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Chapter 4 The influence of grinding on the preservation of starch grains from rice

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The influence of grinding on the preservation of starch grains from rice

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Abstract

China is a major centre for rice domestication, where starch grain analysis has been widely applied to archaeological grinding tools to gain information about plant use by ancient Chinese societies. However, few starch grains from rice have been identified to date. To understand this apparent scarcity of starch grains from rice, we carried out dry- and wet-grinding experiments with stone tools on four types of cereals: rice (*Oryza sativa* L.), foxtail millet (*Setaria italica*), Job's tears (*Coix lacryma-jobi* L.), and barley (*Hordeum vulgare* L.). The results reveal that dry-grinding produces significant damage to starches to the point where they may be undetected in archaeological samples, while wet-grinding causes only slight morphological changes to the starch grains. Moreover, rice starch grains have the most substantial alterations from dry-grinding, possibly impeding their identification. These findings provide a possible means to explain the relative scarcity of rice starch grains recovered from archaeological grinding tools, which we now suggest was caused by the use of the dry-grinding technique. We therefore suggest that rice starch grains have been likely underrepresented in the archaeological record, and previous interpretations of starch analyses need to be reconsidered.

Keywords: rice, grinding technique, starch grains, experimental archaeology, Neolithic China

4.1 Introduction

Rice (*Oryza* spp.) is an important crop in Asia, where cooking techniques, eating habits, and feasting rituals have been historically associated with this plant (Cheung and Tan 2007). China has three major regions that are currently considered the earliest centres for rice domestication, which include the lower catchment of the Yangtze River (Jiang and Liu 2006; Liu et al. 2007b; Liu et al. 2007a; Trivers et al. 2009), the middle catchment of the Yangtze River (e.g. Zhao, 1998), and the upper catchment of the Huai River (Wang and Zhang, 1998: Fig. 4.1a). On the basis of the previous research, these regions all possess well-documented evidence for rice domestication during the early Neolithic period (e.g. Liu et al., 2007b, 2007a; Lu et al., 2002; Wu et al., 2014; Yang et al., 2016; Zhao, 2010). For instance, in the upper catchment of the Huai River, at the site of Tanghu (c. 9000-7000 cal. BP) phytolith analysis yielded evidence of rice cultivation as early as 7800 to 4500 BP (Zhang et al. 2012). At the nearby site of Jiahu (9000-7500 cal. BP), domesticated rice macrobotanical remains have been recovered from all three sub-phases (Zhang 1999; Yang et al. 2017; Zhang et al. 2018). Isotope analysis of human skeletons from Jiahu indicates that C3-based foods, potentially including rice, dominated human diets throughout the occupation of Jiahu (Hu et al., 2006). Rice has also been proven to have been used as an ingredient for fermented beverage production 9000 years ago at Jiahu (McGovern et al. 2004).

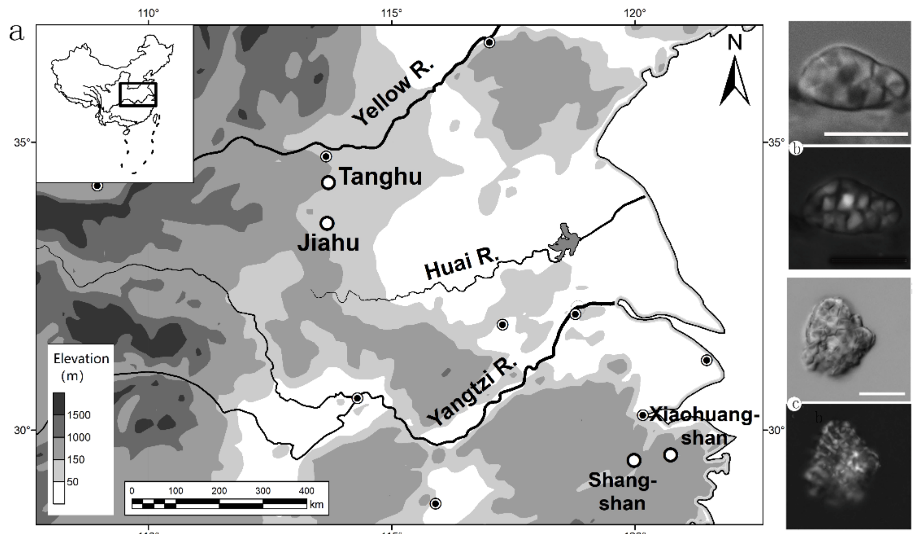

Figure 4.1 The archaeological sites mentioned in the text. (a) and ancient starch grains from rice recovered from grinding tools at the sites of Tanghu (b) and Jiahu (c), scale bar of the starch grains: 20μm. Note the majority of ancient starch grains from rice from these two sites are compound (after Yang et al., 2015b; Zhang, 2015).

Even though the exploitation of rice has been demonstrated by different analytical methods, rice starch grains have seldom been recovered from archaeological grinding tools from these regions (Liu et al., 2010; Yang et al., 2015). In the few cases where rice starch grains have been identified (Yang et al. 2015b; Zhang 2015; Yao et al. 2016), they only account for a very small amount of the total identified starch grains. The ubiquity value (a term used to describe the proportion of samples including a certain type of starch grains against all examined samples; see Li et al., 2018; Yao et al., 2016) of rice is also relatively lower than other plant species, such as plants from the Triticeae tribe, Job's tears (*Coix lacryma-jobi*), and various underground storage organs (e.g. Liu et al., 2014a; Y. Yang et al., 2015; Zhang et al., 2011).

The scarcity of rice starch grains recovered from grinding tools was often used to suggest that rice was not the primary processed material on these tools (Liu et al. 2010b; Yang et al. 2015a; Yang et al. 2015b; Yao et al. 2016). This seems a reasonable hypothesis considering that rice could also have been processed by other ways such as boiling (Yang et al. 2015a; Yao et al. 2016). However, nowadays in China, grinding rice into flour and boiling rice directly are both common culinary practices that could have been passed down through generations from the distant past. If so, rice might have been processed with grinding tools to a larger extent than previously suggested, implying rice starch grains might be underrepresented in samples from archaeological grinding stones. This is possible because studies have suggested that different depositional environments affect the preservation of starch grains (Haslam 2004; Langejans et al. 2012). Indeed, certain starch grains are less resistant than others to amylolysis (Hutschenreuther et al. 2017). Thus, this variation could have led to representation biases on ancient starch grain studies. Similarly, pressure, moisture, and heat involved in different food processing practices could also result in considerable morphological changes (due to starch gelatinization), and even complete destruction of starch grains (Tester and Morrison 1994; Henry et al. 2009; Pagán-Jiménez et al. 2017). Those damage features, especially the loss of extinction crosses, will inevitably have affected starch detection and identification (Lamb and Loy, 2005).

Starch damage features associated with grinding have been investigated in experiments using several types of cereals, such as wheat (*Triticum aestivum* L.), broomcorn millet (*Panicum miliaceum* L.), and maize (*Zea mays* L.) (Babot 2003; Ge et al. 2011; Mickleburgh and Pagán-Jiménez 2012). However, it has been difficult to compare different damage patterns on starch grains from different plant species associated with grinding because the parameters amongst those studies were not always consistent. For instance, the duration of grinding experiments, the tool types, and the grinding technique all varied. Therefore, in this paper, we propose a set of systematic experiments to explore whether starch grains from rice share the same damage features with those from other types of cereals.

Four types of cereals are selected in this experimental study: rice (*Oryza sativa* L.), foxtail millet (*Setaria italica*), Job's tears (*Coix lacryma-jobi* L.), and barley (*Hordeum vulgare* L.) (Table 4.1). These plants were chosen due to their long history of use in China. Rice and foxtail millet are both known to have been cultivated in China at least 10,000 years ago (Zhao 2010; Yang et al. 2012; Wu et al. 2014). Starch grains from Job's tears have been widely recovered from grinding tools at sites dating to at least 8000 years BP in both southern and northern China (Liu 2015; Yao et al. 2016; Liu et al. 2018b). Early evidence for the use of barley has been found on the margins of the Tibetan Plateau 3400 BP (Guedes et al. 2015) and in the Hexi Corridor c. 4000 BP (Flad et al. 2010). Barley belongs to the tribe Triticeae and starch grains from wild Triticeae specimens have been identified on grinding tools dated to the Upper Paleolithic period in northern China (Liu et al. 2018a).

De-hulled cereals were chosen as the processed material because previous studies reveal that Neolithic grinding tools were more likely used to grind cereals into flour rather than for separation of hulls (Liu et al. 2010c; Li et al. 2018). De-hulling was likely carried out using wooden pestles, as such a process was documented in ancient Chinese literature, such as YiJing (from the Western Zhou period, 3000-2750 BP). Wooden pestles were also recovered from the archaeological sites of Bashidang (7540-7100 BP) and Hemudu (c. 7000-5000 BP) in the Yangtze River basin (Peregrine and Ember 2012; Xu 2017).

Table 4.1 Information on the grinding experiments

Exp. No.	Grinding technique	Material	Origin of the cereals	Duration (minutes)	Efficiency
1	dry-grinding	rice	Northeast China	60	1
2	wet-grinding	rice	Northeast China	60	2
3	dry-grinding	foxtail millet	Taiwan	60	1
4	wet-grinding	foxtail millet	Taiwan	60	2
5	dry-grinding	Job's tears	Taiwan	60	2
6	wet-grinding	Job's tears	Taiwan	60	3
7	dry-grinding	barley	Netherlands	60	0
8	wet-grinding	barley	Netherlands	60	0

Note: "Efficiency'' refers to the interpretation of the efficiency to grind the cereals into flour from lower to higher levels 0-3.

4.2 Materials and Methods

In the present study, all the cereals were purchased from Chinese supermarkets in The Hague, Netherlands (Table 4.1). The grinding tools were made from sandstones obtained from the riverbed of the Maas in the southern Netherlands. These stones exhibit great similarity to archaeological grinding tools from Neolithic Chinese sites in terms of their coarseness and texture (Liu et al. 2010c; Cui et al. 2017). The grinding tools all possess at least one flat grinding surface. An example of the experimental grinding tools used in this study is depicted in the supplementary data (Fig. S4.1).

Selected cereals were subjected to dry-grinding (without pre-soaking of the cereals in water) and wet-grinding (by pre-soaking of the cereals in water). These two grinding techniques are both common in present-day societies in Asia (Chiang and Yeh 2002; Kethireddipalli et al. 2002; Suksomboon and Naivikul 2006). The selections of specific grinding techniques are generally based on cultural preferences regarding food texture (e.g. Nout, 2009) or the desire to remove bitterness resulting from pre-soaking (e.g. Hosoya, 2011). For the wet-grinding experiment, each type of cereal was separately soaked in tap water for ten hours. Then, each cereal was ground into flour with a back-and-forth motion (see also Li et al., 2019). An assessment of the efficiency of each grinding experiment was documented using four categories: 0= not effective, very difficult to grind the cereals into flour; 1= moderately effective, cereal can be ground into flour but with a lot of effort; 2= effective; cereal can be ground into flour with some effort; 3= highly effective, very easy to grind cereals into flour (Table 4.1).

After 60 minutes of grinding each type of cereal, the stone tools were sampled following the procedures outlined by previous publications (Torrence and Barton 2006; Cnuts and Rots 2018, with slight modifications). Ultra-purified water was placed on the surface of the stone tools for two minutes. Then the starch grain samples were obtained from the stone surfaces using a pipette. These samples were placed in 2ml micro-centrifuge tubes. The tubes were centrifuged and then mixed with a solution of 50% glycerol in distilled water, a common and suitable method in starch research (Pagán-Jiménez 2007; Hart 2011; Coster and Field 2015; Mariotti Lippi et al. 2015; Liu et al. 2018b). A volume of 40 μL of each sample containing the processed starch grains was placed onto a clean glass slide. Then, cover slides were placed on each slide and were sealed with neutral balsam to prevent the dehydration of starch grains. Microscopic observations were carried out within one month of the slide preparation. Starch grains from unprocessed cereals were studied for comparative purposes. The cereal samples were prepared following the method mentioned in the previously published literature on modern starch research (Wei et al. 2010). First, each specimen was put in a separate 2ml micro-centrifuge tube filled with ultra-purified water. After 8 hours, a disposable pipette was used to press the grain inside the tube in order to release the starch grains. Then the liquid sample containing the released starch grains were transferred to a clean tube for centrifuging. The next steps of the procedure are identical to the one described above for the ground starch samples.

The attributes selected for studying damaged starch grains included (a) starch type (single or compound), (b) shape, (c) size, (d) presence and absence of features on the starch surface (hilum, fissures, and lamellae), and (e) extinction cross morphology (see the description of these starch features e.g. García-Granero et al., 2017; Torrence and Barton, 2006). Following Gong and colleagues (2011), starch grains were divided into three categories based on the characteristics of the extinction crosses. Type I refers to starch grains with clear extinction crosses. This category includes the undamaged starch grains and slightly damaged starch grains. Type II are starch grains with faint extinction crosses, which are still visible under polarized light and dark field view. Type III starch grains are represented by those with non-visible extinction crosses (Fig. 4.3b). All starch samples were observed under a Leica DM2700P microscope with polarizing light and an attached Leica MC170HD camera. When possible, at least 50 single starch grains from each sample were measured and counted (see also Fig. 4.3) using the Leica application suite version 4.8.

4.3 Results

From our experience, dry-grinding of cereals requires more effort than wet-grinding (Table 4.1). Only Job's tears were very easy to dry-grind into flour, which is consistent with the grinding experiment conducted by Liu and colleagues (2018a). In contrast, dry-grinding barley was difficult, an observation that is consistent with previous experiments (Lull et al. 2010). Rice and foxtail millet can both be dry-ground into fine flour with some effort. After soaking, rice, foxtail millet, and Job's tears become much softer and easy to grind, whereas the grinding of soaked barley remains as difficult as in the dry conditions. Detailed morphological changes of starch grains from each type of cereals after dry- and wet-grinding are described below.

4.3.1 Rice

Unprocessed starch grains exhibit various morphologies depending on the plant species, and their morphological types are classified as either compound or single (Tateoka 1962). Both morphological types of starch grains are found in the unprocessed rice samples (Fig. 4.2a and b). The shape of single starch grains is polygonal. The average maximum length of the single rice starch grains is 5.64 ± 1.89μm (Table 4.2). The hilum is centric and closed. No fissures or lamellae are visible. Their extinction crosses are radially symmetrical and cross-shaped. Compound rice starch grains consist of an aggregate of single starch grains. In these cases, the compound structure is oval in shape (Fig. 4.2b) and has diagnostic reflected star-shaped extinction crosses (Fig. 4.2b′).

The dry-grinding technique results in severe damage to the starch grains. The most noticeable change is that few single rice starch grains remained, and more starch aggregates are formed (Fig. 4 2c and d). These starch aggregates have irregular shapes (Fig. 4.2c and d) and their extinction crosses are invisible or become faint and blurry (Fig. 4.2c′ and d′). Only a few compound starch grains survived with observable birefringence (Fig. 4.2c′). A low number of single starch grains that remained completely lost their extinction crosses (Fig. 4.3b). The average maximum length of the single dry-ground starch grains from rice is 8.95 ± 2.58μm (Table 4.2, Fig. 4.3a).

In sharp contrast to dry-grinding, wet-grinding produced no damage signs to rice starch grains. These starch grains are very similar to the unprocessed samples in terms of size (Fig. 4.3a), shape, extinction cross, and characteristics of the starch surface (Fig. 4.2e, e′, f, and f′). The average maximum length of rice single starch grains submitted to wet-grinding is 6.24 ± 1.40μm (Table 4.2, Fig. 4.3a).

4.3.2 Foxtail millet

Starch grains from unprocessed foxtail millet samples consist of both single structures and compound grains (Fig. S4.2a and a′). Single starch grains are polygonal, round, or oval. The mean length of single foxtail starch grains is 10.21± 2.15μm (Table 4.2). The hilum of the starch grains is centric and closed. The extinction cross is clear and cross-shaped. Neither lamellae nor fissures are visible. The compound starch grains from foxtail millet are circular but with some angled flat sides (Fig. S4.3b and b′).

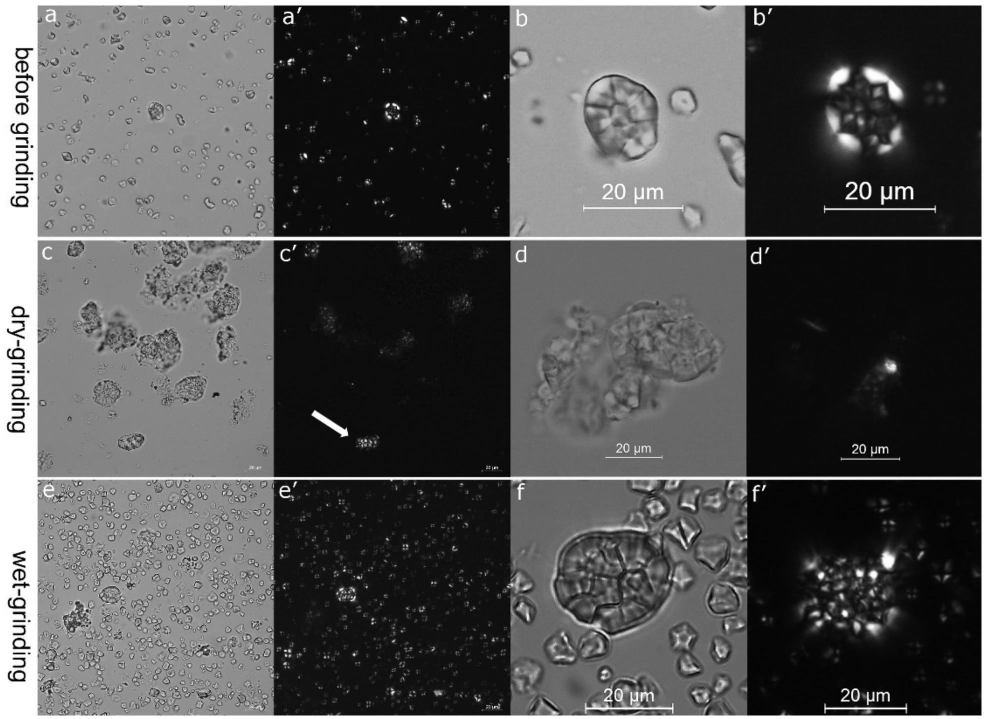

Figure 4.2 Morphological changes of starch grains from rice after dry- and wet-grinding. a, a′: single and compound starch grains coexist in the unprocessed rice samples; b: the oval-shaped compound starch grains under normal light, b′: star-shaped extinction cross of the compound starch grains under polarized light; c, c′: single starch grains nearly invisible after dry-grinding, the only visible compact compound starch grains are marked with the arrow; d, d′: irregular shape aggregates under the normal and polarized light; e, e′, f′ and f′: starch grains perform similar features as the unprocessed samples.

After dry-grinding foxtail millet, more starch aggregates tend to be formed. However, the single starch grains from foxtail millet still remained. Extinction crosses in aggregate starch grain became blurry. Single starch grains had fissures generated on their surfaces and their size enlarged (Fig.4.3a). After dry-grinding, the average maximum length is 13.79 ± 4.42μm (Table 4.2). Most starch grains (43%) display nearly intact extinction crosses; 35% have faint extinction crosses; the rest (22 %) become invisible under polarized light (Fig. 4.3b).

After wet-grinding, the starch grains still have a compact shape and showed clear extinction crosses. The average maximum length of single starch grains is similar to the unprocessed samples (Fig. 4.3a): 10.79 ± 2.47μm (Table 4.2). The most noticeable damage patterns are shallow fissures and radiating striations on the surfaces of the starch grains (Fig. S4.2f and f′).

4.3.3 Job's tears

Starch grains from unprocessed Job's tears consist of polygonal, spherical and oval-shaped single grains. The average maximum length of these starch grains is 10.31 ± 3.77μm (Table 4.2), which is smaller than that reported in previous studies (e.g. 12.4 ± 3.0 μm in Yang and Perry, 2013). This disparity is reasonable, based on studies showing that starch grain size is partially affected by the geographic origins of the source plants (e.g. Perry 2002; Liu et al. 2014b). The starch grain surfaces are smooth, lamellae are not visible, and the hilum is centric. Linear-, V-, Y-shaped, or stellate fissures are present on starch grain surfaces and the extinction cross is mainly cross-shaped.

After dry-grinding, most of the starch grains remain as single structures (Fig. S4.3c and c'). Their size is relatively stable (Table 4.2, Fig. 4.3a) with few dry-ground starch grains found to be enlarged considerably (e.g. Fig. S4.3d and d'). The average maximum length of these single starch grains is 10.74 ± 4.60μm (Table 4.2). Deeper and stellate fissures are observed on the surface of the single starch grains. The majority of the starch grains (68%) remain nearly unchanged in terms of their extinction crosses, though 23% totally lost their birefringence features (Fig. 4.3b); the rest (9%) still possess visible birefringence features under polarized light (Fig. 4.3b).

Wet-grinding resulted in no significant changes to the starch grains (Fig. S4.3e, e', f and f'). The shape, extinction cross, and other surface features of the processed starch grains all resemble the unprocessed samples. The average maximum size of these starch grains increased to 11.29 ± 3.27μm (Table 4.2), which is slightly larger than the unprocessed samples.

4.3.4 Barley

Starch grains from the Triticeae tribe are divided into two types according to their size. Type A refers to starches that were larger than 10μm, while Type B refers to starches less than 10μm in diameter (Lindeboom et al. 2004; Howard et al. 2011). Starch grains from Type B are small with very few diagnostic characteristics, so archaeological identifications of starch grains from the Triticeae tribe are largely based on Type A starch grains (e.g. Y. Yang et al., 2015; Yang et al., 2016b). Unprocessed Type A barley starch grains are oval-shaped. The average maximum length of the barley starch grains measured 21.85 ± 4.43μm (Table 4.2), which is larger than previously suggested (e.g. 18.0 ± 4.1μm in Yang and Perry, 2013). The hilum of these starch grains is centric and closed. Shallow lamellae are visible on some starch grains, usually the bigger ones, and the extinction crosses are thick and "X-shaped" (Fig. S4.4a, a', c and c').

After dry-grinding, lamellae on the starch surfaces of barley became deeper and shallow striations were formed on the surface (Fig. S4.4d). These starch grains also became flatter (Fig. S4.4c). Some of them (43 %) totally lost their birefringence features (Fig. 4.3b), whereas others (36%) still exhibited extinction crosses resembling the ones registered in unprocessed samples (e.g. Fig. S4.4c'). In other cases (21 %), the extinction crosses became faint but still visible under polarized light (Fig. 4 3b). The average maximum length of the dry-ground starch grains is 25.97 ± 8.06μm (Table 4.2).

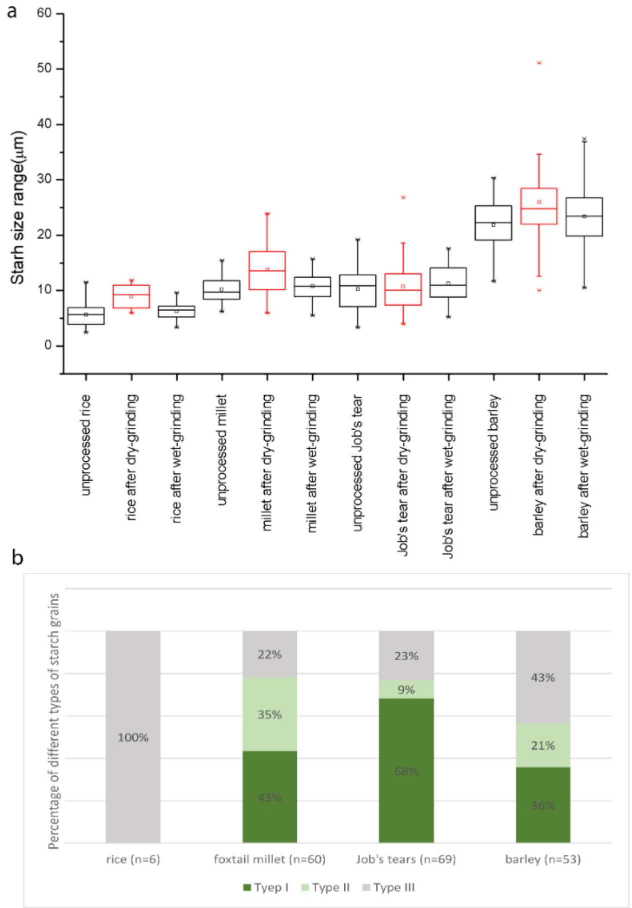

Figure 4.3 The size of starch grains from the unprocessed, dry-grinding (in red colour) and wet-grinding samples. (a) and the proportion of three different categories of damaged starch grains from the dry-grinding samples (b). a: The size of starch grains from the unprocessed, dry-grinding (in red colour) and wet-grinding samples, note the size of the starch grains from the dry-grinding samples is bigger than their unprocessed and wet-grinding samples, except Job's tears. b: Type I, Type II, and Type III refer to three different categories of damaged starch grains. At least 50 single starch grains were measured from each sample except the dry-grinding samples from rice, in which the single grains mostly disappeared, supporting data for Figure 3b can be found in the supplementary data (Appendix II), including Table S4.1 and Figure S4.5- Figure S4.23.

The morphological changes on barley starch grains after wet-grinding are minor in terms of their shape and extinction crosses (Fig. S4.4e, e' and f'). Only some shallow lamellae are observed on starch surfaces under normal light (bright field view, e.g. Fig. S4.4f). The average maximum length of the single wet-ground starch grains is 23.38 ± 5.21μm (Table 4.2).

4.4 Discussion

No damage features are observed on the samples from rice or Job's tears after wet-grinding. Wet-grinding only produced noticeable damage patterns to foxtail millet and barley starch grains. In contrast, dry-grinding results in more extensive starch grain modifications. A formula developed in food chemistry research (see the detailed description in Asmeda et al., 2016) also revealed that starch damage during dry-grinding is the most intense. The present study thus adds an additional line of evidence indicating that the type of observed damage patterns is directly related to the employed grinding techniques, in which the use of water for soaking cereals might result in less damage to starch grains.

Our experimental results also indicate that dry-grinding produced different types of damage patterns depending on the types of cereal. First, the size of Job's tears starch grains remains stable after processing, which is possibly because grinding Job's tears requires only minimal pressure to make flour (Table 4.1). In other words, the seed coat (endosperm) of this species seems to be softer than in the other plants used in the present study. Previous dry- and wet-grinding experiments comparing damage patterns also revealed a similar relationship between starch enlargement and seed coat hardness (Mickleburgh and Pagán-Jiménez 2012). The collated data suggests that the harder the seed coat, the greater the enlargement of the starch grains due to grinding (Table 4.2).

Polarized light and dark field view are often used to recognize, detect, and identify starch grains (e.g. Liu et al., 2011; Lu et al., 2005; Perry and Michael Quigg, 2011; Y. Yang et al., 2015). Our experiments demonstrated that birefringence and extinction crosses of starch grains became invisible after dry-grinding. Moreover, other minerals and organic particles (e.g. fungi and cellulose) also show birefringence and produce optical features similar to extinction crosses (Haslam 2004; Ge et al. 2011). It is thus likely that damaged irregular starch aggregates from rice could have easily been overlooked in the archaeological samples. This phenomenon leads us to reconsider the data from previous research carried out on grinding tools from Chinese Neolithic sites.

Based on the experimental results from the present study, rice starch grains tend to form aggregates after dry-grinding, while inversely many of the single rice starch grains do not remain. The most likely opportunity to detect rice starches after dry-grinding is to locate the few surviving compound grains with intact extinction crosses (Fig. 4.2c'). This is the case at the site of Tanghu, where only one intact compound starch grain has been recovered (Fig. 4.1b) (Yang et al. 2015b). Similarly, the starch grains from rice recovered from Jiahu are mostly compound as well (83%, 69 out of the 83, e.g. Fig. 4.1c) (Zhang 2015). This phenomenon is consistent with the results from our rice dry-grinding experiment. In addition, previous microwear analysis of grinding tools from Jiahu demonstrated that dry-grinding was used for cereal processing (Li et al. 2019).

Starch grain analysis has been carried out at the Neolithic sites of Xiaohuangshan and Shanshan (Liu et al. 2010b; Yao et al. 2016; Yang et al. 2015a) in the Yangtze River basin in Eastern China. This area is presumed to be the one of the earliest areas for rice cultivation and domestication in the world, though only four rice starch grains have been recovered from the grinding tools at the site of Xiaohuangshan (Liu et al. 2010b; Yao et al. 2016), and no starch grains have been identified from grinding tools at the site of Shanshan (Yang et al. 2015a). If dry-grinding was employed at these sites, it might have adversely affected the preservation and potentially the recovery of ancient rice starch grains.

Because no starch grains from rice have been found on the grinding tools from the site of Shangshan, Yang et al. (2015) suggested that there might be an under-representation of rice starch grains. The authors argued that rice single starch grains are normally less than 10μm, which is too small to be easily detected under a microscope. However, a magnification up to 630x has been used for starch research and small size starch grains (less than 5μm) have been revealed in archaeological samples (e.g. Liu et al., 2014c, 2010b; Yang et al., 2016b). Thus, the results of this experimental study provide another possible explanation regarding the scarcity of starch grains of rice on grinding tools, caused by the dry-grinding technique.

Based on observations derived from our experimental research, we need to point out a problematic issue for detecting starch grains in samples obtained from grinding tools by only using polarized light. After dry-grinding, most of the starch grains became more difficult to recognize with polarized light because of the loss of birefringence and their extinction crosses. Therefore, it is imperative to combine normal, white field microscopy to detect such starch grains during the scanning of sample slides. This practice will maximise the chance of detecting both native and damaged starch grains (Pearsall 2016).

Table 4. 2 Characteristics of starch grains before and after grinding

Plant species	Size of starch grains		Main features of starch grains after grinding
	Size range (μm)	Average size (μm)	
Unprocessed rice	1.89-11.45	5.64± 1.89 (n=55)	Dry-grinding results in greater enlargement of the size of starch grains than wet-grinding; Most of the single starch grains disappear after dry-grinding.
Dry-ground rice	5.92-11.88	8.95 ± 2.58 (n=5)	
Wet-ground rice	3.32-9.63	6.24 ± 1.40 (n=66)	
Unprocessed foxtail millet	6.21-15.45	10.21± 2.15 (n=52)	Dry-grinding result in greater enlargement of the size of starch grains than wet-grinding; 22% single starch grains totally lost their extinction crosses after dry-grinding.
Dry-ground foxtail millet	5.87-13.79	13.79 ± 4.42 (n=79)	
Wet-ground foxtail millet	5.50-15.72	10.79 ± 2.47 (n=61)	
Unprocessed Job's tears	3.33-19.16	10.31±3.77 (n=61)	Dry-and wet-grinding both result in slight enlargement of the size of starch grains; 23% single starch grains totally lost their extinction crosses after dry-grinding.
Dry-ground Job's tears	3.92-26.81	10.74 ± 4.60 (n=54)	
Wet-ground Job's tears	5.20-17.54	11.29 ± 3.27 (n=52)	
Unprocessed barley	11.63-30.32	21.85 ± 4.43 (n=75)	Dry-grinding result in greater enlargement of the size of starch grains than wet-grinding; 43% single starch grains totally lost their extinction crosses after dry-grinding.
Dry-ground barley	10.01-51.08	25.97 ± 8.06 (n=68)	
Wet-ground barley	10.46-23.38	23.38 ± 5.21 (n=97)	
Zea mays (mature and hard kernels, control sample)	2-28	13± 3.9 (n=116)	Samples with harder kernels result in the greater the enlargement of the size of starch grains (Mickleburgh and Pagán-Jiménez, 2012)
Zea mays (green and soft)	5-25	12.1±4.7 (n=60)	
Zea mays (mature and hard kernels) *	10-38	23.2± 6.6 (n=60)	
Zea mays (semi-mature and partially hard kernels) *	7-34	20.8±5.7 (n=60)	

Note: Samples marked with * were soaked for one hour and then intensively ground for five minutes. *Zea mays* (maize) control samples were soaked for 24 hours and then ground 15 seconds to avoid overly damaging of starch grains. All the *Zea mays* samples were ground with a marble mortar and pestle (Mickleburgh and Pagán-Jiménez 2012).

4.5 Conclusion

The application of starch grain analysis has grown quickly in China, with increasingly more papers on ancient starch research being been published each year (Yang 2017). Some limitations and issues of this method have been noticed and discussed elsewhere (Haslam 2004; Hutschenreuther et al. 2017; Mercader et al. 2018). These are known taphonomy issues such as soil bacteria, enzymes, fungal degradation, and chemical digestion that may bias representation of starch grains in the archaeobotanical record. In addition, our results indicate different grinding techniques also result in different levels of damage to starch grains and hence different degrees of preservation. Among the different grinding techniques, dry-grinding causes significant morphological changes to starch grains and consequently affects starch grain detection, especially in the case of rice. This result suggests that the scarcity of rice starch grains recovered from grinding tools in Neolithic Chinese sites could be caused by the employment of the dry-grinding technique by ancient communities. Thus, previous interpretations inferring that rice was not the primary cereal processed with Neolithic grinding tools needs to be reconsidered. We have also noticed that the current methodological approach widely applied in starch grain research needs to be adjusted and improved in order to avoid overlooking of damaged starch grains. Overall, by exploring the morphological changes in starch grains caused by different grinding techniques, this research contributes towards a more nuanced interpretation of how rice was processed by past societies.

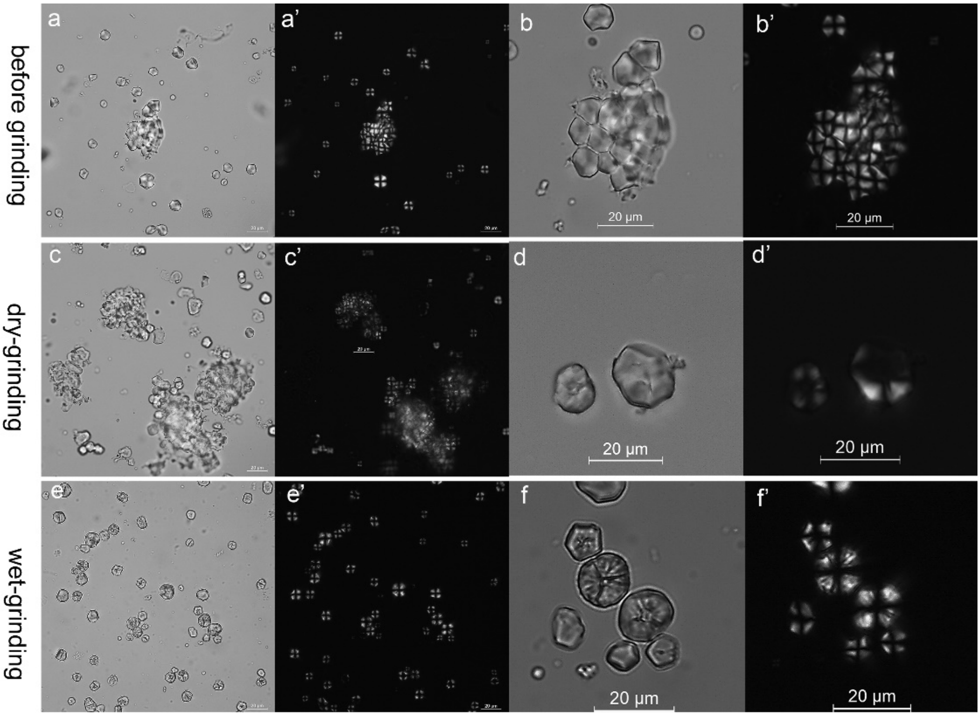

Figure S4.1 Morphological changes of starch grains from foxtail millet after dry- and wet-grinding. a, a': single and compound starch grains coexist in the unprocessed millet samples; b: single and the irregular shape compound starch grains under normal light, b': "+"- shaped extinction cross of the millet starch grains under polarized light; c: single starch grains still exist and more irregular starch aggregates generated in the millet samples, c': the extinction crosses of starch aggregates became blurry; d: irregular fissures on the starch grain surfaces; d': incomplete extinction crosses of the single starch grains; e: single starch grains under the normal and polarized light; f: radical fissures on the starch surfaces; f': arms of the extinction crosses became thicker.

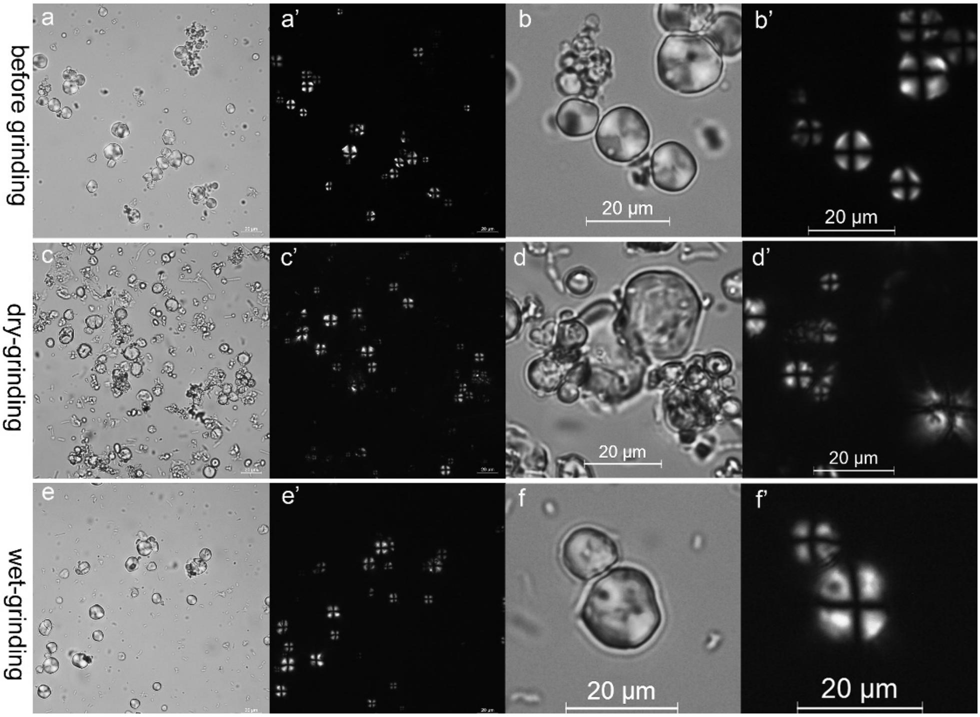

Figure S4.2 Morphological changes of starch grains from Job's tears after dry- and wet-grinding. a, a', b and b': single starch grains in the unprocessed samples from Job's tears; c, c': overview of the single starch grains after dry-grinding, see a majority of these grains still visible under polarized light; d, d': two extremely enlarged single starch grains without extinction crosses; e, e', f and f': starch grains show features resemble the unprocessed samples.

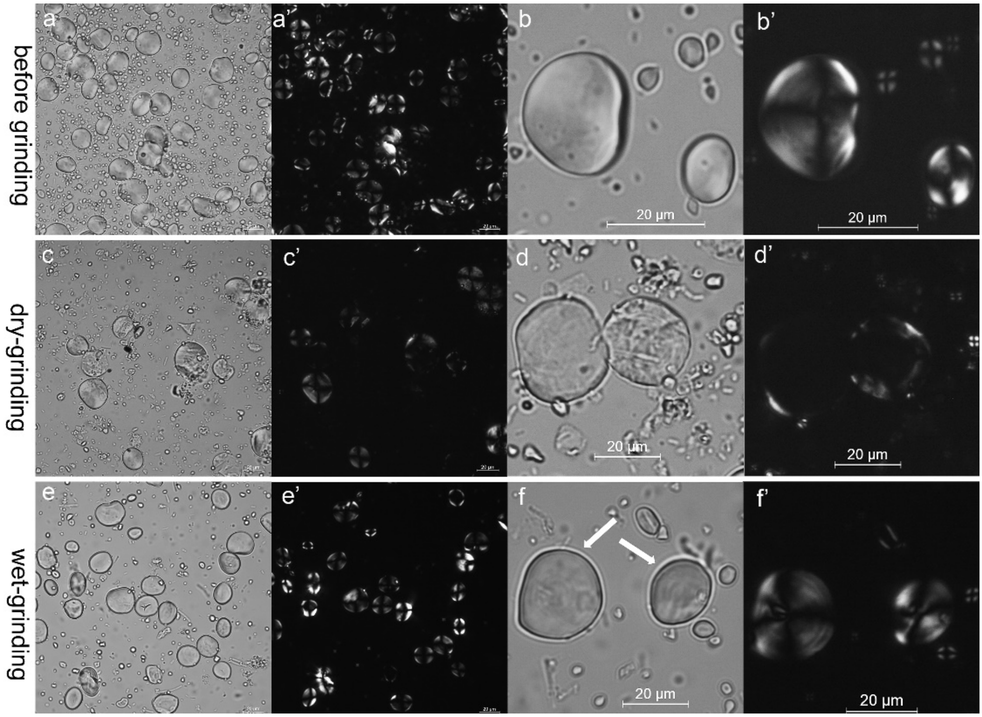

Figure S4. 3 Morphological changes of starch grains from barley after dry- and wet-grinding. a, a': type a and type b starch grains coexist in the unprocessed millet samples; b: barley starch grains with smooth surface; b': "X" -shaped extinction cross of the barley starch grains under polarized light; c, c': overview of the ground barley starch grains, see only very few of them became totally invisible; d: lamellae and shallow striations formed on the starch surface; d': barley starch grains with faint extinction crosses; e, e': overview of starch grains after wet-grinding under normal and polarized light; f: shallow lamellae represent on starch surfaces; f': the extinction crosses resembles the unprocessed samples.

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