

**Evaluation of Food Deterioration by Multivariate
Analysis**

During the development of food products, physicochemical tests, microbial tests, and sensory evaluations are conducted, and the shelf life of the food is set based on their objective indexes. In markets characterized by a large number of products and short product cycles, like at present, safety and quality are adequately ensured during the shelf life of products, but it is difficult to grasp the distinctive features of deterioration of each individual product. In addition, consumers also have various understandings of shelf life after the product is opened. For these reasons, methods for predicting deterioration over time are demanded.

Deterioration of food is caused by microorganisms, water, oxygen, temperature, and light, resulting in decomposition of proteins, carbohydrates, and fats, into low molecular compounds. For example, it is known that proteins decompose into peptides and amino acids and then form amines and organic acids.

The purpose of this article was to track the condition of deterioration of milk after the product was opened. Amino acids, organic acids, sugars, and other hydrophilic components were identified using the Smart Metabolites Database™, and changes over time in those components were analyzed with the Traverse MS multivariate analysis software program.

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■ **Sample Preparation and Analysis Conditions**

Commercially-available milk (paper carton) was prepared as the measurement sample. In order to understand changes due to elapsed time, samples were taken on day 0 (date of opening) and 3, 7, 14, and 21 days after opening. The milk was stored in a refrigerator except when removed for sampling.

For measurements, 100 µL of milk 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. Next, 500 µL of a mixed solvent of water : methanol : chloroform = 1 : 2.5 : 1 was added as an extractant for the hydrophilic components. After shaking for 30 min at 37 °C, the sample was fractionated by centrifugation (4 °C, 3,000 g, 10 min), and 450 µL of the supernatant (water/methanol phase) was collected. 400 µL of ultrapure water was added to the supernatant, centrifugation was conducted 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, and the sample was shaken for 90 min at 30 °C. Following this, 100 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added, the solution was shaken for 30 min at 37 °C. The analysis sample was measured by the Smart Metabolites Database, which contains MRM transitions for 475 metabolites. Table 1 shows the measurement conditions.

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 mode	: 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**

Fig. 1 shows the TICC (total ion current chromatogram) of the milk immediately after opening. A total of 106 components (94 compounds), including lactose and other sugars, amino acids and saturated fatty acids such as palmitic acid, were detected. The detected compounds are listed in Table 2.

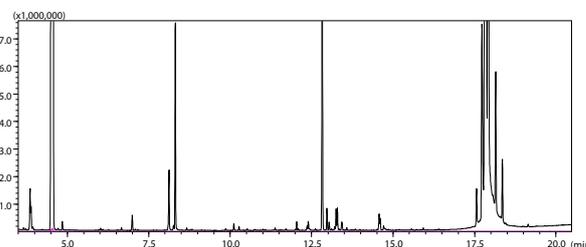


Fig. 1 Analysis Result of Milk Immediately After Opening

Table 2 Compounds Detected from Milk

Sugars/sugar alcohols/sugar acids	2-Deoxy-glucose	Arabinose	Arabitol	Erythrulose	Fructose	Fucose
	Galactose	Galacturonic acid	Gluconic acid	Glucose	Glucuronic acid	Glyceric acid
	Glycerol	Inositol	Lactitol	Lactose	Maltose	Mannose
	N-Acetylmannosamine	Ribitol (internal standard)	Ribonic acid	Ribose	Ribulose	Sucrose
	Threonic acid	Xylitol	Xylose	Xylulose		
Sugar phosphates	3-Phosphoglyceric acid	Dihydroxyacetone phosphate	Fructose 6-phosphate	Glucose 6-phosphate	Glycerol 2-phosphate	Glycerol 3-phosphate
	Mannose 6-phosphate	Ribose 5-phosphate	Ribulose 5-phosphate	Sedoheptulose 7-phosphate		
Amino acids	2-Aminopimelic acid	3-Aminoglutaric acid (β-Glutamic acid)	3-Aminopropanoic acid (β-Alanine)	4-Aminobutyric acid (GABA)	4-Hydroxyproline	5-Oxoproline
	Alanine	Aspartic acid	Creatinine	Cysteine	Dimethylglycine	Glutamic acid
	Glycine	Hypotaurine	Isoleucine	Leucine	Lysine	Phenylalanine
	Proline	Serine	Threonine	Tyrosine	Ureidosuccinic acid	Valine
Saturated fatty acids	Caproic acid	Decanoic acid	Lauric acid	Myristic acid	Nonanoic acid	Octanoic acid
	Palmitic acid	Stearic acid				
Organic acids	2-Ketoglutaric acid	2-Hydroxyglutaric acid	3-Hydroxybutyric acid	3-Hydroxyisovaleric acid	3-Hydroxypropionic acid	Aconitic acid
	Benzoic acid	Citric acid	Fumaric acid	Glycolic acid	Glyoxylic acid	Lactic acid
	Malic acid	Pyruvic acid	Succinic acid			
Others	2-Aminoethanol	Allantoin	Dopamine	O-Phosphoethanolamine	Orotic acid	Pantothenic acid
	Phosphoric acid	Uracil	Urea	Xanthosine monophosphate		

Multivariate Analysis

Traverse MS (Reifycs Inc.) multivariate analysis software is an integrated analysis program for realizing high speed multivariate analysis using MRM data acquired by GC-MS/MS. Traverse MS is the real solution as it allows you to carry out peak integration and identification quickly, accurately, and easily by using a novel peak recognition algorithm developed specifically for MRM analysis data and a highly-operable graphical user interface. In order to evaluate variations in metabolism in vast amounts of MRM data sets, Traverse MS integrates statistical analysis tools including graphical display of peak areas of sample groups, principal component analysis, hierarchical clustering analysis, and metabolic pathway analysis. The qgd files (MRM data) acquired by GCMSsolution™ can also be read directly into Traverse MS.

An analysis by Traverse MS was conducted targeting the above-mentioned 106 components detected from the milk. The analysis was carried out with 3 samples from each sampling day after opening, and the intensities of the compounds detected in each sample were normalized by using Ribitol as an internal standard. Fig. 2 shows the result of PCA (Principal Component Analysis) score plot by Traverse MS. Focusing on the first principal component (horizontal axis, PC1), the results are arranged in the order of day 0, day 3, day 7, day 14, and day 21 from the positive (right) side to the negative (left) side, and the results for days 0 and 3 are distributed on the positive side, while those for days 14 and 21 are on the negative side. It was suggested that the first principal component expresses changes over time.

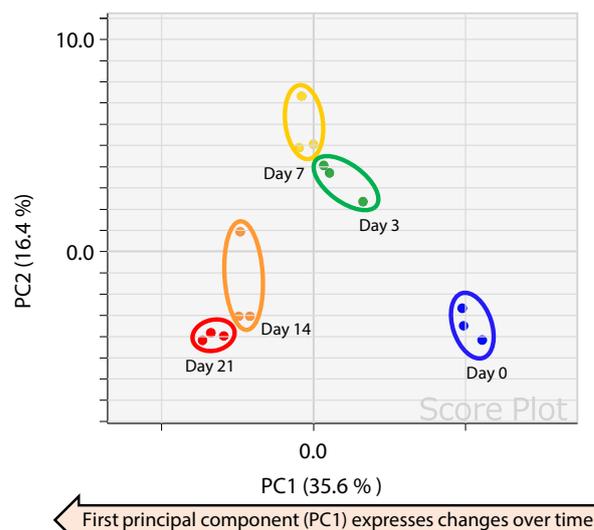


Fig. 2 Distribution of Samples by PCA Score Plot

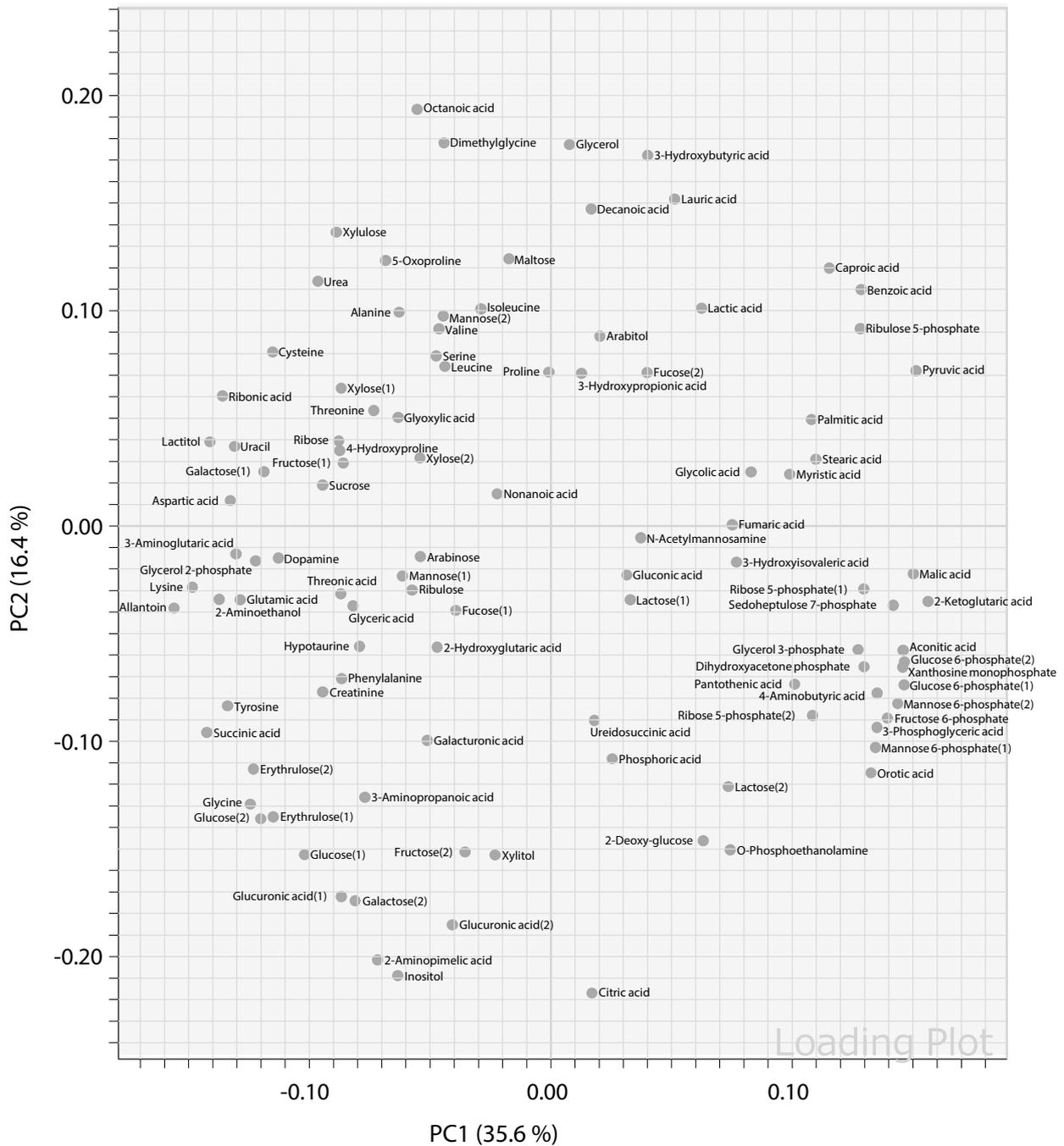


Fig. 3 Distribution of Components by PCA Loading Plot

Fig. 3 shows the PCA loading plot. The components which are positively distributed in the first principal component (horizontal axis, PC1) tend to have large contents in fresh milk, whereas those which are negatively distributed in old milk. The positive components found in PC1 include organic acids such as malic acid, 2-ketoglutaric acid, and pyruvic acid, saturated fatty acids, and sugar phosphates. On the other hand, the negative components found in PC1 include succinic acid, allantoin, amino acids such as glycine, and glucose, erythrulose, and other sugars.

Next, the p-values of the components were calculated based on an analysis of variance (ANOVA). Among the components with p-value < 0.01, Fig. 4 shows some components in which increasing or decreasing trends were confirmed. Here, 2-ketoglutaric acid, malic acid, and glucose 6-phosphate decreased with time, while succinic acid, amino acids such as glycine and lysine, and sugars such as glucose increased. Based on this, it was suggested that, with the passage of time, amino acids increased due to decomposition of some proteins, and some sugar phosphates decomposed into sugar.

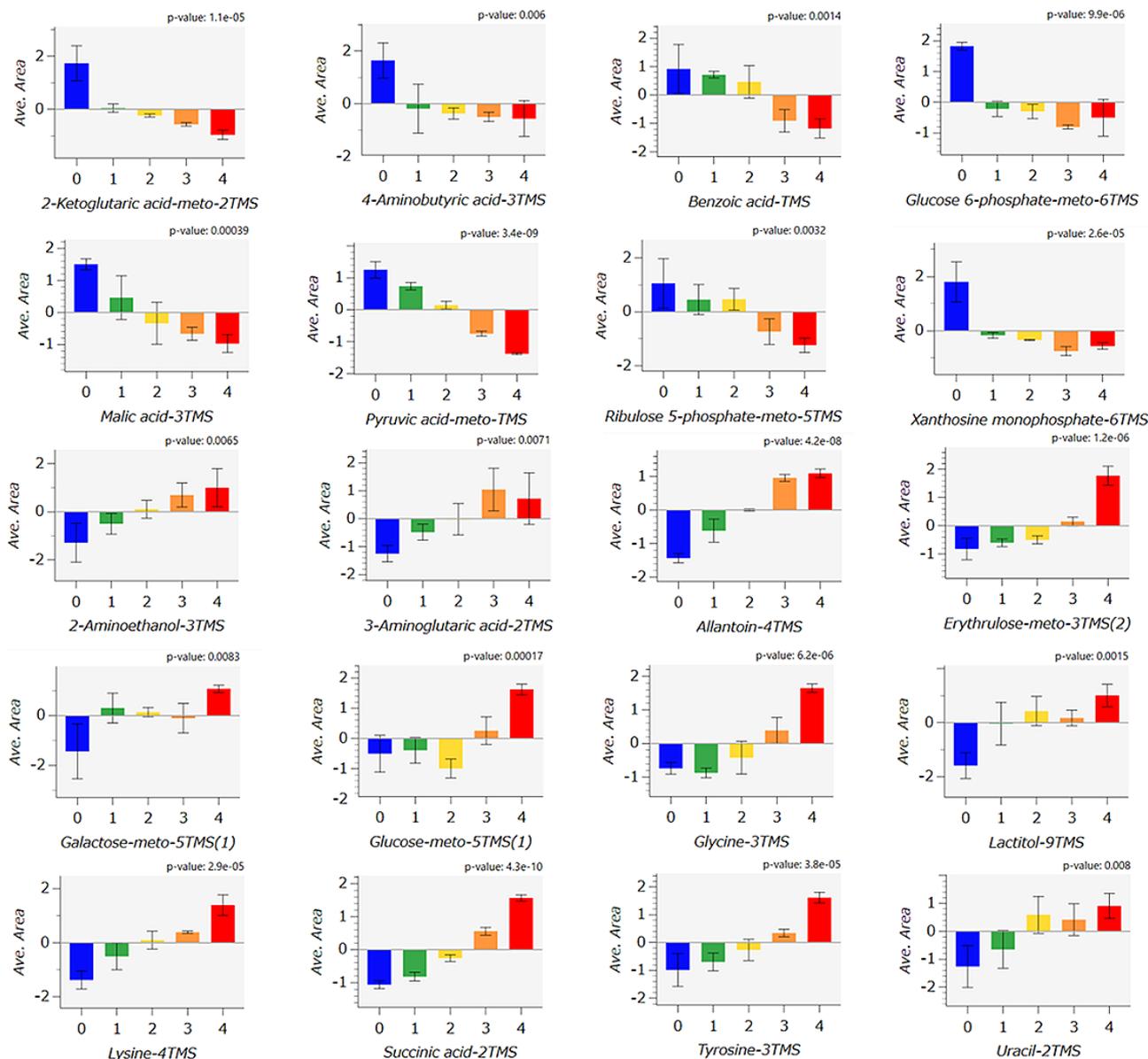


Fig. 4 Results of Analysis of Variance (ANOVA)
0: Time of Opening, 1: 3 Days After Opening, 2: 7 Days After Opening, 3: 14 Days After Opening, 4: 21 Days After Opening
(Only some components with $p < 0.01$ are shown. The bars graphs show the class average, and the error bars indicate the standard deviation in the class.)

Conclusion

A simultaneous analysis of the hydrophilic components in food was possible by using the Shimadzu Smart Metabolites Database, and components that increased or decreased during deterioration could be identified by conducting widely targeted multivariate analysis using the analysis results.

This suggested the possibility that some organic acids, amino acids, and sugars can be used as markers which provide indexes of the freshness or deterioration of foods. Use in combination with objective evaluations for product development is expected to provide a more detailed understanding of the features of food products.

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First Edition: Sep. 2020



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