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Differential analysis of fermented beverage using fast polarity switching TOFMS acquisition with high mass accuracy and multivariate analysis

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Introduction

The metabolomics technique can rapidly bring information about the similarities and differences within a chromatographic dataset. A metabolomic based approach has been established for metabolite profiling and biomarker discovery, however, it is equally applicable to other research fields including industrial chemical product characterization, food analysis and natural product research. Brewing and fermentation product of beer etc contains several polar metabolites such as amino acids, nucleic acids and organic acids, which provide important contribution on the product's quality, flavor and being useful index of a fermentation performance. Profiling studies for several beers using ^{1}H NMR were performed for the requirement of quality control and index compounds were determined [1, 2]. The identification of carbohydrates is easily indicated by NMR but its method may need to be improved in respect of sensitivity. Liquid chromatography-mass spectrometry is one of a widely used tool in the field of metabolomics and metabolite profiling by high sensitivity and selectivity. To obtain the complete profile from a sample, it is necessary to run the LC/MS both positive and negative modes [3].
In this study, we developed an LC-based approach to determine metabolite profiles including polar metabolites and to identify specific endogenous components, aiming at high throughput and comprehensive methods using TOFMS acquisition.

Approach of this study

Analytical equipment

1) "Nexera" ※1 Ultra High Performance Liquid chromatograph
2) "LCMS-IT-TOF" ※1 Hybrid Mass Spectrometer
   Fast scanning, fast polarity switching and formula prediction with high accuracy MS^{n} analysis.
3) "Profiling Solution ver. 1.1" ※1 ,Create an aligned data array.
   "SIMCA-P+ ver. 12" ※2 ,Data mining tool using multivariate statistical analysis.
   "Formula Predictor ver. 1.2" ※1 ,Predicting the molecular formula of target compounds.

※1 Shimadzu, ※2 Umetrics

Fig. 1 Nexera UHPLC and LCMS-IT-TOF.

Strategy of differential analysis using MS-based methods

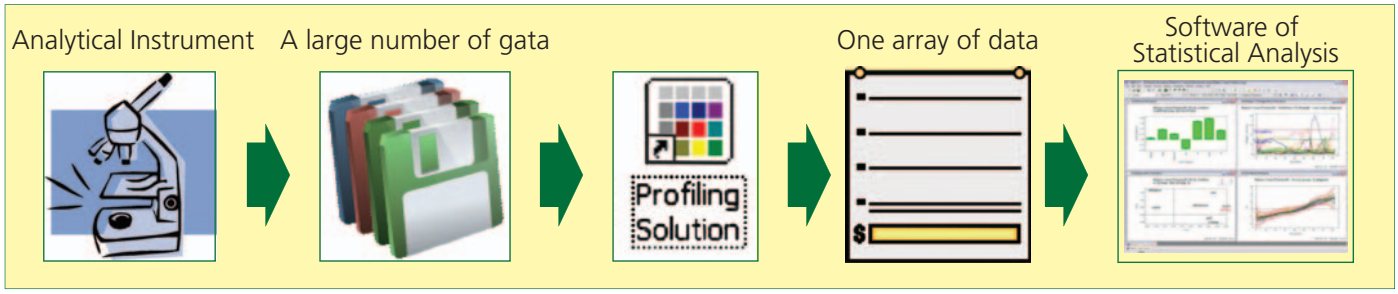

Fig. 2 Work flow of the analysis of metabolites in fermentation products.

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Table 1 Some Characteristics of the Beers analyzed

sample no.	classification *1	type	% alcohol	origin
1	the third beer (no malt)		5.0	Japan
2	the third beer		5.0	Japan
3	the third beer		5.0	Japan
4	the third beer		5.0	Japan
5	the third beer		5.0	Japan
6	the third beer		5.0	Japan
7	low-malt beer	lager	5.5	Japan
8	low-malt beer	lager	5.5	Japan
9	low-malt beer	lager	5.5	Japan
10	beer	lager	5.0	Japan
11	beer	lager	5.0	Japan
12	beer	ale	6.5	Japan
13	beer	lager	5.0	Japan
14	beer	lager	6.0	Japan
15	beer	lager	5.0	Japan
16	beer	lager	4.5	Mexico
17	beer	ale	7.0	Belgium
18	beer	ale	9.0	Belgium
19	beer (high-malt beer)	lager	5.5	Japan
20	beer (high-malt beer)	lager	5.0	Japan
21	beer (high-malt beer)	lager	5.5	Japan
22	beer (high-malt beer)	lager	5.5	Japan
23	beer (high-malt beer)	lager	5.0	Japan
24	beer (high-malt beer)	lager	5.0	Korea
25	beer (high-malt beer)	ale	5.0	Japan

*1: Tax category of Japanese liquor.
Beer: Malt content, 67% or higher.
Low-malt beer: Less than 67% malt (analyzed product contains less than 25% malt).
The third beer: Use malt alternatives, or mix of low -malt beer and another type of spirits.
(High-malt beer: The manufacturer sells the beer of 100% of malt use as high premium beer of added value.)

Table 2 Analytical conditions of LC/MS

Column:	Phenomenex Synergi Hydro-RP 80A (150 mm L. x 2.0 mmI.D., 4.0 um)
Flow rate:	0.2 mL/min
Column temp.:	40°C
Mobile phase:	A) Water containing 0.1% formic acid
Time prog.:	B) 80% Acetonitrile containing 0.1% formic acid 0%B (0-8 min) → 100%B (18-22 min) → 0%B (22.01 min) → STOP 37 min
Injection vol.:	2 uL
Mixer vol.:	0.5 mL
Ionization mode:	ESI positive and negative
Probe voltage:	+4.5 kV/-3.5 kV
CDL temperature:	200°C
BH temperature:	200°C
Nebulizing gas flow:	1.5 L/min
Drying gas flow:	0.1 MPa
CDL,Q-array voltage:	Default value
Scan range:	*m/z* 85-1000

Results

Identification of the isolated compounds

From beer sample, about 60 peaks were detected by peak integration function in positive ion total ion current chromatogram (TICC) and about 30 peaks were detected in negative ion TICC within a 20 min HPLC separation (Fig. 3). When mass accuracy was checked with the known compound such as malic acid and adenosine, it turned out that MS measurement was performed in the accuracy of less than 3 ppm (using external calibration) acquired with fast polarity switching. These detected peaks were verified using formula prediction software that takes into account MSn information, mass accuracy and isotope modeling. Furthermore, tyrosine, phenylalanine, proline, pyroglutamic acid, fumaric acid and hypoxanthine were tentatively assigned by reference to published literature, and identified using authentic standards.

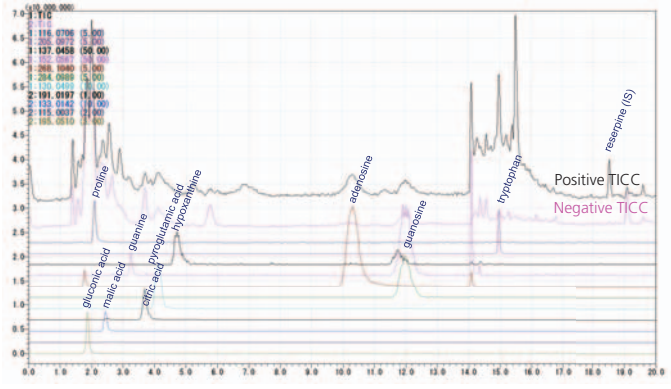

Fig. 3 MS chromatogram of a lager beer No. 20 in Table 1. The important components include amino acids, organic acids and nucleic acids were detected.

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Principal Component Analysis

Pooled QC sample analysis was used to assess the performance of the system by repeatedly injecting it. PCA result of QC samples showed its to be tightly clustered together for both positive and negative ESI data (data not shown). Profiling software produced a data array of *m/z* and retention time pairs. About fifteen hundred ions (1072 ions detected in positive ion, 480 ions detected in negative ion) were detected in common with twenty five beer samples.

PCA of LC/MS data resulted in the separation of samples into six groups: two groups of the third beer, one group of low-malt beer, two groups of beer, and one group of high-malt beer (Fig. 4a). Six groups may be suggested based on the distribution of samples along PC1, which explain most of the variability (41%). These groups of beers roughly are separated according to the malt content: low-malt beers in negative PC1, high-malt beers in positive PC1, and beers characterized by PC1 close to zero. This results suggest that LC/MS approach could be applied to understand the characteristics of sample, for example, label, malt contents and type of beer drinks. The ion of *m/z* 205.0976 in positive mode and *m/z* 191.0199 in negative mode was extracted as a characteristic peak of high-malt beer. These detected peaks were tentatively assigned as tryptophan ($C_{11}H_{12}N_2O_2$) and citric acid ($C_6H_8O_7$) respectively, using formula prediction software, and were further identified by comparison with authentic standards.

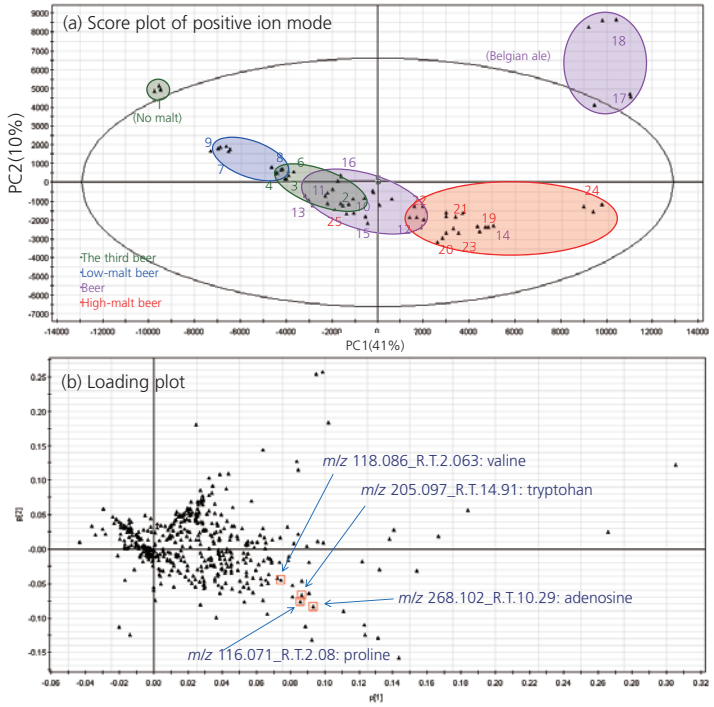

Fig. 4 Multivariate statistics were performed on the aligned data set of positive and negative ions using Umetrics SIMCA-P+ software:
(a) Score plot from 25 beer samples (groups are highlighted by the circles manually drawn).
(b) Loading plot, that indicate each metabolites as *m/z* retention time pairs.

Differential analysis using degradation model sample

In addition, this system was applied to the confirmation of quality deterioration of beer sample. Beers were heated at sixty degrees centigrade for 30 min, 1 hour, 4 hours, 10 hours, 1 day, 3 days, 6 days, and analyzed in the same system. Fig. 5 shows the PCA score and loading plot of one high-malt lager beer (No. 20 in Table 1) of no heat, 4hours, 1 day, 3 days and 6days heated treatment. Sample of heat deterioration were not clearly showed tendency on score plot, since it is considered an imperfect degradation examination. However, several constituents were found as the differentiating components between short term and 6 days treatment (Fig. 6).

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This result suggests that LC/MS approach could be applied to evaluate the content of degradation of several fields such as industrial products. These detected components were tentatively assigned using Formula Predictor software (Fig. 7) and MSn spectra. One of the decreasing intensity of ions by heat treatment was tentatively assigned as deoxyadenosine ($C_{10}H_{13}N_5O_3$).

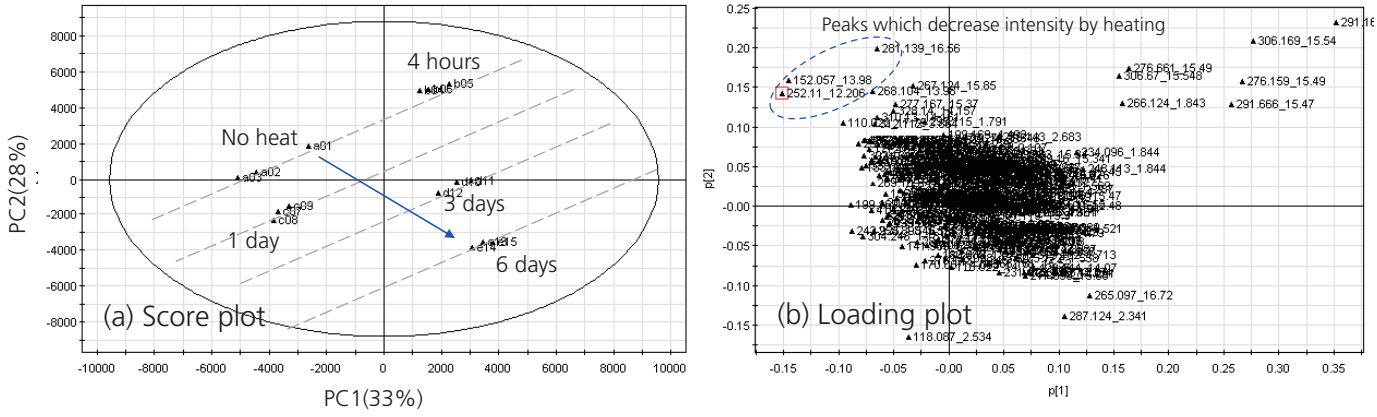

Fig. 5 PCA score plot and loading plot of a lager beer obtained from degradation model data on positive ion mode.

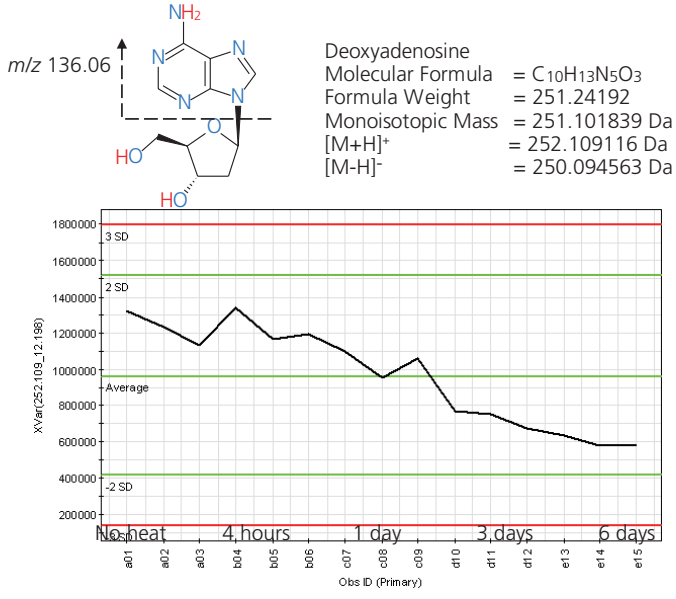

Fig. 6 XVat plot of *m/z* 252.109 eluted at 12.198 minutes. Y axis shows the intensity of detected ion and X axis shows the sample name.

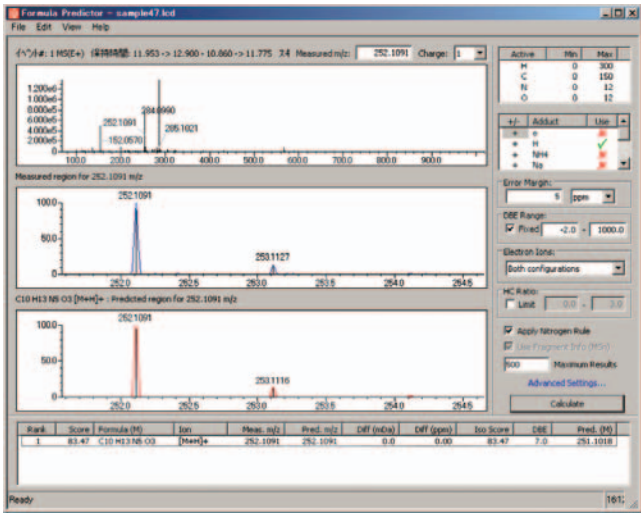

Fig. 7 The formula prediction software results on the *m/z* 252.1091 ion are displayed. The highest score calculated corresponds to the molecular formula $C_{10}H_{13}N_5O_3$.

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Conclusions

- Mass spectrometry-based metabolite profiling was used to identify changes in chemical component levels in fermentation product model using fast polarity switching TOFMS analysis.
- Bioactive marker compounds that belong to the polar metabolites were measured and identified using a combination of high accuracy MS^n data and verified by reference to authentic standards and to internal and external databases.
- Mass spectrometry in combination with multivariate analysis is useful for the rapid determination of subtle differences and exploring the potential markers for quality control not only within the beer products but also in other beverages and biofluids.

References

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