SHIMADZU
Excellence in Science

LCMS-9030 High Performance Liquid Chromatograph Mass Spectrometer

Application News

Non-Targeted Analysis of Metabolites in Beverages Using LCMS-9030

Yutaka Umakoshi and Tetsuo Iida

User Benefits

- An LCMS-9030 system and multivariate analysis software can be used for non-targeted analysis of food samples.
- The content of unknown compounds that differ between samples can be predicted using an LCMS-9030 system capable of accurate mass analysis.
- The same workflow can be used to objectively evaluate the taste, quality, and nutritional value of food.

■ Introduction

In recent years, metabolomics, a technology for comprehensively analyzing metabolites in living organisms, has been attracting attention. It is also used in food research to objectively evaluate the taste, quality, and nutritional value. Metabolomics can be divided into two types: "targeted analysis" and "non-targeted analysis." Targeted analysis measures a specified group of compounds, which makes analysis easier, but may miss important components that were not targeted. Non-targeted analysis is used to comprehensively analyze all metabolites, including unknown components. The quadrupole time-of-flight (QTOF) mass spectrometer is ideal for non-targeted analysis and allows the prediction of unknown compounds that differ between samples.

This article describes a non-targeted analysis of various metabolites in beer and other similar beverages using an LCMS-9030 quadrupole time-of-flight mass spectrometer system (Fig. 1). Multivariate analysis software was used to extract characteristic peaks. As a result of peak identification, it was confirmed that metabolite profiles were based on raw materials and manufacturing methods.

Fig. 1 Nexera™ X3 and LCMS-9030

■ Sample and Pretreatment

The six types of beverages, including beer, low-malt beer, beer-like beverage, and non-alcoholic beer, were used as samples (Table 1). Each sample was pretreated by degassing and then 10-fold dilution with ultrapure water.

Table 1 Sample Information

No	Sample	Description
1	Beer 1	Lager beer (bottom fermentation)
2	Beer 2	Ale beer (top fermentation)
3	Low-malt beer	Purine free
4	Beer-like beverage	Soy protein as ingredients
5	Non-alcoholic beer 1	Made in Japan
6	Non-alcoholic beer 2	Made in Germany

■ Analytical Conditions

Nexera™ X3 UHPLC and LCMS-9030 systems were used as the analytical instruments. The LC method included in "LC/MS/MS Method Package for Primary Metabolites" was used as the method. The data-dependent acquisition (DDA) mode was used to simultaneously acquire precursor *m/z* and MS/MS data. Table 2 shows the analytical conditions.

Table 2 Analytical Conditions

HPLC Conditions (Nexera X3)	
Column:	Reverse-phase column
Column Oven:	40 °C
Solvent A:	0.1 % Formic acid in water
Solvent B:	0.1 % Formic acid in acetonitrile
Mode:	Gradient elution
Flowrate:	0.25 mL/min
Injection Volume:	3 µL
MS Conditions (LCMS-9030)	
Ionization:	ESI, positive
Mode:	Data dependent acquisition (DDA)
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
CID Gas Pressure:	230 kPa

■ Data Analysis

Signpost MS from Reifycs Inc. (Fig. 2) was used for the data analysis. Signpost MS extracts the detected ion information from the acquired data as spots and aligns them based on retention time and *m/z* values. Multivariate analysis based on the aligned peaks makes it easy to extract characteristic peaks for each sample. Compound identification was based on the "Exact Mass Database for Endogenous Metabolites." That database contains retention time and exact mass information for metabolites included in LC/MS/MS series method packages. Retention time and exact mass information enable reliable compound identification. Compounds not included in the Exact Mass Database for Endogenous Metabolites were predicted using the Assign function of LabSolutions Insight Explore™. In order to identify the compounds, the retention times and MS/MS spectrum were confirmed using reference standard samples for the predicted compounds.

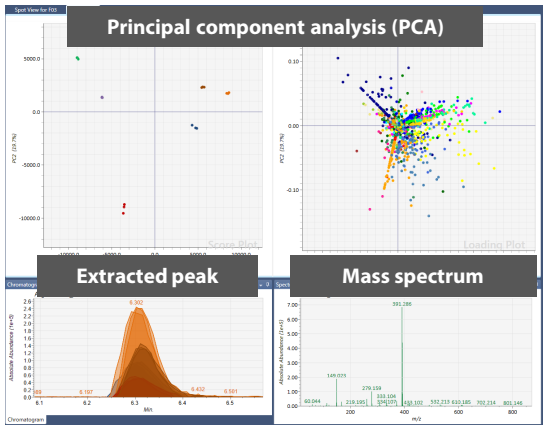

Fig. 2 Screenshot of Signpost MS

■ Comprehensive Analysis of Metabolites

The LCMS-9030 system was used to analyze 6 beverages. Signpost MS extracted 1,698 peaks. Fig. 3 shows the results of principal component analysis based on the height of extracted peaks. Pareto scaling was used to scale the data. The loading plot is color-coded by principal component variable grouping (PCVG). This allows grouping large numbers of spots into related groups, making it easier to interpret the principal components.

On the score plot, beer 1, beer 2, and non-alcoholic beer 2 were plotted in close proximity. Low-malt beer and non-alcoholic beer 1 were also plotted nearby. The beer-like beverage was plotted away from the other samples in the PC2 direction. The peak heights of some metabolites that were characteristic among the samples are shown in Fig. 4.

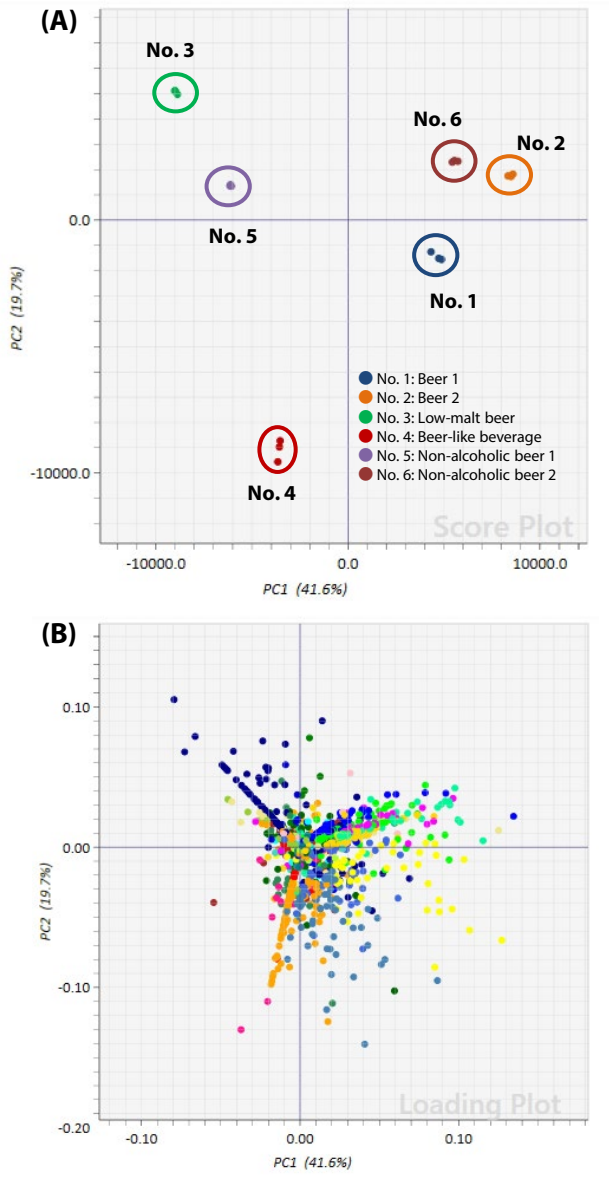

Fig. 3 Principal Component Analysis
(A) Score Plot, (B) Loading Plot

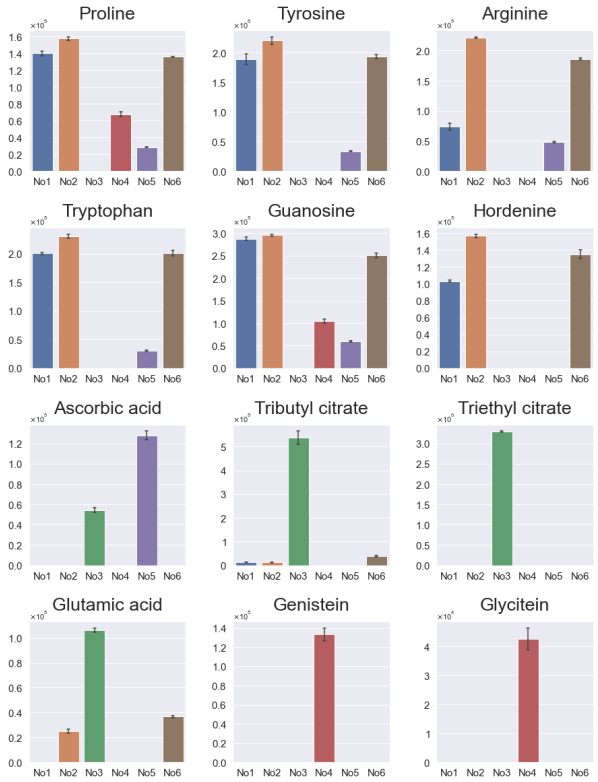

Fig. 4 Characteristic Metabolite Differences Between Samples, with Y-axis: Peak Height (Mean ± SD for n = 3)

Many amino acids (proline, tyrosine, arginine, tryptophan) and nucleic acid metabolites (guanosine) were detected from No. 1, 2 and 6. An alkaloid (hordenine) thought to be derived from barley was also detected. It is presumed that the reason why similar metabolites were characteristically detected from No. 1, 2, and 6 is the fermentation process common to these manufacturing processes. Since No. 6 is produced by fermenting beer ingredients while suppressing alcohol production, it is considered that the metabolic profile is similar to No. 1 and No. 2. An antioxidant (ascorbic acid) was detected from No. 3 and 5. Emulsifying agents (tributyl citrate, triethyl citrate) and an umami component (glutamic acid) were characteristically detected from No. 3. No. 3 and 5 have a different metabolic profile than beer presumably because they are produced by mixing raw materials. Isoflavones (genistein and glycitein) were detected from No. 4. Since No. 4 is made from soybeans, it is possible that metabolites derived from soybeans were detected.

■ Conclusion

In this study, a non-targeted analysis of metabolites in beer was performed using an LCMS-9030 quadrupole time-of-flight mass spectrometer system. Metabolites that were characteristic among the samples were identified and it was confirmed that the metabolite profiles were based on the raw materials and manufacturing methods.

Non-targeted analysis of food samples is possible using an LCMS-9030 system and Signpost MS software. The same workflow can also be used to objectively evaluate the taste, quality, and nutritional value of food.

01-00390-EN First Edition: Jul. 2022

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/