The origins of specialized pottery and diverse alcohol fermentation techniques in Early Neolithic China

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In China, pottery containers first appeared about 20000 cal. BP, and became diverse in form during the Early Neolithic (9000–7000 cal. BP), signaling the emergence of functionally specialized vessels. China is also well-known for its early development of alcohol production. However, few studies have focused on the connections between the two technologies. Based on the analysis of residues (starch, phytolith, and fungus) adhering to pottery from two Early Neolithic sites in north China, here we demonstrate that three material changes occurring in the Early Neolithic signal innovation of specialized alcoholic making known in north China: (*i*) the spread of cereal domestication (millet and rice), (*ii*) the emergence of dedicated pottery types, particularly globular jars as liquid storage vessels, and (*iii*) the development of cereal-based alcohol production with at least two fermentation methods: the use of cereal malts and the use of moldy grain and herbs (*qu* and *caoqu*) as starters. The latter method was arguably a unique invention initiated in China, and our findings account for the earliest known examples of this technique. The major ingredients include broomcorn millet, Triticeae grasses, Job's tears, rice, beans, snake gourd root, ginger, possible yam and lily, and other plants, some probably with medicinal properties (e.g., ginger). Alcoholic beverages made with these methods were named *li*, *jiu*, and *chang* in ancient texts, first recorded in the Shang oracle-bone inscriptions (ca. 3200 cal. BP); our findings have revealed a much deeper history of these diverse fermentation technologies in China.

ancient fermentation methods | starch granules | phytoliths | fungi | millet

China holds the earliest record for pottery production in the world, represented by about 16 sites dating to ca. 20000–10000 cal. BP (1). These incipient vessels are mainly open-mouthed jars or basins with round or flat bases, some of which may have been used for boiling or processing various plant foods (2). By the Early Neolithic Period (ca. 9000–7000 cal. BP), which is defined in this study as the presence of clear evidence for domesticated millet and rice, ceramic vessels became increasingly diverse in type, forming several regional traditions (3). The new vessel types included tall cylindrical jars with or without legs, globular jars, bowls, and cups, of which globular jars are among the most widespread type, distributed over a broad region from the Yellow River to the Yangzi River valleys (Fig. 1) (4). A globular jar is normally constructed with a restricted mouth, a short neck, and a large spherical body. Such a vessel form is suitable for storing liquid and is commonly used for fermenting alcoholic beverages, as its narrow neck can be effectively sealed, to exclude as much air as possible and encourage anaerobic conditions (5). Supporting this hypothesis, chemical analyses of sherds of globular jars from Jiahu in Henan (ca. 9000–7500 cal. BP) have revealed the earliest fermented beverages made of rice, honey, and fruits in China (6). However, we know little about the fermentation methods that were used at this time.

The production of an alcoholic drink from cereals involves two separate biochemical steps: (*i*) saccharification, hydrolysis of the starch in the cereal to fermentable sugars with enzymes called amylases, and (*ii*) fermentation, conversion of the sugars by yeasts to ethyl alcohol and carbon dioxide. Since yeast is ubiquitous in the environments and thus readily introduced incidentally, the key step is saccharification. In ancient China, saccharification may have been achieved by utilizing the amylases in three sources: (*i*) human saliva (mastication; by chewing and spitting out cereals), (*ii*) sprouted grains, and (*iii*) *jiuqu* 酒麴 (or *qu* 麴), a fermentation starter made of moldy cereals, in some cases including herbs (herbaceous plants), which are rich with microorganisms (filamentous fungi, yeasts, and bacteria) (7–9). *Qu* is functional as an agent for simultaneous saccharification and fermentation; during this process, filamentous fungi secrete various enzymes to degrade starch material into fermentable sugars, while yeasts convert sugars to carbon dioxide and alcohol. There are several variants of *qu*, such as *daqu* 大麴, made of wheat (*Triticum* sp.), barley (*Hordeum* sp.), and/or pea (*Pisum sativum*); *xiaoqu* 小麴, made of rice (*Oryza sativa*); and *fuqu* 麩麴, made of bran (the hard outer layers of wheat grain) (10–12). Herbs used as part of *qu* are often referred to as *caoqu* 草麴 (7, 9, 13). Alcohol types first appeared in oracle-bone inscriptions of the Late Shang (ca. 1250–1046 BC), recorded as *li* 醴, *jiu* 酒, and *chang* 鬯 (14). In later texts, such as *Shangshu* (compiled by the fourth century BC), the fermentation methods of these alcohol types were further described as *li* made of *nie* 蘗 (sprouted cereals),

Significance

China is well-known for its distinctive techniques in alcohol fermentation. Here we present archaeological evidence of alcohol making based on analyses of starch granules, phytoliths, and fungi in food residues adhering to 8,000- to 7,000-y-old Neolithic pottery vessels. We demonstrate the earliest association between the wide occurrences of globular jars as liquid storage vessels and the development of two methods of alcohol making: use of cereal malts and use of moldy grain and herbs as starters. The latter method was arguably a unique invention initiated in China. Neolithic people made low-alcohol beverages with broomcorn millet, Triticeae grasses, Job's tears, rice, beans, snake gourd root, ginger, yam, lily, and so forth. Such fermented beverages may have served social, spiritual, and medicinal functions.

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Fig. 1. Globular jars unearthed from major sites in early Neolithic China (9000–7000 cal. BP). *1*, Dadiwan; *2*, Guantaoyuan; *3*, Baijia-Lingkou; *4*, Jiahu; *5*, Shuiquan; *6*, Pengtoushan; *7*, Kuahuqiao; *8*, Xiaohuangshan; *9*, Shangshan; *10*, Houli.

jiu made of *qu*, and *chang* made of millet and herbs. Use of the *qu* method for alcohol production is generally thought to be unique to East Asia, and was initiated in China (7, 9).

Beer made of sprouted millet has been identified in vessels of three Yangshao culture sites in north China (ca. 5700–4700 cal. BP) (15–17), but whether or not *qu* was used in the Neolithic is unknown. To investigate the origins of fermentation technologies in ancient China, we analyzed residues adhering to ceramics from two Early Neolithic sites, Lingkou in Lintong and Guantaoyuan in Baoji, both situated in the Wei River valley in Shaanxi Province (*SI Appendix*, Fig. S1). The analyses revealed abundant starch granules, phytoliths, and fungi. We have conducted a series of experimental studies on starch morphological changes caused by fermentation, using different cereals and tubers (18). We also studied traditional millet beer brewing in north Shaanxi (*SI Appendix*, Fig. S2). The fermented products from these projects were used as comparative references. Here we present the results to address the following issues: (*i*) the relationship between the occurrence of globular jars and the development of alcohol production in Neolithic north China; (*ii*) the regional variations in terms of ingredients in alcohols and brewing methods; (*iii*) the medicinal functions of early alcohol; and (*iv*) the connections between the spread of cereal domestication and early alcohol production.

Lingkou (LK hereafter), excavated in the 1990s, is located on the banks of the Ling River, a tributary of the Wei River. This area is likely to have had abundant water resources, as indicated by the presence of numerous water buffalo remains at the contemporaneous Baijia site in the vicinity (19). The material deposits at LK indicate a long sequence of human occupations from the Early to the Middle Neolithic Period. The pottery types from the earliest deposits include bowls, tripod vessels, flat-based jars, and globular jars, all belonging to the Baijia culture (ca. 7900–7000 cal. BP) (20). We analyzed six vessel sherds, including three globular jars and three tripods; the former is hypothetically related to alcohol making, whereas the latter may have been used for more general culinary purposes (*SI Appendix*, Fig. S1).

Guantaoyuan (GTY hereafter), excavated in the 2000s, is ∼300 km west of LK. It is situated on a high platform in a small basin near the Wei River. The earliest material deposits date to the Baijia Period (7800–6900 cal. BP) (21), from which we sampled 14 pottery and stone objects, which can be classified into three functional groups. Group I (n = 6) consists of vessels hypothetically associated with alcohol making, including five globular jars (POT1–5) and one perforated basin (POT9) likely used as a funnel and steamer (see discussion below). Group II (n = 4) consists of vessels for general food storage and serving, including one tripod (POT6), one jar (POT7), and two bowls (POT11 and 12). Group III (n = 4) consists of four grinding stones (GS1–4) for food processing (*SI Appendix*, Fig. S1). Neither macrobotanical nor pollen analysis has been carried out at the sites, and thus our study of these artifacts provides comparative datasets for general food preparation and consumption. Together, we analyzed residue samples extracted from 20 artifacts, of which the pottery vessels are among the earliest known ceramics in the Wei River Valley. We also analyzed eight control samples.

Results

Starch Remains. A total of 1,128 starch granules were recovered from the artifacts, and 744 of them (66%) can be identified as one of eight types, corresponding to certain plant taxa (Fig. 2, Table 1, and *SI Appendix*, Fig. S3).

Type I: Panicoideae (n = 394; 34.9% of the total; 100% ubiquity), which may include broomcorn millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), and Job's tears (*Coix lacryma-jobi*).

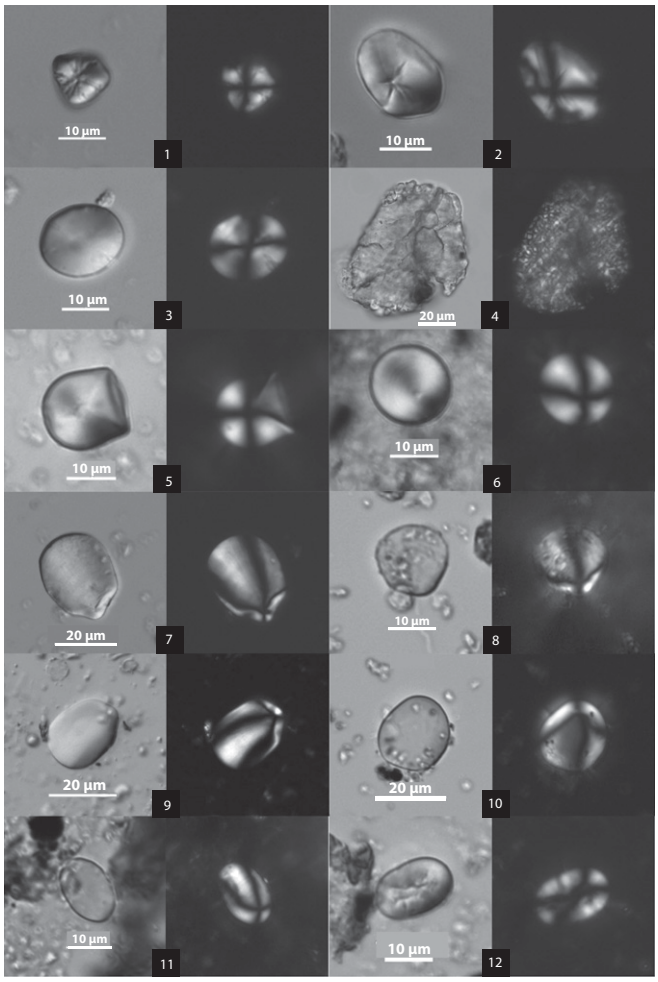

Fig. 2. LK and GTY starch types. *1*, type I, Panicoideae, likely broomcorn millet; *2*, type II, Job's tears; *3*, type III, Triticeae; *4*, type IV, rice; *5* and *6*, type V, snake gourd root; *7* and *8*, type VI, ginger (LK and GTY); *9*, ginger starch from Mijiaya for comparison; *10*, type VII, USO, possibly yam; *11*, type VII, USO, possibly lily; *12*, type VIII, bean (*1–4* and *7*, LK; *5*, *6*, *8*, and *10–12*, GTY) [each starch is shown in DIC/bright-field (*Left*) and polarized (*Right*) views].

Table 1. Lingkou and Guantaoyuan starch record

Taxa	Panicoideae I	Job's tears II	Triticeae III	Rice IV	Snake gourd V	Ginger VI	USO VII	Bean VIII	UNID	Total	Damaged	Gelatinized 1	Gelatinized 2
LK globular (n = 3)	27	4	9	25	3	1			30	99	19	34	
LK tripods (n = 3)	19	10	20					2	54	105	61	35	1
LK total, N	46	14	29	25	3	1		2	84	204	80	69	1
LK total, %	22.5	6.9	14.2	12.3	1.5	0.5		1.0	41.2	100	39.2	33.8	0.5
LK ubiquity, %	100	50	100	16.7	16.7	16.7		16.7	100		100	100	16.7
GTY globular-funnel (n = 6)	216	13	148		23	4	14	4	250	672	234	202	
GTY general pots (n = 4)	55		3				6		7	71	39	9	
GTY grinding stones (n = 4)	77		54				7		43	181	118	11	
GTY total, N	348	13	205		23	4	27	4	300	924	391	218	4
GTY total, %	37.7	1.4	22.2		2.5	0.4	2.9	0.4	32.5	100	42.3	23.6	0.4
GTY ubiquity, %	100	42.9	71.4		28.6	21.4	57.1	28.6	85.7		100	78.6	7.1
LK&GTY, total	394	27	234	25	26	5	27	6	384	1,128	471	292	
LK&GTY, %	34.9	2.4	20.7	2.2	2.3	0.4	2.4	0.5	34.0	100	41.8	25.9	
LK&GTY ubiquity, %	100	40	90	5	25	20	40	25	90	100	100	95	

Type II: Job's tears (n = 27; 2.4% of the total; 40% ubiquity), which show diagnostic characteristics specifically of Job's tears and can be clearly separated from millets (22).

Type III: Triticeae (n = 234; 20.7% of the total; 90% ubiquity), likely wild grasses, such as the genera of *Agropyron*, *Elymus*, and *Leymus*, which are native to north China (23).

Type IV: rice (*Oryza* sp.; n = 25; 2.2% of the total; 5% ubiquity), almost certainly of domesticated form, since no evidence suggests that the Wei River region was within wild rice's (*Oryza rufipogon*) endemic range (24).

Type V: snake gourd (*Trichosanthes kirilowii*; n = 26; 2.3% of the total; 25% ubiquity), commonly found in north China (25); its roots were used as food and medicine in ancient China (26).

Type VI: ginger (*Zingiber* sp.; n = 5; 0.4% of the total; 20% ubiquity), whose roots have been used as spice and medicine since ancient times in China (see discussion below).

Type VII: underground storage organs (USOs; n = 27; 2.4% of the total; 40% ubiquity), which probably include yam (*Dioscorea polystachya*) and lily (*Lilium* sp.), widely distributed in China (27). Most type VII starches are badly damaged, and are not diagnostic to specific taxa.

Type VIII: bean (Fabaceae; n = 6; 0.5% of the total; 25% ubiquity), perhaps wild species of *Vicia* pea. There are 17 species of *Vicia* peas growing in Shaanxi (28). Notably, domesticated pea (*P. sativum*) is one of the ingredients often used for making *daqu* starter today (10) (see *SI Appendix*, Fig. S3 for the description and modern reference images of diagnostic features of each starch type).

Among these plants, starches of millet, Job's tears, Triticeae grasses, snake gourd root, yam, lily, and beans have all been found on grinding stones from Upper Paleolithic and Neolithic sites in north China (29, 30).

A large majority of the starches (n = 763; 67.6%) show signs of morphological alterations, which can be classified into two types, each probably representing different food-processing techniques. The first is damaged starches without gelatinization (n = 471; 41.8% of the total; 100% ubiquity). They show random pitting, deep channels, broken edges, missing or pronounced lamellae, central depression, and/or disappearing extinction crosses under polarized light. These features are consistent with the morphological changes caused by enzymatic attack, which are commonly found on malted cereal starches (Fig. 3, *1*–*3*). Some of these kinds of damage also occur due to grinding, such as broken edges (Fig. 3, *4*). The second is damaged starches with gelatinization (n = 292; 25.9% of the total; 95% ubiquity). The gelatinized starches can be further divided into two groups: (*i*) Most granules exhibit moderate swelling with a hollowed center, resembling a diagnostic damage type caused by low-temperature mashing and fermentation, representing a majority of the granules (Fig. 3, *5* and *6* and *SI Appendix*, Fig. S3, *9*–*13*); this type of modification is different from other food-processing techniques (e.g., steaming and boiling), based on our experimental study (18) and published data (31); and (*ii*) few granules (n = 5; from LK-POT4 and GTY-POT9) show expansion without evidence of enzymatic attack, similar to those from cooked (boiling and steaming) as well as mashed cereals (Fig. 3, *7* and *SI Appendix*, Fig. S3, *14* and *15*). GTY-POT9, a perforated vessel, revealed both types of gelatinized starches. It may have been used as a funnel for processing fermented beverages, but could also be used as a steamer.

Some damaged features of starch could be the result of microbe activity in the postdepositional environment (32), but the damaged starch granules from seven control samples were much lower in frequency (n = 1–5) than residue samples (n = 1–92), and no gelatinized starch was present in five of the control samples (*SI Appendix*, Table S1). To confirm the presence of gelatinized starch, we applied the Congo red staining method (33) on four samples (GTY-POT2, 3, 5, and 9), which all revealed gelatinized starches, characterized by their red color under the bright field, and orange-red or gold-green glow under polarized light (Fig. 3, *8*; see *SI Appendix* for the staining method). More than one-third of the starches (n = 384; 34%) are not identifiable (UNID) due to their lack of diagnostic features, or due to being severely damaged.

Of the eight plant taxa identified, six are present in both the LK and GTY assemblages, including Panicoideae, Job's tears, Triticeae, snake gourd root, ginger, and bean. Panicoideae and Triticeae are the most numerous in count and in ubiquity. Rice was only found in LK, and yam and lily only in GTY (Table 1 and *SI Appendix*, Table S1).

Phytolith Remains. The LK samples yielded a large quantity of phytoliths (n = 389; *SI Appendix*, Table S2), with the majority derived from millet and rice husks. Phytoliths from Paniceae husks predominate the assemblage (n = 217; ubiquity 100%), including 28 η-type phytoliths (Fig. 4, *7*) from broomcorn millet, one Ω-type phytolith (Fig. 4, *6*) from foxtail millet (34), and 188 from undetermined millets (Fig. 4, *14*) (35, 36). Such a pattern suggests that

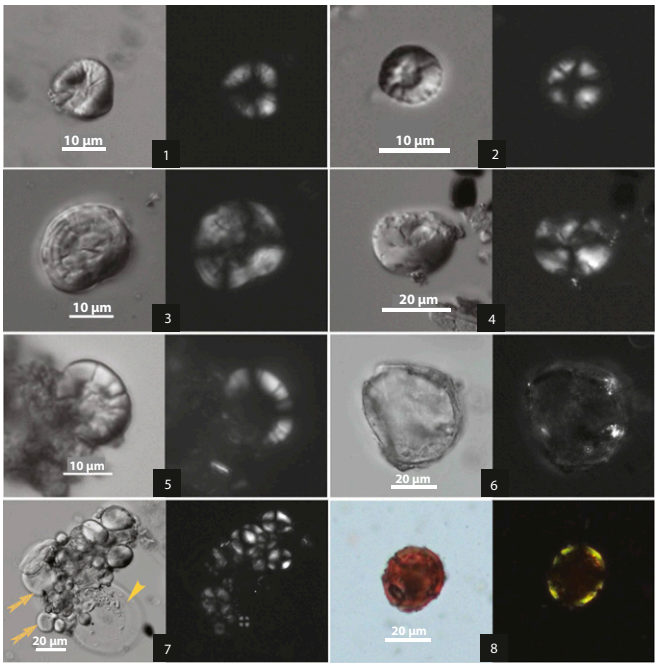

Fig. 3. Damaged and gelatinized starches from LK and GTY pottery. *1*, millet starch with central depression and broken edge; *2*, millet starch with central depression and deep channels; *3*, Triticeae starch with deep channels, pronounced lamellae, and pitting; *4*, Triticeae starch with missing part (perhaps caused by grinding), central depression, and pitting; *5*, UNID starch with central depression, pitting, and partial gelatinization; *6*, fermented starch expanded with hollowed center and birefringent edge; *7*, a cluster of Triticeae starch showing different stages of gelatinization, some with central depression (pointed with stemmed arrow), some flattened and expanded (pointed with nonstemmed arrow), and some still intact; *8*, gelatinized starch stained with Congo red, showing red in bright-field light and golden glow in polarized light (*1* and *2* from LK; others from GTY; each starch is shown in DIC and polarized views).

broomcorn, rather than foxtail, was likely the main type of millet being processed in the LK vessels. Double-peak phytoliths (Fig. 4, *5*) (n = 13) from rice husks also occurred in high ubiquity (66.7%, POT2–5), suggesting that the crop might have been commonly consumed at the site. These observations are consistent with the results of staple isotope analysis of human bones from the nearby Baijia site, indicating that human diets were composed of both C3 and C4 plants (37), such as rice and millet. Other Poaceae family morphotypes mainly include cross, bilobate, rondel, and common bulliform (Fig. 4, *1–4* and *8*). Cross phytoliths show a considerable variation in form and size, some larger than 18 μm in width (Fig. 4, *1*), which are most comparable to the large variant 1 cross type produced by Job's tears (38). One raphide is present (39), likely derived from a USO or external contamination. The profile of LK phytoliths largely corroborates the starch granule assemblage, indicating the presence of millets, rice, Job's tears, and tubers.

The phytolith assemblage from GTY residue samples shows a clearly different profile. Among the 387 recovered phytoliths, none were identified as millet husk, whereas the majority were elongate cell phytoliths from grass stems/leaves (n > 240) and hair cells (n = 59). Raphides (n = 4) were also found, consistent with the presence of USO starches, possibly from yam. One opaque perforated platelet was recovered (POT2), consistent with those from Asteraceae inflorescences (40) (Fig. 4 and *SI Appendix*, Table S3). The absence of husk phytoliths indicates that cereals were dehusked before processing in the pots.

Fungi: Molds and Possible Yeasts. Numerous fungus particles were present in the GTY assemblage, including spores, sporangia/vesicles, hyphae, and possible yeasts. We examined eight residue samples (two globular jars, a tripod, a jar, the funnel-steamer, a bowl, and two slabs) and five control samples, and recorded 1,001 individuals and particle clusters of fungi. Fungi were most abundant in globular jars, the funnel-steamer, and the bowl (range 114–296), and were less common in other vessels and grinding stones (range 9–36), with the lowest frequencies from the control samples (range 2–10). When comparing fungus counts in the residues with those in the controls from the same vessels (two globular jars and one bowl), the ratios are 55:1, 30:1, and 44:1 (*SI Appendix*, Fig. S4*A*). This pattern suggests very high concentrations of fungi associated with the functional areas of these vessels.

Some fungi were consistent morphologically with those from *Aspergillus* and *Rhizopus*, which are among the most commonly used species in modern *qu* starter (10, 11). For example, several fungi show a round head with a long stem, resembling a vesicle with conidiophore from *Aspergillus* (Fig. 5, *1* and *2* compared with *9* and *10* and *SI Appendix*, Fig. S4*B*). Some spores (ca. 10 μm in diameter) are round and still interconnected, likely from a chain of conidia, which is characteristic of *Aspergillus*; another abundant spore form is very small (ca. 3–5 μm), ovoid in shape, consistent with sporangiospores in *Rhizopus* (Fig. 5, *7* and *8* compared with *12–15* and *SI Appendix*, Fig. S4*C*). Some spores appear in germination (Fig. 5, *5*). Hypha fragments were numerous, many entangled, similar to mycelium (Fig. 5, *3*, *4*, and *6* compared with *10* and *11*), but they cannot be identified to the genus level (see *SI Appendix* for further explanation).

Some particles identified as possible yeast cells (n = 21) were present in the globular jars, the funnel-steamer, and the bowl. They are subround or oval in shape, 4.9–11.75 μm in size. Several show a small protuberance on the parent cell. These protuberances are a frequent feature of both budding and mating processes of *Saccharomyces* (41). They are morphologically comparable to wild *Saccharomyces cerevisiae* yeast in the modern millet beer from Shimao, north Shaanxi (3.47–12.16 μm in size), but larger than a cultured *S. cerevisiae* strain (2.64–8.83 μm) in our database (Fig. 5, *17–20* and *SI Appendix*). Further testing with DNA and other diagnostic biomolecules will help verify this tentative identification, as *S. cerevisiae* is the most commonly occurring yeast species in modern Chinese alcohol production (10, 11), and China is likely the center of origin of its domesticated populations (42).

Other types of fungus spores were also found in the GTY samples, some consistent with *Trichothecium* and *Alternaria* (41). However, these are less abundant and do not represent known components of modern *qu*, making interpretation more difficult.

Summary. Millets (mainly broomcorn millet) are present in all artifact groups at both sites with the highest percentage and ubiquity, followed by Triticeae and Job's tears. These plants are likely to have been the main sources of staple food. Snake gourd root, bean, and ginger are also present at both sites, but represented at much lower levels. Rice starch and phytoliths are present only at LK, while yam and lily starches are only found at GTY.

Damaged and gelatinized starch granules are present in all vessels, suggesting that they were in contact with cooked and/or fermented foods. The gelatinized starches are particularly high in percentage among the fermentation vessels (13–70%). On the other hand, grinding stones revealed a very high proportion of damaged starch (44–74%) but a very low percentage of gelatinized ones (0–17%), a composition consistent with their function for grinding (*SI Appendix*, Fig. S5*C* and Table S1). When comparing the starch counts between residue and control samples on the same vessels, the former yielded much greater numbers,

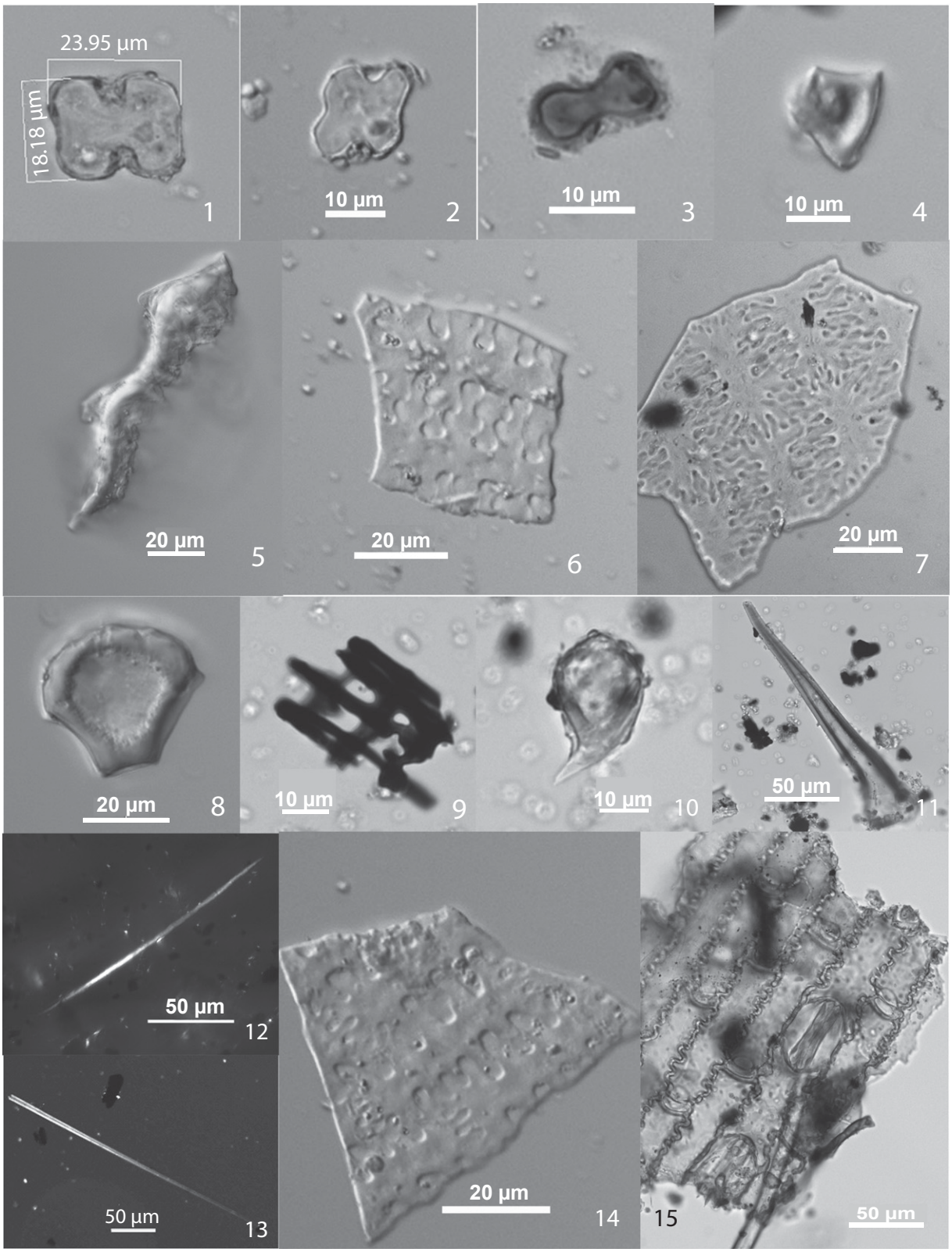

Fig. 4. Phytoliths from LK and GTY. *1*, cross (Job's tears); *2*, cross; *3*, bilobate; *4*, rondel; *5*, rice double peak from husk; *6*, foxtail millet husk; *7*, broomcorn millet husk; *8*, bulliform; *9*, Asteraceae opaque perforated platelets; *10* and *11*, hair cells; *12* and *13*, raphides; *14*, Paniceae husk; *15*, skeleton of long cells from grass leaf (*1–8*, *13*, and *14* from LK; others from GTY).

about 12–22 times of the latter (*SI Appendix*, Fig. S5 *A* and *B*), suggesting that most starch granules in the residue samples are related to the original function of the artifacts.

While cereal husks are absent, fungi are present in abundance at GTY. They are especially numerous in the globular jars, the funnel-steamer, and the bowl, which are likely to have been

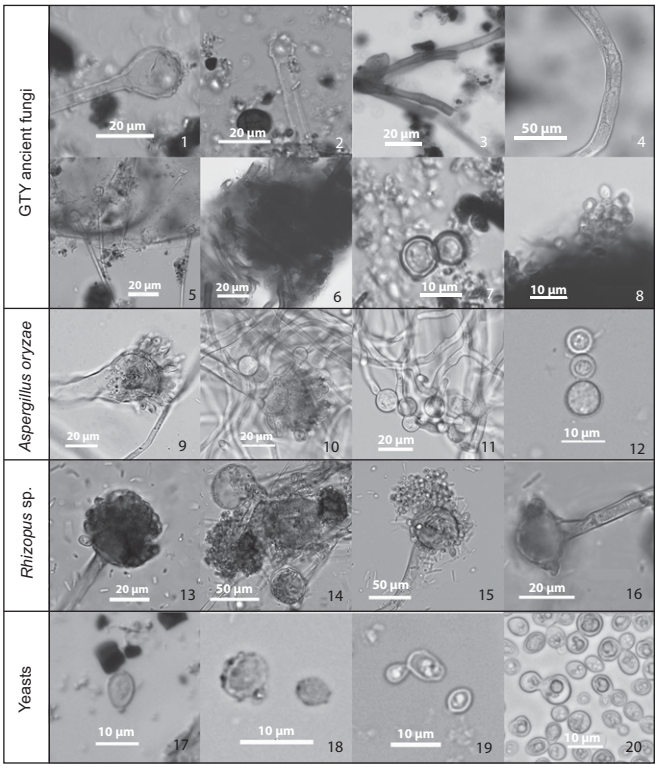

Fig. 5. Molds and possible yeast cells from GTY compared with modern references. GTY samples: *1* and *2*, vesicle/sporangia with stipe, without phialides/spores attached (POT2), compared with *9* and *14*; *3*, cf. sporangiophores growing out of rhizoids (POT1), compared with *Rhizopus* in *SI Appendix*, Fig. S4*C*; *4*, hyphae (POT2), compared with *11*; *5*, cf. spores in germination (POT9); *6*, a cluster of hyphae (POT9), compared with *10* and *11*; *7*, spores in chain (POT2), compared with chained conidia from *A. oryzae* (*12*); *8*, cf. sporangiospores released from sporangia (POT9), compared with *Rhizopus* (*15*); *17* and *18*, possible yeast cells in the initial budding process (POT2 and 9). Modern samples: *9*, *A. oryzae* conidial head; *10*, conidial head with a cluster of hyphae; *11*, a group of conidiophores with vesicles; *12*, conidia in chain; *13*, *Rhizopus* sp. sporangia showing sporangiospores; *14*, a group of sporangia with and without sporangiospores entangled with hyphae; *15*, sporangia releasing small, ovoid sporangiospores; *16*, sporangia without sporangiospores; *19*, wild *S. cerevisiae* yeast in budding, Shimao millet beer; *20*, cultured, domesticated *S. cerevisiae* yeast in various budding forms.

relevant to alcohol fermentation and consumption (*SI Appendix*, Fig. S4*A*). Fungi naturally exist on leaves, and thus the association of fungi with leaf phytoliths explains why grasses were used as *caoqu*.

Discussion

Some marked differences are notable between the fermentation-related vessel group (globular jars and funnel-steamer) and other artifact groups. First, the starches with fermentation-related gelatinization were found in highest proportions in the fermentation group (*SI Appendix*, Fig. S5*C*), suggesting that the former group is preferentially associated with fermentation-based food processing. Second, some plant taxa are present only in the former group, including snake gourd root and ginger, indicating that they may have had a special function related to alcohol making. Finally, fungi (including possible yeasts) are present in greater abundance in GTY globular jars, the funnel-steamer, and the bowl, indicating that they were likely in more frequent contact with fermented foods.

Globular jars (for effective fermentation and storage) and funnel-steamers (for transferring and filtering liquid, as well as steaming grains) appear to have been developed as a set of equipment used for producing fermented beverages, among other culinary functions. The presence of these new pottery types represents a point of departure from making ordinary porridges with open-mouthed pots before 9000 cal. BP. This change parallels the transition in ceramic forms from open-mouthed bowls to globular jars in sub-Saharan Africa, which has been seen as the development of a millet/sorghum beer-brewing tradition (43, 44).

The combination of a globular jar with a single-perforation funnel-steamer predates the more specialized alcohol-making toolkit consisting of *jiandiping* amphora, funnel, and multi-perforation steamer, which was developed during the Yangshao culture (ca. 7000–5000 cal. BP) (7, 15).

Regional Variations in Alcohol Production. There were apparently regional differences in plant ingredients used for alcohol making. The presence of rice only at LK is likely attributable to the different ecological conditions of the sites. LK is situated in the alluvial plain with abundant water resources, an environment more favorable for rice growing than GTY, which is located on the highland plateau region with a cooler and dryer climate (*SI Appendix*, Fig. S1). Samples from additional sites would be beneficial to better establish this contrast.

There were also regional variations in brewing methods, particularly relating to the methods for achieving saccharification. The starch remains from both sites exhibit evidence of likely cereal-based alcohol fermentation, but the phytolith assemblages differ in composition between the two sites. The LK phytolith assemblage consists of a significant proportion of husk phytoliths from Paniceae, broomcorn millet, and rice, suggesting that the fermented beverages were made with unhulled, sprouted broomcorn millet, and probably rice, mixed with additional ingredients (Triticeae, beans, tubers, and probably other materials). The use of sprouted broomcorn millet for beer brewing has indeed been testified to by the residue analyses of pottery vessels from Neolithic Yangshao culture sites at Yangguanzhai, Xinjie, and Mijiaya, all located within an 80-km radius of LK (*SI Appendix*, Fig. S1) (15–17). An ethnographic comparison of millet-based beer making can be found in Yulin, north Shaanxi, where the local residents use sprouted wheat or maize as the saccharifying agent to brew unfiltered, low-alcohol broomcorn millet beer, called *hunjiu* 浑酒 (turbid alcohol) (*SI Appendix*, Fig. S2).

In contrast, the GTY assemblage contains no husk phytoliths, but many from leaves and stems. In addition, GTY samples revealed ubiquitous fungi, some of which are consistent with *Aspergillus* sp. and *Rhizopus* sp., commonly found in *qu* today. During the fermentation process, fungal growth and reproduction (indicated by the development of hyphae and sporangia) require certain conditions, such as the presence of sources of nutrients, a moist environment, and a certain temperature range (10, 12, 45, 46). Such conditions do not naturally occur in normal soil matrix, but may be achieved by using particular types of containers. Therefore, much higher abundancies of fungal particles associated with ubiquitous starches from residues on vessel interiors than in the sediments on exteriors indicate the presence of cereal-based fermentation using *qu* as a starter (*SI Appendix*, Figs. S4*A* and S5 *A* and *B*).

According to ancient Chinese texts and ethnohistoric data, diverse grains and plants were used for making *qu* and *caoqu*. Taiwanese aboriginals, for example, made *qu* by leaving cooked grain in the open for days until it became moldy, and then used it as the starter for making millet or rice alcoholic beverages. They also made *caoqu* with plants from such families as Rutaceae, Fabaceae, Asteraceae, and Chenopodiaceae (7–9, 47). In south China, *Polygonum* spp. are used as *caoqu* for making rice wine (13). Many herbs have also been added as ingredients during the fermentation process, including ginseng (*Panax ginseng*), hemlock parsley (*Lingusticum wallichii*), and ginger (*Zingiber* sp.), among others (7). If *qu*, *caoqu*, and herbs were used for cereal-based fermentation,

we would expect to find damaged and gelatinized starches, phytoliths from leaves, filamentous fungi, and yeasts on the vessel surface. All these are indeed present in the GTY residues, including some herbs (e.g., ginger) traditionally used for fermentation.

Evidently, Early Neolithic people in north China experimented with diverse fermentation methods, which were later elaborated in dynastic times, described in ancient texts as using sprouted cereals (*nie*) to make *li*, moldy grains (*qu*) to make *jiu*, and herbs (*caoqu*) to make *chang* (7, 9). We do not have clear evidence for mastication based on the current data, as this method may not be easily detected archaeologically, though this does not rule out the possibility that it was also used in ancient times. We need to develop methods to test for mastication in archaeological contexts.

Ginger and Medicinal Function of Early Alcoholic Beverages. The ginger starch granules from LK and GTY are nearly identical to the modern cultivated ginger (*Zingiber officinale*) from north China (Fig. 2, *7* and *8* compared with *SI Appendix*, Fig. S3, *6*). The wild progenitor of ginger is unknown, and the origin of its domestication is unclear. However, ginger was under cultivation as a spice and a medicinal plant from ancient times in India and China (48). In China, ginger starch granules have been identified at the Neolithic Dadiwan site (ca. 7800–7300 cal. BP), about 200 km west of GTY (*SI Appendix*, Fig. S1) (49). A ginger starch granule was found in the beer residues on a funnel from Mijiaya (ca. 5000 cal. BP) (Fig. 2, *9*), although it was misidentified as yam in the original publication (figure 3E in ref. 15). The earliest ginger root remains have been unearthed in water-logged Wangshan Tomb 2 in Hubei (50), dating to the end of the Eastern Zhou (770–256 BC) (51). Ginger has been traditionally used as an ingredient for making medicinal alcohol, named *jiangjiu* 薑酒 (ginger alcohol), as recorded in *Bencao Gangmu* in the 16th century (52). It is conceivable that some of the earliest fermented beverages were made for medicinal purposes, among other functions, beginning a long tradition of alcohol use for health benefits in China.

The Spread of Domesticated Cereals and the Emergence of Alcohol-Making Pottery. The domestication processes of millet and rice probably initiated in the Yellow River and Yangzi River valleys, respectively, around 10000 cal. BP (53, 54), predating the first appearance of globular jars. In north China, domesticated millets, both broomcorn and foxtail, became widespread by 8000 cal. BP, while rice was less commonly cultivated. In the region north of the Huai River, the arguably earliest domesticated rice remains have been identified at Jiahu (ca. 9000 cal. BP) (55), where the earliest rice-based beer was also recovered (6). The rice starch and phytoliths on the alcohol-making pottery from LK account for the first appearance of this crop in northwest China. Given its association with fermentation-related vessels, rice's northwestward expansion nearly 8,000 y ago may have been partially related to its importance in alcohol production.

Based on the current data, three material changes occurring in 9000–7000 cal. BP signal the innovation of specialized alcoholic making in some regions in China: (*i*) the spread of cereal domestication (millet and rice), (*ii*) the emergence of dedicated pottery types (globular jar and funnel-steamer, among others), and (*iii*) the development of cereal-based alcohol production with at least two fermentation methods: use of malts and *qu* starters.

There was a period of 11,000 y from the first simple pottery vessels (20000 cal. BP) to the first alcohol-making globular jars, raising the question of whether people had already experimented with alcohol fermentation before 9000 cal. BP. A pre-Neolithic alcohol is not impossible, as exemplified by the discovery of 13,000-y-old wheat/barley-based beer brewing in stone mortars at Raqefet Cave, Israel (56). It is also unclear what other functions the early globular jars may have had, in addition to alcohol fermentation. More functional studies on Paleolithic and Early Neolithic pottery are needed.

Conclusions

The appearance of new pottery types (globular jars and funnel-steamers) around 9000–7000 cal. BP marks the earliest development of specialized alcohol production. The major ingredients of cereal-based alcoholic beverages include broomcorn millet, Triticeae, Job's tears, and rice, which were mixed with snake gourd root, ginger, yam, lily, and other plant additives, some likely used for medicinal properties. Different regional traditions in alcohol production may have already been developed at this time. People utilized diverse local plants and experimented with different brewing methods, such as malting millet, making *qu* with moldy grain, and using herbs as *caoqu*. These ancient beverages were likely very low in alcohol content, similar to the millet beer *hunjiu* made by people in north Shaanxi today, but they were the prototypes of ancient alcohol, recorded in the earliest Chinese writing system as *li*, *jiu*, and *chang*. These beverages may have served social, economic, spiritual, and medicinal functions, helping the development of sophisticated cultural and ritual traditions in ancient China.

Methods

Residue samples were processed with protocols established in the Stanford Archaeology Center (see *SI Appendix* for details). Starch and phytolith identifications are based on our modern reference collection of over 1,100 specimens and published information (34, 40, 57). Fungi in modern *qu* and *koji* reference and ancient samples were identified based on descriptions in published sourcebooks (41, 58, 59). Yeast cells were compared with modern samples identified by DNA testing. In brief, modern reference samples had DNA isolated and the resulting material was high throughput-sequenced to identify species, and these were compared with existing reference samples to determine likely origin (see Dataset S1 for the raw data and *SI Appendix* for more detailed descriptions of methods) (60).

Our laboratory in the Stanford Archaeology Center has been regularly cleaned and checked to prevent starch contamination. We collected eight control samples from artifacts and tested them for starch, phytolith, and fungus. The results show considerably higher counts of microfossils in the residues than the controls, and compositions between the two are often very different, supporting the authenticities of the microfossils in the residue samples (*SI Appendix*, Figs. S4 and S5 and Table S4).

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1. L. Wang, P. Sebillaud, The emergence of early pottery in East Asia: New discoveries and perspectives. *J. World Prehist.* **32**, 73–110 (2019).
2. X. Yang *et al*., Starch grain evidence reveals early pottery function cooking plant foods in north China. *Chin. Sci. Bull.* **59**, 4352–4358 (2014).
3. L. Liu, X. Chen, *The Archaeology of China: From the Late Palaeolithic to the Early Bronze Age* (Cambridge University Press, Cambridge, UK, 2012).
4. L. Liu, Zaoqi taoqi, zhuzhou, niangjiu yu shehui fuzahua de fazhan (Early pottery, porridge, and development of social complexity). *Zhongyuan Wenwu* **2**, 24–34 (2017).
5. I. S. Hornsey, *A History of Beer and Brewing* (The Royal Society of Chemistry, Cambridge, UK, 2003).
6. P. E. McGovern *et al*., Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 17593–17598 (2004).
7. H. T. Huang, *Science and Civilisation in China: Vol. 6, Biology and Biological Technology; part V, Fermentations and Food Science* (Cambridge University Press, Cambridge, UK, 2000).
8. C. Ling, Zhongguo ji dongnanya de juejiu wenhua (The culture of masticating alcohols in China and Southeast Asia). *Bull. Inst. Hist. Philol. Acad. Sin.* **4**, 1–23 (1957).
9. C. Ling, Zhongguo jiu zhi qiyuan (The origins of alcohol in China). *Bull. Inst. Hist. Philol.* **29**, 883–901 (1958).
10. G. Jin, Y. Zhu, Y. Xu, Mystery behind Chinese liquor fermentation. *Trends Food Sci. Technol.* **63**, 18–28 (2017).

11. X.-W. Zheng, M. R. Tabrizi, M. J. R. Nout, B.-Z. Han, *Daqu*—A traditional Chinese liquor fermentation starter. *J. Inst. Brew.* **117**, 82–90 (2011).
12. B. Chen, Q. Wu, Y. Xu, Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese Maotai-flavor liquor. *Int. J. Food Microbiol.* **179**, 80–84 (2014).
13. W. Yu, Niangzao Jiangnan mijiu de caoqu (The herb-starter for making rice wine in southern China). *Dongfang Meishi Xueshuban* **4**, 75–80 (2003).
14. S. Wen, T. Yuan, *Yinxu Buci Yanjiu: Kexue Jishu Pian (A Study of Oracle Bones from Yinxu: Science and Technolgy)* (Sichuansheng shehui kexueyuan chubanshe, Chengdu, China, 1983).
15. J. Wang *et al*., Revealing a 5,000-y-old beer recipe in China. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 6444–6448 (2016).
16. L. Liu, J. Wang, Y. Zhao, L. Yang, Yangshao wenhua de guyajiu: Jiemi Yangguanzhai yizhi de taoqi gongneng (Beer in the Yangshao culture: Decoding the function of pottery at the Yangzhuanzhai site). *Nongye Kaogu* **6**, 26–32 (2017).
17. L. Liu *et al*., Shaanxi Lantian Xinjie yizhi Yangshao wenhua wanqi taoqi canliuwu fenxi: Niangzao guyajiu de xinzhengju (Residue analyses on pottery from the late Yangshao culture site of Xinjie in Lantian, Shaanxi: New evidence of beer brewing). *Nongye Kaogu* **1**, 7–15 (2018).
18. J. Wang *et al*., Identifying ancient beer brewing through starch analysis: A methodology. *J. Archaeol. Sci. Rep* **15**, 150–160 (2017).
19. B. Zhou, "Baijiacun yizhi dongwu yihai jianding baogao (Identification of faunal remains from Baijiacun site)" in *Lintong Baijia*, Institute of Archaeology CASS, Ed. (Bashu Press, Chengdu, China, 1994), pp 123–126.
20. Shaanxi Institute of Archaeology, Ed., *Lintong Lingkoucun* (Sanqin Press, Xi'an, China, 2004).
21. Shaanxi Institute of Archaeology, Archaeological Team of Baoji City, Eds., *Baoji Guantaoyuan* (Wenwu Press, Beijing, China, 2007).
22. L. Liu, S. Ma, J. Cui, Identification of starch granules using a two-step identification method. *J. Archaeol. Sci.* **52**, 421–427 (2014).
23. Z. Y. Wu, P. H. Raven, D. Y. Hong, Eds., Poaceae. *Flora of China* (Science Press, Beijing, China, 2006), vol. 22.
24. D. A. Vaughan, B.-R. Lu, N. Tomook, The evolving story of rice evolution. *Plant Sci.* **174**, 394–408 (2008).
25. Z. Y. Wu, P. H. Raven, D. Y. Hong, Eds., Cucurbitaceae through Valerianaceae, with Annonaceae and Berberidaceae. *Flora of China* (Science Press, Beijing, China, 2011), vol. 19.
26. S. Zhu, Ed., *Jiuhuang Bencao (Herbal for Relief of Famines)* (Shangwu Press, Taipei, Taiwan, 1406).
27. Z. Y. Wu, P. H. Raven, Eds., Flagellariaceae through Marantaceae. *Flora of China* (Science Press, Beijing, China, 2000), vol. 24.
28. Northwest Institute of Botany CAS, Ed. *Qinling Zhiwuzhi* (*Flora of Qinling Mountains*) (Kexue Press, Beijing, China, 1981), vol. 1.3.
29. L. Liu, A long process towards agriculture in the Middle Yellow River valley, China: Evidence from macro- and micro-botanical remains. *J Indo-Pac Archaeol* **35**, 3–14 (2015).
30. L. Liu *et al*., Harvesting and processing wild cereals in the Upper Palaeolithic Yellow River valley, China. *Antiquity* **92**, 603–619 (2018).
31. A. G. Henry, H. F. Hudson, D. R. Piperno, Changes in starch grain morphologies from cooking. *J. Archaeol. Sci.* **36**, 915–922 (2009).
32. A. Hutschenreuther, J. Watzke, S. Schmidt, T. Büdel, A. G. Henry, Archaeological implications of the digestion of starches by soil bacteria: Interaction among starches leads to differential preservation. *J. Archaeol. Sci. Rep.* **15**, 95–108 (2017).
33. J. Lamb, T. Loy, Seeing red: The use of Congo red dye to identify cooked and damaged starch grains in archaeological residues. *J. Archaeol. Sci.* **32**, 1433–1440 (2005).
34. H. Lu *et al*., Phytoliths analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). *PLoS One* **4**, e4448 (2009).
35. A. R. Weisskopf, G.-A. Lee, Phytolith identification criteria for foxtail and broomcorn millets: A new approach to calculating crop ratios. *Archaeol. Anthropol. Sci.* **8**, 29–42 (2014).
36. M. Madella, C. Lancelotti, J. J. García-Granero, Millet microremains—An alternative approach to understand cultivation and use of critical crops in Prehistory. *Archaeol. Anthropol. Sci.* **8**, 17–28 (2016).
37. P. Atahan *et al*., Early Neolithic diets at Baijia, Wei River valley, China: Stable carbon and nitrogen isotope analysis of human and faunal remains. *J. Archaeol. Sci.* **38**, 2811–2817 (2011).
38. N. A. Duncan, J. Starbuck, L. Liu, A method to identify cross-shaped phytoliths of Job's tears, *Coix lacryma-jobi* L., in northern China. *J. Archaeol. Sci. Rep.* **24**, 16–23 (2019).
39. A. Crowther, "Morphometric analysis of calcium oxalate raphides and assessment of their taxonomic value for archaeological microfossil studies" in *Archaeological Science Under a Microscope: Studies in Residue and Ancient DNA Analysis in Honour of Thomas H. Loy*, M. Haslam, G. Robertson, A. Crowther, S. Nugent, L. Kirkwood, Eds. (ANU E Press, Canberra, Australia, 2009), pp. 102–128.
40. D. R. Piperno, *Phytoliths: A Comprehensive Guide for Archaeologists and Paeoecologists* (Altamira Press, Lanham, MD, 2006).
41. G. St-Germain, R. Summerbell, *Identifying Fungi: A Clinical Laboratory Handbook* (Star Publishing, Belmont, CA, 2011).
42. S.-F. Duan *et al*., The origin and adaptive evolution of domesticated populations of yeast from Far East Asia. *Nat. Commun.* **9**, 2690 (2018).
43. D. N. Edwards, Sorghum, beer and Kushite society. *Norw. Archaeol. Rev.* **29**, 65–77 (1996).
44. R. Haaland, Porridge and pot, bread and oven: Food ways and symbolism in Africa and the Near East from the Neolithic to the present. *Camb. Archaeol. J.* **17**, 167–183 (2007).
45. W. Shurtleff, A. Aoyagi, *History of Koji—Grains and/or Soybeans Enrobed with a Mold Culture (300 BCE to 2012): Extensively Annotated Bibliography and Sourcebook* (Soyinfo Center, Lafayette, CA, 2012).
46. Y. Zhu, J. Tramper, Koji—Where East meets West in fermentation. *Biotechnol Adv.* **31**, 1448–1457 (2013).
47. H. Yuasa, *Segawa's Illustrated Ethnography of Indigenous Formosa People: The Bunun* (SMC Publishing, Taipei, Taiwan, 2009).
48. P. N. Ravindran, K. N. Babu, Eds., *Ginger: The Genus Zingiber* (CRC Press, Boca Raton, FL, 2004).
49. J. Wang, X. Zhao, H. Wang, L. Liu, Plant exploitation of the first farmers in northwest China: Microbotanical evidence from Dadiwan. *Quat. Int.*, 10.1016/j.quaint.2018.10.019 (2018).
50. Cultural Relics Team HPBC, Hubei Jiangling sanzuo Chumu chutu dapi zhongyao wenwu (Large amounts of cultural relics unearthed from three Chu tombs in Jiangling, Hubei). *Wen Wu* **5**, 33–39 (1966).
51. Z. Fang, Chulun Jiangling Wangshan Chumu de niandai yu muzhu (On the dates and occupants of the Wangshan Chu tombs in Jiangling, Hubei). *Jianghan Kaogu* **1**, 59–62 (1980).
52. S. Li, *Bencao Gangmu (Materia Medica, Arranged According to Drug Descriptions and Technical Aspects)* (Public Health Press, Beijing, China, 1981).
53. Z. Zhao, Zhongguo gudai nongye de xingcheng guocheng—Fuxuan chutu zhiwu yicun zhengju (The process of origin of agriculture in China: Archaeological evidence from flotation results). *Quat. Sci.* **34**, 73–84 (2014).
54. X. Zuo *et al*., Dating rice remains through phytolith carbon-14 study reveals domestication at the beginning of the Holocene. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 6486–6491 (2017).
55. Z. Zhao, J. Zhang, Jiahu yizhi 2001 niandu fuxuan jieguo fenxi baogao (Report of flotation results from the 2001 season at the Jiahu site). *Kaogu* **8**, 84–93 (2009).
56. L. Liu *et al*., Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: Investigating Natufian ritual feasting. *J. Archaeol. Sci. Rep.* **21**, 783–793 (2018).
57. M. Madella, A. Alexandre, T. Ball, International code for phytolith nomenclature 1.0. *Ann. Bot.* **96**, 253–260 (2005).
58. J. Webster, R. Weber, *Introduction to Fungi* (Cambridge University Press, Cambridge, UK, 2007).
59. J. Wei, *Zhenjun Jianding Shouce (Handbook of Identification of Fungi)* (Shanghai Science and Technology Press, Shanghai, China, 1979).
60. N. Sinnott-Armstrong, DNA sequencing of cultured millet beer. NIH Sequence Read Archive. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA535381. Deposited 15 May 2019.

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Supplementary Information for

The origins of specialized pottery and diverse alcohol fermentation techniques in Early Neolithic China

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This PDF file includes:

Supplementary text
Figs. S1 to S5
Tables S1 to S4
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Dataset S1

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Supplementary Information Text

Extended description of methods and results

1. Sample collecting and processing methods

We collected residue samples from GTY and LK pottery (Figure S1) in the Baoji Museum and the Jingwei Archaeology Station in Xi'an, respectively. The accessibility to the pottery vessels was limited, as it was difficult to locate specific vessels in the storage facilities. The pottery vessels and sherds have been washed after excavation, but some still show visible but often sporadic residues (yellowish substances) on the interior surface, often near the base, which were the targeted sampling spots. Each pottery sample was first cleaned with a new toothbrush to remove loose soil on the surface. Then residues for microbotanical analysis were extracted from pottery by two methods: 1) for small sherds, the artifacts were immersed into an ultrasonic bath for 3 minutes; and 2) for large vessels, an ultrasonic toothbrush was used to clean the vessel's surface for 3 minutes. The residue liquid from each sample was kept in a test tube. The control samples from sediments on the pottery exterior surfaces and the unused side of a grinding stone were processed in the same way as residue samples.

The ultrasonic extraction method helps to remove residues embedded inside porous vessel surface, which are likely related to the pottery function, while the control samples scraped from the surface sediments may contain surrounding soil matrix and later contaminations.

Residue extraction involves two procedures. 1) EDTA dispersion; after centrifuging and decanting supernatant, 4 ml of 0.1% EDTA ($Na_2EDTA•2H_2O$) solution was added to each tube (15 ml). The tubes were placed in an automatic shaker for 2 hours to disperse the sediments, then filled with distilled water and centrifuged for 5 min at 1,500 rpm, and the supernatant was decanted. 2) Heavy liquid separation; 4 ml of SPT (sodium polytungstate) at a specific gravity of 2.35 was added to each tube. The tubes were then centrifuged for 15 min at 1,000 rpm. The top 1-2 mm layer of organics was carefully removed from each tube by a new pipette and then transferred into a new 15 ml tube. The samples were topped off with distilled water and centrifuged for 5 min at 1,500 rpm to concentrate the microfossils (starch, phytoliths, and fungi) at the bottom of the tube, and the supernatant was decanted. The rinse was repeated two more times to remove any remaining SPT.

The Congo Red staining method follows the protocol provided in Lamb and Loy (2005) (1) with modifications, as follows: 1) A small proportion of processed residues (dried) is left in a test tube after most of it was extracted for starch and phytolith analyses; 2) apply 40 mL of Congo Red solution (1 mg ml^{-1} aqueous pH 7) to the tube; 3) agitate the tube bottom with a pipette tip; 4) transfer the Congo Red solution from the tube with pipette onto a microscope slide, cover with a cover slip, and seal it with nail polish on the edges; and 5) wait for 15 min allowing to stain before viewing under the microscope.

Microscopic analysis: 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.

2. Making millet beer in north Shaanxi Province

In order to understand the brewing process of ancient alcohol and the morphological changes of starch granules caused by fermentation, we investigated the brewing methods of millet beer, *hunjiu* (turbid alcohol) in Shimao village, Yulin city, north Shaanxi province. It is a traditional drink made by the local people, often during the Chinese New Year (in the winter) in the past, but it has been more commonly made throughout the year thanks to the availability of refrigeration in recent years. The basic raw materials of the beer are wheat/maize and millet, and the brewing can be divided into five steps as follows.

1) Germination of cereals for making malt: Soaking the wheat or maize in water for one day, drain the water and leave the wheat/maize in a container for three days, sprinkling water in the morning and evening each day until the seeds are sprouting; drying the sprouted seeds either by baking or by sun drying, then grinding into flour to be used as the starter.

2) Steaming millet flour: broomcorn millet, about 5 kg, is dehusked, steeped for 1 hour, and ground into flour. Spreading the millet flour on a steamer to form a thin layer, waiting for a few moments until the flour appears wet, then sprinkling a new layer; repeat the process 6-7 times until all the remaining flour is steamed and forming a cake. The entire steaming process takes about 5-6 minutes (Fig. S2:1-3).

3) Mixing the millet cake with the wheat/maize malt: placing the millet cake on a wooden board, kneading it with the malt flour until fully mixed (some people add a small amount of ground maize flour to reduce the stickiness of the millet cake); the ratio of malt to millet varies among different families, ranging from 1:5 to 1:10 (Fig. S2:4,5).

4) Placing the mixed cake in a mash pot, which has a small mouth, a short neck, and a large body; pouring boiled water into the pot and using a wooden pestle to stir the cake with water into paste (Fig. S2:6,7).

5) Covering the mash pot with a lid, and placing it at a warm spot in the room, often on the heated bed (*kang*); after 24 hours the mash is fermented. The mash is mixed with hot water (1:3 or 1:4 ratio), heated to boiling, and served hot. It is a yellowish, porridge-like, and low-alcoholic beverage, and tastes slightly sour and sweet (Fig. S2:8-9).

This millet beer may be very similar to one of the ancient alcohols, *li*, made of *nie* (sprouted grain), which was said to have a sweet taste (2: 159-162). It is noteworthy that the use of wheat and maize germination for making malt would have been a practice after the introduction of these crops to China from West Asia (during the third millennium BC) and the New World (after 1492), respectively. The early Neolithic people in north China would have utilized native plants, wild and domesticated, for cereal germination. The sprouted wheat/maize and millet for making *hunjiu* are ground into very fine flour with milling machines today, but the early Neolithic people may have ground the cereals coarsely with grinding stones or mortars and pestles.

3. Starch types

Starch types are determined based on our reference data (Fig. S3:1-8).

Type I starch granules are characterized by their round or polygonal shape, V-, Y-, or linear shaped fissure, centric hilum, and extinction cross with straight harms. The size ranges are 6.82-16.5 μm for LK and 4.84-25.58 μm for GTY. These measurements fall into those of undamaged native starch from broomcorn millet (3.03-12.8 μm), foxtail millet (4.84-21.17 μm) and Job's tears (4.54-29.2 μm) in our modern reference (3) (Fig. S3:1,2). However, since many granules are damaged and thus enlarged in size in the ancient assemblages, we cannot employ the criteria for identifying native starch. Therefore, Type I starches may consist of two types of millets as well as Job's tears, all belonging to Panicoideae subfamily.

Type II starch granules are similar to Type I, but exhibit larger size ranges (16.78-24.12 μm for LK and 13.27-26.01 μm for GTY), eccentric hilum, and extinction cross with zig-zag arms. These are typical features of Job's tears (Fig. S3:2), rarely found in millets (3).

Type III starch granules resemble Triticeae. They are characterized by lenticular shape and centric hilum, and many species under Triciceae tribe share such a general form. *Agropyron cristatum*, for example, has a size range of 10.54-38.8 μm (Fig. S3:3). The size range of Type III for LK is 13.96-26.99 μm, but for GTY is 5.02-53.98 μm due to the presence of some very large gelatinized starch granules.

Type IV starch granules are very small (3.63-5.7 μm) and appear in a compound form. Most individual granules are blurry, but when visible, they are polygonal in shape. These forms match rice (*Oryza sativa*) starch in fermented conditions based on our modern reference data (Fig. S3:13).

Type V starch granules (size range 16.15-20.2 μm for LK and 7.39-28.58 μm for GTY) consist of two forms, bell-shaped and spherical; the hilum is often eccentric, and the extinction crosses often have bend arms. These starch forms are found in the roots of snake gourd (*Trichosanthes kirilowii*) (Fig. S3:5).

Type VI starch granules (size range 29.49 μm for LK and 20.67-24.77 μm for GTY) are elongate oval (for larger ones) and nearly triangular (for smaller ones); the hilum is located extremely near the edge with a relief visible under the DIC view, particularly shown on large granules. These characteristics are the most diagnostic features of many *Zingiber* species (Torrence and Barton 2006, Plate 27). In our modern reference, starch assemblages in young ginger (*Zingiber officinale*) roots tend to exhibit more irregular, fan-shaped, and medium-sized granules, while those from mature ginger are predominantly large, elongate granules (size range 8.49-40.31 μm) (Fig. S3:6). Type IV granules match well with the morphological variation of ginger.

Type VII starch granules (size range 7.79-37.15 μm) exhibit general characteristics of underground storage organs, such as oval or spherical shape, very eccentric hilum, and bend extinction cross. Some of them are similar to *Dioscorea polystachya* yam and *Lilium* lily bulb, based on our reference data. Yam are generally characterized by a large granule size range (9-53 μm for domesticated yam and 16-70 μm for wild yam), irregular triangular, an extremely eccentric hilum, the presence of lamellae in most cases, and an extinction cross with bent arms. The possible yam starch from GTY-POT1 has a general shape and size (21.67 μm) consistent with *D. polystachya*, which is one of the four *Dioscorea* species distributed in Shaanxi (4). The lily-like starch granule in Type VII has an elongate oval shape and a size of 16.02 μm, morphologically similar to *Lilium tigrinum* (size 5.75-57.77 μm) and *L. pumilum* (size 4.39-61.24 μm),

which are two of the 12 lily species found in Shaanxi (5). Some granules from yam and lily share similar features, such as elongate ovoid/oval shapes, but yam granules often has a straight edge opposite of the hilum, forming a nearly triangular shape, which is not found in lily.

Yam and lily starches differ from ginger in that they do not exhibit a relief/ridge feature at the edge near the hilum, and the hilum is not extremely close to the edge (Fig. S3:7,8).

Type VIII starch granules (size range 18.27-21.8 μm for LK and 14.01-22.22 μm for GTY) are oval in shape, the fissure appears like a long and darkened depression, and the extinction cross has many arms. These characteristics are typical of beans under the Fabaceae family, particularly similar to a wild pea (*Vicia* sp.) in our reference data (Fig. S3:4). In the poem "Xiaoya·Caiwei" of *Book of Songs* (11^{th}-7^{th} centuries BC), which recorded Western Zhou people's lives in the Wei River valley, the word *wei* refers to a wild pea (6), botanically identified as *Vicia gigantea* Bunge (7), or *Vicia sinogigantea* (8). It is very likely that Type VIII starch granules belong to *Vicia*, but we can't identify them to specific species here.

4. Damaged starch types

In our experimental study, we analyzed the starch morphological changes in many cereal species in order to establish a database for fermented cereal starches, which can be used to compare with ancient residue samples. The results have been partially published (9). Here we discuss the data relevant to the current study, which include wheat, broomcorn millet, rice, and *Elymus* sp. (a wild grass belonging to Triticeae tribe, native to North China (10).

Reference samples from our brewing experiments exhibit two types of gelatinization. The first type is "fermented gelatinization," which show features diagnostic of beer brewing. Experimental starches from mashed broomcorn millet and wheat in two millet beer samples (from Yulin and Shimao, both in Shaanxi) exhibit missing centers and deep channels (Fig S3:9), swelling and hollowed centers with less attacked and birefringent periphery (Fig. S3:10), and missing parts without extinction cross. A similar pattern can be found in the fermented *Elymus* sp. (Fig. S3: 11,12) and rice starches (Fig. S3:13). These damage features are the effects of enzymatic hydrolysis and/or low-temperature mashing during sprouting and mashing, which rarely appear in boiled or steamed starches (9, 11). The second type is "non-fermented gelatinization," which show damage patterns similar to those resulting from boiling and steaming. Starches typical of this category show even expansion and significant swelling, without visible central depression or holes as those present in the fermented starches (Fig. S3:14,15). These two types of gelatinization commonly occur in the same population of fermented starches in our experiments. Since individual starch granules often demonstrate great variation in morphological change during fermentation process, those granules less effected by enzymatic attacks would appear similar to the boiled/steamed starches. The fermented gelatinization would not appear in unfermented, boiled/steamed starches. The presence of fermented and boiled/steamed gelatinization forms in the experimental beer indicates that both types of gelatinization could exist in the ancient residues from beer brewing, a phenomenon which we observed in the LK-GTY assemblage.

5. Fungi

The main microorganisms in traditional *qu* are filamentous fungi (molds), such as *Aspergillus, Rhizopus, Mucor, Monascus* and *Penicillium*, among others. Fungi produce enzymes to degrade the starch material into fermentable sugars under certain conditions, in terms of source of nutrients (e.g., starchy foods), moisture level, and temperature range. Therefore, it is crucial to create favorable conditions which allow fungi to grow during the fermentation process (12-15). It is not uncommon to find spores in soils, but abundant fermentation-related filamentous fungal hyphae and sporangia do not appear in regular sediments/soils.

The primary components of fungi include hyphae and spores. A hypha is a long, branching filamentous structure of a fungus, formed as a tubular cell wall with or without septa. Hyphae grow at their tips; new hyphae are typically formed by emergence of new tips along existing hyphae, leading to the development of a mycelium, an interconnected network of hyphae. A sporangium, usually growing at the tip of specialized hyphae (e.g., sporangiophores), is an enclosure in which spores are formed. Spores allow fungi to be reproduced when dispersed via wind and germinate into haploid hyphae. Filamentous fungi are generally characterized by: hyphae aseptate or few-septate, > 4 μm in diameter; sporangia, merosporangia or sporangioles present; ballistospores or zygospores occasionally present. Spores are highly various in shape (16, 17).

Aspergillus and *Rhizopus* are the most commonly found fungi genera in various modern *qu* starters (13, 18). To establish a comparative reference we analyzed fungi in a *koji* from Japan (made of rice) and a *daqu* from Hebei, China (made of wheat). Koji contains cultured *Aspergillus oryzae* (14), and most fungi in the *daqu* are morphologically consistent with *Rhizopus* sp. (16, 19, 20).

Aspergillus is characterized by its conidiophores terminating in an apical vesicle and, at the opposite end, in a basal foot cell inserted into the supporting hypha. Phialides are attached directly to the vesicle (uniseriate) or on an intervening cell called a metula (biseriate); conidia are formed in chains (Fig. S4b). The identification of species depends primarily on colony color and the form of conidial heads (16:56).

Rhizopus exhibits the following appearances: (1) hyphae broad, not or scarcely septate; (2) rhizoids and stolons present; (3) sporangiophores brown, solitary or in tufts on the stolons, diverging from the point at which the rhizoids form; (4) sporangia rather round; (6) apophysis absent or scarcely apparent; (7) sporangiospores ovoid (Fig. S4c) (16:224).

6. Yeasts

We examined two modern samples of yeast, *Saccharomyces cerevisiae*, for comparison. One sample is from the traditional millet beer *hunjiu* from Shimao, as described above. During the fermentation process, no yeast was intentionally added to the mash; therefore, the yeast cells identified in the *hunjiu* sample are likely wild forms. Numerous yeast cells were visible in this sample under a light microscope, appearing predominantly oval and round in shape. The size range of these cells is 3.47-12.16 μm (n=101). The second modern yeast sample is a liquid media cultured, domesticated strain of *S. cerevisiae (BY4741)*, also predominantly oval and round in shape. The size range is 2.64-8.83 μm (n=85), which is considerably smaller than that of the *hunjiu* assemblage

(Fig. 5:19,20). This variation might reflect differences in culture conditions and domestication status (21-23).

Interestingly, the GTY yeast cells are more comparable morphologically (size) with the wild *S. cerevisiae* in *hunjiu* than the domesticated *S. cerevisiae.* Given potential ambiguity over the origin of these cells, we aimed to use DNA sequencing to determine their composition. DNA sequences are diagnostic of particular species in aggregate, but individual short sequencing reads can be incorrectly assigned as a result of similar sequences in multiple species. An alternative sequencing strategy is to only examine regions of known variability between species, such as the ITS1 sequence (24), but we wanted to capture the whole diversity of sequences and instead employed unbiased sequencing.

We took a sample of the alcohol from the Shimao *hunjiu* and streaked it onto a YPD Agar plate. This plate was incubated for three days at 30C in order to allow fungus and yeast to preferentially grow. Individual colonies of growth were picked and used, along with the original alcohol, as input for the DNA sequencing. The two samples of alcohol consisted of the millet beer itself and the streaked colonies picked from the plate.

Samples of alcohol from the Shimao *hunjiu* and a positive control (cultured domesticated yeast) and negative control (YPD media), were pelleted and directly resuspended in 300uL of lysis buffer from the MasterPure Yeast DNA Purification Kit (Epicentre), incubated at 65C for 15 minutes, and placed on ice. Then 150uL of MPC Protein Precipitation solution was added, vortexed, and pelleted to isolate DNA. The supernatant was purified using a DNA Clean & Concentrate-5 kit (Zymo Research) following manufacturer instructions.

Purified DNA was quantified with NanoDrop (ThermoFisher), tagmented using Nextera Tn5 (Illumina), and amplified with NEBNext (NEB) using distinct primers for each library and the following settings: 63C annealing for 30s, 72C extension for 60s, and 95C melting for 10s (with an initial 5 minute extension at 72C and 30 seconds of melting at 95C). Amplified DNA size was validated and amount quantified using a TapeStation 4200 (Agilent). Additional libraries for other projects were pooled and sequenced on a NextSeq 500 High Output kit (Illumina). Raw data are deposited on the NIH Short Read Archive with project ID PRJNA535381 (see Datasets S1).

Resulting reads were trimmed using Skewer (25) and aligned to the nr sequence resource using blastn. Read ends were processed separately to use as alignment controls. The resulting reads indicated that there were some yeast sequences present, particularly that of *S. cerevisiae* and *Pichia kudriavzevii,* in the *hunjiu* samples and positive control but not negative control (Dataset).

In our analysis, there was limited presence of the expected broomcorn millet (*Panicum miliaceum*) DNA sequences. Upon further investigation, we noted that the whole genome of *P. miliaceum* was not sequenced in the April 2018 release of the NCBI-BLAST nt database used for initial analyses. The China Agricultural University released a draft genome for the Longmi4 cultivar on July 10, 2018 (GenBank accession 6813148). Repeating analyses on the updated NCBI nt database from January 2019 indicated that one of the Shimao millet samples indeed included some *Panicum* chloroplast and nuclear DNA. Mapping this sample directly to the *P. miliaceum* genome using BWA-MEM 0.7.17 confirmed this finding. While the unambiguous millet content was quite low (~0.1% of the sample, which corresponds to ~2% of non-bacterial/aspergillus reads), the

degradation of DNA from fermented starch sources is not fully investigated in this work. In particular, the DNA isolation method is optimized for recovery of yeast and fungus, and using multiple isolation techniques on the same samples is an area for future study.

7. Comparison of starch in residues and control samples

No soil samples were collected during the excavations at the sites, therefore, we managed to obtain eight control samples by scraping off the sediments adhering to the exterior surface of pottery and the unused side of a grinding stone. These include four globular jars (LK-POT4,5; GTY-POT1,2), one bowl (GTY-POT12), one grinding stone (GTY-GS2), and two pottery vessels with unknown function and excluded from the residue analyses (GTY-POT10,14).

We compared starch counts in residue samples (n=9) with control samples (n=6). The ratios between the two groups are 17:1 and 22:1 from two LK globular jars (LK-POT4,5). The comparison between the seven residue samples (GTY-POT1-5,9,12) and four control samples (GTY-POT1,10,12,14) from GTY revealed the similar results: starch counts in the residue group range 20-125, and in the control group range 1-10. The ratios between the two groups from the same vessels (POT1,12) are 13:1 and 12:1, respectively (Fig. S5a,b).

We tested the proportions of damaged starches among three artifact groups (fermentation pots, general pots and grinding stones) from GTY. Since some samples yielded very small numbers of starch, which are not statistically meaningful, we only analyzed the samples with more than 15 granules. The results show that the fermentation pot group exhibits the highest proportions of gelatinized starches (13-70%) but lowest damaged starches (13-43%), whereas the grinding stones show much lower proportions of the gelatinized (0-17%) but higher damaged ones (44-74%). The two samples in the general pot group do not show a consistent pattern, probably due to their diverse functions (Fig. S5c).

We analyzed five control samples from pottery for phytolith. Most of them yielded very low counts. One sample (POT14) yielded only two long cells commonly found in leaves. We compared phytolith compositions between residues and control samples from four artifacts: two globular jars, one bowl, and one grinding stone. In three cases, phytoliths in residues are much more numerous than those in the controls, with ratios of 69:1, 1.8:1, and 2.8:1. In one case (GTY-POT1), there are more phytoliths in the control than in the residue (144:83), but their compositions are very different. The control sample yielded abundant husk phytoliths from millets (n=77; 54%), many identifiable as the η-type broomcorn millet (n=28) and the β-type barnyard (n=2), which are not present in the residue (Fig. S5d). In fact, millet husks were not found in any other GTY samples in this study. Barnyard (*Echinochloa* sp.), a wild grass growing widely in China (26), may have been accidentally harvested with the millet. This finding indicates that millets were processed at the site, but only dehusked millet grains were used for fermentation. POT1 globular jar may have been placed near the millet processing area.

Fig. S1. Locations of major sites mentioned in the text and examples of artifacts analyzed.
1,2: globular jars (GTY-POT3,5); 3: tripod (GTY-POT6); 4: funnel-steamer (GTY-POT9); 5,6: bowls (GTY-POT11,12); 7: tripod leg (LK-POT4); 8: globular jar (LK-POT1); 9: grinding stone (GTY-GS2).

Fig. S2. Production of millet beer (*hunjiu*) in Shimao, Shaanxi.
1: Dehusked broomcorn millet; 2: ground millet into flour; 3: steaming the millet flour, layer by layer, to make a millet cake; 4: the millet cake and the ground malt (made of sprouted maize); 5: kneading the millet cake with the malt flour until well mixed; 6: mixing the cake with hot water in a mash pot; 7: the mash; 8: placing the mash pot on a warm bed *kang*; 9: boiling the mash with hot water (1:4 ratio), the millet beer is ready to serve (Photos 1-8 by Jing Shao, 9 by Li Liu).

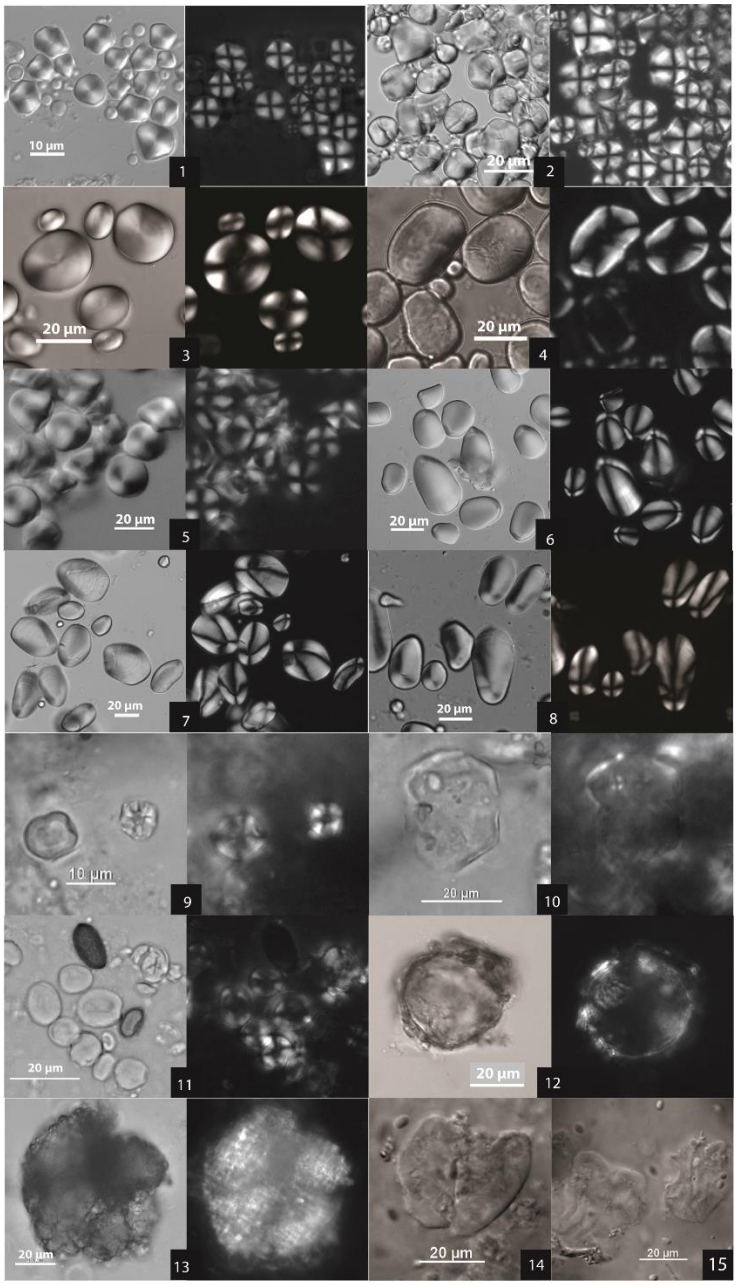

Fig. S3. Modern reference of native starches (1-8) and fermented starches (9-15). 1: Broomcorn millet (*Panicum milieceum*); 2: Job's tears (*Coix lacryma-jobi*); 3: wheatgrass (*Agropyron cristatum*); 4: wild pea (*Vicia* sp.); 5: snake gourd root (*Trichosanthes kirilowii*); 6: ginger (*Zingiber officinale*); 7: yam (*Dioscorea polystachya*); 8: lily (*lilium pumilum);* 9: fermented broomcorn millet starch with deep fissures/channels and central depression; 10: fermented broomcorn millet starch with nearly hollowed center, expanded, but birefringent edges; 11: sprouted *Elymus* starch granules with central depressions, deep channels and disappearing crosses; 12: mashed *Elymus* starch with hollowed center, expanded, and birefringent edges; 13: fermented rice starch with birefringence; 14,15: gelatinized starches in millet beer, flattened, expanded, and completely lost of the extinction crosses, similar to boiling and steaming effects.

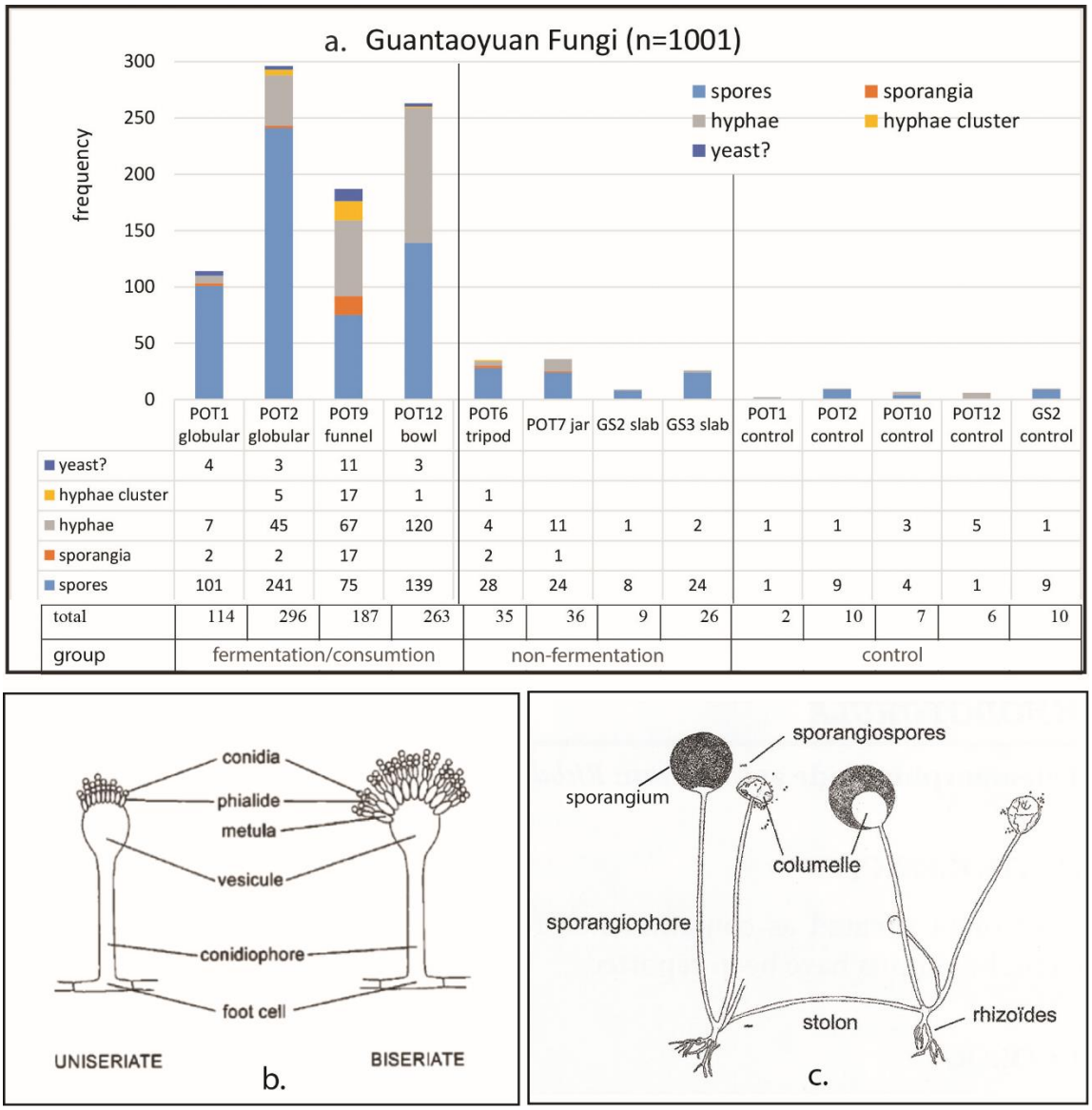

Fig. S4. GTY fungi counts and structures of molds.
a. Compare fungi counts in different vessel types and control samples, showing extremely higher counts of fungi in the fermentation vessels than in the non-fermentation vessels and controls; b. *Aspergillus* sp. uniseriate and biseriate *aspergillary* heads (12:57). c. *Rhizopus* sp. (12:225).

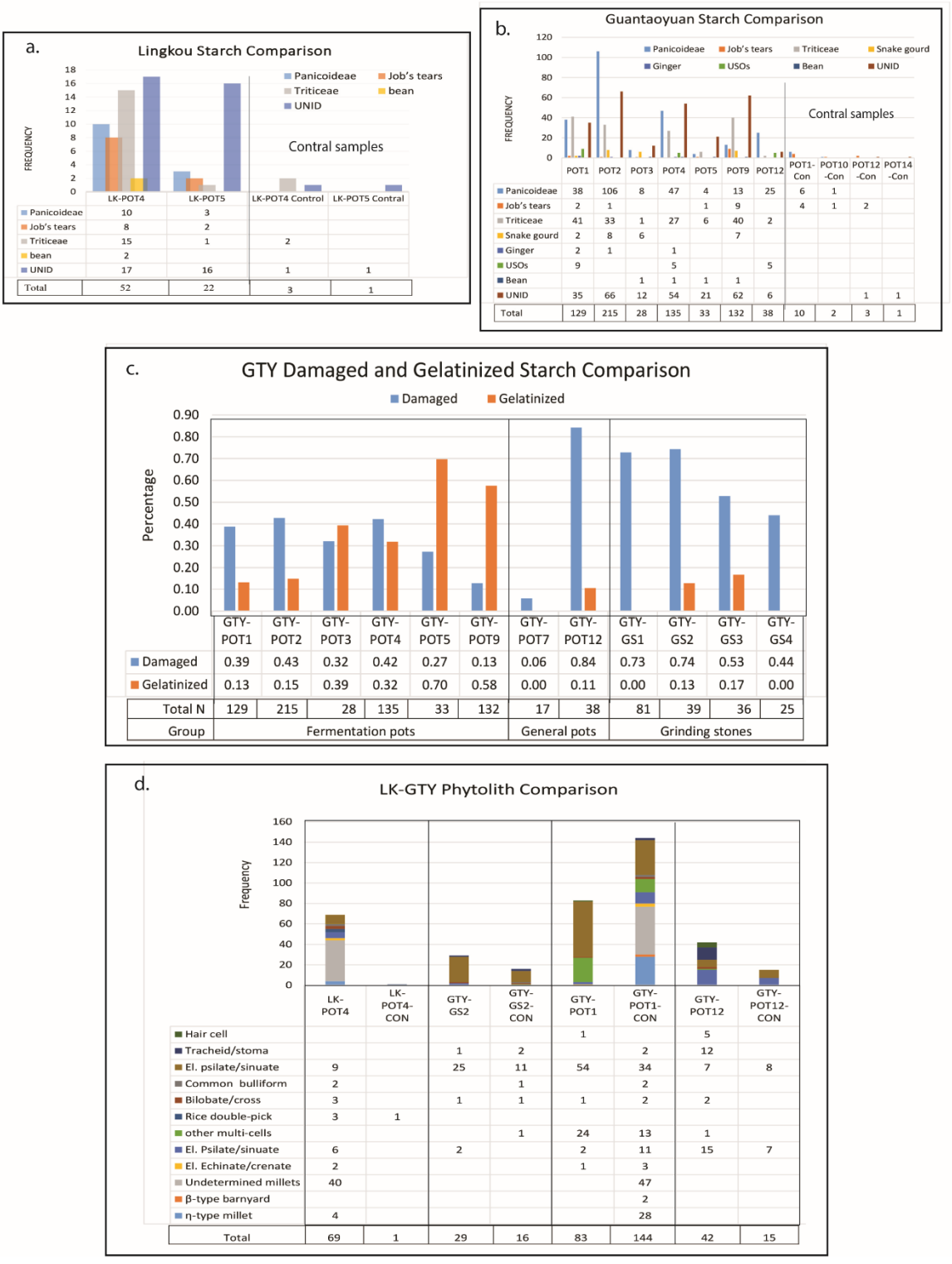

Fig. S5. Comparison of starch and phytolith assemblages between residue and control samples.

Table S1. Lingkou and Guantaoyuan starch counts and size range (in μm)

Taxa	Panico-ideae	Job's tears	Triti-ceae	Rice	Snake gourd	Ginger	USO	Bean	UNID	Total	Dama-ged	Gelati-nized 1	Gelati-nized 2
Starch type	I	II	III	IV	V	VI	VII	VIII					
LK Globular													
POT1	2		2						7	11	1	4	
POT2	17		5	25	3	1			13	64	8	22	
POT3	8	4	2						10	24	10	8	
Total N	27	4	9	25	3	1			30	99	19	34	
Total %	27.3	4.0	9.1	25.3	3.0	1.0			30.3	100	19.2	34.3	
Ubiq. %	100	33.3	100	33.3	33.3	33.3			100		100	100	
LK Tripods													
POT4	10	8	15					2	17	52	41	10	1
POT5	3	2	1						16	22	5	14	
POT6	6		4						21	31	15	11	
Total N	19	10	20					2	54	105	61	35	1
Total %	18.1	9.5	19.0					1.9	51.4	100	58.1	34.3	1
Ubiq. %	100	66.7	100					33.3	100		100	100	33.3
LK total N	46	14	29	25	3	1		2	84	**204**	80	69	1
LK total %	22.5	6.9	14.2	12.3	1.5	0.5		1.0	41.2	100	39.2	33.8	0.5
LK ubiq. %	100	50	100	16.7	16.7	16.7		16.7	100		100	100	16.7
LK min	6.82	16.78	13.96	3.63	16.15	29.49		18.27					
LK max	16.5	24.12	26.99	5.7	20.2	29.49		21.8					
LK mean	11.83	18.96	20.40	4.74	17.51	29.49		20.04					
GTY Globular and funnel-steamer													
POT1	38	2	41		2	2	9		35	129	50	17	
POT2	106	1	33		8	1			66	215	92	32	
POT3	8		1		6			1	12	28	9	11	
POT4	47		27			1	5	1	54	135	57	43	
POT5	4	1	6					1	21	33	9	23	
POT9	13	9	40		7			1	62	132	17	72	4
Total N	216	13	148		23	4	14	4	250	672	234	198	4
Total %	32.1	1.9	22.0		3.4	0.6	2.1	0.6	37.2	100	34.8	29.5	0.6
Ubiquity %	100	66.7	100		66.7	50	33.3	66.7	100		100	100	16.7
GTY General pots													
POT6	5		1						1	7	1	3	
POT7	16						1			17	1		
POT11	8	1								9	5	2	
POT12	25		2				5		6	38	32	4	
Total N	55		3				6		7	71	39	9	
Total %	77.5		4.2				8.5		9.9	100	54.9	12.7	
Ubiquity %	100	50	50				50		50		100	75	
GTY Grinding stones													
GS1	35		26				4		16	81	59		
GS2	19		11				1		8	39	29	5	
GS3	15		6				1		14	36	19	6	
GS4	8		11				1		5	25	11		
Total N	77		54				7		43	181	118	11	
Total %	42.5		29.8				3.9		23.8	100	65.2	6.1	
Ubiquity %	100		100				100		100		100	50	
GTY totl N	348	13	205		23	4	27	4	300	**924**	391	218	4
GTY totl %	37.7	1.4	22.2		2.5	0.4	2.9	0.4	32.5	100	42.3	23.6	0.4
GTY ubi %	100	42.9	71.4		28.6	21.4	57.1	28.6	85.7		100	78.6	7.1
GTY min	4.84	13.27	5.02		7.39	15.31	7.79	14.01	3.64				
GTY max	21.84	26.01	39		28.58	24.77	37.15	22.22	69.7				
GTY mean	13.25	20.73	20.75		13.68	20.61	17.81	17.75	17.25				
Control samples													
LK-POT4-Con	1		2							3	1		

LK-POT5-Con									1	1	1		
GTY-POT1-Con	6	4								10		9	
GTY-POT10-Con	1	1								2	1		
GTY-POT12-Con		2							1	3		1	
GTY-POT14-Con									1	1			
GTY-GS2-Con	1	27							7	35	5		
Taxa	Panico-ideae	Job's tears	Triti-ceae	Rice	Snake gourd	Ginger	USO	Bean	UNID	Total	Dama-ged	Gelati-nized 1	Gelati-nized 2

Table S2. Lingkou phytolith record

		Globular			*Tripods*			*Total*		*Ubiquity*	
Phytolith morphotype	Possible taxonomic attribution	POT1	POT2	POT3	POT4	POT5	POT6	Total N	Total %	Ubiquity N	Ubiquity %
Silica skeletons											
η-type	P. miliaceum (inflorescence)	1	4	7	4	11	1	28	7.2	6	100
Ω-type	S. italica (inflorescence)		1					1	0.3	1	16.7
Undetermined millets	Paniceae (inflorescence)	11	27	38	40	62	10	188	48.3	6	100
Elongate columellate	Poaceae	1	1		2	8	1	13	3.3	5	83.3
Elongate Psilate/sinuate	Poaceae stem/leaf	4	4	10	6			24	6.2	4	66.7
Single-cell phytolith											
Double-peak	*Oryza* husk		1	5	3	4		13	3.3	4	66.7
Phragmite bulliform	*Phragmite* leaf					1		1	0.3	1	16.7
Bilobate	Panicoideae	2	3	4	10	3	1	23	5.9	6	100
Polylobate	Panicoideae	1						1	0.3	1	16.7
Cross/quadra-lobate	Panicoideae			2	3	4		9	2.3	3	50
Saddle	Poaceae			1				1	0.3	1	16.7
Rondel	Poaceae	2	2	3	3	2	1	13	3.3	6	100
Common bulliform	Poaceae leaf		2	1	2	1		6	1.5	4	66.7
Elongate psilate/sinuate	Poaceae stem/leaf	1	1	19	9	13	11	54	13.9	6	100
Trichome		1						1	0.3	1	16.7
Hair cell	Eudicots				2	5		7	1.8	2	33.3
Raphide	USOs				1			1	1.3	1	16.7
Prinkle					5			5	100	1	16.7
Total N		24	46	90	90	114	25	**389**			
Total %		6.2	11.8	23.1	23.1	29.3	6.4	100			

Table S3. Guantaoyuan phytolith record

	Globular and funnel						***General pots***				***Grinding stones***				***Total***		***Ubiquity***	
Phytolith morphotype	POT 1	POT 2	POT 3	POT 4	POT 5	POT 9	POT 6	POT 7	POT 11	POT 12	GS 1	GS 2	GS 3	GS 4	Total N	Total %	Ubiq. N	Ubiq. %
Silica skeletons																		
Undetermined multi-cell				1											1	0.3	1	7.1
Elongate Echinate	1				1		1								3	0.8	3	21.4
Elongate Crenate		1	1												2	0.5	2	14.3
Elongate Psilate/sinuate	2	2		17		8	2	9	21	15	2	2	2	1	83	21.4	12	85.7
Opaque perforated platelets		1													1	0.3	1	7.1
Stoma sheet			1	4			3	2	5	1					16	4.1	6	42.9
Undetermined multi-cell	24			2		1		1							28	7.2	4	28.6
Single-cell phytolith																		
Bilobate		3								2		1			6	1.6	3	21.4
Polylobate				2											2	0.5	1	7.1
Cross/quadra-lobate	1														1	0.3	1	7.1
Saddle											1				1	0.3	1	7.1
Common bulliform				1											1	0.3	1	7.1
Elongate dendriform/echinate /crenate/columellate					1										1	0.3	1	7.1
Elongate psilate/sinuate	54	13		15	6	1		1	2	7	26	25	5	2	157	40.6	12	85.7
Tabular scrobiculate (Cyperaceae achene)								1							1	0.3	1	7.1
Trichome											1				1	0.3	1	7.1
Hair cell	1	2	1	2	1	4	6	2	31	5	2		1	1	59	15.2	13	92.9
Stoma				1				3		12		1			17	4.4	4	28.6
Tracheid								2							2	0.5	1	7.1
Raphide	2												2		4	1.0	2	14.3
GTY Total N	85	22	3	45	9	14	12	21	59	42	32	29	10	4	387	100		
GTY Total %	22.0	5.7	0.8	11.6	2.3	3.6	3.1	5.4	15.2	10.9	8.3	7.5	2.6	1.0	100			

Table S4. Phytoliths and starch granules in control samples

	LK-POT4 exterior surface	LK-POT5 exterior surface	GTY-GS2 unused side	GTY-POT1 exterior surface	GTY-POT2 exterior surface	GTY-POT10 exterior surface	GTY-POT12 exterior surface	GTY-POT14 exterior surface	Total
Phytolith									
Silica skeletons									
η-type				28					28
β-type				2					2
Undetermined millets				47					47
Elongate Echinate				1					1
Elongate Crenate				2					2
Elongate Psilate/sinuate				11		6	7		24
Elongate Irregular			1						1
Opaque perforated platelets				6					6
Stoma sheet				1		1			2
Jigsaw				1					1
Undetermined mult-cell				5					5
Single-cell phytolith									
Rice double-pick	1								1
Cross/quadra-lobate			1	2					3
Common bulliform			1	2					3
Elongate psilate/sinuate			11	34		4	8	2	59
Tracheid			2	2					4
Phytolith Total N	1		16	65		13	18	2	115
Starch									
Panicoideae			1	6		1			8

Triticeae	2		27	4		1	2		36
UNID	1	1	7				1	1	11
Starch Total N	3	1	35	10		2	3	1	55
Damaged starch N	2	1	5			1		1	10
Gelatinized 1 (ferment)				9			1		10
Fungi									
Spores				1	9	4	1	9	24
hyphae				1	1	3	5	1	11
Fungi total N				2	10	7	6	10	35

Additional data: dataset S1 (separate file)

DNA sequencing of alcohol and yeast

References

1. Lamb J & Loy T (2005) Seeing red: the use of Congo Red dye to identify cooked and damaged starch grains in archaeological residues. *Journal of Archaeological Science* 32:1433-1440.
2. Huang HT (2000) *Science and Civilisation in China: Vol 6, Biology and Biological Technology, Part V: Fermentations and Food Science* (Cambridge University Press, Cambridge).
3. Liu L, Ma S, & Cui J (2014) Identification of starch granules using a two-step identification method. *Journal of Archaeological Science* 52:421-427.
4. Ding Z & Gibert MG (2000) Dioscoreaceae. *Flora of China 24*.276-296. http://flora.huh.harvard.edu/china/mss/volume24/DIOSCOREACEAE.published.pdf. (data accessed May 15, 2019)
5. Liang S & Tamura MN (2000) Lilium Linnaeus. *Flora of China 24*.135-149. http://flora.huh.harvard.edu/china/PDF/PDF24/lilium.pdf. (data accessed May 15, 2019)
6. Yuan M (1999) *Shijing Yizhu (Translation of Classic of Poetry)* (Qingdao Chubanshe, Qingdao).
7. Northwest Institute of Botany CAS ed (1981) *Qinling Zhiwuzhi (Flora of Qinling Mountains) Vol. 1.3* (Kexue Press, Beijing).
8. Bao B & Turland NJ (2010) Vicia Linnaeus. *Flora of China 10*.560-572. http://flora.huh.harvard.edu/china/PDF/PDF10/Vicia.pdf. (data accessed May 15, 2019)
9. Wang J, *et al.* (2017) Identifying ancient beer brewing through starch analysis: A methodology. *Journal of Archaeological Science: Reports* 15:150-160.
10. Wu ZY, Raven PH, & Hong DY eds (2006) *Flora of China. Vol. 22 (Poaceae)* (Science Press and Missouri Botanical Garden Press, Beijing and St. Louis).
11. Henry AG, Hudson HF, & Piperno DR (2009) Changes in starch grain morphologies from cooking. *Journal of Archaeological Science* 36:915-922.
12. Chen B, Wu Q, & Xu Y (2014) Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese Maotai-flavor liquor. *International Journal of Food Microbiology* 179:80-84.
13. Jin G, Zhu Y, & Xu Y (2017) Mystery behind Chinese liquor fermentation. *Trends in Food Science and Technology* 63:18-28.
14. Shurtleff W & Aoyagi A (2012) *History of Koji -- Grains and/or Soybeans Enrobed with a Mold Culture (300 BCE to 2012): Extensively Annotated Bibliography and Souurcebook* (Soyinfo Center, Lafayette, CA).
15. Zhu Y & Tramper J (2013) Koji - where East meets west in fermentation. *Biotechnology Advances* 31:1448e1457.
16. St-Germain G & Summerbell R (2011) *Identifying Fungi: A Clinical Laboratory Handbood* (Star Publishing Company, Belmont, CA).

17. Webster J & Weber R (2007) *Introduction to Fungi* (Cambridge University Press, Cambridge).
18. Zheng X-W, Tabrizi MR, Nout MJR, & Han B-Z (2011) *Daqu* – A Traditional Chinese Liquor Fermentation Starter. *Journal of the Institute of Brewing* 117(1):82-90.
19. Shurtleff W & Aoyagi A (2012) *History of Koji - Grains and/or Soybeans Enrobed with a Mold Culture (300 BCE to 2012): Extensively Annotated Bibliography and Sourcebook* (Soyinfo Center, Lafayette, CA).
20. Wei J (1979) *Zhenjun Jianding Shouce (Handbook of Identification of Fungi)* (Shanghai Science and Technology Press, Shanghai).
21. Johnston GC, Ehrhardt CW, Lorincz A, & Carter BL (1979) Regulation of cell size in the yeast Saccharomyces cerevisiae. *Journal of Bacteriology* 137(1):1-5.
22. Turner JJ, Ewald JC, & Skotheim JM (2012) Cell size control in yeast. *Current Biology* 22(9):R350-R359.
23. Smith C, Pomiankowski A, & Greig D (2013) Size and competitive mating success in the yeast Saccharomyces cerevisiae. *Behavioral Ecology* 25(2):320-327.
24. Koljalg U, Nilsson RH, Abarenkov K, & et al. (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22:5271-5277.
25. Jiang H, Lei R, Ding S-W, & Zhu S (2014) Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. *BMC Bioinformatics* 15:182.
26. Chen S & Phillips SM (2006) Echinochloa P. Beauvois. *Flora of China 22*.515-518. http://flora.huh.harvard.edu/china/PDF/PDF22/Echinochloa.pdf. (data accessed May 15, 2019)