Gas chromatographic mass spectrometric detection of dihydroxy fatty acids preserved in the ‘bound’ phase of organic residues of archaeological pottery vessels

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A methodology is presented for the determination of dihydroxy fatty acids preserved in the ‘bound’ phase of organic residues preserved in archaeological potsherds. The method comprises saponification, esterification, silica gel column chromatographic fractionation, and analysis by gas chromatography/mass spectrometry. The electron ionisation mass spectra of the trimethylsilyl ether methyl ester derivatives are characterised by fragment ions arising from cleavage of the bond between the two vicinal trimethylsiloxy groups. Other significant fragment ions are [M−15]+, [M−31]+, m/z 147 and ions characteristic of vicinal disubstituted (trimethylsiloxy) TMSO groups (Δ7,8, Δ9,10, Δ11,12 and Δ13,14; m/z 304, 332, 360 and 388, respectively). The dihydroxy fatty acids identified in archaeological extracts exhibited carbon numbers ranging from C16 to C22 and concentrations varying from 0.05 to 14.05 µg g⁻¹. The wide range of dihydroxy fatty acids observed indicates that this approach may be applied confidently in screening archaeological potsherds for the degradation products of monounsaturated fatty acids derived from commodities processed in archaeological pottery vessels. Copyright © 2011 John Wiley & Sons, Ltd.

Fragments of ceramic containers, namely potsherds, are common artefacts found at archaeological sites, with their inorganic porous ceramic matrix providing a medium conducive to the preservation of biomolecules and their degradation products derived from commodities processed in the vessels, particularly fats, oils, waxes and resinous materials.[1–5] Commonly occurring organic compounds absorbed in potsherds are preserved either as free components, retained via strong absorbative forces trapped within molecule-sized pores, or as chemically bound components, e.g. linked via ester bonds within a polymeric organic or organic-ceramic network.[6] Free compounds are readily extracted from the ceramic potsherd matrix using organic solvents (e.g. CHCl3/MeOH, 2:1 v/v), while the bound components can be released from the potsherd, following solvent extraction, by strong base hydrolysis (e.g. 0.5 M KOH in MeOH/H2O; 9:1 v/v).[7,8] To date, the majority of archaeological studies have focused on freely extractable lipid components and have resulted in the characterisation of organic residues for an impressive range of commodities from around the world.[9] In contrast, complex, ‘bound’ fatty acid oxidation products have been only recently been used as biological marker compounds (biomarkers) for plant oils and terrestrial or marine oils and fats.[3,10,11]

A wide range of oxygenated degradation products has been detected in potsherds as ‘bound’ residues including: fatty acids, mono- and dihydroxy fatty acids, ketoacids and α,ω-dicarboxylic acids.[8] However, thus far, vicinal dihydroxy fatty acids have shown the most promise as biomarkers in ‘bound’ residues, since the position of the additional hydroxyl groups reflects the original double-bond position of the monounsaturated fatty acid precursors present in the original processed commodities.[3,11,12]

The complex mixtures of oxygenated compounds released by base hydrolysis require a subsequent clean-up procedure prior to analysis and full characterisation by gas chromatography/mass spectrometry.[11] Presented herein is an effective analytical protocol based on saponification, esterification, flash silica gel column fractionation, and gas chromatography/electron ionisation mass spectrometry for the detection of vicinal dihydroxy fatty acids preserved as polymeric or adsorbed ‘bound’ residues from potsherds from the archaeological record.

EXPERIMENTAL

Archaeological potsherd samples (n = 12) were supplied by Museu Universitário Professor Oswaldo Rodrigues Cabral – Universidade Federal de Santa Catarina (Florianópolis, Brazil) and corresponded to Itararé tradition vessels associated with the pre-colonial Jê population (ca. 1000 AD). The vessels originated from sites located on the coastal islands of Santa Catarina State (São Francisco do Sul and Santa Catarina Islands), south Brazil.

A thin surface layer of potsherd (ca. 0.5 mm) was removed initially, using an electric hand drill, to avoid traces of external...
contamination. Sub-samples of finely ground sherds (2 g) were then extracted with CHCl₃/MeOH solution (2:1 v/v, 10 mL, 2 × 15 min sonication) to remove ‘free’ lipids. A solvent-extracted potsherd (1 g) containing insoluble residues was then saponified with 0.5 M NaOH (MeOH/H₂O solution, 9:1 v/v, 5 mL, 70 °C, 1 h) to liberate ‘bound’ components. After cooling, the mixture was centrifuged (2500 rpm, 15 min) and the supernatant removed and acidified to pH 3 with 3 M HCl. Solvent-extracted H₂O was added (1 mL) and the hydrolysed lipids extracted with CHCl₃ (3 × 3 mL). The solvent was then evaporated under a gentle stream of nitrogen gas until completely removed. The hydrolysed acid components were methylated using BF₃·MeOH (14% w/v, 100 µL, 70 °C, 1 h), followed by addition of 2 mL of solvent-extracted H₂O from which the methyl esters were then extracted with CHCl₃ (3 × 2 mL).

The methylated extracts were fractionated using a glass column (200 × 5 mm i.d.) incorporating a porous glass sinter to retain the packing of 1 g of activated silica gel (120 °C, 24 h). Packing was achieved by applying a slurry of the silica gel in n-hexane using a positive flow of nitrogen gas. Excess solvent was eluted until the solvent level was 1 mm above the top of the silica gel bed. The methylated extracts were quantitatively transferred onto the column using 1 mL of n-hexane, which was again run to a level of 1 mm above the silica gel bed. The extracts were separated by applying a positive pressure with nitrogen gas to give an elution rate of 15 mL min⁻¹, using the elutropic series of the following four solvent systems: n-hexane (6 mL), n-hexane/dichloromethane (DCM) (9:1 v/v, 2 mL), DCM (6 mL), DCM/MeOH (1:1 v/v, 5 mL). The dihydroxy fatty acids eluted in the DCM/MeOH system. All fractions were collected and solvent removed under a gentle stream of nitrogen.

The dihydroxy fatty acid fraction was derivatised by the addition of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) (30 µL, 70 °C, 30 min). Excess reagent was removed under nitrogen. Internal standard was added (10 µg, n-tetracontane) and the samples redissolved in n-hexane (200 µL) for analysis by GC/MS.

GC/MS analyses were performed using a ThermoFinnigan Trace GC/MS system (Thermo Fisher Scientific, Hemel Hempstead, UK) fitted with a programmable temperature vapourising (PTV) GC injector at 290 °C, and incorporating a quadrupole mass spectrometer. Helium was the carrier gas and the GC oven temperature programme was: 50 to 100 °C at 10 °C min⁻¹ then to 300 °C at 4 °C min⁻¹, isotherm for 10 min; and the column had a ZB1 stationary phase (60 m × 0.32 i.d. × 0.1 µm film thickness; Phenomenex, Macclesfield, UK). The mass spectrometer was operated in electron ionisation (EI) mode with the following parameters: transfer line temperature 300 °C, electron energy of 70 eV, emission current of 300 mA, ion source temperature 170 °C, with the mass analyser scanning the range m/z 50–600 with a cycle time of 1.0 s.

RESULTS AND DISCUSSION

Figure 1 depicts a partial gas chromatogram showing the distribution of lipids typically observed for the DCM/MeOH fraction. The major lipid oxidation products are hydroxy fatty acids and ω,ω-dicarboxylic acids. Vicinal dihydroxy fatty acids were identified by virtue of a specific cleavage between the two carbon atoms bearing the TMSO groups, resulting in fragment ions highly diagnostic of either a specific compound or an isomer bearing the same number of carbon atoms (Scheme 1). For example, the fragment ions at m/z 215 and m/z 287 are generated by derivatised 11,12-dihydroxyarachidic acid, and those at m/z 243 and m/z 259 by derivatised 9,10-dihydroxyarachidic acid (Figs 2A and B, respectively).

Figure 1. Total ion current ion (TIC) chromatogram showing the characteristic range of compounds observed in the DCM/MeOH fraction eluted from a flash silica gel column of an alkaline extract of the polymerised ‘bound’ phase of an archaeological potsherd.
The GC resolution is challenging for isomers bearing the same number of carbon atoms (Fig. 1); thus, the identification of compounds is only possible through the characteristic fragment ions depicted in Scheme 1. The sum of these fragment ions yields the molecular weight, which may be corroborated by the presence of [M–15] and [M–31] fragments (for methyl ester-TMS derivatives; Figs. 2 (A) and 2(B)). Other characteristic fragment ions are those observed at m/z 147 and for vicinal dihydroxy acids substituted at Δ7,8, Δ9,10, Δ11,12 and Δ13,14, at m/z 304, 332,
360 and 388, respectively. The Δ-substituted fragments are rearrangements of mid-chain vicinal dihydroxy TMS methyl esters, with their formation involving a cleavage of the bond between the two vicinal TMSO groups and migration of the eliminated TMS to the ester group. These characteristic fragment ions are shown in Figs. 2(A) and 2(B) for the Δ¹¹,₁₂- and Δ⁹,₁₀-dihydroxyarachidic acids, respectively. The EI mass spectra of the α,ω-dicarboxylic acids were readily characterised by the presence of fragment ions at m/z 98, [M-31]⁺ and [M-73]⁺ (for methyl ester derivatives).

Vicinal dihydroxy fatty acids may be observed in extracts of all sherds with different mixtures of isomers and chain lengths, ranging from C₁₆ to C₂₂, varying between sherds (Fig. 3). Threo and erythro pairs of diastereoisomers of vicinal dihydroxy fatty acids are present in all the chromatograms (Fig. 1), their mass spectra being almost identical. α,ω-Dicarboxylic acids are present in most of the sherds with

Table 1. Semi-quantitative concentrations and maximum and minimum concentrations of the single dihydroxy fatty acid isomers detected

<table>
<thead>
<tr>
<th>Acids Sherds</th>
<th>C₁₆-</th>
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<td>E775 (µg g⁻¹)</td>
<td>0.18</td>
<td>4.11</td>
<td>0.12</td>
<td>8.69</td>
<td>1.13</td>
<td>0.42</td>
<td>0.84</td>
<td>3.91</td>
<td>0.12</td>
<td>1.52</td>
<td>0.3</td>
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<td>L687A (µg g⁻¹)</td>
<td>0.15</td>
<td>1.15</td>
<td>nd</td>
<td>14.05</td>
<td>1.79</td>
<td>nd</td>
<td>1.09</td>
<td>0.09</td>
<td>nd</td>
<td>3.01</td>
<td>0.51</td>
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<td>ME17 (µg g⁻¹)</td>
<td>0.16</td>
<td>2.14</td>
<td>nd</td>
<td>6.18</td>
<td>0.94</td>
<td>0.28</td>
<td>1.47</td>
<td>8.18</td>
<td>nd</td>
<td>6.71</td>
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<tr>
<td>MG19A (µg g⁻¹)</td>
<td>0.05</td>
<td>1.18</td>
<td>nd</td>
<td>2.67</td>
<td>0.27</td>
<td>nd</td>
<td>0.37</td>
<td>2.71</td>
<td>nd</td>
<td>0.67</td>
<td>0.11</td>
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<td>MS51 (µg g⁻¹)</td>
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<td>8.45</td>
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<td>M935 (µg g⁻¹)</td>
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<td>nd</td>
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<td>1.84</td>
<td>0.07</td>
<td>0.69</td>
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<td>range (µg g⁻¹)</td>
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<td>0.27-14.05</td>
<td>0.07-8.18</td>
<td>0.05-3.01</td>
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aDihydroxy fatty acid concentrations were calculated based on the areas of fragment ion ‘e’ mass chromatogram peak (Scheme 1) of the threo-diastereoisomer and the m/z 71 mass chromatogram of the internal standard added before GC/MS analysis (10 µg, n-tetratriacontane).
the processing of commodities containing high abundances of fatty acids and their positional isomers present in the lipid oils and animals fats. The narrow range of vicinal dihydroxy products or marine and terrestrial commodities, such as plant (iii) the processing of mixtures of either different marine 

0.05 to 14.05 µg g−1 (Table 1). The C18 vicinal diol isomers are observed at highest concentrations, with the lowest concentration detected being 0.27 µg g−1. The distribution of variables (concentration × area) for components with the same carbon number appears to be normal within the range; a strong linear relationship (R² >0.98) is seen for all isomers of the same carbon number (Table 1). As already discussed above such a wide range of vicinal dihydroxy fatty acids bearing C16 to C22 carbon atoms and the different positional isomers seen for a given homologue is typical of that expected to arise from the monounsaturated fatty acids commonly observed to occur in marine oils and fats. This is consistent with the coastal locations from which the potsherds were recovered and the other archaeological finds, i.e. the remains of marine fauna.[11,18,19] The experimental approach detailed above has considerable potential value in the analysis of any plant, and marine or terrestrial animal oil/fats likely to contain significant concentrations of monounsaturated fatty acids. Since dihydroxy fatty acids are the direct degradation products of monounsaturated fatty acids, the detection of vicinal dihydroxy fatty acid distributions is a useful means of inferring the original fatty acid composition and thus which commodities were processed in archaeological pottery vessels. α-ω-Alkylphenylalkanoic acids have also been used for detecting the processing of marine oil/fats in archaeological vessels; however, the formation of these compounds is reliant on protracted periods of vessel heating.[20] In the case of vicinal dihydroxy fatty acids, heating is unnecessary since oxidation of monounsaturated fatty acids occurs spontaneously even at room temperature.[16]

Thus, their presence in archaeological pottery vessels is expected to be more common than that of α-ω-Alkylphenylalkanoic acids. Finally, such is the purity of the dihydroxy fatty acid fraction isolated by saponification and silica gel column chromatography that, assuming there is not a large quantity of other dihydroxy fatty acids present, e.g. isomers with ω-hydroxy functionalities, the vicinal dihydroxy fatty acids pattern could be, in principle, determined using GC alone.

CONCLUSIONS

An effective GC/MS-based protocol for the analysis of vicinal dihydroxy fatty acids preserved as polymerised/bound residues in archaeological potsherds was reported. The method comprises saponification, esterification, ‘flash’ silica gel column chromatography, and identification using GC/MS. A total of eleven vicinal dihydroxy fatty acids were detected with carbon numbers ranging from C16 to C22 with vicinal dihydroxy fatty acid diastereoisomers present in all instances. The wide range of dihydroxy fatty acids observed indicates that this approach may be applied confidently in screening archaeological potsherds for the degradation products of monounsaturated fatty acids derived from commodities processed in archaeological pottery vessels.

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REFERENCES


