

**Organic Residue Analyses of
Pottery Vessels from
Willington Quarry**

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1. Introduction

Fats and waxes absorbed and trapped in the ceramic matrix of unglazed pottery vessels during the processing of organic materials are well protected from chemical decay and microbial attack and can be retrieved and identified even after several thousands of years of burial (Heron and Evershed, 1993). The application of modern analytical techniques allow even highly degraded remnants of natural commodities to be characterised and identified (Evershed *et al.*, 1990, 1994, 1997a, 1999). Frequently, compositional information obtained from organic residue analysis provides the only evidence for the exploitation and processing of animal commodities or leafy vegetables, particularly at sites exhibiting a paucity of environmental evidence. To date, the use of chemical information in the reconstruction of vessel use has enabled the identification and classification of a wide range of residues, including those derived from animal fats (Dudd and Evershed, 1998; Copley *et al.*, 2003; Evershed *et al.*, 1992; Needham and Evans, 1987), marine oils (Hansel *et al.*, 2004), beeswax (Charters *et al.*, 1995; Evershed *et al.*, 1997b, 2003), birch bark tar (Charters *et al.*, 1993a), leafy vegetables (Charters *et al.*, 1997; Evershed *et al.*, 1991, 1992, 1994) and plant oils (Condamin *et al.*, 1976; Copley *et al.*, 2001a,b).

The identification of ancient commodities based on organic residues in pottery is inevitably complicated by the degradative processes occurring during vessel use and burial. Reliable identifications, however, can be made based on individual diagnostic components and comparison of lipid profiles with modern reference samples and degraded materials produced in laboratory simulation experiments (Evershed *et al.*, 1995a; Dudd and Evershed, 1998; Dudd *et al.*, 1998). Degraded animal fats are by far the most commonly identified residues found in association with pottery vessels, and are characterised by a readily recognisable distribution of free fatty acids, monoacylglycerols, diacylglycerols and intact triacylglycerols. However, identification of the particular type of animal from which the fat is derived is not straightforward and complicated by chemical and micro-biological alteration (Evershed *et al.*, 1992; Dudd *et al.*, 1998). Distinctions can be made based primarily upon the distributions of free fatty acids present (Needham and Evans, 1987; Rottländer, 1990), however, other approaches are required in order to make unambiguous distinctions between remnant fats derived from different animal species.

The use of stable isotopes in archaeology was first explored by Morton and Schwarcz (1988) who investigated the bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of organic residues thought to originate from the C_4 cereal maize. Hastorf and DeNiro (1985) and Sherriff *et al.* (1995) also used bulk stable isotope measurements to characterise prehistoric carbonised plant and animal remains, respectively; however, interpretations of complex residues can be problematic using this approach. Such problems can be overcome by recording stable carbon isotope values for compounds whose structures can be matched to specific groups of organisms. The first application of compound-specific stable carbon isotope measurements to archaeological samples was reported by Evershed *et al.* (1994). The $\delta^{13}\text{C}$ values obtained for individual components in solvent extracts of pottery vessels from the Raunds area project, Northamptonshire, confirmed the epicuticular wax components being investigated were of C_3 plant origin. The distributions of components were consistent with the lipids in the potsherds having derived from *Brassica* species, such as cabbage. We have developed this approach further, and by utilising fundamental differences in the stable carbon isotope composition of the fatty acid component of fats from the major domesticates, we have been able to make clear distinctions between remnant fats of different origins in archaeological ceramics (Evershed *et al.*, 1997a; Dudd and Evershed, 1998; Mottram *et al.*, 1999; Copley *et al.* 2003). These differences in stable isotope values were coupled with differences in fatty acid composition, although the former are deemed to be diagenetically more robust.

2. Aims

Potsherds from Willington Quarry, South Derbyshire, were submitted for organic residue analysis using gas chromatography (GC) and GC-mass spectrometry (GC-MS). Where degraded animal fat residues were identified, further classification of their origin(s) was performed by comparing compound-specific stable carbon isotope values ($\delta^{13}\text{C}$), determined by GC-combustion-isotope ratio MS (GC-C-IRMS) of their major fatty acids (i.e. palmitic acid, $\text{C}_{16:0}$ and stearic acid, $\text{C}_{18:0}$) with values from modern reference fats.

3. Materials and Methods

Fifty potsherds from the Neolithic occupation at Willington Quarry, South Derbyshire were provided by the Matthew Beamish, Project Officer, University of Leicester Archaeological Services. Details of context are described in Table 1. The site is described in detail elsewhere (Beamish & Ripper, 2000; Beamish, 2001). Lipid analyses were performed using established protocols, which are described in more detail in earlier publications (Evershed *et al.*, 1990; Charters *et al.*, 1993b).

3.1 Solvent extraction of lipid residues

Approximately 2 g samples were taken and their surfaces cleaned using a modelling drill to remove any exogenous lipids (e.g. soil or ‘finger lipids’ due to handling). The samples were then ground to a fine powder, accurately weighed and a known amount (20 µg) of internal standard (*n*-tetratriacontane) added. The lipids were extracted with a mixture of chloroform and methanol (2:1 v/v). Following separation from the ground potsherd the solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Portions (generally one fifth aliquots) of the extracts were then trimethylsilylated and submitted directly to analysis by gas chromatography (GC). Where necessary combined gas chromatography-mass spectrometry (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 GC retention time alone.

3.2 Preparation of trimethylsilyl derivatives

Portions of the total lipid extracts were derivatised using *N,O*-bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (20 µl; 70°C; 20 min; Sigma-Aldrich Company Ltd., Gillingham, UK) and analysed by GC and GC-MS.

3.3 Saponification of total lipid extracts

Methanolic sodium hydroxide (1M) was added to the TLE and heated at 70°C for 1 h. Following acidification to pH 3 to 4, with hydrochloric acid, lipids were extracted into hexane.

3.4 Preparation of fatty acid methyl ester (FAME) derivatives

FAMES were prepared by reaction with BF_3 -methanol (14% w/v; 2 ml; Sigma-Aldrich, Gillingham, UK) at 70°C for 1 h. The methyl ester derivatives were extracted with diethyl ether and the solvent removed under nitrogen. FAMES were re-dissolved into hexane for analyses by GC and GC-C-IRMS.

4 Results

4.1 Lipid residue analysis

GC analyses of the solvent extracts obtained from the Willington Quarry pottery revealed sixteen of the fifty (32%) potsherds examined to contain appreciable lipid concentrations ($> 5 \mu\text{g g}^{-1}$), ranging from 5 to 2690 $\mu\text{g g}^{-1}$ with a mean value of 207 $\mu\text{g g}^{-1}$. By way of example, Figure 1 displays the partial gas chromatogram obtained from lipid residue, WiQ14. Table 1 lists the lipid concentrations and individual lipid components identified from each sample.

Twelve out of the sixteen extracts with significant lipid concentrations contained acylglycerols (mono-, di- and tri-) and free fatty acids with distributions consistent with an animal fat origin (Fig 1). Although containing just free fatty acids, four other residues also likely derive from animal fats, as indicated by their dominant fatty acids, i.e. palmitic acid (hexadecanoic acid; $\text{C}_{16:0}$) and stearic acid (octadecanoic acid; $\text{C}_{18:0}$). Two of the extracts also contained traces of wax esters (WiQ14, WiQ28), which indicates a small contribution from a plant or insect wax (e.g. beeswax) source.

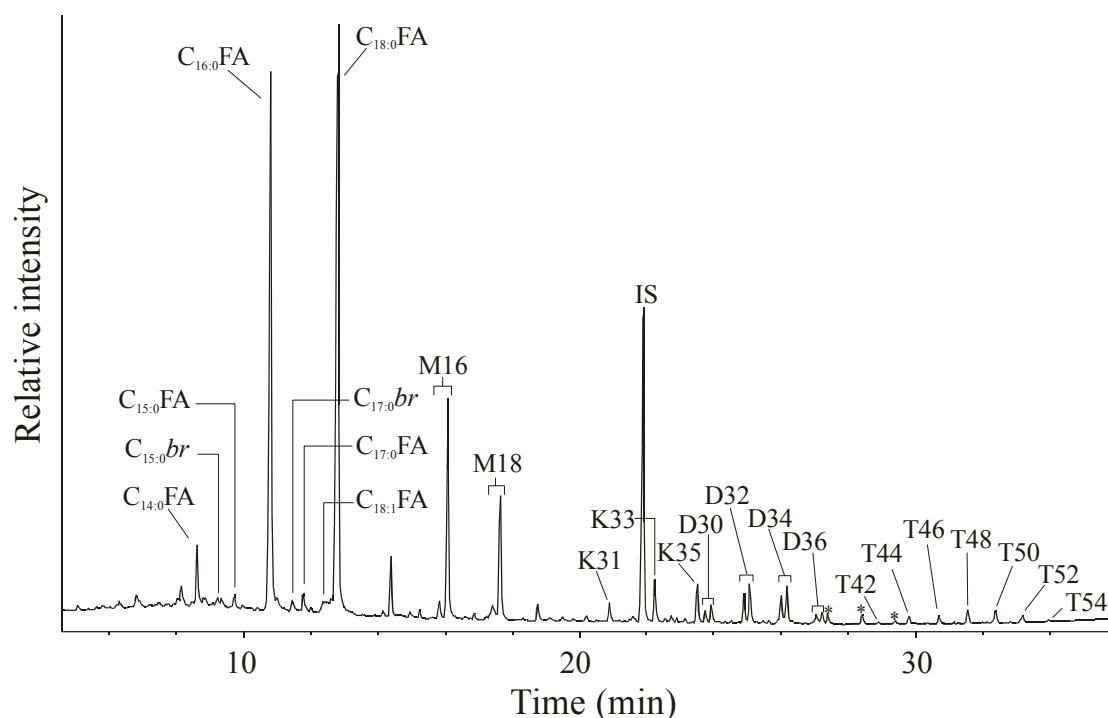


Figure 1: Partial gas chromatogram of the trimethylsilylated lipids extracted from potsherd WiQ14. Chromatographic peak identities are: C_{14:0}FA to C_{18:0}FA, saturated straight chain fatty acids with 14 to 18 carbon atoms, respectively; C_{18:1}FA, mono-unsaturated fatty acid with 18 carbon atoms; C_{17:0}br, branched chain fatty acids with 17 carbon atoms; M16 to M18, monoacylglycerols with 16 to 18 acyl carbons; D30 to D36, diacylglycerols with 30 to 36 acyl carbons; T42 to T54, triacylglycerols with 42 to 54 acyl carbons; K31 to K35, ketones containing 31 to 35 carbons; (*), wax esters; IS, internal standard, *n*-tetratriacontane (*n*-C₃₄).

Appreciable amounts of triacylglycerols were identified within five of the Willington Quarry lipid extracts (Fig. 2). Two residues (WiQ25 and WiQ44) contained triacylglycerol distributions with acyl carbon numbers ranging from C₄₄ to C₅₄, the remaining three (WiQ14, WiQ20 and WiQ49) from C₄₂ to C₅₄ acyl carbon atoms. The detection of low molecular weight C₄₂ triacylglycerols detected in WiQ14, WiQ20 and WiQ49 suggests that these vessels may have originally been used to process ruminant dairy products. The remaining residues with acyl carbon number ranges from C₄₄ to C₅₄ could, however, not be precisely classified, as adipose and dairy fat origins are equally plausible.

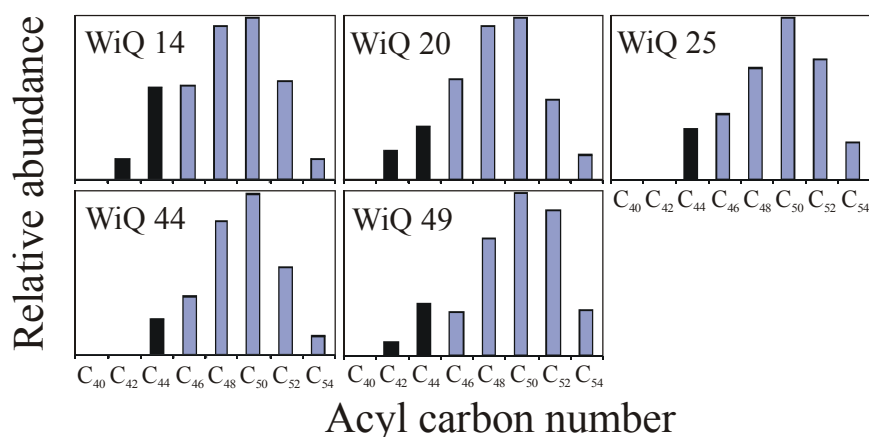


Figure 2: Histograms showing the acyl carbon number distributions of triacylglycerols identified in the lipid.

Five of the residues (WiQ02, WiQ22, WiQ24, WiQ26 and WiQ49) contained mid-chain ketones ranging in chain length from C₂₉ to C₃₅; and a further four (WiQ17, WiQ05, WiQ14, WiQ23) contained mid-chain ketones ranging from C₃₁ to C₃₅. These components form through the clay-catalysed condensation of fatty acids at temperatures typically greater than 300°C (Fig. 3; Evershed *et al.*, 1995b; Raven *et al.*, 1997), though whether the pottery was exposed to these high temperatures during or after use is uncertain.

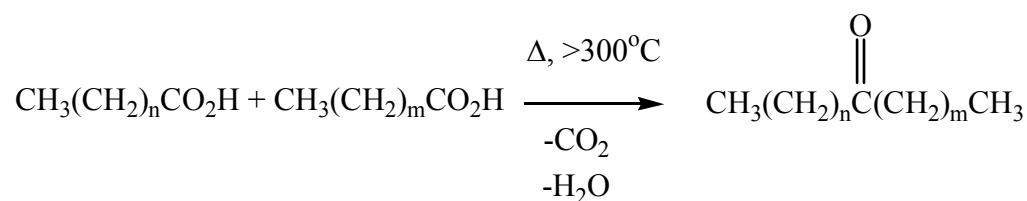


Figure 3: The ketonic decarboxylation of free fatty acids.

Stable carbon isotope ($\delta^{13}\text{C}$) values were obtained for the individual C_{16:0} and C_{18:0} fatty acids from fourteen of the degraded animal fat residues and compared with $\delta^{13}\text{C}$ values obtained from modern reference animals, which were raised on strict C₃ only diets. Figure 4 displays the $\delta^{13}\text{C}$ values for the C_{16:0} and C_{18:0} fatty acids obtained from the archaeological residues as a scatter plot, with confidence ellipses (1 standard deviation) overlaid, corresponding to the modern reference values. Seven of the degraded animal fat residues (WiQ01, WiQ02, WiQ05, WiQ20, WiQ24, WiQ43, and WiQ44) corresponded directly with a ruminant dairy fat origin as they plotted within the reference ellipse; while one residue (WiQ10) was found to be derived from a ruminant adipose origin, plotting directly within that reference ellipse.

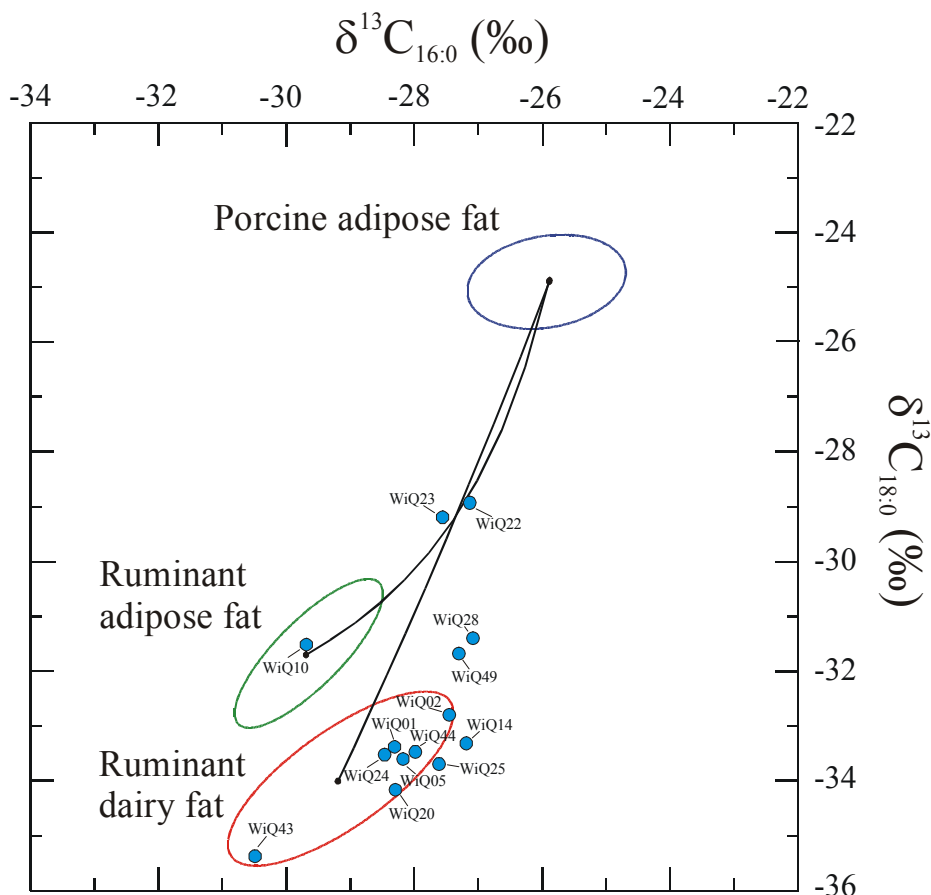


Figure 4: A plot of the $\delta^{13}\text{C}$ values obtained from the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids. The modern reference fats are represented by confidence ellipses (1 standard deviation). Theoretical curves from the porcine adipose fats to the ruminant adipose and dairy fats are plotted to illustrate the $\delta^{13}\text{C}$ values expected through their mixing. All reference $\delta^{13}\text{C}$ values include the addition of 1.2 ‰, to adjust for fossil fuel burning (Friedli *et al.*, 1986).

In addition, four samples (WiQ14, WiQ25, WiQ28, and WiQ49) may also have derived from a dairy fat origin as they plotted in close proximity to the reference dairy fat ellipse. The remaining two residues (WiQ22 and WiQ23) both plotted within the areas expected for mixing of different fats along the theoretical mixing curves.

Instead of this mixing hypothesis, each vessel may have been used to process only one commodity and the observed differences between their $\delta^{13}\text{C}$ values and the reference fat values may be a result of isotopic variations between the diets of the modern reference and prehistoric animals. These dietary ambiguities can be eliminated by comparing the $\Delta^{13}\text{C}$ ($\delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) values calculated for the archaeological residues with the modern reference fats. Using this criterion as many as eleven of the residues may have derived from ruminant dairy fat and three from ruminant adipose fat (Fig. 5). However, as seven of the residues plotted within the reference ruminant

dairy fat ellipse and one plotted within the reference ruminant adipose fat ellipse (Fig. 4), it is clear that the isotopic composition of the diets for the modern reference and prehistoric animals were comparable. As a result, the two residues (WiQ22 and WiQ23) plotting furthest from the ruminant adipose ellipse are more likely to derive from the mixing of ruminant and porcine fats.

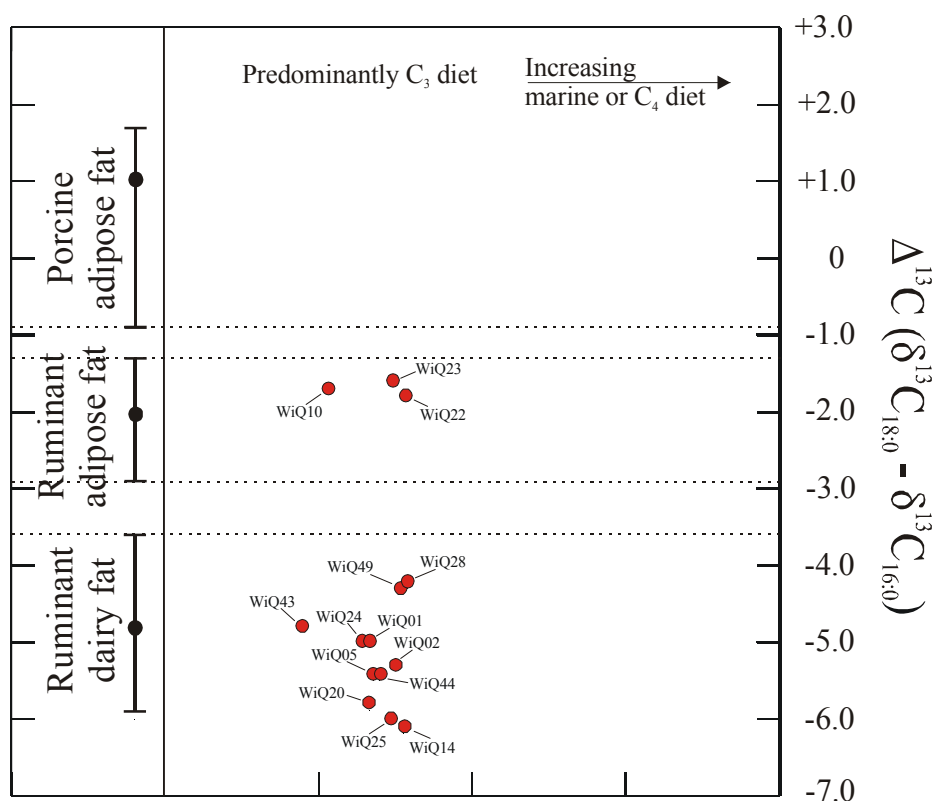


Figure 5: A plot showing the difference between $\delta^{13}\text{C}$ values obtained from the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids ($\Delta^{13}\text{C}$) extracted from the potsherds compared with values obtained from modern reference fats.

Table 1. Major lipid components detected in the Willington Quarry sherds

Sample Name	Vessel Number	Context/Group	Lipid Concentration ($\mu\text{g g}^{-1}$)	Lipid Components
WiQ 01*	10	0226/2541	117	FFA
WiQ 02*	19	0278/0802	36	FFA, K
WiQ 03	26	0297/0802	0	
WiQ 04	45	0390/0809	0	
WiQ 05*	51	0391/0809	60	FFA, K, DAG, TAG(tr)
WiQ 06	53	0402/0810	tr	FFA(tr), TAG(tr)
WiQ 07	68	0028/0803	0	
WiQ 08	70	0028/0803	0	
WiQ 09	78	0036/-	0	
WiQ 10*	91	0080/2518	11	FFA, MAG, DAG, TAG(tr)
WiQ 11	113	0114/4501	0	
WiQ 12	116	0413/0809	0	
WiQ 13	125	0458/0803	0	
WiQ 14*	153	1000/2508	86	FFA, MAG, K, DAG, WE(tr), TAG
WiQ 15	181	1003/3538	0	
WiQ 16	183	1004/2509	tr	FFA(tr)
WiQ 17	191	1040/2508	5	K
WiQ 18	194	1041/2508	0	
WiQ 19	219	1090/2518	0	
WiQ 20*	229	0114/4501	2686	FFA, MAG, DAG, TAG
WiQ 21	247	0117/2507	0	
WiQ 22*	266	0212/4501	19	FFA, K
WiQ 23*	267	0212/4501	18	FFA, K
WiQ 24*	270	0221/2541	85	FFA, MAG, K, DAG, TAG(tr)
WiQ 25*	279	0225/2541	28	FFA, MAG, DAG, TAG
WiQ 26	296	1152/2516	12	FFA(tr), K
WiQ 27	304	1259/2523	0	
WiQ 28*	329	1345/2525	79	FFA, WE(tr)
WiQ 29	348	1416/2504	0	
WiQ 30	352	1416/2504	tr	
WiQ 31	399	1463/2508	0	
WiQ 32	401	1463/2508	0	
WiQ 33	435	1497/2508	tr	
WiQ 34	473	1759/2550	0	
WiQ 35	495	1817/2550	0	
WiQ 36	499	1850/2550	tr	MAG(tr), DAG(tr), TAG(tr)
WiQ 37	504	1864/2518	0	
WiQ 38	521	1934/2541	0	
WiQ 39	533	1973/2541	0	
WiQ 40	550	2022/2525	0	
WiQ 41	562	2063/2550	0	
WiQ 42	577	4000/2522	0	
WiQ 43*	616	0000/2504	9	FFA, MAG DAG(tr), TAG(tr)
WiQ 44*	617	0000/2504	49	FFA, MAG, DAG, TAG
WiQ 45	263	0000/2525	0	

Sample Name	Vessel Number	Context/Group	Lipid Concentration ($\mu\text{g g}^{-1}$)	Lipid Components
WiQ 46	648	0000/2529	0	
WiQ 47	652	0000/2504	tr	
WiQ 48	676	3090/2516	0	
WiQ 49*	715	1002/2509	13	FFA, MAG, K, DAG, TAG
WiQ 50	716	1002/2509	0	

FFA refers to free fatty acids; MAG to monoacylglycerols; DAG to diacylglycerols; TAG to triacylglycerols; K to mid-chain ketones; WE to wax esters; C_x denotes carbon chain length; tr indicates that only trace amounts were present. Samples with an asterisk were selected for further analyses using GC-C-IRMS.

5. Summary

Lipid preservation at Willington Quarry was shown to be good, with appreciable concentrations of absorbed lipid residues being detected in 32 % (16/50) of the potsherds analysed. Initial analyses revealed that these residues were comprised of individual lipid components that were highly indicative of animal fat remains. Two lipid extracts (WiQ14 and WiQ28) also showed a minor plant or insect wax contribution. Thirteen of the potsherds also showed evidence of extreme heating (> 300°C), as indicated by the presence of mid-chain ketones.

In order to classify the origins of these animal fat residues more precisely, stable carbon isotope values ($\delta^{13}\text{C}$) were obtained for the C_{16:0} and C_{18:0} fatty acids from fourteen of the samples and compared with values for modern reference fats. The $\delta^{13}\text{C}$ values from eleven of the archaeological residues were consistent with those expected for ruminant dairy fats; with nine (WiQ01, WiQ02, WiQ05, WiQ14, WiQ20, WiQ24, WiQ25, WiQ43 and WiQ44) plotting directly within or immediately adjacent to the reference ellipse and two (WiQ28, and WiQ49) plotting slightly further away, but assigned a dairy origin on the basis of their $\Delta^{13}\text{C}$ values. Interestingly, three of the residues (WiQ14, WiQ20 and WiQ49) that were assigned ruminant dairy fat origins also contained triacylglycerols with wide distributions (i.e. C₄₂-C₅₄ acyl carbons), which are also indicative of degraded dairy fat residues, thereby further supporting the more robust stable isotope criterion.

The $\delta^{13}\text{C}$ value from another of the residues (WiQ10) was found to compare directly with the values expected for ruminant adipose fat, while the remaining two (WiQ22 and WiQ23) residues plotted outside any reference ellipse but along the theoretical mixing curves. The good agreement between most of the lipid residues and the

reference values indicates that the values of these two residues are most likely to derive from the processing of a mixture of ruminant and porcine fats.

Table 2 lists all the samples containing more than trace lipid residues and the assignment of origin(s) based on: (i) the lipid components present, (ii) their triacylglycerol distributions, (iii) their $\delta^{13}\text{C}$ values, and (iv) their calculated $\Delta^{13}\text{C}$ values.

Table 2. Assignments of origins for the absorbed lipid residues

Sample Name	Lipid residue origin(s) as suggested from:			
	Lipid components present	Triacylglycerol distribution	$\delta^{13}\text{C}$ values	$\Delta^{13}\text{C}$
WiQ 01	Animal fat	-	Dairy	Dairy
WiQ 02	Animal fat	-	Dairy	Dairy
WiQ 05	Animal fat	-	Dairy	Dairy
WiQ 10	Animal fat	-	Ruminant adipose	Ruminant adipose
WiQ 14	Animal fat + trace plant/insect wax	Dairy	Dairy	Dairy
WiQ 20	Animal fat	Dairy	Dairy	Dairy
WiQ 22	Animal fat	-	Mixture: ruminant/porcine fat	Ruminant adipose
WiQ 23	Animal fat	-	Mixture: ruminant/porcine fat	Ruminant adipose
WiQ 24	Animal fat	-	Dairy	Dairy
WiQ 25	Animal fat	Ruminant adipose and/or dairy	Dairy	Dairy
WiQ 28	Animal fat + trace plant/insect wax	-	Dairy?	Dairy
WiQ 43	Animal fat	-	Dairy	Dairy
WiQ 44	Animal fat	Ruminant adipose and/or dairy	Dairy	Dairy
WiQ 49	Animal fat	Dairy	Dairy?	Dairy

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