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Thematic review

Microscopic Analysis of Starch Grains and its Applications in the Archaeology of the Stone Age

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ABSTRACT

Archaeobotanical micro-residuals are today a major focus in artefactual and bioarchaeological investigations. Though starch grains analysis may be regarded as marginal, it can be a useful analysis for archaeological research, being a method suitable for the investigation of stone artefacts and ceramic vessels. Soil samples and dental calculus can also be examined. Through the use of various extraction methods it is possible to answer questions of diet composition and purpose of stone tool use. As documented in recent studies examining the composition of the human diet, starch grain research should be one of the main areas of archaeobotanical investigation. Its applicability can be seen in studies where it is useful to define the role of plants in human subsistence. New evidence of plant use in archaeological contexts in the Stone Age, beginning in the Palaeolithic and ending in the Neolithic, has been presented in recent papers. Current archaeological studies, including those using starch grain analyses, have particularly indicated the higher ratio of plants in the diet during the Palaeolithic period.

1. Introduction

Archaeobotany offers a series of analytical methods (*e.g.* Evans, O'Connor, 1999). These various methods can be sorted according to the function and character of material studied, which may include seeds, stones of fruit, wood, charcoal and other botanical macro-remains on the one hand, and a large group of botanical microremains on the other (Jacomet, Kreuz, 1999). Integrated archaeobotanical approaches, which combine analyses of several biological indicators, began in the 1970s (Scott and Lewis, 1981; Cummings, 1994) and have become widespread due to their effectiveness as a tool for understanding the past environment, human diet, and the function of particular archaeological objects (Pető *et al.*, 2013; García-Granero *et al.*, 2015). The analysis of starch grains is a suitable, though still rather uncommon, archaeobotanical technique in archaeological research. However, starch grain analysis has been employed in archaeological research for more than

the last two decades. Starch grains are part of the group of plant microremains that includes phytoliths, pollen, spores and other “non-pollen” objects. Examination of these plant remains can elucidate changes in the environment, both natural and anthropogenic (Lentfer *et al.*, 2002; Evans, Ritchie, 2005; Roosevelt, 2016). Starch occurs as insoluble, semi-crystalline granules in plant tissue that store energy in specific parts of the plant, such as seeds, roots and tubers (storage organs) (Hardy *et al.*, 2016), and as transitory starch, which is usually not recovered or identified in samples. The analysis of starch grains is connected with investigations into plant use and plant processing in the past and also the composition of the herbaceous component of the human diet (Barton, White, 1993; Hall *et al.*, 1989; Fullager *et al.*, 1998; Henry *et al.*, 2014; Corteletti *et al.*, 2015; Tromp, Dudgeon, 2015; Shillito *et al.*, 2018; López, 2018; Primavera *et al.*, 2018). This technique is also suitable for research into the use and function of artefacts and for deciding issues of plant domestication and vegetation history (Loy *et al.*, 1992; Hardy *et al.*, 2009; Denham *et al.*, 2003; Fuller *et al.*, 2014; López, 2018, Cagnato, 2018; Albert *et al.*, 2018).

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However, damaged starch grains can hinder the use of this particular technique. The results from starch grain analysis are suitable as complementary analyses to other techniques, such as palynology, phytolith analysis or plant macroremains (García-Granero *et al.*, 2015; Pestle, Laffoon, 2018).

The examination of starch grains has improved along with improvements in microscopic technique. Antonia van Leeuwenhoek (1632–1723) was the first scientist to publish an illustration of starch grains. This Dutch scientist and microscopist engaged in the observation of natural materials and created a record of the starch grains of common species of plants such as wheat, barley, rye, oats, beans, peas, rice and corn (Hogg, 1854; Britannica, 2016). The work of

Fritzche continued that of Leeuwenhoek. He also recognized the potential of the heterogeneity of starch grains and its use for determining the genus and species of plants. It was only a short step towards the creation of taxonomic keys and atlases (Torrence, Barton, 2006). The German botanist and cofounder of cell theory, Matthias Jakob Schleiden (1804–1881), created a key with his own classification based on starch shape and hilum position. Karl Wilhelm von Nägeli (1817–1891) continued the study of the structure of starch (Britannica, 2016; Torrence, Barton, 2006). This Swiss botanist built on the work of J. M. Schleiden and created a modification of the starch-grain sorting system (Britannica, 2016), among others we could mention, such as Henry

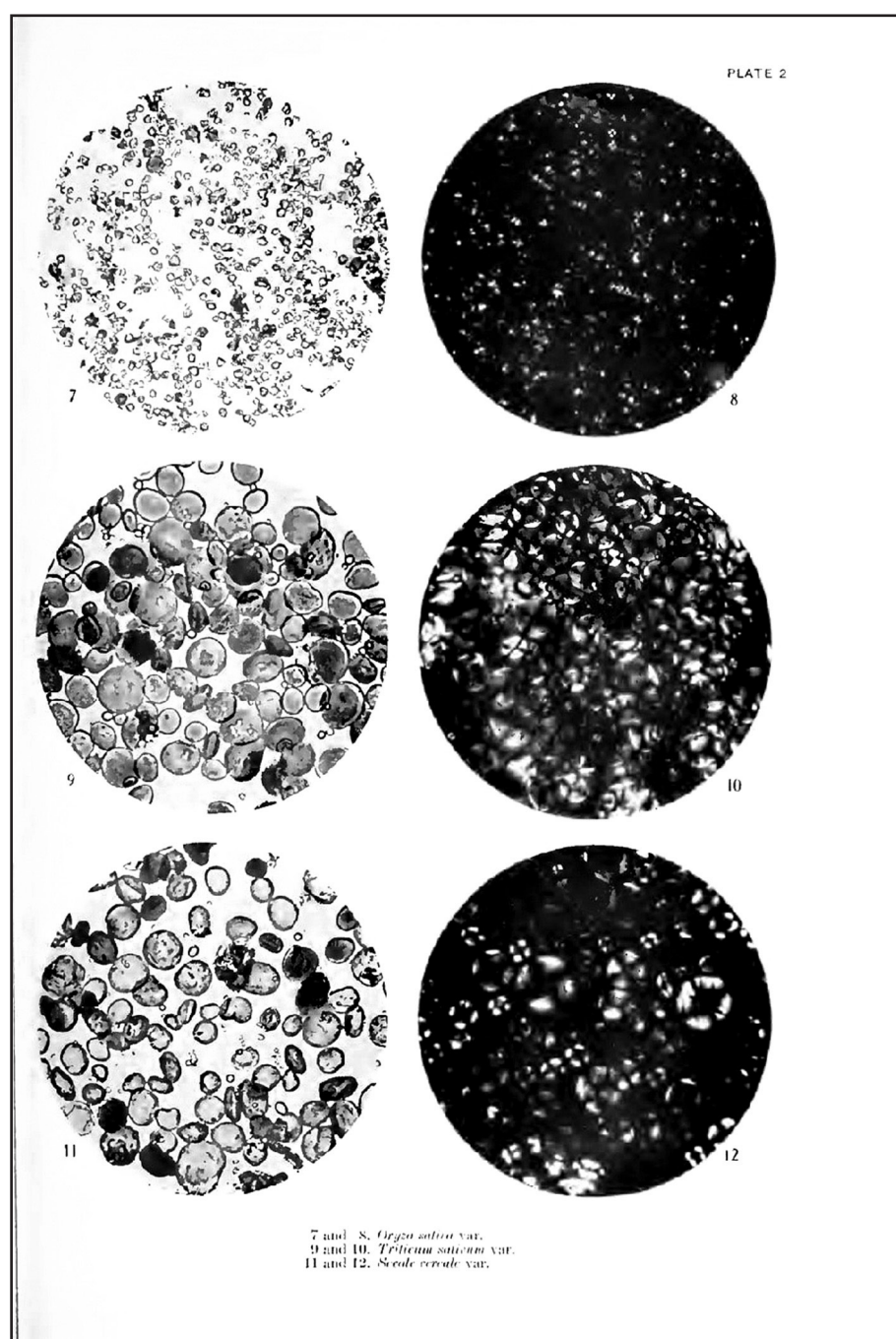


Figure 1. Illustration of *Poaceae* starch grain (Reichert, 1913).

Kraemer (1868–1924) (Kraemer, 1907). Edward Tyson Reichert was a physiologist from Philadelphia who created a comprehensive work summarizing the knowledge of the study of starches (Figure 1), and described their properties and use for starch grain identification (Reichert, 1913; Torrence, Barton, 2006). These works found use in everyday life, especially in the pharmaceutical and food industries, where there was a need to check the quality and origin of food and plant products from medicinal plants. For example, in 1978, William Sedgwick Saunders (London's Medical Officer for Health) inspected the flour sold in London to prevent it being mixed with gypsum (Stevenson, 2014).

Subsequently, starch atlases were created to recognize individual species by noting differences in starch grains (Loy *et al.*, 1992). Starch analysis has then been put into use in archaeological research over the last twenty-five years. In 2006, Robin Torrence and Huw Barton published a comprehensive account of starch analyses in archaeology (Torrence, Barton, 2006). One of the most prominent results of starch analysis connected with Stone Age artefacts can be traced to 2007, when the first results of the human plant diet at the Palaeolithic Gravettian site were published, describing the important role of plants in the Palaeolithic diet (Aranguren *et al.*, 2007). Before Palaeolithic people were usually regarded as only hunters of large animals. In the last thirty years, we can see the expansion of starch studies in archaeology: as described in a recent account by Barton and Torrence (2015).

In this paper, we summarize some basic knowledge of starch analyses from the Palaeolithic, Mesolithic and Neolithic period. Our main focus is on the starch grain itself, its biology and mode of identification of stone implements in particular. A special section summarizes some of the results of starch analyses at specific archaeological sites and with certain objects.

1.1 Starch

Starch is a polysaccharide used as a reserve energy store in the majority of autotrophic plants. The exception is presented by the families Asteraceae, Campanulaceae, Liliaceae, and others, which store inuline as their reserve polysaccharide. Starch is a ready source of glucose for plants, suitable for long storage. It is a composition of two homopolysaccharides (amylose and amylopectine), originating from α -D-glucopyranose. Amylose and amylopectine occur in a weight ratio of 1:3. In some crop plants, cultivars have been bred with an elevated one or the other component of starch (amylose or amylopectine) (Prugar, 2008). Amylose is a linear homoglycan consisting of up to 4500 (more often 1000–2000) glucose units. Amylopectine is a multiple-branched polysaccharide consisting of chains of 50,000–100,000 D-glucose units. Amylose is a linear α -D(1-4)-glucane of disaccharide maltose; the branching of the chain is limited to approximately ten loci per molecule (Velíšek, 1999; Bemiller, Whistler, 2009).

Starch is synthesised in the green parts of the plant – in the chloroplasts. There, small starch grains, about 1 μ m in

diameter, which are called temporary or transitory starch, are created. These are further used or transported. Starch is further stored in special organelles – amyloplasts. Major quantities of starch are stored in reserve organs in specialized cells of the seeds, roots and tubers (Bemiller, Whistler, 2009). Premature fruits also contain starch, but with the ripening process the starch content decreases and in ripe fruits the starch hardly occurs. However, there are exceptions, such as bananas, where high amounts of starch are contained in the fruit (Velíšek, 1999). Starch is stored in amyloplasts in the form of starch grains, which are species-specific and differ in shape, size and polysaccharide ratio. These starch grain characteristics are, for the most part, given genetically, but are also influenced by external influences (Selvam, 2013). According to the crystallinity level of the granules, the starch can be divided into four forms, designated A, B, C and V. The variability is due to the internal spatial arrangement of the molecules. The most stable is form A, which occurs in cereals, and the least stable is form B, which is found in root crops and potatoes. The C form is characteristic of leguminous seeds (it is a mixture of starch form A and B), whereas gelatinized starches occur in the V form. From a chemical point of view, starch grains can also contain small quantities of other substances that occur in plant cells, such as proteins and lipids (Velíšek, 1999; Ahmed *et al.*, 2016; Bemiller, Whistler, 2009).

1.2 Starch grain

Starch granules occur in various shapes and sizes: round, kidney-shaped, oval-elongated and polygonal shapes are common. Granules can be separated or coagulated into aggregates. Starch grains also differ in size. It is possible to distinguish several structures and formations, all of which are used in the identification process. For example, the visibility and position of the hilum is observed: whether it is located in the centre of the starch granule or off-centre (Lentfer *et al.*, 2002). Sometimes lamellas are visible. These are concentric lighter and darker stripes on the granule that are circular from the centre towards the starch edge (Czaja, 1969). They are more recognizable in larger starch grains and are connected to the gradual growth of starch grains. They can be divided into crystalline (a denser part) and semi-crystalline (a softer part with darker colouring). On the surface of starch grains, fissures or other superficial structures can be distinguished. Furthermore, the bevelling of the starch grain can be considered (Gott *et al.*, 2006; Bemiller, Whistler, 2009). The effect of outside, natural or anthropogenic, influences on the starch granule can lead to its injury or even destruction (starch modification, swelling, gelatinization). Starch can be damaged mechanically (*e.g.* broken in the course of milling). A limiting factor for its preservation can be temperatures above 50°C, when gelatinization takes place in the presence of water. The starch grain starts to deteriorate with exposure to enzymes, the effect of the amylase enzymatic group. Other harmful influences are, for example, long water exposure, low temperatures and charring (carbonization) (Messner *et al.*, 2008; Lentger, 2012).

2. Methodology of starch analysis

When the starch grain has a certain optical appearance, the starch can be identified with optical microscopy in polarized light by observing the extinction cross on the starch granule (Figure 2). Further approaches to starch identification encompass chemical methods and staining methods (Haslam, 2004). Lugol's solution stains the starch grains dark blue (Reichert, 1913). Using CongoRed colouring can be useful for identifying starch damaged by processing (e.g. cooking). After the pigment application, the starch turns red according to the degree of damage (Lamb, Loy, 2005). Another staining solution is Trypan blue. Trypan blue stains damaged starch grains and damaged starch that has a damaged natural shape, whereas undamaged starch granules do not stain. (Torrence, Barton, 2006) In some cases of archaeological investigation the various forms of starch do not stain well. The starch of individual (plant) genera and species can also be identified due to different rates of enzymatic decomposition or observing differences in their reaction with acids (Reichert, 1913). For the precise identification of starch grains, a specimen from a reference catalogue should be used (Haslam, 2004). A reference catalogue consists of starch grains from the individual parts

of contemporary vegetation. It should contain samples from crop plants, medicinal plants, commercially-used plants and other important species occurring in the surrounding environment. Fresh samples considered for cataloguing should not be dried at temperatures higher than 35°C. After drying they can be ground and used to create slides (Therin *et al.*, 1997, Lentfer 2009, Hart, 2014). The prepared sample should be examined microscopically and described. Measured and detected values are then stored electronically, and the starch grains are also photographed. Results from the measurement and description of starch grains can then be employed in creating a statistical method of starch identification from archaeological samples, especially for determining statistically-significant traits and the correlation between them (Lentfer *et al.*, 2002).

2.1 Sampling of starch from archaeological objects

Starch extraction is conducted by various means, most often with a pipette directly from the object's surface, or with the use of an ultrasonic bath. In the pipetting method a small quantity of distilled water is placed directly onto the surface of the examined object (Figure 3), though the place needs to be carefully selected. The most promising locations for starch grain conservation are scratches and fissures on the

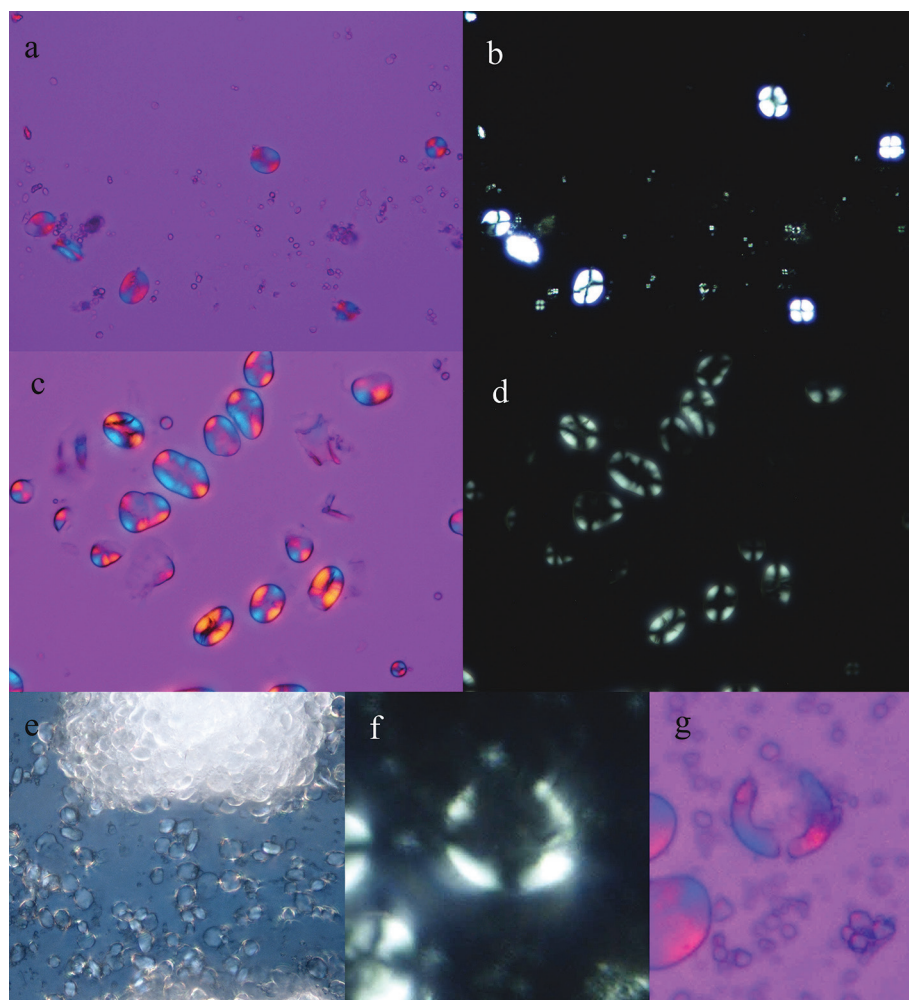


Figure 2. Recent grains under different lights (J. Kovárník) a, b) Starch grains from spring barley (*Hordeum vulgare*) in cross polarised light. c, d) Starch grains of *Pisum sativum* (Fabaceae) in cross polarised light. e) Starch grains from spring barley in normal light. f, g) Starch grain hydrolysed by enzymes.

Figure 3. Grinding stone from Hrdlovka, during sampling in the laboratory. (J. Kovárník).



surfaces of various objects, or pores in the structure of dishes (Messner *et al.*, 2008, Li *et al.*, 2013; Pagán-Jiménez *et al.*, 2015, Copeland, Hardy, 2018). The water drop containing the residues is collected by the pipette, from which it is possible to create a sample for microscopic analysis. This technique is suitable for determining the purpose and use of various objects and their individual parts (Li *et al.*, 2013; Tao *et al.*, 2011; Yang *et al.*, 2014; Yasui, 2015), allowing an examination of an object's specific function or mode of use. The other analytic approach is obtaining the sample from the examined object using sonication. The object is placed into a clean (sterile) beaker/test tube along with distilled water and the container inserted into the ultrasonic bath for five minutes. The distilled water with the relieved sample particles is transferred into a test tube and centrifuged at 2500 rpm for 15 minutes. The excess solvent is then removed. Separation from the denser liquid ($\rho=1.8 \text{ g.cm}^{-3}$) then takes place, the lighter fraction containing the starch grains coming to the

surface with the heavier (mineral) fraction remaining on the test tube bottom. The mixture of sample and dense liquid is centrifuged for 5 minutes. After that the supernatant (approx. 2 ml) is extracted and subsequently diluted to make the starch grains form a sediment on the bottom. The microscopic sample can then be created (Messner *et al.*, 2008; Liu *et al.*, 2010; Marcadert *et al.*, 2007). These techniques can, of course, be combined: it is possible to point-collect samples with the pipette and subsequently extract residues from the whole object in the ultrasonic bath (Lantos *et al.*, 2015; Yang *et al.*, 2015; Copeland, Hardy, 2018).

The extracted samples can be stored in a dry condition or in a tube with an ethanol solution (Therin *et al.*, 1997). One way for making starch samples are by mounting on a microscopic slide using only water (in the case of temporary slides) or by storing dry samples of starch grains on a slide with a cover glass affixed using clear nail polish. This sample can be stored in a dry condition and a water drop can be added under the

cover glass before observation. This method is simple and allows the observation of starch grains in polarized and non-polarized light. It also allows repeated observations after drying and rehydration of the sample. (Piperno, 2006; Coil *et al.*, 2003). Another way is to mount the sample in media to make a permanent preparation. Mounting media suitable for starch samples are glycerol (which can be diluted by water 1:1), *Permout*, Euparal, or immersion oil, etc., and the slide can then be sealed with clear nail polish (Yeung *et al.*, 2015; Coil *et al.*, 2003). However, *Permout*, for example, has some disadvantages: the optical properties of *Permout* do not allow the extinction cross to be observed on small starch grains. Starch placed into this medium for long periods also loses its typical refractions and slowly degrades. Starch has a refractive index of 1.52 to 1.53; for optimal observation in polarized light mounting media with a higher or lower refractive index than starch are suitable (Coil *et al.*, 2003; Piperno, 2006; Wang *et al.*, 2017).

2.2 Sampling of starch from dental calculus

Dental calculus is a layer that forms on teeth, especially on the tooth base. This layer is formed from biological plaque after mineralization. This material also contains other components such as organic materials or chemical components. It is a rich source of the tracks left by an ancient life style (Leclerc *et al.*, 2018, Copeland, Hardy, 2018; Eerkens *et al.*, 2018). Calculus must be carefully pulled down onto metal foil. After collection, the calculus is placed in a test tube with 0.6 M HCL for a short time. This step is for cleaning the calculus and for assuring whether it really is calculus or another sediment type. The next step is similar to the previous treatment, the dental calculus is dissolved in HCL for 5 days at a low temperature (Hardy *et al.*, 2009). To speed up this extraction procedure it is possible to crush the larger parts of dental calculus and work with the resulting powder (Buckley *et al.*, 2014). The final solution is vortexed, centrifuged at 15 min, washed in ultra pure water and centrifuged again (three times) (Hardy *et al.*, 2009). Another extraction possibility is using the ultrasonic bath (Hardy *et al.*, 2012; Madella *et al.*, 2014). A similar method for dental calculus is described by Cristiani *et al.*, 2016.

2.3 Sampling of starch from soil samples

Starch grains can also be extracted from soils. This procedure can be complicated because it is necessary to take the soil samples from a specific place such as a pore, small depression or split/crack. However, in such locations there is a high probability of taking a rich sample. In below-ground conditions it is harder to preserve intact starch grains. In a field experiment that compared the survival rate of starch grains on grinding stones placed on the ground surface and below ground, it was shown that the samples taken from the experimental grinding stones on the soil surface contained better preserved starch grains (Barton, 2009; Vranová *et al.*, 2015). Despite such observations, starch granules are well preserved at many archaeological sites (Fullagar *et al.*, 1998; Therin *et al.*, 1997). The first step for sample preparation is to

dry the soil in the laboratory. After that, the process is similar to phytolith extraction including the heavy-liquid flotation procedure. For separating starch grain from sediment, a heavy liquid such as cesium chloride (CsCl) or sodium polytungstate (SPT) should be used. Other chemicals with a high density can also be used. Starch grain analysis from soil samples can be usefully applied for land-use reconstruction (Dickau, *et al.*, 2007; Balme, Beck, 2002; Parr, Carter, 2003).

3. Starch identification

Identification is carried out by direct observation and by comparison with specimens from the reference collection. In a vast collection the most important part is the thorough description of starch grains, as this will accelerate the comparison process (Cosgrove *et al.*, 2007; Torrence *et al.*, 2004; Hou *et al.*, 2016). Direct observation can be accompanied by a statistical evaluation (Cosgrove *et al.*, 2007; Herzog, 2014; Mayle, Iriarte, 2014), for example multi-dimensional analytic methods, which facilitate starch-grain identification from archaeological findings (Lentfer *et al.*, 2002; Torrence *et al.*, 2004). The next step would be automatic classification of starch grains provided by specialized software with Image analysis modules and statistics outputs. (Arráiz *et al.*, 2016)

The sample must be prepared carefully and accurately in a clean laboratory environment and using only starch-free equipment and chemicals. It is good procedure to: control the level of contamination in the laboratory; add a control sample into the set of samples during the whole procedure; and examine the possibilities of transfer (Buffington *et al.*, 2018). These simple precautions can reduce the risk of contamination, but not completely. Other ways to reduce contamination are complicated and expensive to purchase. Within this group can be included air filtration, sticky mats, restricted access, barrier curtains and airlocks (Crowther *et al.*, 2014; Herzog, 2014; Ma *et al.*, 2017).

3.1 Microscopic structures that can look like starch

In nature there exist several structures with similar optical properties to those of starch. Here is a list of common structures that look like starch: spherulites, fungal spores, bordered pits, damaged pollen, wall thickening, algae (diatoms and coccoliths), and microscopic air bubbles on the slide. Many of these structures are of the same size as a starch granule and make a similar optical signal under polarised light (Torrence, Barton, 2006). Two things that may help in differentiating between starch and non-starch structures are staining the starch grains (iodine staining) and rotating the birefringence extinction cross (Yeung *et al.*, 2015; Moss, 1976).

3.2 Implications of starch analysis in archaeological research of the Stone Age

Perhaps the oldest identification of starch grains from the archaeological context of the Stone Age comes from Qesem

Cave, Israel. The archaeological investigation revealed here eight hominid teeth in the context of the Amudian industry, which belongs to the blade-dominated Lower Palaeolithic Acheulo-Yabrudian Cultural Complex (AYCC) and predates the Middle Palaeolithic Mousterian traditions (400–250 ka, Hershkovitz *et al.*, 2011; 2016). The authors present evidence for potentially inhaled and ingested material in the dental calculus that was extracted from three of these teeth (Hardy *et al.*, 2016). The identification of starch granules and specific chemical compounds in the dental calculus samples from Qesem Cave points to deliberate ingestion of essential dietary components, most likely in the form of concentrated sources such as seeds or nuts. The phytoliths and plant fibres found in the calculus could be the result of non-dietary activities, including raw material processing, oral hygiene or food remains.

Interesting evidence regarding a complex of starch grains was found in Shanidar Cave and Spy Cave (Henry *et al.*, 2011), where the plant residues from tooth calculus originating from Neanderthal teeth were investigated. The two sets of samples were compared. The first came from Shanidar III, the Shanidar Cave in Iraq. The second set of samples came from Spy Cave in Belgium (Spy I and Spy II). In all, seven teeth were sampled: three teeth came from Shanidar III, two from Spy I and two from Spy II. The Shanidar Cave samples contained 73 starch grains, and the Spy Cave samples contained a total of 136 starch grains (Henry *et al.*, 2011). The Spy Cave samples were 36,000 years old and the samples from Shanidar Cave were about 46,000 years old (Reader, 2011). In addition to the starch analysis, phytoliths were also analysed. With the results of both analyses completed, it was found that there had been heat modification of the plant food. The starch granules were typically damaged by heat. It is considered that larger-scale plants were used, based on the seasonality of their maturation. Maturation is an important growing phase for maturing seeds or starch-rich organs, making them suitable for harvesting, storing and processing as food (Henry *et al.*, 2011). An important message from this study is the direct evidence of plant use among the Neanderthals and the role of plants in their subsistence, a group traditionally regarded as strongly dependent on hunted animals. The results show the difference in diet caused by the different environments. But Neanderthals from both the sites had a quite varied diet (grass seeds, plant underground-storage organs, legumes, and dates). Henry found that Neanderthals were adaptable, had good knowledge about their surroundings, and used their environment very effectively. This is highlighted by their complicated food behaviour, including heat preparation of food for better digestibility. The data collected from sediment could not clearly show which plant material was used for food. Calculus micro-analysis can also be used for ancient diet reconstruction. The results from the calculus analysis also showed that samples taken from different teeth from the same person could provide significantly different amounts of information. Two teeth from the same individual were compared: the calculus from the first tooth contained a large

amount of starch grains, while the second tooth was poor in starch grains. This comparison was made at the Spy I site and repeated at Spy II with the same result. The subsistence of Neanderthals seems to be much more balanced given the perspective of starch analysis.

Starch analysis had already been used effectively in a series of entire archaeological studies of the Gravettian population belonging already to *Homo sapiens*. Residue analyses on a grinding tool from Grotta Paglicci in southern Italy (32,614 ± 429 calibrated (cal) BP), recorded rich assemblages of starch grains, most probably of *Avena* (oat) caryopses, and this has substantially enriched our knowledge concerning the food plants of Palaeolithic Europe in the context of the Early Gravettian period (Lippi *et al.*, 2015). The quantitative distribution of the starch grains on the surface of the grinding stone furnished information about the tool being used as a pestle grinder. The particular state of preservation of the starch grains suggests the use of thermal treatment before grinding, possibly to accelerate the drying of the plants, making the following process easier and faster (Lippi *et al.*, 2015). One of the most important results of starch analysis in archaeology has been made in the case of Gravettian (the Upper Palaeolithic period) site Bilancino. The authors have identified starch grains on the surface of a grinding stone from the hunter-gatherer campsite of Bilancino (Florence, Italy), dated to around 25,000 BP. Analysis has identified the remains of starches of the wild plants *Typha* and Graminae cf. *Brachypodium*. The stone can be seen as a grindstone and the starch has been extracted from locally-growing edible plants. This evidence can be claimed as implying the making of flour – and presumably some kind of bread – some 15 millennia before the local “agricultural transition” (Aranguren *et al.*, 2007).

The above-mentioned study from Grotta Paglicci, Bilancino, and other archaeological sites like the Upper Gravettian site Dolní Věstonice, clearly indicate the predisposition of Upper Palaeolithic modern humans for sophisticated plant exploitation long before the spread of agricultural knowledge (Revedin *et al.*, 2010; 2015). The last decade of research has fundamentally changed our view on how the Paleolithic people exploited plants. Phytolith studies, the analysis of starch granules on stone tools, as well as isotopic and nutritional studies, have newly demonstrated the significant role of plant food in their diet (Beneš, 2018).

This picture seems to be confirmed by micro-remains analyses of starch from the Palaeolithic period from other regions of the world. Interesting evidence of ancient starch has been published by Loy *et al.* (1992), who presented residue analysis of stone artefacts from the Solomon Islands. This study described the use of starch analysis on a set of stone tools from Kilu Cave on Buku Island (Solomon Islands in Melanesia). In the Kilu Cave profile, evidence was found of a dual settlement, the oldest occupation being dated at 28,000 years old. Starch analysis was used as direct evidence of the spread of plant species and to specify the use of tools. In all, a set of 47 stone tools and fragments were examined, twenty of them showing no trace of starch grains. Of the

27 tools that contained starch grains, 8 stone tools came from younger layers and 17 stone artefacts from older layers (up to 28,000 years old). Stone artefacts from the older layers contained starch grains derived from plants of the *Colocasia* and *Alocasia* genera (Loy *et al.*, 1992). This micro-remains evidence supports our view of the balanced role of plants in the local Palaeolithic diet in Oceania, despite the fact that the tropical environment of the Solomon Islands favoured a plant-based subsistence in general (Bellwood, 2005).

The Late Palaeolithic and Early Neolithic archaeobotanical knowledge from China concerning starch analysis is very important. China belongs to the centres of agricultural beginnings (Bellwood, 2005; Barker, 2006; Beneš, 2018), where a vast amount of archaeobotanical research has been recorded in the last decade. China has a large area that includes boreal landscape and semi-deserts in the north of the country, temperate deciduous forests, and regions of subtropical forests in southern China. The two key developments of the Epipalaeolithic and Early Neolithic centres were situated in the middle Yangtze basin and in the upper and middle Yellow River Basin of northern China. A functional study of two grinding stones (a slab and a grinding stone) near a burial dated to 9220–8750 BC cal. (before the onset of the Neolithic), at Donghulin, investigated the range of plants exploited during this early occupation period. Starch residues indicate that the grinding stones were used for processing plants, and confirm the processing of acorns, which is consistent with the incidence of oak (*Quercus*) in the pollen record (Liu *et al.*, 2010b). Similar observations concerning a younger period have been made in the case of the Early Neolithic Peiligang culture sites Shigou and Egou in the catchment of the Yellow River, where starch analysis on grinding stones has documented the presence of acorns and other plants, probably *Cyclobalanopsis*, *Lithocarpus*, *Dioscorea*, *Trapa* and others (Liu *et al.*, 2010a). The above-mentioned examples indicate the unchanged plant processing customs of people in the Late Palaeolithic and Early Neolithic period reflecting a continual development from a hunter-gatherer economy to the stages of early agriculture. The use of archaeological artefacts in this context plays an important bioindicative role as indicated by the starch analysis.

In recent years some new observations have been made in the field of Stone Age research concerning Mesolithic and Neolithic Europe. The most interesting evidence associated with starch grains has been made during an investigation of the dental calculus of Mesolithic human teeth at the site of Vlasac in the Danube Gorges of the central Balkans (Cristiani *et al.*, 2016). The research has provided direct evidence that already by 6600 cal. BC, if not earlier, the Late Mesolithic foragers here consumed domestic cereals, such as *Triticum monococcum*, *Triticum dicoccum*, and *Hordeum distichon*. These plant species were the main crops found among the Early Neolithic communities of southeast Europe. This investigation indicates the great value of starch grain identification in the Mesolithic human population and their interaction with the Neolithic people from the southernmost Balkans several centuries before the Neolithic package of

domesticated plants and (probably) Neolithic people reached the inland areas of the Balkans around 6200–6000 BC cal (Kreuz *et al.*, 2005; Brami, 2017).

Another interesting example, again from the Balkan region, combines starch and phytolith analysis. At the late Middle and early Late Neolithic site of Stavroupoli, Thessaloniki, Greece, a series of organic remains from ceramic sherds were studied. The research focused on matter recovered from cooking vessels, where charred food crusts had adhered to the inner walls of 17 late Middle and early Late Neolithic vessels (ca. 5600–5000 BC cal.). Starch grains were very scarce or absent in most of the samples, but samples ST129 and ST192 presented a high concentration of small Panicoideae and Triticeae grains (García-Granero *et al.*, 2017), as well as big and small types of Panicoideae. With supporting phytolith analysis, the research authors concluded that the analysis of starch grains and phytoliths from charred food crust on the ceramics added valuable information to previous studies on the diet in Neolithic Stavroupoli. Especially remarkable is the identification of the use of the potentially wild weed *Setaria* in the diet.

Research of starch remains was also successful in the central European Neolithic context. In Tiszásziget-Agyagbánya in southeastern Hungary, a site belonging to the Late Neolithic Tisza culture (ca. 5000–4500 BC cal.) was investigated. Here in a pit associated with postholes – the remains of a longhouse – three intact vessels and one grinding stone were unearthed. The layout of objects in the pit evokes its ritual character. The unearthed vessels were situated in the hole of the former central post of the building's structure. The subject of interest by microscopic and biochemical analysis was organic matter from the bottom of the vessels. The methods used were complex, including the study of phytoliths, starch and biochemical signals (Pető *et al.*, 2013). Phytolithic morphologies present in the samples have their anatomical origin from the leaf and inflorescence of *Poaceae* (as well as cereals). Among the phytoliths, which indicate the predominance of grasses, five partly-damaged starches were observed. Based on their identification, the starches from the sample most likely represent wheat (*Triticum* sp.).

Starch analysis has been carried out in the case of a set of grinding stones from the Neolithic site of Hrdlovka (Northwest Bohemia, Czech Republic). The samples were taken from grindstones deposited in feature 838 and dated to 4620–4458 BC cal. and were situated in the area of house 8 from the Late SBK period. The deposition of millstones could have a ritual or social meaning connected with the foundation of the house. Altogether 8 grinding stones were analysed for the presence of starch grains and their surfaces revealed 49 starch grains (Beneš *et al.*, 2015). Overall, it was possible to determine 12 circular starch grains as belonging to plants of the family *Poaceae*. Starch grains of an oval shape with the characteristic extinction cross belonged to the family *Fabaceae*. Determination of starch grains from the Neolithic Hrdlovka site represents the first such positive analysis of micro-remains in the Czech Republic.

The last above-mentioned examples from central Europe represent cases of a similar archaeological situation, where artefacts from sunken features dating to the Neolithic period played a central role, as well as a specific, possibly ritual, meaning for both artefact deposits. In both cases starch analysis contributed to specifying their role in the Neolithic community.

4. Conclusion

Starch grain analysis is a useful tool for evaluating archeobotanical specimens from soil samples, artefacts, and from human teeth. Using starch grain analysis, it is possible to identify plants that are in a non-flowering growth phase and would not be identified by pollen analysis. A further great advantage is that humans preferred plants with a large amount of starch. Those plants, and their individual organs with a high starch content, were used as energy sources from human nutrition. People used and processed a lot of material that can be identified in archaeological contexts. However, for the same reason, there is a risk of contamination of the sample from present-day materials containing starch grains. Unfortunately, starch grains are relatively susceptible to environmental conditions, such as those not of optimal temperature and neutral pH. Another disadvantage is the inability to capture plants from the family Asteraceae, as they do not form starch but inulin. Starch grain analysis is more effective in combination with other bioarchaeological methods, such as pollen, phytolith determinations and analysis of botanical macroremains. Hence the method of starch analysis is also notably useful in combination with biochemical analysis targeted at a specific group such as amino acids or lipids.

In general, starch analysis constitutes a useful tool in bioarchaeological investigations. Despite its relatively limited taxonomical power, it is an effective supporting method in the functional determination of artefacts such as millstones and grinders, and a key source of information in the bioarchaeological research of food remains on pottery. It is also possible to point towards the propagation, use and processing of plant species. The anthropological application of this method targeted on dental calculus is also particularly important. Current studies of these biological traces have shed new light on the ratios between meat- and plant-based diets. Some results clearly indicate a higher ratio of plant diets among Palaeolithic human populations than previously thought. In future research, starch analysis – together with pollen and phytolith analyses – will form a crucial basis for functional bioarchaeological interpretation.

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