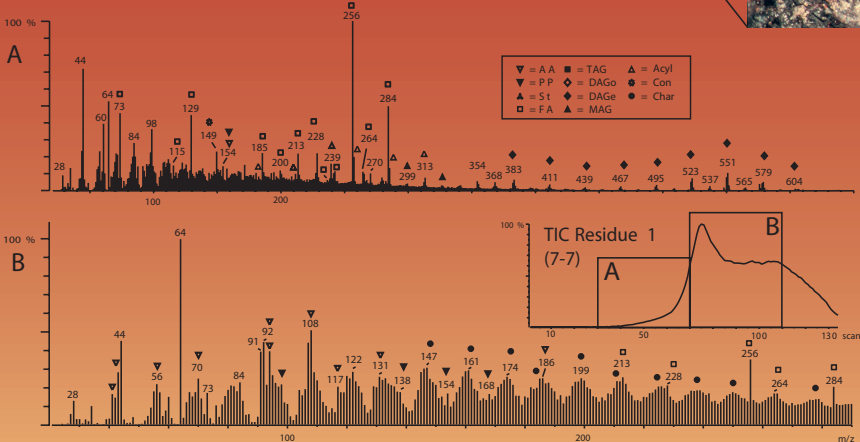
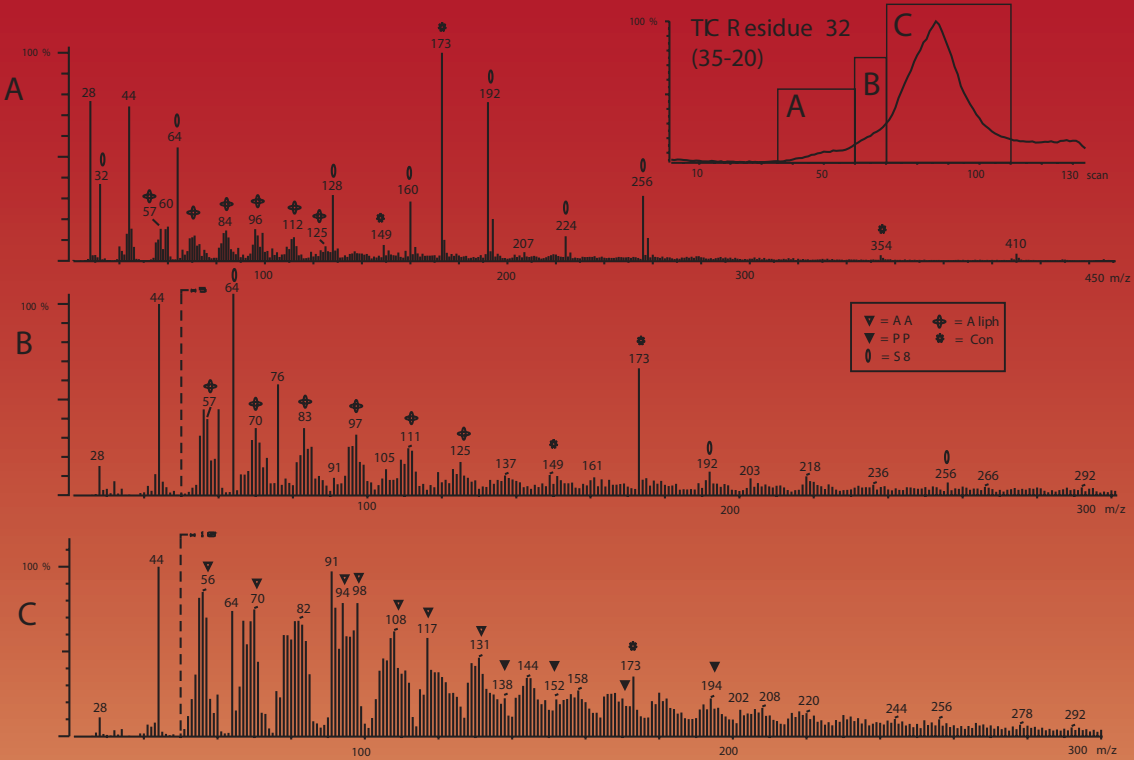
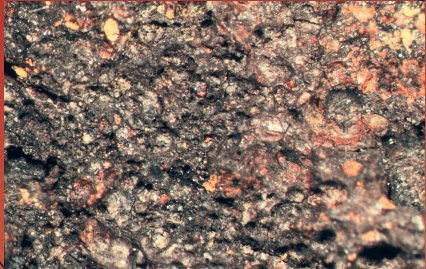


Molecular Studies of Organic Residues Preserved in Ancient Vessels

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Tania F.M. Oudemans



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Tatiana Frederica Margreta Oudemans

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Promotiecommissie:

- Promotores: Prof. Dr. C. C. Bakels
Prof J.J. Boon
FOM Institute for Atomic and Molecular Physics, Amsterdam
University of Amsterdam
- Referent: Prof. Dr. C. Heron
Department of Archaeological Sciences, University of Bradford, UK
- Overige leden: Dr. A. van As
Prof. Dr. M. van Kolfschoten
Prof. Dr. Ir. J. van der Plicht

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*To my parents - for your love and support, your solid conviction that I would always
succeed at what I truly wanted to do, for the gift of education
for your stories of far away countries
for giving me the world to explore*

*To my beloved Denya - for all your love and support, for always believing in me
and standing next to me, for your trust and generosity
for being my match and marrying me
for choosing life with me*

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Chapter 1

General Introduction

This introduction defines organic residue analysis and its importance as supplier of independent information about the actual use of ceramic vessels in the context of functional ceramic studies in archaeology. The main challenges of organic residue analysis lie in the area of sample selection, analytical protocol and the archaeological interpretation of the chemical information obtained. The approach chosen in this thesis is illustrated below by a description of the rationale of the work presented here.

Modified after:

Section 1, 2 and 4: T.F.M. Oudemans, J.J. Boon & R.E. Botto in press, 'FTIR and solid-state ^{13}C CP/MAS NMR spectroscopy of charred and non-charred solid organic residues preserved in Roman Iron Age vessels from the Netherlands', *Archaeometry*.

Section 3: T.F.M. Oudemans & D.W. Erhardt 1996, 'Organic residue analysis in ceramic studies: implications for conservation treatment and collections management', *Archaeological conservation and its consequences*, A. Roy & P. Smith (eds.), The International Institute for Conservation of Historic and Artistic Works, London, Copenhagen, 137-142.

1. Organic residue analysis in ceramic studies

1.1. A functional perspective towards ceramics in archaeology

Pottery assemblages are frequently studied by archaeologists in search of information about a variety of different aspects of past societies, such as socio-economic developments, the organisation of production and trade, and the mechanisms of cultural interaction. Ceramic containers form an optimal source material for archaeological studies because they were used in daily activities, were commonly produced locally (reflecting local values and needs), had a relatively limited use-life, and are frequently preserved well in archaeological context.

In order to make behavioural inferences from the shattered remains of a once thriving community, a clear understanding of the function or use of the original vessel is essential (Hally 1983; Henrickson 1990). The archaeological information stored in any assemblage of artifacts can only be interpreted fully if the actual use of the objects is known. Both the kind of questions that may be addressed (Hally 1986) and the quality and specificity of the archaeological inferences (Skibo 1992, 4-5) would be improved if an accurate assessment could be made of the original vessel use.

1.2. Intended vessel function versus actual vessel use

In spite of this clear rationale, the identification of vessel usage is one of the most difficult problems for those studying archaeological ceramics. It is therefore not surprising that vessel use has received surprisingly little attention among archaeologists until the late 1980's (Van der Leeuw & Pritchard 1984; Henrickson 1990). In the last three decades the systematic investigation of the original function of vessels within assemblages has come to be recognised as an informative strategy for archaeologists interested in learning about a wide range of domestic activities within ancient settlements (Henrickson 1990).

Most archaeological methods to identify vessel function are directed at the study of "intended vessel function" – the job (or range of jobs) the potter had in mind when making the vessel. Such studies are based on the assumption that morphology as well as other physical and technological attributes of pottery can be optimised to suit a particular range of uses (Erickson & Stickel 1973, 356) or are constrained by the intended use context (Braun 1983). The research strategy is primarily aimed at uncovering specific characteristics of vessel morphology and technology that identify functional vessel categories. However, the relationships between morphology, technology and use are not uniform and specifiable but complex and variable, and their study commonly produces only very general use categories (Howard 1981, Table 1.1; Rice 1990) or local ethnographic classifications (Henrickson & McDonald 1983). Although the comparison of cross-cultural data shows that some relationships are almost universally upheld (Henrickson & McDonald 1983; Varien & Mills 1997), many of the predicted relationships between form, technology and function have been found to be somewhat equivocal (Plog 1980, 60-62). In addition, a growing number of archaeological (Woods 1986; Mills 1999; Sinopoli

1999) and ethnoarchaeological studies (Aronson *et al.* 1994; Arnold 2000) provide evidence that a variety of factors including environmental and social factors determine the processes of pottery manufacture and use. One illustrative example of the complex nature of the relationships between vessel form, technology and use, is the study of optimal characteristics for cooking pots. Cooking vessels are generally expected to make efficient use of heat for cooking. It has become generally accepted that cooking vessels have certain forms (rounded rather than angular contours) to avoid thermal damage and permit greater exposure of the vessel base; are relatively thin walled to conduct heat better and prevent thermal stress inside the vessel wall; and are coarse textured, porous, and tempered with materials that have low coefficients of thermal expansion (burned shell, crushed potsherd) to accommodate thermal stress (Rye 1976; Henrickson & McDonald 1983; Rice 1987, 237). Contrary to these general rules a wide range of shapes have been recorded in cooking pots in the past and present (Rice 1987, 239). The study of the archaeological material from Britain shows many flat based cooking vessels and a majority of vessels have sandy fabrics, thus contradicting the theories concerning thermal shock resistance properties of pottery (Woods 1986). A study of factors influencing Kalinga users in their choice of cooking pots, illustrates that although technical arguments (differences in strength and weight of the vessels) play a role, kinship and social affiliation of consumers strongly influence their choice of cooking vessels (Longacre & Stark 1992; Aronson *et al.* 1994).

On the other hand, studies directed at the “actual vessel use” – the way vessels were put to task by the user - avoid trying to untangle these complex relationships and can give independent information about the original utilitarian role of the vessels. The traditional archaeological approach of inferring actual vessel use through the study of recovery context, is usually limited in scope by the small quantities of vessels recovered in their original use-context (Orton *et al.* 1993, 28-30). The most direct and detailed way to identify “actual vessel use” is through the study of “use alterations” – traces of use found in and on the ceramic material. Although the importance of the study of use-wear traces in functional studies of artifacts such as stone tools (Semenov 1964, 6) and ceramics (Matson 1965, 204-208) was already formulated in the mid 1960’s, the term ‘use-alterations’ was only introduced in ceramic studies in the 1980’s (Hally 1983).

1.3. The use - alteration perspective

Following Hally (1983), Skibo (1992, 42-45) defined ‘use-alterations’ as any chemical or physical change that occurs to the surface or substance of ceramics as a result of use, and identified four types of alterations: i) attrition or wear of the ceramic; ii) discolouration of the clay also called fire clouding; iii) soot depositions and iv) organic residues (adhering to either exterior or interior vessel wall or absorbed into the ceramic).

Use-wear of the vessel can be the result of use (stirring or scraping of the content, salt erosion or thermal spalling). Careful study of such phenomena can give indications of the original use (Bray 1982; Hally 1983; Henrickson & McDonald 1983; Hally 1986; Skibo 1992; Beck *et al.* 2002). However, a number of non-use phenomena can also cause these attritions and confusion

must be avoided by comparison of neighbouring shards with different wear patterns and detailed study of old and fresh break surfaces.

Colour changes of the clay caused by changes in the oxidative state of iron in the clay (also called fire-clouding) and soot deposition have been used as indications of cooking over an open fire (Hally, 1983) or for the type of cooking technique employed (Hally 1983; Henrickson 1990; Skibo 1992, 42-49; Kobayashi 1994; Skibo & Blinman 1999; Arthur 2002). Skibo showed that the presence of a light coloured patch on the bottom of the vessel indicates that the vessel was placed in the fire, while vessels with an entirely black bottom were hung over a fire. The study of these discolourations can only be performed successfully on whole vessels and must be employed carefully in order to prevent confusion with accidental secondary heating of vessels after their discard.

The chemical characterisation of organic remains found in direct association with vessels, is one of the more recently developed methods in the functional study of ceramics. Although first applied in the 1920's and 30's (see Rottländer & Schlichtherle 1980 for references), organic residue analysis has only been widely used since the 1980's (and Heron & Evershed 1993 for references; see Evershed *et al.* 1999) as a result of improvements in micro-analytical instrumentation and an increasing interest in functional aspects of archaeological ceramic assemblages.

1.4. Aims

This study is aimed at the molecular characterisation of the organic residues preserved in an assemblage of ceramic vessels originating from an indigenous settlement dating from the Iron Age and Roman period at Uitgeest – Groot Dorregeest in the Netherlands in order to better infer the way the vessels were originally used (see Appendix 1 for a more complete descriptions of the ceramics and residue studied).

Naturally, the study of small amounts of complex organic materials preserved in the ground for thousands of years creates many analytical and methodologically challenges. 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?
- Analytical protocol: What combination of analytical techniques will supply the most useful information or give the most complete answers?
- The interpretation of chemical evidence in terms of archaeological context: To what extent can the original vessel contents be identified? How can the questions of original vessel usage be addressed?

2. Choice of sample material

In the study of organic residues, much research is aimed at absorbed residues – organic matter absorbed into the actual ceramic fabric of the vessel. These studies involve the identification of extractable compounds such as lipids and waxes (Heron *et al.* 1994; Evershed *et al.* 1995; Evershed *et al.* 1999); resinous materials and wood pitches (McGovern *et al.* 1996; Eerkens 2002) and proteinaceous materials (Evershed & Tuross 1996; Buckley *et al.* 1999; Craig & Collins 2000; Craig *et al.* 2000; Craig & Collins 2002; Craig *et al.* 2005). All these studies use selective techniques to extract the absorbed organics from the ceramic material of the vessel.

An alternative approach is the analysis of surface residues - solid organic matter preserved as crusts or films adhering to the interior or exterior surface of ceramic vessels. Although the chemical characterisation of these solid surface residues is analytically complicated by the complexity of the material and limited sample size, there are various methodological arguments for the study of surface residues.

Firstly, archaeologists frequently have no prior knowledge of the actual nature of the original material involved. Choosing the appropriate extraction method is complicated by this lack of knowledge and the extracted sample may not be representative for the residue under study. The overall chemical composition of organic residues needs to be investigated prior to the application of extraction techniques.

Secondly, the study of surface residues makes it possible to sample only one layer of material. Microscopic examination of cross-sections helps to prevent the incorporation of multiple use-phases in one 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 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. Although heating plays an important role in the preservation of surface residues (through charring or condensation), thermal degradation also causes the loss of many distinct chemical characteristics of organic remains (Pastorova *et al.* 1993; Boon *et al.* 1994; Pastorova *et al.* 1994; Braadbaart 2004; Braadbaart *et al.* 2004a; Braadbaart *et al.* 2004b; Oudemans *et al.* in press-b). Extended exposure of foods to temperatures above 300 °C makes identification of biomolecular markers of the original foodstuffs increasingly difficult (Oudemans *et al.* in press-b).

The work presented here is focussed on the study of these solid surface residues (although chapter 5 deals with extractable lipids). The ceramic assemblage from which the samples were taken is illustrated in Appendix 1.

3. A combined spectroscopic approach

The study of small amounts of complex mixtures of degraded organic materials creates many analytical challenges. Although this thesis reflects more than a decennium of developments in organic residue analysis in archaeology, the two basic analytical approaches to obtain chemical information about such materials have not changed (see also Appendix 3). Characteristics of the sample as a whole can be determined and cumulative results obtained that give information on the level of the 'total sample'. These techniques produce chemical 'fingerprints' used for classification of residues or for comparison with reference materials. Alternatively, residues can be separated into fractions each of which can be analysed separately in detail on a molecular level. However, each separation step requires additional sample.

In this study a combined spectroscopic approach is chosen. Looking at the organic residues with different analytical techniques will lead to a multi-faceted picture of a complex mixture of solid and extractable compounds and will illustrate the possibilities and limitations of organic residue analysis in the study of ceramic vessel use to its fullest potential.

3.1. Overall composition - destructive and non-destructive techniques

Elementary and isotopic characterisation of complete samples is obtained by using a destructive technique based on the gas chromatographic analysis of the combustion gas of a small amount of sample. carbon, hydrogen and nitrogen (CHN) elementary composition indicates what fraction of the sample is organic and suggests the chemical composition of the material. The C/N ratio indicates the protein fraction in the material and the C/H ratio illustrates the degree of saturation and condensation of the material. This technique was successfully applied to determine what percentage of the total organic carbon present in ground ceramic material could be released using different preparation steps (Craig *et al.* 2004).

Bulk stable isotope ratio analysis (SIA) of stable nitrogen and carbon isotopes $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$, gives information on what animals, fish or plants may have been used to form the residue (Morton & Schwarcz 2004). However, mixing of foodstuffs often limits the applicability of this technique in organic residue analysis, although it is a useful technique in the study of human remains, bones, dental material and identifiable plant remains. Compound-specific isotope analysis of individual lipids or amino acids using gas chromatography – isotope ratio mass spectrometry (GC-IRMS) has resulted in very interesting identifications of the origin of particular biomarkers (see Section 3.2).

Chemical characterisation of complete samples can also be obtained through the application of non-destructive spectroscopic techniques such as Fourier transform Infrared (FTIR) spectroscopy or solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy.

FTIR is based on the light absorption characteristics of various chemical compounds in a material. Each type of chemical bond or functional group absorbs light of a particular wavelength (or range of wavelengths). The presence or absence of absorption peaks typical for

particular bond types or functional groups provides information of the presence or absence of certain compound classes and the ratio of organic - inorganic material in a given residue. FTIR is a rapid analytical technique ideal for the initial classification of organic residues (Colombini *et al.* 2003; Regert *et al.* 2003). FTIR microscopy has the added advantage that solid organic residues can be analysed by pressing them between two crystals in a diamond anvil cell. General determination of the nature of the samples can be made through comparison with reference materials. FTIR can rarely be used for detailed identification of complex mixtures because increasing complexity of the analysed sample results in decreasing resolution and a loss of identification potential (Hill & Evans 1988, 1989). In addition, FTIR is insensitive to compounds present in smaller quantities (< 5%), and is limited in its capacity to distinguish between samples containing different proportions of similar compounds.

Solid-state ^{13}C NMR spectroscopy has been designed to study the carbon functional group distribution in complex solid organic materials in medicine, biochemistry and geochemistry, and has recently been applied in the field of organic residues analysis (Oudemans *et al.* 1992; Sherriff *et al.* 1995). The identification of carbon functional groups is based on the electronic environment and magnetic susceptibility of the ^{13}C atoms. Each type of carbon bond contributes to a specific chemical shift that can be measured (in ppm) relative to a standard compound. The relative amounts of saturated C-C bonds, unsaturated C=C bonds and C-H bonds provides additional information on the degree of condensation of the organic residue. Although solid-state ^{13}C NMR spectroscopy has the analytical advantage that it provides quantitative results, and is less sensitive to sample inhomogeneity than FTIR, it also requires a much larger sample (10 - 100 mg).

In this thesis CHN analysis is applied for the identification of the overall organic fraction of the material (Chapter 5 and 6), while FTIR and solid-state ^{13}C NMR spectroscopy are combined to quantify the relationship between solid and extractable compounds in the residues and to give information about the organic functional groups present in the residues (Chapter 6).

3.2. Molecular characteristics - extractable & non-extractable compounds

In order to obtain identification of individual compounds present in mixed organic materials, a separation or fragmentation step is often required. Certain compound classes, such as lipids, terpenoids, waxes, hydrocarbons and alcohols can be extracted from a complex mixture with organic solvents. These extractable compounds can be separated and identified using gas chromatography (GC) and gas chromatography mass spectrometry (GCMS) after appropriate derivatisation or preparative separation.

Compounds-specific isotope analysis of individual lipids or amino acids using gas chromatography – isotope ratio mass spectrometry (GC-IRMS) is one of the most recent instrumental innovations that has resulted in very interesting identifications of the origin of particular biomarkers (Fogel & Tuross 2003). GC-IRMS was first applied in organic residue when Evershed and co-workers showed that the $\delta^{13}\text{C}$ values of the alkanes and ketones preserved in early medieval vessels were consistent with those of fresh wild-type Brassica vegetables as were grown in Britain in the medieval period (Evershed *et al.* 1994). It was also

shown that the $\delta^{13}\text{C}$ values of individual fatty acids in medieval oil lamps originated from ruminant animals such as sheep and cattle, while those in 'dripping dishes' placed under roasting spits were consistent with those from monogastric animals such as pigs (Evershed *et al.* 1997b; Mottram *et al.* 1999). Further studies confirmed the use of milk in late Neolithic settlements in Switzerland (Spangenberg *et al.* 2006), the Scottish Atlantic coast in the Iron Age (Craig *et al.* 2005) and Saxon Britain (Dudd & Evershed 1998). Proof of processing of palm fruits in Egyptian Nubia in antiquity was shown by detection of palm fruit lipids in ceramic vessels from Qasr Ibrim (Copley *et al.* 2001) and cooking of maize was shown in absorbed organic residues from a variety of Mississippi Valley potsherds (Reber & Evershed 2004b, a). The application of compound specific isotope ratio analysis is definitely one of the more promising new analytical techniques within organic residue analysis and has the potential to give extended insight in the origin of many organic compounds. A recent study of a two absorbed archaeological milk residues, showed that the $\delta^{13}\text{C}$ values of free and bound C16:0 and C18:0 fatty acids were independent of whether they were released by solvent extraction, alkaline hydrolysis or catalytic hydropyrolysis (Craig *et al.* 2004). This conclusion confirmed that the stable carbon isotope values of extractable lipids can accurately represent the values of the overall C16:0 and C18:0 fatty acid isotope ratios. The only important limitation to compound-specific isotope analysis is that it is limited to the comparison of organic compounds with significant differences in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values. In spite of its great potential, GC-IRMS falls outside of the scope of this thesis. Obtaining detailed molecular information on the chemical composition of the remaining solid, non-extractable, chemically bound or condensed macromolecular fraction of the residue is much more complicated. The remaining compounds may include (partially degraded) proteins and complex sugars, melanoidins, condensed cyclic hydrocarbons and cross-linked drying oils. This solid fraction can only be studied after a fragmentation step. The fragmentation can involve either a chemical fragmentation procedure such as acid treatment, or a controlled thermal fragmentation step such as pyrolysis.

Proteins and complex sugars (such as gums) are most easily analysed after they have converted to their individual monosaccharides or amino acids. Sugars can be derivatised and analysed using GC (Kharbade & Joshi 1995) while amino acids composition is usually determined using high-performance liquid chromatography (HPLC) (Evershed & Tuross 1996; Oudemans *et al.* 1996; Buckley *et al.* 1999). The main limitation of these techniques is the loss of identifying information during the fragmentation process. For instance, the identification of (partly degraded) proteins from their amino acid composition is difficult because many proteins have comparable amino acid compositions. Specific protein markers have recently been shown to be identifiable using immunological techniques after a mild fragmentation and extraction procedures (Craig & Collins 2000; Craig *et al.* 2000; Craig & Collins 2002; Craig *et al.* 2005).

Alternatively, condensed materials such as (partially degraded) proteins, melanoidins, caramelised sugars, cross-linked drying oils and partially carbonised materials may be fragmented using thermal fragmentation. Analytical pyrolysis techniques such as Curie-point pyrolysis mass spectrometry (CuPyMS) or Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGCMS), and Direct Temperature-resolved Mass Spectrometry (DTMS) use a form of controlled heating in an oxygen-free environment. The added thermal energy causes

the macromolecular compounds to split (along the weakest bond in the chain) into fragments specific to the original molecule.

In this study extractable lipids were analysed using GC and GCMS (Chapter 5) in order to compare the results of various kinds of absorbed residues and surface residues. Various analytical pyrolysis techniques (Chapter 2 and 3) and DTMS (Chapter 4 and Appendix 1) were used to obtain detailed molecular information about the solid organic fraction of the residues.

4. Interpretation in an archaeological context

4.1. Definition of 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 possibility of detecting differences in chemical composition between residues and iii) the extent to which the origin of the different compounds can be traced back to prehistoric times.

4.2. Transformation processes

Although the range of organic compounds that can still be detected and the possibility of detecting differences in chemical composition between residues are primarily a matter of analytical protocol, the ability to infer the use of particular biomaterials in prehistoric times through organic residue analysis, is to a large extent determined by a correct understanding of the transformation processes that influence the chemical composition of the remaining residues.

Transformation processes are summarised in Figure 1 and include processes in the original prehistoric context or “systemic context”, the so called C_1 -transforms (cultural transforms), and processes in the post-depositional context, or “archaeological context”, including N -transforms (natural transforms) as well as the C_2 -transforms (cultural transforms) that can take place during and after excavation (Schiffer 1972, 1983). Each of the transformation processes creates a change in the chemical composition of the original organic materials. Some of these chemical changes will complicate recognition of the original materials due to 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).

4.3. Post-firing treatment

The habit of treating ceramic containers after firing but prior to use (for the purpose of surface sealing or enhancing appearance), has frequently been described in ethnographic and ethnoarchaeological studies. 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. Post-firing treatment can result in the formation of residues that may well be interpreted as part of a use residue, although non-edible sealing materials such as plant gums or resins are more easily distinguished from foodstuffs prepared in ceramics. Depending on the sealing material and technique, it is likely that organic components are both absorbed into the ceramic of the vessel and deposited onto the interior (or exterior) of the vessel. Although the thermal regime of cooking vessels may well result in a relatively complete thermal degradation of the original sealing material after a few hours of cooking, both residues extracted from ceramic and residues scraped from the surface of the vessel may well be contaminated with sealing materials. However, microscopic investigation of the surface residues can help prevent mixing of different layers. Extraction technique will never be able to distinguish between post-firing treatments and use residues (if similar materials are used). In short, post-firing treatments with common foodstuffs would be extremely difficult to distinguish from use residues in cooking vessels.

4.4. Food and non-food preparation

Preparation of non-food materials (e.g. glues, dyes, paints, lamp oils, medicines) as well as foodstuffs in ceramic vessels may involve two important chemical processes: mixing without heating and cooking or heating.

Mixing has both preserving and degrading effects. It obscures the original biomolecular characteristics of an organic material making it harder to identify its origin, but it also creates possibilities for the formation of new resistant compounds (for instance melanoidins are formed when proteins and sugars are heated together).

Cooking and heating result in an even more extreme combination of chemical effects. Heating of organic liquids in a ceramic vessel will certainly lead to preservation processes such as impregnation of organics into the ceramic material of the vessel and the formation of chars on the surface of the vessel. On the other hand, possible loss of water-soluble compounds and the thermal degradation that may take place also result in the loss of specific biomolecular characteristics. Charring is one of the most important formation processes that results in preservation of solid organic residues on ceramic vessels. However, the chemical changes that take place during charring are complex and can obscure a lot of the original markers. In order to understand the chemical changes during char formation, heating experiments were

performed with modern foodstuffs. In Chapters 3, 4 and 6 such experimental chars were studied alongside the archaeological residues.

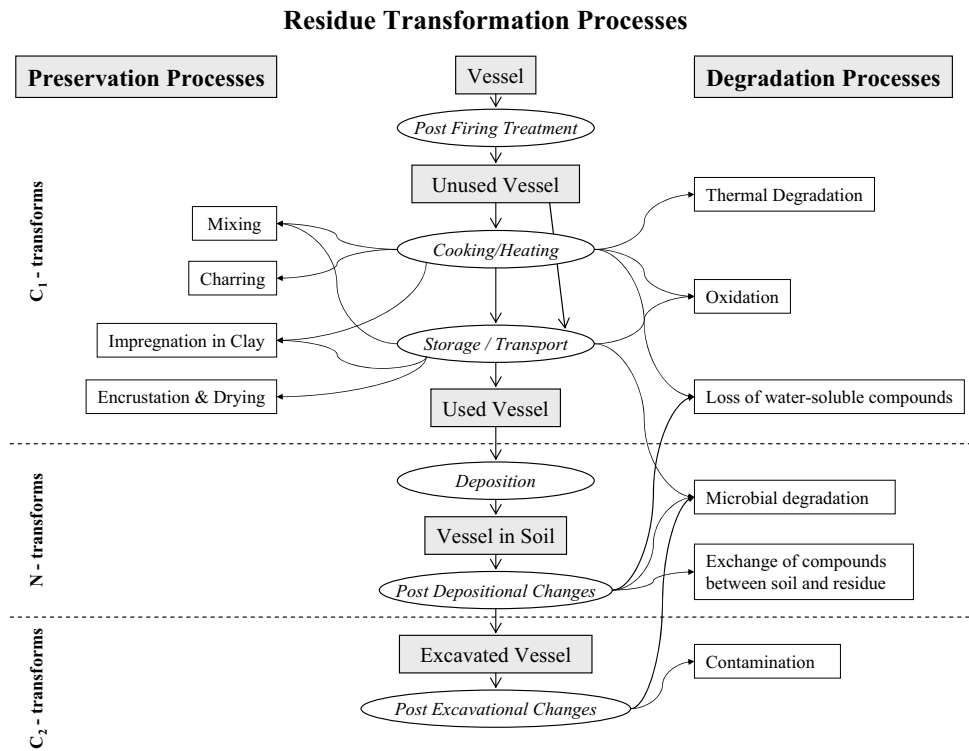


Figure 1: Residue Transformation Processes.

Transformation processes include processes in the original prehistoric context or systemic context C_1 -transforms (cultural transforms), and processes in the post-depositional context or archaeological context including N -transforms (natural transforms) as well as 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 their preservation (preservation processes).

4.5. Storage and transport

Using ceramic vessels for serving, storage or transport of organic materials (whether it is foods or non-foods) may leave residues behind. Liquids especially may be absorbed into the ceramic of the vessels, although sealing materials may have been used to prevent this effect in long-term storage or transport vessels. Examples of recognisable archaeological residues of such liquids are plentiful and include mostly residues of wines or other fermented beverages (Gerhardt *et al.*

1990; McGovern *et al.* 1996; McGovern *et al.* 2004), oils (Condamin *et al.* 1979; Shedrinski *et al.* 1991) and medicines (Gibson & Evans 1985). Storage or transport of solid materials is much less likely to leave residues behind, and may only be indicated by the presence of encrustations or absorbed residues that show no chemical signs of thermal degradation, but show signs of severe oxidation due to long-term exposure to oxygen.

4.6. Post – depositional changes

After a ceramic vessel has been discarded, a number of degradation processes are expected to influence the chemical composition of the organic residues: loss of water-soluble compounds, microbial degradation and the exchange of organic compounds between the residue and soil that surrounds it.

Deposition in (or on) the soil may cause further loss of water-soluble compounds due to the presence of rain or ground water.

Microbial degradation is an important degradation process to take into account. Many studies have already been directed at the microbial degradation of lipid characteristics in buried fats and bog bodies (Den Dooren de Jong 1961; Morgan *et al.* 1973; 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). Charred residues are obviously less susceptible to microbial degradation due to the partial denaturation of the organic materials during charring (Chapter 5).

Exchange of compounds between residue and soil is a clear possibility that has been questioned by others before. Remarkably, no evidence of significant impregnation of soil compounds into the residues has been found as of today (Heron *et al.* 1991; Evershed & Tuross 1996; Oudemans & Boon 1991, 1996). However, it has always remained unclear what was lost during deposition - no evidence can be found for what is no longer present. Careful consideration must be given at all times to compounds that could originate from soils. Comparison with experimentally prepared residues must be made in order to consider missing compounds, that might have been lost during burial. Only further further experimentation may help solve this issue in the future. In this thesis soil samples were analysed alongside the archaeological residues in Chapter 2 and 3, in order to make sure analytical pyrolysis could distinguish between soil samples and residues.

4.7. Post – excavational changes

Post-excavational contamination occurs regularly in archaeological samples. Especially significant are contaminations with human skin fats from the hands of archaeologists and the compounds originating from packaging and cleaning agents. Additional microbial degradation or fungal growth may well take place after excavation. In this study growth of microorganisms and fungi was prevented by dry or cold storage and microscopic inspection of residues prior to sampling.

4.8. Final Composition

How and to what extent transformations take place is partially determined by cultural phenomena specific to the given culture, and partially the result of chemical processes. Optimally, this thesis will lead to a better understanding of the chemical processes that play a role in the preservation and degradation of biomolecular characteristics. 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). Which chemical processes may turn out to play the most significant role in the preservation or degradation of solid organic residues will be described throughout this thesis.

5. Rationale of the Thesis

A wide range of different complementary analytical techniques was used to explore the molecular characteristics of the solid organic residues that have survived the test of time and to identify the processes that may have played a role in their preservation and degradation. Each Chapter of this thesis discusses the application of one or more of these techniques and addresses one or more of the research questions described in section 1.4.

Chapter 2 explores the analytical possibilities of Curie-point pyrolysis mass spectrometry (CuPyMS) as a tool to distinguish between solid organic residues and their surrounding soil. Subsequently, the question is addressed whether the chemical evidence is sufficient to distinguish between different residues, and whether these chemical differences are correlated to the type of vessel in which they were preserved. Multivariate analytical techniques are used for the comparison of chemical CuPyMS ‘fingerprints’.

Chapter 3 focuses on the more detailed identification of compounds preserved in solid surface residues. Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGCMS) is used for its capacity to non-selectively identify a wide range of compounds. Soil samples and experimentally charred modern foodstuffs were analysed alongside the ancient residues for comparison on a molecular level.

In Chapter 4 direct temperature-resolved mass spectrometry (DTMS) is combined with multivariate techniques to group the residues. Their characteristics are subsequently compared to the chemical properties of experimentally charred modern foodstuffs in order to facilitate the determination of the biomolecular origin of the various kinds of residues.

In order to explore all possibilities, extractable lipids from both the surface residues and the directly adjacent ceramic vessel wall are analysed quantitatively in Chapter 5. The methodological argument to study surface residues rather than lipids extracted from the ceramic

fabric of the vessel is tested by comparing lipids from different types of residues and from residues from different periods.

The great challenge in the studies of solid organic residues remains the ability to gain a detailed understanding of the nature of the solid condensed phase and the mechanisms of its formation. In Chapter 6 solid-state ^{13}C magnetic resonance spectroscopy and Fourier transform infrared spectroscopy using a diamond anvil cell, are applied to quantify the relationship between solid and extractable compounds in the residues, to give information about the organic functional groups present, and to give an insight into the degree of condensation of the chars. In addition, the application of these solid-state techniques is used to verify earlier results obtained in analytical pyrolysis studies and to clarify the relationship between the (already thermally degraded) charred residues and the controlled heating fragmentation taking place during analytical pyrolysis and direct temperature-resolved mass spectrometry.

Chapter 7 summarises and reviews the results presented in earlier chapters and discusses some areas for further study.

6. Publication list

This thesis is based on papers published between 1991 and 2006. Although a part of this work is based on data published prior to 2001 (Chapter 2 and 3), this thesis not only contains additional recent publications (Chapters 4, 5, 6, and Appendix 1), but also presents an overall synthesis of the results of the combined spectroscopic study of solid organic residues found in association with ceramic vessels from prehistory and the Roman period (Chapter 1 and 7). The thesis is based on the following publications:

Chapter 1

Section 1, 2 and 4: T.F.M. Oudemans, J.J. Boon & R.E. Botto in press-a, 'FTIR and solid-state ^{13}C CP/MAS NMR spectroscopy of charred and non-charred solid organic residues preserved in Roman Iron Age vessels from the Netherlands', *Archaeometry*.

Section 3: T.F.M. Oudemans & D. Erhardt 1996, 'Organic residue analysis in ceramic studies: implications for conservation treatment and collections management', *Archaeological conservation and its consequences*, A. Roy & P. Smith (eds.), The International Institute for Conservation of Historic and Artistic Works, London, Copenhagen, 137-142.

Chapter 2

T.F.M. Oudemans & J.J. Boon 1996, 'Traces of ancient vessel use: investigating prehistoric usage of four pot types by organic residue analysis using pyrolysis mass spectrometry', *Analecta Praehistorica Leidensia*, vol. 26, 221-234.

Chapter 3

T.F.M. Oudemans & J.J. Boon 1991, 'Molecular archaeology: analysis of charred (food) remains from prehistoric pottery by pyrolysis-gas chromatography/mass spectrometry', *Journal of Analytical and Applied Pyrolysis*, vol. 20, 197-227.

Chapter 4

T.F.M. Oudemans, G.B. Eijkel & J.J. Boon in press-b, 'Identifying biomolecular origins of solid organic residues preserved on Iron Age Pottery using DTMS and MVA', *Journal of Archaeological Science*.

Chapter 5

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', in H. Barnard & J. Eerkens (eds.), *Theory and Practice of Archaeological Residue Analysis*, British Archaeological Reports International Series, Archaeopress, Oxford.

Chapter 6

T.F.M. Oudemans, J.J. Boon, & R.E. Botto in press-a, 'FTIR and solid-state ^{13}C CP/MAS NMR spectroscopy of charred and non-charred solid organic residues preserved in Roman Iron Age vessels from the Netherlands', *Archaeometry*.

Appendix 1

T.F.M. Oudemans, unpublished results

Appendix 2

T.F.M. Oudemans, G.B. Eijkel & J.J. Boon 2005, 'DTMS and DTMS/MS study of solid organic residues preserved on ancient vessels', *Proceedings of the 33rd International Symposium on Archaeometry, 22-26 April 2002, Amsterdam*, H. Kars & E. Burke (eds.), Vrije Universiteit, Amsterdam, 501-505.

Appendix 3

T.F.M. Oudemans & D. Erhardt 1996, 'Organic residue analysis in ceramic studies: implications for conservation treatment and collections management', *Archaeological conservation and its consequences*, A. Roy & P. Smith (eds.), The International Institute for Conservation of Historic and Artistic Works, London, Copenhagen, 137-142.

Chapter 2

Studying Vessel-Use using Curie-Point Pyrolysis Mass Spectrometry and Multivariate Analysis

In this Chapter the chemical composition of solid organic residues on vessels from the Roman Iron Age is characterised by Curie-point Pyrolysis Mass Spectrometry. Soil samples of the surrounding sediment were analysed to check for contamination, but no indications were found for severe post-depositional degradation or contamination with soil components. Discriminant Analysis was used to compare the chemical characteristics of the residues as represented in the CuPyMS spectra, and Complete Link Cluster Analysis was used to group residues in clusters of similar chemical composition. The chemical composition of the residues was correlated to the form and size of the vessels in which they were found. This correlation was interpreted as a distinction in original vessel use between vessels of different form and size.

Modified after:

T.F.M. Oudemans & J.J. Boon 1996, 'Traces of ancient vessel use: investigating prehistoric usage of four pot types by organic residue analysis using pyrolysis mass spectrometry', *Analecta Praehistorica Leidensia*, vol. 26, 221-234.

1. Introduction

1.1. Functional classification of pottery, the use-alteration perspective

Pottery is a find-category frequently studied by archaeologists in search of information about different aspects of past societies, such as: social organisation, the organisation of trade and exchange, demography and subsistence. In spite of the long-standing tradition of ceramic studies in archaeology, it has always remained difficult to assign functions to ancient vessels. The understanding of how pottery was used is however essential to all studies that deduce information from pottery assemblages. The archaeological information stored in any assemblage of artifacts can only be interpreted fully if the actual use of the objects is known (Skibo 1992, 4-5).

Archaeological methods to identify vessel function are either directed at the function intended by the potter, or at the actual use of a vessel. The study of intended vessel functions is based on the assumption that the form, size and composition of pottery and, to a certain extent, its decoration, are constrained by the intended context and conditions of use (Braun 1983). The main difficulty of this approach is the interpretation of observed morphological and technological variation in terms of function (Rice 1987, 207-232; Rice 1996). The relationships between form, function and production technology are complex and variable. The study of intended vessel functions is therefore limited in detail and resolving power and will usually result in a general framework of possible functions (Rice 1990).

Studies directed to the actual use of vessels, on the other hand, can give independent information about the utilitarian role of a vessel. The traditional archaeological approach, the study of recovery context, is usually limited in resolution and open to multiple interpretations. The most direct and detailed way to analyse vessel use is through traces found in and on the ceramics. Hally (1983) was the first to use the term 'use-alterations' for modifications to the ceramic caused by use of the vessel. More recently, other researchers (Henrickson 1990; Skibo 1992, 42-49) have described various types of modifications including: the effects of firing such as, soot deposition and discolouration due to differences in oxidative state (i.e. oxygen discolouration); attrition or 'use-wear' of ceramic vessels (e.g. scratches, dents, chipping); and organic residues present in the vessels. Although each of these types of alterations can give information about vessel use, it must be kept in mind, that none will give information about the complete range of possible vessel uses within a society, but rather, on vessels of a selective range of daily applications.

1.2. Organic residue analysis to study vessel use

Studies of organic residues found in association with ancient pottery have mainly been directed at the identification of extractable compounds such as lipids and resinous materials (Rottländer & Schlichtherle 1980; see bibliographies in Evershed *et al.* 1992; Heron & Evershed 1993). Both

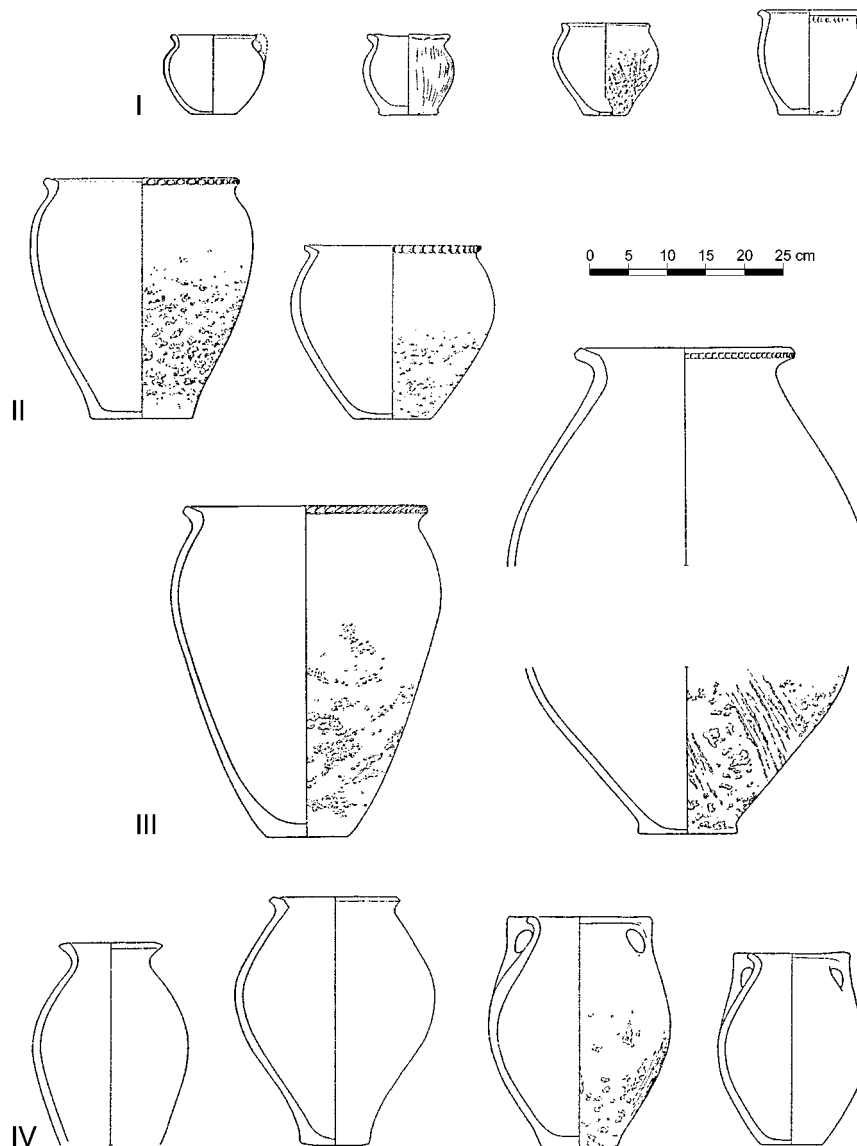


Figure 1: Morphological vessel types.

Morphological vessel types are based on form and size variables (Abbink 1999) such as Greatest Diameter (GD), Rim Diameter (RD), Distance from Rim to Greatest Diameter (DRG). Type I: small, three partite, wide mouthed vessels ($GD/RD < 1.5$, $GD < 170$ mm; $GD/DRG > 2.4$); Type II: medium sized, three partite, wide mouthed vessels ($GD/RD < 1.5$, $180 \text{ mm} \leq GD < 340$ mm; $GD/DRG > 2.4$); Type III: large, three partite, wide mouthed vessels ($GD/RD < 1.5$, $GD > 350$ mm; $GD/DRG > 2.9$); Type IV: Jar-like, three partite, narrow mouthed vessels ($GD/RD \geq 1.5$ but $GD/RD < 2.1$; $GD/DRG < 2.4$). Drawing: J. Hulst, Dutch National Service for Archaeological Heritage (ROB).

visible surface residues and compounds absorbed in the ceramic of the vessel wall are potentially useful sample material for systematic research of vessel use within ceramic complexes. In the literature some disagreement exists concerning the suitability of the different materials. Absorbed residues are probably more universally present (Evershed 1993a) and it is claimed, are more protected against degradation and contamination with soil compounds (Rottländer 1990). Their analysis is, however, limited to specific classes of extractable compounds, such as lipids or resinous materials, and the results are harder to interpret because the compounds are probably accumulated in the ceramic over a longer period of vessel use. Surface residues, on the other hand, are less common but have recently been shown (Oudemans & Boon in press) to contain a better preserved complex lipids profile. In addition, surface residues are probably the result of the last, or one of the last, phases of vessel use. In theory, this increases the likelihood of identification of the original vessel contents. Finally, solid surface residues can be studied for many classes of non-extractable compounds such as proteins and polysaccharides. Since the chemical composition of the total residues is unknown prior to analysis, solvent extracts, as obtained in the study of absorbed residues, may not be considered representative for this composition without further study.

However, the knowledge of complex solid biomaterials is constantly increasing due to the application of new analytical techniques, such as analytical pyrolysis mass spectrometry (Boon 1992). Curie-point Pyrolysis Mass Spectrometry (CuPyMS) has been shown to be a suitable analytical technique for the study of charred natural products (Pastorova *et al.* 1993a; Pastorova *et al.* 1993b) and for the study of archaeological surface residues (Oudemans & Boon 1991, 1996). The advantage of this technique is the capacity to analyse a complex mixture of compounds in one single analysis. Mixtures of soluble compounds and solid materials can be analysed.

In this paper, a series of solid organic residues from an indigenous settlement from the Roman period at Uitgeest-Groot Dorregeest was analysed with CuPyMS to characterise their chemical composition. The mass spectra were compared using multivariate analytical techniques, resulting in clusters of chemically similar residues. The clusters were subsequently correlated to the size and form of the vessel. The hypothesis is tested (Abbink 1985; Abbink 1999, 37 and 339), that variation in form and size of vessels represents a variation in the intended use of the pottery.

2. Experimental

2.1. Samples and sample treatment

The pottery studied was recovered from an indigenous settlement from the Roman period (ca. 0 - 300 AD) at Uitgeest-Groot Dorregeest in the Netherlands (Woltering 1982, 1983). The settlement was situated on the remains of a coastal barrier and a sandy Dunkirk I creek deposit. During the Roman period, the settlement was bordered on SE and SW by a low lying, eutrophic

Table 1 - Residues and Soil Samples

Nr	Find number ^a	Residue ^b	Position ^b	Vessel Type ^c	Sediment ^d
1	7-7	Brown	In	II	Organic clay
2	8-1	Red brown	Ex	III	Organic clay
3	8-2	Brown	In	II	Organic clay
4	8-5	Cream Coloured	In	I	Organic clay
5	14-6-4.2	Brown	In	-	Sandy
6	14-6-4.3a	Brown	In	I	Sandy
7	14-6-4.3b	Black	Ex	I	Sandy
8	14-6-4.4	Brown	In	-	Sandy
9	14-6-4.5	Brown	In	-	Sandy
10	18-3-2.a	Brown	In	I	Organic clay
11	18-3-2.b	Black	Ex	I	Organic clay
12	18-7	Brown	In	II	Organic clay
13	19-7-90.2a	Brown	In	II	Organic clay
14	19-7-90.2b	Brown	In	II	Organic clay
15	20-4	Brown	In	II	Sandy
16	20-4-157	Brown	In	-	Sandy
17	30-12-3(=30-2)	Brown	In	II	Organic clay
18	31-4.a	Brown	In	I	Organic clay
19	31-4.b	Black	Ex	I	Organic clay
20	32-6-18	Brown	In	-	Peat
21	33-5-2.a	Brown	In	-	Peat
22	33-5-2.b	Brown	In	-	Peat
23	33-8-2.a	Brown	In	-	Peat
24	33-8-2.b	Brown	In	-	Peat
25	34-0-12	Brown	In	-	Organic clay
26	34-0-30 (=34-12)	Brown	In	II	Organic clay
27	34-7-62	Red brown	In	II	Organic clay
28	34-7-95.b	Black	Ex	I	Organic clay
29	34-11-3	Brown	In	II	Organic clay
30	35-5-120	Brown	In	II	Sandy
31	35-7-28	Cream Coloured	In	-	Sandy
32	35-20	Cream Coloured	In	IV	Sandy
33	35-21	Brown	In	II	Sandy
34	P1 ^d	Peat		-	Pit 16
35	P2 ^d	Peat		-	Pit 34

^a Find number: first number indicates the excavation pit, letters indicate multiple samples from one vessel.

^b For residue appearance, vessel type and residue position (In = Interior; Ex = Exterior) see Appendix 1.

^c Sediment: the soil type in which the vessel was found.

^d P1 and P2 are peat samples from the excavation found in pit 16 and pit 34 respectively.

peat deposit and cut off on NW and NE by a fresh water gully running in the old course of a salt water creek. In the settlement a number of incomplete three-ided houseplans and about twenty filled-up water wells, dated to the Roman period, could be detected (Abbink 1985; Abbink 1999, 66-67).

The choice of the sample material for CuPyMS analysis was based on three criteria: burial context, vessel morphology and presence of different types of surface residues (see also Appendix 1). The pottery was found in three different types of burial contexts: in sandy creek deposits, in highly organic clay deposits (i.e. filled-up prehistoric wells or ditch fills) and in peat deposits (Table 1). Different morphological vessel types were distinguished in the studied ceramic complex based on several size and form characteristics. Four types of vessels were selected for this study (Fig. 1) of which type II represents the largest number in terms of quantity of recovered shards. Vessel types I, III and IV were much less abundant. The sample set used in this study, contained various different types of surface residues. Most of the residues were dark brown or black, thick (> 1 mm), carbonised crusts situated on the interior of vessels. Although these 'chars' occurred in all four different vessel types, they were very rare in vessels of type IV. A few cream coloured flaky crusts of medium thickness (circa. 1 mm) occurred on the inside of vessels. These particular residues were most frequently found on vessels of type IV. Very rarely, smooth red brown, thin (< 1 mm) residues were discovered on the interior of vessels or situated as streaks or dripping traces on the exterior of vessels. The patterns on the exterior of vessel 8-1 were probably the result of purposeful decoration with a thick liquid. A fourth kind of residues, a pitch black, smooth, thin (< 1 mm) layer could be found on the exterior of several vessels. These black residues were most frequently found on vessels of type I. The selected sample set consists of 17 shards from complete vessels of all four types, and 10 shards that were too small to allow identification of the original vessel type. These shards were included because they contained thick residues.

All shards had previously been washed with tap water. The residue samples were scraped from the ceramic surface with a scalpel (cleaned with Dichloromethane), after removal of the outermost 0.5 mm of the residue. In addition to the residue samples, two samples of the most organic sediments present in the site (P1 and P2) were analysed in order to check for exchange of compounds from the sediment. About 100 μg of each sample was ground with a small glass mortar and pestle. Subsequently a suspension was made by addition of about 50 μl ultra pure water (Millipore Q[®] grade).

2.2. Instrumental

The analyses were performed in triplicate on a fully automated Curie-point pyrolysis mass spectrometer the FOM-autoPyMS originally built at AMOLF in the mid seventies and described in its latest version by Boon and co-workers (1984). Pyrolysis is a thermal fragmentation of large, non-volatile molecules in an inert atmosphere. The smaller, more volatile fragments formed during this rapid heating process are representative of the original (macro) molecules. Curie-point pyrolysis is accomplished by inductive heating of a ferromagnetic wire coated with

the sample material. The thermal energy thus transferred to the sample is used for desorption or pyrolysis of the molecules.

The desorbed molecules and pyrolysis fragments are subsequently ionised and transported to the mass spectrometer where they are separated according to mass. The instrumental conditions were: heating for 0.1 s at a rate of 5000 °C/s up to a Curie-point temperature of 610 °C, the total heating time was 1.0 s. The pyrolysis chamber was set at 180 °C, the ionisation current was 16 eV and the total mass range measured was m/z 20-240 at a scan speed of 10 scans/s with a total number of averaged spectra of 200. The results are visualised in a Mass Spectrum in which the intensity of each mass is plotted on a scale of 1 to 100% relative to the most abundant mass. Mass Spectra (Fig. 2) can therefore be seen as a chemical ‘fingerprint’ of the total organic chemical composition of the sample.

2.3. Multivariate Analysis

In order to facilitate the comparison of mass spectra, a data reduction technique called Discriminant Analysis (DA) was applied. The statistical package used was a modified version of the ARTHUR multivariate analysis package (Hoogerbrugge *et al.* 1983). A number of Discriminant Functions (DFs) were calculated, which express the main similarities and dissimilarities between groups of samples. The DFs are linear recombinations of highly correlated masses. The number of DFs needed to explain the total variance in a set of samples depends on the diversity of the samples and the complexity of the material studied. The total variance in a set of samples can be seen as a summation of variance introduced by systematic changes in sampling or analytical procedure (I_W = within group variance), and variance between the groups of replicate analyses of samples (I_B = between group variance). The Fisher ratio (F) of a given DF, defined as $F = I_B/I_W$, expresses the amount of variance relevant to group separation explained by that DF. The characteristic variance of a sample set is here defined as the sum of the Fisher ratios of all the DFs. Using these Fisher ratio's, a selection of DFs can be made that are most relevant to group separation. Two-dimensional representations of the discriminant space (discriminant maps of two relevant DFs) can be made (Fig. 3 and 4), showing the samples plotted around an origin, which represents the calculated ‘average spectrum’. The Euclidean distance between two sample points in the map expresses the relative difference in chemical composition between two samples (e.g. samples close together are more similar to one another than samples further apart).

In order to outline groups of chemically similar samples, Complete Link Cluster Analysis (CLCA) was employed. Since not all DFs have the same explanatory power, each dimension is weighted prior to cluster analysis. The weighting factor k_i is defined as the percentage of the characteristic variance explained by the given DF_{*i*}. The weighted Euclidean distance D between sample points x_1 and x_2 is defined as:

$$(1) \quad D_{(x_1, x_2)}^2 = \sum_{i=1}^n k_i \cdot (x_{1i} - x_{2i})^2$$

in which i symbolises the dimension or DF and k_i is a weighting factor for dimension i and x_{1i} and x_{2i} are the values for x_1 and x_2 in the dimension i . CLCA was applied to the weighted Euclidean distance between the samples in the discriminant dimensions considered relevant to group separation. The similarity value S between object x_1 and object x_2 is defined as:

$$(2) \quad S_{(x_1, x_2)} = 1 - \frac{D_{(x_1, x_2)}}{\max D_{(x_m, x_n)}}$$

in which $\max D$ represents the maximal weighted Euclidean distance between two points within the set of sample points. Clusters of samples with high similarity values can thus be formulated. In order to explain the chemical differences between the clusters in terms of molecular composition, the sets of correlated masses that define the dimensions of the discriminant space are subsequently chemically interpreted in terms of classes of bio-organic compounds (Fig. 3 and 4).

Table 2 - Clusters of residues and their typical composites

C ^a	Sample ^b	S ^d	Compounds ^c	Markers [m/z] ^c
A	1, 3, 5, 8, 9, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 25, 26, 29, 30, 33	0.62	Charred Polysaccharides [1] Fatty Acids [2] Proteins [3] Elementary Sulphur	146, 147, 160, 161, 174, 175, 188, 189. 60, 73, 101, 115, 129. 92, 94, 117, 131, 154. 32, 34, 60, 64, 96, 128, 160, 192, 224, 226, 256, 258.
B/D	6, 7, 10, 11, 18, 19, 32	0.78/0.93	Phenols PAHs [4] CO ₂ Aliphatic Compounds	78, 91, 92, 105. 128, 142, 156, 170, 178, 192. 44 55, 56, 57, 69, 70, 83, 84, 85, 97, 98, 99.
C	2, 4, 31	0.81	Proteins	92, 94, 117, 131, 154.
E	27, 28	0.76	Elementary sulphur	32, 34, 60, 64, 96, 128, 160, 192, 224, 226, 256, 258.
Soil	P1, P2		Polysaccharides [5] Lignins [6] Fatty acids	43, 55, 60, 72, 96, 98, 110, 112 114, 126, 128. 124, 138, 150, 152, 164, 168, 210, 180, 184, 194. 60, 73, 101, 115, 129.

^a Cluster: cluster A through E. Soil samples are also indicated as a separate unit.

^b Sample numbers: number as indicated in Table 1.

^c Markers: typical pyrolysis markers for certain compound classes.

^d Relative Similarity Values: similarity in chemical compositions (see Formula 2).

^e Compound Classes: chemical compound classes represented by the typical markers.
[1] = Pastorova *et al.* 1993b; [2] = Waller 1972; [3] = Meuzelaar *et al.* 1982, 109 and Munson & Fetterolf 1987; [4] = Medalia *et al.* 1983; [5] = Meuzelaar *et al.* 1982, 16-18 and 115-149 and [6] = Boon *et al.* 1986 and van der Heiden *et al.* 1990.

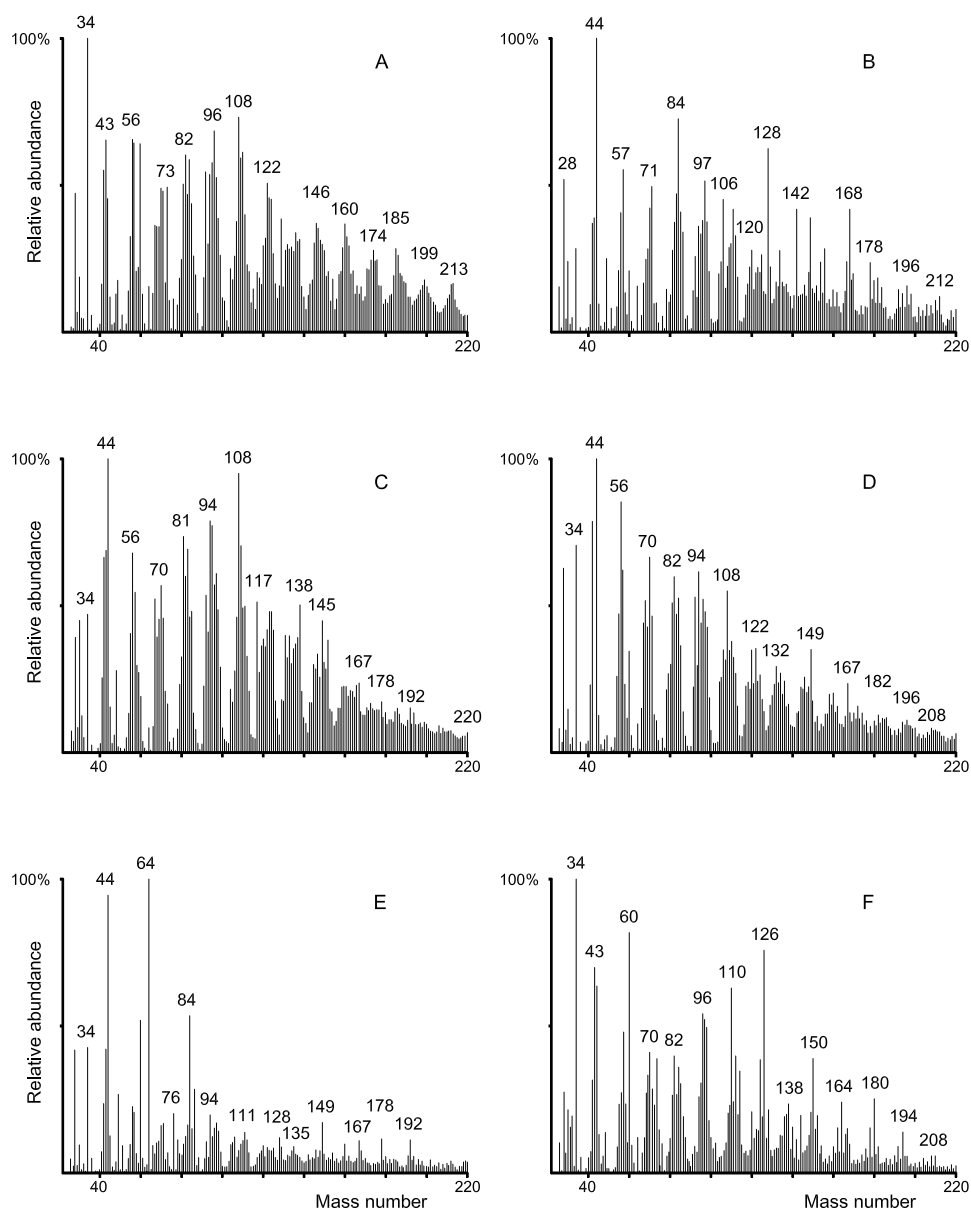


Figure 2: Mass spectra of archaeological residues (5) and a peat sample.

The intensity of each mass is plotted relative to the most abundant mass. Letters A through E indicate clusters of chemically similar residues. A: sample 12 (18-70), a typical dark brown char from vessel type II; B: sample 11 (18-3-2.b), a black soot from the exterior of a vessel of type I; C: sample 31 (35-7-28), a cream coloured residue; D: sample 18 (31-4.a), a typical char from vessel type I; E: sample 28 (34-7-95.b), a black soot contaminated with elementary sulphur; and F: sample 34 (P1), a peat sample from pit 16.

3. Results and Discussion

3.1. Soil samples versus archaeological residues

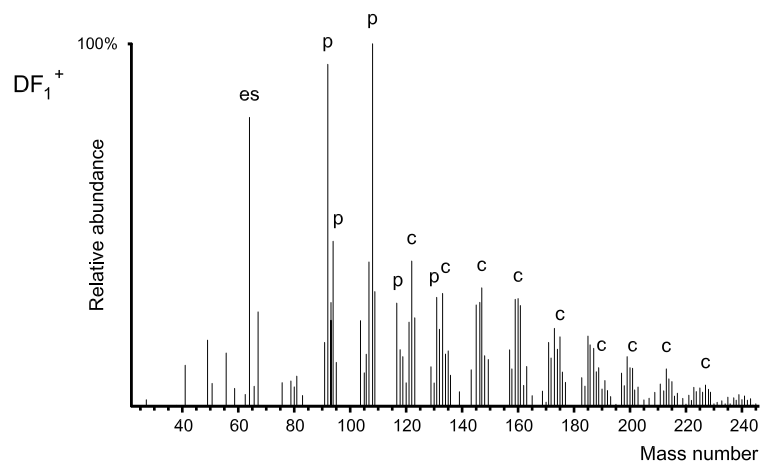
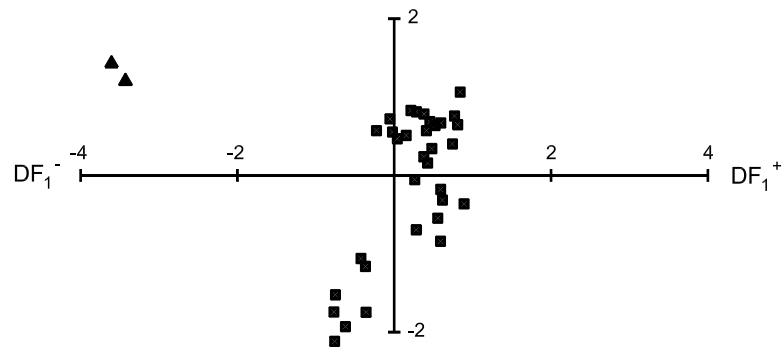
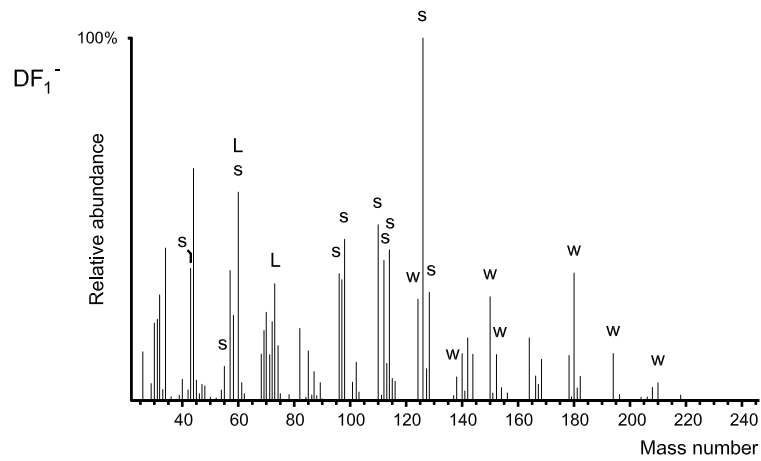
In order to compare the archaeological residues to the surrounding soil in which they were buried for many centuries, the CuPyMS mass spectra of residues and soil samples were included in a DA of the total sample set. A discriminant map of the discriminant functions DF_1 and DF_2 , respectively expressing 41.9% and 28.6% of the characteristic variance (Fig. 3), visualises a clear distinction between these two groups of samples. The soil samples are primarily characterised by mass peaks in the DF_1^- spectrum, whereas most of the organic residues on the vessels are characterised by the DF_1^+ . The residue samples are further characterised and divided by masses in the DF_2 dimension (not shown in Fig. 3).

The spectrum representing the DF_1^- space contains markers for the biomolecular compound classes lignins, polysaccharides and fatty acids (see Fig. 2 and 3, Table 2: Soil) typical in pyrolysates of peat samples (Bracewell *et al.* 1980; Boon *et al.* 1986). The absence of lignin and intact polysaccharide markers in pyrolysates of archaeological residues is also shown before in Curie-point Pyrolysis GC/MS studies (Oudemans & Boon 1991). Although polysaccharides were probably an important component of prehistoric foods, intact polysaccharides have obviously not survived the extreme conditions during cooking or burial in the ground. Lignins originate from the ‘woody’ parts of plants, and are not likely to be a significant constituent of foods. Fatty acids are an important component of human foods. However, the quantity of fatty acids in the peats must be much larger causing the fatty acid markers to appear in the DF_1^- side of the spectrum. The archaeological residues are distinctly different in chemical composition (Fig. 2 and 3) and are primarily typified by fragments indicative of proteins, charred polysaccharides and elementary sulphur (see also Table 2).

Exchange of any significant quantity of compounds between archaeological residues and organic soils, such as P1 and P2, has obviously not taken place, which is in agreement with a study on the exchange of extractable soil lipids by Heron and co-workers (1991). It is therefore not likely that remains of soil, stuck to the surface of ceramics, will ever be mistaken for residues of the original vessel contents. In addition, no correlation could be found between the chemical composition of residues and the type of sediment in which they were preserved. In Fig. 3 samples recovered from different sediment types were situated in various quadrants of the map. In conclusion, it can be stated that the chemical classification of the residues based on CuPyMS data is, therefore, a reflection of the original vessel contents and not an artefact of post-depositional changes in chemical composition of residues during burial.

Figure 3 (on facing page): Discriminant map comparing Residues and Soil samples.

Residues (black squares) and soil samples (black triangles) are plotted according to their similarities and dissimilarities in chemical composition. Triplicates are plotted as one average point. Markers for lignin's (W), polysaccharides (S), fatty acids (L), proteins (P), charred polysaccharides (C) and elementary sulphur (ES) are indicated in the spectra for the DF_1^+ and DF_1^- directions.



3.2. Chemical composition of archaeological residues

After removal of the soil samples from the data set, a second discriminant analysis was performed to ‘zoom in’ on the chemical characteristics of the various residue samples (Fig. 4). Although nineteen DFs were defined to explain the total variance in the data set, only the first three (explaining respectively 44.0%, 16.8% and 13.3% of the characteristic variance) were considered representative of the chemical composition of the residue material. The additional DFs represented minor variations in chemical composition due to fluctuations in analytical circumstances or presence of inorganic components. The second DF was not considered suitable for mapping, because it represents merely the presence or absence of contaminating phthalate esters (indicated by fragments m/z 149 and 167) that could be identified as contaminants. These contaminations probably take place in contact with plastic bags in which ceramics are often stored after excavation. The DF₂ is therefore not relevant to the chemical composition of the original residue.

The weighted CLCA of the distances in the first three dimensions indicated the existence of 5 clusters A through E (Fig. 4). Clusters B and D are situated in the same direction of the map (Fig. 4), and are characterised by the same masses in the given dimensions DF₁ and DF₃. The calculated relative similarity values give a measure for the relative chemical similarity between samples in the clusters (Table 2). The typical chemical characteristics of the clusters can be interpreted as indicators or markers for certain compound classes (Table 2). Features that appear in all samples will, however, not be shown as typical characteristics for either of the clusters. For the absolute chemical composition of the residues, the original mass spectra must be considered as well (Fig. 2).

Cluster A

Cluster A consists of chars containing charred polysaccharides. The combination of markers m/z 110, 146, 147, 160, 161, 162, 174, 175, 188 and 189, is typical for polysaccharides heated under inert circumstances at temperatures over 250 °C (Pastorova *et al.* 1993a; Pastorova *et al.* 1993b). The experiments described by Pastorova and co-workers were designed to resemble charring processes in ceramic vessels. During the cooking of a thick liquid, high temperatures caused by restricted circulation could cause burning and eventually charring against the heated wall and bottom of the vessel. Some of the residues in cluster A contain additional characteristics, such as a strong protein influence (i.e. samples 1, 3, 25, 26, 30 and 33) or the presence of fatty acids in relatively large quantities (i.e. samples 9, 13, 17 and 22).

Clusters B and D

Clusters B and D consist of residues containing aliphatic compounds and/or Polycyclic Aromatic Hydrocarbons (PAHs) and their methyl and ethyl derivatives. The black samples on the exterior of vessels (samples 11, 7 and 9) are characterised by the presence of PAHs and compounds that release CO₂. A detailed identification of these compounds by pyrolysis gas chromatography mass spectrometry (PyGCMS) leads to the interpretation of the residues as

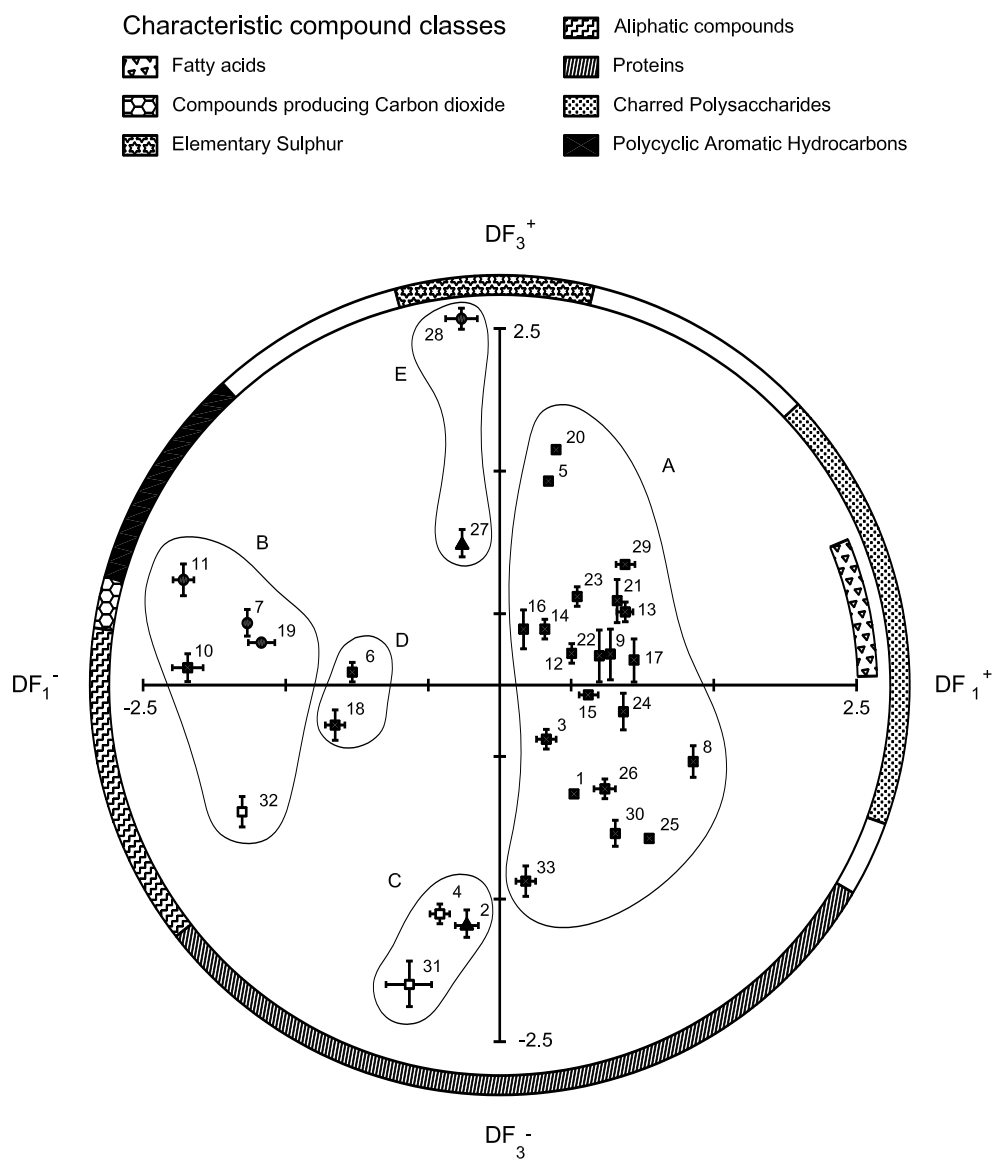


Figure 4: Discriminant map with clusters of residues (A through E) and their chemical characteristics. Standard deviation error bars indicate triplicate measurements. The distance between two sample points expresses the relative difference in chemical composition. Indicated are: brown chars (■); black soot residues on exterior of vessels (●); red brown residues (▲); and cream coloured residues (□). The shadings in the circle indicate the compound classes indicative for various directions in the discriminant map.

smoke condensates or 'soot' from cooking over wood fires (Oudemans & Boon 1991). In addition, several chars and a white residue are classified in the clusters B and D. These residues belong to this cluster due to the presence of markers for aliphatic compounds (not due to the presence of any PAHs). The aliphatic compounds are most likely pyrolysis products of some kind of aliphatic polymeric structure formed from lipids under high temperatures. The presence of similar aliphatic markers in pyrolysates of experimentally obtained chars of recent food stuffs is shown in Chapter 3 (Oudemans & Boon 1991). This aliphatic product may be formed during heating of oils in the vessel in order to make it more waterproof. However, the obvious absence of any intact fatty acids is, in this context, rather strange. One would expect to find remainders of free fatty acids in the residues as well. An additional sample, sample 32 is different in appearance as well as in chemical composition: it contains some protein markers in addition to the aliphatic compounds mentioned earlier. The origin of this residue may be found in a combination of waterproofing and the use of the vessel for storage or processing of proteinaceous materials.

Cluster C

Cluster C contains protein rich residues of different appearance. Although these different samples seem difficult to compare, their clustering indicates similarity in chemical composition. The mass spectra (Fig. 2) show a clear pattern of protein fragments and even include some markers for intact dipeptides such as m/z 154 (Munson & Fetterolf, 1987). It is not clear, whether these three residues are of a similar origin. The absence of fatty acids and charred polysaccharides from the mass spectra, does however suggest a vessel contents of primarily proteinaceous material. These residues show a relatively highly preserved protein pattern, which suggests a lack of heating. The cream coloured residues 4 and 31 are situated on the interior of vessels may have been formed during storage or processing of protein-rich materials. Dairy products seem to be excluded because no significant quantities of fatty acids are present. The red brown sample 2 is situated on the exterior of a vessel and may be a protein mixed with an inorganic pigment used for decorative purposes. The presence of a similar red brown residue (though contaminated with elementary sulphur) sample 27 on the inside of a vessel, suggests that this material may be prepared in a ceramic vessel, causing a smooth residue all over the interior of the vessel.

Cluster E

Cluster E is a cluster with sulphur-containing residues. The markers m/z 32, 34, 64, 128, 160, 192 and 256 indicate the presence of elementary sulphur (S_8). Elementary sulphur is quite rare in nature and the origin of the material is not clear. It is possible that the clay used for the production of these specific vessels contained S_8 . Since the two samples (27 and 28) involved were both very thin, clay particles could have been scraped from the surface of the vessel and mixed with the sample. Bacteria may also produce elementary sulphur during degradation of biological compounds.

3.3. Correlation with Pot Type

When the morphological pot types are plotted in the two-dimensional DF map (Fig. 5), 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 3). Vessels of different size and form were therefore obviously used for different daily uses. Although there is no complete overlap between vessel type and chemical properties of the residues, careful interpretations can be made about possible vessel usage of different vessel types.

Vessel Type I

The origin of the charred residues in this type of vessels is not completely clear. The CuPyMS data indicate that they are quite different in composition from those in vessels of Type II. The absence of charred polysaccharides, protein markers or fatty acids as typical features, is significant. These residues may have been formed during activities other than food preparation. A possible origin may be found in a post-firing water proofing of the vessels by heating of oil in the vessels. The high temperatures may cause cross-linking of the lipids. Interesting in this case is also the frequent presence of soot on the outside of these vessels. One residue (sample 4) is clearly different and belongs to cluster C, which suggests occasional variation in use of the vessel type.

Vessel Type II

The majority of the residues on these vessels are found in cluster A. The origin of these residues can probably be found in the preparation of grains or porridge or other starch-rich stews. In some cases protein-rich material such as meat, fish or pulses may have been added, while in other instances fats may have enriched the mixture. Sample 27 belongs to cluster E because it contains markers for elementary sulphur. This sample is of quite a different nature and may be the result of the preparation or storage of a protein rich material (see for explanation above).

Vessel Type III and IV

Due to the absence of multiple samples of these vessel types, no conclusions can be drawn on the usage of these vessel types as a whole. The MS data of the two samples that were analysed did, however, present interesting evidence explained earlier.

Although larger numbers of complete vessels should be studied, in order to confirm the vessel uses here suggested and check their statistical significance. However, even when the origin of samples cannot be understood completely, it can still be concluded that vessels from Type I and Type II were used in a different way. This supports the original hypothesis that the variation in pot morphology had a utilitarian meaning in the indigenous settlement in Uitgeest-Groot Dorregeest.

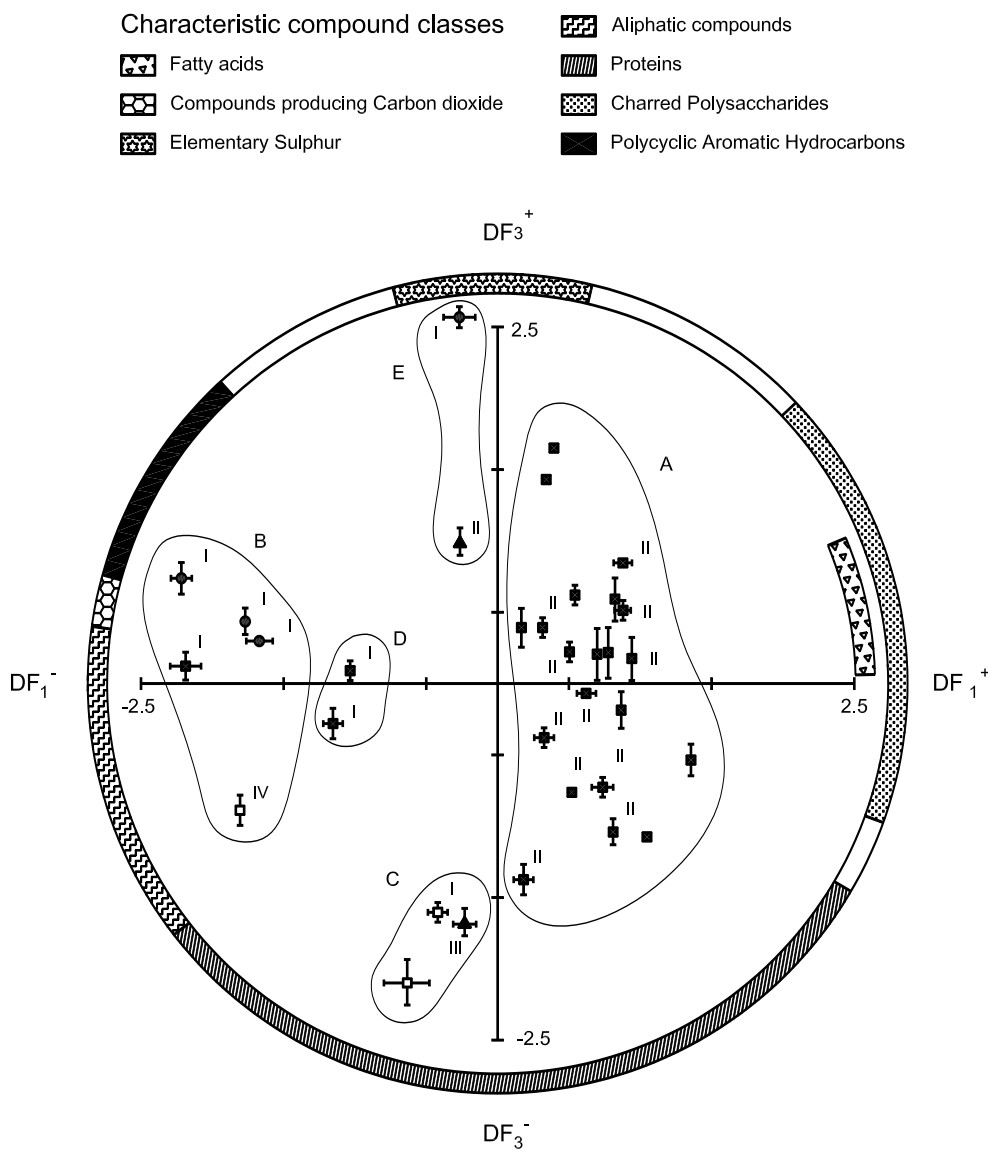


Figure 5: Correlation between vessel types and chemical composition of the residues.

The vessel types are indicated I through IV. Shards of which the vessel type could not be identified are indicated without type number. Clusters of residues (A through E) were derived from the chemical characteristics as expressed in the CuPyMS spectra (see Fig. 4). The distance between two sample points expresses the relative difference in chemical composition. Indicated are: brown chars (■); black soot residues on exterior of vessels (●); red brown residues (▲); and cream coloured residues (□). The shadings in the circle indicate the compound classes indicative for various directions in the discriminant map.

Table 3: 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	B/D	Heated lipids	Water proofing ?
	Black, exterior	3	B/D	Soot	Cooking on wood fires
	Black, exterior	1	E	Contamination	Cooking on wood fires
	Cream coloured, interior	1	C	Protein	Proteinaceous material?
Type II	Char, interior	10	A	Starch	Starch-rich food
	Red brown, interior	1	E	Contamination	Proteinaceous material?
Type III	Red brown, exterior	1	C	Protein	Proteinaceous material?
Type IV	Cream coloured, interior	1	B	Protein	Proteinaceous material?
				Heated lipids	Water proofing ?

^a Vessel Type as indicated in Fig. 1.

^b Residue appearance: as indicated in Table 1.

^c Number: indicates the number of samples.

^d Clusters as indicated in Table 2 and Fig. 4.

4. Conclusions

This paper presents the first systematic study to correlate the chemical composition of solid organic surface residues to the form and size of ceramic vessels in which they were preserved. It has been shown that CuPyMS, in combination with multivariate analytical techniques, is a useful method to systematically and rapidly analyse and categorise solid organic residues found on ancient vessels. The chosen analytical strategy presents not only a measure for similarity or dissimilarity in chemical composition of the samples, facilitating as such an objective classification of the residues, but also highlights the chemical components typical for the various clusters. The chemical classification was shown to be a reflection of the original vessel use, and not an artefact of post-depositional changes in chemical composition of the residues.

Results from the CuPyMS studies give clear evidence to conclude that a correlation does indeed exist between the chemical composition of the surface residues studied and the morphological vessel type of the vessel in which they were found. Vessels of different sizes and forms were, therefore, used for different daily use within the indigenous settlement from the Roman period at Uitgeest-Groot Dorregeest. These results support the usefulness of a morphological vessel classification as a basis for functional studies within this ceramic complex.

The significance of organic residue analysis within archaeological ceramic studies is, however, not limited to testing existing theories concerning the relation between form and function of

Chapter 2

pottery, but also lies in the detailed information about daily use of vessels that cannot be obtained by any other method.

Chapter 3

Molecular Characterisation of Solid Organic Residues by Curie-Point Pyrolysis Gas Chromatography/Mass Spectrometry

In this Chapter analytical pyrolysis techniques are shown to be suitable for molecular characterisation of solid organic residues because of their capacity to non-selectively identify a wide range of compounds. No sample preparation other than grinding was needed and the analysis was performed on very small samples (20-30 μg). Curie-point pyrolysis mass spectrometry was used to rapidly characterise residues and chemical characteristics of the residues were compared using multivariate techniques. Curie-point pyrolysis gas chromatography/mass spectrometry study of four representative residues resulted in detailed identification of preserved compounds. Many bioorganic moieties including fatty acids and characteristic markers for proteins and polysaccharides were detected in residues situated on the inside of vessels. Since no indications could be found for severe post-depositional changes in chemical composition, it is concluded that the composition of the residues is a reflection of the original vessel contents. The refractory nature of the chars is proposed to be the primary cause for the high degree of preservation. Other classes of compounds like polynuclear aromatic hydrocarbons were detected in residues preserved on the exterior of the vessels, and were interpreted as originating from smoke condensates from open cooking fires. A regular alkane/alkene pattern found in many residues and in the ceramic material of the vessel itself, is interpreted as the pyrolysis product of an aliphatic network that was formed from foods under high temperature conditions during cooking. Soil samples and experimentally charred modern foodstuffs were analysed alongside the residues for comparison on a molecular level.

Modified after:

T.F.M. Oudemans & J.J. Boon 1991, 'Molecular archaeology: analysis of charred (food) remains from prehistoric pottery by pyrolysis-gas chromatography/mass spectrometry', *Journal of Analytical and Applied Pyrolysis*, 20, 197-227.

1. Introduction

Amorphous organic (food) remains on pottery from archaeological sites have been noted and studied chemically by archaeologists since the end of the last century (see for references Rottländer & Schlichtherle 1980). These early studies remained incidental and limited to special cases such as wine and beer residues, charred bread and ointments. No systematic approach was ever undertaken until the early seventies when more detailed archaeological questions could be addressed due to improvements in analytical instrumentation. The application of analytical chemistry to the study of organic residues on pottery has, since then, expanded and is concentrated mainly on fatty substances soluble in organic solvents (Morgan *et al.* 1973; Condamin *et al.* 1979; Rottländer & Schlichtherle 1979; Rottländer & Blume 1980; Rottländer & Schlichtherle 1983; Morgan *et al.* 1984; Patrick *et al.* 1985; Hill & Evans 1988, 1989). The samples are prepared by selective chemical methods that focus on the analysis of only a specific part of the original material. An additional challenge is the limited sample size of most archaeological residues. The use of Curie-point pyrolysis in combination with gas chromatographic and/or mass spectrometric techniques helps to overcome these problems because a very small sample (20–30 µg) can be analysed directly in its solid state without any preparation apart from grinding. An advantage of these techniques is their capacity to analyse a complex mixture of compounds almost without discriminating effects (although the conversion of the sample into analysable volatiles is not quantitative). Although the complications concerning archaeological interpretation are not resolved directly by using CuPyMS and CuPyGC/MS, the comparison of very different samples with one another on a molecular level is facilitated. Organic residues, pottery fragments and soil samples can all be analysed and compared to get more detailed information about the presence of different compounds.

The archaeological importance of chemical studies of amorphous residues on pottery lies in the discovery of information on the natural resources used by people in prehistoric times and on the techniques applied to prepare food, dyes, oils and paints. Such studies may also reveal information about the actual use of pottery and as such become an important factor in the determination of the relationship between form, function (actual use) and the production technology of pottery.

Chapter 1 summarises many of the changes archaeological materials may have undergone. The residues can be seen as the remainder of a series of formation processes such as: processes in prehistoric times (including heating, cooking, charring, storing or transportation); processes after use (post-depositional processes including microbial degradation, contamination with soil or leaching of original compounds into the surrounding soil); and finally post-excavational changes and handling by archaeologists (including washing, scrubbing, and contamination with greasy fingers, ink, glue or dust).

The main purpose of the work presented in this chapter is to find out whether CuPyMS and CuPyGC/MS can be applied successfully to study organic compounds hidden in, or grafted on, solid amorphous residues preserved on pottery from archaeological contexts. The success of these studies for archaeological purposes depends on the range of organic compounds that can still be detected, the possibilities to detect differences in chemical composition between the

samples, and the extent to which the origin of the different compounds can be traced back to prehistoric times.

2. Experimental

2.1. Samples and sample treatment

The material studied was found in a settlement from the Late Iron Age and Roman period. The indigenous settlement was situated on the edge of a sandy creek deposit bordered by a peat swamp at Uitgeest-Groot Dorregeest (Woltering 1982, 1983). The shards were found in three different sediments: peat, a sandy creek deposit and in organic rich clay fillings (e.g. filled up water wells from the Roman period or filled up natural creek).

The organic residues, situated on different pots, were of different colour and appearance (Table 1). The residues were mostly charred, dark brown or black crusts (on the inside of pots), but some were white or cream coloured and of flaky substance (on the inside) or red brown and deposited in streaks (on the inside or outside) or pitch black and smooth (on the outside). Samples were taken from morphologically different types of pottery (see also Chapter 2, Fig. 2). These 'types' are based on morphological variables (like diameter and height) as measured and registered by Abbink (1999) and summarised in Appendix 1. All the shards had been washed with tap water and dried prior to sampling. Microscopic examination of the residues with a scanning electron microscope (Oudemans unpublished results) up to a magnification of 500 times, showed a broad variation in visual structure between the samples. Cross sections of each residue were studied to determine the homogeneity of the residues and to make sure only one

Table 1: Experimental and archaeological samples for GC/MS analysis

Nr ^a	Sample ^a	Description ^a	Sediment ^b	Vessel ^a type	Location on Vessel ^a
-	Experiment 1	Flour, protein and fat heated for 5 min at 100 °C	-	-	-
-	Experiment 2	Flour, protein and fat heated for 125 min at 250 °C	-	-	-
26	34-0-30	Brown, 0.2 cm	Humic Clay	IIb	Interior
31	35-7-28	Cream coloured, <0.1 cm	Sand	Shard	Interior
8	14-6-4.4	Brown/black, 0.2 cm	Sand	Shard	Interior
11	18-3-2.b	Black, 0.2 cm	Humic Clay	Ib	Exterior
-	Ceramic from 14-6-4.4	Grey ceramic material	Sand	Shard	-

^a For find number residue appearance, vessel type and residue position see Appendix 1.

^b Sediment: the soil type in which the vessel was found.

layer was sampled (see also Appendix 1). The residues presented here show no visible division in layers so it is assumed that they represent one of the last uses of the vessel. The samples were scraped from the pottery with a solvent cleaned scalpel.

To prevent contamination with organic soil material, the top layer (0.5 mm) of the residue was first removed before the actual sample was taken. Thirty-three samples were analysed by pyrolysis mass spectrometry and twenty-eight by pyrolysis gas chromatography. Four archaeological residue-samples were selected for further pyrolysis gas chromatography/mass spectrometry analysis (Table 1, Figure 1).

The ceramic material (from shard 14-6-4.4) was also sampled and analysed. The sample was taken after removal of the residue and 1 mm of the pottery wall (to prevent mixing with the residue). Soil samples were analysed for comparison. These samples were kept in a dry state for about a year previous to analysis.

Controlled cooking experiments were performed to get information about the chemical composition of different charred materials. Samples were taken from mixtures of fresh foodstuffs (e.g. flour, bovine serum albumin, and plant margarine) that had been heated for 5 min at 100 °C and 125 min at 250 °C respectively in a glass vial on an electric burner.

Sample preparation for all samples was limited to grinding about 100 µg of each sample in a small glass mortar and pestle and subsequently making a suspension of the ground sample in about 50 µl ultra pure water (Millipore Q[®] grade).

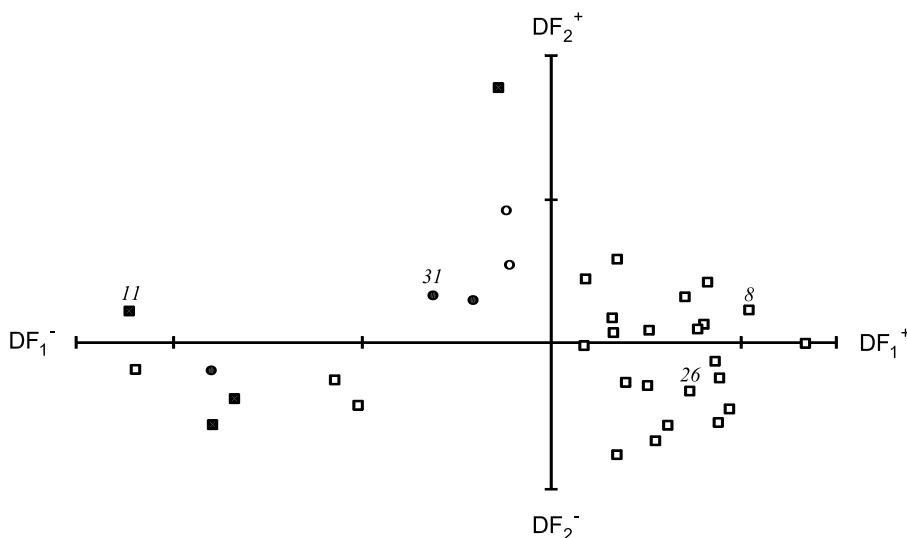


Figure 1: Comparison of chemical composition of 33 archaeological residues after CuPyMS.

Discriminant map of DF_1 versus DF_2 of residue samples (33) from interior and exterior of vessels with four sample selected for further analysis by CuPyGC/MS indicated with numbers (see also Table 1). The Euclidian distance between two sample-points represents the relative difference in chemical composition (e.g. a small Euclidian distance expresses relative similarity between two samples where as a bigger distance indicates a larger difference in chemical composition). Chars on vessel interior (\square); black residues on vessel exterior (\blacksquare); red-brown residues (\circ); and cream-coloured residues (\bullet).

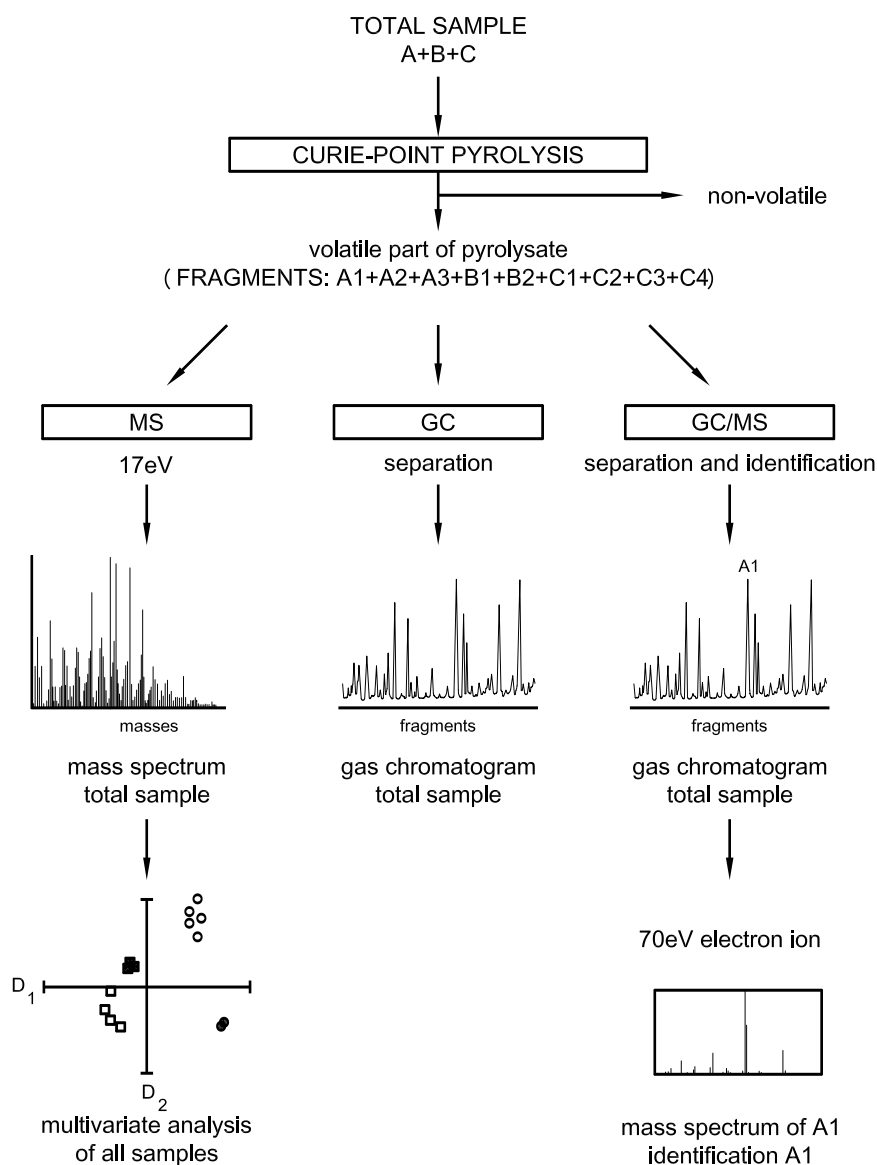


Figure 2: Analytical techniques

Different analytical techniques applied in this study for the characterisation of organic residues. The total sample is fragmented (into A1, A2, B1, B2 etc) using Curie-point pyrolysis after which analysis can be done 1) using MS giving average spectra used for for multivariate analysis, 2) using GC to separate compounds or 3) using GC/MS to separate all compounds and identify each individual compound by its individual spectrum (for instance the mass spectrum of A1)

2.2. Analytical methods and instrumentation

Figure 2 shows an overview of the analytical methods - Curie-point pyrolysis mass spectrometry (CuPyMS), Curie-point gas chromatography (CuPyGC) and Curie-point gas chromatography/mass spectrometry (CuPyGC/MS) - used in this study. Pyrolysis was used in this approach to volatilise absorbed compounds by evaporation and to pyrolyse the organic macromolecular matrix (char) into more volatile fractions, which could then be analysed by MS, GC and GC/MS.

The Curie-point Pyrolysis was carried out in the (FOM 3-LX) pyrolysis unit, designed and produced by the FOM in Amsterdam for the analysis of complex organic materials and most recently described by Boon (1987). About 10 μl of the sample suspension is applied onto a ferromagnetic wire, the sample is dried in vacuo and the analytical probe is placed in a glass liner. This glass lined analytical probe is placed in a heated pyrolysis chamber (180 °C) equipped with a high frequency coil. In CuPyGC analyses this chamber is flushed with helium while CuPyMS takes place in vacuo. The ferromagnetic wire is inductively heated within 0.1 s to its Curie-point in vacuo (PyMS) and up to 2 s in a helium atmosphere (PyGC). The thermal energy is transferred from the wire to the sample that evaporates and pyrolyses. The volatile products are either swept to the beginning of the capillary column by a carrier gas (in CuPyGC and CuPyGC/MS) or expand into an expansion chamber (in CuPyMS) for further analysis.

Pyrolysis mass spectrometry is used for rapid characterisation of complete samples. CuPyMS was carried out on the FOMautoPyMS (Boon *et al.* 1994) which has the capacity to rapidly analyse large numbers of samples and blanks. The pyrolysis chamber and the expansion chamber were heated to 160 °C and 200 °C respectively. The pyrolysis temperature was 610 °C and the total pyrolysis time 1.0 s. To minimise the fragmentation of the pyrolysis products low voltage electron impact ionisation (EI) of 17 eV was used. The mass range used was mass number m/z 23 - 240 and the scan speed was 10 scans/s with a 20 s total data acquisition time. All samples were analysed in triplicate and the CuPyMS spectra shown (Fig. 3) are averaged spectra over the total pyrolysis period. This kind of 'fingerprinting' analysis is very suitable for comparative studies of samples using multivariate analysis, but does not give much information about the nature of the individual components in the spectra because the spectra are cumulative. Multivariate analysis was performed using the FOMpyroMAP package for CuPyMS data (Windig *et al.* 1982; Hoogerbrugge *et al.* 1983; Boon 1992). Pyrolysis gas chromatography and pyrolysis gas chromatography/mass spectrometry are used to further identify the mixture of the pyrolysate by separation and identification of the different compounds involved.

The Curie-point pyrolysis gas chromatography was performed with a Carlo Erba 4200 gas chromatograph equipped with a Flame Ionisation Detector (FID). The column used was a 50 m CP Sil 5 CB fused silica capillary column (ID 0.32 mm, film thickness 1.2 μm). Both injector and detector were kept at 280 °C. The GC oven was kept at 30 °C during pyrolysis and was subsequently programmed to 300 °C at 6 °C/min. The data were recorded with a Nelson 760 interface and an Olivetti M28 PC loaded with Model 2600 Chromatography Software from Nelson Analytical.

Curie-point pyrolysis gas chromatography/mass spectrometry was done on a Packard 438-S gas chromatograph and a JEOL DX-303 double focussing mass spectrometer equipped with the

JEOL data system DA-5000. CuPyGC/MS was done under the same chromatographic conditions and on the same column as the PyGC work. The GC-column ended directly into the ion source of the mass spectrometer. Compounds were ionised at 70 eV electron impact voltage, and the acceleration voltage was 3 kV. Scan speed of the MS was 1 scan/sec over a mass range of m/z 20-1000. Mass calibration was carried out using PFK. In both CuPyGC and CuPyGC/MS helium was used as the carrier gas.

3. Results and Discussion

3.1. Survey with CuPyMS and CuPyGC/MS

The results of the CuPyMS analyses of the total set of 33 residue samples (see also Fig. 3) show clear qualitative differences in the chemical composition of residues. With the use of discriminant analysis, it was possible to quantify these relative differences. Figure 1 shows the total data set in a discriminant map (DF_1 versus DF_2). The distance between two samples represents the relative difference in chemical composition between the samples. The samples on the right side (mainly determined by the DF_1^+) show protein characteristics and free fatty acid, while samples on the far left side of the map (mainly determined by the DF_1^-) show many markers for polynuclear aromatic hydrocarbons (PAHs), alkanes and alkenes. A less clearly defined group of samples is present in the centre. The DF_2^+ axis expresses mainly markers for sulphur-containing compounds. The results of a CuPyGC survey of 28 of these samples confirm this classification. Four samples were selected for more detailed analysis of individual compounds with CuPyGC/MS (Table 1).

3.2. Identification of individual compounds with CuPyGC/MS

The CuPyGC/MS data of four organic residues from the inside and outside of different pots are shown in Figure 4. Many of the peaks in the CuPyGC data could be identified from their mass spectra (Table 2, see end of this Chapter) and many of these compounds could be assigned to bioorganic origins. However, a number of peaks have remained unidentified as can be seen in Table 2 where they are listed together with their characteristic mass peaks. In this section the main compound classes will be discussed.

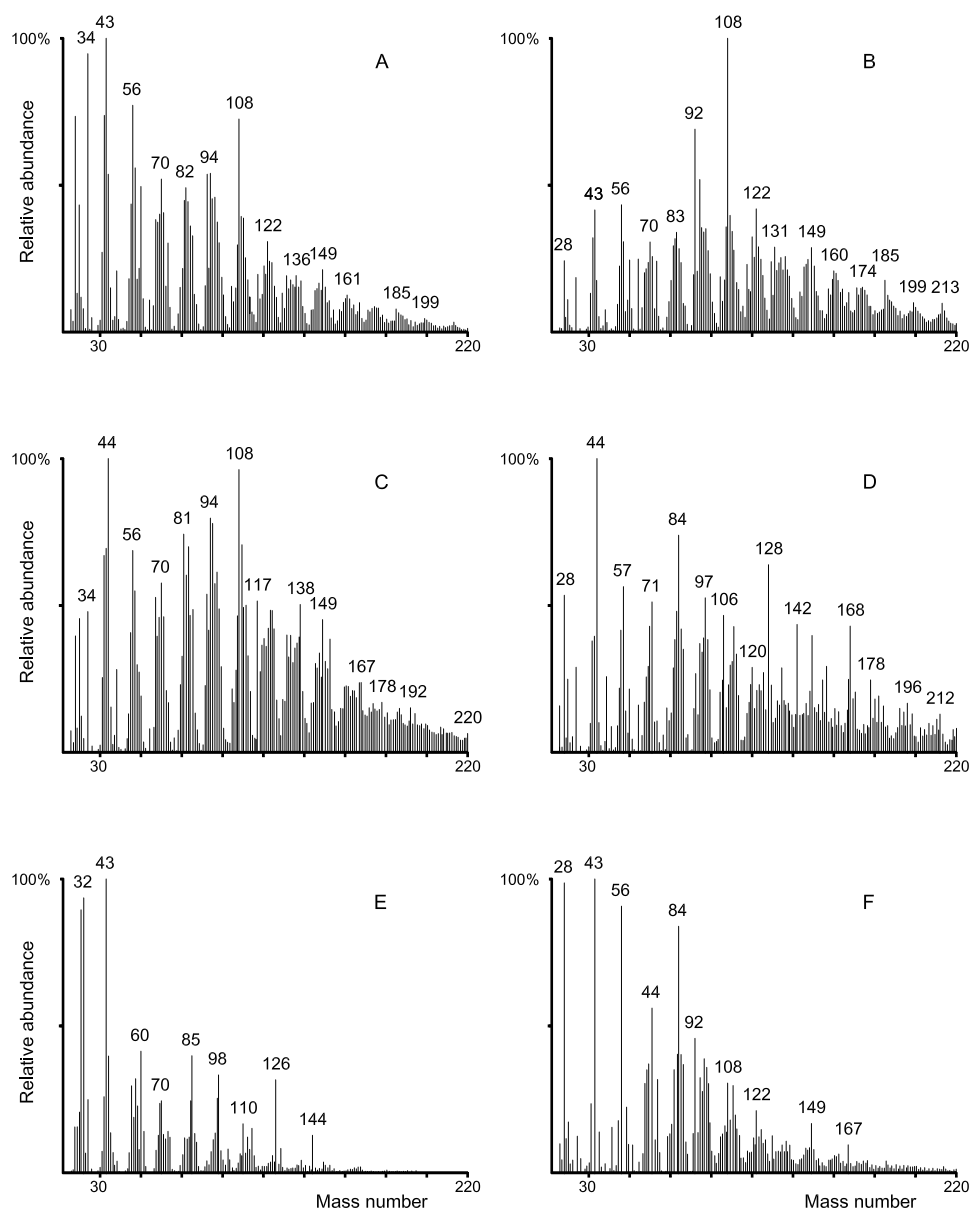


Figure 3: Mass spectra obtained with pyrolysis mass spectrometry (CuPyMS) under 17 eV (EI). Spectra of archaeological residues and experimentally charred modern food mixtures. A = charred residue 34-0-30; B = charred residue 14-6-4.4; C = cream coloured residue 35-7-28; D = black soot residue 18-3-2.b from the exterior vessel wall; E = Experiment 1 - flour, albumin and plant margarine heated for 5 min at 100 °C; F = Experiment 2 – flour, albumin and plant margarine heated for 125 min at 250 °C.

Residues on the outside of the pottery

The CuPyGC/MS results of a black residue (sample 18-3-2.b, Fig. 4d) occurring on the outside of a small pot, show many polynuclear aromatic hydrocarbons like naphthalenes, phenanthrenes and their methylated isomers. Since these compounds were also found in the pyrolysates of low temperature pyrolysis (358 °C), they are not pyrolysis products but the result of desorption of volatile compounds from the sample. Since these PAHs 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 PAHs only occur in residues situated on the outside of the pottery. The alkane/alkene pattern detected in this sample will be explained later.

Residues on the inside of the pottery

Three residues situated on the inside of pots were sampled (samples 34-0-30, 35-7-28 and 14-6-4.4) and the CuPyGC/MS data (Fig. 4a, b, and c) show three compound classes of bioorganic significance: protein remains, remains of polysaccharides, remains of lipids (free fatty acids and fatty amides as well as an alkane/alkene pattern) which are discussed below.

Protein remains of vegetable or animal origin: The CuPyGC/MS results of samples 34-0-30, 35-7-28 and 14-6-4.4 show some specific fragments indicative of charred proteins. Pyrrole, indole, methylindole, toluene, phenol, and cresol detected with CuPyGC/MS are interpreted as 'protein' indicators because these compounds are commonly found in pyrolysates of proteins. As such they are indicative of hydroxyproline, tryptophan, phenylalanine and tyrosine (Meuzelaar *et al.* 1982, 109). Some of the pyrolysis products indicative of adjacent pairs of aliphatic amino acids in intact proteins described by Boon and De Leeuw (1987) and Smith and co-workers (1988) and of the 3,6-piperazinediones described by Munson and Fetterolf (1987) as pyrolysis products of proteins, could be detected in one of our samples. So far it can be tentatively suggested that, although the charring of the food has probably caused severe denaturation of the original peptide chain, the individual amino acid characteristics appear to be preserved in the 'char'. The details of this preservation process are not clear at this time. It is suggested here that a radical reaction (Fig. 6) causes the specific amino acid side chains to be linked chemically to (or to get 'embedded' in) the forming char. About two thousand years later, flash pyrolysis releases these characteristics again. Protein markers occur mostly in samples in combination with free fatty acids and polysaccharide markers. In samples 35-7-28 (Fig. 3c and 4c), however, they occur only in combination with inorganic compounds i.e. carbonates (evidenced by m/z 44 and 28 from CO₂ in the CuPyMS spectrum).

Remains of polysaccharides: In the past it has been stated by archaeological chemists (Rottländer & Schlichtherle 1983) that polysaccharides are unlikely to survive charring because their natural structure is destroyed at temperatures around 190 °C. However, the work of other researchers (Julien *et al.* 1991; Pastorova *et al.* 1993), has shown that low temperature chars of cellulose still retain 'sugar' characteristics. Sugar markers (i.e. methylfuran and dimethylfuran) were detected in charred samples 34-0-30 and 14-6-4.4 but are absent from non-charred sample 35-7-28. These markers are rather unspecific and cannot give any indication of the original type of polysaccharides. The possibility of an archaeological origin is shown by the fact that charring experiments with modern food also render these markers in Curie-point pyrolysates. Apparently

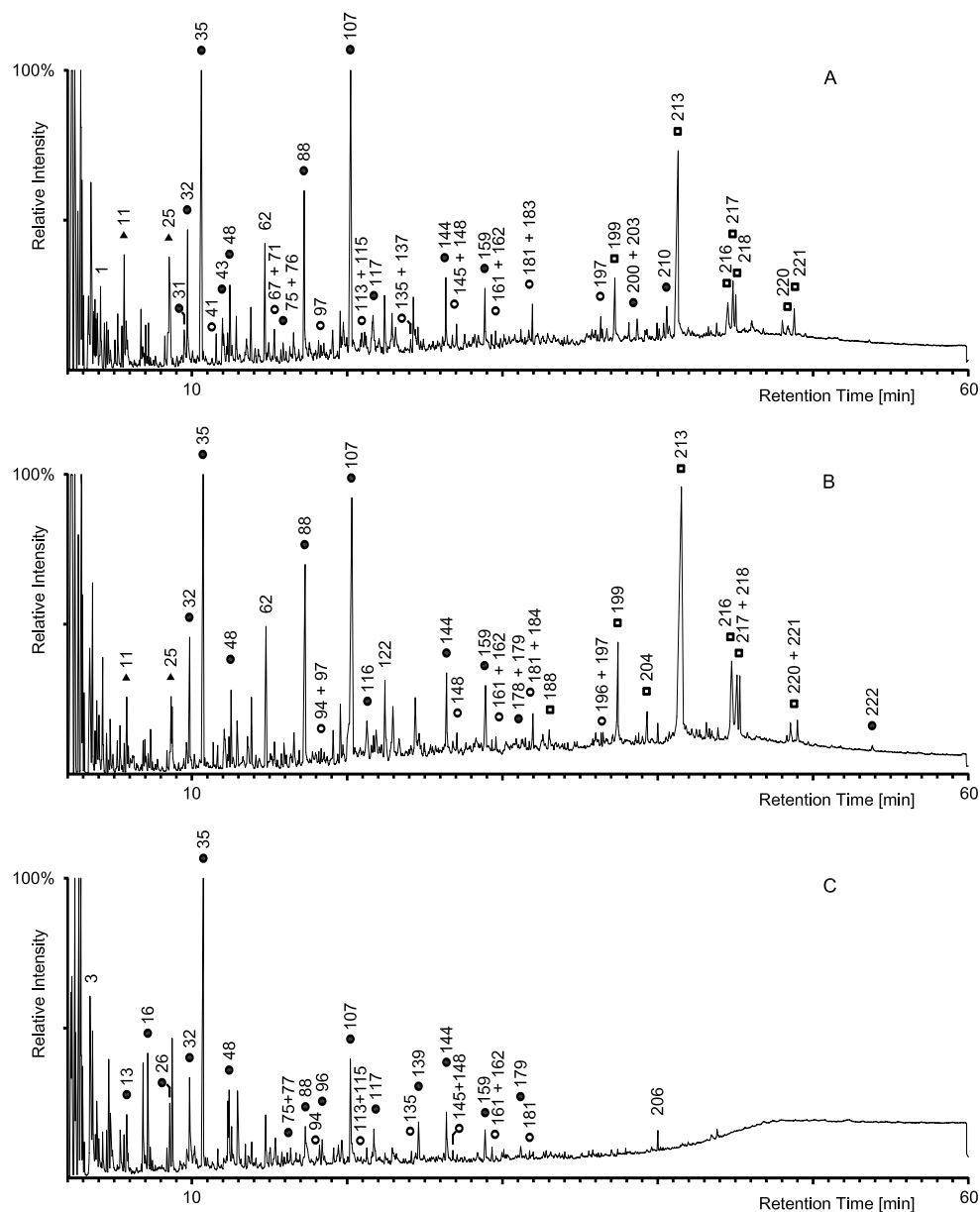
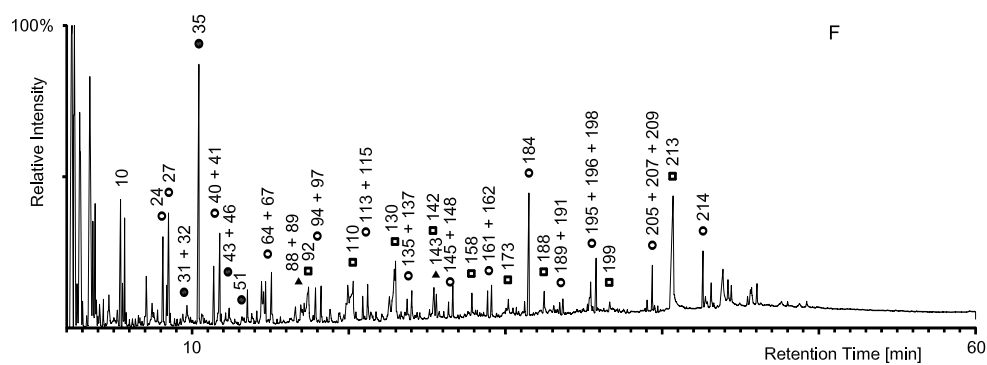
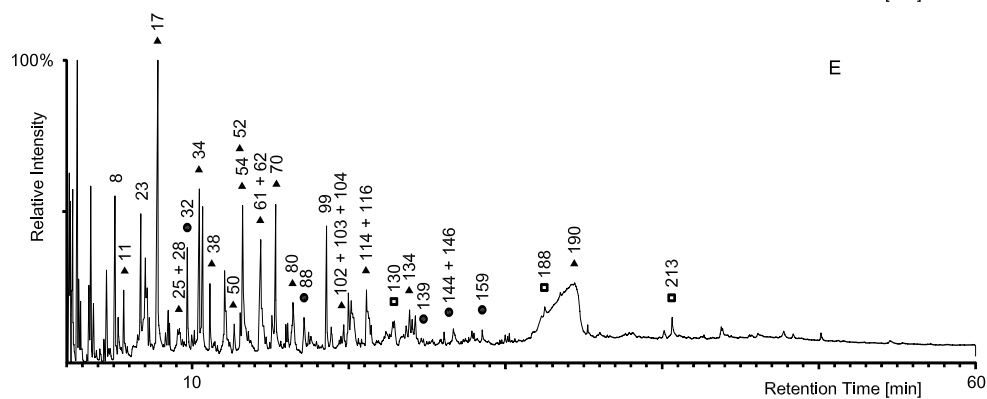
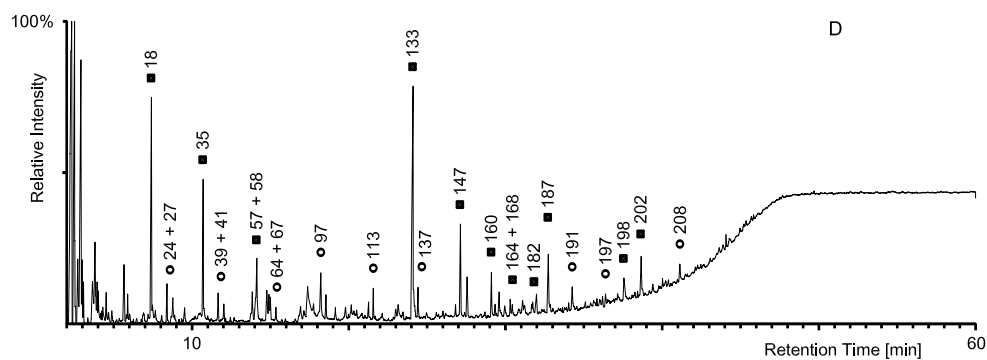


Figure 4 (see also facing page): CuPyGC/MS results of residues and experimentally heated modern foods. Identified peaks are indicated by number (Table 2). Characteristic compound classes are explained in the legend on facing page. A = Charred residue 34-0-30 contains free fatty acids and markers for proteins and polysaccharides; B = Charred residue 14-6-4.4 contains free fatty acids and markers for proteins and polysaccharides; C = Cream coloured residue 35-7-28 mainly shows protein markers; D = Black soot residue 18-3-2.b contains mainly PAHs from smoke condensates; E = Flour, albumin and plant margarine heated for 5 min at 100 °C; F: heated for 125 min at 250 °C.



Characteristic compounds

- Proteins markers
- ▲ Polysaccharide marker
- Fatty acids
- Alkanes / Alkenes
- Polynuclear Aromatic Hydrocarbons

some polysaccharide characteristics remain preserved in low temperature chars (possibly in the form of dehydrated oligosaccharides and melanoidins). Increasing the temperature during charring will reduce the recognisability of the remaining products. No simple assumptions can be made since it is not clear whether the polysaccharide markers originate from charred foods or from oligosaccharides that have impregnated the residues from the surrounding soil. Theoretically, water-soluble saccharides derived from plants or bacterial cell walls could have impregnated the archaeological residue material. Pyrolysis studies of peat samples from the Assendelver Polders (a peat comparable in age) showed a broad range of sugars from polysaccharides (Moers 1989, 89). However, the majority of the sugars in these peats are derived from the remains of vascular plants and occur in the form of water insoluble biopolymers. Such biopolymers would not have impregnated the archaeological residues. The absence of sugar markers from non-charred sample 35-7-28 and soot residue 18-3-2.b does suggest an origin in the charring of polysaccharides originating from the vessel content.

Remains of lipids - Free fatty acids and fatty amides: A number of straight chain saturated fatty acids (C11, C12, C14, C15, C16, C17 and C18:0), one mono-unsaturated fatty acid (C18:1) and three fatty amides (C16:0, C18:0 and C18:1) were detected in samples 34-0-30 and 14-6-4.4. Since free fatty acids also occur in the CuPyGC analyses of low temperature pyrolysis (358 °C), it is clear that these compounds evaporate from the sample. 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 (Fig. 4a and b). The presence of free fatty acids in residues on pottery and in the ceramic of the ancient pottery itself, has been proven numerous times by other researchers who isolated the fatty acids (and sometimes salts of fatty acids) using various extraction methods (see for references also Rottländer & Schlichtherle 1980; Evershed *et al.* 1992). Though the presence of free fatty acid is commonly accepted to be the result of hydrolysis of vegetable or animal fats after deposition (Den Dooren de Jong 1961), the distribution found in some of the organic residues in the pots may have been caused by additional biodegradation processes obscuring the original lipid signature. The identification of original foodstuffs based on the relative distribution of intact lipids (e.i. mono-, di- or triglycerols and sterols) in archaeological samples is a more promising process. Although neither mono, di- nor triglycerides could be detected with the pyrolysis techniques utilised in this study, their presence was confirmed through lipid extraction and presented in Chapter 5 (Oudemans & Boon in press). The fatty amides are most likely produced by heating fatty acids with amines to a temperature of 200 °C (Davidek *et al.* 1990, 183). It is not clear whether this formation happened during the preparation of food in Roman times or during the pyrolysis phase of the analysis.

Remains of lipids - Alkene/alkane pattern: In pyrolysates of many samples, a regular pattern of n-alk-1-enes and n-alkanes ranging from C6 to C18 was detected. These homologous series of alkenes and alkanes occur in residues on the interior of vessels (e.g. samples 34-0-30, 35-7-28 and 14-6-4.4), the exterior of vessels (e.g. sample 18-3-2.b), in experimental chars, and in samples of the ceramic material itself. In the residue-samples they occur in combination with other compounds like fatty acids or protein markers (Table 3). These straight chain alkane/alkene patterns have been reported before in the pyrolysates of the cuticles of modern and fossil plants (Nip *et al.* 1986), of the rootlets of Ericaceae and ericaceae peat (van Smeerdijk & Boon 1987), and in pyrolysates of coals from an early phase of coal diagenesis (Tromp *et al.*

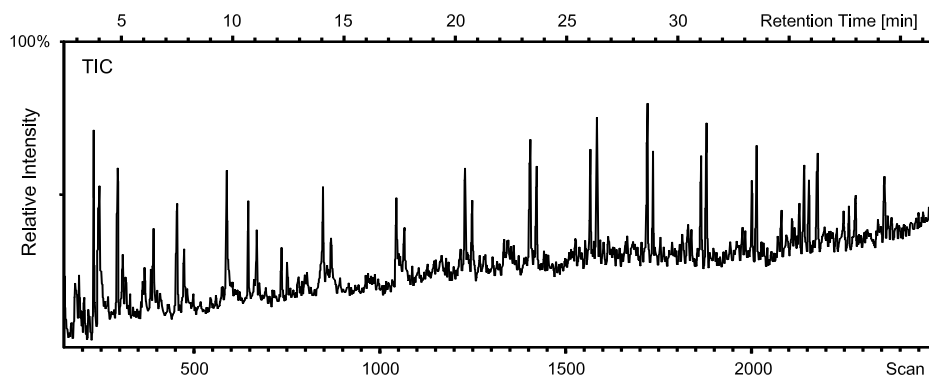


Figure 5: Alkane/Alkene pattern in CyPyGC/MS profile of a sample of archaeological ceramic material.
The total ion current of the ceramic material of the archaeological shard 14-6-4.4 shows a clear pattern of alkanes and alkenes ranging from C6 to C18.

1988, 241). The pattern was interpreted as the pyrolysate of a highly aliphatic biopolymer thought to be present in the cuticles of plants and claimed to be very resistant to biodegradation. The same pattern can be found when polyethylene is pyrolysed. The most important difference with the pattern observed in our samples is the chain length distribution. The occurrence of this pattern in the pyrolysates of prehistoric (food) residues may be caused by pyrolysis of an aliphatic network created by radical polymerisation in the residues and in the wall of the pot under high temperature conditions during cooking. This aliphatic network is likely to be formed from food components. The mechanism of formation of alkene/alkane patterns during pyrolysis is not entirely clear, but Hartgers and co-workers (1995) have suggested a mechanism which explains the formation of the alkenes and alkanes as product of H-radical transfer with primary and secondary alkyl radicals that were created during pyrolysis of silicon-bound hydrocarbons. It is plausible that a similar mechanism is at work during pyrolysis of the aliphatic material described above. According to this model, pyrolysis of long chain aliphatic components that are bound to some larger structure will result in homologous series of alkenes and alkanes leading up to the C number of the longest chain minus one. Shedrinski (1991) reported on the formation of a similar alkene/alkane patterns from PyMS data of salts of fatty acids. This origin might play a role in some of the prehistoric residue samples. However, charring experiments (e.g. sample 1 and 2) show the same alkene/alkane pattern after pyrolysis (Fig. 4f), and as such support the possible origin of the aliphatic compounds. The presence of fats seems a pre-requisite since charring experiments with only water, flour and albumin do not give this alkene/alkane pattern.

Ceramic material

The ceramic from several shards was analysed by CuPyGC and the results show a pattern of straight chain n-alk-1-enes and n-alkanes ranging up to C18. In the ceramic samples these compounds form the majority of the organic fraction (Fig. 5 shows the TIC of the

CuPyGC/MS analysis of a ceramic sample from vessel 14-6-4.4). It is possible that the higher temperature reached in the wall of the pot during cooking and charring lead to the formation of aliphatic network polymers from lipids that were absorbed in the shard when the pot was in use. This seems to be the only organic material present in any significant amount.

Heating experiments

Experimental heating and charring of modern foods was performed (e.g. Experiments 1 and 2) to obtain more information about the changes in composition of different materials before and after charring (Fig. 4e and 4f). The results obtained with CuPyMS and CuPyGC/MS confirmed the fact that many characteristics of polysaccharides and proteins and fatty acids can still be found in the experimentally carbonised materials. The results from these heating experiments show many of the components that are also detected in the archaeological material.

Soil samples

Dried peat samples from the site have been analysed and show the type of pattern (polysaccharide markers, lignin markers and high molecular weight lipid markers) which are typical for peat samples in the west of the Netherlands (van Smeerdijk & Boon 1987). Though the total pyrolysis product profile from the peat samples is very different from those of the archaeological samples, it cannot be excluded that the surrounding soil matrix partly determines the remaining chemical composition of the residues. Contamination with soil particles and ground water or specific degradation processes could have an effect on the chemistry of the residues. It is therefore useful to determine the relationship between the chemical composition of the analysed residues and the type of sediment around the shards. As shown in Chapter 2, a study of this relationship by CuPyMS failed to show a direct correlation (Oudemans & Boon 1996). Also, the large difference in composition between soil, charred residue, and ceramic material suggests that exchange of soil components is very limited in the archaeological site at Uitgeest – Groot Dorregeest.

Table 3: Presence of different compound classes in reported samples.

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 14-6-4.4	-	-	-	-	+	-		Sand

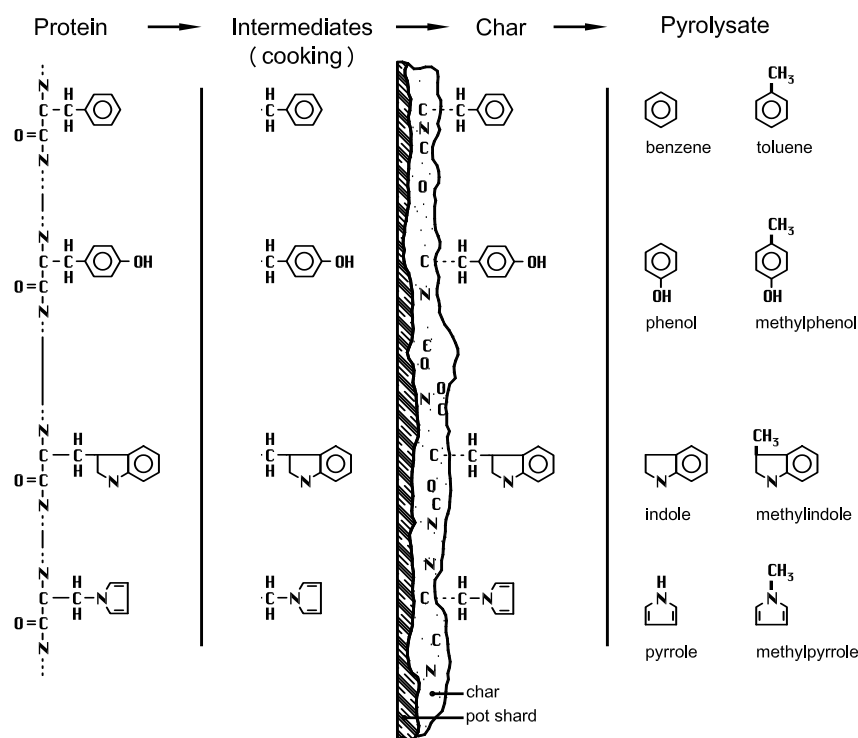


Figure 6: Suggested mechanism for the preservation of protein characteristics in charred residues.

Contaminating materials

In pyrolysates of many samples, markers for plasticisers (m/z 149, 167) were found (Fig. c and 3d). These markers probably originate from the plastic bags in which the pottery was kept for over a year prior to analysis.

3.3. Chemical data as evidence for vessel use in prehistoric times

The CuPyMS and CuPyGC/MS results show clear differences in the chemical composition of the residues (Table 3). The chemical composition of samples scraped from vessels cannot provide direct information about original use because the sample is the result of a series of formation processes that took place in time, such as processes in prehistory (cooking, mixing, storing, cleaning of pots), post-depositional processes (biodegradation, impregnation of soil or ground water, leaching), and post-excavational processes (excavation and handling by archaeologists and restoration people). Yet information about prehistoric use of the pottery

can be deduced from the chemical results after careful consideration is given to the influence of the formation processes on the chemical composition of the residues:

Handling by archaeologists

Excavation and handling of pottery can result in contamination of the samples, with for example plasticisers from packaging materials. It is also possible that other cases of contamination took place during washing, drying and handling (contamination with dust, paper from drying of the pottery on newspaper and fats from archaeologists' hands). When dealing with archaeological materials it is preferable to freeze (-20 °C) the samples within 24 hours after excavation to prevent any additional bacterial degradation or fungal growth. Unfortunately, the material reported here was not treated with such caution. Despite this drawback, the degree of detectable contamination is very limited. This is most likely due to the refractory nature of the charred materials and the encapsulation of indicative compounds in the macromolecular structure of the char. It is however, not unthinkable that working with washed shards means that some soft and/or soluble materials were removed during washing of the shards and therefore never studied.

Biodegradation

Biodegradation on and in the ground has a strong selective effect on preservation of different biological materials. Fresh foods especially are vulnerable to biological attack, as the digestibility of foods goes hand in hand with sensitivity to biodegradation. Only rarely will the remains of food preparation or 'home-chemistry' be preserved as a residue on broken pottery. Very specific circumstances like an anaerobic climate, aridity, extremely low temperatures, charring, carbonisation or the presence of preserving chemicals (like waxes, resins, tannins, acids, alkalis, metal oxides or salt) are needed to preserve foods in an archaeological context (Gilbert & Mielke 1985, 2). The residues that archaeologists find on pottery should therefore not be seen as representing all uses of pottery but rather as rare cases that have survived for a specific reason. The residues represented here are mostly the result of charring (with the exception of three light coloured residues such as sample 35-7-28). Charring is known to reduce the microbial degradation (and possibly also the impregnability for water) in such a way that many traces of bioorganic compounds are still present. The chemical composition of the residues therefore clearly represents processes that took place in prehistoric times.

Processes in prehistory

Many processes in prehistory will have had an obscuring effect on the evidence of the last usage. For instance the use of a vessel for different functions could complicate the interpretation of the results. Visual inspection of the chars under the scanning electron microscope showed the absence of layers in the residues (Oudemans unpublished results). For the indigenous settlement in Uitgeest-Groot Dorregeest one can thus assume that people did not cook or prepare food in 'dirty' pots. The residues are considered to represent the last use of the pot. The cooking temperature also has its effect on the chemical composition of the residue. Although the charring has a positive effect on the preservation of the residue, the denaturation

has a negative effect on the possibilities for identification of the original material. The heating temperature and heating time play a role in the chemical composition of the final residue (see also Chapter 6). Mixing of materials is something one has to take for granted in most food preparation and 'home-chemistry'. The position of the residue on the pot also determines the chemical composition of the residue that one might find. Black residues on the outside of vessels have distinctively different chemical composition from those on the inside. For the determination of the original use of a vessel, it is important to show that differences in chemical composition are correlated to archaeological variables such as pot morphology (Oudemans & Boon 1996). More work on the identification of the bioorganic origin of the solid surface residues is presented in Chapters 4, 5 and 6.

4. Conclusions

Pyrolysis mass spectrometric techniques are useful for the chemical characterisation of organic residues on archaeological pottery. The use of CuPyMS, CuPyGC and CuPyGC/MS has resulted in the detection of many bioorganic moieties in the charred and non-charred residues including characteristic markers for proteins and polysaccharides and other compounds like fatty acids, polynuclear aromatic hydrocarbons and aliphatic polymers.

The existence of clear differences in chemical composition between the residues has been demonstrated. The chemical composition of the residues depends on whether residues are situated on the exterior or the interior vessel wall, and on whether they are charred or not.

The chemical characteristics of peat samples from the surrounding matrix are in no way similar to the results from the residues. No indications were found to assume that the chemical difference between residues is the result of post-depositional influences. It is therefore inferred that the chemical composition of these solid organic residues is a reflection of the original use in the indigenous settlement in Uitgeest-Groot Dorregeest.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr.	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26 char	31 beige	8 char	11 soot	
1	70	3'34	methyl-2-butene	-	-	x	x	-	-	
2	66	3'43	cyclopentadiene + CS ₂	x	x	x	x	-	x	
3	72	3'55	2-methylpropanal	x	-	-	x	-	-	Pro
4	68	3'60	cyclopentene	-	x	-	-	-	x	
5	55	4'02	cycloethane	-	-	x	x	x	-	Pro
6	86	4'19	? <i>m/z</i> 43, 41, 71, 42, 69, 84	-	-	x	-	x	-	
7	84	4'19	2-methyl-2-pentene	-	-	x	-	x	-	
8	86	4'21	2,3-butanedione	x	-	-	-	-	-	Ps
9	72	4'40	2-butanone	x	x	x	x	x	x	
10	84	4'46	hexene	-	x	x	x	-	x	
11	82	4'53	methylfuran	x	x	x	-	x	-	Ps
12	86	4'58	hexane	-	x	x	x	-	x	
13	69	5'08	2-methylcyanopropane	-	-	-	x	-	-	Pro
15	86	5'58	3-methylbutanal	x	x	x	x	-	x	Pro
16	74	6'07	propanol	x	-	-	-	-	-	Ps
17	69	6'13	cyanobutane	-	-	-	x	-	-	Pro
		6'15	2-methylbutanal	-	x	x	x	-	x	
18	78	6'20	benzene	-	x	x	x	x	x	
19	84	6'29	2-methyl-1-buten-3-one	x	-	-	-	-	-	
20	80	6'34	cyclohexadiene	-	x	-	-	-	-	
21	82	6'57	cyclohexene	-	x	-	-	-	-	
22	100	7'00	pentane-2,3-dione	x	-	-	-	-	-	Ps
23	44	7'01	acetaldehyde	x	x	x	-	x	-	
24	98	7'23	heptene	-	x	x	x	-	x	A
25	96	7'38	2,5-dimethylfuran	x	x	x	-	x	-	Ps
26	83	7'40	2-methylcyanobutane	-	-	-	x	-	-	pro
27	100	7'43	heptane	-	x	x	-	-	x	A
28	96	7'45	? <i>m/z</i> 43, 41, 39, 27, 68	x	-	-	x	-	-	
29	80	8'14	pyrimidine or pyridazine	x	-	-	-	-	-	Pro
30		8'30	2-methylpentanal	-	-	x	-	-	x	
31	79	8'50	pyridine	-	x	x	-	-	-	Pro
32	67	9'00	(1H)-pyrrole	x	x	-	x	x	-	Pro
33	84	9'12	? <i>m/z</i> 43, 84, 55, 27, 58	x	-	-	-	-	-	
34	102	9'31	methylpyruvate	x	-	-	-	-	-	Ps
35	92	9'37	toluene	x	x	x	x	x	x	
36	84	9'54	pentenone	-	x	-	-	-	-	
37	98	9'57	methylthiophene/pentenone	-	-	-	x	-	-	
38	84	10'01	(2H)-furan-3-one	x	-	-	-	-	-	Ps
39	112	10'36	octene	-	x	x	x	-	x	A
		10'36	trialkylfuran ?	-	-	x	-	-	-	
40	110	10'37	dimethylhexadiene	-	-	x	-	-	-	A

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1

? *m/z* = unknown compound with characteristic main peaks (given according to relative abundance)

Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr.	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26 char	31 beige	8 char	11 soot	
41	114	11'01	octane	-	x	x	x	-	x	A
42	112	11'07	? <i>m/z</i> 112, 83, 70, 55	-	x	-	-	-	-	
43	93	11'07	2-methylpyridine	-	x	x	-	-	-	Pro
44		11'15	? <i>m/z</i> 82, 39, 94, 54, 53	x	-	-	-	-	-	
45		11'20	? <i>m/z</i> 57, 41, 29, 55, 39	-	-	-	x	-	-	A
46	97	11'23	? <i>m/z</i> 112,83,70,55	-	x	-	x	-	-	
47	96	11'24	2-furaldehyde	x	-	-	-	-	-	Ps
48	81	11'37	2 of 3-methyl-(1H)pyrrole	-	-	x	x	x	-	Pro
49		11'38	benzylalcohol + ? <i>m/z</i> 110, 81, 67, 54	-	x	-	-	-	-	
50	72	11'50	methyltetrahydrofuran-3-one	x	-	-	-	-	-	Ps
51	81	12'00	2 of 3-methylpyrrole	-	x	x	x	x	-	Pro
52	116	12'14	acetyloxypropane-2-one	x	-	-	-	-	-	Ps
53		12'22	? <i>m/z</i> 43, 70, 55, 57, 56	-	-	-	x	-	-	
54	98	12'24	2-hydroxymethylfuran	x	-	-	-	-	-	Ps
55		12'36	? <i>m/z</i> 60, 98, 43, 41, 97	x	-	-	-	-	-	
56	94	12'39	? <i>m/z</i> 60, 41, 54, 43, 94	-	x	-	-	-	-	
57	106	12'47	ethyl-benzene	x	x	x	x	x	x	
58	106	12'58	dimethylbenzene (p or m-xylene)	-	-	-	-	-	x	
59	106	13'04	dimethylbenzene (p or m-xylene)	-	x	x	x	-	x	
60	114	13'27	2-heptanone	-	x	-	-	-	-	
61	95	13'32	(5H)-furan-2-one	x	-	-	-	-	-	Ps
62	104	13'43	vinylbenzene	x	x	x	x	x	x	
63	106	13'54	dimethylbenzene (o-xylene)	-	x	x	-	-	x	
64	126	13'59	1-nonene	-	x	-	x	-	x	A
65	110	14'02	2-acetylfuran	x	-	-	-	-	-	Ps
66	102	14'08	pentanoic acid C5:0	-	x	-	-	-	-	FA
67	128	14'23	nonane	-	x	x	-	-	x	A
		14'24	dimethylpyrrole	-	-	x	-	-	-	
68	98	14'25	2,5-dihydro-5-methylfuran (β-ang. lact.)	x	-	-	-	-	-	Ps
69		14'29	? <i>m/z</i> 80, 95, 94, 53, 107	-	-	-	x	-	-	
70	98	14'32	2,3-dihydro-5-methylfuran (α-ang. lact.)	x	-	-	-	-	-	Ps
71		14'35	alkylketone + dimethylpyrrole	-	-	x	-	-	-	
72		14'38	? <i>m/z</i> 94, 95, 83, 109, 28	-	-	-	x	-	-	
73	126	14'45	? <i>m/z</i> 126, 97, 56, 55	-	x	-	-	-	-	Ps?
74	124	14'49	? <i>m/z</i> 54, 60, 41, 95, 124	-	x	-	-	-	-	
75	95	14'52	dimethylpyrrole	-	-	x	x	-	-	Pro
76	107	14'56	2,4-dimethylpyridine	-	-	x	-	-	-	Pro
77	95	15'12	1H-ethylpyrrole	-	-	-	x	-	-	Pro
78	130	15'19	4-hydroxymethyltetrahydropyran-3-one	x	-	-	-	-	-	Ps
79	130	15'24	2,3-dihydroxyhex-1-en-4-one	x	-	-	-	-	-	Ps
80	110	15'45	5-methyl-2-furaldehyde	x	-	-	-	-	-	Ps

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1
 ? *m/z* = unknown compound with characteristic main peaks (given according to relative abundance)
 Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr.	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26 char	31 beige	8 char	11 soot	
81	120	15'54	propylbenzene	-	x	x	-	-	x	
82		16'00	? <i>m/z</i> 82,41, 28, 55, 57, 83	-	x	-	-	-	-	
83	120	16'07	alkyl(C3)benzene	-	-	-	-	-	x	
84	103	16'21	cyanobenzene	-	x	-	x	-	-	
85	103	16'23	pyridine derivative ?	-	-	-	x	-	-	
86	113	16'27	? <i>m/z</i> 85, 57, 41, 100	-	x	-	-	-	-	
87	144	16'28	? <i>m/z</i> 111, 101, 126, 43, 43, 144	x	-	-	-	-	-	
88	94	16'38	phenol	x	x	x	x	x	-	
89		16'45	C3-benzene 4-hydro dihydropyranone	-	x	-	-	-	-	Ps
90		16'50	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one	x	-	-	-	-	-	Ps
91		17'04	? <i>m/z</i> 94, 93, 73, 109	-	-	x	-	-	-	
92	116	17'05	hexanoic acid C6:0	-	x	-	-	-	-	FA
93	120	17'14	ethylmethylbenzene	-	-	x	-	x	x	
94	140	17'15	1-decene	-	x	-	x	x	-	A
95		17'28	108, 107, 57, 41, 70, 94, 121	-	-	x	-	-	-	
96	109	17'30	2,3,5-trimethylpyrrole	-	-	-	x	-	-	Pro
97	142	17'37	decane	-	x	x	-	x	x	A
98		17'44	? <i>m/z</i> 57, 41, 29	-	-	x	-	-	-	
99	112	17'54	2-hydroxy-3-methylcyclopentene-1-one	x	-	-	-	-	-	
100	118	18'11	alkyl(C3)benzene	-	-	-	-	x	-	
101	120	18'18	alkyl(C3)benzene	x	-	-	-	-	x	Pro
102	108	18'58	methylphenol (o-cresol)	x	-	x	x	x	-	
103	128	19'07	? <i>m/z</i> 128, 43, 57, 85	x	-	-	-	-	-	Ps
104	134	19'12	butylbenzene	-	-	-	-	-	x	
105	120	19'17	? <i>m/z</i> 81, 82, 39, 54, 97	x	-	-	-	-	-	
106	134	10'32	methylpropylbenzene	-	-	-	-	-	x	
107	108	19'35	methylfenol (p/m-cresol)	x	x	x	x	x	-	
108		19'46	? <i>m/z</i> 68, 107, 108, 110, 69	-	x	-	-	-	-	
109	134	19'55	alkyl(C4)benzene	-	-	-	-	-	x	
110	130	19'59	heptanoic acid C7:0	-	x	-	-	-	-	FA
111	134	20'05	alkyl(C4)benzene	-	-	-	-	-	x	
112	154	20'07	C11-alkene	-	-	-	x	-	-	A
113	154	20'21	undecene	-	x	x	x	-	x	A
114	126	20'36	3-dihydro-2-methyl-(4H)-pyran-4-one	x	-	-	-	-	-	Ps
115	156	20'41	undecane	-	x	x	x	-	x	A
116		20'46	? <i>m/z</i> 99, 59, 56, 28, 72	x	-	-	-	x	-	
117	117	20'52	benzylcyanide	x	x	x	x	-	-	Pro
118		21'01	? <i>m/z</i> 200, 154, 69, 57	-	x	-	-	-	-	
119	134	21'06	alkyl(C5)benzene	-	-	-	-	-	x	
120		21'08	? <i>m/z</i> 42, 70, 113, 41	-	-	x	-	x	-	

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1
 ? *m/z* = unknown compound with characteristic main peaks (given according to relative abundance)
 Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr.	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26	31	8	11	
						char	beige	char	soot	
121	152	21'13	? <i>m/z</i> 132, 117, 82, 54	-	x	-	-	-	-	A
122	122	21'42	dimethylfenol	-	x	x	-	x	-	
123		21'51	? <i>m/z</i> 44, 43, 144, 101, 125	x	-	-	-	-	-	
124		22'01	? <i>m/z</i> 70, 113, 43, 42, 130, 115	-	-	x	-	-	-	
125		22'12	? <i>m/z</i> 43, 41, 69, 107, 70, 57, 85	x	-	-	-	-	-	
126	148	22'13	pentylbenzene	-	x	-	-	-	-	
127	122	22'14	ethylphenol	-	-	x	x	x	-	
128		22'26	? <i>m/z</i> 113, 43, 41, 50, 100, 85, 69	-	x	-	-	-	-	
129	148	22'32	1-methyl-4-isobutylbenzene	-	-	-	-	-	x	
130	144	22'37	octanoic acid C8:0	x	x	-	-	-	-	FA
131	156	22'52	decanone	-	x	-	-	-	-	
132		22'54	? <i>m/z</i> 41, 98, 99, 28, 58, 70	x	-	-	-	-	-	
133	133	23'04	naphthalene	-	-	-	-	-	x	PAH
134	144	23'13	1,4:3,6-anhydro- α -D-glucopyranose	x	-	-	-	-	-	Ps
135	168	23'15	1-dodecene	-	x	x	x	-	-	A
136	136	23'22	? <i>m/z</i> 73, 71, 43, 97, 44, 29, 55, 57	x	-	-	-	-	-	
	120	23'22	phenylacetaldehyde	-	-	x	-	-	-	Pro
137	170	23'33	n-dodecane	-	x	x	-	-	x	A
138		23'36	? <i>m/z</i> 71, 69, 73, 97, 56, 44, 41, 29	x	-	-	-	-	-	
139	131	23'45	phenyl cyanopropane	x	-	-	x	-	-	Pro
140	146	23'54	dimethylbenzofuran	-	x	-	-	-	-	
141	134	23'57	aniline derivative	-	-	-	x	-	-	
142	158	25'12	nonanoic acid C9:0	-	x	-	-	-	-	FA
143	162	25'19	butylbenzaldehyde	-	x	-	-	-	-	Ps
144	117	25'47	indole	x	-	x	x	x	-	Pro
145	182	25'59	1-tridecene	-	x	x	x	-	-	A
146		26'02	1,4-dideoxy-D-glycero-hex-1-enopyrano-3-ulose	x	-	-	-	-	-	Ps
147	142	26'13	2-methylnaphthalene	x	-	-	-	-	-	PAH
148	184	26'17	tridecane	-	x	x	-	x	x	A
149	150	26'18	vinylmethoxyphenol	x	-	-	-	-	-	
150	210	26'33	5(2-dimethyl-ethyl)pyrrolidino-3,6 piperazinedione	-	-	-	x	-	-	Pro
151	142	26'40	1-methylnaphthalene	-	x	-	-	-	x	PAH
152	210	27'00	5(1-methyl-propyl)pyrrolidino-3,6 piperazinedione	-	-	-	x	-	-	Pro
153		27'05	unknown	x	-	-	-	-	-	
154	160	27'06	trimethylbenzimidazol ?	-	-	-	-	x	-	
155	134	27'07	alkyl(C5)benzene	-	-	-	-	x	-	
156		27'18	? <i>m/z</i> 55, 98, 69, 60, 138, 153, 195	x	-	-	-	-	-	Pro
157		27'40	? <i>m/z</i> 55, 98, 69, 60, 138, 153, 195	x	-	-	-	-	-	Pro

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1
 ? *m/z* = unknown compound with characteristic main peaks (given according to relative abundance)
 Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26 char	31 beige	8 char	11 soot	
158	172	27'43	decanoic acid C10:0	-	x	-	-	-	-	FA
159	131	28'10	2-methylindole	x	-	x	x	x	-	Pro
160	154	28'15	vinyl-naphthalene	-	-	-	-	-	x	PAH
161	196	28'33	tetradecene	-	x	x	x	x	-	A
162	198	28'48	tetradecane	-	x	x	x	x	x	A
163	156	28'50	ethylnaphthalene	-	-	-	-	-	-	PAH
164	156	29'10	1,3-dimethylnaphthalene	-	-	-	-	-	x	PAH
165		29'10	? m/z 127, 57, 43, 147, 116	-	x	-	-	-	-	
166		29'19	? m/z 139, 43, 41, 69, 81, 99	x	-	-	-	-	-	
167	209	29'28	? m/z 138, 55, 69, 98, 166, 180, 209	x	-	-	-	-	-	Pro
168	156	29'31	dimethylnaphthalene	-	-	-	-	-	x	PAH
169	156	29'38	dimethylnaphthalene	-	-	-	-	-	x	PAH
170	147	29'54	(1H)-isoindole-1,3-(2H)-dione	x	-	-	-	-	-	Pro?
171	260	29'56	? m/z 55, 124, 98, 69, 166	x	-	-	-	-	-	Pro
172	156	30'01	2,6-dimethylnaphthalene	-	-	-	-	-	x	PAH
173	186	30'03	undecanoic acid C11:0	-	x	x	-	-	-	FA
174		30'12	? m/z 82, 67, 83, 81, 57, 95	-	x	-	-	x	-	
175	154	30'14	biphenyl	-	-	-	-	-	x	PAH
176	190	30'23	octylbenzene	-	x	-	-	-	x	
177	147	30'30	2,3-dihydro-4-methylindole	-	-	-	-	x	-	Pro
178	145	30'45	dimethylindole	-	-	-	x	x	-	Pro
179	147	30'46	1,3-dihydro, 3-methylindole	-	-	-	x	x	-	Pro
180	168	30'50	methylbiphenyl	-	-	-	-	-	x	PAH
181	210	30'57	pentadecene	-	x	x	x	x	x	A
182	168	31'05	methylbiphenyl	-	-	-	-	-	x	PAH
183	154	31'05	? aromatic hydrocarbon	-	-	-	-	-	x	PAH
184	212	31'13	pentadecane	-	x	x	-	x	x	A
185	203	31'13	? m/z 129, 116, 130, 101, 103	x	-	-	-	-	-	
186	210	31'33	? m/z 210, 144, 141, 83, 55	-	x	-	-	-	-	A
187	168	31'50	dibenzofuran	-	-	-	-	-	x	PAH
188	224	32'16	hexadecene	-	x	-	-	-	-	A
189	200	32'21	dodecanoic acid C12:0	x	x	-	-	x	-	FA
190	162	33'17	1,6-anhydroglucose	x	-	-	-	-	-	Ps
191	226	33'27	hexadecane	-	x	x	-	-	x	A
192	236	35'01	heptadecadiene	-	-	-	x	-	-	A
193	238	35'02	heptadecene	-	x	-	x	-	-	A
194	222	35'05	? m/z 138, 123, 157, 57, 165, 180	x	-	-	-	-	-	
195	238	35'11	heptadecene	-	x	-	-	-	-	A
196	238	35'16	heptadecene	-	x	-	-	x	-	A
197	240	35'36	heptadecane	-	x	x	-	x	x	A
198	180	36'42	(9H)-fluoren-9-one	-	-	-	-	-	x	PAH

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1

? m/z = unknown compound with characteristic main peaks (given according to relative abundance)

Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Table 2 - Identified pyrolysis products in CyPyGC/MS results

Nr.	M ⁺	RT	Name	Samples						Origin
				Exp 1	Exp 2	26 char	31 beige	8 char	11 soot	
199	228	36'39	tetradecanoic acid C14:0	-	x	x	-	x	-	FA
200	196	37'31	5(1-methyl-ethyl)pyrrolidino-3,6 piperazinedione (cis) derivative	-	-	x	-	-	-	Pro
201	254	37'38	alkane	-	x	-	-	-	x	A
202	178	37'43	phenanthrene	-	-	-	-	-	x	PAH
203	196	38'08	5(1-methyl-ethyl)pyrrolidino-3,6 piperazinedione (trans) derivative	-	-	x	-	-	-	Pro
204	242	38'40	pentadecanoic acid C15:0	-	-	-	-	x	-	FA
205	254	38'55	alkyl ketone ?	-	x	-	-	-	-	A
206	237	39'09	? <i>m/z</i> 97, 43, 110, 57, 42,124	-	x	x	x	x	x	
208	254	39'24	heptadecan-2-one	-	x	-	-	-	-	
209	268	39'37	nonadecane	-	x	-	-	-	-	A
210	194	39'48	5(1-methyl-ethyl)pyrrolidino-3,6 piperazinedione (cis) derivative	-	-	x	-	-	-	Pro
211	194	40'03	5(1-methyl-ethyl)pyrrolidino-3,6 piperazinedione (trans) derivative	-	-	x	-	-	-	Pro
212	149	40'13	phthalate	-	-	x	-	-	-	Cont
213	256	42'14	hexadecanoic acid C16:0	-	x	x	-	x	-	FA
214		42'37	? <i>m/z</i> 122, 136, 69, 164	-	x	x	-	-	-	
215		42'58	? <i>m/z</i> 43, 57, 97, 41, 56	-	-	x	-	-	-	
216	282	43'53	octadecenoic acid C18:1	-	-	x	-	x	-	FA
217	284	44'20	octadecanoic acid C18:0	-	-	x	-	x	-	FA
218	255	44'22	hexadecanoic acid amide	-	-	x	-	x	-	
219		47'12	? <i>m/z</i> 125, 244, 91, 153, 70	-	-	x	-	-	-	
220	281	47'47	octadecenoic acetamide	-	-	x	-	x	-	
221	283	48'23	octadecanoic acetamide	-	-	x	-	x	-	
222	309	53'16	pyrrolidine derivative	-	-	-	-	x	-	Pro

M⁺ = Molecular weight of the molecular ion. RT = Relative GC retention time. Samples as in Table 1
 ? *m/z* = unknown compound with characteristic main peaks (given according to relative abundance)
 Origin of the compound: Ps = Polysaccharides; Pro = Proteins; FA = Fatty acids; PAH = Polynuclear Aromatic Hydrocarbons; A = Aliphatic hydrocarbons (alkanes/alkenes) and Cont = Contaminant.

Chapter 4

Identifying Biomolecular Origins of Solid Organic Residues using Direct Temperature - resolved Mass Spectrometry and Multivariate Analysis

Although several selective analytical techniques have been applied to the analysis of specific classes of compounds, such as extractable lipids, waxes, terpenoids and protein fragments, a non-selective analytical technique is required to characterise and categorise complete solid organic residues. In this study, Direct Temperature-resolved Mass Spectrometry (DTMS) is used for the characterisation of 34 solid residues. Sample preparation is limited to grinding very small samples (5 - 10 µg) and suspending them in 15 - 20 µl water. DTMS analysis of aliquots of this suspension (1 - 2 µl) gave information about a broad range of organic compounds, such as lipids, polynuclear aromatic hydrocarbons, markers for residual proteins and polysaccharides, and for newly formed complex condensed polymers. Multivariate analysis of the DTMS spectra identified five different chemotypes: groups of residues with comparable chemical characteristics. The biomolecular origin of each of these chemotypes is identified by comparison with experimentally charred reference materials. The chemotypes A₁ and A₂ consist of charred residues identified as starch-rich foods (mixed with animal or plant products), chemotype C consists of protein-rich charred animal products without starch, chemotype B contains smoke condensates from wood fires, and chemotype D consists of special protein-rich foods or non-food products containing little or no lipid fraction.

Modified after:

T.F.M. Oudemans, J.J. Boon & G.B. Eijkel in press, 'Identifying biomolecular origins of solid organic residues preserved on Iron Age pottery using DTMS and MVA', *Journal of Archaeological Science*.

1. Introduction

1.1. Organic residue analysis in ceramic study

The chemical characterisation of organic residues found in association with ancient ceramic vessels can provide archaeologists with valuable information about the daily lives of prehistoric people. Organic residue analysis can provide information about foods prepared, stored and served in ceramic vessels, some of which may not be detectable by any other means.

The chemical analysis of organic residues - both of solid surface residues and of residues absorbed into the ceramic of the vessel - has taken place as early as the 1920s, but has greatly expanded in the last two decades due to improvements in analytical instrumentation and an increasing interest in the functional aspects of pottery in ceramic studies (Rottländer & Schlichtherle 1980; Evershed *et al.* 1992; Heron & Evershed 1993; Evershed *et al.* 1999). Many analytical techniques have been applied to the study of specific classes of compounds such as solvent extractable lipids (Evershed *et al.* 1990; Evershed *et al.* 1999); waxes (Heron *et al.* 1994; Evershed *et al.* 1997d; Regert *et al.* 2001); terpenoids (Gianno *et al.* 1990; Charters *et al.* 1993a; McGovern *et al.* 1996; Dudd & Evershed 1998; Eerkens 2002) and amino acids (Evershed & Tuross 1996). The most recent developments include the use of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to identify isotope ratios of individual fatty acids absorbed in prehistoric and medieval ceramics (Evershed *et al.* 1994; Evershed *et al.* 1997b; Mottram *et al.* 1999) and the detection of the most abundant protein in milk (α -casein) using immunological methods (Craig & Collins 2000; Craig *et al.* 2000; Craig *et al.* 2005). Although these studies have facilitated the identification of a whole range of compounds in archaeological residues, and have provided plausible identifications for particular groups of residues, they are limited to specific compound classes. A non-selective technique is required to characterise the overall chemical composition of residues and categorise them accordingly.

1.2. Pyrolysis mass spectrometry in the study of ancient organic residues

Pyrolysis mass spectrometry (PyMS) is a rapid micro-analytical technique particularly suited for the identification of a wide range of natural organic polymers and other complex organic materials (Boon 1992; Moldoveanu 1998). Pyrolysis is the thermal dissociation of large molecules or polymers in an inert atmosphere that produces structurally specific and chemically characteristic fragments (also called 'pyrolysis products' or 'pyrolysates').

In Curie-point PyMS, or flash-pyrolysis, the sample is heated very rapidly to a set temperature dependent on the Curie-point of the metal filament (commonly between 500 °C and 800 °C). The resulting mass spectrum is a chemical 'fingerprint' indicative of the overall chemical composition of the residue. In the first Curie-point PyMS study of solid organic residues from ancient ceramics, indicative markers for a broad range of bioorganic moieties were detected and

subsequently identified using Curie-point PyGC/MS (Oudemans & Boon 1991). Protein remains indicative of severely denatured peptide chains could be detected, as well as polysaccharide remains in the form of various furans, phenols and benzenes. Lipids were detected in the form of fatty acids, and additional compounds formed from lipids under carbonising conditions could be identified. Multivariate studies (Chapter 2) of Curie-point PyMS measurements of a large group of residues showed five distinct groups of residues with specific chemical composition (Oudemans & Boon 1996).

1.3. DTMS – Direct Temperature-resolved Mass Spectrometry

New developments in analytical pyrolysis mass spectrometry have resulted in Direct Temperature-resolved Mass Spectrometry (DTMS) instrumentation. Commercially available DTMS instruments can measure much larger fragments (up to m/z 1000) than the locally developed Curie-point PyMS instrumentation (up to m/z 220). Additionally, DTMS gives temperature-resolved information. By gradually raising the temperature of the sample, a physical separation is achieved between low molecular weight compounds in the evaporation phase, and the cross-linked fraction of a sample in the pyrolysis-phase. The temperature-resolved information facilitates the interpretation of the results.

A collection of solid organic residues was analysed using DTMS in order to determine the chemical composition and identify the original biomaterials involved in their formation. Multivariate analysis was used to classify samples into groups of residues with similar chemical composition, so called ‘chemotypes’. For each ‘chemotype’ identifications of the original biological materials involved are made by detailed comparison with experimentally charred modern reference materials.

2. Materials and Methods

2.1. Settlement

A large-scale excavation at Uitgeest-Groot Dorregeest in the Netherlands (Woltering 1982, 1983) uncovered habitation remains dating back to the Late Iron Age, the Roman Iron Age and the Medieval period. The indigenous settlement at Uitgeest-Groot Dorregeest (1 – 300 AD), was situated about 50 kilometre north of the Roman – German border, on top of a small, relatively dry sandy ridge formed by the remains of a coastal barrier and a sandy deposit from the Dunkirk I period (see also Appendix 1 for further description of the settlement site).

2.2. Samples and sample treatment

The ceramic assemblage of Uitgeest-Groot Dorregeest was studied extensively by Abbink (1985, 1999) and contains primarily simple, wide mouthed, globular or ellipsoid jars with short rim and neck and a maximum diameter equal to, or slightly larger than, the rim diameter. Many of the vessels contain visible surface residues of different kinds (Appendix 1, Table 3). In the assemblage of 147 partial vessels with identifiable morphological vessel 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 'creamy layers' (3%) occurred occasionally. Archaeological residues from all four categories were chosen for analysis (Table 1). Some of the residues originated from the group of partial vessels with identifiable morphological type (14), while others were taken from shards (20). In order to avoid selective sampling, residues recovered from different types of sediments were selected. All ceramics were washed with tap water immediately after excavation. The residue samples were scraped from the ceramic surface with a scalpel, after removal of the outermost 0.5 mm of residue.

In addition to the archaeological samples, a number of biomaterials were chosen as reference materials. Potato amylose (mw over 150000, Janssen Chimica) and a crystallised bovine blood albumin (BDH Biochemicals) were heated from 20 °C up to 250 °C (in 30 minutes) and subsequently progressively charred at 250 °C for 2 to 16.5 hours under a constant flow of nitrogen (100 ml/min). These charring conditions were chosen for two reasons: they were estimated to reflect conditions in cooking vessels on an open fire, and because earlier CuPyMS and CuPyGC/MS studies showed that heating at 250 °C for 2.5 hours were the minimum conditions needed for the formation of a condensed polymeric char network commonly observed in archaeological chars (Oudemans & Boon 1991; Pastorova *et al.* 1993b).

Sample preparation is limited to grinding 5 – 10 µg of residue with a small glass mortar and pestle, and suspending them in 15 – 20 µl ultra pure water (Millipore Q[®] grade). Multiple DTMS measurements (4) were performed on aliquots (1 – 2 µl) of each suspension over a period of three days.

2.3. Analytical procedures and instrumental conditions

DTMS measurements were carried out on a JEOL DX-303 double focussing (E/B) mass spectrometer equipped with a JEOL DA-5000 data system. The sample suspension was applied to the filament (Pt/Rh 9:1, 100 µm) of a direct insertion probe, which is resistively heated at 1 A/min in vacuo to a maximum temperature of ca. 800 °C. Ions were generated under low voltage EI conditions (16 eV) in an ionisation chamber kept at 180 °C and accelerated to 3 kV before being measured over a range of m/z 20 – 1000 at 1 s full range cycle time. Additional analytical work was performed on a selected number of residues using a JEOL JMS-SX/SX 102A with a JEOL MS-MP 9020D data system. In this mass spectrometer, the same filament was heated at a slower rate of 0.5 A/min, and the ions were generated under similar conditions in an ionisation chamber kept at 190 °C and before being accelerated to 8 kV. Measurements were performed over a mass range m/z 20 – 1000 at a 3 s full range cycle time and post-accelerated to 10 kV. The resolution used was 1000.

Table 1: Archaeological residues and their chemical classification

Nr ^a	Find number	Sediment	Vessel Nr ^b	Residue Type	Residue colour	Pos. ^c	Chemo type ^d
1	7-7	Organic clay	7.07	Char	Brown	In	A ₁
2	8-1	Organic clay	8.01	Pigment	Red brown	Ex	C
3	8-2	Organic clay	8.02	Char	Brown	In	A ₂
4	8-5	Organic clay	8.05	Cream coloured	Beige	In	A ₁
5	14-6-4.2	Sandy		Char	Brown	In	A ₂
6	14-6-4.3a	Sandy		Char	Brown	In	D
7	14-6-4.3b	Sandy		Soot	Black	Ex	B
8	14-6-4.4	Sandy		Char	Brown	In	A ₁
9	14-6-4.5	Sandy		Char	Brown	In	A ₁
10	18-3-2a	Organic clay		Char	Brown	In	D
11	18-3-2b	Organic clay		Soot	Black	Ex	B
12	18-7	Organic clay	18.07	Char	Brown	In	A ₂
13	19-7-90.2a	Organic clay	19.18	Char	Brown	In	A ₂
15	20-4	Sandy	20.04	Char	Brown	In	A ₁
16	20-4-157	Sandy		Char	Brown	In	A ₂
17	30-12-3	Organic clay	30.02	Char	Brown	In	A ₂
18	31-4a	Organic clay	31.04	Char	Brown	In	D
19	31-4b	Organic clay	31.04	Soot	Black	Ex	B
20	32-6-18	Peat		Char	Brown	In	A ₂
22	33-5-2b	Peat		Char	Brown	In	A ₁
23	33-8-2a	Peat		Char	Brown	In	A ₂
24	33-8-2b	Peat		Char	Brown	In	A ₂
25	34-0-12	Organic clay		Char	Brown	In	A ₁
26	34-0-30	Organic clay	34.12	Char	Brown	In	C
28	34-7-95b	Organic clay		Soot	Black	Ex	E
29	34-11-3	Organic clay		Char	Brown	In	A ₂
30	35-5-120	Sandy	35.33	Char	Brown	In	A ₁
31	35-7-28	Sandy		Cream coloured	Beige	In	D
32	35-20	Sandy	35.20	Cream coloured	Beige	In	D
33	35-21	Sandy	35.21	Char	Brown	In	D
34	14-6-4.2b	Sandy		Char	Brown	In	C
35	14-6-4.3c	Sandy		Char	Brown	In	A ₁
36	34-7-95a	Organic clay		Char	Brown	In	A ₁
37	33-5-2ab	Peat		Char	Brown	In	A ₁

^a = Number corresponds to Chapter 2 (Oudemans & Boon 1996). Samples 14, 21 and 27 were excluded from this study due to limited sample size.

^b = Vessel numbers are given when profile is of identifiable morphological type, shards have no number (Abbink 1999).

^c = The position of the residue on the ceramic vessel: in = interior of the vessel; ex = exterior of the vessel.

^d = Chemotype defined according to DTMS analysis, see sections 4.1. through 4.7.

The summarised intensity of all ions as a function of time is called the Total Ion Current (TIC). The mass spectrum of each scan was registered separately, giving temperature resolved information. The mass spectra can be averaged over the entire heating time to give an “average mass spectrum” or “overall mass spectrum” which represents the overall chemical composition of the sample and is used as input for the multivariate comparison of samples. An average mass spectrum is calculated prior to normalisation by adding up the mass spectra in the selected scan range and averaging them.

2.4. Multivariate Analysis

The overall mass spectrum of each measurement was included in a dataset that was numerically analysed by Discriminant Analysis (DA) and Complete-linkage Cluster Analysis (CLCA) using the FOMpyroMAP multivariate analysis programme, a modified version of the ARTHUR multivariate analysis package produced by Infometrix Inc. (Seattle, USA, 1978 release). This procedure was used to reveal the main chemical similarities and dissimilarities between the samples and to group them according (Windig *et al.* 1982; Hoogerbrugge *et al.* 1983).

The validity of this kind of classification cannot be estimated easily for there is no cluster “validity measure” for hierarchical clustering techniques. Although theoretically cluster analysis is the most suitable multivariate technique (because no prior knowledge is available about the form or number of expected groups), no objective indicator exists to determine the quality of the proposed partitioning of the data (Lavine 2000). The stability of the combined DA and CLCA results is therefore established by performing a series of 34 test runs on the total sample set, minus one residue. New DFs were defined each time and a new CA was done with the excluded residue in the test set. The consistency of the classification of the residues was indicated by how often the excluded residue was subsequently classified in its original cluster and by the percentage of samples that were classified in their original cluster.

3. Results - Reference Materials

DTMS analysis of modern foods results in very specific mass spectra characteristic for the chemical composition of the original biomaterials. However, residues from archaeological context are often heated or (partially) charred and thus produce very different DTMS spectra. In order to understand the chemical changes during thermal degradation and produce reference materials, two modern foodstuffs (amylose and albumin) were experimentally heated and analysed using DTMS.

3.1. Amylose - Untreated and Experimentally Charred

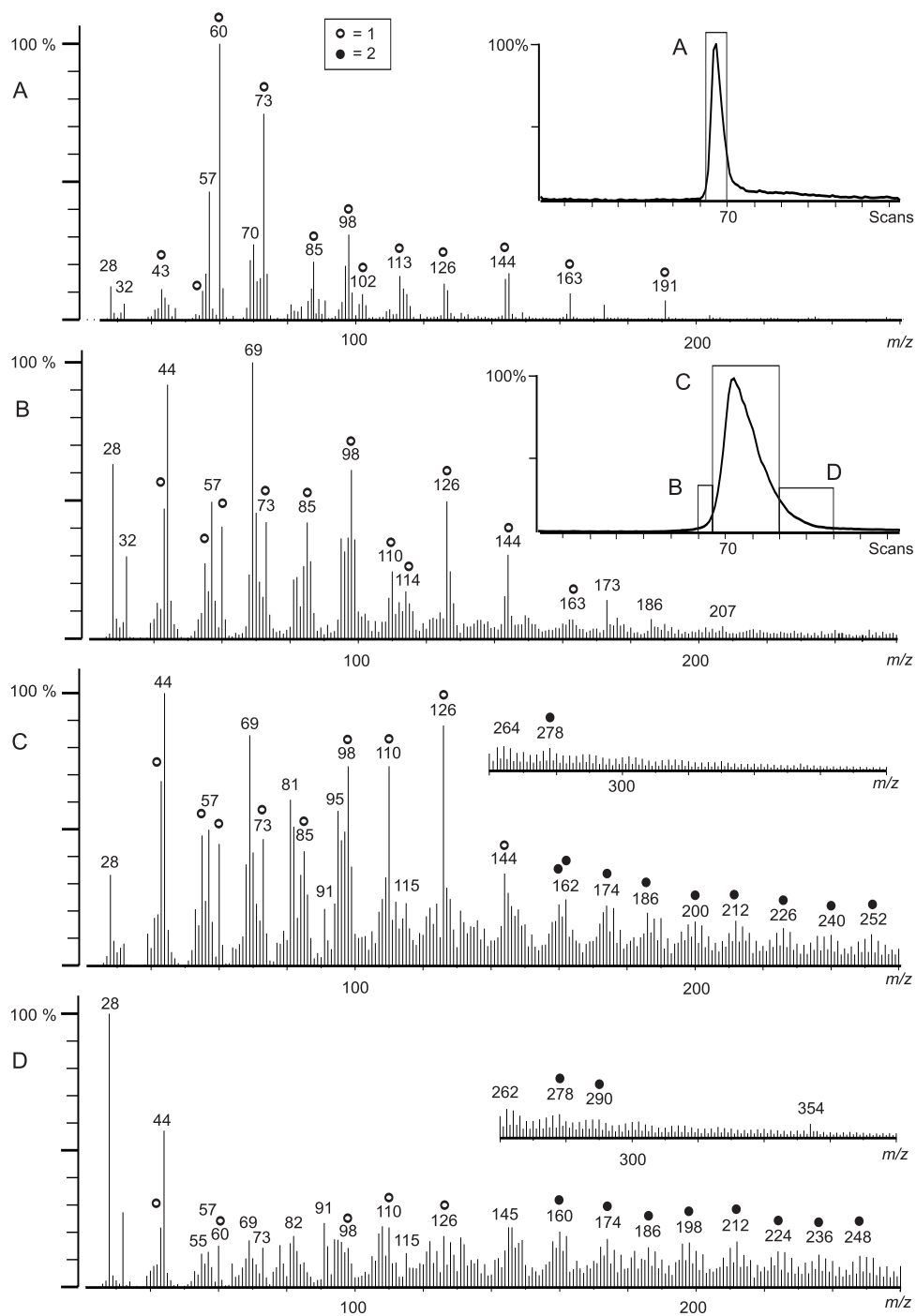
Amylose, one of the major macromolecular components of starch (Wong 1989; Davídek *et al.* 1990), was selected as a reference material to study the chemical effects of polysaccharide charring.

The DTMS spectrum of untreated amylose (Fig. 1A) shows all of the indicative markers for hexose polymers (Table 2) as identified in earlier studies using analytical pyrolysis (Meuzelaar *et al.* 1982; Pouwels *et al.* 1989; Boon 1992; Pastorova *et al.* 1993a). The absence of fragment ions in the higher mass area is indicative of a complete fragmentation of the polymer into smaller fragments and a lack of further ionisation induced fragmentation of the pyrolysis products. The given fragments are all formed around the same temperature (scans 60 – 72) indicating a structurally homogeneous sample.

In general, DTMS results of experimentally charred amylose (Fig. 1B – D and Table 2) are comparable to earlier studies of microcrystalline cellulose heated under anaerobic conditions up to 2.5 hours up to temperatures ranging from 190 to 390 °C (Pastorova *et al.* 1993a; Boon *et al.* 1994; Pastorova *et al.* 1994). Some markers for intact hexose polymers are released from the amylose char (Fig. 1B) at slightly lower temperatures (scans 50 – 65) than in untreated amylose (Fig. 1A scans 60 – 72), which indicates fragmentation of high molecular-weight oligosaccharides into smaller units during the experimental charring. The high temperature part of the TIC (Fig. 1C – D) shows a typical higher mass fraction indicative of the condensed polymeric structure formed during heating of polysaccharides. The apex of the TIC profile has shifted to a higher temperature (from scan 67 to scan 73) and has broadened substantially, confirming the formation of a more thermally stable network polymer.

3.2. Albumin - Untreated and Experimentally Charred

Bovine serum albumin (BSA), a simple protein previously studied using Curie-point PyGC/MS (Munson & Fetterolf 1987), was selected to study the chemical effects of experimental protein charring. Detailed pyrolysis studies of amino acids performed in the 1980s and 1990s (Meuzelaar *et al.* 1982; Tsuge & Matsubara 1985; Chiavari & Galetti 1992) have identified specific pyrolysis products for many individual amino acids (Table 3). However, non-specific complex mixtures of primary and secondary fragmentation-products occur regularly in pyrolysis studies of proteinaceous materials and can vary extensively in composition depending on the pyrolysis conditions (Moldoveanu 1998, 373-397). Pyrolysis of polypeptides or intact proteins commonly reveals two groups of products: i) “amino acid markers” similar to those obtained from pyrolysis of single amino acids, and ii) “peptide & protein markers” indicative of two or more amino acids occurring in their original chain structure. These peptide markers originate from cyclisation of two amino acids prior to pyrolytic cleavage and may include diketopiperazines (DKPs) and their secondary fragmentation products (Smith *et al.* 1988; Stankiewicz *et al.* 1996); substituted 2,4-imidazolindiones (Munson & Fetterolf 1987;



Stankiewicz *et al.* 1996); and di-substituted 3-alkenyl-5-alkyl-pyrrolidin-2,4-diones and their corresponding 2,4-dialkyl-3,5-diketopyrroline isomers (Boon & De Leeuw 1987).

Table 2: Markers for hexose polymers in DTMS (16 eV EI) spectra.

Pyrolysis markers	m/z values	reference
Markers for Intact Hexose Polymers		
hexose polymers (amylose, cellulose, amylopectines)	31, 43, 55, 60, 73, 85, 97/98, 102, 110, 113, 114, 126, 144, 163, 191	[1,2,3,4]
Markers for Charred Hexose Polymers (250 – 310 °C)		
alkylated benzenes	92, 106, 120, 134, 148, 162, 176, 190	[4,5]
alkylated benzofurans	132, 146, 160, 174, 188	[4,5]
dibenzofurans	168, 182, 196, 210, 224, 238	[4,5]
alkyl-benzene aromatics	92, 104	[4,5]
alkyl-phenols	108, 122	[4,5]
Markers for Charred Hexose Polymers (over 310 °C)		
highly condensed aromatic structure	160, 162, 174, 176, 186, 188, 190, 198, 200, 202, 212, 214, 224, 228, 236, 238, 240, 248, 250, 252, 276, 278, 288, 290	[6]
CO and CO ₂	28, 44	[6]

[1] = Meuzelaar *et al.* 1982; [2] = Pouwels *et al.* 1989; [3] = Boon 1992; [4] = Pastorova *et al.* 1993a; [5] = Boon *et al.* 1994; [6] = Pastorova *et al.* 1994.

DTMS results for untreated BSA (Fig. 2A - B) show both groups of pyrolysates (Table 3). Fragments are generated over a narrow temperature range (scans 62 - 80) indicating a structurally homogeneous sample. Intact peptide and protein markers are primarily generated at the beginning of the TIC curve (Fig. 2A), while amino acid markers are liberated over the entire temperature range (Fig. 2A - B).

Earlier DTMS studies of whole peas and of Pisane HD (pea protein isolate) heated under anoxic conditions up to temperatures ranging from 130 to 700 °C for a maximum of 2 hours (Braadbaart 2004; Braadbaart *et al.* 2004a), showed markers for many protein chars (Table 3). The DTMS results for bovine serum albumin heated at 250 °C for up to 17 hours (Fig. 2C - D)

Figure 1 (on facing page): DTMS results for Amylose.

A: Mass spectrum for untreated potato amylose shows markers for (1) intact hexose polymers.

B – D: Mass spectra for potato amylose experimentally heated for 17 hours at 250 °C. B: The partial mass spectrum for low temperatures still includes many markers for (1) residual oligosaccharides. Partial mass spectra for higher temperature phases C and D are increasingly characterised by the presence of (2) a newly formed, thermally stable polymeric structure.

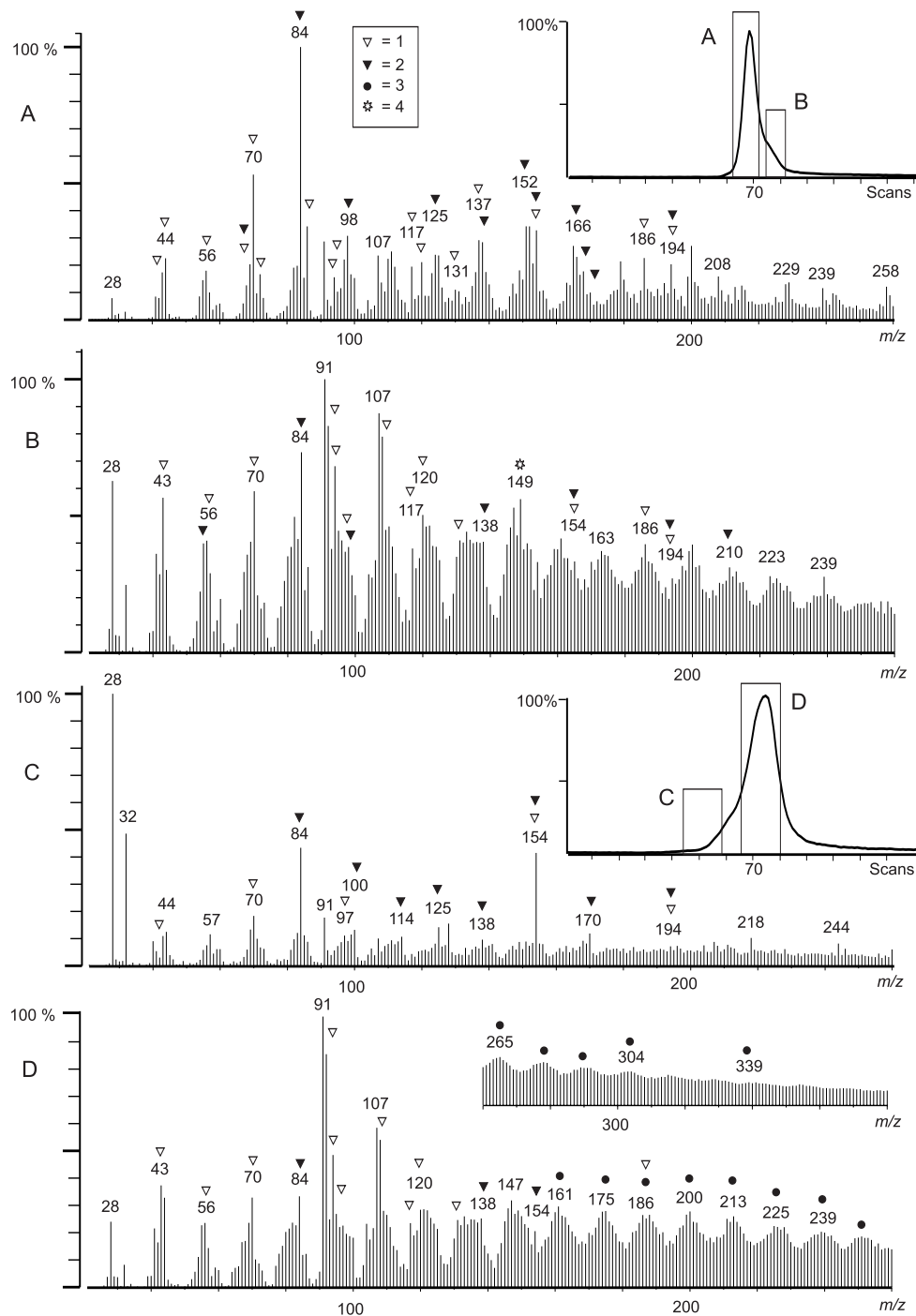
Table 3: Markers for proteins, polypeptides, amino acids in DTMS spectra		
Pyrolysis markers	m/z values	ref.
Markers for Amino Acids		
Arginine	69, 114	[1]
Asparagine	97, 99	[1]
Cysteine	34, 93, 103, 127	[1,2]
Glutamine	67, 83, 97	[1]
Hydroxy-proline	67, 69, 95, 113, 160, 174, 186	[1]
Leucine and Isoleucine	69, 70, 83, 86, 87, 153, 155, 226	[1,3]
Lysine	69, 128	[1]
Methionine	34, 48, 74, 90, 105, 145	[1, 2]
Phenylalanine	92, 104, 106, 117, 121, 182	[1,2,3]
Proline	69, 71, 194	[1]
Tryptophan	117, 131, 143, 145, 156, 160, 170	[1,2,3]
Tyrosin	94, 108, 120, 122, 137	[1,2,3]
Valine	56, 73, 69, 72, 85, 99, 125, 127, 154, 198	[1,3]
Markers for Peptides and Proteins		
Diketopiperazines (DKP) (and additional smaller fragments)	154, 166, 168, 170, 182, 192, 194, 196, 198, 210 (58, 67, 68, 69, 70, 85, 87, 92, 99, 114, 124, 125, 138)	[4,5]
Substituted 2,4-imidazolidindiones (and additional smaller fragments)	100, 114, 142, 156 (29, 45, 57, 72)	[6,7]
3-alkyl-5-alkyl-pyrrolidine-2,4-diones & 2,4-dialkyl-3,5-diketopyrrolines isomers	55, 69, 84, 98, 124, 138, 152, 166, 180, 181, 195, 209	[5,6]
Markers for charred Proteins		
Alkylphenols/alkylbenzenes	91, 92, 94, 105, 107, 108, 119, 122, 131	[8,9]
N-containing heterocyclic compounds	117, 131, 133, 147, 161, 175, 189, 199, 213, 227	[9]
Benzene and Aromatic compounds	78, 146, 160, 174	[9]
HCN	27	[9]
CO/CO ₂ /SO/SO ₂	28, 44, 48, 64	[9]
Benzene/Toluene	78, 92	

[1] = Chiavari & Galetti 1992; [2] = Meuzelaar et al. 1982; [3] = Tsuge & Matsubara 1985; [4] = Smith et al. 1988; [5] = Stankiewicz et al. 1996; [6] = Boon & De Leeuw 1987; [7] = Munson & Fetterolf 1987; [8] = Moldoveanu 1998; [9] = Braadbaart 2004.

Figure 2 (on facing page): DTMS results for Bovine serum albumin.

A – B: Partial mass spectra show a mixture of markers for (1) amino acids; (2) peptides and proteins, including markers for Diketopiperazines (DKPs); 3-alkyl-5-alkyl-pyrrolidine-2,4-diones and their matching 2,4-dialkyl-3,5-diketopyrrolines isomers and (4) a contaminant.

C – D: Bovine serum albumin experimentally heated for 17 hours at 250 °C. C: Partial mass spectrum still includes many markers for (1) peptides and proteins. D: Higher temperature partial mass spectrum also includes markers for (3) a newly formed, thermally stable polymeric structure.



are similar to those from heat-treated Pisane HD and most closely resemble mild thermal degradation products (Braadbaart *et al.* 2004a).

In charred BSA, part of the indicative peptide and protein markers (m/z 100, 114, 154, 170) are still present at temperatures slightly lower than in untreated BSA (Fig. 2C), indicating breaking of peptide bonds and formation of smaller peptides during experimental charring. At higher temperatures (Fig. 2D) the mass spectrum contains few markers for peptides and intact proteins and is dominated by amino acid markers and a wide spectrum of masses indicative of a condensed thermostable polymer. This polymer is characterised by the presence of odd numbered masses (m/z 117, 131, 133, 147, 161, 175, 189, 199, 213, 227). The apex of the TIC profile has shifted to a higher temperature (from scan 68 to scan 75) and has broadened substantially, confirming the formation of a more thermally stable condensed structure.

4. Results - Archaeological Residues

4.1. Multivariate Analysis

DTMS data of archaeological residues do not show such specific or immediately recognisable peak patterns. In order to identify significant combinations of chemical characteristics in DTMS spectra of archaeological residues, multivariate techniques were applied. Discriminant analysis was used to illustrate the differences within the set of samples, and to identify the mass values that determine those differences. Six Discriminant Factors (DFs), explaining 42.4%, 19.9%, 9.3%, 7.6%, 5.4% and 3.9% of the total relevant variance in the dataset respectively, were considered important for the chemical composition of the residue material. The map of discriminant functions DF_1 and DF_2 visualises the chemical similarity and dissimilarity between the residues (Fig. 4a). In the reduced six dimensional discriminant space defined by DF_1 through DF_6 , clusters of chemically similar residues were determined using hierarchical complete-linkage cluster analysis (CLCA). A dendrogram representing the similarity matrix of all 132 mass spectra shows six groups of samples - clusters A_1 , A_2 , B, C, D and E - with similarity values of 0.70 or more (Fig. 3). Cluster A_1 and A_2 are named as subdivision of what used to be one cluster in Chapter 2 (Oudemans & Boon 1996), but are of the same level as the other clusters. Multiple measurements of the same residue gave similarity values of 0.9 or more (with the exception of sample 35).

The stability of the combined DA and CLCA results is established by performing a series of test runs that showed the consistency of the classification of the residues (see Table 4). The overall figures showed how often the test residue was classified within its original cluster and what percentage of total number of residues, were classified in their own cluster. The overall numbers are above 80% and indicate the relative consistency of the classification as defined by this method. Larger clusters are more consistent than the smaller clusters.

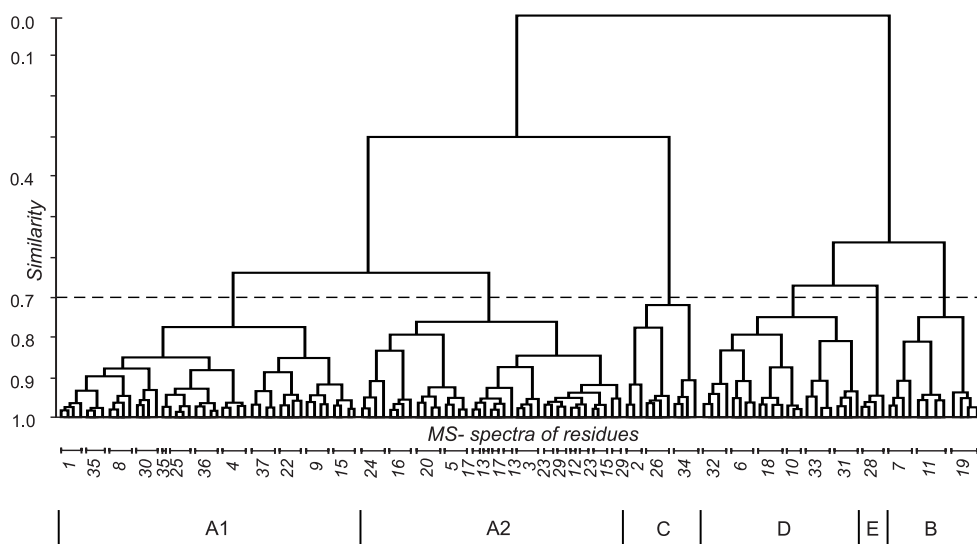


Figure 5: Complete-linkage dendrogram of the measured mass spectra of organic residues.

Residues are indicated with their sample number (see Table 2). Chemotypes show a similarity of 0.70 or higher. Similarity of multiple measurements of one residue is > 0.90 (except residue 35). Although multiple measurements mostly cluster with one another first, exceptions can be seen (residues 35, 17, 13, 23, 29 and 12).

Table 4: Chemotypes and their statistical consistency

Chemotype	A ₁	A ₂	B	C	D	E	Total
N	11	10	3	3	6	1	34
Residues	1, 4, 8, 9, 15, 22, 25, 30, 35, 36, 37	3, 5, 12, 13, 16, 17, 20, 23, 24, 29	7, 11, 19	2, 26, 34	6, 10, 18, 31, 32, 33	28	
Consistency of classification ^a	100%	93%	33%	100%	67%	0%	82%
Cluster Stability ^b	94%	96%	80%	88%	76%	76%	89%
Residues that shift position ^c	4, 25, 35, 36	16, 24	11	26, 34	6, 31, 32, 33	28	

^a Consistency of classifications: percentage of residues from a cluster that classify in the same cluster during their test run.

^b Cluster stability: percentage of residues in a cluster that do not shift position (averaged over 34 test runs). When a test divides a cluster in two subgroups, the cluster was defined as the main group containing the majority of the samples.

^c Residues that shift position: sample numbers of residues that shift position three or more times during 34 test runs.

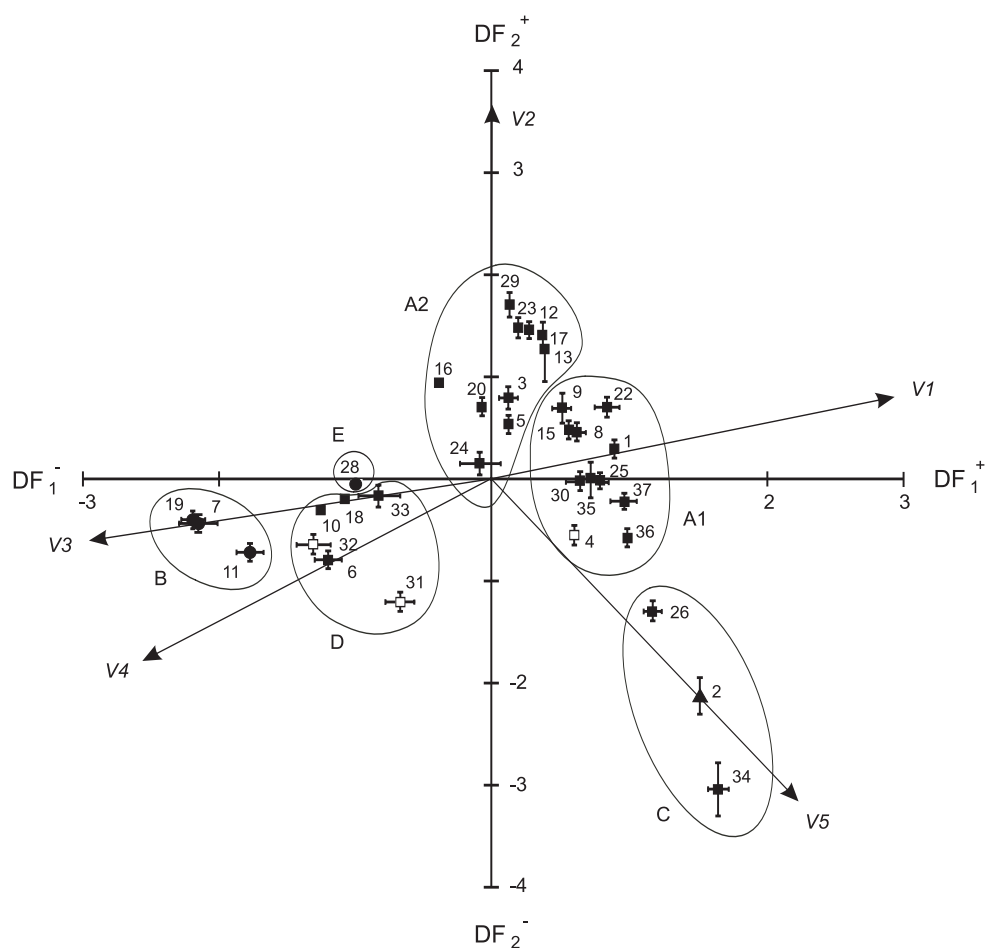
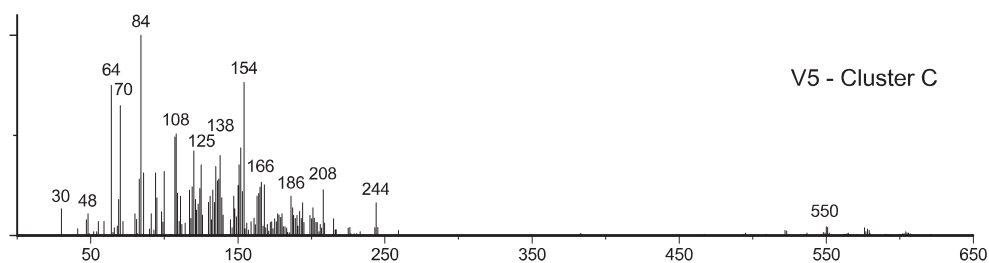
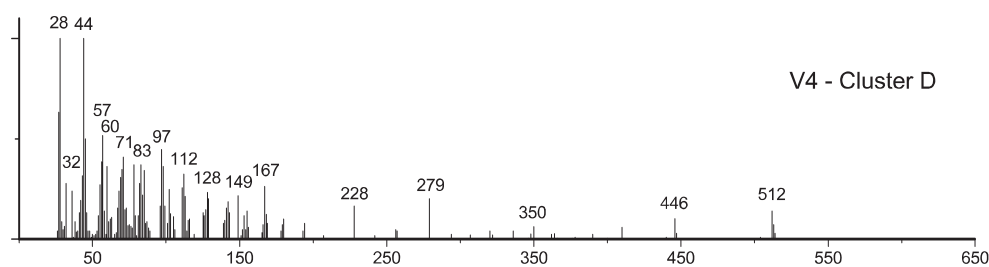
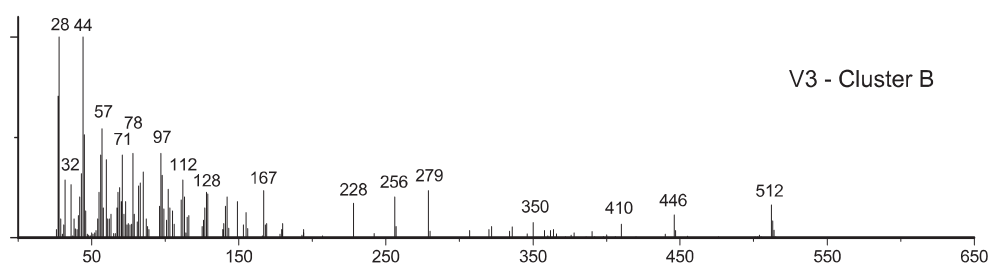
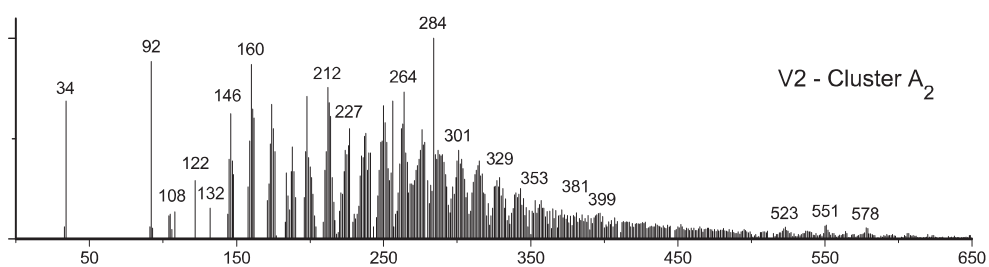
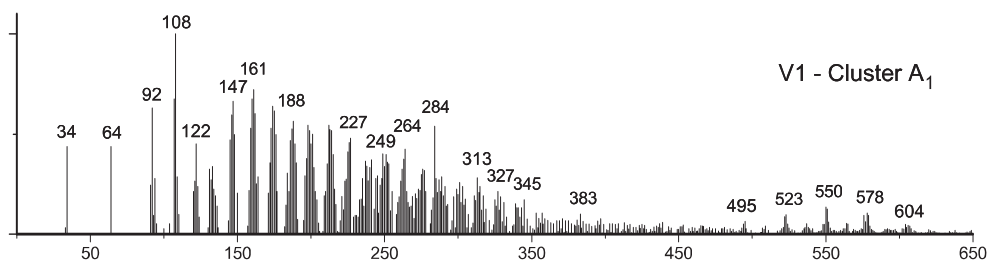


Figure 4: Discriminant map with chemotype clusters (A₁ through E).

A: In the discriminant map of DF_1 versus DF_2 the relative difference in chemical composition between residues, is expressed by their Euclidian distance. Multiple measurements are indicated by standard deviation error-bars. Clusters of samples with a similarity value $S > 0.70$ have been indicated. Each cluster represents a particular chemotype. Chars on vessel interior (■); black residues on vessel exterior (●); red-brown residues (▲); and cream coloured residues (□).

B (on facing page): Vectors representing the typical chemical characteristics of clusters A₁ through D. Each chemotype is characterised by a set of correlated m/λ values depicted in the rotational vectors 1 through 5. The vector spectra depict variations with respect to the zero-point spectrum. Chemotype E is not represented due to its similarity to chemotype B.



4.2. Chemotypes

In Figure 4a each cluster represents a particular chemotype: a group of residues with a recurring combination of chemical characteristics. Each chemotype is characterised by a set of correlated m/z values as represented by the DF scores in the rotational vectors 1 through 5 (Fig. 4b). Chemotype E is not depicted because it is determined primarily by contaminants such as phthalates and elementary sulphur (see also section 4.6.). The vector spectra depict variations with respect to the zero-point spectrum and are defined through graphical rotation (Windig *et al.* 1982). Mass values that appear in all samples will not be shown as typical characteristic for either direction. When studying the complete chemical composition, the original mass spectra must always be considered.

4.3. Chemotype A₁

Chemotype A₁ (Fig. 4b, vector 1) is primarily characterised by: i) a lipid profile with markers for intact lipids, such as diacylglyceryl-fragments and acylium ions (see also Table 5); ii) amino acid markers (Table 3); and iii) an envelope of higher molecular weight compounds including odd numbered compounds indicative of a condensed polymeric aromatic structure (Table 2 and 3). The DTMS data for charred residue 1 (nr. 7-7), a representative sample for cluster A₁, show an evaporation range (Fig. 5A) with intense mass peaks for diacylglyceryl-fragments (DAG 14 - 36) and acylium ions (C12:0 - 18:0). Even numbered DAGs are visible as m/z values for [M-RCOOCH₂]⁺ ions, although their intensity suggests an additional contribution from fragment ions [M-RCOO]⁺ and [M-RCOOH]⁺ of odd numbered DAGs (DAG 15 - 35). The presence of unsaturated triacylglycerides is indicated by [M-RCOO]⁺ and [M-RCOOH]⁺ fragment ions with m/z values 2, or 4 points lower than the saturated ones (DAG 36:1, 36:2, 34:1, 34:2, 32:1, 30:1, 28:1, 26:1, 24:1 and 22:1). Saturated free fatty acids (FA 10:0 - 18:0), unsaturated free fatty acids (C18:1) and cholesterol can be observed in the spectrum.

The pyrolysis range (Fig. 5B) shows few markers for peptides and intact proteins (m/z 138, 154, 168) 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 in the mass range m/z 140 - 300, indicating the incorporation of nitrogen containing compounds into the condensed aromatic material.

4.4. Chemotype A₂

Chemotype A₂ (Fig. 4b, vector 2) is primarily identified by: i) a lipid fraction with some markers for intact lipids, high amounts of free fatty acids and small amounts of phytosterols; and ii) an envelope of higher molecular weight compounds similar to the one in cluster A₁ but with relatively more even numbered mass peaks.

Table 5: Markers for lipids in DTMS spectra.

Pyrolysis markers	m/z values	Ref.
Markers for Triacylglycerides		
M^+ (TAG 34 – 36)	610, 638	[1]
M-18 (TAG 34 – 38)	592, 620, 648	[1]
$[M-RCOOH]^+$ even: DAG 16 – 36	326, 354, 382, 410, 438, 466, 494, 522, 550, 578, 606	[1]
$[M-RCOOH]^+$ odd: DAG 15 – 35	312, 340, 368, 396, 424, 452, 480, 508, 536, 564, 592	[1]
$[M-RCOO]^+$ even: DAG 16 – 36	327, 355, 383, 411, 439, 467, 495, 523, 551, 579, 607	[1]
$[M-RCOO]^+$ odd: DAG 15 – 35	313, 341, 369, 397, 425, 453, 481, 509, 537, 565, 593	[1]
$[M-RCOOCH_2]^+$ DAG 22 – 36	425, 453, 481, 509, 537, 565, 593	[1]
$[RCO + 128]^+$ C12:0 – 18:0	311, 339, 367, 395	[1]
$[RCO + 74]^+$ C10:0 – 18:0	229, 257, 285, 313, 341	[1]
$[RCO]^+$ C12:0 - 18:0	183, 211, 239, 267	[1]
$[RCO-1]^+$ C10:0 - 18:0	154, 182, 210, 238, 266	[1]
Markers for Monoacylglycerides		
$[M-CH_2OH]^+$	187, 215, 243, 271, 299, 327	[1]
$[RCO]^+$ C12:0 - 18:0	183, 211, 239, 267	[1]
Free Fatty Acid Markers		
M^{+*} unbranched FA12:0 – 28:0	200, 228, 256, 284, 312, 340, 368, 396, 424	[2]
M-OH = $[RCO]^+$ FA12:0 - 18:0	183, 211, 239, 267	[2]
$[(CH_2)_nCOOH]^+$ Up to FA20:0	73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241	[2]
$[M-H_2O]^+$ FA16:1 – 18:1	236, 264	[2]
Markers for Sterols		
Cholesterol (C27) animal source	386, 368, 275	
Brassicasterol (C28) plant source	398	[3]
Ergosterol (C28) fungal source	396, 363, 253	
Campesterol (C28) plant source	400, 382	[3]
?-Sitosterol (C29) plant source	414, 396, 399, 329, 303	[3]
Stigmasterol (C29) plant source	412, 394	[3]
Alkylated steradienes (C27, C28, C29)	368, 382, 396	[4]
Markers for Natural Waxes		
Long-chain FA (FA20:0 – 28:0)	312, 340, 368, 396, 424	[5,6,7]
Monoesters ME38 – ME52	536, 564, 592, 620, 648	[5,6,7]

[1] = Odham & Stenhagen 1972b; [2] = Odham & Stenhagen 1972a; [3] = Knights 1967; [4] = Braadbaart 2004; [5] = Nelson & Blomquist 1995; [6] = Kolattukudy 1976; [7] = Bianchi 1995.

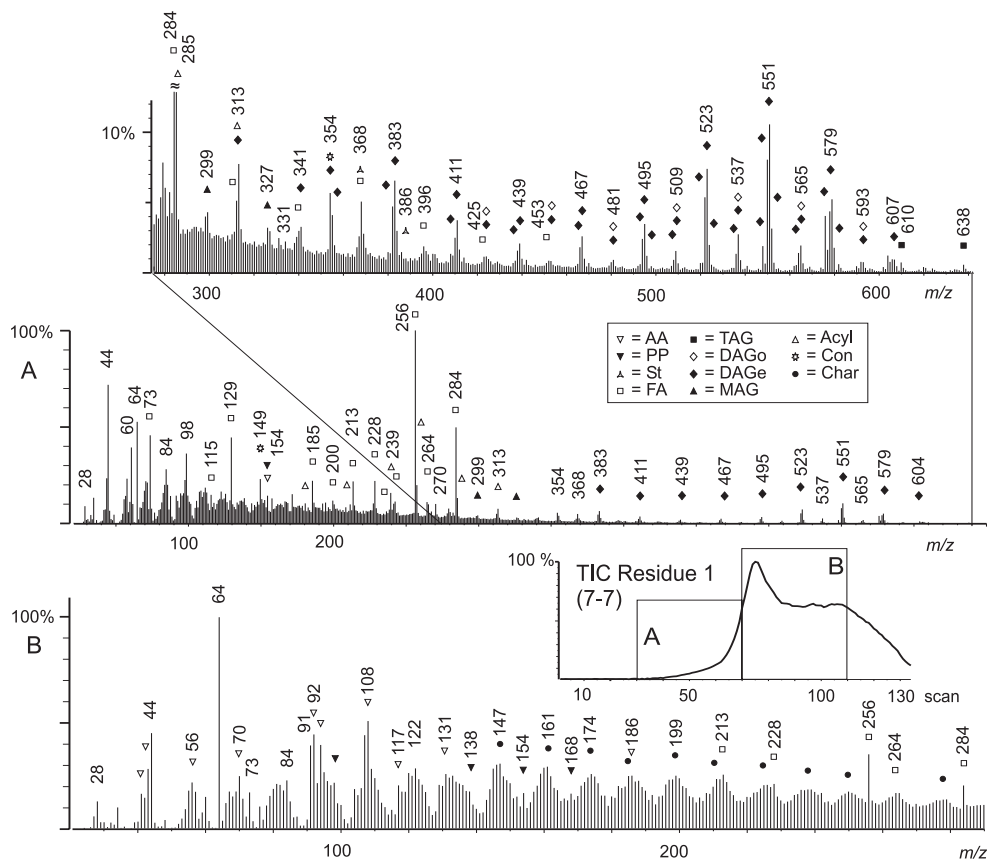


Figure 5: Cluster A₁ - DTMS results for charred residue 1 (nr. 7-7)

A: Partial mass spectrum of the low temperature range shows a mixture of lipid indicators including (FA) fatty acids and their fragments; (TAG) triacylglycerides M⁺ ions; (DAGe) even numbered diacylglyceryl-fragments; (DAGo) odd numbered diacylglyceryl-fragments; (Acyl) acylium-ions; (MAG) Monoacylglycerides; and (St) cholesterol. Some markers can be seen for (PP) peptides and proteins and (AA) amino acids and an occasional contaminant (Con).

B: High temperature partial mass spectrum clearly shows markers for (Char) thermally stable condensed polymeric network.

DTMS results for a representative charred residue 23 (nr. 33-8-2a) are shown in Fig. 6. The lipid profile of charred residue 23 (Fig. 6A) shows high mass peaks for saturated free fatty acids (C10:0 – 28:0); some markers of DAG fragments (DAG 30 – 36) and small amounts of unsaturated free fatty acids (such as m/z 236, 264 for C16:1 and 18:1). An additional series of minor peaks (m/z 312, 326, 340, 354, 368, 382/384, 394/396/398, 410/412/414/416, 424/426/428) indicates the presence of what might be markers for sterols (m/z 368, 380, 382, 386, 394, 396, 400, 412, 414). At the high end of the mass spectrum an occasional peak is visible for what could represent the molecular ion of C42 and 44 wax esters (m/z 620, 648).

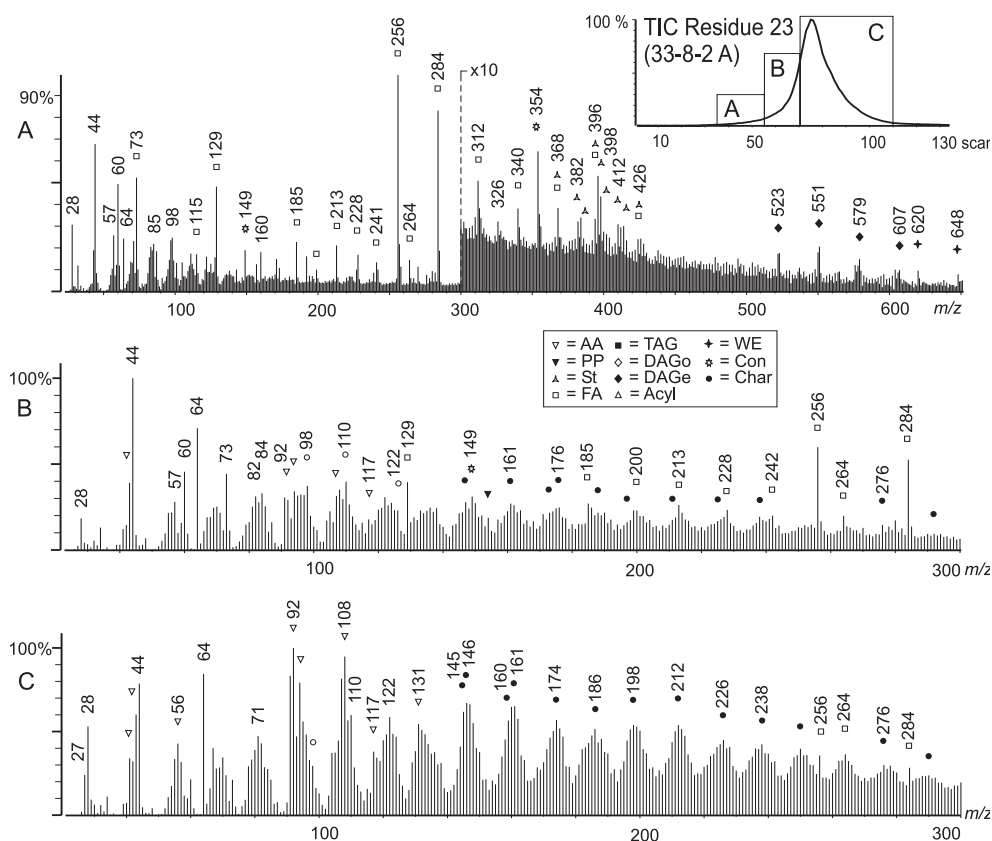


Figure 6: Cluster A₂ - DTMS results for charred residue 23 (nr. 33-8-2a)

A: Partial mass spectrum of the low temperature range shows a mixture of lipid indicators including (FA) free fatty acids; (DAGe) even numbered DAGs; (Acyl) acylium-ions; (St) various sterols including plant sterols and cholesterol; (WE) some markers for wax esters; and (Con) an occasional contaminant. B: At slightly increased temperatures, additional markers can be seen for (PS) residual oligosaccharides; and (Char) a condensed polymeric network. C: The high temperature mass spectrum contains mostly markers for (AA) amino acid and the (Char) thermally stable condensed polymeric network.

In the medium temperature range (Fig. 6B), some markers for intact polysaccharides, functionalised furans and alkyl-furans (m/z 98, 110), for intact peptide or proteins (m/z 154) and for residual fatty acid (m/z 256, 284) can be seen. The pyrolysis range (Fig. 6C) shows series of m/z values indicative of a highly condensed aromatic material. Peaks with both odd and even values are present. The peak for m/z 64 (SO_2) is very prominent in the higher temperature range, which suggests the presence of sulphur-containing inorganic compounds.

Table 6: Markers for other compounds in DTMS spectra.

Pyrolysis markers	m/z values
Markers for Aromatic Hydrocarbons	
Alkylated Benzenes	91, 92, 115, 119
Alkylated Naphthalenes	128, 142, 156, 170
Alkylated Phenantrenes/Antracenes	178, 192, 206, 220, 234
Alkylated Pyrenes	202, 216, 230, 244, 258
Alkylated Chrysenes	228, 242, 256, 270, 284
Markers for other compounds	
Aliphatic compounds	55, 56, 57, 69, 70, 71, 83, 84, 85, 97, 98, 111, 112, 113, 125, 126, 139, 140, 153, 154, 167, 168, 181, 182, 195, 196
CO and CO ₂	28, 44
SO and SO ₂	48, 64
H ₂ S	34
Elementary Sulphur	32, 64, 96, 128, 160, 192, 224, 256
Markers for Contaminants	
Phthalates	149, 167, 279
Squalene	410
Santovac 5 (pentaphenylether)	354, 446

4.5. Chemotype C

Chemotype C (Fig. 4b, vector 5) is characterised by: i) markers for peptides or intact proteins; and ii) amino acid markers.

DTMS results for representative charred residue 26 (nr. 34-0-30) show a lipid profile (Fig. 7A) comparable to that of residue 1 (nr. 7-7), including high peaks for saturated free fatty acids (C8:0 - 18:0) and small amounts of unsaturated free fatty acids (C16:1 and 18:1). Intact lipids are indicated by markers for acylium ions (C10:0 - C18:0); DAG - fragments both saturated (DAG 14 - 36) and unsaturated (DAG 36:1, 36:2, 34:1, 34:2, 32:1, 30:1, 28:1, 26:1, 24:1 and 22:1) as well as odd numbered DAGs (DAG 15 - 35), monoacylglycerol-fragments (MAG 16:0 - 18:0) and cholesterol.

The pyrolysis range (Fig. 7B) shows residual markers for peptides and intact proteins such as diketopiperazines (DKPs) and alkylated pyrrolidinediones and their fragmentation products (m/z 98, 138, 154, 168) and relatively intense mass peaks for amino acid moieties (m/z 91, 92, 94, 107, 108, 117, 131) indicative for a protein fraction comparable to the experimentally charred BSA (Fig. 2D).

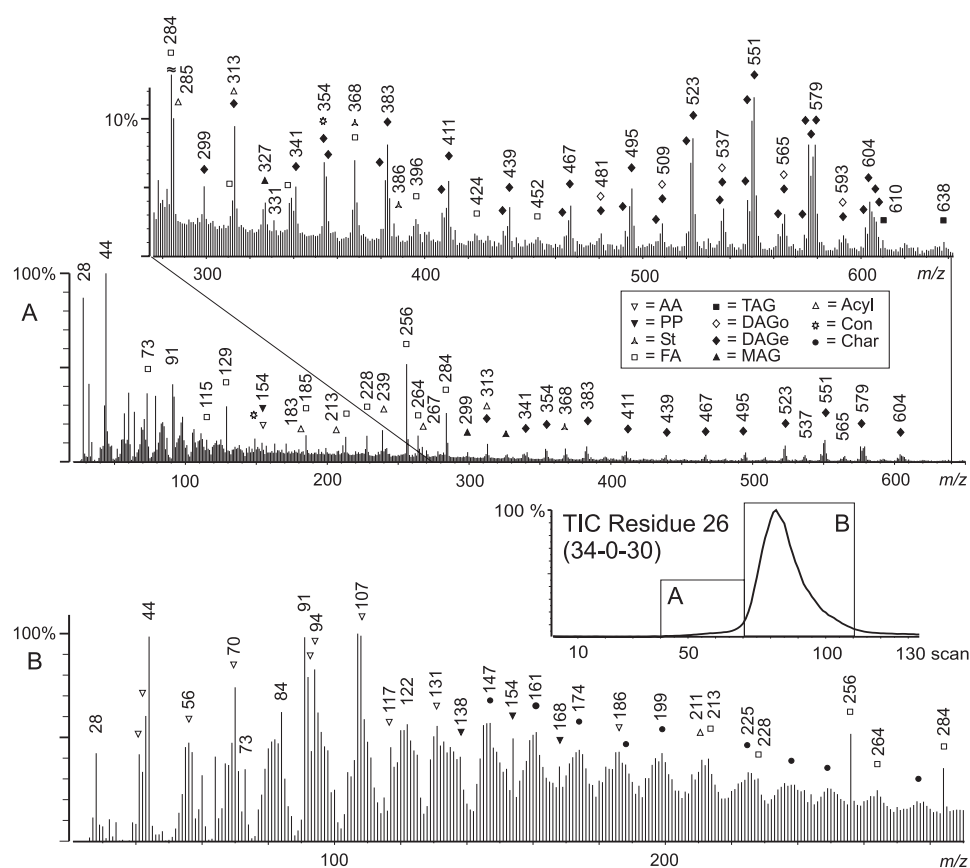


Figure 7: Cluster C - DTMS results for charred residue 26 (nr. 34-0-30)

A: The low temperature mass spectrum shows a mixture of lipid indicators including (FA) free fatty acids and their fragments; (TAG) triacylglycerides M^+ ions; (DAGo) odd numbered and (DAGe) even numbered DAGs and their fragments; (Acyl) acylium-ions; (MAG) MAGs; and (St) cholesterol. Some markers can be seen for (PP) peptides and proteins and (AA) amino acids and an occasional contaminant (Con). B: The high temperature mass spectrum also (Char) markers for a thermally stable condensed polymeric network.

4.6. Smaller Chemotypes B, D and E

The masses characteristic for the clusters B (vector 3), D (vector 4) and E (not depicted) are very similar, which is to be expected from their close association within the DF plot (Fig. 4a). The vectors generally depict the same characteristics: i) high peaks for m/z values 28, 32 and 44 throughout the measurement indicative of the presence of some air background in the ionisation chamber; ii) markers for short chain aliphatic compounds; iii) markers for alkylated

aromatic compounds; iv) some markers for sulphur-containing compounds (m/z 32, 34, 64, 66, 128, 256); and v) a small number of markers for contaminants visible in the low temperature range and the high temperature range (Table 6). All these characteristics indicate a low organic content for these residues, which causes the presence of small amounts of air and contaminants to play a role in the spectra and thus in the determination of their chemotype. The distinction between the smaller chemotypes can be seen more fully when looking at individual measurements in more detail.

Chemotype B

Soot residue 19 (nr. 31-4b) shows m/z values for sulphur-containing compounds and short chain aliphatic compounds in the evaporation range (Fig. 8A). No lipids can be detected. In the pyrolysis range (Fig. 8B) some amino acid markers can still be detected, although the majority of the peaks belong to alkylated aromatic compounds and the overall mass distribution suggests one or more homologous series with varying degrees of saturation such as alkylated benzenes and alkylated naphthalenes. As the pyrolysis-temperature increases (Fig. 8C), only markers for alkylated polyacenes can be seen ranging from naphthalene and phenanthrene to chrysene, in combination with some markers for small fragments (m/z 27, 28, 44, 64).

Chemotype D

Cream coloured residue 32 (nr. 35-20) primarily shows markers for sulphur-containing compounds and a peak representing a nitrogen-containing aromatic compound of unknown origin (m/z 173) in the evaporation range (Fig. 9A). No lipid markers can be detected. At slightly increased temperatures (Fig. 9B) aliphatic compounds become more prevalent. In the pyrolysis range (Fig. 9C) markers for a relatively intact protein fraction become evident. Some markers for peptides or intact proteins could be detected in minor amounts (m/z 138, 152, 154, 170, 194), and many markers for amino acids could be detected in relatively high intensities (m/z 70, 91, 92, 94, 98, 107, 108, 117, 131).

Chemotype E

Soot residue 28 (nr. 34-7-95b) primarily shows 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.

4.7. Comparing to CuPyMS

In general, the classification in five major chemotypes is consistent with earlier CuPyMS results in Chapter 2 (Oudemans & Boon 1996). The primary difference is the division of CuPyMS Cluster A, into two Chemotypes A₁ and A₂. This separation is primarily due to a better transmission of larger fragments that enables the detection of relatively large amounts of diacylglycerol-fragments in residues of chemotype A₁ and larger pyrolysis products from

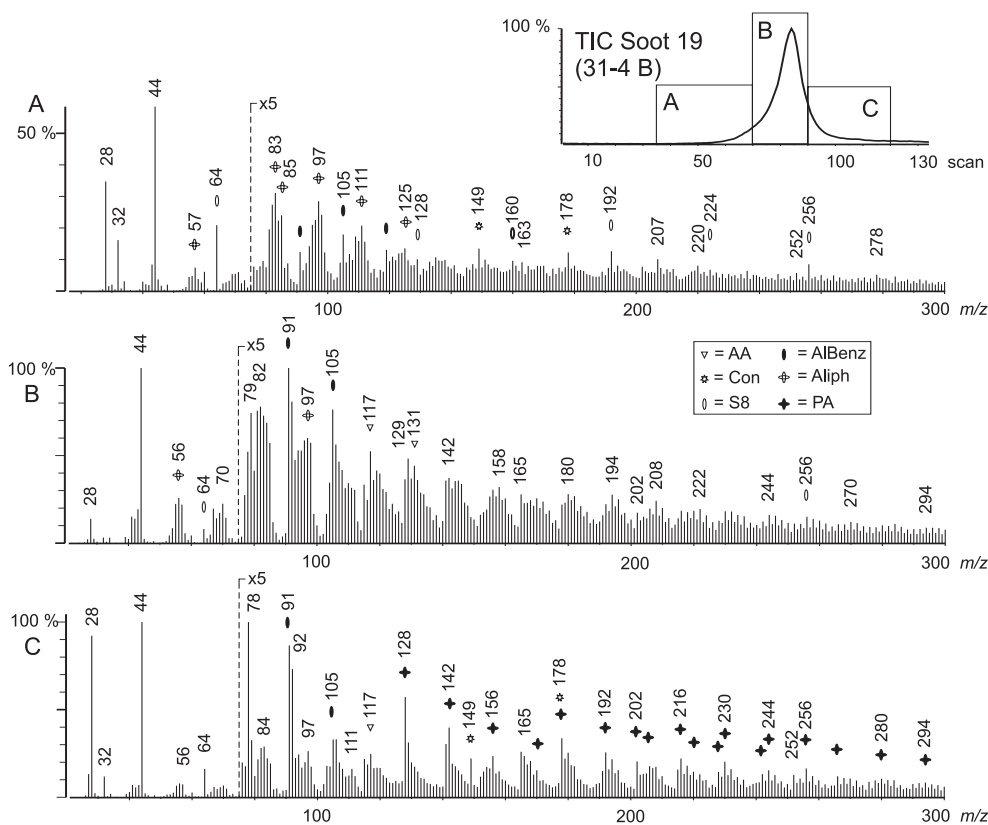


Figure 8: Cluster B - DTMS results for soot residue 19 (nr. 31-4b)

A: The low temperature results show a mixture of (S8) elementary sulphur; (AlBenz) alkylated benzenes; and (Aliph) aliphatic components and (Con) an occasional contaminant. B and C: At higher temperatures, mass spectra also include (AA) a few amino acid markers and (PA) many markers for polyacenes.

polysaccharide chars in chemotype A₂. The chemical characteristics for chemotype C are more or less consistent, and slight differences can be noted when comparing chemotypes B, D and E to the earlier clusters. These chemotypes are relatively less strictly defined and characterised primarily by a low organic content.

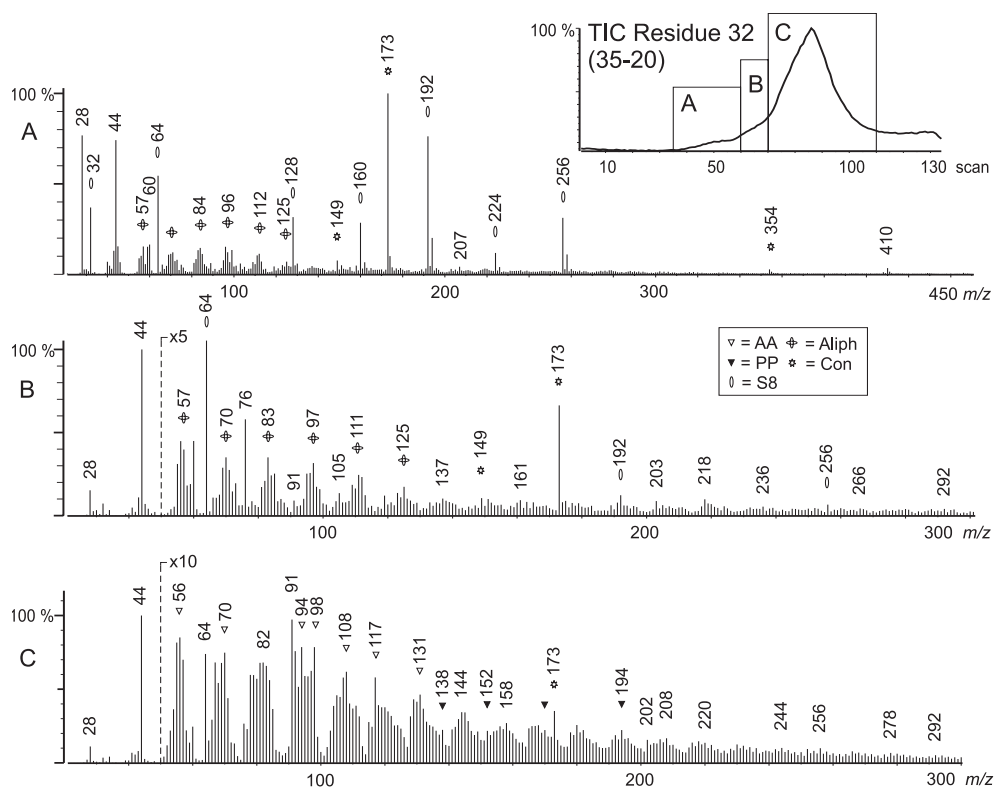


Figure 9: Cluster D - DTMS results for cream coloured residue 32 (nr. 35-20)

A and B: At the lower temperature ranges MS results show a mixture of (S8) elementary sulphur; (Aliph) aliphatic components and (Con) an occasional contaminant. B: The high temperature mass spectrum mostly shows (AA) amino acid markers and (PP) peptide and protein markers.

5. Discussion

5.1. Formation processes

The ability to disseminate the use of particular biomaterials in prehistoric times through organic residue analysis is to a large extent determined by a correct understanding of the formation processes that influence the chemical composition of the remaining residues.

Transformation processes include processes in the original prehistoric context or “systemic context” the so called C₁-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 C₂-transforms (cultural transforms) that can take place during and after excavation (Schiffer 1972, 1983). The formation processes most significant for organic residue analysis are the C₁-transforms “mixing” and “thermal degradation” and the N-transforms “biodegradation” and “exchange or organic compounds between soil and residue” (see also Chapter 1). 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. The chemical processes that might have been significant in the formation and preservation of solid organic residues are discussed in the following subsections.

5.2. Thermal degradation

The thermal degradation that takes place during heating of foods is of great importance to the study of solid organic residues. Charring is one of the most important formation processes that results in preservation of solid organic residues on ceramic vessels. DTMS results of many charred residues show the presence of thermostable polymers with varying degrees of condensation (Oudemans & Boon 1991, 1996; Oudemans *et al.* 2005).

An earlier model for cellulose char formation (Boon *et al.* 1994; Pastorova *et al.* 1994) gives some insight into the way the preservation of biomolecular characteristics might work. The model proposes the formation of a condensed thermostable network polymer consisting of furanoid and (hydroxy) aromatic skeletons, hydrocarbon side chains and a large number of carboxyl and carbonyl groups. The polymer is formed at temperatures between 250 and 310 °C and consists of a slowly growing three dimensional network that combines larger, non-volatile residual structural elements, such as anhydroglucose cores extended with glycol-aldehyde side chains, and small volatile compounds, such as enolic furans and glycol-aldehydes, trapped after their release inside the char (Boon *et al.* 1994). In chars heated over 310 °C, a disproportionation of the furan dominated polymer occurs by loss of CO and CO₂ leading to the formation of a purely aromatic polymer with ever increasing amounts of C=C bonds and loss of hydroxyl groups (Boon *et al.* 1994; Pastorova *et al.* 1994). DTMS results (Fig. 1 A - D) indicate that the chemical mechanisms that take place during charring of amylose are comparable.

Earlier DTMS studies of protein chars have led to the definition of three thermal degradation phases in whole peas and Pisane HD (a pea protein isolate) heated under anoxic conditions up to temperatures ranging from 130 to 700 °C for a maximum of 2 hours (Braadbaart 2004; Braadbaart *et al.* 2004a). The original protein markers could still be observed up to 270 °C. The mild thermal degradation phase - up to temperatures ranging from 270 °C to 310 °C - results in markers for alkylated phenols and benzenes (*m/z* 91, 92, 94, 105, 107, 108, 119, 122), as well as odd numbered markers that can tentatively be attributed to N-containing hetero-cyclic compounds (*m/z* 117, 131, 133, 147, 161, 175, 189, 199, 213, 227), indicating the formation of a material with mainly aromatic compounds. The medium thermal degradation phase -

temperature range from 310 °C up to a transitional stage from 400 to 440 °C - results in similar spectra but was signified by a decrease in odd numbered markers and an increase in even numbered masses (m/z 146, 160, 174). This shift suggests a loss of nitrogen from the newly formed aromatic material, a phenomenon that becomes more pronounced at higher temperatures. The severe thermal degradation phase - temperatures over 440 °C - results in DTMS spectra with ever decreasing yields of pyrolysis products and remaining mass peaks at m/z 27 (HCN), 28 (CO), 44 (CO₂), 48 (SO), 64 (SO₂), 78 (benzene), and 92 (toluene).

Although no model has been formulated for the formation of protein chars, it is clear that both protein- and polysaccharide-chars form condensed thermostable polymers at temperatures between 270 and 310 °C. At these temperatures it is still possible to distinguish pure polysaccharide chars from chars that have a protein component. Pure polysaccharide chars show a predominance of even numbered m/z values and may contain some residual sugar characteristics in the lower temperature range of the DTMS spectrum (Fig. 1 C and D). Protein containing chars often show some residual markers for peptides and amino acids (m/z 154, 117) and show equal intensities for both even and odd numbered m/z values (Fig. 2D). Although the presence of a protein fraction in the original material can thus be identified, it is impossible to exclude the presence of a polysaccharide fraction in charred materials at this time.

As the heating temperature increases to the range between 310 - 400 °C, the homologous series of DTMS markers show distinct similarities between chars originating from proteins or polysaccharides, and masses representing alkylphenols and alkylbenzenes are present in both. An increasingly similar chemical composition is observed, suggesting that the remaining polymer becomes an increasingly C-enriched aromatic product without any other significant functionalities (Boon *et al.* 1994). At such temperatures it becomes increasingly difficult to determine the original biomaterials involved in the char formation. However, under the cooking/charring conditions to be expected from prehistoric cooking practices in ceramic vessels over open fires, temperatures higher than 300 °C are probably not reached inside the vessel.

5.3. Exchange of organic compounds between soil and residue

Although the N-transforms can have serious effects on organic residues, it is significant to notice that the chemical classification in clusters A₁ through D is not related to the kind of sediment in which the residue was buried. Residue classification shows no correlation with the sediment type surrounding the residue (Table 1). An earlier PyMS study compared the chemical composition of organic residues with that of two peat samples from the excavation in Uitgeest-Groot Dorregeest. In Chapter 2, peat samples clustered together and away from all archaeological residues and were characterised by markers absent from archaeological residue samples such as markers for lignins and intact polysaccharides (Oudemans & Boon 1996). More detailed study through PyGC/MS in Chapter 3, confirmed the absence of such peat compounds in the archaeological residues (Oudemans & Boon 1991). PyMS and DTMS results give no indication that significant amounts of organic material was exchanged between soil and residue and no significant difference in degradation could be determined between different

Table 7: Chemotypes and their Biomolecular Origin

Chemo type	n	Residues	Example	Chemical Characteristics	Original Biomaterials
A ₁	11	1, 4, 8, 9, 15, 22, 25, 30, 35, 36, 37	Charred residue 1 (7-7)	<ul style="list-style-type: none"> - Short chain FA and DAGs - Odd numbered DAGs - Cholesterol - Protein/peptide markers - Amino acid markers - Charred protein/polysaccharide 	Charred animal product (most likely 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	Charred residue 23 (33-8-2a)	<ul style="list-style-type: none"> - Short chain FA and DAGs - Unsaturated FA (C16:0 & 18:1) - Plant sterols - Waxes (plant leaf wax) - Residual polysaccharide markers - Some protein/peptide markers - Charred polysaccharide/protein 	Charred combination of (leafy) vegetables and grain, possibly in combination with an animal meat or fat.
B	3	7, 11, 19	Soot residue 19 (31-4b)	<ul style="list-style-type: none"> - Aliphatic compounds - Sulphur-containing compounds - Alkylated polyacenes 	Low organic content – Soot or smoke condensate.
C	3	2, 26, 34	Charred residue 26 (34-0-30)	<ul style="list-style-type: none"> - Markers for intact peptides - Amino acid marker - Short chain FA and DAGs - Odd numbered DAGs - Cholesterol 	Mildly charred animal product without starch (possibly milk).
			Red brown residue 2 (8-1)	<ul style="list-style-type: none"> - Markers for intact peptides - Amino acid marker - No lipids 	Special protein-rich, lipid-free non-food product (used for decoration).
D	6	6, 10, 18, 31, 32, 33	Cream coloured residue 32 (35-20)	<ul style="list-style-type: none"> - Markers for intact peptides - Amino acid markers - Sulphur containing compounds - Aliphatic compounds - No lipids 	Low organic content - Special protein-rich, lipid-free food (egg white), or non-food product (bone or skin glue).
E	1	28	Soot residue 28 (34-7-95b)	<ul style="list-style-type: none"> - Sulphur-containing compounds 	Low organic content.

sediments within Uitgeest-Groot Dorregeest. This is in agreement with a study of extractable lipid in ceramics from Great Britain (Heron *et al.* 1991). At this stage it cannot be excluded that a small amount of organic compounds (short chain fatty acids, or water soluble polysaccharides) were exchanged between soil and residue, but the chemical classification is not affected by any such exchanges.

5.4. Biomolecular Origin of Residues

In spite of the broad range of original biomaterials involved, and the unique series of transformation processes each residue has undergone, tentative identifications of the original biomaterials can be made in many cases (Table 7).

Chemotype A₁ – Charred residue 1 (nr. 7-7)

The low temperature range of charred residue 1 (nr. 7-7) contains a relatively well-preserved lipid fraction with short chain lipids (both in fatty acids and in intact acylglycerides), cholesterol and acylglycerides with odd numbers of carbons. The biomolecular origin of such a lipid profile could be found in ruminant milk fats which commonly contain relatively high amounts of lower saturated acids (Breckenridge & Kuksis 1967, 1968; Marai *et al.* 1969; Christie 1981; deMan 1999). The presence of acylglycerides with an odd number of carbons seems in agreement with a milk fat origin (Murata 1977; Mottram *et al.* 1997; Mottram & Evershed 2001), although a post-depositional bacterial origin cannot be excluded at this time. The condensed polymeric component of the residue (Fig. 5B) is very comparable to charred BSA (Fig. 2D), and shows some residual peptide or intact protein markers as well as a higher mass envelope including both odd and even numbered mass peaks. Either a pure protein char or a protein/polysaccharide mixture could render such results. Residue 1 (nr. 7-7) can be identified as a relatively well-preserved animal product (most likely a ruminant milk) possibly cooked in combination with a starch. A cooked dairy product or a cereal-gruel seems to be the most likely origin of this residue.

Chemotype A₂ – Charred residue 23 (nr. 33-8-2a)

The lipid profile of charred residue 23 (Fig. 6A), shows high peaks for saturated free fatty acids (C10:0 – 28:0), a small amount of unsaturated C16:1 and C18:1 and some markers for DAG fragments in the range DAG30 - DAG36. The origin for the very long chain fatty acids can probably be found in the presence of plant waxes. Common components of either plant epicuticular wax (Kollattukudy 1976; Bianchi 1995) are long chain even numbered fatty acids ranging from C20:0 through 28:0 (m/z 312, 340, 368, 396, 424); markers for wax esters in the range of C38 – C52 (m/z 536, 564, 592, 620, 648); odd numbered long chain n-alkanes (C21 - C35), and even numbered primary alcohols in the C22 - C34 range. Although no indications could be seen for long chain alcohols or alkanes, minor peaks could be seen for wax esters C42 - 44. 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 m/z 396 through m/z 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) have been shown to survive charring at 250 °C for 120 min (Braadbaart 2004), and many studies have shown the survival of cholesterol (Evershed *et al.* 1990; Oudemans & Boon 1991; Evershed *et al.* 1995b) in ancient ceramics. However, plant sterols have only been identified in

soils and coprolites from archaeological context. The medium temperature spectrum (Fig. 6B) shows a mixture of incidental residual polysaccharide markers and markers for intact polypeptides and amino acids. The high temperature spectrum (Fig. 6C) shows some residual polysaccharide markers and a high mass area with an emphasis on the even mass values, indicative for a considerable polysaccharide component. 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.

Cluster B - Soot residue 19 (nr. 31-4b)

Residue 19 (nr. 31-4b) is a residue 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), but may also originate from the soil in which the ceramic lay buried. 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 (Fig. 8B and 8C) suggests a wood smoke condensate or soot, an origin consistent with the situation of the residue on the exterior of the vessel. In the evaporation range (Fig. 8A), markers for short chain aliphatic compounds can be seen, indicating their origin from evaporation rather than pyrolysis. This origin contrasts with earlier interpretation that these compounds originate from pyrolysis of a polymeric networks (Oudemans & Boon 1996). It is possible that the aliphatic compounds are a minor component of the smoke condensate. However, considering the low organic content of the residue, the inclusion of a minor internal source in the mass spectrometer cannot be excluded.

Cluster C - Charred residue 26 (nr. 34-0-30)

The lipid profile of residue 34-0-30 contains a lipid fraction (Fig. 7A) not unlike the lipid fraction in residue 1 (nr. 7-7). The protein fraction shows a high degree of preservation (Fig. 7B) with markers for intact peptides such as diketopiperazines (DKPs) and alkylated pyrrolidinediones and relatively high mass peaks for amino acid moieties. A mildly heated protein source such as a charred milk product might render such a pattern easily, due to the thermal stability of casein. But a similar spectrum was obtained in the heating experiments with BSA (Fig. 2D), so the exact origin of the protein material cannot be established. Although the presence or absence of a starch source cannot be proven at this stage, it is likely that starch is either absent or is only a minor component of the original material. No positive indication for the presence of residual polysaccharide-characteristics can be seen, contrary to what one would expect in a residue with such limited thermal degradation. The origin for residue 34-0-30 is probably a lightly charred animal product, most likely milk.

Cluster C – Red-Brown “pigment” residue 2 (nr. 8-1)

Not all residues in cluster C are charred: residue 2 (nr. 8-1) is a red brown material situated on the exterior of the vessel. The placement of the material suggests the use of an organic material to decorate the vessel. Visually similar materials were seen applied to the exterior of various vessels in the Uitgeest-Groot Dorregeest assemblage in dots, stripes or small patches (Abbink 1999, 233 & 289). It is significant to note, that residue 2 contains absolutely no chemical evidence for the presence of lipids. Its placement in cluster C is based on the presence of markers for a well-preserved protein fraction. The absence of lipids in this residue probably indicates the use of a special protein-rich and lipid free material (possibly mixed with a pigment or other colourant) for decoration without heating the ceramic. It is possible that the material was applied to the surface of the vessel immediately after firing while the vessel is still warm. Most likely biomolecular origin of the residue would be egg white or bone glue (gelatin). The thermal stability of gelatin (Wong 1989) might render a well preserved protein profile even after some minor heating.

Cluster D - Cream coloured residues 32 (nr. 35-20) and 31 (nr. 35-7-28)

The residues 31 and 32 do not only differ from the other residues in visual appearance, they are also rather unusual in their chemical composition. The absence of lipids and the presence of a relatively well-preserved protein profile make these residues very similar in chemical composition to residue 2 (nr. 8-1) in cluster C. A low organic content has placed these residues in cluster D. Residues 31 (nr. 35-7-28) and 32 (nr. 35-20) may be the result of the preparation of a special kind of food (egg whites) or non-food product (bone or skin glue). It is obvious from the absence of markers for a polymeric network structure in the high temperature range (Fig. 9 C) that the residues have not undergone severe charring or may even have been kept in their vessel without heating.

6. Conclusions

Direct temperature resolved mass spectrometry in combination with multivariate analysis is a unique micro-analytical strategy for the characterisation and classification of solid organic residues from archaeological context. Information about the overall chemical composition is combined with a high sensitivity for a wide range of compound classes as diverse as lipids, waxes, polynuclear aromatic compounds, oligosaccharides, small peptides and protein fragments, and a variety of thermally stable (more or less condensed) polymeric char structures. The lack of any sample preparation prior to analysis makes DTMS an optimal technique to analyse extremely small amounts of solid organic mixtures. DTMS can be applied in archaeology in the study of organic residues of any kind, be it organic residues of food or adhesives in ceramics; paint residues from cave paintings, classical statues or ancient wooden objects; hafting residues on stone or bone tools or metal objects; or remainders of

mummification materials, inks, seals or other complex mixed organic materials. Within organic residue analysis in ceramic studies, DTMS is primarily unique in its capacity to rapidly determine overall chemical composition of solids without losing sensitivity for a wide range of compound classes. It prevents the misguided focus on the extractable part of the residues and opens up a wide range of biomolecular sources (such as protein and polysaccharide remains and polymerised lipids) that would otherwise remain undiscovered (especially if denaturation through charring or oxidation would prevent extraction).

The use of DTMS in combination with multivariate analysis, confirmed many of the earlier results obtained using Curie-point pyrolysis mass spectrometry (Oudemans & Boon 1991, 1996), and has resulted in a more detailed classification due to the measurement of a much wider range of masses. Charred residues have been better classified based on the identification of intact lipids and fragments for various condensed aromatic polymers. The temperature-resolved information obtained in DTMS measurements facilitates the interpretation of the mass spectrometric data in terms of chemical structure and illustrates that classifying characteristics are related to particular charred biomaterials. Experimentally “charred” modern foodstuffs (starch and protein) are used as reference materials to identify the biomolecular characteristics preserved throughout the thermal degradation that might take place during cooking and charring in ceramic vessels.

A combination of marker components and temperature-resolved information from the DTMS profile, give indication for the origin of each cluster of residues. Cluster A₁ contains charred animal products (most likely milk), possibly in combination with a starch. Cluster A₂ contains mildly charred (leafy) vegetable and grain mixtures. Cluster B contains only smoke condensates (soot) from the wood fires used for cooking. Cluster C contains a group of fairly diverse residues that represent both residues of mildly charred animal products without starch; and residues of special protein-rich, lipid-free non-food products (possibly used for decoration of vessels). Cluster D contains mildly heated residues of protein-rich, lipid-free foods or non-food products (bone or skin glue).

Although many molecular characteristics of the original foods have been lost as a result of extensive thermal degradation during cooking, some specific characteristics have been preserved within the newly formed, condensed polymeric char-material. Although the level of interpretation remains limited to general food groups, it is the interpretation of these specific “signature” characteristics that can render unexpected and exciting information about the origin of solid organic residues from archaeological context.

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 than 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 °C 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 hydrolysis, 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 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 (Davidek *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 than 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 than 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 nitriles (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 $-\text{CH}_3$ or $-\text{CH}_2-$ 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 ($\text{C}=\text{O}$), such as in carboxylic acids and their salts at 166 - 181 ppm, aliphatic esters $\text{R}-\text{COO}-\text{R}'$ at 169 - 176 ppm; and amides $-\text{CONH}_2$ 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 ($\text{R}-\text{CN}$), 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 c	R-CN Nitril	O-alkyl	C-N Collage n	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 (Davidek *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 (A₁, A₂, 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 A₁ and A₂, 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 A₁ and A₂

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 A₁ and A₂. There are indications that different original materials formed the chars of chemotypes A₁ and A₂ - A₁ being rich in proteins and lipids, and A₂ 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 A₁ 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 few 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 A₂ 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 °C (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 °C 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 A₁. 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 (Davidek *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 than 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	Charred 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 pyrolysis. 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 than 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 archaeological 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 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.

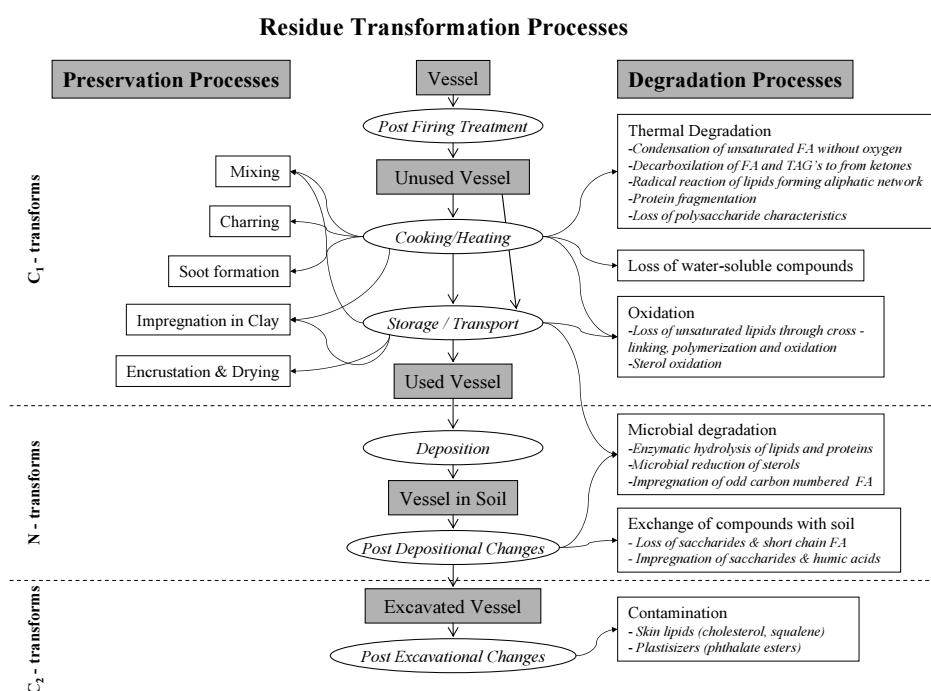


Figure 1: Residue Transformation Processes.

Transformation processes include processes in the original prehistoric context or “systemic context”, also known as C₁-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 C₂-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 influence 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 (Davidek *et al.* 1990, 195); decarboxylation of fatty acids and acylipids forming ketones (Davidek *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 performed 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 than for storage or transport. Some other residues are also found in this vessel type (chemotype A₁) 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 A₁ or A₂. 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 addition 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 than 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.

Appendix 1

Materials

The Dutch National Service for Archaeological Heritage (Rijksdienst voor het Oudheidkundig Bodemonderzoek) excavated the settlement site of Uitgeest-Groot Dorregeest in the early 1980s. The archaeological remains at the site date back to the Late Iron Age, the Roman period and the Early and Late Medieval period and represent what may have been a continuous occupation. In this appendix, the excavation, its geological setting and a brief reconstruction of the original village will be sketched.

The indigenous ceramics for the Iron Age and the Roman Iron Age settlement were extensively studied for variations in technology, form and function by Abbink (1999). The work in this thesis is performed on organic residues originating from the assemblage of indigenous ceramics from Uitgeest-Groot Dorregeest. The selection criteria employed to choose the samples for this study will be clarified and the chosen vessels described and depicted.

1. Uitgeest-Groot Dorregeest

1.1. The Excavation

Uitgeest-Groot Dorregeest is situated just north of the contemporary town of Uitgeest in the province of 'Noord Holland' in the Netherlands (Fig. 1, 2). During the large-scale excavations of this settlement site between the fall of 1980 and the spring of 1983 the Dutch National Service for Archaeological Heritage (ROB) excavated about 3 ha of what once had been a small settlement situated on a dry, sandy coastal barrier (Fig. 1). Under the direction of Dr P.J. Woltering and the field supervision of H. ter Schegget, A.G. Jong and G.R. Tak, habitation remains dating back to the Late Iron Age, the Roman period and the medieval times were uncovered (Woltering 1982, 1983). Though never conclusively proven, the remains may well represent a continuous occupation from the Late Iron Age onwards (Besteman 1990b, a).

Although Uitgeest-Groot Dorregeest was excavated more than 25 years ago, the final site reports have not been published to date (van Heeringen & Koot 2005). Two short reviews have appeared (Woltering 1982, 1983) that include some reconstructed house plans and the situation of a number of water wells. Woltering also discussed the geology of the settlement, and a recent publication (van Geel *et al.* 2003) gives a more complete paleo-environmental reconstruction for the Roman period. In her ceramic study Abbink (1999, 63-80) summarises the geology and archaeological features and proposes a site reconstruction for the Roman period. Additional publications have appeared on imported Roman metals (Zoetbrood 1985), imported Roman ceramics (Erdrich 1996), Roman medicines (Buurman 1988), and medieval materials (Koning 1998) found at the site. Abbink published an extensive study on the relations between form, function and technology in handmade ceramics from Uitgeest-Groot Dorregeest (1985; 1999).

1.2. Geology and Habitation

Uitgeest-Groot Dorregeest is situated in a part of the Netherlands that underwent very diverse regional geological developments and was strongly influenced by the geological events occurring in the former IJ-estuary (Vos 1983; Zagwijn 1986). Although the original image of extreme geological fluctuations during the Holocene as a result of subsequent transgression and regression phases of the North Sea (Zagwijn 1986) has recently been modified (Weerts *et al.* 2000; de Mulder *et al.* 2003), it is clear that the geological development in the Western Netherlands varies depending on the specific location under scrutiny. These ancient 'wetlands' of the Northern and Western Netherlands are currently seen as dynamic containing a rich biodiversity that renders a broad spectrum of subsistence possibilities for people in pre- and protohistoric times (van Heeringen & Koot 2005, 4).

The geological features uncovered at Uitgeest-Groot Dorregeest depict a complexity typical for settlement sites from this period (Woltering 1982, 1983; Abbink 1999, 63-80; van Geel *et al.*

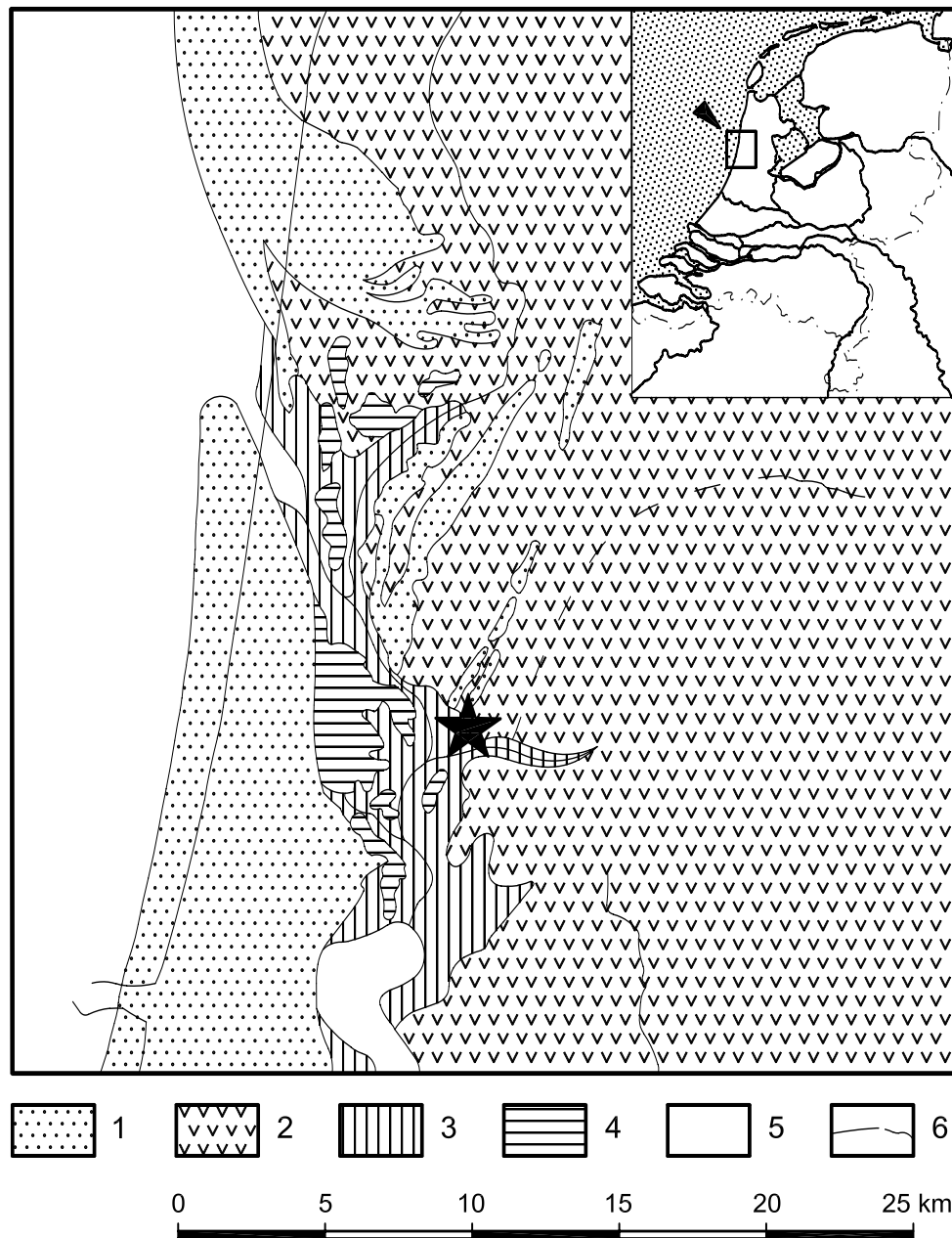


Figure 1: Map showing the location of the indigenous settlement at Uitgeest-Groot Dorregeest and its geological surroundings during the late Iron Age and Roman Period.

Legend shows (1) beach barriers and older dunes; (2) peat; (3) marine deposits, clay; (4) marine deposits, sand and loam; (5) open water and (6) rivulets. Modified after Van Geel and co-workers (2003).

2003). A maze of intersecting deposits showed evidence of many fluctuations in the local water level and the local salinity. A brief summary is given below.

Prior to habitation.

The excavation site at Uitgeest-Groot Dorregeest was located on the relatively dry sandy remains of a coastal barrier (formed during the Calais IV transgression phase). The coastal barrier was surrounded and partly overgrown by peat - the base of which was dated to circa 3900 BP using radiocarbon dating (State Geological Service, unpublished). Both the coastal barrier and the peat deposit were partly eroded by a complex system of continuously changing salt water creeks and sandy deposits from the Duinkirk I transgression phase. The earliest deposits of shells in these salt water creeks at Uitgeest-Groot Dorregeest consistently date back to circa 2400 BP by radiocarbon dating (Koning 1998; Abbink 1999; State Geological Service, unpublished). This gives a terminus post quem for the oldest possible habitation of this site in the Late Iron Age around 450 BC. This geological evidence is in agreement with van Heeringen (1992) who concludes that habitation in the Western Netherlands was mostly absent in the beginning of the Middle Iron Age, and a renewed 'colonisation' of the area took place in the fourth and third century BC. Most of the settlements from this period were located on higher lying river dunes although local fluctuations in geology and landscape did result in variation in habitation patterns (van Heeringen 1992).

First habitation in Late Iron Age.

The earliest signs of habitation are found in the bed of the large creek that borders the north and west of settlement. Figure 1 shows the creek bed at the beginning of the settlement in the Late Iron Age and in its final stage in the Roman period when erosion no longer took place. When the first occupation started, the salt water creek had changed to a fresh water gully draining the water from the peaty hinterland out to the sea. The earliest fills of the gully that actually contained debris of human activity (see Abbink 1999, features 10, 34, 37) were situated on the north side of the settlement. The pottery contained many typical Iron Age forms and lacked imported Roman vessels confirming a Late Iron Age (250 BC - 0) origin (van Heeringen 1992). Because this early material was usually found spatially and stratigraphically separated from the earliest ceramics from the Roman period, and no structural remains from the Late Iron Age were recovered Abbink concludes that the Late Iron Age habitation was probably located to the north or east of the excavated area (Abbink 1999, 63-64).

The settlement during the Roman period.

The indigenous settlement at Uitgeest-Groot Dorregeest (0 - 300 AD), was situated about 50 km north of the Roman - German border, on top of a small, relatively dry sandy ridge formed by the remains of a coastal barrier and a sandy deposit from the Dunkirk I period (Fig. 1, 2). Large raised bogs bordered the settlement on the east side while low marshes intersected by creeks surrounded the settlement on the west, north and north-east side (van Geel *et al.* 2003). The plant, pollen and fungi record showed that the region around the settlement was largely treeless and typical for transitional areas between wet and dry or salt and fresh water conditions

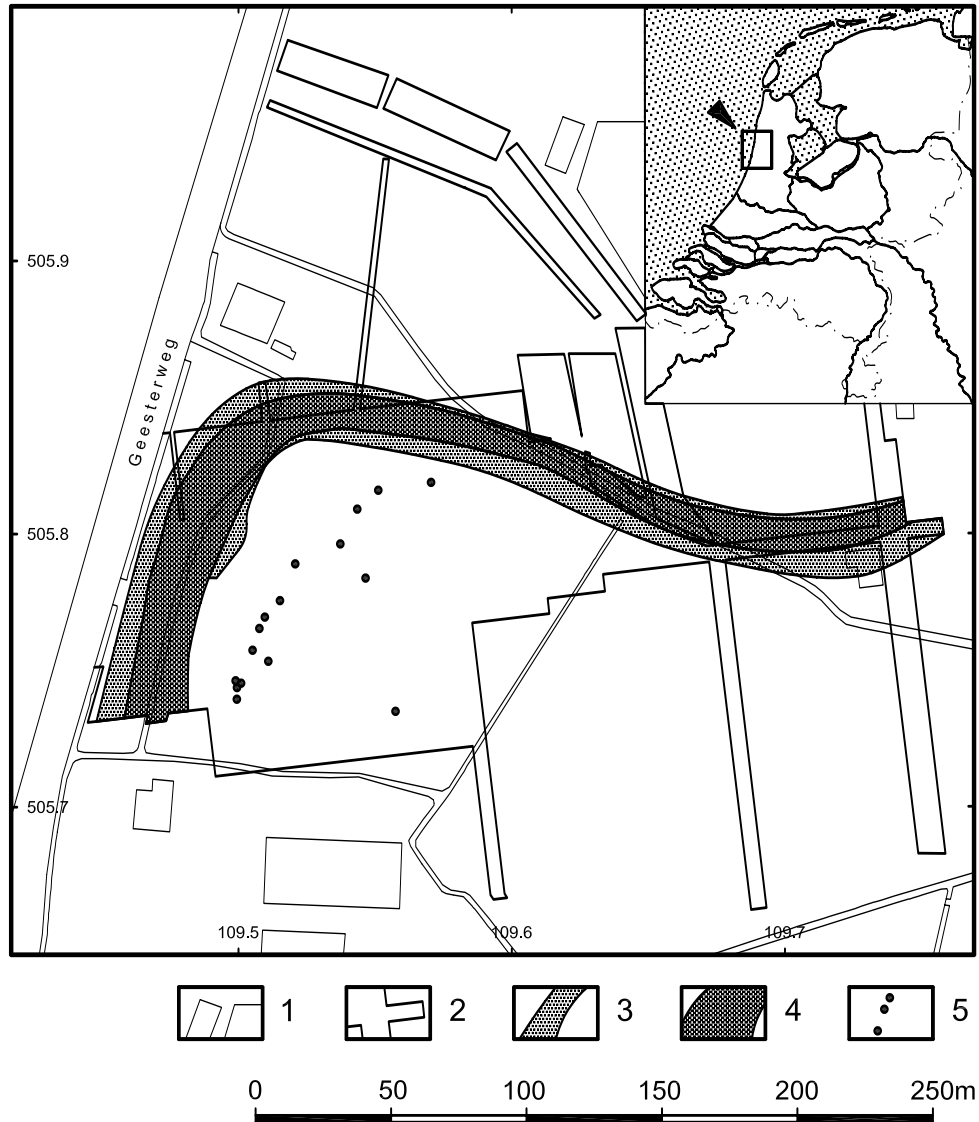


Figure 2: Uitgeest-Groot Dorregeest is situated on the north side of the contemporary town of Uitgeest, in the province of Noord Holland in The Netherlands.

The excavated area (3 ha) is bordered by the Geesterweg on the west side and the Rijksweg A9 on the east side. Legend shows (1) modern ditches; (2) boundaries of excavated area; (3) Dunkirk-1 creek: the course of the creek in the Late Iron Age and Roman period; (4) The course of the creek at the end of the Roman Period to early Medieval times; and (5) wells in the Roman period. After Abbink (1999, Fig. 3.1)

(van Geel *et al.* 2003). Van Geel and co-workers describe that the lower areas surrounding the settlement must have been quite moist, especially during the winter, but large grazing areas for herbivores must have been present on the natural grasslands on the salt marshes.

Radiocarbon dates of the fresh water gully suggest that the residual creek filled up rapidly during the Early Roman period (Koning 1998; Abbink 1999, 64) and contained water only for a brief period (50 - 100 years). Several layers of humic sand and clay containing occupation debris separated by thin layers of wind-blown white sand and imprinted with human footprints and prints of cattle and pigs. However, the Roman imports from the debris layers could all be dated to the 2nd and 3rd century AD (Zoetbrood 1985) suggesting that the creek held water for a much longer period. This discrepancy has not been explained to date.

What the indigenous settlement of Uitgeest-Groot Dorregeest exactly looked like, and how many houses it counted at any given time in the Roman period, may never be completely clear. The extreme density of features, the absence of most of the original floor level and the continuous erosion and re-deposition of sediments during the numerous changes in the course of the creeks, have obscured much of the original pattern from the contemporary eye (Woltering 1983; Abbink 1985; Abbink 1999, 66-67). The west side of the habitation area probably consisted of small number of three-isled farmhouses (between one and four) that were inhabited simultaneously, and periodically rebuild in more or less the same place (Abbink 1999). In addition to the farmhouses, the settlement contained several shed-like structures and several wall-ditch structures similar to those defined in the Assendelver Polders Project (Therkorn & Abbink 1987) and interpreted as dwellings without a stable area. A substantial number of round water-wells were excavated within the habitation area. Although 16 wells with linings of sods could be radiocarbon dated to the Roman period, none could be ascribed to a specific dwelling (Abbink 1999, 66-67). Rows of small posts bordered the settlement on the west and northeast side and are interpreted as a fences to keep domesticated animals away from the farmyards (van Geel *et al.* 2003). Several fragments of field systems on the east side of the settlement indicated agricultural activity close to home (Woltering 1983; Abbink 1999, 63-80). The prosperity of the inhabitants of the settlement at Uitgeest-Groot Dorregeest is clear from the archaeological evidence. Numerous luxury goods and imported materials (Zoetbrood 1985; Buurman 1988; van Heeringen 1992; Erdrich 1996; van Geel *et al.* 2003) were recovered from the site. Van Geel and co-workers propose that the prosperity of the settlement may have been the result of trade in cattle or secondary products of stock breeding such as hides, smoked meats, dairy products and wool with the Roman Empire.

The medieval period.

In the fourth century AD, a renewed period of erosion and re-deposition took place in the same creek system. A complex maze of gullies and ditches was formed. Most of these ditches contained medieval occupation debris from the Carolingian period (300 - 400 AD). Although features from this period frequently intersect older features a thick layer of wind blown sand usually separated them. The archaeological record suggests a continuous occupation from the Late Iron Age through the Roman period and up to the Carolingian Time (Besteman 1990b, a).

2. Ceramic Studies

2.1. Study by Abbink

A systematic study of the technology, from, function and use of handmade pottery from the Roman period settlements at Uitgeest-Groot Dorregeest and Schagen-Muggenburg, including an extensive inventory of technological and morphological characteristics, as well as contextual information and use-alteration characteristics of vessels, was performed by Abbink (1999). The overall goal of the study was to assess the technological know-how of the indigenous potters and to establish in what way they applied this know-how to make vessels with specific properties for specific vessel-use (Abbink 1985, 23).

2.2. On-Site treatment

An enormous amount of ceramic material was uncovered during the excavations at Uitgeest-Groot Dorregeest, as is frequently seen in settlement sites from the Roman period. During Roman times ceramic vessels were common utensils in every indigenous household. Ceramic vessels were obviously more frequently used and more easily discarded than in previous periods. Due to the large quantity of ceramics artifacts the first treatment and selection took place directly on the site at Uitgeest-Groot Dorregeest. Ceramics were washed with water and soft brush, dried on newspaper, and subsequently weighed, sorted and numbered by hand with waterproof ink. After this registration, a selection was made. Roman import ceramics and medieval ceramics were collected and treated separately from the indigenous ceramics (Besteman 1990b, a; Erdrich 1996).

The handmade ceramics from the Late Iron Age and the Roman period were numerous and fairly uniform in style and technology. A rigorous selection needed to be made between ceramics that needed to be documented and preserved for later study and those that could be discarded (Abbink 1999, 67-80). Between 60.000 and 70.000 shards of the total assemblage were selected for preservation based on two criteria: 1) the importance of the shard for documentation of the variation within the assemblage (rims, bases, profiles, decorated materials, handles and other indicative shards) and; 2) the importance of the shard for further study (including the presence of organic surface residues or other use-alterations).

2.3. Vessel Selection and Classification

However, even after an extensive restoration operation, it turned out to be impossible to establish an estimation of the total number of ceramic vessels involved, because very few complete profiles or vessels could be reconstructed. From a 3 ha excavation of extensively inhabited terrain, less than 20 complete vessels could be restored, and most of those were small

in size. Although this may be partially due to the nature of the settlement (much was discarded material), it is probably mainly due to the extreme fragmentation of the ceramic material as a result of the excavation techniques employed. Large-scale excavations using machines and systematic, un-monitored rapid excavating with shovel and pick axe, can not be expected to render much intact ceramic material.

If a complete profile was present or the four most important size variables (i.e. rim diameter, maximum circumference, height and base diameter) could be measured vessel-profiles were selected to be included in the study (Abbink 1999, 67). Due to the poor preservation of pottery and the over-representation of smaller vessels, the sample of selected vessels had to be extended with some incomplete, partial vessel profiles from the larger vessels. These partial profiles usually consisted of rim/upper-wall fragments including the maximum circumference, or base/lower-wall fragments with a large part of the lower wall preserved. The original analysis by Abbink was based on 147 selected vessels (Sample I). Sample I consisted of mostly indigenous ceramics from the Roman period, but also contained some late Late Iron Age vessels. According to Abbink there is little or no difference between the vessel shape and technology in these two periods. An additional test sample (Sample II) of 629 shards was chosen by in order to test the correlations detected in Sample I.

It is clear that the lack of control over the temporal aspects of the ceramic production or the changes in household inventory, the over-representation of vessels with a high breaking index (due to fragility or vessel-use) and the under-representation of larger vessels (due to poor reconstructability), make Sample I far from ideal in composition. It is completely unclear to what extent and in what way Sample I can be related to the overall composition of the ceramic assemblage of Uitgeest-Groot Dorregeest. In spite of these disadvantages Abbink concludes that the sample is adequate to study pottery production and use in prehistoric context (1985, 80).

Table 1: Morphological vessel classification (Oudemans & Boon1991; 1996)

Type	Name	Maximum Diameter [mm]	Rim [GD/RD]	Upper Wall [GD/H1]
I	Small Vessel	GD < 170	Wide GD/RD < 1.5	Short GD/H1 > 2.4
II	Medium Vessel	180 <= GD < 340	Wide GD/RD < 1.5	Short GD/H1 > 2.4
III	Large Vessel	GD > 350	Wide GD/RD < 1.5	Shortest GD/H1 > 2.9
IV	Jar-like Vessel	-	Narrow 2.1 > GD/RD >= 1.5	Long GD/H1 < 2.4
V	Other forms	-	-	-

2.4. Morphological Vessel Classification

The ceramic assemblage of Uitgeest – Groot Dorregeest contains primarily simple, wide mouthed, globular or ellipsoid jars with short rim and neck and a maximum diameter equal to, or slightly larger than, the rim diameter. Abbink's morphological study is based on the variability in size and shape as expressed by six form variables: diameter of rim (Rd); diameter of maximum circumference or greatest diameter (GD); total height (Htot); diameter base (BD); height from rim to the maximum circumference or upper-wall (H1); height from base to the maximum circumference or lower diameter (H2).

Based on measurements of the above six variables a first morphological classification was defined by Oudemans and Boon (1991; 1996) based on a number of relevant relationships: Type I - small, three partite, wide mouthed vessels ($GD/RD < 1.5$, $GD < 170$ mm and $GD/H1 > 2.4$); Type II - medium sized, three partite, wide mouthed vessels ($GD/RD < 1.5$, 180 mm $\leq GD < 340$ mm and $GD/DRG > 2.4$); Type III - large, three partite, wide mouthed vessels ($GD/RD < 1.5$, $GD > 350$ mm and $GD/H1 > 2.9$); Type IV - Jar-like, three partite, narrow mouthed vessels ($GD/RD \geq 1.5$ but $GD/RD < 2.1$ and $GD/H1 < 2.4$) and Type V - containing all other forms.

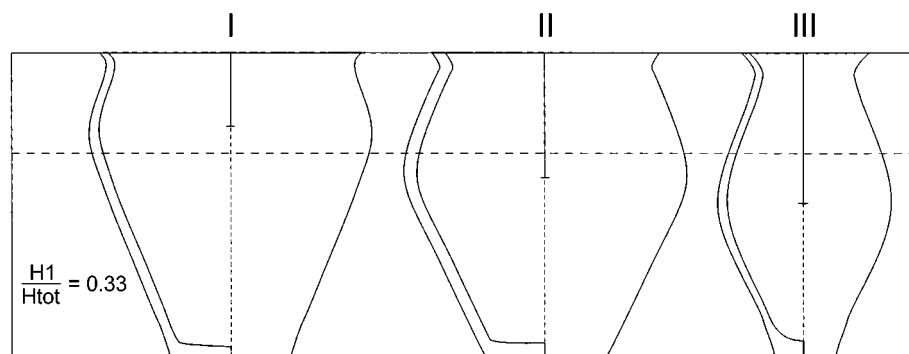


Figure 3: Three basic shapes are present within the three partite form of pottery from Uitgeest-Groot Dorregeest.

The major form (shape 1) is defined by more or less equal rim diameter, maximum diameter and height. The position of the maximum diameter relative to the total height of the vessel defines the difference between shape 1 and 2 and is related to vessel size (Abbink 1999, 284). Shape 2 occurs most frequently in smaller vessels, due to variation in size of the lower wall, while the majority of vessels with shape 1 have a maximum diameter between 250 - 330 mm (group 3). Shape 3 represents pottery in group 4, the jar-like vessels, when the height of the vessel exceeds the size of the maximum diameter, while the opening is small. After Abbink (1999, Fig. 8.25a). The dotted line indicates the ratio $H1/H_{tot} = 0.33$.

Type I, II and III contain three-partite, wide mouthed, globular or ellipsoid jars with short rim and neck and a maximum diameter equal to, or slightly larger than, the rim diameter. The main difference between the vessel types I, II and III is their different size. The maximum diameter proved to be an optimal measure for the overall size of the vessel and is therefore chosen as primary classifying characteristic for the remaining vessels (Table 1). Three size groups are

Table 2: Morphological vessel classification (Abbink 1999, 173, 386 & 299)

	Name	Size [GD]	Rim [GD/RD]	Shape [H1/Htot]& [H1/RD]	Non-metric variables	n	Function
1	Small Vessel	GD < 190	Wide GD/RD < 1.5		Including pedestal bowls	7	
1.1				H1/Htot < 0.33 H1/RD < 0.33	Roughly made; scraped; mostly smooth rim; no handles	7	Heating or storage of special food or non-foods
1.2				H1/Htot > 0.33 0.333 < H1/RD < 0.6	Detailed finish; polished; mostly smooth rim; handles occur regularly	12	Storage of special food or non-foods
2	Medium small Vessel	190 < GD < 295	Wide GD/RD < 1.5			28	
2.1	Small Medium	180 < GD < 280		H1/Htot < 0.33 H1/RD < 0.33	Roughly made; scraped upper wall; mostly decorated rim; often smitten lower wall; some handles	12	Cooking
2.2	Large Medium	GD > 280		H1/Htot > 0.33 0.333 < H1/RD < 0.6	Often smitten lower wall; mostly smooth rim; some handles	6	Special functions
3	Large Vessel	GD > 295	Wide GD/RD < 1.5			-	
3.1				H1/Htot < 0.33	Polished upper wall; often smitten lower wall; commonly decorated rim; no handles	33	Cooking
3.2				H1/Htot > 0.33	Polished upper wall; smitten lower wall; commonly decorated rim; no handles	21	Cooking
4	Jar-like Vessel	-	Narrow GD/RD > 1.5	H1/Htot > 0.33 & GD/RD < 1 H1/RD > 0.6	Always smooth rim; lower wall smitten 50% of the time	13	Cooking or Storage of dry foods
9	Other forms	-			Handles frequent	8	Storage of fluids
Total						147	

defined: Small, Medium and Large. Type I, the small vessels (GD < 170mm) contains a clearly defined size group. The remaining vessels in Type II and III (maximum diameters over 180 mm) show a more or less continuous class, with a less distinct separation between Type II and Type III. However, the very large vessels (Group III) with maximum circumference of 350 mm or more have relatively shorter upper walls as expressed in the GD/H1 ratio of 2,9 or more. Vessels from type Type IV are recognised by their narrow mouthed, tall shape and therefore appropriately called 'jar-like' (GD/RD ratio of 1,5 or more). The significantly tall upper wall is characterised by a ratio of GD/H1 smaller than 2,4.

In her conclusive publication Abbink (1999, 170-234 and 299-305) came to a morphological classification in five vessel groups with subdivisions in shape (Table 2 and Fig. 3). The main difference is the cut-of point between Groups 2 and 3 (at a GD of 295 mm) which differs from cut-of point between Type II and III (at a GD of 340 mm). A new addition to the original type division is Abbink's subdivision based on shape (Fig. 3). The major form (shape 1) is defined by more or less equal rim diameter, maximum diameter and height. The position of the maximum diameter relative to the total height of the vessel defines the difference between shape 1 and 2 (Fig. 3) and is related to vessel size (Abbink 1999, 284). Shape 2 occurs most frequently in smaller vessels, due to variation in size of the lower wall, while the majority of vessels with shape 1 have a maximum diameter between 250 - 330 mm (group 2 and 3). Shape 3 represents pottery in group 4, the jar-like vessels. The height of the vessel exceeds the size of the maximum diameter, while the opening is small.

Table 3: Residue types according to Abbink (1999, Table 8.15)

Residue Name	Description	Position of residue on vessel	Vessels containing residues [n=147]
Soot	Black dull to shiny residues	Exterior	66 vessels (= 45%)
Char	Dark brown or black residues, commonly with cracked or pitted surface	Interior	47 vessels (=32%)
Pigment	Red or dark brown residues in the form of drops or linear stains	Exterior or interior	8 vessels (=5%)
Creamy layers	Cream or light brown coloured layer	Interior	4 vessels (= 3%).

Although, other non-metric technological variables such as rim types, absence or presence of handles and treatment of the exterior surface were also registered, they were not used as meaningful criteria for the classification in vessel groups. These variables do often correlate highly with the pot groups and subgroups (Table 2).

Appendix 1

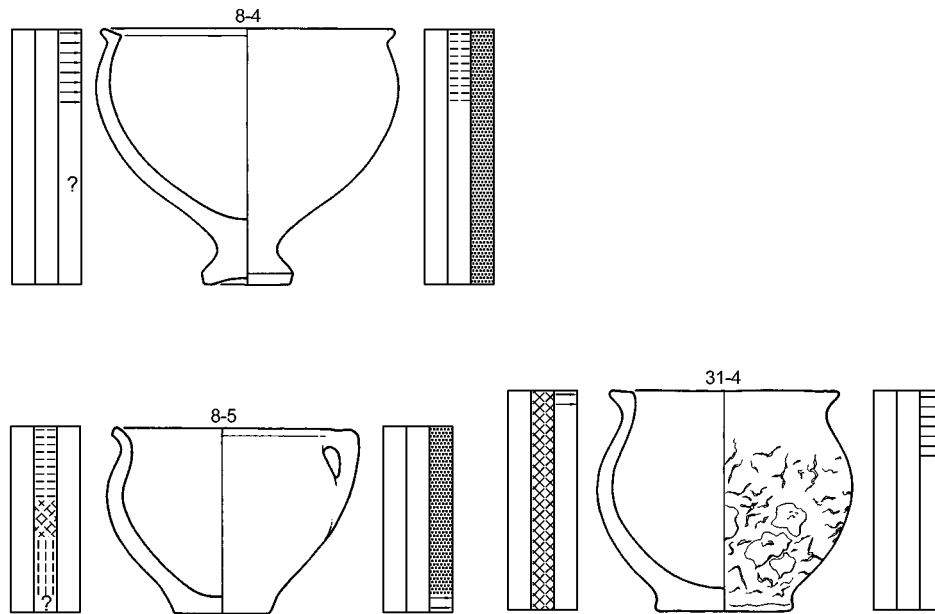


Figure 4a: Vessels from Uitgeest-Groot Dorregeest - Type I (Oudemans & Boon, 1991)

Vessel 8-4 has shape 1 while the two vessels below have shape 2 according to Abbink (1999). Legend for surface treatment can be found on page 193.

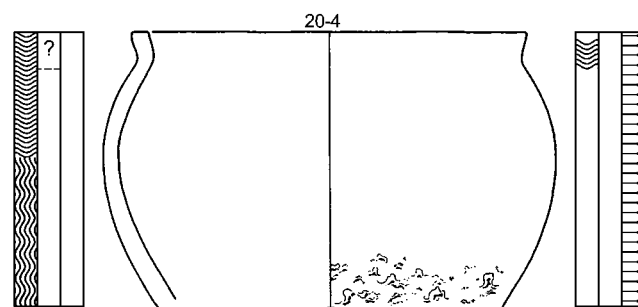


Figure 4b: Vessel from Uitgeest-Groot Dorregeest - Type II (Oudemans & Boon, 1991)

Vessel 20-4 falls in group 2 and has shape 1 according to Abbink (1999). Legend for surface treatment can be found on page 193.

0 50 100 150 mm

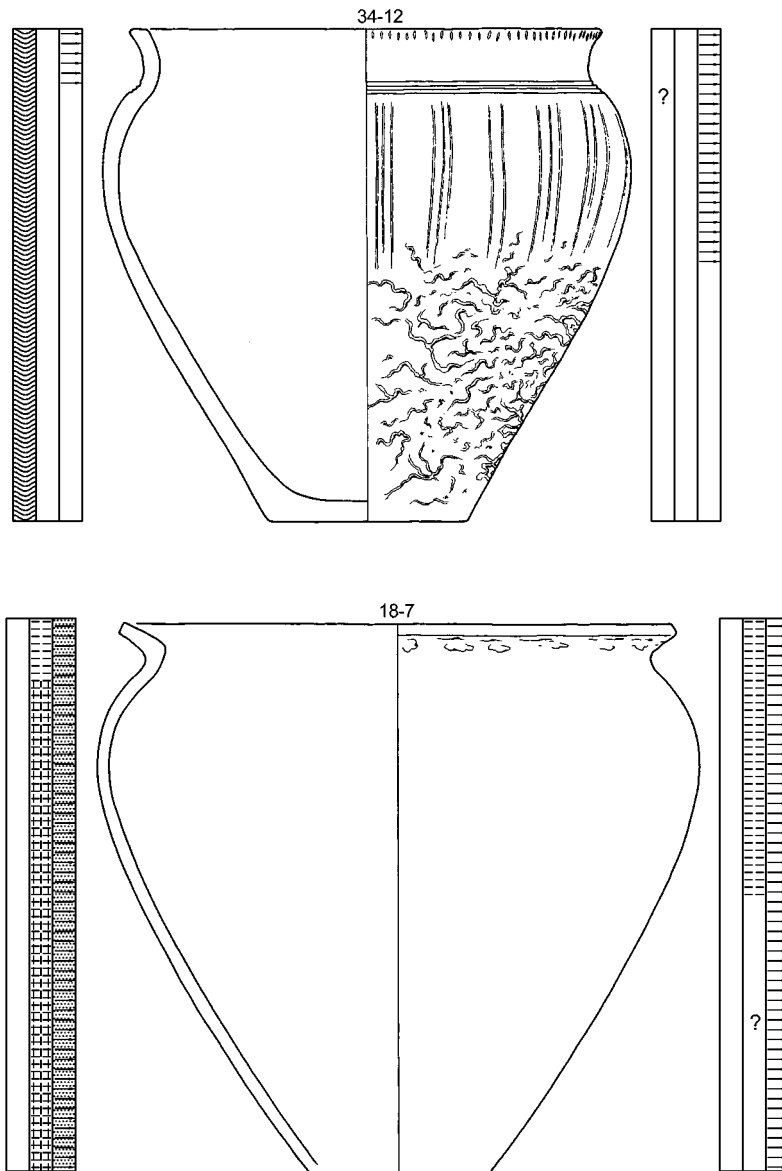


Figure 4c: Vessels from Uitgeest-Groot Dorregeest - Type II (Oudemans& Boon, 1991)

Vessels fall in group 3 (GD < 330 mm) and have shape 1 according to Abbink (1999). Legend for surface treatment can be found on page 193.

0 50 100 150 mm

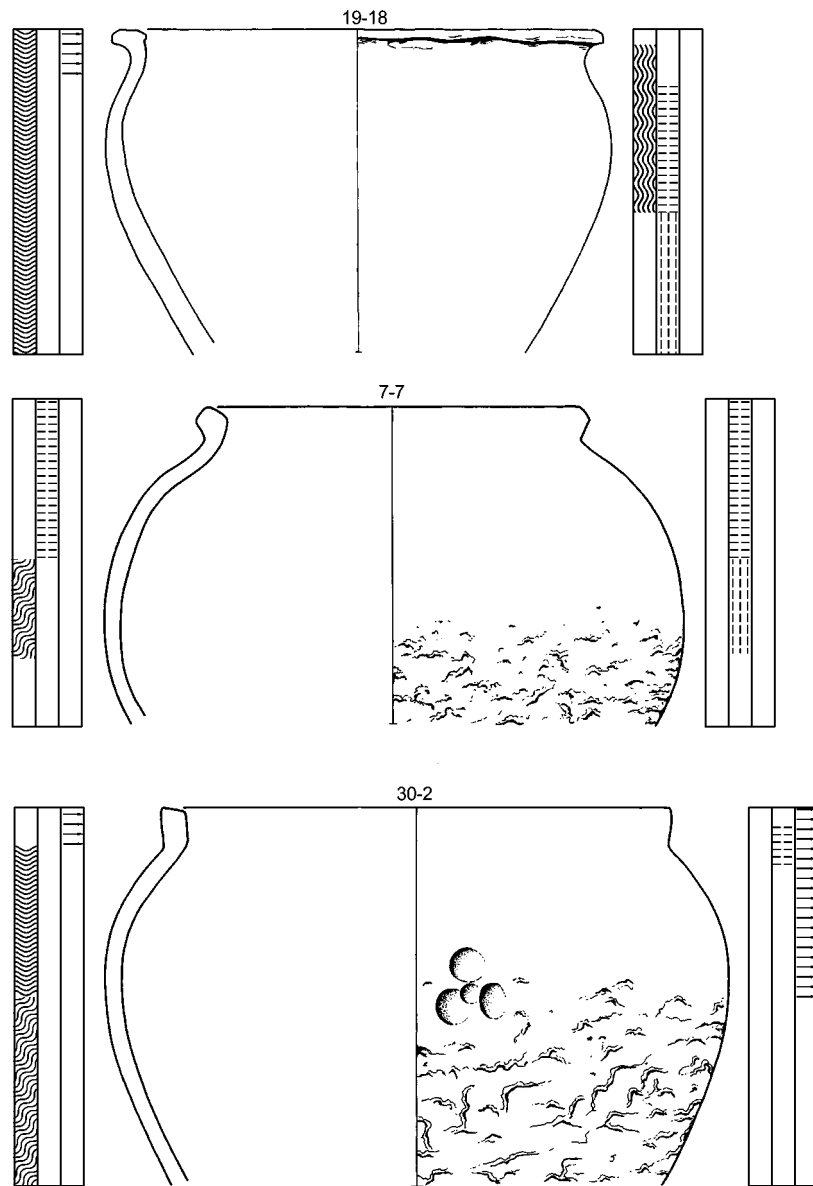


Figure 4d: Vessels from Uitgeest-Groot Dorregeest - Type II (Oudemans & Boon, 1991)

Vessel 19-18 falls in group 3 (GD < 330 mm) and has shape 1; vessel 7-7 falls in group 3 and has shape 2; and vessel 30-2 falls in group 4 (GD > 330 mm) and has shape 1 according to Abbink (1999). Legend for surface treatment can be found on page 193.

0 50 100 150 mm

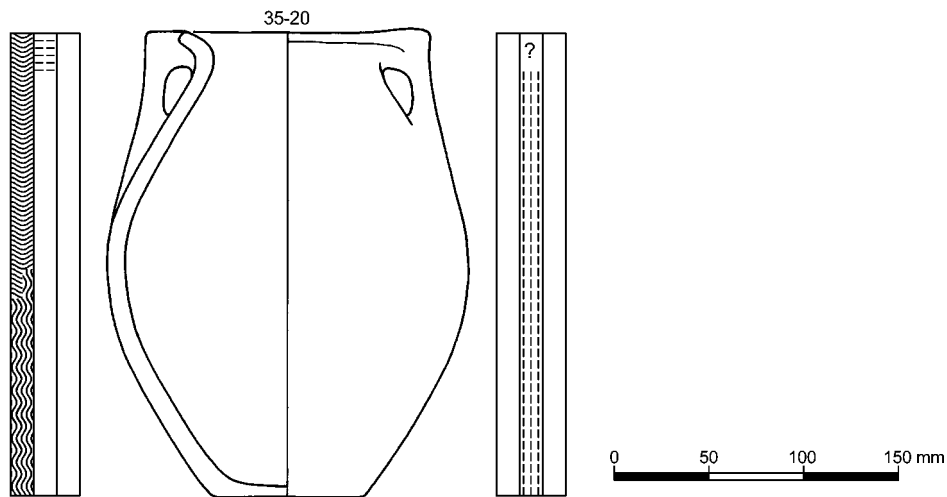


Figure 4e: Vessel from Uitgeest-Groot Dorregeest - Type IV (Oudemans& Boon, 1991)
Vessel 35-20 falls in group 5 and has shape 3 (the so called jar-like shape) according to Abbink (1999).

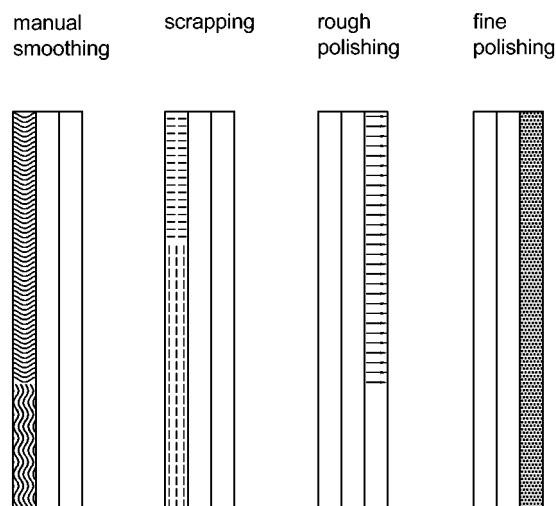


Figure 4f: Explanation of types of surface treatment (according to Abbink 1999, 210).
Block of columns on the left of vessel depict interior surface, on the right the exterior surface.
Within each block, three types of surface treatments are represented by symbols. The extent and the direction of the treatment is indicated in the columns. The symbols represent the above indicated surface treatments.

Although Abbink draws conclusions about vessels based on this last morphological classifications, while this thesis draws conclusions based on the original typology as designed by Oudemans and Boon (1991; 1996), there is no real conflict in results because the main divisions in vessel groups have remained very similar.

2.5. Solid Surface Residues and Soot Deposits

Abbink also registered many use-alterations that appeared on the surface of the ceramics, such as use-wear, soot deposits on the exterior of vessels, and presence of various solid surface residues (Abbink 1999, 285-319). The occurrence of different types of surface residues in sample I are summarised in Table 3. In the assemblage of 147 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 ‘creamy layers’ also called residuetype B1 (3 %) occurred occasionally.

It is significant to note that the frequency of occurrence (Table 3) is not representative for the entire assemblage because the on-site selection of shards involved the selective preservation of all shards with visible residues adhering to them (see Section 2.2. for explanation). The numbers in Table 3 are therefore much higher than to be expected in the overall assemblage.

Table 4: Frequency of occurrence of surface residues of different vessel groups according to Abbink (1999, table 8.15)

Group	n	Total	Soot	% vessels in group	Char	% vessels in group	Pigment	% vessels in group	B1 creamy layer	% vessels in group
1	26	18%	14	54%	8	31%	-	-	2	8%
2	46	31%	26	57%	14	30%	1	2%	-	
3	54	37%	21	39%	21	39%	2	4%	1	2%
4	13	9%	4	31%	1	8%	-	-	2	15%
unknown	8	5%	1	13%	3	38%	1	13%	3	38%
Sample I	147	100%	66	45%	47	32%	4	3%	8	5%

The percentage of vessels in a group containing a particular type of residue (Table 4) suffers from the same fate, and can only be used as an indication of the kind of residues that occur or do not occur regularly on particular types of vessel. Soots and charred residues are primarily detected on ceramics from vessel groups 1, 2, and 3, while group 4 obviously is not used for cooking and or char-forming activities. Although the numbers are small, pigments show only on vessels from group 2 and 3 (not on group 1). Cream coloured residues occur in much higher frequency on vessels from group 4 (Table 4) and are most likely directly related to the morphological vessel type.

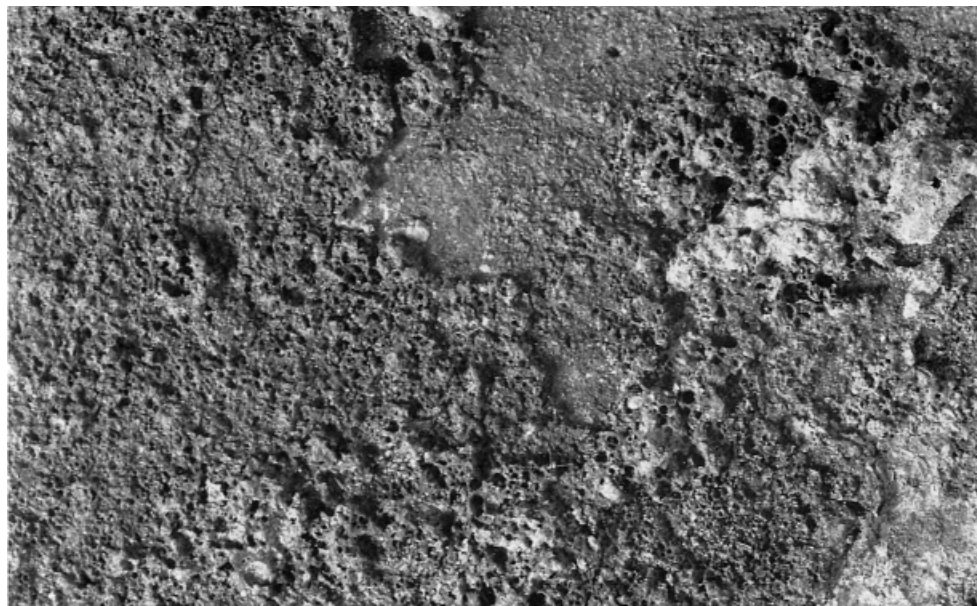


Figure 5a: Charred surface residues on the interior of vessels from Uitgeest-Groot Dorregeest.
Examples of charred surface residues show the variation in visual characteristics of this type of residues. Enlargement is 400%. After Abbink (1999, 287).



Figure 5c: Red-brown residues on the exterior of vessels from Uitgeest-Groot Dorregeest.

Examples of two red-brown residues. The top vessel shows obvious dripping traces, while the bottom vessel shows vague shadowy dripping traces just below the handle. Scale 1:1. After Abbink (1999, 289).



Appendix 1



Figure 5d: Soot residues on the exterior of vessels from Uitgeest-Groot Dorregeest.

Examples of two soot residues. Both examples show black residues situated just under the rim or just above the widest diameter of the vessel. Scale 1:1. After Abbink (1999, 286).

3. Organic Residue Analysis

The nature of this study was to establish an insight into the presence, nature and origin of organic remains detected on ceramic vessels from Uitgeest – Groot Dorregeest and develop a better insight into original vessel use.

3.1. Organic Residues

The organic residues were of different colour and appearance (Table 3) and could roughly be divided into four types: charred residues, soot, cream coloured residues and red-brown residues. The charred residues were most numerous and seemed very well preserved. Chars were all dark brown or black in colour, all situated on the interior vessel wall and all relatively brittle when removed. Char thickness varied from 0.5 mm up to 3 mm, and their surface varied from a soft dark brown, bubbly structure to a hard shiny black surface (Fig. 5a).

Soot deposits were commonly seen on the exterior vessel walls of ceramics from all groups (although significantly less on vessels from group 4). The soot deposits were pitch black and smooth in appearance and relatively thin (> 0.5 mm). The most common place for soot deposits was directly under the rim and just below the greatest diameter of the vessel (Fig. 5d)

Cream coloured residues of tough, flaky substance could occasionally be seen on the interior vessel wall of vessels (Fig. 65a). The thickness varied from very thin (0.3 mm to circa 1 mm thick). Only three such samples were obtained for this study (see Table 5).

Red brown residues (called ‘pigment’ by Abbink) were very rare and only two samples could be collected (Table 5). Although one sample was deposited in a dripping pattern on the exterior vessel wall and one in the interior, their appearance is similar: red brown to dark brown, extremely thin (< 0.1 mm) smooth residues with a sticky texture (Fig. 5c).

3.1. Selection of Residue Samples

The problems addressed in this study are directly related to the choice of residue samples. In order to obtain valuable chemical data from the spectroscopic study, well preserved and adequately large samples are preferable. Because prior to analysis it was unclear what visual characteristics indicated the residues state of preservation a wide range of visually different residues were sampled (Table 5).

In order to correlate the chemical results to the archaeological variables such as form and technology, age and burial circumstances, a number of residues should ideally be sampled to represent each of those variables. However, the number of surface residues in the total assemblage was limited to 59 and the number of soot deposits to 66, resulting in a limited sample choice. The priority was given to obtaining samples from the various different vessel forms. Many of the questions concerning burial circumstances, age and vessel technology will consequently remain unanswered in this particular study.

Table 5: Surface Residue Samples and Soil Samples

Nr	Find number ^a	Residue ^b	Position ^c	Vessel type ^d		Sediment ^e
1	7-7	Char	In	II	3.2	Organic clay
2	8-1	Red brown	Ex	III*	3*	Organic clay
3	8-2	Char	In	II	3.1	Organic clay
4	8-5	Cream Coloured	In	I	1.2	Organic clay
5	14-6-4.2	Char	In	-	1*	Sandy
6	14-6-4.3a	Char	In	I*	1*	Sandy
7	14-6-4.3b	Black	Ex	I		Sandy
8	14-6-4.4	Char	In	-		Sandy
9	14-6-4.5	Char	In	-		Sandy
10	18-3-2.a	Char	In	I*	1*	Organic clay
11	18-3-2.b	Black	Ex	I*	1*	Organic clay
12	18-7	Char	In	II	3.1	Organic clay
13	19-7-90.2a	Char	In	II	3.1	Organic clay
14	19-7-90.2b	Char	In	II	3.1	Organic clay
15	20-4	Char	In	II	2.1	Sandy
16	20-4-157	Char	In	2/3*		Sandy
17	30-12-3 (=30-2)	Char	In	II	4.1	Organic clay
18	31-4.a	Char	In	I	1.2	Organic clay
19	31-4.b	Black	Ex	I	1.2	Organic clay
20	32-6-18	Char	In	-		Peat
21	33-5-2.a	Char	In	-		Peat
22	33-5-2.b	Char	In	-		Peat
23	33-8-2.a	Char	In	-		Peat
24	33-8-2.b	Char	In	-		Peat
25	34-0-12	Char	In	-		Organic clay
26	34-0-30 (=34-12)	Char	In	II	3.1	Organic clay
27	34-7-62	Red brown	In	II*	3*	Organic clay
28	34-7-95.b	Black	Ex	I*	1*	Organic clay
29	34-11-3	Char	In	II*	2/3*	Organic clay
30	35-5-120 (=35-33)	Char	In	II	4.1	Sandy
31	35-7-28	Cream Coloured	In	-		Sandy
32	35-20	Cream Coloured	In	IV	5	Sandy
33	35-21	Char	In	II	3.1	Sandy
34	P1 ^f	Peat	-	-		Pit 16
35	P2 ^f	Peat	-	-		Pit 34
36	34-7-95.a	Char	In	1*		Organic clay
37	33-5-2ab	Char	In	-		Peat
-	14-6-4.3c	Char	In	1*		Sandy

^a Find number: the first number indicates the number of the excavation pit the letters indicate multiple samples from one vessel.

^b Residue appearance

^c Position of the residue on the vessel: In = Interior; Ex = Exterior of the vessel.

^d Morphological vessel type: according to Oudemans & Boon (1996) and according to Abbink (1999) see also Fig 3 and 4.

^e Sediment: the soil type in which the vessel was found. * = an estimated type.

^f P1 and P2 are peat samples from the excavation found in respectively pit 16 and pit 34.

For some of the spectroscopic work in this thesis, samples were taken of the ceramic material itself (Chapter 5). A summary of the ceramic samples taken in the course of this study is given in Table 6.

Table 6: Ceramic Samples from Uitgeest-Groot Dorregeest	
Ceramic samples	
8.4 S	Ceramic
14-6-4.2b S	Ceramic
14-6-4.3c S	Ceramic
14-6-4.4 S	Ceramic
8-1 S	Ceramic
34-0-12 S1-3	Ceramic
35-7-28 S	Ceramic
14-6-4.4 S	Ceramic

In order to study the effect of increased burial time on the preservation of absorbed lipids in charred materials (chapter 5), a number of samples were taken from charred surface residues of recovered from ceramics from a number of other excavations. A summary is given in Table 7.

Table 7: Charred surface Residues from other Excavations	
Other Excavations	
Schagen Mug 79-1-1	Char
Uitgeest 54 226-48	Char
Uitgeest 54 320-17	Char
Hazendonk 32.740	Char
Hazendonk 33.781	Char
NO-polder 14 6745	Char
NO-polder 14 7054	Char

Appendix 2

DTMS and DTMS/MS Study of Solid Organic Residues

Modified after:

T.F.M. Oudemans, G.B. Eijkel, & J.J. Boon 2005, 'DTMS and DTMS/MS study of solid organic residues preserved on ancient vessels', in: *Proceedings of the 33rd International Symposium on Archaeometry, 22-26 April 2002*, H. Kars & E. Burke (eds.), Vrije Universiteit, Amsterdam, 501-505.

1. Introduction

1.1. Organic residue analysis in ceramic studies

The chemical characterisation of solid organic residues found in association with ancient pottery can give direct information about the original prehistoric vessel use. Chemical analysis of solid surface residues and absorbed organic residues alike, has taken place as early as the 1920s (Rottländer & Schlichtherle 1980), and has greatly expanded in the last two decennia (Evershed *et al.* 1992; Heron & Evershed 1993; Evershed *et al.* 1999) due to improvements in analytical instrumentation and an increasing interest in the functional aspects of pottery in ceramic studies. Many analytical techniques have been applied to the analysis of specific classes of compounds such as solvent extractable lipids (Evershed *et al.* 1999), waxes (Heron *et al.* 1994; Evershed *et al.* 1997; Regert *et al.* 2001), terpenoids (Charters *et al.* 1993a; McGovern *et al.* 1996; Dudd & Evershed 1998) and amino acids (Evershed & Tuross 1996) and, more recently, to immunologically detectable proteins (Craig & Collins 2000; Craig *et al.* 2000). Although these studies have facilitated the identification of a whole range of compounds in archaeological residues, and have provided plausible identifications for particular groups of residues, they are limited to a specific group of compounds. For the study of the non-soluble solid macromolecular fraction that forms the matrix of solid organic residues, a non-selective analytical technique is required to characterise the overall chemical composition of residues and to identify the cross-linked components otherwise ignored.

1.2. PyMS studies of solid organic residues

Analytical pyrolysis mass spectrometry (PyMS) has been applied to the study of solid biomaterials from very different origins and has proven to be a fast fingerprinting method particularly suited for the recognition of different classes of compounds in complex materials (Boon 1992).

In earlier studies of residues preserved on ancient vessels, indicative markers for a broad range of bioorganic moieties were detected using Curie point PyMS mass spectra and subsequently identified using Curie point PyGC/MS (Oudemans & Boon 1991). The Curie point PyMS spectra are obtained through very rapid heating of the sample to a set temperature of 610 °C, creating a chemical fingerprint indicative of the overall chemical composition of the residues. A comparative statistical study of these Curie point PyMS fingerprints (up to mass weight m/z 220) showed the chemical composition of the residues to be correlated to the form and size of the vessel. Since no indications could be found for severe post-depositional degradation or contamination of the samples, this correlation reflects a difference in original use between vessels of different forms and sizes (Oudemans & Boon 1996).

1.3. DTMS and DTMS/MS

Developments in instrumentation have opened up new possibilities for the study of solid biomaterials. Direct temperature-resolved mass spectrometry (DTMS) achieves a physical separation between low molecular weight compounds by evaporation and the cross-linked fraction of a sample by pyrolysis, by gradually raising the temperature of the sample on the probe.

DTMS not only makes it possible to chemically categorise small amounts of organic residues on pots, but can also give an insight into the physical conditions that have allowed compounds to survive long-term burial in archaeological context. The temperature at which compounds are freed from the solid, is indicative of the preservation processes - encapsulation in an inert material, adsorption within the pores of a matrix, and cross-linking or condensation - that may play a role in the survival of foodstuffs in ancient vessels. Tentative identifications of the chemical composition of residues can be made by comparison with modern (fresh or experimentally charred) reference materials.

Subsequent tandem mass spectrometry makes it possible to identify individual characteristic ions in the DTMS spectra of residues. This Direct Temperature-resolved Mass Spectrometry/Mass Spectrometry (DTMS/MS) option can thus identify ions and confirm earlier tentative identifications based on comparison with standards.

In this study, a short account is given of the DTMS and DTMS/MS characterisation and comparison of 34 charred and non-charred solid organic residues preserved on exterior and interior of vessels recovered from indigenous settlements from the Roman period (0 - 300 AD) at Uitgeest-Groot Dorregeest (Abbink 1999). A more extended article on this topic is appearing soon (Oudemans *et al.* in press-b).

2. Experimental

2.1. Sample treatment

Samples (5 - 10 µg) for DTMS and DTMS/MS were ground up in 15 - 25 µl of ultra pure water. An aliquot of 1 - 2 µl of this suspension was used per analysis.

2.2. Instrumental

DTMS experiments were carried out on a JEOL DX - 303 double focussing (E/B) mass spectrometer equipped with a JEOL DA - 5000 data system. The sample suspension was applied to the filament (Pt/Rh 9:1, 100 µm) of a direct insertion probe, which is inductively heated at

1A/min to a maximum temperature of 800 °C. Ions were generated by low voltage (16 eV) EI conditions in an ionisation chamber kept at 180 °C and accelerated by 3 kV before being measured over a range of m/z 20 – 1000 at 1 s full range cycle time. Quadrupole measurements were done for each sample.

DTMS/MS experiments were carried out on a JEOL JMS - SX/SX 102A tandem mass spectrometer (B/E/B/E) with a JEOL MS - MP 9020D data system. The filament (Pt/Rh 9:1, 100 μ m) was heated at a rate of 0.5 A/min to an end temperature of about 800 °C. Ions were generated by EI (16 eV) in an ionisation chamber kept at 190 °C and accelerated to 8 kV. Measurements were performed over a mass range appropriate for the ion under scrutiny at a full range cycle time of 3 s and post-accelerated to 10 kV. Collision induced dissociation (CID) was performed in the third field free region using helium as a collision gas (0.5 - 1.0 10^{-3} Pa). The resolution used was 1000.

2.3. Multivariate Analysis

Discriminant analysis was performed using a modified version of the package ARTHUR (infometrix, Seattle) that calculates discriminant functions (DF), linear recombinations of highly correlated masses, that express main similarities and dissimilarities between groups of mass spectra. A hierarchical Q-mode Complete Link Cluster Analysis (CLCA) was applied to the discriminant scores of the samples weighted according to the relative variance they explain.

3. Results and Discussion

3.1. Chemotyping

Multivariate analysis of the DTMS spectra of residues (chars, red-brown residues, cream coloured residues and black residues on exterior vessel walls) shows the occurrence of several 'chemotypes': i.e. groups of residues with similar chemical composition (Fig. 1), which are in general agreement with earlier CuPyMS classifications based on more limited mass range measurements (Oudemans & Boon 1996). The clustering in Figure 1 is based on the discriminating chemical features (masses of characteristic fragments) from the DTMS spectra. In the DTMS spectra of a typical charred residue from Chemotype A₁, sample 7-7 (Fig 2), a distinction between desorption and pyrolysis is clearly visible. The volatile lower molecular weight part of the residues (Fig. 2, spectrum A) includes lower temperature desorption products such as fatty acids (m/z 129, 256 from C16 FA, 284 from C18 FA), sterols (m/z 368 from cholestadiene) and acylglycerols (m/z 523, 550, 551, 577, 579), while the compounds formed at higher temperatures (spectrum B) are derived from native proteins (m/z 108, 117, 131) and

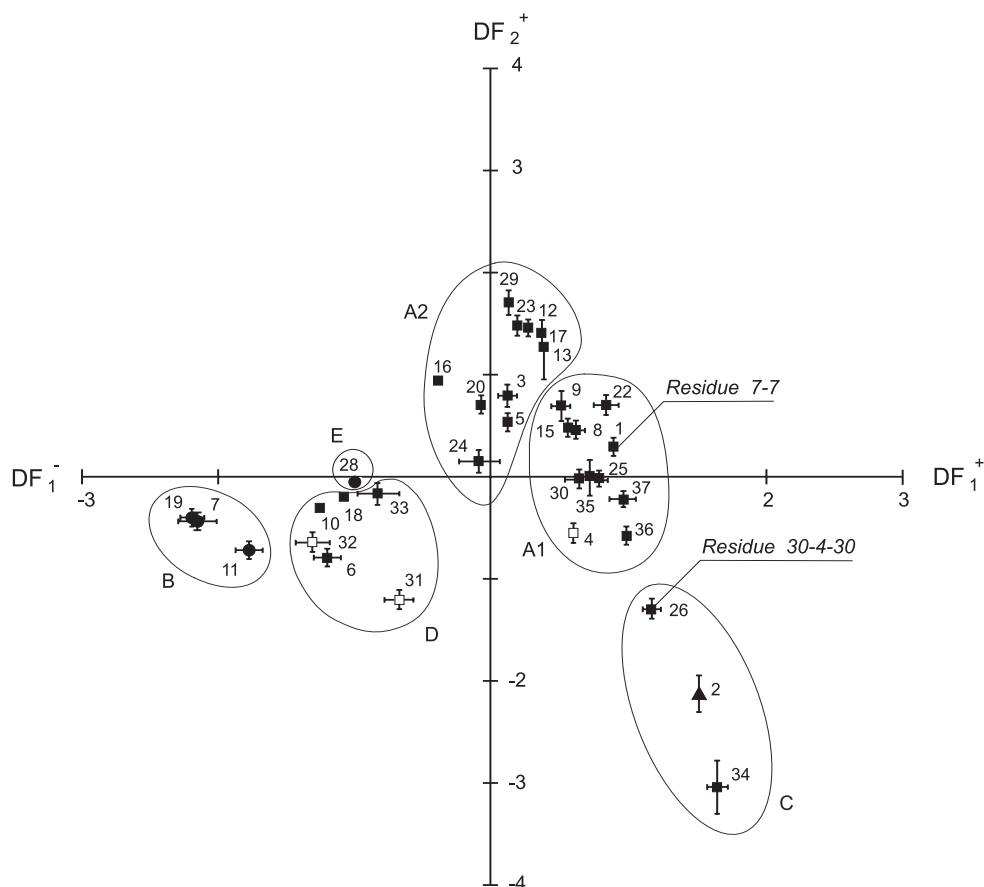


Figure 1: Chemotyping Residues based on MS spectra.

Discriminant analysis and cluster analysis were used to classify residues according to discriminating chemical features (m/z values of characteristic fragments) in the DTMS data. Each cluster represents a particular chemotype. Discriminating features in Chemotype: A: lipids, polysaccharide fragments; B: aliphatic and aromatic hydrocarbons; C: proteins and protein fragments; D: aliphatic and aromatic hydrocarbons; E: contamination. Residue types: Chars on vessel interior (■); black residues on vessel exterior (●); red-brown residues (▲); and cream coloured residues (□).

polysaccharides (m/z 95, 96, 109, 110, 126) and more condensed cross-linked materials evolved during partial charring (unresolved envelope of mass peaks from m/z 100 – 500).

Charring of polysaccharides and proteins leads to a newly formed thermally stable cross-linked network (Boon *et al.* 1994) that can be addressed at higher temperatures with DTMS. The DTMS data show that the char is a solid matrix of cross-linked organic compounds in which lipids are incorporated. Lipids may be incorporated in the charred matrix through absorption into pores in the matrix or by encapsulation within the matrix. Their liberation at evaporation temperatures does indicate they are not covalently bound to the matrix.

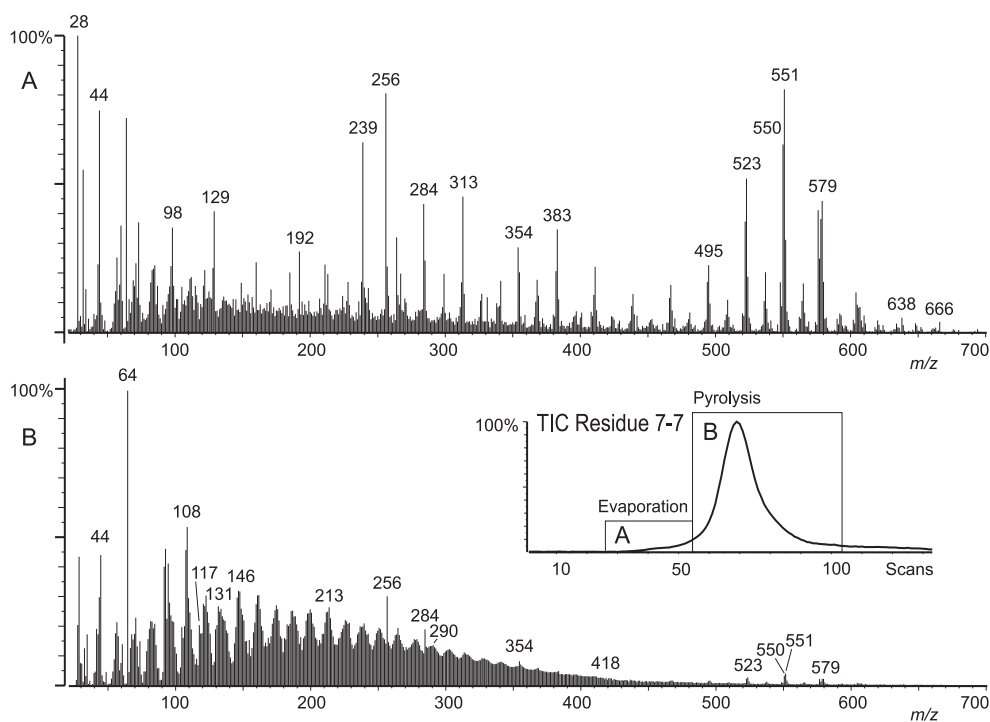


Figure 2: DTMS results for residue 7-7.

Total Ion Current (TIC) and Mass Spectra for A: Evaporation, containing fatty acids and acylglycerol fragments; and B: Pyrolysis, containing markers for cross-linked components created from proteins and polysaccharides during the original food preparation.

Residues situated on the exterior surfaces of vessels Chemotype B, show a different mass spectrum typically containing series of aromatic compounds in the higher temperature region. These aromatic compounds are indicative of wood smoke and soot.

Residues from Chemotype C, predominantly show compounds derived from native proteins (m/z 108, 117, 131) and the condensed cross-linked materials evolved during partial charring (m/z 144, 145, 146, 159, 160, 161, 173, 174, 175, etc). A typical sample from this chemotype, residue 34-0-30, is a charred residue preserved on the interior of a vessel. The DTMS spectrum of the high temperature pyrolysis fraction (Fig. 3) shows such a pattern in detail. The liberation temperature of these compounds indicates that the fragments indicative of proteins such as indole (m/z 117) and methyl indole (m/z 131) are incorporated in a network of cross-linked compounds and are chemically bound to the matrix.

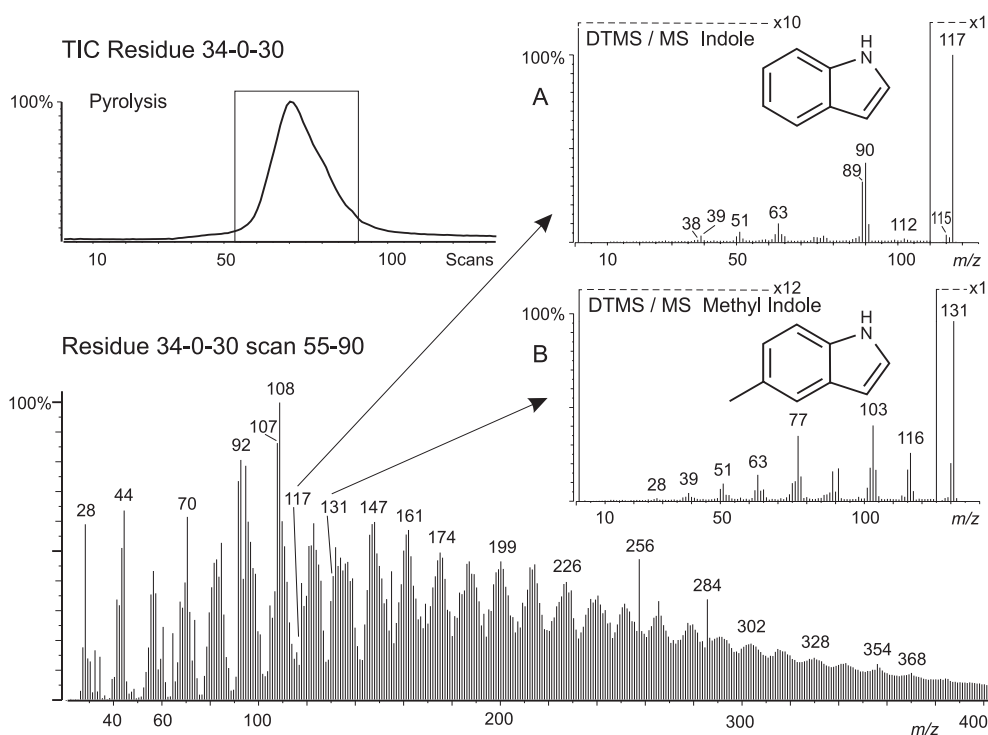


Figure 3: DTMS spectrum of the pyrolysis fraction of residue 34-0-30.

DTMS/MS identifications for two DTMS/MS spectra for the ion peaks of A: indole (m/z 117) and B: methyl-indole (m/z 131).

3.2. Identification

Although the spectra of samples were compared to the spectra of fresh biological materials such as Amylose and Bovine Albumin and their experimentally charred counterparts (Oudemans *et al.* in press-a), DTMS/MS provided the tool for more solid identification of compounds. A typical Chemotype C sample, 34-0-30 (Fig. 3) illustrates the identifying power of DTMS/MS. During the DTMS/MS experiment, a preselected ion was isolated and introduced into a collision chamber to collide with helium. The fragments were detected in the second sector MS to give a mass spectrum of the fragmented ion. Figure 3 shows two MS/MS spectra for indole (m/z 117) and methyl-indole (m/z 131), two fragments originating from the tryptophane (Tsuge & Matsubara 1985) in the native proteins.

4. Conclusions

DTMS is providing a rapid analytical technique to obtain information about a broad range of chemical compounds in solid organic samples. Compounds as varied as lipids, proteins, polysaccharides, polynuclear hydrocarbons, and fragments of complex cross-linked compounds were detected in microgram amounts of archaeological samples.

In addition, DTMS gives information about the chemical composition of the sample and renders new understanding of the kind of chemical complexes under study. Solid organic residues are now proven to consist of a matrix of cross-linked compounds (probably consisting of a combination of partially heated proteins and polysaccharides) in which lipids are incorporated either through adsorption or through encapsulation within the matrix, but without being chemically bound to this matrix.

The addition of DTMS/MS greatly increases the identifying potential of DTMS techniques and gives a more definite character to the tentative identifications obtained through comparison with standards and known (fresh or experimentally charred) biomaterials.

Appendix 3

Organic Residue Analysis in Ceramic Studies - Implications for Conservation Treatment and Collections Management

In this appendix a review is presented of the possibilities and limitations of various analytical techniques. The application of organic residue analysis in ceramic studies raises many questions concerning conservation treatment of ceramics during and after excavation, as well as the long-term storage of ceramic vessels. An organic residue preservation protocol is presented for conservators in the field and the museum, and sampling strategies are discussed. Ceramics that contain organic residues should be treated as organic/inorganic composites rather than as exclusively inorganic materials.

Modified after:

T.F.M. Oudemans & D.W. Erhardt 1996, 'Organic residue analysis in ceramic studies: implications for conservation treatment and collections management', *Archaeological conservation and its consequences*, A. Roy & P. Smith (eds.), The International Institute for Conservation of Historic and Artistic Works, London, Copenhagen, 137-142.

1. Introduction

1.1. Organic residues

Organic residues found in association with ancient ceramic vessels can be seen as the result of human activity that took place hundreds, if not thousands, of years ago. These residues supply an insight into the type of organic products people cooked, stored or otherwise prepared, and also illustrate which vessels were used for these tasks. The complex of vessel/residue/burial context is therefore a perfect example of 'behaviour' fossilised in material remains.

Organic residues can occur as bulk residues (organics contained in closed ceramics), as surface residues (solid crusts or films adhering to the interior or exterior of a vessel) or as absorbed residues (organics absorbed into the ceramic fabric of the pot). Bulk residues are rarely discovered in large quantities at a site, except in special burial circumstances such as graves, shipwrecks, natural catastrophes (volcanic eruptions, earthquakes) or caches of treasure. Surface residues are much more common. Many types have been described (Oudemans & Boon 1991) including black sooty residues, black or dark brown 'carbonised' crusts or chars, red-brown smooth layers, and cream-coloured or yellowish crusts. Although organic residues are frequently mentioned, no systematic description of the visual characteristics has been proposed, nor has a uniform terminology been adopted for this purpose. Results from a small number of diverse ceramic assemblages suggest that 1.0 - 0.5 % of the shards contain visible surface residues (Oudemans unpublished results). Absorbed residues are invisible and can be detected only by extraction and analysis. The frequent detection of extractable organics such as lipids and terpenoids suggests these residues are commonly present in ceramic vessels (Rice 1987, 233-234). However, there are no records on the frequency of occurrence of absorbed residues in ceramic complexes.

1.2. Degradation and preservation

The state of degradation varies widely both between and within excavations. Burial circumstances can have different effects on various classes of chemical compounds. For example, the presence of water and lack of oxygen will cause anaerobic degradation, which may change the lipid profile and degrade sugars present in the residues. The presence of acidic water (as in peat bogs) will change the composition of the proteinaceous materials quite extensively due to acidic denaturing. Arid conditions preserve structural elements such as lignin and proteins, but have a strong oxidising and cross-linking effect on the lipids.

Empirical results show that some processes such as carbonisation of residues and the absorption of organics into the ceramic fabric of vessels seem to help preserve organic compounds such as lipids and proteins. Although many hypotheses have been postulated to explain this phenomenon, the chemical mechanisms are not fully understood.

2. Analytical Techniques - Potential and Limitations

The study of small amounts of complex mixtures of degraded organic materials creates many analytical challenges. Two approaches can be taken to obtain chemical information from such materials. Characteristics of the mixture as a whole can be determined giving information on the level of a 'total sample'. Usually these techniques result in a chemical 'fingerprint' that can be compared with fingerprints of other reference materials. The information obtained with fingerprinting techniques is commonly used to compare and classify samples and to determine further analytical strategies.

Alternatively, the sample can be separated into fractions and each fraction can be analysed in more detail on a molecular level (Erhardt *et al.* 1988). However, each separation and preparation step requires additional sample, and some separation steps result in a loss of information due to incomplete separation, sample loss, or undesired chemical change during separation. Rather than preparative fractionation, analytical micro-pyrolysis applies online thermal fragmentation prior to analysis. This technique has the advantage that small samples can be analysed for a broad spectrum of compounds in a single analysis.

2.1. Elemental and isotope characterisation using CHN analysis and SIA analysis

The organic elemental composition of a residue can be determined by analysis of the amounts of carbon (C), hydrogen (H) and nitrogen (N) present in the combustion gas of a small sample. The CHN results indicate what fraction of the sample is organic and the ratios suggest the chemical composition of the material. The C/N ratio indicates the protein fraction present and the C/H ratio illustrates the degree of saturation and condensation of the material.

Stable isotope analysis (SIA) gives information on the ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in the sample. Since these ratios depend on the metabolic system of the organisms involved, they can be seen as an indication of the type of original material present in the residue. However, mixing of foodstuffs generally limits the applicability of this technique in organic residue analysis. Compound specific SIA is a newly developed analytical technique that is discussed in Chapter 1.

2.2. Chemical characterisation of mixtures using FTIR and NMR spectroscopy

Fourier transform infrared spectroscopy (FTIR) is based on the light absorption characteristics of various chemical compounds in a material. Each type of chemical bond (or functional group) absorbs light of a particular wavelength or range of wavelengths. The presence or absence of

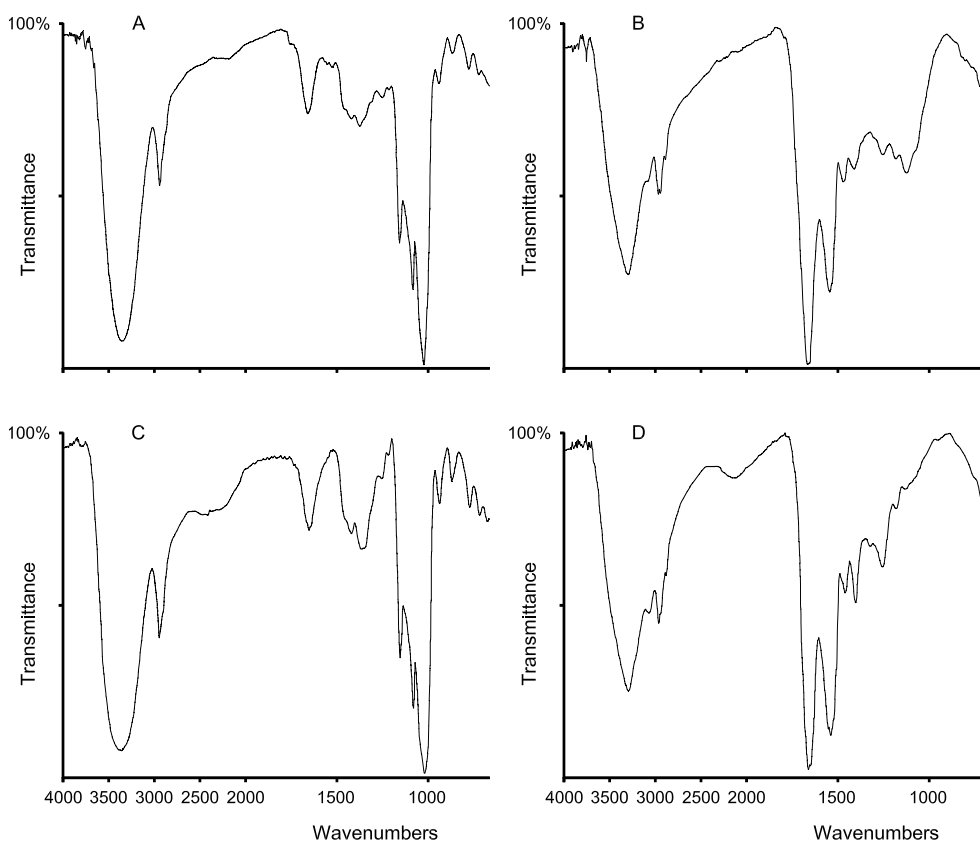


Figure 1: FTIR spectra of charred surface residues from Kalinga cooking pots and fresh reference materials. Residues were taken from two oppaya (meat and vegetable cooking pots) collected while in use at a Kalinga village (Guina-ang, The Philippines) by Skibo (1992): (a) Small oppaya (no. 88-77-44), (b) Medium sized oppaya (no.88-77-20), (c) Corn starch, (d) Bovine albumen. Correlation coefficient (over wavelengths 700-1900 cm⁻¹ and 2400-3750 cm⁻¹) between (a) and (c) was 0.98 and between (b) and (d) 0.97. Spectra were recorded with a Matson 4326 Upgrade FTIR spectrophotometer (Oudemans & Hopwood unpublished results).

absorption peaks typical for particular bond types or functional groups provides information on the absence or presence of certain compound classes in the given sample (Fig. 1).

FTIR is a rapid analytical technique ideal for the initial classification of organic residues into groups with broadly comparable chemical composition (Oudemans & Hopwood unpublished results). General determination of the nature of the samples can be made through comparison with reference spectra of known materials (Fig. 1). FTIR can rarely be used for identification of complex mixtures because increasing complexity of the analysed sample results in decreasing resolution and a loss of identification potential. Other limitations of FTIR as an analytical tool

for organic residue analysis include its relative insensitivity to compounds present in smaller quantities (< 5%) and its limited capability to provide quantitative results when distinguishing between samples containing various proportions of similar compounds. An advantage of the technique of combined FTIR microscopy is its ability to analyse solid samples by pressing them into a thin layer between two crystals (diamond or inorganic salt crystals), although this technique is sensitive to sample inhomogeneity.

Solid-state ^{13}C nuclear magnetic resonance spectroscopy (NMR) has been designed to study the carbon functional group distribution in complex solid organic materials in medicine, biochemistry and geochemistry, and has recently been applied in the field of organic residue

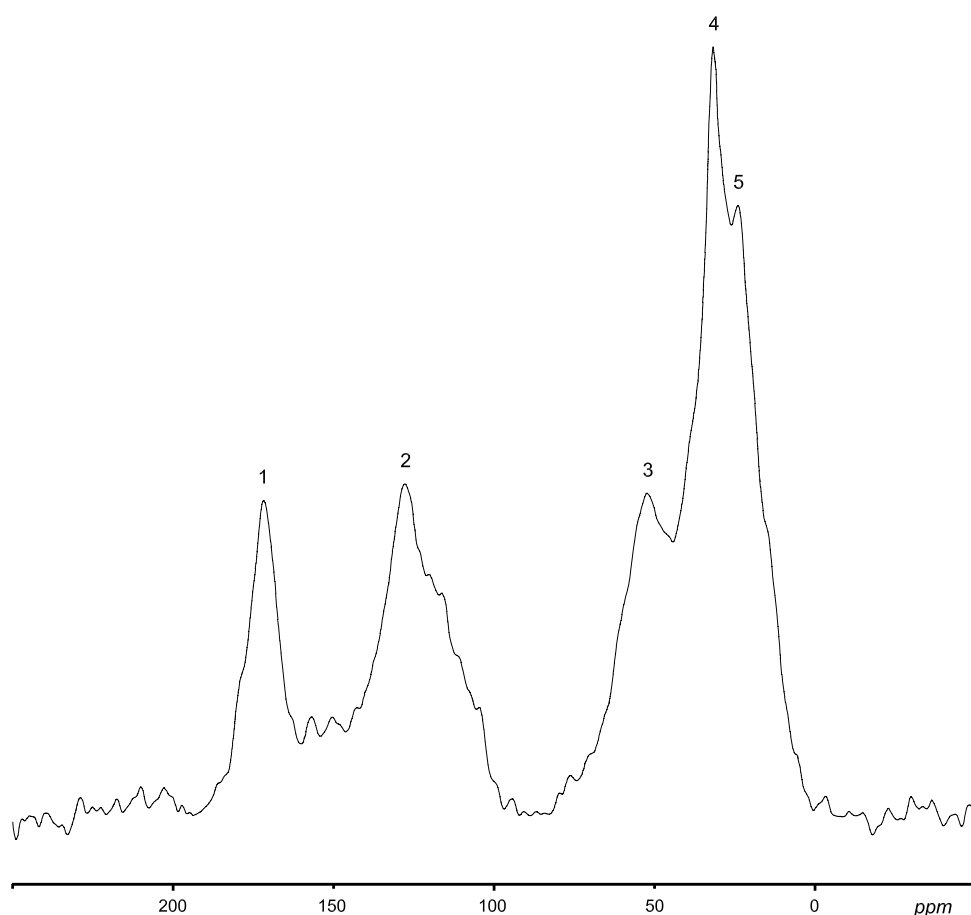


Figure 2: Example of a solid-state ^{13}C NMR spectrum of charred surface residue from the Roman period. from a vessel (no. 34-7-95 A) found in an indigenous settlement in Uitgeest-Groot Dorregeest, the Netherlands. The residue contains 31% carbon and the spectrum shows carbon functional group distribution: (1) carboxyl groups $-\text{CO}_2\text{H}$; (2) aromatic structures; (3) carbon-nitrogen bonds in proteins $-\text{C}(\text{N})\text{CO}_2\text{H}$; (4) $-\text{C}(\text{H}_2)-$; (5) methyl groups $-\text{CH}_3$. The spectrum was recorded on a Brüker CXP-100 (2.3 Tesla) spectrometer at the Argonne National Laboratory, Argonne, Illinois (Oudemans *et al.* in press-a).

analysis (Sherriff *et al.* 1995; Oudemans *et al.* in press-a). The determination is based on the electronic environment and magnetic susceptibility of the ^{13}C atoms in an organic material. Each different type of carbon bond contributes to a specific type of chemical shift that can be measured (in ppm) relative to a standard compound (Fig. 2). The ratios between saturated C-C bonds, unsaturated C=C bonds and C-H bonds provide information on the degree of condensation of the organic residue. CP/MAS (cross-polarisation/magic angle spinning) NMR has some clear advantages over FTIR since it provides quantitative results, is not affected by the inhomogeneity of the sample and is less affected by loss of sensitivity due to sample complexity than FTIR. The disadvantages are that a larger sample is required (100 mg) and the analytical procedure is much more time-consuming and expensive. However, CP/MAS NMR is the only analytical technique that gives quantitative results that make it possible to obtain information on the relative amounts of extractable and non-extractable compounds present in the sample.

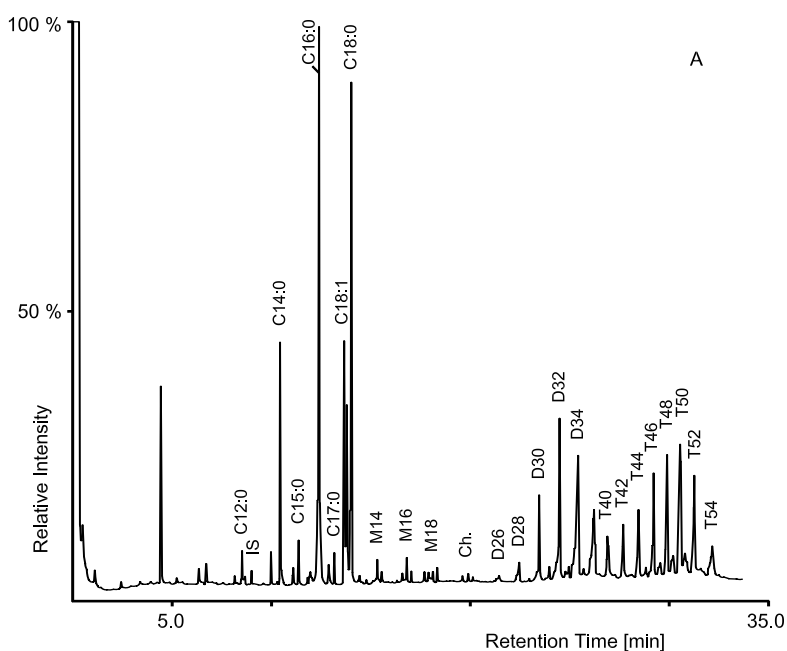


Figure 3a: GC/MS of the TMS derivative of a total lipid extract from a charred surface residue.

Residue was preserved on a vessel (no. 14-6-4.22 R) found in an indigenous settlement in Uitgeest-Groot Dorregeest, the Netherlands (Oudemans & Boon in press). The profile shows different classes of compounds such as fatty acids, monoacylglycerols, cholesterol, diacylglycerols and triacylglycerols. The internal standard is indicated as IS.

2.3. Molecular characterisation of extractable compounds by GC/MS

Because many samples are complex mixtures of similar compounds, more detailed identifications can be made only after a separation step is conducted. Certain compound classes, such as lipids (fatty acids, acyl lipids, sterols, waxes), terpenoids, alcohols and hydrocarbons, can be extracted with organic solvents. These extractable compounds can be separated and identified by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) after appropriate derivatisation or preparative separation. GC separates all volatile compounds present in the derivatisation extract, based on chemical characteristics. GC separates all volatile compounds present in the derivatisation extract, based on chemical characteristics (Fig. 3a). Some individual compounds can be identified by comparison with the retention times of standard compounds. However many peaks cannot be identified by GC alone. GC/MS combines a separation technique (GC) with an identification technique (mass spectrometry) so that the individual peak that represents a compound can be identified without use of standards (Fig. 3b).

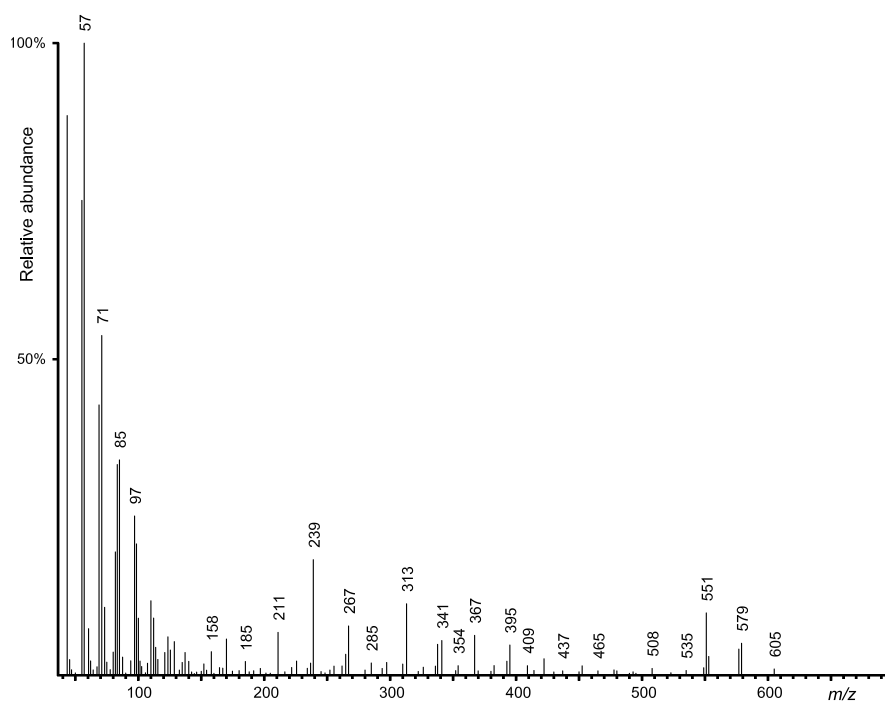


Figure 3b: The mass spectrum of a selected saturated triacylglycerol TAG 50:0 (see Fig. 3a for elution time). The molecular ion is not visible but different sets of indicative fragments can be seen: the RCO^+ ions (m/z 211, 239, 267, 295) and the M-RCOOH^+ ions (m/z 523, 551, 579, 605).

2.4. Molecular information on non-extractable compounds

It is much more complicated to obtain detailed information on the chemical composition of the remaining non-extractable, solid, chemically bound, condensed macromolecular fraction of the residue. The remaining compounds include proteins, complex sugars, melanoidins, condensed cyclic hydrocarbons and cross-linked drying oils, and can be studied best after a fragmentation step.

Fragmentation by hydrolysis.

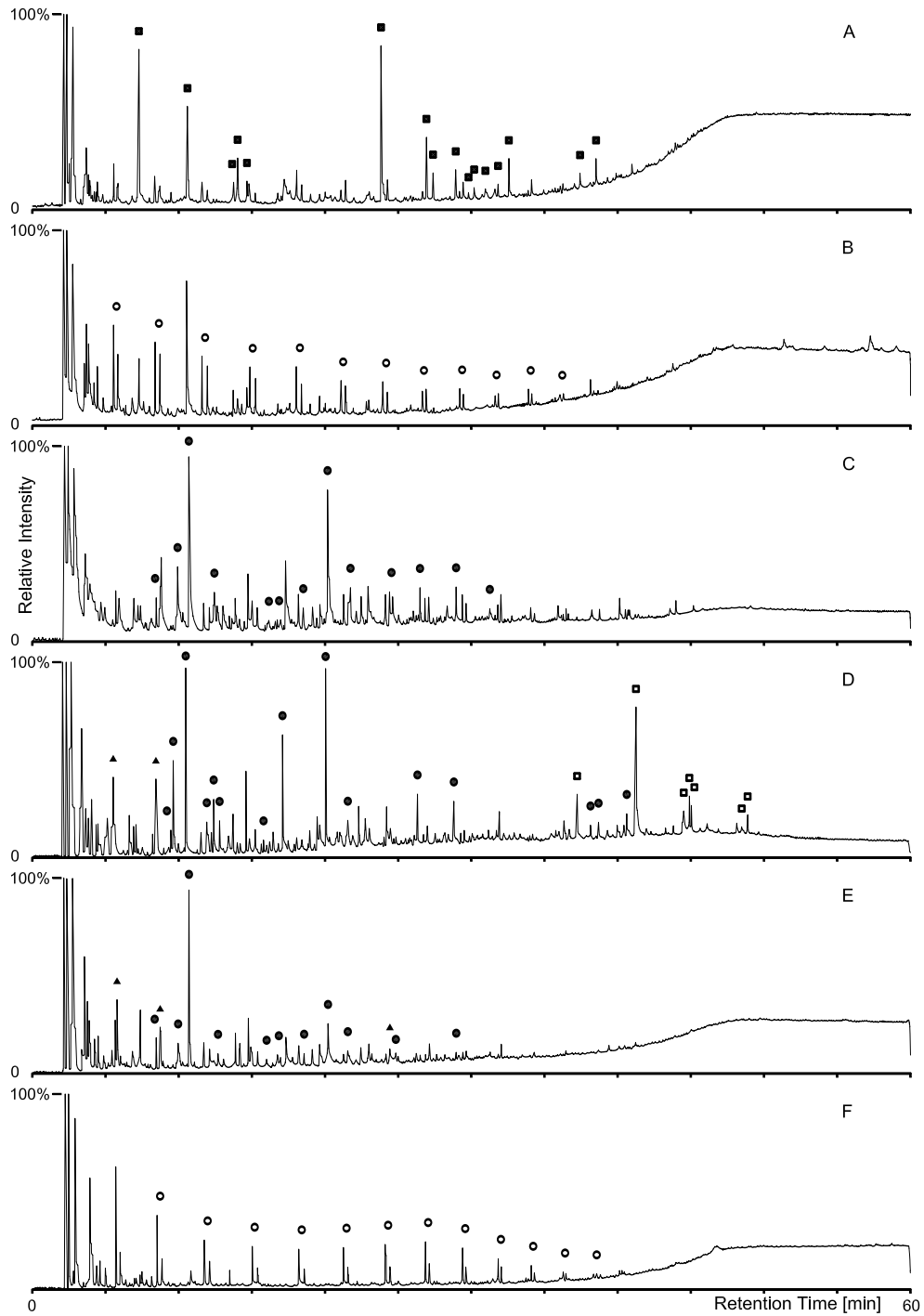
Proteins and complex carbohydrates (such as gums) can best be analysed after they have been converted into their individual amino acids or sugars, which is usually achieved by acid or alkaline hydrolysis. Sugars can be derivatised and volatilised for GC analysis, while amino acid composition is usually determined by high-performance liquid chromatography (HPLC). The main disadvantage of these techniques is the loss of identifying information during the fragmentation procedure. The identification of complex sugars such as plant gums seems much less hindered than the identification of protein polymers, since the relative quantities of the various sugar units in gums are more characteristic of their origin (Kharbade & Joshi 1995). The identification of proteins and (partly) degraded proteins is limited since many proteins have a similar amino acid composition. One possible exception to this is gelatin, which has a very distinctive amino acid profile (Evershed & Tuross 1996).

Fragmentation by pyrolysis.

Another way to fragment condensed materials, such as proteins, melanoidins, caramelised sugars, cross-linked drying oils and other condensed macromolecular materials, is through analytical pyrolysis (Oudemans & Boon 1991, 1996). Pyrolysis consists of rapid heating under oxygen-free conditions. The added thermal energy causes the macromolecular compounds to split (along the weakest bond in the chain) into fragments specific for the original molecule. Analytical pyrolysis is often combined with MS analysis (Fig. 4).

Figure 4 (on facing page): Pyrolysis GC-MS plots of a soot residue (A), four solid surface residues (B - E), and a sample of the ceramic material of the vessel wall (F).

Various indicative fragments can be seen, such as, polynuclear aromatic hydrocarbons (■) indicative of smoke condensates; alkanes and alkenes indicative of an aliphatic polymer such as cross-linked lipids (○); markers indicative for proteins (●), polysaccharides (▲) and free fatty acids (□). Samples were collected from vessels found in an indigenous settlement in Uitgeest-Groot Dorregeest, the Netherlands (Oudemans & Boon 1991).



3. Conservation Treatment and Collections Management

Recent advances in organic residue analysis have created a need for re-evaluation of many traditional treatment procedures for ceramic artefacts. Ceramics, whether or not they contain visible organic residues, should no longer be viewed as exclusively inorganic materials but rather as a composite material with both an inorganic and an organic component.

Treatments and preservation protocols written specifically for degradable organic materials can also be applied to organic residues (Mills & White 1987). However, there may be a discrepancy between the purpose of conservation treatments usually directed at preservation and consolidation of the physical, structural and optical qualities of an artefact and treatments for organic residue analysis, primarily directed at the preservation of chemical characteristics of the original material. Many consolidation treatments traditionally applied to ceramics will affect the chemical composition of residues in more or less serious ways. It is advisable to review and evaluate the conservation protocols for ceramic artefacts with regard to their possible effects on organic components.

3.1. Sampling strategies

When organic residue analysis (ORA) is conducted to obtain information concerning diet and food preparation techniques, a number of criteria can be used to select the appropriate sampling strategy:

- The food groups under consideration should have chemically distinct characteristics that are detectable with the chosen analytical technique(s).
- The food processing technique must be likely to leave a residue (for example, storage of a dry food is less likely to leave a residue than storage of an oil).
- Food groups that rarely leave any other kind of direct archaeological evidence, such as leafy plants and legumes (Evershed *et al.* 1991) are the most challenging examples.

The sampling strategy for diet-specific information will prioritise samples based on their state of preservation. The best-preserved residues will have the best potential to help identify the original food. Often a particular type of ceramic vessel will be chosen for sampling. To obtain conclusive results, chemical evidence for a particular food should be present in more than one vessel.

When ORA is employed to study vessel use of one or more types of vessels in a ceramic assemblage, the sampling strategy is more complex and involves a much larger selection of samples, because statistically significant numbers of samples from each type of vessel must be available in order to draw conclusions about use of a particular type of vessel. A number of criteria can direct the sampling strategy:

- The vessel types must be clearly distinguishable.
- A number of intact profiles of each vessel type should be available for sampling.
- The presence of surface residues on the intact profiles enhances the potential of a ceramic assemblage for ORA (for example, it is difficult to select vessels on the basis of invisible, absorbed residues).

Combined study of both absorbed and surface residues will improve the interpretability of results, because a better insight can be obtained into the relationship between vessel use and residue formation.

Clearly, an ORA project should be undertaken only if an archaeological question can be posed that leads to a hypothesis testable with the described techniques. It is essential to appreciate the many methodological problems concerning residue formation and preservation that are still being studied and to develop an insight into the scope and limitations of this new discipline. It is through close cooperation between conservators, both in the field and in museums, and ORA specialists in the laboratory that these problems can best be addressed.

3.2. Conservation protocol

Organic residue analysts generally prefer to conduct the sampling themselves, or at least to be present during sampling, to ensure that the sampling methods provide representative, uncontaminated samples. Choosing samples can also be very difficult because surface residues frequently look like 'burnt porridge' or 'soot' to someone with an untrained eye. Residues are frequently hard to distinguish from secondary deposits or soil remains. Knowledge of the ceramic assemblage is required to choose representative ceramic pieces.

The main problems involved in organic residue sampling are contamination and degradation following excavation. Contamination is a serious problem, because organic residues are usually present in small quantities and frequently have a low organic content (varying typically between 5 and 50%). Contamination often occurs unnoticed during handling, storage or transportation of the ceramics (fingerprints, paper traces, mineral oil from instruments, plasticisers from plastic bags and vials, mould growth and so on), making them hard to prevent. General contamination through handling should be limited as much as possible by wearing non-powdered latex or nitril gloves.

Other steps can be taken to limit the chances of contamination and post-excavation degradation:

- Registering the organic surface residues. The location of a residue on the vessel, and the colour, texture and thickness of the residue should be recorded. Photographs (high magnification) of the intact surface residue are very helpful in future evaluation of results.

- Selecting samples before washing. When surface residues are visible before washing of the shards, or when sampling for absorbed residues (there are no visual indications of the absence or presence of absorbed organics), ceramic pieces can be selected before washing. Pottery should be wrapped in solvent-cleaned aluminium foil, and stored in a polyethylene zip-lock bag or in a glass container with a Teflon-lined cap at -20 °C.
- Selecting samples after washing and drying. If surface residues are not visible without washing, or cold storage is impossible, cleaning is required before selecting samples. Washing ceramics gently under running tap water or with pressurised tap water should be done as soon as possible after excavation to prevent degradation and mould growth. Scrubbing, brushing and excessive handling should be avoided. Rapid drying is advisable to prevent mould growth and other degradation processes. Unfortunately, even mild treatments such as washing and drying can affect the organic material present, causing the loss of brittle surface residues. After the ceramic is completely dry, it should be wrapped in solvent-cleaned aluminium foil and packed in a polyethylene zip-lock bag or a glass container with a Teflon-lined cap. Store in a cool dark place where no condensation can take place.
- Sampling the residue. Scanning electron microscopy (SEM) study of the surface residue can visualise previously undetected contaminations such as mould growth. After this visual inspection, but prior to sampling, it is advisable to remove the outer surface (about 1 mm) of the residue in order to reduce the risk of contamination by soil components. Surface residues are scraped from the ceramic with a solvent-cleaned scalpel. Absorbed residues are extracted from a piece of the ceramic cut or drilled out of the vessel. The residue (or the piece of shard) is subsequently ground up and stored in a glass vial with Teflon-lined cap.
- Taking additional samples. To conduct an organic residue study of an excavation, the chemical composition of the soil surrounding the residues should be determined. Soil samples (about 10 g) should be taken directly adjacent to the ceramics under study, and from the exterior of the vessel (soil on the interior may contain part of the original vessel contents), with a solvent-cleaned scalpel and stored at -20°C in glass vials with Teflon-lined caps.

3.3. Long-term storage and collections management

If organic residue analysis will be conducted on a ceramic collection in the future, storage conditions and collections management protocol should be adapted to prevent contamination, reduce further degradation as much as possible, and avoid consolidation of the ceramics.

- Contamination. Storage materials and containers should be inert and contain no volatile materials. Plasticisers present in many polymers, such as polyvinyl chloride (PVC, or 'vinyl'), can volatilise and contaminate samples stored over long periods. Even seemingly

innocuous and easily overlooked items such as vinyl cap-liners of glass containers should be avoided. Gore-tex, a Teflon-coated textile, can be used to pack large vessels without contaminating them.

- Further degradation. Store ceramics in a dark, cool and dry place and check regularly for mould growth and other forms of organic degradation. Record all visible changes. Do not spray with anti-fungal chemicals.
- Consolidation. Consolidation with any kind of material precludes further organic residue analysis. No coating can be applied to the ceramic for exhibition. Also avoid mild acid treatments that are sometimes employed for desalination. Efflorescence can be avoided by storage in an environment with low relative humidity. Glues, adhesives, organic compounds and ink should not be applied to any area of a ceramic that might be sampled.

Even when these factors are taken into account, the effects of storage conditions different from those present during burial are not entirely clear. It is possible that organics in the ceramics do not undergo excessive chemical changes when stored in dark, cool, dry places, but more study is still required.

4. Conclusions

Organic residues found in association with ceramic vessels can give direct information about what materials were cooked, stored or otherwise prepared by people in the past and about what vessels they used for each task.

This appendix summarises the potential of various analytical techniques that can be applied to residue studies and reviews the possibilities and limitations of organic residue analysis when applied to the study of prehistoric diet or vessel use.

The application of organic residue analysis in the study of ceramic artefacts leads to many questions concerning conservation treatments in the field and during long-term storage. Guidelines are given to conservators concerning the choice of appropriate ceramic assemblages for organic residue studies, and the prevention of contamination, degradation and consolidation of ceramics stored for future residue analysis following excavation. Although it is made clear that all post-excavation treatment affects organic residue analysis to some extent, light surface rinsing with tap water and rapid air-drying is the least intrusive method.

The potential of organic residue analysis depends largely on the application of conservation treatments and storage methods designed for organic/inorganic composite materials rather than exclusively inorganic materials. Treatments and storage methods should be reviewed and adapted in regard to their possible effect on the chemical composition of the organic fraction of the composite.

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Summary

Molecular Studies of Organic Residues Preserved in
Ancient Vessels

Summary

It is the archaeologist's job to explain the behaviour of people in the past. The archaeologist tries to discover how people lived, what kind of families they formed, how they collected and prepared food, organised their society, worshipped their higher powers and exchanged goods with neighbouring groups. In contrast to other humanistic disciplines, archaeology only works with the material remains of a society as source material: objects, structural remains of living spaces (e.g. houses, hearths, discard areas and water-wells), fragments of agricultural field systems and remains of special activity areas (e.g. butchering sites, stone workplaces, pottery sheds or kilns).

Pottery assemblages for instance, are frequently studied by archaeologists in search of information about a variety of different aspects of past societies, such as socio-economic developments, the organisation of production and trade, and the mechanisms of cultural interaction. In order to make behavioural inferences from the shattered remains of a once thriving community, a clear understanding of the function or use of the original object is essential. Ceramic vessels were tools produced, used and discarded by people in the past. They were a part of the daily lives of these people. Without knowledge of the function and use of these tools we are blind to the messages they encapsulate. The archaeologist's employment of pottery is comparable to the historian's employment of written texts: the information obtained from the text is hard to interpret if it remains unclear whether one is dealing with a personal diary, a tax document, a popular newspaper or the script of a comedy. Due to an increasing awareness of this necessity, ceramic studies in archaeology now often include functional aspects of pottery assemblages. Although these studies commonly discuss information on recovery context, pot morphology and ceramic technology, the results frequently remain relatively non-specific due to the complex relationships between vessel form, function and technology. Independent information about the original utilitarian role of vessels is needed to estimate the role each of these factors played in the experience and behaviour of people in the past. Such information can be obtained through the study of use alterations - any chemical or physical change that occurs to the surface or substance of ceramics as a result of use. Four types of use alterations are commonly defined: i) use-wear; ii) discolouration of the clay also called "fire clouding"; iii) soot depositions and iv) organic residues (either on exterior or interior vessel wall or absorbed into the ceramic). The chemical characterisation of organic residues found in direct association with vessels is one of the more recently developed methods in the functional study of ceramics.

This thesis describes a systematic study of the molecular characteristics of organic residues preserved in assemblages of ceramic vessels recovered from a number of prehistoric settlements, in order to better infer the way the vessels were used. The main focus of this work is an assemblage of solid organic residues from an indigenous settlement from the Roman period at Uitgeest-Groot Dorregeest in the Netherlands.

This study addresses a number of basic questions concerning the feasibility of molecular organic residue analysis as a tool in the study of ancient vessel use. Naturally, the study of small amounts of complex organic materials preserved in the ground for thousands of years creates many methodological and analytical challenges. The main methodological questions concern the selection of the most representative and well-preserved sample materials, and the development

of an analytical protocol that supplies the most useful information and gives the most complete answer (Chapter 1).

From an archaeological point of view, the most prominent questions concern the interpretation of chemical evidence in terms of original vessel content and vessel use. To what extent can the original vessel content be identified and how can questions of original vessel usage be addressed? The more specific question concerning the assemblage from Uitgeest-Groot Dorregeest was whether groups of vessels with different form and/or size characteristics were actually used in different ways during the Roman period. Organic residues found in association with four morphological vessel types were studied for molecular characteristics illustrative of their original vessel use (Chapter 2 and Chapter 7).

A wide range of different complementary analytical techniques was used to explore the molecular characteristics of the solid organic residues. The following techniques have been applied (Chapter 1): Light microscopy and scanning electron microscopy (SEM), elemental CHN analysis, Curie-point pyrolysis mass spectrometry (CuPyMS), Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGC/MS), direct temperature-resolved Mass Spectrometry (DTMS), gas chromatography/mass spectrometry (GCMS) of extractable lipids, solid-state ^{13}C magnetic resonance spectroscopy (^{13}C CP/MAS NMR) and Fourier transform infrared spectroscopy (FTIR) using a diamond anvil cell.

Thermal fragmentation methods in combination with mass spectrometry were first introduced in Chapter 2. Curie-point pyrolysis mass spectrometry (CuPyMS) was applied to obtain chemical 'fingerprints' of the complete surface residues, including the extractable fraction and the non-extractable solid fraction. CuPyMS in combination with multivariate analytical techniques is shown to be a useful method for systematic and rapid analysis and categorisation of solid organic residues (33) and surrounding soil samples (2). The chosen analytical strategy presents not only a measure for similarity or dissimilarity in chemical composition of the samples, facilitating as such an objective classification of the residues, but also highlights the chemical components typical for the various clusters of residues. The chemical classification was shown to be a reflection of the original vessel use, and not an artefact of post-depositional changes in chemical composition of the residues. No evidence was found for the exchange of any significant quantity of compounds between archaeological residues and organic soils (this was also supported by CuPyGC/MS evidence presented in Chapter 3). In addition, no correlation could be found between the chemical composition of residues and the type of sediment in which they were preserved. Results from the CuPyMS studies give clear evidence to conclude that a correlation does indeed exist between the chemical composition of the surface residues and the morphological vessel type of the vessel in which they were found. The smaller vessels from Type I often showed soot residues on the outside and residues from cluster B/D on the inside, while the majority of the residues in vessels from Type II were charred starch-rich materials from cluster A. Vessels of different sizes and forms were, therefore, used for a different daily use within the indigenous settlement from the Roman period at Uitgeest-Groot Dorregeest. These results support the usefulness of a morphological vessel classification as a basis for functional studies within this ceramic complex.

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Chapter 3 focuses on the more detailed identification of compounds preserved in solid surface residues. Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGC/MS) is used for its capacity to non-selectively identify a wide range of compounds. Soil samples and experimentally charred modern foodstuffs were analysed alongside the residues for comparison on a molecular level. The use of CuPyMS, CuPyGC and CuPyGC/MS has resulted in the detection of many bioorganic moieties in the charred and non-charred residues situated on the interior or exterior surface of vessels including characteristic markers for proteins and polysaccharides and other compounds like fatty acids, polynuclear aromatic hydrocarbons and aliphatic polymers. The existence of clear differences in chemical composition between the residues is demonstrated. The chemical variation is related to the visual appearance of the residue.

Black residues occurring on the outside of vessels, show many polynuclear aromatic hydrocarbons like naphthalenes, phenanthrenes and their methylated isomers. Since these PAHs were found to desorb from the sample and are common in smoke condensates of wood fires, the residues are interpreted as 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. Some specific fragments indicative of charred proteins can be seen in the CuPyGC/MS results. Although thermal exposure has caused severe denaturation of the original peptide chain, some short peptides chains and individual amino acid characteristics are preserved. It is possible that a radical reaction causes the specific amino acid side chains to be linked chemically to (or to become 'embedded' in) the forming char. 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) in non-charred residues. Some specific fragments indicative of charred polysaccharides can be detected in the CuPyGC/MS results. 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. Lipid remains were detected in the form of free fatty acids, fatty amides and alkanes and alkenes. The free fatty acids evaporated from the sample. Fatty amides can be produced by heating fatty acids in combination with amines to a temperature of 200 °C. 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 the residues in DTMS studies (Chapter 4) and lipid extraction studies (Chapter 5).

In Chapter 4 direct temperature-resolved mass spectrometry (DTMS) is combined with multivariate techniques to group the residues into five "chemotypes" and compare their characteristics to the chemical properties of experimentally charred modern foodstuffs in order to facilitate the determination of the biomolecular origin of the various kinds of residues. The study of (34) solid residues by DTMS confirmed many of the earlier results obtained using CuPyMS (Chapter 2 and 3), and has resulted in a more detailed classification due to the measurement of a much wider range of masses. The temperature-resolved information facilitates the interpretation of the results in terms of chemical structure. Experimentally

“charred” modern foodstuffs (starch and protein) are used as reference materials to identify the biomolecular characteristics preserved throughout the thermal degradation of cooking and charring.

A combination of marker components and temperature-resolved information from the DTMS profile, gives indications of the origin of each chemotype of residues. Chemotype A₁ contains charred animal products (most likely milk), possibly in combination with a starch. Chemotype A₂ contains mildly charred (leafy) vegetable and grain mixtures. Chemotype B contains only smoke condensates (soot) from the wood fires used for cooking. Chemotype C contains a group of fairly diverse residues that represent both residues of mildly charred animal products without starch; and residues of protein-rich, lipid-free non-food products (possibly used for decoration of vessels). Chemotype D contains mildly heated residues of protein-rich, lipid-free foods or non-food products (bone or skin glue).

Many molecular characteristics of the original foods have been lost as a result of extensive thermal degradation during cooking but some specific characteristics have been preserved within the newly formed, condensed polymeric char-material. Although the level of interpretation remains limited to general food groups, it is the interpretation of these specific ‘signature’ characteristics that can render unexpected and exciting information about the origin of solid organic residues from archaeological context.

Chapter 5 presents a quantitative study of the extractable lipid composition in charred and non-charred surface residues and lipids absorbed into the ceramic material of the vessels, including fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols, sterols and long-chain alcohols. The methodological argument to study surface residues rather than lipids extracted from the ceramic fabric of the vessel, is tested by comparing extractable lipids from these different sources (absorbed residues versus surface residues) residues and comparing residues from surface residues from the Roman period and the Neolithic period.

Results show 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), 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 when 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 very 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 represent organic decorative materials, or be the result of storage or transport of solid organics. 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 did

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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. Some indication for site-specific degradation can be observed.

In Chapter 6 a combined Fourier transform infrared spectroscopy (FTIR) using a diamond anvil cell and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopic study, supported by elementary CHN analysis, has resulted in a semi-quantitative classification of solid organic residues found on ceramic vessels from Uitgeest-Groot Dorregeest. Three groups of organic residues (one consisting of two subgroups) were defined based on the extent of aromatisation that has taken place within the residue according to the NMR spectra: i) aromatic charred residues (mildly condensed and highly condensed); ii) cream-coloured non-aromatic residues and iii) soot residues containing polynuclear aromatic hydrocarbons. Both elementary CHN composition and FTIR characteristics were in direct agreement with the NMR results – additionally showing the extent and nature of the inorganic fraction of the residues as well as the presence of a limited amount of specific biomolecular characteristics for lipids, peptides and carbohydrates. Charred residues that are mildly condensed contain characteristics for lipid and peptides, while highly condensed chars contain only minimal amounts of lipids and occasional carbohydrates characteristics. Non-charred residues show FTIR spectra indicating the presence of calcium carbonate and a small amount of proteinaceous material without lipid components, which is in agreement with NMR results showing only aliphatic and carboxylic group resonance peaks. FTIR and solid-state NMR data confirm earlier results obtained in analytical pyrolysis studies and supports the application of DTMS in combination with MVA as a rapid strategy for the characterisation and classification of solid organic residues (Chapter 4).

In Chapter 7 the work in this thesis is summarised and discussed and some areas of further study are presented. Organic residue analysis has undergone revolutionary changes since the 1970s. 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 increasing body of knowledge about their origin and use within ancient societies.

In order to make molecular organic residue analysis a powerful tool in the study of ancient vessel use and diet, a number of basic research questions need to be further addressed. Firstly, the identification of the overall molecular composition of many organic remains needs to be improved. Most prominently absent from many studies of organic residues are compounds indicative of carbohydrates or starches, and to a lesser degree remains of proteinaceous materials. Secondly, models for the formation of organic residues in ceramic vessels must be designed and experimentally tested, in order to create a better insight in the mechanisms of preservation and decay of organic residues. Residue formation models play an important role in molecular organic residue analysis. They facilitate the translation of molecular results to original vessel contents, and determine the selectivity of the residue preservation processes and give us a tool to determine to what extent results from organic residue studies can be extrapolated to a larger archaeological context. Without verifiable models organic residue

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analysis will remain a curiosity rather than take its place in the essential archaeological debate about the meaning of use and function in object-oriented sciences such as archaeology. Thirdly, more systematic ceramic use-alteration studies (including organic residue analysis) are needed to put the results of organic residue analyses on individual vessels in the context studies of ceramic function in general. Only then will the results be optimally applied.

Samenvatting

Moleculaire Studies van Organische Residuen
Gepreserveerd in Prehistorisch Aardewerk

Samenvatting

De archeoloog heeft tot taak het gedrag van mensen uit het verleden te verklaren. De archeoloog probeert te ontdekken hoe de mensen leefden, wat voor soort familiebanden ze onderhielden, hoe zij hun voedsel verzamelden en voorbereidden, hun maatschappij organiseerden, hun hogere machten vereerden en goederen met naburige groepen ruilden. In tegenstelling tot andere disciplines in de menswetenschappen werkt archeologie slechts met de materiële overblijfselen van de maatschappij als bronmateriaal: de voorwerpen en de structurele overblijfselen van leefruimten (o.a. huizen, haarden, afvalhopen en waterputten), fragmenten van landbouwsystemen, en de resten van speciale activiteitengebieden (o.a. slachtplaatsen, steenwerkplaatsen, pottenbakkershutten of ovens).

Aardewerk assemblages bijvoorbeeld, worden vaak bestudeerd door archeologen op zoek naar informatie over een verscheidenheid aan verschillende aspecten van samenlevingen uit het verleden zoals socio-economische ontwikkelingen, de organisatie van productie en handel, en de mechanismen van culturele interactie. Om conclusies te kunnen trekken over menselijk gedrag op basis van de gefragmenteerde overblijfselen van een ooit bloeiende gemeenschap, is een duidelijk inzicht in de functie van de originele voorwerpen essentieel. Aardewerke potten zijn werktuigen die zijn gemaakt, gebruikt en uiteindelijk afgedankt door mensen in het verleden. Ze waren een deel van het dagelijkse leven van deze mensen. Zonder kennis over de functie van dit aardewerk, zijn we blind voor de informatie die ze bevatten. De archeoloog gebruikt aardewerk zoals de historicus geschreven teksten gebruikt: de informatie die uit de tekst wordt verkregen is moeilijk te interpreteren als het onduidelijk blijft of men een persoonlijk dagboek, een belastingsdocument, een populaire krant of het script van een komedie bestudeert. Door toenemende bewustwording van dit probleem, omvatten aardewerk studies in de archeologie tegenwoordig vaak ook functionele aspecten. Hoewel deze studies regelmatig context-informatie, morfologie en aardewerktechnologie bespreken, blijven de resultaten vaak beperkt tot relatief algemene en non-specifieke classificaties als gevolg van het complexe verband tussen vorm, functie en aardewerk-technologie. Er is onafhankelijke informatie nodig over de utilitaire rol van aardewerk in haar originele gebruiks-context, om te kunnen bepalen welke factoren belangrijk waren in de belevingswereld van mensen in het verleden. Dergelijk informatie kan worden verkregen door de studie van gebruiks-sporen. Gebruiks-sporen zijn gedefinieerd als alle mechanische of chemische veranderingen van het oppervlak of de matrix van het aardewerk als gevolg van oorspronkelijk gebruik (slijtage van het oppervlak; verkleuring van het aardewerk; roet afzetting en organische residuen zoals aankoeksels aan de binnenkant of buitenkant van de pot of organische stoffen geabsorbeerd in het aardewerk zelf). Eén van de meest recente ontwikkelde onderzoeksrichtingen in het functionele aardewerkonderzoek is organische residuen analyse - de studie van de chemische kenmerken van organische residuen die worden gevonden in directe associatie met aardewerk.

Dit proefschrift doet verslag van een systematische studie van de moleculaire karakteristieken van de organische residuen die bewaard zijn gebleven op aardewerk afkomstig uit een aantal prehistorische vindplaatsen, met als doel het originele gebruik van het aardewerk beter te kunnen bepalen. Het werk is vooral gericht op een collectie vaste residuen die als aankoeksels op het aardewerk van de inheems-romeinse nederzetting Uitgeest-Groot Dorregeest werden aangetroffen.

Deze studie gaat in op een aantal basisvragen betreffende de toepasbaarheid van moleculaire organische residuen analyse als hulpmiddel in de studie van oorspronkelijk aardewerk gebruik. Natuurlijk leidt de studie van kleine hoeveelheden complexe organische materialen die voor duizenden jaren in de grond zijn bewaard, tot veel methodologische en analytische uitdagingen. De belangrijkste methodologische vragen betreffen de selectie van representatieve en goed-bewaarde residuen, en de ontwikkeling van een analytisch protocol dat aangeeft welke analytische technieken de meest bruikbare informatie verstrekken of het meest complete chemische inzicht verschaffen (zie ook Hoofdstuk 1).

Van een archeologisch standpunt betreffen de meest prominente vragen de interpretatie van chemisch resultaten in termen van originele inhoud en origineel gebruik van het aardewerk. In welke mate kan de originele inhoud worden bepaald en hoe kan de kwestie van origineel gebruik worden benaderd? De meer specifieke vraag over het aardewerk van Uitgeest-Groot Dorregeest was, of groepen potten met verschillende vorm en/of grootte op verschillende manieren werden gebruikt in de Romeinse tijd. De organische residuen die in vier morfologische pottypen zijn gevonden werden bestudeerd om de moleculaire kenmerken van hun originele gebruik te bepalen.

Een breed spectrum van complementaire analytische technieken werd gebruikt om de moleculaire kenmerken van de vaste organische residuen te onderzoeken. De volgende technieken zijn toegepast (Hoofdstuk 1): licht microscopie en scanning electron microscopie (SEM), elementaire CHN analyse, Curie-punt pyrolyse massaspectrometrie (CuPyMS), Curie-punt pyrolyse gaschromatografie/massaspectrometrie (CuPyGC/MS), temperatuur-opgeloste massaspectrometrie (DTMS), gaschromatografie/massaspectrometrie (GCMS) van extraheerbare lipiden, vaste stof ^{13}C magnetische resonantiespectroscopie (^{13}C NMR CP/MAS) en Fourier transform infrarood spectroscopie (FTIR) met behulp van een diamantcel.

De thermische fragmentatiemethodes in combinatie met massaspectrometrie worden geïntroduceerd in Hoofdstuk 2. Curie-punt pyrolyse massaspectrometrie (CuPyMS) werd toegepast om chemische ‘vingerafdrukken’ van de complete residuen te verkrijgen met inbegrip van de extraheerbare en niet-extraheerbare fracties. Resultaten laten duidelijk zien dat CuPyMS in combinatie met multivariate analyse een goede methode biedt voor snelle, systematische analyse en categorisatie van vaste organische oppervlakte-residuen (33) en bijbehorende grondmonsters (2). De gekozen analytische strategie geeft niet alleen een maat voor de vergelijkbaarheid van de chemische samenstelling van de residuen (en maakt als dusdanig een objectieve classificatie van de residuen mogelijk), maar accentueert ook de chemische componenten typisch voor de diverse clusters residuen. De chemische classificatie was een aantoonbare afspiegeling van het originele aardewerkgebruik, en niet een artefact van post-depositionele chemische veranderingen. Er zijn geen aanwijzingen gevonden voor enige significante uitwisseling van componenten tussen archeologische residuen en de omringende grond (een conclusie die ook door CuPyGC/MS resultaten wordt bevestigd in Hoofdstuk 3). Bovendien bestaat er geen correlatie tussen de chemische samenstelling van de residuen en het type sediment waarin zij zijn bewaard. De CuPyMS resultaten tonen duidelijk dat een correlatie bestaat tussen de chemische samenstelling van de bestudeerde oppervlakteresiduen en het morfologische pottype waarin zij werden gevonden. De kleinere potten van Type I toonden vaak

roetaanslagen op de buitenkant en residuen van cluster B/D op de binnenkant, terwijl de meerderheid van de residuen in potten van Type II verkoolde zetmeel-rijke materialen van cluster A bevatten. Aardewerke potten van verschillende vorm en/of grootte werden gebruikt voor verschillend dagelijkse taken binnen de inheems Romeinse nederzetting in Uitgeest-Groot Dorregeest. Deze resultaten bevestigen het nut van een morfologische aardewerk-classificatie als basis voor functionele studies binnen dit aardewerkcomplex.

Hoofdstuk 3 concentreert zich op de meer gedetailleerde identificatie van componenten gepreserveerd in de oppervlakte-residuen. Curie-punt pyrolyse gaschromatografie/massaspectrometrie (CuPyGC/MS) heeft de capaciteit een breed spectrum van componenten op niet-selectieve wijze te identificeren. Ook grondmonsters en experimenteel verkoolde moderne levensmiddelen werden geanalyseerd voor vergelijking op moleculair niveau. Het gebruik van CuPyMS, CuPyGC en CuPyGC/MS van verkoolde en niet-verkoolde residuen heeft geresulteerd in de detectie van vele bioorganische componenten, zoals kenmerkende indicatoren voor eiwitten en suikers en andere verbindingen zoals vetzuren, polynucleare aromatische koolwaterstoffen en alifatische polymeren. Duidelijke verschillen in chemische samenstelling tussen de residuen werden aangetoond. De chemische variatie is gerelateerd aan de visuele kenmerken van de residuen.

Zwarte residuen die op buitenzijde van aardewerken potten voorkomen, tonen vele polynucleare aromatische koolwaterstoffen zoals naphthalen en phenanthreen en hun gemethyleerde isomeren. Aangezien deze PAKs van de samples desorberen en vaak in rookcondensaten van houtvuren worden aangetroffen, worden de residuen geïnterpreteerd als resultaat van koken op een open vuur.

De residuen aan de binnenkant van potten tonen drie groepen verbindingen van bioorganische betekenis: indicatoren voor eiwitten, suikers en vetten. Enkele specifieke fragmenten indicatief van verkoolde proteïnen kunnen in de CuPyGC/MS resultaten worden aangetoond. Hoewel de verhitting ernstige denaturatie van de originele peptide-ketens heeft veroorzaakt, zijn sommige korte peptide-ketens en individuele aminozuren bewaard gebleven. Het is mogelijk dat een radicaal-reactie specifieke aminozuur-zijketens chemisch verbind met (of inbed in) het verkoolde materiaal tijdens de vorming van het residu. De eiwit-indicatoren komen vooral voor in combinatie met vrije vetzuren en polysaccharide-indicatoren. In enkele gevallen, komen zij ook voor in combinatie met anorganische componenten (d.w.z. carbonaten) in niet-verkoolde residuen. Enkele specifieke fragmenten indicatief voor verkoolde polysacchariden zijn ook detecteerbaar tijdens CuPyGC/MS. Blijkbaar blijven bepaalde polysaccharide-kenmerken bewaard in verkoolde residuen gevormd bij lage temperatuur (mogelijk als gedehydrateerde oligosacchariden en melanoidinen). Het verhogen van de temperatuur tijdens het verkolen vermindert de herkenbaarheid van de resterende residuen. Lipiden kenmerken werden ontdekt in de vorm van vrije vetzuren, fatty-amiden en alkanen en alkenen. De vrije vetzuren kwamen vrij door simpele verdamping. De vetzuren kunnen worden geproduceerd door vetzuren in combinatie met aminen te verhitten (tot temperaturen van 200 °C of meer). Opvallend is dat de vrije vetzuren en de vetzuren vaak voorkomen in combinatie met eiwit-indicatoren en soms met indicatoren voor polysacchariden. Mono-, di-, of triacylglycerolen werden niet gedetecteerd met de gebruikte pyrolysetechnieken, maar werden aangetoond in de residue met DTMS (Hoofdstuk 4) en als extraheerbare lipiden (Hoofdstuk 5).

In Hoofdstuk 4 wordt temperatuur-opgeloste massaspectrometrie (DTMS) met multivariate technieken gecombineerd om de residuen te groeperen in vijf “chemotypes” en hun kenmerken te vergelijken met de chemische eigenschappen van experimenteel verkoolde moderne levensmiddelen ter bepaling van de biomoleculaire oorsprong van residuen. De studie van 34 vaste residuen met DTMS bevestigde veel van de eerder verkregen CuPyMS resultaten (Hoofdstuk 2 en 3), en heeft geresulteerd in een meer gedetailleerde classificatie (gevolg van het meten van een breder massa-bereik). De temperatuur-opgeloste informatie vergemakkelijkt de interpretatie van de resultaten in termen van chemische structuur. De experimenteel verkoolde moderne levensmiddelen (zetmeel en eiwit) werden gebruikt als referentiemateriaal om de biomoleculaire kenmerken te identificeren die tijdens de thermische degradatie van het koken en verkolen bewaard blijven. Een combinatie van indicatieve componenten en temperatuur-opgeloste informatie van het DTMS profiel, geven aanwijzingen voor de oorsprong van elk chemotype. Chemotype A₁ bevat verkoolde dierlijke producten (waarschijnlijk melk), mogelijk in combinatie met een zetmeel. Chemotype A₂ bevat mild verkoolde (blad) groente gemengd met graan. Chemotype B bevat enkel rookcondensaten (roet) van de houtvuren. Chemotype C bevat een groep vrij diverse residuen representatief voor zowel residuen van mild verkoolde dierlijke producten zonder zetmeel; als residuen van speciale eiwit-rijke, lipide-vrije oneetbare producten (die misschien voor decoratie van aardewerk werden gebruikt). Chemotype D bevat licht verwarmde residuen van eiwit-rijke, lipide-vrije voedingsstoffen of oneetbare producten (beender- of huidlijm). Vele moleculaire kenmerken van het originele voedsel zijn verloren gegaan als resultaat van uitgebreide thermische degradatie tijdens het koken, maar sommige specifieke kenmerken zijn bewaard binnen het nieuwe gevormde verkoolde gecondenseerde polymere materiaal. Hoewel het niveau van interpretatie tot algemene voedselgroepen beperkt blijft, is het de interpretatie van deze specifieke kenmerken die onverwachte en opwindende informatie over de oorsprong van organische residuen uit archeologische context kunnen opleveren.

Hoofdstuk 5 geeft een kwantitatieve studie van de extraheerbare lipiden in verkoolde en niet-verkoolde oppervlakte-residuen en lipiden geabsorbeerd in het ceramisch materiaal van potten zelf inclusief vetzuren, monoacylglycerolen, diacylglycerolen, triacylglycerolen, sterolen en lange-keten alcoholen. Het methodologische argument om oppervlakte residuen te verkiezen als sample-materiaal boven geabsorbeerde residuen (Hoofdstuk 1), werd getest door lipiden van verschillende soorten residuen te vergelijken en door lipiden van verkoolde residuen van verschillende ouderdom te vergelijken.

Resultaten tonen een hogere graad van preservatie van lipiden in oppervlakte-residuen dan in de direct aangrenzende ceramiek van het aardewerk. Niet alleen is in oppervlakte-residuen (vooral verkoolde residuen), de totale lipide-opbrengst per gram sample veel hoger, de hoeveelheid intacte acyl-lipiden en onverzadigde vetzuren is ook hoger in oppervlakteresiduen. Dit verschil in preservatie is waarschijnlijk het resultaat van een hogere temperatuur exposie van lipiden binnen de aardewerke potwand en van de resistente aard van de verkoolde oppervlakteresiduen (vooral wanneer ze ook eiwitten bevatten). Deze ontdekking kan belangrijke gevolgen voor bemonsteringsstrategieën in organische residue-analyse hebben.

Samenvatting

De lipiden extracten van verkoolde en niet-verkoolde oppervlakte-residuen zijn ook zeer verschillend van samenstelling. De verkoolde oppervlakte-residuen tonen de hoogste opbrengsten (in mg/g sample). De niet-verkoolde residuen tonen daarentegen veel kenmerken die een andere aardewerkgebruik suggereren (laag percentage organisch materiaal, een lagere hydrolyse graad, weinig of geen bacteriële degradatie en weinig of geen geabsorbeerd lipidemateriaal in het direct aangrenzende aardewerk). Waarschijnlijkst zijn deze organische residuen blootgesteld aan de lucht zonder ernstige verhitting te hebben ondergaan. De niet-verkoolde residuen zijn mogelijk het resultaat van decoratie van het aardewerk met organische materialen, of het resultaat van opslag of vervoer van vaste organische producten.

De lipiden van verkoolde oppervlakte-residuen van twee Neolithische vindplaatsen (ca. 5000 jaar oud) werden vergeleken met verkoolde residuen van drie inheems Romeinse nederzettingen (ca. 1800-2000 jaar oud). Hoewel de Neolithische verkoolde residuen niet beduidend veel lagere lipide-opbrengsten opleverden, bevatten lipideprofielen meer vrije vetzuren en een meer materiaal van bacteriële oorsprong. Dit fenomeen is waarschijnlijk het resultaat van een voortdurende lage graad van microbiële degradatie in de grond. Enkele aanwijzingen voor vindplaats-specifieke degradatie kunnen ook worden waargenomen.

In Hoofdstuk 6 is Fourier transform infrarood spectroscopie met diamantcel (FTIR) gecombineerd met vaste stof ^{13}C nuclear magnetic resonance (NMR) spectroscopie, ondersteund door elementaire CHN analyse. Een semi-kwantitatieve classificatie van vaste organische residuen uit Uitgeest-Groot Dorregeest kon worden gemaakt. Drie groepen organische residuen (één die uit twee subgroepen bestaat) werden gedefinieerd, gebaseerd op de mate van aromatisatie van het residu volgens de NMR spectra: i) aromatische verkoolde residuen (matig gecondenseerd en hoog gecondenseerd); ii) roomkleurige non-aromatic residuen en iii) roet residuen die polynucleare aromatic koolwaterstoffen bevatten. Zowel de elementaire C/H/N samenstelling als de FTIR kenmerken waren in directe overeenkomst met de NMR resultaten - en gaven extra informatie over de aard en omvang van de anorganische fractie en de aanwezigheid van een beperkte hoeveelheid specifieke biomoleculaire kenmerken voor lipiden, peptiden en koolhydraten. De matig gecondenseerde verkoolde residuen bevatten kenmerken voor lipiden en peptiden, terwijl de hoog gecondenseerde verkoolde residuen slechts minimale hoeveelheden lipiden en occasionele koolhydratenkenmerken bevatten. De niet-verkoolde residuen tonen FTIR spectra indicatief voor de aanwezigheid van calciumcarbonaat en een kleine hoeveelheid eiwit materiaal zonder lipidecomponent, hetgeen in overeenstemming is met de NMR resultaten die enkel pieken voor alifatische en carboxyl-groepen tonen. FTIR en NMR gegevens bevestigen eerdere resultaten die in analytische pyrolyse studies werden verkregen en ondersteunen de toepassing van DTMS in combinatie met MVA als snelle strategie voor de karakterisering en classificatie van vastere organische oppervlakteresiduen (Hoofdstuk 4).

In Hoofdstuk 7 wordt het werk in dit proefschrift samengevat en bediscussieerd en enkele suggesties voor verdere studie worden gepresenteerd. Organische residuen analyse heeft revolutionaire veranderingen ondergaan sinds 70er jaren. Voortdurende instrumentele innovaties in de analytische chemie hebben het mogelijk gemaakt steeds kleinere organische samples in steeds meer detail te bestuderen. De studie van de moleculaire samenstelling van

extraheerbare componenten, zoals lipiden, harsen en wassen hebben een grote hoeveelheid kennis over hun gebruik in het verleden opgeleverd.

Om moleculaire organische residuen analyse daarentegen te maken tot een krachtig hulpmiddel in de studie van aardwerkgebruik en oude diëten, moeten een aantal basale onderzoeksvragen worden opgelost. Ten eerste, moet vaker de chemische samenstelling van complete organische residuen worden bepaald. In de meeste organische residuen studies schitterend koolhydraten of zetmeel en, in mindere mate eiwitten in afwezigheid.

Ten tweede, moeten er modellen worden geformuleerd voor de vorming van organische residuen in aardewerk en vervolgens experimenteel getest, opdat een beter inzicht kan worden verkregen in de mechanismen van conservering en degradatie van organische residuen. Deze modellen spelen een belangrijke rol in moleculaire organische residuen analyse. Zij vergemakkelijken de vertaling van chemische resultaten op moleculair niveau naar originele potinhoud, en bepalen de selectiviteit van de residue conservering. De modellen geven ons een hulpmiddel om te kunnen bepalen in hoeverre resultaten van organische residuen analyse te extrapoleren zijn naar een bredere archeologische context. Zonder testbare modellen zal organische residueun analyse beperkt blijven tot een soort curiositeit in plaats van haar rechtmatige plaats in te nemen in de essentiële discussie over de betekenis van functie en gebruik in object-gerichte wetenschappen zoals archeologie.

Ten derde, moet er meer aandacht komen voor systematische gebruiksporen studies (met inbegrip van organische residuen-analyse) binnen de ceramische studies in de archeologie. Het is van groot belang om organische residuen analyse in te zetten binnen een archeologische probleemstelling opdat resultaten kunnen worden benut binnen de context van functionele aardewerkstudies. Alleen dan kunnen de resultaten tot hun volle recht komen.

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My other promotor and scientific example was Corrie Bakels. Her dissertation was the first I ever read and for many years I wanted my dissertation to be just as thorough as hers. Corrie, thank you for your many years of support, your combination of blunt honesty and polite reserve have always been familiar to me. After all is said and done, I have to admit that my dissertation is probably not half as thorough as yours. But it was a good thing to strive for.

For many years I worked within the context of the AMOLF, and I have come to realise that it is a place of great scientific luxury. Not only is the AMOLF equipped with the best scientific instruments, it also provides the help of a capable and enthusiastic support staff, and gives beginning researchers a once-in-a-lifetime start in science.

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Curriculum Vitae

About the Author

Tania Oudemans was born on April 21st, 1962 in Upper Fern Tree Gully (Victoria) in Australia. In 1980 she obtained her VWO degree at the 'Kruissheren College' in Uden, the Netherlands and left her residence in rural Brabant to become a student at the University of Leiden. Studying Biology (Bachelors in 1985) and Prehistoric Archaeology she obtained her Masters in 1989 at that same University. After a three month and 15000 mile road-trip around the United States, she was ready to start her PhD studies at the AMOLF in January of 1990 under the guidance of Prof. Jaap Boon (AMOLF, University of Amsterdam) and Prof. Corrie Bakels (University Leiden). This multi-disciplinary project continued through the spring of 1994, when Tania left for a 18 month Post-Doctoral position as a Research Fellow and Visiting Fulbright Scholar at the Museum Conservation Institute (formerly known as Conservation Analytical Laboratory) of the Smithsonian Institute in Washington DC in the USA. Here she continued her work on the analysis of solid surface residues and extractable lipids from ceramics in collaboration with Dr. David Erhardt, Dr. David Von Endt and Walter Hopwood. A collection of ethno-archaeological ceramics originating from a Kalinga Village in the Philippines was studied. During an additional six months in 1995 and 1996 Tania was a Visiting Research Fellow at the Geophysical Laboratory of the Carnegie Institute, Washington DC in the group of Dr. Ed Hare. After her return to the Netherlands in 1996 (and a long spell of disabling RSI), work was found in forensic trace-analysis and dactyloscopy for the police department of 'Hollands Midden' in Leiderdorp in 1998 and as a University Lecturer in the International Masters program of the Design Academy in Eindhoven (1998 through 2000) where she taught Research Methodology, and Research Design and Management. Tania has continued to work for the police and has held the job of Crime Analyst since 2002. After her complete recovery, Tania returned to AMOLF Institute on a part-time basis to update and renew her work on the study of solid organic residues in indigenous Roman ceramics, and to complete the thesis that is currently in front of you.

List of Publications

This Thesis is based on the Following Publications:

T.F.M. Oudemans & J.J. Boon 1996, 'Traces of ancient vessel use: investigating prehistoric usage of four pot types by organic residue analysis using pyrolysis mass spectrometry', *Analecta Praehistorica Leidensia*, vol. 26, 221-234, (Chapter 2).

T.F.M. Oudemans & J.J. Boon 1991, 'Molecular archaeology: analysis of charred (food) remains from prehistoric pottery by pyrolysis-gas chromatography/mass spectrometry', *Journal of Analytical and Applied Pyrolysis*, vol. 20, 197-227, (Chapter 3).

T.F.M. Oudemans, G.B. Eijkel & J.J. Boon in press-b, 'Identifying biomolecular origins of solid organic residues preserved on Iron Age Pottery using DTMS and MVA', *Journal of Archaeological Science*, (Chapter 4).

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', in H. Barnard & J. Eerkens (eds.), *Theory and Practice of Archaeological Residue Analysis*, British Archaeological Reports International Series, Archaeopress, Oxford, (Chapter 5).

T.F.M. Oudemans, J.J. Boon, & R.E. Botto in press-a, 'FTIR and solid-state ^{13}C CP/MAS NMR spectroscopy of charred and non-charred solid organic residues preserved in Roman Iron Age vessels from the Netherlands', *Archaeometry*, (Chapter 6).

Other publications related to this Thesis:

T.F.M. Oudemans, G.B. Eijkel & J.J. Boon 2005, 'DTMS and DTMS/MS study of solid organic residues preserved on ancient vessels', *Proceedings of the 33rd International Symposium on Archaeometry, 22-26 April 2002, Amsterdam*, H. Kars & E. Burke (eds.), Vrije Universiteit, Amsterdam, 501-505.

T.F.M. Oudemans & D. Erhardt 1996, 'Organic residue analysis in ceramic studies: implications for conservation treatment and collections management', *Archaeological conservation and its consequences*, A. Roy & P. Smith (eds.), The International Institute for Conservation of Historic and Artistic Works, London, Copenhagen, 137-142.

T.F.M. Oudemans, D. Erhardt & D.W. von Endt 1996, 'Organic Residues as Use Indicators: Comparative Spectroscopic Studies of Contemporary and Archaeological Kalinga Ceramics', *International Symposium on Archaeometry*, 80.

T.F.M. Oudemans, J.J. Boon & R.E. Botto 1992, 'Tracing vessel use by combined spectroscopic studies of solid organic residues on prehistoric pottery', *Archaeometry '92 Conference Abstracts*, 25.

I. Pastorova, T.F.M. Oudemans & J.J. Boon 1993, 'Experimental polysaccharide chars and their "fingerprints" in archaeological food residues', *Journal of Analytical and Applied Pyrolysis*, vol. 25, 63-75.

Propositions

part of

Molecular Studies of Organic Residues Preserved in Ancient Vessels

1. Contrary to popular belief amongst archaeologists solid organic residues preserved on ancient pottery are not, in fact, objectionable ‘crud’ that needs to be removed from the surface of precious vessels as fast as possible, but the carriers of valuable information about original diet and vessel use. *This thesis*

2. The molecular study of solid organic surface residues preserved on ceramic vessels can give direct evidence of the preparation and consumption of starches, proteins and lipids by people in the past. *This thesis*

3. Organic residue analysis is one of the most powerful tools in the study of actual ancient vessel use, and as such can help archaeologists determine the function of technologically and morphologically different vessel types. *This thesis*

4. In spite of thousands of years of direct contact, surprisingly little evidence can be found for the exchange of organic compounds between solid surface residues and the soil that surrounded it during burial. *This thesis*

5. Although according to Ratty “There is nothing - absolutely nothing half so much worth doing as simply messing about in boats,” messing about in the ceramic lab runs a close second as is proven by the extreme growth in the number of publications in the field of ceramic analysis in archaeology since the mid 1980’s.

Ratty in ‘The Wind in the Willows’ by Kenneth Grahame, 1908
Prudence M. Rice in ‘Journal of Archaeological Research’, 1996

6. The ‘Neolithic Revolution’ first described by Gordon Childe in the 1920’s, has long since been accepted as a ‘revolution’ not so much in the sense that its introduction was rapid, but rather to denote its importance and the degree of change it brought about. However, with recent archaeological discoveries of earlier and more diverse signs of domestication it is becoming increasingly clear that these changes were so gradual and varied that the term ‘Neolithic Experimentation’ seems more appropriate - including its

association with learning by trial and error.

Neolithic people in Britain preferred meat over grain, Richards et al. 'British Archaeology', 1996
11,400 year old domesticated figs from the Jordan Valley, Kislev et al. 'Science', June 2006

7. A profound investment in the quality of our national education system - including better education and increased salaries for teachers, more money for remedial teaching in schools, more practical training for students in professional education, and greater investment in our higher education - is of utmost importance to prevent the rise of a nation of poor, illiterate and bigoted people. Poverty, illiteracy and bigotry can simultaneously be reduced by raising the level of education the people enjoy.

8. Because the complete integration of a minority into society at large often leads to a decreased visibility of that minority, the gay and lesbian movement in the Netherlands has clearly overshot its goal - the result of 60 years of gay and lesbian liberation is not the celebration of our society's diversity but a new form of homophobia resulting from a renewed invisibility.

'COC 60 – Wapenfeiten', <http://www.coc.nl/>

9. Mutual consent ought to be the basis for all laws about sexual conduct between adults.

10. The high number of anti-terrorist laws instituted in the Netherlands since 9/11 is threatening to disturb the democratic balance between the human right to privacy and our government's power to breach it.

11. The first article of the Dutch constitution formulates in beautifully clear language one of the basic principles of our democracy: 'All persons in the Netherlands shall be treated equally in equal circumstances. Discrimination on the grounds of religion, belief, political opinion, race, or sex or on any other grounds whatsoever shall not be permitted.' All that is left to us is to live according to this principle.

*Allen die zich in Nederland bevinden worden in gelijke gevallen gelijk behandeld.
Discriminatie wegens godsdienst, levensovertuiging, politieke gezindheid,
ras, geslacht of op welke grond dan ook, is niet toegestaan.
Artikel 1, Grondwet voor het Koninkrijk der Nederlanden*

12. The miracle is not to walk on water. The miracle is to walk on the green earth, dwelling deeply in the present moment and feeling truly alive.

Thich Nhat Hanh in 'Peace is Every Step: The Path of Mindfulness in Everyday Life', 1992

Tania Oudemans

November 2006