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Comparison of dry ashing and wet oxidation methods for recovering articulated husk phytoliths of foxtail millet and common millet from archaeological soil

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ABSTRACT

Phytolith research on foxtail and common millets represent one of the keys to explore early agricultural activities in the Yellow River basin in China. However, the phytoliths of these two millets easily disintegrate during burial and the extraction process. In this paper, both dry ashing and wet oxidation methods were applied to determine the effects on phytolith extraction from archaeological soil samples. The results indicated that the dry ashing method had two significant advantages over wet oxidation: (1) the morphology of husk phytoliths was retained to a greater extent; and (2) nearly all the charcoalified tissues were removed successfully. The dry ashing method proved to be a better method for phytolith extraction of both foxtail millet as well as common millet from archaeological samples.

1. Introduction

To date, foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*) are regarded as staple foods in Northern China during the Neolithic period, and were initially domesticated in the Yellow River valley of China (Zhao, 2005; Crawford et al., 2006; Hunt et al., 2008; Barton et al., 2009). Foxtail millet and common millet can be identified by their morphological characters, which rely on exceptional conditions of burial at archaeological sites. Unlike organic plant remains, phytoliths are abundant at most archaeological sites, and are chosen as the preferred method when the macroremains are not well preserved (Crawford, 2005; Harvey and Fuller, 2005). The husk phytoliths of millets are easily preserved and display unique anatomical characters, five of which were selected to distinguish between foxtail millet and common millet (Lu et al., 2009). Recently, there has also been a report about prominent diagnostic differences in the phytoliths of foxtail millet (*S. italica*) and green foxtail (*Setaria viridis*) (Zhang et al., 2011). In all studies, large phytoliths with undulate extremities tend to be fragmented after long burial and during complicated extraction processes. It is clear that if the structures of the husk phytoliths of millets from the soil of archaeological sites can be retained intact, the identification of both millets will take less time and be more secure.

At present, there are two basic methods for extracting phytoliths from modern plants, viz. dry ashing and wet oxidation. It has been suggested that both these methods may modify the resulting samples in different ways (Rovner, 1983; Parr et al., 2001; Emma, 2009; Wu et al., 2012). For most archaeological soil samples, in order to remove sand, clays, carbonates, etc., the procedures involve sieving, wet oxidation and then heavy liquid extraction. The method varies according to the preference of the analyst, although the full impact of these procedures on the resulting phytolith assemblage remains unknown (Emma, 2009). Studies on the comparison of different methods have been focused on plant domestication in West Asia by using modern samples. However, the difference between phytoliths of foxtail millet and common millet extracted from archaeological soil samples, has rarely been studied. These samples tend to contain abundant charcoal, especially from

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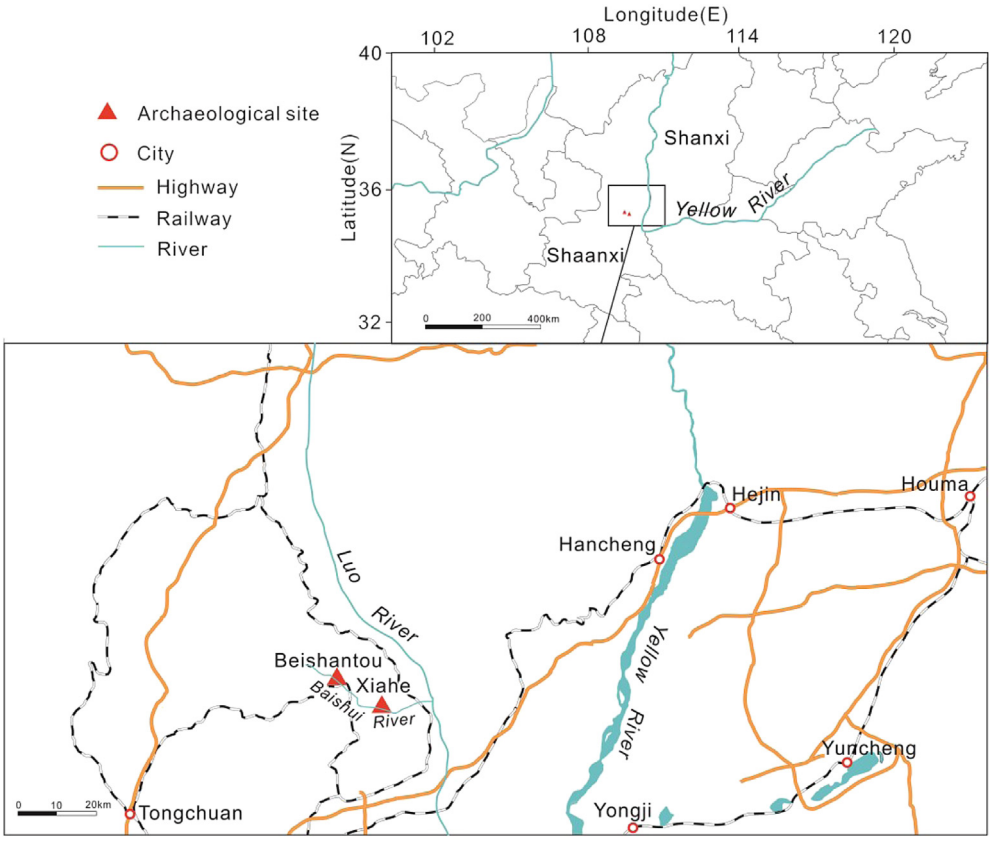

Fig. 1. Map showing the sites referred to in the paper.

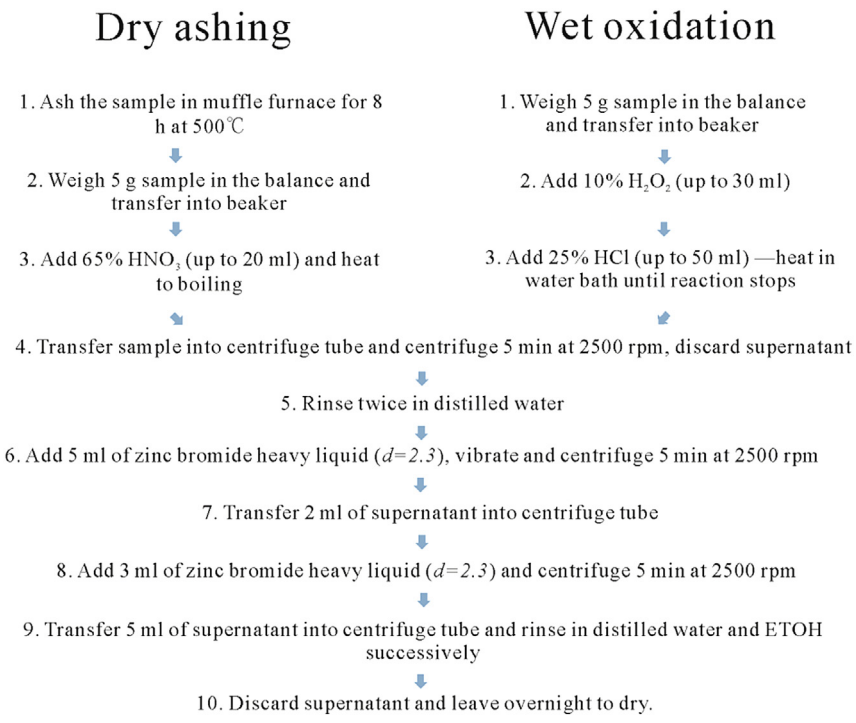

Fig. 2. Description of dry ashing and wet oxidation method.

Table 1
Comparison of the percent of phytoliths found in each category using the different processing methods.

	MD	T1		T2		T3	
		Dry ashing	Wet oxidation	Dry ashing	Wet oxidation	Dry ashing	Wet oxidation
Sample 1	< 60 μm	12.0%	46.3%	11.7%	29.7%	35.3%	75.3%
	> 60 μm	88.0%	53.7%	88.3%	70.3%	64.7%	24.7%
Sample 2	< 60 μm	31.0%	44.0%	17.3%	29.3%	25.0%	47.0%
	> 60 μm	69.0%	56.0%	82.7%	70.7%	75.0%	53.0%

sites of intense human activities (Li et al., 2006). The stable structure and low specific gravity of charcoal prevents it from being removed from phytoliths by chemical reagents or heavy liquid. In order to find a good procedure for phytolith extraction of both millets, we will compare the effects and results of different extraction techniques by using archaeological soil samples.

2. Materials and methods

The soil samples were collected from two late-Neolithic sites in the Baishui River valley of Shaanxi Province (Fig. 1). Beishantou site (35°10′56.8″N, 109°33′42.1″E) was found in 2012. The main of the site was a storage pit (H1) with clean charred grains. There was a ca. 15 cm thick layer of charred grains of foxtail millet and common millet at the bottom of H1. The charred grains, some with husk, were clean and with little else. Above the layer of grains there were some pottery shards and pellets of burnt soil. Xiahe site (35°08′06.5″N, 109°41′17.9″E) was excavated in 2012 by Shaanxi Provincial Institute of Archaeology. There were 3 houses, 13 tombs, 55 pits, 1 kiln and 8 ditches in 700 m^2 excavated area. Remains of the Miaodigou culture, Late Yangshao culture, Miaodigou II culture and Keshengzhuang II culture have been brought to light. The Holocene soils in the area are slightly alkaline, with the pH values varying between 7.4 and 8.3 (Liu et al., 1996) and the content of $CaCO_3$ between 0 and 12.4% (He et al., 2004). It has been reported that, phytoliths are generally preserved in large amounts in loess (Lu et al., 1991).

Sample 1 was collected from H1 storage pit of the Beishantou site. Between this layer and the bottom of the pit, there were grey soils consisting of decayed crop ash and charred grains, which were also sampled in the present study. Sample 2 was collected from H20 storage pit of the Xiahe site. A large number of charred grains, pieces of charcoal, as well as some pottery shards and pellets of burnt soil were discovered inside the pit. The soils were sampled in the middle of H20.

The two samples were processed using two extraction methods, dry ashing and wet oxidation, respectively. The experiment was repeated for three times (T1, T2, T3). The dry ashing method was based on the reference of Sun et al. (2012), while the wet oxidation method mainly followed Lu et al. (2009). Later heavy liquid was added to the products of both methods to isolate phytoliths from any remaining material. A summary of the two extraction methods is provided in Fig. 2. After extraction the phytoliths were mounted on a microscope slide using Canada balsam. The slides were measured and counted using a Nikon ECLIPSELV100 POL microscope at 500×. Measurements were taken of the maximum diameter (MD). Phytoliths were counted according to the MD and the following broad counting categories were used: <60 μm, >60 μm. The reason for choosing 60 μm as dividing point is that the average width of the ΩIII undulating patterns of epidermal long cells is about 60 μm in foxtail millet (Zhang et al., 2011). Phytoliths less than 20 μm were not counted as most of them cannot be identified.

3. Results

In the present study, we measured maximum diameter (MD) values for 300 fragments of phytoliths for each sample and

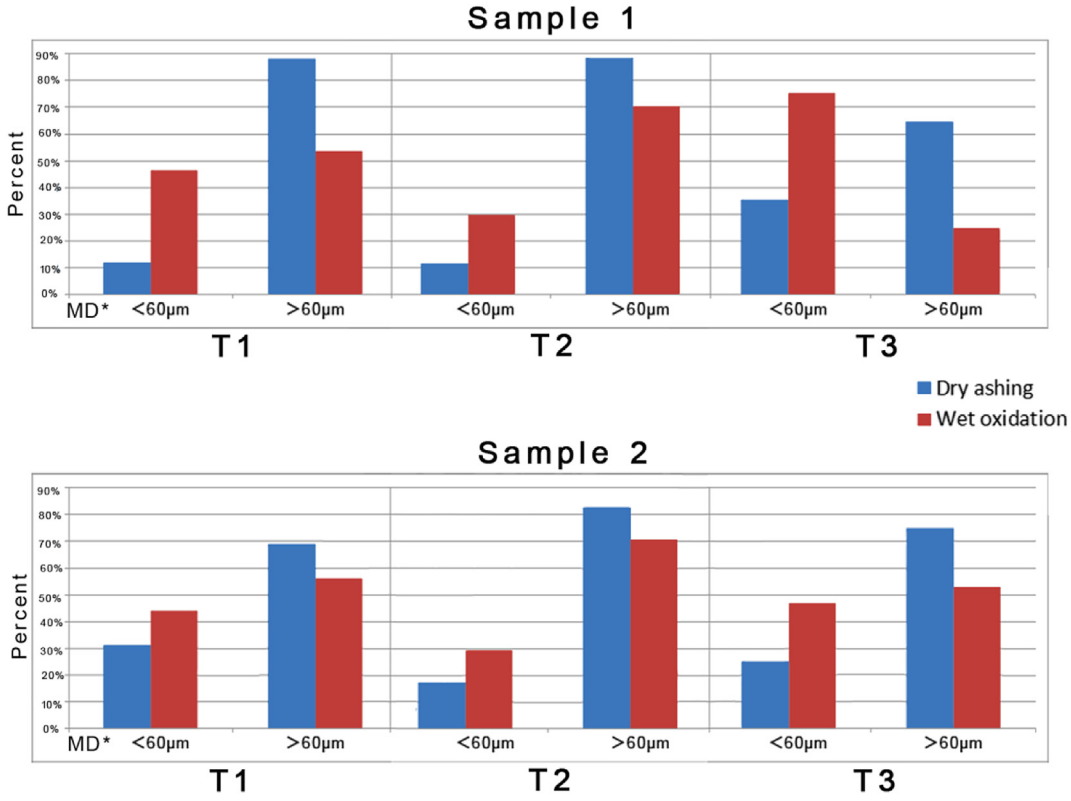

Fig. 3. Comparison of the percentage of phytoliths found in each category using the different processing methods. *MD: maximum diameter.

Table 2
Comparison of the means of maximum diameter of phytoliths using the different processing methods.

Means of MD (μm)	T1		T2		T3	
	Dry ashing	Wet oxidation	Dry ashing	Wet oxidation	Dry ashing	Wet oxidation
Sample 1	104.16	71.78	113.23	84.11	77.24	51.43
Sample 2	79.48	69.17	101.51	85.03	92.03	68.39

experimental process, respectively and 3600 totally. The results are shown in Table 1 and Fig. 3. For Sample 1, the dry ashing method produces more phytoliths with MD >60 μm than those obtained by wet oxidation. In the wet oxidation process, the percentage of phytoliths with MD <60 μm is 46.3%, 29.7% and 75.3% successively, while the size-class >60 μm represents about 53.7%, 70.3% and 24.7% of all phytoliths. For the dry ashing method, the percentage of phytoliths with MD <60 μm is 12.0%, 11.7% and 35.3%, while the category >60 μm is about 88.0%, 88.3% and 64.7%, which is higher than that using the wet oxidation method.

For Sample 2, the statistics/results are similar to those of Sample 1. The dry ashing method produces a greater percentage of phytoliths with MD >60 μm than that using wet oxidation. There are 44.0%, 29.3% and 47.0% small phytoliths (MD <60 μm) in each wet oxidation experiment, but only corresponding 31.0%, 17.3% and 25.0% employing the dry ashing method. The percentage of phytoliths with MD >60 μm is about 69.0%, 82.7% and 75.0% using the dry ashing method, but only 56.0%, 70.7% and 53.0% employing the wet oxidation method. Table 2 shows that, both Sample 1 and Sample 2, means of the MD of phytoliths using dry ashing are generally bigger than those using wet oxidation. In Fig. 3 images of phytoliths from the two processing methods can be seen, which provides a good visual demonstration of the differences in the impurities. The dry ashing method removed almost all the carbonized tissues. In contrast, phytoliths are less clear and hard to identify due to the coating of carbonized tissues when the wet oxidation method is employed.

4. Discussion

To date, much research has been undertaken to distinguish foxtail millet and common millet using the surface Ω- and η-undulated patterns, respectively (Fig. 5). In addition, characteristics of the dendriform epidermal long cell endings like cross wavy/finger types are also diagnostic (Lu et al., 2009). Comprehensive patterns of phytoliths (epidemical cells) of the husk make the identification more precise. However, burial and extraction processes may both cause the phytoliths to disintegrate. The present study

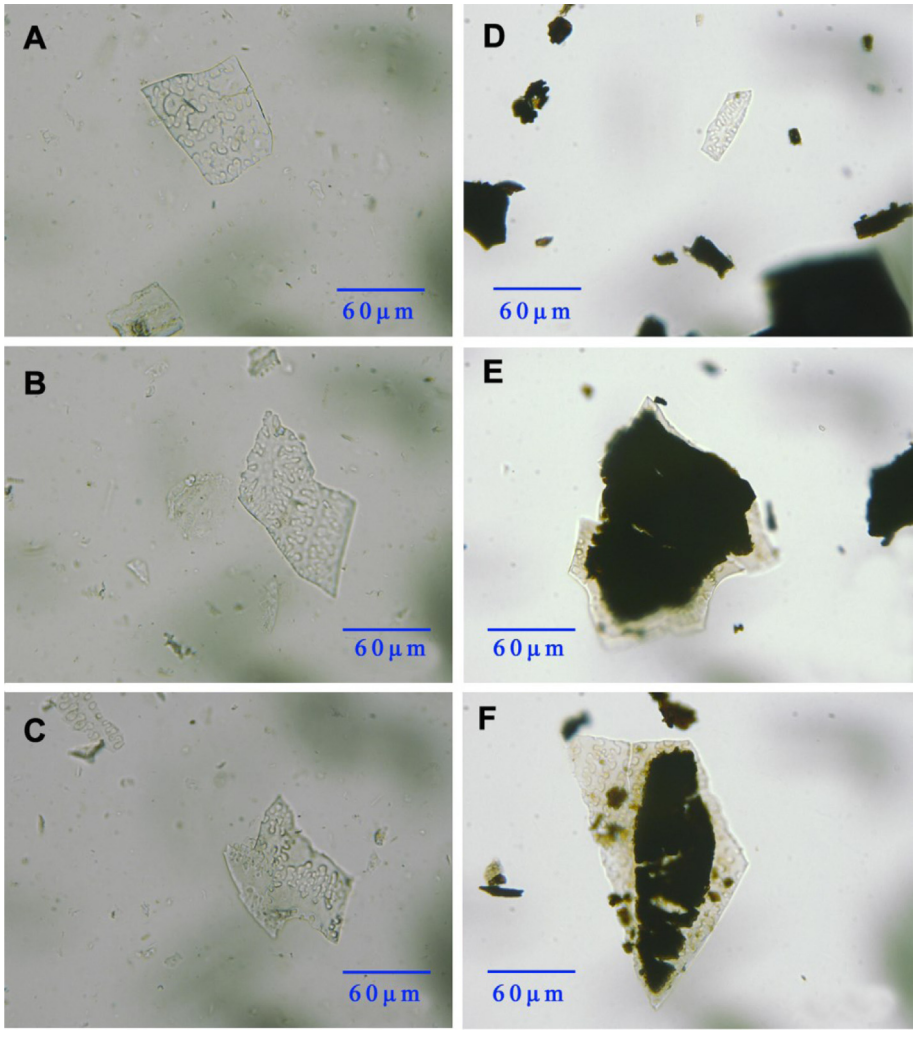

Fig. 4. Images of phytoliths produced by using the two processing methods: A, B and C are from dry ashing method, while D, E and F are from acid extraction method.

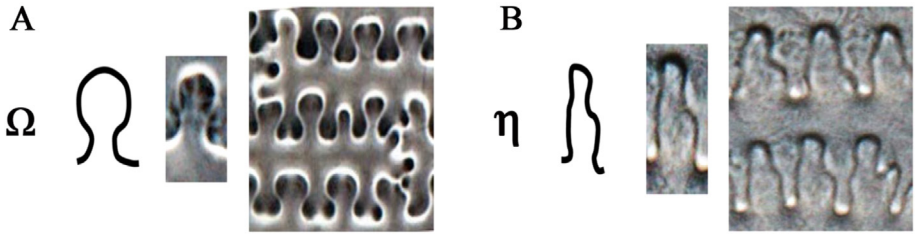

Fig. 5. The undulated patterns of foxtail and common millets (Modified from Lu et al., 2009). The epidermal long cell walls are (A) Ω-undulated in foxtail millet, and (B) η-undulated in common millet.

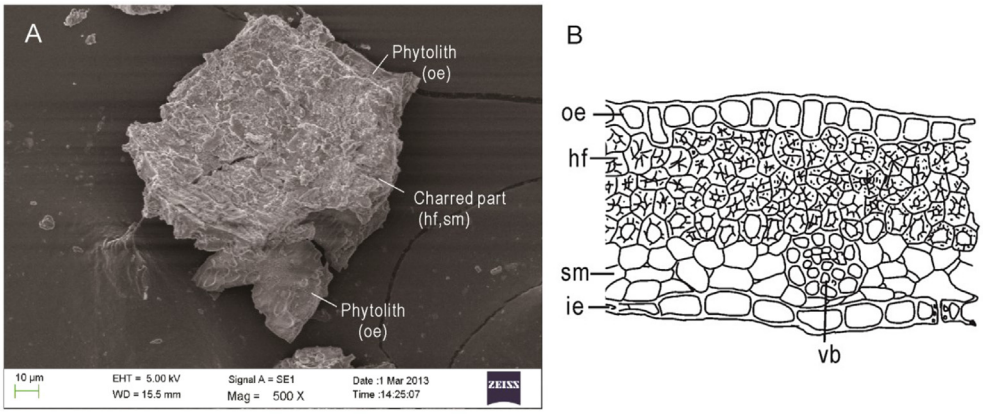

Fig. 6. (A) SEM photo of phytoliths with charcoalified tissues of millet. (B) Cross section of a husk of broom corn* showing the following: oe: outer epidermis, hf: hypodermal fibres, vb: vascular bundle, sm: spongy mesophyll, and ie: inner epidermis (Modified from Winton and Winton, 1932). * Cross section of the husk of foxtail and common millets is similar to that of broom corn.

demonstrates that with less chemical treatment, the phytoliths recovered from the dry ashing method were generally larger than those obtained by the wet oxidation method. This conclusion confirms the findings that the dry ashing method produces more conjoined cell phytoliths or multicellular phytoliths than those using the wet oxidation method (Jones and Milne, 1963; Emma, 2009; Sun et al., 2012).

Apart from the size of the phytoliths, another important matter that affects the identification process is the presence of charcoal. Usually, there is abundant charcoal among the archaeological samples because of the utilization and management of fire by ancient humans. As products of combustive activities, charred remains usually occur in large quantities in archaeological soil samples, which interfere with the phytolith observation. The presence of plenty of blackish, non-transparent charcoal in thin sections will retard/complicate identification. When observing the phytoliths produced by wet oxidation method, we also find a number of phytoliths covered by charcoalified tissues (Fig. 4E and F). By observing the assemblages under a scanning electronic microscope (SEM), we found that they belonged to parts of the lemma or palea (Fig. 6A). Since the phytoliths are produced by the outer epidermis (Sangster et al., 1983), the charcoalified part must represent hypodermal fibres and spongy mesophyll (Fig. 6B). Both of these are rich in lignin, and could be charred easily. In samples with a mass of charred remains, the dry ashing method is to be preferred. In the process the charred parts are removed, so enough transparent phytoliths are available for identification.

5. Conclusion

The present study has shown that the dry ashing method yielded better results than the wet extraction technique. The former produces large phytoliths and removed the charcoalified tissues successfully, which is more than can be said of the wet oxidation technique. However, the method of extraction varies according to differences in the soil samples. There is no method, not even dry ashing, which can be applied to all samples. Nevertheless, by reducing the damage to the phytoliths and effectively removing the charred remains, the dry ashing method facilitates the identification of both foxtail millet and common millet. Moreover this method has led to the discovery of more diagnostic differences between the phytoliths produced by these two millets.

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