Mediterranean Archaeology and Archaeometry
Vol. 20, No 1, (2020), pp. 127-143
Open Access. Online & Print.

www.maajournal.com

DOI: 10.5281/zenodo.3674091

THE DIET OF ROMANS DURING 2ND PUNIC WAR IN THEIR CAMP CERRO DE LAS ALBAHACAS (SANTO TOMÉ, SPAIN) FROM ORGANIC RESIDUE ANALYSIS IN CERAMIC VESSELS BY GC-MS, HPLC-APCI-MS AND GC-C-IRMS METHODS

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Received: 17/01/2020
Accepted: 18/02/2020

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ABSTRACT

This paper shows the results of chemical analyses performed on a set of ceramic vessels retrieved from the Roman camp built after the battle of Baécula (208 b.C.) on the site known as Cerro de Las Albahacas (Santo Tomé, Jaén, Spain). The paper also assesses the sampling strategy and its influence on the interpretation of the results. A set of 16 ceramic vessels of Iberian typology used by the Roman army was studied in order to analyse the chemical markers associated with their contents. The method consisted in joint use of GC-MS, HPLC-APCI-MS and GC-C-IRMS. The chemical markers identified beeswax and vegetable lipid, mainly in amphorae, while non-ruminant fat is associated with a different type of ceramic vessels. Based on the products identified, the paper puts forward interpretation on the types of food or beverages consumed by Italian-Roman soldiers at the time of the 2nd Punic War.

KEYWORDS: Roman, Baécula, beeswax, vegetable lipid, pottery, GC-MS, HPLC-APCI-MS, GC-C-IRMS

1. INTRODUCTION

The battlefield of Baécula lies on what is known today as Cerro de las Albahacas (Santo Tomé, Jaén, Spain). This elevation of over 1500 ha. overlooks a good part of the high valley of river Guadalquivir and marks the site where the Carthaginian army led by general Asdrubal and the Roman army under the command of Scipio Africanus collided in the Iberian peninsula during the 2nd Punic War (208 b.C.) (Polybius X 38, 7 to 40; Livy XXVII 18, 10 and 11). The research on this context has also allowed to develop a complex method comprehensive of various analysis techniques for the study of a highly specific site, both for its unique profile and for its short timespan of barely a few days (Bellón et al., 2015) (as shown in Figure 1).

Almost a decade's research has made available an archaeological record of specific battlefield-related material. Within this body of archaeological evidence, hundreds of metal items stand out for their relevance (Quesada et al., 2015). Retrieved from a 500 ha. surface, they mark the main battleground. As a result of this sustained research campaign, two camps were identified on the hilltop of Cerro de las Albahacas (Rueda et al., 2015a):

1.- Camp A: This camp was built by the Carthaginian army before the battle. Spanning 940 m. north to south and ca. 630 m. at its widest point, it covers ca. 55 ha. (Bellón et al., 2016).

2.- Camp B: Following the Roman victory in 208 b.C., Scipio Africanus' army built a new camp with the remains of the former camp and enlarged it westward. Of a similar size across, it must have spanned 1423 m. along the longitudinal axis and must have covered 76 ha. (Bellón et al., 2017a).

The high amount of late Iberian ceramics that spreads densely over the area of Camp B contrasts sharply with the extremely rare occurrence of Roman items, which date back to the late 3rd ct. b.C. This may be due to the supply needs of a high number of individuals (Rueda et al., 2015b). it is well-known that marching Roman armies relied on a limited amount of ceramic vessels, so the amount of Iberian ceramics recorded has been explained in terms of the neighbouring Iberian *oppidum* of Baécula (today's Paraje de Los Turruñuelos, ca. 3 km. northwest). After the battle, and once the new camp had been built, the Roman army must have made use of this oppidum's vessels and resources.

The survey of one of the areas with the highest concentration of ceramics, camp B, identified ca. 20 structures related to the Second Punic War dug into the geological base. Markers of their relation with the war context recorded in the structures include remains of weapons (a whole spearhead), Hispanic-Carthaginian and Roman coins of the late 3rd ct. b.C. (post-211 b.C.), molten metal, slag, and a large amount of native Iberian storage ceramics. The latter were destroyed intentionally, as evidenced by large pieces spread over the structures and by chips that attest forcible destruction. The destruction and accumulation of sediment in the structures related to the camp area took place at the same time in all the structures and may, therefore, be attributed to the Roman army's abandonment and intentional destruction of the camp (Bellón et al., 2017b).

Three accumulations of ceramics (groups 21, 22, 23) were also recorded during the survey alongside the former group of structures on a small soil level above the geological base (as shown in Figure 2). They were mainly Iberian storage vessels, but also smaller vessels like platters, bowls and dishes. They do not hold a direct stratigraphic relation with the structures dug into the bedrock, but their properties are identical as regards amphora ware, metalware and numismatics. This paper focuses on those three ceramic groups.

Chemical analysis of the ceramic fragments proves especially relevant for their amount, for their clear spatial relatedness, for their Iberian typology, for the importance of the event with which they are related (the Battle of Baécula), and for their connection with the Roman army's maintenance. The analysis of lipid markers in this group of vessels allows an approach both to the Roman soldiers' consumption food and to the productive capacity of the Iberian population that presumably supplied the vessels and their contents.

The above interpretation is worth describing as much as the following related methodological considerations are too. The need for prompt sampling of ceramic vessels so as to avoid marker degradation and the need for keeping them unwashed, even if that hindered easier reconstruction, required a procedure whereby the minimum vessel sampling size was identified according to shapes, colours, textures and thicknesses. This paper assesses the efficiency of this sampling method according to whether the fragments researched represent adequately the final number of vessels identified after reconstruction, according to whether the sample size is too large and unnecessary or, in the latter case, according to whether such sampling supplies such relevant information that it compensates for the high sample size.

The chemical analysis method used relies on joint and coordinate use of gas chromatography-mass spectrometry (GC-MS), liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS) and gas chromatography-combustion-isotope ratio mass spectrometry

(GC-C-IRMS). Archaeological analysis as well as carpological and anthracological data obtained from the Iberian *oppidum* of Turruñuelos, i.e. from the surroundings and the closest settlement to the battleground, were used as a complement and as feedback of the chemical data (Montes and Pradas, 2014).

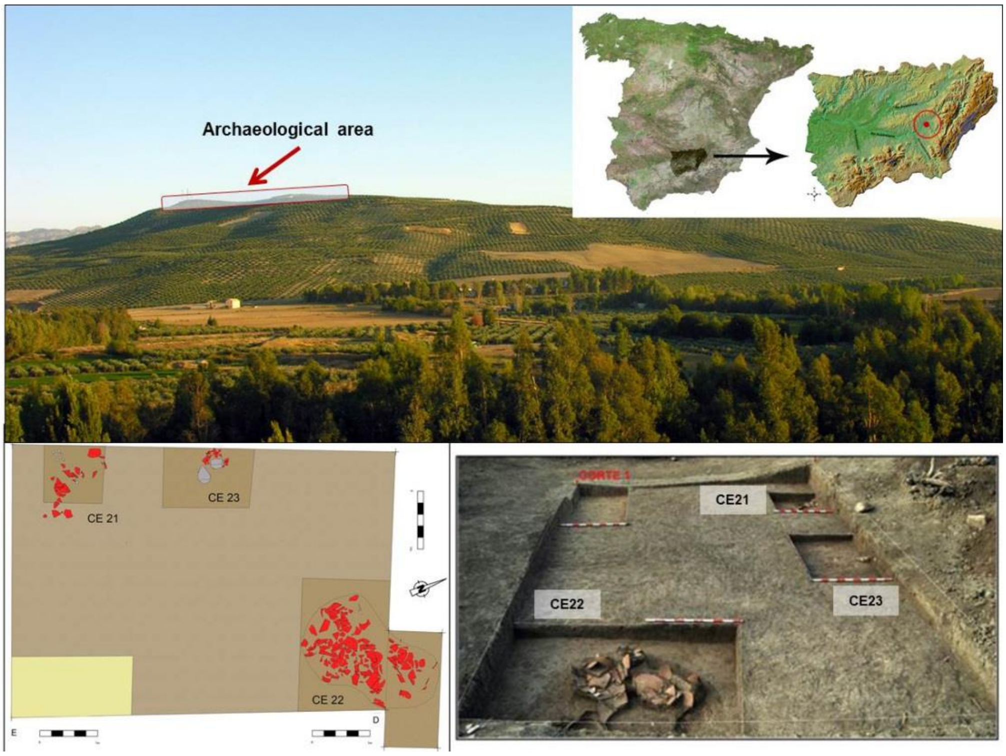

Figure 1. Cerro de Las Albahacas. Location of the ceramic groups 21, 22 and 23 of sector 7

Figure 2. Ceramic groups 21, 22 and 23

GC-MS lipid and wax analysis of ceramic vessels has become a widespread research resource, especially after the trimethylsilyl (TMS) derivatives research method was added in the 90s. This method allows the analysis of a range of highly relevant markers in archaeology, such as sterols, wax esters, and mono-, di- and tri-acylglycerols (Evershed et al., 1990, 1991, 2008; Garnier et al., 2003; Regert et al., 2001, 2003; Colombini et al., 2005; Parras et al., 2011, 2015a, 2015b; Van de Velde et al., 2019; Sánchez et al., 2019). Subsequent methodological improvements equipped archaeometric research further with analysis of stable carbon isotope composition. Using a highly sensitive device such as a gas chromatograph-combustion-isotope ratio mass spectrometer and plotting the $\delta^{13}C$ values of methyl palmitate ($\delta^{13}C_{C16:0}$) against those of methyl stearate ($\delta^{13}C_{C18:0}$) or $\Delta^{13}C$ value ($\delta^{13}C_{C18:0}$-$\delta^{13}C_{C16:0}$) plotted against $\delta^{13}C_{C16:0}$, terrestrial animal fat of various origins (ruminant, non-ruminant, and milk) can be separated from sea animal fat (Regert, 2011; Craig et al., 2011; Heron et al., 2013; Cramp et al., 2014; Manzano et al., 2015; Whelton et al., 2018; Dunne et al., 2019).

HPLC-APCI-MS, a chromatographic variant used in this paper, is also used successfully for the analysis of other artifacts. Use of APCI as an ionization source has been proved to contribute to relevant data collection in AAB and ABC TAGs mass spectrometry. The data are not only about which fatty acids are in each TAG, but also about the position in which the fatty acids estearify each of the three positions of the glycerol skeleton. This allows an estimate of the regioisomeric composition of TAGs based on the contrast in the relative abundance of diacylglycerol [M-RCO2]+ fragments. This regioselective analysis leads to the identification of a fatty acid in the sn-2 position of glycerol (Saliu et al., 2011; Kimpe et al., 2002; Romanus et al., 2007; Evershed et al., 2002; Regert, 2011; Parras et al., 2015). This is crucial for the analysis of contents in view of its direct association with the type of residue present. Vegetable lipid can thus be distinguished from animal fat. In the latter case, it may be even possible to tell ruminant from nonruminant fat based on the proportion of palmitic and stearic acid in the sn-2 position. Ruminant fats show a P:S ratio in the sn-2 position of ca. 60:40, whereas the ratio is closer to 95:5 in nonruminants (Evershed et al., 2002).

2. MATERIALS AND METHODS

The chemical analysis method consists of a three-step procedure. First, the general lipid profile of the ceramic group is analysed by GC-MS. Second, the samples that display chemical markers related with animal or vegetable lipid, whether in isolation or alongside other residues like beeswax, are analysed by HPLC-APCI-MS in order to identify the types of TAG and, where applicable, to calculate the P/S index in the sn-2 position. Finally, GC-C-IRMS analysis of ^{13}C stable isotopes is carried out on the samples that show a clear lipid profile associated with animal fat, in order to separate adipose fat (ruminant and non-ruminant) from milk fat.

2.1. *SAMPLING*

The ceramic stock under research consists in 134 fragments of the ceramic groups 21, 22 and 23, retrieved from cutting 1 of sector 7 of the Roman camp B after the battle.

The high number of ceramic fragments and the low number or the complete lack of rims and/or bases made it notoriously difficult to identify the number of vessels of the three groups. Restoration of the three groups for precise identification was not advisable, because restoration entailed washing and prolonged exposure and, consequently, a risk of sample manipulation and contamination by various external agents. Sampling was therefore carried out according to criteria based on the identification of bases whenever possible, and on the shapes, colours and thicknesses recorded, even if this entailed that the final sample would overestimate the actual number of vessels of the site. This was even more so in the case of larger vessels, where the contrasts between fragments increased. At the same time, this sampling procedure had the advantage to allow comparison of the actual number of fragments of each vessel, once they had been restored and their actual number had been identified (as shown in Table 1 and Figure 3).

The amount of the sample taken, between 2 and 9 g, was according to the size of the vessel. The fragments of archaeological vessels selected for analysis were wrapped each in a piece of dark paper and stored in a freezer at least at -20 °C until their analysis. The fragments were then taken out of the freezer and a sample was collected. Any remains of soil were removed with an electric hand-drill to avoid contamination (Heron et al., 1991; Mayyas, 2018). The sample was then grinded to the appropriate size (0.25 mm) in an agatha mortar. Two g were taken for the analysis.

Table 1. Sample numbers and distribution

Groups	Types of vessels Identified	Structures	N° fragments analysed
G1	Amphora 1	21, 22	**22**
	Amphora 2	21, 22	**13**
	Amphora 3	22	**21**
	Amphora 4	22	**33**
	Amphora 5	22	**10**
	Amphora 6	23	**4**
	Non determined	22	**21**
G2	4 platters	22	**5**
	1 bowl	22	**1**
	1 bottle	22	**1**
	1 dish	22	**1**
	1 unguentarium	22	**1**
	1 pot	21	**1**

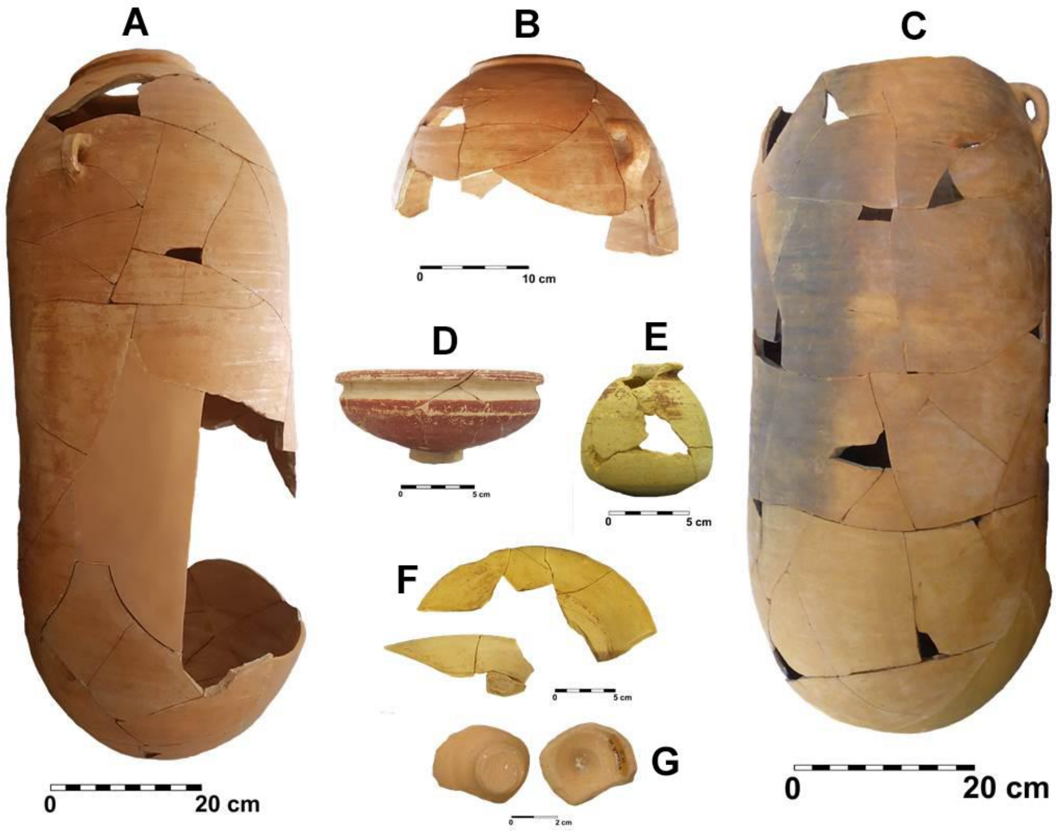

Figure 3. Selection of the analysed ceramic ensemble: amphora 1 (A), amphora 2 (B), amphora 4 (C), platter (D), bottle (E), dish (F), unguentarium (G)

2.2 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

2.2.1 LIPID EXTRACTION

Extraction is in accordance with the procedure described in Evershed et al. (1990). Ten µL of tetratriacontane (internal standard) and 10 mL of the mixture chloroform/methanol ($CHCl_3$:MeOH) (2:1 v/v) were added to 2 g of the ground ceramic fragment. Lipids were extracted with ultrasound for 15 minutes. The solution was centrifuged (3500 rpm, 5 minutes) eliminating the remaining ceramics and removing the supernatant where the lipids are solved. This process was repeated twice. The extract portions were combined into one for solvent evaporation under a N_2 flow. The dry extract was solved again in 500 µL of $CHCl_3$. The extracts were stored in a deep freeze (-20 °C) until required for GC-MS, HPLC-APCI-MS and GC-C-IRMS.

2.2.2 DERIVATISATION (TMS COMPOUNDS)

A 100 µL aliquot was removed and transferred to a smaller vial. This volume was evaporated to dryness under a N2 flow. N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) was used as a derivatisation agent. The derivatisation reaction took place with 20 µL of this reagent at 70 °C for 30 to 40 minutes. When the reaction was over, the vial was cooled and the remaining derivatising agent was evaporated under a N2 flow. The sample was then solved again in 50 µL of cyclohexane. An amount of 1 µL of the sample was injected into the chromatograph.

2.2.3 GC-MS ANALYSIS

The analyses were performed using a gas chromatography equipment (model Thermo Trace GC Ultra) coupled to a Thermo DC Q II mass spectrometer. The samples were introduced by on-column injection into a 15 m x 0.25 mm I.D. fused silica capillary column, coated with poly(dimethylsiloxane) stationary phase with 0.1 µm film thickness. Helium was used as the carrier gas (purity 99.99%) at a flow speed of 1.2 ml/min. The GC oven temperature program was as follows: initially at 50 °C, held for 2 min, ramp to 350 °C at 10°C/min, held for 10 min.

The mass spectrometer conditions were an emission current of 100 µA, an electron energy of 70 eV and an ionization source temperature of 300° C. Total ion measurements were obtained in the mass spectrometer that scanned from m/z 50-900 at a scan rate of 1,15 scan/s. The GC-MS capillary interface was maintained at a temperature of 350°C.

2.3 GAS CHROMATOGRAPHY-COMBUSTION-ISOTOPE RATIO MASS SPECTROMETRY (GC-C-IRMS)

This isotopic analysis technique requires addition of a small amount of HCl-activated Cu during chlroform/methanol extraction. This is done in order to eliminate the native sulphur (S8) residue that may remain in the vessels and may not only affect the measurement and the results, but also damage the equipment.

2.3.1 DERIVATISATION (FAMES)

Formation of methyl esters of fatty acids (FAMEs) in the sample is required to obtain the complete record of palmitic and stearic acids. Both acids occur in high concentrations in animal fat, and have a specific 13C isotope concentration that is used for identification of the origin of the type of fat.

For methylation of fatty acids, a 250 µL aliquot of the extract was taken and dried under a N2 flow. 1,5 mL of a 5% (v/v) mixture of H2SO4 in MeOH was added at 5%, and the reaction took place at 70 °C for 1h. The sample was then diluted with 5 mL distilled water and was extracted three times (multiple extraction) with 2 mL ethyl ether each, thus obtaining the methyl esters of the fatty acids in the sample. After evaporation of ethyl ether under a N2 flow, the sample was redissolved in 0,5 mL (500 µL) hexane or cyclohexane, and was thus ready for 1 µL injection in the chromatograph.

2.3.2 C-C-IRMS ANALYSIS ($\delta^{13}C_{C16:0}$, $\delta^{13}C_{C18:0}$, $\Delta^{13}C$)

The GC-C-IRMS combustion reactor is intended for oxidation of the organic compounds separated by GC (FAMEs) into CO_2. CO_2 results from combustion of palmitic and stearic acids. IRMS then measures the relative abundance of ^{13}C and ^{12}C (equation 1). The results are expressed as:

$$\delta^{13}C\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}}\ R = {}^{13}C/{}^{12}C \text{ in per mil.}$$

Equation 1

An additional source of C atoms results from the MeOH used in the reaction during the formation of the methyl esters of the palmitic and stearic acids, so the $\delta^{13}C$ values of FAMEs (equation 2) must undergo correction by a mass balance equation,

$$\delta^{13}C_{FA} = \frac{\{[(n+1)(\delta 13C_{FAMEs})] - \delta 13C_{H2SO4\ in\ MeOH}\}}{n},$$

Equation 2

where $\delta^{13}C_{FA}$ is the corrected value for each fatty acid, $\delta^{13}C_{FAME}$ is the value obtained for the fatty acid with an n number of C atoms, and $\boldsymbol{\delta^{13}C_{H2SO4\ in\ MeOH}}$ is the correction factor of the derivatising agent.

The values of $\delta^{13}C_{C16:0}$, $\delta^{13}C_{C18:0}$ and $\Delta^{13}C$ were obtained with a gas chromatography equipment (model Thermo Trace GC Ultra) coupled to a MAT 253 (Thermo) mass spectrometer using a GC-C III combustion interphase. This equipment is the property of the Laboratory for Stable Isotope Analysis (LAIE) of the Institute for Environmental Science and Technology (ICTA) of the Autonomous University of Barcelona (UAB).

The compounds were separated and identified with a 60 m x 0.25 mm (inner diameter) DB-5 MS-UI chromatography capillary column and a (5%-phenyl)-methylpolisiloxane stationary phase of 0.25 µm thickness. Helium was used as the carrier gas. The equipment's accuracy was ± 0.3 ‰. The samples were injected under the following experimental conditions: injector temperature, 310 ^{0}C; initial temperature, 80 ^{0}C for 1 min; temperature gradient 1, 30 ^{0}C / min to 120 ^{0}C; temperature gradient 2, 6 ^{0}C / min to 320 ^{0}C; final temperature, isothermal at 320 ^{0}C for 21 min; combustion reactor temperature, 940 ^{0}C; sample injection volume, 0,5 – 1 µL.

2.4 *HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-MASS SPECTROMETRY (HPLC-APCI-MS)*

A 100 µL aliquot was removed and transferred to a smaller vial, evaporated to dryness under a N2 flow and solved again in 200 µL of isopropanol. No previous derivatisation is needed. An amount of 20 µL of sample was injected into the chromatograph.

The analyses were performed on an Agilent 1290 Infinity HPLC system. The final HPLC method for the analysis of TAGs used the following conditions: one chromatographic column Zorbax Eclipse Plus C18 (150 mm x 4.6 mm, 1.8 µm particle size), column temperature of 30ºC and the mobile phase gradient, at a flow rate of 0.4 mL/min, of 3 min 50% B (2-propanol), 10 min 90 % B, 20 min 100% B, held for 5 min. The mobile phase was prepared fresh every day before the analyses.

The HPLC system was connected to an Agilent 6220 TOF (time-of-flight) mass spectrometer equipped with an APCI interface operated in the positive ionization mode, using the following operation parameters: capillary voltage, 3000 V; vaporizer temperature: 375 ºC; nebulizer pressure, 60 psi; drying gas flow rate, 5 L/min; gas temperature, 325 ºC; corona current: 4 µA. The time-of-flight analyser was used in the mass range m/z of 100-1300, with a scan time of 1 s.

3. RESULTS AND DISCUSSION

Positive results were obtained from GC-MS analysis in 58 out of 134 fragments (43%). Vessel restoration after sampling and analysis identified 15 vessels, classified as two groups. The first group (G1) comprehends storage vessels (amphorae) and a number of fragments that could not be identified as specific vessels. The second group (G2) comprehends smaller vessels (mainly platters and bowls). Careful sampling without specific vessel identification results in an inordinate hypothetical number of vessels. However, the analysis of multiple samples of the same vessel safeguards proper identification of contents and, as will be shown below, the identification of various fat patterns for the same vessel.

Vessel restoration confirmed a tendency recorded for other areas of the camp: some vessels were destroyed and their fragments were purposely scattered as several accumulations, most probably in accordance with some ritual. This applies to amphorae 1 and 2, both arranged as two separate accumulations (E21 and E22).

Within the set of 54 fragments that yielded positive results after GC-MS analysis, ten fragments showed chemical markers of lipids and, therefore, qualified for HPLC-APCI-MS analysis. Five of these displayed a TAG profile associated with a vegetable lipid. Only one fragment showed a fat profile appropriate for GC-C-IRMS analysis.

Overall, the chemical markers found in the samples identify beeswax, vegetable lipid and non-ruminant animal fat. Beeswax is recorded in four vessels (four amphorae) and in the undetermined set (U), alone and with vegetable lipid. In the two remaining vessels (amphora 3 and platter), the chemical markers signal vegetable lipid and animal fat, respectively (as shown in Table 2).

The chemical markers associated with beeswax recorded in amphorae 1, 2, 5 and 6 and in the undetermined set (U) match partially or completely the set of chemical markers that is indicative of this substance (Regert et al., 2001; Colombini and Modugno, 2009; Mayyas and Khaled, 2015; Parras et al., 2015a; Roffet-Salque et al., 2015; Pollard et al., 2017; Sánchez et al., 2019) (as shown in Figure 4), namely:

- A series of C23 to C33 n-alkanes displaying a unimodal distribution with a strong odd-over-even predominance.
- A series of W40-W54 carbon number palmitic acid wax esters with a main constituent that contains 46 carbon atoms.
- Long-chain alcohols, resulting from hydrolysis of wax esters, with an even carbon number, usually from C24(OH) to C34(OH), maximizing at C30.
- A series of W(OH)40-W(OH)50 carbon number hydroxymonoesters. The low degradation of beeswax components in the extracts can also be concluded from the occurrence of hydroxymo-

noesters, whose degradation could be attributed to leaching with ground water, rainfall or washing the fragment.

The hydrophobic nature of beeswax makes it relatively resistant to degradation. Hence, if protected from extensive microbial attack and/or exposure to high temperature during anthropogenic manipulation, the aforementioned chemical properties can be used in assessing its occurrence (Roffet-Salque et al., 2015).

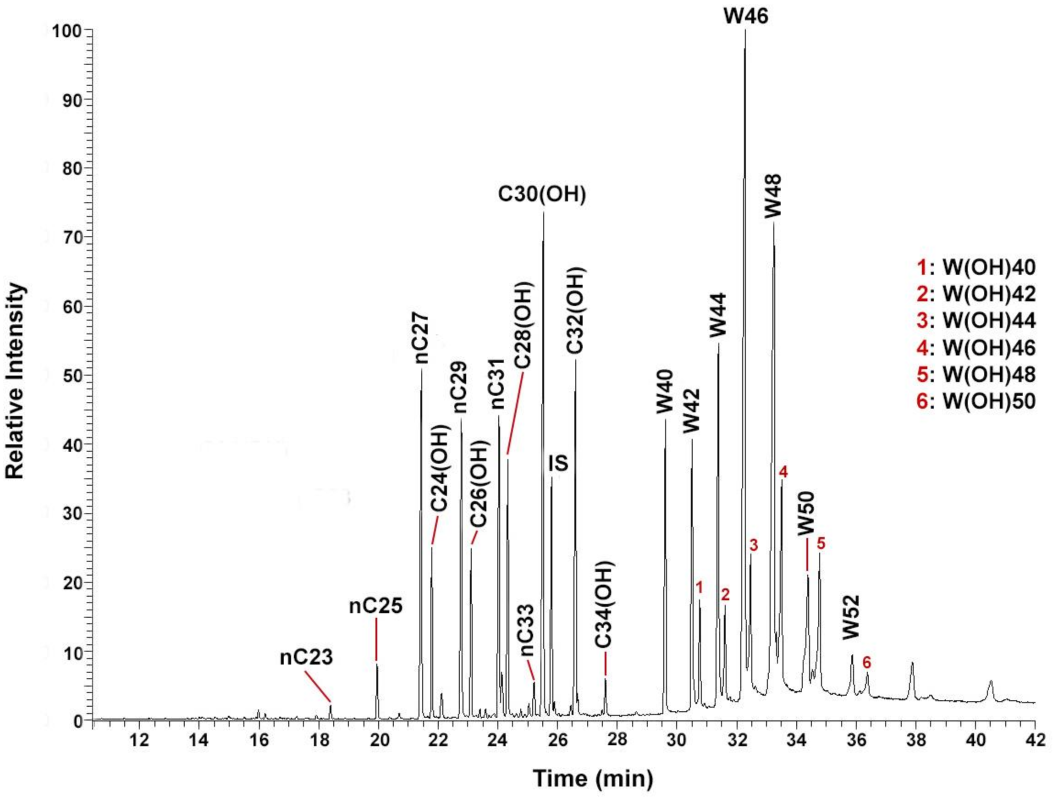

Figure 4. GC-MS analysis of amphora 2: beeswax

Vegetable lipid was clearly identified in amphora 1 alongside beeswax, and in amphora 3 as the only remaining residue. This identification is based on the abundance of chemical markers clearly associated with this type of fats: oleic (C18:1) and linoleic unsaturated fatty acids (C18:2), vegetable sterols such as β-sitosterol and stigmasterol, and a range of triacylglycerols composed mainly of mono- and di-unsaturated fatty acids identified by HPLC-APCI-MS: LLO, PLL, PLO, OPL, OLO, POP, POO, OOO (as shown in Table 2).

Table 2. GC-MS, HPLC-APCI-MS and GC-C-IRMS results: P: palmitic acid, S: stearic acid, O: oleic acid, L: linoleic acid, W: wax ester, W-(OH): wax hydroxymonoester, nC: n-alkanes, C(OH): fatty alcohols, NDSV: Non Determined Storing Vessel

Vessel	**Group**	**Chemical markers (GC-MS, HPLC-APCI-MS, GC-C-IRMS)**	**Content**
Amphora 1	**21, 22**	nC23, nC25, nc27, nC29, nC31, nC33, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50, W(OH)40, W(OH)42, W(OH)44, W(OH)46, W(OH)48	**Beeswax 17 fragments**
		Profile 1. nC23, nC25, nc27, nC29, nC31, nC33, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50, ***C12:0, C16:0, C18:0, C18:1, C18:2***, **sitosterol, stigmasterol**	**Beeswax + vegetable lipid 1 fragment**
		Profile 2. C16:0, C18:0, nC23, nC25, nc27, nC29, nC31, nC33, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50, W(OH)40, W(OH)42, W(OH)44, W(OH)46, W(OH)48, ***LLO+PLL, OPL, PLO, LOP, OLO, POP, OPO, POO, OOO, POS, OSO***	**Beeswax + vegetable lipid 4 fragments**

Amphora 2	**21, 22**	nC23, nC25, nC27, nC29, nC31, nC33, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50, W(OH)40, W(OH)42, W(OH)44, W(OH)46, W(OH)48, W(OH)50	**Beeswax 12 fragments**
Amphora 3	**22**	**Profile 1**. C12:0, C16:0, C18:0, C18:1, C18:2, sitosterol, stigmasterol	**Vegetable lipid 1 fragment**
		Profile 2. C16:0, C18:0, C18:1, LLO+PLL, OPL, PLO, LOP, OLO, OPO, POO, OOO, OSO,	**Vegetable lipid 1 fragment**
Amphora 5	**22**	nC23, nC25, nC27, nC29, nC31, nC33, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50	**Beeswax 9 fragments**
Amphora 6	**23**	nC23, nC25, nC27, nC29, nC31, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50	**Beeswax 2 fragments**
ND	**22**	nC23, nC25, nC27, nC29, nC31, C24(OH), C26(OH), C28(OH), C30(OH), C32(OH), W40, W42, W44, W46, W48, W50, W(OH)40, W(OH)42, W(OH)44, W(OH)46	**Beeswax 8 fragments**
Platter	**22**	C16:0, C18:0, C18:1, PP, PS, SS, PMP, OLO, PPP, PPO, POP, OOO, PSP+PSO, SPS, SSS. P/S ratio *sn*-2: 82/18. $\delta^{13}C_{C16:0}$: -25.21 and $\delta^{13}C_{C18:0}$: -23.88 ($\Delta^{13}C$: 1.33)	**Non ruminant fat**

A finer analysis of the type of fatty acids and TAGs of the two vessels followed up the identification of vegetable lipid contents. Two vegetable lipid profiles were identified:

- A profile characterized by stigmasterol, sitosterol, lauric acid (C12:0) and linoleic acid (C18:2) that is compatible with the fat of nuts high in lauric and linoleic acids (almonds, walnuts) (Pollard et al., 2017; Skibo et al., 2009). This fat is recorded alongside beeswax in amphora 1, and alone in amphora 3 (as shown in Figures 5 and 7).
- A profile associated with vegetable oils of fleshy fruits (olive) with a high content in triacylglycerols of oleic acid (OOO, OOP…) and a lack of sterols and linoleic (C18:2) and lauric acids (C12:0) (Mottram et al., 1997; Kimpe et al., 2001). This fat is recorded alongside beeswax in amphora 1, and alone in amphora 3 (as shown in Figures 6 and 8).

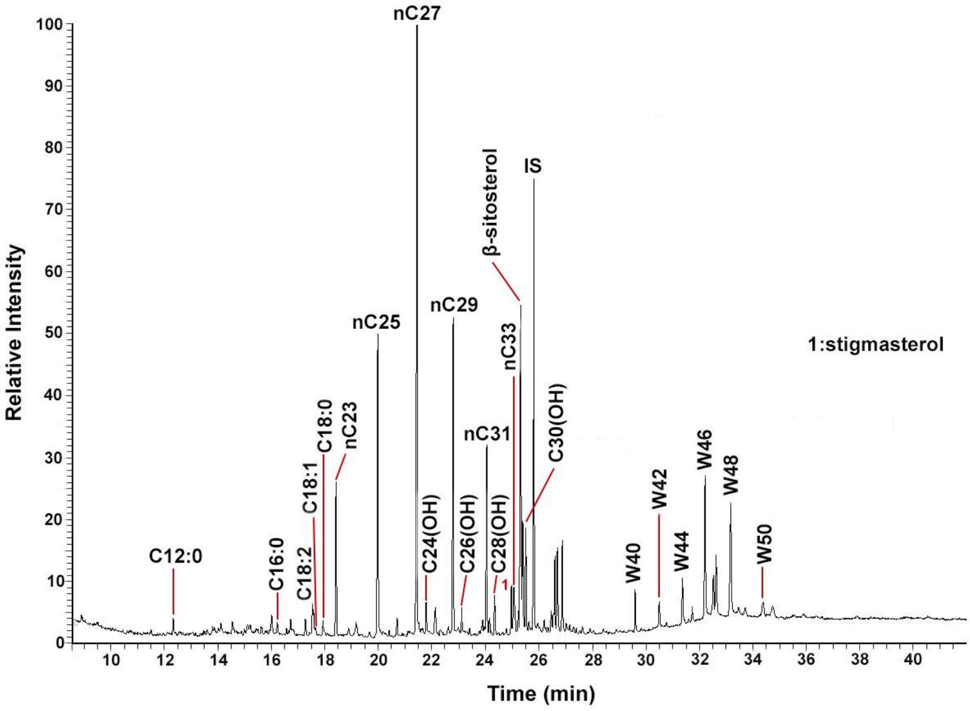

Figure 5. Profile 1. GC-MS analysis of amphora 1: beeswax + vegetable lipid

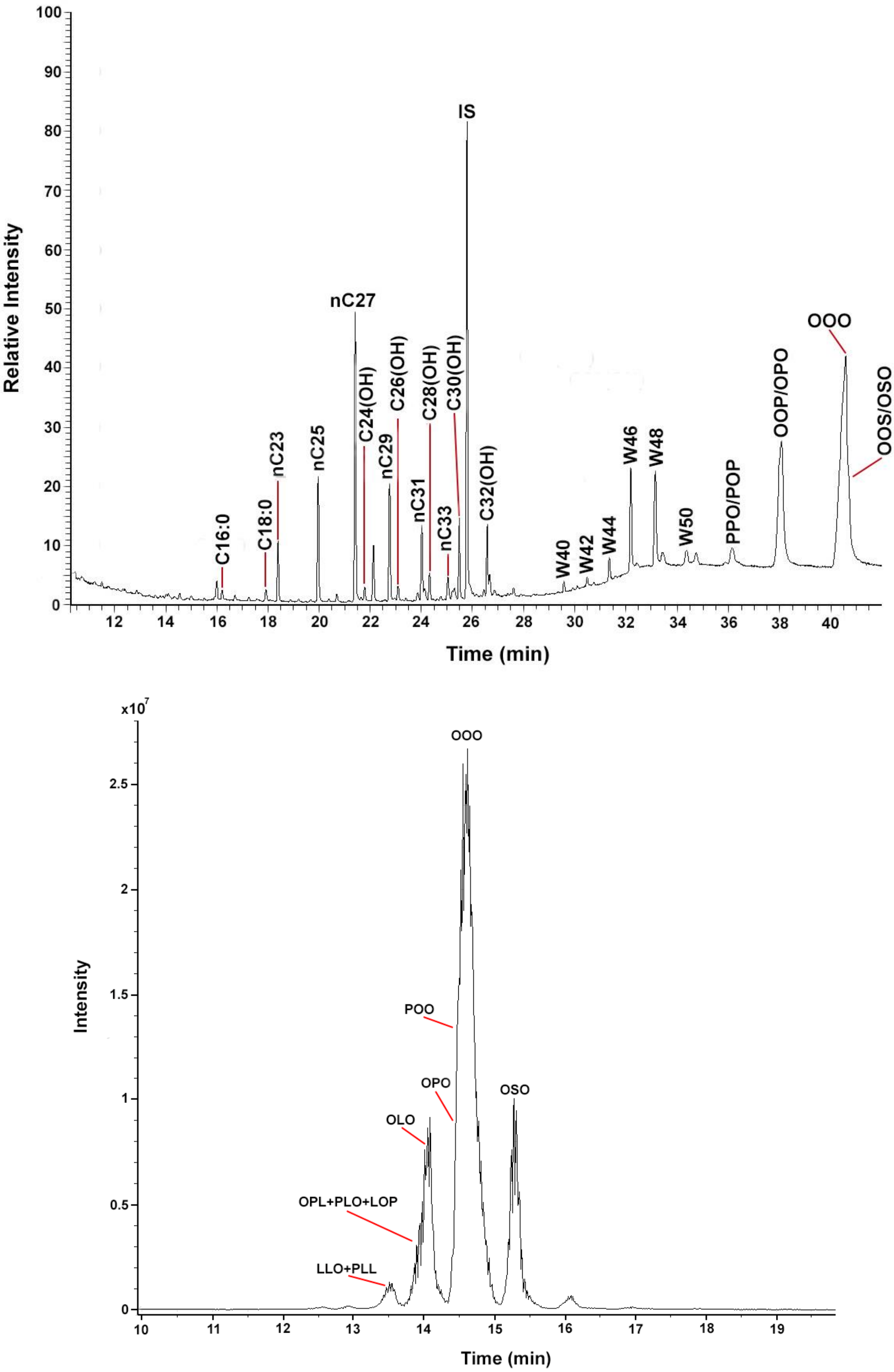

Figure 6. Profile 2. GC-MS (A) and HPLC-APCI-MS (B) analysis of amphora 1: beeswax + vegetable lipid

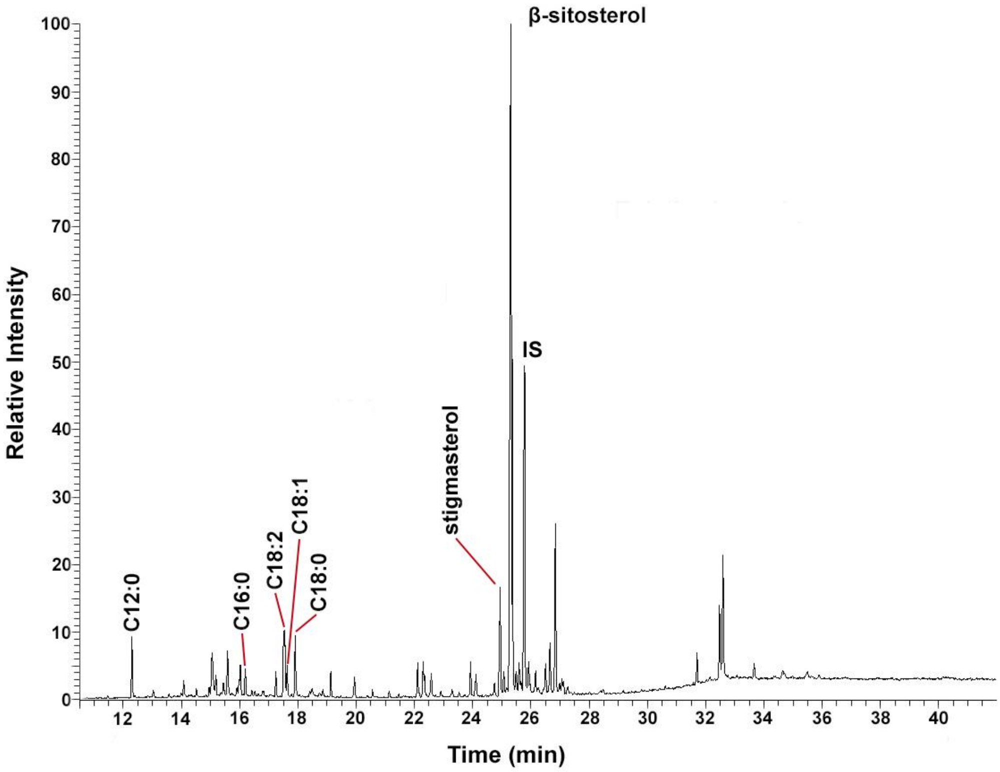

Figure 7. Profile 1. GC-MS analysis of amphora 3: vegetable lipid

The lipid profile of the remaining vessel, a platter, matches that of animal fat, with saturated fatty acids C16:0 and C18:0 and the dominance of triacylglycerols of palmitic, stearic and oleic acids (PMP, PPP, PPO, POP, OOO, PSP, PSO, SPS, SSS) (as shown in Figure 9). HPLC-APCI-MS analysis of triacylglycerols allowed to identify quantitatively the non-ruminant origin of the residue, especially in view of the low amount and intensity of chemical markers obtained from GC-MS analysis. This interpretation relies on the dominant occurrence of a range of triacylglycerols formed with the oleic (C18:1) and palmitic (C16:0) fatty acids, with a high amount of tripalmitin, by contrast with saturated triacylglycerols formed with palmitic and stearic acids (C18:0), which are more closely associated with ruminant animal fat (Regert, 2011). The semiquantitative analysis of the palmitic and stearic acids in the sn-2 position of TAG reveals a P/S index of 82/18. This places the fat identified here at the characteristic interval of non-ruminant fat (95/5 or 85/15) (Evershed et al., 2002).

GC-C-IRMS analysis confirmed the origin of this animal fat. The δ^{13}C data (defined as 13C/12C) and the Δ^{13}C value (defined as $\delta^{13}C_{C18:0}$ - $\delta^{13}C_{C16:0}$) are a robust criterion for the separation of ruminant from non-ruminant fat. The comparison of the experimental data obtained δ^{13}C (-25.21‰; -23.88‰) and Δ^{13}C (1.33) with samples of present-day fat for reference (Mottram et al., 1999; Copley et al., 2003; Gregg et al., 2009; Whelton et al., 2018) led to the conclusion that the isotopic profile identified belongs to pig adipose fat (non-ruminant).

3. CONCLUSIONS

The results obtained confirm the efficiency of GC-MS, HPLC-APCI-MS and GC-C-IRMS analysis. The lipid profile obtained, especially when it is not sufficiently representative, is supplemented with HPLC-MS analysis of TAG and with GC-C-IRMS. The former is particularly useful for the separation of animal and vegetable lipid, and the latter for the identification of animal fat (ruminant, non-ruminant and milk).

Once the number of vessels of the ceramic groups is known, furthercheck of the sampling used shows that selection criteria based on the fragments of bases, thicknesses, sizes or outer textures tend to overestimate the number of vessels. This is also the case with small vessels, only to a lesser extent. The high number of fragments under study allows a more thorough spatial analysis, as it covers more areas of the vessels. This is particularly important, because chemical markers decay unevenly according to the position of the residue inside the vessel. The increased number of fragments also increases the chance to attest the occurrence of various fat contents or profiles (amphorae 1 and 3).

Overall, content analysis identified a range of food items consumed by the Roman army and, very probably, by the dwellers of the nearby Iberian oppidum of Los Turruñuelos, a supply area for the Roman army camp of Cerro de las Albahacas. The carpological and anthracological analysis of this *oppidum* (Montes and Pradas, 2015) reveals the occurrence of almonds and olives, and these may be the origin of the two types of vegetable lipid of amphorae 1 and 3.

The identification of just beeswax in amphorae 2, 5, 6 and in the unclassified group of fragments is open to interpretation: as an inside covering or as honey, which was a common food among the Roman army (Menéndez, 2002). It may also be related with posca, a most widespread drink among the Roman soldiers for being refreshing. Posca was made with a sort of vinegar diluted and mixed with water and, sometimes, also with honey (Monzo, 2010). The consumption of honey in the Iberian peninsula has been attested since the 7th millennium. In the case of the Iberians, it has been inferred from the attestation of vessels adapted for honey storage, for the abundance of beeswax in contexts of the 6th to 4th ct. b.C. and for the identification of ceramic beehives from the late 3rd ct. b.C. (Bonet and Mata, 1995; Parras et al., 2015; Morais, 2006). It can therefore be assumed that some amphorae may have contained honey for the dwellers of the Iberian *oppidum* of Los Turruñuelos' storage and consumption, and that they were requisitioned by the Roman army for various purposes.

The results obtained from amphorae 1 and 3 are harder to interpret, both because several successive uses may be represented and for the combinations that can be obtained from the three substances recorded, namely two types of vegetable lipid and beeswax.

The profile of the above fats is compatible with olive oil in one case and with almond oil in the other. The low fat yield of the latter type of oil and the large size of the amphorae allows to discard the hypothesis of storage of almond oil or of any other nuts.

Amphora 1 may signal nut consumption by emulsifying ground seeds with a piece of cloth and then mixing them with water to produce a drink. If beeswax is discarded as an inner covering, honey may also have been mixed with the drink obtained from almonds in order to add honey's sugars and other beneficial properties. Before or after the above, amphora 1 must have contained olive oil. The attestation of beeswax in a high number of fragments of amphora 1 may have resulted from the disintegration of the chemical markers that are characteristic of vegetable lipids. Amphora 3 evidences the twofold fat use too, except that without attestation of beeswax. This suggests storage of vegetable (presumably, olive) oil and of the abovementioned water and almond drink, only without honey in this case.

Smaller and thicker, amphora 4 is typologically different. Not any results were obtained from the analysis of 33 fragments. This cannot be attributed to any different conditions under which it may have been buried, or to associate degradation factors, because it was under the same conditions as the five amphorae of which results were obtained. Therefore, the lack of chemical markers of contents must be interpreted in terms of different contents: water, solid matter or, simply not any use.

Whereas the identification of vegetable lipid contents and beeswax seems to have a recursive typology classification in amphorae, this is otherwise in the other vessels under study: only one of the smaller vessels (four platters, two bowls, one dish, one pot and one unguentarium) showed a completely different fat pattern. Specifically, GC-C-IRMS analysis identified non-ruminant animal fat (most probably, pig), considering the $\delta^{13}C$ (-25.21‰; -23.88‰) and $\Delta^{13}C$ (1.33) values obtained. Pork was part of the diet of both the Italic population and of the Roman army, as bacon but maybe also roasted or boiled in stews and soups (King, 1999; Menéndez, 2002; Costa and Casal, 2009).

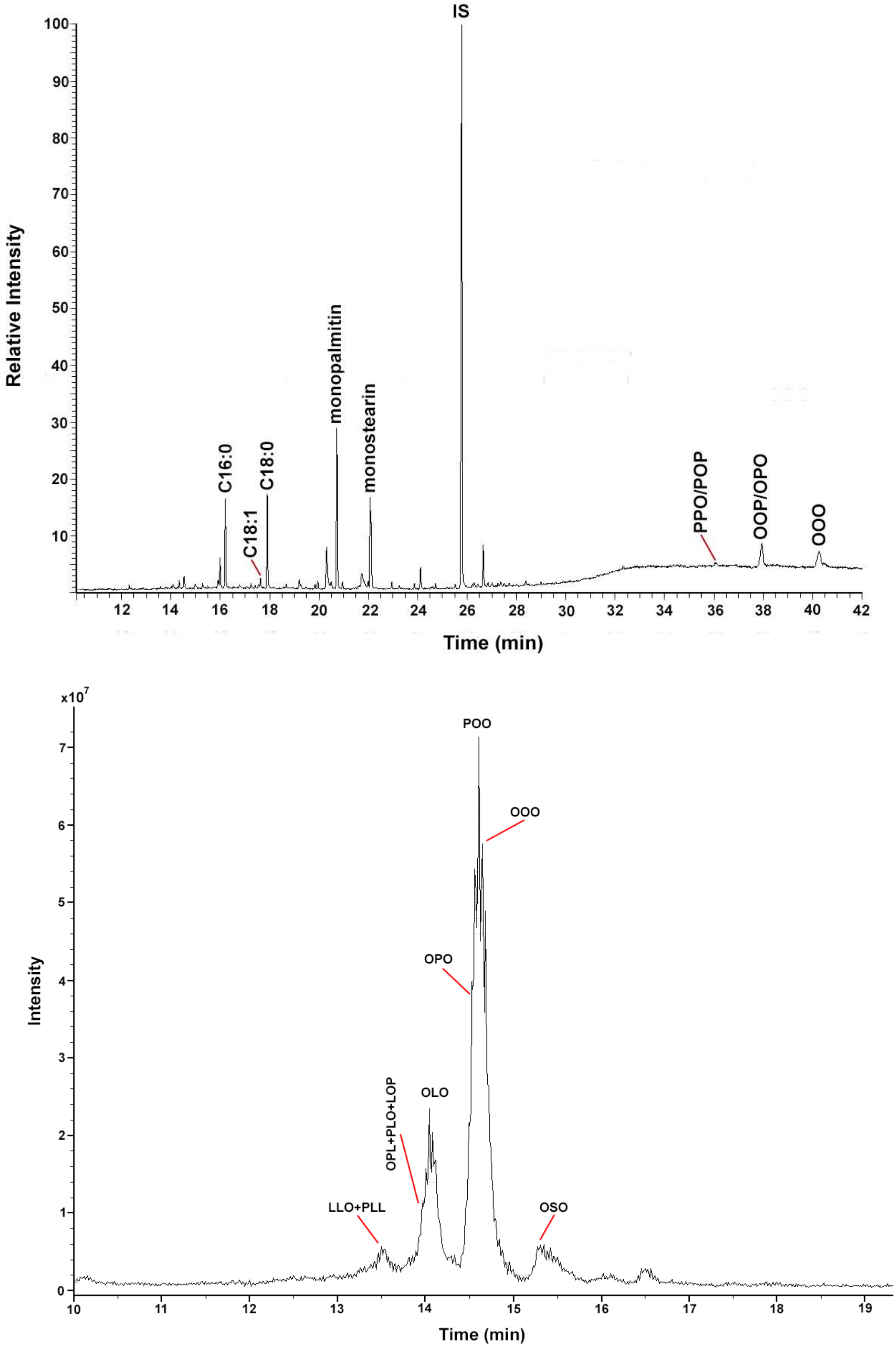

Figure 8. Profile 2. GC-MS (A) and HPLC-APCI-MS (B) analysis of amphora 3: vegetable lipid

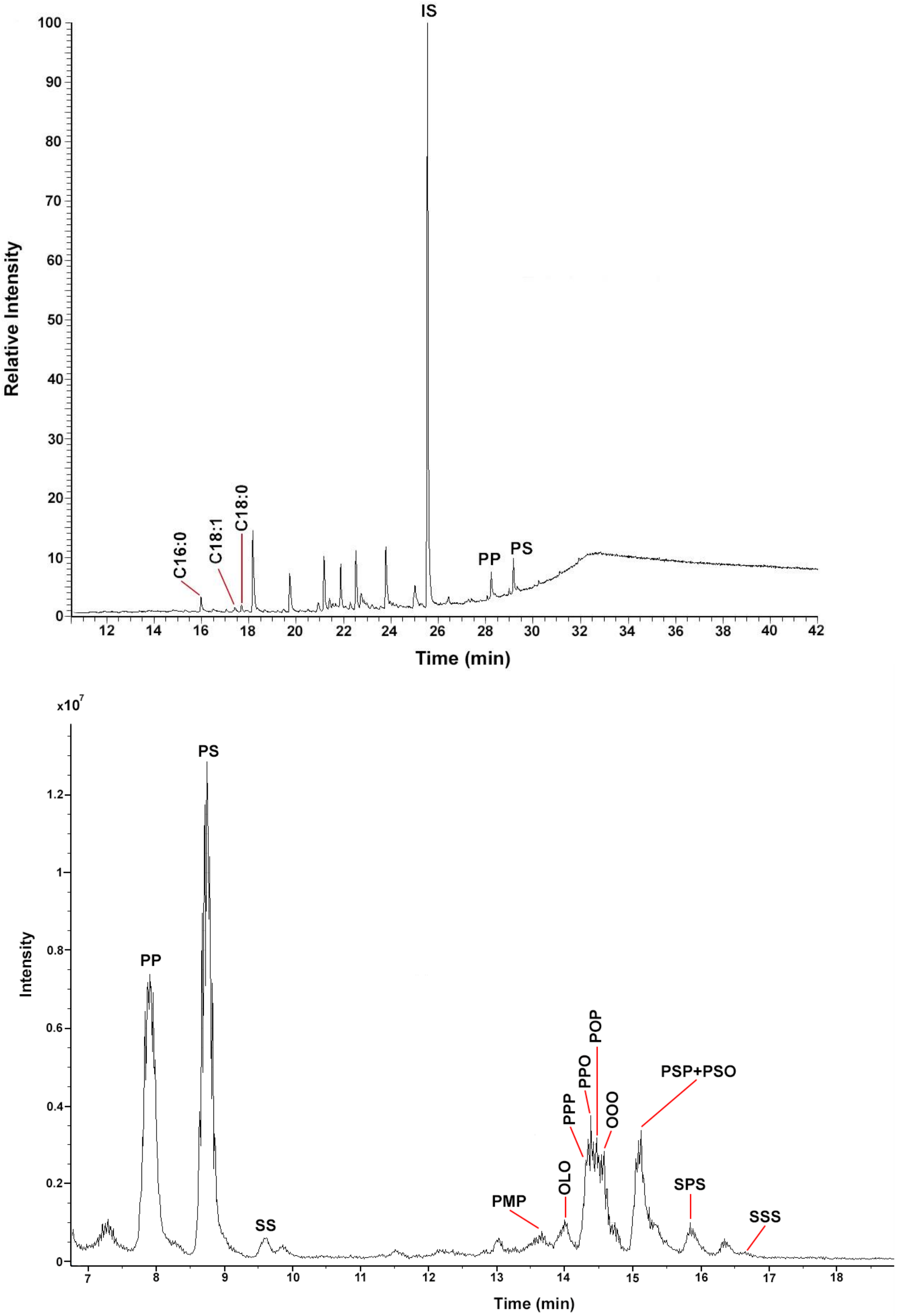

Figure 9. GC-MS (A) and HPLC-APCI-MS (B) analysis of the platter base: non-ruminant animal fat

ACKNOWLEDGMENTS

We would like to thank the support given by the University Research Institute for Iberian Archaeology, the Department of Physical and Analytical Chemistry, the CICT of the University of Jaén, the Laboratory for Stable Isotope Analysis (LAIE) of the Institute for Environmental Science and Technology (ICTA) of the Autonomous University of Barcelona and the Project Arqueología de lo Invisible (HAR2015-73959-JIN). The manuscript was translated into English by Salvador Valera.

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