Direct evidence for the existence of dairying farms in prehistoric Central Europe (4th millennium BC)

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The molecular and isotopic chemistry of organic residues from archaeological potsherds was used to obtain further insight into the dietary trends and economies at the Constance lake-shore Neolithic settlements. The archaeological organic residues from the Early Late Neolithic (3922–3902 BC) site Hornstaad-Hörnle IA/Germany are, at present, the oldest archaeological samples analysed at the Institute of Mineralogy and Geochemistry of the University of Lausanne. The approach includes 13C/12C and 15N/14N ratios of the bulk organic residues, fatty acids distribution and 13C/12C ratios of individual fatty acids. The results are compared with those obtained from the over 500 years younger Neolithic (3384–3370 BC) settlement of Arbon Bleiche 3/Switzerland and with samples of modern vegetable oils and fat of animals that have been fed exclusively on C3 forage grasses. The overall fatty acid composition (C9 to C24 range, maximizing at C14 and C16), the bulk 13C/12C and 15N/14N ratios (δ13C, δ15N) and the 13C/12C ratios of palmitic (C16:0), stearic (C18:0) and oleic acids (C18:1) of the organic residues indicate that most of the studied samples (25 from 47 samples and 5 from 41 in the δ13C18:0 vs. δ13C16:0 and δ13C18:0 vs. δ13C18:1 diagrams, respectively) from Hornstaad-Hörnle IA and Arbon Bleiche 3 sherds contain fat residues of pre-industrial ruminant milk, and young suckling calf/lamb adipose. These data provide direct proof of milk and meat (mainly from young suckling calves) consumption and farming practices for a sustainable dairying in Neolithic villages in central Europe around 4000 BC.

Keywords: carbon-13; compound-specific isotope analysis; dairying farms; fatty acids; food; Germany; Neolithicum; nitrogen-15; organic residues; potsherds; Switzerland

1. Introduction

Since around 4300 cal BC neolithic farmers in central Europe used the shore of Lake Constance and other Alpine lakes as settlements [1–7]. They erected houses on piles and developed a mixed ecology community based on agrarian, breeding, shepherd, gathering, hunting and fishing activities, as well as clearance of woodland [8–16]. This communication is a contribution to a long-term project, with the aim of understanding the environment, ecology and economy of these Neolithic
villages in central Europe, and in particular the prehistoric strategies to obtain and prepare food [11]. We performed a molecular and isotopic characterisation of the fat preserved in the unglazed ceramic recovered from the Neolithic settlements Hornstaad-Hörnle IA (Germany) and Arbon Bleiche 3 (Switzerland) on the shore of Lake Constance (Bodensee) (Figure 1).

The oldest archeological samples studied come from the early Late Neolithic (3922–3902 BC) settlement of Hornstaad-Hörnle IA at the point of the peninsula Höri on the western shore of Lake Constance, Baden-Württemberg, Germany (Figure 1) [17–18] Eight houses built between 3922 and 3902 BC (dendrochronological and 14C dating) were found during the excavation from 1983 to 1993 [9, 17]. During the excavations, artifacts associated with farm activities, abundant fragments of unglazed ceramic and well-preserved vessels were found [8, 9, 17, 19–21]. The other site is Arbon-Bleiche 3, on the southeastern shore of Lake Constance in Canton Turgau, Switzerland [11, 22, 23]. From 1993 to 1995, rescue excavations conducted by the cantonal Archaeological Survey of Thurgau found 27 houses built in a time span of 14 years in a later phase of the Late Neolithic (3384 and 3370 BC, dendrochronological and 14C dating). Several other Neolithic and Bronze Age lake-shore dwellings lie on the shore of Lake Constance, however, Hornstaad and Arbon are by far the best investigated ones in terms of an interdisciplinary approach, applying botanical, zoological, mineralogical and chemical methodologies.

The cultural layer in Hornstaad-Hörnle IA and Arbon Bleiche 3 lies in the zone of water-logging, and therefore the preservation of the organic materials is excellent. Dark brown to black consolidated organic residues were recognised in the interior of the pottery vessels recovered in both archeological sites, indicating that they were used for food storage and cooking. Some cereals, other plant tissues and very rare fish bones were identified in the organic residues within the pottery vessels, but no structures of tissues or bones of other animals were found [9, 23–25]. This paper presents the results of the chemical and isotopic investigations of the organic residues from Hornstaad-Hörnle IA and compared them with the published data on organic residues from Arbon Bleiche 3 [26]. The chemistry of the organic residues preserved in this archaeological pottery (ancient dirty dishes) provides further insight into the farming activities and the diet at the Late Neolithic lake villages.

2. The organic biochemical approach

The main food constituents are water, sugars, proteins and fat. Water is evaporated and mixed during food storage and cooking, and later during burial of the vessel in the archaeological site. Sugars are water-soluble and are leached during early diagenesis. Proteins are hydrolysed
to water-soluble amino acids during very early diagenesis. Fats are hydrophobic and therefore may be preserved in archaeological pottery during burial. The animal and plant fats are composed principally of triglycerides in which three fatty acids are attached to a glycerol by ester bonds. The most common fatty acids found in plant and animal lipids have 4–20 carbon atoms (abbreviated as C
\(_{x:y}\)
, where ‘\(x\)’ is the number of carbon atoms and ‘\(y\)’ the number of double C–C bonds in the chain). The more abundant saturated fatty acids are the lauric (C
\(_{12:0}\)
), myristic (C
\(_{14:0}\)
), palmitic (C
\(_{16:0}\)
) and stearic (C
\(_{18:0}\)
) acid, and the unsaturated acids are palmitoleic (C
\(_{16:1}\)
), oleic (C
\(_{18:1}\)
), linoleic (C
\(_{18:2}\)
) and linolenic (C
\(_{18:3}\)
). The ester bonds in the triglycerides can be broken by hydrolysis during degradation of fats. Once released from the triglyceride, the short-chain fatty acids are appreciably more water-soluble and volatile than the long-chain fatty acids. So degraded fat is identified by high concentrations of palmitic and stearic acids.

The preservation of lipids in many archaeological materials is well documented, and the carbon isotope composition of the main fatty acids preserved in archaeological pottery appears unaffected by diagenetic alteration during prolonged burial [27, 28]. So, they provide a highly robust criterion to distinguish between the most important categories of domestic animals and vegetable material used in pre-history, including among others, animal fats [29, 30], milk [26, 31, 32], plant oils [33], plant leaf waxes [34], and beeswax [35].

3. Materials and methods

3.1. Samples

Fifteen potsherds of different geometry were selected from the material recovered in Hornstaad-Hörnle IA for this study (Table 1). All sherds, except sample HO-611M, contain a dark, black brownish amorphous solid deposited in the interior of the pottery vessels, supposed to have been used for storage and cooking of food.

Samples were prepared and analysed at the Institute of Mineralogy and Geochemistry of the University of Lausanne using modified procedures described previously [25]. The potsherds were

Table 1. Carbon and nitrogen concentrations (in weight percent) and bulk carbon (\(\delta^{13}\)C vs. VPDB) and nitrogen (\(\delta^{15}\)N vs. Air-N\(_2\)) isotopic compositions of organic residues in potsherds from Hornstaad-Hörnle IA.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Description</th>
<th>Organic residue</th>
<th>C (wt.%)</th>
<th>N (wt.%)</th>
<th>C/N (at.)</th>
<th>(\delta^{13})C (‰, VPDB)</th>
<th>(\delta^{15})N (‰, AIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-24C</td>
<td>Vessel with handle, AH 2</td>
<td>Crust</td>
<td>4.5</td>
<td>1.2</td>
<td>3.65</td>
<td>−27.5</td>
<td>3.5</td>
</tr>
<tr>
<td>HO-89C</td>
<td>Pot, AH 3</td>
<td>Crust</td>
<td>45.2</td>
<td>1.4</td>
<td>31.34</td>
<td>−24.8</td>
<td>9.5</td>
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<tr>
<td>HO-89M</td>
<td></td>
<td>Matrix</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HO-124C</td>
<td>Pot, AH 3</td>
<td>Crust</td>
<td>20.7</td>
<td>1.0</td>
<td>20.86</td>
<td>−24.4</td>
<td>2.4</td>
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<td>Pot, AH 3</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<td>Vessel with handle, AH 2</td>
<td>Matrix</td>
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<tr>
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<td>Crust</td>
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<td>AH 3</td>
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<td>42.33</td>
<td>−24.6</td>
<td>1.0</td>
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<tr>
<td>HO-456C</td>
<td>Pot, AH 3</td>
<td>Crust</td>
<td>15.1</td>
<td>1.3</td>
<td>11.61</td>
<td>−25.4</td>
<td>4.2</td>
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<td>Vessel with handle, AH 3</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HO-718M</td>
<td>Pot, AH 3</td>
<td>Matrix</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Arbon Bleiche 3 (n = 30) 35.0–64.3 3.4–12.8 5.7–19.0 −27.9—24.3 0.2–9.6

Note: Data for Arbon Bleiche 3 from ref [26].

–, not determined.
cleaned of visible foreign material and the organic residues sampled by scraping the potsherds with an organic solvent cleaned sharp blade or a very low-speed driller. Following removal of exogenous material with cleaned SS-forceps, the samples were ground, homogenised, weighed and stored in screw-cup sealed vials at $-20^\circ$C in the dark until use.

### 3.2. Sample preparation and analysis

All the solvents used were of a quality suitable for chromatography (Fluka, Switzerland) and were glass-distilled shortly before use. All the reagents were tested for purity by gas chromatography–mass spectrometry (GC–MS). All the glassware used for sample handling was thoroughly washed, rinsed with distilled water and heated at 480°C for 4 h before use. An aliquot ($\sim0.1$–$0.2$ g of organic crust and $\sim0.2$–$21$ g of ceramic matrix) of the samples was refluxed with dichloromethane for 2 days, with change of solvent after the first 24 h. The solvents containing the extracted lipids were combined and reduced to 2 ml by rotary-evaporation, and then gently evaporated to dryness in a good ventilated fume-hood. Carboxylic acids were isolated from the extracted lipids by alkaline hydrolysis with 1 N aqueous ethanolic potassium hydroxide at 60°C for 3 h. After cooling to room temperature, non-saponifiables were removed by washing with hexane aliquots. The sample was then acidified with 1 N HCl, and the acid lipids separated with hexane. The acid fraction was methylated (14% BF$_3$-methanol Fluka, 60°C, 8 min) and the fatty acid methyl esters (FAMEs) purified by elution with hexane. The FAMEs were stored with 1 ml hexane in 2 ml vials with PTFE-lined screw-caps at +4°C until gas chromatographic analysis [36, 37].

### 3.3. Gas chromatography–mass spectrometry

Chemical characterisation of the lipids was performed with an Agilent (Palo Alto, USA) gas chromatograph 6890 coupled to an Agilent 5973 quadrupole mass selective detector (GC–MS). The system was equipped with an Agilent free fatty acids phase fused silica capillary column (50 m length, 0.20 mm i.d.) coated with nitroterephthalic acid modified polyethylene glycol stationary phase (film thickness 0.33 μm). A sample aliquot was injected splitless at a temperature of 200°C. Helium was used as carrier gas (1 ml/min flow rate). After an initial period of 2 min at 100°C, the column was heated to 240°C at 5°C/min followed by an isothermal period of 30 min. The MS was operated in the electron impact mode at 70 eV, source temperature of 250°C, emission current of 1 mA and multiple-ion detection with a mass range from 50 to 600 amu. Compound identifications were based on comparison of standards, GC retention time and mass spectrometric fragmentation patterns. The abundances of FAME in the organic residues relative to total FAME were calculated from total ion chromatogram peak areas.

### 3.4. Isotopic analysis of bulk organic residue

The carbon and nitrogen isotope compositions, along with the percentage of C and N (and then the C/N atomic ratio), were determined by flash combustion on a Carlo Erba 1108 (Milan, Italy) elemental analyser connected to a Thermo Fischer (former Thermoquest/Finnigan, Bremen, Germany) Delta S isotope ratio mass spectrometer (IRMS) that was operated in the continuous helium flow mode via a Thermo Fischer Conflo III split interface. Flash combustion was in an O$_2$ atmosphere in a quartz reactor at 1020°C packed with Cr$_2$O$_3$ and (Co$_3$O$_4$)Ag to form CO$_2$, N$_2$, NO$_x$ and H$_2$O. The gases were then passed through a reduction reactor containing elemental copper and copper oxide at 640°C to remove excess of oxygen and to reduce the non-stoichiometric nitrous products (NO$_x$) to N$_2$. Water was subsequently removed by anhydrous Mg(ClO$_4$)$_2$, and N$_2$ and CO$_2$ were then separated in a gas chromatograph with a packed column (Pora-PLOT Q, 5 m length, 1/4 inch i.d.) at 70°C and were then analysed for their isotopic composition on
the IRMS. Reference N₂ and CO₂ gases were inserted in the He carrier flow as pulses of pure standard gases. The stable isotope composition of carbon and nitrogen is reported as $\delta^{13}C$ and $\delta^{15}N$ values in per mil ($\%e$) = $(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \times 1000$, where $R$ is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. For carbon, the standard is Vienna Pee Dee Belemnite limestone (VPDB) and for nitrogen is air-N₂. The calibration and assessment of the reproducibility and accuracy of the isotopic analysis are based on replicate analyses of laboratory standard materials (glycine: $\delta^{13}C = -26.0%e$ and $\delta^{15}N = 2.7%e$; pyridine: $\delta^{13}C = -29.2%e$ and $\delta^{15}N = 3.2%e$; urea: $\delta^{13}C = -43.1%e$ and $\delta^{15}N = -1.4%e$) and international reference materials (USGS-24 graphite: $\delta^{13}C = -16.05%e$; IAEA-PEF1 polyethylene foil: $\delta^{13}C = -32.15%e$; NBS-22 oil: $\delta^{13}C = -30.03%e$; IAEA-NO₃ potassium nitrate: $\delta^{15}N = +4.7%e$). The $\delta^{13}C$ and $\delta^{15}N$ values reproduce to 0.1%e (1 $\sigma$). The carbon and nitrogen concentrations were determined from the peak areas of the major isotopes. Calibration was done periodically with organic standards. The repeatability was better than 0.2 wt.% for both carbon and nitrogen.

3.5. Isotopic analysis of individual fatty acids

The compound specific stable carbon isotope analyses ($\delta^{13}C$ values) of the fatty acids were obtained by the use of an Agilent 6890 GC coupled to a Thermo Fischer Delta S isotope ratio mass spectrometer by a combustion (C) interface III (GC-C-IRMS) under a continuous helium flow [39]. The combustion interface consists of two ceramic furnaces. An oxidation reactor with CuO/NiO/Pt wires at 940 °C and a reduction reactor with Cu wires at 600 °C. Water was removed from the effluent gas by passing a Nafion tube (Perma Pure, Toms River, NJ, USA) with an annular back-flow of He. The IRMS ion source pressure is lower than 3 bar. The GC was operated with the same type of column and temperature programme used for GC–MS analyses. The background subtraction and $\delta^{13}C$ values were calculated using the Thermo Fischer ISO-DAT 7.2 software. The repeatability and intermediate precision of the GC-C-IRMS procedure, and the performance of the GC and combustion interface were evaluated every five analyses by injection of an in-house mixture of n-alkanoic acids (UNIL-FAME MIX) of known isotopic composition ($\delta^{13}C_{10:0} = -31.1%e$, $\delta^{13}C_{12:0} = -29.7%e$, $\delta^{13}C_{14:0} = -29.2%e$, $\delta^{13}C_{16:0} = -29.6%e$, $\delta^{13}C_{18:0} = -29.2%e$, $\delta^{13}C_{12:0} = -27.6%e$, $\delta^{13}C_{20:0} = -29.4%e$, $\delta^{13}C_{21:0} = -28.6%e$, $\delta^{13}C_{22:0} = -28.4%e$), and at least three replicate analyses of the Hornstaad-Hörnle IA samples. The standard deviations for repeatability ranged between 0.05 and 0.4%e for the main FAME and for intermediate precision between 0.3 and 1.1%e. The accuracy of the GC-C-IRMS analyses was checked every 10 analyses by injection of a FAME isotope standard (icosanoic acid methyl ester, $\delta^{13}C_{20:0} = -25.4%e$) prepared by A. Schimmelman from the Biogeochemical Laboratories at Indiana University, USA. The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by a mass balance equation [36]:

$$\delta^{13}C_{\text{FAME}} = f_{\text{FA}}\delta^{13}C_{\text{FA}} + f_{\text{MeOH}}\delta^{13}C_{\text{MeOH}}$$

where $\delta^{13}C_{\text{FAME}}$, $\delta^{13}C_{\text{FA}}$ and $\delta^{13}C_{\text{MeOH}}$ are the carbon isotope compositions of the fatty acid methyl ester, the fatty acid and the methanol used for methylation of the fatty acid, respectively, and $f_{\text{FA}}$ and $f_{\text{MeOH}}$ are the carbon fractions in the fatty acid methyl ester due to the alkanoic chain and methanol, respectively.

4. Results and discussion

4.1. Carbon and nitrogen contents and bulk isotope composition

The C and N concentrations and the bulk $\delta^{13}C$ and $\delta^{15}N$ values for the organic residues are given in Table 1. The median C and N concentrations of the organic residues are 20.6 wt. %
(range: 4.5–45.5 wt.%) and 1.2 wt.% (range: 0.5–1.4 wt.%), respectively. The relatively high total nitrogen content (C/N = 3.6–42.3, median = 26.1) suggests processed/altered protein-rich food.

The C and N isotope composition of the organic remains in pottery vessels used for cooking or storage of food may reflect the average δ¹³C and δ¹⁵N values of the degraded food material during vessel use and burial. This may provide insights into the types of food consumed, and particularly whether the diet was predominantly animals or plants (C₃, C₄) based. The range (average and standard deviation) for organic crust (n = 6) in potsherds from Hornstaad-Hörnle IA are −27.5 to −24.0‰ (−25.1 ± 1.2‰) and 1.0 to 9.5‰ (+3.7 ± 3.0‰) for δ¹³C and δ¹⁵N, respectively (Table 1). These values are (statistically) similar to those measured in Arbon Bleiche 3 (δ¹³C = −26.1 ± 0.8‰, and δ¹⁵N = −3.7 ± 1.8‰)²⁶. Terrestrial C₃ plants have δ¹³C values between −30 and −23‰ and δ¹⁵N between −7 and 6‰ [40]. Thermal degradation and microbial reworking of organic matter may cause selective loss of more reactive organic compounds, creating an isotopic shift of 1–5‰ [41]. The small variations of the δ¹³C values (between −27.9 and −24.3‰) in Hornstaad-Hörnle IA potsherds within the range expected for degraded animal and plant tissues are consistent with the archaeological evidence of C₃ plants and animals, whose subsistence was mainly based on C₃ plants.

4.2. Fatty acid distribution

The organic residues from Hornstaad-Hörnle IA potsherds contain a significant amount of extractable lipids, consisting almost entirely of free and bonded fatty acids identified by their methyl ester (FAME) mass spectra (Figure 2). The total ion gas chromatograms show a series of FAME of straight chain carboxylic acids in the C₉ to C₂₄ carbon number range, excluding C₂₁ and C₂₃ (Figure 2). The main saturated fatty acids are the lauric (C₁₂:0), myristic (C₁₄:0), pentadecanoic (C₁₅:0), palmitic (C₁₆:0), margaric (C₁₇:0) and stearic (C₁₈:0) acids, maximizing at C₁₆ and C₁₈. These fatty acid distributions are typical of degraded fats and have a strong biological signature, maximizing at C₁₆:0 with generally clearly greater abundance than C₁₈:0. Small to trace amounts of capric (C₁₀:0), hendecanoic (C₁₁:0), tridecanoic (C₁₃:0), arachidic (C₂₀:0) and behenic (C₂₂:0) acids occur in all samples. The only unsaturated acids identified and quantified were palmitoleic (C₁₆:1) and oleic (C₁₈:1). Terminally, branched iso and anteiso b-C₁₅:0 acids elute between C₁₄:0 and C₁₆:0. Apart from trace amounts of linoleic (C₁₈:2), no polyunsaturated fatty acids were detectable. This is to be expected since the polyunsaturated fatty acids would have decomposed under the oxidative

Figure 2. GC–MSD total ion chromatogram of the fatty acid methyl esters in lipids extracted from organic residues of potsherds from Hornstaad-Hörnle IA (see text).
conditions during vessel use and subsequent burial at the archaeological site. The short C-chain fatty acids (C_{10} to C_{15}), which are more soluble and volatile, are relatively better preserved into the ceramic matrix (Figure 2). The phenols and benzoic carboxylic acids in the extracts of the ceramic matrix may be related to oxidative/thermal degradation of the indigenous fatty acids, or a contamination of the pottery during burial. The relative concentrations of the fatty acids compare favourably with those from the potsherds of Arbon Bleiche 3, having a general pattern similar to vegetable and animal fats (figure not shown for brevity).

4.3. Stable carbon isotope composition of individual fatty acids

The δ^{13}C values of the main fatty acids of the lipids extracted from Hornstaad-Hörnle IA potsherds (organic crust and ceramic matrix) vary between −39.0 and −23.9‰ (Table 2). Since the precision, including the overall analytical error for sample preparation and isotopic analyses is <1.2‰, the predominant factor in the δ^{13}C deviations of the individual fatty acids is real archaeological variability. Most δ^{13}C_{16:0} values are higher than the δ^{13}C_{18:0} values (Table 2 and Figure 3). The differences between the δ^{13}C values of the main fatty acids (∆^{13}C_{18:0−16:0} and ∆^{13}C_{18:0−18:1}) indicate different biological sources and degrees of thermal and microbial degradation of the organic residues (Figures 3A and B).

4.4. Origin of lipids in the organic residues

The animal and plant fats show a distinctive distribution in the carbon isotopic composition of the main fatty acids, reflecting their different biosynthetic origin. In a previous study, fat samples of modern animals that have been fed exclusively on C_{3} forage grasses and vegetable oil samples were analysed in order to test the origin of the organic residues in the archaeological ceramics [26]. Thus, the origin of lipids preserved in the archaeological ceramics can be assessed by comparison

Table 2. Carbon isotope composition (δ^{13}C) of main fatty acids from lipids extracted from potsherds (matrix and organic crust) of Hornstaad-Hörnle IA.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Description</th>
<th>Myristic (C_{14:0})</th>
<th>Penta-decanoic (C_{15:0})</th>
<th>Palmitic (C_{16:0})</th>
<th>Palmitoleic (C_{16:1})</th>
<th>Heptadecanoic (C_{17:0})</th>
<th>Stearic (C_{18:0})</th>
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<tr>
<td>HO-24C</td>
<td>Vessel with handle, AH 2</td>
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<td>−28.0</td>
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<td>−29.3</td>
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Abbreviations. *C, organic crust; M, matrix of the pottery. –, not measurable.
Figure 3. Carbon isotope composition of stearic acid ($\delta^{13}C_{18:0}$) vs. palmitic ($\delta^{13}C_{16:0}$) and oleic ($\delta^{13}C_{18:1}$) acids of the organic residues from Hornstaad-Hörnle IA.

with reference samples of modern edible vegetable oils and animal fats [26, 36, 37]. All the reference fats show a distinctive distribution in the carbon isotopic composition of the main fatty acids, reflecting their different biosynthetic origin. The fatty acids in the animal adipose originate mainly from the diet, and plot near the 1-to-1 $\delta^{13}C_{16:0} - \delta^{13}C_{18:0}$ line. Exceptions are the fat from deer and fish, which is explained by the diversity of the diet of these animals, and different metabolism of aquatic organism. The milk and milk products are isotopically distinct from the adipose samples. This fractionation is explained, considering the biochemistry and physiology of milk production in ruminant animals [26].

Most samples of organic residues in archaeological vessels from Hornstaad-Hörnle IA plot in similar $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:0}$ and $\delta^{13}C_{18:1}$ vs. $\delta^{13}C_{18:0}$ fields of Arbon Bleiche 3, corresponding to modern milk products, and calf/lamb and pig adipose (Figures 4A and B). Several samples are between the fields of plants and animal fats, suggesting admixture of fats of different origins (different metabolic route) and different degrees of degradation.

No sample plot in the fields of fish fats. The differences in isotopic composition between the individual animal fatty acids may be due to isotopic fractionation occurring during biosynthesis (including synthesis de novo by bacterial and protozoa in the rumen) and different rates of their metabolic turnover [26].

In the $\delta^{13}C_{18:1}$ vs. $\delta^{13}C_{18:0}$, the archaeological samples plot outside the fields for modern calf or lamb, towards isotopically heavier oleic acid (Figure 4B). This merits a short explanation. Oleic acid has an unsaturation (a double C−C bond). This more ‘reactive’ group in the carbon skeleton of the carboxylic acid may break during thermal (e.g., cooking, fires in the settlement) or diagenetic degradation, releasing preferentially isotopically light moieties. The residual oleic acid is enriched in $^{13}C$. This transformation processes do not affect the palmitic and stearic acids.

The comparison of the carbon isotopic composition of modern and archeological lipids has some problems. Some differences of the $\delta^{13}C$ values may be apparent, and explained by (1) different $\delta^{13}C$ value of the primary carbon source, (2) change(s) of the $\delta^{13}C$ values during cooking by the prehistoric people and (3) diagenetic alteration of the organic residues during burial at the archeological site [26]. Evershed et al. [28] have shown that the isotopic composition of lipids absorbed into the porous structure of the archeological pottery appears unaffected by diagenetic alteration during burial. The pre-industrial atmospheric CO$_2$ was isotopically heavier (by $\sim 1.6\%$) than in present time [42]. The carbon isotopic composition of plant and animal (primary producers and consumers) fats depends on the $\delta^{13}C$ value of the atmospheric CO$_2$ fixed into organic compounds by photosynthesis. Therefore, if the isotopic fractionation in the pre-industrial (e.g., 230 AC)
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Figure 4. Carbon isotope composition of stearic (δ¹³C₁₈₀) vs. palmitic (δ¹³C₁₆₀) and oleic (δ¹³C₁₈₁) acids of the organic residues from Hornstaad-Hörnle IA and Arbon Bleiche 3 with those of modern (A,B) and pre-industrial (C,D) animal and plant fats. The fields for present-day European C₃-vegetable oil lipids is from refs. [26, 36, 37].

biogeochemical carbon cycle was determined by similar photosynthetic mechanisms and metabolic routes as those known today, we can safely assume that the δ¹³C₁₆₀ vs. δ¹³C₁₈₀ and δ¹³C₁₈₀ vs. δ¹³C₁₈₁ fields for pre-industrial plants and consumers were shifted by ∼1.6‰ towards more positive δ¹³C values. With this adjustment, almost all samples from Hornstaad-Hörnle IA are included in the isotopic fields for pre-industrial dairying products, calf/lamb adipose, cattle adipose and C₃ plants.

It is very difficult to assess the isotopic fractionation of fatty acids during cooking in Neolithic time. The temperature achieved by wood–fire cooking outdoors may be very variable, depending among others on wood type, weather conditions and wind direction and may vary between 100 to above 450 °C. Furthermore, the pre-historic settlers did not (thoroughly) wash the vessels and dishes before use. Therefore, the studied archeological organic remains are most probably admixtures of fats of different origin and different degree of thermal alteration.

A preliminary study of the changes of the δ¹³C values of the main fatty acids during cooking of cow, goat and sheep milk at 100 °C (5 h) and 250 °C (1 h) has shown a ¹³C enrichment of C₁₈₀ and C₁₈₁ during heating. We have explained this isotopic shift by preferential cleavage of the ¹²C–¹²C single or double bonds and loss of ¹²C-rich moieties, with loss of C₂ moieties [26]. The proposed cracking pathway was mainly based on the δ¹³C changes in heated cow and goat milk. Here, we re-evaluate the proposed cracking pathway including a key reference thrown from petroleum systems. For thermal cracking, the variations in proton-affinities and bond strengths of
Figure 5. Bulk and fatty acids carbon isotope composition of goat raw and heated milk (5A) data from [26]. The thermal degradation pathway of C₁₈ fatty acids during heating (5B).

the C−C bonds in straight-chain \textit{n}-alkanes determined using quantum chemistry computations indicate that the most favourable initiation step is the breaking of the $\beta$-bond to create a C₂ (ethyl) radical and a residual chain of C$\textit{n}$−₂ length [43]. The oxidation of the released alkyl-intermediary to a C$\textit{n}$−₂ carboxylic acid may proceed readily during cooking in an oxygenated environment. A pathway in C$\text{₁₈}$, C$\text{₁₈}$₀ and C$\text{₁₆}$₀ degradation involving chain shortening with loss of an ethyl radical and oxidation of the alkyl moiety explain the $^{12}$C-enrichment of short-chain (C₁₆, C₁₄) fatty acids in heated milk (Figure 5).

5. Conclusions

Fat residues from calf/lamb adipose (lactating animals) and ruminant milk from a C₃ plant ecosystem were chemically and isotopically identified in almost all potsherds from the early Late Neolithic lake shore settlement of Hornstaad-Hörnle IA, Germany. This indicates meat consumption (e.g., from young suckling calves) and farming practices for sustainable dairying in young Neolithic (ca 4000 cal BC) settlements in central Europe.

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