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Application News

No. C238

Liquid Chromatography Mass Spectrometry

Food Metabolomics Analysis of Wines Using LCMS™-8060NX Triple Quadrupole Mass Spectrometer (Part 2)

Metabolomics is a scientific field in which the differences in multiple sample groups are clarified by comprehensively analyzing low molecular metabolites such as amino acids and organic acids which are produced by cellular activity. This technology is also applied in the field of food products and is used as a technique (food metabolomics) for searching for nutritional value and quality in foods.

In recent years, nonalcoholic beverages have attracted considerable attention, particularly in the beer market. Together with improvement of the manufacturing process, this trend has also heightened the importance of component analysis of fragrance and taste components in order to impart a taste and aroma closer to those of the real product. In the wine industry as well, interest in nonalcoholic wines which are closer to real wines has also increased, while on the other hand, adulterated wines are viewed as a problem. Here, "adulterated wine" refers to wine which is produced by mixing low-cost wine with a high class wine and marketed with only the label changed.

Application News No. C226 introduced an example in which wines from different producing regions and grape varieties were analyzed by food metabolomics, demonstrating that this technique can be applied to evaluations of the relationship between the fermentation process and flavor of wine. The present article introduces an example of a food metabolomics analysis using a high performance liquid chromatograph-mass spectrometer (LC/MS/MS) to clarify the differences between real wine and nonalcoholic wine, and the differences between quality (rated) wine and table (daily) wine.

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Samples and Sample Preparation

The sample wines used here were a nonalcoholic wine, quality wine, table wine, and a mixed wine consisting of the quality wine and table wine mixed at a ratio of 50 : 50.

Samples were prepared by filtering the wines with a membrane filter, followed by dilution by 100× with ultrapure water. During dilution, 2-Morpholinoethanesulfonic acid was added as an internal standard so as to obtain a concentration of 1 µmol/L.

Analysis Conditions

The ion-pair free LC/MS/MS method included in the Shimadzu LC/MS/MS Method Package for Primary Metabolites Ver. 2 was used. This method enable simultaneous analysis of 97 hydrophilic metabolites such as amino acids, organic acids, nucleosides, and nucleotides. Table 1 shows the HPLC and MS analysis conditions. An LCMS-8060NX mass spectrometer was used. The IonFocus™ unit of the LCMS-8060NX (Fig. 1) utilizes ion-transport focus electrodes to introduce only ions into the mass spectrometer with greater efficiency while expelling unnecessary neutral particles. As a result, it is possible to satisfy both high sensitivity analysis and instrument robustness, even with samples with a large matrix content, such as food samples and samples of biological origin. For details, please refer to Application News No. C226 and C233.

Table 1 Analysis Conditions

[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)
Probe position	: +3 mm
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

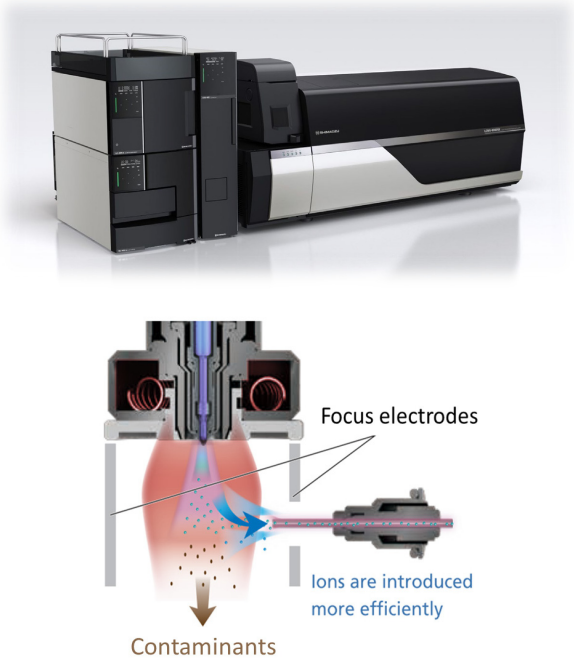
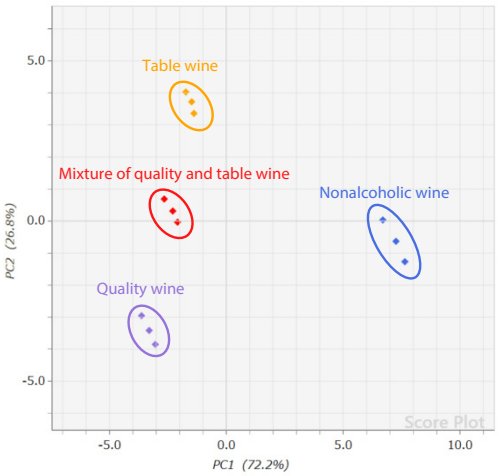


Fig. 1 Appearance of LCMS-8060NX and Concept of IonFocus Unit

Metabolome Analysis

As a result of the LC/MS/MS analysis, 67 components were detected, centering on amino acids, organic acids, and nucleic acid-based metabolites. A principal component analysis (PCA) was conducted with the Traverse MS software (Reifycs Inc.) using the peak area ratios of each component with respect to the internal standard. Fig. 2 shows the score plot and the loading plot. The nonalcoholic wine and real wines could be differentiated on the first principal component (PC1) axis. The nonalcoholic wine had large contents of arginine, proline, 4-Hydroxyproline, and other amino acids and citric acid. It is generally said that proline in wine displays bitterness or sweetness. The real wines had large contents of lactic acid and succinic acid. The difference between the quality wine and the table wine could be distinguished on the second principal component (PC2) axis, and the mixture of the quality wine and table wine was positioned between the plots of those two wines.

(a)



(b)

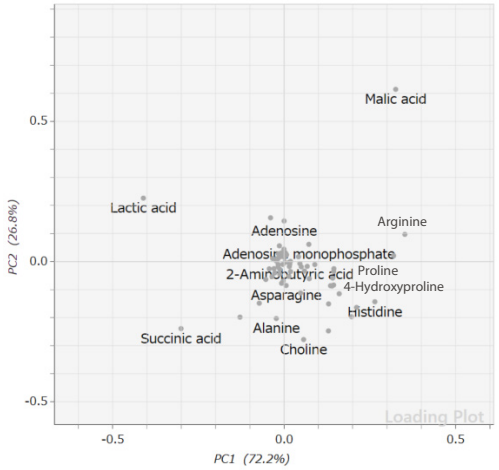


Fig. 2 Results of Principal Component Analysis (a) Score Plot, (b) Loading Plot

Fig. 3 shows the peak area ratios of lactic acid and malic acid in each of the wine samples. Normally, malolactic fermentation occurs after alcohol fermentation in the wine fermentation process, and the malic acid in the fruit juice and wine is decomposed to lactic acid and carbon dioxide by lactobacilli. Malolactic fermentation is generally said to change the acid taste of wine to a smooth and mellow taste. The quality wine contained a higher proportion of lactic acid than malic acid, while the nonalcoholic wine and table wine both had larger contents of malic acid than lactic acid. Based on these results, it was suggested that malolactic fermentation had proceeded in the quality wine, but was slight in the other two wines.

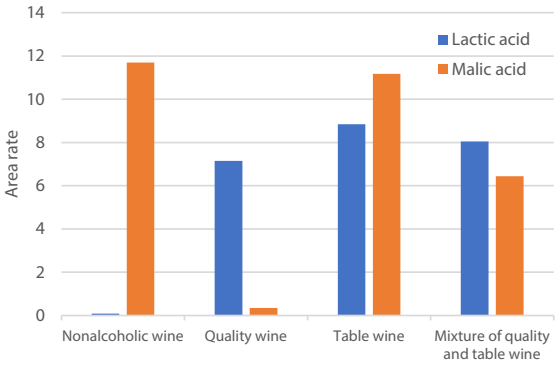


Fig. 3 Peak Area Ratios of Lactic Acid and Malic Acid

Conclusion

As described above, it was possible to clarify the differences between nonalcoholic wine and real wine and between quality wine and table wine by a comprehensive analysis of the hydrophilic metabolites in the wines. This analysis technique is also considered to be useful for improving nonalcoholic wines and distinguishing adulterated wines from quality wines.

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