Molecular studies of organic residues preserved in ancient vessels

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Chapter 7

General Discussion

In this final Chapter the main results of this study are discussed in the context of the research questions defined in Chapter 1. Topics of sample selection, analytical protocol, and interpretation of chemical evidence in terms of archaeological context will be addressed, and well as the implications of this study for organic residue analysis in general and the ceramics from Uitgeest-Groot Dorregeest in specific.

1. Aims and Research Questions

1.1. Aims

This study is aimed at molecular characterisation of organic residues preserved in an assemblage of ceramic vessels in order to better understand the way these vessels were used in the Roman period. The main research questions concern the following topics:

- The selection of samples: What organic residue samples best represent the original vessel use? What residues have the best preservation potential for biomolecular marker compounds?
- Analytical techniques: What combination of analytical techniques will supply the most useful information or give the most complete answer? What range of organic compounds can be detected and identified?
- Classification of residues: What differences in chemical composition between residues can be identified? Can we classify the residues based on chemical characteristics?
- The interpretation of chemical evidence in terms of archaeological context: 1) To what extent can the origin of the different compounds be traced back to ancient times? 2) To what extent can the original vessel contents be identified? 3) How to address the questions of original vessel use?

2. Visible solid surface residues versus absorbed residues

This thesis is focused on the study of visible solid surface residues preserved on ceramic vessels recovered from an indigenous settlement dated to the Later Iron Age and Roman period in Uitgeest-Groot Dorregeest. The study was inspired by the work of Abbink on the technology, morphology, and function of the same ceramic assemblage (Abbink 1985, 1999). The assemblage consisted of 147 partial or complete vessels of which a fairly high percentage contained visible surface residues of different types such as soot (45%), chars (32%), red brown "pigment" stains or splatters (5%), and cream coloured crusts (3%). A rich source of visible surface residues originating from one clearly defined assemblage made it possible to perform structural analysis of organic biomarker compounds that remained behind in different types of vessels.
This study focussed on visible solid surface residues preserved as crusts or films adhering to the interior or exterior surface of ceramic vessels. There are various methodological arguments that make these residues more attractive for organic residue analysis then the, more commonly used,

organics absorbed into the ceramic material of the vessel itself. The methodological advantages for using solid surface residues as sample material are threefold. Firstly, archaeologists frequently have no prior knowledge of the actual nature of the original materials involved. Choosing the appropriate extraction method is complicated by this lack of knowledge and the extract may not be representative for the residue under study. Secondly, the study of visible surface residues makes it possible to limit the sample to one single layer of material. Microscopic examination of cross-sections helps to prevent the incorporation of multiple use-phases in a single sample. Absorbed residues are a combined deposit of multiple use-phases, possibly including primary and secondary use remnants. Mixing of different use-phases in one extraction may hinder the interpretation of chemical results. Extractions of absorbed residues may also include post-firing sealing products, complicating the results even more. A final strong argument for the study of surface residues is the fact that absorbed residues have usually been exposed to a more severe thermal regime (both in time and in temperature) than residues situated on the interior surface of the vessel. Extended exposure of foods to temperatures above 300 ºC makes identification of biomolecular markers of the original foodstuffs increasingly difficult (Oudemans *et al.* in press-b).

Most of the analytical techniques that were applied to the surface residues were also applied to one or more ceramic samples in order to check these theoretical considerations.

CHN elemental analysis was combined with FTIR and NMR spectroscopy to study a ceramic sample (sample 33-8-2 S) for overall chemical composition. The FTIR spectrum resembles most closely the spectrum of silica in addition to the presence of saturated aliphatics without carboxylic or alcohol functional groups. NMR results show the presence of two main resonance areas for two broad carbon functional groups: aliphatic and aromatic structures. The fraction carbon aromaticity of the ceramic material resembles that of the most highly condensed surface residues ($f_a > 0.70$). No resonance peak for carbonyl groups (C=O) was identified. In summary, the 'whole sample' approach shows that the ceramic material contains little or no (< 5%) organic material, except for some highly condensed semi-aromatic structure with a clear aliphatic component (possibly aliphatic side-chains).

A more detailed analysis of this condensed material was obtained through CuPyGC/MS study of four ceramic samples (samples 14-6-4.4 S, 14-6-4.2 S, 8-5 S and 8-3 S) that show a pattern of straight chain n-alk-1-enes and n-alkanes ranging from C6 to C18 (Chapter 3). Little or no free fatty acids or other organic compounds were detected in most of the ceramic samples. A small amount of free fatty acids were detected in one sample (14-6-4.4 S). It was proposed that the high temperatures reached inside the ceramic material of the vessel wall during cooking, led to the production of an aliphatic network as a result of radical polymerisation of lipids that were absorbed into the ceramic. This mechanism would explain the chain length distribution of the alkane/alkene pattern observed in the archaeological material. It must be noted, that similar alkane/alkene patterns are detected in surface residues from the interior and exterior of vessels, and in experimental chars produced in glass containers in the laboratory (Chapter 3). According to a model proposed by Hartgers and co-workers (1995) pyrolysis of long-chain aliphatic components that are bound to some larger structure, will result in homologous series of alkanes and alkenes leading up to the C-number of the longest chain minus one. Shedrinski (1991) reported a similar alkane/alkene pattern in PyMS data of salts of fatty acids. This origin may

play a role in some archaeological residues, although the attempts to extract the insoluble salts of fatty acids from ceramics through acidic extraction released only very small amounts of the "recalcitrant" fatty acids (Stern *et al.* 2000) and led the researchers to conclude that such non-extractable lipids were probably bound as cross-linked macromolecules. It is worth noting that in experimental chars the presence of lipids was a prerequisite for the formation of the alkane/alkene patterns. Experimentally charred protein and starch combinations did not form alkane/alkene patterns. A study of free and covalently bound lipid organic compounds in archaeological ceramic samples by Craig and co-workers (2004) concludes that even after solvent extraction, alkali saponification and catalytic hydropyrolysis, a significant amount of residual carbon (> 50 wt % of the total organic carbon) remained present in the ceramic sample in the form of a 'recalcitrant' polymeric phase. The researchers confirmed the presence of a condensed polymeric structure that they infer to be of highly aromatic nature. They propose the formation of this material via gradual polymerisation/aromatisation of food residues in repeated cooking phases, and possibly through additional diagenetic structural transformation after burial (Craig *et al.* 2004).

The fact that some ceramics contain a certain amount of absorbed extractable lipids is well known in organic residue analysis (Evershed *et al.* 1990; Heron & Evershed 1993). In this thesis extractable lipids from several surface residues were compared to the directly adjacent ceramic material of the vessel (Chapter 5) in order to determine the similarities and differences between the two kinds of sample material. Ceramic samples of vessels containing non-charred residues contain extremely low concentrations of lipids (< 0.01 mg/g) that might easily be dismissed as blanks. The four ceramics containing charred surface residues (34-0-12 S, 14-6-4.4 S, 14-6-4.2 S and 14-6-4.3C S) also yielded extractable lipids from the ceramic material of the vessel wall in varying amounts (0.02 – 0.16 mg/g). However, in only one case (sample 14-6-4.3C S)was an almost identical lipid profile is obtained from the ceramic material and the surface residue. In the other pairs, extractable lipid profiles from surface residues and ceramic material are quite different. The most significant difference is an increased saturation of fatty acids and TAGs combined with a complete hydrolysis and an increased percentage of odd carbon number fatty acids. Lipids extracted from charred surface residues are obviously in a better state of preservation than those extracted from the directly adjacent ceramic material of the vessel. This difference in preservation is likely the result of a combination of chemical mechanisms. Most importantly, absorbed lipids will have been exposed 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 (Davídek *et al.* 1990, 186) - and the high degree of saturation due to heat induced polymerisation. The increase in the relative amount of odd carbon number fatty acids is hard to interpret. The extractable lipids may have resulted from complete hydrolysis of odd numbered TAGs (indicative of ruminant milk fats), or may indicate an increased bacterial activity in the post-depositional phase. The relatively polar porous ceramic material is likely to be more permeable to bacteria then the relatively apolar charred material of the residues causing an increase in bacterial debris inside the ceramic matrix in the post-depositional phase.

In conclusion, the methodological choice of solid surface residues as a more attractive sample material for the identification of original vessel contents, is supported by experimental results

as shown in this thesis. 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 may be very different and may depend on the kind of vessel use. Data presented in this thesis 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/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 studied.

3. Analytical techniques - possibilities on a molecular level

The most important question about analytical protocol concerns the potential of various analytical techniques to supply useful information about original vessel use. The possible range of chemical characteristics detected and identified using different analytical techniques are summarised here.

3.1. CHN analysis

Elemental CHN analysis was shown to be a useful technique for the initial identification of the organic content and the basic organic composition of the organic fraction of a sample (Chapter 5 and 6). A distinct difference in organic content between charred residues (27 - 70%), non-charred residues (4 - 9%) and the ceramic material of the shard (< 5%) can be seen (Table 1). Non-charred residues (both cream coloured crusts and red-brown residues) obviously consist mainly of inorganic material and hardly contain more organic material then the ceramic material itself. However, the organic fraction of such non-charred residues presents a completely different elemental composition than the charred residues. The low C/H ratios show an obvious lack of condensation, confirming that the residues were not severely heated.
Even among the charred residues from Uitgeest-Groot Dorregeest there is a considerable variation in elemental composition (Table 1). The total organic fraction varies from 27 - 70% (average 50%); the C/H ratios vary from 9 - 18 (average 13) indicating a less aliphatic and more condensed nature of the material as the ratio goes up. The C/N ratios vary from 6 – 11 (average 8) indicating a decrease in the amount of nitrogen present in the material as the ratio goes up. The charred residues from other Roman Iron Age settlements show a similar picture. Chars from the Neolithic (Chapter 5) do not differ significantly in overall organic fraction, but show an increased C/N and C/H ratio indicating an increase in condensation and a decreased

presence of nitrogen containing compounds in the chars, possibly caused by extensive thermal exposure.

Table 1 – Results for CHN elemental analysis

		n	Total Organic [%]		C/N ratio		C/H ratio	
Excavation	Type residue		Range	Average	Range	Average	Range	Average
Uitgeest -GD	Non-charred	2	4-9	7	8 - 20	14	2 – 6	4
	Charred	9	27 – 70	50	6 – 11	8	9 – 18	13
	Ceramic material	1	4	-	18	-	6	-
Other RIA sites	Charred	3	51 – 61	58	5 – 10	7	9 – 13	12
Neolithic sites	Charred	4	49 – 62	56	7 – 14	11	15 – 32	20

3.2. FTIR Spectroscopy

FTIR Spectroscopy was applied to identify the overall composition of functional groups in complete samples (Chapter 6), and to compare them to experimentally charred modern reference materials like amylose and albumin.
Experimental chars were analysed to identify typical characteristics of thermal degradation in polysaccharide and protein chars. The amylose chars produced at 250 °C show increased aromatisation and a loss of intact pyranose units after 2,5 hours of heating, but still show some characteristics indicative of ketone presence after 17 hours of heating. These experimental results show the possibility that some characteristics for polysaccharide origins of chars may be preserved after significant thermal exposure in environments without oxygen (such as a boiling food). The albumin chars show signs for dehydration and protein fragmentation after 2,5 hours of heating, but retain some of the obvious indicators for proteinaceous material (the Amide I and Amide II bands from the peptide backbone). However, after heating for 17 hours the picture changes dramatically and reflects increased dehydration and the loss of almost all protein characteristics. Signs for increased condensation and aromatisation are then found in the char. A new transmission band becomes visible around 2225 cm^{-1} that is ascribed to organic nitrilles (probably resulting from the dehydration of amines or other condensation reactions taking place during extensive periods of heating). In summary, FTIR spectroscopy has shown that increased periods of thermal exposure (even at the same temperature) reduce the number of characteristics of polysaccharides and proteins to a minimum, and produce chars that are chemically more and more similar. This effect limits the potential for identification of the original vessel contents after extended periods of heating.
FTIR spectra of archaeological residues are characterised by the presence or absence of a relatively limited number of broad absorption bands. FTIR spectra can clearly distinguish

between cream coloured crusts, soot residues and charred residues. The FTIR spectra of the charred residues closely resemble those of experimentally heated modern foodstuff (amylose and albumin), give ample confirmation of the aromatic nature and indicate the relative amount of dehydration that has taken place in the ancient chars. Functional groups prevalent in lipids can also be identified in spectra of chars as well as some small amounts of functional groups indicative of mildly heated carbohydrates or intact peptides. The FTIR spectra confirm that the cream coloured residues are non-carbonised residues with low organic content consisting primarily of inorganic salts such as calcium carbonate. The organic component consists primarily of proteinaceous material while lipid indicators are absent. The FTIR results of the soot residue resemble the charred residues to some extent, except the aromatic absorption bands are located in a slightly different place, indicating the presence of six- membered aromatic rings that are further conjugated – a feature typical for the polynuclear aromatic hydrocarbons (Williams & Fleming 1966, 67) which suggest an origin of smoke condensates.

3.3. NMR spectroscopy

The NMR studies have resulted in a semi-quantitative classification of solid organic residues and show the presence of a limited amount of specific biomolecular characteristics for lipids, peptides and carbohydrates. Solid-state NMR also confirms results obtained in analytical pyrolysis studies.

Most of the solid-state CP/MAS ^{13}C NMR spectra of archaeological residues reveal two main resonance areas assigned to two broad carbon functional groups: aliphatic and aromatic structures. Aliphatic structures show up as a broad resonance band between 10 - 60 ppm, demonstrating individual resonance peaks for $-CH_3$ or $-CH_2-$ in some of the residues. Aromatic structures and alkenes can be seen in a second broad area formed by two overlapping resonance bands, 100 - 150 ppm for alkenes, and 110 - 140 ppm for aromatic carbons respectively. In addition, more specific resonance peaks can be seen in some of the residues. Firstly, a clear resonance peak at 160 - 180 ppm is assigned to a combination of various carbonyl carbons (C=O), such as in carboxylic acids and their salts at 166 - 181 ppm, aliphatic esters R-COO-R' at 169 - 176 ppm; and amides $-CONH_2$ at 162 - 179 ppm. Secondly, the presence of proteinaceous material in one of the residues is visible as a broadening of the aliphatic resonance area between 50 and 60 ppm assigned to a C-N resonance in proteins (Sherriff *et al.* 1995) and to a more distinct collagen resonance at 50 - 55 ppm. In residue 34-7-95.a this effect is combined with a broadening of the aromatic resonance in the area of 114 - 124 ppm, assigned to the presence of a nitril resonance (R-CN), indicating the presence of proteinaceous material in this particular residue. Finally, an indicator for residual sugar components appears at very low intensity in residue 14-6-4.2 as a resonance peak centred at 73 ppm, indicates the presence of oxygen-substituted carbons in carbohydrates. Another important marker for charred polysaccharides is a resonance peak at 155 ppm that indicates the presence of furanyl, or possibly phenolic, C-O functionalities (Pastorova *et al.* 1994). Although no distinct resonance peaks can be observed in the NMR spectra in this study, a certain broadening of the aromatic

area can be observed in the highly aromatic sample 14-6-4.2 and possibly in sample 33-8-2.b and 33-5-2.a.
The degree of condensation in the solid residues can be quantified by calculating the fraction carbon aromaticity (Hayatsu *et al.* 1986; Botto *et al.* 1987). The fraction carbon aromaticity f_a varies from 0.33 to 0.73 for charred solid residues, while the cream coloured residue does not show any aromatic resonance signal. The archaeological residues can be divided into three groups depending on their fraction carbon aromaticity: highly condensed chars with f_a >0.50 comparable to various experimentally charred starch-rich; mildly condensed chars with and f_a of 0.30 - 0.40 comparable to experimentally charred fatty fish, and non-aromatic residues have no measurable fraction carbon aromaticity.

Table 2: Resonance in NMR spectra

Nr [a]	Sample nr [b]	Type [c]	Carbonyl groups	Furanyl/ Phenolic C-O	Aromatic	R-CN Nitril	O-alkyl	C-N Collagen	Aliph	f_a [d]	DTMS Chemo-type
ppm			160-180	155	100-150	114-124	70-75	50-60	10-60		
36	34-7-95.a	C	++	-	+	±	-	+	++	0.33	A1
8	14-6-4.4	C	+	-	+	-	-	-	++	0.40	A1
24	33-8-2.b	C	+	?	++	-	-	?	++	0.54	A2
5	14-6-4.2	C	-	±	++	-	±	-	+	0.72	A2
21	33-5-2.a	C	±	?	++	-	-	-	+	0.73	-
-	33-8-2 S	S	-	-	+	-	-	-	+	-	-
31	35-7-28	L	+	-	-	-	-	?	±	0.00	D

Resonance intensity on a nominal scale: absent (-), trace (?), low (±), present (+) and high (++).
[a] Sample numbers refer to earlier publications (Oudemans & Boon 1996; Oudemans et al. in press-b)
[b] Find number: Archaeological registration code - the first 2 digits indicate the excavation pit
[c] Type residue: C = Char, S = Shard , L = cream coloured crust
[d] f_a = fraction carbon aromaticity (accuracy ± 0.02)

3.4. CuPyGC/MS

The suitability of analytical pyrolysis techniques for the chemical characterisation of surface residues was also studied. Four residues were analysed in detail with Curie-point pyrolysis gas chromatography/mass spectrometry (Chapter 3).
Many bioorganic moieties are detected in the residues situated on the interior or exterior surface of vessels, including fatty acids and characteristic markers for proteins and polysaccharides. Black residues occurring on the outside of a vessel show many polynuclear aromatic hydrocarbons like naphthalenes, phenanthrenes and their methylated isomers in the CuPyGC/MS data. Since these PAHs were found to desorb from the sample and are common

in smoke condensates of wood fires (Medalia *et al.* 1983), the residues are probably the result of cooking on an open fire.

The residues situated on the inside of vessels, show three compound classes of bioorganic significance: markers for proteins, polysaccharides, and lipids. Fragments indicative of charred proteins are seen in the CuPyGC/MS results. Pyrrole, indole, methylindole, toluene, phenol, and cresol are interpreted as 'protein' indicators for hydroxyproline, tryptophane, phenylalanine and tyrosine respectively (Meuzelaar *et al.* 1982, 109). Some pyrolysis products indicative of adjacent pairs of aliphatic amino acids in intact proteins (Boon & De Leeuw 1987) and some of the 3,6-piperazinediones (Munson & Fetterolf 1987) described as pyrolysis products of proteins, could be detected in one of the samples. Although thermal exposure has caused severe denaturation of the original peptide chain, some short peptides chains as well as individual amino acid characteristics are detected. It is possible that a radical reaction causes the specific amino acid side chains to be linked chemically to (or to get 'embedded' in) the forming char. Thousands of years later, Curie-point pyrolysis releases these characteristic amino acid side chains. Protein markers occur mostly in samples in combination with free fatty acids and polysaccharide markers. However, they also occur in combination with inorganic compounds i.e. carbonates (as in sample 35-7-28).

Table 3: Results for CuPyGC/MS

Nr	Sample Type	Proteins	PS	FFA	FA	A/A network	PAH	Location on Vessel	Sediment
-	Experiment 1	+	+	+	-	-	-		
-	Experiment 2	+	+	+	-	+	-		
26	34-0-30	+	+	+	+	+	-	Interior	Humic Clay
31	35-7-28	+	-	-	-	+	-	Interior	Sand
8	14-6-4.4	+	+	+	+	+	-	Interior	Sand
11	18-3-2.b	-	-	-	-	+	+	Exterior	Humic Clay
-	Ceramic 14-6-4.4S	-	-	-	-	+	-		Sand

Fragments indicative of charred polysaccharides are detected in the CuPyGC/MS results. These identifications are in agreement with other studies that have shown low temperature chars of cellulose still retain 'sugar' characteristics (Julien *et al.* 1991; Pastorova *et al.* 1993a; Boon *et al.* 1994; Pastorova *et al.* 1994). Markers such as methylfuran and dimethylfuran were detected in some of the archaeological residues. The detected markers are rather unspecific and cannot give any indication of the original type of polysaccharides. Experimentally charred food (flour, albumin and vegetable margarine heated for 125 min at 250 °C) shows similar markers in Curie-point pyrolysates. Apparently some polysaccharide characteristics remain preserved in low temperature chars (possibly in the form of dehydrated oligosaccharides and melanoidins).

Increasing the temperature during charring reduces the recognisability of the remaining products.
It is not clear whether all the polysaccharide markers detected in the chars are actually part of the original vessel content. In theory some of them could originate from oligosaccharides that impregnated the residues from the surrounding soil during burial. Pyrolysis studies of peat samples from the Assendelver Polders by Moers (1989, 89) have shown that sugars from polysaccharides could also be present in pyrolysates of peats from Uitgeest-Groot Dorregeest. The majority of the sugars in the study by Moers were derived from the remains of vascular plants and occur in the form of biopolymers that are non-soluble in water. However,. it is possible that some water-soluble saccharides derived from plants or bacterial cell walls may have impregnated the archaeological material. In practice no indications have been found to support this theoretical possibility.
Lipid remains were detected in the form of free fatty acids, fatty amides and alkanes and alkenes. Straight chain saturated fatty acids (C11:0 – C18:0), unsaturated fatty acids (C18:1) and fatty amides (C16:0, C18:0 and C18:1) were detected with CuPyGC/MS. The free fatty acids were evaporated from the sample. The fatty amides can be produced by heating of fatty acids with amines to a temperature of 200°C (Davídek *et al.* 1990, 183). It is not clear whether this formation happens during the preparation of food in prehistoric times or during the pyrolysis phase of the analysis. It should be noted that free fatty acids and fatty amides are often observed in combination with protein markers and sometimes with markers for polysaccharides. Mono-, di- or triacylglycerols could not be detected with the pyrolysis techniques utilised, but were proven to be present in the residues in DTMS studies (Chapter 4) and lipid extraction studies (Chapter 5).

3.5. GC/MS of extractable lipids

A quantitative study was performed of the extractable lipid composition in charred and non-charred surface residues and of lipids absorbed into the ceramic material of vessels (Chapter 5) and included fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols, sterols and long-chain alcohols.
Results show an apparently greater degree of lipid preservation 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), the amount of intact acyl lipids and unsaturated fatty acids is also 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 (especially those containing proteins). 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 the highest yields (in mg/g sample) of extractable lipids. However, non-charred residues show many characteristics (low overall organic contents, a lower degree of hydrolysis, little or no bacterial degradation and a directly adjacent vessel wall that contain little or no absorbed lipid material) that suggest a different kind of vessel use. Most

likely these organic residues are the result of a longer period of exposure to oxygen without having undergone severe heating. Non-charred residues may result from organic decorative materials, or from remains of organics stored or served in the vessels.
Lipids from charred surface residues from two Neolithic sites (ca. 5000 years old) and from three native Roman settlements (ca. 1800-2000 years old) were compared. Although Neolithic chars did not produce significantly lower lipid yields, the lipid profile contained relatively more free fatty acids and a higher proportion of material of bacterial origin. This phenomenon is proposed to be the result of ongoing low-level microbial degradation in the ground during burial.

4. Chemical classification of residues - chemotypes and their origin

Thermal fragmentation and mass spectrometry were applied to obtain chemical 'fingerprints' of the complete residues including the extractable fraction and the non-extractable solid fraction. The mass spectra were used to classify the residues based on chemical composition using multivariate analytical techniques (discriminant analysis and cluster analysis). A study of CuPyMS data using MVA (Chapter 2) resulted in a first classification which was later refined in a study applying Direct Temperature-resolved Mass Spectrometry and MVA (Chapter 4). DTMS could measure a much wider range of masses including of intact lipids and fragments for various condensed aromatic polymers. The DTMS results confirmed many of the earlier results of the CuPyMS study and resulted in a classification of the surface residues in six chemotypes (A_1, A_2, B, C, D, and E). Each chemotype contains a group of residues with similar chemical composition.
Results from other analytical techniques were used to fill in the picture and elaborate on the particular details of these chemical compositions. Notwithstanding the large number of spectroscopic techniques employed, the chemical classification to a large extent follows the original visual classification made by the ceramic specialist. Charred residues, containing starches, are mostly found in chemotypes A_1 and A_2, chemotype B contains soot residues, while three particularly well-preserved protein residues (one pigment and two charred residues) can be found in chemotype C. All residues with little or no organic content (including two of the three cream coloured residues and a number of charred residues) are found in chemotype D while chemotype E contains one severely contaminated soot residue. The main chemical characteristics and the possible origin of the chemotypes are discussed here.

4.1. Charred Residues - Chemotypes A_1 and A_2

Charred residues obtained from the interior of ceramic vessels have a high overall organic content of 27 - 70%. FTIR spectra show that the inorganic fraction of the chars is limited and consists primarily of silica. FTIR spectra of charred residues closely resemble those of experimentally heated modern foodstuffs (both amylose and BSA) and give clear evidence of the aromatic nature of the material. Functionalities prevalent in lipids can be identified in all chars, and a few functionalities indicative of mildly heated carbohydrates can be seen in residue 14-6-4.2, while intact peptides are indicated in residues 34-7-95.a and 34-0-30.

The chars can be divided into two subgroups: Chemotype A_1 and A_2. There are indications that different original materials formed the chars of chemotypes A_1 and A_2 - A_1 being rich in proteins and lipids, and A_2 primarily consisting of starches. These different biomaterials have a different tendency to form condensed chars. Starchy materials condense at relatively low temperatures, while other materials (for instance fatty fish or fat-rich meats) are much less sensitive to condensation. Although many archaeological questions would be best served with an exact determination of the precise food types used, this is very difficult after extensive thermal degradation.

Chemotype A_1 consists of 11 residues most of which are charred residues (except for cream coloured residues 8-5). The NMR data for chars 14-6-4.4 and 34-7-95.a show mildly condensed chars with a low fraction carbon aromaticity ($f_a < 0.50$). The C/H ratios of these chars (between 10 - 11) reflect limited condensation in comparison with those of the highly condensed category. The overall organic fraction of the residues is between 38 – 49% and the preservation of carbonyl group resonance peaks (most likely originating from lipids) and markers for proteinaceous materials are well represented in these chars. The DTMS data for chars 7-7 show a relatively well-preserved lipid fraction with short chain lipids (both in fatty acids and in intact acylglycerols), cholesterol and acylglycerols with odd numbers of carbons. The biomolecular origin of such a lipid profile could be found in ruminant milk fats that commonly contain relatively high amounts of smaller saturated acids. The presence of acylglycerols with an odd number of carbons seems in agreement with a milk fat origin. The pyrolysis range shows fe. markers for peptides and intact proteins and is dominated by amino acid markers and a wide range of masses indicative of a condensed polymeric structure. The spectrum shows the presence of both even and odd numbered peaks, indicating the incorporation of nitrogen containing compounds into the condensed aromatic material, which is comparable to charred BSA. Either a pure protein char or a protein/polysaccharide mixture could render such results. Example residue 1 (nr. 7-7) can be identified as a relatively well-preserved cooked animal product (most likely a ruminant milk) possibly prepared in combination with a starch. A cooked dairy product or a milk-based cereal-gruel seems to be the most likely origin of this residue. Although some variation occurs within the chemotype (especially in the amount and kind of lipids preserved in the chars, the total organic content (average 47%), C/H ratio (average 13) and the relatively low C/N ratio (average 8) were consistent with an interpretation of mildly charred protein or protein/starch mixture.

Chemotype A_2 consists of 10 charred residues. The NMR data for chars 14-6-4.2 and 33-8-2.b show highly condensed chars with a high fraction carbon aromaticity ($f_a > 0.50$). The C/H ratios (between 13 – 16) indicate that progressive condensation has taken place in these residues and the NMR results closely resemble experimental cellulose chars heated for 150 minutes at 270 - 290 ºC (Pastorova *et al.* 1993b). The organic content of these residues is relatively high (between 57-67%) but few specific biomolecular characteristics can still be traced in these residues. The NMR results show little or no indication of the presence of lipids or protein remains, and the only markers that can be detected are very reduced indications of the presence of residual carbohydrate characteristics (in residue 14-6-4.2). However, the DTMS results for charred residue 33-8-2.a include a lipid profile with high peaks for saturated FAs (C10:0 – 28:0), a small amount of unsaturated FAs, and some markers for DAG fragments and minor peaks for wax esters C42 - 44. The origin for the very long chain fatty acids and the wax esters can be found in the presence of plant waxes (Kolattukudy 1976; Bianchi 1995). Plant leaf waxes have been detected before in organic residues preserved in association with ceramic vessels (Evershed *et al.* 1991; Charters *et al.* 1995). The additional series of minor peaks in the range 396 t/m 424, indicates the presence of what might be markers for a sterol mixture. Dehydrated sterols such as C27 (m/z 368), C28 (m/z 382) and C29 (m/z 396) can survive charring at 250 ºC for 120 min. The pyrolysis spectra show some residual polysaccharide markers and a high mass area with an emphasis on the even mass values, indicative for a considerable polysaccharide component. Markers for intact polypeptides and amino acids are also present. Residue 23 is a mildly charred starch (with a minor protein component) with a partially hydrolysed lipid fraction including plant waxes. A combination of (leafy) vegetables with grain could be the origin of this residue. A small amount of animal material (meat or meat fat) could also have been included. Although some variation occurs within this chemotype, the total organic content (average 62%), C/H ratio (average 14) and the relatively higher C/N ratio (average 11) are consistent with an interpretation of a highly condensed originally starch-rich material.

4.2. Well preserved protein residues – Chemotype C

Three residues were classified as chemotype C, two charred residues (samples 34-0-30 and 14-6-4.2b) and one red-brown 'pigment' residue (sample 8-1). Although these residues are visually different, they share a low total organic fraction (9 - 37%), a low degree of condensation (average C/H = 7) and a high amount of nitrogen in the residues (average C/N = 7). No sample of this category was submitted to NMR spectroscopy due to limited sample size, but FTIR of char 34-0-30 shows a well preserved protein signal with some remaining resonance in the Amide I band. The DTMS spectrum for charred sample 34-0-30 shows a lipid profile not unlike the lipid fraction in chemotype A_1. The protein fraction shows a high degree of preservation with markers for intact peptides and relatively high mass peaks for amino acid moieties. A mildly heated protein source might easily render such a pattern, although the exact origin of the protein material cannot be established. A similar DTMS spectrum was seen in experimentally heated albumin, but a lightly charred milk product could easily render a similar pattern (due to the thermal stability of casein). Although the absence of a starch source cannot be proven, no

positive indication for the presence of residual polysaccharide characteristics can be detected (contrary to what one would expect in a residue with such limited thermal degradation). It is therefore likely that starch is either absent or is only a minor component of the original material. The origin of residue 34-0-30 is probably a lightly charred animal product (possibly milk). The lipid analysis of the other char in this chemotype (sample 14-6-4.2b) comes to a comparable conclusion: a well-preserved lipid profile, probably originating from a ruminant milk fat.
However, not all residues in cluster C are charred: residue 2 (sample 8-1) is a red-brown material situated on the exterior of the vessel. Its placement in cluster C is based on the presence of markers for a well-preserved protein fraction. It is significant to note, that the DTMS spectra lack chemical evidence for the presence of lipids. However, the lipid analysis shows a very small amount of extractable lipids are present. The lipids are completely saturated (average I sat = 1.0) indicating exposure to oxidising conditions. The effects of hydrolysis are very limited (I hydr = 0.10) resulting in a well-preserved TAG profile lacking cholesterol and lacking odd carbon numbered TAGs. Hydrolysis of lipids is greatly enhanced by heating in the presence of water (Davídek *et al.* 1990, 186) which would suggest that this vessel was not used for cooking or boiling of fatty substances in water. The 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. It is possible that this residue was regularly exposed to the air during the use life of the vessel. Residue 8-1 may have been applied as a decoration to the exterior of the vessel. The placement of the material confirms such a decorative purpose. Visually similar materials were registered on the exterior of various vessels in the Uitgeest-Groot Dorregeest assemblage in dots, stripes or small patches (Abbink 1999, pp. 233 & 289).

4.3. Soot residues – Chemotype B

This chemotype contains three similar looking residues from the soot category. None of these samples were submitted to CHN analysis, NMR spectroscopy or the GC/MS study of extractable lipids. However, results from CuPyGC/MS, DTMS and FTIR all give a consistent picture. FTIR spectroscopy of sample 31-4.b indicates a residue with a medium amount of organic material (comparable to residue 14-6-4.4 at 49%). FTIR results show the presence of aliphatic moieties and aromatics with further conjugated six-membered rings typical for polynuclear aromatic hydrocarbons. The DTMS results of sample 31-4.b are most clearly characterised by the absence of markers for edible biomaterials. The presence of sulphur-containing compounds is most likely caused by a contamination of the sample with a small amount of ceramic material from the vessel wall (accidentally included when the sample was scraped from the vessel surface during sampling). The presence of intense mass peaks m/z 28 and 44 in the early part of the temperature range, indicating decarboxylation of organic compounds and the presence of alkylated aromatic compounds and long-chain aliphatic compounds in the higher temperature ranges suggests a wood smoke condensate or soot. In the evaporation range, markers for short chain aliphatic compounds can be seen, indicating their origin from evaporation rather then pyrolysis. It is possible that the aliphatic compounds are a

minor component of the smoke condensate. However, considering the relatively limited organic content of the residue, the inclusion of a minor internal contamination in the mass spectrometer cannot be excluded. The origin of these samples as wood smokes is confirmed by the detailed results of the CuPyGC/MS study. Results of residue 18-3-2.b show many polynuclear aromatic hydrocarbons like naphthalenes, phenanthrenes and their methylated isomers. Since these PAH's are common in smoke condensates of wood fires (Medalia *et al.* 1983), these residues are probably the result of cooking on an open fire. It is notable in this context that the PAH's only occur in residues situated on the outside of the pottery, a place consistent with their origin as smoke condensates.

4.4. Residues with low organic content – Chemotype D

This chemotype contains four chars and two cream coloured residues (35-7-28 and 35-20). Chemotype D is determined not so much by the presence of typical chemical markers, but by the markers it lacks. DTMS spectra of these residues (Chapter 4) show many characteristics that indicate the low organic content of the residues. Only the presence of a small amount of air and some contaminants determine their chemotype. And although these residues all share a low organic content, a clear distinction can be seen between the cream-coloured residues in this cluster and the chars in this cluster. Both groups of residues will be discussed below.
Cream coloured residues obtained from the interior of ceramic vessels have a very low overall organic content according to their CHN analysis (4 – 5%). Although the percentage organic material present in the residues is similar to that of the ceramic material, the chemical composition of the organic material is quite different. FTIR and NMR results show an absence of aromatic signals, which is confirmed by the low C/H ratio (3 - 6) and indicates that these residues are not the result of carbonisation of foods in cooking vessels. FTIR results also show an overwhelming presence of precipitated calcium carbonate in the residues, as well as the presence of silica. FTIR results of the organic fraction show only a small amount of well-preserved, unheated (or lightly heated) proteinaceous material, while lipids seem absent. DTMS results show that these residues lack markers for lipids and primarily contain markers for a relatively intact protein fraction and some additional contaminating compounds (aliphatics and sulphur containing compounds). Some markers for peptides or intact proteins could be detected in minor amounts and many markers for amino acids could be detected in relatively high intensities. The presence of a relatively well-preserved protein profile makes these residues very similar in chemical composition to residue 2 (nr. 8-1) in cluster C. Only the very low organic content has placed these residues in cluster D. In spite of the agreement of FTIR and DTMS about the absence of lipid characteristics, the presence of extremely small amounts of lipids in some of the samples is shown through lipid extraction techniques. Cream coloured sample 35-7-28 contain a small amount (total lipid yield 1.32 mg/g) of completely saturated lipids with a limited degree of hydrolysis (I hydr = 0.39). A well-preserved TAG profile (without odd carbon numbered TAGs) and the presence of cholesterol are seen. This lipid profile suggests an animal material that has been extensively exposed to oxygen without extensive heating. The absence of odd carbon numbered FAs or TAGs shows that bacterial growth has occurred only to a very

Table 4: Chemotypes and their chemical characteristics

	n	Residues	Example	Chemical Characteristics	Biomolecular origin
A_1	11	1, 4, 8, 9, 15, 22, 25, 30, 35, 36, 37 10 chars 1 cream coloured residue	Charred residue 7-7	-Organic fraction = 47% -Mild aromaticity fa < 0.5 -Low C/N ratio = 8 -Short chain FA and DAGs -Odd numbered DAGs -Cholesterol -Protein/peptide markers -Amino acid markers -Polysaccharide/protein char	Mildly condensed protein or protein/starch mixture. Rich in well-preserved lipids Charred animal product (most likely ruminant milk), possibly in combination with a starch. Cooked dairy product or cereal gruel.
A_2	10	3, 5, 12, 13, 16, 17, 20, 23, 24, 29 10 chars	Chared residue 33-8-2.a	-Organic fraction = 62% -High aromaticity fa > 0.5 -High C/N ratio = 11 -Short chain FA and DAGs -Unsaturated FA -Plant sterols -Waxes (plant leaf wax) -Residual polysaccharides -Protein/peptide markers -Polysaccharide/protein char	Highly condensed starch-rich material. A charred starch mixed with (leafy) vegetables and possibly a small amount of an animal meat or fat. Cooked grain and vegetable stew or cereal gruel.
B	3	7,11,19 3 Soots	Soot residue 31-4.b	-Organic fraction ± 50% -Aliphatic compounds -Sulphur compounds -Alkylated polyacenes	Smoke condensates
C	3	2,26,34 2 chars 1 pigment	Charred residues 34-0-30 14-6-4.2b	-Organic fraction = 37% -Low aromaticity C/H = 9 -Low C/N ratio = 6 -Markers for intact peptides -Amino acid marker -Short chain FA and DAGs -Odd numbered DAGs -Cholesterol	Lightly charred animal product rich in well-preserved lipids (possibly ruminant milk). Probably without starch.
			Red-brown residue 8-1 on exterior of vessel	-Organic fraction = 9% -Low aromaticity C/H = 5 -Low C/N ratio = 8 -Markers for intact peptides -Amino acid marker -Lipids (sat., limited hydrolysis) -No cholesterol	Non-charred protein rich product. Probably exposed to oxygen. Possibly decorative material made with plant oils and proteins.
D	6	6, 10, 18, 31, 32, 33		-Organic fraction = 4-5% -Low aromaticity C/H = 3-6 -High C/N ratio = 18-20 -Markers for peptides & AA -Sulphur & Aliphatic compounds -Lipids (sat., limited hydrol.) -Cholesterol	Inorganic crust of $CaCO_3$ with residual well-preserved proteins, small amount of oxidised animal lipid. Residue of storage or transport of solids/liquids with low organic content.
E	1	28	1 soot 34-7-95.b	-Sulphur compounds	Low organic content

limited extent. The DTMS results confirm that the residues have not undergone severe charring. In summary, these residues do not only differ from the other residues in visual appearance, they also differ in chemical composition. They are primarily inorganic crusts containing a small amount of well-preserved proteins and minute traces of oxidised animal lipids. It is clear that these residues were not the product of heating or cooking of foods. It is possible that these vessels may have been used for storage or transport of solid materials or liquids with low organic content. Obvious exposure to air can be seen in the DTMS results and the extracted lipids. The origin of the very low amounts of relatively well-preserved proteinaceous material may reflect one of two things: i) a proteinaceous waterproofing material was applied to the ceramic vessel prior to use (possibly milk) after which the vessel was used as container for solids of low organic liquids (water storage vessel) or ii) cream coloured residues may be the result of the storage or cold preparation of a special kind of protein-rich food or non-food (bone or skin glue).

Four chars classify in chemotype D although the exact origin of these charred residues is not completely clear. The CuPyMS data indicate that neither charred polysaccharides, nor protein markers, nor fatty acids are present. Only a clear alkane/alkene pattern is observed. This would suggest that the residues were formed from lipids that have been exposed to extended periods of high temperature (Chapter 3). The high temperatures would cause radical reactions and form a cross linked aliphatic network that would produce alkanes and alkenes during purolysis. One explanation for the formation of such a residue may be found in a post-firing treatment for waterproofing. An alternative explanation is the use of the vessel as a container for roasting or drying of special foods such as nuts, roots, or grains. Some fat may have been added to prevent burning if the foods did not contain lipids. Interesting in this case is also the frequent presence of soot on the outside of these vessels, suggesting heating of the vessel above an open fire, rather then use of the vessel for storage or transportation.

4.5. Sulphur contamination - Chemotype E

DTMS results of soot residue 28 (sample 34-7-95.b) primarily show m/z values for sulphur-containing compounds and aliphatics. No lipids can be detected, but phthalates are present. This residue contains so little organic material, that it is not further discussed.

5. Interpretation of chemical information in an archaeolgical context

5.1. Definition of Archaeological Goals

The final archaeological value of the work presented in this thesis, depends on i) the range of organic compounds that can be detected, ii) the possibilities to detect differences in chemical composition between residues, iii) the extent to which the origin of the different compounds can be traced back to prehistoric times and, iv) how original vessel use can be addressed.

5.2. Interpretation of final composition

The range of organic characteristics and compounds identified, and the differences in chemical composition detected between residues are described above. The interpretation of the chemical composition of organic residues in terms of original vessel use is like resolving the chemical puzzle of transformation processes in reversed order. In order to prove the use of particular biomaterials in prehistoric times, the transformation processes that influence the chemical composition of the remaining residues need to be disentangled and the results translated back to the their possible original materials.
The residue transformation processes are summarised in reversed order in Figure 1 and include processes in the post-depositional context or "archaeological context" including the so called C_2-transforms (cultural transforms) that can take place during and after excavation as well as the N-transforms (natural transforms) (Schiffer 1972, 1983), and the processes in the original prehistoric context or "systemic context", also known as C_1-transforms (cultural transforms). All these transformation processes have potentially created a change in the chemical composition of the residue - some of these chemical changes will complicate the chance of recognising the original materials due to the degradation of specific chemical characteristics (degradation processes), while other chemical changes will enhance the preservation of such typical chemical characteristics of the original materials (preservation processes).

5.3. Transformation processes

The way in which these transformations take place is partially determined by cultural phenomena specific to the given culture, and partially the result of chemical processes. Here the chemical processes that have played a role in the preservation and degradation of biomolecular characteristics will be summarised. The remaining variation in chemical characteristics can then be understood and interpreted as resulting from variation in prehistoric behaviour (for instance in the use of ceramic vessels or the choice of foodstuffs cooked in the vessels).

5.4. Post – excavational changes

Post-excavational contamination can easily occur in archaeological samples. Both contamination with skin lipids (cholesterol and squalene are the best known) and contamination with various packing materials (phthalate esters prevalent in plasticisers) are likely events in the daily practice of archaeological excavation and post-excavational ceramic treatment and handling. The most common way to prevent such contaminations is to take residue samples only after removal of the outer layer of the residue (ca. 1 mm).
Additional microbial degradation or oxidation of organics could take place after excavation. In this study growth of micro-organisms and fungi was limited by dry or cold storage and microscopic inspection of residues prior to sampling. One or two residues were excluded because fungal remains were visible during microscopic inspection. The details of these post-excavational degradations have not been further studied.

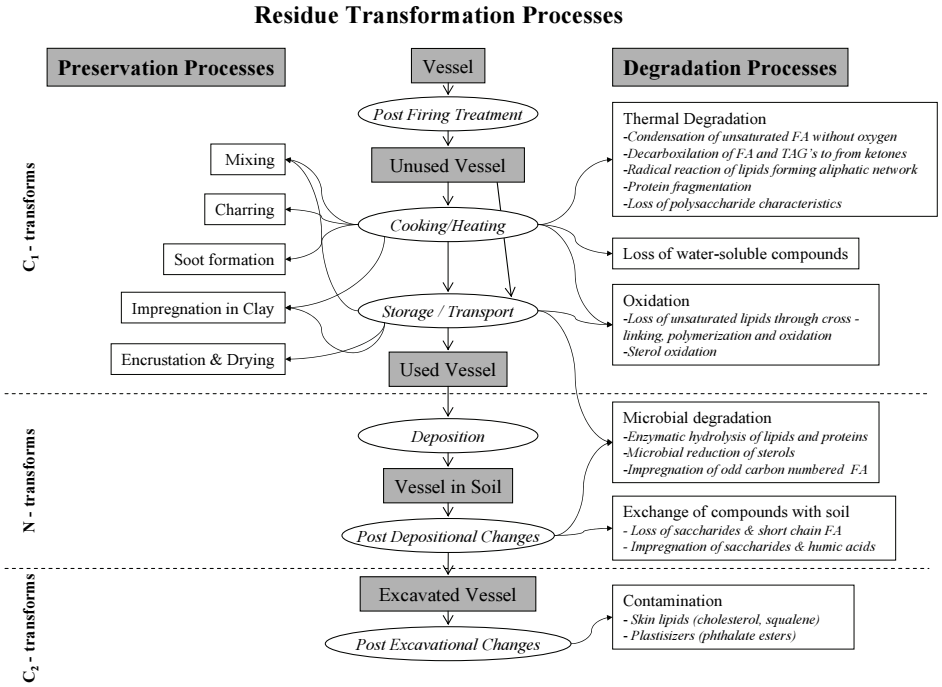

Figure 1: Residue Transformation Processes.
Transformation processes include processes in the original prehistoric context or "systemic context", also known as C_1-transforms (cultural transforms), and processes in the post-depositional context or "archaeological context" including the so-called N-transforms (natural transforms) as well as the C_2-transforms (cultural transforms) that can take place during and after excavation. Each of the transformation processes creates changes in the chemical composition of the original organic materials in the vessels. Some of these changes cause the degradation of specific chemical characteristics (degradation processes), while other chemical changes will enhance the preservation of such typical chemical characteristics of the original materials (preservation processes).

5.5. Post-depositional changes

A large number degradation processes are expected to have influenced the chemical composition of the organic residues during the long period of burial. The most significant changes are probably caused by microbial degradation and the exchange of organic compounds between the residue and soil.

Exchange of compounds between residue and soil could involve the selective loss of water-soluble compounds from the residue, or could involve the impregnation of certain soil compounds into the residues.

Loss of water-soluble compounds could not be shown to have taken place in this study. However, few water-soluble compounds were detected in the residues, which would indicate a possible loss of compounds over time. No small intact saccharides, water soluble amino acids or short chain free fatty acids were detected in the residues. Selective loss of short chain fatty acids can occur as a result of hydrolysis of acyl lipids during the use of the vessel and after deposition. The presence of short acyl fragments in intact acyl lipids shows their presence as part of the original material, and their loss may be due to post-depositional selective loss. However, the lipid extraction method could also have caused this loss due to the enhanced volatility of short chain free fatty acids. Alkaline environments can also enhance the transformation of free fatty acids to salts of fatty acids and can produce salts of various kinds. Some of these salts are relatively soluble in water and can cause fatty acids to leach out of their original matrix. However, the excavations under scrutiny in this study were all situated in mildly acidic soils. Pyrolysis markers for amino acids and saccharides were detected in these studies but mostly seem to originate from partially or severely charred materials, not from the water-soluble free monosaccharides or amino acids. Although these compounds could have been part of the original material they are not found in the residues.

Although impregnation of the residue with water-soluble compounds from the soil could theoretically happen, no evidence has been found to support this actually happening in this study. Even though peat contains many intact polysaccharides and pyrolysis markers for polysaccharides are visible in CuPyMS and CuPyGC/MS studies (Chapter 2 and 3), most of the polysaccharides present in peat are found in insoluble form and most markers in the residues indicated charred polysaccharides.

In conclusion, no evidence has been found for the exchange of any significant quantity of compounds between archaeological residues and organic soils. This is in agreement with other studies (Heron *et al.* 1991; Evershed & Tuross 1996). In addition, no correlation could be found between the chemical composition of residues and the type of sediment in which they were preserved. However, careful consideration must be given at all times to compounds that could originate from soils (such as polysaccharides and humic acids), and comparison need to be made to experimentally prepared residues in order to consider missing compounds that might have been lost during burial.

Microbial degradation is an important degradation process to take into account. Many studies have already been directed at the microbial degradation of lipids in buried fats and bog bodies (Den Dooren de Jong 1961; Morgan *et al.* 1984; Morgan & Titus 1985; Evershed 1991, 1992), and some experimental studies have assessed the microbial lipid contribution to degraded fats

and oils in absorbed residues (Dudd *et al.* 1998). It is for this reason that the composition of lipid extracts need to be carefully considered before conclusions can be drawn about their origin (see also Chapter 5). It is obvious from this thesis that most charred residues have a high organic fraction and contain some of the best-preserved lipid profiles. Charred residues are known to be less susceptible to microbial degradation due to the partial denaturation of the organic materials during charring and the refractory nature of the resulting material. But even in these residues biodegradation may influences the chemical composition. 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. This phenomenon is proposed to be the result of ongoing low-level microbial degradation during burial.

5.6. Processes in original use-context

The processes taking place in the original ancient context are the main focus of our study. The two transformation processes most important in the formation of organic residues in vessels are the process of cooking or heating of organics, and the process of storage or transport of organics in ceramic vessels. Cooking and heating cause impregnation of compounds in the clay and may result in charring and the formation of crusts on the interior vessel surface. The formation of smoke condensates on the exterior of the vessel is also a secondary result. Storage and transport may also cause impregnation of compounds into the clay (Kimpe *et al.* 2004) and may result in formation of dried crusts on the interior vessel surface. Both processes may cause mixing of many different biomolecular compounds.

Evidence of cooking and heating of non-food materials as well as foods was shown in this study. The clearest evidence that cooking or heating has taken place is the presence of charred organic residues. Although the char formation has a clear preserving effect, the process of condensation also has a severe degrading effect. Thermal degradation causes many severe changes in the original material. During thermal exposure in reducing circumstances, lipids undergo profound changes such as condensation of unsaturated fatty acids to form cyclic hydrocarbons or acyclic polymers (Davídek *et al.* 1990, 195); decarboxylation of fatty acids and acyllipids forming ketones (Davídek *et al.* 1990, 184); and radical reactions of lipids forming aliphatic networks as is proposed in this thesis. In addition, both proteins and polysaccharides fragment into small subunits and subsequently condense into more complex systems. This leads to an overall decrease in typical characteristics (Pastorova *et al.* 1993a; Pastorova *et al.* 1994; Braadbaart 2004).

In this study it is shown that mild heating and charring could preserve many characteristics of the original ancient material. Some peptide indicators could still be found in mildly condensed chars and cream coloured residues (Chapter 6), indicating a severe, but not complete, denaturation of the peptide chain. The individual amino acid characteristics are proposed to be preserved as a result of a radical reaction causing the specific amino acid side chains to be linked chemically to (or to get 'embedded' in) the forming char (Chapter 3). Protein markers occur

mostly in samples in combination with free fatty acids and polysaccharide markers, however, they occur also in combination with inorganic compounds i.e. carbonates (Chapter 3). The exact biomolecular origin of the proteins could however not be determined. Recent studies by Craig and co-workers have shown the possibility to use immunological methods for the identification of milk proteins in ancient vessels (Craig & Collins 2000).
Polysaccharide chars were shown to be present in many of the charred materials although the indicative characteristics of the kind of polysaccharide involved are not traceable. However, the mere fact that starch and starch-rich materials could be identified as having been prepared and cooked within ancient ceramic vessels has never been proven before.
Many charring experiments were performed in the context of this study and more were performed by others researchers using similar techniques (Pastorova *et al.* 1993a; Pastorova *et al.* 1994; Braadbaart 2004) in order to identify the chemical effects of thermal degradation and char formation. It has become clear that the chars discovered in archaeological contexts are surprisingly similar to those produced under controlled circumstances in the laboratory. And although many identifying characteristics are lost, others were preserved to be discovered thousands of years later. It was shown that the more extreme the thermal exposure of the residues (in temperature or time) the fewer the number of identifiable characteristics that could be detected. Increasingly similar condensed materials were formed.
Using ceramic vessels for serving, storage or transport of organic materials (whether foods or non-foods) may leave residues behind. Residues of storage and transport could be identified by a severe degree of oxidation that had taken place in the material while lacking aromatisation as a result of charring. Lipids undergo a so-called chemical 'drying' process, a loss of unsaturated lipids, through cross-linking, polymerisation and auto-oxidation.
Non-charred residues of three kinds were studied in this thesis: soot, cream-coloured residues and pigments. The pigment residue (sample 8-1) was shown to consist of a red-brown non-charred protein-rich material with some plant oils. Although the organic content was low, markers for intact peptides indicated a high degree of preservation in the material. The cream coloured residues in chemotype D are mainly of inorganic composition with a small amount of well-preserved non-heated (or lightly heated) proteins and some animal lipid (exposed to oxygen) mixed in. These residues could be the result of storage or transport of solids or of liquids with low organic content.
Naturally the storage or transport of some organic liquids (oils, fats, resins) could result in very large amounts of absorbed residues, but no residues like that were found in the vessels under study.

5.7. Post-firing treatment

Post-firing treatment with mixtures of organic components is common among 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, bitumen, various resins and other plant materials (Arnold 1985, 139-140; Kobayashi 1994; Diallo *et al.* 1995). Most commonly the treatment involves the application

of an organic liquid or paste to the pots while they are still hot from firing. No obvious evidence has been found for the presence of post-firing treatments in Uitgeest-Groot Dorregeest. However, cooking pots tend to seal themselves even after a single cooking phase (Charters *et al.* 1997), so no need for sealing seems necessary. If sealing was performed with common foodstuffs, it would be impossible to distinguish those residues from common use residues.

6. Implementation in Uitgeest-Groot Dorregeest

The main focus of this thesis was the ceramic assemblage of Uitgeest-Groot Dorregeest (Abbink 1985, 1999) which contains primarily simple, wide mouthed, globular jars with short rim and neck and a maximum diameter equal to, or slightly larger than, the rim diameter (see Chapter 2, Figure 1 and Appendix 1). Many of the vessels contain visible surface residues of different kinds (see Appendix 1, Table 3). In the assemblage of 147 partial vessels with identifiable morphological type, soot residues occurred most commonly (45%); charred residues occurred on about every third vessel (32%); and other residues such as 'pigment' residues (5%) and 'cream coloured crusts' (3%) occurred occasionally according to Abbink (1999, 396-397 and 165-166, Table 8.15)
Archaeological residues from all four categories were chosen for analysis using various spectrometric techniques preformed in the context of this thesis. Some of the residues originated from the group of partial vessels with identifiable morphological type (16), while others were taken from shards (22). In order to avoid selective sampling, residues recovered from different types of soil were analysed.

6.1. Vessel use

When the different vessel types were compared to the kind of residues they contain, it becomes obvious that there is a correlation between the chemical composition of the residue and the original size and form of the vessel on which the residue was found (Table 5). Although there is no complete overlap between vessel type and chemical properties of the residues, careful interpretations can be made about possible vessel use of different vessel types. Vessels of different size and form were clearly utilised for different daily uses.
Vessels of Type I often contain soot on the exterior and a char of chemotype D on the inside. The origin of the charred residues in chemotype D is not completely clear. The CuPyMS data indicate that charred polysaccharides, protein markers or fatty acids are absent, and only a clear alkane/alkene pattern is present. This would suggest that the vessel was exposed to extended high temperatures in the presence of fatty material. The high temperatures would cause radical reactions and form an aliphatic network. One explanation may be found in a post-firing treatment for waterproofing. An alternative explanation is the use of the vessel as a container

Table 5: Summary of possible origins of residues per type vessel

Vessel [a]	Residue [b]	n [c]	C [d]	Origin	Possible vessel use
Type I	Char, interior	3	D	Heated lipids	Water-proofing, roasting
	Char, interior	2	A_1	Mildly condensed protein or protein/starch mixture. Rich in well-preserved lipids	Charred animal product (most likely ruminant milk), possibly in combination with a starch. Cooked dairy product or cereal gruel.
	Black, exterior	4	B/E	Soot	Heating on wood fires
	Cream coloured, interior	1	A_1	Protein or protein/starch mixture	Proteinaceous material?
Type II	Char, interior	5	A_2	Highly condensed starch-rich material.	A charred starch mixed with vegetables and possibly a small amount of an animal meat or fat.
	Char, interior	3	A_1	Mildly condensed protein or protein/starch mixture. Rich in well-preserved lipids	Charred animal product (most likely ruminant milk), possibly in combination with a starch. Cooked dairy product or milk based cereal gruel.
	Char, interior	1	D	Heated lipids	Water-proofing, roasting
	Char, interior	1	C	Well-preserved protein and well-preserved lipids (possibly ruminant milk).	Lightly charred animal product rich in well-preserved lipids (possibly ruminant milk). Probably without starch.
	Red brown, interior	1	E	Contamination	
Type III	Red brown, exterior	1	C	Non-charred protein rich product. Probably exposed to oxygen.	Possibly decorative material made with plant oils and proteins.
Type IV	Cream coloured, interior	1	D	Inorganic crust (calcium carbonate) with some well-preserved non-heated proteins and some animal lipid (exposed to oxygen).	Possibly residue of storage or transport of solids or of liquids with low organic content.

[a] Vessel Type as indicated in Chapter 2.

[b] Residue appearance: as indicated in Table 5.

[c] Indicates the number of samples.

[d] Clusters as indicated in Chapter 2.

for roasting or drying of special foods such as nuts, roots, or grains. Some fat may have been added to prevent burning if the foods did not contain lipids. Interesting in this case, is the frequent presence of soot on the outside of these vessels which would support the use of these vessels for roasting or drying foods over an open fire rather then for storage or transport.
Some other residues are also found in this vessel type (chemotype A_1) indicating the small vessels of vessel Type I were sometimes also used for cooking or boiling milk or milk based cereal gruels or porridge.
The majority of vessels of Type II contain residues of chemotype A_1 or A_2. It is obvious that the vessels of this vessel type are everyday starch cooking vessels. The residues are the result of cooking of milk and grains in porridge or other starch-rich stews. In some cases protein-rich material such as meat, fish or pulses were the main constituents of the food, while in other instances fats may have enriched the mixture. One residue belongs to Chemotype C and is the result of a well-preserved protein-rich material (possibly ruminant milk) cooked in the vessel. One residue belongs to cluster E because it contains a contamination with elementary sulphur. Vessels of Type III and Type IV are under-represented in this study due to a lack of residues on this vessel type. Due to the absence of multiple samples of these vessel types, no conclusions can be drawn about the usage of these vessel types as a whole. The residues that were studied show that the large vessel from Type III was decorated with a mixture of plant oil and proteins. The vessel from Type IV contained a residue that primarily consists of inorganic material (calcium carbonate) in combination with some well-preserved non-heated proteins and some animal lipid (exposed to oxygen). This vase-like vessel was possibly used for storage or transport of solids or for liquids with low organic content. The last explanation seems the most likely in the given context and would indicate a use as water container.
In summary, this thesis shows that vessels of particular morphological types (form and size) are used for specific tasks in the settlement of Uitgeest-Groot Dorregeest. Although the use classification and the morphological classification did not overlap completely, a clear correlation could be seen. The need for a systematic sampling approach was shown to be necessary to determine the actual use of groups of vessels. The distinction between post-firing treatment of vessels with ordinary foods, the primary and the secondary use of a vessel remains very complicated. It has also been shown that only a fraction of the possible uses of ceramic containers are in fact detected during organic residue analysis, and that much remains to be discovered.

7. Further Research

Organic residue analysis, the study of molecular characteristics of organic residues found in association with pottery, has undergone revolutionary changes since the early 1980s. Ongoing instrumental innovations in analytical chemistry have enabled the analysis of ever-smaller organic samples in ever-greater detail. Studies of the molecular composition of extractable

compounds, such as lipids, resins and waxes have created an ever-increasing body of knowledge about their origin and use within ancient societies.
This thesis applied a combined spectroscopic approach that made it possible to detect and identify a range of biomolecular characteristics in surface residues that dramatically extends the extractable compounds commonly analysed in organic residue analysis. This knowledge broadens the study of organic residue analysis to include different types of vessel use.
And although many molecular characteristics of the original foods have been lost as a result of extensive thermal degradation during cooking, and the level of interpretation remains limited to general food groups, a surprising amount of specific characteristics have been preserved within the newly formed, condensed polymeric char-material.

However, in order to make molecular organic residue analysis a powerful tool in the study of ancient vessel use, a number of basic research questions still need to be addressed.
Firstly, the identification of the overall molecular composition of organic residues needs to become a standard practice in organic residue analysis. Most prominently absent from the analysis of organic residues are compounds indicative of starches and proteinaceous materials. With the improving knowledge of the survival of carbohydrates, starches and proteinaceous materials in organic residues, more attention needs to be directed to the analysis of these major components of the human diet.
Secondly, models for the formation of organic residues in ceramic vessels must be designed and tested, in order to provide a better insight in the mechanisms of preservation and decay of organic residues. Residue formation models play an important role in two distinct aspects of molecular organic residue analysis. Firstly, formation models can facilitate the translation of molecular results to original vessel content. Secondly, models will illustrate to what extent preservation processes work selectively: enhance the preservation of certain kinds of residues while other decompose. Clear models can provide a tool to estimate the applicability of our conclusions in the larger context of human behaviour in the past. In additional to models theoretical models, heating experiments may also help us to understand the effects of multiple use phases in ceramics. It is essential for organic residue analysis to acquire a better insight into the processes that play a role in the impregnation of the ceramic material with organic compounds, the formation of a insoluble macromolecular structure, and the subsequent thermal degradation of this structure during ongoing thermal exposure in the ceramic wall of the vessel.
Thirdly, systematic use alteration studies need to be performed in order to put the results of organic residue analysis on individual vessels in a larger archaeological context. It needs to be kept in mind that not all uses will ever be 'visible' through organic residue analysis, as some uses will not leave behind detectable residues of any kind. In the context of general ceramic use-alteration studies, this variation in vessel use may become clear and illustrate a more diverse employment of ceramic vessels then is currently detectable. However, not all assemblages will lend themselves to such studies, for a large number of relatively intact vessel profiles is needed to give significant information about vessel use on an assemblage scale.