Katholieke Universiteit Leuven

Faculteit Bio-ingenieurswetenschappen

DISSERTATIONES DE AGRICULTURA

Doctoraatsproefschrift nr. 798 aan de faculteit
Bio-ingenieurswetenschappen van de K.U.Leuven

Residue analysis on organic remains in archaeological artefacts

Promotoren:
Prof. D. De Vos, K.U.Leuven
Prof. M. Waelkens, K.U.Leuven

Leden van de examencommissie:
Prof. C. Michiels, voorzitter
Prof. P. Jacobs, K.U.Leuven
Prof. J. Poblome, K.U.Leuven
Prof. W. Van Neer, K.U.Leuven
Prof. G. Giorgi, University of Siena

Proefschrift voorgedragen tot het behalen van de graad van Doctor in de Bio-ingenieurswetenschappen

door

Kerlijne ROMANUS

Juni 2008

Dankwoord

Neen, een doctoraat maak je niet alleen…

Zonder dagelijkse begeleiding sta je nergens… dankjewel Dirk voor al je goede ideeën, je ervaring, je wijsheid en hulp. Dit werk zou zonder jou niet zijn wat het nu geworden is. Ik wens je nog heel veel succes en plezier met alles wat je doet op het COK en heel veel geluk daarbuiten.

Zonder onderwerp en zonder financiering sta je nergens… dankjewel prof Waelkens om me de kans te geven aan dit fantastische project een scherfje bij te dragen.

Zonder jury sta je nergens… dankjewel allemaal voor het kritisch nalezen en geven van zinvolle opmerkingen.

Zonder labo-omgeving sta je nergens… dankjewel prof Jacobs, Hilda, Chris, Inge, Katrien, Lieve, Ines, Hilda, Sabina, Hugo, Mia, Johan, Stef en Walter voor alle mogelijkheden en bijstand op het COK. En dankjewel prof Verbeke, Anja en Els voor de stabiele isotopenanalysen die stuk voor stuk met veel toewijding zijn gebeurd.

Zonder archeologisch team sta je nergens… dankjewel iedereen die met veel zorg scherven in zilverpapier heeft ingepakt.

Zonder kennis van specialisten sta je nergens… dankjewel Jeroen, Patrick en Wim om altijd mijn (waarschijnlijk heel on-archeologische) vragen te beantwoorden en om me te voorzien van steeds weer interessante en waardevolle stalen.

Zonder collega's sta je nergens… dankjewel allemaal voor de supertoffe tijd hier op het COK, voor alle wetenschappelijke en 'iets minder' wetenschappelijke gesprekken. Het was 'goeie boel'! En een speciaal dankjewel voor de bureaugenootjes Pieter, Sabina, Angi, Bogdan en 1/2Luc... ik ga jullie missen!

Zonder hulp in het labo sta je nergens... dankjewel Sabina voor alle hulp die ik van jou gehad heb; LCMS analysen, extracties, effe tussendoor een injectie op de GCMS... noem maar op. Ik kon het allemaal aan jou vragen en er was nooit iets teveel. Uiteraard was er ook altijd tijd voor een babbeltje, al dan niet met bijhorend lachsalvo ;-).

Zonder thesisstudenten sta je nergens… dankjewel Marie en Jan voor jullie enthousiasme, motivatie en goed werk. Ik vond het met jullie allebei echt heel fijn samenwerken.

Zonder vrienden sta je nergens… dankjewel allemaal (nee, ik ga niet iedereen opnoemen ;-)) voor jullie niet te missen vriendschap, leuke (soms hilarische) momenten, die ongeremdheid ;-), reisjes (onder andere naar Sagalassos ;-)), spelletjesavonden, weekendjes en zovéél meer.

Zonder familie sta je nergens… dankjewel moeke en vake, broers en zussen, nichtjes en neefje, en mijn schoonouders voor het altijd welkome en warme nest.

Zonder Tom sta ik nergens… jeweetwel.

Kerlijne

List of publications

Romanus, K., Poblome, J., Verbeke, K., Luypaerts, A., Jacobs, P., De Vos, D., and Waelkens, M., 2007, An evaluation of analytical and interpretative methodologies for the extraction and identification of lipids associated with pottery sherds from the site of Sagalassos, Turkey in *Archaeometry* 49(4): 729-747.

Romanus, K., Van Neer, W., Marinova, E., Verbeke, K., Luypaerts, A., Accardo, S., Hermans, I., Jacobs, P., De Vos, D., and Waelkens, M., 2008, Brassicaceae seed oil identified as illuminant in Nilotic shells from a first millennium AD Coptic church in Bawit, Egypt in *Analytical and Bioanalytical Chemistry* 390(2): 783-793.

Romanus, K., Poblome, J., Demarcke, M., Degryse, P., Jacobs, P., De Vos, D., and Waelkens, M. (*in press*), Assessing the content of local/regional fabric 4 amphorae from Sagalassos, SW Turkey in *J. Poblome, P. Monsieur, F. Vermeulen and M. Waelkens (eds) From Amphorae to Modelling the Late Roman Economy (International ROCT Workshop, 5-6 December 2005, Ghent) (FACTA. A Journal of Roman Material Culture Studies. Supplement 1) Pisa-Rome.*

Poblome, J., Corremans, M., Bes, P., Romanus, K., and Degryse, P., (*in press*), It is never too late… The late Roman initiation of amphora production in the territory of Sagalassos, in *I. Delemen, S. Cokay-Kepçe and A. Özdibay (eds) Prof Dr Haluk Abbasoglu'na 65. Yas Armagan. Studies presented to Prof Dr Haluk Abbasoglu, Istanbul*.

Romanus, K., Poblome, J., Accardo, S., Degryse, P., Jacobs, P., De Vos, D., and Waelkens, M. (*submitted*) Wine and olive oil permeability in pitched and non-pitched ceramics correlated with results from archaeological amphorae from Sagalassos, Turkey in *Journal of Archaeological Science.*

Romanus, K., Degryse, P., De Clercq, W., Poelman, H., Walton, M., Jacobs, P., De Vos, D., and Waelkens, M. (*submitted*) An excavated 16th century ointment identified as lead plaster mixed with beeswax in *Analytical Chemistry.*

Table of contents

Abstract

Because of our fascination for the ancient way of life, archaeological science tries to answer as many historical questions as possible. An important issue in this context is the diet of our ancestors. Indeed, food not only is a matter of survival, but also of social and economical significance. Food remains, preserved in potsherds during centuries, may deliver key elements in elucidating our own history. Such organic residues are inherently biochemically complex and therefore demand accurate and powerful chemical techniques for their analysis. The present work focuses on archaeological finds from the ancient city of Sagalassos, Turkey. Yearly excavations, led by the Katholieke Universiteit Leuven, already exposed loads of ceramic potsherds. Also a few valuable artefacts from other archaeological sites were investigated.

A first goal of the present research was to initiate compound-specific stable carbon isotope analysis, as this technique was never assessed on Sagalassos material before. In the meanwhile, we thought it would be opportune to compare these results with those acquired from triglyceride and fatty acid methyl ester analyses, as all three aim at identifying the animal meat prepared in a cooking pot. For the first time, dairy fats were recognized in ceramic material from Sagalassos. Concerning the comparative study, consistency between the three techniques was acknowledged.

A second objective was to develop and optimize a technique to reliably detect wine in archaeological amphorae. Classical authors mention them to be used for storage and transport of wine, oil or fish sauce. Nevertheless, the functionality of amphorae remains a considerable gap in archaeological knowledge although their importance for agriculture, social affairs and last but not least economics is substantial. So far, residue analysis lacks a good quality procedure to recognize wine. Indeed, we are faced with many difficulties here due to the high hydrophilicity, and thus susceptibility to leaching, of wine ingredients. The development and optimization of a method to extract and identify polyphenols, typical wine compounds, was succeeded through a Folin Ciocalteu colorimetric reaction. Subsequently, a series of 31

Sagalassos amphorae was subjected not only to this novel technique but also to standard lipid analyses. Only from two sherds a significant amount of polyphenols could be retrieved. This was a surprisingly low number as in 23 amphorae traces of pitch were identified, a material known to be exclusively used in wine amphorae. Another remarkable result was the recovery of walnut oil next to pitch; classical authors wrote down that pitched amphorae are associated with wine storage. The above contradictions were the catalyst in initiating a laboratory degradation experiment to evaluate the behavior of wine and oil in pitched ceramic material.

For this purpose, small ceramic pots were made, covered with pitch and, after a period of drying, filled with wine or olive oil. Layers of 1 mm were scraped from each pot and one by one analysed for lipid and polyphenol content. Their respective concentration profiles proved the inevitability to pitch a vessel intended for oil storage and the ability of wine to infiltrate through the pitch layer. The acquired results validated our outcome from the Sagalassos amphorae.

Residue analysis also aims at investigating extraordinary archaeological finds to add to our knowledge not only of ancient diet but also of the complete way of living. For example, in the Macellum in Sagalassos, many unguentaria were excavated; among them, two contained a visible greasy residue. Of course, this was an excellent opportunity to investigate the functionality of those small glass bottles. Both residues contained beeswax next to negligible amounts of inorganic material. We presume these residues are leftovers from beeswax stoppers.

Other very interesting study subjects were Nilotic shells filled with greasy burned deposits found in a Coptic church in Egypt. The archaeological question was twofold; first to substantiate the hypothesis that the shells had been used as lamps and secondly to recognize the origin of the illuminant. We succeeded on both levels through the application of four chromatographic techniques. The residues pointed towards vegetal oil and revealed hydroxylated substances characteristic for fried oil. The fuel could be identified as oil from Brassicaceae seeds like rapeseed or radish. Distinction between the two was made by means of stable carbon isotope analysis; and radish seed oil was acknowledged as the illuminant used in these particular Egyptian lamp shells.

The final chapter in this work presents the results of chemical analyses on a greasy substance recovered from an excavated medieval ceramic vessel in the Castle of Middelburg, Belgium. The organic fraction was proven to consist out of beeswax and vegetal oil while the inorganic fraction comprised lead soaps, iron, gypsum,

leadsulfates and others. The formulation of this ointment indicated that a lead plaster had been mixed with beeswax. Lead plasters are suitable for treating bruises while beeswax probably was added to facilitate the application on and the hydration of the skin.

Samenvatting

Archeologie tracht in te spelen op onze nieuwsgierigheid naar hoe de mens in de oudheid geleefd heeft. Een belangrijk aspect in dit verhaal is het voedingspatroon van onze voorouders. Eten is immers niet enkel een kwestie van overleven, maar heeft een minstens even belangrijke sociale en economische impact. Overblijfselen van voedsel, bewaard in potscherven gedurende eeuwen, kunnen de sleutel zijn in het ontrafelen van onze eigen geschiedenis. Dergelijke organische residu's zijn in wezen complex en vereisen precieze en krachtige chemische analysetechnieken. Dit doctoraatsonderzoek spitst zich voornamelijk toe op archeologische vondsten van de antieke stad Sagalassos, gelegen in Turkije. Jaarlijkse opgravingen, onder leiding van de Katholieke Universiteit Leuven, brachten al hele ladingen potscherven aan het licht. Ook werden enkele artefacten van andere archeologische sites onderzocht.

Een eerste doel was het opstarten van stabiele koolstof isotopen analysen omdat deze techniek nog nooit eerder werd toegepast op materiaal van Sagalassos. Daarnaast leek het ons zinvol ook een vergelijkende studie te maken met twee andere technieken om de diersoort, klaargemaakt in een kookpot, te achterhalen. Deze twee technieken zijn triglyceride en vetzuur methylester analyse. Voor de eerste maal werden sporen van zuivelproducten in potscherven van Sagalassos gedetecteerd. Met deze vergelijkende studie kon vastgesteld worden dat de drie technieken dezelfde resultaten opleveren.

Een tweede luik in dit doctoraat was het ontwikkelen en optimaliseren van een techniek voor het detecteren van wijn in amforen. Volgens antieke auteurs worden amforen gebruikt voor het transport en de opslag van wijn, olie en vissaus. Toch blijft de functie van amforen bij archeologen voor raadsels zorgen ondanks hun belang in landbouw, sociale aangelegenheden en niet te vergeten handel en economie. Tot nu toe was er in residuanalyse geen betrouwbare methode om wijnresten te analyseren. Dit is immers een enorme uitdaging omdat alle ingrediënten van wijn gekenmerkt worden door een hoge wateroplosbaarheid waardoor ze een lange bewaartijd waarschijnlijk niet zullen overleven. Toch slaagden we erin een techniek te ontwikkelen voor het opsporen van polyfenolen, een typische wijncomponent, door

het toepassen van een Folin Ciocalteu kleurreactie. Daaropvolgend werden 31 Sagalassos amforen aan deze methode onderworpen, alsook aan standaard vetanalysen. Slechts twee scherven vertoonden een significante hoeveelheid polyfenolen. Dit was veel minder dan verwacht aangezien in 23 stuks, sporen van teer werden geïdentificeerd, wat volgens de klassieke hypothese kenmerkend is voor wijnamforen. Een ander opmerkelijk resultaat was de vondst van walnotenolie in een amfoor waar ook teer in aangebracht was. Dit is in tegenspraak met klassieke schrijvers. Al deze bevindingen brachten ons bij het opstarten van een laboratorium degradatie-experiment om het gedrag van wijn en olie in combinatie met teer te evalueren.

Daartoe werden kleine keramiekpotjes gemaakt waarin teer werd aangebracht. Na een droogtijd werden de potjes gevuld met wijn of olijfolie. Van elk potje werden lagen van ongeveer 1 mm afgeschraapt en in elke laag werd de concentratie aan vetten en polyfenolen bepaald. De gegenereerde permeatieprofielen brachten aan het licht dat het aanbrengen van een teerlaag noodzakelijk is in een olieamfoor en dat wijn, ondanks de teer, toch kan infiltreren in de keramiek. Deze uitkomst bevestigde onze resultaten van de Sagalassos amforen.

Residuanalyse heeft niet enkel tot doel om het vroegere dieet maar ook alle aspecten van het leven in de oudheid te achterhalen. Bijvoorbeeld in het Macellum te Sagalassos werden tientallen unguentaria opgegraven; in twee werd een zichtbaar residu opgemerkt. Dit was een uitgelezen kans om de functie van deze kleine glazen flesjes te onderzoeken. Beide residu's bestonden voor het grootste deel uit bijenwas en slechts voor een heel kleine fractie uit anorganisch materiaal. We veronderstellen dat een stopsel, gemaakt van bijenwas, diende om deze flesjes af te sluiten.

Andere interessante studieobjecten waren schelpen, gevuld met een verbrande vettige brij, opgegraven in een Koptische kerk in Egypte. De archeologische vraagstelling was tweeledig; ten eerste diende bevestigd te worden dat deze schelpen als lamp hadden gediend en een tweede doel was om de oorsprong van de gebruikte brandstof te bepalen. Door vier chromatografische technieken toe te passen zijn we daarin geslaagd. De residu's wezen op de aanwezigheid van plantaardige olie en vertoonden gehydroxyleerde triglyceriden die kenmerkend zijn voor verhitte olie. De olie kon geïdentificeerd worden als afkomstig van zaden van Brassicaceae planten, zoals koolzaad of radijs. Volledig uitsluitsel kwam er dankzij de stabiele koolstofisotopen

analysen waarbij olie van radijszaden werd herkend als brandstof in deze Egyptische lampschelpen.

Het laatste hoofdstuk van dit doctoraat stelt de resultaten voor van chemische analysen op een zalf die bewaard was gebleven in een middeleeuws keramiekpotje opgegraven in het Kasteel van Middelburg te België. De organische fractie van de zalf bestond uit bijenwas en plantaardige olie terwijl de anorganische fractie loodzepen, ijzer, loodsulfaat, gips en anderen bevatte. De samenstelling van deze medische zalf toonde aan dat het hier ging om een loodplaaster gemengd met bijenwas. Loodplaasters zijn gekend voor het behandelen van kneuzingen, en bijenwas werd waarschijnlijk toegevoegd om de plaaster gemakkelijker aan te brengen en voor de hydratatie van de huid.

1 Introduction

1.1 Residue analysis

Archaeological disciplines seek at satisfying our curiosity about the ancient ways of life. In this wide-ranging research domain, the diet of our ancestors is one of the topics people were always especially interested in. Residue analysis on archaeological potsherds aims at filling gaps in our knowledge about ancient diet exploiting chemical techniques. Organic residues, which seem to be well preserved in ceramics, still contain valuable information about cooking or storage of commodities. Nevertheless, a range of physical, microbial and chemical alterations took place during food preparation, deposition and centuries of burial. The consequential complexity together with the minuscule concentrations of organic residues makes it a difficult challenge to draw exact and reliable conclusions. So far, lipids were the heart of these investigations because of their non-polar character and their abundance in many goods [5,16,19]. It was proven that a ceramic matrix plays, to some extent, a protective role against microbial, oxidative and hydrolytic reactions on lipids [27,28]. Lately also polyphenols and proteins became a subject matter of residue analysis in archaeology [2,12,24,25]. The difficulty here is their high polarity and thus susceptibility towards water logging phenomena.

1.2 Residue analysis on a time scale

The first experiments in residue analysis on ceramic pottery were attempted by Condamin *et al.* in the seventies [5]. They wanted to establish scientific criteria to allow identification of amphora content. In first instance, the focus was on the detection of oil after extraction, saponification and gas chromatographic analysis. They proved for the first time that traces of oil were preserved after thousands of years of burial [5]. In a second paper also different types of archaeological ceramics

were analysed but by means of thin layer chromatography together with high performance liquid chromatography. Traces of 9,10-dihydroxystearic acid were found in a fifth century oil lamp, suggesting the former presence of rancid oil. In a Gaza-Rilled I amphora, indications of sesame oil were found [33].

A new era in residue analysis started with a publication of Evershed *et al.* [19]. That paper formulates the necessity of combining different analysis techniques in residue analysis in order to obtain sufficient and complementary information. Afterwards, a range of publications on this subject emerged exploring new techniques and with very promising results [14,16,17,20,21,27,32]. Discovering the power of compound-specific stable carbon isotope analysis was another huge step forward. It was proven that $\delta^{13}C$ values of fatty acids provide the means to distinguish between porcine and ruminant adipose fat because of differences in their metabolism and physiologies. As the C18:0 fatty acid in dairy fat is significantly more depleted in ^{13}C than the corresponding compound in carcass fats, also a difference can be told between dairy and adipose fat (Figure 1.1). For this kind of analyses, a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS) is used in order to establish the $\delta^{13}C$ values of individual compounds [13,32]. Since then, stable carbon isotope analyses have been widely applied in residue analysis on ceramic material [6,7,8,9,17,21].

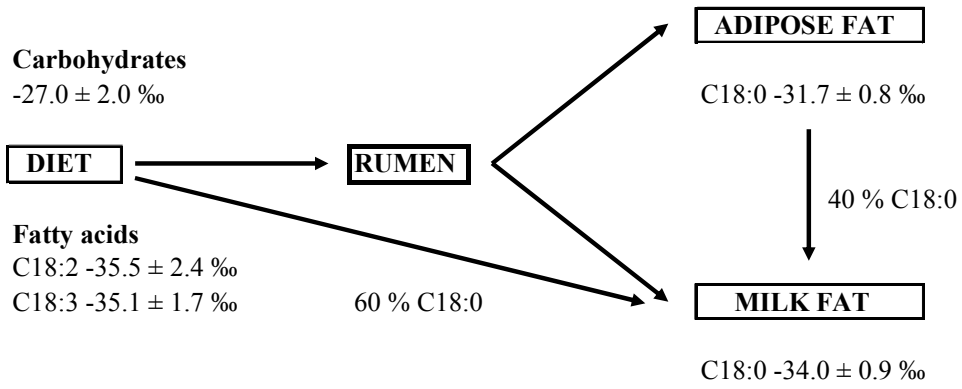


Figure 1.1: Schematic presentation of dietary routing of fatty acids and carbohydrates and its consequences for the isotopic composition of dairy against adipose fats [6]. About 60 % of the C18:0 in the rumen appears to be directly incorporated from the diet, after biohydrogenation of unsaturated fatty acids in the rumen and reflects the inability of the mammary gland to biosynthesize C18:0.

Only recently, research on other biomolecules than lipids started to succeed. For example, polyphenol analysis might solve the question about the presence of wine in ceramic vessels. The difficulty is the high polarity of these compounds. Therefore, they are very susceptible to hydrolysis and oxidation. A very dry environment and moderate preservation temperatures are necessary for polyphenols to resist degradation. Amphorae, recovered from a shipwreck, were kept in an anoxic environment and indeed, they still enclosed small amounts of polyphenols. The amphorae under study were a Dressel 1-type amphora from the shipwreck *Madrague de Giens* dated to the Iron Age (70-60 BC) and a Haltern 70-type amphora from the shipwreck Port-Vendres II dated to the Claudius reign (41-54 AD) [24]. Also a jar, found in an Egyptian tomb of king Tutankhamun and dated to Dynasty XVIII, seemed to have contained polyphenols and thus probably wine [25].

Another newly explored area is the study on protein residues. The first investigations on pottery indicated that only a tiny amount of proteins were absorbed (and preserved in the ceramics of Late Saxon/early Medieval date and collected from the West Cotton site, Raunds, Northamptonshire, U.K.. No more than a small quantity of gelatin was found, a proteinaceous material known to be present in animal tissue [23]. This was to be expected as proteins lack the hydrophobicity of lipids and their infiltration in the pores of the ceramic material is less likely because of their higher molecular mass. Despite these obstacles, milk proteins seemed to have survived in coarse ware cooking vessels, dated to the middle of the first millennium BC and collected from South Oist, U.K., as they were detected by an immunological detection method [11]. Other protein research mainly focused on archaeological bone remains and archaeological cultural objects rather than on pottery [2].

1.3 Challenges in residue analysis

1.3.1 Contamination

A serious difficulty that we need to be aware of in this kind of research is the possibility of contamination. Such interferences might lead to false conclusions and thus jeopardizing archaeological repercussions. Of course, there are a number of

potential sources of pollution as the residue goes through an elaborate life cycle. Figure 1.2 gives a simplified scheme of such a life cycle with a few critical points of contamination. Sometimes, for example in the case of modern contaminants, like for example phthalates, it is very obvious and straightforward to distinguish them from authentic ancient material. On the contrary, grease from hands is a lot more difficult to differentiate because of its resemblance with animal adipose fats.

Experiments have been conducted to study the influence of soil surrounding a particular potsherd. This is indeed a realistic problem as soil organic matter comprises a significant amount of lipids which might migrate into the pores of the ceramic material [14,27,30]. In a first study where the migration of soil lipids was investigated, researchers concluded that the soil lipid profile showed clear distinction with the lipid profile found in archaeological potsherds from St Neots-type and Stanion-type ware cooking vessels and jars from Raunds, Northamptonshire; thus excluding possible infiltration of soil lipids in a vessel [27]. A second paper reports the investigation of microbial lipid contributions in replica pottery vessels by means of a laboratory degradation experiment. They showed that microbial lipids contributed only to a very limited extent to the total lipid content in pottery [14].

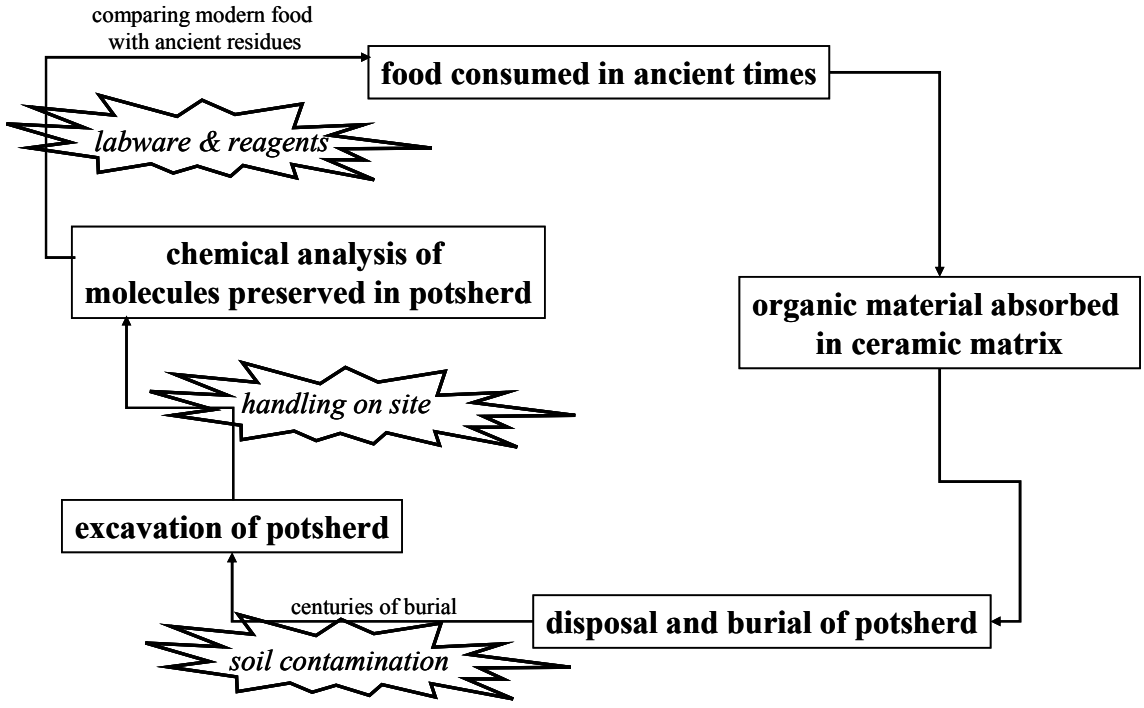


Figure 1.2: The life cycle of an organic residue where at any moment contamination might occur. 'Absorbed' means that the molecules entered in the pores of the ceramic matrix.

Another study also examined the migration of soil lipids in ceramic material, mainly water pipes and cooking pots from Sagalassos (Turkey) during burial. They were able to reproduce the results from the first study and thus confirm the trustworthiness of the lipid profile in archaeological potsherds [30].
Another type of contamination might occur during excavation of the potsherd: human skin secretes lipids, which are very similar with animal adipose fats. In that way researchers might conclude that animal fats were prepared in that vessel while instead the potsherd only has been touched by the archaeologist. To avoid this problem, these days, a sherd which is intended for residue analysis, needs to be wrapped in acid free paper from the moment of excavation. Also right before chemical analysis, the outer layer of the potsherd needs to be removed by a dental drill.
Modern contamination might turn up through the contact of plastic material with the ceramic material, for example when the excavated potsherds are transported in plastic bags. Also during chemical treatment specific pollutants may arise. Using plastic instead of glass containers, phthalates, important additives in plastic industry, will appear in the total lipid extract. Therefore, care must be taken to use glass tools at all times during sample pretreatment. Even solvents, used for extraction, can cause phthalate contamination if they were stored in plastic vessels.

1.3.2 Chemical alterations

Chemical alterations of original commodities already start during food preparation and vessel use. When chemical compounds are subjected to high temperatures, several reactions take place. For example, heating meat induces the development of grayish-brown color, protein coagulation, release of juices due to the decrease in water holding capacity, increase in pH, development of a typical cooked or roasted meat aroma and softening induced by the shrinking and partial conversion of collagen to gelatin [3]. In the case of vegetables, strong heating causes hydrolysis of pheophytins, releasing carbonic acid monomethylester which decomposes into CO_2 and methanol. Also a *Maillard* reaction might occur between amino compounds and carbohydrates. In general, vitamins will disappear because of temperature instability and sensitivity towards hydrolysis, while proteins will undergo denaturation because of heating [3].

Laboratory degradation experiments were conducted, by Evershed *et al.* [22], heating triglycerides in contact with fired (800 °C) marl clay in order to evaluate the formation of chemical compounds. It was concluded that long-chain ketones were formed during heating, probably by a free radical mechanism involving decarboxylation and dehydration over the clay surface. As long-chain ketones are also synthesized by higher plants, one must be very careful in drawing correct conclusions [22]. Studies about the formation of oxidation products during frying of oils show that many chemical reactions occur at such elevated temperatures. Thermal and oxidative degradation of triglycerides takes place next to hydrolytic cleavage reactions [29]. One study postulated the formation of ω-(o-alkylphenyl)alkanoic acids as a result of heating marine food [26].

Not only temperature plays a major role in inducing chemical reactions but also oxidation of unsaturated fatty acids is a crucial factor during vessel use, recycling and burial. Indeed, various α,ω-dicarboxylic acids are known to be formed through the formation of hydroperoxide intermediates via radical reactions with the double bond of a fatty acid [3]. These polar diacids are generally only encountered in archaeological pottery excavated in an arid climate like for example in Egypt [4,10]. Of course, they are very susceptible to elution because of their low molecular weight and their polar acid groups. Nevertheless, a study proved that these oxidation products can be covalently bound to ceramic material. By an alkaline treatment, they can also be extracted and analysed [35]. In some cases, oxidation products can be as diagnostic as the original product. For example, in Egyptian ceramic oil lamps, from the North Necropolis at Antitoe (Egypt) dating back to the fifth to seventh centries AD, a range of oxidation products was found and in that way researchers could prove Brassicaceae oil had been used as fuel [4,10]. As rapeseed oil and radish seed oil contain high amounts of gondoic and erucic acid, their respective oxidation products, namely 11,12-dihydroxyeicosanoic acid and 13,14-dihydroxydocosanoic acid, are indicators of equal value [4,10]. Since fish contains mainly polyunsaturated fatty acids, oxidation reactions take place at high rate, altering substantially the original fatty acid profile of marine food. For that reason, recognizing fish deposits remains the most difficult topic in archaeological residue analysis [18,26].

Another critical point is the occurrence of hydrolysis reactions during vessel use, recycling or burial. Or, when the excavated potsherds are not properly -in too light or too humid conditions- stored, additional oxidation and hydrolysis reactions might take

place. Hydrolysis can arise in two ways: by a chemical or by an enzymatic reaction. It is known for triglycerides to form free fatty acids, monoglycerides and diglycerides, a pattern frequently observed in archaeological potsherds (Figure 1.3) [18]. The hydrolysis products are hardly ever a reliable indication for a specific foodstuff. Nevertheless, their relative amounts proved to be useful in defining the origin of the lipids [15,31]. The main concern is that hydrolysis products, because of their lower molecular weight and higher polarity, are more susceptible to leaching phenomena.

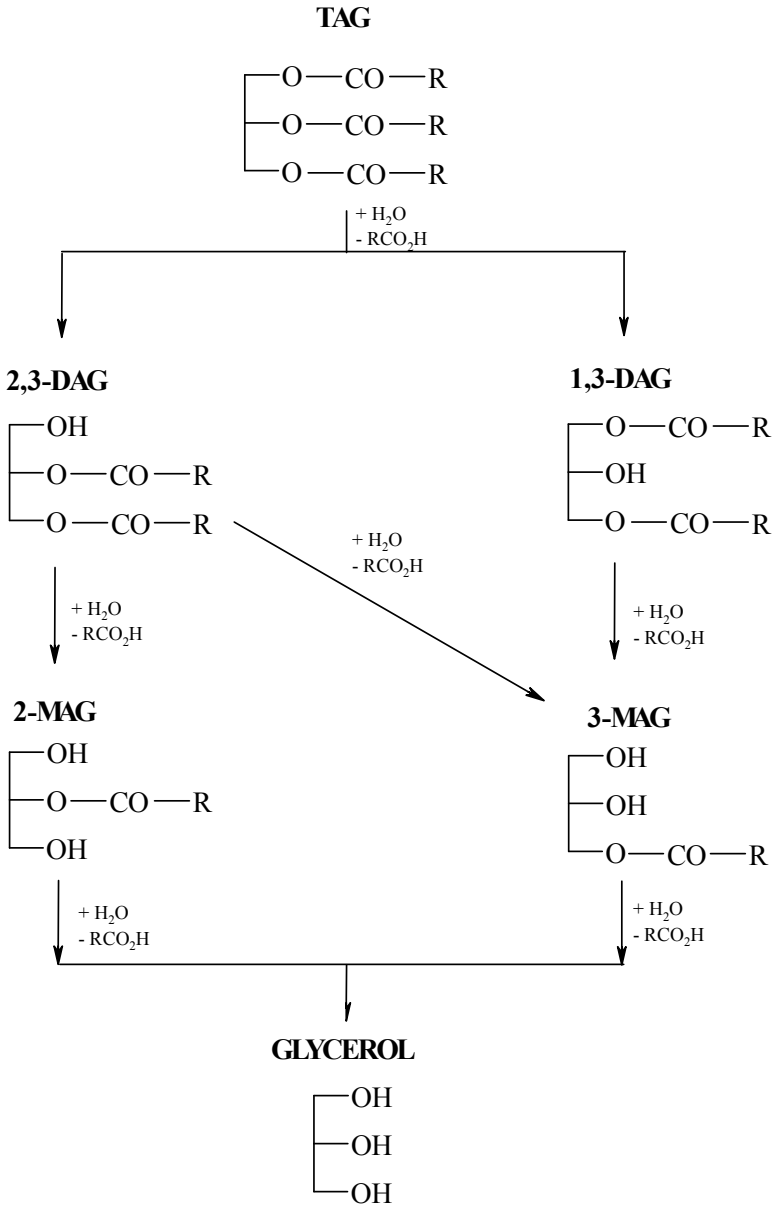


Figure 1.3: Hydrolytic degradation of triglycerides (TAG) resulting in the formation of diglycerides (DAG), monoglycerides (MAG) and glycerol.

Finally, degradation of triglycerides can also be caused by microbiological and thus enzymatic impact. The most well known mechanism of fatty acid metabolism under aerobic conditions is β-oxidation resulting in a stepwise degradation of fatty acids yielding acetyl-CoA [3,18] (Figure 1.4).

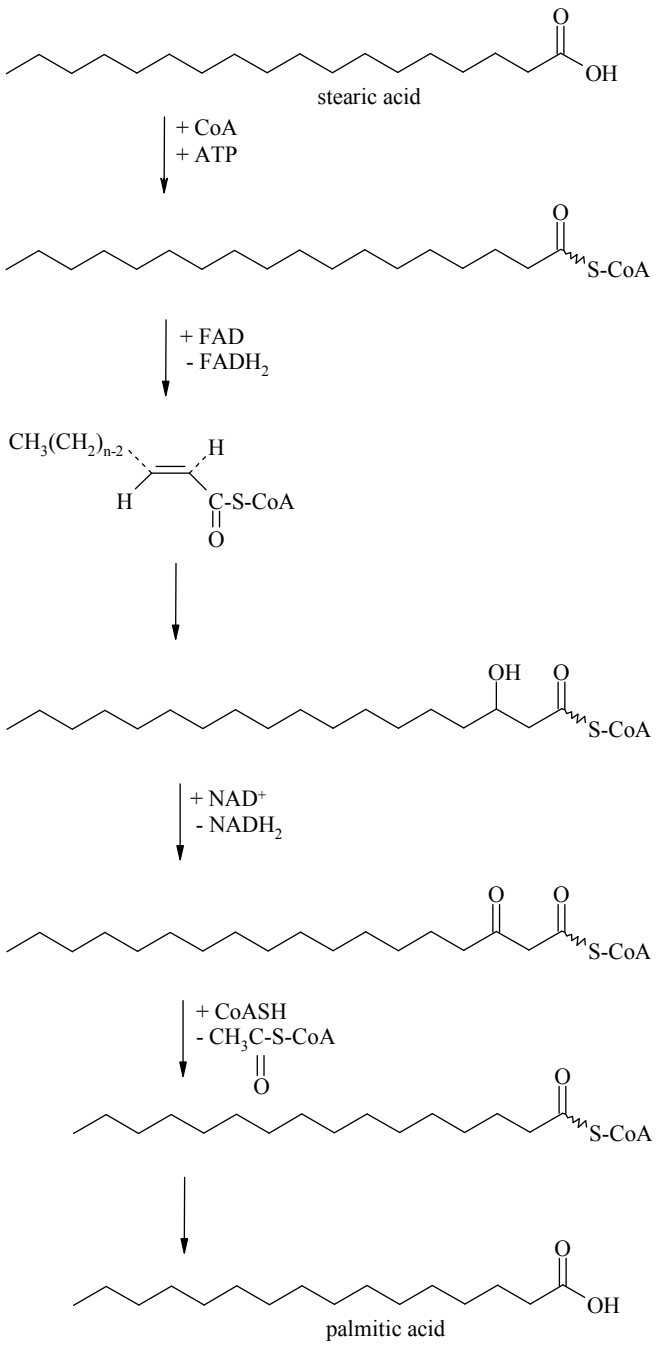


Figure 1.4: The β-oxidation pathway of stearic acid degradation forming palmitic acid.

1.3.3 Interpretation

Of course, enough caution must be addressed while interpreting acquired results. A nice illustration is the paper of Barnard *et al*. in which a potsherd containing heated camel milk was sent to seven labs with the purpose of identifying the original content [1]. Lipid analysis showed that the camel milk was highly degraded resulting in a range of non-diagnostic fatty acids. Proteins were not detected by the applied method, being cross-over electrophoresis, probably because of the degradation of the product due to cooking. Stable isotopic analyses on carbon and nitrogen were performed also. The increase in nitrogen and decrease in atomic C/N ratios beginning 22 mm below the rim indicates that protein-rich seed or a whole milk product were absorbed in lower parts of the vessel. The results were able to give a number of indications but the exact source of the foodstuff was not identified by any laboratory. Although disappointing, it should be hardly surprising because the techniques used nowadays only look at molecules and not at an entire foodstuff. Furthermore, additional archaeological and historical data are necessary to pinpoint a highly specific foodstuff like for example camel milk. This proves collaboration with archaeologists is indispensable in order to draw correct conclusions. Archaeological residue analysis can therefore never be more than complementary in reconstructing the diet of ancient people.

Many problems and difficulties are the reason for complicating the interpretation. Firstly, most probably, a cooking vessel is used for the preparation of multiple meals resulting in a mixture of foodstuffs. As a consequence, it is impossible to draw straightforward conclusions. Furthermore, by cooking and frying, many oxidation and hydrolysis reactions occur, changing the original composition of the food. During burial, degradation of the commodities arises, resulting in very low residual concentrations. The consequential life cycle of pottery is complex and can comprise manufacture, distribution, prime use, reuse, maintenance, recycling, discard and reclamation [34]. Therefore, this research domain demands the need for highly sensitive analytical methods. Finally, the available archaeological material is precious, imposing non-destructive techniques, and limited, consequentially less residual material can be analysed.

1.4 Scope of this work

1.4.1 Comparative study of methodologies in lipid residue analysis

So far, compound-specific stable carbon isotope analyses have not been performed on ceramics from Sagalassos. Also the lack in literature of a critical study comparing the results, interpretations and conclusions from different methodologies in lipid research concerning ceramic residue analysis is striking. Considering this essential information, it seems clear to blend both challenges in one comparative study.
Firstly, it was necessary to select an appropriate set of Sagalassos cooking pots for this experiment. Secondly, we needed to optimize a methodology for triglyceride analysis with liquid chromatography and mass spectrometry to establish the origin of the fat prepared in the vessel. Thirdly, the analyses on the gas chromatograph combustion isotope ratio mass spectrometer (GC-C-IRMS) required testing and optimization for the analysis on archaeological material from Sagalassos.

1.4.2 Did amphorae contain wine, oil or fish products?

The ancient written and documentary records on the functional use of amphorae are few and not necessarily straightforward to interpret [36]. Therefore, chemical analysis of organic residues might provide the answer to the simple archaeological question whether an amphora contained oil, fish products or rather wine, if not something different altogether. This question may seem simple, but so far utterly few successful reports are known. In that way, this topic comprises two main goals. The first one was to develop and optimize new methodologies to detect polyphenols as an indicator for the storage of wine in the past. And the second goal was to find new and reliable techniques to prove the presence of fish and fish products in ceramics.

1.5 References

1. **Barnard H., Ambrose S., Beehr D., Forster M., Lanehart R., Malainey M., Parr R., Rider M., Solazzo C., and Yohe R., 2007**, Mixed results of seven methods for organic residue analysis applied to one vessel with the residue of a known foodstuff in *Journal of Archaeological Science* 34: 28-37

2. **Barnard H., Shoemaker L., Craig O., Rider M., Parr R., Sutton M. and Yohe R., 2007**, Introduction to the analysis of protein residues in archaeological ceramics in *BAR International Series 1650 edited by Barnard H. and Eerkens J.*

3. **Belitz, H.D., and Grosch, W., 1999**, Food chemistry (second edition), Berlin

4. **Colombini M, Modugno F., and Ribechini E., 2005**, Organic mass spectrometry in archaeology: evidence for Brassicaceae seed oil in Egyptian ceramic lamps in *Journal of Mass Spectrometry* 40:890-898

5. **Condamin J., Formenti F., Metais O., Michel M., and Blond P., 1976**, The application of gas chromatography to the tracing of oil in ancient amphorae in *Archaeometry* 18(2): 195-201

6. **Copley M., Berstan R., Dudd S., Docherty G., Mukherjee A., Straker V., Payne S., and Evershed R., 2003**, Direct chemical evidence for widespread dairying in prehistoric Britain in *Proceedings of the National Academy of Sciences* 100(4): 1524-1529

7. **Copley M., Berstan R., Dudd S., Straker V., Payne S., and Evershed R., 2005a**, Dairying in antiquity. I. Evidence from absorbed lipid residues dating to the British Iron Age in *Journal of Archaeological Science* 32: 485-503

8. **Copley M., Berstan R., Straker V., Payne S., and Evershed R., 2005b**, Dairying in antiquity. II. Evidence from absorbed lipid residues dating to the British Iron Age in *Journal of Archaeological Science* 32: 505-521

9. **Copley M., Berstan R., Mukherjee A., Dudd S., Straker V., Payne S., and Evershed R., 2005c**, Dairying in antiquity. I. Evidence from absorbed lipid residues dating to the British Neolithic in *Journal of Archaeological Science* 32: 523-546

10. **Copley M., Bland H., Rose P., Horton M., and Evershed R., 2005**, Gas chromatographic, mass spectrometric and stable carbon isotopic investigations of organic residues of plant oils and animal fats employed as illuminants in archaeological lamps from Egypt in *The Analyst* 130:860-871

11. **Craig O., Mulville J., Parker Pearson M., Sokol R., Gelsthorpe K., Staceyll R., and Collins M., 2000**, Detecting milk proteins in ancient pots in *Nature* 408:312

12. **Craig O., Taylor G., Collins M., and Parker Pearson M., 2005**, The identification of prehistoric dairying activities in the Western Isles of Scotland: an integrated biomolecular approach in *Journal of Archaeological Science* 32: 91-103

13. **Dudd S., and Evershed R., 1998**, Direct demonstration of milk as an element of archaeological economies in *Science* 282: 1478-1481

14. **Dudd S., Regert M., and Evershed R., 1998**, Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds in *Organic Geochemistry* 29: 1345-1354

15. **Eerkens, J.W., 2005**, GC-MS analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America in *Archaeometry* , 47, 1, 83-102

16. **Evershed R., 1993**, Biomolecular archaeology and lipids in *World Archaeology* 25: 74-93

17. **Evershed R., Dudd S., Copley M., Berstan R., Stott A., Mottram H., Buckley S., and Crossman Z., 2002**, Chemistry of archaeological animal fats in *Accounts of Chemical Research* 35: 660-668

18. **Evershed R., Heron C., Charters S., and Goad L., 1992**, The survival of food residues: new methods of analysis, interpretation and application in *Proceedings of the British Academy* 77: 187-208

19. **Evershed R., Heron C., and Goad LJ., 1990**, Analysis of organic residues of archaeological origin by high temperature gas chromatography and gas chromatography mass spectrometry in *The Analyst* 115: 1339-1342

20. **Evershed R., Heron C., and Goad LJ., 1991**, Epicuticular wax components preserved in potsherds as chemical indicators of leafy vegetables in ancient diets in *Antiquity* 65: 540-544

21. **Evershed R., Mottram H., Dudd S., Charters S., Stott A., Gibson A., Conner A., Blinkhorn P., and Reeves V., 1997**, New criteria for the identification of animal fats preserved in archaeological pottery in *Naturwissenschaften* 84: 402-406

22. **Evershed R., Stott A., Raven A., Dudd S., Charters S., and Leyden A., 1995**, Formation of long-chain ketones in ancient pottery vessels by pyrolysis of acyl lipids in *Tetrahedron Letters* 36(48): 8875-8878

23. **Evershed R., and Tuross N., 1996**, Proteinaceous material from potsherds and associated soils in *Journal of Archaeological Science* 23: 429-436

24. **Garnier N., Richardin P., Cheynier V., and Regert M., 2003**, Characterization of thermally assisted hydrolysis and methylation products of polyphenols from modern and archaeological vine derivatives using gas chromatography-mass spectrometry in *Analytica Chimica Acta* 493: 137-157

25. **Guasch-Jané M., Ibern-Gómez M., Andrés-Lacueva C., Jáuregui O., and Lamuela-Raventós R., 2004**, Liquid chromatography with mass spectrometry in tandem mode applied for the identification of wine makers in residues from ancient Egyptian vessels in *Analytical Chemistry* 76: 1672-1677

26. **Hansel F., Copley M., Madureira L., and Evershed R., 2004**, Thermally produced ω-(o-alkylphenyl)alkanoic acids provide evidence for the processing of marine products in archaeological pottery vessels in *Tetrahedron Lette*rs 45: 2999-3002

27. **Heron C., Evershed R., and Goad L., 1991**, Effects of migration of soil lipids on organic residues associated with buried potsherds in *Journal of Archaeological Science* 18: 641-659

28. **Heron C., Evershed R., Goad L., and Denham V., 1989**, New approaches to the analysis of organic residues from archaeological remains in *Archaeological Sciences 1989: proceedings of a conference on the application of scientific techniques to archaeology, Bradford, September 1989 (eds. P. Budd, B. Chapman, C. Jackson, R. Janaway and B. Ottaway), 332-339, Oxbow Books, Oxford*

29. **Houhoula D., Oreapoulou V., and Tzia C., 2003**, The effect of process time and temperature on the accumulation of polar compounds in cottonseed oil during deep-fat frying in *Journal of the Science of Food and Agriculture* 83: 314-319

30. **Kimpe K., 2003**, Chemical analysis of the lipid fraction from ancient ceramics of Sagalassos, PhD Thesis, Katholieke Universiteit Leuven

31. **Malainey M., Przybylski R., and Sherriff B., 1999**, The effects of thermal and oxidative degradation on the fatty acid composition of food plants and animals of Western Canada: implications for the identification of archaeological vessel residues in *Journal of Archaeological Science* 26: 95-103

32. **Mottram H., Dudd S., Lawrence G., Stott A., and Evershed R., 1999**, New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats in archaeological pottery in *Journal of Chromatography A* 833: 209-221

33. **Passi S., Rothschild-Boros M.C., Fasella P., Nazzaro-Porro M., and Whitehouse D., 1981**, An application of high performance liquid chromatography to analysis of lipids in archaeological samples in *Journal of Lipid Research* 22: 778-784

34. **Peña, J., 2007,** Roman pottery in the archaeological record, Cambridge

35. **Regert M., Bland H., Dudd S., van Bergen P., and Evershed R., 1998**, Free and bound fatty acid oxidation products in archaeological ceramic vessels in *Proceedings of the Royal Society London B* 265: 2027-2032

36. **Rothschild-Boros M., 1981**, The determination of amphora contents, Archaeology and Italian Society, Prehistoric and Medieval Studies, in *Papers in Italian Archaeology II, BAR International Series 102*

2 Experimental

2.1 Lipids

2.1.1 Extraction

The extraction procedure was based on the work of Kimpe [3] and was further optimized and adapted to new equipment. In order to avoid contamination, the potsherds were cleaned with a dental drill. Afterwards, they were crushed in a mortar with pestle to increase the extraction performance due to the higher contact surface. Before extraction, approximately 0.5 mg of an internal standard, *n*-heptadecane, was added to the ceramic material. About 5 g of the milled sherd was submitted to a Soxtec extraction with 60 ml chloroform : methanol (2:1). The apparatus used was a Soxtec Avanti and was programmed to boil for 45 min followed by rinsing for 2 h. The solvent was partially evaporated in the Soxtec apparatus and the remaining solvent was removed under a stream of nitrogen. The dried total lipid extracts were stored at –18 °C until further analysis.

2.1.2 Derivatization

Derivatization reactions are used to convert compounds which are not suitable for analysis with gas chromatography into their analyzable derivates. To investigate the fatty acid profile of an archaeological potsherd, free fatty acids and acylglycerides need to be converted into esters. On the other hand, when looking at the total lipid fraction, a silylation reaction is required. For analysis of triglycerides with liquid chromatography, it is not necessary to derivatize.

The dried total lipid extract was used for the preparation of the fatty acid methyl esters (FAME). Two known methods were applied for methanolysis of only the

acylglycerols (AG) [3], or for conversion of both acylglycerols and free fatty acids (FFA) to the methyl esters (method 2) [4]. Remark that acylglycerols (AG) comprise triglycerides (TAG), diglycerides (DAG) and monoglycerides (MAG).

In method 1, which was based on previous work of Kimpe [3], for the transesterification of only AG, 300 µl of KOH in methanol (0.45 g KOH in 8 g methanol) was added to the total lipid extract dissolved in 200 µl diethylether. After shaking for three minutes, 1 ml cyclohexane and 200 µl bidistilled water were added. After centrifugation, the cyclohexane phase was separated, washed with 200 µl bidistilled water and dried. The fatty acid methyl esters were dissolved again in 100 µl cyclohexane and analysed on a polar phase gas chromatograph (PPGC) and a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS).

In method 2, which was based on literature [1] and adapted for lipids from archaeological pottery, both AG and FFA were transesterified. Therefore, the total lipid extract was dissolved in 1 ml BF_3 in methanol (50 wt%), was kept for 1 h at 70 °C. After cooling, 2 ml of bidistilled water was added and the mixture was extracted twice with 2 ml cyclohexane. After evaporation of cyclohexane, the dried extract was subjected to all steps of method 1.

Prior to analysis on a gas chromatograph with mass spectrometer (GC-MS) the total lipid extracts were subjected to a silylation derivatization. It involves the replacement of an acidic hydrogen on the compound with an alkylsilyl group. The derivatives are generally less polar, more volatile and more thermally stable. For the silylation, 50 µl N-methyl-N-(trimethylsilyl)-trifluoroacetamide (97 %) (MSTFA) was added to the lipid extract which was dissolved in 100 µl toluene. This mixture was held at 60 °C for 1 h before the solvent was removed under a stream of nitrogen. The silylated lipids were dissolved in 50 µl toluene before analysis.

For analysis on the high performance-liquid chromatograph with mass spectrometer (HPLC-MS), one fifth of a dried total lipid extract was dissolved in isopropanol: diethylether (80:20) prior to injection without derivatization. This combination of solvents was used as it proved to give the best results for dissolution and ionisation of the triglycerides.

2.1.3 Polar phase gas chromatography

For quantitative analyses of the FAME from the potsherds, 1 µl of the extract obtained by method 1 or method 2 was automatically injected on a Hewlett Packard 6890 GC with automatic integrator HP 3365. The instrument was equipped with a capillary 60 m fused silica column with an internal diameter of 0.32 mm, and with a polar BPX70 stationary phase (SGE, film thickness of 0,25 µm). The sample was injected twice at a split ratio of 1:100 at 250 °C. The oven temperature was held at 180 °C for 32 min, increased to 250 °C at 3 °C/min followed by an isothermal 5 min hold at 250 °C. A flame ionisation detector (FID) was used at 260 °C. Compound identification was accomplished by comparison with retention times of known methyl esters. The individual FAME were quantified by using appropriate response factors. To establish these sensitivity factors (SF), a known amount of a commercial methyl ester together with a known amount of internal standard (IS), *n*-heptadecane, was injected on the PPGC and calculated using following equation:

$$SF = \frac{C_{FAME} \times A_{IS}}{C_{IS} \times A_{FAME}}$$

with C_{FAME} being the concentration of the FAME, A_{FAME} being the area of the chromatographic peak of the FAME, C_{IS} being the concentration of the IS and A_{IS} being the area of the chromatographic peak of the IS.

2.1.4 High temperature gas chromatography

Analyses of the silylated samples on a high temperature gas chromatograph (HT-GC) with on-column injection were performed on a Shimadzu 2010 GC with flame ionisation detector (FID). This technique was used because a broad range of chemical compounds needs to be analysed here. The high oven temperature is needed to elute high molecular weight compounds while the on-column injection is a way to evaporate molecules with very high boiling points. The column used was a VB5 column of 15 m with an inner diameter of 0.32 mm and film thickness of 0.10 µm. One µl of the sample was automatically injected in the programmable temperature vaporizer (PTV) inlet system programmed at 155 °C and heated at 200 °C/min to

430 °C. The column temperature was held at 140 °C for 1 min, increased to 240 °C at 5 °C/min followed by an increase to 325 °C at 4 °C/min and held at 325 °C for 15 min followed by a last ramp to 350 °C at 2 °C/min. The detector temperature was programmed at 370 °C.

2.1.5 Gas chromatography – mass spectrometry

To be able to identify unknown chemical compounds present in the lipid fraction of an archaeological potsherd, the silylated total lipid extract was injected on a gas chromatograph with mass spectrometer (GC-MS). In mass spectrometry, an evaporated molecule gets fragmented and ionized through electron ionisation. These ions are accelerated and separated in a quadrupole due to their differences in mass. The fragmentation pattern of each molecule is different and characteristic. In that way, identification of unknown compounds is possible. The GC-MS analyses were performed on an Agilent 5973 Network Mass Selective Detector coupled to an Agilent 6890N GC with a 30 m HP5MS capillary column with an internal diameter of 0.25 mm. One µl of the sample was injected in the splitless mode at a temperature of 290 °C. The oven temperature was held at 140 °C for 2 min, increased to 325 °C at 4 °C min^{-1} and kept there for 5 min. Afterwards a second step of 1 °C min^{-1} going to 340 °C was programmed. The mass spectrometer was programmed at a temperature of 340 °C. Mass spectra were taken between *m/z* 50 and *m/z* 800 with *m/z*, according to the IUPAC definition, being the mass to charge ratio used to denote the dimensionless quantity formed by dividing the mass number of an ion by its charge number.

2.1.6 Gas chromatography – combustion – isotope ratio mass spectrometry

A method which has never performed on Sagalassos material before is the assessment of compound-specific stable isotope analysis on a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS). This highly advanced technique is an excellent approach to identify the source of animal fats, as already proved in previous

work [4]. $\delta^{13}C$ values of fatty acids are able to distinguish between ruminant (e.g. sheep and cattle) and porcine adipose fats because they reflect the differences in their metabolism and physiologies. Discrimination can also be made between ruminant adipose and dairy fats via the same $\delta^{13}C$ values. It is known that lipids are more depleted in ^{13}C than carbohydrates. As lipids in ruminant adipose fat originate from carbohydrate fermentation in the rumen and dairy fats are mainly derived from dietary unsaturated fatty acids; the C18:0 fatty acid in dairy fat is more depleted in ^{13}C than the corresponding compound in adipose fat. This results in more negative $\delta^{13}C_{C18:0}$ values for dairy fats than for ruminant adipose fats.

On a gas chromatograph, the separation of the molecules in the sample occurs. The compounds eluting from the chromatographic column then pass through a combustion reactor (an alumina tube containing Cu, Ni and Pt wires maintained at 940 ºC) where they are oxidatively combusted forming CO_2 and water. Water is then removed in a water separator by passing the gas stream through a tube constructed from a water permeable nafion membrane. The sample is then introduced into the ion source of the MS by an open split interface. Ionisation of the analyte gases is achieved using electron ionisation. The ionised gases are separated in a single magnetic sector analyser by virtue of their momentum and are detected by an array of Faraday cups the output from which is used to calculate the final stable isotope ratio.

This method was tested and optimized for ceramics from Sagalassos. For GC-C-IRMS analysis of the FAME, an Interscience Trace gas chromatograph with a DB-5 column was used (30 m; 0.25 mm and film thickness of 0.25 μm, from J&W Scientific) coupled to a Finnigan MAT Delta-S isotope ratio mass spectrometer via a modified Finnigan MAT combustion interface with commercial available catalysts. The FAME were introduced via a split injector at 250 °C. Hydrogen was used as the carrier gas. The GC oven temperature program was as follows: 2 min held at 80 °C, ramping at 6 °C/min to 250 °C, then from 250 to 300 °C at 10 °C/min and a 10 min hold at 300 °C. Chromatographic analyses were performed in duplicate. The carbon isotope ratios ($\delta^{13}C$ values) are the ratios of ^{13}C to ^{12}C, and are expressed relative to an international standard reference material called Vienna Pee Dee Belemnite (vPDB). The original vPDB material was a sample of fossilized shells of an extinct organism called a belemnite collected decades ago from the banks of the Pee Dee

River in South Carolina. In order to take into account the additional methyl group, the $\delta^{13}C$ values of the FAME were corrected by using a mass balance equation:

$$\delta^{13}C_{FA} = \frac{(((n+1)(\delta^{13}C_{FAME})) - \delta^{13}C_{KOHinMeOH})}{n}$$

with $\delta^{13}C_{FA}$ the corrected value for the fatty acid, n the carbon chain length, $\delta^{13}C_{FAME}$ the value obtained for the fatty acid of carbon chain length n, and $\delta^{13}C_{KOHinMeOH}$ the correction factor for the derivatising agent [1].

2.1.7 High performance liquid chromatography – mass spectrometry

Triglycerides are difficult to analyse with gas chromatography because they are high in carbon number and have a high boiling point. Therefore high performance liquid chromatography (HPLC) is a more suitable technique for their investigation. As unknown compounds can be present also, the HPLC system is coupled to a mass spectrometer (MS). The MS is equipped with an ion trap and is used in the atmospheric pressure chemical ionisation (APCI) mode. In APCI, the analyte solution is introduced into a pneumatic nebulizer and desolvated in a heated quartz tube before interacting with the corona discharge creating ions. The corona discharge produces primary N_2^+ and N_4^+ by electron ionisation. These primary ions collide with the vaporised solvent molecules to form secondary reactant gas ions, e.g. H_3O^+ and $(H_2O)_n^{H+}$. These reactant gas ions then undergo repeated collisions with the analyte resulting in the formation of analyte ions. The high frequency of collisions results in a high ionisation efficiency and thermalisation of the analyte ions. This results in spectra of predominantly molecular species and adduct ions with little fragmentation.
A new method was optimized to analyse triglycerides extracted from archaeological potsherds. HPLC-MS analyses were performed in duplicate on a SpectraSystem HPLC, coupled to a Finnigan LCQ DECA mass spectrometer. The reversed phase column was a 15 cm Varian OmniSpher C18 column with a diameter of 3 mm, which was held at 60 °C. A full 10 µl loop of the sample was injected. The mobile phase was a mixture of methanol and isopropanol (85:15); with the column used, this solvent mixture gave better peak separation than propionitrile. The system was operating in the isocratic mode for 30 min. Atmospheric pressure chemical ionisation (APCI) was

used in the positive mode. The temperatures of the capillary and vaporizer were 200 and 400 °C, respectively. The mass spectrometer was set to scan from m/z 200 to 1300. As regio-isomers are not well separated, data given refer to sums of the triglycerides with a given fatty acid composition.

2.2 Polyphenols

2.2.1 General

During the wine making process, grape components, and among them polyphenols, migrate into the wine. Therefore, they seem ideal compounds to be used as biomarkers for wine in archaeological ceramics. As no standard analytical procedure was available for this kind of research, we developed a new method to extract and detect polyphenols preserved in ancient amphorae.

2.2.2 Extraction

For the extraction, the following conditions were chosen after careful optimization of each parameter in the procedure: 2.5 g of the milled potsherd was stirred in 10 ml of methanol : water : acetic acid (4.5 : 4.5 : 1) for 1h at 70 °C. After centrifugation, the solvent phase was removed from the mixture and the ground material was extracted a second time following the same procedure. Solvents from both extractions were pooled and the solvent was removed with a rotary evaporator.

2.2.3 Folin Ciocalteu colorimetric reaction

The detection method was based on a standard procedure to quantify polyphenols but needed adaptation and optimization for this particular application. To prepare the Folin Ciocalteu reagent, 700 ml deionized water, 100 g sodium tungstate, 25 g

phosphomolybdic acid, 100 ml concentrated hydrochloric acid and 50 ml 85 % orthophosphoric acid were refluxed for 10h before adding 150 g lithium sulphate and a few drops of liquid bromine. The excess bromine was boiled off from an open flask before diluting to 1 l with deionized water. The dried extracts were then dissolved in 3 ml distilled water from which 2 ml was used in the Folin Ciocalteu colorimetric reaction based on a procedure from Waterman and Mole [5]. Three minutes after adding 125 µl of the Folin Ciocalteu reagent, 250 µl of a saturated sodium carbonate solution (20 g anhydrous sodium carbonate in 100 ml water) was added. After 2h the absorbance of the reactants was recorded at 760 nm. A calibration curve was prepared with gallic acid and the concentration of polyphenols in the extract was expressed as equivalent mg gallic acid. Because of potential interference by reduced iron in the Folin Ciocalteu reaction, total iron concentrations in the extracts were determined by means of a Solar 969 Atomic Absorption Spectrometer.

2.3 References

1. **Aldai, N., Murray, B.E., Nájera, A.I., Troy, D.J., and Osoro, K., 2005**, Derivatization of fatty acids and its application for conjugated linoleic acid studies in ruminant meat lipids in Journal of the Science of Food and Agriculture, 85, 1073-1083.

2. **Jones, D.M., Carter, J.F., Eglinton, G., Jumeau, E.J., and Fenwick, C.S., 1991**, Determination of δ13C values of sedimentary straight chain and cyclic alcohols by gas chromatography/isotope ratio mass spectrometry in Biological Mass Spectrometry, 20, 641-646.

3. **Kimpe, K., 2003**, Chemical analysis of the lipid fraction from ancient ceramics of Sagalassos, PhD Thesis, Katholieke Universiteit Leuven.

4. **Mottram H., Dudd S., Lawrence G., Stott A., and Evershed R., 1999**, New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats in archaeological pottery in *Journal of Chromatography A* 833: 209-221.

5. **Waterman, P., and Mole, S., 1994**, Analysis of phenolic plant metabolites, Oxford.

3 An evaluation of analytical and interpretative methodologies for the extraction and identification of lipids in Sagalassos cooking pots

3.1 Introduction

Not only physical ceramic characteristics such as vessel fabric and morphology, but also chemical analysis of the organic compounds, preserved within the ceramic matrix, gives information about food prepared in the past [9,13]. In order to detect a broad variety of lipid compounds, residue analysis uses different derivatization and chromatographic techniques. High temperature gas chromatography (HT-GC) is used to examine the total lipid profile by analysing silylated compounds, while a mass spectrometer coupled to a gas chromatograph (GC-MS) is used to look for biological markers like cholesterol. Using a highly sensitive device like a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS) [30], and plotting the $\delta^{13}C$ values of methyl palmitate against those of methyl stearate, assignments have been proposed for different animal species [17,18]. An attractive technique for triglyceride (TAG) identification is high performance liquid chromatography-mass spectrometry (HPLC-MS); animal assignments have been proposed applying this method also [23,24].

While the usefulness of each of these approaches separately has been proven beyond any doubt in previous work, it is clear that a comparison between results obtained by different techniques can strongly contribute to corroborating the conclusions. In a groundbreaking study, Dudd and Evershed [12] compared $\delta^{13}C$ values with TAG composition as analysed by HT-GC. Using the combination of both techniques, they proved the presence of dairy residues in Iron Age or Romano-British vessels. With a similar aim, the present study evaluates the results obtained by various lipid analysis techniques on samples excavated at the ancient city of Sagalassos. Three different lipid analysis techniques are used, viz. polar phase gas chromatography (PPGC), HPLC-MS for obtaining TAG profiles, and GC-C-IRMS for the quantification of stable carbon isotope contents. GC-C-IRMS has not previously been applied to

samples from Sagalassos. While GC-C-IRMS is usually performed on methyl ester derivatives, HPLC-MS analyses triglycerides. In comparing several methods, special attention should therefore be given to the sample preparation and derivatization.

3.2 Sagalassos

Sagalassos, a classical city in Southwest Turkey, has been the subject of archaeological research by the Katholieke Universiteit Leuven (Belgium) since 1990. After a few preliminary research projects, archaeological research started in 1986 and from 1989 onwards, professor Marc Waelkens from the Katholieke Universiteit Leuven took the lead in a complete excavation program. The city is laid out on south facing terraces in Turkey's western Taurus Mountains at an altitude of 1490 to 1600 m. In the early stages of the project, the main objective was to obtain a clear view of the history and development of the city mainly through excavation. Since 1993, survey studies started on the city and territory, in order to understand why the site was selected for settlement, why and how it developed and declined, and what influences the city had on the territory. The research now specially focuses on the human impact on and the relationship of the city with the environment [37,39].

The city was perhaps first mentioned in the 14th century BC when the name Sallawassa was used to describe a settlement in the Taurus mountains. A century later another name, Sallusa, was used for what could have been the same city. In the second century AD, Arrianos wrote that Alexander the Great conquered Sagalassos in 333 BC. After his government, during the Hellenistic times, the city was governed by a series of Hellenistic kings. Finally, in 133 BC, King Attalos III of Pergamon offered his kingdom, including Sagalassos, to the Romans. At the beginning of the first century BC, Pisidia became part of the province of Cilicia, where in 48 BC again, it goes back to Asia. In 39 BC, the Romans handed the northern part of Pisidia, containing Sagalassos, over to Amyntas, a Celtic aristocrat. After his death in 25 BC, the Romans took control over his kingdom and changed it to a province called Galatia.

The Roman empire introduced a flourishing period for Sagalassos, which developed to the most important economic centre of the region. Part of its prosperity found its

origin in the large potters' quarter known for its export of tableware in the Roman East. Other export products were possibly grain and timber. Thanks to these economic successes, Sagalassos was able to show its richness by building huge monuments throughout the whole city during the first centuries after Christ. From the 3rd century AD on, Pisidia encountered for the first time some difficulties because of Isauric rebellion groups and later Ostrogothic soldiers. Despite these troubles, Sagalassos kept on flourishing due to its contributions in supplying the Roman troops in the East. The potters' quarter kept on producing into the 8th century AD. The construction of several churches from the 5th century onwards is indicative for the Christian influence at that time.

The eventual decline of Sagalassos was probably initiated by catastrophes like an epidemic and an earthquake. A huge earthquake estimated around 540-620 AD and Arabic invasions in the 7th century made people leave the city. Still, isolated hamlets kept on existing until the 11th century AD and from the 12th until the 13th century a Mid-Byzantine fortress was built on the Alexander's hill.

Because of its location and a protective layer made of erosion material, the city and its monuments kept preserved during ages until Paul Lucas in 1706 discovered some remains. A century later, Francis Arundell decoded an inscription that mentioned the name Sagalassos.

In 2005, during the annual archaeological surveys in the peri-urban zone of Sagalassus (Pisidia, southwest Anatolia), the remains of an extensive Archaic-Classical settlement were identified at Tepe Düzen, 1.8 km southwest of Sagalassos. It is clear that the (proto-)urban settlement at Tepe Düzen, densely inhabited during the 8th to 4th centuries BC, was the predecessor of Hellenistic to Early Byzantine Sagalassos. The site is the first of its kind to shed light on the material culture of the ancient inhabitants of the region, the 'Pisidians'. Three seasons of inter-disciplinary research at Tepe Düzen have clearly resulted in a radically altered view on Pisidian society before the conquest of Alexander the Great. The region near later Sagalassos sustained a large, densely populated urban site, which functioned as a local centre for a small region. The settlement was heavily fortified and consisted of an 'acropolis' and a 'lower town' where habitation units were arranged in irregular clusters along a northwest-southeast axis. Craft specialization seems to be attested, while social ranking cannot as yet be attested. It seems that some centuries before this region came

into sustained contact with the Greek world, an indigenous trend towards urbanisation had firmly taken root [35].

The ancient town of Sagalassos and its territory form the subject of a multi-strategy archaeological project aiming to understand the genesis, evolution and devolvement of the urban framework and its hinterland, in strong collaboration with geo- and biosciences [38]. Much attention is hereby paid to the study of the pottery found during the annual excavations, extensive prospecting and intensive surveying campaigns, not in the least because the larger part of the 4th/3rd century BC to the 8th century AD pottery assemblage was locally or regionally produced [32]. Sagalassos red slip ware or the local tableware formed the most visible aspect of the pottery repertoire, strongly linked to urban production facilities [31]. A wide range of common wares was also produced, however, including cooking vessels of sorts [11]. Their production is most probably linked to estates, hamlets or villages in the wider territory of the site, in function of agricultural production [32].

The territory is located in an area containing a vegetation of mixed cold-deciduous and evergreen shrubs, mixed broad- and needle-leafed evergreen forests, a mixed formation of evergreen needle-leafed forest resistant to cold and an area with cold-deciduous broad-leafed woodlands without evergreens. Coniferous forests up to a height of 1000 m are mainly constituted of *Pinus brutia* stands, at higher altitudes stands of *Pinus nigra* will prevail. The current forest map of the region indicates that the present landscape in the territory consist of a mixture of degraded and overgrazed maquis, extensively or intensively exploited agricultural areas, degraded herbaceous vegetation, steppe-like vegetation at high elevations, remnants of natural forests and reforested areas [3].

The meat supply of Sagalassos was based mainly on the slaughter of domestic animals, namely cattle, pig, sheep and goat. If all studied assemblages are considered together, the ovicaprine remains are the most abundant (41.1 %), followed by cattle (35.2 %) and finally pig (22.9 %). This distribution does not merely reflect the culinary preferences of the inhabitants of Sagalassos, but is the result of the suitability of different animals for the local environment. Therefore, the abundance of the different species represents the composition of the livestock during the Roman period. Also fish remains were found at Sagalassos reflecting how the waters, both inland and marine, were exploited by man and how these products were traded. The number of the remains from mollusks and fishes is 47 and 334 respectively. Together they form

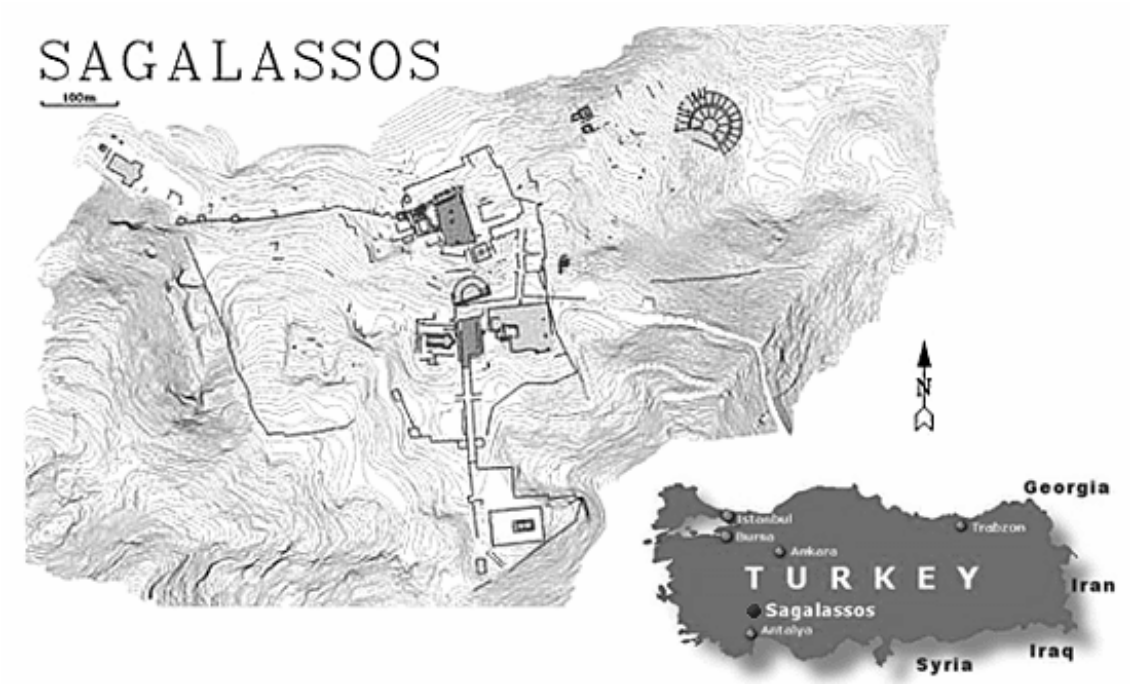


Figure 3.1: Location and map of Sagalassos.

only 0.6 % of the complete studied collection. It is however, very difficult to estimate the contribution of fishing to the food supply of the inhabitants of Sagalassos, based on the number of fish bones. It is probable that fish was more frequently consumed than is implied by the number of their remains. Nevertheless the low number of fish bones, the species diversity is rather high, namely 16 different fish species. They represent local freshwater fish, marine fish and exotic freshwater fish. This can be explained by the location of Sagalassos, namely in an area with many rivers and lakes. Considering the small distances between the town and the fishing areas it is probable that freshwater fishes were sold fresh, but this does not exclude the sale of salted, dried and smoked fish as well. Although the town of Sagalassos is located at a distance of about 110 km from the coast, marine fishes and imported freshwater fishes were represented at the site as well as local freshwater fish [10].

3.3 Samples

Sherds of twenty-six different cooking pots were selected in order to compare the three different analytic methods (Table 3.1). Cooking pots were chosen as these have

Table 3.1: Summary of the samples of the cooking pots here analysed mentioning the room and layer of excavation together with the estimated age.

Sample number	Room	Layer	Date
SA-2003-LA2-57	3	4	6th century AD
SA-2003-LA2-57	3	4	6th century AD
SA-2003-NEG-51A	2	4A	6th century AD
SA-2003-NEG-51	2	4	6th century AD
SA-2003-NEG-51A	2	4A	6th century AD
SA-2003-NEG-51C	2	4C	6th century AD
SA-2003-NEG-51C	2	4C	6th century AD
SA-2003-LA1-71A	2410-2350	10	6th-7th century AD
SA-2003-DA-166	39	5	end 6th-beginning 7th century AD
SA-2003-DA-166	39	5	end 6th-beginning 7th century AD
SA-2003-DA-267	21	7	7th century AD
SA-2003-DA-267	21	7	7th century AD
SA-2003-NEG-59A	2	5A	7th century AD
SA-2003-NEG-68	2	6	7th century AD
SA-2003-NEG-68	2	6	7th century AD
SA-2003-NEG-68	2	6	7th century AD
SA-2003-NEG-59	2	5	7th century AD
SA-2003-NEG-59	2	5	7th century AD
SA-2003-AH-46		3	12th-13th century AD
SA-2003-AH-46		3	12th-13th century AD
SA-2003-AH-46		1	12th-13th century AD
SA-2003-AH-54		1	12th-13th century AD
SA-2003-AH-61		2	12th-13th century AD
SA-2003-AH-19		3	12th-13th century AD

previously been proven to contain higher amounts of extractable lipids [21,22]. The material was collected at different excavation loci within Sagalassos, being the palatial mansion (DA)(Figure 3.2), the East Portico of the Lower Agora (LA1/LA2), the Early Byzantine Complex northeast of the Upper Agora (NEG) and the mid Byzantine fortress on the so-called Alexander's Hill (AH) [38]. The first three sites

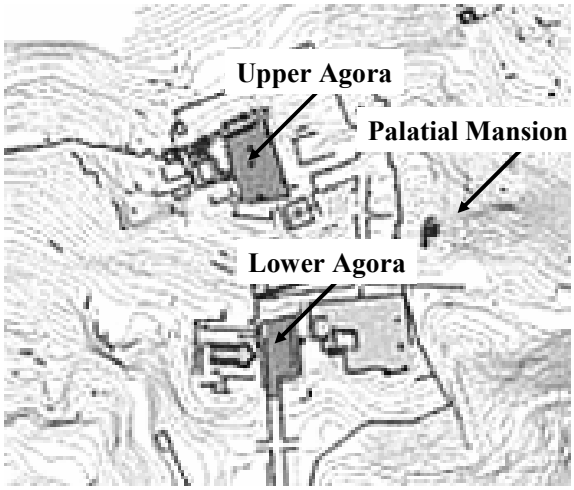


Figure 3.2: Map detail of Sagalassos with arrows indicating the different excavation loci being the Early Byzantine Complex northeast of the Upper Agora (NEG), the complex of the palatial mansion (DA) and the Lower Agora (LA).

represent mostly 6^{th}-7^{th} century AD material; the latter 12^{th}-13^{th} century remains, enabling an interesting chronological comparison. Each of the chosen fragments could be determined as belonging to a cooking vessel based on a combination of the specific local/regional fabric used for these wares, and of the specific typology, which clearly differs from other functional types of vessels [11].

3.4 Methylation methods

Initially, the substrate specificity of the different methylation methods was evaluated (see chapter 2 §1 for the experimental setup). Commercial acylglycerols (AG) and free fatty acids (FFA) were used in these experiments as model compounds for the organic constituents in the potsherds (Figure 3.3). As can be expected, based on the mechanisms of (trans)esterification [26], the use of KOH/MeOH, as in method 1, does not result in any methylation of FFA, while AG are completely converted into fatty acid methyl esters (FAME). When method 2 is used, comprising consecutive BF_3/MeOH and KOH/MeOH treatments, both AG and FFA are methylated, perfectly in agreement with earlier research [1,4]. Experiments on mono- and diglycerides showed that these react in the same manner as the triglycerides (TAG).

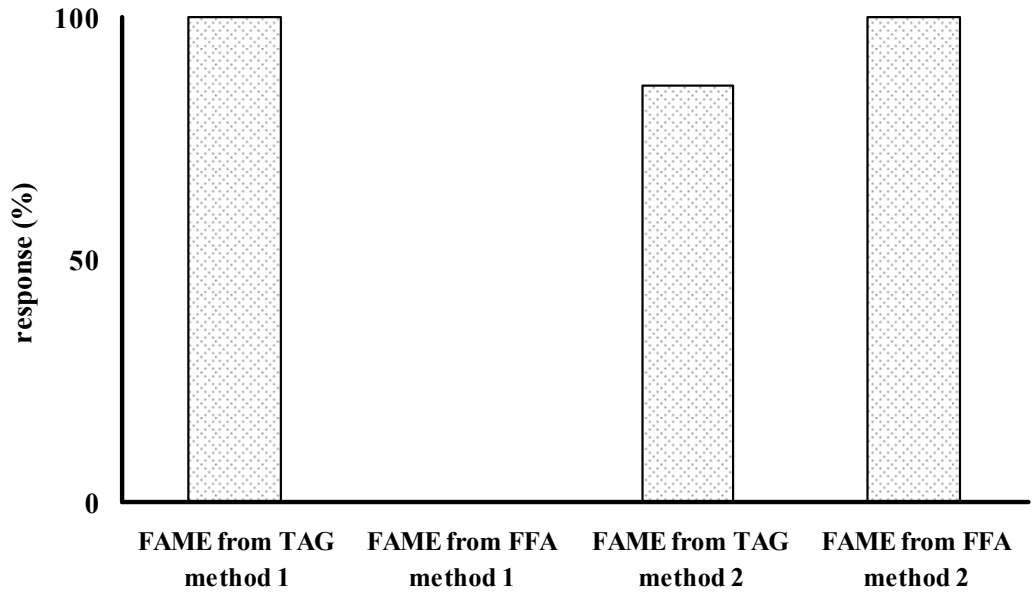


Figure 3.3: Response of the formation of fatty acid methyl esters, measured by PPGC, after transesterification of the same molar amount of pure TAG or FFA, using methods 1 or 2.

3.5 Fatty acid ratios

Because of the limited amount of material in some cases, a number of only 15 sherds could be analysed by the two methylation methods (see chapter 2 §1 for the experimental setup). Figure 3.4 presents the results for one particular potsherd. Generally, the same FAME are detected when applying both methods, except for the minor constituents C16:1 and C15:0, which were for this sherd only detected in the FFA fraction. This is not surprising, since free fatty acids are abundantly present in archaeological potsherds [18]; hence the C16:1 and C15:0 concentrations in the AG fraction are probably too low to be detected. The similar FAME patterns in the AG and AG + FFA fractions indicate that the FFA are formed as hydrolysis products from AG. This is in agreement with earlier research [18]. Detailed analysis of the FAME concentrations for saturated fatty acids, such as palmitic and stearic acid, and for unsaturated fatty acids, like oleic acid, proves that these molecules behave differently during burial. For the specific sherd studied in Figure 3.4, the methyl palmitate yield via method 2 is almost four times that using method 1.

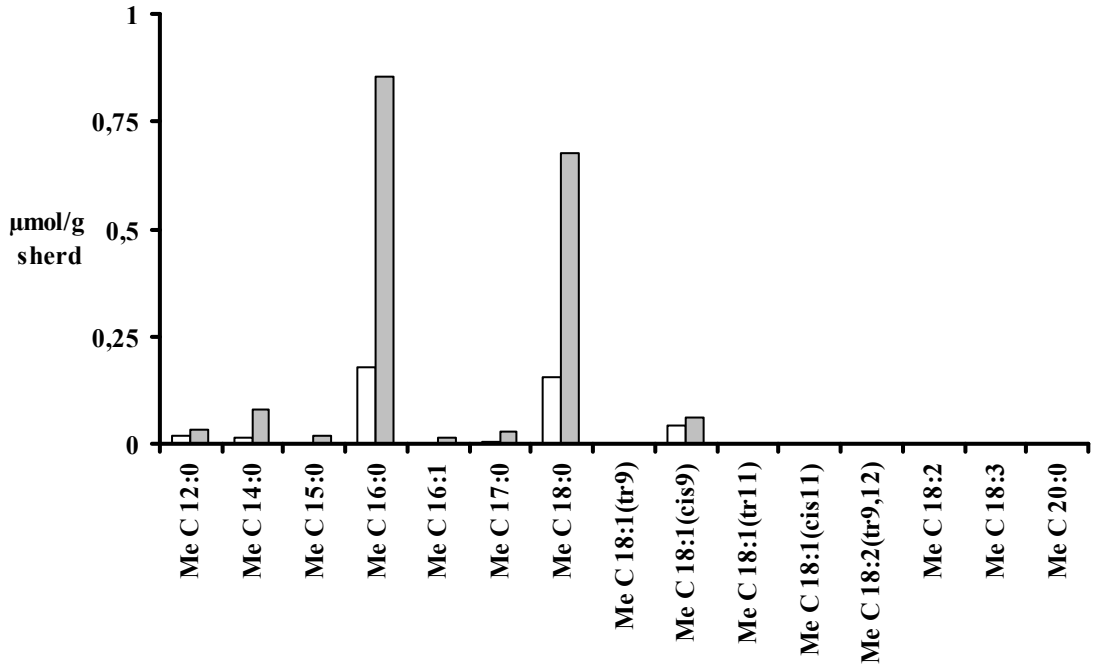


Figure 3.4: FAME concentration in the AG fraction (method 1) and in the pooled AG + FFA fraction (method 2) of an early Byzantine cooking pot (SA-2003-NEG-51A). The gray bars represent the AG+FFA fraction while the white bars represent the AG fraction.

By contrast, almost the same methyl oleate yields are obtained using either method, implying that there is hardly any free oleic acid in the sample left. A fair explanation is that oleic acid is more readily oxidized than saturated fatty acids during burial [2,25].

Evaluation of fatty acid ratios in the AG and AG + FFA fractions occurred for the same series of 15 potsherds. When plotting the ratio of methyl palmitate to methyl stearate (P/S) for the AG + FFA fraction against the P/S ratio for the AG fraction, most points plot on a straight line through the origin (Figure 3.5a).The two samples that appear under this line are depleted in palmitate in the AG + FFA fraction; probably they were subjected to a more pronounced percolation, resulting in preferential leaching of palmitate [2]. Remark that snowfall is usually rather heavy during Sagalassos winters. For the two samples above the line, there is some stearate depletion in the AG + FFA fraction, which can tentatively be ascribed to the faster oxidation of longer-chain fatty acids. Indeed, palmitic acid is the primary product of stearic acid degradation via β-oxidation (reaction scheme in chapter 1) [15,17,25]. However, most sherds seem to have practically identical P/S ratios in both fractions. This indicates that, at least in the burial conditions of this particular archaeological site, palmitate and stearate exhibit similar susceptibility to leaching. Therefore, for the

excavations studied in this work, the P/S ratio might be a trustworthy indicator for the ruminant or non-ruminant origin of fats. Indeed, ruminant adipose fat contains high concentrations of stearic acid in comparison to non-ruminant fat.

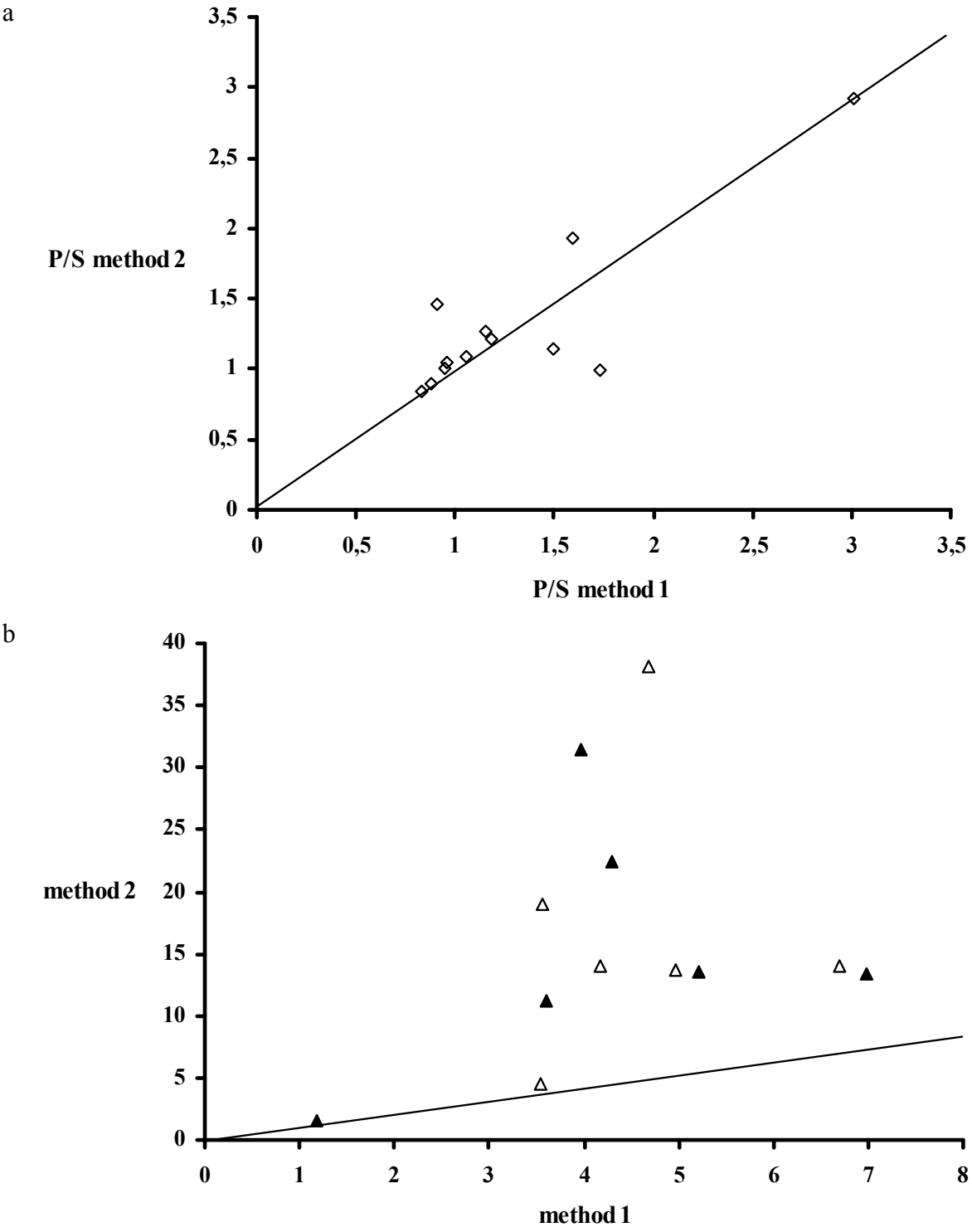


Figure 3.5 FAME ratios in the pooled AG + FFA fraction (method 2) vs. ratio in the AG fraction (method 1). Results were collected from a series of 15 Sagalassos sherds: (a) P/S ratio. (b) P/O and S/O ratios where △ represents P/O ratio and ▲ the S/O ratio

Experiments revealed that a P/S ratio below 1.3 can be considered as indicative for ruminant adipose fat [21]. At contrast, P/S ratios ranging from 4.0 to 9.4 were found for a few commercial olive oils; milk from bovine and ovine origin gives values of 2.9 and 4.9 respectively.

Table 3.2 shows P/S ratios, acquired by method 1, for lipid AG samples from Sagalassos cooking pots. The ratios allow to tentatively assigning the lipid residues to fat of ruminant or non-ruminant origin.

An analogous analysis is not possible when an unsaturated FA, like oleic acid, is considered. For a plot of the methyl palmitate to methyl oleate ratios (P/O) (Figure 3.5b), the P/O ratio in the AG + FFA fraction is often much higher than in the AG fraction. This points to a depletion of free oleic acid. The same pattern arises when S/O ratios are plotted. Oleic residues and other unsaturated FA are more readily degraded than saturated residues [25]. The ratios of unsaturated fatty acids will therefore not be applied further in this study.

3.6 Stable carbon isotope analyses

A characteristic GC-C-IRMS chromatogram, obtained for a Sagalassos cooking pot, is shown in Figure 3.6 (see chapter 2 §1 for the experimental setup). Figure 3.7 shows the results of these analyses of the methyl esters for all potsherds analysed. In this graph, the corrected $\delta^{13}C$ values (‰) for palmitate are plotted against those of stearate; allowing for animal fat identification by comparison with the isotopic composition of reference animal fats such as sheep, beef, porcine and poultry adipose fat [27]. While our values for modern porcine adipose and sheep adipose fat agree with literature, a difference is observed for cattle adipose fat [12,13,6]. The less negative $\delta^{13}C$ values for cattle adipose fat are likely due to the fact that the animal was raised on a diet of C_4 plants like maize [34]. A different position is obtained when considering samples from animals fed with C_3 plants, as in previous archaeological work [6,7,8,27]. The reference values for adipose tissue (-29.8; -31.9) and milk (-29.2; -34.0) from C_3-fed cattle have been added to Figure 3.7 and Figure 3.8 [7].

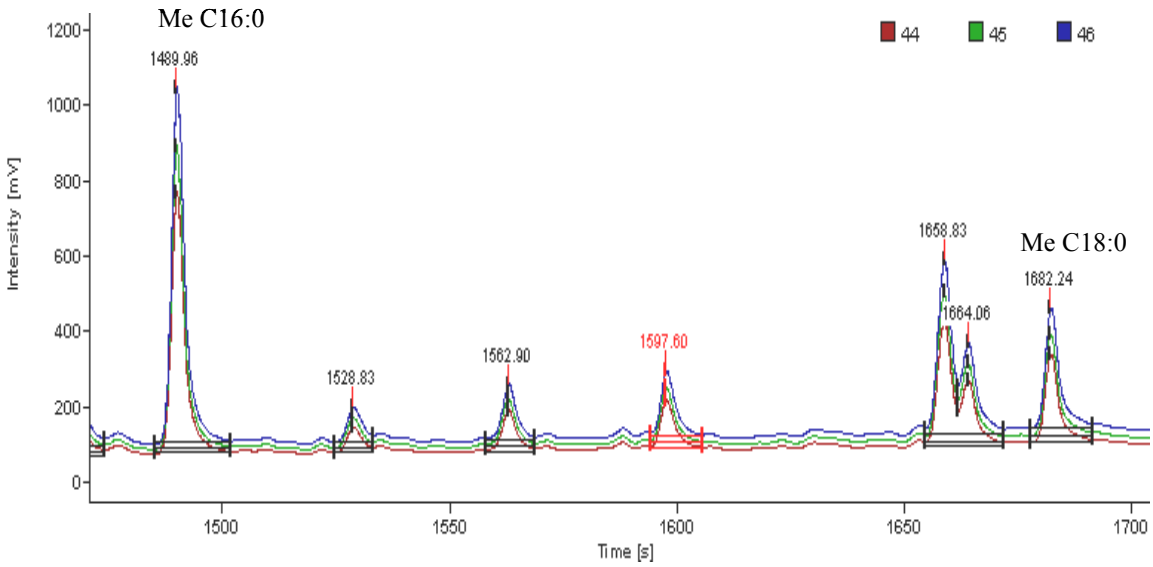


Figure 3.6: GC-C-IRMS chromatogram of fatty acid methyl esters from a representative Sagalassos cooking pot. Peaks at 1489 and 1682 s are assigned to methyl palmitate (Me C16:0) and methyl stearate (Me C18:0), respectively. The three lines, each with different color, represent the CO_2 molecules with different masses depending on the isotopes present. The red line, showing mass 44, stands for a CO_2 molecule with a ^{12}C and two ^{16}O molecules; the green line, showing mass 45, stands for a CO_2 molecule with ^{13}C and two ^{16}O molecules and a CO_2 molecule with a ^{12}C, a ^{16}O and a ^{17}O molecule; and the blue line, showing mass 46, stands for a CO_2 molecule with a ^{12}C, a ^{16}O and a ^{18}O molecule.

In interpreting the values obtained for the Sagalassos sherds, it is important to consider whether the livestock was fed on C_3 or C_4 plants [27,34]. Based on extensive pollen analysis of well-dated archaeological samples and on the current vegetation pattern in the Sagalassos region, there is strong evidence that animals exclusively consumed C_3 plants, such as e.g. wild grasses [3,33,36]. Figure 3.7 shows that few of the Sagalassos sherds can be classified in a straightforward way on the $\delta^{13}C_{18:0}$ versus $\delta^{13}C_{16:0}$ plot. Most of the samples lie in a zone between porcine or poultry adipose and ovine or bovine adipose fat and their assignment remains therefore uncertain. Only in a single case, the $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ value is indicative of ruminant dairy fat.

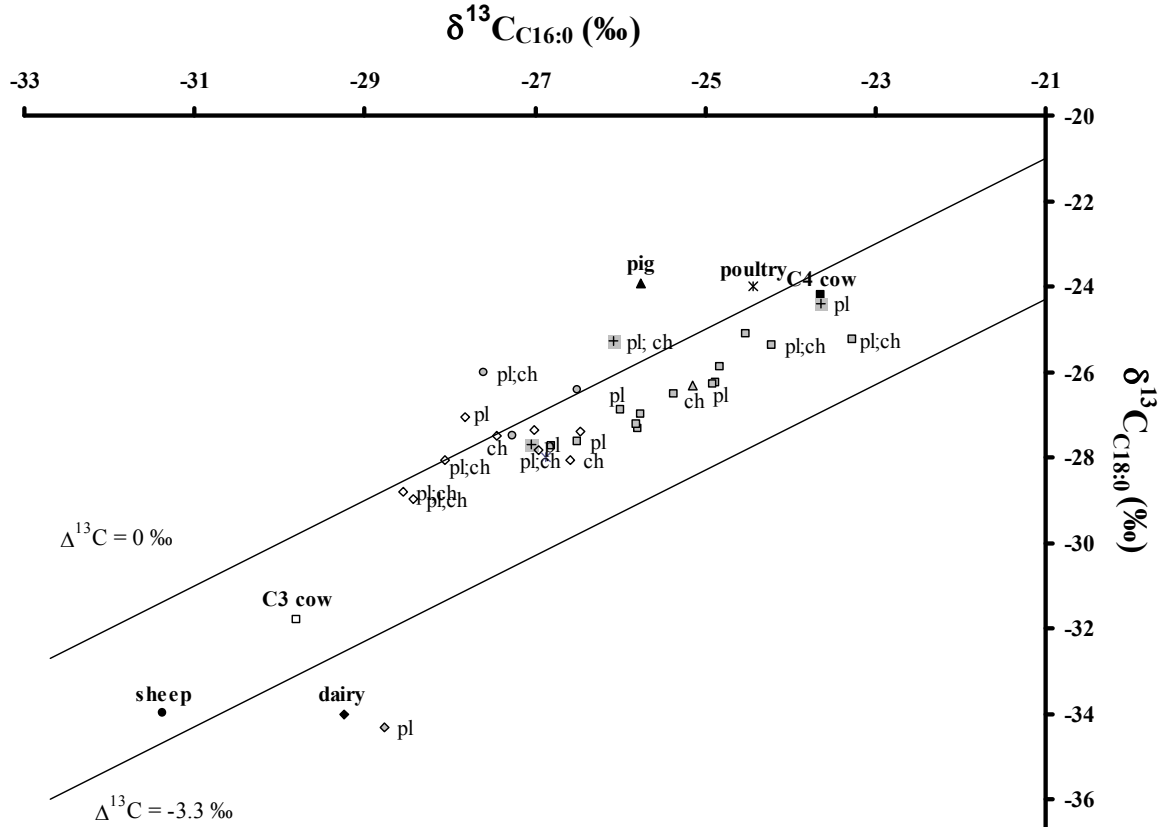


Figure 3.7: GC–C–IRMS results of FAME analysis on all Sagalassos potsherds analysed; $\delta^{13}C_{18:0}$ *vs.* $\delta^{13}C_{16:0}$ plot. The samples in which TAG could be analysed and identified by HPLC-MS are labeled as ruminant (■), non-ruminant (▲), mixture (●), dairy (♦) and non-specific (+), depending on the HPLC-MS identification. Sherds in which HPLC-MS did not detect TAG are labeled as (◊). Recent reference fats are porcine fat (▲), poultry fat (*), cattle raised on a C_4 plants (■), or on a C_3 plants diet (□), sheep fat (●), cow milk fat (♦). When plant biomarkers (pl) and/or cholesterol (ch) were present, the label was added to the plot.

The elimination of dietary effects can be obtained when plotting the $\Delta^{13}C$ value (‰) versus $\delta^{13}C_{C16:0}$ (‰) (Figure 3.8) with the $\Delta^{13}C$ value being:

$$\Delta^{13}C \text{ value} = \delta^{13}C_{C18:0} - \delta^{13}C_{C16:0} \text{ (‰)}$$

^{13}C enrichment due to carbohydrate consumption ($\delta^{13}C \cong 27.0$ ‰) is the same for C16:0 and C18:0 in adipose tissue, but is different in the mammary gland because the latter is not able to synthesize C18:0 [5]. Based on the $\Delta^{13}C$ value, a distinction between non-ruminant adipose, ruminant adipose and ruminant dairy fats can be made more accurately, as proposed by Evershed and co-workers [5]. Sample SA-2003-LA2-57A, with a $\Delta^{13}C$ value lower than -3.3 ‰ likely contained dairy products from ruminants. $\Delta^{13}C$ values between -3.3 and 0 are most frequently

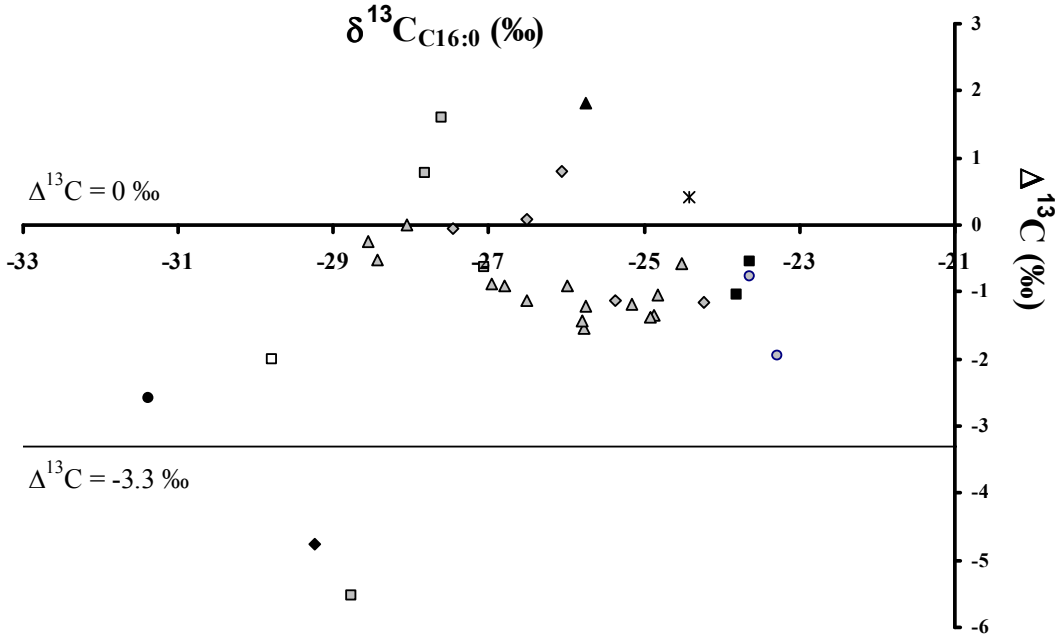


Figure 3.8: $\Delta^{13}C$ *vs.* $\delta^{13}C_{16:0}$ plot for all Sagalassos sherds analysed from four excavation areas in the city. These sites are Alexander's Hill (●); Domestic Area (♦); Lower Agora (■); North East Gate (▲). Modern fats such as porcine fat (▲), poultry fat (*), cattle raised on a C_4 plants diet (■), cattle raised on a C_3 plants diet (□), sheep fat (●), cow milk fat (♦) are also plotted. Interpretation is based on the following characteristics: $\Delta^{13}C > 0$ ‰, porcine or poultry fat; $\Delta^{13}C$ between -3.3 and 0 ‰, ruminant adipose tissue; $\Delta^{13}C < -3.3$ ‰, dairy fat.

observed (Figure 3.8); and based on the assignments made, these values can be ascribed to ruminant adipose tissue [5,6]. Finally, a few points have $\Delta^{13}C > 0$ ‰, and most probably contained non-ruminant adipose fats, such as porcine or poultry fats. In this case, four samples may be assigned as a mixture because the $\Delta^{13}C$ value is close to the limiting value between non-ruminant and ruminant meat ($\Delta^{13}C = 0$ ‰). If cooking pots are, as expected, used repeatedly, they may contain not only meat mixtures but also plant-animal mixtures. Biomarkers for animal residues, e.g. cholesterol, or for plants, e.g. β-sitosterol, can be identified by GC-MS in the silylated lipid extracts, and labels have been added accordingly to Figure 3.7. The joint appearance of animal and plant biomarkers indeed proves mixed use of cooking pots. On the $\Delta^{13}C$ vs. $\delta^{13}C_{16:0}$ plot of Figure 3.8, the different symbols describe the different excavation sites of the samples within the Sagalassos area. All sherds analysed from the North East Gate complex seem to have contained ruminant adipose fats while pots from the east portico of the Lower Agora and from the palatial mansion show more scattered values. As can be seen, assignments for Sagalassos

sherds, based either on the $\Delta^{13}C$ vs. $\delta^{13}C_{C16:0}$ plot, or the $\delta^{13}C_{C18:0}$ vs. $\delta^{13}C_{C16:0}$ plot do not always agree. As the $\Delta^{13}C$ value excludes dietary influences, it was decided to base further classifications on the $\Delta^{13}C$ vs. $\delta^{13}C_{C16:0}$ plot (Table 3.2) [5].

To finish, for 13 Sagalassos potsherds, that were on hand in sufficient amounts, the $\delta^{13}C$ values of the FAME of the TAG fraction and the FAME of the TAG + FFA fraction were compared. Figure 3.9 shows that, with few exceptions, the values are in close or even very close agreement. The largest deviations were observed for samples with very low lipid concentrations. The general trend emerging from these data is that the isotopic enrichments are similar, whether one considers only the AG fraction, or whether one includes the free fatty acids as well.

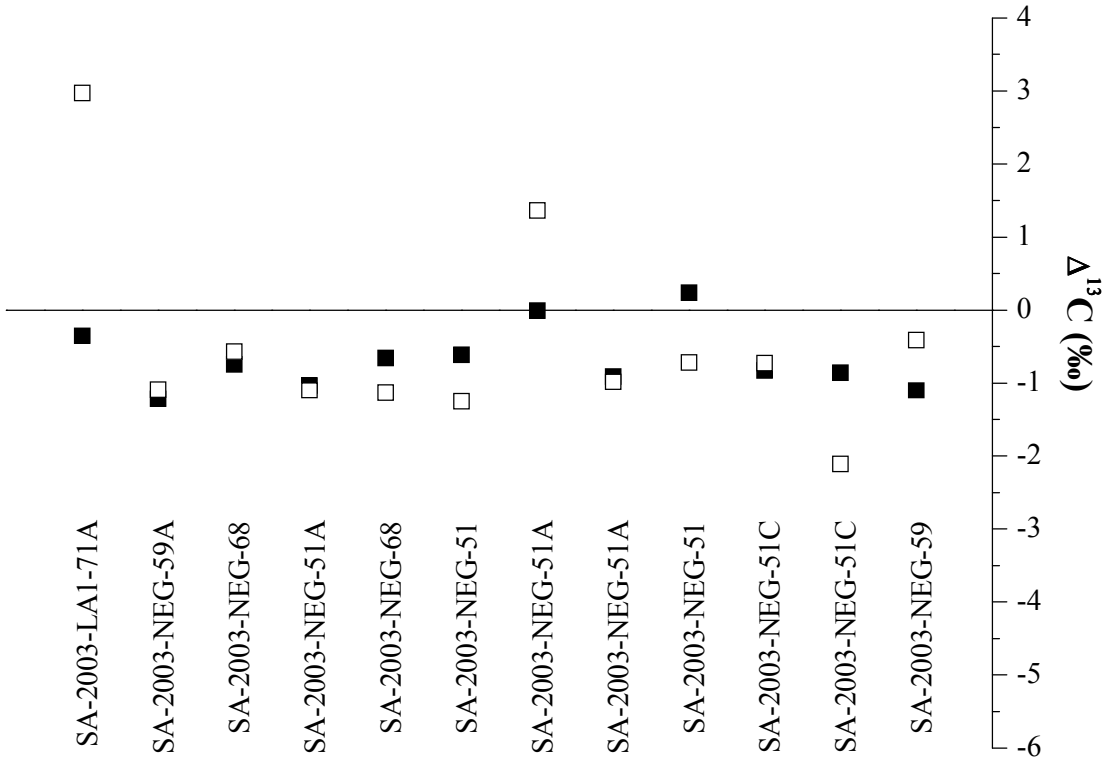


Figure 3.9: $\Delta^{13}C$ values for FAME from only TAG-fraction (■) compared with FAME from the pooled TAG and FFA-fraction (□).

Table 3.2: Comparison of results from various lipid analysis methods. The samples are denoted by their respective archaeological context numbers. Each entry corresponds to a different pot.

	P/S ratio		$\Delta^{13}C$ *vs.* $\delta^{13}C_{C16:0}$	LC-MS
SA-2003-DA-267	0.9	ruminant	ruminant	ruminant
SA-2003-DA-267	1.4	non-ruminant	non-ruminant	non-specific
SA-2003-DA-166	0.8	ruminant	ruminant	ruminant
SA-2003-DA-166	1.5	non-ruminant	mixture ($\Delta^{13}C$ = 0.08)	mixture
SA-2003-LA1-71A			ruminant	non-specific
SA-2003-LA2-57	1.5	non-ruminant	non-ruminant	mixture
SA-2003-LA2-57	1.4	non-ruminant	dairy	dairy
SA-2003-NEG-59A	0.8	ruminant	ruminant	ruminant
SA-2003-NEG-68	1.2	ruminant	ruminant	ruminant
SA-2003-NEG-68	1.3	ruminant	ruminant	ruminant
SA-2003-NEG-51A	1.2	ruminant	ruminant	ruminant
SA-2003-NEG-68	1	ruminant	ruminant	ruminant
SA-2003-NEG-51	1.1	ruminant	ruminant	ruminant
SA-2003-NEG-51A	3	non-ruminant	mixture ($\Delta^{13}C$ =-0.01)	
SA-2003-NEG-51A	1	ruminant	ruminant	ruminant
SA-2003-NEG-51	2.6	non-ruminant	mixture ($\Delta^{13}C$ = 0.23)	
SA-2003-NEG-51C	1	ruminant	ruminant	ruminant
SA-2003-NEG-51C	1.7	non-ruminant	ruminant	non-ruminant
SA-2003-NEG-59	0.9	ruminant	ruminant	ruminant
SA-2003-NEG-59	1.1	ruminant	ruminant	ruminant
SA-2003-AH-46	2.3	non-ruminant	mixture ($\Delta^{13}C$ =-0.22)	mixture
SA-2003-AH-46	4.3	non-ruminant	not detectable	non-ruminant
SA-2003-AH-46	2.5	non-ruminant	not detectable	non-ruminant
SA-2003-AH-54	4.4	non-ruminant	ruminant	non-ruminant
SA-2003-AH-61	1.6	non-ruminant	ruminant	non-specific
SA-2003-AH-19	1.8	non-ruminant	ruminant	ruminant

3.7 Triglyceride analyses

Typically, triglycerides are preserved in very small concentrations in archaeological material, nevertheless they are quite inert, and thus essentially undegraded lipid compounds. Therefore, it is worthwhile to attempt their analysis by HPLC-MS, even if not all samples contain detectable amounts of TAG. In the specific preservation context of Sagalassos, a large fraction of the samples contains sufficient amounts of TAG. TAG do not need to be derivatised before analysis (see chapter 2 §1 for the experimental setup). As can be seen in the HPLC chromatogram of Figure 3.10, co-elution of several TAG occurs, e.g. distearyl-palmityl-glycerol (PSS) and distearyl-oleyl-glycerol (SSO). Even within an overlapping peak, the individual compounds can be identified based on the observation of the pseudomolecular ion [MH^+] and on the known fragmentation pattern. While the intensity of the [MH^+] ion is low, several fragment ions are present in high abundance, allowing reliable quantification of the TAG.

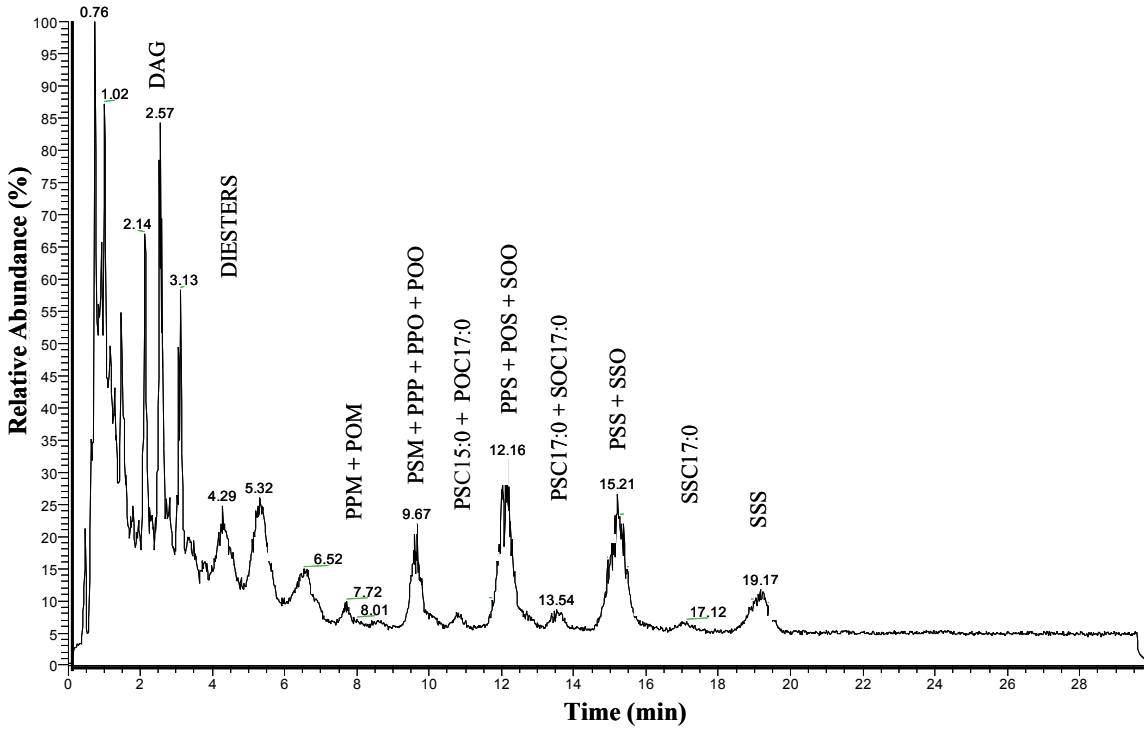


Figure 3.10: HPLC-APCI-MS chromatogram of the lipid extract from Sagalassos potsherd SA-2003-NEG-51A. Peaks before 5.32 min are assigned to diglycerides and diesters. Peaks of even and odd-numbered triglycerides have been labeled on the chromatogram. This result is characteristic for ruminant adipose fat.

Triglycerides from contemporary reference fats from ruminants, like sheep and cattle, and non-ruminants, like pork (Figure 3.11) and poultry, show significant differences between the two groups. For example, while tristearin (SSS) is detected in all ruminant adipose fat, it is absent or present, in only very small quantities, in the TAG profiles of non-ruminant adipose fat (Figure 3.12) [28]. A similar situation is encountered for odd numbered fatty acids on the glycerol backbone of triglycerides [40]. Their presence is limited to ruminant meat because the rumen of these animals contains micro-organisms synthesizing these odd-numbered fatty acids. Odd-numbered fatty acids may also be present due to microbial soil contamination. However, previous research proved that microbial lipids only contribute to a minor extent to the overall lipid distribution of the absorbed lipids [14]. Another indication for the presence of ruminant adipose fat is the occurrence of larger quantities of distearyl-oleyl-glycerol (SSO). This molecule can also be found in non-ruminants, but to a much lower extent than in bovine or ovine fat. For example, we found 1.1 and 2.0 % SSO in pork and chicken meat respectively, against 8.5 and 14.7% SSO in beef and sheep respectively. Similar results were reported in literature [28]. On the other hand, positive markers to distinguish non-ruminant adipose fat from ruminant adipose fat are the larger quantities of LiLiM, LiPP, LiOP and LiOO, where M and Li stand for myristic and linoleic acid. However, the latter compounds can also be found in milk fats or in a vegetable oil such as olive oil (Figure 3.13). Care must be taken when using unsaturated TAG as markers because they are readily oxidized and therefore less likely to survive burial. Nevertheless considerable amounts of these molecules were recovered from the samples here analysed.

Based on the occurrence of specific triacylglycerols, a classification of the potsherds was attempted. Halve of the sherds show clear evidence for the presence of ovine or bovine adipose fat. This profile, as shown in Figure 3.14, is especially frequent among potsherds excavated in the North East Gate area. The relative concentrations of saturated and non-polar TAG, such as SSS, tend to be higher in the archaeological samples than in the modern fats. The enrichment in SSS, PSS or SSO implies that highly unsaturated TAG, such as LiOO or LiOP, may be degraded or leached over time. Nevertheless, matching profiles of non-ruminant adipose fat were observed several times, especially in samples from Alexander's Hill. A third group of sherds may well have contained a mixture of ruminant and non-ruminant adipose fat, for instance with the simultaneous presence of substantial amounts of LiOP, LiOO and

SSS. In order to be observed, non-ruminant fat should be present in sufficient amounts because it might easily be overlooked in case ruminant fat is also present.

Only one sample gave a TAG profile similar to that of milk fats (SA-2003-LA2-57)(Figure 3.15 vs. Figure 3.13) [29]. This matches the outcome of the GC-C-IRMS analysis for this particular potsherd from the east portico of the Lower Agora. Apart from some sherds that did not have detectable amounts of TAG, a final group presented a non-specific profile. While the presence of e.g. POS or PSS suggests that the lipids are of animal origin, specific marker molecules, such as SSS or odd numbered fatty acids, are absent. The classifications of the Sagalassos potsherds based on HPLC-MS analysis are gathered in Table 3.2

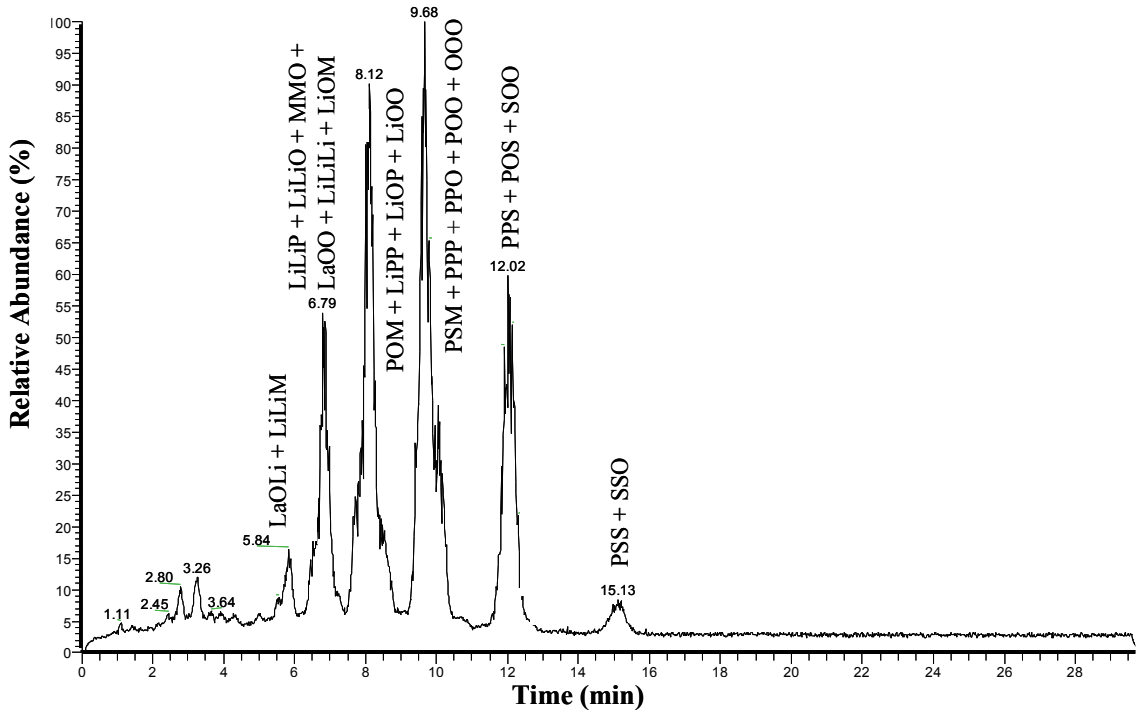
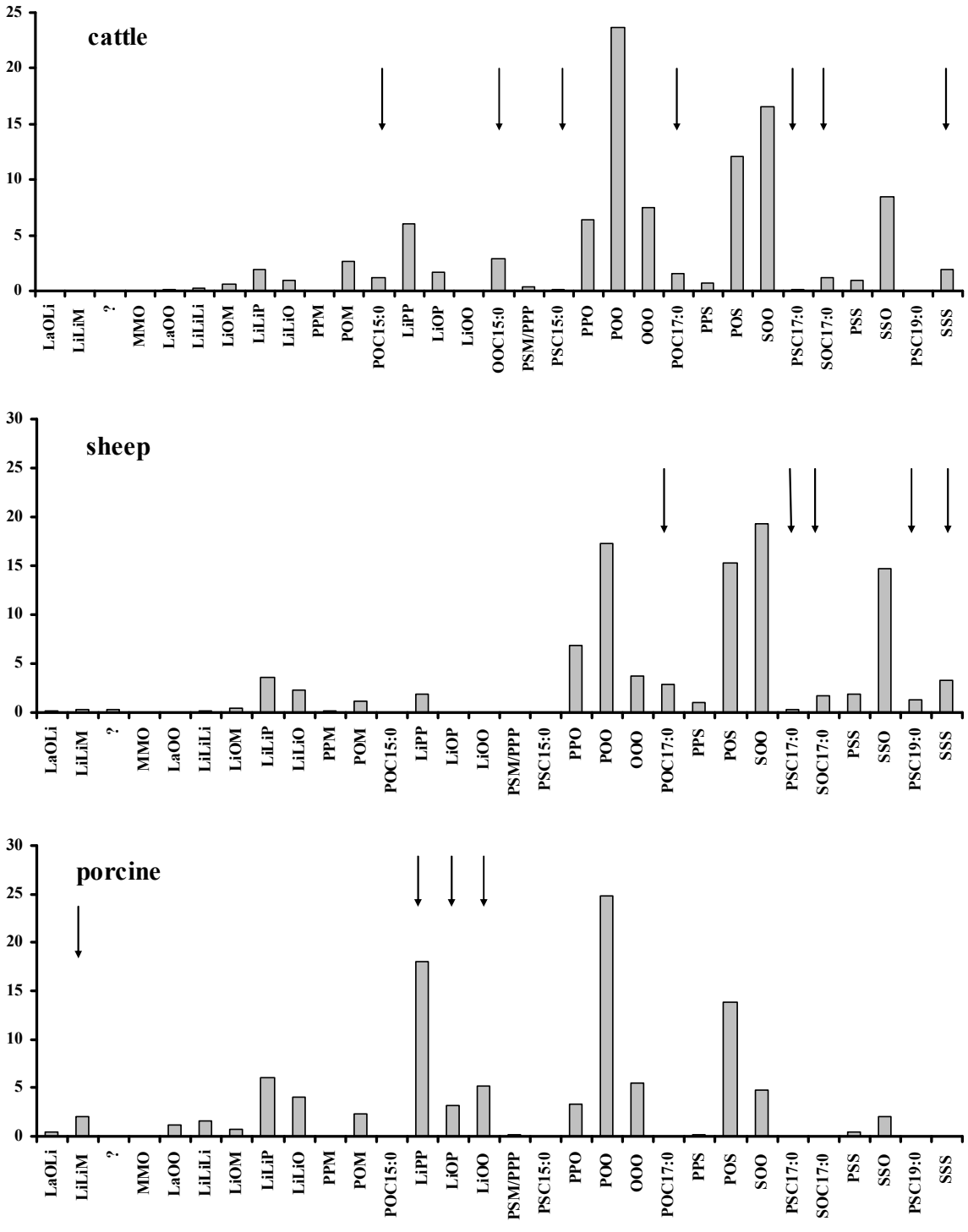


Figure 3.11: HPLC-MS chromatogram of lipid extract from modern porcine adipose fat. Peaks of odd-numbered triglycerides and tristearin are not detected.


cattle
sheep
porcine
LaOLi
LiLiM
?
MMO
LaOO
LiLiLi
LiOM
LiLiP
LiLiO
PPM
POM
POC15:0
LiPP
LiOP
LiOO
OOC15:0
PSM/PPP
PSC15:0
PPO
POO
OOO
POC17:0
PPS
POS
SOO
PSC17:0
SOC17:0
PSS
SSO
PSC19:0
SSS

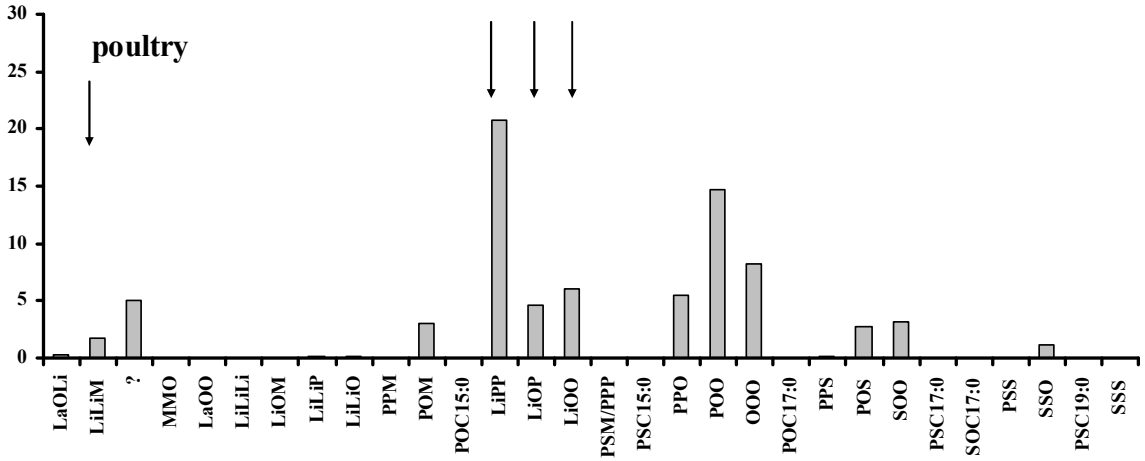


Figure 3.12: TAG profiles of modern reference fats (cattle, sheep, pork and poultry) acquired on the HPLC-MS. Arrows indicate diagnostic TAG.

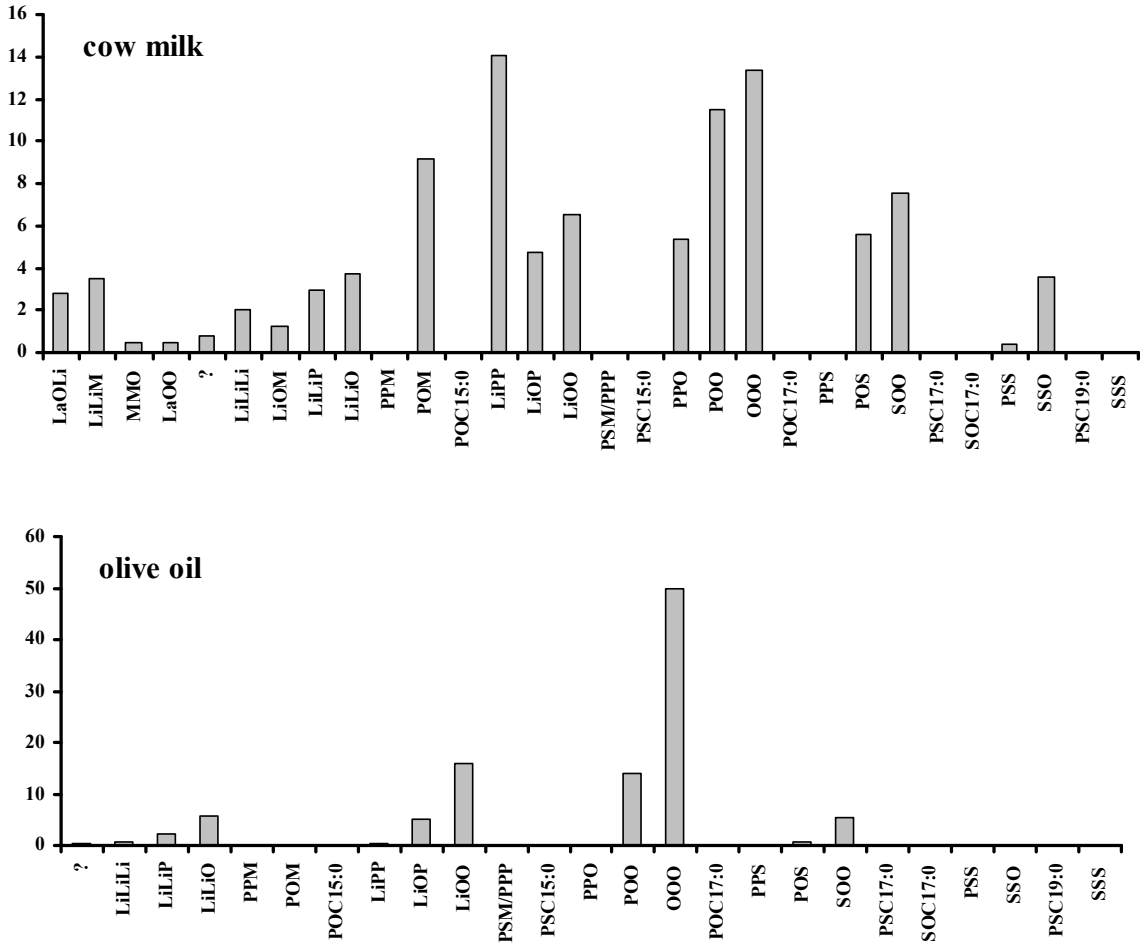


Figure 3.13: TAG profile of modern milk fat and olive oil.

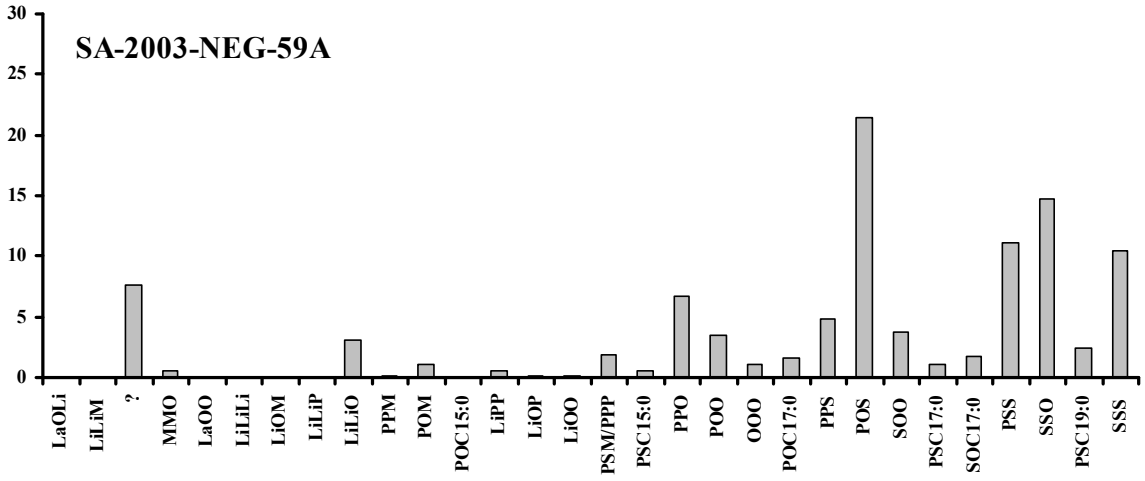


Figure 3.14: TAG profile from Sagalassos cooking pot SA-2003-NEG-59A, with characteristic profile of ruminant adipose fat.

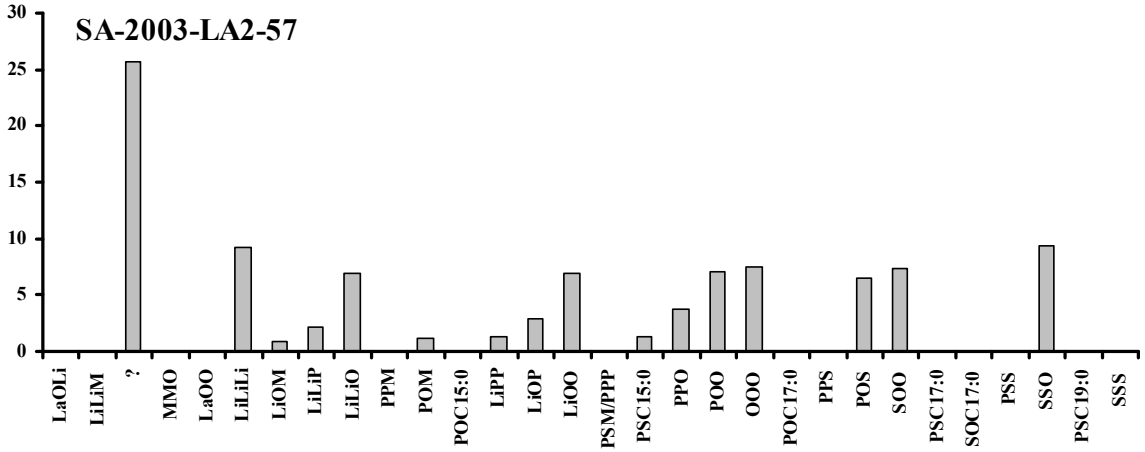


Figure 3.15: TAG profile from Sagalassos cooking pot SA-2003-LA2-57, with a TAG profile similar to the profile of milk.

3.8 The three techniques compared

The results for the three analytical methods (P/S ratio, $\Delta^{13}C$ vs. $\delta^{13}C_{C16:0}$ for AG-fraction and HPLC-MS) are gathered in Table 3.2, and are compared for the 26 potsherds here analysed. For 23 sherds, results for each method are available. 17 out of this subset give congruous results.

For the detailed comparison between GC-C-IRMS ($\Delta^{13}C$ vs. $\delta^{13}C_{C16:0}$ for AG-fraction) and HPLC-MS, 20 out of the 22 available results were in agreement. For the preparation of FAME for GC-C-IRMS, a method was applied that only converts the acylglycerol compounds using KOH in methanol. Hence, free fatty acids did not contribute to the GC-C-IRMS, and this makes it more straightforward to compare results from FAME analysis using GC-C-IRMS with the results of TAG analysis by HPLC-MS. On the isotope ratio plot of Figure 3.7, the points are labeled according to results acquired with HPLC-MS. Particularly for assignment of ruminant adipose tissue, agreement between the two methods is excellent (Table 3.2).

It is gratifying to see that for one sample, both HPLC-MS and GC-C-IRMS gave a positive identification of dairy products. Finally, for a group of samples, the TAG concentrations were insufficient to be detected by HPLC-MS, while data were obtained for the isotope ratio analysis of the derived methyl esters. Curiously, the latter points appear in a cluster on the isotope ratio plots, with rather low $\delta^{13}C_{C16:0}$ and $\delta^{13}C_{C18:0}$ values between -27 and -29 ‰ (Figure 3.7).

The results summarized in Table 3.2 indicate that the HPLC-MS technique is a trustworthy method to gather information on the origin of TAG in archaeological potsherds because the results are in agreement with isotope analysis results. The main inconvenience is that in a few potsherds scarce amounts of TAG were detected. As many archaeological cooking pots might have contained mixtures, HPLC-MS analysis can deliver supporting information in comparison to isotope analysis, for instance by identifying SSS. A possible drawback of the GC-C-IRMS method is that some valuable information may be lost when only the $\Delta^{13}C$ value is considered instead of $\delta^{13}C_{18:0}$ versus $\delta^{13}C_{16:0}$. Indeed, in our data set, many samples show a simultaneous increase of the $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$ values with respect to what is expected for C_3-based ruminant adipose fat, even if the $\Delta^{13}C$ and HPLC-MS results suggest that this is the correct assignment. The reason for these increased $\delta^{13}C_{18:0}$ and $\delta^{13}C_{16:0}$ values so far is unclear.

Finally, for the present samples with their specific history, it seems that the P/S ratio is a rather good first indicator for the nature of the animal fat, even if the method lacks the refinement to unequivocally recognize dairy samples. Nevertheless, one should be cautious to apply this parameter at other sites with a different burial environment.

3.9 Implication of the results in the context of Sagalassos

The cooking pots, here analysed, come from different locations within ancient Sagalassos. Generally, the preponderance of ruminant adipose fat is in agreement with archaeozoological findings on livestock composition at ancient Sagalassos based on the relative number of identified bones. Cattle, ovicaprines and pig are represented for 25, 48 and 27% respectively [10]. For the first time, there is evidence that dairy fats were processed in cooking pots excavated in Sagalassos (SA-2003-LA2-57). Additionally, vessels excavated in the North East Gate area predominantly contain lipids of ruminant adipose origin. Non-ruminant adipose profiles prevail in the samples collected on the so-called Alexander's Hill. Finally, samples from the Lower Agora and the palatial mansion present a mixed pattern. The three early Byzantine excavation areas are associated with urban occupation. In contrast, the mid Byzantine fortress on Alexander's Hill was essentially a military camp, and was destroyed in the later 12th-early 13th century AD. It is of interest to note that the mid Byzantine food pattern of the military on Alexander's Hill is somewhat different from, at least, that of the early Byzantine civic occupation in the North East Gate area. Archaeozoological research further demonstrated that, in contrast to other excavation areas, food provisioning to the mid Byzantine military was especially focused on animals with a high meat yield, including mainly beef, followed by pork. Deer bones were also relatively abundant on Alexander's Hill (unpublished data).

3.10 Conclusion

The comparison of P/S analysis, GC-C-IRMS of methyl esters and HPLC-MS analysis of triacylglycerols was evaluated in this study. For the twenty-six cooking pots here analysed, the three methods appeared to be complementary and generally in agreement. P/S analysis in the methyl ester fraction is a quick test, but it can only be reliable if the P/S ratios are not affected by selective leaching or oxidation. GC-C-IRMS seems so far the best option for unequivocal identification of dairy residues. Whereas the origin of the absolute values of $\delta^{13}C_{C16:0}$ and $\delta^{13}C_{C18:0}$ requires

further research, the method based on the difference $\Delta^{13}C$ often gives clear answers. Finally, HPLC-MS is a compound-specific technique, and can be particularly useful to identify specific triacylglycerols. Its main limitation is that TAG may at many sites not survive burial, even if their preservation at Sagalassos was sufficient for analysis.

3.11 References

1. **Aldai, N., Murray, B.E., Nájera, A.I., Troy, D.J., and Osoro, K., 2005**, Derivatization of fatty acids and its application for conjugated linoleic acid studies in ruminant meat lipids in *Journal of the Science of Food and Agriculture*, 85, 1073-1083.

2. **Belitz, H.D., and Grosch, W., 1999**, Food chemistry (second edition), Berlin.

3. **Beuls, I., 2004,** Design of odontological tools to elucidate small ruminant herd management at Sagalassos (SW-Turkey) in the Roman-Byzantine period (0-650 AD), PhD thesis, Katholieke Universiteit Leuven.

4. **Carvalho, A.P., and Malcata, F.X., 2005**, Preparation of fatty acid methyl esters for gas-chromatographic analysis of marine lipids: insight studies in *Journal of Agricultural and Food Chemistry*, 53, 5049-5059.

5. **Copley, M.S., Berstan, R., Dudd, S.N., Docherty, G., Mukherjee, A.J., Straker, V., Payne, S., and Evershed, R.P., 2003**, Direct chemical evidence for widespread dairying in prehistoric Britain in *Proceedings of the National Academy of Sciences*, 100 (4), 1524-1529.

6. **Copley, M.S., Berstan, R., Dudd, S.N., Straker, V., Payne, S., and Evershed, R.P., 2005a**, Dairying in antiquity. I. Evidence from absorbed lipid residues dating to the British Iron Age in *Journal of Archaeological Science*, 32, 485-503.

7. **Copley, M.S., Berstan, R., Straker, V., Payne, S., and Evershed, R.P., 2005b**, Dairying in antiquity. II. Evidence from absorbed lipid residues dating to the British Bronze Age in *Journal of Archaeological Science*, 32, 505-521.

8. **Copley, M.S., Berstan, R., Mukherjee, A.J., Dudd, S.N., Straker, V., Payne, S., and Evershed, R.P., 2005c**, Dairying in antiquity. III. Evidence from absorbed lipid residues dating to the British Neolithic in *Journal of Archaeological Science*, 32, 523-546.

9. **Craig, O., Mulville, J., Parker Pearson, M., Sokol, R., Gelsthorpe, K., Stacey, R., and Collins, M., 2000**, Detecting milk proteins in ancient pots in *Nature*, 408, 312.

10. **De Cupere, B., 2001**, Animals at ancient Sagalassos. Evidence of the faunal remains (Studies in Eastern Mediterranean Archaeology 4) Turnhout.

11. **Degeest, R., 2000**, The common wares of Sagalassos (Studies in Eastern Mediterranean Archaeology 3) Turnhout.

12. **Dudd, S.N., and Evershed, R.P., 1998**, Direct demonstration of milk as an element of archaeological economies in *Science*, 282, 1478-1480.

13. **Dudd, S.N. and Evershed, R.P., and 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 in *Journal of Archaeological Science*, 26, 1473-1482.

14. **Dudd, S.N., Regert, M., and Evershed, R.P., 1998**, Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds in *Organic Geochemistry*, 29, 1345-1354.

15. **Eerkens, J.W., 2005**, GC-MS analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America in *Archaeometry* , 47, 1, 83-102.

16. **Evershed, R.P., Berstan, R., Grew, F., Colpey, M.S., Charmant, A.J.H., Barham, E., Mottram, H.R., and Brown, G., 2004**, Formulation of a roman cosmetic in *Nature*, 432, 35-36.

17. **Evershed, R.P., Dudd, S.N., Copley, M.S., Berstan, R., Stott, A.W., Mottram, H., Buckley, S.A., and Crossman, Z., 2002**, Chemistry of archaeological animal fats in *Accounts of Chemical Research*, 35 (8), 660-668.

18. **Evershed, R.P., Heron, C., Charters, S., and Goad, L.J., 1992**, The survival of food residues: new methods of analysis, interpretation and application in *Proceedings of the British Academy*, 77, 187-208.

19. **Evershed, R.P., Heron, C., and Goad, L.J., 1990**, Analysis of organic residues of archaeological origin by high temperature gas chromatography and gas chromatography mass spectrometry in *The Analyst*, 115, 1339-1342.

20. **Jones, D.M., Carter, J.F., Eglinton, G., Jumeau, E.J., and Fenwick, C.S., 1991**, Determination of $\delta^{13}C$ values of sedimentary straight chain and cyclic alcohols by gas chromatography/isotope ratio mass spectrometry in *Biological Mass Spectrometry*, 20, 641-646.

21. **Kimpe, K., 2003**, Chemical analysis of the lipid fraction from ancient ceramics of Sagalassos, PhD Thesis, Katholieke Universiteit Leuven.

22. **Kimpe, K., Drybooms, C., Schrevens, E., Jacobs, P.A., Degeest, R., and Waelkens, M., 2004**, Assessing the relationship between form and use of different kinds of pottery from the archaeological site Sagalassos (Southwest Turkey) with lipid analysis in *Journal of Archaeological Science*, 31, 1503-1510.

23. **Kimpe, K., Jacobs, P.A., and Waelkens, M., 2001**, Analysis of oil used in late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat in *Journal of Chromatography A*, 937, 87-95.

24. **Kimpe, K., Jacobs, P.A., and Waelkens, M., 2002**, Mass spectrometric methods prove the use of beeswax and ruminant fat in the late Roman cooking pots in *Journal of Chromatography A,* 968, 151-160.

25. **Malainey, M.E., Przybylski, R., and Sherriff, B.L., 1999**, The effects of thermal and oxidative degradation on the fatty acid composition of food plants and animals of Western Canada: implications for the identification of archaeological vessel residues in *Journal of Archaeological Science*, 26, 95-103

26. **March, J., 1985**, Advanced Organic Chemistry, 3^{d} Edition, John Wiley & Sons, New York.

27. **Mottram, H.R., Dudd, S.N., Lawrence, G.J., Stott, A.W., and Evershed, R.P., 1999**, New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats preserved in archaeological pottery in *Journal of Chromatography A*, 833, 209-221.

28. **Mottram, H., Crossman, Z. and Evershed, R.P., 2001a**, Regiospecific characterisation of the triacylglycerols in animal fats using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry in *The Analyst*, 126, 1018-1024.

29. **Mottram, H.R., and Evershed, R.P., 2001b**, Elucidation of the composition of bovine milk fat triacylglycerols using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry in *Journal of Chromatography*, 926, 239-253.

30. **Mottram, H.R., and Evershed, R.P., 2003**, Practical considerations in the gas chromatography/combustion/isotope ratio monitoring mass spectrometry of ^{13}C-enriched compounds: detection limits and carryover effects in *Rapid Communications in Mass Spectrometry*, 17, 2669-2674.

31. **Poblome, J., 1999**, Sagalassos red slip ware. Typology and chronology (Studies in Eastern Mediterranean Archaeology 2), Turnhout.

32. **Poblome, J., 2006**, Made in Sagalassos. Modelling regional potential and constraints in *Territorio e produzioni ceramiche. Paesaggi, economia e società in età romana, Menchelli, S. and Paquinucci, M. (eds), Instrumenta 2, Pisa University Press, 355-363 and 420-421*.

33. **Sage, R.F., and Monson, R.K., 1999**, C4 plant biology, Academic Press, New York.

34. **Tieszen, L.L., 1991**, Natural variations in the carbon isotope values of plants: implications for archaeology, ecology and paleoecology in *Journal of Archaeological Science*, 18, 227-248.

35. **Vanhaverbeke, H., Waelkens, M., Vyncke, K., De Laet, V., Aydal, S., Mušič, B., De Cupere, B., Braekmans, D., Degryse, P., Marinova, E., Poblome, J., Verstraeten, G., Van Neer, W., Šlapšak, B., Medaric, I., Ekinci, H.A., and Erbay M.O., submitted**, Emerging urbanism in Pisidia (southwest Anatolia). The origins of Sagalassus at Tepe Düzen in *Anatolian Studies.*

36. **Vermoere, M., Vanhecke, L., Waelkens, M., and Smets, E., 2001**, Modern pollen studies in the territory of Sagalassos (Southwest Turkey) and their use in the interpretation of a Late Holocene pollen diagram in *Review of Palaeobotany and Palynology*, 114, 29-56.

37. **Waelkens M., 2002**, Ontdekking van het verloren Sagalassos. Uitgeverij Davidsfonds

38. **Waelkens, M., 2006**, Sagalassos, in W. Radt (ed.) Stadtgrabungen und Stadtforschung in Westlichen Kleinasien (Byzas 3) Istanbul, 325-358.

39. **Waelkens M., Paulissen E., Vermoere M., Degryse P., Celis D., Schroyen K., De Cupere B., Librecht I., Nackaerts K., Vanhaverbeke H., Viaene W., Muchez P., Ottenburgs R., Deckers S., Van Neer W., Smets E., Govers G., Verstraeten G., Steegen A., and Cauwenberhs K., 1999**, Man and environment in the territory of Sagalassos, a classical city in SW Turkey in *Quaternary Science Reviews* 18: 697-709

40. **Zubay, G.L., 1998**, Biochemistry, Wm. C. Brown Publishers, Dubuque.

4 Assessing the content of fabric 4 amphorae from Sagalassos

4.1 Introduction

Amphorae are of great archaeological value not only because of their numerical preponderance at many late Roman sites around the Mediterranean, but also because of the diversity of the regional production and of the scale of circulation. As a result, amphorae are a very useful methodological tool in documenting socio-economic patterns in antiquity. Notwithstanding the complex history of amphora research, especially in the Roman East, with a resulting jungle of nomenclature, many archaeologists and ancient historians have considered the wider implications of the amphora field in their research.

Particular areas of growth in amphora research can still be identified, such as the study of provenance, production infrastructure and organization, or the determination of the varying and fluctuating scales of distribution. However, the ancient written and documentary records on the functional use of amphorae are few and not necessarily straightforward to interpret [19]. Therefore, chemical analysis of organic residues might represent a most valuable avenue of research, providing the answer to the simple archaeological question whether an amphora contained oil, fish products or rather wine, if not something different all together. These questions may seem simple, but we are only beginning to grasp the ramifications of the scientific answers.

In residue analysis, the chemical composition of vegetal oils is easily recognized. A low stearate content is characteristic for all oils, while the percentage of unsaturated fatty acids such as oleic acid is high [12]. Another greasy food that is typically contained in amphorae, is fish products. Recovering the latter after many years of burial is more complicated, because fish fatty acids are polyunsaturated. They are therefore very sensitive to oxidation and what's more, these degradation products are easily washed out. In a rare report, hexadecenoic acic (C16:1),

eicosenoic acid (C20:1) and docosenoic acid (C22:1) were detected together in ceramics from the Iron Age settlement in Old Scatness in Shetland; these fatty acids were considered as biomarkers for cod and herring [3]. Hansel *et al.* [10] detected ω-(*o*-alkylphenyl)alkanoic acids in 10th century AD pottery from Southern Brazil; they suggested that these stable compounds are formed upon heating the triunsaturated fatty acids in fish. It should be noted, however, that preparation of *garum* does not always comprise a heating step. Clearly, the search for unambiguous and well-preserved fish or fish sauce biomarkers is ongoing.

Wine completes the triad of typical amphora-related products. Although wine was an ordinary aspect of daily life in antiquity, this fermented beverage was rarely the point of attention in residue analysis. Biomarkers for wine could be tartaric acid or polyphenols because they are both present in high quantities in wine [2]. Earlier research attempted the use of liquid chromatography with mass spectrometry in tandem mode to detect tartaric acid in an ancient Egyptian wine jar from Tutankhamun's tomb [14]. Michel *et al.* [15] and Badler *et al.* [1] claimed the presence of tartaric acid based on Fourier transform-infrared spectroscopy and the Feigl spot test. As a second possible biomarker for wine, polyphenols were also investigated in a few studies. For example, Petit-Domínguez *et al.* [17] determined tannin concentration in 5th century BC Iberian amphorae of the R1 Phoenician type with a Folin Denis colorimetric reaction, while others successfully applied an *in situ* tetramethylammonium hydroxide treatment followed by thermally assisted hydrolysis and methylation gas chromatography-mass spectrometry (GC-MS) on Roman wines. The latter were preserved in sealed amphorae from shipwrecks, the first being a Dressel 1-type amphora found on the *Madrague de Giens*; the second one was a Haltern 70 type transported by the *Port-Vendres II* [13]. An alternative approach is alkaline fusion of polyphenols containing *e.g.* malvidin; the syringic acid which is released is easily identified [14]. Closely related to direct detection of wine molecules, is the analysis of resins and pitches that were used as a wine additive, or as an inner coating for the amphorae [4,6,9].

This study aims at providing new insights in the functional use of a typical range of amphorae excavated in Sagalassos in 2004. The amphora sherds were subjected to standard lipid analyses in order to investigate potential storage of vegetal oils or fish sauce. Next to 7 Late Roman 1 and a few Late Roman 3, Late Roman 4 and Phoenician amphorae, a range of 17 local/regional fabric 4 amphora sherds were

analysed. Three different lipid analysis techniques were used: polar phase gas chromatography (PP-GC), high performance liquid chromatography-mass spectrometry (HPLC-MS) for obtaining triglyceride (TAG) profiles, and GC-MS for searching biomarkers like for example methyldehydroabietic acid and cholesterol. Another goal of this PhD work was to develop a new methodology for the detection of polyphenols and to test it on the same range of pottery.

4.2 Samples

31 sherds of different amphorae (Table 4.1) were selected for lipid and polyphenol analyses. The material was collected from different excavation sites within Sagalassos: room 25 in the palatial urban mansion (DA = domestic area), and rooms 3, 5 and 7 of the early Byzantine complex northeast of the Upper Agora (NEG = north eastern gate) [21]. The ceramic assemblage throughout the stratigraphy in the DA mainly belonged to Phase 8 in the relative chronology of the local tableware, *i.e.* 450/75-550/75 AD while the NEG material was dated 550/75-650 AD in room 5, and 450/75-550/75 AD and 350/75-450/75 AD in room 7. Each of the analysed fragments was determined as belonging to a local/regional Fabric 4, a Late Roman 1, a Late Roman 3, a Late Roman 4 or a Phoenician amphora, based on the macroscopic fabric identification and the particular typology, which clearly differs from other types of vessels in this fabric, such as cooking wares and jugs.

As amphorae are known to have contained wine, oil or fish products, this set of ceramics were chosen because we wanted to validate our newly developd method for polyphenol analysis.

Table 4.1: Summary of analysed amphorae from Sagalassos with part, fabric, room, layer and age.

Sample number	Part	Fabric	Room	Layer	Date
SA-2004-NEG-57	handle	fabric 4	7	16	4th-5th century
SA-2004-NEG-74	shoulder	LR 4	7	8	4th-5th century
SA-2004-NEG-66	handle	fabric 4	7	7	4th-5th century
SA-2004-NEG-57	handle	fabric 4	7	16	4th-5th century
SA-2004-NEG-57	shoulder	fabric 4	7	16	4th-5th century
SA-2004-NEG-46	shoulder	Phoenician	3	5	5th-6th century
SA-2004-NEG-46	shoulder	Phoenician	3	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21	shoulder	LR 1	25	5	5th-6th century
SA-2004-DA1-21		LR 1	25	5	5th-6th century
SA-2004-NEG-83	rim	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	rim	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	shoulder	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	shoulder	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	shoulder	unknown	5	6	6th-7th century
SA-2004-NEG-83	shoulder	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	shoulder	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	foot	LR 3	5	6	6th-7th century
SA-2004-NEG-83	handle	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	handle	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	handle	fabric 4	5	6	6th-7th century
SA-2004-NEG-99	base	fabric 4	5	7	6th-7th century
SA-2004-NEG-83	base	unknown	5	6	6th-7th century
SA-2004-NEG-83	handle	unknown	5	6	6th-7th century
SA-2004-NEG-83	handle	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	handle	fabric 4	5	6	6th-7th century
SA-2004-NEG-83	shoulder	fabric 4	5	6	6th-7th century

4.3 Polyphenol analyses

As the Folin Ciocalteu reagent is an oxidant, reducing metal ions such as Fe(II) might give a false positive result (see chapter 2 §2 for experimental setup). Indeed, when plotting the response curve for Fe(II) oxidation by the Folin Ciocalteu reagent, a linear plot is obtained (Figure 4.1). Because ceramic material is known to contain iron oxides, it might release Fe(II) ions during extraction, especially since acetic acid is used in the extraction solvent. Therefore Fe(II) contents must be determined or estimated at all times to exclude false positive interpretations. Because of the limited amount of archaeological sample from Sagalassos, it was not possible to directly assess the exact Fe(II) content by means of titration.

In order to estimate the maximal Fe(II)/total Fe ratio for the Sagalassos amphorae, we subjected ceramic material with a known high Fe(II) content to the same *modus operandi*. The dark gray colored Gallo-Belgic tableware not only is rich in Fe(II); it is also devoid of polyphenols. Therefore, four 2nd century AD sherds of Gallo-Belgic tableware were used for polyphenol analyses. Samples 13 and 14 are ceramics from the Oude Vlissingseweg in Middelburg; sample 35 is a ceramic from Poortvliet on the isle of Tholen, and sample 41 was found in Borssele - Ellewoutsdijk in Zuid-Beveland. All these samples were excavated in the province of Zeeland in the Netherlands. As expected, high absorbance values were obtained, from which Fe(II) concentrations were calculated, using the response curve of Figure 4.1. Comparison of the total iron concentration as measured with Atomic Absorbance Spectroscopy (AAS), with the Fe(II) content determined by the Folin Ciocalteu test, showed that maximally ~10 % of the iron occurs as Fe(II) (Figure 4.2). For all amphorae excavated in Sagalassos, it can be safely assumed that the Fe(II)/total Fe ratio is much lower, as their red color is indicative of a more oxidized state of the Fe in comparison with the iron in the dark gray Gallo-Belgic tableware. In further analytical work, it is therefore safely assumed that of the Fe in the Sagalassos ware, less than 10 % is available in the divalent state.

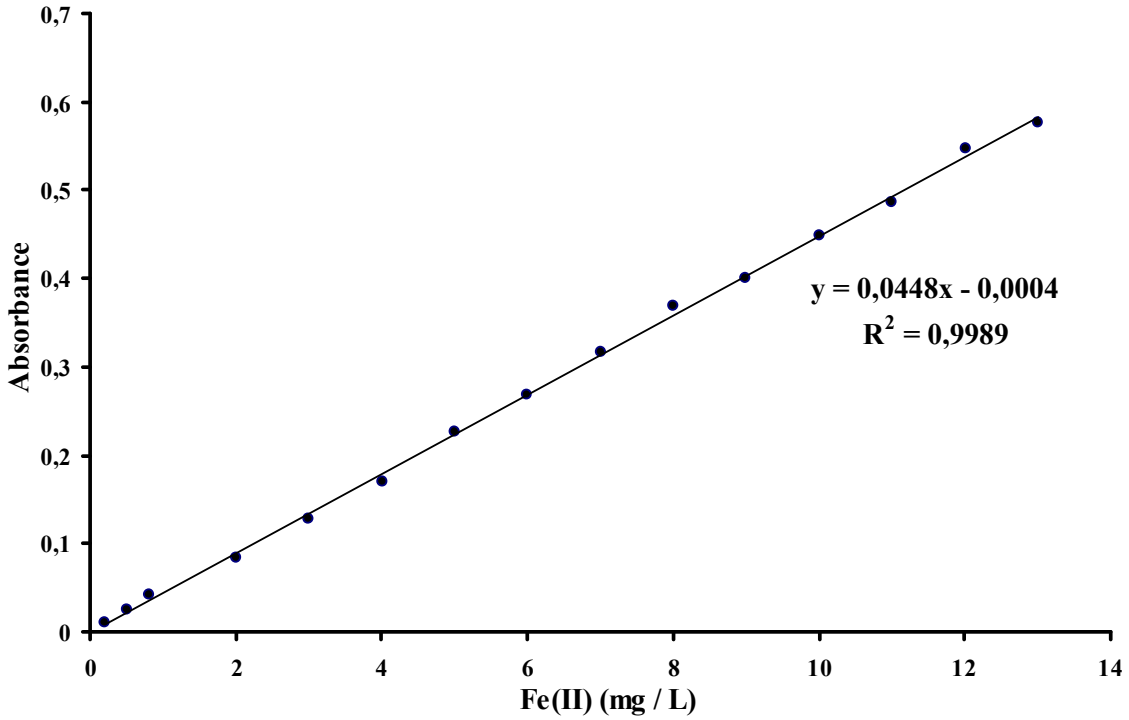


Figure 4.1: Calibration curve of Fe(II) as analyte in the Folin Ciocalteu reaction.

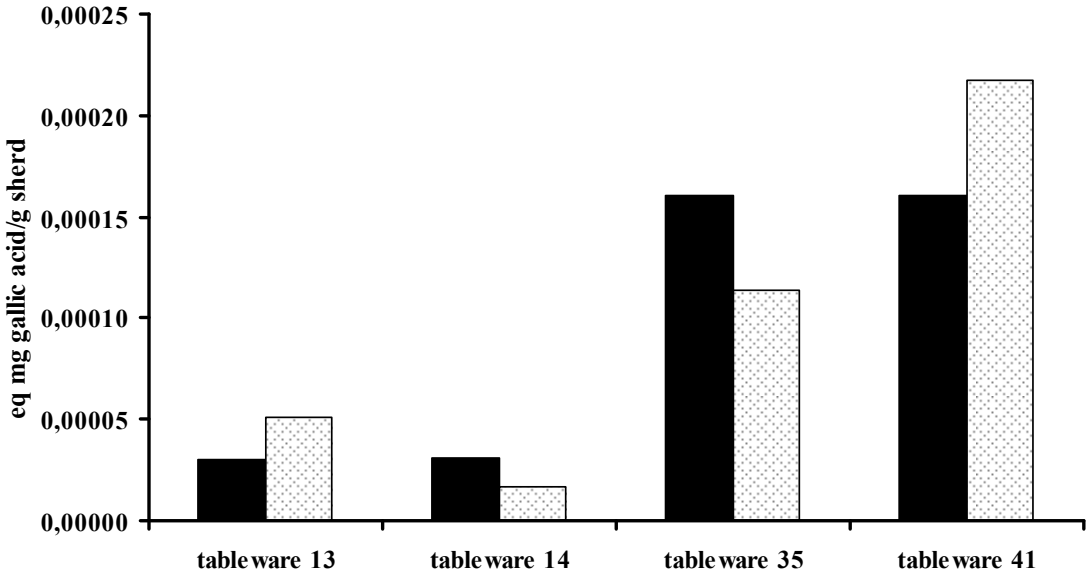


Figure 4.2: The black bars represent the response in the Folin Ciocalteu test, expressed as equivalent mg gallic acid per g Belgian Tableware material. The white dotted bars show the (false positive) response that Fe(II) would induce in the Folin Ciocalteu test if 10 % of the total Fe were present as Fe(II), based on the calibration curve of Figure 4.1.

Taking into account the iron interference in the Folin Ciocalteu reaction, only the rim of one Fabric 4 amphora (SA-2004-NEG-83) and the base of one Late Roman 3 amphora (SA-2004-NEG-83) contained a significant amount of polyphenols (Figure 4.3 and Table 4.2). And so, the latter most probably contained wine. A number of only 2 out of 31 amphorae who showed wine residues was unexpectedly small, as amphorae are believed to have contained wine frequently. A reasonable explanation is that wine, together with its polyphenols, was not able to penetrate through the pitch layer on the inside of the amphorae, and therefore can not be detected in the majority of the 31 Sagalassos amphorae. The occurrence of these pitches will be discussed below. Nevertheless, this newly developed method seems to be able to extract and detect polyphenols in a ceramic matrix.

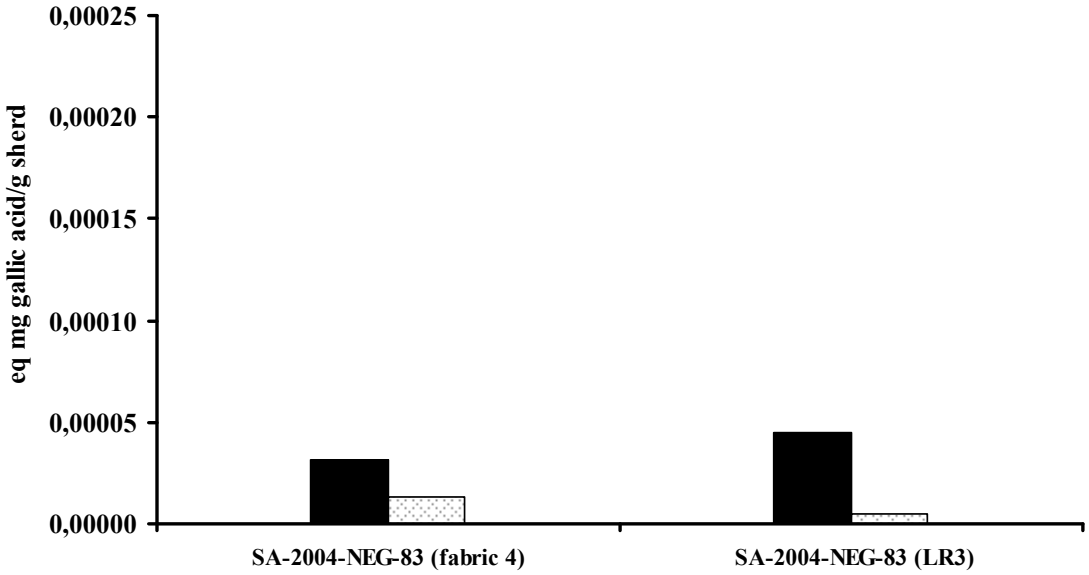


Figure 4.3: Two amphorae contain a significant amount of polyphenols. The black bars represent the extracted amount of polyphenols, expressed in equivalent mg gallic acid per gram sherd, measured with the Folin-Ciocalteu reaction after extraction. The white dotted bars represent the false positive response that would be induced by Fe(II), supposing that 10 % of the total iron, as measured with Atom Absorption Spectroscopy, would be present as Fe(II).

4.4 Lipid analyses

When observing a representative fatty acid profile of a local/regional Fabric 4 amphora (Figure 4.4), the difference with a profile from a cooking pot in the same type of fabric is clear (Figure 4.5) (see chapter 2 §1 for experimental setup). In cooking pots, a larger amount of lipid residues remains preserved while in amphorae no more than small amounts of fatty acids were found (see also previous chapter). Still we managed to extract a significant amount of fatty acid residues from 25 out of 31 amphorae, and from 15 out of the 17 Fabric 4 amphorae (Table 4.2). Another difference with cooking pots is the larger fraction of mono- and polyunsaturated fatty acids which points in the direction of vegetal oils.

In addition to fatty acid profiles, fatty acid ratios were established for each amphora. These ratios are useful to tell the origin of the lipids (see chapter 3). A high stearic acid content is an indication for animal fat, while unsaturated fatty acids are characteristic for vegetal oils [12]. Additionally, earlier degradation experiments proved that palmitic acid can be a degradation product of oleic acid [8]. High values of P/S and low values of S/O are thus indicative for plant oils. There is chemical evidence to say that oil was possibly preserved in 14 out of the 31 amphorae: the characteristics of vegetal oil were clearly found in 8 of the 17 Fabric 4, in 3 of the 7 LR1, in 1 of the 2 Phoenician amphorae, in the single LR3 amphora and in one of unknown fabric.

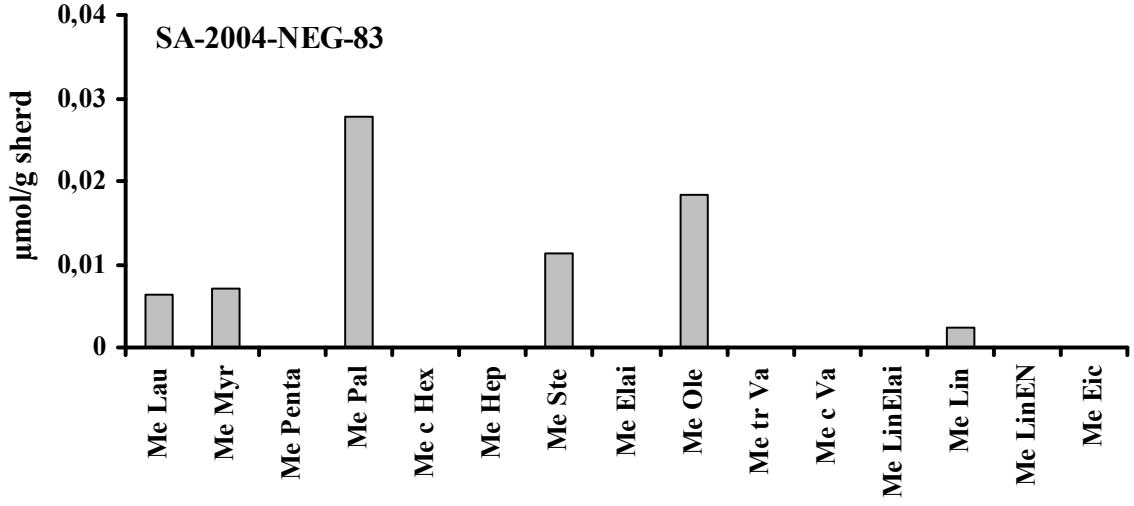


Figure 4.4: Fatty acid profile of amphora SA-2004-NEG-83.

This suggests that amphorae frequently contained oily substances. In 6 amphora samples, no FAME were detected and in 8 samples the fatty acid ratios did not indicate the presence of oils (Table 4.2). The three remaining samples were not assigned because of the absence of stearate.

Analysis of silylated derivates on GC-MS revealed valuable information about the presence of sterols and pitch compounds in the sampled amphorae (Figure 4.6 and Table 4.2). Pitches and tars were manufactured by heating natural resins to obtain denser and stickier materials; they were applied to make porous ceramics tight for storage and transport of liquids like wine or fish sauce. More viscous commodities, for example olive oil, were never stored in sealed vessels according to classical authors like Cato and Columella [11]. The heating treatment of the resin has a great influence on its chemical composition. Terpenoid compounds undergo aromatization, demethylation and decarboxylation reactions, with the formation of new compounds of lower molecular mass. Characteristic pitch compounds, such as oxidized and aromatized abietic acid and pimaric acid are gathered in Figure 4.8 and Table 4.3. Retene and other dehydrogenated tricyclic hydrocarbons are biomarkers for pitch. When pitch is prepared by heating pine resin in the presence of wood, methyldehydroabietic acid (Figure 4.7) is formed as well [4]. Both biomarkers were found in 23 of the 31 amphorae from Sagalassos, indicating that the pitch was always prepared by heating *Pinaceae* resin in the presence of wood (Table 4.2). Pitch was found in 12 out of the 17 Fabric 4, in 5 out of the 7 LR1, in the two Phoenician amphorae, in the single LR3 amphora and in the 3 amphorae with unknown fabric.

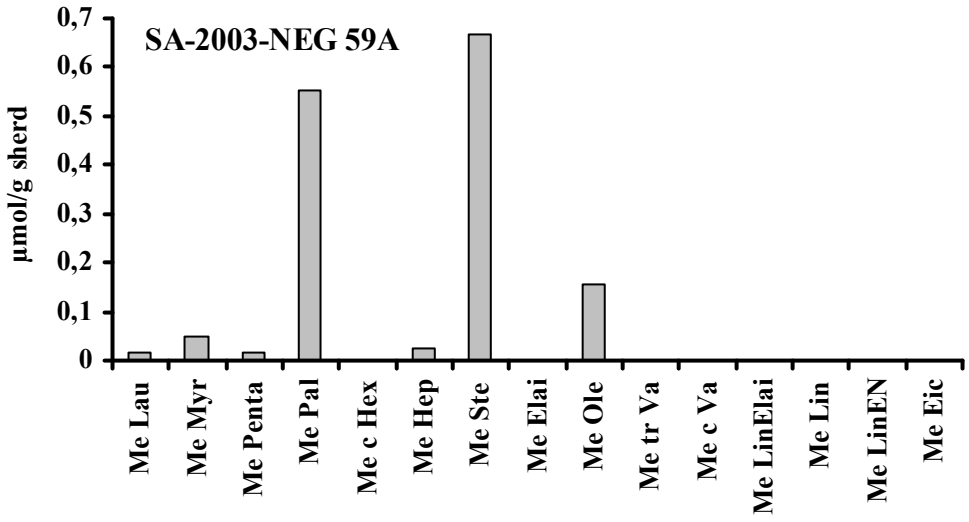


Figure 4.5: Fatty acid profile of cooking pot SA-2003-NEG 59A.

Table 4.2: Summary of the results for the 31 Sagalassos amphorae where LR stands for Late Roman.

	Fabric	Wine	FAME	Oil	Pitch
SA-2004-DA1-21	LR 1		no		yes
SA-2004-DA1-21	LR 1		no		yes
SA-2004-DA1-21	LR 1		yes	no	yes
SA-2004-DA1-21	LR 1		yes	yes	no
SA-2004-DA1-21	LR 1		yes	yes	yes
SA-2004-DA1-21	LR 1		yes	yes	yes
SA-2004-DA1-21	LR 1		yes	no	/
SA-2004-NEG-83	Fabric 4		yes	no	yes
SA-2004-NEG-83	Fabric 4	yes	no		no
SA-2004-NEG-83	Fabric 4		yes	no	no
SA-2004-NEG-83	Fabric 4		no		yes
SA-2004-NEG-83	unknown		no		yes
SA-2004-NEG-83	Fabric 4		yes	yes	yes
SA-2004-NEG-83	Fabric 4		yes	/	yes
SA-2004-NEG-83	LR 3	yes	yes	yes	yes
SA-2004-NEG-83	Fabric 4		yes	yes	yes
SA-2004-NEG-83	Fabric 4		yes	no	yes
SA-2004-NEG-83	Fabric 4		yes	no	yes
SA-2004-NEG-99	Fabric 4		yes	yes	yes
SA-2004-NEG-83	unknown		yes	yes	yes
SA-2004-NEG-83	unknown		yes	/	yes
SA-2004-NEG-46	Phoenician		yes	/	yes
SA-2004-NEG-46	Phoenician		yes	yes	yes
SA-2004-NEG-57	Fabric 4		yes	yes	no
SA-2004-NEG-57	LR 4		no		no
SA-2004-NEG-66	Fabric 4		yes	yes	yes
SA-2004-NEG-57	Fabric 4		yes	yes	yes
SA-2004-NEG-57	Fabric 4		yes	yes	yes
SA-2004-NEG-83	Fabric 4		yes	yes	no
SA-2004-NEG-83	Fabric 4		yes	no	no
SA-2004-NEG-83	Fabric 4		yes	no	yes

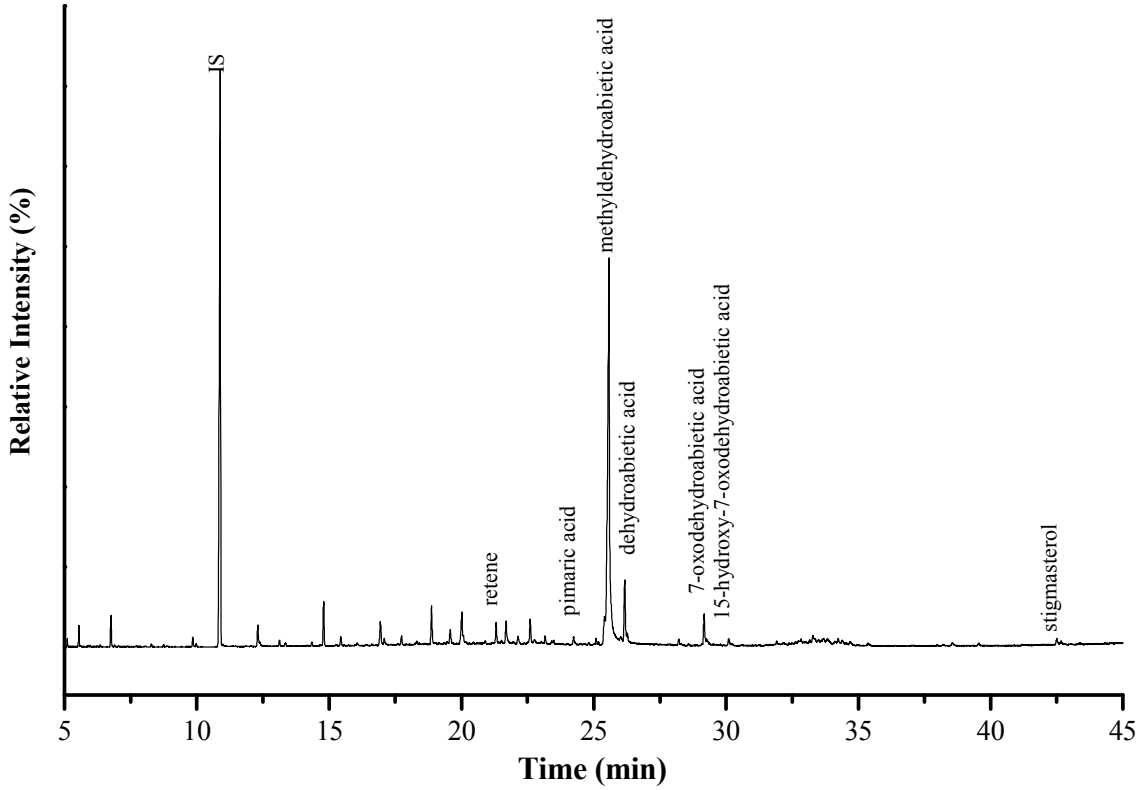


Figure 4.6: GC-MS chromatogram of LR1 amphora SA-2004-DA1-21.

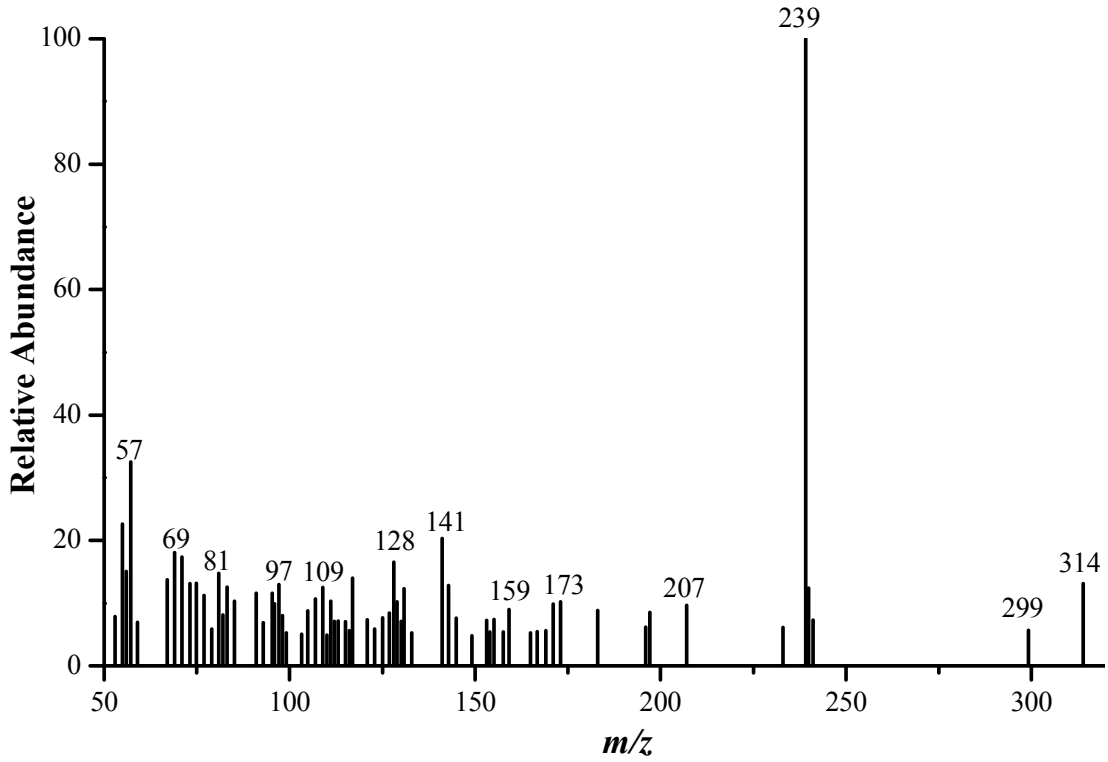


Figure 4.7: Mass spectrum, acquired by GC-MS, of methyldehydroabietic acid.

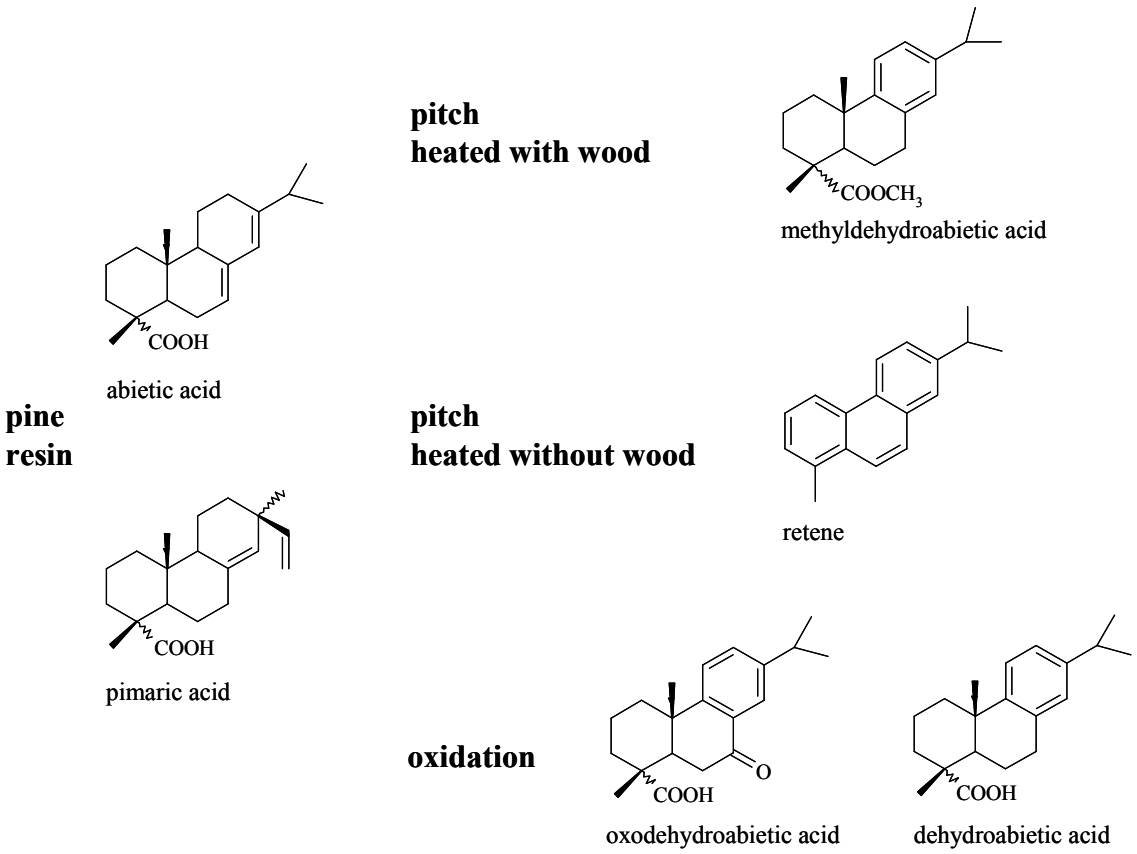
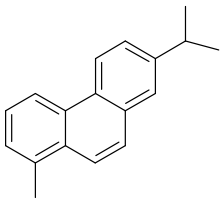
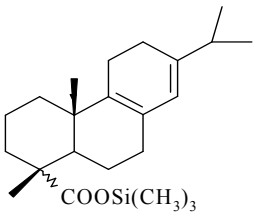
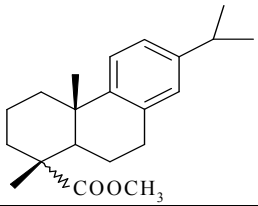
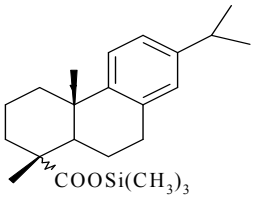
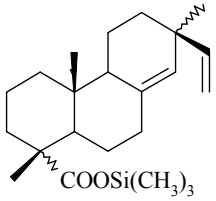
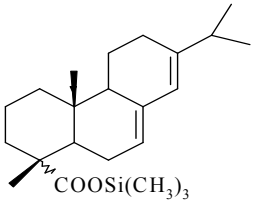
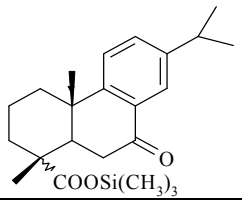
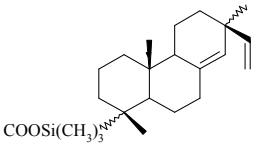


Figure 4.8: Resin and pitch compounds

Based on the occurrence of specific triacylglycerols (see chapter 3), a classification of the potsherds was attempted. Of the 31 amphorae analysed, only 2, both LR1, exhibited a detectable amount of triglycerides. This observation confirms the FAME analysis in which low amounts of fatty acids, originating from TAG, were found. Figure 4.9 shows the TAG profile from amphora SA-2004-DA1-21, from which large amounts of linoleic acid residues were recovered. Care must be taken when using such unsaturated TAG as markers because they are readily oxidized and therefore less likely to survive burial (see also previous chapter). Nevertheless considerable amounts of these molecules were recovered from the samples analysed. Based on the abundant presence of linoleic acid, the assumption that walnut oil had been stored in this vessel was done. This seems reasonable because modern pollen studies revealed that *Juglans regia* was cultivated in Sagalassos at that time [19]. The second amphora in which TAG were detected showed a similar pattern.

Table 4.3: Summary of resin biomarkers [16,4]. TMS stands for trimethylsilylated.

Product	Origin	Product	Origin
Retene 	***Pine pitch produced in the absence of wood***	Palustric acid TMS 	***Abietane compound from pine resin***
Methyldehydroabietic acid 	***Pine pitch produced in the presence of wood***	Dehydroabietic acid TMS 	***Can occur naturally and can be an oxidation product of abietane compounds from pine resin***
Pimaric acid TMS 	***Pimarane compound from pine resin***	Abietic acid TMS 	***Abietane compound from pine resin***
Oxodehydroabietic acid TMS 	***Oxidation product of abietane compounds from pine resin***	Isopimaric acid TMS 	***Pimarane compound from pine resin***

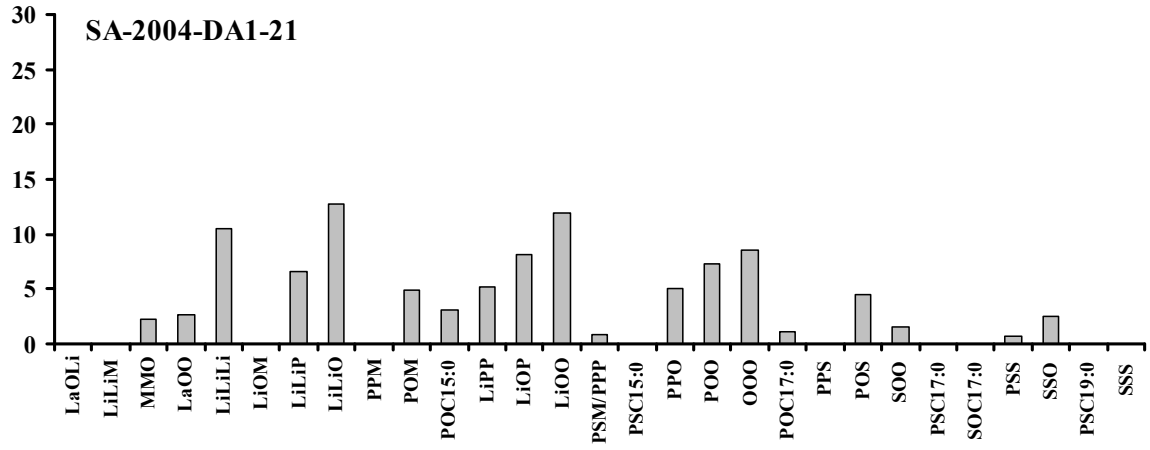


Figure 4.9: Triglyceride profile of LR1 amphora SA-2004-DA1-21, in which a walnut oil profile can be recognized.

4.5 Comparison of the results

Conflicting with our expectations, residue analysis of 31 Sagalassos amphorae (Table 4.2) revealed only in two cases the presence of polyphenols as biomarkers of wine. This observation might be ascribed to the application of pitches in wine amphorae inhibiting wine, and consequently polyphenols, to penetrate into the ceramic matrix. Nevertheless, in these two cases the presence of polyphenols in the sherd may be caused by an imperfect pitch layer, or by the fact that the top part of the vessels was not pitched. This possibility was confirmed by the chemical absence of pitch at the rim of the local/regional Fabric 4 amphora (SA-2004-NEG-83). The reason why the Late Roman 3 amphora (SA-2004-NEG-83) contained detectable polyphenols is probably that the base part of an amphora is in continuous contact with its liquid content. This higher contact time may have increased the opportunity for wine components to diffuse through the ceramic fabric, even if pitch was applied. Pitch was found in 23 of the 31 amphorae. Additionally, lipids originating from vegetal oils were found in vessels in which also pitch compounds were detected. This is in contradiction with the traditional archaeological approach of amphora content, which is based on ancient authors. In this vision, a close link is advocated between pitching vessels and transporting wine in them [11]. Our results therefore shed a new light on the chemical evidence for the use of amphorae. It might be possible that in the 4th century AD, some landholders in the area of Sagalassos wanted to specialize and intensify part of their agricultural production. Unfortunately, their efforts have only come to us in particular amounts of very broken amphorae. Indeed, not only do we still lack strong proof for wine as intended content, but, more importantly, we have next to no indication of the distribution pattern of the Fabric 4 amphorae beyond the immediate study region. In this way, we are not in the position to measure the success of the Sagalassos landholders [18].

4.6 Conclusion

Amphorae are known to have contained mainly wine, vegetal oils or fish products. By means of residue analysis, we have tried to identify the vessel content for a set of amphorae originating from Sagalassos.

At first, a new analytical procedure was optimized to detect polyphenols as a biomarker for wine. Therefore, polyphenols were quantified by means of the Folin-Ciocalteu colorimetric reaction. Although an interference with reduced iron could be observed, a significant amount of polyphenols was found in two amphorae; demonstrating the presence of wine.

Also lipid traces from food products, like vegetal oil and fish products, may be preserved in the ceramic matrix. Therefore lipid residues were examined by a combination of three analytical techniques. Triacylglycerols were detected by means of a liquid chromatograph with a mass spectrometer. Although the very low concentrations of triglycerides in the majority of amphorae, it was possible to recognize the triglyceride profile of walnut oil in two cases. For all amphorae, methyl esters of the triglycerides were analysed and quantified on a polar phase gas chromatograph column. High fatty acid ratios for palmitate to stearate and for oleate to stearate were found, which point in the direction of vegetal oils. A third technique, in which silyl derivates were injected in a gas chromatograph coupled to a mass spectrometer, supplied information about fatty acids, alcohols, sterols and resins present. The latter are found as by-products from abietic acid, originating from the inner pitch coatings of the amphorae. The chemical composition of this pitch showed that it had been prepared by heating tar in the presence of wood.

At first we assumed that we would be able to find pitch together with polyphenols in amphorae, used for wine storage. Nevertheless, this was not always the case. Maybe because of the coating, it was impossible for wine to migrate into the ceramic matrix. On the other hand, it is reasonable that on less accessible places no pitch adhered and that precisely on these places polyphenols could intrude into the ceramic fabric. Additionally, lipids were found together with resins. This might point in the direction of a double use of amphorae while the latter is not in agreement with archaeological literature. Therefore, we felt the need to initiate a laboratory experiment to evaluate the behavior of wine and oil in pitched ceramics.

4.7 References

1. **Badler, V.R., McGovern, P.E., and Michel, R.H., 1990**, Drink and be Merry! Infrared Spectroscopy and Ancient Near Eastern Wine in *Museum Applied Science Center for Archaeology* 7, 25-36.

2. **Belitz, H.D., and Grosch, W., 1999**, Food chemistry (second edition), Berlin.

3. **Brown, L.D., and Heron, C., 2004**, Exploring links: preliminary investigations into marine resources and ceramics from Old Scatness, Shetland in *Atlantic Connections and Adaptations, Symposia of the Association for Environmental Archaeology 21*.

4. **Colombini, M.P., Modugno, F., and Ribechini, E., 2005**, Direct exposure electron ionisation mass spectrometry and gas chromatography/mass spectrometry techniques to study organic coatings on archaeological amphorae in *Journal of Mass Spectrometry* 40, 675-687.

5. **Degeest, R., 2000**, The common wares of Sagalassos (Studies in Eastern Mediterranean Archaeology 3), Turnhout.

6. **Eerkens, J.W., 2002**, The preservation and identification of piñon resins by GC-MS in pottery from the Western Great Basin in *Archaeometry* 44, 1: 95-105.

7. **Eerkens, J.W., 2005**, GC-MS analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America in *Archaeometry* 47, 1: 83-102.

8. **Evershed, R.P., Heron, C., Charters, S., and Goad, L.J., 1992**, The survival of food residues: new methods of analysis, interpretation and application in *Proceedings of the British Academy* 77, 187-208.

9. **Hamm, S., Bleton, J., and Tchapla, A., 2004**, Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples in *Journal of Separation Science*, 27, 235-243.

10. **Hansel, F.A., Copley, M.S., Madureira, L.A., and Evershed, R.P., 2004**, Thermally produced ω-(o-alkylphenyl)alkanoic acids provide evidence for the processing of marine products in archaeological pottery vessels in *Tetrahedron Letters*, 45, 2999-3002.

11. **Heron, C., and Pollard, A.M., 1988**, The analysis of natural resinous materials from Roman Amphoras in *Science and archaeology, Proceedings of a conference on the application of scientific techniques to archaeology, Oxford.*

12. **Kimpe, K., 2003**, Chemical analysis of the lipid fraction from ancient ceramics of Sagalassos, PhD Thesis, Leuven.

13. **Garnier, N., Richardin, P., Cheynier, V., and Regert, M., 2003**, Characterization of Thermally Assisted Hydrolysis and Methylation Products of Polyphenols from Modern and Archaeological Vine Derivatives Using Gas Chromatography – Mass Spectrometry in *Analytica Chimica Acta*, 493, 137-157.

14. **Guasch-Jané, M.R., Ibern-Gómez, M., Andrés-Lacueva, C., Jáuregui, O., and Lamuela-Raventós, R.M., 2004**, Liquid chromatography with mass spectrometry in tandem mode applied for the identification of wine markers in residues from ancient Egyptian vessels in *Analytical Chemistry*, 76, 1672-1677.

15. **Michel, R. H., McGovern, P.E., and Badler, V.R., 1993**, The First Wine and Beer; Chemical Detection of Ancient Fermented Beverages in *Analytical Chemistry*, 65(8), 408-413.

16. **Mills, J., and White, R., 1977**, Natural resins of art and archaeology. Their sources, chemistry, and identification in *Studies in conservation*, 22, 12-31.

17. **Petit-Domínguez, M.D., García-Giménez, R., and Rucandio, M.I., 2002**, Chemical Characterization of Iberian Amphorae and Tannin Determination as Indicative of Amphora Contents in *Microchimica Acta*, 141, 63-68.

18. **Poblome, J., Corremans, M., Bes, P., Romanus, K., and Degryse, P., (*in press*)**, It is never too late… The late Roman initiation of amphora production in the territory of Sagalassos, in *I. Delemen, S. Cokay-Kepçe and A. Özdibay (eds) Prof Dr Haluk Abbasoglu'na 65. Yas Armagan. Studies presented to Prof Dr Haluk Abbasoglu, Istanbul*.

19. **Rothschild-Boros, M., 1981**, The determination of amphora contents, Archaeology and Italian Society, Prehistoric and Medieval Studies in Papers in *Italian Archaeology II, BAR International Series 102*.

20. **Vermoere, M., Vanhecke, L., Waelkens, M., and Smets, E., 2001**, Modern pollen studies in the territory of Sagalassos (Southwest Turkey) and their use in the interpretation of a Late Holocene pollen diagram in *Review of Palaeobotany and Palynology*, 114, 29-56.

21. **Waelkens, M., 2006**, Sagalassos, in W. Radt (ed.) Stadtgrabungen und Stadtforschung in Westlichen Kleinasien (Byzas 3) Istanbul.

22. **Waterman, P.G., and Mole, S., 1994**, Analysis of phenolic plant metabolites, Oxford.

5 Wine and olive oil permeability in pitched and non-pitched ceramics correlated with results from Sagalassos amphorae

5.1 Introduction

In the previous chapter, we applied residue analysis to study the content of a set of late Roman amphorae originating from Sagalassos. As amphorae are known to be used for mainly vegetal oil, fish products or wine, not only standard lipid analyses but also polyphenol extractions were performed. The majority of the amphorae showed biomarkers of resins, cited by classical authors to seal wine amphorae [4]. Nevertheless half of the sherds showed a lipid profile of vegetal oil whereas two amphorae had chemical evidence for storage of wine. These data seem to contradict the generally accepted observation that pitch was mostly, if not exclusively used for wine amphorae. In that perspective we wanted to assess a long term experiment to investigate the permeability of wine and oil in ceramic material whether or not with a pitch layer. We also wanted to investigate the necessity to seal an oil amphora.

5.2 Samples

Ophiolite clay ceramic bowls were manufactured for this experiment. This type of clay was chosen because the local/regional Sagalassos amphorae, the main body of archaeological samples in the previous chapter, were produced with ophiolitic clays and to imitate the original chemistry and more importantly porosity of the ancient storage wares (smectite-illite, tempered with quartz and crushed ceramics) [2]. The interior bottom surface (± 28 cm²) of twelve bowls was covered with a known amount (± 1 g) of commercial pine pitch (Auson, Sweden) and stayed for drying during

45 days (Figure 5.1). This particular pitch has the same chemical composition as the antique material; confirmed by GC-MS analysis. During the first day after application, the bowls were tilted at an angle of ~5° and slowly rotated, in order to realize a homogeneous pitch cover layer. Three empty bowls and three pitch bowls were filled, each with 1 ml wine, ± 1 g olive oil or both. Because of the lower density of oil, wine will form a lower layer in any case; hence, in those cases where both wine and oil were applied, the wine was added first. Three bowls, with and without pitch, were kept for blank analyses. The bowls were placed on thick filter paper, and stored in a well-vented and clean environment. After a period of 45 days, the bowls were visually dry. Next, consecutive cylindrical segments of 1 mm high and ± 4 cm diameter were scraped from the bottom of the bowls using a chisel mounted on a lathe. In the following text, bowls covered with pitch or without pitch will be denoted as P or N respectively.

5.3 Results

5.3.1 Pitch permeation

To evaluate quantitatively the extent to which pitch infiltrated in the bowltery, silylated samples were analysed on a high temperature gas chromatograph (HT-GC) with FID detector (see chapter 2 §1 for experimental setup). As retene is one of the major components of the pitch, it was used as a marker for pitch permeation. The

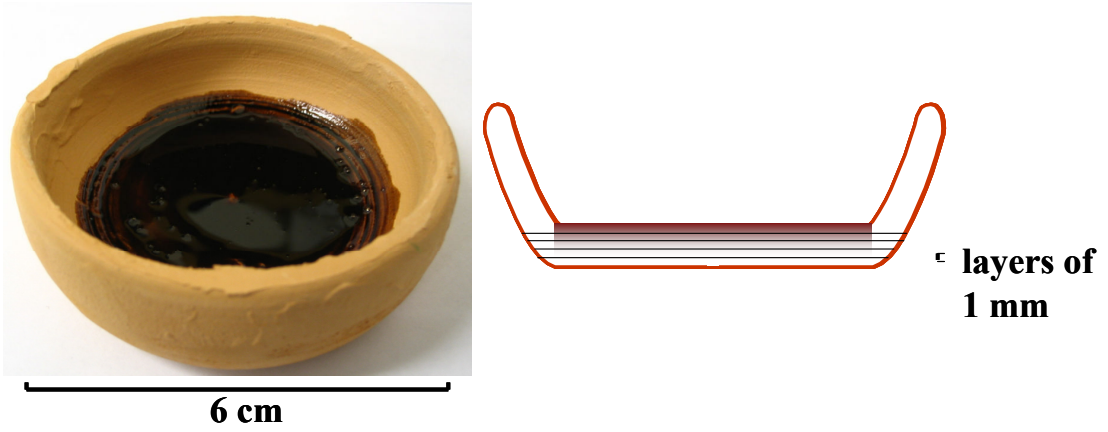


Figure 5.1: Picture of a ceramic bowl filled with pine pitch together with scheme of the scraping of the layers being 1 mm high and having 4 cm of diameter

amount of retene was determined by using the appropriate sensitivity factor. Figure 5.2 shows the amount of retene in each layer of a ceramic bowl with a pitch layer (P) expressed in mg retene per gram sherd. As expected, a significant amount of the pitch infiltrated in the ceramic. Retene was approximately absent in the base layers.

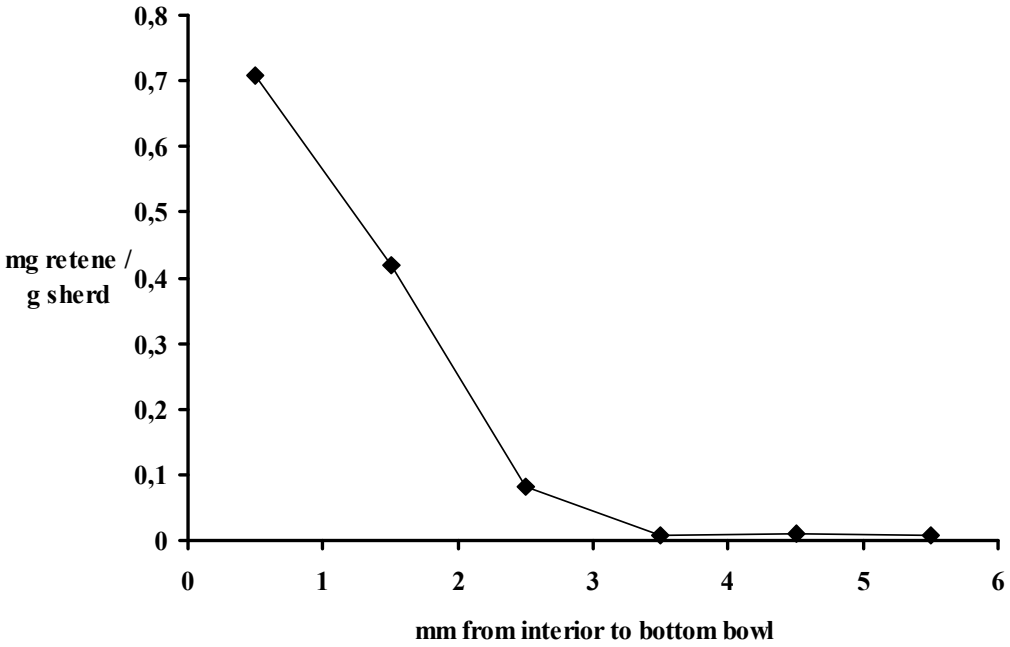


Figure 5.2: Pitch permeability profile for P (♦). The curve is based on the amount of retene in each layer analysed by high temperature gas chromatography and is expressed in mg retene per gram sherd. The X-axis is indicative for the ceramic layers, each being 1 mm thick, and beginning from the interior (0 mm) towards the bottom (6 mm) of the bowl.

a b

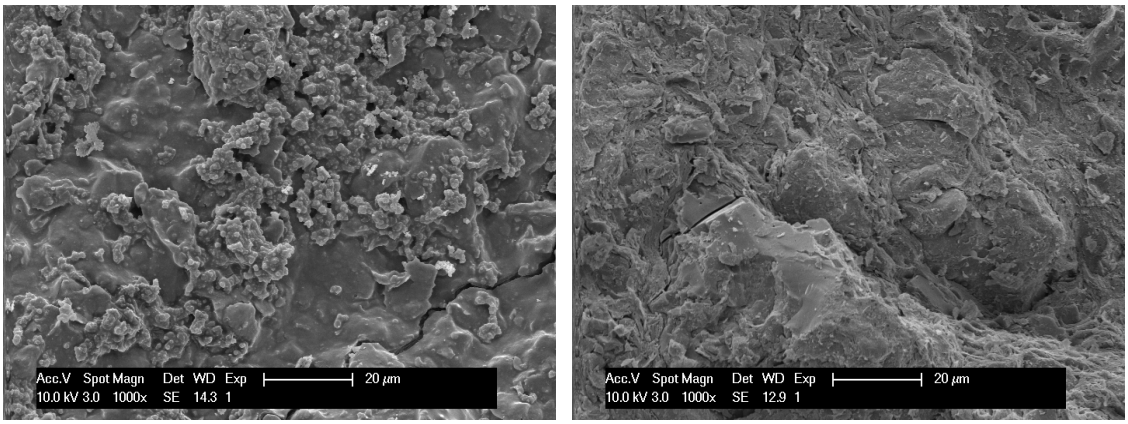


Figure 5.3: SEM pictures of a bowl with pitch layer (a) and a bowl without pitch application (b).

In order to check the microscopic appearance of the pitch layer, scanning electron microscopy (SEM) was performed on a ceramic with and without pitch (Figure 5.3). The scanning electron micrographs were taken on a XL30 SEM-FEG electron microscope from FEI-Philips. Before analysis, samples were coated with gold. The SEM micrograph of the bowl with pitch (Figure 5.3a) shows a layer of molecules covering the underlying pores which are visible on the SEM picture of the blank ceramic material (Figure 5.3b).

Thin sections of 30 µm thick were made of the fresh ceramic containers and of a cross section of the bottom part of a ceramic container treated with pitch. These samples were examined under the polarising microscope. Thin section analysis showed that the small ceramic bowls are made up of a fine clay, light brown under parallel polaroids, with evenly distributed angular to sub-angular quartz grains of maximum 100 µm in diameter as mineral aggregates, next to crushed ceramics (grog), unevenly distributed throughout the fabric of the ceramics, rounded and up to maximum 500 µm in diameter. This fabric is very similar to the original ancient wares found at Sagalassos [2]. The porosity of the fresh ceramics shows two populations. One type of porosity comprises small pores in the fired clay matrix, around 50 µm in diameter, often angular, which do not form an open network in the ceramics.

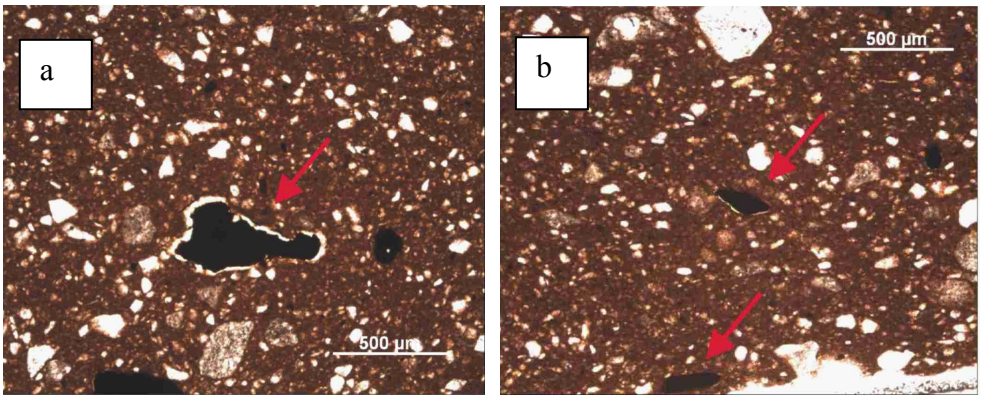


Figure 5.4: a) Thin section of the fabric of the experimental ceramics, showing the light brown fired clay matrix, angular to sub-angular quartz grains (white) and grog (black, opaque). Around the larger piece of grog, indicated with the red arrow, the larger pore network in the ceramic can be identified. b) Thin section of the same fabric, made from the bottom of a bowl treated with pitch. The red arrows indicate the closed porosity and permeability around the fragments of grog, no longer showing an open network.

Another type of porosity is the large, often elongated pores around the grog fragments in the fabric of the ceramic, which seem to be interconnected and in contact with the outer surface of the ceramics, forming pathways of permeability in the fabric. It is clear from the comparison of the thin section of the untreated and treated ceramics that the latter pores are filled with pitch when the ceramics were treated, blocking the network of pores (Figure 5.4).

5.3.2 Wine infiltration

For each bowl, permeability profiles for wine were established by performing a Folin Ciocalteu colorimetric reaction to assess the polyphenol concentration in each layer (Figure 5.5) (see chapter 2 §2 for experimental setup). When the ceramic matrix has been in contact neither with wine nor with pitch, the polyphenol readings are essentially zero (N in Figure 5.5). In the containers without pitch (N_{wine} and $N_{oil+wine}$), an increased polyphenol concentration was seen towards the bottom. Without the hindrance of a pitch layer, it seems that the wine completely infiltrates and percolates through the ceramic matrix. Consequently, the highest polyphenol concentration is measured in the bottom layer (N_{wine}). The higher concentration of polyphenols in $N_{oil+wine}$ can be explained by a retarding factor of the hydrophobic oil behaving like a stopper.

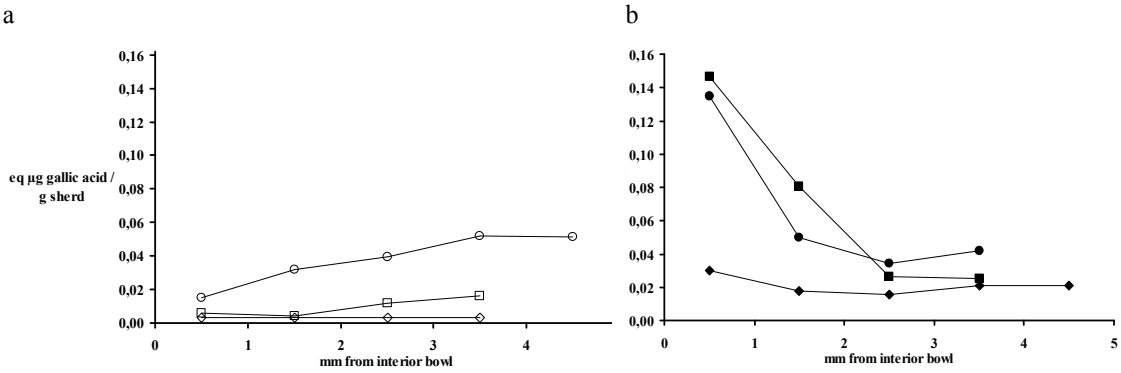


Figure 5.5: Permeability profiles of wine for a) bowls without pitch: N_{wine} (□); $N_{oil+wine}$ (○); N (◊); and b) bowls with pitch: P_{wine} (■); $P_{oil+wine}$ (●) and P (♦). The curves are based on polyphenol concentration in each layer assessed by the Folin Ciocalteu reaction and are expressed as eq µg gallic acid per g sherd. The X-axis is indicative for the ceramic layers, each being 1 mm thick, and taken from the interior (0 mm) towards the bottom (6 mm) of the bowl.

In the vessels with a pitch layer (P_{wine} and $P_{oil+wine}$), a dissimilar pattern is observed. The pitch layer clearly hampered the infiltration, causing an accumulation of the polyphenols in the upper layer. However in this case, the polyphenol penetration rate is not affected by the addition of oil. When only pitch is applied (P) also a significant amount of polyphenols is measured by the Folin Ciocalteu reaction. This can be rationalized by the occurrence of phenolic structures in the pine pitch as confirmed with GC-MS.

5.3.3 Oil permeation

A quantification of the infiltrated olive oil was made, based on the assessment of fatty acid methyl esters (FAME) (Figure 5.6) (see chapter 2 §1 for experimental setup). The latter are transmethylation products of triglycerides. The highest concentration of methyl oleate was observed in the vessel without pitch (N_{oil}). In that case, the pores of the ceramic material were completely open and the oil had the possibility to permeate almost without hindrance. When adding wine, the latter filled the pores first and less oil was able to penetrate ($N_{oil+wine}$). Figure 5.7a confirms this assumption by comparing the oil and wine penetration profiles in sample $N_{oil+wine}$.

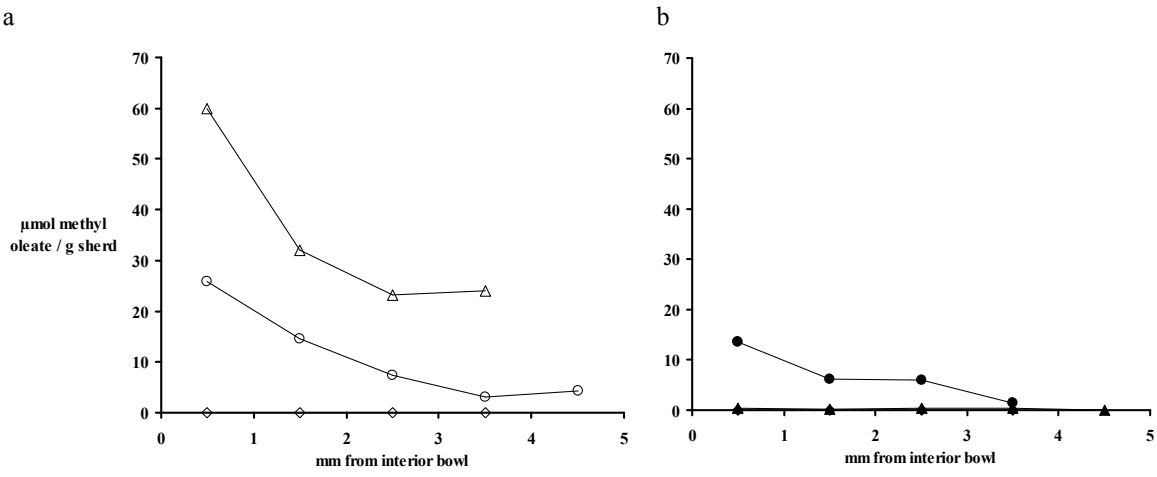


Figure 5.6: Permeability profiles for olive oil for N_{oil} (△); $N_{oil+wine}$ (○); N (◊); P_{oil} (▲); $P_{oil+wine}$ (●) and P (♦). The curves are based on data of fatty acid methyl ester analysis and are expressed as µmol methyl oleate per g sherd. The X-axis is indicative for the ceramic layers, each being 1 mm thick, and taken from the interior towards the bottom of the bowl.

While the polyphenols are concentrated towards the bottom of the ceramic layer, methyl oleate is enriched in the top layer, proving that oil infiltrates much more slowly than wine. Furthermore, the wine was unable to evaporate or percolate due to the oil film above. The pitch layer completely hampered the permeation of olive oil since nearly no FAME were detected in vessel P_{oil}. Not only the number of pores decreased but also the pore diameter diminished, resulting in a more difficult pathway for the viscous oil. This result also suggests that the oil is not capable to dissolve the pitch. A remarkable feature is the high concentration of methyl oleate when wine was added ($P_{oil+wine}$). There could be a few explanations for this behavior. Pitch might be partially dissolved by the alcohol-containing wine resulting in more and wider pores. This hypothesis can be confirmed by the polyphenol profile of P_{wine} and $P_{oil+wine}$ in which some penetration of wine is observed despite the application of a pitch layer. In an alternative explanation, oil droplets trapped in the aqueous layer might be entrained by the flowing wine, and thus follow a trajectory similar to that of the wine. The latter hypothesis is supported by the similar relative permeation profiles of oil and wine in $P_{oil+wine}$ (Figure 5.7b).

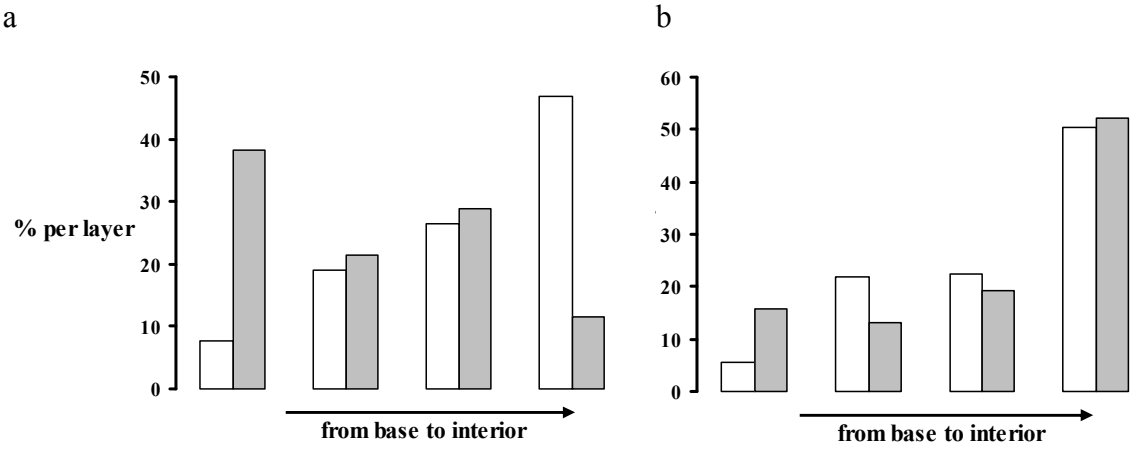


Figure 5.7: Evaluating the similarity in infiltration speed for wine and olive oil in a) $N_{oil+wine}$ and b) $P_{oil+wine}$; white bars represent the relative percentage of methyloleate, while the grey bars represent the relative percentage of polyphenols infiltrated per layer

5.3.4 Search for new biomarkers

Analyses of silyl derivatives by GC-MS were performed in search for biomarkers to prove the presence of wine together with resins (see chapter 2 §1 for experimental setup). Careful investigation of the obtained chromatograms did not reveal new products which might be useful as biomarker.

5.4 Discussion

In the previous chapter, we studied the functionality of a series of late Roman amphorae excavated in Sagalassos. Retene and methyldehydroabietic acid were both found in twenty-three out of thirty-one analysed amphorae. These molecules are biomarkers for pitch and their coexistence designated that this substance was manufactured by heating tar together with wood [1,3]. According to ancient authors, pitch was preferentially used in vessels for the transport of wine [4]. This observation is inconsistent with our findings as only in two amphorae a significant amount of polyphenols was detected while in twenty-five sherds traces of vegetal oil were observed. Because we expected to find pitch together with wine, instead of oil, evaluating the permeability of pitched or non-pitched bowltery for wine and oil was considered required to ratify our previous results and measure their impact in the amphora debate.

The first point of attention was to investigate the need to seal an amphora intended to use for vegetal oil storage. When the oil is too viscous to infiltrate in the ceramic, there would not be the necessity to seal the ceramic. Evaluating the results for N_{oil}, it is clear a significant amount of oil is present in the base layer (Figure 5.6). This indicated that a pitch layer was required in order to avoid too much oil waste. When applying pitch, no infiltration of oil was observed (P_{oil}). This makes sense when comparing with the results from the late Roman local/regional Fabric 4 amphorae where markers for pitch were found together with vegetal oil. For example for a LR1 amphora excavated in room 25 of the palatial mansion and dated to the 5^{th}-6^{th} century (SA-2004-DA1-21), a chromatogram is shown where retene and

methyldehydroabietic acid are present in high concentration. In the same amphora, triglyceride analysis with liquid chromatography coupled to mass spectrometry (LC-MS), demonstrated a characteristic profile of walnut oil (see previous chapter). Indeed, *Juglans regia* has been reported to be widely cultivated at the time in Sagalassos [6]. It is very well possible that such is the result of the specific ophiolitic fabric constitution of the local/regional Fabric 4 amphorae, but surely the wider occurrence of this type of clay and its use in the ceramic production of the Roman East, does not necessarily make this fabric exceptional. Further research is required to compare such physical aspects of these and other fairly open fabric constitutions, typically selected for amphorae.

Another important question was why the archaeological material showed only in two cases a significant amount of polyphenols. There could be a couple of reasons for this observation. First, wine was not able to pass the pitch barrier. Figure 5.5 shows this is not the case as there is a significant infiltration of polyphenols through the pitch layer (P_{wine}). Of course, the permeation speed decreased considerably in contrast to N_{wine} where a complete drain of polyphenols is observed. A second reason for the lack of polyphenols might be their inability to resist during burial. Nevertheless, their detection in a few archaeological samples makes us believe their conservation is to be expected in some cases. A long term degradation experiment to evaluate this hybowlhesis could be useful. The fact that wine, and thus polyphenols, flowed through the pitch layer and that preservation is likely, are important considerations for data interpretation in archaeological residue analysis. Both imply that when polyphenols are absent, we can presume that that particular vessel probably had not been intended for wine storage.

The aspect of re-use of amphorae is also a fundamental archaeological issue. That is why we investigated the influence of mixing oil with wine. As shown in Figure 5.7b, there is a simultaneous infiltration of wine and oil when pitch is applied. In Figure 5.7a, a fast drain of wine is noticeable because the hindering factor of the pitch is lacking. These graphs confirm the earlier discussion that the pores are completely filled with wine and thus inhibiting the oil to infiltrate. Because oil does not dissolve pitch (P_{oil} is plane), it might have been possible to use an oil amphora for wine storage afterwards.

5.5 Conclusion

To our awareness, a tentative/experimental setup to evaluate the permeability of wine and oil in ceramics with pitch application has never been done before. Results from this long term experiment shed new light on data interpretation of archaeological residue analysis on amphorae. As lipid permeability profiles showed an easy oil infiltration in ceramics without pitch application, a pitch barrier seems advisable for oil storage to avoid too much waste. Although such could be specifically linked to the specific fabric characteristics, still, this observation is incongruent with ancient sources indicating that pitched amphorae were preferentially used for the transport of wine. Nevertheless, earlier research on late Roman amphorae from Sagalassos showed the coexistence of vegetal oil traces and pitch biomarkers.

Another incongruity is the infiltration of wine through the pitch layer as observed in the polyphenol measurements. When analysing thirty-one amphorae, only in two a significant amount of wine residues were detected. At first, we assumed wine had no ability to pass the pitch barrier and thus never reached the ceramic matrix. The present results showed this not to be the case. The most plausible explanation is that mainly the local/regional Fabric 4 amphorae, presumably produced in different pockets on the territory of Sagalassos, were used for another purpose than wine [5].

5.6 References

1. **Colombini, M.P., Modugno, F., and Ribechini, E., 2005**, Direct exposure electron ionisation mass spectrometry and gas chromatography/mass spectrometry techniques to study organic coatings on archaeological amphorae in *Journal of Mass Spectrometry* 40, 675-687.

2. **Degryse, P., Poblome, J., Donners, K., Deckers, S., and Waelkens, M., 2003**, Geoarchaeological investigations of the ‘potters' quarter’ at Sagalassos (SW Turkey) in *Geoarchaeology* 18, 2: 255-281.

3. **Eerkens, J.W., 2002**, The preservation and identification of piñon resins by GC-MS in pottery from the Western Great Basin in *Archaeometry* 44, 1: 95-105.

4. **Heron, C., and Pollard, A.M., 1988**, The analysis of natural resinous materials from Roman Amphoras in *Science and archaeology, Proceedings of a conference on the application of scientific techniques to archaeology, Oxford*.

5. **Poblome, J., Corremans, M., Bes, P., Romanus, K., and Degryse, P., (*in press*)**, It is never too late… The late Roman initiation of amphora production in the territory of Sagalassos, in *I. Delemen, S. Cokay-Kepçe and A. Özdibay (eds) Prof Dr Haluk Abbasoglu'na 65. Yas Armagan. Studies presented to Prof Dr Haluk Abbasoglu, Istanbul*.

6. **Vermoere, M., Vanhecke, L., Waelkens, M., and Smets, E., 2001**, Modern pollen studies in the territory of Sagalassos (Southwest Turkey) and their use in the interpretation of a Late Holocene pollen diagram in *Review of Palaeobotany and Palynology*, 114, 29-56.

6 Beeswax stoppers in glass *unguentaria* from Sagalassos

6.1 Introduction

Intact small glass bottles, called *unguentaria*, were found throughout the whole city of Sagalassos. The vessel type in itself however, is fairly common in the Roman and late Roman-early Byzantine period throughout the empire. In Turkey, the first blown examples were found in Priene and, based on associated numismatic evidence, dated to the reign of L. Caesar as 'Consul designatus' (2-11 AD) [17]. Due to the easy manufacturing techniques, the type knew a rapid spread. Throughout Asia Minor examples are found in Sardis [24], the Isparta region [4], Bodrum [15], Amorium [11,14], Adana [23], Cilicia [7] and Elaiussa Sebaste [10].

In the Macellum (Sagalassos), twenty of them were excavated and among these, two showed the presence of a greasy residue (Figure 6.1). The two bottles had following find numbers: SA-2006-MAC-00048-00100 and SA-2006-MAC-00064-00173. Most of the *ampullae* in Sagalassos are dated to the 4^{th} - 7^{th} century AD and in sharp contrast with most of the published examples, the Sagalassos finds were not retrieved from burials, but came out of dump contexts, maybe indicating a different function. Although these miniature bottles are commonly called 'tear bottles' or *lacrimaria* - mourners supposedly filled small glass bottles or cups with their tears and placed them in burial tombs as symbols of respect- the exact purpose is still unclear and analysing the remains might mean a step forward in elucidating their functionality. Similar studies were undertaken on *unguentaria* dated to the 5^{th} to the 7^{th} century unearthed in the Oplontis Villa B at Pompeii (Italy) [1] and in Jerusalem [2], indicating that the studied vessels were used as oil or unguent containers.

6.2 Samples

The two residues under study had a cylindrical shape with a length and diameter of about respectively 1 cm and 0.5 cm, and had a dark brown to almost black color (Figure 6.2). The context of SA-2006-MAC-00048-00100 consisted of a very heterogeneous layer, characterized by light brown to brown sandy silt containing a large amount of rubble stones, larger pieces of rock, small and large architectural fragments. The origin of the deposit seemed to be partly from collapse, partly from erosional nature. In the portico and the courtyard, it included the large amount of architectural fragments originating from the portico, the central tholos and the walls of the shops. The erosion and collapse deposits could have resulted from a very gradual decay process already started prior to the proper collapse of the structures while the SA-2006-MAC-00064-00173 sample is from room 3, to the south of room 2 at the back of the Macellum, which was completely covered by a collapse/erosion layer. A dump covered an actual walking level/floor substrate (locus 65/64). The latter was obviously laid down to fill the irregularities of the natural bedrock substrate. It had a dark, clayish appearance and its surface was clearly beaten. Its thickness varied from ca. 0.15 m to ca. 0.30 m in the deepest zones. The ceramic evidence from both layers (locus 34 and 64) presented the characteristics of secondary deposition. Their dating clustered around AD 580/590.

a b

Figure 6.1: Pictures of two *unguentaria* (SA-2006-MAC-00064-00173 (a) and SA-2006-MAC-00048-00100 (b)) containing a visible residue.

a b

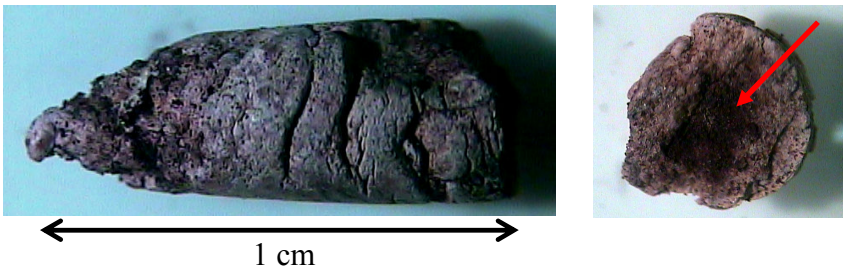


Figure 6.2: A microscopic picture where the residue from the *unguentarium* with sample number SA-2006-MAC-00048-00100 is shown from side view (a) and top view with the red arrow indicating a finger imprint (b).

6.3 Results

6.3.1 Extraction

The results for the lipid extraction are assembled in following table (Table 6.1). It is clear that the majority of both residues consisted out of fatty material, namely 93 %.

To assess the inorganic content in the sample, a thermogravimetric analysis was performed on the remainder after extraction. The amount of minerals seemed to be very low. Indeed, only 5 % of the whole sample seemed to be present as inorganic material.

Table 6.1: Assembled results for the extracted residues. The amounts for lipids and inorganic material are expressed in mass percentages of the complete residue.

	Mass (g)	**Lipids (%)**	**Inorganic material (%)**
SA-2006-MAC-00024-00046	0.15	93	4.8
SA-2006-MAC-00064-00173	0.08	93	5.4

6.3.2 Analysis of silylated lipid extract

As shown in Figure 6.3, analysis on a gas chromatograph with mass spectrometer (GC-MS) generated a chromatogram containing high amounts of free fatty acids ranging from 16 to 34 carbon atoms with the highest concentration for tetracosanoic acid. Also a series of odd numbered alkanes are abundantly present with the highest concentration for heptacosane. Alkanols are visible in smaller concentrations and range from 24 to 32 carbon atoms with triacontanol showing the biggest peak. Towards the end of the chromatogram, a series of mono-esters from 40 to 46 carbon atoms is detectable. These results are very characteristic for beeswax. The complete sample seems to consist out of beeswax as only its compounds and their degradation products are detectable in the chromatogram [9,18,19]. Exactly the same result was obtained for the second residue.

6.3.3 Wax ester analysis

Both lipid extracts were analysed on a liquid chromatograph with mass spectrometer (LC-MS) in the atmospherical pressure chemical ionisation mode. The results for the two residues were equivalent and are shown in Figure 6.4 and Table 6.2. The presence of high amounts of wax esters is confirmed.

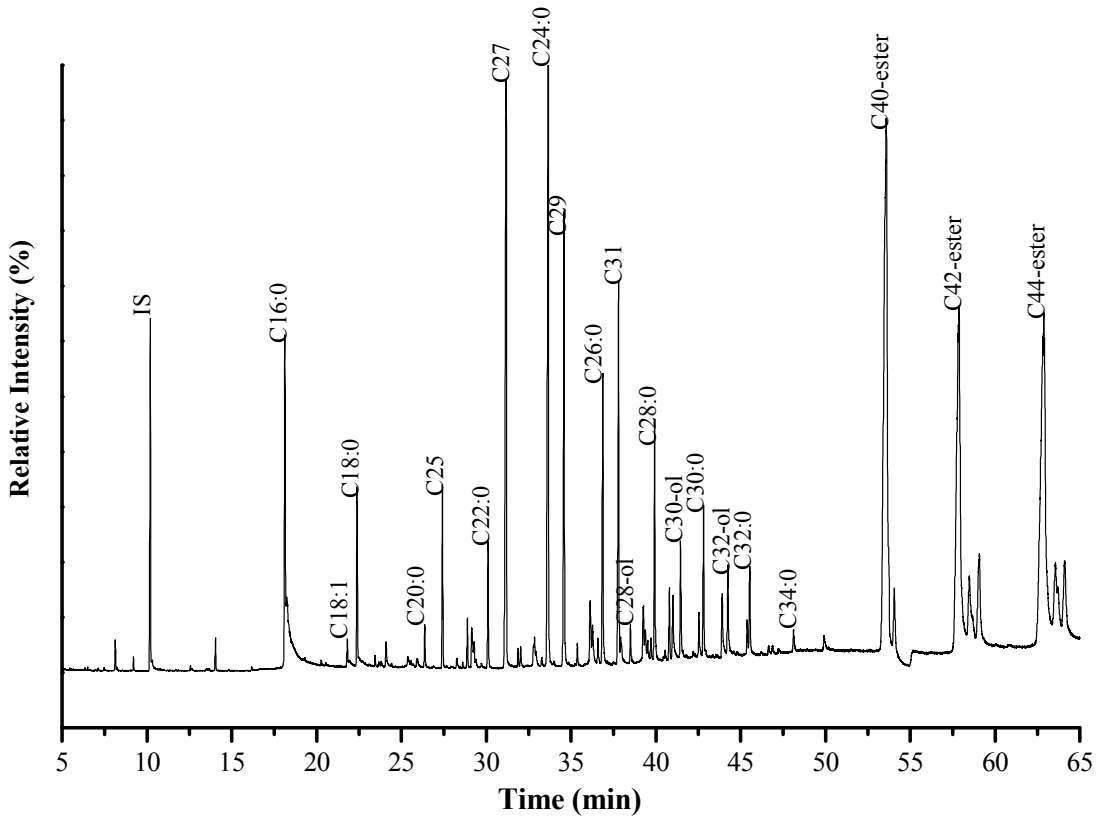


Figure 6.3: Reconstructed GC-MS chromatogram of the silylated lipid fraction of the residue from sample SA-2006-MAC-00064-00173. All functional groups are present in their trimethylsilyl form. IS stands for internal standard, Cn:m stands for a fatty acid with n carbon number and m the amount of double bonds, Cn represents an alkane with n carbon atoms, Cn-ol is the symbol of an alkanol with n carbon atoms and Cn-ester stands for a mono-ester with n carbon atoms.

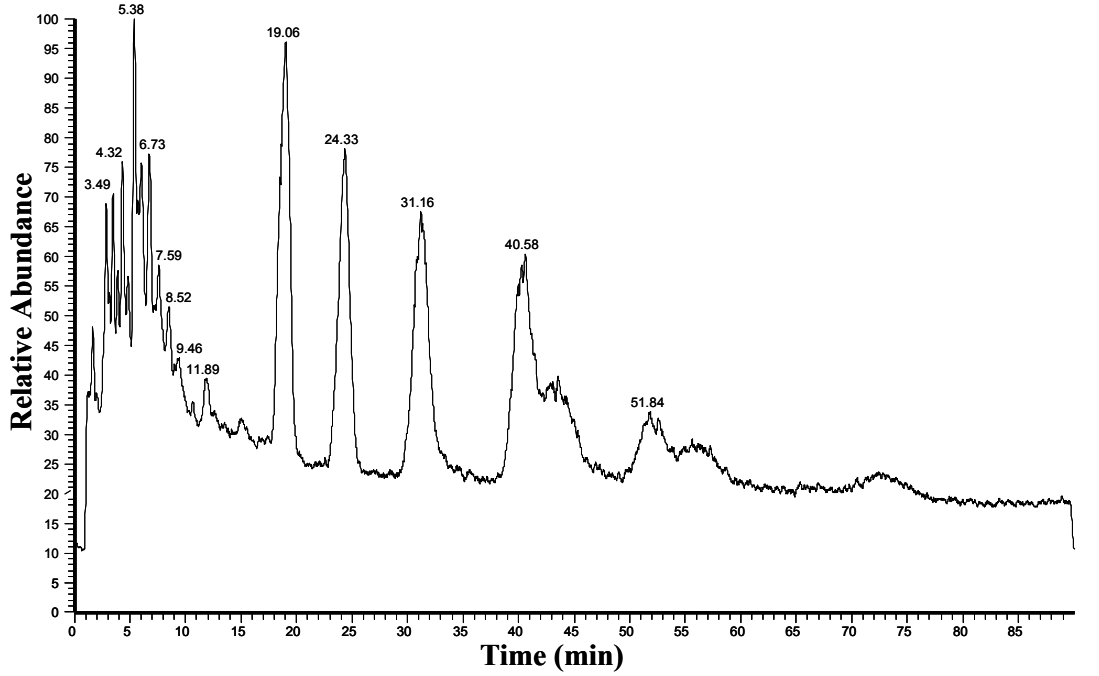


Figure 6.4: HPLC-MS chromatogram of the residue in *unguentarium* SA-2006-MAC-00024-00046.

Table 6.2: Summary of the retention times in minutes (RT) of the wax esters detected in the residue in *unguentarium* SA-2006-MAC-00024-00046 together with their characteristic mass fragments (expressed in *m/z*) obtained with a liquid chromatograph with mass spectrometer.

RT (min)	Wax ester	$[MH]^+$	$[MH-H_2O]^+$	[MH-RCOOH]+
2.8	C40 hydroxymonoester	609	591	
3.4	C42 hydroxymonoester	637	619	
4.3	C44 hydroxymonoester	665	647	
5.3	C46 hydroxymonoester	693	675	
6.0	C56 hydroxydiester	863		591
6.7	C48 hydroxymonoester	721	703	
7.6	C58 hydroxydiester	891		619
8.5	C60 hydroxydiester	919		645
11.8	C62 hydroxydiester	947		675
19.0	C56 diester	847		591
24.3	C58 diester	875		619
31.1	C60 diester	903		647
41.1	C62 diester	931		675
52.1	C64 diester	959		703

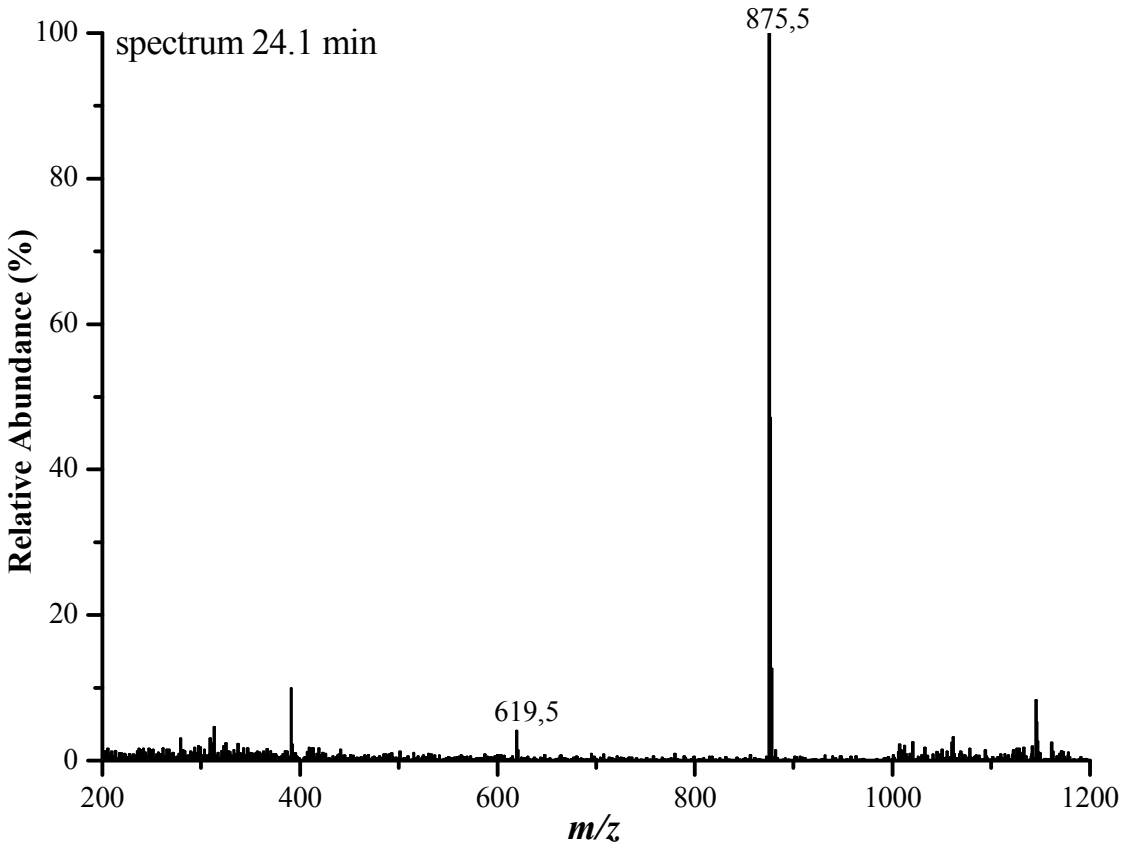


Figure 6.5: APCI mass spectrum of C58 diester.

A series of hydroxymono-esters is detected from 40 to 48 carbon atoms next to hydroxydiesters from 56 to 62 carbon atoms and diesters from 56 to 64 carbon atoms. They were identified by their respective mass fragments. Hydroxymono-esters are characterized by a fragment with the loss of a water molecule $[MH\text{-}H_2O]^+$ while diesters show fragments with the loss of a palmitic acid moiety $[MH\text{-}RCOOH]^+$ (Figure 6.5). The presence of these series of wax esters is diagnostic for beeswax and thus confirmed the results of the GC-MS analysis [9,18,19].

6.4 Discussion

In order to elucidate the functionality of *unguentaria* in Sagalassos, it is crucial to gather answers about their content in ancient time. In that way, the exceptional find of greasy residues, still encapsulated in two bottles, presented an excellent opportunity for delivering these answers. Chemical analyses on both residues revealed that 93 % of them consisted out of beeswax and about 5 % seemed to be inorganic material.

Earlier research proved the processing of beeswax in cooking pots of Sagalassos [12]. Researchers considered the beeswax was intended for candle making. As the size and shape of *unguentaria* are inappropriate for burning a candle, the data presented here demand an expansion of that hypothesis. Because of its presence and processing in Sagalassos, we have indications that beeswax was not exceptional merchandise in the city. As the bottles were found in high amounts throughout the whole city of Sagalassos, they couldn't have been luxurious goods.

There are a few options for the functionality of *unguentaria*. Maybe the bottles contained a salve or lip balm. Nevertheless, the shape of the bottles is not practical to get a sticky substance out. Moreover, the residue would have covered the inner walls of the bottle instead of being compact, cylindrical and positioned in the neck of the bottle. Also the low amount of inorganic material is contradictory to the presence of a salve.

A second option is that a liquid was kept in the bottles and that a beeswax stopper was used for sealing. This perfectly explains the conical shape of the residue and its position in the neck of the *unguentaria*. On the microscopic pictures of the residue (Figure 6.2), one can also see an impression of a finger. Various materials are known to have been used in Roman times as stoppers for glass vessels. Lathe-turned glass,

dated to the first century, were found in Cologne, Marienburg [8]. Glass stoppers for tubular glass kohl jars dating from the mid-third to mid-fourth centuries are known [20,22], both with a bird-like element as finial. A lead stopper for a glass aryballos was excavated in a first-century tomb at Monasteriaki Kephala, Crete [5]. In a sarcophagus dated to the first half of the third century at Cologne a wooden stopper was found [13]. Grass was also discovered to close a first-century glass *unguentarium* from the Egyptian Fayum [6]. An upside down pine cone was employed to stop a Roman glass bottle in the Athenian Agora [16]. Dried figs were used as stoppers for ceramic vessels in the Bronze Age shipwreck at Ulu Burun [3] and the Tosephta Shabbath mentions the use of papyrus as a stopper: «who takes a sheet in order to wind it over the mouth of a small flask of spikenard» [21].

As beeswax is an easy manipulatable product, it must have been easy for use as a stopper but probably it was not convenient to take the closure back out. As day after day opening probably was impracticable, we assume that a ritual or a practice that required the content to be emptied in one instant instead of a daily used fluid was kept in an *unguentarium*. Indeed, maybe people collected water which had flown over bone remains from a divine person and carried those tiny bottles on them as an amulet for protection.

6.5 Conclusion

In Sagalassos, two excavated *unguentaria* were shown to contain a visible greasy residue. As the functionality of those small glass bottles is still unclear, the purpose of this study was to elucidate this issue by means of chemical analyses. Both residues contained 93 % beeswax and only 5 % inorganic matter. Because of the conical shape and their position in the bottle, one can assume a stopper made of beeswax was used to close an *unguentarium*. In that case, these bottles were probably filled with a liquid substance. Nevertheless, it does not seem practical to use such a stopper day after day as beeswax is an easily deformable substance. That is why we exclude the hypothesis that a daily used liquid was kept in these small bottles. Unfortunately, it is not possible to make any statement about the true origin of the fluid. Maybe the content was intended to be used once or maybe a ritual fluid was kept in these *unguentaria*.

6.6 References

1. **Baraldi P., Ciarallo A., Colombini M., Modungo F. and Ribechini E., 2006**, An investigation on the organic residues in archaeological glass vessels from Pompeii and Oplontis (Italy), *unpublished poster hand-out, 17th conference of the International Society for the History of Glass, Antwerp, 4-8 september 2006.*

2. **Basch A., 1972**, Analyses of oil from two Roman glass bottles in *Israel Exploration Journal* 22:27-32.

3. **Bass G., 1986**, A Bronze Age shipwreck at Ulu Burun (Kaş): the 1984 campaign. *American Journal of Archaeology* 90: 285.

4. **Bayram F., and Dönmez H**. (ed) Ispartia Müzesi, Ankara, 58-59.

5. **Carrington Smith J., 1982**, A Roman chamber tomb on the south-east slopes of Monasteriaki Kephala, Knossos, *Annual of the British School at Athens* 77: 282, nr 77, pl.40a, b.

6. **Edgar C.C., 1905**, *Graeco-Egyptian glass: catalogue général des antiquités du musée du Caire*. Cairo (reprint 1974): 55, nr 32661, pl. VIII.

7. **Erten Yağci E., 1999**, Kilikia'da cam in Olba 2, 172.

8. **Fremersdorf F., 1958**, Römisches Buntglas aus Köln (Denkmäler des römischen Köln 3). Cologne: 55, pl. 124h.

9. **Garnier N., Cren-Olive C., Rolando C., and Regert M., 2002**, Characterization of archaeological beeswax by electron ionisation and electrospray ionisation mass spectrometry in *Analytical Chemistry* 74: 4868-4877.

10. **Gençler C., 2004**, Vetri, in Equini Schneider E., (ed) Elaiussa Sebaste II. Un porto tra oriente e occidente, Rome: nr 150.

11. **Gill M., with contributions by Lightfoot C.S., Ivison E.A., and Wypysky M.T., 2002**, Amorium reports, finds I: The Glass (1987-1997), *BAR International Series 1070*, Oxford, nr 280.

12. **Kimpe K., Jacobs P., and Waelkens M., 2002**, Mass spectrometric methods prove the use of beeswax and ruminant fat in late Roman cooking pots in *Journal of Chromatography A* 968: 151-160.

13. **La Baume P., 1960**, Neue Funde Römischer Sarkophage in Köln ,in: *Mouseion, Studien aus Kunst und Geschichte für Otto H. Förster*. Cologne: 82, fig. 26.

14. **Lightfoot C., 2005**, Glass Finds at Amorium in *Dumbarton Oaks Papers* 59, 174.

15. **Özet A., 2000**, Sparkles from the deep. Glass vessels of the Bodrum Museum of underwater archaeology, Istanbum, 66-68.

16. **Pers. Communication** of G.D. Weinberg to E.M. Stern.

17. **Platz-Horster G., 1979**, Zu Erfindung und Verbreitung der Glasmacherpfeife in *Journal of Glass Studies* 21: 27-31.

18. **Regert M., Colinart S., Degrand L., and Decavallas O., 2001**, Chemical alteration and use of beeswax through time: accelerated ageing tests and analysis of archaeological samples from various environmental contexts in *Archaeometry* 43: 549-569.

19. **Regert M., Langlois J., and Colinart S., 2005**, Characterization of wax works of art by gas chromatographic procedures in *Journal of Chromatography A* 1091: 124-136.

20. **Smith R., 1957**, Glass from the ancient world. The R.W. Smith Collection, exh. Cat the Corning Museum of Glass, Corning N.Y.: 155-157, nr 321.

21. **s.n., 1978**, Function and design in the Talmudic Period, exh. Cat. Haaretz Museum, Tell Aviv: 53.

22. **s.n., 1979**, The Constable-Maxwell Collection of ancient glass, auc.cat., Sotheby Parke Bernett (London, June 4-5, 1979) 68, nr 109.

23. **Stern E., 1989**, Glass vessels exhibited in the Bölge Museum-Adana in Belleten 153, 590.

24. **von Saldern A., 1980**, Ancient and Byzantine glass from Sardis, Archaeological Exploration of Sardis Monograpphs, no. 6. ed. Hanfmann G. and Scott J., Cambridge, Massachusetts: Harvard University Press, nrs 113 and 564.

7 Brassicaceae seed oil recognized as lamp fuel in Nilotic shells from a first millennium AD Coptic church in Bawit, Egypt

7.1 Introduction

As already demonstrated in the previous chapter, not only the origin of foodstuff can be identified by residue analysis, but also information on numerous other commodities or luxury goods, such as ointments and other personal care products [11] or lighting fuels [17] can be obtained. For example, in studies of ceramic oil lamps, a variety of illuminants has been identified such as vegetal oils, ruminant fat or beeswax [18]. In a 5th century oil lamp AD from Northern Africa, Passi [30] found 9,10-dihydroxystearic acid, suggesting that the lamp may have contained an oxidized, rancid oil. In ancient oil lamps from Sagalassos, researchers found olive oil as fuel together with traces of animal fat, based on analysis of the triglycerides [17]. Intensive studies were carried out on Egyptian ceramic oil lamps from the North Necropolis at Antitoe (Egypt) dating back to the fifth to seventh centries AD, in which monocarboxylic acids, α,ω-dicarboxylic acids, and long-chain dihydroxylated acids were found, indicating the presence of Brassicaceae oil [4,6].

This section proclaims the first study on archaeological lamp fuels in a non-ceramic matrix. The residues studied were recovered from *Chambardia rubens* shells, supposedly used as lamps in Egypt during the early Islamic period [19]. The aim of this investigation is not only to prove that these shells were used as a lamp, but also to identify the source of the fuel. A wide range of methodologies is applied like fatty acid methyl ester analysis on GC, identification of silylated compounds on GC-MS, determination of $\delta^{13}C$ values (‰) of FAME by GC-C-IRMS and LC-MS.

7.2 Samples

Excavations carried out in the site of Bawit, Egypt, since the early 20th century by both French and Egyptian teams revealed the presence of two churches, habitation quarters and a large number of cells provided with oratories, often decorated with wall paintings [21,28,29]. This site is a Coptic monastery that was founded at the end of the 4th century AD and abandoned in the 10th century and is located on the west bank of the Nile, about 280 km south of Cairo. During the on-site identifications of the faunal remains from the 2003-2005 excavations of the northern church of Bawit one of the archaeologists noted the presence of numerous large shells of the Nile bivalve *Chambardia rubens arcuata*, a taxon previously mentioned in the literature as *Aspatharia rubens* and sometimes *Spathopsis rubens* [7]. The greatest length of the completely preserved valves varied between 9 and 14 cm. It was striking that 19 of the 37 valves were burned and that many of them had a black crust adhering at the interior side (Figure 7.1). The abundance of the shells in the church and their burned aspect suggest that these valves were used as containers for an illuminant. Other lamps have been found during the previous excavations in 1901-1913 (some of them are kept in the Louvre) and during the recent excavations [19]. Sample of black crust was taken from the interior of eight shells for lipid analysis.

Figure 7.1: Picture of a lamp shell from the northern church.

7.3 Extraction

Table 7.1 presents the sample weights and corresponding yields of extracted lipids, together with the analysis types performed on each sample (see chapter 2 §1 for experimental setup). Because of the small sample size and the ensuing impossibility to perform two derivatisations on one sample, half of the samples were subjected to FAME and GC-C-IRMS analysis, while to the remaining ones GC-MS and LC-MS analysis was applied. As all samples were collected in the same church, we assume that their usage and fuel have been similar. While it cannot be excluded that the shells were re-used, the fact that they are much less precious than *e.g.* ceramic lamps makes re-use less plausible.

7.4 FAME analyses

The concentrations of saturated or unsaturated C_{12}-C_{18} fatty acid residues can be quantified by standard FAME analysis after their conversion to respective methyl derivatives (see chapter 2 §1 for experimental setup). Hence, using this method, metabolites of these acyl residues cannot be identified with certainty, as will be addressed below. The similarity of the fatty acid profiles from all four samples confirms the initial assumption that they indeed have the same origin. The concentrations of the fatty acid methyl esters (FAME), as analysed by PPGC, are

Table 7.1**:** Results of lipid extraction and outline of the analyses performed for each sample.

	Weight (g)	**Lipids (%)**	**Analyses performed**
ASP1	0.62	6.2	PPGC & GC-C-IRMS
ASP2	1.58	0.3	GC-MS & LC-MS
ASP3	0.37	0.6	GC-MS & LC-MS
ASP4	0.85	0.8	GC-MS & LC-MS
ASP5	0.57	11.7	PPGC & GC-C-IRMS
ASP6	0.22	37.5	GC-MS & LC-MS
ASP7	0.40	4.6	PPGC & GC-C-IRMS
ASP8	1.59	0.8	PPGC & GC-C-IRMS

strikingly low; the four samples contained only 0.3 - 1 µmol FAME per g sample. It should be noted that the FAME were exclusively obtained by transesterification of triglycerides, and not by methylation of free fatty acids. The latter will be studied in more detail in the silylated form by GC-MS. The low FAME concentrations thus are related to low concentrations of intact triglycerides. A representative fatty acid profile is shown in Figure 7.2. Relatively high amounts of methyl oleate and methyl *cis*-hexadecenoate were observed. This is unexpected because unsaturated fatty acids are more sensitive to autoxidation [3,9,20].

An alternative method of interpreting PPGC results is based on evaluating fatty acid ratios rather than absolute values. When an animal fat would have been used rather than a vegetal product, high amounts of methyl stearate, a saturated fatty acid, would be detected. Consequently, a low ratio of methyl palmitate to methyl stearate (P/S) and a high ratio of methyl stearate to methyl oleate (S/O) would be observed [9,16] (see also chapter 3). Here, on the contrary, high P/S ratios are observed. This excludes the possibility that an animal fat was used as the major fuel compound. Additionally, the S/O ratios are low, with comparable amounts of stearate and oleate (Table 7.2). Taking into account the high degradability of oleic acid, it is to be assumed that the original product had a high oleic acid content, characteristic for vegetal oil.

Table 7.2: Overview of fatty acid ratios from the Egyptian lamp shells and some reference fats. P/S, P/O and S/O stand for the ratio of methyl palmitate to methyl stearate; methyl palmitate to methyl oleate and methyl stearate to methyl oleate respectively.

	P/S	**P/O**	**S/O**		**P/S**
ASP1	3.3	3.8	1.2	**ruminant adipose fat**	< 1.3
ASP5	3.3	4.2	1.3	**ruminant dairy fat**	2.9
ASP7	4.1	5.1	1.3	**non-ruminant adipose fat**	> 1.3
ASP8	1.9	4.5	2.4	**rapeseed oil**	3.1
				radish seed oil	3.5
				olive oil	4.0

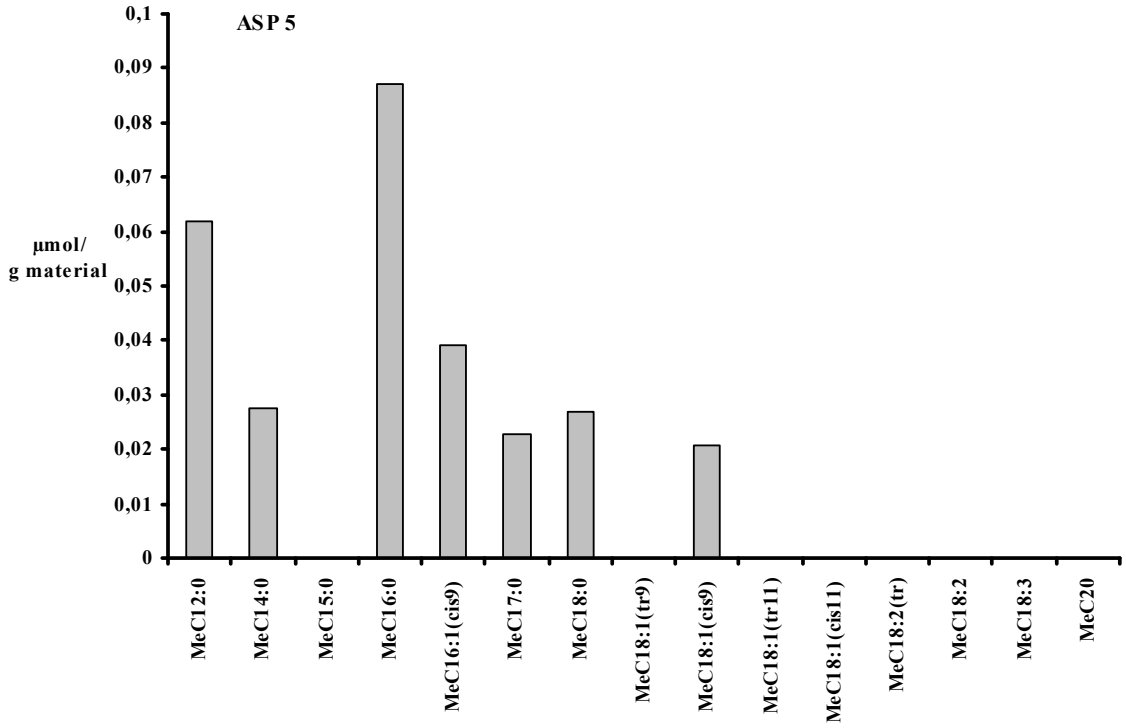


Figure 7.2: Fatty acid profile of sample ASP 5. The amounts of FAME are expressed in µmol FAME per g original sample.

7.5 Stable carbon isotope analyses

As also mentioned in previous chapters, the animal origin of a lipid sample can be elucidated by evaluating the $\delta^{13}C$ values (‰) of FAME because each species has its characteristic isotopic signature in the stearic and palmitic residues (Figure 7.3) [25,5]. As the PPGC results rather point towards a vegetal than towards an animal source, it is necessary to consider also reference data of vegetal oils, as is done in Table 7.3 and on Figure 7.3.

The $\delta^{13}C$ values of our samples are clustered, as shown in Figure 7.3; and the $\Delta^{13}C$ values ($\Delta^{13}C = \delta^{13}C_{C18:0} - \delta^{13}C_{C16:0}$) are all close to 0 ‰. Such values can not be assigned to a particular animal lipid type, but may well correspond to a vegetal oil. Isotopic analysis is not capable of discriminating between various C_3 vegetal oils, because of their similar isotopic signatures (Table 7.3) [15]. Differentiating between C_4 and C_3 plants is much easier because C_4 plants follow the Calvin cycle, resulting in relatively depleted $\delta^{13}C$ values (‰) like for example $\delta^{13}C_{C16:0} = -14$ ‰ [35,33,34].

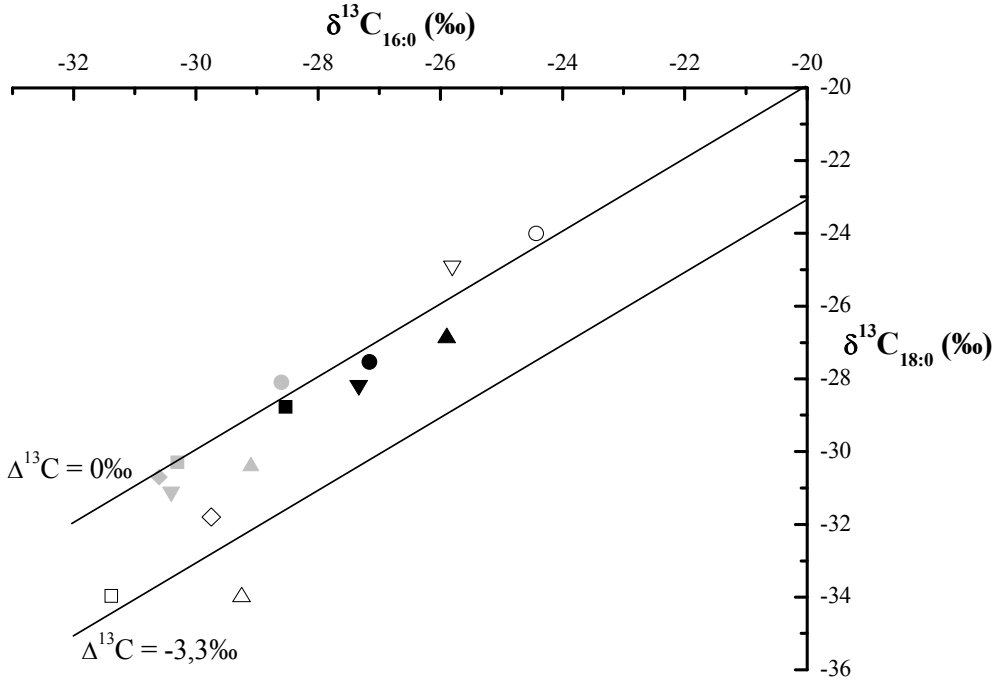


Figure 7.3: $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ plot (expressed in ‰) with ■, ●, ▼ and ▲ representing samples ASP1, ASP5, ASP7 and ASP8 respectively. The other symbols stand for sheep (□), cattle raised on a C_3 plants diet (◊), poultry (○), porcine (▽), dairy fats from cows raised on a C_3 plants diet (Δ), palm oil (▼), groundnut oil (▲), sunflower oil (♦), rapeseed oil (■) and radish seed oil (●). All values were obtained with modern tissue. Reference values for adipose tissue (-29.8; -31.9) and milk (-29.2; -34.0) from C_3-fed cattle have been taken from literature [28, 32].

This excludes the possibility that a C_4 plant oil was used as a fuel here, in agreement with the fact that all the plants traditionally used for oil production in Egypt are C_3 species. When reference $\delta^{13}C$ values (‰) for rapeseed oil [15] and radish seed [27] are plotted on Figure 7.3, it can be seen that the values of the archaeological samples rather resemble that of radish seed oil than that of rapeseed oil, but more evidence for such an assignment will only be supplied based on GC-MS results as will be discussed in the following paragraphs.

Table 7.3: δ13C values (‰) of a few modern vegetal oils [28, 32].

	($\delta^{13}C_{C16:0}$; $\delta^{13}C_{C18:0}$)(‰)	**$\Delta^{13}C$ value (‰)**
groundnut oil	(-29.1; -30.4)	-1.3
palm oil	(-30.4; -31.1)	-0.7
rapeseed oil	(-30.3; -30.3)	0
radish seed oil	(-28.6; -28.1)	0.5
sunflower oil	(-30.6; -30.7)	-0.1

7.6 Triglyceride analyses

Triglycerides (TAG) can be analysed by HPLC-MS with APCI not only for identifying animal fats [24] but also vegetal oils. Although TAG are sensitive to hydrolysis during burial of archaeological samples, in some cases a fraction remains preserved which can be of great value in finding the origin of fats (see previous chapters). In the HPLC-MS analyses of the lipid extracts from the shells, the focus was first on TAG containing the saturated or unsaturated C_{12}-C_{18} fatty acids. Concentrations of such TAG were generally below the detection limit, except in sample ASP6. In this sample, very small amounts of glycerol-dioleate-linoleate and glycerol-dioleate-palmitate were identified, in agreement with the results of the FAME analysis.

In addition to these TAG, a number of other, higher molecular weight compounds of unknown structure showed up at longer retention times in the LC-MS chromatograms (Figure 7.4). As the analysis was performed on a silylated sample, and the silylating agent introduces a large amount of nitrogen into the sample, it is not surprising that TAG can appear in the mass spectrum as $[M+NH_4]^+$ ions, with an even mass for the base peak instead of the usual odd values for $[M+H]^+$. This was confirmed by an independent analysis of trilaurin, against a background of silylating agent and its decomposition products. Additionally, all high molecular weight compounds contain at least two and even three $(CH_3)_3SiO$-groups, as evidenced by two or three consecutive losses of mass 90 ($-(CH_3)_3SiOH$) in the fragmentation patterns. As a result, all unknown compounds could be identified as TAG with 46 or 48 C atoms in the acyl chains, and with two or three -OH groups that are silylated during derivatisation. Peaks resulting from $(CH_3)_3SiOH$ loss are very intense, making it difficult to assign precise molecular structures to the ions observed. For instance, the ion at m/z = 1000.5 may arise not only from glycerol-laurate-myristate-(13,14-di(trimethylsilyloxy)docosanoate), but also from glycerol-myristate-palmitate-(9,10-di(trimethylsilyloxy)octadecanoate). In any case, the presence of dihydroxylated fatty acids in the triglycerides is beyond doubt. Similar fatty residues will be found by GC-MS (see following paragraph).

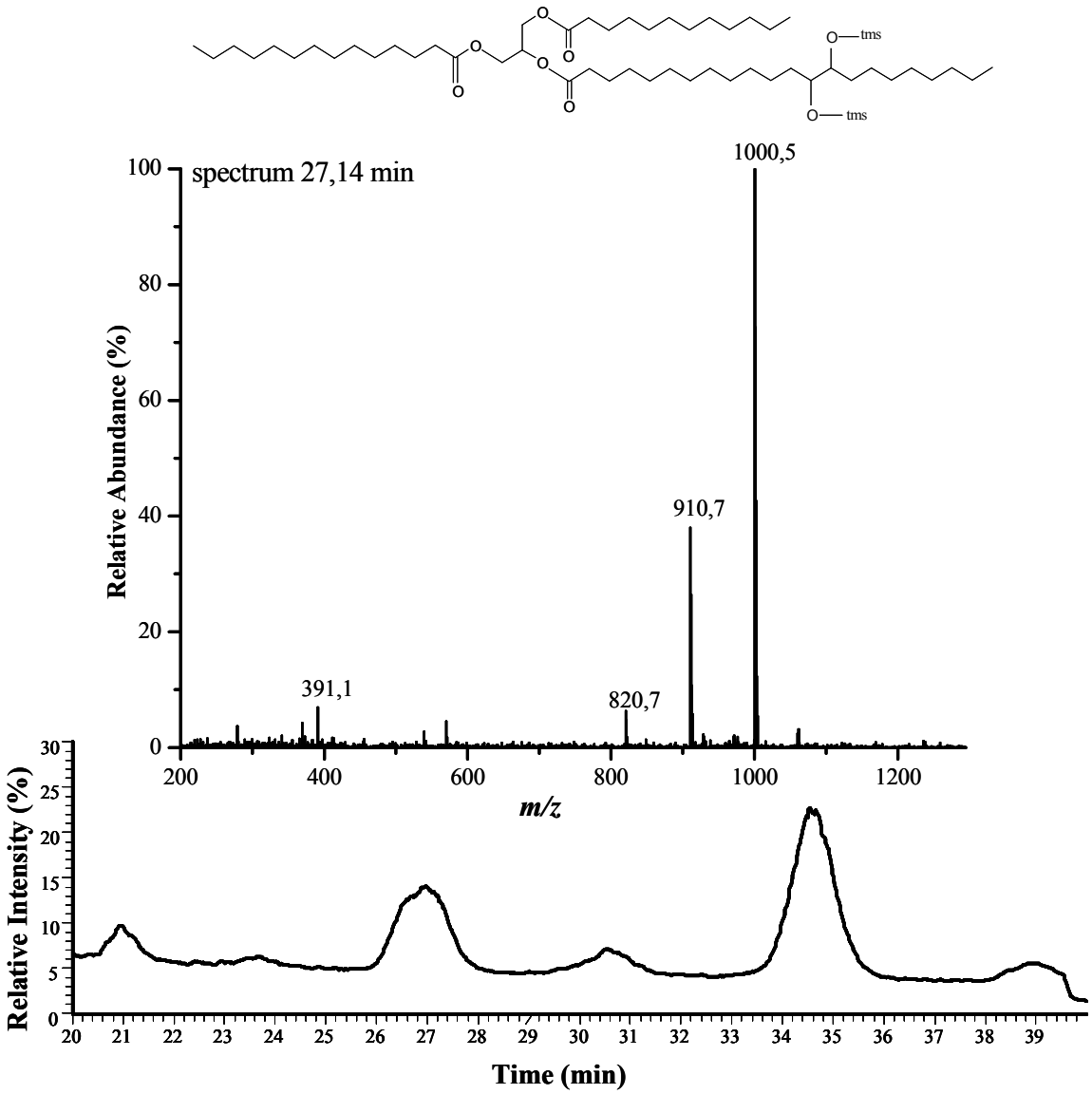


Figure 7.4: Partial APCI HPLC-MS chromatogram of sample ASP6, containing very small concentrations of intact TAGs but considerable amounts of silylated dihydroxylated or trihydroxylated TAGs; the mass spectrum of a disilylated dihydroxylated compound eluting at 27.14 min is shown as an inset.

7.7 Analysis of silylated derivates

Results from GC-MS analyses delivered the identification of three groups of characteristic compounds. The first group, found in three of the four samples, consisted out of a range of short chain dicarboxylic acids (Figure 7.5). The latter are

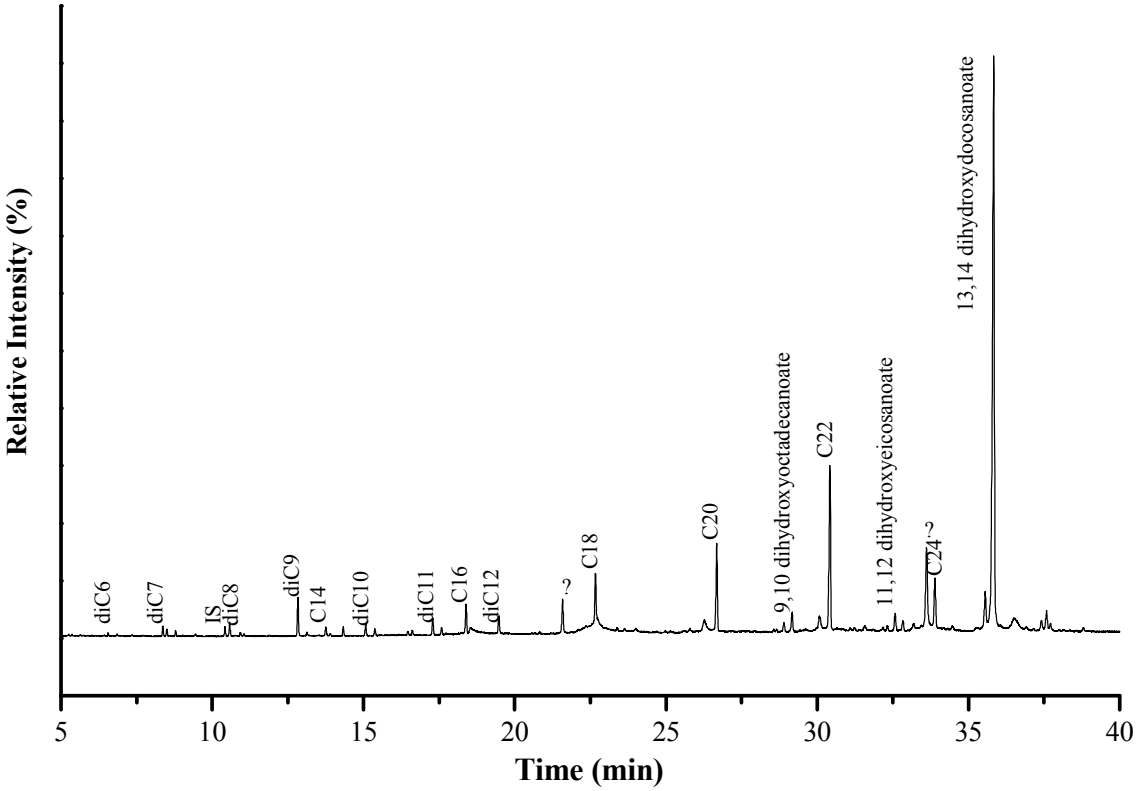


Figure 7.5: Reconstructed total ion GC-MS chromatogram of sample ASP4. DiCn represents a diacid with n carbon atoms, Cn a saturated fatty acid with n carbon atoms, while IS stands for n-heptadecane being the internal standard. All acids and hydroxylated compounds are in the trimethylsilylated form.

oxidation products of unsaturated fatty acids formed during combustion or burial of the oil [4,6]. They range from six up to thirteen carbon atoms, azaleic acid (diC9 in Figure 7.5) being the most abundant. The mass spectra of the identified diacids are characterized by the fragment ions $[M-15]^+$ and $[M-131]^+$.

In all samples, saturated fatty acids with up to 24 carbon atoms were identified. The highest concentrations were found for docosanoic or behenic acid (C22:0). Because of its high concentration, behenic acid must originate from the fuel itself. Indeed, it can not be a degradation product from erucic acid (C22:1(13)) because the reduction reaction, needed here, is implausible to occur during the oxidizing burial conditions [3].

Trisilylated 13,14-dihydroxydocosanoate appears to be the base peak of the chromatogram in Figure 7.5 (at 35.8 min). Together with 11,12-dihydroxyeicosanoate, these dihydroxylated fatty acid molecules are oxidation products of erucic acid and gondoic acid (C20:1(11)), respectively. The latter fatty acids are known to be richly present in oils from seeds of Brassicaceae plants

containing up to 60 % erucic acid and 20 % gondoic acid [4,6]. The presence of 9,10-dihydroxyoctadecanoate was also confirmed in the GC-MS spectra, although in lower concentrations. Vicinal dihydroxyacids are formed after dihydroxylation of the double bond of monounsaturated acids. As two peaks with near-identical mass spectra are detected in the chromatograms, the presence of their *threo* or *erythro* stereoisomers is confirmed (Figure 7.6).

Supplementary information is contained in the isomer composition of the dihydroxylated fatty acids. The elution order of the *threo* and *erythro* isomers was assigned performing the stereospecific Os-catalyzed *cis*-dihydroxylation of double bonds [1]. Indeed, *cis*-dihydroxylation of *cis* fatty acids such as oleic or erucic acid yields the *erythro* isomers, while *cis*-dihydroxylation of *trans* fatty acids such as elaidic or brassidic acid yields the *threo* isomers. Analysing the silyl derivates of the synthesized dihydroxyacids on GC-MS, it was proven the *threo* isomer elutes before the *erythro* isomer. Additionally, it was confirmed that nor the derivatisation step, nor the injection in the GC result in epimerization of the dihydroxyacids.

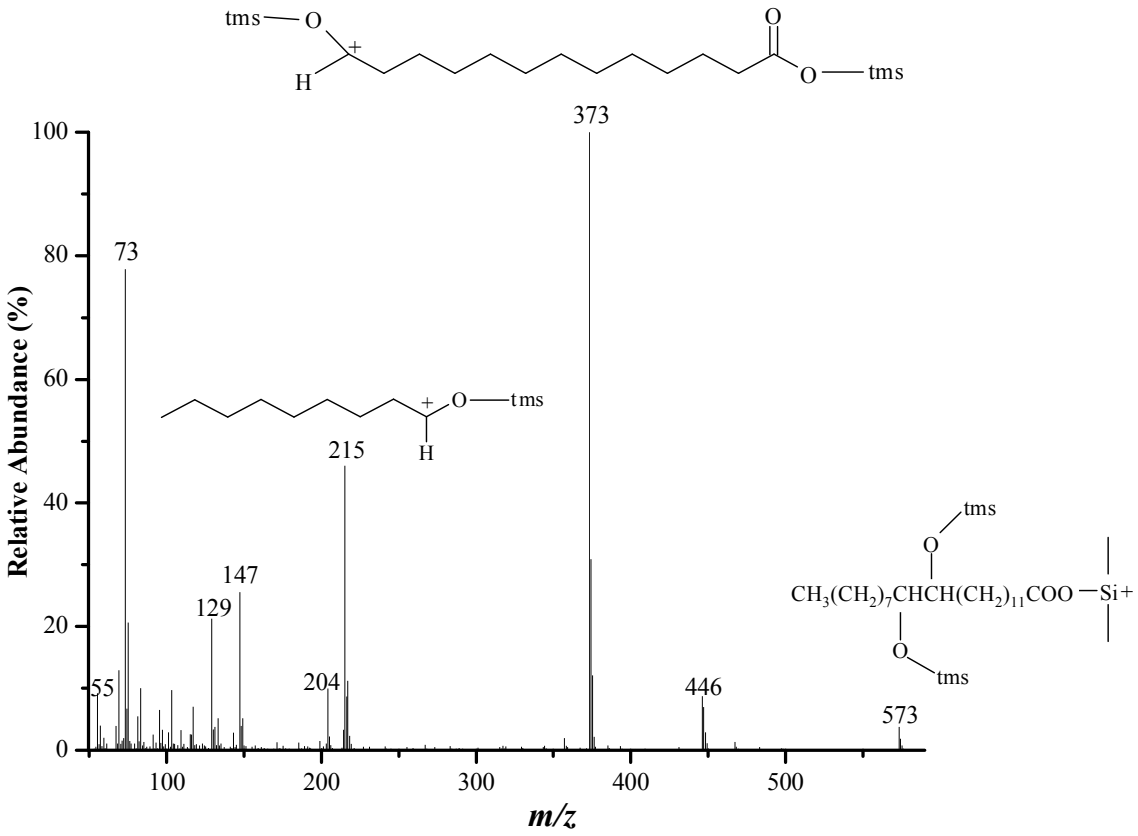


Figure 7.6: Mass spectrum acquired by GC-MS of 13,14-dihydroxydocosanoate in the trimethylsilylated form together with important mass fragments.

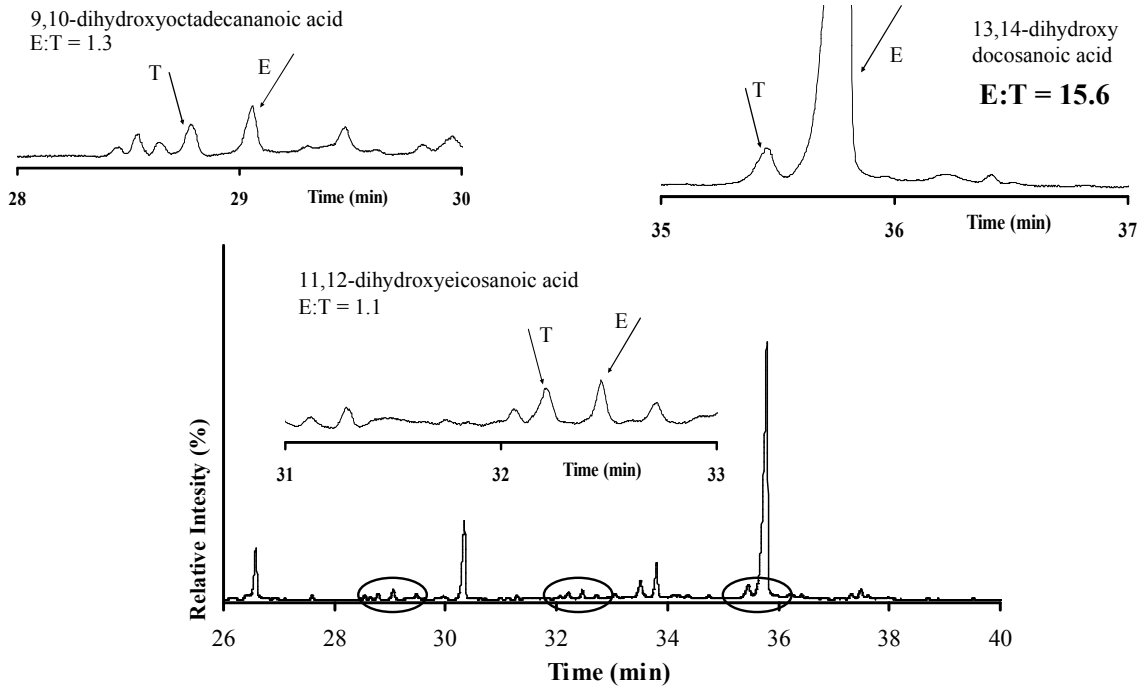


Figure 7.7: Partial GC-MS chromatogram (based on total ion count) of ASP6 with focus on the erythro (E) and threo (T) isomers of the silylated dihydroxylated fatty acids together with their erythro:threo ratio (E:T).

In the archaeological samples of the present study, two distinct situations were encountered. For the C_{18} and C_{20} dihydroxyacids, comparable amounts of both isomers were found. In such case, there usually was a slight preference for the *erythro* isomer, with *erythro*:*threo* ratios between 1 and 3. However, especially when a large amount of the C_{22} dihydroxyacid was found, a clear stereospecificity was observed, with a strong preference for the *erythro* compound. In 3 out of 4 samples, the *erythro:threo* ratio in the C_{22} dihydroxyacid fraction was 15 or higher (Figure 7.7).

The GC-MS chromatogram for sample ASP3, was unlike the other samples in that dicarboxylic acids, 9,10-dihydroxyoctadecanoate, 11,12-dihydroxyeicosanoate and 13,14-dihydroxydocosanoate were absent. However, the long chain fatty acids were present, with a similar ratio of C22:0 to C24:0 (= 3 ± 0.5) as in the three other samples. This indicates that similar oil was used in all cases; however, this particular sample may have been subject to elution of the polar compounds, resulting in the absence of diacids and dihydroxylated acids.

In sample ASP6 the plant-derived ß-sitosterol was detected. In none of the shells, cholesterol or its oxidation products were detected, excluding the possibility that

animal fats were used as an illuminant. This also confirms the results of the fatty acid ratios from which high P/S and low S/O ratios were calculated.

7.8 General Discussion

The burned greasy deposits from eight *Chambardia* shells from a church at Bawit, Egypt, were analysed with a variety of techniques, with the aim of confirming the function of the shells as lamps and of identifying the illuminant that had been used. Literary sources, such as Greek papyri, document the use of several plant oils in Egypt as an illuminant from the Ptolemaic period onwards [23]. These include species such as radish (*Raphanus sativus*), castor (*Ricinus communis*), olive (*Olea europaea*), flax (*Linum usitatissimum*), safflower (*Carthamus tinctorius*) and sesame (*Sesamum indicum*) although in many texts it is difficult to identify the exact nature of the oil. Besides plant oil, also animal fat could be used as an illuminant, a fuel type for which 'open lamps' such as the bivalve shells were more suitable than a typical ceramic lamp with a small filling hole. Palm fibers, papyrus, reeds, linen, may have been used as lamp wick [26], but no macroscopic traces of them were observed in the shells.

The results of the fatty acid methyl ester fraction analysed by PPGC lead to a first assumption that the illuminant used in the shells from Bawit was of vegetal origin. Indeed, significant amounts of unsaturated fatty acyl groups still could be recovered from the TAG fraction by transmethylation. As these compounds seem to have persisted through an extended burial time, they must have been present in very high concentrations in the original fuel. As animal fats mainly comprise saturated rather than unsaturated fatty acids, animal fat can be excluded as being the fuel used here. The absence of cholesterol or other animal markers from the GC-MS chromatograms confirms this hypothesis.

Assessment of the stable carbon isotope values of two important fatty acid methyl esters, *viz*. methyl palmitate and methyl stearate, supports the idea that plant rather than animal fat has been burned in these shells. The $\delta^{13}C$ values (‰) are clustered, and comparable to those from C_3 vegetal oils [15,27]. This excludes the possibility that oil from a C_4 plant was used as an illuminant here. The $\delta^{13}C$ values of the

samples are close to those of radish oil; further confirmation of this hypothesis will be given below.

Intact, non-oxidized triglycerides were detected in very small quantities by APCI-LC-MS only in one sample. The recovery of few intact triglycerides, indicates that the lipids have been exposed to hydrolysis and/or oxidation, either during burning of the oil [6], or during years of preservation [12]. The fatty acids on the glycerol backbone display a high degree of unsaturation, in agreement with the vegetal origin of the lipids.

With both GC-MS and LC-MS, a series of lipid oxidation products was detected. The major compound classes detected by GC-MS are C_6-C_{13} diacids and vicinal dihydroxyacids (C_{18}, C_{20} and C_{22}). It is well known that such polar compounds generally are lost from archaeological material due to elution with water, unless they are strongly bound to ceramics [32], or the samples are preserved in unusually dry conditions [6]. The latter situation clearly applied here, as Bawit, at the edge of the Libyan Desert, experiences an arid climate. Particularly 13,14-dihydroxydocosanoic acid and 11,12-dihydroxyeicosanoic acid, derived from erucic acid (C22:1(13)) and gondoic acid (C20:1(11)), respectively, are very clear biomarkers for seed oil from Brassicaceae plants such as radish (*Raphanus sativus*). Similar observations have been made previously for lipids extracted from ceramic Egyptian oil lamps [4,6]. The results are also in line with archaeobotanical findings and written sources. Indeed, radish occurs frequently in documents from the Roman period onwards [2,22]. Around AD 1050, radish and turnip seed oil are the most common vegetal oils used [13]. Although there are identification problems due to the morphological similarities of the seeds of the various Brassicaceae, it appears also from the archaeobotanical record that there was an emphasis on radish especially in Late Roman to early Islamic times [27,8,10,14].

The dihydroxylated C_{18}, C_{20} and C_{22} fatty acids occur as a mixture of *threo* and *erythro* isomers with essentially identical fragmentation patterns. The latter confirm that they are stereoisomers, rather than positional isomers. The most likely intermediates in formation of diols are epoxides. If the epoxidation is due to a free radical process, one may expect formation of *cis* and *trans* configured epoxides from the *cis* double bond in the acyl residue. In a next, stereospecific hydrolysis step, the *cis*-epoxide is opened in an SN2 reaction to form the *threo* isomer, while the *trans*-epoxide yields the *erythro* isomer (Figure 7.8). As the *trans* configuration is

somewhat more stable than the *cis* configuration, it is expected that the *erythro* form prevails over the *threo* form when the oxidation is dominated by free radical chemistry. In the overall transformation of the *cis* fatty acid to the diols, both *syn* and *anti* dihydroxylation occur.

Similar mixed stereoselectivities have previously been observed in archaeological samples, or in deliberately aged Brassicaceae oil [4,6]. However, this does not yet explain the differences among the various dihydroxyacids, and particularly the high stereospecificity encountered for *erythro*-13,14-dihydroxydocosanoic acid. As a hypothesis, we propose that the almost stereospecific oxidation of erucic acid to the *erythro* diol must have occurred at much lower temperatures than the less stereospecific oxidation of the C_{18} and C_{20} unsaturated fatty acids. This hypothesis is based on calculating the *erythro* to *threo* ratios (E:T) via a thermodynamic approach. If the reaction scheme of Figure 7.8 is correct, the E:T ratios reflect the relative stabilities of the *cis* and *trans* epoxide intermediates. At high temperature, the stability difference between the two epoxides is smaller, and this leads to a lower E:T ratio, as demonstrated in Table 7.4.

Such relatively low E:T ratios are observed for the dihydroxylated C_{18} and C_{20} fatty acids. At contrast, if the free radical oxidation proceeds at lower temperature, the larger difference between the energies of the *trans* and *cis* epoxides results in a larger E:T value, *e.g.* of 15 at 300 K. This is exactly the ratio that is observed experimentally in the chromatogram of Figure 7.7.

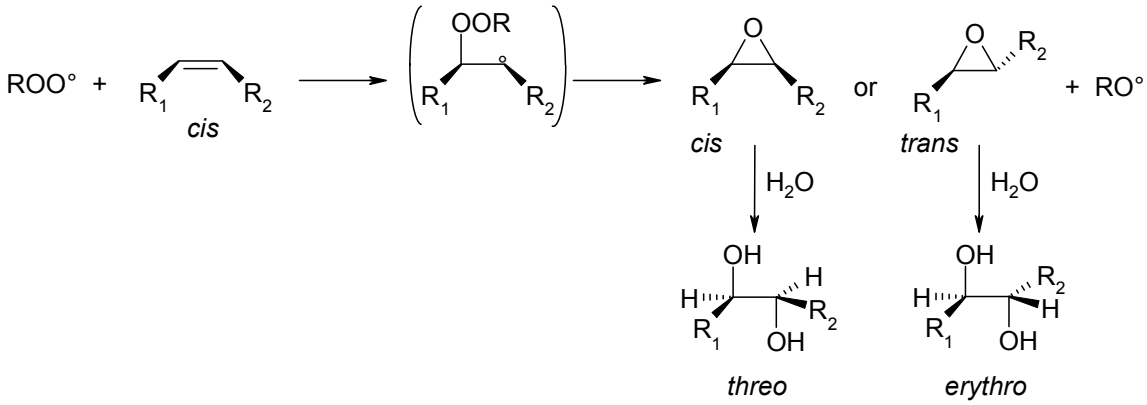


Figure 7.8: Reaction scheme where threo and erythro diol isomers are formed out of a cis double bond together with their epoxide intermediates. Possible oxygen-transferring species ROO° are acylperoxy or alkylperoxy radicals.

Summarizing, the oxidation of C_{18} and C_{20} unsaturated fatty acids seems to have happened at higher temperature than that of the C_{22} compound. In view of the higher melting points and lower vapor pressures of higher molecular weight compounds, it is reasonable to assume that the heaviest oil fractions, containing the C_{22} fatty acids, will gradually be enriched during burning of the oil. This explains the large amount of erucic acid left in the sample, while much smaller amounts of the C_{18} and C_{20} acids were left. The E:T ratios indicate that for C_{18} and C_{20} acids, oxidation must largely have happened during combustion or immediately after it, in the hot oil; the oxidation of the large amount of erucic acid left after quenching of the flame must have taken place in much milder conditions, in the buried, cooled oil and over a much longer period.

In an alternative hypothesis, the formation of *erythro*-13,14-dihydroxydocosanoic acid and related diols might be ascribed to microbial enzymatic processes during burial. Some soil bacteria are known to produce Rieske dioxygenases, which are non-heme mono-iron enzymes that initiate the bio-degradation of unsaturated compounds by *cis*-dihydroxylation [31]. However, *cis*-dihydroxylation of predominantly *cis* fatty acids should yield the *threo* isomers, while the *erythro* isomers are the most abundant in the present samples. So, there is no direct evidence for a microbial origin of diols.

During burial, hydrolysis and oxidation of triglycerides will occur. While unsaturated acyl residues can be detected in the TAG fraction by both PPGC and LC-MS, they are completely absent from the free fatty acid fraction as studied by GC-MS, in agreement with previous studies on Brassicaceae lamp fuel [4,6]. By contrast, significant amounts of the saturated fatty acids are identified by GC-MS, proving that there has been considerable triglyceride hydrolysis. The absence of unsaturated fatty acids demonstrates that once the unsaturated fatty acids are released by hydrolysis from the triglycerides, they are prone to fast oxidation.

Table 7.4: Theoretically calculated percentages of the erythro and the threo form for oxidation via epoxide intermediates.

Temperature (K)	***threo* intermediate (%)**	***erythro* intermediate (%)**	**E:T ratio**
300	6	94	15
400	10	90	9
500	14	86	6
600	18	82	4

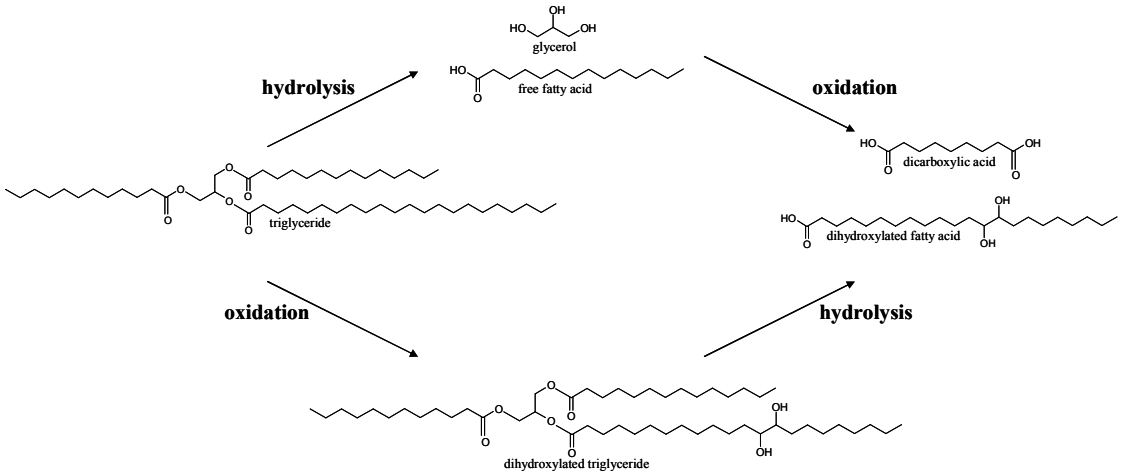


Figure 7.9: Formation of hydroxylated fatty acids from triglycerides via consecutive hydrolysis/oxidation. The identification of hydroxylated triglycerides with LC-MS proves that oxidation took place before hydrolysis.

While the previous data are in line with consecutive triglyceride hydrolysis and oxidation of the unsaturated fatty acids, LC-MS unexpectedly shows that this sequence can also be reversed (Figure 7.9). Indeed, the unambiguous identification of a series of C_{46}-C_{48} triglycerides with 2 or 3 alcohol functions proves that in these molecules, oxidation of the lipid chain must have preceded hydrolysis.

While Brassicaceae oil has been previously identified in samples from Egypt, several features are apparent from the present work. First, the study is likely the first to prove that lipids such as lighting fuel residues can be preserved even in non-porous, i.e. non-ceramic matrices, provided preservation conditions are favorable. Secondly, the use of LC-MS has revealed that unsaturated triglycerides can be degraded not only by hydrolysis followed by oxidation, but that the sequence can be reversed. Finally, the varying isomer ratios in the diol compounds can only be explained by assuming that oxidation can take place in harsh conditions, associated with combustion of the oil, or in mild conditions, after quenching of the flame.

7.9 Conclusion

For the first time, archaeological residues from Egyptian bivalve *Chambardia rubens arcuata* shells, probably used as lamps, are chemically analysed, proving that a ceramic matrix is not necessarily required for the preservation of organic compounds. The distribution of the fatty acid methyl esters indicates that a triglyceride type oil with a high degree of unsaturation, and thus of vegetal origin, was used. The results of the stable carbon isotope analyses on methyl palmitate and methyl stearate, confirm this hypothesis and correspond well to the characteristics of radish seed oil (*Raphanus sativus*). Using liquid chromatography coupled to mass spectrometry, we were able to identify mono, di and trihydroxylated triglycerides, highly oxidized compounds reported to be formed in fried oil. Using a gas chromatograph coupled to a mass spectrometer, oxidation products like α,ω-dicarboxylic acids and dihydroxyacids were recognized. Dihydroxyacids such as 11,12-dihydroxyeicosanoic acid and 13,14-dihydroxydocosanoic acid, are oxidation products of gondoic and erucic acid respectively, which are important fatty acids from seed oil of Brassicaceae plants. Evaluating the ratios between the *threo* and *erythro* isomers of the dihydroxyacids, it became clear that dihydroxylation of the C_{18} and C_{20} acids occurred in conditions different from those of the oxidation of the C_{22} acid.

Combining the results from the four analytical techniques on the eight lamp shells, allows assuming that seed oil from a Brassicaceae plant, in particular from radish (*Raphanus sativus*), was used as an illuminant. This is both a confirmation of the initial assumption that the shells might have been used as lamps and delivers an answer to the question what oil had been used.

7.10 References

1. **Ahrgren L., and Sutin L., 1997**, Sharpless dihydroxylation on an industrial scale in Organic Process Research and Development 1:425-427.

2. **Bagnall R., 1993**, Egypt in *Ancient Antiquity*, Princeton University Press, Princeton.

3. **Belitz H., and Grosch W.,1999**, Food chemistry (second edition), Berlin.

4. **Colombini M, Modugno F., and Ribechini E., 2005**, Organic mass spectrometry in archaeology: evidence for Brassicaceae seed oil in Egyptian ceramic lamps in *Journal of Mass Spectrometry* 40:890-898.

5. **Copley M., Berstan R., Dudd S., Docherty G., Mukherjee A., Straker V., Payne S., and Evershed R., 2003**, Direct chemical evidence for widespread dairying in prehistoric Britain in *Proceedings of the National Academy of Sciences* 100(4):1524-1529.

6. **Copley M., Bland H., Rose P., Horton M., and Evershed R., 2005**, Gas chromatographic, mass spectrometric and stable carbon isotopic investigations of organic residues of plant oils and animal fats employed as illuminants in archaeological lamps from Egypt in *The Analyst* 130:860-871.

7. **Daget J., 1998**, Catalogue raisonné des mollusques bivalves d'eau douce africains, Backhuys Publishers, Leiden/ORSTOM, Paris.

8. **de Vartavan C., and Amoros V., 1997**, Codex of ancient Egyptian plant remains, Triade Exploration, London.

9. **Eerkens J., 2005**, GC-MS analysis and fatty acid ratios of archaeological potsherds from the Western Great Basin of North America in *Archaeometry* 47(1):83-102.

10. **El Hadidi M., and El Fayoumi H., 1997**, Catalogue of the archaeobotanical specimens in Cairo University Herbarium. 1. Abu Sha'ar site, Red Sea coast - Egypt; season 1991 in *Taeckholmia* 17:47-60.

11. **Evershed R., Berstan R., Grew F., Copley M., Charmant A., Barham E., Mottram H., and Brown G., 2004**, Formulation of a Roman cosmetic in *Nature* 432: 35-36.

12. **Evershed R., Heron C., Charters S., and Goad L., 1992**, The survival of food residues: new methods of analysis, interpretation and application in *Proceedings of the British Academy* 77:187-208.

13. **Gil M., 1975**, Supplies of oil in medieval Egypt: a Geniza study in *Near Eastern Studies* 34:63-73.

14. **Hopf M., and Germer R., 1998**, Pflanzliche Reste aus Nag'el-Scheima in M. Bietak and M. Schwarz Nag' el-Scheima. Eine befestigte christliche Siedlung und andere christliche Denkmähler in Sayala - Nubien. Ägyptischen Kommission der Österreichischen Akademie der Wissenschaften. *Berichte des Österreichischen Nationalkomitees der UNESCO-Aktion für die Rettung der nubischen* Altertümer 9, Wien, 545-554, pl 2-3.

15. **Kelly S., Parker I., Sharman M., Dennis J., and Goodall I., 1997**, Assessing the authenticity of single seed vegetable oils using fatty acid stable carbon isotope ratios in *Food Chemistry* 59:181-186.

16. **Kimpe K., 2003**, Chemical analysis of the lipid fraction from ancient ceramics of Sagalassos, PhD Thesis, Katholieke Universiteit Leuven.

17. **Kimpe K., Jacobs P., and Waelkens M., 2001**, Analysis of oil used in Late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat in *Journal of Chromatography A* 937:87-95

18. **Kimpe K., Jacobs P., and Waelkens M., 2002**, Mass spectrometric methods prove the use of beeswax and ruminant fat in Late Roman cooking pots in *Journal of Chromatography A* 968:151-160.

19. **Lyon-Caen C (in press)** Les lampes de Baouit, in C. Louis (ed.), Études coptes (Actes de la XIIIe Journée d'études coptes à Marseille, 7-9 juin 2007), *Cahiers de la Bibliothèque copte.*

20. **Malainey M., Przybylski R., and Sherriff B., 1999**, The effects of thermal and oxidative degradation on the fatty acid composition of food plants and animals of Western Canada: implications for the identification of archaeological vessel residues in *Journal of Archaeological Science* 26:95-103.

21. **Mathieu B., 2004**, Travaux de l'Institut français d'archéologie orientale en 2003-2004 in *BIFAO* 104 :671-673.

22. **Mayersons Ph., 2001**, Radish Oil: A Phenomenon in Roman Egypt in *Bulletin of the American Society of Papyrologists* 38:109-117.

23. **Mossakowska M., 1994**, Les huiles utilisées pour l'éclairage en Égypte (d'après les papyrus grecs) in *The Journal of Juristic Papyrology* 24 :109-131.

24. **Mottram H., Crossman Z., and Evershed R., 2001**, Regiospecific characterization of the triacylglycerols in animal fats using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry in *The Analyst* 126:1018-1024.

25. **Mottram H., Dudd S., Lawrence G., Stott A., and Evershed R., 1999**, New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animals fats preserved in archaeological pottery in *Journal of Chromatography A 8*33:209-221.

26. **Nicholson P., and Shaw I. (eds), 2000**, Ancient Egyptian materials and technology, University of Cambridge Press, Cambridge.

27. **O'Donoghue K., Clapham A., Evershed R., and Brown T., 1996**, Remarkable preservation of biomolecules in ancient radish seeds in *Proceedings Royal Society London B* 263:541-547.

28. **Pantalacci L., 2005**, Travaux de l'Institut français d'archéologie orientale en 2004-2005 in *BIFAO* 105:440-443.

29. **Pantalacci L. 2006**, Travaux de l'Institut français d'archéologie orientale en 2005-2006, *BIFAO* 106:365-369.

30. **Passi S., Rothschild-Boros M.C., Fasella P., Nazzaro-Porro M., and Whitehouse D., 1981**, An application of high performance liquid chromatography to analysis of lipids in archaeological samples in *Journal of Lipid Research* 22: 778-784.

31. **Que L., Feng Y., and Ke C., 2007**, in Abstract of Papers, 234th ACS National Meeting, Boston, MA, United States.

32. **Regert M., Bland H., Dudd S., van Bergen P., and Evershed R., 1998**, Free and bound fatty acid oxidation products in archaeological ceramic vessels in *Proceedings Royal Society London* 265:2027-2032.

33. **Sage R., and Monson R., 1999**, C4 plant biology, Academic Press, New York

34. **Tieszen L., 1991**, Natural variations in the carbon isotope values of plants: implications for archaeology, ecology and paleoecology in *Journal of Archaeological Science* 18:227-248.

35. **Woodbury S., Evershed R., and Rossell J., 1998**, δ13C analyses of vegetable oil fatty acid components, determined by gas chromatography-combustion-isotope ratio mass spectrometry, after saponification or regiospecific hydrolysis in *Journal of Chromatography A* 805:249-257.

8 An excavated 16th century ointment identified as lead plaster mixed with beeswax

8.1 Introduction

In exceptional cases archaeologists have the opportunity to excavate an ancient vessel still containing its original content. For example, Egyptian make-up powders, dating from between 2000 and 3000 BC, were recovered from their original containers. Analysis showed that they contained apart from natural minerals also synthetic compounds. In this way, it was proven that the Egyptians knew the practice of wet chemistry already from 2000 BC onwards [14,18]. Another rare archaeological find was a small tin canister in a Roman temple precinct in London containing a white cream dated to the middle of the 2th century AD. Evershed *et al.* [10] found that ruminant fat had been mixed with starch and SnO_2 to prepare a cosmetic balm. The reconstruction of this formulation led to a white cream having a pleasant texture when rubbed into the skin. Probably Roman women used this emulsion as a foundation layer. Using synchrotron Fourier transform infrared spectroscopy, Cotte and coworkers [3] analysed a 33 centuries old Egyptian cosmetic preserved in a reed container. They could observe that the core of the particles consisted of lead soaps, while phosgenite ($Pb_2CO_3Cl_2$) was discovered at their surface. As they could not prove whether the production of lead soaps occurred during the production of the cosmetic of afterwards during preservation, they set up a kinetic study of oil saponification by lead salts [4], with the conclusion that the reaction can be significantly accelerated by heating and adding water. Very recently the content was analysed for 44 samples, found in the barber-surgeon's cabin during the under-water excavations of the wreck of the Mary-Rose, King Henry VIII's prestigious battle-ship which sunk in 1570. Most of the ointment samples contained pine resins or a rosin oil where some of them were diluted with beeswax. A few samples were mixed with

inorganic material like sulphur, zinc, lead compounds. Another group of ointments consisted out of animal fat blended with a lead-based compound [7,9].
A substantial amount of ancient greasy material preserved in its original ceramic container was excavated in the Castle of Middelburg, close to the Belgian-Dutch border. The time of discard was estimated around 1550-1600 AD, thus in late medieval times. A first archaeological question was to elucidate the initial function of the fatty material, more precisely to find out if the ointment was intended for cosmetic or medicinal purpose, for instance in treating wounded soldiers. Another point of interest is to reveal its formulation and way of preparation.

8.2 The sample and its find context

A substantial amount of ancient greasy material preserved in its original ceramic container was found during the excavations at the Castle of Middelburg (Maldegem, province of East-Flanders, Belgium) at the border with The Netherlands. The castle and the city of Middelburg (nowadays a small border-village) were founded in 1448 by the governor-general of finances of the Duke of Burgundy, Pieter Bladelin. He built the castle and city to express his elite status [5]. After the death of Bladelin and of his successor William Hugonet, chancellor of Burgundy, the city and castle lost their top-eliterian status. From 1560 onwards, it further degenerated as a consequence of military activities. Because of its short-lived, though turbulent and important history, and because of the very good archaeological preservation, the site was protected as the first Flemish Archaeological Monument by the Flemish government.
The container with the preserved content was found at the base of the bottom layer of the castle moat at 2.50 m below actual surface (ca. 10.5 m below actual sea-level) in permanent waterlogged conditions. The matrix of the sediment in which it was buried consisted of highly organic clayish mud of ca. 50 cm thick, containing many other archaeological objects dating from the late 16th and early 17th century. This layer was sealed by ca. 2 m of debris, consisting mainly of brick, shale, limestone and mortar from the castle, dumped in the moat during the destruction of the site (ca. 1750).
The container in which the sample was found is typologically known as a “gallipot” or “albarello”, a ceramic vessel form which was originally used for containing salves

or ointments. Typo-chronologically and based on the find context, the form can be dated in the second half of the 16th, early 17th century. At that time, the castle of Middelburg had lost its eliterian Burgundian status and had become an important military anchor-point in the Spanish-Dutch Wars, or Eighty-Years War, in which the Catholic Armies of the Spanish King Philip II fought with the Dutch Protestant Armies of the Van Nassau dynasty. Two gardeobe-chutes of the castle, filled up ca. 1600, contained a large amount of culture and paleo-ecological remains of military origin and even revealed an exceptionally large amount of gallipots, identical in form and fabric to the one from which the sample described originates. This unusual find was interpreted as the material proof of the presence of a military-surgeon cabinet at the castle during the late 16th century [6]. It is indeed to be expected that armies engaged in constant battle brought along their own surgeons and encamped them with the troops at the headquarters.

8.3 Results

8.3.1 Organic analyses

An average of 24 ± 3 % lipids on weight basis was extracted from the ointment, indicating that a high amount of fatty material was present in the sample.

GC-MS analysis of the silylated lipid fraction of the ointment revealed a range of even and odd numbered free fatty acids containing nine to twenty-six carbon atoms, with a dominant peak for palmitic acid (Figure 8.1). Next to fatty acids, a range of n-alkanes with carbon numbers between 27 and 34 was identified with the highest concentrations for heptacosane, nonacosane and hentriacontane. Alkanols with carbon numbers between 24 and 32 were present in important amounts with the highest concentration for triacontanol and dotriacontanol. The final portion of a chromatogram from a sufficiently concentrated sample showed the presence of wax esters, namely mono-esters from 40 to 44 carbon atoms with the highest amount for the mono-ester with 42 carbon atoms. Nowhere in the chromatogram was any type of sterols detected. As palmitic acid is an important hydrolysis product of beeswax, all

these observations together are diagnostic for the presence of beeswax in the ointment [16,17]. Diols and hydroxypalmitic acid are also hydrolysis products of beeswax but because of their high polarity, they washed out during burial and consequently are no longer visible in the chromatogram.

Isomers of octadecenoic acid and 9,10-dihydroxyoctadecanoic acid were also found, the latter known to be an oxidation product of fatty acids with a double bond at position nine [1]. These compounds do not originate from beeswax, indicating that an additional greasy ingredient had been mixed in the ointment. The origin of this ingredient has to be assessed by the following lipid analyses techniques. The presence of unsaturated fatty acids with a trans-configuration is characteristic for microbial activity. The latter can be indicative for mixing ruminant fat in the salve or for bacterial spoilage after its use [19].

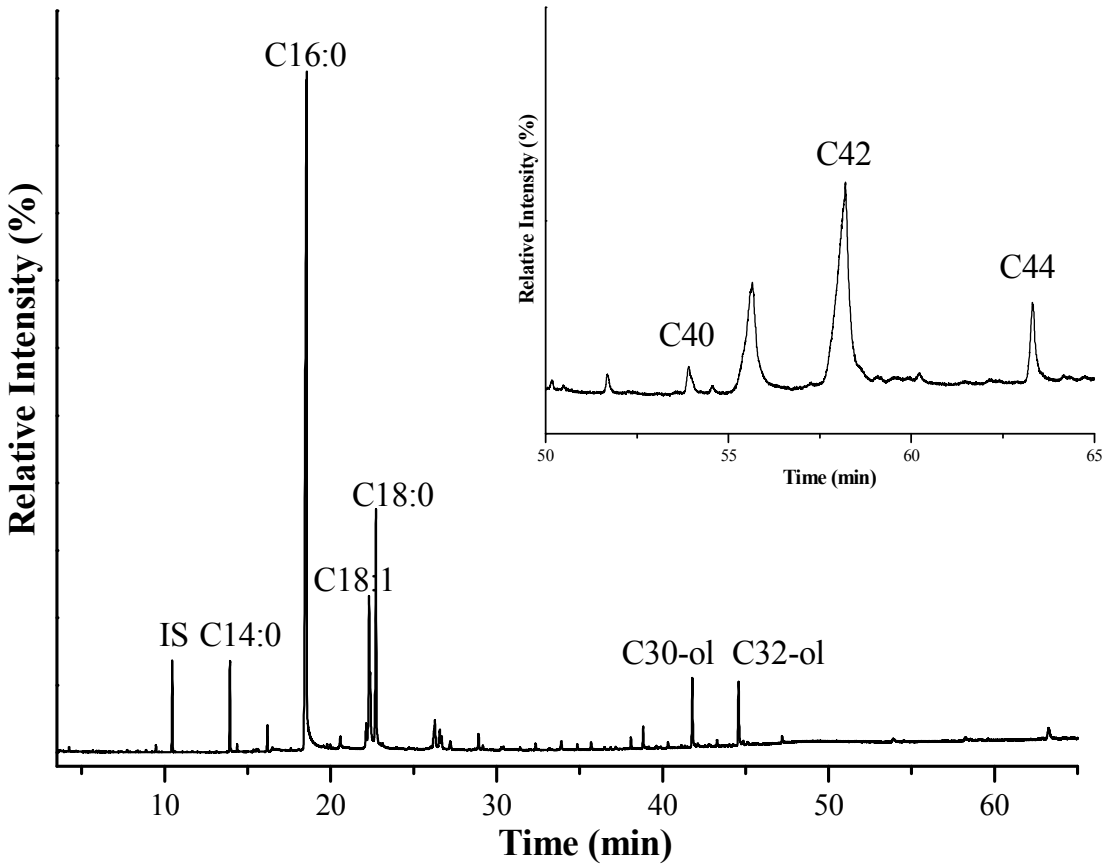


Figure 8.1: Reconstructed GC-MS chromatogram of the silylated total lipid extract injected with a split ratio of 1:50. All molecules with active hydrogen atoms are present in their trimethylsilylated form. The internal standard is marked by symbol IS, free fatty acids are marked by Cn:m with n the number of carbon atoms and m the number of double bonds and alkanols are symbolized by Cn-ol with n the number of carbon atoms. The inset represents a detail (50-65 min) of a more concentrated sample injected with splitless mode where Cn stands for a monoester with n being the carbon number.

The following graphs represent fatty acid methyl ester profiles for the acylglyceride (AG) fraction ($FAME_{AG}$) (Figure 8.2) and for the free fatty acid (FA) plus AG fraction ($FAME_{AG+FA}$) (Figure 8.3). An important remark is the disability to produce FAME out of wax ester by means of the transesterification method established here. This implicates that while evaluating the AG fraction, we are not looking at beeswax. Of course the free fatty acids are hydrolysis products from beeswax and also from the additional ingredient. The amount of free fatty acids is significantly higher than the amount of fatty acids sequestered in acylglycerides. This observation indicates that major degradation occurred due to the fabrication process of the ointment or afterwards during burial. The same free fatty acids as in the GC-MS chromatogram were identified. Also the presence of free fatty acids with a double bound in the trans-configuration is confirmed. Because of their absence in the acylglyceride fraction, they are presumably from microbial origin. Mould growth was observed on the ointment, which might have been responsible for these particular fatty acids.

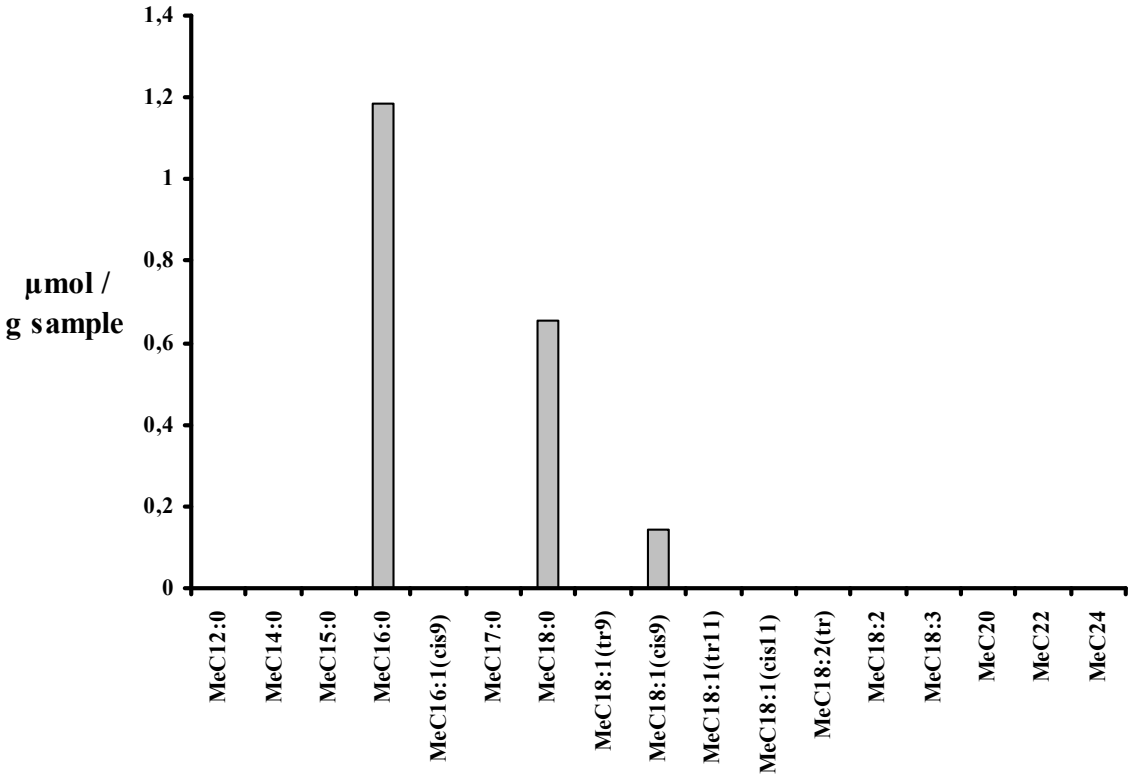


Figure 8.2: Fatty acid methyl ester profile of the triglyceride fraction ($FAME_{AG}$). The quantities of the obtained methyl esters are expressed in µmol / g sample.

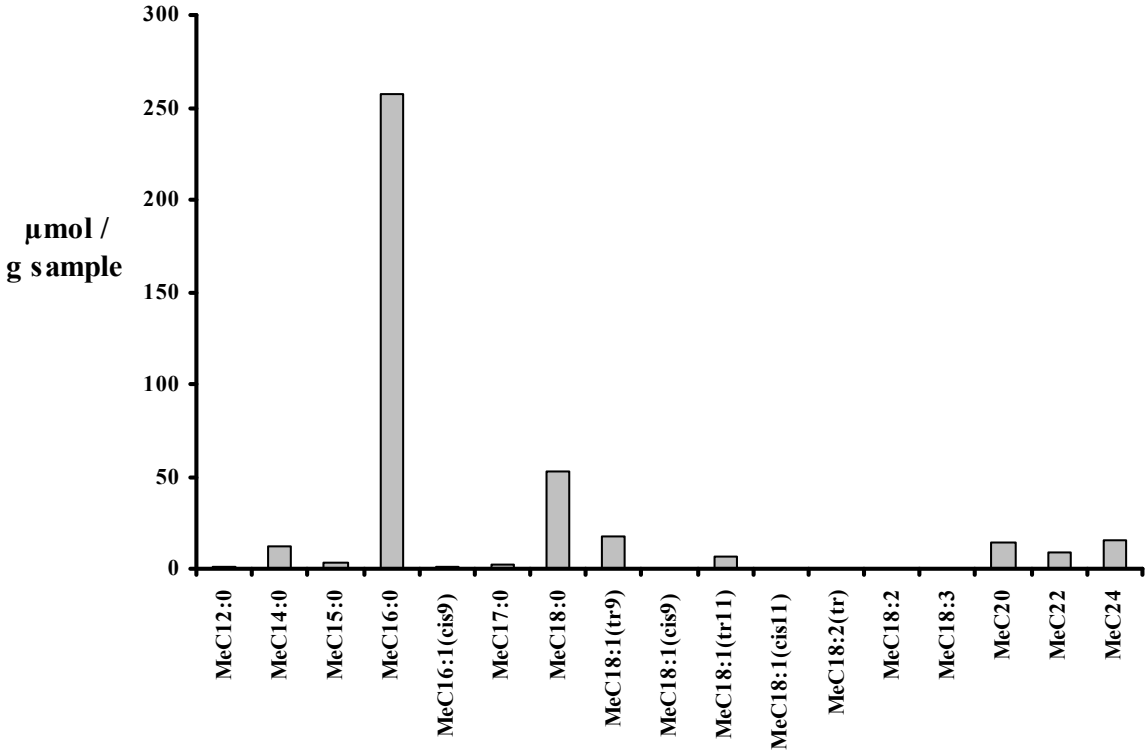


Figure 8.3: Fatty acid methyl ester profile of the triglyceride plus free fatty acid fraction ($FAME_{AG+FA}$). The quantities of the obtained methyl esters are expressed in µmol / g sample.

In order to recognize the additional ingredient next to beeswax, it was necessary to investigate the acylglyceride fraction. Detection of oleic acid in this fraction points in the direction of vegetal oil and also its palmitate to stearate ratio (P/S), being 1.4, does not support the assumption that a ruminant fat was mixed with beeswax (see chapter 3). The high concentration of palmitic acid in the sample causes a shift in the P/S ratio of $FAME_{AG+FA}$ to 3.9. This shift is due to palmitic acid being the main hydrolysis product of wax esters from beeswax [9].

Figure 8.4 shows the results of the stable carbon isotope analysis of the fatty acid methyl esters from the ointment sample. There is a clear shift in the $\delta^{13}C_{16:0}$ values when comparing the results for the two fractions namely $FAME_{AG}$ (■) (-28.5; -29.8) (in ‰) next to $FAME_{AG+FA}$ (●) (-27.5; -29.8) (in ‰). This difference can be attributed to the high amount of free palmitic acid coming from beeswax esters. Furthermore, it confirms that the origin of the acylglycerides is from an additional ingredient in the salve.

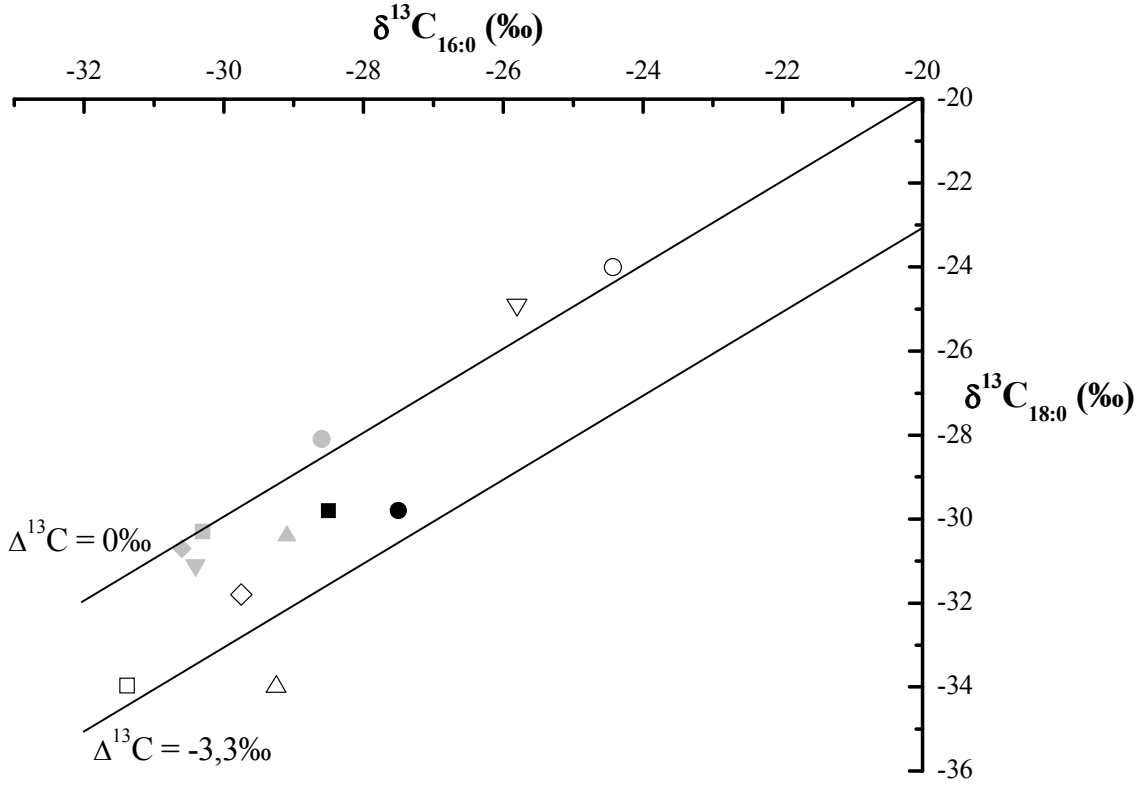


Figure 8.4: $\delta^{13}C_{18:0}$ vs. $\delta^{13}C_{16:0}$ plot (expressed in ‰) with ■ and ● representing samples $FAME_{AG}$ and $FAME_{AG+FA}$ respectively. The other symbols stand for sheep (□), cattle raised on a C_3 plants diet (◇), poultry (○), porcine (▽), dairy fats from cows raised on a C_3 plants diet (△), palm oil (▼), groundnut oil (▲), sunflower oil (♦), rapeseed oil (■) and radish seed oil (●). All values were obtained with modern tissue. Reference values for adipose tissue (-29.8; -31.9) and milk (-29.2; -34.0) from C_3-fed cattle have been taken from literature.

The obtained data points fall in the range of ruminant adipose fat. This conclusion would be remarkable as GC-MS analysis did not reveal the presence of cholesterol, a biomarker for animal fat. On the other hand, the delta values (in ‰) of the ointment fall in the area of a few modern vegetal oils (Figure 8.4). The latter seems more reasonable as it nicely corresponds to the results for the other techniques where oleic acid and its oxidation products were detected.

Analysing the archaeological salve on a liquid chromatograph with mass spectrometer (LC-MS) allows to separate and identify the high molecular weight lipid compounds. This method was applied to search for intact triglycerides and to confirm the presence of wax esters that were recognized on GC-MS.

The majority of the chromatogram consisted of wax esters and this confirmed the hypothesis that beeswax was the main ingredient of the salve (Figure 8.5). Wax esters

in beeswax typically contain palmitic acid esterified with a carbon chain of varying carbon number [17,19]. This also explains the high amount of free palmitic acid and long-chain alcohols in the sample due to hydrolysis phenomena [9]. Long-chain wax esters exhibit a characteristic mass spectrum in a mass spectrometer with ion trap used in the atmospheric pressure chemical ionisation (APCI) mode (Figure 8.5). The protonated molecule mass ($[MH]^+$) appears together with fragments that have lost water ($[MH\text{-}H_2O]^+$) or palmitic acid ($[MH\text{-}RCOOH]^+$) (Table 8.1). Although visible in the GC-MS chromatogram, no mono-esters were detected by LC-MS. This can be explained by a very fast elution. Indeed, the hydroxymono-esters have retention times of only 5.7 minutes.

A few peaks of wax esters showed co-elution with a small amount of triglycerides. For example, C58 hydroxydiester co-elutes with tripalmitin (PPP) and palmityl-stearyl-myristyl glycerol (PSM) while C60 hydroxydiester co-elutes with dipalmityl-stearyl glycerol (PPS) and C62 hydroxydiester with distearyl-palmityl glycerol (PSS). Because of possible overlap, it cannot be excluded that triglycerides with an oleate moiety are present although not visible. The presence of intact triglycerides points again in the direction of mixing beeswax with an additional oil or fat in the ointment. All indications suggest that the formulation consists out of beeswax mixed with vegetal oil.

Table 8.1: Summary of the retention times in minutes (RT) of the wax esters detected in the ointment together with their characteristic mass fragments (expressed in *m/z*) obtained with a LC-MS.

RT	**Wax esters**	**$[MH]^+$**	**$[MH\text{-}RCOOH]^+$**	**$[MH\text{-}H_2O]^+$**
5.7	C44 hydroxymonoester	665.4		647.5
7.2	C46 hydroxymonoester	693.5		675.9
7.6	C56 hydroxydiester	863.6	591.5	
8.9	C48 hydroxymonoester	721.5		703.7
9.8	C58 hydroxydiester	891.5	619.5	
12.2	C60 hydroxydiester	919.5	647.5	
15.4	C62 hydroxydiester	947.6	675.6	
19.6	C64 hydroxydiester	975.5	703.4	
24.2	C56 diester	847.5	591.4	
30.9	C58 diester	875.5	619.5	
39.4	C60 diester	903.4	647.5	

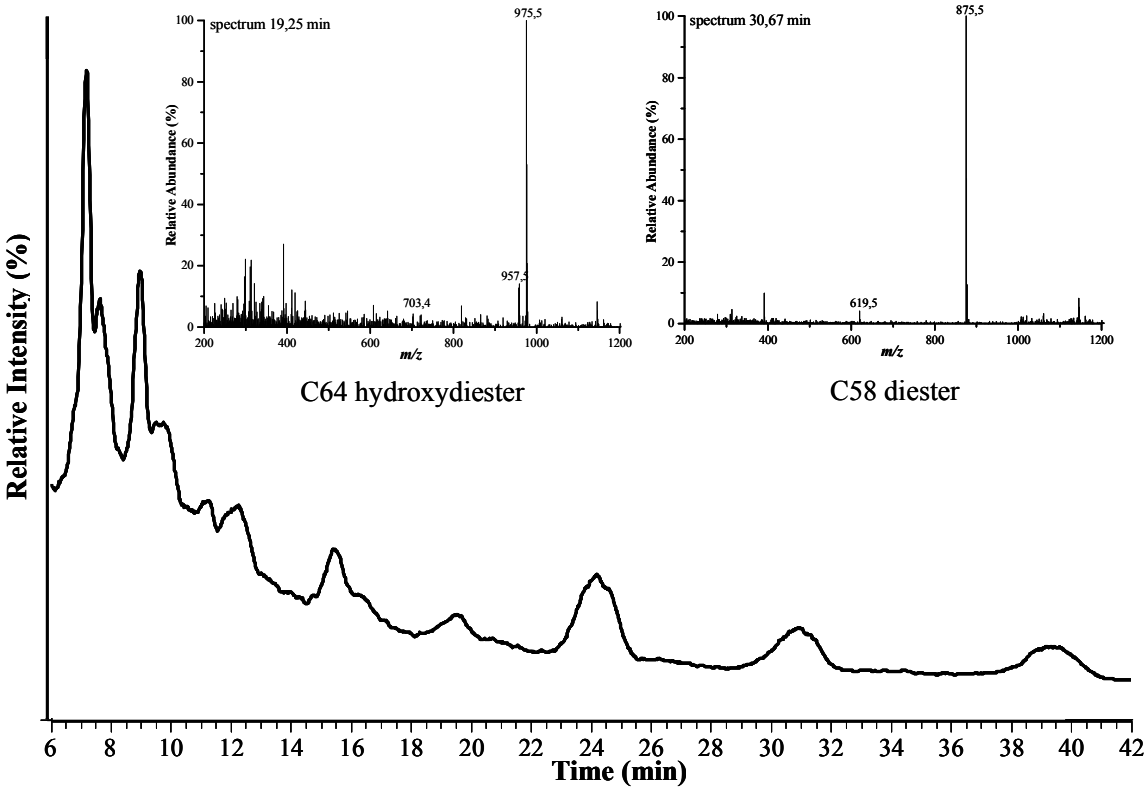


Figure 8.5: Reconstructed partial chromatogram of the ointment acquired by a liquid chromatograph with mass spectrometer with insets of the mass spectrum of C64 hydroxydiester taken at 19.25 min and the mass spectrum of C58 diester taken at 30.67 min.

Attenuated-total-reflectance Fourier transform infrared spectra (ATR-FTIR) showed the presence of lead soaps as indicated by carbonyl bands at 1539 and 1517 cm^{-1}, suggesting a small amount of lead oxide was also incorporated in the material and reacted with free fatty acids within the matrix [3,4].

8.3.2 Inorganic analyses

A known amount of the ointment (0.0215 g) was characterized with thermogravimetric analysis (TGA) on a TA Instruments Q500 under flowing O_2, programmed at 25 °C, followed by an increase to 900 °C at 10 °C/min. Performing this analysis, it was proven the sample contained about 24.6 % inorganic material.

The mineralogy of any inorganic crystalline fraction in the ointment was identified through X-ray diffractometry (XRD). A small amount of sample was mounted on a graphite plate. Operational parameters of the measurement were: Cu Kα radiation,

graphite monochromator, 45 kV, 30 mA, automatic divergence slit, and receiving slit of 0.1°. The X-ray diffractogram of the ointment revealed the presence of calcium sulfate (gypsum) and lead sulfate as main inorganic crystalline components in the sample. A high background in the measurement can be observed in the diffractogram, due to the presence of large quantities of non-crystalline material in the sample studied. This high background is likely to conceal any other mineral components added to the mixture. The detection limit of the XRD technique under the circumstances of this study lies around 3 % of the bulk material.

Elemental analysis by an electrothermal-vaporization inductively coupled plasma time-of flight mass spectrometer (ETV-ICP-TOFMS) was performed on a GCI's GBC 9500 Optimass. Also X-ray fluorescence (XRF) was applied to the sample, using a GCI's Keymaster X-ray fluorescence spectrometer with a Re tube at 40 kV and 1 μA for a 60 s accumulation. Elemental analysis by ETV-ICP-TOFMS and XRF analysis revealed Si and Ca as major components respectively ~80 and ~20 %. Both methods exposed lead and iron as minor elements and many trace elements like Na, Al, Mn, Ni, Cu. The minor and trace elements have concentrations below 1 % per weight.

The X-ray photoelectron spectroscopic (XPS) measurements were performed on the residue of a Soxhlet extracted sample, meaning that mainly inorganic material was measured here. XPS analyses were performed on the residue of the ointment after lipid extraction, using a Perkin Elmer PHI ESCA 5500 system with a monochromatic 450 Watt AlKα source equipped with a Multipak 6.1A program. To survey the complete range of binding energies in the sample, an analysis at low resolution was performed from 0 to 1400 eV during 120 minutes at 220 Watt. For more detailed knowledge of the binding energies of C1s, O1s, S2p and Pb4f, analyses were performed during 21, 21, 108 and 54 min respectively. The homogeneity of the sample was checked by establishing four measurements. In the range of the bindings energies from 250 to 750 eV, intense peaks for O and C are present next to clear peaks for Ca and N and small peaks for Fe and Pb. In the area of the lowest binding energies, lines for S, Si, Pb and Ca could be observed next to Fe and Al in lower amounts. The average of four atomic concentration measurements is summarized in Table 8.2. Even if all organic molecules had been extracted out of the sample, apparently high amounts of carbon and oxygen stayed behind.

Detailed examination of the peak positions of Pb4f, S2p and C1s, revealed for Pb4f a peak at ~138.5 eV and at ~143.4 eV which may correspond to leadoxide (PbO),

leadsulfide or leadsulfate (Figure 8.6). For S2p two separate peaks were found at ~162 and ~170 eV with different intensities respectively 30 % and 70 %. The peak at ~162 eV can be ascribed to sulfides for example PbS while the peak at ~170 eV most probably indicates the presence of sulfates. Next to the reference value for C1s, being 284.6 eV, a small peak at ~287.5 eV is present, corresponding to a carbon oxygen bond (one double or two single bonds).

Table 8.2: The average together with standard deviation of four concentration measurements with XPS of the main elements in the salve expressed in at %.

	C1s	**O1s**	**Ca2p**	**S2p**	**N1s**	**Si2p**	**Pb4f**	**Fe2p3**	**Al2p**
Atomic concentration (%)	72.15	20.82	3.06	1.31	1.10	1.01	0.21	0.17	0.16
Standard deviation	1.62	1.07	0.20	0.24	0.02	0.43	0.08	0.03	0.06

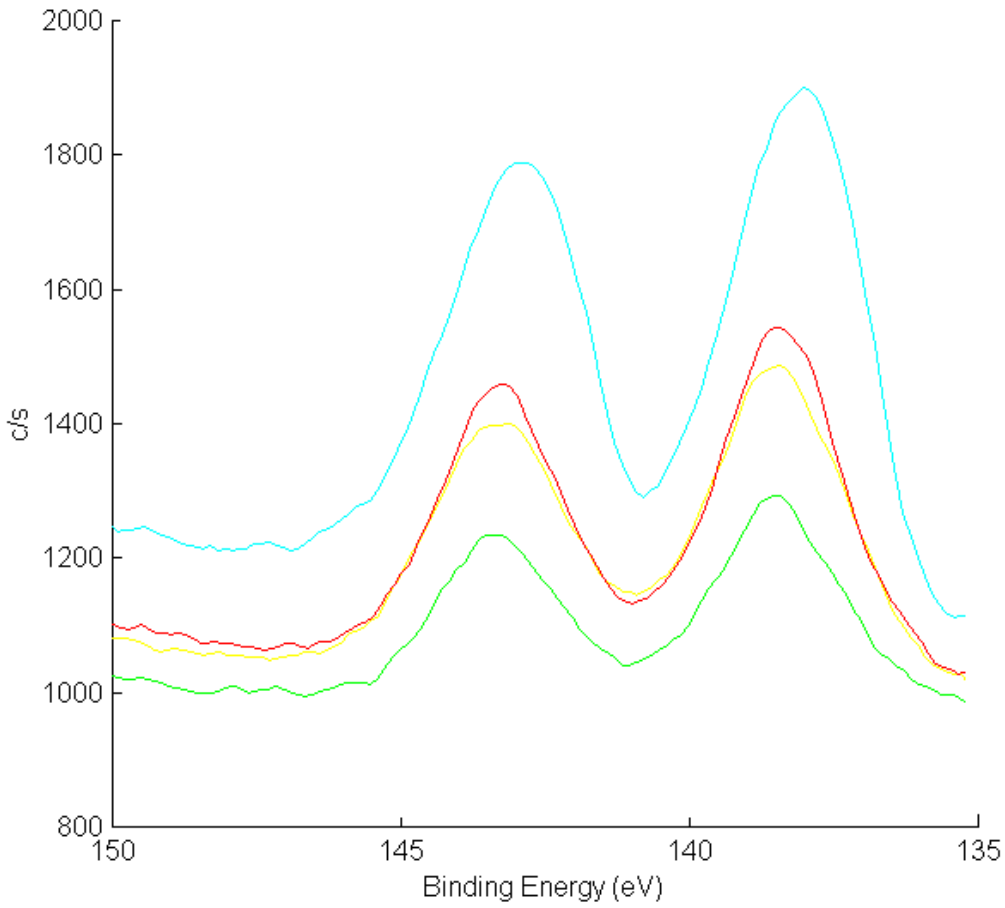


Figure 8.6: Scheme with binding energies for Pb4f acquired by XPS showing peaks at 138.5 eV and 143.4 eV.

To evaluate the presence of starch, 0.5 g ointment was subjected to a colorimetric reaction with 1 mL of an iodine-KI solution (0.05 mol/l). As no colorimetric reaction could be observed, no starch seemed to be present in the sample. This was to be expected as the ointment was found in a waterway and starch consists out of easily hydrolysable carbohydrate polymers.
To evaluate the presence of peptide bonds and thus proteins, 0.5 g ointment was subjected to a Biuret reaction by addition of 2 mL of a solution containing 50 parts of reagent A (sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) and 1 part of reagent B (4 % cupric sulfate)(BCA^{TM} protein Assay Kit, Pierce). The Biuret test on the ointment exhibited a deep purple color proving the presence of a significant amount of proteins in the sample. This explains the presence of nitrogen, measured by XPS.

8.4 Discussion

A high amount of palmitic acid next to alkanes, alcohols and wax esters in the lipid fraction are diagnostic for beeswax used in the formulation of the ointment. Triglyceride and fatty acid methyl ester analyses proved that an additional ingredient was mixed in the salve. Indeed, a significant amount of oleic acid was found in the methylated triglyceride fraction, pointing in the direction of vegetal oil. Also, an oxidation product of oleic acid, being an important ingredient of vegetal oils, namely 9,10 dihydroxyoctadecanoate was found. As a part of the oleic acid fraction is captured in the triglyceride structure, the remainder probably was oxidized exhausting free oleate. In addition, the isotopic delta values also fall in the area of vegetal oils. ATR-FTIR measurement revealed the presence of lead soaps. This means lead compounds in the ointment reacted with fatty acids from vegetal oil. Whether this happened during preparation of the salve or during burial is not distinguishable. In conclusion, we can hypothesize that the salve contains a very high amount of beeswax next to lower amounts of vegetal oil. There is evidence that the beeswax was never subjected to a heating process as no sublimation of the beeswax alkanes occurred [17]. Nevertheless, extensive hydrolysis of the beeswax compounds and triglycerides happened during preparation or burial of the ointment. There were no traces of starch

present in the sample. On the contrary, a Biuret test showed a high amount of proteins enclosed in the ointment.

Analysing the inorganic fraction of the sample by a variety of techniques, elemental analyses revealed the presence of Ca and Si next to Pb and also trace elements like Fe, Al, Na, Mn, Cu and Ni were detected in very low concentrations. Traces of Si, Fe and Al may be due to dissolution of residual clay material from the soil around the ceramic vessel. In order to characterize the configuration of some chemical elements present in the sample, the ointment was examined by XPS. These analyses showed that lead was present as leadoxide, leadsulfide or leadsulfate. Only leadsulfate was detected in XRD, next to gypsum, explaining the presence of higher amounts in Ca. Whether gypsum was added as a separate component is unclear, but this component may have been used as a whitener or as a specific compound of the medicinal mixture. Gypsum and lead in different forms (litharge, cerusa, burnt lead, lead plates…) are described as basic components necessary for medicinal preparations for treatment of several diseases (e.g. the Simples of John de Vigo or Compounds of de Vigo and Brunschwig, two lists describing essentials in medicine in the early sixteenth century).

A reasonable hypothesis for the functionality of this salve is that it was intended for use as a medicinal plaster. Indeed, checking the assembly for endoplastrum simplex in a pharmaceutical guide shows that PbO, together with other lead compounds and heavy metals, was mixed with medicinal oil, a blend called lithargyrum. The latter was mixed with water and needed to be heated in a copper vessel inducing a saponification reaction forming lead soaps and glycerol [8]. This formulation explains nicely the appearance of lead soaps, the traces of vegetal oil and the range of trace elements in the ointment analysed here. The heating process also explains the absence of sterols in the ointment. Lead plasters were recommended by barber-surgeon Thomas Gale in his book from 1563. They were used to treat severe bruising and contusions next to treating external problems such as skin infections, ulcers and burns [7]. One manuscript about therapeutics, dated 1562, written by Italian friars also mentioned ointments to cure leg sores. They were all based on zinc oxide, lead carbonate or lead oxide mixed with wax or vegetal oil [15]. Probably the beeswax was added to the lead plaster to enhance its application and maybe also to hydrate the skin during treatment. Remarkably, a sample of a medicinal plaster found in the Mary Rose shipwreck (sample 12, 80A1538) is very close in composition to the ointment

described in this study. It is composed of a mixture of beeswax, plant oil (likely poppy seed) and lead although no gypsum component is described [7]. Finding such medicine in a military context is therefore not unusual. In its application, the ointment was melted, applied and then allowed to set forming a protective dressing also suitable for open wounds [7]. Maybe gypsum was added for whitening or to fortify the initial plaster. The exceptional find of a ceramic vessel still filled with a significant amount of ointment made it possible to try a range of chemical techniques to find the true origin of the preparation. Such results were never published before and this study elucidates a piece of history behind medicinal practices in Late-Medieval to Early-Modern Times.

8.5 References

1. **Colombini, M.P, Modugno, F., and Ribechini, E., 2005**, Organic mass spectrometry in archaeology: evidence for Brassicaceae seed oil in Egyptian ceramic lamps in *Journal of Mass Spectrometry* 40:890-898.

2. **Copley M., Berstan R., Straker V., Payne S., and Evershed R., 2005**, Dairying in antiquity. II. Evidence from absorbed lipid residues dating to the British Iron Age in *Journal of Archaeological Science* 32: 505-521.

3. **Cotte, M., Dumas, P., Richard, G., Breniaux, R., and Walter, Ph., 2005**, New insight on ancient cosmetic preparation by synchrotron-based infrared microscopy in *Analytica Chimica Acta* 553: 105-110.

4. **Cotte, M., Checroun, E., Susini, J., Dumas, P., Tchoreloff, P., Besnard, M., and Walter, Ph., 2006**, Kinetics of oil saponification by lead salts in ancient preparations of pharmaceutical lead plasters and painting lead mediums in *Talanta* 70: 1136-1142.

5. **De Clercq, W., Dumolyn, J., and Haemers, J., 2007**, Vivre Noblement. The material and immaterial construction of Elite-Identity in Late-Medieval Flanders in *Journal for Interdisciplinary History* 38: 1-31.

6. **De Clercq, W., Caluwe, D., Cooremans, B., Deforce, K., De Buyser, F., De Groote, K., Ervynck, A., Lentacker, A., Mortier, S., Pype, P., Van Neer, W., Vandenberghe, S., and Wouters, H., 2007**, Living in times of War. Waste of ca. 1600 from two garderobe-chutes from the Castle of Middelburg-in-Flanders (Belgium) in *Post-Medieval Archaeology* 41-1:1-63.

7. **Derham, B., 2005**, Analysing the barber-burgeon's medicines and ointments in *The Archaeology of the Mary Rose Volume 4; Before the Mast: Life and Death Abroad the Mary Rose edited by Gardener J.*

8. **Dequeker, R., 1954**, De artsenijvormen van de Belgische Pharmacopee IV.

9. **Edwards, H., Sibley, M., Derham, B., and Heron, C., 2004**, Raman spectroscopy of archaeological samples from the Barber-Surgeon's medicine chest on the Mary Rose in *Journal of Raman Spectroscopy* 35: 746-753.

10. **Evershed, R.P., Berstan, R., Grew, F., Copley. M.S., Charmant, A., Barham, E., Mottram, H., and Brown, G., 2004**, Formulation of a Roman cosmetic in *Nature* 432:35-36.

11. **Garnier, N., Cren-Olive, C., Rolando, C., and Regert, M., 2002**, Characterization of archaeological beeswax by electron ionisation and electrospray ionisation mass spectrometry in *Analytical Chemistry* 74: 4868-4877.

12. **Kelly S., Parker I., Sharman M., Dennis J., and Goodall I., 1997**, Assessing the authenticity of single seed vegetable oils using fatty acid stable carbon isotope ratios in *Food Chemistry* 59:181-186.

13. **O'Donoghue K., Clapham A., Evershed R., and Brown T., 1996**, Remarkable preservation of biomolecules in ancient radish seeds in *Proceedings Royal Society London B* 263:541-547.

14. **Martinetto, P, Anne, M, Dooryhée, E, Drakopoulos, M, Dubus, M, Salomon, J, Simionovic,i A, and Walter, Ph., 2001**, Nuclear Instruments and Methods in *Physics Research B* 181: 744-748.

15. **Norton, S., 2003**, Experimental therapeutics in the Renaissance in *The journal of pharmacology and experimental therapeutics* 304: 489-492.

16. **Regert M., Colinart S., Degrand L., and Decavallas O., 2001**, Chemical alteration and use of beeswax through time: accelerated ageing tests and analysis of archaeological samples from various environmental contexts in *Archaeometry* 43: 549-569.

17. **Regert M., Langlois J., and Colinart S., 2005**, Characterization of wax works of art by gas chromatographic procedures in *Journal of Chromatography A* 1091: 124-136.

18. **Walter, P., Martinetto, P., Tsoucaris, G., Bréniaux, R., Lefebvre, M., Richard, G., Talabot, J., and Dooryhee, E., 1999**, Making up in ancient Egypt in *Nature* 397:483-484.

19. **Zubay, G.L., 1998**, Biochemistry, Wm. C. Brown Publishers, Dubuque

9 General conclusions

Residue analysis aims at assisting the archaeological discipline through chemical evidence; immediately emphasizing the interdisciplinary character of this research field. Both chemical and technical aspects of the analytical procedures are a huge challenge. And in addition, the interpretation at archaeological level continues to be particularly difficult. Therefore, the need for close collaboration between the diverse disciplines remains crucial. The results of this PhD thesis confirmed but also refuted some general accepted awareness's in archaeology. On top of that, new insights in ancient way of life were put forward. Taking those facts into account, the scientific significance of residue analysis has been demonstrated once more.

In first instance, a critical study was undertaken to evaluate and compare the results from three different lipid analysis techniques to identify the origin of animal fat. Therefore, an optimization of the methodology on a gas chromatograph-combustion-isotope ratio mass spectrometer for the stable carbon isotope analysis on fatty acid methyl esters was required as this type of analyses was never performed on Sagalassos material before. This technique was compared with triglyceride analysis on a liquid chromatograph with mass spectrometer and the evaluation of fatty acid ratios after a transmethylation step. The three methods were applied on twenty-six cooking pots from Sagalassos to evaluate and compare their reliability. Agreement between the three is demonstrated, in particular in detecting ruminant adipose fat. Also for the first time, the use of dairy fats in a Sagalassos cooking pot has been recognized.

The second aim was to investigate the functionality of amphorae as little is known about their content in ancient times. Classical authors state that amphorae contained wine, vegetal oil or fish sauce. Residue analysis proved to be successful in detecting and identifying vegetal oil but so far it lacked the ability to unambiguously recognize wine and fish remains in ceramic material. Indeed, polyphenols, characteristic compounds of wine, are very susceptible to degradation and oxidation processes, and thus are easily lost after centuries of burial. We encounter a similar situation with fish

lipids as they are of polyunsaturated nature and thus quickly oxidized and lost. For these reasons, the focus of this work lied on developing new methodologies for the detection of polyphenols and fish biomarkers. We succeeded in creating a reliable technique for the extraction and quantification of polyphenols in ancient amphorae. Although many efforts were devoted to the search for fish biomarkers, it turned out to be an impossible challenge to recover marine lipids from archaeological ceramic material, at least for the preservation contexts that we had access to.

After development and optimization of the polyphenol detection method, thirty-one Sagalassos amphorae were, next to standard lipid analyses, subjected to this particular technique. Although we expected a high number of wine amphorae, as pitch was detected in twenty-three, only two sherds showed a significant amount of polyphenols. Furthermore, pitch was found in amphorae in which traces of vegetal oil, for example walnut oil, could be detected. The latter is incongruent with reports from ancient authors as they claim that it was out of place to store oil in a pitched amphora. These results generated more questions than answers and a permeation experiment with pitch, wine and olive oil emerged to be obvious in the research domain of residue analysis.

For that particular experiment, small ceramic pots were made and covered with a pitch layer in which, after drying, a known amount of wine or olive oil was deposited. Layers of 1 mm were abraded starting from the bottom. Each layer was then analysed for lipid and polyphenol content gathering their respective penetration profiles. It was exposed that, even with a pitch layer, wine could infiltrate in the ceramic matrix beneath. Consequently the absence of polyphenols in archaeological amphorae is not due to their inability to reach the ceramic under the pitch. Furthermore, as oil proved to easily infiltrate in the ceramic pores, the application of pitch seems indispensable when storing oil in an amphora. Our results contradict the common view about the use of pitch but confirm the earlier outcome from Sagalassos amphorae.

This work also incorporates chemical analyses on a few exceptional archaeological finds. For instance, in Sagalassos, two excavated *unguentaria* showed the presence of a visible greasy residue. So far, the exact function of those small glass bottles remained unclear, and therefore these findings created a great opportunity in elucidating that matter. Careful investigation showed that both residues comprised principally beeswax next to inorganic material in a small amount. Because of their conical shape, their position in the neck of the bottle and a macroscopically visible

finger imprint, the residues are assumed to originate from beeswax stoppers. This implies that *unguentaria* contained a liquid and as it seems impractical to use a waxlike closure every day, we prefer the hypothesis of a ritual over a daily used fluid. Indeed, maybe people carried a sacred liquid on them for protection.

Another extraordinary archaeological find are burned greasy residues in lamp shells, excavated in a first millennium AD Coptic church in Bawit, Egypt. The objective here was to recognize the origin of the illuminant in the bivalve *Chambardia rubens* shells. A variety of chromatographic techniques was used in an attempt to obtain satisfactory results. Fatty acid methyl ester profiles exhibited relatively high amounts of unsaturated fatty acids suggesting the use of vegetal oil. This assumption was confirmed by stable carbon isotope analysis on the same fatty acid methyl esters. A gas chromatograph mass spectrometric chromatogram of the silylated lipid fraction demonstrated a series of dicarboxylic acids next to high concentrations of 11,12-dihydroxyeicosanoate and 13,14-dihydroxydocosanoate. The latter are known to be oxidation products of two important constituents of seeds from Brassicaceae plants, namely gondoic and erucic acid. Analysis on a liquid chromatograph with mass spectrometer generated unknown spectra which we could identify as arising from hydroxylated triglycerides; these compounds have never been associated with archaeological residues before. These results all together attest that oil from radish seed had been used as fuel in these Egyptian lamp shells.

During excavations at the Castle of Middelburg, Belgium, archaeologists had the opportunity to excavate a Late Medieval ceramic pot still containing a greasy substance. A series of chemical techniques was assessed on what was presumed to be an ointment in order to reveal its origin and function. The organic fraction, analysed by chromatographic and mass spectrometric techniques showed the presence of beeswax next to smaller amounts of vegetal oil. Infrared analyses proved a certain amount of lead soaps. The inorganic fraction, about 24.6 %, was also investigated by a series of techniques. An X-ray diffraction measurement showed calcium sulfate (gypsum) and lead sulfate. X-ray fluorescence and inductively coupled plasma analyses detected high quantities of calcium and silicon next to smaller amounts of iron and lead. X-ray photoelectron spectroscopic results confirmed the elevated concentrations of calcium, sulfur and silicon next to minor elements like lead and iron. The ensemble of these data points in the direction of a medicinal formulation of a lead plaster, used for treating bruises, mixed with beeswax, added for easy

application on and hydration of the skin. Perhaps gypsum was added to whiten or to strengthen the plaster.

In the context of amphora functionality, a few issues remain worthwhile to investigate in the future. As we established the unfeasibility to use lipids as biomarkers for fish sauce, it might be useful to explore the potential of protein research in this area. Proteins can be diagnostic molecules and in some cases they proved to survive burial. A few approaches are possible; through an immunological detection, protein hydrolysis followed by the identification of the individual amino acids or through mass spectrometric analysis techniques. Another interesting topic might be the use of, and further optimization of the methodology for analysing fecal biomarkers. These particular markers are able to differentiate between the origins of dung, whether coming from humans, bovines, pigs etc. Next to being helpful in determining whether and where farming practices took place, they can be a reliable tool to establish the time of ruralization in the ancient city of Sagalassos.

10 List of abbreviations

AAS	Atomic Absorbance Spectroscopy
AG	Acylglycerols
AD	Anno Domini
AH	Alexander's Hill
APCI	Atmospherical Pressure Chemical Ionisation
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared
BC	Before Christ
BF_3	Boron trifluoride
$C_{12:0}$	Lauric acid
$C_{14:0}$	Myrictic acid
$C_{16:0}$	Palmitic acid
$C_{16:1}$	Hexadecenoic acid
$C_{18:0}$	Stearic acid
$C_{18:1\ (cis9)}$	Oleic acid
$C_{18:1\ (tr9)}$	Elaidic acid
$C_{18:1\ (cis11)}$	Vaccenic acid
$C_{18:1\ (tr11)}$	*trans*-vaccenic acid
$C_{18:2}$	Linoleic acid
$C_{18:3}$	Linolenic acid
$C_{20:0}$	Eicosanoic acid
$C_{20:1\ (cis9)}$	Eicosenoic acid
$C_{20:1\ (cis11)}$	Gondoic acid
$C_{22:0}$	Docosanoic acid
$C_{22:1\ (cis9)}$	docosenoic acid
$C_{22:1\ (cis13)}$	Erucic acid
$C_{24:0}$	Tetracosanoic acid
DA	Domestic Area
DAG	Diacylglycerols

$\delta^{13}C$ values	Stable carbon isotope values
$\Delta^{13}C$	$\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$
ETV-ICP-TOFMS	Electrothermal Vaporization-Inductively Coupled Plasma-Time of Flight Mass Spectrometer
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acids
FID	Flame Ionisation Detector
GC	Gas Chromatography
GC-C-IRMS	Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometer
GC-MS	Gas Chromatography - Mass Spectrometer
HPLC-MS	High Performance Liquid Chromatography - Mass Spectrometer
HT-GC	High Temperature - Gas Chromatography
IS	Internal Standard
KOH	Potassium hydroxide
LA	Lower Agora
LR	Late Roman
MAC	Macellum
MAG	Monoacylglycerols
MeOH	Methanol
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
N	Bowl without pitch
NEG	Northeast of upper Agora
P	Bowl with pitch
PP-GC	Polar Phase Gas Chromatography
P/O	Palmitate to Oleate ratio
P/S	Palmitate to Stearate ratio
PTV	Programmable Temperature Vaporizer
RT	Retention Time
SA	Sagalassos
SEM	Scanning Electron Microscopy
S/O	Stearate to Oleate ratio
TAG	Triglycerides

TGA	Thermogravimetric Analysis
TMS	Trimethylsilyl
vPDB	vienna Pee Dee Belemnite
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffractometry
XRF	X-ray Fluorescence