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Case report

Identification of a disinterred grave by molecular and stable isotope analysis

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ABSTRACT

Confirmation of a potential disinterred grave was sought by GC and GC/MS analyses of lipid extracts of whole soils and white particulate matter. Fatty acid profiles and concentrations determined for three of the soils correlated with the deposition of a large amount of exogenous organic matter, most likely adipocere and/or decomposed body fat. Determination of $C_{16:0}$ and $C_{18:0}$ fatty acid $\delta^{13}C$ values by GC/C/IRMS revealed the input to be isotopically distinct from common British domesticated animals, plotting closely to values determined for adipose fat obtained from of a murder victim. By considering the difference between $\delta^{13}C$ values ($\Delta^{13}C_{18:0-16:0}$) a potential isotopic proxy for identifying the source of adipocere (human) and adipose tissue was proposed.

1. Introduction

Following death, in the absence of any impeding environmental factors, a cadaver will begin to decay after approximately 4 min [1]. Fuelled by the nutrient rich fluids released by autolysis, microorganisms (bacteria, fungi and protozoa), derived largely from the intestinal tract, proceed to consume the soft tissue of the cadaver giving rise to the process known as putrefaction. One of the major processes known to occur during this period is the decomposition of adipose tissue. At 60–85%, lipids constitute the largest proportion of adipose tissue and between 90 and 99% of this lipid fraction comprises triacylglycerols [2–4]. Triacylglycerol decomposition is mediated by a suite of tissue lipases and proceeds via hydrolytic cleavage of ester bonds to yield a mixture of free fatty acids (as their sodium and potassium salts) dominated by oleic acid and, to a lesser extent, linoleic, palmitoleic and palmitic acid [4,5]. When left in a suitable environment (e.g. buried, submerged) the sodium and potassium ions may be displaced by calcium and magnesium ions thereby forming the insoluble, white, greasy substance commonly referred to as adipocere; this is the saponifcation theory of adipocere formation [5,6]. As early as 1875, Ebert recognized that adipocere also contained a hydroxy-fatty acid which subsequent studies have shown to be 10-hydroxyoctadecanoic acid, a microbially mediated hydration product of the oleic acid that is the major component of human adipose fat [5,7–15].

A large number of factors influence the formation of adipocere following the onset of decomposition, these include: pre-mortem body conditions, the cause of death, the time elapsed between death and burial, the method of burial, environmental conditions of the burial site and also any anthropogenic effects wrought on the site. A comprehensive review of the factors that influence the formation of adipocere has been presented by Fiedler and Graw [16]. Of particular importance to this study is the depositional environment and whether it was conducive to adipocere formation. A high level of moisture and anoxic conditions are two factors that have been shown repeatedly to favour adipocere production, hence significant deposits are often found associated with submerged bodies [17–19]. Extensive formation of adipocere in warm, tropical environments has been reported to occur after a burial period of less than 4 days [20]. In another study, Simonsen reported formation of adipocere within 22 days in a body, rapid for a temperate climate where adipocere is known to usually take between 3 and 6 months to develop; this anomalous discovery was ascribed to the extraordinarily hot summer of 1975 [21]. The effects of burial method and environment have been revisited recently by Forbes and others who concluded that adipocere formation is promoted by a mildly alkaline pH, warm temperatures, anaerobic conditions, a sand/silty sand soil type and, of particular relevance to this study, the direct burial of a body in soil (as opposed to a coffin or plastic coverings) [22–24]. To date there have been relatively few studies concerned with the characterisation of soils amended with an input of adipose tissue/adipocere. Fiedler and others conducted soil morphological analyses as well as physical, chemical and microbiological assays of grave soils obtained from a cemetery in the Central Black Forest [25]. Whilst the associated corpses had been preserved through the formation of adipocere the results revealed that

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phosphorous, dissolved organic carbon and cadaverine had all leeched from the graves into the surrounding soil. Interestingly, microbial activity and biomass were observed to be higher in the control soil, this was ascribed to the inert character of adipocere. Indeed, under near anoxic to anoxic conditions degraded adipose tissue may exist indefinitely as adipocere [26–29] requiring the presence of gram-positive bacteria and oxygen for further degradation and eventual mineralization (complete conversion of organic matter to simple substrates such as H_2O, CO_2, NO_3^- etc.) to occur [30].

This study was instigated to assess soils suspected of having comprised a temporary grave being inspected as part of a missing persons investigation. White particulate matter, intimately mixed with some of the soil, had been morphologically identified as adipocere and mtDNA analysis conducted on a sample of the particulate matter had established a maternal link to a relative of the missing person (Metropolitan Police Service, personal communication). Whilst this was a highly specific result qualitatively it still did not establish, unambiguously, the nature of the matter and whether it was present in the soil *at levels consistent with there having been a body or body parts buried there*. This was achieved by applying a range of elemental and molecular analyses, including GC and GC/MS of lipid extracts, to detect high concentrations of adipocere derived lipid that were distinct from the chemical background of soil. In an additional, more speculative aspect to the study, compound-specific stable isotope analyses were undertaken, using GC/C/IRMS, to investigate the potential of this technique for determining the source of any adipocere residues found.

2. Experimental method

2.1. Soils and reference materials

Soils and putative exogenous inputs observed in the soils were collected from the crime scene and provided for analysis by the Metropolitan Police Force (Table 1). A section of adipose tissue, excised from the torso of the victim, was later provided; this was sub-sampled a total of three times. Additional adipose fats sampled from eight recently deceased humans were supplied by the University of Tennessee Forensic Anthropology Center (University of Tennesseee, Knoxville, TN, USA) for further reference purposes.

2.2. Isolation and pre-treatment of lipid compounds

Prior to extraction all soils were ground (<1 mm) using a mortar and pestle. Randomly selected samples (~2–6 g) of each soil were then placed in pre-extracted cellulose thimbles that were each inserted into a Soxhlet apparatus and extracted for 24 h using a dichloromethane/acetone solvent system (9:1, v/v, 200 ml) [31]. Solvent was removed from soil extracts under reduced pressure, using a rotary evaporator, to yield a total lipid extract (TLE). Lipids from individual white particles isolated from the soils, adipose fat from the suspected victim and the reference fats obtained from the USA were extracted by ultrasonication for 10 min using a chloroform/methanol solvent system (2:1, v/v, 5 ml) [32]. Solvent was removed by evaporation under a gentle stream of nitrogen to yield TLEs.

In order to quantify lipid components extracted from the soil, a known mass of an internal standard (*n*-nonadecanoic acid) was added to an aliquot of each soil TLE. Each aliquot was then saponified for 60 min using NaOH dissolved in methanol/double distilled water (9:1 v/v, 0.5 M, 2 ml, 70 °C). Saponified TLEs were extracted into diethylether and excess solvent removed under a gentle stream of nitrogen. Prior to analysis TLE residues were heated with a BF_3–MeOH complex (14% w/v) [33] to methylate any carboxylic acid functional groups. The double bond positions of monounsaturated components were determined after Scribe and others [34]. Since soil extracts are also likely to contain a large quantity of hydroxylated lipids the saponified and methylated TLEs were also heated for 30 min with *N*,*O*-bis(trimethylsilyl)trifluroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS; 30 µl, 60 °C) to ensure trimethylsilylation of any hydroxyl functionalities. Excess derivatizing agent was removed under a gentle stream on nitrogen. Derivatized samples were redissolved into hexane prior to analysis.

2.3. Instrument analysis

Elemental analyses were performed on a Carlo Erba CHN EA1108 elemental analyzer.

Derivatized fractions were analyzed using a Hewlett-Packard 5890 series II gas chromatograph (GC) equipped with a fused-silica capillary column (Varian Chrompack CPSil-5CB, 50 m length × 0.32 mm i.d., film thickness 0.12 µm). Samples (dissolved in hexane) were injected (1.0 µl) on-column. The temperature was programmed from 40 °C (2 min isothermal) to 300 °C at a rate of 10 °C min^{-1} (10 min isothermal). The flame ionisation detector (FID) temperature was held at 300 °C. Hydrogen was used as carrier gas (10 psi head pressure).

Gas chromatography/mass spectrometry (GC/MS) was performed using a ThermoFinnigan TraceMS equipped with a fused silica capillary column (Phenomenex, ZB1; 60 m length × 0.32 mm i.d., film thickness 0.1 µm). Samples were introduced using a programmable temperature vaporising (PTV) injector with a transfer line maintained at a temperature of 300 °C. The source temperature was held at 200 °C and ionisation energy was set at 70 eV with the quadrupole analyser scanning the range m/z 50–650 with a cycle time of 0.6 s. The temperature program used was: 40 °C (2 min isothermal) to 300 °C at 10 °C min^{-1} (10 min isothermal). Helium was employed as carrier gas with a constant flow of 2 ml min^{-1}.

GC/C/IRMS analyses were performed on a Varian 3400 GC, equipped with a fused silica capillary column (Varian Chrompack, Factor Four VF-23ms; 60 m length × 0.32 mm i.d., film thickness 0.15 µm). The temperature program used was: 40 °C (2 min isothermal) to 240 °C at 10 °C min^{-1} (16 min isothermal). Helium was employed as carrier gas with a constant pressure of 10 psi. The column was connected via an extensively modified type I Finnigan MAT combustion interface to a Finnigan MAT Delta S; the combustion reactor contained CuO/Pt and was set to a temperature of 850 °C. $\delta^{13}C$ values were calibrated against a CO_2 reference of known isotopic composition (previously calibrated against five independent standards of known isotopic composition relative to vPDB) introduced directly into the source three times at the beginning and end of every run. All samples were run in duplicate and the average of the two values taken. Where compounds of interest in the same sample occurred at very different concentrations samples were reanalysed at an appropriate dilution to ensure a ~2000–5000 mV response. $\delta^{13}C$ values were corrected for exogenous carbon added by the derivatization of compounds for GC analysis [35]. The precision of the method was determined using the $\delta^{13}C$ values obtained for the $C_{16:0}$ FAME component of soil IJH8 from 10 sequential analyses. The standard

Table 1
Summary of the soils (and associated particulate matter) obtained from the scene of crime

Sample name	Description
A	Upper top soil taken from the suspect grave
B	Lower top soil taken from the suspect grave at the perceived limit of adipocere deposition
C	White residue mixed with soil taken from the suspect grave
D	Sample of soil taken from the spoil heap associated with the excavation of the suspect grave
E	White, greasy, particulate matter mixed with soil taken from the suspect grave
F	White debris with hair attached taken from the suspect grave
G	Top soil taken from an undisturbed part of the scene of crime away from the suspect grave to be used as a control

deviation obtained for these analyses was 0.09‰. When combined with the error associated with the methyl group added during derivatization (0.10‰, determined by EA-IRMS) the overall error associated with precision was calculated to be ±0.13‰. Since this was lower than the manufacturer's specified precision for the GC/C/IRMS instrument (±0.3‰) all measurements have been quoted with a precision of ±0.3‰.

3. Results

3.1. Elemental analysis

Sub-samples were taken of white particulate matter that could be clearly identified by eye in two of the soils (C and E) and from an additional sample recovered from the suspected grave where similar white debris attached to hair was identified (F). Fig. 1 summarises the data obtained from elemental (C,H and N) analysis of these particles. The data are presented as average ($n = 5$) percentage compositions for each of the three sub-samples. The %C values obtained for sub-samples taken from C and F are very similar (12.3 and 14.5%, respectively) whilst that obtained from the sub-sample of soil E is markedly higher (41.7%). The C:N ratios calculated for samples C and F are relatively close to each other (137 and 220, respectively) compared with that obtained for E (1450).

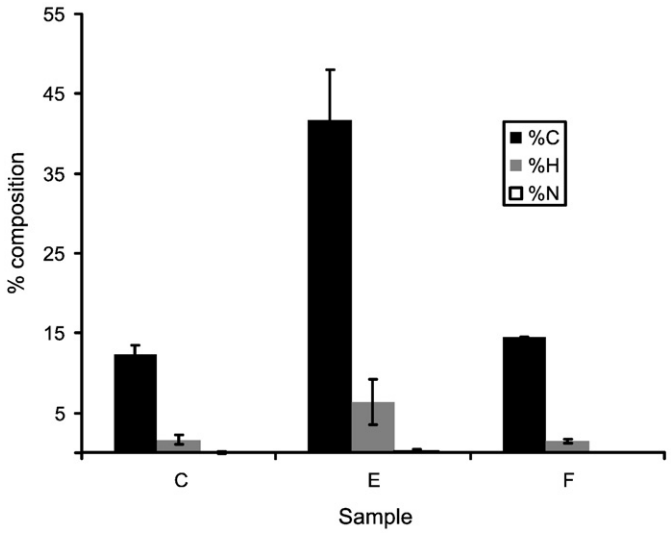

Fig. 1. Histogram displaying the percentage composition (w/w) of C, H and N in the white particles collected from C, E and F.

3.2. Total lipid extracts

A distributional and quantitative assessment of the lipid content of the soils provides the ideal means for determining any large inputs of exogenous organic matter such as that which might have arisen from the decay of a buried body. The hydrolysed lipid extracts from a wider range of soils, obtained in and around the putative grave, were analysed by GC and GC/MS. In each case the data obtained falls into one of two discrete categories. The first, represented by soils A, C and E (Fig. 2a, c and e), is characterized predominantly by a narrow distribution of lipids largely comprising saturated fatty acids in the C_{14} to C_{18} carbon number range. Additionally, C_{16} and C_{18} monounsaturated analogues are observed as are diunsaturated, hydroxy and keto analogues of the C_{18} fatty acid. Interestingly, the distribution derived from E is dominated by a $C_{18:1\Delta9}$ fatty acid whilst those observed for A and C are dominated by palmitic acid, stearic acid, a $C_{18:0}$ monohydroxy fatty acid and a relatively lower proportion of the $C_{18:1\Delta9}$ component. Whilst, on close inspection, other compounds may be observed, their abundance is insignificant relative to the major fatty acid components. The concentration of solvent extractable lipid was calculated from gas chromatographic data and found to be 1.7, 2.1 and 17.1 mg $g^{-1}{}_{dry\ weight}$ for soils A, C and E, respectively. The second category is represented by soils B, D and G (Fig. 2b, d and f). In this case the observed distribution of lipids describes a wider and more diverse range. It is dominated by a series of *n*-alkanols (C_{22}–C_{34}) and fatty acids (C_{16}–C_{34}). In addition, there is a relatively high abundance of $C_{18:1\Delta9}$ and $C_{22:0}$ ω-hydroxy fatty acids in each soil. In this case the concentrations of total solvent extractable lipid were calculated to be 0.1, 0.5 and 0.5 mg $g^{-1}{}_{dryweight}$ for soils B, D and G, respectively. The stark contrast between these soils may be demonstrated more easily by calculating the summed concentration of the $C_{16:0}$ and $C_{18:0}$ fatty acid components in each soil (Fig. 3). The difference in concentration between the categories of soil for these two components is extreme with soils A, C and E ranging in concentration from ~1000 to 4200 μg $g^{-1}{}_{dry\ weight}$ whilst soils B, D and the control soil G all yield concentrations in the sub 20 μg $g^{-1}{}_{dry\ weight}$ range. At its most extreme this represents a ~500 fold difference in the concentration of the $C_{16:0}$ and $C_{18:0}$ fatty acid components between soil E and the control soil G.

GC/MS analysis of the hydrolysed TLE obtained from white particulate matter associated with sample E yielded a narrow distribution of only a few lipid components (Fig. 4a). The distribution comprises even chain length fatty acid homologues in the C_{14} to C_{18} range. Unsaturated, monohydroxy and keto analogues of the saturated C_{18} component are also observed to occur. Fig. 4b depicts the chromatogram generated by GC/MS analysis of the hydrolysed TLE derived from adipocere fat obtained from the remains of the victim. The range of compounds that occur is identical to that observed for the above white, particulate matter apart from the occurrence of a $C_{16:1\Delta9}$ fatty acid and a shift in the relative abundance of some components, principally an increase in the saturated and unsaturated C_{18} fatty acids and a concomitant decrease in the abundance of the C_{18} monohydroxylated fatty acid.

3.3. Compound-specific stable carbon isotope analysis

$\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids in each sample were obtained readily by GC/C/IRMS with full baseline resolution (Fig. 5). Fig. 6 depicts a cross-plot of the $\delta^{13}C$ values obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acid components derived from each of the soils (A, C and E) and an associated white particle that exhibited abnormally high concentrations of extractable lipid (sub-sampled from E). Furthermore, values obtained from the human adipocere reference fats (USA) and the three samples taken from the adipocere fat of the victim have been plotted. Additional confidence ellipses (1σ) have been included to enable further comparisons with the values usually obtained from the fat of common British domesticated animals to be made [36,37]. Values for the North American human adipocere reference fats plot over a wide range with the most depleted values plotting around −24‰ for both fatty acids and the least depleted values plotting at −16‰ for the $C_{16:0}$ component and −18‰ for the $C_{18:0}$ component. In direct contrast, the values obtained for the soils, white particle from E and the adipose fat taken from the remains of the victim all plot closely at around −28‰ for both the $C_{16:0}$ and $C_{18:0}$ fatty acids. Moreover, none of the values obtained plot within the confidence ellipses denoting the boundaries for values obtained from the fats of common domesticated animals, fed on a strict C_3-carbon only diet.

4. Discussion

4.1. Elemental composition

The results reveal a clear disparity between the compositions of those obtained from C and F and those obtained from E. The percentage C, H, N compositions observed for white, particulate matter from the former two samples are very similar although they are too low to be

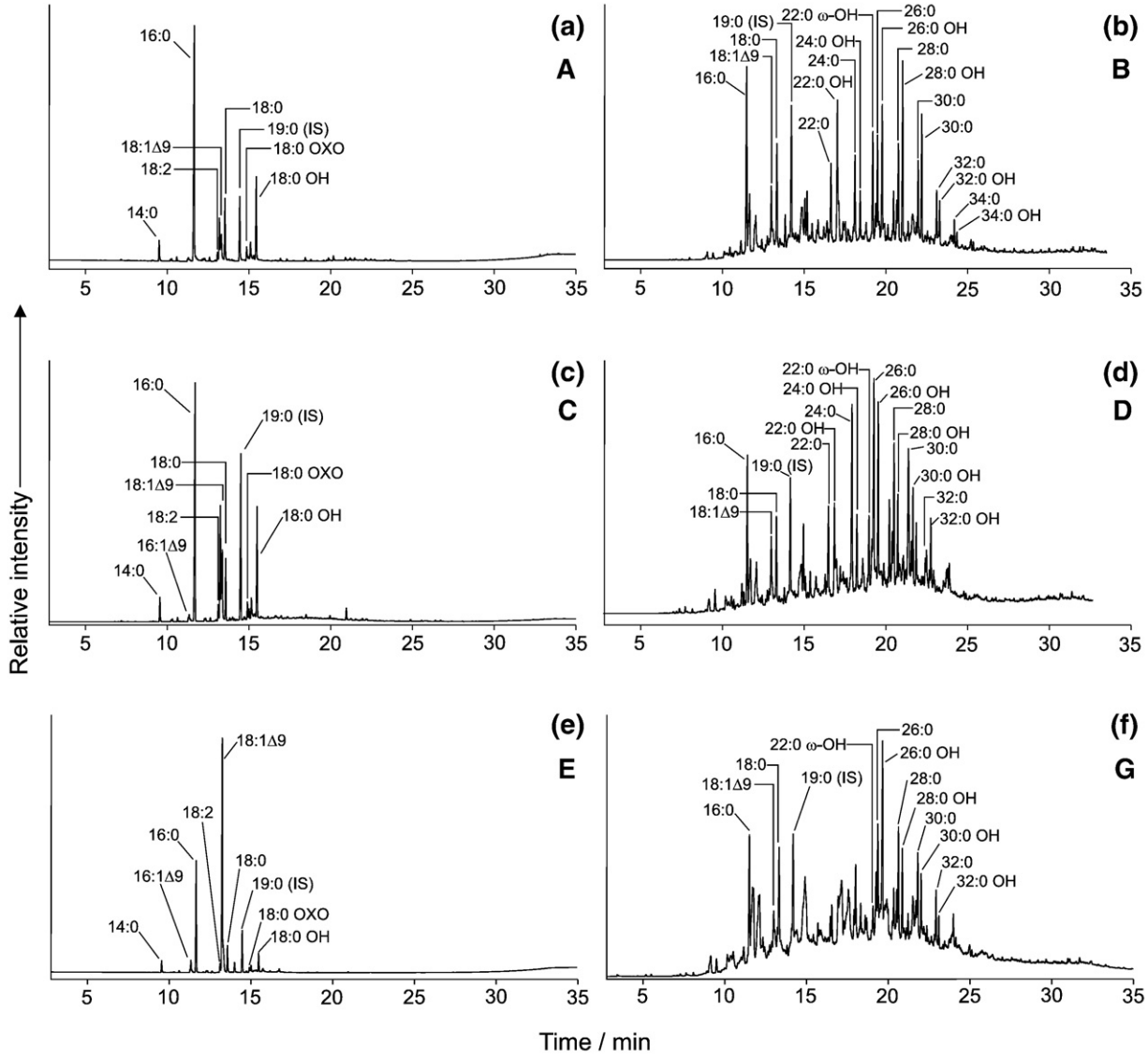

Fig. 2. Partial gas chromatograms (GC–FID) of fatty acids extracted from soils: (a) A, (b) B, (c) C, (d) D, (e) E and (f) G (control). Chromatographic peak identities are: **14:0** to **34:0**, saturated straight chain fatty acids with 14 to 34 carbon atoms; **16:1** and **18:1**, monounsaturated fatty acids with 16 and 18 carbon atoms, respectively; **18:2**, diunsaturated fatty acids with 18 carbon atoms; **18:0 OH** and **18:0 OXO**, hydroxy- and oxo-fatty acids, respectively, with 18 carbon atoms; **26:0 OH** to **32:0 OH**, long-chain alcohols with 26 to 32 carbon atoms, respectively; IS, internal standard, *n*-nonadecanoic acid.

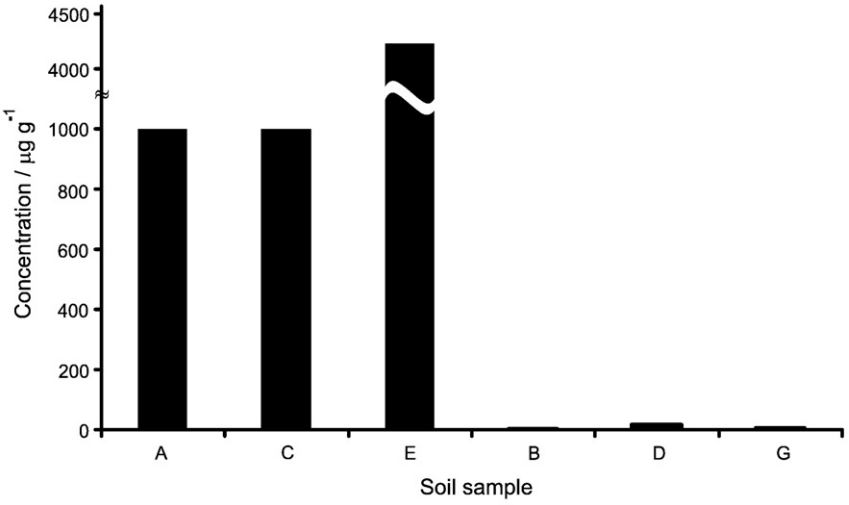

Fig. 3. Histogram showing the combined concentration of the $C_{16:0}$ and $C_{18:0}$ fatty acids present in each soil sample.

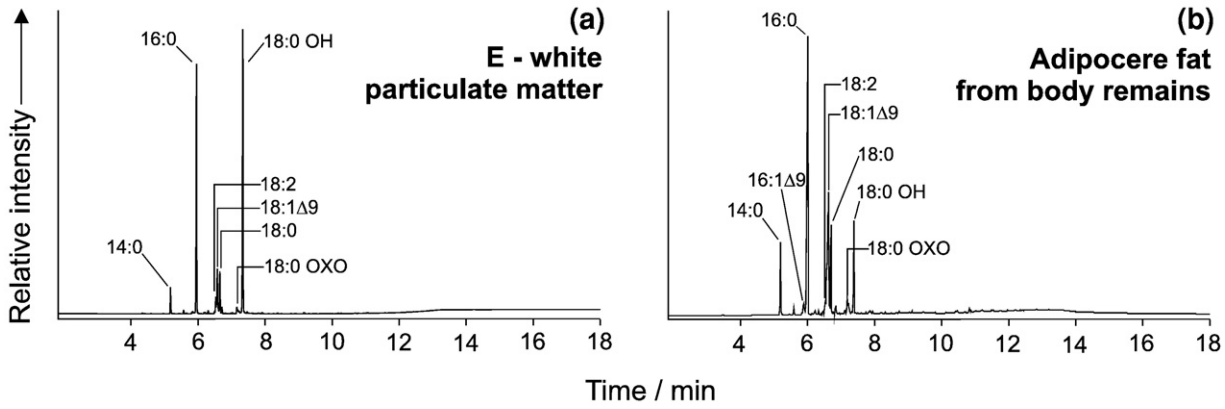

Fig. 4. Partial gas chromatograms (GC/MS) of fatty acids extracted from: (a) white particulate matter sub-sampled from E and (b) adipose fat from the recovered body remains of the victim.

considered characteristic of a source that is predominantly lipid, i.e. adipocere. Analogous compositions obtained for the particles derived from E (~42% C and ~6% H) are comparatively higher and are more consistent with a lipid origin; this is also mirrored in the substantially higher C:N ratio observed for E. Whilst the percentage carbon content of pure, human fat can be assumed to be ~77%, by considering the major lipid components of a pure adipocere wax, a lower content of ~68% may be estimated [3,8]. Although this is still high relative to the average value obtained for the particles associated with E, in terms of elemental composition they can be considered comparatively closer to that of adipocere than the other particles analysed. This conclusion is substantiated by two further pieces of observational evidence. Particles sub-sampled from E were soft and pliable and dissolved readily in organic solvent (chloroform/methanol, 2:1 v/v), after ultrasonication, to yield a clear solution (again consistent with a predominantly lipid composition). In complete contrast, particles sub-sampled from C and F were more durable requiring significant pressure to deform them. Furthermore, they were insoluble in both organic and aqueous solvents remaining as a fine, white suspension after ultrasonication. Combined with the elemental compositions these observations are more consistent with a predominantly non-lipid origin for particles sub-sampled from C and F, e.g. paint, although further analyses would be required to substantiate an exact origin.

4.2. Distributional and quantitative analysis of lipids

Whilst lipid distributions obtained for soils A and C are consistent with the lipid composition of adipocere, as determined by several previous studies, that obtained for soil E does not agree with a predominant input of this type [5,10,11,12,38]. The lipid distribution obtained from this latter soil does correlate fairly well with that observed for lipids derived from adipose tissue [6,22–24]. The high concentration of the $C_{18:1\Delta9}$ fatty acid, in particular, is very characteristic of fresh mammalian fat indicating a surprisingly high level of preservation of organic matter in this soil. By comparison, the extracts obtained from A and C appear to have been afforded a lower level of preservation with the presence of relatively high concentrations of a monohydroxylated $C_{18:0}$ fatty acid presumably having arisen from the oxidation of the corresponding $C_{18:1\Delta9}$ component generated during the transformation of adipose tissue to adipocere by bacteria [5,10–15,38]. However, it should be noted that the concentration of

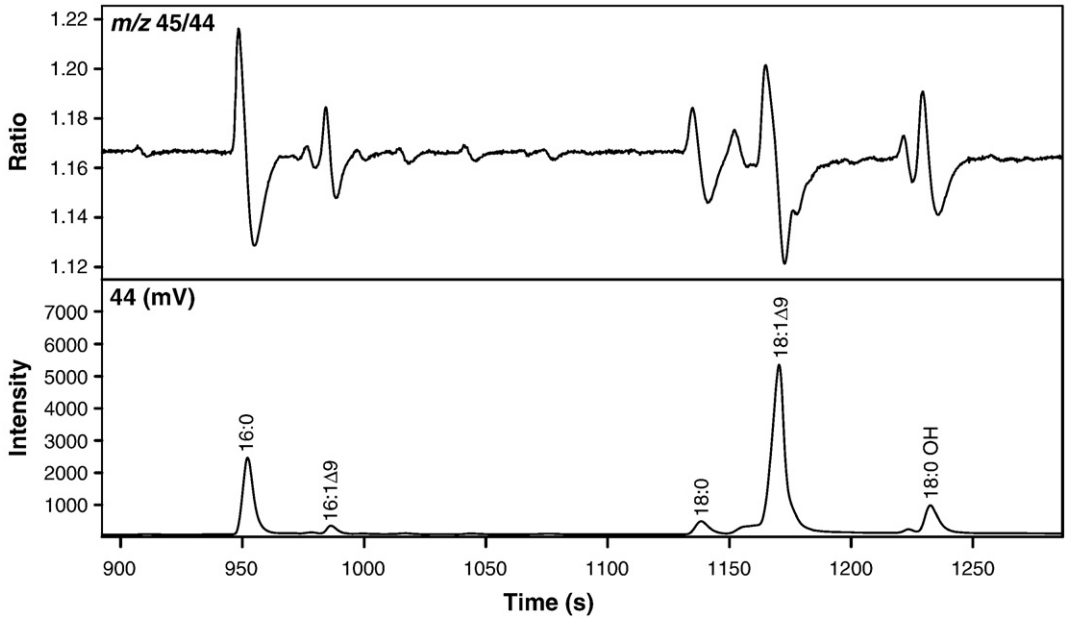

Fig. 5. A partial gas chromatogram (GC/C/IRMS) of fatty acids extracted from soil E. The upper trace depicts the ratio of m/z 45 to m/z 44 with the characteristic isotope 'swings' associated with each chromatographic peak. The lower trace depicts the output of the Faraday cup monitoring m/z 44 (in mV) and clearly shows the baseline separation achieved for the $C_{16:0}$ and $C_{18:0}$ fatty acid components.

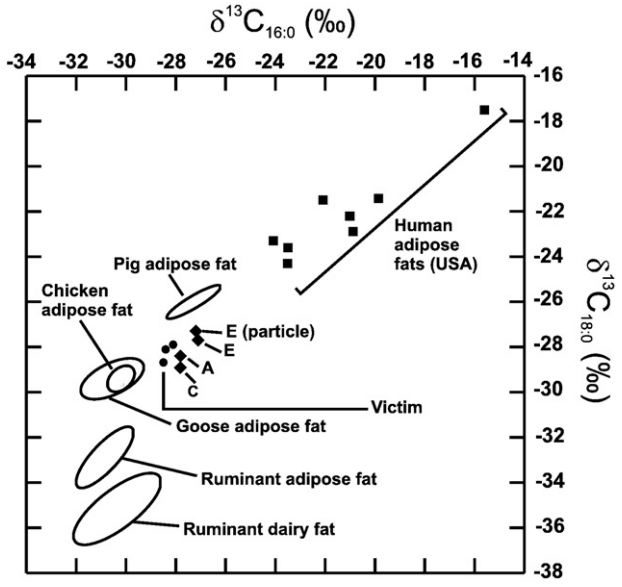

Fig. 6. A plot of the $\delta^{13}C$ values determined for the $C_{16:0}$ and $C_{18:0}$ fatty acids extracted from soils, white particulate matter, adipose fat from the victim and reference human adipose from North America. Reference fats are plotted as confidence ellipses (1σ), corresponding to ruminant dairy fat ($n=13$), ruminant adipose fat ($n=18$), and further adipose fats from chicken ($n=3$), goose ($n=4$) and pigs ($n=8$) obtained from domesticated animals reared on strict C_3 carbon diets. Each point has a precision of ±0.3‰ or less.

monohydroxylated $C_{18:0}$ fatty acid observed in E is indicative of at least some adipocere formation in this soil. The wider and more diverse distributions of lipids derived from soils B, D and G are consistent with those typically obtained from mineral soils that have not been contaminated by large amounts of organic matter [40]. The lipid components comprising the observed distributions arise primarily from overlying vegetation as well as the excretions, exudates, sloughed material and general organic detritus from soil flora and fauna [41]. Since G is a control soil, at this point it may be safely concluded that A, C and E all contain relatively high concentrations of an exogenous substance, most likely decomposing adipose tissue or adipocere, whilst B and D do not. The large differences observed between the concentrations of total lipid extracted from the two soil groups (A, C and E *vs* B, D and G) also attest to an unnaturally large input of exogenous organic matter to the former soils, especially E which at 17.1 mg $g^{-1}_{\text{dry weight}}$ contains at least eight times the concentration of extractable lipid compared with A and C and at least thirty four times the concentrations observed to occur in B, D and G. This is demonstrated further by the huge difference in total $C_{16:0}$ and $C_{18:0}$ fatty acid concentration observed between A, C and E when compared with B, D and G. The concentrations observed for the former set of soil samples are vastly greater than anything one might reasonably expect to observe in a typical mineral soil indicating that there has been a substantial input of exogenous organic matter to E and, to a lesser degree, A and C [42].

The partial chromatogram obtained from GC/MS analysis of white particulate matter sub-sampled from E describes a distribution of components entirely consistent with that of adipocere [5,10,11,12,38,39]. Interestingly, the concentration of the $C_{18:0}$ monohydroxylated fatty acid is very high relative to the other observed components in contrast to the $C_{18:1\Delta9}$ fatty acid that dominates the distribution obtained from the parent soil, E. This may be best rationalised by there being a substantial reservoir of relatively unaltered adipose fat within the soil matrix of E that is still in the process of being transformed to adipocere whereupon the higher melting point of the predominant $C_{18:0}$ monohydroxylated fatty acid combined with the formation of insoluble Ca^{2+} and/or Mg^{2+} salts results in the formation of the observed white particulate matter [5,6].

The results obtained from identical analyses conducted on a sample of adipose fat obtained from the victim yield a distribution of fatty acids very similar to those obtained for A and C indicating that adipocere formation had continued unabated within the whole body parts although not to the same extent as the white particulate matter mixed throughout E.

4.3. Compound-specific stable carbon analysis

Whilst, in this case, mtDNA evidence combined with lipid distributions concentrations provides compelling evidence supporting the transient burial of the victim's remains other complementary methods of analysis warranted investigation in order to assess their potential for determining the source of adipocere deposits. Moreover, having latterly received adipose fat from the victim the possibility of determining a further link between the victim and the adipocere deposits also warranted further investigation. An additional level of information was sought from the extracted lipid components through the use of compound-specific light stable isotope ratio monitoring mass spectrometry or, more commonly, compound-specific isotope analysis (CSIA). Although molecular biological techniques, such as mtDNA profiling, undoubtedly provide a more unambiguous result when identifying sources of organic matter, in this case, this was only possible due to the occurrence of distinct white particulate matter, most likely adipocere, that could be removed by hand from soil E. However, in the event of adipocere formation having not progressed to the same extent, be it due to a shorter period of deposition or more arid conditions [22], CSIA of the extractable lipids might possibly provide a useful, albeit less specific, means of determining the origin of such exogenous organic matter.

In order to ensure high accuracy and confidence in data obtained by GC/C/IRMS it is imperative components of interest are resolved chromatographically to the baseline [43]; this was routinely achieved for the fatty acid components of interest to this study. A crossplot of $\delta^{13}C$ values obtained for $C_{16:0}$ and $C_{18:0}$ fatty acids has previously proven useful in determining the mammalian source of fat derived archaeological residues commonly absorbed in ancient ceramics [36,44]. The $C_{16:0}$ and $C_{18:0}$ fatty acid components extracted from A, C and E (and the associated white particulate matter) will comprise both endogenous and exogenous fatty acids. However, the concentration of these compounds derived from the exogenous input of adipose tissue and/or adipocere in these samples, is so large, relative to the concentrations usually observed in mineral soils, as to render any change to the isotopic signature derived from the input negligible. The $\delta^{13}C$ values obtained for the A, C and E (and the associated white particulate matter) all plot in the region of −28‰, remarkably close to those points similarly generated by plotting $\delta^{13}C$ values obtained from the adipose tissue of the victim. In contrast the $\delta^{13}C$ values obtained from the eight reference adipose tissues obtained from the USA plot over a much wider range of about 8‰ for both the $C_{16:0}$ and $C_{18:0}$ fatty acids. These results, whilst peculiar on initial inspection, are easily explained by the varied but increased proportion of C_4 derived dietary carbon constituting the dietary norm in the USA; by comparison a European diet is dominated by a carbon input that is predominantly of C_3 origin [45]. Nevertheless, this does highlight the absolute need for reference values, such as those used to construct the British domesticate confidence ellipses, to be of a geographical context suitable for comparison with the samples under investigation if such crossplots are to be used. However, even these reference values may have the potential to prove misleading due to the incorporation of unspecified quantities of C_4 derived carbon into the diets of contemporary domesticated livestock. Clearly a better way of interpreting these values, independent of any variation in diet, is required.

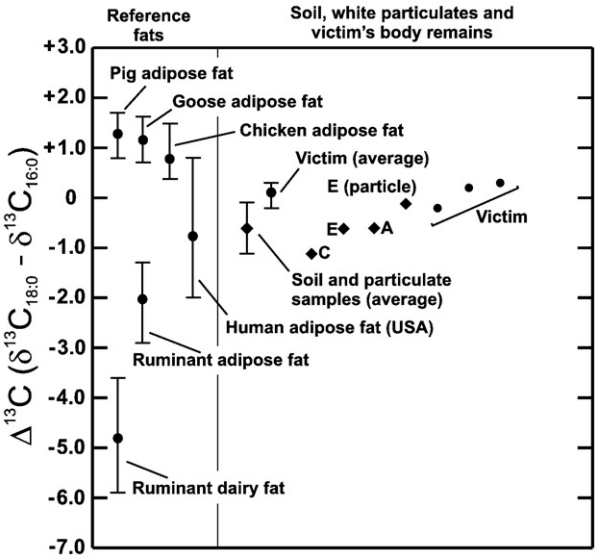

Fig. 7. A plot showing the $\Delta^{13}C$ ('$\delta^{13}C_{18:0}$–$\delta^{13}C_{16:0}$') values for the lipids extracted from the soils: A, C, E and E-white particle and the three sub-samples of the adipose fat from the recovered body remains of the victim. The average ranges of $\Delta^{13}C$ values are also displayed for these samples. The average ranges corresponding to the reference fats (including North American human adipose, $n=8$) are displayed in the left hand column.

Evershed and co-workers [46,47] have shown that calculation of $\Delta^{13}C_{18:0-16:0}$ (defined as $\delta^{13}C_{18:0}$–$\delta^{13}C_{16:0}$) values enables the source of the $C_{16:0}$ and $C_{18:0}$ fatty acids to be investigated on the basis of isotopic fractionation wrought solely by the differing physiologies of potential source organisms and therefore independent of any effects introduced by diet that may obfuscate attempts to source samples of unknown origin. Fig. 7 is a plot of the average and absolute $\Delta^{13}C_{18:0-16:0}$ values calculated for A, C, E (and the associated white particulate matter) and the adipose tissue obtained from the victim. Analogous average ranges have also been calculated using the values obtained from the reference British domesticate and human adipose (USA) fats. As observed previously, A, C, E (and the associated white particulate matter) all plot close to the $\Delta^{13}C_{18:0-16:0}$ values determined for the adipose tissue of the victim whilst remaining significantly separate from those ranges derived from the common British domesticate reference value sets. The fact that none of the values plot within the ranges constructed previously from fat samples of common British domesticated animals indicates that the input of organic matter has not arisen from deposition of animal fats commonly associated with household waste [46]. This is supported by the fact that, other than the white particulates observed in C and E (and F, a hair sample isolated from the dig) there were no discernable remains that might indicate deposition of household waste or burial of a household pet. Moreover, they now plot within the range determined for the human adipose (USA) fats thereby indicating that the $\Delta^{13}C_{18:0-16:0}$ values calculated for A, C, E (and the associated white particulate matter) are consistent with an input of adipose tissue and/or adipocere of human origin to these soils. Whilst still preliminary in nature it is envisaged that this initial study may be further developed [e.g. analysis of more components, combination with compound specific δD and/or $\delta^{15}N$ (for N containing compounds) analyses] and, in time, become a useful and robust means for sourcing organic inputs (e.g. adipocere) to environmental matrices of forensic interest in circumstances where more specific molecular biological techniques such as DNA and mtDNA profiling are rendered unviable.

5. Conclusions

This study was conducted in order to determine the qualitative and quantitative lipid content of a series of soils suspected of having comprised a temporary burial site for the remains of a murder victim. In particular, it was critical to assess the concentration of an exogenous input to the soil identified as white particulate matter and determined, by mtDNA profiling, to be maternally linked to the victim. This was necessary to establish whether this matter was present within the soil at a concentration consistent with a body or body parts having been buried there. Additionally, light stable isotope monitoring MS was used to determine $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids derived from the same soils and particulate matter. In order to assess the potential of this technique as a means of determining the origins of fat residues in soils. The primary outcomes of this study are as follows:

(a) Elemental (C, H, N) analysis of white particulate matter associated with soils C and E and additional white matter attached to a hair (F) revealed that the matter sampled from E comprised a high percentage of carbon (47%) and was most likely of biological origin, e.g. adipocere, whilst the white particulate matter sampled from C and F exhibited comparatively lower carbon contents and were thus more likely of an alternative origin.
(b) Distributional and quantitative analysis of extractable soil lipids identified three soils that had been subjected to an substantial input of exogenous organic matter identified as being consistent with the lipid distribution of adipocere (A and C) and an adipocere (minor)/adipose tissue (major) mix (E).
(c) Identical analyses conducted on white particulate matter sub-sampled from E showed it to possess a lipid distribution consistent with that of adipocere thereby implying that soil E actually contained a greater concentration of relatively less degraded organic matter with a distribution consistent with that of adipose tissue. The white particulate matter sub-sampled from soil E is most likely adipocere.
(d) A crossplot of the $\delta^{13}C$ values obtained from the $C_{16:0}$ and $C_{18:0}$ fatty acids extracted from the samples demonstrated that the soils, white particulate matter from E and the adipose tissue obtained from the remains of the murder victim all plotted close to one another outside of the confidence ellipses determined for British domesticated animals.
(e) $\Delta^{13}C_{18:0-16:0}$ values provide a potential means of identifying inputs of human adipocere to soils irrespective of the dietary history of the body responsible for its generation.

Future work shall seek to: (1) assess the chemical and microbiological effects that the deposition of adipose tissue and adipocere has on the actual soil environment, and (2) develop further the application of compound-specific stable isotope methodologies to the identification of grave sites and human remains.

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References

[1] A.A. Vass, S.-A. Barshick, G. Sega, J. Caton, J.T. Skeen, J.C. Love, J.A. Synstelien, Decomposition chemistry of human remains: a new methodology for determining the postmortem interval, J. Forensic Sci. 47 (2002) 542–553.
[2] A.E. Reynold, G.F. Cahill, Handbook of Physiology: Adipose tissue, American Physiological Society, Washington, 1965.
[3] S.B. Heymsfield, M. Waki, J. Kehayias, S. Lichtman, F.A. Dilmanian, Y. Kamen, J. Wang, R.N. Pierson Jr., Chemical and elemental analysis of humans in vivo using improved body composition models, Am. J. Physiol. 261 (1991) E190–E198.

[4] B.B. Dent, S.L. Forbes, B.H. Stuart, Review of human decomposition processes in soil, Environ. Geol. 45 (2004) 576–585.
[5] T. Takatori, Investigations on the mechanism of adipocere formation and its relation to other biochemical reactions, Forensic Sci. Int. 80 (1996) 49–61.
[6] S.L. Forbes, B.H. Stuart, I.R. Dadour, B.B. Dent, A preliminary investigation of the stages of adipocere formation, J. Forensic Sci. 49 (2004) 1–9.
[7] Ebert, Ber. Deut. Chem. Ges. VIII (1875) 775.
[8] R.F. Ruttan, M.J. Marshall, The composition of adipocere, J. Biol. Chem. 29 (1917) 319–327.
[9] J. Boldingh, in: P. Desnuelle (Ed.), The Enzymes of Lipid Metabolism, Pergamon Press, Oxford, 1961, p. 277.
[10] T. Takatori, A. Yamaoka, Mechanism of adipocere formation. 1. Identification and chemical properties of hydroxy-fatty acids in adipocere, Forensic Sci. 9 (1977) 63–73.
[11] T. Takatori, A. Yamaoka, Mechanism of adipocere formation. 2. Separation and identification of oxo fatty acids in adipocere, Forensic Sci. 10 (1977) 117–125.
[12] T. Takatori, K. Terazawa, K. Nakano, H. Matsumiya, Identification of 10-hydroxy-12-octadecanoic acid in adipocere, Forensic Sci. Int. 23 (1983) 117–122.
[13] T. Takatori, N. Ishiguri, H. Tarao, H. Matsumiya, Microbial production of hydroxy and oxo-fatty acids by several microorganisms as a model of adipocere formation, Forensic Sci. Int. 32 (1986) 5–11.
[14] T. Takatori, H. Gotouda, K. Terazawa, K. Mizukami, M. Nagao, The mechanism of experimental adipocere formation — substrate-specificity on microbial production of hydroxy and oxo fatty acids, Forensic Sci. Int. 35 (1987) 277–281.
[15] H. Gotouda, T. Takatori, K. Terazawa, M. Nagao, H. Tarao, The mechanism of experimental adipocere formation — hydration and dehydrogenation in microbial synthesis of hydroxyl acid, Forensic Sci. Int. 37 (1988) 249–257.
[16] S. Fiedler, M. Graw, Decomposition of buried corpses, with special reference to the formation of adipocere, Naturwissenschaften 90 (2003) 291–300.
[17] H.W. Bernard, Adipocere in the bodies of the drowned, Br. Med. J. (1917) 252.
[18] G.E. Cotton, A.C. Aufderheide, V.G. Goldschmidt, Preservation of human tissue immersed for five years in fresh water of known temperature, J. Forensic Sci. 32 (1987) 1125–1130.
[19] P.F. Mellan, M.A. Lowry, M.S. Micozzi, Experimental observations on adipocere formation, J. Forensic Sci. 38 (1993) 91–93.
[20] A. Powell, Early formation of adipocere, Br. Med. J. (1917) 842–843.
[21] J. Simonsen, Early formation of adipocere in temperate climate, Med. Sci. Law 17 (1977) 53–55.
[22] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of burial environment on adipocere formation, Forensic Sci. Int. 154 (2005) 24–34.
[23] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of soil type on adipocere formation, Forensic Sci. Int. 154 (2005) 34–43.
[24] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of the method of burial on adipocere formation, Forensic Sci. Int. 154 (2005) 43–52.
[25] S. Fiedler, K. Schneckenberger, M. Graw, Characterization of soils containing adipocere, Arch. Environ. Contam. Toxicol. 47 (2004) 561–568.
[26] A.K. Mant, Adipocere — a review, J. Forensic Med. 4 (1957) 18–35.
[27] J. Henderson, Factors determining the preservation of human remains, in: A. Boddington, A.N. Garland, R.C. Janaway (Eds.), Death, decay and reconstruction: approaches to archaeology and forensic science, Manchester University Press, Manchester, 1987, pp. 43–54.
[28] R.P. Evershed, Chemical composition of a bog body adipocere, Archaeometry 34 (1992) 253–265.
[29] R. Berstan, S.N. Dudd, M.S. Copley, E.D. Morgan, A. Quye, R.P. Evershed, Characterisation of 'bog butter' using a combination of molecular and isotopic techniques, Analyst 129 (2004) 270–275.
[30] S. Pfeiffer, S. Milne, R.M. Stevenson, The natural decomposition of adipocere, J. Forensic Sci. 43 (1998) 368–370.
[31] K. Lehtonen, M. Ketola, Solvent-extractable lipids of *Sphagnum*, *Carex*, *Bryales* and *Carex-Bryales* peats: content and compositional features *vs* peat humification, Org. Geochem. 20 (1993) 363–380.
[32] R.P. Evershed, C. Heron, L.J. Goad, Analysis of organic residues of archaeological origin by high-temperature gas chromatography and gas chromatography–mass spectrometry, Analyst 115 (1990) 1339–1342.
[33] I.D. Bull, I.A. Simpson, S.J. Dockrill, R.P. Evershed, Organic geochemical evidence for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney, Org. Geochem. 30 (1999) 535–556.
[34] P. Scribe, J. Guezennec, J. Dagaut, C. Pepe, A. Saliot, Identification of the position and the stereochemistry of the double bond in monounsaturated fatty acid methyl esters by gas chromatography/mass spectrometry of dimethyl disulfide derivatives, Anal. Chem. 60 (1988) 928–931.
[35] G. Rieley, Derivatization of organic-compounds prior to gas-chromatographic combustion-isotope ratio mass-spectrometric analysis — identification of isotope fractionation processes, Analyst 119 (1994) 915–919.
[36] R.P. Evershed, H.R. Mottram, S.N. Dudd, S. Charters, A.W. Stott, G.J. Lawrence, A.M. Gibson, A. Conner, P.W. Blinkhorn, V. Reeves, New criteria for the identification of animal fats preserved in archaeological pottery, Naturwissenschaften 84 (1997) 402–406.
[37] S.N. Dudd, Molecular and Isotopic Characterisation of Animal Fats in Archaeological Chemistry. PhD Thesis, University of Bristol, 2000, 396p.
[38] S.L. Forbes, B.H. Stuart, B.B. Dent, The identification of adipocere in grave soils, Forensic Sci. Int. 127 (2002) 225–230.
[39] K. Saito, Occurrence of 10-hydroxystearic acid in adipocere, J. Biochem. 59 (1966) 487–494.
[40] P.F. van Bergen, C.J. Nott, I.D. Bull, P.R. Poulton, R.P. Evershed, Organic geochemical studies of soils from the Rothamsted Classical Experiments-I. Total lipid extracts, insoluble residues and humic acids from Broadbalk Wilderness, Org. Geochem. 29 (1998) 1779–1795.
[41] M. Cresser, K. Killham, T. Edwards, Soil Chemistry and its Applications, Cambridge University Press, Cambridge, 1993. 192 pp.
[42] I.D. Bull, P.F. van Bergen, P.R. Poulton, R.P. Evershed, Organic geochemical studies of soils from the Rothamsted Classical Experiments-IV. Preliminary results from a study of the effect of soil pH on organic matter decay, Org. Geochem. 28 (1998) 11–26.
[43] W. Meier-Augenstein, Applied gas chromatography coupled to isotope ratio mass spectrometry, J. Chromatogr. A 842 (1999) 351–371.
[44] H.R. Mottram, S.N. Dudd, G.J. Lawrence, A.W. Stott, R.P. Evershed, New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats preserved in archaeological pottery, J. Chromatogr. A 833 (1999) 209–221.
[45] A.J.M. Wagenmakers, N.J. Rehrer, F. Brouns, W.H.M. Saris, D. Halliday, Breath $^{13}CO_2$ background enrichment during exercise: diet related differences between Europe and America, J. Appl. Physiol. 74 (1993) 2353–2357.
[46] M.S. Copley, R. Berstan, S.N. Dudd, G. Docherty, A.J. Mukherjee, V. Straker, S. Payne, R.P. Evershed, Direct chemical evidence for widespread dairying in prehistoric Britain, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1524–1529.
[47] R.P. Evershed, S. Payne, A.G. Sherratt, M.S. Copley, J. Coolidge, D. Urem-Kotsu, K. Kotsakis, M. Ozdoğan, A.E. Ozdoğan, O. Nieuwenhuyse, P.M.M.G. Akkermans, D. Bailey, R-R. Andeescu, S. Campbell, S. Farid, I. Hodder, N. Yalman, M. Ozbaşaran, E. Bıcakcı, Y. Garfinkel, T. Levy, M.M. Burton, Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding, Nature 455 (2008) 528–531.