Characterisation of 'Bog Butter' using a Combination of Molecular and Isotopic Techniques

Robert Berstan[a], Stephanie N. Dudd[a], Mark. S. Copley[a], E. David Morgan[b], Anita Quye[c] and Richard. P. Evershed*[a]

[a]*Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK.*

[b]*Department of Chemistry, University of Keele, Keele, Staffordshire, ST5 5BG, UK.*

[C] *Department of Conservation & Analytical Research, National ~~Laboratory, Department of Museum Services, Royal~~ Museums of Scotland, Chambers Street, Edinburgh, EH1 1JF, UK.*

Formatted: Superscript

* Author for correspondence: r.p.evershed@bristol.ac.uk

Abstract

The chemical analyses of 'bog butters' recovered from peat bogs of Scotland were performed with the aim of determining their origins. Detailed compositional information was obtained from 'bog butter' lipids using high temperature gas chromatography (HTGC) and GC-mass spectrometry (GC/MS). The results indicate the degree to which 'bog butters' have undergone diagenetic alterations during burial to form an adipocere like substance, consisting predominately of hexadecanoic (palmitic) and octadecanoic (stearic) acids. GC-combustion-isotope ratio MS (GC-C-IRMS) was used to determine $\delta^{13}C$ values for the dominant fatty acids present, revealing for the first time that 'bog butters' were derived from both ruminant dairy fats and adipose fats. The results are compared and contrasted with modern reference fats and adipoceres produced *in vitro*.

Introduction

Extensive peat cutting in Scotland and Ireland, has resulted in the discovery of large quantities of white waxy substances known as 'bog butters'. In excess of 270 of these finds, often associated with wooden containers, trays or animal skins have been recovered from the peat, with the earliest known example being radiocarbon dated to 400-350 cal BC.[1] Peat bogs exhibit exceptional preservation qualities, due to the cool temperature and anaerobic conditions that exist in their waterlogged environments. It is clear that the communities of the bog lands were aware of these properties and buried the 'bog butters' intentionally to simply preserve or, possibly, to modify the properties of the original material to provide a more desirable commodity. Historical records, including Butler's Irish Hudibras from the 17th century stated that 'butter to eat with their hog was seven years buried in a bog', while in the 1670s Debes described the inhabitants of the Faeroe islands burying sheep tallow,[2] suggesting that 'bog butter' might also derive from an animal adipose fat.

Previous analytical work[3-7] generally concluded that 'bog butter' was a fat of animal origin, that had been diagenetically altered to resemble adipocere. Adipocere (Latin: *adeps* – fat, *cera* – wax) was first recognised on the remains of bodies buried at the Cemetery of Innocents, Paris[8] and has more recently been identified on bog bodies,[9-12] corpses recovered from the sea[13] and seal carcasses from an Eskimo midden.[14,15] A wet anaerobic environment are the conditions necessary for adipocere formation and this has been demonstrated by its *in vitro* production from human fat, olive oil,[16] mutton fat and butter.[7] The 'bog butters', like adipocere are almost entirely saponified, thereby comprising predominately free saturated fatty acids, in particular palmitic ($C_{16:0}$) and stearic ($C_{18:0}$). Previous work was unable to assign the precise origin(s) of the 'bog butter', due to compositional changes that occurred during burial.

Compound-specific stable carbon isotope measurements on fatty acids have been employed to unambiguously distinguish between degraded ruminant fat (e.g. cattle and sheep) and non-ruminant fat (e.g. pigs) extracted from archaeological pottery.[17,18] The difference in $\delta^{13}C$ values reflects the fundamental differences between the diets, metabolisms and physiologies of the different species of animal. This technique has also been successful in distinguishing between degraded dairy fat and adipose fat from cattle and sheep.[19,20] A distinct difference between the $\delta^{13}C$ values of the $C_{18:0}$ fatty acids from ruminant adipose and milk fat is evident due to the their respective carbon sources. Fatty acids in ruminant adipose tissue are mainly biosynthesised from acetate, formed predominantly from dietary carbohydrates fermented in the rumen.[21] In contrast, the mammary gland is only capable of synthesising fatty acids up to C_{16} in carbon chain length, with the $C_{18:0}$ fatty acid in milk fat deriving mainly from the remobilisation of adipose fatty acids and from unsaturated C_{18} fatty acids (i.e. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) directly from the diet after biohydrogenation in the rumen.[22] Reflecting the contribution from dietary plant lipids (i.e. C_{18} fatty acids), which are depleted in ^{13}C relative to their carbohydrates, hence, the $\delta^{13}C$ values for $C_{18:0}$ fatty acids in ruminant milk fat are ~2.3 ‰ less than their adipose fat equivalents.[19,20]

The origins of degraded fats as determined by the isotopic composition of their major fatty acids can often be supported by the distributions of surviving triacylglycerols. The fatty acids present in these acyl lipids that endure archaeological timescales are generally saturated in nature, indicating the preferential loss of the unsaturated components. Silver ion thin layer chromatography (Ag^+ TLC) has been used to isolate saturated triacylglycerols from modern domesticates in order to provide carbon number distributions for those components most likely to be preserved (the unsaturated components being inherently more labile and thus more susceptible to a variety of degradation reactions). Analysis of these triacylglycerol fractions by high temperature gas chromatography (HTGC) revealed the ruminant adipose fats (cattle and sheep) as having wider acyl carbon distributions than the non-ruminant porcine fat.[23,24] In dairy fat there is an abundance

of short chain fatty acids (C_4 to C_{14}) resulting in broader triacylglycerol distribution than that observed in adipose fat.[25,26] However, during degradation there is a preferential loss of these lower molecular weight moieties as the ester linkages are less sterically hindered and therefore more prone to hydrolysis. If a high level of degradation has occurred, the distribution of triacylglycerols in dairy fat often resembles that of adipose fat. Fortunately, however, the heavier of these low molecular weight triacylglycerols frequently survive archaeological timescales in sufficient abundances for a distinction to be drawn between adipose and dairy fat.[19,20,24]

Previous reports[6,7,27] showed that 'bog butters' were degraded animal fats, possibly from a dairy origin, however diagenetic alterations during burial only allowed tentative conclusions to be made. In this paper we present the results from the chemical analyses of nine Scottish 'bog butters' in an attempt to establish, for the first time, the precise origin(s) of these enigmatic deposits. HTGC and GC-mass spectrometry (GC/MS) were employed to identify the lipid components present, while GC-combustion-isotope ratio MS (GC-C-IRMS) afforded the diagnostic compound-specific stable carbon isotope values ($\delta^{13}C$ values) for the major fatty acids.

Material and Methods

Samples

Sub-samples from nine 'bog butters' from various locations around Scotland were supplied by the National Museum of Scotland, Edinburgh; a full description of these samples are given in Table 1. Synthetic adipoceres were produced as described by in Morgan *et al.*[7] Briefly, fresh mutton adipose and butter were placed in conical flasks together with stream water and nutrients. The mix was inoculated with microorganisms by the addition of soil and sealed with a rubber bung, pierced with a glass capillary plugged with cotton wool to allow pressure equilibration without allowing oxygen to diffuse inwards. For stable carbon isotope analyses, the modern reference fats

(ruminant adipose and milk), from animals reared on strict C_3 diets (archaeologically comparable) were supplied by Manor Farm, Bristol.

Lipid extraction

Lipids were extracted/dissolved by the ultrasonication (20 min) of the 'bog butter' samples (*ca.* 1 mg) in a mixture of chloroform and methanol (2:1 v/v; 10 ml). Following separation of the undissolved matter via centrifugation, the solvent was evaporated under a gentle stream of N_2 to obtain the total lipid extract (TLE).

Preparation of trimethylsilyl derivatives

Portions of the total lipid extracts were derivatised using *N,O*-bis(trimethylsilyl)trifluroacetamide (BSTFA; 30 μl; 70ºC; 30 min). Prior to analyses by HTGC and GC-MS, the BSTFA was evaporated under N_2 and the samples redissolved in an appropriate volume of *n*-hexane.

Preparation of fatty acid methyl ester (FAME) derivatives

Intact acyl lipids present in the samples were hydrolysed via the heating (70ºC; 1 h) of the total lipid extracts using sodium hydroxide dissolved in methanol and double distilled water (9/1 v/v; 0.5 M; 2 ml). Following acidification to pH 3-4, the lipids were extracted into hexane (3 x 1 ml) with the solvent removed under a gentle stream of N_2. The FAMEs were prepared by reaction with BF_3-methanol (14% w/v; 100 μl; 70ºC; 1 h). After cooling the reaction mixture was quenched with double-distilled water (1 ml) and the methyl ester derivatives extracted with diethyl ether (3 x 1 ml). The solvent was then removed under N_2 and the FAMEs redissolved in an appropriate volume of *n*-hexane for analysis by GC and GC-C-IRMS.

Preparation of dimethyldisulphide (DMDS) derivatives

A portion of the FAMEs in *n*-hexane (100 μl) was derivatised further by the addition of dimethyl disulphide (100 μl) and iodine in diethyl ether (6% w/v, 2 drops). The mixture was left in darkness at room temperature for 24 h after which the excess iodine was removed by the addition of aqueous sodium thiosulphate (5%, 500 μl). The DMDS derivatives were then extracted into *n*-hexane (3 x 1 ml) and analysed by GC and GC-MS.

High temperature gas chromatography (HTGC)

HTGC analyses were performed on a Hewlett Packard 5890 series II GC coupled to an Opus V PC with HP Chemstation software. The samples were injected into a J & W Scientific fused silica capillary column (15 m x 0.32 mm i.d.) coated with a dimethyl polysiloxane stationary phase (DB1, 0.1 μm film thickness). The temperature program consisted of a 2 min isothermal period at 50ºC followed by an increase to 350ºC at 10ºC min^{-1}, the temperature was then held at 350ºC for 10 min. Hydrogen was the carrier gas and a flame ionisation detector was employed to monitor the column eluent.

Gas chromatography-mass spectrometry (GC-MS)

Electron ionisation HTGC-MS analyses of the TLEs were performed on a Carlo Erba 5160 Mega series GC interfaced to a Finnigan 4500 quadrupole MS (electron energy 70eV; scan range 50-800; scan time 1.5 s). The column and temperature program used were the same as those for GC analyses, with helium as the carrier gas. Data were acquired using an INCOS data system and processed on a DECstation (Digital) 5000/125.

GC-MS analyses of the FAME/DMDS derivatives were achieved using a ThermoFinnigan Trace MS (electron energy 70eV; scan range m/z 50-850; scan time 0.8 s). The samples were injected into a fused silica capillary column (60 m x 0.32 mm i.d.) coated with a dimethyl polysiloxane

stationary phase (Phenomenex; ZB1, 0.1 μm film thickness). The temperature program consisted of an initial temperature of 50ºC, which was held for 1 min before rising to 300ºC at 10ºC min^{-1}, the temperature was then held at 300ºC for 10 min. Data were acquired and processed using an Excalibur data system (Version 1.2).

Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS)

Analyses were performed using a Varian 3400 GC coupled to a Finnigan MAT Delta-S IRMS via a modified Finnigan MAT combustion interface [Cu and Pt wires (0.1 mm o.d.) in an alumina reactor (0.5 mm i.d.)]. The reactor temperature was maintained at 860ºC and the MS ion source pressure was 6 x 10^{-6} mbar. The GC column was a fused silica capillary column (50 m x 0.32 i.d.) coated with a dimethyl polysiloxane stationary phase (CP Sil-5-CB, 0.25 μm film thickness). The temperature program consisted of a 1 min isothermal period at 50ºC followed by an increase to 240ºC at 10ºC min^{-1}, then a further increase to 300ºC at 4ºC min^{-1} with an isothermal period of 10 min. The carbon isotope ratios were expressed relative to VPDB (*Belemnitella americana*), $\delta^{13}C$ (‰) = 1000 $[(R_{sample} - R_{standard})/R_{standard}]$, where R is the $^{13}C/^{12}C$ ratio. Analytical error is ± 0.3 ‰.

Results and Discussion

Investigation of the 'bog butters' comprised three major aspects: (i) assessment of lipid composition by means of GC and GC/MS; (ii) Comparison of the composition of 'bog butter' with those of synthetic adipoceres, and (iii) determination of compound-specific $\delta^{13}C$ values for $C_{16:0}$ and $C_{18:0}$ to confirm the origins of 'bog butters'.

Fatty acid compositions

Lipid extracts from the 'bog butters' and the synthetically produced adipoceres were analysed following trimethylsilylation using HTGC. Figure 1 shows the partial gas chromatograms for two synthetically produced adipoceres derived from butter and mutton fat and two 'bog butter'

extracts from Dumfries and Ross-shire. Free fatty acids ranging from C_{12} to C_{20} (even-over-odd-carbon number predominance) were the principal components of 'bog butters', with palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids predominating. Triacylglycerols and sometimes diacylglycerols were also observed, indicating that hydrolysis of the acyl moieties during burial was incomplete. Analysis of sub-samples from the interior and exterior of each 'bog butter' mass, revealed similar lipid distributions, showing that the extent of hydrolysis/degradation was largely homogeneous throughout. In agreement with previous investigations,[6,7] the lipid distributions, particularly the high abundance of $C_{18:0}$, indicate that 'bog butters' are clearly derived from animal fat.

Odd carbon number fatty acids ($C_{15:0}$ and $C_{17:0}$) were observed in all 'bog butters' as straight and branched (*iso*- and *antiso*-) chains in abundances of up to 2.0% and 1.5% of the total free fatty acids, respectively. It is known that micro-organisms present in the rumen of ruminant animals (e.g. cattle and sheep) synthesise these odd and branched chain fatty acids, resulting in increased abundances in ruminant animals relative to non-ruminants.[~~27~~28] Caution, however, must be exercised if assigning the origin of a degraded animal fat solely using this criterion, as the magnitude of diagenetic contributions from bacteria active during burial is unknown.

Monounsaturated octadecenoic acids ($C_{18:1}$) were present in appreciable abundances in each 'bog butter' (between *ca.* 2.0 and 8.0 % of the total free fatty acids). Further GC-MS analyses were performed on these compounds as dimethyl disulphide adducts of their methyl ester derivatives to determine the range of positional isomers and stereoisomers present. The characteristic fragmentation patterns[~~28~~29] revealed a range of positional isomers from Δ^9 to Δ^{16}, with Δ^{11} (11-octadecenoic acids) being the most abundant in all bog butters. The *trans* (*E*) stereoisomer of each positional isomer was more abundant than the corresponding *cis* (*Z*) configuration, with the exception of Δ^9, where each stereoisomer was present in similar abundances. Figure 2 shows, by way of example, the gas chromatogram and mass chromatograms obtained from 'bog butter' SB5.

Approximately 95% of the octadecenoic acids in fresh ruminant fat are present as *cis* (*Z*) stereoisomers,[29~~30~~] with a range of positional isomers from Δ^7 to Δ^{12}, dominated by the Δ^9 isomer. The less abundant, but more stable *trans* (*E*) stereoisomer has a wider distribution of positional isomers, consisting Δ^7 to Δ^{16}, with the majority being Δ^{11}.[~~30~~31] In fresh porcine fat (non-ruminant), unsaturated fatty acids exist almost exclusively with a *cis* (*Z*) configuration, with a much lower abundance of *trans* isomers compared with ruminant fats.[~~31~~32] Comparison of the range of positional isomers and stereoisomers present in the 'bog butters' with fresh animal fats revealed that the 'bog butter' distributions, dominated by *trans* (*E*) stereoisomers, are consistent with the *trans (E)* stereoisomers in ruminant fat, with both having a maximum at Δ^{11}. This suggests the more labile *cis* (*Z*) stereoisomers may have been lost due to reaction (e.g. oxidation or hydration) during burial.

10-Hydroxyoctadecanoic acid (and to a lesser extent 9-hydroxyoctadecanoic acid) were identified in seven out of nine 'bog butters' and the synthetic adipocere from mutton fat in abundances of 0.2% and 67.4% of the total free fatty acids. Figure 3a displays the mass spectrum of the trimethylsilyl derivative of the hydroxyoctadecanoic acids in 'bog butter' SB4. Although no molecular ion was present, the diagnostic ions at *m/z* 215 and 331 and also *m/z* 229 and 317 (resulting from α-cleavage at either side of the carbon atom bearing the hydroxyl group) indicate both 10- and 9-hydroxyoctadecanoic acids are present, respectively. Hydroxyoctadecanoic acids (in particular 10-hydroxyoctadecanoic acid) have frequently been identified in adipocere, e.g. as components of corpses immersed in the sea for 3-6 months[13] and bog bodies recovered from the peat bogs,[10,12] and are believed to derive from *Z*-9-octadecenoic acid (most abundant isomer in fresh ruminant fat) after microbial oxidation.[~~32~~33] Oxo-octadecanoic acid has also previously been identified in adipocere,[~~33~~34] demonstrating further oxidation has occurred. GC-MS analysis of one of the 'bog butters' SB4 revealed trace quantities of 10-oxo-octadecanoic acid (Fig. 3b), with the

position of the ‘oxo-’ group on the carbon chain identified by the characteristic *m/z* 215 ion, occurring as a result of β-cleavage.

Triacylglycerol distributions

Triacylglycerols were identified in both ‘bog butters’ and synthetic adipoceres, revealing the hydrolysis to diacylglycerols, monoacylglycerols and free fatty acids had not proceeded to completion. Figure 4 displays triacylglycerol distributions, as histograms, of a selection of the synthetic adipoceres and ‘bog butters’, with the abundance of each component calculated by integrating their peak areas in the HTGC profile. The synthetic adipoceres from mutton fat (Fig. 4a), New Zealand butter (Fig. 4b) and Farmhouse butter all resembled a degraded adipose fat profile. Closer inspection of the lower molecular weight triacylglycerols (C_{42} and C_{44}) revealed them to be present in higher abundance in the butter adipoceres compared with the mutton fat, which contained no C_{42} and only very low abundance of C_{44}. There was an absence of C_{42} or C_{44} triacylglycerols in the ‘bog butters’ SB2 (Fig. 4c), SB6, SB7 and SB9, suggesting a ruminant adipose origin. Similar to the mutton fat adipocere (Fig. 4a), ‘bog butters’ SB1, SB3, SB4 and SB8 contained no detectable C_{42} and only trace amounts of C_{44}, this again might suggest an adipose origin, however, a dairy origin cannot be discounted as hydrolysis of the low molecular weight fatty acyl moieties may have occurred to a greater degree than in the synthetically produced adipoceres. One sample, SB5 (Fig. 4d), can confidently be assigned a dairy fat origin as both C_{42} and C_{44} were detected in abundances of 0.7% and 2.3% of the total triacylglycerols, respectively.

Compound-specific stable carbon isotopes

Even though the HTGC profiles showed that extensive degradation had occurred during burial, the measurement of stable carbon isotope values from individual fatty acids allows species identification in diagentically altered fat samples. The $\delta^{13}C$ values for the individual $C_{16:0}$ and $C_{18:0}$ fatty acids from the ‘bog butters’ were plotted against the same fatty acids derived from

modern reference fats from domesticated cattle and sheep grouped within confidence ellipses of one standard deviation (Fig. 5). Interestingly, the 'bog butters' fell into two distinct groups, one comprising three different 'bog butters' that correlated with an adipose fat origin and a second group consisting of six different 'bog butter' samples that corresponded to a dairy fat origin. SB2, SB6 and SB7 were all assigned a ruminant adipose fat origin; significantly, this was supported by triacylglycerol distributions, suggesting the burying of tallow was practised in Scotland. The remaining 'bog butters': SB1, SB3, SB4, SB5, SB8 and SB9 were confidently assigned a dairy fat origin on account of the relatively depleted $\delta^{13}C$ value for their $C_{18:0}$ fatty acid. The wide triacylglycerol distribution observed in specimen SB5 supported the dairy fat identification, however due to the preferential hydrolysis of the diagnostic lower molecular weight triacylglycerols in the other 'bog butters', distinctions could not be made on this basis between a dairy or adipose fat origin. This demonstrates the usefulness of stable isotopes in identifying the origins of highly degraded animal fats when distributions have been highly altered.

It was suggested in previous work[6,7] that the formation of adipocere results in an increased abundance of $C_{16:0}$ fatty acid, deriving possibly from $C_{18:1}$ fatty acid.[16] The different isotope values confirm that the $C_{16:0}$ and $C_{18:0}$ fatty acids have different origins, i.e. the $C_{16:0}$ fatty acid in milk is biosynthesised in the mammary gland while the $C_{18:0}$ is derived from a combination of dietary fatty acids and adipose fat. If the $C_{16:0}$ fatty acid was derived from the C_{18} fatty acids to any appreciable extent their respective $\delta^{13}C$ values would be more comparable.

Conclusions

Although previous work was unable to elucidate the precise origin of these enigmatic 'bog butters', it was generally agreed that they most likely were derived from a butter or dairy fat origin. Most historical records supported this conclusion, the exception being the burying of ruminant tallow on the Faeroe Islands. Determination of the lipid distributions using HTGC and

GC/MS revealed the ‘bog butters’ to resemble adipocere of a ruminant animal origin, with the triacylglycerol profiles in some of the specimens providing a tentative means of differentiating between the dairy and adipose fat origins. However, compound-specific stable carbon isotope determinations make it possible for the first time to assign a precise origin for each ‘bog butter’. Six of the ‘bog butters’ from Morvern (SB1), Glenurquhart (SB3), Skye (SB4), Ross-shire (SB5), Durness (SB8) and Shetland (SB9) were indeed found to be of a dairy fat origin, with the remaining three from Dumfries (SB2), Rhiconich (SB6) and Loch Carron (SB8) unambiguously classified as having an adipose fat origin. These results demonstrate that the burying of both butter/dairy fat and tallow/adipose fat in peat bogs was a widespread practise in Scotland during the late Iron Age to Medieval periods.

Acknowledgements

We thank the National Museum of Scotland for providing the ‘bog butter’ samples. We also thank Jim Carter and Andy Gledhill for their assistance with GCMS and GC-C-IRMS. We are also grateful to NERC for mass spectrometry facilities (Grant No. F14/6/13).

References

1. C. Earwood, *The Journal of Irish Archaeology*, 1997, **VIII**, 25-42.

2. W. Bergmann, in *Organic Geochemistry*, ed. I. A. Breger, Pergamon Press, 1963, vol. 16, pp. 503-542.

3. W. I. Macadam, *Proc. Soc. Antiq. Scot.*, 1882, **16**, 204-223.

4. P. S. Arup, *Analyst*, 1932, **57**, 300-302.

5. J. Ritchie, *Proc. Soc. Antiq. Scot.*, 1941, **75**, 5-22.

6. M. D. Thornton, E. D. Morgan and F. Celoria, *Science and Archaeology*, 1970, **2/3**, 20-25.

7. E. D. Morgan, C. Cornford, D. R. J. Pollock and P. Isaacson, *Science and Archaeology*, 1973, **10**, 9-10.

8. E. F. Fourcroy, *Annales de Chimie*, 1790, **5**, 154-185.

9. R. P. Evershed and R. C. Connolly, *Naturwissenschaften*, 1988, **75**, 143-145.

10. R. P. Evershed, *Archaeometry*, 1990, **32**, 139-153.

11. R. P. Evershed, in *Archaeological Sciences 89,* ed. P. Budd, B. Chapman, R. Janaway and B. Ottaway, Oxbow Books, 1991, pp. 352-361.

12. R. P. Evershed, *Archaeometry*, 1992, **34**, 253-265.

13. T. Takatori and A. Yamaoka, *Forensic Sci*., 1977, **9**, 63-73.

14. E. D. Morgan, L. Titus, R. J. Small and C. Edwards, *Artic*, 1983, **36**(4), 356-360.

15. E. D. Morgan, L. Titus, R. J. Small and C. Edwards, *Archaeometry*, 1984, **26**(1), 43-48.

16. L. E. Den Dooren de Jong, *Anton. van Lee. J. M. S*., 1961, **27**, 337-361.

17. 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 and V. Reeves, *Naturwissenschaften*, 1997, **84**, 402-406.

18. H. R. Mottram, S. N. Dudd, G. J., Lawrence, A. W. Stott and R. P. Evershed, *J. Chromatogr. A*, 1999, **833**(2), 209-221.

19. S. N. Dudd and R. P. Evershed, *Science*, 1998, **282**, 1478-1481.

20. M. S. Copley, R. Berstan, S. N. Dudd, G. Docherty, A. J. Mukherjee, V. Straker, S. Payne and R. P. Evershed, R.P., *P. Natl. Acad. Sci. U.S.A.*, 2003, **100**, 1524-1529.

21. R. G. Veron, in *Progress in lipid research supplement No.1 Lipid metabolism in the mammary gland of ruminant animals*, ed. W. W. Christie, Pergamon Press, 1981, pp. 279-362.

22. J. H. Moore and W. W. Christie, in *Progress in lipid research supplement No.1. Lipid metabolism in the mammary gland of ruminant animals*, ed. W. W. Christie, Pergamon Press, 1981, pp. 227-277.

23. S. N. Dudd, PhD Thesis, University of Bristol, Bristol, 1999.

24. S. N. Dudd, R. P. Evershed and A. M. Gibson, *J. Archaeol. Sci.*, 1999, **26**, 1473-1482.

25. D. T. Davies, C. Holt and W. W. Christie, in *Biochemistry of Lactation*, ed. T. B. Mepham, Elsevier Science Publishers, Amsterdam, 1983, pp. 71-117.

26. P. McDonald, R. A. Edwards and J. F. D. Greenhalgh, *Animal Nutrition*. Longmann, 1988.

27. A. Quye, Analytical Report No. 92/28, National Museums of Scotland, 1992

2~~7~~8. G. F. Spencer, S. F. Herb and P. J. Gormisky, *J. Am. Oil Chem. Soc.*, 1976, **53**, 745-765.

2~~8~~9. P. Scribe, J. Guezennec, J. Dagaut, C. Pepe and A. Saliot, *Anal. Chem.*, 1988, **60**, 928-931.

~~29~~30. W. W. Christie and J. H. Moore, *J. Sci. Food. Agr.*, 1971, **22**, 120-124.

3~~0~~1. J. D. Hay and W. R. Morrison, *Lipids*, 1973, **8**, 94-95.

3~~1~~2. W. R. H. Duncan and G. A. Garton, *J. Sci. Food. Agr.*, 1967, **18**, 99-102.

3~~2~~3. T. Takatori, *Forensic Sci. Int.*, 1996, **80**, 49-61.

3~~3~~. T. Takatori and A. Yamaoka, *Forensic Sci*., 1977, **10**, 117-125.

3~~4~~. C. Earwood, *Proc*. *Soc*. *Antiq*. *Scot*., 1991, **121**, 231-240.

3~~5~~. C. Earwood, *Proc*. *Soc*. *Antiq*. Scot., 1993, **123**, 355-362.

3~~6~~. H. Friedli, H. Lotscher, H. Oeschger, U. Siegenthaler and B. Stauffer, *Nature*, 1986, **324**, 237-238.

FIGURE CAPTIONS

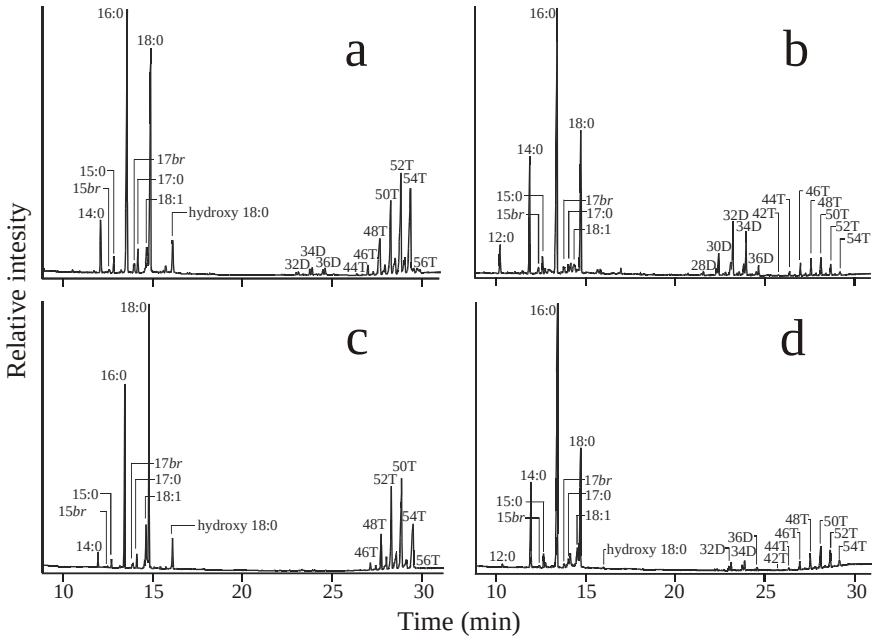
Figure 1. Partial gas chromatograms displaying the lipid distributions from synthetically produced adipoceres from a) Mutton fat and b) New Zealand Butter and 'bog butters' from c) Lochar Moss, Dumfries (SB2) and d) Ross-shire (SB5). Peak identities are: 12:0, 14:0, 15:0, 16:0, etc., corresponding to straight chain fatty acids containing 12, 14, 15 and 16 carbon atoms, respectively; 15*br* and 17*br* corresponding to closely eluting *iso*- and *anteiso*-branched fatty acids with 15 and 17 carbon atoms, respectively; 18:1, mono-unsaturated C_{18} alkanoic acid; hydroxy 18:0, hydroxyoctadecanoic acid (predominantly 10-hydroxyoctadecanoic acid); 28D, 30D, 32D, etc., diacylglycerols containing 28, 30, 32, etc. acyl carbon atoms, respectively (1,2-isomer eluting before the 1,3-isomer); 42T, 44T, 46T, 48T, etc., triacylglycerols with 42, 44, 46, 48, etc. acyl carbon atoms, respectively.

Figure 2. A partial gas chromatogram showing the lipid extract from 'bog butter' SB5 (methyl ester / dimethyl disulphide derivatised). Peak identities are as for Figure 1. The major peaks in the chromatogram have been expanded off scale to reveal detail of the peaks relating to the dimethyl disulphide (DMDS) adducts of the $C_{18:1}$ fatty acid methyl ester (FAME). The inset displays the total ion current (TIC, *m/z* 50-850) and selected mass chromatograms that correspond to characteristic fragment ions conveying the positional isomers that were present in the octadecenoic acid. Peak identities are: *E*, *trans* stereoisomer*; Z*, *cis* stereoisomer; Δ^X, position of the double-bond on the carbon chain, where X corresponds to the carbon atom counting from the carboxyl carbon end.

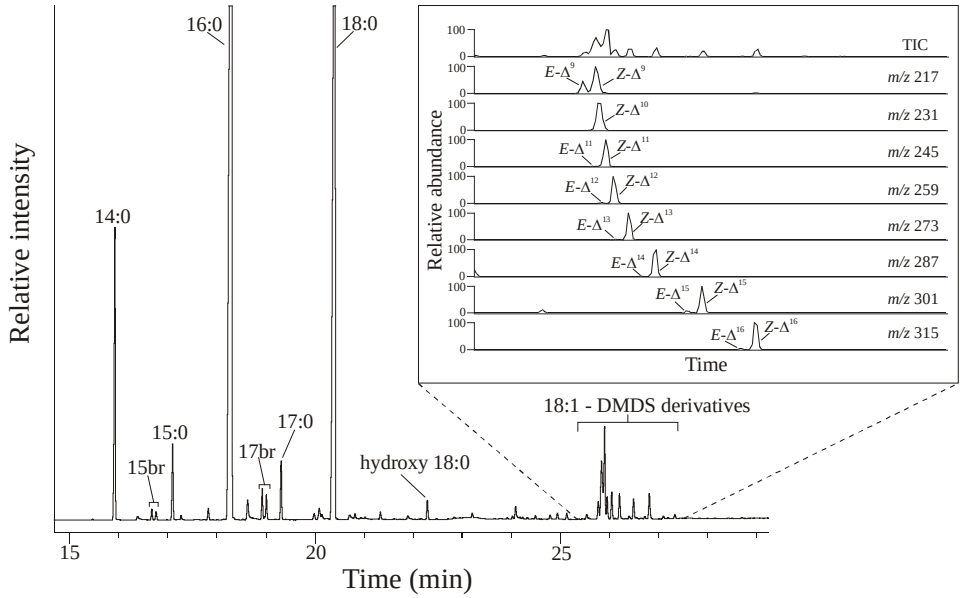
Figure 3. Electron ionisation (70eV) mass spectra of trimethylsilyl derivatives of a) 9- and 10-hydroxyoctadecanoic acids and b) 10-oxo-octadecanoic acid obtained through GC-MS analysis of 'bog butter' SB4 from Kyleakin, Skye.

Figure 4. Histograms representing the acyl carbon number distributions of triacylglycerols identified in the *in vitro* produced adipoceres from: a) mutton fat, and b) New Zealand butter, and 'bog butters' from: c) Lochar Moss**,** Dumfries (SB2) and d) Ross-shire (SB5).

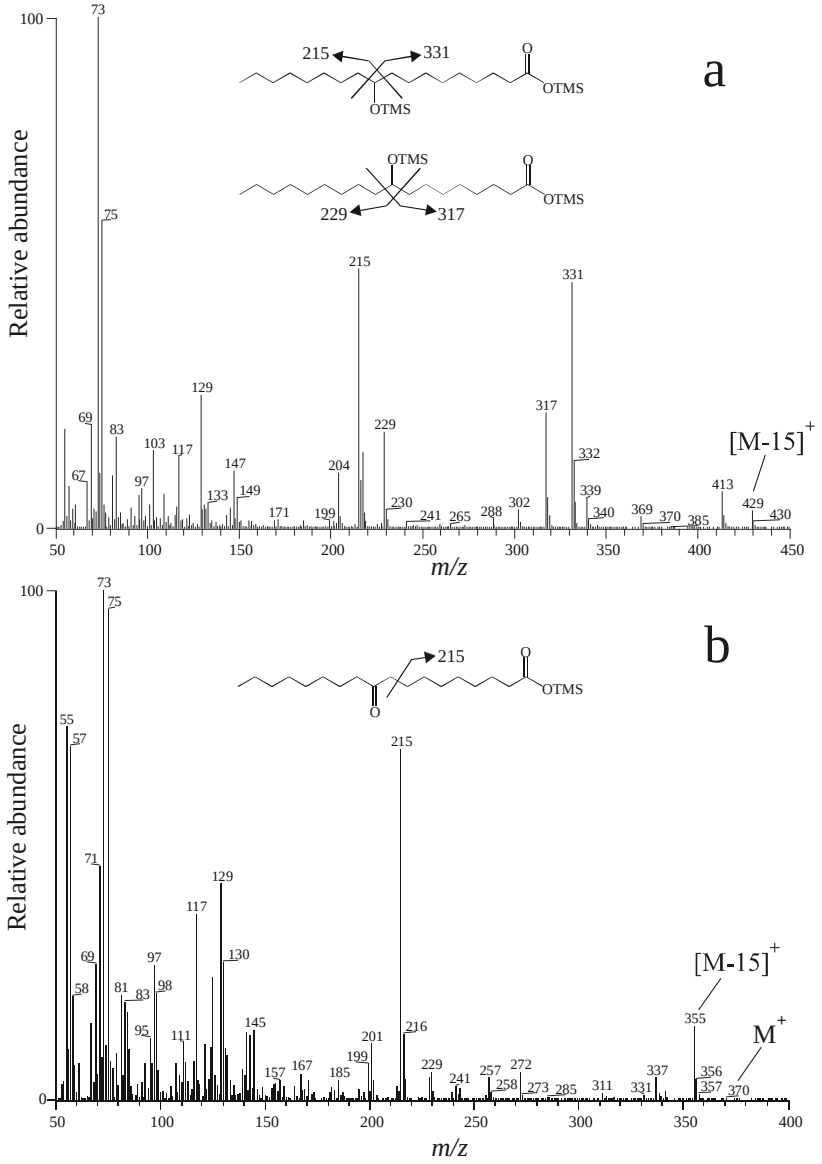
Figure 5. A plot of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids extracted from 'bog butters'. The confidence ellipses (1σ) correspond to modern reference ruminant animal fats (adipose and dairy), corrected for the post-industrial revolution effects of fossil fuel burning by the addition of 1.2 ‰.[36] a) Plots sub-samples from six different 'bog butters' corresponding with a dairy fat origin: ●, Gleann Geal, Morvern (SB1); ✖, Bunloit, Glenurquhart (SB3); ○, Cunnister, Shetland (SB9); ▲, Ross-shire (SB5); □, Kyleakin, Skye (SB4); ■, Durness, Sutherland (SB8). b) Displays sub-samples from three 'bog butters' with a ruminant fat origin: ●, Lochar Moss, Dumfries (SB2); ○, Rhuvoult, Rhiconich (SB6); ✖, Plockton, Loch Carron (SB7). Numbered labels refer to a particular sub-sample from a 'bog butter' mass and the 'int' and 'ext' labels corresponding to sub-samples taken from the interiors and exteriors of the 'bog butters'.



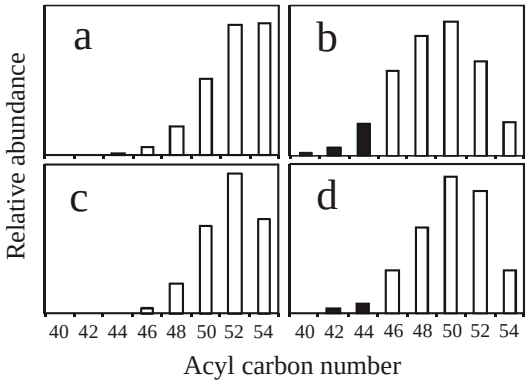
Berstan *et al*. Figure 1



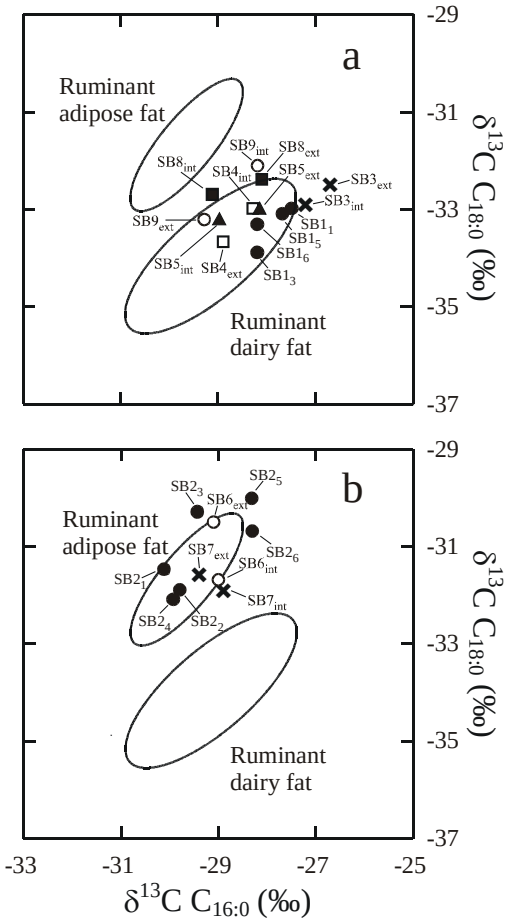
Berstan *et al*. Figure 2



Berstan *et al*. Figure 3



Berstan *et al*. Figure 4



Berstan *et al*. – Figure 5

Table 1. 'Bog butter' sample codes, origins and descriptions.

Laboratory number	Location of find	Sample code[a]	Description
SB1	Glen Gell, Morvern	ME 166	In an alder keg, 1.4 m (h) x 0.4 m (d). Radiocarbon date: 1802+/-35BP; 140-247 cal AD, (UB-3185)[34]
SB2	Lochar Moss**,** Dumfries	-	No container
SB3	Bunloit, Glenurquhart	ME 360	Discovered in a wooden container with dimensions of 0.46 m (h) x 0.29 m (d)
SB4	Kyleakin, Skye	ME 167	Discovered in an alder keg, 0.36 m (h) x 0.33 m (d). Radiocarbon date: 1730 +/- 35 BP; 246-346 cal AD, (UB-3186)[34]
SB5	Ross-shire (unprovenanced)	ME 171	No container
SB6	Rhuvoult, Rhiconich	ME 689	Packed inside an intestinal membrane
SB7	Plockton, Loch Carron	ME 174	Cattle hair discovered on 'bog butter' mass. Size: 0.46 m (h) x 0.28 m (d)
SB8	Durness, Sutherland	SCC9	Inside an oak trough, 0.73 m (l) x 0.28 m (w). Radiocarbon date of the trough: 940+/-80 BP; 960-1260 cal AD, (OxA-3010)[35]
SB9	Cunnister, Shetland	ME 222	Found within an oblong wooden dish

[a] Museum accession number