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Profiling of oligosaccharides and polysaccharides in alcoholic beverage using single quadrupole LC-MS

2022 AOAC Annual Meeting

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1. Overview

Simultaneous analysis of oligosaccharides and polysaccharides was achieved using a single quadrupole LC-MS. Up to 36-mer polysaccharides (average molecular weight 5855.09) were detected in beer as the trivalent ion (m/z 1949.63). As the results of principal component analysis and relative comparison, profiling of oligosaccharides and polysaccharides in six types of alcoholic and non-alcoholic beers was successfully performed.

2. Introduction

In recent years, attention has been focused on the technology of metabolomics, which is defined as the comprehensive analysis of metabolites in vivo. 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.

Beer is made mainly from fermented malt and contains compounds derived from malt and compounds produced during fermentation. Some of these compounds affect the taste and flavor of beers. Therefore, it is important to analyze these compounds comprehensively for evaluation. This poster describes the profiling of oligosaccharides and polysaccharides in alcoholic beverages.

*Putri, Sastia Prama, et al.: Application of gas chromatography-mass spectrometry-based metabolomics in food science and technology, J. Biosci. Bioeng., 133, 425-435 (2022).

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 (zero carbs) and beer D is made from soy protein, not barley. The manufacturing process of non-alcoholic beer E and F is different. Table 1 shows the details of the samples.

Table 1 Sample Details

Sample	Description
Beer A	Lager beer (bottom fermentation)
Beer B	Ale beer (top fermentation)
Low-malt beer C	Zero carbs
Beer D	Soy protein as ingredients
Non-alcoholic beer E	Zero carbs, 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.

3. Materials and Methods

3-3. Analytical Conditions

LC/MS analysis was performed using a Nexera™ XR HPLC system coupled with an LCMS-2050 single-quadrupole mass spectrometer (Shimadzu Corporation, Japan, Figure 1). The target compounds were malto-oligosaccharides and polysaccharides (up to 40-mer) that are considered to be contained in beer. Polysaccharides with a molecular weight of 1500 or more were detected as polyvalent ions from the viewpoint of measurable mass range and sensitivity.

Analytical condition

UHPLC (Nexera XR system)	
Column:	Shodex Asahipak NH2P-40 3E (250 mm x 3.0 mm I.D., 4.0 µm)
Mobile Phases	A: 2.5 mmol/L Ammonium bicarbonate aq. B: 25 mmol/L Ammonium bicarbonate aq. / Acetonitrile=10:90
Time Program:	70%B (0 min)→ 40%B (25 min)→ 70%B (25.01-30 min)
Flow Rate:	0.3 mL/min
Injection Vol.:	5 µL
Column Temp.:	40 °C
MS (LCMS-2050)	
Ionization:	ESI/APCI (DUIS™), Negative mode
Mode:	SIM (40 events)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	400 °C
DL Temp.:	150 °C

Figure 1 Nexera™ XR and LCMS-2050

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4. Results

Figure 2 shows a SIM chromatogram of a mixed standard solution of 10 mg/L malto-oligosaccharides (DP1-DP10). In addition to DP1-DP10 oligosaccharides, polysaccharides of up to 24-mer were detected.

By using a highly sensitive mass spectrometer as a detector for LC, it was possible to detect trace amounts of oligosaccharides and polysaccharides that cannot be detected by LC-RID and LC-ELSD. The concentration ranges of calibration curves, coefficients of determination (r^2), and repeatability are shown in Table 2. Good linearity was confirmed over a wide concentration range and the repeatability was also good.

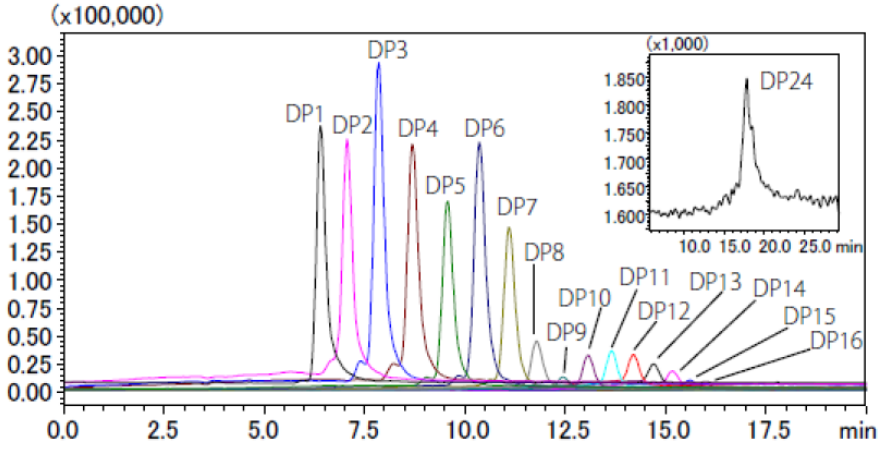

Figure 2 Chromatogram of Mixed Standard Solution

Table 2 Calibration Curves and Repeatability

Compound	Conc. Range (mg/L)	r^2	%RSD (R.T.)	%RSD (Peak area)
Glucose	0.05-100	0.998	0.25	4.49
Maltose	0.05-10	0.999	0.20	5.09
Maltotriose	0.01-10	0.999	0.40	8.33
Maltotetraose	0.01-50	0.995	0.36	6.46
Maltopentaose	0.01-50	0.997	0.52	5.66
Maltohexaose	0.05-50	0.997	0.23	4.35
Maltoheptaose	0.05-100	0.997	0.37	6.65

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Table 3 shows the number of oligosaccharides and polysaccharides detected in each sample. In beer A, beer B, and non-alcoholic beer F, polysaccharides which are thought to be polymers of glucose are detected in addition to various malto-oligosaccharides such as maltose. Figure 3 shows a SIM chromatogram of beer B. Up to 36-mer polysaccharides (average molecular weight 5855.09) were detected as the trivalent ion (m/z 1949.63).

Table 2 Calibration Curves and Repeatability

Beer A	Beer B	Low-malt beer C	Beer D	Non-alcoholic beer E	Non-alcoholic beer F
36	36	4	36	15	36

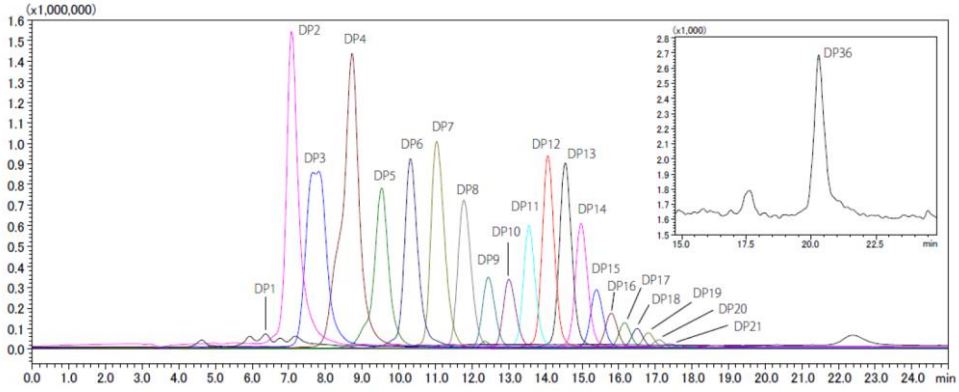

Figure 3 Chromatogram of Beer B

Principal component analysis (PCA) was conducted by Multi-omics Analysis Package (Shimadzu Corporation, Japan) using the peak area of each compound. Figure 4 shows the result of PCA. From the score plot, it was found that "beer A and beer B" and "low-malt beer C and non-alcoholic beer E" had similar tendencies. In the loading plot, many oligosaccharides and polysaccharides were plotted on the left side of the first principal component (PC 1). That suggests that PC 1 shows the malt ratio in ingredients.

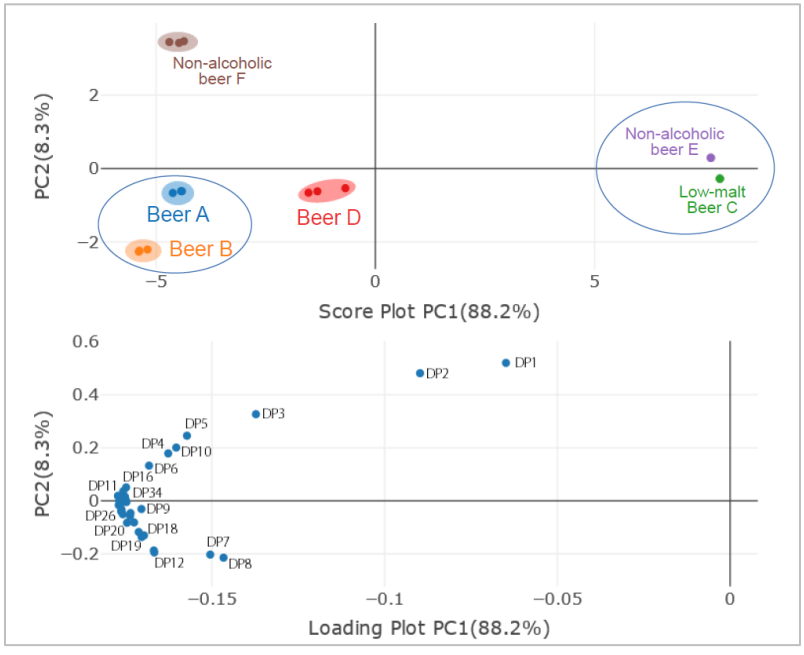

Figure 4 PCA Results for Beers

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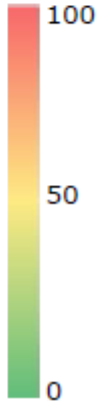
The relative peak areas (maximum 100) for each oligosaccharide and polysaccharide were heat-mapped (Table 4). Beer A and Beer B contained a large amount of oligosaccharides and polysaccharides which seemed to be derived from malt. Non-alcoholic beer E and non-alcoholic beer F had different tendencies. Non-alcoholic beer E is made by seasoning wort without fermentation for zero alcohol and carbs. Therefore, non-alcoholic beer E had less oligosaccharides and polysaccharides. DP1 (glucose) and DP2 (maltose) were more abundant in non-alcoholic beer F. It is considered that glucose and maltose remain undecomposed due to the manufacturing method that suppresses alcoholic fermentation.

Table 4 Relative comparison of oligosaccharides and polysaccharides contained in beer (relative peak area)

	Beer A	Beer B	Low-malt beer C	Beer D	Non-alcoholic beer E	Non-alcoholic beer F
DP1	4.0	2.6	1.5	3.9	3.9	100.0
DP2	25.2	22.2	0.2	31.9	24.3	100.0
DP3	75.8	31.1	0.3	51.8	15.1	100.0
DP4	95.2	56.4	0.2	57.3	3.8	100.0
DP5	79.5	47.5	0.0	47.1	2.2	100.0
DP6	82.0	69.0	0.0	77.6	2.5	100.0
DP7	73.0	94.3	0.0	100.0	1.9	49.6
DP8	70.8	90.6	0.0	100.0	1.6	45.1
DP9	72.7	100.0	0.0	87.2	1.8	86.7
DP10	53.2	64.3	0.0	64.6	1.3	100.0
DP11	94.6	100.0	0.0	68.1	1.0	98.2
DP12	80.3	100.0	0.0	55.3	0.6	51.6
DP13	71.8	100.0	0.0	55.9	0.5	52.6
DP14	68.0	100.0	0.0	61.3	0.4	65.6
DP15	73.1	100.0	0.0	60.2	0.3	90.1
DP16	83.9	99.1	0.0	52.5	0.0	100.0
DP17	95.0	100.0	0.0	46.2	0.0	80.5
DP18	88.0	100.0	0.0	44.1	0.0	62.4
DP19	80.4	100.0	0.0	46.8	0.0	61.2
DP20	80.3	100.0	0.0	54.2	0.0	72.6
DP21	83.8	100.0	0.0	57.9	0.0	91.4
DP22	91.4	100.0	0.0	54.3	0.0	99.3
DP23	95.0	100.0	0.0	48.5	0.0	89.8
DP24	95.8	100.0	0.0	48.7	0.0	78.6
DP25	95.2	100.0	0.0	47.9	0.0	73.5
DP26	98.5	100.0	0.0	72.8	0.0	92.1
DP27	88.1	100.0	0.0	68.5	0.0	95.5
DP28	93.0	100.0	0.0	61.6	0.0	92.8
DP29	95.8	100.0	0.0	57.9	0.0	81.7
DP30	92.6	100.0	0.0	58.0	0.0	83.5
DP31	95.7	100.0	0.0	60.1	0.0	85.8
DP32	100.0	98.7	0.0	70.5	0.0	92.2
DP33	100.0	94.9	0.0	77.8	0.0	92.5
DP34	100.0	90.4	0.0	78.9	0.0	94.5
DP35	100.0	98.0	0.0	80.1	0.0	95.7
DP36	98.7	100.0	0.0	79.1	0.0	97.3



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5. Conclusions

- An easy and comprehensive method to simultaneously analyze oligosaccharides and polysaccharides using a single quadrupole LC-MS was developed.
- Profiling of oligosaccharides and polysaccharides in alcoholic beverage was successfully performed.

First Edition: August, 2022

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