Application News

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Liquid Chromatography Mass Spectrometry

# Food Metabolomics Evaluation of Japanese Rice Wine Types Using LC/MS/MS

Metabolomics is an increasingly well-known technology for comprehensively analyzing in vivo metabolites. Food metabolomics is an application of this technology. Conventionally, sensory analysis conducted by human assessors to evaluate tastes, aroma, deliciousness, grades, etc. has been the main method of food evaluation. Food metabolomics is used to scientifically "evaluate/predict the quality" of food and "explore functional ingredients" by comprehensively analyzing the metabolites in food and then analyzing the relationship between the analysis results and the results of evaluations conducted by humans such as sensory analysis.

As an example of food metabolomics, this article introduces an analysis of five types of specially designated Japanese rice wine (sake). In this study, we comprehensively analyzed the hydrophilic components of sake samples with a high performance liquid chromatograph mass spectrometer (LC/MS/MS) and verified the characteristics of each sake type.

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## Samples and Pretreatment

Using five types of sake made by a single manufacturer, we prepared samples by ultra-filtering the supernatant of sake obtained by centrifugation. It was expected that the differences according to manufacturing method could be directly identified since the five types of sake were basically made with the same ingredients, e.g., water and rice. The main categories of specially designated sake are summarized in Table 1 and the sample details are given in Table 2. The term "origarami" in Table 2 refers to sake including "ori" (lees), a mixture of rice and yeast, which settle while the sake is stored in a tank after wringing the liquid from "moromi" (raw unrefined sake).

**Table 1 Classification of Specially Designated Sake**

| Special Designation | Ingredients | Rice Polishing Ratio | Percentage of Koji Rice | Aroma, Flavor, etc. |
|---|---|---|---|---|
| Ginjoshu | Rice, malted rice, brewer's alcohol | 60% or lower | 15% or higher | Ginjo-zukuri method<br>Unique aroma and flavor<br>Good color and clarity |
| Daiginjoshu | Rice, malted rice, brewer's alcohol | 50% or lower | 15% or higher | Ginjo-zukuri method<br>Unique aroma and flavor<br>Especially good color and clarity |
| Junmaishu | Rice, malted rice | – | 15% or higher | Good aroma, flavor, color, and clarity |
| Junmai-ginjoshu | Rice, malted rice | 60% or lower | 15% or higher | Ginjo-zukuri method<br>Unique aroma and flavor<br>Good color and clarity |
| Junmai-daiginjoshu | Rice, malted rice | 50% or lower | 15% or higher | Ginjo-zukuri method<br>Unique aroma and flavor<br>Especially good color and clarity |
| Tokubetsu-junmaishu | Rice, malted rice | 60% or lower or a special production method | 15% or higher | Especially good aroma, flavor, color, and clarity |
| Honjozoshu | Rice, malted rice, brewer's alcohol | 70% or lower | 15% or higher | Good aroma, flavor, color, and clarity |
| Tokubetsu-honjozoshu | Rice, malted rice, brewer's alcohol | 60% or lower or a special production method | 15% or higher | Especially good aroma, flavor, color, and clarity |

* Source: Japan Sake and Shochu Makers Association

**Table 2 Sample Details**

| | Sample |
|---|---|
| 1 | Junmai-daiginjoshu |
| 2 | Junmai-daiginjoshu (origarami) |
| 3 | Junmaishu |
| 4 | Junmaishu (origarami) |
| 5 | Junmaishu<br>(manufactured with a yeast strain different from that of 3) |

## Analysis Conditions

Using the free ion-pair LC/MS/MS method of the LC/MS/MS Method Package for Primary Metabolites Ver. 2, the analysis was conducted with LCMS™-8060. This analysis method enables simultaneous analysis of 97 hydrophilic metabolites including amino acids, organic acids, nucleosides, and nucleotides. These are key metabolites for metabolome analyses in the field of life science. The HPLC and MS analysis conditions are shown in Table 3.

**Table 3 Analysis Conditions**

| | |
|---|---|
| [HPLC conditions] (Nexera™ X2) | |
| Column | : Reversed-phase column |
| Mobile phases | : A) 0.1% Formic acid in water<br>B) 0.1% Formic acid in acetonitrile |
| Mode | : Gradient elution |
| Flow rate | : 0.25 mL/min |
| Injection volume | : 3 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 |

## Metabolome Analysis

Each sake sample was measured by LC/MS/MS. Subsequently, a principal component analysis was conducted with SIMCA™ software using the area ratio of each component to the internal standard. The score plot is shown in Fig 1. It was confirmed that plots vary according to the difference between junmai-daiginjoshu and junmaishu as well as the difference in yeast strains. The loading plot demonstrating the difference in primary main components is shown in Fig. 2, indicating that Junmaishu tends to contain a higher amount of amino acids such as glutamic acid, leucine, threonine, isoleucine, and serine. Conversely, it was confirmed that junmai-daiginjoshu tends to contain a higher amount of organic acids including malic acid and pyruvic acid.

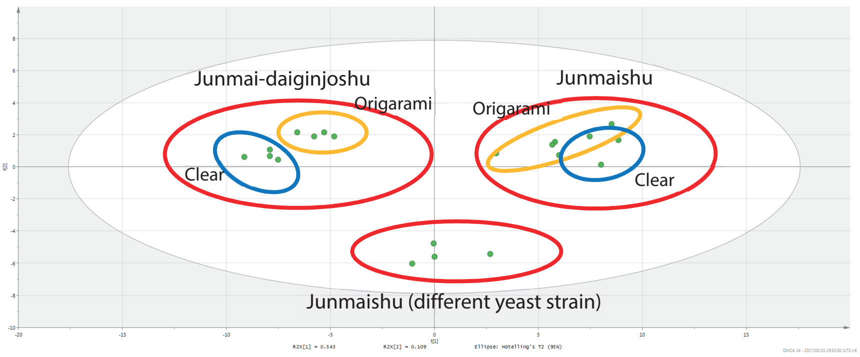


**Fig. 1 Score Plot: Analysis Results of the Main Components of Five Sake Types**

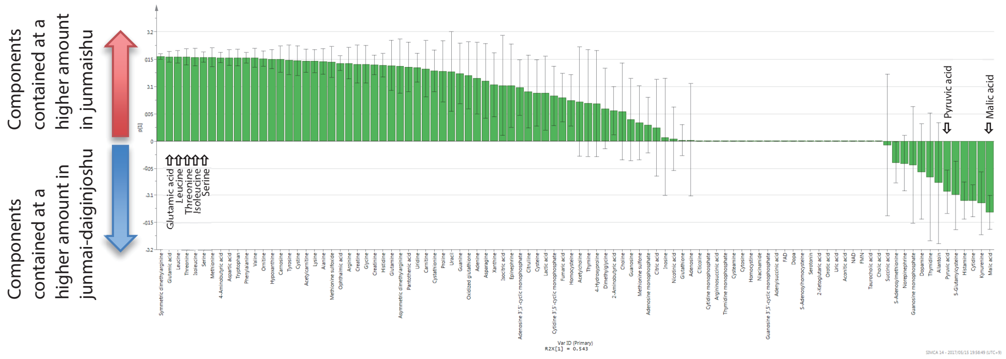


**Fig. 2 Loading Plot: Comparison of Junmai-daiginjoshu and Junmaishu (Primary Main Components)**

As confirmed in this study, it is well known that the amount of amino acids tends to be higher in junmaishu and lower in junmai-daiginjoshu. This is due to the rice polishing ratio. While the rice polishing ratio of the junmai-daiginjoshu sample was 45%, that of the junmaishu sample was 60%. Since protein is removed from the surface of rice when it is highly scraped off, junmai-daiginjoshu contains a lower amount of amino acids. It is generally said that food and beverage items taste mild when they contain an insufficient amount of amino acids and taste too strong when they contain an excessive amount of amino acids. Although we were not able to confirm an association with sensory analyses, from the results junmai-daiginjoshu is predicted to taste light and refreshing compared to junmaishu since it contains a lower amount of amino acids and a higher amount of organic acids such as malic acid. In addition, we compared the difference between origarami and clear junmai-daiginjoshu samples. Although the results are not shown, it was confirmed that origarami tends to contain a higher amount of amino acids. This result suggests that the savory taste (umami) of rice and yeast is stronger in origarami than in the clear one.

Meanwhile, the difference of yeast strains used for manufacturing sake was demonstrated by the secondary main components. The loading plot of secondary main components is shown in Fig. 3. It was confirmed that junmaishu made with a different yeast strain tended to contain a higher amount of glutathione, citric acid, and lactic acid. Glutathione is one of the antioxidants that are said to prevent alcoholic fatty liver disease. The metabolomics approach enables science-based product differentiation with explanations using, for example, the detected components that affect deliciousness or the content of functional ingredients.

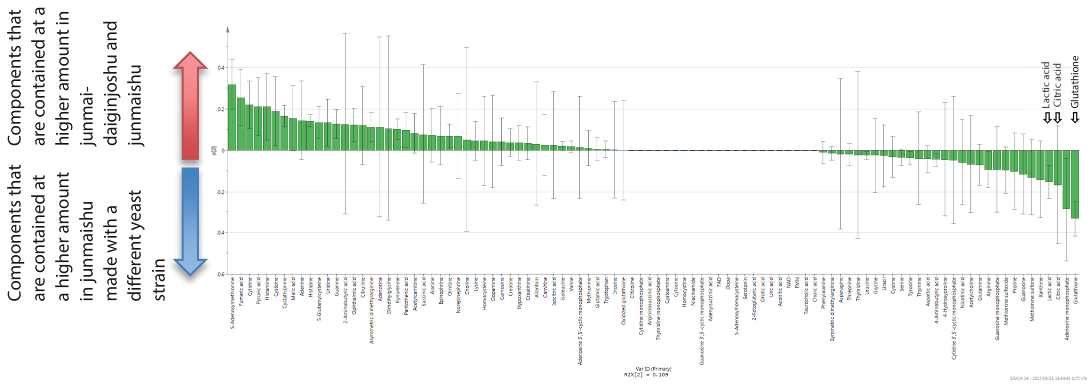


**Fig. 3 Loading Plot: Comparison of Two Junmaishu Types Using Different Yeasts (Secondary Main Components)**



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