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Archaeological Evidence for the Dietary Practices and Lifestyle of 18th Century Lisbon, Portugal—Combined Steroidal Biomarker and Microparticle Analysis of the Carbonized Faecal Remains

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Abstract: The study of the urban context in the contemporary center of Portugal's capital city uncovered traces of daily lives that were abruptly interrupted and utterly transformed by the Great Lisbon Earthquake on the morning of 1 November 1755. Charred organic residue was recovered from a cylindrical vessel excavated from the storage area of the town house at the Rossio square. The archaeological sample was studied through a multi-analytical approach based on microstructural, elemental and biomolecular characterization by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR), variable pressure scanning electron microscopy coupled to energy dispersive X-ray spectroscopy (VP-SEM-EDS), and gas chromatography coupled with mass spectrometry (GC-MS). The residue was identified as human faeces collected in the ceramic vessel for disposal, and further analysis provided additional information about diet and the living conditions in the 18th century.

Keywords: paleofaeces; organic residue analysis; The Great Lisbon Earthquake; Modern Period

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1. Introduction

The study of amorphous remains from archaeological contexts has often been linked to dietary habits [1–4]. Comprehensive studies have identified charged amorphous remains as food preparations, products or cooking residues [5–8]. However, for the samples without a defined structure, chemical analysis might be necessary as it can provide the decisive information, considering that the archaeological context is, without a doubt, crucial in the final interpretation. To enable a holistic reconstruction of the past diet and lifestyle, a multi-analytical approach can yield extensive and versatile datasets enabling an in-depth study. Here, the results of the microstructural, elemental and chemical analysis are presented, identifying the unknown amorphous residue as carbonized faeces.

Archaeological faeces have often been described as coprolites, a term originating from paleontology, and referring to the faeces that have been fossilized [9,10]. Still, archaeological material is more often preserved through desiccation or partial mineralization [11,12]. Here, the organic remains, identified as human waste, were preserved due to carbonization through charring under oxygen-poor conditions. The most important factors that influence

this simultaneous degradation–preservation are temperature and duration of exposure, as well as chemical content and amount of moisture in the organic remains [13]; nonetheless, high temperatures do irreversibly alter the structure and the composition of the original material, through different mechanisms than other modes of preservation. To address and study archaeological waste without simultaneously describing a specific mode of preservation, a more suitable term of palaeofaeces, or archaeological faeces, has been used for the archaeological material [14–18] and will be employed in this study.

As a well-preserved organic archaeological material, found at, or in the proximity of, the habitation areas, paleofaeces may contain partly digested remains of the ingested food, recognizable to the naked eye or under the microscope [19–22]. They also demonstrate a specific chemical profile that include lipids and steroids. Thus, they are an abundant well of information as it is a direct product of the diet of the individuals. Alongside well-formed, cylindrical paleofaeces, recognized as coprolites or dung [23,24], chemical analyses have been successfully employed in the studies of latrine and occupational deposits [25–28] and trash middens [29–31], as well as on intestinal contents [32,33], even when individual formed faeces are not identifiable in the soil matrix. Steroidal studies combined with morphological multiproxy approaches have been incorporated into environmental and archaeological research, providing insights into past agricultural practices and the former presence of animals [34].

Sterols, a class of compounds commonly found in faeces, can be indicative of the original biogenic source, and since they are relatively stable and resistant during diagenesis, they are well suited as a faecal biomarker. The detection of faecal markers from archaeological sediments has often been linked to sewers, fuel material or manuring practices and connected to the increase in population density [26,35–39], but with lower values in comparison with a direct analysis of waste, gut content and coprolites [33,40–42]. Faecal steroids include sterols and stanols, which are excreted as faeces, after the intestinal microbes in the digestive tract, under anoxic conditions, modify sterols from plant and animal tissues. The differentiation among soils and gut content can be established depending on sterol stereochemistry [29,43]. Furthermore, since the relative ratio of sterols (Figure 1) such as coprostanol, cholesterol and sitosterol vary depending on the diet, digestive processes and gut bacteria, distinction between the herbivores, carnivores and omnivores can be asserted [34,44,45]. As paleofaeces provide information about dietary practices of a limited time interval, the results of the analyses present the diet focused on specific short-term events [46]. On the other hand, microparticle analysis is interlinked with environmental conditions and provides additional information about living standard, hygiene, and the lifestyle.

During the archaeological excavation in 2017 at the Rossio square (Praça D. Pedro IV) in the center of Lisbon (Portugal), a ground floor of an 18th century town house was discovered. The glimpse into urban context under one of the main squares of contemporary Lisbon uncovered the remains of daily lives that were abruptly interrupted and utterly transformed by the Great Lisbon Earthquake. The Earthquake struck on the morning of 1 November 1755, a Saturday of All Saints' Day, followed by a tsunami and fires across the city [47–50]. The amorphous organic remains, recovered from inside of a vessel, were preserved due carbonization, and through combined biomarker and microparticle analysis provided an insight into dietary habits and lifestyle of the Lisbon's residents.

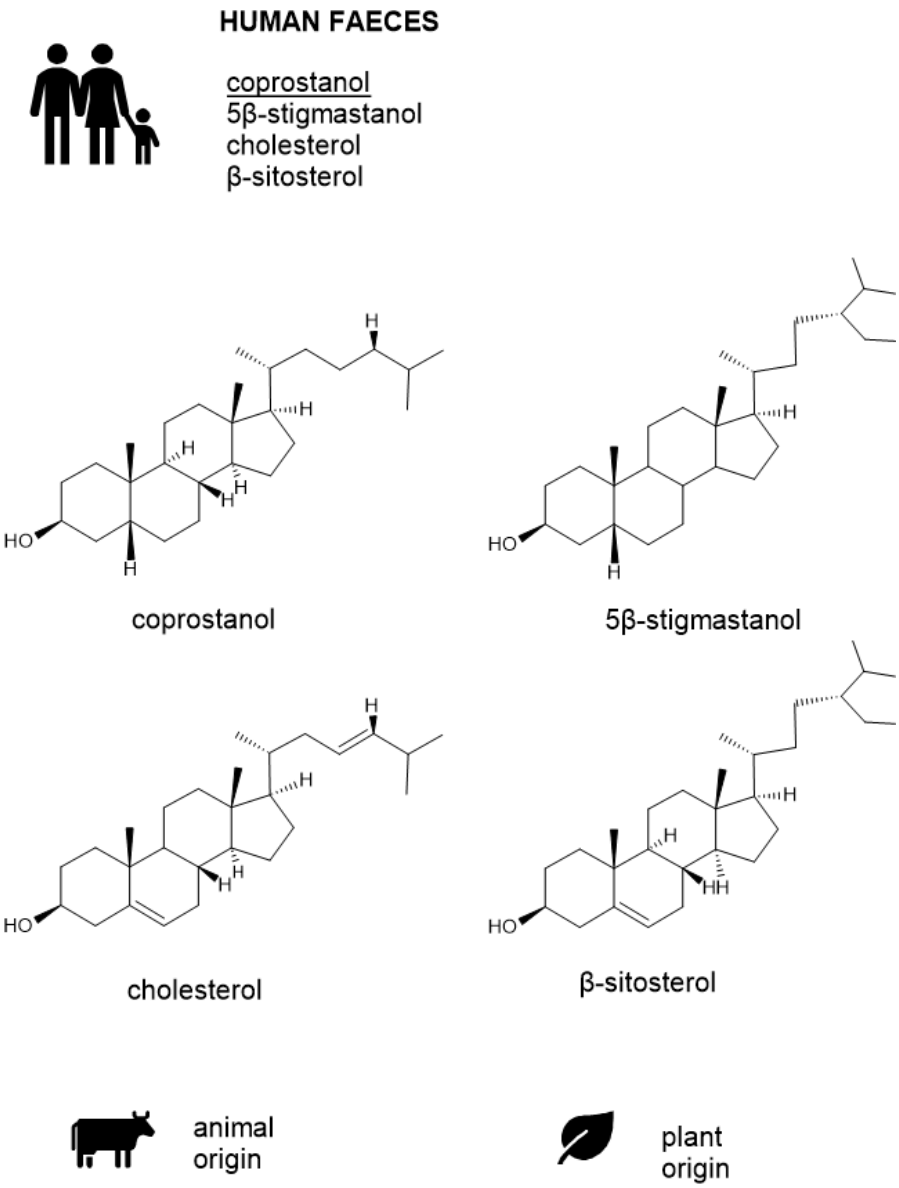

Figure 1. Indication of the major sterols in human faeces (according to Prost et al. 2017 [34]).

2. Materials and Methods

Microstructural analysis of coprolitic material was carried out under optical, digital and variable pressure scanning electron microscopy (VP-SEM), while elemental composition was obtained by VP-SEM coupled to energy dispersive X-ray spectroscopy (EDS). The state of preservation of the organic matter in the archaeological sample was determined by Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR). The chemical composition of the organic matter was revealed by gas chromatography coupled with mass spectrometry (GC-MS). Non-pollen palynomorphs (NPPs) and parasite eggs were identified under optical microscope.

2.1. Description of Archaeological Sample

The uncovered housing unit was located in the southwest corner of one of the three blocks which, before the earthquake, defined a side of Rossio square. Excavations exposed different sections of the same property [51], including the kitchen, storage, and courtyard/stable area with adjacent sewer system of the city (Figure 2a). The area identified as a storage space was located south of the kitchen and divided by plastered walls to the north

and west. The floor was brick tiled, indicating the indoor space of the house. Even though the uncovered space was relatively small, due to the parameters of the excavated area, it yielded an abundance of archaeological finds including organic remains found inside the tall cylindrical ceramic vessel (Figure 2b).

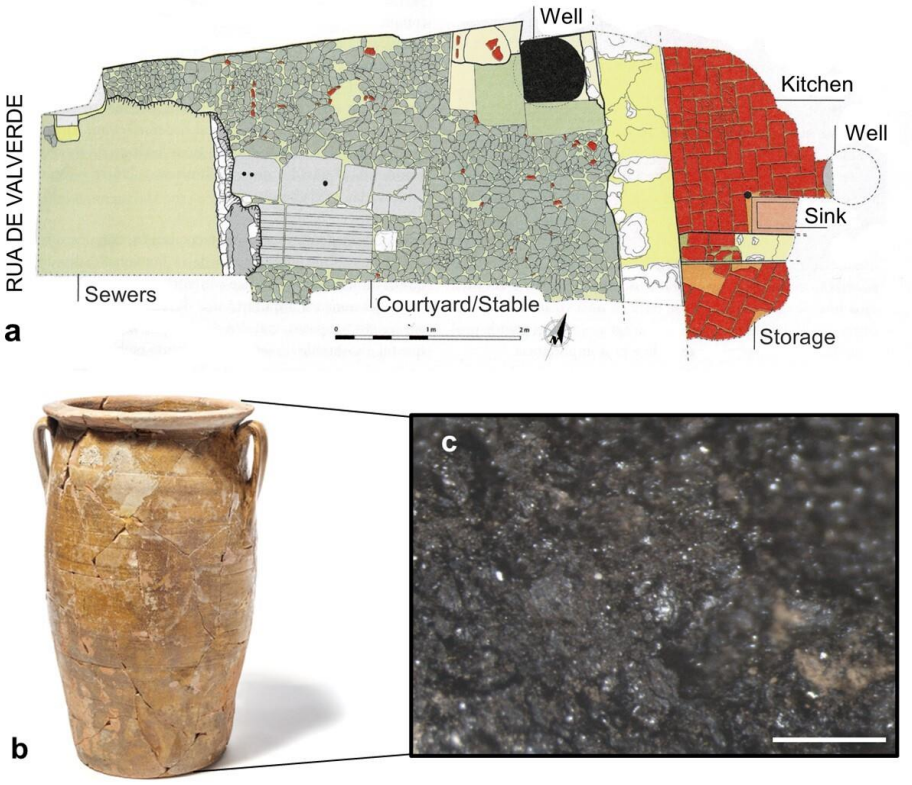

Figure 2. (**a**) Plan of the excavated sectors of the house destroyed by the Great Lisbon Earthquake [51]; (**b**) cylindrical vessel (PDPIV'17.16) recovered from the storage area (© CML | DMC | DPC | José Vicente 2020); height: 46 cm, width: 25 cm; (**c**) organic residue found in the vessel.

The amorphous residue (Figure 2c) was preserved due to prolonged exposure to high temperatures during the fires that engulfed Lisbon residential area following the Earthquake, resulting in carbonization. Therefore, the residue is dark, from dark brown to almost black. Although no recognizable shape was retained, it is well preserved due to closed deposition in a vessel and carbonization, but the time and high temperatures also affected its structure, making the archaeological material hard but friable.

2.2. Microscopy

The sample was documented with stereomicroscope LEICA M205C, equipped with a digital camera (Leica MC170 HD) under low (1× to 100×) magnification. Cross sections were examined under stereo and 3D Digital Microscope HRX-01 with wide range (20× to 140× magnification).

2.3. ATR-FT-IR

A preliminary assessment carried out using Fourier transform infrared spectroscopy of the archaeological samples was done at room temperature and ambient humidity with a Bruker™ Alpha spectrometer coupled with a single-reflection diamond ATR module. The archaeological material collected from the vessel was subsampled and analyzed after documentation and prior to other analyses. All spectra were acquired in the absorbance mode, in the range from 4000 to 400 cm^{-1}, from a total of 128 scans at 4 cm^{-1} resolution. Spectra were recorded and analyzed using Bruker OPUS software (version 6.5), subsequently

normalized and averaged using the SpectraGryph software (Version 1.2.14). Three FT-IR spectra were performed on one subsample, and a representative spectrum is presented in this study.

2.4. VP-SEM-EDS

Variable pressure SEM-EDS analysis was carried out using a Hitachi S3700N SEM coupled to a Bruker XFlash 5010 SDD EDS Detector. The sample was analyzed at low vacuum (40–50 Pa) and with an accelerating voltage of 20 kV and a ca. 10 mm working distance. The VP-SEM images were acquired in the backscattering mode [52].

2.5. GC-MS

For the chemical analysis of the residue by GC-MS, organic compounds were extracted with chloroform/methanol [53]. Analysis was performed with Shimadzu GC2010 coupled with a Shimadzu GCMS-QP2010 Plus. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm × 15.0 m, 0.10 µm film thickness) was used for separation, with helium as carrier gas, adjusted to a flow rate of 152.5 mL/min and velocity of 62.4 cm/s. An amount of 1 µL of the sample was injected in splitless mode, with the injector set at 250 °C and a column flow of 1.5 mL/min. The GC temperature program was set at 50 °C for 2 min, ramped up to 150 °C, then to 250 °C, and finally increased to 350 °C at which point it was held for 2 min for a total run time of 67 min. The mass spectrometer was programmed to acquire data between 40 and 850 m/z. The temperature of the ion source was 240 °C and the interface temperature was 280 °C. All obtained results were processed with Automated Mass spectral Deconvolution and Identification System (AMDIS). The chromatograms were interpreted in comparison with the NIST library database. As a reference material, beta-Sitostanol (Matreya LLC, 97+% purity, 50 mg) was analyzed under the same conditions.

All solvents and reagents used in this research were ultrapure or GC–MS grade. Chloroform ($CHCl_3$) was obtained from ITW Reagents, methanol (MeOH) and n-hexane (C_6H_{14}) were obtained from Fisher Scientific™. Sulfuric acid (H_2SO_4) (99.9%) was purchased from Sigma-Aldrich (St. Louis, MI, USA). Internal standard n-tetratriacontane (C34), and derivative reagent N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS), were also obtained from Sigma-Aldrich. A Milli-Q Integral Water Purification system (Millipore®) was used to produce ultrapure water.

2.6. Non-Pollen Palynomorphs (NPPs) Extraction

To examine preserved microfossils the material was subsampled and weighted. Non-pollen palynomorphs (NPPs) and parasite remains were examined after standard pollen extraction [54,55] with HCl (37%), HF (40%) and NaOH (10%), and after the dissolution in 0.5% aqueous solution of trisodium phosphate for 48 h [56,57]. The extracts were stored in glycerol and mounted on glass slides to examine under light microscope (with 400–640× magnification) and identified using existing databases [58,59].

Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich, while hydrofluoric acid (HF) was obtained from ITW Reagents. Trisodium phosphate (Na_3PO_4) was packed by SPD. Glycerol was obtained from Farmax. A Milli-Q Integral Water Purification system (Millipore®) was used to produce ultrapure water.

3. Results

Based on the obtained data, the analyzed amorphous residue was determined to be mostly organic in nature, with organic and inorganic inclusions. Spectroscopic and elemental analysis indicated the unknown charred substance as a faecal matter, while chromatographic techniques were used to examine the composition, nature, and the origin of the waste. The residue was identified as human faeces, and further analysis provided additional information about diet and the living conditions at the time of the Great Earthquake.

3.1. ATR-FT-IR

The organic origin of the substance was established by the initial ATR-FT-IR analysis of the charred residue retrieved from the cylindrical vessel (Figure 3). The absorbance spectrum shows the presence of decayed organic material (in the 1300 to 4000 cm^{-1} region), while in the 500 to 1100 cm^{-1} region phosphates present in the substance are detected. The wide band around 3300 cm^{-1} is attributed to the O-H stretching of water molecules, while specific infrared bands characteristic for aliphatic C-H stretching vibrations of methyl/methylene functional groups are recorded at 2921 and 2850 cm^{-1} and assigned to fats and lipids. Additionally, the spectrum shows absorption characteristic for the organic matter, at 1565 cm^{-1} and 1375 cm^{-1}, most likely connected to nitro compounds. The band at 1565 cm^{-1} can be assigned to N-H bending and C-N stretching vibration [60], while the band at 1375 cm^{-1} corresponds to N-O stretching of the unconjugated nitro group [61]. However, overlapping might occur with the O–H bending of the carboxyl group and C-H rocking vibrations of CH_3 group [62]. It should be emphasized that the original composition of carbonized organic residue was altered due to the physical and chemical changes caused by heating [63–65], making band assignment in the fingerprint region more complex and uncertain. However, asymmetrical P-O stretching was assigned to the band at 1025 cm^{-1}, while the bands at 547 and 600 cm^{-1} were ascribed to P-O bending mode, typical for phosphate-rich samples [66,67]. As in the complex mixtures, the more intense stretching may often overlap with other intense bands, butthe presence of a clear doublet at 600/547 cm^{-1} provides the best diagnostic test for phosphate in the infrared spectrum [68]. The FT-IR spectra of the charred archaeological residue presents a profile analogous to coprolites [69], manure or organic sewer waste [60,70].

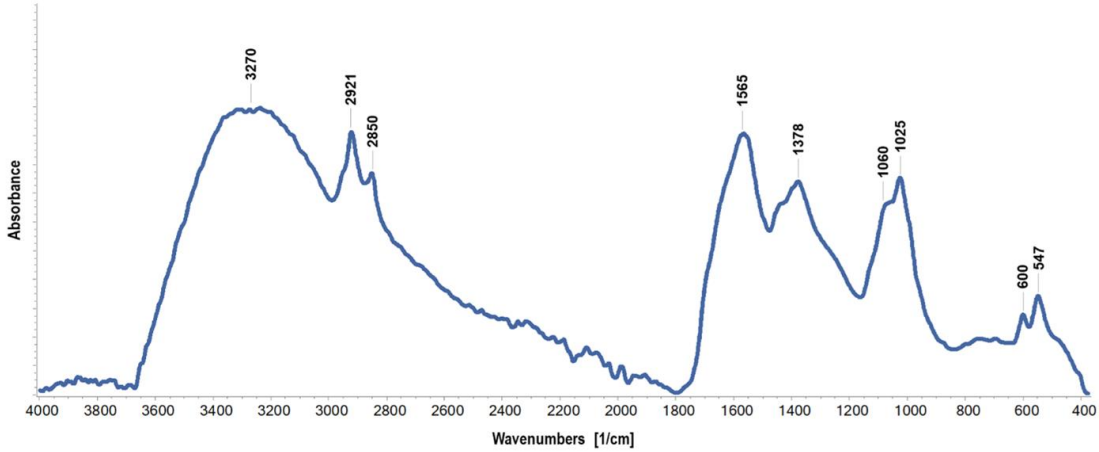

Figure 3. ATR-FT-IR spectra (4000–400 cm^{-1}) of an archaeological sample from the cylindrical vessel.

3.2. Microscopy and VP-SEM-EDS

The sample examined under microscope with lower magnifications showed a heterogeneous and slightly porous structure with unevenly distributed visible white inclusions, ca. 200 µm in size, embedded in a charred matrix (Figure 4a,b). To verify whether the observed IR bands are related to organic matter or to inorganic material, the elemental composition of the sample was also ascertained using VP-SEM-EDS. The results of elemental analysis on the cross section of the archaeological sample determined a carbon matrix, and the major elements identified include calcium, and phosphorus, followed by magnesium, silicon, iron and aluminum. Smaller amounts of sodium, potassium, manganese and sulfur were also detected. This is in accordance with coprolite studies [71–74]. Calcium and phosphorus are noted as main elements detected in both herbivore and carnivore coprolites [75,76]. In the charred archaeological sample, calcium alongside with phosphorus can be associated with bacteria activity and the individual's diet, since elemental distribution is also influenced by the presence of inclusions found within the faecal matrix. In fact, as seen in Figure 4f,h,

based on their composition these inclusions can be divided in two types—calcium-rich, and silicon-predominant inclusions—and are likely fragments of partially digested food of animal and plant origin. Point analysis showed that the most abundant inclusions, first observed as white particles under digital microscope, are composed of calcium and phosphorus, and therefore could be identified as micro fragments of animal bones. Iron-rich inclusions of 50–80 µm size can also be found throughout the faecal sample (Figure 4d). These inclusions may be either remnants of ingested food or evidence of the intestinal and digestive secretions. However, elemental analysis and high-resolution microscopy, while useful in indicating dietary preferences, could not reliably distinguish the biogenic origin of organic waste, especially for heterogenous samples.

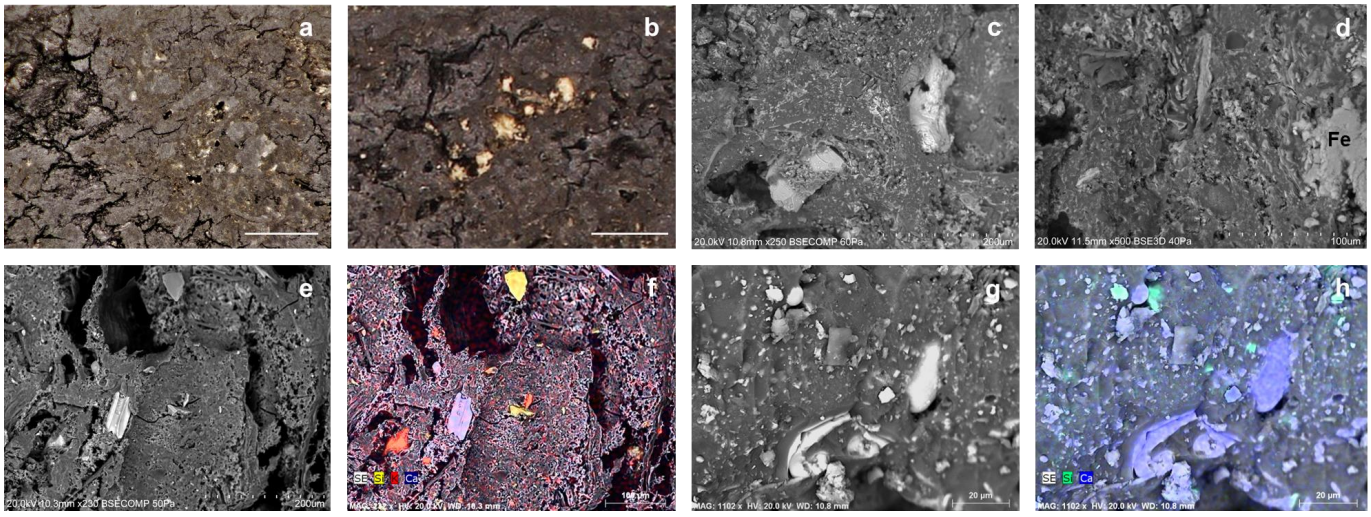

Figure 4. Micrographic and elemental analysis of the carbonized faeces. (**a**–**d**): Visible porosity and inclusions; (**e**–**h**): elemental mapping showing calcium-rich and silicon-rich inclusions.

3.3. GC-MS

Chemical analysis by GC-MS revealed a complex profile with abundance of fatty acids, alkanes, alcohols and steroids (Figure 5, Table 1). Presence of monoacylglycerols (MAGs), monopalmitin and monostearin, as well as monounsaturated octadecenoic acid, supports a good preservation of organic material. The most abundant fatty acids are stearic and palmitic, followed by icosanoic (arachidic), and heptadecanoic (margaric) acid. The very long chain fatty acids, docosanoic (behenic) and tetracosanoic (lignoceric) acids are also present. Fatty alcohols ranging from C14:OH to C22:OH were detected, as well as even and odd numbered alkanes (C17–C29). Still, it must be noted that high molecular weight alkanes are present in relatively low amounts and lack a clear odd-over-even chain length preference [77] to distinguish the plant matter. Since palmitic and stearic acid are detected in almost equal abundance, with the presence of the odd fatty acids, namely C15:0, pentadecanoic and C17:0, heptadecanoic acid, a predominant presence of ruminant animal fat is likely [78–80]. However, the amounts of palmitic and stearic acids, as well as specified odd fatty acids, should be interpreted with caution considering the sample is a heterogeneous mixture, the lipid content has been altered through thermal degradation, and even though the archaeological conditions were favorable for preservation post-deposition degradation influenced the chemical composition of the sample [81,82]. Microbial contamination could have affected the chemical profile of the archaeological sample, contributing to the abundance of odd-numbered fatty acids, while bacterial activity could have occurred even after excavation.

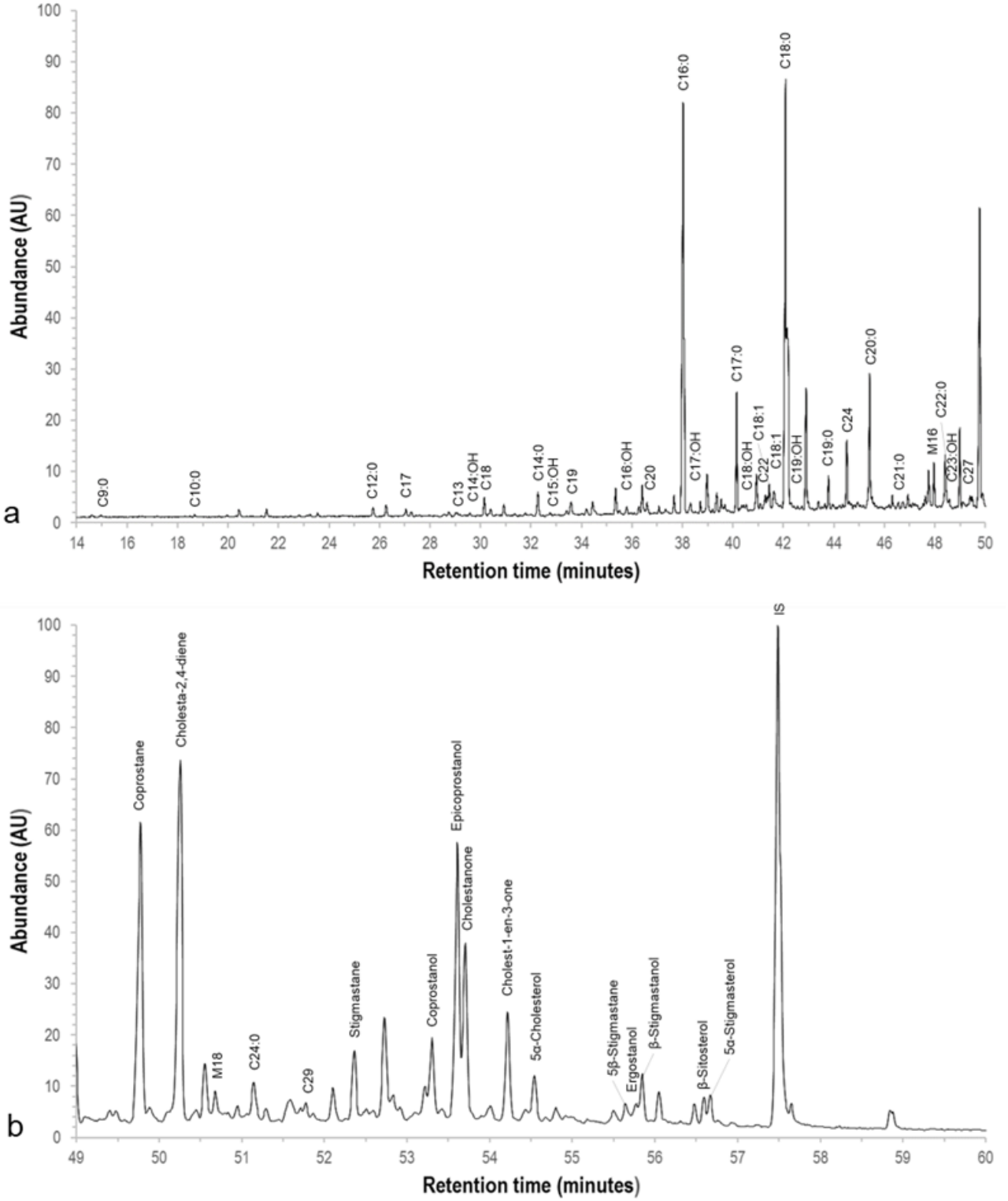

Figure 5. GC-MS chromatogram of organic residue, divided into 2 segments. (**a**) from 14–50 min; (**b**) from 49–60 min. Marked are the trimethylsilyl (TMS) derivatives of relevant compounds detected. Cx alkane with x carbon atoms, Cx:OH linear alcohol with x carbon atoms, Mx monoacylglycerol with x carbon atoms, Cx:y fatty acid with x carbon atoms and y double bonds, IS internal standard.

Table 1. List of identified organic compounds from organic sample recovered from Rossio square (Praça D. Pedro IV) *.

	Compound	Retention Time (Minutes)		Compound	Retention Time (Minutes)
Steroids	Coprostane	49.758	Fatty acids	Pelargonic acid (C9:0)	14.966
	Cholesta-2,4-diene	50.258		Capric acid (C10:0)	18.675
	Stigmastane	52.362		Lauric acid (C12:0)	25.733
	Coprostanol	53.38		Tridecylic acid (C13:0)	29.078
	Epicoprostanol	53.61		Myristic acid (C14:0)	32.279
	Cholestanone	53.706		Palmitic acid (C16:0)	38.024
	Cholest-1-en-3-one	54.221		Margaric acid (C17:0)	40.148
	5α-Cholesterol	54.542		Octadecenoic acid isomer (C18:1)	41.442
	5β-Stigmastane	55.642		Octadecenoic acid isomer (C18:1)	41.608
	Ergostanol	55.775		Stearic acid (C18:0)	42.142
	β-Stigmastanol	55.842		Nonadecylic acid (C19:0)	43.783
	β-Sitosterol	56.583		Arachidic acid (C20:0)	45.415
	5α-Stigmasterol	56.675		Heneicosylic acid (C21:0)	46.932
Alcohols	Myristyl alcohol (C14:OH)	29.583		Behenic acid (C22:0)	48.407
	Pentadecyl alcohol (C15:OH)	32.79		Lignoceric acid (C24:0)	51.143
	Palmityl alcohol (C16:OH)	35.792	Alkanes	Heptadecane (C17)	27.042
	Heptadecyl alcohol (C17:OH)	38.327		Octadecane (C18)	30.408
	Stearyl alcohol (C18:OH)	40.425		Nonadecane (C19)	33.56
	Nonadecyl alcohol (C19:OH)	42.325		Eicosane (C20)	36.59
	Arachidyl alcohol (C20:OH)	45.608		Docosane (C22)	41.075
	Tricosyl alcohol (C23:OH)	48.567		Tetracosane (C24)	44.608
Acylglycerols	Monopalmitin	47.96		Heptacosane (C27)	49.142
	Monostearin	50.676		Nonacosane (C29)	51.858

* The trimethylsilyl (TMS) derivatives of compounds detected.

The distribution of sterols was dominated by coprostanol and its sterol precursor, cholesterol, while in lower amounts of β-Sitosterol and its reduction product stigmastanol were noticed, along the presence of ergostanol. Epicoprostanol, product of post-depositional epimerization in the faecal waste, has also been detected.

The pattern of fatty acids, long chain alkanes and alcohols, and faecal steroids can be related to human diet based on the animal and plant products consumed. The faecal steroids [34,43,45], which include sterols, stanols and stanones, are produced by the reduction of the sterols of animal origin (cholesterol) and plant origin (such as sitosterol, campesterol and stigmasterol). The microbial reduction in the intestine yields stanols but, in the higher intestine of mammals, as humans are, only the 5β(H) stanols are formed, while the 5α(H) analogues are formed during deposition by soil bacteria. In human faeces [83–85] coprostanol is the mostly commonly detected 5β(H) stanol, formed from both ingested and de novo biosynthesized cholesterol. Detection of faecal steroids, namely coprostanol, in addition to archaeological information derived from vessel typology [86] from which organic matter was retrieved, allows the interpretation of the organic material transformed through the digestive tract, or accumulated faecal matter.

3.4. Microparticle Analysis

Since microscopy and elemental mapping revealed silicon-rich inclusions, analysis of microremains was conducted to gain additional information. Pollen grains, besides the added standard of *Lycopodium* spores, were not detected. It is important to note that the absence of contemporary pollen grains suggests that the sample was not contaminated post-excavation. However, various non-pollen palynomorphs (NPPs) [87,88] were identified in the sample such as plant remains, coprophilous fungi spores, larvae and tissues.

Plant remains (Figure 6a–d) visible in the sample include plant tissues and vessels. Elongate, polylobate and dendric tissues are characteristic of grass family, Poaceae, which includes cereals [89,90]. However, the prevalent microremains in the sample are coprophilous fungi spores [91,92]. Non-coprophilous fungal spore of *Glomus* type is also present (Figure 5e,f). The development of both types of fungi occurred outside the gut on the deposited faeces. This indicates that remains were deposited for some time, possibly as an accumulation from more than one bowel movement.

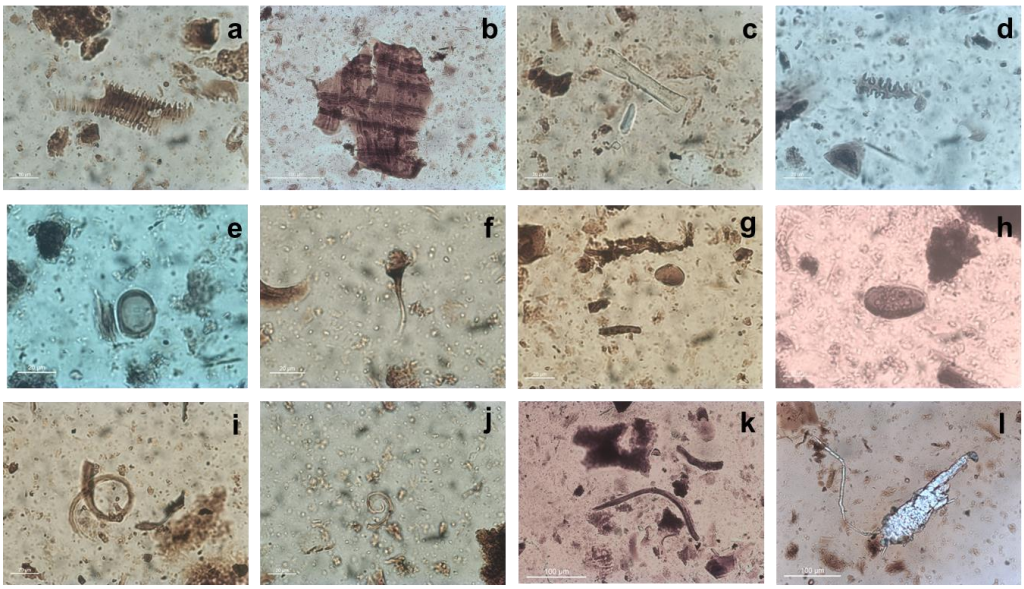

Figure 6. Micro-particles present in the carbonized faeces: (**a**,**b**): unknown tissue fragments; (**c**): elongate plant tissue; (**d**): dendric plant tissue; (**e**): fungal spore of *Glomus*-type (HdV-1103); (**f**): a spore filament; (**g**): cf. HdV-123; (**h**): cf. HdV-368; (**i**–**k**): unknown elongated remains; (**l**): remains of a crustacean.

The faecal sample also contained unidentified microparticles that might have belonged to nematode, or roundworm larva (Figure 6i–k), but the state of preservation does not allow a certain determination [93,94]. These remains are elongated, up to 200 µm long, fragmented and often coiled. However, the remains of crustacean specimens (Figure 6l) also present in the faecal sample may indicate that these arthropods acted as carriers for parasitic worms.

4. Discussion

Analyses of paleofaeces and coprolites present a very specific study of consumed and partly digested plant and animal materials. They provide a picture of a diet concerning only from a few hours to a maximum of a few days before defecation, therefore reflecting only a short-term dietary practice [95]. However, the information that can be obtained from faecal material is abundant and unique. Biomolecular analysis can be a particularly powerful tool to discover the food choices and health status of both animals and humans [9,43,46]. Since lipid molecules are part of all living organisms and have a very poor solubility in water, especially the more complex ones, they are fairly resistant to post-depositional

degradation. However, and despite this apparent stability, it is important to account for the diagenetic transformation expected for the different lipid biomolecules when analyzing faecal data [41]. When the data are combined, and distribution of sterols, long chain alkanes and alcohols is considered, it can be related to the dietary choices. In the case of humans, the relative number of compounds derived from the cholesterol and plant sterols can provide an idea about the correlations of animal/plant products consumed by an individual. Herbivores ingest large amounts of plant-derived sterols; therefore, the faecal profile should show prevalence of sitosterol and stigmastanols [96–98]. On the other hand, omnivore diet results in a much higher representation of choresterol and coprostanol in relation to stigmastanol [23,33,42,99]. Carnivore faeces is unlikely to be recovered in this context, but in that case steroidal profile should be characterized by very high levels of cholesterol and only trace amounts, or even absence, of coprostanol and epicoprostanol [42,44,100].

In humans, cholesterol-derived coprostanol (Figure 7), is the major 5β-stanol in human faeces, accounting for up to or more than half of the total sterol content [43]. As previously stated, this is due to formation from both ingested and synthesized cholesterol, while other stanols represent direct dietary input since humans do not synthesize plant sterols [24]. However, the effectiveness of the conversion of cholesterol-derived 5β-stanols may vary among the individuals [101], as it is dependent on the gut bacteria present in the intestinal tract [102] and dietary choices [103]. Post-deposition, after the microbial hydrogenation in the gut, exposure to environmental conditions outside the gut and diagenetic processes in general lead to the formation of epicoprostanol, often detected in sewers [104].

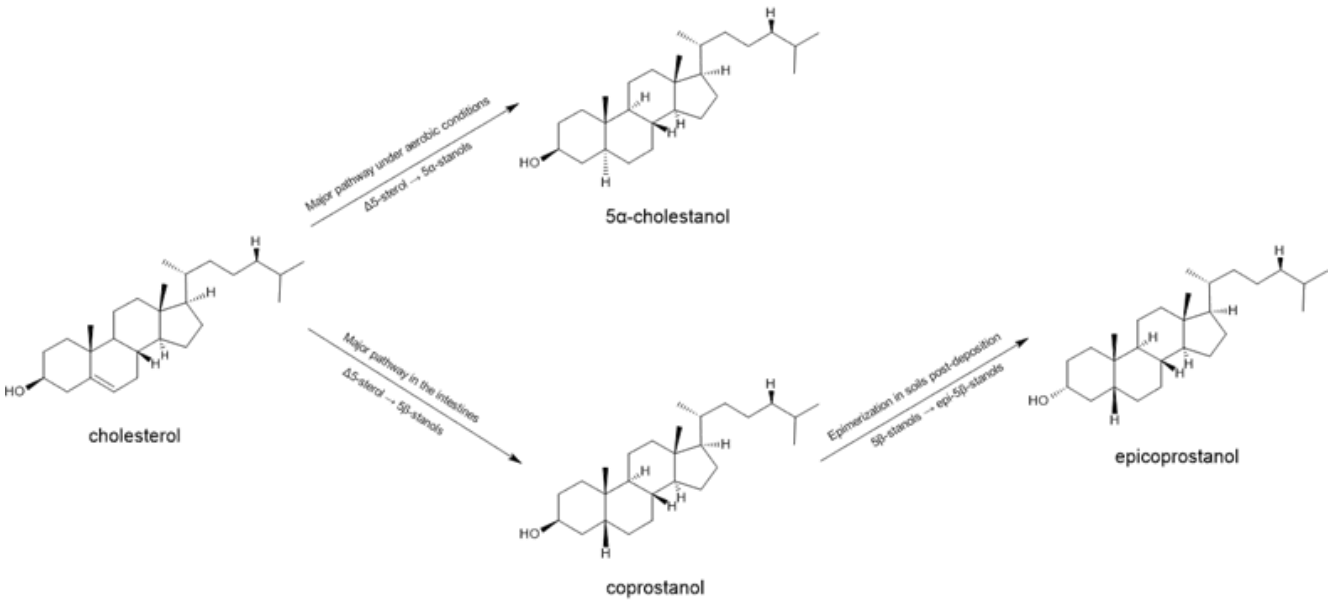

Figure 7. Simplified schematic of the biochemical pathway of the Δ5-sterol (cholesterol) reduction to 5α-stanols and 5β-stanols in the natural environment and inside the gut (according to Bull et al., 2002 [43], Sistiaga et al., 2014 [24], Prost et al., 2017 [34]).

The pattern of faecal steroids reveals that the individuals residing in the house of Rossio square probably had a diet rich in animal protein complemented by some plant products, as the total peak areas of the stanols and stanones derived from cholesterol are higher than those derived from plant steroids. The detected large amounts of coprostane and cholestane, both diagenetic products of coprostanol and cholesterol, respectively, are likely due to the effect of the extreme heat at which the paleofaeces was subjected due to the fire after the earthquake. The importance of the animal products in the diet is further confirmed by the pattern of long chain alkanes and alcohols. These compounds arise from the plant waxes [105], and they should be abundant in coprolites produced by plant-based

diet. In omnivores, the size of these peaks can give an indication of the relative amount of plant products consumed, which does not seem to be very large for this individual.

Furthermore, since structural and elemental analysis, substantiated with the IR spectrum, demonstrates faeces fairly dense with inclusions of animal origin, probably micro fragments of animal bones, it can be concluded that the diet of the occupants of this house was abundant in meat, or at least that the last meals before the Earthquake struck were plentiful in meat product. However, since those micro fragments are hard to digest and the sample is heterogeneous, there might be a bias towards animal products consumption on the expense of plant-based food. Nonetheless, without a doubt meat was readily available, prepared and consumed in this household.

However, meat based, protein rich diet also influenced the health of the individuals consuming it. While intake and availability of meat had its health benefits, and points to abundance and comfortable lifestyle, long-term fatty diet could also have adverse consequences [106]. Inadequately prepared meat dishes could also advance parasitic infections [107]. Therefore, in the densely populated urban context of 18th century Lisbon, proper sanitation was substantial to the overall health of the population. Practice of collecting the faeces in designated ceramic vessels [86], their separation and removal, as it was done in better-off Lisbon households of the 18th century, is a testament of the importance of hygiene standards by itself. This is furthered by the value that was given to the water systems in Portugal, where water supply has a long history and the sanitation water treatment structures, based on Roman and Islamic traditions, are documented since the Middle Ages [108]. In Lisbon, at the end of 15th and in the 16th centuries, urban sanitation and public health policies were implemented that included street cleaning by residents, designation of public latrines, garbage collection and disposal sites, removal of polluting activities from the city center, pavement of streets, and the installation of sewerage pipes and covering of open-air ditches [109]. In terms of public concern with cleanliness, the infrastructure and the policies put Lisbon among the forefront of major contemporary European cities of the Modern Era [110]. Part of the sewage network that covered the city was unearthed in the west part of the Rossio house courtyard, constructed at the end of the 15th or the beginning of the 16th century, which supports the notion that sanitation was important and accessible. Excavation exposed up to 2.20 m in length and 1.10 m in depth, showing that it was constructed with a bricked arch ceiling, laying on roughly cut stone walls [51]. Additionally, in 18th century Lisbon, the *Águas Livres* Aqueduct, constructed by 1748, was another systematic solution that secured fresh clean water to the urban population [111]. However, the presence of parasitic species in the faeces should not be so surprising, since the animals were kept in close proximity to the tenants on the ground floor of the same house, where a part of it served as stables [51]. Nonetheless, these infections have the potential to be harmful to health, nutrition, and well-being, especially if they persist or went untreated [112]. Conveniently, natural treatments of the 18th century against infestation are well-documented in Lusitanic traditional medicine [113,114], as it was likely a common condition in Portugal as well in other major European communities in the transition from Early to Late Modern Period [115].

5. Concluding Remarks

Recovering carbonized human faecal matter in a ceramic vessel is truly unusual, since charred remains usually tend to be remains of foodstuff. Biomolecular approach was necessary to determine the origin of the amorphous visible residue, while integrating archaeological and chemical information allowed the identification of the vessel and its organic content. The vessel was recognized as *calhandro*, a word used to describe a tall, cylindrical vase, intended for collecting waste and other filth [85]. Once the faecal matter was collected from the residents of the house in this big cylindrical pot, it would be transported to the river front and disposed. Even though activities associated with waste collection and disposal are common and a relevant part of an everyday life, finds like these are not frequently recognized in the archaeological context.

Microstructural, elemental and chemical analysis of carbonized faeces provided information about dietary habits and health of the 18th century individuals and their lifestyle. The presence and abundance of human derived 5b-stanols, such as coprostanol, was strong evidence for the identification of the remains as human excrement. Good preservation of organic matter, even after the carbonization and the presence of sterols, stanols and stanones, confirms the high resistance of these compounds to degradation in the natural environment and makes them suitable as a biomarker in the archaeological context.

The observed nondigested food remains demonstrate a mixed diet based on both animal and plant sources. Microfragments of bones detected also contributed to the resistance of the matter, since the consumption of bone elevates the levels of phosphorous and calcium in excrement and advances preservation [116].

Elemental analysis and biomolecular information also support an omnivore diet. The abundance of detected calcium in the analyzed sample points to a calcium-rich diet that can come from animal products, dairy and dairy products, but also from sardines and leafy greens. Biomolecular analysis, furthermore, pointed towards a predominance of animal food sources. The plant-based component of the diet is represented by long chain alkanes and alcohols, along with sitosterol and its products from gut bacteria activity. The presence of coprophilous fungi spores and epicoprostanol in the faecal remains affirm that the material was deposited over a certain amount of time. Therefore, the analysis supports that the cylindrical vessel, discovered in the storage area was used to accumulate human waste. The possible occurrence of parasitic roundworms is not surprising, and provides an idea about environmental interactions, sanitation and health in the developing city.

Results presented here demonstrate that interpretation of decayed organic remains can be ambiguous, and that biomolecular analysis is necessary for the correct interpretation. Combining steroidal biomarkers with other proxies can help verify the interpretation but may also raise new questions for further investigation, since archaeological faeces as a copious well of information can be approached from different avenues of study. Moreover, this research reinforces the notion that archaeological faeces provide excellent evidence of diet, health and lifestyle of the past societies.

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