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Application News

High Performance Liquid Chromatograph Mass Spectrometer
Statistical Analysis Software eMSTAT Solution™

Comparison of Metabolites in Rice from Different Production Areas Using LC-MS/MS

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User Benefits

- eMSTAT Solution enables multivariate analysis of chromatogram data with intuitive operations.
- Multivariate analysis can identify the characteristic compounds in samples.
- Metabolite profiling using LC-MS and multivariate analysis can be performed easily and efficiently.

■ Introduction

Metabolomics is a research approach for comprehensively analyzing metabolites and is utilized across various fields. In the food industry, qualities such as taste, aroma, and nutritional value are determined by multiple components. By comprehensively analyzing these components, it is possible to evaluate food quality. Environmental factors such as climate and soil, which vary by production region, influence the metabolite profiles of agricultural products. As a result, metabolomics is gaining attention as a scientific tool for assessing regional characteristics. Rice, one of the most representative grains, is primarily composed of starch and protein. It also contains sugars, amino acids, organic acids, lipids, and secondary metabolites, all of which are believed to significantly contribute to rice quality through its metabolite profile.

This article presents the results of comprehensive metabolite analysis conducted on rice of the same cultivar grown in different production areas. Measurements were performed using the LCMS-8060RX and the LC/MS/MS Method Package for Primary Metabolites Ver. 3. Data processing was carried out with LabSolutions Insight™, and statistical analysis was conducted using eMSTAT Solution.

■ Sample and Pretreatment

In this analysis, eight commercially available Koshihikari rice samples produced in six prefectures of Japan (Niigata 1, Niigata 2, Toyama 1, Toyama 2, Kagawa, Chiba, Shiga, and Ibaraki) were used. Approximately 10 mg of freeze-crushed rice was weighed and pretreated as shown in Fig. 1. Methionine sulfone was used as the internal standard (IS). Each sample was weighed and pretreated (n = 5).

- Rice 10 mg
- IS (10 nmol/mL) in MeOH 200 µL

↓ Vortex (1 min)
↓ Centrifuge (15,000 rpm, 10 min, 4 °C)

- Supernatant 40 µL
- Water 160 µL

Fig. 1 Pretreatment

■ Analytical Conditions

The measurement conditions are summarized in Table 1. Using the LC/MS/MS Method Package for Primary Metabolites Ver. 3, a total of 141 compounds were analyzed. Additionally, MRM transitions targeting hexose and sucrose were added.

Table 1 Analytical Conditions

[HPLC Conditions] Nexera™ X3	
Column:	Shim-pack™ GIST PFPP (150 mm × 2.1 mm I.D., 3 µm)*1
Column Temp.:	40 °C
Solvent A:	Water + 0.1 % Formic acid
Solvent B:	Acetonitrile + 0.1 % Formic acid
Rinse:	Water + 0.1 % Formic acid
Flowrate:	0.25 mL/min
Injection Volume:	3 µL
[MS Conditions] LCMS-8060RX	
Ionization:	ESI (CoreSpray)
Mode:	MRM
Nebulizing Gas:	2.0 L/min
Drying Gas:	10.0 L/min
Heating Gas:	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C
CID Gas Pressure:	270 kPa

*1 P/N: 227-30858-07

Data processing was performed using LabSolutions Insight, software designed to support quantitative analysis of multiple samples. By using LabSolutions Insight, data processing for multiple samples and compounds can be carried out more easily and efficiently. The peak areas of detected compounds were normalized using the sample weight and the peak area of the internal standard. The resulting area ratios were used for subsequent analysis. These corrected area ratios were further analyzed using eMSTAT Solution. The overall workflow from sample pretreatment to data analysis is shown in Fig. 2.

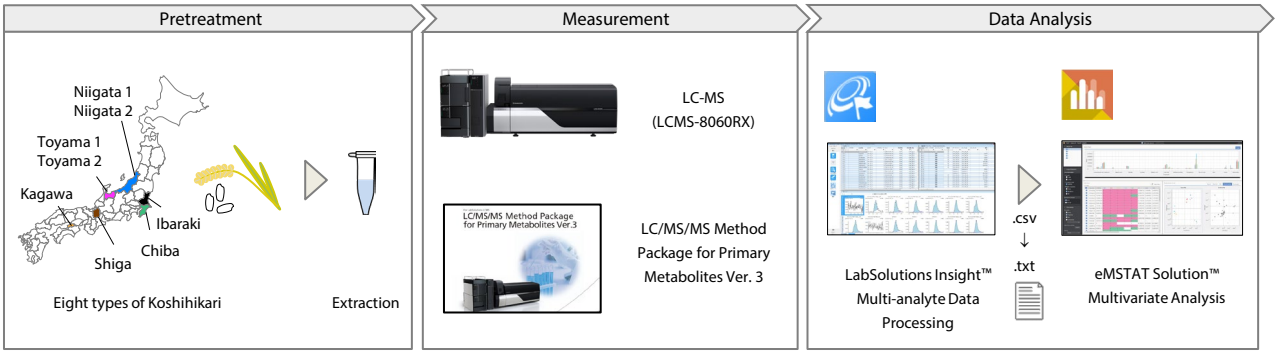

Fig. 2 Workflow from Pretreatment to Data Analysis

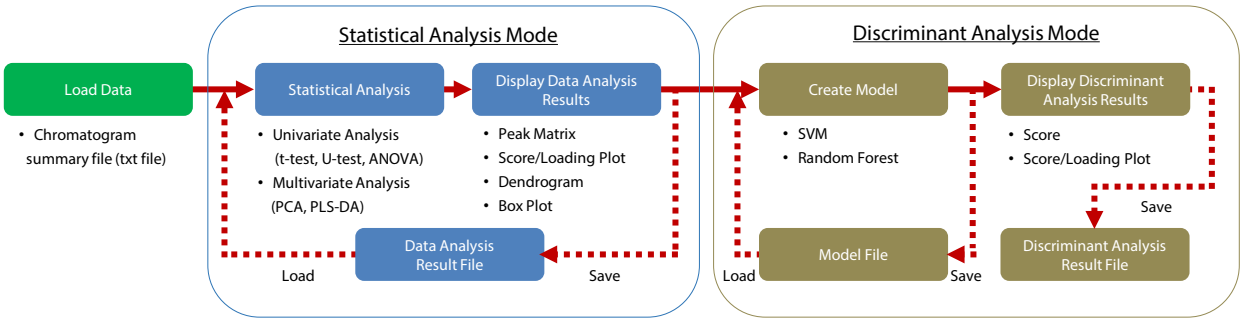

Fig. 3 Workflow for Data Analysis Using eMSTAT Solution

■ eMSTAT Solution

eMSTAT Solution is statistical analysis software featuring an intuitive and user-friendly interface. It enables both univariate and multivariate analyses for marker discovery using analytical data obtained from liquid chromatography and gas chromatography. Furthermore, discriminant analysis of unknown samples can be performed by models based on known sample data (training data). The data analysis workflow is shown in Fig. 3, and an example of the analysis interface is shown in Fig. 4.

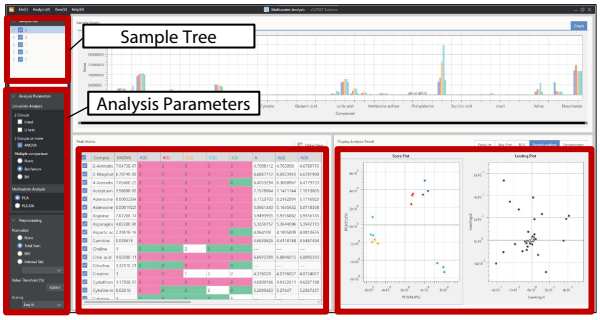

Fig. 4 Example of the Analysis Interface in eMSTAT Solution

■ Detected Compounds

Using LabSolutions Insight, a total of 49 compounds, including amino acids, nucleic acids, and organic acids, were detected in the samples. A representative chromatogram obtained from the analysis is shown in Fig. 5. Hexose and sucrose, for which additional MRM transitions were configured, were also detected in the samples. A list of the detected compounds is shown in Table 2.

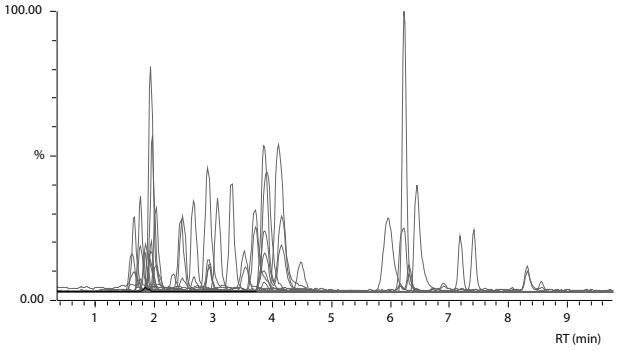

Fig. 5 Representative Chromatogram
(Some chromatograms have been enlarged for display.)

Table 2 Detected Compounds

No.	Compound Name	Classification
1	4-Aminobutyric acid	Amino acids
2	4-Hydroxyproline	
3	Alanine	
4	Arginine	
5	Asparagine	
6	Aspartic acid	
7	Asymmetric dimethylarginine	
8	Glutamic acid	
9	Glutamine	
10	Glycine	
11	Histidine	
12	Isoleucine	
13	Leucine	
14	Lysine	
15	Methionine	
16	Methionine sulfoxide	
17	Phenylalanine	
18	Proline	
19	Serine	
20	Symmetric dimethylarginine	
21	Threonine	
22	Tryptophan	
23	Tyrosine	
24	Valine	

No.	Compound Name	Classification
25	Glutathione	Amino acid derivatives
26	S-Adenosylhomocysteine	
27	Serotonin	
28	Adenine	Nucleobases
29	Cytosine	
30	Uracil	
31	Adenosine	Nucleosides
32	Cytidine	
33	Guanosine	
34	Inosine	
35	Uridine	
36	4-Hydroxybenzoic acid	Organic acids
37	Ferulic acid	
38	Malic acid	
39	Nicotinic acid	
40	Ophthalmic acid	
41	Pantothenic acid	
42	Pyruvic acid	
43	Salicylic acid	
44	Succinic acid	
45	p-Coumaric acid	
46	Hexose	Sugars
47	Sucrose	
48	Allantoin	Others
49	Choline	

■ Comparison of Regional Characteristics Using Multivariate Analysis

Corrected peak area values for 49 compounds were analyzed using eMSTAT Solution. Analysis of variance (ANOVA) revealed statistically significant differences ($p < 0.05$) in 45 out of the 49 compounds. Principal component analysis (PCA) was performed, and the score plot and loading plot are shown in Fig. 6. Samples were clearly separated on the score plot according to their respective production regions.

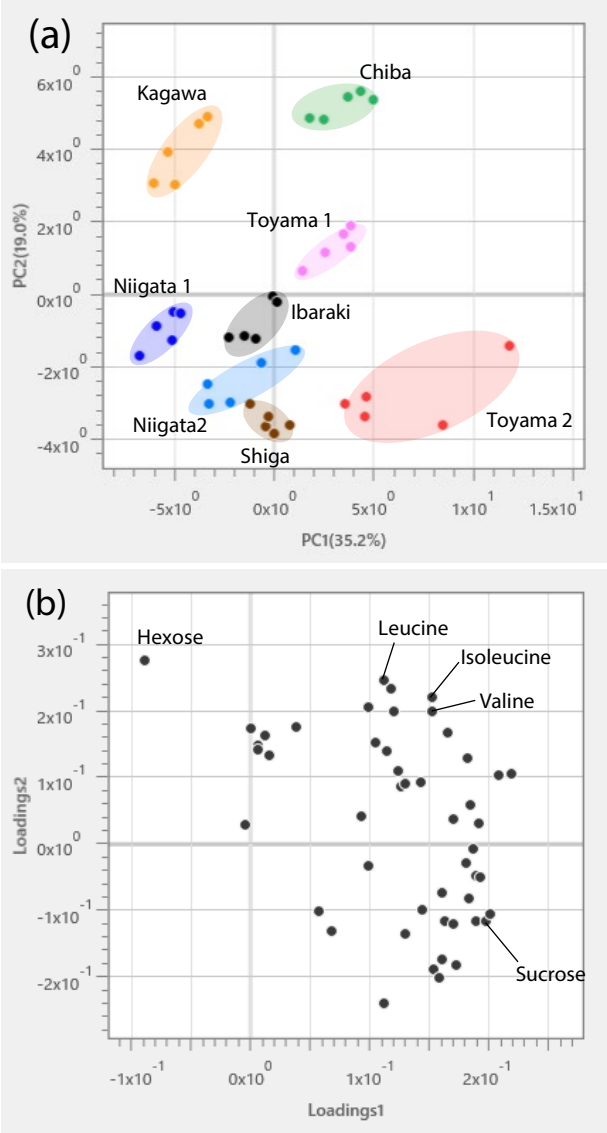

Fig. 6 Results of Principal Component Analysis
(a) Score Plot, (b) Loading Plot (Scaling: Unit Variance)

Fig. 7 presents metabolites that exhibited distinctive patterns across samples. Hexose (a monosaccharide) and sucrose (a disaccharide) were plotted at distant positions on the loading plot, suggesting substantial differences in sugar profiles among production regions. In contrast, the branched-chain amino acids such as leucine, isoleucine, and valine were plotted closely, indicating similar behavior across samples.

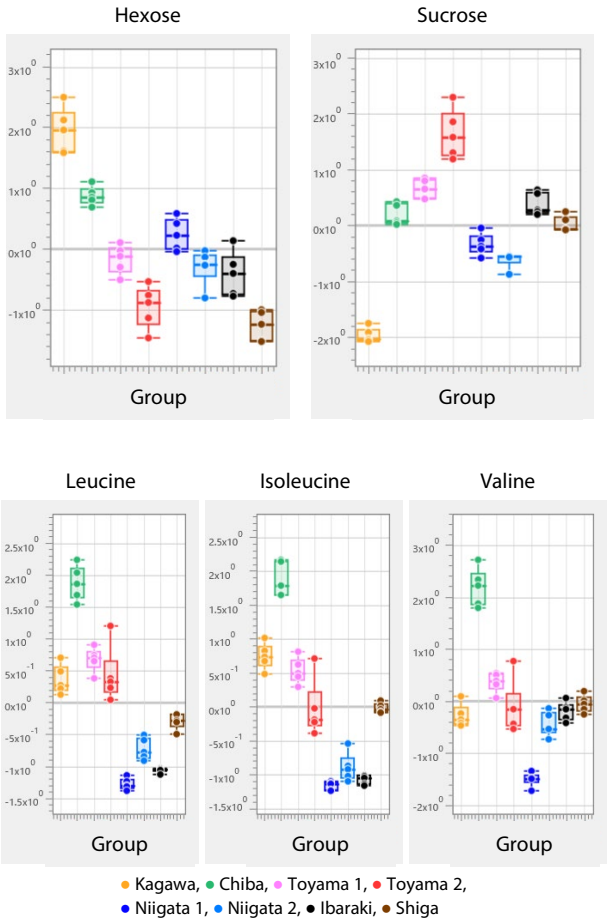

Fig. 7 Box Plots of Characteristic Metabolites Among Samples
(Vertical axis: Scaled peak area values)

■ Conclusion

In this study, comprehensive analysis of hydrophilic metabolites was conducted on rice samples of the same cultivar grown in different production regions. Principal component analysis (PCA) revealed clear separation of samples according to their respective regions in the score plot. Several compounds exhibiting distinctive behavior across samples were also identified.

By utilizing the LC/MS/MS Method Package for Primary Metabolites Ver. 3, LabSolutions Insight, and eMSTAT Solution, the workflow from metabolome analysis to multivariate statistical evaluation can be performed easily and efficiently.

<Related Applications>

1. Comparison of Metabolites in Rice from Different Production Areas Using GC-MS/MS Application News No. 01-01000-EN

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