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Technical Report

Science-Based Evaluation and Visualization of Sake Flavors and Providing this Information to Consumers

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Abstract:

One of the appeals of Japanese sake is the wide variety of tastes and aromas (flavors) offered by sake brands and individual products. Sake makers want to be able to describe to consumers the unique flavor characteristics of sake brewed within a specific "terroir" in a way that is also easy to understand. A visual representation of evidence-based data that describes the flavor characteristics of sake can help consumers select the sake products that best suit their preferences.
This article reports on an attempt to analyze aroma components in sake by gas chromatography-mass spectrometry and taste-related components by liquid chromatography-mass spectrometry, respectively, and use a statistical analysis of these data to create a visual distribution of sake flavors. This article also creates an example flavor map that can be used to position sake based on flavor profiles.
Keywords: **Foodomics, sensory evaluation, taste, smell, flavor, aroma, aroma components, multivariate analysis, principal component analysis, liquid chromatography-mass spectrometry, LC/MS, gas chromatography-mass spectrometry, GC/MS**

1. Background

"Terroir" is a French word encompassing the environmental factors unique to a region, such as its soil, climate, and people. Terroir is considered a determining factor in the flavor of wines and other fermented products and is used to identify regions of origin as product brands. In recent sake production, sake makers deal directly with rice farmers to secure a rice variety, quality, and quantity of ingredients, and produce a wide variety of sake influenced by local ingredients (rice and water) and climate.
The sensation called "flavor" experienced when drinking sake is a combination of many sensual elements such as aroma, taste, texture, and temperature. Various surveys have shown that flavor plays an important role in sake purchases for the majority of people[1)]. The ability to convey the flavor characteristics of sake that have been brewed with advanced techniques and within a complex "terroir" in simple and widely recognizable terms would allow consumers to select the products that best suit their preferences.
Of the factors that make up "flavor," this report focuses on the composition and content of taste-related components and aroma components in sake and attempts to visualize and develop a science-based representation of the flavor characteristics of sake brewed at three sake breweries.

2. Sake Samples

Eleven sake brewed at three Kamikawa Taisetsu breweries in Hokkaido (located in Kamikawa, Obihiro, and Hakodate, Fig. 1) with high-quality natural water and Hokkaido-grown rice suitable for sake brewing were analyzed. The rice remaining (40 to 70 %), koji (yellow koji or white koji), rice variety, and other brewing conditions used to produce each sake are shown in Table 1.

3. Sake Analysis and Data Analysis

Aroma components were analyzed using a gas chromatograph-mass spectrometer and taste-related components were analyzed using a liquid chromatograph-mass spectrometer. To identify which components impart samples with their characteristic flavor, instead of analyzing each data point individually, all data were analyzed together using principal component analysis to visualize the variation present in the data set. This data analysis was performed using eMSTAT Solution™ statistical analysis software. (Reference: C190-E290 Technical Report, Investigation of Components that Affect Flavors and Visualizing Differences in Tastes)

4. Aroma Component Analysis

10 mL samples of sake were added to a 20 mL headspace vial and sealed. The sealed headspace vial was then heated at 60 °C for 10 minutes before purging and trapping the headspace and flowing it into a gas chromatography system. The Smart Aroma Database™ was used to detect and identify up to 24 aroma components. The area data for each component was then used in principal component analysis.
Principal component analysis is an analytical technique that takes a large dataset of multidimensional observations and efficiently aggregates and expresses them in graphical forms. All observations are presented together in a two-dimensional representation that enables visual interpretation. Within this representation, a new coordinate axis (the first principal component [PC1, x-axis]) is created that best accounts for the variability present in this dataset of multidimensional observations. Another coordinate axis, the second principal component (PC2, y-axis), is also created that accounts for much of the variability that remains in this dataset but was not fully explained by the first principal component.

Table 1 Sake Brewing Conditions

Product Name	Brewery	Water	Koji	Rice	Rice remaining
Kamikawa Taisetsu Junmai Daiginjo	Ryokkyugura	Soft water	Yellow koji	Kitashizuku	40 %
Kamikawa Taisetsu Junmai Ginjo				Ginpu	50 %
Kamikawa Taisetsu Tokubetsu Junmai				Suisei	60 %
Junmai Kamikawa				Suisei	70 %
Yamahai Kamikawa Taisetsu			White koji	Ginpu	50 %
Tokachi Junmai Daiginjo	Hekiungura	Medium-hard water	Yellow koji	Kitashizuku	45 %
Tokachi Junmai Ginjo				Ginpu	55 %
Junmai Tokachi				Suisei	70 %
Goryo Junmai Daiginjo	Goryonokura	Soft water	Yellow koji	Suisei	45 %
Goryo Junmai Ginjo				Ginpu	55 %
Junmai Goryo				Suisei	70 %

Fig. 1 Three Brewery Locations

1. Shimadzu Corporation, 2. College of Gastronomy Management, Ritsumeikan University, 3. Kamikawa Taisetsu Sake Brewery

On score plots, points that appear close to each other are considered similar to one another, allowing the identification of products with similar (or dissimilar) characteristics. Loading plots are also used to verify which components in the samples account for the differences visualized in the score plot. For samples on the score plot in the same relative position as components on the loading plot, components positioned further from the origin on the loading plot have the greatest influence on these samples.

Examining the score plot of aroma components (Fig. 2) reveals three Junmai Daiginjo-type sake that are grouped close to each other. According to the loading plot (Fig. 3), ethyl hexanoate has a strong influence on these three sake. The score plot and loading plot also show the aroma characteristics of Yamahai Kamikawa in the top left region are influenced by isoamyl acetate and ethyl acetate, and the aroma characteristics of Kamikawa Tokubetsu Junmai in the bottom right region are influenced by 2-phenylethanol.

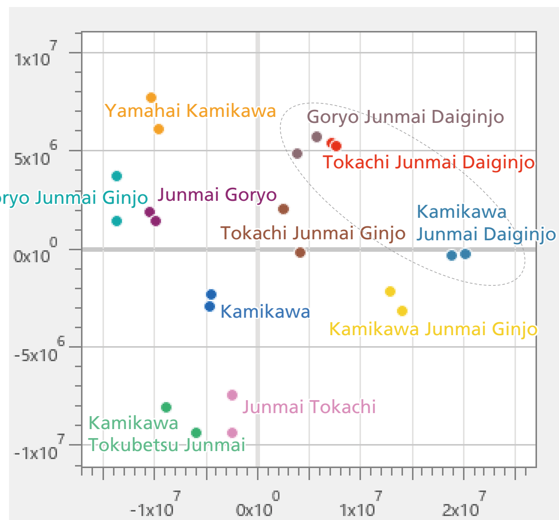

Fig. 2 Score Plot (PCA) of Aroma Components

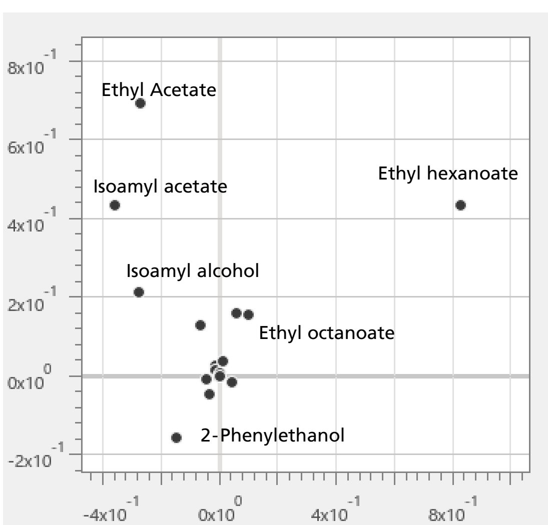

Fig. 3 Loading Plot (PCA) of Aroma Components

Aroma is the combined effect of a wide range of chemical substances. To survey which component groups impart aroma characteristics to particular sake, an analysis of variance (ANOVA) was used to identify differences in aroma components between groups of three or more sake. This analysis was performed on aroma components with *P*-value < 0.05 (Table 2).

Table 2 Aroma Components that Differ Between Sake (*P*-value < 0.05)

Compound Name	Aroma Characteristic
Ethyl hexanoate	Apple-like ginjo aroma
Isoamyl acetate	Banana-like ginjo aroma
Isoamyl alcohol	Base note
Isobutanol	Base note
2-Phenylethanol	Base note

Ethyl hexanoate, which has an aroma likened to fresh, acidic fruits such as green apples and pears, and isoamyl acetate, which has an aroma likened to fruits with a full-bodied sweetness such as bananas and melons, are called "ginjo" aromas. These aroma components can also be found in fruit. Ginjo aromas were detected in large amounts in Junmai Daiginjo, Junmai Ginjo, and Yamahai sake types (Fig. 4). Kamikawa Junmai Daiginjo, Tokachi Junmai Daiginjo, and Kamikawa Junmai Ginjo contain a greater proportion of ethyl hexanoate than isoamyl acetate and have a high fragrance. Yamahai Kamikawa sake contains a similar total amount of ginjo aroma components to these three sake but has a greater proportion of isoamyl acetate than ethyl hexanoate giving it a smooth ginjo aroma.

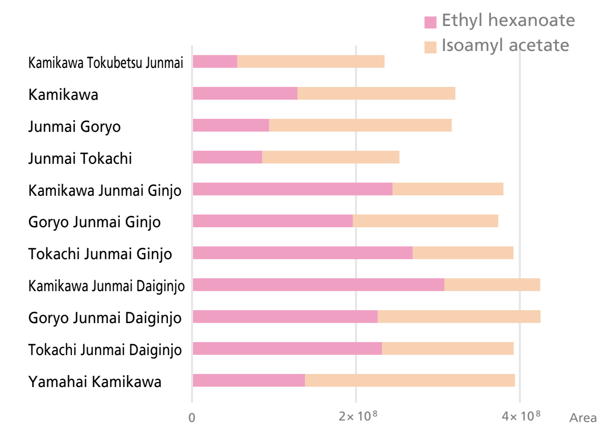

Fig. 4 Ginjo Aroma Compound Content of 11 Sake

The aroma of isoamyl alcohol, isobutanol, and 2-phenylethanol (which imparts a rose-like aroma) are described as base note that are sensed from sake in the mouth[2,3]. These three compounds underpin the aroma of sake and were detected in large amounts in Yamahai and Junmai sake (Fig. 5). Goryo Junmai Ginjo, which is brewed using conditions intended to produce an easy-drinking Ginjo-type sake, contained the least amount of ethyl hexanoate and the greatest amount of base note compounds of all Ginjo sake (Fig. 4), which confirmed the conditions used to brew Goryo Junmai Ginjo achieved their intended purpose.

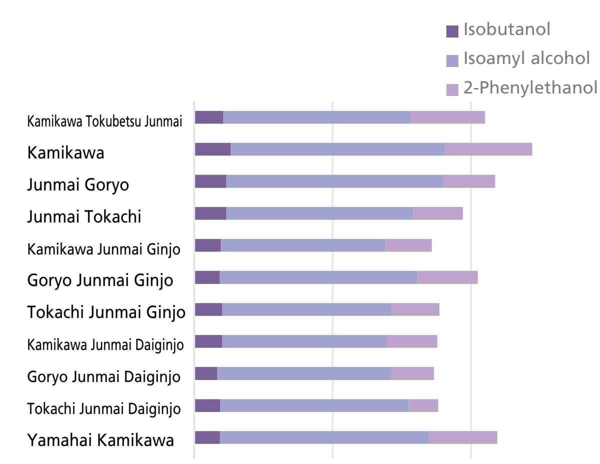

Fig. 5 Base Aroma Compound Content of 11 Sake

5. Analysis of Taste-Related Components

Samples were carried out a simultaneous LC/MS analysis for 151 hydrophilic metabolites that were used to represent taste-related components and included sugars, amino acids, organic acids, nucleosides, and nucleotides. Principal component analysis was then performed on area data as a ratio of an internal standard. The resulting score plot is shown in Fig. 6 and the loading plot in Fig. 7.

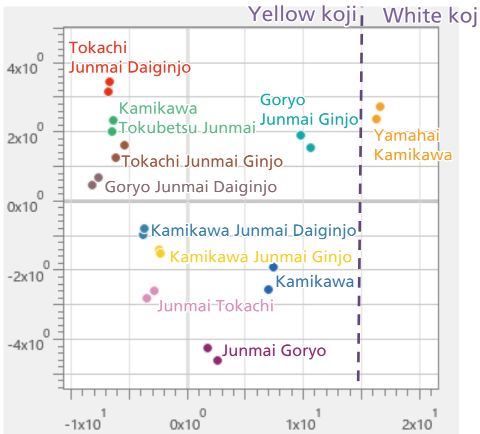

Fig. 6 Score Plot (PCA) of Taste-Related Components

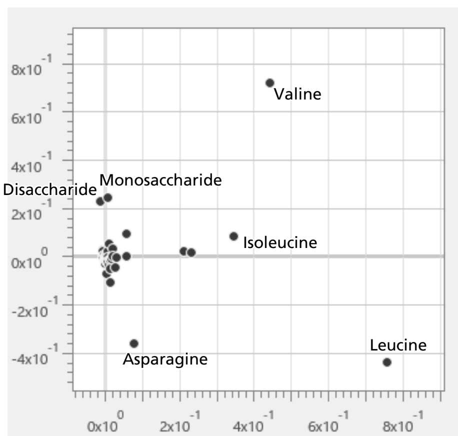

Fig. 7 Loading Plot (PCA) of Taste-Related Components

The score plot separated sake based on the koji used in brewing, with Yamahai Kamikawa made with white koji on the right side and sake made with yellow koji on the left side of the plot. Grouping all yellow koji sake and looking for components that differ from those in white koji Yamahai Kamikawa sake revealed that Yamahai Kamikawa contains a uniquely large amount of citric acid (Fig. 8).

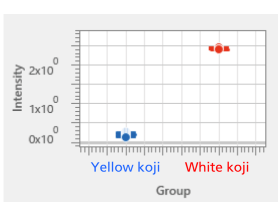

Fig. 8 Box Plot of Citric Acid Content

Sake on the score plot also formed into groups according to rice remaining with the general order of Junmai Daiginjo-type sake (smaller rice remaining), followed by Junmai Ginjo-type sake, then Junmai-type sake (larger rice remaining) from the negative to the positive ends of the x-axis. Tokachi (Hekiungura Brewery) products made with medium-hard water tended to be on the negative side of the x-axis, while Kamikawa (Ryokkyugura Brewery) and Goryo (Goryonokura Brewery) products made with soft water are widely distributed throughout the x-axis.

The loading plot in Fig. 7 shows that sake taste is imparted by amino acids such as leucine, sugars, and organic acids. The loading plot also shows the further toward the positive side of the x-axis the greater the variety and quantity of amino acids. Taste-related components with a *P*-value < 0.05 (Table 3) were examined in greater detail by ANOVA.

Table 3 Taste-Related Components that Differ Between Sake (*P*-value < 0.05)

	Compound Name	Taste Characteristic
Sugars	Monosaccharides	Sweet
	Disaccharides	Sweet
	Trisaccharides	Sweet
Amino Acids	Alanine	Sweet
	Proline	Sweet
	Glutamine	Sweet
	Lysine	Sweet
	Glutamic acid	Acid and Umami
	Aspartic acid	Acid and Umami
	Isoleucine	Bitter
	Leucine	Bitter
	Phenylalanine	Bitter
	Histidine	Bitter
	Tyrosine	Bitter
	Valine	Bitter
Organic Acids	Citric acid	Acid
	Malic acid	Acid
	Lactic acid	Acid
	Succinic acid	Acid

Regardless of the brewery, there were generally more monosaccharides in Ginjo and Daiginjo type sake, which use a lower rice remaining, than in Junmai-type sake, which uses a higher rice remaining (Fig. 9). Comparing sake with equivalent rice remaining showed that Goryo (Goryonokura Brewery) products tend to contain more monosaccharides. The results also show that Goryo products contain more disaccharides and trisaccharides that give a smooth, sweet taste.

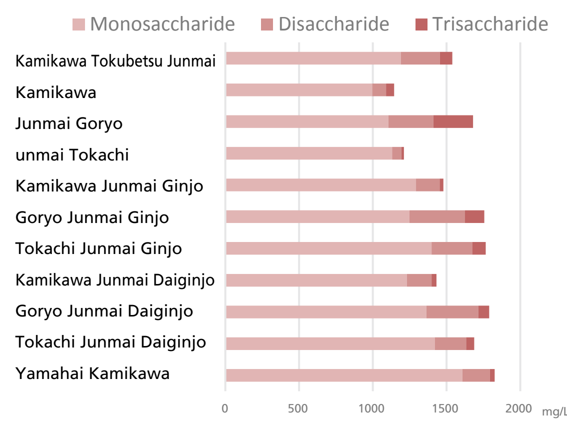

Fig. 9 Sugar Content of 11 Sake

The organic acid content of sake is compared in Fig. 10. Organic acids are known for their fresh, full and rich flavor which impart sake with body[4]. The higher the rice remaining is, the more organic acids tend to be present in sake.

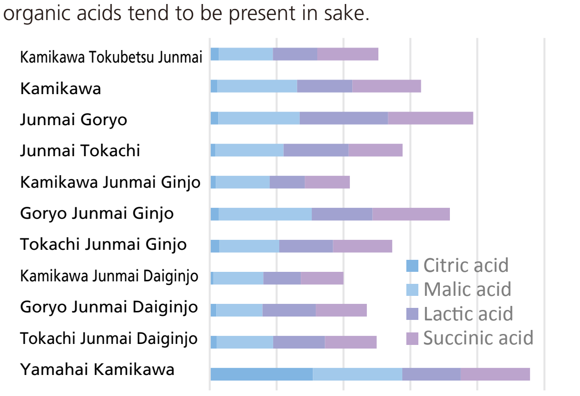

Fig. 10 Organic Acid Content of 11 Sake

Acidity and sweetness are said to cancel each other out while a proper blend of each is thought to impart a rich taste[5]. The sweet taste of Ginjo and Daiginjo-type sake seems to be due to their high sugar content and also low organic acid content. Junmai Goryo and Yamahai Kamikawa have a high total organic acid content, which explains their characteristically strong umami taste and body. Acidic qualities also differ based on the organic acid, with malic acid and citric acid having a pleasant cooling acidity and succinic acid a distinctive umami taste. Junmai Tokachi and Yamahai Kamikawa contain more malic acid than succinic acid, where the malic acid imparts a crisp and refreshing quality.

The amino acid content of sake is compared in Fig. 11. A high amino acid content is said to impart richness and a strong umami taste, while a low amino acid content imparts a light body. Kamikawa (a Junmai-type sake), Goryo Junmai Ginjo and Yamahai Kamikawa (a type of Junmai Ginjo sake that use a rice remaining of 50 to 55 %), contained high quantities of amino acids, which probably impart richness and a strong umami taste to these sake.

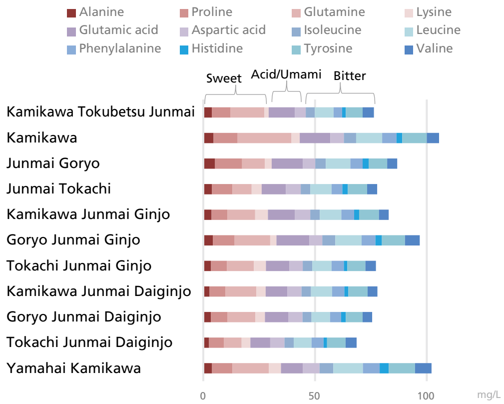

Fig. 11 Amino Acid Content of 11 Sake

The sake fermentation process is affected by the hardness of the brewing water, where harder water (such as water in the Nada district of Kobe) results in a quicker fermentation process and crisp aftertaste, and softer water (such as water in the Fushimi district of Kyoto) results in a smoother fermentation process and mellow taste. Comparing the brewing water used to produce three Junmai-type sake brewed with the same rice variety and rice remaining (Kamikawa, Junmai Goryo, and Junmai Tokachi) showed the sake brewed with soft water contained more organic acids and amino acids. The brewing water used by those breweries is presumed to affect fermentation and produce richness in the sake.

6. A Flavor Map of Characteristic Sake Tastes and Aromas

Up to this point, this report has described the characteristics of taste-related components and aroma components. Since flavor is created by a synergistic effect of taste and aroma, an attempt was made to combine these flavor elements and visualize characteristic sake flavors using a "Flavor Map" that places depth of taste and aroma notes on its axes.

The x-axis of the principal component analysis of taste-related components (the first principal component, Fig. 7) principally reflects the amounts of amino acids and organic acids and the compositional balance of these components in sake, thus the x-axis may be described as showing the depth of taste. The x-axis of the principal component analysis of aroma components (the first principal component, Fig. 2) principally reflects top notes (orthonasal) aroma components that are sensed when sake is brought near the nose and base note components that are released in the mouth and sensed through the retronasal route, thus the x-axis may be described as representing these sake "fragrant" (high/less or orthonasal/retronasal). A flavor map based on these two axes (Patent Application 2023-008000) is shown in Fig. 12.

The flavor map describes the taste and aroma of sake in graphical form and shows a distribution of products compared in terms of depth of taste and aroma. The flavor map shows that the 11 sake tested in this study exhibit a wide range of flavor characteristics, from the light body and high fragrant of Kamikawa Junmai Daiginjo to the rich taste and less fragrant of Yamahai Kamikawa, and demonstrate a product range that meets the needs of a variety of consumers.

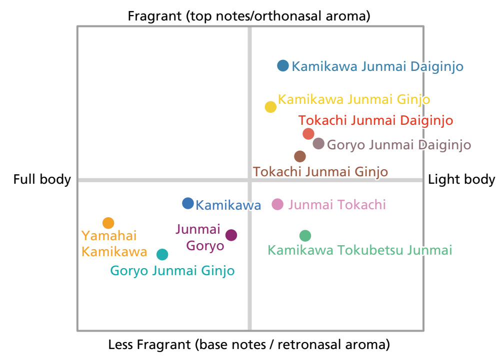

Fig. 12 Sake Flavor Map

7. Conclusion

The flavor characteristics of sake were visualized using taste-related and aroma components in the literature. It's shown that has been associated with specific sake flavors. The component profile of each sake product can vary depending on the year of harvest of the brewing rice and flavor maps that can be updated to reflect these types of changes are useful. Kamikawa Taisetsu Sake Brewery, Otaru University of Commerce, and Shimadzu Corporation are pursuing collaborative research based on the concept of "visualizing the flavor characteristics of food to promote sales of locally produced foods and contribute to regional development." This report attempted to develop a science-based explanation for the flavor characteristics of sake based on taste-related component and aroma components, and further visualizations based on a more comprehensive analysis that incorporates texture and other elements of sake flavor are also planned.

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