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ORGANIC RESIDUES: ORIGINS, ANALYSIS AND SCOPE – AN OVERVIEW FOR THE ARCHAEOLOGICAL CERAMICIST

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The capacity of ceramic sherds to retain and preserve organic residues is now widely recognised and residue analysis is increasingly specified as a component of ceramic assemblage studies. This article aims to introduce the range of organic residues that are typically encountered in association with archaeological ceramics, highlighting factors that can affect their preservation and outlining the most usual approaches to analysis as well as considering the types of information that can be reasonably inferred from such data.

Origins and distribution

Organic residues on ceramics are usually regarded as remnants of the original vessel contents or the accumulated remnants of multiple uses, but they may also be present as intended components of the vessel itself, as applied decoration (Connan *et al.* 2004; Urem-Kotsou *et al.* 2002), surface sealants (Steele *et al.* 2008;

Beck, Smart & Ossenkop 1989; Heron & Pollard 1988) or as adhesive for stoppers or repairs (Charters *et al.* 1993a). Pots with extant original contents are very rare, although examples are known (Charrie-Duhaut *et al.* 2007; Condamin *et al.* 1976; Garnier *et al.* 2003; Serpico & White 1996), but residues can sometimes be visible as deposits on sherd surfaces, for example the chars and soots associated with cooking vessels. Even apparently 'clean' sherds often contain residue absorbed by the porous fabric and these invisible residues are usually better preserved than surface deposits because the ceramic microstructure protects them from microbial degradation (Dudd, Regert & Evershed 1998). The porosity of the ceramic fabric can therefore be an important factor governing preservation. Unglazed coarse fabrics seem to offer the best potential for organic residue retention and survival, although to date there has been no systematic study of the influence of fabric characteristics or of surface treatments such as slipping or burnishing (but see Reber (2007)). Clearly there are implications for the comparisons that can be made between residues from different vessel and/or fabric types within an assemblage that will affect qualitative evaluation of residue composition (in terms of preservation) as well as comparative residue yield.

Mode of vessel use is a further factor affecting residue deposition and penetration. Absorption of lipid residues within cooking vessels has been shown to vary according to mode of use. Higher concentrations of residue accumulate at the top of the vessel during boiling of fatty food due to the fat rising to the surface of the water (Charters *et al.* 1993b). However, such absorption differentials may be more useful for selecting promising sherds for lipid analysis than for inferring vessel use. Experiments have shown that, although compared to boiling, roasting of meat in ceramic vessels leads to wider distribution of lipid in the body of the vessel, the most extensive absorption is still in the upper regions of the vessel (Evershed 2008). Factors such as vessel shape and use life will modify or blur these absorption patterns further and consideration must also be given to other culinary practices that could lead to similar distributions (for example sealing of stored foodstuffs with fat). It is important also to note that distribution patterns will be different for non-lipid food constituents such as proteins. Thus the properties of the residue components are as important as vessel use in determining absorption distributions. Factors affecting the distribution of charred surface deposits may be different again and these have been explored through ethnoarchaeological and experimental studies to show the relationship between vessel use life and the area and thickness of surface residues (Kobayashi 1994). The many variables affecting absorption and deposit patterns should be given careful consideration when using these parameters to select analytical samples or to examine issues of vessel use.

Degradation and loss

As well as influencing their absorption, the properties of the different classes of residue components are perhaps the greatest factor determining their survival for detection in the archaeological record. Lipids are the most enduring components of absorbed food residues, on account of their hydrophobicity and greater resistance to structural modification compared to proteins and carbohydrates. Resins, bituminous materials and waxes are particularly robust and often survive as visible surface deposits.

Degradation and loss of residue begins during the use-life of the pot (through cleaning, heating etc.) but the greatest losses take place in the burial environment after discard (see Figure 1). Experimental studies have shown around 99% loss of extractable lipid from food residues, principally through microbial action (Evershed 2008). The surviving material is thought to be protected in micropores or by chemical interactions with the ceramic surface. The latter seem to be particularly significant in the survival of more polar lipid compounds which are otherwise readily lost by leaching (Regert *et al.* 1998) and are probably a key mechanism in the survival of protein residues (Craig & Collins 2002). The duration of burial is probably less important for residue survival than the prevailing conditions within the burial environment. Viable residues have been obtained from ceramics of more than 5000 years old (Craig 2004) while experimental studies have demonstrated the variability of lipid preservation with burial environment (Reber & Evershed 2004b) and, perhaps unsurprisingly, that anoxic conditions favour organic residue preservation (Evershed 2008). The many variables affecting burial conditions mean that, on the basis of current knowledge, residue preservation is not necessarily predictable and pilot studies are usually necessary to assess the potential of an assemblage for residue work.

Lipid analysis for food residues

The archaeological durability of lipids has made them the main focus of research for food residue studies to date and molecular analysis of lipid residues by gas chromatography – mass spectrometry (GC/MS) is usually the first step in any initial assessment of an assemblage. Analysis is necessarily destructive, typically involving solvent extraction of a portion of powdered sherd to release the absorbed lipid. Alkaline hydrolysis may also be undertaken to release bound lipid fractions which can provide additional information in the form of more polar degradation products (Regert *et al.* 1998; Copley *et al.* 2005a). Lipid profiles obtained by GC/MS analysis represent a mixture of original lipid components and their degradation products (plus any contamination from the burial environment or subsequent handling, see Figure 1 and later discussion). Even with good preservation, interpretation of the origin of a residue on the basis of this data will usually be limited to general classifications such as animal (possibly ruminant or non-ruminant), plant or marine

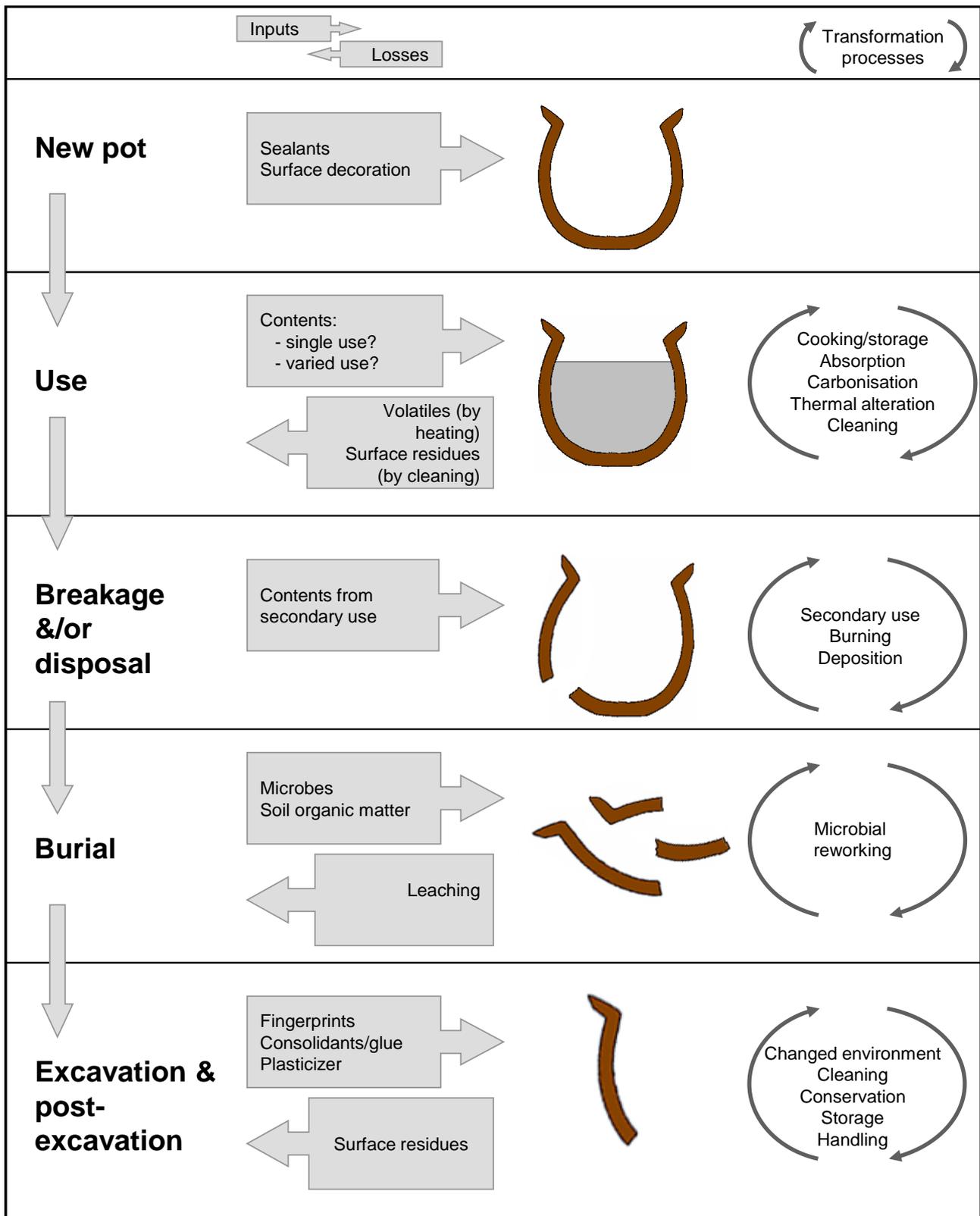


Figure 1: Schematic showing the principal inputs, losses and transformation processes affecting the survival and composition of organic residues in archaeological ceramics.

derived fat. These classifications are inferred from the range and relative abundance of the compounds detected (such as fatty acids, acylglycerols and sterols) along with their degradation products. Interest in developing methodologies for more specific classification of residues on the basis of fatty acid ratios persists (e.g. Malainey 2007) in spite of the very wide range of factors affecting the fatty acid profiles seen in archaeological material. Fatty acid ratios can sometimes be instructive but should be treated with caution: the pitfalls are highlighted by the results of a recent 'round robin' blind test involving multiple laboratories (Barnard *et al.* 2007a).

Occasionally, however, specific biomarkers are preserved which enable more precise interpretations to be made with some confidence. Compounds indicative of plant leaf waxes (Evershed *et al.* 1991) for example, offer a rare opportunity to identify particular vegetable contributions to food residues, evidence for which is more usually limited to non-specific plant derived sterols. Caution is essential when evaluating the different contributions in mixed residues: the relative abundance of lipid from different sources will be a function both of the lipid richness of the source foodstuff (thus meat will tend to dominate over vegetables, see Evershed 2008) and the degradation resistance of components from different sources. Mixtures of very similar source materials – such as ruminant and non-ruminant fats – are impossible to resolve on the basis of molecular profiles alone. It is now usual to turn to gas chromatography combustion isotope-ratio mass-spectrometry (GC-C-IRMS) to achieve more diagnostic determinations of residue source. This method measures compound specific ratios of light (^{12}C) and heavy (^{13}C) carbon which vary depending on the origin of the carbon from which the molecule was synthesised. The technique has proved particularly valuable for the identification of dairy products (Dudd & Evershed 1998), which are indistinguishable from ruminant carcass fats on molecular evidence due to the absence of unique biomarkers for milk (although given favourable preservation conditions dairy products can sometimes be inferred from triacylglycerol distributions (Berstan *et al.* 2004)). GC-C-IRMS is also showing promise for the classification of plant-derived fats (Copley *et al.* 2001) and has been used with success to demonstrate the presence of maize in New World food residues (Reber & Evershed 2004a).

Although the molecular profiles obtained by GC/MS alone have limited scope for the identification of food residue origins they are nevertheless an important and instructive source of information about the degradative history of the residue. While most of the degradation occurs after deposition, changes in composition which occur during the use-life of the vessel can endure and yield useful information about vessel function. Long-chain ketones are commonly found as constituents of residues from cooking vessels; they are thermal degradation products formed *in situ*

during cooking activities when the residue is repeatedly heated in excess of 350 °C and are thus useful indicators of cooking (Raven *et al.* 1997). Similarly, a range of alkyl phenols formed by thermal alteration of unsaturated fatty acids are important indicator compounds for the presence of marine, fish and plant oils (Evershed *et al.* 2008). Unsaturated fatty acids are particularly vulnerable to degradation, only being preserved in exceptional preservation conditions (Solazzo & Erdhardt 2007). Alkyl phenols therefore present important evidence for the original presence of unsaturated fatty acids in more degraded residues, as well as offering insights into culinary practice.

Alternative approaches for residue analysis

Alternative analytical approaches are necessary for the identification of commodities which contain little or no lipid. Perhaps the most important such example likely to be associated with ceramic vessels is wine. Recent research has demonstrated the potential of liquid chromatography with tandem mass spectrometry (LC/MS/MS) for the characterisation of ancient wine residues using biomarkers for grapes (tartaric acid) and red grape pigment (as syringic acid), (Guasch-Jané *et al.* 2006 & refs therein). A different approach, using pyrolysis-GC/MS to identify phenolic markers in wine (derived from flavanoid and tannin constituents) has been applied with success to Roman wine residues (Garnier *et al.* 2003). Both of these methods detect grape derivatives rather wine *per se* and thus do not differentiate between juice, wine or vinegar. It is also worth noting that both of these studies tested excellently preserved archaeological material: the former coming from the arid conditions of Egyptian tombs, the latter being the contents of sealed Roman amphorae from Mediterranean shipwreck sites.

In recent years interest in the non-lipid compounds that may be present in food residues has grown. Proteins in particular offer the opportunity for more specific identification of food products because they are synthesised against the DNA template of the source organism. Moreover, they are capable of surviving over geological timescales and are major constituents of many important foodstuffs so should make ideal biomarkers. Their principal drawback is their vulnerability to microbial degradation. Nevertheless successful identifications have been achieved in recent years using two different analytical approaches: immunological and proteomic. Immunological methods are based on the detection of specific compounds by labelled antibodies. The approach can be both sensitive and specific but requires foreknowledge of the compounds of interest and can be hampered by the effects of degradation. Proteomic techniques use mass spectrometry to identify peptides according their chemical structure and thus can be used for the analysis of 'unknowns' (Barnard *et al.* 2007b). Immunological methods have proved successful in the detection of dairy products in residues on ceramics from the Hebrides (Craig *et al.* 2000) while proteomic analysis has

enabled specific identification of marine mammal fats in Iñupiat potsherds from Alaska (Solazzo *et al.* 2008). Again preservation is key; the latter study was of material from a frozen burial environment with exceptional accompanying lipid residues (Solazzo & Erdhardt 2007) and well preserved lipids were also extracted from the residues in the Hebridean material (Craig *et al.* 2005b). Notwithstanding these examples, it is unclear to what extent lipid preservation is a reliable indicator for the preservation of other compound classes. This is a particularly interesting question given that lipid analysis by GC/MS is usually the first step in assessing the residue potential of an assemblage.

Although visible surface deposits are often less well preserved than absorbed residues they can nevertheless yield interesting insights. Pyrolysis-GC/MS of charred surface deposits has revealed polymerised remains of carbohydrate and proteinaceous material that would not normally be seen in extracted residues (Oudemans & Boon 1991; Pastorova, Oudemans & Boon 1993). Direct temperature-resolved mass spectrometry (DTMS) has been used in a similar way to provide information on general classes of organic material present in surface deposits, detecting polysaccharide, protein and lipid material in a single analysis (Oudemans, Eijkel & Boon 2007). Surface residues may also preserve structural (cellular) information that can be archaeobotanically identified, often with a high degree of precision (e.g. to genus), using SEM or light microscopy (Cartwright 2002; Shishlina *et al.* 2007). Archaeobotanical approaches have proved particularly useful in the characterisation of beer residues which are challenging to detect by chemical methods (Samuel 1996).

Soot deposits occurring on the exterior of cooking vessels may offer scope to examine fuel sources if fuel biomarkers are preserved in soot condensates. Little research has been undertaken to address the feasibility of such analysis (although see Bonfield 1997), perhaps because soot deposits tend to be scanty and are easily removed by post-excavation cleaning.

Natural products (or non-food residues)

Natural products are often associated with ceramics as sealants and adhesives e.g. (Stern *et al.* 2008), but may also appear as residues from the manufacturing or processing of such materials (Regert *et al.* 2003). Alternatively, residues of these substances may be the remains of the original vessel contents either transported or stored therein (Colombini *et al.* 2005; Stern *et al.* 2003). As already noted, some natural products such as resins, waxes and bituminous materials survive well in archaeological contexts. They are readily identified from their molecular composition using GC/MS and often contain diagnostic biomarkers that enable them to be linked to a source with some precision.

For resins, the biomarkers are terpenoid compounds, and the higher (di and tri-) terpenoids that survive best in archaeological material can usually be

linked to botanical family of origin and sometimes even to genus (depending on preservation and botanical specificity) (see, for example (Eerkens 2001; Lampert *et al.* 2002; Stern *et al.* 2003)). Characteristic thermal alteration products of resins enable the identification of tars and pitches produced from them (or more usually from the corresponding timber), although the botanical origin may be less readily determined due the extent of thermal alteration (Reunanen, Ekman & Hafizoglu 1996).

Beeswax is by far the most common wax product encountered on archaeological ceramics and is readily identified from its characteristic combination of wax ester, hydrocarbon and fatty acid components (Heron *et al.* 1994; Regert *et al.* 2001). The presence of beeswax is sometimes interpreted as proxy indicator for honey in residues, although in the absence of additional evidence such interpretations cannot be supported above alternative explanations for beeswax such as surface sealants (Porter *et al.*, in press).

Bitumen is characterised by the presence of hopanoid and sterane hydrocarbons; these biomarkers, combined with carbon and nitrogen isotopic data, enable identification of the geological source (Connan 1999 and refs therein).

Contamination: managing and minimising

Concerns are often raised about the impact of contamination on residue analysis especially when considering the suitability of a previously excavated assemblage. Handling of sherds presents potentially the most significant problem as this introduces animal (human) fats which could be mistaken for residue components; it is thus wise to keep handling of any sherd collection destined for residue analysis to a minimum, using gloves where necessary. Probably the most ubiquitous contaminants seen in residues are phthalate plasticizers from plastic bags. These can be avoided or reduced by wrapping sherds in acid free tissue but in fact plasticizers rarely confound interpretation of analytical data as they are usually readily distinguishable from residue components. Washing of sherds will lead to losses, especially of surface deposits, and soaking, acid washing and desalination should be avoided as all will remove organic residues. Nevertheless, good results have been obtained from washed sherds so a washed assemblage is not necessarily unsuitable for residue work. In ideal circumstances sherds should be presented for analysis unwashed with, hopefully, some adhering soil since best practice is to analyse samples of soil from the relevant context to monitor for contamination during burial (soil contamination of sherd residues has received considerable attention and is not considered to be a significant problem, see Dudd, *et al.* 1998; Heron, *et al.* 1991). In non-ideal conditions contamination problems are typically mitigated by removing the surface 1 mm of sherds prior to sampling or comparing internal and external sherd portions (on the assumption that most of

the contents residue will be absorbed through the inner surface of the vessel).

History and scope

Archaeological residue analysis is a relatively young field. The earliest analyses took place in the first half of the 20th century (e.g. Gill 1906) but systematic research only took off in the late 1970s and early 1980s. Food residue studies from this period resulted in many claims for the identification of substances as specific as milk, grapeseed oil, fish oil, wild boar fat, and butter. The majority of this early food residue work has been discredited as more has been learned about preservation mechanisms and degradation pathways (whereas early natural product studies, based on biomarkers, have proved more enduring e.g. Mills & White 1977). Since the early 1990s emphasis has shifted away from the 'fingerprinting' approach to molecular profiles towards a more cautious and thorough interrogation of the origin of individual compound classes always backed up by experimental research (Evershed 2008). Observations and interpretations from lipid profiles can now be tested and refined using isotope analysis and perhaps increasingly by protein analyses as well (see, for example, Craig *et al.* 2005b). The rise of these new techniques, together with the increasing application of chemometric data analysis, has hugely extended the scope of residue research.

Expectations have changed too. The reconstruction of ancient 'recipes' is now recognised as an unhelpful and unrealistic goal and single vessel analyses, especially for food residues, are unlikely to yield instructive results. Attention has turned to the use of residues to tackle wider archaeological questions and publications are increasingly reflecting this with research in recent years addressing questions such as the rise of dairying (Copley *et al.* 2005b; Copley *et al.* 2005c; Copley *et al.* 2005d; Craig *et al.* 2005a), the extent of exploitation of specific commodities (Mukherjee *et al.* 2007) and the function and/or circulation of particular vessel types (Knappett *et al.* 2005). These studies show the importance of evaluating residue data alongside the full range of complementary archaeological evidence (zoological, botanical, ceramic etc.) and illustrate how residue analysis is at its most powerful when used comparatively on large sample numbers to understand resource exploitation (intra- or inter-site) and differential vessel use.

R. J. Stacey

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