SHIMADZU
Excellence in Science

Application News

No. M310

GC-MS GCMS-TQ™8040 NX

Quality Evaluation of Food Products Using Multivariate Analysis (1): Analysis of Metabolites in Tomato Juice

Y. Kawakita, Y. Sakamoto

User Benefits

- Smart Metabolites Database™ enables highly sensitive simultaneous analysis of hydrophilic metabolites.
- Distinctive components between different samples can be identified by multivariate analysis.
- Multivariate analysis using MRM data is easily possible by Traverse MS software.

■ Introduction

In the food and beverage markets, many products that offer some form of added value in addition to taste have been developed and are marketed as foods with health claims, which capture health-oriented needs, and various products in the high-end price range, which allow consumers to enjoy a sense of closer to the original materials, i.e., more natural, high-class feeling or luxury. Product differentiation is conducted by using materials, manufacturing process, and distribution process which are different from those of general products.

Components such as sugars, nucleic acids, amino acids, organic acids, and fatty acids contained in food products differ depending on the type of product and the manufacturing process, and influence the quality of the products, including their taste, functional components, and shelf life.

In this article, the objective was to identify the distinctive components in three types of commercially-available tomato juice. A simultaneous analysis of hydrophilic components was carried out by using the Smart Metabolites Database, and differences in the components of the samples were analyzed by the Traverse MS multivariate analysis software. The results are introduced in the following.

■ Sample Preparation and Analysis Conditions

Three types of commercially-available tomato juice were prepared as samples. These products were using only tomatoes or tomatoes and common salt (table salt) as raw materials.

Fig. 1 shows the workflow of sample preparation. First, 100 µL of the tomato juice was measured out in a 1.5 mL microtube, and 10 µL of aqueous ribitol (0.2 mg/mL) was added as an internal standard. 500 µL of a mixed solvent consisting of water : methanol : chloroform = 1 : 2.5 : 1 (extraction solvent) was added to the tube. The mixture was shaken for 30 min at 37 ˚C, then separated by centrifuging (4 ˚C, 3000 g, 10 min), and 450 µL of the supernatant (water/methanol phase) was taken. 400 µL of ultrapure water was added, and the solution was mixed well and then centrifuged again for 5 min, and 500 µL of the supernatant was taken. After vaporizing the methanol with a centrifugal evaporator, the sample was sufficiently exsiccated by freeze-drying. 200 µL of a methoxyamine-pyridine solution (20 mg/mL) was added to the residue after exsiccation, and the mixture was shaken for 90 min at 30 ˚C. Following this, 100 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, and the mixture was shaken for 30 min at 37 ˚C and then used as the analysis sample. The Smart Metabolites Database, which contains MRM transitions for 475 metabolites, was used in the GC-MS/MS measurements. Table 1 shows the measurement conditions.

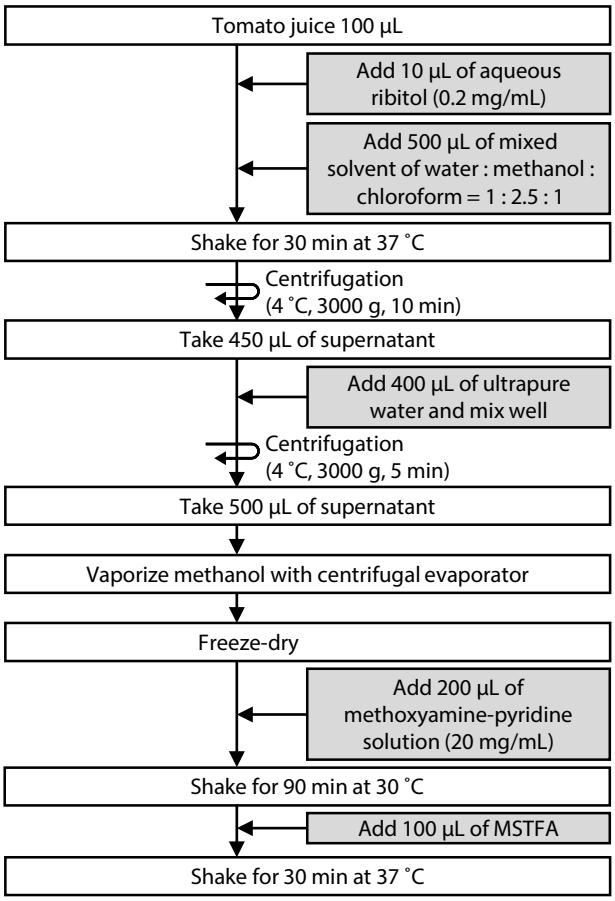

Fig. 1 Workflow of Sample Preparation

Table 1 Measurement Conditions

GC-MS	: GCMS-TQ™8040 NX
Autoinjector	: AOC™-20i + s
Column	: BPX-5 (length 30 m, 0.25 mm I.D., df = 0.25 µm)
[GC]	
Vaporizing chamber 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	: 39.0 cm/s (constant linear velocity)
Injection volume	: 1 µL
[MS]	
Ion source temp.	: 200 ˚C
Interface temp.	: 280 ˚C
Data sampling mode	: MRM
Loop time	: 0.25 s

■ Results

As a representative example, Fig. 2 shows the chromatogram of Sample 1. As shown in Table 2, 113 components were detected from each of the tomato juice samples, including glucose, fructose and other sugars, amino acids such as GABA and others, organic acids, nucleic acids, and saturated fatty acids.

A multivariate analysis was conducted using the Traverse MS multivariate analysis software (Reifycs Inc.). The analysis was carried out using data acquired 3 times for each sample, and the intensities of the compounds detected in each sample were normalized by using ribitol as an internal standard. The tomato juices used here were labeled Sample 1 to 3. Table salt was added to only Sample 1, and Sample 3 was made from the high-grade variety of tomato.

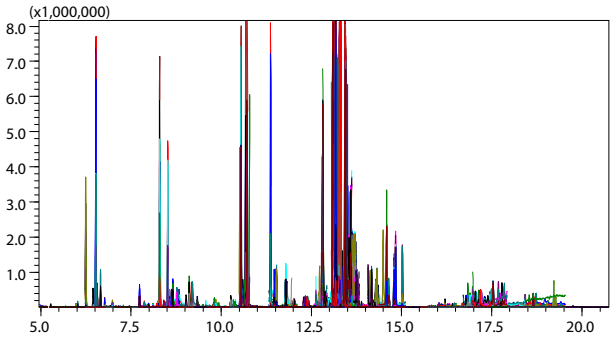

Fig. 2 Analysis Result of Tomato Juice (Sample 1)

Table 2 Components Detected in Tomato Juice Samples

Sugars	**Amino acids**	**Others (organic acids, amines, etc.)**
Arabitol	2-Aminobutyric acid	2-Aminoethanol
Erythritol	2-Aminopimelic acid	2-Hydroxyglutaric acid
Erythrulose	3-Aminoglutaric acid	2-Ketobutyric acid
Fucose	3-Aminoisobutyric acid	2-Ketoglutaric acid
Fructose	3-Aminopropanoic acid	3-Hydroxybutyric acid
Galactose	4-Aminobutyric acid (GABA)	3-Hydroxyisovaleric acid
Galacturonic acid	5-Oxoproline	3-Hydroxypropionic acid
Glucaric acid	5-Aminovaleric acid	Aconitic acid
Glucosamine	Alanine	Allantoin
Glucose	Anthranilic acid	Benzoic acid
Glucuronic acid	Arginine	Caproic acid
Glyceric acid	Asparagine	Citramalic acid
Glycerol	Aspartic acid	Citric acid
Inositol	Glutamic acid	Fumaric acid
Lyxose	Glycine	Glycolic acid
Maltose-meto	Histidine	Hypotaurine
Psicose	Homoserine	Isocitric acid
Rhamnose	Isoleucine	Lactic acid
Ribulose	Leucine	Lauric acid
Trehalose	Lysine	Maleic acid
Xylose	Methionine	Malic acid
Xylulose	O-Acetylserine	Malonic acid
	Ornithine	Mesaconic acid
Sugar phosphates	Phenylalanine	Methylsuccinic acid
3-Phosphoglyceric acid	Proline	Niacinamide
Dihydroxyacetone phosphate	Saccharopine	Nicotinic acid
Fructose 6-phosphate	Serine	O-Phosphoethanolamine
Glucose 6-phosphate	Threonine	Oxalic acid
Glycerol 3-phosphate	Tyrosine	Palmitic acid
Mannose 6-phosphate	Tryptophan	Phosphoric acid
Ribose 5-phosphate	Valine	Protocatechuic acid Putrescine
Sedoheptulose 7-phosphate		Pyrogallol
	Nucleic acids	Pyruvic acid
	Adenine	Ribonolactone
	Adenosine	Stearic acid
	Adenosine monophosphate	Succinic acid
	Guanine	Tartaric acid
	Guanosine	Threonic acid
	Hypoxanthine	Tryptamine
	Inosine	Urea
	Uracil	Uric acid
	Uridine	
	Xanthosine	

Fig. 3 and Fig. 4 show the results of a principal component analysis (PCA) and a hierarchical clustering analysis (HCA), respectively. The PCA score plot and the dendrogram by HCA confirmed that the three samples can be clearly distinguished.

Furthermore, the heat map of the hierarchical clustering analysis showed that many components tend to have relatively large contents in Sample 3 in comparison with the other two samples, and tend to have relatively small contents in Sample 1. The heat map also revealed that the distinctive components tend to have relatively large contents in samples 1 and 2.

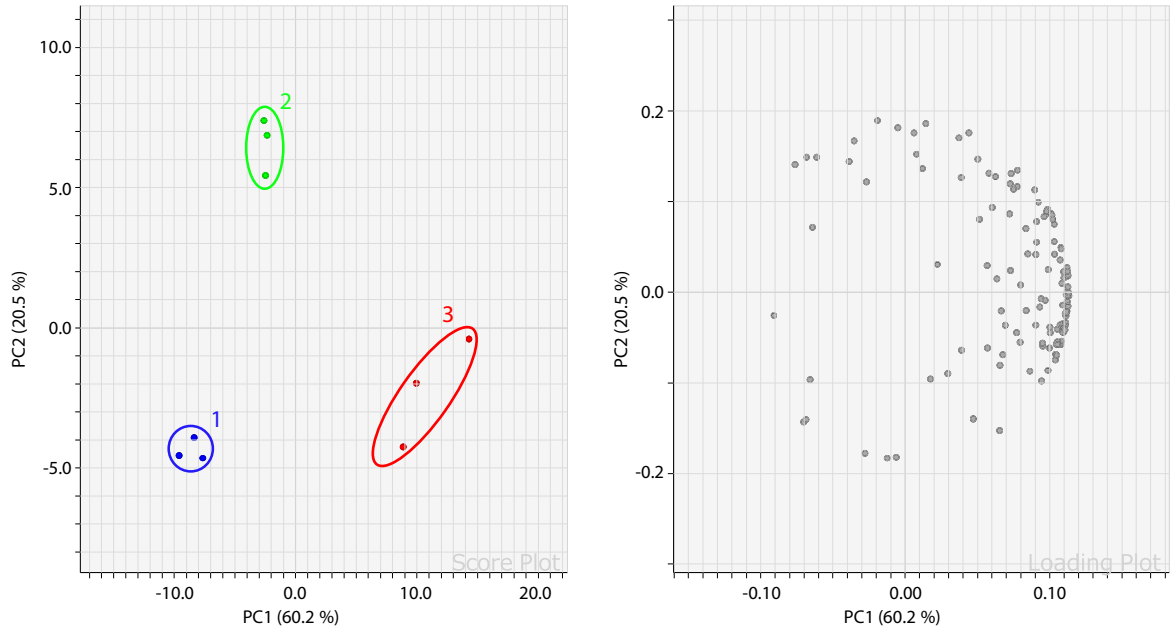

Fig. 3 Principal Component Analysis (PCA)
Left: Score Plot, Right: Loading Plot

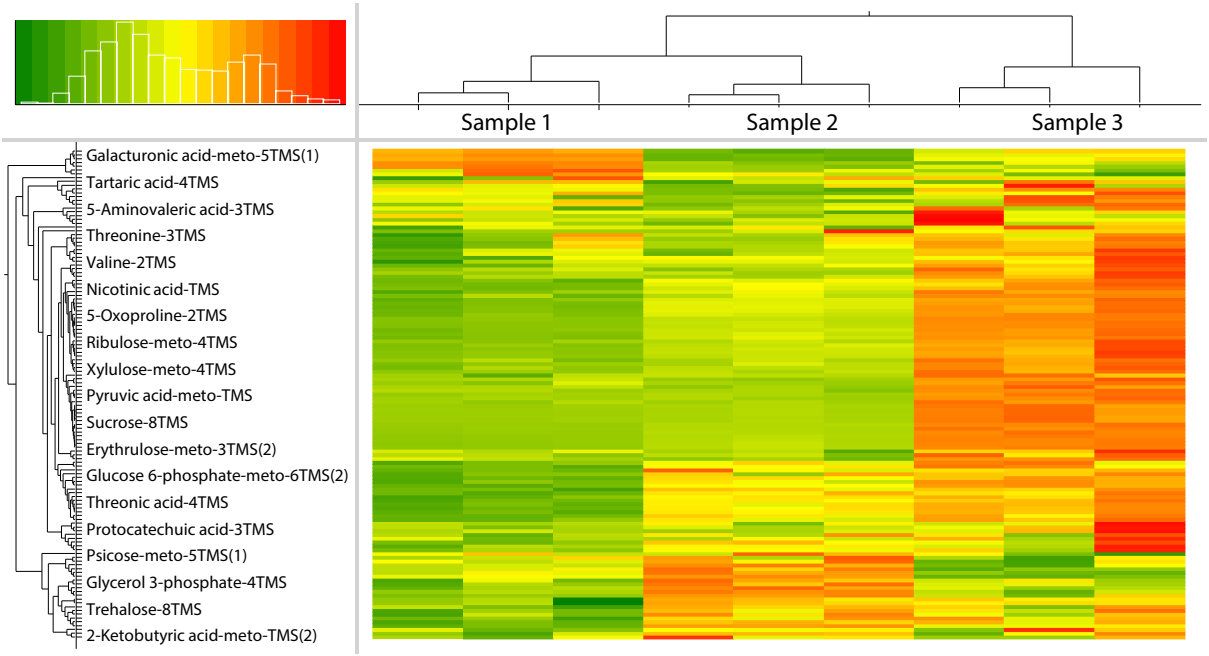

Fig. 4 Dendrogram and Heat Map by Hierarchical Clustering Analysis (HCA)
The relative detected amounts of each component are shown by color gradation, where redder colors indicate larger amounts and greener colors indicate smaller amounts.

In order to verify the distinctive components of each sample in detail, ANOVA (analysis of variance) was conducted. Fig. 5 shows the results of an analysis of the distinctive components in each sample for components with a p-value < 0.01. In Sample 1, the content of galacturonic acid, which is known to be constituent components of plant cell walls, tended to be relatively larger than that in the other samples. In Sample 2, the contents of adenosine monophosphate, glycine and asparagine, which is a taste component, tended to be larger.

Sample 3, in which a large number of components were detected to be large relatively, contained remarkably large amounts of sucrose (sweet taste) and citric acid (acidic taste). 4-aminobutyric acid (GABA), glucosamine, ornithine and vitamin B3 (nicotinamide and nicotinic acid), which are well known to be functional components were detected in all the tomato juices, and Sample 3 also contained the largest amounts of these components.

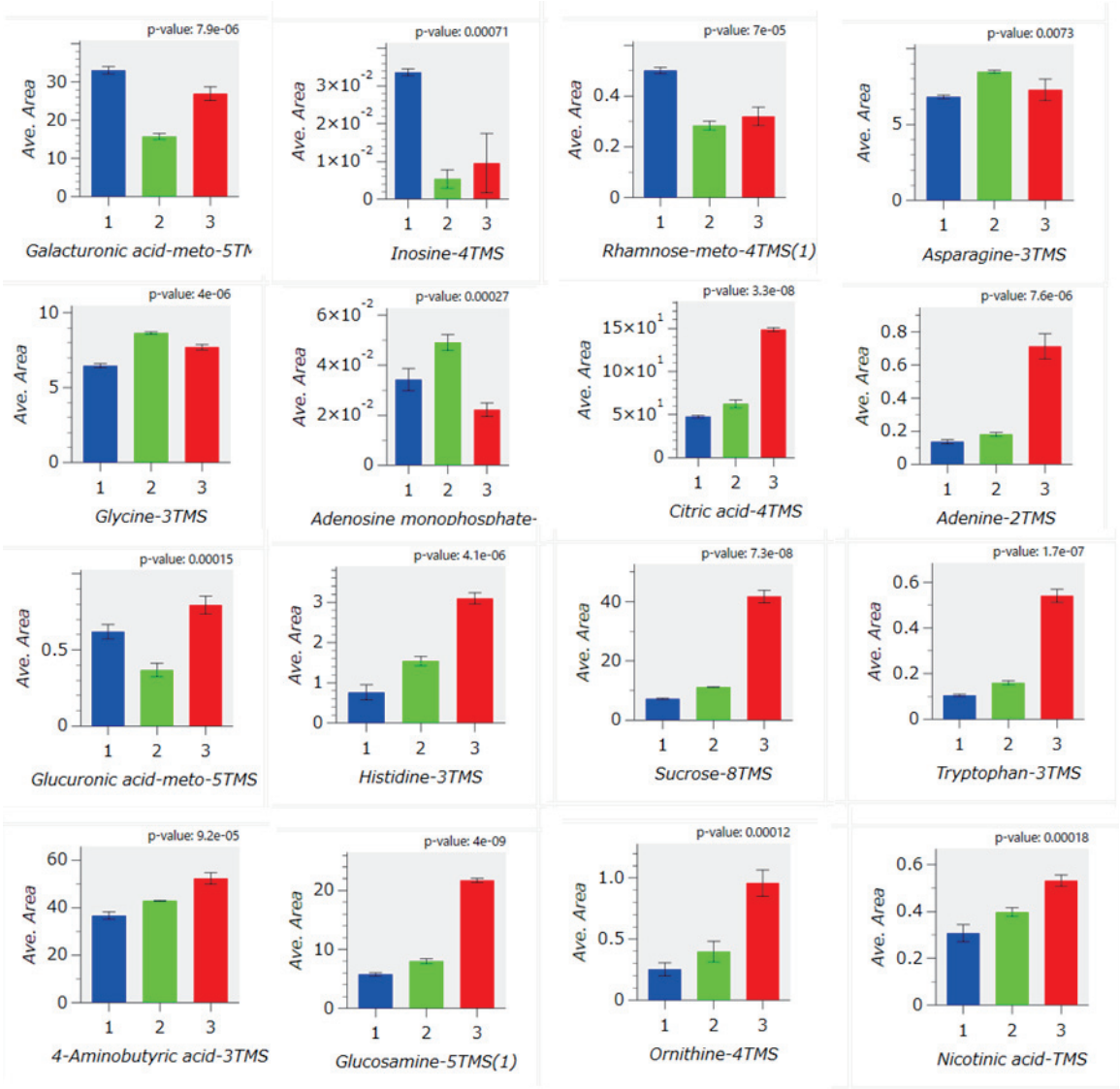

Fig. 5 Results of Analysis of Variance (ANOVA)
(Only some components with p < 0.01 are shown.
The bar graphs show the class average, and the error bars indicate the standard deviation within the class.)

■ Conclusion

It was possible to search for distinctive components between tomato juice products by a simultaneous analysis of hydrophilic components using the Smart Metabolites Database and a multivariate analysis. Use of this technique in combination with a sensory evaluation of taste and flavor has the potential to be a useful tool for development of products matched to market needs.

First Edition: Jan. 2021

SHIMADZU
Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/