Historic England

Organic Residue Analysis and Archaeology

Supporting Information

Summary

This document contains supporting information to help readers understand terms and concepts used in organic residue analysis (ORA) and developed in *Organic residue analysis and archaeology: guidance for good practice*. It also includes a research agenda and strategy for future ORA. This document comprises four sections:

1 Lipids, analytical techniques and preservation

2 Research agenda and strategy

3 Reporting, publishing and digital archiving

4 Further reading and thematically organised bibliography

Section 1 includes detailed information on lipid analysis (not included in the printed guidance booklet), and describes the analytical techniques used in ORA. Factors affecting the preservation and decay of organic residues are also discussed.

The second section focuses on a research agenda and strategy for ORA. This section gives details on areas where the application of organic residue analysis would help address specific archaeological questions regarded as priorities by pottery research groups and/or described within regional frameworks. Guidance is given about areas where specific 'themes' for research can be targeted at site level, in order to address site- or region-specific questions and where these might contribute to large-scale archaeological questions (regional and national). This is intended to both enhance the value of individual projects and also, importantly, to maximise the collective research potential of datasets.

Section 3 briefly covers reporting, publishing and digital archiving of ORA material, and Section 4 gives detailed suggestions for further reading and includes a thematically organised bibliography, intended to provide guidance on literature relating to ORA.

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HistoricEngland.org.uk/advice/technical-advice/archaeological-science/

Front cover
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Contents

1 Lipids, Analytical Techniques and Preservation

1.1 Lipids

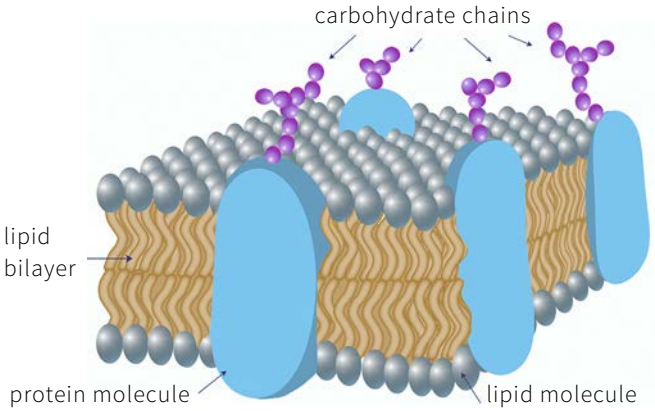
(Terms in **bold** are defined in the Glossary included in *Organic residue analysis and archaeology: guidance for good practice*.)

Lipids are the **fats, waxes** and **resins** of the natural world. Diagnostic lipid markers can survive, often over long timescales, at their site of deposition because of their **hydrophobic** nature, which means they will not readily dissolve in water. The stability of these biomolecules makes their survival more likely, in relative terms, in comparison to other biomolecules such as DNA, proteins or carbohydrates (Figure 1).

Lipids<carbohydrate≈lignin<protein<nucleotides

Figure 1
The preferential survival of biomolecules.

Lipids are a group of naturally occurring organic molecules (originating from living organisms) that are mainly composed of carbon, hydrogen and oxygen. These molecules form a range of structures, owing to the ability of carbon to bond to carbon in a wide variety of arrangements, including linear, branched and cyclic carbon skeletons, normally fully substituted with hydrogen atoms (known as saturated). These include **fatty acids, waxes, sterols** (including **cholesterol**), **phospholipids, mono-, di-** and **triacylglycerols**. Together with carbohydrates and proteins, lipids are the main constituents of plant and animal cells, making up the building blocks of their structure and function (Figure 2).



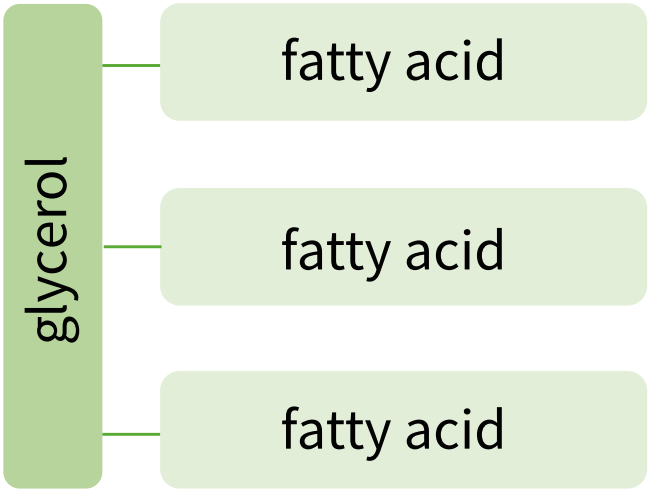


Figure 2 (top)
Cell membrane structure showing the lipid bilayer.

Figure 3 (above)
Basic structure of a triglyceride (triacylglycerol).

1.1.1 Lipids found in food residues

The three major classes of lipids are **triglycerides** (known as triacylglycerols, or TAGs), **phospholipids**, and **sterols**. The sterols can be derived from animals (zoosterols), the most common being cholesterol, or plant (phytosterols) sources. Phytosterols such as sitosterol, stigmasterol and campesterol predominate in plants, but the sterol composition of plants can be very complex. Each type of lipid fulfils a variety of structural, metabolic and physiological roles in living organisms. For example, one of the functions of cholesterol is as a structural component of cell membranes.

The triacylglycerols, the main form of lipid found in living organisms and in the human diet, are made from a glycerol molecule joined by three **fatty acid** chains (Figure 3). **Mono**- and **diacylglycerols** (DAGs) have one and two fatty acids respectively. These fatty acids consist of a **carboxylic acid** (–COOH) group on one end of a carbon chain and a methyl group ($-CH_3$) on the other end. Fatty acids (often known as FFAs, free fatty acids) can differ from one another in two important ways: carbon chain length and degree of unsaturation.

Most importantly, these fatty acids, constituents of triglycerides, are the major components of fats and oils, and it is these lipids that form the backbone of research in organic residue analysis. Triglycerides make up *c* 95 per cent of lipids in our diet and are found in most foods in varying abundances, including meats, fish, dairy products, seeds, nuts and plants (Figure 4).

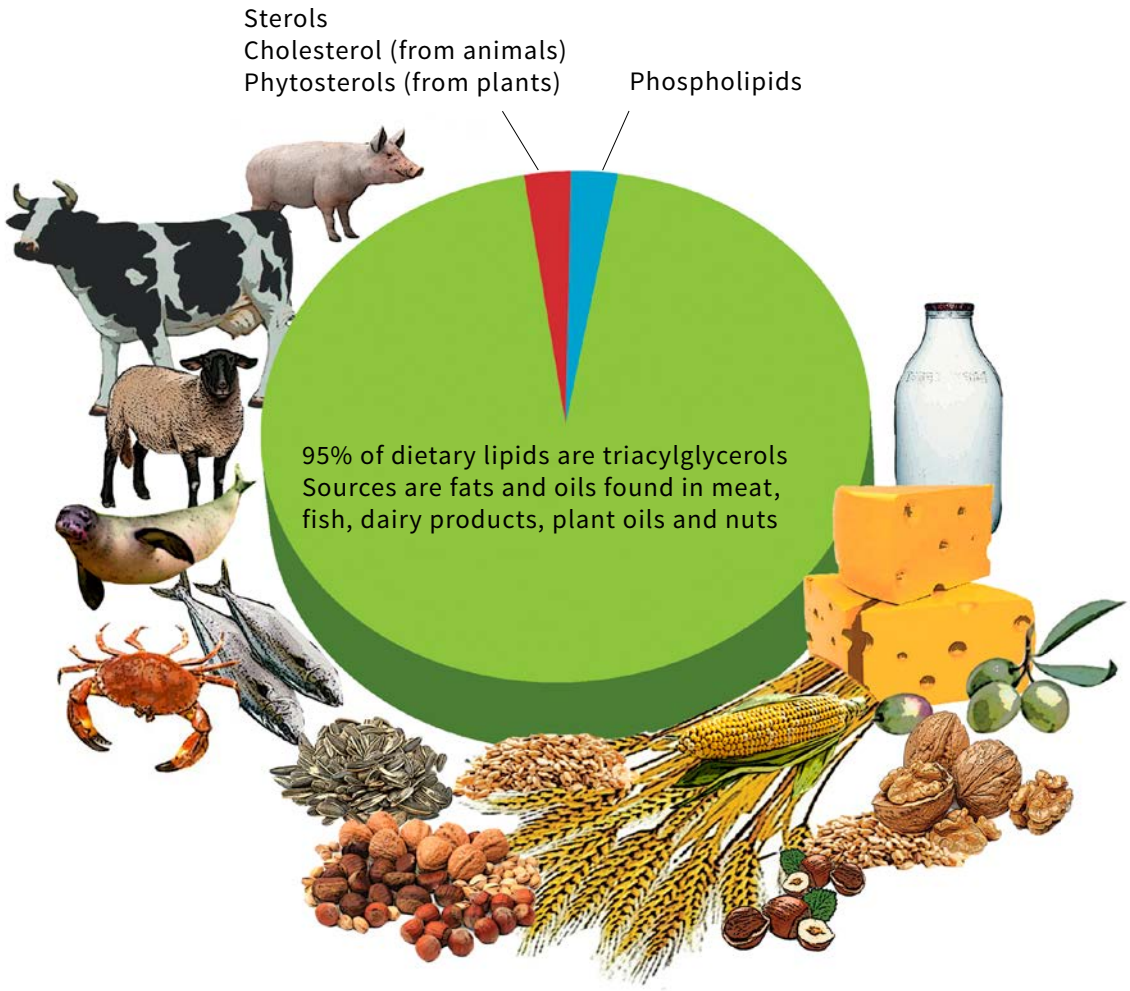


Figure 4
Sources of dietary lipids (from triacylglycerols).

1.1.1.1 Fats and oils

The difference between fats (for example, butter and lard) and oils (for example, sunflower oil) from animals and plants is that fats are lipids that are solid at room temperature, whereas oils are liquid. This is because the fatty acids making up fats and oils come in two major types. **Saturated fatty acids** (Figures 5 and 6), found in high abundance in animal products (including dairy) and palm and coconut oils, are saturated to capacity with hydrogen atoms, joined by a single bond; thus if each available carbon bond holds a hydrogen atom, we call this a saturated fatty acid chain. These chains may also be **monounsaturated**, containing one double bond, or **polyunsaturated** fats containing two or more double bonds (known as points of unsaturation). **Unsaturated** fats have a lower melting point and are liquid at room temperature. **Monounsaturated** fatty acids (Figure 5) are found in plant oils such as olive oil, sesame oil and sunflower oil, and in avocados and nuts (including almonds, peanuts, pecans, walnuts and cashews). **Polyunsaturated fattys acids** (Figure 5) are found mainly in plant-based foods, oils and fish. Common sources of both types of fats are nuts (almonds, walnuts, peanuts and hazel), soybean oil, corn oil, safflower oil, flaxseed oil, canola oil and fish (salmon, herring and trout).

Although more than 1,000 naturally occurring fatty acids have been identified, only a relatively small number are found in significant amounts. The most abundant saturated fatty acids in both animal and plant tissues are straight-chain compounds with 16 and 18 carbon atoms (known respectively as palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acid. Whilst these are the most common **saturated** fatty acids in animal and plant tissues, there can also be one or more double bonds.

FATTY ACIDS

Most contain an even number of carbon atoms, in a linear chain. Carbon chain length may vary from 4-30 carbon atoms (commonly 12-24)

Classification based on degree of unsaturation

SATURATED	MONOUNSATURATED	POLYUNSATURATED
No double bonds are present in the carbon chain. Meat, milk, butter, lard, tallow, palm and coconut oils	One double bond is present in the carbon chain. Nuts, olive oil, sesame oil, sunflower oil	Two or more double bonds are present in the carbon chain. Soybean oil, corn oil, nuts, fish (for example salmon, herring and trout)

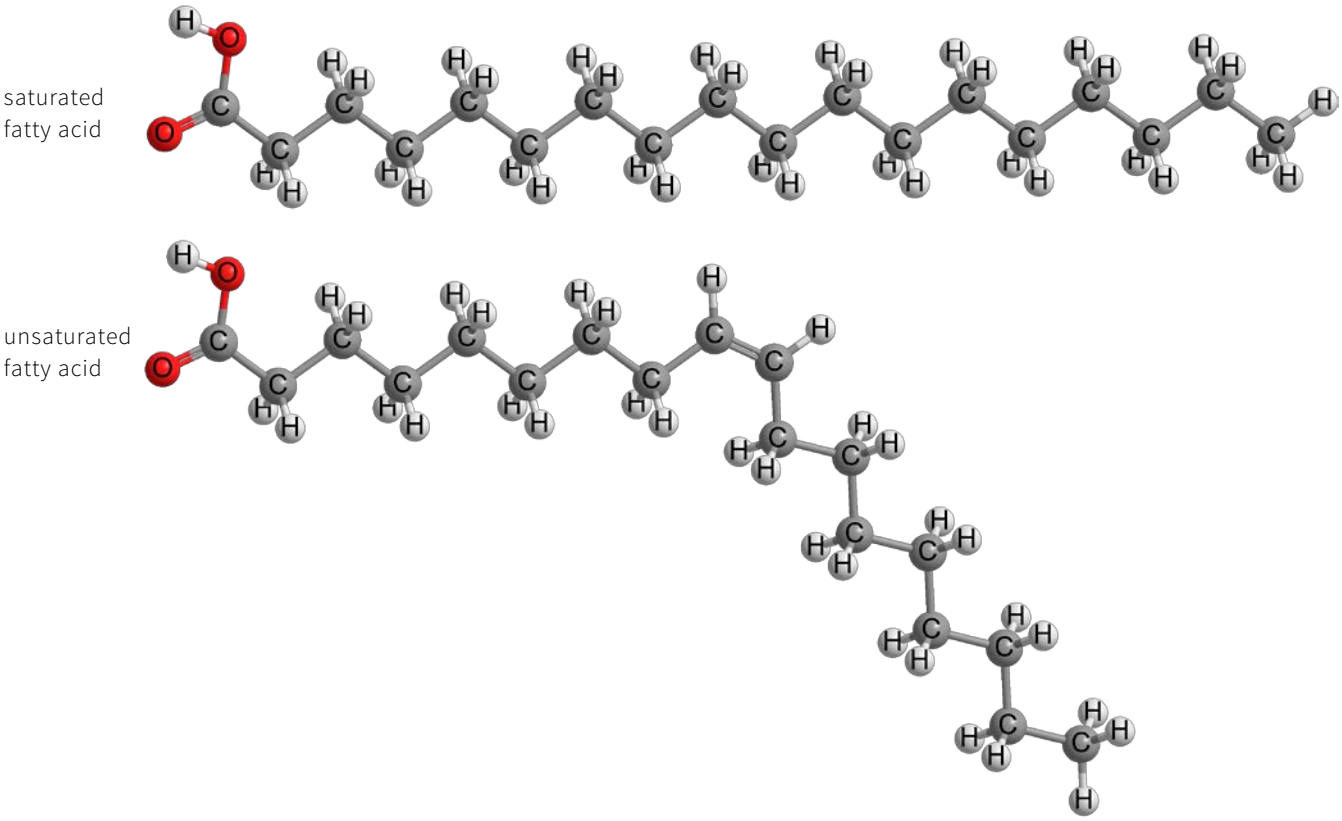


Figure 5 (top)
Types of fatty acids: saturated, monounsaturated and polyunsaturated.

Figure 6 (above)
The chemical structure of saturated and unsaturated fatty acids.

Plant oils, for example, usually contain significant quantities of **mono-**, **di-** and **polyunsaturated** fatty acids. The numbers in their notations ($C_{16:0}$ and $C_{18:0}$) denote the number of carbon atoms in the fatty acid chain (first number) and the number of double bonds (after the colon). Thus, zero denotes that there are no double bonds, making it a saturated fat; whereas a $C_{18:1}$ fatty acid is monounsaturated, as it has one double bond, and a $C_{18:2}$ fatty acid is diunsaturated, as it has two double bonds.

Commercially important fats and oils of animal and plant origin consist almost exclusively of fatty acids, although the fatty acid composition of oils and fats from different organisms can vary significantly. Consequently, different lipid profiles detected in archaeological vessels can aid identification of particular types of organisms. For example, in animal fats the $C_{16:0}$ and $C_{18:0}$ fatty acids are the most abundant, whereas in plant oils the $C_{16:0}$ fatty acid and C_{18} unsaturated fatty acids ($C_{18:1,}$ $C_{18:2}$ and $C_{18:3}$) tend to predominate. Nontheless, the palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids are the most commonly observed fatty acids in archaeological residues, since **unsaturated fatty acids** tend to readily degrade and are lost.

Specific 'fingerprints' of these compounds, often combined with stable carbon isotope analysis, enable us to identify the processing of different commodities in archaeological vessels. This analysis includes predicting degradative processes affecting the original fatty acid composition. For example, lipid profiles containing long-chain **ω-(*o*-alkylphenyl) alkanoic acids** (APAAs), formed from the transformation during cooking of diagnostic polyunsaturated fatty acids with up to six double bonds, suggest an aquatic origin.

Compound-specific stable carbon isotope analysis

A range of chemical criteria, including saturated fatty acid and triacylglycerol distributions and compositions, double bond positions and $\delta^{13}C$ values of the major saturated fatty acids (palmitic ($C_{16:0}$) and stearic ($C_{18:0}$)), have been used to assign the origin of ancient animal fats to tissues from ruminant animals such as domesticated cattle, pigs, sheep and goats. In fresh animal fats, different distributions of lipids – for example triacylglycerols – can be indicative of origin, for example, dairy or carcass fats. However, these are generally altered by degradative processes over archaeological timescales and, consequently, even if present, cannot reliably be regarded as being diagnostic of fat type. Nevertheless, using **gas chromatography-combustion-isotope ratio mass spectrometry** (GC-C-IRMS, see Section 1.2.2) determinations of the two major fatty acids that do survive (palmitic ($C_{16:0}$) and stearic ($C_{18:0}$)) enables robust separation and identification of carcass and milk fats (Figure 7), based upon different sources of carbon in the organism's biosynthetic process.

This approach uses what is known as the **stable carbon isotope proxy** ($\delta^{13}C$ proxy). Ruminant carcass fats can also be separated from those of non-ruminants because of their metabolic differences. For example, by comparing $\Delta^{13}C$ values of the palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids from two morphologically distinct vessel containers,ruminant animal fat, such as sheep or goat, was found in 'lamps' and non-ruminant animal fat, such as pig, was identified in 'dripping dishes' (Mottram *et al* 1999).

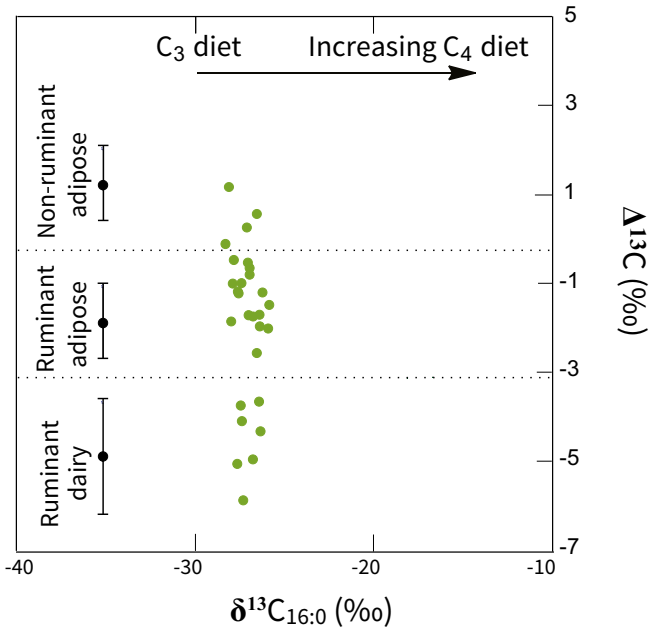


Figure 7
Stable carbon isotope proxy ($\Delta^{13}C$ proxy) that enables determination of the origins of animal fats, for example ruminant or non-ruminant, carcass or dairy fats.

Marine and freshwater fats can also be determined through this isotopic approach (Cramp and Evershed 2014).

1.1.1.2 Waxes

Other lipids found in organic residues extracted from ceramic vessels that might originate from food products include natural waxes produced by insects (for example, beeswax and/or honey, Figure 8) and waterproof coatings on the outer surface of plants (leaf or **epicuticular waxes**). Their composition comprises a range of lipids including fatty acids, *n*-**alkanes**, *n*-alkanols and **wax esters**. These compounds, or combinations thereof, have been used to identify the processing of beeswax (possibly associated with honey exploitation) and plants, such as vegetables, in vessels.

1.1.1.3 Lipids from non-food residues, including waxes, resins and tars

Lipids from natural products not used for dietary purposes have been found in both visible and absorbed residues. These lipids originate from resins, tars and bitumen; and waxes from beeswax have also been found to have been used for non-dietary purposes. These commodities may have been stored or processed in vessels, or used in their manufacture as sealants, decoration, or for repair (as adhesives). Typical lipids from these commodities include di-, tri- and sesquiterpenoid biomarkers from resins. The distillation of resins produces biomarkers, such as methyl dehydroabietic acid, resulting from the manufacture of tars and pitches.

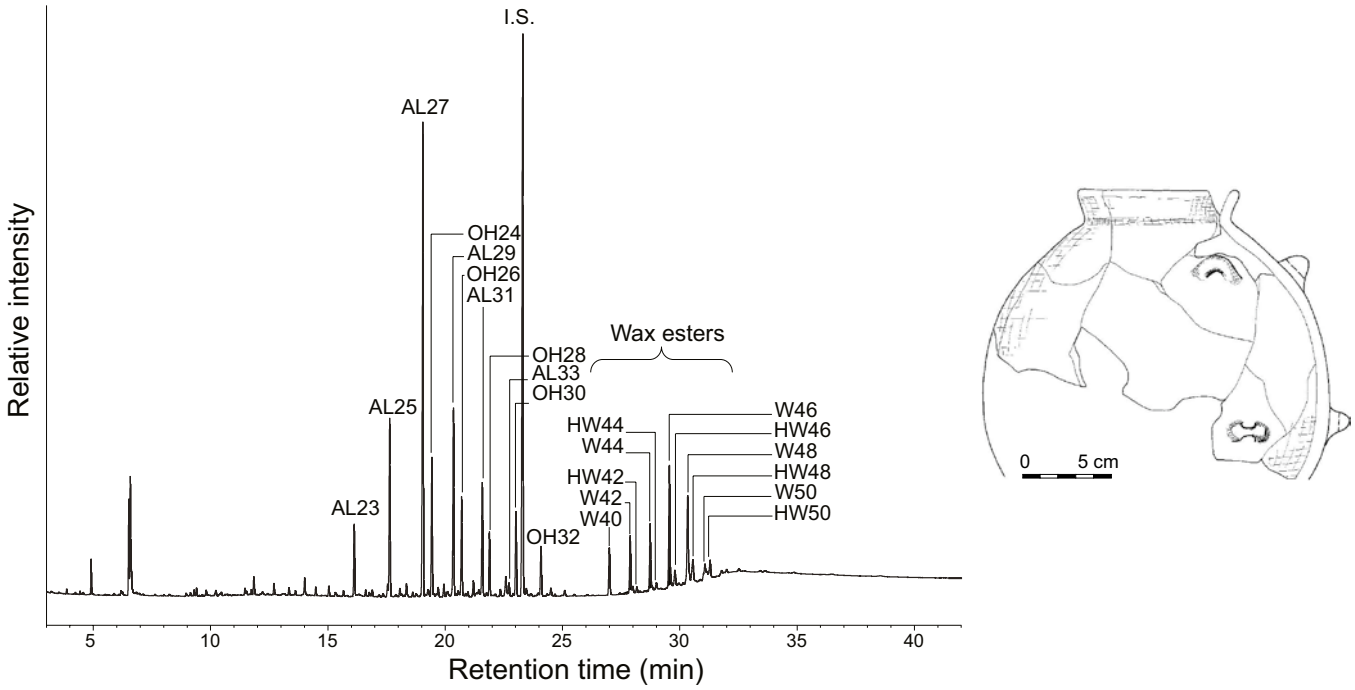


Figure 8
Chromatogram showing the distinctive lipid distribution typical of beeswax (from Salque *et al* 2013 fig 2), including distributions of *n*-alkanols (OHX), *n*-alkanes (ALX), palmitate wax esters (WX) and hydroxy wax esters (HWX), where X denotes the carbon chain length.

1.2 Analytical techniques

During the last few decades, various analytical techniques have been utilised to identify and characterise different compound classes from visible and absorbed lipid residues extracted from archaeological ceramics. These include:

- pyrolysis-GC (Py-GC)
- pyrolysis-GC-mass spectrometry (Py-GC-MS)
- high performance liquid chromatography
- infrared spectroscopy
- solid state ^{13}C nuclear magnetic resonance spectroscopy
- direct inlet electron ionisation mass spectrometry
- bulk stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$ values)

However, the main analytical techniques used to identify and characterise different compound classes from visible and absorbed lipid residues extracted from archaeological ceramics are:

- gas chromatography (GC), including high temperature GC (HT-GC)
- gas chromatography-mass spectrometry (GC-MS)
- gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)

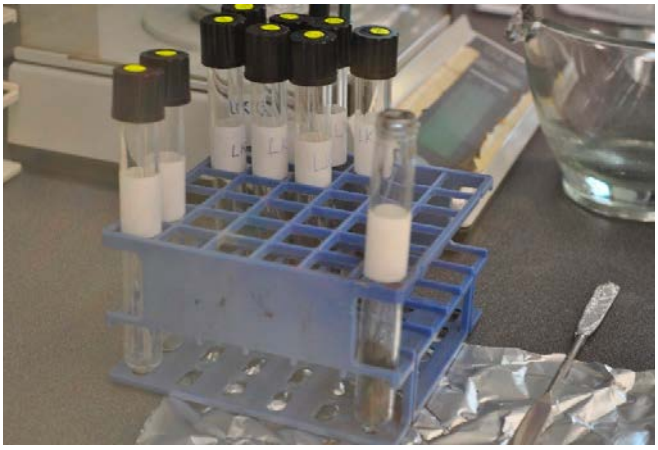
1.2.1 GC and GC-MS

The main analytical technique used within the field is the instrumental technique of gas chromatography coupled with a mass spectrometer (GC-MS), which is an extremely powerful tool enabling the separation, identification and quantification of complex mixtures of chemicals. In simple terms, compounds present within the mixture are separated (gas chromatography) and individual peak identities confirmed by their fragmentation patterns (mass spectrometry). This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds, and the small quantities of analyte often recovered from sample sizes found in archaeological and environmental materials.

Samples to be analysed by GC-MS are first solvent extracted and subjected to various 'wet chemical' techniques before they can be analysed (Figure 9). In order to analyse a compound by GC-MS it must be both sufficiently volatile and thermally stable. In addition, functionalised compounds may require chemical modification (known as derivatisation) before analysis, to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained.

Once the extraction procedure has been carried out the sample solution is injected into the GC inlet, where it is vaporised and swept onto a chromatographic column (a very long narrow glass tube with a polymer coating) by a carrier gas (usually helium). The sample flows through the column and the compounds comprising the mixture of interest are separated by virtue of their differences in volatility and relative intereaction with the polymer coating of the column (known as the stationary phase) and the carrier gas (mobile phase). This means that the different compounds take different amounts of time to reach the end of the column. Each constituent emerges from the column and enters the detector where it is recorded as a peak, the size of which is relative to its abundance in the sample. The graph produced is called a chromatogram, which is effectively a 'chemical fingerprint' showing the separation of components within the sample.







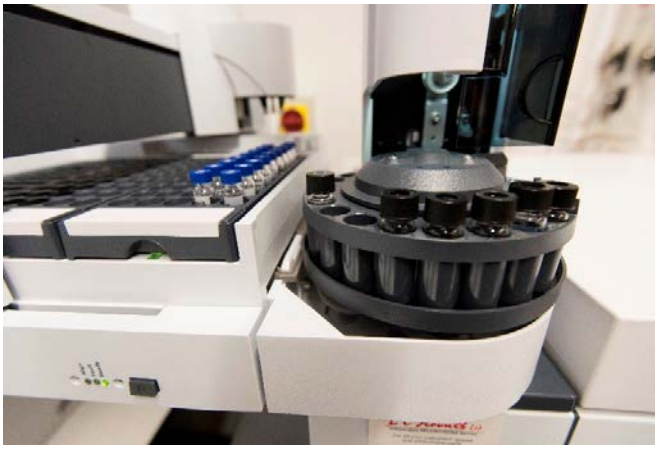
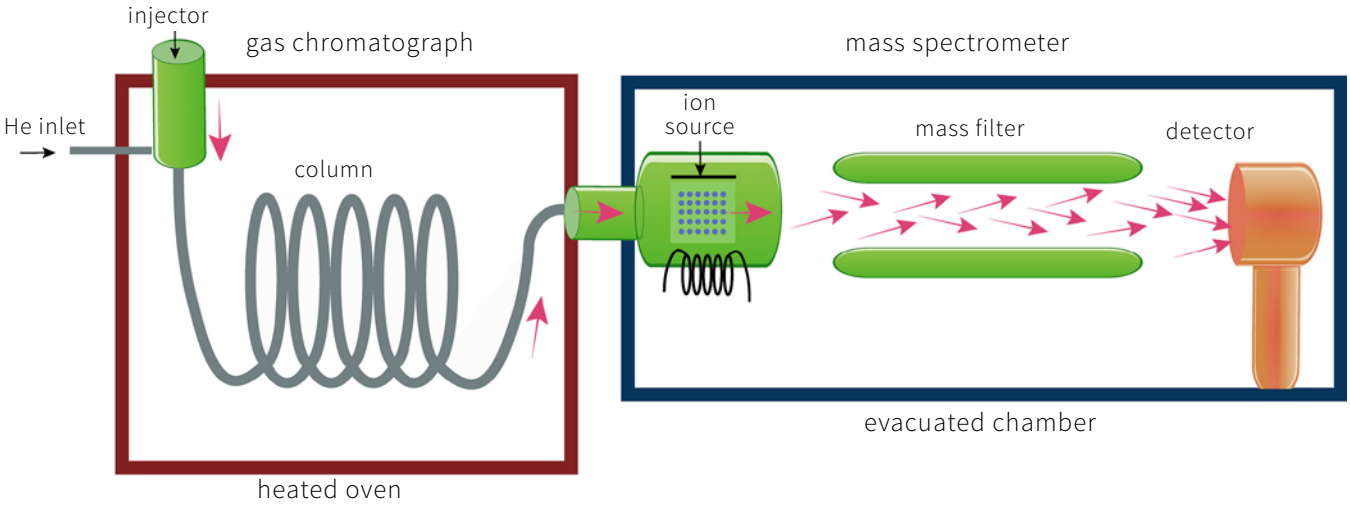


Figure 9
Laboratory processes used in ORA.
images from universities of Bristol and York.

As the compound exits the end of the GC column it moves into the mass spectrometer part of the instrument (Figure 10). Here, at the end of the column, compounds undergo ionisation whereby the sample molecules usually lose one electron. A molecule with one electron missing is called the molecular ion and is represented by $M^{+\cdot}$ (a radical cation). When the resulting peak from this ion is seen in a mass spectrum, it gives the mass-to-charge (m/z) ratio of the compound. Due to the large amount of energy imparted to the molecular ion, it usually fragments, producing further smaller ions with characteristic relative abundances that provide a 'fingerprint' for that molecular structure (Figure 11). Unknown components of mixtures can then be identified by comparison to reference mass spectral libraries.



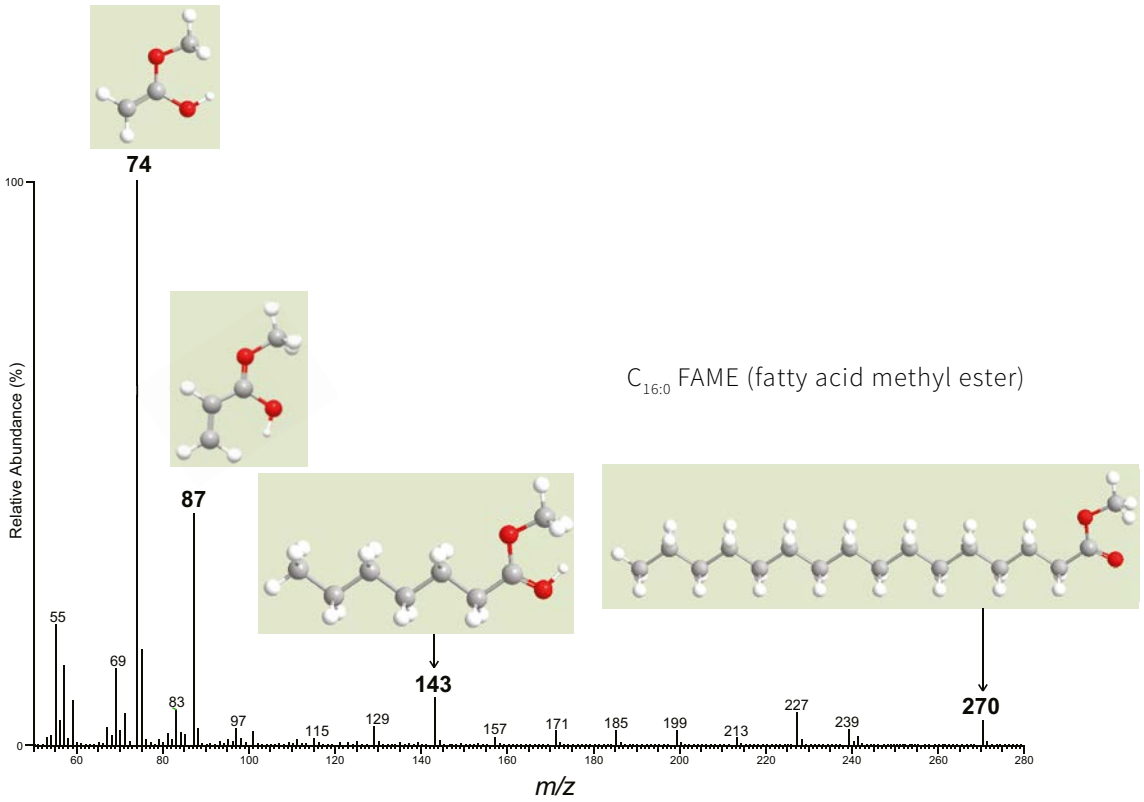


Figure 10 (top)
Components of a gas chromatograph-mass spectrometer.

Figure 11 (above)
Fragmentation of a fatty acid methyl ester into smaller ions.

1.2.2 GC-C-IRMS

GC-C-IRMS is an instrumental technique used to determine the relative ratio of stable isotopes of carbon ($^{13}C/^{12}C$) in individual compounds separated from often complex mixtures of components. In this case, it is used to ascertain the $\delta^{13}C$ values for the individual $C_{16:0}$ and $C_{18:0}$ fatty acids separated using GC and GC-MS.

1.2.3 Understanding chromatograms

To help those from a non-chemical background to better understand and interpret the analytical results shown in these guidelines, some detail is provided here on what a chromatogram depicts (Figure 12).

The chromatogram is a two-dimensional plot in which the ordinate (y) axis gives the relative concentration (amount) of particular components eluted from the column (that is, the area under a peak generated is proportional to the concentration that generates it), and the x-axis represents time (the amount of time it takes a certain compound to pass through a GC column is called the retention time). Each peak on a chromatogram represents an individual component present in the sample. The identities of the components are indicated by standards (containing typical compounds such as $C_{16:0}$ and $C_{18:0}$ fatty acids) injected under the same operating conditions, through matching of retention times. This identity is then structurally confirmed by the mass spectrum produced for each peak via GC-MS.

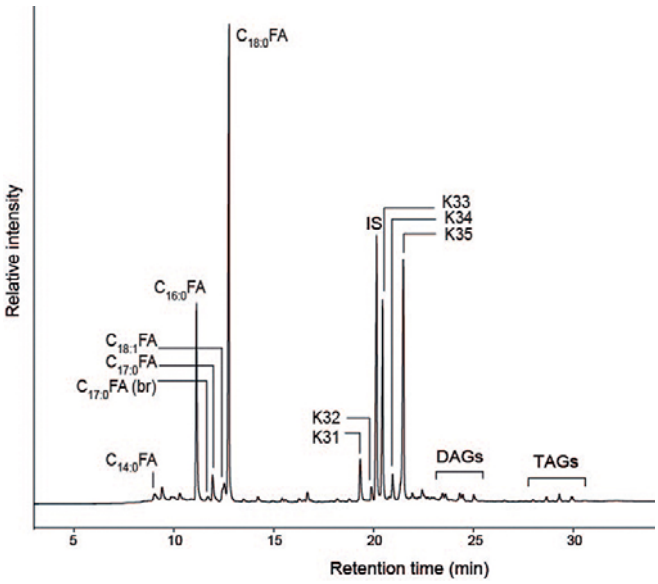


Figure 12
Typical chromatogram showing a heated animal fat. $C_{x:y}$ is a free fatty acid with carbon chain length x and degree of unsaturation y; KX are mid-chain ketones with carbon chain length X, and I.S. is the internal standard (C_{34} *n*-alkane).

The concentrations of lipids present can be determined by the addition of the known quantity of internal standard, added at extraction stage. When interpreting the results, it should be noted that the intensity of the internal standard (IS) can be used as a rough indicator of the concentration of the lipids present in a sample. For example, a small IS peak denotes a concentrated sample. The same amount of internal standard is commonly added to all samples being processed.

The chromatogram shown in Figure 12 shows typical components $C_{16:0}$ FA and $C_{18:0}$ FA, diacylglycerols (DAGs) and triacylglycerols (TAGs), all indicating a degraded animal fat. The K denotes ketones, with a distribution that is indicative of cooking at high temperatures. Consequently, this chromatogram suggests that animal fats were cooked in this vessel, probably through boiling at high temperatures.

1.3 Factors affecting the preservation and decay of organic residues

The porous nature of the ceramic fabric is regarded as an important factor in the preservation of organic residues in archaeological sherds. For this reason lipid analysis is only carried out on unglazed vessels, as the presence of slips or glazes inhibits the mobilisation of lipids into the ceramic matrix. The absorption of lipids into vessels during storage or cooking is likely to be dictated by the size and shape of the ceramic pores (Figure 13), which are determined by the nature of surface treatments and firing conditions during vessel fabrication. This entrapment of organic residues in the protecting environment of the ceramic pore structure probably limits their loss by water leaching. Certainly, the highest concentrations of lipid residues have been observed in very arid geographical areas. Furthermore, it is thought that pore spaces are inaccessible to enzymes produced by degrading microbes, thus limiting microbial degradation. At the other environmental extreme, the burial of artefacts under waterlogged (anoxic) conditions is also favourable for the survival of organic residues.

1.3.1 Use and deposition

Lipids are deposited within the ceramic fabric of a vessel during storage or processing of their contents during the full life history of the vessel. During this time, and in their subsequent deposition in the burial environment, lipids may undergo compositional chemical change through processes such as bacterial action, pyrolysis and autoxidation – for example, in the mixing of different commodities during cooking or the burning of fuel in lamps (Figure 14). It is crucial to match the 'chemical fingerprint' of the

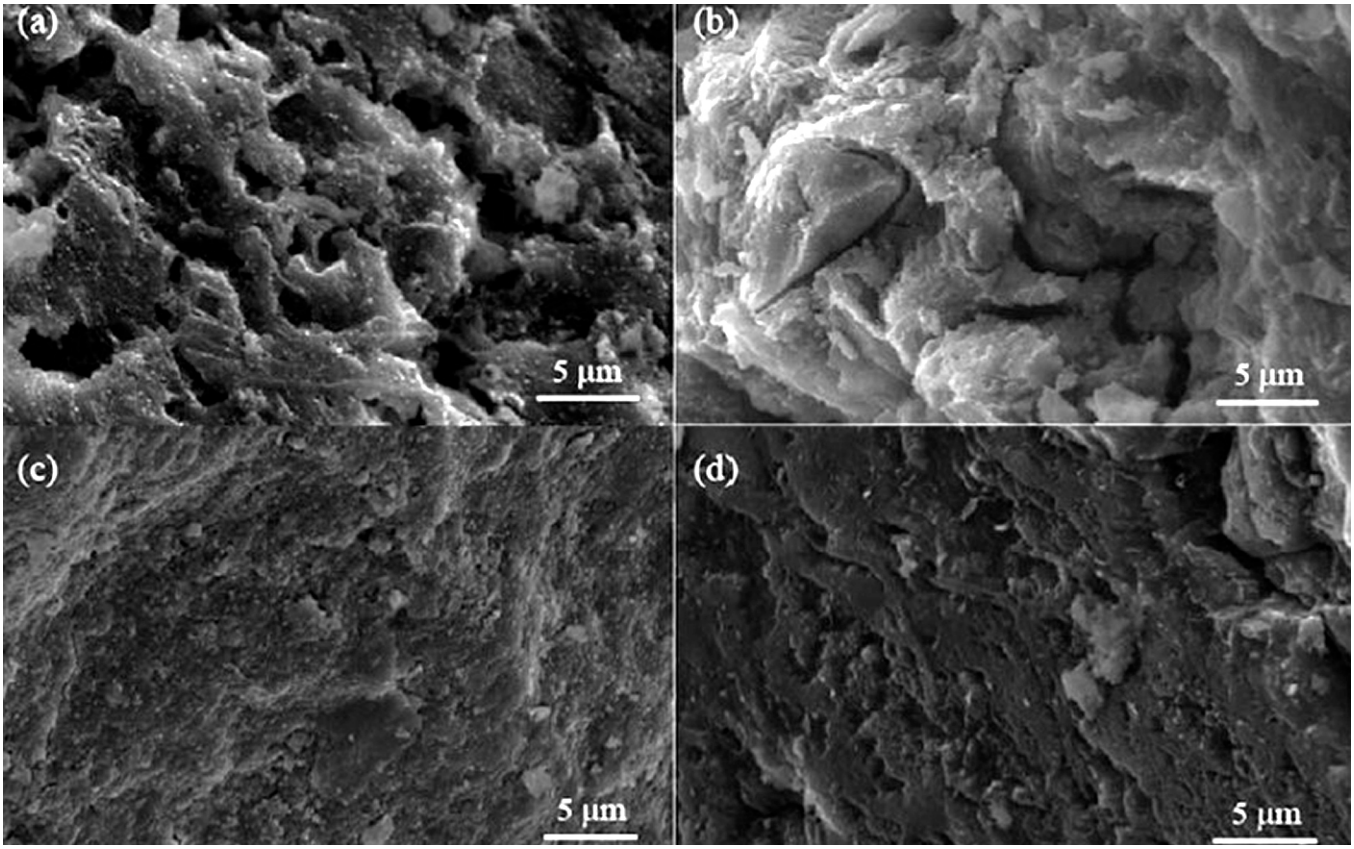


Figure 13
Scanning electron micrographs showing the macro-porosity of archaeological potsherds.
From Correa-Ascencio and Evershed 2014, fig 5.

Reproduced from Analytical Methods 6, Correa-Ascencio, M and Evershed, R P, 'High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction'. 2014, Page No.s 1330–40, with permission of The Royal Society of Chemistry.

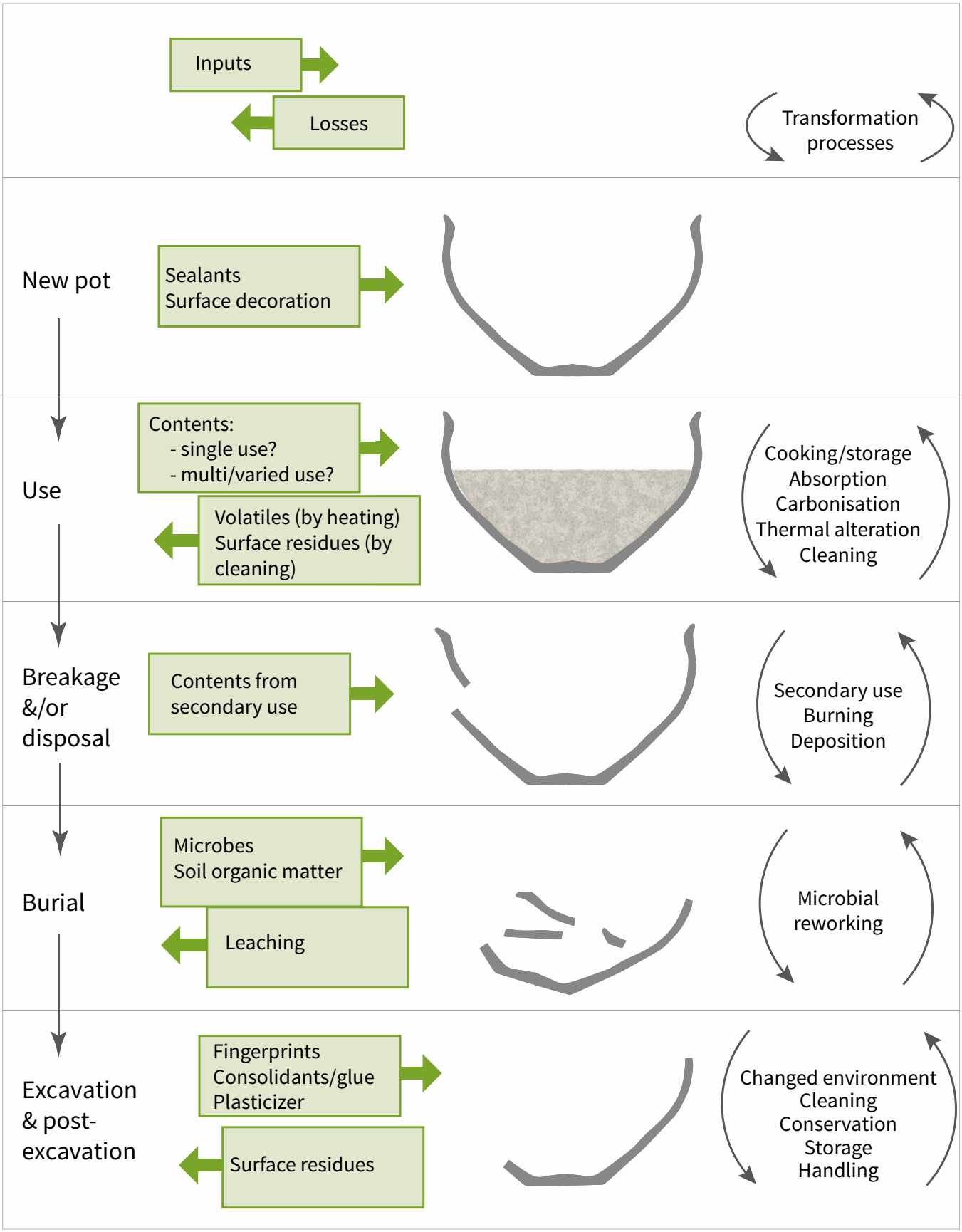


Figure 14
Lipids activity within the life-cycle of a pot.
From Stacey 2009, fig 1. Reproduced with permission from The Old Potter's Almanack.

structures and their distribution to those of biological materials that might have existed in the past. Consequently, structural alteration of the lipids that occurs during these processes must be taken into account when interpreting the origin of biomarkers.

In the burial environment, lipid survival can be influenced by a number of factors: the acidic or alkaline conditions of the soil (soil pH), temperature, light exposure, degree of waterlogging and oxidation-reduction (redox) conditions. For example, it has been demonstrated that extremes of waterlogging and desiccation are often conducive to the survival of residues.

1.3.2 Possible sources of contamination

1.3.2.1 Soil contamination

The first source of contamination of pottery in the burial environment might reasonably be surmised to originate from organic matter present in soil, reflecting the input of animal (such as insects and worms) and plant material, together with microbial synthesis from bacteria and fungi, and subsequent degradative processes (for example, Figure 15 shows potsherds and part vessels in context). However, comparison of lipids (by GC and GC-MS) absorbed in late Saxon/early medieval sherds excavated from the Raunds Area Project at West Cotton, Northamptonshire, with those from soils adhering to the sherds demonstrated that there were clear differences between them. This comparison suggests that contamination within the burial matrix is not a serious problem (Heron *et al* 1991).

1.3.2.2 Contamination arising from excavation and post-excavation practices

As with any type of analysis, chemical tests of organic archaeological remains can be impaired by improper find recovery, handling, treatment or storage. However, in recent years archaeologists have become increasingly aware of the potential problems this can cause, and have abandoned many former common excavation practices, such as brushing artefacts, which destroys surface residues.

The first possible source of contamination might come from human contact during excavation, cleaning, handling and storage of the potsherd. Potentially, the most significant problem arises from handling by excavators and curators. Handling introduces lipids on human hands that can be mistaken for animal fat components preserved in the vessels. Potential contaminants from human fingerprints might include cholesterol or squalene. Cholesterol is known to survive over archaeological timescales, although it is unusual to find it in significant quantities; but squalene degrades much more rapidly, therefore if it were identified in chromatographic profiles it would be assumed to be the result of modern handling.

Chemical compounds from lotions, perfumes, sunscreens and insect repellant have also been detected in organic residue analyses. Compounds such as phthalate plasticisers from plastic bags used to store the potsherds are commonplace, although these are easily chemically identifiable and will not necessarily impede the interpretation of analytical data. Pottery stored in wooden drawers in museums have been found to contain biomarkers known as terpenoids, which indicate the presence of conifer resin.

Figure 15
Clusters of late Iron Age pottery from Middleton Quarry, Norfolk, shown in context.
Archaeological Project Services.

Finally, contamination could also be inadvertently introduced during the preparation of the sample extract in the laboratory, through analyst error, or possible adulteration or impurity of solvents.

The process of conservation and curation can also generate possible sources of contamination, for example, traces of labels applied during curation or reference and site details directly written on potsherds in ink or other media (Figure 16). However, if the available potsherds are a small sample set that includes potsherds with such attachments, and they are not of historical significance, then the top layer of the potsherds can be cleaned with a modelling drill to remove such contaminants.

Another possible contaminant is the glue used by conservators to reconstruct part or complete vessels (Figure 16); therefore sampling from pottery that includes glue or similar products should be confined to sections that are free from these additives.

It has long been common practice for museum conservators to clean potsherds thoroughly and sometimes to wash them in hydrochloric acid solution to remove calcitic encrustations. Where possible, this practice should be avoided for potsherds likely to undergo organic residue analysis.

Potsherds are also often handled without wearing gloves, although these also are a source of contamination – both from the plasticisers that they are made from and from the powder sometimes contained within them. Using nitrile (powder-free) gloves is recommended.

However, note that excellent ORA results have been obtained from numerous cleaned potsherds from museum collections, and therefore such existing collections should not be precluded from analysis.

Figure 16
Example of contaminants that would affect ORA sampling.

2 Research Agenda and Strategy

Applying organic residue analysis to pottery assemblages has the potential to help address specific archaeological questions regarded as priorities by pottery research groups and/or described within regional frameworks. Summaries of regional and thematic research framework documents are shown in Table 1. A full list of regional and thematic research frameworks can be found online.

Some, but not all, regional research frameworks give high prominence to studies of organic residues in pottery, for example East Midland Research Framework, research objectives 3J, 4G and 5E.

The research strategy here is intended as a tool to develop research and is not meant to be prescriptive. Rather, guidance is given to areas where specific 'themes' for site-level research can be targeted in order to address site- or region-specific questions. Suggestions are also provided where these might contribute to large-scale archaeological questions (national and international). This is intended to both enhance the value of individual projects and also, importantly, to maximise the collective research potential of datasets.

Note that the ceramic record constitutes a crucial aspect of archaeological research and issues of form, fabric, function, and techniques of manufacture and distribution remain central themes in ceramic research. ORA can provide meaningful insights into these research areas, consequently helping in broader interpretations of material culture and in social and cultural relations and transformations.

Table 1 (pages 16-17)
Summary details of regional or thematic research framework documents.

Regional Documents	Name	Framework	Date	Available on-line
East of England	East of England Research Framework	Resource Assessment Research Agenda and Strategy Revised Research Framework	1997 2000 2011	www.eaareports.org.uk/
East Midlands	East Midlands Research Framework	Resource Assessment and Research Agenda Updated	2006 2012	http://archaeologydataservice.ac.uk/researchframeworks/eastmidlands/wiki/
London	London Archaeological Research Framework	Resource Assessment Research Agenda Research Strategy	2000 2002 2010 (not publ)	www.museumoflondon.org.uk
North East	North East Research Framework	Resource Assessment, Research Agenda and Strategy	2006	http://www.durham.gov.uk/research
North West	An Archaeological Research Framework for North West England	Resource Assessment Research Agenda and Strategy	2006	http://www.liverpoolmuseums.org.uk/mol/collections/archaeology/arf/
South East – Solent Thames	Solent Thames Research Framework	Resource Assessments and Research Agendas	2008–2010, published 2014	https://library.thehumanjourney.net/2597/
South West	South West Research Framework	Resource Assessment and Research Agenda Research Strategy	2007 2012 2012–2017	www1.somerset.gov.uk/archives/hes/swarf/publications.htm
West Midlands	West Midlands Research Framework	Resource Assessment, Research Agenda and Strategy	2011	http://archaeologydataservice.ac.uk/archives/view/wmrrf_he_2016/ hardcopy www.oxbowbooks.com/dbbc/the-archaeology-of-the-west-midlands.html
Yorkshire	Yorkshire Archaeological Research Framework	Resource Assessment Research Agenda	2005 2007	https://www.historicengland.org.uk/images-books/publications/yorks-arch-res-framework-agenda/

Thematic research frameworks				
Prehistoric Ceramics Research Group	Prehistoric Ceramics Research Group Research Framework: Agenda and Strategy	Research Agenda and Strategy	2016	[forthcoming]
Roman Pottery in Britain	A Research Framework for Post-Roman Ceramic Studies in Britain	Resource Assessment, Agenda and Strategy	2013	http://mprgframework.info/
Post-Roman Ceramic Studies	Research Strategy and Updated Agenda For the Study of Roman Pottery in Britain	Research Agenda and Strategy	2011	http://romanpotterystudy.org/SGRPPublications/strategy/Strategy.pdf

2.1 Theme 1: Archaeological deposits and finds assemblages

Pottery often forms a significant part of an archaeological deposit, particularly as part of redeposition processes. The identification and interpretation of structured deposits might be aided by a consideration of what role the form, function, size and decoration of vessels plays in their selection for structured deposition. Using ORA in site-specific research on the function of vessels, and whether there is a relationship between form and function, could feed into questions of how the occurrence and nature of deposition practices varied regionally and chronologically. For example, pits associated with Neolithic Grooved Ware frequently reveal a greater complexity and formality in terms of the objects placed within them, and often contain 'exotic' artefacts such as polished stone tools, boar tusk blades and bone pins (Pollard 2002). An organic residue comparison between Grooved Ware from formal pit depositions and different contexts – such as house and settlement deposits, chambered tombs, enclosures, henge and cursus monuments – may shed light on cultural, social and political aspects of Neolithic food consumption and discard. Different vessel types and inter- and intra-site variations can also be investigated.

An ORA programme that involves sampling specific assemblages could be used to address archaeological questions over specific temporal and spatial frameworks. For example, Roman black-burnished ware is an instantly-recognisable cooking ware, used both by military garrisons and civilians, and was widely exported across much of Roman Britain from the 2nd century to the end of Roman occupation. It is therefore an ideal artefact for residue analysis examining possible variations in, and changes to, diet and cooking methods across the province through time. Comparison of Carinated Bowl assemblages from Early Neolithic sites in Britain and Ireland with those from the adjacent areas of France and Belgium provides another example.

2.2 Theme 2: Chronology

Pottery is key for dating, and the identification, recovery and detailed analysis of diagnostic groups in stratigraphic sequences are of fundamental importance for most regions in all periods, particularly in prehistoric pottery studies. However, many questions remain regarding the ceramic sequences of most regions of Britain and the relationships between regional- and national-period chronologies. A programme combining both radiocarbon dating and organic residue analysis on an assemblage that contains well-stratified sequences of pots with burnt residues can provide information both on dietary practices across time and establishing secure chronologies for the sequence.

Although currently experimental, Stott *et al* (2001, 2003) and Berstan *et al* (2008) have attempted direct dating of archaeological pottery by compound-specific radiocarbon analysis of **absorbed** lipids. Once this technique is fully validated, the production of radiocarbon dates for lipids will further refine chronologies and could be used directly to link commodities processed in the vessels during their use. This development could also provide absolute dates for addressing archaeological questions relating to dietary and subsistence practices.

2.3 Theme 3: Functions of pottery

The study of function in pottery has proved useful for information on a range of aspects of past societies, including production, trade and exchange, and technological change and specialisation. It is generally assumed that the technology, form and size of a vessel are constrained by its intended use, but the relationships between form, function and technology are complex and variable. The fabrication and style of vessels are often related to the commodities processed or stored within them. Consequently, organic residue analysis can be used to examine the relationships between form and function. Vessel capacity and shape are likely to relate closely to different potential functions for the various styles of pottery. For example, narrow, high-mouthed jars and jugs were probably used to store and handle liquids, whereas broader bowl-shaped pots were more likely used to prepare solid food. Consideration of use-wear and analysis of residues – such as variations between vessels of different forms, sizes and decorative styles – are critical factors to determine how such vessels were actually used. For example, changing patterns in food consumption in later prehistory could be examined by comparing vessel types, capacities, decoration and use from Late Bronze Age, Early Iron Age and Middle to Late Iron Age domestic structures (Morris 2002).

Other research might focus on organic residue analysis from vessels from funerary contexts. Such analyses can help indicate whether cremation vessels were previously used as cooking vessels or whether they were manufactured solely for special purposes, as Boast (1995) has suggested for Beakers. Similarly, specific pots may have been produced for a variety of other special purposes, such as the very large globular urns from a number of Bronze Age sites in central southern England associated with feasting episodes (Ladle and Woodward 2003).

2.4 Overarching archaeological research themes

Organic residue analyses on ceramics have also proven to be of great value in answering large-scale questions across temporal and spatial scales. Some specific examples are set out below. For example, ORA was used to investigate pottery assemblages from the north-east Atlantic archipelagos, ranging from the Early Neolithic to the Viking/Norse period. This revealed an immediate shift away from the exploitation of aquatic resources in the earliest Neolithic, later followed by a gradual return to the inclusion of marine products over subsequent millennia, maximising in the Late Norse period (Cramp *et al* 2014).

2.4.1 Settlement organisation

Organic residue analysis might usefully be applied to studies of settlement organisation, especially with regard to spatial use. For example, spatial variability in the quantity, forms and condition of pottery at a site can be used to interpret patterns of activity, and thus help to understand how particular areas within a site, or even sites themselves, were used (Woodward 2002). ORA can further refine this understanding by revealing areas of food preparation, cooking or storage – specific patterns that may not be readily apparent from ceramic distribution patterns alone.

2.4.2 Diet and subsistence practices

ORA can also contribute significantly to key questions that arise about diet and subsistence practices, particularly over transition periods, such as the Mesolithic–Neolithic transition, between the Bronze and Iron Ages, and from the Late Iron Age to the Romano-British period. For example, the transition from the Bronze Age to the Iron Age represents a time of considerable development and intensification of agricultural practices. Consequently, specific questions might address how diet and land-use varied over time and between different ecological zones. In this instance, ORA of absorbed residues from pottery can help identify specialist pastoralist zones and elucidate coastal resource exploitation strategies.

Similarly, ORA can be valuably integrated into investigation of aspects of continuity or change in dietary practices between the Iron Age and Roman period in Britain, a transition still poorly understood. There are also still many unanswered questions regarding subsistence in Roman Britain. For example, what is the evidence of diet for people of high and low status in Romano-British society? Are there differences between rural and urban settlements? Does diet differ at military sites? ORA can help address these questions.

Data derived from ORA can also be integrated into broader-scale projects, such as those concerned with Roman agricultural practices. For example, faunal evidence suggests a clear reliance on cattle as a primary source of meat, yet the faunal assemblage from Romano-British York shows an abundance of pig. Here ORA can be applied to determine whether this anomaly is reflected in the organic residues, and thereby begin to address the nature of the relationship between food preparation and faunal remains preserved in the archaeological record.

Additionally, historical sources suggest that dairying in Roman Britain was minimal and did not generally play a large part in Roman subsistence. A multi-site ORA project can assess the validity of the literary evidence, and thereby help refine our understanding of the written records for Roman Britain compared to the archaeological evidence. Comparisons with assemblages from other Roman territories could then help shed further light on the role of Roman Britain within the Empire.

3 Reporting, Publishing and Archiving

3.1 Reporting, publishing and digital archiving

A report on organic residues should include the stated aims and objectives of the project (the specific research questions to be answered), details of the samples analysed, methodology, results and conclusions. The level of interpretation provided by an organic residues report will depend on the type of analysis carried out – for example, if stable carbon isotope analysis ($\delta^{13}C$) was carried out on the lipids.

It is recommended that the ORA report is integrated with other post-excavation analyses and incorporated into the overall site discussion, for inclusion in the publication report. This does not preclude the ORA results being published as a specialist article.

All digital material produced during the ORA report should be included in the archive for the whole project. Current best practice is for digital material to be curated at a Trusted Digital Repository, such as the Archaeology Data Service.

3.2 Archiving ceramics to be used for ORA – guidance for museum curators and conservators

These brief guidelines provide advice for the storage and conservation of ceramic material for museum curators and conservators, in order to best maximise research potential.

- Where possible, ceramic material identified as having future research potential, which has been agreed to form part of the museum archive, should be stored separately from the main archive – documented, packed and labelled appropriately
- Museum curators should be made aware that the cleaning, packaging and marking of such material are likely to differ from those required by their general conditions of acceptance, and asked to accommodate this where possible
- Provision should be made to ensure that replacement packaging materials that might subsequently be used in museum stores (post-deposition) should be capable of continuing to preserve future research potential for as long as possible

- Museum curators should also be aware that handling, conservation practice and preparation of materials for display may result in contamination with regard to ORA sampling; a balance may need to be drawn between providing public access to archaeological material and future research

As mentioned, ORA is a destructive technique. Consequently, it is unlikely that there will be any remaining material for curation after analysis of the sample has taken place. However, usually potsherds larger than the recommended 2–3g are supplied for analysis, which means that there is likely to be residual material that can be returned to the project commissioning body. In case they are required for further analysis, such returned material should be well-labelled and stored separately from the main ceramic assemblage in the project archive.

4 Further Reading

There are good overviews of archaeological chemistry, and, specifically, organic residue analysis, for the non-specialist:

Pollard, A M, Batt, C M, Stern, B and Young, S M M 2007 *Analytical Chemistry in Archaeology*. Cambridge: Cambridge UP

Pollard, A M, and Heron, C 1996 *Archaeological Chemistry*. London: The Royal Society of Chemistry

Useful guides to lipids and lipid analysis:

Christie, W W 1981 *Lipid Metabolism in Ruminant Animals*. Oxford: Pergamon Press

Gunstone, F D, Harwood, J L and Dijkstra, A J 2007 *The Lipid Handbook*, 3 edn. Abingdon: Taylor and Francis Group

Gurr, M L, Harwood, J L and Frayn, K N 2008 *Lipid Biochemistry: An Introduction,* 5 edn. London: John Wiley & Sons

Hilditch, T P and Williams, P N 1964 *The Chemical Constitution of Natural Fats*. London: John Wiley & Sons

Other useful published books and articles:

Heron, C and Evershed, R P 1993 'The analysis of organic residues and the study of pottery use'. *Archaeol Method and Theory* **5**, 247–84

Mills, J S and White, R 1994 *The Organic Chemistry of Museum Objects*. London: Butterworth and Co. Ltd

Stacey, R J 2009 'Organic residues: origins, analysis and scope – an overview for the archaeological ceramicist'. *The Old Potter's Almanack* **14**, 1–8

Roffet-Salque, M, Dunne, J, Altoft, D T, Casanova, E, Cramp, L J E, Smyth, J, Whelton, H and Evershed, R P 2016 In Press 'From the inside out: Upscaling organic residue analyses of archaeological ceramics'. *J Archaeol Sci: Reports*.

Information on the technique and data analysis of GC-MS:

McMaster, M and McMaster, C 2008 *GC-MS. A Practical Users Guide*, 2 edn. London: John Wiley & Sons

Smith, R M and Busch, K L 1999 *Understanding Mass Spectra – A Basic Approach*. London: John Wiley & Sons

Archaeometry and *The Journal of Archaeological Science* are two of the main journals that contain publications relating to organic residue analysis. Further information on publications which cover the identification of particular biomarkers or specific archaeological questions can be found in the thematically organised bibliography below.

4.1 Thematic bibliography

For a comprehensive overview of the subject of organic residue analysis:

Evershed, R P 2008 'Organic residue analysis in archaeology: the archaeological biomarker revolution'. *Archaeometry* **50**, 895–924

Beeswax

Evershed, R P, Vaughan, S J, Dudd, S N and Soles, J S 1997 'Fuel for thought? Beeswax in lamps and conical cups from late Minoan Crete'. *Antiquity* **71**, 979–85

Evershed, R P, Dudd, S N, Anderson-Stojanovic, V R and Gebhard, E R 2003 'New chemical evidence for the use of combed ware pottery vessels as beehives in Ancient Greece'. *J Archaeol Sci* **30**, 1–12

Frith, J, Appleby, R, Stacey, R and Heron, C 2004 'Sweetness and light: chemical evidence for beeswax at tallow candles at Fountains Abbey'. *Medieval Archaeol* **XLVIII**, 220–7

Heron, C, Nemcek, N, Bonfield, K M, Dixon, D and Ottaway, B S 1994 'The chemistry of Neolithic beeswax'. *Naturwissenschaften* **81**, 266–9

Kimpe, K, Jacobs, P A and Waelkens, M 2002 'Mass spectrometric methods prove the use of beeswax and ruminant fat in late Roman cooking pots'. *J Chromatography A* **968**, 151–60

Namdar, D, Neumann, R, Goren, Y and Weiner, S 2009 'The contents of unusual cone-shaped vessels (cornets) from the Chalcolithic of the southern Levant'. *J Archaeol Sci* **36**, 629–36

Regert, M, Colinart, S, Degrand, L and Decavallas, O 2001 'Chemical alteration and use of beeswax through time: accelerated ageing tests and analysis of archaeological samples from various environmental contexts'. *Archaeometry* **43**, 549–69

Roffet-Salque, M, Regert, M, Evershed, R P, Outram, A K, Cramp, L J E, Decavallas, O, Dunne, J, *et al* [there are 58 more authors] 2015 'Widespread exploitation of the honeybee by early Neolithic farmers'. *Nature* **527**(7577), 226–30

Stacey, R J 2011 'The composition of some Roman medicines: evidence for Pliny's Punic wax?' *Analyt Bioanalyt Chem* **401**, 1749–59

Bitumens, pitches and tars

Connan, J 1999 'Use and trade of bitumen in antiquity and prehistory: molecular archaeology reveals secrets of past civilizations'. *Philosoph Trans Roy Soc London* **354**, 33–50

Connan, J and Nissenbaum, A 2003 'Conifer tar on the keel and hull planking of the Ma'agan Mikhael Ship (Israel, 5th century BC): identification and comparison with natural products and artefacts employed in boat construction'. *J Archaeol Sci* **30**, 709–719

Connan, J, Nieuwenhuyse, O P, Van As, A and Jacobs, L 2004 'Bitumen in early ceramic art: bitumen-painted ceramics From Late Neolithic Tell Sabi Abyad (Syria)'. *Archaeometry* **46**, 115–24

Connan, J, Nissenbaum, A, Imbus, K, Zumberge, J and Macko, S 2006 'Asphalt in iron age excavations from the Philistine Tel Miqne-Ekron city (Israel): Origin and trade routes'. *Organic Geochem* **37**, 1768–86

Dudd, S N and Evershed, R P 1999 'Unusual triterpenoid fatty acyl ester components of archaeological birch bark tars'. *Tetrahedron Lett* **40**, 359–62

Evershed, R P, Jerman, K and Eglinton, G 1985 'Pine wood origin for pitch from the Mary Rose'. *Nature* **314**, 528–30

Grünberg, J 2002 'Middle Palaeolithic birch-bark pitch'. *Antiquity* **76**, 15–16

Stern, B, Connan, J, Blakelock, E, Jackman, R, Coningham, R A E and Heron, C 2008 'From Susa to Anuradhapura: Reconstructing aspects of trade and exchange in bitumen-coated ceramic vessels between Iran and Sri Lanka from the Third to the Ninth Centuries AD'. *Archaeometry* **50**, 409–28

Urem-Kotsou, D, Stern, B, Heron, C and Kotsakis, K 2002 Birch-bark tar at Neolithic Makriyalos, Greece. *Antiquity* **76**, 962–7

Coprolites, manuring and soils

Bethell, P H, Goad, L J, Evershed, R P and Ottaway, J 1994 'The study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material'. *J Archaeol Sci* **21**, 619–32

Bull, I D, Simpson, I A, Dockrill, S J and Evershed, R P 1999 'Organic geochemical evidence for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney'. *Organic Geochem* **30**, 535–56

Bull, I D, Simpson, I A, van Bergen, P F and Evershed, R P 1999) 'Muck "n" molecules: organic geochemical methods for detecting ancient manuring'. *Antiquity* **73**, 86–96

Bull, I D, Evershed, R P and Betancourt, P P 2001 'An organic geochemical investigation of the practice of manuring at a Minoan site on Pseira Island, Crete'. *Geoarchaeol* 16, 223–42

Bull, I D, Elhmmali, M M, Roberts, D J and Evershed, R P 2003 'The application of steroidal biomarkers to track the abandonment of a Roman wastewater course at the Agora (Athens, Greece)'. *Archaeometry* **45**, 149–61

Evershed, R P, Bethell, P H, Reynolds, P J and Walsh, N J 1997 '5ß-Stigmastanol and related 5ß-Stanols as biomarkers of manuring: analysis of modern experimental material and assessment of the archaeological potential'. *J Archaeol Sci* **24**, 485–95

Hjulström, B and Isaksson, S 2009 'Identification of activity area signatures in a reconstructed Iron Age house by combining element and lipid analyses of sediments'. *J Archaeol Sci* **36**, 174–83

Shillito, L-M, Bull, I D, Matthews, W, Almond, M J, Williams, J M and Evershed, R P 2011 'Biomolecular and micromorphological analysis of suspected faecal deposits at Neolithic Çatalhöyük, Turkey'. *J Archaeol Sci* **38**, 1869–77

Diet and subsistence practices

(including identification of ruminant and non-ruminant carcass products, dairy, aquatic and plant resources)

Baeten, J, Jervis, B, De Vos, D and Waelkens, M 2013 'Molecular evidence for the mixing of meat, fish and vegetables in Anglo-Saxon coarseware from Hamwic, UK'. *Archaeometry* **55**, 1150–74

Copley, M S, Rose, P J, Clapham, A, Edwards, D N, Horton, M C and Evershed, R P 2001 'Detection of palm fuit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia'. *Proc Biol Sci* **268**, 593–7

Copley, M S, Berstan, R, Dudd, S N, Docherty, G, Mukherjee, A J, Straker, V, Payne, S and Evershed, R P 2003 'Direct chemical evidence for widespread dairying in Prehistoric Britain'. *Proc Nat Acad Sci USA* **100**, 1524–9

Copley, M S, Berstan, R, Dudd, S N, Straker, V, Payne, S and Evershed, R P 2005a 'Dairying in antiquity. I. Evidence from absorbed lipid residues dating to the British Iron Age'. *J Archaeol Sci* **32**, 485–503

Copley, M S, Berstan, R, Straker, V, Payne, S and Evershed, R P 2005b 'Dairying in antiquity. II. Evidence from absorbed lipid residues dating to the British Bronze Age'. *J Archaeol Sci* **32**, 505–21

Copley, M S, Berstan, R, Mukherjee, A, Dudd, S N, Straker, V, Payne, S and Evershed, R P 2005c 'Dairying in antiquity. III. Evidence from absorbed lipid residues dating to the British Neolithic'. *J Archaeol Sci* **32**, 523–46

Copley, M S, Berstan, R, Dudd, S N, Aillaud, S, Mukherjee, A J, Straker, V, Payne, S and Evershed, R P 2005d 'Processing of milk products in pottery vessels through British prehistory'. *Antiquity* **79**, 895–908

Craig, O E, Chapman, J, Heron, C, Willis, L H, Bartosiewicz, L, Taylor, G, Whittle, A and Collins, M 2005 'Did the first farmers of central and eastern Europe produce dairy foods?' *Antiquity* **79**, 882–94

Craig, O E, Taylor, G, Mulville, J, Collins, M J and Parker Pearson, M 2005 'The identification of prehistoric dairying activities in the Western Isles of Scotland: an integrated biomolecular approach'. *J Archaeol Sci* **32**, 91–103

Craig, O E, Forster, M, Andersen, S.H, Koch, E, Crombe, P, Milner, N J, Stern, B, Bailey, G N and Heron, C P 2007 'Molecular and isotopic demonstration of the processing of aquatic products in northern European prehistoric pottery'. *Archaeometry* **49**, 135–52

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Cramp, L J E, Evershed, R.P and Eckardt, H 2011 'What was a mortarium used for? Organic residues and cultural change in Iron Age and Roman Britain'. *Antiquity* **85**, 1339–52

Cramp, L J E, Jones, J, Sheridan, A, Smyth, J, Whelton, H, Mulville, J, Sharples, N and Evershed, R P 2014a 'Immediate replacement of fishing with dairying by the earliest farmers of the northeast Atlantic archipelagos'. *Proc Royal Soc B: Biol Sci* **281**(1780), DOI: 10.1098/rspb.2013.2372 [online journal]

Cramp, L J E, Evershed, R P, Lavento, M, Halinen, P, Mannermaa, K, Oinonen, M, Kettunen, J, Perola, M, Onkamo, P and Heyd, V 2014b 'Neolithic dairy farming at the extreme of agriculture in northern Europe'. *Proc Roy Soc B: Biol Sci* **281**(1791), DOI: 10.1098/rspb.2014.0819 [online journal]

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Dudd, S N, Evershed, R P and Gibson, A M 1999 'Evidence for varying patterns of exploitation of animal products in different prehistoric pottery traditions based on lipids preserved in surface and absorbed residues'. *J Archaeol Sci* **26**, 1473–82

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Isaksson, S and Hallgren, F 2012 'Lipid residue analyses of Early Neolithic funnel-beaker pottery from Skogsmossen, eastern Central Sweden, and the earliest evidence of dairying in Sweden'. *J Archaeol Sci* **39**, 3600–9

Mukherjee, A J, Gibson, A M and Evershed, R P 2008 'Trends in pig product processing at British Neolithic Grooved Ware sites traced through organic residues in potsherds'. *J Archaeol Sci* **35**, 2059–73

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Smyth, J R and Evershed, R P 2015 'Milking the megafauna: using organic residue analysis to understand early farming practice'. *Environmental Archaeol* DOI: 10.1179/1749631414Y.0000000045 [online journal]

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Evershed, R P 1992 'Chemical composition of a bog body adipocere'. *Archaeometry* **34**, 253–65

Evershed, R P and Connolly, R C 1994 'Post-mortem transformations of sterols in bog body tissues'. *J Archaeol Sci* **21**, 577–83

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Styring, A K, Sealy, J C and Evershed, R P 2010 'Resolving the bulk δ^{15}N values of ancient human and animal bone collagen via compound-specific nitrogen isotope analysis of constituent amino acids'. *Geochimica et Cosmochimica Acta* **74**, 241–51

Mummification practices

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5 Where to Get Advice

Within Historic England, the first point of contact for general archaeological science enquiries, including those relating to organic residue analysis, should be the HE regional science advisors, who can provide independent, non-commercial advice. For contact details see HistoricEngland.org.uk/scienceadvice

Specific advice can be sought from laboratories carrying out organic residue analysis, based at the Universities of Bradford, Bristol and York:

School of Chemistry
University of Bristol
Cantock's Close
Clifton
Bristol BS8 1TS

Contact: Richard Evershed
R.P.Evershed@bristol.ac.uk

Archaeological Sciences
University of Bradford
Bradford
West Yorkshire BD7 1DP

Contact: Ben Stern
B.Stern@bradford.ac.uk

Department of Archaeology
University of York
King's Manor
York YO17EP

Contact: Oliver Craig
oliver.craig@york.ac.uk

5.1 Contact Historic England

East Midlands
2nd Floor, Windsor House
Cliftonville
Northampton NN1 5BE
Tel: 01604 735460
Email: eastmidlands@HistoricEngland.org.uk

East of England
Brooklands
24 Brooklands Avenue
Cambridge CB2 8BU
Tel: 01223 582749
Email: eastofengland@HistoricEngland.org.uk

Fort Cumberland
Fort Cumberland Road
Eastney
Portsmouth PO4 9LD
Tel: 023 9285 6704
Email: fort.cumberland@HistoricEngland.org.uk

London
1 Waterhouse Square
138-142 Holborn
London EC1N 2ST
Tel: 020 7973 3700
Email: london@HistoricEngland.org.uk

North East
Bessie Surtees House
41-44 Sandhill
Newcastle Upon Tyne NE1 3JF
Tel: 0191 269 1255
Email: northeast@HistoricEngland.org.uk

North West
3rd Floor, Canada House
3 Chepstow Street
Manchester M1 5FW
Tel: 0161 242 1416
Email: northwest@HistoricEngland.org.uk

South East
Eastgate Court
195-205 High Street
Guildford GU1 3EH
Tel: 01483 252020
Email: southeast@HistoricEngland.org.uk

South West
29 Queen Square
Bristol BS1 4ND
Tel: 0117 975 1308
Email: southwest@HistoricEngland.org.uk

Swindon
The Engine House
Fire Fly Avenue
Swindon SN2 2EH
Tel: 01793 445050
Email: swindon@HistoricEngland.org.uk

West Midlands
The Axis
10 Holliday Street
Birmingham B1 1TG
Tel: 0121 625 6870
Email: westmidlands@HistoricEngland.org.uk

Yorkshire
37 Tanner Row
York YO1 6WP
Tel: 01904 601948
Email: yorkshire@HistoricEngland.org.uk

6 Acknowledgements

Contributors

This document was written and compiled by Julie Dunne with contributions from Lucy Cramp, Richard Evershed, Carl Heron, Oliver Craig, Ben Stern, David Knight, Alistair Barclay, Rob Perrin, Vanessa Straker, Sylvia Warman, Gail Boyle, Rebecca Stacey, Rhea Brettell, Jessica Smyth and Simon Hammann.

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