SHIMADZU

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

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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 beer was successfully performed.

2. Introduction

Recently, increasing attention has been devoted to metabolomics using a mass spectrometer in the food industry. Objective evaluation of taste and search for functional components in food products are expected using metabolomics. Beer is made mainly from fermented malt, so it contains malt-derived and fermentation-derived compounds. Some of these compounds affect the taste and flavor of beer. Therefore, it is important to analyze these compounds comprehensively for the evaluation of beer. This poster describes the profiling of oligosaccharides and polysaccharides in alcoholic beverages

3. Methods

Seven f beverages were used in this study. Table 1 shows the detailed sample information. All beverages used in this study were 10-fold diluted with water. 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.

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

Table 2 Analytical Conditions

[HPLC conditions]	:Nexera XR
Column	:Shodex Asahipak NH2P-40 3E (250 mm x 3.0 mm I.D., 4.0 µm)
Flow rate	:0.3 mL/min
Mobile phase	: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)
Column temp.	:40 ˚C
Injection volume	:5 µL
[MS conditions]	: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

4. Results

By using a highly sensitive mass spectrometer as a detector for LC, trace determination of oligosaccharides and polysaccharides was able to be performed, whereas impossible employing LC-RID or LC-ELSD method. The concentration ranges of calibration curves, coefficients of determination (r^2), and repeatabilities are shown in Table 2. Good linearity over a wide concentration range was confirmed as well as repeatability at lowest concentration for each compound.

Table 2 Calibration Curves and Repeatabilities

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

Table 3 shows the number of oligosaccharides and polysaccharides detected in each sample. In beer A, B, and F, polysaccharides that are thought to be polymers of glucose were detected in addition to various malto-oligosaccharides such as maltose. Figure 2 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).

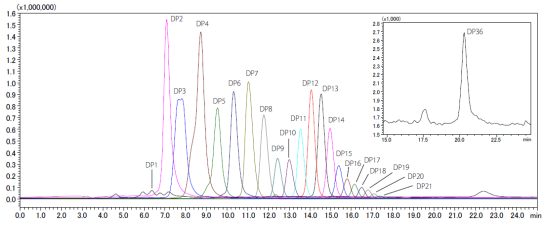

Figure 2 Chromatogram of Beer B

Table 3 Number of Detected Compounds

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

Principal component analysis (PCA) was conducted by Multi-omics Analysis Package (Shimadzu Corporation, Japan) using the peak area of each compound. Figure 3 shows the result of PCA. From the score plot, it was found that "beer A and B" and "beer C and E" are thought to be in same categories. In the loading plot, many oligosaccharides and polysaccharides are plotted on the left side of the first principal component (PC 1). That suggests that PC 1 shows the remained amounts of saccharides in beverages.

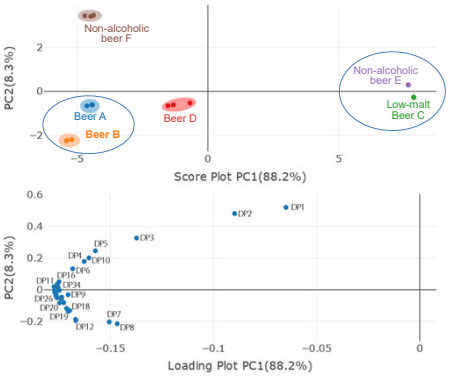

Figure 3 PCA Result for Beer

The relative peak areas (maximum 100) for each oligosaccharide and polysaccharide were heat-mapped (Table 4). Beer A and Beer B contained large amounts of oligosaccharides and polysaccharides that 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 seasoned wort without fermentation for zero alcohol and carbohydrates. Therefore, non-alcoholic beer E has 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

5. Conclusions

An easy and comprehensive method for simultaneous analysis of oligosaccharides and polysaccharides using a single quadrupole LC-MS was developed.
Profiling of oligosaccharides and polysaccharides in alcoholic beverages was successfully performed.