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# Wine or Beer? A reinvestigation of residues from bronze vessels from the Beibai'e cemetery, Shanxi China

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**Abstract**

The Beibai'e cemetery is a high-status noble tomb group from the early Spring–Autumn period (770 B.C–476 B.C). Three sealed bronze vessels with mud and liquid residues were excavated from the M1 tomb. In a previous investigation, it was concluded that the residues were fruit wine since syringic acid was detected. However, this finding contradicts the grain-based brewing traditions prevalent in the central plains region of China since the Neolithic era. In the previous study, syringic acid was considered a unique biomarker for fruit wine. In this study, multiple analytical techniques, including microfossil analysis, HPLC—MS and FTIR were applied. The results indicated that the residue was beer rather than fruit wine. This study demonstrated that comprehensive analysis and multiple pieces of evidence are necessary in wine residue research.

**Keywords** Bronze vessels, Residue, Plant microfossil analysis, FTIR, HPLC–MS, Beer

## Introduction

Alcoholic beverages have significantly shaped the course of human history. Due to different climatic environments and dietary traditions, the raw materials and brewing techniques have varied considerably in different regions, giving rise to various alcoholic beverages in different parts of the world, such as beer and liquor in China, wine in the Mediterranean world, and koumis in nomadic peoples [1, 2]. China has a long history of beer making [3, 4]. Sufficient evidence indicates that alcoholic beverages have been intentionally made in ancient China since the Neolithic era, and traditions related to drinking beer and distilling liquor have existed for thousands of generations according to archaeological evidence and historical documents [5–7].

The Beibai'e cemetery is a tomb group for high-status nobles from the early Spring–Autumn period (770 B.C–476 B.C.) from a district of the Eastern Zhou Dynasty in the central plains region of China (35°12' N, 111°44' E) (Fig. 1a) [8]. Nine tombs of nobles from the Beibai'e cemetery were excavated in 2020, from which numerous bronze vessels were unearthed (Fig. 1c) [9, 10]. According to the archaeological typology analysis, these vessels were probably used as beer or wine containers [11]. In a recent study, Li et al. detected organic residues in these vessels by GC–MS and HPLC–MS/MS [10]. Their study revealed that the residues could be classified as alcoholic beverages based on discovered volatile organic compounds such as organic acids, alcohols, esters, and sugars. Due to the relatively high content of syringic acid, the authors concluded that the residues were from wine.

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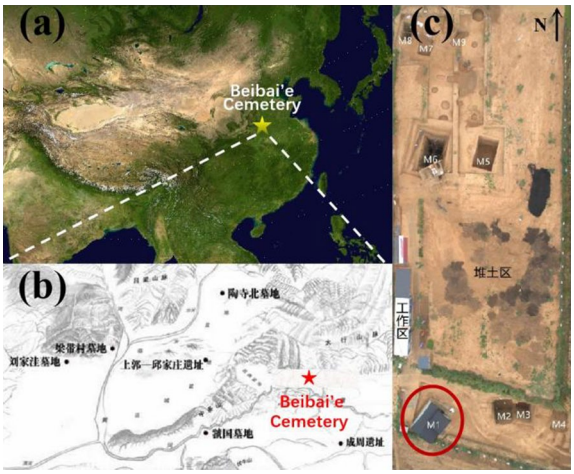


**Fig. 1** **a** Location of the Beibai'e site; **b** Topographic map of the Beibei'e site and surrounding sites; **c** Orthographic projection of the Beibei'e site excavation area in 2020

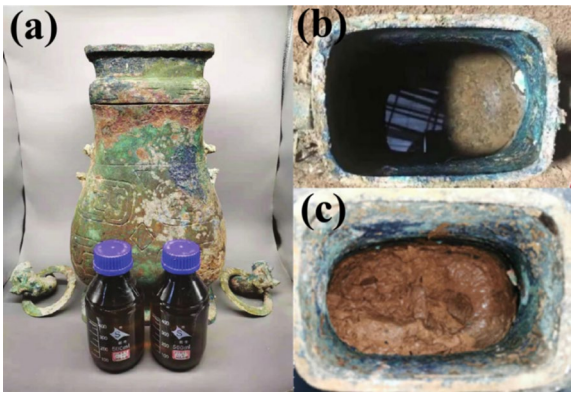


**Fig. 2** **a** Front view of the M1:45 bronze vessel; **b** liquid residue in the M1:45 bronze vessel; **c** saturated mud residue in the M1:44 bronze vessel

If this conclusion is valid, these samples predate the first wines in ancient China by more than 500 years.

However, China has a tradition of brewing alcoholic beverages from cereals rather than fermented fruit juices as described above. Furthermore, there are clear records in historical documents that brewed alcoholic beverages were used in sacrificial activities during the Spring–Autumn period [12–14]. On the other hand, fruit wine was not a mainstream beverage in ancient China, and no historical records or archaeological materials indicated that fruit wine was a funerary object. Even up to the Han Dynasty (202 B.C–220 A.D.), there is no definite evidence of fruit wine brewing in China [15–17]. Therefore, it is rather surprising that fruit wine was found in such a high-status noble tomb group in the Eastern Zhou Dynasty, an era when beer and distilled liquor brewing technology was highly developed and prevalent.

In this study, we reinvestigate the residues in the bronze vessels, as well as liquid and mud samples unearthed from the Beibai'e cemetery. Multiple analytical techniques were applied for identification, including plant microfossil analysis, high-performance liquid chromatography–mass spectrometry (HPLC–MS), and Fourier transform infrared spectrometry (FT-IR). It was vital to conduct a comprehensive analysis to determine the types of ancient liquor residues present based on multiple pieces of evidence.

## Samples and experiments

### Samples

Three samples unearthed at the Beibai'e cemetery were examined, which consisted of two clear liquid samples collected from M1:45 and M1:48 bronze vessels and one mud sample from the M1:44 bronze vessel (Fig. 2).

### Analysis and experiments

#### *Plant microfossil analysis*

*Plant microfossil experiment in the laboratory at Northwest University (NWU)* This experiment was carried out in the Key Laboratory of Cultural Heritage Research and Conservation, Ministry of Education, China. A certain amount of sample was placed in an ultraclean container, 6% $H_2O_2$ was added to remove the organic matter in the residue, the sample was washed with ultrapure water to neutral pH, and then CsCL heavy liquid with a specific gravity of 1.8 g/cm$^3$ and $ZnCl_2$ heavy liquid with a specific gravity of 2.3 g/cm$^3$ were applied to float the starch particles and phytoliths, respectively, and their morphology were observed. The details of this method are shown in previously published papers [18, 19].

*Plant microfossil experiment in the laboratory at Xiamen University (XMU)* The experiment was carried out in the Laboratory of Archaeometry, Xiamen University, Xiamen, China. Four grams of mud samples were taken with a clean disposable suction head and then placed in two 5 mL clean, disposable, plastic-covered centrifuge tubes, with 2 g samples in each tube. Deionized water was added to the 4 mL mark in the 2 tubes, and then the tubes were centrifuged at 2000 rpm for 5 min. The supernatant was discarded, sodium polytungstate heavy liquid with a specific gravity of 2.0 was added to the 4 mL mark, and the mixture was agitated for 30 s via a contact oscillator. However, the mud was too sticky to disperse well, so it was shaken manually to disperse the sample. Then, the samples were centrifuged at 2000 rpm for 5 min. Approximately 1 mm of the upper layer, which was rich in organic mat-

ter, was transferred to a new 1.5 mL clean plastic-covered centrifuge tube, deionized water was added to 1.5 mL, and the tube was centrifuged at 5000 rpm for 5 min. This step was repeated 3 times to thoroughly elute the heavy solution. The supernatant was removed, and approximately 100 μL of the liquid was retained, the liquid was agitated several times with a liquid extractor to blend the precipitate evenly, and it was transferred to a clean slide, with approximately 20 μL for each slide. The slides were air-dried. Fifty microliters of 50% glycerol was added to the dried sample area, the area was covered with cover glass and sealed with transparent nail polish. A total of 10 sample slides were made, numbered from SYB-1 to SYB-10. The prepared slides were examined by a biological microscope (Zeiss scope A1) equipped with a polarizing module and photographed by an AxioCam MRc digital camera connected to a computer, and the data were recorded and measured by Zeiss software.

#### *HPLC–MS*

Seven kinds of organic acids (oxalic acid, lactic acid, fumaric acid, succinic acid, malic acid, D-(-)-tartaric acid, and citric acid) were selected, which are typically applied as biomarkers in ancient liquor residue analysis. A concentration gradient of eight standard samples was prepared to establish the standard curve for quantitative analysis.

Three pieces of unearthed archaeological residues were analyzed, including two refined liquid samples from the M1:45 and M1:48 bronze vessels. The third sample was an extract of the mud in the M1:44 bronze vessel. The three samples were unearthed from the Beibai'e Cemetery M1. The data acquisition system included ultrahigh-performance liquid chromatography (Vanquish, UPLC, Thermo, USA) and high-resolution mass spectrometry (Q Exactive, Thermo, USA).

Five hundred microliters of liquid samples were collected from two bronze kettles, M1:45 and M1:48, and were directly injected into the test system to preserve the original information to the extent possible. The extraction processes of the mud were as follows. A 300 μL solution (methanol: chloroform = 7:3) was added to a 50 mg mud sample, and the mixture was placed in an ice bath for 30 min. Then, 200 μL deionized water was added to the mixture and mixed thoroughly. Then, the mixture was centrifuged at 12000 rpm for 10 min to extract the supernatant. The extraction was repeated once. The supernatants obtained from the two extracts were mixed and freeze-dried. The residue was dissolved by adding 200 μL methanol. The supernatant was obtained by centrifugation at 12000 rpm for 10 min. Ten microliters of 0.1 M EDC and 10 μL of 0.1 M 3NPH were added to the supernatant of the mud extract, and the reagent was incubated at 40 °C for 30 min for derivation.

The test system included ultrahigh-performance liquid chromatography and high-resolution mass spectrometry. The test parameters and test conditions are shown in Table 1.

#### *FTIR and Fisher discriminant method*

Five types of modern alcoholic beverages were selected, including wine, Shaohsing wine, rice wine, beer and liquor, and the details are shown in Table 2. The ancient samples to be tested were = in liquid form, including two samples taken directly from the bronze vessels and the supernatant of the mud after extraction and centrifugation. The extraction process for the mud sample was as follows: 3 ml methanol was used to extract the organic residues in the mud, the samples were vortexed for 5 min to evenly mix the solid and liquid and centrifuged at 9000 rpm for 20 min, and the supernatant was extracted. Dry KBr powder was pressed to form a uniform tablet approximately 1 mm thick, three drops of each sample were added onto the KBr tablet, and two parallel tablets were made for each sample. The test parameters were as follows: scanning wavenumber 400–4000 $cm^{-1}$, resolution 4 $cm^{-1}$, and repeated scanning 16 times. A total of 5 sets of spectral data were collected for each sample (Bruker Company, Germany, ALPHA·II) after drying at 40 ℃ for 20 min.

To synthesize comparable information from infrared data to facilitate cluster analysis, an infrared Fisher discriminant model was established. The model building indicators were selected from the infrared characteristic peak wavenumbers of all samples, and the Fisher discriminant function was established. The data were processed by IBM SPSS Statistics 25, Thermo Scientific Omnic 7.3, OPUS 7.5, and Origin 2018.

## Results

### Plant microfossil analysis

To explore the raw materials in the liquid in the bronze vessels, the plant microfossils present in the mud sample in the M1:44 bronze vessel were analyzed first in the laboratory of NWU. By taking the collected morphological data for plant microfossils from more than 50 genera and more than 200 species as the database [20–22], the phytoliths, starch granules, and pollen extracted from the residues were identified. A total of 5 starch granules and 1 sporopollenin were extracted, and 38 microremains of suspected *Saccharomyces cerevisiae* were also observed (Fig. 3).

In this study, 5 starch granules were extracted, and their identifiable forms could be mainly divided into two categories, type A and type B. The microfossils that could not

**Table 1** Instrument parameters

| **Liquid chromatography parameters** | |
|---|---|
| **Chromatographic column** | **Waters BEH C18(50*2.1 mm, 1.8 μm)** |
| Mobile phase | Phase A: 0.1% formic acid aqueous solution<br>Phase B: 0.1% formic acid acetonitrile solution |
| Flow rate | 0.35 mL/min |
| Column temperature | 40 ℃ |
| Injection volume | 2 μL |
| Elution gradient | 0.0 min A/B(90:10 V/V)<br>12.0 min A/B(10:90 V/V)<br>14.1 min A/B(90:10 V/V)<br>16.0 min A/B(90:10 V/V) |
| Mass spectrometric parameters | |
| Ion source | Electrospray ionization (ESI) |
| Sheath gas | 40 arb |
| Auxiliary gas | 10 arb |
| Ion spray voltage | − 2800 V |
| Temperature | 350 ℃ |
| Ion transfer tube temperature | 320 ℃ |
| Scan mode | Fullms-ms$^2$ Mode |
| Ion mode | Negative Ion Mode |
| Data processing software | TraceFinder |

**Table 2** Modern standard sample information

| **Sample type** | **Brand information** |
|---|---|
| Wine | Great Wall Dry Red Wine, CofCO Wine Co., LTD., 750 mL |
| Shaohsing Wine | Guyue Longshan, Zhejiang Guyue Longshan Shaoxing Liquor Co., Ltd, 500 mL |
| Rice wine | Sheng Long Lao Rice Wine, Hubei Sheng Long Qing Rice Wine Co., LTD., 888 mL |
| Beer | Tsingtao Draft Beer, Tsingtao Beer Co., LTD., 250 mL |
| Liquor | Xifeng Jiu, Shaanxi Xifeng Wine Co., LTD., 500 mL |

be identified were classified as type C. We only observed one particle belonging to type A, which has a double lens shape with an umbilical point in the middle (Fig. 3a, b), and the maximum particle size is 19.4 μm. These starch granules are suspected to be from wheat plants. One particle belong to Type B was found (Fig. 3c-d); this type of starch granule is polyhedral with obvious cracks along the long axis, which has rounded, two-dimensional triangles. It is likely to be a starch granule from millet. Three particles belonging to Type C were found (Fig. 3e–j); the inner walls are collapsed, leaving only the external outline and the internal pit. The edges show features of enzymolysis and ablation and have no identifiable characteristics due to severe morphological damage [26–28].

One pollen particle was also found in this experiment, which is nearly round, with obvious pores on the surface, and its particle size is greater than 20 μm (Fig. 3k). These are the characteristics of gramineous pollen. In addition, we also observed 38 microremains of suspected *Saccharomyces cerevisiae* (Fig. 3l, m), which are highly similar to modern *Saccharomyces cerevisiae* in morphology and size (Fig. 3n).

Another independent experiment was carried out in the laboratory of XMU to verify the reliability of the results, and analogous results were observed (Fig. 4). A total of 3 wheat starch granules were found, which are round and have no extinction cross under polarized light (Fig. 4a, b, d). The size of these granules ranges from 43.52 μm to 62.61 μm, which is larger than that of standard starch granules from modern wheat and barley [23]. Two of the three granules showed clearer lamellae on their surfaces than the raw starch granules. These granules display features of thermal swelling and gelatinization according to comprehensive analysis and previous works on ancient starch granules [23, 24]. In this work, 8 pollen grains were found with a size of more than 50 μm,

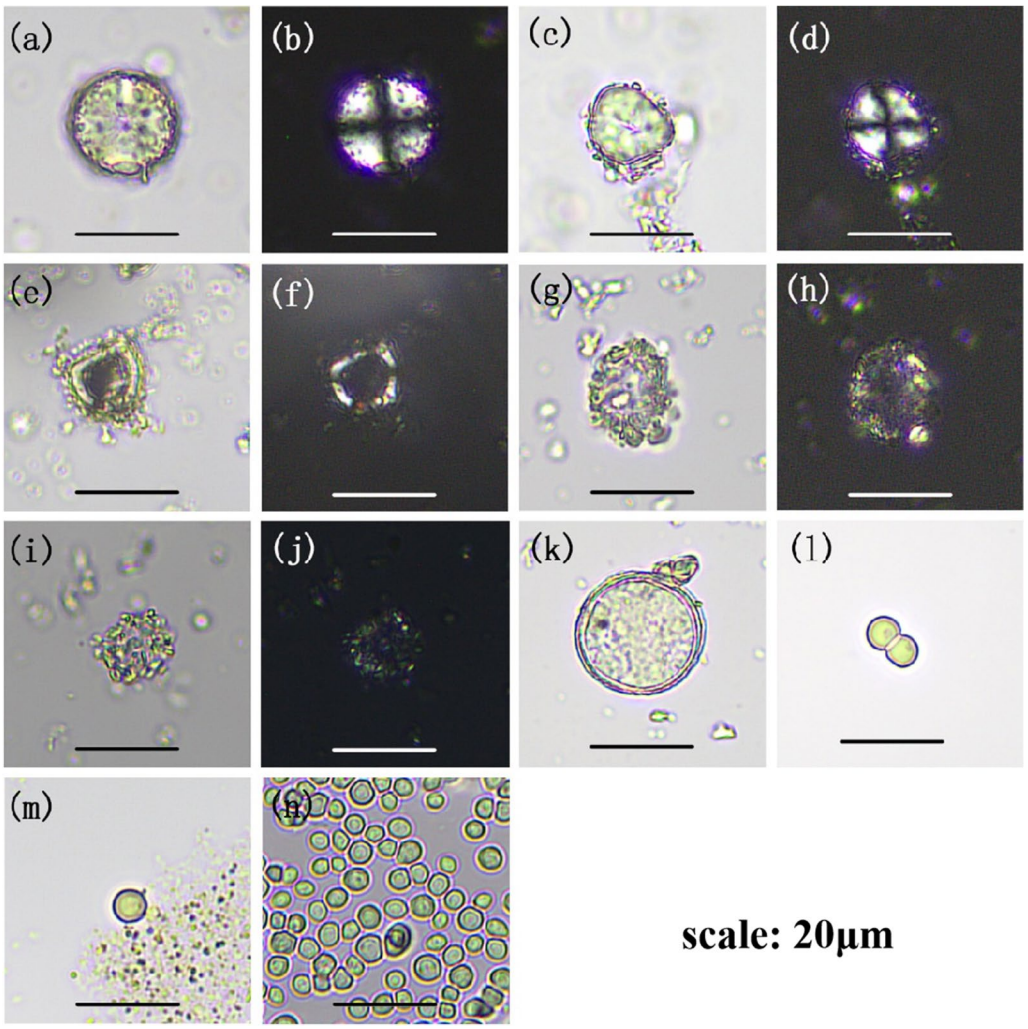


**Fig. 3** Microremains extracted from the M1:44 mud residue and a comparison diagram (laboratory at NWU), **a**, **b**: starch granules of Triticeae; **c**, **d**: millet starch granules; **e**, **j**: damaged starch granules; **k**: Gramineous pollen; **l**, **m**: suspected *Saccharomyces cerevisiae*; **n**: modern *Saccharomyces cerevisiae*

and some were more than 100 μm in size (Fig. 4a, c, e, f). Based on the characteristics of the connecting portion between the saccus and the body and the mesh size on the surface of the saccus, they may have come from different genera of Pinaceae [25].

In addition, 202 yeast cells were observed in a region measuring approximately 760*320 μm on the SYB-8 slide(Fig. 4g). These yeast cells are green, in agreement with the result from the NWU laboratory, which may be attributed to the diffusion of $Cu^{2+}$ from the bronze vessel. The length of these cells ranges from 7.5 to 11.63 μm, with an average length of 9.35 μm. The width ranges from 5.27 to 7.74 μm, with an average width of 6.3 μm. In addition, clear nuclei can be observed after magnification = (Fig. 4h). The morphology of these yeast cells is very similar to that of *Saccharomyces cerevisiae*, but the size is slightly larger, which may be ascribed to interspecific differences [7, 26]. These cells were found on the same slide and showed centralized distribution even after strong oscillation and shaking during the sample preparation process. It can be inferred that they were originally clustered.

Table 3 shows the results from the analysis of plant microfossils in the two laboratories.

#### Biomarker analysis by HPLC–MS

The FTIR analysis showed preliminary distinctions, and the three suspected liquor residue samples were subsequently analyzed by HPLC–MS. The same kind of liquor residue might be present in the three bronze vessels,

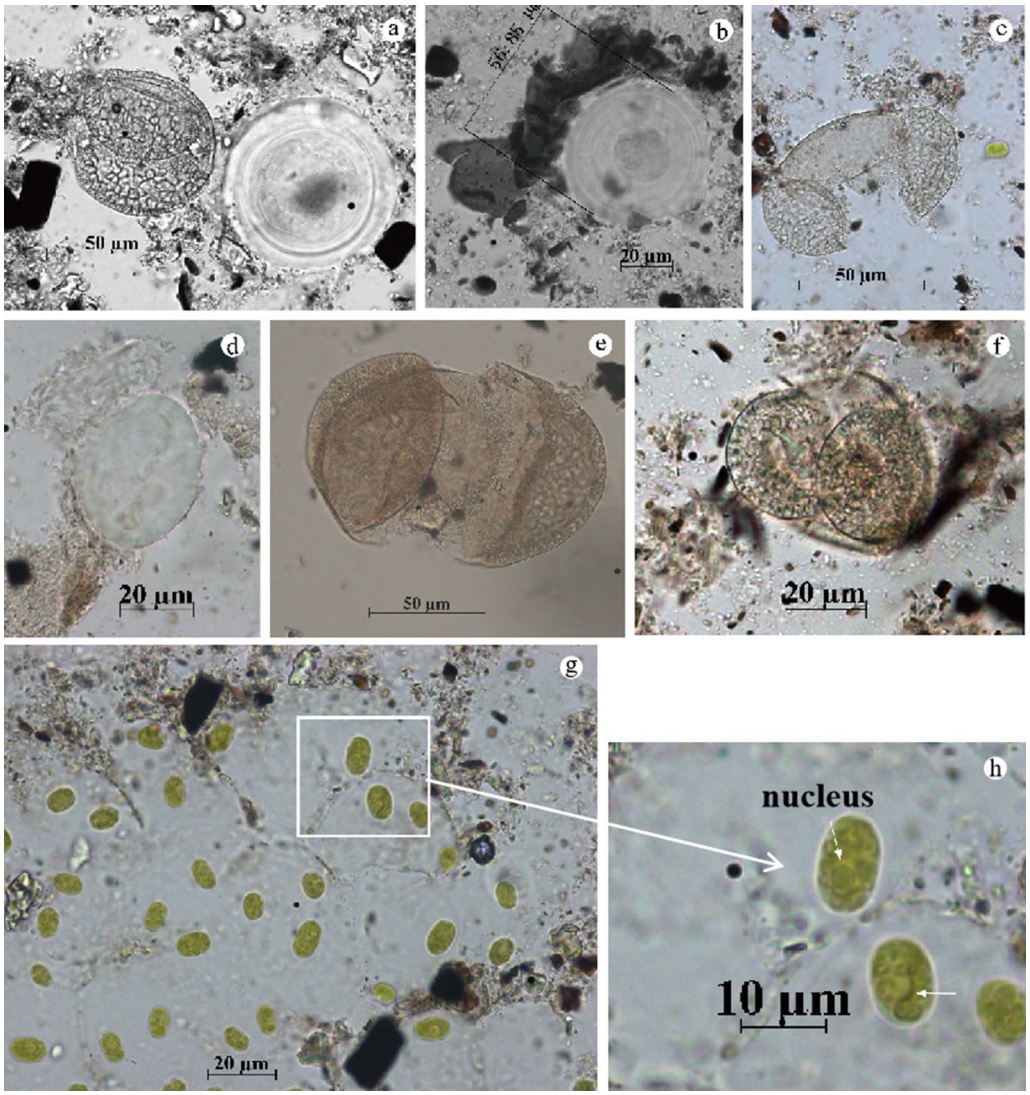


**Fig. 4** Microremains extracted from the M1:44 mud residue and comparison diagram (laboratory in the XMU), **a**, **b**, **d**: wheat starch granules; **a**, **c**, **e**, **f**: pollen grains from different genera of Pinaceae; **g**, **h**: yeast cells

**Table 3** Plant microfossil analysis results from the two laboratories

| | Starch Grain | | | Pollen Grain | *Saccharomyces cerevisiae* |
|---|---|---|---|---|---|
| | Triticeae | Millet | Unidentified | Gramineous pollen | |
| NWU | 1 | 1 | 3 | 1 | 38 |
| XMU | 3 | | 0 | 8 | 202 |

as indicated by the roughly similar peak positions and shapes of the total ion diagram (Fig. 5).

A quantitative analysis of seven target organic acids with correlations with ancient wine residues was carried out. External standards were used for quantification. Seven standard solutions of organic acids with a mass concentration range of 1–1000 ng/mL (1, 10, 50, 100, 250, 500, 800, 1000 ng/mL) were injected into the HPLC–MS instrument from low concentration to high concentration. After the peak areas of the different organic acid standard solutions were obtained, a standard curve was constructed with mass concentration as the abscissa and peak area as the ordinate, and the regression equation and linear fit of the data were calculated. The results showed excellent linearity with a 0.9915–0.9995 linear correlation coefficient ($R^2$) in Table 4.

Seven organic acids were detected in the three archaeological samples, and the data were compared

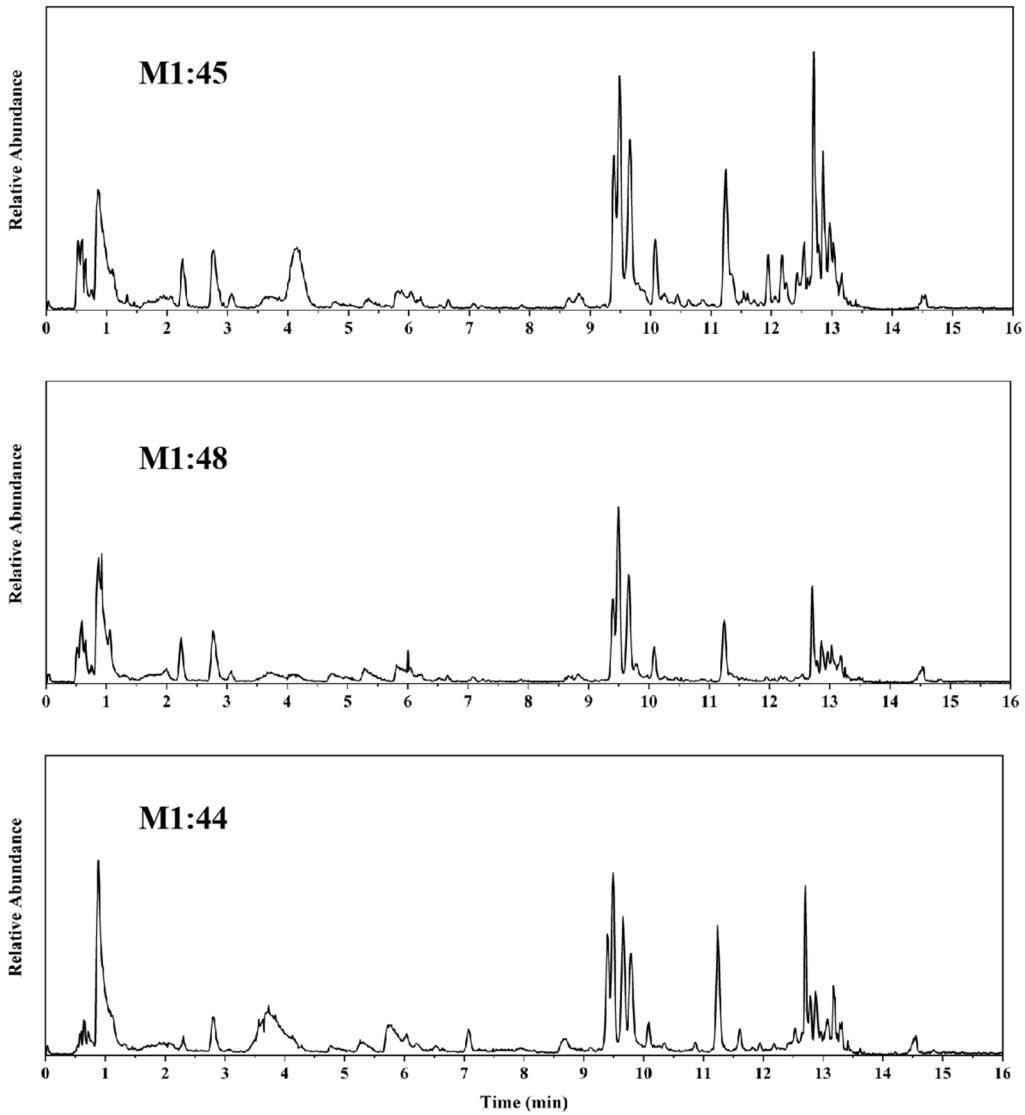


**Fig. 5** Liquid chromatograms of three ancient samples (TICs)

**Table 4** Quantitative results of organic acid standard samples

| Compound | Retention Time (min) | Linear regression equation | linear correlation coefficient ($R^2$) | Detection Limit(ng/mL) | Quantitation Limit(ng/mL) |
|---|---|---|---|---|---|
| Lactic acid | 3.690 | Y = 1.395e5X | 0.9967 | 1 | 10 |
| D-(-)-Tartaric acid | 7.480 | Y = 4.417e5X | 0.9972 | 0.5 | 1 |
| Malic acid | 8.140 | Y = 1.489e5X | 0.9974 | 0.5 | 1 |
| Succinic acid | 8.610 | Y = 2.08e4X | 0.9956 | 0.5 | 1 |
| Fumaric acid | 9.410 | Y = 3.514e5X | 0.9995 | 0.5 | 1 |
| Oxalic acid | 9.740 | Y = 6.329e3X | 0.9980 | 0.5 | 1 |
| Citric acid | 10.670 | Y = 1.742e3X | 0.9915 | 0.5 | 1 |

with the established standard curves to obtain quantitative data. The results show that a total of six organic acids were detected in the three ancient residues, and fumaric acid was not detected in the liquid samples. The quantitative data of the six organics are shown in Table 5.

### FT-IR Spectra and Fisher Discriminant model analysis

The FTIR data of the ancient samples were compared with that of modern samples to preliminarily identify the three ancient samples. The IR data of the samples are shown in Fig. 6. On the whole, the peaks of different types of samples were quite different and complex. Clustering analysis of archaeological samples is highly subjective and prone to deviations when it is based solely on IR peak data. As a result, it is challenging to cluster ancient samples by directly comparing their IR spectra with those of modern samples. Therefore, it is essential to further process the data in combination with statistical analysis methods.

To perform the cluster analysis efficiently, the Fisher discriminant function was established by selecting the model establishment index from the infrared characteristic peak wavenumbers of all samples to maintain similar information and the dispersion of heterogeneous information. Sixty-two wavenumbers were selected from the data for all samples. The details are shown in Table 6. We found that an absorption peak at 1645.05 $cm^{-1}$ existed in all samples, and

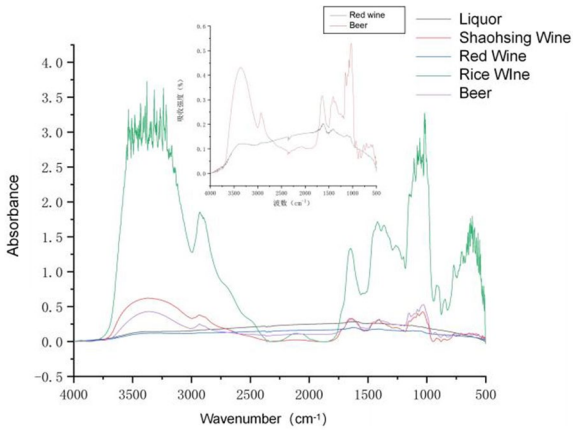


**Fig. 6** Infrared spectra of five standard reference samples

**Table 5** Quantitative results for target organic acids in the ancient samples

| Sample | Retention time (min) | Peak area response | Detected mass (m/Z) | Target organic acid | Quantitative results (ng/mL) |
|---|---|---|---|---|---|
| M1:45 liquid residue | 3.713 | 51821188 | 224.07 | Lactic acid | 371.507 |
| | 7.496 | 165731 | 419.10 | D-(-)-Tartaric acid | 0.375 |
| | 8.134 | 7106568 | 403.10 | Malic acid | 47.929 |
| | 8.605 | 5518540 | 387.11 | Succinic acid | 265.313 |
| | N/F | N/F | N/F | Fumaric acid | N/F |
| | 9.737 | 11557001 | 359.08 | Oxalic acid | 1825.975 |
| | 10.663 | 372423 | 596.15 | Citric acid | 213.777 |
| M1:48 liquid residue | 3.713 | 51821188 | 224.07 | Lactic acid | 371.507 |
| | 7.496 | 165731 | 419.10 | D-(-)-Tartaric acid | 0.375 |
| | 8.134 | 7106568 | 403.10 | Malic acid | 47.929 |
| | 8.605 | 5518540 | 387.11 | Succinic acid | 265.313 |
| | N/F | N/F | N/F | Fumaric acid | N/F |
| | 9.737 | 11557001 | 359.08 | Oxalic acid | 1825.975 |
| | 10.663 | 372423 | 596.15 | Citric acid | 213.777 |
| The extract of M1:44 mud residue | 3.703 | 99687315 | 224.07 | Lactic acid | 714.66 |
| | 7.497 | 138741 | 419.10 | D-(-)-Tartaric acid | 0.314 |
| | 8.134 | 2450600 | 403.10 | Malic acid | 16.459 |
| | 8.605 | 42719778 | 387.11 | Succinic acid | 2053.824 |
| | 9.404 | 544143 | 385.09 | Fumaric acid | 1.548 |
| | 9.740 | 6108079 | 359.08 | Oxalic acid | 965.06 |
| | 10.664 | 317987 | 596.15 | Citric acid | 182.529 |

N/F refers to not detected

the relative absorption intensity was obtained by calculating the ratio of the absorption intensity at other locations relative to that at 1645.05 $cm^{-1}$. Using a relative absorption intensity to carry out subsequent analyses can reduce errors caused by substance concentration and operating mistakes. Fifteen data samples were selected from each sample to establish the Fisher discriminant function. Fifteen wavenumbers were selected randomly from each sample to establish the Fisher discriminant function, and the results are shown in Table 7. We took the first two discriminant functions of the two major categories and made scatter plots. The verification conditions are shown in Table 8, which indicates that the samples were well separated. As shown in Fig. 7a–c, the scatter plots achieved good clustering, and the centroid of each group with similar composition is close. When the ancient residue sample data were entered into the established Fisher discriminant model, the results showed good agreement with data for beer.

## Discussion

This study involved a comprehensive analysis of three ancient organic residue samples from the Beibai'e cemetery, including two clarified liquid residues and a mud sample from a bronze vessels dated to the Spring–Autumn period. To further explore the brewing ingredients, the microfossils in the mud sample were analyzed. The same samples were analyzed by two independent laboratories (at NWU and XMU) to ensure the accuracy of the results.

Starch granules of wheat and millet were observed, and it is worth noting the morphological characteristics, such as gelatinization, size enlargement, central collapse, and missing parts, related to the brewing process [27, 28] (Figs. 3,4). In addition, we found relatively abundant amounts of *Saccharomyces cerevisiae*, which presents

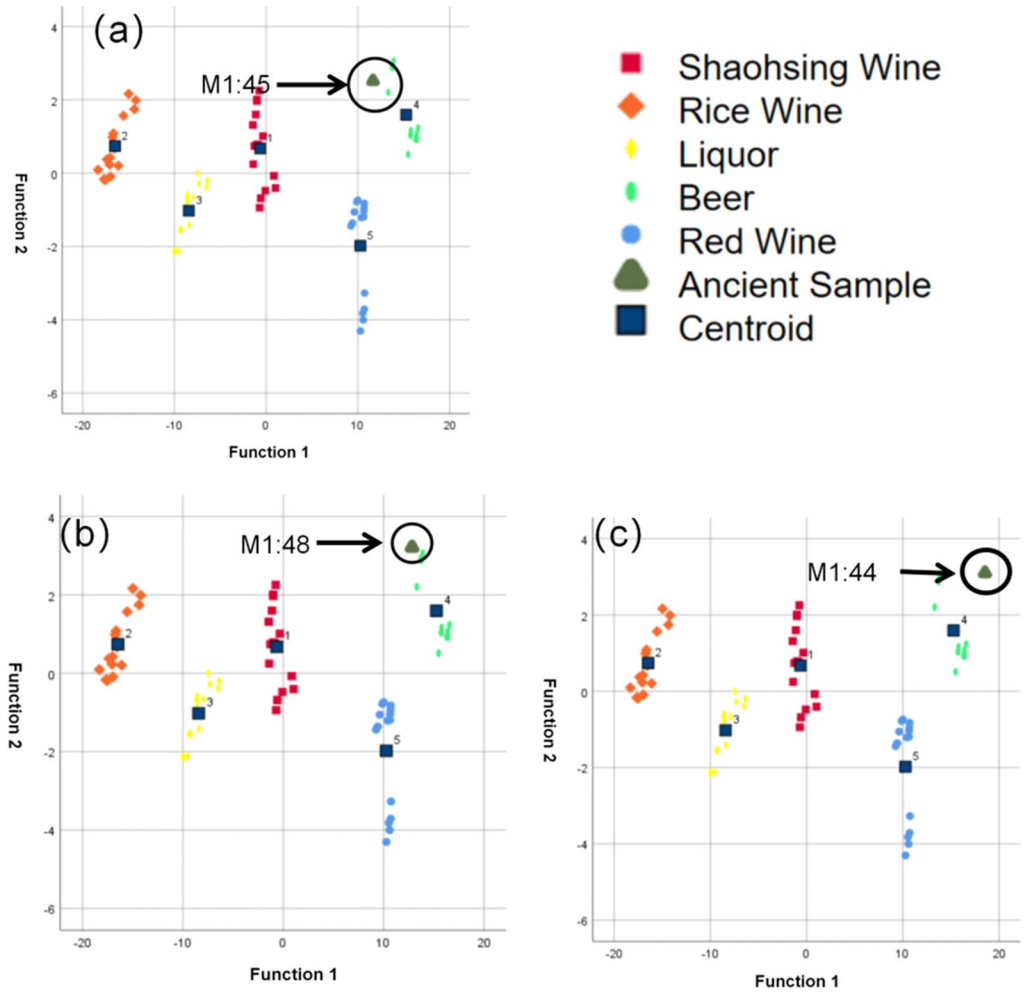


**Fig. 7** Scatter diagram of ancient residue sample classification

strong evidence for the brewing process [27]. It is particularly interesting that *Saccharomyces cerevisiae* is concentrated in the distribution area, indicating that it was originally clustered.

Seven nonvolatile organic acids commonly used as ancient alcohol biomarkers were selected for targeted quantitative analysis to subdivide the sample types. Six of the targeted organic acids were detected by HPLC–MS. The ingredients in the three samples were roughly similar (Fig. 5, Table 5), which further indicates that the three bronze vessels contained the same inclusions. The relative content of oxalic acid was the highest, which would be produced in the process of grain soaking, saccharification, and brewing [27], and it is more likely to appear in beer. However, the leaves of some other plants may also introduce oxalic acid during the brewing process, so based only on oxalic acid content, it cannot be concluded that the liquid was beer [27]. The content of lactic acid is second only to that of oxalic acid, which is the main biomarker of beer. The Mucor and Rhizopus in Distiller's yeast mainly produce lactic acid [27]. Compared with oxalic acid, lactic acid has a stronger correlation with beer. Succinic acid is produced by yeast during its metabolic activities in the fermentation process, so the presence of a high content of succinic acid further indicates that the residue in the ancient bronze vessels is a fermentation product [31]. Tartaric acid accounts for a large proportion of red wine and other fruit wines [27–29]. Nevertheless, the content of tartaric acid in the three ancient samples is extremely low, indicating that the samples are not likely to be fruit wine. The amounts of citric acid and malic acid in grain liquor are moderate and irregular, and their correlation with alcohol type is not good, which further proves that the samples are alcohol. In summary, although the forms of the three samples in the three bronze vessels are different, they are likely to be the same ancient alcohol residue and are likely to be beer.

In previous work on the same residues analyzed in this study, syringic acid was considered strong evidence of fruit wine [10]. However, syringic acid has a wide range of natural sources, including fruits, vegetables, honey, herbs, and grains, such as Panicum miliaceum [27]. Therefore, based only on syringic acid content, the residues cannot be concluded to be fruit wine. In addition, in the early Spring–Autumn period, fruit production was still relatively limited in the central plains region of China. It was not until the Warring States period (475–221 B.C.) that fruit production and processing technology improved significantly [27]. In contrast, the processing and production of grain crops such as millet and wheat were highly prevalent in the Spring–Autumn period in the central plains [27, 28]. Despite the grain brewing traditions mentioned before in ancient China, it is far more likely that beer rather than fruit wine was brewed based on the natural resources available in the region [27–29]. However, the possibility that fruit was used to alter the fragrance of the liquor cannot be excluded, although there is no evidence to support it.

Finally, through infrared spectrum analysis combined with Fisher's discriminant analysis of three ancient samples and five standard reference liquid samples, the ancient samples were identified as belonging to the beer category.

## Conclusion

Multiple analytical techniques were applied to analyze liquid and mud residues excavated from the high-status noble tomb group at the Beibai'e site, Shanxi, China. Wheat and millet starch particles with damage characteristics related to the brewing process were observed in plant microfossil analysis, and abundant *Saccharomyces cerevisiae* were clustered together, which may be attributed to the use of "beer yeast," which is associated with beer brewing techniques. To further explore the type of residues, seven organic acids were selected as biomarkers, which are commonly used in ancient alcohol studies, and targeted quantitative analysis was performed by HPLC–MS. Six organic acids were detected, and the quantitative results illustrate that the samples are more likely to be grain liquor rather than fruit wine based on the relatively high lactic acid and oxalic acid contents and extremely low content of tartaric acid. In addition, the IR results combined with Fisher discriminant analysis revealed that the samples are likely to be beer.

N/F refers to not detected.

## Appendix

See Tables 6, 7 and 8.

**Table 6** The wave number of the IR spectrum characteristic peaks ($cm^{-1}$)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1873.874 | 1847.075 | 1816.153 | 1737.817 | 1721.326 | 1686.281 | 1678.281 | 1651.236 | 1645.051 |
| 1636.805 | 1620.314 | 1583.207 | 1570.839 | 1550.224 | | | | |
| 1533.732 | 1511.056 | 1492.503 | 1473.96 | 1461.581 | 1453.335 | 1438.905 | | |
| 1424.474 | 1410.044 | 1395.614 | 1377.061 | 1344.077 | 1313.155 | 1261.618 | | |
| 1203.897 | 1166.791 | 1148.238 | 1119.377 | 1107.008 | 1092.578 | 1076.085 | | |
| 1045.164 | 1032.795 | 1020.427 | 995.689 | 929.722 | 917.3532 | 892.6156 | | |
| 869.9394 | 851.3862 | 783.3578 | 775.1119 | 764.8046 | 723.5752 | 711.2064 | | |
| 700.8991 | 682.3459 | 672.0385 | 663.7927 | 639.055 | 622.5633 | 612.256 | 601.9486 | 581.3339 |
| 566.9037 | 554.5349 | 527.7358 | 508.1000 | | | | | |

**Table 7** Fisher discriminant function relation

$$F(x)_1 = 74.338X_1 - 80.682X_2 + 0.249X_3 + \cdots - 16.641X_{19} + 32.248$$
$$F(x)_2 = 39.229X_1 - 26.338X_2 + 0.038X_3 + \cdots - 18.163X_{19} - 3.628$$
$$F(x)_3 = -4.259X_1 + 10.329X_2 + 0.009X_3 + \cdots - 24.031X_{19} + 13.008$$
$$F(x)_4 = 2.88X_1 + 8.117X_2 + 0.113X_3 + \cdots + 2.718X_{19} + 5.997$$

**Table 8** Wilk's Lambda Verification

| Wilk's Lambda | value |
|---|---|
| F1 | 0 |
| F2 | 0 |

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**Author contributions**
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**Availability of data and materials**
All data generated or analysed during this study are included in this published article [and its supplementary information files].

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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