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Starch granules, dental calculus and new perspectives on ancient diet

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ABSTRACT

Recent work in various parts of the world has suggested the possibility of ancient starch granules surviving and adhering to archaeological artefacts. Often this information is used to infer aspects of diet. One additional source for recovery of archaeological starch granules is dental calculus. The presence of plant food debris in dental calculus is well known but has not been not widely investigated using archaeological material. The extraction of starch granules from dental calculus represents a direct link to the consumption of starchy food by humans or animals. Using dental calculus also sidesteps many other questions still inherent in using starch granules to reconstruct aspects of ancient diet, such as the effects of diagenesis on their morphology; as the starches are trapped inside a concreted matrix they are less likely to alter over time. We used amylase digestion by a starch-specific enzyme to confirm the material as starch.

Starch-based foods constitute 50-70% of the energy intake of most humans today (Atkins and Bowler, 2001). Evidence in the form of a combination of archaeological remains and ethnographic records suggests that starchy food also had an important role in pre-agricultural human diet. But evidence for starchy foods such as tubers, roots and seeds can be difficult to find on some archaeological sites and the recovery and analysis of starch granules found in archaeological contexts have shed new light on far reaching questions surrounding plant use and domestication (e.g. Aranguren et al., 2007; Fullagar, 2006; Horrocks and Nunn, 2007; Perry et al., 2006; Piperno et al., 2000, 2004; Samuel, 1996; Van Peer et al., 2003).

Starches used for archaeological studies are extracted from residual material that is found in places such as adhering to the edges of flaked stone tools; as material that has accumulated in the pores of the coarse granular structure of stones used for grinding; inside pots and in sediment (Hardy, 2008a; Iriarte et al., 2004; Perry et al., 2006; Piperno et al., 2000, 2004; Van Peer et al., 2003). But this assumes the function of certain tool types; for example grinding tools are assumed to have been used for food preparation. While this is very probable in many cases, Baysal and Wright (2006) have demonstrated that grinding tools may also have other non-food-related uses. Similarly, with regard to flaked stone tools, while some of these may have been used for collection and processing of food, they had many other uses notably in relation to raw material preparation (Hardy, in press-a; Hardy and Sillitoe, 2003).

The use of dental calculus to extract starch granules for dietary reconstruction offers a direct link to consumption. The presence of plant food debris in archaeological dental calculus has been known about for some time (Cummings and Magennis, 1997; Dobney and Brothwell, 1986, 1988; Gobetz and Bozarth, 2001; Hardy, in press-b; Lieverse, 1999; Lilley et al., 1994) but has not been not widely investigated. Assuming that things recovered from the mouth are likely to have been consumed, (though there are exceptions, for example inner bast fibres can be chewed to soften them, Hardy, 2008b), the extraction of starch granules from dental calculus represents a direct link to the consumption of starchy food by humans or animals.

Edible starchy plants can be found in most environments, for example over 60 indigenous edible starchy plant species can be found in Britain alone. Starch is a reserve polysaccharide of plants, an end product of carbon fixation by photosynthesis. It is present in most green plants and can be found in every type of plant tissue including leaves, stems, roots fruit, seeds and even pollen grains. It is the major carbohydrate and energy reserve in seeds and plant tubers. Most cereal starch is located in the endosperm which is the central and largest part of the grain, while starch granules are the dominant component of tuberous root crops such as potatoes.

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Starch is composed of a mixture of two glucose polymers – amylose and amylopectin – which together form discrete and morphologically identifiable granules (Evers et al., 2004; Zobel, 1997). Starch has a very low osmotic pressure and this provides plants with a way of storing large reserves of glucose without influencing water relations within their cells. Starch granules are relatively stable structures, but they are also able to be readily decomposed to water-soluble sugars by digestive enzymes (Radley, 1968).

Though starch is normally susceptible to enzymatic attack, it is semicrystalline and can survive for long periods of time in a stable environment, for example in micro-cracks in flaked and ground stone tools, within fragments of plant cell walls or within dental calculus.

1. Evidence of pre-Neolithic plant consumption

Understanding ancient diet is a key component of studies into early prehistory even though there is often little direct surviving evidence. Pre-Neolithic diet is studied using different techniques including counts of bones and shellfish, stable isotope analysis, assessments of calorific values and residues in pots, but with a few exceptions (Clark, 1976; Zvelebil, 1994), reconstructions of pre-Neolithic diet tend to be based largely around meat or seafood with little attention paid to plant foods except hazelnuts. However, there is extensive evidence for starchy plants on pre-agricultural sites in Europe and the Near East (Aura et al., 2005; Hardy, 2007; Moore et al., 2000; Weiss et al., 2004).

We need plants to live (Hladik and Pasquet, 2002). Apart from the vitamins and minerals that plants provide, starch is a macronutrient and an important source of carbohydrate for dietary energy. Starch occurs as granules which are broken down into glucose in the digestive system. Today dietary starch comes mainly from foods such as bread, pasta, potatoes and rice. While the proportion of starchy foods may not have been as high in a hunter gatherer society, a completely starch free diet is also unlikely.

Humans have a basic metabolic need for high energy carbohydrates and it is very likely that starchy plants were important possibly even as far back in evolutionary time as the ability to exploit underground storage organs may be linked to the Last Common Ancestor's (LCA) ability to survive in savannah environments (Laden and Wrangham, 2005). Though a reduction in starch intake is advocated in some modern diets, the long-term implications of an absence of starch in the diet may be negative, while the amount of carbohydrate from animal tissue is too small to be considered quantitatively important (Garrow et al., 2000).

One human group that has managed to survive with a low carbohydrate input is the high Arctic population as their traditional diet was based almost exclusively on fish and seal products. They would have replaced carbohydrates as their predominant dietary energy source with protein and fat. The negative physical consequences of this diet, such as excessive urea production necessitating a high intake of water, mean that it is unlikely that this would have been followed unless there was no alternative. However the incorporation of large amounts of fat in the diet does suggest a way in which people could survive in periglacial environments where few plants might have been available.

Ethnographic evidence indicates almost every known hunter gatherer group included plants in their diet. Percentages of plant consumption for hunter gatherers range from 6–15% at high latitudes to 45–55% in tropical grasslands and desert scrub (Cordain et al., 2000) (Fig 1). Additionally, there is a small but significant number of pre-Neolithic sites where charred remains of edible tubers, seeds and legumes have been found (e.g. Aura et al., 2005; Clark, 1954; Kubiak-Martens, 1996, 1999, 2002; Mason and Hather, 2000; Moore et al., 2000).

Fig. 1. Processing wild seeds Central Australia.

The domestication of plants and cultivation of crops is the control and management of starch sources. Examples include cereal grains in the Near East, rice and millet in East Asia, maize and potatoes in the Americas and underground plant storage tissues in SE Asia. The fact that starch-based foods were adopted so widely across the world as a primary food source suggests that human consumption of starch has a very long heritage.

2. Ancient starch analysis

Analysis of ancient plants from the remains of starch granules has been expanding rapidly as a research area in archaeology, but a number of outstanding issues remain.

2.1. Starch identification

Starch granules from archaeological contexts are identified on the basis of the size and shape of granules, through the use of stains (Torrence and Barton, 2006) and by the birefringent Maltese cross pattern which is clearly visible under the light microscope in polarized light. Birefringence is a characteristic commonly found in materials with crystalline layers arranged in a concentric pattern. Together these tests give a good indication of whether a material is indeed starch, but none is sufficiently specific to confirm it.

The only unequivocal test for starch is to degrade it with an alpha amylase, an enzyme that is specific for the chemical linkages contained in starch and that uniquely degrades starch. Pure preparations of this enzyme may be obtained commercially; the currently favoured source for starch analysis is from *Bacillus licheniformis* (see Section 3).

2.2. Cooking and gelatinization

Starch is insoluble in cold water but when heat is applied the granules go through a process called gelatinization. Temperatures of gelatinization vary but gelatinization does not normally occur below 60 °C. The rate and extent of gelatinization is influenced by the application of shear forces (mixing, stirring) with heating. Gelatinization results in the disruption of molecular order, leading to granular swelling and loss of birefringence caused by disruption of the semicrystalline structure. Retrogradation, the process in which the starch chains begin to reassociate in an ordered structure, can occur once the gelatinized starch cools. This can result in a starch