Molecular studies of organic residues preserved in ancient vessels

Oudemans, T.F.M.

Chapter 5

A Comparative Study of Extractable Lipids in the Shards and Surface Residues of Ceramic Vessels from Neolithic and Roman Iron Age Settlements

In this Chapter lipids extracted from charred and non-charred surface residues are quantitatively compared to lipids absorbed into the ceramic material of the vessel. The emphasis is on ceramic vessels originating from Uitgeest-Groot Dorregeest, but charred surface residues from other Roman Iron Age settlements and from Neolithic sites were analysed for comparison using Gas Chromatography and Gas Chromatography Mass Spectrometry.
Both the total yield of extractable lipids and the relative lipid composition of the extracts were studied quantitatively. Three indices were formulated and used as tentative, comparative measures for the state of preservation of the various samples (i.e. degree of saturation of fatty acids; the degree of hydrolysis; and the proportion of odd carbon number fatty acids).
The results show considerable variation in composition and preservation between different types of samples. Surface residues clearly contain a more intact acylglycerol profile than lipids extracted from the ceramic fabric of the vessel. These differences are probably caused by repetitive heating of the absorbed lipids inside the vessel wall. The refractory nature of charred materials is proposed to be an additional important factor in the high degree of preservation of lipids in surface residues.

Modified after:
T.F.M. Oudemans & J.J. Boon in press, 'A comparative study of extractable lipids in the shards and surface residual crusts of ceramic vessels from Neolithic and Roman Iron Age settlements in the Netherlands', *Theory and Practice of Archaeological Residue Analysis*, H. Barnard & J. Eerkens (eds.), British Archaeological Reports International Series, Archaeopress, Oxford.

1. Introduction

1.1. Lipid analysis in ceramic studies

Pottery assemblages are a rich and durable source of information for those who want to study the daily behaviour of people in the past. In order to assess the value of the information obtained from these assemblages, the actual use of ancient vessels is an essential pre-requisite. The identification of organic remains of the 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 (Rottländer & Schlichtherle 1979; Rottländer & Schlichtherle 1980; Mills & White 1987; Evershed *et al.* 1992; Heron & Evershed 1993; Pastorova *et al.* 1993b; McGovern *et al.* 1996; Oudemans & Boon 1996; Evershed *et al.* 1999; Craig *et al.* 2000; Regert & Rolando 2002; Oudemans *et al.* 2005). The study of organic residues has focused primarily on fatty materials. Lipids are optimal for organic residue studies due to their easy retrieval with solvent extraction and the continuous development of analytical techniques such as gas chromatography, gas chromatography/mass spectrometry and gas chromatography isotope ratio - mass spectrometry. Lipids also have obvious potential as diagnostic markers for the original vessel use due to their overall chemical stability (Eglinton & 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 immobilisation of the molecular debris considered crucial to long term preservation on a molecular level (Eglinton & Logan 1991). Post-depositional exchange of lipids between residues and their surrounding soil has been shown to be absent or very limited (Heron *et al.* 1991; Oudemans & Boon 1991).

1.2. Types of residues

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

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 shard (Needham & Evans 1987; Regert *et al.* 2001). A number of additional methodological advantages have been formulated for the study of surface residues (Oudemans & Boon 1991; Oudemans *et al.* in press-a; Oudemans *et al.* in press-b). 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. Extractions of absorbed residues may also include post-firing sealing products which will complicate results even more. Post-firing surface sealing with organic mixtures is common amongst traditional potters and is performed with a variety of materials including common foodstuffs such as milk and various starch-rich foods (see references in Rice 1987, 163-164), as well as less edible materials such as beeswax, various resins and other plant materials (Arnold 1985, 139-140; Kobayashi 1994; Diallo *et al.* 1995). Stern and co-workers 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 (Stern *et al.* 2000). An additional reason to use surface residues is the relatively higher degree of thermal degradation that has likely taken place in absorbed residues in cooking vessels. Absorbed residues have usually been exposed to more severe heating regimes (both in time and in temperature) than residues situated on the interior surface of the vessel. Although numerous quantitative studies have been performed on lipids extracted from different residue types no quantitative comparison of lipids extracts was ever published.

1.3. Aims

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

2. Experimental

2.1. Sample material and sample treatment

Organic residues from five different prehistoric contexts in the Netherlands were analysed (Table 1). The main focus of the 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 residues and non-charred residues were chosen for analysis. Non-charred surface residues from this settlement can appear as cream coloured 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) 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 Uitgeest-Groot Dorregeest, Schagen-Muggenburg (Abbink 1999; Therkorn 2004) 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 aluminium foil and stored at -20 °C.

Surface residues (ca. 5 - 10 mg) were scraped from the ceramic with a solvent cleaned scalpel, after removal of the upper 0.5 mm of the residue. Ceramic samples (ca. 2 g) were cut out of the vessel with a solvent cleaned scalpel, after removal of any surface residue and an additional thin layer (1 mm) of ceramic. Samples were crushed and ground in an agate mortar and stored in glass vials. Samples were prepared according to Evershed and co-workers (1990). In short, an internal standard (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 and removal of the supernatant, the samples were dried in a round-bottomed flask by rotary evaporation at 50 °C (in vacuo). A small amount (100 μl) of solvent (chloroform/methanol, 2:1 v/v) was added to transfer the total lipid extract to a vial. One fifth (20 μl) of this extract was transferred to a second screw-topped vial and silylated with 25 μl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane and heated at 60 °C for 10 min directly prior to analysis. All analytical grade solvents were distilled before use.

Table 1: Archaeological samples

Site	Period[a]	Sample nr[b]	Sample Type	N [%]	C [%]	H [%]	Total organic [%]	C/N	C/H
Uitgeest GD	RIA	34-0-30	Char	5.93	41.16	3.72	50.81	6.94	11.06
Uitgeest GD	RIA	35-7-28	Cream coloured	0.18	3.52	1.20	4.90	19.56	2.93
		35-7-28 S	Ceramic						
Uitgeest GD	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 GD	RIA	8-1	Red brown	0.79	6.55	1.25	8.59	8.29	5.24
		8-1 S	Ceramic						
Uitgeest GD	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 GD	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 GD	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-Mug.	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

[a] Period: RIA = Roman Iron Age, Neo = Neolithic period

[b] Sample nr. = Find number and number of residue taken from ceramic R = Surface residue, and S = ceramic material from vessel wall.

2.2. Instrumentation

CHN analysis was performed on all surface residues in order to get a rough indication of the overall organic composition of the sample. Elemental composition was determined after

samples were dried, weighted and analysed in duplo using a Carlo Erba 1500 CHN analyser. Elemental composition is 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 calculate from their weight percentages (not on a molar basis).
The analytical GC work was performed during a research internship at the Department of Biochemistry of the University of Liverpool under the supervision of Dr. R.P. Evershed. The GC analyses were performed on a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionisation detector and a Hewlett-Packard 3396A computing integrator/plotter. On-column injection was used to introduce samples into a 60 cm x 0.32 mm inner diameter (i.d.) retention gap of de-activated fused silica, connected to the analytical column, a polyamide clad analytical column of 12 m x 0.22 mm i.d., coated with a BP1 stationary phase (OV-1 equivalent, 0.1 μm film thickness), via a stainless-steel union of 0.8 mm i.d. (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 (70 eV) mode with a source temperature of ca. 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.

2.3. Quantification

The quantitative data were derived from the peak areas measured using gas chromatography (GC) with a flame ionisation detector (FID). The peak areas were corrected for compound specific response with use of the Effective Carbon Number (ECN) per compound (Table 2). The ECN's were 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 is decreased with 0.1 per double bond (Scanlon & Willis 1985). Because saturated and unsaturated forms of DAGs and TAGs co-elute under current conditions, the effect of double bonds of acylglycerols (varying from 0.6 % for D40:2 to 1.1 % for T54:6) 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)$_i$ expresses the relative amount of a component i necessary to obtain the same response (in area measured) as the reference component IS (Kaiser 1969, 99-103; Scanlon & Willis 1985) and defined as:

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

where ECN_{is} indicates the calculated Effective Carbon Number for the Internal Standard (17.00 for heptadecane) and ECN_i indicates 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:

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

where A_{is} expresses the known amount of Internal Standard 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 derivatisation, the normalised weight percentage WP_i of the original compounds is calculated according to:

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

where $MW_{i\,(underiv)}$ indicates the molecular weight of compound i in underivatised form in mg and n is total number of compounds in the sample. The total lipid yield (TLY) of the extraction procedure is defined in units of mg/g according to:

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

where W_s indicates the amount of sample used for extraction in gram. This calculation is based on the assumption that the extraction is equivalent per lipid species and that a 100 % extraction of the added internal standard from the ground material is achieved.

2.4. Comparing lipid profiles

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 defined in formula (3). The saturation index I sat of the free fatty acids serves to express the proportion of saturated even carbon numbered fatty acids in the extract. The saturation index I sat is defined as:

(5) $$\text{I sat} = \frac{\sum(WP_i \text{ saturated even FA})}{\sum(WP_i \text{ all even FA})}$$

This value is a tentative measure for the degree of polymerisation 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 numbered free FAs relative to all even carbon numbered acyl fragments in TAGs or free FAs. The hydrolysis index I hydr is defined as:

(6) $$\text{I hydr} = \frac{\sum(WP_i \text{ even FA})}{\sum(WP_i \text{ even FA and all TAG})}$$

This parameter provides a measure for the degree of hydrolysis that has taken place in a sample. Acyl fragments can be hydrolysed by microbial activity (enzymatic hydrolysis) and under alkaline or acidic conditions (chemical hydrolysis).
The odd carbon number FA index I o/e corresponds to the proportion of odd carbon number free FAs in the total FA and is defined as:

(7) $$\text{I o/e} = \frac{\sum(WP_i \text{ odd FA})}{\sum(WP_i \text{ all FA})}$$

Since the C15:0 and C17:0 FAs 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.

3. Results

3.1. CHN Analysis

Elemental CHN analysis (Table 1) shows a distinct difference in total organic content between 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-a). Chars from Uitgeest – Groot Dorregeest showed more variation in total organic content than chars from other sites which all fell fel within a range of 49 - 62% (Table 1). Amongst the charred residues there is a considerable variation in elemental composition. The C/H ratios of the total

Table 2: Compounds detected in GC/MS

Compounds	Mass Peak	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 *	$[M-15]^+$	$[M-CH3]^+$	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.

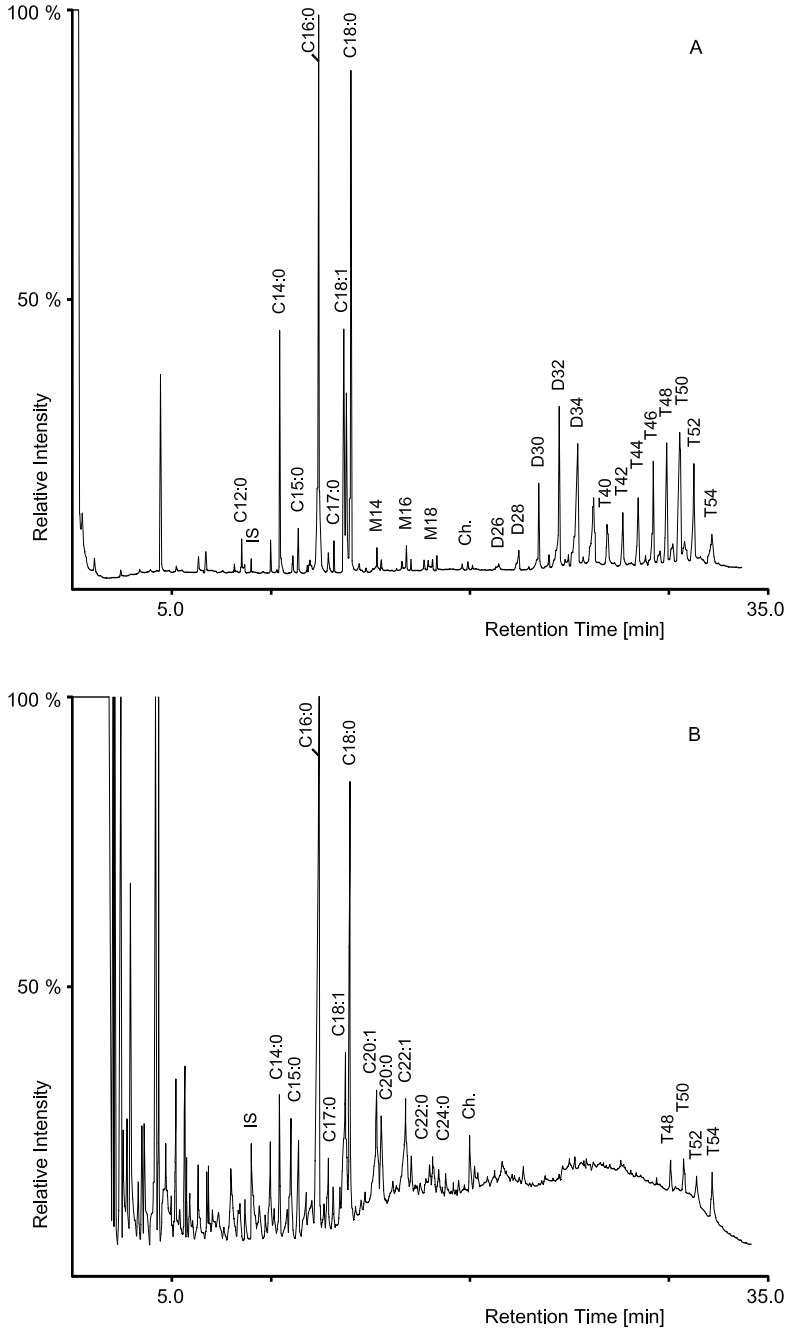


Figure 1: Lipids from charred residues from Roman (Utg-GD 14-6-4.2b) and Neolithic period (Haz. 33.781). Identified: C = fatty acid; M = Monoacyglycerol; D = Diacylglycerol; T = Triacylglycerol; Ch = Cholesterol; Ph = Phthalate esters; IS = Internal Standard. Numbers represent the total number of carbon atoms in the acyl moieties.

char collection vary from 9.12 – 32.76 indicating a less aliphatic and more condensed nature of the material as the ratio goes up. 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 goes up.

3.2. Qualitative Lipid Analysis

The compounds identified by GC/MS analysis are summarised in Table 2 and further illustrated in the GC traces (Fig. 1, 2). The identity of the compounds in the total lipid extracts 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; 1972b) and Waller and co-workers (1972; 1980).
Although isomers of the C18:1 fatty acid and diacylglycerols (positional) were detected in the GC traces, no isomer specific identification can be given under the analytical conditions employed. Isomers of C15:0 and C17:0 fatty acids (normal-, iso- and anteiso-) and monoacylglycerols (1-, and 2- forms) were identified in some of the samples, but have been summarised in the quantitative results. In GC/MS the EI mass spectra of triacylglycerols display such weak molecular ions M^+,. and fragment ions $[M-18]^+$ that they are of little diagnostic

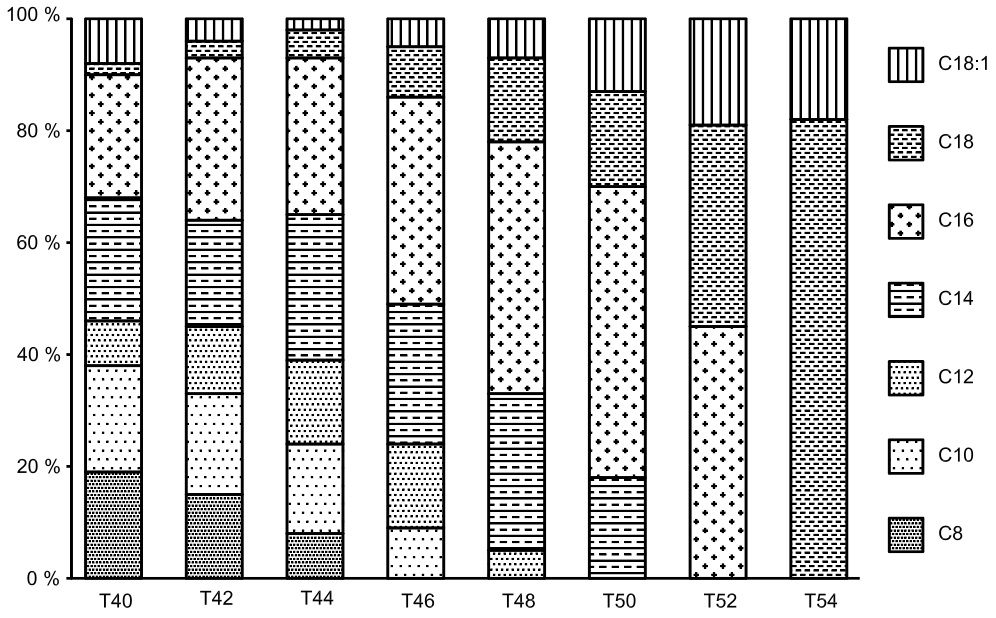
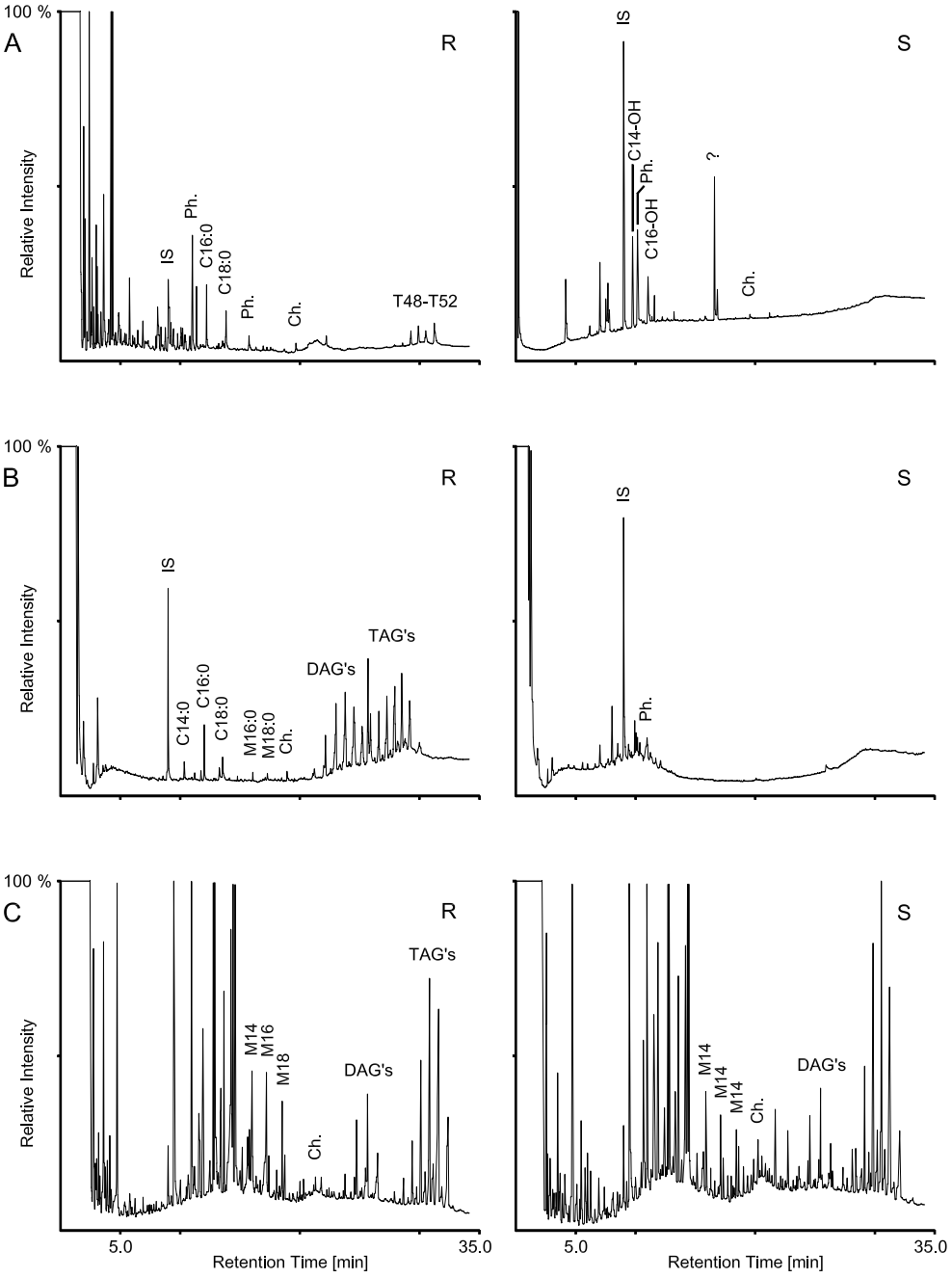


Figure 3: Relative composition of acyl fragments in MS spectra of intact TAGs of char Utg-GD 34-0-30 R.
The percentages are relative numbers based on the intensities of $RCOO^+$ fragments in the EI mass spectra of each TAG. When one assumes that fragmentation is equivalent for all acyl chains, these figures can be seen as representing the acyl composition in intact triacylglycerols.


100 %
A
R
S
Relative Intensity
IS
Ph.
C16:0
C18:0
Ph.
Ch.
T48-T52
IS
C14-OH
Ph.
C16-OH
?
Ch.
100 %
B
R
S
Relative Intensity
IS
C14:0
C16:0
C18:0
M16:0
M18:0
Ch.
DAG's
TAG's
IS
Ph.
100 %
C
R
S
Relative Intensity
M14
M16
M18
Ch.
DAG's
TAG's
M14
M14
M14
Ch.
DAG's
5.0
35.0
Retention Time [min]
5.0
35.0
Retention Time [min]

value. Hence the total carbon number of the triacylglycerols was established by comparison of the GC 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 triacylglycerols (Fig. 3). All triacylglycerols of a given total carbon number co-elute on the stationary phase as employed in this study. Hence, the identification of all triacylglycerol is limited to molecular species.

Due to the high complexity of the mixtures analysed, it was not always possible to identify all the peaks visible in the high temperature GC traces. Some of the minor components (including alkanes, wax esters, 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 spectrum. The total measured peak area of all identified compounds (including the internal standard) in the high temperature GC is summarised in Table 3 and varied between 51% and 100% with an average of 84% for surface residues (although in 2 samples only 53% was identified) and 69% for ceramic samples (although 2 samples contained no identifiable lipids and one sample only 5% that could be identified).

3.3. Calculation of the Total Lipid Yield (TLY)

A first assessment of a sample is made by calculation of the total lipid yield (Table 3) according to formula (4). The calculation of the TLY includes all compounds identified in the GC runs with an ECN which could be calculated (Table 4). Phthalate esters were not included because they were considered contaminating compounds. The Total Lipid Yield (TLY) per sample (Table 3) shows considerable variation between samples, but general trends are visible. Firstly, 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 TLY's between 0.47 and 27.52 mg/g, while the adjacent ceramic samples yield TLY's between 0.00 and 0.16 mg/g. Secondly, most charred surface residues (9 of the 12 samples) produce lipid yields 5 - 50 times higher than non-charred surface residues (average of 1.71 mg/g). And finally, charred residues from different excavations vary considerably in lipid yield. Chars from Schagen-Muggenburg and Uitgeest-54 gave relatively high TLY's (between 43.43 and 139.56 mg/g) while the Neolithic chars exhibit lower yields (between 1.77 and 19.59 mg/g) with an average TLY comparable to that from Uitgeest-Groot Dorregeest.

Figure 2 (on facing page): Comparing GC traces of lipids extracted from surface residues (R) and from the directly adjacent ceramic material of the vessel (S).
A: Cream coloured crust Utg-GD 34-7-28. B: Red brown residue Utg-GD 8-1. C: Charred residue Utg-GD 14-6-4.3b. Identified compounds: C = fatty acid; M = Monoacyglycerol; D = Diacylglycerol; T = Triacylglycerol; Ch = Cholesterol; Ph = Phthalate esters; IS = Internal Standard. Numbers represent the total number of carbon atoms in the acyl moieties of the lipid.

3.4. Comparing Lipid Composition

In order to provide a quantitative assessment of the highly complex lipid extracts, the normalised weight percentage of each identified lipid was calculated in underivatised form (Table 4) according to formula (3). The GC trace of the charred residue Utg-GD 14-6-4.2b shows (Fig. 1a) several classes of compounds including free fatty acids (C), monoacylglycerols (M), diacylglycerols (D), triacylglycerols (T), cholesterol (Ch) and phthalate-esters (Ph). Although some variation can be seen between charred residues from Uitgeest-Groot Dorregeest, most samples were found to be of comparable lipid composition (Fig. 2c R).
However, the lipid composition of non-charred surface residues from Uitgeest – Groot Dorregeest was significantly different. These lipid profiles showed no odd FAs and relatively low percentages of free FAs as can be seen in the GC traces of cream colour residue 35-7-28 (Fig. 2a R) and red-brown residue 8-1 (Fig. 2b R).
Absorbed residues from Uitgeest-Groot Dorregeest yield lower proportions of intact DAGs and TAGs, and relatively more odd carbon number FAs than those of the surface residues (Table 4, Fig. 2). Vessels with non-charred residues yield little or no absorbed lipids from the ceramic (Fig. 2a S and 2b S). The comparison between lipid extracts of vessel fabrics and their directly adjacent surface residues was performed on six vessels, of which four contained chars and two contained non-charred residues. The lipid profile of the lipids absorbed in the vessel fabric does not necessarily reflect that of the solid residue situated on the vessel surface. Only in two cases (e.g. Utg-GD 14-6-4.3c and 34-0-12) were very similar profiles observed (Fig. 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 TLY's (Table 4). The interior section showed a lipid profile similar to the surface residue directly adjacent to it.
The charred surface residues from the Neolithic sites rendered lipid GC traces that contained a relatively high percentage of free FAs including long-chain fatty acids up to C24, no detectable MAGs and DAGs (Fig. 1b) and higher proportions of odd FAs.

4. Discussion

4.1. Lipid quantification

Most earlier quantitative lipid studies have been based on the assumption that all compounds exhibit similar responses in the FI detector of the GC. Although this assumption is valid where closely related compounds are being investigated, differences in response of the FID may be observed (Kaiser 1969, 99-103) when compounds show widely varying chemical properties. In this investigation, rather than assume equivalent responses for all the components in the total

Table 3: Total Lipid Yields and Preservation Indices

Site	Sample nr.[a]	Sample type	TLY correct. [mg/g][b]	TLY uncorr. [mg/g][c]	Identif. peak area [%][d]	I sat[e]	I hyd[f]	I o/e[g]
Uitgeest GD	34-0-30	Char	27.52	25.07	98 %	0.78	0.51	0.07
Uitgeest GD	35-7-28	Cream Col.	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 GD	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 GD	8-1	Red brown	2.10	2.30	100 %	1.00	0.10	0.00
	8-1 S	Ceramic	-	-	-	-	-	-
Uitgeest GD	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 GD	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 GD	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-M.	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-P14	6745	Char	11.86	13.96	84 %	1.00	0.43	0.33
NO-P14	7054	Char	1.77	2.80	53 %	1.00	0.53	0.64

[a] Sample nr. = Find number and number of residue taken from ceramic.

[b] TLY corrected is calculated according to formula 4 and expressed in mg/g sample.

[c] TLY uncorrected is calculated assuming linear response for all compounds in the FI-detector and expressed in mg/g sample.

[d] Identified peak area indicates the percentage of the total peak area that could be ascribed to a known compound (see also table 4).

[e] I sat was calculated according to formula 5.

[f] I hyd was calculated according to formula 6.

[g] I o/e was calculated according to formula 7.

lipid extracts, corrected response factors for each compound were calculated in order to enhance quantitative precision.

Although differences between TLY's and the traditional uncorrected total yields are shown to be considerable (10 - 20%) especially when dealing with low overall yields (Table 3 and in Table

4a 34-0-30 Δ %), they are not in the same order of magnitude as the differences between total lipid yields calculated for lipid extracts originating from different excavations (or even between different kinds of residues within a one excavation). For instance, the ceramic material from Uitgeest - Groot Dorregeest shows uncorrected lipids yields between 0.02 and 0.18 mg/g, while lamps and dripping dishes from the medieval site at Raunds (UK) reportedly frequently contain yields between 0.1 and 1 mg/g (Evershed *et al.* 1991; Charters *et al.* 1993b; Evershed *et al.* 1999), and amphorae from the Late Bronze Age in the Western Isles of Scotland contain between 0.025 and 0.3 mg/g lipid (Craig *et al.* 2005). These large differences completely overshadow the smaller differences due to corrected FID response factors.
The approximation of equivalent response is probably sufficiently precise to allow general comparisons of extractable lipids in soil and potshards (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.* 1993b).
However, the use of corrected FID response factors for each compound is especially relevant when comparing relative lipid compositions. Discrepancies of plus or minus 10 - 15% for various compounds can be seen (Table 4a). When quantifications 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.

4.2. Lipids as 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 numbered free FAs (C4-C24), MAGs, DAGs and TAGs occur commonly in plant and animal fats (Hillditch & Williams 1964, 6-25), not all different compound classes are equally suitable as taxonomic markers. In this investigation only TAGs, sterols and free FAs are used as diagnostic chemotaxonomic markers. Both 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 and can be derived from microbial activity. Although the same is valid for free FAs, they may be diagnostic in specific cases as is discussed below.
TAGs are not produced by micro-organisms and therefore strongly 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 surrounding soil. However, oils will undergo a 'drying process' a combination of cross-linking, polymerisation and oxidation if sufficient di- or tri-unsaturated acyl fragments are present in TAG mixtures in the presence of oxygen (Mills & 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 ºC 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 the ceramic

of cooking vessels and cause an overall 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 origins (sitosterol and campesterol). Sterols have low solubility in water and are not easily damaged through overheating (damage will occur around 280 – 300 ºC), but are relatively easily oxidised in fats and oils (Davídek *et al.* 1990, 204 and 216). The interpretation of cholesterol as indicator for animal origin must be made with caution due to the possibility of post-excavational contamination with cholesterol through handling of the potshards. Some oxidation products of cholesterol have been detected in Saxon oil (Evershed *et al.* 1992). Microbial reduction of Δ5-sterols (like cholesterol) to 5α(H)- and 5b(H)-stanols occurs commonly under anaerobic conditions in the intestines of humans and animals and during diagenesis in sediments (Evershed *et al.* 1997a, Mackenzie *et al.* 1982). This process may also be take place in the context of the original residue.

Although free FAs are abundantly present in most organic residues, they also illustrate clearly the difficulty in assigning degraded lipids to a specific source. FAs may be extracted from different sources, mixing the remains of the original vessel contents with the secondary products of microbial activity. In addition, a wide range of degradative pathways exists for free FAs, causing the overall free FA composition to become an unreliable chemotaxonomic indicator.

Firstly, selective degradation of unsaturated FAs can occur as a result of oxidation or autoxidation in fresh materials (Davídek *et al.* 1990, 201-204). Additional condensation processes taking place during heating or cooking of lipids (Malainey *et al.* 1999a). When heated up to 270 - 300 °C with limited access to oxygen unsaturated lipids (primarily the polyunsaturated FAs 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 ºC in the presence of calcium salts (Evershed *et al.* 1995c; 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 shard samples (Oudemans & Boon 1991). Anoxic conditions and temperatures up to 300 °C may well have been present in the ceramic wall of the vessel during cooking, and in some of the surface residues during severe charring. Stern and co-workers confirm the hypothesis that hard to extract fatty acids may indeed be present in the ceramic, 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 and after deposition. The enhanced volatility and water solubility of short-chain FAs may result in the selective loss. Enzymatic degradation of intact fatty acids by micro-organisms through β-oxidation (Leninger 1977) can also play a role in this process through loss of one or more pairs of C atoms from the acyl chain. This effect is commonly observed in bog bodies and buried fats such as bog butter (Thornton *et al.* 1970; Evershed 1992).

Table 4a: Extracted Lipids from Surface Residues and Absorbed Residues												
Site		Uitgeest-Groot Dorregeest										
Find number		34-0-30			35-7-28		34-0-12			8-1	14-6-4.4	
Residue type		R char			R cream coloured crust	S	R char	S3	S1	R red brown crust	R char	S
TLY[mg/g]			27.52		1.32	0.01	0.47	0.02	0.02	2.10	14.77	0.01
Lipid	ECN	Wp_i [%]	Δ [%]	X_i [%]								
FFA												
C12:0	14.00	0.63	6.6	0.68							0.29	1.99
C13:0	15.00											
C14:0	16.00	3.78	6.8	4.12			4.39	9.04	24.57	1.00	5.34	12.10
C15:0	17.00	0.50	6.9	0.55				7.93			0.72	
C15:0	17.00	0.75	6.9	0.82				5.05			1.21	5.35
C16:1	17.90						1.45				0.44	4.23
C16:0	18.00	15.84	7.0	17.39	25.15		29.56	21.64	49.38	3.55	27.69	40.36
C17:0	19.00	0.65	7.1	0.71			4.08				1.46	
C17:0	19.00	0.52	7.1	0.57				2.74			1.37	3.98
C18:2	19.80	0.24	7.6	0.27								
C18:1	19.90	8.56	7.4	9.38			16.78		2.30		19.63	9.10
C18:0	20.00	9.58	7.2	10.48	13.20		35.35	23.80	23.75	2.06	12.81	21.94
C19:0	21.00	0.09	7.3	0.10								
C20:1	21.90											
C20:0	22.00	0.45	7.3	0.49							1.61	
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 4b: Extracted Lipids from Surface Residues and Absorbed Residues												
Site		Uitgeest-Groot Dorregeest				Sch	Utg54	Hazendonk			P14	
		14-6-4.3b		14-6-4.2b		79-1	226	320	32	33	6745	7054
		R char	S	R char	S	R char	R char	R char	R char	R char	R char	R char
TLY [mg/g]		4.71	0.16	9.97	0.04	139.56	52.48	43.43	19.59	7.38	11.86	1.77
Lipid	ECN											
FFA												
C12:0	14.00	0.50	0.67	0.46		0.30	1.77	1.33	2.32	9.53		
C13:0	15.00		0.60	0.07								
C14:0	16.00	4.35	5.47	2.76	13.22	2.01	5.26	4.48	3.15	4.17		14.21
C15:0	17.00	1.54		0.39					6.32	3.14		
C15:0	17.00	1.71	2.39	0.65	14.95	2.30	5.99	4.30	1.37	1.10	11.89	22.80
C16:1	17.90	0.46								0.54		
C16:0	18.00	26.20	27.80	12.11	37.74	9.01	16.04	14.29	27.56	27.44	22.24	14.40
C17:0	19.00	1.68	1.32	0.54					4.46	3.15		
C17:0	19.00	1.84	1.73	0.48		0.63				1.13		
C18:2	19.80											
C18:1	19.90	7.04	5.25	6.52	7.13	12.00		1.31	6.22	12.78		
C18:0	20.00	30.82	24.56	12.48	26.96	7.42	8.74	9.46	13.51	12.43	14.05	7.08
C19:0	21.00	0.34										
C20:1	21.90								2.13	5.25		
C20:0	22.00	0.70	1.22	0.38					1.77	4.64		
C22:1	23.90									3.10		
C22:0	24.00			0.43						2.10		
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 4a Continued: Extracted Lipids from Surface and Absorbed Residues												
Site		Uitgeest-Groot Dorregeest										
Find number		34-0-30			35-7-28		34-0-12			8-1	14-6-4.4	
Residue type		R char			R cream coloured crust	S	R char	S3	S1	R red brown crust	R char	S
TLY[mg/g]			27.52		1.32	0.01	0.47	0.02	0.02	2.10	14.77	0.01
Lipid	ECN	Wp_i [%]	Δ [%]	X_i [%]								
MAG												
M14:0	20.93	0.14	5.5	0.15								
M16:0	22.93	0.45	5.8	0.49				1.32			0.29	
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						2.91	1.10	
D31	33.69	0.27	-7.4	0.26								
D32	34.69	4.79	-5.4	24.62						8.81	2.82	
D33	35.69	0.30	-6.7	0.29								
D34	36.69	5.55	-5.6	5.35						11.56	3.39	
D35	37.69	0.12	-5.9	0.11								
D36	38.69	4.58	-5.7	4.41						11.19	0.53	
TAG												
T40	38.45	2.66	-15.7	2.29						7.17		
T42	40.45	2.44	-14.7	2.12						7.22	0.59	
T43	41.45											
T44	42.45	2.72	-13.8	2.39						6.48	1.11	
T45	43.45	1.02	-13.4	0.90								
T46	44.45	3.52	-13.0	3.12						7.51	2.09	
T47	45.45	0.42	-12.6	0.38								
T48	46.45	4.79	-12.3	4.29	8.59					9.81	4.12	
T49	47.45	1.18	-11.6	1.06								
T50	48.45	7.19	-11.5	6.45	14.19					13.36	6.63	
T51	49.45	1.71	-11.2	1.55								
T52	50.45	6.86	-10.9	6.23	6.16					7.38	4.77	
T53	51.45											
T54	52.45	3.05	-10.3	2.80	29.94							
Other												
C9-diacid	13.00											
Chol.	29.19	0.20	15.1	0.23	2.77		5.07	3.92				0.95
Total		100		100	100	100	100	100	100	100	100	100

Table 4b Continued: Extracted Lipids from Surface and Absorbed Residues												
Site		Uitgeest-Groot Dorregeest				Sch	Utg54	Hazendonk			P14	
		14-6-4.3b		14-6-4.2b		79-1	226	320	32	33	6745	7054
		R char	S	R char	S	R char	R char	R char	R char	R char	R char	R char
TLY [mg/g]		4.71	0.16	9.97	0.04	139.56	52.48	43.43	19.59	7.38	11.86	1.77
Lipid	ECN											
MAG												
M14:0	20.93			0.66		0.57						
M16:0	22.93	1.29	1.18	1.07		0.97	1.92					
M18:1	24.83					0.52			15.63			
M18:0	24.93	1.05	0.83	0.94		0.90						
DAG												
D28	30.69	0.19		0.90		1.50	7.85	5.24				
D29	31.69			0.07								
D30	32.69	0.39	0.51	3.65		3.26	4.17	3.48				
D31	33.69			0.88		0.64						
D32	34.69	0.99	1.32	7.45		6.56	6.95	7.08				
D33	35.69			1.32		0.69						
D34	36.69	1.54	1.45	8.40		2.46	8.25	7.62				
D35	37.69											
D36	38.69	0.98	0.63	4.76								
TAG												
T40	38.45			0.26		0.63						
T42	40.45	0.18	0.23	0.91		1.64		2.93				
T43	41.45			0.19								
T44	42.45	0.38	0.55	1.60		2.47	4.15	1.66				
T45	43.45	0.07				0.44						
T46	44.45	0.82	1.57	2.85		4.18	4.13	3.20				
T47	45.45	0.26	0.68	1.13		0.78						
T48	46.45	1.78	3.28	4.52		8.09	7.14	7.55	2.07	1.12	6.28	5.43
T49	47.45	0.54	0.88	1.80		1.70						
T50	48.45	3.52	4.98	6.55		12.14	3.81	10.21	4.55	1.73	13.00	9.20
T51	49.45	1.15	1.01	1.85								
T52	50.45	4.36	4.65	6.15		11.27	10.84	10.21	1.79	1.93	4.53	
T53	51.45	0.34		0.82								
T54	52.45	2.46	2.46	2.02		4.69	2.98	4.09	4.96	3.12	23.65	17.03
Other												
C9-diacid	13.00											9.84
Chol.	29.19	0.20	0.47	0.62		0.29		1.55	2.22	1.60	4.41	
Total		100	100	100	100	100	100	100	100	100	100	100

Thirdly, alkaline environments enhance the transformation of free FAs to salts of FAs and can produce salts of various nature. Transformation of fatty acids into insoluble salts occurs commonly in fresh fat buried in the ground during the formation of adipocere which consists mainly of fatty acids and their calcium salts (Eglinton & Logan 1991). Some of these salts are relatively soluble in water and can cause FAs 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 indicated to be aware of this mechanism (Condamin *et al.* 1979; Rottländer & Schlichtherle 1979), and this conversion was shown to occur under arid conditions in an Egyptian oil stored in a sealed stone vessel of which 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 common to fatty materials buried in the ground are the formation of hydroxy fatty acids formed through hydration of double bonds in adipoceres (Den Dooren de Jong 1961; Evershed 1991, 1992) and the formation of isomers of mono-unsaturated FAs observed in bog bodies (Evershed 1991, 1992). No hydroxy FAs were detected in the lipids extracted in this study, so this pathway obviously was not active. Although the formation of isomers of mono-unsaturated FAs may have taken place, isomers of C18:1 were not separated in this analysis, so no conclusions may be drawn about this process.

4.3. Operational parameters from lipid preservation and degradation

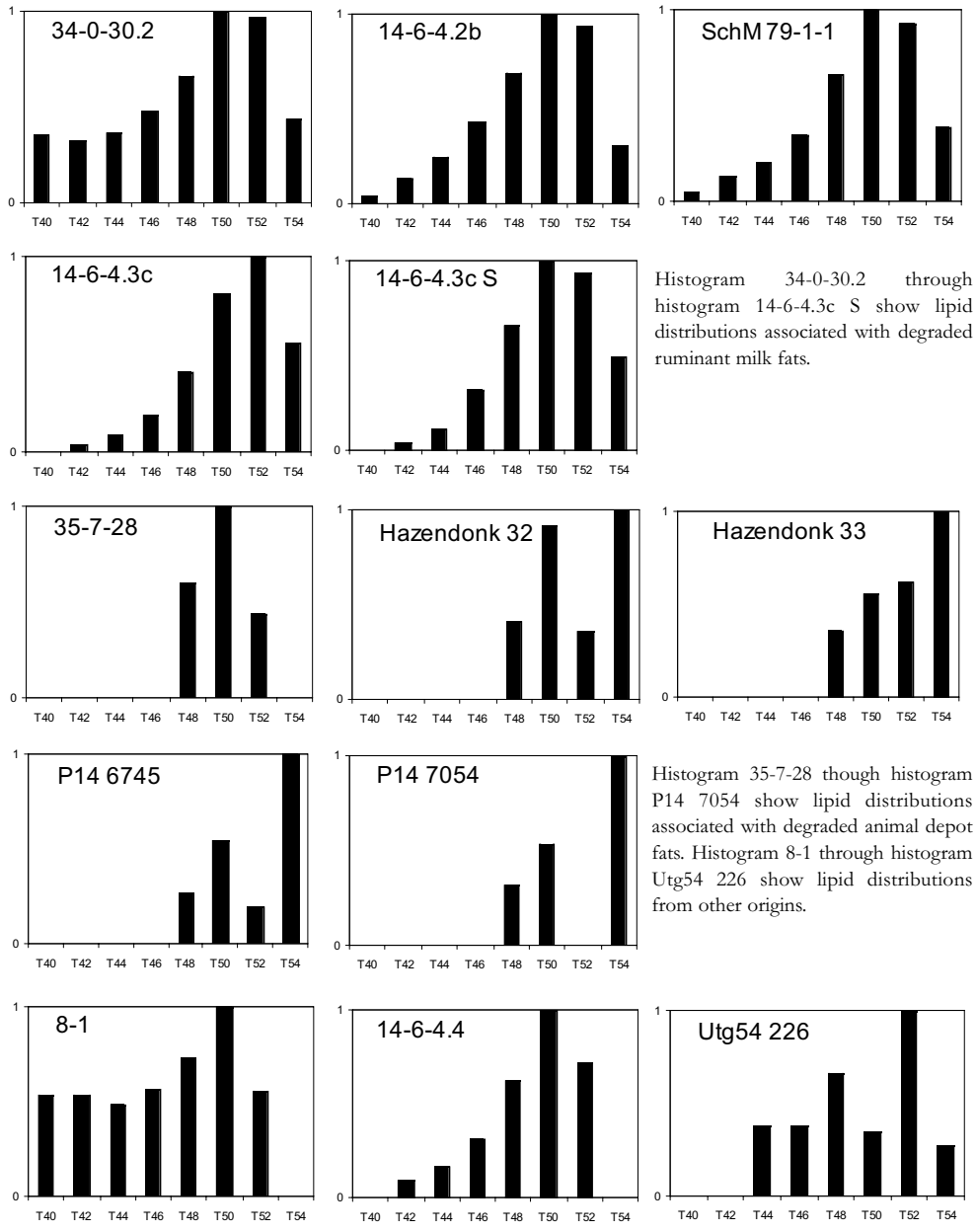
Some significant aspects of lipid preservation and degradation can be studied using the operational parameters defined in the experimental Section (Table 3).
The saturation index I sat expresses the proportion of saturated even carbon numbered fatty acids in the residue and is a tentative measure for the degree of polymerisation 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 polymerisation in FAs) and the C/H ratio (a measure of the overall condensation in residue). This suggests that the oxidation without heating plays an additional prominent role in the degree of saturation of the extractable lipids.
The hydrolysis index I hydr provides a measure for the degree of hydrolysis that has taken place in a sample. Acyl fragments can be hydrolysed by microbial activity (enzymatic hydrolysis) and 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 FAs may be present in the form of insoluble salts, which excludes them from extraction. However, under acidic conditions free FAs will be preserved in their free form in the original matrix (Eglinton & Logan 1991), unless subsequent degradation pathways have effected their preservation (e.g. selective loss of short-chain or unsaturated FAs). It must be noted that the hydrolysis index does not appear to be correlated to total lipid yields in this study. This would indicate that the degree

of hydrolysis does not determine the overall lipid preservation and lipids are commonly preserved even after hydrolysis.
The odd carbon number FA index I o/e corresponds to the proportion of odd carbon number free FAs in the total free FA. In the extracts under investigation C15:0 and C17:0 FAs are major contributors to the total weight in the numerator of this index. Since these FAs 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 directly into the residue as part of a ruminant milk fat during the original vessel use. Since ruminant milk fat is know to contain odd carbon numbered TAGs (Breckenridge & Kuksis 1967; Murata 1977) which can produce odd carbon number FA after hydrolysis. 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 (Shaw 1974; Nes & Nes 1980, 135). However, only when no odd numbered TAGs are present originating from the original vessel context (and when lipid hydrolysis is not complete), is a high I o/e index 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 increasing 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.* in press-a). Severe heating (over 250 ºC) over a longer period of time (over 2 hours) was shown to create progressively condensed materials with high C/H ratio's (between 13 - 16), high overall organic contents (between 57 - 67%) and relatively few remaining biomolecular characteristics, such as nitrogen containing compounds or lipid characteristics. Because part of the chars under investigation fall within these parameters (Table 1), low lipid yields were expected from these residues. However, the TLY data show clearly that the highest amounts of lipid are extracted from chars with both a high C/H and a high C/N, de facto modifying the above model of condensation. Although a certain amount of condensation is obviously desirable for preservation, the char usually contains lipids when a nitrogen component is also present. This correlation suggests that the presence of lipids is either determined by the presence of original biomaterials containing protein (meat, fish, fat-rich seeds) or that the preservation of lipids is strongly determined by the presence of nitrogen containing compounds contributing to char formation. The last effect could be caused by the occurrence of the Maillard reactions known to produce highly insoluble materials of strongly refractory nature.

4.4. Possible origin of lipids

It is obvious from the above that the total extractable lipid composition in archaeological materials cannot be proposed to 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 here. The relative proportion of unsaturated FAs in the residues is relatively low for plants with the exception of the presence of very long chain free FAs (C26-C30) known to be

the hydrolytic degradation products of wax esters (Kolattukudy 1976) combined with the presence of long chain alcohols in Utg-GD 34-0-12 S3. This combination suggests this sample was probably, at least partly, derived from plant material.
The presence of cholesterol leads to the tentative conclusion that several of these 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 value of cholesterol must however be used with caution, for this compound also occurs in the surface lipids of human skin. If squalene is detected in the same extract it must be considered contaminated during preparation of the sample. The isoprenoid unsaturated hydrocarbon squalene also occurs in human skin fats. However, no squalene was detected in the extracts under consideration.
Triacylglycerols 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 Sch-M 79-1-1 (see the first five histograms in Figure 4). Free short chain FAs (C4 - C12), reported to be characteristic for dairy products (Hillditch & Williams 1964, 144-145; Breckenridge & Kuksis 1968) are absent from the extracts. However, 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 acids moieties in some intact TAGs (as illustrated for residue 34-0-4 in Fig. 3 and 4) is rather significant in this respect.
A high abundance if C40 to C46 components in the distribution of even carbon numbered TAGs was shown to be correlated to the presence of degraded ruminant (i.e. cows, goats, sheep) milk fats in ceramics from the Iron Age and Roman period in Stanwick in the UK (Dudd & Evershed 1998). A comparable TAG distribution pattern is clearly visible (Fig. 4.) in the five charred residues. The additional presence of cholesterol leads to the conclusion that the lipids in these chars are, at least partially, derived from ruminant milk fats.
TAG distribution patterns without high abundance if C40 to C46 components, lacking odd carbon numbered TAGs but containing cholesterol, can be interpreted as (at least partially) derived from animal depot fats (Fig. 4). Non-charred residue 35-7-28 and chars Utg54 320-17, NO-P14 6745, Hazendonk 32.740 and Hazendonk 33.781 fall within this category.
Odd carbon numbered free FAs 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 chars from Utg-54 and NO-P14. No odd numbered TAGs were present in these residues and lipid hydrolysis is incomplete (average I hydr = 0.48 from NO-P14 and I hydr = 0.45 for Utg-54). In these residues bacterial growth took place during post-depositional degradation. Chars 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 numbered FA could not be ascribed with certainty to post-depositional bacterial degradation with as much certainty.



Histogram 34-0-30.2 through histogram 14-6-4.3c S show lipid distributions associated with degraded ruminant milk fats.

Histogram 35-7-28 though histogram P14 7054 show lipid distributions associated with degraded animal depot fats. Histogram 8-1 through histogram Utg54 226 show lipid distributions from other origins.

Figure 4: Histograms of the relative abundance [%] of even carbon numbered TAGs (T40 – T54) in total lipid extracts from residues in ceramics from Neolithic and Roman Iron Age settlements in The Netherlands.
The identification of the origins is supported either by the presence if odd carbon numbered TAGs and cholesterol (in extracts shown on the first two lines), by the presence of cholesterol (in extracts shown on line three and four), or by the absence of both (in extracts shown on the bottom line).

4.5. Charred surface residues from Uitgeest-Groot Dorregeest

Charred surface residues from Uitgeest-Groot Dorregeest exhibit an I hydr with an average value of 0.73 with char 34-0-30 being less hydrolysed then average, and char 34-0-12 completely hydrolysed. The low I o/e with an average value of 0.07, indicates the presence of some odd numbered free FAs but no higher then 7% of the total corrected lipid yield. The low average I sat of 0.08 indicates limited bacterial activity and a relatively highly preserved lipid profile. All chars (except 34-0-12) contain extractable lipids that can be ascribed to ruminant milk fats. Comparison of these results with those obtained from non-charred residues suggests a difference in original material and/or in mode of formation.

4.6. Non-charred surface residues from Uitgeest-Groot Dorregeest

The non-charred residues are completely saturated (average I sat = 1.0) indicating a greater exposure to oxidising conditions. The effects of hydrolysis are very limited (average I hydr 0.25) in these residues, resulting in well-preserved TAG profiles completely lacking in odd carbon numbered TAGs. Because non-enzymatic hydrolysis of lipids is greatly enhanced by heating in the presence of water (Davídek *et al.* 1990, 186), the results suggest that these vessels were not used for cooking or boiling of fatty substances in water. The complete absence of odd carbon numbered FAs shows that bacterial growth has occurred only to a very limited extent, suggesting the formation of a denatured material prior to deposition in the soil, possibly as a result of polymerisation or network formation. Although the two residues are visually and chemically different it is possible that these residues were both regularly exposed to the air during their use life. The vessels 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 use of the vessel. It is clear that these vessels were not used as cooking vessels.

4.7. Lipids absorbed in the ceramic

The ceramic samples of two vessels containing non-charred residues yield extremely low concentrations of extractable lipids (<0.01 mg/g) that might easily be dismissed as blanks (Fig. 2a S and 2b S). 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 alcohols (C12, 14, 16, 18) that are, as yet, unexplained.
The four vessels containing charred residues always yield extractable lipids from the ceramic material directly adjacent to the residues. In one case (14-6-4.3c S) an almost identical lipid profile is obtained from the ceramic material reflecting the same origin as the surface residue (Fig. 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 are quite different. The most significant difference

is an increased saturation (average I sat = 0.92) in the absorbed residues, combined with a complete hydrolysis (average I hydr = 1.0), and an increased odd carbon number FA 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 importantly, 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 polymerisation. The increase of I o/e is hard to explain. 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 origin of the odd numbered FAs. Although this explanation seems counter-intuitive because absorption of lipids in the ceramic of the vessel wall would seem to reduce external influences and prevent further degradation, it is clear form the results that the refractory nature of the charred material obviously prevents bacterial degradation even more profoundly. It has been proposed before (Evans 1990; Oudemans & Boon 1991) 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 char. The mechanisms of encapsulation are as yet unknown.

4.8. Charred surface residues from different sites

Extractable lipid profiles from Neolithic chars vary from those from the Roman period in that they often lack MAGs and DAGs and that no odd carbon numbered TAGs are preserved from this period. All of the chars from the Neolithic contain cholesterol and present even carbon numbered TAG distribution patterns that can be interpreted as originating from animal depot fats (Fig. 4). The average degree of hydrolysis (average I hydr = 0.67) and saturation (average I sat = 0.90) are very comparable to those of chars from the Roman period (average I hydr = 0.71 and average I sat = 0.94). The only profound difference is an increase in the I o/e in the Neolithic chars (average I o/e= 0.33) compared to chars from the Roman period (average I o/e = 0.12). Because of the absence of indicators for milk fats, this increase is interpreted as a higher degree of bacterial growth as a result of a longer period of burial in the ground.
Some combinations of index values appear to be typical for a specific settlement. For example, settlement P14 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 are of Neolithic age, the index values of P14 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 or differences in vessel use. Because the number of samples studied is relatively small, no definitive conclusions can be presented here.

4.9. 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 therefore 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 types 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/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, examples of both surface residues and absorbed organics are studied in order to enhance the overall understanding of vessel use based on organic residue analysis.

5. Conclusions

The quantitative study of the extractable lipid composition (including fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols, 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 total lipid yield per gram sample much higher in surface residues (especially charred surface residues), but also the amount of intact acyl lipids (as expressed in the hydrolysis index) and the amount of unsaturated fatty acids are 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 discovery may have important consequences for sampling strategies in 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 ratio's ranging between 9 and 33) indicating more or less severe thermal degradation due to cooking or heating of organic materials. In spite of this thermal degradation, charred surface residues show the highest yields (in mg/g sample) of extractable lipids. Non-charred residues show many characteristics - low overall organic contents, a lower degree of hydrolysis, little or no bacterial degradation and little or no absorption of lipids into the directly adjacent ceramic vessel - that suggest a different vessel use. Most likely these organic residues are the result of a use-life with

longer period of exposure to oxygen without having undergone 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 chars from three native Roman settlements (ca. 1800-2000 years old). Although Neolithic chars showed comparable lipid yields, the lipid profile contained a relatively higher proportion of material of bacterial origin. In spite of some indication for site-specific degradation, this phenomenon is proposed to be the result of ongoing low-level microbial degradation in the ground.

An important consideration for future work in organic residue analysis is the mechanism responsible for the deposition, or accumulation, of residues in and on vessels. The results show that surface residues are a more attractive sample material for the identification of 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, examples of both surface residues and absorbed organics preferably sampled from the same vessels should be studied.