SHIMADZU
Excellence in Science

Application News

No. C226

LC-MS

Food Metabolomics
Analysis of Wines Using LCMS™-8060NX Triple Quadrupole Mass Spectrometer

In recent years, attention has been focused on the technology of metabolomics, which is defined as the comprehensive analysis of metabolites in vivo. Metabolomics is an academic field that comprehensively analyzes low-molecule metabolites, such as amino acids and organic acids, generated by the activities of cells to clarify differences among multiple sample groups. It is said that it permits exhaustive analysis more easily than other "omics" because the number of target components is small. Originally, it was a technique that has been developed with the expectation of results in the medical field, for example, in searches for diagnostic markers using clinical samples and etiological analyses using model animals. This analytical method is recently used more and more in the industrial and food fields to make comparisons among the products of different manufacturers, as well as to compare raw materials from different sources. The application of metabolomics not to living organisms but to food as covered here is called "food metabolomics".

This article covers an example of food metabolomics using wines of different types of grapes or origins. To comprehensively analyze the hydrophilic components in wines, analysis was performed by two methods using a high performance liquid chromatograph mass spectrometer (LC/MS/MS).

T. Hattori, Y. Yamada

Samples

As samples, we prepared six types of red wines of different types of grapes or origins. Table 1 shows the details of the samples.

Table 1 Details of Samples

	Producing regions	Grape varieties
Wine A	France	Cabernet Sauvignon
Wine B	France	Merlot
Wine C	France	98 % Pinot Noir, 2 % Pinot Beurot
Wine D	U.S.A	Cabernet Sauvignon
Wine E	Chile	Cabernet Sauvignon
Wine F	Australia	Cabernet Sauvignon

Pretreatment

For analysis of short-chain fatty acids/organic acids, the samples were derivatized by 3-nitrophenylhydrazine (3-NPH) to improve the retention of samples in an ODS column and the sensitivity of MS detection. To wines filtered with a membrane filter, 3-NPH (derivatizing agent), pyridine (catalyst), carbodiimide (condensing agent) and 2-ethylbutyric acid (internal standard) were added, and allowed to react with each other at a room temperature for 30 minutes. After the reaction, the samples were diluted 5-fold with methanol solution containing formic acid.

For simultaneous analysis of hydrophilic metabolites, wines were filtered with a membrane filter, and diluted 100-fold with ultra-pure water. When diluting the samples, 2-morpholinoethanesulfonic acid (MES) was added to make a concentration of 1 µmol/L as the internal standard.

Analytical Conditions

Using the LC/MS/MS Method Package for Short Chain Fatty Acids, short-chain fatty acids/organic acids were analyzed with LCMS-8060NX. The analytical method enables the analysis of 6 short-chain fatty acids, such as acetic acid, propionic acid and butyric acid, and 16 organic acids related to the central metabolic pathway. Tartaric acid, a large amount of which is contained in wine, was also added to this method. Table 2 shows the analytical conditions for HPLC and MS.

Table 2 Analytical Conditions (Analysis of Short-Chain Fatty Acids/Organic Acids)

[HPLC conditions] (Nexera™ X3)		
Column	:	Mastro™ C18 (150 mm×2.0 mm I.D., 3.0 µm)
Mobile phases	:	A) 0.1 % Formic acid in water B) Acetonitrile
Mode	:	Gradient elution
Flow rate	:	0.35 mL/min
Injection volume	:	3 µL
[MS conditions] (LCMS-8060NX)		
Ionization	:	ESI (Positive and negative mode)
Mode	:	MRM
Nebulizing gas flow	:	2.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL temp.	:	250 °C
Block heater temp.	:	400 °C
Interface temp.	:	300 °C

For simultaneous analysis of hydrophilic metabolites, the LCMS-8060NX was used in conjunction with the ion-pair free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 2. This analytical method enables simultaneous analysis of 97 hydrophilic metabolites, such as amino acids, organic acids, nucleosides, and nucleotides, which are important in metabolome analysis in the life sciences field. Table 3 shows the analytical conditions for HPLC and MS.

Table 3 Analytical Conditions (Simultaneous Analysis of Hydrophilic Metabolites)

[HPLC conditions] (Nexera X3)		
Column	:	Reversed-phase column
Mobile phases	:	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Mode	:	Gradient elution
Flow rate	:	0.25 mL/min
Injection volume	:	3 µL
[MS conditions] (LCMS-8060NX)		
Ionization	:	ESI (Positive and negative mode)
Mode	:	MRM
IonFocus voltage	:	±2 kV
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL temp.	:	250 °C
Block heater temp.	:	400 °C
Interface temp.	:	300 °C

IonFocus™ Unit Improves the Sensitivity

IonFocus Unit (Figure 1) for LCMS-8060NX introduces ions only into the mass spectrometer efficiently with its focus electrodes for ion transportation, and expels unnecessary neutral particles. This feature satisfies the high sensitivity and high robustness using even samples which contain a large amount of matrices, such as in vivo/food samples. The effect of IonFocus unit on the hydrophilic metabolites detected from wines to evaluate its effect was tested. Of the components tested, about 90% showed an approximately 1.5-fold increase in sensitivity. Figure 2 shows an example of improved sensitivity by IonFocus unit.

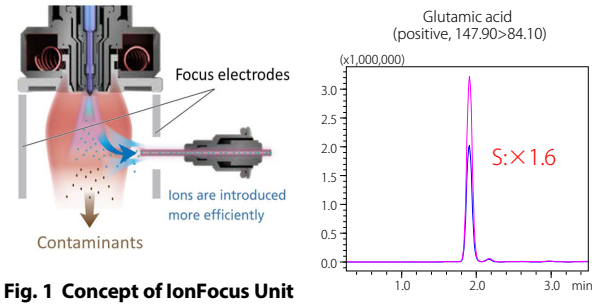

Fig. 1 Concept of IonFocus Unit

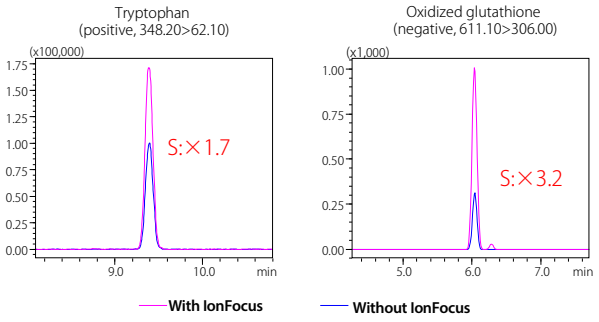

Fig. 2 Improved Sensitivity by IonFocus Unit

Peak Processing Using Peakintelligence™

The detected peaks were processed by Peakintelligence, the optional software for LabSolutions Insight™. Peakintelligence has learnt peak processing skills from experienced users so that the AI can process data with the same skill level. Peakintelligence can detect peaks/process the data correctly even when the SN ratio is low and peaks from different components are present nearby, as shown in Figure 3. This can reduce the number of peaks incorrectly detected/undetected and shorten the data processing time for checking and correcting the peak processing results.

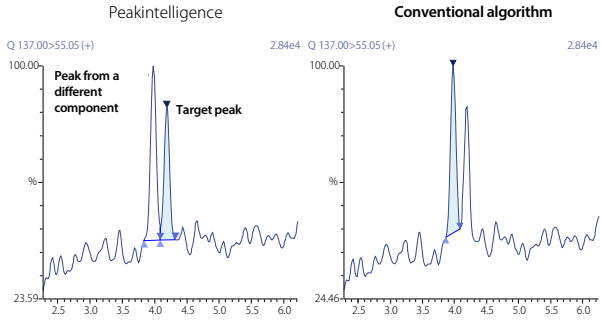

Fig. 3 Peak Processing by Peakintelligence

Metabolome Analysis

The analysis of short-chain fatty acids/organic acids detected 5 short-chain fatty acids and 13 organic acids. Using the peak height ratio of each component with respect to the internal standard substance, principal component analysis was performed by SIMCA®16 software. Figure 4 shows a score plot and loading plot. There were differences in the first principal component between French wines and Non-French wines. It was found that French wines contain a higher amount of tartaric acid or succinic acid; however, the American wine contains a higher amount of malic acid. Meanwhile, the Chilean and Australian wines were confirmed to have similar patterns of short-chain fatty acids/organic acids.

As a result of simultaneous analysis of hydrophilic metabolites, 60 components, such as amino acids, organic acids, and nucleic acid metabolites, were detected. Using the peak area ratio of each component with respect to the internal standard substance, principal component analysis was performed. Figure 5 shows a score plot and loading plot. The components were divided by type of grape into the three clusters. The figure showed that Cabernet Sauvignon wines contain higher amounts of proline and 4-hydroxyproline. Proline in wine is said to cause bitterness or sweetness. A Merlot wine was found to contain higher amounts of phenylalanine, leucine and lysine. A Pinot Noir wine was confirmed to have a higher amount of alanine. Alanine is said to be the source of sweetness or the umami taste in wine.

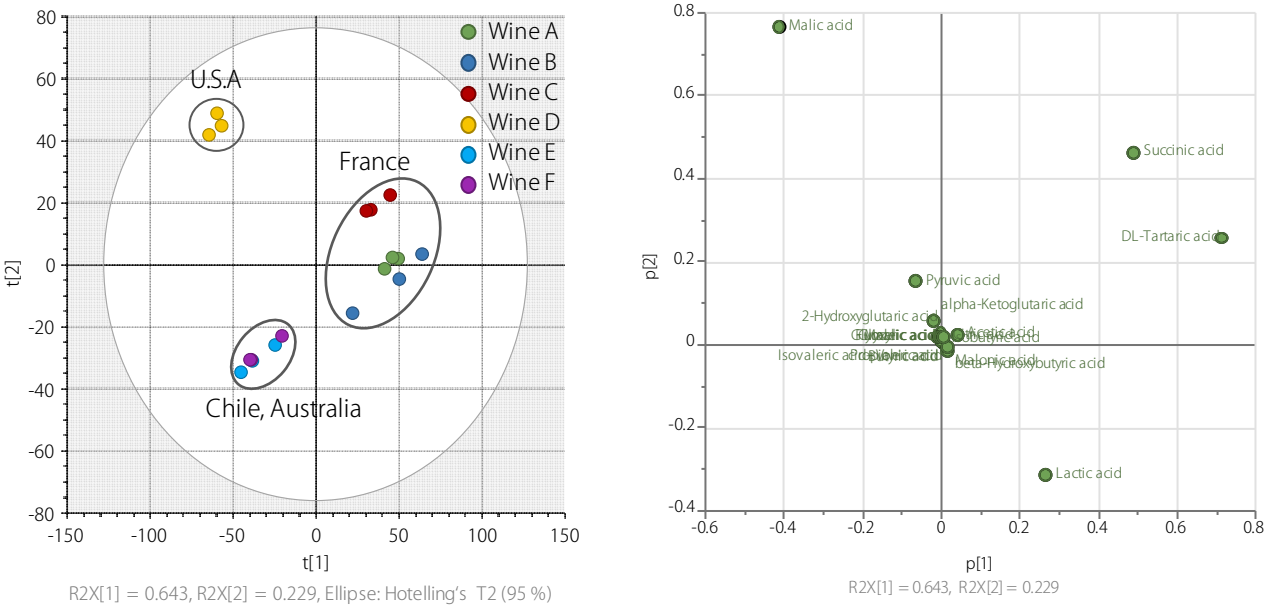

Fig. 4 Principal Component Analysis in Analysis of Short-Chain Fatty Acids/Organic Acids

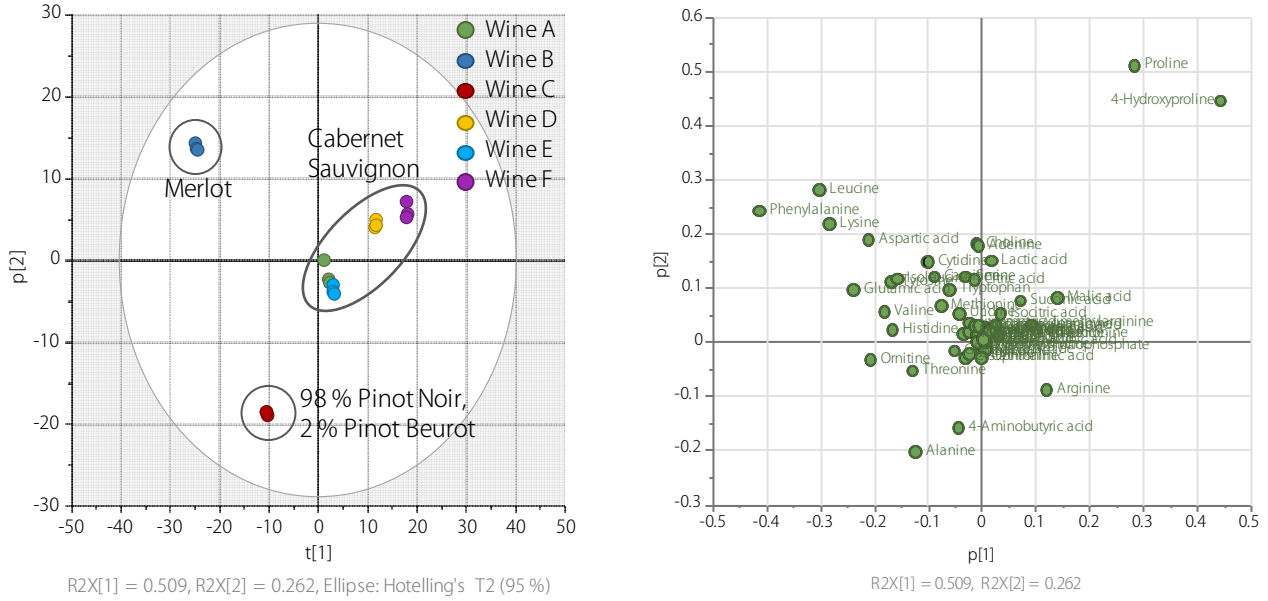

Fig. 5 Principal Component Analysis Results in Simultaneous Analysis of Hydrophilic Metabolites

As mentioned above, it was suggested that hydrophilic metabolites (specifically, amino acids) are affected by type of grape, but that short-chain fatty acids or organic acids are affected by not type of grape but origin of the wine (such as the soil and climate where the grapes are grown, and the winemaking process). The winemaking process is known to affect the short-chain fatty acids or organic acids in wine. In the winemaking process, malolactic fermentation normally occurs after alcohol fermentation. During malolactic fermentation, malic acid in juice or wine is converted to lactic acid and carbon dioxide by lactobacilli. Malolactic fermentation is said to round the acidity of wine. Figure 6 shows the percentages of short-chain fatty acids/organic acids in the wines.

The figure showed that the percentage of lactic acid is higher than that of malic acid in French wines A, B and C, Chilean wine E and Australian wine F, suggesting that malolactic fermentation progresses well. In American wine D, however, the percentage of malic acid is higher than that of lactic acid, suggesting that malolactic fermentation does not progress well.

Conclusion

As mentioned above, comprehensive analysis of short-chain fatty acids/organic acids in wine and hydrophilic metabolites can be used to evaluate the fermentation process or relationship between the process and flavors. This analytical method can be useful for production of more tasty and higher-quality wines.

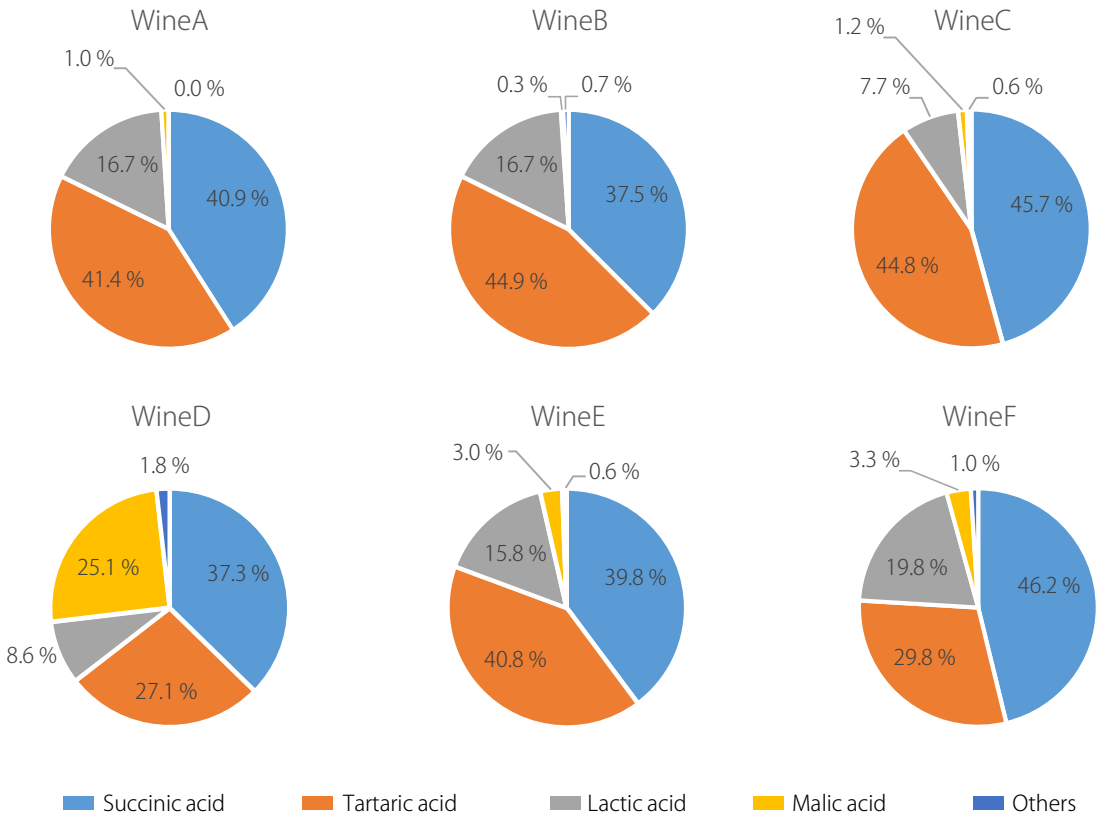

Fig. 6 Profiles of Short-Chain Fatty Acids/Organic Acids in Wines

First Edition: Sep. 2020

Shimadzu Corporation
www.shimadzu.com/an/