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The scientific analysis of the bronze mous excavated from Wushan, Chongqing, China: new perspectives from alloy composition analysis and proteomic method

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Abstract

The bronze mou was an important artifact commonly used in the Ba-Shu region (now in Chongqing and Sichuan Province) during the Spring and Autumn Period and the Han Dynasty. Although extensive archaeological study of the bronze mou has been conducted, scientific analysis of the bronze mou has been limited, and no organic residue analysis has been performed on the bronze mou. In this study, four bronze mous dated to the Han dynasty (206 BC–220 AD) with organic residues unearthed in three archaeological sites in Wushan, Chongqing, were selected for analysis by portable X-ray fluorescent spectrometer (P-XRF) and liquid chromatograph-tandem mass spectrometry (LC/MS/MS). The results of the alloy composition indicated that all the samples are copper-tin-lead ternary alloys. The proteomic analysis results confirmed that the bronze mou had been used as cooking utensils or containers. At the Tuchengpo Cemetery, soybeans were found in both bronze mou M32:27 and M38:39, and vigna was discovered in M32:27. No useful protein was discovered in the bronze mou from the Shennvmiao Cemetery. Proteins of ginkgo and chicken were found in M16:12 at the Gaotangguan Cemetery. The discovery indicated that residents in Wushan, Chongqing, had access to a diverse range of food sources around 2000 years ago. Furthermore, this study demonstrates that proteomic analysis is highly effective in studying archaeological organic residues.

Keywords Proteomics, Bronze mou, Vessel function, Han Dynasty

Introduction

During the Spring and Autumn Period and the Warring States Period (770 BC–221 BC), significant changes occurred in the bronze wares of the Ba-Shu region, leading to the emergence of new typical artifacts. As one of the new typical artifacts, the bronze mou (鋈) originated in the Chengdu Plain of the Ba-Shu region. During the middle of the Western Han Dynasty, the Ba-Shu bronze mou was replaced by the Qin-style mou. From the late Western Han Dynasty to the Three Kingdoms Period, the number of the bronze mou had decreased. Based on the existing typological studies, the bronze mou was likely to have originated from the bronze fu (釜), with functions for cooking and containing food. The bronze fu and the bronze mou are similar in shape, but the bronze fu generally lacks ears, and the bronze mou has a single or double ear [1]. If the bronze mou was used to store or process food, some organic substances from plants and animals may have remained or been deposited in it. Even though they were buried for a long time, they can usually be preserved today. These organic substances

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that have persisted until now are referred to as organic residues. Organic residue analysis involves extracting organic substances from artifacts containing residues, using quantitative scientific detection methods to conduct quantitative analysis, and identifying the source of organic residues. This process provides valuable information about the utilization of ancient animals and plants and the function of related carriers [2–4]. Invisible traces of organic residues are commonly found in vessels, such as pottery and bronze wares, which are the primary focus of organic residue analysis. Extensive research has been conducted on organic residues found in pottery [5–8]. In contrast, insufficient research has been conducted on organic residues discovered in porcelain and bronze wares [9, 10].

However, organic residues are prone to deterioration during burial, which makes it difficult to determine their precise composition in archaeological remains. Identifying the plant or animal species used by ancient human communities is of great interest, both rigorous methodological and theoretical approaches must be employed to obtain optimal results from organic residues [11]. Lipids are less specific than proteins when it comes to taxonomic or tissue identification [8, 12]. However, lipidomic analyses have been extensively used to identify organic remains from archaeological samples [13–15]. Since food products made from plants contain higher levels of proteins than lipids, proteins analysis enables more accurate detection of plant-based products [8]. The analysis of proteins in archaeological samples offers a complementary analytical method for determining resource utilization and enables more precise classification. Proteomic techniques based on high-resolution mass spectrometry have proven to be sensitive and accurate, requiring only a very minimal amount of material to identify the origin of organic residues [16–18]. Additionally, proteomic approaches based on high-resolution mass spectrometry have been developed and employed in recent years for the identification of archaeological organic residues in archaeological samples [19–21].

Currently, academic research on the bronze mou primarily focuses on its origin, utility, and typological analysis [1, 22]. However, there is a lack of scientific and technological research on the bronze mou. The current scientific and technological research mainly focuses on analyzing corrosion products before restoring the bronze mou [23], but there is no analysis of the remains found in the bronze mou. Additionally, the majority of current scientific research focuses on the bronze mou of the Warring States Period [24], while the bronze mou of the Han Dynasty has received little attention.

In this paper, we propose a multi-method approach for the scientific study of the four bronze mous of the Han Dynasty. We used the typological analysis to examine the dates and cultural affiliations of the four bronze mous, and employed P-XRF to analyze the alloy composition of the bronze mous. In order to determine the exact origins and investigate information about the use of animals and plants, a mass spectrometry-based proteomic approach was employed to analyze the organic residues taken from these bronze mous. The bronze mous excavated from Wushan, Chongqing, provide valuable insights into the lifestyle and social activities of ancient residents during the Han Dynasty.

Sample background

Sites and bronze mous

Four bronze mous with residues unearthed from three archaeological sites in Wushan, Chongqing, were chosen for analysis. The information of these bronze mous is shown in Table 1. The location map of these relevant archaeological sites is shown in Fig. 1. Tuchengpo Cemetery, Gaotangguan Cemetery, and Shennvmiao Cemetery are not far from each other, and all influenced by the Ba-Shu culture and the Qin-Han culture, which provide a relatively detailed background for discussing the vessel function and ritual practice.

Two different bronze mous of the Han Dynasty excavated from Tuchengpo Cemetery were selected. Their archaeological numbers are 2004WTIIM32:27 (M32:27) and 2006WTVIM38:39 (M38:39) respectively. Tuchengpo Cemetery is located in Wushan, Chongqing, near the Daning River, a tributary of the Yangtze River, to the east, and approximately 1000 m away from the Yangtze River to the south. During 2004–2006, the majority of the Tuchengpo Cemetery was excavated by the Wuhan Municipal Institute of Cultural Relics and Archaeology and the Wushan Relics Administrative Office. During the excavation, many funerary bronze mous of the Han Dynasty were uncovered [25]. Since the excavation records of M32 and M38 at Tuchengpo Cemetery have not yet been published, the exact dates of M32 and M38 during the Han Dynasty remain unknown.

There was one bronze mou of the Han Dynasty collected from Gaotangguan Cemetery, with the

Table 1 Sample information

Sample number	Archaeological number	Archaeological site	Date
1	2004WTIIM32:27	Tuchengpo cemetery	Han Dynasty
2	2006WTVIM38:39	Tuchengpo cemetery	Han Dynasty
3	2001WGM16:12	gaotangguan cemetery	Han Dynasty
4	2004WSM23:3	shennvmiao cemetery	Han Dynasty

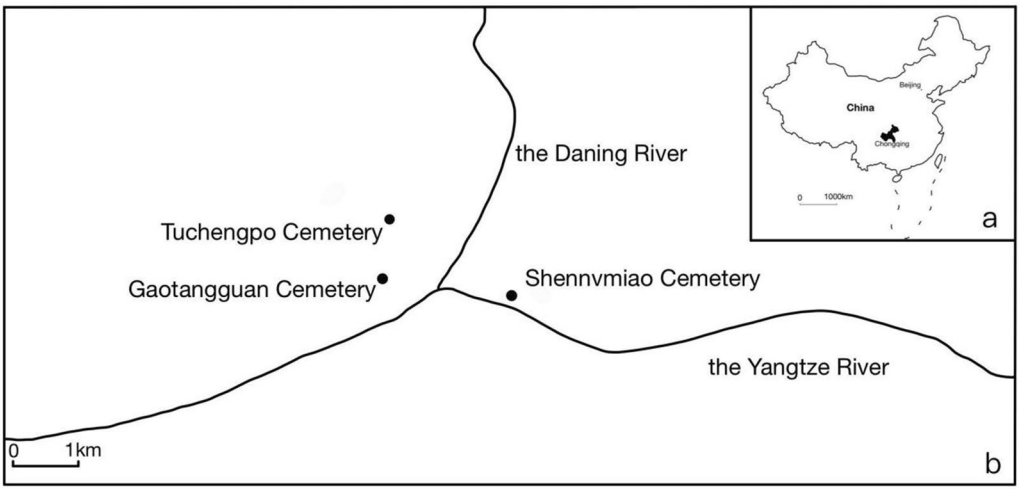

Fig. 1 The location map of archaeological sites mentioned. **a** Location of Chongqing in China; **b** Location of the study sites in Chongqing

archaeological number 2001WGM16:12 (M16:12). Gaotangguan Cemetery is located in Wushan, Chongqing, near the northern bank of the Yangtze River. Fifteen tombs dating back to the Han Dynasty were excavated by the Hunan Provincial Institute of Cultural Relics and Archaeology in 2001. Five bronze mous of the Han Dynasty were unearthed from five tombs at Gaotangguan Cemetery [26].

The last selected bronze mou of the Han Dynasty was discovered in Shennvmiao Cemetery, and its archaeological number is 2004WSM23:3 (M23:3). Based on the excavation records (not yet published), Shennvmiao Cemetery is located approximately 2 km to the east of Tuchengpo Cemetery in Wushan, Chongqing. In 2004, the Yichang Museum conducted an archaeological excavation of Shennvmiao Cemetery, uncovering 27 tombs dating back to the Han Dynasty. Based on the type of art excavated from M23, it can be dated to the late Eastern Han Dynasty.

Residue samples description

The black granular residue was found at the bottom of the interior of all the four bronze wares. Four residue samples were taken from the four bronze mous, with one sample collected from each vessel. In total, there are two samples from Tuchengpo Cemetery, one from Gaotangguan Cemetery, and one organic residue sample from Shennvmiao Cemetery.

Typological analysis

All the four bronze mous have folded shoulders, a flat abdomen, and two symmetrical ring-shaped ears, which completely accord with the characteristics of the bonze mous in the Han Dynasty (206 BC–220 AD). The images of the four bronze mous are shown in Fig. 2. According to the published archaeological reports, M16 excavated at Gaotangguan Cemetery is dated to the Xinmang period (9 AD–24 AD), so bronze mou M16:12 should also be dated to the Xinmang period. M23 of Shennvmiao Cemetery is dated to the late period of the Eastern Han Dynasty (190 AD–220 AD), so the bronze mou M23:3 also dates back to the late period of the Eastern Han Dynasty. The other two bronze mous from Tuchengpo Cemetery both have a large angle between the shoulder and abdomen, and the abdomen diameter is larger than the height of the artifact. However, the abdomen of M32:27 is broader and flatter than that of M38:39. According to the existing typological analysis of the bronze mous of the Han Dynasty [22], M32:27 may be dated to the early Eastern Han Dynasty (25 AD–105 AD), while M38:39 could be dated to around the late Western Han Dynasty (48 BC–8 AD). Since the dates of M32 and M38 have not yet been published, the analysis of the dates of the two bronze mous from Tuchengpo Cemetery in this paper is provided for reference.

Scientific analysis

P-XRF analysis

The portable X-ray fluorescence spectrometer (P-XRF) was used to analyze the alloy composition of the four bronze mous. The test instrument was a ThermoFisher NITON XL3t955-HE energy-dispersive X-ray fluorescence spectrometer, using the quantitative analysis Standard FP method. The portable X-ray fluorescence spectrometer was set to the general metals mode, and the surface of bronze with minimal corrosion was chosen for composition analysis. For the test, the spot diameter

Fig. 2 The images of the bronze mous. **a** M16:12; **b** M23:3; **c** M32:27; **d** M38:39

was 8 mm, the test time was more than 60 s, and the test angle was 0°.

LC/MS/MS analysis

Protein extraction

The protein extraction procedure was modified from published references and successfully conducted on these four residue samples [19, 20]. A small amount of samples were obtained with a scalpel and then ground with a mortar. 200 mg of the residue sample was mixed with 1 ml of extraction solution (tris–HCl, pH 8.0, 10 mM dithiothreitol, 10% sodium dodecylsulfate, and 0.0025% bromphenol blue). Following three rounds of 15-min ultrasonic baths, the mixture was incubated for one hour at 56 ℃. It was then sonicated for 15 min and centrifuged at 12,000*g* for 15 min.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The extracted proteins were separated and purified by electrophoresis. 45 µl of the supernatant was heated at 95 ℃ for 5 min while being mixed with 5 µl of glycerol. After cooling to room temperature, the mixture was loaded onto gels (5% stacking gel, 12% separating gel), with 25 µl of the mixture being placed into per well. When the sample reached the separating gel, the electrophoresis device's connection to the power supply was changed from 80 to 120 V. The sample was run on the separating gel for about 3 cm before the power was cut off and the gel was taken off. Following the protocol for microwave-assisted Coomassie Blue staining, the gel immersed in 0.25% Coomassie Blue w/v, 50% ethanol, and 10% acetic acid was placed in a microwave oven and heated to medium–low temperature for 45 s before being slowly shaken for 10 min. The staining agent was then poured out. The destaining solution (25% ethanol, 8% acetic acid) was added to the gel after several rounds of washing with distilled water, and it was slowly shaken overnight to reveal the blue-stained protein region. To prevent the horizontal carryover of proteins from different samples, each sample was run on a separate gel [27].

In-gel digestion

As the proteins smeared along the gel, which is typical of ancient remains, the entire blue-stained protein region on the gel slab was collected and divided into 1 mm^3 sized pieces. Proteins were reduced using 5 mM dithiothreitol (DTT) and alkylated using 11 mM iodoacetamide (IAM). In-gel digestion was conducted with sequencing grade-modified trypsin in 50 mM ammonium bicarbonate and incubated overnight at 37 °C. Make sure that the liquid completely covers the gel particles. The peptides were extracted twice with 1% trifluoroacetic acid (TFA) in 50% acetonitrile aqueous solution [28]. The peptide extracts were then centrifuged in a SpeedVac to reduce the volume.

LC/MS/MS

Peptides were separated using a Thermo-Dionex Ultimate 3000 HPLC system, which was directly connected to a Thermo Orbitrap Fusion Lumos mass spectrometer, for a 125-min gradient elution at a flow rate of 0.300 μl/min for LC/MS/MS analysis. The analytical column was an Acclain PepMap RSLC C18 capillary column (75 μm × 150 mm Thermo Fisher Scientific). Mobile phase B contained 100% acetonitrile and 0.1% formic acid, while mobile phase A contained 0.1% formic acid. A single full-scan mass spectrum in the Orbitrap (300–1500 m/z, 120,000 resolution) was performed in the data-dependent acquisition mode using Xcalibur 4.1 software. This was followed by three seconds of data-dependent MS/MS scans in the Orbitrap (30,000 resolution) at 30% normalized collision energy (HCD).

During the entire procedure, several specific measures have been implemented to avoid laboratory contamination according to the guideline proposed by Hendy, et al. (2018) [29]. Initially, sample preparations were conducted in a dedicated "clean room" designed for ancient protein analysis. Protective clothing, pure chemicals, clean laboratory glassware, and equipment were required to minimize the risk of contamination. Furthermore, blank washings were included during the LC–MS/MS analysis to prevent carryover between different samples. Additionally, consumables were not reused to avoid cross-contamination.

Database search

Using Mascot software version 2.5 (Matrix Science, UK), the MS/MS spectra were searched against the NCBInr database (downloaded in April, 2016, 87,376,087 sequences). Trypsin was selected as the enzyme with a maximal of 2 missed cleavages. Four modifications, including Acetyl (Protein N-term), Deamidation (NQ), Gln- > pyro-Glu (N-term Q), and Oxidation (M), were set as variable modifications, whereas Carbamidomethylation (C) was set as a fixed modification. A precursor mass tolerance of 10 ppm and a product ion mass tolerance of 0.07 Da were used in the searches. The data were then filtered with a 5% FDR (PSM homology). Protein matches with two or more peptides were accepted.

After removing any protein that originates from the common "contaminant" datasets reported in the previous publication [30], the remaining proteins were kept as potential endogenous proteins. To validate the species-specificity of the sequences, each peptide from these proteins was extracted and BLAST analyzed against the latest NCBInr database using the online tool "blastp suite" (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The BLAST taxonomic results of all the assigned peptides were integrated to confirm the accurate taxonomy of each protein. Only reliable proteins with specific origins were reported.

Results

Alloy composition

The P-XRF results of the four bronze mous are shown in Table 2. The elemental analysis results show that the four bronze mous of the Han Dynasty are copper-tin-lead ternary alloys. In addition, previous scholars have analyzed the alloy composition of the bronze mous of the Warring States Period in Chongqing [24], revealing that they were also copper-tin-lead ternary alloys, which may indicate a continuity in the alloy composition over time. "Kaogongji" explores the connection between the alloy composition and the type of bronze ware. It is widely accepted that the copper-tin-lead ternary alloy is primarily used for ritual and practical utensils [31].

The lead-tin bronzes have a wide range of lead and tin content, ranging from 11 to 33% Sn and 8 to 49%

Table 2 Alloy composition (wt%) of the bronze mous

Archaeological site	Archaeological number	Location	Cu	Sn	Pb	Fe
Tuchengpo Cemetery	2004WTIIM32:27	Rim	36.404	26.918	33.217	1.602
		Shoulder	36.065	25.369	35.399	1.207
		Bottom	71.311	10.922	16.461	0.569
	2006WTVIM38:39	Bottom	25.024	30.835	42.419	0.466
		Bottom	34.13	24.452	40.041	0.223
		Bottom	34.031	16.543	48.557	0.465
Shennvmiao Cemetery	2004WSM23:3	Shoulder	79.679	11.65	8.066	0.085
		Rim	74.021	13.048	12.283	0.092
		Bottom	71.687	17.472	10.363	0.029
Gaotangguan Cemetery	2001WGM16:12	Spacer on the shoulder	38.786	32.96	25.873	0.441
		Shoulder	33.188	28.088	37.317	0.266

Pb. The surfaces of the four bronze mous are severely rusted, so the data is for reference only. The Cu content will be lower, while the Sn and Pb content will be higher than the actual alloy composition due to the influence of corrosion. Only the results of the bronze mou M23:3 and the results of the bottom of the bronze mou M32:27 can accurately reflect the alloy composition. The alloy composition of these bronze mou of the Han Dynasty should be approximately 75% Cu, 15% Sn, and 10% Pb. The alloy composition of bronze mous of the Warring States Period has been analyzed, and the result is about 72% Cu, 17% Sn, and 11% Pb [24], which is close to the result in this paper. This may mean that from the Warring States Period to the Han Dynasty, the bronze mou was generally produced with an average ratio of about 70–80% Cu, 10–20% Sn, and 5–10% Pb. From the Warring States Period to the Han Dynasty, there were no significant changes in the alloy composition of the bronze mou. It is noteworthy that the Fe content in the bronze mou M32:27 is more than 1%, which may be higher than the actual iron content due to corrosion.

Proteomic analysis

After the SDS-PAGE, all the four samples showed blue protein bands, indicating that the method of extracting proteins from the samples, separating and purifying the proteins through gel electrophoresis was effective. However, the trailing phenomenon of protein bands was obvious, which may be attributed to the degradation of proteins in archaeological samples. Furthermore, the protein detection limit of mass spectrometry analysis in the electrophoresis bands is lower compared to the Coomassie Blue staining method. Therefore, follow-up mass spectrometry was performed on the four samples.

Only the proteins with two or more detected peptides were included to ensure the reliability of the results. After this filtration, sample M23:3 contains only some common contamination proteins, which may have been introduced during burial processes and laboratory operations, such as keratins and some microbial proteins from the soil. After removing the common contamination proteins, some dietary proteins were found in the remaining three samples (sample M32:27, sample M38:39, and sample M16:12). Detailed information on the identified proteins and their specific peptides is displayed in Table 3.

It showed that the glycinin from *Glycine sp.* and basic 7S globulin from *Vigna sp.* in the bronze mou M32:27, derived from soybean seeds and vigna seeds in Table 3, indicating that soybeans and vigna were stored or processed in the bronze mou M32:27. Glycinin from *Glycine sp.* and seed storage protein from sunflower were detected in M38:39, which indicated that the bronze mou M38:39 was used to store or process soybeans and sunflower seeds. In addition to plant protein (seed globulin from ginkgo biloba), the collagen alpha-2(I) chain precursor of chicken was also found in M16:12. The predominant presence of collagen type I in tendons, ligaments, and bones indicates that chicken meat (or chicken bones) had been stored or processed in the bronze ware M16:12.

Discussion

Proof of the bronze mou as cooking utensils

Some bronze mous found in tombs have soot traces on the bottom and abdomen, or with the remains of animals visible to the naked eye inside, which supports that the bronze mous were used for cooking [25]. Chen also believed that the bronze mou was an artifact that could be used as a ritual vessel, and it was supported by numerous archaeological excavations [1]. Furthermore, some scholars have discovered that some bronze mous were inscribed with markings that indicate their capacity, suggesting that the bronze mou was used for measuring [32]. The primary function of the bronze mou is as a cooking utensil, with additional uses as a military pot and wine warmer [1].

Based on the aforementioned previous studies on the bronze mou, it is evident that the bronze mou was utilized as a cooking vessel. The absence of direct evidence of the original food contents means that most of the information about its function has been inferred from vessel typology, inscriptions carved on the bronze mou, historical records from later eras, and occasionally preserved bones in the bronze mou. Direct evidence of the function of bronze mous through organic residue analysis was provided for the first time in this study. Proteomic analysis of organic residues in the bronze mou collected from three archaeological sites in Wushan directly proves that the bronze mou was an important component of cooking utensils in the daily life of the local ancestors. In addition, soot traces were observed on the abdomen or bottom of all the four bronze mous. It proved that the bronze mou was used as a practical cooking utensil (Additional files 1, 2, 3, 4).

Diverse utilization of plants and animals in Wushan

It's warm and humid in Wushan. The abundant vegetation in the environment undoubtedly supports the development of agriculture. Soybeans were found in both M32:27 and M38:39. According to the literary records and archaeological findings, soybeans were cultivated throughout the country during the Han Dynasty. It was a common crop during the Han Dynasty and was one of the five essential grains [33]. Therefore, the soybeans found in two bronze mous excavated at Tuchengpo Cemetery are likely to have been locally cultivated.

Table 3 The dietary proteins and peptides identified in this study

Sample	Protein	NCBI Accession number	Taxa	Tissue	Number of identified peptides (unique)	Number of peptide spectral matches	Identified peptides	Modifications	Score	Homology threshold	BLAST taxonomic hit
M32:27	Glycinin G1	gi|121,276	*Glycine sp.*	Seed	2(2)	4	VLIVPQNFVVAAR		80	46	*Glycine soja/Glycine max/Lotus japonicus*
							SQSDNFEYVSFK		72	39	*Glycine soja/Glycine max*
	basic 7S globulin	gi|920,711,617	*Vigna sp.*	Seed	2(2)	6	AVAPFELCFHSK		52	39	*Vigna angularis/Vigna radiata* var. radiata
							QLEENLVVFDLAK	Gln- > pyro-Glu (N-term Q)	80	55	*Vigna angularis*/Vigna unguiculata/*Vigna radiata* var radiata/*Phaseolus vulgaris*
							QLEENLVVFDLAK		66	55	*Vigna angularis/Vigna unguiculata/Vigna radiata* var radiata/*Phaseolus vulgaris*
M38:39	11S globulin seed storage protein G3	gi|112,676	*Helianthus annuus*	Seed	2(2)	4	TNDNAMIANLAGR		91	63	*Helianthus annuus/Smallanthus sonchifolius/Lactuca sativa/* Cichorium/*Ambrosia artemisiifolia*
							TNDNAMIANLAGR	Oxidation (M)	92	62	Helianthus annuus/*Smallanthus sonchifolius/* Lactuca sativa/ Cichorium/*Ambrosia artemisiifolia*
							FFLAG-NPQAQAQSQQQQQR		75	59	*Helianthus annuus/ Helianthus petiolaris*
	Glycinin G1	gi|121,276	*Glycine sp.*	Seed	2(2)	2	VLIVPQNFVVAAR		61	48	*Glycine soja/Glycine max/Lotus japonicus*
							SQSDNFEYVSFK		60	38	*Glycine soja/Glycine max*
M16:12	ginnacin	gi|575,943	*Ginkgo biloba*	Seed	2(2)	4	ETLNPNALSLPR		54	39	*Ginkgo biloba*
							YTNTPTMAYVVEGEGR	Oxidation (M)	71	41	*Ginkgo biloba*
	collagen alpha-2(I) chain precursor	gi|206,597,434	*Gallus gallus*	Tendon, ligaments and bones	6(6)	12	GDVGPVGR		59		Conserved(include *Gallus gallus / Penelope pileata*)

Table 3 (continued)

Sample	Protein	NCBI Accession number	Taxa	Tissue	Number of identified peptides (unique)	Number of peptide spectral matches	Identified peptides	Modifications	Score	Homology threshold	BLAST taxonomic hit
							VGPIGPAGNR		53	45	Aves (include *Gallus gallus /Penelope pileata*)
							AGVMGPAGNR	Oxidation (M)	64	48	Conserved(include *Gallus gallus / Penelope pileata*)
							GLPGESGAVGPAG-PIGSR		76	46	Aves(include *Gallus gallus*)
							GEIGPAGNVGPTG-PAGPR		88	43	Aves(include *Gallus gallus/Penelope pileata*)
							GDPGPVGPVGPAGAF-GPR		77	41	*Gallus gallus / Penelope pileata*

There are 16 species, 3 subspecies, and 3 varietas of vigna in China, and they are widely cultivated throughout the country, although they are primarily produced in the southeastern, southern, and southwestern regions [34]. Vigna has been discovered at an archaeological site of the Warring States Period in Hubei. Additionally, the cultivation method of adzuki beans of vigna was mentioned in the ancient documents from the Western Han Dynasty [35]. So, there is a possibility that the vigna in M32:27, excavated at Tuchengpo Cemetery, was locally grown.

It's proven that the ancient residents in Wushan raised chickens as poultry as early as the Spring and Autumn Period by archaeological discoveries [36]. During the Han Dynasty, in addition to agriculture, animal husbandry and fishing also played significant roles in the livelihood mode of ancient residents in the Wushan area. Proteins of gallus were discovered in M16:12, revealing that the main meat source for the ancient residents of the Han Dynasty in the Gaotangguan Cemetery may have been produced by the livestock economy, with chicken being one of them.

It is widely believed that the sunflower was introduced to China during the Ming Dynasty. Some scholars have analyzed that the "kui" (葵) recorded in bamboo slips of the Han Dynasty is likely the present sunflower [37]. That one seed protein of sunflower was found in M38:39 implying that the Chinese ancestors may have utilized sunflower since the Han Dynasty. However, when considering cross-species proteomics [38], it is important to note that species that are underrepresented in databases may be incorrectly assigned a species-level taxonomic classification due to protein sequence similarities across species. This issue is particularly problematic in samples that may contain multiple species, such as cooking-generated organic residues. Thus more evidences, such as the possible routes of introduction of sunflower in China, are needed to confirm its presence during the Han Dynasty.

It is difficult to determine when ginkgo was first cultivated as a fruit tree in China. However, there are records about ginkgo cultivation in the Western Han Dynasty, and some scholars believe that from the Han Dynasty to the Song Dynasty, ginkgo was increasingly cultivated in the Jiangnan area (the region south of the Yangtze River) [39]. The presence of ginkgo seed globulin in the bronze mou (M16:12) excavated from the Gaotangguan Cemetery probably came from locally cultivated ginkgo trees, indicating that ginkgo was likely one of the daily foods at that time.

Although the detected proteins are quite limited in this study, it can provide insight into the utilization of plants and animals by ancient residents at that time to some extent by the presence of soybean protein found in the bronze mou excavated from Tuchengpo Cemetery, and the proteins of ginkgo biloba and chicken found in the bronze mou excavated from Gaotangguan Cemetery. In order to address the relevant issues comprehensively and thoroughly, more detailed research is needed in the future.

Conclusion

In recent years, proteomic methods have been applied in archaeology, opening up new avenues for analyzing residues. The approaches have been effective in identifying archaeological fibers and textiles [40, 41], residues in archaeological artifacts [42, 43], binders in paintings [44, 45], organic additives in building materials [46, 47], and organic binders in papers [48], etc. In this paper, proteomic analysis is employed to analyze the composition of the organic residues in the four bronze mous dated to the Han Dynasty, which were unearthed from three archaeological sites: Tuchengpo Cemetery, Shennvmiao Cemetery, and Gaotangguan Cemetery in Wushan, Chongqing. Before that, typological analysis was used to determine the exact burial dynasty of the four bronze mous of the Han Dynasty. The portable X-ray fluorescence spectrometer (P-XRF) was used to determine the alloy composition of the four bronze mous. The P-XRF results showed that the four bronze mous are all made of copper-tin-lead ternary alloy, with a possible alloy composition of 70–80% Cu, 10–20% Sn, and 5–10% Pb. The proteomic analysis results revealed that food with soybean or vigna was found in the two bronze mous unearthed from Tuchengpo Cemetery and ginkgo biloba and chicken (chicken bone) were found in the bronze mou unearthed from Gaotangguan Cemetery. This work not only reflects the function of the bronze mou as cooking vessels but also reflects the abundant food sources and diverse agricultural and animal husbandry systems in Wushan. Different species of plants and animals can be identified in the bronze mous excavated from tombs, likely indicating the original plant and animal products used in ritual practices. This study provides crucial insights for further exploration of the social life and funeral customs of ancient residents in Wushan, Chongqing.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40494-024-01203-7.

Additional file 1: Table S1. All the protein and peptide details in Sample M32:27.

Additional file 2: Table S2. All the protein and peptide details in Sample M38:39.

Additional file 3: Table S3. All the protein and peptide details in Sample M16:12.

Additional file 4: Table S4. All the protein and peptide details in Sample M23:3.

Author contributions
Liwei Tan: methodology, formal analysis, investigation, data curation, writing—original draft, visualization. Xiaopan Fan: methodology, formal analysis, project administration, writing—review and editing. Huiyun Rao: analysis, writing—review and editing. Hui Zhang: sample information, writing—review and editing. Yimin Yang: resources, writing—review and editing.

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Availability of data and materials
Raw MS/MS data from 4 samples have been uploaded to the ProteomeXchange Consortium via the PRIDE partner repository under Accession code PXD048792.

Declarations

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

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