

# A protocol for the analysis of lipid residues in connection with prehistoric food habits

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This paper presents an analytical protocol for the study of past food cultures, both in general and within the SIV project in particular. The article describes the chemical analysis of organic remains, concentrating on lipids, in ceramic and soil samples. It discusses chemometric analysis, the effects of decomposition on analysis, experimental reconstructions to understand these effects, and the use of other evidence in the research of food cultures.

## Introduction

In the recently started SIV (*Svealand in the Vendel and Viking Periods*) project the analysis of social rank will be a major aim (Arrhenius & Herschend 1995). As food and eating habits are strong inter- and intra-cultural markers, they will be among the topics studied (Hansson & Isaksson 1995:32-33; Lidén 1995:26). Much of what has been published on Late Iron Age eating habits in Sweden tend to be more or less based on ethnological work, transferring historical conditions to the past (e.g. Genrup 1983; 1988; Lagerqvist & Åberg 1994). A reason for this is of course the lack of reliable data. What ethnology and

anthropology have shown is that eating habits form a strong thread in the web of culture (e.g. Douglas 1978), and their analyses contribute to the understanding of the social rank in the past.

## Organic residues of food

Alongside finds associated with culinary practices, organic remains of cooking provide the possibility of direct evidence for eating habits. When organic residues lack clearly discernible morphological features for classification, chemical analysis offers the only opportunity for analysis. The analysis of organic residues has developed

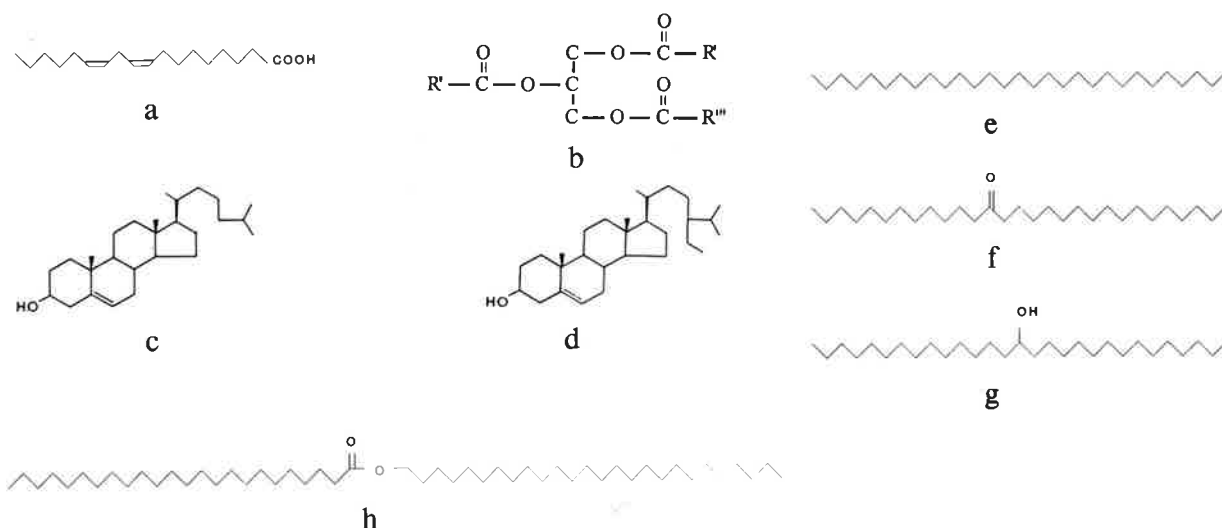


Figure 1. Structures of some lipid compounds of archaeological interest: (a) fatty acid, (b) triacylglycerol, (c) cholesterol (animal), (d) sitosterol (plant), (e) n-alkane, (f) ketone, (g) fatty alcohol, (h) wax ester.

dramatically during the last few decades, aiming at the classification of residues into natural products and the determination of their biological origin. Methods developed in fields like chromatography make the analysis of trace-amounts of organic compounds possible. The limitations today are not mainly methodological, but lie in the nature of the archaeological material. Sound remarks on these limitations have called for a critical attitude, and a development towards analysing more stable and larger molecules, sometimes called biomarkers, is discernible (Evershed et al. 1992; Heron & Evershed 1993). The simpler and larger, the better is the potential preservation of a compound. But with lesser functional groups in a compound (i.e. simpler and more stable), the less specific it is. In other words, the compounds that are most likely to be preserved are the ones that tell us the least.

A multitude of organic materials of archaeological interest are analysed today. Concerning food-related analyses, visible remains in pots are the more readily analysed. Quite recently organic compounds extracted from

ceramics have been analysed (Evans 1990; Evershed et al. 1990; Skibo 1992). Organic material is able to enter the pores of a ceramic during use or manufacture, and ceramic proves to be a relatively good matrix for preservation. Some "pioneer-work" on soil samples has been done, aiming to help in the interpretation of features (Bethell et al. 1994; Nolin et al. 1994; Isaksson 1996). Experiences in organic geochemistry are now being utilized, and questions on settlement organization and land-use are treated (Bull et al. 1995). The group of compounds most commonly targeted is lipids, that is, natural fats, oils and waxes (fig. 1). The reason for this is that the properties of these compounds offer relatively good preservation and characterization opportunities, in comparison with proteins and carbohydrates, for example.

### Analytical methods

The extraction of the organic compound from its deposition matrix is the first step. By using different solvents

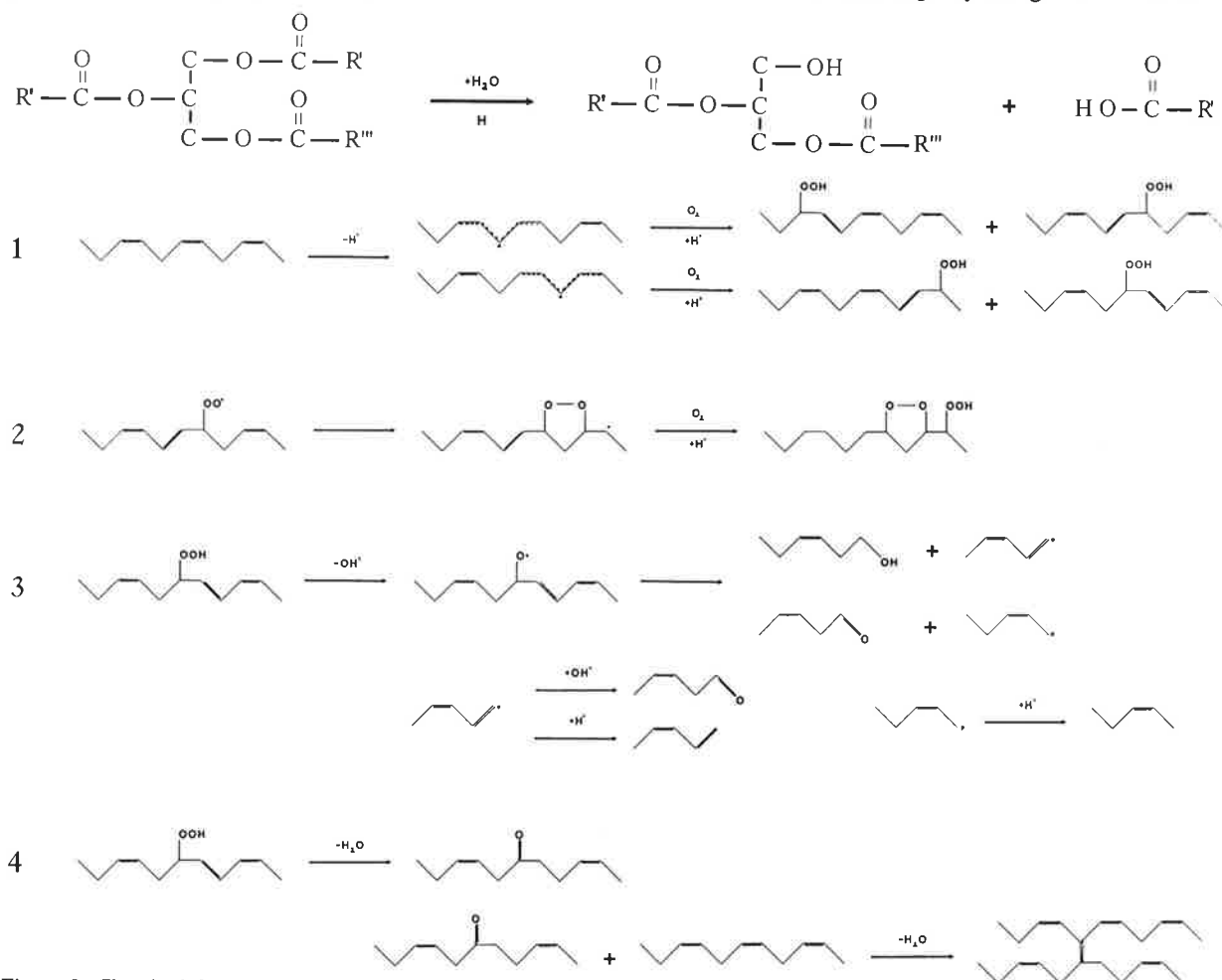


Figure 2. Chemical decomposition of acylglycerols and fatty acids.

(a) Hydrolysis. The ester bond between a fatty acid and its glycerol backbone is broken. If the hydrolysis is complete the triacylglycerol is split into glycerol and free fatty acids.

(b) Autoxidation. Some of the more important processes involved in autoxidation are: (1) free radical abstraction of a hydrogen atom finally resulting in hydroperoxides; (2) 1,3-cyclization leading to hydroperoxy epidioxides; (3) homolytic cleavage of monohydroperoxide, finally resulting in aldehydes, ketones and alcohols; (4) decomposition of hydroperoxides leading to highly conjugated species via the condensation of conjugated ketone. (Kumarathasan et al. 1992)

with varying polarity it is possible to separate individual components or classes, which can then be identified (Hillman et al. 1993:94–121). The preferred methods used for separation and identification of extracted components are gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). This preference is due to the ability of these methods to resolve individual components from complex mixtures, and, in the case of GC/MS, the opportunity of characterizing the separated compounds. Curie-point pyrolysis gas chromatography and GC/MS (CuPy-GC/MS) are now being developed, and will probably prove to be powerful analytical tools, as is isotope ratio monitoring GC/MS (Bull et al. 1995; Heron & Evershed 1993). Simpler methods, like high-performance thin-layer chromatography (HPTLC) and solid phase elution (SPE), are also being used for separation of lipid classes, but mainly for preparative purposes and screening (Nolin et al. 1994).

### Chemometrics in archaeological science.

The chemical analysis will provide a large set of data, suitable for statistical evaluation. By defining natural groupings in multiple dimensions or variables and interpreting them in terms of known behaviour, this approach

may form the basis for classification.

Statistical evaluation of analytical data has been used within archaeological science for a wide range of purposes and with varying success. Examples are chronological analysis of coins (Carter 1993), provenance and other analyses of pots and vessels (Mommsen et al. 1992; Mommsen et al. 1994; Pollard & Hatcher 1994), and classification of soil samples (Linderholm & Lundberg 1994).

When applied to chemical data and methods, this approach is called chemometrics. Easy-to-use computer programs have increased the accessibility of this approach. One possible drawback to this accessibility is that it may be tempting to take a “black box” approach to using these statistics, feeding in numbers and coming up with “The Answer”. All statistical procedures make assumptions about the data and have restrictions on what they can and cannot do. If these assumptions and restrictions are violated, the results could be meaningless (Kovach 1986).

Chemometrics has found its way into lipid analysis (Kaufmann 1990; 1992). Used for experimental design, optimization and data analysis, chemometrics is becoming a standard method at many laboratories. The identification of modern natural oils has been proven successful

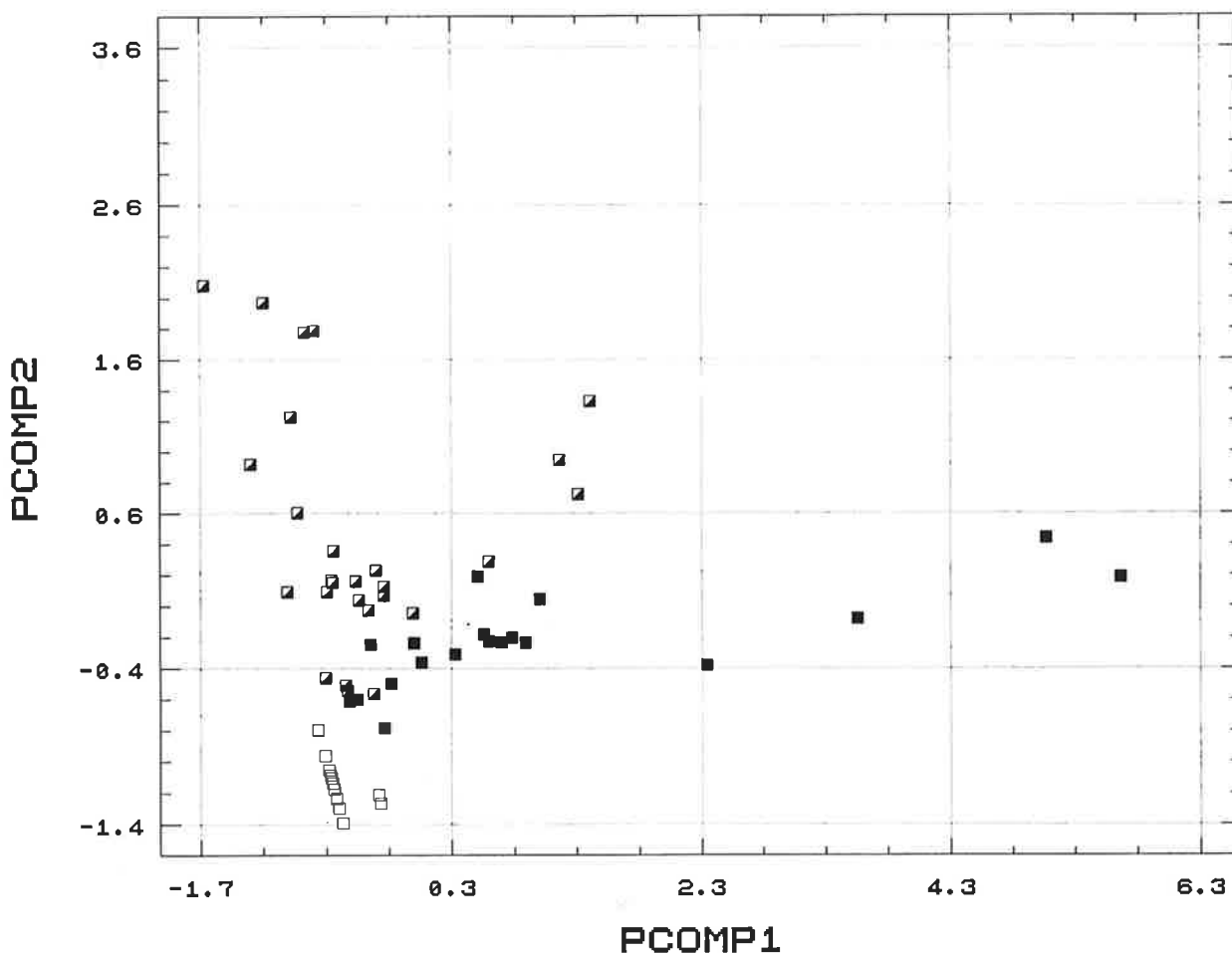


Figure 3. First two principal components of 59 food-stuff standards.

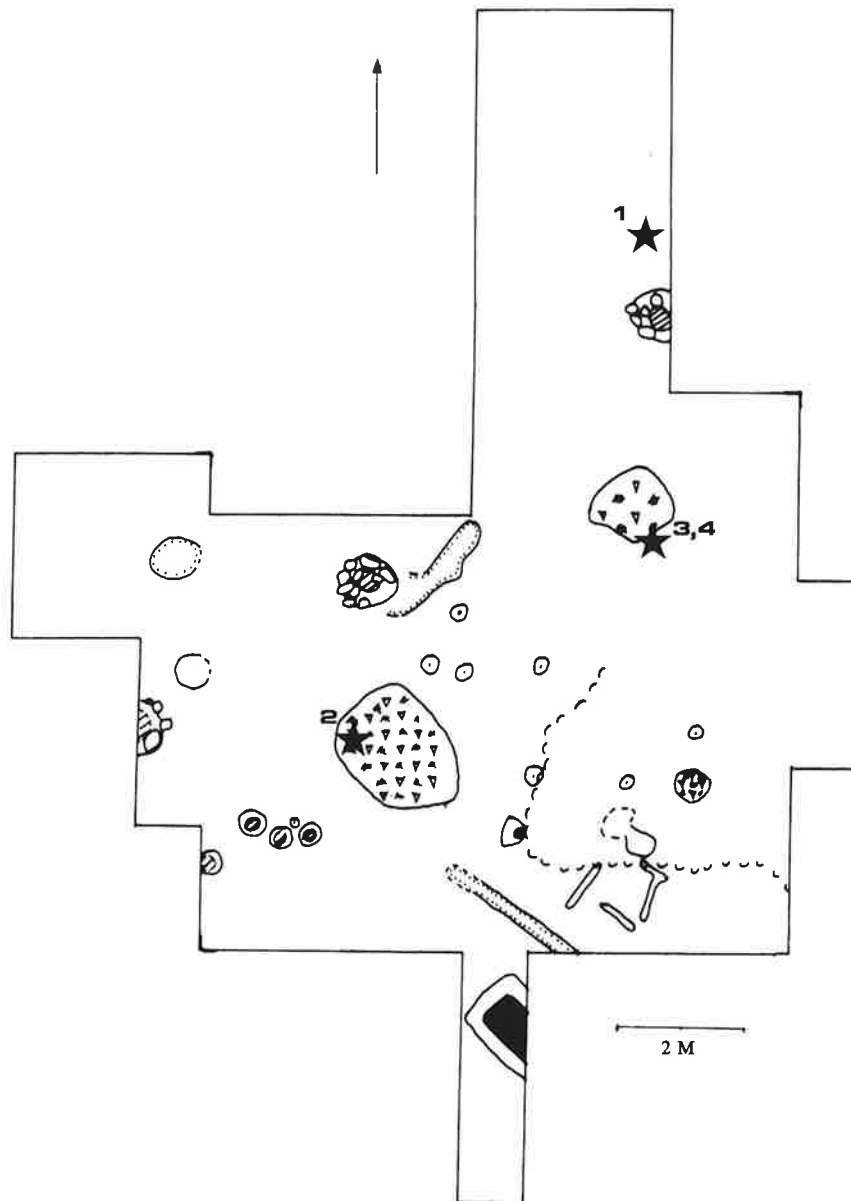


Figure 4. Location of samples 1-4 at site Vendel, Vendel par., Uppland, Sweden.

through work by Kaufmann and Herslöf (1990). They analysed the fatty-acid composition and triacylglycerol pattern of a number of natural vegetable oils was analysed. Utilizing these experimentally determined sets of variables, and principal component analysis (PCA), it was possible to discriminate between different groups of oils. They were clearly separated from one another, thereby forming a basis for an objective means of identification and classification of oils (Kaufmann & Herslöf 1990).

The identification and classification of analysed lipids from prehistoric organic residues are the major concern of this paper, and here chemometrics may be of help. However, owing to the nature of archaeological materials, chemometric methods must take into account the effects of decomposition in their analysis. The problems posed by decomposition have been acknowledged and ex-

periments are running at several laboratories, including the Archaeological Research Laboratory, in order to overcome them. At the Archaeological Research Laboratory models on decomposition in different environments are being designed. These reveal how the material to be examined will change over time given the circumstances of its preservation. The basis of this approach is to include a limited number of the factors involved in decomposition in the model, to test the model through experiments, and to modify the model, introducing new factors into it as a result of the experimental testing. In this way the model is built and tested step by step. Some preliminary results are presented below.

The first model tested considered the effects of chemical decomposition of acylglycerols. Acylglycerols are the major constituent of animal and vegetable fats and oils; thus they form the obvious basis for chemometric analysis seeking to detect and classify these substances. However, acylglycerols are subject to decomposition, so chemometric analysis of archaeological samples cannot search for them directly. Such an analysis can only be carried out if there is a trace product

from the process of decomposition which reliably signals the original presence and nature of the acylglycerols themselves.

One major process at work in the decomposition of acylglycerols is hydrolysis (Mills & White 1994:34), although the extent of hydrolysis depends on conditions under which the decomposition takes place. The effect of hydrolysis is to break the ester-bonds that join fatty acids to their glycerol backbone (fig. 2a), forming free fatty acids (hence the dominant presence of such acids in, for example, bog body adipocere, see Evershed 1992). These products are then subjected to a second major process of chemical decomposition, autoxidation (fig. 2b). Autoxidation, however, will only effect unsaturated fatty acids, those polyunsaturated autoxidizing rapidly at room temperature, those monounsaturated at elevated temperatures (Kumarathan et al. 1992); saturated fatty acids

escape autoxidation completely. Within the confines of the model, which deals only with two dominant forces in decomposition, this means that the best chance of tracing the original nature of acylglycerols is to analyse the actual presence in any archaeological sample of saturated fatty acids, affected by hydrolysis and unaffected by autoxidation.

Fully aware of the incompleteness of the model, I used it as the basis for an attempt to classify natural fats and oils by means of saturated fatty acids. Data used were collected from food composition tables (*Statens Livsmedelsverk* 1988). To remove the influence of different amounts of fatty acids in different fats and oils, and the influence of different units, ratios of the three most common saturated fatty acids were used, attaining unitless values. The fatty acids used are myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids, in the ratios C14:0/C16:0, C14:0/C18:0 and C18:0/C16:0. These variables were used in a principal component analysis (PCA), using the *Statgraf 3.0* software package. Each of these variables were allowed to represent a coordinate axis in a k-dimensional space (k=number of variables), often called measurement space (M-space). Each object (food-reference) is described by a vector containing the values of the variables and hence can be seen as a point in this space. A set of objects with the same measured variables form a swarm of points in M-space and objects similar to each other will be near one another in this space. Distance therefore constitutes a measure of similarity/dissimilarity. By PCA the swarms in M-space may be projected down onto a two dimensional plane, where the natural groupings are more readily distinguished. Hence, PCA can be seen as solving the eigenvalue-eigenvector equation for a given matrix of objects described by a set of variables, comprising a k-dimensional space. However, a quite low precision was obtained, only separating between general categories of food (fig. 3). The most clearly distinguishable are the vegetable lipids. Most terrestrial-animal lipids are well separated from the marine lipids.

The model was tested on some archaeological samples (table 1, figs. 4-5). Fatty acids from extracted lipids were analysed as their corresponding methyl esters on a GC. The methyl esters were produced by acid-catalysed esterification and transesterification (fig. 6). With this method both free fatty acids and o-acyl lipid fatty acids are targeted. Results obtained are shown in figure 7.

Table 1. List of samples. For more data on sample 1-4 see Donkow (1995) and on sample 5 Trotzig (1991).

Sample no.	Location and find data	Context/find	Dating	Lipid burial matrix
1	Up. Vendel par. Find no. 34:2	Potsherd in settlement layer	Late Iron Age	Ceramic
2	Up. Vendel par. Find no. 125	Potsherd in settlement layer	Late Iron Age	Ceramic
3	Up. Vendel par. Find no 134:1.	Sherd from burial urn	AD 850-900	Ceramic
4	Up. Vendel par. Find no 134:2	Sherd from burial urn	AD 850-900	Ceramic
5	Go. Grötlingbo par. SHM 27778:13762:1	Bronze-vessel in grave	Viking Period	Cu-corrosion

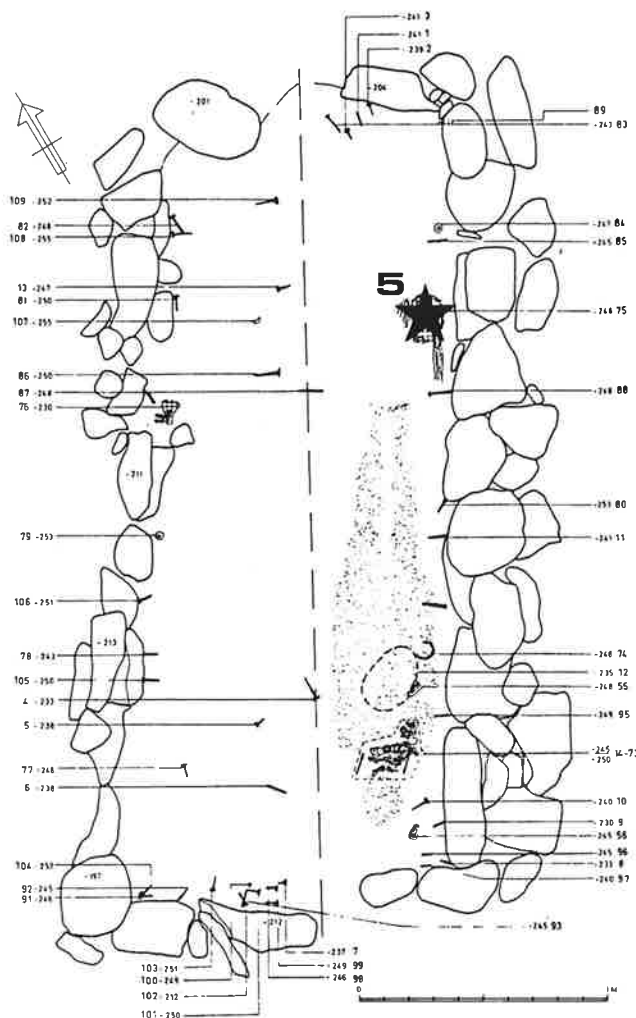


Figure 5. Viking Age female inhumation-grave. Sample 5. Grötlingbo par., Gotland, Sweden. From Trotzig (1991).

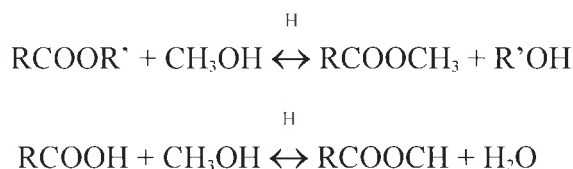


Figure 6. Acid-catalysed esterification and transesterification. The extracts were treated by 1% sulphuric acid in methanol at 60°C for 12 hours.

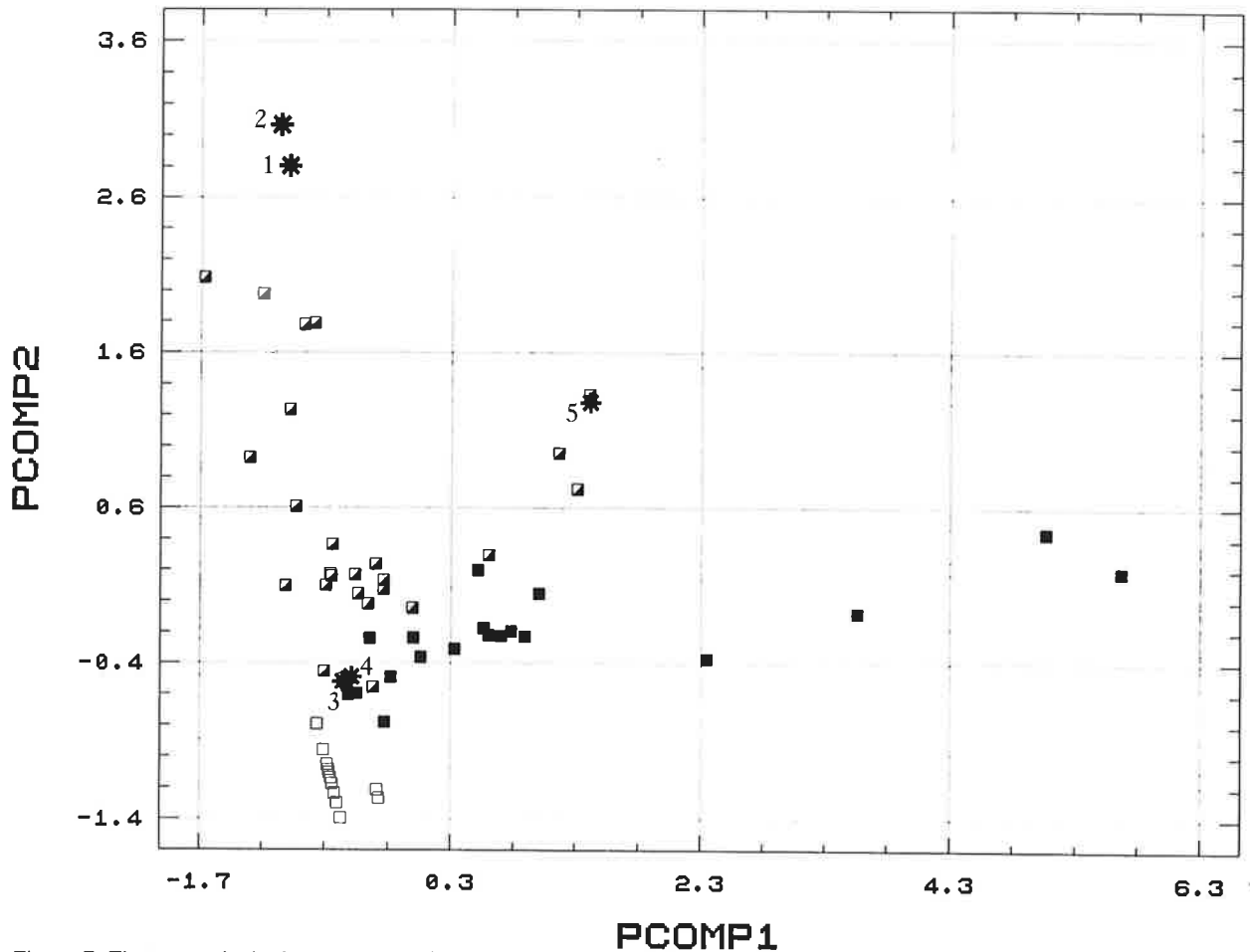


Figure 7. First two principal components with samples.

Some samples could not be classified, though, which shows that the model is incomplete. The use of fatty acid ratios in classifications of archaeological materials has been justly questioned (Heron & Evershed 1993:269), due to the complexity of decomposition under certain conditions. For example, the conversion of oleic acid (C18:1) to palmitic acid (C16:0), has been shown to take place under waterlogged conditions by the influence of soil micro-organisms (den Dooren De Jong 1961). So the next step was to design decomposition experiments to reveal extra decompositional effects under controlled conditions, like anaerobic and aerobic, humid and dry. These experiments must take into account factors specific to the archaeological investigation. These pertain firstly to the environment in which decomposition takes place and secondly to the particular substances decomposing. The interaction between these two classes of parameter produces a unique pattern of decomposition characteristics of a particular substance in a particular environment.

#### Environmental effects in decomposition

In the analysis of decompositional environment microbiological activities in the soil are a crucial factor. In-

cluded in the SIV project are field-campaigns concentrated on certain sites in middle Sweden, and to ensure that the experiments are closely connected to these sites, on-site soil has been collected to serve in them. In this way the microbiological fauna and flora of the site of interest are used in the experiment.

#### Decomposing substances

Having included unique site conditions in the model, it remained to select the food-stuffs whose decomposition should be traced. The choice of these food-stuffs was based on the archaeological questions at issue in the project, and on an analysis of circumstantial evidence including written sources, runic inscriptions from the periods of interest, and existing archaeological research (Isaksson forthcoming). In the models to be tested both pure and mixed samples of these food-stuffs are investigated. These natural materials are placed in soil from the site and exposed to different environments. The first results are currently being evaluated. This experiment provides us with a sort of worst-case scenario, revealing the maximum effects of decomposition. Organic compounds stand a better chance of survival if encapsulated in some way. For example, if organic remains are charred, pro-

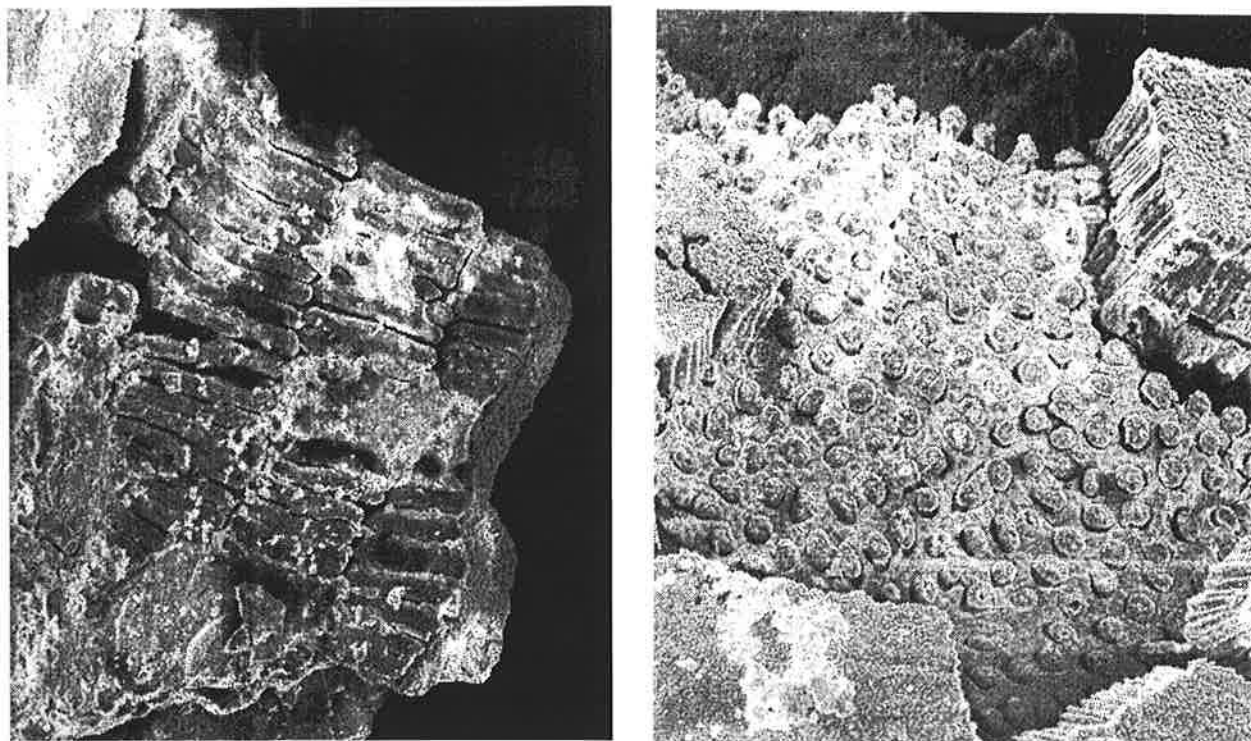


Figure 8. Scanning electron micrographs of (left) barley and (right) pea in sample no. 5, identified by Ann-Marie Hansson. Photo by the author.

viding a protective wall of carbon, or if protected by a ceramic matrix, less decomposition occurs (Evans 1990).

The aim of the experiments is to modify and extend the original simple decomposition model, by evaluating the microbiological effect on lipid classes of interest, that is fatty acids, acylglycerols, sterols, alkyl compounds, wax esters (fig. 1) and their corresponding decomposition products. This involves evaluating and looking for biomarkers, and searching out recognizable patterns which can be used in chemometric data analysis and classification. In the classification of natural vegetable oils quoted above it was found to be necessary to take account of both fatty acid composition and triacylglycerol composition to obtain valid class-models (Kaufmann & Herslöf 1990:116).

At the same time as conducting these experiments we are investigating deposition of lipids in ceramics and features. Reconstructed pottery and features are used, investigating where and how lipids are deposited (Isaksson 1996). From the data obtained it will be possible to develop the protocol for on-site sampling. The experiments will also provide insight into the effects of different uses, such as cooking, storage and fermenting of food and drink. We will also investigate the effects of multi-use vs. single-use of ceramics on the composition of deposited lipids. The reconstruction of pottery will also help in evaluating the survival of organic matter in the clay during manufacturing.

## Conclusion

The major aim is to develop a protocol for sampling and analysis of ceramics and soil samples within the SIV project. All pottery sherds of workable size are collected for analysis. Care is taken to avoid handling them with bare hands and samples are taken from any soil directly adhering to the sherds surface in order to investigate any exchange with the environment. The importance of this factor has been pointed-out by Heron et al. (1991:641–659). Soil samples are also collected from each square metre of each layer of the excavated trenches. Finds and features are registered and digitalized successively using the *Microstation Field* software, which enables quick references to be made to up-to-date chorological relations. In this way it is easy to distinguish areas showing increased traces of culinary activities, i.e. concentrations of potsherds and features like hearths, ovens and cooking-pits. In these areas the soil-sampling is intensified. All samples are deep-frozen as soon as possible.

Archaeobotanical investigations of soil are part of the project as well, although they are not a topic of this paper. However, the chemical investigation will be supported by macro- and microscopical examinations of organic remains and potsherds, by searching for preserved structures, botanical and faunal remains (fig. 8), and by documenting manufacturing technique and use-ware.

Led by the questions at issue, screening methods for quick evaluation of samples are being developed. This is especially important for the analysis of soil samples as

these will be in great number. HPTLC might be the method of choice, for its visuality and multi-sample possibilities, or SPE for high efficiency. For quick screening of certain samples FT-IR might be useful. By developing such a screening method it will be possible to pick out which samples are of interest for further analysis.

For the analysis of lipid classes GC/MS is the method of choice. Providing structural data of separated compounds, this has been developed as the standard method for archaeological samples. It will be used as the last analytical step, on samples deemed interesting from the screening.

The classification of analysed lipid residues will rest on the decomposition experiments. By tracing biomarkers and classifying stable lipid compounds through chemometrics, the origins of lipid residues will be interpreted. A major issue to investigate from the experiments is the possible class-precision accessible by the chemometric analysis.

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