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Application News

Untargeted Metabolomics of Vinegar Using Quadrupole Time-of-Flight LC-MS

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User Benefits

- The differences in the types and contents of flavor compounds and functional compounds in foods can be clarified using untargeted metabolomics with the LCMS-9030 quadrupole time-of-flight (QTOF) LC-MS.
- The high mass accuracy of MS and MS/MS data, a feature of Shimadzu QTOF, enables highly accurate compound identification.

■ Introduction

Vinegar is a liquid condiment that is manufactured from saccharide-containing ingredients by alcoholic and acetic fermentation. Vinegar is made all over the world using various ingredients and manufacturing methods. Vinegar contains numerous compounds, including amino acids, saccharides, and organic acids. These compounds not only affect the flavor of vinegar but are also involved in biological regulation functions. Untargeted metabolomics, a method for comprehensively analyzing metabolites including unknown compounds, can reveal differences in the types and contents of flavor compounds and functional compounds in foods.

This Application News describes an example of untargeted metabolomics of vinegar using the LCMS-9030 quadrupole time-of-flight (QTOF) LC-MS and the MS-DIAL program for untargeted metabolomics/lipidomics.

■ Samples and Sample Pretreatment

The 14 types of vinegar shown in Table 1 were analyzed. Each vinegar sample was diluted 10-fold with ultrapure water, and *p*-chlorophenylalanine was added to each sample (the final concentration was 2.5 nmol/mL) as an internal standard.

Table 1 Sample Details

No.	Sample	Country of Origin	Acidity (%)	Type
1	Rice vinegar	Japan	4.5	Rice vinegar
2	Black rice vinegar A	Japan	4.2	Black rice vinegar
3	Black rice vinegar B	Japan	4.5	
4	Black rice vinegar C	Japan	4.2	
5	Malt vinegar	U.K.	5.0	Grain vinegar
6	Grain vinegar	Japan	4.2	
7	Balsamic vinegar A	Italy	6.0	Grape vinegar
8	Balsamic vinegar B	Italy	4.5	
9	Balsamic vinegar C	Italy	6.0	
10	Red wine vinegar	France	7.0	
11	White wine vinegar	France	7.0	
12	Apple cider vinegar A	Italy	5.0	Apple cider vinegar
13	Apple cider vinegar B	Japan	4.5	
14	Apple cider vinegar C	Japan	5.0	

■ Analytical Conditions

Analysis was performed using the Nexera™ X3 UHPLC system and the LCMS-9030 QTOF LC-MS (Fig. 1). The analytical conditions of HPLC and MS are shown in Table 2.

The non-ion pairing HPLC conditions from the LC/MS/MS Method Package for Primary Metabolites Ver. 3 were adopted to use the retention time information for compound identification. MS data was acquired using the Data-Dependent Acquisition (DDA) mode. In DDA mode, multiple ions with high intensity are selected from all ions detected by MS analysis and analyzed by MS/MS. Unknown compounds are identified by accurate mass and MS/MS spectra.

Fig. 1 Nexera™ X3 and LCMS-9030

Table 2 Analytical Conditions

[HPLC Conditions] (Nexera X3)	
Column:	Discovery HS F5 (150 mm × 2.1 mm I.D., 3 µm)
Column Temp.:	40 °C
Flow Rate:	0.25 mL/min
Mobile Phases:	A) 0.1 % Formic Acid in Water B) 0.1 % Formic Acid in Acetonitrile
Mode:	Gradient Elution
Injection Volume:	2 µL
[MS Conditions] (LCMS-9030)	
Ionization:	ESI Positive or Negative
Mode:	Data Dependent Acquisition (DDA)
TOF-MS:	MS *m/z* 50-1000 MS/MS *m/z* 10-1000
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
Interface Temp.:	300 °C
DL Temp.:	250 °C

■ Data Analysis

Data analysis was performed using the MS-DIAL program (Ver. 5.1.230912). The MS-DIAL program enables peak detection, alignment, data table creation, and statistical analysis (e.g. principal component analysis, PCA) from LC-MS and GC-MS data. The 141 metabolites that are the target compounds in the non-ion pairing method from the LC/MS/MS Method Package for Primary Metabolites Ver. 3 were identified by their retention time information and exact mass data. Other metabolites were identified using MS-DIAL metabolomics MSP spectral kit.

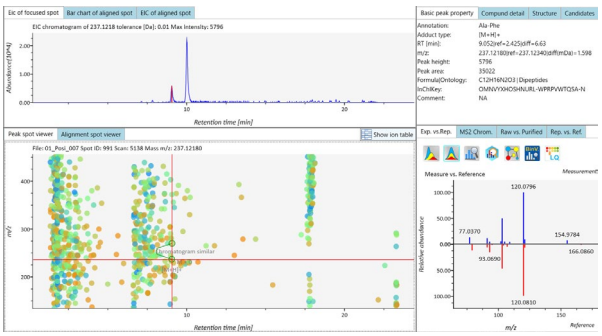

Fig. 2 MS-DIAL Program

■ Analysis Results

As a result of analysis using QTOF LC-MS and MS-DIAL program, 5,309 peaks in positive mode and 10,386 peaks in negative mode were detected. After correction by internal standard, PCA was performed. Fig. 3 and 4 show the results of PCA for positive mode and negative mode, respectively. Three clusters ((1) black rice vinegars A and B, (2) balsamic vinegars A, B, and C, and (3) other vinegars) were formed in the score plot of both positive and negative modes. In the loading plot of positive mode and negative mode, there were many plots on the right side, indicating many characteristic compounds in black rice vinegars A and B.

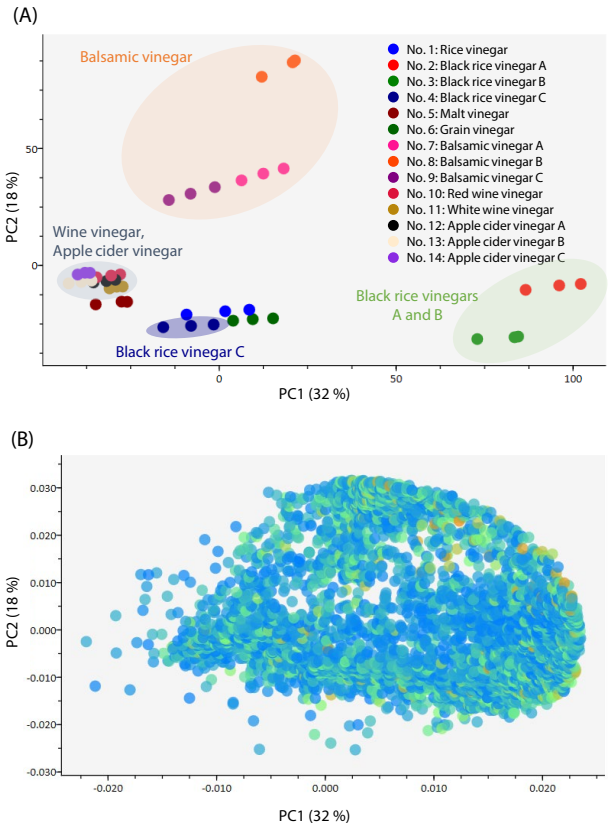

Fig. 3 PCA Results (Positive Mode)
(A) Score Plot, (B) Loading Plot

The balsamic vinegar plots showed that balsamic vinegar B tended to differ from balsamic vinegars A and C, with separate distributions along the second principal component (PC2) axis, particularly in positive mode. The biased loading plot distribution also indicates the presence of numerous characteristic compounds for balsamic vinegar B. This difference may have been due to fermentation-derived metabolites given that balsamic vinegar B had been aged for 6 years.

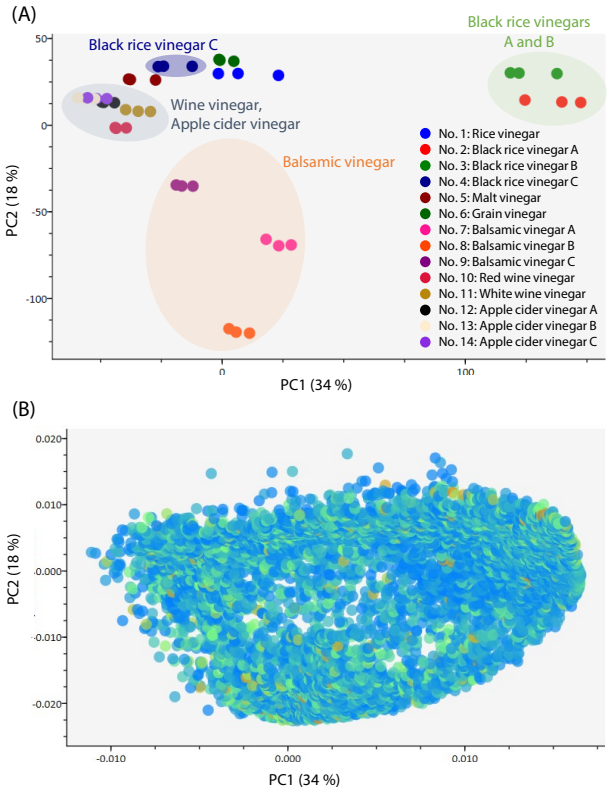

Fig. 4 PCA Results (Negative Mode)
(A) Score Plot, (B) Loading Plot

An example of compound identification using the MS/MS spectrum is shown in Fig. 5. The top spectrum is the measurement MS/MS spectrum of citric acid. The bottom spectrum is the reference MS/MS spectrum of citric acid in the MS-DIAL metabolomics MSP spectral kit. The pattern of the measurement MS/MS spectrum was largely consistent with that of the reference MS/MS spectrum. The difference between the theoretical *m/z* and the measured *m/z* was less than 1 mDa. The compound identification with high accuracy could be performed.

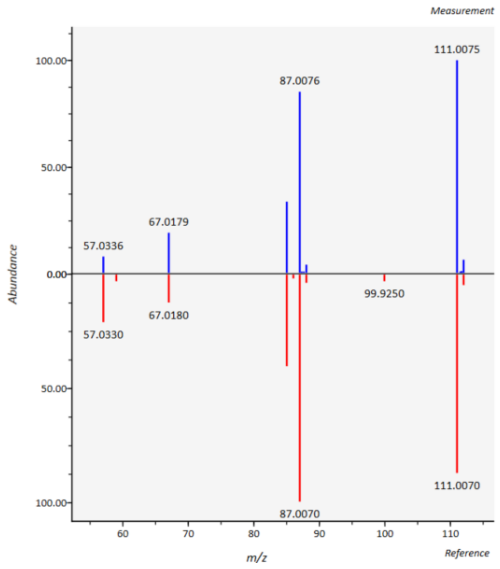

Fig. 5 Comparison of Measurement (Top) and Reference (Bottom) MS/MS Spectra for Citric Acid

Amino acids and dipeptides with high concentration were detected in black rice vinegars A and B (Sample Nos. 2 and 3). Hydroxytyrosol, which was detected in high concentrations in the balsamic vinegars (Sample Nos. 7–9), is a polyphenol that is known to have antioxidant properties. Similarly, quercetin, which was detected in high concentrations in the apple cider vinegars (Sample Nos. 12–14), is also a polyphenol with antioxidant properties. The peak height ratios of characteristic metabolites in the samples are shown in Fig. 6.

■ Conclusion

In this study, untargeted metabolomics of vinegar using the LCMS-9030 QTOF LC-MS and the MS-DIAL program were performed. Many peaks containing flavor compounds such as amino acids and dipeptides, and functional components such as polyphenols were detected, revealing differences of metabolic profile in each vinegar. When searching a database for unknown peaks, highly accurate mass data is useful for significantly refining the number of candidate compounds. The LCMS-9030 can acquire not only highly accurate MS data but also highly accurate MS/MS data. The high mass accuracy of MS and MS/MS data, a feature of Shimadzu QTOF, enables highly accurate compound identification in untargeted metabolomics.

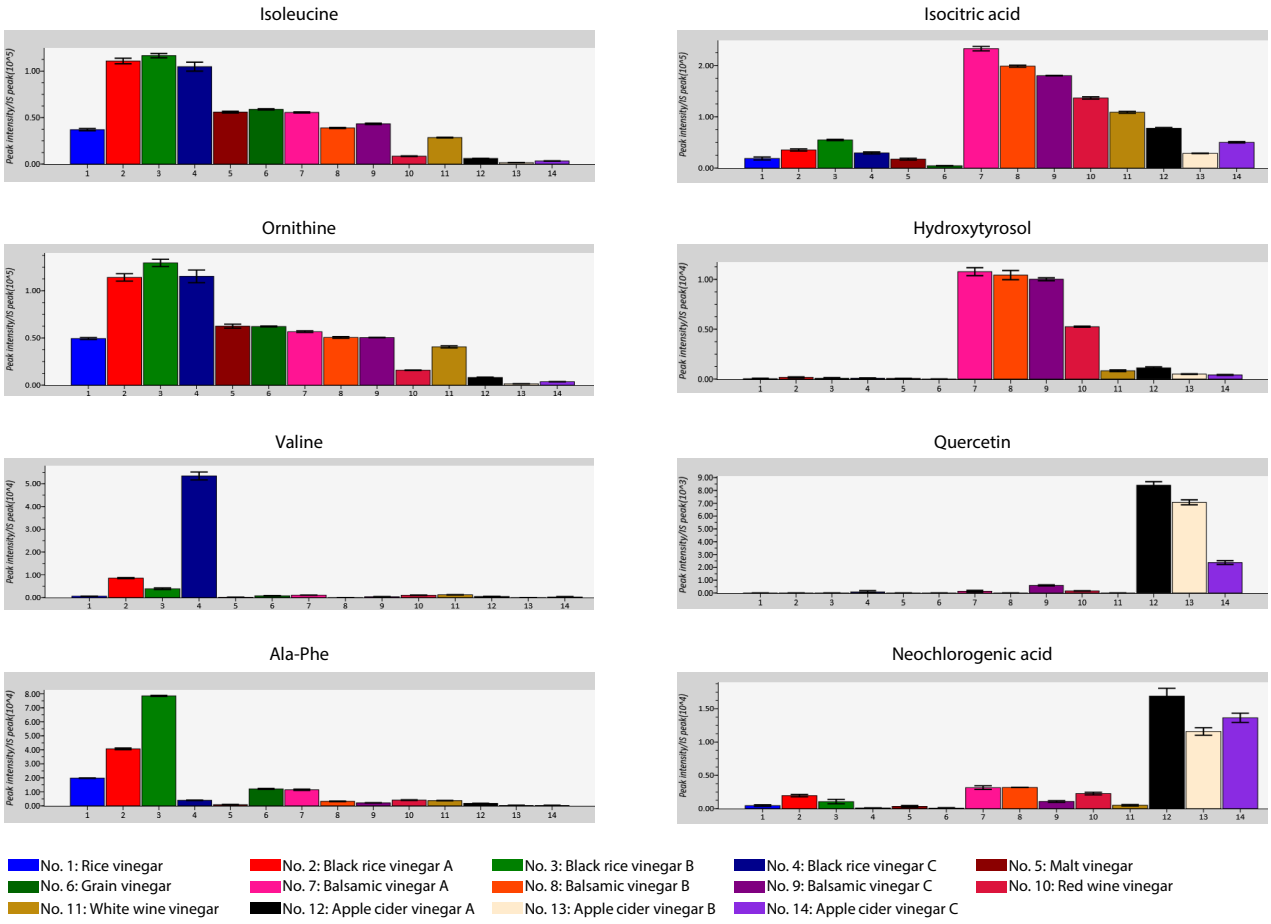

Fig. 6 Characteristic Metabolites among the Samples

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