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Utilising phytanic acid diastereomers for the characterisation of archaeological lipid residues in pottery samples

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ABSTRACT

Phytanic acid diastereomers, 3*S*,7*R*,11*R*,15-phytanic acid (*SRR*) and 3*R*,7*R*,11*R*,15-phytanic acid (*RRR*), were determined by GC–MS in extracts of archaeological ceramic. The *SRR*% was higher in pottery from coastal sites corresponding with ^{13}C enriched *n*-alkanoic acid corroborating a predominantly marine origin for the food residues. Conversely, low *SRR*% and ^{13}C depleted *n*-alkanoic acid were found at inland sites, which are most likely derived from ruminant products. These observations are explained by differences in the bacterial transformation of phytol to phytanic acid between ruminant and aquatic organisms and allow these products to be easily distinguished in archaeological contexts.

Introduction

Molecular and stable isotope analyses of organic residues preserved in archaeological pottery provide valuable insights into the cooking practices and diet of past societies, and have become pivotal to the investigation of economic and cultural changes in the past. Organic matter (particularly lipids) is trapped within the clay matrix of ceramic vessels during food manipulation (e.g., cooking, storage) and occasionally forms a carbonized residue on their surfaces. A wide range of analytical procedures have been applied to characterise ancient biomolecules in pottery vessels but in recent years, gas chromatography mass spectrometry (GC–MS) and gas chromatography combined with isotope ratio mass spectrometry (GC-C-IRMS)[1,2] have become the methods of choice. These techniques have been routinely used to discriminate various mammal fats,[3] fish oils,[4,5] dairy products[6,7] and edible plants[8] based on the identification of lipid biomarkers and/or the isotopic criteria of less diagnostic compounds, such as *n*-alkanoic acids.

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a tetramethyl-branched isoprenoid fatty acid that is readily extracted from archaeological ceramics. This compound has been identified in some of the oldest pottery vessels in the world.[4,5,9–12] The presence of phytanic acid, together with other isoprenoid and ω-(o-alkylphenyl)alkanoic acids (APFAs) has been used to infer the processing of aquatic organisms.[4,5,9–12] Phytanic acid can also be found at high concentrations in ruminant carcass tissues and dairy products and is also a minor component of other food sources such as rabbit meat.[13] Phytanic acid originates from phytol[14] (Fig. 1), a constituent of chlorophyll. In ruminants, phytanic acid is formed in the rumen through bacterial oxidation and hydrogenation of phytol (3,7*R*,11*R*,15-tetramethylhexadec-2-en-1-ol).[14,15] In freshwater and marine organisms, it is formed from the digestion of phytoplankton chlorophyll by zooplankton and other invertebrates.[16] Phytanic acid also has the potential to be transmitted through the terrestrial and marine food chains[17–20] and often accumulates in lacustrine and marine sediments.[21]

Phytanic acid has three chiral centres at carbon positions 3, 7 and 11 (Fig. 1). In nature, the configuration of the stereocentre at position 3 may be (*S*) or (*R*) (diastereomers), while the other chiral centres are observed only in the (*R*) configuration, as in phytol.[22,23] Crucially, the ratio of 3*S*,7*R*,11*R*,15-phytanic acid (*SRR*) and 3*R*,7*R*,11*R*,15-phytanic acid (*RRR*) varies between organisms and depends on differences in its biosynthesis and dietary precursors. Higher *SRR*/*RRR* ratios have been reported in marine animal tissues[24–26] compared to terrestrial, and more subtle differences in the diastereomer ratios have been used as a means to authenticate organic ruminant milk and dairy products.[27–32] Based on this research, herein, we investigate the utility of using phytanic acid diastereomers recovered from archaeological pottery as a novel biomarker to distinguish aquatic and ruminant products. As

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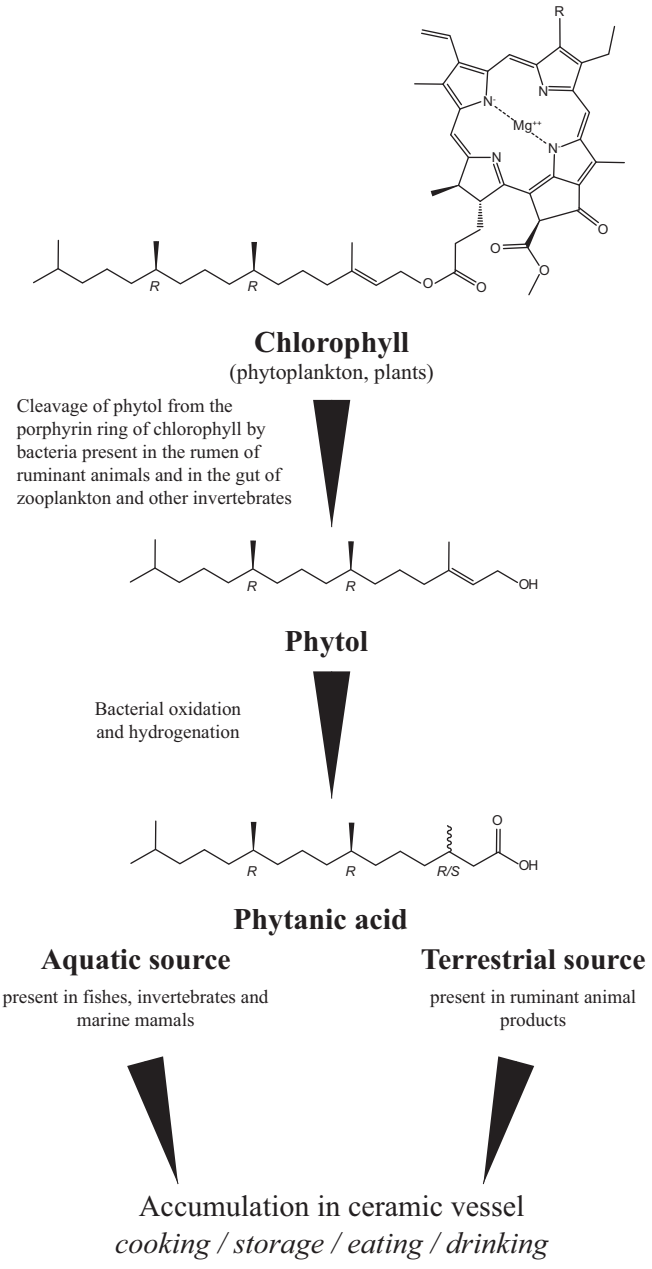

Figure 1. Schematic representation of the origin of phytanic acid in ceramic vessels.

phytanic acid is frequently found in archaeological cooking vessels, distinguishing its origin maybe extremely important in the absence of other more diagnostic compounds.

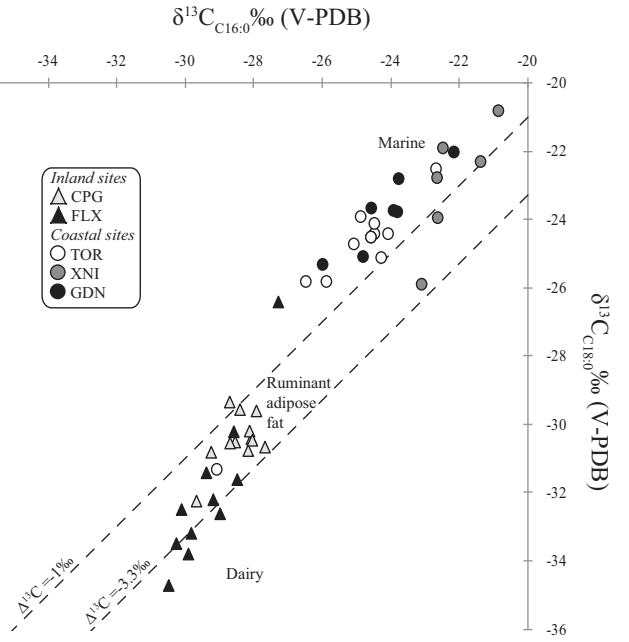

Figure 2. Fatty acid stable carbon isotope ratios – plot of the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from the archaeological residues extract considered in this study.

Results

The diastereomers of phytanic acid were determined in 48 pottery samples from 5 archaeological sites (Table 1); chosen due to their geographical location (coastal vs. inland) and associated economic activity (hunter–fisher–gatherer vs. farming). The contribution of the *SRR* isomer in total phytanic acid (*SRR*%) separates the pottery samples into two significantly different groups ($p < 0.001$; One-Way ANOVA). The first group where *SRR* dominates over the *RRR* are all from coastal sites occupied by hunter–fisher–gatherers in Japan and Alaska (TOR, XNI and GDN). The means *SSR*% of these groups are 81.5% (TOR), 88.9% (GDN) and 90.9% (XNI). Vessels from the second group with more similar relative abundances of *SRR* and *RRR*, come from two inland British medieval sites Britain (FLX, CPG). Here, the mean *SRR*% are 46.8% (CPG) and 54.8% (FLX). The various extracts yielded a diverse range of lipid concentrations (0.02–4.33 μg mg^{-1}), however no statistically significant correlation was found between the *SRR*% and the concentration ($r_s = -0.28$, $p < 0.06$; Spearman's *rho*).

To provide independent corroborative evidence regarding the source of residues in these pots, GC-C-IRMS was undertaken to determine the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ *n*-alkanoic acids (Fig. 2). The $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ acids ranged from −30.5‰ to −20.9‰, and −34.7‰ to −20.8‰, respectively. With the exception of one sample from Torihama, the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ for the coastal sites are greater than −26.5‰ and

Table 1
Summary of the results

Code	Site	Location	Context	No. samples analysed	No. samples yielding Aqu. Bio.	Average *SRR* (%)	Average value $\delta^{13}C_{16:0}$ (‰)	Average value $\delta^{13}C_{18:0}$ (‰)
TOR	Torihama	Japan	Coastal, HG	12	11	81.6 (±7.1)	−25.1 (±1.6)	−25.1 (±2.1)
GDN	Nunalleq	USA	Coastal, HG	7	6	88.9 (±3.8)	−24.2 (±1.2)	−23.8 (±1.2)
XNI	Nash Arbor	USA	Coastal, HG	6	4	90.9 (±2.3)	−22.2 (±0.9)	−22.9 (±1.8)
CPG	Coppergate	UK	Inland, Far	12	0	46.8 (±11.3)	−28.5 (±0.6)	−30.4 (±0.8)
FLX	Flixborough	UK	Inland, Far	11	0	54.8 (±7.8)	−29.3 (±0.9)	−32.0 (±2.2)

Hunter–fisher–gatherer (HG), Farming (Far). Aquatic biomarkers (Aqu. Bio.) are defined by the presence of a combination of specific compounds, C_{20} or C_{22} ω-(o-alkylphenyl) alkanoic acids associated with at least one isoprenoid fatty acid.[33] *SRR* (%) is the percentage contribution of *SRR* diastereomer in total phytanic acid.

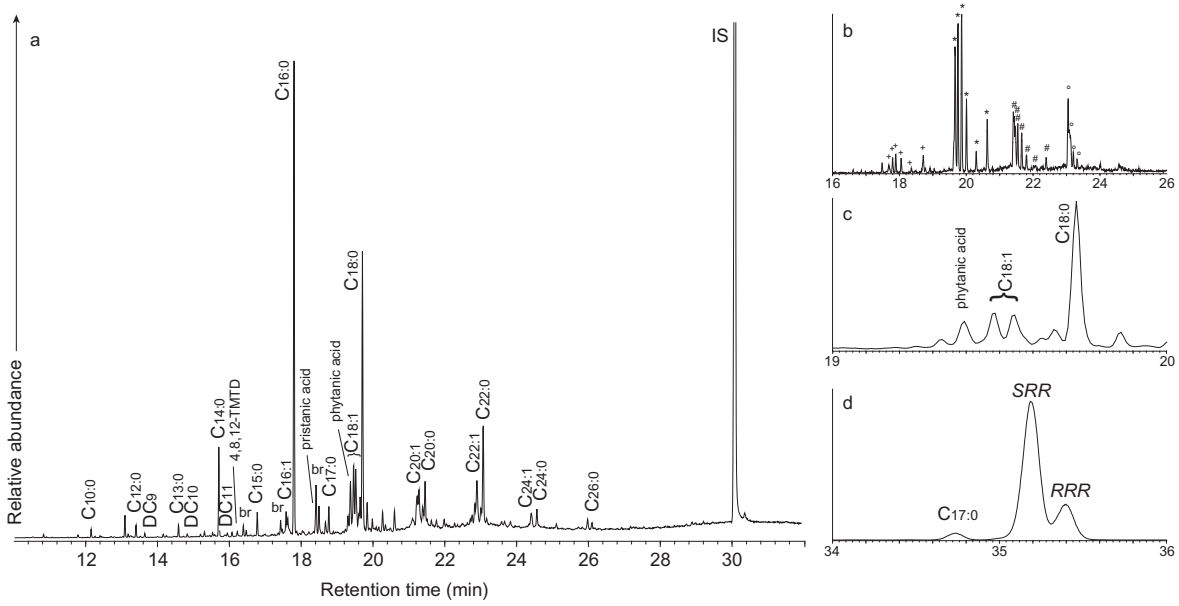

Figure 3. Partial gas chromatograms of a lipid extract from a charred deposit adhering to a Torihama potsherd (TOR136) – (a) The total ion chromatogram is characteristic of a degraded aquatic oil and is dominated by medium- and long-chain saturated and mono-unsaturated fatty acids and isoprenoid fatty acids, 4,8,12-TMTD (4,8,12-trimethyldecanoic acid), pristanic and phytanic. $C_{n:x}$, fatty acids with carbon length n and number of unsaturations *x*; DC_n, α,ω-dicarboxylic acids with carbon length *n*; br, branched chain acids; IS, internal standard (*n*-hexatriacontane). (b) The *m*/*z* 105 ion chromatogram shows the presence of ω(*o*-alkylphenyl)alkanoic acids with 16 (+), 18(∗), 20(#) and 22 (o) carbon atoms. The presence of the latter two components, thermally produced from C_{20} and C_{22} polyunsaturated fatty acids, and the distribution of their isomers confirm that this residue is derived from aquatic organisms.[33] (c) The partial total ion chromatogram shows that phytanic acid appears as a unique peak under GC–MS screening condition. (d) The diastereomers of phytanic acid are resolved under improved GC–MS conditions as shown in the *m*/*z* 101 ion chromatogram.

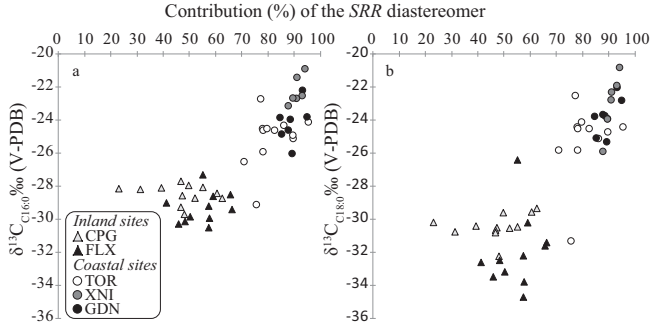

Figure 4. Relation between fatty acid stable carbon isotope ratios and SRR% diastereomer in total phytanic acid. Plots are of the $\delta^{13}C$ values of $C_{16:0}$ (a) and $C_{18:0}$ (b) *n*-alkanoic acids against *SRR%* from archaeological residues extract considered in this study.

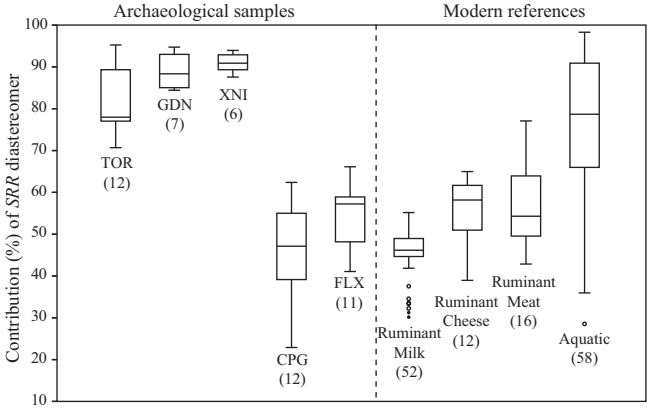

Figure 5. Boxplots of the *SRR%* diastereomer in total phytanic acid in archaeological samples and modern references. Plots represent median, ranges and quartiles with outliers marked. Data are from this study and those previously published.[18,19,24,25,27-29,35,36]

consistent with lipid originating from marine resources.[9,10] A high proportion of these samples also contained lipid biomarkers diagnostic of aquatic organisms,[33] such as other isoprenoid alkanoic acids (4,8,12-trimethyltridecanoic acid – 4,8,12,TMTD, and 3,7,11,15-tetramethylhexadecanoic acid, pristanic acid) and a wide array of ω-(o-alkylphenyl)alkanoic acids (Fig. 3, Table 1). In contrast, inland samples generally have $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ lower than −27‰, which are typically associated with terrestrial mammals, dairy products and plants. The absence of aquatic biomarkers in these samples, combined with the lower $\delta^{13}C$ values of $C_{18:0}$ compared to $C_{16:0}$ (expressed as $\Delta^{13}C$; <−1‰), in all but one FLX and one CPG sample, indicate that the main source of lipids were from ruminant meat and/or dairy products.[34]

Discussion

The presence of diagnostic molecular biomarkers and the stable isotope ratios of alkanoic acids extracted from coastal and inland pottery discussed in this study, clearly discriminate between the two main sources of animal products: aquatic and ruminant. The frequency of the two isomers of phytanic acid, *SRR* and *RRR* also differentiates between these two food groups. For example the *SRR%* show a strong positive linear correlation with the $\delta^{13}C$ values of palmitic acid and stearic acid ($r = 0.83$ and 0.84, respectively) (Fig. 4). This demonstrates that the ratio of phytanic acid stereoisomers to a large extent reflects the dominant source of animal products processed in the pottery.

Phytanic acid isomers have been studied in a variety of marine or freshwater fish, zooplankton, reptiles and mammals.[18,19,24,25,29,35,36] Variability of *SRR%* can be observed in aquatic ecosystems (e.g., 28.6–98.3%) and is related to the inherent complexity of phytanic acid synthesis and transmission in marine organisms.[17] Despite such variability, the *SRR* isomer generally

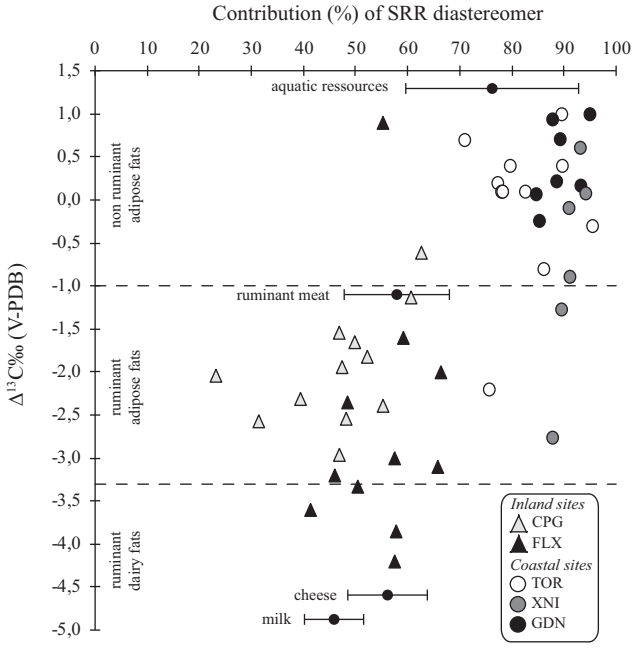

Figure 6. Plot of the $\Delta^{13}C$ values against the contribution of the *SRR* diastereomer in archaeological samples – $\triangle^{13}C$ ($=\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) ranges according the average ± 1 s.d. of a global database of modern reference animal fats,[34] *SRR%* according to average ± 1 s.d. of data available in the literature and results from this study.

predominates with an average value of 76% (±16.6, $n = 58$) (ESI, Supplementary Table 1). The results obtained from the coastal pottery are consistent with these modern aquatic data (Fig. 5). The majority of these samples also have $\Delta^{13}C$ values greater than −1‰, which is typically observed in non-ruminant mammals, plants and fish (Fig. 6).

Recent studies have demonstrated that the ratio of diastereoisomers in ruminant fat is directly related to feeding practice and in turn the composition of rumen bacteria. For example, phytanic acid directly synthesised in the laboratory by chemical hydrogenation and oxidation from plant phytol appears to have almost an equivalent abundance of both diastereomers.[23,35] A similar ratio has been encountered in organic milk, whereas conventional milk shows a clear dominance of *SRR*[29] linked to increased amount of maize silage in diet.[27,28,30,37]

Interestingly, organic cheese products also show a systematic increase of the *SRR%*.[28,38] The incorporation of increased silage in the feed of cattle for semi-hard cheese has been proposed as an explanation for this phenomena,[28] but samples of moose milk and cheese from the same farm also show an increase of the *SRR* %.[29] Thus, the preferential reduction of the *RRR* isomer, most likely during bacterial enzymatic transformation during the cheesemaking process, may serve as useful way for distinguishing cheese from milk in archaeological vessels. In total, five FLX samples have $\triangle^{13}C$ values consistent with ruminant dairy fats (i.e., $\triangle^{13}C < -3.3‰$[34]). Two of these have *SRR%* that are compatible with modern cheeses (Fig. 6). Clearly however, the effect of soil bacteria on alterations to the *SRR%* also needs to be considered, especially during the early stages of diagenesis. Nevertheless, our data suggest that meaningful *SRR%* is retained despite extensive exposure to the burial environment.

Conclusion

The ratio of the two naturally-occurring diastereomers of phytanic acid preserved in archaeological pottery distinguishes marine and ruminant food sources. The contribution of *SRR* correlates with stable isotope data from the same samples and so does not appear to be grossly affected by food cooking or post-depositional process. Nevertheless subtle differences occur between residues derived from milk and cheese and the impact of food preparation needs to be explored further. Moreover, as phytanic acid is also found in lipid extracts from archaeological bone,[39] the *SRR%* could offer new insights into cattle husbandry, seasonality or even human diet.[40]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.011.

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