Analysis of plant micro-remains and organic acid residues reveals the dietary conditions at the Chengyan site during the early Yangshao Culture in western Henan, central China

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Analysis of plant micro-remains and organic acid residues reveals the dietary conditions at the Chengyan site during the early Yangshao Culture in western Henan, central China

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ABSTRACT

The early Yangshao period (*ca.* 7.0–6.0 ka BP) is a pivotal transition stage for prehistoric human subsistence strategies from hunting and gathering to farming and husbandry. The western Henan Province constitutes the core area of Yangshao Culture. To investigate the dietary conditions of the early Yangshao ancestors in this area, analysis of plant micro-remains (starch grains and phytoliths) and organic acid residues was conducted on 34 pottery samples unearthed at the Chengyan Site in Lingbao City, western Henan Province, central China. The results revealed that the plant foods of the Chengyan people included Triticeae, Job's tears (*Coix lacryma-jobi*), rice (*Oryza sativa*), millet (*Setaria italica* and *Panicum miliaceum*), lotus root (*Nelumbo nucifera*), snake gourd root (*Trichosanthes kirilowii*), yam (*Dioscorea*), lily (*Lilium brownii*), legumes (Fabaceae), and acorn (*Quercus*), in which, some species served as raw materials for brewing fermented beverages. These findings demonstrate that botanical resource exploitation during this period in central China exhibited remarkable diversity, with foraging maintaining its significance as an essential subsistence strategy for early human populations in acquiring plant foods. The presence of rice at the Chengyan site indicates its spread to western Henan during the early Yangshao period, and a rice-millet mixed farming, dominated by foxtail millet and broomcorn millet, had developed in the area. This study provides valuable insights into the dietary patterns and agricultural production trajectories of the early Yangshao communities in the Central Plains region.

1. Introduction

Dietary behavior is one of the most important survival activities for early human-beings (Xie, 2017). Studying the diet of prehistoric humans can inform us of their food structure, crop cultivation, as well as cultural and social development situations (Chen, 2013). The period of 7.0–5.0 ka BP was a crucial transitional time in the Neolithic China, during which not only human subsistence strategy changed from gathering and hunting to farming and animal husbandry, but also early society evolved from simple human groups to complex states (Su, 1965). The Yangshao Culture (7.0–5.0 ka BP) was one of the most important Neolithic archaeological cultures in China. With broad area of Shanxi, Shaanxi, and Henan provinces as its core, it had the widest geographic coverage and largest population, exerting a strong impact on its surrounding cultures at that time. As time went on, it accumulated substantial population and material foundations for the formation of Chinese civilization (Li, 2021; Zhao, 2000). In view of the great significance of Yangshao Culture in the development of prehistoric Chinese society, the dietary conditions of Yangshao people have consistently been a key focus of academic research.

The western Henan Province, connecting the Guanzhong and Luoyang Basins, served as the core area of Yangshao Culture. This region

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attained its climactic phase of sociocultural development during the Miaodigou cultural horizon (*ca.* 5.9–5.4 ka BP), manifested through settlement proliferation, demographic intensification, and the emergence of complex settlement hierarchies, constituting a pivotal transition toward early social complexity in the Central Plains (Bi et al., 2016). The studies of human diets during the Yangshao period in this area can provide important information for understanding the utilization characteristics of animal and plant resources, the evolution of crop structure, the development of the agricultural economy and its relationship with the complexity of early society in the Central Plains. It should be noted that to date, only the Xipo site among Yangshao culture sites in the western Henan region has undergone systematic zooarchaeological analyses. At the site, osteological remains of domesticated pigs and dogs have been identified, yet their quantities remain subdominant to wild faunal assemblages (Ma, 2007). Given the paucity of zooarchaeological assemblages from the Yangshao cultural horizon in western Henan region, this study focuses on the status of human's plant resource utilization.

Archaeological literature review reveals that prior to 1990s, a sand-tempered jar exhibiting distinct impressions of foxtail millet on its interior surfaces was recovered from early Yangshao cultural deposits (*ca.* 7.0–6.0 ka BP) at the Wangwan site (Li and Yan, 1961). In addition, a pottery cup belonging to the early and middle Yangshao periods (approximately 6.0 ka BP) was also found at Xigaoya site, with impressions of rice husks at the edge of its mouth (Zeng and Zhu, 1981). Since the 1990s, with the gradual popularization of plant archaeological research in China, scholars have carried out analyses of carbonized plant remains, phytolith, and pollen at three sites, namely Nanjiaokou (Wei et al., 2000), Xipo (Zhong et al., 2020), and Yangshaocun (Wang, 2015) in the western Henan. The carbonized plant remain analysis showed that, during the mid-Yangshao period, people exploited both foxtail millet and rice at the Nanjiaokou site. And at the Xipo site, a dry farming model dominated by foxtail millet and broomcorn millet, with a small amount of rice has been established during the Miaodigou period. Pollen and phytolith analyses for Yangshaocun showed that a similar farming model with Xipo prevailed during the middle and late Yangshao cultural periods (6.0–5.0 ka BP) (Zhong et al., 2020). The above studies demonstrate that middle-late Yangshao populations in western Henan predominantly engaged in millet-based agriculture (foxtail millet and broomcorn millet), supplemented by sporadic rice utilization. However, significant lacunae exist in the understanding of early Yangshao dietary systems, particularly regarding the crop structure and agricultural production level in this pivotal region of the Central Plains.

The Chengyan site (34°28′31″N, 111°1′7″E) is located south of Chengyan Village, Chuankou Town, Lingbao County, Sanmenxia City, China (Fig. 1). In 2019, for the construction of a new railway, the Henan Provincial Institute of Cultural Heritage and Archaeology conducted an archaeological excavation at the site, uncovering over 4600 m^2. According to the characteristics and types of unearthed artifacts, excavators suggest that the cultural attribute of the Chengyan site belongs to the Dongzhuang type of the early Yangshao culture (*ca.* 6.2–6.0 ka BP). During the excavation, nearly 30 house foundations, 50 tombs, and 100 urn coffin burials were discovered. Additionally, more than 20 pottery kilns containing abundant plant ash and fired clay were found at the site

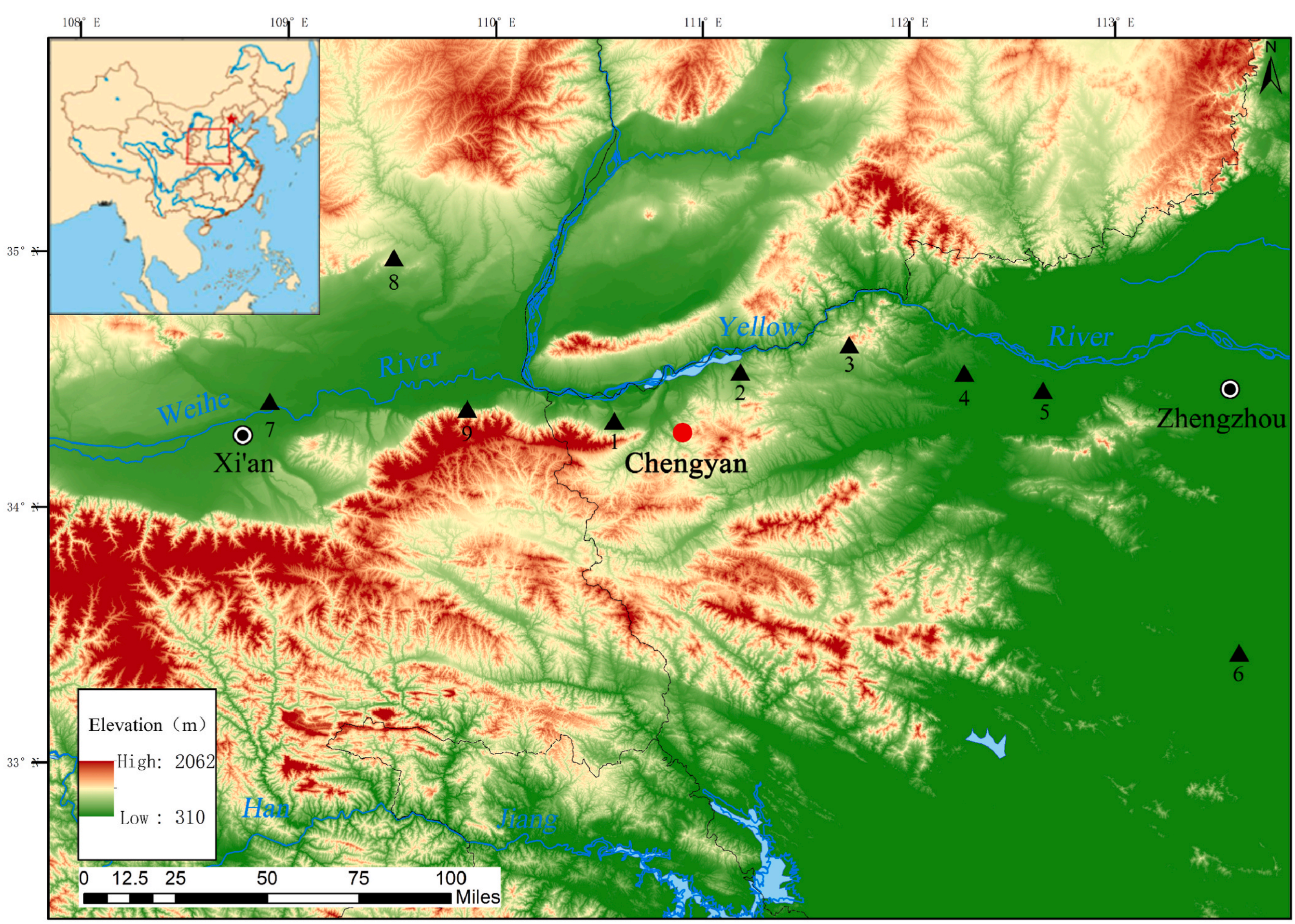

Fig. 1. Locations of Chengyan and other relative sites
1. Xipo 2. Nanjiaokou 3. Yangshaocun 4. Wangwan 5. Xigaoya 6. Jiahu 7. Yangguanzhai 8. Xiahe 9. Xinglefang.

(Fang, 2020). The discovery of multiple pottery kiln remains indicates that specialized pottery production may have occurred in the early Yangshao period. These findings at Chengyan site provide important information for understanding the production activities and lives of ancestors, as well as cultural and social development situation in the early Yangshao period in western Henan, central China.

To elucidate human's dietary patterns, crop structure, and transitional processes from foraging economies to agricultural systems during the early Yangshao period in the Central Plains region, partial typical pottery shards from the Chengyan site were selected for starch grain analysis, phytolith analysis, and organic acid residue analysis in this paper.

2. Materials and methods

2.1. Experimental materials

The experimental samples consisted of unwashed, intact pottery artifacts and pottery fragments excavated from the Chengyan site in 2019 and 2022. The samples were packaged in woven bags and stored in a repository to prevent contamination prior to analysis. A total of 34 pottery samples were selected for this study (Fig. 2). Based on the pottery types, these samples are believed to be associated with food processing (Liu et al., 2021a; Sun, 2022). The selected samples included one large-mouthed round-bottomed pot, 13 pot's bottom fragments, 13 *jiandiping*s, one cup, one basin, and five artifacts' mouth rims.

2.2. Experimental methods

All selected samples were taken back to the Bioarchaeology laboratory of University of Science and Technology of China for residue extraction and analysis. To prevent contamination from the experimental environment, instruments, or reagents, a blank control sample was prepared and treated using the same protocol.

Starch grains and phytoliths on the pottery surfaces were extracted by ultrasonic cleaning. The obtained liquid samples were subjected to

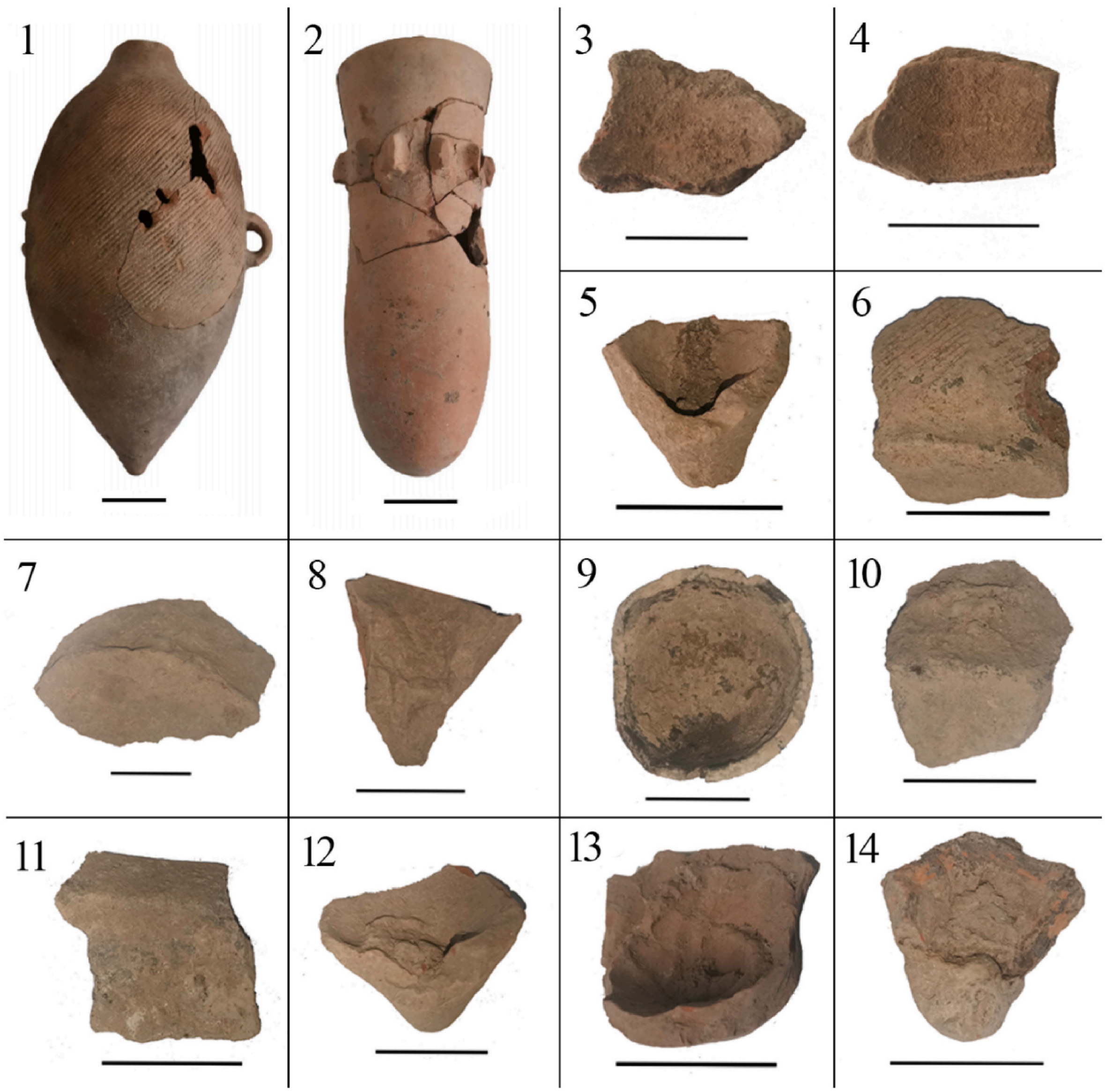

Fig. 2. Partial sampled pottery and pottery fragments from the Chengyan site (scale: 5 cm) (1) *Jiandiping* 2019LCT1530⑤; (2) Large-mouthed round-bottomed pot 2019LCT2119H599; (3) Pot 2022LCT3016②; (4) Rim 2022LCT3016②; (5) *Jiandiping* 2022LCTG2③; (6) Pot 2022LCTG2④:2; (7) Pot 2022LCT3217⑤; (8) *Jiandiping* 2022LCT3217⑤; (9) Cup 2022LCT2917H1595; (10) Pot 2022LCT2917H1595; (11) Rim 2022LCTG2④:2; (12) *Jiandiping* 2022LCTG2④:2; (13) *Jiandiping* 2022LCT2917H1595; (14) *Jiandiping* 2022LCTG2H1545.

anti-flocculation treatment, then 10 % HCl and Hydrogen peroxide were added to remove the carbonate impurities and organic matter in the samples. The starch grains were separated and extracted with heavy liquid cesium chloride (CsCl), according to the analytical methods described by Yang et al. (2022) and Liu et al. (2017). Based on Piperno et al. (PIPERNO, 1988) and Sun et al. (2024), phytoliths can be extracted using zinc bromide ($ZnBr_2$). The extracted ancient starch grains were mounted on glass slides in 50 % (v/v) glycerol and 50 % (v/v) distilled water, and observed under an optical microscope (Leica DM4500P) with 90° polarizing filters and differential interference contrast (DIC) optics at 600 × magnification. The phytoliths were mounted in glycerol on glass slides and observed under the same optical microscope with a bright-field model at 200 × or 400 × magnifications. The morphological description and species identification of ancient starch grains and phytoliths in the experiment were mainly based on a database of modern plant starch grains and phytoliths established by our laboratory, as well as some relevant published research literatures (Wang and Lv, 1993; Yao, 2016; Yang et al., 2010a; Torrence and Barton, 2016).

After the observation under the microscope, a large number of starch grains with fermentation damage characteristics were found. According to current literatures, these damaged starch grains were likely related to alcoholic beverages making (Liao et al., 2022a; Liu et al., 2021b). In this case, liquid chromatography-mass spectrometry (LC-MS) analysis was conducted to verify whether human at the site had already engaged in wine-brewing activities. For LC-MS analysis, ten common organic acids in fermented drinks were selected as detection targets including lactic, acetic, butyric, succinic, tartaric, pyruvic, fumaric, malic, citric, and oxalic acid (Gong, 2023). During the experiment, standard reagents were also tested with the same procedure to ensure all tests were not contaminated in the laboratory. The specific organic acid reagent used were as follows: L-lactic acid (85 %, AR), glacial acetic acid (99 %, AR), butyric acid (99 %, AR), succinic acid (99 %, AR), tartaric acid (L (+) -tartaric acid, 99 %, AR), pyruvic acid (98 %, AR); fumaric acid (99 %, AR); DL-malic acid (99 %, AR); citric acid (99 %, AR) acid (99 %, AR). The tartaric acid was produced by Shanghai McLin Biochemical Technology Co., Ltd., the DL-malic acid was produced by Tianjin Fuchen Chemical Reagent Co., Ltd., and all remaining organic acid reagents were produced by the State Pharmaceutical Group Chemical Reagent Co., Ltd.

Appropriate amounts of various standard reagents were weighed, dissolved in a KH_2PO_4 buffer, and diluted to 100 mL to obtain a mixed standard solution with concentration of 20 mg/mL lactic acid, 20 mg/mL acetic acid, 10 mg/mL butyric acid, 20 mg/mL succinic acid, 40 mg/mL tartaric acid, 20 mg/mL pyruvate, 30 mg/mL fumaric acid, 20 mg/mL malic acid, 10 mg/mL citric acid, and 10 mg/mL oxalic acid and stored at 4 °C. The archaeological samples were centrifuged at 3000 rpm for 15 min and then transferred to new 15 ml centrifuge tubes. Subsequently, samples were filtered by 0.45 μm microfiltration membrane into 5 ml sample bottles. Next, a vacuum centrifugal concentrator was used to centrifuge at 1000 r/min and concentrate to 100 μl at 30 °C.

The samples were sent to the Physical and Chemical Experiment Center of the University of Science and Technology of China for detection together with the ancient samples. The detection and analysis methods were described by Li (2017a) and Wen, R. et al. (Wen et al., 2018). The experimental instrument used was a liquid chromatography-mass spectrometer (Thermo Fisher Scientific), and the test results were analyzed using Thermo Xcalibur 2.2.42.

The chromatographic conditions were as follows: column (Waters Atlantis T3 column 1.8 μm, 2.1 × 50 mm); mobile phase (A phase: water, B phase: acetonitrile); column temperature (30 °C; injection volume: 10 μL); flow rate (0.15 mL/min, constant speed); and elution conditions (linear gradient). The mass spectrometry conditions were as follows: ion source (negative ion mode electrospray ionization source); scanning mode (full scan); scanning range (50−600 m/z); spray voltage (4 k V); tube lens (110 V); capillary (voltage:35 V); temperature (275 °C); sheath gas (N_2); and flow rate (5 arb).

3. Results

3.1. Starch grain analysis

Among the 34 pottery samples collected from the Chengyan site, seven pottery samples did not yield any starch grains. Additionally, some starch grains were extracted from another two pottery samples' outer surfaces which might be caused by contamination during burial or excavation processes because no grains were found on their inner surfaces. Therefore, the abovementioned nine pottery samples were excluded in the further analysis. No starch grains were detected in all blank samples, suggesting that the analysis was not contaminated during the experiment. A total of 422 starch grains were extracted from the inner surfaces of the remaining 25 pottery samples. 77 starch grains could not be identified owing to a lack of morphological characteristics or serious damage. The remaining 345 starch grains were classified into 11 categories according to their morphological characteristics: rice (*Oryza sativa*), Job's tears (*Coix lacryma-jobi*), millet (*Setaria italica* and *Panicum miliaceum*), Triticeae, lotus root *(Nelumbo nucifera)*, snake gourd root (*Trichosanthes kirilowii*), yam (*Dioscorea*), lily (*Lilium brownii*), legume (Fabaceae), acorn (*Quercus*), and unidentified underground storage organs (Fig. 3, Supplementary Material 1).

Type A: Rice starch grains (n=8; size range: 5.1–11.6 μm). The grains exhibited polygonal shape, centric hila. Their surfaces were smooth without lamellas; no cracks were observed. The extinction arm was X-shaped under orthogonal polarization with sharp angles. Combined with Yang Xiaoyan's discussion on the single rice starch granule from the Kuahuqiao site (Yang and Jiang, 2010), we concluded that the A-type starch granules were from rice (Fig. 3a)

Type B: Job's tears starch grains (n=26; size range: 13.3–24.9 μm). Most starch grains exhibited nearly circular polyhedral morphologies with smooth surfaces lacking lamellae, and the hila were located at or near the centers. Some grains displayed "-", Y-shaped, or radial cracks at the hilum. Extinction arms were vertically oriented under orthogonal polarization, and some exhibited Z-shaped bending at the end of the extinction arm. According to modern reference samples, starch grains of gramineous plants (e.g., *Setaria italica*, *Panicum miliaceum*, and *Coix lacryma-jobi*) are generally polyhedral, and the extinction arms are vertical. However, the particle sizes of *Setaria italica* and *Panicum miliaceum* are generally smaller, averaging 7.6 ± 1.4 μm and 9.2 ± 2.8 μm, respectively (Ge et al., 2010). In contrast, the Job's tears starch grains have larger maximum and average particle sizes, and their extinction arms were mostly Z-shaped (Liu et al., 2014a). Based on this, we identified Job's tears as the source of the Type B starch grains (Fig. 3b).

Type C: Millet starch grains (n=16; size range: 15.1–25.1 μm). The starch grains exhibitedpolyhedral structureswith centrally located hila, and some hila were depressed. There were "-"-shaped, T-shaped, Y-shaped, or radial cracks at the hilum. Compared with modern reference samples, the collected grains' morphological characteristics were similar to those of gramineous starch grains (Yang et al. (2010b). Analysis of *Setaria italica*, *Panicum miliaceum*, and their wild relatives revealed that Type C starch grains extracted from the samples lacked diagnostic specificity, precluding further taxonomic classification. Based on the above analysis, we concluded that Type C starch grains originated from millet (Fig. 3c).

Type D: Triticeae starch grains (n=24; size range: 11.3–30.1 μm). The frontal projection appears round or oval, while the side projection is thin and long, and nearly olive-shaped. The hilum is located at or near the center. There is no cracks at the hilum. The extinction arms are either vertical or X-shaped under orthogonal polarization, exhibiting diffuse extinction. Some larger starch grains have clear lamellae (Fig. 3d). smaller granules are nearly circular or polygonal with a centered hilum, and there is no cracks at the hilum (Fig. 3e). By comparing with modern reference samples preserved in the laboratory and referencing the criteria for identifying Triticeae starch granulesestablished by Liu et al. (2014b) and Yang et al. (2016a), this study identified that the type D

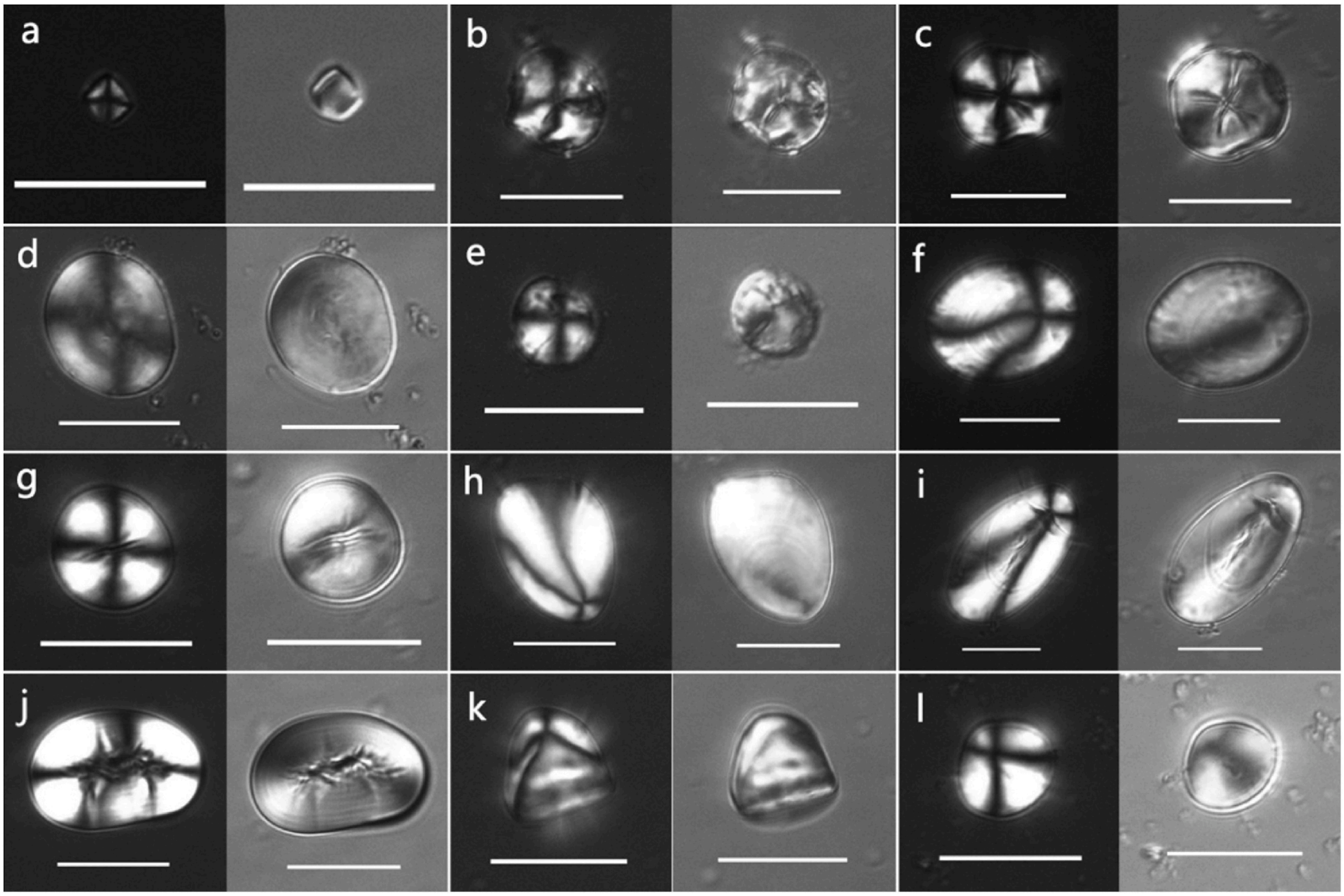

Fig. 3. Starch grains extracted from the pottery surfaces at the Chengyan site (scale: 20 μm)
a: rice, b: Job's tears, c: millet, d, e: Triticeae, f: lotus root, g: snake gourd root, h: yam, i: lily, j: legume, k: acorn, l: unidentified underground storage organs.

starch grains originated from Triticeae plants.

Type E: Lotus root starch grains (n=3; size range: 17.1–32.0 μm). These starch grains were elongated, oval, or nearly circular in shape, and their particle sizes differed considerably. The hilum was eccentric in the oval-shaped grains, with some exhibiting "-" or "+"-shaped fissures. By comparing our samples with modern reference samples preserved in the laboratory and referencing the research work of Wan et al. (2011), we identified that the source of Type E starch grains as lotus root (Fig. 3f).

Type F: Snake gourd root starch grains (n=11; size range: 12.1–23.3 μm). Most starch grains were round and a few were bell-shaped or semicircular. Based on the relevant identification data and characteristics of starch grains from the roots of *Trichosanthes kirilowii* reported by Ge et al. (2010) and Yang et al. (2016b), this study preliminarily identified the source of Type F starch grains as snake gourd root (Fig. 3g).

Type G: Dioscorea starch grains (n=9; size range: 12.5–28.0 μm). The starch grains were oval or quadrangular-oval in shape, and the hilum was eccentric. According to the morphological data of modern Dioscorea starch grains in our laboratory and studies by Wan et al. (2011) on starch grains of modern tubers, this study concluded that the source of the Type G starch grains was Dioscorea (Fig. 3h).

Type H: Lily starch grains (n=3; size range: 19.5–41.9 μm). These starch grains exhibited elongated oval shapes with eccentrically positioned hilum. Under cross-polarized light, extinction arms appeared either crossed or near-cruciform, with partial curvature. The hila were generally larger than the distal ends. Based on morphological data of modern lily starch grains from our laboratory, combined with studies on starch grains of modern root tuber plants by Wan et al. (2011), this studydetermined that Type H starch grains originated from lily (Fig. 3i).

Type I: Legume starch grains (n=9; size range: 12.5–31.8 μm). These starch grains were kidney-shaped, with a centrally positioned hilum, and there were radial fissures at the hilum. Following Wang et al.'s (Wang et al., 2013) analysis of starch grains from common edible legumes in China, this study concluded that Type I starch grains more likely derived from legumes (Fig. 3j).

Type J: Acorn starch grain (n=1; size: 15.7 μm). The starch grain exhibited a triangular-ovate morphology, with a centrally positioned hilum and no observable lamellae. Under cross-polarized light, the extinction arm was X-shaped. Starch grains of nuts (e.g. *Castanea* and *Quercus* etc.) are mostly teardrop-shaped or triangular-oval. Notably, starch grains of *Castanea* with a long axis exceeding 10 μm generally exhibite lamellae, whereas starch grains of *Quercus* lack this feature (Yang et al., 2009). Therefore, this type of starch grain was identified as originating from Acorn (Fig. 3k).

Type K: Unidentified underground storage organs starch grains (n=5; size range: 13.1–22.6 μm). These starch grains were predominantly oval and triangular-oval in shape, and some of them had short "-"-shaped fissures, unclear lamella, "X"-shaped extinction cross, and eccentric hila. Due to the lack of significant morphological specificity, the source of Type K starch grains was identified as unidentified underground storage organs (Fig. 3l).

In addition, a total of 182 starch grains experienced fermentation damage, which will be explained in the Discussion section below.

3.2. Phytolith analysis

The pottery samples for phytolith analysis were from 12 different cultural units. No phytoliths were detected in blank control sample, suggesting that the experiment was not contaminated. A total of 6607 phytoliths were recovered from 12 samples, according to Wang and Lv (1993) and other related published researches (Liu et al., 2017), these phytoliths can be divided into 20 categories. Phytoliths derived from crops were identified, including η-type from husks of broomcorn millet, Ω-type from husks of foxtail millet, and three different types of phytoliths were recognized in rice: BULLIFORM, DOUBLE-PEAKED, and BILOBATE with a scooped-end parallel arrangement. Furthermore, the morphological types identified as CUNEIFORM BULLIFORM, BILOBATE, BLOCKY, RECTANGLE, SMOOTH-ELONGATE, and ELONGATE-ECHINATE exhibited siliceous vessels. The types categorized as SHORT SADDLE, LONG SADDLE, WAVY TRAPEZOID, ACICULAR, RONDEL, and POLYHEDRONS displayed conical projections. SCUTIFORM-BULLIFORM was obtained from *Phragmites* (reeds). Other microfossil remains including sponge spicules, diatoms, and charcoal (Fig. 4; Supplementary material 1).

The results of statistical analysis show that the relative content of rice phytoliths in the crop structure was approximately 25.22 %, and those of the foxtail millet husk and broomcorn millet husk phytoliths in the crop structure account for about 4.59 % and 70.17 %, respectively. The broomcorn millet husk phytoliths occupies a dominant position. Among the rice characteristic phytoliths, the DOUBLEPEAKED phytoliths mainly from rice glume account for approximately 77.65 %.

3.3. LC-MS analysis

The LC-MS detection chromatogram of the organic acid standard sample show that the separation of the ten target organic acids was optimized under these detecting conditions (Fig. 5; Supplementary material 2). Table 1 shows the mass spectrometry analysis results for the organic acid standard sample. As can be seen, the error values were all less than 5 ppm.

Through simulation experiments, Liu et al. (Wang et al., 2017a) demonstrated that the morphology of starch grains exhibited damage characteristics after fermentation, such as the disappearance of birefringence, center cracking, and hilum invagination. In this study, starch grains with above damage characteristics were recovered from ten pottery samples. To further confirm whether these potteries had been used for brewing wine, liquid samples from these ten pots were selected for LC-MS testing. During the experiment, ultrasonic water samples from the external surfaces of some pots, along with laboratory blank water samples, were used as control samples. The organic acids in the liquid samples were identified by comparing retention times and MS data with those of standard samples. It is worth noting that the retention times of some organic acids in the archaeological samples deviated from those in the standard samples. However, all identical organic acids within the archaeological samples shared consistent retention times. We attribute these deviations to the unique nature of the archaeological samples. To ensure accuracy, data with significant deviations were excluded during analysis. In total, 15 samples (including five control samples) were analyzed. Six types of organic acids—acetic, butyric, tartaric, malic, pyruvic, and oxalic—were detected (Supplementary material 1 and 2). No organic acids were found in the blank water samples, leading to the conclusion that the extracted organic acids were not derived from the laboratory or the surrounding soil environment.

The results showed that oxalic acid was ubiquitous in the external ultrasonic washing of multiple samples, which may have been caused by oxalic acid pollution in the soil. Samples 2, 6, 8, and 9 only detected acetic acid and butyric acid, while the other six samples detected multiple organic acids.

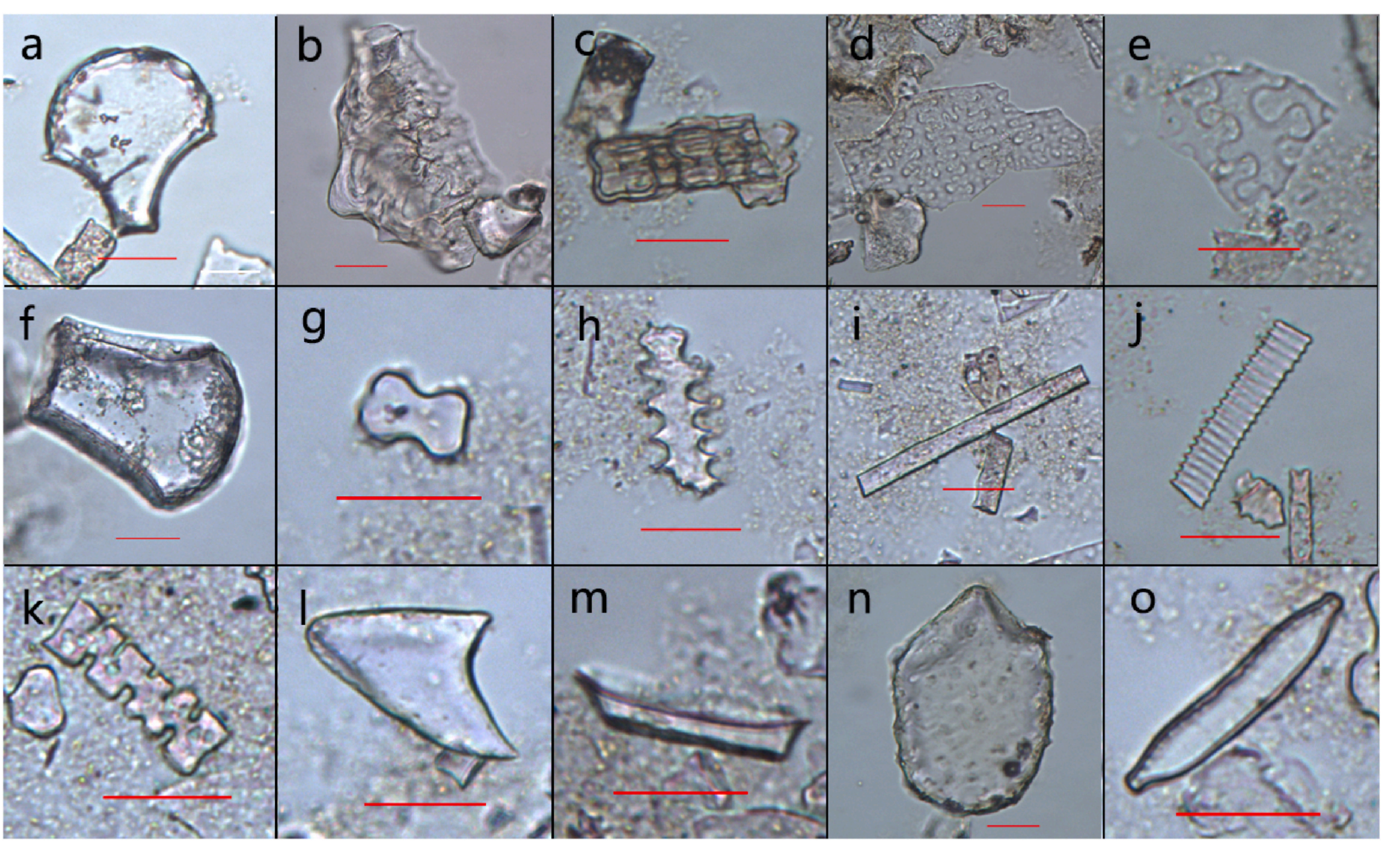

Fig. 4. Main phytolith morphotypes and other microfossils found at the Chengyan site (scale:20 μm)
A: BULLIFORM from rice; b: DOUBLE-PEAKED from rice; c: BILOBATE with scooped ends paralleled arrangement from rice; d: η-type from husks of broomcorn millet; e: Ω-type from husks of foxtail millet; f: CUNEIFORM BULLIFORM; g: BILOBATE; h: ELONGATE-ECHINATE; i: SMOOTH-ELONGATE; j: siliceous vessel; k: TOOTH-SHAPED; l: ACICULAR; m: LONG-SADDLE; n: SCUTIFORM-BULLIFORM was from *Phragmites* (reeds); o: diatoms.

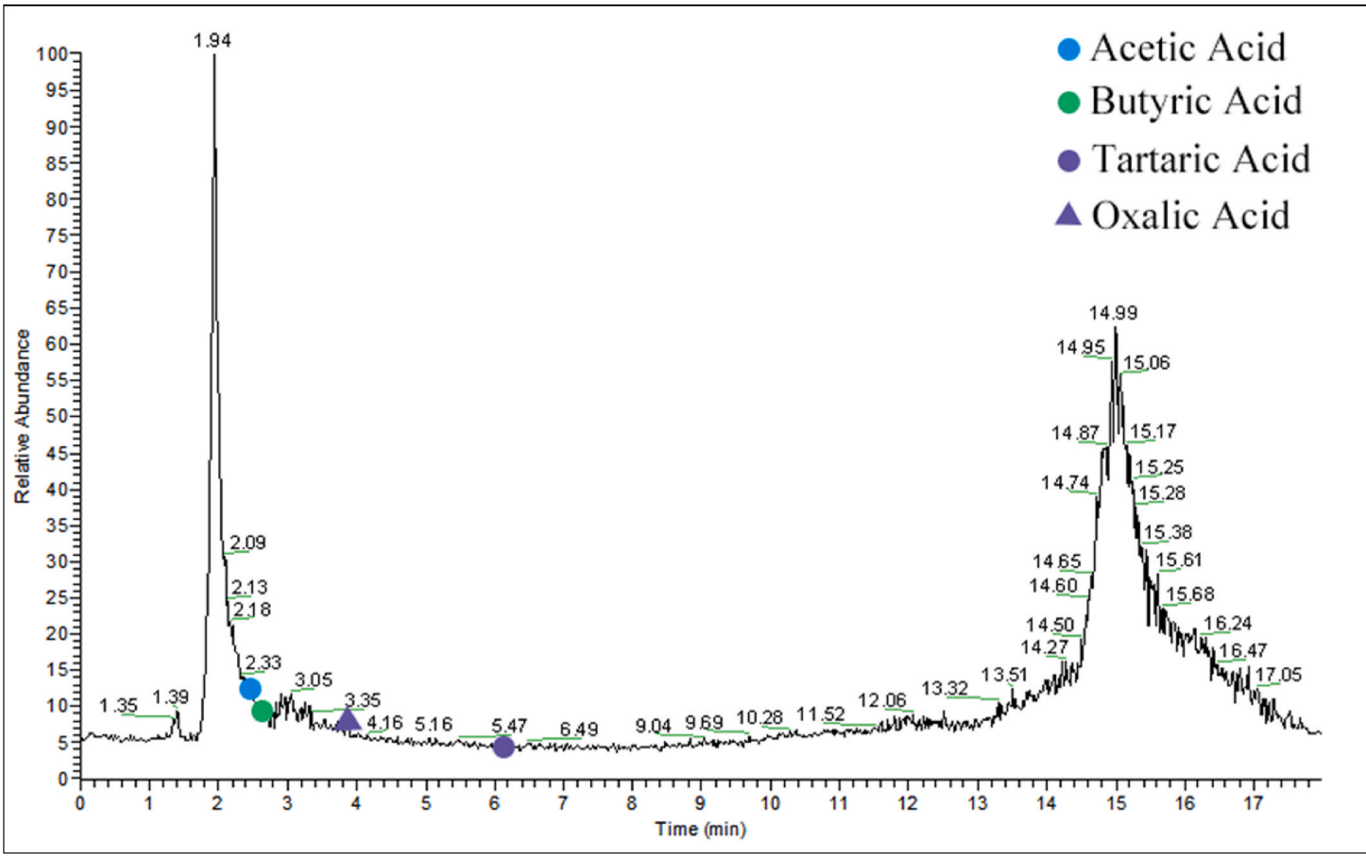

Fig. 5. Chromatogram of the archaeological Sample (Sample 8).

Table 1
Mass spectrometry analysis results of organic acid standard sample.

No.	Organic acid	RT (min)
1	LA	5.21
2	AA	2.49
3	BA	2.53
4	SA	1.68
5	TA	7.86
6	PA	3.74
7	FA	1.62
8	MA	6.42
9	CA	3.14
10	OA	2.65

4. Discussion

4.1. Utilization of edible plant resources in the early yangshao period at the chengyan site

To investigate the importance of different plant food resources in ancient people's dietary structure, two methods (i.e., absolute quantity and ubiquity/frequency), are generally used to quantitatively analyze the experimental results. Considering that the absolute number of extracted residual starch grains can be affected by various human or natural factors (Henry and Piperno, 2008), this paper only analyzed the ubiquity of various types of starch grains to discuss the relationship between Chengyan people and these plant foods (Fig. 6).

In total, 422 starch grains were extracted from 25 pottery samples unearthed at the Chengyan site. The grains were divided into 11 types, including cultivated crops (e.g., rice, foxtail millet and broomcorn millet) and multiple wild plant resources such as Job's tears, yams, legumes, lilies, and tuber plants which might come from people's

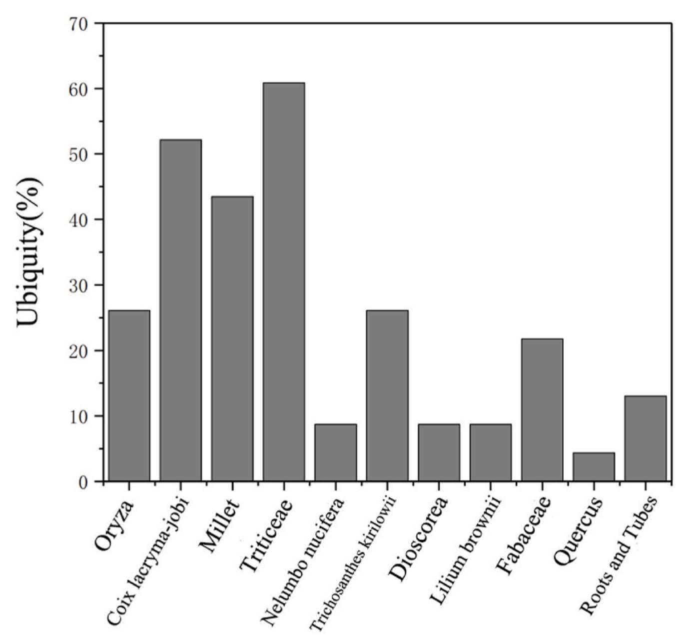

Fig. 6. Ubiquity of starch grains at the Chengyan site.

gathering activities. This result shows that the utilization of plant food resources at the Chengyan site has significant diversification characteristics.

The result of the statistical analysis show that the ubiquities of rice and millet starch grains were 24 % and 44 %, respectively. In addition to crop's starch grains, Job's tears, Triticeae, had higher ubiquities of starch grains, whose ubiquity reached 56 % and 60 %, respectively. Yi et al. (2021) once acquired starch grains, such as Job's tears, Triticeae, snake gourd root, lily, yam, and Vigna from the early Yangshao pottery of the Zhangwangzhuang site in Wuyang, Henan Province. The ubiquity of Job's tears at that site is also higher than that of millet and rice starch grains, which is similar to the results of this study. Liu et al. extracted starch grains, such as Job's tears, snake gourd root, and legumes from the early Yangshao pottery at Dingcun site in Henan (Liu et al., 2021c), Banpo site and Jiangzhai site in Shaanxi (Liu et al., 2021d). Combined with the results of this experiment, Job's tear, snake gourd root, legume, yam, and root tuber were widely exploited as important plant food resources for early Yangshao ancestors in the Central Plains.

The above results show that although the agricultural economy of the Chengyan site has greatly developed, the gathering of wild plants was still an important way for ancestors to obtain plant food resources. At present, there exists a paucity of investigation regarding the utilization of plant resources of the early Yangshao ancestors in the western Henan Province. Expanding the geographical scope from western Henan to encompass southern Shanxi and eastern Guanzhong regions—collectively constituting one of the two core zones of the Central Plains—reveals that systematic analysis of carbonized macro-botanical remains at the Yuhuazhai site in Xi'an has been conducted for its early Yangshao cultural deposits. Quantitative analysis revealed that domesticated crops accounted for 17.3 % of the total botanical assemblage, with wild plant resources constituting the remaining 82.7 %, reflecting a transitional subsistence economy during the early Yangshao period. Archaeobotanical investigations at the Chengyan and Yuhuazhai sites demonstrate that the early Yangshao period was a critical transitional phase from gathering economy to agriculture economy in the Central Plains core zone. Notably, previous plant archaeological research at Nanjiaokou (Zeng and Zhu, 1981), Xipo (Wei et al., 2000), and Yangshao Village (Zhong et al., 2020) sites in the middle-late Yangshao periods indicate that millet and rice mixed farming has always been the main form of agricultural economy in the western Henan Province, and agricultural production has become the main way for humans to obtain plant-based food after entering the middle Yangshao Culture.

A large number of phytoliths from crops of foxtail millet, broomcorn millet, and rice were also found in this experiment. To understand the crop structure of the Chengyan site, the numbers of phytoliths from three kinds of crops were analyzed (Fig. 7). The result show that the relative percentage of characteristic phytoliths from crops at the Chengyan site was approximately 24.7 %, and in which, the percentage of dryland crops, represented by husk phytoliths from foxtail millet and broomcorn millet, was approximately 75.3 %, indicating that dryland crops occupied a major position in the crop structure at the Chengyan sie. This result is consistent with previous studies on the crop structure of many other sites during the middle and late Yangshao periods in Western Henan, which indicates that millet was always the main crop of the Yangshao period in this area.

Rice originated in the middle and lower reaches of the Yangtze River 10,000 years ago, but existing data show that as early as 9000 years ago, it has spread to the southwestern Huanghuai Plain represented by rice remains found at Jiahu site (Zhang et al., 2018). Subsequently, in the early Peiligang Culture period, approximately 8000 years ago, it further spread northward to the Songshan area with higher latitude and altitude (Zhang et al., 2017; Wang et al., 2018; Bestel et al., 2018). However, owing to a lack of archaeobotanical evidence, it is not known whether rice continued to spread to the western Henan and Guanzhong plain in Shaanxi during the Peiligang period. According to the existing literatures (Zeng and Zhu, 1981), since the middle Yangshao culture, around 6000 years ago, a small amount of rice remains have been found at many sites in Western Henan. Coincidentally, in the Guanzhong area, a few rice remains were also discovered at sites such as Xinglefang (Liu et al., 2013), Yangguanzhai (Zhang et al., 2010), Anban (Zhang et al., 2017), and Xiahe (Zhang et al., 2018), which all belongs to the middle and late Yangshao period. The discovery of rice remains at the Chengyan site indicates that rice has spread to Western Henan in the early Yangshao Culture period, 6000 years ago. This finding provides new evidence for understanding the spatiotemporal process of rice spreading westward in Neolithic China.

4.2. The brewing of alcoholic beverages at the chengyan site

To investigate the production and consumption situation of alcoholic beverages at the Chengyan site, ten pottery samples with damaged starch grains relating to wine brewing were chosen for organic acid residue analysis in this study. The result shows that except for oxalic acid, Samples 2, 6, 8, and 9 only detected acetic acid and butyric acid. As these two organic acids are widely present in fruits, vinegar, and various condiments, it is very difficult to determine their sources. Therefore,

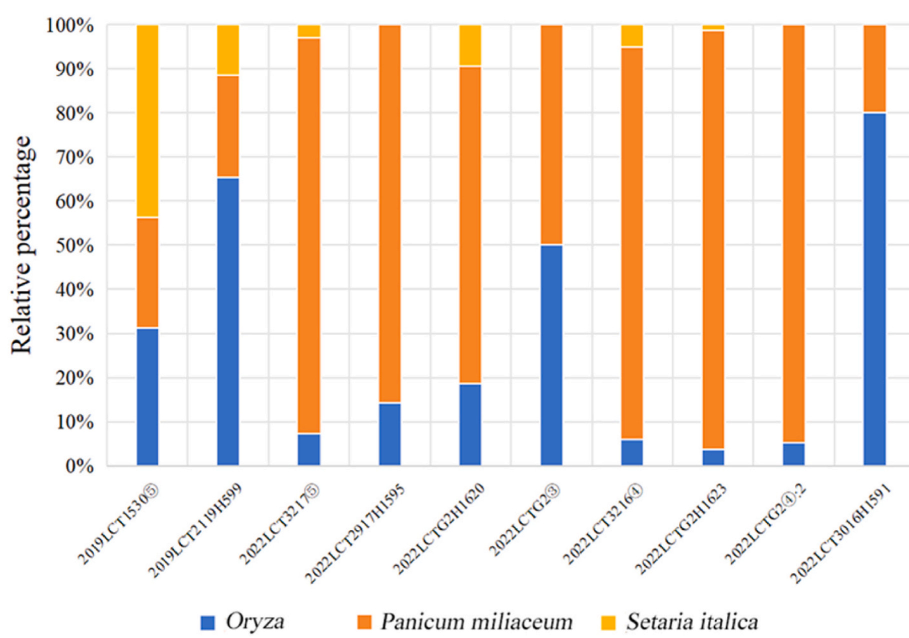

Fig. 7. The relative percentage of phytoliths in *Oryza*, *Panicum miliaceum,* and *Setaria italica* at the Chengyan site.

whether these four potteries are related to fermented drinks was not discussed in this paper. Notably, multiple organic acids were detected from the other six potteries. Among them, acetic and butyric are common in various fermented beverages, and they originate from different organic substances through acetic acid bacteria's fermentation (BARATA et al., 2012). Tartaric acid is an iconic organic acid existing widely in grain or fruit wine (Ding and Xia, 2014), and malic acid is very common in fermented drinks except distilled liquor (Li, 2017b). Combined with the result of starch grain analysis, it can be concluded that five pottery *Jiandiping* (Sample 1: 2022LCT3217⑤; Sample 3: 2022LCT2917H1595; Sample 4: 2022LCTG2H1620; Sample 5: 2022LCTG2③; Sample 7: 2022LCT3216④) should to be related to alcoholic beverages. For sample 10 (pottery cup: 2022LCT2917H1595), it is difficult to determine whether the organic acid residues came from alcoholic beverage because only malic acid was found in the cup apart from acetic and butyric. In addition, in the starch grain analysis on two potteries (one is *Jiandiping* numbered 2019LCT1530⑤, the other is a large-mouth round bottom jar numbered 2019LCT2119H599) which were both unearthed at Chengyan site in 2019, 164 starch grains with fermented damage characteristics were observed (Wang et al., 2017a) (Fig. 8), and these damage features included disappearance of partial edge (Fig. 8a), small pits on the surface (Fig. 8b), blurred cross extinction arm (Fig. 8c), recessed hilum (Fig. 8a and b), and ring-shaped rim (Fig. 8d,e,f) (Liao et al., 2022a). The statistical result shows that the damaged starch grains accounted for 60.27 % and 54.79 % of the total starch grains from each pottery sample, respectively. Combined with the simulation experiment by Liu et al. (2021a), it can be inferred that these two artifacts were also related to alcoholic drinks. Thus, it is believable that the ancestors at the Chengyan site in the early Yangshao period has begun to use crops and wild plants for brewing alcoholic beverage.

According to the result of starch grain analysis, eight kinds of starch grains coming from rice, millet, Triticeae, yam, snake gourd root, Job's tears, lily, and lotus root, were discovered in seven pottery artifacts related to alcoholic drinks. This discovery indicates that the Chengyan people once exploited the above plants as raw materials for wine brewing. Previous researches indicate these starch grains have been found in many sites in Henan Province (Lu, 2017; Sun, 2018), and were human's common edible plant resources during the Yangshao period. Simultaneously, they were also widely used for wine brewing in the Neolithic Yellow River valley. For example, at the Yangguanzhai site' Liu et al. found that ancient people exploited millet, Triticeae, Job's tears, snake gourd root, yam, and lily as raw materials for wine making during the middle-late Yangshao period (Yang et al., 2022). Similar phenomena were also discovered in other sites such as Lingkou (Liu et al., 2020), Jiangzhai (Yang et al., 2009), Banpo (Sun, 2018), Mijiaya (Wang et al., 2017b) and Xinjie (Liu et al., 2018b) in the Guanzhong region. In addition, at the Qingtai site in central Henan, Liao et al. found that raw materials for wine brewing during the late Yangshao period included Triticeae, millet, Job's tears, legume, lotus root, rice, and acorn based on starch grain analysis and organic acid residue analysis (Liao et al., 2022b). These studies indicate that multiple edible plant resources including crops and wild plants were used for wine making in the middle Yellow River region during the Yangshao period. The research results of this paper provide new data for confirming the universality of early Yangshao ancestors' wine making behavior in the Central Plains region.

In this study, seven pottery artifacts are believed to be related to alcoholic drinks, of which, six are *Jiandiping* and one is a round-bottomed jar. After conducting analysis on plant micro-remains and use-wear, Liu (2017) proposed that *Jiandiping* was used for brewing and drinking wine in the early and middle stages of Yangshao Culture, which

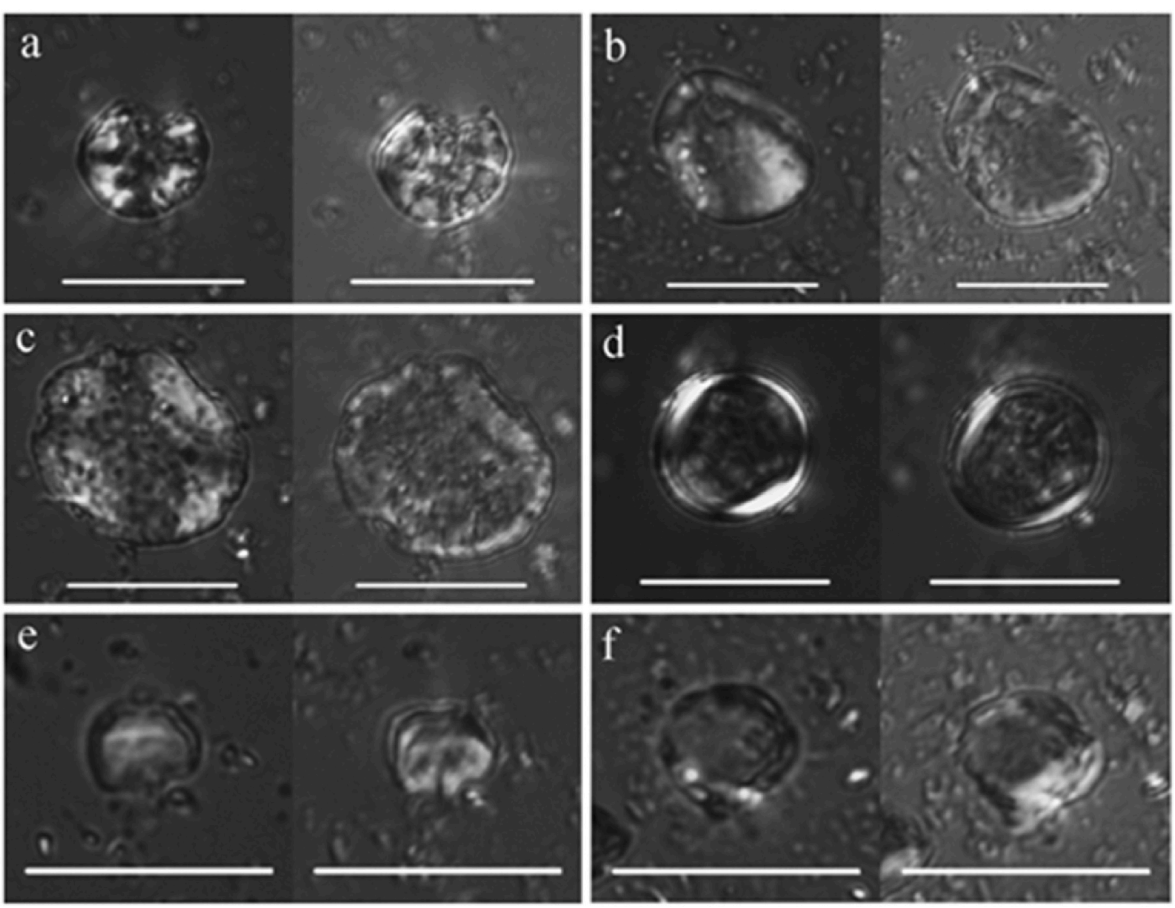

Fig. 8. Damaged starch grains from the Chengyan site (scale: 20 μm).

aligns with the experimental results of this paper. Previously, at the sites of Yangguanzhai (Yang et al., 2022), Lingkou (Yang, 2017), Jiangzhai (Yang et al., 2009), Banpo (Sun, 2018), Mijiaya (Liu et al., 2018a), and Xinjie (Sun, 2018), *Jiandiping* were also confirmed to be related to wine brewing or drinking. It should be noted that the above sites are all located in the Guanzhong Plain. Combined with the research results of this paper, it can be inferred that in Guanzhong Plain and its surrounding areas, *Jiandiping* were once widely used as wine vessels. However, Liao et al. (2022b) did not find damaged starch grains related to wine making in seven *Jiandiping* unearthed from the Qingtai site belonging to the mid-late Yangshao period in central Henan Province. Therefore, whether the *Jiandiping* has different functions in different regions in the Central Plains still needs to be further confirmed.

5. Conclusions

In this study, analysis of starch grain, phytolith, and organic acid residue on 34 pottery samples were conducted to investigate the dietary practices at the Chengyan site during the early Yangshao culture in western Henan Province, central China. The analysis of plant microremains revealed that the plant foods of the Chengyan inhabitants included Triticeae, Job's tears (*Coix lacryma-jobi*), rice (*Oryza sativa*), millet (*Setaria italica* and *Panicum miliaceum*), lotus root *(Nelumbo nucifera*), snake gourd root (*Trichosanthes kirilowii*), yam (*Dioscorea*), lily (*Lilium brownii*), legumes (Fabaceae), and acorn (*Quercus*), which indicates that plant food utilization showed high diversity during the early Yangshao period in western Henan, with gathering playing a significant role in food acquisition. Furthermore, the results suggest that the Chengyan site had developed a mixed rice-drought farming model dominated by foxtail millet and broomcorn millet, supplemented by a smaller amount of rice. The discovery of rice remains at the Chengyan site demonstrates that rice had spread to western Henan in the early Yangshao cultural period, 6000 years ago. This finding provides valuable evidence for understanding the spatiotemporal process of rice's westward spread in Neolithic China. Additionally, organic acid residues from pottery containing starch grains with fermentation-related damage characteristics were analyzed. The results indicate that the ingredients used in fermented beverages were derived from the plant foods in daily life. The associated pottery types suggest that in the Guanzhong Plain and its surrounding areas, *Jiandiping* are widely used as wine containers. This study offers new data supporting the universality of brewing and drinking practices in the early Yangshao ancestors in the Central Plains region.

CRediT authorship contribution statement

Yingxue Gong: Writing – original draft. **Yuzhang Yang:** Writing – review & editing. **Xingtao Wei:** Resources, Investigation. **Jingwen Liao:** Methodology, Formal analysis. **Binggui Sun:** Methodology, Investigation. **Yuchun Wang:** Methodology. **Juzhong Zhang:** Resources.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quaint.2025.109848.

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