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Summary

Molecular Studies of Organic Residues Preserved in Ancient Vessels

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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 area's and water-wells), fragments of agricultural field systems and remains of special activity area's (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.

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 been 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 A_1 contains charred animal products (most likely milk), possibly in combination with a starch. Chemotype A_2 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 then 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 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 polynucelar 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 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.