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Application News

No.M271

Gas Chromatography Mass Spectrometry

Investigating Food Quality Evaluation: Complete Analysis of Aroma Compounds and Metabolites in Food

There are a wide variety of methods of ensuring food quality evaluation, and which method is used depends on the food type and purpose of examination. An evaluation method widely implemented in recent years has been to analyze all various compounds present in food, then to use multivariate analysis to find trends in these compounds that can be linked to food quality.
Much research is being conducted into the measurement of all aroma compounds and metabolites present in food, using these results as an indicator of food qualities such as flavor, functionality, and deterioration.
This Application News presents the results of an experiment that set out to measure aroma compounds and metabolites present in sake, and investigated which of these compounds could be used to distinguish between different sake types. Three commercially available sakes, including a normal quality sake (*futsu-shu*), a sake made with rice and malted rice (*junmai-shu*), and sake made with the rice polished to at least 50 % (*daiginjo-shu*), were analyzed for aroma compounds and metabolites. The main compounds found in these sakes were then identified and analyzed further. We were able to clearly separate a pattern of detected compounds in these three types of sake. Combining this method of quality control with conventional methods such as sensory evaluation will allow for the collection of more precise and revealing quality control data.

1. Analysis of Aroma Compounds

Sample

Three sakes of different brands were obtained as samples.
To these sakes were added ultrapure water and 1 mg/mL of an aqueous solution of 3-octanol to prepare samples that contained 10 % ethanol and 0.5 mg/L of 3-octanol. From each sample prepared in this manner was taken 1 mL, which was added to a headspace sampler vial, to which was added 0.5 g of sodium chloride. The samples were confirmed to be saturated with sodium chloride. These vials were then inserted into a headspace sampler and used for analysis.

Analytical Conditions

Table 1 Analytical Conditions for Aroma Compound Analysis

Headspace sampler	: HS-20
Triple quadrupole gas chromatograph mass spectrometer	: GCMS-TQ8040
HS	
Mode	: Trap
Trap Tube	: Tenax GR
Number of Multi-Injections	: 5
Oven Temperature	: 70 °C
Sample Line Temperature	: 150 °C
Transfer Line Temperature	: 150 °C
Vial Pressurization Gas Pressure	: 100 kPa
Vial Warming Time	: 10 min
Vial Pressurization Time	: 2 min
Pressurization Equalization Time	: 0.1 min
Loading Time	: 1 min
Loading Equalization Time	: 0.1 min
Injection Time	: 2 min
Needle Flush Time	: 5 min
Sample Charged Volume	: 1 mL
GC	
Column	: HP-INNOWax (60 m × 0.25 mm I.D., 0.25 µm)
Carrier Gas	: He
Control Mode	: Linear velocity (25.5 cm/sec)
Injection Method	: Split
Split Ratio	: 3
Oven Temperature	: From 40 °C (5 min) by (3 °C/min) to 240 °C (15 min)
MS (EI Method)	
Ion Source Temperature	: 200 °C
Interface Temperature	: 200 °C
Tuning Mode	: Standard
Measurement Mode	: Scan (*m/z* 35 to 350)
Event Time	: 0.3 seconds

Results

Samples of the three sake types were labeled as *futsu-shu*, *junmai-shu*, and *daiginjo-shu*. Taking the results from analysis, peak identification was performed based on the NIST 14 library and quantitative ions, reference ions, and retention indices mentioned in previous articles*. The numbers of compounds identified are shown in Table 2. The 86 compounds detected by this analysis are also listed in Table 3.

Table 2 Numbers of Compounds Detected by Aroma Compound Analysis

	Futsu-shu	*Junmai-shu*	*Daiginjo-shu*
Detected compounds	78	76	86

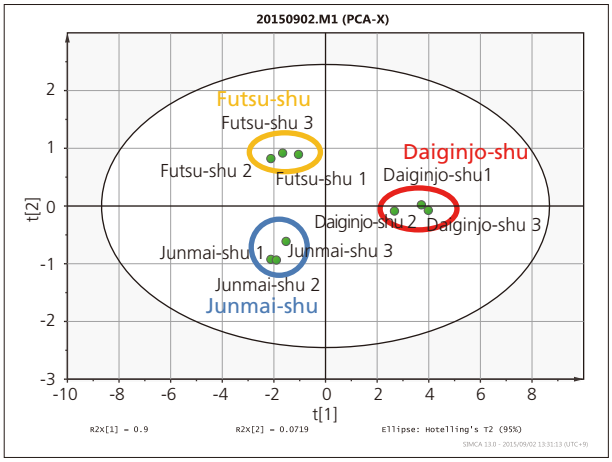

Fig. 1 Score Plot of Aroma Compound Analysis

Principal Component Analysis (PCA) was performed for the 76 compounds detected in all samples. A score plot of this analysis is shown in Fig. 1. The three different sake types are clearly separated on the score plot. A loading plot of this analysis is shown in Fig. 2. Compounds characteristic to each sample were identified from these results.
The results suggest that performing a complete analysis of aroma compounds and subsequent multivariate analysis of identified compounds may be useful for food quality evaluation.

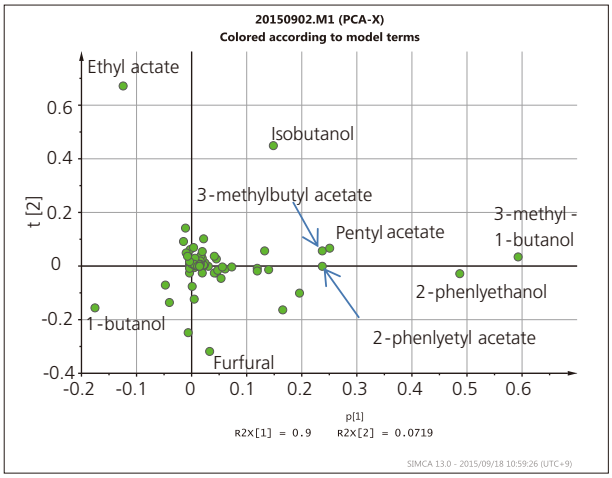

Fig. 2 Loading Plot of Aroma Compound Analysis

Table 3 List of Compounds Detected by Aroma Compound Analysis (86 Compounds)

ethyl acetate	3-methylbutyl propanoate	2-ethyl-1-hexanol	1-decanol
3-methylbutanal	4-pentenyl acetate	decanal	β-citronellol
2, 4, 5-trimethyl-1,3-dioxolane	3-methyl-1-butanol	2-nonanol	diethyl pentanedioate
ethyl propanoate	ethyl hexanoate	ethyl 3-hydroxybutanoate	ethyl phenylacetate
ethyl 2-methylpropanoate	3-octanone	benzaldehyde	2-phenylethyl acetate
propyl acetate	styrene	ethyl 2-hydroxyhexanoate	2- (2-butoxyethoxy) ethyl acetate
2, 3-butanedione	hexyl acetate	propanoic acid	hexanoic acid
isobutyl acetate	2-octanone	1-octanol	benzyl alcohol
ethyl butanoate	octanal	3-methylbutyl methoxyacetate	diethyl hexanedioate
1-propanol	acetoin	ethyl 3-methylthiopropanoate	butylated hydroxytoluene
ethyl 2-methylbutanoate	2-heptanol	ethyl decanoate	2-phenylethanol
ethyl 3-methylbutanoate	3-methyl-1-pentanol	butyrolactone	heptanoic acid
butyl acetate	ethyl heptanoate	1-nonanol	phenol
DMDS	ethyl lactate	acetophenone	dehydromevalonic lactone
1- (1-ethoxyethoxy) pentane	1-hexanol	phenylacetaldehyde	octanoic acid
isobutanol	3-ethoxy-1-propanol	furanmethanol	ethyl hexadecanoate
3-methylbutyl acetate	2-nonanone	ethyl benzoate	decanoic acid
ethyl pentanoate	ethyl octanoate	diethyl succinate	2-phenylethyl octanoate
1-butanol	1-heptanol	(Z)-3-nonen-1-ol	benzoic acid
ethyl 2-butenoate	3-methylbutyl hexanoate	3-methylthio-1-propanol	dodecanoic acid
pentyl acetate	acetic acid	pentanoic acid	
2-heptanone	furfural	naphthalene	

2. Analysis of Metabolites Present in Foods

Sample

Next, metabolites present in foods were extracted from each sample, derivatized, and analyzed by GC-MS.
We took 20 µL of each sample, added 60 µL of an aqueous solution of ribitol (0.2 mg/mL) as an internal standard solution, and dried this mixture thoroughly in a centrifugal concentration device. To the dried residue was added 100 µL of a methoxyamine hydrochloride/pyridine solution (20 mg/mL), and this mixture was shaken at 30 °C for 90 minutes. Subsequently, 50 µL of *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, and the mixture was shaken at 37 °C for 30 minutes. This sample was then added to a GC-MS vial and used for analysis.

Analytical Conditions

Table 4 Analytical Conditions for Analysis of Metabolites Present in Foods

Triple quadrupole gas chromatograph mass spectrometer	: GCMS-TQ8040
Optional software	: Smart Metabolites Database
GC	
Column	: BPX5 (30 m × 0.25 mm I.D., 0.25 µm)
Carrier Gas	: He
Control Mode	: Linear velocity (39.0 cm/sec)
Injection Method	: Split
Split Ratio	: 30
Oven Temperature	: From 60 °C (2 min) by (15 °C/min) to 330 °C (3 min)
MS (EI method)	
Ion Source Temperature	: 200 °C
Interface Temperature	: 280 °C
Tuning Mode	: Standard
Measurement Mode	: MRM
Loop Time	: 0.25 seconds

Table 5 Numbers of Compounds Detected by Analysis of Metabolites Present in Foods

	Futsu-shu	*Junmai-shu*	*Daiginjo-shu*
Detected compounds	147	140	149

Results

Taking the results from analysis, peak identification was performed for compounds registered in the Smart Metabolites Database based on their quantitative ions, reference ions, and retention indices. The numbers of compounds identified are shown in Table 5. The 149 compounds detected by this analysis are also listed in Table 6.
Principal Component Analysis (PCA) was performed for the 138 compounds detected in all samples. A score plot of this analysis is shown in Fig. 3. The three different sake types are clearly separated on the score plot. A loading plot of this analysis is shown in Fig. 4. Compounds characteristic to each sample were identified from these results.
The results suggest that performing a complete analysis of metabolites and subsequent multivariate analysis of identified compounds may be useful for food quality evaluation.

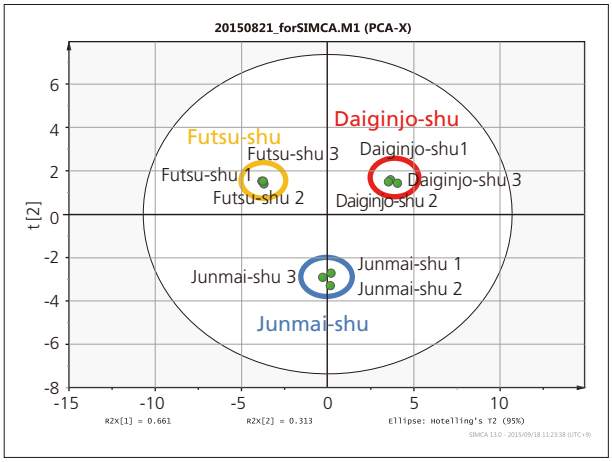

Fig. 3 Score Plot of the Analysis of Metabolites Present in Foods

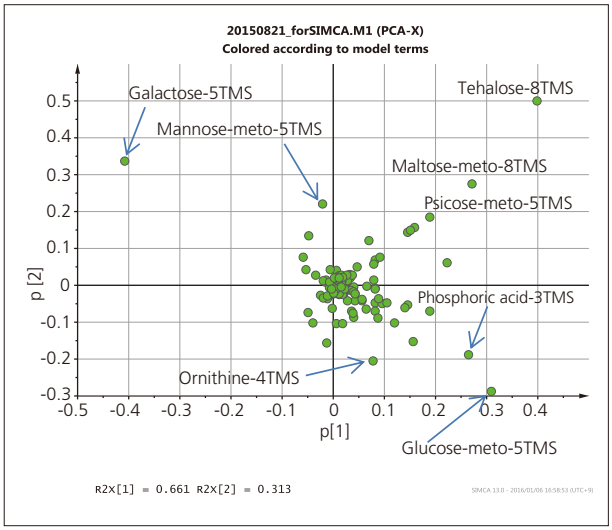

Fig. 4 Loading Plot of the Analysis of Metabolites Present in Foods

Table 6 List of Compounds Detected by Analysis of Metabolites Present in Foods (149 Compounds)

2-Aminobutyric acid	Aspartic acid	Histidine	Ornithine
2-Aminoethanol	Batyl alcohol	Homocysteine	Palmitic acid
2-Aminopimelic acid	Benzoic acid	Homoserine	Pantothenic acid
2-Deoxy-glucose	Cadaverine	Hydroxylamine	Phenylacetic acid
2-Hydroxybutyric acid	Caproic acid	Hypotaurine	Phenylalanine
2-Hydroxyglutaric acid	Citramalic acid	Hypoxanthine	Phenylpyruvic acid
2-Hydroxyisocaproic acid	Citric acid	Indol-3-acetic acid	Phosphoric acid
2-Hydroxyisovaleric acid	Cystamine	Isocitric acid	Proline
2-Isopropylmalic acid	Cystathionine	Isoleucine	Psicose-meto
2-Ketoglutaric acid	Cysteine	Lactic acid	Putrescine
3-Aminoglutaric acid	Cystine	Lactitol	Pyridoxamine-4TMS
3-Aminopropanoic acid	Cytidine	Lactose	Pyruvic acid
3-Hydroxy-3-methylglutaric acid	Cytosine	Lauric acid	Ribitol
3-Hydroxybutyric acid	Decanoic acid	Leucine	Ribose
3-Hydroxyglutaric acid	Dihydroxyacetone phosphate	Lysine	Saccharopine
3-Hydroxyisobutyric acid	Dopamine	Lyxose	Serine
3-Hydroxypropionic acid	Eicosapentaenoic acid	Maleic acid	Stearic acid
3-Methoxy-4-hydroxybenzoic acid	Elaidic acid	Malic acid	Succinic acid
3-Phenyllactic acid	Fructose	Maltitol	Tagatose
4-Aminobutyric acid	Fumaric acid	Maltose	Threitol
4-Hydroxybenzoic acid	Galactose	Mannito	Threonic acid
4-Hydroxyphenylacetic acid	Galacturonic acid	Mannose 6-phosphate	Threonine
4-Hydroxyproline	Glucose	Mannose	Thymine
5-Aminolevulinic acid	Glucuronic acid	Margaric acid	Trehalose
5-Aminovaleric acid	Glutamic acid	meso-Erythritol	Tryptophan
5-Methoxytryptamine	Glutamine	Methionine	Tyramine
5'-Methylthioadenosine	Glutaric acid	Methylsuccinic acid	Tyrosine
5-Oxoproline	Glyceric acid	Mevalonic lactone	Uracil
Acetylglycine	Glycerol 2-phosphate	Myristic acid	Urea
Aconitic acid	Glycerol 3-phosphate	N6-Acetyllysine	Uridine
Adenine	Glycero	N-Acetylmannosamin	Valine
Alanine	Glycine	Nicotinic acid	Xanthine
Allose	Glycolic acid	Nonanoic acid	Xylito
Arabinose	Glycyl-Glycine	Norvaline	Xylose
Arabitol	Glyoxylic acid	Octanoic acid	Xylulose
Arginine	Guanine	Octopamine-4TMS	
Ascorbic acid	Hexanoylglycine	Oleic acid	
Asparagine	Histamine	O-Phosphoethanolamine	

[References]
* Natsuki Mimura, Atsuko Isogai, Kazuhiro Iwashita, Takeshi Bamba, and Eiichiro Fukusaki.
Gas chromatography/mass spectrometry based component profiling and quality prediction for Japanese sake
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