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Organic Residue Analysis of the Longstone Edge Barrow pottery

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Introduction

Lipid residues of cooking and the processing of other organic commodities have been found to survive in archaeological pottery vessels for several thousand years as components of surface and absorbed residues. The components of the lipid extracts of such residues can be identified and quantified through solvent extraction and using a combination of analytical techniques capable of achieving molecular level resolution, i.e. high temperature-gas chromatography (HTGC), GC/mass spectrometry (GC/MS; Evershed *et al*., 1990) and GC-combustion-isotope ratio MS (GC-C-IRMS; Evershed *et al.*, 1994), Characterisation of lipid extracts to commodity type is only possible through detailed knowledge of diagnostic compounds and their associated degradation products formed during vessel use or burial. For example, triacylglycerols (TAGs) are found in abundance in fresh animal fats, however, they are degraded to diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids during vessel use and burial, such that in archaeological pottery the free fatty acids tend to predominate; this has been observed in numerous pottery vessels (Evershed *et al*., 2002) and verified through laboratory degradation experiments (e.g. Charters *et al*., 1997; Dudd and Evershed, 1998; Evershed, 2008). An increasing range of commodities is being detected in pottery vessels, including animals products (e.g. Evershed *et al*., 1992, Copley *et al*., 2003), leafy vegetables (Evershed *et al*., 1991, Evershed *et al*., 1994), specific plant oils (Copley *et al*., 2005a) and beeswax (Evershed *et al*., 1997).

Animal fats are by far the most common class of residue identified from archaeological pottery with compound-specific stable carbon isotope analysis allowing detailed characterisation of their source. GC-C-IRMS allows the carbon stable isotope ($\delta^{13}C$) values of individual compounds to be determined within a mixture. We have found that the $\delta^{13}C$ values for the principal fatty acids ($C_{16:0}$ and $C_{18:0}$) can be used for distinguishing between different animal fats, e.g. ruminant and non-ruminant adipose fats and dairy fats (Evershed *et al*., 1997a, Dudd & Evershed, 1998), as well as in the identification of the mixing of commodities (Evershed *et al.*, 1999, Copley *et al*., 2001). Recently we have demonstrated that dairy products were important commodities in Prehistoric Britain, as illustrated through the persistence of dairy fats in prehistoric pottery (Copley *et al*., 2003, 2005b). For an overview of the use of compound specific stable isotopes in archaeology, see Evershed *et al*., 1999 and Evershed 2008b.

Materials and Methods

Lipid analyses were performed using established protocols which are described in detail in earlier publications (Evershed *et al*., 1990; Charters *et al*., 1993b) and in the Appendix of this document. The identification of individual compounds was based upon eluting order, comparison of retention times to standards and comparing the mass spectra with known fragmentation patters and NIST spectra library.

Longstone Edge barrow and Early Bronze Age pottery

In 1996 a Bronze Age barrow situated on the escarpment at Longstone Edge was excavated by English Heritage prior to further damage from the quarrying operations.

Barrow 1 exhibited several phases of use in the Neolithic and Early Bronze Age, and was reused in later prehistory and the Romano-British period. Pre-dating the mound was roughly levelled subsoil, which formed a possible excarnation platform, with fragmentary human remains found on it. Several cists, lined with limestone slabs were dug into the subsoil and in-filled with sediment. One cist contained disturbed remains of two individuals and fragments of a Beaker. A later cremation burial containing remains of two individuals and a Food Vessel was dug into the mound, before the construction of the barrow. Another two Food Vessels were found in the central cist grave of the adjacent Barrow 2 in 19th century (Andrews & Fernandez-Jalvo 2012, Last, *in press*).

Two Early Bronze Age potsherds deriving from Longstone Edge barrow were received for organic residue analysis. The details of a Beaker and Food Vessel samples are listed in Table 1.

Table 1. Details of the potsherd submitted for organic residue analysis

Eng.Her. Sample No.	*Bristol Sample No.*	*Stratigraphy:*	*Weight [g]:*	*Description:*
472 Longstone Edge	DER 09	1055, 72541	1.990	Beaker, brownish-red, AOC, body
472 Longstone Edge	DER 10	3030, 72649	1.618	Food Vessel, reddish-brown, fingernail-impressed chevrons forming a herringbone pattern, body

Figure 1. Early Bronze Age potsherds from Longstone Edge barrow submitted for analysis: Beaker DER 09 (left) and Food Vessel DER 10 (right).

Results and discussion

Table 2 lists lipid concentrations detected in Longstone Edge barrow potsherds and the assignments of the broad commodity groups based on the molecular data retrieved.

Table 2: Summary of the results of the organic residue analyses.

Bristol sherd number	*Lipid concentration ($\mu g\ g^{-1}$)*	*Lipids detected*	*$\delta^{13}C_{16:0}$ ± 0.3 (‰)*	*$\delta^{13}C_{18:0}$ ± 0.3 (‰)*	*Predominant commodity type*
DER 09	4.10	Phthalates –modern contamination	n/a	n/a	n/a
DER 10	283.43	FA14, FA15br, FA15, FA16, FA17br, FA17, FA18:1, FA18, FA20-FA26, MAGs, DAGs, TAGs	-27.06	-32.95	Ruminany dairy fat

Key: FA refers to free fatty acids; MAGs to monoacylglycerols; DAGs to diacylglycerols; TAGs to triacylglycerols.

Early Bronze Age pottery from Longstone Edge barrow displayed good preservation with high lipid concentration detected in only one of two potsherds analysed. While it wasn't possible to detect any preserved organic residues in the Beaker DER 09, the Food vessel DER 10 displayed a fairly high concentration of preserved lipids (283.43 μg g^{-1} potsherd). This uneven lipid preservation is not uncommon, since it is heavily influenced by degradative alterations that may occur during vessel use or due to post-burial conditions in the soil (Evershed *et al.*, 1999; Evershed 2008).

Figure 2 shows a partial gas chromatogram for the total lipid extract (TLE) of the absorbed residue from Longstone Edge Food Vessel, indicating the compounds detected, namely: free fatty acids, with high abundances of saturated $C_{16:0}$ and $C_{18:0}$ components. The presence of mono-, di- and triacylglycerols indicates a relatively good level of preservation, most likely due to the favourable post-burial conditions in the soil.

Preserved organic residues within the Longstone Edge Food Vessel show a distribution typical of partially degraded animal fat. The chromatogram also shows traces of odd carbon number saturated fatty acids ($C_{15:0}$, $C_{17:0}$) with their *iso*- and *anteiso*-branched varieties ($C_{17:0br}$), which generally indicate ruminant lipid source (Mottram *et al*., 1999; Evershed *et al*., 2002).

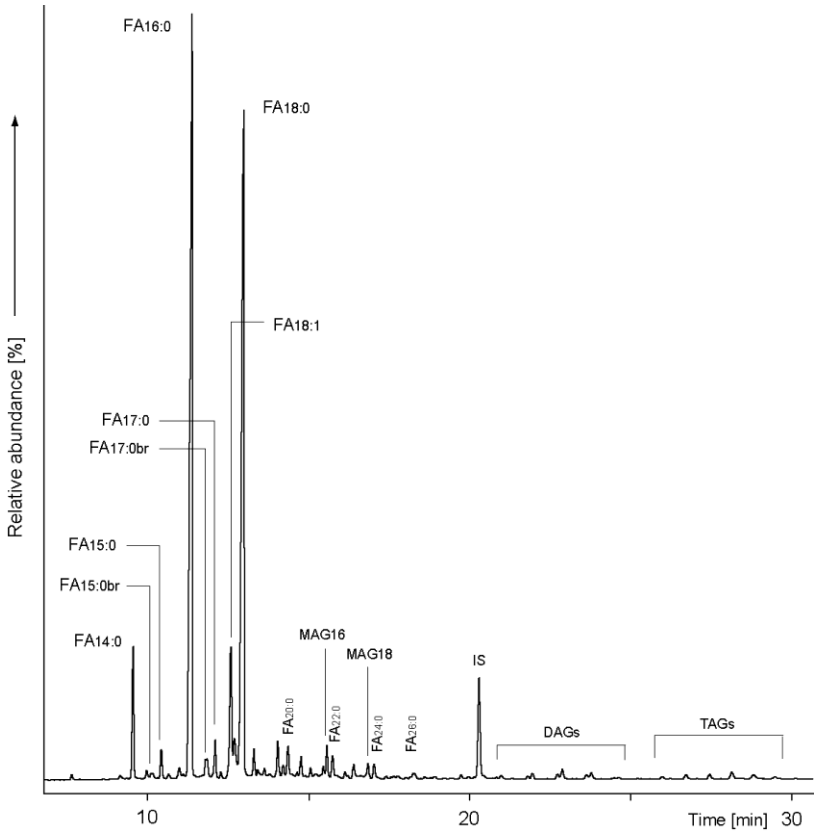

Figure 2. Partial HTGC profile of the trimethylsilylated total lipid extract from Longstone Edge Food Vessel, illustrating the distribution of compounds characteristic of degraded animal fat. Key: $FA_{X:0}$ are saturated free fatty acids of carbon length x and degree of unsaturation y. IS is the added internal standard (C_{34} *n*-alkane). MAGs are monoacylglycerols; DAGs are diacylglycerols; TAGs are triacylglycerols.

The parent triacylglycerols (TAGs) present in fresh adipose fats and plant oils quickly degrade into their constituent fatty acids, with palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids persisting in highest abundance, with minor contributions from unsaturated and shorter chain saturated fatty acid components. Many archaeological lipid extracts are usually only comprised of free fatty acids, indicating that complete hydrolysis of the precursor TAG components had taken place (Evershed, 2008). Long chain fatty acids (LCFA) in the carbon range C_{20} to C_{26} were also identified in the Food Vessel lipid extract. These fatty acids are not particularly useful biomarkers, unless accompanied by other compounds, which can then indicate either a marine lipid source (Copley *et al*., 2004b; Hansel *et al*., 2004; Evershed *et al*., 2008d) or degraded plant waxes (Tulloch, 1976; Bianchi, 1995).

Despite their predominating occurrence, the $C_{16:0}$ and $C_{18:0}$ fatty acids posses only limited biomarker potential. Broad groups of commodities can only be alluded to by the investigation of the $C_{16:0}$ vs. $C_{18:0}$ fatty acid ratio (P/S ratio). A higher concentration of palmitic fatty acid could indicate a plant source, while higher stearic acid content usually indicates the presence of animal derived fat (Dudd, 1999; Mottram *et al*., 1999; Copley *et al*., 2005a; Romanus *et al.*, 2007). Previous investigation of P/S ratios in modern fats and oils has provided some additional proxies, however, interpretations of these have to be used with great caution and only in combination with other chemical and isotopic evidence. P/S ratio calculated for Longstone Edge Food Vessel was 1.1 which indicates the presence of of ruminant fats.

As mentioned previously, triacylglycerols (TAGs) are the most abundant constituents of fresh fats and get degraded quickly through microbial degradation and weathering. Comparison of the TAG

distributions with those of modern reference fats has shown that specific distributions can be linked to different lipid sources and allow preliminary differentiation of their origins from the two major classes of domestic animals (ruminant and non-ruminant/ porcine) and between ruminant dairy and ruminant adipose fats. Ruminant animals show a characteristic distribution of TAGs with carbon numbers ranging from C_{44} to C_{54} with a maximum concentration at C_{52}; where as non-ruminant animals display a slightly shorter distribution with carbon numbers between C_{46} and C_{54} with a low concentration at C_{46} and C_{54} and a maximum again at C_{52}. Dairy fats show the widest TAG distribution with carbon numbers range C_{42} until C_{54}, usually with two maximums at C_{50} and C_{52} (Evershed et al. 1997; Dudd & Evershed 1998; Mottram et al. 1999). Distribution of TAGs observed in the analysed Food Vessel can be seen on Figure 3.

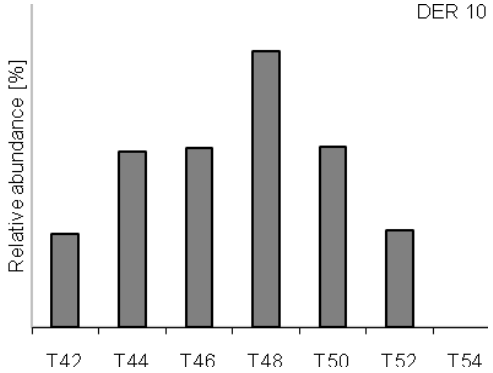

Figure 3. The distributions of triacylglycerols detected in the Longstone Edge barrow Food vessel lipid extract.

A wide TAG distribution with a range of carbon numbers between C_{42} and C_{52} could most likely be derived from ruminant dairy fat residue, as already suggested by the presence of odd-carbon number saturated fatty acids ($C_{15:0}$, $C_{17:0}$) and their branched homologues ($C_{15:0br}$, $C_{17:0br}$).

However, laboratory experiments have shown that triacylglycerol distributions can be skewed by degradation; the wide TAG distribution characteristic of fresh ruminant dairy fat can be considerably narrowed due to preferential degradation and solubility of compounds with lower carbon numbers, and thus can come to resemble the narrower TAG distribution seen in ruminant adipose fat (Dudd et al., 1998).

For further confirmation of the presence of ruminant dairy fats, the total lipid extract from Food Vessel DER 10 was submitted for further analysis by GC-C-IRMS; these values are plotted in Figure 4.

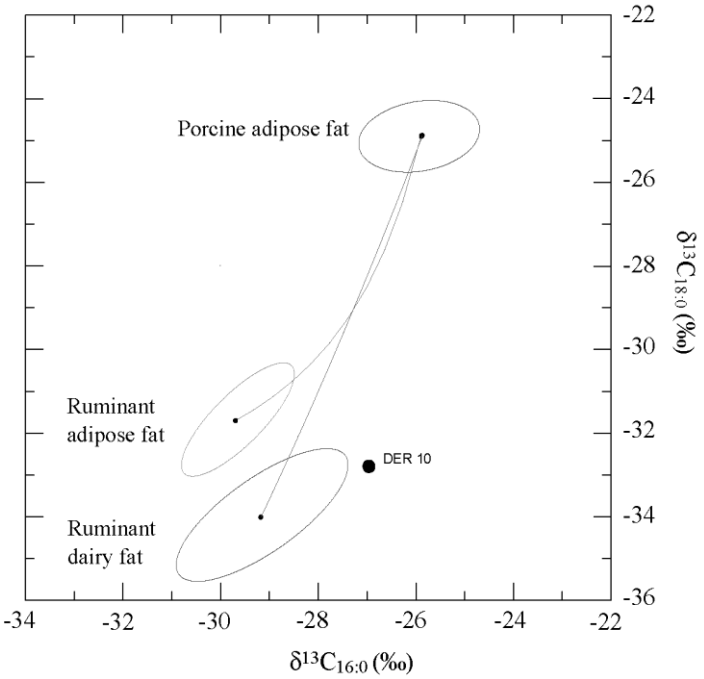

Figure 4. Scatter plot showing the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids prepared from total lipid extract of the Longstone Edge Food Vessel. The values of modern reference fats are represented by confidence ellipses (1 standard deviation). All $\delta^{13}C$ values obtained for modern reference animal fats have been adjusted for the post-Industrial Revolution effects of fossil fuel burning, by the addition of 1.2 ‰. Lines connecting the ellipses represent theoretical $\delta^{13}C$ values obtained through the mixing of these fats.

The $\delta^{13}C$ values obtained for modern reference animal fats from the major domesticated animals exploited in British prehistory are grouped within confidence ellipses, onto which the values from Longstone Edge pottery extract have been plotted. The $\delta^{13}C$ values for the $C_{18:0}$ fatty acid are more depleted in milk fats than in ruminant adipose (meat) fats, thereby enabling distinctions to be drawn between milk and adipose fats of ruminant animals, such as cattle, sheep and goat (Dudd & Evershed, 1998). This is witnessed in the *c.* 2.5 ‰ shift between centroids of the reference ruminant adipose fat and ruminant dairy fat ellipses. The less depleted $\delta^{13}C$ values seen for the fatty acids in non-ruminant fats (e.g. porcine fats) compared to equivalent components in ruminant fat are due to differences in diet and in the metabolic and biochemical processes involved in the formation of body fats. The $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids from Food Vessel potsherd extract DER 10 plots adjacent to the ruminant dairy fat reference ellipse, confirming earlier interpretations of a strong presence od dairy products within the vessel. The modern fats used to construct the reference isotope plot were reared on a strict C_3 diets of forages/fodders and cereals. The slight displacement of $\delta^{13}C$ isotopic values outside the dairy confidence ellipse may be due to the fact that the animals in prehistory were reared on diets, which varied in $\delta^{13}C$ values compared to today's values of environmental influences.

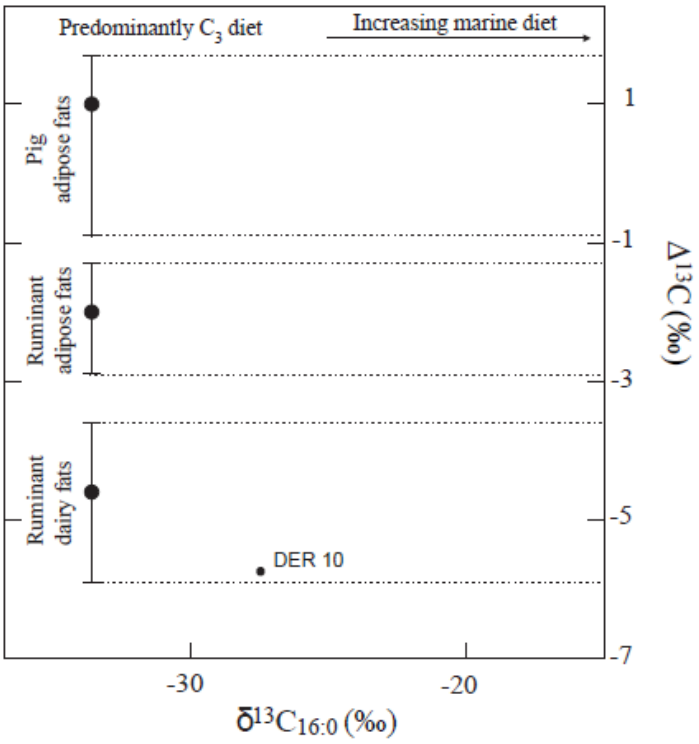

Figure 5. Plot showing the difference between $\Delta^{13}C$ values ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) and $\delta^{13}C$ values obtained from the $C_{16:0}$ fatty acids extracted from the Longstone Edge Food Vessel potsherd. The ranges for the modern reference fats are plotted to the left of the diagram with the indicated standard deviation.

$\Delta^{13}C$ values ($\delta^{13}C_{16:0}$ - $\delta^{13}C_{18:0}$) which reflect only the differences in metabolisms of animals are therefore an useful indicator of lipid origin when variations in isotope values occur. Figure 5 displays the $\Delta^{13}C$ values plotted against $\delta^{13}C_{16:0}$ values for the Longstone Edge Food Vessel potsherd extract. The ranges on the left side of the plot belong to the modern reference fats. $\Delta^{13}C$ value obtained for the Food Vessel again strongly confirms the presence of ruminant dairy lipids.

4. Conclusions

Lipid residue analyses of submitted potsherds from Longstone Edge barrow a partial preservation of organic residues. While the Beaker vessel contained no organic molecules, the Food Vessel displayed a strong presence of ruminant dairy fats. These were characterized by the presence of predominant free fatty acids, namely palmitic and stearic, together with odd-carbon number fatty acids with their branched homologues. The latter are predominantly produced by bacteria living in the rumen.

Good lipid preservation was also indicated by the presence of intact triacylglycerols, whose wide carbon number distribution further indicated the presence of ruminant dairy fats. Dairy origin of lipid extract was confirmed by the $\delta^{13}C$ values of extracted palmitic and stearic fatty acids, which plotted adjacent to the modern dairy fats reference values.

The Food Vessels that have been analysed in the past couple of years as part of a doctoral research project have revealed a very similar picture with a high percentage (79%) of analysed vessels containing ruminant dairy fat residues. The $\delta^{13}C$ values have confirmed a strong presence of dairy product consumption, which is mainly traced in the vessels that have been deposited in the graves.

The domestic pottery on the other hand displays a slightly broader use of pots, which is not mainly focused on dairy products, but also incorporates processing of ruminant meat, porcine animal products as well as plant material. The connection of dairy residues with funerary pottery has also been witnessed in other British Early Bronze Age funerary pottery, including all different pottery types, such as Collared Urns, Food Vessels, Trevisker pottery and Beakers (Šoberl, unpublished).

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6. Appendix – Analytical protocols

6.1 *Solvent extraction of lipid residues*

Lipid analysis of the potsherd involved taking a 2 g samples and cleaning the surface using a modelling drill to remove any exogenous lipids (e.g. soil or finger lipids due to handling). The sample was then ground to a fine powder, accurately weighed and a known amount (20 μg) of internal standard (*n*-tetratriacontane) added which enables later quantification of lipid concentration. The surface residues were not cleaned due to their fragile nature, but were sub-sampled and ground to a fine powder and weighed; again an internal standard was added. The lipids were extracted with a mixture of chloroform and methanol (2:1 v/v). Following separation from the ground sample the solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Portions (generally one third aliquots) of the extracts were then trimethylsilylated and submitted directly to analysis by HTGC. Where necessary combined GC/MS analyses were also performed on trimethylsilylated aliquots of the lipid extracts to enable the elucidation of structures of components not identifiable on the basis of HTGC retention time alone.

6.2 *Preparation of trimethylsilyl derivatives*

Portions of the total lipid extracts were derivatised using *N,O*-bis(trimethylsilyl) trifluoroacetamide (40 μl; 70°C; 60 min; T-6381; Sigma-Aldrich Company Ltd., Gillingham, UK) and analysed by HTGC and GC/MS).

6.3 *Saponification of total lipid extracts*

Methanolic sodium hydroxide (5% v/v) was added to the TLE and heated at 70°C for 1 h. Following neutralisation, lipids were extracted into chloroform and the solvent reduced under gentle stream of nitrogen.

6.4 *Preparation of methyl ester derivatives (FAMEs)*

FAMEs were prepared by reaction with BF_3-methanol (14% w/v; 100μl; Sigma-Aldrich, Gillingham, UK) at 70°C for 1 h. The methyl ester derivatives were extracted with chloroform and the solvent removed under nitrogen. FAMEs were re-dissolved into hexane for analysis by GC and GC-combustion-isotope ratio MS (GC-C-IRMS).