

ORGANIC RESIDUE ANALYSIS IN CERAMIC STUDIES: IMPLICATIONS FOR CONSERVATION TREATMENT AND COLLECTIONS MANAGEMENT

Tania F.M. Oudemans and David Erhardt

ABSTRACT

The chemical characterization of organic residues found in association with ceramic vessels can provide direct information on original vessel use and on diet and cooking practices of people in the past. Major improvements in analytical instrumentation have enabled the study of ever smaller samples of complex organic materials on a molecular level. Various analytical techniques have been applied to study the chemical composition of organic residues. Volatile, non-chemically bound classes of compounds such as lipids and terpenoids have been selectively extracted and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Other studies have been directed at the characterization of the condensed matrix of solid organic residues (for example, carbonized or cross-linked compounds). Non-destructive techniques such as nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FTIR) have been used to characterize materials based on the absence or presence of functional groups. More destructive techniques have been employed to fragment the condensed macromolecular matrix by partial hydrolysis or analytical pyrolysis. A review is presented of the possibilities and limitations of various analytical techniques. The application of organic residue analysis in ceramic studies raises many questions concerning conservation treatment of ceramics during and after excavation, as well as the long-term storage of ceramic vessels. Ceramics that may contain original organic residues should be treated as organic/inorganic composites rather than as exclusively inorganic materials. In this paper an organic residue preservation protocol is presented for conservators in the field, and sampling strategies are discussed.

INTRODUCTION

Organic residues

Organic residues found in association with ancient ceramic vessels can be seen as the result of human activity that took place hundreds, if not thousands, of years ago. These residues supply an insight into the type of organic products people cooked, stored or otherwise prepared, and also illustrate which vessels were used for these tasks. The complex of vessel/residue/burial context is therefore a perfect example of 'behaviour' fossilized in material remains.

Organic residues can occur as bulk residues (organics contained in ceramics), as surface residues (solid crusts or films adhering to the interior or exterior of a vessel) or as absorbed residues (organics absorbed into the ceramic fabric of the pot).

Bulk residues are rarely discovered in large quantities at a site, except in special burial circumstances such as graves, shipwrecks, natural catastrophes (volcanic eruptions, earthquakes) or caches of treasure. Surface residues are much more common. Many types have been described [1] including black sooty residues, black or dark brown 'carbonized' crusts, red-brown smooth layers, and cream-coloured or yellowish crusts. Although organic residues are frequently mentioned, no systematic description of the visual characteristics has been proposed, nor has a uniform terminology been adopted for this purpose. Results from a small number of diverse ceramic assemblages suggest that 1-5% of the sherds contain visible surface residues [2].

Absorbed residues are invisible and can be detected only by extraction and analysis. The frequent detection of extractable organics such as lipids and terpenoids suggests these residues are commonly present in ceramic vessels [3]. However, there are no records on the frequency of occurrence of absorbed residues in ceramic complexes.

Degradation and preservation

The state of degradation varies widely both between and within excavations. In addition, burial circumstances can have different

effects on various classes of chemical compounds. For example, the presence of water and lack of oxygen will cause anaerobic degradation, which may change the lipid profile and degrade sugars present in the residues. The presence of acidic water (as in peat bogs) will change the composition of the proteinaceous materials quite extensively due to acidic denaturing. Arid conditions preserve structural elements such as lignin and proteins, but have a strong oxidizing and cross-linking effect on the lipids.

Empirical results show that some processes such as carbonization of residues and the absorption of organics into the ceramic fabric seem to help preserve organic compounds such as lipids and proteins. Although many hypotheses have been postulated to explain this phenomenon, the mechanism is not fully understood.

APPLICATION OF ORGANIC RESIDUE ANALYSIS IN CERAMIC STUDIES

Organic residues as indicators of diet

Many classes of organic compounds, including lipids (fatty acids, acyl lipids, sterols, leaf waxes) [4], sugars and carbonized sugars [1, 5], amino acids [6], terpenoids [7, pp 109-122] and beeswax [8], have been detected and identified in organic residues on ceramics. Often these studies were directed at understanding diet in societies in the past. However, the identification of the original diet from organic residues is complicated by several factors.

Organic residues may not originate from food remains because vessels were often used to prepare or store other types of materials, such as organic adhesives, paints, coatings and medicines. Many of these are chemically indistinguishable from foods. Foods are often a mixture, increasing the complexity of the chemical composition of the residue. In addition, many foods contain common chemical compounds that are not species-specific or even indicative of particular food groups. Generally, only broad classifications of foods are feasible, such as vegetable oil, mammal fat, fish fat, dairy fat or plant wax. Exceptions are specific identifications such as beeswax, caffeine, theobromine and oxalic acid (from wine).

More specific identifications will probably be possible in the future, but at present organic residue analysis for the identification of diet is limited. Many aspects of diet can probably be studied more successfully using other evidence from the archaeological record.

Organic residues as indicators of vessel use

Ceramic studies are increasingly directed at functional aspects of pottery assemblages. Knowledge of the utilitarian aspects of pottery is needed before ceramic artifacts can be used to draw conclusions which relate to other aspects of the society [9]. Although ceramic studies commonly include information on recovery context, pot form and ceramic technology, the conclusions on functional classification are seldom very specific due to the complex relationships between form, function and technology.

The study of the chemical characteristics of residues provides independent information regarding the use of vessels that cannot be obtained through any other technique [10].

ANALYTICAL TECHNIQUES, THEIR POTENTIAL AND LIMITATIONS

The study of small amounts of complex mixtures of degraded

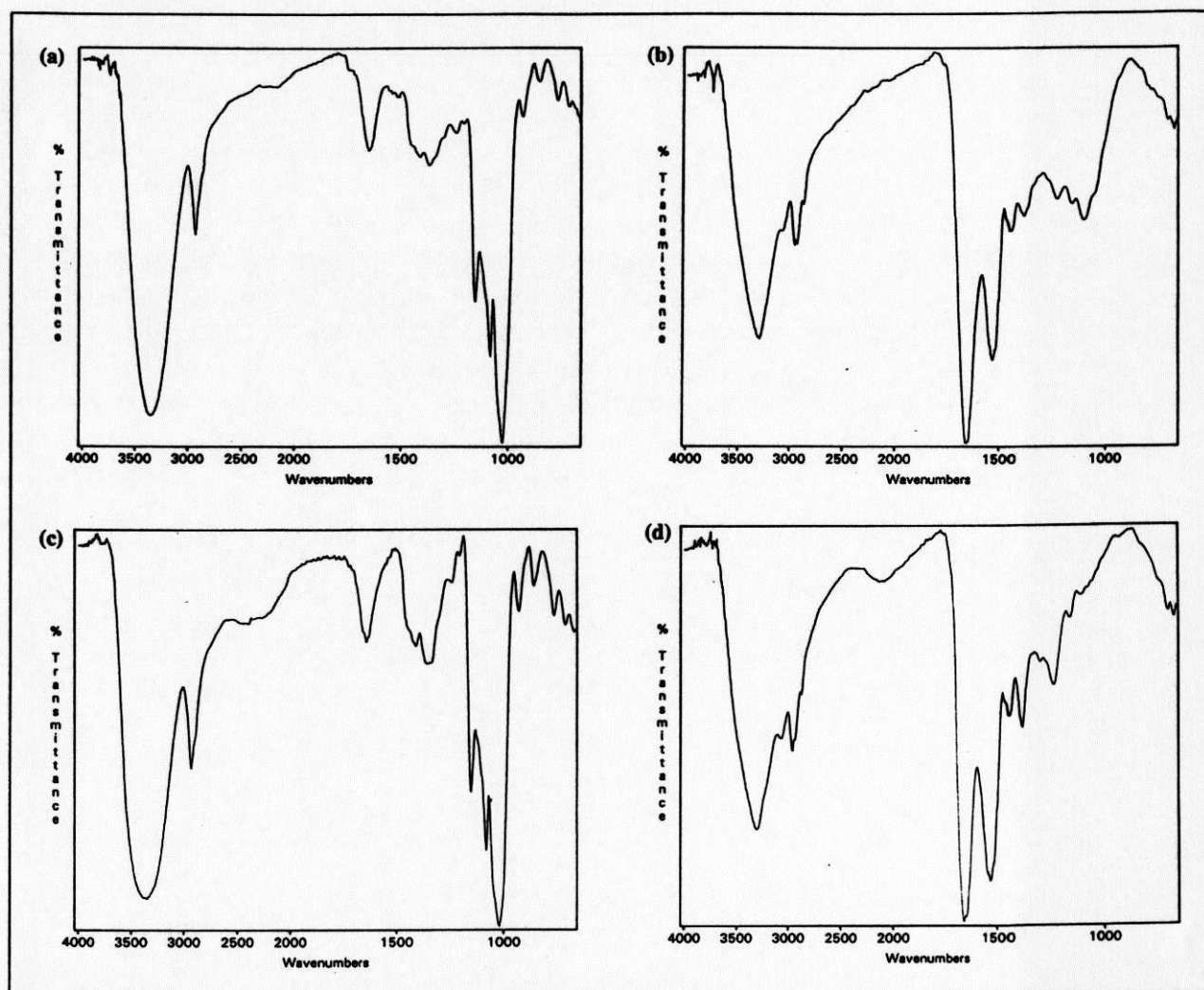


Fig. 1 FTIR spectra of black surface residues from two *oppaya* (meat and vegetable cooking pots) collected while in use at a Kalinga village (Guina-ang, The Philippines) by Skibo [9], and two reference materials. (a) From small *oppaya* (no. 88-77-44). (b) From *oppaya* (no. 88-77-20). (c) Corn starch. (d) Bovine albumen. Correlation coefficient (over wavelengths 700-1900 cm^{-1} and 2400-3750 cm^{-1}) between (a) and (c) was 0.98 and between (b) and (d), 0.97. Spectra were recorded with a Matson 4326 Upgrade FTIR spectrophotometer [12].

organic materials creates many analytical challenges. Two approaches can be taken to obtain chemical information on such materials. Characteristics of the mixture as a whole can be determined and a cumulative result obtained that gives information on the level of a 'total sample'. Usually these techniques result in a chemical 'fingerprint' that can be compared with fingerprints of other known materials. The information obtained with fingerprinting techniques is commonly used to compare and classify samples and to determine further analytical strategies.

Alternatively, the sample can be separated into fractions and each fraction can be analysed in more detail; for further analytical strategies, see [11]. However, each separation and preparation step requires additional sample, and some separation steps result in a loss of information due to incomplete separation, sample loss, or undesired chemical change during separation.

Elementary and isotopical characterization of mixtures using C/H/N analysis and SIA analysis

The organic elemental composition of a residue can be determined by analysis of the amounts of carbon (C), hydrogen (H) and nitrogen (N) present in the combustion gas of a small sample. The C/H/N results indicate what fraction of the sample is organic and the ratios suggest the chemical composition of the material. The C/N ratio indicates the protein fraction present and the C/H

ratio illustrates the degree of saturation and condensation of the material.

Stable isotope analysis (SIA) gives information on the ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in the sample. Since these ratios depend on the metabolic system of the organisms involved, they can be seen as an indication of the type of original material present in the residue. However, mixing of foodstuffs generally limits the applicability of this technique in organic residue analysis.

Chemical characterization of mixtures using FTIR and NMR spectroscopy

Fourier transform infrared spectroscopy (FTIR) is based on the light absorption characteristics of various chemical compounds in a material. Each type of chemical bond (or functional group) absorbs light of a particular wavelength or range of wavelengths. The presence or absence of absorption peaks typical for particular bond types or functional groups provides information on the absence or presence of certain compound classes in the given sample (Fig. 1).

FTIR is a rapid analytical technique ideal for the initial classification of organic residues into groups with broadly comparable chemical composition [12]. General determination of the nature of the samples can be made through comparison with reference spectra of known materials (Fig. 1). FTIR can rarely be used for

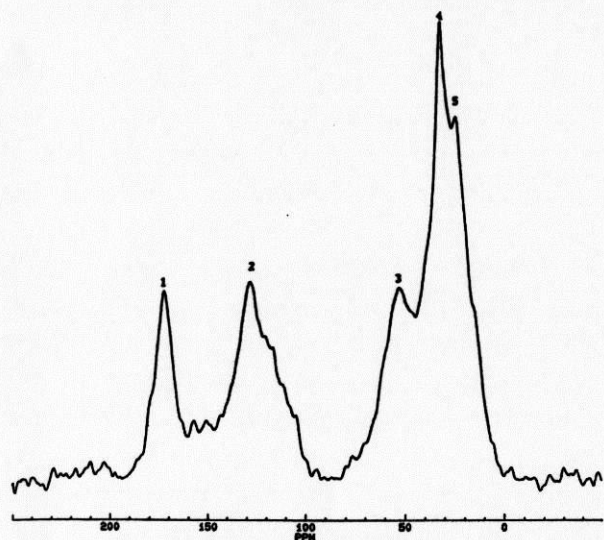


Fig. 2 Example of a solid state ^{13}C NMR spectrum of solid charred surface residues from an Iron Age vessel (no. 34-7-95 A) found in a settlement in Uitgeest-Groot Dorregeest (The Netherlands). The residue contains 31% carbon and the spectrum shows carbon functional group distribution: (1) carboxyl groups $-\text{CO}_2\text{H}$; (2) aromatic structures; (3) carbon-nitrogen bonds in proteins $-\text{C}(\text{N})\text{CO}_2\text{H}$; (4) $-\text{C}(\text{H}_2)-$; (5) methyl groups $-\text{CH}_3$. The spectrum was recorded on a Bruker CXP-100 (2.3 Tesla) spectrometer at the Argonne National Laboratory, Argonne, Illinois, by Dr R.E. Botto [14].

identification of complex mixtures because increasing complexity of the analysed sample results in decreasing resolution and a loss of identification potential. Other limitations of FTIR as an analytical tool for organic residue analysis include its relative insensitivity to compounds present in smaller quantities (<5%) and its limited capability to provide quantitative results when distinguishing between samples containing various proportions of similar compounds. An advantage of the technique of combined FTIR microscopy is its ability to analyse solid samples by pressing them into a thin layer between two crystals (diamond or inorganic salt crystals), although this technique is sensitive to sample inhomogeneity.

Solid state ^{13}C nuclear magnetic resonance spectroscopy (NMR) has been designed to study the carbon functional group

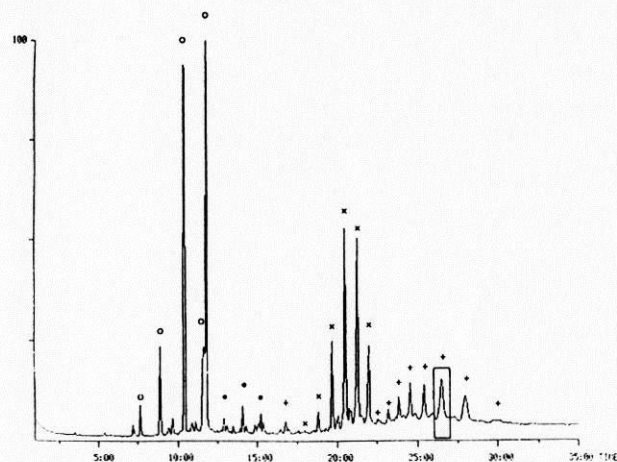


Fig. 3a GC-MS plot of the tetramethylsilylate derivative of a total lipid extract from a brown-black surface residue on an Iron Age vessel (no. 14-6-4.22 R) found in Uitgeest-Groot Dorregeest (Netherlands) [1]. The profile shows different classes of compounds such as fatty acids (o), monoacylglycerols (*), cholesterol (\pm), diacylglycerols (x) and triacylglycerols (+).

distribution in complex solid organic materials in medicine, biochemistry and geochemistry, and has recently been applied in the field of organic residue analysis [13, 14]. The determination is based on the electronic environment and magnetic susceptibility of the ^{13}C atoms in an organic material. Each different type of carbon bond contributes to a specific type of chemical shift that can be measured (in ppm) relative to a standard compound (Fig. 2). The ratios between saturated C-C bonds, unsaturated C=C bonds and C-H bonds provide information on the degree of condensation of the organic residue. CP/MAS (cross-polarization/magic angle spinning) NMR has some clear advantages over FTIR since it provides quantitative results, is not affected by the inhomogeneity of the sample and is less affected by loss of sensitivity due to sample complexity than FTIR. The disadvantages are that a larger sample is required (100mg) and the analytical procedure is much more time-consuming and expensive. However, CP/MAS NMR is the only analytical technique that gives quantitative results that make it possible to obtain information on the relative amounts of extractable and non-extractable compounds present in the sample.

Molecular characterization of extractable compounds by GC and GC-MS

Because many samples are complex mixtures of similar compounds, more detailed identifications can be made only after a separation step is conducted. Certain compound classes, such as lipids (fatty acids, acyl lipids, sterols, waxes), terpenoids, alcohols and hydrocarbons, can be extracted from a complex mixture with organic solvents. These extractable compounds can be separated and identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) after appropriate derivatization or preparative separation. GC separates all volatile compounds present in the derivatized extract based on chemical characteristics (Fig. 3a). Some individual compounds can be identified by comparison with the retention times of standard compounds. Many peaks, however, cannot be identified by GC alone. GC-MS combines a separation technique (GC) with an identification technique — mass spectrometry (MS) — so that the individual peak that represents a compound can be identified without use of standards (Fig. 3b).

Molecular information on non-extractable compounds

It is much more complicated to obtain detailed information on the

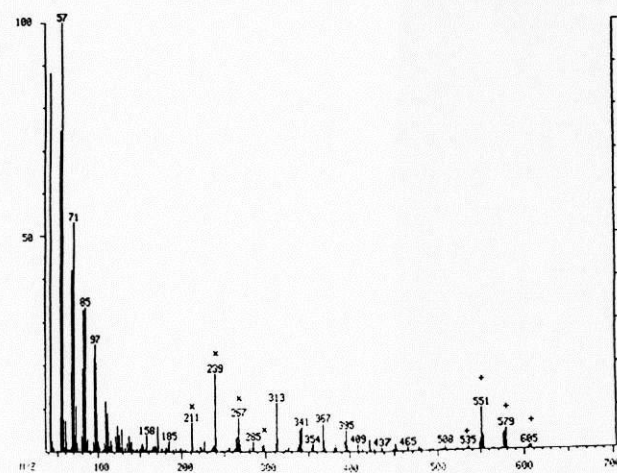


Fig. 3b The mass spectrum of a selected compound: a triacylglycerol with 50 carbon atoms in the fatty acid part of the molecule (see Fig. 3a for elution time). The molecular ion is not visible; two sets of indicative fragments can be seen: the RCO^+ ions (x) and the M-RCOOH^+ ions (+).

chemical composition of the remaining non-volatilizable, solid, non-extractable, chemically bound, condensed macromolecular fraction of the residue. The remaining compounds include proteins, complex sugars, melanoidins, condensed cyclic hydrocarbons and cross-linked drying oils, and can be studied best after a fragmentation step.

1 Fragmentation by hydrolysis

Proteins and complex carbohydrates (such as gums) can best be analysed after they have been converted into their individual amino acids or sugars, which is usually achieved by acid or alkaline hydrolysis. Sugars can be derivatized and volatilized for GC analysis, while amino acid composition is usually determined by high-performance liquid chromatography (HPLC). The main disadvantage of these techniques is the loss of identifying information during the fragmentation procedure. The identification of complex sugars such as plant gums seems much less hindered than the identification of protein polymers, since the relative quantities of the various sugar units in gums are more characteristic of their origin [15]. The identification of proteins and (partly) degraded proteins is limited since many proteins have a similar amino acid composition. One possible exception to this is gelatin, which has a very distinctive amino acid profile [16].

2 Fragmentation by pyrolysis

Another way to fragment condensed materials, such as proteins, melanoidins, caramelized sugars, cross-linked drying oils and other condensed macromolecular materials, is through analytical pyrolysis [1,10]. Pyrolysis consists of rapid heating under oxygen-free conditions. The added thermal energy causes the macromolecular compounds to split (along the weakest bond in the chain) into fragments specific for the original molecule. Analytical pyrolysis is often combined with MS analysis (Fig. 4).

CONSERVATION TREATMENT AND COLLECTIONS MANAGEMENT

Recent advances in organic residue analysis have created a need for re-evaluation of many traditional treatment procedures for ceramic artifacts. Ceramics, whether or not they contain *visible* organic residues, should no longer be viewed as exclusively inorganic materials but rather as a composite material with both an inorganic and an organic component.

Treatments and preservation protocols written specifically for degradable organic materials can also be applied to organic residues [7]. However, there may be a difference in the purpose of conservation treatments, usually directed at preservation and consolidation of the physical, structural and optical qualities of an artifact, and treatments for organic residue analysis, primarily directed at the preservation of chemical characteristics of the original material. Many consolidation treatments traditionally applied to ceramics will affect the chemical composition of residues in more or less serious ways. It is advisable to review and evaluate the conservation protocols for ceramic artifacts in regard to their possible effects on organic components.

Sampling strategies

When organic residue analysis (ORA) is conducted to obtain information concerning diet and food preparation techniques, a number of criteria can be used to select the appropriate sampling strategy:

- 1 The food groups under consideration should be chemically distinctive and detectable with the chosen analytical technique(s).
- 2 The food processing technique must be likely to leave a residue (for example, storage of a dry food is less likely to leave a residue than storage of an oil).

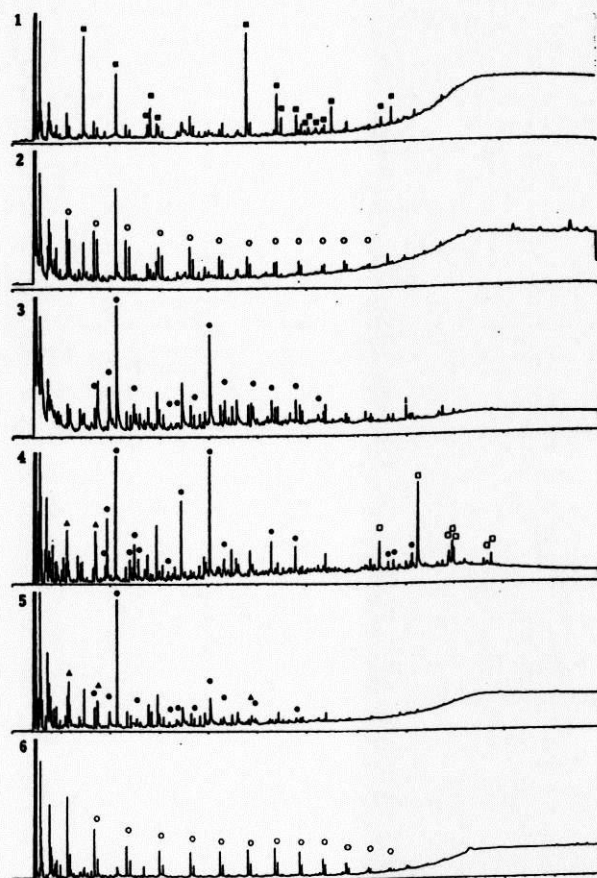


Fig. 4 Pyrolysis GC-MS plots of (1) a soot residue, (2-5) four solid surface residues, and (6) a sample of the ceramic of the vessel fabric. Various indicative fragments can be seen, such as polynuclear aromatic hydrocarbons (●), indicative of smoke condensates; alkanes and alkenes, indicative of an aliphatic polymer such as cross-linked lipids (○); markers indicative for proteins (▲), polysaccharides (▲) and free fatty acids (★). Samples were collected in an Iron Age village excavated at Uitgeest-Groot Dorregeest (Netherlands) [1].

- 3 Food groups that rarely leave any other kind of direct archaeological evidence are the most challenging (for example, evidence of grain is available more frequently than evidence of leafy plants and legumes [17]).

The sampling strategy for diet-specific information will prioritize samples based on their state of preservation. The best-preserved residues will have the best potential to help identify the original food. Often a particular type of ceramic vessel will be chosen for sampling. To obtain conclusive results, chemical evidence for a particular food should be present in several vessels.

When ORA is employed for functional studies of one or more types of vessel in a ceramic assemblage, the sampling strategy is more complex and involves a much larger selection of samples, because statistically significant numbers of samples from each type of vessel must be available in order to draw conclusions about use of a particular type of vessel. A number of criteria can direct the sampling strategy:

- 1 The vessel types must be clearly distinguishable.
- 2 A number of intact profiles of each vessel type should be available for sampling.
- 3 The presence of surface residues on the intact profiles enhances the potential of a ceramic assemblage for ORA (for example, it is difficult to select vessels on the basis of invisible, absorbed residues).

- 4 Combined study of both absorbed and surface residues will improve the interpretability of results, because a better insight can be obtained into the relationship between vessel use and residue formation.

Clearly, an ORA project should be undertaken only if an archaeological question can be posed that leads to an hypothesis testable with the described techniques. It is essential to appreciate the many methodological problems concerning residue formation and preservation that are still being studied and to develop an insight into the enormous scope as well as the limitations of this new discipline. It is through close cooperation between conservators, both in the field and in museums, and ORA specialists in the laboratory that these problems can be best addressed.

Conservation protocol

Organic residue analysts generally prefer to conduct the sampling themselves, or at least to be present during sampling, to ensure that the sampling methods provide representative, uncontaminated samples. Choosing samples can also be very difficult because surface residues frequently look like 'burnt porridge' or 'soot' to someone with an untrained eye. The residues are frequently hard to distinguish from secondary deposits or soil remains. Knowledge of the ceramic assemblage is required to choose representative ceramic pieces.

The main problems involved in organic residue sampling are contamination and degradation following excavation. Contamination is a serious problem, because organic residues are usually present in small quantities and frequently have a low organic content (varying typically between 3 and 50%). Contamination often occurs unnoticed during handling, storage or transportation of the ceramics (fingerprints, paper traces, mineral oil from instruments, plasticizers from plastic bags and vials, mould growth and so on), and this makes it hard to prevent. General contamination through handling should be limited as much as possible by wearing unpowdered latex or nitrile gloves.

Other steps can be taken to limit the chances of contamination and post-excavation degradation:

- 1 *Registering the organic surface residues.* The location of a residue on the vessel, and the colour, texture and thickness of the residue, should be recorded. Photographs (under high magnification) of the intact surface residue are very helpful in future evaluation of results.
- 2 *Selecting samples before washing.* When surface residues are visible before washing of the sherds, or when sampling for absorbed residues (there are no visual indications of the absence or presence of absorbed organics), ceramic pieces can be selected before washing. Pottery should be wrapped in solvent-cleaned aluminium foil, and stored at -20°C in a polyethylene zip-lock bag or in a glass container with a Teflon-lined cap.
- 3 *Selecting samples after washing and drying.* If surface residues are not visible without washing, or cold storage is impossible, cleaning is required before selecting samples. Washing ceramics gently under running tap-water or with pressurized tapwater should be done as soon as possible after excavation to prevent degradation and mould growth. Scrubbing, brushing and excessive handling should be avoided. Rapid drying is advisable to prevent mould growth and other degradation processes. Unfortunately, even mild treatments such as washing and drying can affect the organic material present, causing the loss of some water-soluble compounds and of brittle surface residues. After the ceramic is completely dry, it should be wrapped in solvent-cleaned aluminium foil and

packed in a polyethylene zip-lock bag or a glass container with a Teflon-lined cap. Store in a cool dark place where no condensation can take place.

- 4 *Sampling the residue.* Scanning electron microscopy (SEM) study of the surface residue can visualize previously undetected contaminations such as mould growth. After this visual inspection, but before sampling, it is advisable to remove the outer surface (c. 1mm) of the residue in order to reduce the risk of contamination by soil components. Surface residues are scraped off the ceramic with a solvent-cleaned scalpel. Absorbed residues are extracted from a piece of the ceramic cut or drilled out of the vessel. The residue (or the piece of sherd) is subsequently ground up and stored in a glass vial with Teflon-lined cap.
- 5 *Taking additional samples.* To conduct an organic residue study of an excavation, the chemical composition of the soil surrounding the residues should be determined. Soil samples (c. 10g) should be taken directly adjacent to the ceramics under study, and from the exterior of the vessel (soil on the interior may contain part of the original vessel contents), with a solvent-cleaned scalpel and stored at -20°C in glass vials with Teflon-lined caps.

Long-term storage and collections management

If organic residue analysis will be conducted on a ceramic collection in the future, storage conditions and collections management protocol should be adapted to prevent contamination, reduce further degradation as much as possible, and avoid consolidation of the ceramics.

- 1 *Contamination.* Storage materials and containers should be inert and contain no volatile materials. Plasticizers present in many polymers, such as polyvinyl chloride (PVC, or 'vinyl'), can volatilize and contaminate samples stored over long periods. Even seemingly innocuous and easily overlooked items such as vinyl cap-liners of glass containers should be avoided. Gore-tex, a Teflon-coated textile, can be used to pack large vessels without contaminating them.
- 2 *Further degradation.* Store ceramics in a dark, cool and dry place and check regularly for mould growth and other forms of organic degradation. Record all visible changes. Do not spray with anti-fungal chemicals.
- 3 *Consolidation.* Consolidation with any kind of material precludes further organic residue analysis. No coating can be applied to the ceramic for exhibition. Also avoid mild acid treatments that are sometimes employed for desalination. Efflorescence can be avoided by storage in an environment with low relative humidity. Glues, adhesives, organic compounds and ink should not be applied to any area of a ceramic that might be sampled.

Even when these factors are taken into account, the effects of storage conditions different from those present during burial are not entirely clear. It is possible that organics in the ceramics do not undergo excessive chemical changes when stored in dark, cool, dry places, but more study is still required.

CONCLUSIONS

Organic residues found in association with ceramic vessels can give direct information about what materials were cooked, stored or otherwise prepared by people in the past and about what vessels they used for each task.

This paper summarizes the potential of various analytical techniques that can be applied to residue studies and reviews the possibilities and limitations of organic residue analysis when applied to the study of prehistoric diet or vessel use.

The application of organic residue analysis in the study of ceramic artifacts leads to many questions concerning conservation treatments in the field and during long-term storage. Guidelines are given to conservators concerning the choice of appropriate ceramic assemblages for organic residue studies, and the prevention of contamination, degradation and consolidation of ceramics stored for future residue analysis following excavation. Although it is made clear that all post-excavation treatment affects organic residue analysis to some extent, light surface rinsing with tap-water and rapid air-drying is the least intrusive method.

The potential of organic residue analysis depends largely on the application of conservation treatments and storage methods designed for organic/inorganic composite materials rather than exclusively inorganic materials. Treatments and storage methods should be reviewed and adapted in regard to their possible effect on the chemical composition of the organic fraction of the composite.

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