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ORIGINAL PAPER

Brassicaceae seed oil identified as illuminant in Nilotic shells from a first millennium AD Coptic church in Bawit, Egypt

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Abstract Burned greasy deposits were found inside shells of the large Nile bivalve *Chambardia rubens*, excavated in an eight- to tenth- century AD church of the Coptic monastery of Bawit, Egypt, and supposedly used as oil lamps. The residues were subjected to a combination of chromatographic residue analysis techniques. The rather high concentrations of unsaturated fatty acids, as analysed by gas chromatography (GC) in the methylated extract, suggest the presence of a vegetal oil. Analysis of the stable carbon isotopes (δ^{13}C values) of the methyl esters also favoured plants over animals as the lipid source. In the search for biomarkers by GC coupled to mass spectrometry on a silylated extract, a range of diacids together with high concentrations of 13,14-dihydroxydocosanoate and 11,12-dihydroxyeicosanoate were found. These compounds are oxidation products of erucic acid and gondoic acid, which are abundantly present in seeds of Brassicaceae plants. Liquid chromatography coupled to mass spectrometry analysis showed low concentrations of unaltered triglycerides, but revealed sizeable amounts of triglycerides with at least one dihydroxylated acyl chain. The unusual preservation of dihydroxylated triglycerides and α,ω-dicarboxylic acids can be related to the dry preservation conditions. Analysis of the stereoisomers of the dihydroxylated fatty acids allows one to determine whether oxidation took place during burning of the fuel or afterwards. The results prove that the oil of rapeseed (*Brassica napus* L.) or radish (*Raphanus sativus* L.) was used as illuminant in early Islamic Egypt, and that not only ceramic lamps but also mollusk shells were used as fuel containers.

Keywords Residue analysis · Egyptian lamp shells · HPLC-MS · GC-C-IRMS · GC-MS · Brassicaceae seed oil

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Introduction

Organic-chemical residue analysis of archaeological material gained progressively more attention during the past decade. Indeed, chemical recognition of ancient food remains might add key elements to archaeological knowledge [1]. Lipids are often at the heart of such investigations.

Indeed, the pores of ceramic material seem to be an ideal conservation matrix for these nonpolar compounds, even in wet conditions [2, 3]. While apolar lipids are generally the only recoverable compounds in samples from humid climates, more polar molecules might be preserved in arid regions because of the limited exposure to water and hydrolysis [4, 5]. Such compounds include polyphenols, as indicators for wine storage in amphorae [6–8], but characteristic polar metabolites of lipid compounds may be identified as well.

A broad range of analytical tools are at hand to establish nature and origin of lipids. The main technique is gas chromatography (GC) coupled to different detection types like flame ionization detection (FID), mass spectrometry (MS) and isotope ratio mass spectrometry (IRMS) [9]. For high molecular weight lipids, liquid chromatography with mass spectrometry (LC-MS) is a frequently used technique [10, 11]. For examination of archaeological material, appropriate extraction and derivatization procedures are required because the sample size is restricted and lipid concentrations are often close to the detection limit [3]. Even within this well-studied domain of lipid analysis, several open questions remain. For instance, it proved difficult to unambiguously propose biomarkers for fish, or for processed fish products like the *garum* condiment [12].

Residue analysis is not only able to identify the source of food products, but can also deliver information on numerous other commodities or luxury goods, such as ointments and other personal care products [13] or lighting fuels [14]. In studies of ceramic oil lamps, a variety of illuminants have been identified such as vegetal oils, ruminant fat or beeswax [15]. In a fifth-century oil lamp from Northern Africa, Passi [2] found 9,10-dihydroxystearic acid, suggesting that the lamp may have contained an oxidized, rancid oil. In ancient oil lamps from Sagalassos, Turkey, researchers found olive oil as fuel together with traces of animal fat, based on analysis of the triglycerides with LC-MS [14]. Intensive studies were carried out on Egyptian ceramic oil lamps in which monocarboxylic acids, α,ω-dicarboxylic acids, and long-chain dihydroxylated acids were found, indicating the presence of Brassicaceae oil [16, 17].

In this work, we look for the first time at archaeological lamp fuels in a non-ceramic matrix. The residues studied were recovered from *Chambardia rubens* shells, supposedly used as lamps in Egypt during the early Islamic period [18]. The aim of this research is not only to prove that these shells were used as lamps, but also to identify the source of the fuel. A wide range of methodologies are applied like fatty acid methyl ester (FAME) analysis by GC, identification of silylated compounds by GC-MS, determination of $\delta^{13}C$ values (‰) of FAME by GC-C-IRMS and LC-MS with atmospherical pressure chemical ionization (APCI).

Experimental

Samples

The study concerns the site of Bawit, a Coptic monastery that was founded at the end of the fourth century AD and abandoned in the tenth century. It is located on the west bank of the Nile, about 280 km south of Cairo. Excavations carried out here since the early twentieth century by both French and Egyptian teams revealed the presence of two churches, habitation quarters and a large number of cells provided with oratories, often decorated with wall paintings [19–21]. During the on-site identifications of the faunal remains from the 2003–2005 excavations of the northern church of Bawit one of us noted the presence of numerous large shells of the Nile bivalve *Chambardia rubens arcuata*, a taxon previously mentioned in the literature as *Aspatharia rubens* and sometimes *Spathopsis rubens* [22]. The greatest length of the completely preserved valves varied between 9 and 14 cm. It was striking that 19 of the 37 valves were burned and that many of them had a black crust adhering to the interior side (Fig. 1). The abundance of the shells in the church and their burned aspect suggested that these valves were used as containers for an illuminant. Other lamps have been found during the previous excavations in 1901–1913 (some of them are kept in the Louvre) and during the recent excavations [18]. Lipid analysis has been carried out on samples of black crust taken from the interior of eight shells.

Lipid analyses

Standard lipid analysis on the eight samples started with a chloroform/methanol (2:1) solvent extraction of the material. Four of the total lipid extracts were subjected to a selective transesterification of the triglycerides prior to analysis of the fatty acid methyl esters by polar-phase gas chromatography (PPGC) and GC combustion isotope ratio

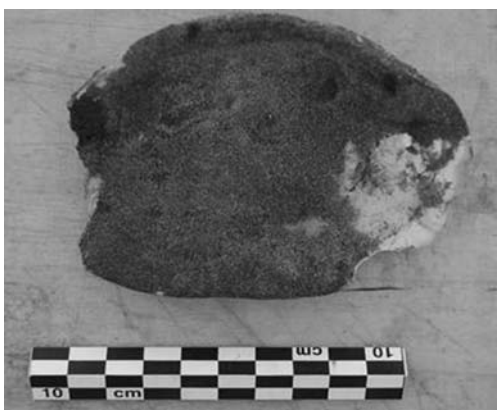

Fig. 1 Picture of a lamp shell from the northern church (ASP2)

mass spectrometry (GC-C-IRMS). For more detailed information, we refer to previous work [8, 11]. The four other extracts were subjected to silylation derivatization, prior to analysis by gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS). For the silylation, 50 μL *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (97%) (MSTFA) was added to the lipid extract and dissolved in 100 μL toluene. This mixture was held at 60 °C for 1 h before the solvent was removed under a stream of nitrogen. The silylated lipids were dissolved in 50 μL toluene before analysis. GC-MS analyses were performed on an Agilent 5973 Network Mass Selective Detector coupled to an Agilent 6890N GC with a 30 m HP5MS capillary column with an internal diameter of 0.25 mm. A 1 μL aliquot of the sample was injected in the splitless mode at a temperature of 290 °C. The oven temperature was held at 140 °C for 2 min, increased to 325 °C at 4 °C min^{-1} and kept there for 5 min. Afterwards a second step going to 340 °C at 1 °C min^{-1} was programmed. The mass spectrometer was programmed at a temperature of 340 °C. Mass spectra were taken between *m/z* 50 and 800 with *m/z*, according to the IUPAC definition, being the mass to charge ratio used to denote the dimensionless quantity formed by dividing the mass number of an ion by its charge number. The silylated lipid extracts were also analysed on an HPLC-MS with atmospheric pressure chemical ionization (APCI) to obtain a triglyceride profile [11]. The *m/z* detection range was set at 200–1,300 in the positive mode.

Results

Extraction

The sample weights and corresponding yields of extracted lipids are presented in Table 1, together with the analysis types performed on each sample. Because of the small sample size and the ensuing impossibility to perform two derivatizations on one sample, half of the samples were subjected to FAME and GC-C-IRMS analysis, while GC and LC-MS analysis was applied to the remaining ones. As all samples were collected from the same spot, we assume that their usage and fuel were similar. While it cannot be excluded that the shells were re-used, the fact that they are much less precious than e.g. ceramic lamps makes re-use less plausible.

FAME analysis

The standard FAME analysis aims to quantify the concentrations of saturated or unsaturated C_{12}–C_{18} fatty acid residues after conversion to their methyl derivatives. Hence, using this method, metabolites of these acyl residues cannot be identified with certainty, as will be addressed below. The similarity of the fatty acid profiles from all four samples confirms the initial assumption that they indeed have the same origin. The concentrations of the fatty acid methyl esters (FAMEs), as analysed by PPGC, are strikingly low; the four samples contained only 0.3–1 μmol FAME per gram sample. It should be noted that the FAMEs were exclusively obtained by transesterification of triglycerides, and not by methylation of free fatty acids. The latter will be studied in more detail in the silylated form by GC-MS. The low FAME concentrations thus are related to low concentrations of intact triglycerides. A representative fatty acid profile is shown in Fig. 2. Relatively high amounts of methyl oleate and methyl *cis*-hexadecenoate were observed. This is unexpected because unsaturated fatty acids are more sensitive to autoxidation [23–25].

Another way of interpreting PPGC results is based on evaluating fatty acid ratios rather than absolute values. When an animal fat had been used rather than a vegetal product, high amounts of methyl stearate, a saturated fatty acid, would be detected. Consequently, a low ratio of methyl palmitate to methyl stearate (P/S) and a high ratio of methyl stearate to methyl oleate (S/O) would be observed [25, 26]. Here, on the contrary, high P/S ratios are observed. This excludes the possibility that an animal fat was used as the major fuel compound. Additionally, the S/O ratios are low, with comparable amounts of stearate and oleate (Table 2). Taking into account the high degradability of oleate, it is to be assumed that the original product had a high oleate content, like a typical vegetal oil.

Table 1 Results of lipid extraction and outline of the analyses performed for each sample

Sample	Weight (g)	Lipids (%)	Analysis
ASP1	0.62	6.2	PPGC & GC-C-IRMS
ASP2	1.58	0.3	GC-MS & LC-MS
ASP3	0.37	0.6	GC-MS & LC-MS
ASP4	0.85	0.8	GC-MS & LC-MS
ASP5	0.57	11.7	PPGC & GC-C-IRMS
ASP6	0.22	37.5	GC-MS & LC-MS
ASP7	0.40	4.6	PPGC & GC-C-IRMS
ASP8	1.59	0.8	PPGC & GC-C-IRMS

GC-C-IRMS gas chromatography combustion isotope ratio mass spectrometry, *GC-MS* gas chromatography mass spectrometry, *LC-MS* liquid chromatography mass spectrometry, *PPGC* polar-phase gas chromatography

Stable carbon isotope ratios

$\delta^{13}C$ values (‰) of FAME can help in elucidating the animal origin of a lipid sample because each species has its characteristic isotopic signature in the stearic and palmitic residues (Fig. 3) [9, 27]. These highly specific $\delta^{13}C$ values

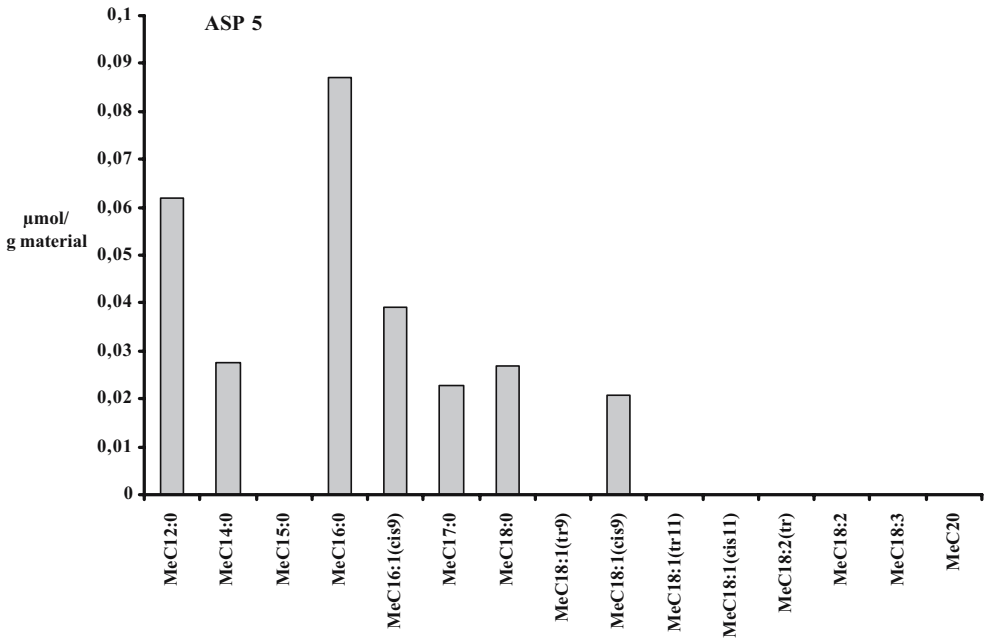

Fig. 2 Fatty acid profile of sample ASP5. The amounts of FAME are expressed in µmol FAME per gram original sample

(‰) are the result of the dissimilar routing of dietary carbon and fatty acids during synthesis of fats in the body. For example, dairy fats can be recognized because the $\delta^{13}C$ values (‰) of C18 fatty acids in dairy products are 2.3‰ lower than in ruminant adipose tissue. This difference can be ascribed to the fact that the mammary gland lacks the ability to synthesize C18:0, to the bacteria in the rumen which induce the biohydrogenation of unsaturated fatty acids and to differences in the $\delta^{13}C$ values (‰) of the plant dietary fatty acids and carbohydrates [27]. As the PPGC results rather point towards a vegetal rather than an animal source, it is necessary to also consider reference data of vegetal oils, as is done in Table 3 and Fig. 3.

Figure 3 shows that the $\delta^{13}C$ values of our samples are clustered; the $\Delta^{13}C$ values ($\Delta^{13}C = \delta^{13}C_{C18:0} - \delta^{13}C_{C16:0}$) are all close to 0‰. Such values cannot be assigned to a particular animal lipid type, but may well correspond to a vegetal oil. Isotopic analysis is not capable of discriminating between various C_3 vegetal oils, because of their similar isotopic signatures (Table 3) [28]. Differentiating between C_4 and C_3 plants is much easier because C_4 plants follow the Calvin cycle, resulting in relatively depleted $\delta^{13}C$ values (‰) like for example $\delta^{13}C_{C16:0} = -14‰$ [29–31]. This excludes the possibility that a C_4 plant oil was used as a fuel, in agreement with the fact that all the plants traditionally used for oil production in Egypt are C_3 species. When reference $\delta^{13}C$ values (‰) for rapeseed oil [28] and radish seed [32] are plotted in Fig. 3, it can be seen that the values of the archaeological samples rather resemble that of radish seed oil than that of rapeseed oil, but more evidence for such an assignment will only be supplied based on GC-MS results (vide infra).

Triglyceride profiles

HPLC-MS with APCI is capable of analysing triglycerides (TAGs) not only in animal fats [10] but also in vegetal oils. Although TAGs are sensitive to hydrolysis during burial of archaeological samples, in some cases a fraction remains preserved which can be of great value in finding the origin of fats [8, 11]. In the HPLC-MS analyses of the lipid extracts from the shells, the focus was first on TAGs containing the saturated or unsaturated C_{12}–C_{18} fatty acids. Concentrations of such TAGs were generally below the detection limit, except in sample ASP6. In this sample, very small amounts of glycerol-dioleate-linoleate and glycerol-dioleate-palmitate were identified, in line with the results of the FAME analysis.

Table 2 Overview of fatty acid ratios from the Egyptian lamp shells and some reference fats

Sample/reference fat	P/S	P/O	S/O
ASP1	3.3	3.8	1.2
ASP5	3.3	4.2	1.3
ASP7	4.1	5.1	1.3
ASP8	1.9	4.5	2.4
Ruminant adipose fat	< 1.3		
Ruminant dairy fat	2.9		
Non-ruminant adipose fat	> 1.3		
Rapeseed oil	3.1		
Radish seed oil	3.5		
Olive oil	4.0		

P/S, *P/O* and *S/O* stand for the ratio of methyl palmitate to methyl stearate, methyl palmitate to methyl oleate, and methyl stearate to methyl oleate, respectively

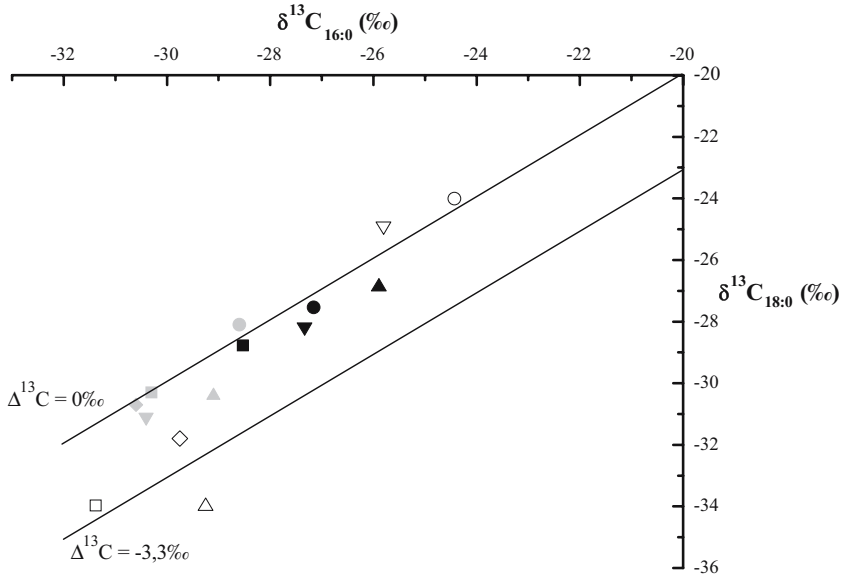

Fig. 3 $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ plot (expressed in ‰) with ■, ●, ▼ and ▲ representing samples ASP1, ASP5, ASP7 and ASP8 respectively. The other symbols stand for sheep (□), cattle raised on a C_3 plants diet (◇), poultry (○), porcine (▽), dairy fats from cows raised on a C_3 plants diet (△), palm oil (▼), groundnut oil (▲), sunflower oil (♦), rapeseed oil (■) and radish seed oil (●). All values were obtained with modern tissue. Reference values for adipose tissue (−29.8; −31.9) and milk (−29.2; −34.0) from C_3-fed cattle have been taken from literature [28, 32, 33]

Besides these usual TAGs, a number of other, higher molecular weight compounds of unknown structure showed up at longer retention times in the LC-MS chromatograms (Fig. 4). As the analysis was performed on a silylated sample, and the silylating agent introduces a large amount of nitrogen into the sample, it is not surprising that TAGs can appear in the mass spectrum as $[M+NH_4]^+$ ions, with an even mass for the base peak instead of the usual odd values for $[M+H]^+$. This was confirmed by an independent analysis of trilaurin, against a background of silylating agent and its decomposition products. Additionally, all high molecular weight compounds contain at least two and even three $(CH_3)_3SiO$ groups, as evidenced by two or three consecutive losses of mass 90 ($-(CH_3)_3SiOH$) in the fragmentation patterns. As a result, all unknown compounds could be identified as TAGs with 46 or 48 C atoms in the acyl chains, and with two or three –OH groups that are silylated during derivatization. Peaks resulting from $(CH_3)_3SiOH$ loss are very intense, making it difficult to assign precise molecular structures to the ions observed. For instance, the ion at *m/z* 1,000.5 may arise not only from glycerol-laurate-myristate-(13,14-di(trimethylsilyloxy) docosanoate), but also from glycerol-myristate-palmitate-(9,10-di(trimethylsilyloxy)octadecanoate). In any case, the presence of dihydroxylated fatty acids in the triglycerides is beyond doubt. Similar fatty residues will be found by GC-MS (see below).

Table 3 $\delta^{13}C$ values (‰) of a few modern vegetal oils [28, 32]

Oil	($\delta^{13}C_{C16:0}$; $\delta^{13}C_{C18:0}$) (‰)	$\Delta^{13}C$ value (%)
Groundnut	(−29.1; −30.4)	−1.3
Palm	(−30.4; −31.1)	−0.7
Rapeseed	(−30.3; −30.3)	0
Radish seed	(−28.6; −28.1)	0.5
Sunflower	(−30.6; −30.7)	−0.1

Analysis of silylated derivates

The GC-MS chromatograms provided strong evidence for three groups of characteristic compounds. First, in three of the four samples, a range of short-chain dicarboxylic acids were found (Fig. 5). The latter are oxidation products of unsaturated fatty acids formed during combustion or burial of the oil [16, 17]. They range from six up to thirteen carbon atoms, azaleic acid (C9) being most abundant. The mass spectra of the identified diacids are characterized by the fragment ions $[M-15]^+$ and $[M-131]^+$. In all samples, saturated fatty acids with up to 24 carbon atoms were identified. The highest concentrations were found for docosanoic or behenic acid (C22:0). Because of its high concentration, behenic acid must originate from the fuel itself. Indeed, it cannot be a degradation product from erucic acid (C22:1(13)) because the required reduction is improbable during the oxidizing burial conditions [23].

The base peak of the chromatogram in Fig. 5 (35.8 min) can be assigned to trisilylated 13,14-dihydroxydocosanoate. Together with 11,12-dihydroxyeicosanoate, these dihydroxylated fatty acid molecules are oxidation products of erucic acid and gondoic acid (C20:1(11)), respectively. The latter fatty acids are known to be abundant in oils from seeds of Brassicaceae plants containing up to 60% erucic acid and 20% gondoic acid [16, 17]. The presence of 9,10-dihydroxyoctadecanoate was also confirmed in the GC-MS spectra, although in lower concentrations. Vicinal dihy-

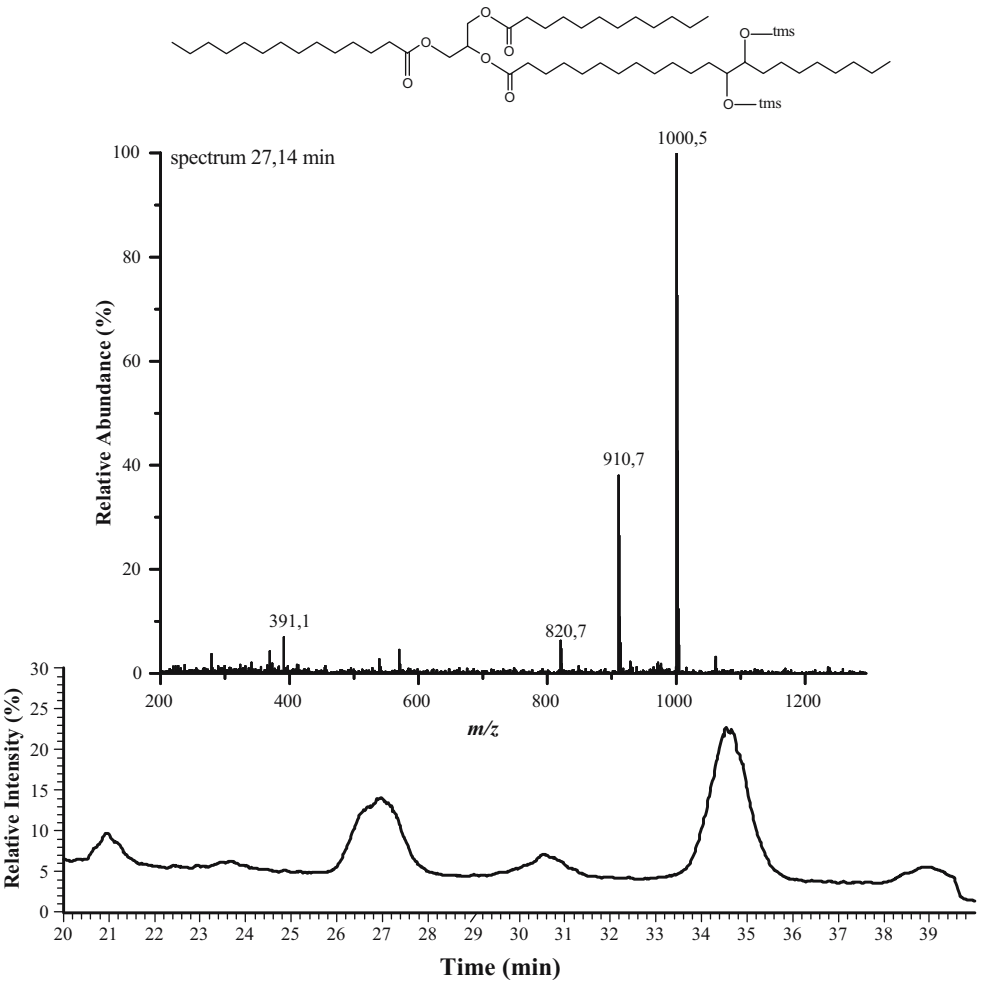

Fig. 4 Partial APCI HPLC-MS chromatogram of sample ASP6, containing very small concentrations of intact TAGs but considerable amounts of silylated dihydroxylated or trihydroxylated TAGs; the mass spectrum of a disilylated dihydroxylated compound eluting at 27.14 min is shown as an *inset*

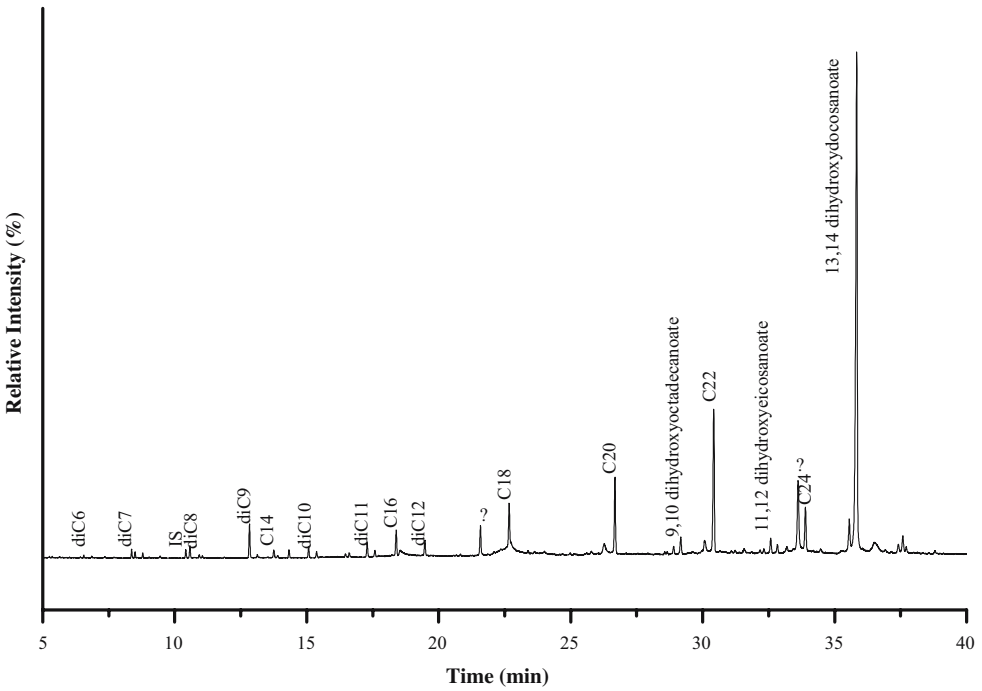

Fig. 5 Reconstructed total ion GC-MS chromatogram of sample ASP4: *diCn* represents a diacid with *n* carbon atoms, *Cn* a saturated fatty acid with *n* carbon atoms, while *IS* stands for *n*-heptadecane (the internal standard). All acids and hydroxylated compounds are in the trimethylsilylated form

droxyacids are formed through dihydroxylation of the double bond of monounsaturated acids. As they can exist as *threo* or *erythro* stereoisomers, the chromatograms usually contained two peaks with near-identical mass spectra (Fig. 6).

Additional information is contained in the isomer composition of the dihydroxylated fatty acids. The elution order of the *threo* and *erythro* isomers was assigned performing the stereospecific Os-catalysed *cis*-dihydroxylation of double bonds [34]. Indeed, *cis*-dihydroxylation of *cis* fatty acids such as oleic or erucic acid yields the *erythro* isomers, while *cis*-dihydroxylation of *trans* fatty acids such as elaidic or brassidic acid yields the *threo* isomers. Analysis of the silyl derivates of the synthesized dihydroxyacids by GC-MS, proved that the *threo* isomer elutes before the *erythro* isomer. Additionally, it was confirmed that neither the derivatization step nor the injection in the GC result in epimerization of the dihydroxyacids. In the archaeological samples of the present study, two distinct situations were encountered. For the C_{18} and C_{20} dihydroxyacids, comparable amounts of both isomers were found. In such cases, there usually was a slight preference for the *erythro* isomer, with *erythro*/*threo* ratios between 1 and 3. However, especially when a large amount of the C_{22} dihydroxyacid was found, a clear stereospecificity was observed, with a strong preference for the *erythro* compound. In three out of four samples, the *erythro/threo* ratio in the C_{22} dihydroxyacid fraction was 15 or higher (Fig. 7).

For sample ASP3, the GC-MS chromatogram was different in that dicarboxylic acids, 9,10-dihydroxyoctadecanoate, 11,12-dihydroxyeicosanoate and 13,14-dihydroxydocosanoate were absent. However, the long-chain fatty acids were present, with a similar ratio of C22:0 to C24:0 (= 3 ± 0.5) as in the three other samples. This indicates that similar oil was used in all cases; however, this particular sample may have been subject to elution of the polar compounds, resulting in the absence of diacids and dihydroxylated acids.

In sample ASP6 the plant-derived β-sitosterol was detected. Cholesterol or its oxidation products were not detected in any of the shells, excluding the possibility that animal fats were used as an illuminant. This also confirms the results of the fatty acid ratios from which high P/S and low S/O ratios were calculated.

General discussion

The lipid remains in eight *Chambardia* shells from a church at Bawit, Egypt, were analyzed with a variety of techniques, with the aim of confirming the function of the shells as lamps and of identifying the illuminant that was used. Literary sources, such as Greek papyri, document the use of several plant oils in Egypt as an illuminant from the Ptolemaic period onwards [35]. These include species such as radish (*Raphanus sativus*), castor (*Ricinus communis*),

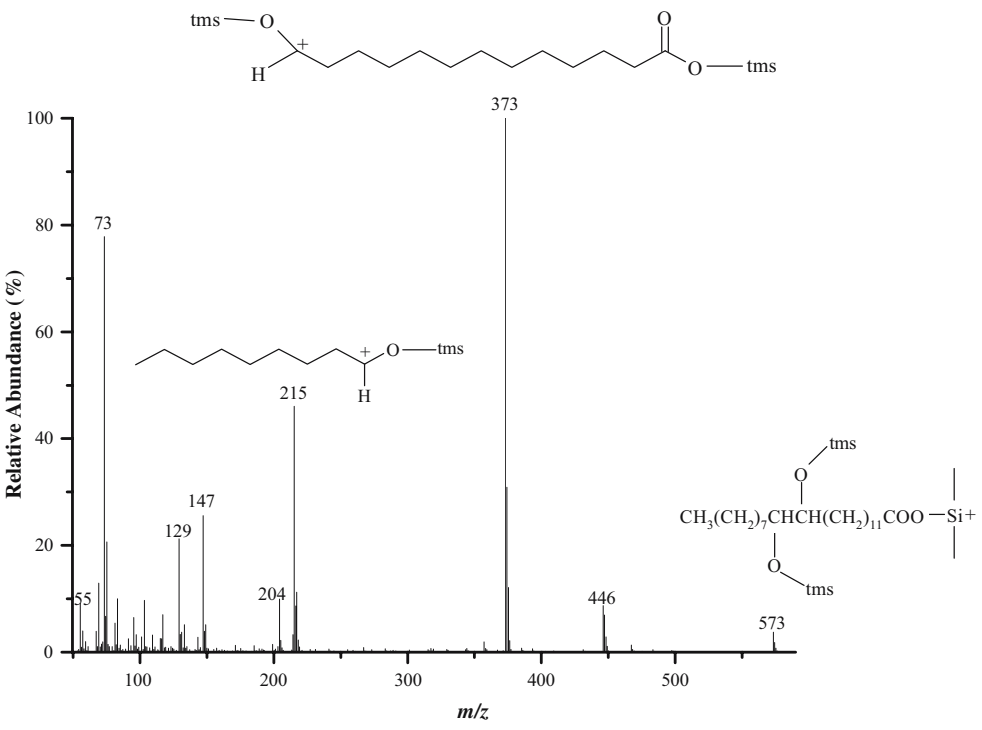

Fig. 6 Mass spectrum acquired by GC-MS of 13,14-dihydroxydocosanoate in the trimethylsilylated form together with important mass fragments

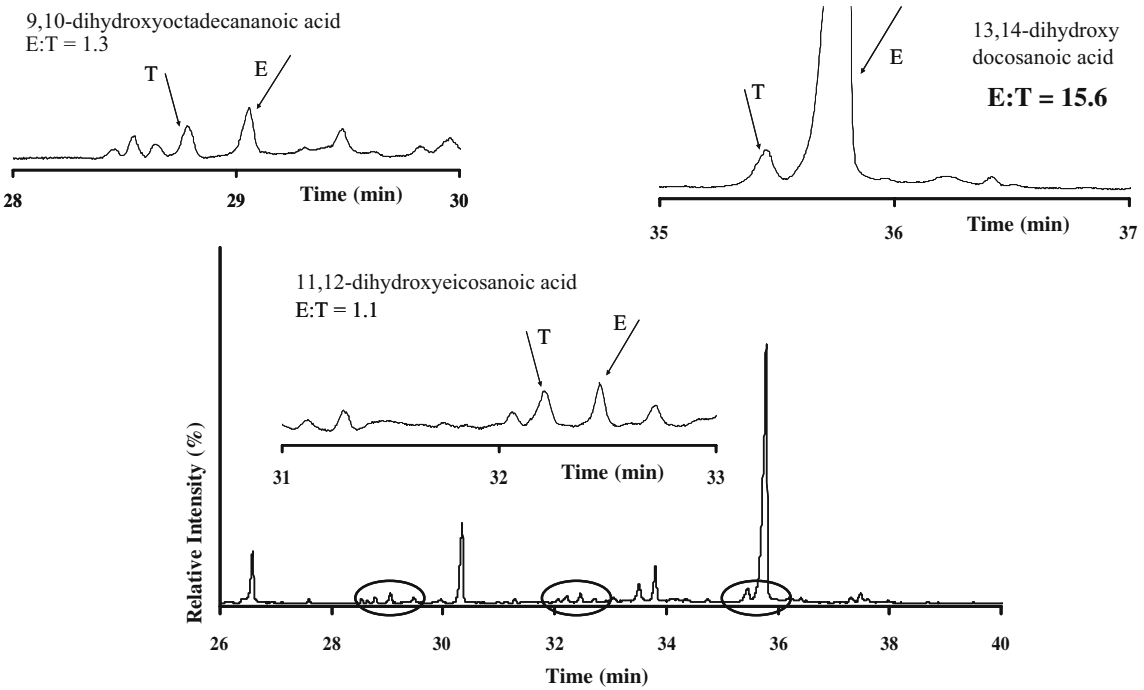

Fig. 7 Partial GC-MS chromatogram (based on total ion count) of ASP6 with focus on the *erythro* (*E*) and *threo* (*T*) isomers of the silylated dihydroxylated fatty acids together with their *erythro/threo* ratio (*E*/*T*)

olive (*Olea europaea*), flax (*Linum usitatissimum*), safflower (*Carthamus tinctorius*) and sesame (*Sesamum indicum*) although in many texts it is difficult to identify the exact nature of the oil. Besides plant oil, animal fat could also be used as an illuminant, a fuel type for which 'open lamps' such as the bivalve shells were more suitable than a typical ceramic lamp with a small filling hole. Palm fibres, papyrus, reeds and linen may have been used as lamp wick [36], but no macroscopic traces of them were observed in the shells.

The analysis of the fatty acid methyl ester fraction by PPGC leads to a first assumption that the illuminant used in the shells from Bawit was of vegetal origin. Indeed, significant amounts of unsaturated fatty acyl groups still could be recovered from the TAGs fraction by transmethylation. As these compounds seem to have persisted through an extended burial time, they must have been present in very high concentrations in the original fuel. As animal fats mainly comprise saturated rather than unsaturated fatty acids, animal fat can be excluded as being the fuel used here. The absence of cholesterol or other animal markers from the GC-MS chromatograms confirms this hypothesis.

Assessment of the stable carbon isotope values of two important fatty acid methyl esters, viz. methyl palmitate and methyl stearate, supports the idea that plant rather than animal fat was burned in these shells. The $\delta^{13}C$ values (‰) are clustered, and comparable to those from C_3 vegetal oils [28, 32]. This excludes the possibility that oil from a C_4 plant was used as an illuminant here. The $\delta^{13}C$ values of the samples are close to those of radish oil; further confirmation of this hypothesis will be given below.

Intact, non-oxidized triglycerides were detected in very small quantities by APCI-LC-MS only in one sample. The recovery of few intact triglycerides, indicates that the lipids have been exposed to hydrolysis and/or oxidation, either during burning of the oil [17], or during years of preservation [37]. The fatty acids on the glycerol backbone display a high degree of unsaturation, in agreement with the vegetal origin of the lipids.

With both GC-MS and LC-MS, a series of lipid oxidation products were detected. The major compound classes detected by GC-MS are C_6–C_{13} diacids and vicinal dihydroxyacids (C_{18}, C_{20} and C_{22}). It is well known that such polar compounds generally are lost from archaeological material due to elution with water, unless they are strongly bound to ceramics [4], or the samples are preserved in unusually dry conditions [17]. The latter situation clearly applied here, as Bawit, at the edge of the Libyan Desert, experiences an arid climate. In particular, 13,14-dihydroxydocosanoic acid and 11,12-dihydroxyeicosanoic acid, derived from erucic acid (C22:1(13)) and gondoic acid (C20:1(11)), respectively, are very clear biomarkers for seed oil from Brassicaceae plants such as radish (*Raphanus sativus*). Similar observations have been made for lipids extracted from ceramic Egyptian oil lamps [16, 17]. The results are also in line with archaeobotanical findings and written sources. Indeed, radish occurs fre-

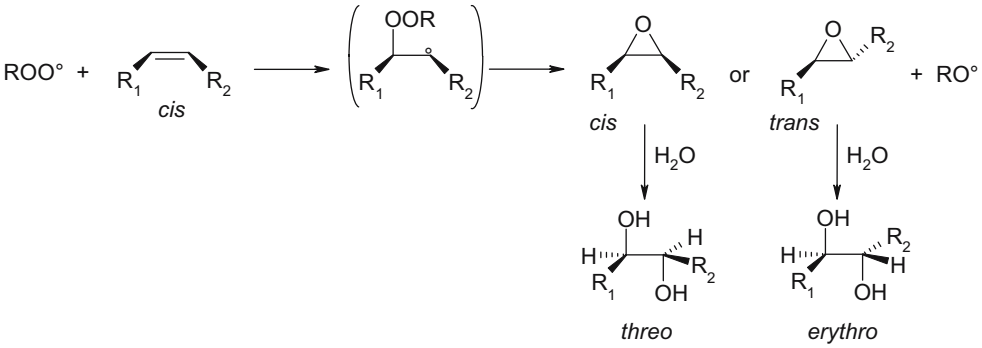

Fig. 8 Reaction scheme where *threo* and *erythro* diol isomers are formed out of a *cis* double bond together with their epoxide intermediates. Possible oxygen-transferring species ROO· are acylperoxy or alkylperoxy radicals

quently in documents from the Roman period onwards [38, 39]. Around AD 1050, radish and turnip seed oils are the most common vegetal oils used [40]. Although there are identification problems due to the morphological similarities of the seeds of the various Brassicaceae, it also appears from the archaeobotanical record that there was an emphasis on radish especially in Late Roman to early Islamic times [32, 41–43].

As observed by previous workers, the dihydroxylated C_{18}, C_{20} and C_{22} fatty acids occur as a mixture of *threo* and *erythro* isomers with essentially identical fragmentation patterns. The latter confirm that they are stereoisomers, rather than positional isomers. The most likely intermediates in the formation of diols are epoxides. If the epoxidation is due to a free radical process, one may expect formation of *cis* and *trans* configured epoxides from the *cis* double bond in the acyl residue. In a subsequent, stereospecific hydrolysis step, the *cis* epoxide is opened in an S_N2 reaction to form the *threo* isomer, while the *trans* epoxide yields the *erythro* isomer (Fig. 8). As the *trans* configuration is somewhat more stable than the *cis* configuration, it is expected that the *erythro* form prevails over the *threo* form when the oxidation is dominated by free radical chemistry. In the overall transformation of the *cis* fatty acid to the diols, both *syn* and *anti* dihydroxylation occur.

Similar mixed stereoselectivities have previously been observed in archaeological samples, or in deliberately aged Brassicaceae oil [16, 17]. However, this does not yet explain the differences among the various dihydroxyacids, and particularly the high stereospecificity encountered for *erythro*-13,14-dihydroxydocosanoic acid. As a hypothesis, we propose that the almost stereospecific oxidation of erucic acid to the *erythro* diol must have occurred at much lower temperatures than the less stereospecific oxidation of the C_{18} and C_{20} unsaturated fatty acids. This hypothesis is based on calculating the *erythro* to *threo* ratios (E/T) via a thermodynamic approach. If the reaction scheme of Fig. 8 is correct, the E/T ratios reflect the relative stabilities of the *cis* and *trans* epoxide intermediates. At high temperature, the stability difference between the two epoxides is smaller, and this leads to a lower E/T ratio, as demonstrated in Table 4. Such relatively low E/T ratios are observed for the dihydroxylated C_{18} and C_{20} fatty acids. At contrast, if the free radical oxidation proceeds at lower temperature, the larger difference between the energies of the *trans* and *cis* epoxides results in a larger E/T value, e.g. of 15 at 300 K. This is exactly the ratio that is observed experimentally in the chromatogram of Fig. 7.

Summarizing, the oxidation of C_{18} and C_{20} unsaturated fatty acids seems to have happened at higher temperature than that of the C_{22} compound. In view of the higher melting points and lower vapour pressures of higher molecular weight compounds, it is reasonable to assume that the heaviest oil fractions, containing the C_{22} fatty acids, will gradually be enriched during burning of the oil. This explains the large amount of erucic acid left in the sample, while much smaller amounts of the C_{18} and C_{20} acids were left. The E/T ratios indicate that for C_{18} and C_{20} acids, oxidation must largely have happened during combustion or immediately after it, in the hot oil; the oxidation of the large amount of erucic acid left after quenching of the flame must have taken place in much milder conditions, in the buried, cooled oil and over a much longer period.

In an alternative hypothesis, the formation of *erythro*-13,14-dihydroxydocosanoic acid and related diols might be ascribed to microbial enzymatic processes during burial. Some soil bacteria are known to produce Rieske dioxygenases, which are non-heme mono-iron enzymes that initiate the biodegradation of unsaturated compounds by *cis*-

Table 4 Theoretically calculated percentages of the *erythro* and the *threo* form for oxidation via epoxide intermediates

Temperature (K)	*threo* (T) intermediate (%)	*erythro* (E) intermediate (%)	E/T ratio
300	6	94	15
400	10	90	9
500	14	86	6
600	18	82	4

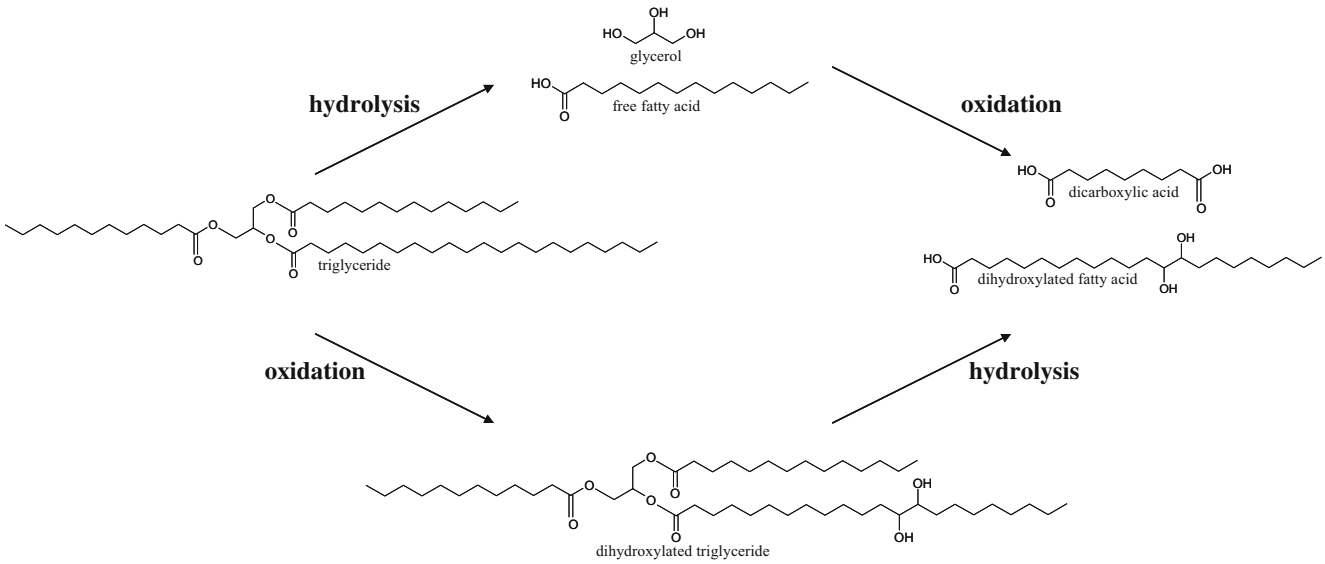

Fig. 9 Formation of hydroxylated fatty acids from triglycerides via consecutive hydrolysis/oxidation. The identification of hydroxylated triglycerides with LC-MS proves that oxidation took place before hydrolysis

dihydroxylation [44]. However, *cis*-dihydroxylation of predominantly *cis* fatty acids should yield the *threo* isomers, while the *erythro* isomers are the most abundant in the present samples. Hence, there is no direct evidence for a microbial origin of the diols.

Hydrolysis and oxidation of triglycerides will occur during burial. While unsaturated acyl residues can be detected in the TAGs fraction by both PPGC and LC-MS, they are completely absent from the free fatty acid fraction as studied by GC-MS, in agreement with previous studies on Brassicaceae lamp fuel [16, 17]. By contrast, significant amounts of the saturated fatty acids are identified by GC-MS, proving that there has been considerable triglyceride hydrolysis. The absence of unsaturated fatty acids demonstrates that once the unsaturated fatty acids are released by hydrolysis from the triglycerides, they are prone to fast oxidation.

While the previous data are in line with consecutive triglyceride hydrolysis and oxidation of the unsaturated fatty acids, LC-MS unexpectedly shows that this sequence can also be reversed (Fig. 9). Indeed, the unambiguous identification of a series of C_{46}–C_{48} triglycerides with two or three alcohol functions proves that in these molecules, oxidation of the lipid chain must have preceded hydrolysis.

While Brassicaceae oil has been previously identified in samples from Egypt, several features are apparent from the present work. Firstly, this study is likely the first to proof that lipids such as lighting fuel residues can be preserved even in non-porous, i.e. non-ceramic, matrices, provided preservation conditions are favourable. Secondly, the use of LC-MS has revealed that unsaturated triglycerides can be degraded not only by hydrolysis followed by oxidation, but that the sequence can be reversed. Finally, the varying isomer ratios in the diol compounds can only be explained by assuming that oxidation can take place in harsh conditions, associated with combustion of the oil, or in mild conditions, after quenching of the flame.

Conclusion

For the first time, archaeological residues from Egyptian bivalve *Chambardia rubens arcuata* shells, probably used as lamps, are chemically analyzed, proving that a ceramic matrix is not necessarily required for the preservation of organic compounds. The distribution of the fatty acid methyl esters indicates that a triglyceride-type oil with a high degree of unsaturation, and thus of vegetal origin, was used. The results of the stable carbon isotope analyses on methyl palmitate and methyl stearate confirm this hypothesis and correspond well to the characteristics of radish seed oil (*Raphanus sativus*). Using liquid chromatography coupled to mass spectrometry, we were able to identify mono-, di- and tri-hydroxylated triglycerides, highly oxidized compounds reported to be formed in fried oil. Using a gas chromatograph coupled to a mass spectrometer, oxidation products like α,ω-dicarboxylic acids and dihydroxyacids were recognized. Dihydroxyacids such as 11,12-dihydroxyeicosanoic acid and 13,14-dihydroxydocosanoic acid are oxidation products of gondoic and erucic acid, respectively, which are important fatty acids from seed oil of Brassicaceae plants. By evaluating the ratios between the *threo* and *erythro* isomers of the dihydroxyacids, it became clear that dihydroxylation of the C_{18} and C_{20} acids occurred in conditions different from those of the oxidation of the C_{22} acid.

Combining the results from the four analytical techniques applied to the eight lamp shells confirms that seed oil from a Brassicaceae plant, in particular from radish (*Raphanus sativus*), was used as an illuminant. This is both a confirmation of the initial assumption that the shells might have been used as lamps and an answer to the question of the nature of oil used.

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