Journal of Archaeological Science: Reports 10 (2016) 142–146

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Methods to isolate and quantify damaged and gelatinized starch grains

Amanda G. Henry [a,*], Cynthianne Debono Spiteri [b], Thomas Büdel [a], Antje Hutschenreuther [a], Simone Schmidt [a], Jörg Watzke [a]

[a] Plant Foods in Hominin Dietary Ecology Research Group, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany
[b] Institute of Prehistory, Early History and Medieval Archaeology, Eberhard Karls Universität Tübingen, Schloss Hohentübingen, 72070 Tübingen, Germany

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 9 September 2016
Accepted 12 September 2016
Available online xxxx

Keywords:
Methods testing
Starch grains
Starch granules
Gelatinization
Heavy liquid flotation
Density separation
Filtration

ABSTRACT

Damaged and gelatinized starch grains recovered from artifacts and sediments have the potential to provide valuable information about past food processing behaviors. Because these particles have different physical properties from native undamaged starches, it is unclear if the methods used to recover them from archeological contexts are effective. Here we present tests of several laboratory methods for isolating starches, with the hope of identifying the best method for quantifying total starch numbers and recovering gelatinized starches. Our results indicate that no methods can provide total recovery of starch grains, and that most methods strongly under-represent gelatinized starches.

1. Introduction

Starch grains recovered from stone tools and from sediment samples have been used as a marker of dietary behavior across many geographic regions and historical time periods. Based on their distinctive and often diagnostic morphologies, native (undamaged) starches can record the presence of particular plant taxa or plant parts. Moreover, researchers are often interested in how these plants were used, and several studies have examined how damaged or gelatinized starches can indicate intentional processing of plant foods, such as grinding or cooking. Babot and colleagues (del P. Babot, 2003; del P. Babot and Apella, 2003) subjected a variety of New World plants to various processing methods, including drying, freezing, roasting, charring, and milling, and noted that these processes produced a variety of distinctive damage to the starch grains, ranging from cracking to loss of organization at the hilum, and alteration of the extinction crosses. Henry et al. (2009) noted similar distinctive changes to starches from domesticated food plants of Near Eastern origins, which depended on the type, duration, and temperature of cooking. Messner and Schindler (2010) reported similar patterns among cooked wild plants from eastern North America. In subsequent studies, these distinctive damage markers have been used to identify processing in the archeological record (e.g., Henry et al., 2011).

Despite the potential of gelatinized and damaged starches for recording processing behavior, several authors have expressed concerns about the utility of this technique. Some have argued that processes other than intentional processing can also damage or gelatinize starches. For example, aging of the starch grain over archeological time periods could cause the degradation of hydrogen bonds that hold the starch together, leading to gelatinization-like damage (Collins and Copeland, 2011; Henry, 2015). Others have noted that taphonomic factors can damage starches, or bias an assemblage toward over or under representation of damaged or gelatinized starches (Debono Spiteri et al., 2014). These concerns about the origins of gelatinized starch grains are confounded by methodological questions. Gelatinized starch has significantly different properties than native starch, including a lower density (Marousis and Saravacos, 1990) and greater affinity for clay particles (Correa de Araujo, 1988). It is possible that the methods currently used in archeological starch research, which were developed for the analysis of native starches, may not be appropriate for the analysis of damaged and gelatinized starches. This methodological concern prevents researchers from accurately assessing damaged and gelatinized starches in their assemblages, thus preventing a coherent study of potential taphonomic biases.

As part of a larger project to assess the long-term preservation of native and gelatinized starches on stone tools buried in various sediment types (Debono Spiteri et al., 2014), we were particularly concerned that the methods we used would correctly recover native and gelatinized or damaged starches from sediments. We tested a variety of published methods specific for starch research, and others that we modified from other fields. Though some methods provided a final proportion of native and gelatinized that matched the starting proportion,

* Corresponding author.
E-mail address: amanda_henry@eva.mpg.de (A.G. Henry).

http://dx.doi.org/10.1016/j.jasrep.2016.09.003
2352-409X/

all of the methods lost starches throughout the processing steps, with some losing a significant amount of the starting starch concentrations. Many of the methods strongly biased the results against gelatinized starches. These results indicate that more work is needed to develop a new method that allows both the assessment of overall starch content as well as the proportion of native to gelatinized starches.

2. Methods

We tested several standard and some novel methods for isolating starch grains from starch:sediment suspensions as well as from pure starch suspensions. Our first tests (group A) aimed to assess whether any standard laboratory procedures (e.g., centrifuging, vortexing) caused morphological damage to gelatinized starches. The next series of tests (group B) examined how well the two most commonly used heavy liquid preparations (sodium polytungstate and cesium chloride) isolated starches, with a variety of pre-treatment steps. Finding these unsatisfactory, we attempted a vacuum-based separation method (group C), then a density gradient method used in histological preparations (group D), before reverting to a filtration method (group E). Finally, a thorough reassessment of our methods indicated that some of the exact steps we had used in Group B, including centrifugation speed and time, were different from what have been used in previous publications (e.g., Therin and Lentfer, 2006), so we did a short series of experiments to test whether varying these parameters would change the results (group F).

For these experiments we used prepared starch powders from wheat (Weizenin wheat starch produced by UniLever, batch L322503804) and potato (Kartoffelmehl potato starch produced by RUF, batch L348976). We chose these starches because they are among the best-understood starch types from a chemical and physical point of view (e.g., BeMiller and Whistler, 2009; Douzals et al., 1996), they are easy to acquire in large quantities, they represent two of the three main types of crystal structure found in starches [A type and B type; (Buléon et al., 1998)], they come from two different plant organs (seed and tuber), and finally, because they have been important in archeological studies of human dietary behavior (e.g., del P. Babot, 2011; Piperno et al., 2004). In one test (B1) we also examined cattail (*Typha* cf. *angustifolia*) root starches, which we isolated from wild-growing stands around the Cospudener See in Leipzig.

A brief note on our terminology: though we use the term gelatinized to describe starches that have been damaged by heat and water, we are referring only to starches that are partially gelatinized and thus retain some of their shape and other features that allow us to identify them as starches, and not to completely gelatinized starch pastes. The methods are described in general terms below, but detailed methods for each group are included in Supplementary Tables 1–6.

In the cases where we created starch:sediment mixes, the sediments were collected from Leipzig, dried, sieved through a 1 mm mesh, and autoclaved with a Tuttnauer Systec 40l 3150 EL at 121 °C under 101 kPa pressure for 20 min to remove any endogenous starch and starch-consuming bacteria. For most tests (except group A) we created gelatinized starch suspensions that were designed to have a mixture of both native and gelatinized starches. Therefore we cooked starch suspensions at temperatures lower than the average gelatinization temperature. For potato, gelatinization begins at roughly 55 °C and half of the granules are gelatinized at 61 °C (Shiotsubo, 1984). We chose 56 °C as a temperature that would give slightly more native than gelatinized starches. Wheat starches begin gelatinizing at roughly 52 °C and half of the granules are gelatinized at 60 °C (Eliasson and Karlsson, 1983). We wanted again to have slightly more native than gelatinized starches and so chose 52 °C, but wheat starches reacted unpredictably and most of the starches in the cooked wheat samples were gelatinized (see the full Data Table for counts). This is probably because the milling process to create the isolated starch powder somewhat damaged the granules, making them more susceptible to gelatinization.

2.1. Group A methods — standard laboratory processes

A 10% w/v solution of wheat starch was gelatinized, and subjected to a variety of tests that were meant to replicate what starches might experience during their recovery from the surface of a stone tool, including drying, scratching or scraping, vortexing, diluting, and centrifuging (Supplementary Table 1). These tests assessed only the morphological changes to gelatinized starch, and not the ratio of native to gelatinized starches.

2.2. Group B methods — heavy liquid flotation

The second series of tests was designed to ascertain the ability of the two most commonly used heavy liquids, cesium chloride (CsCl) and sodium polytungstate (SPT), to isolate native and gelatinized starches. We generally followed the protocols in previous publications (as described in Torrence and Barton, 2006, chap. 8), though we omitted steps designed to remove organics. As a preliminary step, we first determined how long native starches could stay in CsCl before becoming visually damaged (Test B1, Supplementary Table 2). Previous work (Torrence and Therin, 2006) has suggested that starches are stable in CsCl up to an hour, but these tests only measured overall starch concentration and did not document whether the state of the starch granules changed before they were removed from the record. We then performed floats of pure starch solutions and starch:sediment mixes, using both raw and cooked wheat and potato starches. We performed one set of flotations with initial deflocculation with sodium polymetaphosphate (also called sodium hexametaphosphate or SHMP). We also tested whether an initial "clay removal" step, in which the samples were floated in 1.2 g/ml heavy liquid, changed the results. These tests assessed the ratio of native to gelatinized starches, and examined where starches may have been lost during processing.

We also considered that the behavior of gelatinized starches in heavy liquid flotations may be affected by the water trapped within the gelatinized starches making them 'sticky' and therefore more likely to create clumps with other starches and sediment particles. Therefore we performed several methods where starch:sediment solutions were dried prior to heavy liquid flotation. We used acetone, ethanol, and freeze drying. The dried samples were then floated in SPT at 1.8 g/ml.

2.3. Group C methods — vacuum aspiration

Based on the observation that starches swell when hydrated, and particularly that damaged starches absorb more water and become less dense (Dengate et al., 1978; Haine et al., 1985; Marousis and Saravacos, 1990), we estimated that gelatinized wheat and potato starches should be slightly more dense than 1.2 g/ml. Combining this with our observation that these gelatinized starches averaged about 250 μm in maximum width, we calculated using Stokes law that they would settle at a rate of 0.68 cm/s when in water. By contrast, native starches, with a published density of 1.6 g/ml (Lisinska and Leszczynski, 1989) and average diameter of 40 μm, should settle at 0.05 cm/s. We attempted to use the differential settling rates of starches and sediments to separate them (Supplementary Table 3). By placing starch/sediment mixtures in tall graduated cylinders, we could use a vacuum pump system to remove the top layers in which we expected to find the starches, leaving larger sediment particles to fall out faster. The large amount of clays and sands that remained in this sample required us to then use a heavy-liquid flotation method to isolate the starches, which proved unsatisfactory.

2.4. Group D methods — density gradient

Based on our results from the previous methods, it became clear that gelatinized starches did not behave as predicted by their measured density, and that the behavior of native starches in mixed solutions was