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Olive oil or lard?: Distinguishing plant oils from animal fats in the archeological record of the eastern Mediterranean using GC-C-IRMS

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Keywords:	GC-C-IRMS, plant oils, animal fats, archaeological residues

Abstract:	Distinguishing animal fats from plant oils in archaeological residues is not straightforward. Characteristic plant sterols, such as β-sitosterol, are often missing in archaeological samples and specific biomarkers do not exist for most plant fats. Identification is usually based on a range of characteristics such as fatty acid ratios, all of which indicate that a plant oil may be present, none of which uniquely distinguish plant oils from other fats. Degradation and dissolution during burial alter fatty acid ratios and remove short chain fatty acids, resulting in degraded plant oils with similar fatty acid profiles to other degraded fats. Compound specific stable isotope analysis of $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$, carried out by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), has provided a means of distinguishing fish oils, dairy fats, ruminant and non-ruminant adipose fats but plant oils are rarely included in these analyses. For modern plant oils where $C_{18:1}$ is abundant, $\delta^{13}C_{18:1}$ and $\delta^{13}C_{16:0}$ are usually measured. These results cannot be compared with archaeological data or other modern reference fats where $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$ are measured, as $C_{18:0}$ and $C_{18:1}$ are formed by different processes resulting in different isotopic values. Nine samples of six modern plant oils were saponified releasing sufficient $C_{18:0}$ to measure the isotopic values, which were plotted against $\delta^{13}C_{16:0}$. The isotopic values for these oils, with one exception, formed a tight cluster between ruminant and non-ruminant animal fats. This result complicates the interpretation of mixed fatty residues in geographical areas where both animal fats and plant oils were in use.

Olive oil or lard? : Distinguishing plant oils from animal fats in the archaeological record of the eastern Mediterranean using GC-C-IRMS

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Abstract

Distinguishing animal fats from plant oils in archaeological residues is not straightforward. Characteristic plant sterols, such as β-sitosterol, are often missing in archaeological samples and specific biomarkers do not exist for most plant fats. Identification is usually based on a range of characteristics such as fatty acid ratios, all of which indicate that a plant oil may be present, none of which uniquely distinguish plant oils from other fats. Degradation and dissolution during burial alter fatty acid ratios and remove short chain fatty acids, resulting in degraded plant oils with similar fatty acid profiles to other degraded fats.

Compound specific stable isotope analysis of $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$, carried out by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), has provided a means of distinguishing fish oils, dairy fats, ruminant and non-ruminant adipose fats but plant oils are rarely included in these analyses. For modern plant oils where $C_{18:1}$ is abundant, $\delta^{13}C_{18:1}$ and $\delta^{13}C_{16:0}$ are usually measured. These results cannot be compared with archaeological data or other modern reference fats where $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$ are measured, as $C_{18:0}$ and $C_{18:1}$ are formed by different processes resulting in different isotopic values.

Eight samples of six modern plant oils were saponified releasing sufficient $C_{18:0}$ to measure the isotopic values, which were plotted against $\delta^{13}C_{16:0}$. The isotopic values for these oils, with one exception, formed a tight cluster between ruminant and non-ruminant animal fats. This result complicates the interpretation of mixed fatty residues in geographical areas where both animal fats and plant oils were in use.

Keywords: archaeological residues, fats, oils, GC-C-IRMS

Background

The use of gas chromatography-mass spectrometry (GC-MS) in the analysis of organic residues and the use of biomarkers to identify the original source material(s) which formed those residues are well established techniques in archaeological science[1-8]. However distinguishing between plant oils and animal fats in archaeological residues has proved to be more complicated and, since the 1990s, compound specific carbon stable isotope analysis, carried out using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), has been used to identify different fatty materials[1-2, 5, 9-18].

The difficulties in distinguishing the source material of degraded fats arise primarily from the lack of unique biomarkers for specific fats or oils and the effects of degradation on fatty material. Fatty acids are present in all fats and oils[8, 19] and identify the presence of fatty material in archaeological residues, but specific biomarkers which are unique to particular fats and oils are rare. General classes of biomarkers are present in different types of fresh fats; for example unsaturated fatty acids in plant oils[19-20], long chain polyunsaturated fatty acids in fish oils[3, 19, 21], short chain fatty acids in dairy fats[3, 5, 19], cholesterol in animal fats[19] and plant sterols (in particular β-sitosterol) in plant oils[19]. However these biomarkers are not necessarily specific to only one group of fats (*e.g.* the occurrence of β-sitosterol in shell fish[22-24]) and are affected by degradation processes both before and after burial. The action of heat, light, bacteria and water can degrade fats during the formation of residues, for example during cooking, and burial in the soil exposes fats to further degradation and dissolution processes. Unsaturated fatty acids are chemically reactive and degrade easily[20, 25-28], short chain fatty acids are soluble in water[5, 9, 13, 17], while complex acylglycerols may be hydrolysed to release free fatty acids [2-3, 6, 9, 20, 26, 28-29]. These processes produce archaeological residues which are dominated by hexadecanoic ($C_{16:0}$, palmitic) and octadecanoic ($C_{18:0}$, stearic) acids regardless of their original source material[1-2, 7, 30].

Attempts have been made to identify degraded fats in archaeological residues by comparing simple or complex ratios of fatty acids derived from the analysis of these residues with the equivalent ratios in fresh fats[31-32]. However preferential degradation and dissolution before, during and after burial will affect these ratios. For example the ratio $C_{16:0}/C_{18:0}$ cannot be regarded as constant over archaeological time as $C_{16:0}$ is twice as soluble in water at 20°C as $C_{18:0}$ (see Table 1)[33-34] and $C_{16:0}$ may be preferentially leached from the residues at all but the driest of burial sites. Fatty acid ratios are also affected by both the solvents used and the exact method employed to extract and analyse the residue[30, 35]. For example the solubility of $C_{16:0}$ is 2.5 times that of $C_{18:0}$ in chloroform, 4.4 times greater in ethanol and 37 times greater in methanol at 20°C (see Table 1)[33-34] leading inevitably to changes in fatty acid ratios in the extracted samples. As a result fatty acid ratios cannot be considered diagnostic for the degraded fats found in archaeological samples[1-2, 7, 30].

Other methods for identifying degraded fats rely on the analysis of intact triacylglycerols or the presence of plant sterols[36-37]. However un-degraded triacylglycerols are rarely present in archaeological material as they are easily hydrolysed in the presence of water, and plant sterols are also rarely identified in archaeological residues and may be present in other fats (see above).

GC-C-IRMS has been used successfully to distinguish degraded ruminant dairy fats, ruminant and non-ruminant adipose fats and fish oils by measuring the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ [2-4, 9-16, 18, 21, 25-26]. There is evidence that $\delta^{13}C$ values are preserved, even after significant degradation of the original material, and comparison with modern reference fats and oils allows unique identification of these fats[3, 15]. However plant oils have rarely been included in this type of analysis although GC-C-IRMS of modern oils has become a routine procedure for identifying adulteration or geographical source of olive oils[38-41]. In fresh olive oil, and most other fresh plant oils, $C_{18:1}$ is very abundant while $C_{18:0}$ is present in very low abundances[19] and as a result isotopic measurements of modern oils utilise $\delta^{13}C_{18:1}$ rather than $\delta^{13}C_{18:0}$ [38-39]. In consequence these results cannot be compared easily with the values for archaeological fats where $C_{18:1}$ may not be present or is only present at very low concentrations, and $\delta^{13}C_{18:0}$ is generally measured.

The aim of this research was to determine whether $\delta^{13}C_{18:0}$ could be measured in modern plant oils and to establish a small database of modern standards. This database was then used to determine whether an archaeological residue contained a plant oil or an animal fat.

Materials

Modern materials:

Eight modern plant oils were prepared for GC-C-IRMS analysis. These comprised one sample each of almond oil (*Hermitage Oils, 20 Clifford Avenue, Kingston Upon Hull, HU8 0LU*), argan oil (*Belazu, The Fresh Olive Company Ltd, 7 Barretts Green Road, London, NW10 7AE, UK* Moroccan argan oil, cold pressed), moringa oil (*EssentialOilsOnline at www.essentialoilsonline.co.uk*), sesame oil (*Meridian Foods Ltd, The Estate Office, Sutton Scotney, SO21 3JW, UK* Mexican unrefined, cold pressed), and walnut oil (*Sainsbury's, 33 Holborn, London, EC1N 2HT, UK, own label*) together with three samples of olive oil (*Carapelli Firenze SpA, Via Leonardo da Vinci, 31 Tavarnelle Val di Pesa, Florence, Italy, I-50028,* Italian extra virgin, cold pressed; *Sainsbury's own label,* Greek extra virgin, cold pressed; *Agrovim SA, 6th Km National Road, Kalamata – Messini, P. O. Box 134, Kalamata 24100, Greece, Iliada Kalamata,* Greek extra virgin, cold pressed). All the above are C_3 plant oils chosen primarily on the basis that there is archaeological evidence that most these plants were in use during the Late Bronze Age (LBA) in the eastern Mediterranean[42-47] and therefore useful comparisons for the archaeological material. The exception is argan oil, which, although of Mediterranean origin, was not in use during the LBA and was included as another example of a C_3 plant oil. A secondary consideration was the availability of oils for sampling. Although several supermarket samples were included, efforts were made to obtain the least processed, unheated samples for analysis.

Archaelogical samples

The archaeological residue exemplifies the problems associated with identifying fats and oils in archaeological residues. It is a visible residue still attached to a sherd of Red Lustrous Wheelmade (RLWm) ware collected as a surface find from the necropolis at Saqqara in Egypt[48-50]. RLWm ware is a ceramic type widely distributed across the eastern Mediterranean during the LBA[51-53] and is particularly significant as the consistency of the ceramic fabric points to manufacture in one location, as yet unidentified[48, 50-51, 54-58]. Its relative rarity and strong association with burials indicates a valuable commodity or commodities[51, 59], probably the contents of the vessels rather than the vessels themselves[51].

The residue is a brown, sticky but rubbery mass approximately 1cm in thickness adhering to the interior surface of the sherd (see Figure 1). Closer inspection of this residue with a hand lens reveals that it is not uniform but varies in colour. Two samples were therefore taken from different areas of the residue to see if the variation in appearance equates to differences in composition. Several samples of this residue had been examined by GC-MS with all the analyses producing similar results[49-50, 60]. The residue produced a chromatogram in which $C_{16:0}$ was the main constituent (378 mg/g of residue), as well as two isomers of 9,10-dihydroxyoctadecanoic acid (18 mg/g and 278 mg/g) and $C_{18:0}$ (151 mg/g) (Figure 2a). Closer

inspection revealed α,ω-dicarboxylic acids (C_4 to C_{10}) with the highest abundances of C_8 (173 mg/g) and C_9 (azeleic acid) (110 mg/g), a series of saturated fatty acids ranging from $C_{9:0}$ to $C_{24:0}$, small abundances of $C_{18:1}$ (7 mg/g), a trace of $C_{18:2}$, and a peak formed by the co-elution of $C_{19:0}$ and 12-hydroxy-octadec-9-enoic acid (ricinoleic acid) (3 mg/g) (Figure 2b). Dicarboxylic acids and short chain fatty acids along with mono- and dihydroxy fatty acids are the most widely reported degradation products of unsaturated fatty acids[20, 25-28, 61]. This indicates an original source material rich in unsaturated fatty acids which is typical of many plant oils[5, 19, 39]. The presence of ricinoleic acid indicates that the original contents of the vessel contained at least some castor oil[19-20]. The GC-MS results therefore identify the main constituent(s) of this residue as degraded fatty material but, apart from the presence of some castor oil, the original material(s) cannot be uniquely identified. Bulk $\delta^{13}C$ analysis of the residue gave a mean value of -26.2‰ ± 0.3‰ which is within the range for C_3 plant tissues (approximately -23‰ to -30‰)[62] but also within the range of ruminant animal fats (approximately -23.5‰ to -30.0‰)[26, 63-64].

In addition to the biomolecular evidence, there is some archaeological evidence that RLWm ware vessels contained a commodity which was liquid at room temperature. The three main forms of RLWm ware – spindle bottle, pilgrim flask and arm-shaped vessel – all have very narrow openings at the neck[51] making it unlikely that these vessels were used for storing any material which would be solid at normal ambient temperatures. In the case of this residue the archaeological evidence would therefore indicate a plant oil rather than an animal fat.

However none of this evidence uniquely identifies this residue as a degraded plant oil.

Methods

Rationale

Only very small amounts of free $C_{18:0}$ are present in fresh plant oils, the majority being bound into triacylglycerols which can be released by saponification[19]. The key question is whether this liberated $C_{18:0}$ can legitimately be used in GC-C-IRMS analysis to provide a modern analogue for the $C_{18:0}$ present in archaeological oils. In modern oils isotopic effects may occur during the formation of fatty acids and their incorporation into triacylglycerols, and sample preparation may also potentially lead to isotopic fractionation. Similarly any archaeological oil samples will have been subject to many chemical reactions over time. The question which must be asked is whether the 'life histories' of modern and archaeological samples are equivalent in terms of isotopic effects and the resultant isotopic fractionation.

Studies of plant physiology indicate that all $C_{18:0}$ in plant tissue, whether free or bound fatty acid, is manufactured by the same biosynthetic pathway. Synthesis is usually *de novo* from acetyl-coenzyme A (acetyl-CoA) and involves the action of acetyl-CoA carboxylase and fatty acid synthetase[65-67]. Incorporation of all fatty acids into acylglycerols is achieved by the action of fatty acid transferases via the Kennedy pathway[65-68]. These processes are the same for all plants; the different isotope effects observed in the tissues of C_3, C_4 and CAM plants occur during photosynthesis, and not during the formation of fatty acids or acylglycerols[8, 62, 69-71]. Both archaeological and modern oils have been formed by these processes and it is reasonable to assume that plants produced oils of similar molecular composition in the past. So the isotopic signature of both modern and ancient oils would be similar allowing for the differences in pre- and post-industrial atmospheric carbon.

This leaves the question of isotope effects introduced during degradation of archaeological samples and the sample preparation of modern oils. Isotope effects can only be introduced in kinetic reactions or when a closed system reaches dynamic equilibrium[62]. Kinetic isotope effects only occur when a process is incomplete and unidirectional (*e.g.* evaporation, dissociation, diffusion and many biological processes)[62]. During sample preparation for this study a saponification method was used which ensured the complete hydrolysis of all acylglycerols. This process was verified by analysing all saponified samples by GC to ensure no acylglycerols were present. Therefore this was a reaction taken to completion in a closed system and no fractionation should result. In one of the few studies to compare modern oils with archaeological fats[18] saponification was used for the preparation of the modern oils. These modern oils were not analysed specifically to compare with archaeological samples but had been analysed during an earlier study which looked at the authentication of oils[39]. The implications of the preparation methods were not discussed in either study.

The fractionation of archaeological fats arising from degradation processes taking place in the burial environment is poorly researched but is generally considered to be negligible on the basis of the one published study[4]. This is a problem common to all GC-C-IRMS analysis of archaeological fats and the accepted approach is to assume that no fractionation occurs as the result of burial or sample preparation[3, 5, 9-12, 14-15, 18, 21, 29, 72]. If archaeological samples are saponified to ensure the complete hydrolysis of any remaining acylglycerols, no significant isotopic effects will be present due to sample preparation. This was verified by GC-MS analysis of the saponified archaeological samples to ensure no acylglycerols were present. On this basis, the comparison of $\delta^{13}C$ values for fatty acids in saponified modern oils and archaeological fats should be a valid exercise.

All the modern samples in this study were corrected for the industrial burning of fossil fuels by a value of +1.6‰ to allow a comparison with archaeological fats[18, 73-74]. Similarly data from the literature on animal fats had also been corrected, most using a correction of +1.2‰ according to the source papers[5, 15, 75] while the fish oils were corrected by an unknown amount[21]. Although different corrections had been made, the measurements of these values were made at different times separated by least 10 years during which time the $^{13}C/^{12}C$ ratio in atmospheric CO_2 will have changed considerably. This is apparent from the steep fall in values of $\delta^{13}C$ recorded by Friedli *et al.*[73]. This may be a source of uncertainty when comparing stable isotope ratios of modern materials with archaeological samples, particularly as it is not always recorded in the literature how this correction is made or if it has been made at all.

Sample preparation

The modern oil samples were saponified by heating *c.* 20-30 mg (two drops) of each oil at 70°C with 4ml 5% methanolic sodium hydroxide (NaOH) for two hours in a sealed tube. After cooling the samples were acidified with 6M hydrochloric acid (HCl) and the free fatty acids extracted with 3 aliquots of 2mL of hexane; the solvent was then removed under a stream of dry nitrogen with gentle heating. This method was sufficient to hydrolyse all acylglycerols present in the fresh oils thus preventing any isotope effects being introduced during the saponification process by taking the reaction to completion.

The saponified extracts were methylated by heating in a sealed tube with *c.* 2ml boron trifluoride-methanol complex (BF_3) at 70°C for one hour. Extraction with hexane and

solvent removal was carried out as above and the samples were stored in a freezer at -17°C until sent for analysis.

The archaeological samples for GC-C-IRMS were prepared by taking two samples of the residue (6.4 mg and 7.3 mg) and saponifying them by heating with 4mLl aqueous methanolic (1:9 v/v) 0.5M NaOH for 1 hour in a sealed tube at 70°C. Acidification was carried out with approximately 2mL 0.5M HCl and extraction with hexane carried out as above. Methylation was achieved with 100µl BF_3 and heating for 20 minutes at 70°C. After heating a few drops of deionised water were added to quench the reaction. Extraction of the fatty acid methyl esters (FAMES) was carried out as above. This less vigorous method using aqueous methanolic NaOH was used with the archaeological samples to prevent transesterification taking place during saponification. Significant levels of transesterification would result in the formation of FAMES incorporating carbon atoms of unknown and unquantifiable $\delta^{13}C$ values, potentially affecting the measured $\delta^{13}C$ values.

GC-C-IRMS analysis

GC-C-IRMS of archaeological and modern samples was carried out at the Stable Isotope Facility at the CEH Lancaster. Analyses were carried out on an Agilent 6980 gas chromatograph (*Agilent, UK, 610 Warfedale Rd, Winnersh Triangle, Wokingham, RG41 5TP*) connected to an Isoprime mass spectrometer (*Isoprime Ltd, Isoprime House, Earl Road, Cheadle Hulme, Cheadle, SK8 6PT*) via a platinum/copper oxide combustion furnace heated to 850°C with the interface at 300°C. The GC was fitted with a 50m, 0.32mm inside diameter column with a 0.25µm thick coating of Carbo Wax stationary phase. The carrier gas was helium and the GC oven was programmed as follows: 50°C isothermal for 5 minutes, 50° - 170°C at 10°min^{-1}, 170° - 300°C at 3°min^{-1}, 300° - 320° at 15°min^{-1}, isothermal at 320° for 15 minutes. Post combustion water was removed by a water permeable nafion membrane. Six pulses of carbon dioxide of known isotopic composition were fed into the ion source from a reference gas injector box during each run. A FAME standard containing $C_{16:0}$ and $C_{18:0}$ of known isotopic value was run prior to each batch of analyses to ensure that the combustion furnace and instrument were functioning correctly. The standard deviation of the standard fatty acid methyl ester mixture was better than or equal to 0.3‰ for all analyses. Each sample was run in triplicate.

A correction was made to all results to allow for the carbon atom added during methylation using the mass balance equation[76]:

$$\partial^{13}C_{FA} = \frac{\left((n+1)\partial^{13}C_{FAME}\right) - \partial^{13}C_{BF3}}{n}$$

where $\delta^{13}C_{FA}$ is the corrected value for the free fatty acid, n is the number of carbon atoms in the unmethylated fatty acid, $\delta^{13}C_{FAME}$ is the value for the methylated fatty acid and $\delta^{13}C_{BF3}$ is the isotopic value of the BF_3 methanol complex. A sample of the batch of BF_3 used in the methylation process was analysed at the same time as the samples.

Results

The results of the GC-C-IRMS analysis of modern oils are shown in Figure 3 together with the generally accepted ranges for ruminant and porcine adipose fats, ruminant dairy fats and

fish oils taken from the literature. All values have been corrected for the difference between pre- and post-industrial revolution isotopic values of atmospheric carbon to allow comparison with archaeological samples. With the exception of moringa oil all the samples clustered within a narrow range of $\delta^{13}C_{16:0}$ values (-28.4 to -27.2‰) and a slightly greater range of $\delta^{13}C_{18:0}$ values (-29.6 to -27.3‰). The sample of moringa oil showed more enriched isotopic values with $\delta^{13}C_{16:0}$ at -25.9‰ and $\delta^{13}C_{18:0}$ at -25.5‰. The standard deviation for $\delta^{13}C_{16:0}$ was better than or equal to 0.3‰; for $\delta^{13}C_{18:0}$ better than or equal to 0.6‰.

The results for the two samples of the archaeological residue are also shown in Figure 3. Sample 1 showed $\delta^{13}C_{16:0}$ of -28.3‰ and $\delta^{13}C_{18:0}$ of -29.0‰ and sample 2 produced similar results ($\delta^{13}C_{16:0}$ -27.6‰; $\delta^{13}C_{18:0}$ -28.8‰). The standard deviation for $\delta^{13}C_{16:0}$ was 0.5‰ and for $\delta^{13}C_{18:0}$ 0.2‰. The results place this residue within the measured range for plant oils.

The results are also presented as a plot of $\Delta^{13}C$ (where $\Delta^{13}C = \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) against $\delta^{13}C_{16:0}$ (Figure 4). $\Delta^{13}C$ shows difference in depletion between $C_{18:0}$ and $C_{16:0}$ fatty acids giving a measure of the different biosynthetic pathways producing these two acids from different fat sources. From this plot it can be seen that all the plant oils, with the exception of almond oil lie within the range of values generally accepted for porcine adipose fats. Almond oil lies within the range for ruminant adipose fats. The archaeological samples again plot in the same area as the majority of the plant oils.

Discussion and Conclusions

The first aim of this project was to determine whether $\delta^{13}C_{18:0}$ could be measured in modern plant oils allowing them to be used as a comparison for archaeological residues. Although the concentration of $C_{18:0}$ in fresh plant oils is low, even after saponification, it proved possible to measure $\delta^{13}C_{18:0}$ using a polar column and an appropriate temperature programme for the GC when carrying out GC-C-IRMS analysis.

The second aim was to establish a small database of modern samples of plant oils for comparison with archaeological fatty residues. Although only eight modern plant oils were analysed it is encouraging that seven of these samples lie relatively close together on the plot of $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ normally used for distinguishing fats from different source materials. Similarly seven samples also plot closely together on the plot of $\Delta^{13}C$ vs. $\delta^{13}C_{16:0}$, although the outliers are different in each case. From this limited database the plant oils are distributed in the area between ruminant adipose and porcine adipose on the standard $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ plot and have isotopic signatures different to the other main groups of fats (fish oils, ruminant milk and ruminant adipose).

An archaeological residue was also analysed to determine whether it could be uniquely identified as a plant oil by comparing its isotopic signature with the database of modern oils. In this case this proved possible, both samples from the residue lying within the range for plant oils.

The results reported here for modern plant oils introduce a significant complication to the interpretation of results for fats from archaeological residues. Results plotting in this area of the standard $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ graph are usually considered to represent mixtures of ruminant and porcine adipose fats[4-5, 13]. This will not present a problem in geographical areas or periods of history where no plant oils were in use. However in areas such as, for example, the Mediterranean during Late Bronze Age, when there is archaeological evidence for the use

of plant oils, the situation is no longer clear. In these cases the interpretation of isotopic signatures within in this range of values as mixtures of animal fats can no longer be considered valid. The plot of $\Delta^{13}C$ vs. $\delta^{13}C_{16:0}$ does not differentiate plant oils and porcine adipose/ruminant adipose fats. In data analysed and presented in this way plant oils are essentially invisible and will always be interpreted as animal fats if there is no indicative biomolecular or archaeological evidence.

It is clear that further work on both a database of modern plant oils and on archaeological material is necessary. Eight samples are not sufficient to establish a clear database for the isotopic values of modern oils. In particular further analyses of moringa and almond oil are needed to determine whether they differ from other C_3 plant oils or whether the two samples analysed here are within the natural variation produced by differing growing conditions and/or during extraction and processing of the oils. It is also clear that the biomolecular, archaeological and isotopic evidence will need to be considered together when interpreting the results of analyses on archaeological residues.

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Number of C atoms	Solubility in g acid per 100g solvent at 20°C			
	water	chloroform	methanol	acetone
12	0.0055	83	120	60.5
14	0.0020	32.5	17.3	15.9
16	0.00072	15.1	3.7	5.38
18	0.00029	6.0	0.1	1.54

Table 1: Solubility of fatty acids in four common solvents. Data collated from Hoerr & Ralston (1944)[33] and Ralston & Hoerr (1942)[34]

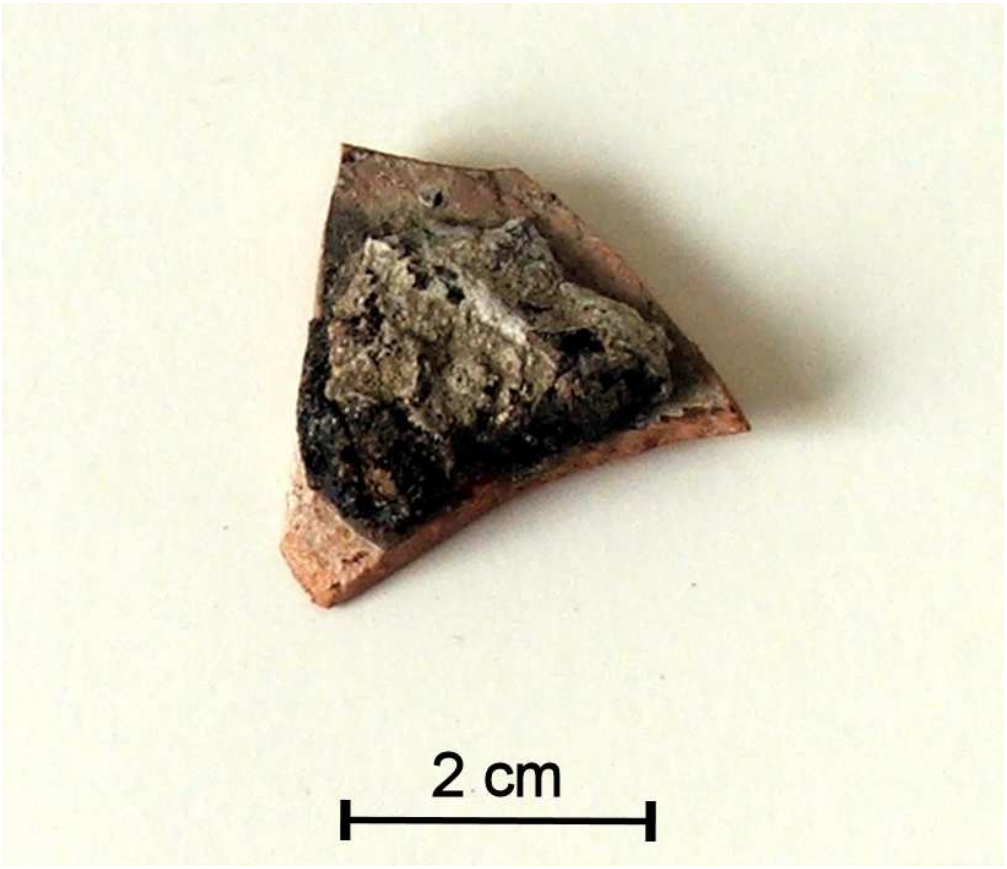


Figure 1: The archaeological residue attached to a Late Bronze Age sherd of Red Lustrous Wheelmade Ware from Saqqara, Egypt
69x59mm (300 x 300 DPI)

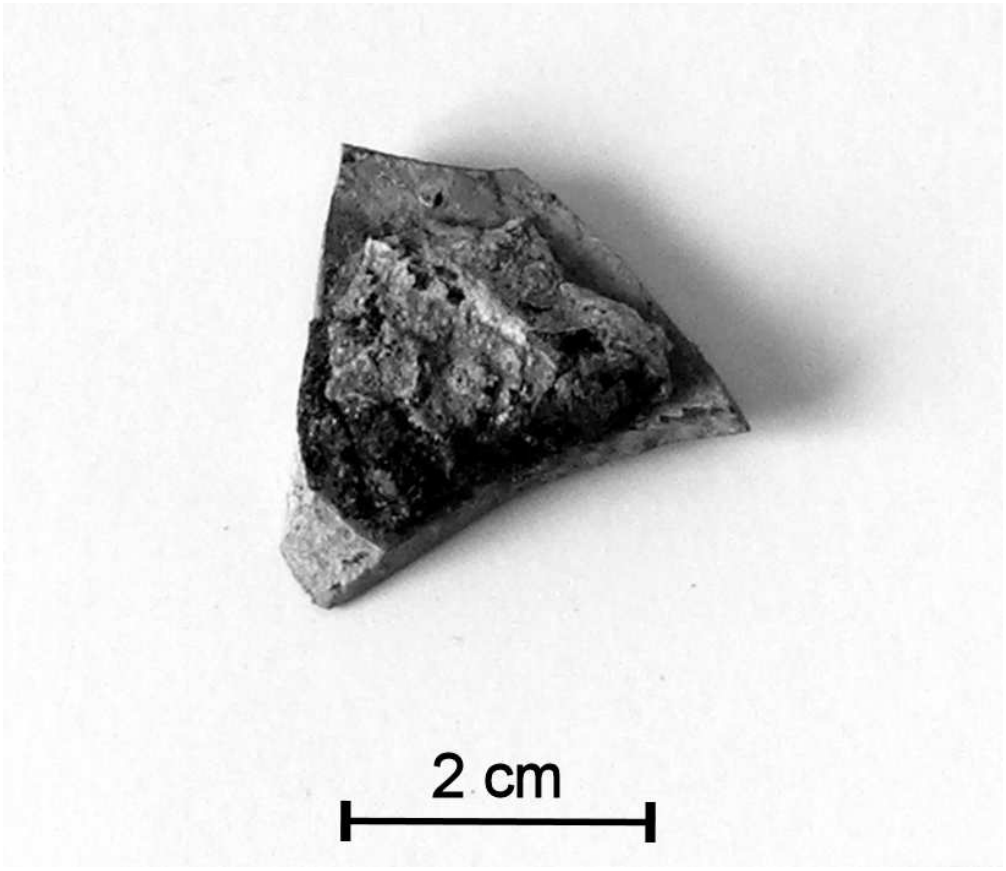
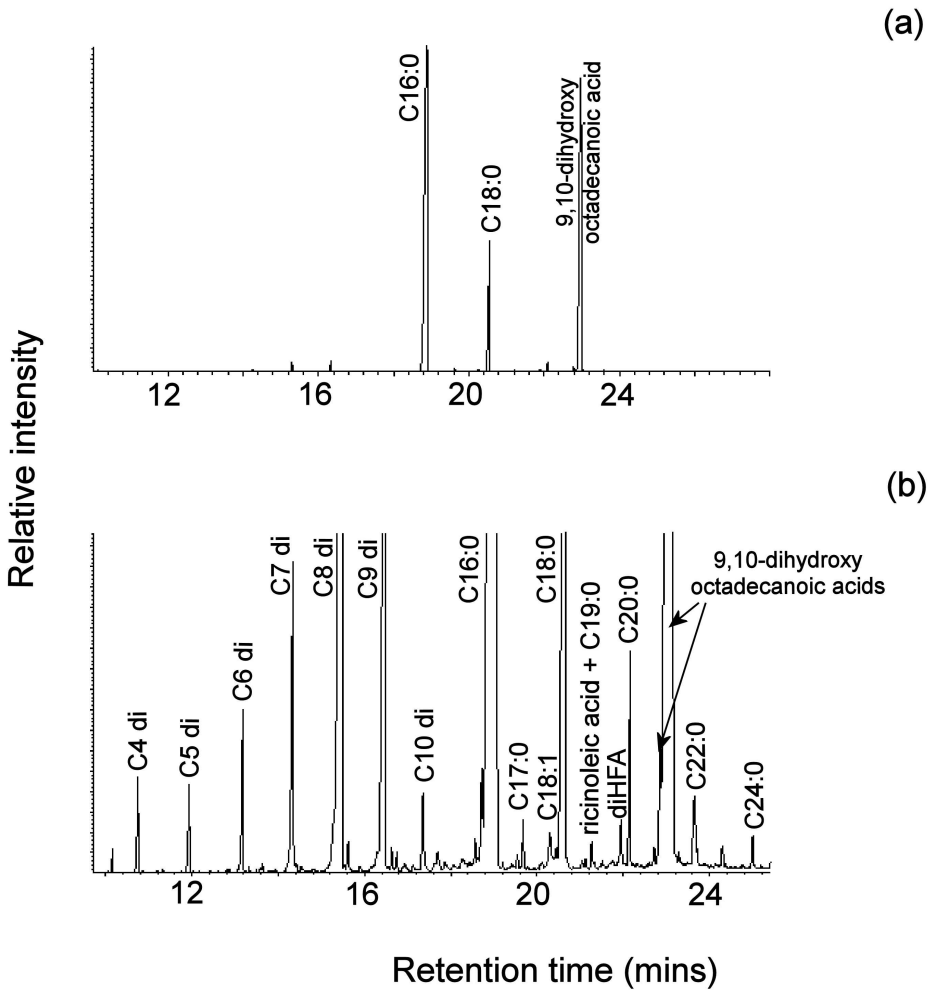


Figure 1: The archaeological residue attached to a Late Bronze Age sherd of Red Lustrous Wheelmade Ware from Saqqara, Egypt.
69x59mm (300 x 300 DPI)



Key: Cx:y - fatty acid with x carbon atoms and y double bonds
Cx di - dicarboxylic fatty acid with x carbon atoms
diHFA - dihydroxy fatty acid

Figure 2: (a) chromatogram produced by the archaeological residue from Saqqara, Egypt. (b) enlargement of the chromatogram between 10 and 25 minutes
120x148mm (600 x 600 DPI)

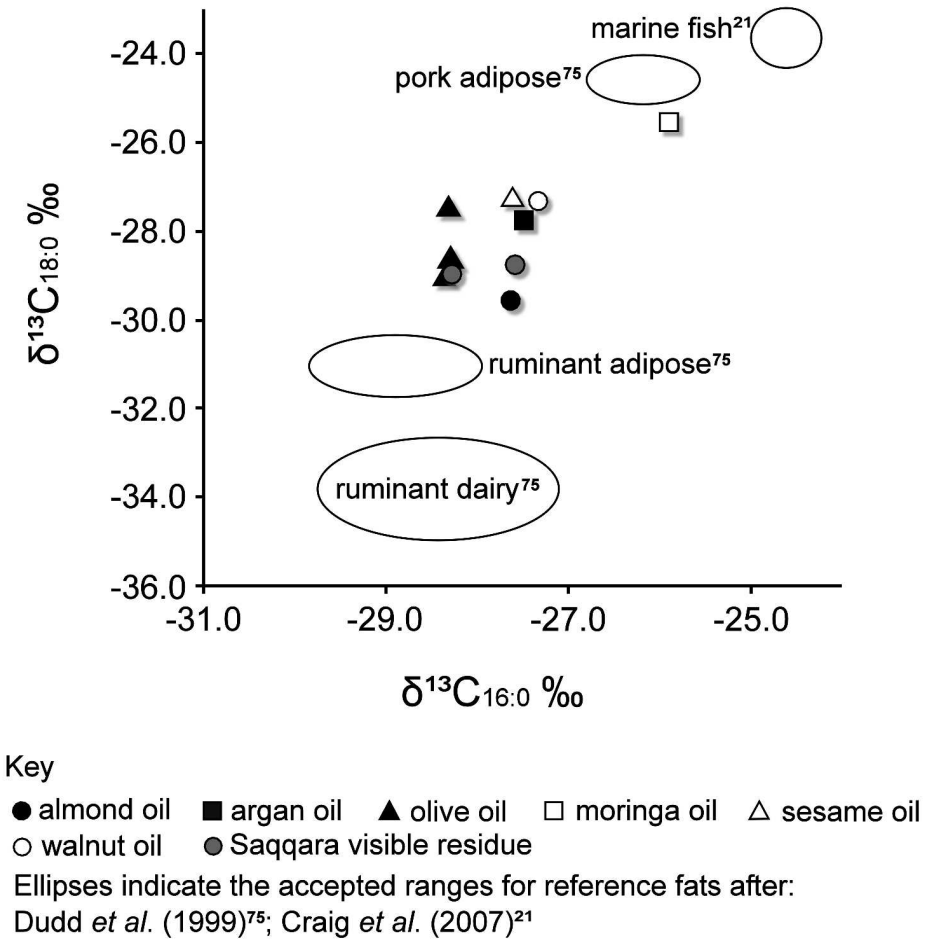


Figure 3: Results of compound specific stable isotope analyses of eight modern oils and the archaeological residue from Saqqara, Egypt.
199x235mm (600 x 600 DPI)

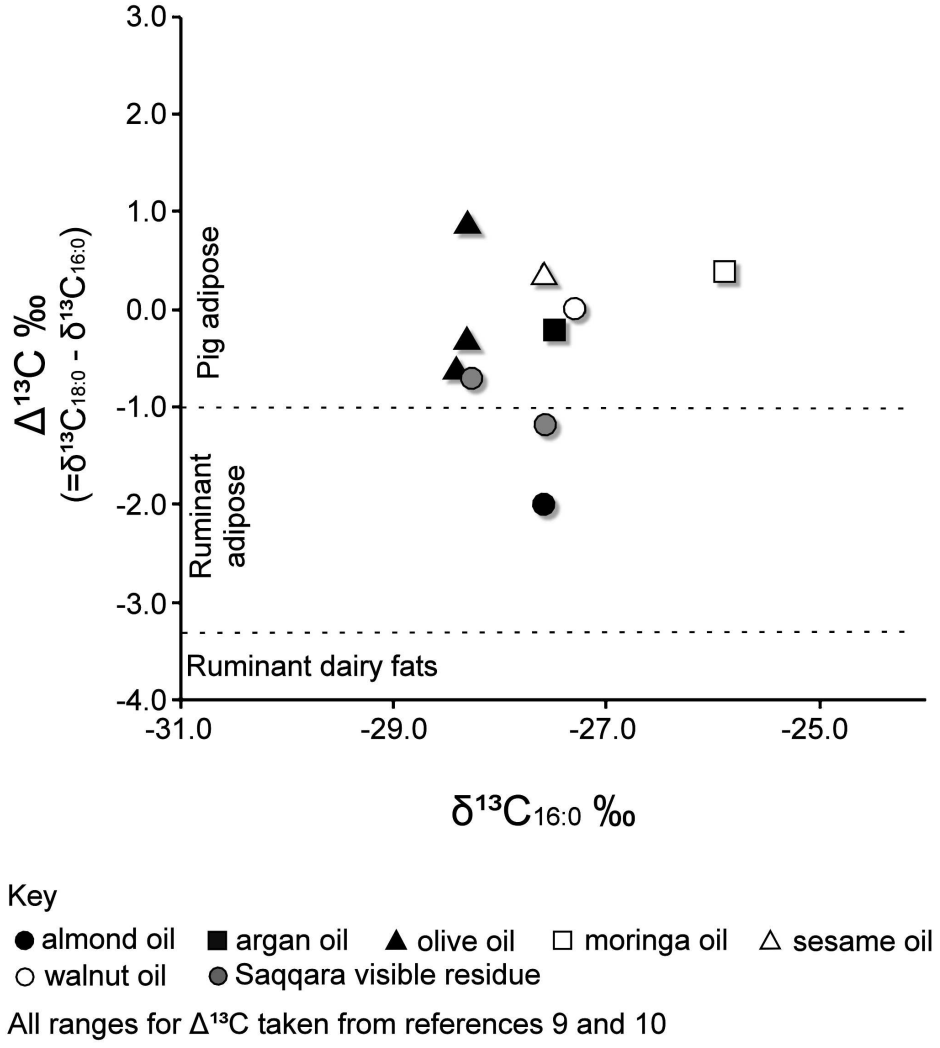


Figure 4: Plot of $\Delta^{13}C$ against $\delta^{13}C_{16:0}$ for eight modern oil samples and the archaeological residue from Saqqara, Egypt, where $\Delta^{13}C = \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$
185x204mm (600 x 600 DPI)