Pots and lipids: molecular and isotope evidence of food processing at Maharski prekop

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ABSTRACT – The pottery assemblage from the Maharski prekop site was analysed to obtain insights into vessel use and husbandry practices. Total lipid extracts of pottery samples were subjected to gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) and soft ionisation electrospray mass spectrometric techniques ESI Q–TOF MS and ESI Q–TOF MS/MS. The charred organic deposits on vessels were AMS ¹⁴C dated. The results show that some vessels were used for cooking ruminant meat, while in other traces of mixed non–ruminant and ruminant meat or plants and animal meat cooking were identified. Some vessels were used for milk processing.

Introduction

Organic residues survive in two principal forms on archaeological pottery: as charred deposits encrusted on ceramic vessels, and/or as absorbed residues preserved in low concentrations in highly degraded and complex matrices in vessel walls. Both are closely associated with the chaîne opératoire of food storage, and food preparation, cooking and consumption.

In this article, we present direct chemical evidence for what we may recognise as preserved traces of cooked, boiled, stored or processed food through lipid characterisation using gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) and soft ionisation electrospray mass spectrometric techniques ESI Q–TOF MS and ESI Q–TOF MS/MS.

The analyses were carried out on organic residues extracted from the Eneolithic vessels sampled from the Maharski prekop pottery assemblage. The analy-
ses focused on 20 potsherds (Tab. 1), covering well-defined groups of vessel size and related fabrics (see Mlekuž et al. this volume). Charred organic deposits on the surface of 22 vessels from the Maharski prekop assemblage were directly dated by the AMS radiocarbon method (Fig. 1). The dates demonstrate a distribution of probabilities, with periods of intensive occupation dating between 4400 and 4000 and between 3800 to 3550 calBC. While the charred deposits on pottery dating has been broadly accepted, Anders Fischer and Jan Heinemeier (2003; see also Craig et al. 2007) have noted that a number of dates obtained from vessel residues from prehistoric sites in northern Europe are too old with respect to the ‘old’ carbon effect, which was introduced into the residue through the processing of marine and freshwater organisms in vessels. However, we found no evidence that the pottery at Maharski prekop was used for freshwater fish, crayfish or mollusc processing.

The Maharski prekop site is located beside a palaeo-river channel in the Ižica flood plain. During the excavation in the 1970s, 2432 vertical wooden piles were recorded at the site. The arrangement of piles can be interpreted as the remains of nine houses with dimensions of around 10 x 3.5–4.5m arranged in parallel. The settlement is divided into two building phases. The pottery distribution within the ‘cultural layer’ is clustered in at least three distinct concentrations. However, we cannot confirm any direct relation between houses and pottery (see Mlekuž et al. this volume).

Palinological data indicate that the floodplain forest was mixed, comprising predominantly Quercus, Corylus, Fagus and Alnus, with some coniferous elements and open ground herbaceous taxa. In addition, the presence of cereal pollen is attested from at least 4000 calBC. A sharp decline in arboreal pollen and an expansion of herbaceous taxa, particularly Poaceae, and carbonised grains of Hordeum sp., support the notion of arable fields surrounded by forest (Culiberg, Sercelj 1991.252; Gardner 1999.130, 165, 168; Sercelj 1975.121; 1981–1982.104; see also Andrič 2007). The composition of the animal bone assemblage shows that domestic ruminant animals prevailed in local subsistence. The domestic package consists of sheep and goat (29.7%), cattle (12.9%) and pig (5.1%). In addition, dog represents 12%. In the wild animal assemblage, red deer (18.1%), roe deer (3.4%), boar (11.8%) and birds (3.0%) predominate (Drobné 1975a; 1975b).

Material and methods

We selected 20 pottery fragments for a pilot chemical study encompassing lipid distribution including fatty acids, stable isotope composition (bulk δ13C or δ15N, and δ13C of individual fatty acids) and the di- and triacylglycerols (DAGs and TAGs) distribution of organic residues. Within the assemblage, three samples (MP85, MP158A and MP181) were obtained from charred organic residues from vessel surfaces (Tab. 1). The sherds were surface cleaned to remove any exogenous lipids. The sub-samples were then ground to a fine powder. First, the isotopic composition of carbon and nitrogen was determined on the bulk samples (Tab. 1). Each sample was weighed in duplicate into tin capsules and analysed using a Europa Scientific isotope ratio mass spectrometer with ANCA–SL preparation module for solid and liquid samples (PDZ Europa Ltd, Crewe, UK). Stable isotope results are presented as δ13C or δ15N values in per mil (‰) relative to the VPDB and AIR international standard, respectively.

Lipid analyses were performed using the extraction procedure described in detail by Richard Evershed et al. (1994). Two grammes of fine powder samples were transferred into a 50ml glass vial. Lipids were extracted with a mixture of chloroform and methanol (2:1 v/v), and 20µl of an internal standard (n-tetratriacontane, 1mg/ml in n-hexane) was added to determine lipid concentrations. The solvent was extracted by ultrasonication (2 x 15min) and evaporated to dryness under a gentle stream of nitrogen to obtain the total lipid extract (TLE). One portion of the TLE was derivatised using BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide, 40µl; 70°C, 60min), evaporated to dryness and re-dissolved in n-hexane for GC and GC–MS analysis.

Further aliquots of the TLE were methylated using BF3/methanol to obtain fatty acid methyl esters (FAMEs) (14%, w/v; 100µl; Sigma Aldrich, Gillingham, UK; at 70°C for 1h). The methyl ester derivatives were extracted with hexane, and the solvent removed under nitrogen. FAMEs were re-dissolved in hexane for analysis by GC and GC–C–IRMS. GC–C–IRMS analyses were performed using an Isoprime GV system (Micromass, Manchester, UK).

The third TLE aliquot was used to identify the TAG content following the procedure described by Sigrid Mirabaud et al. (2007). The silica solid-phase extraction (SPE) cartridges (500mg, 3ml, Isolute, Biotage) were rinsed with 2ml of n-hexane, 2ml of...
CH₂Cl₂/CH₃OH (2:1 v/v), and then 4ml of n-hexane. The first fraction of the sample containing hydrocarbons was eluted with 1ml of hexane, while the second fraction, which contains the TAGs, was eluted with 1ml of CH₂Cl₂. The fractions with TAGs were evaporated to dryness and then analysed. Mass measurements were run on a hybrid quadrupole time of flight mass spectrometer (Q–TOF Premier) provided with an orthogonal Z-spray ESI interface (ESI–MS; Waters Micromass, Manchester, UK). The doping reagent of LiCl (Merck, Darmstadt, Germany) was added to the methanol sample solution for more efficient electrospray ionisation of long chain lipids. For ESI–MS/MS, the lithiated molecular ions [M+Li]⁺ were selected in the first quadrupole, accelerated to an energy of 3kV, dissociated by using 10 to 30eV collision energy and analysed with the TOF analyser.

Results and discussion

Bulk C and N isotope composition

The carbon and nitrogen isotope composition of the organic remains in pottery vessels may reflect the average δ¹³C or δ¹⁵N values of the degraded food during vessel use and burning. It may provide important information, particularly on whether the food was based primarily on animals or plants (C₃ and C₄). The determination of the isotopic composition of C and N was possible in only 15 samples. The average and standard deviation from potsherd samples are –30.7 ± 1.3‰ and +1.0 ± 3.8‰ for δ¹³C and δ¹⁵N, respectively (Tab. 1). These data fall in the range expected for degraded animal and plant tissues whose subsistence was based mainly on C₃ plants.

Three charred organic residue samples from Maharski prekop (MP158A, MP85, and MP181) exhibit higher δ¹⁵N values ranging from +6.2 to +9.8‰. We suggest that these pots were used to process low-protein fat and/or high-fibre food (Spannengerg et al. 2006; Craig et al. 2007). However, the GC–MS analysis gives a more precise identification of food content. The C₁₆:₀/C₁₈:₀ fatty acid ratios of ≥2.0 and the presence of a homologous series of long chain n-alkanes from C₁₆–C₃₅ (odd-over-even carbon number predominance) indicate the use of plants and the presence of degraded vegetable oils in samples MP85 and MP181. In sample MP158A, on the other hand, the low C₁₆:₀/C₁₈:₀ ratio of 0.1, and the presence of cholesterol and stable isotope data with ∆¹³C value of −2.4‰ show the presence of ruminant adipose fat. These data match well with the results obtained for lipids in the bulk pottery sample MP158 from the same vessel, as we discuss below. The differentiating parameter between the two samples of a single vessel is the high δ¹⁵N value of +9.8‰ determined in the charred organic residues on the inner surface of this pot (MP158A). It is worth pointing out that thermal degradation and microbial reworking of organic matter could significantly alter the δ¹⁵N values, making this analysis less reliable, and should be used only as a general indicator prior to more specific chemical and molecular analysis such as lipid identification and stable isotope data.

Fig. 1. Radiocarbon dates of charred organic deposits on the surface of 22 vessels from Maharski prekop.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (BP) ± Error</th>
<th>δ13C (%)</th>
<th>δ15N (%)</th>
<th>Fatty acids</th>
<th>Commodity type</th>
</tr>
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<tr>
<td>MP1 Poz-48522</td>
<td>5280 ± 40</td>
<td>-31.7</td>
<td>1.7</td>
<td>C14&gt;0, C15&gt;0, C16&gt;0, C16&gt;1</td>
<td>Odd carbon numbered mixture</td>
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<tr>
<td>MP2 Poz-48659</td>
<td>4750 ± 35</td>
<td>n/a</td>
<td>n/a</td>
<td>C18&gt;1, C18&gt;0, C20&gt;0, C22&gt;0</td>
<td>C26–C34, C29 H60 mixture</td>
</tr>
<tr>
<td>MP17 Poz-48514</td>
<td>4900 ± 40</td>
<td>n/a</td>
<td>n/a</td>
<td>C10&gt;0, C12&gt;0, C15&gt;0, iso-C15&gt;0</td>
<td>2.0–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
</tr>
<tr>
<td>MP25 Poz-48510</td>
<td>5340 ± 40</td>
<td>-29.9</td>
<td>3.8</td>
<td>C10&gt;0, C12&gt;0, C15&gt;0</td>
<td>Isododecanoic acid, possible goat milk</td>
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<tr>
<td>MP45 Poz-48512</td>
<td>5210 ± 40</td>
<td>-30.4</td>
<td>-4.0</td>
<td>C12&gt;0, C16&gt;0, C18&gt;1, C18&gt;0</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP46 Poz-48513</td>
<td>4980 ± 40</td>
<td>n/a</td>
<td>n/a</td>
<td>C16&gt;0, C18&gt;1, C18&gt;0, C20&gt;0</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP96 Poz-48516</td>
<td>5270 ± 40</td>
<td>-29.7</td>
<td>2.2</td>
<td>C10&gt;0, C12&gt;0, C13&gt;0</td>
<td>10.7–30.4, 30.1–30.2</td>
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<tr>
<td>MP100 Poz-48502</td>
<td>5470 ± 35</td>
<td>-32.0</td>
<td>-0.4</td>
<td>C12&gt;0, C16&gt;0, C18&gt;2, C18&gt;1</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP121 Poz-48503</td>
<td>5760 ± 40</td>
<td>-32.0</td>
<td>3.5</td>
<td>C9&gt;0, C10&gt;0, C16&gt;0,</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP123 Poz-48521</td>
<td>4790 ± 35</td>
<td>-30.8</td>
<td>-0.2</td>
<td>C9&gt;0, C10&gt;0, C16&gt;0,</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP144 Poz-48504</td>
<td>5105 ± 35</td>
<td>n/a</td>
<td>n/a</td>
<td>C9&gt;0, C10&gt;0, C16&gt;0,</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<td>MP151 Poz-48526</td>
<td>5000 ± 40</td>
<td>-29.6</td>
<td>2.1</td>
<td>C10&gt;0, C14&gt;0, C16&gt;0,</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<tr>
<td>MP158 Poz-48506</td>
<td>4860 ± 40</td>
<td>-30.0</td>
<td>9.5</td>
<td>C16&gt;0, C18&gt;0, C22&gt;0,</td>
<td>0.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
</tr>
<tr>
<td>MP172 Poz-48508</td>
<td>4940 ± 40</td>
<td>-28.9</td>
<td>-5.2</td>
<td>C16&gt;0, C18&gt;0, C22&gt;0,</td>
<td>1.1–2.9, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
</tr>
<tr>
<td>MP174 Poz-48517</td>
<td>5310 ± 40</td>
<td>-33.8</td>
<td>-2.3</td>
<td>C9&gt;0, C10&gt;0, iso-C15&gt;0,</td>
<td>2.4–3.1, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
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<td>MP177 Poz-48518</td>
<td>4700 ± 40</td>
<td>n/a</td>
<td>n/a</td>
<td>C9&gt;0, C10&gt;0, C14&gt;0, iso-C15&gt;0,</td>
<td>2.4–3.1, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
</tr>
<tr>
<td>MP181 Poz-48508</td>
<td>4940 ± 40</td>
<td>-25.2</td>
<td>6.2</td>
<td>C14&gt;0, C16&gt;0, C18&gt;0,</td>
<td>2.4–3.1, 3.1–3.2, 3.9–3.0, 4.0–4.1</td>
</tr>
</tbody>
</table>

**Table 1.** A summary of the analyses of the potsherds and organic residues from Maharski prekop.
Lipid identification in potsherds

The lipid residue analyses were performed with the GC and GC–MS methods. Lipid preservation was very good, with 75% of potsherds yielding an appreciable lipid concentration. Lipid concentrations of less than 5µg g⁻¹ were considered unreliable and excluded from further interpretation (Tab. 1).

The well-preserved extractable lipids in 15 samples consisted mainly of free and bounded fatty acids identified by their methyl esters (FAME) mass spectra. The most common distribution found in our samples was dominated by a high abundance of the C₁₆:₀ and C₁₈:₀ fatty acids derived from degraded animal fats (Fig. 2). The presence of degraded animal fats can be further recognised in the relative abundances of C₁₆:₀ and C₁₈:₀. In 11 samples (or 73%; numbers MP1, MP25, MP45, MP100, MP121, MP123, MP151, MP158 with MP158A, MP172, MP211) the C₁₆:₀/C₁₈:₀ ratios of fatty acids range between 0.1 and 2.0 (Fig. 3). They indicate ruminant adipose fat (Copley et al. 2005). In 2 samples (or 13%; numbers MP96 and MP85) the ratios are greater than 3.0 and consistent with degraded vegetable oils (Copley et al. 2005). The other two samples, MP174 and MP181, have C₁₆:₀/C₁₈:₀ ratios of 2.4, indicating the possible presence of dairy fats.

The C₁₆:₀/C₁₈:₀ ratios that range from 0.9 to 2.0 in 6 of the 11 potsherds extracts (MP1, MP25, MP45, MP100, MP158, MP211) and the ratio of >2.0 in MP174 are all consistent with degraded animal fats and clearly show evidence of fatty acid preservation. The main acids in these extracts are C₁₂:₀, C₁₄:₀, C₁₅:₀, iso-C₁₅:₀, C₁₆:₀, C₁₇:₀, iso-C₁₇:₀, C₁₈:₀, C₁₈:₁, C₂₀:₀ and C₂₂:₀. Other fatty acids were less frequently observed (C₉:₀, C₁₀:₀, C₁₃:₀). The isomers of C₁₈:₁ with two different double bond positions at 9 and 11 were found in all 7 pottery samples mentioned above. The presence of branched iso-fatty acids with 15 and 17 carbon atoms was observed in samples MP1, MP25 and MP174. The presence of these acids together with two double bonds positional isomers of C₁₈:₁ indicates ruminant animal fats that have been biosynthesized in the gut and rumen (Christie 1981; Dudd et al. 1999; Mottram et al. 1999; Regert 2011). The parallel biomarkers, i.e., the cholesterol and its derivatives, were detected in four samples (MP158 with MP158A [samples relate to the same vessel], MP100 and MP174), confirming the presence of degraded animal fats (Tab. 1 and Fig. 2). A single C₁₈:₁ isomer that reflects non-ruminant monogastric animal fat, such as porcine (Enser 1991; Evershed et al. 1997), was not detected in any of the samples from Maharski prekop.
A homologous series of long chain \( n \)-alkanes from \( C_{16} \)-\( C_{33} \) (odd-over-even carbon number predominance) that originate from epicuticular waxes of vascular plants (Bianchi 1995) was present in 9 samples (numbers MP1, MP96, MP100, MP123, MP158 with MP158A, MP174, MP85, MP181) (Tab. 1). The highest abundance of \( n \)-alkanes associated with the highest abundance of \( C_{16:0} \) in sample MP96 suggests the presence of plants as well. The correlation of long chain \( n \)-alkanes, the highest abundance of \( C_{16:0} \) and the highest \( C_{16:0}/C_{18:0} \) ratio of 10.7 indicate that the vessel was used for preparing or storing plant foods. The appearance of both animal and plant biomarkers observed in the other 8 samples (numbers MP1, MP100, MP123, MP158 with MP158A, MP174, MP85 and MP181) suggests that the pots were used for mixed food processing and storage (Tab. 1).

**Stable carbon isotope composition of fatty acids**

The \( \delta^{13}C \) values determined by the GC–C–IRMS method in fatty acids in modern edible oils and fats can be used to access the origin of lipids preserved in archaeological ceramics (see Copley et al. 2003; Spangenberg et al. 2006; Craig et al. 2007). The \( \delta^{13}C \) values of the major fatty acids in oils from \( C_3 \) plants are plotted near the 1:1 line, while modern dairy fats plot below the \( \Delta^{13}C = -3.3\% \) line (Evershed et al. 2002; 2008; Copley et al. 2003; 2005; Dunne et al. 2012) (Fig. 4a). Seven samples of Maharski prekop plot near the 1:1 line, while the other five samples fall below the 1:1 line in a zone of ruminant adipose fats. No evidence of fish, porcine or ruminant dairy fat was found in the samples when observing the \( \delta^{13}C \) values.

A more precise differentiation between non-ruminant adipose, ruminant adipose and ruminant dairy fats can be seen in the diagram where \( \Delta^{13}C \) values (\( \delta^{13}C_{16:0}-\delta^{13}C_{18:0} \)) are plotted against the \( \delta^{13}C_{16:0} \) values (Fig. 4b). The comparison of the \( \Delta^{13}C \) values of modern reference animal fats with those of the Eneolithic pottery residues show that 42% of these plot in the range for ruminant adipose fats. A further 58% of the samples fall close to the limiting value between non-ruminant and ruminant meat (\( \Delta^{13}C = 0\% \)). However, the later samples may not be assigned to meat mixture exclusively, but also to a mixture of plant-animal fats. The \( \delta^{13}C \) values indicate that three pottery vessels (MP100, MP151, MP158 with MP158A) could contain sheep/goat and cattle fats. None of the \( \delta^{13}C \) values determined in our samples plot below the \( \Delta^{13}C = -3.3\% \) line, which was used as a criterion for the determination of dairy foods (Evershed et al. 2002; 2008; Copley et al.
Distinguishing animal fats by ESI Q–TOF MS

DAG and TAGs are indicative lipids of degraded animal fats. Previous work has shown that the TAG distribution could help to differentiate between fats of ruminant and non-ruminant animals and ruminant dairy fats (Kimpe et al. 2002; Mirabaud et al. 2007; Regert 2011). We applied the ESI Q–TOF MS and ESI–MS/MS methods to obtain information about the structure of TAGs in the Maharski prekop pottery samples. A major advantage of this method is its sensitivity, which overcomes one of the major difficulties raised by the low occurrence of TAGs in complex archaeological samples. A similar approach including the nanoESI–MS method was successfully applied to a series of modern reference fats and archaeological samples (see Mirabaud et al. 2007; Garnier et al. 2007; Regert 2011). The TAG analysis of modern fats of ruminants (i.e., sheep, goat, and cattle), and non-ruminants (i.e., pig) has shown clear differences between these two groups. The ruminant adipose fats contain TAGs that range from T42 (for cattle) or T44 (for sheep) to T54 (Dudd et al. 1999; Mukherjee et al. 2007). The non-ruminant adipose fats also show a narrow distribution that range from T44 to T54, but with low quantities of T46 and T48. A broad distribution that ranges from T26 to T54 is recognised in dairy fats (Mirabaud et al. 2007; Regert 2011). It should be mentioned, however, that even if these distributions could partially resist degradation, they are modified in TAGs compositions to varying extents over time (Dudd et al. 1999; Mukherjee et al. 2007; Regert 2011).

The distribution of TAGs in five Maharski prekop samples shows that samples MP45 and MP123 have a large TAG distribution from T26 to T54. In the other three samples, a narrower distribution runs from T26 to T40 (sample MP25) or from T40 to T54 (samples MP100 and MP158) (Fig. 5). Along the saturated TAGs, two T50:1 and T54:3 (sample MP45) four T50:1, T52:1, T54:5, and T54:2 (samples MP100 and MP158) unsaturated TAGs were detected. We compare these distributions with modern reference fats. We recognise the TAG distribution in samples MP45 and MP123 as indicators of dairy fat products (Fig. 5). The comparison of the TAG and DAG distributions in the identification of goat versus cow milk favours goat milk (Mirabaud et al. 2007). More precise identification could be obtained from fatty acid distribution in T4:0 using ESI–MS/MS fragmentation. This fragmentation was not possible in the Maharski pre-
Maharski prekop samples MP100 and MP158 show evidence of the presence of cattle adipose fats, since the TAG distribution starts at T₄₀. This is in accordance with the Δ¹³C values for these particular samples (Fig. 4B). Some samples (MP25) show only low molecular TAGs and DAGs, which suggest that lipids are of animal origin, although more precise determination was not possible (Fig. 5).

On the other hand, the TAGs and DAGs distributions in the Maharski prekop samples (MP45 and MP123) do not correlate well with the Δ¹³C values. The values of -0.3 and 0.2‰ are higher than expected for cow or even goat milk (Fig. 4B). One of the possible explanations for this could be milk processing, since it was observed that C₁₆:₀ and C₁₈:₀ fatty acids are enriched in ¹³C during heating, and thus the difference in the δ¹³C values between the two acids becomes lower (Spangenberg et al. 2006). Further investigation is needed, therefore, to understand the influence of milk processing (i.e., warming and boiling) on the isotopic composition of fatty acids.

Conclusions

The results obtained from the lipid analysis of pottery from Maharski prekop indicate dietary trends involving animal and plant tissue. None of the total lipid extracts contained porcine or freshwater fish adipose fats or ruminant dairy fats. A good correlation in the interpretation of ruminant adipose fats, based on lipid identification and stable isotope analysis, was obtained in pottery samples MP100, MP151, MP158 with MP158A and MP172. Furthermore, the TAG distributions in MP100 and MP158 samples correlate well with the presence of cattle adipose fats recognised in the discriminating criteria found in modern fats. In other samples, the presence of different mixed fats either from non-ruminant and ruminant meat or from a plant-animal fats mixture was observed. Nevertheless, the TAG distribution in two samples (MP45 and MP123) showed the residues of dairy products that probably derived from goat milk. These data do not match the outcome of the stable isotope analysis completely, since the Δ¹³C values in those samples are higher than expected for goat milk. One of the possible explanations for this distribution could be milk processing and dairy food production. Further investigation is needed to understand the inconsistencies that appear in the results obtained by different methods.

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