Survival, Extraction and Identification of Starch Granules at Kaman-Kalehöyük, Turkey

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INTRODUCTION

Starch-based foods today constitute about one third of the global dietary food intake. Evidence in the form of a combination of archaeological remains and ethnographic records suggests that starchy food also had an important role in past human diet. But evidence for starchy foods such as tubers, roots and seeds, can be difficult to find on archaeological sites. Recent work in various parts of the world has suggested the possibility of starch granules surviving in archaeological contexts (e.g. Fullagar 2006; Perry *et al*. 2006; Piperno *et al*. 2004, 2000; Samuel 1996; Van Peer *et al.* 2003).

Starch is the major carbohydrate and energy reserve in seeds and plant tubers where it is usually found as granules. 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. Starch is composed of a mixture of two polymers- amylose and amylopectin- which together form discrete granules (Evers *et al*. 2004; Radley 1968), their packaging promotes stability, but they are also able to be readily decomposed enzymatically to water soluble sugars.

Though starch is normally susceptible to enzymatic attack, it is an inherently stable molecule and can survive for long periods of time in a stable environment. For example, micro-cracks in flaked and ground stone tools afford protection for starch granules locked inside. Likewise, starch granules locked inside larger pieces of residual material such as fragments of cellulose are also protected. Starch also survives in dental calculus as the polysaccharides in dental plaque appear to protect it from the salivary amylase that would normally break it down.

The potential for starch granule analysis to answer far reaching archaeological questions related to plant use has been demonstrated in many high profile publications (*e.g.* Perry *et al.* 2006; Piperno *et al.* 2004; Van Peer *et al*. 2003).

AIM

The aim of this preliminary assessment was to determine whether micro residues survive in the archaeological deposits at Kaman-Kalehöyük and what the potential for future more detailed study is. Sample collection reflected this aim; samples were taken from as many different context types as possible and no attempt at consistency was made, it was simply a question of taking a chance on what was there, seeing if residual material could be found and if so where.

FIELDWORK

Samples from a range of contexts were selected to examine the potential for residue survival here. Samples were placed in sealed bags or where rehydration was necessary to extract the samples they were placed in ependorff tubes and suspended in ethanol. Samples of dental calculus were also taken to explore the possibility of starch extraction from here.

NON STARCH RESIDUES

Phytoliths were observed in several samples.

STARCH

Samples were taken from a number of locations where residues were apparent or thought likely to occur. These included grinding stone surfaces, the insides of pots, mudbrick, pits and soil.

EXTRACTION METHOD FOR ALL RESIDUE EXCEPT DENTAL CALCULUS

A small amount (normally around 0.50-0.8 µls) of sample were placed in centrifuge tubes with 200 ml of the heavy density liquid Sodium Polytungstate, at a density of between 1.7 and 1.8. Starch has a density of 1.55 so this allowed for a wide margin of error in order to lose none of the samples. Samples were centrifuged at 1000 r.p.m for 15 minutes. The starch was then extracted from the surface and washed three times in ultra pure water then twice more in acetone. Samples were then dried. For rehydration and storage, tiny amounts of 70% ethanol were placed into each sample container. Samples were placed on microscope slides and mounted in Karo Corn syrup for optical observation.

An optical microscope (Meiji) was used with crossed polarized light to identify the presence and absence of starch their overall morphology, size and distribution. Slide scanning was carried out using a x20 objective while more detailed analysis was undertaken at x40. Once all samples had been examined and consistent morphological types had been identified, certain samples were selected for more detailed study and photography. This was carried out using a Zeiss confocal microscope. All photography and detailed analysis were carried out using a x63 oil immersion lens and all photography was undertaken using DIC (Differential Incident Contrast).

DENTAL CALCULUS

Starch was found in almost all samples of dental calculus. Dental calculus is made up of calcium phosphates which are deposited in plaque as salts. If plaque is not cleaned off teeth, this mineralizes and turns into calculus. As calculus is mineralized, it can survive for extended periods of time.

Saliva differs across species in its mineral and chemical components. Even among different human groups the plaque and calculus structure can differ at the mineral and microbial levels. It is not clear to what extent differences in calculus among human groups is dependent on genetic or dietary difference. However, it does not appear that these differences have an effect on the survival of starch in calculus. Calculus is common among non industrialized communities in the past probably due to the less extensive microbial communities which are linked to a low consumption of refined sugars.

Dental calculus can be found either in the supra gingival area, that is above the gum line, or in the sub gingival area, below the gum line in the gingival crevice. The area of the gingival crevice is protected to a large extent from salivary amylase and may well therefore form an area of preferential survival of starch particularly as microbial communities here are proteolitic rather than sacrolitic (that is they break down proteins more readily than sugars).

Dental calculus builds up at different rates in different individuals; therefore an absence of calculus does not necessarily indicate a different diet. However, the causes of individual difference in calculus build up is not well understood though it is thought to be genetically based and linked to different ways of interacting with microbes.

Proteins and amino acids may also survive in dental calculus.

EXTRACTION METHOD

As little work has been undertaken to extract starch from dental calculus, no clear method has been developed. Preliminary tests were undertaken with modern starch samples, using Hydrchloric Acid and also EDTA (ethylenediaminetetraacetic acid) to dissolve the calculus. Both methods proved to be equally effective. As HCl is a much faster method to dissolve calculus, this was selected.

Small amounts of calculus from each sample were placed inside tubes with 20mls of 0.6%M solution of HCl and rotated continuously at 4deg C. for 3-5 days.

Samples were then vortexed and centrifuged at 3000r.p.m for 15 minutes, and washed in ultra pure water three times. Samples were placed on microscope slides and mounted in Karo Corn syrup for optical observation.

RESULTS

Starches vary considerably in their shape and size (Figures 1 and 2). Consequently, it is possible through a combination of optical and scanning electron microscopy to determine the origins of the starches to genus level.

A range of morphologically different starches was identified at Kaman-Kalehöyük though the significance of these, in terms of different plant types, needs to be explored further. Starches were placed in preliminary groups based on their gross morphology. The aim of this was to determine whether preliminary indications of differential presence or absence could be identified based either on the location of the finds in the site or of the types of finds from where the starch samples were taken. Starches were split into six groups:

Type 1 – 10 – 15 µm hexagonal or square shaped
Type 2 – 20 – 25 µm elongated
Type 3 – 40 µm and over elongated
Type 4 – 20 – 30 µm round
Type 5 – Large and small starches (type A and B) together (some of the best known starches, these are characteristic of certain types of cereal such as wheat)
Type 6 – clouds of very small granules (1-5 µm).

Though types 5 and 6 were present they were recognised infrequently.

Starch was found in most contexts (Table 1). It is interesting to note that the samples from mud-brick and unused surfaces of grinding stones contained the least abundant samples of starch while samples from pits and the insides of pots had the most abundant populations. No link was made between relative abundances of starches and different areas or stratigraphic levels at the site.

With regards to an interpretation of starches, type 1 is common almost everywhere. It is therefore likely that this is an environmental indicator rather than anything to do with patterns of food consumption.

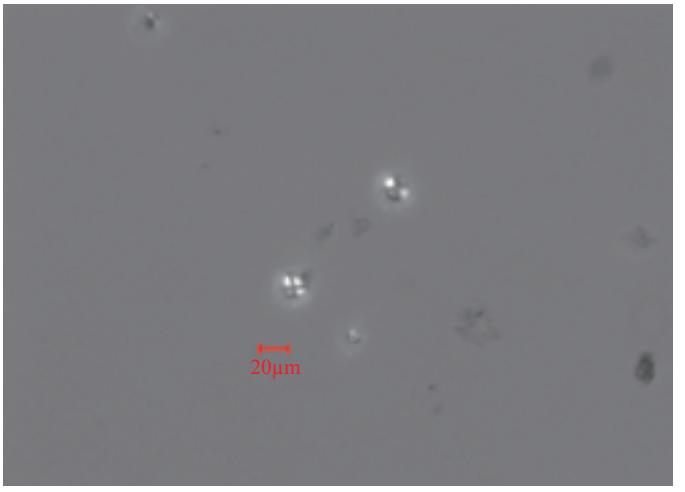


Fig.1 Type 1 starches (ST 048 scale bar = 20 microns)

The most significant differences in starch populations occur in the pots where there is a very high number of type 4 starches and in the human dental calculus which contained a very low number of all starches except type 3.

Dental calculus samples contained around 90% of the whole type 3 population. Type 3 starch is a large elongated starch. Examination by starch biochemists have identified type 3 starch as being most likely from a type of tuber, currently unknown. Though this is a very preliminary result, it does suggest that Starches found in dental calculus may offer a more direct link to consumption.

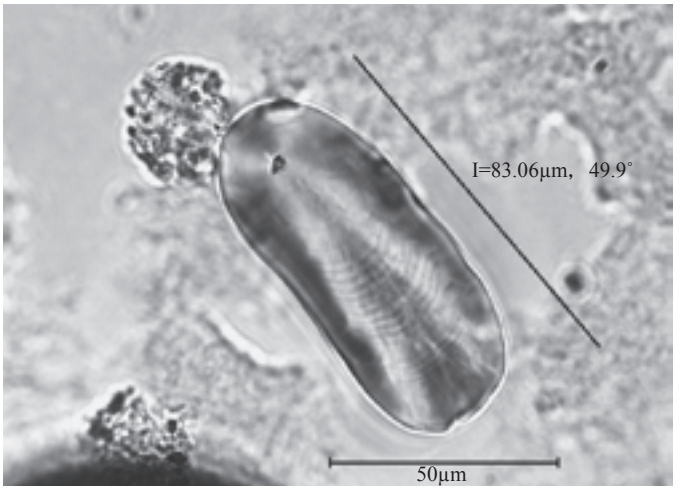


Fig.2 Type 3 starch. (ST 052 scale bar = 50 microns)

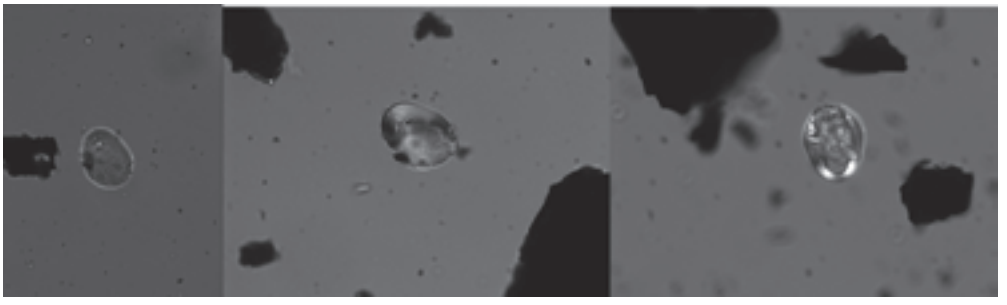
ENZYMATIC DEGRADATION OF STACHES

Starch in archaeological samples is usually identified using a range of morphological criteria however as the survival of starch in archaeological deposits is not well understood, further tests to confirm the identity of starch as starch is necessary. To confirm the identification of starch, a sample was subjected to a thermostable alpha amylase test.

Two samples of starch from Kaman-Kalehöyük were selected and a small amount of the alpha –amylase enzyme, uniquely destructive to starch, was injected onto a microscope slide containing this starch. The sample was then heated to encourage the amylase to become active. The degradation of the starches was then recorded photographically.

DISCUSSION

Starch survives in the deposits of Kaman-Kalehöyük. Starch survival into archaeological time is still not well understood. Though many plants produce starch and many of these are edible, it is not possible to be sure of their consumption simply because the starch is present on the archaeological site. In this study by comparing the starch that is present in dental calculus with that present in a range of contexts linked to living areas and food preparation items, a picture can be built up of which starches represent plants that were definitely eaten and which starches represent plants that may have been eaten or may have been used in a different capacity. One starch type (type 3) is predominant in the dental calculus samples and not common elsewhere. Its shape suggests it is a tuber of some sort.



Sample number 06000654. (ST003) Grinding stone.

Table 1 Location and abundance of starch granules according to sample source

Source	Total no of samples	No of samples containing starch	Type 1 starch (>10 per sample)	Type 2 starch (no of samples)	Type 3 (actual nos.)	Type 4 (actual nos.)
Grinding stone	4	3	0	1		
Grinding stone soil	1	1	1 (over 100)			3
Mudbrick	3	2	0			
Pit	3	3	3	3		7
Pot	8	6	5	3	1	26
Soil inside pot	3	3	1			1
Soil	2	2			1	
Human tooth calculus	18	17	1	1	25	4
Herbivore calculus	5	5	1	1	3	
White floor	1	1		1		2

FUTURE WORK

The ultimate aims of this study are to be able to reconstruct the component of starchy food in the diet of the Kaman-Kalehöyük inhabitants and also to link the starch evidence to other paleo-botanical information from the site to assist in environmental reconstruction.

A combination of optical properties and examination of starch using scanning electron microscopy (SEM) has been identified as the most secure method to reach identification to genus level. Currently no SEM work has been carried out on the starch samples from Kaman-Kalehöyük, this will be a future focus of research.

A preliminary impression of the likely origin of some starches can sometimes be gained without recourse to a detailed reference collection. For example large elongated starches are often linked to tubers, while bimodal starch populations (called type A and type B) are known to be uniquely identifiable as cereals. However more detailed identification of starches can only be conducted by comparison with known reference material. In order for this work to move to the next step of actual identification of genus types, a modern reference collection needs to be made of starchy plants likely to have been available to the inhabitants of Kaman-Kalehöyük. An investigation followed by collection of modern samples, based on modern ethnographic work, known plant origins and trajectories and paleo-botanical information from the site is required to create a reference collection of local available edible starchy food.

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