Non-Targeted Analysis of Metabolites in Alcoholic Beverages Using LC-QTOFMS

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1. Introduction

Metabolomics, a technique that analyzes metabolites in living organisms, is increasingly being applied in the food industry for product development and quality control. A particularly powerful approach is non-targeted metabolomics, which aims to analyze not only known metabolites but also unknown compounds without prior assumptions to reveal unexpected chemical differences between samples. Quadrupole time-of-flight mass spectrometry (QTOFMS), with its accurate-mass capability, is well suited for this purpose. However, the large volume of generated data creates challenges for feature identification and data comparison.

This poster presents a case study of analyzing compounds in various beverages, primarily beer, using the non-targeted analysis software. Using this, we performed peak detection, alignment, and library searching across multiple data sets. Analysis of peaks showing differences between samples confirmed that these profiles were based on the raw materials and manufacturing methods.

2. Methods

2-1. Sample and Pretreatment

Six commercially available beer-type 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.

2-2. Analysis Conditions

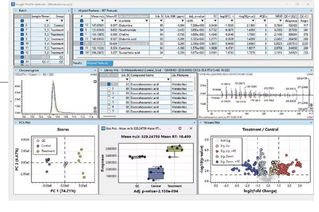
Shimadzu Nexera™ X3 UHPLC and LCMS-9030 systems were used as the analytical instruments. The LC method included in the LC/MS/MS Method Package for Primary Metabolites was used as the method. The data-dependent acquisition (DDA) mode was used to simultaneously acquire precursor *m/z* and MS/MS data. Table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

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	Contains soy protein
5	Non-alcoholic beer1	Made in Japan
6	Non-alcoholic beer2	Made in Germany

Table 2 Analysis Conditions

[HPLC conditions] （**Nexera X3**）		
Column	:	Reversed-phase column
Column oven	:	40℃
Solvent A	:	0.1% Formic acid in water
Solvent B	:	0.1% Formic acid in acetonitrile
Mode	:	Gradient elution
Flow rate	:	0.25 mL/min
Injection volume	:	3 µL
[MS conditions] （**LCMS-9030**）		
Ionization	:	ESI, Positive
Mode	:	Data Dependent Acquisition（DDA）
MS event	:	*m/z* 50-1000, 0.1 sec
MS/MS event	:	*m/z* 10-1000, 0.1 sec × 5
Collision Energy	:	35.0 ± 17.0 V
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL temp.	:	250℃
Block heater temp.	:	400℃
Interface temp.	:	300℃



3. Results

3-1. Data Analysis

Shimadzu non-targeted software LabSolutions Insight™ Profiler was used for data analysis. Alignment results, chromatograms, and spectra are linked with PCA and volcano plots, facilitating the interpretation of complex datasets. For library searches, the NIST 23 Mass Spectral Library, which contains approximately 50,000 compounds, was used. Scores were calculated based on MS/MS spectral matching.

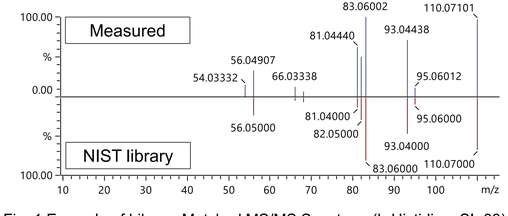


Fig. 1 Example of Library-Matched MS/MS Spectrum (L-Histidine, SI=83)

3-2. Results of Alignment and Library Search

The LC-QTOFMS system was used to analyze six types of beer and beer-like beverages in three repeat measurements. LabSolutions Insight Profiler detected 4,695 features. A NIST 23 Mass Spectral Library search of the MS/MS spectral features detected 403 compounds with a similarity score of 50 or higher, including 214 compounds with a score of 80 or higher. Fig. 1 shows the library-matched MS/MS spectrum for the feature with an average detected *m/z* of 156.077 and a retention time of 1.9 min, identified as L-Histidine with a similarity score of 83.

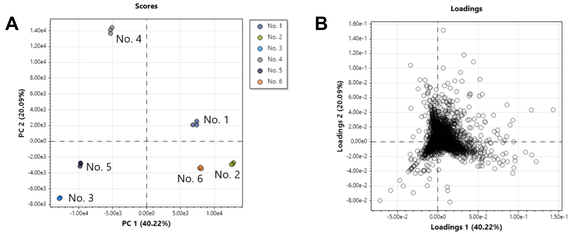


Fig. 2 Results of Principal Component Analysis
(A) Score Plot, (B) Loading Plot

3-3. Principal Component Analysis of 6 Types of Beer

Fig. 2 shows the results of principal component analysis for peak area values observed among the samples. Pareto scaling was used for data scaling. On the score plot, Beer 1 (No. 1), Beer 2 (No. 2), and Non-alcoholic beer 2 (No. 6) were plotted close together. Low-malt beer (No. 3) and Non-alcoholic beer 1 (No. 5) were also plotted close together. Beer-like beverage (No. 4) was plotted in a different position from the other samples along the PC2 axis.

Fig. 3 shows the area values of certain compounds that were characteristic among the samples. Amino acids (histidine, phenylalanine, tyrosine, and tryptophan) and nucleic acid metabolites (cytidine and guanosine) were detected in large quantities in samples No. 1, 2, and 6. An alkaloid (hordenine), thought to be derived from barley, was also detected. The detection of similar characteristic compounds in samples No. 1, 2, and 6 is presumed to be due to common raw materials and fermentation processes used in their manufacturing methods. Although No. 6 is a non-alcoholic beer, it is manufactured using beer ingredients and a fermentation method that suppresses alcohol production, resulting in a similar profile to No. 1 and 2. Isoflavones (daidzin and genistein) were detected in No. 4. Since No. 4 is manufactured using soybeans as a raw material, the detected compounds are presumably derived from soybeans.

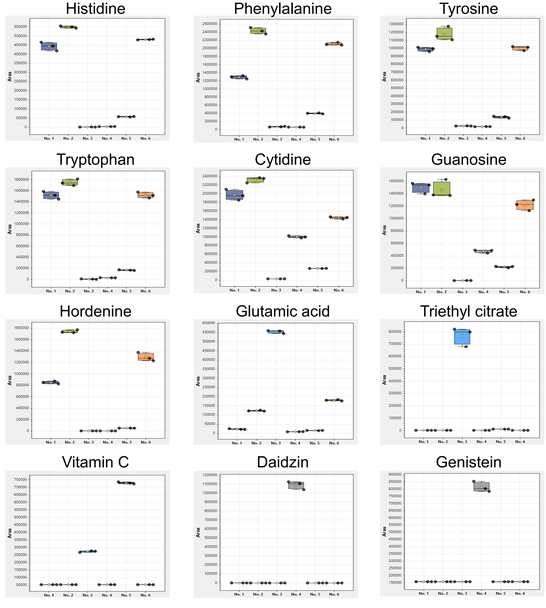


Fig. 3 Box Plots of Characteristic Compounds Among Samples
(Y-Axis: Area Value, n = 3)

4. Conclusions

In this study, compounds in various beer and beer-like beverages were analyzed by non-targeted analysis using an LC-QTOFMS system. Analyzing compounds characteristic of each sample with non-targeted analysis software confirmed that the resulting profiles reflected differences in raw materials and manufacturing methods. This analytical method is expected to enable the objective evaluation of the taste, quality, and nutritional value of foods.