Widely targeted metabolomics of hydrophilic compounds in wine using two LC-MS/MS methods: Comparison of different types and producing regions

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1. Overview

・We performed widely targeted metabolomics of wine using LC-MS/MS with a newly-developed ionization unit, IonFocus Unit.
・Hydrophilic metabolites in wines were comprehensively analyzed by two LC-MS/MS methods to investigate the difference of wines.

2. Introduction

Recently, increasing attention has been devoted to the metabolomics using mass spectrometer in the food industry. Objective taste evaluation of food products and search for functional ingredients in food products are expected using metabolomics. Wine is an alcoholic beverage made mainly from fermented grape juice. Wine contains compounds derived from grape juice and compounds produced during the fermentation process and these compounds affect the taste and flavor. In this study, we comprehensively analyzed hydrophilic compounds in wines using two LC-MS/MS methods (97 hydrophilic compounds and 23 short chain fatty acids and organic acids) to search compounds that are characteristic of wines that are different grape varieties and producing regions. We also evaluated the newly developed ionization unit.

3. Materials and Methods

3-1. IonFocus Unit

To improve the trade-off between sensitivity and robustness, we have newly developed an IonFocus Unit (Figure 1). In this ionization unit, the focus electrodes introduce only ions into the MS with greater efficiency while expelling contaminants. Therefore, the IonFocus Unit keeps the sensitivity even if the ion spray away from the inlet of the MS to decrease the matrix effect and increase the robustness. The IonFocus Unit achieves both high-sensitivity analysis and high instrument robustness. Therefore, IonFocus Unit is useful in food metabolomics because the food samples contains a large amount of matrix.

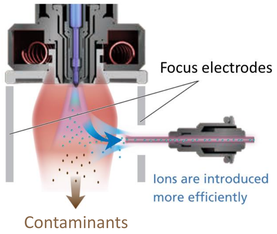


Figure1 The concept of the IonFocus Unit

3-2. Sample

As samples, 6 of red wines were analyzed. Table 1 shows the details of the samples.

Table 1 Sample Details

	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

3-3. Sample Preparation

For analyses of 97 hydrophilic compounds, the wines were centrifuged at 14,000 g for 15 minutes and the supernatants were filtered. The filtrates were diluted 100-fold with water and analyzed by LC-MS/MS.

For analyses of short chain fatty acids and organic acids, the wines were derivatized. Before the derivatization, the wines was centrifuged at 14,000 g for 15 minutes and the supernatants were filtered. 50 µL 3-nitrophenylhydrazine (50 mmol/L, in 75% methanol), 50 µL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (50 mmol/L, in 75% methanol), pyridine (7.5%, in 75% methanol), and 50 µL 75% methanol were added to the 50 µL filtrates. They were mixed at room temperature under the shade for 30 min to derivatize. The derivatized samples were diluted 5-fold with 75% methanol containing 0.5% formic acid and analyzed by LC-MS/MS.

3-4. Analytical Conditions

■Analysis for Short Chain Fatty Acids and Organic Acids

UHPLC (Nexera X3™ system)

Column: Mastro C18 (150 mmL. × 2.0 mmI.D., 3.0 µm)
Mobile phase A: 0.1% Formate/water
B: Acetonitrile
Flow rate: 0.35 mL/min
Injection vol.: 3 µL
Column temp.: 40°C

MS (LCMS-8060)

Ionization: ESI (Positive/Negative, MRM mode)
DL temp.: 250°C HB temp.: 400°C
Interface temp.: 300°C Nebulizing gas: 2.0 L/min
Drying gas: 10 L/min
Heating gas: 10 L/min

■Analysis for 97 Hydrophilic Compounds

UHPLC (Nexera X2™ system)

Column: Discovery HS F5 (150 mmL. × 2.1 mmI.D., 3.0 µm)
Mobile phase A: 0.1% Formate/water
B: 0.1% Formate/acetonitrile
Flow rate: 0.25 mL/min
Injection vol.: 3 µL
Column temp.: 40°C

MS (LCMS-8060NX)

Ionization: IonFocus (ESI, Positive/Negative, MRM mode)
DL temp.: 250°C HB temp.: 400°C
Interface temp.: 400°C Nebulizing gas: 3.0 L/min
Drying gas: 10 L/min Focus voltage: ±2 kV
Heating gas: 10 L/min

Figure 2 Nexera X3 and LCMS-8060NX

4. Result

4-1. Effect of IonFocus on the Sensitivity

We investigated the effect of the IonFocus Unit on sensitivity of hydrophilic compounds. The standard solutions that contains 36 of hydrophilic compounds (10 µmol/L) was used as a sample and 1 µL of the sample was injected without the column. The focus voltage was set to ±0 to ±5 kV. Figure 3 shows the relationship between the focus voltage and the peak intensity. For most hydrophilic compounds, the peak intensity was highest when the focus voltage was ±2 kV. Therefore, the focus voltage was set to ±2 kV in the subsequent experiments.

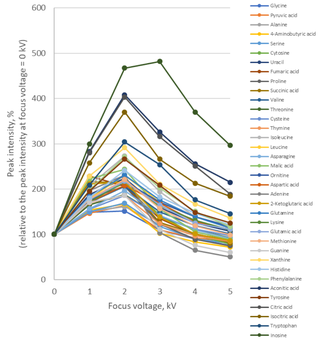


Figure 3 The relationship between focus voltage and peak intensity

4-2. Widely Targeted Metabolomics of 6 wines

Acids in wine not only affect the color, balance and taste of the wine, but are also involved in yeast growth during fermentation and protection from bacteria. Therefore, it is important to analyze the acid in wine for the evaluation and improvement of wine and the control of winemaking process. Wine mainly contains organic acids and short chain fatty acids such as tartaric acid, malic acid, lactic acid, succinic acid, acetic acid, and citric acid.
In analyses of short chain fatty acids and organic acids, we analyzed 6 short chain fatty acids and 17 organic acids. 6 short chain fatty acids and 14 organic acids were detected in wines. Principal component analysis (PCA) was conducted by SIMCA 16 software (Umetrics, Sweden). As a result of the PCA, the clusters were separated into 3 groups according to the producing regions (Figure 4). PC1 showed the difference between French and non-French wines. The French wine contained more tartaric acid and succinic acid. The United States wine contained more malic acid and pyruvic acid. Chile and Australian wines have similar characteristics.
Figure 5 shows the peak area rate of malic acid and lactic acid. The French wine contained more lactic acid and less malic acid. Therefore, French wine has more malolactic fermentation than wines of other countries and is considered to have less acidity.

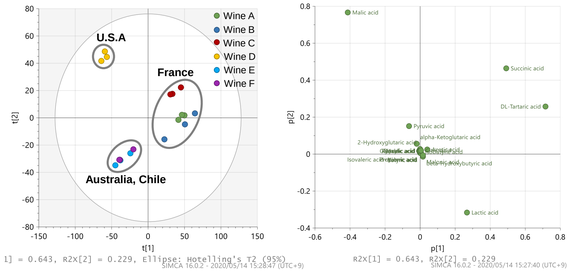
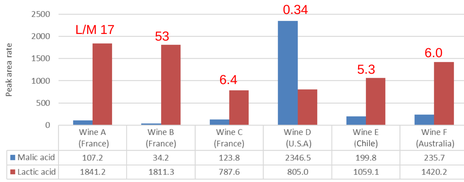


Figure 4 Score plot and loading plot in analysis of short chain fatty acids and organic acids



	Wine A (France)	Wine B (France)	Wine C (France)	Wine D (U.S.A)	Wine E (Chile)	Wine F (Australia)
Malic acid	107.2	34.2	123.8	2346.5	199.8	235.7
Lactic acid	1841.2	1811.3	787.6	805.0	1059.1	1420.2

Figure 5 Peak area rate of malic acid and lactic acid

Grape juice contains amino acids, polypeptides, proteins, and ammonium ions as main nitrogen compounds. It is known that the amount of these compounds changes depending on grape varieties and producing regions. The amount of amino acids is related to the growth and fermentability of wine yeast. Therefore, it is also important to analyze amino acids in wine for the evaluation and improvement of wine and the control of winemaking process.
In comprehensive analyses of hydrophilic metabolites containing amino acids, we analyzed 97 metabolites in wines. 60 hydrophilic metabolites were detected. Main compounds of the metabolites were amino acids, organic acid, and nucleic acid-related substances. As a result of the PCA, the clusters were separated into 3 groups according to the grape varieties (Figure 6). Cabernet Sauvignon and Merlot contained more proline and 4-hydroxyproline. Proline exhibits bitterness and sweetness. Merlot wine contained more phenylalanine, leucine, and lysine. The Pinot Noir wine contained more alanine than wines of other grape varieties. Alanine is evaluated as an amino acid that exhibits sweetness and umami.

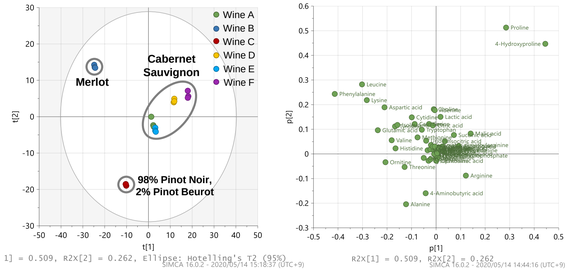


Figure 6 Score plot and loading plot in analysis of hydrophilic compounds

5. Conclusions

・A Newly-developed ionization unit, IonFocus Unit enabled highly sensitive analysis of hydrophilic compounds.
・Widely targeted metabolomics using LC-MS/MS was applied to find the differences of wines with different grape varieties and producing regions.
・As a result of PCA using the data of short chain fatty acids and organic acid, the characteristic of wines derived from producing regions was revealed.
・As a result of PCA using the data of hydrophilic compounds, the characteristic of wines derived from grape varieties was revealed.
・This analytical method seems to be useful for the evaluation and improvement of wine and the control of winemaking process.