

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 (<i>m/z</i> 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

| | <i>Futsu-shu</i> | <i>Junmai-shu</i> | <i>Daiginjo-shu</i> |
|--------------------|------------------|-------------------|---------------------|
| Detected compounds | 78 | 76 | 86 |

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.

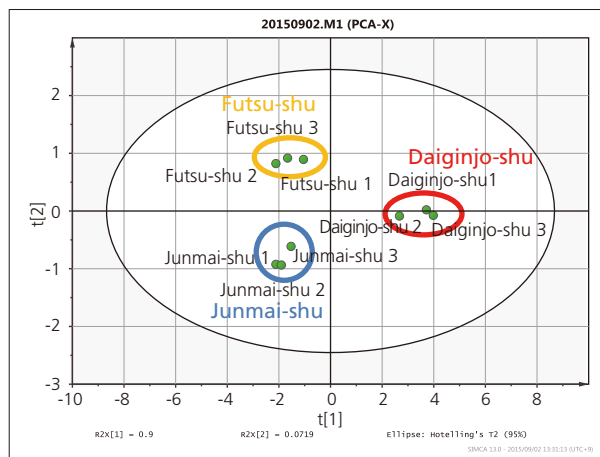


Fig. 1 Score Plot of Aroma Compound Analysis

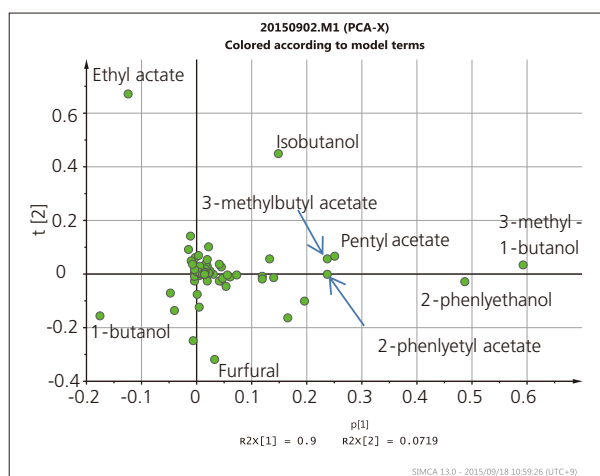


Fig. 2 Loading Plot of Aroma Compound Analysis

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-butenolate | 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 $^{\circ}\text{C}$ for 90 minutes. Subsequently, 50 μL of *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, and the mixture was shaken at 37 $^{\circ}\text{C}$ 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 \times 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 $^{\circ}\text{C}$ (2 min) by (15 $^{\circ}\text{C}/\text{min}$) to 330 $^{\circ}\text{C}$ (3 min) |
| MS (EI method) | |
| Ion Source Temperature | : 200 $^{\circ}\text{C}$ |
| Interface Temperature | : 280 $^{\circ}\text{C}$ |
| Tuning Mode | : Standard |
| Measurement Mode | : MRM |
| Loop Time | : 0.25 seconds |

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

| | <i>Futsu-shu</i> | <i>Junmai-shu</i> | <i>Daiginjo-shu</i> |
|--------------------|------------------|-------------------|---------------------|
| 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.

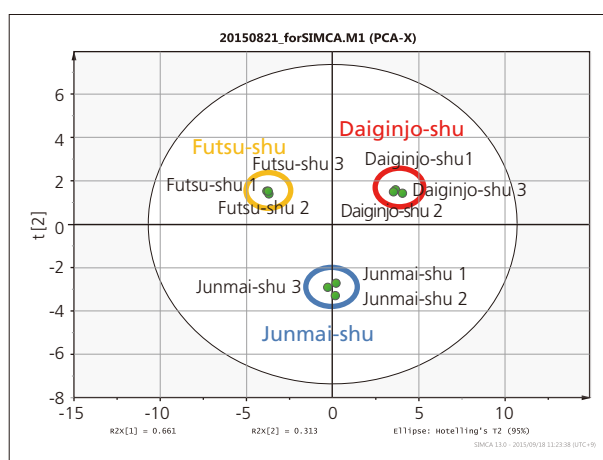


Fig. 3 Score Plot of the Analysis of Metabolites Present in Foods

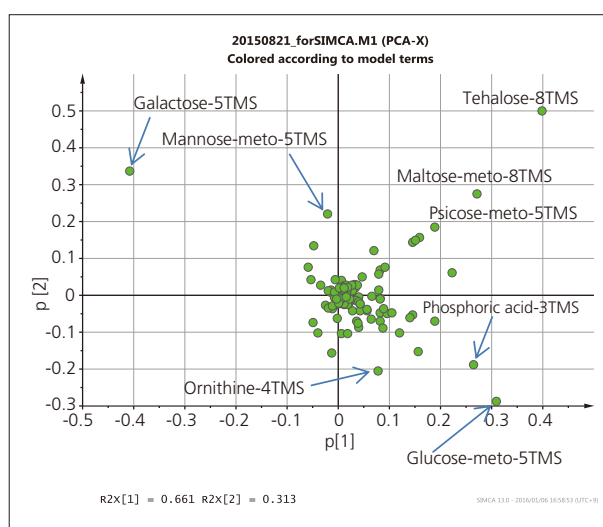


Fig. 4 Loading Plot of the Analysis of Metabolites Present in Foods

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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