

The Survival of Food Residues: New Methods of Analysis, Interpretation and Application

R. P. EVERSLED, C. HERON,* S. CHARTERS & L. J. GOAD

*Department of Biochemistry, University of Liverpool, P.O. Box 147,
Liverpool L69 3BX, U.K.*

Summary. In order to gain a better understanding of the nature of organic matter of archaeological interest, chemists are faced with the task of characterising a wide range of natural products. Gas chromatography (GC) and combined GC/mass spectrometry (GC/MS) are the methods of choice for the analysis of complex lipid mixtures. Application of these techniques to the investigation of amorphous residues preserved in potsherds would appear to hold considerable promise for use in studies of diet and vessel function. Modern capillary GC columns achieve complex mixture separation of a wide range of compound classes, e.g., intact acyl lipids, free fatty carboxylic acids, sterols and hydrocarbons. Computerised data-handling techniques are essential for processing the large amount of gas chromatographic and mass spectral data which arises from potsherd analyses. A large number of potsherds are being analysed in an effort to investigate patterns of vessel usage. The characteristic compounds, or groups of compounds that are revealed correspond to 'markers' or 'indicators', and provide a 'fingerprint' that can be used chemotaxonomically, to deduce the nature of foodstuffs associated with ceramic vessels and classify vessel function. Interpretations are supported by the analysis of lipid extracts of contemporary foodstuffs, and by the results of degradation and cooking simulation experiments.

* Current address: Department of Archaeological Sciences, University of Bradford, Bradford BD7 1DP, UK.

Read 14 February 1991. © The British Academy 1992.

1. Introduction

Insights into the diets of early societies can be gained, indirectly, from cultural evidence (artefacts related to food procurement, preparation and consumption) and human skeletal remains (Gilbert and Mielke 1985). However, more direct evidence for dietary constituents derives from the identification of intact plant and animal remains, e.g., charred seeds and grain, pollen and animal bone (Dimbleby 1978; Parmalee 1985; Carbone and Keel 1985). This approach inevitably introduces a bias toward more durable materials, with the result that the question of 'missing foods' exist in many attempts to characterise dietary components (Hall 1987; Hillman 1989). Problems of this nature emphasise the need for a multidisciplinary approach to palaeonutritional and dietary studies.

One technique involves the examination of the vessels and implements used in the storage, preparation and consumption of food since the form of a ceramic vessel or artefact may have a functional relationship to a specific cultural practice (see Rice (1987) for an overview of vessel form and use-related properties). A perhaps obvious, and potentially more specific, approach to deducing diet, is to examine the amorphous remains of foodstuffs associated with artefacts. Organic residues adhering to the surface, of say a flint cutting tool, or absorbed into the porous fabric of an unglazed ceramic cooking vessel, should provide information regarding usage and diet.

Potsherds occur ubiquitously in the archaeological record, and thus offer particular promise for systematic study. The basis of our approach is founded on the phenomenon of transfer of organic matter between foodstuffs and the vessels, and other utensils, used in the preparation and consumption of the food. Our objective is to detect the food remnants, adhering onto, or absorbed into, the wall of a ceramic vessel, during its usage, and relate their chemical composition to a specific faunal or floral origin. This paper discusses our approach to the study of preserved organic matter associated with buried potsherds, with particular focus on the derivation of dietary information.

2. Analytical strategy

The interdisciplinary analytical strategy adopted in this work is summarised schematically in Figure 1. A close collaboration between analytical chemists, biochemists and archaeologists is fundamental in the delineation of problems to be addressed. Moreover, the archaeological expertise is essential in assisting in the sampling of individual potsherds for investigation and in providing conventional ceramic interpretations, contextual and environmental information.

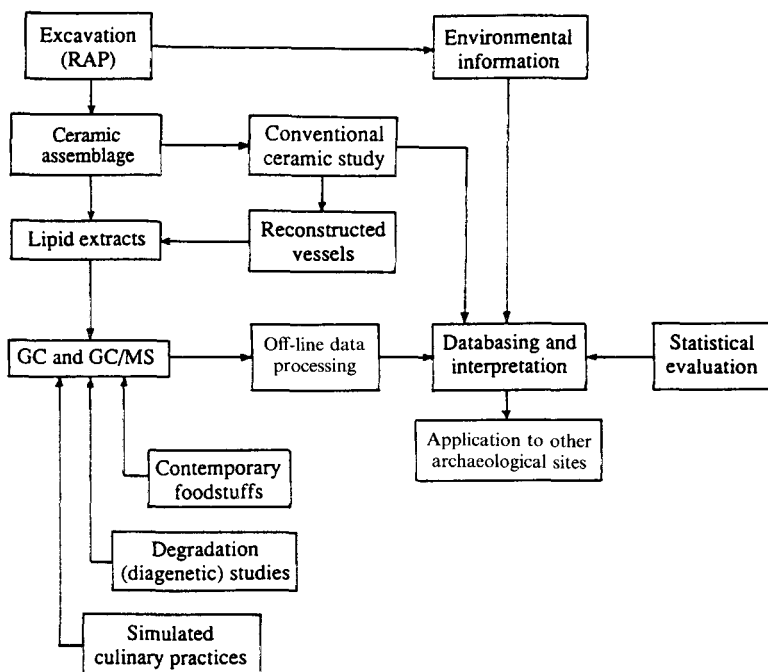


Figure 1. Analytical strategy adopted for the investigation of organic residues preserved in potsherds. RAP refers to the Raunds Area Project, which has provided the majority of the potsherds we have investigated to date.

Until now the analysis of organic matter associated with potsherds has been attributed little more than novelty value. A contributory factor to this situation has been the tendency for analyses to be performed upon single vessels or small groups of vessels of particular form or function. The approach we are developing involves carrying out analyses of large numbers of potsherds. In this way it will be possible to determine whether or not the organic residue found in a particular vessel is 'typical' or 'atypical' of its type, date or context. Thus, we will be able to judge the significance of the use of one vessel, or a series of vessels, against a background knowledge of general use (Heron *et al.* 1991a).

In our experience, the occurrence of visible surface residues is atypical. Hence, in order to provide a technique of potentially wide application to archaeology, we have concentrated on developing the investigation of organic matter absorbed into the pot wall. Clearly, this approach may be limited in the case of well-glazed pottery, but as much early pottery is unglazed or poorly glazed there is considerable scope for this analytical approach.

Few of the investigations of the preserved organic matter associated with potsherds, or intact vessels, have succeeded in confidently identifying food constituents (see examples given in Jones 1986). Failure to achieve positive identifications in early work arose from a lack of analytical techniques capable of detecting and characterising the low levels of organic matter preserved in many samples. Additionally, even in some more recent studies, there has been a tendency to over-simplify the analytical approach required to characterise the complex organic residues, especially in the light of pre- and post-depositional chemical changes that will have ensued (see section 5).

It is our contention that characterisation of the organic residues associated with buried potsherds must begin with the identification of the individual compounds present. Only in this way is it possible to recognise and differentiate between preserved compounds, and compounds whose structures have altered, either by cooking or post-depositional diagenetic processes. In essence we are addressing a problem of biochemical phylogeny or chemotaxonomy, for which precise structural information is a prerequisite (Nes and Nes 1980). The characterisation of organic residues relies on matching a specific compound or mixture of compounds to that of a contemporary plant or animal natural product likely to have been exploited in antiquity. Mills and White (1987) provide a useful review of the chemical composition of some appropriate natural products.

Although we are considering all classes of molecule comprising foodstuffs as potential chemical 'indicators' or 'markers' of diet, our investigations have focused initially on the lipid components. Lipids offer considerable potential for use in the classification of the organic residues in general, owing to the huge body of biochemical data that exists regarding their: (i) structure; (ii) distribution in natural materials; (iii) biosynthesis, and (iv) degradative pathways. In addition, lipids have hydrophobic properties, which will limit their translocation from potsherds, particularly by dissolution in waterlogged environments (*cf.* Evershed and Connolly 1988; Evershed 1990, 1991), unless severely structurally altered or degraded. Moreover, analyses of potsherds and adhering burial soil, recovered from Raunds, Northamptonshire, U.K., indicate that lipids migrating from the burial soil are not a serious source of interference (Heron *et al.* 1991b). The analysis of soil samples to serve as controls should be a routine procedure in any organic residue analysis programme. Contamination by microbial lipids does not appear to be serious; however, this subject is poorly understood and worthy of further investigation.

The following discussions are restricted solely to the techniques used in the investigation of lipids occurring in potsherds, and their application to the determination of diet and vessel usage. Presented here is an overview of the analytical protocol used in our laboratory for the investigation of lipids from

potsherds. Our approach to the interpretation of the compositional information derived from GC and GC/MS analyses is summarised. Central to interpretation is a consideration of the decay processes known to occur in the lipid constituents of foodstuffs. We have therefore taken this opportunity to introduce some of the more commonly occurring degradative pathways of lipids, with examples drawn from our recent work.

3. Experimental methods

3.1 Sample preparation

Methods for the analysis of lipids from biological materials are well developed (see Kates (1989) for a compilation of general methods) and readily applied to the examination of potsherds. Owing to the small amounts (often < 1 mg/g) of lipid generally present in potsherds, refinement of the standard analytical approach is required to avoid contamination of the endogenous lipids. Many of the substances used in subsequent interpretations occur widely in nature and handling of the sherds should be avoided to minimise contamination with lipids from the hands. A less obvious source of contamination are laboratory reagents and glassware. All glassware must be scrupulously clean and reusable glassware should be washed with powerful oxidising agents (e.g., concentrated nitric acid) prior to use; washing with organic detergents must be avoided. Disposable glassware, e.g., vials and pipettes, should be used wherever possible, to avoid cross-contamination between samples and all new glassware must be washed before use. All reagents and solvents must be of high grade. Where doubts exist concerning the purity of solvents they should be distilled before use.

Lipids are obtained by solvent extraction (chloroform/methanol, 2:1) of powdered sherds (typically 2 g samples) or visible surface residues (sample sizes vary according to availability). Ultrasonication to aid the extraction process is preferred to other techniques, e.g., Soxhlet extraction, as smaller sample sizes are readily accommodated. Moreover, the solvent volumes should be kept small (*ca.* 10 ml) and extractions carried out at ambient temperature, so minimising potential problems of contamination and degradation of extracted lipids.

3.2 High-temperature gas chromatography

Evaporation of the extracting solvent affords the total lipids. Gas chromatography has been employed previously to investigate lipid extracts derived from potsherds. Until now analyses have been performed on low resolution packed columns (e.g., Condamin *et al.* 1976). Capillary GC columns offer spectacular improvements in resolution compared to packed columns; thus

greatly enhancing the compound identification and compositional information that can be derived from the small amounts of complex lipid mixtures obtained from potsherds. In addition, the analysis of these extracts is greatly simplified by the availability of high-temperature stable crosslinked polysiloxane stationary phases (Evershed *et al.* 1990a). Columns coated with these phases allow the direct analysis of the wide range of lipid species preserved in many potsherds, including low volatility species, such as triacylglycerols, without the need to perform chemical or enzymatic degradations used in earlier investigations. The only chemical treatment of the lipid extract required prior to analysis, is trimethylsilylation of protic sites (e.g., hydroxyl moieties present in free fatty carboxylic acids, monoacylglycerols, diacylglycerols, sterols, etc.) using *N,O*-bis(trimethylsilyl)trifluoroacetamide (or other reagents) to enhance GC properties (e.g., Myher and Kuksis 1984). For routine GC work an apolar dimethyl polysiloxane (OV-1 type) stationary phase is sufficient, while availability of capillary columns coated with high-temperature stable polarisable (65% phenyl 35% methyl) polysiloxane stationary phases, raises possibilities for the separation of high molecular weight intact acyl lipid species according to the degree of unsaturation. This facilitates the detailed assessment of the level of fat degradation in experimental and archaeological samples. Compound identifications by GC involve retention time comparisons to compounds of known structure, and an understanding of the chromatographic behaviour of homologous and other closely related structures. Owing to the small amount of lipid present unambiguous compound identifications must rely on combined GC/MS and there will rarely be sufficient material available for other analytical techniques such as ^1H and ^{13}C nuclear magnetic resonance spectroscopy.

3.3 Gas chromatography/mass spectrometry (GC/MS)

Combined GC/MS is used routinely for the analysis of lipids extracted from a wide variety of biological, environmental and geological materials. GC/MS is regarded as a 'natural combination' as both techniques deal with volatile or semi-volatile compounds, and attain optimum performance with sample sizes in the nanogram (10^{-9} g) range. The high absolute sensitivity of GC/MS means it is well-suited to the study of the small amounts of lipid derived from potsherds. GC/MS work in our laboratory is performed on a double-focusing magnetic sector mass spectrometer. Electron ionisation at 70 or 20 eV is most commonly used to generate molecular (M^+) and fragment ions. Negative ion chemical ionisation, using ammonia as reagent, can also be used to rapidly assess the nature of fatty acid moieties in intact acyl lipids, e.g., triacylglycerols (Evershed *et al.* 1990b). By operating the mass spectrometer at low resolution (typically $m/\Delta m = 1000$), mass measurement to nominal

mass is readily achieved. At this level of performance the mass spectra recorded can be used to identify the majority of lipid species encountered so far in this work.

4. Data analysis and interpretation

Analysis of the raw data derived from the GC/MS analysis of the lipid extracts of potsherds is performed according to well-established procedures. Briefly, the identification of peaks displayed in the total ion current (TIC) chromatogram relies on an assessment of the recorded mass spectra and GC elution orders. The mass spectra of the individual components of the lipid mixtures are interpreted either manually, on the basis of known fragmentations of organic compounds, or automatically, by computer searching of mass spectral databases.

For example, Figure 2 shows the GC profile obtained from the high-temperature GC analysis of a total lipid extract of a Late Saxon/Medieval potsherd from Raunds. The normalised chromatogram suggests a relatively simple lipid composition, the major components, identified from their EI mass spectra, being the free fatty carboxylic acids palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acid. However, closer examination of the expanded (x-axis) GC profile, obtained using computerised data handling facilities, reveals nearly 100 components in all, including other fatty carboxylic acids, monoacylglycerols, diacylglycerols, triacylglycerols, long-chain alkyl compounds and sterols. Other samples reveal a wide range of other compound types, such as wax esters, hydrocarbons, ketones and alcohols.

The various distributions of compounds that are observed represent the net result of a range of pre- and post-depositional and post-excavational processes. Hence, the organic extracts of potsherds can contain:

- i) substances absorbed by the vessel during usage, and preserved in their native (undegraded) state;
- ii) substances absorbed by the vessel during usage, which are structurally altered (degraded), initially through a particular culinary practice, or subsequently, during vessel use;
- iii) substances absorbed by the vessel during use which are structurally altered, chemically or enzymatically, during burial (natural decay);
- iv) substances arising through migration from the burial environment, or microbial and fungal activity, leading to lipid deposition within the potsherd, and,
- v) substances transferred to potsherds due to post-excavational activities (contamination, e.g., plasticisers and lipid from hands).

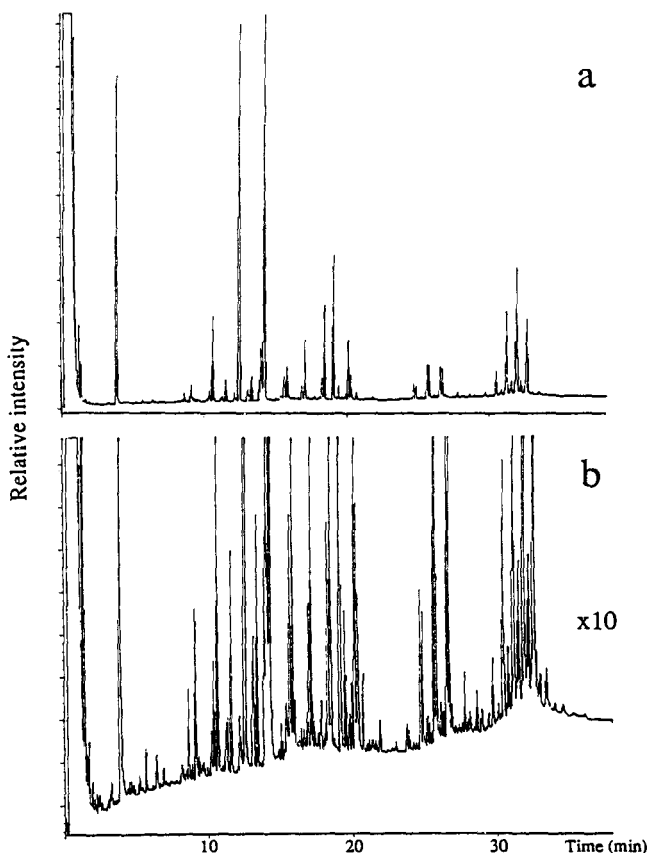


Figure 2. GC profiles obtained for the total lipid extract of a potsherd from a Late Saxon/Early Medieval cooking vessel showing (a) the chromatogram normalised to the major component (stearic acid) and (b) expansion ($\times 10$) of the x-axis to reveal nearly one hundred components in all. The peaks were identified from their electron ionisation mass spectra obtained by combined GC/MS. The compounds present comprise mainly acyl lipids, ranging from free fatty carboxylic acids (as their trimethylsilyl (TMS) ester derivatives) at short retention time (12.5 mins), to intact triacylglycerols eluting at longer retention times (30 mins). The peak identities of the major components are given in Figure 2 of Evershed *et al.* 1990b. The analysis was performed on a $12\text{ m} \times 0.22\text{ mm}$ i.d. fused silica capillary column coated internally with BP-1 stationary phase (immobilised dimethyl polysiloxane, $0.1\ \mu\text{m}$ film thickness). Following on-column injection at 50°C , the GC oven temperature was held at that temperature for 2 mins, programmed to 350°C at 10°C , then held at that temperature for 10 mins. The carrier gas was helium at a column head pressure of 25 psi. A flame ionisation detector was used at a temperature of 350°C .

In addition, the identification of food residues must take account of a range of non-culinary activities, such as the use of natural organic sealants (for example, resins and other organic extracts; *cf.* Rice 1987, 163) that might affect lipid composition and lead to misinterpretations.

5. Chemical changes in the lipid constituents of foodstuffs

A substantial body of information exists concerning the changes that occur in the lipid constituents of contemporary foodstuffs as a result of chemical and/or microbial decay. This information can assist in unravelling the complex mixtures often observed in potsherd extracts. The most commonly occurring of these decay processes are discussed briefly below, with examples drawn from our recent investigations; these discussions are also relevant to the interpretation of data derived from the analysis of other organic remains of archaeological interest.

5.1 Hydrolysis

The bulk of the extractable lipid in foodstuffs, corresponds to the fatty carboxylic acids present in acyl lipids, e.g., triacylglycerols and phospholipids. These acyl lipids will be susceptible, during cooking, storage and burial, to both chemical and enzymatic hydrolysis. The hydrolysis of triacylglycerols is presented schematically in Figure 3. We have succeeded in producing a slow hydrolysis of an animal fat in the laboratory to yield a distribution of acyl lipids comparable to that observed in many of the potsherd extracts. Figure 4a shows the distribution of acyl lipids derived from the partial hydrolysis of a contemporary animal (beef) fat. The distribution obtained through high-temperature GC analysis of the intact lipids clearly reflects the compositional changes anticipated from the degradative pathway presented in Figure 3. An analogous pattern of degradation would be expected for other animal fats and vegetable oils. Further experiments aimed at simulating triacylglycerol degradation in the archaeological context are currently in progress.

Distributions of acyl lipids comparable to those presented in Figure 4a have been observed in lipid extracts of potsherds (e.g., Figure 4b) dating back to the Bronze Age (Evershed, Heron, Charters and Goad, unpublished results). These profiles suggest usage of the original vessel in the processing of a fatty foodstuff. More specific assignment to a plant or animal origin would rest on sterol analysis. For example, GC/MS analysis of the lipid mixture shown in Figure 4b showed cholesterol to be the only sterol present. This observation is consistent with this acyl lipid mixture having arisen from an animal fat. Vegetable oils would be expected to yield phytosterols, possessing additional alkylation at the C-24 position of the sterol side-chain, e.g., campesterol, sitosterol and stigmasterol (note also the presence of a Δ^{22} double-bond). The structures of these commonly occurring animal and plant sterols are presented in Figure 5 together with their EI mass spectra.

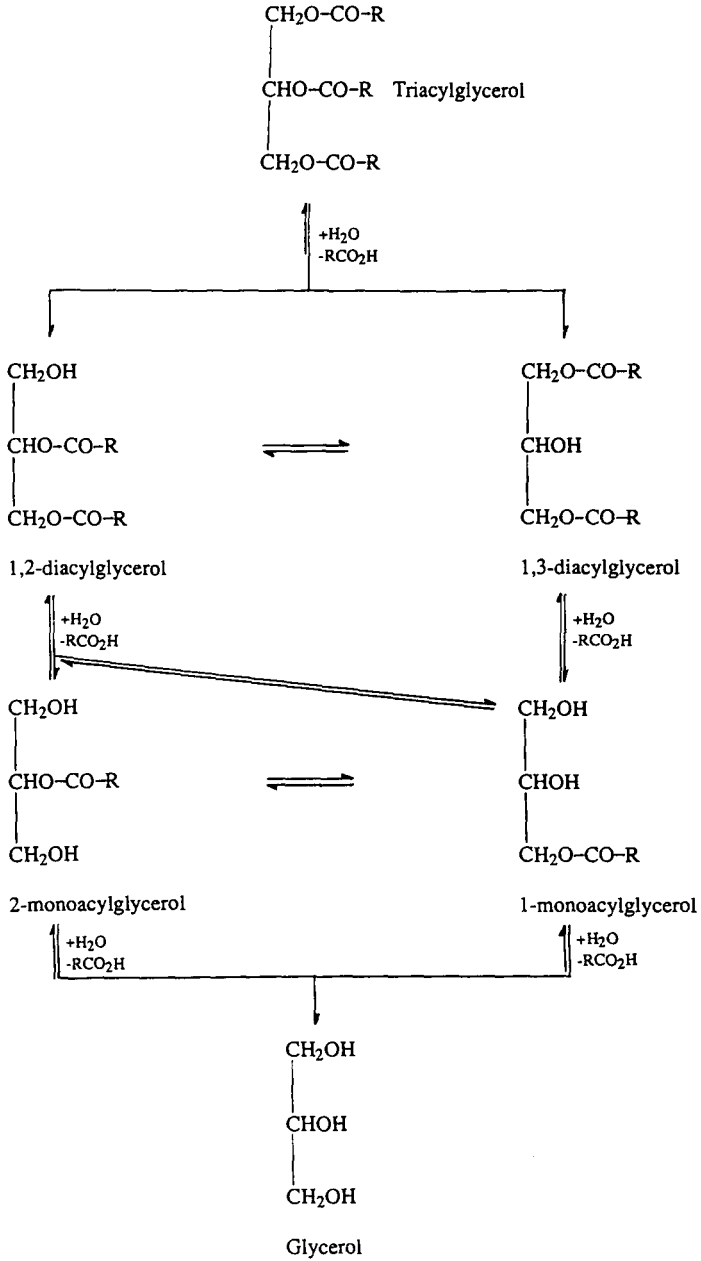


Figure 3. Hydrolysis of triacylglycerols.

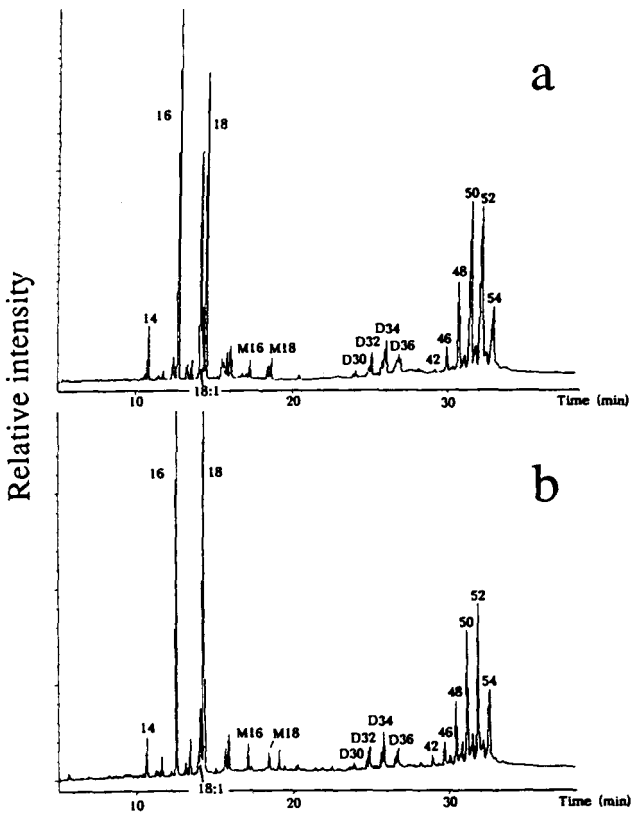


Figure 4. GC profiles of the acyl lipids derived from (a) beef subjected to slow hydrolysis (deionised water, room temperature, 12 months) and (b) the total lipid extract of a Late Saxon/Early Medieval cooking vessel. The experimental conditions are identical to those given in the caption to Figure 2.

5.2 Oxidation

As certain functional groups present in lipids are particularly sensitive to oxidation, this is amongst the most frequently observed degradative reaction of lipids in food materials (Chan 1987). The oxidation of unsaturated lipids occurs most readily with singlet oxygen produced in the atmosphere or directly in the food or vessel wall.

The most likely mechanisms of degradation involve the formation of endoperoxides, through direct attack across double-bonds, which subsequently rearrange into hydroperoxides (e.g., Figure 6). These hydroperoxides are reactive species that can: (i) react further with singlet oxygen to produce hydroxyepidioxy and dioxalane derivatives; (ii) combine to give peroxy dimers by recombination of peroxy radicals; (iii) disproportionate into hyd-

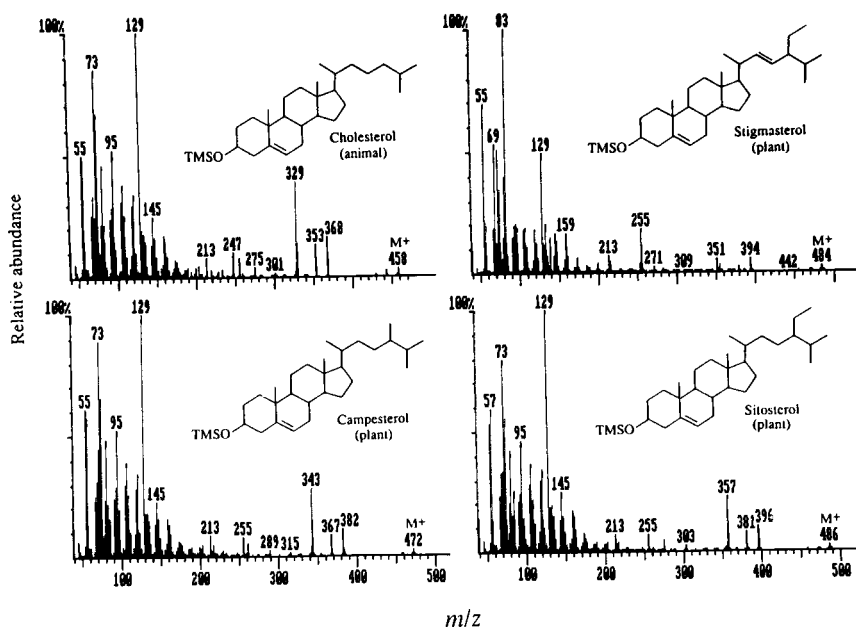


Figure 5. Structures and electron ionisation mass spectra of commonly occurring animal and plant sterols as their TMS ether derivatives. The mass spectra clearly reflect the minor structural differences in the sterol side chain.

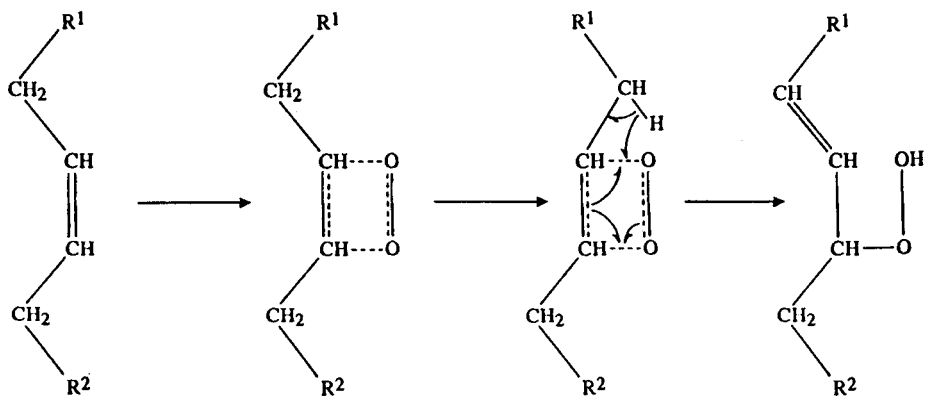


Figure 6. Mechanism of oxidation of isolated double-bonds by singlet oxygen.

roxylic and oxodienoic derivatives; (iv) produce epoxides by inter- and intramolecular reactions; (v) cleave to give low molecular weight aldehydes, alkenes, alcohols and alkanes, and (vi) form covalent bonds with other food components, e.g., proteins, to produce compounds which are insoluble in lipid-extracting solvents.

All the above oxidative reactions serve to deplete the unsaturated fatty acid components commonly observed in lipid extracts of contemporary biological materials. The outcome of these reactions is clearly demonstrated in our archaeological samples. For example, polyunsaturated fatty acids are so sensitive to oxidation that they are rarely observed in archaeological materials. Although linoleic acid ($C_{18:2}$) is widespread in animal and plant tissues we have yet to observe it, either as the free compound, or in intact acyl lipids, in any potsherd extract. However, oleic acid ($C_{18:1}$) is frequently observed, albeit in widely varying abundance; this variability presumably reflects the source foodstuff and/or the level of oxidative/reductive damage experienced by the lipid.

As alluded to above, sterols are amongst the most important of the minor components of fats and oils used diagnostically in classifying the lipid extracts of potsherds. In the pure form, sterols are relatively resistant to autoxidation, but are much more susceptible to oxidation in peroxidising fats and oils (Maerker 1987). By analogy with other lipids, the autoxidation of sterols is enhanced by contact with atmospheric oxygen at elevated temperatures. The pathway for the autoxidation of cholesterol is shown in Figure 7. The oxidation of cholesterol proceeds by hydrogen abstraction, predominantly at the C-7 position, and leads initially to the formation of allylic hydroperoxides. These unstable hydroperoxides decompose to yield various hydroxylic, oxo and epoxy derivatives. We have observed cholesterol autoxidation products in a number of lipid extracts. The chromatogram reproduced in Figure 8 shows the presence, albeit in low abundance, of cholesterol, and its 7-hydroxy and 7-one oxidation products in a mixture which is consistent with a highly degraded animal fat. This extract was derived from an Anglo-Saxon lamp sherd, which would presumably have been fuelled with animal tallow—an obvious by-product of meat processing. The detection of these oxidation products, together with degraded acyl lipids may indicate heating of the fat either during cooking (*cf.* Bascoul *et al.* 1986) or subsequently during the use of the lamp. Further investigations, particularly cooking simulation experiments using unglazed pottery vessels, will shed further light on the nature of sterol autoxidation products arising from a range of plant and animal foodstuffs.

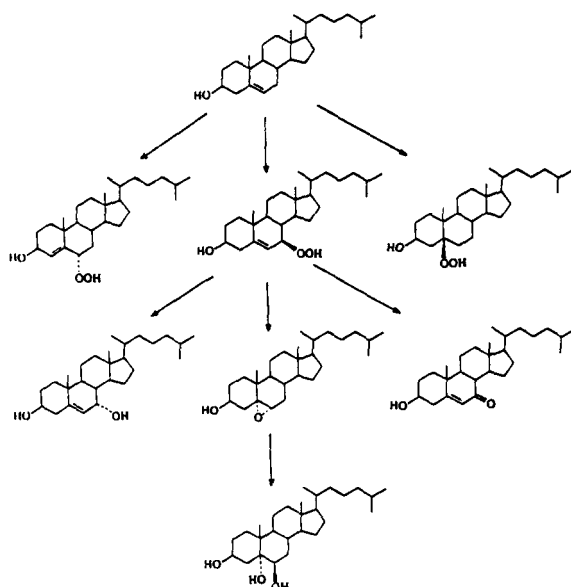


Figure 7. Oxidation products of cholesterol resulting from attack by singlet oxygen (compiled from Smith 1981).

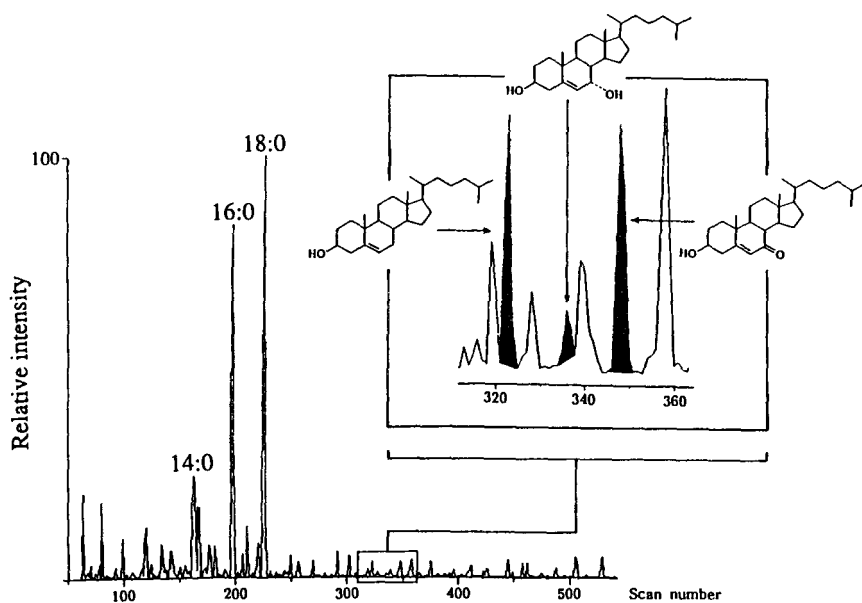


Figure 8. Total ion chromatogram (TIC; equivalent to a GC profile but derived from combined GC/MS) of the total lipid extract of Late Saxon/Early Medieval lamp sherd showing the major free fatty acids (as TMS ester derivatives), and an expansion of the sterol elution region, to reveal cholesterol oxidation products.

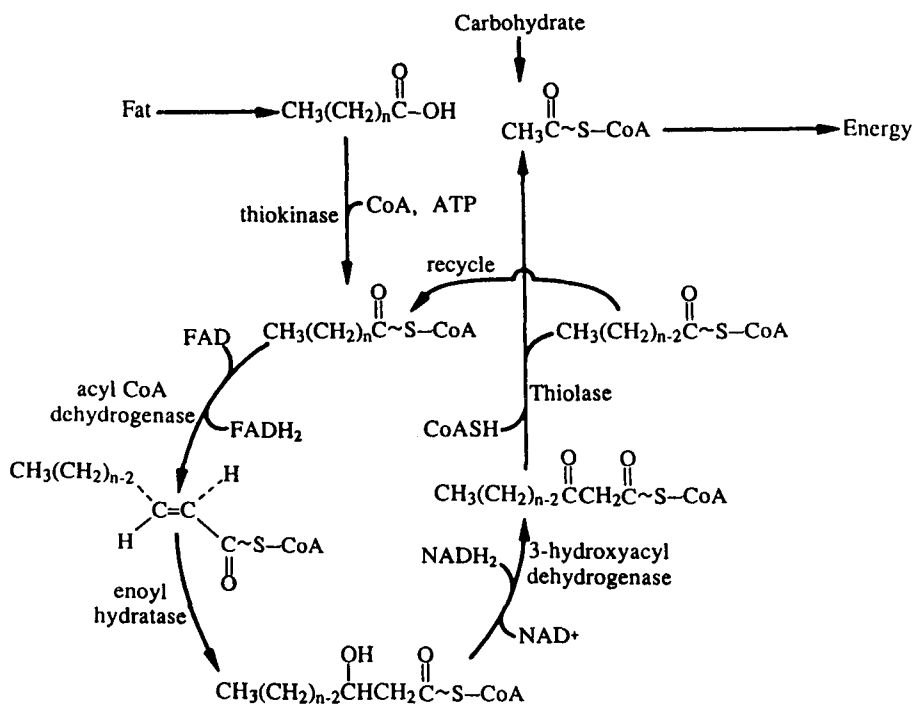


Figure 9. The β -oxidation pathway of fatty acid degradation.

5.3 Microbiological degradation

Buried organic matter, unless protected in some way, will be open to the full impact of decay by microorganisms active in the burial matrix. The population of microorganisms will be governed by the conditions prevailing in the burial environment, i.e., climate, degree of aeration, humidity and pH.

Enzymatic hydrolysis has already been discussed briefly in relation to the degradation of animal fats and vegetable oils. The microflora and microfauna that exist in soil contain enzymes that are capable of mediating a wide range of other structural changes in lipids. The most well known mechanism of fatty acid metabolism under aerobic conditions is β -oxidation (Figure 9). β -Oxidation is the stepwise degradation of fatty acids to yield acetyl-CoA (a building block of many essential natural substances) and metabolic energy. Microbial β -oxidation may also occur under anaerobic conditions (Gurr and James 1975), and evidence for this process in archaeological specimens has come from studies of adipocere formation (den Dooren de Jong 1961; see also below). This metabolic route will very rapidly deplete the acyl lipid content of the organic matter absorbed in potsherds. Straight-chain, branched, saturated and unsaturated fatty acids alike are potential substrates for the

β -oxidation pathway. Other oxidative pathways are also known which could also be involved in the degradation of lipids absorbed in potsherds during burial, e.g., lipoxygenases are widely distributed in microorganisms and capable of producing site-specific hydroperoxy derivatives of essential fatty acids (Gurr and James 1975).

Anaerobic environments are generally more conducive to the preservation of organic matter. In the case of food residues preserved in potsherds, anaerobic decay will only occur under certain burial conditions. Reduction is a well-recognised degradative process that can occur in lipids, mediated by microorganisms active in anaerobic environments. Structural alterations of this nature obviously rely on the availability of lipid species to the microorganisms.

The microbial reduction (hydrogenation) of Δ^5 -sterols to stanols under anaerobic conditions is a well-established process in sediments (Gaskell and Eglinton 1975; Mackenzie *et al.* 1982). 5α -Stanols have been detected by GC/MS in a number of lipid extracts of potsherds. However, their co-occurrence together with the corresponding Δ^5 -sterols, indicates that microbial reduction of the sterols has not proceeded to completion. As this microbial reduction is generally a relatively rapid process, it would appear that not all the lipid is available as substrate to the microorganisms active in the burial matrix (in this instance the soil), or that cessation of microbial activity has occurred for some reason. Assuming no enzyme specificity exists microbial reduction should affect all the absorbed Δ^5 -sterols equally, hence the sterol:stanol ratio should be constant at each carbon number, i.e. both cholesterol (C_{27}) and sitosterol (C_{29}) should be equally affected. If this is not the case then post-excavational contamination must be suspected, e.g., through handling of potsherds which would introduce cholesterol from skin lipids.

The double bonds in fatty acids will also be susceptible to biohydrogenation under anaerobic conditions. We have not yet fully assessed whether or not the acyl lipids absorbed in potsherds are readily reduced by microbes. However, there are indications from analyses of ancient adipocere, and studies of the anaerobic degradation of whole animal and plant fats in the laboratory, that reduction does occur (den Dooren de Jong 1961; Morgan *et al.* 1973; Thornton *et al.* 1970). Both these groups found that fatty materials decomposing anaerobically, under waterlogged conditions, are susceptible to hydrolysis, hydration, reduction and carbon-carbon bond scission reactions. In the case of triacylglycerols containing predominantly oleic acid, relatively rapid (two months incubation at 25°C) degradation occurs to yield predominantly palmitic acid. For palmitic acid to be formed from oleic acid, hydrogenation of the mid-chain double-bond, and shortening of the alkyl chain by two methylene groups, must be invoked. Chain shortening is thought to proceed via β -oxidation (den Dooren de Jong 1961), but this has not been

established unambiguously using radio- or stable isotope labelling techniques. The formation of 10-hydroxystearic acid (Evershed 1990) and scrambling of the double-bond position in monoenoic fatty acids (Evershed 1991) present in human adipocere, suggests that the mechanism of fat degradation in a waterlogged anaerobic environment is not straightforward. Fatty materials absorbed in potsherds buried in waterlogged anaerobic contexts are also presumed to be susceptible to degradation in this way.

6. Identification of food residues

Although considerable promise exists for the identification of food residues from the analysis of preserved lipids, these interpretations by necessity must proceed cautiously, taking full account of the decay processes that will have occurred over archaeological time. For example, lipid profiles of the type shown in Figure 4b occur with reasonable regularity, and provide a clear indication of the vessel having a function associated with the preparation and/or storage of a fatty food. More definite assignment to either a plant or animal source can be made through the analysis of the trace sterol components. In this case, the finding of cholesterol as the major sterol indicated the preserved fatty residue was of animal origin. Precise assignment of this residue to a particular animal species is problematical, as a result of the similarity of the acyl lipid composition of various species. Moreover, degradation of the dominant unsaturated fatty acids will complicate interpretations. Species identifications based on the distribution of the saturated fatty acid components should be made with caution, owing to the possibilities for double-bond reduction and chain shortening of fatty acids seen under anaerobic conditions (see above). In some cases, preserved acyl lipid distributions can be very diagnostic. For example dairy product residues are readily recognisable by the presence of short-chain fatty acids, with less than twelve carbon atoms, in the free fatty acid and intact acyl (mono-, di- and triacylglycerol) lipid fractions (Rottländer and Schlichtherle 1979; Evershed, Heron, Charters and Goad unpublished data).

The abundant long-chain alkyl compounds found in plant epicuticular waxes are of particular use in deriving dietary information relating to the preparation and consumption of leafy vegetables. Compounds of this type have been detected, sometimes in substantial quantities (> 3 mg/g of the sherd), in a number of unglazed Late Saxon/Early Medieval vessels recovered from the West Cotton site in Northamptonshire. The distribution of compounds detected in these samples included nonacosane, nonacosan-15-one and nonacosan-15-ol, provides strong evidence for *Brassica* vegetables, most likely *B. oleracea* (cabbage), or possibly *B. rapa* (turnip leaves) being a

significant component of the diet of the community at West Cotton (Figure 10; Evershed *et al.* 1991). This new method of identifying preserved epicuticular leaf waxes should have wide application, since the exploitation of many leafy vegetables by early communities remains undetected by traditional palaeobotanical techniques (Hall 1987).

In the light of the potential of this approach we have recently been establishing a database of epicuticular leaf compositions for comparison with total lipid extracts from potsherds. By this means we have derived evidence for the consumption of leeks (*Allium porrum*). The epicuticular leaf wax of leek is composed predominantly of hentricontan-16-one, and the detection of

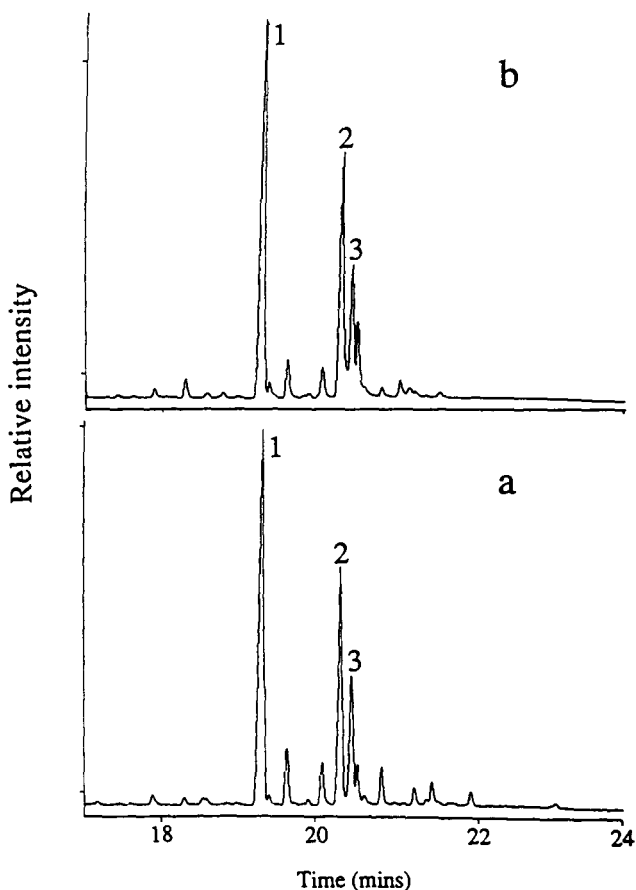


Figure 10. Partial GC chromatograms for (a) the total lipid extract of a potsherd from a Late Saxon/Early Medieval cooking vessel, and (b) the hexane soluble fraction of the epicuticular leaf wax of *Brassica oleracea* (cabbage). The numbers on the peaks correspond to the structures given in Figure 11. GC conditions were the same as those given in the caption to Figure 2.

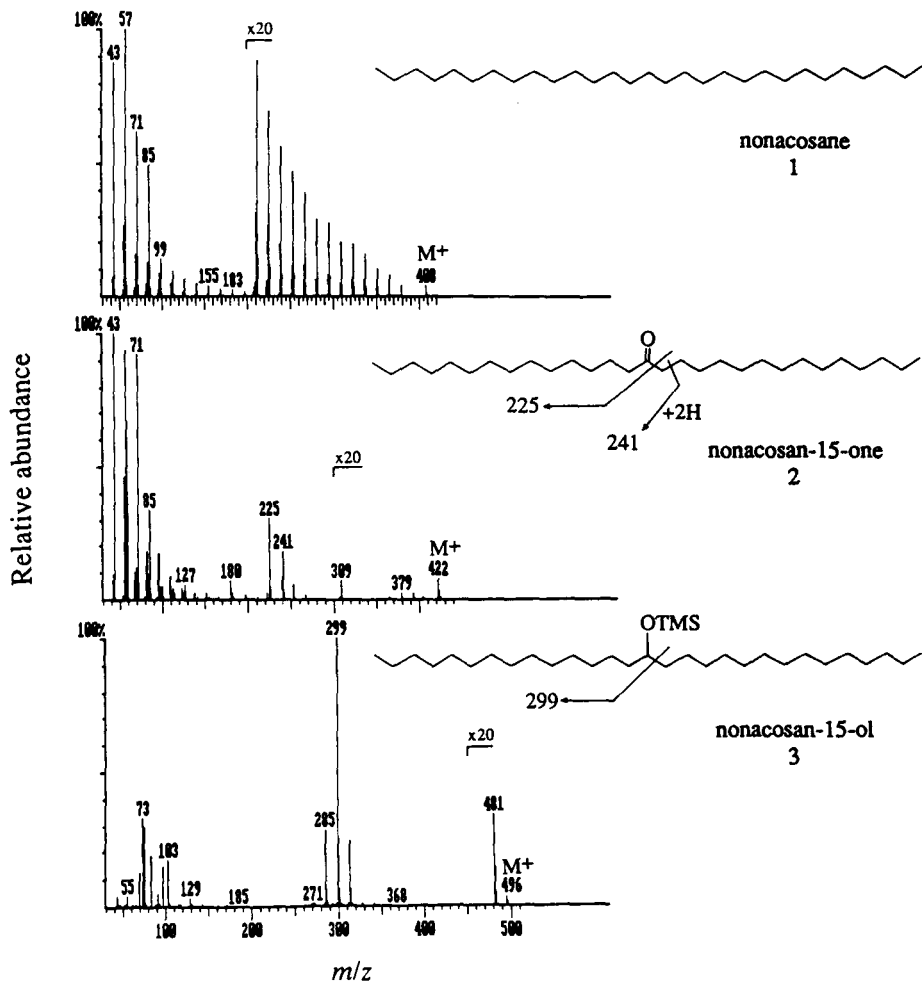


Figure 11. Electron ionisation (70 eV) mass spectra, obtained by combined GC/MS, of the major long-chain alkyl components of the partial GC chromatograms presented in Figure 10. Note that the nonacosan-15-ol was analysed as its TMS ether derivative. All other experimental details are the same as those given in the caption to Figure 2.

this compound in a significant number of potsherds is consistent with leeks being a dietary component at this site. Again, as is often the case, palaeobotanical evidence for the utilisation of leeks at this site has, until now, been lacking. This finding is in complete agreement with documentary evidence for the widespread cultivation of leeks during the Medieval period (Hallam 1988). We would again add a note of caution here, in that the identification of dietary components on the basis of a single marker compound is an

uncertain practise, in view of the large number of plant species that remain to be investigated.

7. Summary

A wide range of mechanisms exist for the decay of organic matter in archaeological contexts. However, by application of modern chromatographic and mass spectrometric methods we have demonstrated that sufficient organic matter remains preserved in potsherds for deductions to be made concerning the identity of a variety of constituents. Lipids appear to be relatively well-preserved and hold particular promise for use as chemotaxonomic markers of food remains. The data that has been obtained shows that extensive decay of the original organic matter occurs during burial, hence, the lipid distributions ('fingerprints') of potsherd extracts rarely match those of contemporary foodstuffs. For this reason we are performing laboratory simulations of decay of selected foodstuffs, in order to obtain suitable reference material to aide in interpretations. The distributions are also complicated by factors such as non-specific usage, consequently the lipid profiles will represent contributions from a variety of foodstuffs. The use of a range of ingredients in recipes will also yield complex lipid profiles reflecting such mixtures. In an effort to deconvolute data arising from these practices, we are carrying out simulation experiments with contemporary foodstuffs cooked in replica ceramic vessels. Special consideration is also being given to the possibility of post-firing treatments being employed by early potters to improve appearance or decrease permeability of unglazed vessels (Rice 1987). Modern ethnographic practices include: the application of resinous coatings, or the scalding of milk to seal the interior of new unglazed vessels. The implications of these practices to interpretations based on the analysis of preserved organic matter are obvious, and should be given due consideration when making deductions concerning diet and vessel function based on chemical analysis. Provided due consideration is given to these factors in the interpretation of the analytical data, there are clear indications that potential exists for the study of food preparation and consumption, and pottery function.

Acknowledgements

In presenting this paper we would like to thank Mark Prescott for invaluable assistance with GC/MS analyses and Ann Leyden for assistance in sample preparations and GC analyses. Varian Denham and Paul Blinkhorn are thanked for valuable discussions and ceramic interpretations. Thanks are

also due to Mark Robinson and Gillian Campbell for environmental information. We are indebted to the Northamptonshire Archaeology Unit for co-operation in provision of samples from the Raunds Area Project. The financial support of the Science and Engineering Research Council Science-based Archaeology Committee (SERC-SBAC) and English Heritage Historic Buildings and Monuments Commission (HBMC) for England are gratefully acknowledged.

References

- Bascoul, J., Domergue, N., Olle, M. and Crastes De Paulet, A. 1986: Autoxidation of cholesterol in tallows heated under deep frying conditions: evaluation of oxysterols by GLC and TLC-FID. *Lipids* 21, 383–387.
- Carbone, V.A. and Keel, B.C. 1985: Preservation of plant and animal remains. In Gilbert, R.J. and Mielke, J.H. (editors), *The Analysis of Prehistoric Diets* (London, Academic Press) 2–19.
- Condamine, J., Formenti, F., Metais, M.O., Michel, M. and Blond, P. 1976: The application of gas chromatography to the tracing of oil in ancient amphorae. *Archaeometry* 18, 195–201.
- Chan, H.W.S. (editor) 1987: *Autoxidation of Unsaturated Lipids* (London, Academic Press).
- den Dooren De Jong, L.E. 1961: On the formation of adipocere from fats. *Antonie van Leeuwenhoek Journal of Microbiology and Serology* 27, 337–361.
- Dimbleby, G. 1978: *Plants and Archaeology* (London, John Baker).
- Evershed, R.P. 1990: Lipids from samples of skin from seven Dutch bog bodies: preliminary report. *Archaeometry* 32, 139–153.
- Evershed, R.P. 1991: Bog body lipid taphonomy. In Budd, P., Chapman, B. Jackson, C., Janaway, R. and Ottaway, B. (editors), *Archaeological Sciences 1989* (Oxford, Oxbow Monograph 9) 352–361.
- Evershed, R.P. and Connolly, R.C. 1988: Lipid preservation in Lindow Man. *Naturwissenschaften* 75, 143–145.
- Evershed, R.P., Heron, C. and Goad, L.J. 1990a: Analysis of organic residues of archaeological origin by high-temperature gas chromatography/mass spectrometry. *Analyst* 115, 1339–1342.
- Evershed, R.P., Prescott, M.C. and Goad, L.J. 1990b: High-temperature gas chromatography/mass spectrometry of triacylglycerols with ammonia negative-ion chemical ionisation. *Rapid Communications in Mass Spectrometry* 4, 345–347.
- Evershed, R.P., Heron, C. and Goad, L.J. 1991: Epicuticular wax components preserved in potsherds as chemical indicators of leafy vegetables in ancient diets. *Antiquity* 65, 540–544.
- Gaskell, S.J. and Eglinton, G. 1975: Rapid hydrogenation of sterols in a contemporary lacustrine sediment. *Nature* 254, 209–211.
- Gilbert, R.I. and Mielke, J.H. (editors) 1985: *The Analysis of Prehistoric Diets* (Orlando, Academic Press).
- Gurr, M.I. and James, A.T. 1975: *Lipid Biochemistry* (London, Chapman and Hall).
- Hall, A. 1987: In pursuit of the uneatable (some thoughts on the search for archaeological evidence of vegetables). *Archaeology in York: Interim* 12, 1–7.
- Hallam, H.E. 1988: *The Agrarian History of England and Wales Vol. 2: 1042–1350* (Cambridge, Cambridge University Press).
- Heron, C., Evershed, R.P., Goad, L.J. and Denham, V. 1991a: New approaches to the analyses of organic residues from archaeological ceramics. In Budd, P., Chapman, B. Jackson, C., Janaway, R. and Ottaway, B. (editors), *Archaeological Sciences 1989* (Oxford, Oxbow Monographs 9) 332–339.

- Heron, C., Evershed, R.P. and Goad, L.J. 1991b: Effects of migration of soil lipids on organic residues associated with buried potsherds. *Journal of Archaeological Science* 18, 641–659.
- Hillman, G.C. 1989: Late Palaeolithic plant foods from Wadi Kubbaniya in Upper Egypt: dietary diversity, infant weaning, and seasonality in a riverine environment. In Harris, D.R. and Hillman, G.C. (editors), *Foraging and Farming: the Evolution of Plant Exploitation* (London, Unwin Hyman) 207–239.
- Jones, R.E. 1986: Identification of materials carried by ceramic vessels. In Jones, R.E. (editor), *Greek and Cypriot Pottery: A Review of Scientific Studies* (Athens, British School at Athens Fitch Laboratory Occasional Paper No.1) 839–847.
- Kates, M. 1989: *Techniques of Lipidology* (Amsterdam, Elsevier).
- Mackenzie, A.S., Brassell, S.C., Eglinton, G. and Maxwell, J.R. 1982: Chemical fossils: the geological fate of steroids. *Science* 217, 491–504.
- Maerker, G. 1987: Cholesterol autoxidation—current status. *Journal of the American Oil Chemists Society* 64, 388–392.
- Mills, J.S. and White, R. 1987: *The Organic Chemistry of Museum Objects* (London, Butterworths).
- Morgan, E.D., Cornford, C., Pollock, D.R.J. and Isaacson, P. 1973: The transformation of fatty material buried in soil. *Science and Archaeology* 10, 9–10.
- Myher, J.J. and Kuksis, A. 1984: Determination of plasma total lipid profiles by gas-liquid chromatography. *Journal of Biochemical and Biophysical Methods* 10, 13–23.
- Nes, W.R. and Nes, W.D. 1980: *Lipids in Evolution* (New York, Plenum Press).
- Parmalee, P.W. 1985: Identification and interpretation of archaeologically derived animal remains. In Gilbert, R.I. and Mielke, J.H. (editors), *The Analysis of Prehistoric Diets* (Orlando, Academic Press) 61–95.
- Rice, P.M. 1987: *Pottery Analysis: A Sourcebook* (Chicago, University of Chicago Press).
- Rottländer, R.C.A. and Schlichtherle, H. 1979: Food identification of samples from archaeological sites. *Archaeo-physika* 10, 260–267.
- Smith, L.L. 1981: *Cholesterol Autoxidation* (New York, Plenum Press).
- Thornton, M.D., Morgan, E.D. and Celoria, F. 1970: The composition of bog butter. *Science and Archaeology* 2–3, 20–25.