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Stable carbon isotopic characterisation of free and bound lipid constituents of archaeological ceramic vessels released by solvent extraction, alkaline hydrolysis and catalytic hydropyrolysis

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Abstract

A sequential degradation scheme, involving solvent extraction, alkaline saponification and catalytic hydropyrolysis (open-system pyrolysis assisted by high hydrogen gas pressure), was used for recovering both free and covalently-bound lipid organic compounds from two archaeological ceramic sherds sampled from the interior of cooking vessels and from a ceramic control sample used for cooking modern milk. Various organic molecular products released by this regime were identified and quantified using gas chromatography–mass spectrometry (GC–MS), and the stable carbon isotopic ($\delta^{13}C$) values of the major products were measured using gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS). Significant amounts of fatty acids and fatty acid-derived hydrocarbons could be released by alkaline hydrolysis and hydropyrolysis, respectively, following initial solvent extraction of the ceramic. No significant lipid signal bias, in terms of both carbon number distributions of compounds or stable carbon isotopic signatures, could be discerned though between free, hydrolysable and tightly-bound molecular components in this study. So, conventional analysis of free fatty acid components appears, from this limited data set, to provide an accurately representative insight into the total fatty acid composition in archaeological pots. Even after catalytic hydropyrolysis, a significant amount of residual carbon (>50 wt.% of the total organic carbon) remained on the archaeological sherds in the form of highly condensed aromatic polymers, with much lower (6 wt.%) levels of residual carbon persisting in the control pot. This aromatic macromolecular

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phase on the vessel interior most probably originates from repeated use of the vessels for cooking, via gradual polymerisation/aromatisation of food residues, rather than being derived from smoke condensates produced from cooking on open wood fires. The co-existence of preserved aliphatic fatty acids and a thermally-stable aromatic macromolecular phase within the same ceramic matrix suggests that the preserved lipid components were introduced into the vessel after the formation of the bulk of the char, and so fatty acid analyses most probably provide information concerning the later uses of archaeological pots prior to burial.

Keywords: Hydropyrolysis; Residue analysis; Archaeology; Fatty acids; Stable isotope analysis

1. Introduction

Insights into the use of ceramic vessels by humans in the past can be gauged from analysis of their residual molecular lipid content. This is an important resource for archaeologists, especially when other organic remains, such as human, faunal and botanical specimens, are poorly preserved. Organic residues on ceramics have been used to study ancient economies, diet and consumption practices [1–4] and, more recently, as a substrate for radiocarbon dating [5]. Preserved fats and waxes, resulting from a variety of human activities, can be released from ceramic vessels by extraction with organic solvents, even after thousands of years of exposure to the depositional environment. By using analytical methods such as high temperature-gas chromatography (HT-GC) and gas chromatography–mass spectrometry (GC–MS), compounds derived from a range of commodities have been identified, including beeswax [6], epicuticular plant waxes [7] and pine resins [8].

Degraded animal fats, characterised by high concentrations of *n*-alkanoic acids, monounsaturated *n*-alkenoic acids and lesser amounts of monoacyglycerols (MAG), diacyglycerols (DAG) and triacyglycerols (TAG), are by far the most frequently detected residues reported from solvent extraction of archaeological cooking vessels [9]. Unfortunately, changes in the distribution of these compounds due to diagenetic alteration, prevent any further classification of the origin of these fats using conventional chromatographic methods [10]. Recently, compound-specific stable carbon ($\delta^{13}C$) isotopic measurements of the most abundant *n*-alkanoic acids ($C_{16:0}$, $C_{18:0}$) have provided new criteria for distinguishing ruminant adipose, ruminant dairy and non-ruminant fats [10,11]. These same acids have been the compounds of choice for compound-specific radiocarbon dating, after isolation by preparative gas chromatography [5]. In these studies, absorbed lipids are liberated from the powdered ceramic matrix, after removal of any extraneous surface residue, by ultrasonically-aided solvent extraction (2:1, (v/v), chloroform/methanol). This method is preferred to other techniques, e.g. soxhlet extraction [12], as smaller sample sizes are readily accommodated [3].

It has been shown, however, that solvent extraction is unable to release all the lipid content from archaeological sherds and that saponification, through alkaline hydrolysis, or treatment with aqueous (*m*-trifluromethylphenyl)trimethylammonium hydroxide (TMTFTH) releases additional saturated and unsaturated fatty acids [13], often in greater abundance [14,15]. In these studies, both the soluble and the insoluble fractions have similar compositions

of fatty acids, suggesting that they have originated from the same source. But whether these insoluble fatty acids are tightly associated with the ceramic matrix or are molecular constituents of an organic macromolecular phase (a preserved bio- or geo-polymer) is not known. Even after saponification, it has been demonstrated, through the application of elemental microanalysis, that a residual organic fraction is still present in archaeological cooking vessels [15]. In some cases, this 'non-extractable' faction can account for 65–75% of the total organic carbon in the original ceramic sample [15], values consistent with 'bound' organic carbon sequestered in recent sediments in the form of kerogens [16].

Pyrolysis methods allow characterisation of the total lipid content of archaeological sherds and provide additional insight into the mechanisms of preservation. Molecular fragments of organic residues released from the matrix of archaeological cooking vessels by pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) are dominated by a range of straight chain hydrocarbons, comprising predominantly *n*-alk-1-enes and *n*-alkanes [17]. This product distribution was interpreted as resulting from fragmentation of an aliphatic network polymer formed by polymerisation of adsorbed lipids, most likely formed during the cooking process rather than post-depostional. Yet the degree of polymerisation that had occurred in these samples is not clear, as similar hydrocarbon distribution patterns may also be obtained by flash pyrolysis of simple model silicon-bound hydrocarbons [18]. Also, the presence of an insoluble organic fraction has been observed in storage amphorae [14], which would suggest that fatty acids could equally become ester bound to the ceramic matrix during post-deposition or by stronger covalent linkages, as it is unlikely that these particular sherds were exposed to excessive heat.

Although methodological advances provide new scope for the identification and characterisation of ancient organic residues, little is known about their formation, either during pottery use or during exposure to the burial environment. As it is likely that only a limited set of reaction conditions may actually lead to the successful absorption and long-term preservation of organic residues in ceramic vessels, understanding formation processes is crucial for evaluating evidence within an archaeological context. For very degraded samples, especially, the recovery and analysis of a recalcitrant bound lipid fraction may yield the only diagnostic organic signal relating to the original use of the host vessel. Furthermore, with increasing number of isotopic measurements that are being made on fatty acids components in archaeological pottery, it is important to assess whether the method of analytical extraction used and the mode of incorporation of the lipid signals (free or bound) released can lead to significant variation in the isotopic signatures recorded.

Isotopic fractionation may occur by a number of processes; as a result of mass selective partitioning of lipids between organic solvent and the ceramic surface during extraction recovery, from molecular interactions (ionic and covalent) between organics and the ceramic surface during use and subsequent burial, and from polymerisation and degradation (both biotically- and abiotically-mediated) reactions of organic matter occurring during use, deposition and subsequent diagenesis. Small $\delta^{13}C$ fractionations ($\leq$0.5%) have even been observed when highly volatile organic acids are physically adsorbed on to solid non-polar organic phases from aqueous and vapour phases [19,20]. Therefore, inefficient extraction may potentially result in compounds appearing in the analytical window that are either isotopically depleted or enriched in ^{13}C compared to the original food input.

Here we report on the first use of a unique pyrolysis technique, catalytic hydropyrolysis, for recovery of lipid constituents from archaeological potsherds which are inaccessible to solvent extraction and, in some cases, saponification. Hydropyrolysis involves temperature-programmed pyrolysis assisted by high hydrogen gas pressures and a sulphided molybdenum catalyst, and the treatment is performed in a well-swept, open-system reactor configuration [21,22]. This method has successfully been applied previously to efficiently fragment insoluble organic matter (kerogen) in sediments to generate, principally, a dichloromethane–soluble tar product, with high conversions of typically greater than 85 wt.% being achieved for immature (pre-oil window) kerogen [22–24]. Unlike other open-system pyrolysis techniques that have been used to fragment organic residues from ceramics and which employ high temperatures (>600 °C), such as *flash pyrolysis* in the form of Py–GC–MS and Py–MS [17,25], hydropyrolysis is excellent at preserving important structural and stereochemical features in hydrocarbon products. Secondary reactions which can occur during pyrolysis (cracking and isomerisation) are minimised during hydropyrolysis due to the relatively low temperatures required for covalent bond cleavage (generally over a 200–450 °C temperature window) and due to the efficient removal of volatile products from the hot zone of the reactor (with residence times being of the order of only a few seconds).

The thermal reactivity of different carbon functionalities in lipid structures under hydropyrolysis conditions has been modelled in detail previously by Snape and co-workers using suitable solid calibrants [26–29]. Importantly, for convenient and accurate determination of the $\delta^{13}C$ signatures of lipids which are not accessible to solvent extraction, it is known that only minor levels of decarboxylation accompanies reductive cleavage of the oxygen atoms in both free and bound saturated fatty acids under typical hydropyrolysis conditions i.e. a $C_{18:0}$ *n*-alkane is the dominant product generated from hydropyrolysis of a bound $C_{18:0}$ *n*-alkanoic acid [29]. Thus, the bulk of the carbon isotopic information in lipid molecules is preserved during hydropyrolysis treatment and no derivatisation needs to be performed since the principal products are released in hydrocarbon form, which is simple to directly analyse using gas chromatography. In order to investigate possible isotopic and compositional differences that may exist between bound and free fatty acids species in archaeological cooking pots; the distribution and $\delta^{13}C$ signatures of hydrocarbon products released by hydropyrolysis from pre-extracted and saponified ceramics are here compared with those obtained for fatty acid compounds released by the solvent extraction and alkaline saponification steps. The analytical methodology associated with the overall sequential degradation scheme is outlined in Fig. 1.

2. Experimental

2.1. Samples

Archaeological materials consisted of two sherds taken from the interior of cooking vessels that were obtained from two excavation sites. The first sherd (Easingwold, Ew) was obtained from the Iron Age site of Easingwold, North Yorkshire, UK and the second (Vendel, Ve) from the Early Medieval site of Vendel, Svealand, Sweden. Both these sherds were obtained from much larger pottery assemblages that have previously been investigated

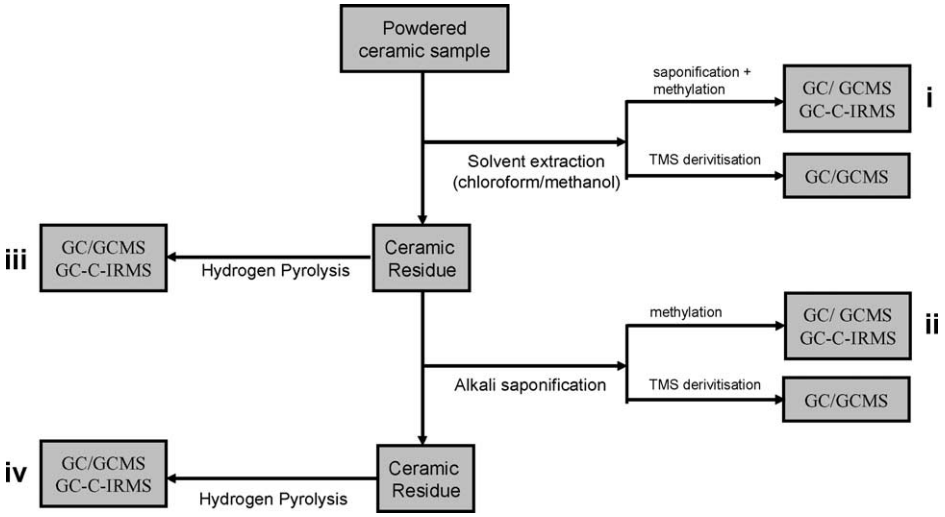

Fig. 1. Schematic representation of the methodology and analytical procedure used in this study. Roman numerals correspond to the samples analysed by GC–C–IRMS.

using residue analysis [15,30]. Controls of replica ceramic that had been 'open' fired were also included in the analysis. The negative control (Ne) was obtained immediately after firing and had no organic input. An identical replica pot (modern milk pot, Mp) was used to heat bovine milk on three separate occasions (each for 1 h) over an open wood fire. Samples of the milk used in the experiment were recovered and retained and the empty pot was stored at −20 °C prior to analysis. Further details of construction, firing and use of the control sherds can be found elsewhere [31]. The first 2 mm layer of ceramic of each sherd was discarded by drilling from the interior surface to eliminate contamination from extraneous surface material. A further 3 g of ceramic was then removed from the newly exposed inner pot surfaces to a depth no greater than 5 mm of the original surface. These samples were sealed in pre-extracted glass vessels and freeze dried.

2.2. Solvent extraction and saponification

The powdered sherd samples (ca. 3 g) were solvent extracted with 10 ml of a 2:1 (v/v) mixture of chloroform and methanol by ultrasonification (2 × 15 min). The dissolved products were removed, after centrifugation, and the process was repeated twice. The total solvent extracts were combined and reduced to a small volume by rotary evaporation, transferred to a small vial and gently dried under nitrogen. The ceramic residue that remained after solvent extraction was dried under a nitrogen gas flow. A portion of this residue (1.5 g) was saponified with 4 ml of sodium hydroxide (5% (w/v) in methanol) for 4 h at 70 °C. The solution was neutralised with 6 M hydrochloric acid (HCl) and liberated lipid components were then extracted five times with hexane. The hexane extracts were combined and gently dried under nitrogen.

A portion of the solvent extracts were saponified with 2 ml of NaOH (5% (w/v) in methanol) at 70 °C to release free fatty acids. After 1 h, the extracts were cooled and the solution was neutralised by the addition of concentrated HCl solution (6 M). Free fatty acids were extracted three times with hexane and dried under nitrogen. Both these and the extracts that had been saponified directly from the ceramic were methylated with a solution of boron trifluoride–methanol (14%, (w/v) complex at 70 °C for 1 h. The resulting methyl esters were extracted with diethyl ether and dried under nitrogen for analysis by GC–C–IRMS. Another portion of the solvent extracts and the saponified extracts were treated with 20 μl of *N,O*-bis(timethylsilyl) tetrafluroacetamide containing 1% (v/v) chlorotrimethylsilane at 65 °C for 30 min to produce trimethylsilyl derivatives which were then dried under nitrogen. These derivatized extracts were analysed by GC and GC–MS.

2.3. Catalytic hydrogen pyrolysis (hydropyrolysis)

The ceramic residues remaining after solvent extraction along with those which had undergone subsequent saponification were dried under nitrogen and impregnated with an aqueous solution of ammonium dioxydithiomolybdate ($(NH_4)_2MoO_2S_2$), resulting in a nominal molybdenum loading of 1 wt.%. The samples were then dried in vacuo, placed in a stainless steel reactor tube and heated from 200 to 420 °C at 8 °C min^{-1} under high hydrogen pressure (15.0 MPa H_2). A continuous flow of hydrogen sweep gas of 6 dm^3 min^{-1}, as measured at ambient temperature and pressure, through the reactor bed ensured that residence times of generated volatile products in the heated reactor zone were kept very short (order of a few seconds). The total pyrolysis run time was of the order of only ca. 30 min. The pyrolysates were collected in a dry-ice cooled trap and recovered in dichloromethane for subsequent fractionation. Any bound/trapped *n*-alkanoic acids in the ceramic matrix were reductively converted into the *n*-alkane form by this hydropyrolysis treatment, without significant loss of carbon in the molecule through decarboxylation.

The hydropyrolysis products, in dichloromethane solution, were treated with activated copper turnings to remove traces of elemental sulphur (produced from thermal dissociation of the catalyst) and then separated by silica gel adsorption chromatography in short Pasteur pipette columns into aliphatics, aromatics and polars (or N, S, O compounds) by successive elution with *n*-hexane, *n*-hexane-dichloromethane (3:1, (v/v) and dichloromethane-methanol (3:1, (v/v), respectively. All aliphatic and (selected) aromatic fractions were analysed in detail using gas chromatographic methods (GC–FID, GC–MS and GC–C–IRMS).

2.4. Gas chromatography of aliphatic and aromatic hydrocarbons

GC was performed with a Carlo Erba 5160 HRGC instrument equipped with a flame ionisation detector (FID). Aliphatic hydrocarbon products generated from hydropyrolysis experiments were analysed on a Zebron fused silica capillary column (30 m × 0.25 mm i.d.) coated with a ZB-1 stationary phase (100% dimethylpolysiloxane; 0.25 μm film thickness) using on-column injection. Aromatic hydrocarbon products were analysed using a fused silica capillary column coated with a HP-5 stationary phase. In both cases, the GC oven was

temperature programmed from 50 °C (held 2 min) to 300 °C (held 20 min) at 4 °C min^{-1} and the carrier gas used was hydrogen.

2.5. Gas chromatography–mass spectrometry of functionalised lipids and hydrocarbons

Mass spectral characterisation of derivitised functionalised lipid products was performed using combined gas chromatography–mass spectrometry on a Hewlett-Packard 5890 II GC with split–splitless injector interfaced to a Hewlett-Packard 5972 quadrupole mass selective detector (MSD) (ion source temperature, 280 °C; filament current, 0.35 mA; electron voltage, 35 eV; accelerating voltage, 2 kV). Samples (1 μl of ca. 0.1 mg ml^{-1} solution) were introduced onto the column from a HP 7673 auto-sampler and the split was opened after 1 min. Separation of compounds was performed on a J&W Scientific fused silica capillary column (15 m × 0.32 mm i.d) coated with DB-1HT stationary phase (100% dimethylpolysiloxane; 0.1 μm film thickness). The stationary phase was a non-polar siloxane–carborane co-polymer with an upper temperature limit of 370 °C. Helium was used as the carrier gas at a column head pressure of 53.9 kPa. The temperature program employed was from 50 °C (held for 2 min) to 350 °C at a constant heating rate of 10 °C min^{-1}. The GC–MS interface temperature was kept at 345 °C and spectra were recorded over the mass range, *m/z*, 50–850 at 1.5 scan s^{-1}. Data acquisition was controlled by a HP Vectra 486 chemstation computer. Compound identifications were based on relative retention times and with comparisons with the spectral reference library. Peaks areas were quantified by comparison with a known amount of an internal standard (C_{36} or C_{34} *n*-alkane, ca. 20 μg), which was introduced to the sample prior to solvent extraction and saponification.

GC–MS analyses of aliphatic and aromatic hydrocarbons produced from hydropyrolysis were carried out on a HP 6890 gas chromatograph, fitted with a SGE BPX35 fused silica capillary column (25 m × 0.22 mm i.d.; 0.25 μm film thickness), coupled to a HP 5973 mass selective detector. Sample injection was performed in splitless mode at a temperature of 325 °C. The oven temperature was held at 50 °C for 2 min, ramped at 12 °C min^{-1} to 350 °C and then held isothermal for 10 min. Helium was used as the carrier gas at a constant flowrate of 2.0 ml min^{-1}. Mass spectral analyses were performed in electron ionisation (EI) mode at 70 eV, using an ion-source temperature of 230 °C and an interface temperature of 350 °C. The mass filter (quadrupole) was set to scan between *m/z* 50 and 710 at 2.26 scan s^{-1}. Chromatograms and mass spectra obtained were analysed with the HP Chemstation A.003.00 software. Individual products were quantified relative to a C_{34} *n*-alkane standard.

2.6. Gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS)

All GC–C–IRMS measurements were performed on a Hewlett-Packard 5890 gas chromatograph attached to a PDZ Europa Ltd. Geo-isotope ratio mass spectrometer via a Orchid II combustion interface (PDZ Europa Ltd., UK). For fatty acid methyl esters (FAMES), analyses were performed using a SGE fused-silica column (30 m × 0.32 mm i.d.) coated with

BPX70 stationary phase (immobilised 70% cyanopropyl (eq.) polysilphenylene-siloxane; 0.25 μm film thickness). The temperature program used was as follows: 130 °C (2 min) to 190 °C (2 min) at 4 °C min^{-1}. Analyses of aliphatic hydrocarbon fractions from hydropyrolysis were performed using a SGE fused-silica capillary column (30 m × 0.32 mm i.d.) coated with a BPX5 stationary phase (0.5 μm film thickness). In both cases, helium was used as the carrier gas. The combustion furnace was maintained at 860 °C and the mass spectrometer source pressure was 1.01×10^{-3} Pa.

The $\delta^{13}C$ values measured for fatty acid analytes were corrected for the methylation derivatisation process by comparing with off-line measurements (using a ANCA S/L Prep unit attached to a PDZ Europa 20/20 isotope ratio mass spectrometer) made on a number of standard *n*-alkanoic acids and their methyl esters after methylation using the same reagents as for the samples. Carbon isotopic values were expressed in per mil (%) notation relative to the Pee Dee Belemnite (PDB) standard. Isotope measurements were made on the C_{16} and C_{18} *n*-alkanoic acids (as methyl esters) for solvent extracts and saponified products and on the C_{16} and C_{18} *n*-alkanes for hydropyrolysis experiments. Extracts from all samples were run at least in triplicate.

2.7. TOC determination on hydropyrolysis residues

Carbon contents were determined using a UIC CO_2 coulometer. A sample (typically 20 mg) was weighed into a ceramic boat and then placed into a ladle containing a magnet. The ladle was then introduced into the pre-combustion tube outside the furnace, and the sample was purged with oxygen using a flow rate of 10 cm^3 min^{-1} for 2 min to remove any adsorbed interfering gases (mostly CO_2). Prior to entering the pre-combustion tube, the oxygen stream was passed through a scrubber to remove any residual CO_2. The ladle was then moved into the combustion furnace (950 °C) using a magnet, and the sample was combusted for 10 min in the oxygen stream. The combustion products passed through different scrubbers to remove halogens, sulphur, nitrogen oxides and water. The resulting gases were bubbled through a coulometer cell containing ethanolamine and a colorimetric pH indicator. The CO_2 from the gas stream reacted with the ethanolamine, causing the indicator colour to fade. The coulometer photometer recognises this condition and starts the electrochemical generation of base, returning the solution to its original colour. The applied current, which is proportional to the CO_2 concentration, was integrated and digitally displayed in microgram C. The detection limit was 1 ppm C.

2.8. Solid state ^{13}C NMR spectroscopy

Solid state ^{13}C NMR spectra using a Bruker DSX200 instrument equipped with a double-bearing probe for cross polarisation (CP) and magic angle spinning (MAS). The resonance frequency for ^{13}C was 50 MHz, and the sample rotor was spun at the magic angle with a frequency of 6.0 kHz. Typically 20,000 scans were accumulated with high power ^{1}H decoupling for the CP experiments, using a contact time of 1.0 ms and a relaxation delay was 1.5 s. The spectra were processed with a line broadening factor of 50 Hz.

3. Results and discussion

3.1. Analysis of lipids

3.1.1. Solvent-extractable lipids

Solvent extraction of each pot released appreciable quantities of lipid material (0.5–6.0 mg g^{-1} of ceramic, see Table 1). In the archaeological samples, most of the lipid constituents present had been hydrolysed into fatty acids during burial, but small amounts of triacylglycerols, diacylglycerols or monacylglycerols were also found suggesting that this degradation process was incomplete (Fig. 2). The very high relative abundance of saturated species, principally *n*-hexadecanoic ($C_{16:0}$) and *n*-octadecanoic ($C_{18:0}$) acids, in these samples suggested that the lipids were derived from animal adipose tissue [32], although contributions from degraded milk fats could not be ruled out. The modern milk pot contained a complex range of triacylglcerols (containing between 28 and 52 acyl carbon atoms) and lesser amounts of *n*-alkanoic acids (containing between 8 and 20 carbon atoms), *n*-alkenoic acids (principally $C_{18:1}$) and branched chain acids, consistent with the lipid distribution for fresh milk [33]. There was no evidence of any hydrolysis products of the TAGs in the modern milk pot, (i.e. DAGs and MAGs), despite the prolonged cooking events. Total fatty acid abundances and distributions (comprising free fatty acids plus the acid components of glyceryl esters) were obtained by alkali hydrolysis of the whole extracts to ensure complete cleavage of ester bonds in glyceryl esters and the resulting total acid fractions were methylated and then analysed by GC and GC–MS (Fig. 3).

3.2. Insoluble lipids released by saponification

Significant amounts of fatty acids (200 μg g^{-1} of ceramic) were further extracted from the ceramic residues by saponification (Table 1). The absence of internal standard in these extracts, which was introduced initially to the ceramic samples, indicates that the solvent extraction was effective at removing any weakly-adsorbed free lipid components. In the modern milk pot and Vendel samples, the remaining insoluble fraction accounts for only less than 10 wt.% of the total amount of acids released by solvent extraction, but in the older Easingwold sample, the lipid yield obtained by alkaline saponification was more than double that released by conventional solvent extraction. In the Ew sample, since the yield of extractable fatty acids was an order of magnitude lower than for Ve or Mp, this suggests

Table 1
Total amounts of straight-chain fatty acids (μg g^{-1} of sherd) extracted from modern and archaeological potsherds after solvent extraction and subsequent saponification (detection limit <0.1 μg g^{-1} sherd)

Sample	Solvent extraction: total fatty acids (μg g^{-1})	Saponification: total fatty acids (μg g^{-1})	Percentage of fatty acids that are solvent-extractable
Modern milk pot (Mp)	6302	528	92
Easingwold pot (Ew)	410	835	33
Vendel pot (Ve)	4224	215	95

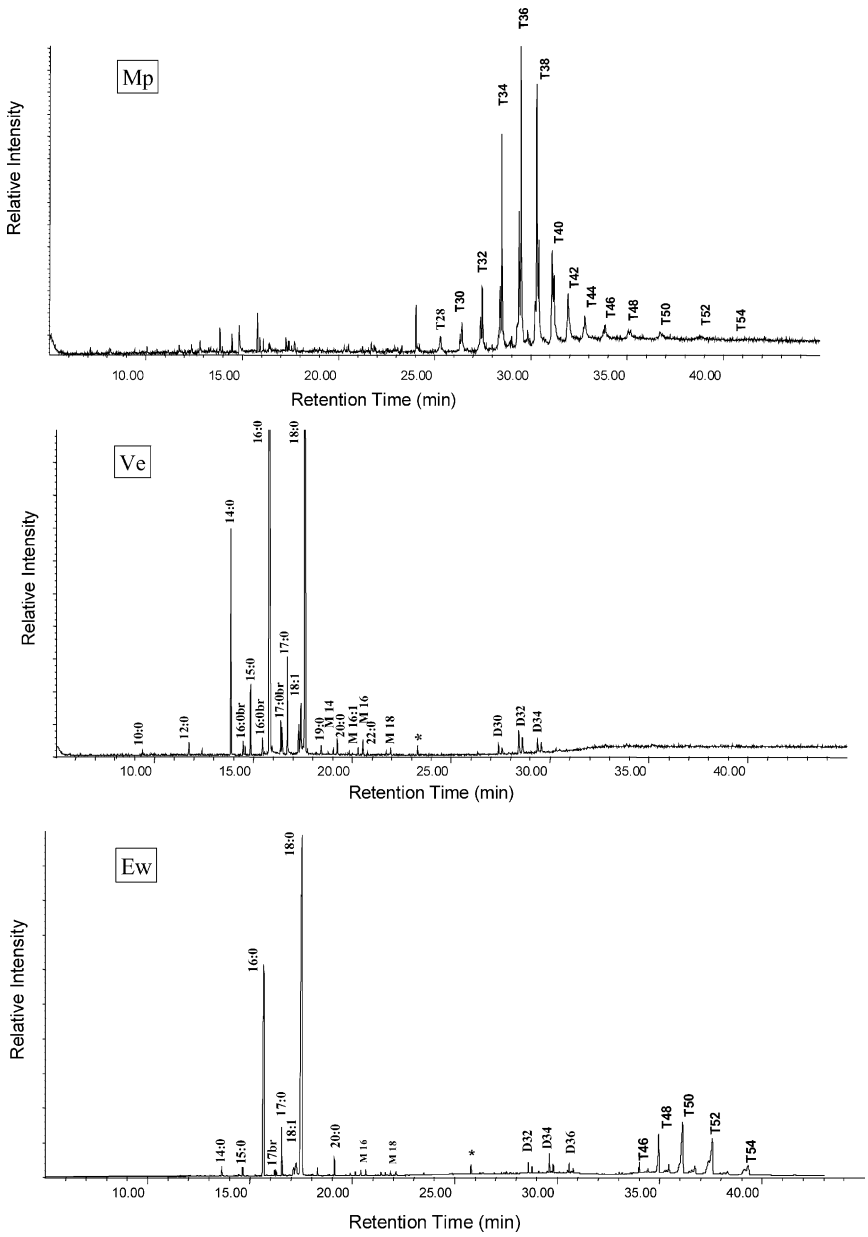

Fig. 2. High temperature-gas chromatograms of the total solvent extracts recovered from an early Medieval cooking vessel from Vendel, Sweden (Ve); an Iron Age cooking pot from Easingwold, UK (Ew) and a modern ceramic pot used to cook milk (Mp). Peaks were identified by GC–MS. Numbers (*X*:*Y*) refer to chain lengths (*X*) and number of saturations (*Y*) in the TMS derivatives of the fatty acids acids; br: branced chain acids; M: monoacylglycerols; D: diacylglycerols; T: triacylglecerols with numbers referring to the number of acyl carbon atoms; *: internal standard. No peaks, apart form the internal standard, were observed in the negative control.

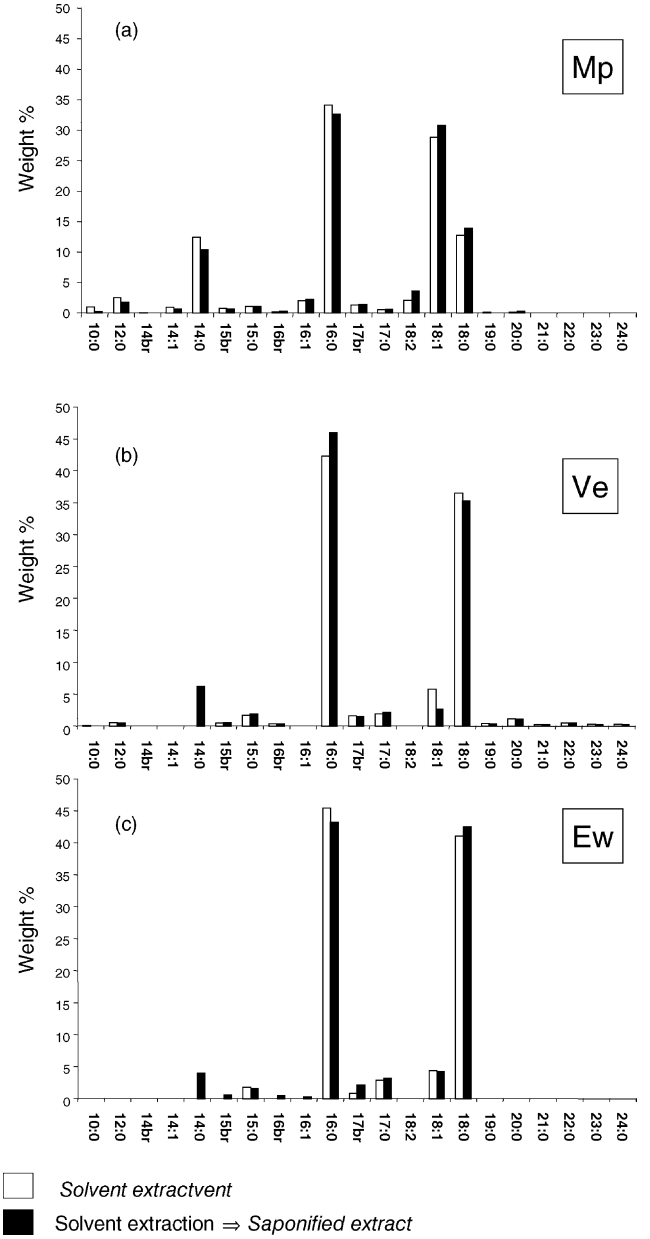

Fig. 3. Distribution of fatty acids released after solvent extraction and subsequent saponification from an early Medieval cooking vessel from Vendel, Sweden (Ve); an Iron Age cooking pot from Easingwold, UK (Ew) and an modern pot used to cook milk (Mp). Fatty acids were analysed as their methyl esters. No peaks were observed in negative control. Peaks were identified by GC–MS. Numbers (*X*:*Y*) refer to chain lengths (*X*) and number of saturations (*Y*) in the TMS derivatives of the fatty acids acids; br: branched chain acids.

that preferential loss of weakly-adsorbed free fatty acids and fats may have occurred during diagenesis following burial.

The composition of fatty acids in the solvent and saponified extracts (Fig. 3) were very similar for each of the samples. This finding is consistent with a previous report [14] and suggests that both soluble and insoluble fractions might originate from a similar source. Analysis of the modern milk pot implied that an insoluble fraction was formed during cooking although the mode of incorporation of residual lipids is uncertain. Measurements using thermocouples on interior and exterior surfaces of identical pots during the boiling of liquids on an open fire [31], suggests that this relatively quick process (<2 h of heating) occurred at a temperature not greater than 200 °C. As much of the soluble lipid in this sample was present as intact TAGs, and therefore unable to form strong interactions with the ceramic matrix, degraded DAGs, MAGs and free fatty acids must preferentially adsorb or covalently-bind to the residue or ceramic surface. Contrary to the findings of Regert [13], fatty acid oxidation products such as hydroxy-fatty acids, were not detected in the solvent extracts nor in the saponified extracts of any of the samples analysed. The absence of these species suggests that either they not formed during cooking or, in the case of the archaeological sherds, they were weakly adsorbed and lost from the sherds during groundwater leaching.

The nature of this insoluble organic material was investigated further by performing hydropyrolysis on the residue remaining after solvent extraction. Although accurate quantitation was not performed on hydropyrolysis products due to a significant loss of volatiles ($\leq C_{18}$) hydrocarbons during product trapping, considerable amounts (>100 $\mu g\, g^{-1}$ ceramic) of pyrolysis products were recovered for GC and compound-specific $\delta^{13}C$ analyses. A new trap design has since been formulated and constructed for future applications that can efficiently recover milligram and sub-milligram quantities of pyrolysate (Snape, pers. commun.). The recovered products here were assumed to be sufficiently representative, structurally and isotopically, of the total hydropyrolysate and consisted mainly of straight chain *n*-alkanes (Fig. 4) at similar relative abundance to their fatty acid precursors obtained by solvent extraction and saponification. It can be noted that only minor levels of decarboxylation or alkyl chain cracking must occur during HyPy treatment and this explains why a distinct even-over-odd predominance (EOP) of carbon chain lengths was preserved in the hydrocarbon products and that the ratio of $C_{16:0}$ and $C_{18:0}$ species was broadly maintained (Figs. 3 and 4). Notably though 9-octadecene the product anticipated from pyrolysis of 9-octadecenoic acid (oleic acid) which was present at significant levels in the solvent and saponified extracts of the modern milk pot, was present in only trace levels. The increased relative abundance of octadecane in this pyrolysate, demonstrates that hydrogenation of the alkyl chain of unsaturated fatty acids occurred during the pyrolysis process (Fig. 4a).

As a check, both stearic ($C_{18:0}$) and oleic acid ($C_{18:1}$) model compounds, adsorbed on a silica gel support, were separately subjected to catalytic hydropyrolysis treatment in the same session of experiments as the archaeological sherds. Fig. 5 shows the total product distributions obtained as determined by GC–FID analyses. It can be seen that *n*-octadecane was by far the dominant product generated for both model compounds, although smaller amounts of shorter-chain hydrocarbons were formed as by-products for the unsaturated acid, oleic acid, by a β-cleavage process. So reductive removal of oxygen atoms without appreciable cleavage of any constituent carbon atoms is the favoured process operating

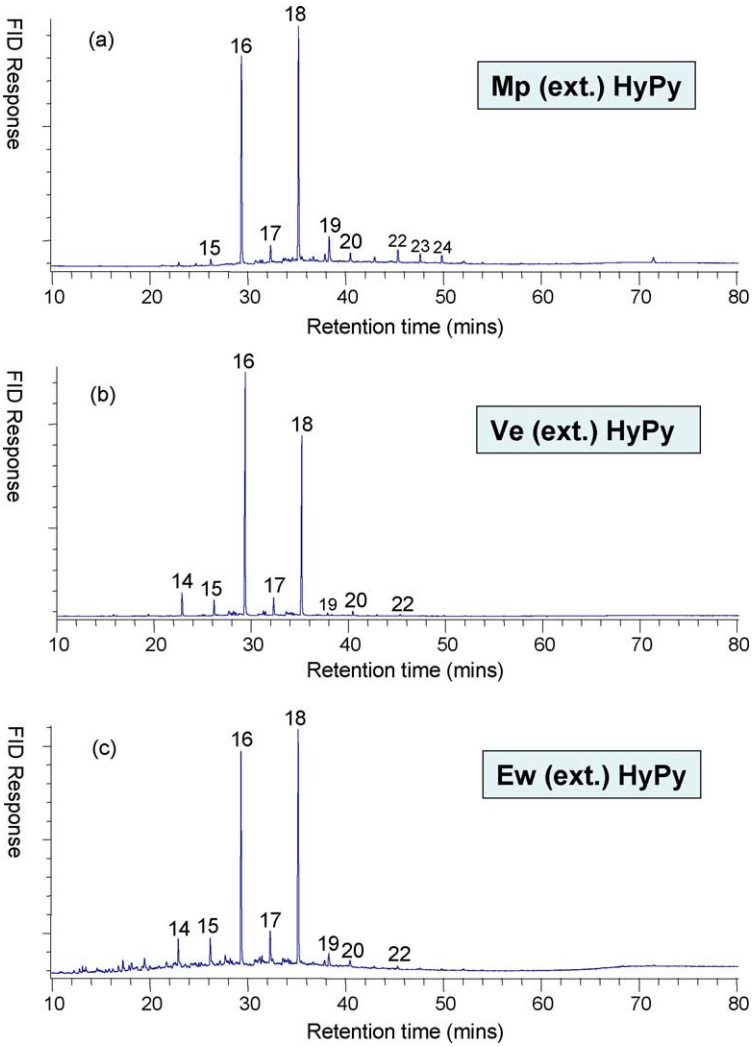

Fig. 4. GC–FID traces showing the total hydrocarbon product distributions generated from catalytic hydropyrolysis of solvent-extracted sherd residues. Numbers refer to alkyl chain lengths of *n*-alkane products.

when hydropyrolysis is applied to free or bound fatty acids, as opposed to decarboxylation reactions. This means that all the carbon isotopic information is largely preserved in the hydrocarbon skeletons produced from hydropyrolysis.

Significantly, a complete series of *n*-alkane (and *n*-alk-1-enes) doublets, usually comprising a smooth modal or biomodal carbon number distribution and extending over a considerable carbon number range (e.g. from C_{12} up to C_{35} and higher), previously observed from Curie-point and hydropyrolysis of preserved aliphatic biopolymers [25,34,35] were absent. In contrast, the carbon number distribution of liberated products are indicative

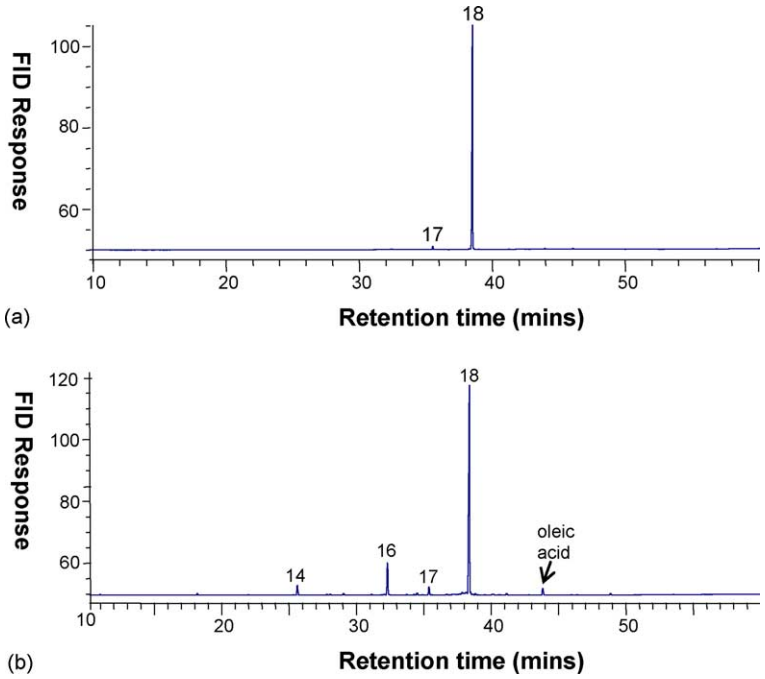

Fig. 5. GC–FID traces showing the total product distributions obtained from catalytic hydropyrolysis of fatty acid model compounds: (a) stearic acid and (b) oleic acid, which had been pre-adsorbed on silica gel. Numbers refer to alkyl chain lengths of *n*-alkane products.

of these being derived predominantly from discrete bound C_{16} and C_{18} acids/alcohols and not characteristic of molecular components of highly cross-linked macromolecular organic matter. These sequestered C_{16} and C_{18} acids and/or alcohols were most probably ester- or ether-bound to insoluble polymeric organic material tightly associated with the ceramic surface and/or linked to the ceramic surface itself, possibly involving strong interactions between the organic functional group (e.g. carboxylate) in the lipids with metal cations [36,37].

3.3. 'Non-extractable, non-saponifiable' lipid content

Hydropyrolysis was also used to characterise any residual 'non-extractable', 'non-saponifiable' lipid content remaining on the pottery after extraction and then saponification. Small but significant amounts of cleaved aliphatic hydrocarbons ($>10\,\mu g\,g^{-1}$ ceramic) were released from the extracted/saponified archaeological samples while only extremely low amounts of aliphatics ($<1\,\mu g\,g^{-1}$ ceramic) were recovered from the extracted/saponified modern milk pot. The aliphatic fractions, in each case, were dominated by straight chain alkanes (Fig. 6), again with a similar distribution to the fatty acids present in the solvent and saponified extracts (Fig. 3) and the alkanes in the previous hydropyrolysates (Fig. 4).

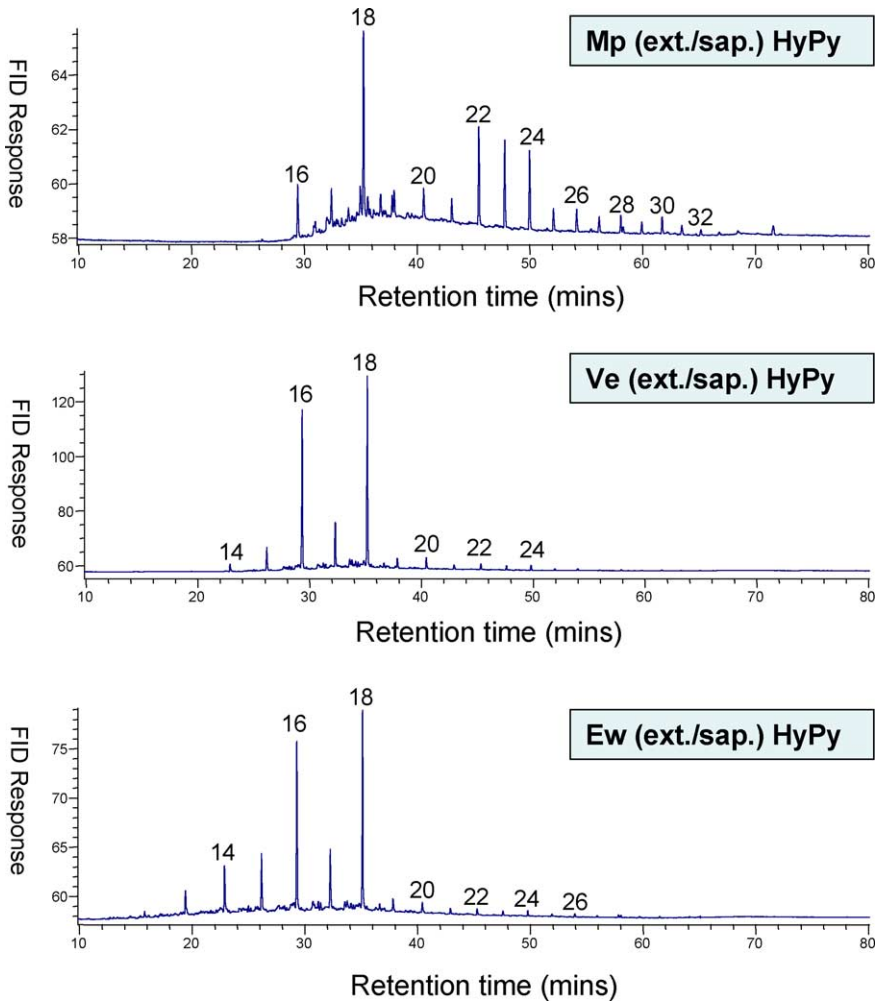

Fig. 6. GC–FID traces displaying the total aliphatic hydrocarbon product distributions generated from catalytic hydropyrolysis of pre-extracted and pre-saponified sherd material. Numbers refer to carbon chain lengths of *n*-alkane products.

This material is most likely derived from intact fatty acid/alcohol precursors which were tightly incorporated within a large macromolecular organic structure, and thus inaccessible to reaction by alkali hydrolysis due to steric hindrance effects.

In addition to the usual C_{14}–C_{18} *n*-alkane signal exhibiting a distinct even-over-odd carbon chain length predominance, smaller relative amounts of *n*-alkanes with longer alkyl chain lengths between 14 and 32 carbon atoms displaying a slight even-over-odd carbon chain length predominance can also be detected in hydropyrolysates of pre-extracted and saponified pot sherds (Fig. 6). This feature is not an artefact of the hydropyrolysis procedure

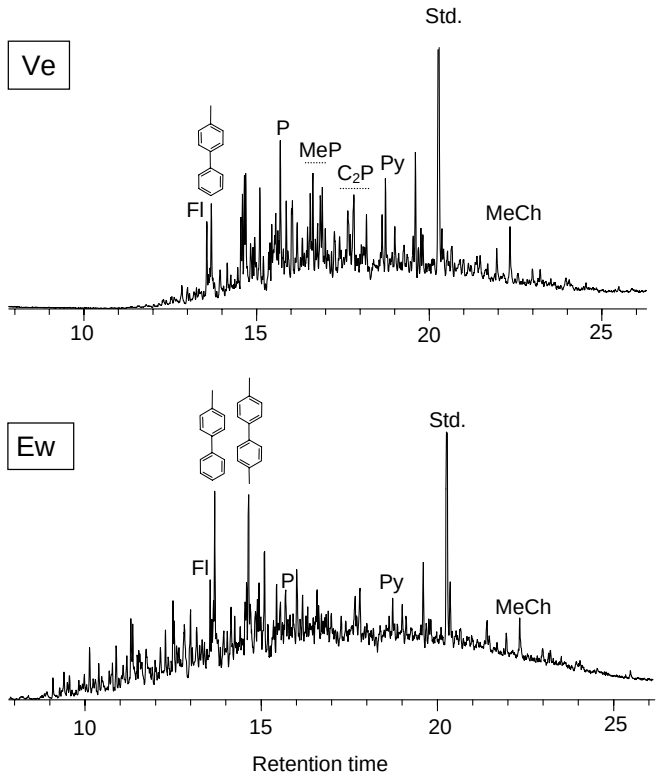

Fig. 7. Total ion chromatograms (TICs) showing aromatic hydrocarbon distributions generated from catalytic hydropyrolysis of pre-extracted and pre-saponified sherd material. Key; Fl: fluorene; P: phenanthrene; MeP: methylphenanthrenes; C_2P: C_2-alkylated phenanthrenes; Py: pyrene; MeCh: methylchrysene.

since no alkyl chain lengths greater than *n*-C_{18} were produced from hydropyrolysis of either stearic or oleic acid model compounds. These longer-chain *n*-alkanes (>C_{18}) have not been observed before in pyrolysates of archaeological potsherds or experimental chars and are more typical of molecular signals produced from pyrolysis of aliphatic biopolymers or immature geo-polymers [35]. Their origin is uncertain but the presence in the modern milk pot implies they can be formed rapidly during cooking (and charring) events, as previously suggested [17], as well from diagenetic reactions occurring in the geo-sphere after burial. The ceramic surface of the pot itself may play an active catalytic role in promoting the formation of macromolecular organic phases which can lead to extensions in original alkyl chain lengths due to secondary reactions.

A complex array of polynuclear aromatic compounds (two- to five-ring PAH) containing a variety of alkylation patterns (which largely elute as an unresolved complex mixture in gas chromatograms), were also released by hydrogen pyrolysis from the archaeological samples (Fig. 7, and see discussion below), but these were only minor components of products released from the modern pot. These complex PAH species constitute products derived from

the thermal transformation of protein, carbohydrate and lipid parent biochemicals, and a wide range of PAH and polar compounds have been detected previously in archaeological and experimentally-produced chars [17,25,38,39]. The low abundances of PAH compounds in the modern pot is, most likely, because this sample had not been exposed to prolonged use.

3.4. Evidence for a recalcitrant and highly aromatic macromolecular residual organic phase in archaeological pot interiors

The residual carbon contents of the powdered sherds were determined (Table 2) after being subjected to the complete sequential degradation treatment involving solvent extraction, alkaline saponification and, then finally, catalytic hydropyrolysis. The carbon values quoted are taken to accurately quantify the organic carbon concentrations since the inorganic carbonate content of these pots was negligible. It can be seen that the residual carbon contents are significant for the two archaeological samples studied (>50 wt.% of the total carbon content remained after sequential degradation), being extremely high in the case of the Vendel pot (>90 wt.%) but much lower for the Milk pot (ca. 6 wt.% of initial carbon remains). It would appear then that the combination of repeated use of vessels and/or diagenesis of organic residues in the geo-sphere can lead to the gradual formation of resistant polymeric residue over time. A further contribution may come from accumulation and cross-linking of PAH from smoke particles resulting from cooking on an open fire [17], although it should be noted that only inner pot surfaces were used in this study, so this explanation seems less likely in this case. Table 2 indicates then that, quantitatively, the bulk of the total organic matter content of the two archaeological pots is present predominantly in the form of a recalcitrant residue; most likely an aromatic-rich macromolecular phase, often termed as "char" [17]. Direct evidence for the presence of a recalcitrant aromatic-rich residue comes from the detection of significant contributions of polynuclear aromatic hydrocarbons (PAHs) compounds in the hydropyrolysates obtained from pre-extracted and pre-saponified archaeological samples. The PAH compounds in hydropyrolysates consist predominantly of a complex distribution of two- to five-ring PAH molecular species (Fig. 7), resulting from a low partial conversion of the recalcitrant phase into soluble aromatic hydrocarbon fragments, as revealed by GC–MS analyses.

Table 2
Organic carbon contents of the different vessels, before and after the extraction procedures were applied (as determined by elemental analysis)

Sample	Description	Carbon content (wt.%)	Percentage recalcitrant carbon
Modern milk pot (Mp)	Untreated	6.28	
	Hydropyrolysis residue[a]	0.39	6.2
Vendel pot (Ve)	Untreated	6.50	
	Hydropyrolysis residue[a]	6.10	93.8
Easingwold pot (Ew)	Untreated	3.30	
	Hydropyrolysis residue[a]	1.95	59.1

[a] After solvent-extraction, saponification and catalytic hydropyrolysis sequential treatment.

Solid-state ^{13}C NMR experiments, using a cross polarisation pulse sequence and with magic angle spinning of samples, were performed on the hydropyrolysis residues of the Ew and Ve archaeological pots to try and directly characterise the bulk carbon chemical structure of the recalcitrant polymeric organic phase. Unfortunately, no significant ^{13}C NMR signal could be obtained on either of these residues even after 20,000 scans had been accumulated. This is likely to be due to the presence of inorganic and/or (more likely) organic radicals causing the NMR probe to detune after only a few scans and also due to protons having very short rotating frame relaxation times (^1H $T_1\rho$) such that effective polarisation transfer from proton to carbon spins could not be achieved [40]. If the residual organic material contains significant *n*-alkyl carbon chains then this aliphatic signal should have been easily detectable using CP/MAS sequences. This was not the case. Conversely, it is known that highly aromatic char material usually contains high concentrations of paramagnetic organic species which can often result in low carbon spin counts being recorded from NMR experiments, especially when CP pulse sequences are employed [40]. So, this was further indirect evidence that the recalcitrant organic material remaining after the complete sequential degradation procedure is likely to consist of a highly condensed aromatic polymer.

The co-existence of preserved aliphatic fatty acids and a thermally-stable aromatic macromolecular phase within the same archaeological pots suggests that the preserved lipid components were introduced into the vessel after the bulk of the char phase had formed and so fatty acid analyses most probably provide information concerning the later uses of archaeological vessels. To our knowledge, no other reports of significant levels of residual carbon in the interior of cooking vessels, in the form of an aromatic polymeric phase, have been published previously. This most probably reflects the fact that results of elemental analysis have rarely been reported in previous archaeological studies to monitor and quantify levels of residual organic carbon persisting on sherds after these have been subjected to solvent extraction, chemical or thermal treatment in the laboratory.

3.5. *Stable carbon ($\delta^{13}C$) isotopic analysis of free and bound lipids*

The stable carbon isotope ($\delta^{13}C$) values of the *n*-hexadecanoic acid (16:0) and *n*-octadecanoic acid (18:0) released by solvent extraction and saponification were measured, after a correction had been made for the derivatisation process (methylation), and compared (Fig. 8). The isotopic signatures measured for the analogous products from hydropyrolysis experiments, here released as *n*-hexadecane and *n*-octadecane, are also plotted for comparison (Fig. 8). A similar approach has been used previously to distinguish different modern reference adipose fats (e.g. equine, porcine and ruminant) and ruminant milk fats [11]. The sources of these *n*-alkanoic acids can be broadly determined by calculating the offset (Δ) between the individual carbon isotope values of each acid, so that $\Delta = \delta^{13}C_{16:0} - \delta^{13}C_{18:0}$. Ruminant fats can be distinguished from non-ruminant fats as the *n*-octadecanoic acid is partially derived directly from the diet, and hence ^{13}C-depleted in comparison with the *n*-hexadecanoic acid which is predominantly produced de novo from acetate. During lactation, the routing of *n*-octadecanoic acid directly from dietary sources is even more enhanced [11,41,42]. Analysis of a range of reference fats obtained from animals fed on different diets, have shown that the value of Δ is typically 0% for non-ruminant

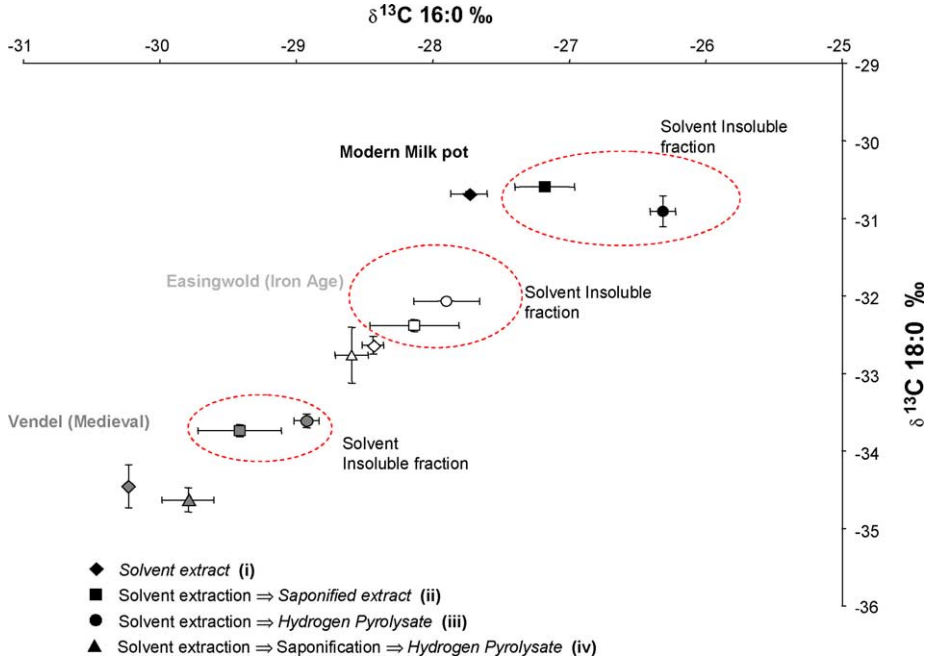

Fig. 8. Plot of the stable carbon isotope values of single compounds (hexadecanoic acid/hexadecane (16:0) and octadecanoic acid/octadecane (18:0)) obtained by different analytical methods applied to the three pots. Roman numerals refer to the stages of the analytical procedure schematically outlined in Fig. 1 (the modern milk pot yielded insufficient compounds for isotopic analysis by hydrogen pyrolysis following saponification). Error bars indicate the machine precision. Ellipses indicate the solvent insoluble fractions. Measurements made on the modern milk pot (Mp) were corrected for contamination with post-industrial carbon [43].

fats, between 0 and 3% for ruminant fats and >3% for ruminant dairy fats [11,42], with the absolute values of the carbon isotopic signatures measured dependent on the animal's diet.

The average value of for solvent extracts of the Ve and Ew samples were 4.52 (S.D., 0.29) and 4.20 (S.D., 0.40) respectfully, implying that these pots had, at least, a significant ruminant dairy input. Similar values of Δ were obtained for the hydrolysis and hydropyrolysis products. The inference of a ruminant origin of the lipid in these sherds is also supported by the presence of odd numbered, branched chain acids as well as positional isomers of octadecenoic acid in solvent extracts [32]. The absence of low molecular weight triacylglycerides and corresponding short chain fatty acids, which characterise milk fat [33], is a result of their increased susceptibility to hydrolysis and loss by leaching and is consistent with previous observations made on laboratory-degraded milk fats [11].

Fig. 8 shows that the differences in $\delta^{13}C$ signatures for C_{16} and C_{18} aliphatic acids/hydrocarbons generated by the different analytical treatments for any particular sample were generally within ca. 1% of each other. For both the archaeological pots, it appears that the bound lipid components of the solvent-insoluble organic fractions were slightly ^{13}C-enriched in comparison to the extractable fatty acid lipids. This stable carbon isotopic pattern mirrors

that generally found for free and bound lipid components, derived from a common biogenic origin, in the soluble and insoluble organic phases of sedimentary organic matter. The larger spread in carbon isotope signals for lipids generated from the modern milk pot substrate by the different analytical procedures probably relates to the fact that the amounts of bound lipid products recovered by hydrolysis and hydropyrolysis were low, and so the limits of accuracy in $\delta^{13}C$ values obtained for these bound components was poorer than average (probably accurate within ±0.8%, as opposed to typically ±0.5%). A small amount of the observed isotope variation may be explained by conversion of the abundant $C_{18:1}$ component to the $C_{18:0}$ alkane during hydrogen pyrolysis of the modern milk pot, such as difference is not evident in the archaeological samples, which are dominated by saturated species. Considering that reference fats from similar sources (e.g. bovine milk) can show a standard deviation in stable carbon isotope signatures greater than 1.0% [11], we believe that the variation in the $\delta^{13}C$ values between free, hydrolysable and strongly-bound lipid components observed in this study is not significant within analytical limits of accuracy so and we choose not to risk over-interpreting trends observed in the isotope dataset. It is also apparent for the archaeological samples that the value for Δ, indicating the source of lipid residue, remains constant regardless of the treatment used, i.e. solvent extraction, alkaline hydrolysis or catalytic hydropyrolysis. So, it appears then that there is no conclusive evidence for significant bias in stable carbon isotopic signatures for the dominant free and bound molecular aliphatic components of the archaeological ceramic vessels used in this study.

3.6. Summary

Open-system catalytic hydropyrolysis (performed at 15 MPa H_2 pressure) provides a rapid means to recover covalently-bound molecular lipid species, predominantly in hydrocarbon form with excellent preservation of molecular features, from archaeological ceramic materials which are not amenable to conventional solvent extraction and alkaline hydrolysis treatments. Detailed molecular and isotopic analyses of products revealed however that no significant lipid signal bias, in terms of both carbon number distributions of compounds or stable carbon isotopic ($\delta^{13}C$) signatures, could be detected in this study for the dominant free and bound molecular aliphatic components of the two archaeological ceramic vessels used in this study. This is reassuring for archaeological scientists since analyses of molecular constituents of conventional solvent extracts and hydrolysis products do appear to give accurate insights into the total lipid distributions present in archaeological vessels and can generate representative $\delta^{13}C$ values for the principal lipid components. A significant amount of the total organic carbon in the archaeological sherds obtained from vessel interiors (>50 wt.%) was present in the form of a recalcitrant highly-aromatic polymeric phase. The most likely origin of this aromatic organic material was from polymerisation of foodstuff residues through repeated use of the vessels and from diagenetic structural transformation of food residues occurring after burial. Preserved fatty acid components were most probably derived from foodstuffs, largely ruminant dairy produce, that were introduced into the vessel after the formation of the bulk of the aromatic residue and so fatty acid analyses may give insights into the later uses of archaeological pots.

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