Chromatographic analysis of honey ceramic artefacts

César Oliveira [1,2] · Alfredo Araújo [1] · Adriana Ribeiro [1] · Cristina Delerue-Matos [1]

Abstract

Honey was the main sweetening agent in the ancient world; it is mentioned in literary sources and archaeologically documented all over the Mediterranean basin. Its production extended throughout different parts of the Roman Empire, with important economic impacts on family economies, both as a foodstuff and as a commercial product. This paper focuses on the chromatographic analysis of Roman Iberian honeypots and ceramic beehives, identifying tracer characteristics from ancient honey in sherds from seven honeypots and three beehives. The obtained results present an important contribution to the knowledge of the local Roman Iberian economy, particularly relating to the production, transport and trade of honey in locally manufactured ceramic containers.

Keywords Archaeometry · Honeypots · Ceramic beehives · Molecular biomarkers · Gas chromatography coupled with mass spectrometry (GC/MS)

Introduction

Honey has been consumed since the very beginning of man's existence and was, for very long time, the only sweetener used by prehistoric individuals. The earliest records of the hunting and consumption of wild honey were located in Anatolia and are dated from the seventh millennium cal BC (Roffet-Salque et al. 2015). They were also found in the Iberian Peninsula rock paintings representing honey hunters climbing long rope ladders as they harvest sections of honeycomb, with a chronology not completely established but varying from the end of the Mesolithic until the end of the Neolithic (Bonet Rosado and Mata Parreño 1995, 1997; Crane 1983; Crane 1997; Crane and Graham 1985; Kritsky 2017).

The oldest traces of beehive products in the Mediterranean basin area are from the Iron Age. In fact, Rageot and co-workers reported for that period evidences of the exploitation of beehive products in Corsica (Rageot et al. 2016), while

Regert and co-workers detected at the site of Grand Aunay (located near Le Mans, France) a sherd containing an organic visible residue composed by a mixture of beeswax with birch bark tar, probably used for coating (Regert et al. 2003).

Due to their numerous uses and high demand, humans have learned how to manage colonies of bees by providing them with hives to more easily access the honey.

The earliest representation of man-made hives dates back to an Egyptian temple from 2400 BC, where cylindrical hives are represented in a horizontal position (Crane 1983; Crane and Graham 1985; Jones et al. 1973; Kritsky 2017). The Egyptian beekeeping technology seems to have spread initially to Greece, where 2000-year-old horizontal clay hives have been excavated, and later to Rome (Crane 1983; Crane and Graham 1985). Honey production extended throughout different parts of the Empire, achieving a position of utmost importance in family economies, both as a foodstuff and as a commercial product (Bonet Rosado and Mata Parreño 1997; Crane 1983; Crane and Graham 1985; Crane 2013; Morais 2011).

Several Roman manuscripts describe the existence of horizontal hives and the skilful operations needed to care for the bees and collect honey with minimum disruption. In fact, Columella (De re rustica IX, VI) refers to these pottery beehives as *getting sunburnt with the heat of the summer and frozen with the cold of the winter*. The economic impacts of

✉ César Oliveira
cesar.oliveira@graq.isep.ipp.pt

[1] REQUIMTE/LAQV, Polytechnic of Porto, School of Engineering (ISEP), Porto, Portugal

[2] Department of Heritage Studies, Porto University, Porto, Portugal

beekeeping were so evident to Roman legislators that Roman civil law considered bee's in a hive the property of the hive's owner and their unauthorized removal a theft condemned by law (Crane 2013).

In the ancient world, traditional beehives were made of different materials such as mud, wood, cork, clay or skeps and were mostly horizontally positioned. In addition, terracotta beehives were also popular, as they were durable, versatile (easy to couple two tubular beehives to construct a bigger hive) and easily transported. The most ancient apiary yet discovered is dating to the tenth to early ninth centuries BCE and is located at Tel Rehov, in the middle Jordan valley in northern Israel (Bloch et al. 2010; Kritsky 2017). These ceramic beehives were usually placed on the ground or on small platforms, installed individually or in stacks. The inner surfaces were striated to promote the adherence of the honeycombs (Bonet Rosado and Mata Parreño 1995), and the beehive ends were closed with caps manufactured with materials such as cork, wood, pottery or dried mud. The front cap had a small opening to allow the bees to fly in and out, while the back end was used for harvesting the beehive (Bloch et al. 2010). In that process, the apiarist could insert smoke through the end of the hive, forcing the bees to move to the front, inducing many of them to leave through the flight hole (Bonet Rosado and Mata Parreño 1995, 1997; Crane 1983; Crane and Graham 1985; Harissis 2014; Jones et al. 1973; Morais 2006). Ceramic hives are known in the Iberian Peninsula for pre-Roman times (Bonet Rosado and Mata Parreño 1997; Jardón Giner et al. 2009; Quixal Santos and Jardón Giner 2016).

The first evidence of containers used to transport honey dates back to the Bronze Age, as is pictured on Egyptian New Kingdom tombs dated to around 1500 BC (Crane 2013) and on some Mycenaean Linear B tablets from approximately the same period (Hornblower et al. 2014; Palmer 1955; Weilhartner 2012). Some papyri of the Ptolemaic period refer to other types of containers used to transport honey (Bortolin 2008). The containers most frequently recovered by archaeologists are Roman and Byzantine, their identification as honeypots made possible due to inscriptions (graffiti and *tituli picti*) (Peña 2007). As well as selling honey in amphorae and in other medium-long distance transport containers, this product was also stored and sold at a local or regional level, in household instruments called *instrumenta domestica* (Persano 2016).

Although it is difficult to recognize the containers used to transport and preserve honey, there are some specific forms well documented in the Iberian Peninsula, in North Central Italy and throughout the Mediterranean basin, including Greek vases especially adapted for this purpose (Morais 2014; Persano 2016). These honeypots are characteristic containers due to a particularly pronounced shoulder (sometimes two) in the shape of a rim or "eyelash", generally placed about one third from the top of the vase or near their mouth (examples in Fig. 1). According to ethnographic parallels documented both in the Iberian Peninsula (several examples from Spain and Portugal are presented by Morais (2006) and Monserrat (2013)) and in Crete (Crane 1983), this particularity seems to occur for two practical reasons: (i) to create a water channel around the containers mouth and therefore prevent insects such as ants from reaching the product and (ii) to avoid leakage down the sides of the vessel (Monserrat 2013; Morais 2006; Persano 2016).

In this study, the organic residues present in ceramic fragments of honeypots (Fig. 1a–g) and beehives (Fig. 2a–c) were determined by gas chromatography coupled to mass spectrometry (additional data on the analysed materials is presented in Table 1). As the purpose of this study, we took into consideration that honey is a complex mixture of natural products, composed of about 200 different compounds, with high amounts of monosaccharides, such as fructose and glucose, and minor traces of disaccharides, trisaccharides and tetrasaccharides (Consonni et al. 2013); about 0.57% of organic acids such as gluconic, pyruvic, malic, citric, succinic and fumaric; minerals; vitamins; proteins; amino acids like proline (the dominant), arginine, tryptophan and cysteine; and lipids and phenolic acids such as gallic, *p*-coumaric, caffeic, ellagic, ferulic, chlorogenic, syringic, cinnamic, vanillic and *p*-hydroxybenzoic (Ciulu et al. 2016; Pereira 2008). One of its most characteristic compounds is 5-hydroxymethylfurfural, formed by the dehydration of hexoses in acidic media (Fallico et al. 2008; Khalil et al. 2010; Kujawski and Namieśnik 2008). This compound is usually absent in fresh honey but its concentration tends to increase during storage even at low temperatures (Biluca et al. 2014).

The identification of honey residues was achieved by searching for biomarkers of honey. Archaeological biomarkers are "substances occurring in organic residues that provide information relating to human activity in the past" (Evershed 2008). However, while some chemical compounds offer a robust means of assigning their presence as components of organic residues, others do not present a univocal source. When such ambiguities exist, the true nature of organic residues can only be reached by the convergence of different biomarkers, providing robust evidences to support conclusions.

Despite present in aged honey, 5-hydroxymethylfurfural can also be produced during the initial stages of the Maillard reaction between amino compounds and reducing sugars from cooked foods processed at high temperature, as meat or milk (Tamanna and Mahmood 2015). Nevertheless, its detection reinforces the evidence of honey residues.

Besides beeswax tracers, biomarkers of honey have rarely been reported in archaeological contexts due to their high susceptibility to degradation under most environmental conditions. Therefore, their detection is usually indirectly made
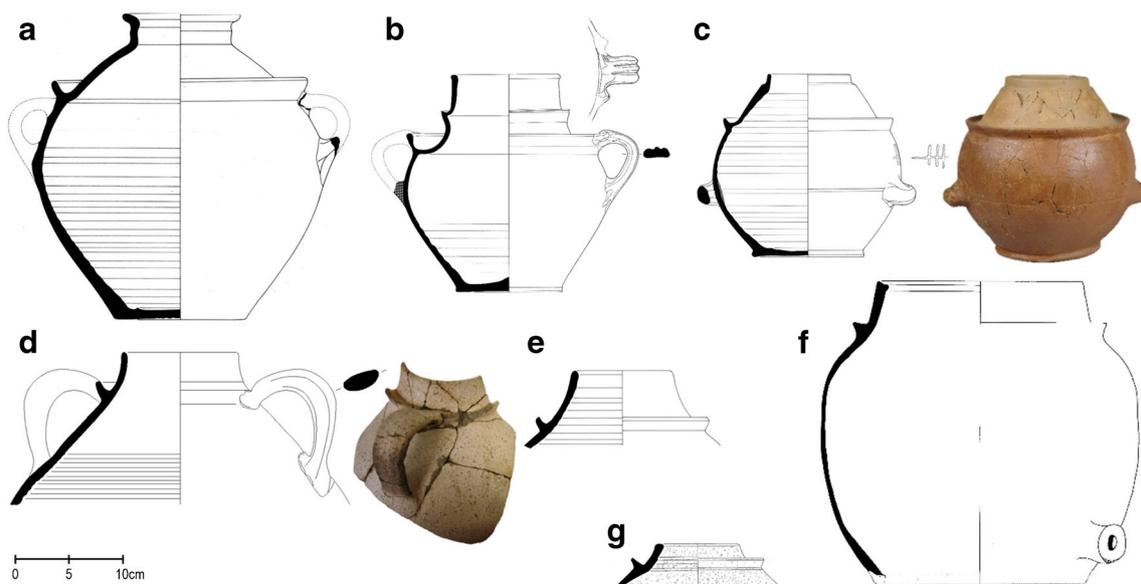
Fig. 1 Studied Portuguese honeypots. **a**–**c** Braga. **d** Chaves. **e** Matosinhos. **f**, **g** Conímbriga

by the presence of beeswax markers (Evershed et al. 2003; Evershed et al. 1997; Garnier et al. 2002; Heron et al. 1994; Needham and Evans 1987; Regert et al. 2001; Roffet-Salque et al. 2015).

In an attempt to better identify the chromatographic pattern of ancient honey, an experimental approach was used to simulate their natural degradation. Despite the interesting results obtained with the identification of honey chromatographic profile, we are aware that there are certain limitations on this experimental methodology as the past archaeological events cannot be fully replicated by experiments in the present (McGovern and Hall 2016).

The analytical methodology followed does not intend to identify traces of honey based on the detection of wax esters and long-chain alcohol characteristics of beeswax (Evershed et al. 2003; Evershed et al. 1997) but on the identification of carbohydrate compounds (Sanz et al. 2004) and other biomarkers of aged honey such as hydroxymethylfurfural, organic acids, amino acids and, eventually, biomass burning tracers

(one of the important conclusions of this manuscript). Monosaccharides (glucose and fructose) and trisaccharide compounds were mostly identified as separated peaks, whereas the disaccharide fraction was detected as an extensive co-elution of molecules (unresolved complex mixture) (de la Fuente et al. 2007; Kuhnert et al. 2013). In fact, disaccharides having glucose, galactose or mannose as their reducing moiety produce two well-resolved peaks with an area ratio of 3:1 to 10:1, those having fructose give two peaks with identical areas, while non-reducing saccharides as sucrose or trehaloses originate a single peak corresponding to an octakis-TMS derivative (Sanz et al. 2004). This profusion of different peaks per disaccharide resulted in compound co-elution.

Materials and methods

Measures were adopted to reduce manipulation and prevent sample contamination. Before use, all glassware was

Fig. 2 Studied ceramic beehives. **a** Braga. **b**, **c** Martinhal, Sagres
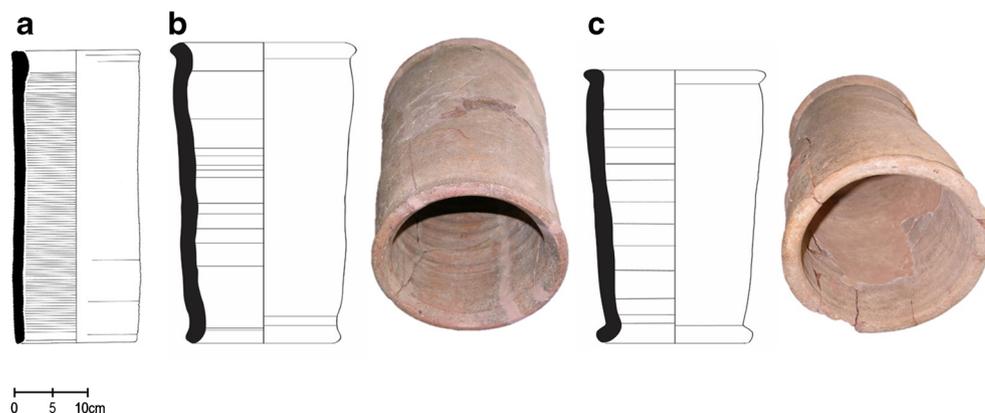
Table 1 Studied honeypots and ceramic beehives

Archaeological site	Inventory number and dimensions	Museum	Brief description	Chronology
Bracara Augusta Braga northwest of Portugal	a) 1991.2000 (maximum width 32.9 cm; height 29.7 cm) b) 1991.2270 (maximum width 23 cm; height 21 cm) c) 1991.2210 (maximum width 20.1 cm; height 17 cm)	D. Diogo de Sousa Archaeology Museum (Permanent Exhibition)	Honeypots. Common ware. Local production ceramic.	Third century AD
	d) 2004.0200 (diameter 17.4–13 cm; length 42 cm)		Ceramic beehive. Common ware. Local production ceramic.	
Aquae Flaviae Chaves North of Portugal	e) Chaves (maximum diameter 32 cm; height 15 cm)	Municipality of Chaves Archaeology Department	Honeypot fragment. Common ware. Local production ceramic.	Second to third century AD
Castrum Quiffiones Matosinhos Northwest of Portugal	f) Monte Castêlo (maximum diameter 19.4 cm; height 7.5 cm)	Municipality of Matosinhos Archaeology Department	Honeypot fragment. Late grey common ware. Local production ceramic.	Third century AD
Municipium Conimbriga Conímbriga Centre of Portugal	g) 65.GVIII 41-3—Fouilles V. n. 862 (maximum diameter 30 cm; height 30 cm) h) 69.R3-2 ≈ F.V.862 (maximum diameter 6.4 cm; height 5.2 cm)	Conímbriga Monographic Museum	Honeypots. Late grey common ware. Local production ceramic.	Fifth century AD
Roman pottery workshop in Martinhal Sagres South of Portugal	i) Mart/11 9-03 001 (diameter 26.3–20.8 cm; length 42.5 cm) j) Mart/11-9-03 002 (diameter 25.0–19.5 cm; length 38.3 cm)	Municipality of Vila do Bispo	Ceramic beehives. Common ware. Local production ceramic.	Fourth century AD

previously submerged for 24 h in chromosulfuric solution and rinsed with deionized water. New sterilized scalpels were used for each sample and the working surfaces of the laboratory benches were routinely decontaminated with ethanol 70%. New sterilized gloves were used before the manipulation of sherds to prevent the samples' contamination with human fingerprints.

In a brief description of the adopted procedure, analysed sherds were mechanically cleaned with a scalpel before sampling to eliminate possible environmental contaminations (Kałużna-Czaplińska et al. 2016 present an interesting review on the different analytical procedures used on the study of organic residues in archaeological ceramic materials by GC-MS). After the first cleaning procedure, about 0.2 g of sherd was collected from each sample's surface and the materials crushed into fine powder on a pre-cleaned agate mortar to increase the contact area and improve the efficiency of extraction. The organic remains were extracted sequentially with dichloromethane and methanol in Soxhlet apparatus, filtered with 0.20-μm PTFE syringe filters and concentrated on a rotary evaporator to about 2 mL. The extraction periods were restricted to about 1 h *per* extract, being the maximum temperature limited to solvent boiling points, therefore minimizing thermal decomposition.

The concentrated extracts were transferred to vials, dried with a gentle nitrogen flow, dissolved in pyridine and derivatized with *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA): trimethylchlorosilane (TMCS) 99:1 for the analysis of the most polar compounds (Fox et al. 2000). No internal standards were used due to the heterogeneity of the samples, as the distribution of organic residues throughout the sample clay is not homogeneous, i.e. the porosity is not equally distributed along the sherd's structure and therefore, a quantification *per* gram of sherd is meaningless.

The chromatographic analyses were performed with an ion trap analyser VARIAN 4000 Performance device that was operated in full scan mode with the following experimental conditions: (a) column ZB-5MSi, 30 m × 0.25 mm × 0.25 μm using helium as carrier gas with a constant flux of 1 mL min^{-1}; (b) injection volume of 1 μL; (c) injector temperature of 250 °C; (d) heating programme 60 to 150 °C (10 min^{-1}), 150 to 290 °C (5 °C min^{-1}), 290 °C (27 min); (e) ionisation mode, electronic impact at 70 eV; (f) interface and ionic source at 290 °C; and (g) scanned masses from *m/z* 50 to 600. Compound identification was based on the GC/MS

spectra libraries Wiley6 and NIST08, co-injection with authentic standards and analysis of fragmentation patterns (Oliveira et al. 2017).

This study included seven honeypots (Fig. 1) and three ceramic beehives (Fig. 2) from the Western Iberian Peninsula (Table 1). Samples were selected without any other criteria as being classified by archaeologists as honeypots or beehives from the roman period, recovered within the Portuguese national territory.

The composition of fresh honeys was also evaluated before and after accelerated processes of thermal degradation and dehydration, as an attempt to mimic the degradation of honey absorbed in ceramic vessels over archaeological timescales. This study was performed on two Portuguese monofloral honeys acquired to local beekeepers: honey from *Campanula* spp. flowers (from Castelo Branco region, centre of Portugal) and from *Calluna vulgaris* flowers (from Peneda-Gerês National Park, north of Portugal) and a multifloral honey (from unknown origin) bought in a local supermarket. The adopted procedure followed a similar protocol to the one conducted by Teodor and co-workers (Teodor et al. 2014) as an attempt to simulate the natural degradation and ageing of wine in ceramic vessels. In fact, a local potter from the city of Braga used local clays and roman similar pottery production techniques to model three reproductions of Fig. 1b honeypot (one example in Fig. 3). Each one of those modern ceramic vessels was filled for 2 days with a different honey type, decanting the excess honey after that time period. The containers with the walls impregnated with honey were then kept for 30 days in darkness at a constant temperature of 80 °C to simulate their degradation and dehydration though time. The vessels were subsequently fragmented into small pieces that followed the same analytical procedure as the sample sherds. This work sought to better identify biomarkers characteristic of the degraded honey. However, despite valuable, authors recognize that this methodology has limitations, as artificial ageing does not take into account the biological action in altering the sugar profile.

Sherds of two Roman open oil lamp specimens (Fig. 4 presents one of the analysed open oil lamps) recovered in the same archaeological context as the studied specimens from *Bracara Augusta* were also analysed to evaluate for possible post-depositional contamination with carbohydrates. As these two Roman open oil lamps were found in association with four of the analysed honeypot specimens (see Table 1), they were all subjected to the same environmental influence and therefore exposed to the same possible environmental contaminants. Therefore, the potential detection of carbohydrates in the sherds of these open oil lamp specimens would suggest a contamination of all the ceramic fragments recovered in that archaeologic context.

Fig. 3 One of the reproductions of Fig.1b honeypot used to mimic the degradation of honey absorbed in ceramic vessels over archaeological periods

Main results and discussion

Figures 5 and 6 present typical chromatograms of honeypots (Fig. 5a), beehives (Fig. 5b), an artificially aged honey (Fig. 5c) and an open oil lamp (Fig. 6).

Derivatized sugar GC profiles can be very hard to interpret as the presence of different tautomeric forms in solution gives rise to distinct derivatives for each tautomeric form of the sugar, producing a large number of chromatographic peaks per sugar, co-elution problems and complex chromatograms (Dhakal and Armitage 2013; Ruiz-Matute et al. 2011; Sanz et al. 2004). This behaviour frequently results in peak
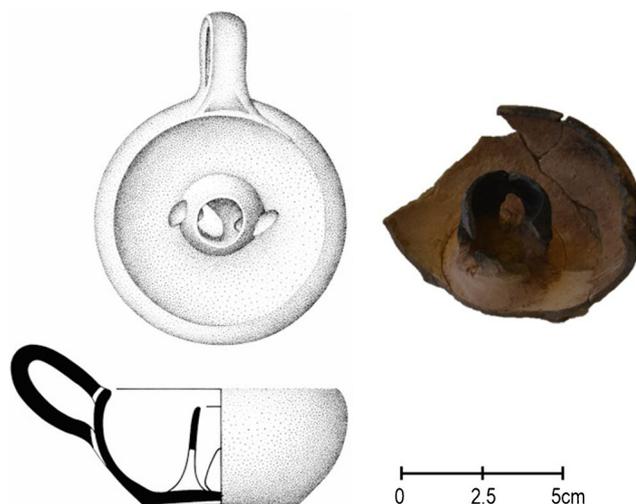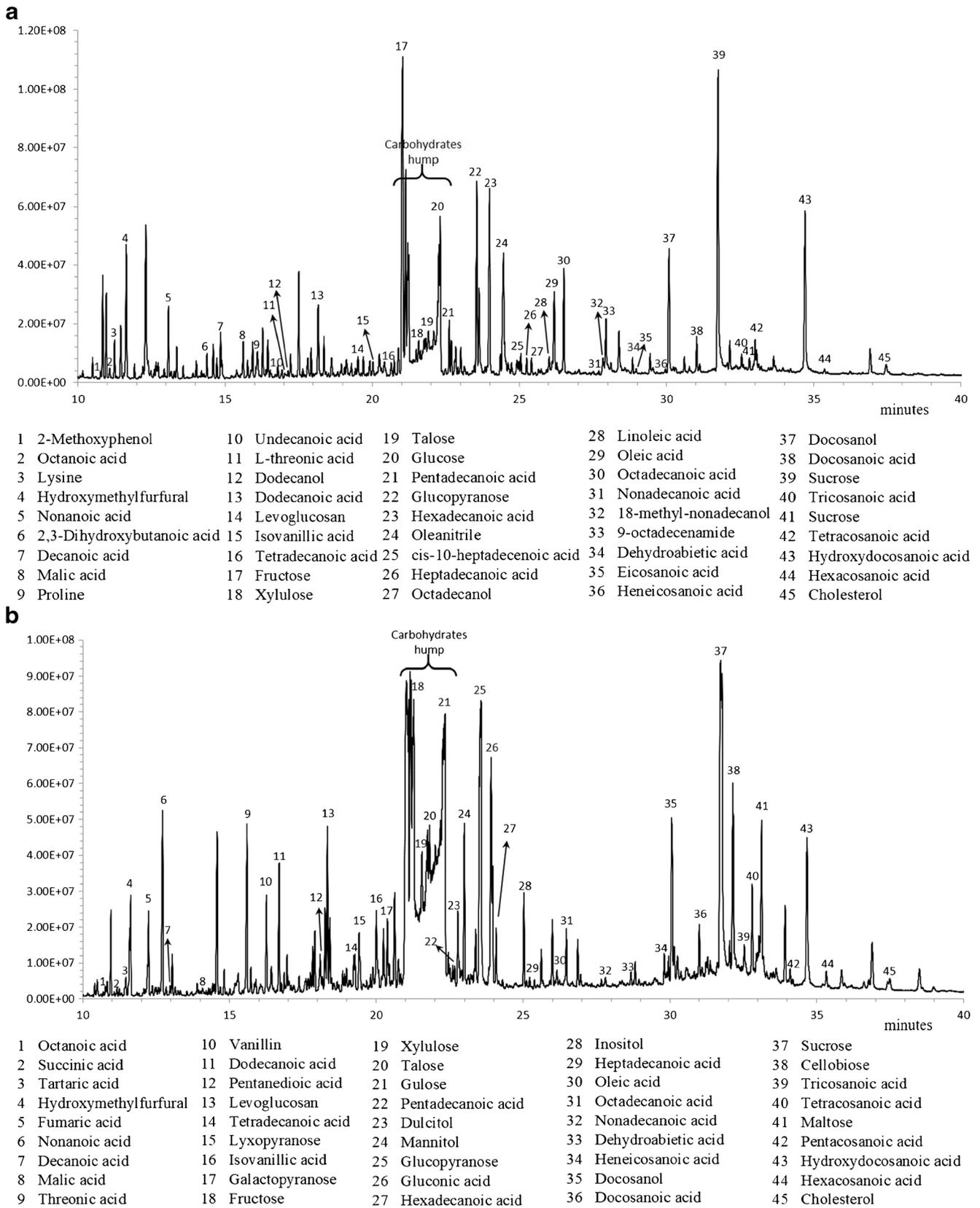

Fig. 4 Roman open oil lamp recovered during *Bracara Augusta* excavations at the same archaeological context as the analysed *Bracara Augusta* honeypots and ceramic beehive

a

#	Compound	#	Compound	#	Compound	#	Compound	#	Compound
1	2-Methoxyphenol	10	Undecanoic acid	19	Talose	28	Linoleic acid	37	Docosanol
2	Octanoic acid	11	L-threonic acid	20	Glucose	29	Oleic acid	38	Docosanoic acid
3	Lysine	12	Dodecanol	21	Pentadecanoic acid	30	Octadecanoic acid	39	Sucrose
4	Hydroxymethylfurfural	13	Dodecanoic acid	22	Glucopyranose	31	Nonadecanoic acid	40	Tricosanoic acid
5	Nonanoic acid	14	Levoglucosan	23	Hexadecanoic acid	32	18-methyl-nonadecanol	41	Sucrose
6	2,3-Dihydroxybutanoic acid	15	Isovanillic acid	24	Oleanitrile	33	9-octadecenamide	42	Tetracosanoic acid
7	Decanoic acid	16	Tetradecanoic acid	25	cis-10-heptadecenoic acid	34	Dehydroabietic acid	43	Hydroxydocosanoic acid
8	Malic acid	17	Fructose	26	Heptadecanoic acid	35	Eicosanoic acid	44	Hexacosanoic acid
9	Proline	18	Xylulose	27	Octadecanol	36	Heneicosanoic acid	45	Cholesterol

b

#	Compound	#	Compound	#	Compound	#	Compound	#	Compound
1	Octanoic acid	10	Vanillin	19	Xylulose	28	Inositol	37	Sucrose
2	Succinic acid	11	Dodecanoic acid	20	Talose	29	Heptadecanoic acid	38	Cellobiose
3	Tartaric acid	12	Pentanedioic acid	21	Gulose	30	Oleic acid	39	Tricosanoic acid
4	Hydroxymethylfurfural	13	Levoglucosan	22	Pentadecanoic acid	31	Octadecanoic acid	40	Tetracosanoic acid
5	Fumaric acid	14	Tetradecanoic acid	23	Dulcitol	32	Nonadecanoic acid	41	Maltose
6	Nonanoic acid	15	Lyxopyranose	24	Mannitol	33	Dehydroabietic acid	42	Pentacosanoic acid
7	Decanoic acid	16	Isovanillic acid	25	Glucopyranose	34	Heneicosanoic acid	43	Hydroxydocosanoic acid
8	Malic acid	17	Galactopyranose	26	Gluconic acid	35	Docosanol	44	Hexacosanoic acid
9	Threonic acid	18	Fructose	27	Hexadecanoic acid	36	Docosanoic acid	45	Cholesterol

Fig. 5 Typical chromatograms. **a** Honeypots (sample 1991.2210; Fig. 1c). **b** Ceramic beehives (sample Mart/11 9-03 001; Fig. 2b). **c** Recent honey after accelerated ageing
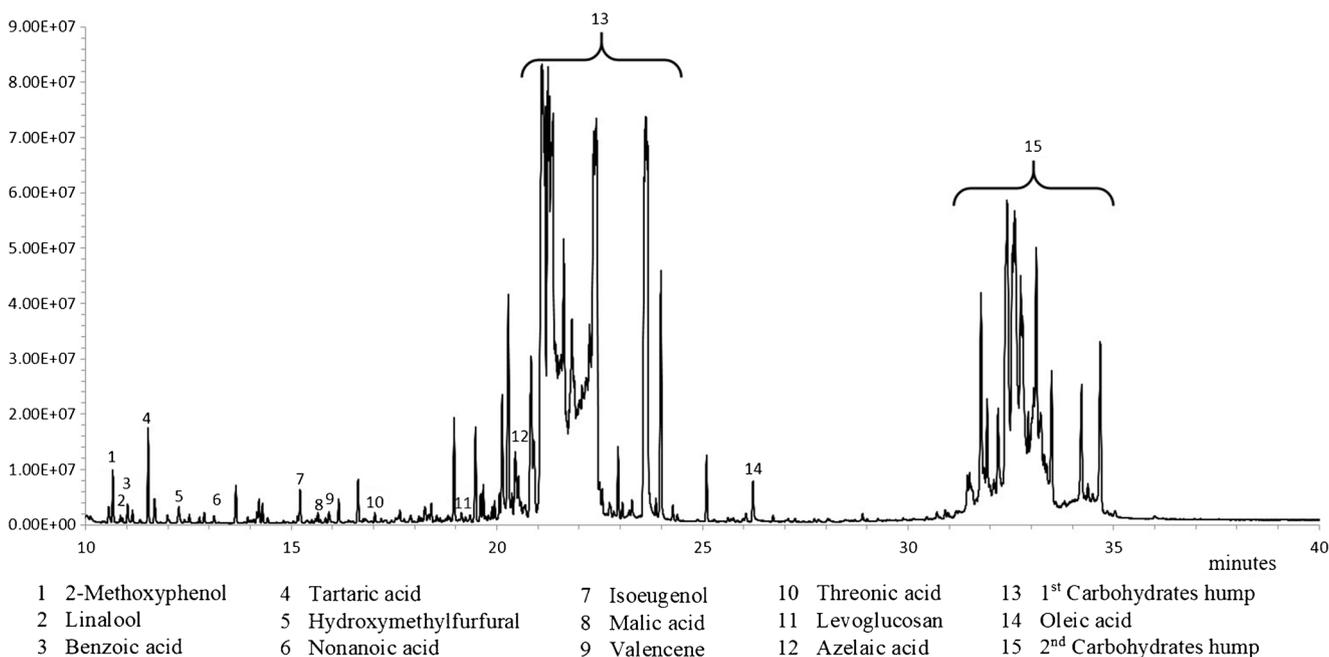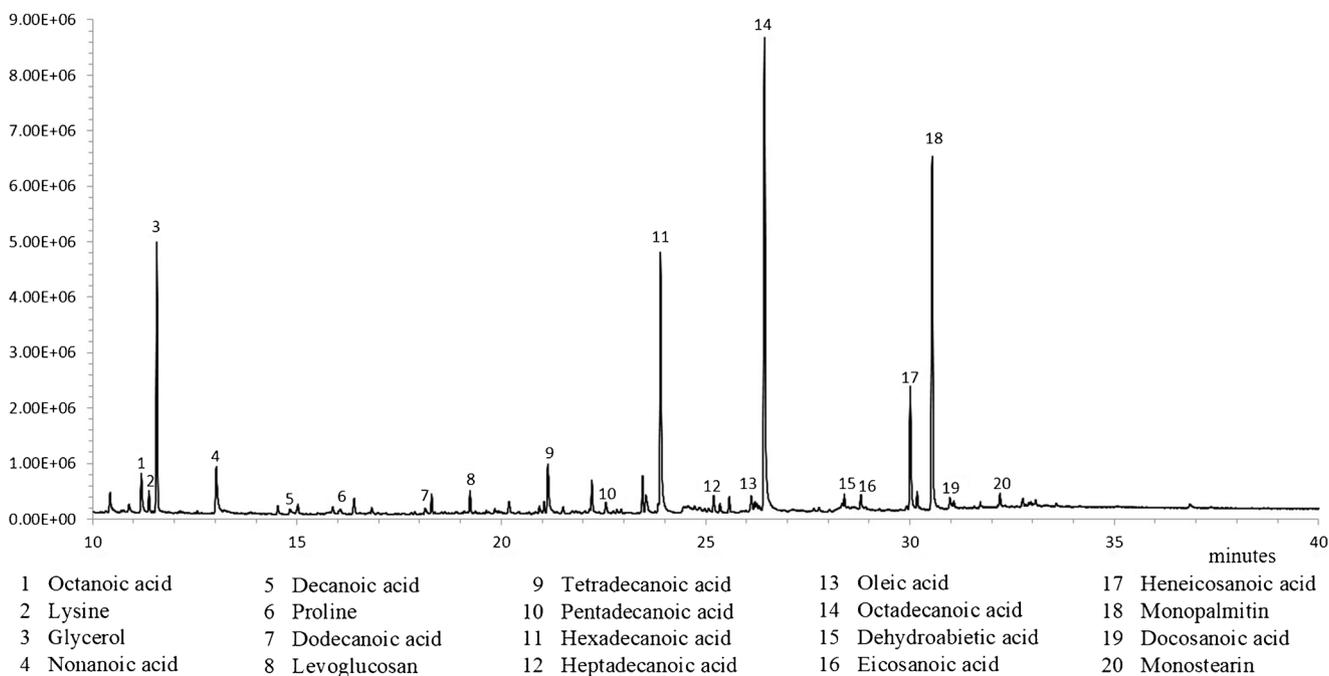
1	2-Methoxyphenol	4	Tartaric acid	7	Isoeugenol	10	Threonic acid	13	1st Carbohydrates hump
2	Linalool	5	Hydroxymethylfurfural	8	Malic acid	11	Levoglucosan	14	Oleic acid
3	Benzoic acid	6	Nonanoic acid	9	Valencene	12	Azelaic acid	15	2nd Carbohydrates hump

Fig. 5 (continued)

broadening, as seen in Fig. 5, that limits the identification of carbohydrates eluted with the same retention time (Sanz et al. 2004). In fact, all samples presented high amounts of carbohydrates, particularly monosaccharides such as fructose, glucose, galactopyranose, talose and xylulose, and lower intensities of disaccharides such as sucrose or lactose (Aliferis et al. 2010; de la Fuente et al. 2007; Feás et al. 2010; Kujawski and Namieśnik 2008). It is important to highlight the hump

(Kuhnert et al. 2013) detected from 21.50 to 22.40 min that was found in all the analysed honeypots, ceramic beehives and artificially aged honey samples, corresponding to an unresolved mixture of carbohydrates that co-elute (Fig. 5a–c). In fact, the average mass spectra (21.50 to 22.40 min) of the analysed honeypots and ceramic beehive humps (Fig. 7a) are almost overlapping with the average mass spectra acquired at identical time period for the artificially aged honey samples

1	Octanoic acid	5	Decanoic acid	9	Tetradecanoic acid	13	Oleic acid	17	Heneicosanoic acid
2	Lysine	6	Proline	10	Pentadecanoic acid	14	Octadecanoic acid	18	Monopalmitin
3	Glycerol	7	Dodecanoic acid	11	Hexadecanoic acid	15	Dehydroabietic acid	19	Docosanoic acid
4	Nonanoic acid	8	Levoglucosan	12	Heptadecanoic acid	16	Eicosanoic acid	20	Monostearin

Fig. 6 Typical chromatogram of an open oil lamp sherd recovered in the same archaeological context as the studied specimens from *Bracara Augusta*

Fig. 7 Comparison of mass spectra (average spectra acquired from 21.50 to 22.40 min). **a** Honeypot (sample 1991.2210; Fig. 1c). **b** Recent honey after accelerated ageing. An identical pattern was obtained on all the analysed honeypots and ceramic beehive chromatograms
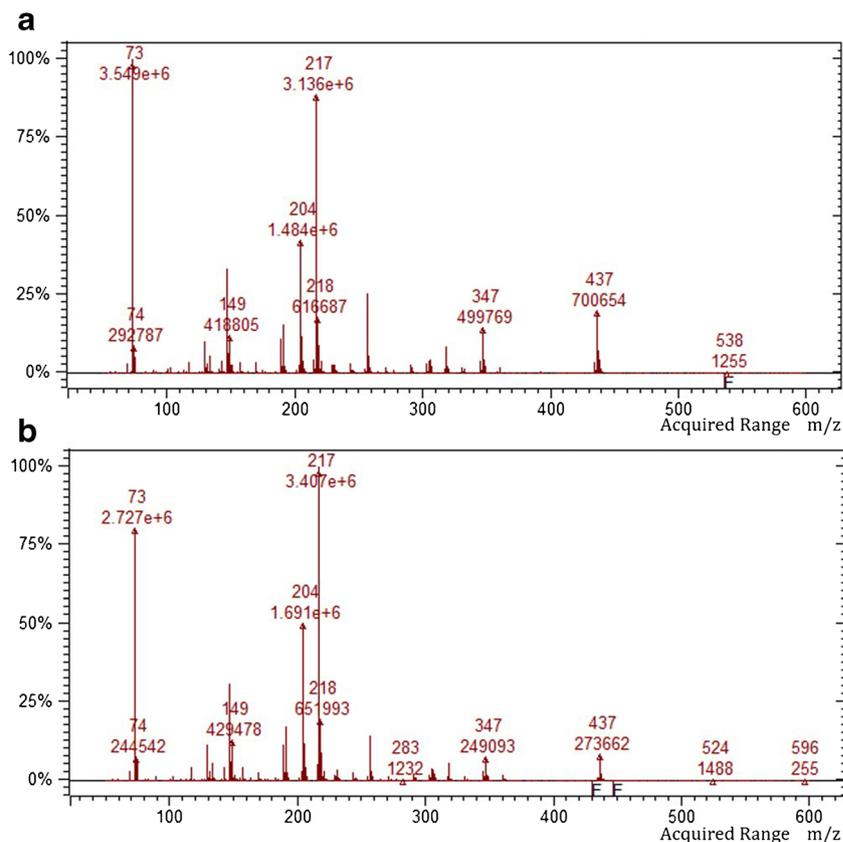
(Fig. 7b). This point strongly suggests a similar chemical composition among them and supports the hypothesis of the presence of honey residues on the ancient sherds.

Carbohydrates are unlikely to survive in buried archaeological material, as they are water soluble and therefore easily leached or assimilated by microorganisms. Therefore, their detection in the analysed sherds has to be evaluated with caution to prevent for sample contamination. To better explain their occurrence in the analysed sherds, fragments of two other ceramic objects—two Roman open oil lamp specimens—recovered in the same archaeological context as the studied specimens from *Bracara Augusta* were also studied to evaluate for possible post-depositional contamination with carbohydrates (one example is presented in Fig. 6). Both chromatograms revealed the presence of palmitic, stearic and oleic acids (Kimpe et al. 2001), together with levoglucosan (compounds characteristic of the fuel used) (Heron et al. 2016), the absence of any carbohydrate hump nor any remarkable carbohydrate peak and a lack of cholesterol which would indicate human contamination. These data are extremely important to more comfortably assign the carbohydrate hump to the vessels content and not to any external contamination.

This possibility is also supported by the detection of hydroxymethylfurfural (HMF) in all chromatograms from honeypots, ceramic beehives and recent honey after accelerated ageing (acquired mass spectrum is presented in Fig. 8a).

In fact, HMF is a compound naturally synthetized by the cyclization of hexoses such as glucose, mannose or galactose and achieved by the chemical and physical phenomenon of dehydration (Chua et al. 2014; de la Fuente et al. 2007; Senila et al. 2012; Teixidó et al. 2006; Zappalà et al. 2005). The detection of HMF is one of the most used indexes to detect low-quality honey, its overheating and/or its ageing (Chernetsova et al. 2011; Fallico et al. 2008; Zappalà et al. 2005).

Some of the studied samples revealed the presence of proline and lysine. Proteins and amino acids are important components of honey and can present both animal and vegetal origin. In fact, amino acids represent about 1% of honey, with proline the most abundant amino acid (Baldi Coronel and Baldi Coronel 2001), signifying more than half of the total amino acids present, together with small amounts of lysine, tyrosine and phenylalanine in a total of 26 different amino acids (Hermosín et al. 2003). Proline has its major source in the salivary secretions of honeybees during their conversion of nectar into honey (da Silva et al. 2016; Hermosín et al. 2003; Kaškonienė and Venskutonis 2010). In fact, the amount of proline in honey has been suggested as an indicator of honey maturity (da Silva et al. 2016; von der Ohe et al. 1991).

Organic acids represent less than 0.5% of honey's constituents, being the honey's total acidity used as an indicator of its

Fig. 8 Acquired mass spectra (TMS derivatives). **a** Hydroxymethylfurfural. **b** ω-Hydroxydocosanoic acid. **c** Dehydroabietic acid
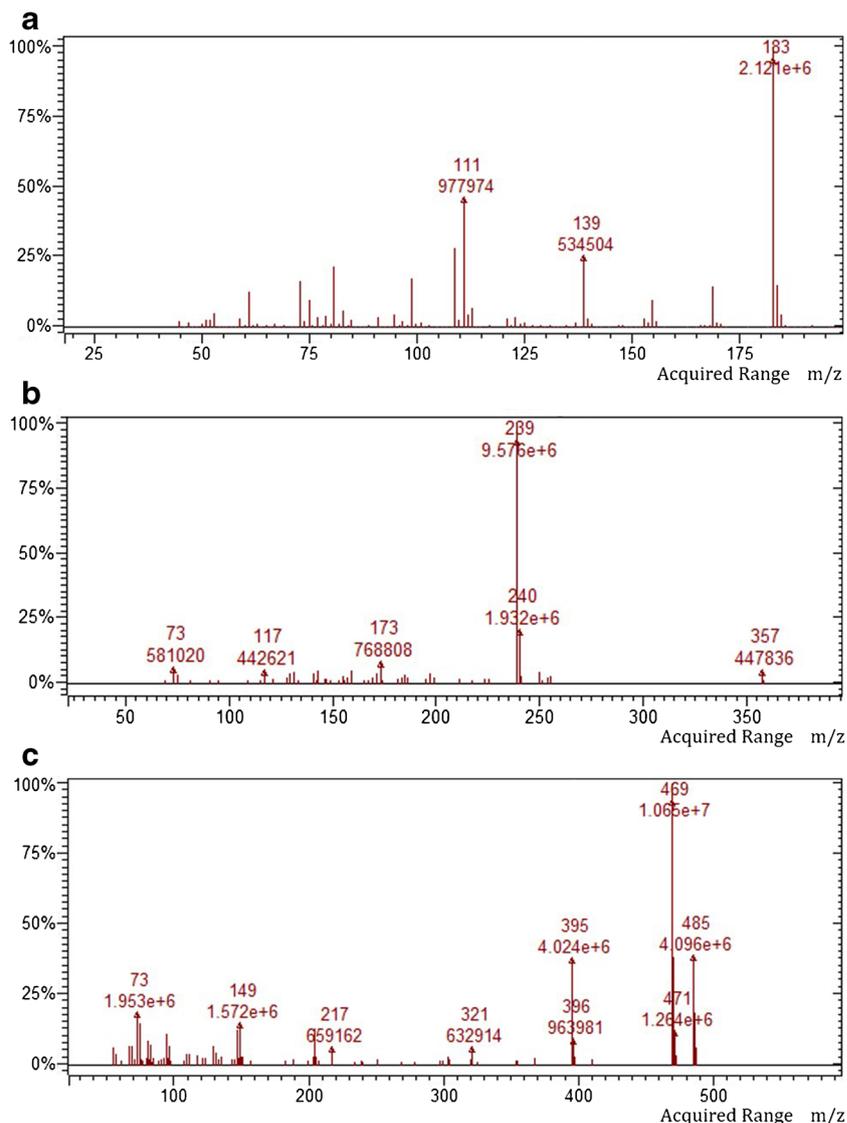
deterioration due to storage, ageing or even to measure its purity and authenticity (Suarez-Luque et al. 2002). The analysed samples revealed small amounts of minority organic acids such as succinic, gluconic, threonic, malic and tartaric acids (Nozal et al. 2003), these being organic acids compatible with the presence of honey residues, among other matrices (Hermosín et al. 2003).

One of the novelties of this study was the detection of levoglucosan (LG) and dehydroabietic acid (DHA). In fact, LG is a well-known fire biomarker for the burning of cellulosic materials like woods or plants (Caseiro and Oliveira 2012; Fabbri et al. 2009; Fraser and Lakshmanan 2000; Gao et al. 2016; Kirchgeorg et al. 2014; Kuo et al. 2008; Simoneit 2002; Simoneit et al. 1999), while DHA is a diterpenic compound produced by the heat of abietic acid throughout a dehydrogenation process, and one of the main components in resins of *Pinaceae* origin

(Jerković et al. 2011). Both LG and DHA (Fig. 8b presents the DHA experimental mass spectrum) were ubiquitously present through all the analysed honeypots and beehive sherds but not in the artificially aged honey samples, supporting the hypothesis of contamination by smoke, probably with the combustion of biomass products (wood or foliage) from trees of the *Pinaceae* family (Jordan et al. 2006). In our opinion, this fact could suggest two possible and/or cumulative sources:

1. Smoke released during the process of harvesting to calm the bees and allow a more secure access to honey (Crane 1983; Crane 1997; Harissis 2014; Jones et al. 1973). With this procedure, honey would be adulterated with smoke residues.
2. Smoke released during the coating of the vessels for waterproofing purposes.

The possible contamination of honey with traces of biomass burning can be easily understood by better understanding the process of harvesting. In fact, it is still common nowadays to use a fumigation device to calm the bees and allow safer access to the honey. However, strong smoking is unnecessary in modern hives with removable combs, and therefore modern beekeepers are able to use a minimum of smoking (Jones et al. 1973). In antiquity, the fumigation was performed by burning vegetal material such as wood and foliage (Bonet Rosado and Mata Parreño 1997; Crane 1992; Harissis 2014; Mason and Mason 1984), resulting in the release of biomass burning tracers that would impregnate the honey (Caseiro and Oliveira 2012; Oliveira et al. 2014; Simoneit 2002; Simoneit et al. 1999).

The additional presence of intense ω-hydroxydocosanoic acid peaks (acquired mass spectrum presented in Fig. 8c) on both honeypots and ceramic beehive chromatograms (Fig. 5a, b) does support a diagnosis of honey ceramic containers. In fact, beeswax is rich in ω-hydroxy acids, particularly those with an even number of carbons ranging from C12 to C32 (Bonaduce and Colombini 2004; Bonaduce et al. 2016; Colombini et al. 2009; Evershed et al. 1997; Regert et al. 2001; Tulloch 1971).

Another curious marker was cholesterol, widely considered a biomarker for the presence of fat residues. In fact, some of the studied fragments exhibited traces of cholesterol, but no squalene was detected. The two compounds are found on human fingerprints, and although cholesterol subsists over archaeological time, squalene rapidly degrades (Heron et al. 2010). Therefore, the absence of squalene indicates cholesterol as a sample component and not a result of more recent handling. The possible source of cholesterol was investigated, analysing artificially aged recent honeys (a typical chromatogram is presented in Fig. 5c). However, the data revealed the absence of cholesterol even after their artificial ageing. Major developments were obtained by complementing the chemical study with ethnographical considerations. In fact, modern and ancient beehives present very different shapes, as the ancient hives are long tubular containers while the modern hives use boxes of wooden mobile frames (for combs) equally spaced (Crane 1997). With that system, harvesting is possible without affecting the queen and brood. The tubular shape of ancient hives required pressing the honeycombs from their ceramic container, sometimes combining the process with the use of boiling water as recommended by Pliny (HN 21.83-84) and Columella (Rust. 9.16.1), particularly in the presence of large amounts of wax that needed to be separated from honey. By using that method, some bees and larvae still inside their honeycombs could be crushed together with honey and beeswax and contaminate honey with traces of cholesterol (Ferreira-Caliman et al. 2012). In fact, following Columella's description, the crushed beeswax and honey

material were put inside a large container that had to be partially filled with water. The water should be brought to boil and the mixture strained through grass to remove particles such as parts of bees, before being boiled again (Kritsky 2015). It is important to stress that bees use sterols in a variety of key metabolic pathways, as the production of their moulting hormones (Joshi and Agarwal 1977; Svoboda et al. 1986; Vanderplanck et al. 2011). Therefore, in the absence of any other explanation, one plausible hypothesis to justify the existence of cholesterol in ancient honey is the presence of parts of bees that would contaminate the honey (Ferreira-Caliman et al. 2012; Ikekawa et al. 1993; Joshi and Agarwal 1977).

Final remarks

The chromatographic analysis using a GC/MS technique performed on Iberian Roman honeypots and ceramic beehives confirmed their use for identical purpose as their ethnographical parallels. The presence of cholesterol in ancient beehives was justified using complementary data from ethnographic parallels.

To our best knowledge, this is the first work where chromatographic techniques where applied to the study of Roman Iberian ceramic beehives and honeypots. Conducting chemical analyses on these container sherds is of the utmost importance to archaeology as it provides scientific evidence that corroborates classical sources, while also providing first-hand information on the function of these ceramic artefacts. In fact, one of the recurrent archaeological problems has been to accurately assess what products were used, transported and traded in certain ceramic vessels, given that the absence of chemical evidence forces archaeologists to conjecture based on stereotypes created by their ceramic typology classifications according to their physical characteristics.

The obtained results validate archaeologists' suspicions about the function of containers with recent ethnographic parallels, also revealing the economic value of honey and their use as a sweetener in the westernmost part of the Roman world.

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