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Wide-Target Analysis of Yogurt Using Triple Quadrupole LC-MS/MS

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1. Introduction

The application of metabolomics technology to foods is called "food metabolomics," and in recent years, along with the development of mass spectrometry technology, it has been used for various purposes, such as quality assessment and quality prediction of foods, improvement of manufacturing and storage processes, and evaluation of functionality. Food contains a great many metabolites, but previous research has revealed many of those responsible for flavor, quality and functionality. Therefore, in food metabolomics, target analysis is often performed to determine the target compounds. In addition, by focusing on the important compounds and analyzing them exhaustively, useful results can be obtained efficiently. In this poster, we report a comprehensive analysis of primary metabolites in yogurt using triple-quadrupole LC-MS/MS.

2. Methods

2-1. Sample Preparation

Eight commercial yogurts were used as samples. Well-stirred yogurt was freeze-dried. Then, mix solvent (ultrapure water: methanol: chloroform = 1: 2.5: 1) was added, agitated, and centrifuged. Finally, the supernatant was concentrated by ultrafiltration and redissolved in 10 µM internal standard solution (2-Morpholinoethanesulfonic acid) to make an analytical sample for LC/MS/MS. Figure 1 shows the detailed pretreatment procedure.

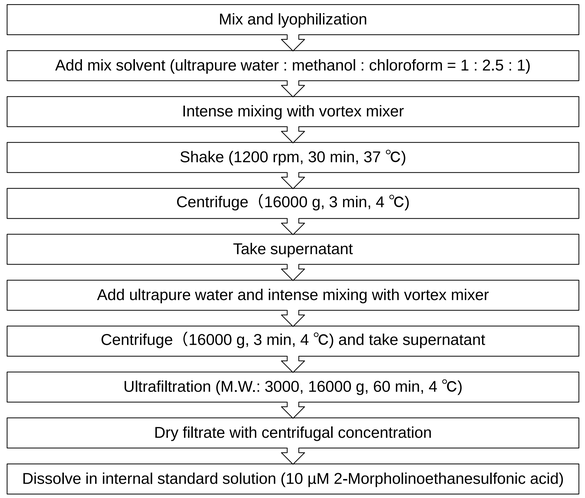

Fig. 1 Workflow for Sample Preparation

2-2. Analysis Conditions

For the analysis of primary metabolites in yogurt, two methods were used: one using ion-pairing reagents (ion-pairing method) and the other without ion-pairing reagents (non-ion-pairing method), which are included in the LC/MS/MS method package primary metabolite Ver. 3 (Shimadzu Corporation). Nexera X3 system and LCMS-8060NX (Shimadzu Corporation) were used for the analysis (Fig.2). Table 1 shows the analysis conditions for the ion-pairing method and non-ion-pairing method.

Fig. 2 Primary metabolites analysis system

Table 1 Analysis Conditions

ion-pairing LC-MS/MS method	
UHPLC (Nexera X3 system)	**MS (LCMS-9030)**
Column: Mastro2 C18 (150 mmL.×2.0 mmI.D., 3.0 µm, Shimadzu GLC)	Ionization: IonFocus ESI Negative
Mobile phase A: 10 mM Dipentylamine, 15 mM Acetate/water	Mode: MRM
B: Methanol	DL temp.: 250 °C
Mode: Gradient elution	HB temp: 400 °C
Flow rate: 0.3 mL/min	Interface temp.: 270 °C
Column temp.: 40 °C	Drying gas: 10 L/min
Injection vol.: 3 µL	Nebulizing gas: 2.0 L/min
	Heating gas: 10 L/min
Non-ion-pairing LC-MS/MS method	
UHPLC (Nexera X3 system)	**MS (LCMS-9030)**
Column: Shim-pack GIST PFPP (150 mmL.×2.1 mmI.D., 3.0 µm, Shimadzu)	Ionization: IonFocus ESI Positive/Negative
Mobile phase A: 0.1% Formate/water	Mode: MRM
B: 0.1% Formate/acetonitrile	DL temp.: 250 °C
Mode: Gradient elution	HB temp: 400 °C
Flow rate: 0.25 mL/min	Interface temp.: 270 °C
Column temp.: 40 °C	Drying gas: 10 L/min
Injection vol.: 3 µL	Nebulizing gas: 3.0 L/min
	Heating gas: 10 L/min

3. Results

3-1. Simultaneous Analysis of Primary Metabolites in Yogurt by LC-MS/MS

Simultaneous analysis of hydrophilic metabolites by both the ion-pairing method and the non-ion-pairing method revealed that the ion-pairing method detected more than 68 compounds, mainly glycolytic and pentose phosphate pathway metabolites, while the non-ion-pairing method detected more than 81 compounds, mainly amino acids, organic acids, and nucleic acid metabolites, and more than 110 compounds in the 2 methods combined. Peak detection was performed by automatic waveform processing by Peakintelligence (Shimadzu Corporation) followed by visual confirmation and correction. Table 2 shows the number of metabolites detected in each yogurt. The results indicate that LC/MS/MS method package for primary metabolite Ver. 3 and Peakintelligence are useful for comprehensive analysis of primary metabolites in yogurt.

Table 2 Number of detections in each yogurt

	Ion-pair	Non-ion-pair	Both
Yogurt1	73/112	82/141	117/198
Yogurt2	71/112	82/141	114/198
Yogurt3	68/112	84/141	111/198
Yogurt4	70/112	81/141	110/198
Yogurt5	69/112	88/141	118/198
Yogurt6	71/112	84/141	116/198
Yogurt7	71/112	87/141	119/198
Yogurt8	73/112	81/141	114/198

3-2. Analysis with the Multi-omics Analysis Package

Principal component analysis was performed using the peak area ratio of metabolites detected in each yogurt to internal standards. Principal component analysis was performed by the Multi-omics Analysis Package (Shimadzu Corporation). The results of the principal component analysis are shown in Figure 3. The Score Plot showed the differences between different yogurts.

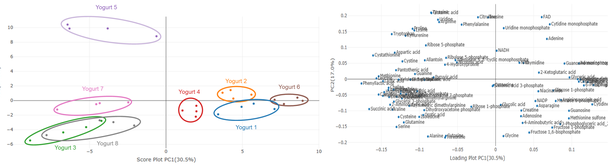

Fig. 3 Results of principal component analysis

For further analysis, compound factor loadings in each principal component are shown. The eight metabolites with positive and negative factor loadings (high impact) in the first and second principal components (PC1 and PC2, respectively) are shown in Table 3 and Table 4, and the eight metabolites with positive and negative factor loadings in PC1 and PC2, respectively, are shown in Table 5 and Table 6. The 8 components with the positively high compound factor loadings in PC1 included nucleic acid-related compounds (6 compounds), as well as the pentose-phosphate pathway (1 compound) and the urea circuit (1 compound), The 8 components with positive compound factor loadings in PC2 included amino acids (4 compounds), nucleic acid-related compounds (3 compounds), and coenzymes (1 compound), amino acids (4 compounds), glycolytic system (1 compound), pentose phosphate pathway (1 compound), sugar phosphate (1 compound), and nucleic acid-related compounds (1 compound). The peak area ratios were then visualized in Figures 4 and 5 using a multi-omics analysis package for the 8 components with positive and 8 components with negative PC1 compound factor loadings, respectively. Figure 4 shows that Yogurt 2 and Yogurt 6 had higher area ratios of nucleic acid-related compounds than the other yogurts. Figure 5 shows that Yogurt 3, Yogurt 5, Yogurt 7, and Yogurt 8 have higher area ratios of metabolites of Cysteine and methionine metabolism (Cystathionine, Methionine, Methionine sulfoxide) The area ratios of Cysteine and methionine metabolism metabolites (Cystathionine, Methionine, Methionine sulfoxide) were higher in Yogurt 7 and Yogurt 8.

Table 3 Top 8 compounds with positive factor loading in PC1

Ranking	Name	PC1 Factor loading
1st	UDP-glucose	0.12856
2nd	Cytidine	0.12682
3rd	Thymidine diphosphate	0.12082
4th	6-Phosphogluconic acid	0.11588
5th	Guanosine diphosphate	0.11561
6th	Thymidine monophosphate	0.11149
7th	Adenosine monophosphate	0.10937
8th	Ornithine	0.10923

Table 4 Top 8 compounds with negative factor loading in PC1

Ranking	Name	PC1 Factor loading
1st	Phenyllactic acid	-0.17606
2nd	Succinic acid	-0.16592
3rd	Cystathionine	-0.16081
4th	Leucine	-0.15640
5th	Carnitine	-0.15354
6th	Methionine	-0.15346
7th	Methionine sulfoxide	-0.14784
8th	Lactic acid	-0.14779

Table 5 Top 8 compounds with positive factor loading in PC2

Ranking	Name	PC2 Factor loading
1st	Tyrosine	0.20975
2nd	Glutamic acid	0.20854
3rd	FAD	0.19570
4th	Citrulline	0.19526
5th	Inosine	0.19490
6th	Uridine	0.18752
7th	Arginine	0.18008
8th	Cytidine monophosphate	0.17299

Table 6 Top 8 compounds with negative factor loading in PC2

Ranking	Name	PC2 Factor loading
1st	Threonine	-0.18452
2nd	Alanine	-0.18128
3rd	Glycine	-0.18003
4th	Cytosine	-0.17945
5th	Fructose 1,6-bisphosphate	-0.16887
6th	Fructose 1-phosphate	-0.15299
7th	Serine	-0.15109
8th	3-Phosphoglyceric acid_2-Phosphoglyceric acid	-0.14218

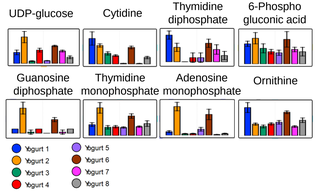

Fig. 4 Area rate of top 8 compounds with positive factor loading in PC1

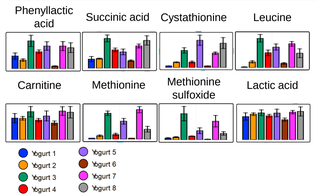

Fig. 5 Area rate of top 8 compounds with negative factor loading in PC1

4. Conclusion

- ✓ Yogurt was analyzed by LC/MS/MS using the LC/MS/MS Method Package Primary Metabolites Ver. 3, and a total of more than 110 metabolites were detected.
- ✓ Principal component analysis of the peak area ratios of the detected compounds using the Multi-omics Analysis Package confirmed the differences between the different yogurts.
- ✓ Using the Multi-omics Analysis Package, the peak area ratios of the detected compounds could be easily visualized on a metabolic map.
- ➢ **A series of workflows using the LC/MS/MS Method Package Primary Metabolites Ver. 3, Peakintelligence, and the Multi-omics Analysis Package are powerful tools in food metabolomics**