



Application of a non-targeted metabolomics approach to identify the aging time of yellow rice wine



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ABSTRACT

During the aging process of yellow rice wine (Chinese rice wine), many chemical and physical changes are produced by the interaction of various components in the wine body, which improves its taste and stability. To further explore these changes, a non-targeted metabolomics method was developed using the X500R QTOF system from SCIEX to study how aging time affects the composition of yellow rice wines produced in the city of Shaoxing, China. SCIEX OS software was used to acquire and analyze the data. Small-molecule metabolites from 5 yellow rice wine samples that were aged for different lengths of time were identified and analyzed. These metabolites are helpful indicators for the aging time of yellow rice wine.

INTRODUCTION

Yellow rice wine has a long history in China, with thousands of years of tradition in Chinese culture and industry. It is known, along with beer and wine, as one of the world's ancient alcoholic drinks. Yellow rice wine is a low-alcohol original wine made from grains (such as glutinous rice) and fermented by a variety of microorganisms. It is popular with the public because of its low alcohol content, full body, rich nutrients and unique flavor. The city of Shaoxing in China is best known for its yellow rice wine. Aging time (i.e., wine storage time) is the main indicator of the quality of yellow rice wine. The identification of wine storage time is one of the many challenges currently faced by the yellow rice wine industry. Some producers label their wine with a misleading age, which can have a negative impact on yellow rice wine promotion and sales. To address this issue, clear indicators for aging time are necessary to help ensure quality. In this study, a non-targeted metabolomics method was developed using the X500R QTOF system from SCIEX to study the effects of aging time on the composition of Shaoxing yellow rice wine. Small-molecule metabolites—which are helpful indicators for the aging time of yellow rice wine—from 5 yellow rice wine samples that were aged for different lengths of time (1, 4, 8, 11 and 13 years) were identified and analyzed.

MATERIALS AND METHODS

Sample preparation:
Five yellow rice wine samples—aged for 1, 4, 8, 11 and 13 years—were acquired from Shaoxing, China, and 5 parallel samples were prepared for each group. Samples were diluted 5-fold using 50% methanol in water and filtered through a PTFE filter (0.22 µm). A representative quality control (QC) sample was prepared by pooling together 10 µL aliquots for each wine sample.

HPLC conditions:
An ExionLC AD system was used, and a 20-min gradient on a Waters HSS T3 column (2.1 x 100 mm, 1.8 µm, 100 Å) at 40° C was developed for chromatographic separation. The composition of the mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile at a flow rate of 400 µL/min. The injection volume was set to 5 µL.

MS/MS conditions:
An X500R QTOF system from SCIEX with a Turbo V ion source and an electrospray ionization (ESI) probe was used. TOF MS and MS/MS data were acquired in a single injection using data-dependent acquisition (DDA) with dynamic background subtraction (DBS) enabled. The MS source conditions were as follows: 30 psi for curtain gas (CUR); 7 psi for collision gas (CAD); 50 psi for nebulizing gas (GS1); 50 psi for heater gas (GS2); 5500 V / -4500 V for ion spray (IS) voltage; 500° C for source temperature. Each sample was injected in both positive and negative polarity. The QC sample was acquired every 5 samples.

RESULTS

In this study, TOF MS data and MS/MS data were acquired from samples using the X500R QTOF system in a single injection for each polarity. The total ion chromatogram (TIC) of the 11 QC samples showed excellent

reproducibility (Figure 1). The stable signal intensity and retention time of the data indicated that the acquired large-scale metabolomics data were reliable and could be used for metabolomic analysis. Using instrument processing software, MS/MS libraries from SCIEX and the Global Natural Products Social Molecular Networking (GNPS) knowledge base, a total of 208 metabolites were quickly identified in 5 different samples of yellow rice wine. Of these 208 metabolites, 157 were identified using the All-in-One HR-MS/MS spectral library and NIST 2017 MS/MS library bundle from SCIEX. These metabolites could be confirmed automatically to ensure the accuracy and reliability of identification according to a mass error of <5 ppm (most within 2 ppm), a difference isotope ratio of <10 ‰ and a library hit score of > 70 (Figure 2). Smart confirmation search was used as the library search algorithm.

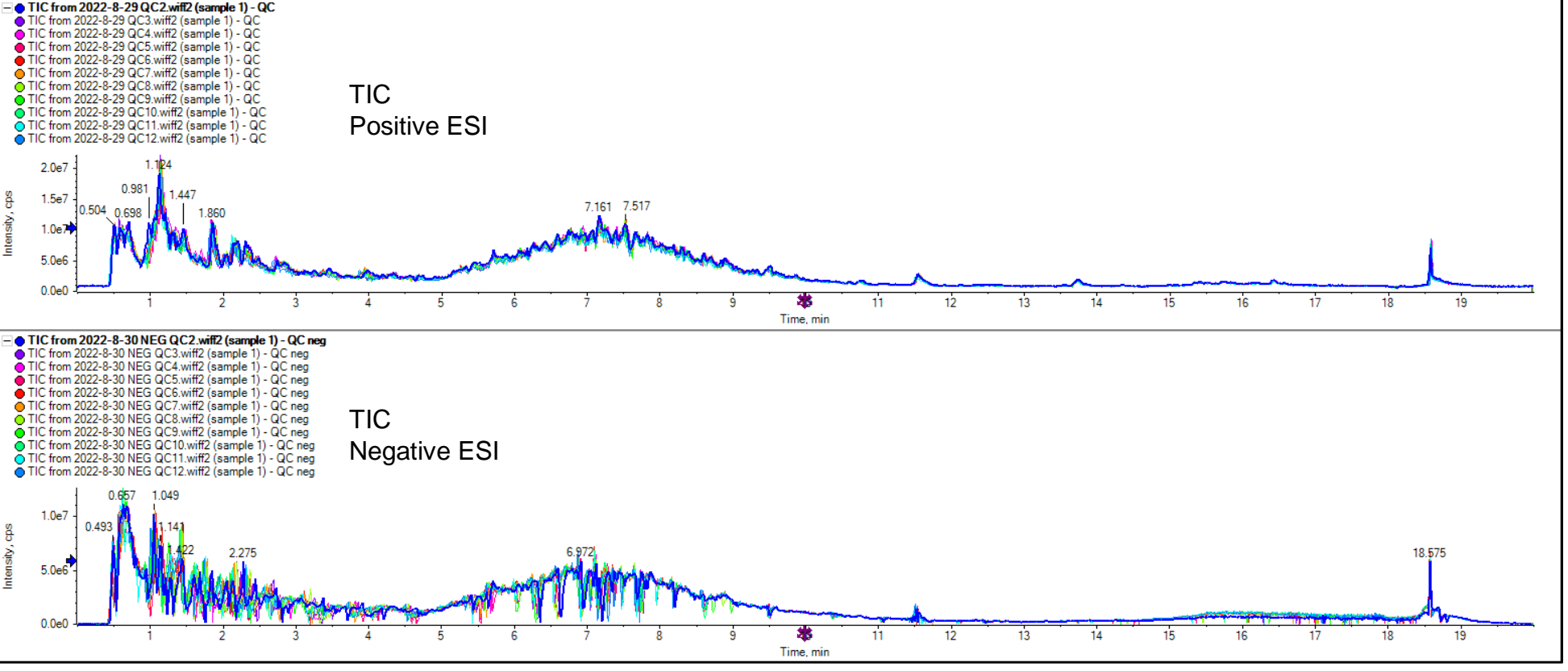


Figure 1. Overlay of the TIC of 11 QC samples in positive (top) and negative (bottom) polarity.

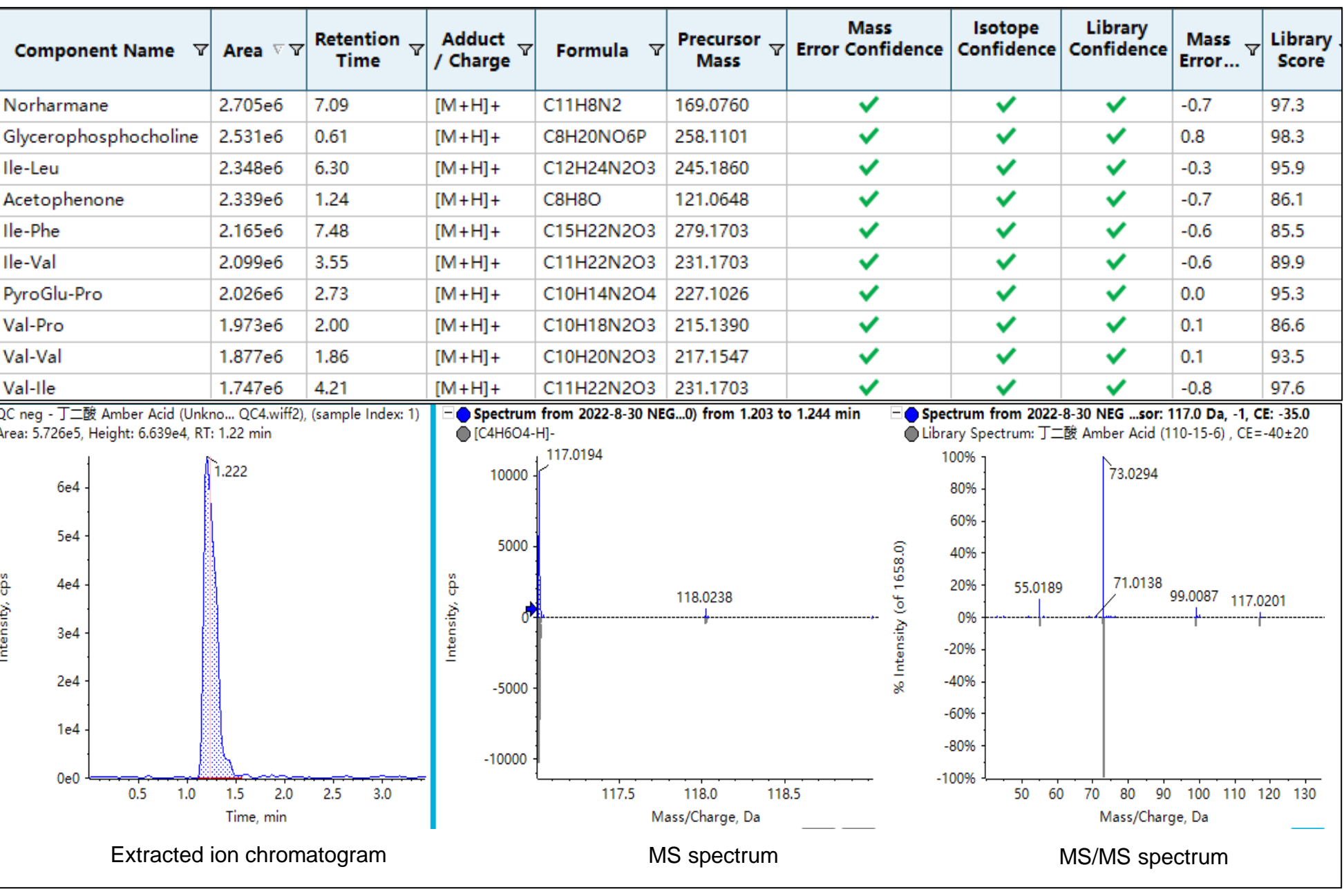


Figure 2. Partial screening results of target compounds by SCIEX OS software and MS spectra of amber acid.

The results demonstrated that amino acids and their derivatives accounted for the highest proportion of all compounds. In addition, phenols, alkaloids, organic acids, nucleotides, carbohydrates, aldehydes, esters and other compounds were detected in the yellow rice wine samples (Figure 3).

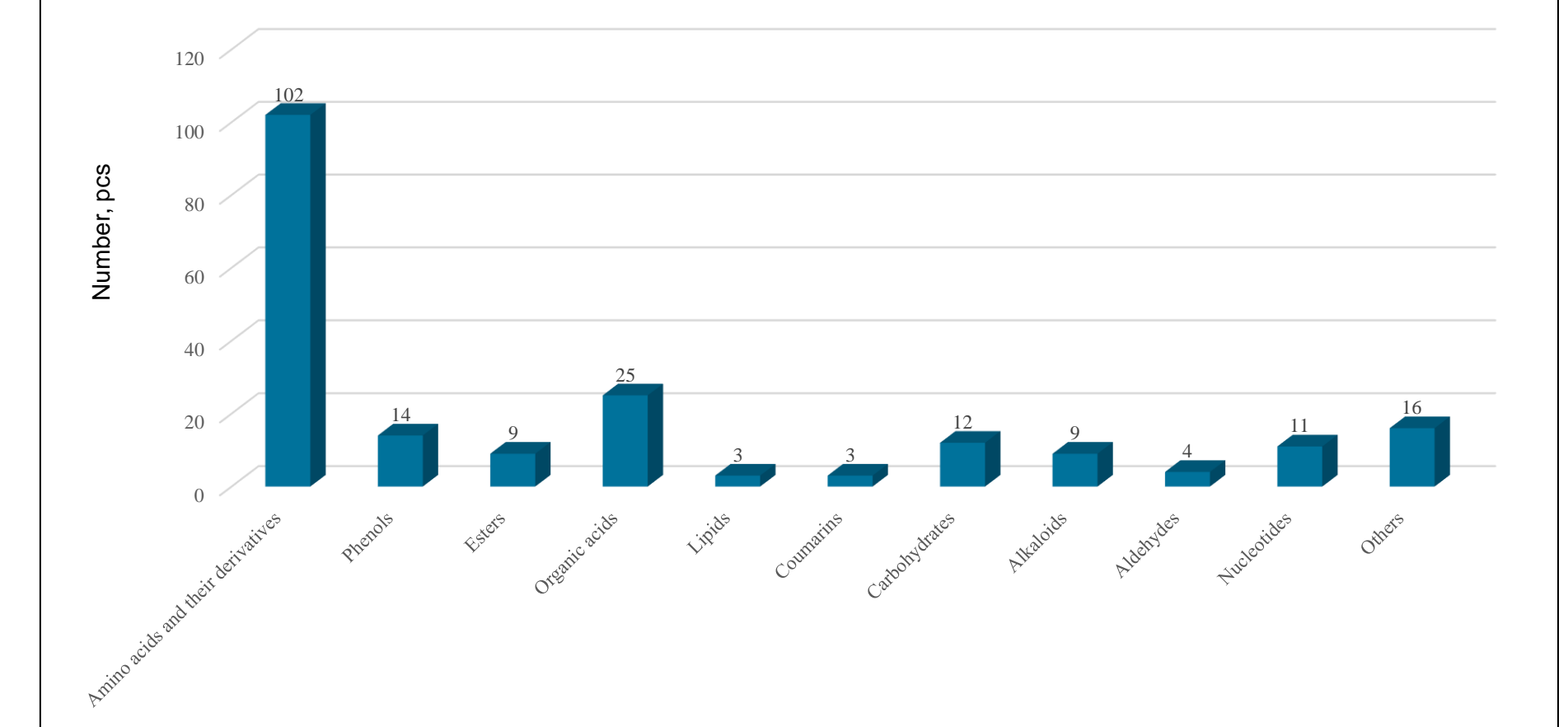


Figure 3. Classification and corresponding number of 208 metabolites identified in the yellow rice wine samples.

The identified metabolites helped to determine a set of differential compounds among different ages of yellow rice wine. Supervised statistical models using partial least squares discriminant analysis (PLS-DA) are shown in Figure 4. There were 80 metabolites that had significant changes using a statistical significance filter of p-value <0.05 and a variable importance in projection (VIP) score of >1.

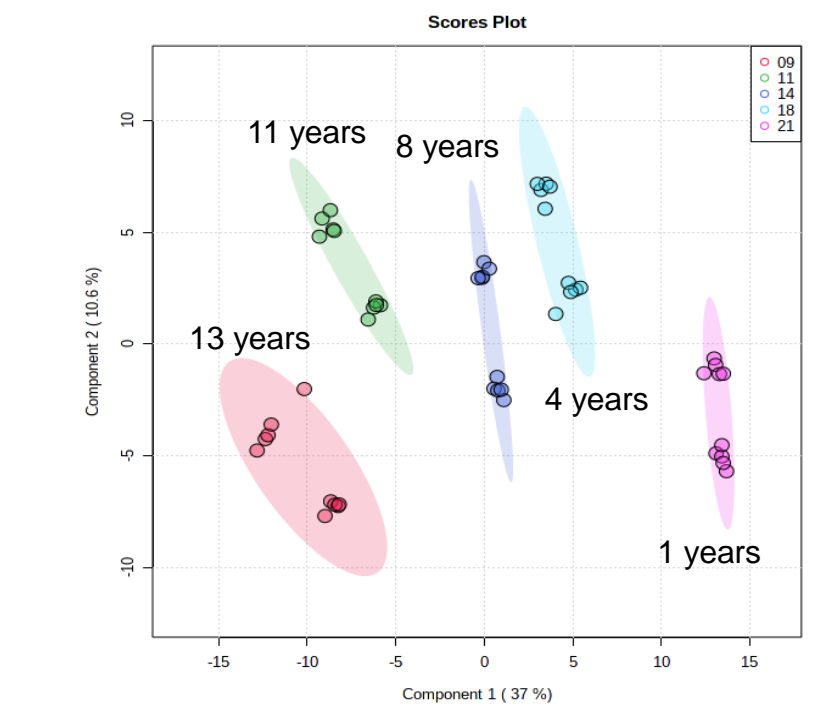


Figure 4. Supervised statistical models using partial least squares discriminant analysis (PLS-DA) are shown (n = 5). A PLS-DA scores plot is shown for 1, 4, 8, 11 and 13 years.

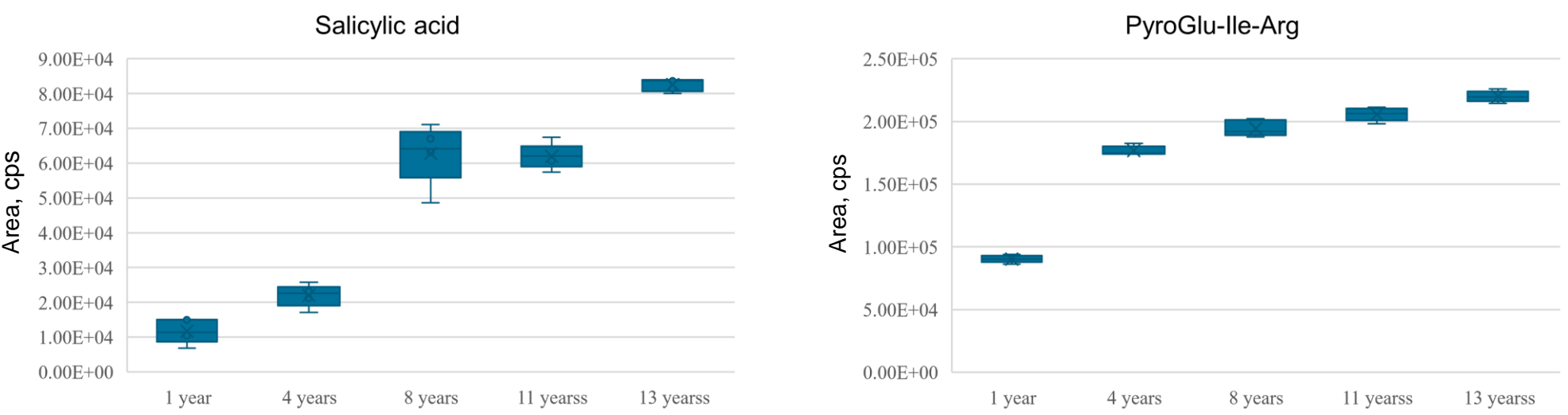


Figure 5. The content of salicylic acid and PyroGlu-Ile-Arg in wine aged for 1, 4, 8, 11 and 13 years.

The content of organic acids, aldehyde and esters—which have been reported as flavor substances in the aging process of Shaoxing yellow rice wine—increased when brewing years increased. In addition, different amino acids and small peptides showed up- and down-regulation trends when brewing years increased. Most of all, the content of 2 functional peptides—cyclic dipeptides and pyroglutamyl (PyroGlu) peptides—in the yellow rice wine sample aged for 13 years was significantly higher than the content of those in the sample aged for 1 year (Figure 5). For example, the p-value of PyroGlu-Ile-Arg among the 5 yellow rice wines was 4.43E-25. Seven characteristic compounds were detected in wine aged for 1 year, which could be considered as the basis for the identification of yellow rice wine age (Table 1).

| Index | Name |
|-------|-----------------------------------|
| 1 | Tricin |
| 2 | Methionine |
| 3 | Kaempferol 3-glucoside-7-xyloside |
| 4 | 5'-S-Methyl-5'-thioadenosine |
| 5 | 3-Indoleacrylic acid |
| 6 | 4-Methylumbelliferone |
| 7 | Phenylacetic acid |

Table 1. Seven compounds were detected in wine aged for 1 year.

CONCLUSIONS

A non-targeted metabolomics method for studying the effects of aging time on yellow rice wine was established and markers for identifying different aging times were found. A total of 208 metabolites were quickly identified in 5 different samples of yellow rice wine using SCIEX OS software and an MS/MS library bundle from SCIEX. There were 80 metabolites that had significant changes among yellow rice wine of different ages.

TRADEMARKS/LICENSING

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