Appendix

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GC-MS analysis of organic residues in the potsherd samples from Vantaa Maarinkunnas

ABSTRACT

In order to examine the origin of organic residues in pot sherd samples, analyses for fat, protein, carbohydrates, and selected minor components were carried out. A total of eleven pot sherd samples were analysed. No protein or carbohydrate residues were detected in the samples. The samples, however, did contain residues of fatty acids, their decomposition products, cholesterol, and benzoic acid identified and quantified by GC-MS analysis. Cholesterol identified in the samples indicates the presence of animal fat. Further, the fatty acid composition, in particular the ratios of 16:0/18:0 and 16:1/18:1 fatty acids, as well as the presence of phytanic acid in the samples, suggest that the fat present is of marine origin. Benzoic acid, a new finding in archaeological contexts, was detected in all eleven samples, suggesting the presence of cranberry or cowberry in the pottery. However, the origin of benzoic acid in archaeological samples requires further research.

Introduction

Even though organic materials in prehistoric contexts have gone through major changes due to microbial growth or their low chemical stability, residues of the original components are often still detectable. GC-MS analysis is a sensitive and highly specific method that provides a powerful tool for analysing organic residues present at minor levels in prehistoric samples.

This study was designed to detect and identify organic compounds in the charred residues in samples from pot sherds, in order to gain information about the origin of the organic material stored or cooked in the vessels.

Sample description

The sherds to be sampled for charred food remains (food crust) were not selected before the pottery went through the routine processes of cleaning and cataloguing. The cleaning involves gentle brushing to remove excess sand from the surfaces of

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the artifacts. Touching the sherds and, especially, the crust unnecessarily was avoided as far as possible. The same rule has been followed while the sherds have been residing in their cardboard boxes – the normal manner of storing the finds in the collections of the National Museum of Finland (NM).

For this study, eleven sherds were selected. The selection was based on the presence of a sufficient amount of crust for the analyses, and also on the assignability of the sherds to one or other of the recognized Comb Ceramic styles. The samples were scraped with a scalpel from the inner surface of the sherds. Sample size varied, depending on the amount of organic residue on the sherd surface. From some sherds, several square centimetres of charred material was obtained. The first three sherds were sampled by Markku Reunanen in the spring of 1999 at Åbo Akademi in Turku; the remaining samples were procured by Petro Pesonen at the National Board of Antiquities in the autumn of the same year.

Analytical procedure

The samples from the pot sherds were ground to crumbs. A 50 mg portion of ground sample was placed in a test tube and 0.2 mg of heneicosanoic acid was added as an internal standard. The sample was first hydrolysed by mixing it with 5 ml of hydrolysis solution. The hydrolysis solution contained 0.5-M KOH in ethanol:water (90:10% v/v), and the sample was allowed to hydrolyse at 70°C for two hours. After the completion of the hydrolysis, the solution was centrifuged, and the supernatant was diluted with 10 ml of distilled water. The water solution was acidified with 0.5-M sulphuric acid to pH 2, and then extracted with three 2 ml doses of diethyl ether. The ether extracts were combined and evaporated to dryness. Finally, the dry extract was dissolved in 200 µl of pyridine, and silylated by adding 150 µl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 µl of trimethylchlorosilane (TMCS). The silylation was completed at 70°C for a period of 15 minutes.

The analysis continued with the separation and identification of the silylated components by gas chromatography-mass spectrometry (GC-MS). The GC-MS was executed with an HP 6890-5973 GC-MS instrument equipped with a HP-1MS methyl polysiloxane column (15 m x 0.25 mm i.d., 0.25 µm film thickness). The GC oven was temperature programmed from 60°C to 290°C, with a 8°C/min. heating rate. The split-injector and detector transfer line temperatures were held at 260°C and 280°C, respectively. Helium was used as the carrier gas at a constant flow rate of 1 ml/minute. Mass spectra were recorded at 70 eV electron impact energy. Spectral identifications were obtained from mass spectral libraries, quantitative data from the GC-peak areas of the components. The quantity calculations were based on uncorrected peak areas relative to the internal standard.

Figure 1 shows an example of a GC-MS analysis result from a pot sherd sample from Vantaa Maarinkunnas.

Two of the samples (NM 30464:12554 and NM 30464:14065) were analysed for carbohydrates and peptides. 50 mg of ground sample containing 0.2 mg of adipic acid as the internal standard was hydrolysed at 70°C with 5 ml of 1-M hydrochloric

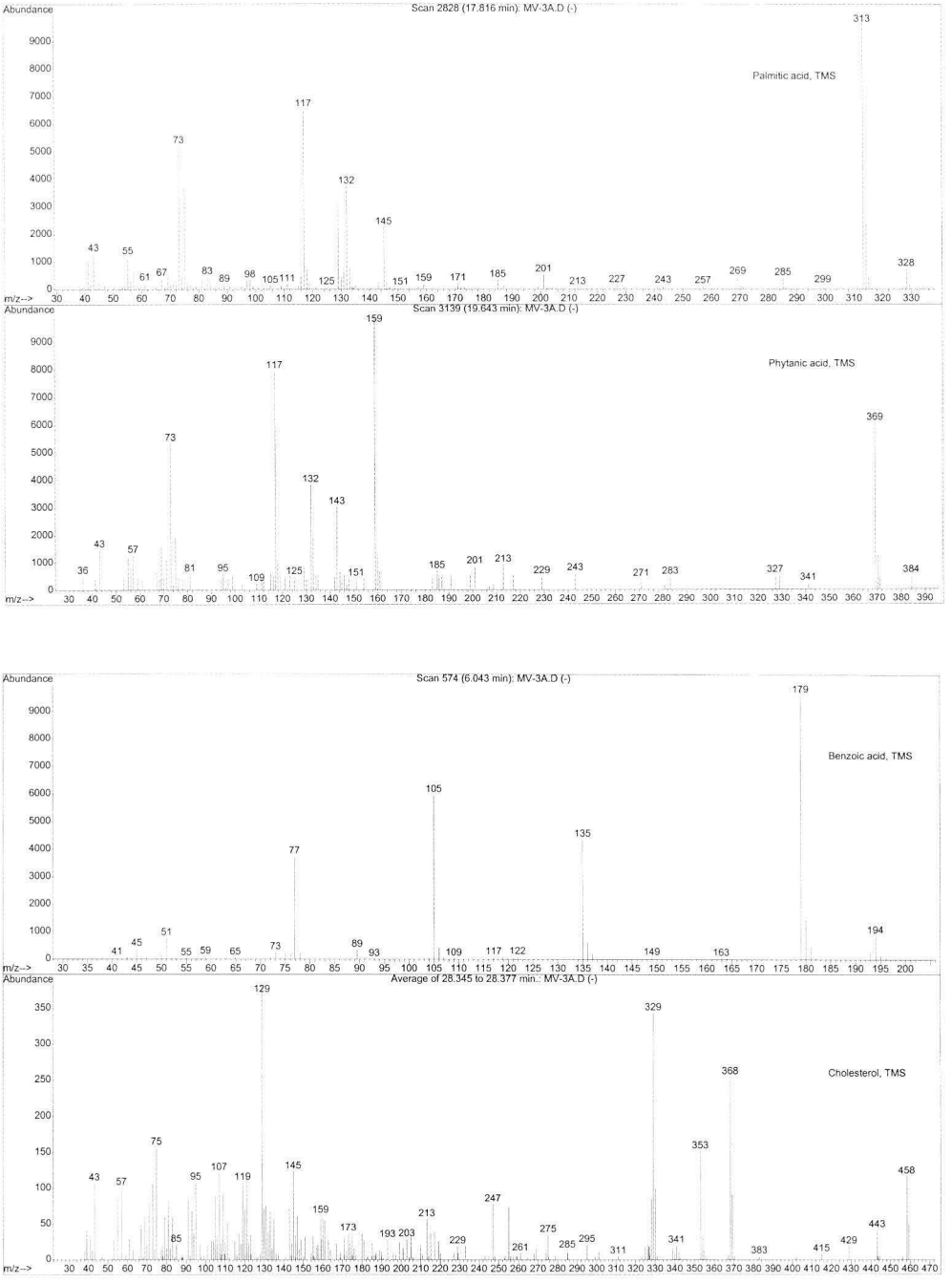

Fig. 1a and b. GC-MS chromatogram of a sample from a pot sherd (NM 30464:12554) from Vantaa Maarinkunnas. For the analytical procedure, see text (Analytical procedure).

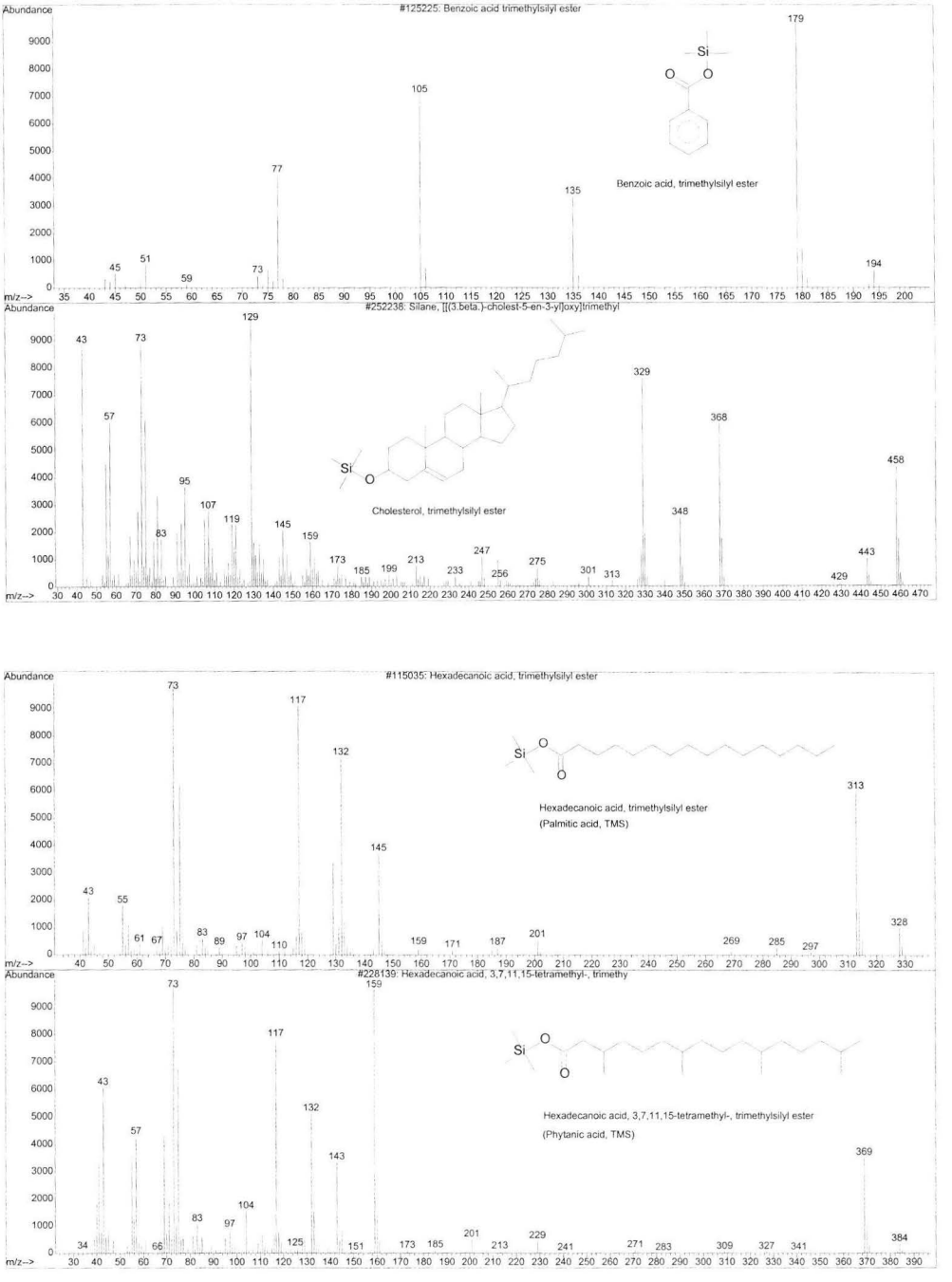

Fig. 1c and d. GC-MS chromatogram of a sample from a pot sherd (NM 30464:12554) from Vantaa Maarinkunnas. For the analytical procedure, see text (Analytical procedure).

acid solution. After four hours of hydrolysis, the sample tube was centrifuged. The supernatant was divided in two equal portions, which were then evaporated to dryness. The dried residues were dissolved in 200 µl of pyridine. For an analysis of monosaccharides, one of these portions was silylated by adding 200 µl of hexamethyldisilazane (HMDS) and 100 µl of TMCS. The mixture was allowed to stand for 30 min. before analysis by GC-MS under the same conditions as described above. The second portion was silylated by adding 300 µl of N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylchlorosilane (TBDMSCl). This sample was analysed for amino acids by the GC-MS. However, no monosaccharides or amino acid residues were detected in the samples.

Results and discussion

The GC-MS analyses showed that most of the samples contained residues of fatty acids and their decomposition products, as well as cholesterol and benzoic acid (Tables 1, 2, and 3).

Total fatty acid content:

The total fatty acid content of the samples was low, varying from 0.2 to 3.65 mg/g (Table 1). Four samples (:12833, :1249, :12554, and :14065) out of eleven contained relatively high levels (more than 1mg/g) of fatty acids (Fig. 2). The fatty acid compositions of these four samples are shown in Figure 3.

The bulk of the fatty acids was made up of the two saturated fatty acids, palmitic and stearic acid, which in these four samples represented 67–80% of all the fatty acids identified. Saturated fatty acids are the most stable of the fatty acids and, as expected, their levels in the samples were the highest. Palmitic (16:0) and stearic (18:0) acids are typical saturated fatty acids found in a wide range of foods. Palmitic acid is the most common fatty acid in fats, and both marine and terrestrial animals contain a considerable amount of it. Stearic acid is less common in edible samples than palmitic acid, but especially prominent in tallow and ruminant animal fats (Padley & al. 1994).

The ratio of stearic to palmitic acids (18:0/16:0) has been used to determine the origin of fat in archaeological samples (Isaksson 1992 and 1996; Hansson & Isaksson 1994). Reference ratios above 0.8 are claimed to indicate fat of terrestrial mammal origin, while lower values indicate marine origin. The fatty acid composition data of current food items (Table 4) also suggest that a higher value (0.6–0.7) of 18:0/16:0 is characteristic of food of terrestrial origin, whereas a low value (0.1–0.2) indicates a marine origin for the fat (fish or sea mammals). In this study the ratio varied from 0.2 to 0.4 in all eleven samples (Table 1). This suggests that the fats in the samples are of marine origin.

Table 1. Fatty acid composition of samples from pot sherds.
Chemical analysis of potsherd samples Markku Reunanen, Åbo Akademi Anu Hopia, HY

Sample	Fatty acid (mg/g) 7:0	8:0	9:0	10:0	11:0	12:0	13:0	14:0	15:0	16:0	17:0	18:1	18:0	18:3	16:0 (anteiso)	16:0 (iso)	20:0	16:1	18:1	15:0 (anteiso)	15:0 (iso)	Phytanoic acid	TOTAL (mg/g)	% Palmitic	% Stearic	18:0/ 16:0	16:1/ 18:1
KM30464:13178	0	0,02	0,02	0,01		0,02		0,03	0,01	0,4	0,01	0,03	0,1									+	0,65	61,5	15,4	0,3	0,0
KM30464:5694	0		0,03	0,01		0,01		0,02	0,03	0,38		0,02	0,06									–	0,56	67,9	10,7	0,2	0,0
KM30464:16650	0		0,02					0,01		0,1		0,02	0,02	0,03								–	0,2	50,0	10,0	0,2	0,0
KM:30464:13300	0	0,03	0,02	0,01		0,03		0,04	0,02	0,38		0,03	0,1									+	0,66	57,6	15,2	0,3	0,0
KM:30464:12889	0					0,07		0,05	0,03	0,56		0,07	0,09									–	0,87	64,4	10,3	0,2	0,0
KM30464:12833	0	0,01	0,02	0,01		0,02	0,01	0,05	0,05	0,87	0,02	0,06	0,28		0,01	0,01	0,01					+	1,43	60,8	19,6	0,3	0,0
KM30464:12606	0	0,04	0,04			0,02		0,03	0,01	0,56	0,01		0,19		0,01	0,01						+	0,92	60,9	20,7	0,3	
KM30464:1249	0,13	0,13	0,11	0,13		0,05		0,11	0,08	1,8	0,04	0,09	0,58		0,05	0,06	0,07	0,05				+	3,48	51,7	16,7	0,3	0,6
KM30464:12554	0,04	0,04	0,02	0,01		0,01	0,01	0,11	0,02	0,85	0,01	0,12	0,28		0	0,01	0,03	0,04	0,03	0,01	0	++	1,64	51,8	17,1	0,3	0,3
KM30464:14065	0,03	0,04	0,04	0,02	0,02	0,03	0,08	0,52		1,88	0,04	0,14	0,58		0,01	0,03	0,02	0,04	0,06	0,05	0,02	+	3,65	51,5	15,9	0,3	0,3
KM30464:12601	0,01	0,01	0,01					0,01	0,01	0,19	0,02		0,08									+	0,34	55,9	23,5	0,4	

Table 2. Fatty acid decomposition products in samples of pot sherds.
Chemical analysis of potsherd samples Markku Reunanen, Åbo Akademi Anu Hopia, HY

Sample	Glycerol (mg/g)	Hydrocarbons (mg/g) 4:0	6:0	8:0	20:0	Hydroxy acids (mg/g) Dihydroxy acetic acid	2,3-dihydroxy butyric acid	9,10-dihydroxy palmitic acid	9,10-hydroxy stearic acid	Dicarboxylic acids (mg/g) 2-hydroksi-3-methyl pentanoic acid	Propanoic	Butanoic	Pentanoic	Hexanoic	Heptanoic	Octanoic	Nonanoic	Decanoic	TOTAL (mg/g)
KM30464:13178	0,02		0,06	0,06	0,03		0,01		0,02					0,01					0,21
KM30464:5694	0,03		0,06	0,07	0,05														0,21
KM30464:16650			0,03	0,04	0,02			0	0					0					0,09
KM:30464:13300	0,02		0,03	0,04	0,03		0,01							0,01					0,14
KM:30464:12889	0,29			0,08	0,06	0,62	0,15												1,2
KM30464:12833		0,01	0,04	0,04	0,02			0,02	0,05										0,18
KM30464:12606		0,03	0,07	0,05	0,02			0,02	0,03										0,22
KM30464:1249	0,13			0,06		0,12	0,06	0,07	0,15							0,03	0,08	0,06	0,76
KM30464:12554	0,02						0,01	0,38	0,49	0,01	0,01	0,02	0,02	0,11	0,04	0,07	0,09	0,02	1,29
KM30464:14065	0,07						0,01	0,38	0,79	0,03	0,02	0,08	0,01	0,03	0,02	0,05	0,06	0,03	1,58
KM30464:12601	0,03						0,01			0,01	0,01	0,02							0,08

Table 3. Minor components in samples from pot sherds.

Chemical analysis of potsherd samples Markku Reunanen, Åbo Akademi Anu Hopia, HY

Sample	Benzoic acid	Pyrrole carboxylic acid	Phtalic acid	Phyta-noic acid	Penta-deca-nitrile	Hepta-deca-nitrile	Choles-terol	Squa-lene	Benzo-phenone
KM30464:13178	0,08	0,01	0,03	+					
KM30464:5694	0,11	0,02		–					
KM30464:16650	0,06	0,01		–					
KM:30464:13300	0,22	0,05		+					
KM:30464:12889	0,69								
KM30464:12833	0,06	0,02	0,01	+	0,04	0,01	0,04		
KM30464:12606	0,26	0,03		+					
KM30464:1249	0,32	0,11	0,09	+			0,03	0,05	
KM30464:12554	0,18			++			0,06		0,03
KM30464:14065	0,1			+			0,01		0,02
KM30464:12601	0,1			+					

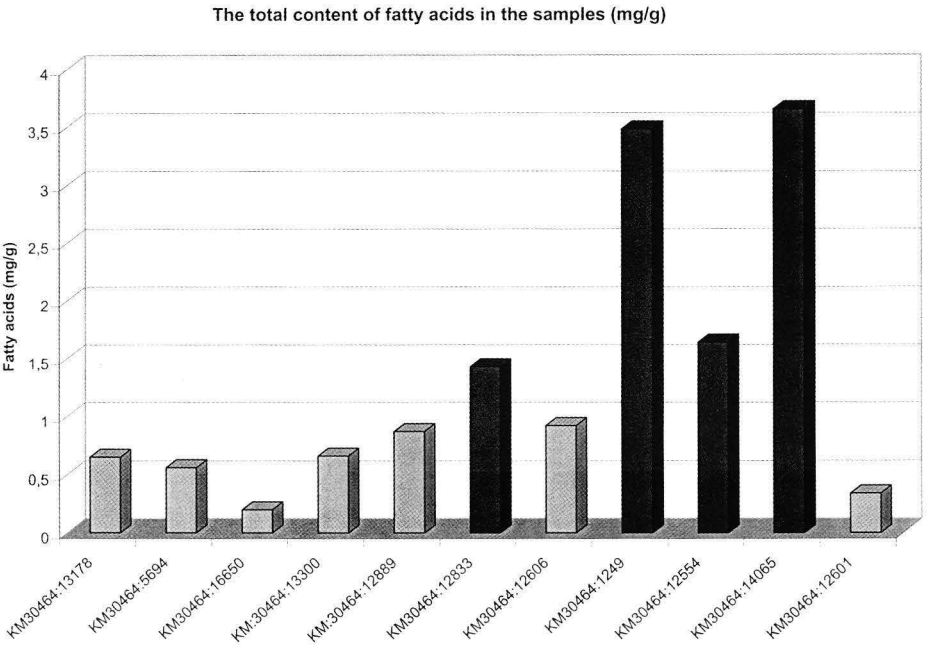

Fig. 2. The total content of fatty acids in the samples.

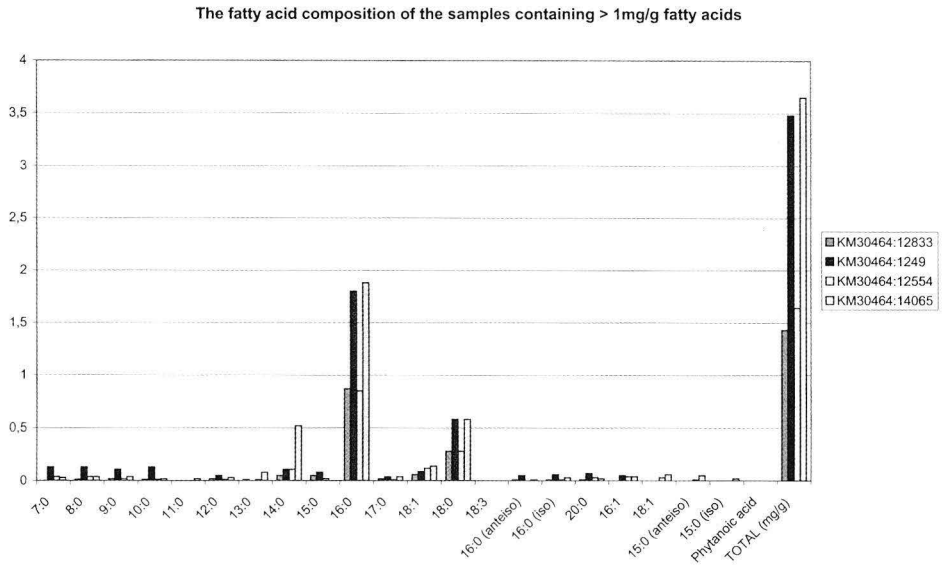

Fig. 3. The fatty acid composition of the samples containing >1 mg/g fatty acids.

Branched-chain fatty acids:

Fatty acids with a branched chain (anteiso and iso fatty acids) were also identified in the samples at levels of up to 0.11 mg/g. Branched-chain fatty acids are present at significant levels in bacterial lipids, butter fat, and tallows and waxes (Padley & al. 1994). Since their presence may indicate microbial growth that is very likely to have occurred in the sample material, using these fatty acids to interpret the origin of sample fat is of limited value. The hydroxy fatty acids that in this study have been classified as decomposition products may also have been formed through microbial growth in the sampled material.

Phytyl-based fatty acids differ in their origin and synthetic pathway from other branched-chain fatty acids. Their structure is based on diterpene phytol derived from chlorophyll. Phytanic acid, which was detected in the samples, is among the most common homologues of these fatty acids (Fig. 4). This fatty acid occurs widely in marine and terrestrial herbivore animal fats (Masters-Thomas & al. 1980). High levels – up to 700 mg/100 g – have been reported especially in some fish oils. Phytanic acid has also been identified in ancient sediments (Gunstone & al. 1994).

Unsaturated fatty acids:

The level of unsaturated fatty acids in the samples was low (Table 1). Unsaturated fatty acids oxidize and decompose easily in the presence of heat, oxygen, and

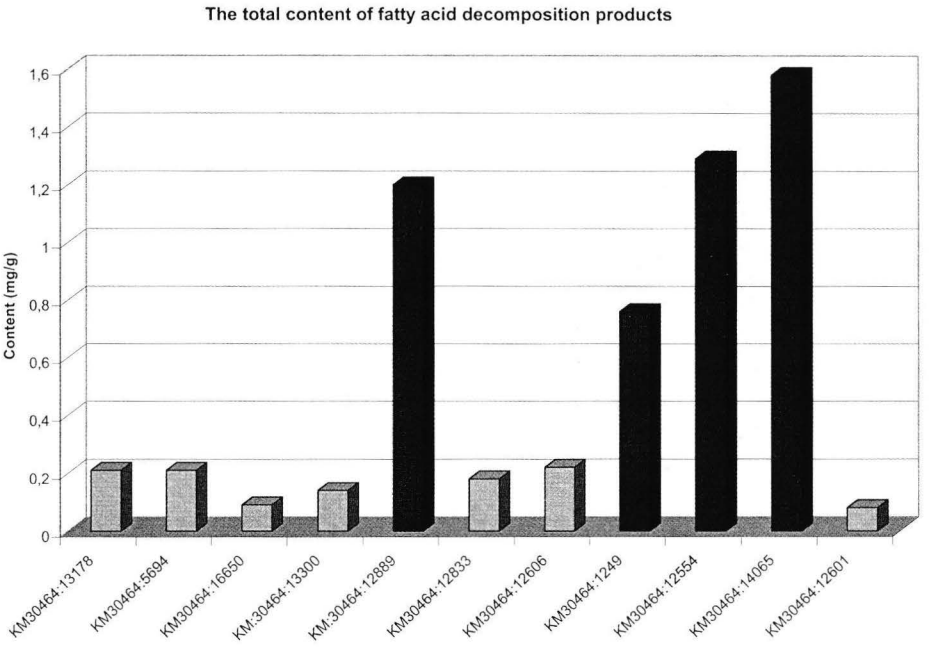

Fig. 4. The total content of fatty acid decomposition products.

metals (Chan 1988). Thus, the low level of these fatty acids in archaeological samples was expected. Oleic acid (18:1) was identified in nine of the eleven analysed samples, and palmitoleic acid (16:1) in three. In addition, one sample contained traces of linoleic acid.

The ratio of palmitoleic to oleic acids (16:1/18:1) has been used to evaluate the origin of fat in archaeological samples (Isaksson 1992 and 1996). According to Isaksson, a low 16:1/18:1 value indicates a terrestrial origin for the fat. Food composition tables of current foods indicate that the ratio is very low (0.1) for terrestrial animals, and somewhat higher (0.2–0.9; average 0.6) for foods of marine origin (Table 4). In the samples of the present study, the 16:1/18:1 ratio ranged from 0.3 to 0.6, which suggests a marine origin for the fat and thus supports the interpretation of the 18:0/16:0 ratio.

Decomposition products of fatty acids:

Unsaturated fatty acids decompose under light, oxygen, heat, and trace metals into a variety of products, including aldehydes, ketones, dicarboxylic acids, hydroxy acids, and hydrocarbons (Chan 1988). Although saturated fatty acids are more stable, it is likely that they have also gone through marked deterioration in the archaeological samples. The fatty acid decomposition products identified in this

Table 4. 18:0/16:0 and 16:1/18:1 ratios of foods.

18:0/16:0 and 16:1/18:1 ratios of selected foods
Data selected from Gunstone 1994 "The Lipid Handbook"

	Fatty acid g/100 g edible part					
Food sample	18:0	16:0	16:1	18:1	**18:0/16:0**	**16:1/18:1**
Butter fat	12,1	27,8	1,84	30,3	**0,4**	**0,1**
Lard (pork)(average)	14,5	26	3,35	48,5	**0,6**	**0,1**
Beef tallow (average)	23	28,5	4,75	38	**0,8**	**0,1**
Mutton tallow	30,5	25,8	1,5	30	**1,2**	**0,1**
Herring (average)	1,6	15,2	7,1	18,45	**0,1**	**0,4**
Trout	4	17,9	11,2	21,2	**0,2**	**0,5**
Salmon	3,6	10,7	5	24,5	**0,3**	**0,2**
Perch	4,7	20,3	7,9	9,1	**0,2**	**0,9**
Pike	3,3	18,9	9,4	18,8	**0,2**	**0,5**
Whale (average)	1,26	12,9	15,9	17,5	**0,1**	**0,9**
Harbour seal	1	11	15	29	**0,1**	**0,5**
Harp seal	1	7	9	27	**0,1**	**0,3**
Data from US Department of Agriculture: Nutrient Database for Standard Reference						
Beef tallow	18,9	24,9	4,2	36	**0,76**	**0,1**
Beef, chunk	2,5	4,87	0,99	7,8	**0,51**	**0,1**
Veal, shoulder	0,32	0,51	0,1	0,86	**0,63**	**0,1**
Ham	2,16	4,06	0,53	7,7	**0,53**	**0,1**
Lard	13,5	23,8	2,7	41,2	**0,57**	**0,1**
Horse	0,16	1,08	0,31	1,3	**0,15**	**0,2**
Elk	0,14	0,35	0,13	0,23	**0,40**	**0,6**
Rabbit	0,11	0,52	0,08	0,54	**0,21**	**0,1**
Chicken	6,2	20,6	6	35,9	**0,30**	**0,2**
Lamb	0,005	0,047	0,002	0,115	**0,11**	**0,0**
Deer	0,51	0,41	0,03	0,63	**1,24**	**0,0**
Pearch	0,04	0,18			**0,22**	
Eel	0,191	1,585	1,255	2,772	**0,12**	**0,5**
Pike	0,018	0,078	0,056	0,079	**0,23**	**0,7**
Flounder (kampela)	0,071	0,158	0,08	0,12	**0,45**	**0,7**
Rainbow trout	0,139	0,421	0,203	0,614	**0,33**	**0,3**
Pikepearch (kuha)	0,06	0,16			**0,38**	
Bream (lahna)	0,12	0,43			**0,28**	
Salmon, oil	4,25	9,84	4,82	16,98	**0,43**	**0,3**
Burbot (made)	0,037	0,12	0,026	0,098	**0,31**	**0,3**
Vendace (muikku)	0,1	0,44			**0,23**	
Whitefish	0,198	0,599	0,52	1,347	**0,33**	**0,4**
Herring oil	0,818	11,704	9,642	11,955	**0,07**	**0,8**
Herrin/Whole	0,109	1,353	0,622	1,516	**0,08**	**0,4**
Trout	0,148	0,815	0,701	1,44	**0,18**	**0,5**
Cod	0,03	0,091	0,016	0,061	**0,33**	**0,3**
Mackerel	0,423	2,125	0,727	2,283	**0,20**	**0,3**
Butter	9,83	21,33	1,82	20,4	**0,46**	**0,1**
Sunflower oil	3,5	5,4	0,2	45,3	**0,65**	**0,0**
Peanut oil	2,2	9,5	0,1	44,8	**0,23**	**0,0**
Soybean oil	3,8	10,3	0,2	22,8	**0,37**	**0,0**
Wheat	0,014	0,283	0,066	0,236	**0,05**	**0,3**
Oat	0,065	1,034	0,013	2,165	**0,06**	**0,0**
Rye	0,009	0,271	0,01	0,28	**0,03**	**0,0**
Barley	0,017	0,411	0,006	0,241	**0,04**	**0,0**

study are hydrocarbons, hydroxy fatty acids, and dicarboxylic acid. The glycerol detected in the samples most likely originates in triacylglycerols and other lipids with a glycerol backbone. The level of decomposition products in the samples varies from 0.2 to 1.6 mg/g (Table 2). In general, samples with a high fatty acid content also have a high level of decomposition products (Figure 4), and their presence confirms the results of the fatty acid analyses.

Cholesterol and other sterols:

In addition to fatty acids, fats and oils also contain a number of minor components characteristic to their origin. It was possible to analyse and identify sterol compounds through the GC-MS analysis used in this study. The sterol composition of archaeological samples has been used to identify the origin of organic material (Isaksson 1998). The only sterol present in the samples of this study was cholesterol (Table 3), which is only present in animal tissue. Microbes, as well as vegetable fats and oils, have their own characteristic sterols other than cholesterol. Thus, the presence of cholesterol in the samples :12833, :1249, :14065, and :12554 can be used as evidence of animal fat stored or cooked in the pots.

Benzoic acid:

Benzoic acid was present in all of the samples, and its level varied from 0.1 to 0.7 mg/g (Table 3, Fig. 5). This finding is new – the presence of benzoic acid has not been previously reported in the literature. Although it is possible that benzoic acid is a decomposition product of other phenolic compounds originally present in the samples, it is an important discovery, since the only significant sources of benzoic acid in the modern diet are cowberry (lingonberry; *Vaccinium vitis-idaea*) and cranberry (*Vaccinium oxycoccos/ microcarpum*). Its presence in the samples, thus, suggests that cowberry or cranberry may have been stored in the studied pottery vessels. However, further confirmation of the origin of benzoic acid in the samples is required before more conclusions can be drawn.

High levels of benzoic acid are known to inhibit microbial growth in food, and it is used as a food preservative (food additive E210) in modern food industry (Lindsay 1996).

Conclusions

The presence of fatty acid residues and cholesterol in the samples from pot sherds indicates that the vessels have contained animal fat. Further, the ratios of 16:0/18:0 and 16:1/18:1 fatty acids suggest that the fat has been of marine origin, deriving from fish or sea mammals, such as seals or whales. The presence of phytanic acid supports this inference, although it is also found in terrestrial animals. The benzoic acid detected in the samples suggests that, in addition to animal products, the

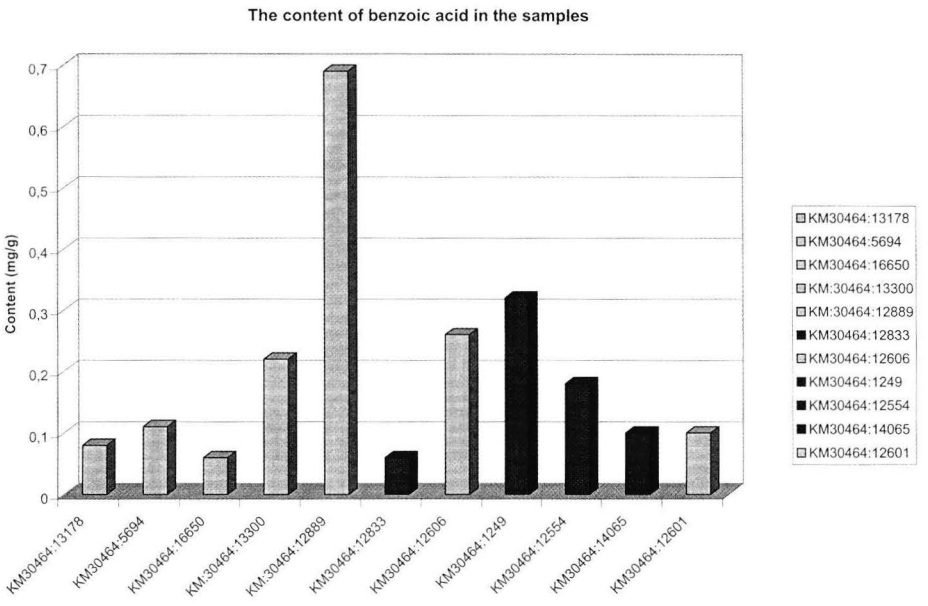

Fig. 5. The content of benzoic acid in the samples.

pottery may have contained cranberry or cowberry. This is the first study reporting benzoic acid in archaeological samples. The finding, therefore, needs further research to confirm it. High levels of benzoic acid would inhibit microbial growth, such as fermentation or the development of mould in the food.

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