3. Background to the techniques

3.1. Introduction.

A high level of identification detail at the level of genus, species or food product is an implicit necessity for the investigation of cuisine. In addition, the breadth of food types across the range of plant and animal contributors is a must, if there is to be hope of interpreting or evidencing food *combinations* and traditions of vessel use. This chapter outlines and evaluates methods that exist to produce detailed datasets of past foods, based on investigations of the ceramic fabric and associated foodcrust.

Many potential methods exist across a range of sub-disciplines; some are well-established but rapidly developing such as those in lipid residue analysis, whilst plant microfossil analyses such as starch investigations are still emerging. As a result some of the lipid residue analyses such as Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Combustion-Isotope Ratio Mass Spectromemetry (GC-C-IRMS) have been applied very successfully to similar artefactual media, and come to represent a mainstay of modern residue analysis. In contrast there is potential for novel methodological developments in the field of plant microfossil analysis, especially in carbonised deposits. There is also a need for *combined* application of these techniques to archaeological problems. Collaborative strategies can hope to engage with very basic but important issues of cuisine such as the relative importance of plants versus animal foods which has eluded systematic investigation to date (Zvelebil, 1994).

Evaluation begins in Section 3.2 with methods for lipid residue analyses. Four areas are assessed: the potential for *identification* detail is very important, but the conditions of *preservation* to generate this necessary detail (subsection 3.2.1.), the risks of *contamination* to residues (subsection 3.2.2.), and the experimental *replication* of chemical signatures to understand mixing or re-use substantiate the identifications (subsection 3.2.4.). All four areas have witnessed developments in the strategies to manage or improve them, with interesting applications. Of all of them identification methods have been most rapidly developing and these are dealt with in detail in sub-section 3.2.3.

Despite significant strides in the resolution of food identifications, a weakness of lipid residue approaches has been the representation of plants in diet and foodways. These limitations are explained in 3.2.3.5. This deficit is significant for cuisine investigations and in response the potential of plant microfossil analysis is explored in Section 3.3. Plants host a variety of useable microfossils that vary in their taphonomic durability and taxonomic specificity. In subsections 3.3.1-3.3.5 phytoliths, starches, calcium oxalates, pollen and spores are addressed for their suitability to carbonised deposit analysis, and the degree to which their taxonomic specificity has been established.

It is shown that phytoliths and starches have generated the major contribution to debates about domestication and global plant dispersals. For this reason, their collaborative ability to represent potentially important plant species and food parts, and their evidential durability to charring, starches and phytoliths are selected for investigation of the Baltic foodcrusts. Recent programming techniques developed for automated starch classification have not been applied to archaeological questions, and this is an area where significant but achievable methodological development pertains. Likewise, phytolith investigations have been extremely limited in northern Europe because a temperate climate is less conducive to their formation in plants. This is poorly founded on reference collections however, so a preliminary phytolith investigation is proposed.

3.2. Lipid residue analysis.

3.2.1. Preservation.

Casual observation of Danish and northern German material suggests an incredibly large number of pots preserve visible residues up to six or seven thousand years old, which is remarkable. Whilst this is a fantastic resource, the benefit of biomolecular techniques is that both visible residues *and* absorbed residues that have been incorporated into the fabric of the vessel can be studied. This means no limits need be placed on sample selection from a preservation point of view, and the selection concerns can turn to interpretive consideration; that is, choosing to represent the processes at play in the past, not just the novelty of what remains in the present.

Of all the classes of compounds that make up food, lipids are perhaps the most robust largely because of their insolubility (Barnard *et al.,* 2007). Hydrophobic or amphiphilic small molecules broadly define the term 'lipid', and may be subdivided into classes (Fahy *et al.,* 2005). The transference of lipid on to the pot surface during cooking or other activities, and its subsequent incorporation into the fabric aids in the preservation of the biomolecule. Experimental boiling of lamb in replica unglazed vessels led to relatively large amounts of lipid being deposited in rim fabric, the maximum amount being 2.8mg g^{-1} (Evershed, 2008a). Adsorption on to the clay surface limits the availability of substrate for enzyme action (Evershed, 2008a; Heron and Evershed, 1993). In addition, there is evidence to suggest that both sherd fabric and residue matrix may act as a molecular trap, sieving out and inhibiting the access of degrading micro-organisms (Evershed *et al.,* 1997; Evershed, 2008b).

In a recent preliminary analysis of some sherds from sites within the Baltic region (Craig and Heron, 2006) it was shown that lipid preservation is exceptionally good at underwater sites such as those highlighted in dark blue in figure 3.1. It is a likely consequence of the hydrophobia of triacylglycerols and long chain aliphatic compounds- the major components of meat fat and leaf wax respectively (Evershed, 2008a) -aiding the clustering and retention of the molecules within the sherds, which allows successful lipid extraction figures to hit a hundred percent in some cases. At shell middens the success rate for extractions is lower relative to the underwater sites, but still extremely good.

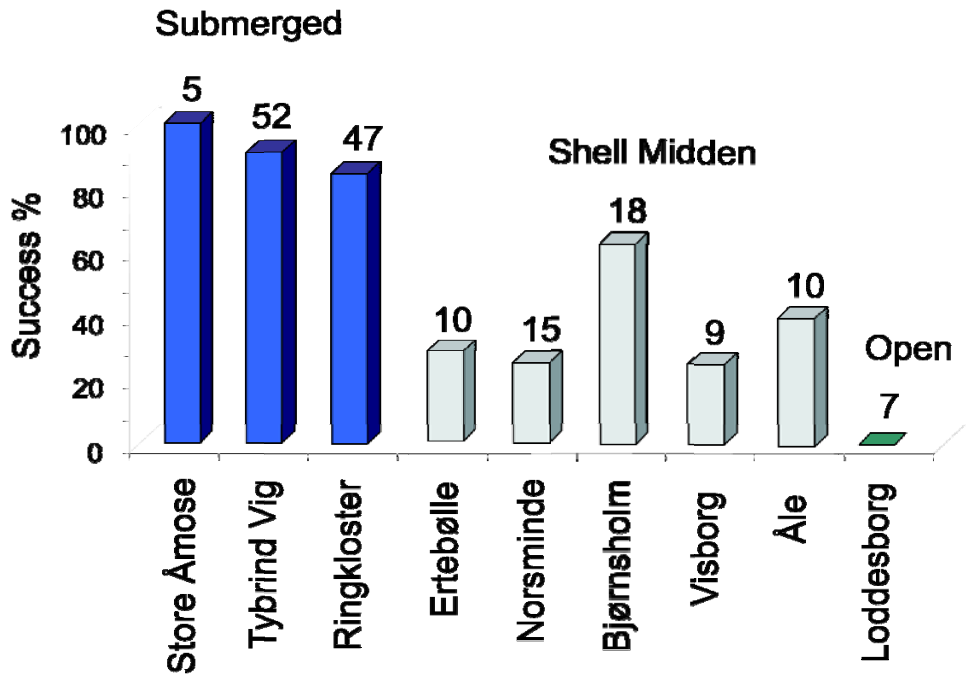


Figure 3. 1. A graph showing the success rate for the recovery of lipid from ceramics in a preliminary study of Baltic pottery (Craig and Heron, 2006).

As a result of degradation there is a preferential loss over time of unsaturated or unusual short or long-chain fatty acids (Heron *et al.,* 2007). This results in a progressive chemical homogenisation of the preserved lipid. The greater number of double bonds on monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids makes them more chemically reactive and prone to oxidation. As they oxidise and leach, the samples become dominated by saturated fatty acids which make the identification of substances with predominantly unsaturated lipid content, like many plants and fish, more challenging. But, the Gas Chromatography (GC) and Mass Spectrometry (GC-MS) techniques that have been applied to the study of lipids can make *use* of the oxidation and hydrolysis products like fatty acids to aid in identification of the precursor lipid.

In modern simulations of cooking processes lipid profiles typical of archaeological samples have been reproduced from a forty year old tsoukali vessel from Metataxades in Greece that had been used to cook pork. Triacylglycerols (TAGs) had degraded to di- and monoacylglycerols (DAGs and MAGs), and free fatty acids (Evershed, 2008a). Preservation experiments testing the effect of aerobic and anaerobic environments found that the hydrolysis of TAGs in oxic conditions was well advanced after only two days, making them virtually undetectable by gas

chromatography after 100 days. Anoxic conditions produced 10-25 times as much lipid as oxic conditions (Evershed, 2008a). These favourable conditions parallel those at many of the proposed archaeological sites for study.

3.2.2. Contamination.

There is a risk of contamination from the burial environment when doing lipid residue analysis, and multiple ways of managing this have been developed. The clearest way of evidencing a use-related lipid signature is by finding unique or distinctive carbon skeletons that directly result from food processing activities as opposed to microbial lipids. These biomarkers are particularly desirable findings because their occurrence as a consequence of heating, for example, confidently evidences the original use of the pot. Their discussion is more fully dealt with in subsection 3.2.3.2., on food identification, but as markers that exclude the possibility of contamination whilst simultaneously informing on vessel contents they have become a focus of recent research (eg., Craig *et al.,* 2007; Evershed, 2008b; Isaksson *et al.,* 2010).

Experimental work suggests that microbial contributions to absorbed lipid residues are limited. Dudd *et. al.,* (1998) found that there was less than 2% incorporation of bacterial and fungal markers in absorbed lipids after the decay of milk and olive oil. It is a common strategy now to also compare the proportions of preserved lipid from interior samples and exterior soot deposits, and soil when working with pottery (e.g., Craig *et al.,* 2005) as a measure of background lipid.

3.2.3. Identification.

3.2.3.1. Gas chromatography and fatty acid ratios.

Fatty acids make up the most extensively studied class of lipids. Early investigations used gas chromatography on its own to separate the fatty acids so that the ratios of hexadecanoic acid ($C_{16:0}$, palmitic acid) to octadecanoic acid ($C_{18:0}$, stearic acid) could be calculated (Barnard *et al.,* 2007; Regert, 2007; Malainey, 2007). Large amounts of C_{18} and C_{16} fatty acids relative to other constituents are good indicators of animal products.

On its own, this technique is not a secure classifier of lipid food sources. Oleic acid converts to palmitic acid in waterlogged, anaerobic conditions (Heron and Evershed,

1993; Eerkens, 2007) skewing the ratios, and $C_{16:0}$ is twice as soluble in water at 20°C as $C_{18:0}$. This may be especially pertinent for the Baltic region where the isostatic uplift and rising sea levels, as well as many bogs result in lots of anaerobic sites to draw residues from. The point is illustrated by analyses of pottery residues from a coastal site on the Vrendenburg Peninsula, South Africa which calculated a $C_{18:0}/C_{16:0}$ ratio of 2.28 which compared favourably to published values for Grey Atlantic seal (Patrik *et al.* 1985). Yet experimental cooking with seal produced ratios of 4.91 and 4.97 (Patrik *et al.* 1985), suggesting a preservation factor was causing the disparity.

Some forays have been made into the ratios of other fatty acids that decompose at similar rates. However, most of the candidates such as $C_{16:1}$ and $C_{18:1}$, or isomers of the same fatty acid $C_{18:1}\omega9$ and $C_{18:1}\omega7$ (Eerkens, 2007), that are monounsaturated, cannot be relied upon to preserve. If they decompose at similar rates it is often a factor of their having similar functions within the species they come from too, meaning they are produced in similar amounts (Eerkens, 2007) rendering their ratios less sensitive identifiers. As a result the direction of research has swung in the direction of other complementary techniques that can be added to the toolkit, generating an assemblage of data that provides different perspectives of the lipid chemistry.

3.2.3.2. Mass spectrometry and chemical biomarkers.

A number of variations of mass-spectrometry methods have been used on ceramic residues; as rapid molecular screening measures (Evershed *et al.,* 2003), for better separation of molecules with a similar molecular mass (Kimpe *et al.,* 2004) and in the face of particularly challenging preservation situations. Gas chromatography followed by nanoelectrospray mass spectrometry was applied to late Neolithic vessels from Chalain 4 and Clairvaux XIV in Jura, France to distinguish between different original species for dairy and subcutaneous fat (Mirabaud *et al.,* 2007). The low flow rates generated more complete ionisation which was sensitive enough to precisely identify low TAG presence to sheep fat, goat fat and cow milk (Mirabaud *et al.,* 2007). However, these efforts are often unnecessary if standard GC-mass spectrometry is combined with single compound isotope measurement, to give an extra dimension to identification, and will not be incorporated.

Detecting fish and other aquatic products in cuisine is a necessity in southern Scandinavian prehistory where other archaeological evidence has created controversy over the degree of reliance Mesolithic and Neolithic peoples had on the sea (Milner *et al.,* 2004; Richards *et al.,* 2003a; Richards *et al.,* 2003b; Schulting, 1998). The identification of marine products has spurred investigations into biomarker research: altered 'chemical skeletons' (Hansel and Evershed, 2009) brought about by the condensations, oxidations, and polymerisations, hydrolyses, isomerizations and cyclizations of the cooking process (Hansel *et al.,* 2004). Isoprenoid acids (4,8,12-trimethyltridecanoic acid (TMTD), phytanic acid and pristanic acid), and ω-(*o*-alkylphenyl)alkanoic acids have been found to indicate the heating of aquatic commodities (Craig *et al.,* 2007) to over 270ºC (Evershed, 2008b).

The distribution of *n*-alkanoic acids has traditionally been attempted to distinguish animal products from one another, but both fish and plant *n*-alkanoic distributions are dominated by the $C_{16:0}$ acid (Olsson and Isaksson, 2008). As well as incorporating the above isoprenoid acids, information on the positional isomers that generate the ω-(*o*-alkylphenyl)alkanoic acids are useful. For the formation pathway of these biomarkers, the higher the carbon number of the precursor fatty acid, the greater the number of positional isomers that will be produced (Hansel *et al.,* 2004), that is, the more arrangements of double bonds there will be. Plants are lacking in C_{20} compared to oily marine products. When analysing vessel samples from pre-contact Santa Catarina Island, Brazil, it was possible to evidence marine resources because of the array of C_{20} isomers as well as those from C_{16} and C_{18}, a result of precursor $C_{16:3}$, $C_{18:3}$ and $C_{20:3}$ fatty acyl marine components (Hansel *et al.,* 2004).

Using the same principle of identifying variation in the positional isomers it is possible to specify whether the marine food is a mammal as opposed to lower tropic orders such fish. The suite of aquatic biomarkers including isoprenoid fatty acids and ω-(*o*-alkylphenyl)alkanoic acids were identified from 'cemented' residues in northern Norwegian 'slab-lined pits' (Heron *et al.,* 2010). Crucially though, positional isomers on the dihydroxy fatty acids indicate that the thermally altered precursor fatty acid was cetoleic acid, which is the most abundant $C_{22:1}$ isomer found in marine oils (Heron *et al.,* 2010). Although the rendering of blubber on such a large scale may not have been for consumption as much as illumination or other activity,

the example illustrates the potential for sensitive identifications of possible marine mammal foods in regions and substrates similar to our Baltic residues.

3.2.3.3. Bulk isotopes.

Bulk isotope IRMS measurements are made on the total carbon and nitrogen content of foodcrusts, from carbohydrate, protein and lipid constituents of the original foods. They are a blunt tool for identifying the food contents of ceramics, but are useful in combination with other techniques and multiple authors advocate their incorporation into multi-disciplinary frameworks (Craig *et al.,* 2007; Hart *et al.,* 2007; Spangenberg *et al.,* 2006). In contrast, single compound isotope analyses are performed on single fatty acid contributions which can give a higher resolution picture of what the compound represents, but only of the lipid contribution to diet, playing down protein and carbohydrate dominated food sources.

Philippsen (2009) used experimentally created foodcrusts of known ingredients to compare to predicted bulk isotope values of food mixtures from crusts. Measures of the $\delta^{13}C$ and $\delta^{15}N$ of the known weights of ingredients were taken before cooking. The expected bulk isotope values were an average of the isotope values for the ingredients with their percentage contribution to the stew (based on their weight), and their contribution to the carbon and nitrogen fraction (based on the amount of carbon and nitrogen in the ingredient) (Philippsen *pers. comm.*). The results of the predicted bulk isotope values deviated from the actual measures of the foodcrusts, suggesting complex contributions from food classes contributing nitrogen and carbon, and intervening processes that can alter C/N ratios.

Foodcrusts can be contaminated from background isotope signals in the burial environment (Craig *et al.,* 2007). The removal of macroremains such as fish scales is an important first step in reducing any effects of contamination, especially in marine sediments. The burial environment has also been shown to be a locus for the microbial reworking of nitrogen (Lehmann *et al.,* 2002), that were found to lead to isotopic shifts of 1-5‰ on modern plant experiments (DeNiro and Hastorf, 1985). The boiling, roasting and carbonisation involved in generating the crusts has been found to bring about variations of +/- 3‰ in both $\delta^{13}C$ and $\delta^{15}N$ of modern plants (DeNiro and Hastorf, 1985). Bulk isotope measures must therefore be employed with acknowledgement of the limits of their resolution. In conjunction with other

lines of evidence though, they can produce useful detail to interpretations (Craig *et al.,* 2007).

^{13}Carbon indicates the relative contribution of terrestrial and marine foods to foodcrusts. Fischer and Heinemeier (2003) suggest that relatively light carbon isotopes <-28‰ indicate a freshwater contribution, whereas heavier carbon isotopes >-26‰ are the range for marine foods. Shifts of +1 to +2‰ in δ^{13}C can occur between the flesh of a consumer and its food source (Spangenberg *et al.,* 2006). These shifts are affected to a degree by biological variations in the δ^{13}C values of the different biomolecules contributing to the bulk measure (Barnard *et al.,* 2007). However, δ^{13}C measures have been useful at distinguishing freshwater fish from marine resources at different sites in prehistoric Denmark (Craig *et al.,* 2007), as well as eliminating freshwater fish containing food residues from selection for radiocarbon dating (Craig, 2004) because of the reservoir effect. The herbivores used to reference the δ^{13}C findings in Late Neolithic potsherds from Arbon Bleiche 3 in Switzerland including cow, sheep, goat and deer ranged from values of -30.7 to -26.7‰ (Spangenberg *et al.,* 2006).

^{15}Nitrogen contributions to food are useful because they fractionate according to the position of the consumer in foodwebs, and so they can add important detail to interpretations that are not possible by other means reported here. There is a 2-4‰ enrichment in ^{15}N for each stage of the foodchain (Minagawa and Wada 1984). Terrestrial plant proteins have a value of 3‰ (+/- 1‰) (Craig *et al.,* 2007). The δ^{15}N of several lacustrine fish species from across four lakes in Europe and Russia gave a range +7‰ to +15‰ (Dufour *et al.,* 1999). Piscivorous marine fish have δ^{15}N values between +11‰ to +16‰ (Craig *et al.,* 2007), whereas other marine foods values range from c. +8‰ for shellfish and molluscs, up to +18‰ for marine mammals (Richards and Hedges, 1999). Terrestrial herbivores are variable but most should not exceed +7‰ in northern Europe, except for pigs which can be a slightly enriched compared to other herbivore species (Craig *et al.,* 2007).

3.2.3.4. Single compound isotopes.

The addition of isotope analyses to the repertoire has bolstered successes in discriminating between previously indistinguishable food items. Evidence suggests that δ^{13}C values preserve even if the original residue is degraded (Steele *et al.,* 2010).

Detailed identifications can be made between different sub-cutaneous fats derived from terrestrial meat sources using carbon isotopes ($\delta^{13}C$). GC-MS is of limited value as a technique on its own because adipose degradation results in only high saturated C_{16} and C_{18} and *n*-alkanoic acids values (Mottram *et al.,* 1999). Acyl lipids like triacylglycerol and phospholipids hydrolysed in laboratory degradation experiments (Evershed *et al.,* 1997), limiting the sensitivity of the GC-MS fingerprint. The resolution of fatty acid ratios is only great enough to separate different subcutaneous fats in situations of excellent preservation.

Applied to Medieval pottery from West Cotton in Northamptonshire it was possible to separate the functions of two pottery types; 'lamps' and 'dripping dishes' (Mottram *et al.,* 1999). The $\delta^{13}C$ values of the $C_{16:0}$ fatty acids were plotted against those of the $C_{18:0}$. In the 'lamps' the $C_{16:0}$ was enriched in ^{13}C relative to the $C_{18:0}$. In the 'dripping dishes' however $C_{16:0}$ was relatively depleted (Evershed *et al.,* 1997). Importantly these values correlated with modern reference adipose fats from comparable isotope ecologies. The reason for this is related to differences in the way ruminant and monogastric animals synthesise fatty acids from dietary carbohydrate.

The lamps were consistent with a ruminant source such as sheep or cow, whilst the dripping dishes were from a monogastric source like pig (Evershed *et al.,* 1997). This case study illustrates the importance of *combining* techniques though because the ruminant $\delta^{13}C$ values displayed similar characteristics to some vegetable oils. It was possible to refer back to the GC-MS data to evaluate the structures and distributions of fatty acids in the two vessel types, and review the sourcing from a different perspective.

Both types of vessels contained a high saturated alkanoic acid content indicating an animal source. In addition, the distribution of minor lipid components was different for the two vessels, and distinguishes them both from plant. Analysis of the monounsaturated acids in lamp extracts revealed a mixture of positional isomers of octadecenoic acid (Evershed *et. al.,* 1997). Essentially, ruminant sources generate compounds with the same atomic composition, but different structure (Crowe *et al.,* 2006). In this case the differences in the structure relate to double bonds distributed variously at carbon atoms 9, 11, 13, 14, 15 and 16 (Dudd and Evershed, 1999). Analogy to modern examples established this as a feature of the biohydrogenation of

dietary fats in the rumen. Monogastric sources only contain a single isomer, Z-9-octadecenoic acid (Evershed *et al.,* 1997).

There are some aspects of food consumption in the past that can *only* really be known through chemical means, and milk dairying certainly falls into this category. Milk is a clear signal that intensive management practices of animals are occurring, and is considered an indicator of domestication. Again difficulties are present in distinguishing milk products from adipose fats. Degraded milk sees a rapid loss of shorter chain fatty acids less than 14carbons long (Dudd *et al.,* 1998), as they become more water soluble and volatile after hydrolysis (Dudd and Evershed, 1998).

This degradation can be characteristic to an extent, but not enough to be relied upon. Milk has a greater abundance of lower carbon-number triacylglycerols than ruminant and non-ruminant fats, when degraded. Ruminant TAGs distribute within the range C_{40} to C_{42} and C_{54} and non-ruminant TAGs plot from C_{44} to C_{54}, with less C_{44} and C_{46} (Dudd and Evershed, 1999), both of which are higher carbon numbers than milk at between C_4 to C_{14} (Dudd and Evershed, 1998).

So, carbon isotopes are, again, a useful additional technique for discrimination. Single compound $\delta^{13}C$ of the $C_{18:0}$ fatty acid reveal it to be characteristically depleted in ^{13}C (Craig *et al.,* 2005). This comes about because the mammary glands are incapable of synthesising $C_{18:0}$ fatty acids. Biohydrogenation (bacterial reduction) of dietary $C_{18:2}$ and $C_{18:3}$ to $C_{18:0}$ occurs as a lactation response, and the $C_{18:0}$ is routed to milk (Copley *et al.,* 2005). Graphically, we can see from figure 3.2 (Copley *et al.,* 2005, 526) that ruminant adipose and dairy fats display ellipses close together, but separable.

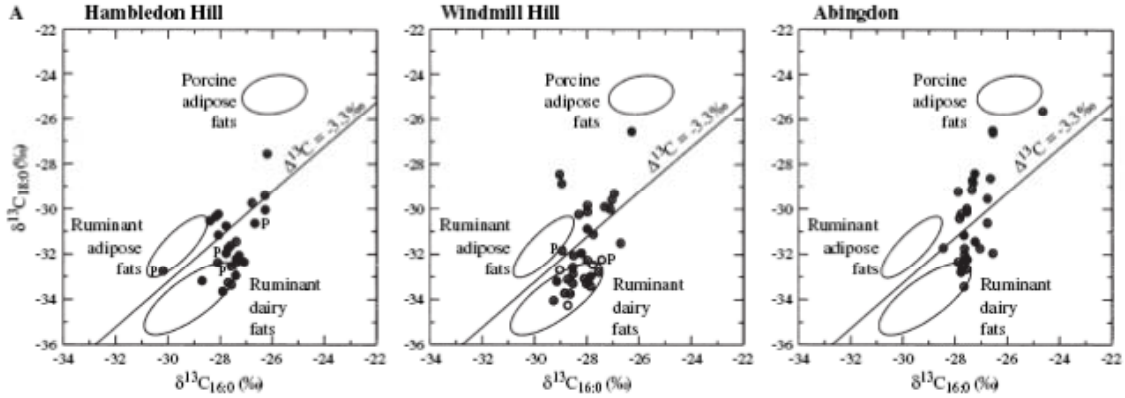


Figure 3. 2. The single compound isotope measurements from three Brisish Neolithic sites, showing the high number of samples that fall in the range of ruminant dairy fats (Copley et al. 2005, 526).

Measuring the change in $\delta^{13}C$ values between $C_{18:0}$ and $C_{16:0}$ (written as $\Delta^{13}C$ above) affords a boundary measure where milk values and adipose values separate. For this isotope ecology, values lower than 3.3‰ are considered milk (Copley *et al.,* 2005). Those samples that fall on the line were considered mixtures of milk and animal fat. But it is clear that for these Neolithic ceramics from six southern English sites, milk was an important commodity. At a number of these sites, such as Runneymede Bridge and Eton Rowing Lake a respective 35% and 32% of the sherds showed evidence of milk absorption (Copley *et al.,* 2005). This identification criterion is crucial for samples from Baltic prehistory, where early evidence for the adoption of domesticated foods into cuisine may be marked by dairy foods.

3.2.3.5. The plant lipid deficit.

Categorizing plant material from animal fats is difficult to do (Steele *et al.,* 2010), and must be considered a weakness of current chemical approaches, that under-represent the presence of floral foods. This is partly because plants *contain* fewer lipids in their undegraded state than animal foods. On top of that the problem that degradation acts severely on many plant lipids because their composition is dominated by unsaturated lipid, and β-sitosterol is often missing from archaeological samples compounds the situation. Fatty acid ratios are characteristics that are usually used to identify the presence of plants but dissolution of short chain fatty acids in burial alters their ratios. This homogenises their signature to become more like animal fats (Steele *et al.,* 2010), and mixtures can over-shadow and reduce the

visibility of any plant compound peaks. In addition, lower quantities of plant lipids are transferred to ceramics during cooking (Evershed *et al.,* 1995).

In the face of these challenges, the importance of investigating for the presence of specific molecular evidence has been highlighted (Steele *et al.,* 2010; Stern *et al.,* 2008). The response has been an emphasis on biomarker research, using a diverse range of mass spectrometry techniques. Despite the preservation problems associated with unsaturated lipids, phytosterols are a diverse class (Isaksson *et al.,* 2010) suggesting a high potential for sensitive identifications if these preserve. The problem for northern European research is that most of these biomarkers do not refer to native temperate species, or plants that play a significant role in debates in prehistory. For example, radish oil which is distinctively characterised by an abundance of longer fatty acid chains up to 24 carbons, of both monounsaturated and saturated components (O'Donoghue *et al.,* 1996). Likewise oil from date and don palm (*Phoenix dactylifera* L. and *Hyphaena thebacia* (L.) Mart.,) could be identified on the basis of saturated carboxylic acids in the C_{12} to C_{18} range (Copley *et al.,* 2001). The findings are from vessels from Qasr Ibrim in Egyptian Nubia however, posing limited usefulness in a northern European context.

Investigations have understandably focused on those species that are most likely to produce the most lipids, like olives. Roman amphorae from the Mediterranean demonstrated the presence of olive oil fatty acid indications when subjected to gas chromatography (Condamin *et al.,* 1976). The C_{16} fatty acids mainly comprise palmitic acid and palmitoleic acid, whilst the C_{18} fatty acids are stearic acid, a monoethylenic acid like oleic acid and linoleic acid (Condamin *et al.,* 1976). The concentrations of these fatty acids alter geographically so potential was envisaged for tracking trade routes. However, the reliability of the techniques sensitivity was not securely established.

Plant lipid analysis is certainly an area with potential for growth, and calls have been made for more imaginative techniques for plant finding (Reber and Evershed, 2004). The application of high-performance liquid chromatography (HPLC) coupled with atmospheric-pressure chemical ionisation mass spectrometry (APCI MS) to Preclassic (900 BC to AD 250) Mayan pottery from Colha, Belize successfully targeted residual cacao signatures. Three out of fourteen vessels exhibited the cacao

marker theobromine (Hurst *et. al.,* 2002). The technique works pressures high enough to give good separation to smaller compounds, and was probably aided by the specialised use of the vessels *solely* for preparing chocolate beverages.

Similarly, liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) has identified the product used to make the enigmatic ancient Egyptian drink *shedeh*. This remarkably allowed investigators to identify not only a grape source for the drink, because of tartaric acid markers, but also to narrow this to *red* grapes based on the presence of syringic acid (Guasch-Jané *et. al.,* 2006). There is a large research potential for this finding; *shedeh* was a valuable commodity in trade because of its divine association with the god Ra, and knowledge of its constituents means we can begin to relate networks of production with consumption.

Starchy plants present further problems, because underground storage organs are designed to minimise lipid content in favour of carbohydrates. Species specific biomarkers for starchy foods are few. Exceptions in maize where the long chain alcohol *n*-dotriacontanol, and plant waxes may be unique to this important crop species (Reber and Evershed, 2004), but require further comparative studies to measure their distinctiveness. Of significance for the transition to agriculture is the identification of ergosterol (5,7,22-ergostatrien-3β-ol) as a possible biomarker for fermentation using cereals (Isaksson *et al.,* 2010). In tests using Swedish Neolithic, Bronze Age and early Iron Age vessels ergosterol was not found in any of the Neolithic examples, but was present in 6 of the later vessels when agriculture was being practised more intensively (Isaksson *et al.,* 2010). One explanation for the adoption of cereals has been their use in fermented beverages (Dineley and Dineley, 2000), and this poses an important contribution to the assessment of whether cereals really had an impact in Denmark and northern Germany at the beginning of the Funnel Beaker period. It is both a proxy for fermented beverages, and baking with fermented dough.

Perhaps one of the most pertinent successes in plant lipid analysis is the findings of leafy vegetables like northern European *Brassica* species in pottery. GC-MS spectra of Late Saxon-Medieval (9^{th}-13^{th} centuries AD) pottery from West Cotton, Northamptonshire displayed peaks of nonacosane, nonacosan-15-one, and nonacosan-15-ol, which are components in epicuticular leaf wax (Evershed *et. al.,*

1991). The proportions of these indicators were closely associated with those in fresh examples tested (Charters *et. al.,* 1997). For the purposes of analysing southern Scandinavian pottery this is important because identifying leafy vegetables is a particular challenge for *any* technique. Wild *Brassica* species have also been found to produce similar proportions of epicuticular leaf wax components (Evershed *et. al.,* 1991).

Only recently have investigations been made into the single compound $\delta^{13}C$ isotope values of $C_{16:0}$ and $C_{18:0}$ fatty acids in plants for archaeological residue analysis (Steele *et al.,* 2010). Conventional approaches for plant identification using single isotopes exploit the $C_{18:1}$ fatty acid which is present in higher concentrations. This prevents comparison to other archaeological foods such as ruminant adipose, porcine, dairy and marine products which are identified by their $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ signatures. By extracting these comparable fatty acids from a range of modern plant oils (almond, argan, moringa, sesame, walnut and olive), it was possible to identify a $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ range that falls tightly *between* porcine products and ruminant adipose (Steele *et al.,* 2010). This raises a problem for studies of mixtures because data points falling into this area are normally considered to be a mixture of pork and ruminant, not plants. Whilst these data are a substantial leap forward in plant lipid residue identification, hesitancy still exists over their use in a northern European context as native lipid rich foods such as hazelnut (*Corylus avellana*) and acorn (*Quercus* sp.) are not catalogued in the analysed reference material. Thus, in order to understand *mixing* of plants and animal foods, secure identifications would benefit from additional types of data such as plant microfossils, to add extra collaborative dimensions to interpretation.

3.2.4. Replication.

Although studies have developed to identify *what* was in the vessel for the important animal foods, what we are less sure about is what those ingredients represent, and how they were processed. It is unclear what the use-life of a vessel may have been, and whether the role it played changed a number of times before ultimate discard. A suite of products we find in archaeological pots may be from the first use of the vessel, the last use, or even be an accumulation of different uses. These sorts of questions are amenable to experimental replication, but have only seen limited investigation.

When lamb was repeatedly cooked in unglazed vessels the maximum mean lipid concentration was 21.8 mg g^{-1} in the rim, but it was only 13.5 mg g^{-1} for olive oil, so the product being processed has a bearing on the capacity of the fabric to absorb large quantities of lipid (Evershed, 2008a). So, certain lipids preferentially absorb into vessels better than others, and we have seen that plants do not.

Run consecutively, with *Brassica* leaves being boiled 10 times followed by a single cooking of lamb, concentrations of *both* foods were present in the chromatogram (Evershed, 2008a). Interestingly though, the concentration of *Brassica* was the same before and after the cooking of lamb, so no overprinting is in evidence with these foods after multiple cooking episodes, and accumulation of absorbed residue was apparent.

When sequential degradations including extraction, saponification and hydropyrolysis were carried out on two archaeological sherds from Iron Age Yorkshire and Early Medieval Sweden it was discovered that remaining organic carbon that *survives* these degradations was composed of a resistant polymeric residue (Craig *et al.*, 2004). Preserved aliphatic fatty acids that *were* extractable from the interior surface must have been the result of cooking episodes that happened after the bulk of the resistant char had formed, evidencing the *later* phases of cooking (Craig *et al.,* 2004). So although experimental work suggest that extracts evidence accumulative cooking (Evershed, 2008a), after a certain phase of charring these early use lipid compounds become polymerised and entrapped beyond the range of extraction.

What is also unclear is where surface deposits fit into this scheme which describes only absorbed residues. Surface deposits may evidence an incremental accumulation over the vessel history, or they may wax and wane depending on what's being cooked and how this affects the adherence of the carbonised material to the surface. So, surface deposits could be evidencing an almost complete history, or they may indicate the last few uses of the pot. It seems likely though that foodcrusts are from the later use of a vessel, and that their accumulation could contribute to the reason for discard in some situations, as the charring would affect the taste of foods.

It is also unclear *how* the products were being processed or treated in the vessels. Carbonisation and thermally induced biomarkers are a good indication that cooking

was occurring, but the form of cooking activities is difficult to assess. In absorbed lipid analysis the distributions in different parts of the vessel profile can suggest how the contents were cooked. In boiling experiments with *Brassica* the highest quantities of lipid were in the rim (Charters *et al.,* 1997; Evershed, 2008a). This is logical considering the hydrophobic properties causing lipids to float on the surface of the liquid. Whilst there is scope for interpreting the cooking activity from the profile, little work has been done on other vessel-related activities such as milk processing for cheese etc., and for example to look for both biomarker evidence and concentration differences in profiles. The same is certainly true for visible residues where no work has been done on analysing for microscopic indicators of different cooking activities, and other processing activities.

In sum, as current information stands absorbed residues are likely to be accumulations of cooking episodes but from *later* uses of the pots. This is because progressive charring from re-use causes lipid polymerisations which resist degradation and extraction procedures (Craig *et al.,* 2004). Likewise, based on the observation of foodcrust formation in unglazed vessels it seems likely that these surface adherences are accumulations from later uses of the vessel since they can become very thick very quickly and taint the contents with char. Whilst this narrows down the use time-range of the extracts being analysed, they still consist of accumulated re-uses, so the investigation of cuisine *must* still work from an interpretive basis.

It has already been noted that in theoretical terms cuisine can be described using only a single food type, since from a theoretical perspective what is being mixed and combined in pots are the *values* associated with foods. However, mixing of multiple food types can be explored interpretively by investigating patterns of use and combinations of foods in assemblages of samples to look for persistence and traditions, in light of the experimental work that has been done.

3.3. Analysing plant microfossils.

There is a deficit of multi-disciplinary work that combines the techniques of organic-geochemistry with microscopic analyses of archaeoflora. There is only one case of the combined use of stable isotope analysis, trace element analysis, starch and phytolith analyses (Boyd *et al.,* 2008), which was adopted because of the challenges

of investigating maize (*Zea mays*) adoption when the knowledge about wild prairie species' microfossil content was so slight. As a C4 plant, maize has a distinctive isotope signal compared to C3 plants, with respective averages of $\delta^{13}C$ -12.5‰ and $\delta^{13}C$ -26.5‰ (Seinfeld *et al.,* 2009). This allowed corroboration of maize findings in the context of wild species. Plant microfossil analysis poses significant promise for the representation of the plant component of foods in northern European pottery contexts. As an additional line of evidence it may add perspectives on organic-geochemical techniques such as IRMS, assisting in explaining the bulk values by suggesting whether a significant carbohydrate contribution exists.

3.3.1.1. What are phytoliths?

Phytoliths are microscopic silica bodies produced by plants, especially the grasses where they can make up to 10% of the dry weight of the plant (Elbaum and Weiner, 2003). The development of phytoliths in plants is dependent on a number of factors. Those external to the plant include its climatic environment of growth, the amount of soluble silica in soil, the amount of water in the soil, and the age of the plant (Piperno, 2006). These factors feature as variables in the evapo-transpiration system, which is a mechanism controlling the distribution of silica in shoots (Feng Ma and Yamaji, 2006; Rosen, 1992).

Phytoliths can have a taxonomic resolution up to genus and sometimes species level, because the silicon dioxide which is drawn from the soil is laid down in the inter- and extra-cellular spaces, generating inverse impressions of the cells they inhabit or surround. These impressions are the phytoliths themselves, and their diagnostic value becomes a product of the plant's choice of where to deposit silica, combined with the taxonomic *uniqueness* of the cell or tissue structures that it's depositing it around. 'Multiplicity' (Rovner, 1971) describes how a range of phytolith shapes and abundances can usefully occur within a single species, whilst 'redundancy' (Rovner, 1971) describes the way one type of phytolith can occur in many families.

Phytoliths can endure temperatures up to 600°C without significant alteration to their morphology (Elbaum and Weiner, 2003). They are susceptible to extreme pH ranges <3 and >9 however (Piperno, 2006), so a highly alkaline environment such as a shell midden may not be conducive to their preservation. The durability of phytoliths has meant they have survived in coprolites (Horrocks 2004; Reinhard and Danielson,

2005), within dental calculus (Henry and Piperno, 2008; Lalueza Fox and Pérez-Pérez, 1994; Lalueza Fox *et al.,* 1996), in association with stone tool residues (Dominguez-Rodrigo *et. al.,* 2001; Kealhofer *et. al.,* 1999) and within sediments (Donohue and Dinan, 1993; Huang and Zhang, 2000; Lu *et. al.,* 2002; Mercader *et. al.,* 2000; Piperno, 1993). Partly because of the wide adoption of the technique in the Americas where hunter-gatherer pottery use was extensive, phytoliths are also one of the most widely used plant microfossil for the study of ceramic residues (Jones, 1993; Lusteck and Thompson, 2007; Staller and Thompson, 2002; Thompson and Mulholland, 1994; Zarillo *et al.,* 2008). Phytoliths charred by cooking have been found to be more resilient to destruction by high pH (Piperno, 2006).

The main issue for the successful application of phytolith analysis to a southern Scandinavian archaeological context is not the *durability* of phytoliths as much as the possibility for their production in the first place. A temperate climate is considered to be non-conducive to the formation of phytoliths because the process is linked to the evapo-transpiration system and evaporation rates are low in northern Europe (Madella *pers. comm.*). As a result only one application of phytolith analysis has been applied in a northern European context (Powers *et al.,* 1989), and the results dealt with generalised categories of phytolith types to suggest activities such as peat cutting, manuring and possible grazing systems on sand dunes in Northwest Britain in prehistory (Powers *et al.,* 1989), rather than high resolution taxonomic classifications.

As a result very little is known about the potential of phytolith formation in temperate Europe; whether known phytolith producers *can* silicify their cells, the *extent* of silicification, whether reduced silicification results in reduced durability to heat, which northern European species *do* produce phytoliths and how taxonomically significant they are. If there is a reduced overall capacity for the production of phytoliths in temperate Europe, this could in fact *reduce* the problem of redundancy as any phytoliths that are generated will be from a more limited number of successful producers. A major thrust of the background for this microfossil will be on the potential for phytolith production in northern Europe. This will be based on the collation of published material about which families native to northern Europe have been found to be good phytolith producers in other parts of the world. In this way

one can assess the likely contribution of phytoliths to the study of cuisine, and the scale at which such research should be conducted.

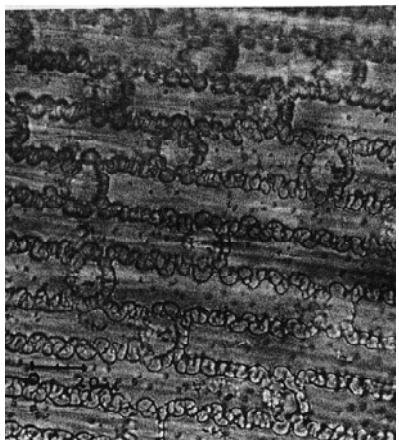
3.3.1.2. Phytoliths and domesticated plant debates.

Phytoliths have been used to study the domestication of many economically important species including rice (*Oryza sativa*) (Harvey and Fuller, 2005; Huang and Zhang, 2000; Itzstein-Davey *et. al.,* 2007; Lu *et al.,* 2002; Pearsall *et. al.,* 1995; Zhao and Piperno, 2000; Zheng *et. al.,* 2003), squashes and gourds from the family Cucurbitaceae in the tropics (Bryant, 2003; Hart *et al.,* 2007; Piperno *et. al.,* 2000a; Piperno and Stothert, 2003), and maize from the Americas (Boyd *et. al.,* 2006; Boyd *et. al.,* 2008; Hart *et al.,* 2007; Umlauf, 1993). One of their most successful applications has been in the context of cereal cultivation in the Near East, because of successful genus level identifications.

Rosen (1992) found that the anatomical features of the culm (stalk) and the husk are distinctive. Her original work focused on distinguishing domestic species from one another; and she had convincing success differentiating the genus of wheat (*Triticum sp.*), barley (*Hordeum sp.*). The anatomy of husks from grasses is similar in its broad morphology (Warnock, 1998, 240). 'Husk', is a catch-all term describing highly silicified modified leaves that enclose a grass floret made up bracts called the palea, lemma and glume (Rosen, 1992). Often silica makes up to 20% of husk dry weight (Tubb *et al.,* 1993 *, 537*).

The epidermis cells of the husk are composed of long-cells, and these cells display changing characteristics (Rosen, 1992) in terms of the sinuosity of the cell walls (wave shape/amplification), the thickness of the cell walls, the number of papillae, ornamentation of the papillae by pitting and minute surface projections, and width of the long cells. Figure 3.3 shows some of the characteristics of grass bracts under electron and light microscopy. Dendriform phytoliths are literally the silicified cell walls of epidermal long-cells, and SEMs (a) clearly shows their cilia-like protrusions interlocking in a 3D fashion. Image (b) gives an impression of a light microscope image. The terminal ends of the long cells are bordered by round spaces which are in fact the papillae, and the small black spots within the cells are bordering pits. The recovery of a high proportion of these dendritic cells from cave sediments at Amud

in Israel attests to the presence of mature seed panicles, suggesting the deliberate gathering of grass seeds by Middle Palaeolithic Neanderthals (Madella *et al.,* 2002).



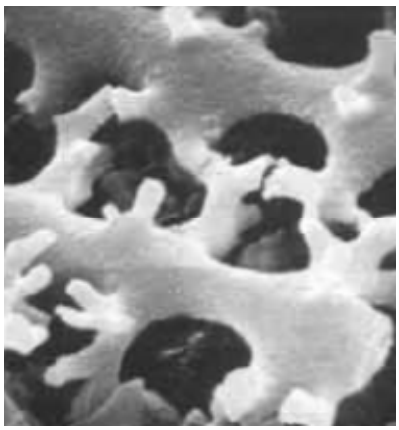


Figure 3. 3. a) an SEM micrograph of dendriform phytoliths x5400 (Hayward and Perry 1980, 549)., b) a light microscope image of grass silica skeletons. Emmer wheat (T. dicoccum). Bar is 20 microns (Rosen, 1992, 133).

Long-cell walls tend to be much thicker in the husk than in other parts of the plant, but there are differences in the thickness *within* the structure of the bract too. There are slightly thinner cell wall examples depending on whether the cells are from the upper, middle, or lower husk, and inner or outer portion (Rosen, 1992). It is important therefore to appreciate differences in all of these husk portions, and combine all available criteria of those mentioned features, such as papillae number and papillae ornamentation. Albert *et al.,* (2008) used assemblages of these cereal phytolith morphotypes to identify activity areas at the Late Bronze and Early Iron Age site of Tel Dor in Israel. Proportions of dendritic cell phytoliths were used to indicate areas of domesticated grass use on site, as the percentage of these cells is consistently 7-8% of the morphotypes. In areas where domesticated cereals were in evidence, morphological analysis revealed them to be likely examples of *Triticum aestivum* (Albert *et al.,* 2008) based on a mean dendritic cell length >17μm and a mean trichome phytolith diameter >22.5μm (Ball *et al.,* 1999). In association with enclosures and calcitic spherulites is was possible to say that the areas were for the foddering and holding of animals (Albert *et al.,* 2008).

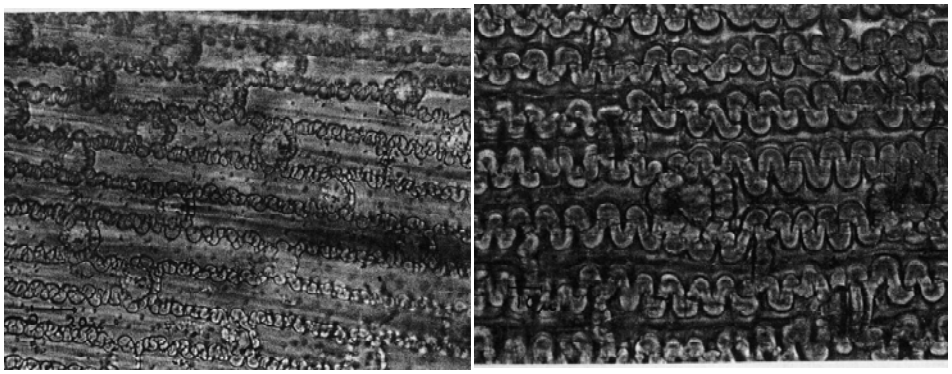


Figure 3. 4., a) The long cell structure from emmer wheat closest to the rachis., b) the long cell structure from the middle portion of the emmer husk (Rosen, 1992, 135). Bar 20 microns.

In analysing emmer wheat (*Triticum dicoccum*) Rosen (1992) notes that long cells are ca 18-23 microns wide in the portion closest to the rachis. The thick cell-walls display very low blunted wave patterns (figure 3.4a), but in the thin-walled version they are often squarer (*ibid. 135*). The wave heights are the length of the cell-wall 'creases', or 'folds'. In the thin waves of emmer wheat they are ca 4 microns, and in the thick waves they are 5-8 microns (*ibid. 135*). In middle portion of the husk (fig 3.4b) the thin and thick cell-walls of long cells have tall, curved waves that are quite irregular in amplitude or crease length (Rosen, 1992, 135). Thin versions are roughly 10 microns; thick are roughly 15.5 microns (*ibid. 135*). There is some overlap between the size of papillae between domestic emmer (22-30 microns), and wild emmer (*T. dicoccoides*, at 21-43 microns), and also einkorn (*T. monococcum*, 25-50 microns). It is these sorts of overlaps that warrant the use of statistical techniques and image analysis software for quantitative clarification. Ball *et al.,* (1999) were able to use the morphometric *means* of a population to significantly distinguish *T. monococcum* from *T. dicoccum,* and *T. aestivum*, based on silica cell and dendriform inflorescence phytoliths. Fig 3.5 summarises their analyses, showing a staged process of elimination. *T.monococcum* has the smallest silica cell length and the smallest silica cell perimeter. These measurements get progressively larger in *T. dicoccon,* and larger still in *T. aestivum.*

Morphometric description	Species
1. Dendriform phytoliths present. Mean length of the top of the surface of silica cells less than 14.6µm (*Nmin*=25). Mean perimeter of the top surface of silica cells less than 41.5µm (*Nmin*=25).	Triticum monococcum.
Dendriform phytoliths present. Mean length of the top of the surface of silica cells greater than 14.6µm (*Nmin*=25). Mean perimeter of the top of the Surface of silica cells greater than 41.5µm (*Nmin*=25).	2.
2. Mean length of dendriform phytoliths less than 68µm (*Nmin*=25). Mean narrowest width of dendriform phytoliths less than 4.5µm (*Nmin*=35).	Triticum dicoccon.
Mean length of dendriform phytoliths greater than 68µm (*Nmin*=25). Mean narrowest width of dendriform phytoliths greater than 4.5µm (*Nmin*=35).	Triticum aestivum.

Figure 3. 4. The process of elimination for distinguishing between the *Triticum* and *Hordeum* genus (after Ball et al., 1996).

As well as wheat varieties, we may also encounter types of barley. Naked six-rowed barley (*Hordeum vulgare*) is known in Denmark from Funnel Beaker contexts at Sarup, on south west Fyn and also Stengade and Spodsbjerg, on Langeland Island (Zohary and Hopf, 2000). The long cells of six-row barley are ca 12-15 microns in the middle portion of the wheat husk (Rosen, 1992, 136). The wave shape of thick cell walls is often squarish (fig 3.6a) and of even amplitude ca 10 microns, unlike the thinner counterparts that have more pointed ends and wave lengths ca 7 microns (*ibid*., 136) (fig 3.6b).

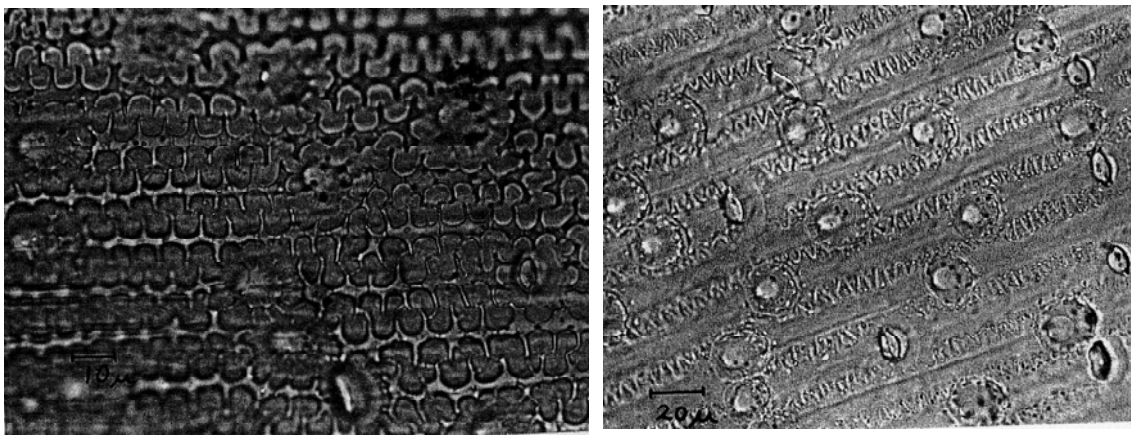


Figure 3. 5., a) the thick long-cells of the middle portion of naked barley husk., b) the thin long-cells of the same species (Rosen, 1992, 138).

There is some overlap in the number of pits that are to be found circling the papillae in all these species. Wild emmer *(T. dicoccoides*) is clearly separable with 16-18 pits (Rosen, 1992, 136). However, domesticated emmer (*T. dicoccum*) and two row barley (*Hordeum distichon)* both have 10-12 pits, but are distinguishable on the basis of papillae size, since barley has much smaller papillae diameter (*ibid.,* 143). Einkorn (*T. monococcum*) on the other hand has 12-14 pits (*ibid.,* 143), meaning overlap with domestic emmer, and also the overlap of papillae size, as we have seen. So, methods for confidently distinguishing these two remain in the realms of population statistics.

Phytolith research has contributed important findings to Near Eastern plant domestication stories, at both the site and regional scale. In a survey of sediments from Late Epipaleolithic (c. 12,000-10,000 BP) to Neolithic (c. 10,000-6,500) sites in the Levant multi-celled phytoliths from cereals took on higher proportions through the sequences, as did the percentage of cereal phytoliths, concurrent with decreases in wild plants such as *Phragmites* sp. (Rosen, 1993). The first appearance of wheat inflorescence silica bodies was from the earliest Natufian layers at Hatula in central Israel, for which there are no other macroremains of cereals at the site, and very few in the region (Rosen, 1993). Phytoliths in the Pre-Pottery Neolithic A layers at Hatula are mainly from cereals, including mostly wheat. And the increased evidence of dendritic cells means that it was inflorescences/seeds that were the object of exploitation. During the Pre-Pottery Neolithic B period percentages of cereals for three sites in the area are consistently in the region of 15%, showing that cereal use was becoming important across a wide area (Rosen, 1993). Were it not for phytoliths

the accumulating knowledge about the processes of domestication would not be possible, poor preservation of macroremains simply does not allow for resolution on these issues.

So, in the Near East phytoliths are important contributors to agriculture adoption debates, but they have had little impact in northern Europe. The research that has been done on the Poaceae family may be of limited breadth in terms of other potentially important plant foods to southern Scandinavian hunter-gatherers, but it does target evidence that will contribute to a core of debates about the origin and spread of these crops. The lack of reference material for *other* plants that could have contributed to cuisine poses a challenge, which will involve negotiating a tight scaling to the investigations. The combined use of phytoliths with other plant microfossils promises a greater corroboration of findings though, so they are worthy of further investigation.

3.3.1.3. Phytoliths in carbonised foodcrusts.

The thermal durability of phytoliths makes them eminently suitable microfossils for studying carbonised foodcrusts. Compared to other microfossils phytoliths have been utilised the most in the context of pottery residues (Boyd *et al.,* 2008; Hart *et al.,* 2003; Staller and Thompson, 2002; Thompson *et al.,* 2004) especially in the Americas where research into maize cultivation and the phytolith record have been pioneered by Dolores Piperno. To a certain extent however, the focus on important domesticated crops that has been evident in phytolith analyses, imposes restrictions on what the techniques can be used to say in the context of pottery. Maize domestication occurred first in south-western Mexico around 7,000-9,000 years ago (Thompson, 2006), but pottery was not involved in the original dispersal, becoming important several thousand years later. Since the record of other food plants is relatively sparse by comparison, the engagements with questions about the social construction of foods, and socialising as related to consumption activities which are driven from broader archaeological quarters, have been very limited.

In a study of three vessels from sites in Ecuador, Thompson (2006) was able to suggest that once particular varieties of maize were introduced to an area, traditions of use for that particular variety persisted for thousands of years. In statistical cluster analyses measurements of rondel phytolith assemblages- the proxy for *Zea* mays-

were found to group into three. Archaeological phytolith samples from foodcrusts from the sites of La Emergencia and Palmitopamba in Ecuador were found to be most consistent with one particular cluster that included *canguil* and *pisankalia* varieties. These two sites are separated by a period of 1400 years (Thompson, 2006), evidencing a regional tradition. In the absence of indicators of other plants though, or without multi-disciplinary data that could suggest an animal food component, it is difficult to tie these findings into a culinary narrative, to explore the value of maize in its social and economic dimensions. Ceramic residues are excellent media for debates about the timing of certain processes as key features in their explanation though. This is because ceramics themselves are often part of well-established typologies, and where the reservoir effect is not an issue the foodcrusts themselves can be directly dated by AMS. In southern Scandinavia the relationship between pottery and early domesticates remains to be established by direct means, so phytolith analysis could be very valuable in this context.

It is fortunate that Old World cereals have a secure genus level identification that can be deployed without recourse to statistical measures of assemblages, although these are advocated where possible (Albert *et al.,* 2008; Ball *et al*., 1999). The reason is that it is unclear how long the residues from southern Scandinavian vessels have been accumulating for. Some of the sites such as Åkonge and Stenø span the transition to agriculture (c.4100-3950 BC), and their foodcrusts may be a palimpsest of wild and domesticated plant foods. It is likely they would respond poorly to statistical measures of wild or domesticated varieties of cereals based on dendritic long cell length (Ball *et al.,* 1999) if they were a mixture.

3.3.1.4. The production of phytoliths in northern European plant families.

Although most temperate European species have not received attention from phytolith research phytolith production is largely under genetic control which establishes a precedent for predicting which plant families will consistently silicify their tissues (Wallis, 2003; Piperno, 2006; Feng Ma and Yamaji, 2006). Appendix I cross references identification research done in non-temperate regions with those same families known to be native to northern Europe. It is hoped that this method can give an early indication of the families prone to silicification, as a forerunner to reference material accumulation and comparison.

Of the 113 families of herbaceous angiosperm, trees, sedges and grasses recorded 5 are given a rating of 1 by Piperno (2006), which means the family is a high producer, and phytoliths can often be identified to family and genus/species. These include families with small numbers of edible species in northern Europe such as the Urticaceae family which contains edible nettles (eg. *Urtica dioica*), the Orchidaceae family which includes a number of species of edible orchid such as pyramidal orchid (*Anacamptis pyramidalis*) and early purple orchid (*Orchis mascula*), both of which have edible underground organs. The Cucurbitaceae family is another important producer, but in northern Europe only the plant black bryony (*Bryonia dioica*) is classified to it. Black bryony does have edible seeds however.

Two families are of particular significance for being grade 1 phytolith producers and having a large number of edible northern European plants classified to them. The Asteraceae family includes many herbaceous plants such as dandelion (*Taraxacum officinalis*), yarrow (*Achillea millefolium*) which has been known historically as a flavouring agent, and burdock (*Arctium minus*). Within this family are also many of the daisy-like plants such as chamomile (*Anthemis arvensis*), and the edible thistles (eg., *Onopordum acanthium*). Between these families a significant proportion of potentially edible and widely available woodland and meadow plants would seem to show a high chance of silicifying their tissues. These plants are not only edible, but in some cases have medicinal qualities too.

Of the remaining families in Appendix I the vast majority are not known to be poor phytolith producers, rather they have not been studied. One important exception is the Rosaceae family which includes many fruiting species such as plum (*Prunus* sp.), hawthorn (*Cratageus* sp.), rowan (*Sorbus aucuparia*) and dog rose (*Rosa canina*). This family is part of Piperno (2006) grade 5, which is those plants that produce badly or which simply have not been studied well for their taxonomic value to be stated.

3.3.1.5. Summary and evaluation of potential for cuisine.

The durability of phytoliths is an important requisite for their survival in ceramic residues, and based on the applications of this technique across the Americas it seems likely that northern European phytoliths will also have endured since prehistory. Their durability is also important for their recovery from the residue

matrix, and means phytoliths have been extracted using the same techniques as for starches. For example, 6% sodium hydroxide (H_2O_2) with mechanical disaggregation allowed for the dispersion of starches and phytoliths into solution. These were both then extracted by heavy-density liquid separation (Zarillo *et al.,* 2008).

It has been shown that there is really an absence of attempts to use phytoliths in northern Europe. This is justifiable considering the less than optimum conditions for their production, but this fact could work in the favour of identifications because less *possibility* for production also reduces redundancy in the overall population. So, if phytoliths *are* found they are from a more limited number of species. Considering this deficit in research, and the large numbers of *possible* plants that could have been important not only for food, but also such things as medicine, a scaled approach to reference collection and study is essential. Investigations carried out here into northern European phytoliths from plant foods must be considered preliminary and evaluative, to judge whether phytolith research can really contribute to pottery residue research in this climate. Well-published identification keys for cereal crop species justifies the technique for interrogating the relationship between pottery and the adoption of cereals, and their relative importance to wild foods though.

3.3.2. Starches.

3.3.2.1. What are starches?

Starch grains are semi-crystalline, water insoluble granules (Miles *et. al.,* 1985) that are produced by plants as a carbohydrate store (Cortella and Pochettino, 1994; Hardy, 2007), and can account for 16-24% of the total weight of a root or tuber crop (Hoover, 2001). Starches are mainly stored in underground storage organs; roots, tubers, corms, rhizomes, but also seeds, grains, nuts, as well as being present as transitory grains in most tissues (Gott *et al.,* 2006). This latter type has a simple structure that is easily moved around to storage areas within the plant. Starch grain morphology is related to the plant species and plant part it comes from, and intra-species variability is the basis for taxonomic keys. These keys are in a state of constant update, but have focused on important domesticated economic species, to the detriment of wild plants. Compared to well-established pollen analysis for example, starch taxonomic keys are still in a state of emergence (Horrocks, 2004).

Reducing the complexity and variety of granules to the description 'starch' is a misnomer. There is a huge range in the details of molecular and granular structures that have great bearing on the susceptibility of starches to taphonomic processes, both in positive and negative favour. Gelatinisation is the process of catastrophic molecular disorder that occurs with the application of excessive heat (Ratnayake and Jackson, 2006). A temperature of 50°C is often cited as the point at which gelatinisation of the granules begins to happen (Gott *et al.,* 2006), and whilst this is true in general, it also implies that when preservation *does* occur it is the exception rather than a rule(s) to be explained and understood. The durability of starches to heating and cooking activities is a major subject in what follows in the evaluation of starches for cuisine investigations.

Recent methodological advances have led to the increased recovery and identification of starches from challenging substrates, such as dental calculus (Hardy *et al.,* 2009; Henry and Piperno, 2008), stone tool residues (Perry, 2004; Piperno and Holst, 1998; Piperno *et al.,* 2000b; Piperno *et al.,* 2004; *Yang et al.,* 2009), coprolites (Horrocks, 2004; Horrocks *et. al.,* 2004) and food residues associated with ceramic vessels (Boyd *et al.,* 2008; Crowther, 2005; Staller and Thompson, 2002). This is not to mention the well established and successfully applied techniques for microfossil extraction from sediments (Balme and Beck, 2002; Horrocks, 2004; Horrocks and Nunn, 2007; Pearsall, 2002).

This improved recovery has gone hand-in-hand with a greater appreciation of the durability of starch, and has led into more experimental work on the complex issue of starch taphonomy. In its turn, greater appreciation has developed for the observable changes in starches that have been subject to taphonomic alteration, and conventional characteristics for identifying starch such as its birefringent extinction cross, lamellae, size and shape characteristics, hilum form and surface architecture (Gott *et al.,* 2006) are also being described for altered granules (Baker and Hobson, 1952; Henry *et al.,* 2009; Horrocks, 2006; Pilar Babot, 2006; Lamb and Loy, 2005; Samuel, 2006).

3.3.2.2. Documenting plant dispersals and domestication with starches.

We now know that starch preservation spans many climatic regions, aiding in the documentation of maize (*Zea mays* L.) dispersal in the Americas (Dickau *et. al.,*

2007; Zarillo *et al.,* 2008), the domestication of taro (*Colocasia esculenta*), yam (*Dioscorea esculenta*), manioc (*Manihot esculenta*), and arrowroot (*Maranta arundinacea*) (Horrocks and Nunn, 2007; Piperno *et al.,* 2000b), and the prehistoric use of cereals (Piperno *et al.,* 2004; Valamoti *et al.,* 2008). In some cases these microfossils are the *only* surviving plant remains, and as such have made important contributions where conventional archaeobotany could not.

The transition to agriculture is a key period in human history, and in the Near East the process is considered to occur 9800-9000 cal BC (Willcox, 2004), based on the size increase of cereal grains and alteration of rachis elements (Hillman *et al.,* 2001; van Zeist and Roller, 1994; Willcox, 1996; Willcox, 2004). Interpretations of cereal domestication have relied heavily on the preservation of intact grains (Warnock, 1998), but with the application of starch analysis to grinding tools from early layers at Ohalo II in Israel the use of wild cereals was in evidence several thousand years before conventional knowledge supposed (Piperno *et al.,* 2004). Starch analysis has led to the re-evaluation of both when human groups began exploiting wild wheat and barley, and also when foragers first employed grinding implements; both of which began in the Upper Palaeolithic 12,000 years before domestication could be evidenced from whole grains (Piperno *et al.*, 2004).

This potential for the recovery of starches from very old residues comes about partly because artefacts can offer micro-environments in pores and cracks, conducive to starch preservation (Barton and Matthews, 2006). This relationship is important because artefacts such as southern Scandinavian pottery have well-established typologies, creating quite high resolution periodisations for the interpretation of plants in direct association with them. By extension, artefacts like ceramics with a high potential for protecting plant microfossils are extensive in time and space making them eminently suitable for the study of site and regional variations in plant values through time.

In the realisation of this potential, economically important plants like cereals have been intensively studied for starch characteristics, knowledge that is transferable from its Near Eastern development centre to southern Scandinavian debates about the timing of cereal introductions and their relative importance compared to wild plant foods. All cereal starch has a bimodal size distribution and this is related to

differences in the granule structures at the molecular level. *Triticum* and *Hordeum* are the two most important genuses in the context of the southern Scandinavian transition to agriculture. In figure 3.7 (Piperno *et al.,* 2004, 671) *Triticum dicoccoides* starch is shown in the top left (a) with the surface craters characteristic to this genus indicated by the arrow. In the top right picture (b) both lamellae and surface depressions characterise the large Type A granules from *Hordeum spontaneum*. More diverse characteristics are recorded from the *Aegilops* genus with *Aegilops genticulata* (c) showing a central protuberance, and *Aegilops peregrina* (d) with very visible lamellae (Piperno *et al.,* 2004).

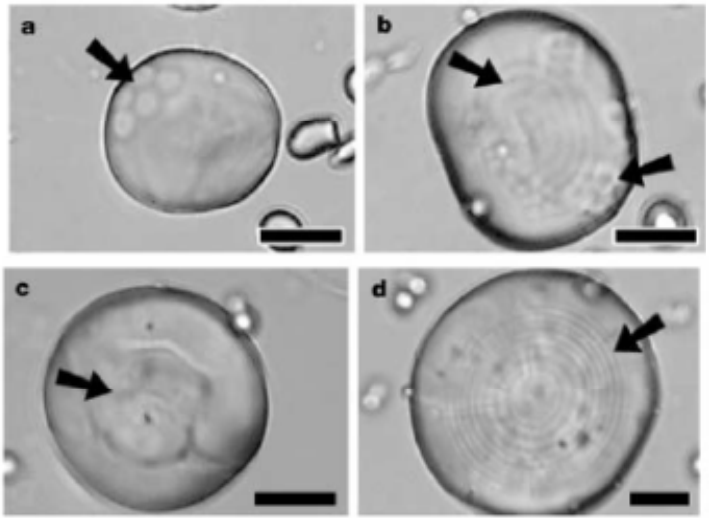


Figure 3. 6., a) *Triticum dicoccoides* showing surface craters, b) *Hordeum spontaneum* with lamellae and surface depressions specific to this species, c) *Aegilops genticulata* shows a central protruberance, d) *Aegilops peregrina* has visible lamellae. Scale bar 10µm (Piperno *et al*., 2004, 671).

Though the description of these economically important crops and their wild precursors is comprehensive, the same is not true of other potentially important wild plants. Recent attempts have been made to collate disparate reference collections into central databases of images and descriptions however, such as a project funded by the Irish Heritage Council which is cataloguing northern European edible plant species (Karen Hardy *pers. comm.*). These databases were unavailable at the time of writing however, and so starch analysis on ceramic residues from southern Scandinavia will require the collection of modern reference plants, that can contribute images to these programmes.

This lack of published comparative reference material beyond only important domesticates (with the exception of Reichert, 1913) has meant that starch analysis has been restricted to a relatively limited groups of workers, with access to reference collections. As a consequence it seems the thrust of research has been on this very specialised documentation of plant dispersals and origin-finding of the earliest domesticated varieties. Whilst important, this forms an almost parallel palaeobotanical agenda to a lot of other archaeologically oriented questions. Aside from the already mentioned lack of discussion of the relative importance of wild versus domestic plant foods (Zvelebil, 1994), little attention has been paid to incorporating the results of starch research into such investigations as the sociality of food production and consumption, the way plant management traditions may have generated group or sub-group identities, and how plant lore was transmitted through ideological means. There is certainly a need for plant microfossil research generally to be incorporated into more interpretive archaeologically-driven research initiatives, and expand from traditional palaeo-economic schools.

3.3.2.3. The durability of starches.

Starch clearly survives in a diversity of situations: the recovery of fossilised starches of fossil mangrove (*Ceriops cantiensis*) from Eocene dated London Clays of Herne Bay and Sheppey, Kent show that some taphonomic pathways can even *increase* the durability of grains (Wilkinson, 1983). Archaeological starches have contributed to debates about the importance of plant foods from context as early as the Palaeolithic at c.11 380 +/- 95 BP in Poland where starches were discovered in preserved parenchyma tissue (Kubiak-Martens, 1996). Remarkably starches have been reported to endure since the Pleistocene c.180, 000 years ago at Sai Island in northern Sudan, in association with pounding implements (Van Peer *et al.,* 2003). Documentation of grains in cooked Egyptian breads, in unaltered forms (Samuel, 1996) shows that the complex physico-chemical properties of such diverse grain morphologies means degradation does not necessarily follow from cooking.

Several factors interplay in explaining the varied responses of starch to heat; the primary mode of degradation in carbonised ceramic residues. This directly bears on whether this microfossil type is suitable for analyses of cuisine in the context of pottery. In general starch morphology can be observed to respond to heat from 50°C upwards (Hoover, 2001; Tang *et al.,* 2001). Within a threshold temperature range a

majority of starch will undergo catastrophic molecular disordering, or gelatinisation. This results from the swelling and loss of crystallinity under conditions of heat (Morris, 1990), which is exacerbated by a hydrous state. The *speed, extent* and to a certain extent *form* that gelatinisation takes depends on variations in the molecular ordering of starches between species.

Two structurally distinct polysaccharides can be extracted from starch: amylose and amylopectin (Morris, 1990). Amylose is an essentially linear polymer containing ~4000 glucose units, whereas amylopectin is longer at ~100,000 glucose units, and is multi-branched giving it supportive properties in the structuring of crystalline regions of granules (Buleon *et. al.,* 1998). Amylose polymers are more linear without the branch points seen on amylopectin molecules. The branches on amylopectin molecules are the primary cause of granule structure. Three types (figure 3.8); A, B and C branches, define different regions of the granule with A and B branches being more numerous than C chains, and cross-linking to extend through the granule. C chains and the branch points that *establish* the cross-linked A and B chains form amorphous layers in the granules, whilst the A and B branches generate alternating crystalline layers. The more linear amylose polymers are woven into the amylopectin structure, and although they branch less they can extend further through the crystalline regions of the granule, contributing to structural integrity. Figure 3.8 illustrates how the structure of amylose and amylopectin contribute to granule morphology.

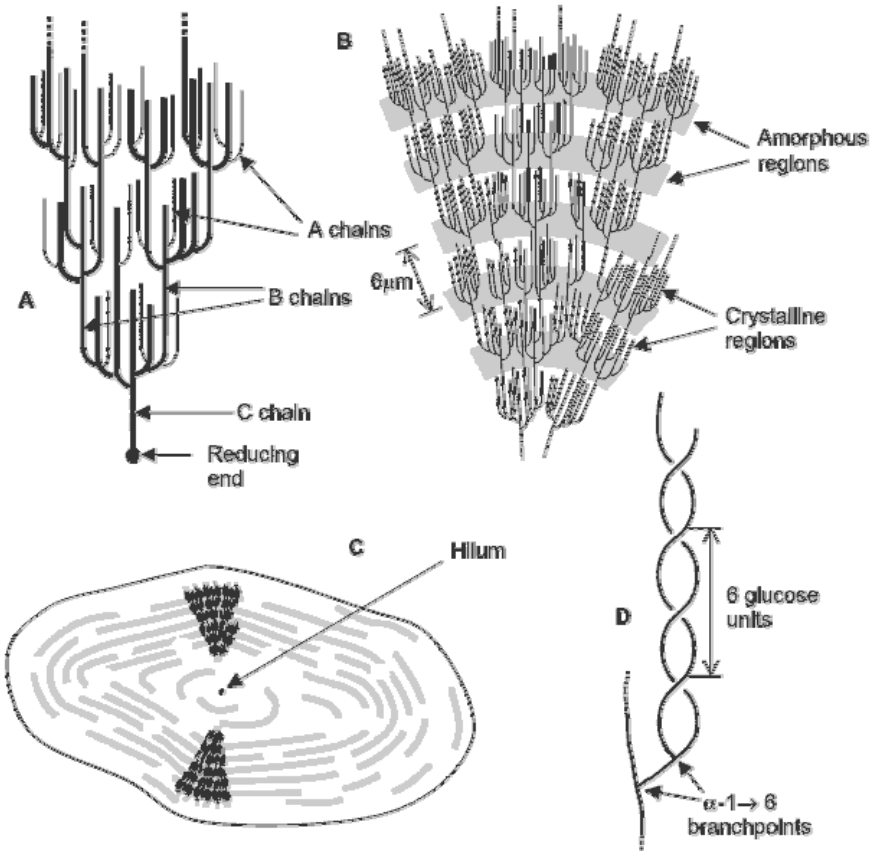


Figure 3. 7. The molecular structure of a starch granule. Linear amylose interweaves with branching polymers of amylopectin. The branch-points equate with regions of greater crystallinity which radiate outwards from the hilum (Sajilata et al., 2006, 3).

Crucially the amylose:amylopectin ratio varies between starch grains, affecting the underlying structural arrangement and stability. Although it is generally considered to be about 20% (w/w) amylose this can vary. Maize is only about 1% (w/w) amylose (Morris, 1990). Higher amylose content lowers swelling capacity, buffering the granules from gelatinisation (Frederiksson *et al.,* 1998; Tester and Morrison, 1990). This is because the amylopectin crystalline regions have a greater capacity to absorb water than amylose, and will function to retain granule molecular order (including characteristic birefringence) if the ratio falls in their favour. So, with a greater capacity to swell and take on water the granules are buffered against catastrophic disordering, the gelatinisation temperature is higher.

Since the amylose:amylopectin ratio changes between plant species as well as between starches in an intra-species granule assemblage, the gelatinisation responses to cooking are diverse. Characteristic gelatinisation morphologies have been recorded for 10 Old World plant foods including four legumes and six grains from

the relevant Poaceae tribe (Henry *et al*., 2009). Characteristic damage can be seen to wheat starches in figure 3.9, where images on the left show fresh granules with characteristic 'crater-like' depressions of the *Triticum* genus and a distinct extinction cross, contrasting to the faded and diffuse extinction cross on the right after boiling for 10 minutes (Henry *et al.,* 2009).

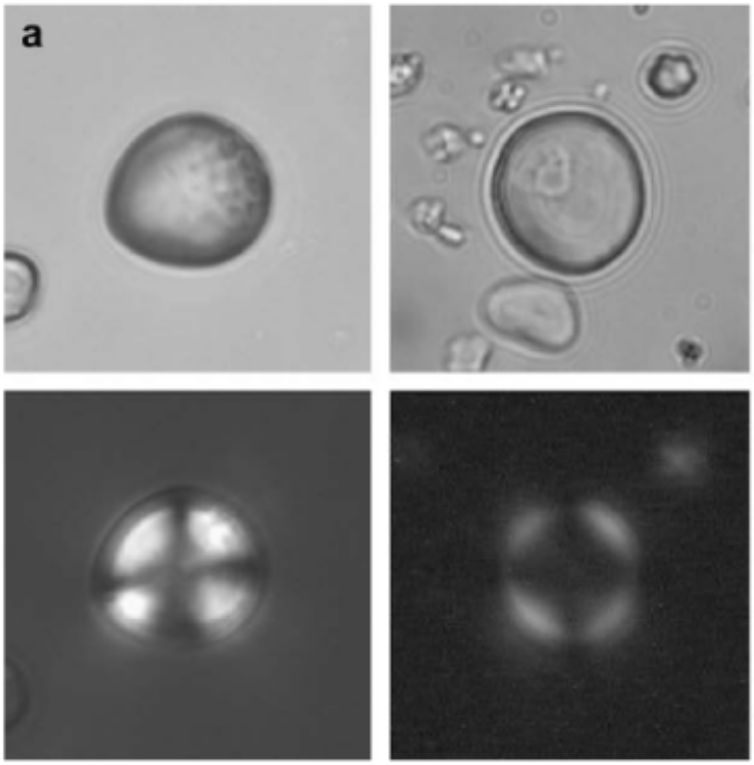


Figure 3. 8. Starches of the Triticum genus. On the left healthy starches with defined characteristics and clear extinction cross. On the right cooked granules have a diffuse extinction cross (Henry et al., 2009, 918).

Some food processing techniques produce unique changes to certain species, but the complex array of molecular ordering in starches from different species means that a single method of processing will not elicit identical responses from different starches (Henry *et al.,* 2009). In general soaking was found to swell the granules only very slightly whilst dimming the extinction cross in cereal starch (figure 3.10. Heat in the absence of external water caused extensive swelling and some loss of water leading to cracking of the granules (figure 3.10). Baker and Hobson (1952) found that air-baked starch changes colour from yellow to brown, and then black. Boiling or baking with water caused granules to swell, lose their extinction cross and appear to soften (Henry *et al.,* 2009). Some granules exhibited very specific responses to heat, such as sorghum (*Sorghum bicolor*) which began to degrade from the hilum which

became depressed after ten minutes of boiling (Henry *et al,.* 2009). These characteristic degradations follow from molecular *re-ordering* as opposed to catastrophic disarrangement (Ratnayake and Jackson, 2006), and since they are a function of species-specific granule composition they cannot easily be predicted. This is especially true considering we know so little about these features in most wild northern European species.

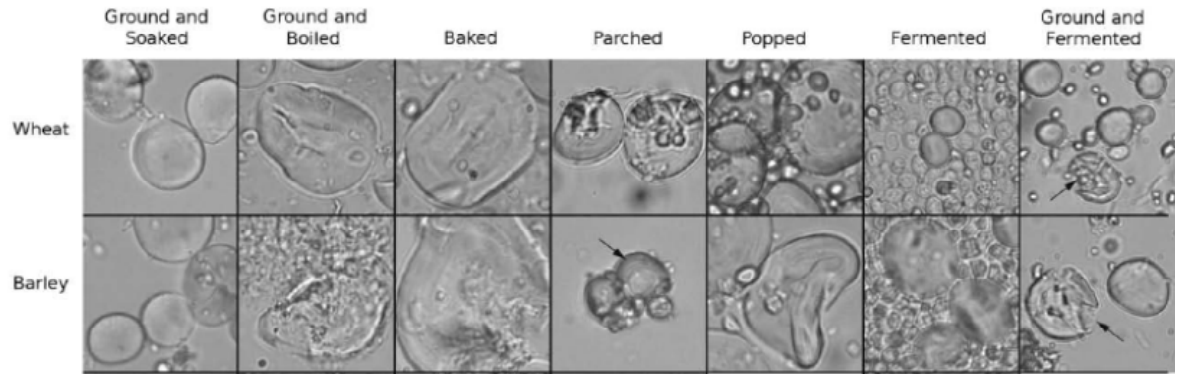


Figure 3. 9. Barley and wheat respond differently to varying processing activities (Henry et al., 2009, 919).

Grain size is related to starch hydration (Torrence and Barton, 2006), with water molecules inhabiting the crystalline regions of the amylopectin molecules (Ratnayake and Jackson, 2006). Small grains generally have less swelling power, or water-binding capacity, meaning gelatinisation occurs relatively quicker than for larger grains, so in theory a granule with a high amylose:amylopectin ratio could counteract gelatinisation by being larger. By implication there is scope for the preservation of unaltered forms of granule if conditions such as not heating at excessive temperatures, the thermal protection of granules at the centre of endosperms or storage organs (Henry *et al.,* 2009), and a high amlyose content are met.

A survival potential is also posed by retrograded grains, a form of resistant starch which derive from crystallized amylose that has reordered on cooling after heating and swelling (Sajilata *et al.,* 2006). Experiments with cooked tubers showed that in samples with gelatinised starch a proportion of the granules were resistant grain varieties, and resisted all thermal alterations to morphology (Gott *et al.,* 2006). Similarly Henry *et al.,* (2009) found a portion of granules in both the *Triticum* and

Hordeum genus' remained unaltered alongside completely gelatinised examples even after ten minutes of boiling.

There are different types of resistant starch (RS), of which retrograded forms (RS_3) are one example. The granule becomes completely hydrated and amylose leaching into solution as a random coiled polymer (Sajilata *et al.,* 2006). When cooling occurs the polymers re-associate with each other and amylopectin as more stable double helices (Wu and Sarko, 1978). This helix re-structuring is very dense and possesses few water molecules, inhibiting the subsequent degradation action of enzymes (Sajilata *et al.,* 2006). In a sample of starches that are at various stages of swelling, amylose leaching and retrogradation it should be possible to expect a range of swollen retrograded forms that have partially substituted their amorphous amylose regions with stable crystalline amylose, as well as resistant granules with unaltered morphologies. This is especially likely considering the repeated uses of vessels that introduces granules at different degrees of alteration, resistance and reception to re-crystallization with double helix amylose.

Resistant $starch_3$ formation has been found to increase significantly with long, low-temperature heating and cooling (Berry, 1986), consistent with the repeated use of ceramics in prehistory. Similar increased RS_3 starch was obtained with repeated heat and moisture treatments (Sievert and Pomeranz, 1989), as in boiling with ceramics. Although RS_3 becomes more stable after thermal alteration, there are other resistant starch types (RS_2) that have an intrinsic resistance due to their molecular organisation. RS_2 types are composed of tightly packed granules that are relatively dehydrated (Sajilata *et al.,* 2006). All types of resistant starch can occur naturally in plant storage organs, in varying quantities. These quantities are undocumented for species relevant to hunter-gatherer diets, making this an important emerging field of starch research, and one that deserves further exploration.

3.3.2.4. Starches in charred deposits.

The application of starch analysis in pottery residues to archaeological problems have been limited, with New World maize (*Zea mays*) agriculture and Australasian taro (*Colocasia esculenta*) domestication being the main regions where an impact on research questions has been made. On the North American Great Plains early maize cultivation was found to be widely practised well before European contact, a date

established on the basis of pottery residue analyses of starch (Boyd *et al.,* 2006). A follow-up study of foodcrust starch extended the impact of maize into much earlier Late Woodland sites on the eastern Canadian Plains around 700 AD (Boyd *et al.,* 2008). In New Ireland Lapita pottery from Papua New Guinea, starches consistent with the Ariod family have suggested the presence to taro (*Colocasia esculenta*) c. 3300 BP (Crowther, 2005).

So far the few applications to archaeological ceramic residues have described the *earliest* findings of certain domestic or non-native plant varieties. The ceramic *context* for the findings has not been discussed; the significance of specific plants to processing in vessels, the reason the *earliest* findings of these species is in a ceramic context, and what this says for innovative relationships between vessels and their contents. This is probably because controversy has existed about the mere existence of starches in carbonised deposits, and despite multiple reports since early in the 20th century (Samuel, 1996), these were sceptically received. Resistant starch forms were only recognised as a phenomenon in the early 1980s (Englyst *et al.,* 1982) in the food science research arena. Confidence in the use of starch analysis for archaeological carbonised deposits has been slow to filter through the disciplines.

3.3.2.5. Methods of analysing starches.

Extraction of starches from the carbonised matrix is a delicate operation considering many of the forms are more susceptible to chemical attack following cooking and burial. In the case of the Lapita pottery residues from New Ireland residues were observed by incident light microscopy (Crowther, 2005), rather than transmitted light. The benefit of this is that it doesn't destroy the foodcrust as no chemical extraction is involved. However, visibility of granules is restricted to those that adhere to the surface of the residue. This not only poses the problem that what is being observed is contamination from the burial environment, but also if starches do represent use-wear and the residue is a layered accumulation what is being observed is only the very last uses of the vessel. The latter is both a pro and a con, compromising high time resolution with loss of other information that is bound into the deeper matrix.

Zarillo *et al.,* (2008) used a 6% solution of hydrogen peroxide (H_2O_2) with gentle agitation for 10 minutes to oxidise the carbonised matrix and release starch grains.

Heavy-density liquid separation was performed with Sodium Polytungstate ($(Na_6(H_2W_{12}O_{40}))$) at specific gravity (sg) 1.3 allowing material with a n sg <1.3 to be siphoned off after centrifuging. This was repeated with Sodium Polytungstate solution at sg 1.7, leaving starches with a specific gravity of ~1.5. The process was repeated at higher concentrations of hydrogen peroxide to remove phytoliths too.

Following extraction of the granules, a limited number of researchers working with artefacts have raised the importance of ruling out residue loading from contaminant starches from the burial environment. Crowther (2005) counted granules on the interior and exterior residues, and compared these to counts on the broken edges to suggest that insignificant contributions of starches from the burial environment were made to the broken edges, pointing to a similar low contamination to the foodcrusts. However, residues on the exterior of the pot should also be considered of a non-food origin as they arise from sooting in most cases. This allows for the possibility of comparing interior and exterior deposits on ceramics.

With other small finds such as stone tools contamination has been discredited by comparing the counts of granules in adhering residues to counts in associated soils. In blind tests of stone tools with residues from Papua New Guinean sites, the number of starches in the residues was compared to associated sediments adhering to the artefacts. The frequency of starch grains was correlated significantly with the use of the stone tools and not with the burial environment in all cases (Barton *et al.,* 1998). Such contamination studies are not always adopted, and this oversight compromises what are otherwise sometimes high profile results (e.g. Piperno *et al.,* 2004).

In order to accurately assign a starch to taxon accurate measures of consistency must be made between archaeological and modern reference starches. Redundancy hampers the identification of starches to their plant origin; this is the morphological overlap of starches from different sources. Only very recently have objective techniques arisen to measure overlap and redundancy accurately. The majority of starch analysis is carried out by observation of the starches in transmitted light, and documentation of their optical properties.

Image analysis techniques in archaeological starch analysis were initiated using multivariate statistical measures (including linear, logistical and nearest neighbour analyses) to classify modern granules to their proper taxon using 15 species native to

Papua New Guinea. This first attempt at automated classification recorded 18 variables, mostly based on surface area and volume to discriminate morphologies (Torrence *et al.,* 2004). The matrix in figure 3.11 shows how many granules were correctly classified and to which species the incorrect classifications were made. The diagonal number series are correctly classified. Only three of the fifteen samples had <50% correctly classified granules, in one case classification was 100% correct. Torrence *et al*., (2004) noted that the starch types are significantly different from each other, and that multivariate methods do classify them accurately. The development of this study to classify Australasian plant species holds little potential for direct application to northern European archaeological contexts however.

	No. of cases	Barringtonia asiatica	Caryota rumphiana	Colocasia fallax	Dioscorea alata	Dioscorea bulbifera	Dioscorea nummularia	Dioscorea pentaphylla	Dioscorea rotunda	Hernandia nymphaelfolia	Hibiscus manihot	Ipomoea batatas	Metroxylon sagu	Musa sp.	Syzygium malaccense	Zingiber officinale
Barringtonia asiatica	50	39	0	0	0	0	0	2	0	0	1	8	0	0	0	0
Caryota rumphiana	100	0	65	8	0	0	0	0	1	4	8	0	0	0	14	0
Colocasia fallax	48	0	11	24	0	2	1	2	1	3	4	0	0	0	0	0
Dioscorea alata	50	0	0	0	46	0	0	0	4	0	0	0	0	0	0	0
Dioscorea bulbifera	50	0	1	3	1	36	6	0	0	1	0	0	0	0	0	2
Dioscorea nummularia	50	0	0	0	1	4	39	0	6	0	0	0	0	0	0	0
Dioscorea pentaphylla	50	1	0	3	0	1	0	33	0	1	5	6	0	0	0	0
Dioscorea rotunda	50	1	1	2	1	2	11	2	26	0	2	0	2	0	0	0
Hernandia nymphaelfolia	50	0	11	2	0	0	0	0	0	33	2	0	0	0	2	0
Hibiscus manihot	50	1	10	2	0	0	0	4	0	1	27	1	0	0	4	0
Ipomoea batatas	50	9	0	0	0	0	0	5	0	0	0	36	0	0	0	0
Metroxylon sagu	100	0	0	0	0	0	0	2	0	0	0	0	98	0	0	0
Musa sp.	50	1	0	0	0	0	0	0	0	0	0	0	0	49	0	0
Syzygium malaccense	50	0	13	0	0	0	0	0	0	1	1	0	0	0	35	0
Zingiber officinale	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50

Figure 3. 10. The matrix shows the number of starches that were correctly classified by multivariate statistical analysis. In general the classifications are highly accurate (Torrence et al. 2004, 525).

The most recent automated-classification method for starch analysis was developed using 26 morphological variables which gives greater sensitivity to differences between classes (Wilson *et al,*. 2010). These variables are described in greater detail in section 5.5.8., of the methodology (Chapter 5). Several hundred starches were analysed from 9 species of economically important crop plants from around the world to test the effectiveness of the classification criteria and the classifier

algorithm used. The trained programme was run on a sub-set of the modern starches that it had not been trained on, as a blind-test. Eight of the nine samples scored >30% correct classifications. The reduced success rate compared to Torrence *et al,.* 2004 is a feature of the species being studied, and their greater redundancy, than a measure of the classification programme, which is sensitive to pixel resolution on a greater number of morphological variables than Torrence *et al.,* (2004). Neither of these methods have been applied to archaeological material, and are poised to make a novel contribution to archaeological starch analysis.

3.3.2.6. Summary and evaluation of potential for cuisine.

The recent acknowledgement that starches can potentially survive incorporation into charred deposits such as foodcrusts means there is scope for these analyses contributing important information to the plant component of cuisine in southern Scandinavian prehistory. The lack of investigations that have linked starch analysis to archaeologically driven questions about the role of plant foods, their contribution to sociality through production, processing and consumption, means this potential is of significant importance. By incorporating starch data into a multi-disciplinary analysis of cuisine, it may work to expand debates that are currently focused on finding the 'origins' of important crop plants. Whilst this is important in the context of the transition to agriculture in southern Scandinavia too, it is not possible to explain any changes by isolating a very limited number of species for study.

The accurate automated classification methods developed by Wilson *et al.,* (2010) require application to archaeological material. Sensitive measures of redundancy are a problem for starch analysis, where observational 'manual' identifications prevail, and correct classification cannot be measured. For starch analysis to build momentum as a viable sub-discipline within archaeobotany, these are problems that *must* be addressed, and it is only by tacking archaeological questions that their value can be established.

3.3.3. Calcium oxalate crystals.

3.3.3.1. What are calcium oxalate crystals?

Both monocotyledonous and dicotyledonous plants produce calcium oxalate crystals (CaOx) to regulate calcium production, and protect against herbivory (Franceschi and Nakata, 2005). Although calcium oxalates can precipitate in a variety of

environments, their biomineralization in plants is not a random process. Calcium oxalates precipitate in both intracellular and extracellular situations within plant tissue, but it is genetically regulated so the *forms* the crystals take in plants are distinctive. They are formed within a membrane-lined 'crystal-chamber' in crystal idioblast cells which acts as a mould determining their shape (Franceschi and Nakata, 2005).

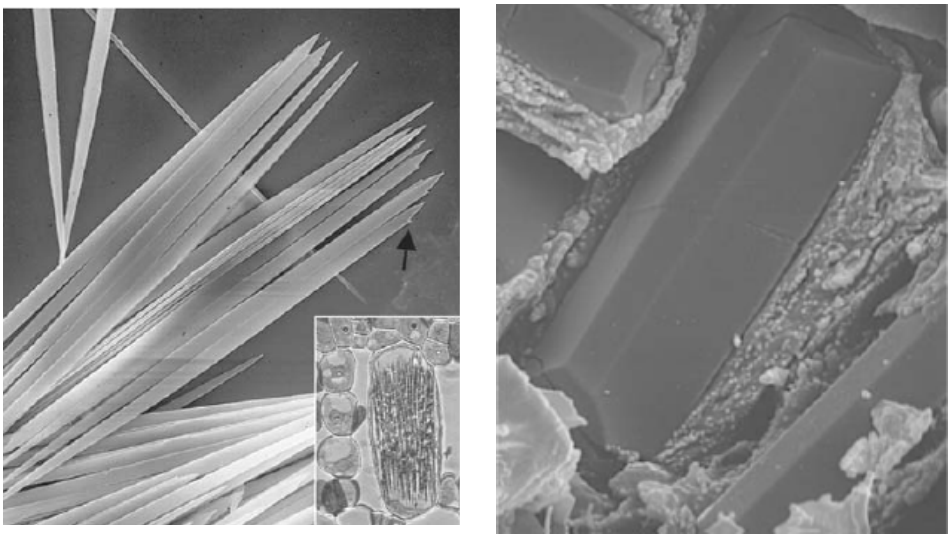


Figure 3. 11. On the left elongated raphide calcium oxalate crystals in SEM. Right, prismatic styloids embedded in the cell wall in SEM (Franceschi and Nakata, 2005, 43).

There are four categories of plant CaOx; raphides, styloids, druses and crystal sand. Raphides form as elongate bundles of needles (figure 3.12) and can irritate the digestive tract if eaten unprocessed. The rhomboid or prismatic shape of styloids (figure 3.12) is the only form to be produced in cell walls. Druses are multi-faceted crystals thought to be a conglomeration of multiple smaller crystals around a nucleation site (Prychid and Rudall, 1999). Finally, crystal sand is a mass of tiny crystals deposited in a single cell.

The latter two types are the most difficult type to identify correctly in light microscopy because of the overlapping similarity in their morphology to crystalline structures precipitated in the absence of a plant 'crystal-chamber'. Druses are common in dicotyledonous plants but relatively rare in monocotyledons (Prychid and Rudall, 1999), giving them some taxonomic potential.

Raphides and styloids are more reliably identifiable in light microscopy. Raphide producing plants engineer size differences in the crystals, but there are also reports of

shape modifications. Most raphide crystals are four-sided in cross-section (Prychid and Rudall, 1999), but at least six sides appear in taxa like *Yucca, Agave and Cordyline* (*ibid.*). In *Typhus angustifolia* crystals are hexagonal towards their ends and octagonal in the centre (*ibid.*). Raphides in the Araceae family are grooved along their longitudinal axis (Ledbetter and Porter, 1970). Styloids may have pointed ends, squared ends, be elongated or squat cuboid (Prychid and Rudall, 1999). In *Allium* they are twinned interpenetrated styloids (Arnott, 1981).

3.3.3.2. Calcium oxalate crystals in ceramic residues.

Calcium oxalate crystals are rarely used in archaeological case studies as an indicator of plant taxa. One exception is the collaboration of starch analysis with calcium oxalate analysis in Lapita pottery residues from Anir, New Ireland (Crowther, 2005) to lend credence to the interpretation of taro (*Colocasia* esculenta) processing in the pottery. As a microfossil in their own right they are lacking the research that would consolidate their usefulness; systematic investigation of morphological differences across a range of taxa being one example. However, there is enough published information of presence and absence (Franceschi and Horner, 1980; Prychid and Rudall, 1999) in northern European families for them to be useful supportive evidence for other microfossils. Their insolubility except in conditions of extremely low pH makes them likely survivors of taphonomic processes (Crowther, 2005).

3.3.3.3. Summary of the potential of calcium oxalates.

The recovery of calcium oxalate crystals is a secondary consequence of starch extraction procedures involving 6% hydrogen peroxide (H_2O_2) soaks, and so calcium oxalates require no extra preparation. The limited morphological distinctiveness means that singly they have only limited taxonomic potential. In collaboration with other microfossils such as starches though, they can be corroborative or unsupportive of interpretations that would otherwise suffer from the pitfalls of redundancy.

3.4. Conclusion.

This chapter has discussed a number of techniques for the investigation of absorbed residues, as well as carbonised surface adherences. The high potential for survival of lipids in well-preserved forms was demonstrated by Craig and Heron (2006) for the same study areas as are proposed for this research. With this in mind, the techniques of Gas Chromatography, Gas Chromatography-Mass Spectrometry, and Gas

Chromatography-Combustion-Isotope Ratio Mass Spectrometry are suitable to the analyses of absorbed and 'foodcrust' residues. These techniques have been demonstrated to offer adequate column separation of lipid molecules from foods that are of interest in southern Scandinavia; aquatic marine, freshwater, ruminant adipose, porcine, ruminant dairy. In combination, the three methods separate lipid compounds and provide identification information from molecular weights of those compounds, plus registering a single compound isotope signature. The three-pronged strands of enquiry can offer mutually reinforcing evidence for the processing of a food in a pot, or contradictions that would suggest food combinations. The benefit of using Gas Chromatography-Mass Spectrometry is also that it registers the presence of known biomarkers for important foods, such as fish. These biomarkers are molecules unique to a food product, or brought about by a specific way of processing foods, and are useful for confirming identifications and suggesting processing activities in the past.

There is one area where lipid residue analysis is challenged, and this is in the identification of which plants were consumed from ceramics. The reasons are the mainly unsaturated lipids of which plants are composed which preserve badly, the relative lack of lipid in plants compared to animals and to a certain extent a research bias in favour of animal foods that has led to a deficit in extensive studies of plant biomarkers. In instances where plants have been targeted for research this is usually for the study of key economic species, such as grapes (*Vitis* sp.) and olive oil. As has been shown, plant lipid analyses often include novel approaches that would extend the three-pronged repertoire of GC, GC-MS and GC-C-IRMS. The potential returns for a methodological extension of this sort are likely to be low. Although techniques such as mass spectrometry coupled with Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APCI MS) offer the potential to separate very small molecules more effectively, the subsequent identification of these molecules to northern European wild plant species is unsupported by reference materials. Within the triple method approach suggested for this project however, leaf wax from the Brassicaceae family can be identified, giving a point of corroboration for other plant identification techniques.

Such techniques for finding and identifying plants in the past include primarily starch and phytolith analyses on foodcrust deposits. Starch analysis has had limited

application to carbonised residues, and is an area where important improvements can be made to methods. The possibilities for the preservation of starches are evaluated, and it is suggested that they have complex physico-chemical structural variations that mean granules respond differently to the introduction of heat. The existence of retrograded starch forms and other resistant starch types in most plants helps to explain the numerous documented instances where starches survive extremes of heat and pH, contrary to what traditional descriptions of their characteristics would postulate.

Phytolith analysis has been little used in temperate Europe, and so its potential contribution to the study of plants in foodcrusts is difficult to assess. Preservation of silica bodies is likely to be good as they are thermally durable, but the main issue is whether phytolith-producing plants inhabit northern Europe, or whether the climate is conducive to their production. An evaluation for phytolith production is made of plant families that coincide in both tropical and temperate climates, to suggest a *potential* for successful application of this technique. The findings suggest that an experimental trial of phytolith analysis is worthwhile. In addition, the incidental recovery of calcium oxalate crystals will be recorded as these have correlated starch evidence for the processing of taro (*Colocasia esculenta*) in pottery (Crowther 2005).