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Orthogonal optimization of extraction and analysis for red wine residues in simulated and archaeological materials using LC/MS and HPLC methods

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ABSTRACT

Identification of red wine residues in archaeological potteries can yield good insight into the development of red wine culture and the history of winemaking. In this work, the ultra- sonication extraction method was developed to extract the organic acids in simulated red wine residues, while the optimization of extraction condition was studied through orthogonal methods. Moreover, the availability of the proposed research method in archaeological research was also investigated by extracting and analyzing two real archaeological samples. The results obtained from the liquid chromatography/mass spectrometry (LC/MS) proved that the tartaric acid can be acted as the biomarker of red wine. The range analysis of orthogonal methods indicated that the temperature is the main factor on the extraction efficiency of tartaric acid, followed by the proportion of methanol in the solvent, and finally is the extraction time. More importantly, tartaric acid can be identified in the real archaeological sample using the proposed extraction and analysis method, indicating its high verifiability and reliability. This work would provide a complete set of analysis method to identify the traces of wine organic residues in archaeological samples, thus understanding the production and conservation of wine or even tracking back to the start brewing process of wine.

1. Introduction

In ancient Egypt, red wine is often compared to the blood of God Osiris, the first resurrection of human, and symbolizes of the rebirth of the dead [1]. Moreover, red wine also is a kind of very important beverage in the Mediterranean area [2]. Many ceramic containers, which have been used to store and serve wine, are associated with the production, consumption and trade of wine. It is thus necessary to study the traces of archaeological wines to determine whether these vessels actually contain red wines or their derivatives, and provide a direct archaeological evidence of red wine culture, the history of winemaking even the people's living habits in prehistory [3, 4].

In recent years, the advancement of modern analytical techniques over past two decades, has broaden boundaries of archaeological residues research and reached a new dimension [5–11]. And various methodologies for the analysis of organic residue in wines have been successfully developed [12–16]. Among these analysis techniques, the most effective approach is the analysis of molecular marker through chemical methods, which could help to verify the existence of wine and thus track back to some cultural and economic activities in ancient times [17]. Garnier et al. have studied the possibility of using polyphenols as biomarkers of ancient fermented beverages and applied thermally assisted hydrolysis and methylation/gas chromatography/mass spectrometry(THM/GC/MS) to identify the presence of wine in archaeological samples [18]. Buonasera et al. have employed a biomarker approach and analyzed organic residues from several central California milling tools [19].Wang et al. have identified the oxalic acid in pottery solid residues using ion chromatography and thought oxalic acid presumably derived from calcium oxalate, which was a by-product of malt wine brewing, and this was also a direct evidence of the earliest origins of beer in China [20]. However, to date, few systematic studies for the extraction and identification of red wine biomarker have been reported due to the low detectable characteristics of residual species in solid archaeological vessels.

The aim of this study is to propose an effective methodology for the extraction and analysis of red wine residues in archaeological materials. In the current research of red wine residues, tartaric acid is usually reported as the biomarker because tartaric acid can be directly obtained from grapes. Pecci et al. have identified tartaric acid and other biomarkers of wine in experimental and archaeological materials using gas chromatograph mass spectrometry [16]. Guasch-Jane et al. have chosen tartaric acid and syringic acid as a biomarker and studied

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archaeological residues from Egyptian vessels using liquid chromatography with mass spectrometry in tandem mode [21]. McGovern et al. have revealed the direct evidence of the fermented beverages in ancient Chinese culture and found that the wine residues of pottery jars contains a certain amount of tartaric acid [22, 23]. From the above analysis, it is beyond doubt that the tartaric acid can be feasibly chosen as biomarker of red wine. However, tartaric acid is abundant not only in wine, but also in grape juice, syrup or other wine derivatives. It is not accurate enough to only choose tartaric acid as the biomarker for the identification of red wine in archaeological materials. Recently, the tartaric acids and malic acids were reported to account for nearly over 90% of the total organic acid content in non-muscadine wines, and tartaric acids and succinic acids were the predominant acids of the muscadine wine [24]. Therefore, in addition to the tartaric acid, the organic acids including malic acid and succinic acid, as the characteristics of red wine, should also be considered to identify the traces of wine in archaeological potteries.

In this work, the ultra-sonication extraction (USE) was used to extract organic acids from archaeological vessels, because the method can produce high pressures and temperatures, accelerate acid molecule motion and operate much more simply [25]. In addition, the optimal extraction conditions of tartaric acid were also investigated by orthogonal methods, the extracted tartaric acid was quantitatively analyzed by high performance liquid chromatography. Finally, the availability of this extraction and analysis method was further verified by two real archaeological samples. The work of this study is not only suitable for the analysis of organic acids in red wine, it also allows this kind of archaeological samples containing red wine to be analyzed and detected.

2. Material and experimental method

2.1. Reagents and samples

Deionized water and chemical reagents of analytical grade were used in all experiments. Their purities were as follows: KH_2PO_4(≥99.5%),L(+)-tartaric acid(≥99.5%),oxalic acid(≥99.5%), malic acid(≥98.5%),acetic acid(≥99.5%), lactic acid (≥85%), citric acid(≥99%), succinic acid(≥99.5%). Methanol and acetonitrile of chromatographic grade were used. Tartaric acid, oxalic acid, malic acid, citric acid and succinic acid were prepared at concentration of 100 mg/L. KH_2PO_4 solutions were prepared at concentration of 0.08 mol/L. Lactic acid and acetic acid were prepared at concentration of 10 mL/L.

Simulated samples were made as potteries with a diameter of 5 cm, and calcined in a furnace at 800 °C. Then all potteries were immersed in Changcheng red wine for more than 6 months to simulate the adsorption residues process of archaeological wine containers. All simulated samples were stored at 4 °C before use to reduce the volatilization of the residues.

Archaeological materials were originated from ship-sunken salvage in the Mediterranean area from French. Materials were at archaeological levels and may be related to wine storage or production.

2.2. Ultrasonic extraction process

Approximately 1 g fragments of simulated sample was pulverized and dried at ambient temperature, and then the powder was extracted with 5 mL water/methanol under the various extraction conditions according to Table 4. Finally, the formed samples are obtained with centrifugation at 10,000 rpm for 16 min with methanol/water solution for several times, and the supernatant was restored at 4 °C for further utilization.

2.3. Analytical method

2.3.1. Ultra-performance liquid chromatography-mass spectrometry

The first step of qualitative analysis for organic acids recovery was performed on UPLC system ACQUITY (Waters, Massachusetts, USA) with a binary pump and mass spectrometry(Waters, USA)with electrospray ionization(ESI) interface. An ACQUITY UPLC BEH C18 analytical column (2.1 × 50 mm i.d., 1.7 μm) was used at 40 °C. A constant flow rate of 300 μL/min was chosen and the injected volume was 15 μL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile with 0.1% formic acids (solvent B). The elution program was set as: 0–3.5 min: 100% A; 6.5–8.5 min: A/B (80:20); 10.5–15.5 min: A/B (50:50); 16.5–18.0 min: A/B (90:10). The mass spectrometer was a single level quadrupole MS system (SQD2, America) and was operating in negative-ion mode for monitoring ions of deprotonated molecules $[M-H]^-$.

2.3.2. Reversed phase high-performance liquid chromatography

Shimadzu HPLC system (LC-10AT HPLC Series, Shimadzu, Kyoto, Japan) was applied for the quantitative analysis of tartaric acid in this study. HPLC was based on Class-VP software and equipped with a binary pump system, a UV/Vis detector (SPD-20A).The column used in this experiment was Agilent ZORBAX SB-C18 column (4.6 × 150 mm i.d., 5 μm). The column temperature was 30 °C. The mobile phase was prepared by 0.08 mol/L KH_2PO_4 aqueous solution which was adjusted by phosphoric acid solution until pH = 2.3. A volume of 10 μL was injected into the instrument for analysis at the flow rate of 1.0 mL min^{-1}. Peak detection at 215 nm was accomplished using the UV/Vis detector. The column temperature was kept at 30 °C. Tartaric acid was identified by comparing the retention times of authentic standards. It should be noted that the low pH of the mobile phase in this operation method may pose a negative effect on column stability over time. Therefore, before and after each test, it is necessary to flush column with a large amount of methanol aqueous solution at different flow rates. Then, the column is washed with methanol or acetonitrile to protect it in the organic phase.

3. Results and discussion

3.1. The selection of extracting agents

The methanol, diethyl ether, phosphoric acid, metaphosphoric acid, ethanol, and pure water are usually considered as the extracting agents for the organic acids extraction from archaeological potteries [26]. Among these extracting agents, the diethyl ether with high toxicity may damage archaeological samples. And the acid ions produced by phosphoric acid or metaphosphoric acid may interfere with the detection of organic acids. Thus, methanol, ethanol and water can be acted as the preferred extracting solution in this work. Table 1 listed the solubility of tartaric acid and malic acid in different solution. It can be seen that both of tartaric acid and malic acid have the highest solubility in water, so pure water is the most ideal extracting agents for organic acids. However, it was reported that malic acid might be partially converted into lactic acid when using pure water to recover organic acids from fruits [26]. In order to avoid this problem, a mixed aqueous solution with methanol or ethanol is proposed to be as the extracting agents in this work. In addition, Table 1 shows that the solubility of tartaric acid

Table 1
Solubility of solute in 100 g Solvent at 20 °C.

Solute solvent	L-Tartaric acid	Malic acid
Water	133	55.8
Methanol	58	12.6
Ethanol	33	–