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INFLUENCE OF GRINDING ON THE PRESERVATION OF STARCH GRAINS FROM RICE*

W. LI†
Material Culture Studies, Faculty of Archaeology, Leiden University, NL-2333CCLeiden, the Netherlands

J. R. PAGÁN-JIMÉNEZ
World Archaeology, Archaeology of the Americas, Faculty of Archaeology, Leiden University, NL-2333CCLeiden, the Netherlands

C. TSORAKI
School of Archaeology and Ancient History, University of Leicester, LeicesterLE1 7RH, UK

L. YAO
Hubei Provincial Institution of Cultural Relics and Archaeology, Wuhan City, Hubei, China

and A. VAN GIJN
Material Culture Studies, Faculty of Archaeology, Leiden University, NL-2333CCLeiden, the Netherlands

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 rice starch grains have been identified to date. To understand this apparent scarcity of starch grains from rice, dry- and wet-grinding experiments with stone tools were carried out 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 it is suggested was caused by the use of the dry-grinding technique. Therefore, it is suggested that rice starch grains have been likely underrepresented in the archaeological record, and previous interpretations of starch analyses need to be reconsidered.

KEYWORDS: RICEGRINDING TECHNIQUESTARCH GRAINSEXPERIMENTAL ARCHAEOLOGYNEOLITHIC CHINA

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 (Liu *et al*. 1981; Jiang and Liu 2006;

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†Corresponding author: email w.li@arch.leidenuniv.nl

Liu *et al.* 2007; Trivers *et al.* 2009), the middle catchment of the Yangtze River (e.g., Zhao 1998), and the upper catchment of the Huai River (Zhang and Wang, 1998, fig. 1a). On the basis of the carried out research, these regions all possess well-documented evidence for rice domestication during the early Neolithic period (e.g., Lu *et al.* 2002; Liu *et al.* 2007; Zhao 2010; Wu *et al.* 2014; Yang *et al.* 2016a). 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 7800–4500 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 subphases (Zhang 1999; Yang *et al.* 2017; Zhang *et al.* 2018). Isotope analysis of human skeletons from Jiahu indicates that C_3-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 BP at Jiahu (McGovern *et al.* 2004).

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.* 2010a; Yang *et al.* 2015a, 2015b). In the few cases where rice starch grains have been identified (Zhang 2015; Yang *et al.* 2015b; 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; Yao *et al.* 2016; Li *et al.* 2018) 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., Zhang *et al.* 2011; Liu *et al.* 2014a; Yang *et al.* 2015b).

The scarcity of rice starch grains recovered from grinding tools was often used to suggest that rice was not the primary processed material of these tools (Liu *et al.* 2010b; Yang *et al.* 2015a,

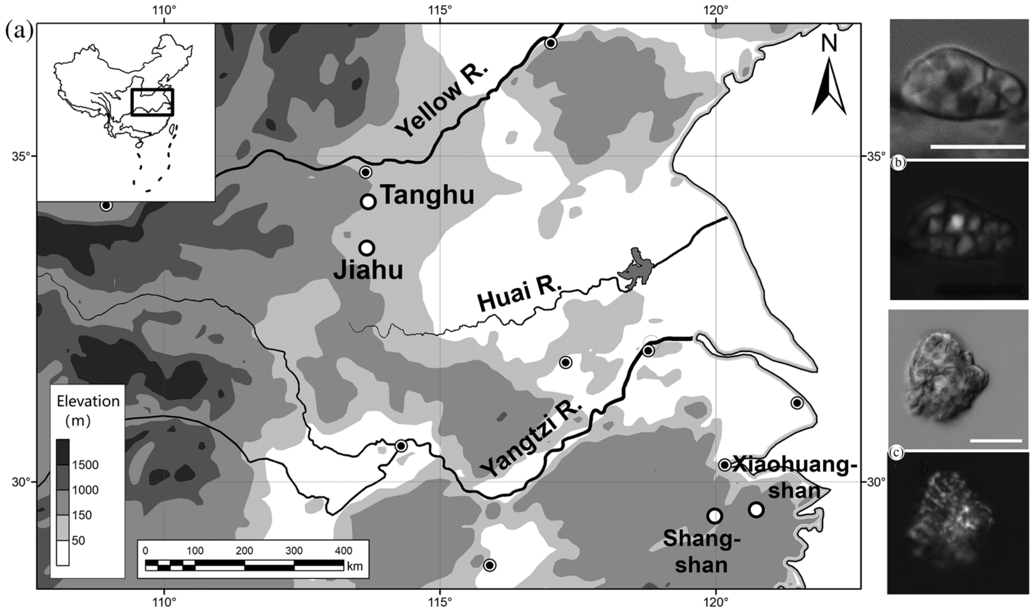

Figure 1 *Archaeological sites mentioned in the text (a) and ancient starch grains from rice recovered from grinding tools at the sites of Tanghu (b) and Jiahu (c). Scale bar of the starch grains = 20 µm. The majority of ancient starch grains from rice from these two sites are compound (after Yang et al. 2015b; Zhang 2015).*

2015b; Yao *et al*. 2016). This seems a reasonable hypothesis considering that rice could also have been processed by other means, 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 the 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, 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.) (del 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, tool types and 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 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 BP (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 years BP (Guedes *et al*. 2015) and in the Hexi Corridor *c*.4000 years BP (Flad *et al*. 2010). Barley belongs to the tribe Triticeae and starch grains from wild Triticeae specimens have

Table 1 *Information on the grinding experiments*

Experiment no.	*Grinding technique*	*Material*	*Origin of the cereals*	*Duration (min)*	*Efficiency**
1	Dry-grinding	Rice	North-east China	60	1
2	Wet-grinding	Rice	North-east China	60	2
3	Dry-grinding	Foxtail millet	Taiwan	60	1
4	Wet-grinding	Foxtail millet	Taiwan	60	2
5	Dry-grinding	Job's tear	Taiwan	60	2
6	Wet-grinding	Job's tear	Taiwan	60	3
7	Dry-grinding	Barley	Netherlands	60	0
8	Wet-grinding	Barley	Netherlands	60	0

*'Efficiency' refers to the interpretation of the efficiency to grind the cereals into flour from lower to higher levels 0–3.

been identified on grinding tools dated to the Upper Palaeolithic 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 years BP). Wooden pestles were also recovered from the archaeological sites of Bashidang (7540–7100 years BP) and Hemudu (*c.*7000–5000 years BP) in the Yangtze River basin (Peregrine and Ember 2012; Xu 2017).

MATERIALS AND METHODS

In the present study, all the cereals were purchased from Chinese supermarkets in The Hague, the Netherlands (Table 1). The grinding tools were made from sandstones obtained from the riverbed of the Maas River 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 additional supporting information Fig. S1.

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 selection of specific grinding techniques is 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 10 h. Each cereal was then 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; and 3 = highly effective, very easy to grind cereals into flour (Table 1).

After 60 min of grinding each type of cereal, the stone tools were sampled following the procedures outlined by Torrence and Barton (2006) and Cnuts and Rots (2018). Ultra-purified water was placed on the surface of the stone tools for 2 min. The starch grain samples were then obtained from the stone surfaces using a pipette. These samples were placed in 2 ml microcentrifuge 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. Cover slides were then placed on each slide and sealed with neutral balsam to prevent the dehydration of starch grains. Microscopic observations were carried out within one month of slide preparation. Starch grains from unprocessed cereals were studied for comparative purposes. The cereal samples were prepared following the method mentioned in previously published literature on modern starch research (Wei *et al.* 2010). First, each specimen was put in a separate 2 ml microcentrifuge tube filled with ultra-purified water. After 8 h, a disposable pipette was used to press the grain inside the tube in order to release the starch grains. The liquid sample containing the released starch grains was then 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 (1) starch type (single or compound), (2) shape, (3) size, (4) presence and absence of features on the starch surface (hilum, fissures and lamellae) and (5) extinction cross-morphology (for the description of these starch features, see, e.g., Torrence and Barton, 2006; and García-Granero *et al.* 2017). Following Gong *et al.* (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. 3, b). 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 (Fig. 3) using the Leica application suite version 4.8.

RESULTS

From our experience, dry-grinding of cereals requires more effort than wet-grinding (Table 1). Only Job's tears were very easy to dry-grind into flour, which is consistent with the grinding experiment conducted by Liu *et al.* (2018a). In contrast, dry-grinding barley was difficult, an observation consistent with previous experiments (Lull *et al.* 2010). Rice and foxtail millet can both be dry-ground into a 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 dry conditions. Detailed morphological changes of starch grains from each type of cereals after dry- and wet-grinding are described below.

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. 2, a, b). The shape of single starch grains is polygonal. The average maximum length of the single rice starch grains is 5.64 ± 1.89 μm (Table 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. 2, b) and has diagnostic reflected star-shaped extinction crosses (Fig. 2, b´).

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. 2, c, d). These starch aggregates have irregular shapes (Fig. 2, c, d) and their extinction crosses are invisible or become faint and blurry (Fig. 2, c´, d´). Only a few compound starch grains survived with observable birefringence (Fig. 2, c´). A small number of single starch grains that remained completely lost their extinction crosses (Fig, 3, b). The average maximum length of the single dry-ground starch grains from rice is 8.95 ± 2.58 μm (Table 2 and Fig. 3, a).

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. 3, a), shape, extinction cross and characteristics of the starch surface (Fig. 2, e, e´, f, f´). The average

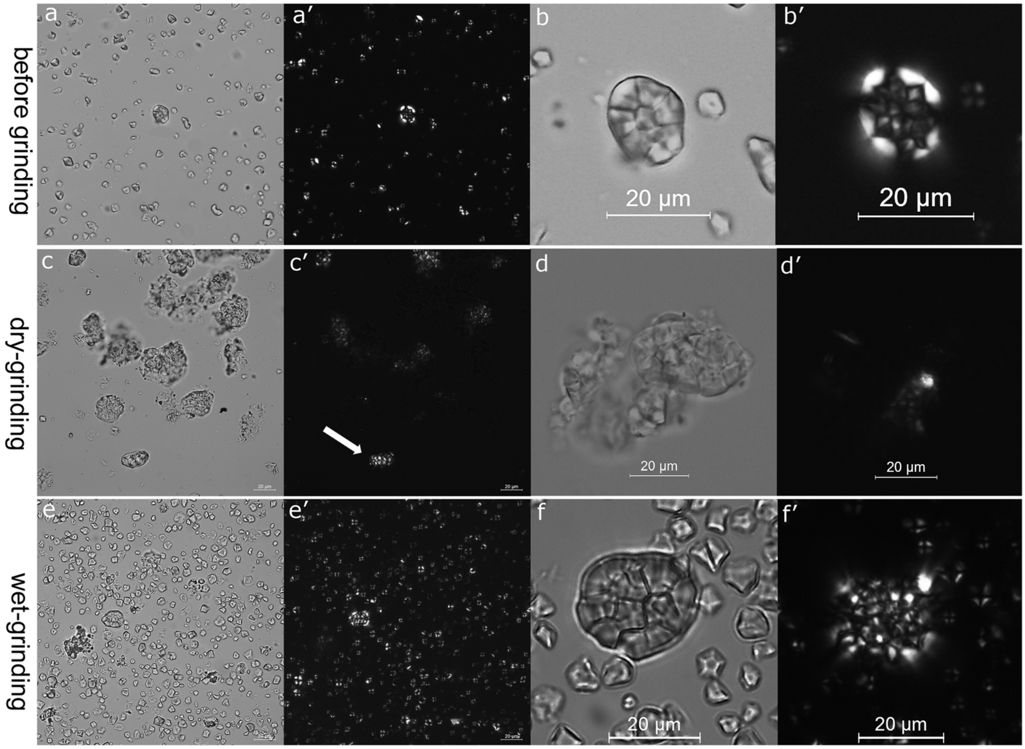

Figure 2 *Morphological changes of starch grains from rice after dry- and wet-grinding: (a, a´) single and compound starch grains coexist in the unprocessed rice samples; (b) the oval-shaped compound starch grains under normal light; (b´) star-shaped extinction cross of the compound starch grains under 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; and (e, e´, f, f´) starch grains perform similar features as the unprocessed samples.*

maximum length of rice single starch grains submitted to wet-grinding is 6.24 ± 1.40 μm (Table 2 and Fig. 3, a).

Foxtail millet

Starch grains from unprocessed foxtail millet samples consist of both single structures and compound grains (additional supporting information Fig. S2, a, a´). Single starch grains are polygonal, round or oval. The mean length of single foxtail starch grains is 10.21 ± 2.15 μm (Table 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 (additional supporting information Figure S3, b and b´).

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. 3, a). After dry-grinding, the average maximum length is 13.79 ± 4.42 μm (Table 2). Most

Table 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)
Z. mays (green and soft)	5–25	12.1 ± 4.7 ($n = 60$)	
Z. mays (mature and hard kernels)*	10–38	23.2 ± 6.6 ($n = 60$)	
Z. mays (semi-mature and partially hard kernels)*	7–34	20.8 ± 5.7 ($n = 60$)	

*Samples were soaked for 1 h and then intensively ground for 5 min. *Z. mays* (maize) control sample were soaked for 24 h and then ground 15 s to avoid overly damaging the starch grains. All the *Z. mays* samples were ground with a marble mortar and pestle (Mickleburgh and Pagán-Jiménez 2012).

starch grains (43%) display nearly intact extinction crosses; 35% have faint extinction crosses; the rest (22%) become invisible under polarized light (Fig. 3, b).

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. 3, a): 10.79 ± 2.47 μm (Table 2). The most noticeable damage patterns are shallow fissures and radiating striations on the surfaces of the starch grains (additional supporting information Fig. S2, f, f´).

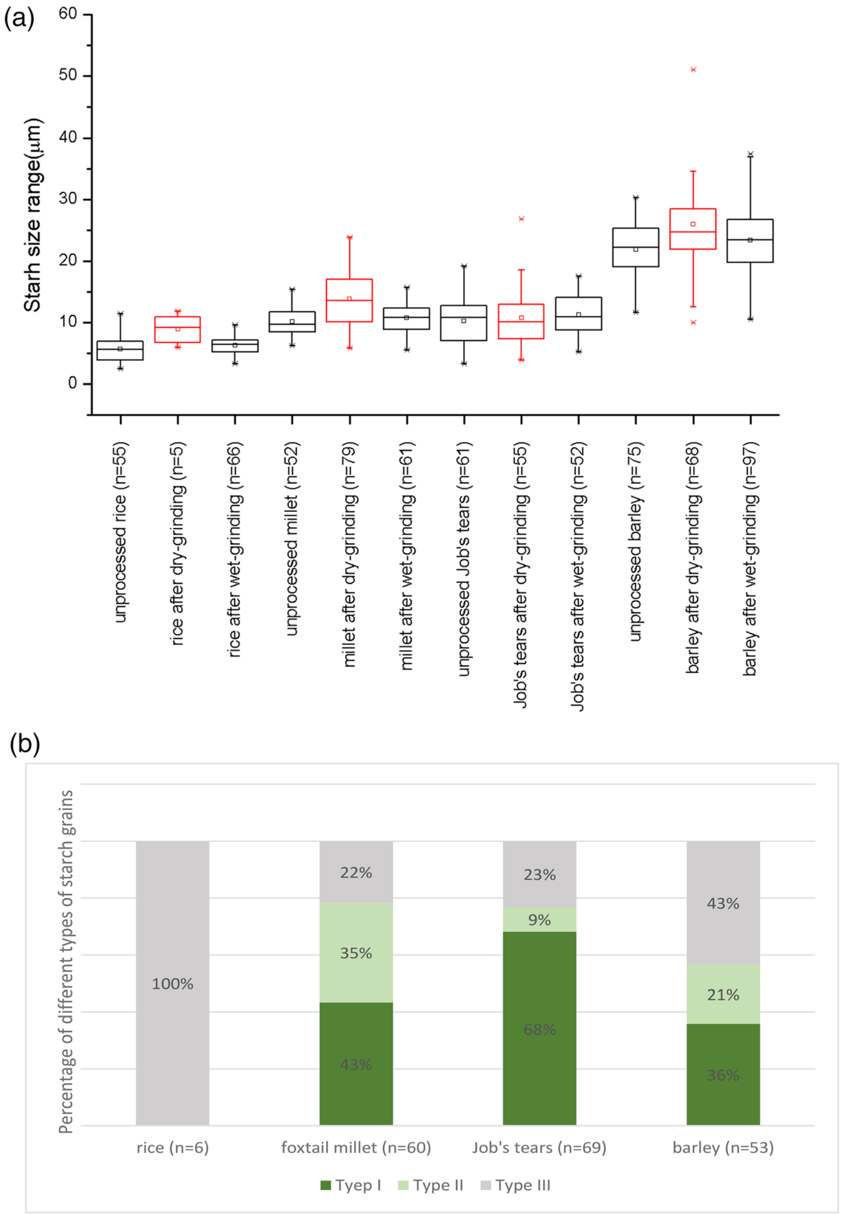

Figure 3 *(a) Size of starch grains from the unprocessed, dry-grinding (red) and wet-grinding samples; note the size of the starch grains from the dry-grinding samples is bigger than their unprocessed and wet-grinding samples, except Job's tears. (b) Proportion of three different categories of damaged starch grains from the dry-grinding samples type I–III refer to three different categories of damaged starch grains. At least 50 single starch grains were measured from each sample, except for the dry-grinding samples from rice in which the single grains mostly disappeared; for supporting data for (b), see additional supporting information Table S1 and Figures S5–S23. [Colour figure can be viewed at wileyonlinelibrary.com]*

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 ± 3.77 µm (Table 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 geographical origins of the source plants (e.g., Perry 2002, 2006; 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 (additional supporting information Fig. S3, c, c´). Their size is relatively stable (Table 2 and Fig. 3, a) with few dry-ground starch grains found to be enlarged considerably (e.g., additional supporting information Figure S3, d, d´). The average maximum length of these single starch grains is 10.74 ± 4.60 µm (Table 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. 3, b); the rest (9%) still possess visible birefringence features under polarized light (Fig. 3, b).

Wet-grinding resulted in no significant changes to the starch grains (additional supporting information Figure S3, e, e´, f, 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 ± 3.27 µm (Table 2), which is slightly larger than the unprocessed samples.

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 10 µm, while type B refers to starches < 10 µm 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., Yang *et al.* 2015a, 2016b). Unprocessed type A barley starch grains are oval shaped. The average maximum length of the barley starch grains measured 21.85 ± 4.43 µm (Table 2), which is larger than previously suggested (e.g., 18.0 ± 4.1 µm 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 (additional supporting information Fig. S4, a, a´, c, c´).

After dry-grinding, lamellae on the starch surfaces of barley became deeper and shallow striations were formed on the surface (additional supporting information Fig. S4, d). These starch grains also became flatter (additional supporting information Fig. S4, c). Some of them (43%) totally lost their birefringence features (Fig. 3, b), whereas others (36%) still exhibited extinction crosses resembling the ones registered in unprocessed samples (e.g., additional supporting information Fig. S4, c´). In other cases (21%), the extinction crosses became faint but still visible under polarized light (Fig. 3, b). The average maximum length of the dry-ground starch grains is 25.97 ± 8.06 µm (Table 2).

The morphological changes on barley starch grains after wet-grinding are minor in terms of their shape and extinction crosses (additional supporting information Fig. S4, e, e´, f´). Only some shallow lamellae are observed on the starch surfaces under normal light (bright field view;

e.g., additional supporting information Fig. S4, f). The average maximum length of the single wet-ground starch grains is 23.38 ± 5.21 μm (Table 2).

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. This is possibly because grinding Job's tears requires only minimal pressure to make flour (Table 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 suggest that the harder the seed coat, the greater the enlargement of the starch grains due to grinding (Table 2).

Polarized light and dark field view are often used to recognize, detect and identify starch grains (e.g., Lu *et al.* 2005; Liu *et al.* 2011; Perry and Michael Quigg 2011; Yang *et al.* 2015a). 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. 2, c´). This is the case at the site of Tanghu, where only one intact compound starch grain has been recovered (Fig. 1, b) (Yang *et al.* 2015b). Similarly, the starch grains from rice recovered from the site of Jiahu are mostly compound as well (83%, 69/83) (e.g., Fig. 1, c) (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 sites of Xiaohuangshan (9000–7000 cal. BP) (Liu *et al.* 2010b; Yao *et al.* 2016) and Shangshan (Yang *et al.* 2015a) in the Yangtze River basin in the 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 Shangshan (Yang *et al.* 2015a). If dry-grinding were 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*. (2015a) suggested that there might be an underrepresentation of rice starch grains. They argued that rice single starch grains are normally $< 10\,\mu m$, which is too small to be easily detected by microscope. However, a magnification up to 630× has been used for starch research and small size starch grains ($< 5\,\mu m$) have been revealed in archaeological samples (e.g., Liu *et al*. 2014a, 2014b; 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 maximize the chance of detecting both native and damaged starch grains (Pearsall 2016).

CONCLUSIONS

The application of starch grain analysis has grown quickly in China, with increasingly more papers on ancient starch research being been published (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 taphonomic 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.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Supporting information.

Figure S2. 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; € single starch grains under the normal and polarized light; (f) radical fissures on the starch surfaces; and (f´) arms of the extinction crosses became thicker.

Figure S3. Morphological changes of starch grains from Job's tears after dry- and wet-grinding: (a, a´, b, b´) single starch grains in the unprocessed samples from Job's tears; (c, c´) overview of the single starch grains after dry-grinding; a majority of these grains are still visible under polarized light; (d, d´) two extremely enlarged single starch grains without extinction crosses; and (e, e´, f, f´) starch grains show features resemble the unprocessed samples.

Figure S4. Morphological changes of starch grains from barley after dry- and wet-grinding: (a, a´) types a and b starch grains coexist in the unprocessed millet samples; (b) barley starch grains with a smooth surface; (b´) 'X'-shaped extinction cross of the barley starch grains under polarized light; (c, c´) overview of the ground barley starch grains; only a 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 the starch surfaces; and (f´) the extinction crosses resembles the unprocessed samples.