PO-CON21024

SHIMADZU
Excellence in Science

Widely targeted metabolomics of 142 hydrophilic compounds in beer using liquid chromatography-single quadrupole mass spectrometer

2022 AOAC Annual Meeting

Natsuki Iwata[1], Takanari Hattori[1], Eberhardt Kuhn[2], Shinnosuke Horie[3], Yusuke Inohana[1]
1 Shimadzu Corporation, Kyoto, Japan; 2 Shimadzu Scientific Instruments, Inc., Columbia, MD;
3 Shimadzu Europa GmbH, Inc., Duisburg, Germany

Widely targeted metabolomics of 142 hydrophilic compounds in beer using liquid chromatography-single quadrupole mass spectrometer

1. Overview

Simultaneous analysis of 142 compounds including amino acids, organic acids, nucleic acid metabolites, and functional components was achieved using a single quadrupole LC-MS. This method was utilized for a comprehensive analysis of various metabolites related to the flavor, quality, and functionality of beers. Principle component analysis result classified the six beers used in this study into two groups based on the hydrophilic compounds analysis results. The grouping result correlates the differences in ingredients and manufacturing processes between different beers.

2. Introduction

Recently, increasing attention has been devoted to metabolomics using mass spectrometry in the food industry. The link between the objective taste profile and the functional components in food products could be found by metabolomic analysis. Beer is an alcoholic beverage made mainly from fermented malt. Beer contains compounds both derived from malt and produced during the fermentation process. Some of these compounds, known as functional components, affect the taste and the flavor of beer. Some of these functional components also have antioxidant effect. Therefore, it is important to analyze these compounds comprehensively for the evaluation of foods. In this study, 142 hydrophilic compounds were analyzed for a widely targeted metabolomics analysis of beers by a single quadrupole LC-MS.

Single quadrupole mass spectrometer LCMS-2050

3. Materials and Methods

3-1. Sample

Four types of beers (A-D) and two types of non-alcoholic beers (E and F) were analyzed. Beer A is a lager beer and beer B is an ale beer. Beer C is a low-malt beer and purines are removed. Beer D is made from soy protein instead of barley. The two non-alcoholic beers (E and F) are differentiated by their manufacturing processes. Table 1 shows the details of the samples.

Table 1 Sample Details

Sample	Feature
Beer A	Lager beer (bottom fermentation)
Beer B	Ale beer (top fermentation)
Low-malt beer C	Purine free
Beer D	Soy protein as ingredients
Non-alcoholic beer E	Made in Japan
Non-alcoholic beer F	Made in Germany

3-2. Sample Pretreatment

All beverages used in this study were diluted 10-fold with water. At dilution, 1 µmol/L 2-morpholinoethanesulfonic acid was added as an internal standard (IS).

3. Materials and Methods

3-3. Analytical Conditions

LC-MS analysis was performed by Nexera™ XR system coupled with a LCMS-2050 single quadrupole mass spectrometer (Shimadzu Corporation, Japan, Figure 1). The analytical conditions for the single quadrupole LC-MS method were developed by referring to the analytical conditions of the ion-pair free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 3.

Analytical condition

UHPLC (Nexera XR system)		
	Column:	Shim-pack™ GIST PFPP (2.1 mmI.D. x 150 mmL., 3.0 µm)
	Mobile phase	A: 0.1% Formic acid in water B: 0.1% Formic acid in acetonitrile
	Flow rate:	0.25 mL/min (17-19 min, 0.5 mL/min)
	Injection vol.:	3 µL
	Column temp.:	40 °C
MS (LCMS-2050)		
	Ionization:	ESI/APCI (DUIS™), Positive/ Negative mode
	Nebulizing gas:	3.0 L/min
	Drying gas:	5.0 L/min
	Heating gas:	7.0 L/min
	Desolvation temp.:	500 °C
	DL temp.:	250 °C

Figure 1 Nexera™ XR and LCMS-2050

Widely targeted metabolomics of 142 hydrophilic compounds in beer using liquid chromatography-single quadrupole mass spectrometer

4. Results

Simultaneous analysis of 142 hydrophilic metabolites such as amino acids, organic acids, nucleosides, and nucleotides, which are important in food analysis, was achieved. As a result, 82 compounds were detected. The main metabolites were amino acids, organic acids, and nucleoside metabolites. Table 2 shows the number of metabolites detected in each sample. More than 70 compounds were detected in beer A, beer B, and non-alcoholic beer F, but only 22 compounds were detected in low-malt beer, showing a different trend.

Table 2 Number of Detected Compounds

Beer A	Beer B	Low-malt beer C	Beer D	Non-alcoholic beer E	Non-alcoholic beer F
76	78	22	57	44	77

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted by Multi-omics Analysis Package (Shimadzu Corporation, Japan) using the area ratio of each compound with IS.

As a result of the PCA, low-malt beer C and non-alcoholic beer E were plotted close together on the score plot and had similar trends in the number of hydrophilic compounds (Figure 2). It was suggested that the PC1 shows the difference of ingredients. As a result of the HCA, the clusters were separated into 2 groups (Figure 3). Non-alcoholic beer E and F were classified in different groups. Non-alcoholic beer E is made by seasoning wort without fermentation. Non-alcoholic beer F is made from the same ingredients as beer and fermented in a way that suppresses the production of alcohol. It was suggested that the difference in ingredients and manufacturing processes affects trends of hydrophilic compounds in non-alcoholic beer E and F.

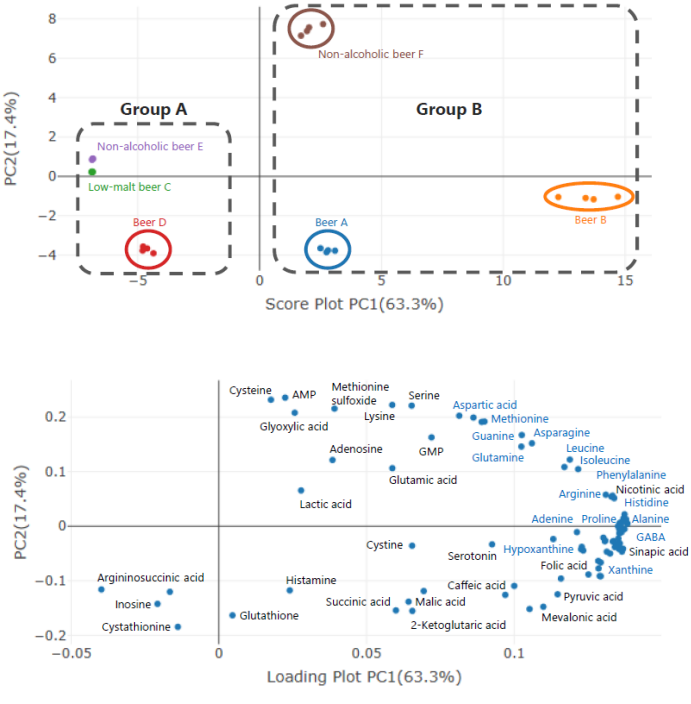

Figure 2. PCA Results for Beers

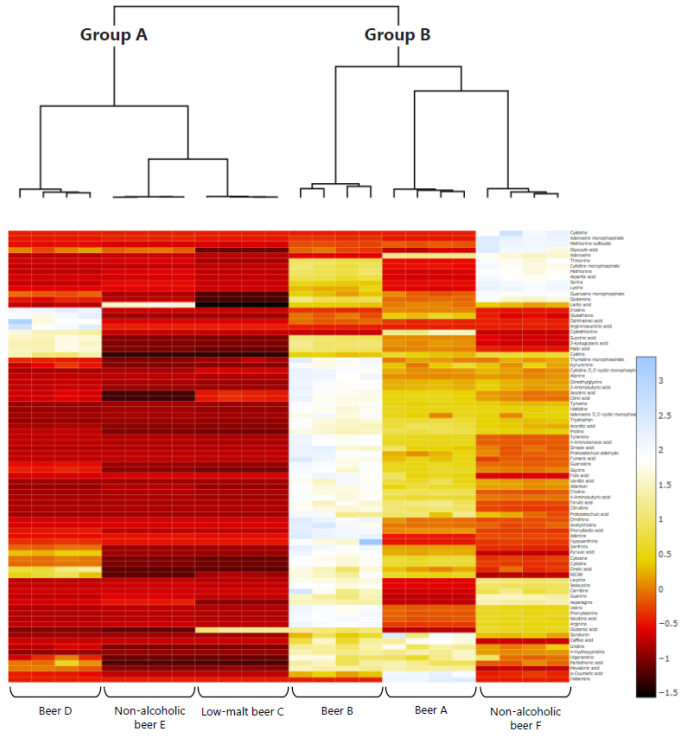

Figure 3. HCA Results for Beers

Widely targeted metabolomics of 142 hydrophilic compounds in beer using liquid chromatography-single quadrupole mass spectrometer

The results of PCA and HCA showed significant differences in nucleoside metabolite, so compounds related to purine in each sample were compared. Figure 4 shows the sum of the peak area ratios of each compound. It was found that beer B contains the most compounds related to purine. Low-malt beer C, beer D, and non-alcoholic beer E do not contain much of the purine related compounds. It is worth noting that these compounds were hardly detected in low-malt beer C (purine free).

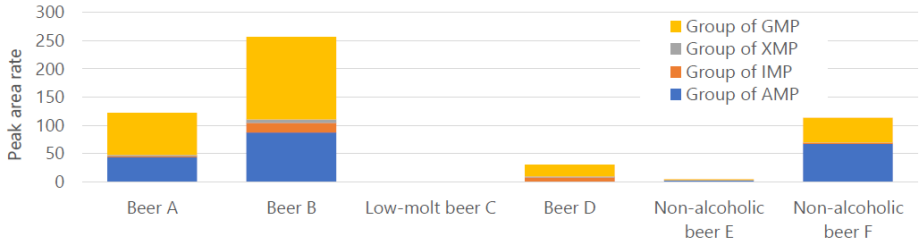

Figure 4. Purines in Beers

In addition, functional components such as GABA (γ-aminobutyric acid), ferulic acid, vanillic acid, sinapic acid, and caffeic acid were also detected. Beer A, B, and non-alcoholic beer F are rich in these functional components. Ferulic acid and vanillic acid are the main antioxidants in beer and are known to be contained in malt. Because the proportion of malt is high in the ingredients of beer A, beer B, and non-alcoholic beer F, more of these functional components were detected as expected. (Figure 5)

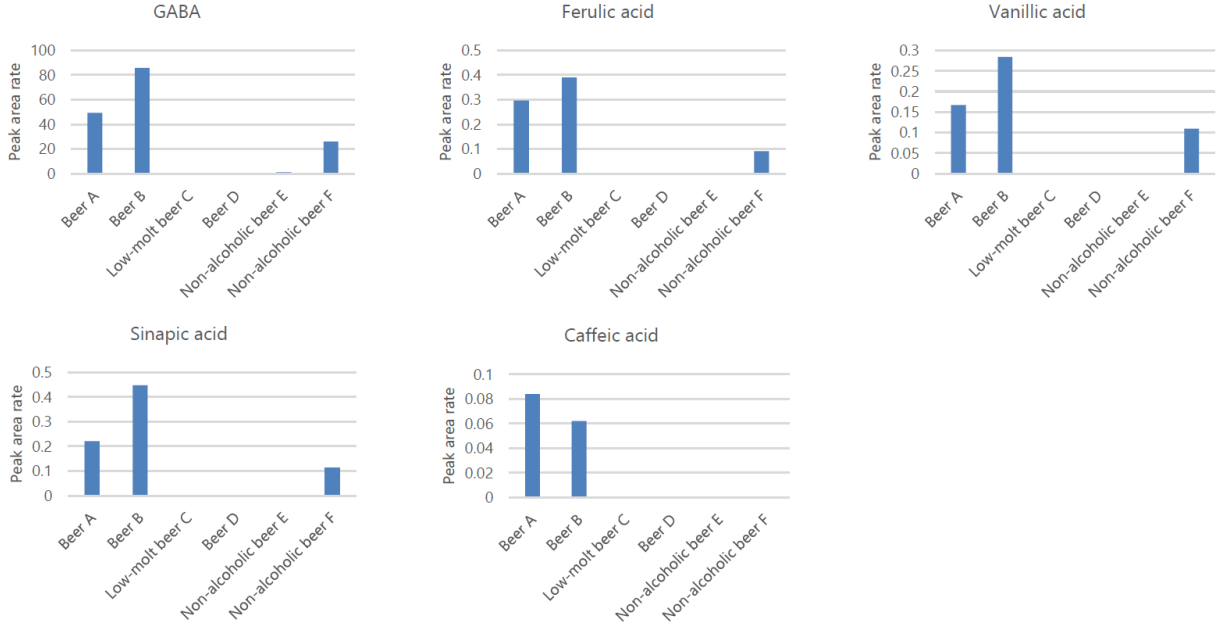

Figure 5. Functional Components in Beers

Widely targeted metabolomics of 142 hydrophilic compounds in beer using liquid chromatography-single quadrupole mass spectrometer

5. Conclusions

- An easy and comprehensive method to simultaneously analyze 142 hydrophilic compounds using a single quadrupole LC-MS was developed.
- Widely targeted metabolomics analysis of beers was successfully performed.

First Edition: August, 2022

Shimadzu Corporation
www.shimadzu.com/an/