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Widely-targeted Metabolomic profiling for wines by LC-MS/MS and GC-MS/MS measurement

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Overview

Sensory tests have generally been performed on the flavor and taste of foods as an approach to quantitatively evaluate food quality. Recently metabolome technique is also expected to find out the components contributing significantly to food flavor and taste. Here we report simultaneous analysis for four types of wine by LC-MS/MS or GC-MS/MS measurement and statistical analysis for the acquired data.

Introduction

In food science, targeted metabolome analysis or global metabolome analysis, is increasingly applied to a number of value-added food production fields including food-safety assessment, quality control, food authenticity, origin and processing. In this study, a widely-targeted metabolomics approach with LC-MS/MS and GC-MS/MS was applied to differentiate and classify four types of wine (different wine grape and location). GC-MS/MS and LC-MS/MS techniques enables to evaluate the level of amino acids, organic acids, sugar and nucleic acid-related compounds which are known to be the components associated with sweetness, bitterness and perceived flavor in food and beverages. Furthermore, to clearly classify four wines by the different level of multicomponent, statistical analysis by PCA and HCA was performed for the detected peaks.

Methods

An aliquot of wine (0.2 mL) was transferred into 1.5 mL tube and then the internal standard was added. After shaking, 0.2 mL was transferred into a centrifugal filter (MWCO 3kDa). Samples were centrifuged and then the filtrates were recovered. A part of the filtrate was diluted in purified water. Diluted sample solution was measured by LC-MS/MS. The remaining filtrate was evaporated. Then the methoximated and trimethylsilylated derivatization procedure was carried out for GC-MS/MS measurement. Using statistical software, the detected peaks intensities were calculated and statistical analysis (PCA and HCA) was performed to evaluate the level of target molecules between four types of wine.

LC conditions (Nexera system)	
Column	: Discovery HS F5 150 mm×2.1 mm, 3.0 µm
Mobile phase	: A: 0.1% Formic acid/water, B: 0.1% Formic acid/acetonitrile
Flow rate	: 0.25 mL/min
Time program	: B conc.0%(0-2.0 min) -25%(5.0 min) - 35%(11.0 min) - 95%(15.0.-20.0 min) – 0%(20.1-25.0 min)
Injection vol.	: 3 µL
Column temperature	: 40°C

MS conditions (LCMS-8060)					
Ionization	: Positive/Negative, MRM mode				
DL Temp.	: 250°C	HB Temp.	: 400°C	Interface Temp.	: 400°C
Drying Gas	: 10 L/min	Nebulizing Gas	: 2.0 L/min	Heating Gas	: 10 L/min

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GC conditions	
Column	: BPX-5 (30 m, 0.25 mm I.D., df=0.25 mm)
Glass insert	: Split insert
Oven temp.	: From 60°C (2 min) by (15°C/min) to 330°C (3 min)
Injection method	: Split
Split ratio	: 30
Control mode	: Linear velocity (39.0 cm/sec)
Injection vol.	: 1 µL

MS conditions (GCMS-TQ8040)			
Ion source temp.	: 200°C	Interface temp.	: 200°C
Measurement mode	: MRM	Loop time	: 0.25 sec

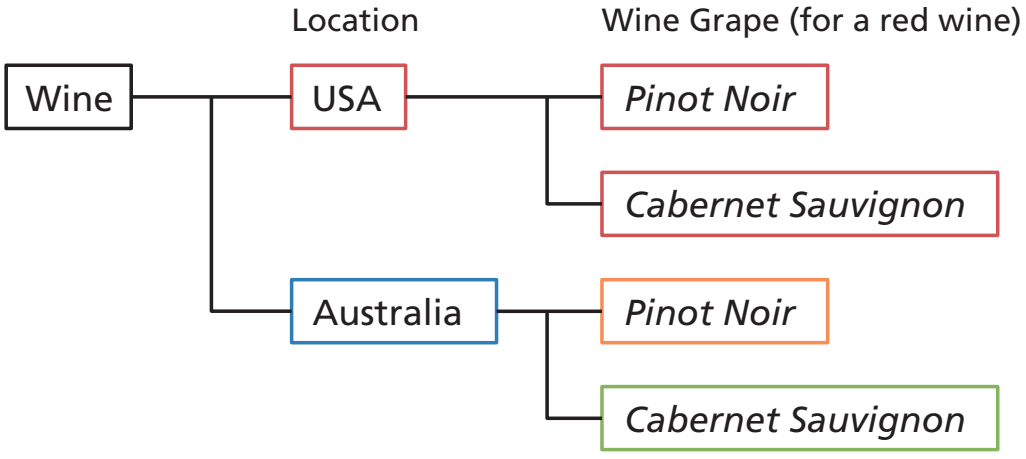

Figure 1 Four types of wine for simultaneous analysis of primary metabolites

Results

Simultaneous analysis by LC-MS/MS of primary metabolites in four types of wine

Comprehensive analysis for 97 compounds (n=3) was performed to find out the different components between four types of a red wine (*Pinot Noir or Cabernet Sauvignon* as a wine grape in USA and *Pinot Noir or Cabernet Sauvignon* as a wine grape in Australia). Figure 1 shows a location and wine grape type of four wines. Here pentafluorophenylpropyl column was used to separate metabolites in 3. Methods. The 63 peaks including amino acids, organic acids and nucleic acid-related compounds were detected in this experiment. Figure 2 shows the typical MRM chromatogram of four types of wine. As shown in the MRM chromatograms, the results depended to the type of wines and indicated the difference of the components in each wine. And in all MRM chromatograms, we can see a peak from proline at a greatly higher concentration compared to other amino acids. Generally proline can not be assimilated on the fermentation process of wine. Therefore, proline can be detected at a greatly higher concentration in compared with other amino acids. Out of the 4 types of wine, only in *Cabernet Sauvignon* from USA, relatively small amounts of amino acids were observed on MRM chromatogram.

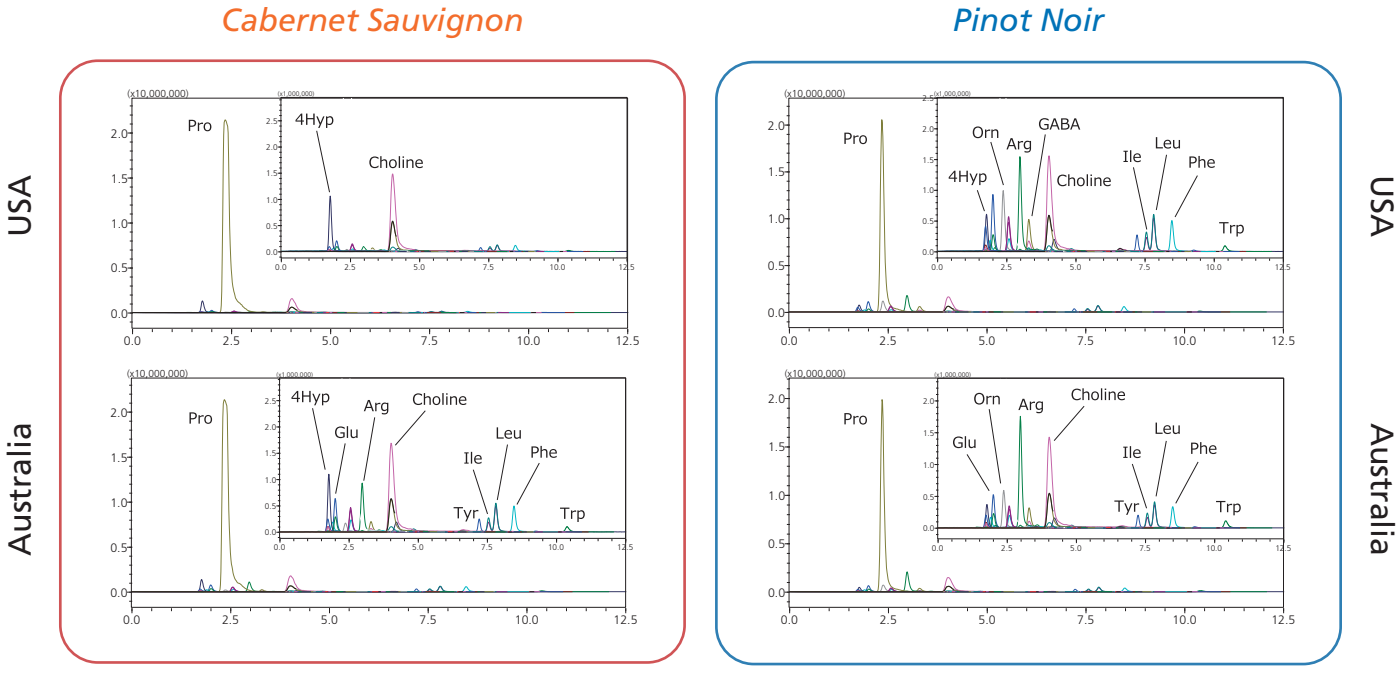

Figure 2 MRM chromatograms of four types of wine

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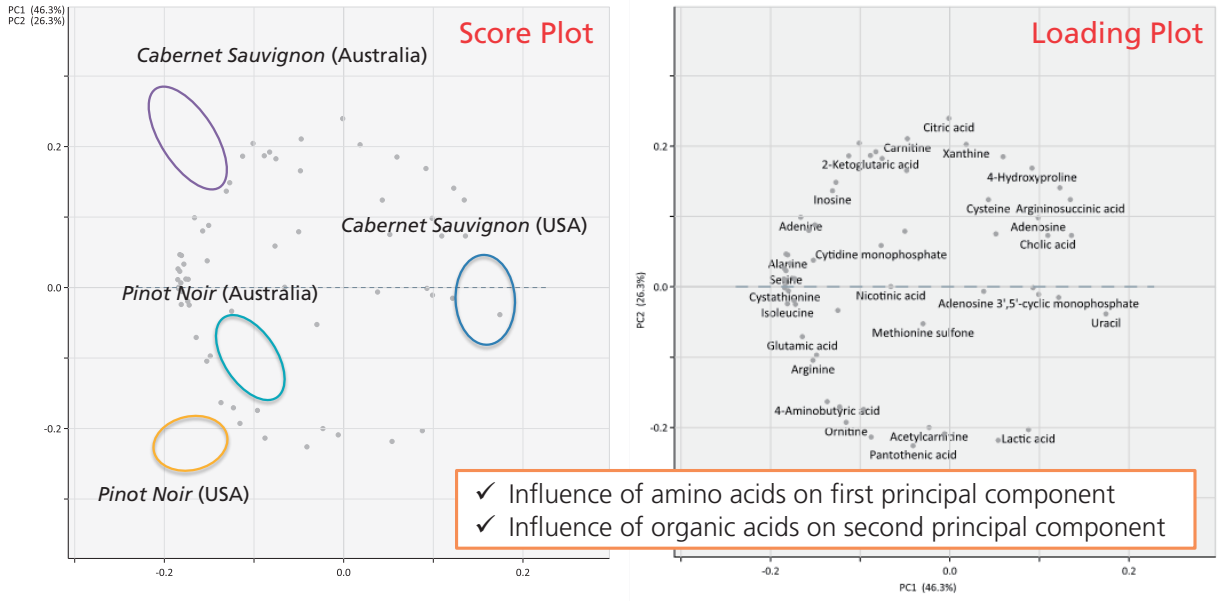

Figure 3 Principal component analysis for the detected metabolites

Figure 4 Hierarchical cluster analysis for the detected metabolites

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Using area ratio on internal standard, principal component analysis (PCA) was performed by Traverse MS software. As a result of PCA, the four types of wine were successfully classified by difference of components in each wine. Figure 3 shows the score plot and loading plot. As Figure 2 shows, a red wine from *Cabernet Sauvignon* in USA had relatively small amounts of amino acids and arranged on score plot away from other wines. Amino acids seem to mainly influence on first principal component and organic acids seem to influence on second principal component. Amino acids are greatly related with wine grape and increase/decrease of organic acids could be especially influenced by fermentation process. These results suggest that there is a possibility to evaluate the differences in fermentation process or grapes by monitoring an increase/decrease of components. Figure 4 shows the result of hierarchical cluster analysis (HCA) and we can see the difference of components between four types of wine in this figure.

Simultaneous analysis by GC-MS/MS of primary metabolites in four types of wine

Then we performed principal component analysis for the components detected in four types of wine by GC-MS/MS measurement. This time, area ratio of the detected peaks (>300 components) was calculated and statistical analysis was carried out for these values. As for the results of LCMS measurements, red wine from *Cabernet Sauvignon* in USA was away from other wines on a score plot (Figure 5). Thus, a combination of analysis by LC/MS/MS and GC/MS/MS with a statistical analysis could become a powerful tool to find out the components that greatly affect the taste of food and to evaluate its quality.

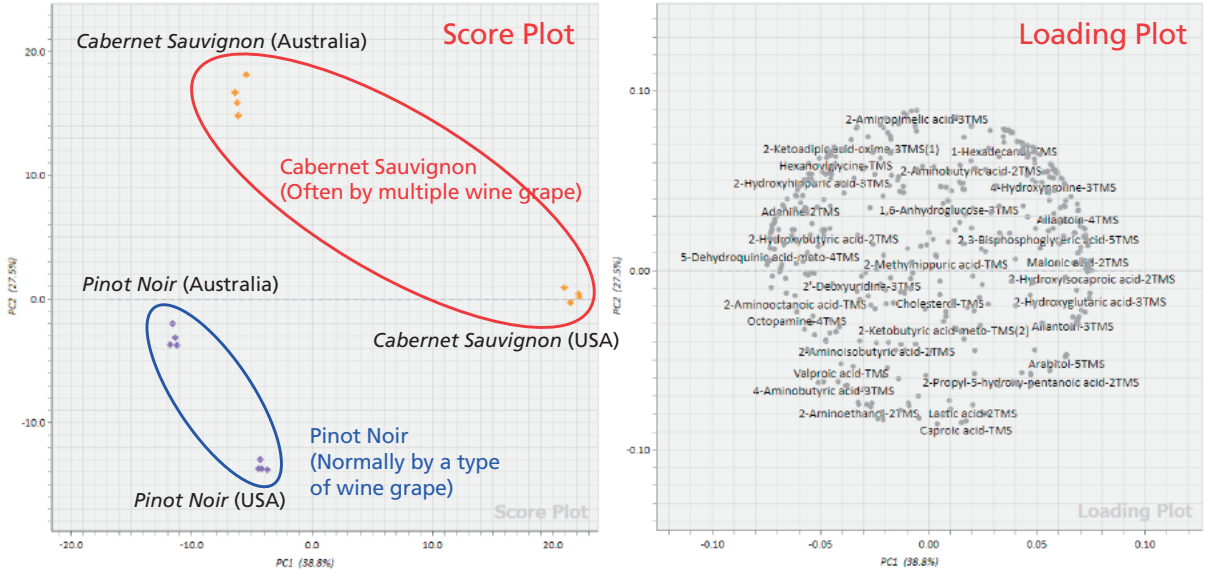

Figure 5 Principal component analysis for the detected metabolites

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Conclusions

- Complementary analysis was performed for four types of wine by LC-MS/MS and GC-MS/MS.
- Principal component analysis (PCA) for the detected metabolites successfully categorized four types of wine and found out some characteristic components.
- PCA for the data acquired by LC-MS/MS and GC-MS/MS indicated a similar trend: red wine from Cabernet Sauvignon in USA was arranged away from the other three wines.

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