



## The impact of mineral composition on the yield and preservation of selected fatty acids in replicate archeological ceramics

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**Abstract:** The process by which clay influences the preservation and retention of absorbed lipids in archaeological pottery is not well understood. Although properties such as porosity and calcium content are frequently suggested as primary factors in lipid retention, their exact influence remains unclear. To investigate this, replicate clay ceramics were manufactured using varying proportions of illitic/illite-smectitic clay, mixed with two different tempers (sand and chalk), and fired at 800°C in an oxidizing atmosphere. This study aimed to assess the impact of these compositional variations on the preservation and retention of selected fatty acids, palmitic acid (C<sub>16:0</sub>) and oleic acid (C<sub>18:1</sub>), often discovered in archaeological lipid residue analysis, in both heat-degraded (100°C for 14 h) and undegraded forms. Gas chromatography-mass spectrometry (GC-MS) was used for quantification, employing calibration curves generated from standard solutions of fatty acid methyl esters (FAME). Two methylation techniques were utilized: acid-catalyzed methylation (ACM) and solvent extraction using a mixture of dichloromethane (DCM) and methanol (MeOH), followed by m-(trifluoromethyl)phenyltrimethylammonium hydroxide (TMTFTH) methylation. A comparison of the two techniques revealed that prior solvent extraction using a combination of DCM-MeOH may have limited the amount of C<sub>16:0</sub> and C<sub>18:1</sub> methylated by TMTFTH, causing inconsistent results in quantification compared to ACM. From the ACM technique, results showed that sand-tempered briquettes, whether degraded (for C<sub>16:0</sub>) or non-degraded (for C<sub>16:0</sub> and C<sub>18:1</sub>), have minimal influence on fatty acid yield while degradation significantly affects the yield of C<sub>18:1</sub>. In contrast, C<sub>16:0</sub> and C<sub>18:1</sub> recovery from chalk-tempered briquettes is proportional to clay content and inversely proportional to the chalk content. This holds both for degraded and non-degraded briquettes. This work highlights the importance of considering and investigating the mineralogical composition of clay and non-clay minerals for predicting the lipid yield from ancient ceramics.

**Keywords:** Fatty acids; organic residues; lipid residue analysis; FAME quantification; derivatization methods; clay; porosity. © 2024 ACG Publications. All rights reserved.

### 1. Introduction

A diverse range of archaeological artefacts may contain traces of organic compounds derived from decomposition of food remains or from the raw materials used in the manufacture of vessels in antiquity. Studies have shown that these organic compounds are often well preserved in pottery artefacts, either through adsorption within the pores of inorganic clay matrix or entrapment as visible charred

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surface deposits [1-3]. This ceramic matrix forms a protective environment that reduces the effects of post-depositional environmental and microbial degradation, facilitating the identification of the original parent organic molecules [4]. Organic residue analysis (ORA) of compounds absorbed into archeological pottery, using various extraction techniques and analytical methods, has yielded significant evidence to answer long-standing archaeological questions in areas ranging from major economic transitions and subsistence strategies to social foodways [5-8]. Lipids have typically been the main target of ORA, and the chemical analysis of various lipid classes and their degradation products has made it possible to differentiate animal fats (both terrestrial and marine) [9-11], identify different waxes (plant and beeswax) [5,12], and detect various adhesives [13-14].

The porosity of unglazed archeological pottery is considered a primary factor in facilitating the absorption and eventual preservation of lipids within the vessel walls, often resulting from various past cooking events or prolonged food storage [15]. The impact of porosity has been quantitatively assessed using mercury intrusion porosimetry, revealing a correlation between pore volume and the amount of lipids extracted and identified through gas chromatography-mass spectrometry (GC-MS) technique [15-16]. Another factor that could influence lipid preservation and retention is the presence of calcium carbonate inclusions in archeological pottery and the formation of calcified deposits on the pottery surface [17-18]. These factors suggest that calcium-containing minerals within the pottery may have reacted with the fatty acids, forming fatty acid salt precipitates, which enhances lipid preservation [18]. Porosity and mineralogical composition are dictated by the raw materials used in the pottery, such as the type of clay and the addition of tempers, as well as the firing temperature, which influences the formation of specific post-firing minerals [2,19]. Understanding how these factors influence lipid retention in pottery necessitates the use of replicate clay ceramics in experimental cooking or food processing studies [16,20-22].

These experimental vessels are typically either commercially produced from a random mixture of clays and clay minerals [23-29] or custom-made, incorporating tempers such as shell, sand, or straw in varying proportions, while utilizing traditional manufacturing techniques [23,30]. Firing temperatures for these experimental ceramics are generally maintained below 1,000°C [18,31-33], with specific studies using temperatures like 825°C [34] or 777°C [22]. Despite these well-established experimental protocols, previous research has primarily focused on the nature and overall quantity of organic compounds recovered, often overlooking the crucial role that a more precise inorganic composition of the clays and tempers would play in the retention and preservation of organic residues.

In this study, a rigorous analysis of the impact of the clay and temper composition of pottery on the preservation and retention of two archaeologically relevant fatty acids, palmitic acid ( $C_{16:0}$ ) and oleic acid ( $C_{18:1}$ ), was conducted. The experimental setup involved manufacturing replicate clay briquettes, which mimicked archeological pottery by their composition, using varying ratios of illitic/illite-smectitic clay, mixed with different amounts of sand and chalk ( $CaCO_3$ ) tempers, and firing them at 800°C in an oxidizing environment. The aim was to estimate the effect of clay matrix composition and the changes it undergoes through the different proportions of added temper on the retention of  $C_{16:0}$  and  $C_{18:1}$  in non-degraded and degraded forms. To provide a more precise estimation of lipid yield, a fatty acid methyl esters (FAME) calibration curve for absolute quantification was applied. FAME quantification is a common technique used in oil and lipid analysis in foods or in paint materials [35] but is not commonly employed in archeological ORA. Two methylation methods were applied: acid-catalyzed methylation (ACM) and the use of solvent extraction (i.e. mixture of dichloromethane and methanol) followed by *m*-(trifluoromethyl)phenyltrimethylammonium hydroxide (TMTFTH), also known as Meth-Prep II derivatization. ACM is a conventional methylation technique frequently applied to ORA in archeological pottery [36-39], while TMTFTH derivatization, typically used for historical paint media, varnishes and textile dyes [35,40-43], has been largely unexplored in the context of ORA in pottery [44].

## 2. Experimental

### 2.1. Preparation and Firing of Briquettes

Experimentally produced pottery briquettes were used as model archeological ceramics for the current investigation. Various proportions of a dominant illite and mixed layered illite-smectite (about 50

wt. %) clay type sourced from natural Quaternary clay formations near Joosu Manor in southeastern Estonia were mixed with different ratios by weight of sieved (#35 US Standard Mesh Number; finer grain size) natural Quaternary sand (S) (about 75 wt.% quartz) from Toome Hill in Tartu, Estonia, and commercial powdered chalk (CH) (about 99% calcite) (Bang & Bonsomer Group Oy, Helsinki, Finland), representing shell inclusions. A briquette without added tempers was also tested.

Each briquette, weighing ca 30.0 g, was individually prepared by manually kneading the clay or clay and temper according to the ratios in Table 1. Milli-Q water (approximately 8 to 10 mL; Milli-Q® Advantage A10 system, Merck Millipore, Darmstadt, Germany) was added to form a paste, which was molded by hand into rectangular pieces (3.0-3.5 cm x 5.0-6.0 cm; thickness 0.8 cm to 1.0 cm). These pieces were air-dried for 12 h at room temperature, then placed in a drying oven for 24 h at 105°C (MOV-112F, Sanyo, Osaka, Japan). Thereafter, the briquettes were fired in an oxidizing environment in a muffle furnace (KL-22, Kerako, Tallinn, Estonia; electronic programmable controller, ST315A, Stafford Instruments, Stafford, United Kingdom) at 800°C. The temperature program included equilibration at room temperature for 10 min, ramping to 800°C within 1.5 h, and held isothermally at 800°C for another 2 h. The fired briquettes were then allowed to cool (Figure 1). A comprehensive X-ray diffraction (XRD) and N<sub>2</sub> gas adsorption data to evaluate the mineralogical content and porosity produced in the briquettes, respectively, was reported in [45].

**Table 1.** Percentage by mass of clay, and sand and chalk tempers used to form the pottery briquettes

Clay and Sand Temper			
Sample Code	Clay	Temper Sand (S)	
S-75	25%	75%	
S-50	50%	50%	
S-25	75%	25%	
S-0	100%	0%	

Clay, Sand and CaCO <sub>3</sub> Temper			
Sample Code	Temper		
	Clay	Sand (S)	CaCO <sub>3</sub> (CH)
CH-45	25%	30%	45%
CH-30	50%	20%	30%
CH-15	75%	10%	15%
CH-5	90%	5%	5%



**Figure 1.** The reference pottery briquettes. The briquette codes and the mixture components are shown in Table 1

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### 2.2. Spiking of Palmitic and Oleic Acid Standards

A fraction from each briquette was homogenized and powdered using a mini ball mill (Mini-mill Pulverisette 23, Fritsch GmbH, Germany) before spiking with fatty acid standard solutions. Approximately 0.25 g of the milled powder was designated for the ACM experiment, while 0.5 g was allocated for the TMTFTH derivatization experiment. A larger sample size for the TMTFTH derivatization method was chosen to ensure sufficient amount of fatty acids can be extracted for accurate quantification of yields. The powdered clay samples were placed in individual clear glass tubes that can be sealed with a PTFE-lined screw caps.

Fatty acid stock solutions of standard palmitic acid ( $C_{16:0}$ ) (certified reference material, TraceCERT<sup>®</sup>, 99.6%, Sigma-Aldrich, Switzerland) and oleic acid ( $C_{18:1}$ ) (ROTICHRON<sup>®</sup> GC,  $\geq 99\%$ , Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were prepared separately in 10 mL volumetric flasks at a concentration of approximately 1.0  $\mu\text{g}/\mu\text{L}$  in hexane (puriss p.a. ACS reagent,  $\geq 99\%$  (GC), Sigma-Aldrich, Co. St. Louis, MO, USA).  $C_{16:0}$  and  $C_{18:1}$  were selected for this study due to their frequent representation in archaeological pottery and usefulness in distinguishing between pottery used for e.g., cooking different animal- or plant-derived food substances [7]. Additionally, a previous study have suggested that the presence of unsaturated bonds in fatty acids, such as in  $C_{18:1}$ , may influence their interactions with minerals in clay [22]. Hence, a comparative analysis between the yield of  $C_{16:0}$  and  $C_{18:1}$  was conducted.

Spiking the clay briquette powders with fatty acids involved adding 25  $\mu\text{L}$  each of  $C_{16:0}$  and  $C_{18:1}$  to the samples designated for ACM, while 50  $\mu\text{L}$  each of  $C_{16:0}$  and  $C_{18:1}$  were added to the samples for TMTFTH derivatization. The specified amounts of fatty acids were first mixed in separate clear glass vials and diluted with approximately 1.0 mL of hexane. This mixture was then carefully transferred to the corresponding briquette powder to ensure uniform soaking. Hexane was allowed to evaporate completely at room temperature, leaving behind the dried fatty acids at their original concentrations absorbed on the clay briquettes.

Two equivalent sets of spiked clay briquette powders were prepared: one for non-degraded samples and another for degraded samples. For the degradation experiment, powdered clay samples were heated in a laboratory oven (ECOCELL<sup>®</sup> 22 – ECO line, MMM Group, München, Germany) for 14 h at 100°C. This temperature was selected based on previous studies indicating that pure, unmixed  $C_{16:0}$  and  $C_{18:1}$  would begin to produce minor oxidation products at 90°C, with  $C_{16:0}$  forming these products at a lower concentration [46-47]. The combination of these two fatty acids together with clay aims to show the effects of the clay matrix during prolonged heating. Furthermore, for comparison, a similar experimental setup was used, following the temperature parameters reported in [33], to study degradation under conditions that mimic potential low-heat cooking events.

### 2.3. Derivatization Methods

#### 2.3.1. Acid-Catalyzed Methylation (ACM)

The ACM was carried out according to the method in [36]. Methanol (4 mL) was added to the degraded and non-degraded powdered clays containing the adsorbed  $C_{16:0}$  and  $C_{18:1}$ . The resulting heterogenous mixture was sonicated (SONOREX, Bandelin Electronic, Berlin, Germany) for 15 min with occasional vortex (VWR International, Germany) mixing to fully disaggregate the clay lumps. Then, 800  $\mu\text{L}$  of concentrated sulfuric acid (analytical reagent, AnalaR NORMAPUR, 98%, VWR Chemicals BDH Prolabo, Leuven, Belgium) was added dropwise, and sealed with a PTFE-lined screw cap. The mixture was heated for 4 h at 70°C (Reacti-Therm<sup>™</sup> III heating module, Thermo Scientific, USA). After cooling to room temperature, the liquid layer was separated by centrifugation (Frontier<sup>™</sup> 5714, Ohaus, Germany) at 3000 rpm for 5 min and extracted three times with 2 mL of hexane with the aid of a vortex mixer. Each hexane extract was filtered through a glass pipette containing a silanized glass wool (Supelco Analytical, Sigma-Aldrich, USA) plug and potassium carbonate (ACS reagent,  $\geq 99.0\%$ , Sigma-Aldrich, USA). The combined extracts were collected and evaporated to dryness under a gentle stream of  $\text{N}_2$  gas. Prior to GC analysis, the dried extracts were reconstituted with 15  $\mu\text{L}$  of 0.4190 mg/g 10% hexadecane ( $\geq 99.0\%$ , Sigma-Aldrich, USA) in toluene (puriss p.a., ACS reagent,  $\geq 99.7\%$ , Honeywell) internal standard solution, and was diluted further with another 140  $\mu\text{L}$  of toluene. The internal standard was prepared by

taking a 10% by volume aliquot from a stock solution of hexadecane in toluene, followed by additional dilution with toluene to achieve a lower concentration.

### 2.3.2. Solvent Extraction and TMTFTH Derivatization

The C<sub>16:0</sub> and C<sub>18:1</sub> absorbed in the degraded and non-degraded powdered clays were initially extracted using a 2:1 dichloromethane (puriss p.a., ACS reagent, ≥ 99.9%, Honeywell, USA): methanol (Chromasolv™ for HPLC, ≥ 99.9%, Honeywell, USA) (DCM:MeOH, v/v) solution as described in [48]. The clay powders underwent three extraction cycles, each with 5 mL of the solvent mixture. During each cycle, the mixture was sonicated for 15 min with occasional vortex mixing, followed by centrifugation at 3000 rpm for 10 min to separate the liquid from the clay solids. The combined extracts were then concentrated to dryness under a gentle stream of N<sub>2</sub> gas.

The dried extracts were derivatized with TMTFTH (m-(trifluoromethyl) phenyltrimethylammonium hydroxide, 5% w/v in methanol, Thermo Scientific, Kandel, Germany) reagent following the optimized procedure in [35]. A 50 μL aliquot of the 5% TMTFTH solution in methanol was added to the dried extracts. The mixture was sonicated for 30 min and then kept at room temperature for 24 h to ensure complete derivatization. Following derivatization, 30 μL of 10% hexadecane in toluene (0.4022 mg/g) internal standard solution was added to the mixture, and was further diluted with 230 μL of toluene for GC-MS analysis.

### 2.4. GC-MS Instrumentation and Parameters

For the analysis, an Agilent Technologies 6890N GC system equipped with an Agilent 5973 mass spectrometric (MS) detector was used. Compound separation was achieved using a 30 m Agilent J&W HP-5ms capillary column ((5%-phenyl)-methylpolysiloxane, film thickness of 0.25 μm, capillary inner diameter of 0.25 mm). Helium (purity 6.0, 99.9999%) was used as carrier gas. Agilent GC-MSD/Enhanced software was used to operate the instrument.

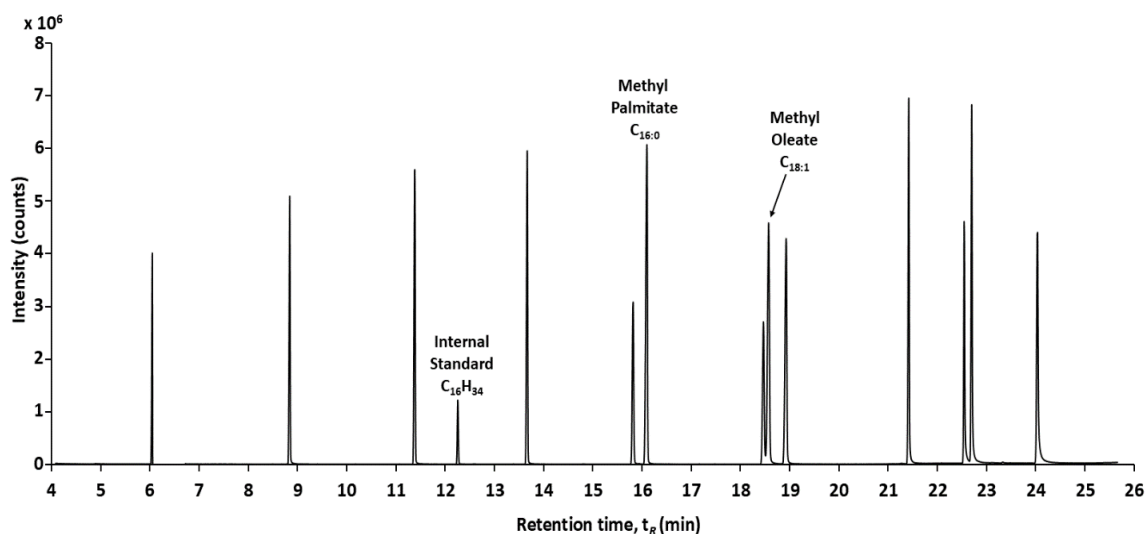
The initial oven temperature was set to 80°C and held isothermally for 2 min. It was then increased to 200°C at a rate of 10°C per min, where it was held for 1 min. This was followed by an increase to 220°C at a rate of 5°C per min, with another 1 min hold time. Subsequently, the temperature was further increased to 280°C at a rate of 30°C per min and maintained isothermally for 3 min. Finally, the program was raised to the final temperature of 300°C at a rate of 30°C per min. The total run time for this temperature program was 25.67 min.

A 1 μL aliquot of the derivatized samples and the FAME calibration standards were injected into the gas chromatography (GC). The GC inlet temperature was maintained at 300°C and operated in split mode with a 2:1 split ratio and a split flow rate of 2.3 mL/min. The MS transfer line temperature was set at 280°C and the ion source temperature at 230°C. MS data acquisition was performed in scan mode, covering the *m/z* range of 30-600, with a solvent delay of 4.00 min. The electron ionization (EI) energy was 70 eV.

### 2.5. Quantitative Analysis by GC-MS

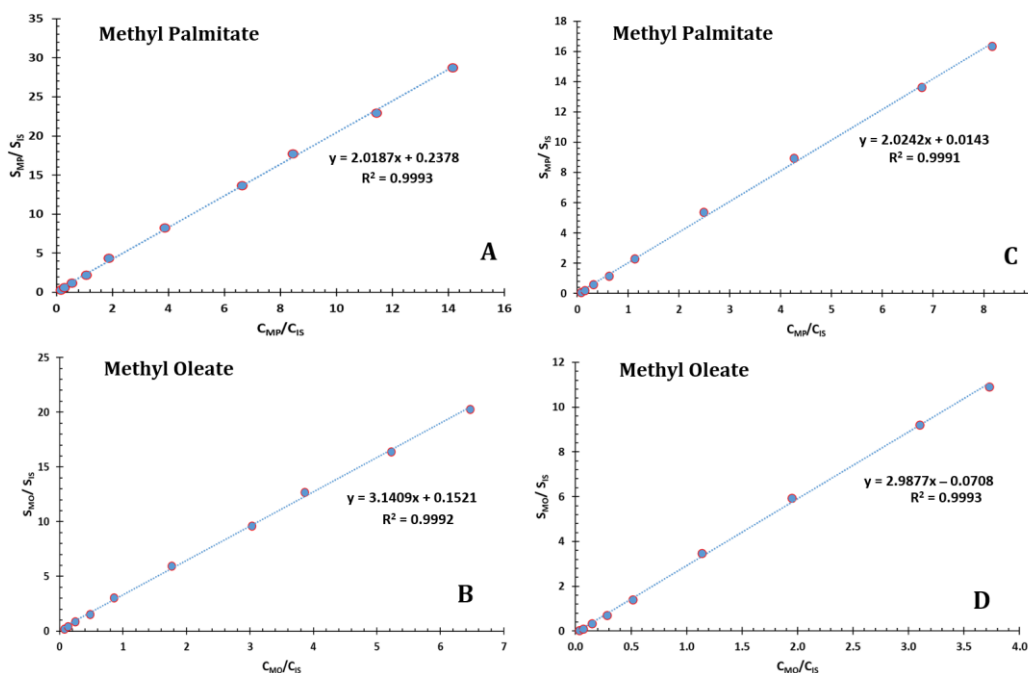
Absolute quantification of C<sub>16:0</sub> and C<sub>18:1</sub> in the clay briquettes was accomplished using standard calibration curves. These curves were constructed from a series of standard solutions prepared from a certified reference material (CRM18918, Supelco, Sigma-Aldrich, USA) containing a mixture of 14 different fatty acid methyl esters (FAME) ranging from C<sub>8</sub>-C<sub>24</sub>. For the quantification process, only the concentrations of methyl palmitate (10.9%) and methyl oleate (4.98%) were considered. The stock FAME mixture solution was initially prepared at a concentration of 6.1102 mg/g in toluene, which corresponds to 0.6660 mg/g of methyl palmitate and 0.3043 mg/g of methyl oleate. Figure 2 shows a typical GC-MS chromatogram of the FAME standard solution, where methylated C<sub>16:0</sub> and C<sub>18:1</sub> are separated from other methylated fatty acids, facilitating the identification and quantification of the peak area ratios relative to the hexadecane (C<sub>16</sub>H<sub>34</sub>) internal standard.

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**Figure 2.** Total Ion Chromatogram (TIC) of one of the FAME calibration standards where the target analytes (methyl esters of  $C_{16:0}$  and  $C_{18:1}$ ) and the internal standard (hexadecane,  $C_{16}H_{34}$ ) are denoted. The remaining peaks are methyl esters of other  $C_8$ - $C_{24}$  fatty acids

The calibration curves were designed to cover the expected amount of  $C_{16:0}$  and  $C_{18:1}$  extracted and methylated from the clay briquette powders. For the ACM method, a ten-point calibration curve was constructed to quantify the derivatized  $C_{16:0}$  and  $C_{18:1}$  using the prepared FAME stock solution (Figure 3). The calibration range for methyl palmitate was set between 0.0065 mg/g and 0.5690 mg/g while for methyl oleate, it ranged from 0.0030 mg/g to 0.2600 mg/g (Figure 3A and B). Due to the lower overall concentration of the derivatized  $C_{16:0}$  and  $C_{18:1}$  that was obtained from the TMTFTH procedure compared to ACM, a nine-point calibration curve was prepared instead, with methyl palmitate concentrations ranging from 0.003 mg/g to 0.316 mg/g and methyl oleate concentrations from 0.001 mg/g to 0.144 mg/g (Figure 3C and D). To all prepared calibration solutions, 60  $\mu$ L of 10% hexadecane in toluene (0.4190 mg/g) internal standard solution was added.



**Figure 3.** Representative calibration curves utilized for quantifying (A) methyl palmitate, and (B) methyl oleate used for the ACM samples. For the TMTFTH methylated samples, (C) and (D) correspond to methyl palmitate and methyl oleate, respectively

The peak area ratios of methyl palmitate ( $S_{MP}$ ) or methyl oleate ( $S_{MO}$ ), relative to the internal standard, hexadecane ( $S_{IS}$ ), were plotted against their concentration ratios, labelled as  $C_{MP}$  for methyl palmitate,  $C_{MO}$  for methyl oleate, and  $C_{IS}$  for the internal standard, respectively. The concentrations of  $C_{16:0}$  and  $C_{18:1}$  in the clay powders were then calculated using the linear equations derived from the FAME mixture calibration curves (Figure 3).

The chromatograms were analyzed using the Agilent Technologies MassHunter Workstation, Qualitative Analysis Version 10.0. Peak areas were evaluated using the extracted ion chromatogram (EIC) mode set to  $m/z$  value of 239.2 for methyl palmitate,  $m/z$  of 264.2 for methyl oleate, and  $m/z$  of 226.0 for hexadecane, respectively. The peaks were identified using the NIST 23 Mass Spectral Library.

## 2.6. Validation of Derivatization and Separation Methods

Methylated stock solutions of  $C_{16:0}$  and  $C_{18:1}$  were analyzed with GC-MS without being spiked on the clay briquette powders, ensuring that no other degradation products were present and that the standards are pure. Similarly, blank clay briquettes were tested and confirmed to be free of any fatty acids.

In each GC-MS batch run, an extraction solvent blank and an analyte control sample containing only the mixture of methylated stock solutions of  $C_{16:0}$  and  $C_{18:1}$  (without clay briquettes) was always included. The concentration of the methylated  $C_{16:0}$  and  $C_{18:1}$  in the control sample was used to determine the percentage yield (recovery) of the analytes in each extraction batch. The percentage yield was calculated as: (concentration of  $C_{16:0}$  or  $C_{18:1}$  / concentration of the control sample without clay)  $\times$  100. This calculation accounts for any random and systematic errors that may have occurred during spiking, extraction and preparation of the samples. The whole set of calibration solutions were also run together with the samples and were arranged randomly in the autosampler.

## 2.7. Data Analysis

Two replicates for both the degraded and non-degraded samples were performed on different days. Average yields were determined from the replicates for every  $C_{16:0}$  and  $C_{18:1}$ , every clay type and separately for degraded and non-degraded samples (Rows "S-X" and "CH-X" in Tables 2 and 3, respectively). Overall average yields were calculated for both  $C_{16:0}$  and  $C_{18:1}$  for clay types (with sand and with chalk), separately for the non-degraded and degraded briquettes by pooling together the 8 yields found for the four different compositions in duplicate (Row "Average" in both tables). Pooled standard deviations were calculated for the average recoveries (jointly for of  $C_{16:0}$  and  $C_{18:1}$ ) and the 95% confidence intervals were calculated using the Student coefficient with 7 degrees of freedom. The statistical significance of differences between yields was evaluated using Student's t-test at 95% confidence level. The statistical significance of correlations was evaluated by comparing the absolute values of the slopes with the half-widths of 95% confidence intervals of the slopes (assuming two degrees of freedom). The raw data used for the calculations can be seen in Table S1 and S2 in the Supporting Information.

# 3. Results and discussion

## 3.1. Quantification Using FAME Calibration Curves

In typical archaeological pottery ORA, quantification is usually achieved without the use of standard calibration curves and rely instead on the relative concentrations of the target analytes in comparison with the internal standard, estimated from their corresponding peak area ratios from either GC-MS or GC-FID. While this approach effectively estimates the amount of organic molecules extracted in pottery, the use of calibration curves is essential for accurately determining the yield of different components, in this case  $C_{16:0}$  and  $C_{18:1}$ , and comparing them across the various clay-temper composition based on the original concentrations spiked.

The calibration curves prepared from the same FAME stock solution in each batch run exhibited very good linearity for both  $C_{16:0}$  and  $C_{18:1}$  with coefficients of determination ( $R^2$ ) at least 0.998 (see Figure 3). However,  $C_{16:0}$  exhibited slight susceptibility to concentration-related changes, particularly at higher concentrations (Figure 3A), as indicated by the higher intercept ( $b = 0.238$ ) compared to the lower

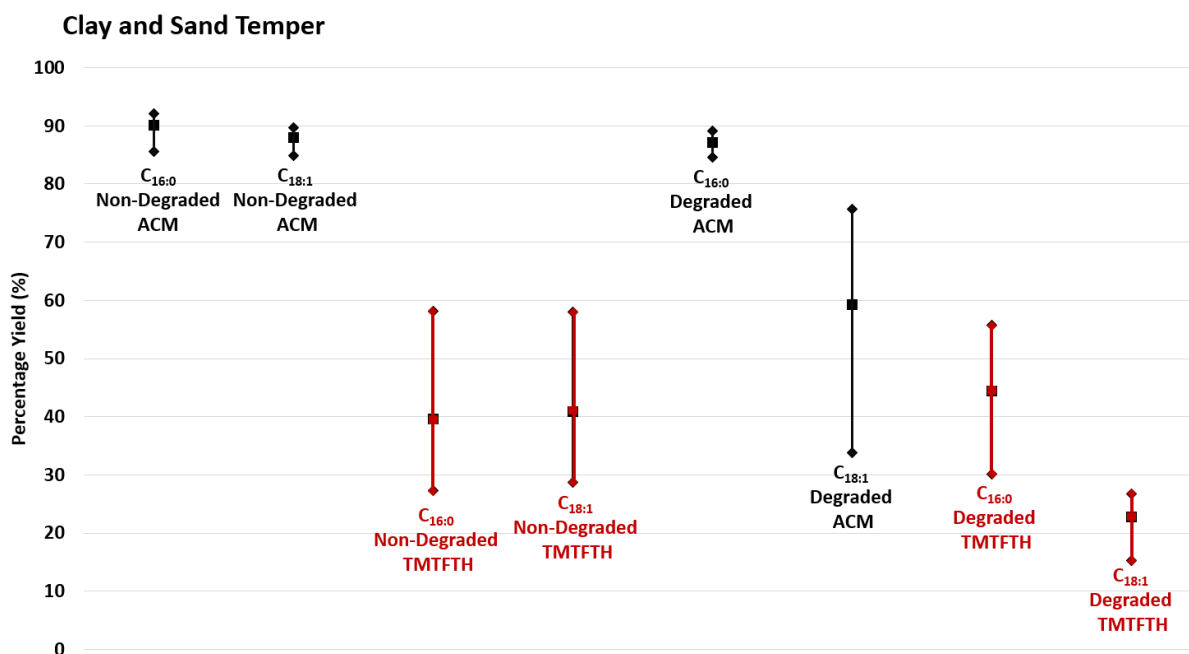
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concentration calibration curves (Figure 3C and D). Nonetheless, the values obtained indicate minimal overall variance, suggesting that the GC-MS response for  $C_{16:0}$  and  $C_{18:1}$  fits well with the estimated model in the regression analysis. This demonstrated that the produced FAME calibration curves were robust and replicable.

#### 3.2. Comparison of Acid-Catalyzed Methylation (ACM) and TMTFTH Methylation

Significant differences in the recovery of spiked  $C_{16:0}$  and  $C_{18:1}$  were observed between the ACM and TMTFTH methylation methods. The ACM method consistently demonstrated substantially higher extraction yield for both fatty acids compared to the TMTFTH methylation from the DCM-MeOH extracts, resulting in nearly two-fold higher concentrations in the sand-tempered briquettes (Figure 4). For instance, the average yield of non-degraded  $C_{16:0}$  and  $C_{18:1}$  in ACM is 90% and 88% (Table 2), while TMTFTH yielded 40% and 41% (Table S3 in the Supplementary Information), respectively under the same conditions. Similarly, for the chalk-tempered briquettes, the difference in yield is more pronounced with a more than six-fold higher concentration yield observed in ACM compared to the TMTFTH method for both fatty acids (Figure 5). As an example, the average yield of degraded  $C_{16:0}$  and  $C_{18:1}$  in ACM is 76% and 71% (Table 3), while 12% and 9% was obtained from the TMTFTH (Table S4 in the Supplementary Information), respectively. Thus, the overall recovery of  $C_{16:0}$  and  $C_{18:1}$  indicated that ACM method was able to methylate more  $C_{16:0}$  and  $C_{18:1}$ , irrespective of the amount of clay, the type of added temper, or the degradation state (non-degraded or degraded) of the fatty acids in the briquettes.

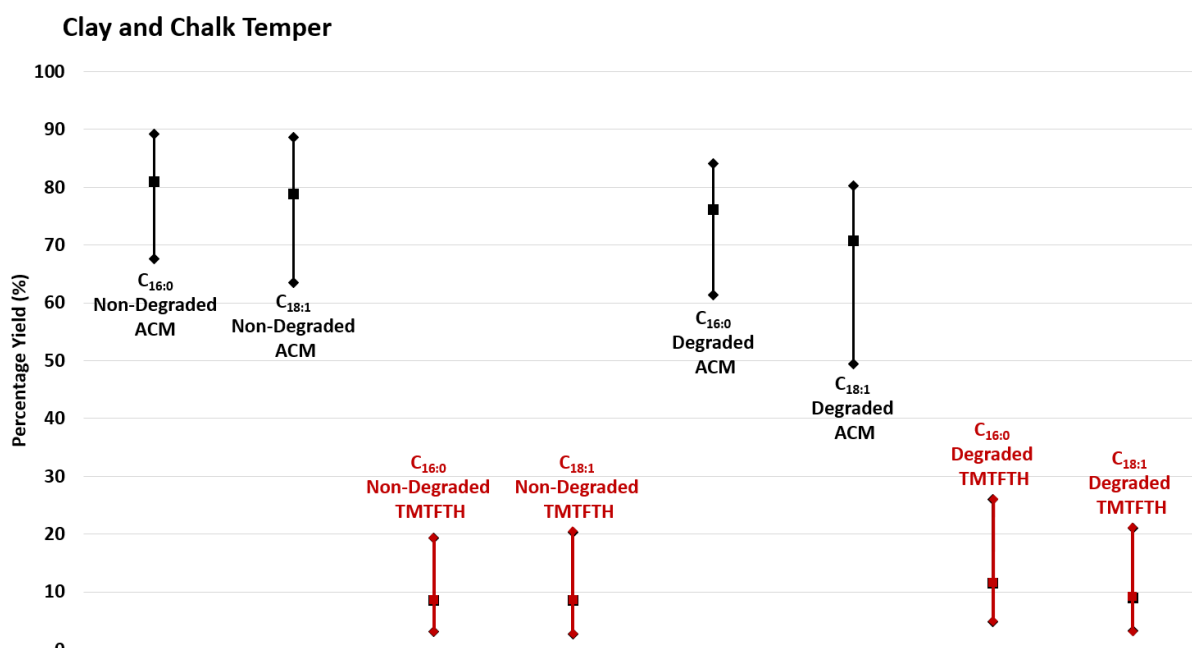
Despite being fired at 800°C, the clay minerals in the briquettes, which belong to the illite/illite-smectite group, still retained some of the physical properties of the original raw clays, particularly their charged surfaces. X-ray diffraction (XRD) analysis from a previous study on the briquettes indicated that the 2:1 layered clays have not fully transformed into an amorphous phase and have not undergone complete dehydroxylation [45]. Consequently, the charged surface of the briquettes can form strong electrostatic attractions with the polar functional groups of  $C_{16:0}$  and  $C_{18:1}$ . This suggests that the acid-catalyzed reaction is more effective in liberating the fatty acids from the inorganic clay sample.



**Figure 4.** Average percentage yields of  $C_{16:0}$  and  $C_{18:1}$  in ACM and TMTFTH for the non-degraded and degraded clay and sand-tempered briquettes. The box represents the average percentage yield, and the diamonds are the minimum and maximum values



In contrast, solvent extraction using a DCM:MeOH (2:1 v/v) mixture followed by TMTFTH methylation resulted in limited recovery of methylated fatty acids, particularly in chalk-tempered briquettes, with average yields of < 12% for both degraded and non-degraded briquettes (see Table S4 in the Supplementary Information). This finding suggests that, in addition to the interactions with the charged clay surfaces, chalk ( $\text{CaCO}_3$ ) may have reacted with the fatty acids, forming the respective calcium salts, which are not well extracted with the solvents used in the current work, and thereby, further limiting the amount of fatty acids methylated by TMTFTH. These results are consistent with previous studies showing that the combined extraction-derivatization approach of ACM results in much higher lipid yields in comparison with solvent extraction followed by separate derivatization [36-37,39]. The discussion henceforth will focus on the results obtained using ACM, while the data obtained from TMTFTH are provided in Table S2, S3 and S4 in the Supplementary Information.



**Figure 5.** Average percentage yields of C<sub>16:0</sub> and C<sub>18:1</sub> in ACM and TMTFTH for the non-degraded and degraded clay and sand/chalk-tempered briquettes. The box represents the average percentage yield, and the diamonds are the minimum and maximum values

### 3.3. Comparison of Average Extraction Recoveries of C<sub>16:0</sub> and C<sub>18:1</sub> from clays with Different Tempers

Besides the carbon chain length, the main difference between the two fatty acids used in this study is the presence of a carbon-carbon double bond in C<sub>18:1</sub>. Both fatty acids interact similarly with the surface of the pores of the clay and non-clay minerals in the briquettes through their carboxyl groups and differ from the minor electrostatic interaction of the unsaturated bond in C<sub>18:1</sub>. Generally, ACM yields for C<sub>16:0</sub> and C<sub>18:1</sub> from non-degraded briquettes are higher in sand-tempered clays compared to the chalk-tempered clays (see Tables 2 and 3). This is manifested by the statistically significant differences between the average recoveries 90% (4%) and 81% (3%) for C<sub>16:0</sub> and 88% (4%) and 79% (3%) for C<sub>18:1</sub>, respectively (here and in similar comparisons below, half-widths 95% confidence intervals are in parentheses).

These results indicate that composition differences between the clay matrices significantly influence the binding of fatty acids to the clay surface. The chalk (or CaO formed at 800°C) present as temper reacts with fatty acids forming Ca<sup>2+</sup> salts. Although the strongly acidic ACM mixture can liberate fatty acids from these salts and enable their methylation, this process is not 100% efficient. Stoichiometric calculations also confirm that in all chalk-tempered briquettes, the amount of H<sub>2</sub>SO<sub>4</sub> exceeded more than 10 equivalents, ensuring that the acid added (800 μL) was present in sufficient excess to fully react with the chalk. While the recoveries for C<sub>16:0</sub> are marginally higher than those for C<sub>18:1</sub>, this difference is not statistically significant.

### Impact of mineral composition on the yield and preservation of selected fatty acids

For degraded samples, a similar situation was observed for C<sub>16:0</sub>, with statistically different recoveries of 87% (5%) in sand-tempered clays and 76% (3%) in chalk-tempered clays. However, for C<sub>18:1</sub> in degraded samples, the trend reverses, with recoveries of 61% (5%) in sand-tempered clays and 71% (3%) in chalk-tempered clays. It is possible that oleic acid is more stable against degradation in the form of Ca<sup>2+</sup> salt, compared to the free fatty acid form. Thus, chalk may hinder the extraction of C<sub>18:1</sub>, but at the same time appears to enhance its preservation. In degraded sand-tempered clays, the average recovery of C<sub>16:0</sub> is statistically significantly higher than that of C<sub>18:1</sub> (87% vs 61%). In degraded chalk-tempered clays, the average recovery of C<sub>16:0</sub> is also nominally higher than that of C<sub>18:1</sub> (76% vs 71%) but this difference is not statistically significant (See Table 2 and 3).

**Table 2.** Average percentage yield of C<sub>16:0</sub> and C<sub>18:1</sub> in clay and sand-tempered briquettes for non-degraded and degraded samples using ACM

Clay with sand (S) temper in %	Non-Degraded		Degraded	
	C <sub>16:0</sub>	C <sub>18:1</sub>	C <sub>16:0</sub>	C <sub>18:1</sub>
S-75 <sup>a</sup>	91.6 (3.0)	89.3 (2.7)	88.8 (2.5)	75.6 (0.5)
S-50 <sup>a</sup>	92.0 (3.7)	89.7 (7.4)	89.1 (5.1)	68.1 (0.2)
S-25 <sup>a</sup>	85.6 (4.5)	84.8 (0.6)	85.8 (1.6)	59.8 (11.0)
S-0 <sup>a</sup>	91.2 (4.5)	88.3 (6.6)	84.6 (10.2)	33.8 (7.8)
Average <sup>b</sup>	90 (4)	88 (4)	87 (5)	61 (5)
<i>m</i> <sup>c</sup>	-0.030	-0.032	-0.064	-0.436
95% CI of <i>m</i> <sup>c</sup>	0.269	0.186	0.079	0.294
Pooled standard deviation	4.6		6.3	

<sup>a</sup> Values in parentheses represent the standard deviations of two replicate measurements.

<sup>b</sup> Half-width of the 95% confidence interval in parentheses.

<sup>c</sup> Slope (*m*) of the regression line between the average yield of C<sub>16:0</sub> or C<sub>18:1</sub> relative to the percentage of clay in the briquettes and its half-width of 95% confidence interval.

**Table 3.** Average percentage yield of C<sub>16:0</sub> and C<sub>18:1</sub> in clay and chalk-tempered briquettes for non-degraded and degraded samples using ACM

Clay with chalk (CH) temper in %	Non-Degraded		Degraded	
	C <sub>16:0</sub>	C <sub>18:1</sub>	C <sub>16:0</sub>	C <sub>18:1</sub>
CH-45 <sup>a</sup>	67.7 (4.8)	63.5 (3.6)	61.4 (5.0)	49.5 (2.4)
CH-30 <sup>a</sup>	80.6 (5.4)	78.1 (5.6)	76.7 (1.4)	75.4 (1.6)
CH-15 <sup>a</sup>	86.3 (3.8)	85.1 (4.1)	82.6 (4.1)	80.3 (3.9)
CH-5 <sup>a</sup>	89.2 (1.5)	88.6 (3.1)	84.1 (0.7)	77.6 (6.3)
Average <sup>b</sup>	81 (3)	79 (3)	76 (3)	71 (3)
<i>m</i> <sup>c</sup>	0.326	0.380	0.345	0.427
95% CI of <i>m</i> <sup>c</sup>	0.233	0.249	0.341	0.789
Pooled standard deviation	4.2		3.7	

<sup>a</sup> Values in parentheses represent the standard deviations of two replicate measurements.

<sup>b</sup> Half-width of the 95% confidence interval in parentheses.

<sup>c</sup> Slope (*m*) of the regression line between the average yield of C<sub>16:0</sub> or C<sub>18:1</sub> relative to the percentage of clay in the briquettes and its half-width of 95% confidence interval.

#### 3.4. Comparison of Extraction Recoveries of C<sub>16:0</sub> and C<sub>18:1</sub> Between Degraded and Non-Degraded Briquettes

The degradation of C<sub>16:0</sub> and C<sub>18:1</sub> is influenced by temperature (100°C), heating time (14 h), mineralogical composition of the briquette, and saturation of the fatty acid (i.e., the presence or absence of double bond). Under the thermal conditions employed in this study, C<sub>16:0</sub> experiences minimal degradation. The differences in average recoveries between degraded and non-degraded briquettes (90% and 87% for sand temper, 81% and 76% for chalk temper) are not statistically significant in the case of C<sub>16:0</sub> (see Tables 2 and 3).

In contrast, the average recoveries of C<sub>18:1</sub> from degraded briquettes (61% and 71% for sand and chalk temper, respectively) are statistically significantly lower than those from the non-degraded briquettes (88% and 79% for sand and chalk temper, respectively), see Tables 2 and 3. Importantly, in line with the findings above, chalk temper appears to suppress the degradation of C<sub>18:1</sub>.

### 3.5. Trends Between Extraction Recovery and the Amount of Temper

The presence or absence of a statistically significant trend between the amount of temper and extraction recovery was determined by evaluating whether the slope ( $m$ ) of the regression line between the average yield of C<sub>16:0</sub> or C<sub>18:1</sub> and the percentage of temper in the briquettes is statistically significant from zero, i.e., whether the absolute value of  $m$  is larger than the half-width of its 95% confidence interval (CI).

#### 3.5.1. Sand-Tempered Briquettes

For the non-degraded sand-tempered briquettes, the regression analysis revealed that  $m$  values of -0.030 for C<sub>16:0</sub> and -0.032 for C<sub>18:1</sub> are not statistically significantly different from zero, relative to the half-width of its 95% CI of 0.269 for C<sub>16:0</sub> and 0.186 for C<sub>18:1</sub>, respectively (Table 2). Additionally, the overlapping standard deviations of the average fatty acid yields across different briquettes indicate no substantial differences in recovery when the fatty acids are non-degraded. Mineralogically, a previous X-ray diffraction (XRD) analysis of the sand-tempered clay briquettes used in this study reported that 2:1 clay mineral content (illite/illite-smectite) ranges from 56.4 wt.% in S-0 to 13.8 wt.% in S-75, while quartz, the dominant non-clay mineral, increases from 32.6 wt.% in S-0 to 71.4 wt.% in S-75 [45]. Despite this substantial variation in mineral composition, the average amount of recovered non-degraded C<sub>16:0</sub> and C<sub>18:1</sub> in S-0 (i.e. 91.2 % in C<sub>16:0</sub> and 88.3 % in C<sub>18:1</sub>) and S-75 (i.e. 91.6% in C<sub>16:0</sub> and 89.3% C<sub>18:1</sub>) remained mostly the same (see Table 2). At the firing temperature of 800°C used to manufacture the clay briquettes, the 2:1 clay minerals had not fully converted to their high-temperature mineral phases, thereby retaining some of their original physical characteristics, particularly their porosity. This inherent porosity possibly played a role in the retention of the spiked C<sub>16:0</sub> and C<sub>18:1</sub> within the ceramic matrix, allowing for effective absorption when not degraded by heat. Consequently, these results suggest that no clear trend can be attributed to variations in clay or sand content, and it cannot be concluded that altering these factors significantly influences the preservation of C<sub>16:0</sub> and C<sub>18:1</sub>.

Constant heating at 100°C for 14 hours did not result in any noticeable degradation products of C<sub>16:0</sub> in the sand-tempered briquettes. Previous studies have reported significant degradation of C<sub>16:0</sub> at temperatures starting around 140°C, leading to the formation of short-chain fatty acids [47]. However, in this study, the temperature was maintained at 100°C to avoid the excessive (possibly complete) degradation of C<sub>18:1</sub>, which is more prone to thermal transformations at temperatures higher than 100°C to volatile and short-chain compounds [49]. This temperature setting allowed for more accurate quantification of the recovered C<sub>18:1</sub> from the briquettes.

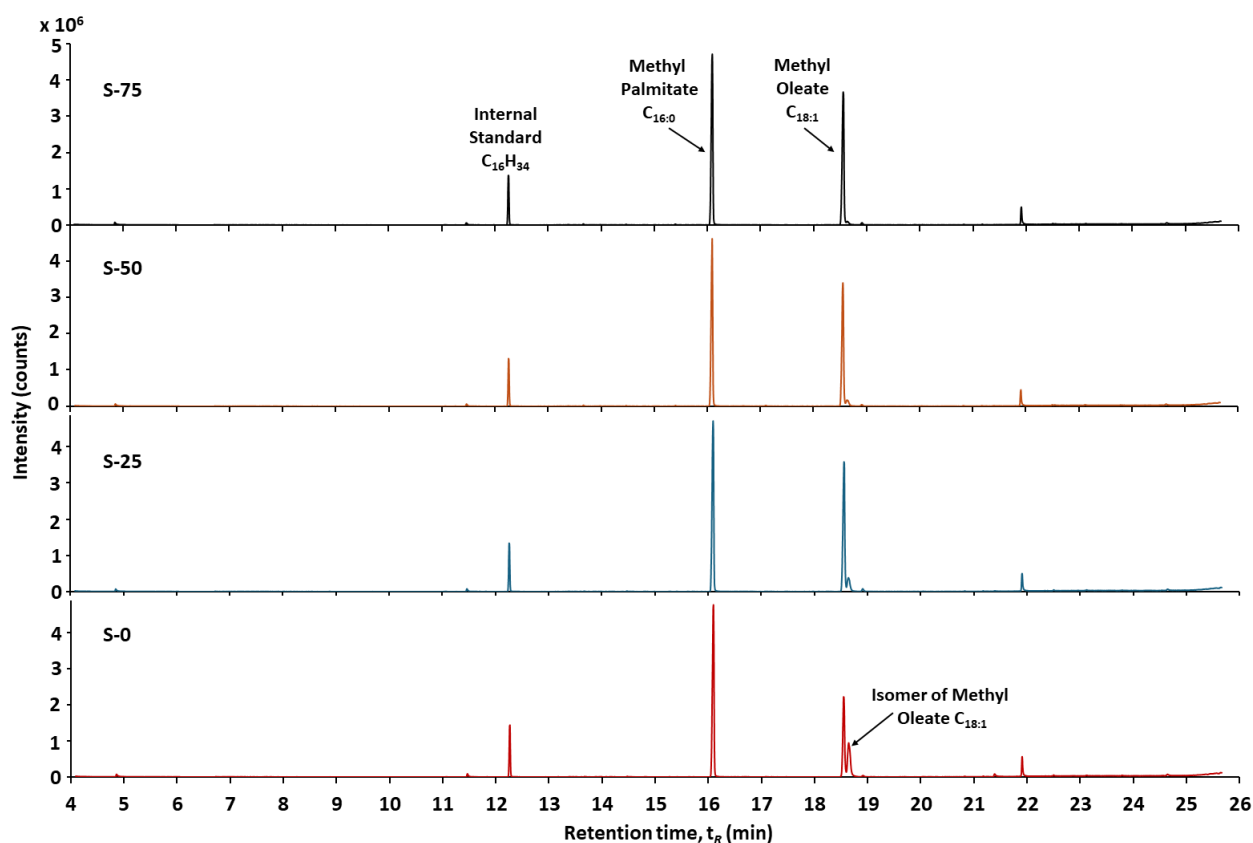
C<sub>16:0</sub> and C<sub>18:1</sub> behaved differently in the degraded sand-tempered briquettes. The regression analysis indicates that  $m$  value of -0.064 for the recovered C<sub>16:0</sub> after degradation and the amount of clay is not statistically significant to zero with half-width of its 95% CI of 0.079 (Table 2). This result suggests that the different amount of clay and sand-temper in the briquettes has no significant effect on the recovery of C<sub>16:0</sub>, and that no clear trend can be attributed to these changing factors. Hence, this may explain how C<sub>16:0</sub> is considered as one of the most well-preserved and ubiquitous saturated fatty acids found in archaeological pottery (regardless of its composition), and commonly identified in both vegetable and animal products [50].

In contrast, the average recovery of degraded C<sub>18:1</sub> from the sand-tempered briquettes exhibited a statistically significant correlation with the amount of clay used to make the briquettes, with an  $m$  value of -0.436 compared to half-width of its 95% CI at 0.294 (Table 2). The data shows significant differences in the degradation of C<sub>18:1</sub> across briquettes with varying clay content. Specifically, the briquette composed of 100% clay (S-0) exhibited the lowest C<sub>18:1</sub> recovery, retaining only 33.8%, compared to more than double that amount at 75.6%, in the briquette containing only 25% of clay (S-75) (Table 2). This trend clearly demonstrates a decrease in C<sub>18:1</sub> yield as clay content increases, and conversely, an increase in C<sub>18:1</sub> yield as the proportion of sand increases.

### Impact of mineral composition on the yield and preservation of selected fatty acids

The substantial reduction in  $C_{18:1}$  or *cis*-9-octadecenoic acid yield in the S-0 briquette is at least in part attributed to the formation of its *trans* isomer, elaidic acid or *trans*-9-octadecenoic acid, identified using the NIST Mass Spectral Library, during the degradation process (Figure 6). A previous study has reported that the conversion of *cis*- $C_{18:1}$  to its *trans* isomer occurs in the presence of oxygen and can begin at temperatures between 90°C and 120°C, with the rate of isomerization increasing with temperature [51]. Isomerization of these types proceeds via a free radical mechanism, which can be catalyzed and initiated by metals [52]. The S-0 briquette has the highest clay mineral content (56.4% of 2:1 clay mineral group) within the briquette series and has 43.5% total of non-clay minerals (i.e. quartz, K-feldspars, plagioclase and hematite) as identified by XRD analysis in [45]. This higher amount of clay would also contain more alkali-metal oxides (fluxing agents), typically associated with illitic clay types [53], suggesting that these would have likely supplied the necessary ions to catalyze the isomerization process observed.

As the amount of clay decreases in the sand-tempered briquettes, such as in the case of the least clay briquette, S-75, with 13.8 wt.% 2:1 clay mineral based on XRD analysis in [45], it appeared to correlate with a reduced level or absence of isomerization at 100°C. These results indicate that the amount of clay minerals is crucial in promoting the formation of certain degradation products that can serve as biomarkers identified from archeological pottery, such as the isomers of  $\omega$ -(*o*-alkylphenyl) alkanolic acids (APAAs) resulting from the degradation of tri-unsaturated fatty acids [25,54]. Traces of stearic acid ( $C_{18:0}$ ) were also observed from the briquettes which were not quantified in this study (See Figure S1 in the Supplementary information).



**Figure 6.** Representative chromatograms of fatty acid methyl esters from the degraded sand-tempered briquettes (S-0 to S-75) derivatized by ACM

#### 3.5.2. Chalk-Tempered Triquettes

A statistically significant correlation was observed between the average yield of  $C_{16:0}$  and  $C_{18:1}$ , with the amount of clay in non-degraded chalk-tempered briquettes, with  $m$  values of 0.326 for  $C_{16:0}$  and 0.380 for  $C_{18:1}$ , relative to the half-widths of their 95% CI of 0.233 for  $C_{16:0}$  and 0.249 for  $C_{18:1}$ , respectively (Table 3). The positive slope of the regression line indicated a direct proportionality between the amount

of clay in briquettes and the recovery of fatty acids. Briquettes with high chalk content, particularly those containing 30% or more chalk (CH-30 and CH-45), tend to strongly bind C<sub>16:0</sub> and C<sub>18:1</sub> more, even if not having undergone thermal degradation.

This observation can be rationalized by examining the mineralogical transformations occurring in the briquettes. XRD analysis from a previous study [45] revealed that the chalk in the briquettes, originally composed of 98.8 wt. % calcite (CaCO<sub>3</sub>), has mostly decomposed at 800°C, leaving ≤ 1.0 wt. % of CaCO<sub>3</sub> in each briquette. The decomposition products, along with their reaction to other minerals present in the clay and sand, led to the formation of calcium-silicate minerals and other calcium-containing minerals such as portlandite (Ca(OH)<sub>2</sub>), and lime (CaO) in varying weight ratios [45]. Specifically, briquettes with a chalk content of 30% or more (CH-30 and CH-45) contain approximately 10 wt. % to 23 wt. % of Ca(OH)<sub>2</sub> and CaO, compared to only 1.5 wt. % to 3.5 wt. % in CH-5 and CH-15, respectively. The high amount of available calcium ions and the basic nature of Ca(OH)<sub>2</sub> and CaO is capable of neutralizing the acidic C<sub>16:0</sub> and C<sub>18:1</sub>, leading to the formation of calcium salts of the fatty acids. These salts can deposit on the surface of the powdered clays even in the absence of heat degradation at 100°C [18,22]. Furthermore, it has been reported that the carboxylate and carbonate groups have similarities in their structure and that the fatty acid long chains may have contributed to an additional stabilizing effect between the interaction of the fatty acids and CaCO<sub>3</sub> [22]. Hence, the yield of the two fatty acids in a particular clay and chalk temper briquette is similar, and the addition of more chalk enhances the binding of C<sub>16:0</sub> and C<sub>18:1</sub>.

The same trend was also observed for C<sub>16:0</sub>, in the degraded briquettes where the average recovery is positively correlated with the amount of clay in the chalk-tempered briquettes with *m* value of 0.345 and a half-width of its 95% CI at 0.341 (Table 3). This positive correlation is statistically significant (but still weak), indicating that the amount of clay and the corresponding ratio of chalk added, affects the preservation of C<sub>16:0</sub> during degradation at 100°C for 14 h. No additional degradation products of C<sub>16:0</sub> were identified in the GC-MS chromatograms.

As in the sand-tempered briquettes, also in the chalk-tempered briquettes, the degradation of C<sub>18:1</sub> is more pronounced than the degradation of C<sub>16:0</sub>, particularly when comparing the yield for the CH-5 and CH-45 briquettes. The briquette with 5% chalk (CH-5) resulted in an average C<sub>18:1</sub> yield of 77.6%, whereas the briquette with 45% chalk (CH-45) produced a lower average yield of 49.5%. In contrast, the yield of C<sub>16:0</sub> is 84.1% in the 5% chalk (CH-5) and 61.4% in the 45% chalk (CH-45), respectively. This observation implies that the fatty acids bind more strongly to the briquettes that contain more chalk. Similarly, archaeological marl clay potsherds tempered with ground limestone or calcite have been reported to have the highest quantity of preserved total lipids as compared to potsherds composed of granitic or glauconitic earth [15]. Although the extraction methods and the target organic compounds are different from this current study, the presence of CaCO<sub>3</sub> seems to be a crucial factor that can influence the yield of fatty acids in clay pottery.

Results show that the relationship between the amount of clay and the recovery of degraded C<sub>18:1</sub> is not statistically significant with *m* value of 0.427 and a half-width of its 95% CI at 0.789 as seen in Table 3. Specifically, when the average yield between CH-5 and CH-15 briquettes is compared, i.e., 77.6% for CH-5 and 80.3% for CH-15. Despite the inconsistency, their standard deviations overlap, and this can be attributed to insufficient fatty acid neutralization, given that CH-5 showed no detectable Ca(OH)<sub>2</sub> in the XRD analysis [45].

### 3.6. Effect of Micro- and Mesoporosity in the Briquettes

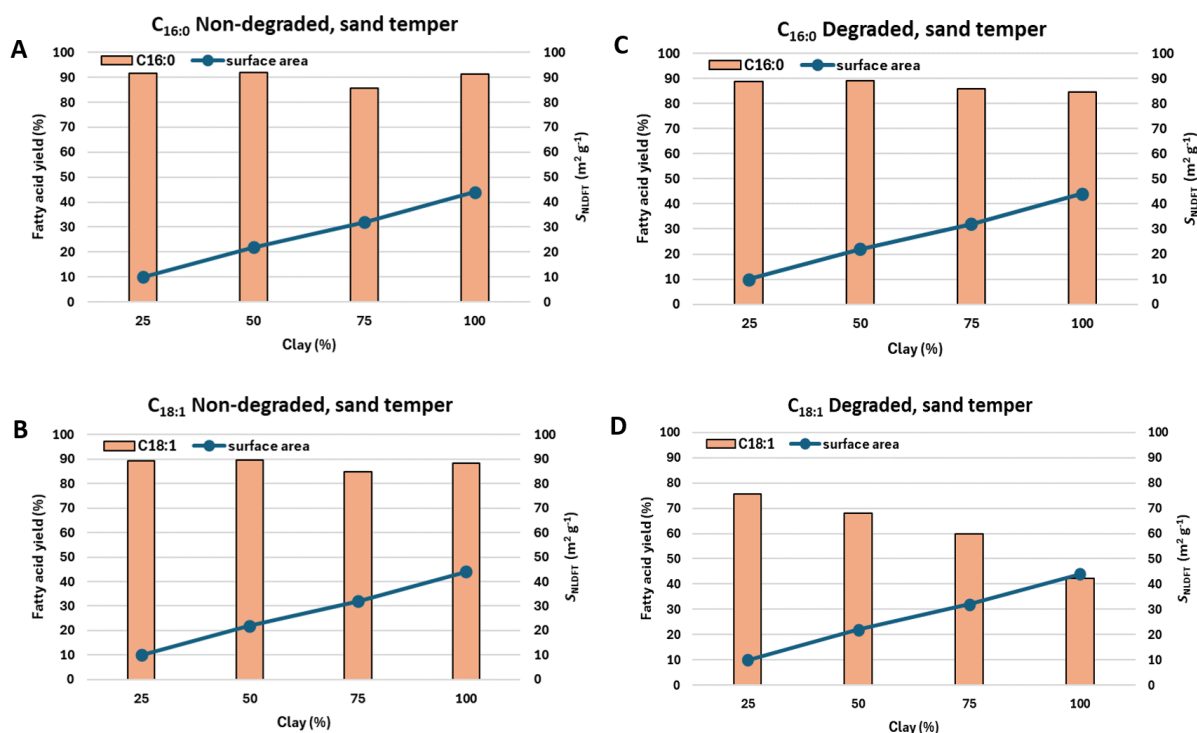
The formation of pores of varying sizes during the manufacturing process of archaeological pottery, and the subsequent adsorption of food residues in the pores of the ceramic matrix, has been associated with the preservation of organic compounds during diagenesis. Mineralogical transformations of the illitic clay and added tempers at the firing temperature of 800°C, as used in this study, significantly influence the development of porosity in the briquettes. This effect is particularly evident when the CaCO<sub>3</sub> from the chalk temper begins to decompose at this temperature.

A previous study investigating the pore size distribution of the fired sand- and chalk-tempered briquettes used in this study reported a substantial volume of micropores (width < 2 nm) and mesopores (width from 2 nm to 50 nm) [45]. The results report that the micro- and mesoporosity formed upon firing

### Impact of mineral composition on the yield and preservation of selected fatty acids

at 800°C are proportional to the clay content in the briquettes. One porosity parameter reported in the study is the specific surface area ( $S_{\text{NLDFT}}$ ), which quantifies the exposed surface area of the clay-temper matrix and was computed from the  $\text{N}_2$  adsorption isotherms using the non-local density functional theory (NLDFT) model for pillared clays. Pillared clays are clays that have stable pillars in-between their interlayer region which enables it to retain their porosity during the hydration and dehydration processes [55]. The  $S_{\text{NLDFT}}$  values for the sand- and chalk-tempered briquettes were graphed together with the average yields of  $\text{C}_{16:0}$  and  $\text{C}_{18:1}$ , and the clay content to show possible relationships between these different parameters (Figure 7 and 8).

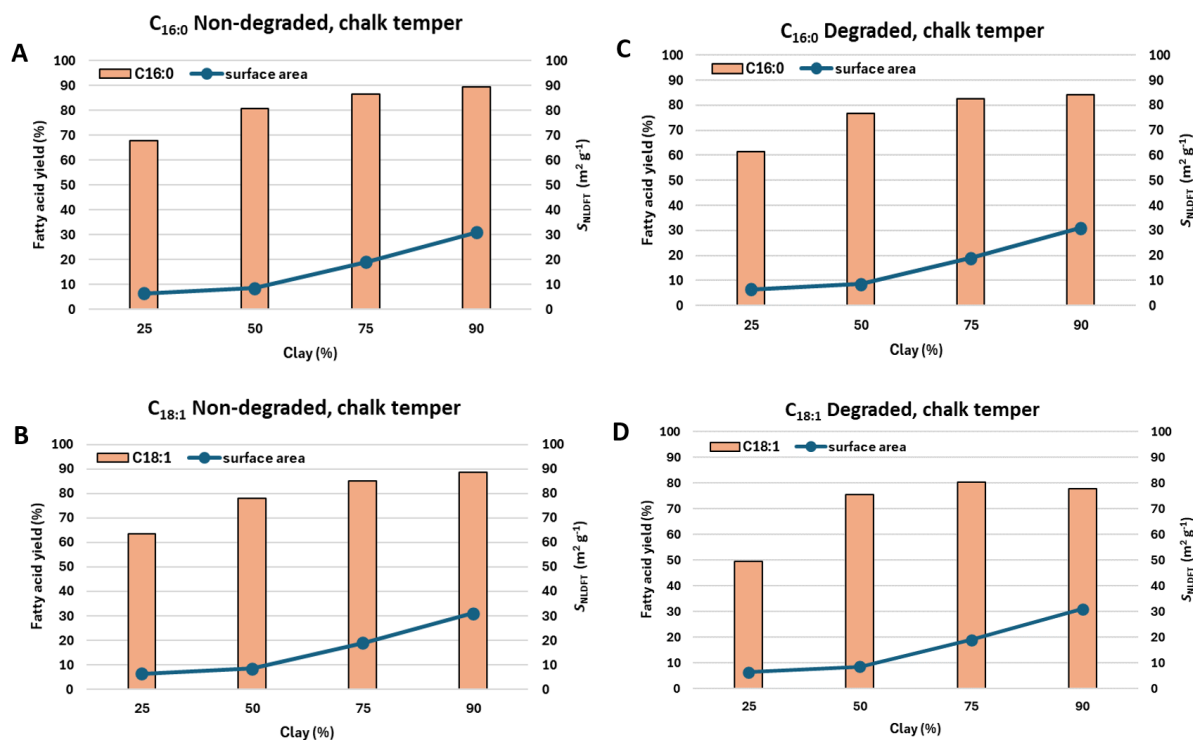
The  $S_{\text{NLDFT}}$  of the sand- and chalk tempered briquettes reported in [45], ranged from 10  $\text{m}^2/\text{g}$  for S-75 to 44  $\text{m}^2/\text{g}$  for S-0, and from 6.4  $\text{m}^2/\text{g}$  for CH-45 to 31  $\text{m}^2/\text{g}$  for CH-5, respectively. The difference in porosity is largely due to the formation of non-porous calcium-silicate minerals and  $\text{Ca}(\text{OH})_2$  in the chalk-tempered briquettes. In both non-degraded (for both fatty acids) and degraded (for  $\text{C}_{16:0}$ ) sand-tempered briquettes, no distinct trend was observed between the recovered fatty acids and the  $S_{\text{NLDFT}}$  (Figure 7 A, B, and C). The average yield of each fatty acid is roughly the same, ranging between 84% to 92%, despite a decrease in  $S_{\text{NLDFT}}$  from 100% clay (S-0) to 25% clay (S-75). In the case of degraded  $\text{C}_{18:1}$ , an inverse correlation appears to exist, wherein a lower recovery of  $\text{C}_{18:1}$  was observed at higher  $S_{\text{NLDFT}}$  (Figure 7D). However, this correlation is not conclusive enough to confirm that micro- and mesopores are solely responsible for the retention of  $\text{C}_{18:1}$  in the degraded sand-tempered briquettes.



**Figure 7.** Correlations between the specific surface area ( $S_{\text{NLDFT}}$ ), the yield of  $\text{C}_{16:0}$  and  $\text{C}_{18:1}$  recovered, and the clay content in the sand-tempered briquettes. The non-degraded briquettes spiked with  $\text{C}_{16:0}$  (A) and  $\text{C}_{18:1}$  (B), while the degraded briquettes are (C) for  $\text{C}_{16:0}$  and (D) for  $\text{C}_{18:1}$ .

In contrast, for the chalk-tempered briquettes, the amount of recovered  $\text{C}_{16:0}$  and  $\text{C}_{18:1}$  appeared to be directly proportional to  $S_{\text{NLDFT}}$ , regardless of whether the briquettes were degraded or non-degraded (Figure 8 A, B, C and D). Higher  $S_{\text{NLDFT}}$  for micro- and mesopores seem to suggest an increased recovery of  $\text{C}_{16:0}$  and  $\text{C}_{18:1}$ . Although previous studies have shown that pores with diameters of  $< 1 \mu\text{m}$  ( $< 1000 \text{nm}$ ) are particularly effective in preserving total lipid content ( $< 20 \mu\text{g}/\text{g}$ ) in archaeological potsherds compared to samples with fewer of such pores [15], the effect of macro-sized pores (width  $> 50 \text{nm}$ ) was not considered in the present study. Furthermore, the direct correlation between micro- and mesopores and the lipid content has not been thoroughly explored. Hence, it is possible and in fact probable that the recovery of  $\text{C}_{16:0}$  and  $\text{C}_{18:1}$  was strongly influenced by the presence of chalk, as well as calcium oxide and

calcium hydroxide (portlandite) derived from chalk on firing at 800°C, as these minerals can react with the fatty acids to form calcium salts that are more difficult to extract, rather than being primarily caused by porosity. In general, this suggests that the  $S_{\text{NLDFT}}$  values of the micro- and mesopores in the briquettes may have been sufficient to adsorb and preserve the spiked  $C_{16:0}$  and  $C_{18:1}$ , and that macro-sized pores (width > 50 nm) may have also contributed to the fatty acid adsorption.



**Figure 8.** Correlations between the specific surface area ( $S_{\text{NLDFT}}$ ), the yield of  $C_{16:0}$  and  $C_{18:1}$  recovered, and the clay content in the chalk-tempered briquettes. The non-degraded briquettes spiked with  $C_{16:0}$  (A) and  $C_{18:1}$  (B), while the degraded briquettes are (C) for  $C_{16:0}$  and (D) for  $C_{18:1}$ .

#### 4. Conclusions

This study has demonstrated that different proportions of clay and added temper (i.e. sand and chalk), fired at 800°C affect the preservation and retention of palmitic acid ( $C_{16:0}$ ) and oleic acid ( $C_{18:1}$ ), with or without heat degradation at 100°C for 14 h. Unsaturated fatty acids, such as  $C_{18:1}$ , are particularly sensitive to heat degradation and variations in the mineralogical composition of the clay pottery. Namely, a higher proportion of clay and consequently the presence of more fluxing agents, particularly in high illitic clay content samples, tend to result in greater degradation of  $C_{18:1}$ , and hypothetically, also other unsaturated compounds. Calcium carbonate rich tempers bind both saturated and unsaturated fatty acids more strongly into the ceramic matrix, most likely due to the conversion of calcium carbonate into calcium salts of fatty acids. Notably, unsaturated fatty acids specifically tend to bind better to the mineral matrix in the case of higher chalk-content materials.

Besides the chemical composition, the presence of significant micro- and mesopores in the briquettes, deriving directly from the clay fabric and firing techniques, may have contributed to the overall preservation of  $C_{16:0}$  and  $C_{18:1}$ . These factors can be used to tentatively estimate the extent of fatty acid preservation in archaeological pottery.

Finally, absolute and more accurate quantification of fatty acids can be achieved using certified FAME standard mixtures analyzed in GC-MS. This method can be invaluable in cases where more reliable quantitative analysis is required for identifying the fatty acids in lipids either from archaeological pottery or other complex samples (e.g. historical paintings). As reported in previous studies, acid-catalyzed methylation (ACM) was found to be superior to TMTFTH methylation after DCM-MeOH extraction.



## Impact of mineral composition on the yield and preservation of selected fatty acids

The results of this study, particularly from the degraded samples, have several wider implications for ORA in archaeological ceramics. First, the quantitative data supports the accepted idea that chalk-tempered ( $\text{CaCO}_3$ ) clay ceramics bind fatty acids more effectively than sand-tempered clay ceramics, meaning that there might be higher yields of lipids from pottery with lower chalk content. This finding ought to be taken into consideration when comparing the lipid preservation from different pottery fabrics, but also when determining the universal thresholds of so-called interpretable lipids. Results also suggest that an increase in  $\text{CaCO}_3$  content in pottery ( $\geq 45\%$  chalk) could limit the efficiency of ACM for extracting fatty acids. In such cases, a more suitable extraction methodology might be needed for more accurate results in the future. However, chalk-containing pottery samples seem to preserve unsaturated compounds better, i.e. their degradation (including the formation of saturated compounds), making such pottery fabrics preferable when substances with higher proportions of unsaturated compounds need to be targeted. Thus, chalk-containing pottery may pose both advantages and difficulties for archaeological ORA, be it in terms of full lipid recovery or relative comparisons of (unsaturated and saturated) compound ratios.

Second, the quantitative data indicates that clay, particularly an illite/illite-smectite type, can act as a catalyst in the transformation of unsaturated compounds, such as *cis*- $\text{C}_{18:1}$ . This suggests that a higher clay content can influence the formation of additional degradation products during heating or cooking processes. Hence, pottery with higher percentage of clay and lower amount of added tempers, might be preferred when targeting certain fatty acid degradation products.

All in all, the mineral components of clay ceramics should be given more importance in archaeological ORA studies. Despite the limitation that only two fatty acids were used in this study, which does not fully reflect the rich variety of lipid compounds preserved in archaeological pottery, the results presented here highlight how variations in the amount of clay and temper, but also the specific firing temperature can influence the nature and overall yield of organic compounds extracted from archaeological ceramics. However, due to complicated correlations between different clay types, tempers, firing techniques and chemical properties of different lipid compounds, further work in establishing organic-inorganic interactions in archaeological pottery is needed in the future.

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## Supporting Information

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## CRedit authorship contribution statement

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