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Analysis of oil used in late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat

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Abstract

The lipid fraction of residues in ancient oil lamps found at the archaeological site of Sagalassos (south-west Turkey) was analysed by gas chromatography (GC) coupled to mass spectrometry (MS). The identification of plant sterols and long chain alcohols suggested that a vegetable oil was used in these lamps. The lipid sample was also analysed with reversed-phase liquid chromatography (LC) coupled to MS with atmospheric pressure chemical ionization (APCI). The identification of TAG's detected with LC–APCI–MS showed that predominantly olive oil was used as a fuel for the antique oil lamps. The presence of large quantities of multiply unsaturated triacylglycerol (TAG) and traces of saturated TAG indicated that also other oils and animal fat were added. Summarizing, the analysis of TAG's with LC–APCI–MS in lipid extracts of ancient ceramics proved to be a valuable method to reconstitute the original contents.

Keywords: Olive oil; Animal fat; Atmospheric pressure chemical ionization; Ceramics, ancient; Lipids

1. Introduction

Major and minor chemical compounds of vegetable oils have been used to detect contamination and adulteration [1]. There exist several methods to control the purity of these oils. Analysis of the constituent fatty acids after conversion to fatty acid methyl esters is frequently performed. Additionally, the sterols, triterpene alcohols or wax ester compounds present can be structurally identified using gas chromatography (GC) coupled to mass spectrometry (MS) [2] and quantified using gas chromatographic methods [3–5]. Since the composition of the triacylglycerols (TAG's) reflects the quality and purity of a vegetable oil [1], their analysis by high temperature-gas chromatography (HT-GC) or high-performance liquid chromatography (HPLC) is a prerequisite. Recently good results for the identification of TAG's were achieved by HPLC combined with atmospheric pressure chemical ionization (APCI) [6,7]. In this way milk fat [8], berry oil [9] and several vegetable oils [6] were characterised in terms of the present TAG's. The mass spectrum shows typically protonated molecular ions $[M+H]^+$ and diacylglycerol fragment ions $[MH-RCOOH]^+$

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in most TAG's. However, only diacylglycerol fragment ions were observed in the spectra of TAG containing saturated acyl chains.

Most of the above mentioned methods have also been applied in the analyses of lipid extracts from pottery. The reconstitution of the original contents was done based on identified sterols or waxes [10] or by quantification of dominant fatty acids [11]. Triacylglycerols (TAG's), although being the major constituents of most animal and vegetable fats and oils, are often present in small concentrations in archaeological ceramics due to chemical or enzymatic hydrolysis during burial [12,13]. Nevertheless, it is believed that the remaining TAG's are still the original and unchanged components [14]. Yet few attempts have been made to identify the constituent fatty acids of triacylglycerols [15], although the structural elucidation of the TAG could further clarify the origin of the lipid extract. This study elucidates for the first time the structure of several TAG's retrieved in archaeological pottery and more specific of Roman oil lamps with the use of LC–APCI–MS.

In the present study lipid extracts of seven late Roman to early Byzantine ceramic oil lamps originating from Sagalassos, an archaeological site in southwest Turkey, were analysed to identify the origin of the fuel used. As olive trees were present on the territory of ancient Sagalassos [16], it is presumed that olive oil was used as fuel for these lamps. Olive oil was not only a basic food source, but also the prime lighting fuel in the ancient Mediterranean world [17,18].

2. Experimental

2.1. Reagents and solvents

All reagents and solvents were of analytical or chromatographic grade. Methanol and cyclohexane were purchased from Merck (p.a. Darmstadt, Germany), chlorofrom from BDH Laboratory Supplies (Poole, UK), isopropanol from Riedel de Haën (Seelze, Germany) and toluene from Acros organics (Geel, Belgium).

2.2. Extraction

The extraction of the lipids was based on the procedure of Evershed et al. [28]. Accordingly, 5 g of a sample was weighed and 60 μg of *n*-heptadecane (99%, Janssens chimica, Geel, Belgium) was added as an internal standard. The lipids were extracted with a soxtec apparatus (automated soxhlet system) with 30 ml of chloroform and methanol (2:1 v/v) for 4 h (1 h in the boiling mode, 3 h in the rinsing mode). A residue was obtained after evaporation of the extraction liquids by the soxtec apparatus until a volume of 2 ml was reached, followed by evaporation to dryness in a vacuum oven at 40°C. The whole extraction procedure was repeated using another 5 g of a representative sample. The amount of dry extract obtained was around 10–100 μg/g sherd. One sample was used for analysis with HT-GC, GC–MS and LC–MS, the other was transesterified and analysed on a polar phase GC. Also commercially available olives were extracted.

2.3. Trimethylsilylation

The total lipid extracts were redissolved in 50 μl of toluene and derivatized using 50 μl of *N*-methyl-*N*(trimethylsilyl)trifluoroacetamide (98%, Avocado Research Chemicals, Rhodes, France) at 60°C for 1 h. The excess solvent was removed under a slow stream of N_2-gas.

2.4. Transesterification

The dry lipid extract was dissolved in 50 μl of toluene. Subsequently 100 μl of methanolic potassium hydroxide (Merck, Darmstadt, Germany) (5%) was added. After 5 min stirring the reaction was stopped by addition of 200 μl of bidistilled water. Subsequently the fatty acid methyl esters (FAMEs) were extracted with 1 ml of cyclohexane, followed by solvent evaporation in vacuum at room temperature. Afterwards the dried lipid extract was redissolved again in 50 μl of cyclohexane.

2.5. HT-GC

Gas chromatographic analysis of the dry silylated

total lipid extract redissolved in toluene was performed on a Hewlett-Packard 5890 gas chromatograph equipped with flame ionisation detector (FID). Samples were introduced by on-column injection into a 15 m×0.32 mm I.D. fused-silica capillary, coated with CP Sil-8 stationary phase with 0.25 μm film thickness from SGE using nitrogen as a carrier gas. The temperature program consisted of a 5 min isothermal hold at 60°C followed by ramping from 60 to 340°C at 10°C/min. The temperature was then kept at 340°C for 15 min.

2.6. Polar phase GC

The GC analyses of the FAME samples were carried out on a second Hewlett-Packard 5890 gas chromatograph with FID detector using nitrogen as carrier gas. The sample was injected on-column into a 60 m×0.32 mm I.D. fused-silica capillary column from SGE, coated with a 0.25 μm film of BPX70 as stationary phase. The analysis was done isothermally at 180°C.

2.7. GC–MS

Analyses were performed using a quadrupole type MD 800 MS directly coupled to a 8000 Fisons GC equiped with a 30×0.32 μm I.D. CP-Sil 8 column (film thickness 0.25 μm) from SGE. The operating conditions were as follows: ion source, 200°C; emission current, 400 μA and electron energy, 70 eV. The GC–MS capillary interface was maintained at a temperature of 250°C. Spectra were recorded every 500 ms from 40 till 650 m/z. Data acquisition and processing was done with a Masslab data system. The temperature program consisted of a hold of 1 min at 140°C, followed by a temperature increase of 15°/min and a 5 min hold at 340°C. Helium was used as carrier gas. Under these conditions no molecules with molecular mass larger than 650 could be eluted.

2.8. LC–APCI–MS

High-performance liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry). The analyses were performed on a SpectraSystem HPLC, coupled to a Finnigan LCQ DECA mass spectrometer. The samples were analyzed using a reversed-phase column of the type Supelcosil LC-18 (25 cm×4.6 mm, Supelco) with a gradient of methanol and isopropanol as mobile phase at a flow-rate of 0.8 ml/min. The mass spectrometer unit was operated with a vaporizer temperature of 500°C, a capillary temperature of 225°C and a corona current of 5 μA. Spectra were obtained in the range of 422 to 1000 m/z, with a scan time of 0.2 s.

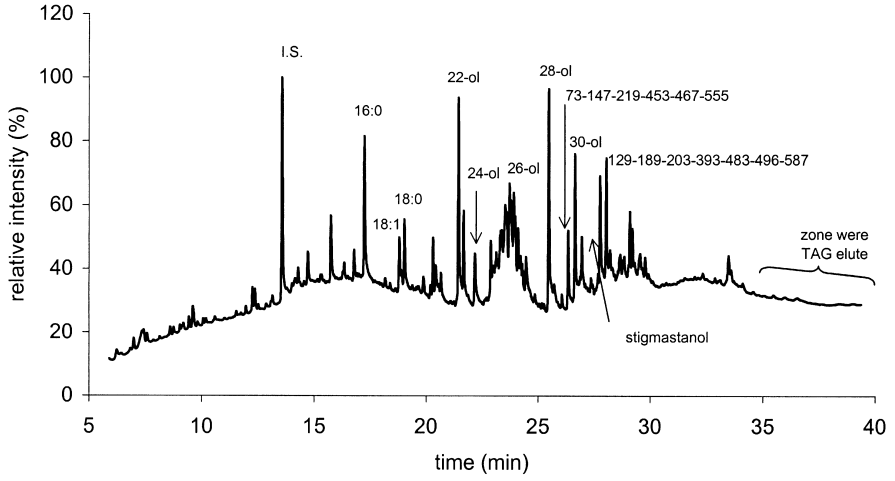

Fig. 1. HT-GC of the trimethylsilylated total lipid extract of antique oillamp sherd. 16:0, 18:1 and 18:1 refer to palmitic, oleic and stearic acid. 22, 24, 26, . . . -ol are n-alkanols with 22, 24 and 26 C atoms.

Table 1
Mass spectrometric data of the analysed lipid compounds (determined as TMS derivatives)

Compound name	Mass spectral data m/z
Myristic acid	285(M^+-15)-145-132-117-73
Palmitic acid	313(M^+-15)-145-132-117-73
Oleic acid	339(M^+-15)-145-132-117-73
Stearic acid	341(M^+-15)-145-132-117-73
Octadecenoic acid methylester	296(M^+)-264-222-180-124-97-83-74-69-88-41
Docosanol	383(M^+-15)-43-57-75-103-111
Tetracosanol	411(M^+-15)-43-57-75-103-111
Hexacosanol	439(M^+-15)-43-57-75-103-111
Octacosanol	467(M^+-15)-43-57-75-103-111
Hentriacontanol	495(M^+-15)-43-57-75-103-111
Dohentriacontanol	523(M^+-15)-43-57-75-103-111
Monopalmitin	459(M^+-15)-371-313-239-147-129-103-73
Monoolein	485(M^+-15)-410-397-339-265-147-129-103-73
Monostearin	487(M^+-15)-399-341-264-147-129-103-73
β-Sitosterol	486(M^+)-471-396-357-255-129-73-57-43
Stigmastanol	488(M^+)-474-432-399-384-305-215-147-107-75
Unidentified component 1	586-496-483-393-203-189-129-103-73
Unidentified component 2	555-527-497-453-229-219-147-129-103-73

2.9. Samples

All sherds were found inside a row of late Roman to early Byzantine (work)shops built along the western edges of the two main squares of Sagalassos. The samples belonged to floor levels arranged after the destruction by an earthquake in 528 AD and were used throughout the 6th century AD or even until the middle of the 7th century AD.

3. Results and discussion

No quantifiable residual TAG's could be detected in the silylated lipid extract of the oil lamps using HT-GC as can be seen in Fig. 1. Only free fatty acids such as palmitic, stearic and oleic acid were abundantly present. This high proportion of free fatty acids in comparison with mono-,di-, and triacylglycerols is caused by extensive hydrolysis and can be seen in most lipid extracts from archaeological sherds [19]. In order to structural identify the eluted compounds, the same sample was injected into GC–MS. The accompanying MS spectra of most significant peaks can be seen in Table 1. Long chain linear fatty alcohols (C22:0–C32:0) together with β-sitosterol and stigmastanol were found. Also two unidentified components were present in all samples, the mass peaks of these unknown components are included in Table 1. No triterpene alcohols were identified. Due to the absence of cholesterol and the presence of β-sitosterol, stigmastanol and large quantities of long chain alcohols, it is assumed that a vegetable oil was used as a fuel for the antique oil lamps [20]. To further elucidate the origin of this

Table 2
Relative amounts of most prevailing fatty acids in the transesterified lipid extract

	Sample number[a]			
	137	165	182	201
C12:0	5	0	0	0
C14:0	1	3	2	3
C16:0	49	64	55	55
cis-C16:1	1	2	1	0
C18:0	19	16	22	19
cis-C18:1 (9)	18	12	14	14
C18:2	2	1	1	1
C20:0	4	2	5	7

Results were obtained by calculating the peak area in the polar phase GC profile.
[a] SA-OO-KK.

vegetable oil, part of the lipid extract was transesterified. Quantification of the FAME compounds, done via polar phase GC, is shown in Table 2. Palmitate (C16:0) had the highest concentration. Although only palm oil contains such high concentrations of palmitic acid, it is improbable that palm oil was used at Sagalassos as palm trees (*Phoenix dactylifera*) did not naturally occur in the area [21,22]. The high concentration of palmitate can be the result of degradation processes of unsaturated fatty acids by microorganisms. Studies of adipocere, which is the result of microbial activity that turns fatty materials into a solid waxy material, showed that this consists mainly of saturated free fatty acids with even-numbered carbon atoms and palmitic acid as the predominant fatty acid [23,24]. The detection

Table 3
Ion species of triacylglycerols in the extracted olive fruit and analysed lipid extracts of antique oil lamps

Ret. time (min)	$[M-RCOO]_1^+$	$[M-RCOO]_2^+$	$[M-RCOO]_3^+$	$[M+1]^+$	TAG name[a]	Mol. wt.	Trivial name[a]
12.39	467	495		nd	14:0–14:0–12:0	695	MMLa
13.43	597	595		877	18:3–18:3–18:2	886	LnLnL
13.63	495			nd	14:0–14:0–14:0	723	MMM
	599			879	18:2–18:2–18:2	878	LLL
14.0	599	601		881	18:2–18:2–18:1	880	OLL
	599	575		855	18:2–18:2–16:0	854	LLP
	573	577		855	18:3–18:1–16:0	854	LnOP
14.41	601			883	18:2–18:1–18:1	882	OLO
	575	577		857	18:2–18:1–16:0	856	LOP
	603	575		857	18:1–18:1–16:1	856	OOPo
14.74	603			885	18:1–18:1–18:1	884	OOO
	603	577		859	18:1–18:1–16:0	858	OOP
15.12	577	551		833	18:0–16:0–16:0	832	PPO
15.28	603	631		913	20:1–18:1–18:1	912	GOO
15.37	605	603		887	18:0–18:1–18:1	886	OOS
15.43	577	579	605	861	18:0–18:1–16:0	860	POS
	551			nd	16:0–16:0–16:0	807	PPP
15.91	603	633		915	20:0–18:1–18:1	914	AOO
	605	607		889	18:0–18:0–18:1	888	SSO
15.93	551	579		nd	18:0–16:0–16:0	835	PPS
16	579	607		nd	18:0–18:0–16:0	863	PSS
17.15	689	603		971	24:0–18:1–18:1	970	LiOO
	605	633	635	917	18:0–20:0–18:1	916	SAO
17.7	607			nd	18:0–18:0–18:0	891	SSS

Abbreviation	Trivial name	Carbon number degree of unsaturation
La	Lauric	12:0
M	Myristic	14:0
Po	Palmitoleic	16:1
P	Palmitic	16:0
O	Oleic	18:1
S	Stearic	18:0
L	Linoleic	18:2
A	Arachidic	20:0
G	Gadoleic	20:1
Li	Lignoceric	24:0

nd, not detected.
[a] Listing of fatty acids is random.

of hydroxystearic acid in some samples confirms this hypothesis, as this is an important fatty acid in adipocere.

In order to check the possibility of small quantities of TAG's still being present in the sherd, the samples were analysed with LC–APCI–MS. The identification of the TAG's is a unique means to identify the originally used oil. The TAG's are remains of the original components of the oil as the contamination with other TAG's is minor. Only TAG migrated from the surrounding burial context or TAG produced by fungi can be possible sources of contamination [25]. However no ergosterol, a sterol typical for fungi, was detected in the silylated extract with the GC–MS indicating that the presence of fungi is probably minimal. Migration of lipids from the soil into the ceramic has been reported to be unimportant [26,27].

TAG standard mixtures with concentrations of 10^{-4} wt.% were successfully detected. Several solvents such as propionitrile, a gradient of methanol and isopropanol and a gradient of methanol and acetonitrile, were used for analysis of the lipid extract of an olive fruit. The best sensitivity was achieved using methanol in combination with isopropanol. The best resolution of the triacylglycerols was obtained with methanol in combination with acetonitrile. Because of the small concentrations of TAG in the oil lamps a better ionisation was preferred over an enhanced resolution. The mass spectra of the TAG's exhibited abundant $[M+H]^+$ and $[M-RCOO]^+$ ions. The fatty acid moieties in the sn-2 position form less abundant $[M-RCOO]^+$ ions than in the sn-1/3 positions [9], which makes it possible to estimate the regioisomeric composition of the triacylglycerols. But as the regiospecific composition would not give us more information on the origin of the oil, these positions were not identified. In Table 3 the detected ions of the identified TAG's are shown. In the oil lamp lipid extracts several TAG's were detected as shown in Fig. 2. The quantities of the identified TAG were estimated using the relative intensity of the mass ions as shown in Fig. 3. Also diacylglycerols and some unknown

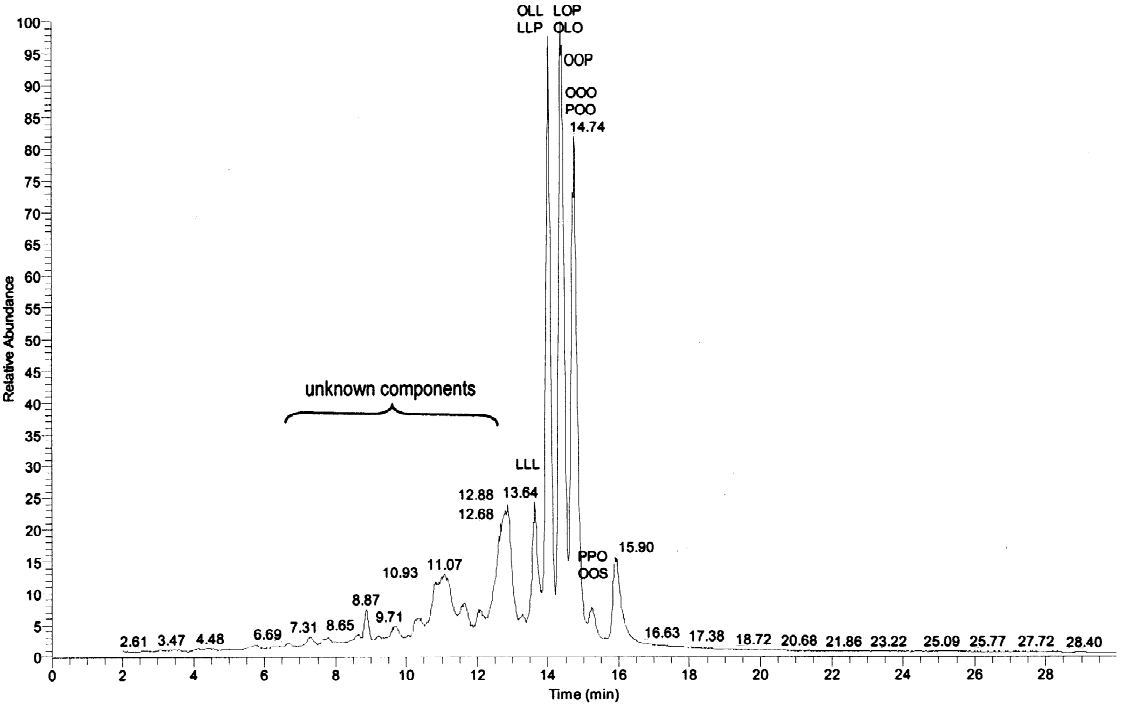

Fig. 2. Ion chromatogram of triacylglycerols of the lipid extract of an antique oil lamp analysed with LC–APCI–MS (for abbreviations see Table 3).

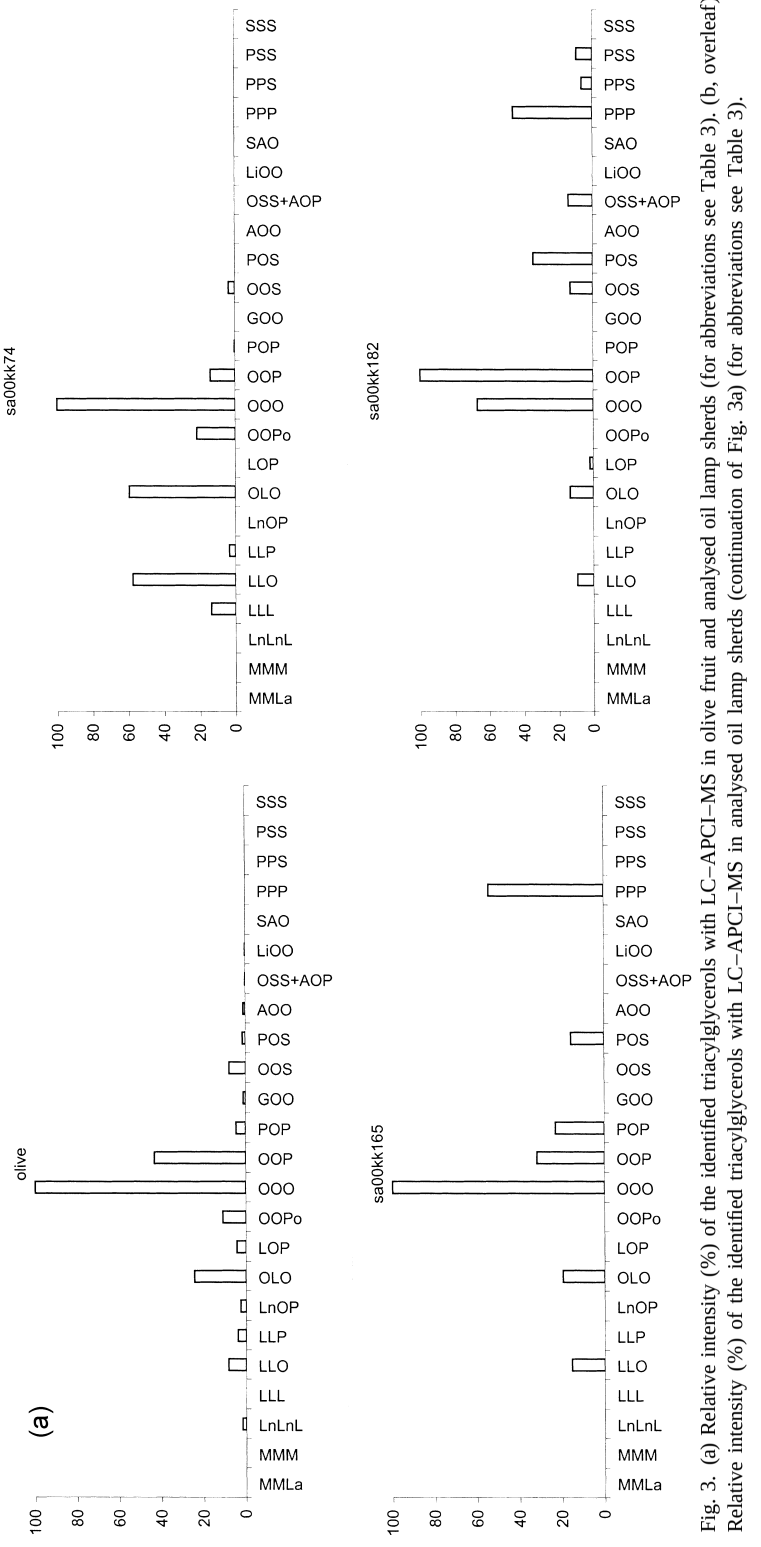

Fig. 3. (a) Relative intensity (%) of the identified triacylglycerols with LC–APCI–MS in olive fruit and analysed oil lamp sherds (for abbreviations see Table 3). (b, overleaf) Relative intensity (%) of the identified triacylglycerols with LC–APCI–MS in analysed oil lamp sherds (continuation of Fig. 3a) (for abbreviations see Table 3).

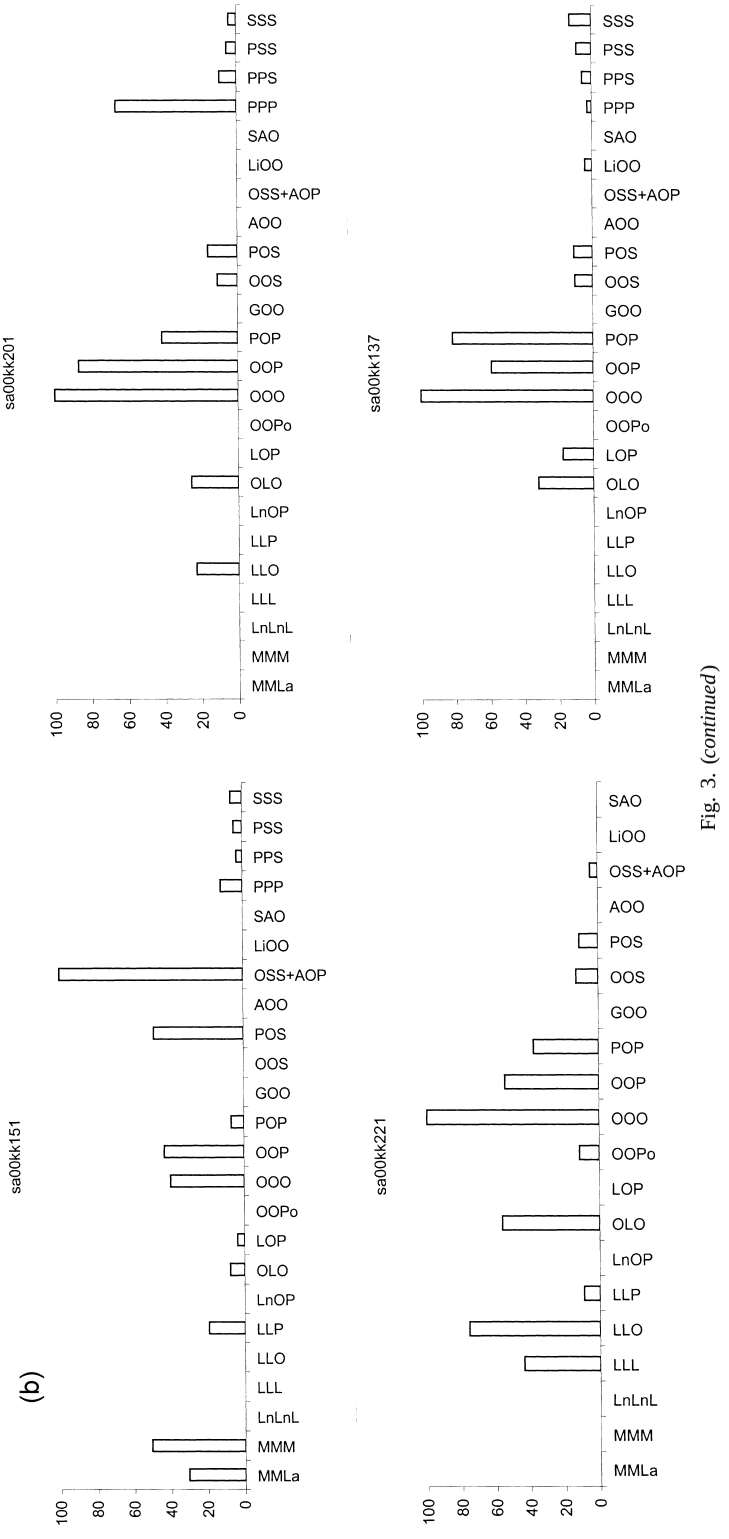

Fig. 3. (*continued*)

compounds were eluted. From the ion chromatogram it can be estimated that the concentrations of the TAG's in the lipid extract were lower than 10^{-4} wt.%. As can be seen from Fig. 3 triolein (OOO) had the largest intensity in most extracts.

Triolein (OOO) is the most important TAG in olive oil. In other possible oils from plants that prevailed around ancient Sagalassos such as sesame oil and safflower oil [21] trilinolein (LLL) and oleoyl-dilineoyl glycerol (OLL) are the most important TAG's, respectively. This indicates that olive oil was used in the oil lamps. Yet in some samples trilinolein and oleoyl-dilineoyl glycerol and dioleoyl-linoleoyl glycerol (OOL) were still abundantly present, indicating that traces of other oils were used as a fuel. Also in one extract, oleoyl-distearyl glycerol (OSS) had the largest intensity whereas in another one dioleoyl-palmitoyl glycerol (OOP) had most intense ions. These results indicate that olive oil was not exclusively used as a fuel. Possibly olive oil was mixed with leftovers of other used oils. In some samples even saturated TAG were present, probably stemming from traces of animal fat. As no cholesterol was detected with the GC–MS, it is believed that the additions of animal fat were minor indeed.

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