CHAPTER SEVEN
Fatty Acid Analysis of Archaeological Residues: Procedures and Possibilities

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Archaeological food residues extracted from areas of fat accumulation in artifacts can be characterized on the basis of relative fatty acid composition. Compositions of ancient residues are compared to experimental residues subjected to periods of oven storage, which simulates the effects of oxidative decomposition over time. Levels of medium and very long chain saturated fatty acids, C18:0 and C18:1 isomers indicate the fat content of the material of origin and probable presence of animal or plant material. This technique performs well in blind tests of decomposed residues of previously unknown foods and identification criteria remain valid over time.

Gas chromatography is an effective and efficient method of examining fatty acids in the form of methyl ester derivatives. Instruments are widely available and relatively inexpensive to obtain and operate. Gas chromatography has long been used to determine the fatty acid composition of archaeological residues. While absolute identifications are not possible, a wide range of archaeological residues can be rapidly categorized. Fatty acid compositions of cooking residues change over time; but these variations can be modeled. In particular, decreases in the relative amounts of C18:1 isomers in decomposing residues strongly correlate with logarithmic curves; the functions can then be used to extrapolate further change. Better than expected preservation of monounsaturated and, occasionally, polyunsaturated fatty acids is observed in the cooking residues of certain plant and plant and meat combinations, likely due to the presence of antioxidants.

With careful selection of archaeological samples for residue analysis, good preservation of residues is possible. The effects of microbes found in parkland, prairie and forest soils appear to be mediated by the reduced availability of oxygen in a burial environment. With a reference collection of decomposed foodstuffs from the region, one can establish possible origins of the residues and eliminate others. If desired, other methods of analysis can be employed to provide more precise identifications by confirming the fatty acid identifications and targeting molecules that can serve as biomarkers.

Previous Research

The major constituents of fats and oils are fatty acids that usually occur in nature as triacylglycerides, consisting of three fatty acids attached to a glycerol molecule by ester-linkages. Their insolubility in water and relative abundance compared to other classes of lipids, such as sterols and waxes, make fatty acids suitable for residue analysis. Since employed by Condamin et al. (1976), gas chromatography has been used extensively to analyze the fatty acid component of archaeological residues (cf. Chapter 5).

The composition of uncooked plants and animals provides important baseline information, but it is not possible to compare modern uncooked plants and animals with highly degraded archaeological residues of prepared foodstuffs. Unsaturated fatty acids, which are found widely in fish and plants, decompose more readily than saturated fatty acids, sterols or waxes. In the course of decomposition, simple addition reactions might occur changing double into single bonds (Solomons 1980), or peroxidation might lead to the formation of a variety of volatile and non-volatile products which continue to degrade (Frankel 1991). Peroxidation occurs most readily in polyunsaturated fatty acids.

Attempts have been made to identify archaeological residues using criteria that discriminate uncooked foods (Marchbanks 1989; Skibo 1992; Loy 1994). Marchbanks' (1989) percent of saturated fatty acids (%S) criteria has been applied to residues from a variety of materials including pottery, stone tools and burned rocks (Marchbanks 1989; Marchbanks and Quigg 1990; Collins et al. 1990). Skibo (1992, 89) could not apply the %S technique and used two ratios of fatty acids: C18:0/C16:0 and C18:1/C16:0. He reported that it was

possible to link the uncooked foods with residues extracted from modern cooking pots used to prepare one type of food. However, the ratios could not identify food mixtures. The utility of these ratios did not extend to residues extracted from archaeological potsherds because the ratios of the major fatty acids in the residue changed with decomposition (Skibo 1992, 97). Loy (1994) proposed the use of a Saturation Index (SI), determined by the ratio:

$$SI = 1 - \left[\frac{(C18:1 + C18:2)}{C12:0 + C14:0 + C16:0 + C18:0)} \right]$$

He admitted that the poorly understood decompositional changes to the suite of fatty acids make it difficult to develop criteria for distinguishing animal and plant fatty acid profiles in archaeological residues. The major drawback of the distinguishing ratios proposed by Marchbanks (1989), Skibo (1992) and Loy (1994) is they have never been empirically tested. The proposed ratios are based on criteria that discriminate food classes on the basis of their original fatty acid composition. The resistance of these criteria to the effects of decompositional changes has not been demonstrated. Skibo (1992) found that his fatty acid ratio criteria could not be used to identify highly decomposed archaeological samples.

In order to identify a fatty acid ratio unaffected by degradation processes, Patrick et al. (1985) simulated the long-term decomposition of one sample and monitored the resulting changes. An experimental cooking residue of seal was prepared and degraded in order to identify a stable fatty acid ratio. Patrick et al. (1985) found that the ratio of two C18:1 isomers, oleic and vaccenic acid, did not change with decomposition and this fatty acid ratio was used to identify an archaeological vessel residue as seal. While the fatty acid composition of uncooked foods must be known, Patrick et al. (1985) showed that the effects of cooking and decomposition over long periods of time on the fatty acids must also be understood.

Development of the Identification Criteria

As the first stage in developing the identification criteria, fatty acid compositions of more than 130 uncooked native food plants and animals from Western Canada were determined using gas chromatography (Malainey 1997; Malainey et al. 1999a). When the fatty acid compositions of modern food plants and animals were subject to cluster and principal component analyses, the resultant groupings generally corresponded to divisions that exist in nature (Table 1). Clear differences in the fatty acid composition of large mammal fat, large herbivore meat, fish, plant roots, greens and berries/seeds/nuts were detected, but the fatty acid composition of meat from medium-sized mammals resembled berries/seeds/nuts.

Cluster A	Subcluster			
	I	II	III	IV
Type	Mammal fat and marrow	Large herbivore meat	Fish	Fish
C16:0	19.90	19.39	16.07	14.10
C18:0	7.06	20.35	3.87	2.78
C18:1	56.77	35.79	18.28	31.96
C18:2	7.01	8.93	2.91	4.04
C18:3	0.68	2.61	4.39	3.83
VLCS	0.16	0.32	0.23	0.15
VLCU	0.77	4.29	39.92	24.11

Cluster B	Subcluster					
	V	VI	VII	VIII	IX	X
Type	Berries and nuts	Mixed	Seeds and berries	Roots	Seeds	Mixed
C16:0	3.75	12.06	7.48	19.98	7.52	10.33
C18:0	1.47	2.36	2.58	2.59	3.55	2.43
C18:1	51.14	35.29	29.12	6.55	10.02	15.62
C18:2	41.44	35.83	54.69	48.74	64.14	39.24
C18:3	1.05	3.66	1.51	7.24	5.49	19.77
VLCS	0.76	4.46	2.98	8.50	5.19	3.73
VLCU	0.25	2.70	1.00	2.23	0.99	2.65