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Technical Report

Investigation of Components that Affect Flavors and Visualizing Differences in Tastes

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Abstract:

Sensory analysis of food is based on the five senses—taste, smell, touch, sight, and hearing. In recent years, there has been a growing need to supplement sensory analysis, which depends on the subjective sensations of assessors, with instrumental analysis used to search for components that affect flavors and to visualize differences in tastes. However, searching for components that contribute to flavors is not easy and the process flow is not well established. Identifying a flavor index based on something ambiguous, other than with the five tastes, is even more difficult.

This report describes the attempt to search for components that contribute to the ambiguous characteristic in sake of "fukurami", which is the sensation of flavor expanding in the mouth, in an effort to establish a process flow for scientifically indicating flavor characteristics and differences in food development. Using partial least squares-discriminant analysis (PLS-DA), specific sugars, organic acids, and aroma components that differed depending on the presence of fukurami were identified.

Keywords: Foodomics, sensory analysis, taste, smell, flavor, aroma, aroma components, multivariate analysis, principal component analysis, discriminant analysis, liquid chromatography-mass spectrometry, LCMS, gas chromatography-mass spectrometry, GCMS

1. Background

Food manufacturers mainly conduct sensory analysis of food products either for quality control purposes by a panel of trained specialists[1)], to determine individual preferences by a panel of consumers or to evaluate prototypes within companies (by an internal company panel)[2)]. Sensory analysis of flavor is performed using the five senses, namely taste (sweetness, saltiness, acidity, bitterness, and umami), smell (fragrance, aroma, and odor), touch (texture and temperature), sight (appearance [color, shape, shininess, and size]), and hearing (sound). Taste is determined by interactions between chemical substances, such as amino acids, organic acids, and nucleic acids, whereas smell is determined by aromatic interactions between volatile substances, such as esters or carboxylic acids. There are a wide variety of compounds that affect flavor, with different compounds for each flavor.

In recent years, there has been a growing need to supplement sensory analysis, which depends on the subjective sensations of assessors, with instrumental analysis used to search for and quantitate components that affect flavors in order to visualize differences or control quality. However, due to the large number of components in food, searching for them is not easy, and the steps involved in that process are not well established. Identifying components in terms of something that is ambiguous, other than with the five tastes, is even more difficult.

This report describes the attempt to search for components that contribute to the ambiguous characteristic in sake of fukurami, which is the sensation of flavor expanding in the mouth, in an effort to establish a process flow for scientifically indicating characteristics of flavors and differences in food development. Considering that fukurami is affected by an interaction between multiple components, this report describes creating a fukurami characterization model using candidate characterization markers and then using the model to predict the results of sensory analysis of unknown samples.

2. Samples and Sensory Analysis

It has been reported[3)] that the higher the alcohol content of sake is, the more the smell of alcohol masks the aroma of the sake. Therefore, the alcohol content of all analytical samples was adjusted to 15 %. Eight different types of samples with different polishing yield rates (the percentage of polished rice remaining after polishing the brown rice) and yeasts were prepared (Table 1). The flavor components were analyzed using a liquid chromatograph mass spectrometer and the aroma components were analyzed using a gas chromatograph mass spectrometer to determine whether they differed from sensory analysis.

Sensory analysis was performed for the ambiguous characteristics of fukurami, such as the aroma and the balance between sweetness and acidity. The results for all the panels were integrated. Each assessor ranked the eight sake samples from one to eight in order of most to least fukurami, with the mean value of all the assessors listed in order in Table 1. Samples ranked by fukurami from one to four were grouped together as "with" fukurami, whereas samples ranked from five to eight were grouped as "without" fukurami. Then the data was analyzed by statistical analysis. Samples 3 and 6 proved difficult to analyze by sensory analysis due to their unique characteristics, so they were not included in the integrated analytical data. A discriminative model was created using the integrated analytical data, and the two samples that were difficult to analyze by sensory analysis were reviewed to determine whether a scientific explanation was possible.

Table 1 Brewing Conditions and Sensory Analysis Results of the Sake Samples

Sample No.	Brewing Conditions		Sensory Analysis of Fukurami		
	Polishing Yield	Yeast	Rank	Consistency	Fukurami
1	A	X	8	◎	Without
2	A	Y	3	○	With
3	B	Y	6	△	Without
4	B	Z	7	○	Without
5	A	X	2	○	With
6	C	X	4	△	With
7	C	X	1	◎	With
8	A	Y	5	○	Without

◎: The samples were ranked unanimously
○: The samples were mostly ranked as consistent
△: The samples were ranked as inconsistent by some assessors

1 Analytical & Measuring Instruments Division

3. Analytical Conditions

Sake samples were diluted 10 times in water. Then the ion-pair-free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 3 was used to simultaneously analyze and identify all 153 target components (with saccharide added), including amino acids, organic acids, nucleosides, nucleotides, and other hydrophilic metabolites, which are important for metabolomic analysis in life sciences. The HPLC and MS analytical conditions are shown in Table 2.

Table 2 Analytical Conditions (Flavor Components)

[HPLC conditions] (Nexera™)	
Column	: Discovery HS F5-3 (2.1 mmI.D. x 150 mmL, 3 µm),
Mobile phases	: A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Gradient	: 0 %(0-2 min)-25 %(5 min)-35 %(11 min)-50 %(12.5-16 min)-95 %(16.01-19 min)-0 % (19.01-30 min)
Flow rate	: 0.25 mL/min (0-17 min、19.01-30 min)、0.5 mL/min (17.01-19 min)
Injection volume	: 1 µL
[MS conditions] (LCMS-8060)	
Ionization	: ESI (Positive and negative mode)
Mode	: MRM
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 °C
Block heater temp.	: 400 °C
Interface temp.	: 300 °C

A 1 mL sample of sake was placed into a 20 mL HS vial and sealed with fluoropolymer-coated silicone rubber septa. After heating it for 30 minutes at 50 °C, 1 mL of the headspace gas was injected into the GC unit. The NIST/EPA/NIH Mass Spectral Library (NIST20) and Wiley Registry of Mass Spectral Data (11th Edition) were used to detect and identify 21 aroma components that were subject to analysis by the method specified by the National Tax Agency and the BCOJ beer analysis method. The analytical conditions are shown in Table 3.

Table 3 Analytical Conditions (Aroma Components)

[HS] (HS-20)	
Mode	: Loop (1 mL)
Oven Temp.	: 50 °C
Sample Line temp.	: 120 °C
Transfer Line temp.	: 150 °C
Vial Stirring	: OFF
Multi-injection	: 1
Vial Pressure	: 50 kPa
Vial Pressurization Time	: 1 min
Equilibration Time	: 0.1 min
Loading Time	: 0.5 min
Load Equilib. Time	: 0 min
Injection time	: 1 min
Needle Flush Time	: 5 min
[GC] (GC-2010 Plus)	
Column	: Rxi-624Sil MS (60 m × 0.32 mm I.D., 1.8 µm)
Carrier gas	: He
Control mode	: Linear Velocity (30.0 cm/s)
Injection mode	: Split (Split ratio : 20)
Oven Temp.	: 40 °C (5 min) → (10 °C /min) → 250 °C (10 min)
[MS] (GCMS-QP2020)	
Ionization mode	: EI
Ion Source temp.	: 230 °C
Interface temp.	: 230 °C
Ionization Voltage	: 70 V
Emission current	: 60 µA
Tuning mode	: Standard
Measurement Mode	: Scan (*m/z* 29 ~ 400)
Event time	: 0.3 sec

4. Analysis of Flavor Component Data

Rather than analyzing each measurement independently, all measurements were comprehensively analyzed using principal component analysis (PCA) to visualize their differences and identify each sample. Area ratio values (with 2-Morpholinoethanesulfonic acid as an internal standard) obtained by LC/MS analysis of the flavor components were analyzed by PCA using eMSTAT Solution™ statistical analysis software. The results are shown in Fig. 1 and 2. The sake samples could be categorized into two main groups, depending on the type of yeast used. The second quadrant was characterized by monosaccharides and disaccharides, whereas the first and fourth quadrants were characterized by amino acids, such as leucine and valine. The amino acids leucine and valine are known to be taken up by yeast, where they contribute to generating the aroma components isoamyl alcohol and isobutanol, respectively, which are fusel oils[4].

These results were clustered into groups on the score plot depending on differences in brewing conditions, but they showed no correlation with sensory analysis results for fukurami.

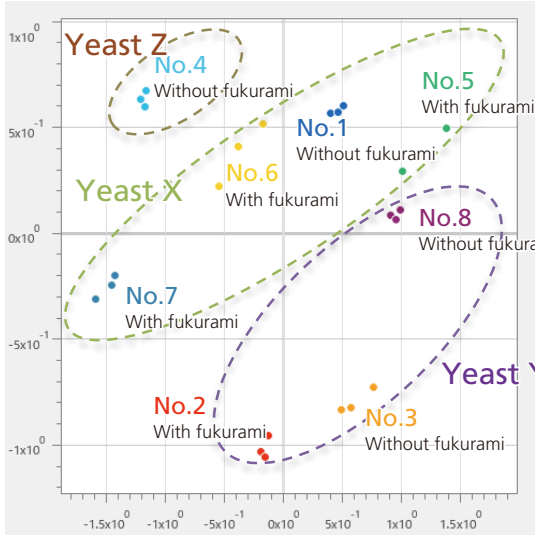

Fig. 1 Score Plot (PCA) of Flavor Components

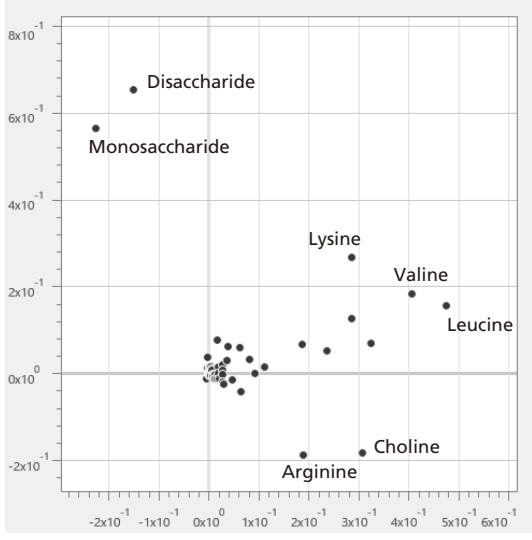

Fig. 2 Loading Plot (PCA) of Flavor Components

Similarly, area values obtained by GC/MS analysis of aroma components were used for PCA. The resulting score plot is shown in Fig. 3 and the loading plot in Fig. 4. Just as with flavor components, the results were grouped depending on the yeast type. Yeast X was characterized by esters and fusel oils such as isobutanol and ethyl acetate. In contrast, yeast Y was characterized by 1-propanol. However, just as with flavor components, the score plot groups did not correlate with the sensory analysis results for fukurami.

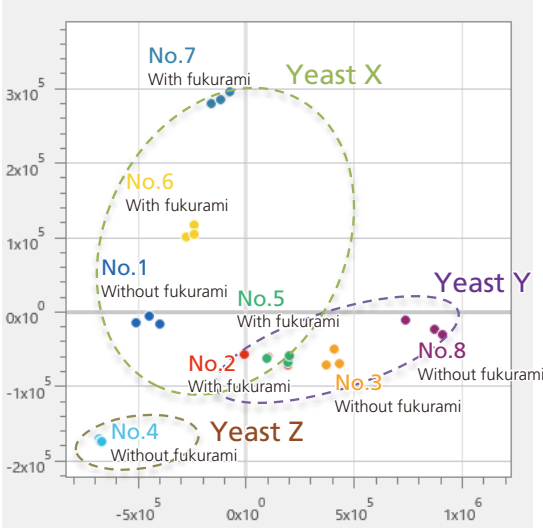

Fig. 3 Score Plot (PCA) of Aroma Components

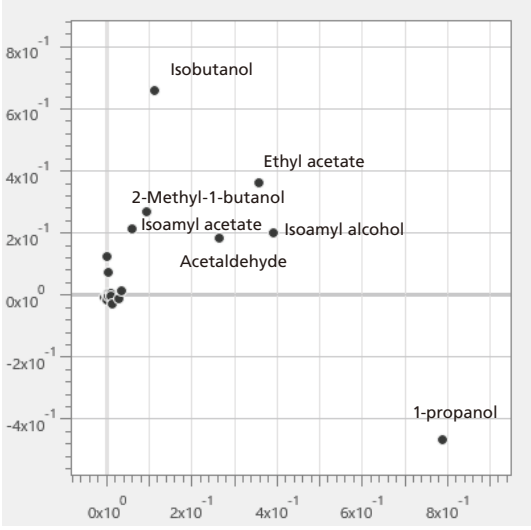

Fig. 4 Loading Plot (PCA) of Aroma Components

5. Evaluation by Integrated Analysis with Sensory Analysis Results and the Discriminative Model

Sensory analysis results (with or without fukurami) and the analytical data for flavor components were used for integrated analysis using PLS-DA with the direction of increasing intergroup differences selected. The resulting score plot is shown in Fig. 5 and the loading plot in Fig. 6. The group without fukurami clustered in the negative region of the score plot (Fig. 5) from the first principal component (PC1). In the loading plot (Fig. 6), amino acids and saccharides appeared in that region and tended to appear more abundantly for samples without fukurami.

The differences between the two groups were analyzed with *t*-test and *u*-test values. The *P*-values (0.05 or less) are shown in Table 4. The components that differed between the two groups included many organic acids, such as malic acid, followed by nucleobases, and amino acids. The box plots were of nucleobase cytidine, organic acid malic acid, saccharide disaccharide, and the amino acid ornithine.

All of the plots included more samples without fukurami than with it. The results suggested that a certain balance between components, such as organic acids (acidity) and saccharides (sweetness), influenced the differences between the groups, rather than whether there was much or little specific component. Assuming a total area value of 100 for the candidate characterization markers in Table 4, Fig. 8 shows the percentage composition of each component. Samples with fukurami had a higher percentage of disaccharides and a lower percentage of malic acid, which means they tended to contain proportionally more sweet components and fewer acidic components. The results also confirmed that they also contained a smaller percentage of the nucleobase cytidine.

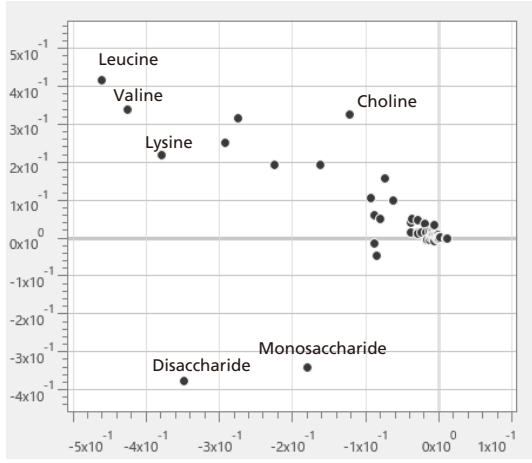

Fig. 6 Loading Plot (PLS-DA) of Flavor Components

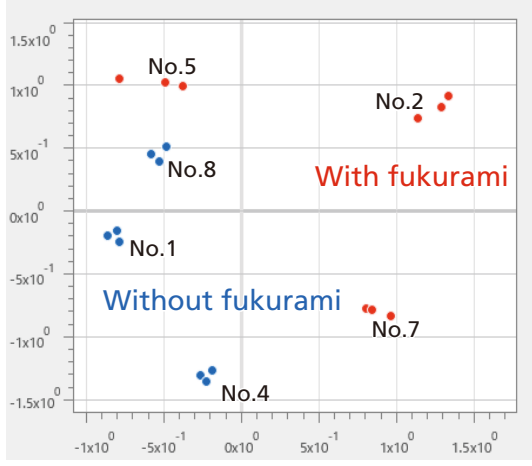

Fig. 5 Score Plot (PLS-DA) of Flavor Components

Table 4 *P*-Values of Flavor Components

Compound (LC/MS Method Package for Primary Metabolites Ver. 3)	*P*-value		Flavor Characteristics
	t-test	*u*-test	
Cytidine	0.000001	0.000041	
Histamine	0.000055	0.000288	
Adenosine	0.001317	0.000494	
Disaccharide	0.002557	0.002756	Sweetness
Ornithine	0.005324	0.007775	
Adenine	0.0062322	0.010407	
Malic acid	0.007597	0.024434	Sourness
Citric acid	0.031563	0.190250	Sourness
Isocitric acid	0.036889	0.093912	
Glyoxylic acid	0.048956	0.113490	

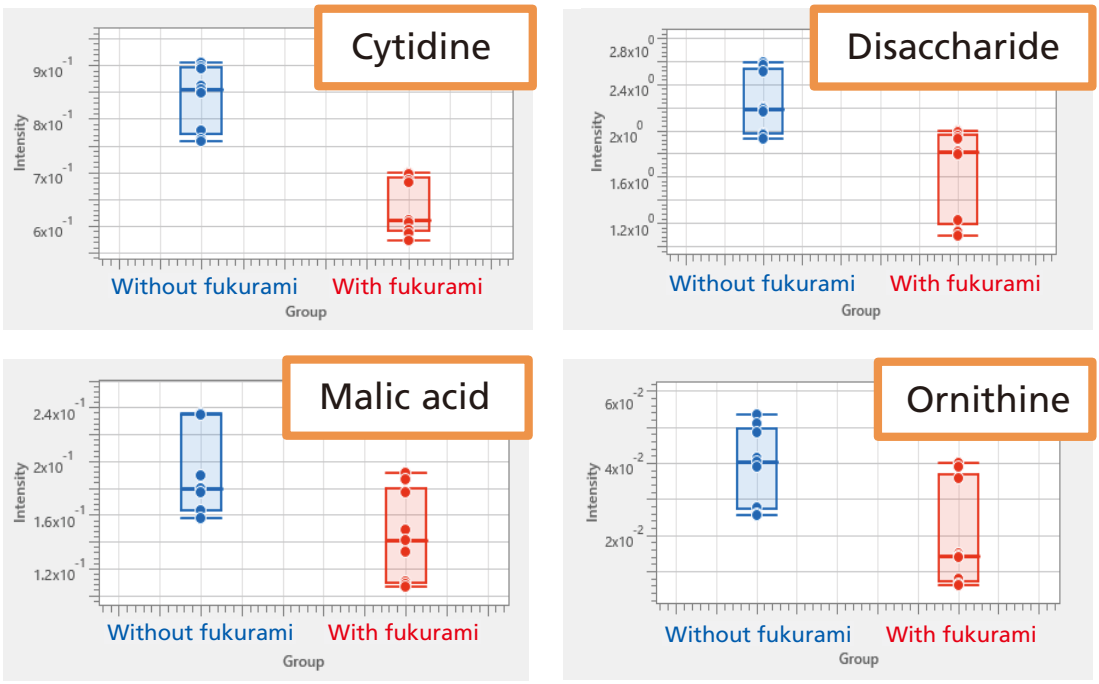

Fig. 7 Box Plot (PLS-DA) of Flavor Components

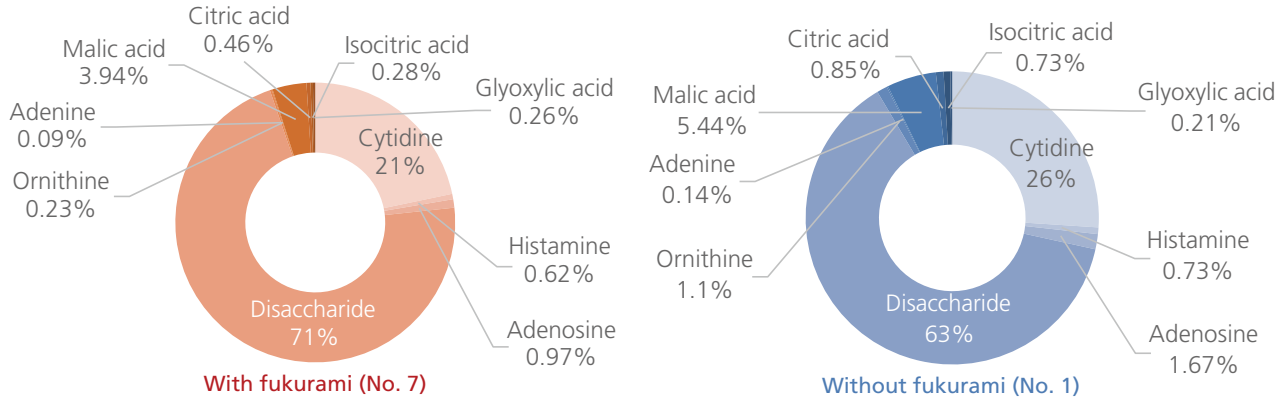

Fig. 8 Percentage Composition of Candidate Characterization Markers for Fukurami

Next, whether or not it is possible to determine from the balance of flavor components if a sample is "with" or "without" fukurami was investigated. Data from known samples (training data) were used to create a discriminant analysis model for determining which group unknown samples belonged to. If a model involves too many explanatory variables it can cause overfitting, so only the candidate characterization markers (Table 4) were used with eMSTAT Solution software to create the discriminative model (the Support Vector Machine algorithm). Excluding the data for samples 3 and 6, which were difficult to characterize by sensory analysis, data from samples 1, 4, and 8 (without fukurami) and 2, 5, and 7 (with fukurami) were used for training. Using the discriminative model that had been created, the groups for sample 3 (without fukurami) and sample 6 (with fukurami) were successfully differentiated by discriminant analysis (Table 5).

Table 5 Discrimination Results for Unknown Samples

Sample	Sample No. 3			Sample No. 6		
Repeat No,	1	2	3	1	2	3
Group (discrimination result)	Without fukurami	Without fukurami	Without fukurami	With fukurami	With fukurami	With fukurami
Score	100	100	100	100	100	100
Cytidine	14439502	14281103	14691370	7713973	7717597	8282329
Histamine	407178	413855	400957	210084	212920	232184
Adenosine	---	---	---	346401	366569	368241
Disaccharide	34008084	34718275	35002609	48041752	48676300	47720288
Ornithine	477553	509141	505657	334852	317064	328420
Adenine	370565	354402	339789	334590	353056	391475
Malic acid	5106722	5013678	5126396	2097430	2207456	2129184
Citric acid	1748690	1870895	1795181	177285	202658	183412
Isocitric acid	1415712	1570172	1412197	112938	112667	112690
Glyoxylic acid	126021	110586	144080	200200	199104	218363

Analytical data for aroma components was used for integrated PLS-DA analysis in the same workflow as for flavor components. The resulting score plot is shown in Fig. 9 and the loading plot in Fig. 10. Components in samples with fukurami were mostly grouped near the first quadrant and characterized by isobutanol, isobutyl acetate, and ethyl acetate, based on the loading plot.

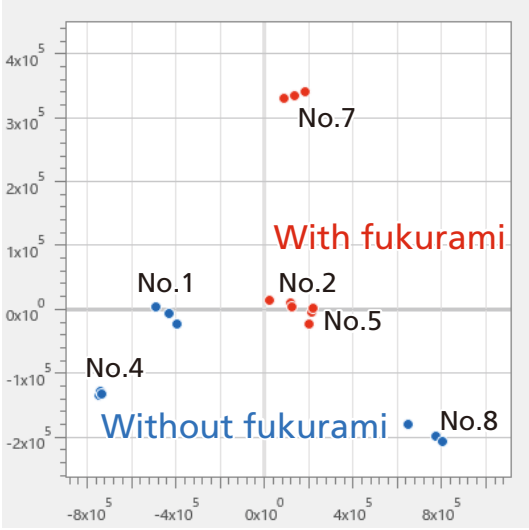

Fig. 9 Score Plot (PLS-DA) of Aroma Components

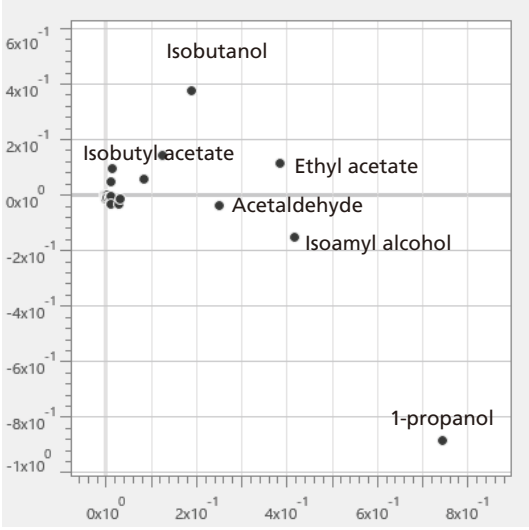

Fig. 10 Loading Plot (PLS-DA) of Aroma Components

Candidate characterization markers for the *P*-values determined from *t*-test and *u*-test analysis (0.05 or less resulted in only one component, so 0.2 or less was specified for the t-test) are shown in Table 6. Box plots (Fig. 11) showed a tendency for higher quantities of isobutanol and isobutyl acetate in samples with fukurami, which confirmed they could be potential candidate components for characterization.

Table 6 *P*-Values for Aroma Components

Compound (GC/MS NIST 20, 11th Edition)	*P*-value		Characteristics of Aroma Components
	t-test	*u*-test	
Isobutanol	0.043052	0.050309	
Isobutyl acetate	0.1046	0.60481	Fruity, banana-like aroma

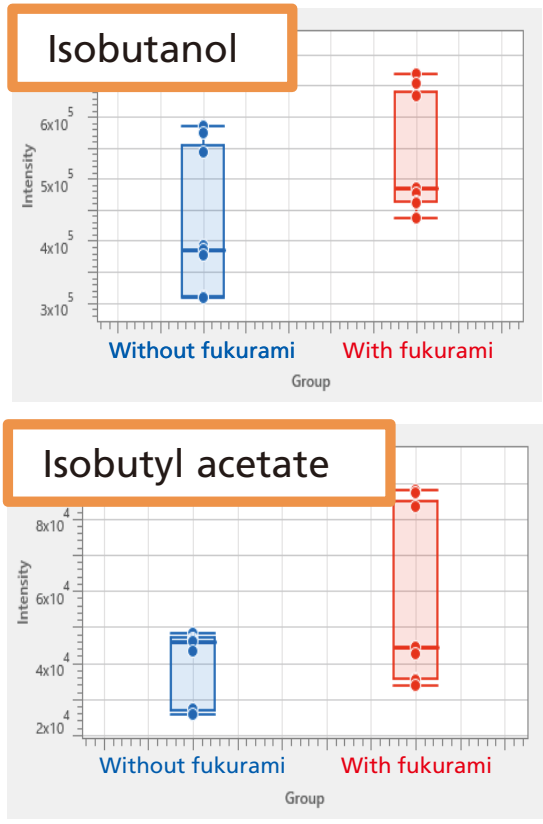

Fig. 11 Box Plot (PLS-DA) of Aroma Components

For the aroma components, the components in Table 6 were used to create a discriminative model. Using this model, sample 3 (without fukurami) and sample 6 (with fukurami) were successfully differentiated by discriminant analysis (Table 7).

Table 7 Discrimination Results for Unknown Samples

Sample	Sample No. 3			Sample No. 6		
Repeat No,	1	2	3	1	2	3
Group (discrimination result)	Without fukurami	Without fukurami	Without fukurami	With fukurami	With fukurami	With fukurami
Score	53	50	51	71	73	74
Isobutanol	459354	471397	467961	561832	572163	577937
Isobutyl acetate	43286	43744	45125	55372	51578	53556

6. Conclusions

- The relationship between the flavor of a sake and its constituent components was investigated. Components related to flavor, such as saccharides, amino acids, organic acids, and components from yeast metabolism were analyzed by LC/MS while the aroma components were analyzed by GC/MS.
- eMSTAT Solution software was used for multivariate (PCA and PLS-DA) integrated analysis of sensory analysis results and for components that affected fukurami.
- From the PLS-DA results, disaccharide (sweetness), malic acid (acidity), and other specific saccharide and organic acids were identified as flavor components that determined whether or not a sake had fukurami. It was also discovered that samples with fukurami tended to contain high levels of the aroma components isobutanol and isobutyl acetate.
- The results suggest that a balance between sweetness and sourness, the presence of specific amino acids or nucleobases, and isobutyl acetate that gives sake a fruity, banana-like aroma, all contribute to producing fukurami, which is sensed as a sweet flavor or sweet aroma with a somewhat lingering aftertaste.
- In addition, those components were used to create discriminative models that can help differentiate between samples that are difficult to analyze by sensory analysis.

Reference
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4) Kiyoshi Yoshizawa, Journal of the Brewing Society of Japan, 75, 6, p. 451–457 (1980)

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