Theory and Practice of Archaeological Residue Analysis

Edited by

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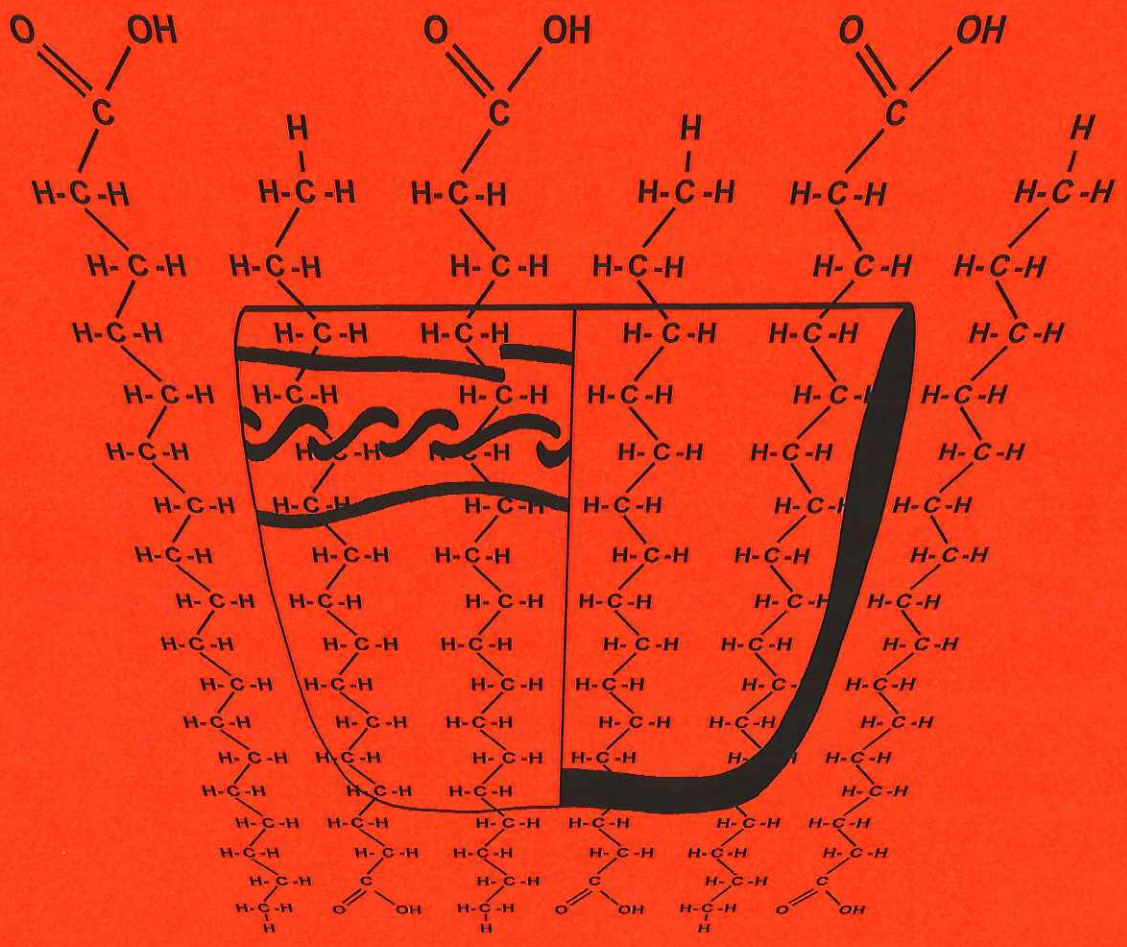

BAR International Series 1650
2007

This title published by

Archaeopress
Publishers of British Archaeological Reports
Gordon House
276 Banbury Road
Oxford OX2 7ED
England
bar@archaeopress.com
www.archaeopress.com

BAR S1650

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ISBN 978 1 4073 0084 9

Printed in England by Chalvington Digital

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CHAPTER NINE
A Comparative Study of Extractable Lipids in the Sherds and Surface Residual Crusts of Ceramic Vessels from Neolithic and Roman Iron Age Settlements in the Netherlands

T.F.M. Oudemans and J.J. Boon

Tania Oudemans; Institute for Atomic and Molecular Physics (AMOLF); P.O.-Box 41883; 1009 DB Amsterdam; the Netherlands; and Faculty of Archaeology; University of Leiden; P.O.-Box 9515; 2300 RA Leiden; the Netherlands; <info@taniaoudemans.com>; and Jaap Boon; Institute for Atomic and Molecular Physics (AMOLF);; P.O.-Box 41883; 1009 DB Amsterdam; the Netherlands. This work is part of the research program of the Faculty for Archaeology of the University of Leiden and Program 49 (BIOMSL) of the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organization for Scientific Research (NWO). We gratefully acknowledge both the FOM and the Faculty for Archaeology, Leiden for financial support of T.F.M. Oudemans. In addition, we would like to thank, T.J. ten Anscher, L.P. Louwe Kooijmans, L.L. Therkorn and the late A.A. Abbink for supplying samples and contextual information about the various sites. We also gratefully acknowledge the extensive technical support and the access to laboratory facilities provided by R.P. Evershed and co-workers. Finally, we would like to thank S.V. Tsygankova, for advice on our graphics, and C.C. Bakels, for critical reading of the manuscript.

Introduction: Lipid Analysis in Ceramic Studies

Pottery assemblages are a rich and durable source of information for the study of the daily behavior of people in the past. In order to assess the value of the information obtained from these assemblages, the use of the ancient vessels is an essential prerequisite. The identification of organic remains of ancient vessel contents can enable the retrieval of information about original vessel use. Since the 1970s, the study of organic residues has shown the preservation of many organic compounds in association with ceramics (Craig et al. 2000; Evershed et al. 1999; Evershed et al. 1992; Heron and Evershed 1993; McGovern et al. 1996; Mills and White 1987; Oudemans and Boon 1996; Oudemans et al. 2005; Pastorova et al. 1993; Regert and Rolando 2002; Rottländer and Schlichtherle 1979; Rottländer and Schlichtherle 1980).

The study of organic residues has focused primarily on fatty materials. Lipids are favored for organic residue studies due to their easy retrieval with solvent extraction and the continuous development of analytical techniques such as GC, GC/MS and gas chromatography isotope ratio mass spectrometry. Lipids also have obvious potential as diagnostic markers for the original vessel use due to their chemical stability (Eglinton and Logan 1991). In contrast to proteins and carbohydrates, lipids possess only a limited number of reactive sites resulting in relatively high resistance to thermal degradation during heating (Davídek et al. 1990, 169). In addition, the aliphatic nature of lipids results in low water solubility and thus enhances the immobilization of the molecular debris considered crucial to long term preservation at a molecular level (Eglinton and Logan 1991). Post-depositional exchange of lipids between residues and their surrounding soil has been shown to be very limited (Heron et al. 1991; Oudemans and Boon 1991; Oudemans, 2006).

Introduction: Types of Residues

In a few rare cases, lipids have been preserved as solidified or liquid substances in sealed vessels (Gibson and Evans 1985; Shedrinski et al. 1991), but most frequently lipids have survived in visible crusts adhering to the interior or exterior surface of a vessel (Hill and Evans 1988; Oudemans and Boon 1991; 1996; Oudemans et al. 2005; 2007; Oudemans and Erhardt 1996; Patrick et al. 1985; Regert and Rolando 2002; Rottländer and Schlichtherle 1979) or absorbed within the ceramic matrix of the vessels (Charters et al. 1995; Charters et al. 1993; Condamin et al. 1979; Dudd et al. 1998; Evershed et al. 1994; Evershed et al. 1990; Evershed et al. 1997; Gianno et al. 1990; Heron et al. 1991; Mottram et al. 1999; Passi et al. 1981; Regert et al. 1998).

The relative suitability of different types of residues for the identification of original vessel content has been discussed by a number of investigators. Although substances in sealed vessels can be in relatively good condition, their sparseness makes them less suitable for systematic study of vessel use. Absorbed lipids may occur more frequently than visible surface residues (Evershed et al. 1991), and have been claimed to be easier to identify due to their better preservation (Rottländer 1990). On the other hand, some researchers detected lipids in surface residues while none were found in the adjacent sherd (Needham and Evans 1987; Regert et al. 2001). A number of additional methodological advantages have been formulated for the study of surface residues (Oudemans and Boon 1991; Oudemans et al. in press). In short, the study of surface residues makes it possible to sample only a limited number of use phases, while absorbed residues represent the accumulated deposits of multiple use-phases in addition to possible post-firing sealing agents. Extractions of absorbed

residues may also include such sealing agents, complicating interpretation even more. Post-firing surface sealing with organic mixtures, the 'seasoning' of the vessel, is common amongst traditional potters and is performed with a variety of materials including common foodstuffs such as milk, oil and various starch-rich foods (Rice 1987, 163-164), as well as less edible materials such as beeswax, various resins and other plant materials (Arnold 1985, 139-140; Diallo et al. 1995; Kobayashi 1994). Stern et al. (2000) confirm that fatty acids extracted from Bronze Age Canaanite amphorae show that the jars were used to hold a lipid product, but that it was impossible to distinguish single use and multiple use. An additional reason to use surface residues in cooking vessels is the relatively higher degree of thermal degradation that has likely taken place in absorbed residues. Absorbed residues have usually been exposed to more severe heating regimes (both in temperature and in time) than residues situated on the interior surface of the vessel. Although numerous quantitative studies have been performed on lipids preserved in different residue types, no quantitative comparison of lipids extracts was ever published.

Introduction: Aims

In this study the extractable lipids of different types of residues are quantitatively analyzed using corrected flame ionization detector (FID) response factors for each compound. Comparisons are made to increase our knowledge of the differences in lipid chemistry between charred and non-charred surface residues; between surface residues and the lipids absorbed in the underlying ceramic material and between charred surface residues from the Roman Iron Age and the Neolithic. In order to facilitate the comparison of the lipid profiles, three operational parameters (the saturation index, the hydrolysis index and the odd carbon number fatty acid index) are defined. The main purpose of this chapter is to address the potential variation in lipid preservation in different sample materials and to discuss the possible biomolecular origin of the extracted lipids.

Experimental: Sample Material and Treatment

Organic residues from five different prehistoric contexts in the Netherlands were studied (Table 1). The main focus of this study was a ceramic assemblage recovered from an indigenous settlement at Uitgeest-Groot Dorregeest dating back to the Roman Iron Age (Abbink 1985; 1999). Both charred and non-charred residues were chosen for analysis. Non-charred surface residues from this settlement can appear as cream-colored crusts adhering to the interior vessel wall, or as red-brown films or dripping patterns on the interior or exterior vessel wall (Table 1). Surface residues were sampled as well as the ceramic fabric of the vessel directly underneath the surface residue. In one case (sample 34-0-12 from Uitgeest-Groot Dorregeest) three longitudinal sections of the vessel wall were sampled and lipids from the interior (S3), middle (S2) and exterior (S1) section of the vessel wall were extracted separately.

Charred surface residues of different age were collected to study the effect of burial time on the preservation of lipids. Residues from the Roman Iron Age settlements Schagen-Muggenburg (Abbink 1999; Therkorn 2004), Uitgeest-Groot Dorregeest and Uitgeesterbroekpolder 54 (Reyers 1985; Therkorn 2004) and from the Neolithic sites NO-Polder 14 (ten Anscher 2000/2001) and Hazendonk (Louwe Kooijmans 1974; 1976) were collected. All ceramic assemblages had roughly comparable burial conditions in peaty soil interspersed with sand and clay layers.

Most ceramics were washed in tap water, dried and stored in plastic bags for different lengths of time (up to 20 years). Ceramics from NO-polder 14 were treated specifically for organic residue sampling: directly after recovery from the field, pottery was wrapped in aluminum foil and stored at -20°C. Surface residues (about 5-10 mg) were scraped from the ceramics with a solvent cleaned scalpel, after removal of the upper 0.5 mm of the residue. Ceramic samples (about 2 g) were cut out of the vessel with a solvent cleaned scalpel, after removal of any surface residue and an additional 1 mm of ceramic. Samples were crushed in an agate mortar and stored in glass vials. Samples were prepared according to Evershed et al. (1990). In short, an internal standard (IS = 20 μg n-heptadecane) was added to each weighed sample, prior to extraction by solvent washing (10 ml chloroform/methanol, 2:1 v/v, 30 min ultrasonication). After centrifuging, the supernatant was dried in a round-bottomed flask by rotary evaporation at 50°C (in vacuum). A small amount (100 μl) of the solvent was added to transfer the total lipid extract (TLE) into a vial. One fifth (20 μl) of this extract was transferred into a second screw-topped vial and silylated with 25 μl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) and heated at 60°C for 10 min directly prior to analysis. All analytical grade solvents were distilled before use.

analytical column, a polyamide clad analytical column of 12 m x 0.22 mm coated with a BP1 stationary phase (OV-1 equivalent, 0.1 µm film thickness), via a stainless-steel union with an inner diameter of 0.8 mm (SGE). The GC oven was programmed from 50°C (2 min isothermal hold after injection) to 350°C at a rate of 10°C/min, after which the temperature was maintained isothermal for 15 min. Helium was used as carrier gas at a constant column head pressure of 1.7 atm. The GC/MS was performed using a similar column in a Pye Unicam 204 GC linked to a VG 7070H double-focusing magnetic sector mass spectrometer. The MS was operated in the EI+ mode (70 eV) with a source temperature of approximately 300°C, an acceleration voltage of 4 kV. The effluent was scanned over the range *m/z* 40-700 in a total cycle time of 3 s. The date acquisition and processing was performed on a Finnigan INCOS 2300 data system.

Experimental: Quantification

The quantitative data were derived from the peak areas measured using a GC with a FID. The peak areas were corrected for compound specific response with using the effective carbon number (ECN) per compound (Table 2), calculated according to Kaiser (1969, 99-103). The contribution of ester bonds was considered to be equal to the sum of an alcohol and a ketone group, being 0.55 for the 1- and 3-position and 0.35 for the 2-position in the acylglycerols (Ackman 1964). The ECN of unsaturated free fatty acids and monoacylglycerols (MAGs) is decreased with 0.1 per double bond (Scanlon and Willis 1985). Because saturated and unsaturated forms of diacylglycerols (DAGs) and triacylglycerols (TAGs) co-elute under current conditions, the effect of double bonds of acylglycerols (varying from 0.6 % for $D_{40:2}$ to 1.1 % for $T_{54:6}$) were neglected. The contribution of trimethylsilyl-groups (TMS) to the ECN of acids (3.0 for the -CO_2-TMS) and alcohols (3.69 for the H-C-O-TMS) were defined according to Scanlon and Willis (1985). Primary and secondary silylated alcohols were assumed to have the same contribution. The ECN of cholesterol was calculated at 29.19, assuming that cyclic C-atoms are comparable to aliphatic C-atoms, minus one double bond equivalent per closed ring, and that the TMS derivative of the 3β-hydroxyl group in cholesterol is comparable to the same group in an alcohol. One double bond in the 5-position was included in the ECN calculation of cholesterol.

The relative molar response factor $F(R\ molar)_i$ (Equation 1) expresses the relative amount of a component i necessary to obtain the same response (in area measured) as the IS (Kaiser 1969, 99-103; Scanlon and Willis 1985) and is defined as:

$$F(Rmolar)_i = \frac{ECN_{is}}{ECN_i} \qquad [1]$$

where ECN_{is} is the calculated ECN for the IS (17.00 for heptadecane) and ECN_i is the calculated ECN for compound i. Therefore the amount of every compound i present in the total sample A_i can now be calculated and expressed in mol:

$$A_i = A_{is} \cdot F(Rmolar)_i \cdot \frac{X_i}{X_{is}} \qquad [2]$$

where A_{is} is the known amount of IS added to the total sample (in mol), X_i is the measured relative peak area for compound i(in percent), and X_{is} is the measured relative peak area for the IS (in percent). In order to calculate the composition of samples before derivatization, the normalized weight percentage WP_i of the original compounds is calculated according to:

$$WP_i = \frac{A_i \cdot MW_{i\ (underivatized)}}{\sum_{i=1}^{n}\left(A_i \cdot MW_{i\ (underivatized)}\right)} \cdot 100\% \qquad [3]$$

where $MW_{i\ (underivatized)}$ is the molecular weight of compound i in underivatized form (in mg), and n is the total number of compounds in the sample.

Site	Period*	Sample number**	Appearance	N [%]	C [%]	H [%]	Total organic content [%]	C/N	C/H
Uitgeest-Groot Dorregeest	RIA	34-0-30	Char	5.93	41.16	3.72	50.81	6.94	11.06
Uitgeest-Groot Dorregeest	RIA	35-7-28	Cream colored	0.18	3.52	1.20	4.90	19.56	2.93
		35-7-28 S	Ceramic						
Uitgeest-Groot Dorregeest	RIA	34-0-12	Char	3.55	21.91	1.55	27.01	6.17	14.14
		34-0-12 S3	Ceramic						
		34-0-12 S2	Ceramic						
		34-0-12 S1	Ceramic						
Uitgeest-Groot Dorregeest	RIA	8-1	Red brown	0.79	6.55	1.25	8.59	8.29	5.24
		8-1 S	Ceramic						
Uitgeest-Groot Dorregeest	RIA	14-6-4.4	Char	5.51	60.13	4.12	69.76	10.91	14.59
		14-6-4.4 S	Ceramic						
Uitgeest-Groot Dorregeest	RIA	14-6-4.3c	Char	4.10	42.46	2.37	48.93	10.36	17.92
		14-6-4.3c S	Ceramic						
Uitgeest-Groot Dorregeest	RIA	14-6-4.2b	Char	4.97	29.19	3.20	37.36	5.87	9.12
		14-6-4.2b S	Ceramic						
Schagen-Muggenburg	RIA	79-1-1	Char	7.08	49.63	4.08	60.79	7.01	12.16
Uitgeest 54	RIA	226-48	Char	7.78	40.04	4.14	51.96	5.15	9.67
Uitgeest 54	RIA	320-17	Char	5.14	51.69	3.92	60.75	10.06	13.19
Hazendonk	Neo	32.740	Char	6.18	43.30	3.13	52.61	7.01	13.83
Hazendonk	Neo	33.781	Char	4.95	55.36	1.69	62.00	11.18	32.76
NO-Polder 14	Neo	6745	Char	4.71	52.68	3.37	60.76	11.18	15.63
NO-Polder 14	Neo	7054	Char	3.02	43.54	2.25	48.81	14.42	19.35
* RIA = Roman Iron Age; Neo = Neolithic									
** R = surface residues S = ceramic material from vessel wall									

Table 1: Overview of the archaeological samples discussed in this chapter.

Experimental: Instrumentation

The amounts of carbon, hydrogen and nitrogen (CHN analysis) were determined for all surface residues in order to get a rough indication of the overall organic composition of the sample. Elemental composition was performed after samples were dried, weighted and analyzed twice using a Carlo Erba 1500 CHN analyzer. Results were referenced in weight percentages using N-phenyl-acetamide or acetanilide (C_8H_9NO) as a standard to determine relative detector response. C/N and C/H ratios are directly calculated from their weight percentages (not on a molar basis).

The analytical GC work was on a Hewlett-Packard 5890A gas chromatograph equipped with a FID and a Hewlett-Packard 3396A computing integrator and plotter. On-column injection was used to introduce samples into a 60 cm x 0.32 mm inner diameter retention gap of de-activated fused silica, connected to the

Compound class	Mass peak (*m/z*)	Diagnostic fragment ions	Range**
Fatty acids*	M^+.		e/s: C8-C30
	$[M-15]^+$	$[M-CH_3]^+$	e/us: C16:1, C18:1,
	m/z 73	$[Si(Me)_3]^+$	C18:2, C20:1, C22:1
	m/z 75	$[HO=Si(Me)_3]^+$	o/s: C9-19, C23, C25
	m/z 117	$[Si(Me)_3OCO]^+$	
Monoacylglycerols *	$[M-15]^+$	$[M-CH_3]^+$	M14:0, M16:0, M16:1
	m/z 129	$[(Me)_3Si-O=CH-CH=CH_2]^+$	M18:0, M18:1
1-monoacyl	$[M-103]^+$	$[M-(CH_2-O-Si(Me)_3)]^+$	
2-monoacyl	*m/z* 218	$[(Me)_3SiO-CH=CH-CH_2-OSi(Me)_3]^+$	
Diacylglycerols *	$[M-15]^+$	$[M-CH_3]^+$	e: D26-D36
	m/z 129	$[(Me)_3Si-O=CH-CH=CH_2]^+$	o: D29-D35
	$[M-RCOO]^+$		
	$[M-(RCOO+1)]^+$	$[M-RCOOH]^+$	
	$[M-(RCOO+14)]^+$	$[M-RCOOCH_2]^+$	
	$[RCO]^+$	acyl fragment ion	
	$[RCO+74]^+$	$[RCOO-CH_2-CH(OH)CH_2]^+$	
	$[RCO+128]^+$	$[RCOO-CH_2-CH(O-C(CH-CH_2)-OCH_2]^+$	
Triacylglycerols	$[M-RCOO]^+$		e: T40-T45
	$[M-(RCOO+1)]^+$	$[M-RCOOH]^+$	o: T43-T53
	$[M-(RCOO+14)]^+$	$[M-RCOOCH_2]^+$	
	$[RCO]^+$	acyl fragment ion	
	$[RCO+74]^+$		
	$[RCO+128]^+$		
Cholesterol *	M^+.		
	$[M-15]^+$	$[M-CH_3]^+$	
	m/z 129	$[(Me)_3Si-O=CH-CH_2=CH]^+$	
	$[M-129]^+$	$[M-(Me)_3Si-O=CH-CH_2=CH]^+$	
Alcohols *	M^+.		e: C12-C18, C24-C32
	$[M-15]^+$	$[M-CH_3]^+$	o: C15
	m/z 103	$[(Me)_3Si-O-CH_2]^+$	
	m/z 75		
Elementary sulphur	*m/z* 64, 128, 256	S_2, S_4, S_8	
Other steroids *	*m/z* 215, 257		Stanols
Alkanes	*m/z* 57, 71, 85		C15-C32
Phthalate esters	*m/z* 149		dibutyl, dimethyl
Squalene	*m/z* 410	M^+.	

* detected in silylated form
** e/s = even numbered, saturated; o/s = odd numbered, saturated; e/us = even numbered, unsaturated

Table 2: Compounds detected by GC/MS.

The total lipid yield (TLY, Equation 4) of the extraction procedure is defined (in mg/g), according to:

$$TLY = \frac{\sum_{i=1}^{n}\left(A_i \cdot MW_{i\ (underivatized)}\right)}{W_s} \quad [4]$$

where W_s is the amount of sample used for extraction in gram. This calculation is based on the assumptions that the extraction is equivalent per lipid species and that a 100% extraction of the added IS is achieved.

In order to facilitate the comparison of lipid profiles, three operational parameters are defined that represent major aspects of lipid preservation and degradation. These indices are based on the relative total weight percentages per compound as calculated in Equation 3. The saturation index I_{sat} of the free fatty acids serves to express the proportion of saturated even carbon number fatty acids in the extract. The saturation index I_{sat} is defined as:

$$I_{sat} = \frac{\sum(WP_i\ saturated\ even\ FA)}{\sum(WP_i\ all\ even\ FA)} \quad [5]$$

This value is a tentative measure for the degree of polymerization that has occurred in the sample as a result of oxidation or heating under anoxic circumstances. The hydrolysis index I_{hydr} represents the proportion of even carbon number free fatty acids relative to all even carbon number acyl fragments in TAGs or free fatty acids. The hydrolysis index I_{hydr} is defined as:

$$I_{hydr} = \frac{\sum(WP_i\ even\ FA)}{\sum(WP_i\ even\ FA\ and\ all\ TAG)} \quad [6]$$

This parameter provides a measure for the degree of hydrolysis that has taken place in a sample. Acyl fragments can be hydrolyzed by microbial activity (enzymatic hydrolysis) and under alkaline or acidic conditions (chemical hydrolysis). The odd carbon number fatty acid index $I_{o/e}$ corresponds to the proportion of odd carbon number free fatty acids to the total fatty acid abundance and is defined as:

$$I_{o/e} = \frac{\sum(WP_i\ odd\ FA)}{\sum(WP_i\ all\ FA)} \quad [7]$$

Because the fatty acids C15:0 and C17:0 are the major contributors to the total weight in the numerator of this index, the $I_{o/e}$ can be interpreted as a reflection of the amount of bacterial material in the sample.

Results: CHN Analysis

Elemental CHN analysis (Table 1) shows a distinct difference in total organic content between the charred residues (27-70%) and the non-charred residue (4-9%). The non-charred residues consist primarily of inorganic compounds and contain hardly more organic material than the ceramic material, which has a total organic content of 4.7% (Oudemans et al. in press). Although the charred residues from Uitgeest-Groot Dorregeest showed more variation than charred residues from other sites, they are roughly comparable in overall chemical contents (Table 1). There is a considerable variation in elemental composition of the charred residues. The C/H ratios vary from 9.12-32.76, indicating a less aliphatic and more condensed nature of the material as the ratio increases. The C/N ratios vary from 5.15-14.42, indicating a decrease in the amount of nitrogen present in the material as the ratio increases.

Results: Qualitative Lipid Analysis

The compounds identified by GC/MS are summarized in Table 2 and further illustrated in Figures 1 and 2. The identity of the compounds in the TLEs were deduced largely from their EI+ mass spectra or from their TMS-derivatives using the characteristic ions (Table 2), given by Odham and Stenhagen (1972a; b) and Waller et al. (1972; 1980). Although isomers of C18:1 and diacylglycerols (DAGs) were detected, no isomer specific identification can be given under the analytical conditions employed. Isomers of C15:0 and C17:0 (normal-, iso- and anteiso-) and monoacylglycerols (1-, and 2- forms) were identified in some of the samples, but have been summarized in the quantitative results. The EI+ mass spectra of TAGs display such weak molecular ions (M^+) and fragment ions ($[M\text{-}18]^+$) that they are of little diagnostic value. The total carbon number of the TAGs was therefore established by comparison of the retention times with those of authentic compounds. Fragment ions representing the loss of one acyl moiety give information about the nature of diacyl fragments. The ions representing the acyl fragments give an indication of the ratio of acyl moieties present in the intact TAGs (Figure 3). All TAGs of a given total carbon number co-elute on the stationary phase as employed in this study. Hence, the identification of all TAGs is limited to molecular species.

Due to the high complexity of the mixtures analyzed, it was not always possible to identify all the peaks produced by high temperature gas chromatography (HTCG). Some of the minor components (including alkanes, wax esters and some steroids) could not be fully

identified due to low signal-to-noise ratios in the GC/MS analyses, or the absence of diagnostic ions in the mass spectra. The total measured peak area of all identified compounds (including the IS) in the HTGC varied between 51-100% (Table 3), with an average of 84% for surface residues (although in two samples only 53% was identified), and 69% for ceramic samples (although two samples contained no identifiable lipids and one sample only 5% that could be identified).

Results: Quantitative Lipid Analysis

A first assessment of a sample is made by calculation of the TLY based on all compounds identified by GC with an ECN which could be calculated (Equation 4, Tables 3 and 4). Phthalate esters were not included because they were considered contamination. TLYs show considerable variation between samples (Table 3), but general trends are visible. First, surface residues always yield more lipids per gram sample than the ceramic directly adjacent to it (20 to 1000 times higher). Surface residues from Uitgeest-Groot Dorregeest produce TLYs between 0.47-27.52 mg/g, while the adjacent ceramic samples yield TLYs between 0.00-0.16 mg/g. Second, most charred surface residues (9 of the 12 samples) produce lipid yields 5-50 times higher than non-charred surface residues (averaging 1.71 mg/g). Finally, charred residues from different excavations vary considerably in lipid yield. Those from Schagen-Muggenburg and Uitgeest 54 gave relatively high TLYs (between 43.43-139.56 mg/g) while those from Neolithic sites exhibit lower yields (between 1.77-19.59 mg/g) with an average TLY comparable to that from Uitgeest-Groot Dorregeest.

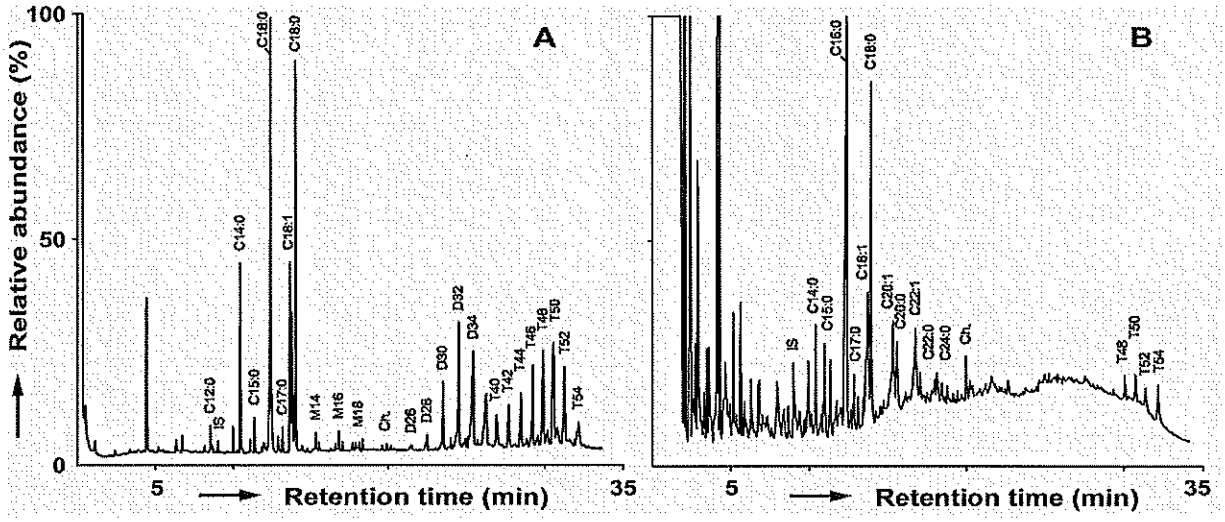


Figure 1: High temperature gas chromatograms of lipids from charred surface residues from sample 14-6-4.2b R from the Roman site Uitgeest-Groot Dorregeest (A) and sample 33.781 from the Neolithic site Hazendonk (B). IS = internal standard; M = monoacylglycerol; D = diacylglycerol; T = triacylglycerol, Ch = cholesterol and Ph = phthalate ester (numbers represent the total number of carbon atoms in the acyl moieties of lipids).

In order to provide a quantitative assessment of the highly complex lipid extracts, the normalized weight percentages of each identified underivatized lipid were calculated (Equation 3, Table 4). The chromatogram of charred residue 14-6-4.2b, from Uitgeest-Groot Dorregeest, shows several classes of compounds including free fatty acids, MAGs, DAGs, TAGs, cholesterol and phthalate esters (Figure 1A). Although some variation can be seen between the charred residues from Uitgeest-Groot Dorregeest, most samples were found to be of comparable lipid composition (Figure 2C R). However, the lipid composition of non-charred surface residues from Uitgeest-Groot Dorregeest appeared significantly different. These lipid profiles showed no odd carbon number fatty acids and relatively low percentages of free fatty acids as can be seen in the chromatogram of cream-colored residue 35-7-28 and red-brown residue 8-1 (Figures 2A R and 2B R).

Absorbed residues from Uitgeest-Groot Dorregeest yielded lower proportions of acyl lipids, and relatively more odd carbon number fatty acids than those of the surface residues (Figure 2, Table 4). Vessels with non-charred residues yielded little or no absorbed lipids from the ceramic matrix (Figures 2A S and 2B S). The comparison between lipid extracts from vessel walls and their directly adjacent surface residues was performed on six vessels, of which four preserved charred and two

preserved non-charred residues. The profile of the lipids absorbed in the vessel walls does not necessarily reflect that of the solid residue situated on the vessel surface. Only in two cases (14-6-4.3c and 34-0-12) very similar profiles were observed (Figures 2C R and S). All vessels with charred surface residues contained absorbed lipids. In sample 34-0-12 the middle section contained no identifiable lipids, while both interior and exterior produced low TLYs (Table 4). The interior section showed a lipid profile similar to the surface residue directly adjacent. The charred surface residues from the Neolithic sites rendered lipid traces that contained a relatively high percentage of free fatty acids, including long-chain fatty acids, up to 24 carbon atoms, no detectable MAGs and DAGs and higher proportions of odd carbon number fatty acids.

Site	Sample number	Appearance	Total lipid yield (mg/g)		Identified peak area [%]	I_{sat} [5]*	I_{hyd} [6]*	$I_{o/e}$ [7]*
			Corrected [4]*	Assuming linear response				
Uitgeest-Groot Dorregeest	34-0-30	Char	27.52	25.07	98%	0.78	0.51	0.07
Uitgeest-Groot Dorregeest	35-7-28	Cream	1.32	2.05	53%	1.00	0.39	0.00
	35-7-28 S	Ceramic	0.01	0.03	5%	-	1.00	0.00
Uitgeest-Groot Dorregeest	34-0-12	Char	0.47	0.58	91%	0.80	1.00	0.05
	34-0-12 S3	Ceramic	0.02	0.04	62%	1.00	1.00	0.21
	34-0-12 S2	Ceramic	-	-	-	-	-	-
	34-0-12 S1	Ceramic	0.02	0.02	92%	0.98	1.00	0.00
Uitgeest-Groot Dorregeest	8-1	Red brown	2.10	2.30	100%	1.00	0.10	0.00
	8-1 S	Ceramic	-	-	-	-	-	-
Uitgeest-Groot Dorregeest	14-6-4.4	Char	14.77	14.42	99%	0.70	0.78	0.07
	14-6-4.4 S	Ceramic	0.01	0.03	85%	0.85	1.00	0.11
Uitgeest-Groot Dorregeest	14-6-4.3c	Char	4.71	4.99	92%	0.89	0.82	0.10
	14-6-4.3c S	Ceramic	0.16	0.18	100%	0.92	0.76	0.09
Uitgeest-Groot Dorregeest	14-6-4.2b	Char	9.97	9.13	96%	0.82	0.54	0.08
	14-6-4.2b S	Ceramic	0.04	0.16	71%	0.92	1.00	0.18
Schagen-Muggernburg	79-1-1	Char	139.56	132.42	94%	0.61	0.39	0.10
Uitgeest 54	226-48	Char	52.48	53.70	74%	1.00	0.48	0.20
Uitgeest 54	320-17	Char	43.43	42.66	86%	0.96	0.43	0.15
Hazendonk	32.740	Char	19.59	22.06	83%	0.85	0.80	0.22
Hazendonk	33.781	Char	7.38	7.79	74%	0.74	0.90	0.12
NO-Polder 14	6745	Char	11.86	13.96	84%	1.00	0.43	0.33
NO-Polder 14	7054	Char	1.77	2.80	53%	1.00	0.53	0.64

Table 3: Total lipid yield and preservation indices. *) Numbers refer to equations in the text.

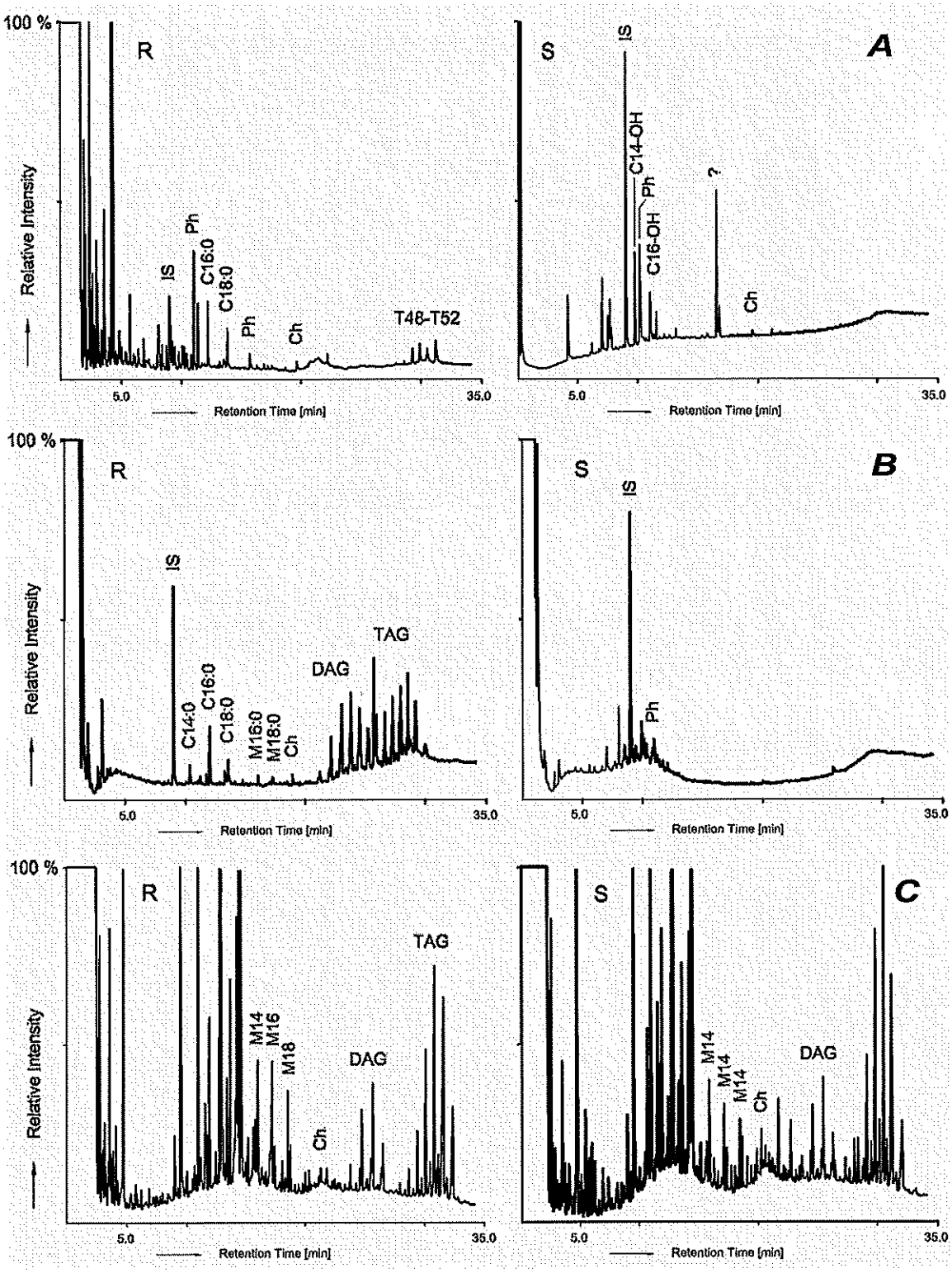


Figure 2: Comparison between lipids from surface residues (R) and lipids absorbed in sherds (S) in cream colored crust 34-7-28 (A), brown residue 8-1 (B) and charred residue 14-6-4.3c (C). IS = internal standard; M = monoacylglycerols; DAG = diacylglycerols; TAG = triacylglycerols, Ch = cholesterol and Ph = phthalate ester (numbers represent the total number of carbon atoms in the acyl moieties of lipids).

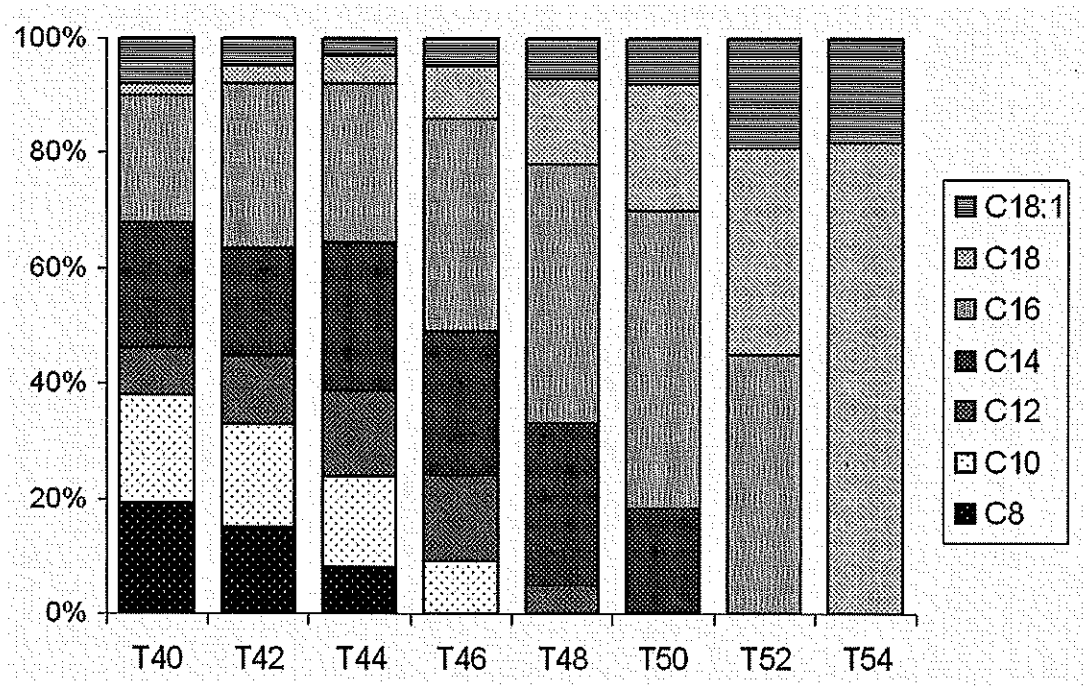


Figure 3: Relative composition of acyl fragments in mass spectra of intact even carbon number TAGs of charred surface residue 34-0-30 from Uitgeest-Groot Dorregeest. The percentages are relative numbers based on the intensities of R-COO^+ fragments in the EI+ mass spectra of each TAG. Assuming that fragmentation is equivalent for all acyl chains, these figures can be seen as representing the acyl composition of intact TAGs.

Discussion: Lipid Quantification

Most earlier quantitative lipid studies have been based on the assumption that all compounds exhibit similar responses in the FID of the GC. Although this assumption is valid when closely related compounds are being investigated, differences in response of the FID may be observed when compounds show widely varying chemical properties (Kaiser 1969, 99-103). In this study, rather than assuming equivalent responses for all components of the TLE, corrected response factors for each compound were calculated in order to enhance quantitative precision. Although differences between such TLYs and the traditional uncorrected TLYs are shown to be considerable (10-20%), especially when dealing with low overall yields (Table 3), they are not in the same order of magnitude as the differences between TLYs calculated for lipid extracts originating from different excavations (or even between different kinds of residues within one excavation). For instance, the ceramic material from Uitgeest-Groot Dorregeest shows uncorrected lipids yields between 0.02-0.18 mg/g, while lamps and dripping dishes from the medieval site at Raunds in the UK frequently contained yields between 0.1-1.0 mg/g (Charters et al. 1993; Evershed et al. 1999; Evershed et al. 1991), and amphorae from the Late Bronze Age in the Western Isles of Scotland contained between 0.025-0.3 mg/g lipid (Craig et al. 2005). These large differences completely overshadow the smaller differences due to correction of the FID response factors.

The approximation of equivalent response is probably sufficiently precise to allow general comparisons of extractable lipids in soil and potsherds (Heron et al. 1991), or comparisons between excavations, and probably sufficiently precise to demonstrate rough differences in concentration of lipids accumulated in different parts of vessels (Charters et al. 1993). However, the use of corrected FID response factors for each compound is especially relevant when comparing relative lipid compositions. Discrepancies of ± 10-15% for various compounds can be seen (Table 4). When quantification of each compound to microgram precision is needed for comparisons with published lipid compositions of reference materials, or detailed comparison between lipid profiles, correction is highly desirable.

Discussion: Chemotaxonomic Markers

The suitability of lipids as chemotaxonomic markers, or biomarkers, depends on their diagnostic value and their capacity for survival during long-term burial. Although even carbon number free fatty acids, with 4-24 carbon atoms, MAGs, DAGs and TAGs occur commonly in plant and animal fats (Hilldítch & Williams 1964, 6-25), not all different compound classes are equally suitable as

taxonomic markers. In this study only TAGs, sterols and free fatty acids were used as diagnostic chemotaxonomic markers. MAGs and DAGs are excluded because their origin is too ambiguous. They can be part of the original prehistoric lipid profile, be formed during hydrolysis of the original lipids or be the result of microbial activity. Although the same is true for free fatty acids, they can be diagnostic in specific cases.

TAGs are not produced by micro-organisms and therefore highly diagnostic for the plant or animal origin of the residue. Due to their insolubility in water, TAGs are not likely to leach out of their original depositional matrix and are unlikely to be exchanged with the surrounding soil. Oils, on the other hand, will undergo a 'drying process', a combination of cross-linking, polymerization and oxidation, if sufficient di- or tri-unsaturated acyl fragments are present in combination with oxygen (Mills and White 1987, 30-32). This effect will lead to a selective preservation of saturated extractable TAGs. A second chemical change that commonly occurs in TAGs is the chemical or enzymatic hydrolysis of the ester moieties leading to an overall loss of TAGs (Evershed et al. 1995a). Additionally, long-chain carboxylic acids in free fatty acids or TAGs can undergo condensation (though ketonic decarboxylation) when exposed to temperatures around 400°C in the presence of calcium salts (Evershed et al. 1995b; Raven et al. 1997). These condensation processes cause the formation of long-chain ketones in cooking vessels and a loss of TAGs. In short, even when the lipid profile contains adequate amounts of TAGs, they may not exactly reflect the original TAG composition.

Sterols are an important minor class of lipids with diagnostic value in organic residue studies (Evershed et al. 1992). Sterols are diagnostic for animal (cholesterol) or plant (sitosterol and campesterol) products. Sterols have low solubility in water and are not easily damaged by heating (damage will occur around 280-300°C), but are relatively easily oxidized into fats and oils (Davídek et al. 1990, 204, 216). The interpretation of cholesterol as an indicator for animal products must be made with caution because of the possibility of post-excavation contamination with cholesterol through handling of the potsherds. Some oxidation products of cholesterol have been detected in Saxon oil (Evershed et al. 1992). Microbial reduction of 5-sterols (like cholesterol) to 5α(H)- and 5β(H)-stanols occurs commonly under anaerobic conditions in the intestines of humans and animals and during diagenesis in sediments (Mackenzie et al. 1982). This process may also take place in the context of the original residue.

Although free fatty acids are abundantly present in most organic residues, they also illustrate most clearly the difficulty in assigning degraded lipids to a specific source. Extracted fatty acids may result from a mixture of different sources, including the remains of the original vessel content as well as the secondary products of microbial activity. In addition, a wide range of degradative pathways exists for free fatty acids, causing the overall free fatty acid composition to become an unreliable chemotaxonomic indicator. First, selective degradation of unsaturated fatty acids can occur as a result of oxidation or autoxidation in fresh materials (Davídek et al. 1990, 201-204). Additional condensation processes take place during heating or cooking of lipids (Malainey et al. 1999). When heated up to 270-300°C with limited access to oxygen, unsaturated lipids (primarily the polyunsaturated fatty acids typical for plant oils) will form cyclic hydrocarbons or acyclic polymers (Davídek et al. 1990, 195). Long-chain carboxylic acids can undergo condensation (though ketonic decarboxylation) when exposed to temperatures around 400°C in the presence of calcium salts (Evershed et al. 1995b; Raven et al. 1997). Together, all these network forming processes are likely to be responsible for the formation of non-extractable aliphatic structures of which the fragments (alkanes and alkenes) were detected in pyrolysates of some surface residues and most sherd samples (Oudemans and Boon 1991). Anoxic conditions and temperatures up to 300°C may well have been present in the ceramic wall of vessels during cooking, and in some of the surface residues during severe charring. Stern and co-workers confirmed the hypothesis that hard to extract fatty acids may indeed be present in the ceramic matrix, bound as cross-linked macromolecules (Stern et al. 2000).

Secondly, selective loss of short-chain fatty acids can occur as a result of enzymatic and non-enzymatic hydrolysis of acyl lipids during the use of the vessel as well as after deposition. The enhanced volatility and water solubility of short-chain fatty acids may also result in a selective loss. Enzymatic degradation of intact fatty acids by micro-organisms through β-oxidation can also play a role in this process through loss of one or more pairs of C atoms from the acyl chain (Leninger 1977). This effect is commonly observed in bog bodies and buried fats such as bog butter (Evershed 1992; Thornton et al. 1970). Third, alkaline environments enhance the transformation of free fatty acids to salts of fatty acids and can produce salts of various nature. Transformation of fatty acids into insoluble salts occurs commonly in fresh fat buried in the ground, for instance during the formation of adipocere (mortuary wax) which consists mainly of fatty acids and their calcium salts (Eglinton and Logan 1991). Some of these salts are relatively soluble in water and can cause fatty acids to leach out of their original matrix, while others, such as calcium and magnesium salts, are virtually insoluble in either water or organic solvents. Although this prevents leaching out, it also prevents extraction during analysis, resulting in deviant lipid profiles. Some researchers have warned for this phenomenon (Condamin et al. 1979; Rottländer and

Schlichtherle 1979). This conversion was shown to occur under arid conditions in an Ancient Egyptian sealed stone vessel. Of the oil probably stored in this vessel only a mixture of salts of long-chain fatty acids remained (Shedrinski et al. 1991). Stern and co-workers undertook to extract such salts using an acidic extraction of ceramic samples, but only released very low amounts of the 'recalcitrant' fatty acids. The researchers concluded the fatty acids were not salts but bound as cross-linked macromolecules (Stern et al. 2000).

Other known degradative pathways that commonly occur in fatty materials buried in the ground are the formation of hydroxy fatty acids formed through the hydration of double bonds in adipoceres (Den Dooren de Jong 1961; Evershed 1991; 1992), and the formation of isomers of mono-unsaturated fatty acids observed in bog bodies (Evershed 1991; 1992). No hydroxy fatty acids were detected in the lipids extracted in this study, so this pathway was obviously not active. Although the latter pathway may have taken place, isomers of C18:1 were not separated in this analysis, so no conclusions can be drawn about this process.

Discussion: Lipid Preservation and Degradation

Some significant aspects of lipid preservation and degradation can be studied using the operational parameters defined in this chapter (Table 4). The saturation index I_{sat} (Equation 5, Table 3) expresses the proportion of saturated even carbon number fatty acids in the residue and is a tentative measure for the degree of polymerization that has occurred in the sample as a result of thermal or oxidative degradation. Contrary to expectations, no correlation could be found between the saturation index (measuring the amount of polymerization in fatty acids) and the C/H ratio (a measure of the overall condensation in residue). This suggests that oxidation without heating plays a prominent additional role in the degree of saturation of the extractable lipids.

The hydrolysis index I_{hydr} (Equation 6, Table 3) provides a measure for the degree of hydrolysis that has taken place in a sample. Acyl fragments can be hydrolyzed by microbial activity (enzymatic hydrolysis), under alkaline or acidic conditions or as a result of heating in the presence of water (non-enzymatic hydrolysis). It must be kept in mind that, under alkaline conditions, free fatty acids may be present in the form of insoluble salts, which excludes them from extraction. However, under acidic conditions free fatty acids will be preserved in their free form in the original matrix (Eglinton and Logan 1991), unless subsequent degradation pathways have effected their preservation (such as the selective loss of short-chain or unsaturated fatty acids). It was noted that the hydrolysis index does not appear to be correlated to TLY in this study. This could indicate that the degree of hydrolysis does not determine the overall lipid preservation and that lipids are commonly preserved even after hydrolysis.

The odd carbon number fatty acid index $I_{o/e}$ (Equation 7, Table 3) corresponds to the proportion of odd carbon number free fatty acids to the total abundance of free fatty acids. In the extracts under investigation C15:0 and C17:0 are the major contributors to the total weight in the numerator of this index. Since these fatty acids are primarily formed during bacterial growth, the $I_{o/e}$ can be interpreted as a reflection of the relative amount of bacterial matter (directly or indirectly) contributed to the sample. Bacterial matter can be incorporated into the residue as part of a ruminant milk fat, during the original vessel use, as ruminant milk fat contains odd carbon number TAGs that can produce odd carbon number fatty acids after hydrolysis (Breckenridge and Kuksis 1967; Murata 1977). However, bacterial matter can also be incorporated into the residue in an indirect way during post-depositional bacterial degradation. The presence of the typical combination of n-, iso- and anteiso- isomers of C15:0 and C17:0 is diagnostic for bacterial growth (Nes and Nes 1980, 135; Shaw 1974). When odd carbon number TAGs are absent and lipid hydrolysis is not complete, a high $I_{o/e}$ should be considered indicative of post-depositional bacterial degradation.

In the charred residues a rough positive correlation exists between the C/N ratio and the C/H ratio, indicating that an increase in condensation goes hand in hand with a decrease in the amount of nitrogen present in the material. This is consistent with the conclusions from a combined FTIR/NMR study of the solid fraction of surface residues (Oudemans et al. 2007). Severe heating (over 250°C) over a longer period of time (over 2 hours) was shown to create progressively condensed materials with high C/H ratios (13-16), high overall organic contents (57-67%) and relatively few remaining biomolecular characteristics, such as nitrogen containing compounds or compounds with lipid characteristics. Because part of the charred residues under investigation fall within these parameters, low TLYs were expected from these residues (Table 1). However, the data show clearly that the highest amounts of lipid are extracted from charred residues with both a high C/H and a high C/N, thus necessitating modification of the above model of condensation. Although a certain amount of condensation is obviously desirable for preservation, the charred residue needs to have a nitrogen component in order to yield lipids. This correlation suggests that the presence of lipids is either determined by the presence of original biomaterials containing protein (meat, fish, high fat content seeds) or that the preservation of lipids is determined by the presence of nitrogen containing compounds contributing to the formation of the charred residue. The last effect could be caused by Maillard reactions (chemical reactions between amino acids and

reducing sugars, usually requiring the addition of heat), known to produce highly insoluble materials of strongly refractory nature (non-enzymatic browning).

Discussion: Possible Origin of Lipids

It is obvious from the above that the total extractable lipid composition in archaeological materials cannot be diagnostic unless careful consideration is given to all possible degradation mechanisms involved. A plant origin is hard to assign to any of the extracts studied. Although the relative proportion of unsaturated fatty acids is relatively low for plants, the presence of very long chain free fatty acids, with 26-30 carbon atoms, known to be the hydrolytic degradation products of wax esters (Kollattukudy 1976, 12), combined with the presence of long chain alcohols (such as occurred in sample 34-0-12 S3 from Uitgeest-Groot Dorregeest) suggests that this residue probably, at least partly, originated from plant material.

The presence of cholesterol leads to the tentative conclusion that several residues (34-0-30, 35-7-28, 34-0-12, 34-0-12.S3, 14-6-4.3c, 14-6-4.2b, 79-1-1, 320-17, 32740, 33781 and 6745) were, at least partly, of animal origin. The diagnostic use of cholesterol must be treated with caution as this compound also occurs in the surface lipids of human skin. If squalene is detected in the same extract this must be considered contaminated during preparation as the isoprenoid unsaturated hydrocarbon squalene also occurs in human skin fats. No squalene was detected in the extracts under consideration.

TAGs with an odd number of carbons in their acyl chains are know to occur in milk fats from cow milk (Murata 1977). Such TAGs were detected in five charred residues (34-0-30, 14-6-4.3c, 14-6-4.3c S, 14-6-4.2b and 79-1-1). Short-chain fatty acids, with 4-12 carbon atoms, are reported to be characteristic for dairy products (Breckenridge and Kuksis 1968; Hilldítch and Williams 1964, 144-145), but are absent in the extracts under consideration. Their absence can easily be caused by selective evaporation during heating, or selective leaching into the surrounding soil during burial. The presence of short-chain fatty acid moieties in some intact TAGs, as illustrated for residue 34-0-4 in Figure 3, is rather significant in this respect. A high abundance of components with 30-46 carbon atoms among the even carbon number TAGs was shown to be correlated to the presence of degraded ruminant milk fats in ceramics from the Iron Age and Roman period site in Stanwick in the UK (Dudd and Evershed 1998). A comparable TAG distribution pattern is clearly visible in five charred residues in Figure 4 (34-0-30, 14-6-4.3c, 14-6-4.3c S, 14-6-4.2b and 79-1-1). The additional presence of cholesterol leads to the conclusion that the lipids in these charred residues are, at least partially, derived from ruminant milk fats. TAG distribution patterns without a high abundance of components with 40-46 carbon atoms, lacking odd carbon number TAGs, but containing cholesterol, can be interpreted as, at least partially, derived from animal depot fats (Figure 4). Non-charred residue 35-7-28 and charred residues 320-17, 6745, 32.740 and 33.781 fall within this category.

Odd carbon number free fatty acids are primarily formed during bacterial growth and indicate the relative amount of bacterial matter that has become part of the residue. As described above, bacterial matter can be incorporated in the residue as part of ruminant milk fat. Bacterial matter incorporated in the residue as a result of post-depositional bacterial degradation in the soil, is shown in charred residues from Uitgeest 54 and NO-Polder 14. No odd carbon number TAGs were present in these residues and lipid hydrolysis is incomplete (average I_{hydr} = 0.48 for NO-Polder 14 and average I_{hydr} = 0.45 for Uitgeest 54). In these residues bacterial growth took place during post-depositional degradation. Charred residues from Hazendonk show a similar pattern, but due to the higher degree of hydrolysis (average I_{hydr} = 0.85) the origin of the odd carbon number fatty acids could not be ascribed to post-depositional bacterial degradation with any degree of certainty.

Discussion: Lipids from Surface Residues

Charred surface residues from Uitgeest-Groot Dorregeest exhibited an average I_{hydr} = 0.73, with charred residue 34-0-30 being less hydrolyzed then average and charred residue 34-0-12 completely hydrolyzed. The low $I_{o/e}$ with an average value of 0.07, indicates the presence of some odd carbon number free fatty acids, but not higher then 7% of the corrected TLY. The low I_{sat} with an average of 0.08, indicates limited bacterial activity and a relatively well preserved lipid profile. All charred residues except 34-0-12 contained extractable lipids derived from ruminant milk fats. Comparison of these results with those obtained from non-charred residues suggests a difference in original material or in mode of formation.

The non-charred surface residues from Uitgeest-Groot Dorregeest are completely saturated (average I_{sat} = 1.0), indicating a greater exposure to oxidizing conditions. The effects of hydrolysis are very limited in these residues (average I_{hydr} = 0.25), resulting in well-preserved TAG profiles completely without odd carbon number TAGs. Non-enzymatic hydrolysis of lipids is greatly enhanced by heating in the presence of water (Davídek et al. 1990, 186), which would suggest that these vessels were not used for cooking fatty substances in water. The complete absence of odd carbon number fatty acids shows that bacterial growth has occurred to a very limited extent, suggesting the formation of a denatured material prior to deposition in the soil, possibly as a result of polymerization or network

formation. These two residues are visually and chemically different, but it is possible that they were both regularly exposed to the air during their formation. The vessel containing residue 35-7-28 may have been used for storage or transport of solid materials, while residue 8-1 may have been applied as decoration prior to the use of the vessel. It is clear that these vessels were not used as cooking vessels.

Discussion: Lipids Absorbed in Ceramics

The ceramic samples of two vessels containing non-charred residues did yield extremely low concentrations of extractable lipids (<0.01 mg/g) that might easily be dismissed as blanks (Figure 2). The ceramic material 8-1 S contained only traces of a few unidentified aliphatic compounds and contaminants (phthalates), indicating a lack of absorption of lipids into the vessel during use. The ceramic material of 35-7-28 S contained contaminants and some even carbon number alcohols, with 12-18 carbon atoms, that are, as yet, unexplained.

The four vessels containing charred residues all yielded extractable lipids from the ceramic material directly adjacent to the residues. In one case (14-6-4.3c S) a lipid profile was obtained from the ceramic material that was almost identical to that from the charred residue, suggesting the same origin (Figure 2C). In the other sample pairs (34-0-12, 14-6-4.2b and 14-6-4.4) extractable lipid profiles from surface residues and ceramic samples were quite different. The most significant difference is an increased saturation (average I_{sat} = 0.92), combined with a complete hydrolysis (average I_{hydr} = 1.0), and an increased odd carbon number fatty acid index (average $I_{o/e}$ = 0.17). Lipids extracted from charred surface residues are obviously better preserved than those extracted from the directly adjacent ceramic material of the vessel. This difference in preservation is most likely the result of a combination of chemical mechanisms. Most important, absorbed lipids will have been submitted to a more extreme thermal regime due to higher temperatures inside the ceramic vessel wall and repetitive cooking phases. This more extensive thermal exposure may have caused both the complete hydrolysis of lipids, due to heating in the presence of water, and the high degree of saturation, due to heat induced polymerization. The increase of $I_{o/e}$ is hard to explain due to the complete hydrolysis. In the case of 14-6-4.2 S the extractable lipids may have resulted from complete hydrolysis of a milk fat residue (just like its adjacent residue). In the case of 14-6-4.4 and 34-0-12 an increased bacterial influence could be the cause. Although this explanation seems counter-intuitive, because absorption of lipids in the ceramic matrix of the vessel wall would be expected to reduce external influences, the refractory nature of the charred material obviously better prevents bacterial degradation. Evens (1990) and Oudemans and Boon (1991) propose that the preservation of lipids in the charred matrix may be enhanced by means of micro-encapsulation of small amounts of lipids during the formation of the charred residue, although the mechanisms of encapsulation are still unknown.

Discussion: Chars from Other Sites

Extractable lipid profiles from Neolithic charred residues differ from those from the Roman period in that they often lack MAGs and DAGs, and that no odd carbon number TAGs are preserved. All the charred residues from the Neolithic contain cholesterol and present even carbon number TAG distribution patterns that can be interpreted as originating from animal depot fats (Figure 4). Hydrolysis (average I_{hydr} = 0.67) and saturation (average I_{sat} = 0.90) are comparable to those of charred residues from the Roman period, with an average I_{hydr} = 0.71 and an average I_{sat} = 0.94. The only profound difference is an increase in the $I_{o/e}$ of the Neolithic charred residues (average $I_{o/e}$ = 0.33) compared to charred residues from the Roman period (average $I_{o/e}$ = 0.12). Because of the absence of other indicators for milk fats, this increase is interpreted as a higher degree of bacterial growth resulting from a longer period of burial in the ground.

Some combinations of index values appear to be typical for a specific settlement. For example, settlement NO-Polder 14 shows a high saturation index (I_{sat} = 1.0) combined with relatively low degree of hydrolysis (I_{hydr} = 0.5), while Hazendonk shows a low degree of saturation (I_{sat} = 0.8) and a much more extensive hydrolysis (I_{hydr} = 0.9). Although both sites date to the Neolithic, the index values of NO-Polder 14 resemble those of the native Roman site Uitgeest 54 more closely than those of Hazendonk. These site-specific effects may be the result of local preserving conditions at the different sites, although the number of samples studied is too small to draw firm conclusion.

Discussion: Sampling Issues

Quantitative comparison of extractable lipids of surface residues and absorbed residues shows an apparent greater degree of preservation of extractable lipids in surface residues than in the directly adjacent ceramic fabric. Surface residues are a more attractive target material for identification of original vessel contents. An argument can be made for the combined study of both surface residues and absorbed residues. The mechanisms responsible for the formation of surface residues and absorbed residues are clearly dependant on vessel use. Data presented in this study show the virtual absence of absorbed extractable lipids underneath non-charred surface residues, indicating that some kinds of vessel use lead to the accumulation of surface residues and little or no absorbed extractable lipids (such as decoration of

vessels or their use as serving dishes or storage and transport vessel of dry goods). Others uses may cause the accumulation of absorbed lipids but produce little or no surface residues (such as storage or transport of oily or fatty liquids). Another argument for dual studies of surface residues and absorbed residues is the possibility of detecting multiple use-phases in one vessel. For these reasons, ideally, samples of both surface residues and absorbed organics should be studied in order to enhance the overall understanding of vessel use based on organic residue analysis.

Conclusions

The quantitative study of the extractable lipid composition (including fatty acids, MAGs, DAGs, TAGs, sterols and long-chain alcohols), shows an apparently greater degree of preservation of lipids in surface residues than in the directly adjacent ceramic fabric of the vessel. Not only is the TLY per gram sample much higher in surface residues (especially in charred surface residues), but the amount of intact acyl lipids (as expressed in the hydrolysis index) and the amount of unsaturated fatty acids are also much higher in surface residues. This difference in preservation is proposed to be the result of a more severe thermal regime inside the vessel wall and the highly refractory nature of charred surface residues. This observation may have important consequences for sampling strategies in archaeological organic residue analysis.

Lipid extracts of charred and non-charred surface residues are very different in composition. Charred surface residues show varying degrees of condensation (C/H ratios between 9-33), indicating more or less severe thermal degradation due to the heating of organic materials. In spite of this thermal degradation, charred surface residues show the highest yields of extractable lipids per gram sample. Non-charred residues show many characteristics (low overall organic contents, a lower degree of hydrolysis, little or no bacterial degradation and little and no absorption of lipids into the directly adjacent ceramic vessel) that suggest a very different kind of vessel use. Most likely these organic residues are the result of a use with longer period of exposure to oxygen without severe heating. Non-charred residues may be the result of decoration with organic materials, or the residue of solids stored or transported in the vessel.

Lipids from charred surface residues from two Neolithic sites (ca. 5000 years old) were compared to charred residue from three native Roman settlements (ca. 1800-2000 years old). Although Neolithic charred residues showed comparable lipid yields, their lipid profiles contained a relatively higher proportion of material of bacterial origin. In spite of indications for site-specific degradation, this phenomenon is proposed to be the result of ongoing low-level microbial degradation.

An important focus for future work in archaeological organic residue analysis should be the mechanisms responsible for the deposition and accumulation of residues in vessels. Our results show that surface residues are a more attractive sample material for the identification of the original vessel content. Some types of vessel-use were shown to lead to the accumulation of surface residues and little or no absorbed lipids. In theory, others may lead to the accumulation of absorbed lipids without forming any surface residue. For this reason, ideally, sample of both surface residues and absorbed organics, preferably taken from the same vessel, should be studied.

		Uitgeest-Groot Dorregeest							
		34-0-30 *)			35-7-28		34-0-12		
		R			R	S	R	S3	S1
		Charred			Cream-colored		Charred		
TLY [mg/g]:		27.52			1.32	0.01	0.47	0.02	0.02
Lipid	ECN	Wpi [%]	Δ [%]	Xi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
Free fatty acids									
C12:0	14.00	0.63	6.6	0.68					
C13:0	15.00								
C14:0	16.00	3.78	6.8	4.12			4.39	9.04	24.57
C15:0	17.00	0.50	6.9	0.55				7.93	
C15:0	17.00	0.75	6.9	0.82				5.05	
C16:1	17.90						1.45		
C16:0	18.00	15.84	7.0	17.39	25.15		29.56	21.64	49.38
C17:0	19.00	0.65	7.1	0.71			4.08		
C17:0	19.00	0.52	7.1	0.57				2.74	
C18:2	19.80	0.24	7.6	0.27					
C18:1	19.90	8.56	7.4	9.38			16.78		2.30
C18:0	20.00	9.58	7.2	10.48	13.20		35.35	23.80	23.75
C19:0	21.00	0.09	7.3	0.10					
C20:1	21.90								
C20:0	22.00	0.45	7.3	0.49					
C22:1	23.90								
C22:0	24.00	0.20	7.4	0.21					
C23:0	25.00	0.28	7.6	0.31					
C24:0	26.00	0.26	7.5	0.29			1.56	3.96	
C26:0	28.00	0.13	7.6	0.14				6.10	
C28:0	30.00							5.26	
C30:0	32.00							3.44	
Alcohols									
C12-OH	14.69					14.70			
C14-OH	16.69					59.65			
C15-OH	17.69								
C16-OH	18.69					17.97			
C18-OH	20.69					7.68			
C24-i-OH	26.69							3.84	
C26-i-OH	28.69								
C28-OH	30.69								
C30-OH	32.69								
C32-OH	34.69								

Table 4a: Lipid composition extracts from surface (R) and absorbed (S) residues 34-0-30, 35-7-27 and 34-0-12 expressed in relative weight percentages WP_i (in %). ECN is the calculated effective carbon number per compound. *) For sample 34-0-30 R the corrected WP_i and the uncorrected X_i are given per detected compound. The difference is expressed by Δ in percent relative to WP_i.

		Uitgeest-Groot Dorregeest							
		34-0-30 *)			35-7-28		34-0-12		
		R			R	S	R	S3	S1
		Charred			Cream-colored		Charred		
TLY [mg/g]:		27.52			1.32	0.01	0.47	0.02	0.02
Lipid	ECN	Wpi [%]	Δ [%]	Xi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
MAG									
M14:0	20.93	0.14	5.5	0.15					
M16:0	22.93	0.45	5.8	0.49				1.32	
M18:1	24.83	0.49	5.2	0.53					
M18:0	24.93						1.76	1.97	
DAG									
D28	30.69	0.82	-8.8	0.77					
D29	31.69								
D30	32.69	2.27	-7.9	2.13					
D31	33.69	0.27	-7.4	0.26					
D32	34.69	4.79	-5.4	24.62					
D33	35.69	0.30	-6.7	0.29					
D34	36.69	5.55	-5.6	5.35					
D35	37.69	0.12	-5.9	0.11					
D36	38.69	4.58	-5.7	4.41					
TAG									
T40	38.45	2.66	-15.7	2.29					
T42	40.45	2.44	-14.7	2.12					
T43	41.45								
T44	42.45	2.72	-13.8	2.39					
T45	43.45	1.02	-13.4	0.90					
T46	44.45	3.52	-13.0	3.12					
T47	45.45	0.42	-12.6	0.38					
T48	46.45	4.79	-12.3	4.29	8.59				
T49	47.45	1.18	-11.6	1.06					
T50	48.45	7.19	-11.5	6.45	14.19				
T51	49.45	1.71	-11.2	1.55					
T52	50.45	6.86	-10.9	6.23	6.16				
T53	51.45								
T54	52.45	3.05	-10.3	2.80	29.94				
Other									
C9-diacid	13.00								
Cholesterol	29.19	0.20	15.1	0.23	2.77		5.07	3.92	
Total		**100**		**100**	**100**	**100**	**100**	**100**	**100**

Table 4b: Lipid composition extracts from surface (R) and absorbed (S) residues 34-0-30, 35-7-27 and 34-0-12 expressed in corrected relative weight percentages WP_i (in %). The numbers indicating the different DAGs and TAGs represent the total number of carbon atoms in the acyl fragments. ECN is the calculated effective carbon number per compound. *) For sample 34-0-30 R the corrected WP_i and the uncorrected X_i are given per detected compound. The difference is expressed by Δ in percent relative to WP_i.

		Uitgeest-Groot Dorregeest						
		8-1	14-6-4.4		14-6-4.3c		14-6-4.2b	
		R	R	S	R	S	R	S
		Brown	Charred		Charred		Charred	
TLY [mg/g]:		2.10	14.77	0.01	4.71	0.16	9.97	0.04
Lipid	ECN	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
Free fatty acids								
C12:0	14.00		0.29	1.99	0.50	0.67	0.46	
C13:0	15.00					0.60	0.07	
C14:0	16.00	1.00	5.34	12.10	4.35	5.47	2.76	13.22
C15:0	17.00		0.72		1.54		0.39	
C15:0	17.00		1.21	5.35	1.71	2.39	0.65	14.95
C16:1	17.90		0.44	4.23	0.46			
C16:0	18.00	3.55	27.69	40.36	26.20	27.80	12.11	37.74
C17:0	19.00		1.46		1.68	1.32	0.54	
C17:0	19.00		1.37	3.98	1.84	1.73	0.48	
C18:2	19.80							
C18:1	19.90		19.63	9.10	7.04	5.25	6.52	7.13
C18:0	20.00	2.06	12.81	21.94	30.82	24.56	12.48	26.96
C19:0	21.00				0.34			
C20:1	21.90							
C20:0	22.00		1.61		0.70	1.22	0.38	
C22:1	23.90							
C22:0	24.00						0.43	
C23:0	25.00						0.60	
C24:0	26.00				0.36	0.50	0.49	
C26:0	28.00						0.30	
C28:0	30.00							
C30:0	32.00							
Alcohols								
C12-OH	14.69							
C14-OH	16.69							
C15-OH	17.69							
C16-OH	18.69							
C18-OH	20.69							
C24-i-OH	26.69							
C26-i-OH	28.69					0.17		
C28-OH	30.69					0.23		
C30-OH	32.69					0.70		
C32-OH	34.69					0.73		

Table 4c: Lipid composition extracts from surface (R) and absorbed (S) residues 8-1, 14-6-4.4, 16-6-4.3c and 14-6-4.2b expressed in corrected relative weight percentages WP_i (in %). ECN is the calculated effective carbon number per compound.

		Uitgeest-Groot Dorregeest						
		8-1	14-6-4.4		14-6-4.3c		14-6-4.2b	
		R	R	S	R	S	R	S
		Brown	Charred		Charred		Charred	
TLY [mg/g]:		2.10	14.77	0.01	4.71	0.16	9.97	0.04
Lipid	ECN	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
MAG								
M14:0	20.93						0.66	
M16:0	22.93		0.29		1.29	1.18	1.07	
M18:1	24.83							
M18:0	24.93				1.05	0.83	0.94	
DAG								
D28	30.69				0.19		0.90	
D29	31.69						0.07	
D30	32.69	2.91	1.10		0.39	0.51	3.65	
D31	33.69						0.88	
D32	34.69	8.81	2.82		0.99	1.32	7.45	
D33	35.69						1.32	
D34	36.69	11.56	3.39		1.54	1.45	8.40	
D35	37.69							
D36	38.69	11.19	0.53		0.98	0.63	4.76	
TAG								
T40	38.45	7.17					0.26	
T42	40.45	7.22	0.59		0.18	0.23	0.91	
T43	41.45						0.19	
T44	42.45	6.48	1.11		0.38	0.55	1.60	
T45	43.45				0.07			
T46	44.45	7.51	2.09		0.82	1.57	2.85	
T47	45.45				0.26	0.68	1.13	
T48	46.45	9.81	4.12		1.78	3.28	4.52	
T49	47.45				0.54	0.88	1.80	
T50	48.45	13.36	6.63		3.52	4.98	6.55	
T51	49.45				1.15	1.01	1.85	
T52	50.45	7.38	4.77		4.36	4.65	6.15	
T53	51.45				0.34		0.82	
T54	52.45				2.46	2.46	2.02	
Other								
C9-diacid	13.00							
Cholesterol	29.19			0.95	0.20	0.47	0.62	
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**

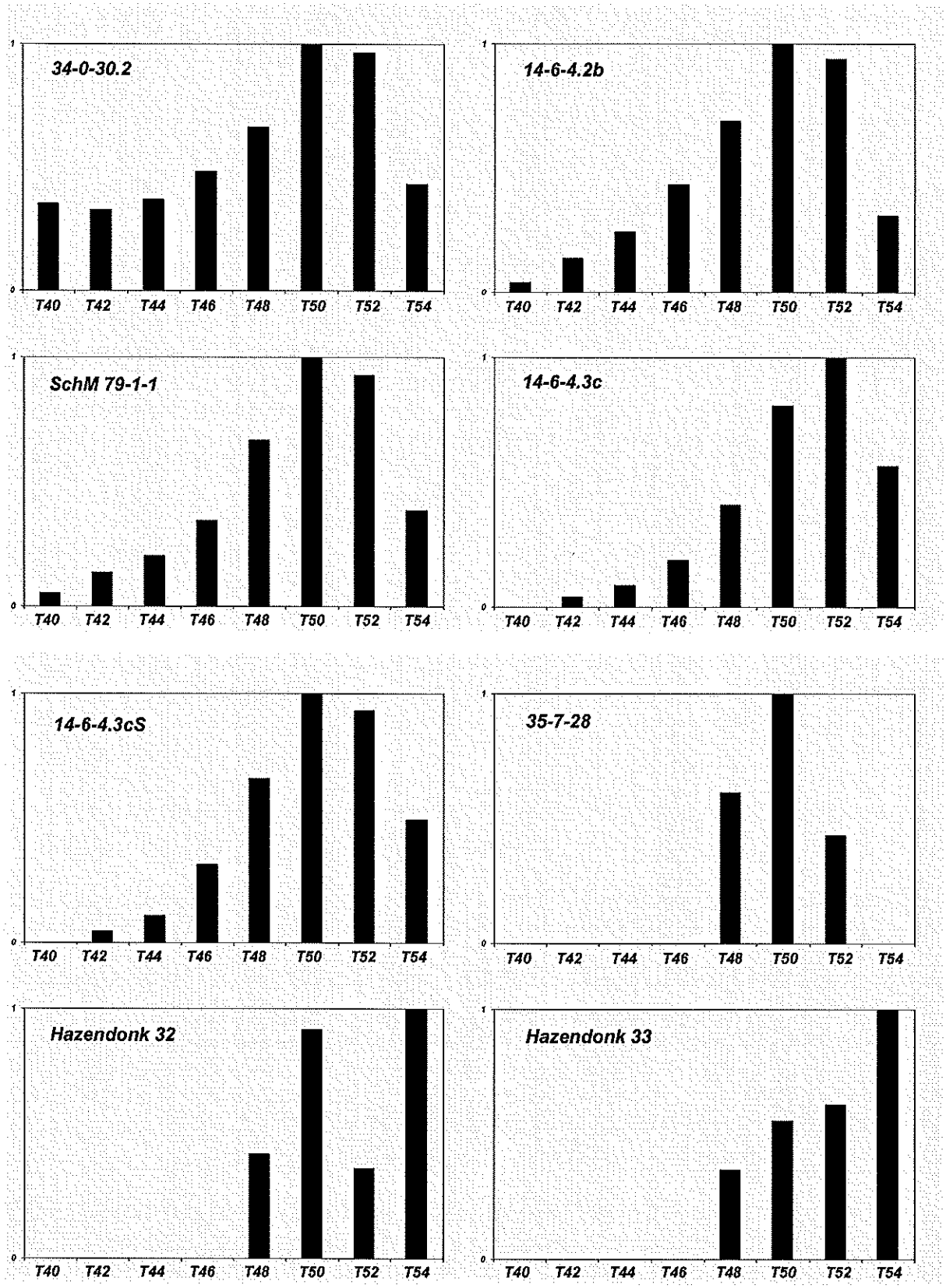
Table 4d: Lipid composition extracts from surface (R) and absorbed (S) residues 8-1, 14-6-4.4, 16-6-4.3c and 14-6-4.2b expressed in corrected relative weight percentages WP_i (in %). The numbers indicating the different DAGs and TAGs represent the total number of carbon atoms in the acyl fragments. ECN is the calculated effective carbon number per compound.

		Sch	Utg54		Haz		P14	
		79-1	226	320	32	33	6745	7054
		R	R	R	R	R	R	R
		Charred	Charred	Charred	Charred	Charred	Charred	Charred
TLY [mg/g]:		139.56	52.48	43.43	19.59	7.38	11.86	1.77
Lipid	ECN	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
Free fatty acids								
C12:0	14.00	0.30	1.77	1.33	2.32	9.53		
C13:0	15.00							
C14:0	16.00	2.01	5.26	4.48	3.15	4.17		14.21
C15:0	17.00				6.32	3.14		
C15:0	17.00	2.30	5.99	4.30	1.37	1.10	11.89	22.80
C16:1	17.90					0.54		
C16:0	18.00	9.01	16.04	14.29	27.56	27.44	22.24	14.40
C17:0	19.00				4.46	3.15		
C17:0	19.00	0.63				1.13		
C18:2	19.80							
C18:1	19.90	12.00		1.31	6.22	12.78		
C18:0	20.00	7.42	8.74	9.46	13.51	12.43	14.05	7.08
C19:0	21.00							
C20:1	21.90				2.13	5.25		
C20:0	22.00				1.77	4.64		
C22:1	23.90					3.10		
C22:0	24.00					2.10		
C23:0	25.00							
C24:0	26.00							
C26:0	28.00							
C28:0	30.00							
C30:0	32.00							
Alcohols								
C12-OH	14.69							
C14-OH	16.69							
C15-OH	17.69							
C16-OH	18.69							
C18-OH	20.69							
C24-i-OH	26.69							
C26-i-OH	28.69							
C28-OH	30.69							
C30-OH	32.69							
C32-OH	34.69							

Table 4e: Lipid composition extracts from surface residues from Schagen-Muggenburg (Sch), Uitgeesterbroekpolder 54 (Utg54), Hazendonk (Haz) and NO-Polder 14 (P14) in corrected relative weight percentages WP_i (in %). ECN is the calculated effective carbon number per compound.

		Sch	Utg54		Haz		P14	
		79-1	226	320	32	33	6745	7054
		R	R	R	R	R	R	R
		Charred	Charred	Charred	Charred	Charred	Charred	Charred
TLY [mg/g]:		139.56	52.48	43.43	19.59	7.38	11.86	1.77
Lipid	ECN	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]	Wpi [%]
MAG								
M14:0	20.93	0.57						
M16:0	22.93	0.97	1.92					
M18:1	24.83	0.52			15.63			
M18:0	24.93	0.90						
DAG								
D28	30.69	1.50	7.85	5.24				
D29	31.69							
D30	32.69	3.26	4.17	3.48				
D31	33.69	0.64						
D32	34.69	6.56	6.95	7.08				
D33	35.69	0.69						
D34	36.69	2.46	8.25	7.62				
D35	37.69							
D36	38.69							
TAG								
T40	38.45	0.63						
T42	40.45	1.64		2.93				
T43	41.45							
T44	42.45	2.47	4.15	1.66				
T45	43.45	0.44						
T46	44.45	4.18	4.13	3.20				
T47	45.45	0.78						
T48	46.45	8.09	7.14	7.55	2.07	1.12	6.28	5.43
T49	47.45	1.70						
T50	48.45	12.14	3.81	10.21	4.55	1.73	13.00	9.20
T51	49.45							
T52	50.45	11.27	10.84	10.21	1.79	1.93	4.53	
T53	51.45							
T54	52.45	4.69	2.98	4.09	4.96	3.12	23.65	17.03
Other								
C9-diacid	13.00							9.84
Cholesterol	29.19	0.29		1.55	2.22	1.60	4.41	
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4f: Lipid composition extracts from surface residues from Schagen-Muggenburg (Sch), Uitgeesterbroekpolder 54 (Utg54), Hazendonk (Haz) and NO-Polder 14 (P14) in corrected relative weight percentages WP_i (in %). The numbers indicating the different DAGs and TAGs represent the total number of carbon atoms in the acyl fragments. ECN is the calculated effective carbon number per compound.


34-0-30.2
14-6-4.2b
SchM 79-1-1
14-6-4.3c
14-6-4.3cS
35-7-28
Hazendonk 32
Hazendonk 33
T40 T42 T44 T46 T48 T50 T52 T54

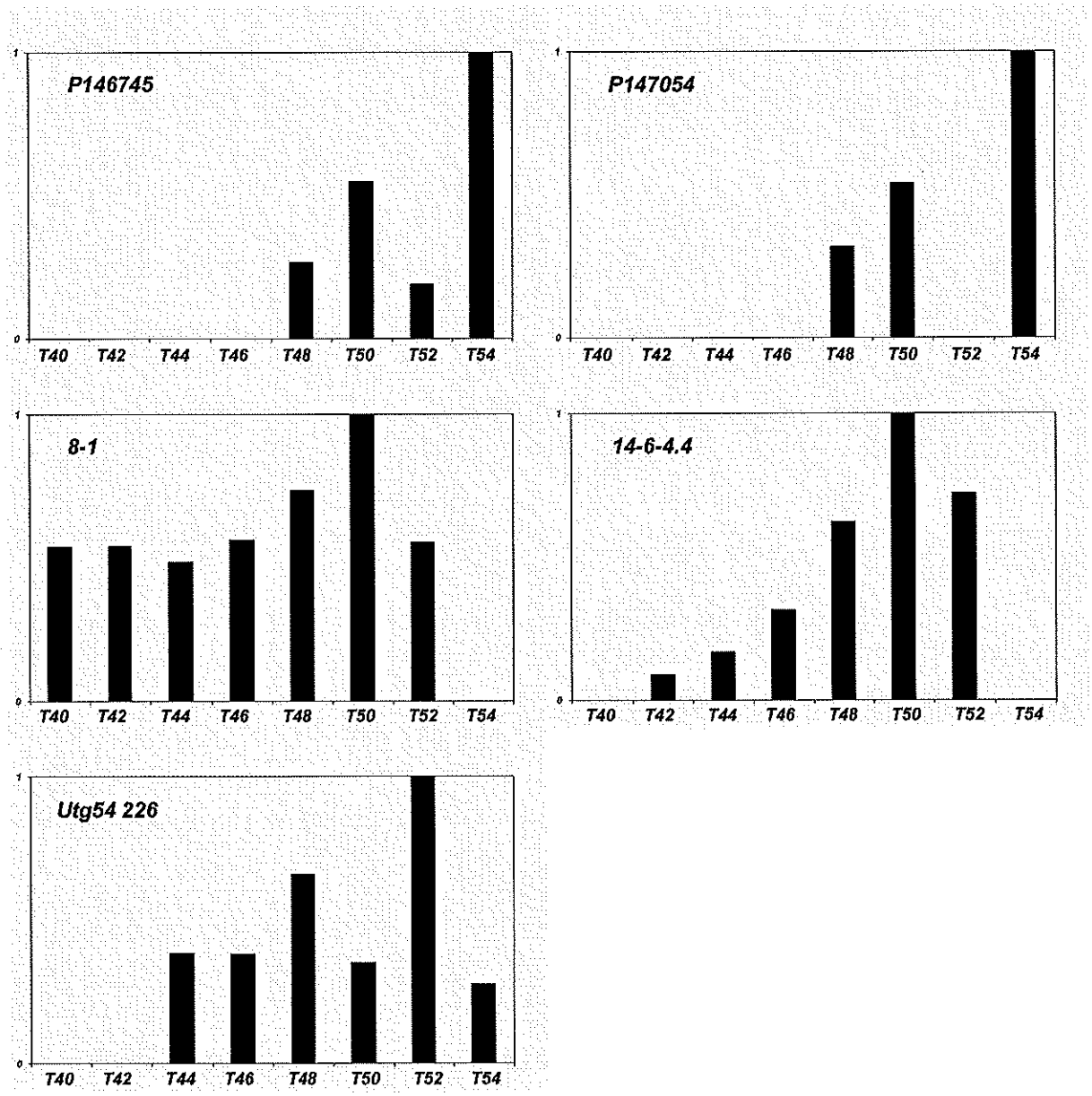


Figure 4 (on this and the previous page): Histograms of the relative abundance (%) of even carbon number TAGs (T40-T54) in total lipid extracts from ceramics from Neolithic and Roman Iron Age settlements in the Netherlands. Histogram 34-0-30.2 through 14-6-4.3C S (on the previous page) show lipid distributions associated with degraded ruminant milk fats. This identification is supported by the presence of odd carbon number TAGs and cholesterol. Histogram 35-7-28 through P147054 (on this and the previous page) show lipid distributions associated with degraded animal depot fats. This identification is supported by the presence of cholesterol. Histogram 8-1 through Utg54 226 (above) show lipid distributions from other origins. This identification is supported by the absence of both cholesterol and odd-carbon TAGs.

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