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Supercritical fluids for higher extraction yields of lipids from archaeological ceramics

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Abstract

The extraction and study of organic residues from ceramics has been a subject of interest for the last 50 years in archaeology and archaeological science. Lipids are among the best-preserved organic substances in archaeological contexts and can provide information about the diets of ancient populations, as well as past environments. Here, we present a method which demonstrates significantly improved extraction of lipids from archaeological pots by replacing liquid organic solvents with supercritical fluids. Optimization of the procedure using response surface methodology (RSM) approach showed that, on our system, optimal conditions for supercritical extraction of lipids from synthetic fired clay ceramics could be achieved using carbon dioxide with 16 volume % of co-solvent EtOH:H₂O (95:5 v/v) in 90 minutes at a flow rate of 2.3 ml/min, for a pressure of 30 MPa and a temperature of 50°C. For all reference and archaeological samples included in this study, lipid yields obtained by supercritical fluid extraction under these optimal conditions were systematically higher than by conventional solvent extraction. This study also highlighted a variability of the ratio of

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3 unsaturated versus saturated fatty acids depending on the extraction method. This can have
4 important implications in the identification of the residue(s). The increased extraction
5 efficiency provided by supercritical fluids, as well as their minimally destructive nature,
6 enable new and refined approaches to residue analysis and dating of archaeological ceramics.
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10 11 12 **Keywords**

13 Supercritical Fluid Extraction (SFE); Residue analysis; Lipid; Archaeological ceramic
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18 Archaeological investigations of lives and lifestyles of human populations in the past are
19 based, almost exclusively, on studies of material remains—stone tools, ceramic vessels, glass
20 or metal artefacts, textiles, and other organic substances (i.e. bone, wood, charcoal, seeds).
21 Archaeologists have always relied on scientific methods to enrich their interpretations of
22 these remains, and over the last few decades, techniques to characterise organic substances at
23 a molecular level, have improved dramatically. This trend is seen clearly in the ever-
24 expanding application of analytical techniques based on the use of chromatography and mass
25 spectrometry (e.g. High Performance Liquid Chromatography (HPLC) and Gas
26 Chromatography / Mass Spectrometry (GC/MS))¹. Although these techniques have many
27 applications in archaeological science, the most common relates to the question of ancient
28 diet and the analysis of ancient food residues preserved within the fabric of ceramic
29 containers²⁻⁴. To exploit this valuable source of information, scientists conventionally
30 pulverise fragments of pottery and apply a combination of liquid organic solvents such as
31 chloroform or dichloromethane and methanol. The extracted residue is then characterised by
32 GC/MS or GC-C-IRMS⁴. This is a time-consuming process which requires toxic solvents and
33 is also destructive as the sherd needs to be crushed to increase the surface interface between
34 ceramic and solvent.
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45 Over the last few decades, the field of analytical chemistry has seen substantial
46 transformations such as with the development and application of new instrumentation using
47 supercritical fluids (SFs) for extraction and chromatographic separation (SFE and SFC,
48 respectively). In the field of archaeological science, the use of supercritical fluids has not yet
49 been fully explored compared to other analytical techniques (e.g. GC/MS or FTIR). There are
50 only few applications of SFE technology published. These examples mostly relate to
51 cleaning, drying, and conservation of delicate archaeological artefacts: actively eroding iron
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3 objects have been cleaned and stabilised using CO₂⁶, SFs have also been used to dry
4 waterlogged corks⁷ or other wooden materials^{8,9} and to clean 18th century silk textiles¹⁰. Two
5 other publications have focused on the use of supercritical fluids prior to radiocarbon
6 dating^{11,12}. Finally, an early publication, which lead to a patent, proposed the use of SC-CO₂
7 for the de-acidification of paper from historical records¹³.
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10 Ongoing research at the Oxford Radiocarbon Accelerator Unit seeks to explore the use of
11 supercritical fluids to extract organic materials from a range of archaeological artefacts. Here,
12 for the first time, we present the results of a pilot study to extract lipids from ceramic samples
13 using supercritical fluids.
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18 **Experimental**

19 **Supercritical Fluid Extraction apparatus**

20 Supercritical fluid extraction tests were performed on a Semi-Prep SFC- SP-2086 instrument
21 from Jasco® equipped with UV detector and fraction collector. The system was modified
22 with support from the company in order to run extractions and not only chromatographic
23 separations in supercritical phase. A new line was installed to bypass the autosampler and the
24 extraction vessel was installed in the oven in place of the column to control the extraction
25 temperature. The extraction vessel was a 10 mL cylindrical extraction vessel provided by
26 Jasco®. Samples were kept in the centre of the vessel by filling it up with solid-glass beads of
27 2 mm diameter (Sigma Aldrich, UK). Tests were also performed, for comparison, on a
28 Waters® MV10 system with 5 mL extraction vessels fitted on 10 parallel lines. The
29 comparative tests did not show any significant differences and therefore results obtained with
30 both instruments are discussed together in this article. Samples were collected in glass vials
31 with cyclone inserts provided by Jasco® to increase the recovery of the co-solvent containing
32 the extracted analytes. Volumes of co-solvent recovered were monitored and compared with
33 the theoretical volume calculated based on the % of co-solvent and the flow rate. All
34 glassware and solid-glass beads were baked at 500°C for 3 h to eliminate any
35 possible contaminants.
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50 **Experimental Design**

51 Ceramic pellets (fired kaolinite) impregnated with olive oil were used for the optimisation of
52 the SFE parameters (preparation of the pellets described in S-1). A number of parameters can
53 be adjusted in SFE including the nature of the solvent and the co-solvent, their relative
54 proportions, the flow rate, the pressure, the temperatures in both the vessel and back pressure
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3 regulator (BPR) and the extraction time. Extractions were performed at the highest
4 temperature (50 °C) and pressure (30 MPa) achievable with the equipment to maximise the
5 extraction yields as reported in the literature¹⁴. The extraction time for SFE, can vary
6 considerably from few minutes up to several hours¹⁵. Various tests performed on the system
7 (data not reported here) demonstrated that an extraction of 90 minutes is sufficient to remove
8 up to 95 % of the olive oil from the clay samples with a large proportion being extracted
9 during the first 30 minutes. It was therefore decided to extract all samples for 90 minutes. The
10 other parameters (polarity of the co-solvent, the proportion of co-solvent and the flow rate)
11 were adjusted using the Response Surface Methodology (details in the S-3). The values tested
12 for optimisation of flow rate, polarity and proportion of co-solvent were chosen based on data
13 reported in literature for the extraction of lipids. The total flow rate (CO₂ + co-solvent) was
14 varied from 1, 2 and 3 mL/min with 10, 15 or 20 vol. % of co-solvent. As each parameter
15 needs to be expressed by numerical values, the nature of the solvent was defined by its
16 polarity: 5.2 (100 % ethanol), 5.39 (ethanol + 5 % water) and 5.58 (ethanol + 10 % water).
17 By determining the maximum of the response function, the following set of ‘optimal’
18 parameters was identified: 16 V% of EtOH:H₂O (95:5) in CO₂ at a total flow rate (CO₂ +
19 modifier) of 2.3 mL/min (Details in S-4 and S-5).
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32 **Total Lipid Extraction**

33 For comparison, the ceramic pellets were also analysed after a conventional extraction
34 procedure. The samples (± 250 mg accurately measured) were crushed using mortar and
35 pestle and then extracted by 3 mL of a mixture trichloromethane : methanol (2:1 v/v). A
36 standard consisting of 5 µL of tridecanoic acid solution in iso-octane (1.87×10^{-6} mol/µL)
37 was also added to assess the extraction efficiency. The solution was vortexed for a few
38 seconds and then sonicated for 30 minutes at 50 °C. The suspension was then centrifuged for
39 10 minutes at 2500 rpm. The supernatant was transferred in a separate 2 mL vial and blown
40 down under nitrogen. This extraction process was repeated 3 times in total (with the
41 supernatant always being blown down into the same 2 mL vial).
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50 **Characterisation by GC/MS**

51 All extracts were derivatised and analyses were performed using an Agilent 7820A gas
52 chromatograph equipped with a Restek Rxi-5ms column (30 m length x 0.25 mm ID x 0.25 m
53 film thickness, 5% diphenyl / 95% dimethylpolysiloxane stationary phase). The mass
54 spectrometer was an Agilent 5975 quadrupole mass spectrometer, operated in electron
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ionization mode (70 eV) and the scan range was m/z 50-650 u.m.a. Detailed analytical conditions are reported in the Supplementary Information (S-2).

Results and Discussion

A total of 12 archaeological samples were selected for residue analysis as part of this pilot study. 7 of these ceramic sherds (samples CFS2, LDS2, SRS4, CFS3, LDS4, SRS3 and SRS2) are from the site of Bobartia Road Midden which is a coastal shell midden near Betty's Bay, southwestern Cape, South Africa¹⁶. The remaining 5 samples come from 3 different prehistoric sites in Eastern Siberia, Russian Federation: Ust' Karenga (Ust2, Ust5), Popovskii Lug (PL2, PL4) and Shamanka II (SHAMII). The first two sites, located within the Upper Vitim and Upper Lena Basins (respectively), are considered as seasonal settlements for hunter-gatherers^{17,18}. The final site is a cemetery complex on the southern shore of Lake Baikal where individuals were buried along with rich material inventory, including ceramic vessels¹⁹. These 12 samples were selected not only as representative of a wide range of geographical, archaeological and chronological contexts, but also as spanning a variety of mineralogical compositions and specific storage histories. Unusually, these samples were available in sufficient quantities to perform both SFE and TLE extractions. This enabled a direct comparison of these methods to establish the absolute lipid yield, as performed in the optimisation procedure, for both the entire residue and for individual organic molecules. Before extraction, all archaeological sherds were first "cleaned" using an electric drill to remove the first mm of the surface, thus eliminating contaminations by soil and handling. Drill heads were cleaned in both milliQ water and acetone by sequentially sonicating them for 15 minutes. For SFE, the "cleaned" sherds were directly extracted without any further pretreatment. For the extraction using conventional organic solvents, the "cleaned" sherds were powdered using mortar and pestle. In both cases around 250 mg of ceramic, precisely weighed, was used. The archaeological samples analysed in this study mainly yielded free fatty acids which can be related to plant and/or animal lipids (Peak identification in S-6). The total amount of free fatty acids recovered per gram of sherd was also calculated for the 12 archaeological samples. In Figure 1, we plot the total FFA yields for archaeological samples by SFE and TLE. The results show that SFE is systematically more efficient than TLE. Similarly, improved efficiencies are also noted for olive oil extraction from reference samples (not shown). We use a logarithmic scale, since the amount of preserved organics varies by several orders of magnitude between the archaeological sherds. This variability can be explained by several

factors. First, the amount of organics is related to the original function of the ceramic. For example, some ceramics may have been used to cook food extremely rich in lipids while others may have been used to store dry food such as cereals. Second, different sherds of the same ceramic object may exhibit heterogeneity which is related to complex mineralogical and thermal variation (e.g. in the case of earthenware pottery used for cooking). Finally, differential degradation of organic residues during burial may result in variability between the amount extracted from different sherds. These factors may explain why the increase of extraction yield with SFE is not identical across the 12 sherds. When we checked SFE using the Waters apparatus, with identical experimental parameters, we observed a similar pattern to the Jasco system, whereby SFE yielded systematically higher yields than TLE. This suggests that the higher extraction efficiencies of SFE are a genuinely reproducible effect, notwithstanding heterogeneities due to differential organic content and preservation.

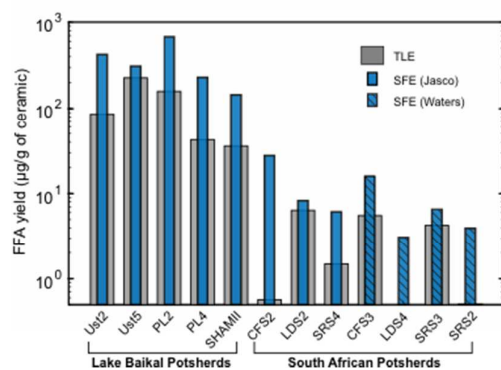


Figure 1. Comparison of the free fatty acid yields from archaeological pottery from Lake Baikal (left) and southwestern Cape, South Africa (right) by SFE and by TLE showing systematic higher yields by SFE for all pottery samples. Note that for samples CFS3, LDS4, SRS3 and SRS2 we tested SFE using a different apparatus, with identical experimental parameters.

The SFE procedure was able to extract in higher quantities the same molecules extracted by TLE but also other molecules such as small free fatty acids which were probably present in quantities too low to be detected after TLE (S-6). Comparing results obtained by SFE and TLE also revealed interesting information regarding the unsaturated free fatty acids. It appears that, for a majority of the samples analysed, the ratio unsaturated / saturated free fatty acids increases with SFE (Table 1, Figure 2). In other words, extracts obtained with supercritical fluids contain more unsaturated free fatty acids than those obtained by TLE. Our hypothesis is that unsaturated free fatty acids, richer in electrons, are more likely to

chemically bind at vacant sites within the thermally-altered clay mineral matrix and form stable organo-metallic complexes. Supercritical fluids are perhaps more efficient at breaking these complexes and increase the extraction of the free fatty acids, relative to TLE. However, not much is currently known about the complex interactions between ceramics and organics, and more investigation is warranted. The identification of unsaturated free fatty acids is very important in understanding the nature of the residue such as differentiating between plant and fish oils^{20,21}. The lower efficiency of TLE may therefore result in a misinterpretation of the function of the ceramics.

Table 1. Amount of saturated and unsaturated free fatty acids (in $\mu\text{g/g}$ of ceramic) extracted with the two different methods (SFE and TLE) on 12 archaeological sherds.

| Sample | Total Saturated FFA by TLE | Total Saturated FFA by SFE | Total Unsaturated FFA by TLE | Total Unsaturated FFA by SFE |
|--------|----------------------------|----------------------------|------------------------------|------------------------------|
| UST2 | 71.43 | 317.45 | 14.96 | 105.50 |
| UST5 | 141.78 | 223.50 | 85.92 | 86.07 |
| PL2 | 102.95 | 519.99 | 55.56 | 155.92 |
| PL4 | 37.19 | 173.11 | 5.39 | 56.96 |
| SHAMII | 24.94 | 123.92 | 10.90 | 20.85 |
| CFS2 | 0.57 | 27.13 | 0.00 | 0.58 |
| LDS2 | 5.31 | 7.66 | 1.07 | 0.62 |
| SRS4 | 1.48 | 5.97 | 0.00 | 0.15 |
| CFS3 | 4.07 | 15.64 | 1.48 | 0.41 |
| LDS4 | 0.00 | 2.85 | 0.00 | 0.22 |
| SRS3 | 4.26 | 6.32 | 0.00 | 0.29 |
| SRS2 | 0.10 | 3.77 | 0.00 | 0.17 |

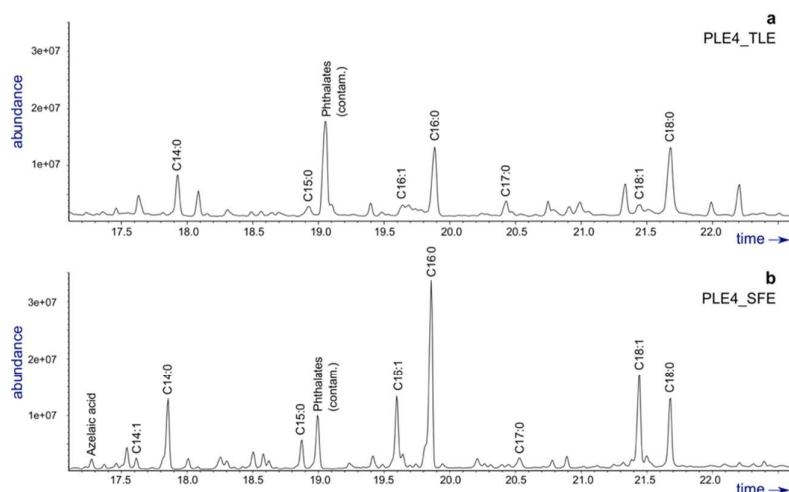


Figure 2. Comparison of the Total Ion Chromatograms showing the free fatty acids identified after a) TLE and b) SFE for the sample PL4. Same amounts of ceramic sherd were used for the two extractions.

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4 Since SFE gives much higher yields, we expect that sufficient quantities may be extracted for
5 (compound specific) radiocarbon dating, which would provide a more reliable way of dating
6 pottery²². The ability of SFE to extract lipids, but also other molecules, as demonstrated for
7 our archaeological samples, also makes this technique a good candidate for decontamination
8 of archaeological samples prior to dating. For radiocarbon dating of bones, the presence of
9 exogenous carbon can substantially affect the obtained date (leading to younger ages if ‘new’
10 carbon is incorporated) and therefore this carbon must be removed²³. Finally, for
11 rehydroxylation (RHX) dating of ceramics the presence of any carbon can significantly affect
12 the dating. Removing organics using SFE would then help with the application of the RHX
13 technique^{24,25}.
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24 **Conclusions**

25 The present work aimed at offering a more efficient alternative to conventional lipid
26 extraction, as performed in archaeology for the last 50 years. This alternative is based on the
27 use of supercritical fluids, rather than chloroform and methanol (or other similar solvents), to
28 perform the extraction.
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30 Ceramic reference samples were produced in the lab in order to optimise the parameters for
31 the supercritical fluid extraction of lipids. When applying those optimised conditions to both
32 lab-made reference samples and archaeological samples, the data generated demonstrated that
33 SFE is a more efficient extraction technique than TLE both in terms of yield and time
34 required for the extraction. The higher yield achieved by SFE can help refine the
35 interpretation of the function of ceramic containers but also raises the possibility of
36 reproducible radiocarbon dating of single compounds from archaeological pottery. As shown
37 in this study, in some cases, the increased yields also allow for some markers to be identified
38 which would remain undetected by TLE. Furthermore, the use of water and ethanol as co-
39 solvent instead of chloroform and methanol, used in TLE, makes it a less toxic and cheaper
40 high-throughput alternative.
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6 team with key samples from the Ust'-Karenga complex. South African materials were
7 excavated under permit from the South Africa Heritage Resources Agency (SAHRA).
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11 12 13 14 **Supporting Information**

15 The file submitted as Supporting Information includes:

- 16 • S-1 Preparation of the reference ceramic samples (Including Figures S-1 and S-2)
 - 17 • S-2 GC/MS Procedure
 - 18 • S-3 Response Surface Methodology (Including Figure S-3)
 - 19 • S-4 Mathematical modelling (Including Table S-1)
 - 20 • S-5 Validation of the mathematical model (Including Figure S-4)
 - 21 • S-6 List of compounds identified in the archaeological samples
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