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Application News

GC-MS GCMS-TQ™ Series and Smart Metabolites Database™

Multivariate Analysis of Tomato Varieties Using Smart Metabolites Database

Y. Sakamoto and Y. Kawakita

User Benefits

- Makes simultaneous analysis to multivariate analysis easy for metabolomics.
- The sample classification filtering function improves the efficiency of data analysis by only analyzing the data for components with a high probability of detection.
- The sugar semi-quantitation method can be used to calculate the approximate concentrations of 24 types of sugars in samples.

■ Introduction

Metabolomics refers to technology used for the comprehensive analysis of all metabolites in organisms. In medical fields, metabolomics is used as an effective way to search for biomarkers that indicate the physiological changes in diseases. In recent years, metabolomics has also been used in a wide variety of food-related applications, such as analyzing differences in the percentage of ingredients, searching for components with functional benefits, establishing methods for quality evaluations, and predicting degradation over time.

GC-MS(/MS) is used in metabolomic analysis to target low molecular weight hydrophilic metabolites such as amino acids, fatty acids, organic acids, and sugars because it can analyze such components simultaneously. However, detecting trace quantities of these components is best performed using SIM or MRM, which require optimization of the MS parameter settings.

Smart Metabolites Database has been compiled specifically for the analysis of metabolic components using GC-MS(/MS). This database has now been upgraded to Version 2.0 to expand its applicability to metabolomics for food applications.

This article describes the use of Smart Metabolites Database with multivariate analysis to identify differences in the components in different tomato varieties and to compare the concentrations of sugars across samples.

■ Smart Metabolites Database Ver. 2

Smart Metabolites Database Ver. 2 includes the analytical condition settings, and optimized SIM and MRM transition settings for metabolic components. The number of registered components in the simultaneous analysis method used to analyze metabolic components for biomarker search applications has been increased to 627 components for SIM and 540 components for MRM. In addition, compound-specific quantitative analysis methods for analyzing sugars have been added to those for amino acids and fatty acid methyl esters (FAMEs).

The database also includes a new sample classification filtering function. By selecting a filter suitable for the sample being measured, measurements can be limited to metabolic components predicted to be in the sample. This can significantly shorten the time required for data processing.

Furthermore, LabSolutions Insight, a software for multianalyte quantitative analysis can be used to easily create lists for multianalyte or multivariate data analysis. These lists can then be output and loaded into the Multi-Omics Analysis Package for principal component analysis (PCA) or hierarchical cluster analysis (HCA) (Fig. 1).

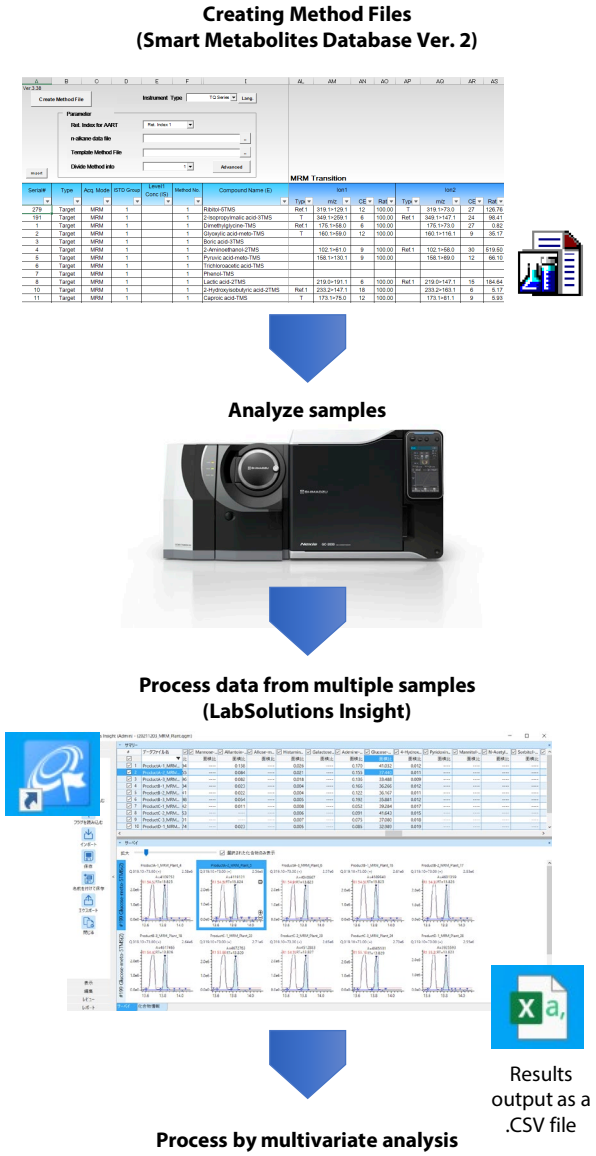

Fig. 1 Process Flowchart for Multivariate Analysis Using Smart Metabolites Database Ver. 2

■ Experiment

Three samples (n = 3) each of 4 commercial tomato varieties were prepared. The tomatoes were cut to a suitable size and then freeze-dried. Components were extracted from 10 mg of the dried samples using a pretreatment protocol based on the Bligh & Dyer method, and were then derivatized by methoximation and trimethylsilylation. Ribitol was used as the internal standard. Smart Metabolites Database Ver. 2 was used for the GC-MS/MS measurements, with the sample classification filter set to "plant." The measurement conditions are listed in Table 1.

Table 1 System Configuration and Analytical Conditions

GC-MS:	GCMS-TQ8040 NX
Auto-injector:	AOC™-30i/20s U
Database:	Smart Metabolites Database Ver. 2
Column:	BPX-5 (30 m, 0.25 mm I.D., 0.25 µm)
[GC]	
Injection Temp.:	250 °C
Column Oven Temp.:	60 °C (2 min) → (15 °C /min) → 330 °C (3 min)
Injection Mode:	Split
Split Ratio:	30
Carrier Gas:	He
Carrier Gas Control:	Linear Velocity (39.0 cm/sec)
Injection Volume:	1 µL
[MS]	
Ion Source Temp.:	200 °C
Interface Temp:	280 °C
Data Acquisition Mode:	MRM

■ Multivariate Analysis Results

The results obtained using the Multi-Omics Analysis Package for principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the data obtained from the 4 tomato varieties (n = 3) are shown in Figs. 2 to Fig. 4.

The samples are clearly differentiated in the PCA score plot and the HCA tree diagram. In addition, the heat map from hierarchical cluster analysis shows that Product A tends to contain more components than the other 3 tomato varieties, including particularly high amino acid levels. The results also show that Product C contains more sugars including inositol, glucose, and fructose.

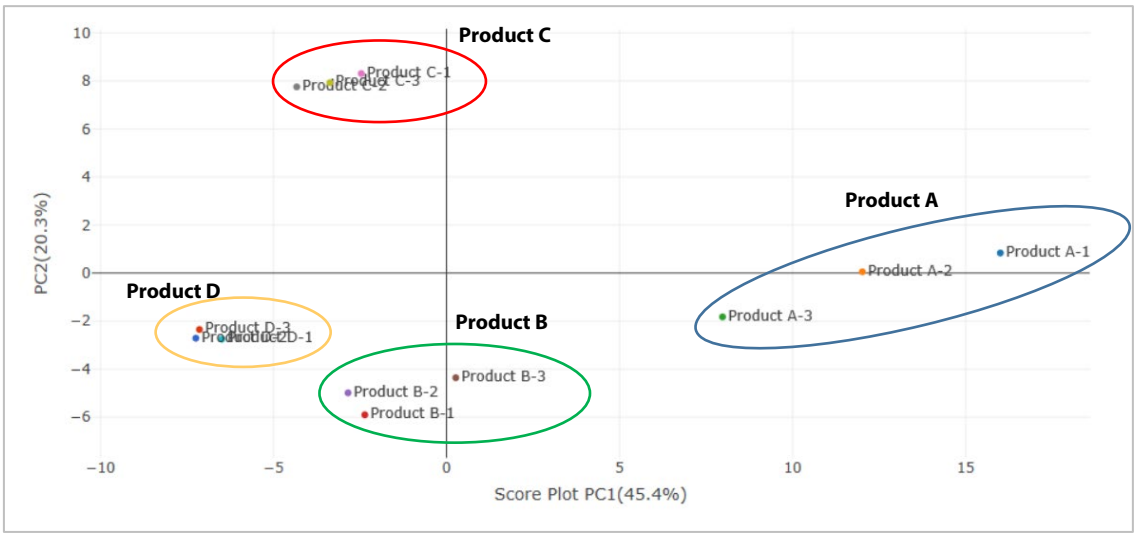

Fig. 2 Score Plot of Metabolic Components Detected in 4 Tomato Varieties

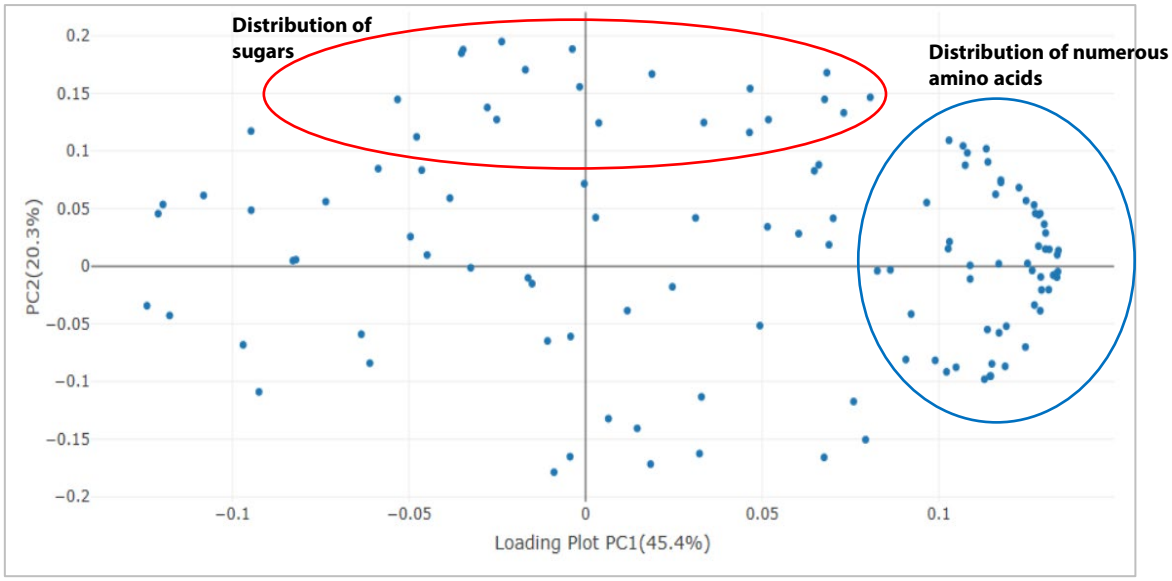

Fig. 3 Loading Plot for the Metabolic Components Detected in 4 Tomato Varieties

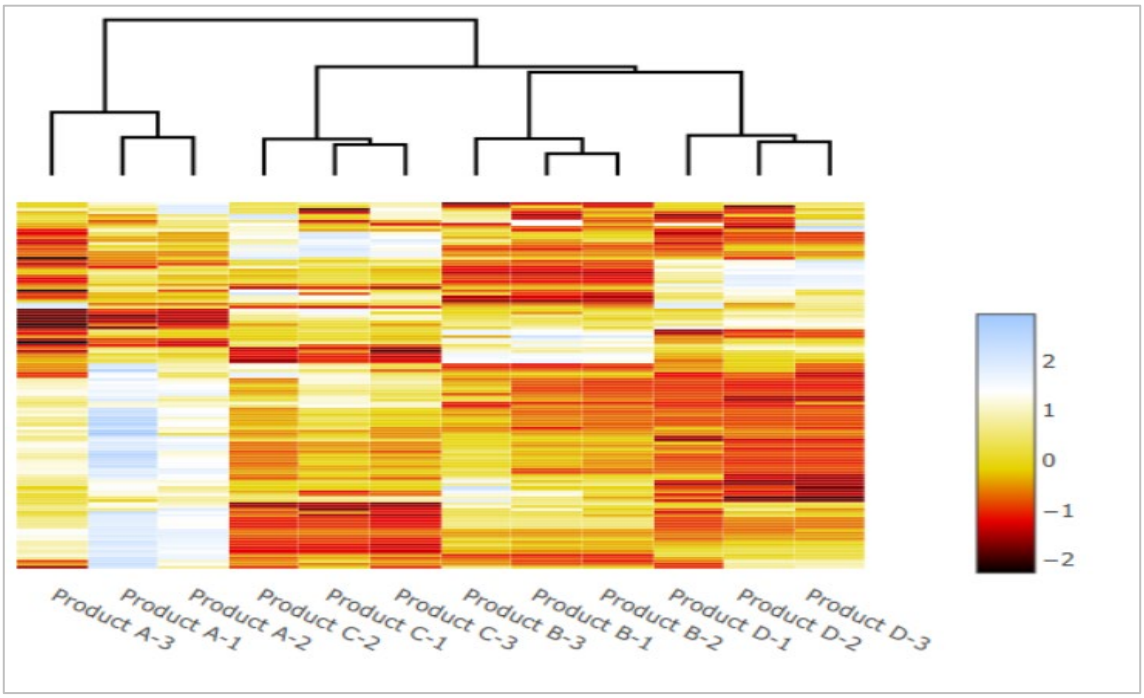

Fig. 4 Hierarchical Cluster Analysis Heatmap (HCA Heatmap) Detected from 4 Tomato Varieties

■ Method for Quantitative Analysis of Sugars

Methoxime-trimethylsilylation (MeOx-TMS) is typically used for derivatization in simultaneous metabolites analysis, but due to the especially reductive characteristic of sugars, methoximation produces chain structures that form two types of geometric isomers. Those two geometric isomers and the change in properties caused by the chain structures make quantitative analysis difficult because the sugar isomers sorbose and fructose and sugar isomers mannose and glucose cannot be separated in chromatograms.

However, the optimized derivatization method settings in the sugar semi-quantitation method included in Smart Metabolites Database Ver. 2 enables the selective detection of sugars. The compound information and calibration curve information necessary for analysis is registered in the database for 24 key sugars. By pretreating the specified sample quantity for simultaneous analysis and then derivatizing the sugars in a portion of the extracted sample, semi-quantitative values can be calculated for the sugars while taking into consideration the extraction efficiency and derivatization efficiency.

Because the results in Figs. 2 to 4 show a difference between the sugars in Product C, the method for the quantitative analysis of sugars was used to evaluate the differences between the tomato varieties. 100 µL of the sample extracted by the Bligh & Dyer method from the sample used for multivariate analysis was collected and derivatized. In addition, ribitol was used as the internal standard. Table 2 lists the analytical conditions for the sugar analysis and Fig. 5 shows the MRM chromatogram for Product C. The results show that the derivatization method provided high selectivity for sugars and that most of the other types of metabolites were not derivatized and not detected.

Table 2 System Configuration and Analytical Conditions

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]	
Injection Temp.:	280 °C
Column Oven Temp.:	150 °C (5 min) → (3 °C/min) → 220 °C → (10 °C/min) → 320 °C (3 min)
Injection Mode:	Split
Split Ratio:	15
Carrier Gas:	He
Carrier Gas Control:	Linear Velocity (34.0 cm/sec)
Injection Volume:	1 µL
[MS]	
Ion Source Temp.:	230 °C
Interface Temp:	280 °C
Data Acquisition Mode:	MRM

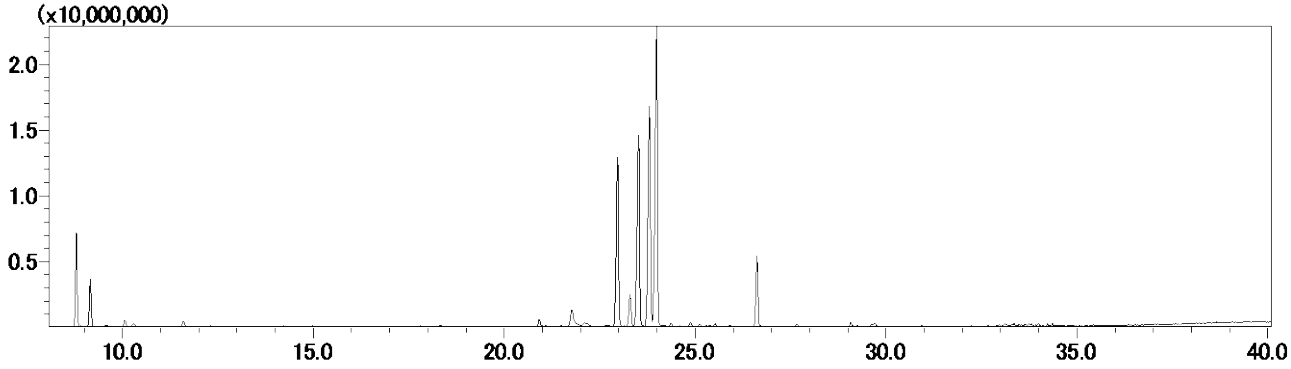

Fig. 5 MRM Chromatogram of Tomato Product C

■ Sugar Analysis Results

Fig. 6 shows the MRM chromatograms for representative components detected in Product C. Semi-quantitative results (concentrations in the freeze-dried tomatoes) calculated from the database for the sugars detected in each tomato are indicated in Table 3 and Fig. 7.

Product C contained more glucose, mannose, fructose, and *myo*-inositol than the other varieties (about 20 to 40 % more), whereas Product B contained mannitol, which was not detected in the other varieties. Product D contained about twice as much sucrose as the other varieties.

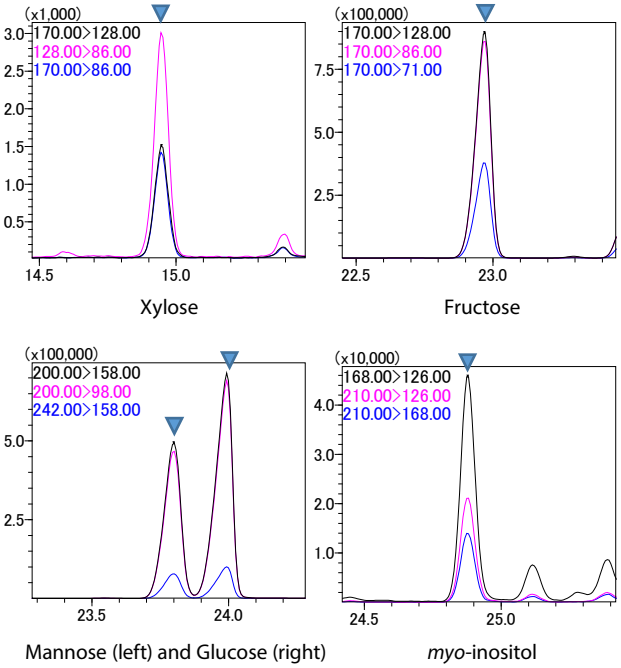

Fig. 6 MRM Chromatograms of Representative Sugars Detected in Tomato Product C

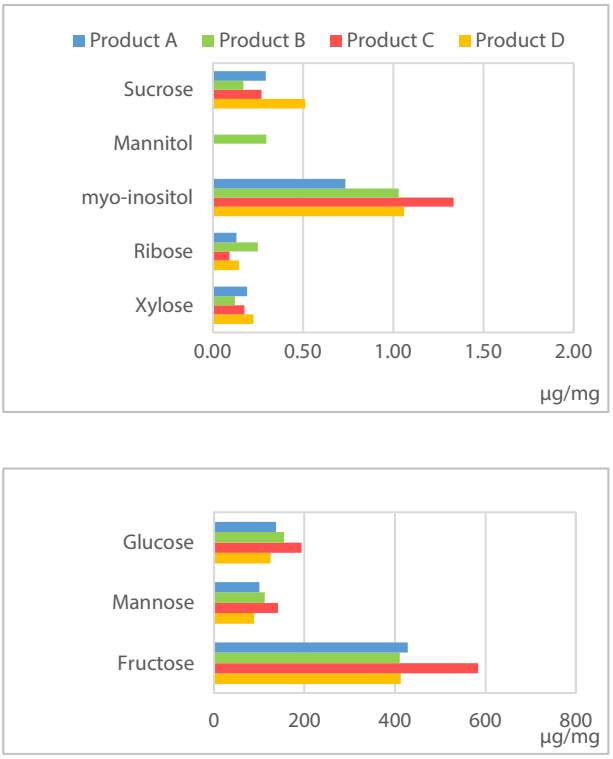

Fig. 7 Comparison of the Semi-Quantitative Results for Sugars Detected in 4 Tomato Varieties
Upper: Low Concentration; Lower: High Concentration

Table 3 Semi-Quantitative Results for Sugars Detected in 4 Tomato Varieties

Unit: µg/mg

Compound	Product A	Product B	Product C	Product D
Xylose	0.19	0.12	0.17	0.22
Ribose	0.13	0.25	0.09	0.15
myo-inositol	0.73	1.03	1.33	1.06
Mannitol	N.D.	0.29	N.D.	N.D.
Sucrose	0.29	0.17	0.27	0.51
Fructose	428	410	584	413
Mannose	101	112	142	89
Glucose	138	155	194	125

■ Conclusion

Multivariate analysis of metabolic components using Smart Metabolites Database Ver. 2 and the Multi-Omics Analysis Package enabled differentiation of metabolites between the tomato varieties. Furthermore, it was possible to examine differences indicated by multivariate analysis in more detail through individual analyses targeting sugars.

The newly developed Smart Metabolites Database Ver. 2 thus provides a useful tool for the metabolomic analysis of foods.

Note: Semi-quantitative results can deviate significantly from the true values depending on the type of sample and pretreatment method used. If accurate quantitative results are required, use standard samples to perform quantitative testing.

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