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Editorial Note

Pakistan Heritage is a double-blind peer-reviewed journal, published annually. This volume included the papers on different aspects of archaeology and history of Pakistan and adjacent regions with subject matter ranging from the Prehistoric to the British Period.

We acknowledge the efforts of the members of the Board of Editorial Advisors, the contributors, the review and colleagues of the Department of Archaeology, Hazara University Mansehra. On the other hand, we are grateful to the worthy Vice Chancellor and management of Hazara University Mansehra for support and encouragement.

Editors

A Methodological Approach to Test Organic Residues in Gandhara Pottery: A Case Study from Taxila Valley (Pakistan)

ABDUL BASIT, ELENA ARGIRIADIS, MARA BORTOLINI, DARIO BATTISTEL AND GHANI UR-RAHMAN

Abstract

The organic residues extracted from ancient pottery can offer valuable insights into the diets of the past, shedding light on historical, cultural, economic, and agricultural practices. In this study, we considered the Taxila (Punjab, Pakistan) and specifically examined the Badalpur site as a case of study. Our investigation focused on five potential food items (long pepper (P. nigrum), masha (V. mungo), masura (L. culinaris), sesamum (S. indicum), and mustard (B. juncea)), which may have been utilized in recipes by Buddhist monks and could had left a chemical signature still preserved in pottery fragments discovered at this site. Using gas chromatography/mass spectrometry analysis (GC/MS) on these food items, we identified a range of compounds that could indicate the presence of these ingredients. We also conducted experiments in controlled conditions, where the food was cooked in pots. Through this investigation, we observed that certain compounds degraded (i.e., amino acids and low molecular weight carboxylic acids), while others were selectively absorbed on the surface of the pots rather than within the interior, such as fatty acids, phytosterols and hydrocarbons. Additionally, we noticed variations in the chemical composition of organic residues across different parts of the pots, such as the base, body, and rim. Despite the complexity of the absorption process and the occurrence of thermal degradation reactions, we successfully identified a set of compounds that remained relatively unchanged during the cooking process, such as piperine, sesamin, amyrin, caryophyllene oxide and pipecolic acid. These compounds proved useful in determining the use of specific ingredients in archaeological pottery. Finally, we applied this methodology to three archaeological fragments recovered from Badalpur, suggesting the use of long pepper (piperine and caryophyllene oxide), sesamum (sesamin) and likely masha and/or masura evidenced by the presence of pipecolic acid. In this paper, we aim to propose a methodological approach to identify and detect chemical compounds that could be indicators of the use of several ingredients in archaeological pottery fragments.

Keywords: Archaeological and Experimental Pottery, Organic residues, GC-MS, untargeted analysis, Taxila, Pakistan

Introduction

In the past decades, several research studies have shown that lipids can be conserved alongside many types of objects found in archaeological sites, among which are pottery and skeletal remains (Briuer 1976, Evershed et al. 1992, Loy 1994, Pollard & Heron 1996, Pollard et al. 2007). Studies in this field have mainly focused on biomarkers and their distribution to investigate the nature and source of ancient diets linked to historical, cultural, economic, and agricultural practices (Philip & Oung 1988, Heron et al. 1994, Evershed et al. 1995a, 1995b and 1999, Boëda et al. 1995, Pecci et al. 2013). The organic residues in archaeological contexts can derive from different activities, such as food processing, food storage, as well

as non-food practices like sealing. These residues may be visible on the artefact surfaces or absorbed into the porous, unglazed walls of vessels, requiring chemical analyses for detection (Heron & Evershed 1993, Irto et al. 2022). Lipids, such as fats and waxes, are commonly examined in archaeological pottery due to their resistance to dissolution and ability to endure in burial environments. However, the preservation of lipids inside ceramic vessels is influenced by environmental conditions, such as dry climates or acidic soils, as well as their entrapment within the ceramic structure (Evershed et al. 2008a and 2008b). Microencapsulation and carbonized residues associated with pottery contribute to lipid conservation, although water and reactive substances can lead to chemical degradation (Hamman & Cramp 2018). In fact, lipids can degrade over time due to the presence of reactive functional groups in their molecular structure. Among lipids, free fatty acids are the most prevalent and extensively studied in archaeological pottery (Evershed 1993, Evershed et al. 2008b). However, only some of them are found in significant quantities, particularly when the ceramic containers were exposed to high cooking temperatures or burial, as chemical reactions like oxidation, hydrolysis, and condensation can alter their composition (Gülacara et al. 1990, Rastogi et al. 2006). In addition, although the distribution of fatty acids depends on the types of food items used in the pot, they cannot be associated to any specific commodity.

The methodology used for the analysis organic residues is based on gas chromatography coupled with mass spectrometry (GC-MS) of the lipid extracts (Evershed 1993, Evershed et al. 2008[a], Colombini et al. 2009, Colonese et al. 2015, Bonaduce et al. 2017). However, determining the origin of these residues poses a challenge due to the four main factors listed below.

First, the chemical fingerprint of the substances used can be ambiguous. Second, the processing of these substances through cooking or firing can result in chemical transformations and/or degradation of the ingredients. Third, the interactions between the chemical compounds and the pottery can vary based on factors such as solubility, volatility of biomarkers, and the heating temperatures involved. As a result, the deposition process of organic materials within the vessel can exhibit different distributions at various points within the vessel. Fourth, mixing of different ingredients in varying proportions affects the lipid composition of food remains. Fifth, the chemical stability of the compounds after disposal and burial can alter their distribution and detectability. All these factors make it difficult to reconstruct ancient cooking recipes or identify the specific usage of archaeological vessels. Therefore, having a reasonable knowledge of the ingredients used in a particular archaeological context can help reduce uncertainty.

This investigation predominantly focuses on the methodological capacity to validate the analysis of organic residues in a number of archaeological pottery from Badalpur, situated in the Taxila Valley (see Figure 1). Taxila valley's early history can be traced back to 558-528 BCE with the conquest of the Achamenians of Persia under the rule of Cyrus the Great (Marshall 1960). Archaeological evidence substantiates Taxila's role as an Indo-Greek capital (Fussman 1993) and indicates continuous habitation since the Mesolithic period (Dani 1986). From the 3rd century BC onward, many Buddhist structures, including monasteries and *stupas*, were constructed, among which is the notable Badalpur Monastery. Badalpur's Buddhist site reveals a diverse ceramic assemblage with varied shapes, styles, and textures, including thin to thick pottery. Excavation yields finely crafted potsherds such as oil lamps, pots, storage jars, dishes, lids, condensers, bowls, basins, cooking pots, flasks, handled pots, jugs, and miniature vessels. (Aiyar 1917, Marshall 1960, Arif et al. 2006 and 2011, Khan et al. 2007 and 2013). A vertical excavation at Badalpur site has revealed eight distinct occupational layers, with structural remains and artifacts, including pottery, metal objects, bones, small finds, and copper coins, providing evidence of four distinct building periods. Period 1: The

earliest occupation (2nd century CE), with a 1.65-meter thick deposit (layers 6-8), featuring red burnished pottery, iron items, and copper coins. Period 2: From the 3rd-4th century CE, identified in layers 4-5 (1.5 meters thick), marked by the reuse and reconstruction of monastery and stupa areas, along with characteristic pottery. Period 3: Spanning the 5th-8th century CE (layers 1-3, 1.85 meters thick), this phase saw the construction of new monastery structures over earlier layers, confirmed by pottery evidence (Khan et al. 2013).

Several studies on Gandhara pottery have examined composition, firing techniques, and slip, with key contributions from Maritan et al. (2018) on golden slip ware from Swat Valley, Olivieri and Iori (2021) on Barikot (Swat Valley) pottery, and Groat (2023) on early distillation technologies in South-Central Asia, including Gandhara. In addition to several studies on Gandhara pottery, there are few that specifically focus on the organic residues, their extraction, and analysis. A notable example is the organic residue analysis of a pottery fragment from a 5th-century CE Buddhist site at Seeraj in Sindh, Pakistan, conducted by Gyulai and Kallay in 1999. The analysis revealed the presence of non-evaporating components like tartaric acid, polyphenols, and minerals—key markers of wine—confirming that the vessel had previously been used to hold wine (Gyulai & Kallay, 1998–1999). This underscores a significant imperative for the implementation of a rigorous and valid methodological approach aimed at scrutinizing organic residues present in Buddhist pottery. Buddhism shares many food practices with distinct elements. The *Lankavatara Sutra* (Chapter Eight) and *Vinayas* (Frauwallner 1956) reports the Buddha's recommendation to consume various grains such as rice, barley, wheat, *mudga* (mung bean), *masha* and *masura* (lentils), as well as ghee, sesame oil, honey, molasses, sugar, fish, and eggs. In adherence to dietary guidelines, a monk was instructed not to make explicit requests for meat, fish, ghee, oil, honey, sugar, milk, or yoghurt, unless experiencing illness (Sen 2014). In addition to the Buddhist literature, archaeobotanical studies of Buddhist archaeological sites in Gandhara also offer valuable insights into the food sources of Buddhist communities. A prominent example is the archaeobotanical study conducted at the Buddhist site in Barikot (Swat, Pakistan) (Spengler et al. 2021), which revealed evidence of a wide variety of crops cultivated within the settlement. These included wheat (*Triticum* sp.), barley (*Hordeum* sp.), rice (*Oryza* sp.), lentil (*Lens culinaris*), field pea (*Lathyrus sativa*), pea (*Pisum sativum*), cowpea (*Vigna* sp.), horsegram (*Macrotyloma uniflorum*), cotton (*Gossypium* sp.), grape (*Vitis vinifera*), along with other cereal crops and legumes.

Drawing upon Buddhist literature as our primary source and incorporating archaeobotanical findings from Gandhara, our investigation concentrated on the analysis of five potential plant-based food items, excluding animal sources, which may have been integral to the dietary practices of Buddhist monks and incorporated into their culinary recipes: *masura* (*Lens culinaris*), *masha* (*Vigna mungo*), long pepper (*Piper longum*), mustard (*Brassica juncea*) and sesamum (*Sesamum indicum*). Initially, we conducted an analysis of the extracts from these food items using untargeted GC-MS to identify the most distinctive chemical compounds. Subsequently, we prepared and cooked these five food items in experimental pots. In the simulation of the cooking experiment, we employed both intact and pulverized iterations of the food items during the experimental procedures. Samples were collected from various points within the experimental pots, including the base, body, and rim, and subjected to GC-MS analysis using the same methodology employed for the extracts of the food items. This investigation allowed us to (a) enable the examination of the validity and potential of the methodological approach employed for analyzing organic residues in experimental pottery samples. (b) identify potential specific biomarkers specific for the food items that

remained largely unaltered during the heating process, (c) assess the absorption mechanism at different points within the vessel, examining where the markers were preferentially absorbed and (d) evaluate the absorption capability of the biomarkers based on the preparation of the initial ingredients (i.e., ground or whole). Finally, we applied the proposed methodology to a set of archaeological samples obtained from Badalpur site, thus demonstrating the applicability and limitations of the methodology outlined in this study. It is worth noting that the current application is limited to a relatively small sample size, serving as a preliminary step for subsequent implementation across a more extensive collection of archaeological samples.

Materials and Methods

Food Commodities and Experimental Pot

Based on the archaeobotanical evidence (Spengler et al. 2021) and references from the Buddhist literature (*Lankavatara Sutra* and *Vinayas*), we considered 5 food commodities that were likely used in the diet of the Buddhist monks: *masura* (*Lens culinaris*), *masha* (*Vigna mungo*), long pepper (*Piper longum*), mustard (*Brassica juncea*), sesamum (*Sesamum indicum*). Original items were purchased in a traditional market in the Rawalpindi district (Pakistan), to minimize any possible geographical variability. About 2 g of each food commodities were ground with a mortar (Retsch RM 200) and stored in aluminum foils until extraction.

Several small pots were made from clay reproducing the local traditional pottery techniques. Two pots were chosen for the cooking experiments (Figure 2-A). The food in the first pot was cooked whole (WFP) (*i.e.*, without being ground), whereas the food in the second pot was cooked ground (GFP). Foods were cooked in each pot for one hour. This process was repeated three times. Figure 2-B shows a typical experimental pot after the cooking experiments. After cooking, different points of the pots (base, body, and rim, see Figure 2-A) were scratched using a rotary tool (DREMEL Model 2050-15) with a sandpaper disposable headpiece. Each sample was divided into two fractions, including the immediate surface layer (S) to be analyzed separately from the underlying internal layer (I) potentially containing absorbed organic residues.

Archaeological Samples

In this study, a total of 3 samples of pottery sherds of cooking pots, collected from the Badalpur site during the excavation campaigns in 2015 and 2016, were considered (see Figure 2-C). The archaeological potsherds were sub-sampled following the same methodology used for the experimental pots. The immediate surface layer was removed to create the first fraction, while the inner underlying layer was removed as a second fraction. The archaeological samples are hereinafter labelled as CPn-j (where n ranges from 1 to 3 and j refers to the fraction collected: surface (S) or internal (I)). Samples CP1 corresponded to the rim of the pot, while CP2 and CP3 refer to the body. The sub-sampling was conducted under a fume hood to ensure a clean environment as well as to avoid the spread of pottery dust generated by drilling. A new sandpaper roller was used for each sample to avoid cross contamination.

All the samples were ground with a mortar. The pestle and mortar were rinsed with water and dried completely after each sample. The rinse was followed by washing three times with methanol (MeOH, pesticide grade, Romil Ltd. Cambridge UK), three times with dichloromethane (DCM, pesticide grade,

Merck KGaA, Darmstadt, Germany) and three times with *n*-hexane (pesticide grade, Romil Ltd. Cambridge UK) to avoid cross contamination. Experimental and archaeological pottery samples were ground obtaining up to 1 g of fine powder that was stored into aluminum foils until extraction.

Sample treatment

The ground samples (*i.e.*, food commodities, experimental and archaeological pots) were extracted in ultrasonic bath, using a mixture of methanol (MeOH, pesticide grade, Romil Ltd. Cambridge UK) and dichloromethane (DCM, pesticide grade, Merck KGaA, Darmstadt, Germany) 2:1 v/v. Before extraction, each sample was spiked with 5 µg of internal standard 5α-androstane (Merck KGaA, Darmstadt, Germany) (later referred to as androstane), adapting the methods proposed in literature (Charters & Evershed 1997, Papakosta et al. 2015, Kaluzna-Czaplinska et al. 2016). The samples were extracted three times with 7 mL of DCM:MeOH (2:1, v/v) mixture for 10 minutes per extraction cycle. The vials containing the pottery powder were centrifuged at 1500 rpm for 5 minutes to separate the fine solid powder. About 20 mL of extract were collected from each sample. Extracts from food samples were filtered on glass wool and anhydrous sodium sulfate to eliminate solid residues and humidity. The volume of the extracts was reduced to about 2 mL using a gentle flow of nitrogen, while the vials were located in a thermostatic bath set at 25 °C. The reduced extract was divided between two new vials (A and B) to analyze both free and bond fatty acids. Extracts in both vials were dried completely and 2 mL of 5% sodium hydroxide (NaOH, Sigma-Aldrich, St. Louis, MI, USA) in MeOH was added to vial B that was heated at 70 °C for 60 minutes for saponification. NaOH was neutralized with 550 µL of 6 M hydrochloric acid (HCl, Sigma-Aldrich, St. Louis, MI, USA). Vial B was then extracted five times with 1 mL of *n*-hexane and each time the upper phase was transferred into the vial A. The resulting ~ 5 mL of liquid extract was dried under a gentle flux of nitrogen.

Chemical Analysis

Before the GC-MS analysis, 100 µL of DCM were added to the dried samples to recover the extract, derivatized with 100 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma-Aldrich, St. Louis, MI, USA) and heated at 70 °C for 60 minutes. The GC-MS analysis was carried out after 24 hours. The derivatized samples were analyzed by an Agilent Technologies 7890A GC coupled with an Agilent Technologies 5975C TAD MSD single-quadrupole. Full scan and single ion monitoring (SIM) mode were used. The GC analysis was carried out in splitless mode, after the injection of 1 µL of solution at 300 °C (Inlet temperature). The temperature program was 70 °C for 1.5 minutes, then a ramp to 150°C at 10°C min^{-1}, 3°C min^{-1} to 300°C, and static at 300 °C for 15 minutes. Helium flow was set at 1 mL min^{-1}. The MSD temperature was set at 150 °C and m/z ratios between 50 and 550 were scanned.

Peak Identification and quantification

The obtained chromatograms were analyzed using the Enhanced ChemStation MSD E.02.00.493 (Agilent Technologies Inc). All peaks of the total ion chromatograms (TIC) that had a signal to noise (S/N) ratio higher than 3 were considered and the corresponding mass spectrum was compared with the NIST library for the identification. The peaks that match the NIST database with a quality level lower than 80% were discarded. Similarly, the peaks that were associated to compounds that can derive from the degradation of

the GC column or the septum were not considered. Finally, a total of six procedural blanks were prepared following the same extraction procedures and GC-MS analysis. Compounds that were detected in the blanks were not considered in the following data treatments.

Although a rigorous quantification of the compounds cannot be achieved, we carried out a semi-quantitative analysis of each compound based on the peak area corresponding to the most intense fragment (m/z target) normalized by the peak area of the internal standard (*i.e.*, androstane) calculated for m/z = 245. This approach does not allow to compare the concentration of different compounds contained in the same sample, but it ensures the comparability between the same compounds found in different samples. In addition, this precaution allows to reduce the background noise and increase the sensitivity of the following analysis that can be carried out in selected ion monitoring (SIM) mode. Moreover, for each compound, we identify a second m/z fragment that can be used as confirmation ion (m/z confirm), following the methodology adopted in Battistel et al. (2015). In Table 1, both m/z target and confirmation ions are reported in conjunction with the ratio between their peak areas (R).

Result and Discussion

Chemical composition of the food commodities

Figure 3A shows typical chromatograms recorded in full scan mode for each of the five food commodities considered. For each chromatographic peak, the corresponding mass spectrum obtained with an ionization energy of 70 eV was compared with the NIST library. A total of 53 compounds out of 61 (not observed in the blanks) were identified with a match quality higher than 90%, while in 5 cases the match quality was between 80 and 90%. In three cases we did not find any match with NIST library. However, these compounds were considered due to their abundance and distinct spectrum. All the compounds were conveniently clustered in 5 groups based on their chemical classification: (a) sesquiterpenes (b) fatty acids (c) aliphatic hydrocarbons (d) phytosterols and (e) other compounds.

(a) As shown in Figure 3A-B, between 16 and 25 minutes, a cluster including sesquiterpenes ($C_{15}H_{24}$) can be recognized. This cluster includes 21 compounds (see also Table 1) that are present only in long pepper (*P. longum*), while they were not detected in the other ingredients. Although we did not perform a rigorous quantification of the compound concentrations (the chromatographic signal should be corrected by the response factor), among sesquiterpenes, long pepper chromatogram indicated a higher content of caryophillene and bisabolene, in agreement with the results reported in Shankaracharya et al. (1997).

(b) Fatty acids are eluted between 44 and 56 minutes apart from lauric and lignoceric acids that have retention times of 29.67 and 66.04 minutes, respectively. Although fatty acids were identified through their mass spectra, their retention times agree with fatty acid tms-derivatives analyzed with an Agilent Technologies HP-5MS GC column (Wan et al. 2007). Differently from sesquiterpenes, the cluster including fatty acids is not specific for any particular commodity. Indeed, as shown in Figure 3C (see also Table 2), the most abundant fatty acids (*i.e.*, palmitic, linoleic, oleic and stearic) are significantly present in all the ingredients.

(c) A number of 10 aliphatic hydrocarbons were mainly detected in *P. longum*, except for H_6 e H_10, that were essentially absent in long pepper. It must be noted that the unambiguous identification of aliphatic hydrocarbons based on their mass spectrum is oftentimes deceptive, due to the common m/z

fragments $(CH_2)_nCH_3^+$ and $(CH)_m(CH_2)_nCH_3^+$ produced during electron ionization for paraffin and olefin, respectively. Therefore, in this paper, we labelled these compounds with the more generic indication of the chemical classification (paraffin *vs* olefin), rather than attempting a possible identification.

(d) A number of 4 phytosterols were mainly detected in *masha* and *masura* (except for PS_1 that was only observed in mustard) at retention times higher than ~64 minutes. Among these compounds, an unambiguous identification was possible only for PS_2 and PS_3 (stigmasterol and b-sitosterol, respectively). The mass spectra of PS_1 and PS_4 match several phytosterols (*e.g.,* campesterol, brassicasterol and avenasterol) without a satisfactory quality. Nonetheless, PS_1 might be tentatively ascribed to brassicasterol (one of the main abundant sterols in *Brassicaceae; e.g.,* mustard) while PS_4 might be ascribed to avenasterol that has retention times higher than b-sitosterol (Xu et al. 2020).

(e) A list of other 18 compounds (labelled as ID#), including aldehydes, amino acids, terpenoids and alkaloids among the others, is reported in Table 1. Among these compounds, ID_6 (Citric Acid, match quality = 84%), ID_9 (Glycerol diacetate laurate, 80%), ID_11 (Palmidrol, 81%), ID_13 (Hexamethyl mellitate, 82%) and ID_14 (Sucrose, 86%) were identified with some uncertainty. They do not match the mass spectra of the NIST library with a quality level higher than 90% likely due to their low concentrations and/or the presence of interfering compounds. Therefore, the identification of these compounds must be carefully considered. In addition, the identification of ID_10 did not provide any quality match values higher than 20% with the NIST library and therefore we indicated it as *unknown*. Although these compounds will be in any case considered in this study, further investigations would be required for a more reliable identification.

Aiming to identify possible biomarkers for the five food commodities considered in this paper, we cross compared the presence and intensity of the 61 compounds considered between the ingredients. In Table 2 the intensity relative to the maximum value are reported.

As shown in Table 2 (see also Figure 3A), all the sesquiterpenes, most of the paraffin (H_2, H_5 and H_9) and the olefin (H_1, H_3, H_4, H_7 and H_8) were detected only in long pepper. Among fatty acids, lauric acid (FA_1) was about 20 times more abundant than in the other food commodities, although not exclusively present. Finally, some compounds, such as ID_3 (benzene propanoic acid), ID_4 (caryophillene oxide), ID_10 (*unknown*) and ID_16 (piperine) are promising candidates as specific markers of the use of long pepper in organic residuals from pottery.

Phytosterols were largely present in *masha* and *masura* (stigmasterol and b-sitosterol). However, phytosterols may be found in many other vegetables, such as turnips among the others (Lepage, 1967). Lynolenyl alcohol (ID_8), Palmidrol (ID_11) and Myo-inositol (ID_12) were significantly found in *masha* and *masura*. M*asha* also contains light carboxylic acids such as citric and succinic acids (ID_1 and ID_6). We also observed a significant presence of glycerol phosphate (ID_5) and sucrose (ID_14) compared to the other ingredients. Nonetheless, these compounds are significantly detected also in most of the other food commodities considered here as well as in a large number of vegetables. Therefore, their specificity should be carefully considered.

Although not specific, the most promising compound that can provide indication of the use of *masura* as ingredient is b-amyrin (ID_18), that is about 5 times higher than in the other food commodities.

Extracts from mustard were less enriched in organic constituents with respect to the other ingredients considered in this study (see the chromatograms in Figure 3A and C). Nevertheless, despite the low content of organics, paraffin (H_10), phytosterol(1) (PS_1) and hexamethyl mellitate (ID_13) (see Table 2) were not detected in the other ingredients, indicating a potential specificity of these compounds to indicate the use of mustard.

The sesamum extracts were characterized by a higher content of the olefin H_6 (about 3 times higher than *masha*) and sesamin (ID_18). The latter is a very promising marker for the presence of sesamum seeds and/or oil in organic residues absorbed on pottery.

Chemical absorption on pottery

Three parts of the experimental pots were analyzed separately. They included base, rim, and body from pots where intact and ground food commodities were prepared. In Figure 4 we reported the chromatograms obtained in the samples. As shown, the number and intensity of the compounds resulted generally higher in the rim with respect to the body that, in turn, had higher and more intense peaks than the base.

More in detail, the signals corresponding to each compound previously identified (corresponding to the m/z target areas), corrected by the androstane area (m/z = 245) and normalized by the weight of the analyzed sample are reported in Table 3. As reported in the table, none of the sesquiterpenes deriving from long pepper (ST_1 – ST_21) was detected in the pot. The absence of sesquiterpenes may be associated to their higher hydrophobicity in conjunction with a possible thermal instability that promotes their degradation while cooking.

Conversely, fatty acids were detected in all the surfaces of the pots, also showing the capability to be absorbed in the interior of the pot. As shown in Figure 5, higher absorption was observed in the superficial rim, when ground ingredients were cooked. On average, we found that the amount of fatty acids absorbed at the surface of the rim is about 1.5 times higher than the body, while in the body it is about 2 times higher than in the base. The same behavior was observed when considering the fatty acids absorbed in the internal part of the pot. In particular, in the interior of the pottery fabric, we found that fatty acids absorbed in the rim are more than 3 times higher than the body that are in turn 9 times higher than the base. These results are in line with the values reported by Evershed (2008) where a larger amount of lipids was found in the rim of the pots, and a lower in the base. Moreover, both for whole and ground food items, the amount of free fatty acids absorbed at the surface with respect to the interior was 45-70, 14-20 and 5-12 times higher for the rim, body, and base, respectively. Indeed, the higher amount of fatty acids absorbed at the rim surface likely enhances the diffusion kinetics into the interior part of the pot.

The fatty acid fingerprint when cooking ground food items is preserved in all the points of the pot (r-Pearson coefficient ranges between 0.987-0.999). Nevertheless, it must be noted that while cooking, fatty acids can undergo oxidation and/or isomerization reactions (Rastogi et al., 2006 and reference therein), thus changing the fatty acid composition of the original food commodity. As a note, we must consider that the heating process may lead to the formation of more volatile compounds or by-products that are not present in the original food item. However, in this paper we only focused on the detection of stable compounds that can persist in the pot attempting to identify the use of one of the food commodities considered here.

Similarly to fatty acids, both hydrocarbons and phytosterols were preferentially found in the surface (mainly rim and body) with a larger presence when the food commodities were ground. Nonetheless, some compounds such as the olefin H_1 and the phytosterol PS_1 were not detected, likely due to their lower concentration in the original food items or to their volatility. Paraffin (H_10) and olefin (H_6), that are possible candidates to detect the presence of mustard and sesamum, respectively, were mainly found in the surface rim, essentially when ground food was cooked.

Carboxylic acids with a low molecular weight, such as succinic acid (ID_1) and citric acid (ID_6) that were particularly abundant in *masha* were not detected in the experimental pot samples, probably due to their high solubility in water or as a consequence of thermal degradation. Interestingly, part of the compounds that could be candidates for the identification of the food commodities considered in this work were detected both in the surface and in the internal part of the pottery.

Based on the results reported in Table 2 and Table 3, we identify a list of compounds that were not lost while cooking. In particular, we considered that: caryophillene oxide and piperine may be used to detect long pepper; glycerol phosphate and pipecolic acid for *masha*, b-amyrin for *masha* and *masura*; hexamethyl mellitate and paraffin (H_10) for mustard; and sesamin and olefin (H_6) for sesamum. It must be noted that high presence of b-sitosterol and stigmasterol may support the use of *masha* and *masura*.

In Figure 6, the distribution of such compounds in the different parts of the pot is reported. As shown, and similarly to fatty acids, the highest concentrations were found in the surface especially when ground food is cooked. Nevertheless, while some compounds such as pipecolic acid, glycerol phosphate, hexamethyl mellitate and olefin (H_6) show the same distribution of the lipids fraction (*i.e.*, a progressive increase from base to rim), other compounds (e.g., caryophillene oxide, piperine, paraffin (H_10), b-amyrin and sesamin, as well as phytosterols) showed maximum values corresponding to the body surface rather than the rim. The different behavior may be related to a combination of factors that affect the absorption process, such as the extraction, solubilization, evaporation and physical-chemical absorption on the pot surface. Therefore, the distribution of chemical compounds on the different parts of the pot is not homogeneous and, although most of the compounds are preferentially absorbed at the rim surface, others were preferentially found on the body. In general, the absorption at the base of the vessel is unfavored.

In Figure 7, the relation between two of the most promising candidates to identify the presence of the food items considered in this study are reported. As shown, for all the food commodities, the ratio between two components of the ingredient changes after cooking. In particular, for long pepper, piperine absorption is on average 1.6 times favored with respect to cayophillene oxide (Figure 7A), although it is worth noting that at the surface of the pottery, piperine is slightly more favorably absorbed than caryphillene oxide; pipecolic acid is absorbed ~4 times more than glycerol phosphate (*masha*) (Figure 7B); hexamethyl mellitate is more than 2500 times higher than paraffin (H_10) (mustard) (Figure 7C); Sesamin is ~3 times higher than olefin (H_6) (sesamum) (Figure 7D). These results significantly complicate the identification of the use of cross ratios between compounds to identify the use of a specific food items; not only because a compound may have multiple sources, but also because different compounds may be more (or less) favorably absorbed and immobilized in the pot surface.

Application to archaeological pot from Taxila Valley

The methodology proposed in this paper was applied to the study of the extracts obtained from 3 archaeological samples from the Badalpur monastery, aiming to test the possibility to identify the use of the food commodities previously considered. These samples included rim (CP1) and body (CP2 and CP3) of three different pots, where internal and superficial material was analyzed in CP1 and CP3, while only the internal part was analyzed in the CP2 sample (in this sample, the extract from the surface was accidentally compromised by laboratory contamination).

In order to increase the instrumental sensitivity, we performed GC-MS analysis in selected ion monitoring (SIM) mode, using the target and confirmation ions monitored at the chromatographic time windows including the retention time of the target compounds. The identification of the compounds was therefore carried out using the retention times and double checked with the R ratio previously identified (see Table 1).

Fatty acids were clearly detected in all the samples. Their presence is not directly indicative of any specific ingredients, but it is interesting to observe that hierarchical cluster analysis performed with the ward method and the r-Pearson distance provided the formation of three clusters including the three samples (see Fig.S1 in supporting material). Although this classification is out of the scope of this paper, we consider that free fatty acids can be successfully employed to quantitatively find associations between pottery samples that may have been employed to cook similar foods or used for similar purposes.

Despite the increased instrumental sensitivity, most of the compounds found in the ingredients and experimental pots were not detected in the archaeological samples, likely due to chemical transformation that occurred during cooking (as previously demonstrated) or to environmental degradation during burial. This latter occurrence has not been specifically investigated in this paper. Nevertheless, based on the retention time (74.07 min) and the R ratio (R=0.30-0.33) we were able to detect sesamin in both CP1 fragments (see Table 1 for comparison), where the signal was higher in the surface than in the internal part. In addition, olefin (H_6) was also detected in CP1-S-rim. Although the ratio H_6: sesamin in CP1-S-rim (0.8) is significantly higher than in the experimental pot (i.e., 0.03), and H_6 was not detected in CP1-I-rim (H_6 was lower than the detection limit), the presence of sesamin detected in CP1 may suggest that some sesamum specie (seeds and/or oil) might be used as ingredient for cooking in this pot. Recent botanical studies indicate that cultivated sesame comes from wild populations in South Asia, specifically the western Indian peninsula and parts of Pakistan, known as Sesamum malabaricum or S. mulayanum. Archaeological evidence shows that sesame cultivation was established in northwestern South Asia during the Harappan civilization, spreading to Mesopotamia by 2000 BC. Sesame cultivation had extended to other parts of India by the end of the 2nd millennium BC, with its introduction to Africa occurring more recently (Fuller 2003). Similarly, b-amyrin (retention time = 79.87 min, and R=3.1-3.3) was detected in CP1 only. The high amounts of b-amyrin in *masha* and *masura*, as well as its persistence in the experimental pots as previously observed, in conjunction with a relatively higher presence of phytosterols, suggest that these two legumes might have been used in CP1 vessel, although we cannot exclude the use of other ingredients come from the same family.

Conversely, in CP3 we were able to detect the contemporary presence of piperine (retention time = 68.32 min, and R=0.70) and caryophillene oxide (retention time = 27.14 min and R=0.70). They were not detected in the internal part of this sample likely because the signal was lower than the detection limit (in agreement

with Figure 6). The ratio piperine : caryophillene oxide in CP3-S-rim (*i.e.*, ~11) is about 15 times lower than the value obtained with experimental pots (i.e., ~ 160 ; see Figure 7). This occurrence may be explained in part considering in part the higher variability associated to the detection of smaller signals in archaeological samples rather than experimental pots, and in part assuming a faster environmental degradation of piperine with respect to caryophillene oxide that reduces the piperine : caryophillene oxide ratio. However, although thermal degradation, instrumental uncertainty and environmental degradation may strongly affect these ratios, the contemporary presence of piperine and caryophillene oxide are consistent with the use of long pepper as ingredient in this pot, or family species of pepper. The simultaneous presence of piperine and caryophyllene oxide is consistent with the utilization of long pepper as an ingredient in this pot, or it could also involve other Piper species like black pepper (Piper nigrum) and various fruits from the Piperaceae family, which encompasses long pepper (Piper longum) (Lee et al. 2020).

Unfortunately, in CP2 we did not detect any peculiar compounds, although this pot shows a higher similarity in terms of fatty acids fingerprint with CP3.

Conclusion

In this paper the GC/MS analysis of the liquid extracts from five food commodities (long pepper, *masha*, *masura*, sesamum and mustard) led to the detection of 61 chemical compounds. The same analysis was performed in two experimental pots, where the same commodities were cooked both whole than ground, demonstrating that some of these compounds degrades during cooking and/or are not persistently adsorbed in the pot. The analyses of pot fragments were carried out in three different parts of the pots (base, body, and rim), demonstrating the occurrence of a differential absorption ability of these compounds. Nevertheless, we were able to identify in the pot some potential biomarkers that might be successfully associated to these ingredients or at least ingredients related to the same family species , such as piperine and caryophillene oxide for long pepper, pipecolic acid, glycerol phosphate and b-amyrin for *masha* and *masura* (in conjunction with a large amount of phytosterols), sesamin and an unidentified olefin (H_6) for sesamum, hexamethyl mellitate (although mass spectrum match was ~ 80% only) and an unidentified paraffin (H_10) for mustard. The analysis performed on archaeological pots demonstrated that we were able to clearly identify sesamin and b-amyrin in one sample, suggesting the utilization of legume species (*masha* and *masura*) along with sesamum species, as well as the presence of piperine and caryophyllene oxide in a second sample, indicates the possible use of long pepper or other related pepper species.. Despite the limited number of commodities considered, we presented here a methodological approach that can be successfully employed to investigate the ingredients used in ancient recipes. As a final remark, we underline the importance of the knowledge of the use of ingredients of the peculiar archaeological context, in order to narrow the range of ingredient candidates. While these biomarkers lack high specificity, they play a crucial role in narrowing down possibilities for identifying ingredients from specific food sources within a particular species. Although absolute specificity is currently unavailable, these markers help refine interpretations. To enhance accuracy, incorporating archaeological information is recommended, providing valuable context for understanding biomarker presence and refining interpretations of ancient dietary practices.

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Reference

Achaya, K.T., 1994. Indian food: a historical companion. Delhi: Oxford University Press.

Aiyar, N. (1917) Frontier Circle for 1916-17: Annual Report of the Archaeological survey of India. Peshawar.

Arif, M., Khattak, M. H Khan. (2006) "Fascinating Discoveries from Buddhist Sanctuary of Badapur, District Haripur, Taxila Valley", Ancient Paksitan, vol XVII:119-126.

Arif, M. et al (2011) "Excavation at the Buddhist site of Badalpur, Taxila Valley (compaign 2005)", Gandharan Studies, vol V: 27-42.

Battistel, D., Piazza, R., Argiriadis, E. et al. GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments. Anal Bioanal Chem 407, 8505–8514 (2015). **https://doi.org/10.1007/s00216-015-8998-2**

Boëda, E., Connan, J., Dessort, D., Muhesen, S., Mercier, N., Valladas, H., & Tisnérat, N. (1996). Bitumen as a hafting material on Middle Palaeolithic artefacts. *Nature*, *380* (6572), 336-338.

Bonaduce, I., Ribechini, E., Modugno, F., Colombini, M.P. (2017). Analytical Approaches Based on Gas Chromatography Mass Spectrometry (GC/MS) to Study Organic Materials in Artworks and Archaeological Objects. In: Mazzeo, R. (eds) Analytical Chemistry for Cultural Heritage. Topics in Current Chemistry Collections. Springer, Cham. https://doi.org/10.1007/978-3-319-52804-5_9

Briuer,F.I. (1976) New clue to stone tool function: plant and animal residues. American Antiquity. 41(4), 478-84.

Charters,S., Evershed, R.P. Quye, A. Blinkhorn, P.W. Reeves V. Simulation Experiments for Determining the Use of Ancient Pootery Vessels: the Behaviour of Epicuticular Leaf Wax During Boiling of a Leafy Vegetable J. Archaeol. Sci., 24 (1997), pp. 1-7, 10.1006/jasc.1995.0091

Colombini, M.P., F. Modugno, M. Giacomelli, and S. Francesconi. 1999. Characterisation of proteinaceous binders and drying oils in wall painting samples by gas chromatography-mass spectrometry. Journal of Chromatography A 846(1–2): 113–124.

Colonese, A. C., Farrell, T., Lucquin, A., Firth, D., Charlton, S., Robson, H. K., Alexander, M. and Craig, O. E. (2015), Archaeological bone lipids as palaeodietary markers. Rapid Commun. Mass Spectrom., 29: 611– 618. doi: 10.1002/rcm.7144.

Evershed, R.P., Heron, C., Charters, S. and Goad, L.J. (1992) The survival of food residues: new methods of analysis, interpretation and application. In A. M. Pollard (ed.), New Developments in Archaeological Science,23, 429-36.

Evershed, R. P. (1993). Biomolecular archaeology and lipids. *World archaeology*, *25* (1), 74-93.

[a]Evershed, R. P., Stott, A. W., Raven, A., Dudd, S. N., Charters, S., & Leyden, A. (1995). Formation of long-chain ketones in ancient pottery vessels by pyrolysis of acyl lipids. *Tetrahedron Letters*, *36*(48), 8875-8878.

[b]Evershed, R. P., Turner-Walker, G., Hedges, R. E., Tuross, N., & Leyden, A. (1995). Preliminary results for the analysis of lipids in ancient bone. *Journal of Archaeological Science*, *22*(2), 277-290.

[a]Evershed, R.P., (2008) Organic Residue Analysis in Archaeology: The Archaeological biomarker Revolution. Archaeometry. 50 (6) 895-924.

[b]Evershed, R. P. (2008). Experimental approaches to the interpretation of absorbed organic residues in archaeological ceramics. *World Archaeology,40* (1), 26-47.

Fuller, D. Q. (2003). Further evidence on the prehistory of sesame. *Asian Agri-History*, *7*(2), 127-137.

Gyulai, F. and Kallay, M. (1998) 'A Drink-Remain from Seeraj (Sindh, Pakistan)', *Ancient Sindh*, 5, pp. 29–34.

Gülacara, F. O., Susini, A., &Klohn, M. (1990). Preservation and post-mortem transformations of lipids in samples from a 4000-year-old Nubian mummy. Journal of archaeological science, 17(6), 691-705.

Hammann, S., & Cramp, L. J. (2018). Towards the detection of dietary cereal processing through absorbed lipid biomarkers in archaeological pottery. Journal of Archaeological Science, 93, 74-81.

Heron, C., &Evershed, R. P. (1993). The analysis of organic residues and the study of pottery use. Archaeological method and theory, 5, 247-284.

Heron, C., Nemcek, N., Bonfield, K. M., Dixon, D., and Ottaway, B. S., 1994, The chemistry of Neolithic beeswax, Naturwissenschaften, 81, 266–9.

Irto, A., Micalizzi, G., Bretti, C., Chiaia, V., Mondello, L., & Cardiano, P. (2022). Lipids in archaeological pottery: A review on their sampling and extraction techniques. *Molecules*, *27*(11), 3451.

Kałużna-Czaplińska, J.; Rosiak, A.; Kwapińska, M.; Kwapiński, W. Different Analytical Procedures for the Study of Organic Residues in Archeological Ceramic Samples with the Use of Gas Chromatography-Mass Spectrometry. Crit. Rev. Anal. Chem. 2016, 46, 67–81. DOI: 10.1080/10408347.2015.1008130.

Khan, M. A. et al. (2007) A preliminary Report of the Third Season (2007-08), Excavation on the Buddhist site of Badalpur, Taxila valley, Museum Journal, National Museum of Pakistan: 39-52.

Khan, M. A. et al. (2013) "Excavation at Badalpur Monastery, District Haripur (Khyber Pakhtunkhwa), Pakistan: A Preliminary Report of Season 2013", Journal of Asian Civilizations Vol. 36, No. 2:65-80.

Lee, J. G., Kim, D. W., Shin, Y., & Kim, Y. J. (2020). Comparative study of the bioactive compounds, flavours and minerals present in black pepper before and after removing the outer skin. *LWT*, *125*, 109356.

Lepage M. Identification and composition of turnip root lipids. Lipids. 1967 May;2(3):244-50. doi: 10.1007/BF02532563. PMID: 17805774.

Loy,T.H.(1994) Methods in the analysis of starch residues on prehistoric stone tools, In J. Hather (ed.),Tropical Archaeobotany. Applications and New Developments. One World Archaeology, 22. London: Routledge,86-114.

Marshall, J. (1960) A Guide to Taxila. Karachi: Sai Corporation.

Papakosta,V., Smittenberg, RH, Gibbs K, Jordan,P, Isaksson, S. (2015) Extraction and derivatization of absorbed lipid residues from very small and very old samples of ceramic potsherds for molecular analysis by gas chromatography–mass spectrometry (GC–MS) and single compound stable carbon isotope analysis by gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS), Microchemical Journal, 123 (196-200). https://doi.org/10.1016/j.microc.2015.06.013.

Pecci, A., Giorgi, G., Salvini, L., & Ontiveros, M. Á. C. (2013). Identifying wine markers in ceramics and plasters using gas chromatography–mass spectrometry. Experimental and archaeological materials. *Journal of Archaeological Science*, *40*(1), 109-115.

Philip, R.P. and Oung,J.N. (1988) Biomarkers: occurrence, utility and detection. Anal.Chem. 60, 887A-896A.

Pollard, A.M. and Heron, C. (1996) Archaeological Chemistry. Washington DC: Royal Society of Chemistry.

Pollard, A., Batt, C., Stern, B., & Young, S. (2007). Analytical Chemistry in Archaeology (Cambridge Manuals in Archaeology). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511607431

Rastogi, P. Mathur, B., Rastogi, S. (2006). Fatty acid oxidation and other biochemical changes induced by cooking in commonly used Indian fats and oils

Sen, C. T. (2014). Feasts and fasts: A history of food in India. Reaktion Books.

Shankaracharya N.B., Jaganmohan Rao L, Pura Naik J.and Nagadakshmi S. (1997), Characterization of chemical constituents of Indian long pepper (Piper longum L.). J. Food Sci. Technol., 34(1), 73–75

Wan, P.J., Dowd, M.K., Thomas, A.E. and Butler, B.H. (2007), Trimethylsilyl Derivatization/Gas Chromatography as a Method to Determine the Free Fatty Acid Content of Vegetable Oils. J Amer Oil Chem Soc, 84: 701-708. **https://doi.org/10.1007/s11746-007-1101-1**

Xu, B., You, S., Zhou, L. et al. Simultaneous Determination of Free Phytosterols and Tocopherols in Vegetable Oils by an Improved SPE–GC–FID Method. Food Anal. Methods 13, 358–369 (2020). **https://doi.org/10.1007/s12161-019-01649-7**

Tables and Figures

Table 1. Characteristics of the compounds identified in the food commodities (long pepper, masha, masura, mustard and sesamum).

Retention time	Label	Compound	Formula	CAS nr.	Match	m/z	m/z	R(±15%)
Sesquiterpenes								
16.41	ST 1	α-cubebene	$C_{15}H_{24}$	17699-14-8	96	161	204	4.65
17.60	ST 2	α-copaene	$C_{15}H_{24}$	3856-25-5	98	161	204	5.11
18.00	ST 3	ß-elemene	$C_{15}H_{24}$	515-13-9	98	161	204	9.82
19.30	ST 4	α-santalene	$C_{15}H_{24}$	512-61-8	99	161	204	0.97
19.67	ST 5	ß-caryophyllene	$C_{15}H_{24}$	87-44-5	99	161	204	4.14
19.84	ST 6	α-bergamotene	$C_{15}H_{24}$	17699-05-7	98	93	119	1.12
20.03	ST 7	ß-cubebene	$C_{15}H_{24}$	13744-15-5	96	161	204	11.2
20.43	ST 8	ß-farnesene	$C_{15}H_{24}$	18794-84-8	97	133	161	1.97
20.68	ST 9	ε-muurolene	$C_{15}H_{24}$	30021-46-6	98	161	204	4.92
21.03	ST 10	Germacrene-D	$C_{15}H_{24}$	23986-74-5	91	161	204	6.25
21.29	ST 11	tetramethylcycloundecatriene	$C_{15}H_{24}$	1000062-61-9	97	147	204	2.68
21.96	ST 12	γ-muurolene	$C_{15}H_{24}$	30021-74-0	99	161	204	1.31
22.52	ST 13	Selinadiene	$C_{15}H_{24}$	1000192-43-5	99	189	204	2.29
22.86	ST 14	γ-selinene	$C_{15}H_{24}$	1000152-04-3	99	161	204	0.68
23.14	ST 15	α-selinene	$C_{15}H_{24}$	473-13-2	97	189	204	0.66
23.34	ST 16	ß-bisabolene	$C_{15}H_{24}$	495-61-4	96	161	204	1.12
23.82	ST 17	α-amorphene	$C_{15}H_{24}$	483-75-0	98	161	204	3.98
23.94	ST 18	δ-cadinene	$C_{15}H_{24}$	483-76-1	98	161	204	1.78
24.11	ST 19	ß-sesquiphellandrene	$C_{15}H_{24}$	20307-83-9	97	161	204	2.02
24.27	ST 20	α-panasinene	$C_{15}H_{24}$	56633-28-4	91	161	204	3.94
24.79	ST 21	α-bisabolene	$C_{15}H_{24}$	29837-07-8	90	93	204	0.25
Fatty Acids								
29.67	FA 1	Lauric Acid	$C_{12}H_{24}O_2$	143-07-7	99	117	257	1.20
44.67	FA 2	Palmitic Acid	$C_{16}H_{32}O_2$	57-10-3	98	117	313	1.15
49.80	FA 3	Linoleic Acid	$C_{18}H_{32}O_2$	60-33-3	99	117	337	0.60
50.01	FA 4	Oleic Acid (cis)	$C_{18}H_{34}O_2$	2027-47-6	99	117	339	1.19
50.17	FA 5	Elaidic Acid (trans)	$C_{18}H_{34}O_2$	112-79-8	99	117	339	0.91
50.75	FA 6	Stearic Acid	$C_{18}H_{36}O_2$	57-11-4	99	117	341	1.14
55.58	FA 7	Eicosenoic Acid	$C_{20}H_{38}O_2$	2462-94-4	90	117	367	0.89
66.04	FA 8	Lignoceric Acid	$C_{24}H_{48}O_2$	557-59-5	90	117	425	1.27
Hydrocarbons								
14.24	H 1	Olefin	C_nH_{2n}	-	97	97	83	0.67
14.54	H 2	Paraffin	C_nH_{2n+2}	-	94	85	71	0.59
30.96	H 3	Olefin	C_nH_{2n}	-	99	97	83	0.81
31.41	H 4	Olefin	C_nH_{2n}	-	99	97	83	0.86
31.91	H 5	Paraffin	C_nH_{2n+2}	-	97	85	71	0.66
38.16	H 6	Olefin	C_nH_{2n}	-	99	97	83	0.67
38.97	H 7	Olefin	C_nH_{2n}	-	99	97	83	0.88
39.29	H 8	Olefin	C_nH_{2n}	-	99	97	83	0.87
39.70	H 9	Paraffin	C_nH_{2n+2}	-	99	85	71	0.64
77.87	H 10	Paraffin	C_nH_{2n+2}	-	95	85	71	0.62
Phytosterols								
64.5	PS 1	Phytosterol(1)	-			357	396	1.09
76.94	PS 2	Stigmasterol	$C_{29}H_{48}O$	83-48-7	96	484	394	1.07
78.92	PS 3	ß-Sitosterol	$C_{29}H_{50}O$	83-46-5	95	357	396	1.18
79.41	PS 4	Phytosterol(2)	-			386	296	1.64
Other Compounds								
15.50	ID 1	Succinic Acid[1]	$C_4H_6O_4$	110-15-6	93	259	147	0.11
16.44	ID 2	Pipecolic Acid[2]	$C_6H_{11}NO_2$	3105-95-1	96	73	156	0.24
18.80	ID 3	Benzene propanoic acid[1]	$C_9H_{10}O_2$	501-52-0	98	104	222	4.68
27.12	ID 4	Caryophillene Oxide[3]	$C_{15}H_{24}O$	1139-30-6	99	109	121	1.49
34.14	ID 5	Glycerol phosphate[4]	$C_3H_9O_6P$	17181-54-3	93	357	299	1.08
36.44	ID 6	Citric Acid[1]	$C_6H_8O_7$	77-92-9	84	273	147	1.1
37.88	ID 7	9.17 Octadecadienal[5]	$C_{18}H_{32}O$	56554-35-9	93	109	95	0.4
38.06	ID 8	Lynolenyl Alcohol[6]	$C_{18}H_{34}O$	506-44-5	93	79	108	2.76
40.35	ID 9	Glycerol diacetate laurate[7]	$C_{19}H_{34}O$	55191-43-0	80	169	73	0.13
45.99	ID 10	Unknown	-	-	-	173	232	1.71
47.75	ID 11	Palmidrol[8]	$C_{18}H_{37}NO_2$	544-31-0	81	85	98	2.62
55.26	ID 12	Myo-Inositol[9]	$C_6H_{19}O_{21}P_5$	55568-91-7	93	387	318	0.34
60.75	ID 13	Hexamethyl mellitate[7]	$C_{18}H_{18}O_{12}$	6237-59-87	82	395	145	2.42
61.01	ID 14	Sucrose[10]	$C_{12}H_{22}O_{11}$	19159-25-2	86	361	437	5.46
65.57	ID 15	Squalene[11]	$C_{30}H_{50}$	7683-64-9	99	81	69	0.54
68.25	ID 16	Piperine[12]	$C_{17}H_{19}NO_3$	94-62-2	99	285	201	0.71
74.03	ID 17	Sesamin[13]	$C_{20}H_{18}O_6$	7076-24-6	96	354	149	0.36
79.83	ID 18	ß-amyrin[3]	$C_{30}H_{50}O$	559-70-6	91	218	203	2.99

[1]carboxylic acid, [2]amino acid, [3]terpenoid, [4]glycerophosphate, [5]aldehyde, [6]alcohol, [7]ester, [8]fatty acid amide, [9]glycerophospholipid, [10]sugar, [11]terpene, [12]alkaloid, [13]polyphenol (lignan)

Table 2. Relative abundance of the compounds in the food commodities relative to the maximum signal corrected by the internal standard per gram of sample

		% relative to maximum				
		Long Pepper	**Masha**	**Masura**	**Mustard**	**Sesamum**
ST_1	α-cubebene	100.0	0.0	0.0	0.0	0.0
ST_2	α-copaene	100.0	0.0	0.0	0.0	0.0
ST_3	β-elemene	100.0	0.0	0.0	0.0	0.0
ST_4	α-santalene	100.0	0.0	0.0	0.0	0.0
ST_5	β-caryophyllene	100.0	0.0	0.0	0.0	0.0
ST_6	α-bergamotene	100.0	0.0	0.0	0.0	0.0
ST_7	β-cubebene	100.0	0.0	0.0	0.0	0.0
ST_8	β-farnesene	100.0	0.0	0.0	0.0	0.0
ST_9	ε-muurolene	100.0	0.0	0.0	0.0	0.0
ST_10	Germacrene-D	100.0	0.0	0.0	0.0	0.0
ST_11	tetramethyl-cycloundecatriene	100.0	0.0	0.0	0.0	0.0
ST_12	γ-muurolene	100.0	0.0	0.0	0.0	0.0
ST_13	Selinadiene	100.0	0.0	0.0	0.0	0.0
ST_14	γ-selinene	100.0	0.0	0.0	0.0	0.0
ST_15	α-selinene	100.0	0.0	0.0	0.0	0.0
ST_16	β-bisabolene	100.0	0.0	0.0	0.0	0.0
ST_17	α-amorphene	100.0	0.0	0.0	0.0	0.0
ST_18	δ-cadinene	100.0	0.0	0.0	0.0	0.0
ST_19	β-sesquiphellandrene	100.0	0.0	0.0	0.0	0.0
ST_20	α-panasinene	100.0	0.0	0.0	0.0	0.0
ST_21	α-bisabolene	100.0	0.0	0.0	0.0	0.0
FA_1	Lauric Acid	100.0	4.7	3.6	1.1	1.2
FA_2	Palmitic Acid	100.0	17.4	10.1	4.2	20.6
FA_3	Linoleic Acid	100.0	6.6	25.5	7.7	64.6
FA_4	Oleic Acid (cis)	100.0	30.9	29.7	9.4	86.2
FA_5	Elaidic Acid (trans)	100.0	11.1	5.5	9.7	12.4
FA_6	Stearic Acid	100.0	38.4	12.5	8.3	52.3
FA_7	Eicosenoic Acid	73.5	4.4	13.9	100.0	6.3
FA_8	Lignoceric Acid	100.0	5.1	3.2	2.8	1.6
H_1	Olefin	100.0	0.0	0.0	0.1	0.0
H_2	Paraffin	100.0	0.0	0.0	0.0	0.0
H_3	Olefin	100.0	0.0	0.0	0.0	0.0
H_4	Olefin	100.0	0.5	0.0	0.1	0.3
H_5	Paraffin	100.0	0.0	0.1	0.0	0.0
H_6	Olefin	0.0	29.6	0.0	0.0	100.0
H_7	Olefin	100.0	0.0	0.3	0.0	0.0
H_8	Olefin	100.0	0.9	0.0	0.0	0.0
H_9	Paraffin	100.0	0.4	0.9	0.0	0.0
H_10	Paraffin	0.0	0.0	0.0	100.0	0.0
PS_1	Phytosterol(1)	0.3	0.0	0.0	100.0	0.0
PS_2	Stigmasterol	32.9	100.0	27.3	0.0	11.1
PS_3	β-Sitosterol	44.3	60.0	100.0	9.9	52.0
PS_4	Phytosterol(2)	10.4	100.0	51.9	0.0	86.9
ID_1	Succinic Acid	0.0	100.0	2.7	0.0	0.0
ID_2	Pipecolic Acid	0.0	100.0	0.0	0.0	0.0
ID_3	Benzene propanoic acid	100.0	0.0	0.1	0.0	0.0
ID_4	Caryophillene Oxide	100.0	0.0	0.0	0.0	0.0
ID_5	Glycerol phosphate	5.3	100.0	44.1	2.8	3.5
ID_6	Citric Acid	0.0	100.0	4.3	0.6	0.4
ID_7	9,17 Octadecadienal	0.0	12.7	30.9	0.0	100.0
ID_8	Lynolenyl Alcohol	0.0	100.0	9.9	0.0	46.6
ID_9	Glycerol diacetate laurate	0.0	100.0	5.9	0.0	22.1
ID_10	Unknown	100.0	0.8	0.1	0.0	0.1
ID_11	Palmidrol	0.0	100.0	85.3	0.0	0.0
ID_12	Myo-Inositol	0.0	100.0	0.12	61.6	0.1
ID_13	Hexamethyl mellitate	7.2	0.0	0.7	100.0	0.0
ID_14	Sucrose	0.4	100.0	26.2	4.2	0.2
ID_15	Squalene	100.0	0.0	14.3	13.7	6.1
ID_16	Piperine	100.0	0.0	0.0	0.0	0.0
ID_17	Sesamin	0.0	0.0	0.0	0.0	100.0
ID_18	β-amyrin	0.0	17.1	100.0	0.0	0.0

Table 3. Abundance of the compounds in the experimental pot samples corrected by the internal standard per gram of sample. Ground and Whole food, Internal and Surface samples are indicated as GFP, WFP, I and S, respectively. Different points of the pot considered are indicated as base, body and rim.

	GFP-I-base	GFP-I-body	GFP-I-rim	GFP-S-base	GFP-S-body	GFP-S-rim	WFP-I-base	WFP-I-body	WFP-I-rim	WFP-S-base	WFP-S-body	WFP-S-rim
ST_1	-	-	-	-	-	-	-	-	-	-	-	-
ST_2	-	-	-	-	-	-	-	-	-	-	-	-
ST_3	-	-	-	-	-	-	-	-	-	-	-	-
ST_4	-	-	-	-	-	-	-	-	-	-	-	-
ST_5	-	-	-	-	-	-	-	-	-	-	-	-
ST_6	-	-	-	-	-	-	-	-	-	-	-	-
ST_7	-	-	-	-	-	-	-	-	-	-	-	-
ST_8	-	-	-	-	-	-	-	-	-	-	-	-
ST_9	-	-	-	-	-	-	-	-	-	-	-	-
ST_10	-	-	-	-	-	-	-	-	-	-	-	-
ST_11	-	-	-	-	-	-	-	-	-	-	-	-
ST_12	-	-	-	-	-	-	-	-	-	-	-	-
ST_13	-	-	-	-	-	-	-	-	-	-	-	-
ST_14	-	-	-	-	-	-	-	-	-	-	-	-
ST_15	-	-	-	-	-	-	-	-	-	-	-	-
ST_16	-	-	-	-	-	-	-	-	-	-	-	-
ST_17	-	-	-	-	-	-	-	-	-	-	-	-
ST_18	-	-	-	-	-	-	-	-	-	-	-	-
ST_19	-	-	-	-	-	-	-	-	-	-	-	-
ST_20	-	-	-	-	-	-	-	-	-	-	-	-
ST_21	-	-	-	-	-	-	-	-	-	-	-	-
FA_1	0.012	3.0	22.2	19.0	42.0	100	1.65	0.92	2.1	1.4	3.1	16.2
FA_2	0.005	4.8	18.0	35.9	78.1	100	0.63	0.48	1.6	9.6	13.6	21.2
FA_3	0.002	5.7	12.7	44.7	100.0	88.6	0.15	0.08	0.8	6.9	11.2	13.5
FA_4	0.005	7.3	21.6	41.0	78.9	100	0.56	0.42	2.2	11.0	15.9	25.0
FA_5	0.003	3.7	18.4	31.7	66.5	100	0.21	0.09	0.6	6.6	10.8	12.2
FA_6	0.008	4.5	17.1	34.3	81.0	100	1.16	1.01	2.5	16.5	24.3	29.0
FA_7	0.001	4.1	17.6	33.1	75.7	100	0.02	0.01	0.1	0.7	1.2	4.3
FA_8	0.001	2.8	12.1	31.7	73.8	100	0.13	0.16	0.4	2.3	3.7	10.7
H_1	-	-	-	-	-	-	-	-	-	-	-	-
H_2	0.039	3.2	12.1	26.9	32.9	100.0	5.1	8.0	3.5	2.9	5.5	4.9
H_3	0.010	1.8	6.4	52.0	89.9	100.0	0.6	0.7	0.5	0.4	0.5	0.5
H_4	-	-	1.8	58.2	100.0	69.2	0.5	0.4	0.3	0.6	0.3	0.5
H_5	0.022	2.8	3.4	58.3	100.0	71.9	0.8	1.0	0.8	1.1	0.7	1.0
H_6	-	-	26.0	51.2	87.7	100.0	-	-	-	-	-	-
H_7	0.017	2.5	5.1	47.6	97.5	100.0	-	-	-	-	-	-
H_8	0.015	2.0	1.9	50.5	100.0	55.1	-	-	-	-	-	
H_9	0.015	1.8	5.4	38.9	67.6	100.0	1.7	2.1	1.3	1.3	1.6	1.9
H_10	-	-	-	71.6	100.0	87.5	17.3	25.7	23.0	23.8	22.9	32.4
PS_1	-	-	-	-	-	-	-	-	-	-	-	-
PS_2	0.00026	3.0	8.9	58.7	100.0	76.7	3.8	3.9	5.2	7.8	14.5	21.5
PS_3	0.00133	4.1	17.4	56.5	100.0	88.9	0.3	0.2	0.9	6.0	10.4	10.5
PS_4	-	3.7	11.0	54.0	100.0	76.5	0.3	-	0.6	6.2	11.2	11.9
ID_1	-	-	-	-	-	-	-	-	-	-	-	-
ID_2	-	-	-	77.9	88.9	100.0	-	-	5.2	-	3.0	6.2
ID_3	0.0015	1.83	8.1	12.8	48.5	15.3	0.042	0.105	100.0	0.2	0.8	8.8
ID_4	-	0.36	21.2	17.0	100.0	42.3	-	-	1.6	-	-	1.1
ID_5	-	0.03	1.1	1.9	45.1	100.0	-	-	-	2.3	5.8	18.5
ID_6	-	-	-	-	-	-	-	-	-	-	-	-
ID_7	-	-	-	-	-	-	-	-	-	-	-	-
ID_8	-	8.01	16.9	71.7	76.6	100.0	-	-	-		0.01	0.01
ID_9	-	1.73	45.0	20.8	59.7	100.0	-	-	-	2.3	13.2	11.2
ID_10	-	-	-	22.4	100.0	53.1	-	-	-	-	0.2	0.4
ID_11	-	-	-	-	-	-	-	-	-	-	-	-
ID_12	-	-	-	52.8	100.0	9.9	-	-	-	-	-	1.7
ID_13	0.0030	8.54	20.8	48.9	77.1	100.0	0.024	0.006	0.1	0.3	0.3	2.8
ID_14	-	-	-	-	-	-	-	-	-	-	-	-
ID_15	0.0010	2.94	4.3	29.6	70.1	100.0	0.233	0.143	0.6	1.1	2.9	5.2

ID_16	0.0008	0.65	1.8	22.8	100.0	44.6	0.210	0.059	1.7	0.7	3.1	3.1
ID_17	0.0010	2.09	10.5	55.9	100.0	73.2	0.681	0.147	1.5	2.3	5.2	3.5
ID_18	-	1.66	5.3	63.1	100.0	61.4	-	-	1.4	3.7	12.8	10.0

Table 4. Abundance of the compounds in the archaeological pot samples corrected by the internal standard per gram of sample.

Label ID	Compound	CP1-I-rim	CP1-S-rim	CP2-I-body	CP3-I-body	CP3-S-body
FA_1	Lauric Acid	0.28	1.24	0.29	0.10	0.84
FA_2	Palmitic Acid	6.94	32.8	8.65	7.72	38.9
FA_3	Linoleic Acid	0.07	0.37	0.003	-	0.06
FA_4	Oleic Acid (cis)	0.38	1.92	0.23	0.29	1.48
FA_5	Elaidic Acid (trans)	0.05	0.23	0.02	0.05	0.19
FA_6	Stearic Acid	4.84	17.4	7.24	7.07	30.4
FA_7	Eicosenoic Acid	-	-	-	-	-
FA_8	Lignoceric Acid	0.04	0.17	0.15	0.17	2.50
H_1	Olefin	-	-	-	-	-
H_2	Paraffin	0.007	0.156	0.048	0.013	0.053
H_3	Olefin	0.004	0.013	0.007	0.005	0.016
H_4	Olefin	0.002	0.007	0.004	0.002	0.004
H_5	Paraffin	-	-	-	-	-
H_6	Olefin	-	0.004	0.001	0.001	0.002
H_7	Olefin	0.005	0.009	0.002	0.001	0.011
H_8	Olefin	0.040	0.614	0.073	0.044	0.178
H_9	Paraffin	-	0.297	0.002	0.002	-
H_10	Paraffin	0.022	0.064	0.018	0.015	0.068
PS_1	Phytosterol(1)	-	-	-	-	-
PS_2	Stigmasterol	0.06	0.08	0.04	0.01	0.04
PS_3	β-Sitosterol	0.26	0.72	0.31	0.12	0.52
PS_4	Phytosterol(2)	0.18	0.07	0.04	0.01	0.06
ID_1	Succinic Acid[1]	-	-	-	-	-
ID_2	Pipecolic Acid[2]	-	-	-	-	-
ID_3	Benzene propanoic acid[1]	-	-	-	-	-
ID_4	Caryophillene Oxide[3]	-	-	-	-	0.016
ID_5	Glycerol phosphate[4]	-	-	-	-	-
ID_6	Citric Acid[1]	-	-	-	-	-
ID_7	9,17 Octadecadienal[5]	-	-	-	-	-
ID_8	Lynolenyl Alcohol[6]	-	-	-	-	-
ID_9	Glycerol diacetate laurate[7]	-	-	-	-	-
ID_10	Unknown	-	-	-	-	-
ID_11	Palmidrol[8]	-	-	-	-	-
ID_12	Myo-Inositol[9]	-	-	-	-	-
ID_13	Hexamethyl mellitate[7]	-	-	-	-	-
ID_14	Sucrose[10]	-	-	-	-	-
ID_15	Squalene[11]	0.021	0.036	0.010	0.016	0.053
ID_16	Piperine[12]	-	-	-	-	0.170
ID_17	Sesamin[13]	0.001	0.012	-	-	-
ID_18	β-amyrin[3]	0.006	0.013	-	-	-

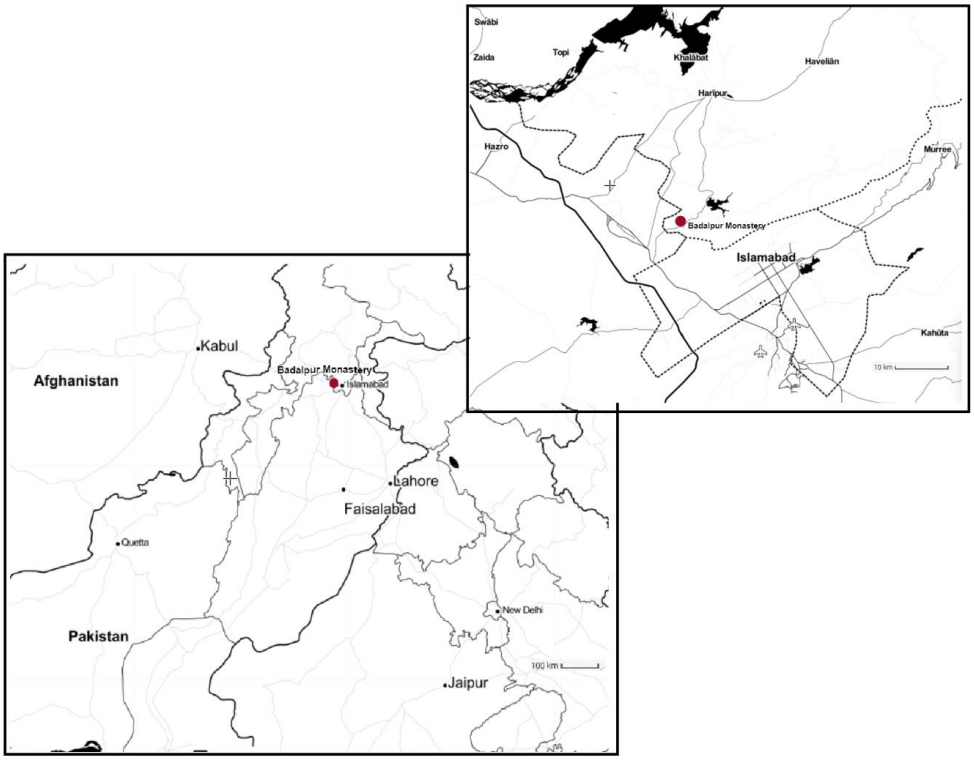

Figure 1. Map of the Badalpur archaeological site.

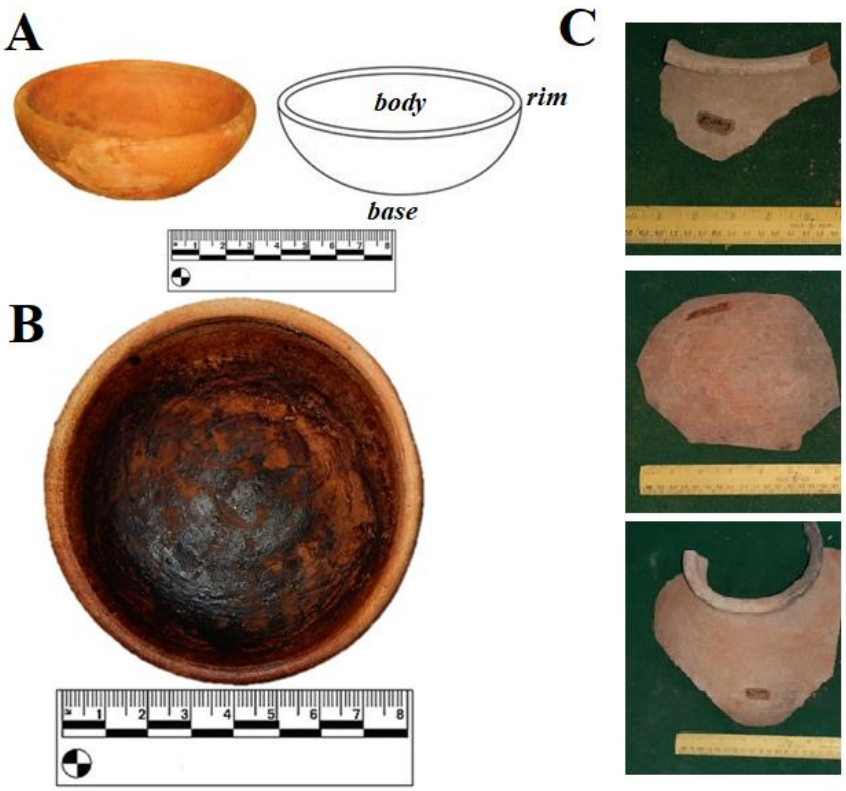

Figure 2. Photographs of the experimental pot before (A) and after (B) cooking. (C) archaeological pottery sherds considered in this study

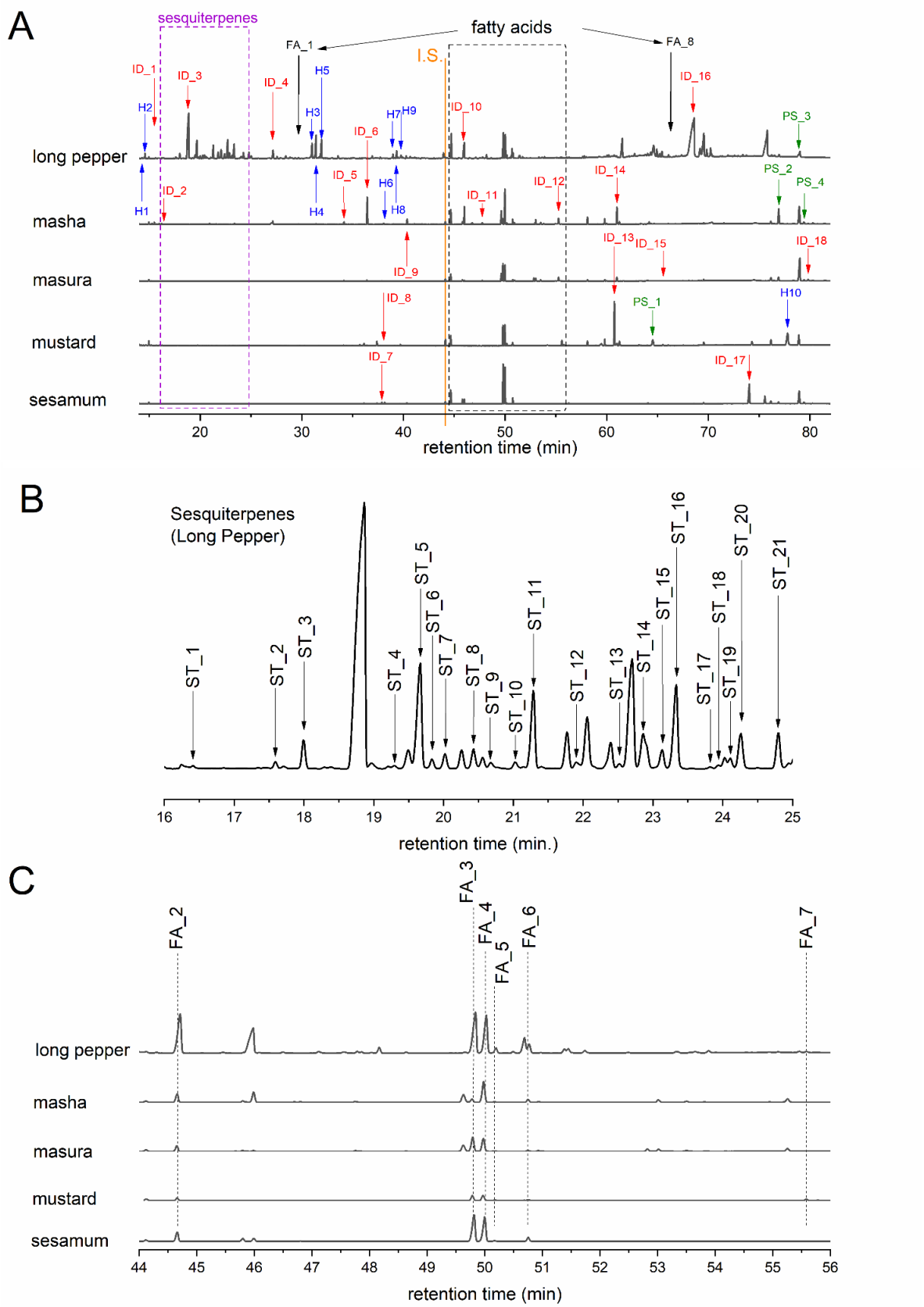

Figure 3. TIC chromatograms of the food commodities extracts (A), sesquiterpenes found in long pepper (B) and fatty acids (C).

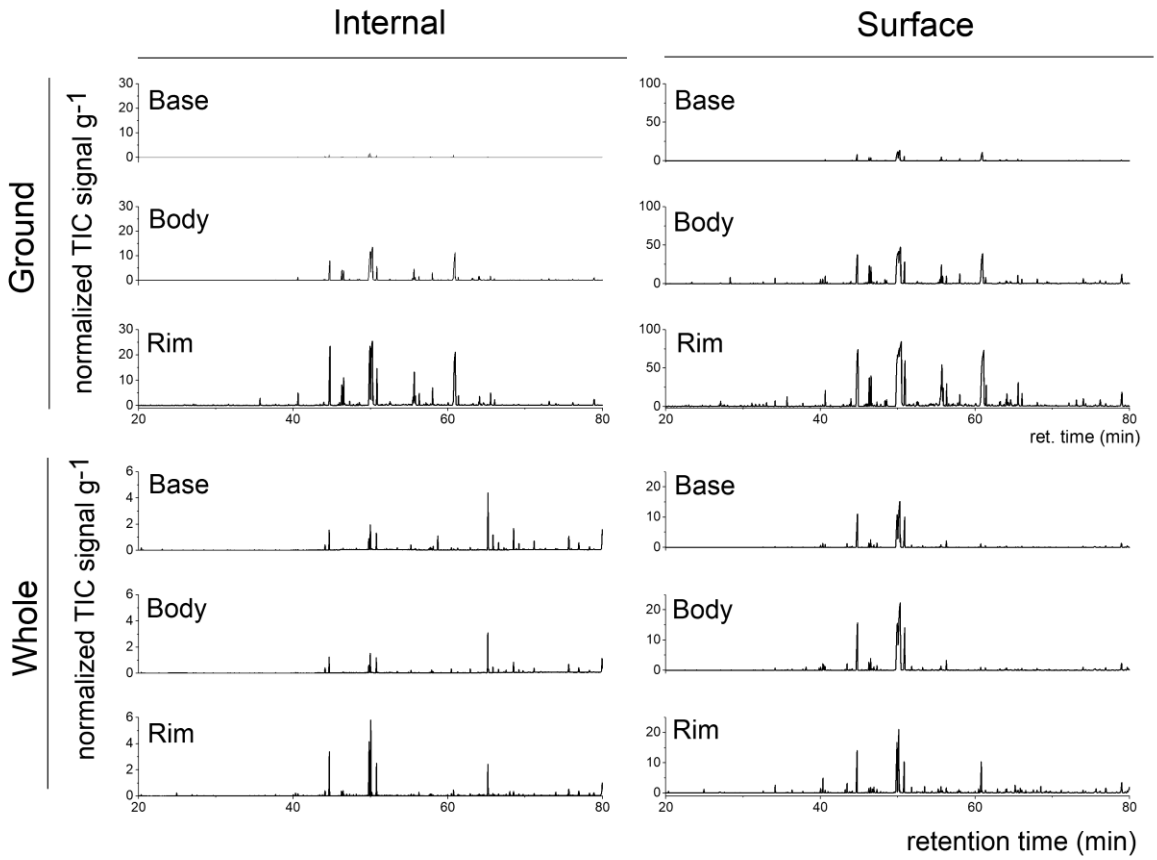

Figure 4. TIC chromatograms obtained from the experimental pot samples

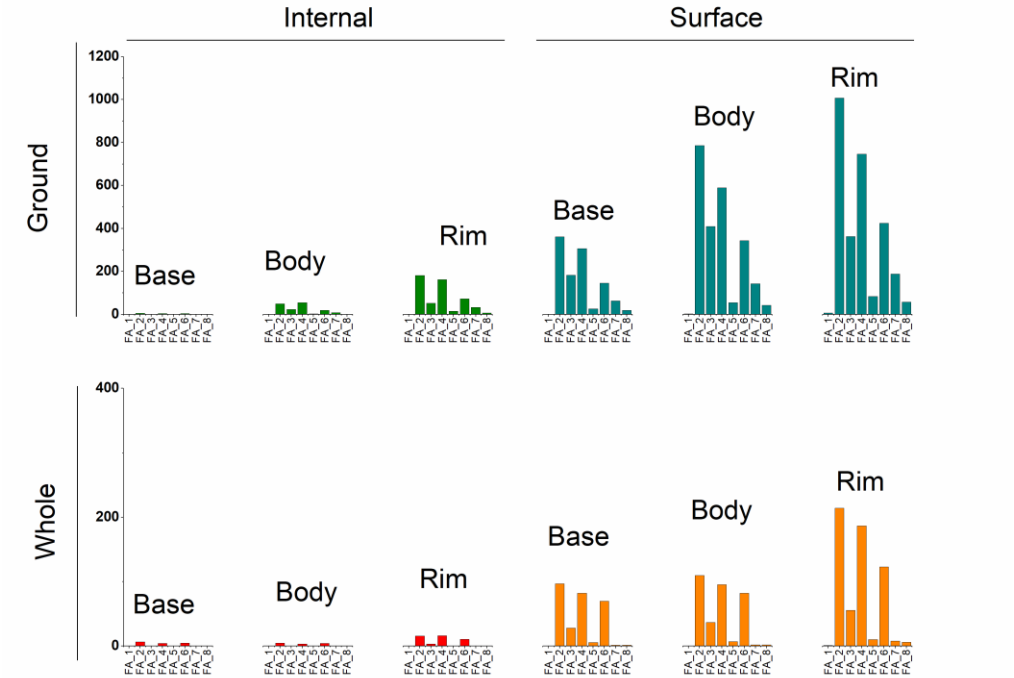

Figure 5. Fatty acids distribution in the different points of the experimental pot collected (base, body, rim) for the internal part and surface, after cooking ground and whole food commodities. Y-scale are reported as m/z target signal corrected by androstane (m/z=245) per gram of sample.

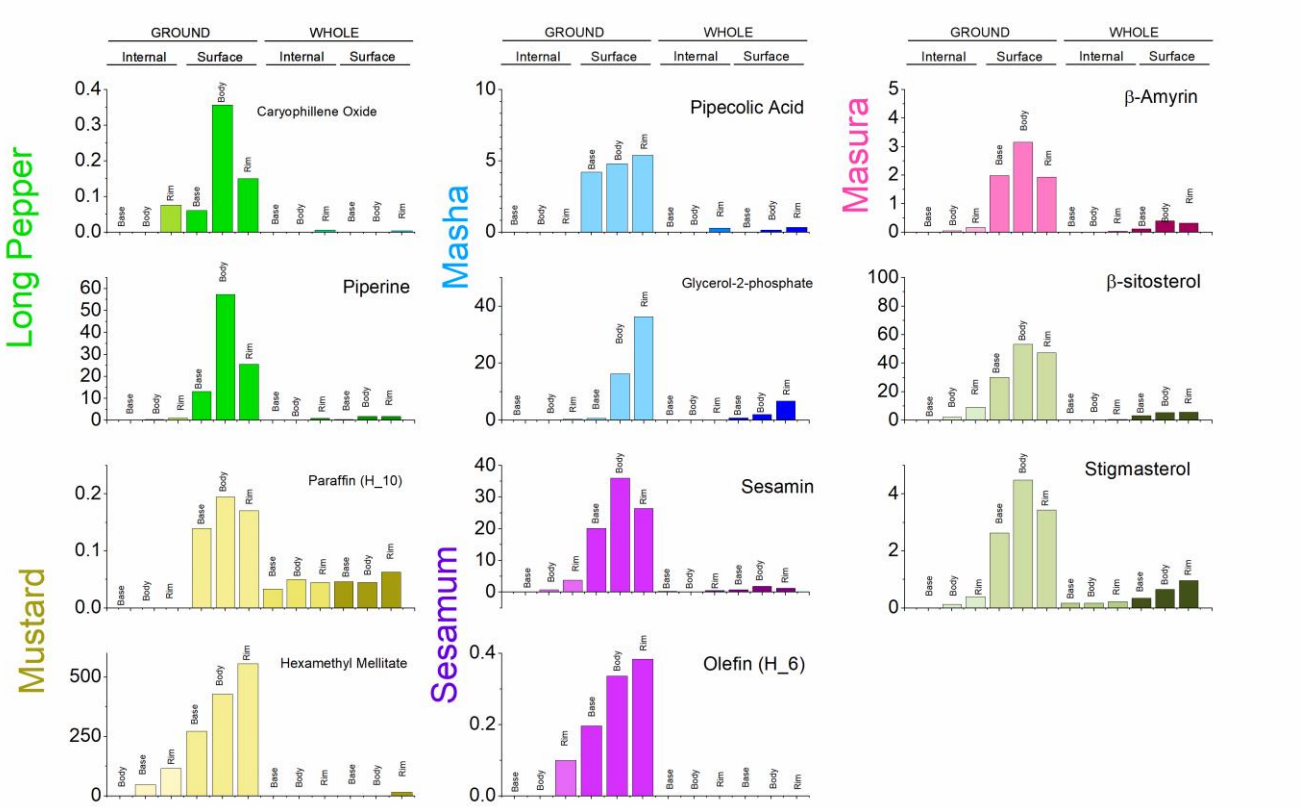

Figure 6. Distribution of the more promising markers for the food items studied in this paper found in the experimental pot.

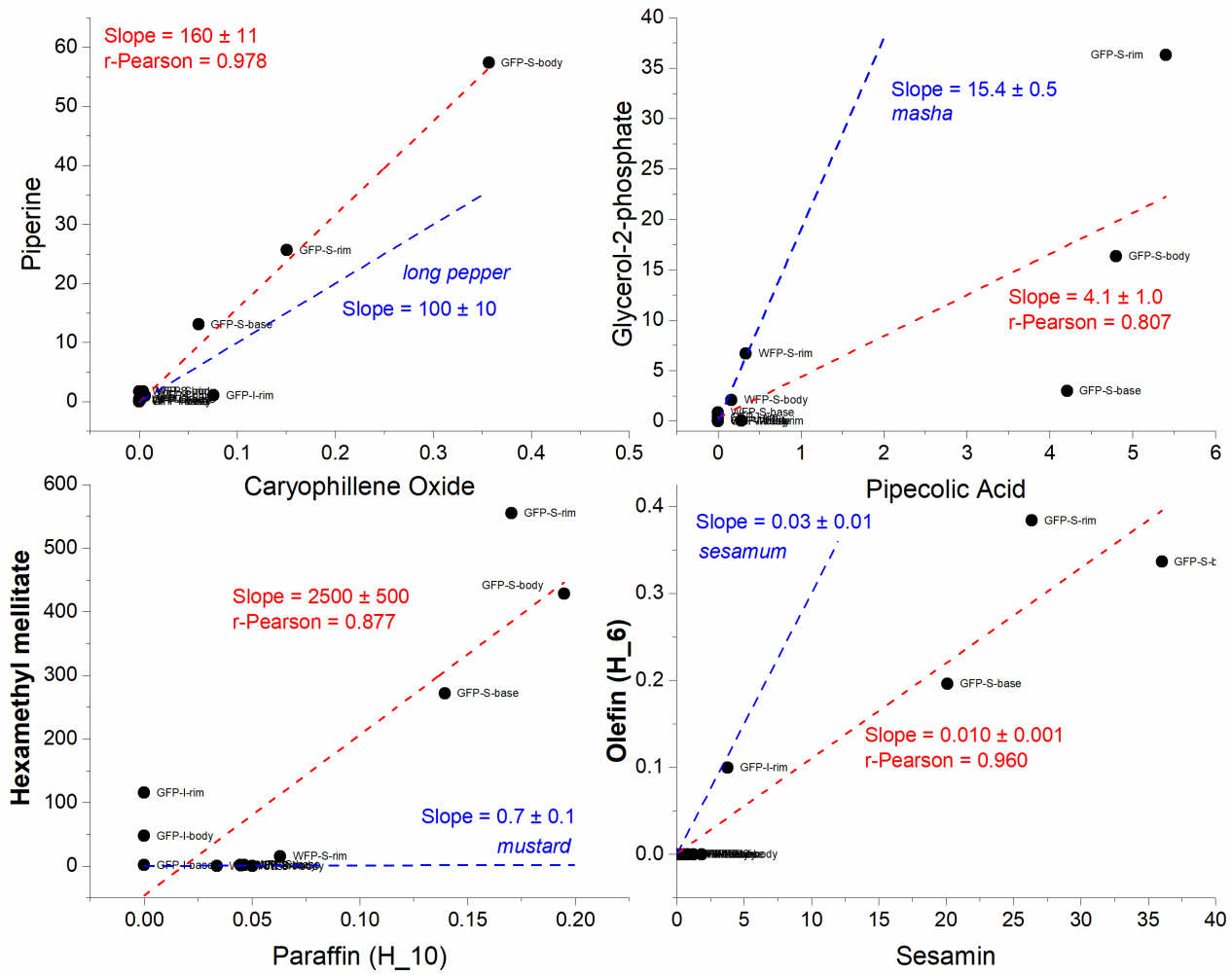

Figure 7. Correlation between food markers (red dotted lines) compared with food items (blue dotted lines).

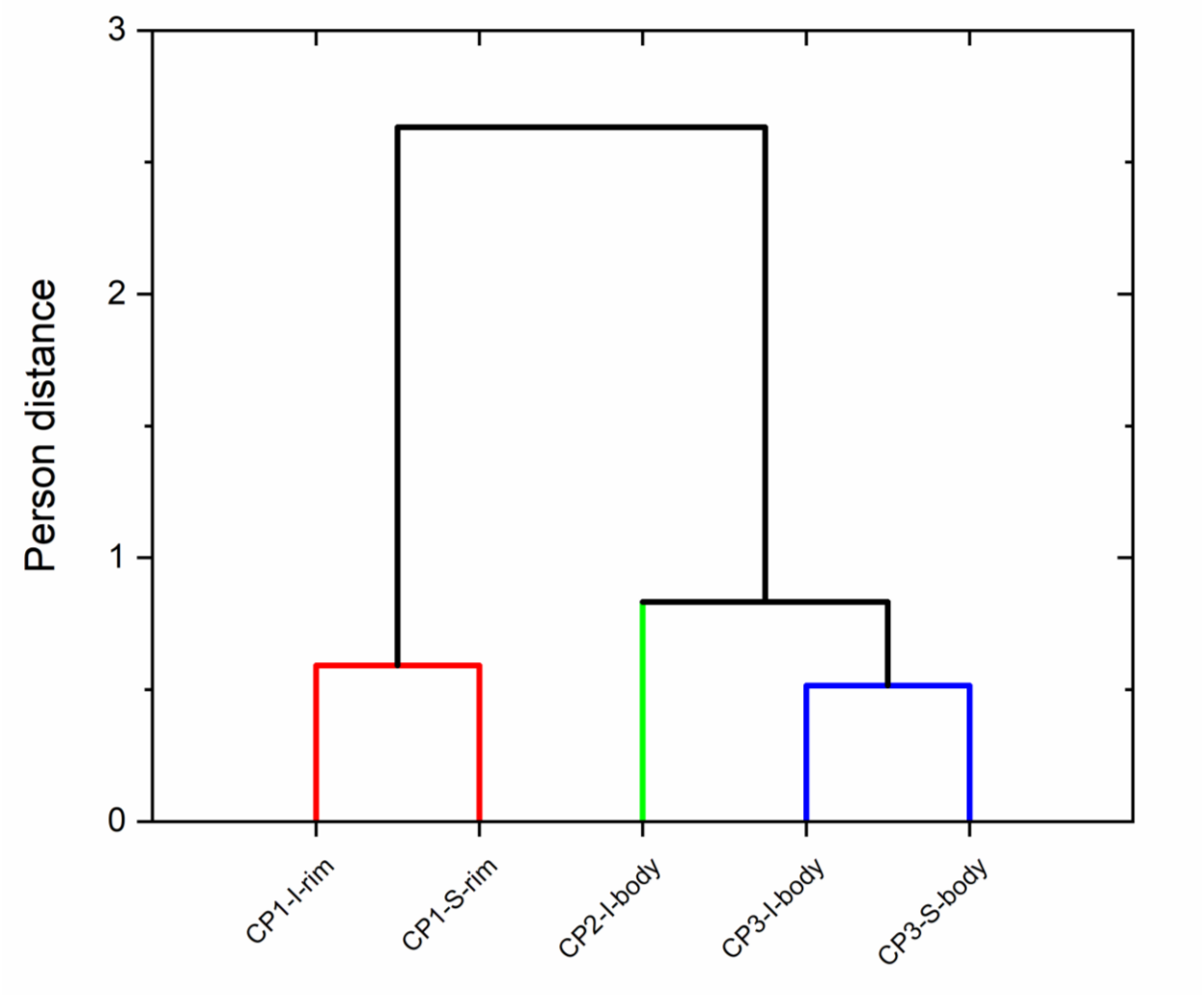

Figure 8. Supporting Material S1