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Application News

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Liquid Chromatography Mass Spectrometry

Food Metabolomics Analysis of Deterioration Characteristics of Alcoholic Drinks Using LC/MS/MS

Recently, metabolomics technology has become a hot topic due to its ability to comprehensively analyze in vivo metabolites. Food metabolomics has grown out of this technology allowing its application to food products. Conventionally, sensory analysis conducted by human assessors to evaluate flavors, aroma, deliciousness, grades, etc. has been the main method used in food evaluation. Food metabolomics is used to more scientifically "evaluate/predict the quality" of food and "explore functional ingredients" by comprehensively analyzing the metabolites in food and comparing the findings against those from evaluations conducted by humans such as sensory analysis.

This report describes an analysis method used to determine the deterioration characteristics of foods based on food metabolomics. The samples, commercially available Japanese rice wine (sake) and white wine, were stored under adverse conditions and then separated by high performance liquid chromatography mass spectrometry (LC/MS/MS), followed by multivariate analysis, to comprehensively investigate the changes in hydrophilic metabolites, including amino acids, organic acids, nucleosides, and nucleotides.

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Samples and Deterioration Experiment

The samples were commercially available alcoholic drinks, including two types of sake (kept refrigerated) and a white wine. The characteristics of these samples are shown in Table 1. To perform accelerated deterioration testing, the samples were stored under each of the test conditions shown in Table 2. Alcoholic drinks are currently distributed domestically and internationally and large volumes are imported and exported. Consequently, the ability to transport these beverages without a negative impact on quality is recognized as very important if the value of the products is to be maintained. The experimental conditions used in this study were designed to reproduce the conditions under which the quality of the products might be adversely affected during transportation, including exposure to the sun, high temperatures, and vibration.

Every sample stored under each of the specified conditions was separated by centrifugation at 12,000 rpm for 5 min, and the supernatant was diluted 100-fold with ultrapure water so it could be analyzed by LC/MS/MS.

Table 1. Characteristics of Test Samples

Samples	
Sake No. 1	Junmai-daiginjoshu, rice-polishing ratio = 50%, Alcohol by volume (ABV) = 15%
Sake No. 2	Ginjoshu, brewer's alcohol added, rice-polishing ratio = 50%, Alcohol by volume (ABV) = 15%
White wine	Produced in Australia, antioxidant (sulfite) added, Alcohol by volume (ABV) = 13%

Table 2. Experimental Conditions for Accelerated Deterioration Testing

Storage Conditions	
A	Stored in a refrigerator protected from light for 2 weeks
B	Stored at room temperature exposed to light for 2 weeks
C	Stored in a refrigerator protected from light for 2 weeks, followed by heating to 50° C while protected from light for 24 hours
D	Stored in a refrigerator protected from light for 2 weeks, followed by shaking at room temperature while protected from light for 24 hours.

Analysis Conditions

Using the ion-pairing free LC/MS/MS method of the LC/MS/MS Method Package for Primary Metabolites Ver. 2, the analysis was conducted with LCMS™-8060 (Fig. 1). The analysis method included in the package enables the simultaneous analysis of the 97 hydrophilic metabolites, which are known to be important in 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
		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

Fig. 1. Nexera™ X2 and LCMS™-8060

Metabolome Analysis

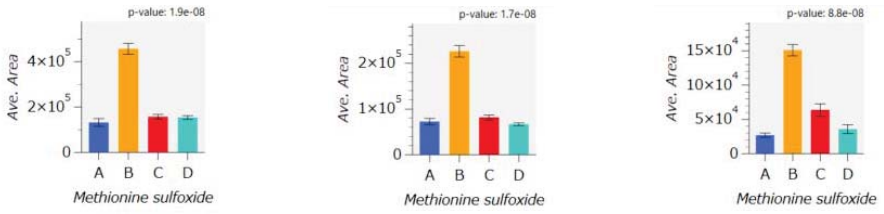
Each sample was measured by LC/MS/MS, and then principal component analysis (PCA) and one-way analysis of variance (one-way ANOVA) were conducted using the areas of each component with Traverse MS software.

When PCA was performed, no apparent difference was observed between the samples stored under different conditions for any of the types of alcoholic drinks tested. In contrast, detailed examination of ANOVA results revealed that some of the components increased or decreased according to the type of alcoholic drink and/or storage conditions. As an example, the results of ANOVA for the effects of storage conditions on sake No. 1 are shown in Fig. 2. The green frames indicate the results for components which showed significant differences ($p<0.05$) between the samples stored under different conditions.

Fig. 2. Results of ANOVA for the Effects of Storage Conditions on the Components in Sake No. 1

In this experiment, some of the components measured in the samples of sake No. 1 stored under condition B were significantly different from those of the samples stored under the other conditions. A similar trend was observed for sake No. 2 and the white wine, showing that some of the experimental conditions, such as heating to 50° C or shaking for around 24 hours, were not sufficient to have a significant impact on hydrophilic compounds, including amino acids and organic acids. It is difficult to draw definitive conclusions based solely on the findings of this study. However, the results suggest that even if the products are accidentally exposed to conditions such as heating and shaking, just for a short period during transportation and/or storage after purchasing the product, this is unlikely to have a significant impact on the quality of the product.

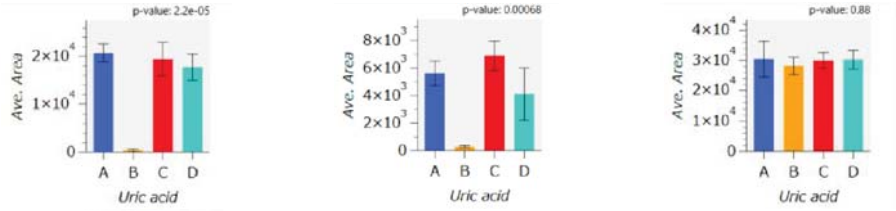
In the samples of sake No. 1, cysteine, methionine sulfoxide, and uric acid were the components which showed significant differences ($p<0.05$) between the samples stored under condition B and those stored under the other conditions. Additionally, there were several other components which showed, for example, a different trend only in the white wine samples. The results of comparing these components in each alcoholic drink tested are shown in Figs. 3 to 6.



Sake No. 1 Sake No. 2 White wine

Fig. 3. Results of ANOVA for Methionine Sulfoxide

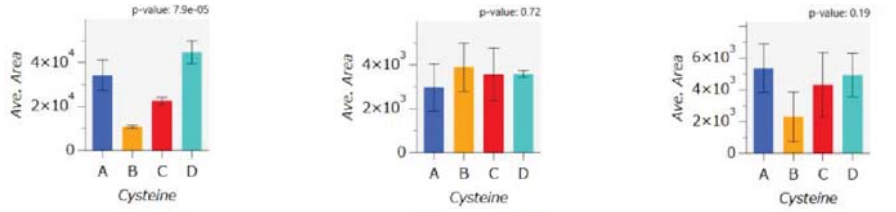
The results of statistical analysis for the methionine sulfoxide contained in each alcoholic drink tested are shown in Fig. 3. The analysis revealed that regardless of the type of alcoholic drink, the level of this component in the samples stored under condition B was markedly higher than those stored under the other conditions. Methionine is known to be an amino acid residue which is more susceptible to aging-associated oxidation and thus considered to be a cause of the increased in vivo oxidative protein damage, and is promptly oxidized to methionine sulfoxide under intracellular oxidative stress conditions. The results of this study suggest the possibility of using methionine sulfoxide as a marker of oxidation of the components of alcoholic drinks.



Sake No. 1 Sake No. 2 White wine

Fig. 4. Results of ANOVA for Uric Acid

Results of statistical analysis for uric acid contained in each alcoholic drink tested are shown in Fig. 4. The analysis revealed that only in the sake samples was the level of this component in those stored under condition B lower than those stored under the other conditions. Uric acid is highly susceptible to oxidation, allowing it to exert a strong antioxidant effect comparable to that of ascorbic acid, which is a known physiological role. It was assumed that uric acid contained in the wine samples was unlikely to undergo oxidation during the storage period because the white wine used in this study had sulfite added as an antioxidant.



Sake No. 1 Sake No. 2 White wine

Fig. 5. Results of ANOVA for Cysteine

Results of statistical analysis for cysteine contained in each alcoholic drink tested are shown in Fig. 5. The analysis revealed that only in sake No. 1 was the level of this component in the samples stored under condition B significantly lower than those stored under the other conditions. Besides methionine, cysteine is known as a precursor of dimethyl trisulfide (DMTS), a major malodorous component of deteriorated sake. Given that the lowered level of cysteine was associated with an increase in DMTS production, it is assumed that addition of brewers alcohol, which was a substantial difference between the samples of sake No. 1 and No. 2, may be a factor that could change the susceptibility of sake to deterioration.

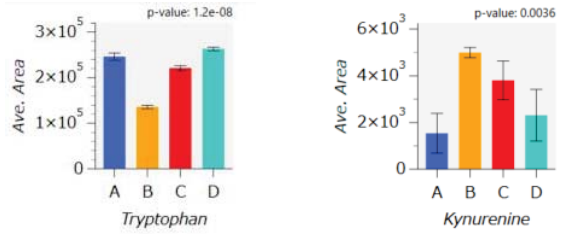


Fig. 6. Results of ANOVA for Tryptophan and Kynurenine in the White Wine Samples

Results of statistical analysis for tryptophan and kynurenine in the white wine samples are shown in Fig. 6. The analysis revealed that the levels of tryptophan and kynurenine in the samples stored under condition B were lower and higher, respectively, than those stored under the other conditions. A similar trend for tryptophan and kynurenine was observed in the samples stored under condition C, although the degree was small. Tryptophan is known to be metabolized to kynurenine through one of its known metabolic pathways, the kynurenine pathway (Fig. 7). Thus, the changes in these components observed in this study appear to correspond to the changes predicted from their relationship to this pathway.

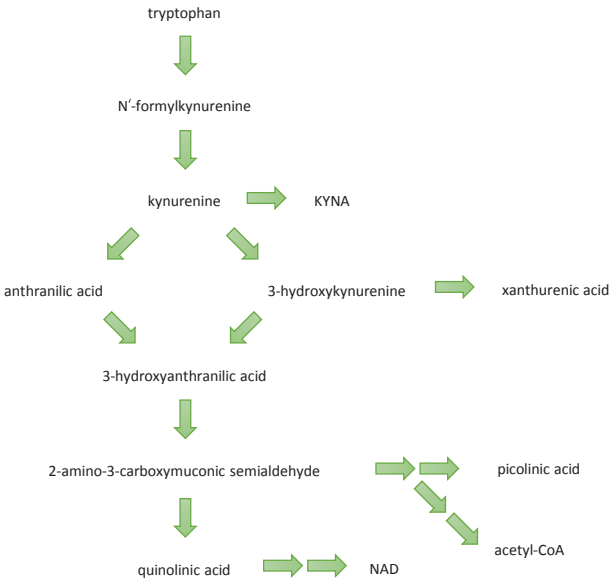


Fig. 7. Kynurenine Pathway

Summary

In conclusion, food metabolomics using LC/MS/MS enabled a comprehensive exploratory analysis of the component(s) that characterize the deterioration of alcoholic drinks.

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