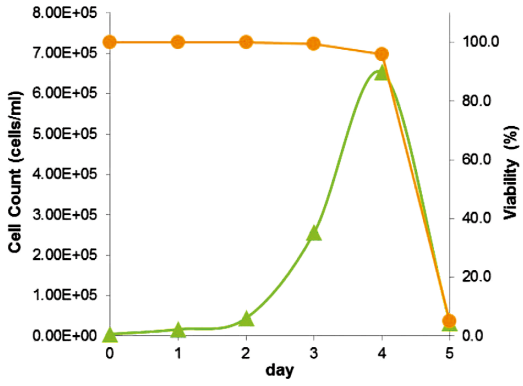


Fig. 21 Growth Curve and Viability of Cell Culture

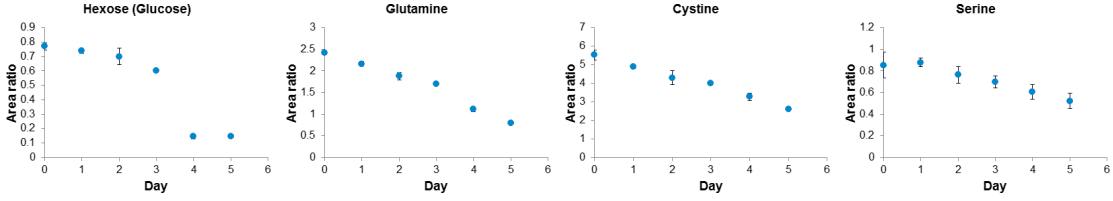
Table 9 Cell Culture Conditions

Cell line:	SJK-287-38 (ATCC CRL-1644)
Medium:	DMEM (Low Glucose) + 10 % FBS + Gln, $NaHCO_3$
Condition:	37 °C, 5 % CO_2, 120 rpm
Scale:	24 mL (N = 4)

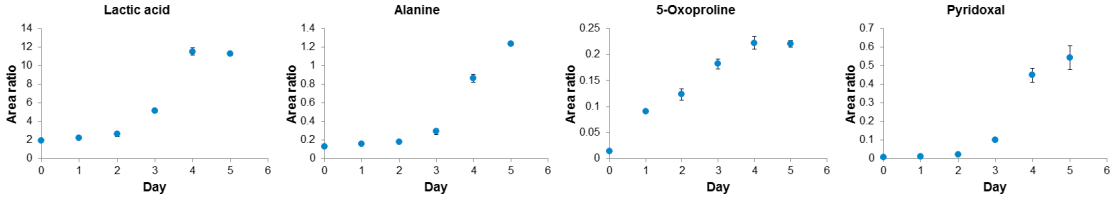
The culture supernatant sample and Fig. 21 was courteously provided by Kyokuto Pharmaceutical Industrial Co., Ltd.

Representative results are shown below. (A) Glucose, glutamine and a few other amino acids, which are the primary sources of carbon and nitrogen, have decreased in abundance with a growing cell number. (B) In contrast, lactic acid increased in abundance over time as a result of glucose consumption for anaerobic respiration. A similar pattern of increase was observed for a few other compounds. (C) No change in relative abundance was observed for essential amino acids and some vitamins.

(A) Example of compounds showing decreasing signal over time.



(B) Example of compounds showing increasing signal over time.



(C) Example of compounds showing no change in signal over time.

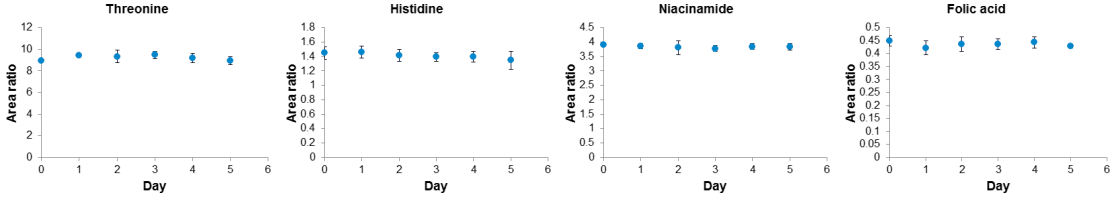


Fig. 22 Monitoring the Change in Culture Supernatant Components with Culture Time

Compression Tests for Cell Aggregates (Micro Compression Testing Machine)

Cultured meat can be produced by culturing meat issue. Various research on cell culturing has been conducted by many laboratories and companies toward the realization of the therapeutic applications of regenerative medicine. As an example, this article introduces the measurement of deformation strength*1 as an equivalent to the hardness of cell aggregates, a model for cultured tissues, using the Shimadzu MCT micro compression testing machine.

*1: Deformation strength: The strength is calculated using the formula shown below. It is defined in JIS Z 8844 (Test method of fracture and deformation strength of a fine particle)

In this study, cell aggregates were tested on three types of cells: HEK293 and iPS cells. HEK293 is derived from human embryonic kidneys and is a general-purpose cell line. iPS cells A and B are iPS cell strains established using the same methods with different cells derived from humans.

Table 10 shows the average sizes for the three cell aggregate types, HEK293 and iPS cells A and B, used in this study. Particle sizes were used to calculate the deformation strength.

Table 10 Average Size of the Cell Aggregate Samples

Cell Aggregate Sample	Average Size (μm)
HEK293	231.31
iPS Cell A	243.13
iPS Cell B	225.59

Fig. 23 shows the appearance of the MCT-510 and a schematic of the testing. With the MCT-510, compression tests can be performed on one particle at a time, and the compression process can be checked on screen using the side observation kit. The formula for calculating the deformation strength is shown below.

$$\sigma_{10\%} = \frac{F_{10\%}}{A} \quad \cdots (1)$$

$$A = \frac{\pi F}{4}$$

$\sigma_{10\%}$: Deformation strength for a 10 % compression displacement of particle size (Pa)

$F_{10\%}$: Force for a 10 % compression displacement of particle size (N)

A : Typical surface area (m^2) (Area equivalent to a circle, found from the particle size measured before compression)

d : Particle size (m)

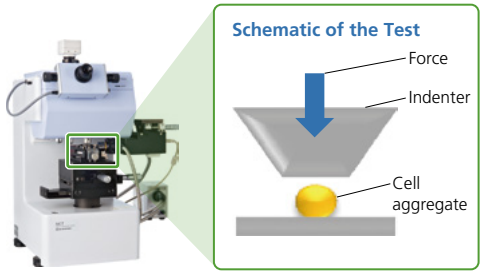
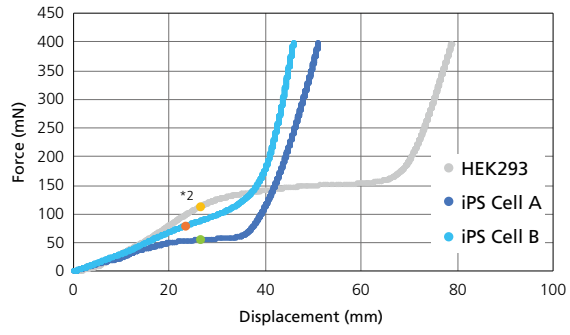


Fig. 23 Appearance of MCT-510 and Schematic of the Test

Fig. 24 shows the force-displacement curve and Fig. 25 shows cell aggregate compression by the MCT-510. The change in force was minimal in the region of about 20 to 60 μm displacement for HEK293 and about 20 to 40 μm for the iPS cells, and the most significant particle deformation occurred in these regions. During the test no clear fracture points were detected. To define the particle strength, a $\sigma_{10\%}$ deformation strength was employed, and it was calculated from the force at a 10 % deformation of the particle (Table 11). A significant difference was found between the HEK293 and iPS cell A, and a difference was also found between the differently derived iPS cells.



*2: The dots indicate 10 % deformation of the particle size.

Fig. 24 Force-Displacement Curve

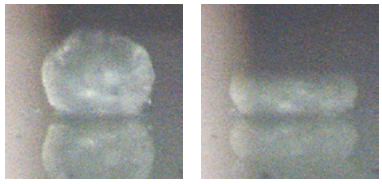


Fig. 25 Compression of Cell Aggregates by the MCT-510

Table 11 Test Results

Sample	HEK293	iPS Cell A	iPS Cell B
Average Size (μm)	271.78	243.13	225.59
Deformation Strength $\sigma_{10\%}$ (MPa)	1.91	1.26	1.77

Related Instruments

Liquid Chromatograph Mass Spectrometer

LCMS-8060NX

Enhanced performance

Sensitivity and Robustness

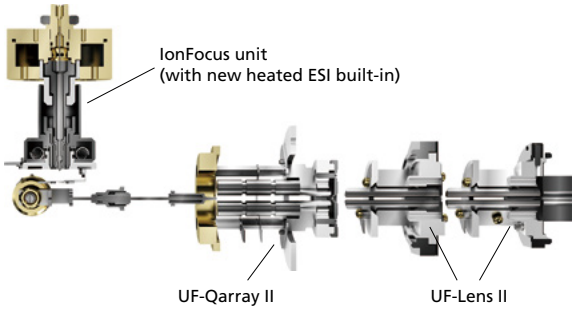
The LCMS-8060NX is a triple quadrupole mass spectrometer with world-class sensitivity and detection speeds. It boasts increased robustness and ease of use as well as Analytical Intelligence functions to maximize your laboratory's output.

ANALYTICAL INTELLIGENCE

Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.

World-class sensitivity and speed

The LCMS-8060NX improves the desolvation efficiency by increasing the ESI heat transfer efficiency and the maximum gas flow rate. Optimum ionization conditions can be set for a wider range of compounds, enabling even higher sensitivity in analysis.



High robustness minimizes downtime

The newly-developed IonFocus unit introduces ions into the mass spectrometer with greater efficiency while expelling unneeded neutral particles, reducing matrix effects and contamination inside the instrument. The new ion guides, UF-Qarray II and the UF-Lens II, increase the robustness of the instrument while maintaining a high ion transmission rate.

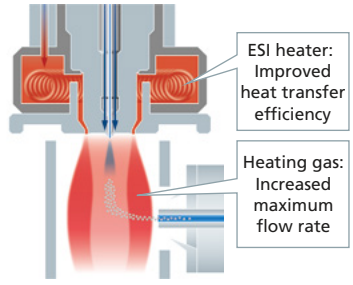
Excellent ease-of-use for greater workflow efficiency

New parameters enable high sensitivity without manual optimization, while features such as automated start-up and shutdown (with LabSolutions Connect MRM) allow unattended operation. Combining the MS with the Nexera series UHPLC provides multiple Analytical Intelligence functions to further increase the efficiency of your overall workflow.

ANALYTICAL INTELLIGENCE

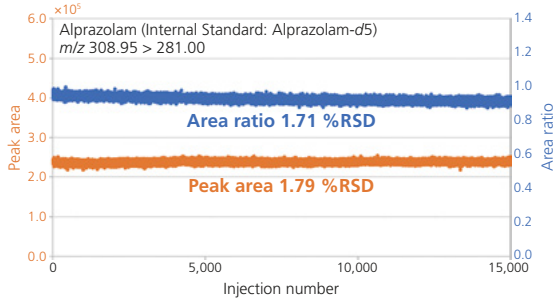
Increased desolvation efficiency for higher sensitivity

A new heat-assisted design improves the desolvation efficiency and dramatically enhances the sensitivity for challenging molecules such as steroid hormones.



Further improved robustness

The excellent robustness of the LCMS-8060NX can be seen most clearly during consecutive analyses of samples of biological origin that tend to contaminate instruments. We performed the consecutive analyses of human blood plasma spiked with alprazolam on the LCMS-8060NX and plotted the resulting area values and area ratios (internal standard material: alprazolam-*d*5). To evaluate the robustness under even more demanding conditions, we performed a total of 15,000 consecutive analyses without using valves to remove impurities. As shown below, the results were extremely stable, with an area value reproducibility of 1.79 %RSD and an area ratio reproducibility of 1.71 %RSD.



Results from Consecutive Analyses of Alprazolam-Spiked Human Blood Plasma

Gas Chromatograph Mass Spectrometer

GCMS-QP2020 NX

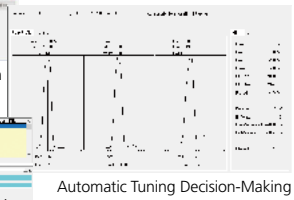
Smart Solutions for Maximizing the Potential of Laboratories

GC-MS systems, which are used in all sorts of fields, have now become general-purpose analytical tool. Consequently, customers are increasingly demanding GC-MS systems that offer higher performance for the cost and enable a better work-life balance for operators. The GCMS-QP2020 NX maximizes the potential of laboratories by offering efficiency improvements for various aspects of analytical work.

Active Time Management

Active time management helps visualize how much time was spent on maintenance, switching between systems, or performing analyses, for example, to help manage the instrument downtime more appropriately. By automating tasks previously performed by users, it enables more efficient system operation.

Automatic System Startup/Shutdown

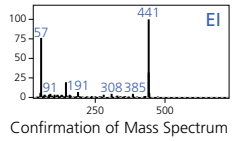


Automatic Tuning Decision-Making

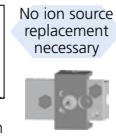
Time Management for Continuous Analysis

Smart EI/CI Ion Source

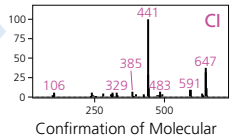
The newly developed Smart EI/CI ion source can be used to acquire CI data without exchanging ion sources or losing the general applicability of EI sensitivity. With the EI mode, even if identification is difficult using a mass spectral library, molecular weight information can be collected from the CI mode data, which is especially useful for predicting unknown compounds.



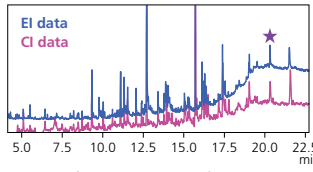
Confirmation of Mass Spectrum



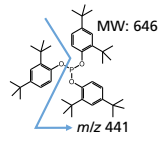
Smart EI/CI ion source



Confirmation of Molecular Weight Information



Example of Analyzing Extract from a Packaging Container (Extracted with 20 % Ethanol)



Based on the EI mass spectrum and CI molecular weight information, the substance is presumed to be tris(2,4-di-*tert*-butylphenyl) phosphite, used as an oxidation inhibitor.

GC-MS Application System

Off-Flavor Analyzer

This analysis system can reliably identify the substances responsible for off-flavor problems. In order to accurately identify substances causing the odor, expertise and experience are required to know what components are responsible for the off-flavor problems, to discriminate the quality of their odors, and to use odor thresholds for those discriminations.

The system provides a database of the major odor-causing substances, as well as sensory information (odor qualities and odor thresholds), for use in combination with GC-MS.

GC-MS Application System

AOC-6000 Plus Multifunctional Autosampler System

The AOC-6000 Plus supports multiple sample injection methods including liquid sample injection, headspace (HS) injection and solid phase micro extraction (SPME). Consequently, it can be used for analyzing samples in a wide range of formats. Furthermore, it can automatically switch between sample injection methods, so that a combination of different sample injection methods can be used within a single sequence of operations. New functions for managing syringe and fiber usage history support accurate analysis.

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