Food and balms: combined botanical and chemical studies from funerary and domestic contexts

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To my father

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Abstract

This study examines plant material preserved in the various archaeological contexts incorporating different approaches, including the study of micro- and macroremains as well as chemical profiles. Identification of microremains – pollen grains, phytoliths, NPPs (non-pollen palynomorphs) and plant tissues – and plant macroremains, like seeds and wood, can be achieved with the aid of microscopy, and depends on plant morphology and taxonomy. Residue analysis, performed on amorphous material, relies on the concept of biomarkers, i.e. organic molecules that can be used as the chemical fingerprinting indicating the origin of the substance.

Combining these approaches, the studied material was selected in accordance with state of preservation and availability. It is important to assure that the artefacts studied are minimally affected by the analytical procedure so they can be used for future research or displayed in exhibitions. Samples were prepared in accordance with the optimum instrumental methods and then studied under light microscope, stereomicroscope, and scanning electron microscope (SEM), depending on specific demands of the material. This methodology enabled the morphological study and identification of plant remains. For chemical analysis, visible organic residue was subjected to non-destructive attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) and minimally destructive pyrolysis gas chromatography mass spectrometry (Py-GC/MS). Organic residues from pottery vessels, identified as carriers for food offerings, were characterized by gas chromatography mass spectrometry (GC/MS). When relevant, the inorganic fraction of the samples was characterized by scanning electron microscope coupled with energy dispersive X-ray spectrometry (SEM-EDS) and X-ray diffraction (XRD). Selection of the appropriate techniques was modified to suit the demands of each case study. All the acquired data was framed in the context of past archaeological and environmental conditions, as well as available natural materials, allowing a comprehensive interpretation of the archaeological plant remains.

Cibo e balsami: combinazione di analisi botanici e chimici per lo studio di contesti funerari e domestici

Riassunto

Questo studio consiste nell'analisi interdisciplinare di micro- e macro-resti vegetali provenienti da vari contesti archeologici e nell'identificazione dei rispettivi profili chimici. Comunemente, l'analisi di micro-resti quali granuli pollinici, fitoliti e NNPs (palinomorfi nonpollinici), e macro-resti vegetali, come semi e legni, avviene per mezzo di indagini microscopiche che permettono di riconoscere la morfologia e tassonomia della pianta di provenienza. Circa l'analisi dei residui dei materiali amorfi, la loro caratterizzazione si basa su biomarcatori, ossia molecole organiche che servono da indicatori chimici, rivelandone l'origine.

Combinando i due approcci, il materiale per la ricerca è stato selezionato basandosi sullo stato di conservazione e sulla disponibilità. Inoltre, dato che il trattamento analitico di manufatti attraverso protocolli di laboratorialo è ridotto al necessario per limitare alterazioni e garantire usi futuri a fini di ricerca o musealizzazione, i campioni sono stati preparati secondo i metodi strumentali appropriati. In rellazione alle delle esigenze specifiche del materiale analizzato, ciascun campione è stato studiato mediante la microscopia ottica, stereo-microscopia e microscopia elettronica a scansione (SEM). Per l'analisi chimica, il residuo organico visibile è stato sottoposto a spettroscopia di trasformata di Fourier a riflettanza totale attenuata non distruttiva (ATR-FT-IR) e spettrometria di massa di gascromatografia a pirolisi minimamente distruttiva (Py-GC/MS). I residui organici recuperati da vasi di ceramica, identificati come contenitori di offerte alimentari, sono stati caratterizzati mediante gascromatografia-spettrometria di massa (GC/MS). Qualora pertinente, la frazione inorganica dei campioni è stata caratterizzata mediante SEM accoppiata con spettrometria a raggi X a dispersione di energia (SEM-EDS) e d diffrattometria a raggi X (XRD). Ciascuna tecnica è stata impiegata a seconda delle esigenze diversificate di ogni caso di studio. I dati acquisiti sono stati contestualizzati alle

precedenti condizioni archeologiche e ambientali, nonché dei materiali naturali disponibili, consentendo un'interpretazione completa dei resti vegetali archeologici.

Alimentos e bálsamos: combinando botânica e análise química para o estudo de contextos funerários e domésticos

Resumo

Este estudo examina o material vegetal preservado nos vários contextos arqueológicos incorporando diferentes abordagens, incluindo o estudo de micro e macroresíduos, e de perfis químicos. A identificação de microrestos e macrorestos vegetais, pode ser obtida com recurso à microscopia, e é baseada na morfologia e taxonomia da planta. A análise de resíduos orgânicos em material amorfo baseia-se no conceito de biomarcadores, ou seja, moléculas orgânicas que podem ser usadas como a impressão digital química indicando a origem da substância. Combinando estas abordagens, o material estudado foi selecionado de acordo com o seu estado de conservação e disponibilidade. É importante assegurar que os artefatos estudados sejam afetados o mínimo possível pelo procedimento analítico para que possam ser utilizados em estudos futuro ou expostos em exposições. As amostras foram preparadas de acordo com os métodos instrumentais ideais e estudadas com recurso a microscopia ótica e microscopia eletrónica de varrimento (SEM) de acordo com as suas características específicas. Esta metodologia possibilitou o estudo morfológico e a identificação de restos vegetais. Para a análise química, o resíduo orgânico visível foi submetido a espectroscopia não destrutiva de reflectância total atenuada no infravermelho por transformada de Fourier (ATR-FT-IR) e espectrometria de massa por cromatografia gasosa de pirólise minimamente destrutiva (Py-GC/MS). Resíduos orgânicos em objetos de cerâmica identificados como contentores de oferendas alimentares foram caracterizados por espectrometria de massas por cromatografia gasosa (GC/MS). Quando relevante, a fração inorgânica das amostras foi caracterizada por SEM acoplada a espectrometria de raios-X de energia dispersiva (SEM-EDS) e difração de raios-X (XRD). A seleção das técnicas apropriadas foi modificada tendo em conta as especificidades de cada estudo de caso. Todos os dados adquiridos foram enquadrados no contexto das condições arqueológicas e ambientais passadas, bem como dos

materiais naturais disponíveis, permitindo uma interpretação abrangente dos vestígios vegetais arqueológicos.

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List of abbreviations

AD	Anno Domini
AMDIS	Automated Mass spectral Deconvolution and Identification System
AMS	Accelerator Mass Spectrometry
ARCHMAT	Archaeological Materials Science
ATR	Attenuated total reflectance
BAOC	Beja-Acebuches Ophiolitic Complex
BC	Before Christ
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
CIZ	Central-Iberian Zone
DNA	Deoxyribonucleic acid
ED-ARCHMAT	The European Joint Doctorate in Archaeological and Cultural Heritage Materials Science
EDS	X-ray Energy Dispersive Spectrometer
FA	Fatty acid
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas chromatography
GC-C-IRMS	Gas chromatography–Combustion–Isotope ratio mass spectrometry
IRMS	Isotope-ratio mass spectrometry
μ-XRD	Micro-X-ray Diffraction
MAGs	Monoacylglycerols
MS	Mass spectrometry
m/z	Mass-to-charge ratio
NIST	National Institute of Standards and Technology
NPPs	Non-pollen palynomorphs

OM	Optical microscopy
OMZ	Ossa Morena Zone
ORA	Organic Residue Analysis
pH	Potential of hydrogen
Py-GC/MS	Pyrolysis – gas chromatography/mass spectrometry
SEM	Scanning Electron Microscope
SEM-EDS	Scanning Electron Microscope coupled with Energy Dispersive X-ray Spectrometry
TAGs	Triacylglycerols
TLE	Total Lipid Extract
TMAH	Tetramethylammonium hydroxide
TPBCSZ	Tomar-Portalegre-Badajoz-Cordoba Shear Zone (TPBCSZ)
VP	Variable pressure
XRPD	X-ray powder diffraction

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Chapter I – Introduction

1. Combining palaeobotanical and chemical approaches to the study of archaeological plant remains

Plant remains uncovered in various types of deposits connected to human activities, reflect the interactions between populations and their natural surroundings through the millennia. Plant remains preserved in archaeological contexts have the potential to reflect agricultural practices, diets, land management, burial rituals, beliefs, networks and trade. In general, these remains indicate exploitation, utilization and/or manipulation of natural assets as well as adaptations to the environment. Analysis of the dynamics of the human-plants relationship has been woven into the archaeological material studies. Archaeobotany (or Paleoethnobotany) is an interdisciplinary field that explores the relationship among plants and people in the past, on the basis of the recovered and recognized botanical remains. Archaeobotany aims to reconstruct past vegetation, human influence and human–plant interrelationships (Miller 1995, Wright 2010).

Since the inception of the discipline in the 19th century, archaeobotanical studies focused on the identification of plant materials and the plant exploitation in ancient societies (Pearsall 2016). In the 20th century, topics of early domestication and cultivation were explored (Helbaek 1959, Clark 1967, Sherratt 1980). In the second half of the 20th century, methodological advancements of flotation technique for recovery of macroremains (Struever 1968) and a greater application of microbotanical studies of archaeological materials (Pearsall 1982, Bryant and Holloway 1983, Rovner 1983, Piperno 1988, Birks and Berglund 2018), furthered the interest in human-environment interactions. In the last decades, new insights into early plant cultivation and domestication (Fuller et al. 2014), population movements, and plant dispersal (Crowther et al. 2016, Spengler 2020) were articulated. This century brought more improvements in methodology and interpretation (Jacomet and Kreuz 1999, Pearsall 2016), including furthering the study of plant microremains (Piperno 2006, Barton and Torrence 2015, Hart 2016). The application of biomolecular and isotopic techniques to plant macrofossils and residues (Evershed et al. 1990, Evershed et al. 1991a, Evershed 1993, Copley et al. 2001, Copley et al. 2005, Fiorentino et al. 2015) opened new pathways into the understanding of plant exploitation and adaptations to the environment. The identification of archaeological plant-based natural products and foodstuffs processed in ceramic vessels, through the analysis of lipids, preserved as surface residues or in the fabric of potsherds, showed specific indication of pottery use that involved plant sources (Evershed 2008a, Roffet-Salque et al. 2017). Developments in organic residue analysis (ORA) are linked to the advances in chromatographic and mass spectrometric techniques. The investigation of organic residues using modern analytical chemical methods began in the 1970s. Further progress in the 1990s enabled accessing stable isotope information from individual chemical compounds (Evershed et al. 1994, Dunne et al. 2017).

The increase in studies focusing on microscopic analysis, contents of vessels, as well as chemical and molecular markers (Regert et al. 2008, Correa-Ascencio et al. 2014, Brettell et al. 2015, Dunne et al. 2019, Pecci et al. 2020, Mariotti Lippi and Giachi 2021) brought to light plant-related remains and products that were often invisible in the archaeological record or detectable only through indirect or contextual evidence. Attempts to bridge this gap in knowledge created a mosaic of diverse approaches in the study of plant materials from the past, usually specializing on the type of organic remains like starch, phytoliths, pollen, seeds, plant tissues and wood, or organic residues (Giachi et al. 2013, García-Granero et al. 2014, Łucejko et al. 2015, Heron and Craig 2015, D'Agostino et al. 2021).

This caused discrepancies in terminologies used by researchers from different backgrounds, conflicting strategies of sampling, loss of information and destruction of potential plant remains, as well as different approach to interpretation of archaeological material. A discussion is necessary to examine the potential and the limitations of the methods utilized. On the basis of the several case studies in the area of plant remains, this work looks into the possibility of a more inclusive workflow, exposes the pitfalls uncovered through research, reviews current terminology and encourages a path towards new lines of research and questioning.

1.1. Preservation of organic material of plant origin

The decay of archaeological materials of plant origin is caused by a complicated interplay between the environment and the matter itself. Excluding pre-depositional activities such as processing or cooking, plant based organic remains are particularly vulnerable to biological, physical, and chemical deterioration after deposition. The variables influencing the survival of plant remains in archaeological context include local pH, temperature, the presence of oxygen, water movement, and microorganisms. The key to good conservation are stable environment conditions with limited oxygen access and water circulation. The most common ways of preservation are defined as carbonization, mineralization, waterlogging, desiccation and freezing, with different extent of preservation achieved according to the environmental conditions, such as the pH (Day 2013). Despite the fact that all of these pathways enable the study of organic materials from the past, they should be considered fragmentary materials representing an incomplete archaeological record, as all modes of preservation lower the resistance, alter, and damage organic matter. Moreover, since the modes of preservation of organic materials are selective and destructive, environmental conditions during and post-deposition highly influence the present archaeobotanical assemblage (Table I.1).

Table I.1 Estimated comparative preservation potential in various types of archaeological sites. Symbols for quality of preservation: *** excellent; ** fair; * poor; - generally not preserved. Adapted from Miksicek 1987.

Differences in the Preservation of Biological Remains in Various types of Environments						
	Frozen sites	Peat bogs (wet, acid)	Waterlogged sites (wet, anaerobic)	Open sites (moist soil)	Open sites (dry soil)	Dry caves
Coprolites	*	*	*	-	-	***
Parasites	**	**	**	-	-	***
Pollen	***	***	***	*	**	***
Phytoliths	***	**	***	**	**	***
Wood	***	***	***	-	*	***
Charcoal	***	***	***	**	**	***
Fresh seeds	***	***	***	-	*	***
Charred seeds	***	***	***	**	**	***
Fibers	***	***	***	-	*	***

Carbonized remains were fossilized through charring under oxygen-poor conditions, irreversibly altering the structure and the composition of the remains. Carbonization is the most common of all archaeobotanical preservation types (Jacomet 2007). Palaeobotanical studies demonstrate that carbonization causes degradation and distortion of plant remains depending on temperature (Boardman and Jones 1990, Märkle and Rösch 2008). The most important factors that influence simultaneous degradation-preservation are temperature and duration of exposure as well as chemical content and amount of moisture in the remains (Stewart and Robertson 1971, Wright 2003). Charred seeds show an increase in width and a decrease in length, as well as structural alterations and the development of protrusions (Braadbaart 2008). Wood demonstrates a reduction in volume, and while mostly keeping its basic structure becomes brittle (Braadbaart and Poole 2008). This means that the dimensions of archaeological and historical carbonized plant remains significantly differ from the source material as well as the modern references. Furthermore, if conditions during carbonization are severe (prolonged exposure, high temperatures), part of the archaeobotanical record is not only damaged, but destroyed.

On the other hand, waterlogging, preservation in wet or humid environments due to anaerobic conditions and still water, is a non-destructive conservation path. In waterlogged conditions, organic materials are preserved as a result of the abundance of static water and the chemical balance of this water, pH and oxidation-reduction potential. Water that excludes air creates reduced oxygen levels and prevents most microorganisms from thriving. In conditions like these, organic materials get saturated with water, thus the form is mostly retained. In an anoxic environment, however, some breakdown might occur due to anaerobic microorganisms such as sulphur-reducing bacteria. Altering stable conditions and exposure to air, by removing the material from its original archaeological context, can cause the further deterioration of organic remains (Fors and Sandström 2006). Sometimes a dual mode of preservation occurs; for example, although charred remains are better preserved in well-drained soil, they can also be recovered in waterlogged conditions (Jacomet 2012), when they are first charred and then submerged. The waterlogged archaeobotanical remains should be stored in wet conditions, because drying will change the water content that sustains their structure and cause destruction of plant remains (Tolar et al. 2010). Deposition in aqueous environments were also determined to be favorable for the preservation of higher concentrations of plant microfossils (Jones et al. 2007, Cabanes et al. 2011).

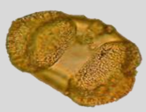
Besides carbonization and waterlogging, mineralization also sometimes occurs in a temperate climate as a form of preserving botanical remains. Mineralization is a naturally occurring complex process in which mineral deposits from the environment surrounding the organic matter, gradually replace plant material. It is reliant on number of factors, including soil type, type of organic remains present, and sufficient circulation of groundwater. This process often occurs through release and absorption of phosphate ions, from a variety of sources including faunal remains, food residues, and excrement, facilitated by fluctuating water levels within the archaeological deposit (Murphy 2014). In addition, mineralization by absorption of calcium carbonate and silica occurs, and this process can sometimes even be caused by metal materials such as bronze and iron (Jacomet 2007). The result is a cast of the organic material called a pseudomorph, which replicates the original form with detail and accuracy (Green 1979). In more severe conditions, preservation of organic remains can occur due to extremely arid or freezing condition that do not allow progression of biodegradation processes. It occurs when moisture levels are too low for the organisms responsible for organic decomposition to survive or be active, is also known as desiccation (Jacomet 2007). It has been demonstrated that desiccated plant remains show good chemical preservation and low signs of microbial alteration (Van Bergen et al. 1997). Desiccated remains should not be re-exposed to humidity, because dry remains will reabsorb the moisture, triggering the degradation processes. In frozen environments, continuous low temperatures hinder activities of microorganisms providing excellent conditions for preservation of organic remains in an outstanding form, preserving food, tissues, even ancient DNA (Rollo et al. 2002).

Even if they are not always recovered in the integral state, plant remains can be preserved in archaeological deposits, structures, soils and vessels, as molecular structures. During partial decomposition, chemical traces can get absorbed into an inorganic matrix (like soil or ceramics), carrying the information about the plant material present or utilized at the site (Hjulström and Isaksson 2009, Pecci et al. 2018). Analysis performed suggests that absorbed organic residues can survive in high percentages of domestic cooking pottery assemblages worldwide (Evershed 2008b), but successful recovery is dependent on conditions of preservation, excavation, and storage. Main organic substances that can be preserved as remnants of plant material are molecules and degradation products of lignin, cellulose, proteins, fats and plant waxes, and resinous material. In association with archaeological soils, vessels and artefacts, even preservation of starch is possible in dry or waterlogged conditions (Pearsall 2016, Copeland and Hardy 2018) that prevent microbiological activities. The degradation of substances of plant origin depends on the environmental conditions, as well as their chemical composition, as not all compounds degrade at the same rate. Natural plant resins, composed of terpenoids, widely utilized since Prehistory, are not only highly resistant to degradation, but can protect and retard biological and chemical degradation of other plant materials. Long-chain lipids are more resistant than carbohydrates and proteins, as they are insoluble and less vulnerable to water leaching and biodegradation (Hillman et al. 1993). Preservation of fats and protein has been reported even with carbonized remains after thermolysis (Hansson and Isaksson 1994), while in waterlogged conditions, lipids and proteinaceous components can be reasonably well preserved (Evershead 1992, Nielsen et al. 2021). Starch degrades more easily than cellulose, while cellulose is more readily broken down then lignin. Of course, when analyzing archaeological material that has been affected by degradation processes, either during processing of organic material or during deposition, fragmentation occurs on the molecular level. So, the original substance is reconstructed on the basis of its building blocks and degradation products. Sometimes, the structure of a single component is sufficient to define the origin of a constituent of an organic residue. However, the analysis of these compounds is not straight forward since organic material can be a complex mixture of ingredients.

1.2. Plant remains in archaeological contexts

Material of plant origin can be preserved via diverse pathways or combinations of them, as described in the section above, enabling them to be studied centuries/millenia later. They can be recovered from the archaeological context and examined on a macro-, micro- and a chemical level (Table I.2).

Table I.2 Review of plant remains in archaeological contexts.

	Plant remains	Occurrence	Methods of analysis	Examples
	Macroremains (fibers, seeds and fruits, wood and other plant tissues)	Grave offerings, part of funerary rituals, storage, kitchen, latrines, wells, pits	Light microscopy, Stereomicroscopy, Scanning electron microscopy, FT-IR, Py-GC/MS, GC/MS, IRMS	Łucejko et al. 2015, Broda and Popescu 2019, Valamoti et al. 2019, Heiss et al. 2020
	Microremains (starch, phytoliths, pollen and non-pollen palynomorphs)	Grave deposits, offerings, balms, vessel coatings, archaeological soil and sediments, latrines, wells, pits	Light microscopy, scanning electron microscopy (SEM)	Konigsson 1989, García-Granero et al. 2014, Saul et al. 2013
	Visible residue (on bones, vessels, tools and artifacts)	Cooking residues, balms, vessel coatings, grave offerings	Light microscopy, scanning electron microscopy (SEM), GC/MS, Py-GC/MS, IRMS	Regert 2003, Colombini et al. 2009, Teetaert et al. 2017
	Absorbed residue in matrix (soil, hearths, tools, ceramics, food utensils, etc.)	Food and beverage in both funerary and domestic context, balms, vessel coatings	GC/MS, IRMS	Copley et al. 2001, Copley et al. 2005, Fiorentino et al. 2015

The most common finds of botanical material on the archaeological site involve plant macroremains, since they are the easiest to detect. Macroremains are all plant remains visible to the naked eye or under minimal magnification, and include seeds, fruits, and wood but can come from all plant parts including roots, branches, leaves and trunk wood (Miller 1995). They are recovered from the archaeological context by using methods of flotation, screening or hand collecting *in situ* during excavation (Wright

2010). This also can refer to plant products, like remains of food preparations, balms, textiles, cordage or basketry. They are studied with an aid of stereomicroscopy at low magnifications (from 10× to 100×), relying on phytomorphology, knowledge of degradation processes that might alter the remains, and taxonomy to identify the correct botanical source of the preserved remains. When necessary, microscopy of greater magnification can be applied such as optical microscopy or scanning electron microscopy.

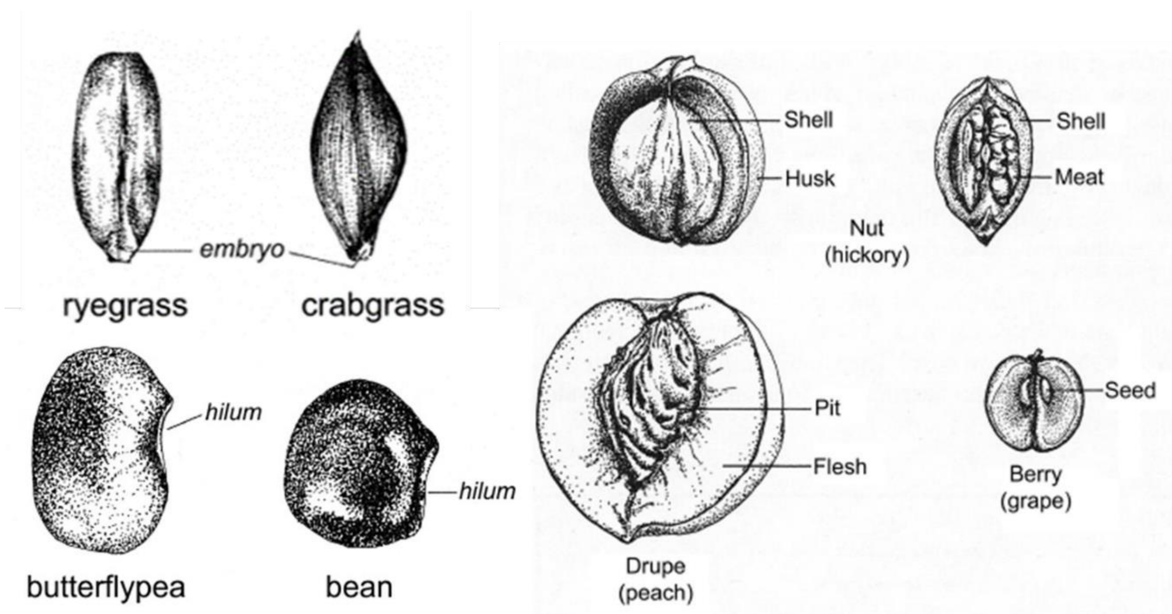


Figure I.1 Some examples of seed and fruit types. Adapted from Pearsall 2018.

These studies include paleocarpology and anthracology. Paleocarpology is a discipline devoted to the study of seeds and fruit remains recovered from archaeological sites and sediments in all modes of preservation. Identification and quantification of carpological remains (Figure I.1) recovered from the archaeological site can provide valuable information about plant-based diet, cultivation, dispersal and human-environment dynamics. On the other hand, anthracology specializes on the analysis and identification of charcoal, ligneous remains preserved after carbonization, based on specific structural characteristics and wood anatomy. Charcoal is charred wood and is preserved due to incomplete combustion by fire that makes it chemically inert and resistant to microbiological degradation especially in a stable or closed environment. As noted above, charred material including wood, undergoes reduction in volume when it is carbonized and is transformed into a friable material. Still, well-preserved anatomical structures (Figure I.2) allow the identification of its botanical origin (Celant and Coccolini 2015). The remains of carbonized wood can give information about paleovegetation, help in the reconstruction of past landscapes, provide data on construction material, exploitation, provenance and trade, as well as the occurrence of natural or anthropogenic paleo-fires.

The identification of the biological origin of plant products, like textiles or cordage, even when recognizable by the naked eye, relies on the study of fibers. Identifying and distinguishing between natural textile fibers of animal source, like wool and silk and textile bast fibers of plant origin like flax, nettle, hemp and jute, is based on surface characteristics, size, shape, cross sections and chemical composition. However, distinguishing among the bast fibers can be a challenging task, since they have similar morphology and chemical compositions (Carr et al. 2008, Bergfjord and Holst 2010).

Furthermore, plant traces can also be preserved in the archaeological record on a micro level (Wright 2010). These remains are not always recognizable, since they are not detectable without a microscope, but because of their highly resistant or inorganic nature might accumulate more readily in favorable condition, like in aired or waterlogged conditions (Jones et al. 2007). Plant microremains include phytoliths, diatoms, starch, microcharcoal and other plant tissues, pollen and non-pollen palynomorphs (NPPs). They can be preserved in soils, floor sediments, on archaeological tools and vessels, entrapped in resins or other organic materials. They differ in chemical composition and are identifiable on a basis of characteristic structure, shape, size and/or surface texture under higher magnification (200×, 400× and 1000×)

using an optical or scanning electron microscope. To retrieve plant source microparticles from the archaeological samples, some form of extraction procedure is necessary. The extraction procedure depends on the targeted plant microremain, since they differ in chemical composition, as well as on the context they are retrieved from.

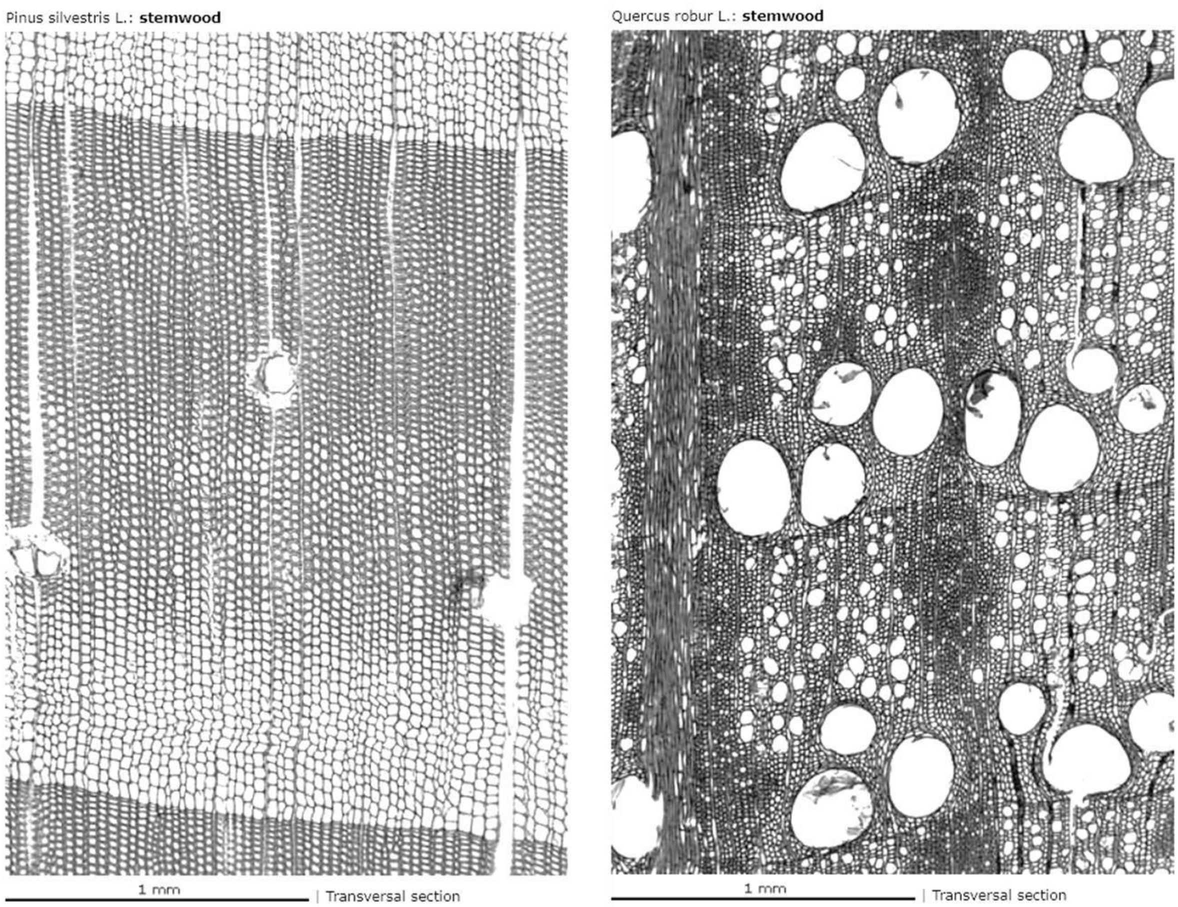


Figure I.2 Transversal sections showing a difference between (a) gymnosperm (softwood) and (b) angiosperm (hardwood) wood (Schoch et al. 2004).

Inorganic remnants of plants can be accumulated and preserved in archaeological context, due to their silica structure. Phytoliths are produced by the deposition of silica from groundwater into plant tissues and since some orders and families show tendencies for silica deposition, they can be useful in recognizing botanical origin (Pearsall 2016). They are identified by morphology, size, and surface characteristics.

Since phytoliths are inorganic, they survive in circumstances under which organic material might decay. They can be deposited into site sediments as well on tools, in vessels or ovens, by natural processes or human actions (Saul et al. 2013). Diatoms, remains of single-celled algae, are mainly recovered from waterlogged environment, and since they are responsive to environmental conditions, when recovered on-site, their identification can provide information about pollution, acidity, or salinity of a water source (Celant et al. 2015). Diatoms can be found in connection with settlements, since small inorganic particles can easily be ingested by organisms using the water source (Stone and Yost 2020).

Starch granules can be recovered in specific contexts related to human presence, like on tools, millstones, in vessels, in dental calculus, and can allow the identification of the processed or stored plants, which can aid in the reconstruction of diet, agricultural practices and technologies (Copeland and Hardy 2018). As starch granules vary between and within species in respect to size and shape, these differences in morphology allow their assignment to a botanical source.

Pollen grains (Figure I.3) are preserved due to the highly resistant exine that contains the chemically inert natural polymer, sporopollenin. Recovery of pollen also depends on the pollination mechanism of the taxa and the deposition processes that occurred (Pearsall 2016). In an archaeological context, pollen accumulates and preserves well in natural anoxic environments and waterlogged archaeological deposits like cesspits and wells, but can also be preserved in natural resins, in hearths and artefacts. Pollen analysis in archaeology uses the same methods as in paleopalynology (Celant et al. 2015), a scientific discipline focused on reconstruction of vegetation, vegetation changes and past environmental conditions, applied to archaeological deposits or objects.

Pollen deposited and preserved at archaeological sites can provide direct information about human activity on local scale, including land use as well as on-site activities, like food processing, plant deposition in graves, etc. (Konigsson 1989). Still, the analysis of pollen in archaeology does not always yield enough data for quantitative analysis of past vegetation and should be combined with paleopalynological studies near the site. Pollen analysis in archaeology is still relatively underrepresented and there is a great potential for further research combining on-site and off-site studies, chemistry and morphology of macrofossil analyses, etc. Non-pollen palynomorphs (NPPs) are remains of a great variety of organisms, including microscopic algae, bacteria, fungi, or other organisms that are recovered from the residue prepared for pollen analysis using classical palynological techniques (Van Geel 2001). Furthermore, microcharcoal and other plant tissues recovered from archaeological sites can give additional information concerning plant use and processing as well record the occurrence of deliberate and natural fires linking the study of human activity with paleoenvironment (Celant et al. 2015).

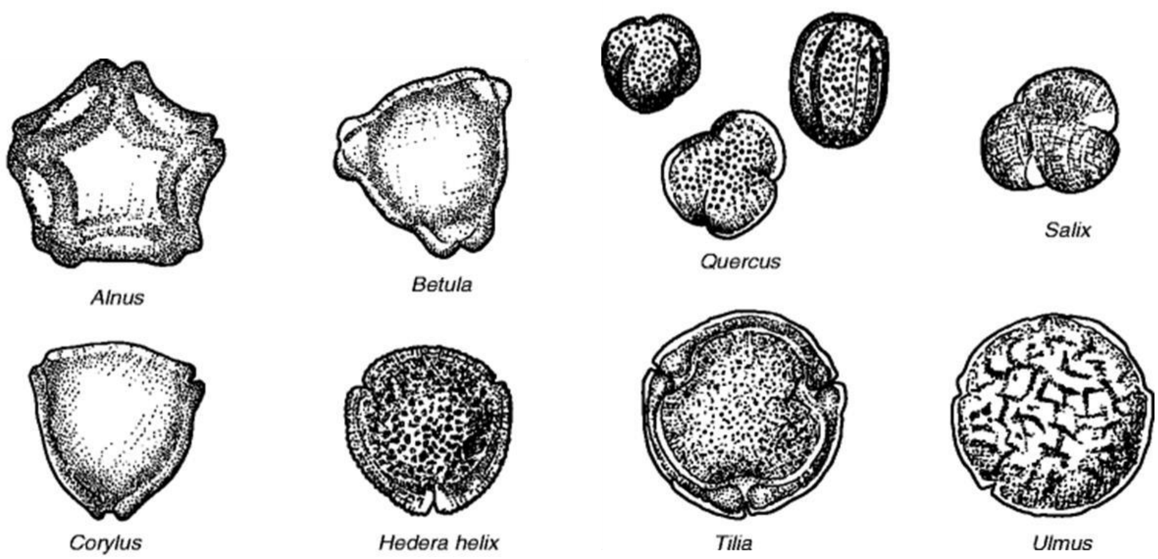


Figure I.3 Different morphology of some pollen grains. Adapted from Reitz and Shackley 2012.

Plant remnants can also be preserved as chemical traces. Organic residues are carbon-based substances of biological origin, and since they are amorphous or even invisible, they do not provide distinguishable morphological features to determine their original biological source. Still, they can be detected and identified with chemical analyses. The high sensitivities of instrumental methods allow microgram (μg) compounds to be detected and identified (Roffet-Salque et al. 2017). The study of organic residues focuses on the traces of the substances recovered from the surface of an archaeological object or absorbed within the matrix of pottery vessels and soil.

Organic residues are composed of organic compounds that remain after decomposition of plants and plant products. Visible organic residues, are organic substances detectable by the naked eye, adhering to the interior or the exterior of a vessel or tool, and could derive from heating, cooking and burning, or from materials used for decoration, impregnation and sealing (Roffet-Salque et al. 2017). They are also sometimes referred by their presumed origin, like foodcrust, adhesives, or incrustation but before the analysis has been conducted, a more general term of visible organic residue is preferable. However, they are susceptible to degradation, can be removed from the archaeological object by post-excavation handling and cleaning and are easily contaminated since they are exposed to the surroundings.

To rise above such difficulties, absorbed organic residues are analyzed. Absorbed residues are organic remains retained and preserved in a matrix, usually of unglazed ceramic vessels but can be preserved in hearths, floors and archaeological soils (Buonasera 2007, Hjulström and Isaksson 2009, Lucquin 2016, Pecci et al. 2018).

Organic residues preserve very well within pottery vessels, since ceramics is a durable and resistant material, found in abundance at archaeological sites, which gives a higher probability for the recovery of absorbed organic matter. The molecules inside the ceramic structure are also protected from light and oxygen that can advance its degradation. Absorbed residues can originate from the storage or processing of plant sources, and it can reflect a single use or an accumulation of events, like cooking.

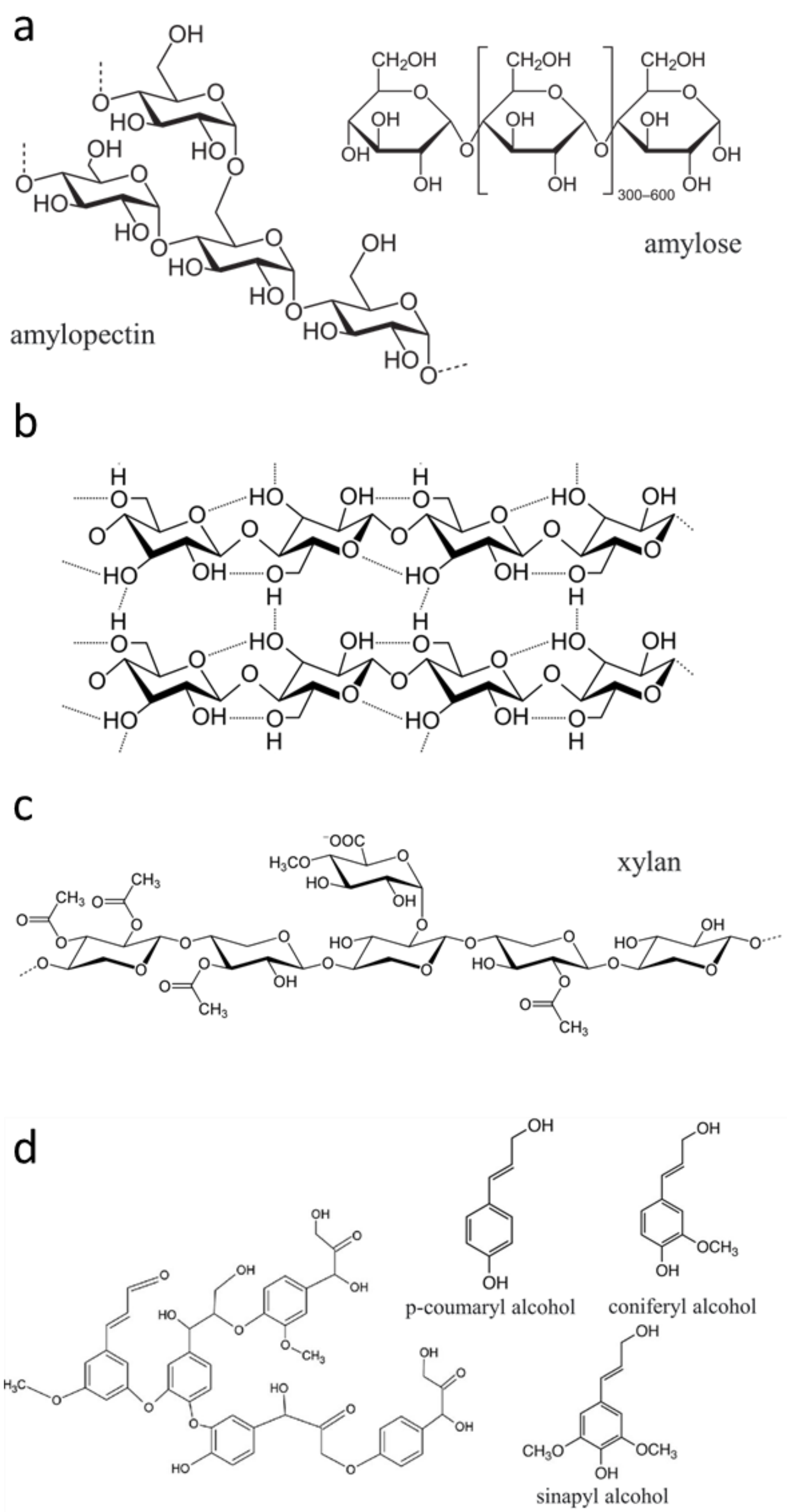


Figure I.4 Structural compounds of plant tissue. (a) starch – amylose and amylopectin, (b) cellulose, (c) hemicellulose (xylan), (d) lignin and aromatic monomers: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol.

The identification of the original plant source is based on a concept of a biomarker (Evershed et al. 1991b, Heron et al. 1993, Evershed 1993, Evershed 2008b, Roffet-Salque et al. 2017). Archaeological biomarkers are chemical compounds that allow the identification of the original plant source material. The more unique the identified structures are, the greater is the certainty a particular constituent of a residue can be confirmed (Evershed 2008b). These chemical indicators include variety of organic compounds including carbohydrates, lipids and proteins.

Basic recognized structures come from cellulose and lignin along with other various proteins and lipids. Biomarkers can rely on structural plant components (Figure I.4) as well on district phytochemicals produced by the original plant source, like polyphenols and terpenes, which can be helpful in the identification of a botanical origin. This allows identification of plant remains such as oils, resins, tars and pitch, oils and waxes, depending on the analytical technique, detected by their characteristic smaller molecular fragments. However, in archaeological residues, original source material can be altered pre-deposition, degraded during and post-deposition, or the amorphous material can be a complex mixture of different biological origins (Steele et al. 2010). As such, the reconstruction of the original botanical source is not a straightforward and simple task. For example, the most widely used biomarkers are fatty acids (Evershed 1993, Evershed et al. 2001a, Colombini et al. 2009, Craig et al. 2020), originating from both plant and animal sources (Craig et al. 2003, Copley et al. 2005, Steele et al. 2010). In mixtures, lipidic compounds from plant products tend to be understated due to the sheer amount of fat originating from animals compared to fats and oils of plant origin (Whelton et al. 2021), therefore it is also important to consider characteristic profiles and abundance of compounds, as well as to compare with contemporary reference material, experimental and available data (Malainey et al. 1999, Eerkens 2005, Miller et. al. 2020). Since unsaturated plant oils, characterized by double bonds between carbon atoms, are chemically reactive and have a higher susceptibility to oxidation than saturated fats

(Steele et al. 2010), a bias in the interpretation of lipidic source favoring animal origin can occur in archaeological samples. On the other hand, chemical analysis can also provide markers of natural or anthropogenic degradation, and thus provide information about the state of preservation (Jia et al. 2008, Łucejko et al. 2015).

Diagnostic lipid markers preserve well in the archaeological contexts because of their hydrophobic nature (Figure I.5). Lipidic compounds can also provide information about food processing, like cooking. Presence of ketones, with odd carbon number distributions of C29:0 to C35:0, indicate that the substance was exposed to high temperatures that exceed 300°C (Evershed et al. 1995, Raven et al. 1997). Recovered traces from plant sources are most often residues of plant oils, waxes, and resins. Degraded plant oils from the archaeological context are characterized by saturated and unsaturated fatty acids, hydroxy fatty acids, and dicarboxylic acids (Regert et al. 1998), while plant waxes can be recognized by even-numbered long-chain fatty acids, long-chain n-alkanes and wax esters (Evershed 1991, Charters et al. 1997, Dunne et al. 2022). Terpenoids allow determination of the natural resins and their products, like tar and pitch, to the botanical family or sometimes even the genus level (Colombini and Modugno 2009, Brettell et al. 2015). Remains of plants can also be confirmed with a detection of plant sterols, like sitosterol, stigmasterol and campesterol, or even be indicative of a specific plant source like brassicasterol (Colombini et al. 2005). Detecting and interpreting compounds originating from plants, their interactions and degradation products can help reconstruct the original material and shed light on its botanical origin.

Since plant remains preserve in archaeological contexts in diverse forms, on a macro-, micro- and chemical level, combining palaeobotanical and chemical approach can contribute significantly to answering questions about plant management and use, and provide new information regarding human-environment interactions.

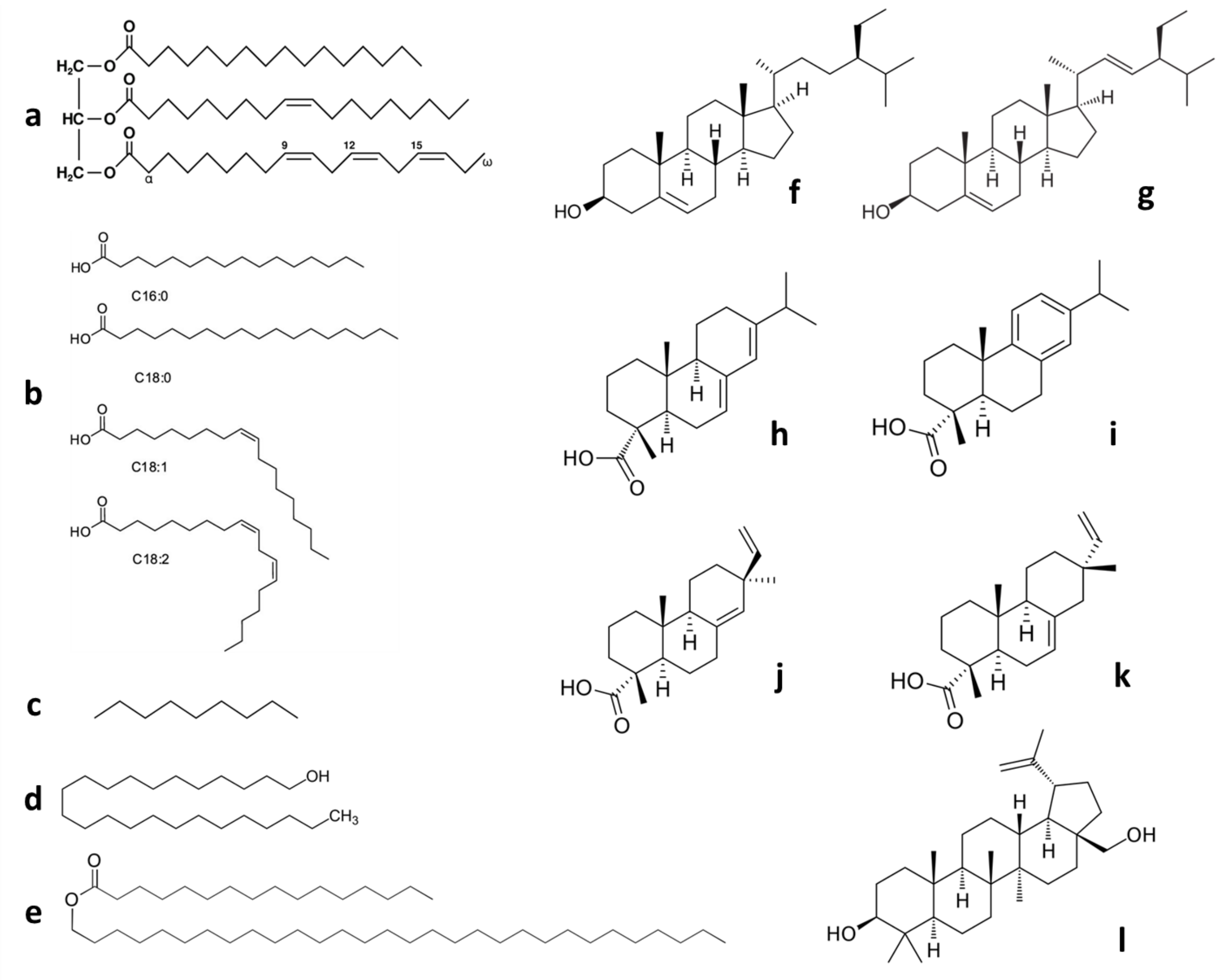


Figure I.5 Some common classes of organic compounds related to plant remains recognized in archaeological context. (a) Triacylglycerols (TAGs); (b) fatty acids (FA): palmitic, stearic, oleic and linoleic acid; (c) alkane (nonane); (d) fatty alcohol (1-tetracosanol); (e) wax ester (triacontanyl palmitate); plant sterols: (f) β-sitosterol; (g) stigmasterol; (h) abietic acid (i) dehydroabietic acid; (j) pimaric acid; (k) isopimaric acid; (l) betulin.

1.3. Importance of looking in the same pot

Both natural processes and human behavior result in the deposition of plant remains at archaeological sites. Since no single category of plant remains provides a full picture of plant exploitation in the past, the presence of all detectable plant remains should be considered, when archaeobotanical studies are integrated into the objectives of an archaeological investigation. Preserved archaeobotanical record only reflects a fraction of the total amount of plant material that was deposited intentionally at the site for various purposes (construction, food, fodder, fuel, etc.) and as products, to which must also be added the plant material unintentionally incorporated to the assemblage (Miller 1995). As such, this record is a partial reflection of the past materials that managed to be preserved and was recovered.

As vessels are often a significant part of archaeological deposits, both in domestic and funerary contexts, they provide a good receptacle for accumulation and preservation of plant material. Furthermore, vessel form, shape and capacity can be linked to the potential function and use. This makes them a good example for the integral approach to plant material combining archaeobotanical and chemical studies. Still, it is necessary to examine the lifecycle of a vessel to study the contents of the vessels and their accumulation (Figure I.6).

When the vessel is created, depending on the style, it can be decorated. These decorations might contain organic plant-based substances (Sánchez et al. 2019). In a connection with the vessel form, it can be sealed or impregnated with natural resins (Regert 2003, Dimitrakoudi 2011). During its use, vessels can be used as a food storage, in food preparation, for cooking or roasting, as well as for preparing, storing, or transporting beverages. These actions, lead to potential preservation of different types of plant remains. Storage vessels often contain macroremains, like seeds or fruit, but the presence of microremains should not be ignored. In the case of vessels that were potentially used for preparation or storage of the beverages, it is even more important to look into possible preservation of microremains, and visible and absorbed residues (Dunne et al. 2017, Dunne et al. 2019, Hammann and Cramp 2018, Evershed et al, 2001b, Reber et al. 2004, Cramp et al. 2012, Pecci et al. 2020). Food preparation can involve elaborate series of steps, resulting in a complex mixture of plant and animal components. Contents of the vessel might undergo thermal processing which can aid preservation of charred macroremains as well as the accumulation and absorption of organic residues, while still altering the original ingredients. Plant material used for the pyre can also be recovered on macro- and micro- level, while chemical evidence from firing might also be preserved. If visible organic residues are recovered, they might reflect a single event, like the final use of the vessel, or a mixture of repeated usage of the vessel (Roffet-Salque et al. 2017). Routine cleaning of vessel influences sensitive organic residues and also removes bigger plant fragments. If the vessel is damaged, it can be repaired and reused, but after the final discard, the vessel and its organic contents are exposed to the deposition environment. These interactions can lead to additional accumulation or displacement of plant material from its surroundings. Furthermore, deposit conditions affect the rate of plant material decomposition depending on temperature, humidity and microbial activities.

The discard patterns can also influence the recovered plant materials (Figure 1.7). The archaeobotanical record can be formed by primary refuse, discarded directly after and at the location of use, and secondary refuse, altered by consequent interventions (Miksicek 1987). The creation of multifaceted complex assemblages containing different types of plant remains can be applied to other archaeological objects and contexts like burials, hearths, disposal pits and cesspits where plant remains can preserve in abundance. Finally, the change of the environment during post-excavation can lead to further deterioration, while handling, cleaning, conservation and improper storage might cause contamination with modern substances (Roffet-Salque et al. 2017). Processes to which preserved plant material is exposed and in what form it preserves should be considered while planning an inclusive study of plant use.

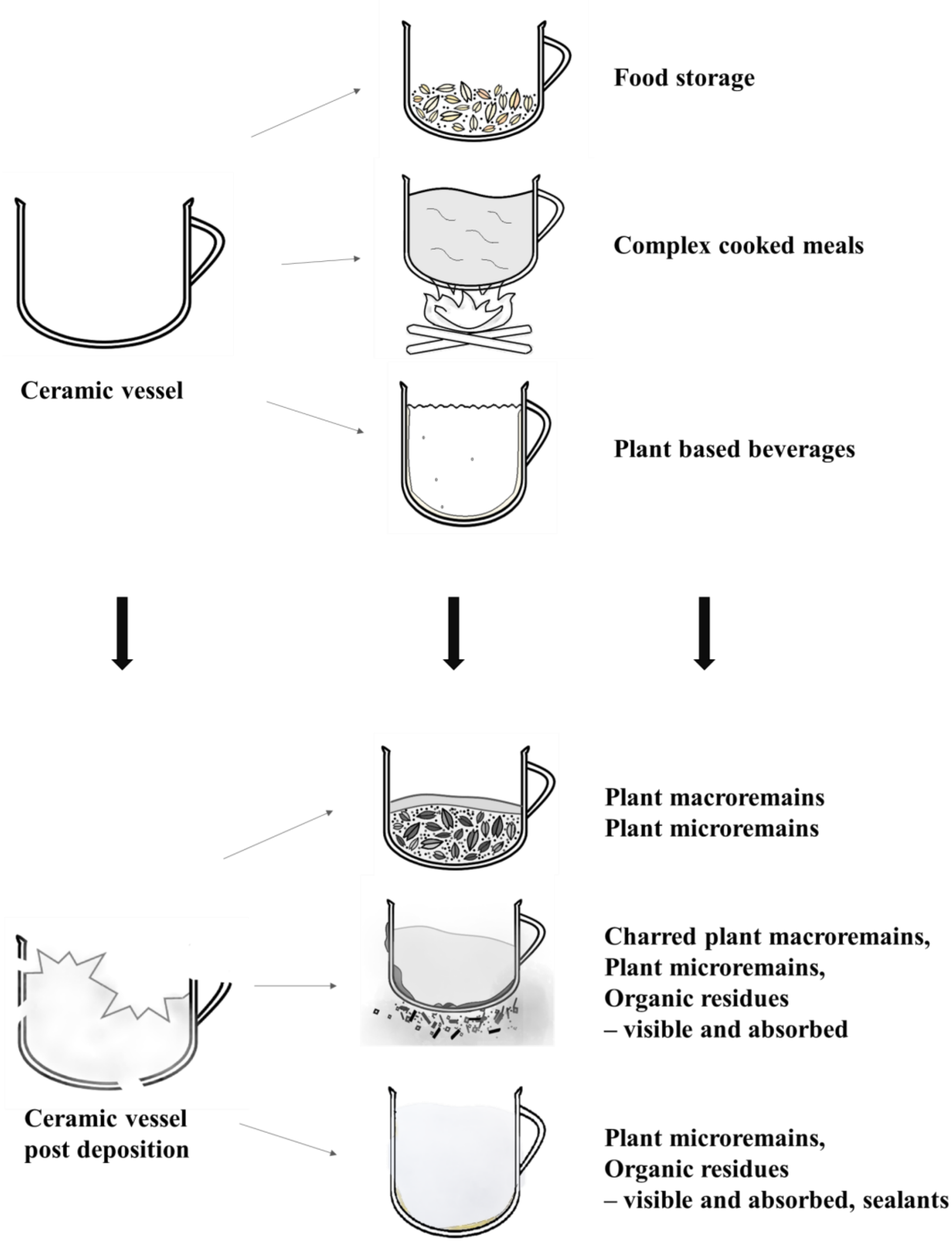


Figure I.6 Deposition of plant remains on the example of a hypothetical ceramic vessel.

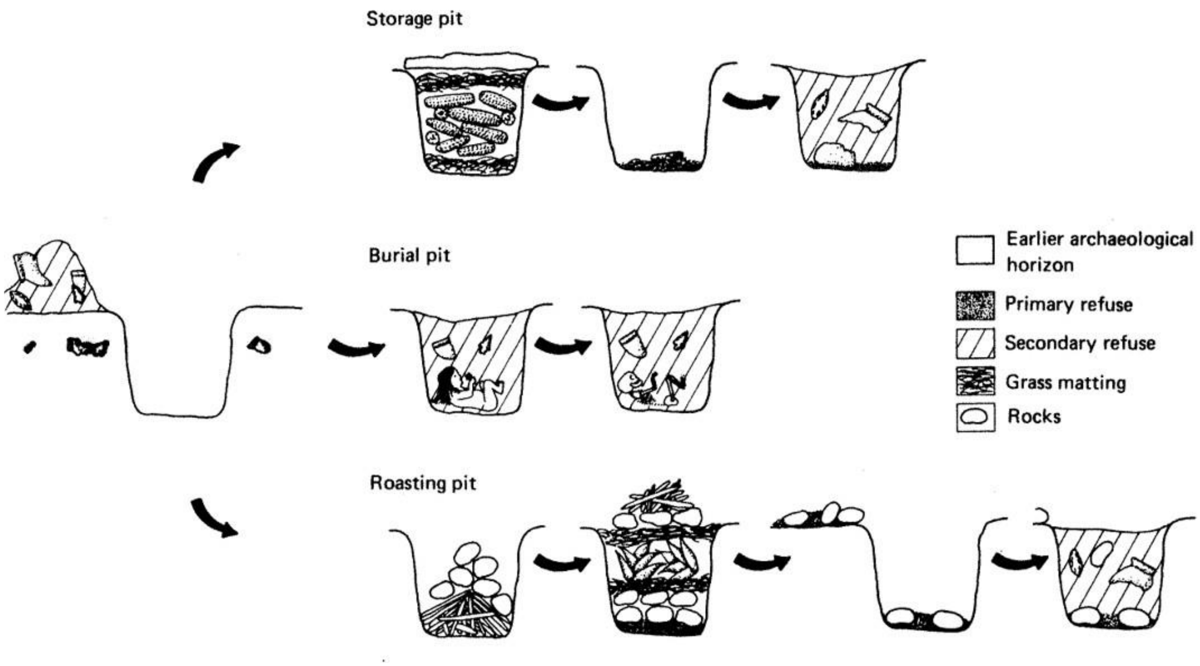


Figure I.7 Deposition of primary and secondary refuse on the example of a hypothetical pit (from Miksicek 1987).

The study of plant remains and the paleoenvironment have been incorporated into both multidisciplinary and interdisciplinary archaeological projects, with a positive trend towards interdisciplinary research (Day 2013). Multidisciplinarity entails different disciplinary perspectives on the same problem using its own methodology and tools, while interdisciplinary approach integrates separate disciplinary data, methods, tools, concepts, and theories in order to create a holistic view or common understanding (Van Den Besselaar and Heimeriks 2001). On the other hand, transdisciplinarity is explicitly formulated and is based on common theoretical understandings, including terminology and methodologies to address the research questions, crossing the boundaries of two or more disciplines (Stock and Burton 2011). As its purpose it is described as orientated toward real-world problems, intervention and change, co-generating knowledge that is solution-orientated, and relevant to both practice and science (Tolk et al. 2021). New technological developments allow study design to lean more forward into transdisciplinary research. Such an approach can provide unity of intellectual frameworks beyond the disciplinary perspectives (Jensenius 2012), providing a systematic theoretical structure for comprehensive analysis of the past human-environment interactions (Rosenfield 1992, Milek 2018).

Ideally, such individualized approach to the subject should allow more in-depth interpretations and understanding of technology, materials, processes, chronology, interactions, preservation mechanisms, among others. However, this approach does have its impediments and barriers, that need to be circumvented. For example, often the communication between archaeologists and scientists of different backgrounds, is not articulated in terms that are recognized to every party, hampering a fruitful cooperation. Terminology, research goals, sampling strategies, ascribed meanings and values, but also approach to permissions, budgets, publications and visibility have to be discussed and elaborated in detail within the comprehensive framework of the research. The effort required for reciprocal understanding is substantial from every side, in all interdisciplinary and transdisciplinary studies, but it is also necessary to achieve meaningful results (Van der Leeuw and Redman 2002, Drennan at al. 2011, Nilsson Stutz 2018) and if implemented with care, is worth the effort.

Just on the basis of a single pot recovered with plant material, theoretically, it is possible to recover and analyze plant macroremains, multiple types of microremains, as well as visible and absorbed residues. This enables the collection of complementary data, that when compiled can give a more inclusive interpretations of the contents of a vessel and tell a story about the object's function, use and lifecycle, as well as reflect the natural paleoenvironment where it was recovered from. These objects can, therefore, become archaeological time capsules with the potential to be forthright communicators of the past cultures. While plants were ubiquitous resource in past societies, because of their fragility and selective preservation, they are underrepresented when compared to inorganic materials like vessels. To discover the contents within the ceramic containers, sometimes it is necessary to look beyond what is experienced by the naked eye. The less obvious traces of plant use, evident only on a microscopic level, or in amorphous forms, can be detected and identified as chemical structures. The holistic approach can help overcome, or at least address the biases that come from the archaeological site formation processes and go beyond the absence of visible remains as a proof of presence and use of plant products. Residue analysis can open new avenues into detecting and identifying plant remains and give more nuance interpretation of human interactions with natural environment and plant exploitation. If used deliberately, combining archaeobotanical and chemical approach to plant remains can expand our comprehension of the presence and use of plants and plant products in past societies.

2. Research aims and thesis outline

The main goal of this research is to identify plant source materials found in various archaeological sites from different time periods and to follow changes in the plant use as well as to interpret the function of the plants in the funerary and domestic context. Considering the complexity of organic remains of vegetal origin that can be retrieved, this research intents to:

- identify food products of plant origin in relations to its context;
- determine the presence and composition of balms and identify which plants were used in these complex mixtures;
- examine organic remains of vegetal origin and their role in funerary rituals, everyday diet, and technological practices;
- place archaeological material in the context of past environmental conditions and available natural materials.

The research was carried out in the Laboratory of Palaeobotany and Palynology of the Department of Environmental Biology at Sapienza University of Rome, Italy and in the HERCULES Laboratory, University of Évora, Portugal. The data obtained have been systemized and compiled with the aim of publishing and presenting the results as individual case studies in specialized conference and international peer-reviewed journals.

Following this introductory chapter, the dissertation is subdivided into three parts, followed by final remarks and aims for future work:

- Chapter I – Introduction establishes the state and a brief history of research, introduces the types of plant remains and their preservation in the archaeological contexts and explains relevant terms.

- Chapter II – Transdisciplinary methods is dedicated to the integration of botanical and chemical methodologies that can be used to study plant remains from archaeological context and explains what techniques have been used in this work.
- Chapter III – Funerary contexts and the study of organic materials for reconstructing ritual practices and diet presents organic material retrieved from the megalithic tomb of Zambujeiro (Manuscript 1) and the funerary vessel from Monte da Comenda (Manuscript 2) in Portugal, and plant traces from the soils of Etruscan funerary area in Cerveteri, Italy (Manuscript 3).
- Chapter IV – Plant remains reflecting everyday life: botanical and chemical studies from domestic context explores plant remains preserved in three different settlements: an Iron age underwater pile-dwelling in Central Italy (Manuscript 4), an Islamic storage from Medieval Lisbon (Manuscript 5) and housing unit in the urban setting of the 18^{th} century Lisbon (Manuscripts 6).
- Chapter V – Final remarks and future research, stems from this research and addresses avenues that can still be explored within the study of archaeobotanical remains.

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Chapter II – Transdisciplinary methods

1. Introduction

Organic materials of plant origin are a great source of information for the detection of changes in dietary habits, rituals, technological practices, trades, and past environmental conditions in different archaeological contexts. Since they are more susceptible to decay and biological oxidation, organic materials are not always preserved in their integral state and can be overlooked during archaeological excavation. However, this knowledge is not lost, being "hidden" as micro-remains and organic residues. Developing new technologies, using cultural heritage science and cross-disciplinary approaches, allows the well of information to be unearthed in laboratories, museums and conservation centers. This potential should not be ignored but encouraged to build a comprehensive mosaic of human diet in the past, as well as to shed a light on the organic materials employed in sacred rituals. The role of plants has been studied using combined approaches, including both plant micro-remains and macroremains, and sometimes even some chemical analysis (e.g. Hansson and Bergström 2002, McGovern et al. 2013, García-Granero et al. 2015, Dunne et al. 2019, Pecci et al. 2018, Mariotti Lippi and Giachi 2021). Studies of archaeological balms, on the other hand, have been mostly focused on chemical analysis of residues (Buckley and Evershed 2001, Brettell et al. 2015).

Combining botanical and chemical approaches in transdisciplinary studies can yield a more rounded picture about the past use of plant material. This chapter covers sampling techniques, review of nondestructive analysis on organic and inorganic matter, chromatographic techniques used for analysis of organic residues and extraction procedures for recovery of plant microremains from archaeological samples. An inclusive analytical approach is intended to maximize the information obtained from the archaeological samples while preserving the integrity of the remains for future analysis, exhibitions, or presentations. Since each case study is specific, a general approach was adapted to best suite demands of the material (Table II.1).

Table II.1 Overview of the methods used in different case studies.

		Case studies					
Methods		**Manuscript 1**	**Manuscript 2**	**Manuscript 3**	**Manuscript 4**	**Manuscript 5**	**Manuscript 6**
Collection of plant macroremains	Dry picking			*	*		
	Water separation				*		
	In situ recovery - subsampled in the laboratory					*	*
Microscopy and imaging	Stereo microscope			*	*	*	*
	Light microscope		*	*	*		*
	VP-SEM				*	*	*
Characterization of inorganic materials	VP-SEM-EDS				*		*
	XRPD		*	*			
	μ-XRD		*				
Identification of organic materials	ATR-FT-IR			*	*		
	GC/MS	*	*				*
	Py-GC/MS			*	*		

2. Sample selection

To avoid the loss of material and information carefully planned sampling strategy is essential to obtain representative samples. If possible, sampling should occur *in situ* during the archaeological excavations. In ideal conditions plant macro- and microremains as well as sediment, residue and the object (e.g. the ceramic vessel) should be retrieved together and analyzed integrally. For the material retrieved from museums, condition of storage and post-depositional history of the object should be well documented, because storage conditions or possible restoration can affect the results. All existing information about the context of the material should be gathered. The samples should be labeled, documented and stored in the stable conditions. Favorable conditions depend on the type of archaeobotanical remains and mode of preservation; for example, carbonized remines that are fragile should be preserved in dry firm container, while waterlogged remains that are susceptible to the loss of structure due to drying out, should be kept in a wet environment. To avoid chemical contamination, organic remains should be stored in material that does not transfer to the object itself, like sterilized aluminum foil, while plastic packaging or contemporary organic containers that can affect future analysis should be avoided. Documentation includes creation of a database, photography, weighing and measuring. After reviewing the analysis plan, samples can be subsampled in laboratory for specific analytical techniques. It is important to keep in mind the way certain analyses affect the sample, for instance, is the analysis destructive or can it be repeated. The rest of the sample should be stored in the appropriate conditions for analysis replication or for different future analysis and research. Since archaeobotanical remains differ depending on a type, preservation modes and archaeological context, careful and targeted planned sampling is crucial for the well-rounded and correct interpretation of the data (Celant et al. 2015).

Techniques for recovering macroremains from the archaeological context include *in situ* collection, screening techniques and water recovery techniques (Pearsall 2016). The best recovery approach should be chosen based on the individual case study, since they all have limitations and certain recovery bias. *In situ* recovery is limited to remains that are detected during the excavation, which can limit the sampling, by overseeing and excluding the smaller particles. Screening techniques, including dry picking and dry sieving, involve processing bulk soil from excavation which allows a more systematic recovery of macroremains. Dry picking includes examining the soil sample and retrieving the plant remains under magnification. This technique is precise and effective but very time-consuming so it cannot be applied to large scale voluminous samples. Dry sieving is a technique that allows the elimination of the mineral component, and the separation of plant remains through the mesh sieves with decreasing diameter. In this case, the primary limiting factor for recovery is the screen mesh size that determines the size of the remains collected.

Water flotation is a recovery technique that separates plant remains from the soil matrix using differences in density of organic and inorganic material. This technique is very efficient but can damage fragile remains, and smaller seeds/fruits can remain trapped in the soil matrix while some waterlogged remains can be too heavy to rise to the surface. Water separation or wet sieving is an approach that combines the use of meshes in water and flotation increasing recovery precision and efficiency (Pearsall 2016).

For the case studies presented in this work that contained macro remains, dry picking (Manuscript 3 and 4), water separation (Manuscript 4) and *in situ* recovery (Manuscripts 5 and 6), were used, depending on the mode of preservation (Table II.1).

For the recovery of plant microparticles and organic residues from soils, soil samples can be recovered from archaeological layers, vessels or by performing coring at the archaeological site. Sampling for microfossils is done without certainty that the soil samples contain any plant remains, but a good planning strategy allows the selection of the locations that are most likely to be abundant in plant remains. It is important to avoid contamination with contemporary materials and to secure the soil samples before treating them in a laboratory setting (Celant et al. 2015).

The soil samples used in the case studies presented in this work were retrieved from archaeological layers (Manuscript 3) and vessels (Manuscripts 3, 4 and 6; Table II.1).

Visible organic residues are retrieved after documentation by carefully removing them with sterile metal tools. They can be analyzed directly without extraction from the soil or ceramic matrix. On the other hand, residues absorbed in ceramic vessels, require mechanical removal of potsherd surfaces to eliminate contamination before powdering the sub-surface fabrics (Heron et al. 1991). This approach is applicable to both freshly excavated sherds and those from museum collections.

Subsampling 2 g of clean powdered potsherd is sufficient for analysis of organic residues, and if sampled with care leaves archaeological vessel structurally intact. A concern when working with organic residues is the potential for them to become contaminated by post-burial or post-excavation contamination due to their exposed nature (Evershed 2008). Ideally, choosing and taking the samples for analyses should take place at the excavation site so that contamination can be reduced to a minimum. Handling, storing and washing the sample will affect the results of analysis. Contamination with plasticizers, glues, and skin lipids are commonly encountered and can interfere with analysis (Figure II.2). Squalene and cholesterol are among the lipid components of human skin, so their simultaneous occurrence in a lipid extract may suggest contamination by handling. Washing and brushing of sherds is also likely to result in loss of information. By experimental approach it has been showed that lipid residues are best preserved in the rim and the body of a vessel (Charters et. al. 1993, Charters at al. 1997, Evershed 2008). Still, spatial distribution of lipids in pottery vessels might be related to the function of the vessel, e.g., used for cooking, roasting, processing or storage (Evershed 2008). Furthermore, as absorption of lipids by the ceramic matrix is generally not homogeneous throughout the wall of the vessel so taking samples from different portions of the vessel is recommended when possible.

Post-excavation contamination with lipids is particularly significant especially when no prior intention or plans have been made for residue analysis, prior to excavation and post-excavation analysis. By choosing pottery for analysis on the site, in advance, contamination from their storage in the museum can be avoided. These samples should be handled as little as possible, air-dried together with any adhering soil and wrapped in an aluminum foil and then, if possible, stored in a paper bag, avoiding plastic containers. To recognize lipid contamination from the soil, samples of sediment collected at the same time as the object itself should be analyzed. Inappropriate storage, conservation and restoration procedures, can also result in contamination of samples (Whelton et al. 2021), but the information about those processes can help correct interpretation.

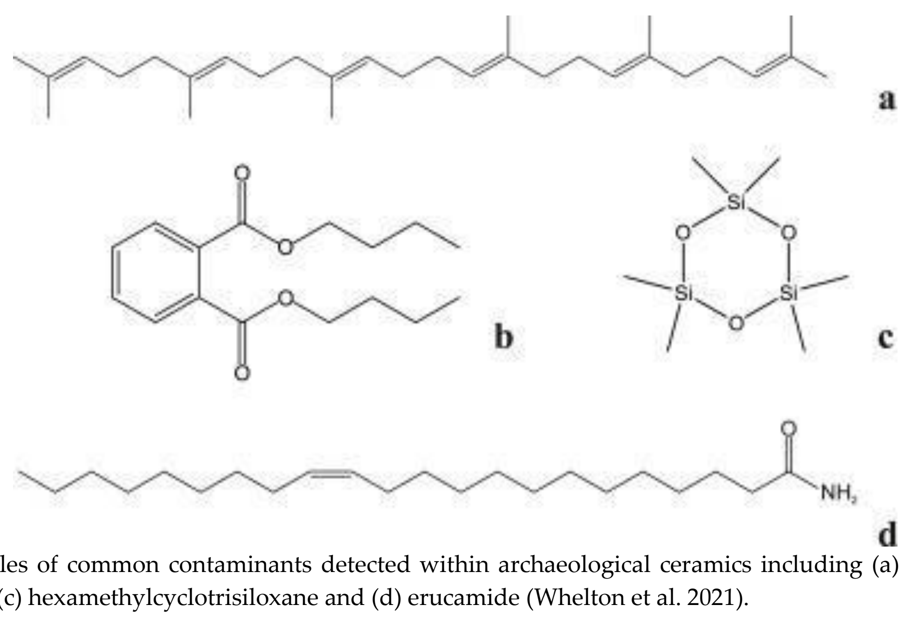

Figure II.1 Examples of common contaminants detected within archaeological ceramics including (a) squalene, (b) dibutyl phthalate, (c) hexamethylcyclotrisiloxane and (d) erucamide (Whelton et al. 2021).

3. Analytical techniques

3.1. Optical microscopy (OM)

Optical microscopy was used for the separation, sorting and identification of plant macroremains (Manuscript 3, 4, 5 and 6; Table II.1). Seeds, fruit and plant fragments were identified based on morphology using a Stemi 508 (Carl Zeiss) stereomicroscope. The fragile remains were gently picked and sorted with a fine paintbrush. The remains were photographed using a mounted digital camera (HAYEAR) under different magnifications (63-200×) depending on the size of the remains and digitally measured. Diagnostic characteristics such as shape, size, color and texture were considered. The identification was made by comparison with the reference collection of the Sapienza University of Rome, as well as with anatomical atlases (e.g. Jacquat 1988, Jacomet et al. 2006, Neef et al. 2012, Cappers and Bekker 2013). The remains were counted after the identification. All intact seeds were counted as one, while 3 fragments of the same taxa were counted as a minimal number of units. Relative abundance was used in order to observe relationships among plant taxa or different plant categories.

The identification of plant microremains (Manuscripts 3, 4 and 6; Table II.1), pollen grains and Non-Pollen Palynomorphs (NPPs), was carried out by light microscopy using ZEISS Axiolab A1 microscope under 200–1000 magnifications. Identification of pollen taxa was done using pollen morphology atlases and existing databases (Beug 2004, Martin and Harvey 2017, Weber and Ulrich 2017, Albert et al. 2016, Shumilovskikh et al. 2021).

To examine pottery fabrics (Manuscript 2; Table II.1) polarizing microscope Leica DM2500P equipped with a digital camera (Leica MC170 HD) was used for the microscopic analysis. The thin sections were examined and documented under plane-polarized and cross-polarized transmitted light with 100× and 200× magnification.

3.2. Variable pressure scanning electron microscope coupled with energy dispersive X-ray spectrometry (VP-SEM-EDS)

Scanning electron microscopy coupled with energy dispersive X-ray spectrometry (SEM-EDS) has proved to be versatile and helpful in the studies involving archaeological organic materials (Milanesi et al. 2009, Colombini et al. 2009a, Valamoti 2011, Giachi et al. 2013, Valamoti et al. 2019, Heiss et al. 2020) because it combines the high image resolution of the electron microscope with analysis of the characteristic X-ray produced when the sample is bombarded with electrons. This allows investigation of micro-morphological features of the sample, including cross sections of plant remains and amorphous residues. Beam of accelerated electrons is used as a source to excite the specimen, usually under vacuum to avoid beam scattering. This interaction produces various signals that contain information about the sample's surface topography and composition, which are recorded depending on the detector (secondary electrons, backscattered electrons, X-ray photons) (Figure II.2). The addition of an X-ray energy dispersive spectrometer (EDS) enables discovery of the elemental composition of the samples analyzed (Frahm 2014).

In this study, data was acquired using variable pressure SEM-EDS, allowing to obtain good resolution images at high magnifications with SEM and elemental composition of the inorganic fraction of the sample in a backscatter mode (Manuscripts 4, 5 and 6; Table II.1). VP-SEM-EDS analyses were carried out using a Hitachi™ S3700N SEM coupled to a Bruker™ XFlash 5010 SDD EDS Detector® with an energy resolution of 124 eV in the MnKα line. The samples were analyzed at low vacuum (40-60 Pa) and with an accelerating voltage of 20 kV. The compositional data was acquired using the Esprit1.9 software and a standardless quantification. Variable pressure (VP) and big sample chamber allowed carbon based (mostly organic) samples to be studied directly, without sample preparation or destruction.

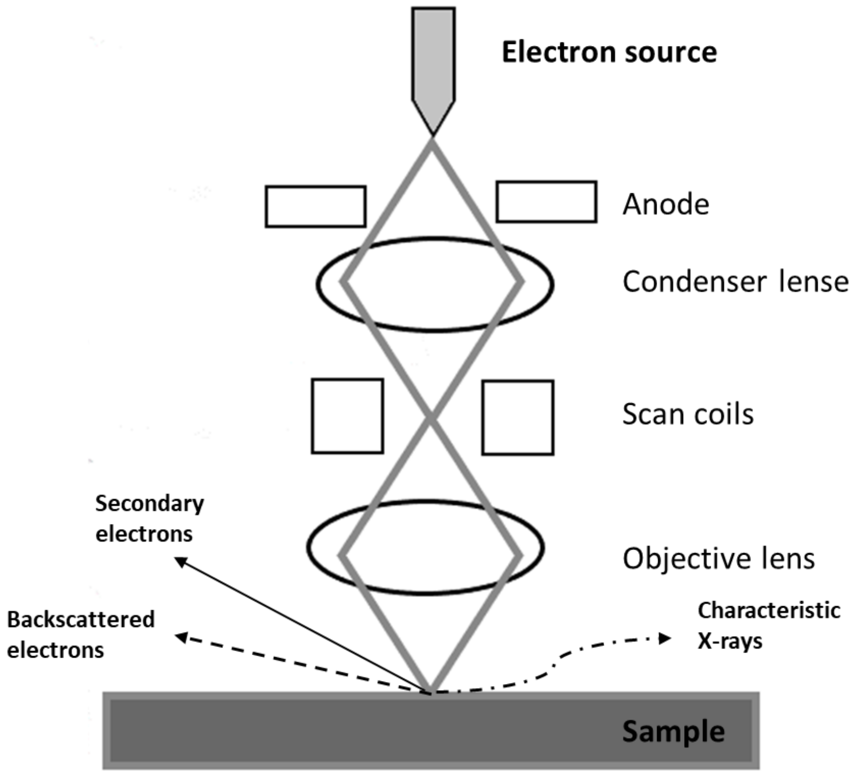

Figure II.2 Schematic diagram of basic components of an SEM and some of the secondary signals that are produced due to the interaction of an electron beam with atoms at or near the surface of the sample (adapted from Nanakoudis 2019).

3.3. X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a powerful technique for the determination of crystalline structures and can be used to acquire information about the mineralogical composition of the inorganic fraction of the archaeological material, sediments and soils (Giachi et al. 2013, Tallón-Armada et al. 2014, Christidis et al. 2020). When a sample is irradiated with monochromatic X-rays of a known wavelength, part of the rays is diffracted by the regular crystal structure. Considering the Bragg' law, two waves reach the crystal at an angle θ and are reflected at the same angle by two close atomic layers. Constructive interference of the rays occurs at an angle θ if the path length difference is equal to a whole number of wavelengths (Figure II.3). Therefore, the diffracted X-rays produce a diffraction pattern dependent on the crystal structure of the sample and can be used to identify clay minerals and mineral inclusions in the sample (Stuart 2007). The intensity of the diffraction peaks is related to the abundance of the compound in the material, allowing a semi-quantification of the crystalline compounds in a sample. To identify the composition of the material, obtained diffractogram is compared with the diffraction patterns in mineral databases.

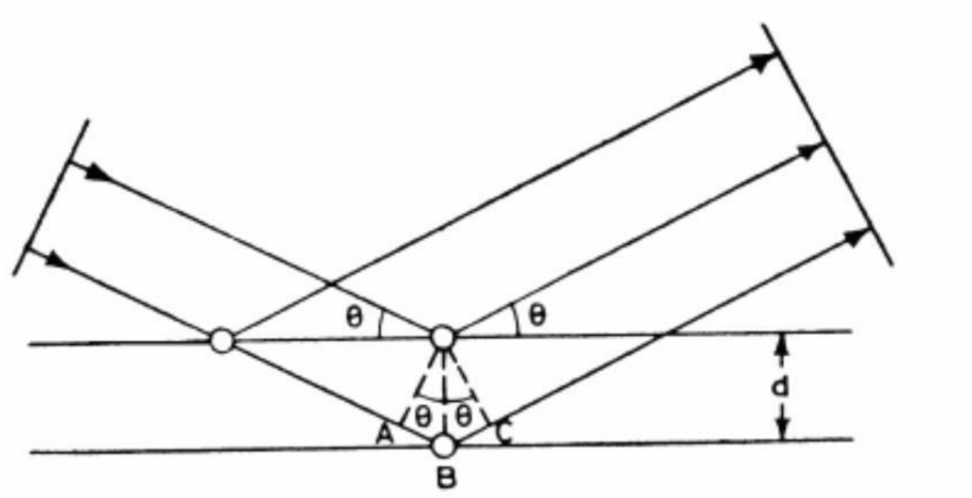

Figure II.3 Parallel X-rays strike the surface at an angle, and are reflected from successive planes of crystals of interplanar spacing d. The path difference between reflections from successive planes is equal to 2dsinθ. Constructive interference of the rays occurs at an angle θ if the path length difference is equal to a whole number of wavelengths (Pollard and Heron 1987).

Micro-X-ray Diffraction (μ-XRD) uses X-rays converged into a spot size of tens of microns in diameter with a collimator, does not require sample preparation, and as a non-invasive technique is suitable for analyzing archaeological material, including soils and clays. X-ray powder diffraction (XRPD) the analyzed material is finely grounded, homogenized, and average bulk composition is determined.

In this work, when possible μ-XRD was used (Manuscript 3) since it is a non-invasive technique. For the ceramics and loose earth (Manuscript 2 and 3), the samples were hand grinded in the agate mortar, and X-ray powder diffraction (XRPD) was used to identify the mineral composition, under same conditions (Table II.1). XRD patterns were recorded with a Bruker™ D8 Discover® instrument equipped with a Cu Kα source and a LYNXEYE linear detector. All measurements were performed over a range from 3° to 75° 2θ, using a 0.05º increment with measuring time of 1 s/step. The identification of crystalline phases was performed with the DIFFRAC.SUITE EVA® software and the International Centre for Diffraction Data PDF-2 database.

3.4. Attenuated total reflectance Fourier-Transformed Infrared Spectroscopy (ATR-FT-IR)

Fourier Transform Infrared Spectroscopy (FT-IR) is suitable as an initial prospective screening technique as it is quick and affordable and gives information about the functional groups of both organic and inorganic compounds. In the study of archaeological plant remains and plant products it has been widely employed to detect the presence of organic substances (Colombini et al. 2009a), to detect the plant-based material, and on the identified matter, estimate state of preservation and detect degradation (Broda and Popescu 2018, Tamburini et al. 2017). Attenuated total reflectance (ATR) enables samples to be examined directly in the solid or liquid state without further preparation which make this technique non-destructive or minimally invasive and suitable for Cultural Heritage and archaeological samples. An infrared spectrum represents a fingerprint of an analyzed sample, dependent on absorption bands in a frequency range 600–4000 cm^{-1}, which correspond to the frequencies of vibrations of the bonds between atoms constituting the material. Since different materials consist of unique combination of atom bonds, characteristic infrared spectrum is produced. By identifying functional groups in the substance through comparison with available databases, composition of original material can be deducted. Still, because FT-IR cannot separate different compounds, when analyzing archaeological samples that are often complex mixtures, chromatographic techniques need to be used to identify the individual compounds.

Throughout this work, the ATR mode allowed a fast and nondestructive analysis of the archaeological samples (Manuscript 3 and 4; Table II.1) and standards. Infrared analysis was carried out at room temperature and ambient humidity with a Bruker™ Alpha spectrometer coupled with a single-reflection diamond ATR module. All spectra were acquired in the absorbance mode, in the range from 4,000 to 400 cm^{-1}, from a total of 128 scans at 4cm^{-1} resolution. Spectra were recorded and analyzed using Bruker OPUS software (version 6.5).

3.5. Gas chromatography/mass spectrometry (GC/MS)

Gas chromatography/mass spectrometry (GC/MS) is the preferred technique for the analysis of lipid extracts of absorbed residues from archaeological contexts (Evershed 2008). The sample must be solubilized for injection into the GC by dissolution or extraction using organic solvents (Sneddon et al. 2007). Gas chromatography separates compounds, and the individual components pass directly into the mass spectrometer so that individual mass spectra can be recorded, and separated compounds identified compared to the database. The coupling of GC with MS makes it a powerful tool for investigation of the complex mixtures and aged organic materials. GC/MS is an excellent technique for qualitative and quantitative determination of volatile and semi-volatile organic compounds in a wide variety of samples (Sneddon et al. 2007).

Gas chromatography (GC) separates the individual molecules in a mixture on the basis of their interaction with the stationary phase of the column (Figure II.4). Before residues from potsherds can be analyzed, preserved compounds must be extracted from the fabric of the pottery fragment and dissolved in an organic solvent (Colombini et al.

2009b). With a use of different extraction procedures, complementary results can be obtained. Samples are then injected into a carrier gas known as the mobile phase that passes through a long, silica-coated column in a temperature-controlled oven, where organic compounds are retained by the stationary phase. Compounds of different molecular weight and polarity elute at different times. The retention times and relative abundances of the different molecules in a mixture are then measured and recorded (Pollard et al. 2007).

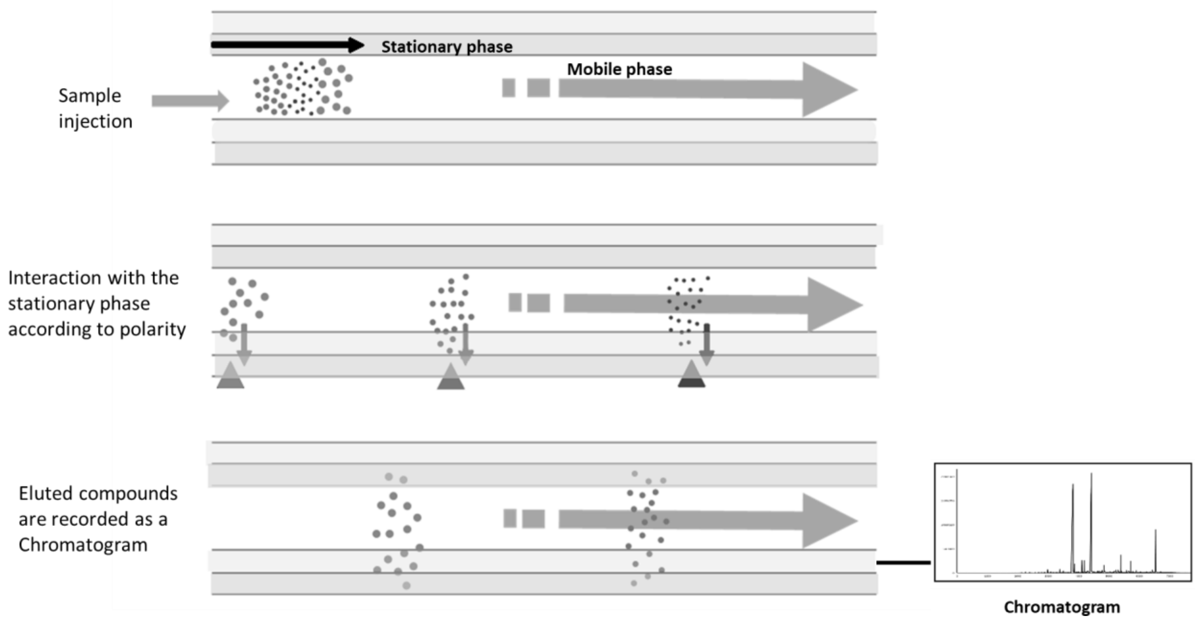

Figure II.4 Separation of compounds by GC within the column. The components passing through the column are transported by the mobile phase (gas) while being partitioned from and adsorbed into the stationary phase (liquid phase and solid phase). Adapted from Shimadzu 2020.

Mass spectrometry (MS) combined with GC is used to identify compounds by their molecular weight and ion fragmentation. Particles are ionized and separated from one another on the basis of their mass-to-charge ratio (m/z) (Pollard et al. 2007). The detector records intensities of ions of different m/z ratios. The most intense peak in the spectrum is called the base peak, while the intensities of all other peaks are given relative to it. Mass spectra of an individual molecular components can then be matched to known compounds using a database like NIST (National Institute of Standards and

Technology – http://www.nist.gov/nvl/) mass spectra library.

GC/MS analysis of the material in the case studies examined in this work (Manuscript 1, 2 and 6; Table II.1) was performed with Shimadzu GC2010 gas chromatographer coupled with a Shimadzu GCMS-QP2010 Plus Mass Spectrometer. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm x 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. 1 μL of the sample was injected in splitless mode, with the injector set at 250 ºC 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 minutes. The mass spectrometer, equipped with a 70 eV electron ionization source, 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. The obtained results were processed with Automated Mass spectral Deconvolution and Identification System (AMDIS). The chromatograms were interpreted in comparison with the NIST library database.

3.6. Pyrolysis – gas chromatography/mass spectrometry (Py-GC/MS)

Organic residue analysis (ORA) is a complex process which necessarily demands sophisticated and sensitive analytical techniques for the proper identifications of a wide range of chemical compounds. Organic materials like natural polymers, resins, proteins or plant gums, have macromolecular nature, which makes them suitable for analytical pyrolysis (Bonaduce and Andreotti 2009). Very small samples (< 1 mg) and/or involatile residues are not suitable for residue analysis that demand sample preparation (Chiavari and Prati 2003, Whelton et al. 2021) but can be analyzed with pyrolysis – gas chromatography/mass spectrometry (Py-GC/MS). Pyrolysis-GC/MS is a fast and efficient approach for identifying organic materials, especially in visible residues. Coupling of the pyrolizer to the injection port of a GC/MS, allows fast sample preparation, separation of the compounds and identification by mass spectrometry. Since only few micrograms are necessary for the analysis, this makes Py-GC/MS, minimally destructive, appropriate for the analysis of archaeological materials and in line with European Standard for Conservation of Cultural property (CSN 2012).

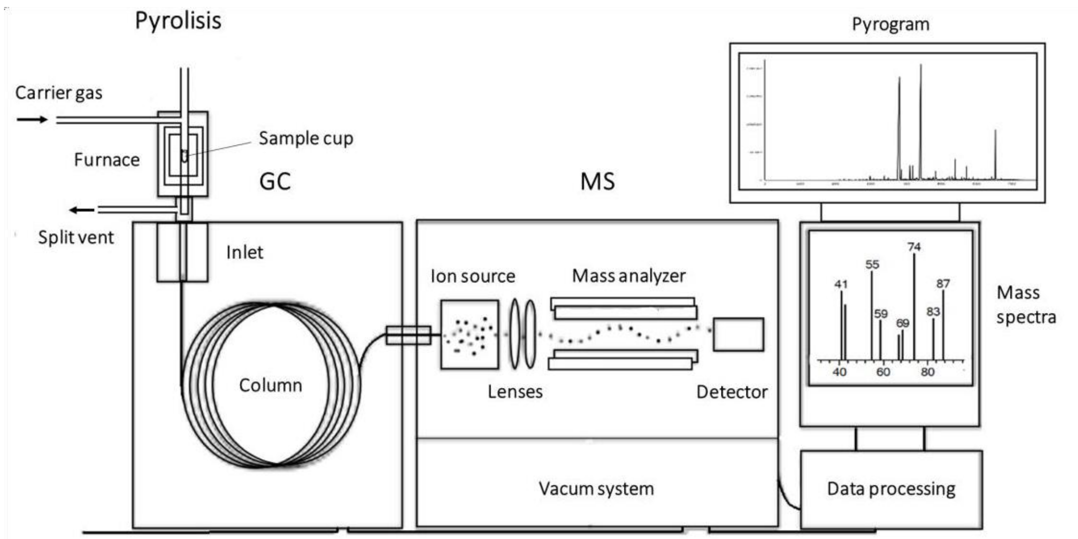

Figure II.5 Schematic diagram of basic components of Py-GC/MS. Adapted from Frontier Lab 2022.

Pyrolysis – gas chromatography/mass spectrometry (Py-GC/MS) is a method in which the sample is rapidly heated at high temperatures under an inert atmosphere (Figure II.5). The thermal process breaks the larger molecules at their weakest bonds, producing smaller more volatile fragments, that are separated by gas chromatography and identified using mass spectrometry. The pyrograms obtained are usually complex, because of high fragmentation and the formation of a wide range of different decomposition products (Chiavari and Prati 2003). To increase the volatility, methylating reagents are added to a sample before pyrolysis, including tetramethylammonium hydroxide (TMAH) as one of the most commonly used reagents. Pyrolysis in the presence of TMAH gives rise to the hydrolysis of ester bonds and leads to the formation of the corresponding methyl derivatives (Bonaduce and Andreotti 2009). Complex archaeological samples, that can be amorphous unknown substances, often composed of more the one material and can contain an inorganic fraction, can further complicate the interpretation of the data.

In the case studies presented below (Manuscript 3 and 4; Table II.1), for the Py-GC/MS analyses, samples were derivatized with 3 μl of tetramethylammonium hydroxide (TMAH) in a 50-μl Eco-cup capsule. The samples were pyrolyzed using a single-shot method at 500 °C for 20 seconds. Analysis was performed with a Frontier Lab PY-3030D single-shot pyrolyzer, coupled to a Shimadzu GC2010 gas chromatographer and to a Shimadzu GCMS-QP2010 Plus mass spectrometer. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm x 30.0 m, 0.50 μm film thickness) was used for separation, with helium as carrier gas, adjusted to a flow rate of 1.50 ml min^{-1}. The split/splitless injector was operated at a temperature of 250 °C in the splitless mode. Gas chromatography temperature program started at 35 °C during 1 min, ramped at 60 °C min^{-1} until 110 °C, then to 240 °C at 14 °C min^{-1}, and finally increased to 280 °C at 6 °C min^{-1}, at which point it was held for 10 min, resulting in 30 min of total run time. Source temperature was placed at 240 °C, and the interface temperature was maintained at 280 °C. The mass spectrometer was programmed to acquire data between 40 and 850 m/z. The obtained results were processed with Automated Mass spectral Deconvolution and Identification System (AMDIS). The chromatograms were interpreted in comparison with the NIST library database.

4. Extraction procedures

4.1. Solvent extraction for the organic residue analysis by GC/MS

As described in sample selection (Chapter II, 2.), to identify the absorbed residue from ceramic residues (Manuscript 1 and 2), before extraction, surfaces of pottery shards were cleaned using a hand drill to remove soil and handling contamination. Samples were grounded in an agate mortar into a fine powder and accurately weighed (2 g of every sample for each extraction procedure). Organic compounds were extracted with chloroform/methanol (Method 1) and with sulfuric acid in methanol (Method 2) (Evershed et al. 1990, Correa-Ascencio & Evershed 2014, respectively), before the analysis with GC/MS (Chapter II, 3.5).

Method 1: Following the addition of 20μl of internal standard (n-tetratriacontane) to 2g of shard powder, sample was extracted with 10mL of chloroform/methanol mixture (2:1,v/v) using ultrasonification for 15 minutes. After centrifugation for 15 minutes at 2500 rpm, the supernatant was carefully removed to a clean vial and evaporated under stream of nitrogen. This step was repeated. To obtain total lipid extract (TLE), chloroform/methanol (250μl, 2:1, v/v) was added to the dry extract, and 50μL was separated in a 1.5 ml glass vial and dried with nitrogen (Evershed et al. 1990).

Method 2: After the addition of 20μl of internal standard (n-tetratriacontane), 5 mL of 2% sulfuric acid in methanol (H_2SO_4/MeOH, 2%, v/v) was added to the shard powder, vortexed for 2 h at 70°C and then centrifuged for 10 minutes at 2500rpm. Supernatant was moved to the clean vial (vial 2) and 2 mL of ultrapure H_2O was added. pH was measured to confirm acidity of the solution (pH< 3). 3 mL of hexane was added to potsherd residue and centrifuged for 5 minutes at 2500 rpm. Hexane supernatant was added to vial 2 and vortexed. This step was repeated 3 times. Hexane fraction, which separated from the aqueous fraction, was removed to another clean vial (vial 3). Furthermore, 2 mL of hexane was added to the remaining H_2SO_4/MeOH/H_2O solution (vial 2) and vortexed. Hexane fraction was again removed to the vial 3. Clear supernatant in vial 3 was evaporated under stream of nitrogen. To obtain total lipid extract (TLE), 250 μl of hexane was added to the dry extract, and 50μL was separated in a 1.5 mL glass vial and dried with nitrogen (Correa-Ascencio & Evershed 2014).

4.1.1. Derivatization of extracted organic residue

Each sample was redissolved in 50 μl of hexane and derivatized with 50 μl of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylclorosyloxane inside of microwave for 30 seconds at 700W (Mirabaud et al. 2007, Mitkidou et al. 2008). The extract was dried under stream of nitrogen. Prior to GC/MS analysis 100μL of hexane was added to the lipid extract.

4.2. Protocol for simultaneous recovery of multiple microfossils

Pollen analysis is a commonly used method for paleoenvironmental reconstruction and in when applied to archaeological samples it employs the same methods as paleopalynology. Removal of organic and inorganic matter from a sample to make plant microremains visible, recognizable and identifiable under microscope requires extraction procedures. Part of the challenge in extracting microremains, is separating diagnostic particles from siliceous mineral matter of similar size and digesting other organic fragments. Using standard procedure for pollen extraction can damage or dissolve other plant remains (Moore and Webb 1987, Magri and Di Rita 2015), since it is quite aggressive and targets carbonates, silicates and organic matter. To ensure preservation of particles less resistant to this type of extraction, the standard palynological extraction was adapted, depending on the targeted microremain while still keeping the same workflow. Microremains were identified with an aid of a light microscope under (400–640 × magnification) using existing databases, experimental data and contemporary references (Beug 2004, Martin and Harvey 2017, Weber and Ulrich 2017, Albert et al. 2016, Shumilovskikh et al. 2021). Microparticle extraction was employed in several case studies (Manuscripts 3, 4, and 6), with various degrees of success.

4.2.2. Pollen extraction

Extraction of pollen and non-pollen palynomorphs (NPPs) from sediments consists of the removal of organic and inorganic matter from a sample, with the aim of making the grains visible under the microscope, while preserving their structure and enabling their identification and quantification. Extraction of pollen and NPPs from archaeological samples was in accordance with the standard method of preparation for palynological analysis (Moore and Webb 1987, Magri and Di Rita 2015). In order to remove carbonate fraction, 1 g of dried sample was dissolved in 30 ml of 37 % HCl. One tablet of *Lycopodium* spores was added as an external standard. Silicates were removed with 30 ml of 48% HF (72 h). Finally, the organic fraction was dissolved in 25 ml of 10 % NaOH in a heat bath for 10 minutes at 100°C. Samples are mounted on glass slides in glycerol.

4.2.3. Phytolith extraction

Samples were treated according to the standard pollen extraction procedure omitting the HF step to preserve silica phytolith structure. Carbonates were removed by dissolving 1 g of sample in 30 ml of 37 % HCl and organic matter was dissolved in 25 ml of 10 % NaOH in a heat bath for 10 minutes at 100°C. One tablet of *Lycopodium* spores was added as an external standard. Samples are mounted on glass slides in glycerol.

4.2.4. Extracting intestinal parasite eggs from archaeological fecal remains

The coprolite sample treated for the examination of pollen grains (method described under Chapter II, 4.2.1.) showed presence of non-pollen palynomorphs (NPPs) including possibility of intestinal parasite eggs (Manuscript 6). This method proved to be effective also when applied to the study of parasite eggs (Warnock & Reinhard 1992). Still, to ensure better preservation of parasite remains a less invasive approach was selected. According to the standard practice for the recovery of parasites from coprolites (Reinheart et al 1986) the carbonatized faeces were treated with trisodium phosphate. 1 g of dried sample was disaggregated by adding 5 ml of a 0.5% aqueous solution of trisodium phosphate for 48 h. Sample was periodically mobilized with gently stirring with a glass rod. According to a simplified procedure (Anastasiou & Mitchell 2013) the extraction was also attempted with a 1g of sample disaggregated in 5 ml of distilled water for the same time period. After their rehydration both subsamples were treated with ultrasound for 15 min.

5. Analytical approach

Throughout this research (Manuscripts 1-6), a generalized workflow combining botanical and chemical analyses was employed allowing a multianalytical and inclusive approach of plant remains and traces from archaeological contexts (Figure II.6). After the selection of the material for the analysis, according to period, availability and preservation state, samples were obtained with a permission of authority responsible for archaeological remains. Sediments and site deposits were examined under stereomicroscope, photographed, and subsampled for the microparticle analysis. Plant macroremains were separated by appropriate techniques, depending on their preservation form, photographed, and measured under stereomicroscope. When possible, to help with the identification and provide more details about the material, macroremains, visible residues and site deposits were examined under SEM. The identification of plant macro- and microremains was done by comparison with anatomical atlases, existing databases, experimental data and reference collections.

Visible residues and sediments were screened for the presence of organic matter by ATR-FT-IR. If the spectra showed presence of organic material, archaeological samples were subjected to more sensitive chromatographic techniques to obtain chemical composition of the remains and identify their natural origin. Sample preparation was done keeping in mind requirements of each technique, minimizing the possibility of contamination, and enabling reproducibility for further research or future studies. The absorbed residues, preserved in pottery walls, after appropriate extractions, were analyzed using GC/MS. When possible, visible residues and sediments were analyzed by Py-GC/MS, requiring only few μg of a sample. VP-SEM-EDS and XRD were used to acquire elemental and/or mineralogical composition for the inorganic fraction of the archaeological material, providing clearer determination of the complex materials. Botanical and chemical information obtained was used to provide in depth interpretation of the archaeological plant remains. Selection of suitable techniques was modified to suit the demands of each case study. All the acquired data was framed in the context of past archaeological and environmental conditions, as well as available natural materials, allowing a comprehensive interpretation.

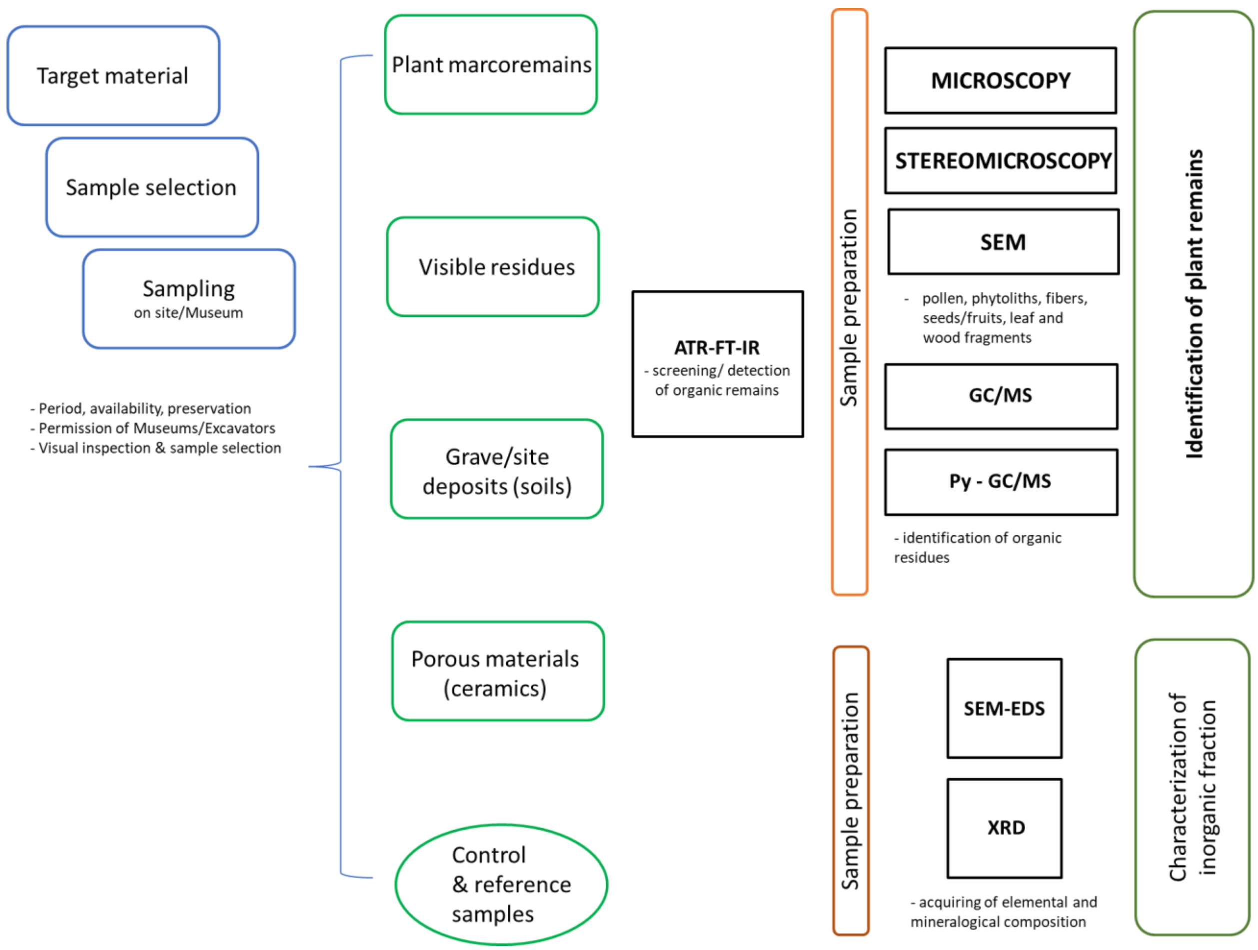

Figure II.6 Workflow for the inclusive analysis of plant remains from an archaeological context combining botanical and chemical analysis.

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Chapter III – Funerary contexts and the study of organic materials for reconstructing ritual practices and diet

1. Introduction

In this chapter evidence for plant use is presented from three Prehistoric funerary sites, mostly based on chemical evidence. Funerary practices, primarily focused on the treatment and the disposal of human body, hold a great potential to reveal much about the lived social context, identity, dietary habits of the living, their relationship towards death and afterlife, as well as rituals employed to honor the ones who passed away. Mortuary evidence is an extremely valuable archaeological resource, representing the direct and purposeful behavior, rather than its incidental debris. Furthermore, burial conditions often provide excellent preservation of organic materials. Grave offerings can include foods and beverage for the dead, while plant sources can also be used for the treatment of a body for ritual and medical purposes as in the case of embalming. Furthermore, during the ritual, flowers and herbs can be used for decorating the dead. Still, materials of organic nature, even if they are more susceptible to decay and biological oxidation, including body itself, food and beverages, raw and construction materials, natural products, and manufactured substances etc., play an essential role in diverse funerary rites. Analytical methods, from microscopic analysis to molecular-based approach of organic residues, allow the identification of organic materials of plant origin, placed as offerings. Additionally, synergetic research of mortuary archaeology, through materials of different sources and use, allow a deeper reconstruction of all the processes involved in dealing with death, often permeated with symbolic meaning, as a reflection of the living.

The analysis of the absorbed residues recovered from the ceramics of Zambujeiro Dolmen, gives an insight into the food for the dead and the celebration of afterlife (Chapter III, 2). The Zambujeiro Dolmen is the biggest chambered tomb in Alentejo region of Portugal, integral to a broader European phenomenon and a part of an impressive prehistoric network of megalithic structures covering the Iberian Peninsula.

The content of the vessels provided evidence for the use and processing of animal and plant products, while the detection of beeswax indicates that populations on the Iberian Peninsula actively participated in the widespread exploitation of the honeybee products by agrarian communities that was documented across Europe.

Regarding the treatment of the dead in the end of 4th and the 3rd millennium BC, in Central and Southern Portugal, the examined hypogeum burial of Monte da Comenda 3 (Chapter III, 3), shows changes in the votive component, namely introduction of ceramics, arrowheads, the replacement of the customary sheep phalanges by deer bones, and zoomorphic representations (Valera 2020). The extraordinary ceramic vessels were composed of two parts, a vessel and nozzle on the top of the vessels mouth. The results of the chemical analysis of the content of the ceramic vessel found beside the Individual 1 of Monte da Comenda 3, show that the organic offerings were a lipidic substance, most likely plant oil.

Finally, remnants found in association with archaeological funerary sites are death assemblages (thanatocoenoses), composed of remains which may not have been requisite for the living, and often originate from site surroundings containing altered or introduced by human actions. Recovering of plant traces from the clay deposits of the Etruscan funerary area of Cerveteri in Italy (Chapter III, 4), indicates anthropogenic grassland environment of the area in the 8th and 7th century BC, translating landscapes of the living to the landscapes for the dead.

The results represented in this Chapter reveal that plants and plant products were integral part of burial and ritual practices of Prehistoric communities, even if the plant remains cannot be detected with a naked eye. Therefore, combined archaeobotanical and chemical approach is necessary to complete the picture of human nature interactions.

2. Manuscript 1: The prehistoric exploitation of bee products in the Iberian Peninsula: chemical evidence from the megalithic tomb of Zambujeiro Dolmen

2.1. Introduction

As part of a broader European phenomenon, Iberian megalithic structures, have long been used as a mirror for social dynamics of the early farming communities (Renfrew 1983). During the Neolithic, on today's territory of Portugal, ancient populations resorted to a new set of burial practices, and for the first time, used massive stone slabs to create megalithic monuments for the dead. Dolmens, funerary structures consisting of a polygonal chamber, were constructed by large vertical stone slabs covered by a capstone and may have been complemented with a corridor formed by vertical stones, smaller than those from the chamber, arranged in parallel rows and also covered (Martínez-Torres and Martínez-Fernández 2014). They were used for multiple burials, and could safeguarded remains of numerous individuals, uniting the community through generations even after death.

The Alentejo region of Portugal is known for a high concentration of Neolithic megalithic monuments that show great diversity (Rocha et al. 2015), with the Zambujeiro Dolmen being one of the most important and intensely studied megalithic tomb in the region. Since, the Neolithic of the Iberian Peninsula and the Western Mediterranean has traditionally been characterized by the simultaneous appearance of pottery, domestic plants and animals, as well as the appearance of new settlements and burial practices (Cruz Berrocal 2012), the examination of organic content of the vessels that were placed in the Zambujeiro, can provide an insight into the relationship of the agrarian communities had with their natural environment and help to understand the use of natural products.

Across Europe the early beekeeping has been linked to the onset of agriculture (Roffet-

Salque et al. 2015), implying domestication of the honeybee. Beeswax, one of the major products of the honeybee, is a complex lipid-based organic compound, secreted by wax glands of a worker bee, and transformed to a construction material for building the honeycomb, creating an outstanding hexagonal cell structure that serves as food storage and as a brood-rearing compartment of the bee colony (Svečnjak et al. 2019). Human exploitation of beeswax has been documented trough centuries, utilized for various purposes (Crane 1999). These uses can be attributed to the distinct characteristics of beeswax - it is one of the natural products available since millions of years, it has natural and pleasant aroma, and it burns very slowly (Moullou et al. 2015, Faraday 2002). Furthermore, it possesses antimicrobial and therapeutic properties (Fratini et al. 2016). Since it is hydrophobic, has a relatively low melting point and possesses plasticity and flexibility at room temperature, it is malleable and resistant to degradation (Bernal et al. 2005). However, the evidence for early bee product exploitation on the Iberian Peninsula, until now has been limited. The chemical data from Zambujeiro vessels can attest to the use of beeswax in the Iberia to at 4/3 millennia BC.

2.2. Archaeological context

Zambujeiro Dolmen (*Anta Grande do Zambujeiro*), the biggest chambered megalithic tomb in Alentejo region of Portugal, is a part of an impressive prehistoric network of megalithic structures covering the Iberian Peninsula (Figure III.1). Located on the left bank of Ribeira de Valverde stream, near the village of Valverde in the municipality of Évora, of Alentejo region. The archaeological site still has a preserved burial chamber with a hall, grater part of a burial mound and two large stelae-menhirs on its periphery (Soares & da Silva 2010), making it an attractive visitor's location and a significant mark in the cultural landscapes of Portugal (Calado & Rocha 2008). The tomb consists of a polygonal chamber, constructed with 7 vertical slabs, inclined towards the interior, and a long corridor. The height of the chamber is about 5 meters, contrasting with the smaller slabs of the corridor. The diameter of the inside of the chamber is up to 5.70 m, while the corridor is about 1.5 m wide and 12 m long. Entrance of the corridor is oriented towards the sunrise at the equinoxes (108.5º). The stone used for construction were exploited from granite outcrops (Soares & da Silva 2010). It was used as burial grounds for collective inhumations with high quality grave offerings, that together with the imposing appearance of the monument, suggests honoring a group of distinguished individuals. It was constructed at the end of the 4th and the transition to the 3rd millennium BC but was still utilized until the Bronze age. A second ritual deposit, at the entrance of the monument, was dated to the late Chalcolithic (2480-2290 BC) (Soares & da Silva 2010).

The stone structure was identified in the 1960s by Henrique Leonor Pina, and has been excavated between 1964 and 1968, only to be recognized as a National Monument in the 1974. In the 1980s, due to degradation of the monument, partial conservation and further archaeological research were conducted (Soares & da Silva 2010). The abundant excavated material has been stored in the Museum of Évora (Rocha 2015), and in the last decade, the research interest been revigorated, resulting in several multidisciplinary studies of the monument and burial goods. The studies were conducted on amber beads, red pigments, lithic arrowheads and selected ceramics (Santos & Rocha 2015, Rocha et al. 2020, Costa et al. 2021, Rocha 2015, Rocha et al. 2018, Manhita et al. 2014).

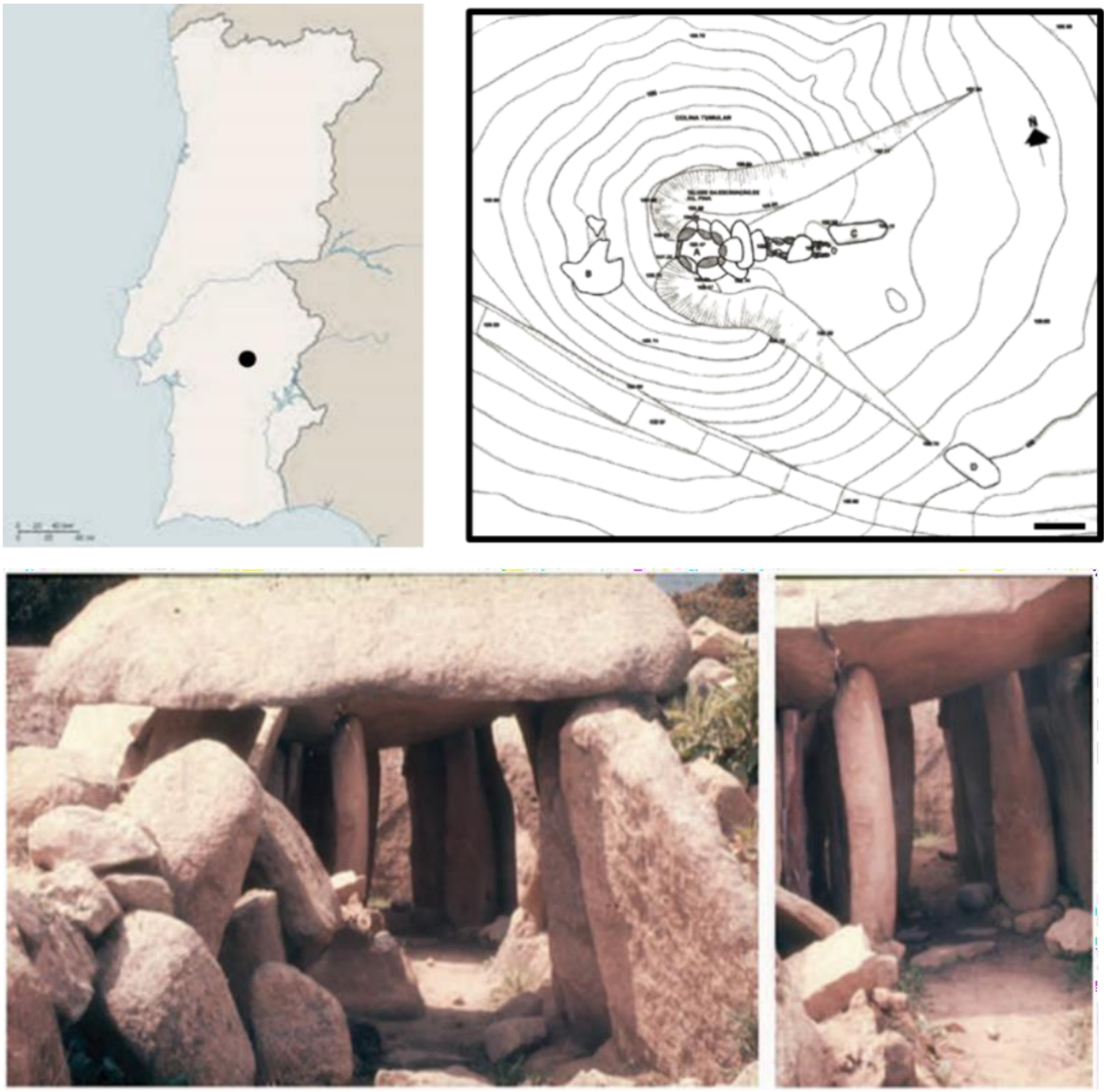

Figure 0.1 The Great Zambujeiro Dolmen (Soares & da Silva 2010).

2.3. Materials and methods

The material analyzed in this work was provided by Museum of Evora. The typology, state of preservation, measurement and documentation was done by Leonor Rocha (Rocha 2015). To identify the original contents of the vessels, after preliminary study of 7 pottery fragments (Manhita et al. 2014), more inclusive analysis was conducted on 27 selected ceramic shards (Table III.1) belonging to 24 distinct vessels. The selected pottery includes different forms such open conical bowls, cups and pots. All the vessels used in this study are typologically attributed to the end of the 4th millennium BCE or rather in the Late Neolithic period of Iberian Peninsula.

Table 0.1 List of analyzed pottery samples.

Vessel number	Sample	Vessel form	Vessel number	Sample	Vessel form
1	Z-3293	fragment	13	Z-4072	pot
2	Z-3694	cup	14	Z-4104	pot
3	Z-3718	pot	15	Z-4114	cup
4	Z-3720	cup	16	Z-4143	pot
5	Z-3947	cup	17	Z-4157	cup
6	Z-3952	open conical bowl	18a	Z-4162	pot
7	Z-3968	pot	18b	Z-4162x	pot
8a	Z-4011	open conical bowl	19	Z-4298	pot
8b	Z-4011x	open conical bowl	20	Z-4303	pot
9	Z-4014	pot	21	Z-4306	pot
10a	Z-4040	pot	22	Z-4309	fragment
10b	Z-4040x	pot	23	Z-4362	fragment
11	Z-4041	cup	24	Z-4365	fragment
12	Z-4044	open conical bowl			

The samples were selected, photographed, ands surfaces of pottery sherds were cleaned using a hand drill to remove soil and handling contamination. Approximately 4 g of samples were taken and then grounded in an agate mortar into a fine powder and accurately weighed. Total Lipid Extract was obtained using sulfuric acid in methanol (2%, v/v), and to gain additional information 12 samples were selected for chloroform/methanol (2:1,v/v) extraction (Chapter II, 4.1.). The acidic extraction, a more recent procedure using H_2SO_4 and methanol proposed by Correa-Ascencio and Evershed, yields high recovery of organic compounds, but the acidic medium promotes the hydrolysis of any ester molecule present in the sample (Correa-Ascencio and Evershed 2014). The loss of compositional information, can be mitigated by reexamining the powdered sample using chloroform:methanol extraction (Correa-Ascencio and Evershed 2014, Roffet-Salque et al. 2017).

The more commonly used methodology, involving the use of a mixture of chloroform : methanol (2:1, *v/v*), is efficient in removing lipids and other non-polar compounds, like terpenes, long chain alcohols and alkanes, from the ceramics (Evershed et al. 1990), but slightly more polar compounds, such as hydroxy-acids and diacids, are usually poorly recovered with such methodology, as they form stronger interactions with the ceramic components and require harsher conditions to be recovered. To get more complete profiles of organic compounds preserved in archaeological sample both extractions were used to complement each other. N-tetratriacontane was used as an internal standard. Samples were derivatized with BSTFA. 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. Internal standard, *n*-tetratriacontane (C34), and the derivative, *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.

Analysis was performed with Shimadzu GC2010 gas chromatographer coupled with a Shimadzu GCMS-QP2010 Plus Mass Spectrometer. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm x 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. 1 μL of the sample was injected in splitless mode, with the injector set at 250 ºC 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 minutes. The mass spectrometer, equipped with a 70 eV electron ionization source, 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. The obtained results were processed with Automated Mass spectral Deconvolution and Identification System (AMDIS). The chromatograms were interpreted in comparison with the NIST library database.

2.4. Results

Organic compounds were retrieved from all the pottery sherds (Table 3.2) yielding a wide range of molecules with good abundance. The main compounds present in the samples from Zambujeiro are fatty acids, alcohols and alkanes. Overall, the samples exhibit good preservation as unsaturated fatty acids and monoacylglycerols (MAGs) were detected in most pottery shards analyzed. Degradation products of fatty acids, diacids and hydroxy acids are present, as to be expected from the organic residue preserved in the archaeological material.

Samples Z-3694, Z-3947, Z-4157, Z-3718, Z-3968, Z-4040x, Z-4072, Z-4162, Z-4162x, Z-4298, Z-4011, Z-4011x, Z-4309 and Z-4362 have a prevalence of saturated palmitic acid over stearic acid (C16:0 > C18:0) (Figure III.2a). However, the relative abundance of these acids in most of the samples is proximate, with the exception of the sample Z-4040, Z-4104 and Z-4306 (Figure III.2b). Furthermore, monopalmitin is more abundant then monostearin in all the samples, except in Z-4040. Unsaturated fatty acids are detected in the samples include octadecenoic acid (C18:1), and in small amounts hexadecenoic acid isomer (C16:1). Octadecenoic acid (C18:1) is identified in all the samples, except Z-4040, Z-4162x, Z-4298, Z-4303, Z-4306, Z-4011x and Z-3293. The presence of unsaturated fatty acids in the original content of the vessels is further confirmed by the detection of azelaic diacid, a degradation product of C18:1 unsaturated fatty acid. The polyunsaturated C18:2 fatty acid, detected in the samples Z-3947 and Z-4011 is exceptional for the prehistoric archaeological samples, since polyunsaturated fatty acids easily undergo oxidation processes breaking the double bonds and forming the lower molecular weight species (Evershed 2008, Colombini et al. 2005). Very long chain fatty acids were detected in most sample except Z-4041 and Z-3293, indicating a possible presence of plant epicuticular waxes (Charters et al. 1997, Copley 2005, Cramp et al. 2011). Both plant and animal sterols were not detected in the samples with the exception of β-sitosterol identified in Z-3694.

Six samples, Z-3947, Z-3968, Z-4072, Z-4011, Z-4011x and Z-4362 have specific profiles characterized by the presence of 15-hydroxypalmitic acid, odd-numbered alkanes (C27-C31), fatty alcohols (C22:OH-C32:OH, peaking at C30:OH) and even-numbered, very long chain fatty acids (C22:0-C32:0), with lignoceric acid (C24:0) being most abundant (Figure III.3). This distribution of hydrocarbons is characteristic for aged beeswax (Heron et al. 1994, Regert et al. 2001, Garnier et al. 2002).

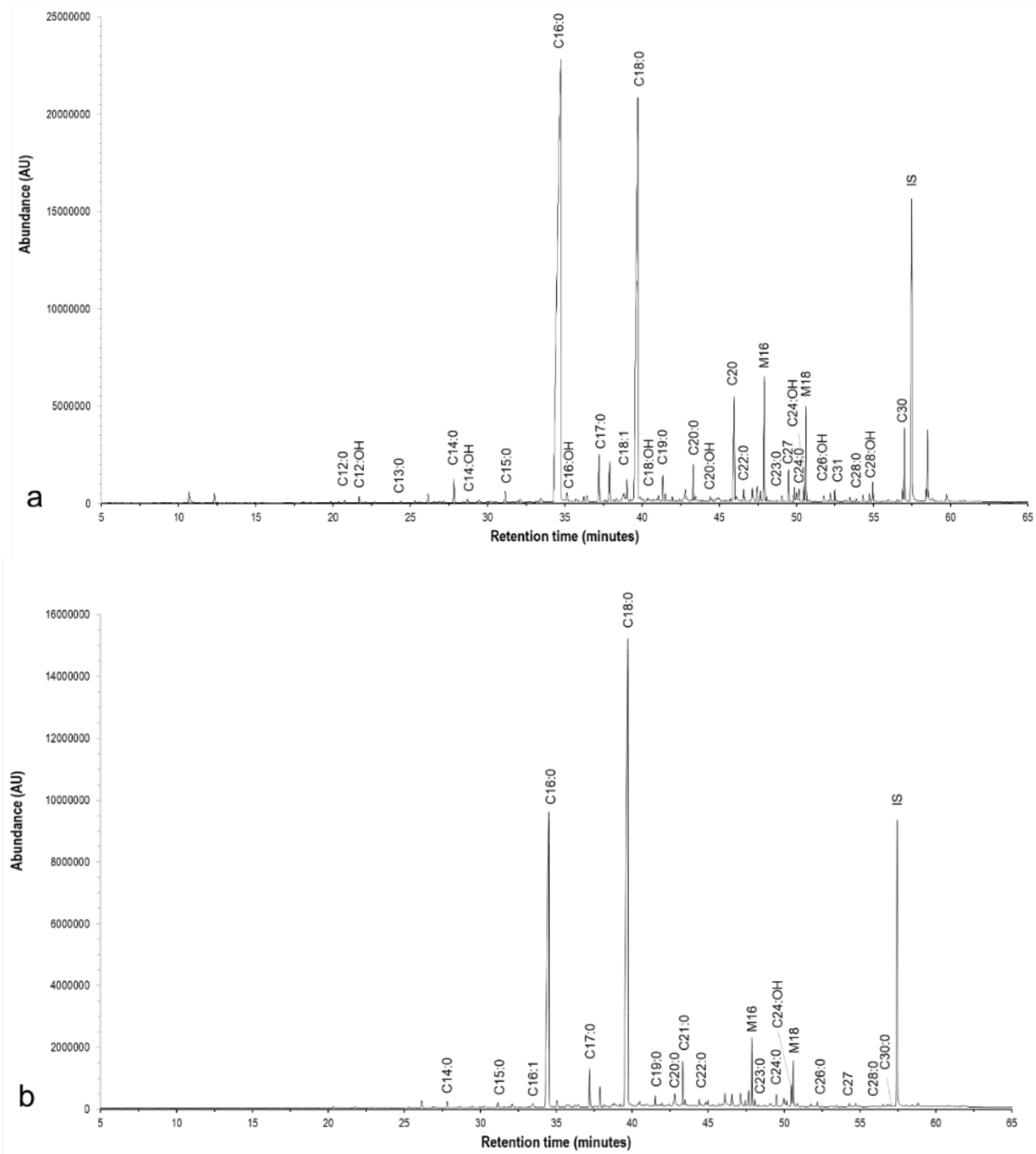

Figure 0.2 (a) GC/MS chromatogram of the sample Z-3718, (b) chromatogram of the sample Z-4040 showing predominance of stearic acid, obtained by acidic extraction. Marked are the trimethylsilyl derivatives and and methyl ester derivatives of relevant compounds detected. Cx n-alkane with x carbon atoms, 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.

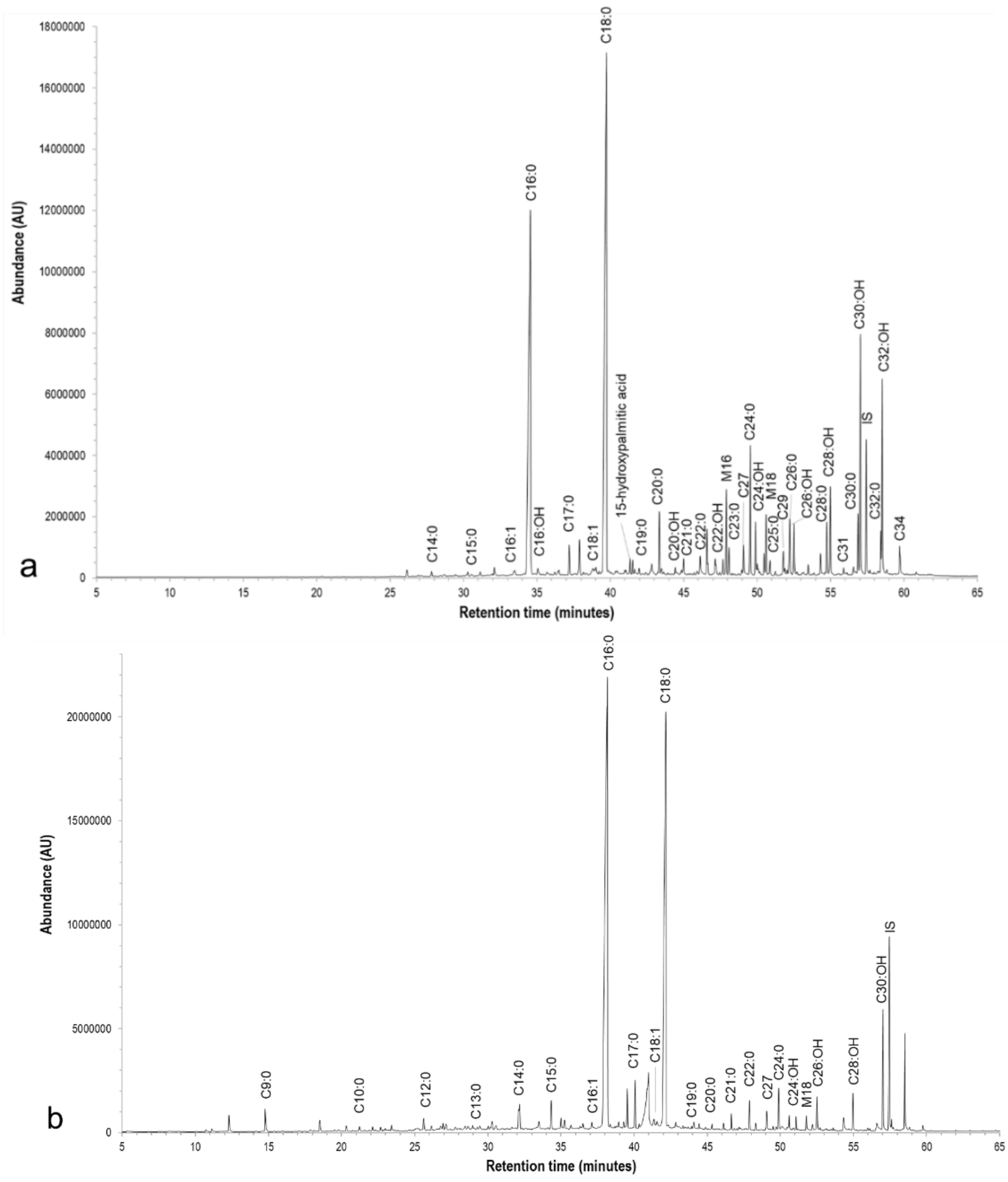

Figure 0.3 GC/MS chromatogram of the sample Z-3968 showing presence of beeswax (a) obtained by acidic extraction; (b) obtained by chloroform:methanol extraction. Marked are the trimethylsilyl derivatives and and methyl ester derivatives of relevant compounds detected. Cx n-alkane with x carbon atoms, 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.

The chromatographic profiles with abundance of palmitic acid, presence of unsaturated fatty acids, very long chain fatty acids and absence of cholesterol and detection of plant sterol, suggest that the vessels were previously utilized for processing of plant material. In the absence of specific biomarkers, it was not possible to establish botanical source of the residue. Even though, only C16:0/C18:0 ratio is not sufficient for the determination of the residue origin (Barnard et al. 2007, Colombini et al. 2009), and might be the result of the fat degradation, in the samples Z-4040, Z-4104, Z-4306 of ceramic pots, combining animal and plant products remains a possibility. On the other hand, beeswax was identified in all three forms of vessel, in two open conical bowls, two pots and one cup. Beeswax was also detected on one typologically undefined fragment.

2.5. Discussion

The results of absorbed residue analysis provided evidence for the exploitation of plant and animal products in all defined vessel forms, pots, cups and open conical bowls. Plant epicuticular waxes present in the samples, implies processing of leafy vegetables in the vessels. As epicuticular waxes are highly hydrophobic solid materials at ambient temperature, the detection of appreciable quantities of residue absorbed into potsherds, indicates that the wax was mobilized from the leaf surface during cooking or plant processing, and therefore free to migrate into the fabric of pot wall (Evershed et al. 1991). However, despite the data confirming presence of substances of plant origin in the studied pottery fragments, it is not possible to establish its botanical origin because specific biomarkers indicating particular taxon have not been detected.

On the other hand, beeswax, largely undetectable to the naked eye, was identified absorbed in five distinct vessels, proving the evidence for the exploitation of bee products during Late Neolithic/Chalcolithic on the Iberian Peninsula. Although the most obvious reason for exploiting the honeybee (*Apis* sp.) would be for honey, beeswax was used for various technological, ritual, cosmetic and medicinal applications (Colombini et al. 2003, Frade et al. 2014, Frith et al. 2004, Amir et al. 2021, Baeten et al. 2010, Bernardini et al. 2012). Direct detection of beeswax in the fabric of unglazed archaeological pottery vessels has been interpreted as a sealant and adhesive (Regert et al. 2003, Duce et al. 2015), used for waterproofing (Charters et al. 1995, Salque et al. 2013), utilized as a fuel (Evershed et al. 1997), or as a residue of honey used for cooking or evidence of processing wax combs (Dunne et al. 2021, Evershed et al. 2003).

The detection of beeswax in archaeological context, relies on its hydrophobic nature that makes it relatively resistant to bio- and thermal degradation, preserving since prehistoric times entrapped in the pottery matrix. However, what is identified is not the equivalent to the chemical composition of the fresh beeswax, but rather aged degradation products of the original substance. Fresh beeswax, with are more than 300 individual components comprises a complex mixture of aliphatic compounds including medium-chain alkanes, carboxylic acids, long chain alcohols and fatty acyl wax esters (Tulloch 1971, Namdar et al. 2007, Was et al. 2014). The wax produced by European honeybee (*Apis mellifera*) has the C27 alkane above 5%, in the C23 to C31 range (Aichholz and Lorbeer 1999). Archaeological samples reflect the original wax composition, providing a profile with long-chain even saturated fatty acids with 22 to 32 carbon atoms, with lignoceric acid followed by cerotic, montanic and melissic acids. Found together with higher odd number alkanes with 21-31 carbon, maximizing at heptacosane, and a presence of long chain alcohols, with melissyl alcohol being most prominent, and detection of methyl 15-hydroxyhexadecanoate (Figure III.4), presence of beeswax can be concluded (Heron et al. 1994, Regert et al. 2001).

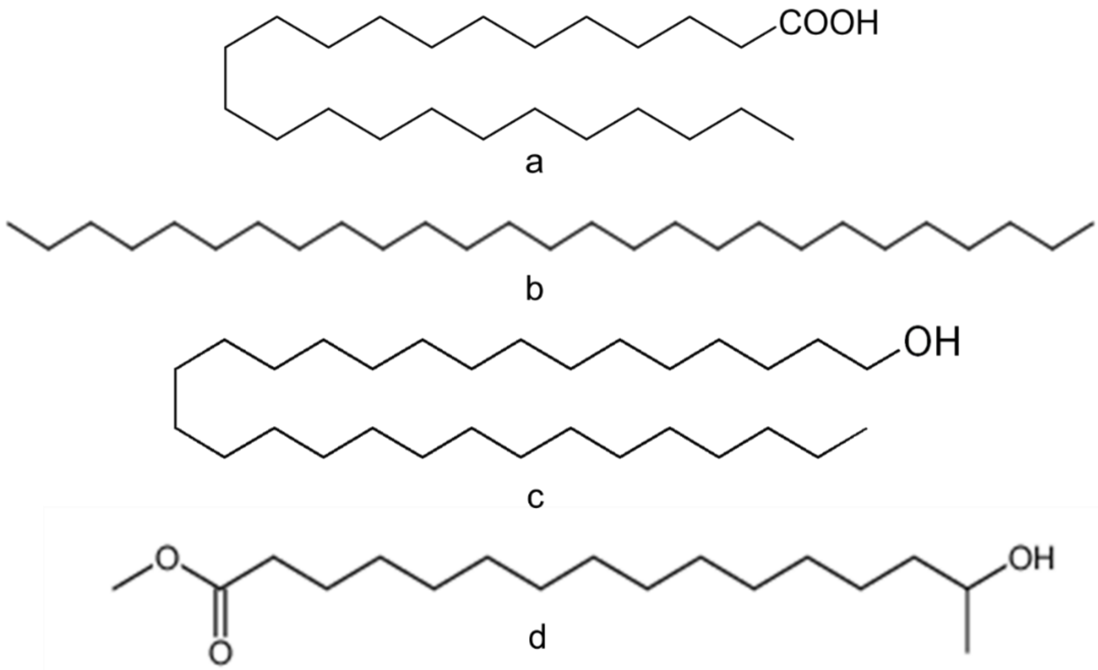

Figure 0.4 Compounds detected in samples Z-3947, Z-3968, Z-4072, Z-4011, Z-4011x and Z-4362 of Zambujeiro Dolmen ceramics: (a) lignoceric acid (C24:0), (b) heptacosane (C27), (c) melissyl alcohol (C30:OH); (d) methyl 15-hydroxyhexadecanoate.

The close association of Neolithic farming communities with the honeybee has been demonstrated across Europe, linking onset of agriculture in Europe with possible evidence for *Apis* domestication and exploitation of its products (Roffet-Salque et al. 2015). Interestingly, intensified human-animal interactions led to management and reliance of bees on humans (Oldroyd 2012), but the introduction of hives did not imperil genetic diversity in honeybees (Harpur et al. 2012). The most ample evidence for the use of bee products comes from Southeastern Europe and the Balkans, connecting the use of beeswax to the Neolithic communities in the $6^{th}/5^{th}$ millennia BC (Regert et al. 2001, Roumpou et al. 2021, Urem-Kotsou et al. 2018, Stojanovski et al. 2020, Hulina 2020).

At the same time chemical evidence of bee exploitation has been recorded across Central Europe, in Italian Alpine region, Slovenia, Austria, Germany and Poland (Cristiani et al. 2009, Bernardini et al. 2012, Šoberl et al. 2014, Heron et al. 1994, Roffet-Salque et al. 2015, Salque et al. 2013). In France, the beeswax has been detected during the second half of the 5th millennium and in 4th millennium (Regert et al. 2001, Mirabaud et al. 2015, Drieu et al. 2020, Rageot et al. 2021). Even if some evidence of Neolithic beeswax has been detected in Britain (Copley et al. 2005), there is no conclusive evidence for Ireland, Scotland and Fennoscandian Peninsula (Smyth and Evershed 2015, Aveling and Heron 1999, Cramp et al. 2014a, Cramp et al. 2014b). Denmark (Heron et al. 2007, Robson et al. 2021) and the southern Baltic region (Heron et al. 2015) seem to be the northern limit for Neolithic bee exploitation, while the distribution to the Northeast, according to the available data, seems to be limited by Eurasian Steppe (Roffet-Salque et al. 2015, Bondetti et al 2020, Courel et al. 2021).

For Northern Africa and Iberian Peninsula, until now, the evidence for the early bee exploitation has been scarce. A single well-preserved beeswax residue has been reported from the from the Algerian site of Gueldaman dated to the 5th century BC (Roffet-Salque et al. 2015). In the Iberian Peninsula, even though beeswax has been detected in later archaeological periods (Cañabate Guerrero and Sánchez Vizcaíno 1997, Bonet Rosado and Mata Parreño 1997, Frade et al. 2014, Parras et al. 2015, Oliveira et al. 2019), the paucity of data might be connected state of research and preservation conditions. The earliest evidence for the interactions of Iberian communities with bees has been connected to Mesolithic rock shelter paintings representing bees and honey hunting scenes in eastern Spain (Dams 1978). Traces of beeswax were identified in the two small bowls from in Gavà Mines located in Catalonia on the northeast of the Iberian Peninsula, south of the Pyrenees Mountain range and dated to Middle Neolithic (Tarifa-Mateo et al. 2021). The wider connection of honeybee products with pottery has been indicated only during the Bell Beaker culture (Ramírez et al. 2005, Guerra-Doce 2006).

The clear and abundant evidence of exploitation of bee products by the Late Neolithic communities discovered at Zambujeiro Dolmen, is a great incentive to review and explore the spatial and temporal distribution of the honeybee in the Iberian Peninsula and its connections to Northern Africa. The future research can uncover the extent of *Apis mellifera* product usage throughout Prehistory of Iberia, as well as dynamics of this specific human/nature interaction.

2.6. Conclusion

Organic residue analysis (ORA) of the remains absorbed into pottery sherds, retrieved from the biggest chambered megalithic tomb of Alentejo region, Zambujeiro Dolmen, provided evidence for the use and processing of animal and plant products. Residues of fats and oils of plant origin stored in the studied fragments, were most likely a result of processing as supported by presence of plant waxes. At the same site, clear evidence of beeswax was detected in pots, conical open bowls as well as in one cup. These results indicate that populations on the Iberian Peninsula actively participated in the widespread exploitation of the honeybee products by agrarian communities that was documented across Europe. This places the beginning of the Iberian apiculture to at least the end of 4^{th} and the beginning of the 3^{rd} millennium BC.

Table 0.2 List of identified compounds.*

Organic compounds	**Samples**													
Vessel number	**2**	**4**	**5**	**11**	**15**	**17**	**3**	**7**	**9**	**10a**	**10b**	**13**	**14**	**16**
Sample number	**Z-3694**	**Z-3720**	**Z-3947**	**Z-4041**	**Z-4114**	**Z-4157**	**Z-3718**	**Z-3968**	**Z-4014**	**Z-4040**	**Z-4040x**	**Z-4072**	**Z-4104**	**Z-4143**
	Cups						Pots							
Saturated fatty acids														
Caprylic acid (C8:0)	-	-	-	-	-	X	-	-	-	-	-	-	-	-
Pelargonic acid (C9:0)	X	X	-	X	-	X	-	X	-	-	-	X	-	-
Capric acid (C10:0)	-	X	-	-	-	-	-	X	-	-	-	X	-	-
Lauric acid (C12:0)	X	-	X	X	-	X	X	X	-	-	X	X	-	X
Tridecylic acid (C13:0)	X	-	-	-	-	X	X	X	-	-	-	X	X	
Myristic acid (C14:0)	X	X	X	X	-	X	X	X	-	X	X	X	X	X
Pentadecylic acid (C15:0)	X	X	X	X	X	X	X	X	-	X	-	X	X	X
Palmitic acid (C16:0)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Margaric acid (C17:0)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Stearic acid (C18:0)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nonadecylic acid (C19:0)	X	X	X	-	-	X	-	X	X	X	X	X	X	-
Arachidic acid (C20:0)	X	X	X	-	-	X	X	X	X	X	X	X	X	X
Heneicosylic acid (C21:0)	-	X	X	-	-	-	-	X	X	X	-	X	X	-
Behenic acid (C22:0)	X	X	X	-	-	X	X	X	X	X	X	X	X	X
Tricosylic acid (C23:0)	X	X	-	-	-	X	X	X	X	X	X	X	-	X
Lignoceric acid (C24:0)	X	X	X	-	X	X	X	X	X	X	X	X	X	X
Pentacosylic acid (C25:0)	-	X	X	-	-	-	-	X	X	-	-	X	-	-
Cerotic acid (C26:0)	-	X	X	-	-	X	X	X	X	X	X	X	-	-
Carboceric acid (C27:0)	-	-	-	-	-	-	-	X	-	-	-	-	-	-
Montanic acid (C28:0)	-	X	-	-	-	-	X	X	X	X	-	X	-	-
Melissic acid (C30:0)	-	X	-	-	-	X	X	X	X	X	-	X	X	-
Lacceroic acid (C32:0)	-	-	-	-	-	-	-	X	-	-	-	-	X	-
C16:0 > C18:0	X	-	X	-	-	X	X	X	X	-	X	X	-	-
Unsaturated fatty acids														
Palmitelaidic acid (C16:1, cis-Δ9)	-	X	X	-	X	X	-	X	X	-	X	X	-	-
Linoleic acid (C18:2 cis-Δ9,12)	-	-	X	-	-	-	-	-	-	-	-	-	-	-
Oleic acid (C18:1 cis-Δ9)	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table III.2 (cont.) List of identified compounds.*

Organic compounds	**Samples**													
Vessel number	**2**	**4**	**5**	**11**	**15**	**17**	**3**	**7**	**9**	**10a**	**10b**	**13**	**14**	**16**
Sample number	**Z-3694**	**Z-3720**	**Z-3947**	**Z-4041**	**Z-4114**	**Z-4157**	**Z-3718**	**Z-3968**	**Z-4014**	**Z-4040**	**Z-4040x**	**Z-4072**	**Z-4104**	**Z-4143**
	Cups						Pots							
Acylglycerols														
1-Monopalmitin	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2-Monopalmitin	X	X	X	X	X	X	X	X	-	X	X	X	X	X
1-Monostearin	X	X	X	X	X	X	X	X	-	X	X	X	X	X
2-Monostearin	-	X	X	X	X	X	X	X	-	-	X	X	X	X
Diacids														
Suberic acid	-	-	-	-	-	X	-	-	-	-	-	X	-	-
Azelaic acid	X	X	X	-	-	X	X	X	-	-	X	X	-	-
Sebacic acid	X	X		-	-		X	X	-	-	X	X	-	-
Undecanedionic acid			X	-	-	X	X	X	-	-	X	X	X	-
Tridecanedioic acid	-	-	-	-	-	X	-	-	-	-	X	-	-	-
Tetradecanedioic acid	-	-	-	-	-	-	-	-	-	-	X	-	-	-
Thapsic acid	-	-	-	-	-	-	-	-	-	-	X	-	-	-
Hydroxy acids														
Methyl 15-hydroxyhexadecanoate	-	-	X	-	-	-	-	X	-	-	-	X	-	-
Alcohols														
Lauryl alcohol (C12:OH)	X	-	-	-	-	X	X	X	-	-	-	-	-	-
Tridecyl alcohol (C13:OH)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myristyl alcohol (C14:OH)	X	-	-	-	-	X	X	X	-	-	X	-	-	-
Pentadecyl alcohol (C15:OH)	-	-	-	-	-	-	-	X	-	-	X	-	-	-
Palmityl alcohol (C16:OH)	X	-	-	-	-	X	X	X	-	-	-	-	-	-
Stearyl alcohol (C18:OH)	X	-	-	-	-	-	X	X	-	-	-	-	-	-
Arachidyl alcohol (C20:OH)	-	-	-	-	-	-	-	X	-	-	-	X	-	-
Behenyl alcohol (C22:OH)	-	-	X	-	-	-	-	X	-	-	-	X	-	-
Lignoceryl alcohol (C24:OH)	-	-	X	-	-	-	X	X	-	X	-	X	-	-
Pentacosyl alcohol (C25:OH)	-	-	-	-	-	-	-	-	-	-	-	X	-	-
Ceryl alcohol (C26:OH)	-	-	X	-	-	X	X	X	-	-	-	-	-	-
Montanyl alcohol (C28:OH)	-	-	-	-	-	X	X	X	-	-	-	X	-	-
Melissyl alcohol (C30:OH)	X	X	X	-	-	X	X	X	-	-	-	X	-	-
Laceryl alcohol (C32:OH)	-	-	X	-	-	X	X	X	X	-	-	-	-	-

Table III.2 (cont.) List of identified compounds.*

Organic compounds	**Samples**													
Vessel number	**2**	**4**	**5**	**11**	**15**	**17**	**3**	**7**	**9**	**10a**	**10b**	**13**	**14**	**16**
Sample number	**Z-3694**	**Z-3720**	**Z-3947**	**Z-4041**	**Z-4114**	**Z-4157**	**Z-3718**	**Z-3968**	**Z-4014**	**Z-4040**	**Z-4040x**	**Z-4072**	**Z-4104**	**Z-4143**
	Cups						Pots							
Alkanes														
Heptadecane (C17)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nonadecane (C19)	X	X	-	-	X	-	-	-	-	-	-	-	X	-
Eicosane (C20)	X	X	X	-	-	-	X	X	-	-	X	X	-	X
Heneicosane (C21)	-	-	-	-	-	-	-	X	-	-	-	X		
Docosane (C22)	-	-	-	-	-	X	-	-	-	-	X	-	-	-
Tricosane (C23)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetracosane (C24)	-	-	-	-	-	X	X	-	-	-	X	-	-	-
Pentacosane (C25)	-	-	X	-	-	-	-	-	-	-	-	-	-	-
Heptacosane (C27)	-	X	X	-	-	-	X	X	X	X	X	X	X	-
Octacosane (C28)	-	X	-	-	-	-	-	-	-	-	-	-	-	-
Nonacosane (C29)	-	-	X	-	-	-	-	X	-	-	-	X	X	-
Triacontane (C30)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hentriacontane (C31)	-	X	X	-	-	-	X	X	X	-	-	X	-	-
Dotriacontane (C32)	-	-	-	-	-	-	X	-	-	-	-	-	-	-
Tetratriacontane (C34)	-	-	-	-	-	-	-	X	-	-	-	-	-	-
Sterols														
β-Sitosterol	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Presence of beeswax	-	-		-	-	-	-		-	-	-		-	-

* The trimethylsilyl derivatives and methyl ester derivatives of compounds detected.

Table III.2 (cont.) List of identified compounds.*

Organic compounds	Samples												
Vessel number	**18a**	**18b**	**19**	**20**	**21**	**6**	**8a**	**8b**	**12**	**1**	**22**	**23**	**24**
Sample number	**Z-4162**	**Z-4162x**	**Z-4298**	**Z-4303**	**Z-4306**	**Z-3952**	**Z-4011**	**Z-4011x**	**Z-4044**	**Z-3293**	**Z-4309**	**Z-4362**	**Z-4365**
	Pots					Open conical bowls				Fragments			
Saturated fatty acids													
Caprylic acid (C8:0)	-	-	-	-	-	-	-	-	-	-	-	-	-
Pelargonic acid (C9:0)	X	X	X	X	-	X	X	-	-	-	-	X	-
Capric acid (C10:0)	-	-	-	-	-	X	X	-	-	-	-	X	-
Lauric acid (C12:0)	X	X	X	X	-	-	-	-	-	-	X	X	-
Tridecylic acid (C13:0)	X	X	X	X	-	-	-	-	-	-	X	X	-
Myristic acid (C14:0)	X	X	X	X	X	X	X	X	X	X	X	X	X
Pentadecylic acid (C15:0)	X	X	-	X	-	X	X	X	X	X	X	-	X
Palmitic acid (C16:0)	X	X	X	X	X	X	X	X	X	X	X	X	X
Margaric acid (C17:0)	X	X	X	X	X	X	X	X	-	X	X	X	X
Stearic acid (C18:0)	X	X	X	X	X	X	X	X	X	X	X	X	X
Nonadecylic acid (C19:0)	-	-	X	X	X	X	X	X	-	-	X	X	-
Arachidic acid (C20:0)	X	X	X	X	X	X	X	X	-	-	X	X	X
Heneicosylic acid (C21:0)	X	-	-	X	X	X	X	X	-	-	X	X	X
Behenic acid (C22:0)	-	-	X	X	X	X	X	X	-	-	X	X	-
Tricosylic acid (C23:0)	-	-	X	X	X	-	X	X	-	X	X	X	X
Lignoceric acid (C24:0)	X	X	X	X	X	X	X	X	X	-	X	X	X
Pentacosylic acid (C25:0)	-	-	-	X	-	-	X	-	-	-	X	X	X
Cerotic acid (C26:0)	-	-	-	X	X	X	X	X	X	-	-	X	X
Carboceric acid (C27:0)	-	-	-	X	-	-	-	-	-	-	-	-	-
Montanic acid (C28:0)	-	-	-	X	X	-	X	X	-	-	X	X	X
Melissic acid (C30:0)	-	-	-	X	-	X	X	X	-	-	X	X	X
Lacceroic acid (C32:0)	-	-	-	-	-	-	-	-	-	-	-	-	-
C16:0 > C18:0	X	X	X	-	-	-	X	X	-	-	X	X	-
Unsaturated fatty acids													
Palmitelaidic acid (C16:1, cis-Δ9)	-	-	-	-	-	-	X	X	-	-	-	-	-
Linoleic acid (C18:2 cis-Δ9,12)	-	-	-	-	-	-	X	-	-	-	-	-	-
Oleic acid (C18:1 cis-Δ9)	X	-	-	-	.	X	X	-	X	-	X	X	X

Table III.2 (cont.) List of identified compounds.*

Organic compounds	**Samples**												
Vessel number	**18a**	**18b**	**19**	**20**	**21**	**6**	**8a**	**8b**	**12**	**1**	**22**	**23**	**24**
Sample number	**Z-4162**	**Z-4162x**	**Z-4298**	**Z-4303**	**Z-4306**	**Z-3952**	**Z-4011**	**Z-4011x**	**Z-4044**	**Z-3293**	**Z-4309**	**Z-4362**	**Z-4365**
	Pots					Open conical bowls				Fragments			
Acylglycerols													
1-Monopalmitin	X	X	X	X	X	X	X	X	-	X	X	X	X
2-Monopalmitin	X	X	X	-	X	X	X	X	-	X	-	X	-
1-Monostearin	X	X	X	-	X	X	X	X	X	X	-	X	X
2-Monostearin	X	X	X	-	X	X	X	-	-	X	-	X	-
Diacids								-					
Suberic acid	-	-	-	-	-	-	X	-	-	-	-	-	-
Azelaic acid	X	-	X	-	-	X	X	-	-	-	X	-	-
Sebacic acid	X	-	X	X	-	X	-	-	-	-	X	-	-
Undecanedionic acid	X	X	X	X	X	-	-	-	-	-	X	-	-
Tridecanedioic acid	X	X	-	-	-	-	-	-	-	-		-	-
Tetradecanedioic acid	X	-	-	-	-	-	-	-	-	-	X	-	-
Thapsic acid	-	-	-	X	-	-	-	-	-	-	-	-	-
Hydroxy acids													
Methyl 15-hydroxyhexadecanoate	-	-	-	-	-	-	X	X	-	-	-	X	-
Alcohols													
Lauryl alcohol (C12:OH)	-	-	X	-	-	-	-	-	-	-	-	X	-
Tridecyl alcohol (C13:OH)	-	-		-	-	-	-	-	-	-	-	-	-
Myristyl alcohol (C14:OH)	-	X	X		X	-	-	-	-	-	-	X	-
Pentadecyl alcohol (C15:OH)		-	-	-	X	-	-	-	-	-	-	X	-
Palmityl alcohol (C16:OH)	X	-	-	-	X	-	-	-	-	-	-	X	-
Stearyl alcohol (C18:OH)	-	-	X		X	-	-	-	-	-	-	X	X
Arachidyl alcohol (C20:OH)	-	-	-	-	-	-	X	-	-	-	-	X	-
Behenyl alcohol (C22:OH)	-	-	-	-	-	-	X	-	-	-	-	X	-
Lignoceryl alcohol (C24:OH)	-	-	-	-	-	-	X	X	-	-	-	X	-
Pentacosyl alcohol (C25:OH)	-	-	-	-	-	-	X	-	-	-	-	-	-
Ceryl alcohol (C26:OH)	-	-	-	-	-	-	X	-	X	-	-	-	-
Montanyl alcohol (C28:OH)	-	-	-	-	X	-	X	-	-	-	-	X	-
Melissyl alcohol (C30:OH)	-	-	-	-	X	-	X	X	-	-	-	X	-
Laceryl alcohol (C32:OH)	-	-	-	-	-	-	X	-	-	-	-	X	-

Table III.2 (cont.) List of identified compounds.*

Organic compounds	**Samples**												
Vessel number	**18a**	**18b**	**19**	**20**	**21**	**6**	**8a**	**8b**	**12**	**1**	**22**	**23**	**24**
Sample number	**Z-4162**	**Z-4162x**	**Z-4298**	**Z-4303**	**Z-4306**	**Z-3952**	**Z-4011**	**Z-4011x**	**Z-4044**	**Z-3293**	**Z-4309**	**Z-4362**	**Z-4365**
	Pots					Open conical bowls				Fragments			
Alkanes													
Heptadecane (C17)	-	-	-	-	X	X	-	-	-	-	X	X	-
Nonadecane (C19)	X	-	-	-	-	-	-	-	X	-	-	-	-
Eicosane (C20)	-	-	-	X	X	-	X	-	-	-	X	X	X
Heneicosane (C21)	X	-	-			X	-	-	-	-	X	-	-
Docosane (C22)	-	-	-	-	-	-	-	-	-	-	-	-	X
Tricosane (C23)	-	-	-	-	-	-	-	-	X		-	-	-
Tetracosane (C24)	-	-	-	-	-	-	-	-	-	X	-	-	-
Pentacosane (C25)	-	-	-	-	-	-	-	-	-	-	-	X	-
Heptacosane (C27)	-	-	-	X	X		X	X	X		X	X	X
Octacosane (C28)	-	-	-	-	X	-	-	-	-	X	-	-	-
Nonacosane (C29)	-	-	-	-	-	-	X	-	-	-	-	X	-
Triacontane (C30)	-	-	-	-	-	-	-	-	-	-	-	-	-
Hentriacontane (C31)	-	-	-	-	X	X	X	-	-	-	-	X	-
Dotriacontane (C32)	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetratriacontane (C34)	-	-	-	-	-	-	-	-	-	-	-	X	-
Sterols													
β-Sitosterol	-	-	-	-	-	-	-	-	-	-	-	-	-
Presence of beeswax	-	-	-	-	-	-			-	-	-		-

* The trimethylsilyl derivatives and methyl ester derivatives of compounds detected.

3. Manuscript 2: Contents and function of Late Neolithic/Chalcolithic funerary vessel from Monte da Comenda, Portugal

3.1. Introduction

In the Central and South of Portugal, the last decades provided new insights that changed the image on the Late Neolithic and Chalcolithic funerary architecture and practices, traditionally linked to the megalithic phenomena (Soares 2003, Valera et al. 2010, Valera 2012, Boaventura et al. 2014, Andrade 2015, Russo 2020, Valera 2020). In the 3rd millennium, a vast diversity of architectural solutions can be observed (Sousa 2016). Furthermore, different ritual practices seem to coincide with different architecture in the area (Valera 2012). Considerable number of burials in pits and in hypogea necropolis, mostly concentrated in the Alentejo region, offer exciting perspectives of the funerary rites of the period.

Hypogea, the underground structures, described as artificial rock cut caves used for inhumations, protected the integrity of the archaeological site throughout the centuries, and provided good conditions for the preservation of the organic remains. Regarding the layout and the construction of the burial structures several types of hypogea were recognized: lateral pit, with undefined entrance with steps, with an entrance through a side pit and with an access through a short corridor (Valera 2020). During Late Neolithic/Chalcolithic they were generally used as multiple or collective burials, that are often accompanied by small votive sets unevenly distributed in the funerary structures, that include stone blades and tools, animal bones and ivory (Valera 2020), while the finds of ceramics are lacking.

The good preservation of organic remains, with no parallel in megalithic monuments of the region, allowed research to advance in many different directions: the characterization of sex, age at death and body metrics and pathologies of individuals (Silva 2003), the determination of their diets and patterns of mobility through isotopic studies (Soberl et al. 2020), the access to aspects of the ritual practices, such as body positions and orientations, number of individuals, their associations within the tombs and bone manipulation practices (Valera and Costa 2013, Valera 2020). The presence of ceramics in the hypogea burial of Monte da Comenda 3, also allows the investigation of those vessels and their contents that were absorbed into a porous matrix of the ceramics, as well as of the significance of organic burial offerings regarding the treatment of the dead in the 4th and 3rd millennium BC.

3.2. Archaeological context

The burial site of Monte da Comenda 3 is administratively located in the parish of Baleizão, municipality and district of Beja, in the Lower Alentejo region of Portugal (Figure III.5a). It is positioned on a slightly elevated terrain, on a natural platform, just above the ravine of the Guadiana River. The river runs in a north-south direction, seasonally causing floods in times of greater rainfall, to east of the archaeological site. Currently, the location of the archaeological site is an agricultural area, marked by the cultivation of irrigated olive trees (Miguel and Godinho 2009). Geologically, this area belongs to Ossa Morena Zone of the Iberian Massif, with predominantly granites and schists, and the presence of quartzites and metamorphic rocks (Carvalho 2016). The region of Baleizão lays on porphyry bedrock and is surrounded by diorites and metavolcanic rocks (Ferreira et al. 2014). The soils in the area of the archaeological site are mostly acidic (Carvalho 2016).

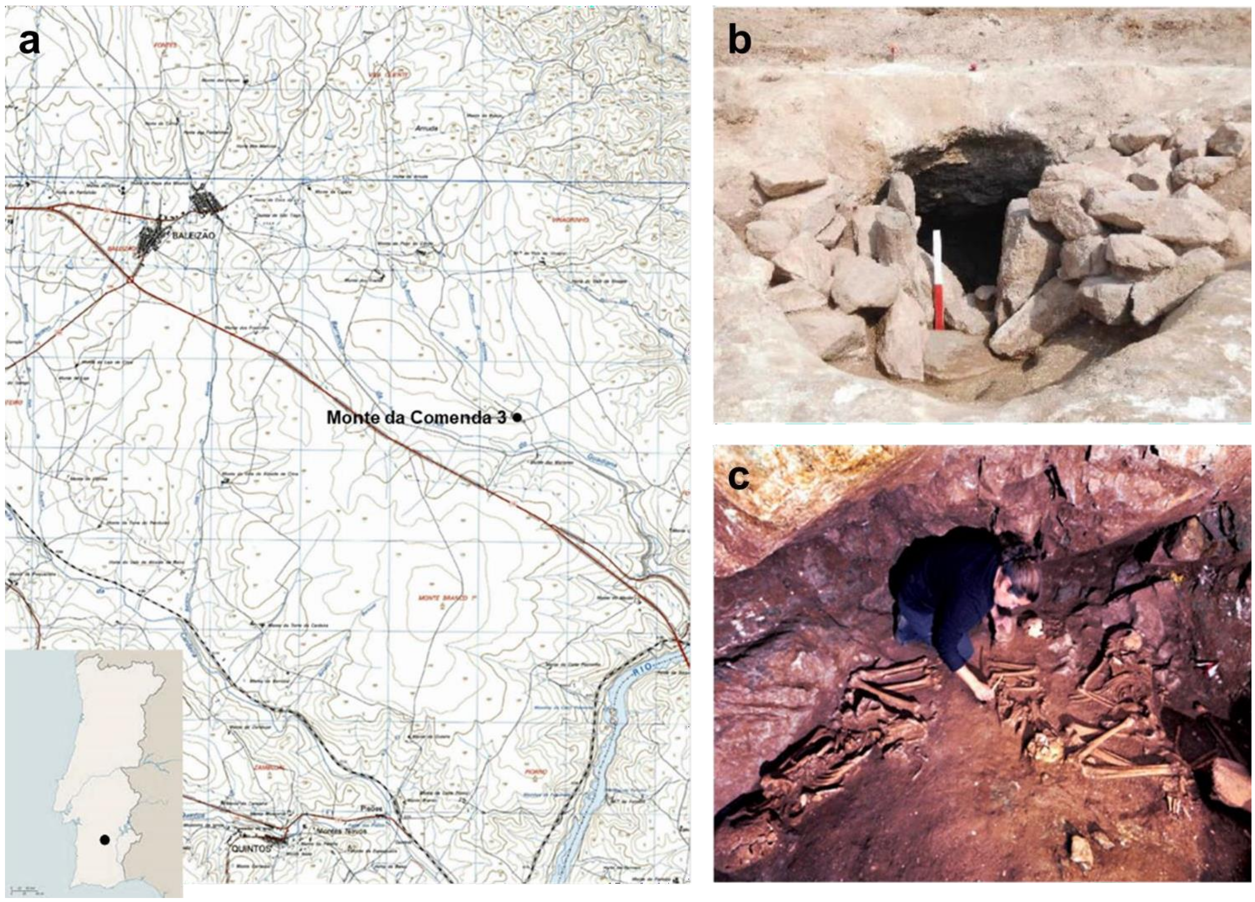

Figure 0.5 (a) Location of the Monte da Comenda 3 archaeological site (adapted from Carvalho 2016); (b) uncovered entrance to the hypogeum (Carvalho 2016); (c) excavation of the burial site (Soberl et al. 2015).

Continuing the Neolithic traditions of hypogea (Valera 2013, Valera and Filipe 2012, Melo and Silva 2016, Valera et al. 2017), the collective burial from Monte da Comenda, represents particular funerary architecture and mortuary practice. Monte da Comenda 3, with its unique typology, is described as hypogeum with a side ramp excavated in the rock, which was later coated with larger stones (Figure III.5b), providing the access to the chamber (Valera 2020). A minimum number of 12 inhumations were identified, 7 subadults and 5 adults, buried alongside votive material associated with specific individuals (Figure III.5c). The remains were radiocarbon dated at 2455-2411 BC (Emslie et al. 2019, Valera 2020).

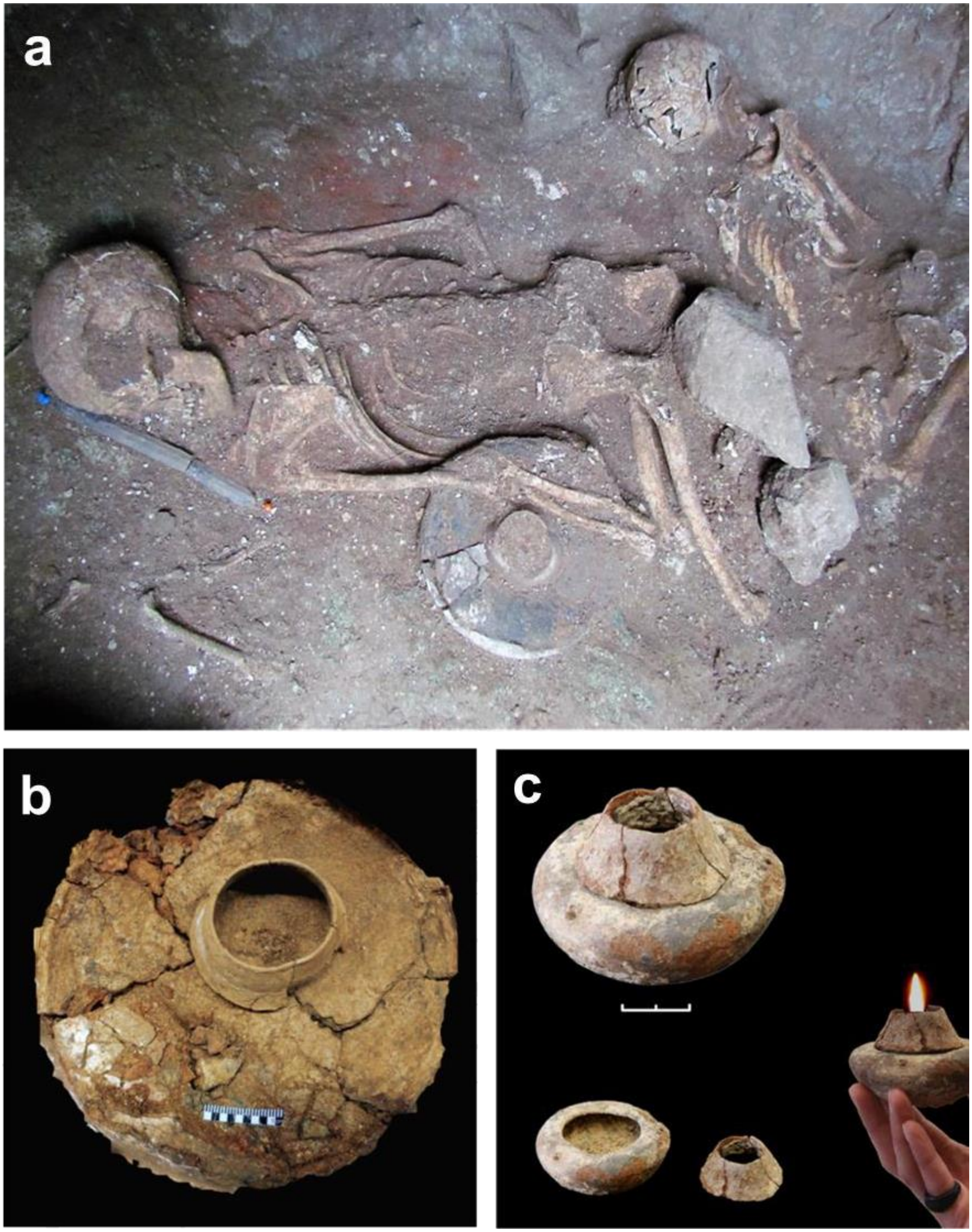

Figure 0.6 (a) Burial of Individual 1 in the of the Monte da Comenda 3 hypogeum (Carvalho 2016); (b) composite ceramic vessel found in association with Individual 1; (c) a small composite vessel associated with Individual 3 and a virtual reconstruction as a lucerne (Valera 2020).

The burial offerings included ceramic vessels, arrowhead and flint blades, deer bones and ivory zoomorphic objects. The Individual 1 and Individual 3 had composite ceramic vessels related with the burial (Carvalho 2016). This type of ceramic containers was interpreted as *lucerna*, an oil lamp (Valera 2020) (Figure III.6c). The larger one, found with Individual number 1, was selected for the organic residue analysis. Individual 1, found in the northwest part of the chamber (Figure III.6a), was an adult female, recovered in a prone position, with remains of the red pigment in the soil around it, a large flint blade, and as mentioned, a large composite vessel with a bone pin found inside (Carvalho 2016, Valera 2020) (Figure III.6.b).

3.3. Material and methods

The ceramic vessel found beside the Individual 1 in the hypogeum was composed of two parts, a vessel and nozzle on the top of the vessels mouth. These pieces were separable but were recovered connected *in situ*, one on top of the other (Figure III.6a,b). This specific typology prompted the investigation about the nature of the vessel and its contents.

Ceramic sample from the bottom of the vessel was taken to examine pottery fabrics and get an insight into mineralogical composition of the ceramics. Polarizing microscope Leica DM2500P equipped with a digital camera (Leica MC170 HD) was used for the microscopic analysis. The thin sections were examined and documented under plane-polarized and cross-polarized transmitted light with 100x and 200x magnification. The minerals were identified on the basis of their optical properties.

X-ray powder diffraction (XRPD) was used to identify the mineral composition of ceramic powder. Analysis was conducted using a Bruker™ D8 Discover® instrument equipped with a Cu Kα source and a LYNXEYE linear detector. All measurements were performed over a range from 3° to 75° 2θ, using a 0.05º increment with measuring time of 1 s/step. The identification of all crystalline phases was performed with the DIFFRAC.SUITE EVA® software using the Powder Diffraction File (PDF-2) X-ray patterns database of the International Centre for Diffraction Data.

The study of the contents of archaeological ceramics, based on absorbed residue analysis, was performed by gas chromatography/mass spectrometry (GC/MS) on the ceramic samples recovered from the bottom, body and rim of the vessel. To discover original contents of the vessel, lipids were extracted from a powdered sample with sulfuric acid in methanol, and to get additional information with chloroform/methanol (Correa-Ascencio & Evershed 2014, Evershed et al. 1990, respectively), as described in Chapter II, 4.1. 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. Internal standard and BSTFA derivative, were also obtained from Sigma-Aldrich. A Milli-Q Integral Water Purification system (Millipore®) was used to produce ultrapure water.

Organic residue analysis was performed with Shimadzu GC2010 coupled with a Shimadzu GCMS-QP2010 Plus Mass Spectrometer. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm x 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. 1 μL of the sample was injected in splitless mode, with the injector set at 250 ºC 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 minutes. The mass spectrometer, equipped with a 70 eV electron ionization source, 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. The obtained results were processed with Automated Mass spectral Deconvolution and Identification System (AMDIS). The chromatograms were interpreted in comparison with the NIST library database.

3.4. Results

The optical investigation under petrographic microscope revealed fine textured ceramic paste of reddish-brown hue, with very poorly sorted, coarse grained temper without preferential orientation (Figure III.7). The fabric data compared to reference charts (Orton et al. 1993) demonstrates relatively high temper to matrix ratio spanning from 30 to 40%. The main tampering minerals identified are quartz found in abundance, followed by feldspars and calcite, as well as presence of amphiboles and opaque minerals. The temper also contained lithoclasts of metamorphic or igneous rocks. Mineral grains are angular with low sphericity, ranging from 20 to 200 μm, while larger rock inclusions can be up to 400 μm. Possible presence of grog in the matrix is also noted.

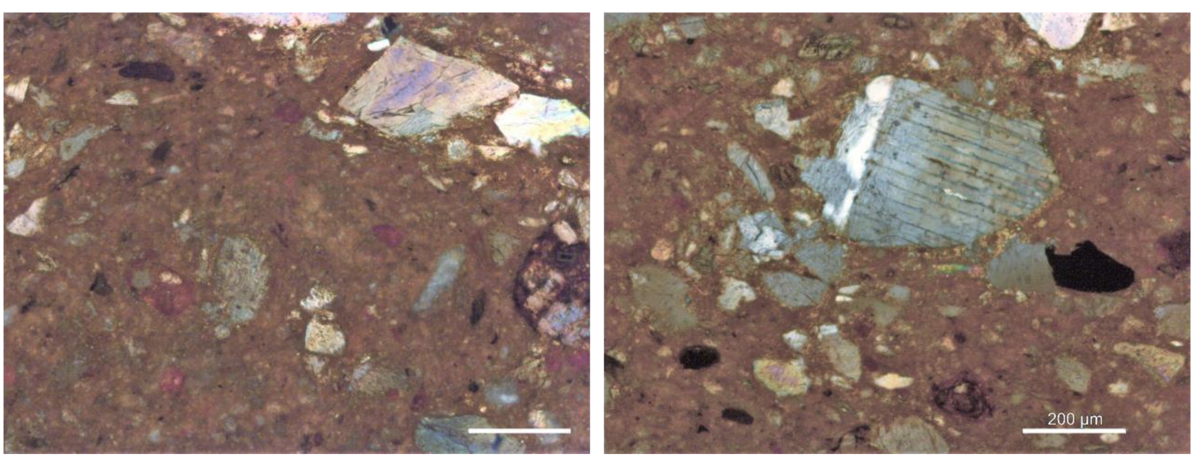

Figure 0.7 Thin-section photomicrographs of pottery fabrics from Monte da Comenda 3.

To assess the results of petrographic analysis and validate mineral phases in the ceramic material, mineralogical composition was determined by XRPD (Figure III.8). Mineral phases detected are quartz, plagioclase and alkali feldspars, clays/micas, calcite, as well as monoclinic amphiboles. The results of XRPD are in accordance with petrographic observation, showing quartz as most abundant, followed by feldspars.

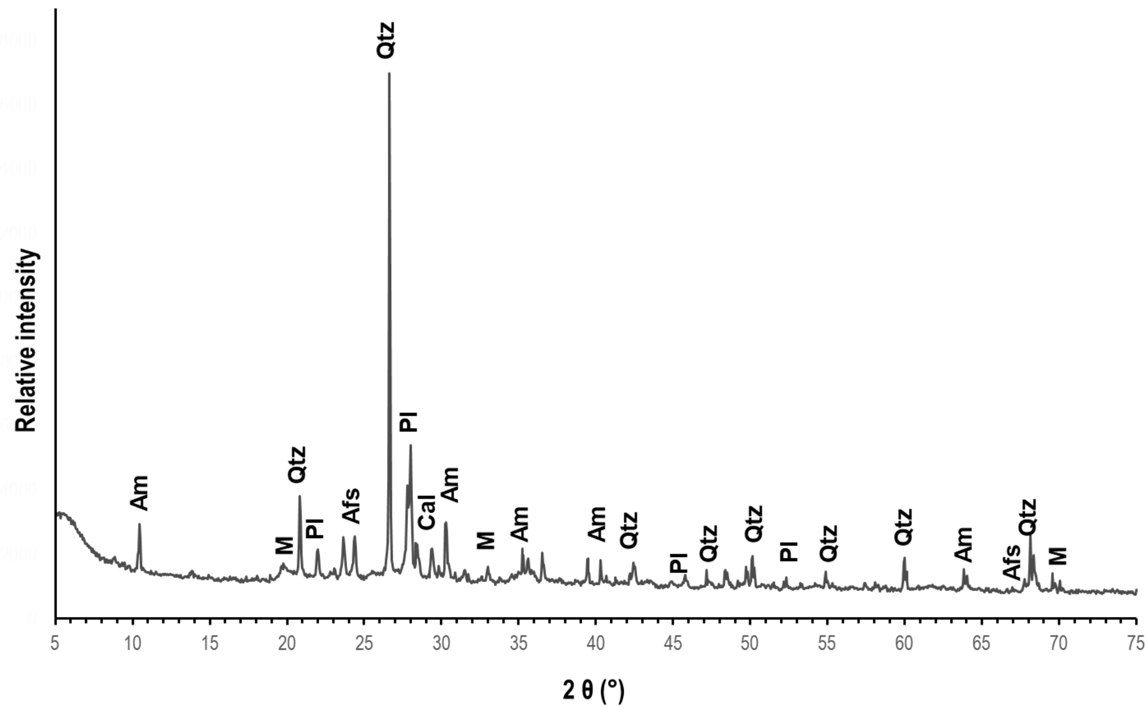

Figure 0.8 Diffractogram of ceramic powder showing detected minerals; Qtz=Quartz, Pl=Plagioclase, Cal=Calcite, Afs=Alkaline feldspar, M=Mica, Am=Amphibole.

Organic residue analysis performed by GC/MS showed good preservation of lipidic content absorbed into the ceramic matrix, composited of fatty acids, acylglycerols, alkanes and fatty alcohols (Figure III.9, Table III.3). The ceramic sherds from the bottom, body and rim of the vessel were analyzed, with the bottom of the vessel yielding the highest relative abundance of organic compounds. All studied ceramic sheds present chromatographic profiles coherent with the presence of oil/fat residues. The most prevalent fatty acids are palmitic and stearic acid. Even numbered lauric and myristic acid are also detected, and possible odd numbered pentadecylic and margaric acid.

Unsaturated fatty acid, octadecenoic acid, together with monoacylglycerols, show excellent preservation of oil/fat. Long chain fatty acids, docosanoic (behenic) and tetracosanoic (lignoceric) acid, were detected only in the bottom part of the vessel. Even number fatty alcohols ranging from C14:OH to C18:OH, and C24:OH to C30:OH were detected in low amounts, as well as even and odd numbered alkanes. Presence of β-Sitosterol, a plant sterol, is probable.

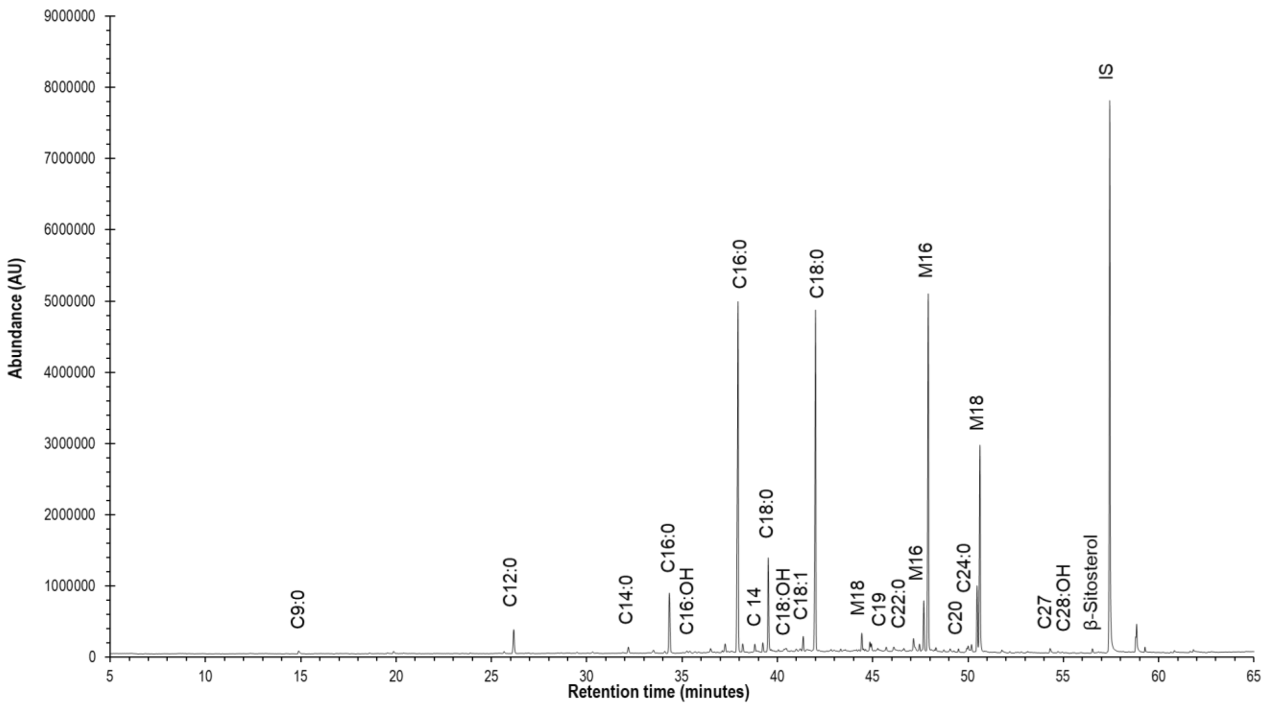

Figure 0.9 GC/MS chromatogram of organic residue. Marked are the trimethylsilyl derivatives and and methyl ester derivatives of relevant compounds detected. Cx alkane with x carbon atoms, Cx:OH linear alcohol with x carbon atoms, Mx monoacylglycerol with x carbon atoms, Cx:y fatty acid with x carbon atoms and y double bonds, IS internal standard.

Table 0.3 List of identified compounds detected in the absorbed organic residue from composite vessel found in association with Individual 1.*

Organic compounds	Samples		
	Bottom	Body	Rim
Saturated fatty acids			
Pelargonic acid (C9:0)	X		
Lauric acid (C12:0)	X	X	X
Myristic acid (C14:0)	X	X	X
Pentadecylic acid (C15:0)	?		X
Palmitic acid (C16:0)	X	X	X
Margaric acid (C17:0)			X
Stearic acid (C18:0)	X	X	X
Behenic acid (C22:0)	X		
Lignoceric acid (C24:0)	X		
C16:0 > C18:0			
Unsaturated fatty acids			
Oleic acid (C18:1 cis-Δ9)	X	X	X
Acylglycerols			
1-Monomyristin	X		
1-Monopalmitin	X	X	X
2-Monopalmitin	X		
1-Monostearin	X	X	X
2-Monostearin	X		
Alkanes			
Tetradecane (C 14)	X		X
Hexadecane (C16)		X	X
Octadecene (C18)		X	X
Nonadecane (C19)	X		
Eicosane (C20)	X		X
Heptacosane (C27)	X	X	X
Hentriacontane (C31)		X	
Alcohols			
Myristyl alcohol (C14:0H)			X
Palmityl alcohol (C16:OH)	X	X	X
Stearyl alcohol (C18:OH)	X	X	X
Lignoceryl alcohol (C24:OH)			?
Ceryl alcohol (C26:OH)		X	X
Montanyl alcohol (C28:OH)	X	X	X
Myricyl alcohol (C30:OH)		X	X
Sterols			
β-Sitosterol	X		X

* The trimethylsilyl derivatives and methyl ester derivatives of compounds detected.

3.5. Discussion

The fabrics of ceramics identified could be indicative of a local clay material source. The pottery sherd besides quartz and feldspars also contains calcite, micas, amphiboles, and lithoclasts from metamorphic and igneous rocks. The western Iberian Peninsula is a very diverse geologically (Pope & Miranda 1999). It has been subdivided in several Zones including Ossa Morena Zone (OMZ) and Central-Iberian Zone (CIZ). The Ossa Morena Zone includes most of the North and Central Alentejo. It is limited to the North by the Tomar-Portalegre-Badajoz-Cordoba Shear Zone (TPBCSZ), and to the South by the Beja-Acebuches Ophiolitic Complex (BAOC), although this division has been under discussion (de San José et al. 2004). OMZ is characterized by folded Paleozoic metamorphic rocks connected mostly with low grade metamorphism. Abundant Precambrian, Cambrian and Carboniferous granitic intrusions are present. The Cambrian sequence has conglomerate deposits at the base and limestone units at the top. In the Late Ordovician syenite and alkaline granite intrusions immerge along the northern zone limit. The Silurian Period is represented by volcanic rocks. Devonian rocks are known in northern parts of the OMZ from Portalegre to north of Cordoba. On the northern TPBCSZ, granite from the Cambrian and Ordovician has metamorphosed to gneiss. The base of the zone shows abundance of migmatites and metamorphosed sediments (de San José et al. 2004, Jesus et al. 2007).

This mineral composition of the ceramics is consistent with sediments of Paleozoic metamorphic terrenes with igneous intrusions found in the municipality of Beja (Ferreira et al. 2014). Still, similar sediments can be observed in a wider region of Ossa Morena Zone, in south-western Spain, respectively (de San José et al. 2004). Elemental analysis is needed to uncover exact chemical composition of the identified aplastic inclusion and characterization of feldspar, amphiboles and mica minerals.

Furthermore, it is important to note that the archaeological site of Monte da Comenda 3 is located besides the Guadiana river flow. Since the mineral grains observed in the ceramic fabrics are subangular with low sphericity, low degree of reworking and short transport distance from the source is probable. These observations indicate that the materials used for the pottery production could come from a local outcrop in general sense. Future investigation of the clay outcrops of the area is necessary to provide additional information to support the hypotheses of local production.

The study of the original contents of the vessel by GC/MS, reviled a residue of plant oils or animal fats. In the absence of specific biomarkers, like sterols (Pecci et al. 2016, Hjulström et al. 2009), or long chain alkyl compounds (Evershed et al.1991, Colombini et al. 2005, Cramp at al. 2011), discerning between animal or plant origin of the fat is based on the profile of the fatty acids present in the archaeological sample (Copley 2005, Colombini et al. 2009). Plant oil residues are characterized by higher amounts of palmitic acid when compared to stearic acid (C16:0 > C18:0), presence of unsaturated fatty acids or their degradation products, hydroxyacids and di-acids, presence of long chain esters originating from plant waxes or their saponification products, long chain alcohols and fatty acids (Eglinton et al. 1962, Evershed 2008, Colombini at al. 2009, Manhita et al. 2020). On the other hand, animal fat has prevalence of the stearic acid (C16:0:C18:0 ratio), and the presence of odd carbon-numbered, straight-chain fatty acids, specifically, pentadecylic acid (C15:0) and margaric acid (C17:0) (Eerkens 2005, Evershed, 2008, Evershed et al. 2002). However, it is necessary to keep in mind that the odd carbon long-chain fatty acids might also be a result of the microbial activity (Dudd et al. 1998). Since animal fat preserves better then plant oils, interpretation biases might occur. Furthermore, palmitic acid is more soluble in water than stearic acid, selective leaching might occur (Steele et al. 2010), complicating the interpretation of the fatty acid profiles and benefiting the interpretation towards degraded animal fat in the archaeological samples.

The absorbed residue from the analyzed vessel from Monte da Comenda 3, does have a minor prevalence of palmitic acid over stearic acid, as well as of monopalmitin over monostearin. Nonetheless, having in mind the degradation processes that might have influences the fatty acid ratio, presence of unsaturated octadecenoic fatty acid and detection of even number fatty alcohols, support the prospect of a plant oil. Finally, a possible presence of long chain fatty acids and plant sterol in the absence of cholesterol, does encourage the interpretation of the original contents of the vessel as plant oil.

These results imply purposeful exploitation of oil rich plants to obtain substantial amounts of oil necessary for the use in the burial ritual. The evidence of oil plants in the Neolithic/Calcholitc period if the western Mediterranean, are defined by cultivation of two species, flax (*Linum usitatissimum*) and opium poppy (*Papaver somniferum*), which marcoremains are often uncovered together (Peña-Chocarro 1999, Antolín et al. 2015, Antolín 2016, Pérez-Jordá et al. 2017). The oil could also have been extracted from wild plants, like wild poppy (*P. somniferum* ssp. *setigerum*), regarded as native to the western Mediterranean (Stika 2005, López Sáez and Guerra Doce 2006, Salavert et al. 2018, Bouby et al. 2020), and milk thistle (*Silybum marianum*), that might have been processed for oil since the early Neolithic on the Iberian Penisula (Antolín and Jacomet 2015). The use of wild olives (*Olea europapea* ssp. *oleaster*) for oil extraction should not be excluded, since harvesting of wild olive fruits is attested in the Neolithic context of the Iberian Peninsula (González Urquijo et al 2000). In the wider area of Southern Europe, gold-of-pleasure (*Camelina sativa*), woolly distaff thistle (*Carthamus lanatus*), turnip (*Brassica rapa*), and common hemp-nettle (*Galeopsis tetrahit*) might have been used as a source of oil (Jesus and Antolín 2022).

Since poppy seed oil has a higher heating value and a lower flesh point then linseed oil (Rahman et al. 2021), which is obtained from the seeds of the flax plant, with availability of both taxa in the Neolithic/Chalcolithic archaeobotanical record, poppy oil would be preferable as an illuminant source. However, there were no chemical evidence of combustion detected, and the vessel does not have traces of charring. Nevertheless, the vessel and the oil in it, might have been used as an illuminant with an organic wick, that would absorb the oil and once lit up burn outside of the vessel. The rest of unconsumed, unburned oil would penetrate into the pores of the vessel and preserve through the millennia enabling this analysis. Alternatively, oil from the vessel might have been used for anointing and treatment of the body during the burial ritual.

3.6. Conclusion

The available data allowed to identification of pottery as handmade production from the wider Beja area. Future studies could provide more information about the technology of Neolithic/Chalcolithic pottery production in the Alentejo region and allow the more accurate identification of the clay outcrops exploited for the ceramic manufacture. The analysis of the organic residue recovered from the vessel reveled to be a lipidic substance, most likely plant oil.

Regarding the treatment of the dead in the 4th and 3rd millennium BC the hypogeum burial of Monte da Comenda 3, shows changes in the votive component, namely introduction of ceramics, arrowheads, the replacement of the customary sheep phalanges by deer bones, and zoomorphic representations (Valera 2020). Even if the tradition of the collective burial continues in Monte da Comenda 3, association of objects to the specific individual is a connection to the ritual of individual burial that emerges in the subsequent period.

4. Manuscript 3: Recovering plant remains from the Etruscan funerary area of Cerveteri, Italy

This section was based on the Report for the Soprintendenza Archeologia Belle arti e Paesaggio per la provincia di Viterbo e per l'Etruria meridionale *(Fundurulic et al. 2020).*

SAPIENZA
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Report on plant remains from the excavation of Onde Marine, Cerveteri, Italy

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The Etruscan Necropolis of *Banditaccia*, dated to VII/VI c. BC, is located on the top of a hill north of Cerveteri. Under the supervision of the *Soprintendenza Archeologia Belle arti e Paesaggio per la provincia di Viterbo e per l'Etruria meridionale*, it is being studied using a multidisciplinary approach. Soil and residue samples (see Table 1) were delivered by Dr. Maria Gilda Benedettini to Dr. Alessandra Celant of the Department of Environmental Biology, Sapienza University of Rome, and will be included in the doctoral studies of Ana Fundurulic, co-tutored by Prof. Donatella Magri, Dr. Alessandra Celant and Prof. Cristina Barrocas Dias (HERCULES Laboratory, University of Évora, Portugal).

4.1. Introduction

The Etruscan Necropolis of Banditaccia, dated to 7th/6th century BC, is located on the top of a hill north of Cerveteri, about 35 km NW of Rome in Central Italy (Figure III.10). The site is a relevant testimony of the Etruscan civilization, which lasted in Northern and Central-Italy from the Iron Age to the first century BC when the Roman civilization assimilated its cultural traditions (Posth et al. 2021). The archaeological excavations started at the end of the 19th century, and the extensive excavations and restoration during the 20th century shaped the ancient landscape (Vagnarelli 2021).

Figure 0.10 (a) Location of the Banditaccia necropolis. (b) View at the contemporary surroundings and natural environment of the Cerveteri necropolises, Google Earth © 2022 image.

While the necropolis of the Banditaccia, is at least partly known, the necropolis of Monte Abatone, located to the southeast of the current urban area of Cerveteri, covering a larger area then that of Banditaccia, has not been through extensive restauration and the plateau is currently used for grazing and cultivation (Dix 2001). The Monte Abatone necropolis contains mostly chamber tombs, alongside tumuli, constructed from the 8th century B.C. to the early Roman Empire. The site has also been investigated since the 19th century and in the 1950s more than 600 graves were excavated during emergency excavations (Martin et al. 2021).

The geological substrate is composed of clays, slits and volcanic material, especially tuffs that were used during the building of the necropolis (Mattias and Ventriglia 1970). The surroundings are dominated by plains and hills, covered by volcanic deposits within the Tolfa-Cerveteri-Manziana volcanic complex and shaped by rivers during millennia (Pinarelli 1991). The characteristic red brown tuff, contains fragments of pumice, and, due to its softness and low permeability, is a workable material (Mattias and Ventriglia 1970). Large parts or the necropolises were carved in the tuff bedrock and covered by stone blocks. Today's landscape is mainly agricultural, characterized by the presence of annual crops, vineyards and olive groves, as well as some abandoned areas and patches of spontaneous vegetation (Cicinelli et al. 2020).

The Etruscan Necropolises of Cerveteri, along with Tarquinia, have been listed among UNESCO World Heritage Sites since 2004, and are characterized by the presence of many tumuli and grave chambers. Research in the area is still ongoing, under the supervision of *Soprintendenza Archeologia Belle arti e Paesaggio per la provincia di Viterbo e per l'Etruria meridionale*, with the necropolises being excavated and studied using multidisciplinary approaches.

4.2. Materials and methods

Soil samples (Table 3.4) from ceramic vessels recovered from the Onde Marine plateau from the Banditaccia and Monte Abatone necropolises were delivered by Dr. Maria Gilda Benedettini to Dr. Alessandra Celant of the Department of Environmental Biology, Sapienza University of Rome. Twelve samples were examined under a ZEISS Stemi 508 stereomicroscope for the identification of plant macroremains (seeds, fruits, wood, plant tissues) under 63-200× magnification. The samples were photographed to compare soil's general characteristics, and the plant remains were digitally measured. Five samples were selected for pollen analysis (n°1, n°3, n°4, n°8, n°11) and n°8 for the detection of phytoliths. The material was weighted, subsampled, and treated with 37% hydrochloric acid (37% HCl) and 10% sodium hydroxide (10% NaOH) following the standard method of preparation for palynological analysis (Moore and Webb 1987, Magri and Di Rita 2015). Sample n°8 was selected for phytolith analysis and the extraction followed modified Kooyman's method with 37% HCl (Kooyman et al. 2015). Identification of pollen taxa was done with a ZEISS Axiolab A1 microscope under 200×, 400× and 1000× magnification.

Sample n°8, on the basis of its visual characteristics, was selected for a chemical analysis. The sample was subjected to the ATR-FT-IR and Py-GC/MS, under conditions described under Chapter II,3.4. and II,3.6. respectively. Micro-X-ray diffraction (μ-XRD) for sample n°8 and X-ray powder diffraction (XRPD) for samples nº1, 3, 4, 9 and 11 were used to identify the mineral composition. Analysis was conducted using a Bruker™ D8 Discover® instrument equipped with a Cu Kα source and a LYNXEYE linear detector. All measurements were performed over a range from 3° to 75° 2θ, using a 0.05º increment with measuring time of 1 s/step. The identification of all crystalline phases was performed with the DIFFRAC.SUITE EVA® software using the Powder Diffraction File (PDF-2) X-ray patterns database of the International Centre for Diffraction Data.

Table 0.4 List of studied samples.

n°	Site	Vessel type	Soil sampled from the part of the vessel	Stratigraphic unit
1	Onde Marine, Banditaccia	pot	bottom	9/1
2	Onde Marine, Banditaccia	pot	middle	9/1
3	Onde Marine, Banditaccia	jug		9/2
4	Onde Marine, Banditaccia	pot	bottom	10
5	Onde Marine, Banditaccia	pot	middle	10
6	Onde Marine, Banditaccia	pot	middle	10
7	Onde Marine, Banditaccia	pot	middle	10
8	Onde Marine, Banditaccia	pot	bottom	36/1
9	Onde Marine, Banditaccia	lid	bottom	36/2
10	Onde Marine, Banditaccia	beaker	middle	14
11	Monte Abatone	pot		SP 4A
12	Monte Abatone	pot		SP 4A

4.3. Results and discussion

In general, the archaeological sediments from Onde Marine were sandy, yellowish to brown in color with visible sand grains and igneous lithoclasts (Figure III.11, nº1-10). The samples from Monte Abatone (n°11, 12), on the other hand, were more compact, darker, and coarser (Figure III.11, nº11-12). The only sample that stood out was sample n°8, from Onde Marine, that looked compact and homogeneous, with a reflecting surface. Since sediments were retrieved from the archaeological vessels it was suspected that they might contain organic remains that would indicate the original contents of the vessel, and their function in votive practices in the Etruscan burials.

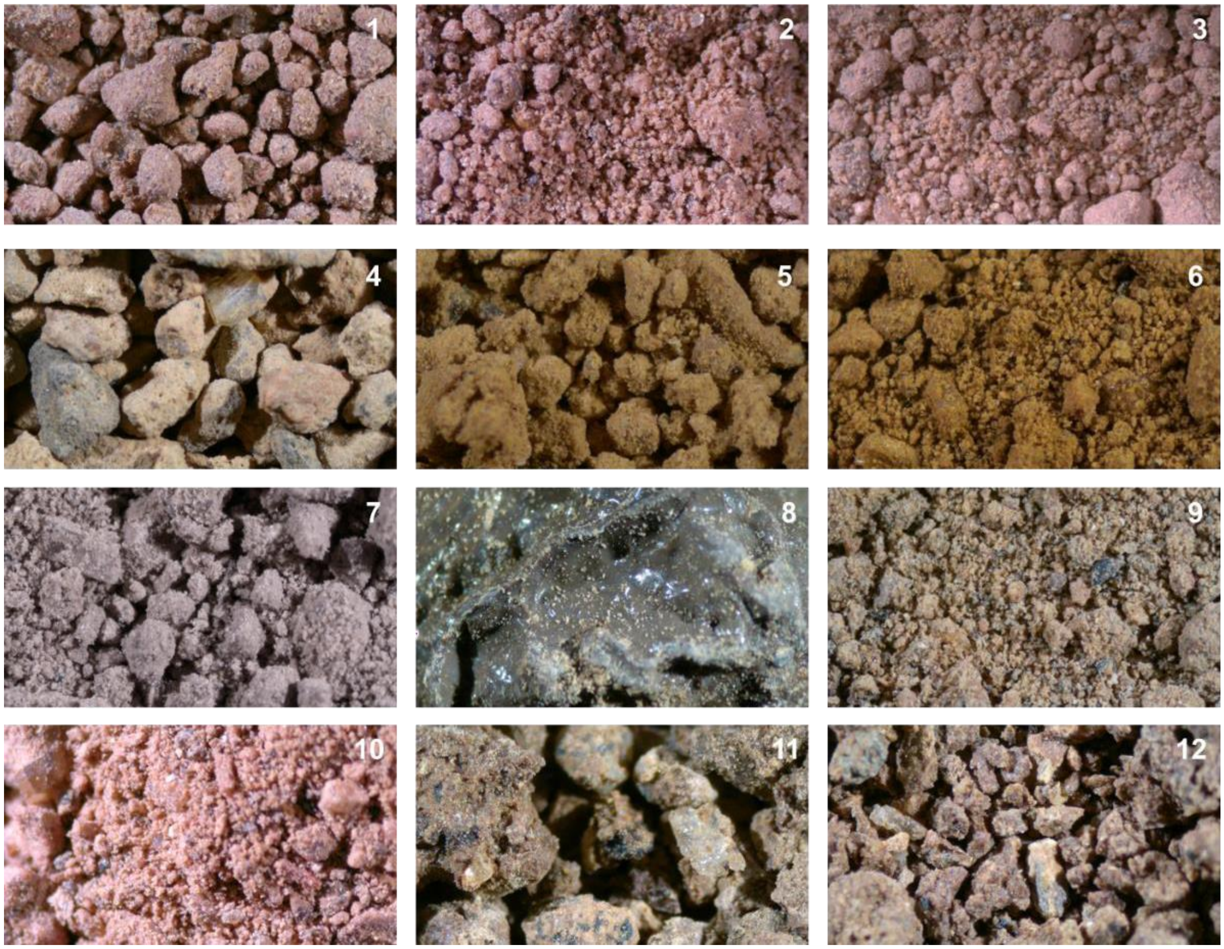

Figure 0.11 Soil samples under 100× magnification. Samples nº 1-10 from Onde Marine; samples nº 11, 12 from Monte Abatone.

Unfortunately, the soil samples were void of plant macro-remains, except for one seed of *Chenopodium album* L. detected in the sample n°4. The seed is dark, flattened round, disk shaped, 1.13 mm in diameter (Figure III.12). Since *C. album*, belonging to the Amaranthaceae family, is a common weed present in the environment, it does not hold a specific meaning for a burial ritual.

Samples had very poor pollen concentration, and despite the strong chemical treatment and 72h of hydrofluoric acid (40% HF), there is a huge mineral component that makes them highly sandy and hard to observe under the microscope, with the exception of n°8 and n°11. Samples n°3 and n°4 did not show any presence of pollen, probably due to lack of preservation in an oxidizing environment, which hinders any interpretation of the surrounding flora. In sample n°1, only four pollen grains were detected, including *Olea*, Cichorioideae, and *Cirsium* type (Figure III.13). A *Polypodium* spore was also detected. This result is too scarce to reach any conclusion, but all the taxa present can be related to human presence.

Figure 0.12 Seed from the soil sample n°4, *Chenopodium album* L.

In sample n°8 four pollen grains of cereals were detected (*Triticum*/*Hordeum*) (Kohler and Lange 1979). The same sample contained pine pollen (*Pinus* cfr *nigra*). Sample n°11 from Monte Abatone, as previously mentioned, was visually different from those of Onde Marine, since it was composed of darker, richer soil. The differences between the samples from the two sites is also evident in the pollen analysis: five taxa were detected in Monte Abatone, including Poaceae, Chenopodiaceae, Asteroideae, Cichorioideae and *Polygonum bistorta* type. This might reflect a surrounding anthropogenic grassland environment. *Polypodium* spore and *Pseudoschizaea* were also detected in sample n°11.
Phytoliths were detected in abundance in the sample n°8, and divided by shape into boxy, triangular, elongate, long saddle and wavy trapezoid (Figure III.14). They indicate a presence of grass (Poaceae) remains (Twiss et al. 1969, Rosen 2008), but plant remains of Fagacae and Rosaceae might also be present.

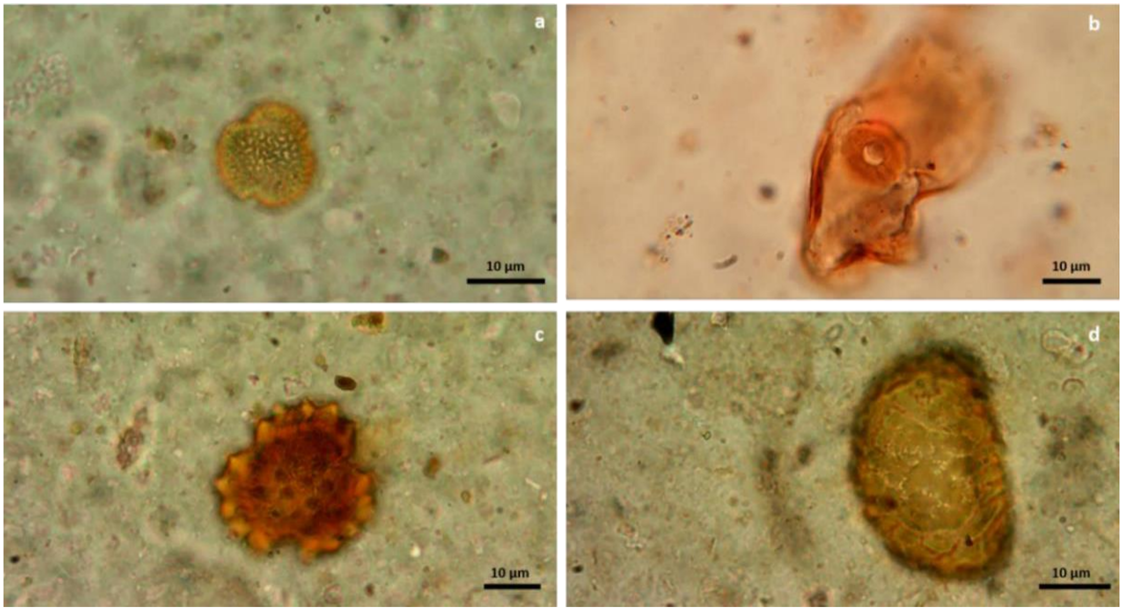

Figure 0.13 Pollen grains of a) *Olea* from sample n°1, b) Cerealia from sample n°8, c) *Cirsium* type and d) *Polypodium* from sample n°1.

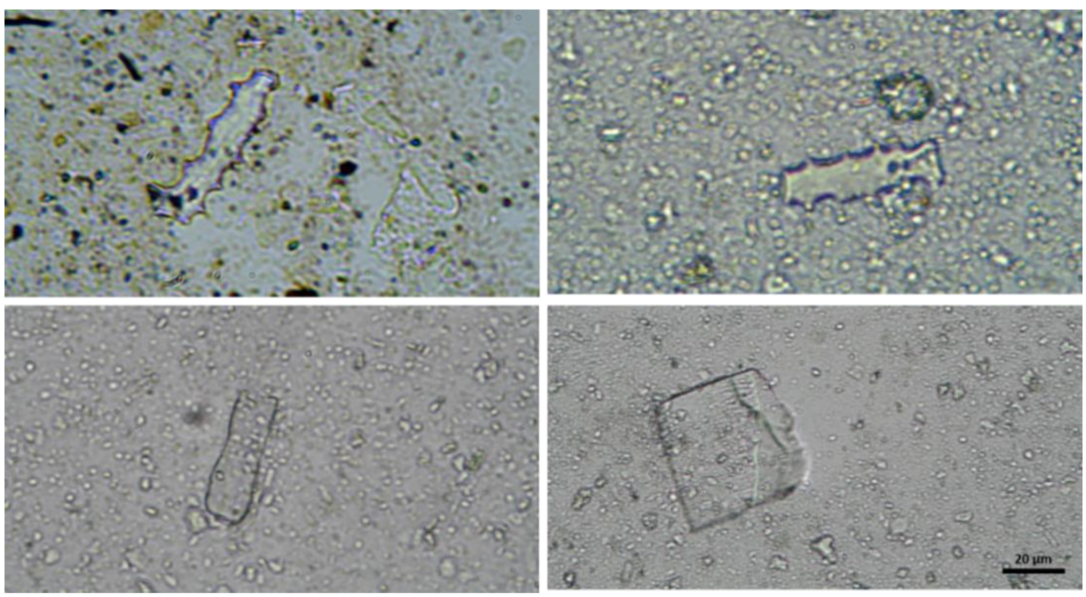

Figure 0.14 Some of the phytolith types present in the sample n°8.

ATR-FT-IR and the more sensitive Py-GC/MS did not provide conclusive information about the presence of the organic matter in sample nº8. The absence of the characteristic bands associated with C-H, C-O, C-C bonds points to the absence of organic matter in the sample analyzed. However, the wide prominent band at 1000 cm^{-1}, characteristic for asymmetric Si-O-Si stretching, together with a band at 908 cm^{-1} associated to Si-O stretching (Derrick et al. 2000), points to the sample being a silica-based inorganic material (Figure III.15).

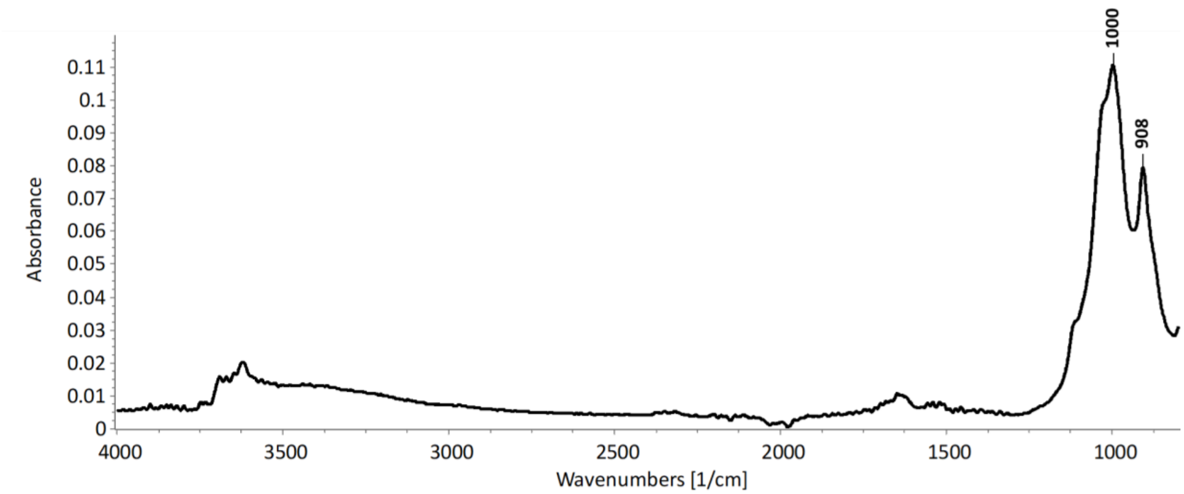

Figure 0.15 ATR-FT-IR spectra (4000–800 cm^{-1}) of soil sample n°8 with bands at 1000 cm^{-1} and at 908 cm^{-1}.

XRD enabled the identification of aluminosilicate phases in sample nº8, as well as in samples nº1, 3, 4, 9 and 11. As seen in the Table 2.2. quartz was detected in all samples along with clay/mica minerals and chabazite. Alkali feldspars or plagioclase were also identified in most samples (Table 3.5). The presence of chabazite in all samples analyzed is consistent with the local geology (Mattias and Ventriglia 1970, Washington 1897, Laurenzi Tabasso et al. 1990).

Differences in crystallinity values, calculated using the DIFFRAC.SUITE EVA ® software should also be noted. Sample n°8 showed lower crystallinity (50.3-54.0 %) compared to other soil samples from Onde Marine (ranging between 70.3% to 74.1%), while sample nº11 from Monte Abatone also displayed relatively low crystallinity (50.3%).

Table 0.5 Identification of the crystalline phases identified by XRD in the soil samples.

	Sample number					
Mineral phase	**1**	**3**	**4**	**8**	**9**	**11**
Quartz, SiO_2	X	X	X	X	X	X
Plagioclase						X
Alkali Feldspar	X	X	X			X
Clay/Mica	X	X	X	X	X	X
Chabazite, $Al_2Si_4O_{12}\cdot 6H_2O$	X	X	X	X	X	X

4.4. Conclusions

To summarize, samples from Onde Marine did not provide abundant preservation of plant remains. Only one seed was detected in n°4, and pollen was detected in samples n°1 and n°8, both showing presence anthropogenic (Cichorioideae, *Cirsium* type, *Polypodium* and *Polygonum bistorta* type) and cultivated species (*Olea* and cereals). The Monte Abatone sample had a higher pollen abundance, but also revealed evidence of domesticated or wild grasses. The suspected organic residue recovered from the vase (n°8) was analyzed for phytoliths, siliceous plant microremains, and showed good abundance, confirming vegetal presence in the dense matrix, but the chemical and mineralogical characterization, identified the sample as a clay deposit connected to local zeolitic tuffs and not an organic residue. However, since the pollen grains are highly resistant, due to their inert chemical structure (Li et al. 2019), and can be preserved even under suboptimal circumstances, provided some information about the environment. The poor preservation of other organic remains might be connected to the specific conditions of the burials and the soils of Cerveteri region (Costantini 2012, Kibblewhite et al. 2015). Obtained results do not clarify the substance of the offerings recovered in

Cerveteri burials, therefore, to uncover the possible absorbed organic content in those vessels, organic residue analysis would be necessary.

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Chapter IV – Plant remains reflecting everyday life: botanical and chemical studies from domestic context

1. Introduction

This chapter presents the archaeobotanical evidence from three occupational sites ranging from Prehistory to the Early Modern Period. Plant remains that were preserved reflect the glimpse into important part of everyday life – plant food selection, storage, and dietary choices. Archaeobotanical materials recovered from pottery vessels originating from now submerged Iron Age site of Gran Carro, located in Central Italy on the shore of Bolsena Lake gave an insight into the cereal consumption, crop selection and food preferences (Chapter IV, 2). Millet finds in a perilacustrine pile-dwelling, suggests that the cultivation of millets, complemented more productive crops of wheat and barley. The results were incorporated into ever-growing mosaic of knowledge concerning millet cultivation and dispersal in Southern Europe.

The results of the study of carpological remains found in silos of Medieval Lisbon attest to importance and incorporation of fruits, during the changing times, when Muslim and Cristian traditions in direct contact, encouraged further agricultural development (Chapter IV, 3). Cultivation of olives and grapes, rooted in Roman traditions, ascertained itself as a symbol of Iberian landscape. As confirmed by the new finds from Largo dos Lóios, plant sourced diet, based on cereals and fruits, in addition to legumes, was a relevant part of the full food consumption of the Medieval Portugal.

Lastly, organic remains that were preserved in an excellent condition due to tragedy that struck Lisbon in 1755, enclosing a fraction of a daily life forever in time. The Great Lisbon Earthquake damaged the residential area, including the house studied, located on the todays Praça de Dom Pedro IV (Rossio), and caused fires across the city, that enabled the preservation of organic matter due to carbonization (Chapter IV, 4). Archaeobotanical data reflected storage strategies of barely grains, most likely intended as an animal fodder. Chemical analysis of organic residue from a large cylindrical vessel, supported by the archaeological context allowed the interpretation as an accumulate human waste and provided information about dietary habits and hygienic practices in the 18th century urban setting.

It is obvious that all of these archaeological sites vastly differ not only in chronology but in geographical location. Still, agricultural practices, cultivation and use of natural resources, storage and food production stay an irreplaceable constant since Prehistory to Modern Period, exposing the extent and nature of human-plant interdependency. The recognition and detailed characterization of the setting from which remains were retrieved is crucial for a correct interpretation of plant use and human activities on the archaeological site. However, combined archaeobotanical and chemical approach can be applied to diverse domestic contexts, yielding interesting and stimulating lines of research, irrespective on the time period.

2. Manuscript 4: A contribution to the cereal consumption in the Central Italian during Iron Age – a new underwater archive from the Villanovan settlement of Gran Carro

This section is based on the article Fundurulic et al. (2022).

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Article

Millets and Cereal Meals from the Early Iron Age Underwater Settlement of "Gran Carro" (Bolsena Lake, Central Italy)

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Abstract: Archeobotanical materials recovered from pottery vessels originating from the underwater archeological site of "Gran Carro", located in Central Italy on the shore of Bolsena Lake, were analyzed to obtain new insight into the agricultural habits present in this Iron Age settlement. The archeobotanical study of cereal remains was combined with analytical data obtained from an amorphous organic residue using optical microscopy, SEM-EDS, ATR/FT-IR and Py-GC/MS. The cereal remains of emmer wheat (*Triticum dicoccum*), barley (*Hordeum vulgare*), broomcorn millet (*Panicum miliaceum*), and foxtail millet (*Setaria italica*) were identified as the preferred crops used for food and/or fodder at the site. The presence of charred millets, which have been directly dated by AMS, confirms consumption at the site and adds to the little-known background of millet use in central Italy. The find of millets in a perilacustrine pile-dwelling during a period when the water level of the Bolsena Lake was several meters lower than at present, attesting to a general dry period, suggests that the cultivation of millets, complementing more productive crops of wheat and barley, may have been favored by the availability of a large seasonally dry coastal plain, characterized by poor and sandy soils unsuitable for more demanding cereals.

Keywords: archeobotany; chemical analysis; villanovan pile-dwelling; Latium; foodstuff; organic residue; *Panicum*; *Setaria*

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2.1. Introduction

In Italy, the transition from the 2nd to the 1st millennium BC reflects cultural and technical changes, while at the same time is still strongly relying on the previously established traditions. Even though Iron Age brought new economic and political values, transforming the structure of the society, most of the commonly exploited food sources, including cereal crops, were introduced to the Italian Peninsula in earlier periods. However, food preferences reveal distinct regional variation with consumption trends that reflect different exchange and cultural networks. Societies of Central Italy, exploiting the fertile volcanic soils, depended on plant sources for multiple purposes, incorporating cereals as both food and animal fodder. To better understand crop selection and farming, storage, processing, and consumption choices, that reflect the inter-play between the environment and culture, a rich new archaeobotanical record from Central Italy, recovered from the Gran Carro underwater archaeological site, was compounded into the mosaic of existing knowledge. Archaeobotanical materials, recovered from pottery vessels, originating from the underwater archaeological site of Gran Carro, located in Central Italy on the shore of Bolsena Lake, were analyzed to obtain new insight into the agricultural habits present in this Iron Age settlement. The results were published in the Special Issue Archaeology of Sustainability and Sustainable Archaeology of Sustainability Journal (Fundurulic et al. 2022)

The archaeobotanical study of cereal remains was combined with analytical data obtained from an amorphous organic residue using optical microscopy, scanning electron microscope coupled with energy dispersive X-ray spectrometry (SEM-EDS), attenuated total reflectance/Fourier-transform infrared spectroscopy (ATR-FT-IR) and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). The cereal remains of emmer wheat (*Triticum dicoccum*), barley (*Hordeum vulgare*), broomcorn millet (*Panicum miliaceum*), and foxtail millet (*Setaria italica*) were identified as preferred crops used for food and/or fodder at the site. The presence of charred millets, which have been directly dated, confirms consumption at the site and adds to the little-known background of millets use in central Italy.

The finds of millets in a perilacustrine pile-dwelling during a period when the water level of the Bolsena Lake was several meters lower than at present, attesting to a general dry period, suggests that the cultivation of millets, complementing more productive crops of wheat and barley, may have been favored by the availability of a large seasonally-dry coastal plain, characterized by sandy soils unsuitable for more demanding cereals.

2.2. Archaeological context

The complex of the Gran Carro is a large site located on the eastern shallow flat shore at S. Antonio of the Bolsena Lake (42.591 N, 11.995 E), in the Lazio region of Central Italy (Figure IV.1). It was founded starting from the Middle Bronze Age (15^{th} century BC), but the most substantial remains are framed at the beginning of the first Iron Age between the 10^{th} and 9^{th} centuries BC, being distinctive for the Villanovan culture in Central Italy. Currently, the site is submerged and is considered to be the first underwater archaeological site in inland waters discovered in Central Italy. This makes Gran Carro unique, not only for abundance of materials found, but also for the excellent state of preservation of this archaeological site.

The Gran Carro settlement was discovered in 1959 by the mining engineer Alessandro Fioravanti and was excavated from the 1960s until the late 1980s in agreement with the Superintendence, also in collaboration with volunteer divers. The excavations mainly focused on the surface finds, but always strived to incorporate a multi-disciplinary approach and environmental information to obtain a well-rounded overview of the archaeological site (Tamburini 1995). After a hiatus, research in the area restarted in 2012, under the supervision of the *Soprintendenza Archeologia Belle arti e Paesaggio per la provincia di Viterbo e per l'Etruria meridionale* in collaboration with underwater archaeologists and specialists merged today in the *Centro Ricerche Archeologia Subacquea*. Recently, it was possible the ascertain that the Gran Carro complex is divided into several functionally distinct sectors (Figure IV.1c) (Barbaro and Severi 2020, Barbaro 2022). The material analyzed was excavated from the currently submerged area, occupied by the remains of pile-dwelling structures mainly attributable to the Early Iron Age (late 10th-9th century BC) built at the time on lands, emerged on the edge of the lake. Adjacent is the so-called Aiola, an elliptical structure formed by unworked stones, first investigated in 2021 and preliminarily interpreted as an open-air ritual place dating from at least the Late Bronze Age (11th century BC), where rites were held that included the lighting of fires in the upper part and the contributing food offerings in pots in the lateral parts. The third area identified is on land near the current shore, in topographical continuity with respect to the submerged part, where materials from the beginning of the Middle Bronze Age (15th century BC) were found.

The remnants of the Iron Age settlement are found on a low flat lakebed that slopes gradually down to a depth of 7.5 m, around 100 meters from the current coastline, indicating that the settlement was in the middle of a broad coastal plain, much larger than the current one (Figure IV.1). Two other extensive underwater settlements coeval to Gran Carro are known from the shores of Bolsena Lake (Barbaro and Severi 2020, Barbaro 2022), documenting the availability of now submerged land around the lake.

The settlement area of pile-dwelling, with stilt houses, is remarkable for its state of conservation, with more than 450 preserved wooden poles arranged in parallel bands set three meters apart, with structures oriented NE/SW, comparable to pile-dwelling settlements in Northern Italy. At the time of its occupation, in the Early Iron Age, this part of the settlement was located in the middle of a vast coastal plain, exploiting the various resources of the lake system and functioning as a link between the coast and the hinterland in the resource exchange system. Regionally, it is a part of the Early Iron Age Bolsena Lake network, that includes settlements and necropolis on, and connected to, the waterfront (Barbaro and Severi 2020).

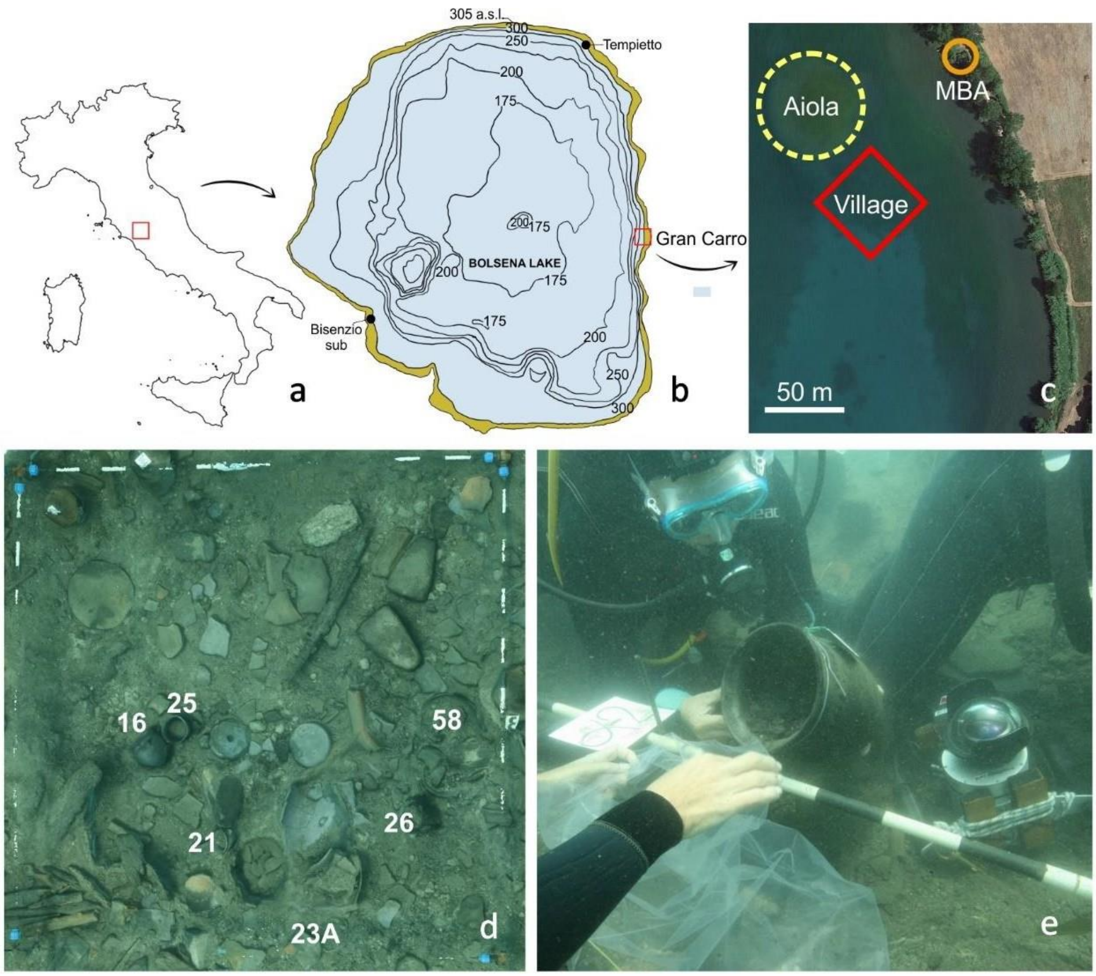


Figure 0.1 Location and underwater photographs of the Gran Carro pile-dwelling: (a) Location of Bolsena Lake; (b) Bathymetry (meters a.s.l.) of Bolsena Lake (in brown, the area emerged at the time of the Gran Carro settlement); (c) Google Earth © 2019 image of the underwater Gran Carro excavation area; (d) Planimetry of the excavation (grid 2 × 2 m); (e) Underwater excavation uncovering Iron Age materials (Vessel 38) (from Fundurulic et al. 2022).

The rise of lake water levels influenced, in part, the gradual displacement of the houses to the higher inland levels, and eventually led to the settlement's abandonment. In the lifecycle of the pile-dwelling, at least five phases of levelling have been recorded, alternating with stages of fire, amounting up to 160 cm in depth of cultural levels. Among the submerged heritage, in addition to wooden structures, other settlement remains were discovered including parts of walls and roofing, bronze objects, remains of ceramic hearths, ceramic remains of looms, wooden artifacts, faunal remains, as well ceramic vessels and pots that contained remains of stored cereals (Barbaro and Severi 2020).

Despite the importance of this underwater archaeological site, which has the potential to provide new significant information on the subsistence economy of the Iron Age communities of Central Italy, only two preliminary papers on carpological remains have been published in the sixty years after the site was discovered (excavation 1974 (Costantini and Costantini Biasini 1995) and excavation 1980 (Hopf 1995)). The newly recovered vegetal contents of ceramic vessels from the domestic area of the pile-dwelling are studied through archaeobotanical and chemical analyses. Even though these vessels were recovered from underwater conditions, their contents show no evidence of having been reworked (Figure IV.1d). The selected vessels were found in domestic contexts in locations abandoned after fires and soon after preserved in waterlogged condition. Thus, the state of preservation of the organic content of the vessels is very favorable to attest to the local consumption of food in everyday life. Moreover, the study of these vessels may, indirectly, provide new information on the agricultural practices of this ancient community during a period when the lake level was much lower than at present, being a testament for environmental and climate conditions different from present.

2.3. Materials and Methods

The material analyzed in this work has been recovered during the excavation campaigns of 2016-2017 directed by Dr. Patrizia Petitti. The plant material collected was entrusted by Dr. Barbara Barbaro, who has been directing excavations since 2019, to the Laboratory of Palaeobotany and Palynology, Department of Environmental Biology, Sapienza University of Rome. The analysis was carried out exclusively on the plant remains recovered from inside ceramic vessels. Traces of burning were observed on the vessels probably as a result of settlement fires after the pots have already been discarded. Cereal remains recovered from eight vessels were studied, along with a food residue recovered from Vessel 26 (Table IV.1). The vessels containing cereals include different forms – open conical bowls, beakers with one handle, rounded bowls with handles, and bigger storage containers. All the vessels that had cereals are typologically attributed to the end of the 10th century BC until the 9th century BC, belonging to the Early Iron Age 1A, representative of the Villanovan culture in the Lazio region.

Table 0.1 List of vessels containing the cereal remains that were analyzed. Scale bar = 5 cm (from Fundurulic et al. 2022).

Number	Photograph	Form
Vessel 16		globular "olletta" with four protrusions
Vessel 21		small, rounded bowl with one perforated handle, on raised leg

Table IV.1 (cont.) List of vessels containing the cereal remains that were analyzed. Scale bar = 5 cm (from Fundurulic et al. 2022).

Number	Photograph	Form
Vessel 23A		conical bowl on four legs
Vessel 23B		biconical jar with encrusted geometric decorations
Vessel 25		beaker with one handle
Vessel 26		small pot with two handles and impressed decoration
Vessel 38		ovoid vessel with corded decoration
Vessel 42		beaker with one ribbon handle

Table IV.1 (cont.) List of vessels containing the cereal remains that were analyzed. Scale bar = 5 cm (from Fundurulic et al. 2022).

Number	Photograph	Form
Vessel 58		conical bowl on four legs

After the sampling of vessel contents in an underwater environment, plant remains were separated by wet-sieving and kept in waterlogged conditions prior to and post analysis (Celant et al. 2015). A subsample of organic sediment from each vessel was analyzed, totaling 3.3 dm^3 wet weight of material. The "water separation" technique (Struever 1968, Jacomet and Kreuz 1999, Persall 2016) was adopted in order to separate cereal remains from the rest of the clayish and organic sediment, using 2.0 mm and 0.5 mm mesh diameter sieves. Charred remains of cereal spikelets and entire caryopses were picked from the first sieve, as they were clearly visible and distinct from the remaining sediment. A stereomicroscope (Zeiss Stemi 508, Carl Zeiss) was used to pick the cereal fragments isolated during the second sieving operation, to carry out morpho-biometric analyses and to take photographs of the macroremains. The reference collection of Sapienza University of Rome and carpological atlases (Jacquat 1988, Jacomet 2006, Neef et al. 2012) were used for the identification of the cereal grains. Pollen analysis of the organic residue of Vessel 26 was carried out following the standard chemical treatment described in Chapter II,4.2.1 with HCl (37%), HF (40%) and NaOH (10%), and stored in glycerol (Magri and Di Rita 2015).

The content of Vessel 38 was dated by Accelerator Mass Spectrometry (AMS) and calibrated using OxCal v3.10 software (Bronk Ramsey 2009) and the IntCal 20 curve (Reimer 2020) at the Centro di Datazione e Diagnostica (CEDAD) of the University of

Salento.

Vessel 26 contained an amorphous residue, visually distinct from the lacustrine sediment infilling the vessel, subsampled by the archaeologists immediately after the recovery and kept at ≤ 0 °C to preserve organic material if present. Since the material seemed homogenous, chemical analysis was conducted to identify the natural source of the residue and examine the possibility of it being a cereal-based product. It was examined and photographed under a stereoscopic microscope, while a scanning electron microscope coupled with energy dispersive X-ray spectrometry (SEM-EDS) was used to obtain high-resolution images and elemental analysis. Attenuated total reflectance/Fourier-transform infrared spectroscopy (ATR-FT-IR) and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) were employed for the chemical characterization of the amorphous material. Variable pressure SEM-EDS analysis was carried out using a Hitachi S3700N SEM coupled to a Bruker XFlash 5010 SDD EDS Detector. The analysis was done using a low vacuum of 40 Pa and an accelerating voltage of 20 kV. FT-IR spectra were obtained using a Bruker ALPHA spectrometer equipped with a universal ATR attachment. Spectra were acquired over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}, and 128 accumulated scans were coadded to produce a spectrum. The instrument was controlled by the Bruker OPUS software. Spectra were normalized and averaged using the SpectraGryph software (version 1.2.14). For Py-GC/MS analysis, four micro-samples were collected and derivatized with 3 μL of tetramethylammonium hydroxide (TMAH, 2.5% (v/v) in methanol) in a 50-μL Eco-cup capsule. The samples were pyrolyzed using a single-shot method at 500 °C. Analysis was performed with a Frontier Lab PY-3030D single-shot pyrolyzer, coupled to a Shimadzu GC2010 gas chromatographer and a Shimadzu GCMS-QP2010 Plus mass spectrometer. A capillary column Phenomenex Zebron-ZB-5HT was used for separation, with helium as carrier gas, adjusted to a flow rate of 1.50 mL min^{-1}. The split/splitless injector was operated at a temperature of 250 °C in the splitless mode. Gas chromatography temperature program started at 35 °C for 1 min, ramped at 60 °C min^{-1} until 110 °C, then to 240 °C at 14 °C min^{-1}, and finally increased to 280°C at 6 °C min^{-1}, at which point it was held for 10 min. Source temperature was placed at 240 °C, and the interface temperature was maintained at 280 °C. The mass spectrometer was programmed to acquire data between 40 and 850 m/z. Compound identification was performed using AMDIS software integrated with NIST-Wiley database. The obtained results were compared to modern reference samples of hand ground cereals, flour, cereal paste and baked cereal product of *Triticum* sp., *Hordeum vulgare* and *Panicum miliaceum*. To identify a possible botanical origin a comparison was made with reference samples based on retention time and m/z values, as well as available published data (Heron 2016, Bordiga 2016, Colonese 2017, Hammann and Cramp 2018). Selected Ion Monitoring (SIM) was employed on specific targeted compounds (m/z 189, m/z 204, m/z 231, m/z 425 and m/z 440 reported for miliacin (Heron 2016); m/z 268 reported for alkylresorcinols (Colonese 2017, Hammann and Cramp 2018).

2.4. Results

Analyzed material yielded cereal carpological remains found in eight distinct vessels (Table 4.2). Emmer (*Triticum dicoccum*) was discovered unthreshed, with a total of 19 caryopses, 15 intact spikelets, and spikelet forks detected in six vessels. Eleven grains of hulled barley (*Hordeum vulgare*) were identified in three vessels, as well as three spikelet forks. In six vessels, a total of 14 millet (*Panicum miliaceum*) caryopses were found in addition to a charred lump of ears (Figure IV.2a). *Setaria italica* is present in all the eight vessels.

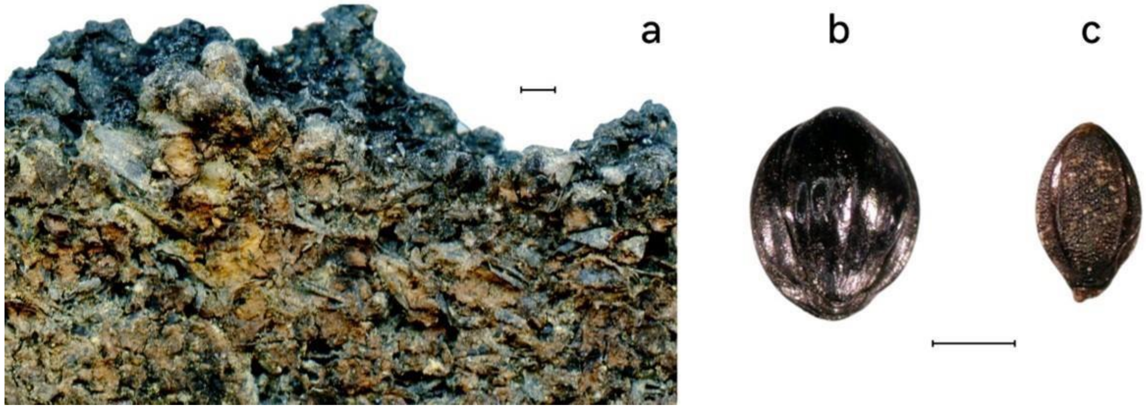


Figure 0.2 Carpological remains from the Gran Carro settlement: (a) agglomeration of *Panicum miliaceum* from the bottom of Vessel 38; (b) caryopsis with lemma and palea of *Panicum miliaceum* (Vessel 21); (c) caryopsis with lemma and palea of *Setaria italica* (Vessel 42). Scale bar = 1 mm (from Fundurulic et al. 2022).

Cultivated cereals are concurrent in most of the vessels, without separation. Two vessels (16 and 58) contained the four cereal taxa. Vessel 23A contained only caryopses of *Panicum* and *Setaria*. Vessel 38 contained aggregated carbonized ears of *Panicum* (Figure IV.2a), shaped like the bottom of the container from which it was recovered. Charred cereal remains are fused together forming an agglomeration, but identifiable fragments, between 0.5 and 1 mm in size, are still clearly visible and distinct. This charred lump of millet has been directly radiocarbon dated between 1195 and 899 cal BC (2 σ), which is within the timeframe of the occupation of the site.

Furthermore, Vessel 26 contained visually distinct amorphous residue. The remains examined under SEM show a fine layered structure consisting of thin sheets and voids (Figure IV.3a,b). The diameter of voids spans from 15 μm to 50 μm. The analytical study of microremains did not reveal identifiable plant tissues or pollen grains. Since it was not certain that the foodstuff was cereal-based, it was necessary to conduct chemical residue analysis.

Table 0.2 List and abundance of identified cereal carpological remains (from Fundurulic et al. 2022).

	Sediment	Type	*Triticum dicoccum*	*Hordeum vulgare*	*Panicum miliaceum*	*Setaria italica*
Vessel 16	0.3 dm³	caryopsis	4	1	7	1
		spikelet	4	-	-	-
Vessel 21	0.13 dm³	caryopsis	3	-	1	4
		spikelet	2	-	-	-
Vessel 23A	0.45 dm³	caryopsis	-	-	4	4
Vessel 23B	0.5 dm³	caryopsis	9	-	-	1
		spikelet	4	-	-	-
Vessel 25	0.5 dm³	caryopsis	1	-	1	2
		spikelet	1	-	-	-
Vessel 26			organic residue			
Vessel 38	0.45 dm³	caryopsis	-	6	agglomeration	4
		Fork	-	2	-	-
Vessel 42	0.4 dm³	caryopsis				9
		spikelet	4	-	-	
		Fork	1	-	-	-
Vessel 58	0.6 dm³	caryopsis	2	4	1	8
		Fork	-	1	-	-
Total number of remains			35	14	14	33

Elemental mapping and point analysis performed by SEM-EDS revealed a carbon-rich matrix, covered in fine-grained arsenic and sulfur-rich particles (Figure IV.3c, d). These particles are also visible under optical microscopy presenting a yellow hue. Given their composition, they are likely the As-sulfide orpiment (As_2S_3), which is known to precipitate through the reaction of aqueous As (III) (H_3AsO_3) and sulfide (H_2S or HS) and is commonly found in low-temperature hydrothermal veins, hot springs and fumaroles (Godelitsas et al. 2015). The high levels of arsenic detected in the lakes of the Viterbo area (Barbieri et al. 2014) and the hydrothermal activity which still occurs in the Bolsena Lake (Lindhorst et al. 2017) can, therefore, explain the formation of As-sulfide precipitates present on the surface of the amorphous residue in Vessel 26.

Initial ATR-FT-IR analysis of the amorphous residue in Vessel 26 confirmed the organic origin of the substance (Figure 4.4a). The absorbance spectra present a characteristic profile for protein-based material (Barth 2007). The wide band around 3300 cm^{-1} is attributed to the O–H stretching of water molecules, overlapping with the N-H stretching. The typical protein bands at 1636, 1542 and 1458 cm^{-1} that can be observed in the spectra are associated with the Amide I, Amide II and Amide III region and arise from C=O stretching, C–N stretching and NH bending. The shape and the maximum of Amide I band at 1636 cm^{-1} could reflect interactions among amide peptide bonds and indicate lower amounts of α-helix structures, which absorb light at 1655–1650 cm^{-1}, and higher content of β-sheets that absorb light 1640–1620 cm^{-1} (van Velzen et al. 2003). The characteristic bands at 1007 and 905 cm^{-1} are associated with the C–O and C–C stretching vibrations that might be assigned to starch molecules (van Velzen et al. 2003, Robertson et al. 2006). Comparison with modern reference samples indicates that the archaeological substance is closest to a simple cereal paste composed of a mixture of finely grounded cereals and water (Figure 4.4b).

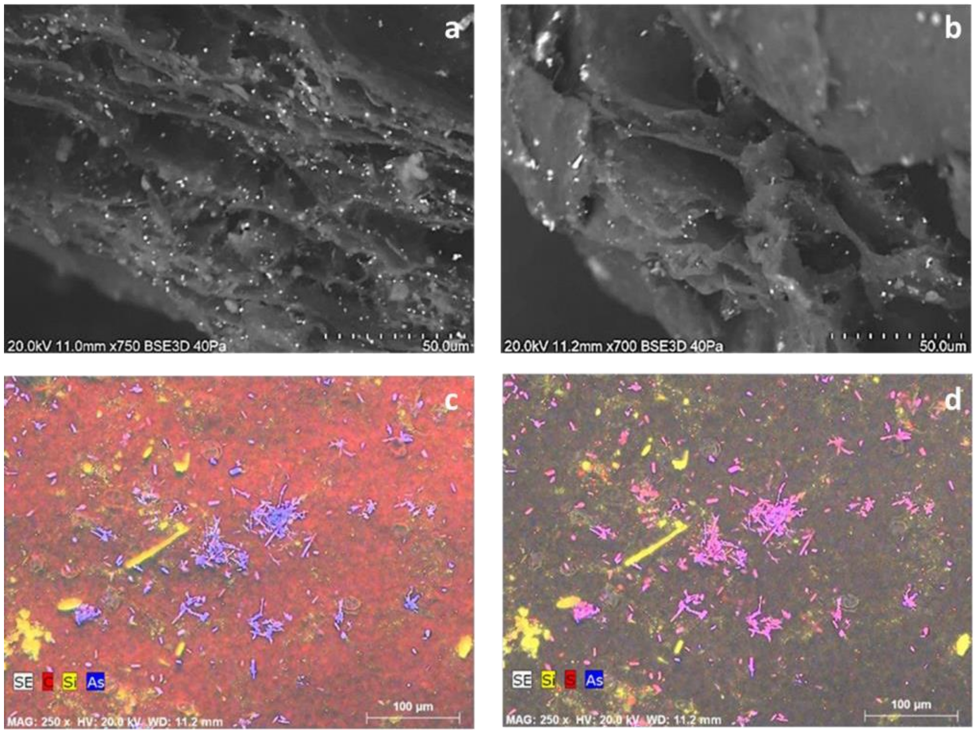


Figure 0.3 (a-b) Microphotographs of the food remains from the Vessel 26 under SEM with a fine layered structure and micro-voids (working distance for SEM images ca. 11 mm); bright particles seen on images are enriched in arsenic and sulfur. (c-d) Elemental mapping of carbon-rich matrix with fine-grained arsenic- and sulfur-rich particles (from Fundurulic et al. 2022).

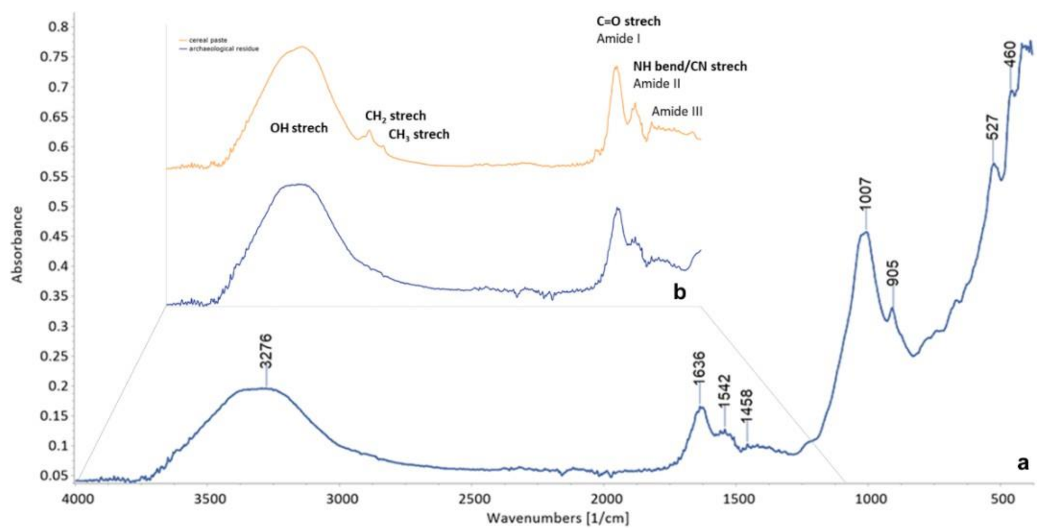


Figure 0.4 (a) ATR-FT-IR spectra (4000-400 cm^{-1}) of an archaeological sample from the Vessel 26; (b) Comparative ATR-FT-IR spectra (4000-1200 cm^{-1}) of an archaeological sample and modern reference of a cereal paste (from Fundurulic et al. 2022).

Table 0.3 List of identified compounds of the organic residue from Vessel 26 by Py-GC/MS (from Fundurulic et al. 2022).

Compounds	RT (min)	Compounds	RT (min)
PROTEIN MARKERS		**CELLULOSE MARKERS**	
1H-Pyrrole, 1-methyl-	2.6508	Cyclopentanone	2.9817
Pyridine	2.7017	Furfural	3.2517
Pyrrole	2.7408	Benzene, 1,3-dimethyl-	3.5175
Toluene	2.8358	2-Cyclopenten-1-one, 2-methyl-	3.7592
1H-Pyrrole, 3-methyl-	3.3367	2(5H)-Furanone	3.8092
Acetamide, N,N-dimethyl-	3.5083	Cyclohexanone	3.8792
Phenol	4.2442	2-Furancarboxaldehyde, 5-methyl-	4.1525
Benzene, 1-methoxy-4-methyl-	4.6083	1,2-Cyclopentanedione, 3-methyl-	4.6608
Phenol, 2-methyl-	4.8325	Maltol	5.3933
Phenol, 3-methyl-	4.9817	Levoglucosan	8.7942
2,5-Pyrrolidinedione, 1-methyl-	5.1400		
Phenol, 2,5-dimethyl-	5.6325	**LIPIDIC COMPOUNDS**	
Phenol, 4-ethyl-	5.7708	Hexadecanoic acid, methyl ester	12.1583
Benzofuran, 2,3-dihydro-	6.2350	Octadecanoic acid, methyl ester	13.8642
1-Methylindole	6.9142	Eicosanoic acid, methyl ester	15.7850
Indole	7.0800	Docosanoic acid, methyl ester	17.8650
L-Proline, 5-oxo-, methyl ester	7.7017	Tetracosanoic acid, methyl ester	19.4967
1H-Isoindole-1,3(2H)-dione, 2-methyl-	8.2900	Hexacosanoic acid, methyl ester	20.7467
L-Proline, 1-methyl-5-oxo-, methyl ester	9.5817	Octacosanoic acid, methyl ester	22.2333
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	11.1192		
9H-Pyrido[3,4-b]indole, 1-methyl-	12.9533	**STEROLS**	
9H-Pyrido[3,4-b]indole	13.0367	Sitosterol	24.9225

Py-GC/MS analysis revealed that the organic residue was a predominantly protein-based substance, characterized by the presence of nitrogen-containing components including N-heterocyclic compounds, pyrroles, pyridines, nitriles, and amines/amides, likely deriving from a plant source. The most abundant molecules detected in the sample are amino acids. Methyl pyroglutamate and methyl ester of L-Proline, 1-methyl-5-oxo, characteristic for the pyrolysis of proline and glutamic acid (Gallois et al. 2007), were identified along with other protein biomarkers (Table 4.3). The high content of proline and glutamic acid is typical for the amino acid composition of gluten, a cereal protein (Woychik et al. 1961, Wieser 2007, Rombouts et al. 2009). Furthermore, cellulose biomarkers, pyrans and furans were also detected (Wang et al. 2012). These compounds, including levoglucosan, maltol and 5-methylfurfural, are abundant pyrolysis products of cereal grains (Galletti et al. 1998, del Río et al. 2013). The prevalent fatty acid is palmitic acid, followed by stearic acid. The ratio between C16:0 and C18:0 is between 1.48 and 2.00, pointing to plant source material. The very long chain fatty acids, docosanoic (behenic) and tetracosanoic (lignoceric) acid, together with sitosterol, along with the absence of cholesterol, confirm the presence of plant material (del Río et al. 2013).

2.5. Discussion

2.5.5. Cereal meals

Cereal food products include cereal fragments, agglomerations and amorphous masses that originated from food preparation procedures (Valamoti et al. 2019). Processing of cereals involves intentional removal or breaking down of the plant tissues in the grains to varying degrees, which leads to improved palatability and digestibility. This includes actions such as crushing/grinding/milling, sieving, soaking, boiling, fermenting, or baking/roasting (Heiss et al. 2017). These products have been classified based on the processing of cereals and the final product as: ground cereals used as such, ground pre-cooked cereals, ground malt and malt products, porridge and bread remains (Valamoti et al. 2019). The agglomeration of *Panicum miliaceum* caryopses, found at the bottom of Vessel 38, could have been the product of the fusing of grain fragments due to charring conditions, since small grains tend to aggregate, or the result of intentional partial processing that was charred post-deposition. Grain agglomerations have at times been interpreted as porridge, a fine meal with coarse inclusions or a coarse meal, generated through charring, but it has been pointed out that, based on morphological features it is not possible to differentiate between accidentally formed lumps and prepared meals (Valamoti et al. 2019). Since Vessel 38 likely served as a storage container, based on its size and shape, it is more like that these joined remains result from the intentional and selective gathering of millet grains. Cereals may have been stored unprocessed to make the grains more resistant to insect and fungal attacks (Sigaut 1988).

At Roca in Southern Italy, a lump of broomcorn millet with glumes, retrieved together with other cereal grains from the sediments of Recent Bronze Age levels, was intentionally charred as part of ritual offerings (Guglielmino and Primavera 2021). The similar assemblage of unprocessed charred millet at Gran Carro might suggest a ritual meaning, also considering the vicinity of the settlement to the ritual structure of the Aiola.

For the amorphous remains, as those recovered from Vessel 26, the terms "cereal preparation" or "cereal product" have been suggested (Heiss et al. 2017). Intensive studies (Valamoti et al. 2019, Heiss et al. 2017, González Carretero et al. 2017, Primavera et al. 2019, Arranz-Otaegui et al. 2018) have been employed to identify and characterize cereal-based products relying on macroscopic/microscopic structure analysis and an archaeobotanical approach, offering new insights into food preparation practices. However, when the structure analysis for uncharred material is inconclusive other methods of investigation become necessary.

Chemical analyses of amorphous food products provide an insight into the composition of substances that have been altered by degradation processes but were preserved due to specific environmental conditions. At Gran Carro, a dual mode of preservation occurs, carbonization and waterlogging. Carbonized cereal remains were fossilized through charring under oxygen-poor conditions, irreversibly altering the structure and the chemical composition of the remains, therefore limiting the results of potential chemical analyses (Hillman et al. 1993). On the other hand, the amorphous food residue in Vessel 26 was preserved by waterlogging, due to anaerobic conditions and still water.

In waterlogged conditions, organic materials preserve as a result of the abundance of static water and the chemical balance of this water's composition, pH and oxidation-reduction potential. Water that excludes air, creates reduced oxygen levels and prevents most microorganisms from thriving. Since organic materials get saturated with water, their form is mostly retained. In an anoxic environment, however, some breakdown might occur due to anaerobic microorganisms such as sulfur-reducing bacteria (Fors and Sandström 2006). Altering stable conditions and exposure to air, by removing the material from its original archaeological context, can cause further deterioration of organic remains.

Preservation of cereals and cereal foods, composed of polysaccharides (starch, cellulose, hemicellulose) and lignin, cereal protein and fats, is affected by their molecular structure. For example, long-chain lipids are more resistant than carbohydrates and proteins as they are insoluble and less vulnerable to water leaching and biodegradation. However, starch decomposed preferentially over cellulose, while cellulose is more easily broken down than lignin and other polyphenols. Degradation of glucose-based products such as starch and cellulose, leads to breaking of the carbohydrate chain, resulting in smaller saccharides more prone to hydrolysis and removal from the residue. A good example is waterlogged wood, where sometimes there is almost no cellulose remaining in the wood and the material can be identified through lignin content (Gelbrich et al. 2008, Colombini et al. 2009). Preservation of fats and protein has been reported in carbonized cereal remains even after thermolysis (Hansson and Isaksson 1994), while in waterlogged conditions, lipids and proteinaceous components can be reasonably well preserved (Evershed 1992, Nielsen et al.2021). The decay of archaeological materials is caused by a complicated interplay between the environment and the matter itself. The key to good conservation of cereals, as shown at Gran Carro, are stable environmental conditions with limited oxygen access and water circulation. This allowed preservation of not only carpological but also of amorphous food remains.

In this case, chemical analysis confirmed the organic origin of the amorphous residue and allowed its identification as a substance based on carbohydrates and protein. Elemental analysis showed a carbon-rich matrix, and a presence of arsenic particles. Since arsenic has an affinity to bind to proteins (Shen 2013), its presence in the lake environment affected the organic protein-rich archaeological sample. Analysis of the IR spectra demonstrated absorption bands that can be mostly assigned to starch, water, and proteins, while the comparison with modern reference samples indicated that the archaeological material is similar to a simple cereal paste composed of a mixture of grounded cereals and water. The spectra results have certainly been affected by waterlogged conditions and the aging of the material (Turksoy et al. 2021), but band assignments could indicate β-sheet content that has been associated with working and processing of gluten protein products (van Velzen et al. 2003, Robertson et al. 2006, Garcia-Valle et al. 2021) forming a melded and palatable meal. Grinded cereals when mixed with water exhibit viscoelasticity that is increased by working, attributed to interactions between aligned β-sheets structures of gluten. During mixing, disulfide bonds break and increase the opportunity for all the gluten proteins to interact and re-structure, resulting in decrease of α-helices and β-turns, and an increase in β-sheets (Robertson et al. 2006).

Even though specific biomarkers do not allow the identification of cereal species, the chemical profile, which is in agreement with reference samples, as well as the abundance of other cereals at the site, support the processing and use of cereals at Gran Carro. The challenge of identifying among cereals in archaeological context through organic residue analysis, lays in the relatively low content of chemically stable compounds, susceptibility to degradation, and lack of distinguishable biomarkers (except for specific cases like miliacin for millet). Still, advancements have been made showing positive results. even on archaeological material (Colonese 2017, Hammann and Cramp 2018), focusing on the presence and ratios of alkylresorcinols. These are compounds that consist of an odd-numbered alkyl chain (C15 to C25), have been reported in fresh samples of wheat and rye at higher levels, and in low amounts in barley, millet, and maize (Ross et al. 2003). However, the analyzed residue from Vessel 26, did not show preservation of alkylresorcinols. Since these compounds are mainly found in the outer layers of cereal grains (Landberg et al. 2008), and are susceptible to decay and affected by processing, they are not detectable in cereal flour and cereal products (Ross et al. 2003).

The possibility of a C4 plant source or a mixture of a C3 and C4 plant in the residue from Vessel 26 was considered. However, targeted GC/MS analysis did not present a biomarker for broomcorn millet, miliacin. Miliacin, the principal pentacyclic triterpene methyl ether, is present in only a small number of the C4-grasses of the Panicoideae subfamily. Selected Ion Monitoring (SIM), that allows the mass spectrometer to detect specific compounds with very high sensitivity, using specific ions (m/z 189, m/z 204, m/z 231, m/z 425, m/z 440) reported for miliacin (Heron 2016), and detected in contemporary millet reference samples, did not demonstrate its presence in the archaeological sample.

As such, the residue from the Vessel 26 could be interpreted as cereal preparation or porridge remains, and the cereals present at the site imply the use of emmer, barley or millet as the botanical origin of this foodstuff, even if those could not be confirmed by chemical analysis.

2.5.6. Millets: Food choices, environmental and socio-cultural dynamics

The representation of emmer wheat and barley recovered from the site is not surprising since both were prevalent, and a staple of plant-based human nutrition in Italy during Late Bronze Age and Iron Age (Costantini 2002, Fiorentino et al. 2004). On the other hand, millets are scarcely found, and higher representation of this cereal from Gran Carro site is noteworthy. Broomcorn millet may have arrived in the Italian Peninsula via Northern Italy, but the exact path and timing of its arrival are unknown (Tafuri et al. 2018). Neolithic finds are uncertain (Rottoli and Castiglioni 2009), and while this region did not take an active part in the domestication of the species, the discovery of remains in Copper Age sites (Tecchiati et al. 2013) indicates its potential use and the knowledge of this species, if not intentional cultivation. However, none of these finds has been directly radiocarbon dated, which is necessary to establish the age of millet remains as small grains may move downwards through stratigraphic sequences (Motuzaite-Matuzeviciute et al. 2013, Herrscher et al. 2018, Filipović et al. 2020, Martin et al. 2021).

Intentional cultivation most likely occurred during the Early Bronze Age through connections with the Eastern Alpine region and Danube-Carpathian agricultural sites (Rottoli and Castiglioni 2009, Filipović et al. 2020, Außerlechner 2021). During the Middle Bronze Age abundant findings of broomcorn millet grains are connected to the Po Plain and the sites of the Terramare Culture (Filipović et al. 2020, Nisbet 1999, Mercuri et al. 2006, Cremaschi et al. 2016, Perego 2017). These findings are supported by isotopic evidence demonstrating direct consumption of millets as well as their use for animal feed (Tafuri et al. 2018, Tafuri et al. 2019, Cavazzuti et al. 2019). The first isotopic results from Central Italy suggest that the introduction of C4 plants in the human diet occurred in the Bronze Age, revealing that millets might have been consumed by certain individuals (Varalli et al. 2015). This data is supported by plant macroremains from Pienza (Castelletti 1976), while the Middle Bronze Age finds of *Panicum* grains at Grotta Misa (Tongiorgi 1947) do not appear sufficiently well documented.

In Southern Italy, during the Bronze Age isotopic analysis of human bone collagen shows lack (Tafuri et al. 2019), or limited evidence (Rumolo et al. 2020) of C4 plant consumption, in opposition to the reconstructed diet trends for the populations of Northern Italy that demonstrate direct and possible indirect consumption of C4 plants like millets (Tafuri et al. 2018). However, archaeobotanical occurrences of *Panicum* were reported from Campania at the Bronze Age sites of Nola–Croce di Papa (Albore Livadie et al. 2019), Capua, Strepparo, and Cento Moggie (Castiglioni and Rottoli 1996) and Oliva Torricella (Delle Donne 2011), and from Apulia, at Roca, where abundant unprocessed grains of *Panicum* were aggregated in a charred lump (Guglielmino and Primavera 2021).

During the Late Bronze Age and Iron Age, in Northern Italy, the incorporation of millets in the regular diet continued and was clearly established (Außerlechner 2021, Nisbet 1990, Nisbet 2021, Rottoli et al. 2016, Sala and Rottoli 2018, Arobba et al. 2003), while the archaeobotanical evidence for its consumption in Central and Southern Italy remains very limited (Bellini et al. 2008). In pre-Roman levels, *Panicum* was detected as a grain imprint in the Early Iron Age Grave T of Forum Romanum in Rome (Helbaek 1956), in Archaic period structures at the Forum and Palatine Hill in Rome (Costantini and Giorgi 2001), and in the Faliscan settlement of Narce (Jarman 1976). In these sites, millet was never found in substantial quantities.

Due to the rarity of discovery in Central and Southern Italy, the finds of *Panicum* in multiple vessels in the Gran Carro village take on added significance, which is further increased by the findings of *Setaria* (foxtail millet), extremely rare in the Italian Peninsula. In many European records *Panicum* and *Setaria* have been found in the same assemblages (Außerlechner 2021, Marinval 1992, Schmidl et al. 2007, Hunt et al. 2008, Dreslerová and Kočár 2013, Reed and Drnić 2016, Alonso and Bouby 2017, Goude et al. 2017). In Northern Italy, *Setaria* has been recorded together with *Panicum* in Bronze Age and Iron Age settlements (Fiorentino et al. 2004, Rottoli and Castiglioni 2009, Außerlechner 2021, Perego 2017, Arobba et al. 2003), as well as in several sites of Roman Age (Rottoli and Castiglioni 2011, Bosi et al. 2020). In Central and Southern Italy, carpological remains of foxtail millet are very rare, being documented in the archaeobotanical assemblage from Insula VI.I of Pompeii, but in smaller quantities than *Panicum* (Murphy et al. 2013), and in Medieval layers (Buonincontri 2017, Primavera et al. 2018).

While information about the wider incorporation of millets in the human diet seems to be lacking, which might in part be due to limited analysis and the difficult preservation of small grains, the evident dichotomy between the agricultural practices of Northern and Central-Southern Italy is indisputable. In fact, the evidence of cultivation, exploitation and use millets in the settlement context of Gran Carro goes against the established pattern of cereal preference of Central Italy. Even though previous archaeobotanical research reported only the presence of *Triticum dicoccum* (4 caryopses from the 1974 excavations (Costantini and Costantini Biasini 1995), and one ear fragment of emmer wheat excavated in 1980 (Hopf 1995), the current research detected the presence of broomcorn millet in six out of eight vessels containing caryopses, including the agglomeration in Vessel 38, accompanied by foxtail millet in all the analyzed vessels, although in low amounts. This indicates the value that millets had in the community, and supports that it was intentionally grown and utilized. Carpological analysis attests to cereal cultivation based on polyculture, combining annual (*Hordeum vulgare*, *Triticum dicoccum*) and single short season (*Panicum* and *Setaria*) crops. These species were staple crops in human diet for the population of Gran Carro settlement and might have also been utilized as animal fodder. However, the vicinity of the settlement to the ritual structure of Aiola, that is currently being excavated and researched, might infuse a new meaning to the significance of cereals in Iron Age ceremonial rites.

Plant food resources were obviously very important for the subsistence of the inhabitants of the Gran Carro site, and therefore cereals played a significant part in human diet and the agricultural economy. Even though nowadays the reports on millets for Central Italian Iron Age suggest that it was not a preferred crop, and it was either sporadically or accidentally cultivated, the cereal findings from Gran Carro shine a different light on the dispersion and value of this small grain crop. Millets were without a doubt present, utilized and bore importance in the diet of Central Italian ancient communities. The discord in distribution compared to Northern Italy is undeniable, and this separation might be attributed to ecological or cultural barriers. Interestingly, similar contrasting geographical patterns have been found in the Balkan and Iberian Peninsula.

In Greece, *Panicum* was more common and abundant in the northern regions of the country, where it is recorded since the Early Bronze Age, than in the south, where only a few grains were identified from the Late Bronze Age levels (Valamoti 2016). According to Valamoti, climate differences between different sections of the country are unlikely to have caused this geographical pattern in millet distribution in prehistoric Greece, which may be better explained by a north-to-south introduction of the crop. Another environmental factor that might have hindered larger millets use is the limiting long-term storage in warm conditions without affecting the flavor and developing a rancid taste (Dvoracek et al. 2010).

In the Iberian Peninsula, sporadic Middle Bronze Age finds have been recorded in the north (Bettencourt 2003, Alonso and Bouby 2017). However, broomcorn millet only became common during the Late Bronze Age in Northern Portugal and Northwestern Spain (Tereso et al. 2016, Peña-Chocarro and Pérez-Jordà 2018), while in Valencia or in Andalusia there is no evidence of millet cultivation until the first millennium BC, despite there being rich archaeobotanical assemblages recorded (Pérez-Jordà et al. 2018). In the Iron Age and during the Roman period, millets spread throughout Iberia, but were still most abundant in the northwest (Peña-Chocarro et al. 2019).

One hypothesis that has not been thoroughly explored is that millets were introduced late in central Italy, as a complement to the more productive and demanding crops of wheat and barley, in relation to a climatic and environmental change. At the time of the Gran Carro village, the vegetation of the Central Mediterranean shows a response to decrease in water availability (Di Rita et al. 2018, Michelangeli et al. 2022), which is confirmed by the low water level of the Bolsena Lake and by similarly low lake level at

Lago dell'Accesa in Southern Tuscany (Magny et al. 2007). This aridity might have favored the complementary cultivation of millets that, as C4 plants, can thrive even under more arid conditions. In addition, the lowering of the Bolsena Lake resulted in the formation of a vast sandy coastal plain around the lake (Figure 4.1), which could be exploited profitably for summer crops, such as those of millets requiring short growing period, between 40 and 90 days. According to ancient sources, millet was grown in areas not suitable for wheat, in sandy or wet soil (Murphy 2016), which could also explain the abundance of *Panicum* in the Terramaras, Bronze Age villages located in the central alluvial plain of the Po Valley characterized by shallow water habitats and seasonal water-level oscillations (Cremaschi et al. 2016), and in the lake-dwelling site of Lavagnone in the Lake Garda area of northern Italy (Perego 2017). The presence of millets in these Bronze Age sites suggests that *Panicum* and *Setaria*, which are typically dry-adapted species, may have found favorable pedological and edaphic conditions in summer-desiccated sandy soils in alluvial areas.

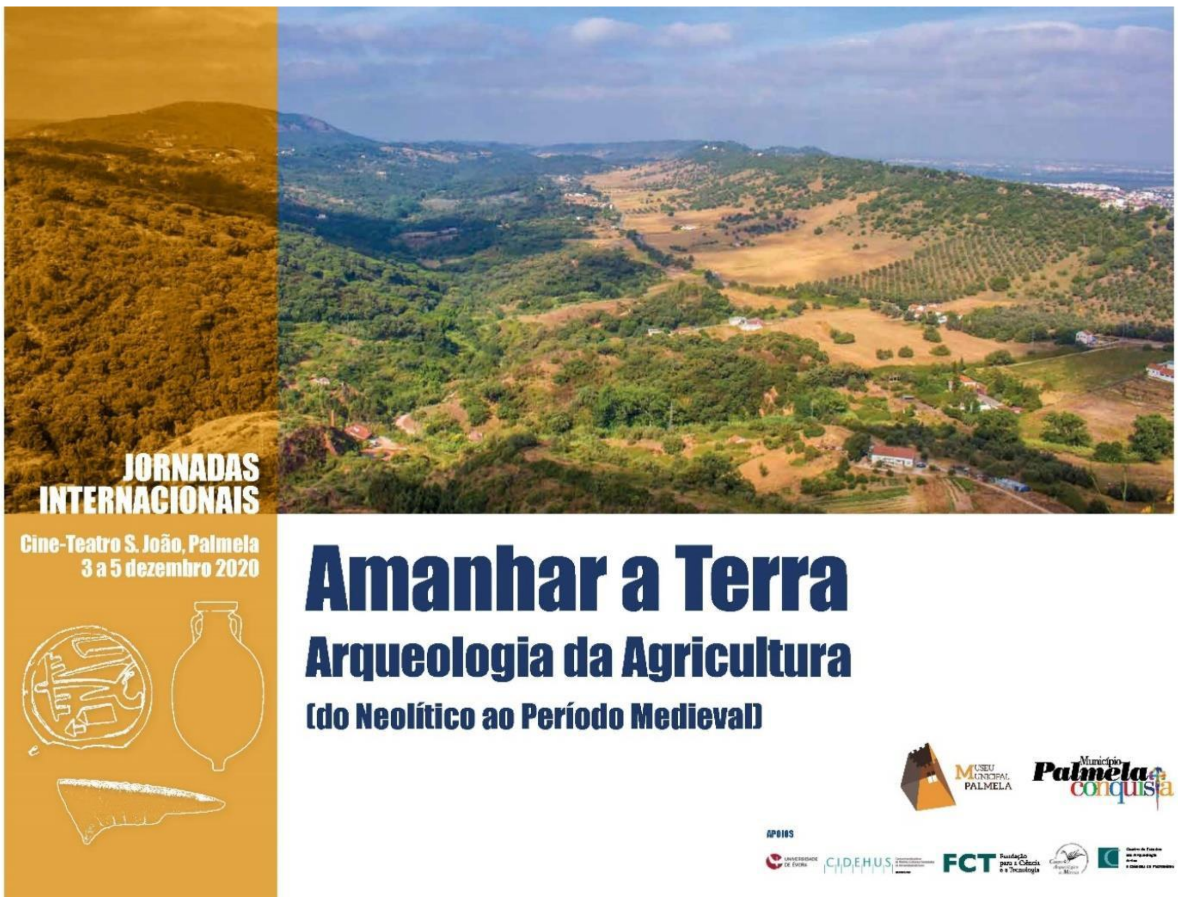
Still, it does not seem plausible that wider utilization in the Central Italy of this fast-growing and adaptable crop was restricted by the changing climate conditions in the later periods for a whole millennium as far as Middle Ages. Keeping in mind the current state of research and preservation of small grain cereals in the archaeological contexts of the Mediterranean, one might look to food preferences, taste and cultural identities reflected in culinary choices, as one of the possible causes for limited evidence of millet consumption.

2.6. Conclusions

The study of cereal grains and of food remains from the vessels of the underwater Gran Carro settlement provides the evidence for agricultural and dietary variability in Italy during Early Iron Age. The analyzed archaeological residue supports the possibility of processing of cereals and consumption of cereal meals at the site. Cultural preferences and taste might have played a role in crop selection during the Iron Age, but the agricultural practices from Northern Italy, active exchange networks, as well as occurrence of broomcorn millet throughout the Peninsula, even if limited, demonstrate that knowledge existed for the successful cultivation and incorporation of this small grain cereal into the human diet. Crop selection at the Gran Carro settlement attest to higher levels of millet production and its economic significance compared to the current known distribution of broomcorn millet in Central Italy. Millet could have been cultivated on sandy soils, in the large coastal plain (100 m width) created by the lowering of the lake level, attesting to a general dry period. This land could be dry and available in summer for a fast cultivation of millet, not disrupting the cultivation of wheat and barley in the fertile soils around the lake. Even though millet was not grown as a main crop in the region, these new finds demonstrate its use and value in the society, offering additional harvest and enriching the diet of the local population. The possible use as an offer in ritual ceremonies could be confirmed by future excavations at Aiola structure. Still, further research is necessary to better understand how social dynamics influenced selection of plant food sources and if they mirror values of a specific population on a local level.

3. Manuscript 5: Carpological remains recovered in two Medieval Islamic silos at Largo dos Lóios, Lisbon

Preliminary results of archaeobotanical analysis were presented at the International Conference Amanhar a Terra – Arqueologia da Agricultura (do Neolítico ao Período Medieval), *June 2021, Palmela, Portugal.*



3.1. Introduction

The arrival of Islamic people from North Africa and the Middle East to the Iberian Peninsula in the 8th century was a cultural and political shift. From the 8th to the 12th century, the area that is now central Portugal became a conjuncture between the Christian and the Muslim populations. Even though the unstable period affected the daily life, communal and seasonal activities continued and developed, as well as exploitation of natural resources. Lisbon area, at the confluence of the River Tagus and the Atlantic Ocean, is surrounded by fertile territory and has a convenient access to the interior by the river, making it appealing location for the settlement of the new population that occupied the city for almost four centuries. Urban growth under Islamic rule began in the 9th century (Torres 1994) linked with demographic expansion, changes in trade and agricultural transformation. Agriculture and diet transformed during the Islamic Period, with the introduction of new cultivars and especially irrigation technologies, with a long-lasting effects, even after the power balance changed in the 12th century (Watson 1974).

The archaeological material from Largo dos Lóios located in today's Alfama historical quarter of Lisbon, was recovered from the storage area of the Medieval Lisbon. On the initiative of the archaeologist Vanessa Filipe, research of the archaeobotanical remains was conducted for the International Conference *Amanhar a Terra – Arqueologia da Agricultura (do Neolítico ao Período Medieval)* that was held in June of 2021 in Palmela, Portugal.

The conference was organized by the Palmela City Council in collaboration with CIDEHUS – *Centro Interdisciplinar de História, Culturas e Sociedades da Universidade de Évora*. The preliminary results were presented in a lecture *Vestígios carpológicos recuperados em dois silos islâmicos. Largo dos Loios (Lisboa)* (Filipe et al. 2021).

3.2. Material and methods

The material was recovered from storage pits dated to from the middle of 10th to the middle of 12th century of the Islamic period. The carpological material was preserved due to carbonization. The survival of plant remains during thermal exposure (Wright 2003, Braadbaart et al. 2005, Van der Veen 2007) is dependent on the temperature to which they had been exposed, but also the time of exposure, and oxidation versus reduction atmosphere. The plant species and its parts also influence the preservation outcomes. For example, presence or absence of glumes on the grains may have an impact, as does the shell of the fruit. The physical conditions of the plants remains before the charring, and the chemical content of the remains affect what will be preserved and what will be destroyed. As a result of carbonization, the physical properties and the chemical structure of seeds and fruit change continuously during charring with increasing temperature (Märkle and Rösch 2008). Therefore, the preserved carbonized remains are just a fraction of the original assemblage of stored food.

The carpological remains recovered were photographed and digitally measured, then sorted by size and outline. The identification of the archaeobotanical material was done using a stereo-microscope under 63-200× magnification by comparison with the reference collection of Laboratory of Palaeobotany and Palynology at Sapienza University of Rome, and with anatomical atlases (Jacomet 2006, Neef et al. 2012, Cappers and Bekker 2013).

To examine the effects of carbonization on seeds, specifically pips of *Vitis vinifera*, contemporary grape pips were carbonized in controlled laboratory conditions. The aim was to examine the change in dimensions, and the state of conservation. The length and the width of fresh grape pips were examined and measured under stereo microscope. Contemporary reference samples of grapes were carbonized at different temperatures

(300 °C, 400 °C, 500 °C, 600 °C) in a Nabertherm high temperature muffle furnace without the airflow. Each temperature program started at room temperature and set to rise for 60 min to the selected temperature, where it stayed for another 60 minutes in stable conditions. The dimensions and preservation state were measured at each temperature.

Microstructural analysis of carpological material was carried out under optical and variable pressure scanning electron microscopy (VP-SEM) at the HERCULES Laboratory of University of Évora. The analysis was done using a low vacuum of 40 Pa and an accelerating voltage of 20 kV and a ca. 11 mm working distance. The VP-SEM images were acquired in the backscattering mode.

3.3. Results

Largo dos Lóios assemblage is dominated with remains of edible fruit, namely *Vitis vinifera*, alongside cereals and weeds. Remains of legume were also detected. The data presented consists of 2984 carbonized seeds and domesticated and wild fruit parts, recognized as nine different plant taxa (Table IV.4).

Of the identifiable plant fragments, 31 seeds belonged to domesticated field crops, 2843 were categorized as fruits, and 109 were determined as weeds (Figure IV.5a). Of the domesticated crops the most represented was barley (*Hordeum vulgare*), followed by wheat (*Triticum aestivum/durum*) and broomcorn millet (*Panicum miliaceum*) (Figure IV.5b). Barley grains composed 71.9 % of all the cereals, while wheat made out 21.9%. Residual presences of broomcorn millet and fragment of vetch (*Vicia* sp.) were also recorded.

Table 0.4 Identified carpological remains from Largo dos Lóios assemblage.

Taxon	Conservation conditions	Type of remain	Min. N. of units	Weight (g)	Average length (mm)	Average width (mm)
Cereals						
Triticum aestivum/durum	charred	caryopsis	7	0.07	4.75	3.56
Hordeum vulgare L.	charred	caryopsis	23	0.46	4.73	2.42
Panicum miliaceum L.	charred	caryopsis	1	0.02	2.71	
Legumes						
Vicia sp.	charred	seed fragment	1	0.01	2.33	1.65
Fruits						
Quercus sp.	charred	acorn	1	0.43	14.00	9.87
Olea europaea L.	charred	stone	4	0.51	10.29	5.11
Vitis vinifera L.	charred	pip	2838	23.93	3.94	2.54
	charred	pericarp fragments				
Weeds						
Galium aparine L.	charred	fruit	99	0.06	2.04	
Sinapis sp.	charred	achene	10	0.01	1.75	

Angular and symmetrical, barley grains are widest at the middle or slightly below, and on average 4.73 mm long and 2.42 mm wide. Wheat caryopses were damaged and fragmented by charring, still the preserved fragments are 4.75 mm in length and 3.56 mm in width on average. The diameter of charred millet grain is 2.71 mm, and the remains of vetch are 2.33 mm in length and 1.65 in width. Other chaff fragments were not recovered together with grains, suggesting processing before storage.

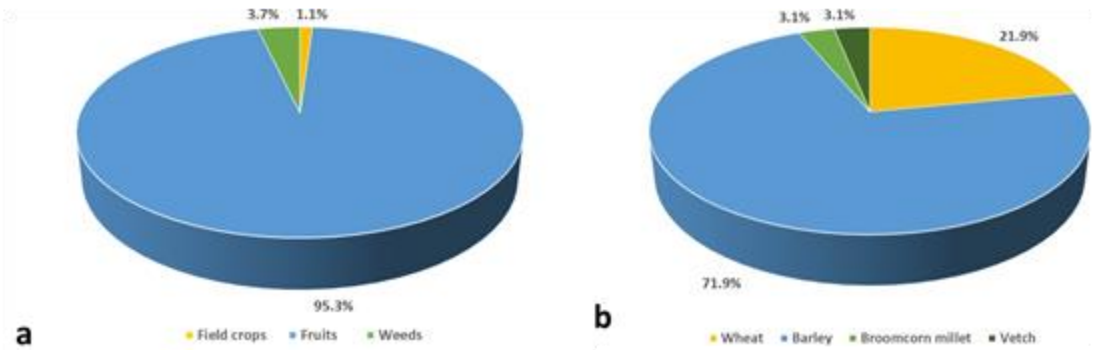


Figure 0.5 (a): Percentage of identified field crops, fruits, and weeds; (b): Domesticated crops.

In addition to cereals and legumes, fruit fragments were identified, including *Quercus* sp. acorn, olive stone (*Olea europaea*) and abundant grape (*Vitis vinifera*) pips. The most represented fruit in the assemblage was grape, accounting for the 99.8% of recovered fruit remains. Fragmented remains of berry pericarp were also preserved, charred, dehydrated, and severally damaged, with some pips still fused with fruit tissue by the heat. Based on measurement of 100 individual seeds, the grape pips examined are relatively small, on average 3.94 mm (±0.65) in length, and 2.54 mm (±1.04), and express considerable morphological variation (Figure IV.6).

The size of recovered charred grape pips was, in part, influenced by preservation conditions. Carpological remains from Largo dos Lóios were preserved by carbonization, due to prolonged exposure to high temperatures, that causes transformation and deformation of charred plant remains, exhibited in weight loss, fragmentation, decrease and/or increase of dimensions, and of course change in chemical structure (Hillman et al. 1993, Smith and Jones 1990, Ucchesu et al. 2016). It alters the archaeobotanical record, preserving and destroying it at the same time, providing only a partial reflection of the true archaeological deposit.

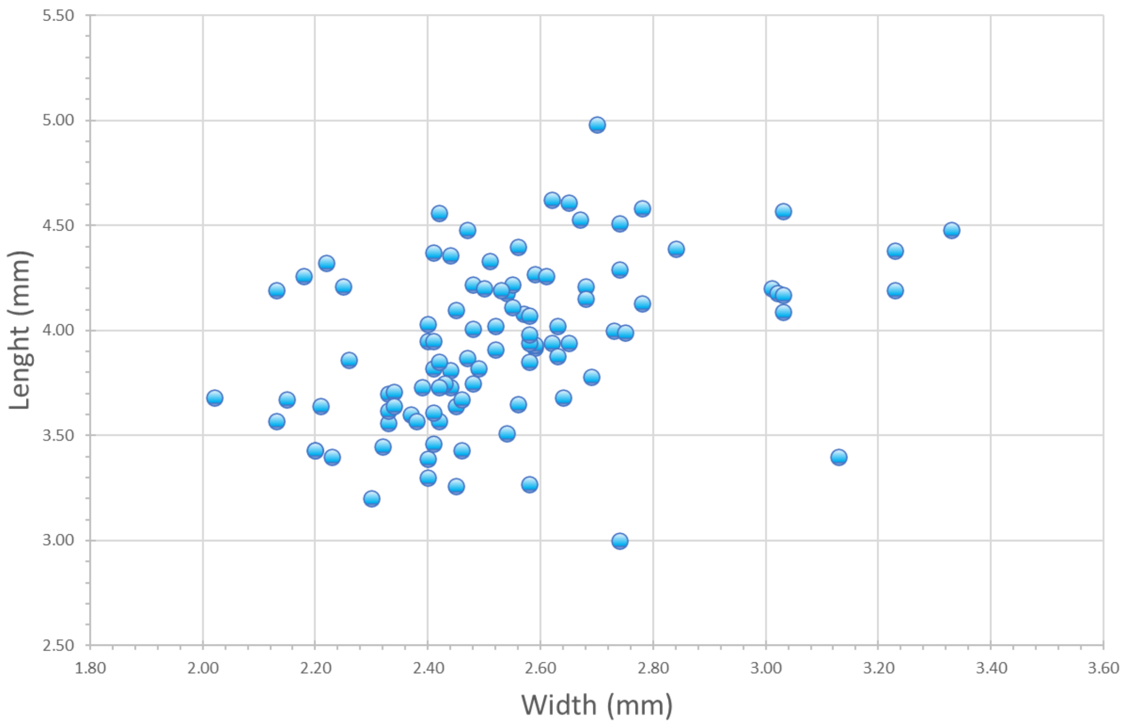


Figure 0.6 Variability in length and width of carbonized *Vitis vinifera* pips from the archaeological context of Largo dos Lóios.

Table 0.5 Preservation state of the contemporary reference of *Vitis vinifera* pips at different temperatures.

Temperature	0°C		300°C		400°C		500°C		600°C	
	L	W	L	W	L	W	L	W	L	W
Dimensions	7.77	4.39	7.39	3.93	6.67	3.79	6.29	3.64	5.48	3.31
Reduction (%)	0	0	4.9	10.5	14.2	13.7	19	17.1	29.5	24.6
Condition	Fresh, uncharred		Charred		Fully charred, good structural consistency		Partly damaged, structural degradation		Whitening of the surface, 40 % destroyed	

Experimental approach with contemporary reference cultivars of grapes, showed that grape pips reduce dimensions during charring depending on the temperature (Figure IV.7). At 300 °C the carbonization is almost complete, and the pips show prominent decrease in width. The chemical conversion of starch, cellulose and oil components into aromatic compounds makes the carbonized seeds more resistant to attacks by micro-organisms when deposited in the soil and increases the chances that archaeobotanical material will be recovered from archaeological sites (Braadbaart 2008).

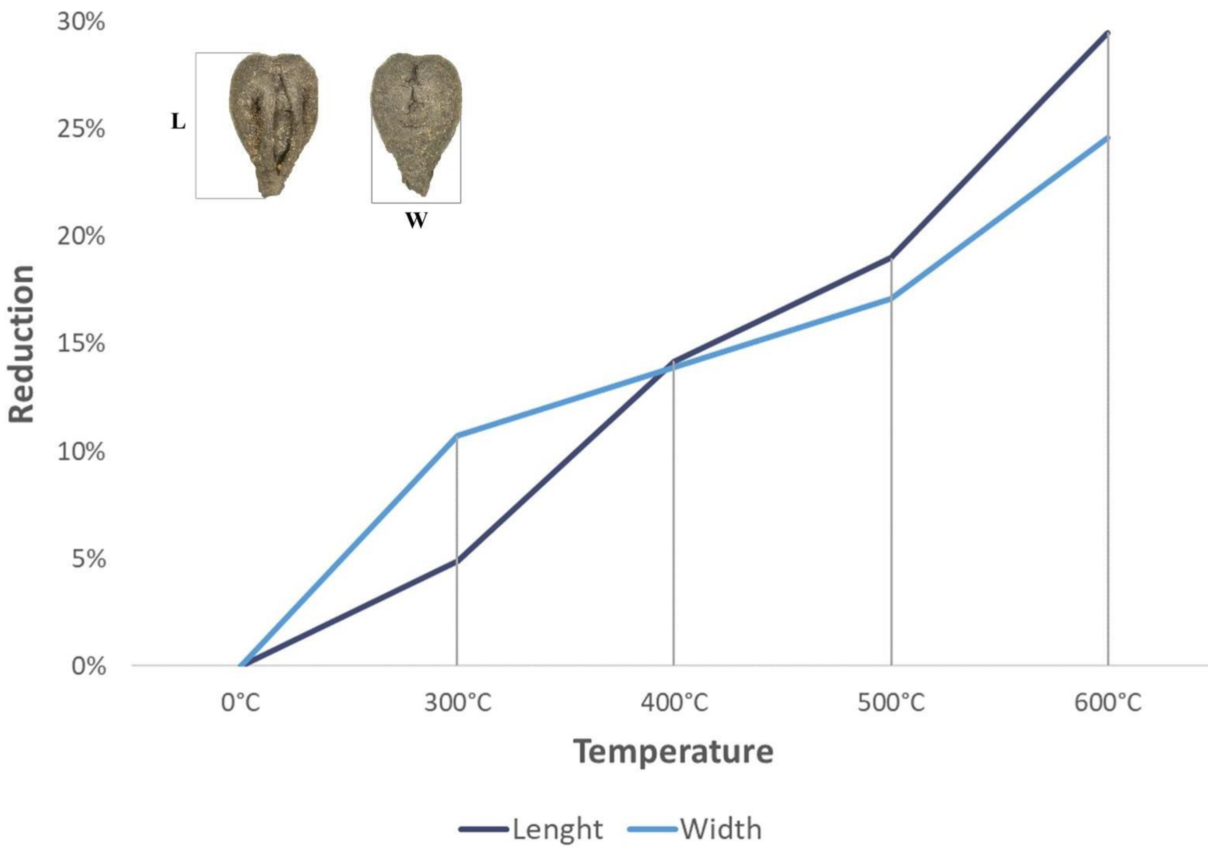


Figure 0.7 Reduction of length and width depending on temperature.

At 400 °C the pip remains are fully charred but show good structural consistency. Both length and width show reduction of ca. 14%. Since the charred remains at this temperature should not undergo any significant chemical changes, it has been suggested as an optimal temperature for a good preservation of carpological remains at the archaeological sites (Braadbaart 2008). This is in line with the results of charring the pips at 500 °C, that show further reduction in size (both in length and width), but also observable structural degradation, that make grape pips fragile and partly damaged in the laboratory conditions. During the formation of archaeobotanical record and excavation of such remains, the likelihood of information loss drastically increases. At 600 °C, the maximum temperature that can be reached with an open fire, almost 40% of the assemblage is destroyed, and the pips have reduced 29.50% in length and 24.60% in width on average (Table IV.5). Since charred grape pips from Largo dos Lóios are fully charred but show good structural consistency, alternative factors could also affect the size of retrieved remains, such as grape variety or prolonged drying prior to charring for production of dried grapes, raisins.

The charred olive stones retrieved from Largo dos Lóios are well preserved but again relatively small, on average 10.29 mm in length and 5.11 mm in width. Furthermore, fragments of acorn (*Quercus* sp.) were found, 14.00 mm in length, and 9.87 mm in width. As the cupule and peduncle of the acorn are not preserved, it was not possible to identify to which species the remains belong to. The assemblage also included rounded dry fruits of cleavers (*Galium aparine*), 2.04 mm in diameter, in a considerable quantity, and several charred mustard seeds (*Sinapis* sp.) with a diameter of 1.75 mm on average.

3.4. Discussion

At the Medieval sites in Portugal cereals represent the most abundant carpological find (Peña-Chocarro et al. 2019). The dominant species are barley and free-threshing wheats, as is confirmed with the cereals retrieved from Largo dos Lóios. However, the absence of chaff remains prevents from distinguishing between *T. aestivum* (bread wheat) and *T. durum* (hard wheat). Naked wheats were highly valued, and had an important role in the Medieval diet, consumed in the form of bread or prepared as porridge for both upper and lower classes, and might have been used as fodder for animals. Barley on the other hand, was considered an excellent fodder, but its frequency and abundance in the archaeobotanical record suggests regular dietary consumption by poorer and part of higher classes (Peña-Chocarro et al. 2019). Barley flour was commonly used through the Middle Ages, mixed with flours from other cereals, nuts or legumes. Since analysis under VP-SEM (Figure IV.8,j) did not show any sign of aleurone cell wall thinning (Heiss et al. 2020) that results from malting process, it can be concluded no germination occurred and the grains were stored in dry conditions.

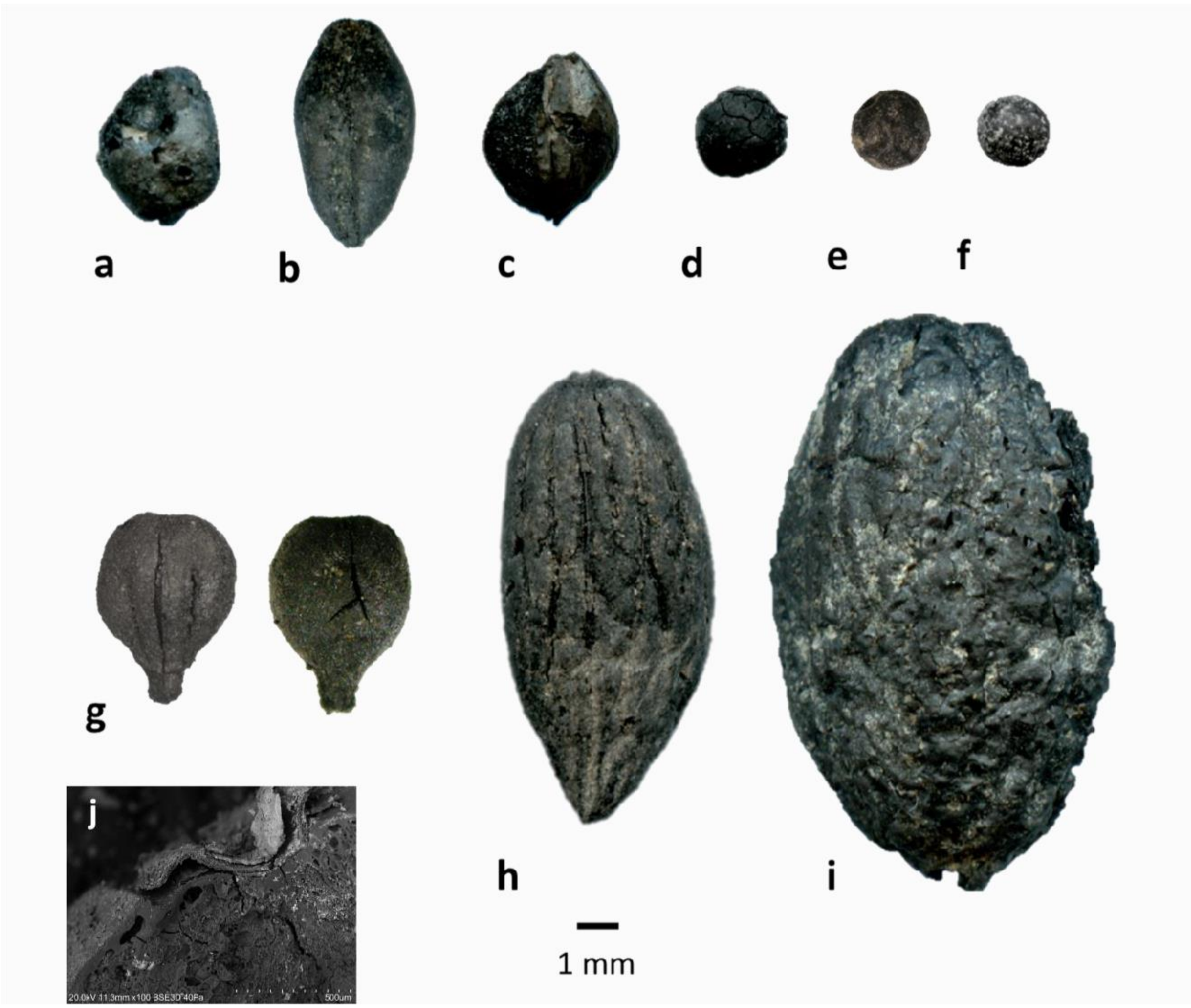


Figure 0.8 (a): *Triticum aestivum/durum*, (b): *Hordeum vulgare* L., (c): *Panicum miliaceum* L., (d): *Vicia* sp., (e): *Galium aparine* L., (f): *Sinapis* sp., (g): *Vitis vinifera* L., (h): *Olea europaea* L., (i): *Quercus* sp., (j): section of *Hordeum vulgare* grain under SEM.

Broomcorn millet gained greater importance throughout Europe in the Medieval times (Castiglioni and Rottoli 2013, Ruas 2005, Brión 2015, Quirós-Castillo 2020). In Portugal remains of millets are more abundant in the north (Oliveira et al. 2017, Peña-Chocarro et al. 2019) but have also been recorded at the *Casa dos Bicos*, Lisbon (Queiroz and Mateus 2011) and *Convento de S. Francisco*, Santarém (Queiroz 2001). As broomcorn millet is a C4 plant, thermophilic and highly drought-resistant, with a short-cycle, it is sown late in Portugal, at the end of June, connected with the feast of St. John (Moreno-Larrazabal 2015). It was valued as fodder but also a practical addition to the human diet that could compensate for the eventual loss of winter-sown cereals. In the Middle Ages consumption of millets was often connected to the lower classes and could have been consumed mixed with other cereals or legumes in a form of bread, porridges or soups (Peña-Chocarro et al. 2019). The joint occurrence of cereals is probably a result of mixed cropping, harvesting, or using the same storage for different harvests, while the choices and preferences of crops may also be related to the interests of the upper class of Medieval Lisbon, reflecting the tax system (Viana 2007, Pinto 2020).

Through the Iberian Peninsula, archaeobotanical records show that legumes are present, but with less abundance compared to cereals, with *Vicia* sp. (including *V. faba*, *V. ervilia* and *V. sativa*) also present (Peña-Chocarro et al. 2019). Still importance of legumes is undeniable, as a rich protein source consumed by humans and animals, as well as taxa that enriches the soil through nitrogen fixation. The scarcity of leguminous remains does not accurately reflect the Islamic diet, since meals based on legumes are established in historical sources (García Sánchez 1996, Rei 2017). This variation might be a result of processing legumes for meals, that does not leave evidence in the form of carpological remains.

Current datasets show that fruits are more abundant at Islamic Mediaeval sites in comparison with Christian sites (Pais 1993, Queiroz and Mateus 2011, Coradeschi et al. 2016, Peña-Chocarro et al. 2019). The grape, together with olive, is the most important cultivated species of the Mediterranean, present in the Iberian Peninsula since Protohistory, and heavily exploited since the Roman times (Buxó 2008, Arruda 2008, Pérez-Jordà et al. 2017, Pérez-Jordà et al. 2021, Peña-Chocarro et al. 2019). During Middle Ages grapes are predominant species recovered at most of the sites, while olive stones have been detected in the Christian context in Lisbon (Queiroz & Mateus 2011). A large amount of preserved pips (2838) would suggest up to 1419 grape berries (Hardie et al. 1996), or around 14 -18 grape clusters. Since there were no remains pedicels or tendrils found, it can be concluded that barriers were picked and cleaned. The size of the seeds is connected to grape variety (Rivera et al. 2007, Bonhomme et al. 2021) and charring conditions of the preservation (Smith and Jones 1990, Ucchesu et al. 2016), like temperature, duration, access to oxygen etc. Information about the effect that temperature has on carpological remains can help recognize and overcome the biases connected to the formation processes that shape organic archaeological materials and influence interpretation. The Medieval period is marked by the unexpected late revival of the use of the wild morphotype (Bonhomme et al. 2021, Freitas et al. 2021), characterized by the smaller seed size and higher width to length index (Rivera et al. 2007) that is not only associated with wild grapevines but also with archaic varieties (Orrù et al. 2013). This post-domestication hybridization event (or events) with local wild grapes occurred in the Iberian Peninsula up to the 500 BP (Freitas et al. 2021), including in the Medieval period. Still, the reduction in size might have occurred prior to abandonment of archaeological materials. During the Middle Ages, on the Iberian Peninsula the dried grapes were produced for consumption and trade (Iriarte-Chiapusso et al. 2017, Jiménez-Cantizano et al. 2012). Drying the grape berries could contribute to dehydration of pips, and consequent reduction in size, prior to carbonization. Preservation of berry pericarps would indicate a possible storage of dried raisins, since they preserve when already dried grapes are charred, as demonstrated trough archaeobotanical experiments (Cartwright 2002, White and Miller 2018.).

The remains of *Vitis* from Largo dos Lóios were most likely intended for consumption, either fresh or dried. However, the viticulture certainly did not disappear from the Iberian Peninsula during the Islamic period (Iriarte-Chiapusso et al. 2017), even though the consumption of alcoholic beverages such as wine were partly prohibited (Adamson 2004). While some vineyards had been uprooted following the Islamic conquests of the eighth century, they appear to have been re-established relatively quickly (Unwin 2005), intended for the fruit production. Since production of table grapes was supported, that were sweeter and had fewer seeds, the wines made from such varieties would be unbalanced and with a high sugar content. Introduction of different stills for distilling and concentrating alcohol for medicinal purposes, as well as perfume production during Islamic period (Cierbide 2007, Adamson 2004), supports that technology was available for wine production, including sweet and fortified wines (Belfrage and Loftus 1993).

Grapes were grown to provide fresh fruit and raisins, vinegars, and the preparation of wine that was permitted for the non-Muslim citizens. Separately from the intention of the grape production, it is inevitable that cultivation continued during the Middle Ages (Peña-Chocarro et al. 2019), and fruit and its products were consumed and traded, relying on the Roman traditions (Ramos-Madrigal et al. 2019, Boso et al. 2020). Climate conditions that lasted from the 900s all the way to 1300s AD (Drake et al. 2012, Moreno et al. 2012), might have favorably influenced the cultivation and the further expansion of *Vitis* sp. to the north (Unwin 2005). An increase of a few tenths of a degree in the average temperature, may seem minimal, but it is significant enough for the grapevine and winemaking, since it would affect the yield, but also grape composition, with changes in sugar and acidity concentrations, as well as polyphenols and aroma compounds (Bonhomme et al. 2021).

Olives, widely spread through Iberia by Phoenicians, never lost their importance in economical and agricultural landscapes of Portugal (Peña-Chocarro and Pérez-Jordà 2018, Peña-Chocarro et al. 2019, Pérez-Jordà et al. 2021, Prata and Cuesta Gómez 2020) and in the Middle Ages growing of olive trees continued to expand, while production of olive oil and table olives was intended for consumption and a valuable export (Barranco and Rallo 2000, Vossen 2007). The size of charred olive stones found at Largo dos Lóios can, again, be attributed to carbonization, as it was demonstrated size shrinkage is equal to 9–10% for variables of distance and 17–19% for surface area of stone outline (Terral et al. 2004). Still, the sample size is limited to know if the olive stones reached their definitive size and maturation before picking. Stones fragmented along the suture line reveal glistening charred remnants of seed oil on the inside. This oleiferous plant kept its high value through Middle Ages, as a symbol of the Mediterranean diet. On the Iberian Peninsula, during Islamic period, studies showed considerable expansion of *Olea* (Van Den Brink and Janssen 1985, Carrión et al. 2007, Pérez-Sanz et al. 2013, Ramos-Román et al. 2018, Ramos-Román et al. 2019), that is attributed to the cultivation and irrigation practices (Terral and Durand 2006) during warmer, and in certain areas dryer climate (Moreno et al. 2012, Moriondo et al. 2013) as a part of agro-pastoral development.

Wild fruits have been gathered and utilized during the Medieval period, with evidence of *Quercus* sp. previously recovered from Islamic context (Peña-Chocarro et al. 2019). The charred remain of *Quercus* spp. acorn demonstrate the use of wild edible fruit at Largo dos Lóios, either for animal feed or as an addition to the human diet. Acorn flour has been utilized on Iberian Peninsula since pre-Roman times and even today this practice is present (Silva et al. 2016). Because of their nutritional value, acorns have been used for human and animal diet, and in medicinal drinks in Middle Ages (Gómez & Sieso 2013). Recovering harvested wild fruits, reflects the relationship medieval society had with their natural surroundings, and provides evidence of woodland use.

Concerning the oaks, they had multiple use in Portugal, most notably is the use of wood and harvesting of cork for utilitarian and decorative objects.

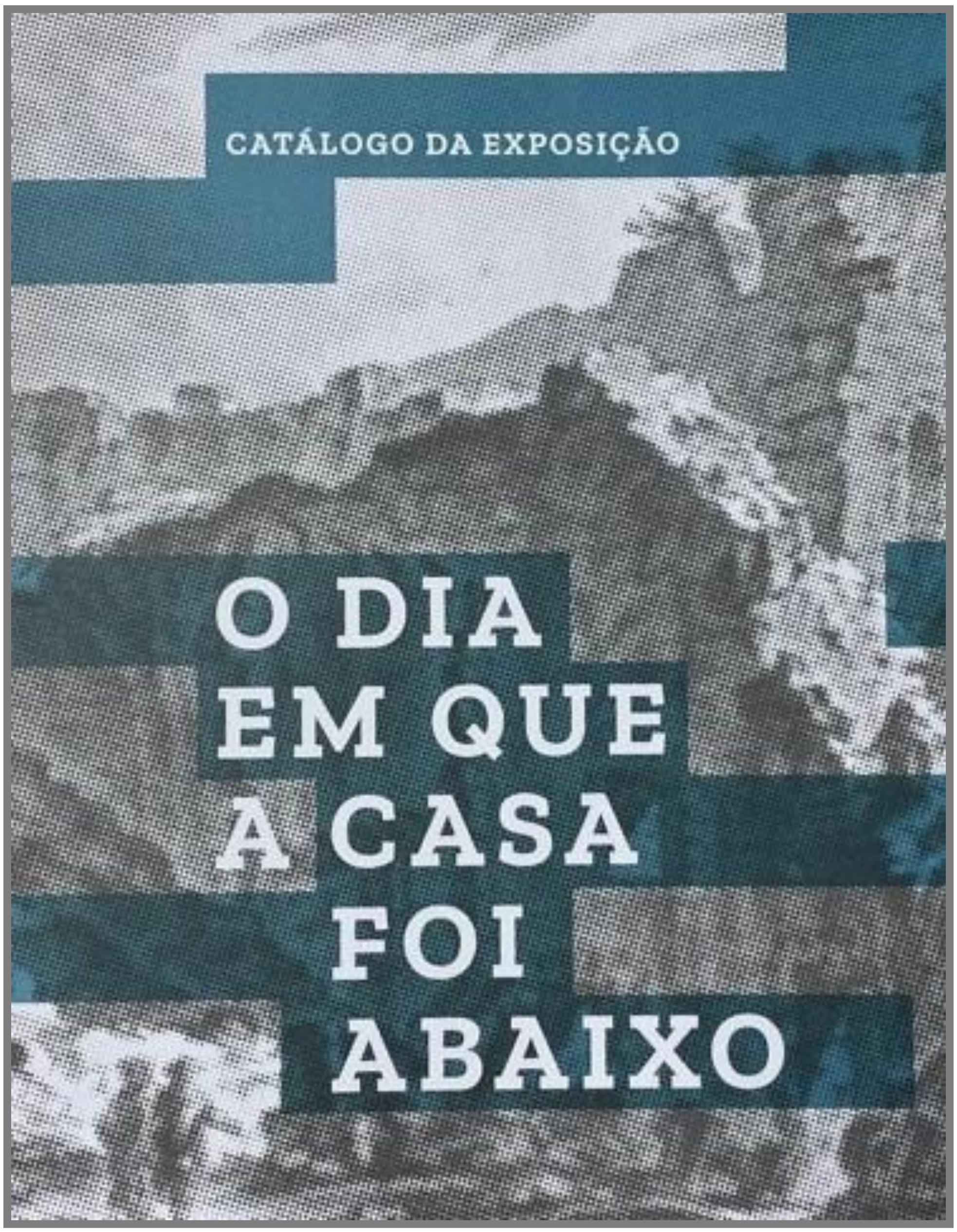
3.5. Conclusions

The basis of Islamic food choices can be translated to the Iberian Peninsula even though, in absence of specific historical resources for the area, it might be suggested that in Portuguese territories, distinct variations in plant food production, consumption and preparations might have developed due to the assimilation of previous culinary traditions of Phoenician, Greek and Roman populations (Adamson 2004) and adjustment to the available natural resources and environmental conditions (Moreno et al. 2012). That is why archaeobotanical remains are essential for the reconstruction of the Medieval diet. Since they are more fragile, they preserve selectively and less well compared to faunal remains, that can lead to underrepresentation when looking at the regional cuisine and use of natural resources. Furthermore, since stable isotope analyses of collagen cannot inform on the fruits and vegetables consumed, only about the contribution of C3 and/or C4 plants (Toso et al. 2019, MacRoberts et al. 2020), these findings are a valuable contribution to explore variety in the medieval diet. Even though storage pits provide abundant information about the cultivation, diet preferences, and storage strategies, they still only represent a fraction of the plant utilization during the Islamic period.

From the archaeobotanical assemblage of Largo dos Lóios, we can conclude that the plant sources, were an integral part of diet in Lisbon during Islamic period, based on cereals and fruits, in addition to legumes. Cereals and wild fruits could have been stored for further processing and used as flour, as a porridge, in soups, transformed into beverages, baked into breads etc., while grapes and olives could have been consumed as tasty and nutritious table fruit, or converted into valuable products like wine, vinegar and oil, intended for trade or consumption. Plant based diet, including vegetables, was a relevant part of the full food consumption in Medieval times, enhanced with dairy products, preserved and fresh meat and fish.

4. Manuscript 6: Archaeobotanical remains and the analysis of carbonized faeces from Praça de Dom Pedro IV, Lisbon

Preliminary results of the analysis were presented in the catalog and incorporated in the exhibition «O dia que a Casa foi abaixo» *at the* Biblioteca das Galveias *in Lisbon, Portugal. (Henriques and Filipe 2020a, Fundurulic et al. 2020).*



4.1. Introduction

During the archaeological excavation in 2017 at the Rossio square (Praça D. Pedro IV) in the center of Lisbon (Portugal), a ground floor of a 18th century town house was discovered. The glimpse into urban context under one of the main squares of contemporary Lisbon uncovered remains of daily lives that were abruptly interrupted and utterly transformed by the Great Lisbon Earthquake. The Earthquake struck on the morning of 1st November 1755, a Saturday of All Saints' Day, followed by a tsunami and fires across the city (Pereira 2009, Santos et al. 2019, Fonseca 2020, Silva et al. 2021). Consequent city reconstructions covered the remains of previous city blocks and molded the current raster of Lisbon Center. The study of the remains found in the house on the Praça D. Pedro IV was done with support of Lisbon Archaeology Center, Office of *Estudos Olisiponenses,* Biblioteca *das Galveias* and Department of Urban Hygiene in collaboration with archaeological company Cota 80.86, HERCULES Laboratory of the University of Évora, the National Museum of Ethnology and the Museum of Lisbon.

The finds and research results were presented in the catalog (Henriques and Filipe 2020a, Fundurulic et al. 2020) and the exhibition «*O dia que a Casa foi abaixo*» presented by the Lisbon Archaeology Center, in collaboration with the Biblioteca *das Galveias* curated by archaeologists José Pedro Henriques and Vanessa Filipe. It intended to evoke the 265th anniversary of the Great Earthquake, through the materiality and excavated finds, in the intersection with written sources, to reconstitute the memory of a housing and its people, that succumbed to this tragic event.

4.2. Archaeological context

The uncovered housing unit was located in the southwest corner of the square as of one of the three blocks that, before the earthquake, defined the side of Rossio square. Excavations exposed different sections of the same property (Henriques and Filipe 2020b), including the kitchen, storage, and courtyard/stable area with adjacent sewer system of the city (Figure IV.9d). The area identified as kitchen had a brick tiled floor, that showed signs of damage and fragmentation endured during the earthquake. To the south of the tiling, a rectangular limestone sink with access to water was uncovered. Associated with the base of the sink, blue and white wall tiles with a motive of acanthus leaf were found. At the southeast end of this area, a larger carbonized layer was observed in which a piece of charred cord was identified. The objects retrieved from the kitchen area were a knife fashioned out of bone, iron and copper, a ceramic vessel, a ceramic measuring container and a cup that was severely been deformed by high temperatures. The area south of the kitchen, divided by plastered walls to the north and west, was interpreted as a storage space (Henriques and Filipe 2020b). The floor is again brick tilled, indicating the indoor space of the house. Even though this area was relatively small, due to the parameters of the excavated area, it yielded an abundance of archaeological finds, including organic remains. These include collection of porcelain fragments decorated in blue and white, characteristic export produced in China during the Qing Dynasty, two large ceramic vessels, several smaller vessels and bowls, fragments of glass and copper. In the same area, in the one of the large cylindrical ceramic vessels, charred organic remains were found. To the west of the kitchen and the storage is the area of the courtyard/stable (Henriques and Filipe 2020b). Since the house collapsed, objects from the upper floors were also found in this area, including ceramic pots, a Chinese female figurine, a bone game piece, parts of adornment and furniture. This ground in this area was laid with cobblestone, usually associated with outside spaces or the transitional sections between the public open space and the private interior. The door frame identified at the northwest corner of the archaeological site would suggest a communication, an entrance, between the street west of the collapsed house, Rua Valverde, and the internal courtyard. The space might have been partly covered as the presence of support stone pillar at the east side of the courtyard would suggest. This area also contained remains of a well, fortified by two rectangular stone slabs. Directly besides the well, on a cobbled pavement, a circular iron rim was recovered, belonging to the wooden bucket, which wooden structure has been completely degraded. Adjacent to the well, was a small rectangular structure, interpreted as a watering hole for livestock. Beneath the courtyard part of the water system vaulted by a brick arch was uncovered. Just next to the stone pillar, rectangular wooden container, a crate, was recovered, containing a large amount of seeds (Figure IV.10). Both the remains of the wooden crate and the seeds inside were completely carbonized. Associated with the crate, bellow the lid, remains of a brush were found. All of these finds led to the interpretation for the use of this area as a stable, which was supported by written sources (Rijo 2020). West of the courtyard a part of the city's sewer system, constructed at the end of the 15th or the beginning of the 16th century, was uncovered. Excavation exposed up to 2.20 meters in length and 1.10 meters in depth, showing that it was constructed with a bricked arch ceiling, laying on roughly cut stone walls.

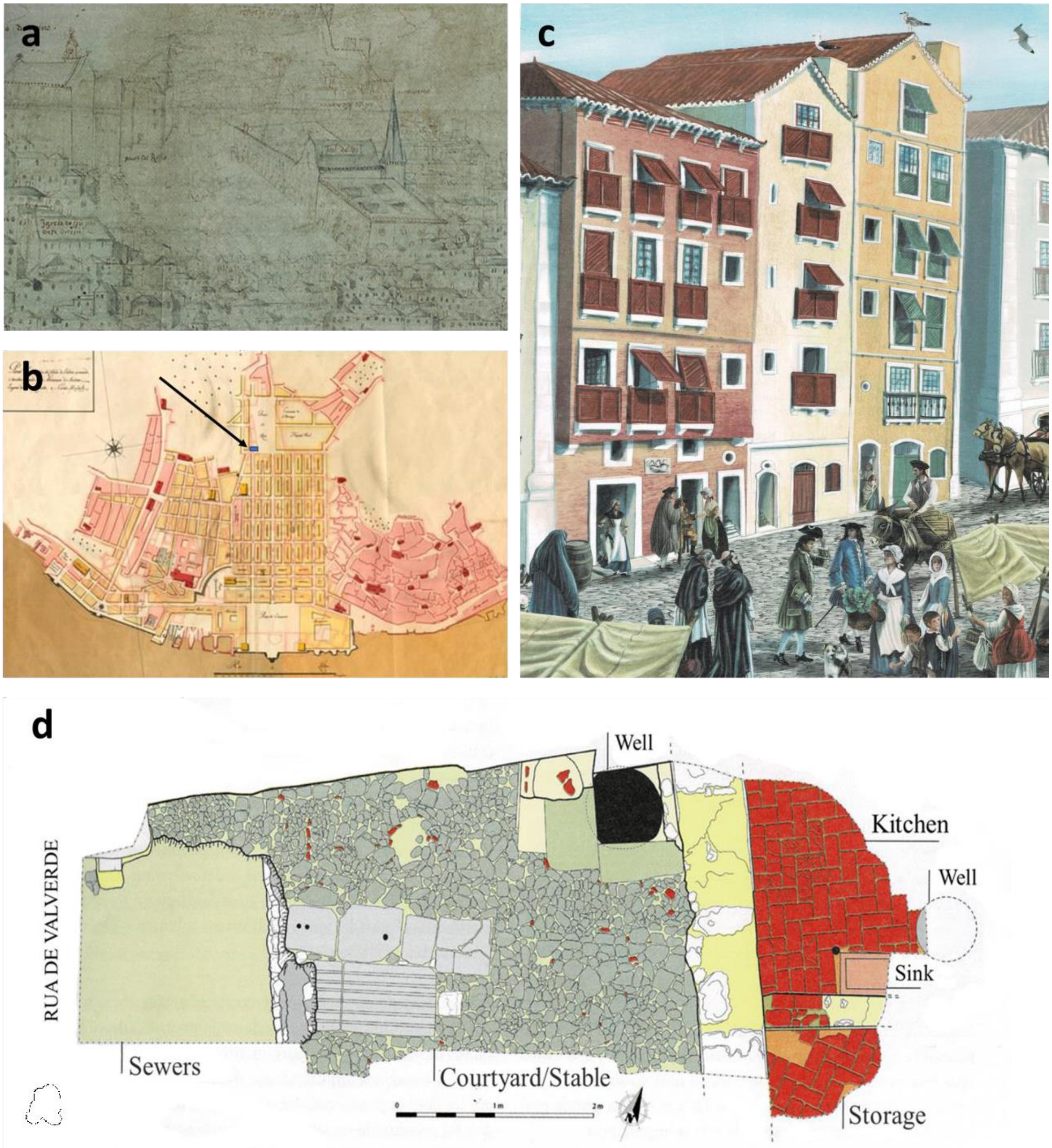


Figure 0.9 (a) Panoramic view of Lisbon with the detail of the Praça de Dom Pedro IV (Rossio). 16th century. Library of the University of Leiden; (b) Topographic map with the approximate location of the house on the square (adapted from Santos et al. 2019). (c) hypothetical reconstruction of the southwest corner and the house researched (Henriques and Filipe 2020a), (d) Plan of the excavated sectors of the house destroyed by the Great Lisbon Earthquake (Henriques and Filipe 2020a).

4.3. Material and methods

In a wooden crate, located near the well in the courtyard on the ground floor of the house at the Praça de Dom Pedro IV, a storage of seeds was discovered (Figure IV.10). The plant remains were thoroughly charred as a consequence of fires that erupted after the Earthquake struck. The deposit was collected at the site and subsampled. 28 g of bulk sediment were analyzed. The carpological remains were photographed and digitally measured, then sorted and divided into groups according to the seed size and outline. The identification of the archaeobotanical material was done using a stereomicroscope under 63-200x magnification by comparison with the reference collection of Laboratory of Palaeobotany and Palynology at the Sapienza University of Rome, and with anatomical atlases (Jacomet 2006, Neef et al. 2012, Cappers and Bekker 2013).

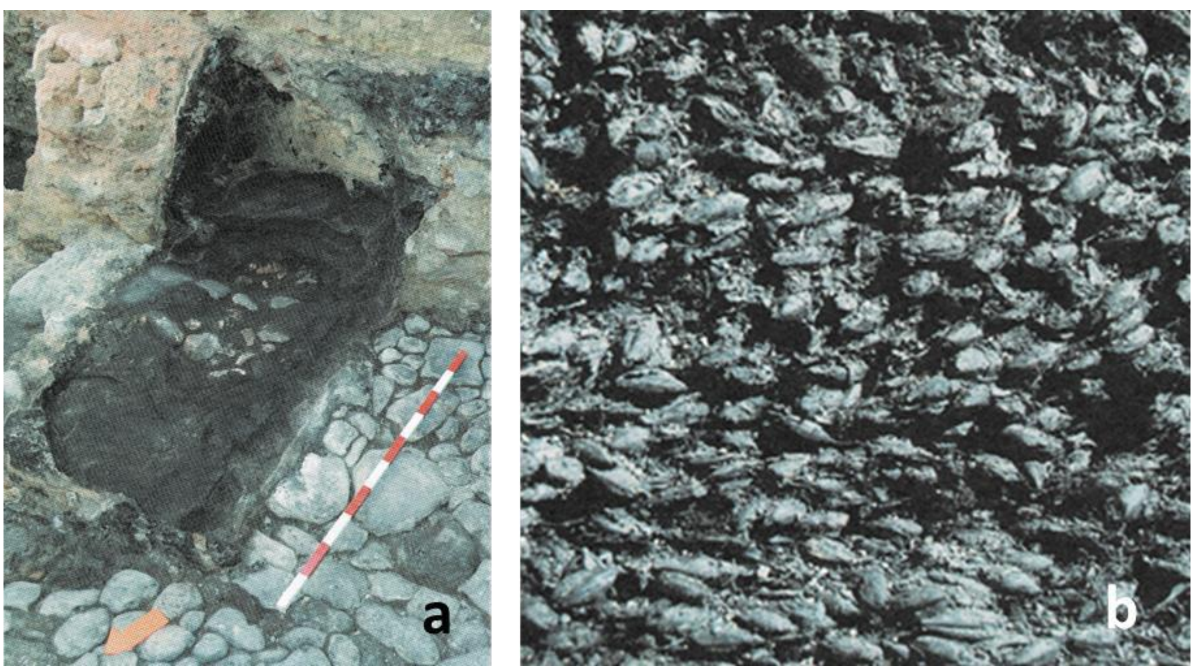


Figure 0.10 (a) Remains of a wooden chest in which cereals were stored; (b) remains of cereals in situ (Henriques and Filipe 2020a).

Amorphous charred organic matter was recovered from the tall cylindrical pottery vessel (Figure IV.11) found in the storage area of the house quarters. Microstructural analysis of carpological material and organic material was carried out under optical and variable pressure scanning electron microscopy (VP-SEM), while elemental composition was obtained by energy dispersive X-ray spectroscopy (EDS). The chemical composition of the organic matter was revealed by gas chromatography coupled with mass spectrometry (GC/MS). Elemental and chemical analysis were conducted in the HERCULES Laboratory, University of Évora in Portugal. 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 analysed 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.

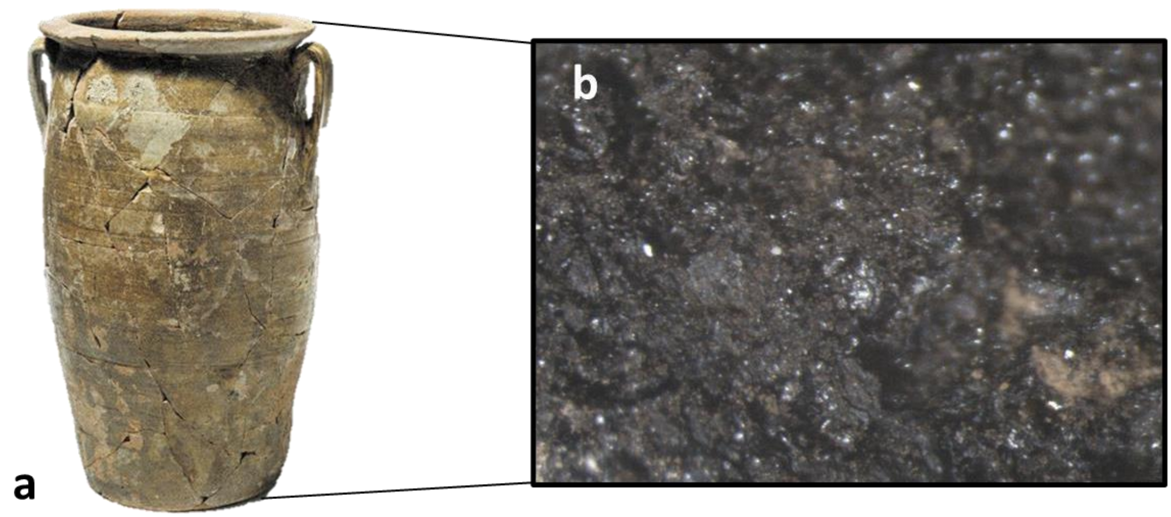


Figure 0.11 (a): Cylindrical vessel recovered from the storage area (Henriques and Filipe 2020a), (b): organic residue found in the vessel.

For the chemical analysis by GC/MS of the residue, organic compounds were extracted with chloroform/methanol (Evershed et al. 1990). Analysis was performed with Shimadzu GC2010 coupled with a Shimadzu GCMS-QP2010 Plus. A capillary column Phenomenex Zebron-ZB-5HT (0.25 mm x 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. 1 μL of the sample was injected in splitless mode, with the injector set at 250 ºC 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 minutes. 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. The 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, Pleasant Gap, PA, USA; 98+%/97+% purity, 50 mg) was analyzed under same conditions. Samples were derivatized with BSTFA. 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. Internal standard, *n*-tetratriacontane (C34), and BSTFA derivative, were also obtained from Sigma-Aldrich. A Milli-Q Integral Water Purification system (Millipore®) was used to produce ultrapure water.

To examine preserved microfossils, the material was subsampled and weighted, and the protocol described in Chapter II, 4.2. was employed. For pollen extraction, standard chemical treatment (Moore et al., 1991, Magri and Di Rita 2015) with HCl (37%), HF (40%) and NaOH (10%) was used. However, to examine the silica phytolith structure, the sample was treated according to the standard pollen extraction procedure while omitting the HF step. Non-pollen palynomorphs (NPPs) and parasite eggs were examined after standard pollen extraction, dissolution in 0.5% aqueous solution of trisodium phosphate for 48 h and distilled water after 48 h. 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 and contemporary references

(Beug 2004, Martin and Harvey 2017, Weber and Ulrich 2017, Albert et al. 2016, Shumilovskikh et al. 2021).

4.4. Results

Organic remains from Praça de Dom Pedro IV were preserved due to prolonged exposure to high temperatures, during the fires that engulfed Lisbon residential area fallowing the Earthquake, resulting in carbonization, i.e., the conversion of organic plant components into charcoal. As displayed in the previous section (Chapter IV, 3.4) carbonization causes transformation and deformation of charred plant remains, exhibited as weight loss, fragmentation, decrease and/or increase of dimensions, and changes in chemical structure. Charring alters the organic matter, preserving and destroying it at the same time, providing only a partial record, a limited reflection of the true archaeological deposit.

The majority of charred carpological remains recovered from the wooden crate were identified as cereal grains, more specifically barley (*Hordeum vulgare*) grains, in a presence of weeds (Table IV.6, Figure IV.12). The barely caryopses were angular, symmetrical, and widest at the middle or slightly below, and presenting, in some cases, the adhering remains of glumes. The seeds are 5.32 mm long and 2.75 mm wide on average, showing variation in size (±1.62 mm for length, ± 0.60 mm for width) dependent on its position of development within the spikelet. As a result of threshing, the seeds are separated from rachis fragments, but some charred grains are still partly covered with husk. Other chaff fragments were not recovered together with the barley grains. The barley grains examined under VP-SEM (Figure IV.12.b), do not show any sign of aleurone cell wall thinning (Heiss et al. 2020) that results from malting process. However, barley was exposed to humid conditions post-excavations, resulting in fungal growth inside of the seed.

Table 0.6 Identified carpological remains recovered from the wooden crate found in a courtyard of the house.

Taxon	Conservation conditions	Type of remain	Min. №. of units	Weight (g)	Average length (mm)	Average width (mm)
Cereals						
Hordeum vulgare L.	Charred	caryopsis	816	14.5	5.32	2.75
Weeds						
Bromus sp.	Charred	caryopsis	4	0.0221	5.77	1.61
Chenopodium album L.	Charred	fruit	2	0.0005	1.33	
Pisum sp. wild type	Charred	seed	4	0.0283	2.44	

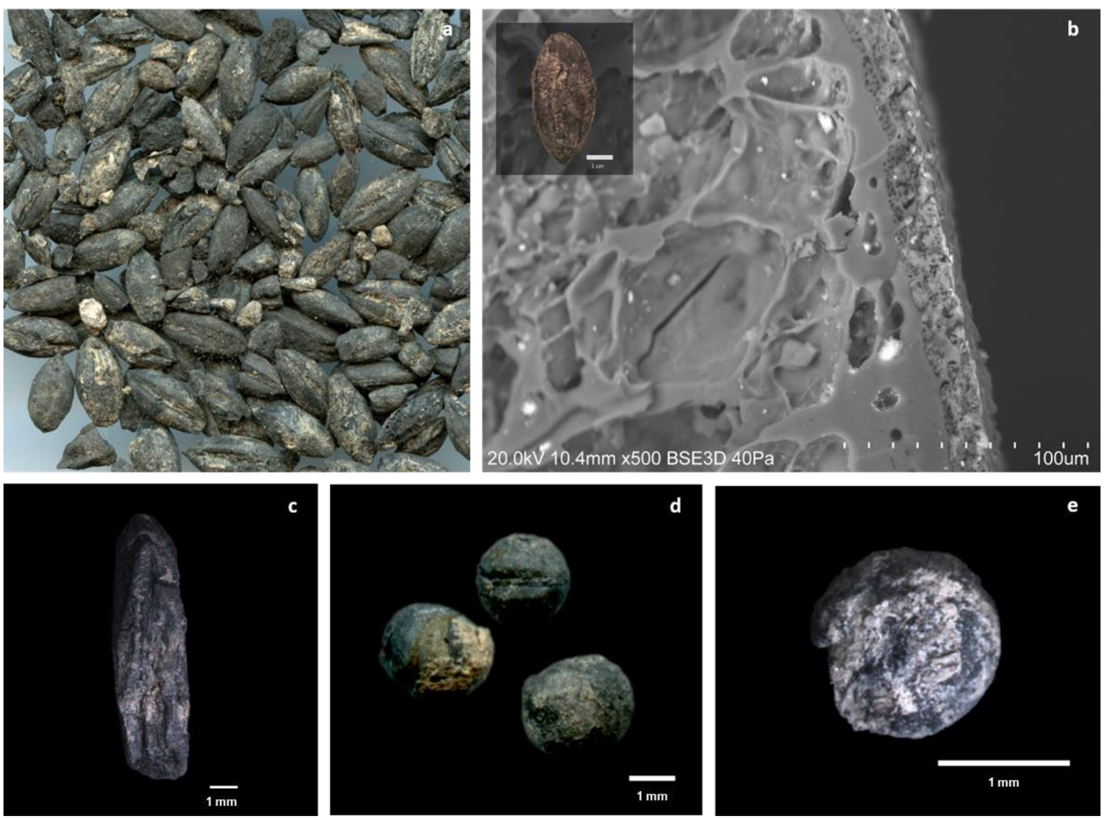


Figure 0.12 (a): Barley deposit (*Hordeum vulgare*), (b): cross section of barley seeds in SEM, (c): brome grasses (*Bromus* sp.), (d): pea (*Pisum* sp.), and (e): fat hen (*Chenopodium album*).

Among the barley grains, associated crop weeds were also identified: brome grasses (*Bromus* sp.), a wild type of pea (*Pisum* sp.) and fat hen (*Chenopodium album*). Charred grains of brome grasses (Figure IV.12c) were 5.77 mm long, and 1.61 mm wide. *Bromus* sp. can be found in fallow lands and in association with crops. *Pisum* sp. seeds (Figure IV.12d) were spherical with a small diameter of 2.44 mm on average and an oval hilum. Based on the sole morphometry of the archaeobotanical remains, it is not possible to reach an identification of the pea macroremains to the species level, but their size indicates a wild type. *Chenopodium album* (Figure IV.12e) is a common weed of almost all cultivated crops, in pastures and strips of uncultivated land, and along roadsides and riverbanks. The seeds found are flattened round, disk shaped, 1.3 mm in diameter. All these weeds are widespread and often appear in areas of human activity.

Amorphous charred organic residue recovered from the large cylindrical vessel (Figure IV.11) displays a heterogeneous and slightly porous structure with visible inclusions. It is well preserved due to closed deposition and carbonization, but the time and high temperatures also affected its structure and chemical composition. Chemical analysis by GC/MS reviled a complex profile with abundance of fatty acids, alkanes, alcohols and steroids (Figure IV.13, Table IV.7). 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. 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 is noted that high molecular weight alkanes are present in relatively low amounts and lack a clear odd-over-even chain length preference (Eglinton et al. 1962) to distinguish the plant matter.

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. Detection of faecal steroids, namely coprostanol, in addition to archaeological information derived from vessel typology (Marques 2020) from which organic matter was retrieved, allows the interpretation of the organic material as faecal matter.

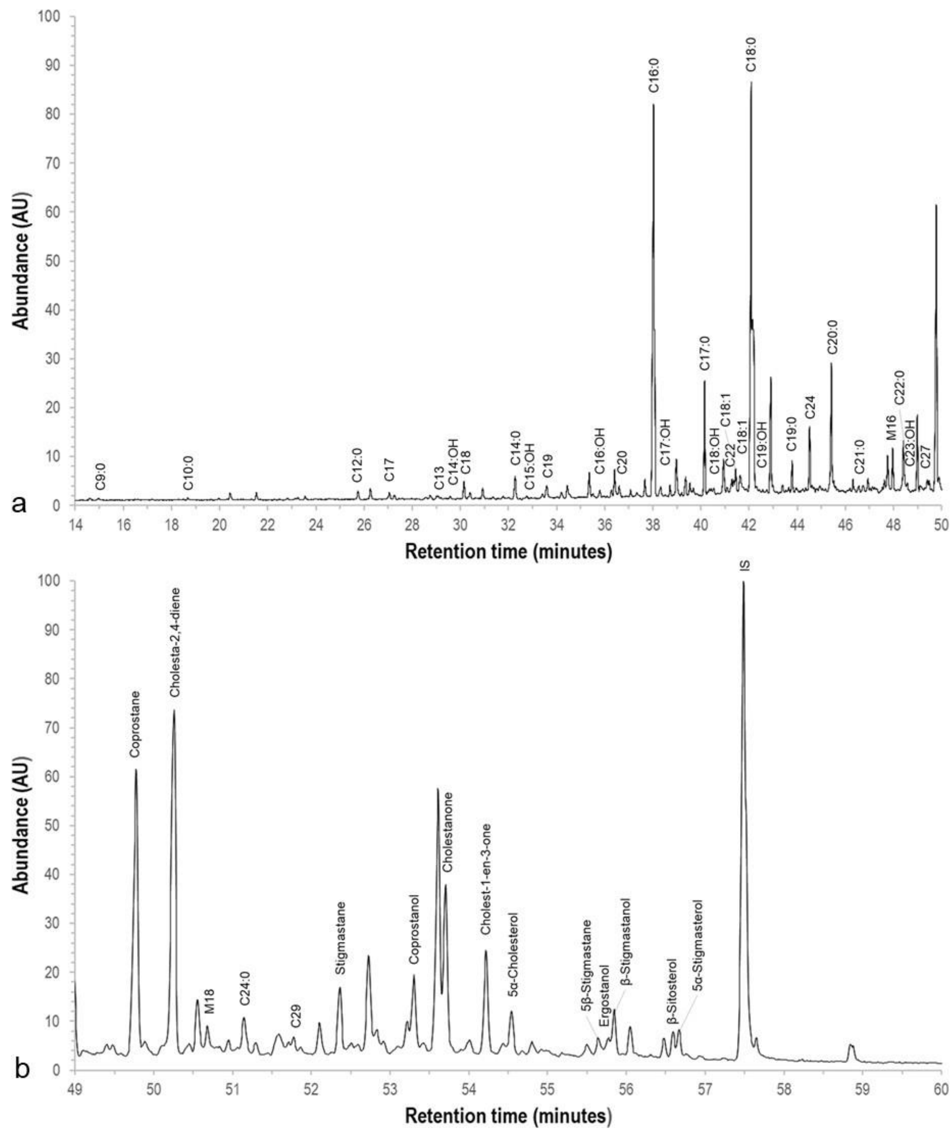


Figure 0.13 GC/MS chromatogram of organic residue (coprolite) recovered from Praça D. Pedro IV, divided into 2 segments. (a) from 14-50 minutes; (b) from 49-60 minutes. Marked are the trimethylsilyl (TMS) derivatives of relevant compounds detected. Cx alkane with x carbon atoms, Cx:OH linear alcohol 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.

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 (Bull et al. 2002, Prost et al. 2007, Harrault et al. 2019) 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 (Figure IV.14). In human coprolites (Ferezou et al. 1978, Furtula et al. 2012, Shah et al. 2007), coprostanol is the mostly commonly detected 5β(H) stanol, formed from both ingested and de novo biosynthesized cholesterol. Therefore, the chemical analysis supports that the cylindrical vessel discovered in the storage area was used to accumulate human waste.

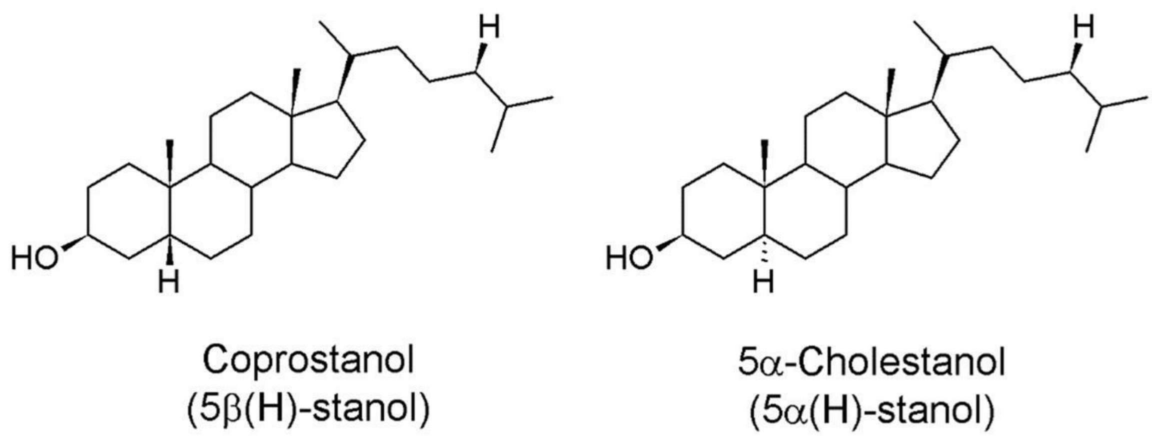


Figure 0.14 Chemical structures of coprostanol and 5α-Cholestanol.

Table 0.7 List of identified organic compounds from coprolite sample recovered from Praça D. Pedro IV, using GC/MS technique. *

	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	53.380		Tridecylic acid (C13:0)	29.078
	Cholestanone	53.706		Myristic acid (C14:0)	32.279
	Cholest-1-en-3-one	54.221		Palmitic acid (C16:0)	38.024
	5α-Cholesterol	54.542		Margaric acid (C17:0)	40.148
	5β-Stigmastane	55.642		Octadecenoic acid isomer (C18:1)	41.442
	Ergostanol	55.775		Octadecenoic acid isomer (C18:1)	41.608
	β-Stigmastanol	55.842		Stearic acid (C18:0)	42.142
	β-Sitosterol	56.583		Nonadecylic acid (C19:0)	43.783
	5α-Stigmasterol	56.675		Arachidic acid (C20:0)	45.415
Alcohols	Myristyl alcohol (C14:OH)	29.583		Heneicosylic acid (C21:0)	46.932
	Pentadecyl alcohol (C15:OH)	32.790		Behenic acid (C22:0)	48.407
	Palmityl alcohol (C16:OH)	35.792		Lignoceric acid (C24:0)	51.143
	Heptadecyl alcohol (C17:OH)	38.327	Alkanes	Heptadecane (C17)	27.042
	Stearyl alcohol (C18:OH)	40.425		Octadecane (C18)	30.408
	Nonadecyl alcohol (C19:OH)	42.325		Nonadecane (C19)	33.560
	Arachidyl alcohol (C20:OH)	45.608		Eicosane (C20)	36.590
	Tricosyl alcohol (C23:OH)	48.567		Docosane (C22)	41.075
Acylglycerols	Monopalmitin	47.960		Tetracosane (C24)	44.608
	Monostearin	50.676		Heptacosane (C27)	49.142
				Nonacosane (C29)	51.858

*The trimethylsilyl (TMS) derivatives of compounds detected.

The results of elemental analysis acquired with VP-SEM-EDS are in accordance with coprolite studies (Sperança et al. 2017), showing the faecal matrix enriched in phosphorus and carbon as a result of bacteria activity, while calcium, alongside with phosphorus, can be associated with the individual's diet. The minor amounts of magnesium, silicon, and iron, followed by sodium, potassium, manganese and aluminium are related to the presence of inclusions found within the faecal matrix. In fact, as seen in Figures IV.15.c-d, based on their composition, these inclusions can be divided in two types – calcium rich, and silicon predominant inclusions – and are likely fragments of digested food of animal and plant origin. Iron-rich inclusions of 50-80 μm in size can also be found throughout the faecal sample (Figure IV.15.b); these inclusions may be either remnants of ingested food, or evidence of the secretion of blood or tissue.

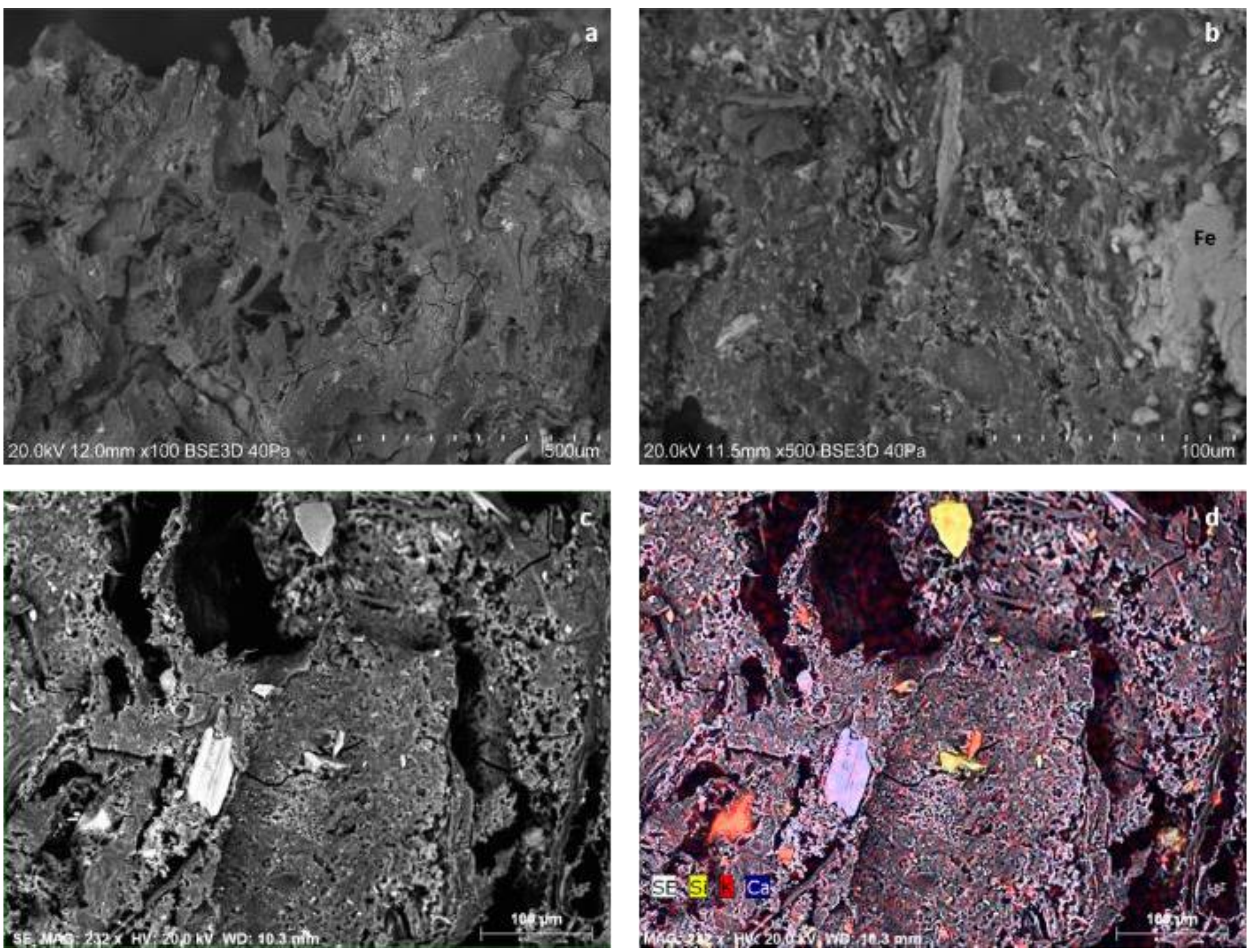


Figure 0.15 Micrographic and elemental analysis of the carbonized faeces, (a-c): Visible porosity and inclusions under VP-SEM, (d): elemental mapping showing calcium rich and silicon rich inclusions.

Pollen grains, besides the added standard of *Lycopodium* spores, were not detected. However, various non-pollen palynomorphs (NPPs) were identified (van Geel 2001, Miola 2012). Other micro-remains present in the examined faecal sample include plant remains, coprophilous fungi spores, larvae and urinary crystals and tissues. It is important to note that the absence of contemporary pollen grains suggests that the sample was not contaminated post-excavation.

Plant remains (Figure IV.16.a-d) visible in the sample include plant tissues and vessels. Elongate, polylobate and dendric tissues are characteristic of grass family, Poaceae, that include cereals (Rosen 2008, Neumann et al. 2019). However, the prevalent micro-remains in the sample are coprophilous fungi spores (Figures IV.16.g-h; Pals et al., 1980, Égüez et al. 2020). Non-coprophilous fungal spore of *Glomus* type (van Geel 2001) is also present (Figures IV.16.e-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.

The faecal sample also contained unidentified micro-particles that might have belonged to nematode, or roundworm larva (Figure IV.16i-k), but the state of preservation and absence of nematode eggs does not allow a certain determination (Brinkkemper and van Haaster 2012, Anastasiou et al. 2018). These remains are elongated, up to 200 μm long, fragmented and often coiled. However, the remains of crustacean specimen (Figure IV.16l), also present in the faecal sample, may indicate that these arthropods acted as carriers for parasitic worms. Urine crystals (Figure IV.16m-p) present in faecal remains were identified as cholesterol and cystine crystals. They occur in the urine of healthy individuals, but high concentration of these crystals can be indicative of a meat dominated diet rich in protein, which affects kidney function and causes formation of kidney stones (Zhang, et al. 2020).

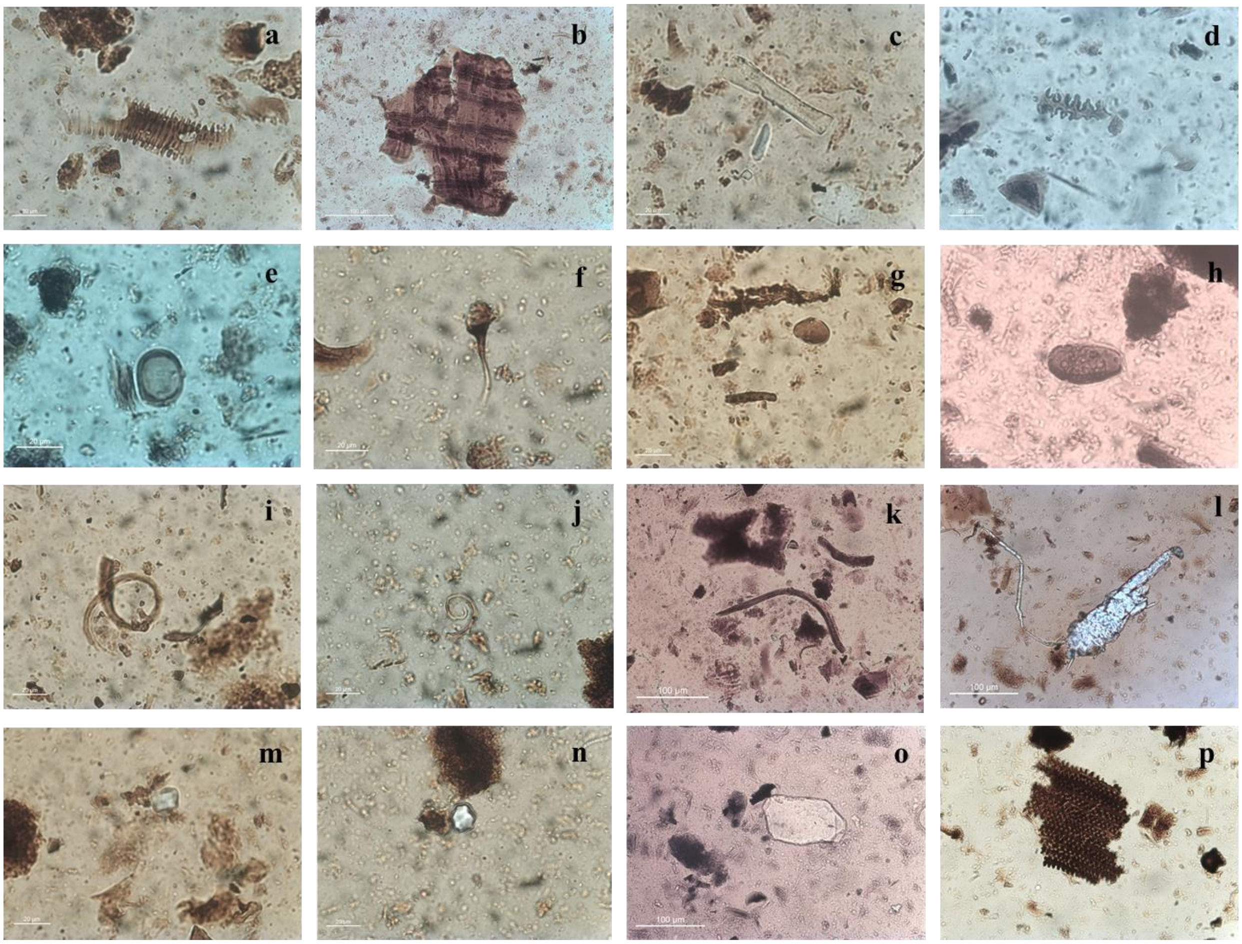


Figure 0.16 Micro-particles present in the carbonized faeces: (a-b): unknown tissue fragments, (c): elongate plant tissue, (d): dendric plant tissue, (e): fungal spore of *Glomus*-type (HdV-1103), (f): a spore filament, (g): cf. HdV-123, (h): fungal spore of *Podospora*-type (HdV-368), (i-k): unknown elongated remains, (l): remains of a crustacean, (m-o): urinary crystals, (p): epidermal tissue.

4.5. Discussion

Barley, alongside naked wheats, has been a dominant crop in the Iberian Peninsula throughout the ages being consumed by both animals and humans (Buxó i Capdevila et al. 1997, Peña-Chocarro et al. 2019). It is generally considered fodder, as a good source of energy and phosphorous and fed to donkeys, mules, and horses together with coarse cereal chaff. As stated, the barley grains were recovered from the wooden crate in the courtyard area, alongside the remains of the bush that might have been used for animal grooming. The archaeological context would suggest that the cereals were intended as animal feed, either for horses kept in the courtyard or smaller poultry like chickens (Henriques and Filipe 2020b). Nevertheless, in a bigger framework of Iberian Peninsula, barley was regularly consumed in various meals (Peña-Chocarro et al. 2019). Nutritionally rich, it is an important source of carbohydrates, dietary fiber, and vegetable protein. Barley flour was commonly used through the Middle Ages, mixed with flours from other cereals, nuts or legumes. Barley bread was revered in the late 15th century, but by the 17th century it was considered inferior to white breads (Gentilcore 2016). In the 1680's *Arte de Cozinha*, Domingos Rodrigues gives recipes for "*Pão de Ló fofo*" and *Pão de Ló torrado*" as well as other sweets made with flour (Rodrigues 1683). He also lists "*Capões cevados, guarnecidos com línguas fritas*" as a second dish for August, likely referring to a barley-fattened animal. Furthermore, considering Lisbon's eight century-long brewing tradition, barley was also grown for production of alcoholic beverages. Still the analyzed barley grains do not show evidence of malting process necessary for beer production. However, barley's adaptability and diversity of use made it an important crop in the agricultural system.

Amorphous charred organic matter, found in the storage area of the house quarters at Praça de Dom Pedro IV, was examined to obtain information regarding the diet and lifestyle of 18th century Lisbon. By the chemical profile it was identified as remains carbonized human faeces. Biomolecular analysis of coprolite can be a particularly powerful tool to discover the food choices and health status of both animals and humans (Reinhard and Bryant 1992, Bull et al. 2002, Shillito et al. 2020a). Lipid analysis that involves the identification of biomarkers in the coprolite, which are organic molecules that can be used as the "chemical fingerprinting" of food products or diseases, and even of the original species that produced the coprolite. Lipid molecules are part of all living organisms and have a very poor solubility in water, which precludes their leaching when the coprolites are buried. Lipids can be very complex molecules and present the advantage, when compared with other biomolecules, of being reasonably stable under adverse burial conditions. However, and despite this apparent stability, it is import to account for the diagenetic transformation expected for the different lipid biomolecules when analyzing coprolite data (Evershed and Connolly 1994). Still, when this information is complemented by the distribution of sterols, long chain alkanes and alcohols, it can be related to the dietary choices.

In the case of humans, the relative amount of compounds derived from the cholesterol and plant sterols can provide an idea about the correlations of animal/plant products consumed by an individual. The detection of the faecal markers is also possible from archaeological sediments and has often been linked to sewers, fuel material or manuring practices and connected to the increase in population density (Bull et al. 2003, Shillito et al. 2011, Baeten et al. 2012, Kaiser and Lerch 2022), but with lower values in comparison with a direct analysis of waste, gut content and coprolites (Evershed and Connolly 1994, Don et al. 2001, Nielsen et al. 2021, Shillito et al. 2020b) as is the case with Lisbon findings. Herbivores ingest large amounts of plant-derived sterols, therefore the feacal profile should show prevalence of sitosterol and stigmastanols (Evershed at al. 1997, Baeten et al. 2012, Sistiaga et al. 2014, Schroeter et al., 2020, Lerch et al. 2022). On the other hand, omnivore diet results in a much higher representation of choresterol and coprostanol in relation to stigmastanol (Bull et al. 2002, Leeming et al. 1996, Bull et al. 2002, Ledger et al. 2019, Shillito et al. 2020b, Zhang et al. 2020, Nielsen et al. 2021).

The pattern of faecal steroids reveals that the individual had probably 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 coprolite was subjected due to the fire after the earthquake. The importance of the animal products in the individual's diet is further confirmed by the pattern of long chain alkanes and alcohols. These compounds arise from the plant waxes and they are usually large peaks in the chromatograms of coprolites from herbivore animals. In the 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.

Integrating archaeological and chemical information allowed the interpretation of the vessel and its organic content. The vessel was recognized as c*alhandro*, a word used to describe a tall, cylindrical vase, intended for collecting waste and other filth (Marques 2020). Even though, activities associated with waste collection and disposal are common and relevant part of an everyday life, finds like these are not frequently recognized in the archaeological context. The tragic chain events caused by the Earthquake brought destruction to the city and its citizens, but intense fires also contributed to good preservation of organic remains. This allowed a glimpse directly into a home life in the 18th century and allowed us to remember and give honor to all lives that suffered on that 1st of November 1755.

4.6. Conclusion

Archaeobotanical analysis reflected agricultural and storage strategies for cereal grains, most likely intended as an animal fodder. Archaeological context including storage of barley in a wooden crate in a courtyard, near the water source and a partially roofed area contributes as well as the find of remains of a brush, supports such interpretation. Nevertheless, the presence of barley gives further information about its cultivation and importance for the 18th century society. The urban – agricultural network of Portugal allowed steady supply of cereals for animal feed as well as to be incorporated in a human diet, as supported by historical records.

Microstructural, elemental and chemical analysis of carbonized faeces provides information about dietary habits, health of the individuals and their lifestyle. The presence and abundance of human derived 5β-stanols, such as coprostanol, provided unambiguous evidence for human excrements. 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 prove a mixed diet based on both animal and plant sources. Elemental analysis and biomolecular information also support an omnivore diet. The abundance of calcium in the analyzed sample points to calcium rich diet that can come from dairy and dairy products, but also from sardines and leafy greens. Biomolecular analysis, on the other hand, 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 urinary crystals in the analyzed material show that the same container was used to deposit faeces as well as urine. Although these crystals are present in urine of healthy individuals, higher concentrations can point to a diet excessively enriched in protein which generally leads to the formation of kidney stones. The presence of coprophilous fungi spores in the faecal remains, affirm that the material was deposited over certain amount of time. Possible occurrence of parasitic roundworms would support the idea of limited hygienic conditions.

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Chapter V – Final remarks and future research

1. Studying food and balms from the archaeological sites

This research aimed to identify food sources of plant origin, as well as to determine the presence and the composition of balms and detect which plants were used in these complex mixtures. Based on the quantitative and qualitative data from six contextually diverse archaeological sites, organic remains originating from plant sources were examined in relations to funerary rituals, everyday diet, and technological practices. By analyzing plant remains on a macro, micro and chemical level, new information was provided regarding human-environment interactions. Application of the combined palaeobotanical and chemical approach contributed to the understanding of plant management and their use. Organic material from both funerary and domestic contexts, reflected the multifaceted role plant and plant products had in the past societies.

These analytical approaches, which combined botanical and chemical analyses, allowed inclusive studies of plant remains and traces from archaeological contexts. Sediments and site deposits were examined under stereomicroscope and subsampled for the microparticle analysis. Plant macroremains were separated by appropriate techniques, depending on their preservation form, and identified under stereomicroscope. When possible, macroremains, visible residues and site deposits were examined under SEM with higher magnification. Visible residues and sediments were screened for the presence of organic matter by ATR-FT-IR. If the spectra showed presence of organic material, archaeological samples were subjected to more sensitive chromatographic techniques to obtain chemical composition of the remains and identify their natural origin. The absorbed residues, preserved in pottery walls, after appropriate extractions, were analyzed using GC/MS. Visible residues and sediments were analyzed by Py-GC/MS, when it was possible, as this technique requires only a few micrograms of sample. VP-SEM-EDS and XRD were used to obtain the elemental and mineralogical composition of the inorganic fraction of the archaeological materials studied.

Considering the diversity of contexts and the archaeological materials, plant remains and state of preservation, following an outlined general workflow, the appropriate techniques were selected for each case study. This provided an abundance of information and in-depth study of the samples recovered from archaeological contexts. The multianalytical research was possible due to collaboration and state of the art infrastructures of the host institutions, Sapienza University of Rome, and HERCULES Laboratory of the University of Evora.

In the funerary contexts presented in the Chapter III, organic residue analysis of the remains absorbed into pottery sherds used in burial practices of the Neolithic–Chalcolithic communities of Central Portugal provided evidence for the use and processing of animal and plant products. The vessels from the biggest chambered megalithic tomb of the Alentejo region, the Zambujeiro Dolmen, contained residues of fats and oils of plant origin, as well as remnants of beeswax. These results indicate that populations on the Iberian Peninsula actively participated in the widespread exploitation of the honeybee products and placed the beginning of the Iberian apiculture to the at least the end of 4th and the beginning of the 3rd millennia BC. Regarding the treatment of the dead in the 4th and 3rd millennium BC, results of the analysis of pottery content from the hypogeum burial of Monte da Comenda 3, revealed that a lipidic substance, most likely plant oil, was used in connection to a specific burial. These results imply purposeful exploitation of oil rich plants to obtain substantial amounts of oil necessary for the use in the burial ritual. On the other hand, while the analysis of soil samples from the Etruscan necropolises of Cerveteri (7th/6th century BC) did not provide abundant plant remains, the detection of pollen grains and phytoliths in these archaeological sites, exposed close human-environmental interactions, reflecting the anthropogenic grassland surroundings. However, to uncover possible further organic content, the analysis of the absorbed organic residue of the vessels recovered from the burials would be necessary.

Analyses of plant remains from domestic contexts described in Chapter IV, that span from Prehistory to the Early Modern Period, illuminate important parts of everyday life: plant food selection, storage, and dietary choices. The study of cereal grains and of food remains from the vessels of the underwater Gran Carro settlement provided the evidence for agricultural and dietary variability in Italy during Early Iron Age. Crop selection demonstrated higher levels of millet production and its economic significance compared to the current known distribution of broomcorn millet in Central Italy. Even though millet was not grown as a main crop in the region, these new finds demonstrate its use and value in the society, offering additional harvest and enriching the diet of the local population. The results of the study of carpological remains found in silos of Medieval Lisbon, on the other hand, attest to importance and incorporation of fruits, during changing times, when Muslim and Cristian traditions, in direct contact, encouraged further agricultural development. As confirmed by the new finds from Largo dos Lóios, plant sourced diet, based on cereals and fruits, in addition to legumes, was a relevant part of the full food consumption of the Medieval Portugal.

The organic remains that were preserved in an excellent condition due to tragedy that struck Lisbon in 1755, the Great Lisbon Earthquake, gave an insight into storage strategies of barely grains. The results described in Chapter IV also provided archaeological material evidence for the urban – agricultural network of Portugal in the 18th century. This network allowed steady supply of cereals for animal feed, as well as cereals to be incorporated in the human diet. Chemical analysis of the organic residue from a large cylindrical vessel allowed the interpretation as an accumulate human waste and provided information about dietary habits and hygienic practices in the 18th century urban setting.

The transdisciplinary approach followed in this work not only permitted plant-based substances to be characterized, but also enabled more intricate questions to be addressed. During this project, since different analytical techniques were accessible, they were utilized on the same archaeological object, in an individualized manner. The choice of the analysis always took into account if its identification or if specific questions could be answered by employing non-destructive or minimally invasive techniques, following European Standard for Conservation of Cultural property. Initial identification was done by optical or scanning microscopy without sample preparation that would alter, contaminate or damage the samples. ATR mode of the FT-IR allowed direct molecular characterization on the solid samples, again without further preparation, and was employed as a screening technique to detect the presence of organic matter, before employing chromatographic techniques – PY-GC/MS and GC/MS – that do require sample preparation. Py-GC/MS in particular is a minimally destructive technique that enables direct analysis, and since there is no need for sample extraction, it is more time and cost effective, has a much shorter sample preparation, limiting sample loss and the possibility of contamination, and has proven to be an excellent tool in characterizing natural polymers such as resin-based vessel coatings or adhesives. However, being a standardless technique, Py-GC/MS only allows relative quantification and is applicable exclusively for residues that do not need extraction procedures, like visible organic residues.

The same principle of employing non-destructive or minimally invasive techniques was applied to the inorganic components of the sample. When possible, μ-XRD was used directly on the sample, instead of the more destructive XRPD, for mineralogical characterization, while SEM-EDS was used under VP conditions without the need of the addition of a conductive material on the surface of the samples. This preserved the samples in their original state and allowed reproduction of the analyses. Still, since chromatographic techniques are highly sensitive, exposing the samples to possible contamination during elemental or mineralogical analysis was avoided, by subsampling for specific technique and/or monitoring the order and sample requirements of each analysis.

Analysis of plant microremains, on samples from the archaeological sites, that included identification of pollen grains, NPPs and phytoliths, required various degrees of extraction. As this is inherently a destructive procedure to various degrees, careful planning, sampling and order of extraction steps were followed, employing the protocol for simultaneous recovery of multiple microfossils, to ensure good preservation of less resistant particles and provide time-effective and efficient extraction.

Throughout this work, versatile knowledge from social sciences and humanities was combined with that of natural sciences, adopting a transdisciplinary approach in the framework of Archaeometry and Cultural Heritage sciences. Focused on specific theme, that of plant remains from the archaeological sites, theoretical understanding, terminologies, and methodologies addressing the research questions were explored, crossing the boundaries of several disciplines and generating new ideas. It is not surprising that in the fields of Archaeology and Archaeobotany, which foundations have been strongly rooted in the interdisciplinary methodologies, with the focus on the dynamics of long-term cultural processes and the operation of human-natural systems and their consequences, strive to achieve an overarching synthesis of disciplines, develop new methodological and theoretical structures. The altering of discipline-specific approaches, a shift from the established disciplinary concentration on reconstructing the past, to a broader exploration of the processes underlying cultural transformation and change has emerged. While inter-, multi- and transdisciplinarity each provide different benefits and limitations, this shift in the approach, is not without its challenges. While transdisciplinarity is encouraged, hopefully beyond just its catchphrase value, and considered a development in tackling present and future global issues, it is still relatively poorly supported and rewarded. The very labels *-trans* and *-disciplinarity*, position this approach as additional to the established scientific disciplines, existing only among recognized institutions and spaces, without a distinct identity. This confines funding opportunities, as well as the employment desirability, hindering the development of such research projects and career paths. Further promoting the paradox of transdisciplinarity, the limitation of funding, access to equipment, time limitations and the required knowledge to acquire and incorporate all the analytical data, do not make these kinds of studies easily available.

International educational programs such as the Archaeological Materials Science (ARCHMAT) Masters program, and The European Joint Doctorate in Archaeological and Cultural Heritage Materials Science (ED-ARCHMAT), that this work is an aftereffect of, provide a space for such research projects, and establish a new category of a researcher, a Transdisciplinary Researcher. Even though, the demands of such formation are extensive, and relate to terminology, research goals, access to reference materials, and relevant bibliography, which can reduce the time and scope of research, it provides the required bridge, with its strength lying in its utilization of theories, methods, practices and interpretations from different fields, bringing the prospective for achievement of a common scientific goal while producing meaningful and impactful results. Hopefully, in time and with a development of more transdisciplinary studies and researchers, such approach will become more established, carving a much-needed area, also within major funding calls.

Since plants were a common resource in previous societies, but due to their fragility and selective preservation, they are underrepresented in comparison to inorganic materials. To discover evidence of plant use, sometimes it is necessary to look beyond what is experienced by the naked eye. The transdisciplinary approach enables the research to go further in the absence of visible, discover the value of dirt and heaps of secrets that it can hold with the material evidence of plant presence and use. Residue analysis can provide a more nuanced interpretation of human interactions within the natural environment. Combining archaeobotanical and chemical approach to the study of plant remains, when used deliberately, can expand our comprehension of the presence, use and meaning of plants and plant products in past societies.

2. Future research

As the work progressed, the questions raised led to ancillary research and revealed a number of other avenues that need to be perused. Future efforts based on strategic sample collection from newly excavated sites, hopefully, will allow for greater integration of the transdisciplinary approach. This methodology would not only allow for the identification of plant remains, but it would also enable more complex questions to be addressed on a wider scale. The advent of gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS) introduced the possibility of accessing stable isotope information from individual biomarker structures, opening up a range of new avenues for the exploitation of organic residues. The major advances in ancient DNA studies though high-throughput sequencing, the continued development of modern DNA databases and improved sample preparation, when applied to archaeological material, allow large scale questions involving plant evolution, taxonomy, domestication, and cultivation to be answered. For example, the possibility of spatial and temporal distribution of the beeswax residues in Iberia, Southern Europe and Northern Africa, in the framework of continuous climatic changes, recorded through relative ratio of hydrogen and oxygen stable isotopes, could uncover the extent of *Apis mellifera* product usage, trough future research. Furthermore, the study on the presence and use of Prehistoric oil plants in funerary context across Iberia can be expanded, incorporating also organic chemical analysis, supported by experimental data. More detailed morphological analysis of seeds and grains show potential for tracking the changes that occurred trough time within human-plant dynamics, especially when combined with experimental data, chemical research and ancient DNA, like for the example for the grape varieties in Southwestern Europe, that participated in post- domestication hybridization with local wild grapes. Through experimental studies, detailed observations of taphonomy could also provide new insights into the formation processes of archaeobotanical assemblages. This also includes studying the formation, identification, and preservation of amorphous remains, like food crusts, cereal food products and coprolites. Finally, the methodology employed here could be rewarded, if targeted at the identification of new biomarkers in degraded plant remains, thus improving a low taxonomic resolution of the organic residue analysis.

Appendix

In collaboration with the Croatian Conservation Institute, the results of the study Chemical characterization of the sacred wood: final resting place of Benedictine Abbots of St. Margaret, Bijela, were presented at, 11º Encontro Nacional de Cromatografia, 2019 in Caparica, Portugal.