Eastern desert ware : traces of the inhabitants of the eastern desert in Egypt and Sudan during the 4th-6th centuries CE

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CHAPTER FOUR
The Use of Eastern Desert Ware as Suggested by Lipid Residues in the Walls of the Vessels

The general appearance of the corpus of 4th-6th century CE ceramic vessels currently identified as Eastern Desert Ware strongly suggests that most of the vessels were intended to be used as serving vessels, or 'table ware' (Chapter 2, Appendix 5). About 65% of the 290 vessels in this study are cups and bowls (types H 1 and H 2 respectively, Appendix 4) with smoothed or burnished (polished) surfaces that are often decorated with incised or impressed decorations, which are frequently enhanced by a partial red slip. Only about 5% of the vessels may have been used for storage or cooking (type H 3, jar or pot). The remarkable shapes of beak-spouted (H 6) and tubular-spouted (H 7) vessels points to rather specialized functions for these vessels. Strouhal (1984) has made a strong case for the use of beak-spouted vessels as feeding bowls for infants (Griffith 1925), although it had previously been suggested that they were oil-lamps (Almagro 1965; Garcia Guinea and Teixidor 1965; Giorgini 1971; Pellicer Catalán 1963), or early Christian liturgical vessels (Wooley and Randall-MacIver 1910). Tubular-spouted vessels appear intended to serve oil, fat or sauce to be poured over a meal. Cups and small bowls were most likely used by a single person, larger bowls may have been intended for communal use. It can also be argued that at least some of the vessels were produced to be used as grave goods specifically as so many vessels were recovered from graves. To understand better the actual use of the vessels, the organic residues trapped in the ceramic matrix of 51 sherds from the Red Sea coast and the *Mons Smaragdus* area were isolated and analyzed.

Organic residues have been extracted and studied from a variety of archaeological materials including ceramics, stone tools, bones, coprolites, cooking stones, grinding stones, paints and wood from shipwrecks. A range of biomolecules has been isolated including lipids, proteins, starches, DNA and plant lignin (Eerkens and Barnard 2007). Lipids preserved in the ceramic matrix of unglazed vessels are relatively well-studied because of their relative propensity to survive archaeologically and their amenable behaviour in laboratory conditions (Charters et al. 1995; Condamin 1976; Eerkens 2002; 2005; Evershed et al. 1991; 1997; Gerhardt et al. 1990; Hill et al. 1985; Malainey et al. 1999a; b; Mills and White 1989; Mottram et al. 1999; Regert et al. 1998; Oudemans and Boon 1991; Patrick et al. 1985; Shimoyama et al. 1995; Skibo and Deal 1995; Stern et al. 2000).

In this chapter the results are discussed of lipid residue analysis in Eastern Desert Ware by gas chromatography combined with mass spectrometry (GC/MS). This is preceded by a short introduction of this method; a glossary of the terminology can be found in Appendix 10.

All 51 sherds that were investigated appeared to have preserved an organic residue, arguing against the production of Eastern Desert Ware as receptacles for water or to be used specifically as grave goods, unless they were filled with food for the deceased or used for a funeral feast before being entered into the grave. Seven vessels were selected to be studied in some detail and were shown to have probably once contained cereals, vegetables or meat stews, indicating that Eastern Desert Ware was indeed most likely used for food. The small number of vessels, both those discussed in this chapter as well as in this study as a whole, does not allow for a statistical correlation of organic residues with vessel form or decoration. The fact that a small tubular-spouted bowl (EDW 87) was apparently used to contain vegetable oil, however, suggests that such a correlation most likely does exist. Most organic residues in Eastern Desert Ware seem to originate from cereals, which is concurrent with the ethno-archaeological data discussed in Chapter 5. Residues from meat or fish were more often encountered in bowls, compared to residues with vegetal origins that were more often seen in cups. Residues from the Mons Smaragdus area were likely to be from food sources richer in animal products, and possibly associated with wealthier lifestyle, than residues from Berenike. Even though lipid residues are not very specifically associated with foodstuffs their analysis can provide valuable information, especially when combined with information from historical, (ethno-) archaeological and experimental sources.

Archaeological Lipids

Lipids are a diverse group of organic molecules that includes, among others, fatty acids, fats (including triacylglycerols), waxes, steroids (including cholesterol) and terpenoids. They are largely hydrophobic molecules, with small polar parts that are hydrophilic. Lipids are ubiquitous in nature and are present in nearly all foodstuffs. The reasons behind the focus on archaeological lipid analysis are that most lipids are stable compounds that are well-studied and relatively easy to analyze (Murphy 1993; Barnard et al. 2007a ;b; c). Furthermore, they appear to get trapped in the ceramic matrix of unglazed pottery where they can remain intact for centuries.

The most commonly analyzed class of lipids are fatty acids. These are strings of CH_2-groups, making up the hydrophobic part of the molecule, with an acidic (hydrophilic) COOH-group attached to the α-carbon (Table 4-1). In unsaturated fatty acids two adjacent H-atoms are replaced by another bond between the two C-atoms, forming a double bond. Unsaturated fatty acids, and especially poly-unsaturated fatty acids (with more than one double bond), are more abundant in food of vegetable origin. Mono-unsaturated fatty acids can oxidize into dicarboxylic fatty acids, which have a COOH-group on both terminal C-atoms. Like unsaturated fatty acids, dicarboxylic fatty acids are more abundant in oils and fats of vegetable origin. One, two or three fatty acids can be attached by an ester bond (R_1-C-O-CO-R_2), to glycerol (CH_2OH-HCOH-CH_2OH) forming a monoacylglycerol (MAG), a diacylglycerol (DAG) or a triacylglycerol (TAG) respectively, generally referred to as fats. These ester bonds can be broken, and the fatty acids released, by strong alkaline (caustic) metal hydroxides like potassium hydroxide (KOH) or sodium hydroxide (NaOH), a reaction referred to as saponification. Small quantities of branched fatty acids, with one or more CH_3-groups attached to the central chain (for instance 3,7,11,15-tetramethyl hexadecanoic or phytanic acid), are present in many plants and animals (especially ruminants and fish), and in much larger concentrations in many bacteria.

Systematic name	Synonyms		Formula	Mass
Dodecanoic acid	C12:0	Lauric acid, Vulvic acid	HOOC-$(CH_2)_{10}$-CH_3	200
Tridecanoic acid	C13:0	---	HOOC-$(CH_2)_{11}$-CH_3	214
Tetradecanoic acid	C14:0	Myristic acid	HOOC-$(CH_2)_{12}$-CH_3	228
Pentadecanoic acid	C15:0	---	HOOC-$(CH_2)_{13}$-CH_3	242
Hexadecanoic acid	C16:0	Cetylic acid, Palmitic acid	HOOC-$(CH_2)_{14}$-CH_3	256
Heptadecanoic acid	C17:0	Margaric acid, Margarinic acid	HOOC-$(CH_2)_{15}$-CH_3	270
Octadecanoic acid	C18:0	Stearic acid, Steric acid	HOOC-$(CH_2)_{16}$-CH_3	284
Nonadecanoic acid	C19:0	---	HOOC-$(CH_2)_{17}$-CH_3	298
Eicosanoic acid	C20:0	Arachic acid, Arachidic acid	HOOC-$(CH_2)_{18}$-CH_3	312
Heneicosanoic acid	C21:0	---	HOOC-$(CH_2)_{19}$-CH_3	326
Docosanoic acid	C22:0	Beheric acid	HOOC-$(CH_2)_{20}$-CH_3	340
Tricosanoic acid	C23:0	---	HOOC-$(CH_2)_{21}$-CH_3	354
Tetracosanoic acid	C24:0	Lignoceric acid	HOOC-$(CH_2)_{22}$-CH_3	368
Pentacosanoic acid	C25:0	---	HOOC-$(CH_2)_{23}$-CH_3	382
Hexacosanoic acid	C26:0	Cerinic acid, Cerotic acid	HOOC-$(CH_2)_{24}$-CH_3	396

Table 4-1: Details of saturated fatty acids with 12-25 C-atoms (mass is integer mass in Daltons).

Some archaeological lipid residues preserve 'biomarkers', molecules that are more or less specific for certain classes of foodstuffs. These can be alkaloids (such as caffeine), steroids (such as cholesterol), terpenoids (a large and diverse group of mostly polycyclic compounds synthesized by plants), but also fatty acids like C22:1 (erucic or docosenoic acid) and phytanic (3,7,11,15-tetramethyl-hexadecanoic) acid. Respectively, these compounds naturally occur in coffee; animal products; certain families of plants (the terpenoids are well-studied and can be very specific, Connolly and Hill 2005, Hanson 2001); members of the *Brassicaceae (Cruciferae)* family (such as mustard, nasturtium or rape seed) or fish; and, finally, in the products of ruminants or, again, fish (Hansel et al. 2004). As is evident from the last two examples the same

molecule is sometimes mentioned as a biomarker for two apparently unrelated classes of residues. Other compounds can mark modern contamination of the sample. This is obvious in the case of man-made organic molecules like phthalates (Kumar 1999), added to plastics to keep them flexible, but must also be considered for naturally occurring compounds like anthraquinones, commonly used as dye in textiles and paper, or 13-docosenamide (erucamide), coated on many plastic objects to prevent them from sticking together. The natural origins of many compounds have been published in the biochemical literature (O'Neil et al. 2001).

Another avenue to match unknown residues with foodstuffs is by comparing the ratios of the abundance of common fatty acids. Results can be sometimes be obtained with a simple C16:0/C18:0 ratio (palmitic acid/stearic acid or P/S-ratio; Malainey et al. 1999a; b) and sometimes only after the two-dimensional plotting on a double logarithmic scale of C16:1/C18:1 versus (C15:0+C17:0)/C18:0 (Eerkens 2002; 2005). Such ratios tend to bring the residues from the same class of foodstuffs together rather well, but often fail to fully separate the different classes. The fact that these ratios may furthermore change over time, as different fatty acids oxidize at different rates, further complicates the application of this method. The previously mentioned lipid biomarkers and fatty acids are usually found with the same analytical techniques, which enables the use of both methods on the same dataset. Additional analytical techniques that have successfully been used to expand the understanding of archaeological lipid residues, include the determination of stable isotope analysis of individual lipid compounds (Copley et al. 2005), or of the complete organic residue (Ambrose 1993; 2000; Morton and Schwarcz 1988; 2004).

The interpretation of archaeological organic residue can only be complete after the results of biochemical analyses are combined with archaeological, historical and ethnographical data. It is assumed that the organic residue found in a ceramic vessel is in some way related to the original use of the vessel. It is often unclear, however, whether these residues represent the first food to come into contact with the ceramics, after which the available binding sites are saturated, or the last, if older residues are continually replaced by new ones. It is also possible that the organic residue represent a combination of all food ever to have been inside the vessel, if the molecules that make up the residue compete for the available binding places (Barnard et al. 2007a; b). Furthermore, most ancient and modern societies have very strict views on what to eat, and on how, when and where to eat it. To learn such views is obviously of great interest to archaeologists, and would be very helpful beforehand, but they may prove very difficult to infer from the study of ceramic vessels alone. On the other hand, the custom to reserve specific vessels for certain foodstuffs or to serve meals in individual portions, each in their own container, is certainly not universal. There are also vessels that are not used for food and food that is not prepared in vessels. Possible sources of organic residues in archaeological pottery include refuse surrounding a discarded vessel, human remains decaying close to a pot included as a grave gift or the micro-organisms digesting anthropogenetic organic residues within the wall of a pot. Ceramic vessels may also have been employed for 'industrial' purposes, such as the preparation of organic dyes or glues, or as coffins, censers, smoking pipes or to store a multitude of things. On the other hand, many foodstuffs never come into contact with ceramic vessels but are eaten raw, roasted over a fire or prepared and consumed in other ways that do not call for ceramics.

Gas Chromatography/Mass Spectrometry

Any mass spectrometer consists of a sample inlet, an ion source (where the molecules in the sample are ionized), a mass analyzer (where ions are separated according to their mass) and an ion detector (Figure 4-1, cf. Chapter 3). Mass analyzers separate ions with a different m/z (mass to charge ratio) by applying electro-magnetic forces, requiring the molecules in the sample to be ionized so that they will respond to such forces. Molecules that do not accept ionization or decompose when ionized will therefore escape analysis. A number of methods to ionize molecules have been developed, each with their own specific advantages and disadvantages. Ionization changes the mass of the original molecule; the ionization process may also cause a molecule to fracture. Analysis of the resulting fragments provides additional information that is helpful in identifying the original molecule. This is especially the case when molecules are ionized in an electron impact ion source (EI^+), a beam of high energy electrons (usually 70 eV), resulting in positive ions when an electron is knocked out of the molecule. The fragmentation patterns that are the result of this method are highly reproducible, much like a fingerprint, which allows comparing the mass spectra generated by an unknown compound with known spectra in one of several digital libraries.

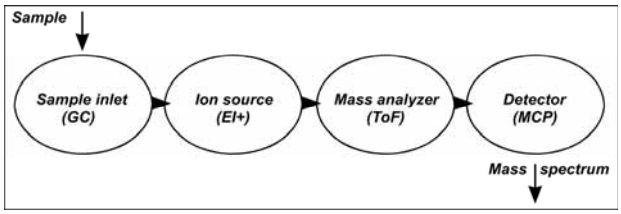

Figure 4-1: Diagram of a GC/MS instrument, consisting of a gas chromatograph (GC), an electron impact ion source (EI^+), a time-of-flight mass analyzer (ToF) and a multi channel plate ion detector (MCP), producing mass spectra (on the right) of the molecules in the sample injected into the inlet (on the left, cf. Figure 3-3).

Analyzers most often used in mass spectrometers are a quadrupole or a time-of-flight (ToF) mass analyzer. A quadrupole consists of four parallel metal rods, two of which carry a high voltage DC-potential, while the other two carry a high frequency AC-potential. Ions entering the space between these four rods follow a complex path because of the constantly changing electro-magnetic fields. Most will at some point leave the space between the rods or crash into one of them. Only ions with a specific m/z will follow a stable trajectory and exit the end of the quadrupole. Changing the voltages on the rods will cause ions with another m/z to reach the detector. Scanning a series of sequential values of m/z must be sufficiently slow to allow ions to travel the length of the rods. A time-of-flight mass analyzer is basically an empty metal tube with an ion accelerator at its beginning and an ion detector at its end. Upon entering the analyzer, the ions in the sample are accelerated, by an electro-magnetic pulse, after which they are allowed to drift towards the detector. The speed of each ion, and consequently the time it needs to complete this journey, depends on its mass and charge state. Different ions will therefore reach the detector, where their arrival is recorded, at different times. The m/z of each ion can be extrapolated from its time of flight.

Ion detectors are designed to amplify the impact of a single ion and turn this into a measurable electric current. This current is then measured and the relative intensity (frequency) of each m/z, during a specific time interval, is plotted on a mass spectrum. Whether or not an ion is recorded by the detector depends on its m/z, not its mass. Ions that carry a double charge will appear to have half their actual mass. Comparatively small organic molecules such as fatty acids, however, usually carry only one charge, especially after the relatively inefficient electron impact ionization (EI^+) that typically gives singly charged ions.

The sample inlet of a GC/MS instrument consists of a gas chromatograph at the centre of which is a long (15-60 m), narrow (0.1-0.5 mm) glass column through which a steady flow of carrier gas is maintained. This column is coated on the inside with a thin layer of liquid to which some molecules in the sample will be attracted. The beginning of the column is attached to an evaporation chamber. The column and evaporation chamber are in ovens of which the temperatures can be carefully controlled. The end of the column is connected to the ion source of the mass spectrometer that makes up the MS-part of the instrument.

Typically 1 µl of the sample is injected into the sample inlet, which is heated to a fixed high temperature (200-300°C). Sample and solvents quickly vaporize and are carried onto the column by the carrier gas. A variable outlet allows regulation of the amount of sample actually passing onto the column. Once the sample is on the column, the mobile phase (the carrier gas) and the stationary phase (the coating inside the column) compete for the molecules in the sample. For each molecule the outcome of this is dependent on the temperature inside the column. When the temperature in the column is slowly raised, the various components of the sample will one by one leave the stationary phase and travel with the carrier gas to the end of the column.

Large molecules (>500-1000 Da), molecules that are too polar and molecules that are not thermally stable, will not pass through a gas chromatograph. In order for more lipids to reach the mass spectrometer they are chemically altered to make them less polar and more stable. This can be achieved by replacing the active hydrogen of the COOH-group by a non-polar group, a process referred to as derivatization. Methods often used include esterification and silylation. To reduce manipulation of the sample, the residue can be extracted into an agent that both saponifies and derivatizes the sample while it evaporates in the gas chromatograph (Stern et al. 2000).

The output of a GC/MS is a combination of a single chromatogram (Figure 4-4) and a large number of mass spectra. Each peak in the chromatogram represents at least one molecule in the sample and a mass spectrum can be created for each of these peaks. With specialized software, mass spectra of unknown compounds can be electronically compared with the spectra of known compounds in a digital library. A visual inspection of the known and unknown mass spectra usually leads to the identification, with a reasonable amount of certainty, of many components in most samples. The lipids detected in Eastern Desert Ware by GC/MS are obviously only those that remain trapped in the ceramic matrix, dissolve in the extraction solvents, survive the sample preparation, pass through the gas chromatograph and ionize in the mass spectrometer.

Sherd (provenance and classification)				Residue (method and type)			
No.	**Site**	**H-class**	**D-class**	**M-1**	**M-2**	**M-3**	**Type**
3	Be	H 1b	D 6	06/02			A
4	Be	H 2a	D 3	06/02	06/02	06/03	D
6	Be	H 2b	D 3	06/02			G
8	Be	H 2a	D 3	06/02	06/03		R
10	Be	H 1a	D 8	06/02			B
12	Be	H 1a	D 6	06/02	06/03		C
13	Be	H 3	D 3	06/02			E
14	Be	H 1a	D 2	06/02			E
15	Be	H 2b	D 0	06/03			P
16	Be	H 2a	D 5	06/02			A
17	Be	H 7	D 3	06/02			E
18	Be	H 3	D 3	06/02			B
21	Be	H 2b	D 2	06/02			G
22	Be	H 2a	D 3	06/02			B
24	Be	H 2a	D 3	06/02			J
25	Be	H 2a	D 2	06/02			J
29	Be	H 1a	D 6	06/02			D
30	Be	H 9	D 6	06/02			B
36	KM	H 2c	D 2	06/03			E
40	KM	H 2b	D 0	06/03			E
43	KM	H 0	D 0	06/03			A
44	KM	H 0	D 8	06/03			E
46	Be	H 10	D 10	06/02			C
47	Be	H 0	D 8	06/02			A
49	Be	H 3	D 7	06/02	06/03		R
50	Be	H 1c	D 0	06/02	06/03		P
51	Be	H 3	D 0	06/02	06/03		B
52	Be	H 2b	D 2	06/02			B
53	Be	H 9	D 2	06/03			E
55	Be	H 7	D 3	06/02			E
56	Be	H 2b	D 6	06/02			A
58	Be	H 1b	D 3	06/02			E
60	Be	H 7	D 3	06/02			E
62	Be	H 2a	D 2	06/02	06/03		F
63	Be	H 2b	D 8	06/02			E
67	MN	H 0	D 0	05/03	05/03	06/03	C
75	MN	H 1	D 3	05/03	06/03		C
76	WS	H 1d	D 5	05/03			E
77	WS	H 2b	D 3	05/03			K
78	WS	H 1d	D 7	05/03			M
82	WS	H 0	D 0	05/03			K
83	WS	H 1d	D 5	05/03	06/03		E
84	WS	H 0	D 0	05/03			A
85	WS	H 0	D 0	06/03	06/03		C
86	WS	H 2c	D 3	05/03			M
87	WS	H 7	D 8	05/03			C
90	WS	H 3	D 5	05/03			K
91	WS	H 2a	D 7	05/03			M
234	WS	H 2c	D 3	08/04	08/04		H
261	WS	H 0	D 0	08/04	08/04		H
262	UH	H 0	D 0	08/04	08/04		H

Table 4-2: Data on the 51 Eastern Desert Ware sherds in this study (Figures 4-2 and 4-8). No. = EDW number; Site = Be: Berenike, KM: Kab Marfu'a; MN: Marsa Nakari; UH: Umm Heiran; or WS: Wadi Sikait (Appendix 5); H-class and D-class = classification of the shape of the vessel and the lay-out of the decoration (Appendix 4); M-1 through M-3: method (Table 4-3); Type = type of residue (Table 4-4).

Eastern Desert Ware

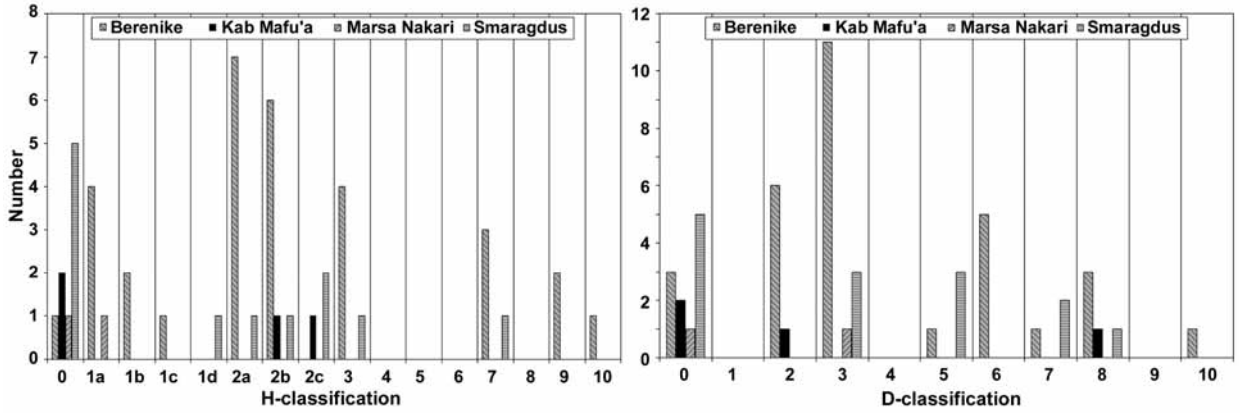

Figure 4-2: H-classification (form) and D-classification (lay-out) of the 51 Eastern Desert Ware sherds in this study (Table 4-2; Figure 4-8; Appendix 5).

	June 2002 (**06/02**)	May 2003 (**05/03**)	June 2003 (**06/03**)	August 2004 (**08/04**)
Pottery quantity	400 mg	400 mg	400 mg	400 mg
Extraction solvents	3 x 2.5 ml chloroform methanol (2/1, v/v)	3 x 2 ml chloroform methanol (2/1, v/v)	3 x 1 ml chloroform methanol (2/1, v/v)	3 x 1 ml chloroform methanol (2/1, v/v)
Sonication	15 min	15 min	30 min	30 min
Solvent evaporation	vacufuge @ 30°C	vacufuge @ 30°C	under N_2	under N_2
Derivatization solvent	---	---	3 x 20 µl ethyl-acetate	3 x 20 µl ethyl-acetate
Derivatization treatment	100 µl methanolic HCL @ 60°C for 1 hour, vacufuge @ 30°C	100 µl methanolic HCL @ 60°C for 1 hour, vacufuge @ 30°C	40 µl BSTFA with 1% TMCS @ 60°C for 1 hour	40 µl BSTFA with 1% TMCS @ 60°C for 1 hour
Injection solvent	40 µl hexane	40 µl hexane	---	---
Internal standard	10 µl 1 mM methyl benzoate in hexane	10 µl 1 mM methyl benzoate in hexane	---	---
Injection volume	1 µl	1 µl	1 µl	1 µl
Temperature ramp	50°C (3 min) 17°C/min (14.7 min) 300°C (6 min)	50°C (3 min) 17°C/min (14.7 min) 300°C (6 min)	50°C (2 min) 10°C/min (30 min) 350°C (10 min)	50°C (2 min) 10°C/min (30 min) 350°C (10 min)

Table 4-3: Overview of the details of the method used to extract and analyze lipid residues in Eastern Desert Ware.

Material

Lipid residues were isolated from 51 sherds found in Berenike, Marsa Nakari and the Mons Smaragdus area (Table 4-2, Figure 4-2) and analyzed by combined gas chromatography mass spectrometry (GC/MS). Berenike and Marsa Nakari (possibly ancient *Nechesia*) are Graeco-Roman harbours on the Red Sea that facilitated the trade between Alexandria and Rome on the one hand and sub-Saharan Africa, *Arabia Felix* and India on the other. Eastern Desert Ware was found here in residential areas and household debris, among sherds of Late Roman Amphora type 1 (LRA-1) and Egyptian red-slipped bowls, produced in the Aswan region (ERSA) and in the Nile Valley north of Aswan (ERSB). These are wheel-thrown vessels that were produced during the 4^{th}-6^{th} centuries CE. The Mons Smaragdus area comprises a series of settlements associated with the main source of beryl (a semi-precious stone) within the Roman Empire. Eastern Desert Ware was found in some of the settlements, again among sherds of LRA-1, ERSA and ERSB (Chapter 2).

Sample Preparation

The first step to isolate ancient lipids is to separate them from the ceramic matrix by extracting them in a suitable solvent. Although not strictly necessary (Gerhardt et al.

1990), a fragment of the sherds is usually ground into a fine powder to maximize contact between any residue and the solvents. From a 15 x 15 mm fragment of each selected Eastern Desert Ware sherd (Table 4-2) the surfaces were removed with an aluminium-oxide grinding stone, to minimize contamination of the sample (Evershed et al. 1990; Heron et al. 1991). The remaining core of the sherd was crushed using an aluminium-oxide mortar and pestle. The resulting pottery powder was stored until use in a sterile glass vial with a Teflon lined cap.

Four slightly different versions (identified as 06/02, 05/03, 06/03 and 08/04) of the following method to extract and identify organic residues in the pottery powder were used (Table 4-3). From each vial 400 mg of pottery powder was transferred into a test tube with a chloroform-methanol mixture (2/1, v/v). Powder and solvent were mixed and sonicated at room temperature. After centrifuging at 2000xg for 15 min, the supernatant was transferred into a second test tube. Fresh solvent was added to the sediment which was again mixed, sonicated, centrifuged and decanted into the same test tube. After three such extractions the depleted pottery powder was discarded. The solvents in the second test tube, in which some of the ancient organic residue had dissolved, were evaporated, after which the dry residue was derivatized.

The initial practice of adding an internal standard, potentially enabling the quantification of the identified molecules, was later abandoned as it did not facilitate the qualitative and semi-quantitative analysis of the samples. To prevent contamination the sample preparation was completed using gloves from which the talcum powder was removed. Residual pottery powder was removed from gloves and tools with three rinses of water, formic acid and acetone respectively. To monitor the procedure, empty sample vials were included as well as vials with the powder of new ceramic vessels or of modern vessels in which known foodstuffs had been prepared.

Analytical Methods

The instrument used to analyze the samples was a gas chromatograph (GC) feeding into an EI/CI time-of-flight mass spectrometer (MS), built by Waters/Micromass, purchased by the Pasarow Mass Spectrometry Laboratory at the University of California, Los Angeles through NSF grant number CHE 0078299. Sample preparation and analysis were performed with the help of Alek Dooley, under supervision of Dr. Kym Faull, director of the Pasarow Mass Spectrometry Laboratory. The injector vials were loaded in the automated injector set to inject 1 µl of each sample into the injector port heated to 250ºC. The vapours released from the samples were carried through the CG-column by a 1.2 ml/min flow of helium. Most samples were first run split 1:50 and, if the response appeared low, repeated at split rates of 1:20, 1:10 and, finally, splitless. The column used was a HP-5MS fused silica capillary, stationary phase 5% methyl silicone, 50 m long, 0.32 mm internal diameter, 0.25 micron film thickness, made by Agilent Technologies.

The resulting chromatograms and mass spectra were stored and studied off-line using MassLynx 4.0 software and the 2002 version of the NIST/EPA/NIH Mass Spectral Library enabling comparison of the spectra of the samples with those of almost 150,000 known molecules. The method of extraction and derivatization (Table 4-3) will preferentially isolate lipids, most of which appeared to be saturated fatty acids (Table 4-1). Small amounts of mono-unsaturated fatty acids and dicarboxylic fatty acids were also seen, as well as acylglycerols (fats) and polycyclic carbohydrates, such as cholesterol and terpenoids.

General Observations

During the collection and study of the data, two observations were made with relevance to the interpretation of these data, as well as to data that may be collected in the future using similar methods. The first, fully expected and explicable, was that longer fatty acids have a longer retention time. The time necessary for a series of fatty acids of increasing length, with an increasing number of CH_2-groups, to pass through a gas chromatograph increases almost linear (Figure 4-3). This phenomenon was used when the comparison of the recovered mass spectra with those in the digital library yielded ambiguous or unlikely results.

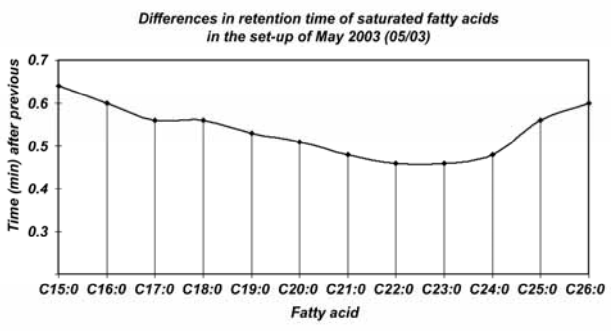

Figure 4-3: Differences in retention time (Y-axis) of a series of saturated fatty acids (X-axis) inferred from twelve chromatogram resulting from a single sample preparation procedure (05/03).

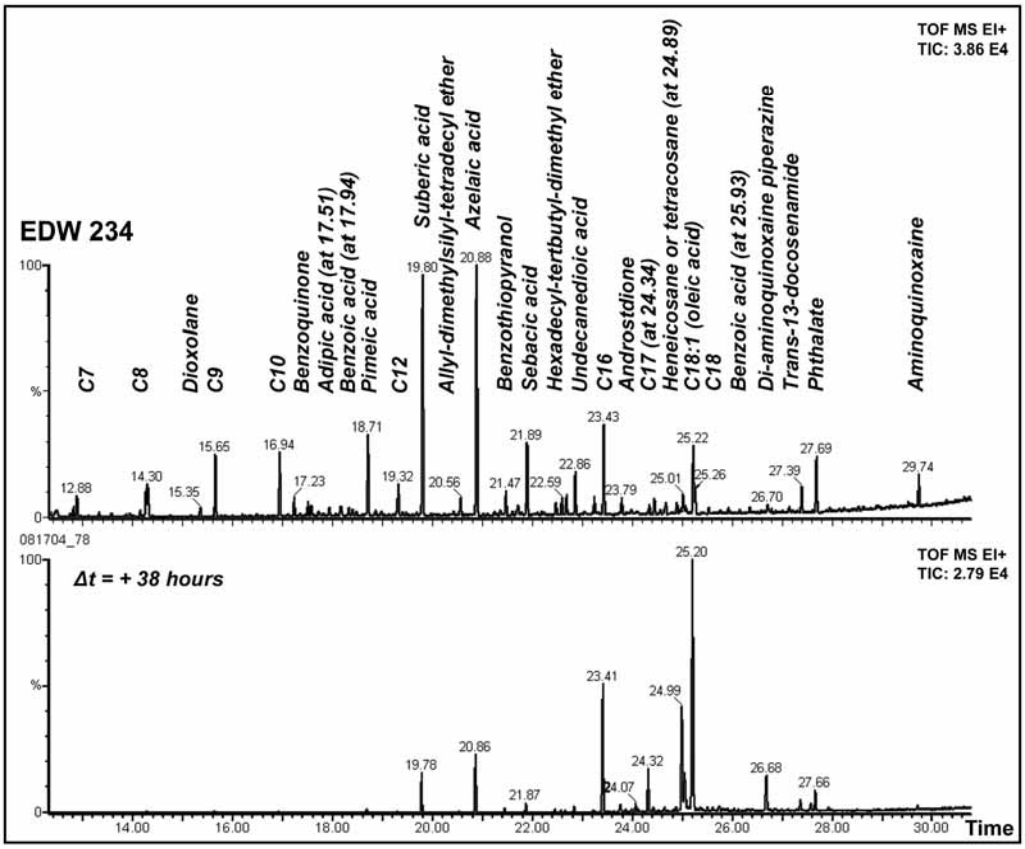

Figure 4-4: Two chromatograms of the same sample (EDW 234) acquired 38 hours apart under similar conditions.

Residue type	Characteristics	
	C16:0 and C18:0	**Additional organic molecules**
Type A	C16:0 = C18:0	none
Type B	C16:0 = C18:0	other saturated fatty acids
Type C	C16:0 = C18:0	other saturated and unsaturated fatty acids
Type D*	C18:0 = C16:0	phytanic acid
Type E	C18:0 > C16:0	none
Type F**	C18:0 > C16:0	acylglycerols
Type G*	C18:0 > C16:0	phytanic acid
Type H	mostly dicarboxylic fatty acids	
Type J*	base peak is phytanic acid	
Type K	C18:0 > C16:0	unsaturated fatty acids
Type M	C18:0 < C16:0	unsaturated fatty acids
Type P**	C18:0 = C16:0	acylglycerols
Type R**	C18:0 < C16:0	other saturated and unsaturated fatty acids as well as acylglycerols
*) Type D/G/J	phytanic acid preserved	
**) Type F/P/R	acylglycerols (MAG, DAG) preserved	

Table 4-4: The residue types found in Eastern Desert Ware. Types D, G and J as well as F, P and R were later united into two larger groups (at the bottom of the table).

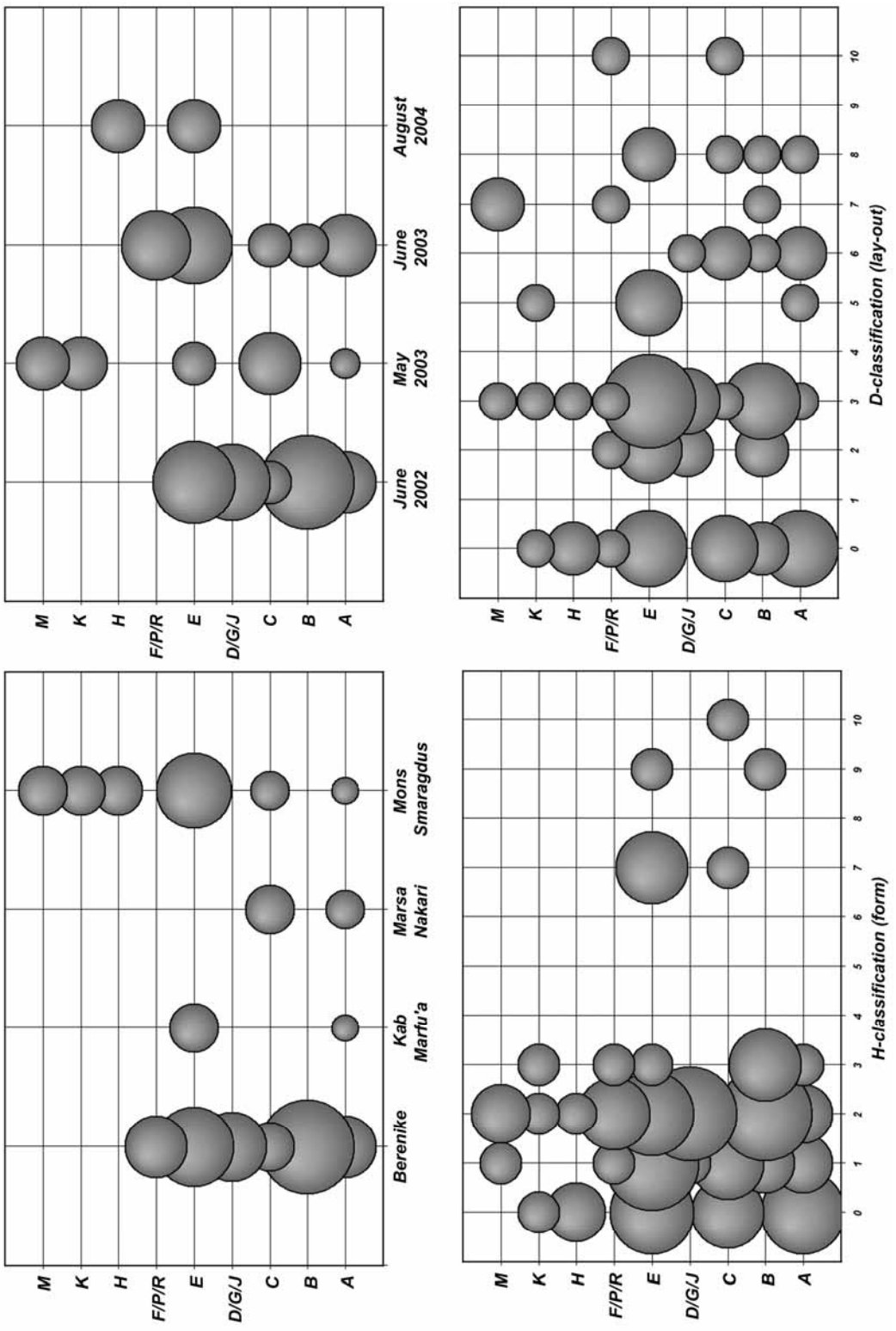

Figure 4-5: Distribution of the nine residue types encountered in Eastern Desert Ware by site, method of analysis, form of the vessel (H-classification) and lay-out of the decoration (D-classification).

A surprising and somewhat enigmatic observation was the difference between the results produced by 'fresh' samples and samples that were injected into the GC/MS again, after having been left untouched in the autosampler of the instrument, at room temperature, for a period of up to 48 hours. The instrument settings were identical during the whole series: the second injection was scheduled beforehand to take place after the first series of runs was completed. A probably relevant difference between the first and the second run of the repeated samples was that the cap of the sample vial was punctured for the first analysis, to extract the 1 µl of the sample to be injected, leaving the sample open to the laboratory atmosphere.

The first chromatogram, of the fresh sample, showed a higher abundance of dicarboxylic fatty acids, oxidation products of mono-unsaturated fatty acids, and a series of short chain fatty acids that was missing from the later chromatogram. As the first chromatogram contains more oxidation products compared to the second, the obvious explanation for the difference, the oxidation of the lipids, is unlikely. A more plausible explanation is that the molecules in the sample somehow lost their derivatization, either because of the time elapsed or under influence of the laboratory atmosphere, and escaped analysis during the second run. This phenomenon warrants further scrutiny, beyond the scope of this chapter, but certainly emphasizes the importance of storing the samples, and especially the derivatized samples, at low temperatures (-20°C) and in an inert environment (under N_2) whenever possible.

Results

All chromatograms were first visually sorted into 13 different types, based on the P/S-ratio (palmitic acid, C16:0, versus stearic acid, C18:0) and other abundant organic molecules. The characteristics of these types are presented in Table 4-4, six types (D, F, G, J, P and R) were later combined into two larger groups (types D/G/J and F/P/R). Very tentatively the resulting nine different types can be associated with fruits and nuts (A), meat (B), nuts or meat (C), fish or milk (D/G/J), oil (E), meat (F/P/R), vegetables or roots (H), roots (K) and vegetables (M). Given the restricted specificity of fatty acids to identify the origin of an organic residue and the expected state of decomposition of the residues in Eastern Desert Ware, the certainty of these identifications is limited (Table 4-5). When the same data are analyzed according to fatty acid ratios, as discussed below, residue type C appears associated with vegetables or roots (rather than or in combination with fruits and nuts), type K with meat (rather than or in combination with roots) and type M with fish rather than or in combination with vegetables). The number of sherds and analyses is obviously too low to allow statistical analysis of the data and the conclusions must necessarily remain preliminary.

A first observation was that there appeared to be a correlation between the method used and the recorded molecules. This is most obvious for method 08/04, which seemed to favour dicarboxylic fatty acids, while method 05/03 seemed to have been the most balanced. However, when represented another way, as discussed below, method 06/03 rather than 05/03 seems to have been the most balanced. As these methods are based on the same principles and the actual differences between them are only slight, this correlation must be attributed to instrument bias caused by variations in the sensitivity of the gas chromatograph and the mass spectrometer.

As can be inferred from Figure 4-5, more residues from sherds from Berenike appeared to be of the B-type (meat?), while more residues from the Mons Smaragdus area were of the E-type (oil?). Mons Smaragdus also had more residues with preserved unsaturated fatty acids or dicarboxylic acids, possible oxidation products of unsaturated fatty acids. These are generally associated with either vegetable food sources or fish. Residues from Berenike preserved more acylglycerols that are usually associated with meat. The residues in sherds from Kab Marfu'a and Marsa Nakari consisted mostly of saturated and mono-unsaturated fatty acids, but the number of sherds from these sites was too small to show any pattern.

Cups (H 1) mostly preserved residues of the E-type (oil?), while bowls (H 2) preserved mostly residues of the C-type (nuts or meat?) and the D/G/J-type (fish, milk or beef?). There also appeared to be a correlation between E-type residues (oil?) and both tubular-spouted vessels (H 7) and vessels decorated in multiple horizontal bands (D 3). No other correlation between the D-class of the vessels and type of residue was apparent.

One way to further interpret the data of archaeological residues is by comparing the ratios of selected fatty acids with those of known foodstuffs, both fresh and artificially aged (Table 4-5, based on Malainey 1999a; b; see also Eerkens 2002; 2005; Barnard 2007). Several sets of ratios have been suggested, mostly for material from the New World. These include long chain (C16:0/C18:0) versus medium chain (C12:0/C14:0) saturated fatty acids, to separate animal from vegetable sources of food, and mono-unsaturated (C16:1/C18:1) versus odd-chain fatty acids ((C15:0+C17:0)/C18:0). For the 51 Eastern Desert Ware sherds in which the organic residue was isolated and analyzed (Table 4-1) these ratios are graphically presented in Figures 4-6 and 4-7.

		Meat	Nuts	Berries	Roots	Fish	Greens
C16:0 / C18:0	Fresh	0-4	0-9	2-6	3-12	4-6	5-12
	Degraded	0-7	0-18	4-12	6-24	8-12	10-24
C16:1 / C18:1	Fresh	0.02-0.2	0-0.3	0-0.08	0.05-0.7	0.2-0.5	0-0.7
	Degraded	0.08-0.8	0-1.2	0-0.32	0.3-2.8	0.8-2.0	0-2.8

Table 4-5: Ratios of the mean saturated and mono-unsaturated fatty acids in various groups of foodstuffs (after Eerkens 2005; Malainey 1999a; b).

Figure 4-6 shows the C16:0/C18:0 versus the C12:0/C14:0 ratios, plotted on a semi-logarithmic scale, with a large ellipse enclosing the ratios associated with seeds and berries (Figure 4-6, top), as well as the C16:1/C18:1 versus the (C15:0+C17:0)/C18:0 ratio, plotted on a double logarithmic scale, with lines connecting the various residue groups (Figure 4-6, bottom). These lines bear no statistical significance. The data points were marked in a way that the correlation between the fatty acid ratios and the residue types identified above could be investigated. This correlation appeared to be small which can be explained by the limited resolution of fatty acid analysis to identify the origins of organic residues. Nevertheless, combining several interpretation methods with archaeological data, as done here for Eastern Desert Ware, may still yield valuable information.

Both graphs in Figure 4-6 show that most residues appear to be of seeds or berries, including cereals. There appeared to be more residues from meat (mammal) than fish, and even fewer from vegetables (greens). The last observation may be partly due to that fact that vegetables will obviously leave fewer lipids than fish or meat. In this context the presence of phytanic acid (3,7,11,15-tetramethyl-hexadecanoic acid), marker for residue type D/G/J, is important as it can be expected in the meat and milk of ruminant animals, as a product of the digestion of chlorophyll by the micro-organisms in their rumen, but also in prepared fish (Hansel et al. 2004). The graph showing the ratios of mono-unsaturated (C16:1/C18:1) versus odd-chain fatty acids ((C15:0+C17:0)/C18:0) appears the most informative (Figure 4-6, bottom). The data points were therefore marked for site, method of analysis, form of the vessel (H-classification) and lay-out of the decoration (D-classification). The results are presented in Figure 4-7.

Represented like this, method 06/03 rather than 05/03 seems to have been the most balanced (the most central and spread out in the graph). There appears to be no correlation between the residue and the form or the decoration of the vessel. The residues from the Mons Smaragdus area tend toward an animal food source, either mammal or fish, compared to the residues from Berenike.

Selected Case Studies

Another way to interpret the organic residues found in archaeological ceramics is to look for biomarkers: molecules more of less specific for certain groups of foodstuffs (Charters et al. 1995, Eerkens 2002, Evershed 1993, Evershed et al. 1991, Heron and Evershed 1993, Mottram et al. 1999). Phytanic acid has been discussed above, other biomarkers that may be found with the described method include other specific fatty acids (such as docosenoic acid, C22:1), but also alkaloids, steroids and terpenoids. Based on the obvious differences in their organic residue (Figure 4-7), five of the 51 sherds were chosen for closer inspection: EDW 4, 46, 67, 77 and 87 (Figure 4-8). The analysis of two more sherds (EDW 8 and 49) has been published elsewhere (Barnard et al. 2005) and is summarized here.

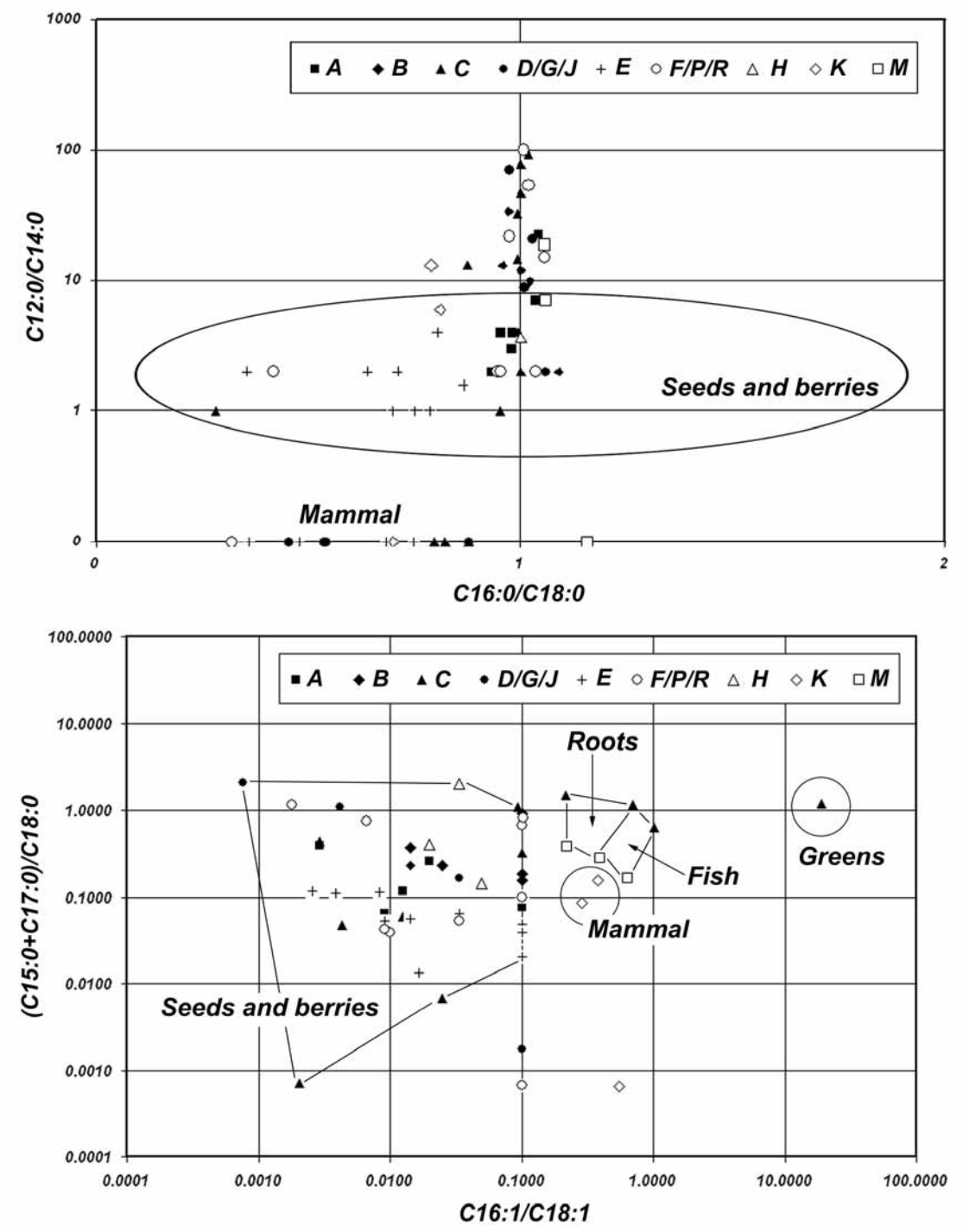

Figure 4-6: Graphic representation of selected fatty acids ratios, with their tentative interpretation (based on Eerkens 2005, Table 4-5), in 51 Eastern Desert Ware sherds (Table 4-1). The data points are marked for residue type (Table 4-4).

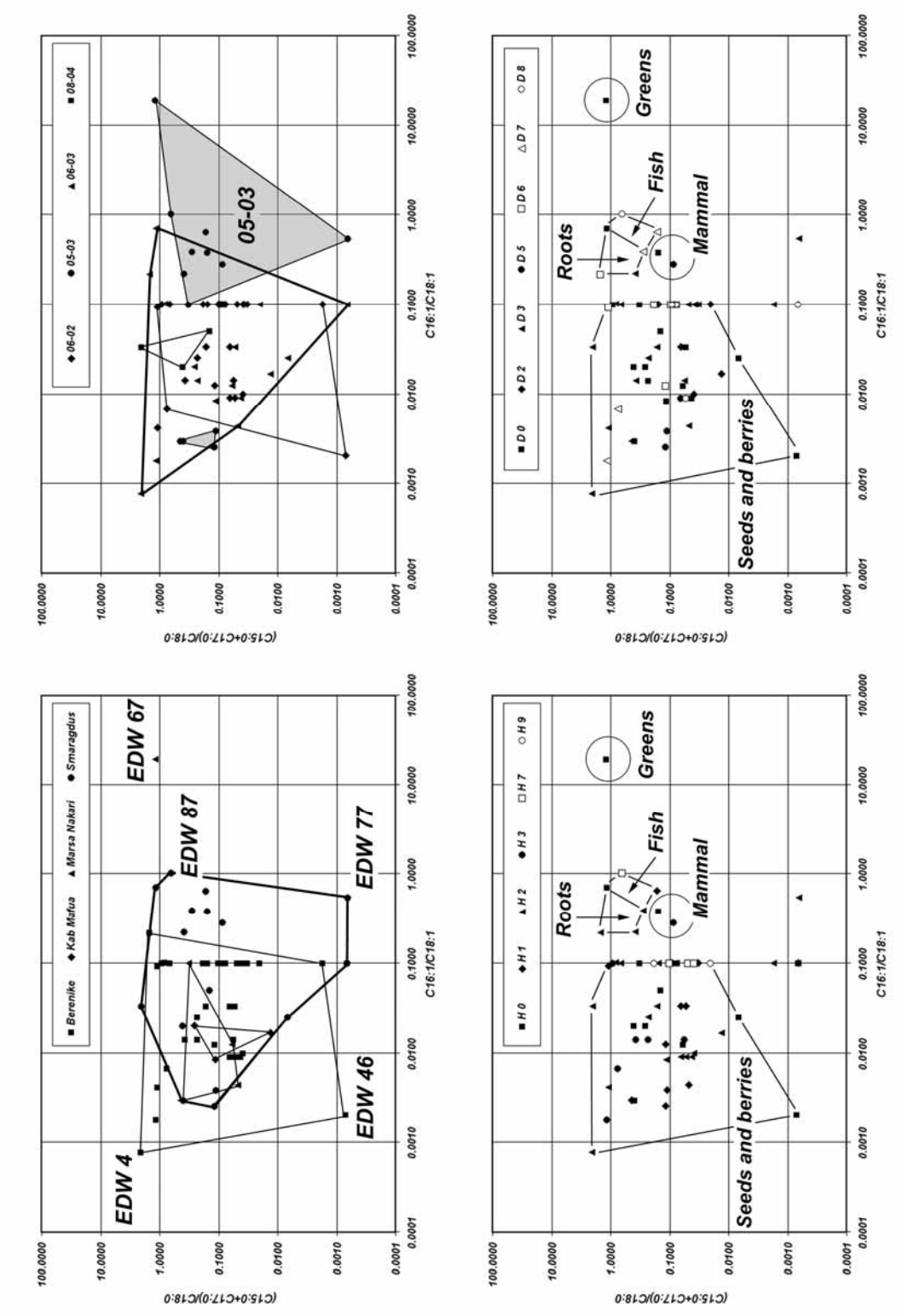

Figure 4-7: Graphic representation of the ratio of mono-unsaturated (X-axis) versus odd-chain fatty acids (Y-axis) in 51 Eastern Desert Ware sherds, marked for site (Table 4-1), method of analysis (Table 4-3), form of the vessel (H-classification) and lay-out of the decoration (D-classification).

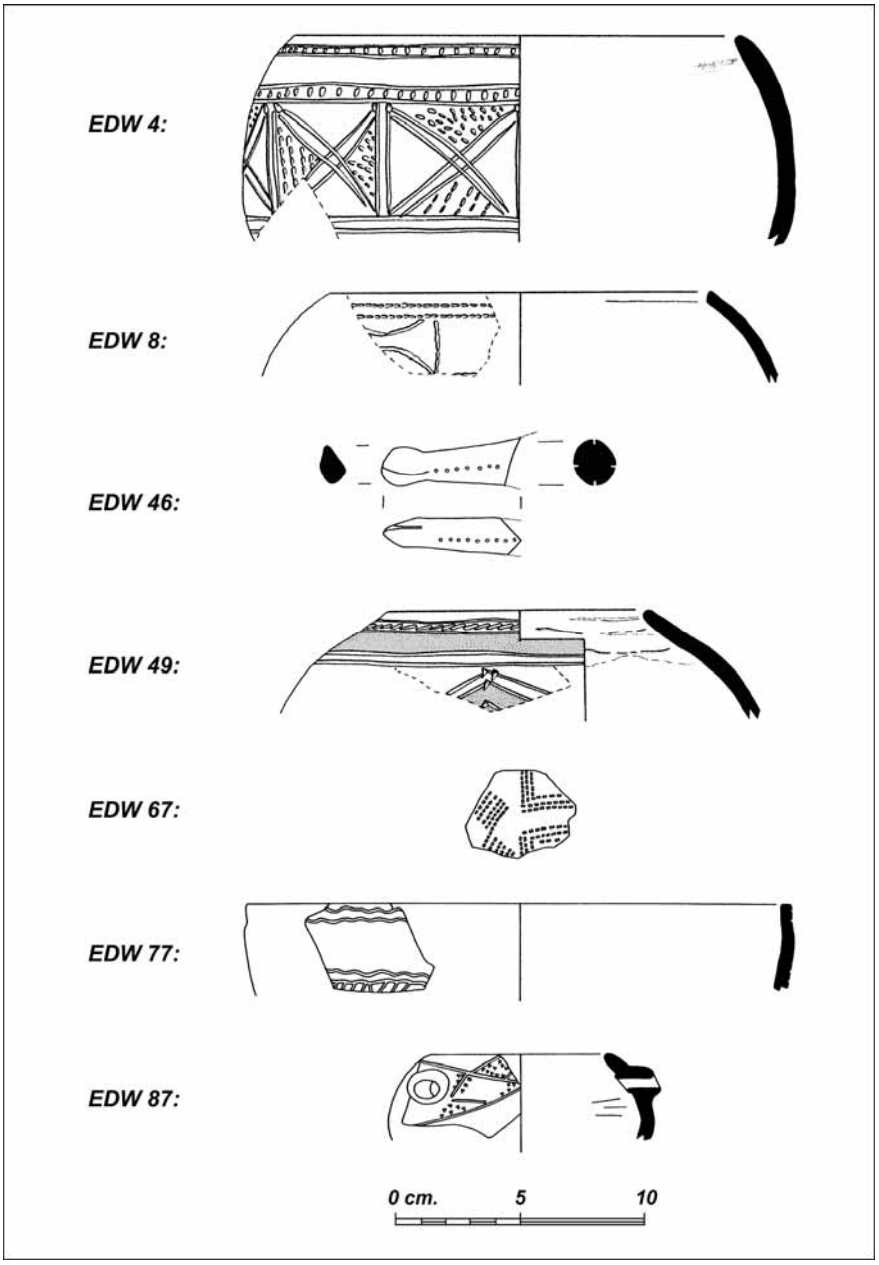

Figure 4-8: The seven Eastern Desert Ware vessels of which the organic residues preserved in their ceramic matrix are discussed in this chapter (Table 4-2; Figure 4-2; Appendix 5). Drawings by P.J. Rose and H. Barnard.

First, the C18:0/C16:0 and C18:1/C16:0 ratios were plotted for each individual sherd and compared with the same rations in six fresh foodstuffs (Figure 4-9, after Barnard et al. 2007a; b). The entry marked 'sample' (Figure 4-9, top-left) is a year-old residue of camel milk cooked in a previously unused vessel. It is obvious that over a relatively short period of time (one year) the C18:0/C16:0 ratio increases while the C18:1/C16:0 ratio decreases, thus dramatically changing the graphic representation of these ratios. This clearly demonstrates one of the central problems of comparing archaeological lipid residues with fresh foodstuffs, or even with relatively fresh residues: next to their limited specificity, the lipid residues change over time. This problem can be addressed by comparing the unknown residues with known residues that are artificially aged, for instance by long-term storage at high temperatures (Malainey et al. 1999a). The analysis of the data with different techniques, including the identification of biomarkers, and combining the results with archaeological information, is another approach to address this problem.

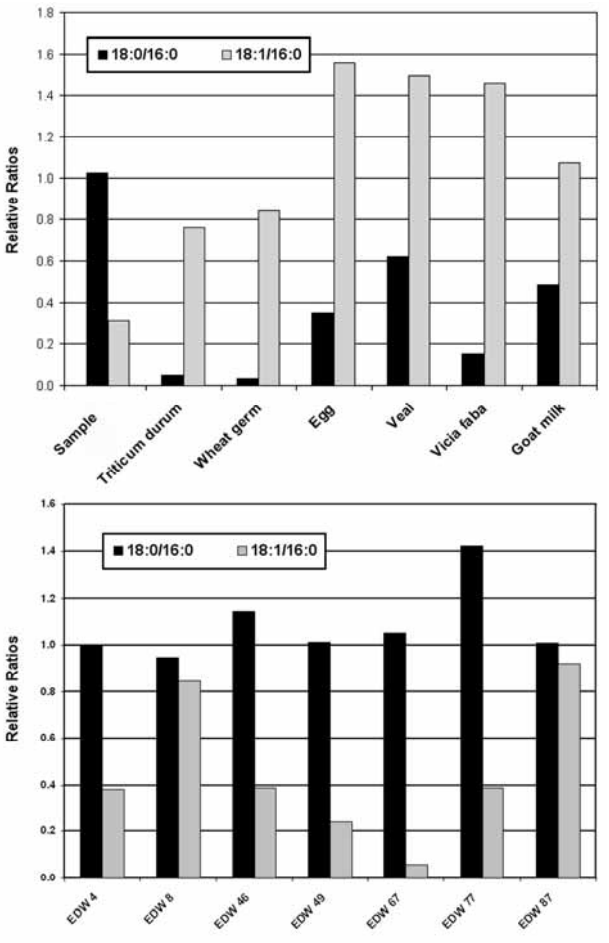

Figure 4-9: Selected fatty acid ratios in fresh foodstuffs (above, after Barnard et al. 2007a; b) and in seven selected Eastern Desert Ware sherds (below).

Additional analytical techniques, such as the determination of the stable carbon and nitrogen isotopes (Ambrose 1993, Barnard et al. 2007a; b; Morton and Schwarz 2004) or the analysis of the preserved proteins in the residue (Colombini and Modugno 2004; Craig et al. 2000; Malar et al. 2000; Yohe et al. 1991; Barnard et al. 2007d), will greatly increase the understanding of the residues. However, as such additional analysis require different samples and instruments, and will therefore be time consuming and costly (in terms of both money and archaeological material), they cannot be routinely applied.

The ratios of the mono-unsaturated fatty acids C16:1/C18:1 and the odd-chain fatty acids (C15:0+C17:0)/C18:0 are presented in Figure 4-10; the annotated chromatograms of EDW 4, 45, 77 and 87 in Figure 4-11; and two chromatograms of EDW 67 in Figure 4-12. Similar chromatograms of EDW 8 and 49 have been published elsewhere (Barnard et al. 2005). EDW 4 (Figure 4-11) preserved many saturated fatty acids, both even-chained and odd-chained, as well as the mono-unsaturated oleic acid (C18:1, 21.53 min after injection). Azelaic and oxysebacic acid (at 17.58 and 24.33 min after injection) are probably the oxidation products of longer mono-unsaturated fatty acids. Both odd-chain and mono-unsaturated fatty acids are more common in food of vegetable origin and thus concurrent with the previous identification of the residue as from 'seeds and berries' (cereals?) by fatty acids ratios. Germanicol and β-sitosterol (at 29.33 and 29.75 min) are plant hormones, but could also have originated from micro-organisms living off the residue after the vessels was discarded. Phytanic acid and cholestanyl (at 21.39 and 27.94 min) are probably of animal origin suggesting a residue of mixed origin. It is possible that some of the fatty acids, such as C16:0 and C18:0, were oversaturated, and subsequently underrepresented when the ratios were calculated, which will have shifted the calculated ratios. Taking all this into consideration EDW 4 may also have been used to contain fish, also relatively high in odd-chained and mono-unsaturated fatty acids as well as phytanic acid and cholestanyl. This would be concurrent with the provenance (Berenike) and shape (bowl) of the vessel. The plant hormones could have been introduced by micro-organisms or by foodstuffs of vegetable origin added to the fish or eaten from the same bowl at during a different meal.

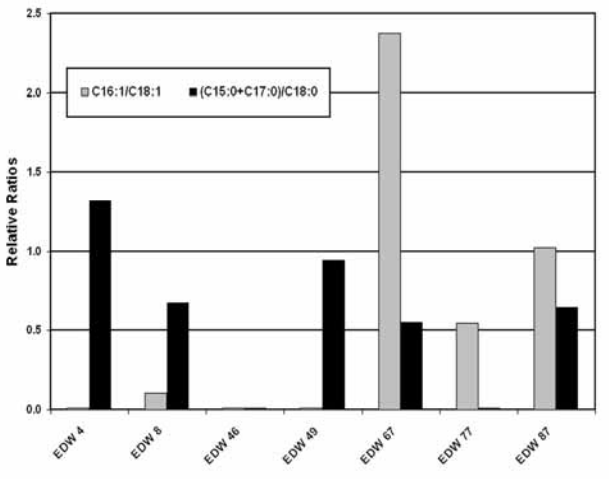

Figure 4-10: Ratios of mono-unsaturated and odd-chain fatty acids in seven selected Eastern Desert Ware sherds.

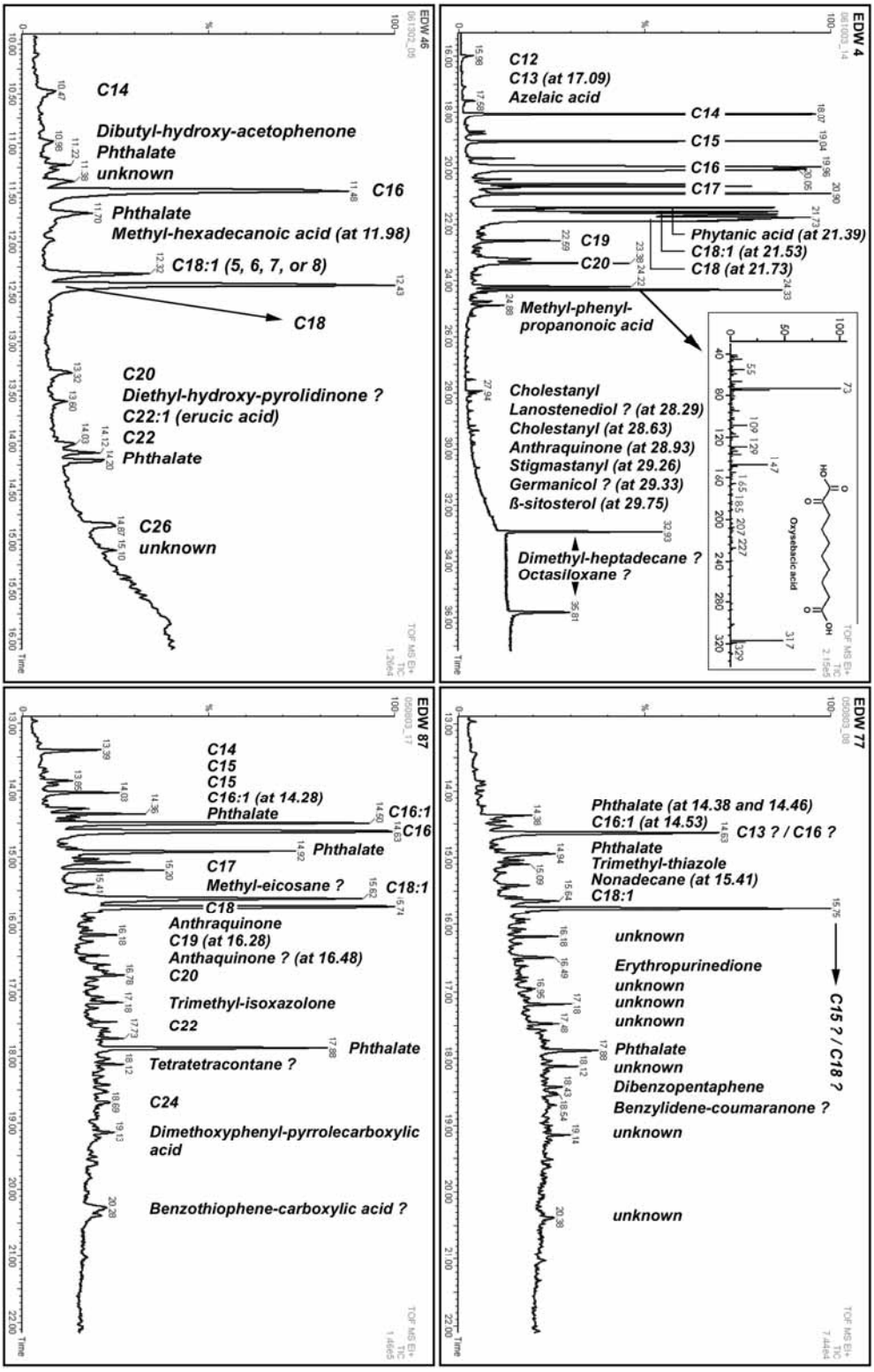

Figure 4-11: Chromatograms showing the organic molecules isolated from EDW 4, EDW 77, EDW 46 and EDW 87 identified by GC/MS (X-axis: retention times; Y-axis: relative intensities; TOF MS: time-of-flight mass spectrometer; EI+: electron impact ionization; TIC: total ion current).

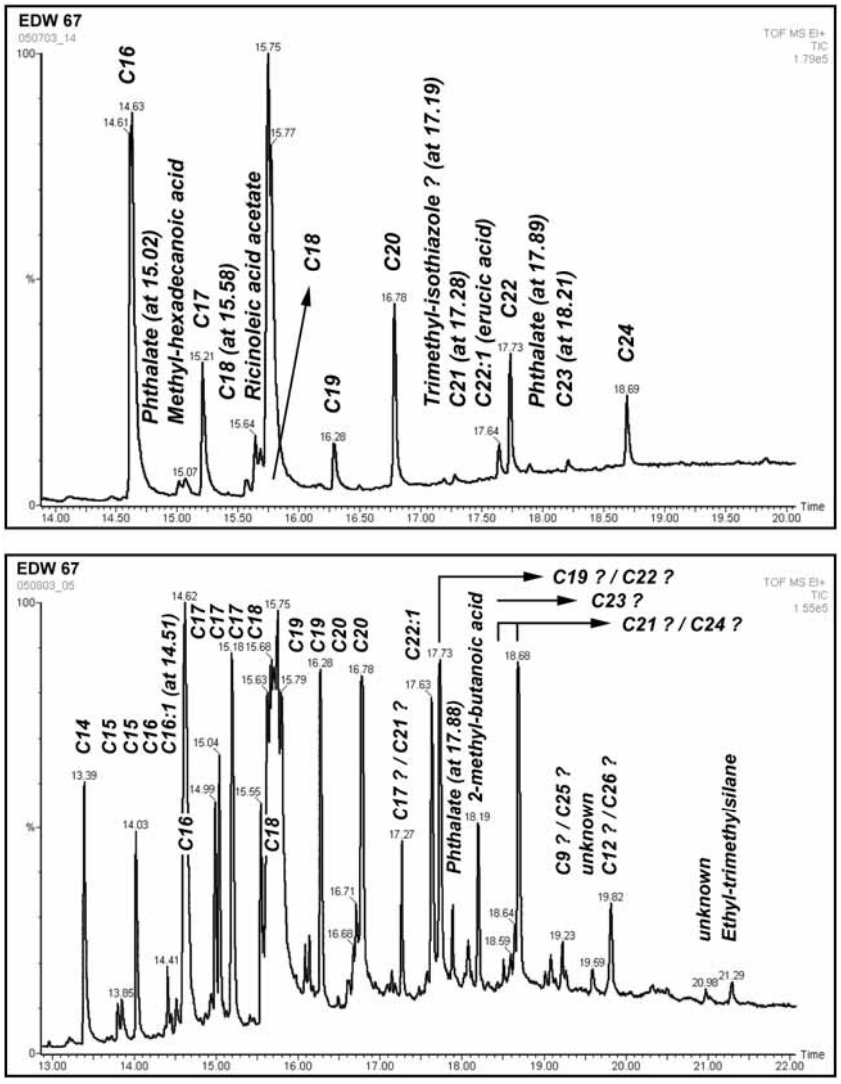

Figure 4-12: Two chromatograms, both acquired by method 05/03, showing the organic molecules isolated from EDW 67 identified by GC/MS (X-axis: retention times; Y-axis: relative intensities; TOF MS: time-of-flight mass spectrometer; EI+: electron impact ionization; TIC: total ion current).

EDW 46 (Figure 4-11) preserved palmitic (C16:0) and stearic (C18:0) acid in almost equal amounts, as well as low amounts of oleic (C18:1) and erucic (C22:1) acid. This is concurrent with the previous identification of the residue as from 'seeds and berries' (cereals?) by fatty acid ratios. As EDW 46 is a handle (Figure 4-8) it may have preserved smaller amounts of lipids compared to the attached vessel. The residue in the handle may also be different from the residue in the vessel, which has not been preserved. EDW 77 preserved low amounts of palmitic (C16:0) and stearic (C18:0) acid, as well as even smaller amounts of palmitoleic (C16:1) and oleic (C18:1) acid. Phthalates (at 14.38, 14.46 and 17.88 min) are man-made molecules introduced in the sample by laboratory plastics. Despite the fact that this residue occurs as an outlier in Figure 4-7, it most likely originated from vegetable foodstuffs naturally low in lipids. Alternatively, because of unfavourable post-depositional circumstances, elements of an originally richer residue may have oxidized below the level of detection. EDW 87 preserved palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic (C18:1) acid in almost equal amounts. Given the absence of other molecules of interest, anthraquinone is naturally present in some plants but is widely used as a dye in paper and textiles and is considered a contamination in this residue, EDW 87 most likely contained vegetable oil. This is concurrent with the shape of the vessel (spouted bowl). The identification of the residue as originating from fish, by fatty acid ratios (Figure 4-7), seems less likely when all data are taken into account.

EDW 67 (Figure 4-12) preserved many saturated and mono-unsaturated fatty acids and little other molecules of interest. This is concurrent with the identification, by fatty acid ratios, as from greens, although a vegetable source richer in lipids (nuts, vegetable oils) or vegetables mixed with oil or fat seems more likely given the abundance of lipids. Again the over-saturation of some of the fatty acids may have altered the fatty acid ratios. EDW 8 and EDW 49 both preserved rich and quite similar organic residues. The residue in EDW 8 was interpreted as originating from a meat stew, the residue in EDW 49 as originating from a vegetable stew (Barnard et al. 2005).

Discussion

The first and most important conclusion from the lipid residue analysis is that all Eastern Desert Ware sherds appeared to preserve organic residues. This indicates that the vessels were most likely used for food, and not solely for water or to serve as grave goods. Vessels found in graves probably belonged to the person or persons buried in the grave (Strouhal 1984). Alternatively, these vessels were used once during a burial feast that may have been part of the interment ritual. Such feasts were common in Ptolemaic Egypt, but have not been described in the region where Eastern Desert Ware was found. The high recovery rate of organic residues in Eastern Desert Ware illustrates the good state of preservation of the organic molecules trapped in the ceramic matrix of these vessels. These residues warrant additional analysis, not only by a combination of the methods described above, but preferably also by other laboratory techniques including stable carbon isotope or protein analysis (Barnard and Eerkens 2007).

Eastern Desert Ware cups (H 1) appear to have mostly contained cereals, vegetables or vegetable oils. Bowls (H 2) seem to have contained more meat and fish dishes. This shows that both were probably used for food and that the cups were not exclusively used for water or wine. Given the size of the bowls they were most likely meant for communal use, which is the usual way to present food in most ancient and many modern societies (Vroom 2003). This observation is concurrent with the presence of meat and fish. The cups may have been used for servings of small side-dishes of broth, porridge or stews, richer in vegetable foodstuffs, or for individual use. The fact that tubular-spouted vessels (H 7) seem associated with vegetable oils is concurrent with the assumptions that these vessels were intended to pour oil, fat or sauce on a meal.

Most of the organic residues in Eastern Desert Ware (including EDW 4 and 46) appear to originate from seeds or berries, most likely from cereals. This is interesting as the staple food of the current inhabitants of the region in which Eastern Desert Ware has been found is flour of sorghum or wheat (Wendrich 2008). This is consumed as a thick porridge (عصيدة , *aseedah*) from a communal bowl (قدح , *qadah* or برمة , *burmah*), or in the form of several kinds of bread (رداف , *ridaaf*, or قبوري , *qaburi*). The latter do not usually come into contact with ceramics after the mixing of the dough (Chapter 5).

The low number of possible fish residues is remarkable given the proximity of the sites to the Red Sea. This finding is concurrent, however, with the observation at Berenike of a shift in dietary patterns between the Ptolemaic and early Roman Periods (200 BCE-300 CE) and the late Roman Period (300-600 CE), the period during which Eastern Desert Ware was used. Archaeo-zoological research did show a decline in the consumption of (imported) beef and pork, as well as (Red Sea) fish, compensated by an increased consumption of ovicaprids (sheep and goats). This was interpreted as a shift in the population of the desert settlements (Sidebotham and Wendrich 1996, Van Neer and Lentacker 1996). The outsiders, originally from the Nile Valley and only temporarily in the area for economical reasons, may have been replaced or joined by a more desert-oriented population, probably the indigenous population of the region.

Another relevant finding is that the residues from the Mons Smaragdus area seem to have preserved more traces of animal sources (meat or fish), compared to those from the Red Sea coast. Such food is usually, but not necessarily, associated with a weathier population. In this case the residues could be indicative of the difference in resources between the population at the privately-owned beryl mines, in the Mons Smaragdus area, and the state-run harbours at Berenike and Marsa Nakari.

Several remarks can be made on the methodology. First is the observation that the samples have to be handled with some care. Not only need they be protected from contamination, by removing the surfaces and wearing gloves at all times, but also from oxidation and other chemical reactions, leading to the loss of molecules of interest, by storage at low temperatures in an inert environment. Second is that ancient lipids residues are not very specifically associated with particular foodstuffs. Their analysis can only yield interesting results when thoroughly interrogated and combined with archaeological, ethno-archaeological and experimental data. Ideally, the information should be complemented with data from other analytical techniques, such as stable isotope or protein analysis (Barnard and Eerkens 2007). For various reasons, such has unfortunately not yet been performed for Eastern Desert Ware.