Use of Thermally Assisted Hydrolysis and Methylation (THM-GC-MS) to Unravel Influence of Pottery Production and Post-Depositional Processes on the Molecular Composition of Organic Matter in Sherds from a Complex Coastal Settlement

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Abstract: Ceramic fragments from the Islet of Guidoiro Areoso (NW Spain), covering a wide range of cultural periods (Neolithic to Late Bronze Age), have been studied by color analysis, elemental analysis of carbon (C) and nitrogen (N), and molecular analysis (thermally assisted hydrolysis and methylation, THM-GC-MS), in order to identify the organic matter (OM) in the prehistoric pottery and reveal information on ceramic production techniques, food remains and post-depositional effects. Results showed that the strong marine influence (sherds recovered from coastal deposits) and microbial activity (recovery from waste deposits, “cuncheiros”) had a profound effect on C/N ratio and molecular composition (N-rich protein and chitin structures). Other organic ingredients originated from the material used for creating the ware (detected as pyrogenic OM) and possibly food remains (fatty acid fingerprints). Dark-colored ware was enriched in both pyrogenic OM from incomplete combustion and non-bacterial fatty acids. Fatty acid patterns could not be related to possible vessel use, and markers of aquatic resources were scarce, or absent. It is argued that THM-GC-MS of pottery fragments is useful for understanding how an archaeological deposit developed in time, what kinds of OM are present, and possibly to make a pre-selection of samples with high potential for more cost-demanding dietary molecular assessments.

Keywords: ceramics; molecular characterization; analytical pyrolysis; THM-GC-MS; color analysis; organic matter; coastal settlement; marine environment

1. Introduction

In spite of the advances in organic chemistry applications for archaeometry, there is still potential to be explored. Molecular research on organic constituents in ceramic materials have traditionally focused on total lipid extracts, aiming to characterize the preserved food residues, for instance by biomarker analysis (liquid and gas chromatography (GC) coupled to mass spectrometry (MS), such as GC-MS) [1–4] or compound-specific isotope ratio MS of lipid extracts from ceramic fragments [5,6], to obtain invaluable dietary information. However, recovered ceramic fragments contain organic matter (OM) from a wide range of possible sources, namely (1) the raw material clay fabric (incomplete burn-off during firing), (2) organic tempers and binders (incomplete burn-off), (3) soot deposited into or onto the ceramic during firing or during use (cooking), (4) decoration and finishing materials (varnishes, pigments, etc.), (5) use (food) residues, (6) post-use accumulations from the burial environment (soil OM, dissolved OM), (7) contamination from handling during excavation or laboratory treatments [7–9]. Identification and characterization of
these sources of OM can reveal information related to ware production parameters (material selection, firing conditions) and vessel use, and therewith assist in the contextualization of pottery fragments. Many of the OM ingredients are macromolecular in nature and cannot be identified by means of molecular analyses of solvent extracts and require chemical or thermal treatment before identification [10].

Analytical pyrolysis techniques are frequently applied for the characterization of non-extractable OM in archaeological artifacts [11]. It has been used to identify paint ingredients, characterize jewelry or organic inclusions therein (resins, wooden artifacts etc.), identify animal tissues (hair, bone collagen, etc.), among others [12–17]. It has significant drawbacks, such as quantitative weakness, secondary reactions, loss of diagnostic features [18] and poor detection of ingredients that are present in trace concentrations. Therefore, analytical pyrolysis has significant limitations for the analysis of ceramics, especially for ingredients of low abundance relative to total OM such as food remains [19]. On the other hand, it has a strong capacity to provide a general screening of organic constituents that can be present, such as soot, charred temper, food residues and OM from the burial environment. Conventional analytical pyrolysis (Py-GC-MS) has been applied to archaeological potsherds with some frequency (e.g., [10,20–23]). Kaal et al. [20] performed statistical data evaluation of Py-GC-MS fingerprints of 24 ceramic samples from various settlements in NW Spain and compared the results with those from color and elemental analyses. Another pyrolysis method is thermally assisted hydrolysis and methylation THM-GC-MS [24], in which a reagent such as tetramethylammonium hydroxide (TMAH) is added to protect polar functional groups against pyrolytic degradation and improve their chromatographic separation [24]. THM-GC-MS has not often been used for ceramic material analysis in general. Shoda et al. [25] recently reported THM-GC-MS data of Neolithic pottery from Korea, using ω-(ω-alkylphenyl) alkanoic acids, isoprenoid fatty acids and triterpenoids as biomarkers of palaeodiet and in particular aquatic resources. Inspired by these results, we aimed to explore the potential of THM-GC-MS for the characterization of OM in a series of well-characterized ceramics recovered from Guidoiro Areoso islet in Galicia (NW Spain). This settlement is suitable as it contains ware from various cultural periods. In addition, being close to the (actual) see level, this set of ceramics has an added complexity of strong marine influences, reflected by for instance mollusks growing on potsherd surfaces. In addition, such coastal environments may be more likely to contain residues of aquatic resources that produce isoprenoid fatty acids [25–27].

The present study explores 50 samples analyzed by THM-GC-MS, color analysis and elemental analysis to determine the amounts of carbon (C) and nitrogen (N). The objective was to identify and characterize different OM components and link their abundances with pottery production, use and burial conditions. This study’s novelty resides not only in the fact that a reasonably large number of potsherds are studied using THM-GC-MS and statistical data evaluation, but also in the unique marine-influenced environment from which the vessels were collected.

2. Materials and Methods

2.1. The Site

The settlement of origin of the ceramics is the megalith “Mámoa Nr. 4” (M4) of Guidoiro Areoso islet (Illa de Arousa, Pontevedra, NW Spain; Figure 1), excavated in 2017. It has a long record of ritual activity, at least from the Middle Neolithic to the Late Bronze Age, divided in five chronological periods [28]. Different forms of burial have been recorded, i.e., megaliths and a cist necropolis [29]. The megalithic activity is subdivided in Middle Neolithic, with many lithic and other artifacts yet ceramics are scarce, and Late Neolithic (bone remains, grave goods) with more fragments. The megalithic funerary activity then ends (stones placed to block entry) and the mound changed in function, becoming some kind of a shell waste deposit (“cuncheiro”, or midden), probably related to a ceremonial role (including banquets/feasts), from the final Late Neolithic stage to the Middle Bronze Age. At the first moment of activity in the midden, Penha and Bell
Beaker Imitation type wares [30] were recovered. This kind of pots are related to Late Neolithic chronologies. The Early Bronze Age is mainly represented by different types of Bell Beaker pottery (undecorated Bell Beakers and some of the regional variations of the Bell Beaker pottery for Galicia [31]). The heterogeneity of Early Bronze Age pottery suggests several moments of activity along this period. During the Middle Bronze Age the midden continues to grow in all directions, containing many in situ potsherds. Then, at the beginning of Late Bronze Age (Late Bronze Age I), ceremonial activities related to the midden halted, and the midden was buried by sediment. On this new soil surface, large ware had been placed on top. Finally, during the Late Bronze Age II, different structures were built on the mounds. Stones, cists, millstones and typical Late Bronze Age pottery were found.

Figure 1. Location of the Guidoiro Areoso islet in the Ría de Arousa (modified after [32]).

2.2. Sample Selection and Preparation

More than 3600 fragments were recovered [33], 145 of which were studied in detail, of which a final selection of 50 samples was made for color, elemental and molecular analyses (Table 1). The selection aimed to obtain a representative number of fragments from each cultural period. Therefore, we selected fragments that allowed for a detailed characterization and well-defined position within the deposit stratigraphy.

The surfaces of the pottery fragments were carefully and gently cleaned from externally bound sediment using distilled water, and the samples were ground to very fine powder (<50 µm).
Table 1. Sample codes, cultural period and possible sources of contamination (VAR1 and VAR2, see Section 3.3). Continuous variables correspond to elemental analysis (%C, molar C/N) and CIELAB color parameters for luminosity (L*), redness (a*), yellowness (b*), chroma (Chr) and hue.

| Sample      | Cultural Period | VAR1                  | VAR2                  | % C   | C:N   | L*   | a*   | b*   | Chr   | Hue  |
|-------------|----------------|-----------------------|-----------------------|-------|-------|------|------|------|-------|------|
| CAM4A-001   | Late Bronze Age I | Intermediate Post waste deposit | 0.76 12.7 53 | 14 | 26 | 30 | 61 |
| CAM4A-002   | Late Bronze Age II | Low Post waste deposit | 1.26 18.9 49 | 10 | 21 | 23 | 65 |
| CAM4A-006   | Late Bronze Age II | Low Marine influence | 0.86 17.6 55 | 8 | 19 | 21 | 69 |
| CAM4A-007   | Late Bronze Age II | Low Marine influence | 1.20 20.1 47 | 3 | 10 | 10 | 74 |
| CAM4A-009   | Late Bronze Age I | Intermediate Post waste deposit | 0.65 15.8 47 | 13 | 26 | 29 | 64 |
| CAM4A-010   | Early Bronze Age | Intermediate Post waste deposit | 1.36 13.6 48 | 6 | 14 | 15 | 69 |
| CAM4A-011   | Middle Bronze Age | Intermediate Post waste deposit | 1.28 12.6 50 | 9 | 22 | 24 | 67 |
| CAM4A-012   | Late Bronze Age II | Low Marine influence | 1.53 17.2 42 | 6 | 15 | 16 | 68 |
| CAM4A-013   | Middle Bronze Age | High Waste deposit | 3.29 33.3 39 | 10 | 19 | 22 | 63 |
| CAM4A-014   | Late Neolithic | High Waste deposit | 1.08 14.0 50 | 5 | 14 | 14 | 71 |
| CAM4A-015   | Early Bronze Age | High Waste deposit | 1.12 14.9 55 | 14 | 26 | 30 | 63 |
| CAM4A-016   | Middle Bronze Age | High Waste deposit | 0.87 14.2 53 | 18 | 31 | 36 | 61 |
| CAM4A-018   | Late Neolithic | Intermediate Post waste deposit | 2.27 34.5 34 | 2 | 11 | 11 | 77 |
| CAM4A-021   | Early Bronze Age | Low Post waste deposit | 2.49 25.9 33 | 2 | 6 | 6 | 74 |
| CAM4A-025   | Late Bronze Age I | Intermediate Post waste deposit | 2.07 18.8 36 | 6 | 14 | 15 | 68 |
| CAM4A-034   | Late Bronze Age II | Low Marine influence | 1.50 21.9 43 | 3 | 11 | 11 | 72 |
| CAM4A-035   | Late Bronze Age I | Intermediate Marine influence | 0.79 16.9 50 | 12 | 24 | 27 | 64 |
| CAM4A-037   | Late Bronze Age I | Intermediate Post waste deposit | 1.89 23.3 38 | 8 | 16 | 18 | 65 |
| CAM4A-040   | Middle Bronze Age | High Waste deposit | 3.12 27.6 33 | 5 | 11 | 12 | 66 |
| CAM4A-045   | Middle Bronze Age | High Waste deposit | 0.82 12.6 48 | 8 | 19 | 21 | 67 |
| CAM4A-046   | Early Bronze Age | High Waste deposit | 1.44 12.7 53 | 6 | 16 | 17 | 69 |
| CAM4A-049   | Early Bronze Age | Low Marine influence | 1.53 14.5 42 | 7 | 17 | 19 | 69 |
| CAM4A-050   | Middle Bronze Age | Intermediate Post waste deposit | 1.03 20.1 58 | 15 | 29 | 32 | 63 |
| CAM4A-058   | Early Bronze Age | High Waste deposit | 2.47 25.4 44 | 5 | 14 | 15 | 69 |
| CAM4A-059   | Early Bronze Age | High Waste deposit | 1.75 14.7 44 | 5 | 14 | 15 | 72 |
| CAM4A-062   | Middle Bronze Age | High Waste deposit | 1.19 14.9 49 | 10 | 21 | 23 | 66 |
| CAM4A-063   | Early Bronze Age | High Waste deposit | 1.39 13.5 54 | 9 | 20 | 21 | 65 |
| CAM4A-065   | Middle Bronze Age | High Waste deposit | 1.77 18.5 42 | 5 | 14 | 15 | 69 |
| CAM4A-066   | Early Bronze Age | Intermediate Post waste deposit | 0.98 13.7 45 | 4 | 14 | 15 | 73 |
| CAM4A-069   | Early Bronze Age | Intermediate Post waste deposit | 1.83 15.9 40 | 5 | 13 | 14 | 68 |
| CAM4A-070   | Late Neolithic | High Waste deposit | 2.05 16.4 37 | 3 | 9 | 10 | 70 |
| CAM4A-071   | Middle Bronze Age | Low Marine influence | 2.05 29.6 39 | 5 | 14 | 14 | 70 |
| CAM4A-072   | Late Neolithic | High Waste deposit | 2.00 20.4 39 | 4 | 10 | 11 | 70 |
| CAM4A-073   | Middle Bronze Age | High Waste deposit | 1.56 18.4 41 | 4 | 12 | 13 | 70 |
| CAM4A-075   | Middle Bronze Age | High Waste deposit | 0.92 16.6 50 | 17 | 30 | 34 | 60 |
| CAM4A-077   | Middle Neolithic | Intermediate Marine influence | 1.49 20.2 42 | 7 | 17 | 18 | 68 |
| CAM4A-080   | Late Bronze Age II | High Marine influence | 4.99 37.1 27 | 2 | 5 | 5 | 68 |
| CAM4A-081   | Late Bronze Age II | High Marine influence | 2.46 14.2 39 | 2 | 8 | 9 | 74 |
| CAM4A-082   | Early Bronze Age | High Waste deposit | 1.26 19.1 45 | 9 | 20 | 22 | 66 |
| CAM4A-085   | Late Bronze Age I | Low Marine influence | 4.56 52.9 27 | 3 | 6 | 7 | 63 |
| CAM4A-091   | Late Neolithic | Intermediate Post waste deposit | 0.95 12.4 48 | 9 | 18 | 20 | 64 |
| CAM4A-092   | Late Neolithic | High Waste deposit | 1.71 11.2 39 | 3 | 10 | 11 | 71 |
| CAM4A-097   | Middle Bronze Age | High Waste deposit | 1.37 23.0 52 | 6 | 17 | 18 | 69 |
| CAM4A-100   | Late Neolithic | Low Marine influence | 1.95 24.6 32 | 5 | 11 | 12 | 67 |
| CAM4A-101   | Late Neolithic | Low Marine influence | 1.04 15.6 57 | 2 | 9 | 7 | 95 |
| CAM4A-102   | Early Bronze Age | High Waste deposit | 1.23 17.6 47 | 8 | 18 | 20 | 66 |
| CAM4A-103   | Middle Bronze Age | High Waste deposit | 1.31 15.9 48 | 5 | 15 | 16 | 71 |
| CAM4A-105   | Middle Bronze Age | High Waste deposit | 2.31 30.1 33 | 4 | 9 | 9 | 66 |
| CAM4A-107   | Late Bronze Age I | Intermediate Post waste deposit | 1.47 19.9 49 | 12 | 24 | 27 | 63 |
| CAM4A-109   | Middle Bronze Age | High Waste deposit | 1.79 28.1 40 | 5 | 14 | 15 | 69 |

2.3. Color and Elemental Analyses

Quantitative color analysis in the CIE L*a*b*C*h (CIELAB) color space was done using a Konica Minolta CR-5 colorimeter [34]. Samples were analyzed in the powder form. L* represents luminosity (L), primary color components (a*: green-red axis, and...
b*: blue-yellow axis) chromaticity (C*) and hue (h). Carbon and nitrogen contents were obtained by combustion of 100 mg of finely milled samples using a Truspec CHNS analyzer (Leco) hosted at the RIAIDT services of the University of Santiago de Compostela.

2.4. THM-GC-MS

An aliquot of 10 µL of tetramethylammonium hydroxide (TMAH, in water, 25%, Sigma-Aldrich, St. Louis, MO, USA) was added to the fire-polished quartz tubes in which 1–2 mg of sample was embedded. The setpoint temperature of the THM reaction was 650 ºC, maintained for 20 s, using a Pyroprobe pyrolyzer (CDS Analytical, Oxford, PA, USA). The pyrolyzer was connected online to an 8860 GC (Agilent, Santa Clara, USA). Analytical-grade helium was the carrier gas (1 mL/min constant flow). The pyrolyzer’s interface oven, the GC inlet and the GC-MS interface were held isothermally at 325 ºC. The GC was equipped with a HP-5MS non-polar column, and was in split mode (1:25). The GC was heated from 60 to 325 ºC at a rate of 20 ºC/min. The initial and final isothermal periods were 5 and 2.5 min, respectively (total runtime 19.75 min). The MS operated in electron ionization (70 eV) mode, scanning in the range of 50 to 500 amu, using a solvent delay period of 5 min to allow the residues of the TMAH and aqueous solvent to elute before the MS was activated. Relative abundances between the THM products (identified by literature and NIST libraries) were based on peak areas of their dominant m/z fragments (Appendix A) and expressed as percentage of total quantified peak area (% TQPA). For background on THM-GC-MS, readers may refer to Challinor [24], and for recent applications in diverse fields, to He et al. [35].

2.5. Data Evaluation

We applied principal component analysis (PCA) on the relative proportions data from THM-GC-MS, after applying a centred logratio transformation (clr) to avoid the close data nature of compositional data. We used one-way analysis of variance (ANOVA) to explore differences between samples of different periods and different depositional features (alleged influence of external factors such as marine influence). All statistical tests were performed in SPSS 20.0 software (IBM, New York, NY, USA).

3. Results and Discussion

3.1. Color and Elemental Analysis

The ceramic fragments analyzed generally had low luminosity (L*, n = 50; 44.1 ± 7.6; Table 1) and chromaticity was low as well (C*, 17.6 ± 7.4). The color components a* and b* had positive values, indicating that the basal color is a mixture of red and yellow, with predominance of yellow over red as the hue exceeds 45º in all measured samples.

The elemental analysis showed that carbon (C) contents ranged from 0.65 to 4.99% (1.68 ± 0.87%; Table 1), while nitrogen (N) content ranged from 0.05 to 0.21% (0.10 ± 0.03%). The C/N molar ratio ranged from 11.2 to 52.9 (19.9 ± 7.8).

Figure 2 shows some relationships between luminosity and elemental composition (%C and C/N). Clearly, dark-colored (i.e., low luminosity value) samples have higher C content and higher C/N than light-colored ceramic fragments. Hence, OM is the main component that decreases luminosity. The negative correlation between L* and C/N indicates that the darkening agent is enriched in C relative to N.
3.2. Thermally Assisted Hydrolysis and Methylation (THM-GC-MS)

The THM-GC-MS chromatograms (Figure 3; Supplementary Material) allowed to identify and semi-quantify 104 compounds (Appendix A). Most of the THM products have a molecular structure based on polymethylene chains (32.5 ± 17.9% of TQPA; Figure 4). The dominant subgroup of these compounds are fatty acid methyl esters (FAMEs), accompanied with fatty diacids (DAMEs), linear alkanes and isoprenoid alkanes. The carbon number of the FAMEs ranges between 7 and 18. These compounds can originate from oils and fats (food remains) but also from fatty acid moieties from plant remains and microbial sources (plankton, fungi, bacteria, etc.). The presence of branched (iso/anteiso) C_{15} FAME probably indicates that at least some of the FAMEs originate from bacterial sources of OM. We identified traces of only one FAME with mid-chain methoxy substitution, probably from cutin (plant cuticles) in selected samples. We searched for some of the markers reported by Shoda et al. [25] and identified a minor trace of a compound that was tentatively ascribed to an isoprenoid pristane-based FAME with m/z 88 and 312 at the expected retention time of pristanic acid methyl ester (ME), and possibly m/z 101 and 326 of phytanic acid ME (too small for peak area calculation). It is concluded that the abundance (relative to total OM that was accounted for in the THM-GC-MS data evaluation) in the studied sherds is much lower than that of the samples studied by these authors [25], or not present at all, and biomarker evaluation for palaeodiet in the present data is not sustained.

![Figure 2. Relationship between luminosity (L*) and carbon (left), C/N molar ratio (center) and the relative proportion of co-eluting THM products phenanthrene and anthracene (right).](image1)

![Figure 3. Example chromatogram (sample CAM4A-100) showing products of fatty acids (FAMEs, in red), polyalkylaromatic compounds (green) and polycyclic aromatic hydrocarbons (black).](image2)
Figure 4. Cont.
Many compounds with at least one atom of nitrogen (N-compounds) were identified (18.2 ± 11.2%; Appendix A), further indicating the contribution of microbial OM to the molecular fingerprints. These compounds include alkylpyrroles, alkylindoles, anilines and succinic acid ME. In addition to microbial N, these compounds (except for succinic acid ME) can also originate from plant protein, but as discussed below, on the basis of statistical data evaluation, this is an unlikely source of N. Finally, the N-compound benzonitrile...
was also identified in the chromatograms of most samples. The most probable source of benzonitrile is pyrogenic OM (PyOM) [20].

This PyOM is also the likely source of polycyclic aromatic hydrocarbons (PAHs; 20.7 ± 9.8%), which are more abundant than is usual for THM fingerprints of biomass. In all likelihood, this can be explained by the substantial portion of charred remains in the OM in the sherds, formed during firing (incomplete burn-off) or cooking (soot, charred food). The first source of PyOM signals is usually dominant for ceramics produced (partially) at low temperature or with truncated firing (reflected by a dark-colored core in contrast to light-colored surfaces, also referred to as “sandwich” morphologies, with a heat-isolated dark-colored nucleus; [8,36,37]).

The compounds with a phenolic moiety include the ME of 4-methoxybenzoic acid (P6), 3,4-dimethoxybenzoic acid (G6) and 3,4,5-trimethoxybenzoic acid (S6). These compounds may originate from plant-derived polyphenols (most importantly, lignin and tannin), which may be detectable due to inclusion of soil OM (from the deposition environment), due to incomplete burn-off of organic temper, or from food remains (fiber). These compounds are scarce (0.4 ± 0.5%).

Monocyclic aromatic products include penta- and hexamethylnaphthalene (14.5 ± 11.4%), which may originate from marine sources [38] and which had not been observed in a study of fragments from non-coastal settlements in NW Spain [20]. This may indicate that some of N-compounds also originate from marine sources such as plankton, as will be discussed later.

Finally, we identified several compounds containing sulfur (pronounced in two samples from the Middle Bronze Age) or phosphorus (most abundant in two samples from the Neolithic period; Figure 4), other compounds from contamination such as \textit{tert}-butylphenol derivatives (plastic additive, surfactant), and compounds which may be formed during the THM reactions as secondary rearrangements such as branched alkanes. We are aware that the THM reaction may generate artifacts, which can be catalyzed by the presence of Fe oxides [39].

In summary, the THM-GC-MS analyses indicate the presence of OM that has a pre-firing origin (temper-derived PyOM, perhaps some of the FAMEs), vessel use origin (soot-derived PyOM, perhaps some food-derived FAMEs) and from after burial (microbial OM from soil and marine fluxes, lignin and FAMEs).

The PCA (Figure 5) provided 3 PCs that explain 60% of total variance. PC1 has elevated negative loadings for the short-chain FAMEs including bacterial FAMEs and positive ones for polyalkyl aromatic compounds. PC1 is not correlated to other variables and is not of major interest for ceramic use identification, as it indicates the balance between different post-depositional inputs and, possibly, also analytical artifacts.

PC2 has high positive loadings for N-containing and lignin products (G6, S6) and negative ones for C\textsubscript{18} FAME. A possible explanation is that PC2 marks the input of post-depositional “exogenous” inputs (from the deposit), such as microbial and plant-derived soil OM constituents.

PC3 is easier to attribute to a specific source of OM, with high loadings for PyOM products (benzonitrile, fluorene, naphthalene, methylphenanthrenes, biphenyl, methylbiphenyl, phenanthrene/anthracene).

Regarding differences between samples, and based on the argued main likely sources of the THM products (Table 2), samples with the highest relative proportion of PyOM are CAM4A-037, -040, -080, -085 and -100, corresponding to various periods. We believe that the most likely source of the PyOM is the incomplete combustion of soil OM from collected raw material and organic binder/temper, either way “pre-firing”. If so, this means that these samples were subjected to a firing regime that facilitated preservation of OM, e.g., due to low temperature or short duration; or other parameters such as use of thick material and/or relatively high loads of temper. Dealing with relative proportions implies that it can also be simply that these fragments are relatively “clean” (few food or post-depositional organic inclusions). A source in surface-bound soot is unlikely due to associations between
PC3 and color analysis (indicating bulk OM contributing to low L*, not effects of a thin layer of surface-adhered soot).

**Figure 5.** PC scores from THM-GC-MS after PCA. Colors indicate cultural periods (red: Neolithic, blue: Early Bronze Age; green: Middle Bronze Age, yellow: Late Bronze Age I, magenta: Late Bronze Age II).

**Table 2.** Main organic matter types identified, with their likely origin (unlikely alternatives within parentheses) and their loadings on PC1-PC3 from the principal component analysis.

| Group          | Fatty Acids | PyOM                | Polyalkyl Aromatic Compounds | Nitrogen Compounds | Polyphenols |
|----------------|-------------|---------------------|-------------------------------|--------------------|-------------|
| Description    | FAMEs       | Pyrogenic organic matter products | Polyalkyl MAH/PAH | Products with nitrogen | Lignin (P6, G6, S6) |
| Origen         | microbial/dietary | Interior ceramic (temper/binder), exterior (soot) | Tentatively marine | Microbial (marine/terrestrial), molluscs | Plant remains |
| Indicator      | Food/abandonment | Firing/cooking | abandonment | abandonment | abandonment |
| PCA            | −PC1        | +PC3                | +PC1                         | +PC2               | +PC2        |
| Operative chain? | Post-depositional/dietary | Temper and soil OM in raw material | Post-depositional | Post-depositional (proteinaceous food) | Post-depositional (temper) |

Part of the variation can indicate the balance between fatty acids and compounds of microbial origin (FAMEs, poly-alkyl MAH and PAH, PC1). If part of the non-microbial FAMEs detected indeed originate from food remains, this would imply a great value for
selecting samples for future analysis. In this regard, samples CAM4A-018 and -081 have signals that may be the most likely associated with food remains. Samples CAM4A-007, -082, -097, -101 and -102 would have relatively strong marine influence. For fragment CAM4A-101 this coincides with a sherd that is colonized by mollusks.

Samples with the highest proportions of lignin and microbial N are CAM4A-014 and -081. The most likely explanation is a relative strong signal from terrestrial post-depositional inclusions (fungi and plant remains). But an effect of decay intensity cannot be discarded as chitin and lignin are probably among the labile sources of OM in the present environment. Bacterial FAMEs are most abundant in CAM4A-010 and -081, and least abundant in -097 and -101. Finally, sample CAM4A-082 has a relatively strong signal of leaf cuticle-derived OM (cutin).

3.3. Comparing THM Data with Color and Elemental Composition

The strongest correlation between the different datasets is the negative link between luminosity (L*) and the PyOM-related PAHs such as naphthalene, fluorene, biphenyls and phenanthrene/anthracene (Figure 2), benzonitrile and PC3. This link between darkness and PyOM is a strong indication of incomplete burn-off as the cause. It was also the main link between color and Py-GC-MS analyses of pottery fragments from non-coastal settlements in NW Spain [20]. It is remarkable that the THM-GC-MS data also highlight such an effect as the main products of PyOM, benzene and toluene, are not detected by THM-GC-MS. The total sum of PAH is not correlated to L* probably because of contributions from non-PyOM, such as plankton, to the poly-alkyl PAHs (mainly polyalkylnaphtalenes and –indenes). Indeed, trimethylindene and hexamethylbenzenes are correlated positively with L*.

There is no apparent effect of cultural period on L* or molecular proxies of PyOM, benzene and toluene, are not detected by THM-GC-MS. The total sum of PAH is not correlated to L* probably because of contributions from non-PyOM, such as plankton, to the poly-alkyl PAHs (mainly polyalkylnaphtalenes and –indenes). Indeed, trimethylindene and hexamethylbenzenes are correlated positively with L*.

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The negative correlations between L*, %C, C/N and PyOM indicates that the PyOM has an effect on L* that the OM from N-rich marine and terrestrial microbial sources does not have. The relationships between other color parameters and the molecular fingerprints are relatively weak and may be due to spurious co-variation effects. Suffice it to mention that the PyOM tends to mitigate the expression of a* (redness) component, probably related to Fe oxides, to a larger extent than b*, C* or h. This negative effect of PyOM on redness may also be related to the association of PyOM with low temperatures (caused by incomplete burn-off) that would also reduce the efficiency of Fe (oxy)hydroxide dehydration to strongly pigmenting red oxides such as hematite. Note that this negative effect makes it unlikely that Fe oxide-catalyzed secondary reactions are the main formation mechanism of PyOM products, lending support to our approach and interpretation of PC3. The %C is negatively correlated with polyalkyl MAH and PAH, probably due to the fact that the THM-GC-MS data is a closed dataset: molecular fingerprints of samples with low OM content will be more strongly affected by marine-derived OM than samples with high OM content. Indeed, we did not find consistent trends between the intensity of the marine signal from THM and position in the deposits or age/cultural period. If this argument is correct, the variations in the fingerprints indicate that not only the PyOM but also the mentioned FAMEs are mainly pre-depositional. Then, the marine, and probably also terrestrial signals (microbial N-rich moieties and plant-derived lignin and perhaps cutin) developed mainly post-depositionally.

The correlations between %N and THM products are different to those for %C. For %N, positive correlations (p < 0.001) were observed for iso-C15 FAME (bacterial lipids), C9-diacid (degraded C18 fatty acids), N compounds (alkylindoles, N-methylsuccinimide) and the phenolic products P6 y G6. This highlights the influence of soil microbes (bacteria and fungi) on OM composition. The association between microbial and lignin products
further supports a post-depositional source of lignin, instead of incomplete burn-off of organic temper or soil OM that was present in the raw material before firing. Perhaps the inclusion of plant signals is stimulated by microbial activity on sherd surfaces, or because enhanced microbial alteration provokes stronger interactions between the ceramic fabric and the soil environment in general. Relationships between THM fingerprint and C/N are consistent, with correlations between C/N with FAMES and PyOM markers.

The ANOVA analysis using cultural period (Neolithic, Early Bronze Age, Middle Bronze Age, Late Bronze Age I and Late Bronze Age II; 7–15 samples/period) revealed few significant differences in molecular composition of the OM. The tentatively identified isoprenoid FAME (pristanic acid ME) is enriched in samples from Late Bronze Age I, which could reflect an effect of different vessel use between periods (marine oils), but in the light of planktonic aquatic sources of OM and the uncertainty in identification of this compound, this observation cannot be considered more than a reason to pursue further analyses using alternative methods of the samples from the Late Bronze Age I in particular. Furthermore, an aliphatic ether is enriched in samples from the Early Bronze Age, and phosphoric acid ME in samples from the Neolithic. Color data are more strongly differentiated on the basis of period, with low values of a*, b* and C* in samples from the Late Bronze Age II and Neolithic, and high values for the other samples, in particular Middle Bronze Age and Late Bronze Age I. For hue, the tendency is reverse. This probably indicates differences in raw material selection and firing conditions, but because these results are not related to OM chemistry, they were not explored further.

An additional ANOVA aimed to identify differences between recovery locations. The midden was expected to coincide with samples with higher likelihood of post-depositional contamination (VAR1; high—intermediate—low) and contamination type (VAR2; waste deposit—post waste deposit—marine influence) than samples from the superimposed layers (Table 1). It appeared that samples with high general contamination likeliness (VAR1) were enriched in N-containing THM products (alkylindoles and -pyrroles), confirming a stronger microbial influence in the midden (including possibly mollusk-derived OM), whereas C16 FAME is enriched in samples with low contamination. There is no color effect on VAR1. For VAR2 (contamination type), samples from the waste deposit have positive PC1 scores from THM (low fatty acid proportions and high microbial influence) whereas the other two categories have negative PC1. Indeed, C16 and C18 FAME are depleted in the samples from the waste deposit. These results might indicate that samples that are prolific in FAMES are good candidates for dietary analyses of the fatty acid profiles using compound-specific IRMS of lipid extracts.

4. Conclusions

The THM-GC-MS analyses have shown that the OM present in the ceramic fragments from Guidoiru Areoso Islet originated mainly from: (1) marine and terrestrial microorganisms (protein, chitin, bacterial fatty acids), (2) charred residues (PyOM, formed during firing, from soil OM or temper/binder), (3) uncharred plant remains (traces of lignin and cutin from post-depositional inclusion of plant-derived soil OM) and (4) fatty acids of unknown source (possibly from food remains). It is clear that the burial environment, with high loads of organic waste and marine influence, caused a relatively complex mixture of OM sources that hampered the unambiguous identification of food remains, let alone identifying precise ingredients. Nevertheless, the study revealed some interesting links between color, elemental and molecular composition of ancient ceramic fragments that allowed for a better understanding of the changes in deposition conditions and will be used to select a subset of samples of which relatively “clean” fatty acid patterns are expected, in particular dark-colored ones, with high C/N and with high non-bacterial FAME proportions. We believe that light-colored fragments, with low fatty acid contents and low C/N, would reveal fatty acid profiles that are too severely affected by microbial inputs.

Supplementary Materials: The THM-GC-MS data are available online at https://www.mdpi.com/article/10.3390/separations8090140/s1.
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Data Availability Statement: We will provide the background data as Supplementary Material.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

List of identified THM products, with the retention time (RT; in minutes), identification (T = tentative), mass/charge ratio of ions used for peak area calculation (m/z) and group allocation. ME = methyl ester; FAME = fatty acid methyl ester; DAME = fatty diacid; PyOM = pyrogenic origin; MAH = monocyclic aromatic hydrocarbon; PAH = polycyclic aromatic hydrocarbon; BCA = benzenecarboxylic acid methyl ester; NCOMP = nitrogen compound; SCOMP = sulfur compound; MCC = polymethylene compounds other than FAMEs and DAMEs).

| RT (min) | Compound | m/z | Group   |
|----------|----------|-----|---------|
| 6.270    | C7 FAME  | 74  | FAME    |
| 7.406    | C8 FAME  | 74  | FAME    |
| 8.340    | C9 FAME  | 74  | FAME    |
| 9.149    | C10 FAME | 74  | FAME    |
| 9.865    | C11 FAME | 74  | FAME    |
| 10.519   | C12 FAME | 74  | FAME    |
| 11.132   | C13 FAME | 74  | FAME    |
| 11.702   | C14 FAME | 74  | FAME    |
| 12.050   | iso-C15 FAME | 74 | FAME   |
| 12.097   | anteiso-C15 FAME | 74 | FAME   |
| 12.247   | C15 FAME | 74  | FAME    |
| 12.771   | C16 FAME | 74  | FAME    |
| 13.259   | C17 FAME | 74  | FAME    |
| 13.731   | C18 FAME | 74  | FAME    |
| 14.224   | C19 FAME | 74  | FAME    |
| 14.618   | C20 FAME | 74  | FAME    |
| 15.428   | C22 FAME | 74  | FAME    |
| 12.662   | C16:1 FAME | 55 | FAME    |
| 13.632   | C18:1 FAME | 55 | FAME    |
| 12.626   | C18:2 FAME | 67 | FAME    |
| 13.632   | C18:2 FAME | 67 | FAME    |
| 8.932    | unidentified alkane | 57 | MCC    |
| 9.679    | unidentified alkane | 57 | MCC    |
| 10.343   | unidentified alkane | 57 | MCC    |
| 10.675   | unidentified alkane (non homologous) | 57 | MCC    |
| 10.960   | unidentified alkane | 57 | MCC    |
| 11.334   | unidentified alkane | 57 | MCC    |
| 11.796   | unidentified alkane | 57 | MCC    |
| 14.359   | N,N-dimethylalkylamide | 87 | NCOMP   |
| 12.353   | unidentified MCC (alkene/ether) | 83 | MCC    |
| 13.311   | unidentified MCC (alkene/ether) | 83 | MCC    |
| 6.477    | C4 DAME  | 59  | DAME    |
| 8.548    | C6 DAME  | 59  | DAME    |
| 10.696   | C8 DAME  | 59  | DAME    |
| 5.912    | benzonitrile | 103 | PyOM   |
| 6.114    | phosphoric acid triME | 110 | OTHER  |
| 6.566    | indene   | 116 | PyOM    |
| RT (min) | Compound | m/z | Group |
|---------|-----------|-----|-------|
| 6.877   | N-methylaniline          | 106 | NCOMP |
| 6.893   | guaiacol (T)            | 109 | OTHER |
| 7.141   | N,N-dimethylaniline      | 120 | NCOMP |
| 7.126   | benzenecarboxylic acid ME | 105 | BCA |
| 7.271   | N-methylsuccinimide       | 113 | NCOMP |
| 7.448   | C3-alkylbenzene          | 119 | MAH |
| 7.494   | tetramethylpyrrole        | 123 | NCOMP |
| 7.515   | unidentified compound (possibly N) | 139 | OTHER |
| 7.806   | methylindene (DP)        | 130 | PyOM |
| 7.889   | N,N,x-x-tetramethylaniline | 149 | NCOMP |
| 8.055   | N,N,x-trimethylaniline   | 134 | NCOMP |
| 8.106   | methylthioanisole (T)    | 138 | SCOMP |
| 8.133   | naphthalene              | 128 | PyOM |
| 8.199   | pentamethylpyrrole       | 136 | NCOMP |
| 8.167   | N,N-dimethyluracil (T) (pyrimidinedione) | 140 | NCOMP |
| 8.341   | 4-methoxybenzoic acid ME (P6) | 135 | Phenolic |
| 8.435   | C3-alkylylpyrroleolcarboxaldehyde (T) | 136 | NCOMP |
| 9.150   | C4-alkylpyrroleolcarboxaldehyde (T) | 136 | NCOMP |
| 9.507   | aromatic compound (C10H14O) | 150 | OTHER |
| 9.512   | C6-alkylbenzene          | 147 | MAH |
| 9.617   | C2-alkylindole           | 144 | NCOMP |
| 9.663   | trimethylindene          | 143 | PyOM |
| 9.715   | biphenyl                 | 154 | PyOM |
| 9.788   | unidentified compound    | 125 | OTHER |
| 9.879   | C2-alkynaphthalene       | 141 | PyOM |
| 10.006  | C2-alkynaphthalene       | 141 | PyOM |
| 10.078  | diphenylmethane/methylbiphenyl | 167 | PyOM |
| 10.111  | 2,6-dissopropylaniline   | 162 | NCOMP |
| 10.099  | 4-tert-butybenzoic acid ME | 177 | OTHER |
| 10.166  | benzenedicarboxylic di ME | 163 | BCA |
| 10.213  | hexamethylbenzene        | 147 | MAH |
| 10.483  | benzenedicarboxylic di ME | 163 | BCA |
| 10.563  | benzenedicarboxylic di ME | 163 | BCA |
| 10.623  | C3-alkynaphthalene       | 155 | PyOM |
| 10.649  | dihydro-C3-alkynaphthalene | 157 | PyOM |
| 10.701  | tert-butylanisole        | 149 | OTHER |
| 10.711  | C3-alkylindole           | 159 | NCOMP |
| 10.960  | C3-alkynaphthalene       | 155 | PyOM |
| 11.080  | C3-alkynaphthalene       | 155 | PyOM |
| 11.002  | 3,4-dimethoxybenzoic acid ME (G6) | 196 | Phenolic |
| 11.116  | fluorene                 | 166 | PyOM |
| 11.360  | C4-alkylindole           | 173 | NCOMP |
| 11.500  | benzenesulfonamide (x,N-dimethyl) | 91 | SCOMP |
| 11.795  | benzenesulfonamide (x,N-dimethyl) | 91 | SCOMP |
| 11.697  | C4-alkynaphthalene       | 169 | PyOM |
| 11.765  | unidentified compound    | 193 | OTHER |
| 11.799  | 3,4,5-trimehtoxybenzoic acid ME (S6) | 226 | Phenolic |
| 11.983  | C4-alkynaphthalene       | 169 | PyOM |
| 12.284  | phenanthrone/antracene   | 178 | PyOM |
| 12.475  | C5-alkynaphthalene       | 183 | PyOM |
| 12.547  | C5-alkynaphthalene       | 183 | PyOM |
| 13.025  | methylphenanthrene       | 192 | PyOM |
| 13.150  | unidentified isoprenoid hydrocarbon | 82 | MCC |
| 13.466  | unidentified isoprenoid hydrocarbon | 82 | MCC |
| 13.518  | unidentified compound    | 212 | OTHER |
| 13.850  | unidentified compound    | 226 | OTHER |
| 14.748  | mid-chain methoxy-FAME (cutin-like) | 201 | OTHER |
| 14.914  | unidentified compound    | 254 | OTHER |
| 12.953  | pristane-based isoprenoid FAME (T) | 88 | FAME |
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