

# Exaggerated expectations in ancient starch research and the need for new taphonomic and authenticity criteria

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## Abstract

Ancient starch research illuminates aspects of human ecology and economic botany that drove human evolution and cultural complexity over time, with a special emphasis on past technology, diet, health, and adaptation to changing environments and socio-economic systems. However, lapses in prevailing starch research demonstrate the exaggerated expectations for the field that have been generated over the last few decades. This includes an absence of explanation for the millennial-scale survivability of a biochemically degradable polymer, and difficulties in establishing authenticity and taxonomic identification. This paper outlines new taphonomic and authenticity criteria to guide future work toward designing research programs that fully exploit the potential of ancient starch while considering growing demands from readers, editors, and reviewers that look for objective compositional identification of putatively ancient starch granules.

**Key words:** biomolecular archaeology, ancient starch, taphonomy, authenticity criteria, elemental and structural characterisation

## Introduction

Ugent et al. (1982) published the earliest account of archaeological starch granules from work at sites north of Lima (Peru) more than 35 years ago. Recovered granules from 4000-year-old desiccated

tubers were identified as potato starch using optical and histological techniques. The authors attributed the exceptional preservation of starch to extreme aridity, and used granule morphology to infer source plant taxonomy, a practice with origins in late 19th and early 20th century biochemistry (Nageli and Nageli 1858; Meyer 1895; Reichert 1913). This approach has persisted until today (Czaja 1978; Jane et al. 1994; Torrence and Barton 2006). During the ensuing decades, the pioneering combination of starch granule identification and studies of early human diets opened the door for an exponential growth of the field and publicity in high impact venues (e.g., Loy et al. 1992; Piperno et al. 2000, 2004, 2009; Perry et al. 2006, 2007; Mercader 2009). These studies focused mainly on sites from the Americas and Australasia, and established the extraction, detection, description, and identification methods currently in use. Since then, dozens of articles have appeared in the field of archaeology on the pervasive, but little understood, presence of starch recovered from tools, sediments, and recently, dental calculus (Henry and Piperno 2008, 2011; Hardy et al. 2009, 2012, 2016; Salazar-García et al. 2013).

## New taphonomic criteria and authentication

Overall, the need for revision stems from multiple challenges common to the discipline including:

1. small recovered assemblages,
2. high contamination risk in both the field (Hart 2011; Laurence et al. 2011; Dozier 2016; Mercader et al. 2017) and the laboratory (Loy and Barton 2006; Crowther et al. 2014; García-Granero et al. 2016),
3. poor understanding of how plant-derived biomolecules and their often complex diagenetic products adsorb onto surfaces,
4. disparity in reporting standards,
5. scarcity of systematically controlled experimentation,
6. lack of a plausible explanation for the millennial-scale survivability of a biochemically degradable polymer,
7. difficulty in authentication, and
8. uncertainty surrounding taxonomic identification (Copeland and Hardy 2018; Mercader et al. 2018a, 2018b).

These gaps in the prevailing starch research perpetuate skepticism and generate inflated notions. This paper is a roadmap organised around two of the aforementioned challenges: taphonomic pathways and authentication, even though there are no standard criteria to address these challenges. This paper provides examples of experimental research that may lead to formal sets of criteria in the near future.

Fresh starch granules are polysaccharide polymers with semi-crystalline and amorphous regions, exhibiting unmodified birefringence from a concentric arrangement of crystalline, helical amylopectin clusters. Customarily, in archaeology, confirmation of starch chemistry uses polarised light microscopy of birefringent granules to display a distinctive “Maltese cross”. The biosynthetic deposition of the crystalline starch granules occurs within plant organelles inside cells surrounded by lignocellulosic walls. When the cells decay, a few of the starch granules may be afforded physical protection through entanglement in the remnant cell wall matrix. Commonly, starch granules do not exist in isolation; they are admixed with non-starch polysaccharides, lipids, and proteins co-occurring in variable proportions, depending on the plant source. Chemical reactions with these co-concurrent molecules must be taken into account when attempting to conceptualise diagenetic processes of starch granules

**Box 1.** Taphonomic pathways.

- Sorption
- Enzymatic inaccessibility
- Microbial modulation
- Silicification
- Phosphatisation
- Non-enzymatic breakdown

in archaeological contexts. Starch is a highly accessible biological energy reserve and the second most ubiquitous polysaccharide after the structural form known as cellulose. Unfortunately, as with many other biopolymers, starch is highly susceptible to hydrolysis over long timescales and the survival of these molecules only occurs under special circumstances (Briggs et al. 2000). The fate of plant biomarkers depends on what happens to the molecule immediately after it is released into the environment (Butts and Briggs 2011), and it is in the early stages of burial that the potential for degradation is greatest. A starch rich plant fragment can contain billions of starch granules, yet can these retain sufficient biochemical functionality and morphology to be identified as starch related thousands of years later?

It can be hypothesised that surfaces where starch granules become enzymatically unavailable, adsorbed to minerals, coated, overgrown, entrapped, or protected from chemical degradation, would be potentially rich locations at which to look for ancient starches (Box 1). Based on existing literature and ongoing experimentation, we outline six potential taphonomic pathways currently being investigated that hold promise for better understanding starch preservation or degradation in common archaeological contexts such as stone tools and dental calculus.

### Sorption to mineral phases

Adherence and bonding to solid substrates is governed by chemical sorption and by previously adsorbed molecules and functional groups (Collins et al. 1995; Kislenco 2002; Demarchi et al. 2016). In this scenario, adsorption delivers a relatively strong bond between the polymer and, for example, the mineral surface of a stone tool. The presence of oxides or other weathering products, surface roughness, pH, and complexation of species through ligand exchange could mediate binding (Dowell 1985; McKnight et al. 1992; Kaiser and Guggenberger 2000). It can be surmised that if starch were to become encapsulated in a mineral phase due to precipitation of the mineral and (or) recrystallisation, this would result in neoformed minerals and micro-structural transformations (e.g., a granule coating) that confer visual properties under polarised light (Lee-Thorp 2002; Briggs 2003) that are clearly distinct from those in native starch. Mineralised granules diffract polarised light in a mosaic pattern that combines strong birefringence (first order crystallisation) with disrupted, isotropic zones (J. Mercader, personal observation, 2017).

### Enzymatic bioavailability

There is vast literature on the mechanism of starch hydrolysis by enzymes under variable water and temperature conditions (Hoover 2000; Singh et al. 2010; Wang and Copeland 2015). Other studies have reviewed the process of gelatinisation due to its central role in food processing (Hoover 2000; Ratnayake and Jackson 2008; Wang and Copeland 2013; Wang et al. 2017). Hydration mobilises

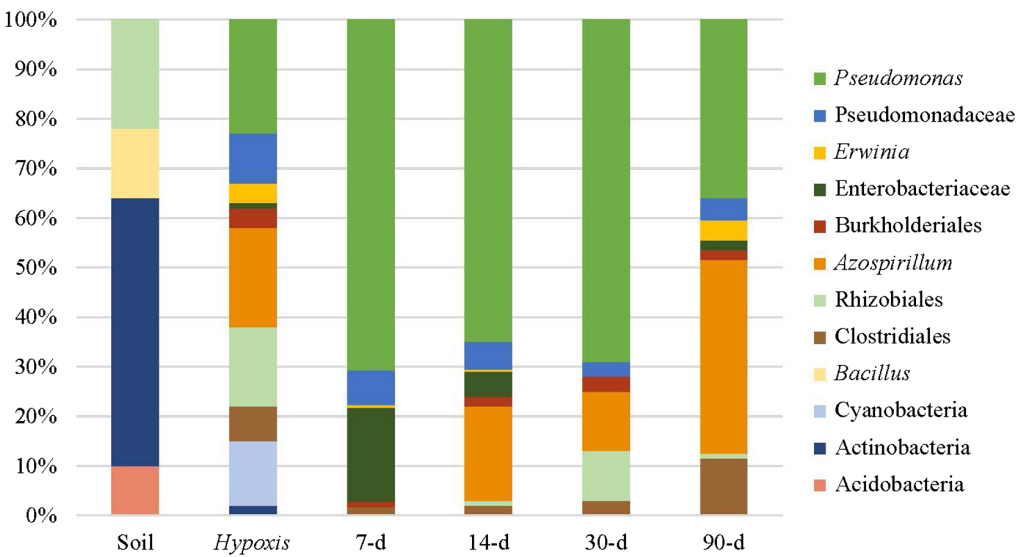
glucose and makes starch highly reactive to molecular degradation and susceptible to consumption by microbiota demonstrating that access to the glucosidic bonds inside the granules is the key to preservation. Mobility and activity of hydrolytic enzymes are limited under low water and temperature conditions, as thermal states permitting gelatinisation allow water molecule diffusion into amylose and amylopectin chains, whereas temperatures below gelatinisation retard water ingress. Water, however, can still move inside granules at low temperatures, for example, in a process known as annealing (Jacobs and Delcour 1998).

Much has been written about archaeological starch granules existing in the sheltered niche of a microscopic artefact crevice (Piperno et al. 2000; Barton and Matthews 2006; Pearsall 2015). This scenario, however, has weak empirical support. Many groups of microorganisms inhabit rock surfaces and form lithobiontic coatings (Dorn 1998), which further support colonisation of the stone's surface, fractures, and pores by bacteria, fungi, and lichen. Many alpha-amylases are exoenzymes (i.e., they are exported out of the cell and work outside the cell). These are much smaller than cells. The smallest known bacteria are 200 nm in diameter (Luef et al. 2015), whereas alpha-amylases range from 40–160 kDa, equivalent to approximately 4–8 nm in diameter (Mehta and Satyanarayana 2016). Therefore, only the enzyme would need to enter a crevice for colonisation, down to a scale of hundreds of nanometers. Moreover, organic material and water commonly co-occur in stone crevices, further accumulating and attracting fungal and bacterial communities to interstitial spaces and microfractures (Golubic and Schneider 2003). Therefore, crevices alone are unlikely to offer protection to starch from hydration or decay, and could, in fact, promote starch degradation.

Research surrounding starch resistance to degradation has seldom been at the forefront of soil and sedimentary analysis. The persistence of carbohydrates in soils, sediments, and surfaces may be due to enzymatic inaccessibility (Cheshire et al. 1969; Cheshire 1977; Dungait et al. 2012). Salts may affect gelatinisation (Gough and Pybus 1973; Jane 1993; Zhu et al. 2009; García-Díaz et al. 2016; Wang et al. 2017), with starch granules close to mineral surfaces possibly having reduced susceptibility to gelatinisation. The chemical and enzymatic lability of organic matter in soils is related to temperature, mineral surface area, encapsulation, heterogeneity, and oxygen (Baldock and Skjemstad 2000; Lützow et al. 2006; Schmidt et al. 2011). Furthermore, laboratory studies show that the rate of enzymatic degradation of starch is affected by molecular organisation (Tester et al. 2006), hydration (Kayisu and Hood 1979), the optimum pH for bacterial and fungal amylases (Pandey et al. 2000; Saranraj and Stella 2013), the presence of Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> salts that impact amylase production (Gupta et al. 2003), and retrogradation. Physico-chemical differences between starches, including variable crystallography, may be another key to understanding the variance in the survivability of the starch polymer (Park et al. 2007).

## Microbial degradation cycles

Despite the archaeological emphasis in studying the geochemical conditions for starch degradation in soils and sediments, little is known about the tempo and mode of microbial decay of starch (Adu and Oades 1978) and the possibility of biofilm-mediated mineralisation (Pacton et al. 2007). Observations over the course of several months, using a diversity of matrices over a range of pH conditions, confirmed the microbial degradation of starch in cycles, where the microbiome clades in the model system dominated hydrolysis at the expense of other clades from the soil itself (P. Dunfield, J. Kim, J. Mercader, personal observation, 2017; Fig. 1, Table 1). This initial observation prompted further experimentation by PD, TA, and JM, whereby soil microbiota under various moisture levels and oxygen conditions hydrolyse different starches. These microcosms are being sampled at regular intervals to study the microbial metabolic activity through gas chromatography, identify the presence or absence of various microbial clades involved in starch degradation through DNA analysis, and record the morphological changes that bacteria trigger in starch granules during alteration under the microscope.



**Fig. 1.** Hydrolysis of starch granules by bacterial and fungal attack. Artificial “soil” composed of nanospheres (clay-/silt-/sand-sized) formed a substrate inoculated with starch from the African potato (*Hypoxis* spp.) in a saturated water environment, progressively drying over several months. The microbial communities that existed in the natural soil and those that form the plant’s own microbiome were profiled through Illumina sequencing (Illumina, San Diego, California, USA), noticing that *Pseudomonas* took over degradation, and that this clade was not detected in the surrounding soil. At 90 d this microbial clade gives way to *Azospirillum*. The pH of the African soil was 6.1, and the pH of the plant itself was 5.3. The pH in the experimental setup changed over time and ranged from 4.9 to 7.

**Table 1.** Substrate, pH, and inoculum variables for experimentation with the model organism, the underground storage organ from *Hypoxis* spp.

<i>Hypoxis</i> sample mass (g)	Experiment type	Substratum	pH	Inoculum
0.3	Single variable	Nanospheres + clay (autoclaved)	5	<i>Trichoderma</i>
0.3	Single variable	Nanospheres + clay (autoclaved)	7	<i>Bacillus</i>
0.3	Single variable	Nanospheres + clay (autoclaved)	5	African soil mixed medium
0.3	Single variable	Nanospheres + clay (autoclaved)	7	African soil mixed medium
0.3	Single variable	Nanospheres + clay (autoclaved)	10	African soil mixed medium
0.3	Control	African soil (natural)	n/a	n/a
0.3	Control	African soil (autoclaved)	n/a	African soil mixed medium
0.3	Control	Nanospheres + clay (autoclaved)	n/a	n/a

A promising model to distinguish the effects of microbial activity on starch granules is found in studies of modern soil surfaces (Haslam 2004; Hutschenreuther et al. 2017; Mercader et al. 2017), where scientists can study and quantify the effects of soil microbes on starch granule shape, size, surface texture, and crystallinity in vivo. Bacterial “preference” for starches from certain plant taxa suggests that the differential survivability of starches is likely to bias archaeological starch records (Hutschenreuther et al. 2017). A recent study of Tanzanian soil surfaces (Mercader et al. 2017)

confirms the impact microbial degradation has on starch architecture in short periods, with granule populations displaying pitting, clefting, slitting, implosion of the hilum, fissuring, and most importantly, pervasive crystallite disruption that results in partial or complete loss of granule birefringence under cross-polarised light. These modifications present us with a problem because the changes inflicted on the granule by natural damage, especially over thousands of years, can overlap with those caused by culinary modification (Henry et al. 2010; Collins and Copeland 2011; Crowther 2012).

## Silicification

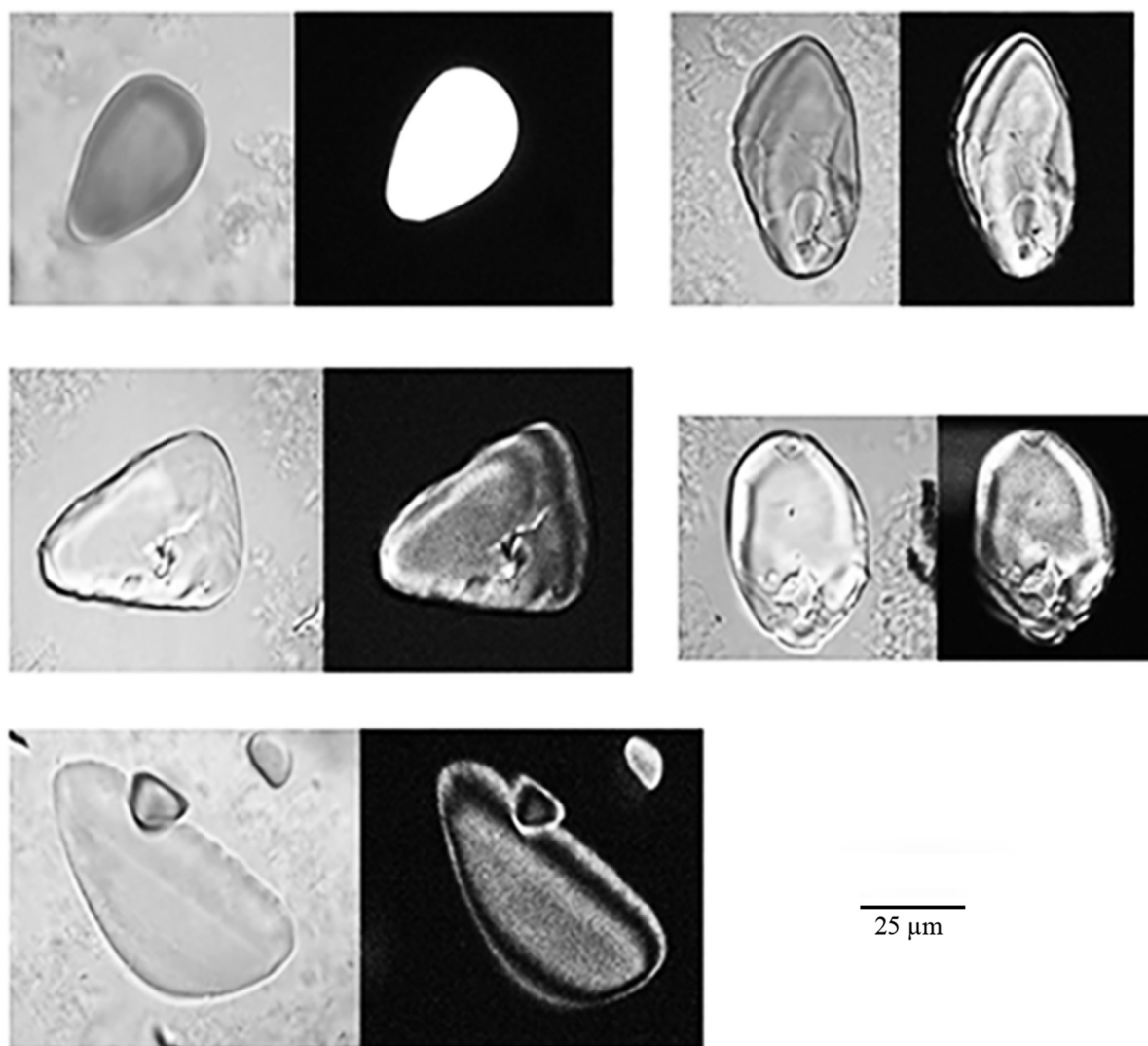
The term “microfossil” has pervaded the literature of ancient starch research, even though there is no evidence that any archaeological starch granule reported in the literature is actually fossilised, unlike palaeontological analogs (Baxter 1964; Wilkinson 1983; Locatelli 2014). The term “exceptional preservation” refers to a fossilisation mode that preserves fragile structures but not their original biochemical features. However, even in cases where exceptional preservation is invoked, any presumption that a microfossil is in its original, unaltered biological form needs to be testable given the generally improbable nature of biological molecule preservation over long time scales (Schweitzer 2011). Normally, ancient and modern molecular counterparts display significant architectural and elemental differences (Gupta and Briggs 2011). During burial, the shielding of labile molecules from complete destruction can take place through aliphatic structural enrichment in the preserved organic matter, a product of diagenesis and kerogen formation (Cody et al. 2011), which results in hydrophobicity (Gupta and Pancost 2004) and chemically resistant, neoformed geo-macromolecules (Gupta et al. 2009).

Fossilisation of starch granules is feasible through the silicification of materials adhered to surfaces when, after burial, they could be permeated by interstitial waters rich in solute minerals that precipitate during wetting and drying cycles. Silicified macrobotanicals are common at famous archaeological localities, such as Olduvai Gorge (Tanzania) (Bamford 2012), and provide independent evidence of silicification as an active fossil forming process in burial environments of interest to archaeologists. Decades of experimental fossilisation studies (Drum 1968; Briggs and Kear 1993; Channing and Edwards 2004; Townson et al. 2014) demonstrate that plant parts permeated by silica-rich solutions undergo fast silicification by the deposition of opaline silica on polysaccharide surfaces and within voids, ideal for mineral nucleation sites (cf. Kealhofer et al. 1999; Smith and Stockey 2002; Butts and Briggs 2011). Under controlled laboratory conditions, authigenic mineralisation of potato, wheat, and corn starch occur by percolating them with sodium silicate, magnesium silicate (Wollast et al. 1968), or colloidal silica solutions (Oehler and Schopf 1971) under different water activity, heat, and pressure regimes over the course of several weeks produced variable silicification modes (J. Mercader, M. Soto, J. Inwood, personal observation, 2018; Fig. 2). Often, the resulting mineralisations have fully polymerised with silica and have disrupted the native amylopectin crystallites, as shown by neoformed crystallisations with strong 1st and 2nd order birefringence and complete disappearance of the “Maltese cross”. These efforts suggest that starch granule morphology can be preserved through such mineral deposition processes.

## Phosphatisation of starch granules in dental plaque

There remain many unsolved questions surrounding the entrapment, co-precipitation, cementation, coating, and mineralisation of starch granules in dental calculus, which is a biogenic calcium phosphate precipitate that often contains microbotanical remains (e.g., Armitage 1975; Fox et al. 1996; Hayashizaki et al. 2008; Hardy et al. 2009; Lee et al. 2013; Power et al. 2014). To understand the potential for the survivability of starch granules from plaque to various aggressors we observed the co-precipitation of calcium phosphate and starch to study artificial calculus under controlled conditions. By replicating calculus formation, we discovered that potato starches can endure high levels of oral amylase and appear to be shielded from the gelatinising effect of sodium hydroxide (Fig. 3),



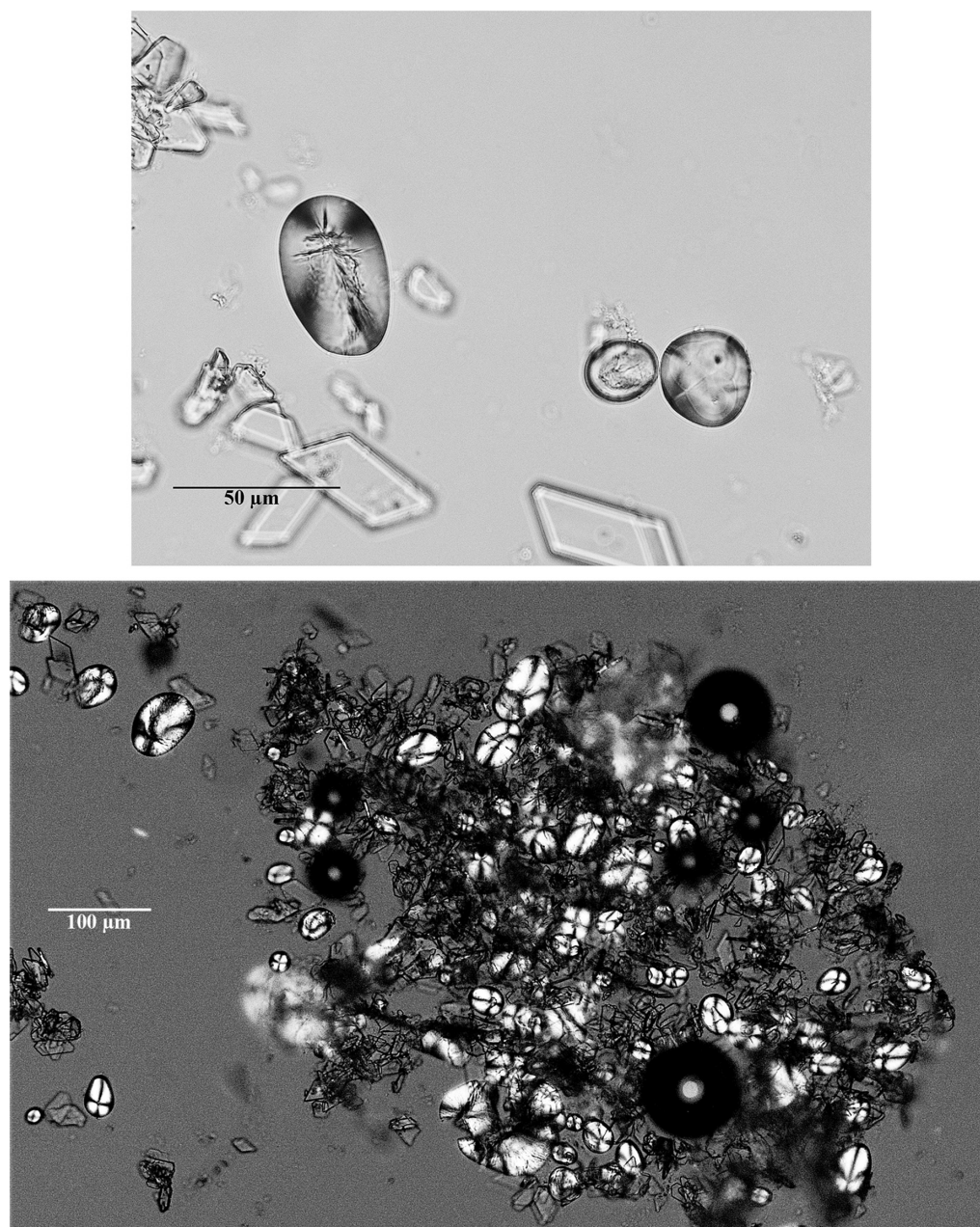


**Fig. 2.** Example of in vitro permineralisation of *Dioscorea praehensilis*. Early silicification modes are patchy, massive, powdery, granulate, and aggregate. Variably silicified casts preserving shape and size develop as early as 7 d and positively by 21 d. Resistance to gelatinising agents increased when the silicifying solution contained NaCl and NaHCO<sub>3</sub>. Some granules gelatinised because of high pH (9–10) but others recrystallised with strong birefringence shown through interference colors from 1<sup>st</sup> order yellowish-white to violet and indigo, in concentric rings. Note the complete disappearance of the Maltese cross.

presumably after mineral exchange with the phosphatising environment has permeated the starch granule surface. This indicates that entombment of organic matter in a calcium phosphate environment has the potential to transform, delay, or even completely suppress microbial action on organic remains (Briggs and Summons 2014). Ongoing studies to build model calculus onto communities of living oral microbiota will further elucidate the manner in which starches become entrapped and protected from amylases and other diagenetic agents.

### Starch breakdown and byproduct preservation

The chemical properties of edible starches, including attributes such as lipid, moisture and ash content, amylose:amylopectin ratio, crystallinity (as indicated by the X-ray diffraction pattern), and



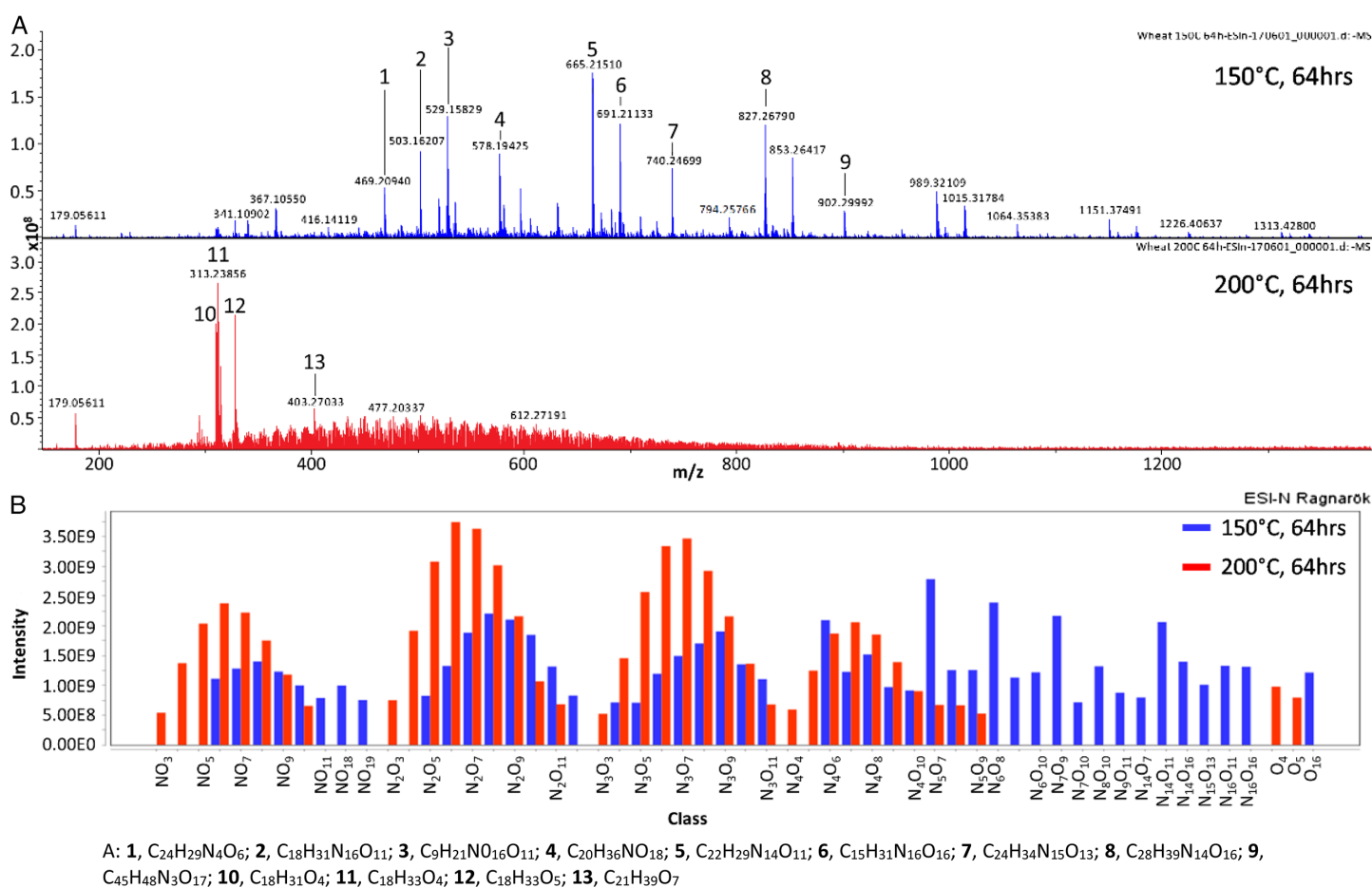
**Fig. 3.** Artificial calculus consisting of calcium phosphate (ammonium phosphate and sodium chloride at 5%) and potato starch granules (equivolume 5%); the starch granules were trapped as the crystals formed. We tested the resistance of these clusters to amylase activity. Amylase at 2% (500–1500 units per mg) has been confirmed to degrade starch granules in as short as 15 min. In the case of artificial calculus (pH 6.8–7), however, starch granules endured >100 h of amylase action (7% solution) at concentrations in the upper range recorded in saliva without changes. The preservation of full birefringence and the Maltese cross demonstrates that the native crystallite structure of amylopectin remained intact for both encapsulated granules and loose specimens. This enhanced protection from enzymatic disruption might have been facilitated by the permeation of calcium phosphate or the formation of nanometer-thick casts.

structural characteristics have been extensively documented ([Hoover 2000](#); [Ancona et al. 2011](#); [Olayinka et al. 2013](#)). To an archaeologist, this information is invaluable for understanding how these molecules react to the process of degradation in taphonomic contexts. It is imperative that these



characterisations be carried out on species of archaeological interest to identify differences in breakdown pathways, and to aid in biomarker identification and the potential neoformation of high molecular mass species (Gupta et al. 2007; Gupta 2015) or other signature recalcitrant products.

In addition to the enzymatic degradation of starch, we must consider non-enzymatic interactions between proteins and starches and their degradation products as a potential route to producing refractory species. The Maillard reaction, the abiotic reaction of amino acid and carbohydrate species, produces complex chemistries proposed as one of the routes of preservation of organic matter in soils and kerogens (Larter and Douglas 1980). This reaction, which occurs under ambient conditions and at high temperatures during cooking, produces a variety of high molecular mass pigmented products or “melanoidins” (Ajandouz and Puigserver 1999; Hill et al. 2005; Hwang et al. 2011). **Figure 4** shows the results of a Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) study. The model melanoidin system was generated by the reaction of glycine and wheat starch in water heated for 64 h at 150 °C and 200 °C, respectively. Although the reaction was carried out at unrealistic



elevated temperatures to promote rapid reaction, the analysis showed that a diverse range of cyclic and multiple heteroatoms are easily produced from simple chemical precursors (Erickson 2009). In addition, an increase in the temperature of the Maillard reaction shows a loss in higher mass species and reduction in the number of heteroatoms per molecule yielding to more recalcitrant products. Although the environmental kinetics and relevance of the so-called Maillard reaction under archaeological conditions are yet to be fully established, the ready conversion of biologically accessible species to more refractory ones suggests that this reaction is worthy of investigation as a route to preserving starch-related carbon.

## Authentication procedures

The notion that a semi-crystalline organic polymer such as starch could be preserved and remain undamaged for hundreds or thousands of years is contrary to chemical and taphonomic assumptions. Thus, claims of residual starch in deep time environments demand solid, multidisciplinary evidence. Authentication procedures should be multipronged (Box 2). Above, we have proposed several taphonomic pathways that may lead to a better understanding of how ancient starch may survive destruction. If mineralisation, chemical attack, microbial degradation, and recrystallisation leave a taphonomic imprint that, optically speaking, separates these preserved granules from their fresh counterparts, what is required in future research is additional analyses encompassing micromorphology, elemental composition, and molecular chemical structure to tease out compositional differences that may exist in putative ancient granules relative to reference materials.

Techniques that can achieve elemental and structural characterisation of discrete starch granule surfaces and microtomed specimens include:

- Raman and Fourier transform infrared spectroscopy,
- energy dispersive X-rays from scanning/transmission electron microscopy (SEM/TEM).

Raman spectroscopy can photo-bleach discreet target granules and therefore detect characteristic G and D bands from amorphous carbon. In addition, performing Raman spectroscopy on microtomed granules can illustrate vibration regions that highlight differences in molecular structure between native and fossil starches. SEM/TEM can reveal features commonly seen in starch such as damaged blocklets and endocorrosion pits (Pérez et al. 2009) as well as hydrolysed shells with channels (Huang et al. 2014). The melting of crystallites and recrystallisation microstructures can also be detected in a granule's cross section by SEM.

Atomic force microscopy is widely used as a technique to probe the surface of starch granules (Gallant et al. 1997; Baldwin et al. 1998; Dang and Copeland 2003). However, degradation products may pose

### Box 2. Authentication traits and procedures.

- Microstructure
- Molecular architecture
- Elemental composition
- Position specific isotopes
- Excavation in decontaminated spaces
- Lab processing of samples in cleanrooms

challenges for effective analysis, as the resolution of this technique requires that samples be extremely clean and free from coatings. It is also worth exploring the new generation of high-resolution coupled imaging and mass spectrometry tools such as secondary ion mass spectrometry (SIMS).

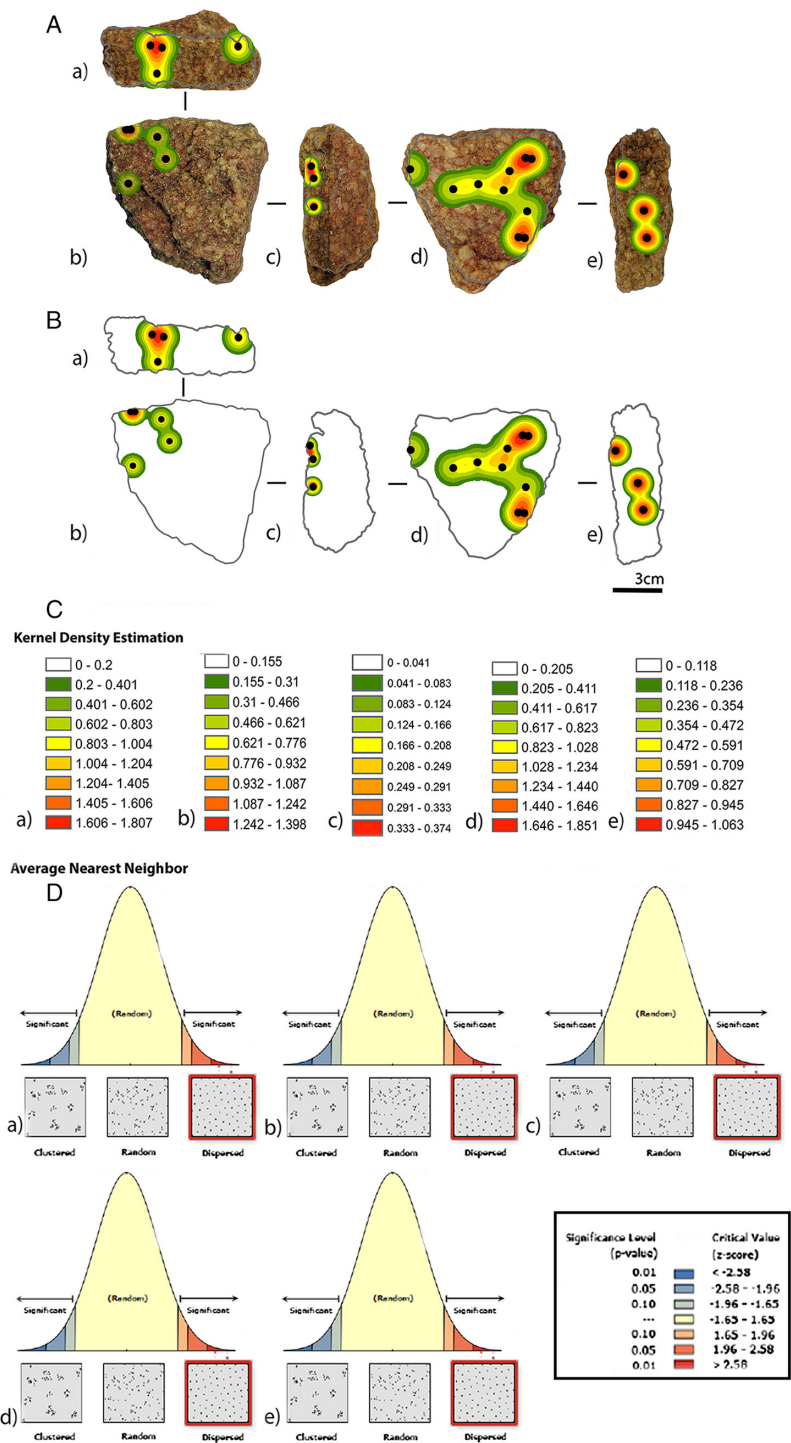
It remains problematic to excavate archaeological artefacts under field conditions that cannot assure the systematic avoidance of contamination (Laurence et al. 2011). Mercader et al. (2017) recommended that field archaeologists characterise the starch contamination landscape that is specific to their study area and utilise dedicated excavation tools that can be cleaned frequently with a solvent or starch gelatinising agent. It can be beneficial to wear disposable clean attire in the excavation area, isolate excavation areas from excessive traffic, and use gloves and sample bags (confirmed to be starch-free) for contact with artefacts. Once retrieval and storage compromise samples, it can be challenging to separate target and contaminant starches. Commonly used decontaminating methods such as airbrushing by compressed air and mild washing (Barton and Torrence 2015) can fail to remove recent intrusions even after several ultrasonic cleaning cycles (Pedergrana et al. 2016; Cnats and Rots 2017; Mercader et al. 2017).

Regarding ancient contamination, functional analysis via use-wear studies and spatial sampling of residues have traditionally buttressed the interpretation of starch granules as genuinely related to ancient utilisation, if it has been demonstrated that the location of the starches was along the used edges of archaeological tools. Unfortunately, recent work has also called this link into question (Rots et al. 2016), as the location of residues does not necessarily indicate that they are use-related (Xhaufclair et al. 2017) or contamination distributed along edges (Pedergrana and Ollé 2017). New experimental research (J. Mercader, M. Soto, personal observation, 2018) is focusing on detecting patterns of microbotanical attachment in natural rocks (lying on modern surfaces and in contact with surrounding soils) to build a baseline whereby residue density estimation by geographic information system (GIS) and nearest neighbour heat maps of plant materials adhering to rock surfaces can be compared with residue scatters from stone tools. Emerging results indicate that naturally attached plant matter consistently presents a dispersed pattern (Fig. 5).

Anti-contamination laboratory protocols remain underdeveloped, under-utilised, and under-reported (Crowther et al. 2014), prompting immediate attention. Contaminant starches are a threat and subpar testing techniques for environmental contamination (e.g., small numbers of passive traps) severely underestimate the magnitude of the problem. It should not be assumed that consumables are starch-free, even when laboratory suppliers state so. The potential for unfiltered, recirculated air to propel large numbers of starch particles around a lab volume has been confirmed (Crowther et al. 2014). It is worth re-evaluating whether archaeological assemblages consisting of small numbers of starch granules could be a false positive. Importantly, this concern about the integrity of the evidence pertains to assemblages derived from all kinds of archaeological materials, including stone tools, dental calculus, sediments, and pottery surfaces.

## Conclusions

Although ancient starch researchers and archaeological residue analysts have reflected on the extraordinary biological, geological, and chemical circumstances that must have occurred if such a fragile biopolymer were to survive on a millennial scale (Barton 2009; Henry 2015), there has been little progress toward characterising starchy materials compositionally or directly investigating the taphonomic and diagenetic challenges for the persistence of starch granules chemically and morphologically. We need a new biogeochemical paradigm (Box 3) to understand changes in deposited starch over time that addresses poorly understood processes of degradation, recrystallisation, and (or) permineralisation. The morphological preservation of a granular structure does not imply the chemical conservation of starch. We recognise starch through imaging techniques because of its



**Fig. 5.** There are natural reasons why starchy materials may adhere to stone tools before burial and during entombment. The plotting of adhered residues on natural rocks provides a baseline to discriminate anthropogenic from natural agency (J. Mercader, M. Soto, personal observation, 2018). (A) and (B) look at the abundance of naturally occurring residue assemblages relative to surface area. These are the bases for statistical analysis through kernel density estimation. (C) is the key to interpret the accumulations plotted per cm<sup>2</sup>. (D) shows the results of the nearest neighbour analysis in which distances are averaged for each plotted residue to its nearest occurrence, whereas the nearest neighbour index is the mean distance divided by the mean distance expected for clustered, random, and dispersed modes, thus calculating the significance level. In the example presented here, all sides of the sample (a)–(e) supported a highly significant dispersed pattern of residue accumulation where there is <1% likelihood that this could be the result of just random chance.



### Box 3. Examples of ongoing efforts.

- Research to assess the impact of microorganisms on starch hydrolysis (Fig. 1)
- Research to understand the silicification of starches in silica-rich environments (Fig. 2)
- Research to examine mineral transfer between starches and artificial dental plaque (Fig. 3)
- Research to evaluate the production and survivability of compound classes in a Maillard reaction of starch and amino acids (Fig. 4)
- Research to investigate the pattern of residue adherence to natural rocks to distinguish them from anthropogenic residues (Fig. 5).

crystallinity. After degradation, how do we detect its altered states if not through optical behaviour? Future work must consider growing demands from readers, editors, and reviewers that look for chemical identification of presumed starch granules and, thus, the need for verified chemical characterisation techniques to secure reliable molecular fingerprints from optically targeted sample sites. Experimentation and theoretical modelling from chemical computation (Damm et al. 1997; Molinero and Goddard 2004; Limbach and Kremer 2006; Fadda and Woods 2010) could help us understand starch degradation over thousands of years. New limits can be explored, and multidisciplinary collaboration with biology, geoscience, and chemistry can help deliver a set of best practices in which data acquisition, authentication, analysis, reporting, and data sharing could lead to realising the full potential (Fenn and Raskino 2008) of a controversial archaeological technique that still holds great promise to shed light on myriad aspects of the past.

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## Author contributions

JM, TA, MB, MB, MJC, LC, AC, PD, AH, JI, MI, J-JK, SL, LL, TO, RP, RS, MS, RT, and HX conceived and designed the study. JM, TA, MB, MB, MJC, LC, AC, PD, AH, JI, MI, J-JK, SL, LL, TO, RP, RS, MS, RT, and HX performed the experiments/collected the data. JM, TA, MB, MB, MJC, LC, AC, PD, AH, JI, MI, J-JK, SL, LL, TO, RP, RS, MS, RT, and HX analyzed and interpreted the data. JM, PD, SL, RS, and RT contributed resources. JM, TA, MB, MB, MJC, LC, AC, PD, AH, JI, MI, J-JK, SL, LL, TO, RP, RS, MS, RT, and HX drafted or revised the manuscript.

## Competing interests

JM is currently serving as a Subject Editor for FACETS, but was not involved in review or editorial decisions regarding this manuscript.

## Data accessibility statement

All relevant data are within the paper.

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