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Application News

LCMS-2050 High-Performance Liquid Chromatograph Mass Spectrometer

Food Metabolomics of Alcoholic Beverage Using Single-Quadrupole Mass Spectrometer

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User Benefits

- Food metabolomics can be performed easily by using single-quadrupole LC/MS.
- Enables simultaneous analysis of 143 compounds including amino acids, organic acids, nucleic acid metabolites, and functional components.
- Even inexperienced metabolomics users can easily perform multivariate analysis by using Multi-omics Analysis Package.

■ Introduction

In recent years, attention has been focused on the technology of metabolomics, which is defined as the comprehensive analysis of metabolites in vivo. Metabolomics is an academic field that comprehensively analyzes low-molecular-weight metabolites, such as amino acids and organic acids, generated by the activities of cells, to clarify differences among multiple sample groups. The application of metabolomics technology to food is called "food metabolomics" and is used for various purposes, such as food quality assessment, quality prediction, improvement of manufacturing and storage processes, and evaluation of functional properties. Food contains a great many metabolites and previous research has revealed many of the metabolites involved in flavor, quality, and functional properties. Therefore, targeted analysis is common in food metabolomics. By focusing on important components and analyzing them comprehensively, metabolomics can efficiently provide useful results.

This article introduces an example of food metabolomics using a single-quadrupole LC/MS system. Compared to triple-quadrupole LC/MS systems, single-quadrupole LC/MS systems are cheaper and involve simpler analytical conditions. So, even those with minimal mass spectrometry experience can easily perform metabolomics.

■ Samples and Pretreatment

For samples, we prepared six types of alcoholic and non-alcoholic beers. Table 1 shows the details of the samples. At dilution, 1 µmol/L 2-morpholinoethanesulfonic acid (MES) was added as an internal standard.

Table 1 Details of Samples

Sample	Description
Beer 1	Lager beer (bottom fermentation)
Beer 2	Ale beer (top fermentation)
Low-malt beer	Purine free
Beer 3	Soy protein as ingredients
Non-alcoholic beer 1	Made in Japan
Non-alcoholic beer 1	Made in Germany

■ Analytical Conditions

LC/MS analysis was performed using a Nexera™ XR HPLC system coupled with an LCMS-2050 single-quadrupole mass spectrometer (Fig. 1). The LCMS-2050 is compact, easy to use, and offers high performance. Equipped with a heated DUIS™ dual ESI and APCI ion source, the system offers a mass range of *m/z* 2 to 2000. These features are especially useful in metabolomics, where metabolites with a wide range of physical characteristics are analyzed.

Fig. 1 Nexera™ XR and LCMS™ -2050

Table 2 shows the analytical conditions for HPLC and MS. Analytical conditions for simultaneous analysis by single-quadrupole LC/MS were developed by referring to the analytical conditions in the ion-pair-free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 3. That enables simultaneous analysis of 143 hydrophilic metabolites, such as amino acids, organic acids, nucleosides, and nucleotides, which are important in food analysis.

Table 2 Analytical Conditions

[HPLC Conditions] (Nexera XR)	
Column:	Shim-pack™ GIST PFPP*1
	(2.1 mmI.D. x 150 mmL., 3.0 µm)
Mobile Phases:	A) 0.1% Formic acid in water
	B) 0.1% Formic acid in acetonitrile
Mode:	Gradient elution
Flowrate:	0.25 mL/min (17 to19 min, 0.5 mL/min)
Injection Volume:	3 µL
[MS Conditions] (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), Positive and negative modes
Mode:	SIM (143 events)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	500 °C
DL Temp.:	250 °C

*1 P/N: 227-30858-07

■ Multivariate Analysis

As a result of simultaneous analysis of hydrophilic metabolites, 82 compounds were detected. The main metabolites were amino acids, organic acids, and nucleoside metabolites. Table 3 shows the number of metabolites detected in each sample. More than 70 compounds were detected in beer 1, beer 2, and non-alcoholic beer 2, but 22 compounds were detected in the low-malt beer, showing a different tendency.

Table 3 Number of Detected Compounds

Beer 1	Beer 2	Low-malt beer	Beer 3	Non-alcoholic beer 1	Non-alcoholic beer 2
76	78	22	57	44	77

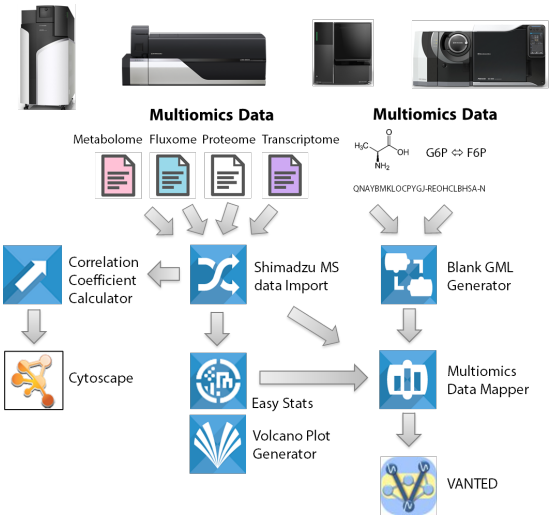

Fig. 2 Multi-omics Analysis Package

The Multi-omics Analysis Package is a metabolic engineering software that can automatically generate metabolic maps and perform a variety of data analyses based on the vast amounts of mass spectrometry data generated in fields such as metabolomics, proteomics, and flux analysis. The Multi-omics Analysis Package can help increase the efficiency of metabolomic data analysis work. The intuitive visualization of data provides powerful support for drug discovery, functionally-enhanced foods, bioengineering, and other life sciences research applications. The package includes gadgets (software tools) for data analysis and gadgets for data processing that are connected to the gadgets for data analysis, making it easy to perform various multivariate analysis tasks, as though using a single software program.

Fig. 3 shows the results of principal component analysis (PCA). From the score plot, the low-malt beer and non-alcoholic beer 1 were plotted close together on the score plot and had similar trends in the amount of hydrophilic compounds. Other beers and non-alcoholic beers were successfully classified and have different features. From the first principal component (PC 1), it was classified into two groups (Group A: low-malt beer, non-alcoholic beer 1, and beer 3; Group B: non-alcoholic beer 2, beer 1, and beer 2). That suggests that the PC 1 shows the difference in ingredients because ingredients that are not used for beer are used for Group A and ingredients that are used for beer are used for Group B.

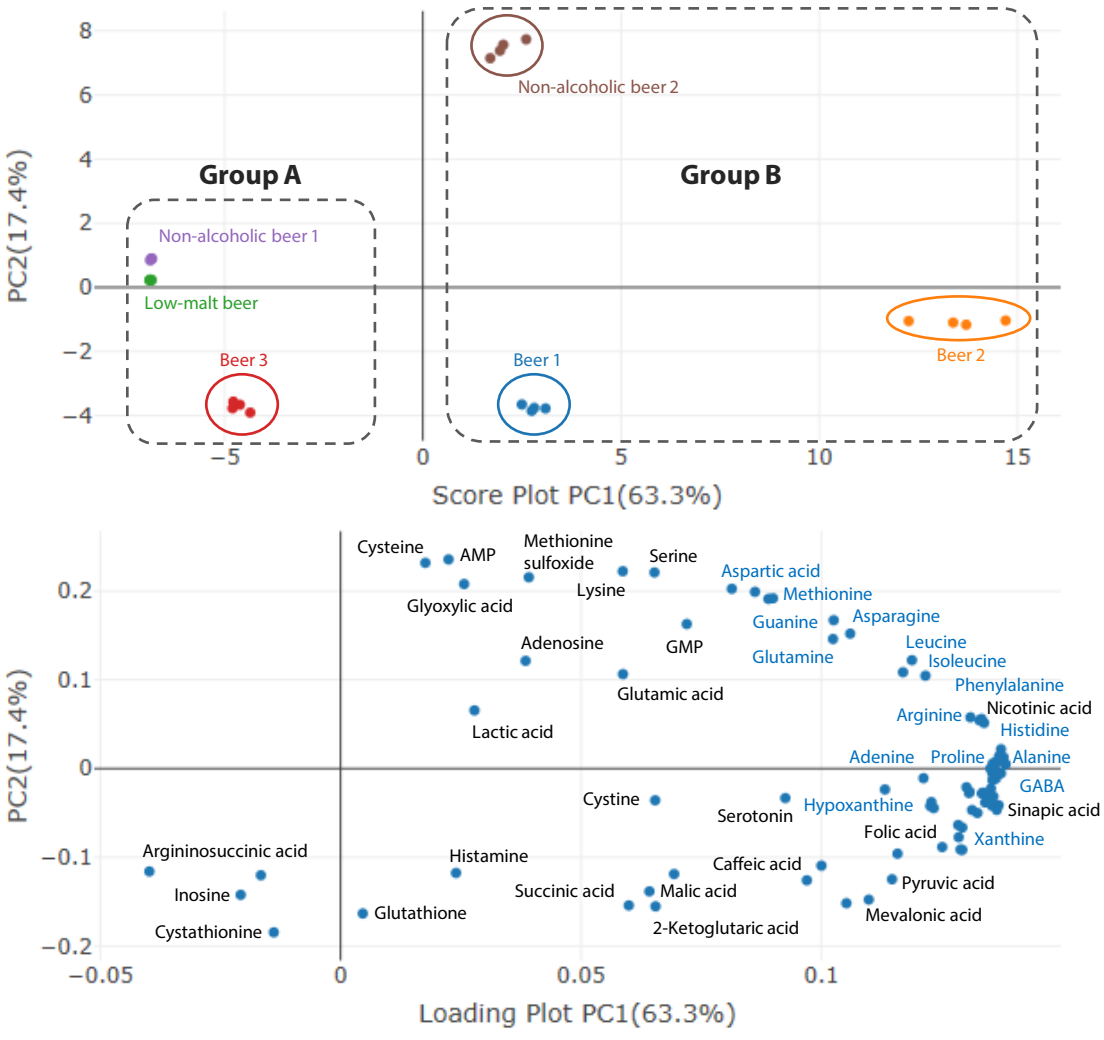

Fig. 3 Results of Principal Component Analysis

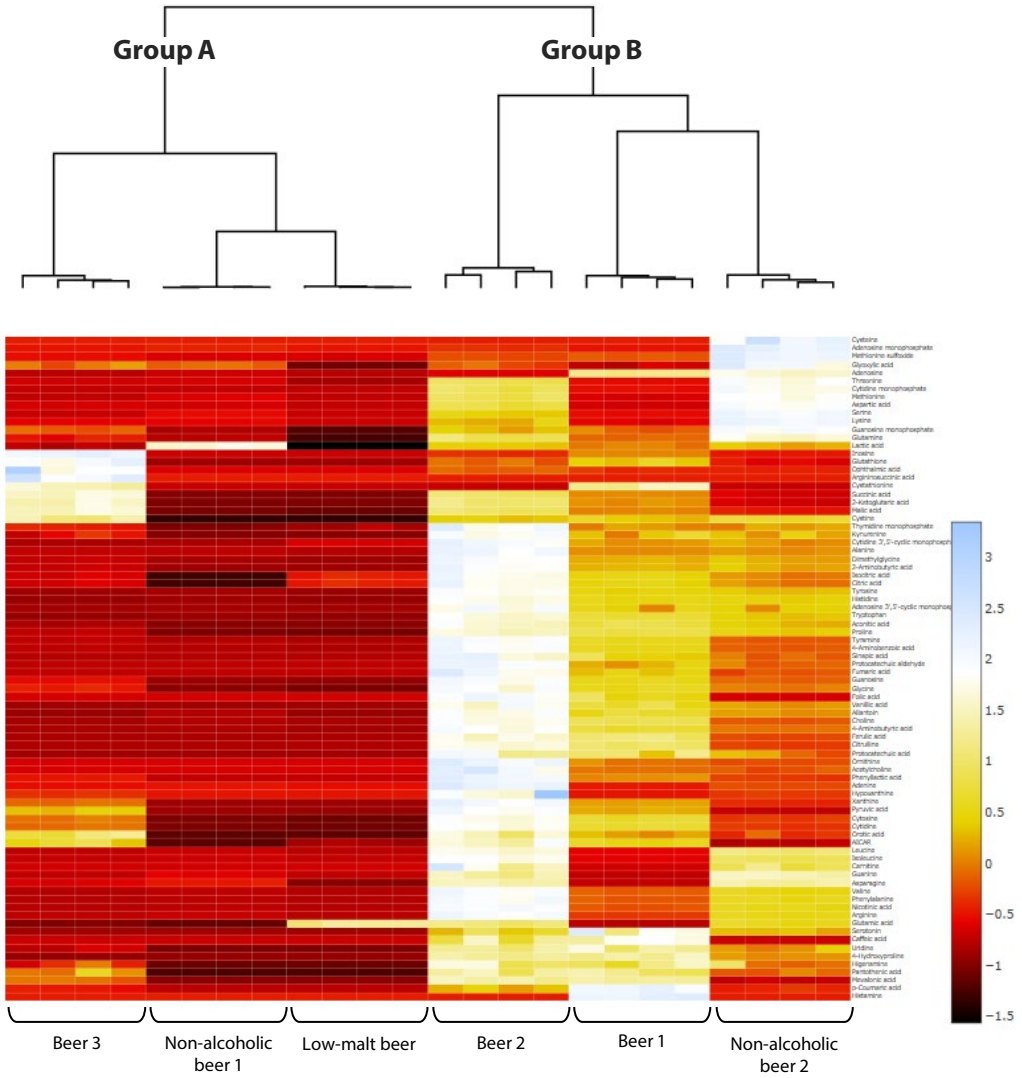

Fig. 4 Results of Hierarchical Cluster Analysis

From the loading plot, the characteristic compounds contained in each sample were identified. Beer 2 contained a lot of amino acids and nucleoside metabolites as shown in blue. PCA makes it easy to classify each sample by feature and find the compounds that cause a difference.

Fig. 4 shows the result of hierarchical cluster analysis (HCA). Similar to the results of PCA, HCA classified samples into two groups (Group A: low-malt beer, non-alcoholic beer 1, and beer 3; Group B: non-alcoholic beer 2, beer 1, and beer 2). Non-alcoholic beer 1 and non-alcoholic beer 2 are non-alcoholic beers, but they are classified in different groups. Non-alcoholic beer 1 made in Japan is made by seasoning wort without fermentation. Non-alcoholic beer 2 made in Germany is made from the same ingredients as beer and fermented in a way that suppresses the production of alcohol. That suggests that the differences in ingredients and manufacturing processes affect the tendencies of hydrophilic compounds in those non-alcoholic beers. Beer 1 and non-alcoholic beer 2 were classified in similar groups. This may be because the ingredients of beer 1 and non-alcoholic beer 2 are beer-based and they are made by bottom fermentation. HCA provides a visual understanding of the degree of similarity between the compounds in each sample.

■ Compounds Related to Purine

The results of PCA and HCA showed significant differences in nucleoside metabolites, so compounds related to purine in each sample were compared. Adenine, adenosine, cyclic adenosine monophosphate, adenosine monophosphate, inosine, hypoxanthine, xanthine, guanine, guanosine, and guanosine monophosphate were detected. Fig. 5 shows the sum of the peak area ratios of each compound. It was found that beer 2 contains the most compounds related to purine, and the low-malt beer, beer 3, and non-alcoholic beer 1 contain few of them. In particular, these compounds were hardly detected in the low-malt beer (purine free).

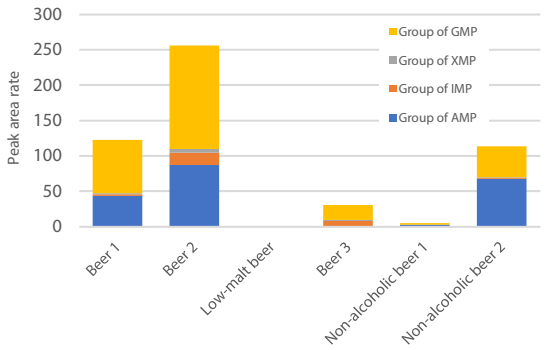

Fig. 5 Purines in Beer Samples

Xanthine differences between samples was quantified. Fig. 6 shows the calibration curve obtained using the standard solution of xanthine. Good linearity with a coefficient of determination (R^2) of 0.999 was obtained for the calibration curve range of 0.1 to 50 µmol/L. Table 4 shows concentrations of xanthine in each sample. Beer 2 contained the highest amount (102 µmol/L) of xanthine.

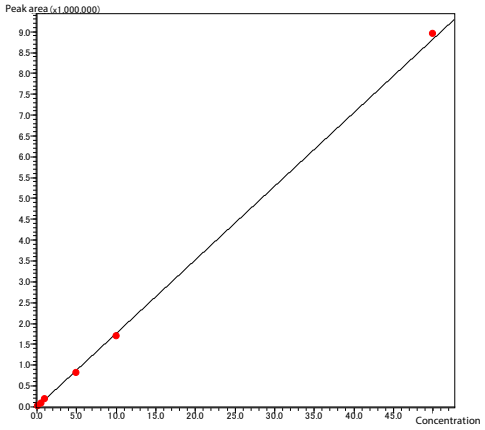

Fig. 6 Calibration Curves of Xanthine

Table 4 Concentration of Xanthine in Beer Samples (µmol/L)

Beer 1	Beer 2	Low-malt beer	Beer 3	Non-alcoholic beer 1	Non-alcoholic beer 2
47	102	N.D.	33	N.D.	27

■ Functional Components

In addition to amino acids, organic acids, and nucleoside metabolites, components with functional properties were also detected in each sample. For example, GABA (γ-aminobutyric acid), which is known to improve blood pressure, relieve stress, and reduce fatigue, and ferulic acid, vanillic acid, sinapic acid, and caffeic acid, which have antioxidant effects, were detected. The peak area ratios of these functional components were compared. As shown in Fig. 7, beer 1, beer 2, and non-alcoholic beer 2 were rich in these functional components. Ferulic acid and vanillic acid are the main antioxidants in beer and are known to be contained in malt. More of these functional components were detected presumably because the proportion of malt is high in the ingredients of beer 1, beer 2, and non-alcoholic beer 2.

■ Conclusion

This article introduces an example of food metabolomics using single quadrupole LC/MS. Although triple-quadrupole LC/MS/MS is normally used for targeted metabolomics, it was found that single-quadrupole LC/MS also has sufficient potential for targeted metabolomics. Compared to triple-quadrupole LC/MS, single-quadrupole LC/MS systems are cheaper and easier to operate, making analysis easier for many people, including those who have no experience with mass spectrometry. The spread of food metabolomics using single-quadrupole LC/MS is expected to lead to further development of technologies and products in the food industry.

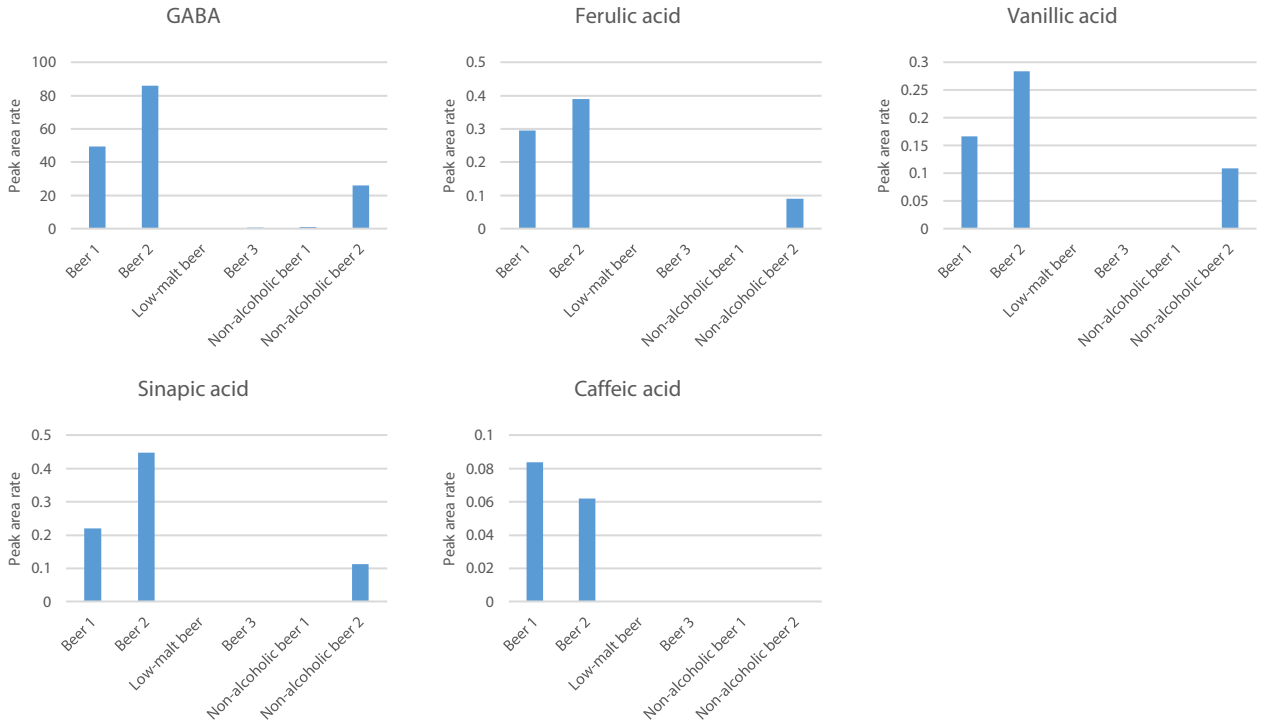

Fig. 7 Components with Functional Properties in Beer Samples

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