CHAPTER FIVE
An Introduction to Archaeological Lipid Analysis by Combined Gas Chromatography Mass Spectrometry (GC/MS)

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As evident from the contributions to this volume, there are several approaches available to examine archaeological organic residues. Many studies concentrate on the interrogation of lipids isolated from archaeological materials while others examine alkaloids or proteins. In time, analyses may include all these classes of compounds, and perhaps others, but currently investigators concentrate on one class of compounds because of limited resources. The reasons behind the focus on lipids are that lipids are relatively stable compounds that are well-studied and easy to analyze (Murphy 1993). Furthermore, the use of methods similar to those employed by other archaeologists enables comparison between different sites and periods. Organic residues have been isolated from ancient objects, using a variety of protocols, including the ceramic matrix of unglazed pottery vessels with a wide range of ages and provenances (Charters et al. 1995; Condamin 1976; Eerkens 2002; Evershed et al. 1991; Gerhardt et al. 1990; Hill et al. 1985; Malainey et al. 1999; Mills and White 1989; Regert et al. 1998; Oudemans and Boon 1991; Patrick et al. 1985; Shimoyama et al. 1995; Skibo and Deal 1995; Stern et al. 2000).

One obstacle encountered by those eager to enter this field is that most publications understandably concentrate on the interpretation of the results and less on the details of the methodology. Another difficulty is the frequent use of jargon, often in the form of abbreviations and acronyms. In this chapter, we explain the basics behind the techniques used to analyze lipids, in archaeological samples, by GC/MS (see also the glossary at the end of this chapter).

Lipids and Fatty Acids

Lipids are a diverse group of organic molecules that includes, among others, fatty acids, fats (including triacylglycerols), waxes, steroids (including cholesterol) and terpenoids. They are largely hydrophobic ('water-hating') molecules, with small polar parts that are hydrophilic ('water-loving'). Lipids will therefore not readily dissolve in water, but rather form micelles or membranes with the hydrophilic parts on the outside and the hydrophobic parts clustering on the inside in an attempt to exclude water from the hydrophobic core (Figure 1, Berg et al. 2002; Voet and Voet 2004).

Lipids are ubiquitous in nature and are present in nearly all foodstuffs. As they appear to get trapped in the ceramic matrix of unglazed pottery, and remain there intact for centuries, they are potential targets for archaeological residue analysis. Downsides to lipid residue analysis are that individual lipids are not specific to one foodstuff and can potentially be introduced to vessels during all stages of use. In addition, there is not one method of analysis suitable for all types of lipids.

The most commonly analyzed class of lipids are fatty acids. These are strings of CH_2-groups, making up the hydrophobic part of the molecule, with an acidic (hydrophilic) COOH-group attached to one end (to the α-carbon).[1] A mix of several systems of nomenclature for these is in use, adding to the confusion of those new to the field. The systematic names of a series of saturated fatty acids, based on the number of C-atoms in Greek, and their synonyms are given in Table 1.

[1] The C-atoms of fatty acids are numbered from the COOH-group, number 2 being the α-carbon. The last C-atom, of the final CH_3-group, is referred to as the ω-carbon (Figure 1).

Systematic name	Synonyms		Formula	Mass
Dodecanoic acid	C12:0	Lauric acid, Vulvic acid	$HOOC-(CH_2)_{10}-CH_3$	200
Tridecanoic acid	C13:0	---	$HOOC-(CH_2)_{11}-CH_3$	214
Tetradecanoic acid	C14:0	Myristic acid	$HOOC-(CH_2)_{12}-CH_3$	228
Pentadecanoic acid	C15:0	---	$HOOC-(CH_2)_{13}-CH_3$	242
Hexadecanoic acid	C16:0	Cetylic acid, Palmitic acid	$HOOC-(CH_2)_{14}-CH_3$	256
Heptadecanoic acid	C17:0	Margaric acid, Margarinic acid	$HOOC-(CH_2)_{15}-CH_3$	270
Octadecanoic acid	C18:0	Stearic acid, Steric acid	$HOOC-(CH_2)_{16}-CH_3$	284
Nonadecanoic acid	C19:0	---	$HOOC-(CH_2)_{17}-CH_3$	298
Eicosanoic acid	C20:0	Arachic acid, Arachidic acid	$HOOC-(CH_2)_{18}-CH_3$	312
Heneicosanoic acid	C21:0	---	$HOOC-(CH_2)_{19}-CH_3$	326
Docosanoic acid	C22:0	Beheric acid	$HOOC-(CH_2)_{20}-CH_3$	340
Tricosanoic acid	C23:0	---	$HOOC-(CH_2)_{21}-CH_3$	354
Tetracosanoic acid	C24:0	Lignoceric acid	$HOOC-(CH_2)_{22}-CH_3$	368
Pentacosanoic acid	C25:0	---	$HOOC-(CH_2)_{23}-CH_3$	382
Hexacosanoic acid	C26:0	Cerinic acid, Cerotic acid	$HOOC-(CH_2)_{24}-CH_3$	396

Table 1: Details of a series of saturated fatty acids. Mass is the integer molecular mass in Daltons (Table 5).

Systematic name	Synonyms	Formula	Mass
9-tetradecenoic acid	C14:1, Myristoleic acid, Myristelaidic acid (trans)	$HOOC-(CH_2)_7-CH=CH-(CH_2)_3-CH_3$	226
9-hexadecenoic acid	C16:1, Palmitoleic acid, Palmitelaidic acid (trans)	$HOOC-(CH_2)_7-CH=CH-(CH_2)_5-CH_3$	254
9-octadecenoic acid	C18:1, Oleic acid, Elaidic acid (trans)	$HOOC-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$	282
11-eicosenoic acid	C20:1, Gondoic acid, trans-Gondoic acid	$HOOC-(CH_2)_9-CH=CH-(CH_2)_7-CH_3$	310
13-docosenoic acid	C22:1, Erucic acid, Brassidic acid (trans)	$HOOC-(CH_2)_{11}-CH=CH-(CH_2)_7-CH_3$	338
15-tetracosenoic acid	C24:1, Selacholeic acid, trans-Selacholeic acid	$HOOC-(CH_2)_{13}-CH=CH-(CH_2)_7-CH_3$	366

Table 2: Details of a selection of mono-unsaturated fatty acids. Mass is the integer molecular mass in Daltons (Table 5). The single syllable that distinguishes the systematic name of an unsaturated fatty acid from that of its saturated counterpart is underlined.