Characterization of lipids preserved in Roman cooking pots by gas chromatography - mass spectrometry (GC-MS)

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Abstract

Lipid extracts of sherds of Roman cooking pots from the Temple of Apollo in Hierapolis (southwest Turkey) were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The high relative abundance of saturated fatty acids, especially stearic acid, together with the presence of cholesterol, suggests that animal fats were processed in these pots. The detection of branched and odd chain fatty acids further indicate a possible ruminant source for these lipids. The data show also the presence of plant-derived products, like campesterol and *b*-sitosterol.

The aim of this research is to illustrate how lipid analysis of pottery vessels, together with the information from archaeological context, can contribute to a better understanding of food habits and ritual activities in past ancient societies.

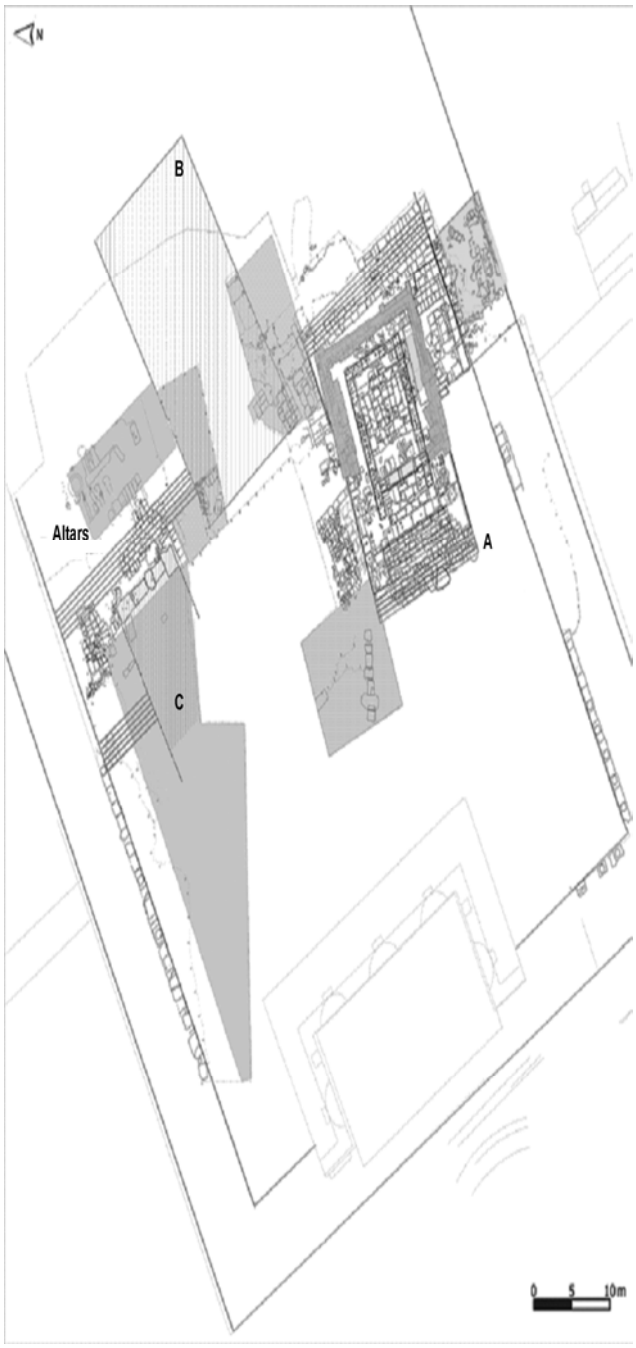

Fig. 1. Plan of the Temple of Apollo with the location of the study area (altars).

INTRODUCTION

Organic residues can be preserved in archaeological ceramics for several millennia after their use either as charred surface deposits or, more commonly, absorbed within the porous structure of the ceramic fabric [1]. The chemical characterization of such residues can provide important information on the original function of the vessels, in particular when the results are combined with contextual data: archaeozoological and palaeobotanical remains, shape and size of vessels, use alteration analysis, pottery distribution.

Identification and characterisation of organic materials in archaeological pottery are usually carried out by means of chromatographic and/or mass spectrometric methods: gas chromatography coupled with mass spectrometry (GC-MS) is the most useful technique for this purpose since it allows to separate and to identify molecular species in complex organic mixtures. Well-established procedures have been developed in the last twenty years in order to characterize the natural compounds in archaeological samples [2-7], including plant resins,

oils, beeswax, degraded animal fats, and to understand the degradation processes that may have changed their original chemical composition.

In this paper the results of chemical analysis of lipid extracts from several sherds of Roman cooking pots from the archaeological site of the Temple of Apollo in Hierapolis (southwest Turkey) are presented.

The ruins of Hierapolis, one of the most important Hellenistic-Roman sites in Asia Minor, are located in the Lycus Valley, a few kilometres north of Laodikea. Excavation and restoration work are carried out by the Italian Archaeological Mission since 1957 [8].

The Temple of Apollo was built inside a sacred area (Fig. 1), about 70 m wide, surrounded by a wall (*temenos*) with a marble colonnade (1st century A.D.). The structures of the temple were built during the 3rd century A.D., later than the *temenos*, but the presence of two Ionic capitals and one Corinthian capital from the 1st century A.D. support the hypothesis that in this area there existed an even more ancient temple. Thanks to epigraphic documents found during the excavation, the marble building has been associated with the worship of the main divinity of Hierapolis, Apollo. The building is positioned at the top of a monumental staircase and near it there is an underground cavity (called the *Plutonion*) from which poisonous gases emerge.

Recent investigations [9] have discovered inside the sacred area an important context that can be associated with ritual practices and animal sacrifice. An extensive area (Fig. 1) is characterized by the presence of ashes, pottery sherds, faunal and botanical remains. The layers of ash covered some holes filled with the remains of ritual activities, in particular vegetable essences. A rectangular marble block with burnt traces and surface ware was found in the same area, whose function seems to be strictly related with animal sacrifice. Many bone fragments were recovered in fact all over the area, together with a great number of ceramic sherds, dating back to the 1st century A.D.: cups and bowls, jugs, cooking vessels.

EXPERIMENTAL SETUP

2.1 Samples

Twenty vessels were sampled for organic residues analysis. The samples belong to cooking

Sample number	Archaeological reference	Pottery type	Shape	Part sampled	Burnt traces
1	HTA03 372/4	Cooking ware	pot	shoulder	no
2	HTA03 372/39		pan	neck	no
3	HTA03 372/40		pot	neck	yes
4	HTA03 372/41		pot	rim	yes
5	HTA03 372/42		pot	rim	yes
6	HTA03 372/43		pot	rim	yes
7	HTA03 372/71		pot	rim	yes
8	HTA03 372/72		pot	rim	yes
9	HTA03 469/10		pan	shoulder	yes
10	HTA04 833/13		pot	shoulder	yes
11	HTA04 917/4		pot	shoulder	yes
12	HTA05 1253/1		pot	rim	yes
13	HTA06 1913/1		pot	rim	yes
14	HTA06 1919/2		pot	rim	yes
15	HTA06 1925/1		pot	shoulder	yes
16	HTA06 1934/1		pot	neck	yes
17	HTA06 1910/4		pot	body	yes
18	HTA06 1910/5		pot	shoulder	yes
19	HTA06 1911/1		pan	shoulder	no
20	HTA06 1912/4		pan	shoulder	no

Table I. Description of samples.

EXPERIMENTAL SETUP

2.1 Samples

Twenty vessels were sampled for organic residues analysis. The samples belong to cooking pots and pans with burnt traces. Ancient cooking pots are in fact the vessel-type of choice for organic residue analyses, because of their capacity to absorb the liquids within the walls of unglazed ceramic vessels [10].

The ceramic containers selected for organic residues analysis were recovered during the excavation of the layers of ash that covered the area of the top terrace of the sanctuary. Full description of the analysed samples are given in Table I.

2.2 Extraction-derivatization

Approximately 2 g of sherd was surface cleaned with a sterile scalpel and crushed in a mortar and pestle. The powdered sherds were solvent extracted and derivatized using established protocols [11,12]. Each powdered sherd was weighed into glass tubes and n-nonadecane (1 ml) was added as internal standard. A chloroform/methanol mixture was used for the extraction (4 ml, 2:1 v/v, 2x20 min. ultrasonication). Following sonication, the testtube was placed in a centrifuge for 15 min to separate the solvent mixture from the inorganic clay particles. Aliquots of the total lipid extract were taken from each sample extract and reduced to a small volume by rotary evaporation, removed to a vial and gently dried under a stream of nitrogen. The dried sample was derivatized by adding BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide, 50 ml) and heated at 80°C for 30 minutes. From the resulting solution 2 ml were used for the GC-MS analysis.

2.3 Gas chromatography/mass spectrometry

Samples were analysed using an Agilent 6850 II series gas chromatograph (column 30 m, internal diameter 0.25 mm, 0.25 μm film thickness) coupled to an Agilent 5973N mass spectrometer operated in the EI mode (70 eV). The GC oven temperature was programmed from 100°C to 280 ° C at 10°C/min, and held at 280°C for 15 min. Helium was used as the carrier gas. Compounds were identified partially by their retention time within the GC, based on comparisons with analysed reference compounds, but mainly by their mass spectra. Mass spectral data were interpreted manually with the aid of the NIST Mass Spectral Library and comparison with spectra reported in literature.

RESULTS

In accordance with the results of GC-MS analysis the samples can be divided into four groups, as summarized in Table II. The samples in the first three groups contain larger concentrations of stearic ($C_{18:0}$) than palmitic ($C_{16:0}$) acid, together with traces of cholesterol (Fig. 2). These data suggest that the cooking vessels were used to process animal derived products [13-15]. In two groups of samples the presence of odd numbered ($C_{15:0}$; $C_{17:0}$), branched chain acids was detected, which

Group number	1	2	3	4
Chemical properties	$C_{18:0}$>>$C_{16:0}$ $C_{18:1}$ (9) low Odd numbered/branched chain fatty acids. Cholesterol[a] Phytosterols[b] $C_{18:1}$(6)[c]	$C_{18:0}$≥$C_{16:0}$ $C_{18:1}$ (9) high Odd numbered/branched chain fatty acids. Cholesterol[a] Phytosterols[b] $C_{18:1}$(6)[c c]	$C_{18:0}$≥$C_{16:0}$ $C_{18:1}$ (9) low Odd numbered/branched chain fatty acids not detected. Cholesterol[a]	$C_{18:1}$ (9)>$C_{16:0}$≥$C_{18:0}$ $C_{18:1}$(6)[c]
Sample number	3[ac] 9[abc] 10[b] 18[b] 20[b]	1[ac] 2[c] 6[c] 7[c] 8[abc] 13[b] 14[b] 15[b] 16	11[a] 17[a] 19	4[a] 5 12[a]
Organic content	Ruminant animal fats; traces of vegetable fats (plants)	Ruminant animal fats; high concentration of vegetable fats (plants)	Non ruminant animal fats?	Vegetable fats (plants)

Table II. Summary of GC-MS results.

are good indicators of ruminant animal fats [16]. Plant sterols (campesterol and b-sitosterol) have been detected in several samples from these groups, indicating that vegetable origin products were also processed in these vessels. Furthermore, in eight samples a particular unsaturated fatty acid, petroselinic acid (cis-6-octadecenoic acid, $C_{18:1}(6)$), was identified. Petroselinic acid is one of the double bond positional isomers of oleic acid (cis-9-octadecenoic acid, $C_{18:1}(9)$) and it occurs at high levels in most seed oils of the *Umbelliferae* family. For example, coriander (*Coriandrum sativum*) contains about 80% of petroselinic acid in its seed oil [17]. Few samples of cooking pots (group 4) show a relative high abundance of unsaturated fatty acids (C18:1(9); C18:1(6)) suggesting that only vegetable origin products were processed in these vessels.

CONCLUSIONS

The lipid content of sherds belonging to cooking pots and pans from the Temple of Apollo in Hierapolis was investigated by GC-MS. The distribution of fatty acids and other biomarkers indicated that predominantly ruminant fat was processed in the vessels. These data can be related with ritual meals based on animal sacrifice, as confirmed also by the faunal remains found in the layers of ash that covered the area of the top terrace of the sanctuary and from the distribution of consuming and serving vessels in the rest of the area. Furthermore, the presence of plant lipids was shown through the identification of plant sterols in several of the pots, indicating that meat and vegetable products have been processed in the same vessel.

REFERENCES

[1] C. Heron, R. P. Evershed, in: M. B. Schiffer (Ed.), Archaeological Method and Theory, Vol. 5, University of Arizona Press, Arizona, 1993, pp. 247–284.

[2] R. P. Evershed, C. Heron, L. J. Goad, Analyst 115 (1990) 1339-1342.

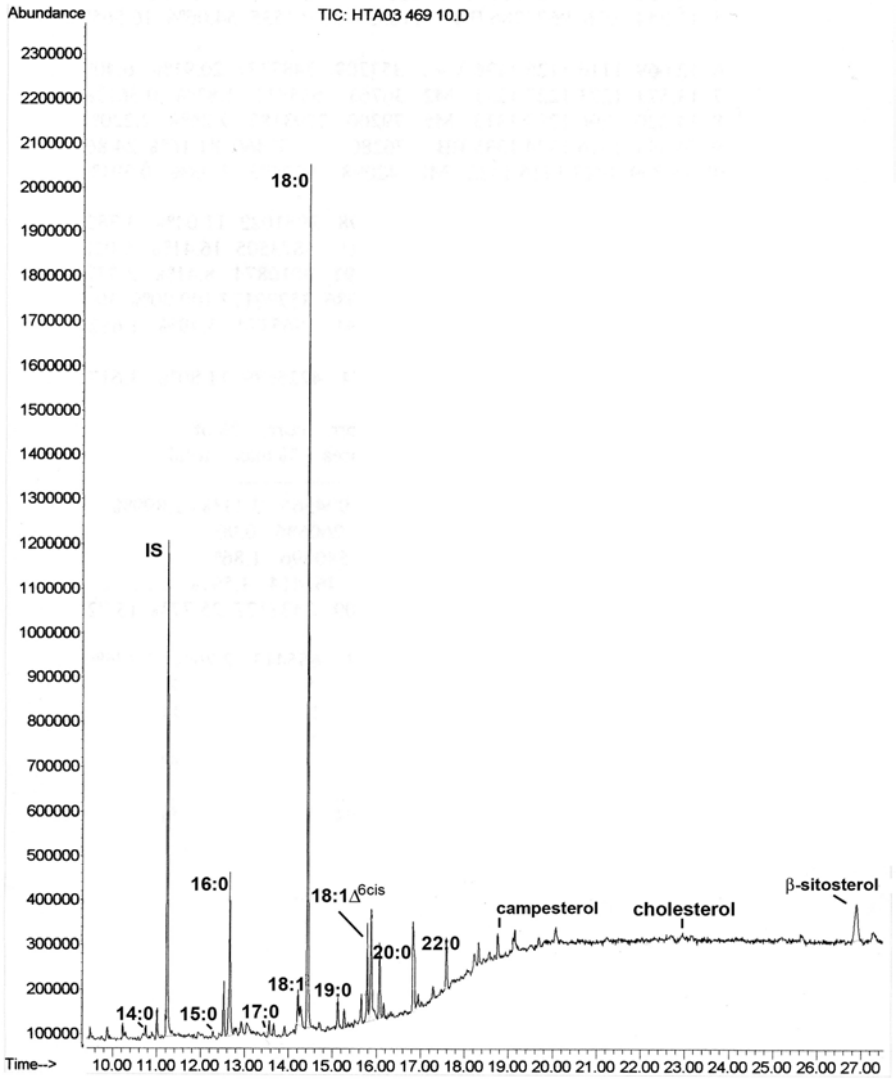

Fig. 2. GC-MS chromatogram of the lipid extract of one sample of cooking vessel (sample 9). IS, Internal Standard (*n*-nonadecane).

[3] R. P. Evershed, S. N. Dudd, M. Copely, R. Berstan, A. Scott, H. Mottram, S. Bulley, Z. Crossman, Accounts Chem. Res. 35 (2002) 660-668.
[4] J. S., Mills, R. White, The organic chemistry of museum objects, Butterworth (2nd ed.), London, 1994.
[5] A. M. Pollard, C. Heron, Archaeological chemistry, Royal Society of Chemistry, Cambridge, 1996.
[6] O. E. Craig, G. D. Love, S. Isaksson, G. Taylor, C. E. Snape, J. Anal. Appl. Pyrolysis 71 (2004) 613–634.
[7] H.R. Mottram, S.N. Dudd, G.J. Lawrence, A.W. Stott, R.P. Evershed, J. Chromatogr. A, 833 (1999) 209–221.
[8] Since 2000 the Italian Archaeological Mission in Hierapolis works under the direction of prof. F. D'Andria, University of Salento; see F. D'-Andria, Hierapolis of Phrygia (Pamukkale). An Archaeological Guide, Istanbul 2003.
[9] G. Semeraro, in: F. D'Andria, M.P. Caggia, (Eds.), Hierapolis di Frigia I. Le attività delle campagne di scavo e restauro 2000-2003, MAIER - Missione Archeologica Italiana a Hierapolis, 2007, pp. 169-209.
[10] M. S. Copley, H. A. Bland, P. Rose, M. Horton, R. P. Evershed, Analyst 130 (2005) 860–871.
[11] S. Charters, R. P. Evershed, L. J. Goad, P. W. Blinkhorn, V. Denham, Archaeometry 35 (1993) 211-223.
[12] S. N. Dudd, R. P. Evershed, J. Archaeol. Sci. 26 (1999) 1473–1482.
[13] M. S. Copley, H. A. Bland, P. Rose, M. Horton, R. P. Evershed, 2005d, Analyst 130 (2005) 865.
[14] M. Regert, N. Garner, O. Decavallas, C. Cren Olivé, C. Rolando, Meas. Sci. Technol. 14 (2003) 1620–1630.
[15] R. P. Evershed, S. N. Dudd, S. Charters, H. Mottram, A. W. Stott, A. Raven, P. F. van Bergen, H. A. Bland, Phil.Trans. R. Soc. Lond. B 354 (1999) 19-31.
[16] O. E. Craig, G. Taylor, J. Mulville, M.J. Collins, M. Parker Pearson, J. Archaeol. Sci., 32 (2005) 97.
[17] W. Thies, Fett Wiss. Technol. 95 (2003) 20-23.