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ORIGINAL PAPER

The brewing function of the first amphorae in the Neolithic Yangshao culture, North China

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Abstract

In recent years, functional study of Neolithic pottery found in the Yellow River valley has shown that globular jars of the pre-Yangshao period (ca. 7000–5000 cal. BC) and *jiandiping* amphorae of the middle and late Yangshao period (ca. 4000–2900 cal. BC) were alcohol fermentation vessels. However, there is a time gap of 1000 years (ca. 5000–4000 cal. BC) between these two sets of vessels, namely the Early Yangshao period when amphorae first appeared. In order to further our understanding of alcohol production in Neolithic China, we employed scientific methods to examine microfossil remains in the residues adhering to the interior walls of eleven among the earliest amphorae from the Banpo and Jiangzhai sites in Shaanxi province. Multiple lines of evidence—taken from starch granules, phytoliths, molds, yeast cells, and rod-shaped calcite crystals found in the residues—indicate that these amphorae were used for brewing alcoholic beverages. The ingredients mainly include broomcorn millet, together with other cereals (foxtail millet, rice and Triticeae), wild peas and tubers (snake gourd roots and foxnut). Two brewing methods have been detected: use of sprouted grain and use of *qu* starter made of moldy grain with herbs. Siphoning through reed straws may have been one of the drinking methods. The results of this research open a new window not only for understanding the long tradition of alcohol production in prehistoric China, but also for investigating alcohol-related social activities of the Yangshao people.

Keywords Banpo · Jiangzhai · Alcohol fermentation · Starch granules · Phytoliths · Fungi

Introduction

China has a very long history of cereal-based alcohol production, and scientific research in recent years has revealed more details about the development of this ancient technology. Most studies have focused on the middle Yellow River region, and the earliest evidence of alcoholic beverages, made of rice and millet among other ingredients, has been found in residues on globular-shaped pottery jars at Jiahu in Henan (McGovern et al. 2004) and Lingkou and Guantaoyuan in Shaanxi (Liu et al. 2019), dating to ca. 7000–5000 cal. BC. These findings predate a later group of fermentation vessels, *jiandiping* 尖底瓶 (meaning pointed-base jar) amphorae, of the middle and late Yangshao culture (ca. 4000–2900 cal. BC), suggested by the residue analysis on 17 vessels from four sites—Mijiaya, Yangguanzhai and Xinjie in Shaanxi, and Dingcun in Henan (Liu et al. 2020; Liu et al. 2018b; Liu et al. 2017; Wang et al. 2016) (Fig. 1a; SM Table 1 for DMS coordinates of sites mentioned in this paper). Based on the current data, there is a 1000-year gap (ca. 5000–4000 cal. BC) between the two groups of brewing vessels, during which the evidence for alcohol production is lacking. This period is known as the early Yangshao culture, when the amphorae first appeared in the region. We cannot understand the alcohol brewing tradition in Neolithic China without knowing the function of the early Yangshao amphorae.

The Neolithic Yangshao culture that flourished in North China (ca. 5000–2900 cal. BC) (Fig. 1a) represents a society that shows increasing social differentiation through time. Its

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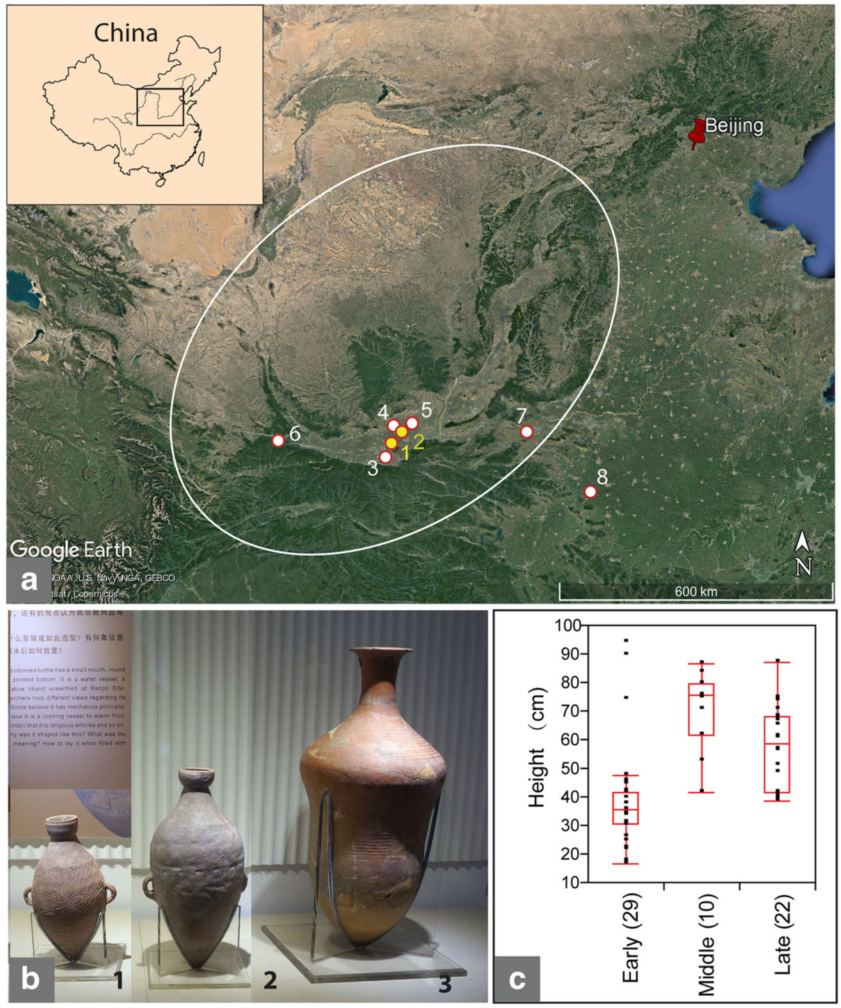

Fig. 1 Location of archeological sites mentioned in this paper and comparison of Yangshao amphorae. **a** White circle: distribution of the Yangshao culture; sites: 1. Banpo; 2. Jiangzhai; 3. Mijiaya & Xinjie; 4. Yangguanzhai; 5. Lingkou; 6. Guantaoyuan; 7. Dingcun; 8. Jiahu. **b** Examples of Yangshao amphorae, showing various forms and sizes: 1 and 2. early Yangshao; 3. late Yangshao (photo taken by L. Liu in Xi'an Banpo Museum). **c** Amphorea' size change from early to late Yangshao periods

early period is characterized by subsistence farming, primarily millet-based. A settlement was typically partitioned into multiple residential groups, each including one large public house surrounded by several small and medium-sized dwellings. This settlement pattern is best represented by the Banpo and Jiangzhai sites in Shaanxi, which are the focus of this study. The early Yangshao is also characterized by an assemblage of distinctive ceramic vessels, among which *jiandiping* amphorae are one of the predominant and iconic types (Liu and Chen 2012: 189–197; Yan 1989). Amphorae have been unearthed from both residential and mortuary contexts in all Yangshao sites. Their typical features include a small mouth and a conical base, with variations in rim form and vessel size. Those in the early Yangshao period often have a "cup-shaped" rim, two loop-formed handles, and a wide range of vessel sizes, mostly smaller by comparison to the amphorae from the middle and late Yangshao, which have mainly "double-lipped" and "horn-shaped" rims, respectively, often without handles, and are generally large in size (Fig. 1b). We analyzed the vessel sizes (based on height shown in site reports) of 62 amphorae from ten Yangshao culture sites (SM Table 2). Among 29 early Yangshao amphorae, 26 examples (90%) fall into the range of 16.8–42 cm, with only three (10%) measuring 74.4–94.5 cm. In contrast, vessel heights from the middle and late Yangshao ($n = 32$) are consistently greater, falling into the range of 39–87.5 cm (Fig. 1c). Apparently, the function of early Yangshao amphorae with different sizes needs to be investigated.

The function of Yangshao amphorae has been the subject of a decades-long debate. The main opinions include water-drawing jar (Gong 2002; Xi'an Banpo Museum et al. 1988), water purifier (Han 2015), ritual vessel for offerings to heaven in the arid surrounding region (Wang 2004), ritual container for alcohol (Sun and Zhao 1988; Zhou and Miao 1986), alcohol fermentation vessel (Bao 2005; Li 1962), and alcohol storage vessel (Wei and Qian 2019). Among these views, water-drawing jar is the most popular one, which has been entered in school textbooks in China (Wang 2004). Most of these interpretations, nevertheless, are hypothetical, based on ethnographic analogies. In particular, it is not practical to draw water with such amphorae, as demonstrated in experimental studies (Sun and Zhao 1988; Zhou and Miao 1986). In this paper, we particularly test the possible function of cereal-based alcohol brewing in association with the early Yangshao amphorae.

Methods of cereal-based alcohol production and their residues

Cereal-based beer brewing involves a biochemical process in two stages. The first stage is saccharification, which converts starch into sugars through the action of enzymes; the second stage is fermentation, which converts sugars into alcohol and carbon dioxide through the action of yeasts. There are two most commonly used methods for brewing alcoholic beverages from starch-rich plants (including cereals and tubers):

1. Use of malts, by first germinating the grains to activate enzymes, then mashing the malts with water in low heat (65–70 C°), and finally adding yeasts for fermentation (Hornsey 2003).
2. Use of *qu* 麴starter made from moldy grains (with or without herbal plants, known as *caoqu* 草麴) which are rich in microorganisms, including molds (to create a variety of enzymes), yeasts and bacteria. *Qu* is added to steamed or boiled grains as the agent to make saccharification and fermentation occur simultaneously. This technique was, arguably, invented in ancient China (Huang 2000; Jin et al. 2017; Ling 1958). These two methods are found in ancient texts, such as *Shujing* (*Book of Documents*, traditionally said to be compiled by Confucius, 551–479 BC), which records "to make *jiu* 酒 and *li* 醴 [alcoholic beverages], use *qu* starter and *nie* 蘖 sprouted grain."

The main difference between these brewing methods is in the saccharification stage, whereas both methods use yeasts to achieve the fermentation. Amphorae have three morphological features that can contribute to effective fermentation. First, yeasts can multiply quickly in an aerobic environment of proper temperature, humidity, and nutrition, but they only convert sugars to alcohol effectively under an anaerobic condition. Therefore, the restricted mouth on brewing vessels would facilitate sealing and thus create an anaerobic environment (Hornsey 2003:18–19). Second, a large amount of dregs can be produced during brewing, such as chaffs from the raw materials and yeasts, and the amphora's conical base is ideal for concentrating the dregs' sedimentation. A parallel design can be found in the cylindroconical fermentation tank in modern beer breweries, which has been tested scientifically as the most logical shape for a beer fermenter (Delente et al. 1969). Third, the height-to-diameter ratio of amphorae is greater than 2:1, a feature that promotes a natural convection current in the vessel during brewing, thereby creating an even temperature distribution in the wort to ensure a consistent quality of the fermented product (Briggs et al. 2004; Liu et al. 2016).

Ethnoarchaeological studies of traditional beer making in Ethiopia indicate that beer jars are produced and used exclusively for beer fermentation. In the Gamo region, the beer jar is used primarily for storing beer in the fermentation process, and vessels are usually not washed after use. Similarly, in the Wallaga region, after fermentation is completed, the beer jar is not washed until the next batch is to be prepared, and the washing is not done completely. Such a continuous use for brewing allows the ingredients of the beer to deposit on, and seep into, the beer jar's walls (Arthur 2002; Wayessa et al. 2015). These practices thereby help to preserve useful microorganisms, such as yeasts, for repeated fermentation in the same vessels. These contemporary reports of a continuing traditional practice suggest the origin of visible organic residues or crust on archeological brewing vessels (McGovern 2009; Samuel 1996). It stands to reason that ancient use of brewing containers as dedicated vessels for production of alcoholic beverages also resulted in accumulation and inoculation of preferred microorganisms, which may be identified under a microscope.

According to these brewing principles and practices, our research strategy was to analyze the composition of microbotanical and microbial remains in amphorae, of various sizes, which show visible food residues on the interior surface. In 2017, we conducted a collaborative project to analyze 11 early Yangshao amphorae excavated at two renowned Neolithic sites, Banpo in Xi'an ($n = 2$; BP hereafter) and Jiangzhai in Lintong ($n = 9$; JZ hereafter), both in Shaanxi province. They represent the first amphorae in Neolithic China (Fig. 2a).

Methods

Residue sample extraction

Banpo was excavated in 1954–1957 (Institute of Archeology 1963), and Jiangzhai was excavated in 1972–1979 (Xi'an Banpo Museum et al. 1988). Unearthed pottery vessels were washed after excavation and have been kept in the storage facilities in the Xi'an Banpo Museum for decades. Both sites have revealed multiple phases of the Yangshao culture deposits, but we only focused on the amphorae of the early period, including both small and large vessels. We particularly selected vessels with visible yellow-white or yellow-black residue traces on their interior surface, for sampling (Fig. 2b, c).

These pottery samples derived from three archeological contexts:

1. Six small amphorae are complete or nearly complete, five of which were found in adult burials as grave goods; they

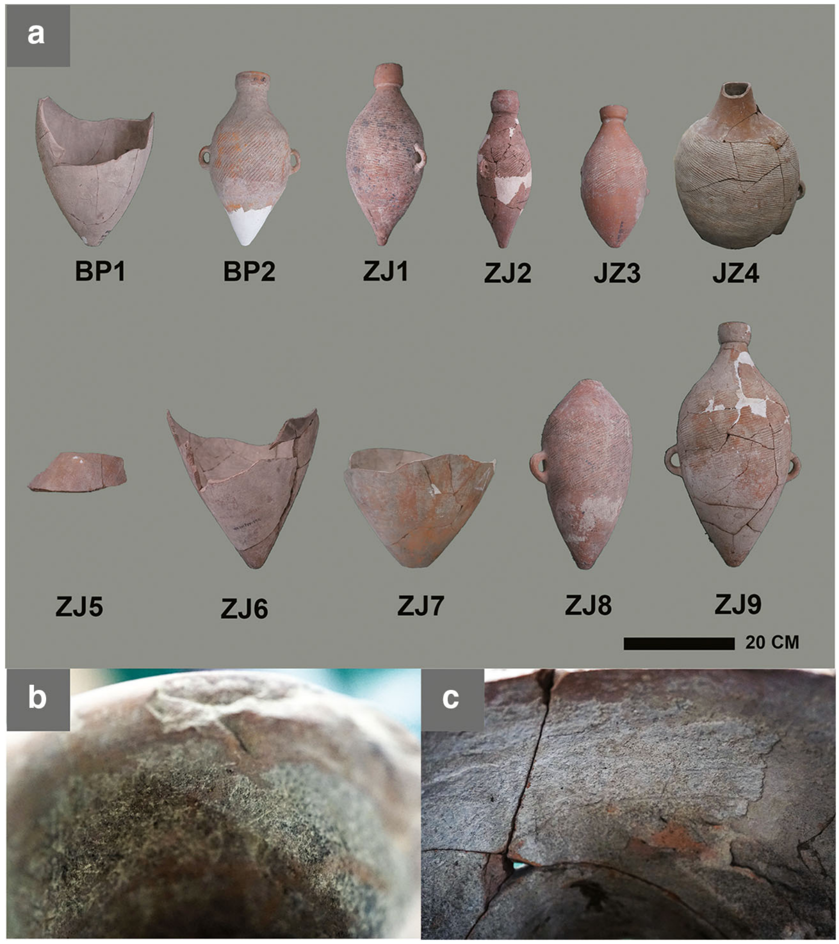

Fig. 2 Banpo and Jiangzhai amphorae analyzed in this study. **a** Amphorae samples analyzed (JZ4 with base missing). **b**, **c** Visible residues adhering to the rim (JZ1) and body (JZ4) interior walls

are likely to have been entered in the burials as functional items to serve the dead in the afterlife.

2. Fragments of large amphorae found in residential areas (house foundations and ash pits), which had been discarded after their useful life.
3. A child burial urn, which was recycled from a large amphora (Table 1). These contextual differences will be compared with the results of residue analyses, in order to evaluate the preservation conditions of various microorganic remains.

The process of collecting and analyzing the residue samples is as follows. Each pottery sample was first cleaned with a new toothbrush to remove loose soil on the interior surface of the vessel. Residues of microbotanical material to be analyzed were then extracted by using an ultrasonic toothbrush and distilled water to clean the same area for 3 min. The residue liquid from each sample was kept in a test tube. We also used clean blades to scrape off visible residue sediments on the vessel's interior surfaces and stored the sediments in small plastic bags. In order to test possible contamination on the vessels from the post-depositional process, we collected a control sample by gathering dust on shelves in the storage room.

Residue samples were processed with protocols established in the Stanford Archeology Center, involving two procedures: EDTA (ethylenediaminetetraacetic acid; 0.1%) dispersion and heavy liquid (sodium polytungstate, density 2.35) separation (Wang et al. 2017). Congo red (0.1%, 1 mg/ml) method (Lamb and Loy 2005) was used to dye a small part of the residue of five samples to determine the presence of gelatinized starch granules. Extractions obtained from residue samples were mounted in 50% (vol/vol) glycerol and 50% (vol/vol) distilled water on glass slides and scanned under a Zeiss Axio Scope A1 fitted with polarizing filters and differential interference contrast (DIC) optics, at × 200 and × 400 for starch, phytoliths, and fungi. Photographs were taken using a Zeiss Axiocam HRc3 digital camera and Zeiss Axiovision software version 4.8.

Identifying alcohol-related microfossil remains

We assessed whether a pottery vessel had been in contact with alcoholic beverages, based on the presence of microbotanical and microbial residues closely linked to alcoholic

Table 1 Starches from Banpo-Jiangzhai amphorae

Sample no.	Artifact no.	Type I Panicoideae	Type II Job's tears	Type III Triticeae	Type IV Snake gourd root	Type V Bean	Type VI Foxnut	UNID	Total	Damaged with gelatinization	Damaged without gelatinization
BP-1	M5(2)	1	2	2	14			12	31	13	9
BP-2	T4606			1			2 (22 granules)	7	30	11	3
BP-Total N		1	2	3	14		2 (22 granules)	19	41	24	12
BP-Total %		2.4	4.9	7.3	34.1		4.9	46.3	100	58.5	29.3
JZ-1	M74:11	7		10		1		5	23	2	9
JZ-2	M228:1	9	3	34	1			18	65	14	27
JZ-3	M87	12		13	1			2	28	13	
JZ-4	H191	5	27	11	2			2	47	1	15
JZ-5	F46	2		2	1			1	6	1	3
JZ-6	F47	20			1			8	29	26	3
JZ-7	W19:1			2				4	6	2	4
JZ-8	M159:3							14	14	14	
JZ-9	M244:6	16	1	28	9	2		13	69	12	31
JZ-Total N		71	31	100	15	3		67	287	85	92
JZ-Total %		24.7	10.8	34.8	5.2	1.0		23.3	100	29.6	32.1
TOTAL		72	33	103	29	3	2 (22 granules)	86	328	109	104
TOTAL%		22.0	10.1	31.4	8.8	0.9	0.6	26.2	100	33.2	31.7
Ubiquity N		8	4	9	7	2	1	11		11	9
Ubiquity %		72.7	36.4	81.8	63.6	18.2	9.1	100		100	81.8
min (µm)		7.13	8.61	4.72	10.2	22.57	23.28 (2.8 granules)				
Max (µm)		19.05	23.03	44.96	32.79	27.4	26.89 (5.41 granules)				
Mean (µm)		13.52	16.18	20.73	16.93	24.90	25.09 (4.14 granules)				

M adult burial, *H* ash pit, *F* house foundation, *T* excavation trench, *W* child burial urn

fermentation. We have developed a methodology to identify diagnostic elements in residues adhering to fermentation vessels. The diagnostic elements which may be present in the residues include (1) starch granules showing typical damage patterns caused by enzyme digestion and low-heat temperature gelatinization due to mashing, (2) yeasts, (3) husk phytoliths indicating use of malted cereals, (4) fungal particles (hyphae, spores, and sporangia) and/or grass phytoliths indicating use of *qu* starter, and (5) starch and phytolith types that mutually corroborate to provide information about ingredients of alcoholic beverages (Liu et al. 2020; Liu et al. 2019; Wang et al. 2016; Wang et al. 2017). In short, the existence of a population of microbotanical and microbial remains related to alcohol fermentation in vessels and their combined characteristics are important evidence for determining whether the vessels were used as brewing containers, as well as for analyzing fermentation methods. Such combinations of microorganisms do not exist in normal soils or on utensils unrelated to alcoholic beverages.

The control sample taken from shelf dust contains numerous fibers and pollens, but only a very small number of starch granules, and the latter show no evidence of fermentation damage. This composition differs very much from that typically seen in pottery residues (see Tables 1, 2, and 3 for details), indicating that the residues from pottery are mostly related to the original function of the vessels.

Starch identifications are based on our modern reference collection of over 1100 plant specimens in the Stanford Archeology Center, a fermented starch database generated by our experimental brewing of many types of plants (Wang

Table 2 Phytoliths from Banpo-Jiangzhai amphorae

Phytolith morphotype	Taxonomic attribution	BP1	BP2	JZ1	JZ2	JZ3	JZ4	JZ5	JZ6	JZ7	JZ8	JZ9	Total	Percent, %
Silica skeletons														
η-Type	*Panicum miliacaeum* husk/inflorescence					25			42		41	3	111	13.03
Ω-Type	*Setaria italica* husk/inflorescence					1							1	0.12
Undetermined Paniceae	Paniceae husk/inflorescence			2	4	25	2	1	72		69	1	176	20.66
Elongate dendriform	Pooideae husk/inflorescence				1								1	0.12
Elongate echinate	Poaceae				1				2	1	7	1	12	1.41
Elongate crenate	Poaceae		1		1				1				3	0.35
Elongate columellate	Poaceae	4	1		6				4		11		26	3.05
Elongate psilate/sinuate	Poaceae		6		4		4	1	2		4		21	2.46
Elongate irregular	Poaceae								1				1	0.12
Undetermined multi-cell		1											1	0.12
Single-cell phytolith														
Double-peak	Oryza husk								1				1	0.12
Phragmite bulliform	Phragmites				5						1		6	0.70
Bilobate	Panicoideae	6		1	11	3	4	4	10			2	41	4.81
Polylobate	Panicoideae	1		1					1				3	0.35
Cross/quadra-lobate	Panicoideae	5			2	1			3		6		17	2.00
Rondel	Poaceae	26			2	1	1	2	6	1			39	4.58
Common bulliform	Poaceae leaf	14	1		4	1		1	1		1	2	25	2.93
Elongate dendriform	Pooideae husk/inflorescence		1			1							2	0.23
Elongate psilate/sinuate	Poaceae	39	36	4	101	20	14	2	61	3	33	7	320	37.56
Papillae cell	Poaceae					1		1					2	0.23
Hair cell	Eudicots	4	3	1	13	3	4	3	4	3	2	1	41	4.81
TOTAL		100	49	9	215	82	29	15	214	10	192	20	852	100

et al. 2017), as well as on published information based on experimental study of morphological change in starch caused by cooking (Henry et al. 2009). Phytolith identification was also based on our reference collection and published information (Lu et al. 2009; Madella et al. 2005; Piperno 2006). Fungi were identified according to our microbial database at Stanford and published sourcebooks (St-Germain and Summerbell 2011; Webster and Weber 2007; Wei 1979).

Results

We found large numbers of starch granules, phytoliths, mold particles (mycelia, hyphae, spores and sporangia), yeast cells, and a few rod-shaped calcite crystals in the amphorae residues.

Starch remains

A total of 328 starch granules were found in the residues of eleven pottery samples, of which 242 (73.8%) were classified into six types. There were 86 (26.2%) starch granules lacking diagnostic characteristics and classified as unidentifiable (UNID) (Table 1; Fig. 3).

Type I is identified as Panicoideae, including foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), and probably small granules from Job's tears (*Coix lacryma-jobi* L.) ($n = 72$; 22% of the total; ubiquity 72.7%); the size range is 7.13–19.05 μm, and the shape is polygonal or near-circular, hilum is centric, and fissures are often present. Starch granules from the three species share these common characteristics, making it difficult to separate them precisely (Liu et al. 2014).

Type II is identified as Job's tears (*C. lacryma-jobi* L.) ($n = 33$; 10.1% of the total; ubiquity 36.4%), and the size range is 8.61–23.03 μm. Its morphology differs from that of millets but has certain characteristics consistent with Job's tears, such as larger size, eccentric hilum, and the extinction cross with zig-zag arm (Liu et al. 2014).

Type III is identified as Triticeae ($n = 103$; 31.4% of the total; ubiquity 81.8%), size range is 4.72–44.96 μm, the shape is lenticular, and the hilum is centric. These features are

Table 3 Fungal elements from Banpo-Jiangzhai amphorae

Fungal element	BP1	BP2	JZ1	JZ2	JZ3	JZ4	JZ5	JZ6	JZ7	JZ8	JZ9	Total	Percent
Yeast-round	1	6		5		2	5	3	1		5	28	6.8
Yeast-ovoid/oval	16	18		8		4	5	3	7		5	66	16.0
Yeast-round/oval budding	2	14				4	11	7	2			40	9.7
Yeast total	19	38		13		10	21	13	10		10	134	32.5
Hyphae (brown or gray)	13	12	3	3	1	7	9	10	1		2	61	14.8
Hyphae (transparent)	7	3	2	74					3			89	21.6
Mycelium				4								4	1.0
Hyphae total	20	15	5	81	1	7	9	10	4		2	154	37.4
Sporangia	5	3	1	3				4				16	3.9
Sporangia with hypha	1	3		53				5	1		1	64	15.5
Sporangia total	6	6	1	56				9	1		1	80	19.4
Spores (round)	1	2	1				6	4	1		3	18	4.4
Spores (oval)	4	1	2			1	5	6	2		5	26	6.3
Spores total	5	3	3			1	11	10	3		8	44	10.7
Molds total	31	24	9	137	1	8	20	29	8		11	278	67%
Fungi total	50	62	9	150	1	18	41	42	18		21	412	100

similar to those of the genus *Agropyron*, *Elymus*, and *Leymus*, which are wild grasses native to northern China (Wu et al. 2006).

Type IV is identified as the root of snake gourd *Trichosanthes kirilowii* ($n = 29$; 8.8% of the total; ubiquity 63.6%), size range 10.2–32.79 μm; the forms include round, bell-shaped, and semi-circular; the hilum is centric or eccentric; and the extinction cross is often curved. This plant is commonly found in China (Wu et al. 2011), and traditionally used as famine food (Zhu 1406).

Type V is identified as bean, probably wild pea *Vicia* sp. ($n = 3$; 0.9% of the total; ubiquity 18.2%). The size range is 22.57–27.4 μm, the form is irregular oval or kidney-shaped, and the extinction cross has multiple arms with a large black area in the center. There are 17 *Vicia* species in the Qinling Mountains region near the sites (Northwest Institute of Botany CAS 1981:98–99).

Type VI is identified as foxnut *Euryale ferox*, which appeared as two round grains (0.6% of the total, ubiquity 9.1%) measuring 23.28 and 26.89 μm in size, respectively. Within each grain, there are many small compound granules, polygonal in shape ($n = 22$; 2.55–4.24 μm). These features resemble foxnut in our reference data. The modern foxnut starches tend to appear in compound form, round, or oval in shape (9.51–32.33 μm in diameter), containing numerous small facetted granules (1.71–3.96 μm in size). The two foxnut grains found in BP2 are connected, with one partially broken. For starch quantitative analysis, we count the large starch grains rather than the small compound granules, to avoid unnecessary inflation in number. Foxnut grows in lakes and ponds and is widely distributed in China (Wu and Raven 2001).

In sum, the starches are derived mainly from millet, Job's tears, and Triticeae, with small numbers from snake gourd roots, wild peas, and foxnut. The starches of these plants have been found on pottery vessels from other Neolithic sites in the Wei River region (Liu et al. 2019; Liu et al. 2018b; Wang et al. 2016). The flotation results at the Yuhuazhai site in Xi'an show that foxtail millet and broomcorn millet were the most important crops in the Yangshao period (Zhao 2017). The starch assemblage of BP-JZ amphorae reflects the plants commonly cultivated and collected in the region during the Yangshao period.

Most starch granules show damage characteristics ($n = 213$; 64.9% of the total) and can be divided into two types: non-gelatinized ($n = 109$; 33.2% of the total) and gelatinized ($n = 104$; 31.7% of the total). The former type is consistent with the damage caused by enzyme digestion, such as random pitting, deep channels, broken edges, missing lamellae, and central depression. The latter type has the characteristics of gelatinization caused by low-temperature heating during mashing and fermentation, such as moderate swelling with a hollowed center, often showing as a birefringent periphery with a dark center; these features have been found in fermented starches subjected to experimental brewing (Wang et al. 2017). There are also a small number of gelatinized starch granules that appear to have been caused by ordinary cooking (boiling and steaming), showing rather evenly expanded surfaces. They are consistent with reported results from experimental cooking (Henry et al. 2009). Such features were also common in our brewing experiments. When the Congo red dye (Lamb and Loy 2005) was applied to five samples (JZ2,4,6,7,9), all revealed gelatinized starches

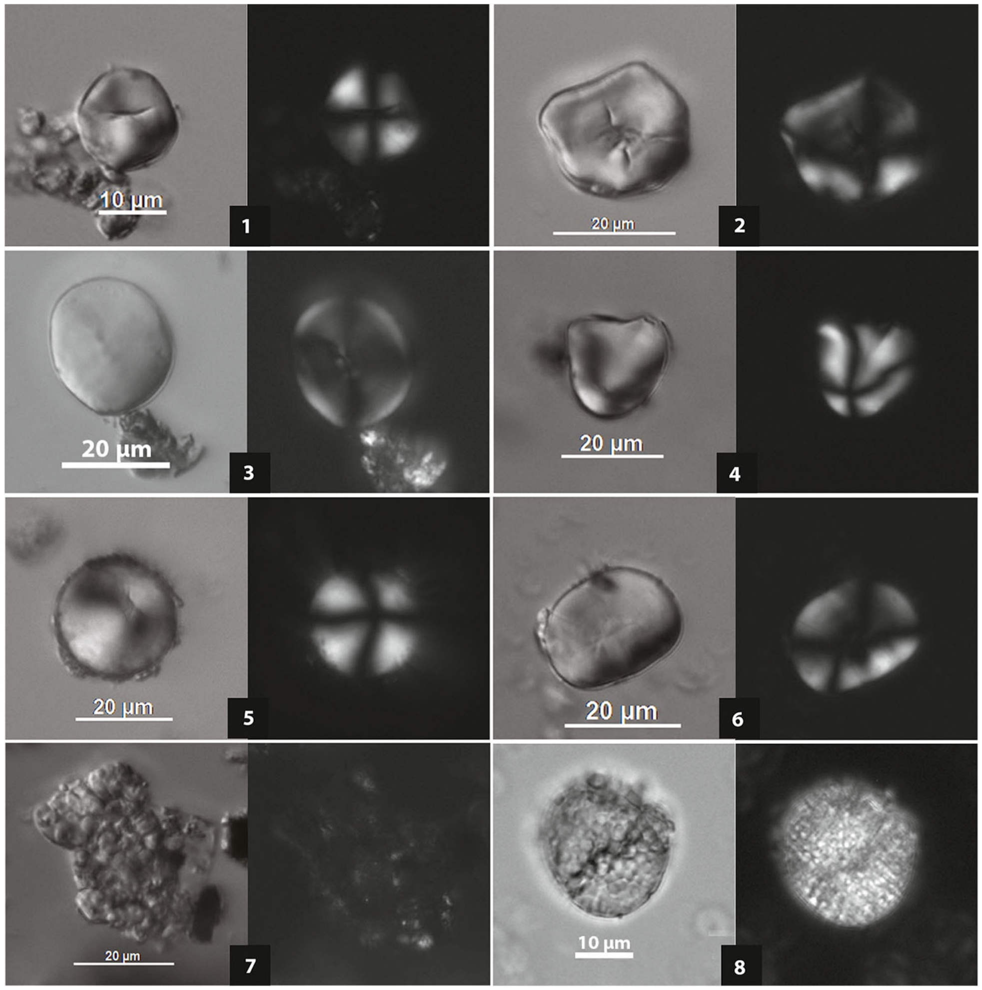

Fig. 3 Types of starch granules in Banpo-Jiangzhai residues compared with modern reference (each starch grain shows images taken by DIC and polarizers). 1. Type I, millet; 2. Type II, Job's tears; 3. Type III, Triticeae; 4 and 5. Type IV, snake gourd root; 6. Type V, wild pea; 7. Type VI, foxnut; 8. Modern foxnut for comparison

that were stained red with orange-red gloss in polarized light (Fig. 4). Taken together, the starch granules overall exhibit various damages consistent with beer brewing.

Phytoliths

A total of 852 phytoliths were recorded. Eight samples contain long-cell undulated phytoliths typical of Paniceae husks (n = 288; 33.8%), including 111 η- undulated type from broomcorn millet, one Ω-undulated type from foxtail millet, and 176 of undetermined type, due to lack of specific identifiable features. Given that most of the husk phytoliths identifiable to the level of genus belong to broomcorn millet, it is likely that most of the undetermined Paniceae husk phytoliths also belong to this species. Bilobate, polylobate, and cross phytoliths commonly found in Panicoideae occur in nine samples (n = 61), most of which may originate from broomcorn millet, except three Variant 1 large crosses from glume or utricle of Job's tears (Duncan et al. 2019). Three dendriform phytoliths are present, which are most likely derived from husks of Pooideae such as Triticeae, but their exact taxonomy cannot be determined. In addition, a double-peak phytolith from rice husk is present. There are also many elongate (psilate/sinuate), bulliform, and rondel phytolith types common in grasses, including six Phragmites bulliforms from reeds. Hair cells (n = 30) found mainly in stems and leaves of eudicots appeared in ten samples. Hair cells come from plants such as Asteraceae, Ulmaceae, Cucurbitaceae, and Urticaceae (Piperno 2006). Snake gourd belongs to Cucurbitaceae, but it is unclear if some of the hair cells come from this plant, and more research is needed in the future. In short, there are a large number of Paniceae husks and a small number of Triticeae and rice husks, coexisting with various grass stems and leaves in the phytolith assemblage (Fig. 5). The presence of Paniceae husks and dendriforms, as well as Variant 1 large cross phytoliths, corroborates the finding of millet, Triticeae and Job's tears in the starch assemblage.

Fungi (mold and yeast)

We found 412 fungal elements or combinations in the residue samples, including yeast cells and molds. In eight samples, a total of 134 yeast cells are present, which are round and oval, with diameters in the range of 3.19–11.9 µm. Forty of them

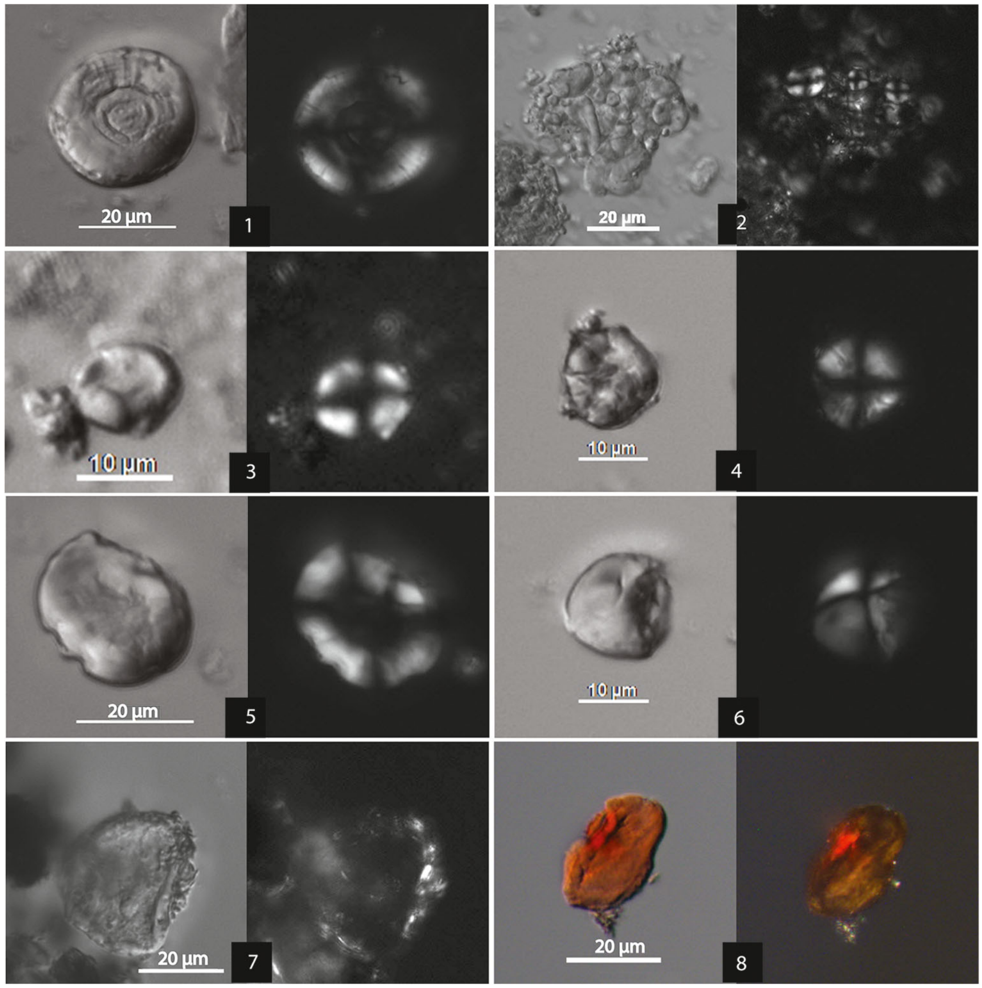

Fig. 4 Damaged starch granules from Banpo-Jiangzhai amphorae residues (each starch sample shows images taken by DIC and polarizers). 1 Triticeae, showing micro-pitting and deep channels; 2 a cluster of Triticeae starches, some granules relatively complete, some appearing expanded and deformed with crosses disappearing, an effect of gelatinization; 3 possibly millet, showing central depression; 4 millet, showing micro-pitting and deep channel; 5 wild pea, central depression and damaged edge, with slight gelatinization; 6 snake gourd root, broken edges; 7 gelatinization due to fermentation, showing swelling and deformation; the center is missing, but the edges remain birefringent; 8 gelatinized starch stained with Congo red

appear in process of budding, indicated by one or more small protuberances on the parent cell or by a smaller cell attached to a bigger parent cell (Fig. 6: 1 ,2). Their morphology is similar to that of *Saccharomyces cerevisiae* (Fig. 7: 8), which is the most commonly used yeast for alcohol fermentation (Boulton and Quain 2001), probably first domesticated in China (Gallone 2016). However, we cannot determine their precise taxonomy based on morphology alone.

All of the samples, except for two (JZ3, JZ8), revealed fungal particles, including 154 hyphae and mycelia, 80 sporangia, and 44 spores. Some of them exhibit features consistent with *Aspergillus, Rhizopus*, and *Mucor*, which are molds commonly found in modern *qu* (Jin et al. 2017; Zheng et al. 2011). *Aspergillus* is characterized by its conidiophores terminating in an apical vesicle (also spelled “vesicule”) and, at the opposite end, in a basal foot cell inserted into the supporting hypha. Phialides are attached directly to the vesicle (uniseriate) or onto an intermediate cell called a metula (biseriate); conidia are formed in chains (St-Germain and Summerbell 2011: 56). *Rhizopus* is characterized by broad hyphae, not or scarcely septate; rhizoids and stolons present; sporangiophores being brown, solitary or in tufts on the stolons and diverging from the point at which the rhizoids form; the sporangia being rather round; and the sporangiospores being ovoid (St-Germain and Summerbell 2011: 224). *Mucor* shows broad hyphae, not or scarcely septate; sporangiophores branched or sometimes unbranched, sporangia with columellas; sporangiospores round or ellipsoidal; and lacking rhizoids and stolons (St-Germain and Summerbell 2011: 190) (Fig. 7: 1–7).

A large number of mold particles ($n = 137$) were found in the JZ2 sample, mostly consistent with *Aspergillus*. At least 42 of them can be identified either as conidiophores connected with rounded vesicles, or as conidia and phialides growing from vesicles. There is also a small amount of *Rhizopus* or *Mucor* in JZ2, indicated by sporangiophores connected to round sporangia with some small, dark-brown sporangiospores visible. Diverged hyphae were found in BP1 and JZ5, and their morphology is similar to that of *Rhizopus* growing from the position opposite the rhizoids (Fig. 6; compare with the corresponding mold morphology in Fig. 7).

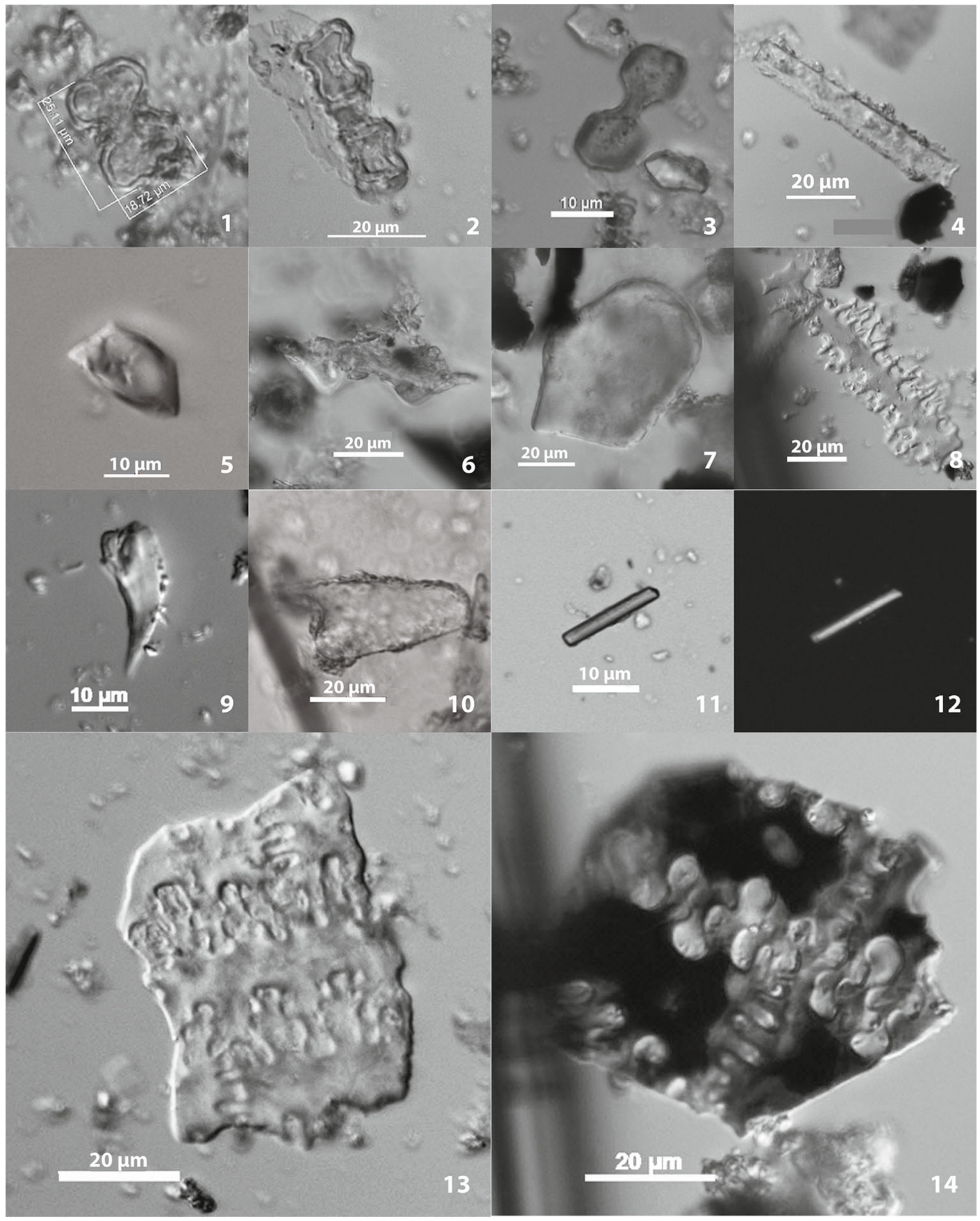

Fig. 5 Examples of phytoliths and rod-shaped calcite crystals in Banpo-Jiangzhai amphorae. 1 Variant 1 cross from Job's tears; 2 cross; 3 bilobate; 4 elongate long cell; 5 rondel; 6 double-peak; 7 Phragmites bulliform; 8 dendriform; 9 hair cell; 10 prinkle; 11 and 12 rod-shaped calcite crystal (bright field and polarized views); 13 η-type from broomcorn millet husk; 14 Ω type from foxtail millet husk

Rod-shaped calcite crystals

A few rod-shaped crystals were found in three samples. Most of them have smooth surfaces, appearing as straight rods with square blunt ends. Birefringent gloss is visible under a polarizer (Fig. 5: 11–12). These characteristics are consistent with the morphology of rod-shaped calcite crystals, which are the result of biomineralization of fungal hyphae. Hyphae release rod-shaped calcite crystals into the surrounding environment after decomposition (Bajnoczi and Kovacs-Kis 2006; Verrecchia 1994). The appearance of such calcite crystals indicates the existence of fungi in the residues, which is corroborated by the identification of fungal hyphae, mentioned above.

Discussion

Based on the appearance of various microbotanical and microbial remains in the residues, we can observe the following phenomena. First, according to the types of starch granules and phytoliths, it can be inferred that the amphorae were used to contain plants, including millet, Job's tears, rice, Triticeae, wild peas, snake gourd roots, and foxnut. The presence of starch granules with damage caused by fermentation indicates that these plants were raw materials for brewing alcohol. Various cereals predominate among the abundant and ubiquitous starches, while wild peas and tubers are fewer. A double-peak phytolith of rice was found in the JZ sample, but no rice starch was present. The granules of rice starch are very small;

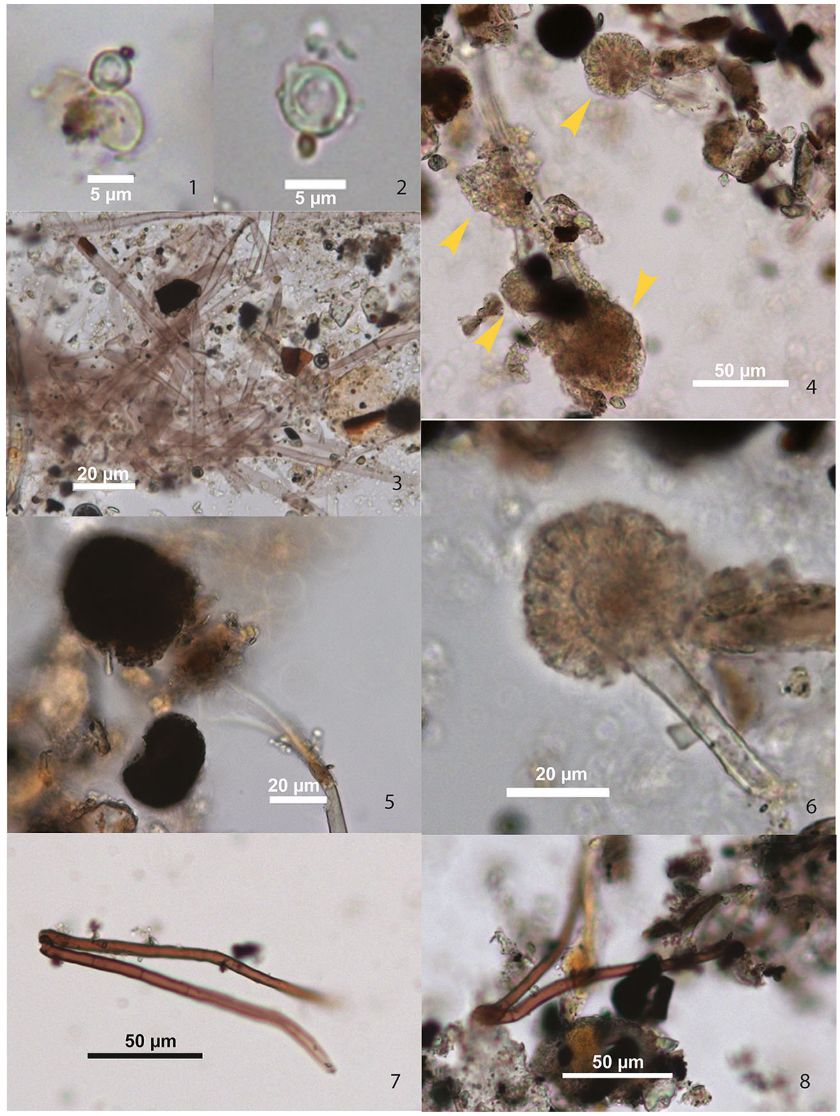

Fig. 6 Examples of yeast cells and molds in the Banpo-Jiangzhai amphorae. 1 and 2 Yeast cells in budding process (BP1, 2); 3 mycelium (JZ2); 4 *Aspergillus*, arrows pointing to vesicle connected with metula/phialide and conidia (JZ2); 5 black sporangia connecting to sporangiophores, probably *Rhizopus* or *Mucor* (JZ2); 6 *Aspergillus* vesicle (enlarged from the vesicle at the upper right corner of 4); 7 and 8 *Rhizopus* (BP1, JZ5) sporangiophores at the junction with rhizoids (missing here)

if they do not appear in a compound form, it is not easy to identify them in ancient residues. Also, according to our brewing experiment, most of the rice starch granules disappeared during the fermentation process. In general, during the brewing process, most starch granules are deformed, making identification difficult. Therefore, we cannot directly calculate the relative proportions of various raw brewing materials from the percentages of starch types in the residues, but, based on ubiquity, we can infer that these plants were the main ingredients of brewing.

Second, the ubiquity of Triticeae starches is rather high. They were found in 9 samples, and three of them (BP2, JZ2, JZ3) coexisted with dendriform phytoliths, which are most likely derived from husks of Pooideae. These starch granules and phytoliths may come from the same plants, but we are unable to provide more precise taxonomy. These remains nevertheless indicate that wild Triticeae seeds were used for food, including alcohol making, prior to the introduction of domesticated wheat and barley to China around third millennium BC.

Third, Paniceae husk phytoliths are present in eight JZ samples, of which two (JZ3,8) contain very high numbers of such husks (51 and 110, respectively), but lack molds (Fig. 8). The presence of cereal husks and the corresponding starch granules with fermentation characteristics can be used as an indicator for brewing with malted cereals. Therefore, these two JZ amphorae may have contained beer with malts as the initiating agent of the brewing process. The earliest evidence of beer made of malts was found in globular jars from the Lingkou site in Lintong, 19 km from Jiangzhai and dating to ca. 5900–5000 cal. BC (Fig. 1a). The brewing ingredients at Lingkou are mainly broomcorn millet and rice (Liu et al. 2019), also similar to Jiangzhai, indicating an ongoing regional tradition.

Fourth, the evidence for the *qu* method used in alcohol brewing is present at both sites. Nine samples contain

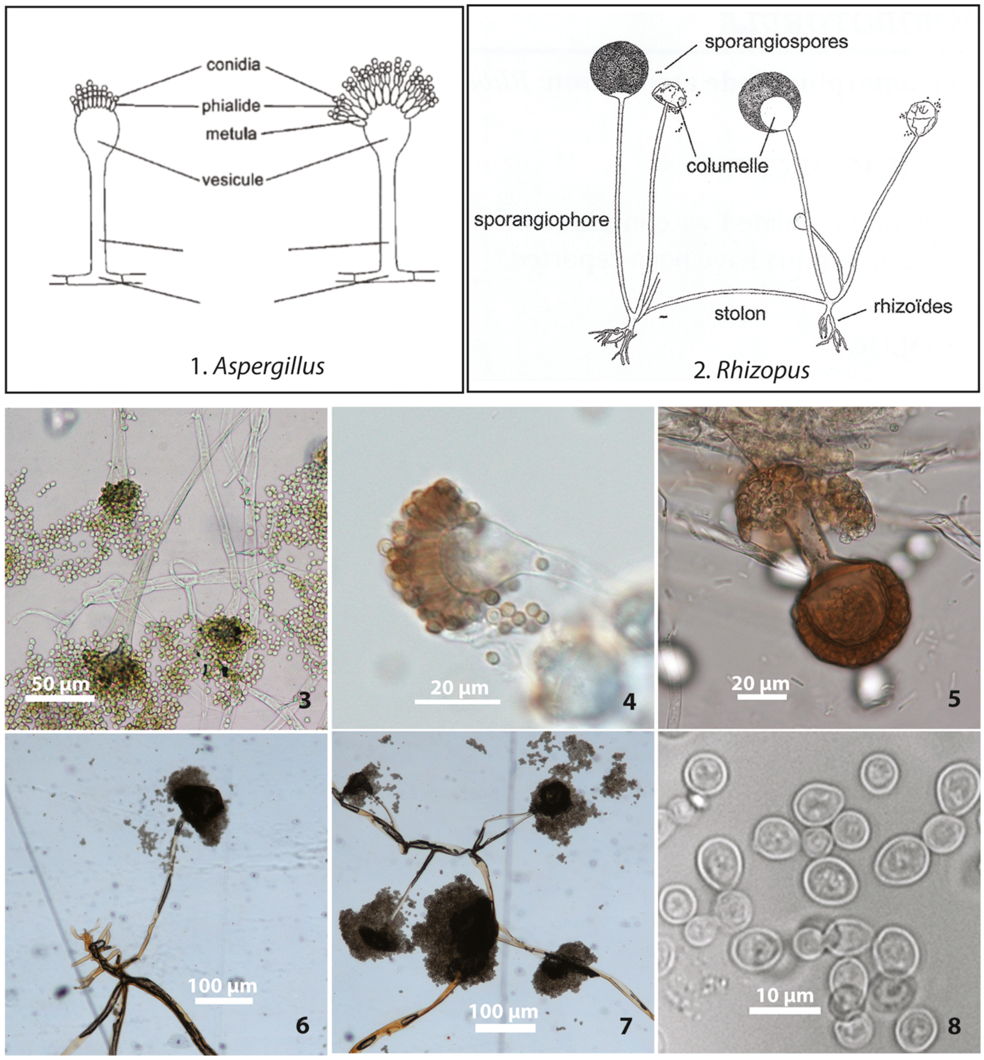

Fig. 7 Comparative reference of modern fungi. 1 *Aspergillus*; 2 *Rhizopus*; 3 *Aspergillus* mycelium; 4 *Aspergillus* vesicle, phialides, and conidia; 5 *Mucor* sporangiophore and sporangium; 6 and 7 *Rhizopus* rhizoids, sporangiophore, sporangia and sporangiospores; 8 *Saccharomyces cerevisiae* yeast

considerable numbers of mold particles (> 8 each), identifiable as *Aspergillus, Rhizopus* and/or *Mucor*. In particular, a large number of mold fragments (n = 137) were found in JZ2, including primarily *Aspergillus*, with a small amount of *Rhizopus/Mucor*. The earliest evidence of using *Aspergillus* and *Rhizopus* for beer brewing was found on pottery at the

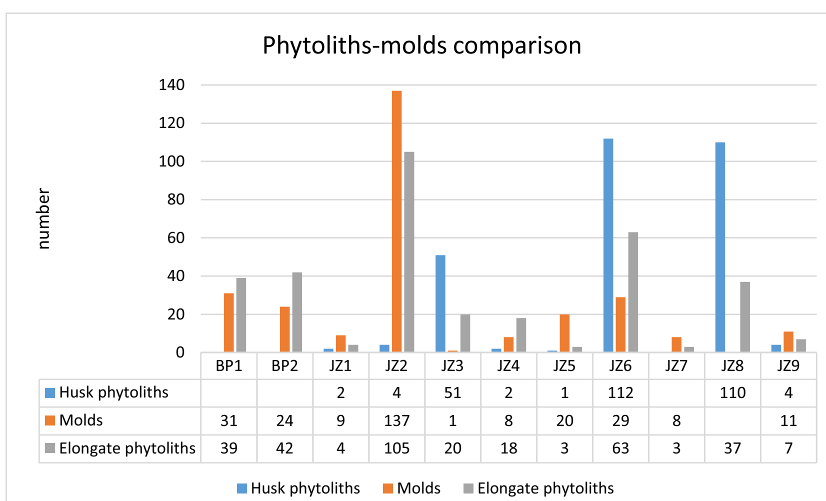

Fig. 8 Inferred brewing methods based on the distribution of phytoliths and molds. BP1, BP2, and JZ7 are *qu*-made alcohol; JZ3 and JZ8 are malted beer; others were probably made by mixing two methods

Guantaoyuan site in Shaanxi (ca. 5800–4900 cal. BC) (Liu et al. 2019), and *Aspergillus* and *Mucor* were identified on amphorae from Dingcun in Henan (ca. 4000–3100 cal. BC) (Liu et al. 2020) (Fig. 1a). As *Aspergillus* and *Rhizopus* are the most recurrent molds in traditional *qu* starters (Huang 2000; Jin et al. 2017), the high concentration of these two molds in one vessel (ZJ2) suggests that without scientific knowledge, the Yangshao people may have intentionally selected desirable microorganisms for fermentation through hundreds of years of trail-and-error experience.

Of the nine samples with molds, three contain no husk phytoliths from malted grains (BP1,2; JZ8), indicating that the alcoholic beverages were likely made by the action of microorganisms in *qu* starter only. In the other six samples, husk phytoliths from malted grains as well as molds were both found (JZ1,2,4-6,9), suggesting possible simultaneous use of the two brewing methods. It must be pointed out that the method of inferring brewing techniques based on the presence of molds and husks in pottery residues has some limitations. For example, if ancient people brewed beer with *qu* starter but failed to completely remove the husks from processed grain, then both molds and a small amount of husk phytoliths may be present in the brewing vessels. In general, the two brewing methods inferred from observed remains may have both existed in the Yangshao sites, but we cannot rule out the possibility that in some cases (such as JZ5 with only one husk phytolith), a small number of husk phytoliths unintentionally entered the ingredients, rather than being the residue of intentionally formed malts, during beer brewing (Fig. 8).

Fifth, elongate psilate/sinuate phytoliths were found in all samples. They are mainly from the stems and leaves of grasses, although also existing in husks (Madella et al. 2016; Weisskopf and Lee 2014). If the *qu* method was used, and certain herbal plants were added as *caoqu*, then molds and elongate phytoliths would be present in the residues. Four samples (BP1,2; JZ2,6) contain large numbers of molds ($n = 24$–137) and elongate psilate/sinuate phytoliths ($n = 39$–105), probably suggesting the use of *caoqu* (Fig. 8).

Sixth, based on use-wear patterns (e.g., vertical striations) on amphorae rims from several Yangshao culture sites, we have suggested that Yangshao people may have used straws made of reeds or bamboo to drink beer from amphorae, referred to as siphonage (Liu 2017). This is a known drinking tradition practiced in the ancient Near East as well as in modern Africa and Southwest China (McGovern 2009). We found bulliform phytoliths from Phragmites' stems and leaves (Fig. 5: 7) in two samples (JZ2,8), supporting the siphonage hypothesis.

Seventh, the alcoholic beverages were made of various domesticated and wild plants commonly consumed at the time, and the provenance and high ubiquity of amphorae suggest that alcoholic beverages may have been commonly consumed by all families in the community, rather than being a luxury item reserved only for elites. The amphorae in various sizes may have been used in different social settings, ranging from household or individual drinking with smaller vessels to community feasting with larger ones. This scenario can be supported by the settlement pattern of Banpo and Jiangzhai, in which a few large public houses were surrounded by many smaller family dwellings. In addition, amphorae's increase in size through time is in parallel with a general trend that the large public houses increased in size from the early (ca. 100 m^2) to late Yangshao (ca. 300 m^2), suggesting a growing scale of public gatherings, likely involving feasting. The association of large public houses with drinking activities has also been revealed by residue analysis of the floors from a mid-late Yangshao large house at the Huizui site (Henan) (Liu et al. 2018a). All these phenomena point to a close relationship between alcohol consumption and the development of social complexity in the Yangshao culture.

Finally, the taphonomical contexts seem to have affected the preservation of microorganic remains, particularly starch granules. Two small, complete amphorae from burials (JZ2 and JZ9) revealed the most numerous starches ($n = 65$ and 69, respectively), and JZ2 also yielded the highest number of molds ($n = 137$), whereas the sherd from a house foundation (JZ5) and a recycled burial urn (JZ7) yielded the lowest starch counts ($n = 6$ each). In terms of sampling strategy, these results suggest that, in addition to targeting brewing vessels with visible residues on the surface, we need to select complete vessels in burials to obtain good evidence of alcohol production and consumption.

Conclusions

Multiple lines of evidence from starch granules, phytoliths, molds, yeast cells, and rod-shaped calcite crystals indicate that the early Yangshao amphorae analyzed here, both large and small, were once beer brewing containers during their use life. These first amphorae may have been invented as a new type of brewing vessels, with a structural design for effective fermentation. The ingredients of the beer include mainly broomcorn millet, together with other cereals (foxtail millet, rice and Triticeae), wild peas, and tubers (snake gourd roots and foxnut), although we are unable to specify the proportion of each ingredient. Siphoning through reed straws may have been one of the drinking methods. Two brewing techniques were detected: use of sprouted grain and use of *qu* starter made of moldy grain with herbs. These two methods were used either independently or simultaneously. Some of the molds for making *qu* are consistent morphologically with *Aspergillus*, *Rhizopus*, and/or *Mucor*, and the yeast cells are similar in form to *Saccharomyces cerevisiae*. Comparable brewing ingredients and fermentation methods have also been identified in the pre-Yangshao and middle-late Yangshao pottery vessels, based on previous studies, suggesting continued regional brewing traditions. The consistent reoccurrence of certain types of fungi over thousands of years suggests that selective accumulation and inoculation of desirable microorganisms may have been achieved through fermentation in specialized and dedicated vessels, including amphorae.

Our new findings help fill the 1000-year time gap in China's Neolithic alcohol fermentation history, allowing us to reconstruct the brewing technologies developed from 9000 to 5000 years ago in the middle Yellow River region. The results of this research open a new window not only for understanding the long technical tradition of alcohol production in prehistorical China, but also for investigating alcohol-related social activities, such as feasting, of the Yangshao people whose material culture spread over an extensive region in the Yellow River valley. Future research needs to analyze the relationship between drinking behavior and the development of social complexity over a broader region and a longer time span in ancient China.

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Author contributions Conceptualization: Li Liu; Methodology, formal analysis, and writing: Li Liu and Jiajing Wang; Resources: Huifang Liu.

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