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Application News

GC-MS GCMS-TQ™8040 NX
Statistical Analysis Software eMSTAT Solution™

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

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User Benefits

- eMSTAT Solution enables multivariate analysis of chromatogram data with intuitive operations.
- Multivariate analysis can identify differences between samples and the characteristic compounds in samples.
- Simultaneous analysis of 502 metabolites and multivariate analysis can be performed easily and efficiently.

■ Introduction

Metabolomics is a research method for comprehensively analyzing metabolites contained in an organism. It is used for a wide variety of fields, such as medical science and food. Because food quality is affected by many components complexly, comprehensive analysis of metabolites can help improve food development and manufacturing processes. Rice contains metabolites, such as sugars, amino acids, and fatty acids. In recent years, development of more flavorful rice has been studied.

This article describes simultaneous analysis of 502 metabolites and multivariate analysis of a variety of rice from different production areas. The GCMS-TQ8040 NX with the Smart Metabolites Database™ Ver. 2 was used for measurement. LabSolutions Insight™ was used for data processing, and eMSTAT Solution was used for multivariate analysis. The process flow, from pretreatment to data analysis, is shown in Fig. 1.

■ eMSTAT Solution

eMSTAT Solution is statistical analysis software that enables multivariate and discriminant analysis of chromatogram data. For multivariate analysis, it can discriminate between samples, and it can search for the marker peaks that contribute to those differences. For discriminant analysis, it can create a discriminant model based on training data and use the model for discriminant analysis. Users can easily switch both modes with just a single click. Workflow of the data analysis is shown in Fig. 2.

■ Pretreatment and Analysis Conditions

Eight Koshihikari samples (n = 3) from six prefectures in Japan (Niigata 1, Niigata 2, Toyama 1, Toyama 2, Kagawa, Chiba, Shiga, and Ibaraki) were prepared. Rice was freeze-crushed. Each 100 mg of crushed rice was extracted by the Bligh & Dyer method, and then derivatized by the methoxime-TMS. 2-isopropylmalate was used as the internal standard substance. Smart Metabolites Database Ver. 2 was used for measurement. Measurement conditions are listed in Table 1.

Table 1 Analysis Conditions

System Configuration	
GC-MS:	GCMS-TQ8040 NX
Autoinjector:	AOC-30i/20s U
Database:	Smart Metabolites Database Ver. 2
Column:	BPX-5 (30 m, 0.25 mm I.D., 0.25 µm)
[GC]	
Sample Injection Temp.:	250 °C
Injection Mode:	Split
Split Ratio:	30
Carrier Gas:	He
Carrier Gas Control:	Linear velocity (39.0 cm/sec)
Column Oven Temp.:	60 °C (2 min) → (15 °C/min) → 330 °C (3 min)
[MS]	
Ion Source Temp.:	200 °C
Interface Temp.	280 °C
Measurement Mode:	MRM

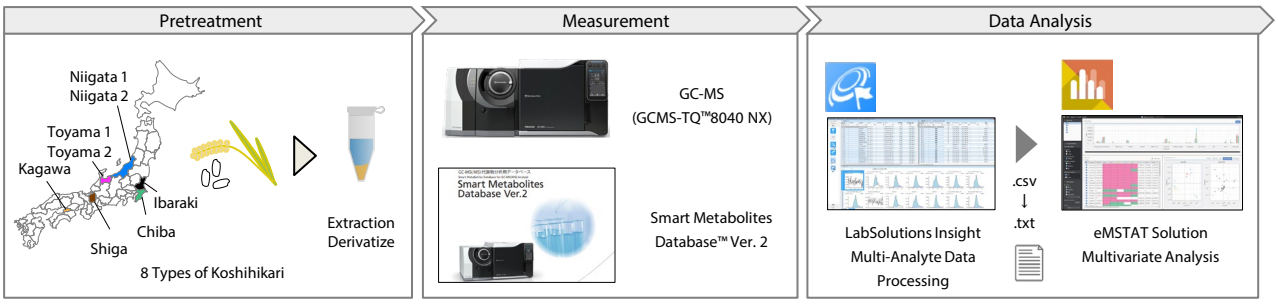

Fig. 1 Workflow from Pretreatment to Data Analysis

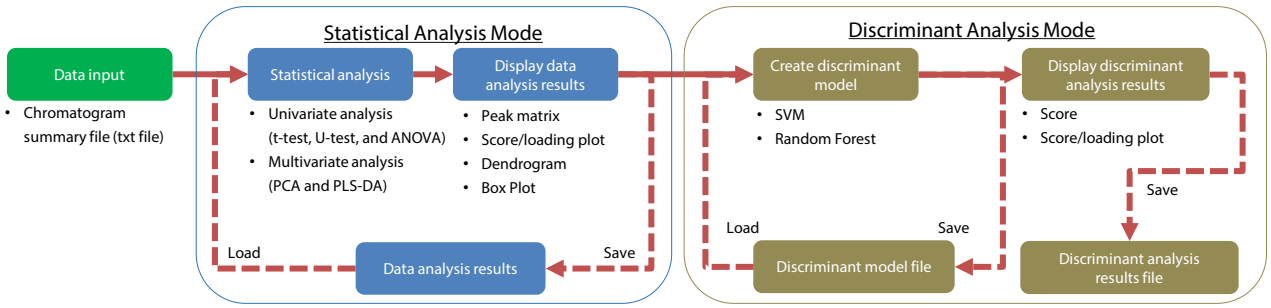

Fig. 2 Workflow of eMSTAT Solution Data Analysis

■ Analysis Results

Examples of MRM chromatograms for detected compounds are shown in Fig. 3, and MRM chromatogram of Niigata 1 is shown in Fig. 4. 65 compounds such as sugars, amino acids, and fatty acids were detected in all samples. Detected compounds are listed in Table 2. Area ratios corrected by the internal standard were used for multivariate analysis. Due to the large number of target components, processing the multi-analyte data would normally take a long time. However, LabSolutions Insight makes data processing easy and efficient. In addition, data input into eMSTAT Solutions can be output (.csv) with a single click.

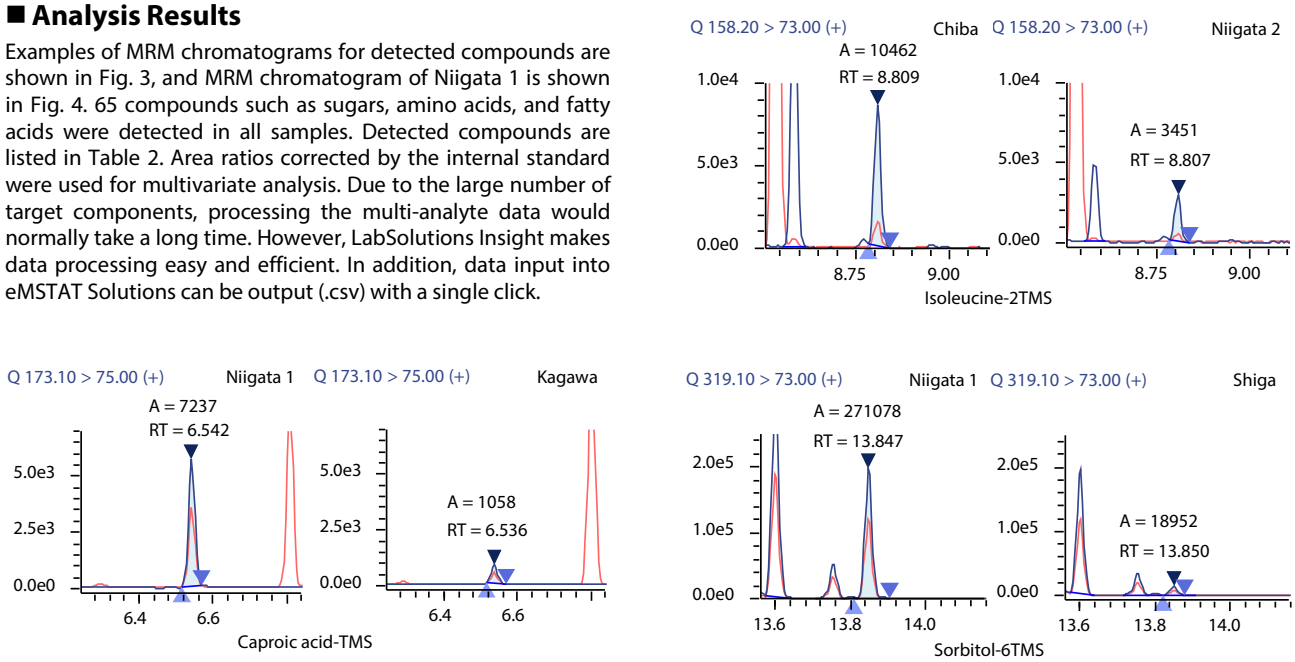

Fig. 3 MRM Chromatograms of Detected Compounds

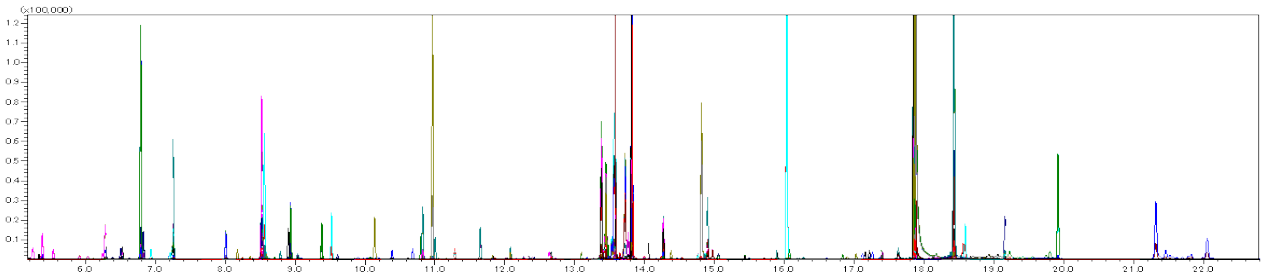

Fig. 4 MRM Chromatogram of Niigata 1 (Overall)

Table 2 List of Detected Compounds

1	Glucose-meto-5TMS(1)	23	2,3-Butanediol-2TMS(1)	45	3-Aminoglutaric acid-2TMS
2	Glucose-meto-5TMS(2)	24	2,3-Butanediol-2TMS(2)	46	4-Aminobutyric acid-3TMS
3	Fructose-meto-5TMS(1)	25	Putrescine-4TMS	47	5-Oxoproline-2TMS
4	Fructose-meto-5TMS(2)	26	Alanine-2TMS	48	2-Aminopimelic acid-3TMS
5	Arabinose-meto-4TMS	27	Valine-2TMS	49	Indol-3-acetic acid-2TMS
6	1,6-Anhydroglucose-3TMS	28	Leucine-2TMS	50	Salicylic acid-2TMS
7	Sucrose-8TMS	29	Isoleucine-2TMS	51	Caproic acid-TMS
8	Raffinose-nTMS	30	Proline-2TMS	52	Glycolic acid-2TMS
9	N-Acetylglucosamine-meto-4TMS	31	Glycine-3TMS	53	Octanoic acid-TMS
10	N-Acetylmannosamine-meto-4TMS(1)	32	Serine-3TMS	64	Nonanoic acid-TMS
11	N-Acetylmannosamine-meto-4TMS(2)	33	Threonine-3TMS	55	Myristic acid-TMS
12	Glycerol-3TMS	34	Aspartic acid-3TMS	56	Palmitic acid-TMS
13	Sorbitol-6TMS	35	Glutamic acid-3TMS	57	Stearic acid-TMS
14	Inositol-6TMS(2)	36	Phenylalanine-2TMS	58	Linoleic acid-TMS
15	Citric acid-4TMS	37	1-Butylamine-2TMS	59	Urea-2TMS
16	Succinic acid-2TMS	38	Hydroxylamine-3TMS	60	Allantoin-4TMS
17	Glyceric acid-3TMS	39	2-Aminoethanol-3TMS	61	Adenosine-4TMS
18	3-Hydroxyglutaric acid-3TMS	40	Cysteine-3TMS	62	Nicotinic acid-TMS
19	Lactic acid-2TMS	41	Tyrosine-3TMS	63	Pantothenic acid-3TMS
20	Glycolic acid-2TMS	42	Tryptophan-3TMS	64	Phosphoric acid-3TMS
21	Benzoic acid-TMS	43	Asparagine-3TMS	65	Glycerol 3-phosphate-4TMS
22	Ferulic acid-2TMS	44	3-Aminopropanoic acid-3TMS		

■ Multivariate Analysis Results

The peak list (.csv) output from LabSolutions Insight was converted into a text file (.txt) and loaded into eMSTAT Solution. The detected 55 compounds with a p-value of 0.05 or less were used for principal component analysis (PCA).

A score plot of the eight types of rice is shown in Fig. 5. The contribution rate from PC1 was 22.0 % and PC2 was 19.4 %, for a total of 41.4 %. Kagawa and Chiba were separated by PC1. Toyama and Niigata were separated by PC2.

Loading plots are shown in Fig. 6. Amino acids such as leucine and valine were more abundant in Chiba, monosaccharides such as glucose and fructose in Kagawa, fatty acids such as octanoic acid and caproic acid in Niigata, and organic acids such as citric acid and 2-amino pimelic acid in Toyama 1 and Toyama 2. Mono and polysaccharides are plotted in opposite by PC2, which suggest sugar profiles are different. Example of box plots for characteristic compounds are shown in Fig. 7.

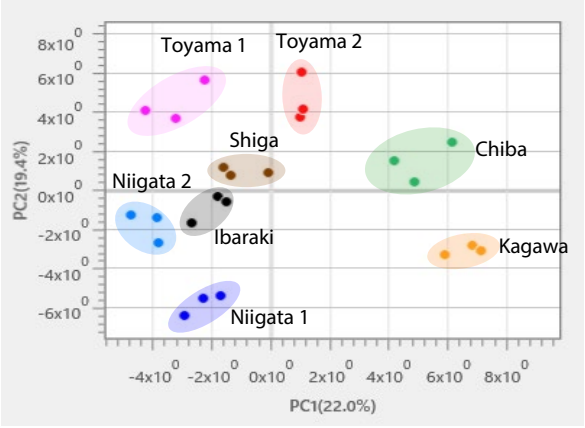

Fig. 5 Score Plot of 8 Types of Rice

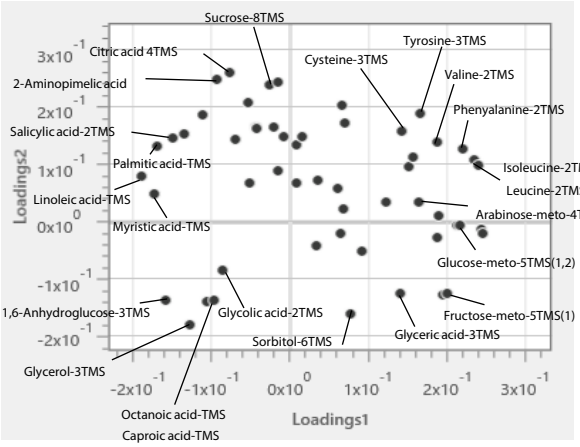

Fig. 6 Loading Plot of 8 Types of Rice

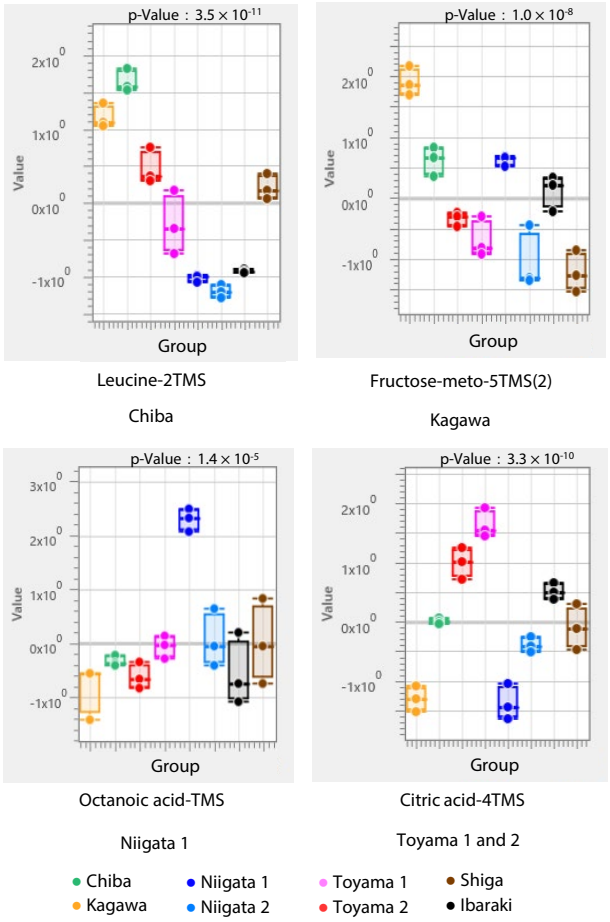

Fig. 7 Box Plots of Characteristic Compounds

■ Conclusion

Metabolites in same variety of rice from different production areas were analyzed, and the differences between samples were identified by multivariate analysis. Score plots were used to visualize differences between the samples, and loading plots were used to confirm the characteristic compounds.

Smart Metabolites Database Ver. 2, LabSolutions Insight, and eMSTAT Solution make metabolites analysis, data processing, and multivariate analysis easy for beginner users.

01-01000-EN First Edition: Aug. 2025

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