Molecular studies of organic residues preserved in ancient vessels

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Chapter 6

FTIR and Solid-State ^{13}C CP/MAS NMR Spectroscopy of Charred and Non-Charred Solid Organic Residues

In this Chapter solid-state ^{13}C magnetic resonance spectroscopy using cross polarisation combined with high powered proton decoupling and magic-angle sample spinning and Fourier transform infrared spectroscopy using a diamond anvil cell, are employed to give information about the organic functional groups present in charred and non-charred solid organic residues and to give an insight in the degree of condensation of the chars. In addition, the application of these solid-state techniques is used for verification of earlier results obtained in analytical pyrolysis studies and 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.

Modified after:
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*.

1. Introduction

1.1. The use-alteration perspective

Pottery assemblages are frequently studied by archaeologists to obtain information about a variety of different aspects of past societies, such as social complexity, the organisation of production, trade and exchange, and the mechanisms of technological change and specialisation. In order to make such inferences from the ceramic remains of an early civilisation, a clear understanding of the original vessel functions is essential (Skibo 1992, 4). The archaeological information stored in any assemblage of artefacts can only be interpreted fully if the actual use of the objects is known.

Archaeological methods to identify vessel function are usually directed at the study of "intended vessel function" – the function the potter had in mind when making the vessel. Such studies are based on the assumption that technology, form and size of a vessel, are constrained by the intended use context (Braun 1983). However, the relationships between form, function and technology are complex and variable, and their study commonly renders only generalised frameworks of vessel functions (Rice 1987, 236-242; Rice 1990). In addition, a growing number of archaeological (Woods 1986; Mills 1999; Sinopoli 1999) and ethno-archaeological studies (Aronson *et al.* 1994; Arnold 2000) provides evidence that a variety of technological, environmental, and social factors determine the processes of pottery manufacture and use.

Studies directed at the "actual vessel use", on the other hand, can give independent information about the utilitarian role of ceramic containers. The traditional archaeological approach, the study of recovery context, is usually limited in scope (not many vessels are found in their original use-context) and its interpretations somewhat equivocal. The most direct and detailed way to identify original vessel use is through the study of "use-alterations" – detectable changes (e.g. scratches, wear, soot deposition, crust formation, cracks) as a result of the use of the vessel (Hally 1983; Henrickson 1990; Skibo 1992, 42-49; Kobayashi 1994; Skibo & Blinman 1999; Arthur 2002). 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 1920s and 30s (see Rottländer & Schlichtherle 1980 for references), organic residue analysis has only been widely applied since the 1980s (see Heron & Evershed 1993; and Evershed *et al.* 1999 for references) as a result of improvements in micro-analytical instrumentation and an increasing interest in functional aspects of archaeological ceramic assemblages.

1.2. Analytical strategies in organic residue analysis

In the study of organic residues found in association with ancient pottery, much research is aimed at the identification of extractable compounds such as lipids and waxes (Heron *et al.* 1994; Evershed *et al.* 1995b; 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). Usually these residues are preserved inside the actual ceramic fabric of the vessel, although resinous materials and wood pitches occurring as adhesives, repair materials or coatings on ceramic vessels have also been studied (Hayek *et al.* 1990; Charters *et al.* 1993a; Dudd & Evershed 1999; Regert *et al.* 2003; Stern *et al.* 2003). All these studies apply selective extraction techniques.

An alternative approach is the analysis of solid organic residues preserved as crusts or films adhering to the interior or exterior surface of ceramic vessels. These solids can be studied through the application of solid-state techniques such as Fourier Transform Infrared (FTIR) spectroscopy and solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy (Sherriff *et al.* 1995), or through molecular characterisation using pyrolytic fragmentation methods (Oudemans & Boon 1991, 1996; Regert & Rolando 2002; Oudemans *et al.* 2005). Although the chemical characterisation is often hindered by the complexity of the material and limited sample size, there are various methodological arguments that advocate the study of surface residues.

Firstly, the study of surface residues makes it possible to sample only one layer of material. Microscopic examination of cross-sections was performed on all residues in this study, and was applied 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. Post-firing surface sealing with organic mixtures is common amongst traditional potters and is performed with a variety of materials including common foodstuffs such as milk and various starch-rich foods (see references in Rice 1987, 163-164), as well as less edible materials such as beeswax, various resins and other plant materials (Arnold 1985, 139-140; Kobayashi 1994; Diallo *et al.* 1995). Inclusion of post-firing materials will complicate the interpretation of results.

Secondly, 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 in organic remains (Pastorova *et al.* 1993a; 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 ºC makes identification of biomolecular markers of the original foodstuffs increasingly difficult (Oudemans *et al.* in press-b).

A final strong argument for the study of surface residues is the fact that the archaeologist frequently has no prior knowledge of the nature of the original materials 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 identified prior to the application of extraction techniques.

1.3. Solid organic residues

Curie-point pyrolysis mass spectrometry (CuPyMS) studies have revealed signature mass spectra for both charred and non-charred residues (Oudemans & Boon 1991; 1996). Clusters of residues with specific chemical compositions could be distinguished, and were shown to be correlated to vessel morphology (Oudemans & Boon 1996). Although a useful approach in the study of actual vessel use and function, archaeological questions concerning the nature of the material kept in vessels could only be addressed through more detailed study. Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGC/MS) was used for identification of a wide range of compounds such as fatty acids, fatty amides and markers for proteins and polysaccharides in charred and non-charred residues. Polynuclear aromatic hydrocarbons and phenolics were detected in black residues interpreted as smoke condensates on the exterior of ceramic vessels (Oudemans & Boon 1991). Direct temperature-resolved mass spectrometry (DTMS) was then used to identify chemotypes – groups of residues with reccurring chemical characteristics – and compare them to experimentally heated modern foodstuffs. Chemotypes with specific groups of biomolecular characteristics could tentatively be identified as representing specific biomaterials (Oudemans *et al.* 2005; Oudemans *et al.* in press-b). An additional GC/MS study rendered detailed information on extractable lipids and helped to compare data on solid and extractable compounds in surface residues and absorbed residues (Oudemans & Boon in press).
Combining PyMS or DTMS techniques with multivariate analysis has thus shown to be a unique micro-analytical strategy for the characterisation and classification of solid organic residues. Information about the overall chemical composition of the solid material is combined with a high sensitivity for a wide range of very diverse compound classes, including many characteristic fragments of the condensed phase. However, the great challenge in micro-analytical studies of solid organic residues remains to gain a detailed understanding of the nature of the solid condensed phase, and the mechanisms of its formation.

1.4. FTIR and solid-state ^{13}C CP/MAS NMR spectroscopy

In this study solid-state ^{13}C NMR spectroscopy and FTIR spectroscopy are employed to quantify the relationship between solid and extractable compounds in residues, to give information about the organic functional groups present and to give an insight in the degree of condensation of the chars. In addition, the application of these solid-state techniques is used for verification of earlier conclusions from PyMS and DTMS studies and clarify the relationship between the, already thermally degraded, charred residues and the controlled heating fragmentation taking place in PyMS and DTMS studies.
FTIR spectroscopy is widely used analytical methods in the study of complex biological solids. The application of FTIR spectroscopy in the investigation of complex organic materials in art and archaeology started in the 1970s when commercial FTIR instruments became available (Low & Baer 1977 and references therein) and has grown in popularity since the introduction

of the FTIR microscope and the development of accessories (such as diamond anvil cells and ATR crystals) that enable the measurement of much smaller solid samples with better overall sensitivity and additional spatial resolution (Learner 1998; Bruni *et al.* 1999; Derrick *et al.* 1999; Van der Weerd *et al.* 2004a). Currently, FTIR is most commonly used in art and archaeology for the initial identification of compound classes in unidentified solid organics such as waterproofing materials (Colombini *et al.* 2003) and prehistoric adhesives (Regert *et al.* 2003); for the determination of the organic content of paints, slips or pigments on ceramics (Maniatis & Tsirtsoni 2002; Wang & Andrews 2002; Van der Weerd *et al.* 2004b); and for the analysis of multi-layer paint films in conservation studies (Van der Weerd *et al.* 2002; Van der Weerd *et al.* 2004a).

Although the application of infrared spectroscopy in organic residue analysis has led to the identification of starch-rich foodstuffs (yam, sweet potato, banana, rice, sago and taro) in surface residues from the Solomon Islands (Hill & Evans 1988, 1989), its application is mostly limited to initial identification of compound classes of unknown solids such as prehistoric adhesives (Regert *et al.* 2003) in ceramic vessels.

There is a vast amount of literature on the application of solid-state ^{13}C NMR spectroscopy to study carbon functional group distribution of biological solids in the fields of medicine, geology and food chemistry (see also for references Dybowski *et al.* 2000; Dybowski *et al.* 2002, 2004). Due to its enhanced sensitivity, the ^{13}C cross polarisation (CP) technique combined with magic angle spinning (MAS) has become one of the more commonly performed solid-state NMR experiments (Taylor 2004). This technique has been used extensively in the study of coals and different types of potential coal precursors in order to evaluate their contributions to low-rank coal formation (Hayatsu *et al.* 1984; Hayatsu *et al.* 1986; Botto 1987). Studies of experimentally obtained chars lead to the formulation of models for cellulose char formation (Boon *et al.* 1994; Pastorova *et al.* 1994) and the elucidation of the chemical structure of charred grains and pulses (Braadbaart 2005).

The application of ^{13}C CP/MAS NMR in archaeology has been the subject of two review papers (Ghisalberti & Godfrey 1998; Lambert *et al.* 2000) and can be summarised as a twofold approach: (1) for the identification of specific chemical compounds such as ancient isoprene rubber, ambrein in ambergris, and beeswax in royal seals; and (2) to obtain specific semi-quantitative carbon functional group distributions of complex solid organic materials such as oriental lacquers, ambers and fossil resins, animal materials (i.e. silk, bone, ivory, dental enamel); pitches and tars; decaying woods; organic food remains; and fossilised plant- and animal materials (i.e. bitumen, asphalt, jet). A more recent publication has appeared on the study of Neolithic soils in Bavaria (Schmid *et al.* 2001).

The application of ^{13}C solid-state NMR spectroscopy to the study of charred organic food residues was first reported in the 1990s (Oudemans *et al.* 1992; Sherriff *et al.* 1995; Oudemans & Erhardt 1996). Sherriff and co-workers combined ^{13}C CP/MAS NMR with ^{13}C and ^{15}N isotope analysis to identify the original foodstuffs cooked in pottery from the Kame Hill complex (Northern Manitoba, Canada) dated to the 10th - 16th century AD. Comparison of three charred residues with experimentally charred modern foodstuffs showed that archaeological residues were similar to those of charred meat and fish and lacked starch characteristics. Starch characteristics were observed in the experimentally charred Wild rice

(Zizania sp.) and Bulrush tuber (Scirpus sp.). The researchers concluded that starchy materials were not cooked in these vessels or that the starch components had degraded over time.

2. Experimental

2.1. Settlement

In the 1980s an excavation at Uitgeest-Groot Dorregeest in the Netherlands uncovered habitation remains dating back to the Late Iron Age, the Roman Iron Age and the Medieval period (Woltering 1982, 1983). The indigenous settlement at Uitgeest-Groot Dorregeest (ca. 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. Large raised bogs and low marshes intersected by creeks surrounded the settlement (van Geel *et al.* 2003). A fresh water gully was running in an old course of a salt-water creek. The west side of the habitation area showed a number of three-aisled houses and a substantial number of round water-wells (with linings constructed of sods) while several fragments of field systems on the east side of the settlement indicate agricultural activity close to home (Woltering 1983; Abbink 1999, 63-80).

Table 1: Residues and reference materials

Nr [a]	Find number[b]	Residue type	FTIR[c]	NMR[c]	CHN[c]
5	14-6-4.2	Char	X	X	X
8	14-6-4.4	Char	X	X	X
21	33-5-2.a	Char	X	X	X
24	33-8-2.b	Char	X	X	X
36	34-7-95.a	Char	X	X	X
31	35-7-28	Cream coloured crust	X	X	X
-	33-8-2.Shard	Ceramic material	X	X	X
1	7-7	Char	X	-	-
19	31-4.b	Soot (from exterior)	X	-	-
23	33-8-2.a	Char	X	-	-
26	34-0-30	Char	X	-	-
32	35-20	Cream coloured crust	X	-	-
-	-	Amylose untreated	X	-	-
-	-	Amylose char 250 ºC 2.5 h.	X	-	-
-	-	Amylose char 250 ºC 17 h.	X	-	-
-	-	Albumin untreated	X	-	-
-	-	Albumin char 250 ºC 2.5 h.	X	-	-
-	-	Albumin char 250 ºC 17 h.	X	-	-

[a] Nr: 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] X = analysis was performed

The plant and pollen 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 (van Geel *et al.* 2003). Although this environment cannot have rendered the most optimal living place, the landscape offered large grazing areas for herbivores.

2.2. Sample preparation and treatment

The indigenous ceramic assemblage from the Roman period 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. In the assemblage of 147 partial vessels with identifiable morphological type, many vessels contained visible surface residues of some sort. Soot residues occurred most commonly (45%); charred residues occurred on about every third vessel (32%); and other residues such as 'cream coloured crusts' (3%) occurred occasionally. Twelve archaeological samples (Table 1) were chosen for FTIR spectroscopy. Eleven samples are taken from various residues and one sample is taken of the ceramic fabric itself (from vessel 33-8-2). Both charred and non-charred (cream coloured) residues situated on the interior wall of various vessels were sampled, and one black soot residue was taken from the exterior of a vessel. Seven of these samples were large enough for NMR analysis (100 mg) and Carbon, Hydrogen and Nitrogen elemental analysis (1 mg). The residue samples were scraped from the ceramic surface with a scalpel, after removal of the outermost 0.5 mm of residue.
Table 1 also shows two sets of experimentally charred modern foods that were analysed by FTIR spectroscopy to illustrate the chemical changes during cooking or heating. Potato amylose (molecular weight over 150000, Janssen Chimica) and crystallised Bovine serum albumin (BDH biochemicals) were heated under laboratory conditions (in glass containers) from 20 ºC up to 250 ºC in 30 minutes, and subsequently progressively charred at 250 ºC for 2 and 16.5 additional hours respectively under a constant flow of nitrogen (100 ml/min). These charring conditions were chosen because they were estimated to reflect conditions in cooking vessels on an open fire, with a lack of oxygen closely reproduce circumstances during a cooking and charring procedure in a ceramic vessel in a solid or highly viscous material (such as a stew or thick soup). Additionally, 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 (Pastorova *et al.* 1993b)

2.3. Instrumental conditions

Elemental Carbon, Hydrogen and Nitrogen Analysis. CHN compositions were determined after samples were dried, weighed and analysed in duplo using a Carlo Erba 1500 CHN analyser. Elemental composition is referenced in weight percentages using N-phenyl-acetamide or acetanilide (C_8H_9NO) as a standard to determine relative detector response. C/N and C/H ratios are directly calculated from their weight percentages.

FTIR Spectroscopy. FTIR measurements are performed by squeezing small solid samples (< 1 μg) between the two diamond windows of a P/N 2550 diamond anvil cell (Graseby Specac, Orpington, Kent, UK). The FTIR single point spectra are acquired on a Bio-Rad Stingray 6000 (Varian, formally known as Bio-Rad, Cambridge, MA, USA), which combines a step-scan Michelson interferometer (Bio-Rad FTS-6000), a Bio-Rad UMA 500 infrared microscope and a mercury-cadmium-telluride (MCT) detector (Van der Weerd 2002). Analysis was carried out in transmission mode (in which the light passes through the sample) and recorded at 4 cm^{-1} spectral resolution, a mirror speed of 5kHz and an undersampling ratio (UDR) of 2. A minimum of 100 spectra was accumulated to obtain a good S/N ratio. The interferometer and the data acquisition were controlled using Win-IR Pro software version 2.5 from Bio-Rad and the resulting spectra were processed with version 2.96 of the same program. Base-line correction and subtraction of a spectrum of the empty anvil cell (with added water vapour) were applied to enhance the spectral quality. Due to the inhomogeneous nature of solid residues, samples were ground with a mortal and pestle and homogenised profoundly prior to use. In addition, multiple spectra were collected from different small amounts of each residue to prevent collection of non-representative data.

Solid-state ^{13}C CP/MAS Nuclear Magnetic Resonance Spectroscopy. NMR measurements with Cross-polarisation (CP) and high-powered proton decoupling and magic-angle sample spinning (MAS), were obtained at a carbon frequency of 25.18 MHz and a proton frequency of 100.13 MHz on a Brüker CXP-100 (B_o = 2.3 Tesla) spectrometer equipped with a double-tuned single coil probe and a dual air-bearing spinning apparatus. The ceramic spinners with an internal volume of 300 μl were packed with the ground up archaeological residue mixed with KBr and spun at approximately 4 kHz. Cross-polarisation (CP) experiments were performed using a contact time of 1.5 ms and a 1 s pulse repetition time (recycle delay time), a 56 kHz proton decoupling field (equivalent to 4.5 μs 90º pulse width), a spectral width of 10 kHz, and an acquisition time of 20 ms. Contact time could not be optimised using archaeological samples due to limited sample size. However, experimental parameters were based on earlier studies with cellulose chars (Pastorova *et al.* 1994). Between 11k and 87k number of scans were obtained for each sample. In a typical experiment, a memory of 200 words was allocated for data acquisition and it was then increased to 4k (2k real data) by zero filling. Before Fourier transformation of the data, the interferogram was apodized using a trapezoidal window function, ramped linearly from the 40th data point to the 400th (and last) data point. Chemical shifts are referenced in parts per million (ppm) from tetramethylsilane (TMS) using tetrakis(trimethylsilyl)silane (TKS) as a secondary reference (Muntean *et al.* 1988).

3. Results and Discussion

3.1. CHN Analysis

Results from CHN elementary analysis are summarised in Table 2. A distinct difference is visible in total organic content between charred residues (38 - 67%), non-charred residue (4%) and the ceramic material (5%). The cream coloured residue obviously consists mainly of inorganic material and the highest overall organic content is found in charred residue 33-8-2.A.
Charred residues show a considerable variation in elemental composition. The C/H ratios vary from 10.2 – 15.5 % indicating a less aliphatic and more condensed nature of the material as the ratio goes up. The C/N ratios vary from 6.3 – 10.8 % indicating a decrease in the amount of nitrogen in the sample as the ratio goes up. Since these two phenomena do not run parallel, multiple factors are influencing the elemental composition of the samples (e.g. the original material involved as well as the extent of charring that has taken place).

Table 2: CHN Elementary composition

Nr [a]	Find number[b]	Sample	C [%]	N [%]	H [%]	Total Organic [%]	C/N	C/H
5	14-6-4.2	Char	48.5	4.6	3.5	56.6	10.5	13.9
8	14-6-4.4	Char	39.6	5.6	3.8	49.0	7.1	10.4
21	33-5-2.a	Char	48.1	7.6	3.1	58.8	6.3	15.5
24	33-8-2.a	Char	57.2	5.3	4.4	66.9	10.8	13.0
31	35-7-28	Cream coloured crust	3.6	0.2	0.6	4.4	18.0	6.0
36	34-7-95.a	Char	30.6	4.3	3.0	37.9	7.1	10.2
-	33-8-2 S	Ceramic material	3.5	0.3	0.9	4.7	11.7	3.9

[a] Nr: 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.

3.2. FTIR Results – Experimental chars

Solid-state FTIR spectra of untreated and experimentally heated amylose and BSA clearly illustrate the severity of the chemical changes taking place during thermal degradation.
Amylose shows FTIR spectra (Fig. 1, Table 3) similar to those observed in earlier charring experiments with cellulose (Pastorova *et al.* 1994). Untreated amylose shows a characteristic polysaccharide pattern (Derrick *et al.* 1999, 180) with a broad and intense O-H stretching band between 3000 - 3600 cm^{-1}, and various intense C-O stretching bands in the area 1000 - 1260 cm^{-1} (including those for intact pyranose-units such as C-O-C skeletal vibrations at 1080 cm^{-1} and

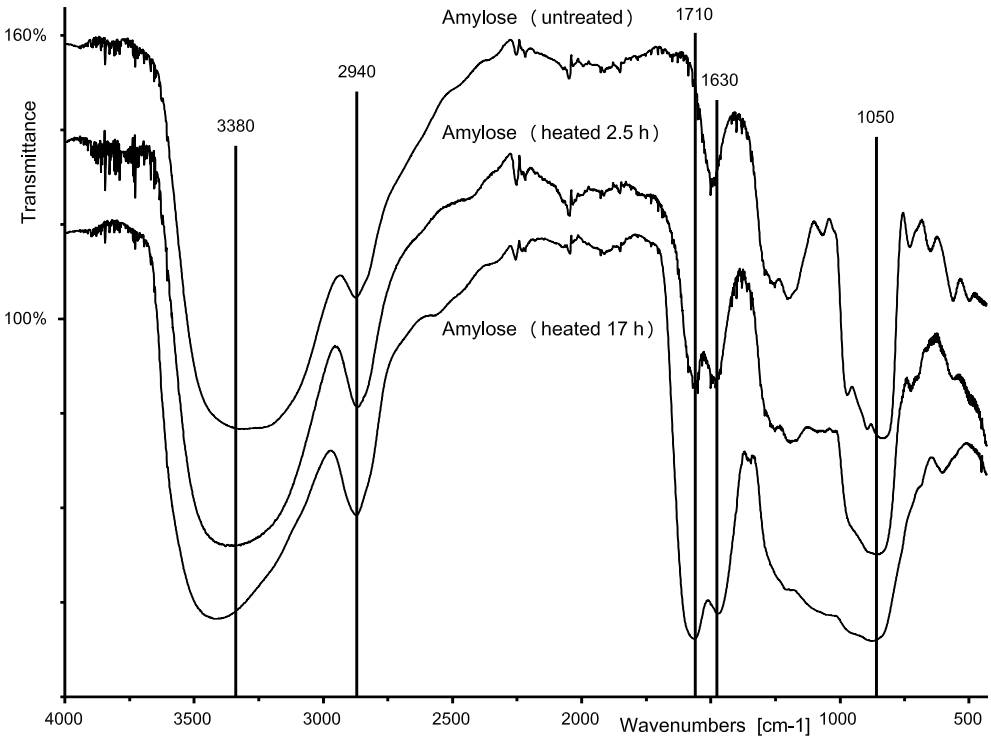


Figure 1: FTIR spectra of amylose.
Amylose is measures in untreated form and after experimental heating under laboratory conditions (in glass containers) from 20 ºC up to 250 ºC in 30 minutes, and subsequently progressively charred at 250 ºC for 2 and 16.5 additional hours respectively under a constant flow of nitrogen (100 ml/min). Spectra are normalised to 100% and shifted by 20% transmittance for visual clarity.

C-O in C-6 skeletal vibrations at 1030 cm^{-1}). After 2.5 hours of heating at 250 ºC, the FTIR spectrum shows a lower O-H stretching band due to dehydration and an increase in the C-H stretching band due to formation of alkylated furans, and alkylated aromatic compounds. In addition, a loss of fine structure in the C-O band area indicating the loss of intact pyranose-units is seen. Two new bands at 1630 and 1710 cm^{-1} assigned to C=C and C=O stretching frequencies begin to appear, and a broad band between 1000 - 1500 cm^{-1} indicates increasing aromatisation (Pastorova *et al.* 1994). After 17 hours of heating at 250 ºC, the O-H stretching band is even more reduced. The bands at 1630 and 1710 cm^{-1} have increased and the fine structure in the aromatic area between 1000 - 1500 cm^{-1} is almost completely lost. However, a change in the relative intensities of the bands at 1630 and 1710 cm^{-1} has not yet occurred, indicating that loss of oxygen as shown in cellulose chars at 310 ºC has not yet taken place (Pastorova *et al.* 1994).

Table 3: Presence of transmission bands in reference materials (Fig. 1,2)

		Amylose			BSA		
Sample		Untreated	Charred 250 ºC 2.5 h.	Charred 250 ºC 17 h.	Untreated	Charred 250 ºC 2.5 h.	Charred 250 ºC 17 h.
FTIR Transmission bands	Region [cm^{-1}]						
O-H (stretch)	3000 - 3600	+	+	+	+	+	+
N-H	3400 - 3200	-	-	-	+	+	+
C-H (stretch)	2800 - 3100						
Sym/Asym. Methyl CH_3	2872/2962						
Sym/Asym. Methylene CH_2	2850/2926						
CN Nitrile (stretch)	2200 - 2250	-	-	-	-	-	+
C=O Keton (stretch)	1700	-	+	+	-	-	+
Amide I: C=O (stretch)	1660 - 1600	-	-	-	+	+	+
O-H (bend)	1650	+	-	-	-	-	-
C=C Aromatic (stretch)	1620 - 1640	-	+	+	-	+	+
Amide II: N-H (bend) & C-N (stretch)	1565 - 1500	-	-	-	+	+	-
C-H (bend)	1300 - 1480	+	+	-	+	+	+
C-O (stretch)	900 - 1260	+	+	-	-	-	-
C=C Aromatic (skel. vibr)	1450	-	+	+	-	-	+
C-H def. vibr in aromatics	1000 - 1200	-	+	+	-	-	+
C-O (antisym bridge stretch)	1170	+	-	-	+	-	-
C-O-C (pyranose skel vibr)	1080	+	-	-	-	-	-
C-O (C-6 skel vibr)	1030	+	+	-	-	-	-

Absence or presence of transmission bands in normalised FTIR transmission spectra:
+ = present and, - = absent

Untreated BSA shows a FTIR spectrum (Fig. 2, Table 3) similar to those of other proteinaceous materials (Derrick *et al.* 1999, 181-183). Three characteristic transmission bands are visible: i) the Amide I band - a strong carbonyl in the area 1650 cm^{-1}; ii) the Amide II band near1550 cm^{-1} attributed to a combination of C-N stretching and N-H bending in secondary amides; and iii) a C-H bending occurring at 1450 cm^{-1} sometimes called Amide III band. A broad band for the O-H stretching can be seen, with the asymmetrical and symmetrical N-H stretching bands appearing as sharper shoulders superimposed upon it. Smaller bands around 2970 cm^{-1} assigned to C-H stretching in aliphatic compounds are also visible. After 2.5 hours of heating at 250 ºC the FTIR spectrum shows relatively little change. A reduction in the O-H band leaves a sharper N-H stretching band in the 3000 - 3600 cm^{-1} area indicating dehydration of the sample. A decrease in the Amide II indicates protein fragmentation (reducing the number of intact C-N peptide bonds). As the heating time increases to 17 hours at 250 ºC the spectrum changes dramatically reflecting increasing dehydration and a loss of most of the protein characteristics

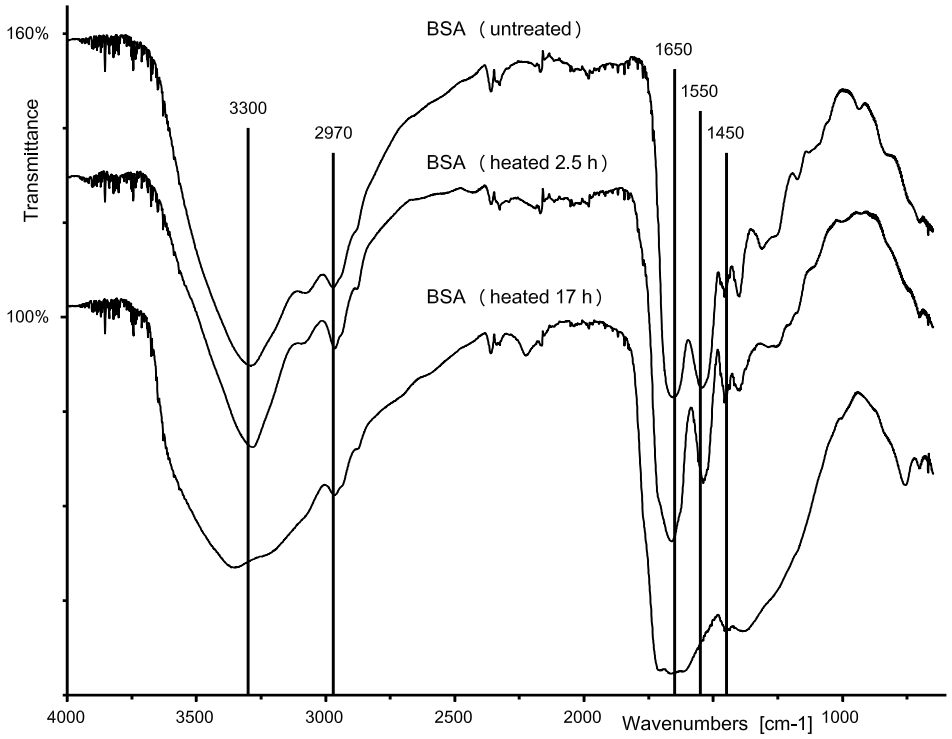


Figure 2: FTIR spectra of crystallised Bovine blood serum albumin (BSA)
BSA was measured in untreated form and after experimentally heating under laboratory conditions (in glass containers) from 20 ºC up to 250 ºC in 30 minutes, and subsequently progressively charred at 250 ºC for 2 and 16.5 additional hours respectively under a constant flow of nitrogen (100 ml/min). Spectra are normalised to 100% and shifted by 30% transmittance for visual clarity.

(Amide II and Amide III bands are mostly gone and the Amide I band is strongly reduced in intensity). An increasing condensation and aromatisation of the sample has taken place. A new band around 2225 cm^{-1} can be ascribed to the presence of organic nitriles, resulting from dehydration of amines or other condensation reactions taking place during extended periods of heating.

3.3. FTIR results – Archaeological residues

The FTIR transmission spectra of the archaeological residues show less resolution and are characterised by the presence or absence of a relatively limited number of broad bands (Fig. 3, Table 4).

Table 4: Relative intensities of various indicative FTIR bands

Nr [a]		36	8	24	5	21	31	1	19	23	26	32	-
Find number [b] Find type C = char, L = cream coloured, B = soot, S = shard		34-7-95.a C	14-6-4.4 C	33-8-2.b C	14-6-4.2 C	33-5-2.a C	35-7-28 L	7-7 C	31-4.b B	33-8-2.a C	34-0-30 C	35-20 L	33-8-2.S S
Transmission bands	Region												
O-H or N-H (stretch)	3000-3600	+	++	+	±	+	±	+++	+++	+++	+++	+	-
C-H (stretch)	2800-3100	+	+++	++	+	±	-	+++	++	++	+++	-	-
CN Nitril (stretch)	2200-2250	-	-	-	-	-	++	-	-	-	-	±	-
C=C Aromatic (stretch)	1600-1700	+	+++	+++	+	++	-	++	-	+++	+++	-	-
C=O (stretch) Amide I-band	1650	+	±	-	-	-	+	±	-	-	+	+	-
Aryl-H in Aromatic 6 -rings	1580	-	-	-	-	-	-	-	++	-	-	-	-
Amide II-band	1565-1500	-	-	-	-	-	+	-	-	-	-	+	-
CO_3^{2-} (stretch)	1490-1370	-	-	±	±	-	+	±	-	±	-	+	-
C=C Aromatic (skel. vibration)	1450	+	++	++	±	±	-	+	+	+	++	-	-
O-H (bend) in alcohol & phenol	1410-1260	-	-	-	-	-	-	-	+	-	-	-	-
C-H def. vibrations in aromatics	1000-1200	++	±	+	++	++	-	+	++	±	++	-	-
Si-O-Si (stretch) in silica	1100-1000	+++	+	±	++	+	+++	±	++	±	±	+++	+++
O-C-O (stretch) in $CaCO_3$	910-850	-	-	±	±	-	++	±	-		-	++	-
Clay indicator (unkn.)	800-770	+++	+	±	++	+	±	-	++	±	-	+	+++
Dehydration [c]		H	M	H	H	H	-	L	L	L	L	-	-
Amount of Clay [d]		XH	M	L	H	M	L	-	H	L	-	M	XH
Amount of $CaCO_3$		-	-	L	L	-	H	L	-	L	-	H	-

Relative intensity of peaks on a nominal scale: not visible (-), very low (±), present (+), high (++), very high (+++).

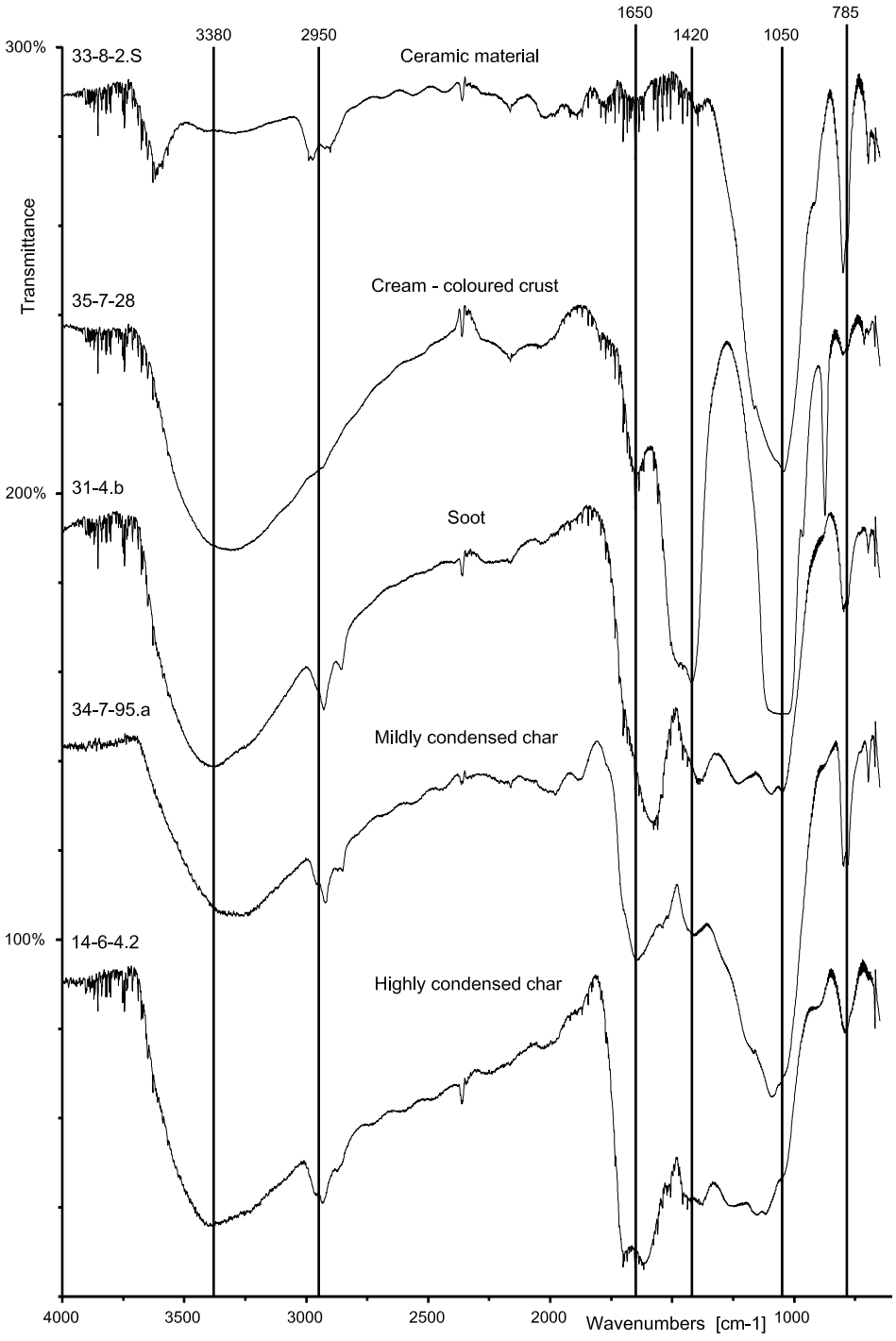
[a] Sample numbers refer to earlier publications (Oudemans & Boon 1996; Oudemans *et al.* in press-b).

[b] Find number: Archaeological registration code (first 2 digits indicate the excavation pit).

[c] Degree of dehydration of the chars is measured as a ratio between the height of the peak at 3300 cm^{-1} and the height of the highest organic peak above 1200 cm^{-1} with - = no measurable amount; L < 0.50; 0.50 < M < 0.80; and H > 0.80.

[d] Amount of clay in the sample is measured as a ratio between the height of the peak at 785 cm^{-1} and the height of the highest organic peak above 1200 cm^{-1} with - = no measurable amount; L < 0.30; 0.30 < M < 0.35; 0.35 < H < 0.50 and XH > 0.50.

[e] Amount of $CaCO_3$ in sample is measured as a ratio between the height of peak at 1050 cm^{-1} and the height of the highest organic peak above 1200 cm^{-1} with - = no measurable amount; L < 0.20 and H > 0.20.


3380
2950
1650
1420
1050
785
300%
33-8-2.S
Ceramic material
Transmittance
Cream - coloured crust
35-7-28
200%
31-4.b
Soot
34-7-95.a
Mildly condensed char
100%
14-6-4.2
Highly condensed char
4000
3500
3000
2500
2000
Wavenumbers [cm-1]
1000

An FTIR spectrum of the ceramic material of the vessel was taken in order to identify the characteristics of the backed clay, prior to measuring organic residues. The spectrum of ceramic material 33-8-2.S (Fig. 3) most closely resembles that of silica (SiO_2) (Derrick *et al.* 1999, 196; Coblentz Society June 2005), and is dominated by a major transmission band 1100 - 1000 cm^{-1} assigned to asymmetric Si-O-Si stretching; and minor transmission bands around 1600 cm^{-1} and 770 cm^{-1}. Although it was expected that the ceramic material would resemble kaolin clay (Derrick *et al.* 1999, 195), it must be noted that not only the O-H stretching bands between 3700 - 3200 cm^{-1} are absent in the ceramic material (dehydration takes place during backing of the clay), but also the transmission between 910 - 830 cm^{-1} (assigned to Si-O stretching) is absent.
The intensity of the FTIR silica peaks gives a measure for the relative amount of ceramic material present in the residues. For instance, the cream coloured residues have a much lower organic content than the charred residues (Fig. 3). In Table 4 the organic content is indicated (rang varying from low, to medium and high) as could be determined using the intensity of the FTIR silica peaks in the residues. Residue 33-8-2b is the char with the highest organic content and 34-7-95.2a with the lowest organic signature. These results confirm the elemental analysis (Table 2).
The FTIR spectra of the organic residues can be divided into three groups that coincide with their appearance: chars, cream-coloured residues and soot.
The spectra of the charred residues (Fig. 3, Table 4) are all characterised by the presence of an undefined broad transmission band between 1500 and 1000 cm^{-1} assigned to aromatic signals. Although this area can be divided into a band around 1450 cm^{-1} assigned to C=C skeletal vibrations in aromatics, and an area between 1200 - 1000 cm^{-1} commonly assigned to a series of C-H deformation vibrations in aromatics, it is often difficult to separate these bands. In strongly aromatic materials the two bands tend to blend. In addition, the transmission at 1100 - 1000 cm^{-1} originating from the ceramic material regularly obscures transmissions from C-H deformation vibrations.
Charred residues also show a broad combined O-H/N-H stretching band in the area 3600 - 3000 cm^{-1}, with superimposed upon it, symmetric and asymmetric methyl and methylene C-H stretching bands in saturated aliphatic compounds between 3100 - 2800 cm^{-1}. This combination is assigned to the presence of lipids or other O-H containing compounds with saturated aliphatic chains. The intensity for the O-H/N-H stretch signal gives a measure of the amount of dehydration that has taken place in the sample due to increasing condensation (as was shown in experimentally heated foodstuffs). The degree of dehydration is summarised in Table 4. Most chars show relatively low dehydration, but residue 33-8-2b shows medium dehydration and residues 33-5-2a, 14-6-4.2 and 34-7-95a show a high degree of dehydration.
A last characteristic of all charred residues is a transmission band at 1700 - 1600 cm^{-1} although its relative intensity in the spectrum varies. Assigning a specific origin to this band is difficult

Figure 3 (on facing page): FTIR spectra of different types of archaeological residues.
Various different types of solid organic residues (cream-coloured crust, soot, and mildly and highly condensed chars) and the ceramic material originating from one of the shards (shard 33-8-2). Spectra are shifted by 50% transmittance for visual clarity.

due to a variety of transmission bands occurring in this particular area: the C=O stretching band in the Amide I peak (in unheated proteins); the O-H stretching in combination with the C=O stretching in ketones (in mildly heated starches); and the aromatic C=C stretching band (typical for aromatic networks) may cause render this effect. However, given the charred nature of the residues it is likely that the C=C stretching band is, at least partly, responsible for the signal in most of the residues. In one charred residue a double transmission band at 1700 - 1600 cm^{-1} is visible, in combination with an intense C-O stretching band in the area 1200 – 950 cm^{-1} (e.g. residue 14-6-4.2). This particular combination indicates the preservation of carbohydrate functional groups in a char. In some residues a more specific Amide I band is visible indicating the presence of intact peptides (e.g. residue 34-7-95a, 14-6-4.4 and 34-0-30)
In short, the FTIR spectra of the charred residues closely resemble those of experimentally heated modern foodstuff and give ample confirmation of the aromatic nature and the degree of dehydration that has taken place in the ancient chars. Functional groups prevalent in lipids can be identified and a small amount of functional groups indicative of mildly heated carbohydrates or intact peptides.
The FTIR spectra of cream coloured residues (one of which is shown in Fig. 3) typically lack both the distinct aliphatic C-H stretches between 2800 - 3100 cm^{-1} (indicative of aliphatic structures such as lipids), and the broad transmission area between 1000 - 1500 cm^{-1} (indicative of aromatic compounds). The spectra of the cream coloured residues are both clearly dominated by an intense peak at 1100 - 1000 cm^{-1} and a medium high peak at 797 cm^{-1}, indicative of the presence of sand or some ceramic material in the sample. In addition, a fairly sharp band at 1490 - 1370 cm^{-1} (CO_3^{2-} stretching band) and a sharp transmission at 870 cm^{-1} (O-C-O bending band) are visible that are ascribed to the presence of precipitated calcium carbonate ($CaCO_3$) (Derrick *et al.* 1999, 194; Maniatis & Tsirtsoni 2002; Coblentz Society June 2005). Superimposed upon these bands are two smaller transmissions at 1650 and 1530 cm^{-1} that can be assigned to Amide I and II bands indicative of proteins and peptides. In residue 35-7-28 a small transmission band at 2239 cm^{-1} (CN nitrile stretching band) is present that can be seen as a characteristic of degraded proteinaceous materials.

In summary, the FTIR spectra show that the cream coloured residues are non-carbonised residues with low organic contents consisting primarily of inorganic salts such as calcium carbonate. The organic component consists primarily of proteinaceous material while lipids are absent from the material. This confirms earlier DTMS results that identified these residues as non-aromatic materials contained no lipids (Oudemans & Boon 1991, 1996; Oudemans *et al.* in press-b).
The soot residue (Fig. 3) resembles the charred residues to some extent, except that the saturated aliphatic C-H signal only shows the methylene group signals (2928 and 2857 cm^{-1}), indicating the absence of methyl end groups. In addition, the aromatic transmission bands are located in a slightly different place. The aromatic Aryl-H bands between 1600 and 1500 show a peak at 1580 indicating the presence of six-membered aromatic rings that are further conjugated – a feature typical for the polyaromatic hydrocarbons (Williams & Fleming 1966, 67) that were shown to be an important components of this residue (Oudemans & Boon 1991).

3.4. Results Solid-state ^{13}C NMR – Archaeological residues

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 (Fig. 4, Table 5). Aliphatic structures (sp^3 hybridised carbons) show up as a broad resonance band between 10 - 60 ppm, demonstrating individual resonance peaks for $-CH_3$ or $-CH_2-$ in some of the residues (e.g. 34-7-95a). Aromatic structures and alkenes (sp^2 hybridised carbons) are visible 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 are visible in some of the residues (Table 5). A clear resonance peak at 160 - 180 ppm is assigned to a combination of various carbonyl carbons (C=O), such as in carboxylic acids and their salts at 166 - 181 ppm; aliphatic esters R-COO-R' at 169 - 176 ppm; and amides $-CONH_2$ at 162 - 179 ppm.

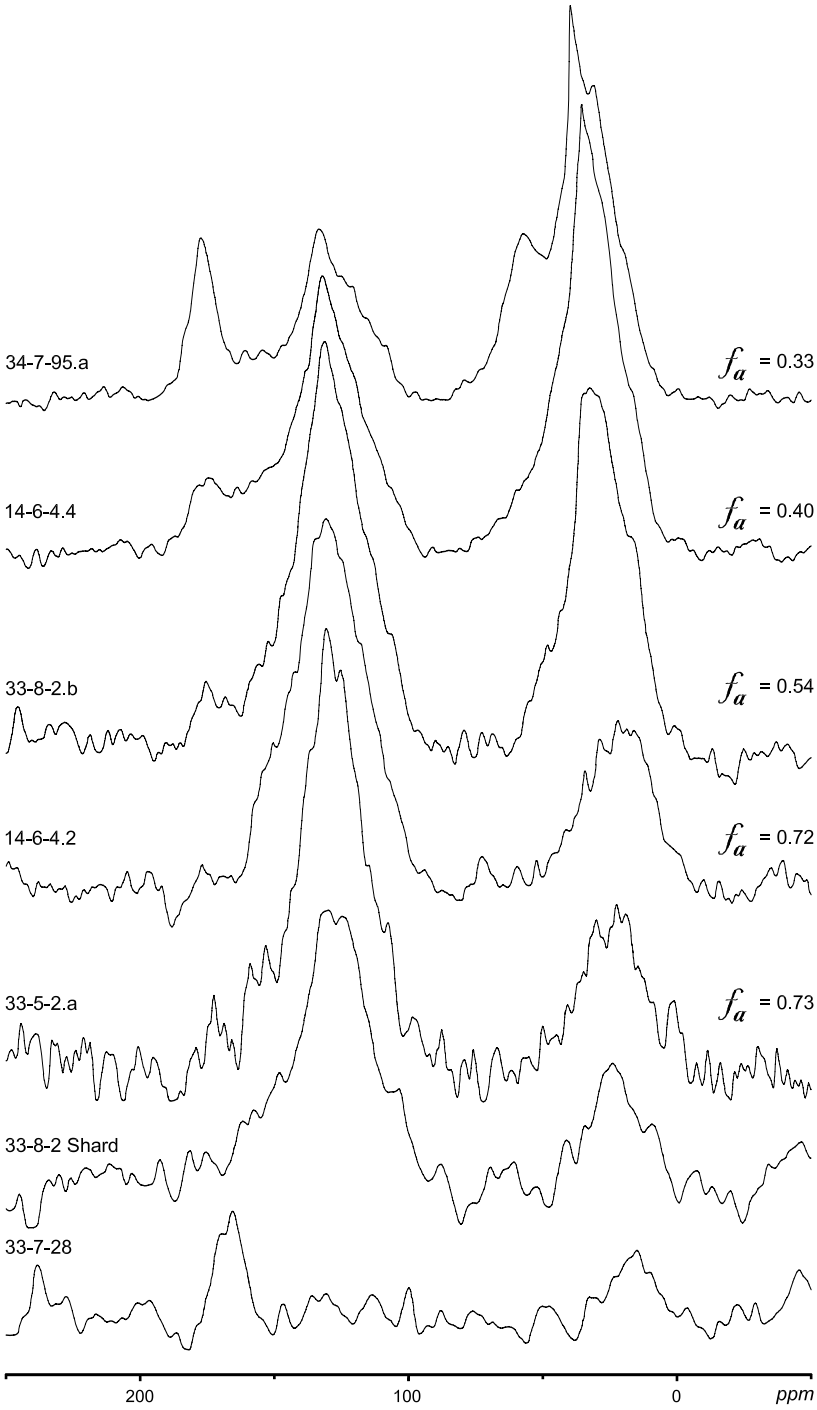
Table 5: Resonance in NMR spectra

Nr [a]	Sample nr [b]	NS [c]	Carbonyl groups	Furanyl/ Phenolic C-O	Arom.	R-CN Nitril	O-alkyl	C-N Collag.	Aliph	f_a	DTMS Chemo -type
ppm			160-180	155	100-150	114-124	70-75	50-60	10-60		
36	34-7-95.a char	73K	++	-	+	±	-	+	++	0.33	A1
8	14-6-4.4 char	87K	+	-	+	-	-	-	++	0.40	A1
24	33-8-2.b char	60K	+	?	++	-	-	?	++	0.54	A2
5	14-6-4.2 char	40K	-	±	++	-	±	-	+	0.72	A2
21	33-5-2.a char	11K	±	?	++	-	-	-	+	0.73	-
-	33-8-2 S ceramic material	57K	-	-	+	-	-	-	+	-	-
31	35-7-28 cream coloured crust	56K	+	-	-	-	-	?	±	0.00	D

Resonance intensity on a nominal scale- absent (-), trace (?), low (±), present (+) and high (++)
[a] Numbers refer to earlier publications (Oudemans & Boon 1996; Oudemans et al. in press-b)
[b] Sample number: Archaeological registration code - the first 2 digits indicate the excavation pit
[c] NS = number of scans.
[d] f_a = fraction carbon aromaticity (accuracy ± 0.02)

A broadening of the aliphatic resonance area between 50 and 60 ppm is 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 34-7-95a this effect is combined with a broadening of the aromatic resonance in the area of 114 - 124 ppm, assigned to the presence of a nitril resonance (R-CN), indicating the presence of proteinaceous material in this particular residue.
A resonance peak centred at 73 ppm is assigned to oxygen-substituted carbons in carbohydrates and is an indicator for residual sugar components. In this study, the resonance peak at 73 ppm only appears in very low intensity in residue 14-6-4.2. 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 broadening of the aromatic area can be observed in the highly aromatic sample 14-6-4.2 and possibly in sample 33-8-2b and 33-5-2a.
Some of the NMR spectra have relatively low S/N ratios, a phenomenon regularly observed in solid condensed materials such as coals (Hayatsu *et al.* 1986). These low S/N ratios can be due to low carbon content of the sample or to the presence of large amounts of free radicals in the material. The relative amount of carbon in the archaeological samples (Table 2) varies extensively and lies between 31% and 58% for charred residues and below 5% for the cream-coloured residue 35-7-28 and the ceramic shard material itself (33-8-2.S). The low organic content of residues 35-7-28 and 33-8-2.S may be the cause for their low S/N ratio, while the difference in S/N between 33-5-2a and the other charred residues, is most likely caused by the lower number of transients measured for this sample (see Table 5). The number of scans (NS) measured for residue 33-5-2a was relatively low (NS = 11K) in comparison with the other residues (NS >40K).
The degree of condensation in the solid residues can be quantified by calculating the fraction carbon aromaticity f_a (Hayatsu *et al.* 1986; Botto *et al.* 1987). Carbon aromaticity is calculated from integrated signal intensities of the aromatic (110 - 160 ppm) and aliphatic (0 - 105 ppm plus >160 ppm) transmission bands. For the aromatic and carbonyl carbons, signal intensities of the spinning sidebands were added to the intensity of the corresponding centre-bands. The fraction carbon aromaticity varies from 0.33 to 0.73 for charred solid residues, while the cream coloured residue does not show any aromatic resonance signal. Although the fraction carbon aromaticity for the organics in the shard material is not calculated, the material is highly condensed and shows an NMR spectrum comparable to cellulose chars produced at temperatures of over 350 ºC (Pastorova *et al.* 1994). The archaeological residues can be divided into three groups depending on their fraction carbon aromaticity: highly condensed chars, mildly condensed chars and non-aromatic residues.

Figure 4 (on facing page): ^{13}C CP/MAS NMR spectra of different types of archaeological residues.
Various solid organic residues were measured as well as the ceramic material originating from one of the shards (33-8-2.S). Three groups of organic residues were characterised based on the extent of aromatisation as expressed in the fraction carbon aromaticity (f_a) with an accuracy of $\pm$ 0.02. Aromatic residues show f_a values of 0.33 - 0.50 in mildly condensed chars (sample 34-7-95 and 14-6-4.4) and f_a values of 0.50 - 0.73 in highly condensed chars (sample 14-6-4.2 and 35-5-2.A). Non-aromatic residues, such as the cream-coloured residue 35-7-28, show no aromatic resonance and thus lack a f_a. The ceramic material shows a relatively highly condensed organic fraction, but the f_a was not calculated for this sample. Chemical shifts are expressed in ppm from TMS.


34-7-95.a
f_a = 0.33
14-6-4.4
f_a = 0.40
33-8-2.b
f_a = 0.54
14-6-4.2
f_a = 0.72
33-5-2.a
f_a = 0.73
33-8-2 Shard
33-7-28
200
100
0
ppm

Highly condensed chars

Highly condensed chars with fraction carbon aromaticity f_a >0.50 show a major aromatic resonance peak (Fig. 4), an aliphatic resonance peak, and occasional indications of residual sugar characteristics such as in residue 14-6-4.2. These residues show NMR results that are comparable to various experimentally charred materials. Microcrystalline cellulose charred for 150 min at 270 and 290 ºC shows similar aromatic and aliphatic resonance areas while still containing indicative sugar resonance peaks at 75 and 105 ppm. Chars produced at 310, 350 and 390 ºC show a progressive loss of oxygen functionalities leaving a major aromatic resonance band, an increasingly reduced aliphatic resonance and a small resonance at 155 ppm for aromatic C-O (Pastorova *et al.* 1994). Wild rice (Zizania sp.) and Bulrush tuber (Scirpus sp.) experimentally charred in replica ceramic cooking vessels over a wood fire, show broad aromatic and aliphatic resonance peaks as well as indicative sugar resonance peaks at 73 and 103 ppm (Sherriff *et al.* 1995). However, neither a charred archaeological specimen of Bulrush tuber, nor the charred organic residues discovered in pottery from the same study, showed any residual carbohydrate characteristics. NMR spectra of intact Emmer-wheat for experimentally charred for 120 minutes, still show some residual polysaccharide resonance around 155 ppm up to 400 ºC but no resonance peaks at 73 and 103 ppm (Braadbaart 2005).

Mildly condensed chars

Mildly condensed chars with fraction carbon aromaticity of 0.30 - 0.40 show major aliphatic resonance peaks, a carbonyl resonance and a sharp resonance peak for sp^2 hybridised carbons. In the least condensed residue 34-7-95a (Fig. 4) the strong aliphatic signal between 10 – 45 ppm in combination with a peak at 130 ppm due to unsaturation (C=C) and a firm carbonyl signal (C=O) at 170 - 175 ppm indicates the presence of lipids. Additional resonance peaks between 50 - 60 ppm and a broadening of the aromatic resonance between 105 - 120 ppm (nitril) give evidence for the presence of a proteinaceous component, with aliphatic amino acids in proteins resonating between 15 - 45 ppm, and aromatic amino acids between 110 - 160 ppm. Residue 34-7-95a is exceptionally well preserved, although a certain amount of condensation has taken place in the residue (protein decomposition is indicated by the reduction of the C-N resonance between 45 - 70 ppm) and the aromatic resonance band is increased relative to fresh proteinaceous materials. NMR spectra of fresh and experimentally charred pickerel (Stizostedion vitreum) illustrate a similar condensation process (Sherriff *et al.* 1995).

Non-aromatic residues

Non-aromatic residues have no measurable fraction carbon aromaticity because no aromatic resonance signal is measured. The presence of a carbonyl group resonance in sample 35-7-28 is assigned to the presence of inorganic carbonate salts rather then to lipids, due to the absence of any resonance at 130 ppm for unsaturated C=C bonds, and the absence of a strong aliphatic signal between 10 – 45 ppm for the aliphatics. No comparable spectrum could be found in the literature.

3.5. Archaeological inferences

Before being able to infer any archaeological information from the organic residues, it is of great importance to address the possible contamination of the archaeological surface residues with compounds from the surrounding soil. Earlier PyMS work compared the chemical composition of organic residues and peat samples from Uitgeest-Groot Dorregeest (Oudemans & Boon 1996). Peat samples were characterised by markers absent from archaeological residues such as markers for lignins and intact polysaccharides. More detailed study through PyGC/MS confirmed the absence of peat compounds from the archaeological residues (Oudemans & Boon 1991). PyMS and DTMS results have given no indication that a significant amount of organic material was exchanged between soil and residue and no significant difference in degradation could be determined between different sediments within Uitgeest-Groot Dorregeest (Oudemans & Boon 1996; Oudemans *et al.* in press-b). This is in agreement with a study of extractable lipid in ceramics from Great Britain (Heron *et al.* 1991).
Studying solid organic residues with different solid-state analytical techniques has thus led to a multifaceted picture indicating that the organic surface residues that appear on the ceramic vessels from Uitgeest-Groot Dorregeest reflect three groups of original residues: Chars (either mildly condensed or highly condensed); cream coloured residues; and soot.

Charred Residues

Charred residues obtained from the interior of ceramic vessels, have a high overall organic content of 38 - 67%. FTIR spectra show that the inorganic fraction of the chars is limited and consists primarily of silica. Whether the silica is an unwanted contamination with ceramic material of the vessel during sampling or originates from the soil is unknown.
FTIR spectra of charred residues closely resemble those of experimentally heated modern foodstuffs (both amylose and BSA) and give clear evidence for the aromatic nature of the material. Functionalities prevalent in lipids can be identified in all chars, and a small amount of functionalities indicative of mildly heated carbohydrates can be seen in residue 14-6-4.2, while intact peptides are indicated in residues 34-7-95a and 34-0-30. The presence of varying amounts of lipids in all charred surface residues in confirmed by a GC/MS study of extracted lipids (Oudemans & Boon in press-b). The chars can be divided into subgroups based on the extent of aromatisation that has taken place in the residue: mildly condensed residues and highly condensed residues.
Mildly condensed chars have a fraction carbon aromaticity (f_a) of less then 0.50 and show a significant NMR resonance for aliphatic structures and less intense aromatic signals. The C/H ratios of these chars (between 10 - 11) reflect limited condensation in comparison with those of the highly condensed category. The overall CHN composition 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 that closely resemble experimentally charred pickerel (Sherriff *et al.* 1995).
Highly condensed chars have a fraction carbon aromaticity of equal to or larger then 0.50 and show an intense NMR resonance peak for aromatics while the resonance for aliphatic structures

is less intense. The C/H ratios (between 13 – 16) reflect the progressive condensation that has taken place in these residues and the NMR results closely resemble experimental cellulose chars heated for 150 min at 270 and 290 ºC (Pastorova *et al.* 1993b). The organic content of these residues is relatively high (between 57% – 67%) but few specific biomolecular characteristics can still be traced in these residues. The NMR results show little indication for presence of lipids or protein remains, and the only markers that can be detected are very reduced signals for the presence of residual carbohydrate characteristics (in residue 14-6-4.2).
The explanation of the difference between these two subgroups of chars could be the increased heat exposure (temperature and heating time) that the highly condensed chars might have undergone in comparison to the mildly condensed chars. In addition, different biomaterials have a different tendency to form condensed chars. Starchy materials condense at relatively low temperatures, while for instance, fatty fish or meat are much less sensitive to condensation. Although, there are indications to propose such differences in original foods between the char groups, it is not possible to determine the exact food type at this time.

Cream coloured residues

Cream coloured residues obtained from the interior of ceramic vessels have a very low overall organic content of about 4%. Although this is about as high as the organic content of the ceramic material, its composition is quite different. Both FTIR and NMR show a complete absence of aromatic signals, which is in agreement with the low C/H ratio (6.0) and confirms earlier results from DTMS studies that these residues are not the result of carbonisation of foods in cooking vessels (Oudemans *et al.* 2005; Oudemans *et al.* in press-b). FTIR results show an overwhelming presence of precipitated calcium carbonate in the residues as well as the presence of silica. FTIR results of the organic fraction showed only a small amount of well preserved, unheated (or lightly heated) proteinaceous material. Due to the extraordinary low amount of organic material present, the explanation for the origin of these materials must probably be found in the storage or preparation of water or water-based liquids. The origin of the very low amounts of relatively well-preserved proteinaceous material may reflect a proteinaceous waterproofing material applied to the ceramic vessel prior to use. GC/MS studies of lipid extracts (Oudemans & Boon in press-b) indicated the presence of a very low amount of lipids in the surface residue 35-7-28 (1.32 mg/g residue) and no lipids in the ceramic directly adjacent to it. The lipid profile of the residue indicated extended exposure to oxidising conditions, and lacked indications of heat-induced hydrolysis or bacterial degradation of lipids. The vessel may have been used for transport or storage or solids materials (Oudemans & Boon in press-b). Because the vessel was not exposed to high temperatures, the proteinaceous material remained relatively unchanged.

Soot residues

Soot residues obtained from the exterior of ceramic vessels have an unknown organic content or elementary CHN composition. However, FTIR results show a medium amount of organic material in the residue (comparable to residue 14-6-4.4 with a organic content of 49%). FTIR shows the presence of aliphatic moieties and aromatics with further conjugated six-membered

rings so typical for polyaromatic hydrocarbons. Although not indicative of any foods cooked inside the ceramic vessel, these chemical characteristics are in complete agreement with DTMS results from earlier studies (Oudemans & Boon 1991; Oudemans *et al.* 2005; Oudemans *et al.* in press-b) and support the interpretation of these residues as smoke condensates originating from wood fires.

4. Conclusions

The combined FTIR and solid-state ^{13}C NMR spectroscopic study supported by elementary CHN analysis, has resulted in a quantitative classification of solid organic residues found on ceramic vessels from Uitgeest-Dorregeest, the Netherlands. Three groups of organic residues were defined based on the extent of aromatisation that has taken place within the residue according to the NMR spectra: aromatic charred residues (mildly condensed and highly condensed); cream coloured non-aromatic residues and soot residues containing polynuclear aromatic hydrocarbons. Both elementary CHN composition and FTIR characteristics were in direct agreement with the NMR results – 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 lipids 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 component, which is in agreement with NMR results showing only aliphatic and carboxylic group resonance peaks.
In addition, FTIR and solid-state NMR results confirm earlier results obtained in analytical pyrolysis studies and support the application of DTMS in combination with multivariate analysis as a rapid strategy for the characterisation and classification of solid organic residues.