# Identification of the lipids on an archaeological vessel from Basel-Gasfabrik, a Celtic site on the border of the River Rhine

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**Materials**

The black carbon (BC) from an archaeological ceramic from the Basel-Gasfabrik site (Inv. No. 1989/5.5102, Kat. 1588) was studied for the isotopic ( $\delta^{13}C$) and molecular compositions of the individual fatty acids. To remove any superficial contamination from handling and recently weathered material, the surfaces of the sherds were washed with analytical-grade and glass-distilled acetone, ethanol, and water. The black carbon coating of the pottery was removed using spatulas and forceps that had been cleaned with solvents, and was homogenized and finely powdered in a pre-washed agate mortar. This sample of black carbon was named J-BC. A piece of the BC-free ceramic was crushed and finely powdered in the agate mortar to form sample J-CE.

Both samples (J-BC and J-CE) were dried at 50 °C for 24 hours, homogenized manually in an agate mortar, and processed as described below.

**Sample preparation**

All of the solvents used were of a quality suitable for chromatography (Fluka, Switzerland) and were glass distilled shortly before use. Both the BC sample (887 mg) and the CE sample (15.24 g) were refluxed with an azeotropic mixture of methanol and dichloromethane for 48 hours, including the replacement of solvents after the first 24 hours, followed by dichloromethane for 24 hours. The solvents were combined and reduced by rotary-evaporation and gently evaporated to dryness. Separation and methylation of the fatty acids from the lipid extracts were performed by alkaline hydrolysis with aqueous ethanolic potassium hydroxide, and extracted with hexane. The fatty acids were esterified with methanolic BF3. The fatty acid methyl esters (FAMEs) were extracted with hexane and washed in a saturated aqueous potassium chloride solution. The FAMEs were stored with 0.5 ml hexane in 2 ml vials with PTFE-lined caps at 4 °C in preparation for the gas chromatographic analysis (1–2).

**Gas chromatography/mass spectrometry detection (GC/MSD)**

Chemical characterization of the lipids was performed with a Hewlett-Packard GC HP 6890 coupled to a HP 5973 quadrupole mass selective detector (GC/MSD). The system was equipped with an HP-FFAP fused silica capillary column (50 m x 0.20 mm i.d.) coated with polyethylene glycol TPA modified as stationary phase (film thickness 0.33 µm). Helium was used as the carrier gas (1 ml/min flow rate), and the splitless injection was done manually at a temperature of 200 °C in order to prevent potential isomerization of the unsaturated fatty acids. After an initial period of 2 minutes, the column was heated to 250° C at 5° C/min followed by an isothermal period of 20 minutes. The MSD was operated in the electron impact mode at 70 eV, a source temperature of 250 °C, an emission current of 1 mA and multiple-ion detection with a mass range of between 50 and 700 amu.

**Isotopic analysis of individual fatty acids by GC/C/IRMS**

The compound specific carbon isotope analyses of the fatty acids from samples OL-residue and OL-soil were obtained by the use of a Hewlett-Packard 6890 GC coupled to a Finnigan MAT Delta S isotope ratio mass spectrometer by a combustion (C) interface III (GC/C/IRMS) under a continuous helium flow. The combustion interface is comprised of a ceramic furnace with copper oxide and platinum catalyst at a temperature of 940 °C. An He-flushed Nafion membrane prevented water from reaching the ion source of the IRMS. The GC was operated with the same type of column and temperature programme used for GC/MS analyses. The performance of the GC/C/IRMS system, including the GC and combustion furnace, was evaluated every 10 analyses by injection of a mixture of FAMEs of known $\delta^{13}C$ values. The background subtraction and $\delta^{13}C$ values were calculated using the ISODAT 7.4 software. The reproducibility assessed from four replicate analyses of the samples ranged between ±0.1 and ±0.4 ‰. The accuracy of the GC/C/IRMS analyses was monitored by co-injection of a laboratory-standard FAME of known isotopic composition. The stable carbon isotope ratios are reported in the delta ( $\delta$ ) notation as the per mil (‰) deviations relative to the Pee Dee Belemnite limestone (PDB) standard:

$\delta^{13}C = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$

where $R = {}^{13}C/{}^{12}C$.

The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by a mass balance equation:

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

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{MeOH}$ 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 $f_{FA}$ and $f_{MeOH}$ are the carbon fractions in the fatty acid methyl ester due to the underivatized fatty acid and methanol, respectively.

**RESULTS**

The gas chromatograms of the fatty acids extracted from the «black carbon» (sample J-BC) and from the BC-free ceramic re-

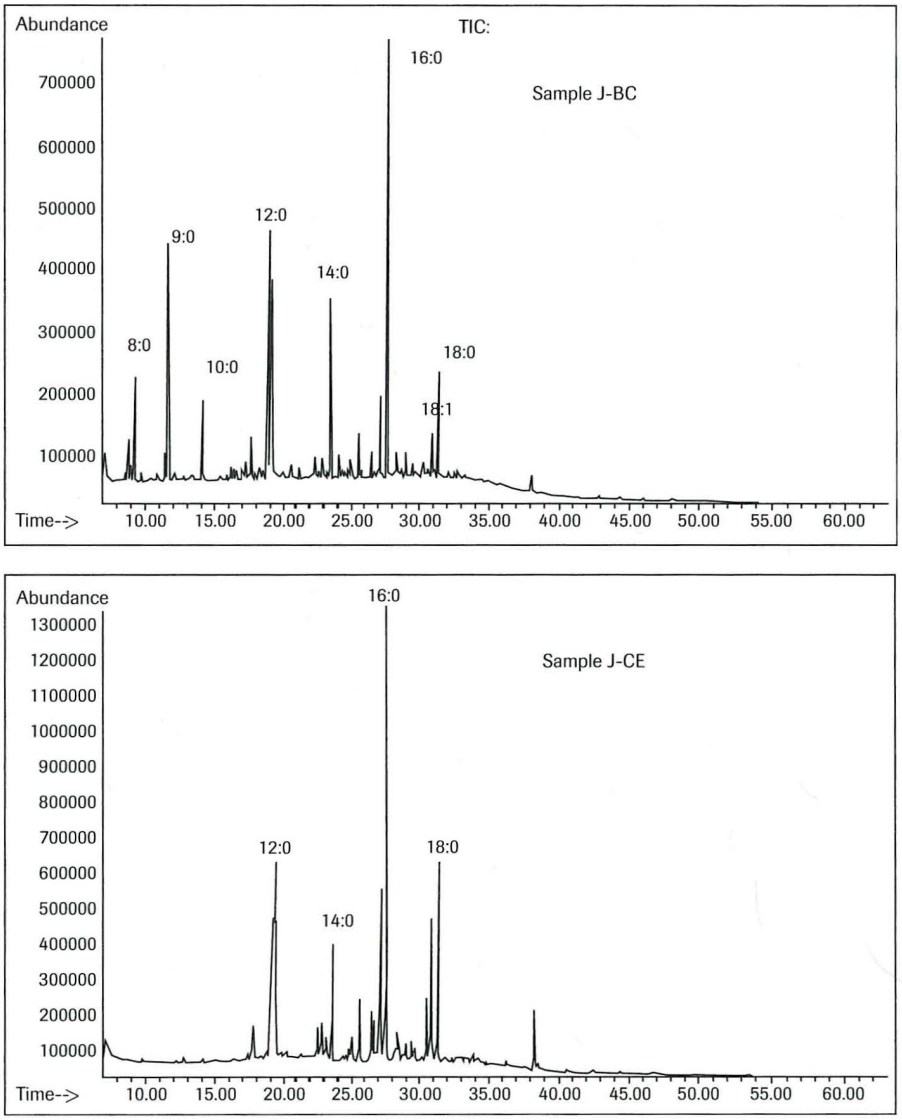


**Figure 1** *GC/MS chromatogram of the fatty acid methyl esters of the lipids extracted from the ceramic from Basel-Gasfabrik (Inv.-Nr. 1989/5.5102): black carbon and ceramic.*

sidue (J-CE) are presented in Figure 1. Both chromatograms are similar for compounds with more than 12 carbon atoms. The saturated fatty acids (n-alkanoic acids) range from 8 to 18 carbon atoms, maximizing at 16:0 (palmitic acid). The main monounsaturated fatty acids are palmitoleic (16:1) and oleic acid (18:1). Alcohols and some branched fatty acids elute (and co-elute) with the short-chain n-alkanoic acids (e.g., 8:0, 12:0).

Only the sample J-BC contained a large enough amount of fatty acids to allow the replicate determination of $\delta^{13}C$ of the major fatty acids (Table 1).

The stable carbon isotopic compositions ( $\delta^{13}C$ values) of the fatty acids extracted from archaeological samples has been shown to provide valuable information on the vessel use, food consumption type, and the origin of dairy residue (e.g., 3–4).

As pointed out by Dudd & Evershed (1998), the co-variation of the carbon isotopic compositions of the palmitic (16:0) and stearic (18:0) fatty acids is a sensitive tool for distinguishing milk, fat, and vegetable based diets. This interpretation follows the approach of Evershed et al. (1999), using $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:0}$ co-variation fields determined for modem vegetable and animal lipids. The carbon isotopic composition of these materials (primary producers and consumers) depends on the $\delta^{13}C$ value of the atmospheric $CO_2$ fixed into organic compounds by photosynthesis. The fatty acids from vegetable fats cover a very broad range, depending on the photosynthetic mechanism used by the plant to fix atmospheric $CO_2$ (e.g., 3). The range for modem European genuine olive oils (-35.4 to -30.7 ‰ for 16:0, -33.5 to -31.3 ‰ for 18:0; 3–4) was included in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:0}$ plot (Fig. 2). The pre-industrial atmospheric $CO_2$ was isotopically heavier (by ~1.6‰ between 1800 and 1980, Freyer, 1986) than today. Therefore, assuming that the isotopic fractionation in the pre-industrial (e.g. AD 230) biogeochemical carbon cycle was determined by today's known photosynthetic mechanisms and metabolic pathways, we could expect the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:0}$ co-variation fields (for plants and consumers, Fig. 2) at that time to have been shifted slightly toward more positive $\delta^{13}C$ values.

The $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:0}$ co-variation of the black carbon residue in the vessel from Basel-Gasfabrik (Inv.-No. 1989/5.5102) plots among animal fat fields, and well outside the field for vegetable lipids (Fig. 2).

| Fatty acid | $\delta^{13}$ (‰, PDB) |
|---|---|
| | J-BC sample |
| n - 9:0 | -26.7±0.1 |
| n - 12:0 | -27.6±0.1 |
| n - 14:0 | -28.7±0.1 |
| n - 16:0 | -29.6±0.1 |
| n - 18:0 | -29.4±0.1 |

**Table 1** *Carbon isotope ratios of the major fatty acids extracted from the ceramic from Basel-Gasfabrik (Inv.-Nr. 1989/5.5102).*

## Conclusion

The isotopic composition of the main fatty acids extracted from the organic residue of the Basel-Gasfabrik archaeological ceramic (Inv.-No. 1989/5.5102) suggests that the fat(s) used in this vessel were of animal origin (ovine/porcine).

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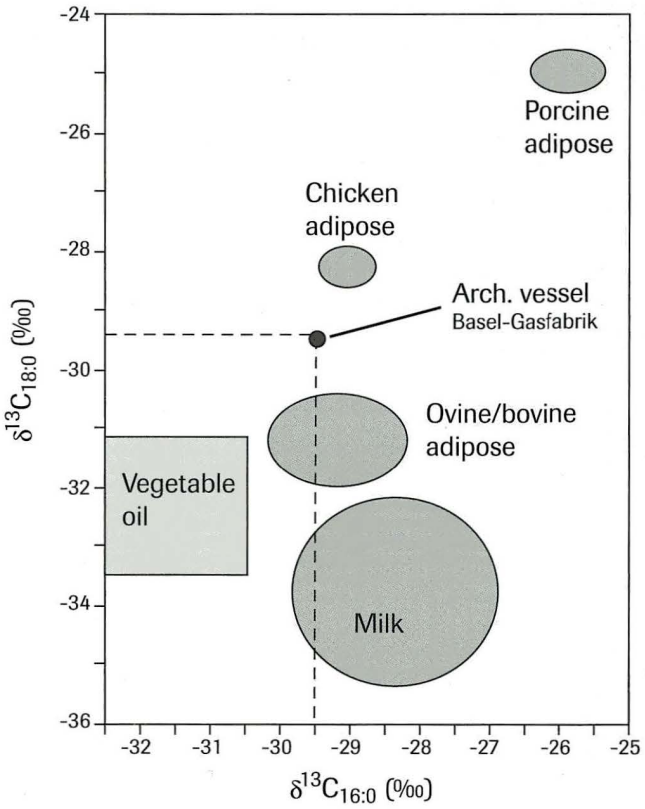


**Figure 2** *Plot of the $\delta^{13}C$ values of the major saturated fatty acids (palmitic = 16:0, stearic = 18:0) from the lipids extracted from the black carbon (Inv.-Nr. 1989/5.5102). The circled fields encompass the ranges for present-day animal fats (data from Evershed et al., 1999). The field for present-day European olive oil lipids is taken from Spangenberg et al. (1998) and Spangenberg and Ogrinc (2001).*