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Contactos e envio de originais:
antoniovalera@era-arqueologia.pt

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ÍNDICE

EDITORIAL

Chegamos, com a presente edição, ao número dez dos volumes publicados da Apontamentos de Arqueologia e Património. Dez números em oito anos, com algum abrandamento e irregularidade nos últimos tempos relativamente aos primeiros. Nestes dez volumes publicaram-se 94 artigos, nos quais foram autores 80 colaboradores, que em vários casos aqui realizaram as suas primeiras publicações.

O projecto inicial, conforme se declarava no editorial do número um da revista, visava a "publicação de pequenos textos informativos ou problematizantes cuja divulgação por outros meios não se justifica por si só ou poderá ser demorada." Pretendia-se "contribuir para a rápida difusão, referenciável e citável, de informações, ideias, pequenos estudos ou análises, cuja disponibilização mais imediata seja importante para o desenrolar da investigação e da actividade arqueológica colectiva", respondendo desta forma às crescentes dificuldades financeiras que se colocavam às edições em papel e à proliferação da actividade arqueológica no âmbito da Arqueologia de Salvamento.

A intenção inicial, porém, viria a ser progressivamente alterada pela realidade. A tradicional tendência para publicar pouco, que sempre caracterizou a Arqueologia portuguesa nos seus mais variados âmbitos, tem mais a ver com uma postura que com qualquer ausência de meios.

Como resultado, a revista acabou por enveredar pela publicação de alguns textos de maior fôlego (que fogem a um Apontamento) a par de outros que melhor respondiam às intenções originais e o seu ritmo de publicação adaptou-se à produtividade daqueles que se disponibilizaram a colaborar.

O resultado, contudo, tem sido positivo, e a julgar pelas citações que, no país e no estrangeiro, os textos da Apontamentos têm merecido, a iniciativa ganhou já o seu espaço no panorama editorial da Arqueologia portuguesa.

Justifica-se, pois, o esforço e, como desde o início, a revista continuará aberta a todos os que com ela queiram colaborar

António Carlos Valera

POTENTIAL OF LIPID ANALYSIS ON PREHISTORIC PORTUGUESE POTTERY

Beatriz Bastos

Resumo:

Potencial da análise de lípidos em cerâmicas pré-históricas portuguesas.

Este projecto foi um estudo piloto com o objectivo de avaliar o potencial da análise de lípidos em cerâmicas arqueológicas Portuguesas. Trinta fragmentos foram analisados, provenientes de dois sítios pré-históricos: Perdigões e Bela Vista 5. Com o intuito de analisar os fragmentos através de GC-MS, 2g de pó de cerâmica foram recolhidos de cada fragmento e de diferentes secções dos mesmos: bordo, base e parede exterior. No total 40 amostras foram analisadas e os resultados comparados entre sítio, tipo de pote, tratamento das paredes e secção do pote. De Perdigões cerca de 1500µg de lípidos por grama de fragmento foram extraídos e 821µg de Bela Vista 5. Vários ácidos gordos livres entre outros lípidos foram identificados na maioria das amostras. Devido a degradação dos lípidos, contaminação moderna e a variabilidade na proporção dos diferentes lípidos, só foi possível identificar o conteúdo de um pote. Os resultados deste projecto demonstraram que lípidos podem ser extraídos de cerâmicas arqueológicas Portuguesas, contudo, também mostraram que a contaminação moderna e a degradação são os maiores problemas para este tipo de investigação.

Abstract:

This projected was a pilot study aiming to assess the potential of lipid analysis on Portuguese archaeological pottery. Thirty potsherds were analysed from two Portuguese prehistoric ditched enclosures: Perdigões and Bela Vista 5. In order to analyse the potsherds through GC-MS, 2g of ceramic powder were drilled from each potsherd and from different sections of the same: rim; base and exterior wall. In total 40 samples were analysed and the results compared through site, type of vessel, ware and section of the vessel. From Perdigões about 1500µg of lipid per gram of potsherd were extracted and 821µg from Bela Vista 5. Several free fatty acids and other lipids were identified in the majority of the samples. Due to lipid degradation, modern contamination and peak ratio variability only the content of one potsherd was identified. The results from this project demonstrated that lipids can be extracted from Portuguese archaeological vessels; however, they also show that modern contamination and degradation are major problems for this kind of research.

1. Introduction

In order to better understand the potential of lipid analysis on archaeological material from Portugal, potsherds from two sites were sampled (both ditched enclosures). With two sets of data from different geographical areas and chronologies it is possible to compare the results from each site, suggest any differences or similarities and propose hypotheses to explain them.

Lipid extraction has been successful on archaeological artefacts from Northern European countries including the United Kingdom and the Baltic (e.g. Evershed *et al.* 1994; Dudd *et al.* 1999; Craig *et al*. 2007). More recently the procedure has been applied in other European countries with warmer climatic environments, including Spain and Italy. (e.g. Evershed *et al.* 2008; Gregg *et al.* 2009; Colombini *et al*. 2005; Pecci *et al.* 2003a and b; Romanus *et al*. 2009; Pérez-Arantegui *et al*. 2011; Schellekens *et al.* 2013).

The objective of this project is to understand the potential and limitations of lipid analysis of Portuguese archaeological artefacts from prehistoric sites, by applying a well-known technique successfully used in other European contexts.

2. The sites

Perdigões is a complex of ditched enclosures, located about 2km northeast of Reguengos de Monsaraz, in Évora district, at the western end of the Álamo valley (Lago *et al*. 1998:48-50; Márquez *et al*. 2011). Due to its location (in a geological depression) with limited visibility to West, South and North, this settlement appears as an amphitheatre, with an entrance orientated to the East (Lago *et al*. 1998:48-50; Valera 2008:120-123; Márquez *et al*. 2011). The site has evidence of occupation between the end of the 4th millennium and the 3rd millennium BC (Late Neolithic and Chalcolithic).

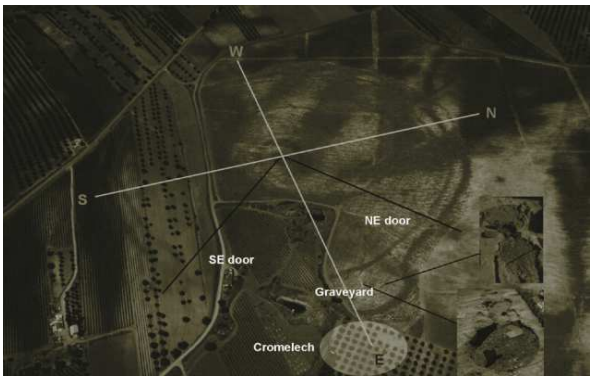

Figure 1 – Site Perdigões and spatial orientation. Source Valera 2008:120.

Bela Vista 5 is also a ditched enclosure settlement, located about 35miles (54km) southwest of Perdigões, in a town called Beringel in Beja district (Valera and Simão 2013:9-10). This settlement was founded in a flat area formed by natural erosion in the left bank of the stream Galego (Valera and Simão 2013:9-10). The surroundings of the site feature some small elevations with natural grooves created by seasonal outflow by rainfall (Valera and Simão 2013:9-10). Radiocarbon dating of the ditches placed the site occupation in the last 300years of the 3rd millennium BC, although there is evidence of earlier and later pits (Valera and Simão 2013:82-83). Both sites were excavated and investigated by ERA Archaeology SA.

Figure 2 – View of the site Bela Vista. Source Valera and Simão 2013:14.

The 15 potsherds selected from Perdigões were all recovered from the same structure: the hypogeum (Valera 2012). The 15 potsherds from Bela Vista 5 were found in one of the sections of ditch 1, the central ditch of the site (Valera and Simão 2013:14-17).

3. Lipids chemistry

Ceramic vessels, if not glazed, have a porous surface, which means that residues from the product contained in the vessels can be absorbed through the walls of the vessels (Evershed *et al.* 1992:188-189; Barnard *et al.* 2007:42-44; Evershed 2008). Thus lipids from foodstuffs or other products cooked or stored in those vessels can get absorbed in the matrix of the ceramic walls and survive full degradation (Heron and Evershed 1993:251-255; Barnard *et al.* 2007:42-44).

The chemistry of lipids and their presence in both animal and plant tissues allows us to enquire the type of foodstuffs that were cooked, stored or used in any way in unglazed ceramic vessels. Thus lipid analysis in archaeological ceramic vessels can provide useful information concerning diet and other cultural practices (e.g. illumination, ointments, and sealants) (e.g. Evershed *et al.* 1990; Charters *et al.* 1993a and b; Heron and Evershed 1993; Evershed *et al.* 2001).

Lipids are compounds that are involved in different biological processes and serve as energy stores in both animal and plant tissues (Perkins 1993: 1-19; Gurr *et al*. 2002:1-3, 93; Christie and Han 2010:3). Since lipids are hydrophobic (insoluble in water) they are more likely to survive in an archaeological context than other soluble compounds or molecules such as proteins or carbohydrates (Perkins 1993:1; Heron and Evershed 1993:251-255; Gurr *et al.* 2002:1-3; Barnard *et al.* 2007:42-44; Christie and Han 2010:4-5).

Within the different molecules that belong to the group lipids, the most studied are: fatty acids (and derivatives), phospholipids, waxes and steroids (Perkins 1993: 1-17; Barnard *et al.* 2007:42-44; Christie and Han 2010:4-5). Fatty acid is a carboxyl acid with a long aliphatic chain that, depending on the carbon-carbon bonds, can be saturated (single bond) or unsaturated (double or triple bonds) (Perkins 1993:1-3,10-17; Gunstone 1992:1-3; Christie and Han 2010:5-11). Unsaturated fatty acids can be divided in two subcategories: monounsaturated (one double bond); and polyunsaturated (more than one bond) (Perkins 1993:10-17; Gunstone 1992:1-3). The proportion of saturated and unsaturated lipids is different between fats and oils: oils have more unsaturated acids than fats (Gunstone 1992:6-8; Gunstone 2004:52-55).

The structure of lipids is directly related to their chemical properties (Gurr *et al.* 2002:1-3). Acylglycerols are the result of the combination of a glycerol (sugar alcohol compound) and a number of fatty acids (Gurr *et al.* 2002:93; Gunstone 2004:66). From those acylglycerols, triacylglycerol (TAG) is one of the main components of natural fats and oils, and results from the esterification of three fatty acids to a glycerol molecule (Perkins 1993:1-3; Gurr *et al.* 2002: 93-99; Christie and Han 2010:11). Less common are mono- and diacylglycerols, which have only one and two fatty acids attached (Gunstone 1967:2; Gunstone 2004:66-67; Christie and Han 2010:11-12). However due to a degradation pathway known as hydrolysis, triacylglycerols can lose fatty acids which results in free fatty acids (fig.3) (Skibo 1992:97-100; Evershed *et al.* 1992:195-197; Evershed *et al.* 2002).

Other important lipids are phospholipids, waxes and steroids. Phospholipids are structural components present in the cell

membranes and waxes have long chain structures of alcohols and fatty acids; and are produced by animals and plants (Perkins 1993: 3-8; Gunstone 1992:9-10; Heron and Evershed 1993:251-255; Gunstone 2004:68-71). Examples of sterols are cholesterol (animal), sitosterol and stigmasterol (plant) (Evershed *et al.* 1992: 195; Perkins 1993:4; Heron and Evershed 1993:251-255; Gunstone 2004:26).

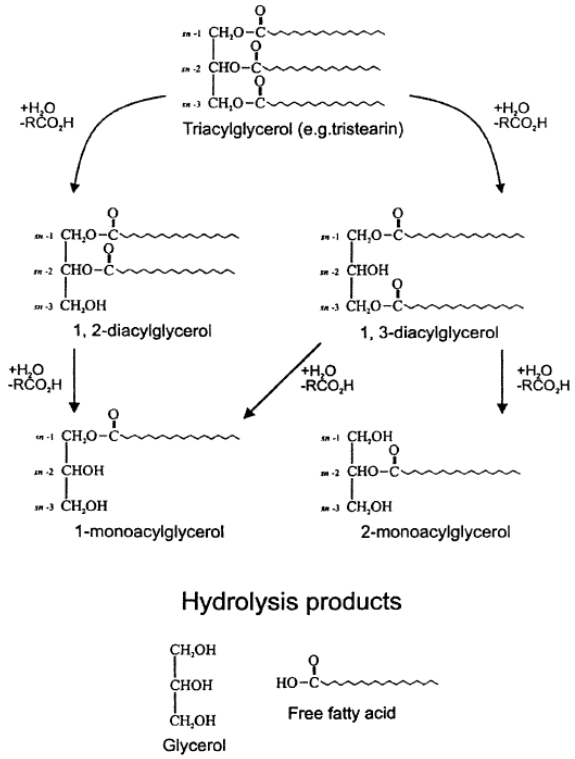

Figure 3 – Hydrolytic pathway for the transformations of triacylglycerols to free fatty acids. Source: Evershed et al. 2002:662.

The detection of sterols in ceramic vessels can determine if animal or plant products were processed in those vessels, however contamination is very common since cholesterol can be transferred to the vessels just by touching it, once cholesterol is one of the lipids present in the human skin (Evershed *et al.* 1992: 202; Stott *et al.* 1999).

4. Method and instrument

From the several techniques developed and applied in archaeological samples in order to extract and interpret the lipid data, gas-chromatography mass-spectrometry (GC-MS) it is the most commonly applied in archaeology, due to the quality of results and the wide range of compounds that can be identified through these instruments (Evershed *et al.* 1990; Evershed *et al.* 1992:191-192).

The GC separates the different molecules within the analysed sample and the MS shows the mass of each molecule allowing the identification of each lipid present in the sample (Evershed *et al.* 1992:191-193; Pollard *et al.* 2007:174-176). Both instruments (GC and MS) can analyse volatile and semi-volatile compounds and samples with small amounts of lipids since they are high sensitive techniques (Evershed 1992; Evershed *et al.* 1992: 191-193; Pollard *et al.* 2007:174-176).

Simulation studies have indicated that lipid degradation occurs and only a small fraction of the total capacity of lipid absorption of a ceramic vessel survives in the archaeological record and is able to be extracted and analysed (Charters *et al.* 1993a; Evershed 2008).

5. Procedure

In total, forty samples were analysed through GC-MS, collected from the thirty potsherds. Different areas of the vessels were sampled aiming to observe the lipid concentration throughout the vessel and possible contamination or degradation related to the burial context. All the pots were sampled in the rim area with the exception of one potsherd with no rim (BV5-461).

The rim was preferred as the main section to be sampled due to the results of previous studies that demonstrated, by comparing data from modern vessels (simulated experiences) with archaeological data, that there is higher concentration of lipids in the rim of the vessels in comparison with the base and middle wall (Charters *et al.* 1993a; Evershed 2008). This indicates that (at least during cooking process) more absorption occurs near the surface of the cooking ingredients resulting in a higher absorption in the rim (Charters *et al.* 1993a; Evershed 2008).

About 2g of ceramic powder were collected by drilling the potsherds on the selected area of the vessel, to a depth of around 2/3mm (fig.4). Prior to this, the surface layer of the vessels were gently removed (with a softer bit) to remove possible contamination[1].

To extract the lipids 10mL of dichloromethane:methanol (2:1 v/v) was added to the ceramic powder, following by ultrasonication for 5 minutes. To achieve separation, the samples were centrifuged at 2000rpm for 5 minutes at room temperature. The product extracted (the 20mL) was then dried until about 2mL under a stream of nitrogen and gentle heat (about 40°C) on a hotplate.

[1] During the laboratory procedures safety measures were always taken (e.g. gloves, goggles). All the instruments and tools used were properly clean at all times. To assess laboratory contamination, blanks were run with every batch of samples (1 per each 7 samples). Each sample was assigned a number, a code related with the type of sample (e.g. BA is a sample from the rim area of the vessel) and a colour associated with the stage of the sample (green: ceramic powder; yellow: solvent extract; blue: derivatised residue). When a ceramic vessel was sampled more than once, a sequential number (showing the order in which it was sampled) was added to the assigned number after a dot (e.g. 3.1; 3.2; 3.3).

Each 2mL of extraction was then transferred into smaller vials, to add the internal standard. The internal standard added was about 10µg of tetratriacontone (C34) to help in the quantification of lipids per sample.

Before derivatisation, the solvent was completely dried under the same conditions stated before. The derivatisation of the samples was achieved by silylation: about 100µl of N,O-bis (trimethylsilyl)trifluoracetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was added. The samples were then heated on a hotplate for thirty minutes (at 40°C) and then completely dried under a stream of nitrogen and gentle heat. Afterwards ten drops of DCM were added to the dried residues, so they could be transferred into the GC vials to be analysed[2].

Figure 4 - Example: Potsherd 153 from Bela Vista 5 after being sampled in the rim and base section.

6. Results

A list of known typologies from Perdigões and Bela Vista 5 was created based in previous publications (e.g. Lago *et al.* 1998:80-90; Valera and Simão 2013).

6.1. Results from Perdigões

In order to confirm if more lipids are absorbed in the rim area than in the base, the potsherds 5202 (BA3.1 and BB3.2) and 5222 (BA4.1 and BB4.2) were sampled in both rim and base section (fig.5 and 6). The results from the potsherd 5202 corroborate the studies mentioned above (e.g. Charters *et al.* 1993a), more lipids were extracted from the rim (BA3.1) that from the base (BB3.2). In the other vessel (5222) there were more lipids in the base (BB4.2) than in the rim section (BA4.1), however the difference is very small (1.31µg lipid g^{-1} potsherd). One possible explanation for this result is the fact that 5222 is a bowl with carination less likely to be used for cooking but instead as "tableware". In this case it is plausible to assert that if the vessel is not subjected to fire action the lipids will less likely be absorbed in the rim. Therefore the lipid absorption can vary between types of pot.

Two samples were taken from the exterior wall of the vessels 5202 and 5158 to assess possible contamination from the soils or other contamination source (fig.7).

The samples from vessel 5202 demonstrate expected results, which is more lipid from the rim that from the exterior wall. However, vessel 5158, bowl with carination, shows the reverse, with a difference of 1.45µg lipid g^{-1} potsherd (grams per potsherd). These outcomes may result from contamination. Heron and colleagues (1991) have demonstrated that contamination by transference of lipids from soil to the ceramic matrix is not likely to occur (Heron *et al.* 1991). Even so contamination by soil or other organic material cannot be completely excluded since no control samples from the soils were collected. Furthermore, the walls of the vessel 5158 were burnished/polished which could influence the absorption of lipids and explain the low amount extracted.

Ceramic typology	
Bowl with carination	Open form, shallow in depth with carination below the rim
Bowl type 1	Open or slightly closed forms with convex or flat base.
Bowl type 2	Open or closed forms with convex or flat base. Deeper than bowl type 1
Globular	Vessel with round shape
Truncated vessel	Vessel with truncated shape
Plate	Open form and shallow in depth with convex or flat base
Carination	Vessel with low carination (near the base)

Table 1 - Typology of the vessels. Adapted from Lago et al. 2007:80-85 and Valera and Simão 2013:68-72.

[2] The instrument used was GC Agilent 7890A Series connected to a 5975C Inert XL mass selective detector (MS).

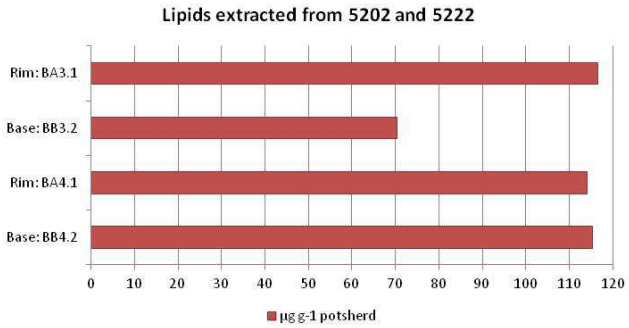

Figure 5 - Lipid extracted from vessels 5222 and 5202 per section (rim and base): BA3.1 – 5202 rim; BB3.2 – 5202 base; BA4.1 – 5222 rim; BB4.2 – 5222 base.

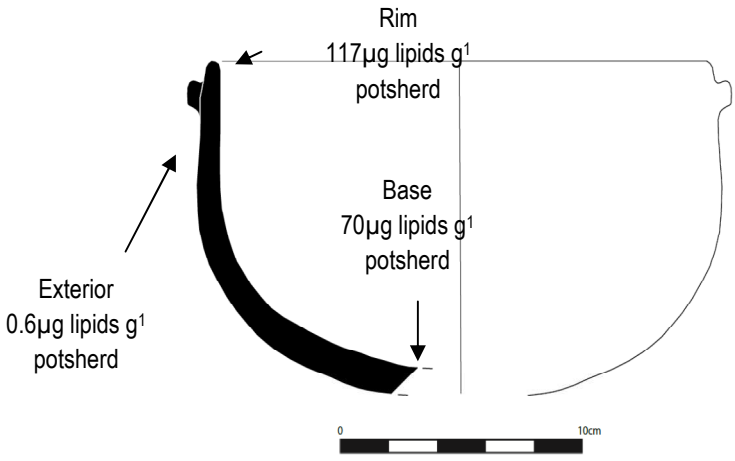

Figure 6 - Vessel 5202 and amount of lipids extracted per section of the vessel

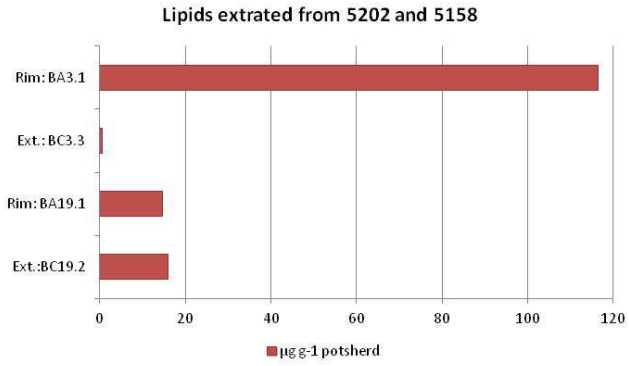

Figure 7 - Lipid extracted from vessels 5202 and 5158 per section (rim and base): BA3.1 – 5202 rim; BC3.3 – 5202 exterior wall; BA19.1 – 5158 rim; BC19.2 – 5158 exterior wall.

Two different types of vessels yielded basically no lipids: a bowl type 1 (5180) and a bowl with carination (5225). The plate 5154 also has low amount of lipids: 0.5µg lipid g^{-1} potsherd. Both interior and exterior walls of this vessel show evidence of intense polishing/burnish. The act of polishing the surface of a vessel turns the ceramic walls more compact, thus, limiting the capacity to absorb lipids into the ceramic matrix. Hence, the low amount of lipids extracted from this plate can be related with this pre-firing treatment or decoration, as with the vessel 5158.

In the case of the two vessels with no lipid extracted (5225 and 5180) this hypothesis cannot be applied as they only show smoothing of the walls. Smoothing will not compact the ceramic particles in the same way as polishing/burnishing. One hypothesis is that these vessels never contained oils or fats, or they experienced complete lipid degradation.

The fourth and fifth vessels with less lipids g^{-1} potsherd were the 5163 (bowl type 2) and 5153 (truncated vessel). Both potsherds have smoothed walls. Due to their size and shape is possible they were used as storage vessels. By being used for storage and not to process foodstuff it is plausible that less lipid absorption would occur. In other case, these vessels could have been used to store water or other liquids that yield little concentrations of lipids.

The bowl type 2 (5202) analysed from Perdigões yielded about 116.5µg lipids g^{-1} potsherd in the rim area, 70.4µg lipids g-1 potsherd in the base, and 0.6µg lipids g^{-1} potsherd in the exterior wall. The interior surface of this potsherd had been just smoothed confirming that this pre-firing treatment does not have a major impact on lipid absorption in comparison with polish/burnish. This vessel yielded more lipids from the rim than from the base section, which could mean that this vessel was used for cooking. The lipid extracted from the other bowl type 2 (5163) was only 7µg lipids g^{-1} potsherd. Both walls of this vessel are covered in concretions which could have affected the preservation/degradation of lipids.

From the bowls with carination only one potsherd yield more than 115µg lipids g^{-1} potsherd (5157) with about 843.3µg lipids g^{-1} potsherd. Unfortunately the vessel 5157 was the only truncated bowl with high carination analysed, so it was impossible to confirm if this variation is related with the subtype of bowl with carination.

6.2. Results from Bela Vista 5

Two vessels, a bowl type 1 (153) and a globular (470) were sampled in the rim and exterior wall (fig.8). The vessel 153 was also sampled in the base section, since it was the only potsherd from this site with a base. The lipids extracted from these samples confirm the results from Charters and colleagues's (1993) study: more lipid concentration in the rim section than in the base (Charters *et al.* 1993a). The exterior samples have lower lipid g^{-1} potsherd which excludes contamination and confirms the findings by Heron *et al.* (1991).

The vessel with carination (461) was found to have fewer lipids. This may be related with the fact that it was sampled in the section of the carination which is in this vessel closer to the base than the rim.

The vessel 414 (bowl type 2) is the second potsherd with a lower concentration of lipids. The surfaces of the vessel walls were just smoothed. Due to the ware and fabric of the vessel it may be argued that it was used as a storage vessel, similar to the vessels 5163 and 5153 from Perdigões.

The vessels type globular (429, 470 and 474) were the second type of vessels with more lipids extracted. However, only one of these vessels (429) had more than 3.5µg lipids g^{-1} potsherd. The potsherd 429 had a total of 418µg lipids g^{-1} potsherd while the other two had 1.8µg (470) and 5µg g^{-1} potsherd (474). These discrepancies may be related with pre- or post-firing treatments. The vessel 429 shows only the smoothing surface treatment, while potsherd 474 seems to have been polished/burnished in the interior walls. The walls of the other globular vessel (470) were subjected to greater erosion, which could mean the loss of the lipids absorbed in the matrix of the ceramic. Another alternative hypothesis is that these types of vessels were primarily used for storage of foodstuff or liquids like water.

The type of vessel with more lipids extracted was the bowl type 1. The vessels 152 and 153 had in total 341µg lipids g^{-1} potsherd: 300µg lipids g^{-1} potsherd and 41µg lipids g^{-1} potsherd respectively. Potsherd 153 has both surfaces polished/burnished, while the walls of 152 were only smoothed ensuring better conditions for lipid absorption, which can explain the different amount of lipids extracted from each bowl.

The plates from Bela Vista 5 can be sub-divided into different types concerning the shape of their body and the type of rim. The plates from this study can be sub-divided in 3 major categories: plate with simple rim (412); plate with thick rim to the interior (154,421,550 and 430); and plate with a flat base (426,541 and 501). The results per sub-type are in fig. 9.

The plates with thick rim to the interior had more lipid absorbed than the other sub-type of plates. Plate 154 was the one with more lipids: 29.4µg lipids g^{-1} potsherd. The other plates from this sub-type yielded less than 2µg lipids g^{-1} potsherd. All the plates from this sub-type have a rough exterior wall, only the interior walls were smoothed (430) and polished (154; 421; 550). These last three plates seemed to have been burnished in the rim section. From the sub-type with a flat base, vessel 426 had more lipids (4µg lipids g^{-1} potsherd), while the other potsherd did not yield more than 1µg lipids g^{-1} potsherd.

No lipids were extracted from the only plate with simple rim (412). The ceramic walls of this plate were very compact and difficult to drill to obtain the ceramic powder, which could have affected the capacity of lipid absorption. The low amount of lipids obtained from the plates confirms that vessels used as tableware absorb less lipids than the vessels used for cooking.

Almost all the potsherds had concretions, especially in the interior walls. To enquire if those concretions would affect lipid preservation/degradation, about 1.7g of concretions (BD11.2) were collected from the vessel 426. This sample did not yield the common fatty acids identified in the other samples (e.g. C16:0 and C18:0). The absence of common fatty acids may reflect that these concretions do not contaminate de vessels since they do not yield lipids. These concretions were confirmed to be of calcareous nature by adding a drop of 2M hydrochloric to a small portion of the sample.

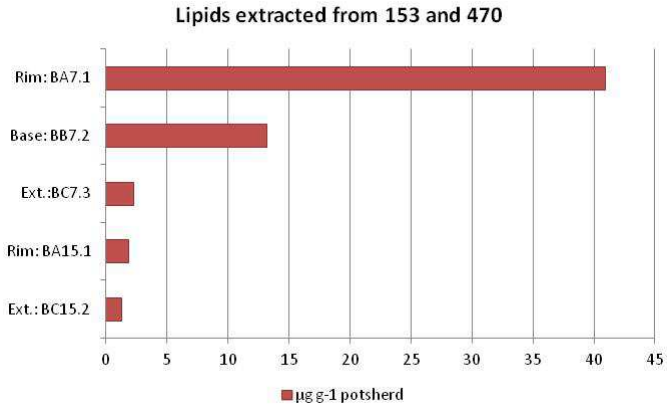

Figure 8 - Lipid extracted from vessels 5202 and 5158 per section (rim and base): BA3.1 – 5202 rim; BC3.3 – 5202 exterior wall; BA19.1 – 5158 rim; BC19.2 – 5158 exterior wall.

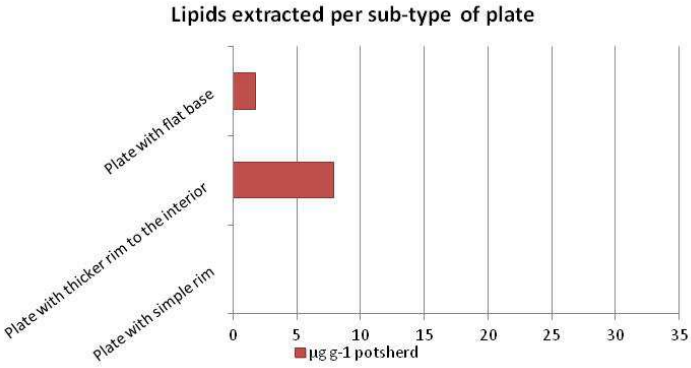

Figure 9 - Average of amount of lipid extracted per sub-type of plate in µg/g^{-1} potsherd.

7. Preservation/degradation between sites

The total amount of lipids extracted from each site was 821µg lipid g^{-1} potsherd from Bela Vista 5 and 1500µg lipid g^{-1} potsherd from Perdigões. Soil characteristics are an important factor for the preservation of organic matter. The paleoenvironmental investigation preformed with soil samples from Perdigões has proved that even in such arid

environments organic matter can be preserved (Wheeler 2010). However, there are no published investigations concerning organic preservation from Bela Vista 5, thus the potential of organic matter preservation is unknown for this site.

Other factor that could influence the preservation of lipids is the depth at which the potsherds were buried. The depth of ditch 1 of Bela Vista 5 varies from 0.20m to 1.5m, while the hypogeum from Perdigões was close to 2m (Valera 2012). If the vessels were closer to the surface it means they undergo more damage: water leaking; human activity (e.g. agriculture; manuring); among others.

Chronology does not justify this difference as it would be logical that the older vessels would have more lipid degradation, and that is not the case since potsherds from Perdigões are from Late Neolithic (Valera and Silva 2011) and Bela Vista 5 from Chalcolithic (Valera and Simão 2013).

From the type of vessels analysed only three were common in both sites (plates, bowl type 1 and 2) which limits the comparison of sites. Nevertheless, plates had low amounts of lipids, which can be related with the use of these vessels as "tableware" and not for cooking, and in both sites pre- and post-firing treatments affected the capacity of lipid absorption of the vessels.

8. Contamination

Plasticisers from plastic bags or laboratory instruments are very common contaminates in archaeological samples. Besides them, treatments of the vessel wall such as sealants, glues and varnishes can leave their own lipid "fingerprint" (Evershed *et al.* 1992: 194; Regert *et al.* 1998). In the samples analysed, plasticisers and cholesterol were the only contaminants identified.

9. Lipid identification and peak ratios

The most common lipids identified during this analysis were the free fatty acids C16:0 and C18:0. The presence of these free fatty acids is very common in archaeological samples due to the degradation pathways of lipids (e.g. hydrolysis) that converts triacylglycerols into mono- and diacyglycerols and then in free fatty acids (Skibo 1992:97-100; Evershed *et al.* 1992:195-197; Evershed *et al.* 2002). In some samples it was possible to identify traces of monoaglycerols (MAGs) which means that some preservation occurred.

Within the free fatty acids identified the majority was saturated (e.g. C16:0 and C18:0), which may result from the degradation through oxidation of the unsaturated fatty acids thus increasing the proportion of saturated acids (Malainey *et al.* 1999a). Even so, some unsaturated acids were identified: C18:1[1] (different isomers) and C16:1. In smaller quantities were also identified: C12:0; C14:0; C15:0. Only traces of the fatty acids with longer carbon chains (≥19) were present in some of the samples. No lipids associated with wax products or other derivatives from plants were identified (e.g. ketones) with exception of sitosterol in one sample (BA27), a truncated vessel from Perdigões (5153).

Cholesterol was identified in 75% of the 40 samples (12 from Perdigões and 18 from Bela Vista 5). However this sterol cannot be directly assessed as an evidence of animal fat since in more than 85% of those samples (with cholesterol) squalene was also identified. Squalene is a natural skin moisturizer which can be transferred to the vessel wall, along with cholesterol, by touching the vessel, which means that the cholesterol identified may be from modern contamination.

Besides lipid identification, other methods have been developed by comparing the peak ratios of modern oils and fats with the archaeological record (Skibo 1992:81-102; Malainey *et al.* 1999b; Eerkens 2005). Some problems related with these methods are: lipid preservation; natural degradation pathways by lipids; chemical changes in the lipid structures conducted during extraction in the laboratory; contamination prior or post excavation; among others (Evershed *et al.* 1992: 188-191,195-206). Normally the peak ratio used is between C16:0 and C18:0 once they usually survive in the archaeological record.

Since the free fatty acids C16:0 and C18:0 were identified in 95% (C16:0) and 93% (18:0) of all samples the ratio between them was calculated. The average of the ratio C16:0/C18:0 for the samples was 1.74 ±0.83 (excluding the sample BA30 that had 20.6). In order to understand and confirm the variability of peak ratios, 12 samples were analysed by the GC-MS twice in a row. From those 12 samples, in only 9 was it possible to calculate the C16:0/C18:0 ratios (table 2).

The results obtained demonstrate that the ratio between free fatty acids can vary, which limits the accuracy of the methods that use peak ratios to identify the source of fats and oils. Even though that variability is only σ±0.23 (excluding the ratios from sample BA30), one common issue with this method is that different animals and plants can have similar C16:0/C18:0 ratios.

Aiming to assess if potsherds from the same vessel would show differences concerning lipids extracted, two vessels (one per site) were sampled in different fragments. The potsherds 5156 and 5164, bowl with carination, (samples BA29 and BA30) from Perdigões are from the same vessel, but during the first stage of identification and separation of the archaeological record each fragment was attributed with a different number. About 65µg lipid g^{-1} was extracted from fragment 5156 and only 19µg lipid g^{-1} potsherd from 5164, minus 46µg than the first fragment.

Besides the variation in the amount of lipids extracted there was a noticeable difference in the lipid composition and the peak ratios. The C16:0/C18:0 ratio from sample BA29 was 1.88 in the first run and 1.98 in the second (σ±0.10). However, the other fragment (sample BA30) had a ratio of 20.59 (first run) and 41.29 (second run), with a difference of

[1] Is important to note that the preservation of the C18:1 is not uncommon in archaeological samples, since it is less prone to degradation than the other unsaturated fatty acids (Skibo 1992:93-94; Malainey *et al.* 1999a).

C16:0/C18:0 ratio				
Site	**Samples**	**Run**	**C16:0/C18:0**	**Difference between runs**
Bela Vista 5	BA13.2	1st	1.80	0.58
Bela Vista 5		2nd	1.22	
Bela Vista 5	BC15.2	1st	1.22	0.08
Bela Vista 5		2nd	1.14	
Perdigões	BC19.2	1st	1.33	0.08
Perdigões		2nd	1.25	
Bela Vista 5	BA25	1st	1.81	0.38
Bela Vista 5		2nd	1.43	
Perdigões	BA27	1st	0.69	0.10
Perdigões		2nd	0.58	
Perdigões	BA28	1st	1.36	-0.08
Perdigões		2nd	1.45	
Perdigões	BA29	1st	1.88	-0.10
Perdigões		2nd	1.98	
Perdigões	BA30	1st	20.59	-20.69
Perdigões		2nd	41.29	
Perdigões	BA31	1st	2.68	0.13
Perdigões		2nd	2.55	

Table 2 - C16:0/C18:0 ratios of nine samples from two different runs.

20.69 between runs. These values not only demonstrate that degradation can vary between fragments from the same vessel, but also how variable free fatty acids ratios can be due to degradation pathways. Hence, fragments from the same pot that undergo different levels of lipid degradation (e.g. due to soil) will display different lipids and ratios resulting in misleading conclusions.

The samples from Bela Vista 5 are from the potsherd 541 that has two fragments. The samples from these potsherds had 0.4µg (BA13.1) and 0.2µg (BA13.2) lipid g^{-1} potsherd, with a difference of just 0.2µg. The C16:0/C18:0 ratio of sample BA13.1 was 1.24, while sample BA13.2 had 1.8 and 1.22 (first and second run) which average is 1.52. In this case the ratio variability does not seem to be very significant (0.28), especially compared with the difference between the samples mentioned before.

Only 4 of the 40 samples had more C18:0 than C16:0 and one of them was the same sample with sitosterol (BA27). Based on the research by Malainey and colleagues (1999a) ≥25% of C18:0 can mean the presence of fat from large herbivores (Malainey *et al*. 1999a). The presence of animal fat does not exclude the possibility of plant residues since other studies have proved that the lipids absorbed in the ceramic matrix do not replace the lipids absorbed before, the lipids are accumulated instead (Charters *et al.* 1993a). Thus, accepting Malainey *et al.* criteria, the vessel 5153 may have contained at the same time, or in different moments of use, animal and plant fats and oils (e.g. soup; stew) (Malainey *et al.* 1999a).

Another study uses both ratios C12:0/C14:0 and C16:0/C18:0 (Eerkens 2005)[2]. Four samples yield both: BA3.1; BB3.2; BA8 and BA14. Comparing the biplot created by Eerkens (2005) (fig.10) with the 4 samples mentioned, the samples show values attributed to terrestrial mammal meat.

Skibo (1992) used other ratios, namely: C18:0/C16:0 against C18:1/C16:0. Eleven samples, including the ones used with Eerkens criteria, had those fatty acids. In this study, high values of C18:1/C16:0 (>1) were attributed to pork and chicken fats, while lower values (≤1) would be from vegetables (Skibo 1992:89-97).

Following these criteria the ratios of the sample BA17 would mean the presence of meat fats. The samples BB3.1, BA4.1, BB4.2, BA23 and BA29 (C18:0/C16:0 <0.6 and C18:1/C16:0 <0.5) have possible vegetable oils. The remaining samples would have both vegetable and meat fats and oils (fig.12).

Vessel 5202 falls within both vegetable and meat (BA3.1) and only vegetables (BB3.2). This shows that even within samples from the same vessels the ratios of free fatty acids can vary and generate problems of interpretation. Sampling criteria (rim or base) must be based in the type of vessel (e.g. cooking pot or plate) to ensure that the section sampled will be the one with more lipids still preserved. However, it is not always possible to select the section to sample once the archaeological pottery is normally fragmented. Further,

[2] Eerkens also used (C15:0+C17:0)/C18:0 against C16:1/C18:1 however, there were no samples with both C16:1 and C18:1 fatty acids (Eerkens 2005).

different fragments of the same vessel may yield more or less lipids, as it was observed with the potsherds 5156 and 5164 from Perdigões (above).

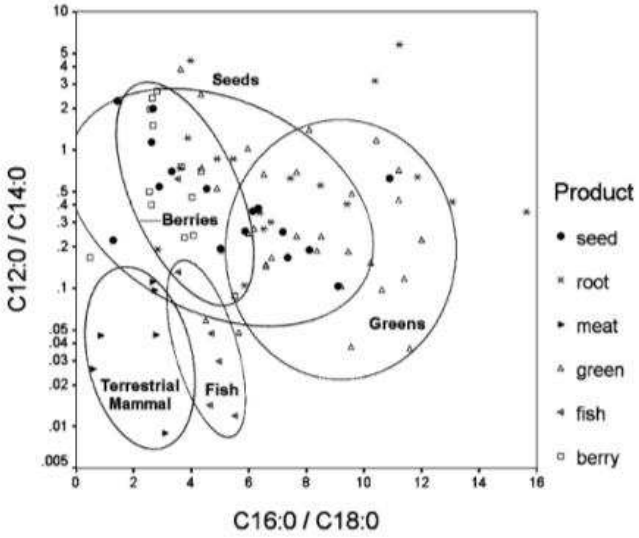

Figure 10 - Biplot of C12:0/C14:0 and C16:0/C18:0. The area highlighted corresponds to the area where the samples BA3.1; BB3.2; BA8 and BA14 would plot. Source: Eerkens 2005:90.

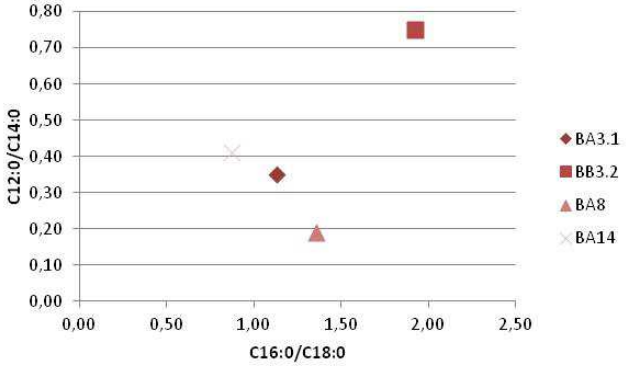

Figure 11 - Biplot of C12:0/C14:0 and C16:0/C18:0 ratios of samples BA3.1, BB3.2, BA8 and BA14.

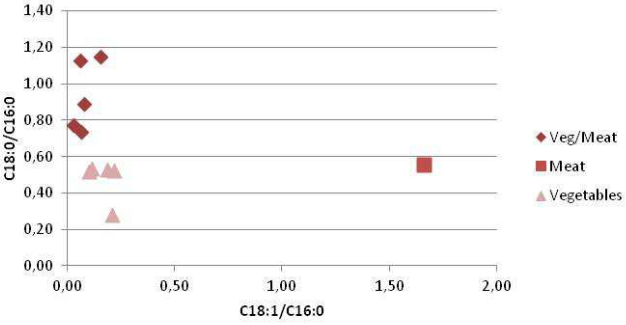

Figure 12 - C18:0/C16:0 and C18:1/C16:0 ratios based in Skibo (1992) ratio criteria.

Comparing Skibo (1992) and Eerkens (2005), criteria (observing the samples used for both: BA3.1. BB3.2, BA8 and BA14), there seems to be some coherence in the data since all four samples fall within the category of meat and or meat/vegetables (fig.11 and 12). However, Eerkens (fig.10) does not consider samples that would present mixed fats and oils (e.g. meat and vegetables) as Skibo does.

Overall it is plausible to argue that the vessel 5153 may have contained plant products (vegetables) due to the presence of sitosterol, however that does not completely exclude the present of animal fats. Unfortunately, this sample did not yield the fatty acids C18:1, C12:0 and C14:0 in order to apply the criteria's used above. Just by looking to the C16:0/C18:0 ratio (mean of 0.63) the lipids would be from seeds and nuts (Erkens 2005). When looking for the amount of C18:0, it would be classified as plant with large herbivore or just large herbivore (≥25%) (Malainey *et al.*1999a). In both situations, plant oil is a possible identification, which is supported with the present of sitosterol.

Besides this vessel, no actual identification of contents from lipid composition is possible to be taken with certainty, as peak ratios have shown to vary between runs, lipid degradation can alter the proportion of free fatty acids, thus ratios as well, and the presence of cholesterol could not be ruled out as contamination.

10. Conclusion

The data obtained from both sites shows that lipid degradation can be extensive in potsherds from Portuguese prehistoric sites. Several potsherds yield basically no lipids which mean that degradation is quite severe. It also showed variation between types of vessels and even within the same typology. The impact of vessel ware, namely polish and burnish was also assessed by this investigation; however, research with more samples will possibly draw more accurate information regarding the impact of those treatments on lipid absorption. It was also possible to observe the variability and limitations of the methods based on peak ratios. More precise techniques such as gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), which provides isotopic composition of each lipid, should be applied in addition with GC-MS to the vessels that yield sufficient amount of lipids in order to rule out contamination and identify the origins of other lipids (e.g. Evershed *et al.* 1994).

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References

BARNARD, H.; DOOLEY, A.N.; FAULL, K.F. (2007), "An Introduction to Archaeological Lipid Analysis by Combined Gas Chromatography Mass Spectrometry" H. Barnard, J.W. Eerkens Eds. *Theory and practice of archaeological residue analysis*, BAR International Series 1650, p. 42-44.

CHARTERS, S.; EVERSHED, R.P.; GOAD, L.J.; LEYDEN, A.; BLINKHORN, P.W.; DENHAM, V. (1993a), "Quantification and distribution of lipid in archaeological ceramics: implications for sampling potsherds for organic residue analysis and the classification of vessel use", *Archaeometry*, 35, p. 211-223.

CHARTERS, S.; EVERSHED, R.P.; GOAD, L.J.; HERON, C.; BLINKHORN, P. (1993b), "Identification of an adhesive used to repair a Roman jar", *Archaeometry*, 35, p. 91-101.

CHRISTIE, W.W.; HAN, X. (2010), *Lipid analysis: isolation, separation, identification and Lipidomic analysis*, Bridgwater, PJ Barnes.

COLOMBINI, M.P.; GIACHI, G.; MODUGNO, F.; RIBECHINI, E. (2005), "Characterisation of organic residues in pottery vessels of the Roman age from Antinoe (Egypt)", Microchemical Journal, 79, p. 83-90.

CRAIG, O.E.; FORSTER, M.; ANDERSEN, S.H.; KOCH, E.; CROMBE, P.; MILNER, N.J.; STERN, B.; BAILEY, G.N.; HERON, C.P. (2007), "Molecular and isotopic demonstration of the processing of aquatic products in northern European prehistoric pottery", *Archaeometry*, 49, p. 135-152.

DUDD, S.N.; EVERSHED, R.P.; GIBSON, A.M. (1999), "Evidence for varying patterns of exploitation of animal products in different prehistoric pottery traditions based on lipids preserved in surface and absorbed residues", *Journal of Archaeological Science*, 26, p. 1473-1482.

EERKENS, J.W. (2005), "GC-MS Analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America", *Archaeometry*, 47,1, p. 83-102.

EVERSHED, R.P.; HERON, C.; GOAD, L.J. (1990),"Analysis of organic residues of archaeological origin by high temperature gas chromatography and gas chromatography/mass spectrometry", *Analyst*, 115, p. 1339-1342.

EVERSHED, R.P. (1992) – Mass spectrometry of lipids, In HAMILTON, R.J.; HAMILTON S eds. (1992), "Lipid analysis: a practical approach", Oxford, Oxford University Press, p. 263-308.

EVERSHED, R.P.; HERON, C.; CHARTERS, S.; GOAD, L.J. (1992), "The survival of food residues: new methods of analysis: interpretation and application", A.M. Pollard Ed. *New developments in archaeological science*, Oxford, Oxford University Press, p. 187 - 208.

EVERSHED, R.P.; ARNOT, K.I.; COLLISTER, J.; EGLINTON, G.; CHARTERS, S. (1994), "Application of isotope ratio monitoring gas chromatography-mass spectrometry to the analysis of organic residues of archaeological origin", *Analyst*, 119, p. 909-914.

EVERSHED, R.P.; DUDD, S.; LOCKHEART, M.J.; JIM, S. (2001), "Lipids in Archaeology", D.R. Brothwell, A.M. Pollard Eds., *Handbook of Archaeological Sciences*, Chichester, Wiley, p. 331–49.

EVERSHED, R.P.; DUDD, S.N.; COPLEY, M.S.; BERSTAN, R. STOTT, A.W.; MOTTRAM, H.; BUCKLEY, S.A., CROSSMAN, Z. (2002), "Chemistry of archaeological animal fats", *Accounts of Chemical Research*, 35, p. 660-668.

EVERSHED, R.P. (2008), "Organic residue analysis in archaeology: the archaeological biomarker revolution", *Archaeometry*, 50, p.895-924.

EVERSHED, R.P.; PAYNE, S.; SHERRATT, A.G.; COPLEY, M.S.; COOLIDGE, J.; UREM-KOTSU, D.; KOTSKIS, K.; ÖZDOĞAN, M.; ÖZDOĞAN, A.E.; NIEUWENHUYSE, O.; AKKERMANS, G.; BAILEY, D.; ANDEESCU, R.R.; CAMPBELL, S.; FARID, S.; HODDER, I.; YALMAN, N.; ÖZBAŞARAN, M.; BIÇAKCI, E.; GARFINKEL, Y.; LEVY, T.; BURTON, M.M. (2008), "Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding", *Nature*, 455, p. 528-531.

GREGG, M.W.; BANNING, E.B.; GIBBS, K.; SLATER, G.F. (2009), "Subsistence practices and pottery use in Neolithic Jordan: molecular and isotopic evidence", *Journal of Archaeological Science*, 36, p. 937-946.

GUNSTONE, F.D. (1967), *An introduction to the chemistry and biochemistry of fatty acids and their glycerides*, London, Chapman and Hall Ltd.

GUNSTONE, F.D. (1992), "Introduction", R.J. Hamilton, S. Hamilton.Eds. (1992*) Lipid analysis: a practical approach*, Oxford, Oxford University Press, p. 1-12.

GUNSTONE, F.D. (2004), *The chemistry of oils and fats: sources, composition, properties and uses*, Oxford, Blackwell Publishing Ltd.

GURR, M.I.; HARWOOD, J.L.; FRAYN, K.N. (2002), *Lipid biochemistry*, 5th edition, Oxford, Blackwell Science Ltd.

HERON, C.; EVERSHED, R.P.; GOAD, L.J. (1991), "Effects of migration of soil lipids on organic residues associated with buried potsherds", *Journal of Archaeological Science*, p. 18641-18659.

HERON, C.; EVERSHED, R.P. (1993), "The analysis of organic residues and the study of pottery use", M.B. SCHIFFER Ed. *Archaeological method and theory*, 5, Arizona, University of Arizona Press.

MALAINEY, M.E.; PRZYBYLSKI, R.; SHERRIFF, B.L. (1999a), "The effects of thermal and oxidative degradation on the fatty acid composition of food plants and animals of Western Canada: 314 implications for the identification of archaeological vessel residues", *Journal of Archaeological Science*, 26, p. 95-103.

MALAINEY, M.E.; PRZYBYLSKI, R.; SHERRIFF, B.L. (1999b), "The fatty acid composition of native food plants and animals of Western Canada", *Journal of Archaeological Science*, 26, p. 83-94.

PECCI, A.; ONTIVEROS, M.C.; VALDAMBRINI, C.; INSERRA, F. (2013a), "Understanding residues of oil production: chemical analyses of floors in traditional mills", *Journal of Archaeological Science*, 40, p. 883-893.

PECCI, A.; GIORGI, G.; SALVINI, L.; ONTIVEROS, M.C. (2013b), "Identifying wine markers in ceramic and plasters using gas-chromatography-mass spectrometry, Exoperimental and archaeological materials", *Journal of Archaeological Science*, 40, p. 109-115.

PÉREZ-ARANTEGUI, J.; RIBECHINI, E.; COLOMBINI, M.P.; ESCUDERO, F. (2011), "Characterization of an ancient 'chemical' preparation: pigments and dugs in medieval Islamic Spain", *Journal of Archaeological Science*, 38, p. 3350-3357.

PERKINS, E.G. (1993), *Analyses of fats, oils, and derivatives*, Illinois, AOCS Press.

POLLARD, M.; BATT, C.; STERN, B.; YOUNG, S.M.M. (2007), *Analytical Chemistry in Archaeology*, Cambridge, Cambridge University Press.

REGERT, M.; DELACOTTE, J.M.; MENU, U.M.; PÉTREQUIN, P.; ROLANDO, C. (1998), "Identification of Neolithic hafting adhesives from two lake dwellings at Chalain (Jura, France)", *Ancient Biomolecules*, 2, p. 81-96.

ROMANUS, K.; BAETEN, J.; POBLOME, J.; ACCARDO, S.; DEGRYSE, P.; JACOBS, P.; DE VOS, D.; WAELKENS, M. (2009), "Wine and olive oil permeation in pitched and non-pitched ceramics: relation with results from archaeological amphorae from Sagalassos Turkey", *Journal of Archaeological Science*, 36, p. 900-909.

SCHELLEKENS, J. BARBERÁ, G.C.; BUURMAN, P. (2013), "Potencial vegetation markers – analytical pyrolysis of modern plant species representative of Neolithic SE Spain", *Journal of Archaeological Science*, 40, p. 365-379.

STOTT, A.W.; EVERSHED, R.P.; JIM, S.; JONES, V.; ROGERS, J.M.; TUROSS, N.; AMBROSE, S. (1999), "Cholesterol as a New Source of Palaeodietary Information: Experimental Approaches and

Archaeological Applications", *Journal of Archaeological Sciences*, 26, p. 705-716.
SKIBO, J.M. (1992), *Pottery Function: A Use-Alteration Perspective*, New York, Plenium Press.
VALERA, A.C.; SILVA, A.M. (2011), "Datações de radiocarbono para os Perdigões (1): contextos com restos humanos nos Sectores I e Q", *Apontamentos de Arqueologia e Património*, 7, p. 7-14.
VALERA, A.C. (2008), "Mapeando o Cosmos", Era, 8, Era/Colibri, p. 112-127.
VALERA, A.C. (2012), ""Ídolos Almerienses" provenientes de contextos neolíticos do complexo de recintos dos Perdigões", *Apontamentos de Arqueologia e Património*, 8, p. 19-28.
VALERA, A.C.; SIMÃO, I. (2013), *Bela Vista 5: relatório final*. Era-Arqueologia.
WHEELER, J. (2010), "Paleoenvironmental assessment of two archaeological sediments, Alentejo region, Portuga"l, *Apontamentos de Arqueologia e Património*, 6, p. 41-45.