Chapter 1 - Introduction

1.1 Introduction

Archaeological residues are commonly defined as the remains of a substance adhering to (Patrick et al. 1985) or preserved within an artefact (Evershed et al. 1992). Archaeological residue research can be broadly divided into two areas. The first is limited to light microscopic identification of remains preserved on artefact surfaces (typically lithics), such as blood cells and starch grains (Barton 2007; Barton et al. 1998; Del Pilar Babot and Apella 2003; Fullagar and Jones 2004; Loy 1993). In the second approach, analysis concentrates on molecules preserved on or absorbed within artefacts. Analysis of ceramic residues has produced significant methodological developments as well as addressing substantive issues such as ancient foodways and trade (Craig et al. 2007; Craig et al. 2005; Eerkens 2001; Evershed et al. 1997; Knappett et al. 2005; Malainey et al. 1999c). Previous archaeological molecular studies have focused on reconstructing residue sources by extrapolating characteristic features of modern organic products (chemotaxonomy) to archaeological remains (Evershed 1993; Evershed et al. 1992).

A key assumption of these studies is that chemotaxonomic features remain stable through cultural and post-depositional processes (Eerkens 2007; Evershed 1993; Evershed et al. 1992). However, the stability of chemotaxonomic features has not been systematically evaluated or proven. A small number of experimental simulations that produced residues by cooking within replica pots and exposing molecules to oxidization have concluded that some key chemotaxonomic features are little affected by cooking or prolonged burial (Charters and Evershed 1997; Malainey et al. 1999b; Patrick et al. 1985).

In this thesis I address how organic products are incorporated into ceramics and transformed into archaeological ceramic residues. The majority of archaeological residue publications focus on the reconstruction of residues to determine their original source products. Surprisingly, few studies have attempted to evaluate residue formation processes. Yet, the identification and evaluation of the formation processes that create the archaeological record have been repeatedly argued as fundamental

(Chase et al. 1994; Child 1995; Coard 1999; Dennell 2005; Jeske and Kuznar 2001; Schiffer 1995). In this study I argue this area of research is an equally essential foundation for reconstructions of original source products from archaeological residues.

Much effort has been spent developing theoretical and methodological frameworks for a systematic approach to archaeological taphonomic processes (Schiffer 1972; Schiffer 1983). Archaeological taphonomic frameworks treat artefacts as part of a complex phenomenon where past human behaviours and natural processes interact to form the archaeological record (Schiffer 1972).

As yet, no systematic attempt has been made to apply the taphonomic methodology to archaeological residue research. This partly reflects a failure to recognise that residues are non-static organic compounds that exist in a constant state of interaction with the environment. It also reflects the nascent state of this research area where developments in instrumentation still drive research agendas.

I adopt the concept and terms of archaeological taphonomy to develop a systematic evaluation of archaeological residue formation processes. From this I identify key factors likely to have significant influences on archaeological residue composition. The taphonomic study of archaeological residues is also concerned with complex interactions between past human behaviours and natural processes as they relate to the preservation of organic molecules. Archaeological taphonomic analysis attempts to identify and evaluate the key cultural and post-depositional processes involved.

For this study I employ two types of instrumentation to assess both non-volatile and volatile residue components. Gas Chromatography - Mass Spectrometry (GC-MS), widely used in the study of archaeological residues (Craig et al. 2007; Eerkens 2005; Evershed et al. 2008; Malainey et al. 1999c; Quigg et al. 2001; Reber and Evershed 2004), is employed to separate and measure a spectrum of volatile molecules, most importantly fatty acid methyl esters (FAMEs). High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS) is used to separate and quantify heavier non-volatile organic molecules (di- and triacylglycerols) (Craig 2004:37). While HPLC-MS has been less frequently used to characterise archaeological residues it is particularly useful for providing an independent measure and in some cases is a unique source of information (Charrié-Duhaut et al. 2007; Craig 2004:79-80; Kimpe et al. 2004).

A series of controlled experiments are conducted to evaluate the effects of taphonomic processes on reference fats and oils (RFOs). These include heating and boiling, reuse of ceramic vessels to cook different products, leaching, microbial alteration, pH, and alteration over time. In the second part of the study I use the results of these experiments to guide the interpretation of archaeological residues. I evaluate a diverse sample from recent archaeological excavations in Central and Western Turkey that date from c. 1450-547 BCE. Samples include a wide range of vessel types (i.e. precious oil containers, cooking pots, large storage pithoi).

My experimental simulations identify the character and likely range of complex interactions involved in archaeological residue formation. The results of this study demonstrate that a taphonomic approach to archaeological residues provides a more systematic means to both understand and evaluate the complex relationships between residues, ceramics, and formation processes.

1.2 Structure and Organisation of the Thesis

Chapter 2 reviews the literature relevant to the three areas of this study: lipid structure and properties, archaeological residue analysis and taphonomy. Section 2.2 describes the composition and structure of lipids and the key chemical and physical interactions that affect them. Section 2.3 discusses molecular archaeological research and details the range of instrumentation that has been used to examine archaeological molecules. Section 2.4 details the assumptions and interpretive methods (biomarkers, ratios, and multivariate statistics) used to interpret highly altered archaeological residues and potential limitations of these methods. Section 2.5 describes archaeological taphonomic frameworks discussing the potential advantages of using systematic interpretive frameworks for understanding archaeological residues.

Chapter 3 presents the analytic rationale of this study by developing a taphonomic model that provides a systematic framework for exploring archaeological residue formation. Section 3.2 details the taphonomic model used for this approach. In Section 3.2.1 and 3.2.2 I develop a cultural taphonomic flow-model based on Schiffer's (1972) *systemic context* flow-model, and define types of residues can form through cultural processes. Section 3.2.3 details the post-depositional processes

examined in this study. Section 3.2.4 describes lipid properties that may influence taphonomic processes. Section 3.2.5 summarises these cultural, post-depositional and chemical processes into a series of queries that form the basis for the experimental component of this study.

Chapter 4 details the experimental methodology and archaeological samples used in this study. Section 4.2 describes the extraction, derivatisation and instrumental procedures used to analyse the experimental and archaeological data. Section 4.3 describes the experimental datasets and the cultural and post-depositional simulations developed in this study. Section 4.4 details the interpretive techniques used to characterise experimental and archaeological samples (divergence indices, biomarkers, ratio analyses and Principal Components Analysis). Section 4.5 describes the archaeological sample and substantive issues that residue analysis can address.

Chapter 5 presents the experimental and archaeological results of this study. Section 5.2 describes the effects of simulated cultural and post-depositional processes on lipid compositions. Section 5.3 applies methods designed to interpret archaeological residues to the experimental dataset to assess the reliability of these techniques for experimentally altered samples. Section 5.4 describes archaeological residue recoveries and presents the archaeological results of this study, first describing residue recoveries (Section 5.4.1) and general molecular features of residues (Section 5.4.2), then applying a range of established interpretive techniques to elucidate residue source (Sections 5.4.3-5.4.6).

Chapter 6 discusses the methodological and substantive implications of this thesis. Section 6.2 considers the implications of the experimental program within the context of the queries posed in Section 3.2.5. Section 6.3 discusses the applicability and reliability of the interpretive techniques used in this study. Sections 6.3.1-6.3.3 reviews the reliability of interpretive techniques when applied to the experiment samples. Section 6.3.4 considers the diagnoses of interpretive techniques applied to archaeological data. Section 6.4 discusses the taphonomic features of archaeological residues identified in this study. Section 6.5 considers the implications of archaeological taphonomic approach developed in this study for archaeological residue interpretation. Section 6.6 presents the substantive conclusions of the archaeological residue analyses.

Chapter 7 provides a summary of the implications of this study and conclusions. Section 7.1 reviews how this study enhanced current understanding of

archaeological residues. Section 7.2 considers the utility of adopting systematic approaches to understanding archaeological residues. Section 7.3 provides suggestions for the most productive directions for future archaeological residue research. Section 7.4 concludes by summarising the main contributions of this study.

Chapter 2 – Literature Review

2.1 Introduction

The purpose of this chapter is to review previous research in the context of three major areas relevant to this study (i.e. physical and chemical properties of lipids, archaeological lipid analyses, and taphonomy). First I describe the chemical and physical properties of lipid molecules and the chief mechanisms of alteration. I then introduce archaeological lipid analysis with a description of the objectives and methodological foundations of the research area. I review the approaches, the instrumentation used, and interpretive techniques applied in archaeological residue research. I outline problems in the conventional interpretative tools used for archaeological residues specifically in relation to interpreting complex archaeological organics. Finally, I describe archaeological taphonomic frameworks and the advantages that this type of systematic approach offers for addressing the formative complexity of archaeological residues.

2.2 Lipid Properties

2.2.1 Structure and behaviour

Fatty acids are characterised as simple lipids, consisting of a carboxyl head group attached to a hydrocarbon (aliphatic) chain (Markley 1960). Hydrocarbon chains occur in a range of configurations, which may contain double bonds (both *cis* and *trans* configurations), branched chains, and ringed structures (Christie 2003:6-12). The length of hydrocarbon chains varies widely, with the number of carbon atoms varying from four to more than 30, although most are between 12 and 20 carbon atoms in length. As carboxyl acids, fatty acids are polar lipids, with polarity decreasing as aliphatic chain length increases.

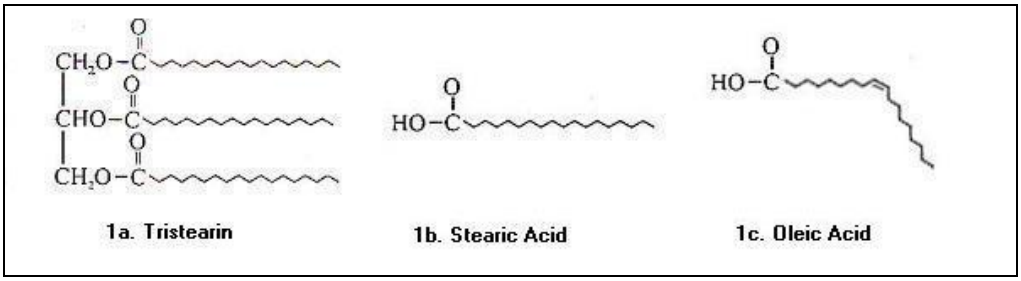

Figure 2.1: Chemical structures of three common lipid species. 1a. Triastearin, 1b. Stearic acid and 1c. Oleic acid (adapted from Evershed et al 2001).

Numerous nomenclature systems have been developed to describe fatty acids. This study utilises two systems interchangeably, the "trivial" (roughly understood as a scientific colloquial descriptors) and the systematic (a more detailed descriptive format the details chain lengths and saturation condition), along with their shorthand forms, as detailed in table 2.1.

Systematic Name	Trivial Name	Shorthand Designation	Trivial Shorthand
Butanoic	Butyric	4:0	-
Pentanoic	-	5:0	-
Hexanoic	Caproic	6:0	-
Heptanoic	-	7:0	-
Octanoic	Caprylic	8:0	-
Nonanoic	-	9:0	-
Decanoic	Capric	10:0	-
Unadecanoic	-	11:0	-
Dodecanoic	Lauric	12:0	-
Tridecanoic	-	C13:0	-
Tetradecanoic	Myristic	14:0	My
Pentadecanoic	-	15:0	-
Hexadecanoic	Palmitic	16:0	P
Hexadecenoic	Palmitoleic	16:1	Po
Hexadecadienoic	-	16:2	-
Heptadecanoic	Margaric	17:0	Ma
Heptadecenoic	-	17:1	Mo
Octadecanoic	Stearic	18:0	S
Octadecenoic	Oleic	18:1	O
Octadecadienoic	Linoleic	18:2	L
Octadecantrienoic	Linolenic	18:3	Ln
Nonadecanoic	-	19:0	-
Eicosanoic	Arachidic	20:0	A
Eicosenoic	-	20:1	-
Eicosatrienoic	dihomo-γ-linolenic	20:3	-
Eicosatetraenoic	arachidonic	20:4	-
Docosanoic	Behenic	22:0	B
Docosenoic	-	22:1	-
Docosadienoic	-	22:2	-
Docosatetraenoic	-	22:4	-
Docosahexaenoic	-	22:6	-
Tricosanoic	-	23:0	-
Tetracosanoic	Lignoceric	24:0	Li
Tetracosenoic	-	24:1	-

Table 2.1: Summary of systematic, trivial, shorthand and trivial shorthand nomenclature systems for the description of common fatty acids.

In natural products most fatty acids occur as components of larger molecules (Christie 2003:22). Approximately 99% of fatty acids are typically bound in larger triacylglycerol molecules. Triacylglycerols consist of three fatty acids attached to a glycerol backbone via ester linkages (Figure 2.1a) (Christie 2003:12-3; Holcapek et al. 2003; Mottram et al. 2001; Mottram et al. 1997). Triacylglycerols are defined by their component fatty acids and the relative positions of fatty acids on the glycerol backbone. Fatty acids attach to glycerol at three sites, two at the ends of the molecule and one in a central position. These are referred to as side-chain numbers (*sn*-), with a shorthand of *sn*-n. Given the symmetry of glycerol, *sn*-1 and *sn*-3 are usually considered interchangeable, and described as *sn*-1(3). Where differentiated, 1,3 and 3,1 triacylglycerols are described as *enantiomeric pairs*, or *mirror image forms*, although these do not affect most lipid analyses (Frankel 2005:4; Mottram and Evershed 1996). An example of trivial description of a triacylglycerol with stearic acid [S] in the *sn*-1(3) positions and oleic acid [O] in the *sn*-2 position is SOS.

The number of potential triacylglycerol species increase exponentially with the number of fatty acid species and can be expressed as $N_{TAG}=N_{FA}^{3}$-M, where N_{TAG} is the number of potential triacylglycerol species, N_{FA} is the number of fatty acids and M is the number of mirror image forms. For example, a mixture of stearic and oleic acid can produce six main triacylglycerols, SSS, OOO, SSO, SOS, OOS and OSO, along with two mirror image forms, SOO(OOS) and OSS(SSO). Mono- and diacylglycerols are also possible and biologically important (Christie 2003:12-3; Evershed 1993). Fatty acids can form esters with other types of molecule, such as steroids, terpenoids, alcohols, and waxes.

Other archaeologically important classes of lipid include the wax esters, terpenes, and steroids. In simple terms, wax esters are long-chain alcohols (hydrocarbons with an –OH functional group) bound to a fatty acid via an ester linkage between alcohol and carboxyl functions.

Terpenoids form a large and diverse class of lipids. Terpenoids are formally defined as head-to-tail dimers of isoprene (Templeton 1969). The molecules are broadly classified based on the number of carbon atoms they possess. Monoterpenoids (Figure 2.2a), sequiterpenoids, diterpenoids (Figure 2.2b) and triterpenoids (Figure 2.2c) contain ten, fifteen, twenty and thirty carbon atoms respectively. (American Chemical Society 1955 Templeton, 1969). Broadly, mono- sesqui- and di- terpenoids

are the main components of scents, the 'essential oils', while di- and especially tri-terpenoids form resinous substances (Adams 2001).

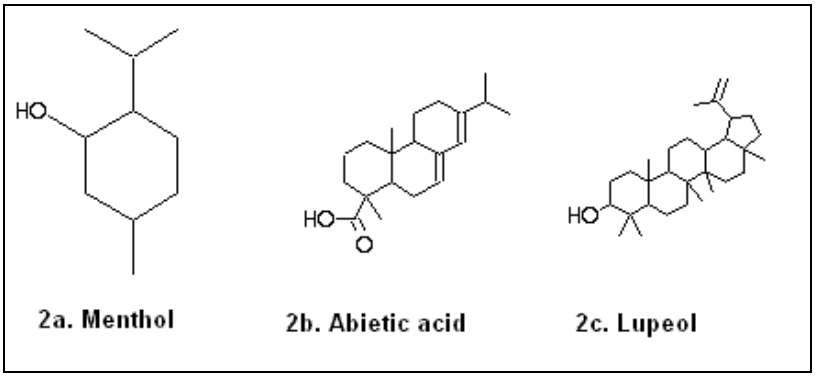

Figure 2.2: Chemical structures of three common terpene species. 2a. Menthol (monoterpene), 2b. Abietic acid (diterpene) and 2c. Lupeol (triterpene) (adapted from NIST 2005).

Terpenoids are further differentiated by their functional groups (alcohols, acids, aldehydes, esters, ketones and ethers), the number of functional groups and their attachment site on the molecules, and the cyclisation and hydrogenation of the base terpenoid hydrocarbon (American Chemical Society 1955; Templeton 1969).

Sterols are a special class of triterpenoid that are used as hormonal precursors in animals (cholesterols (Figure 2.3a)) and plants (campesterols (Figure 2.3b), sitosterols (Figure 2.3c), stigmastanols (Figure 2.3d).

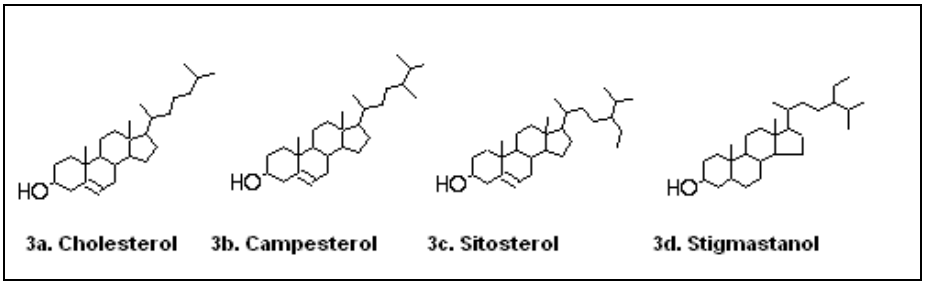

Figure 2.3: Chemical structures of four common sterols. 3a. Cholesterol, 3b. Campesterol, 3c. Sitosterol, 3d. Stigmastanol (adapted from NIST 2005).

2.2.2 Lipid alteration

2.2.2.1 Hydrolysis

Chemical and enzymatic hydrolysis converts triacylglycerols to their component fatty acids and free glycerol (Evershed et al. 2001). The hydrolysis process attacks the ester linkage between fatty acid carboxyl groups and the glycerol backbone, yielding a diacylglycerol and a free fatty acid. The process continues

converting diacylglycerols to monoacylglycerols and monoacylglycerols to glycerol (Figure 2.4). Diacylglycerols and monoacylglycerols are more liable to hydrolysis than triacylglycerols (Evershed et al. 2001). The composition of acylglycerols affects their liability to hydrolysis, with those containing highly unsaturated fatty acid moieties being preferentially hydrolysed.

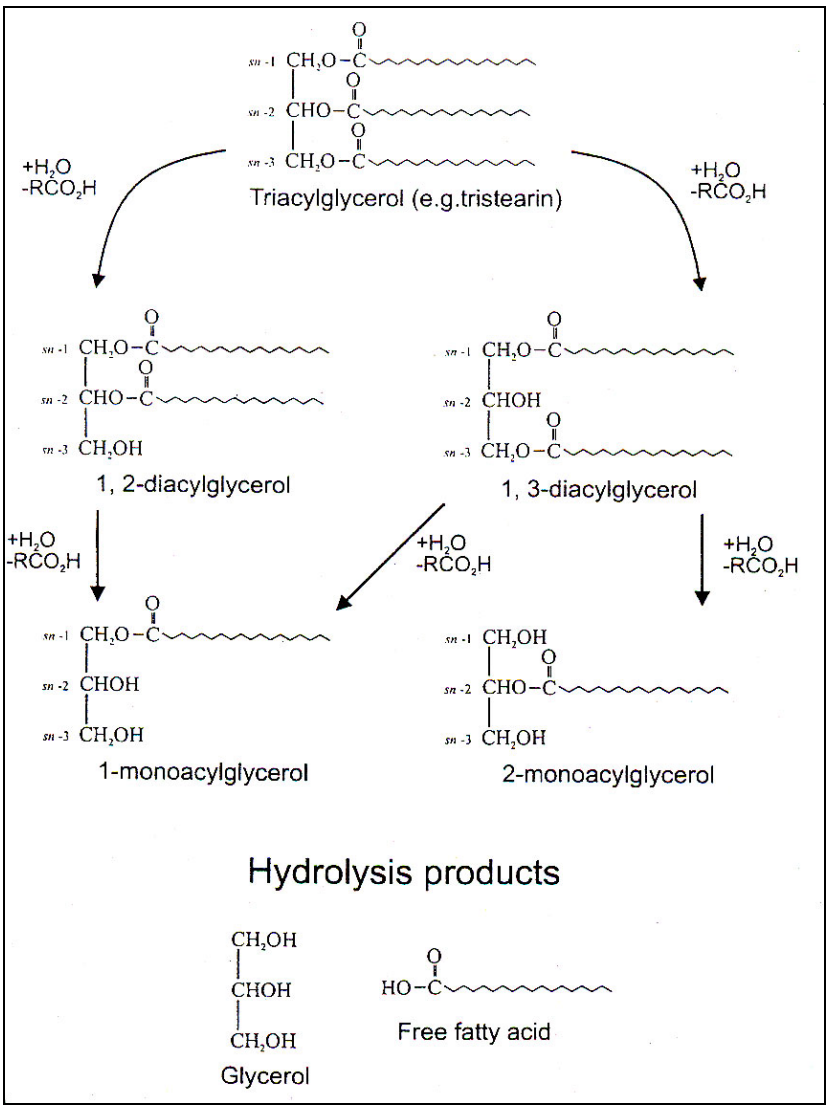

Figure 2.4: Flow chart of chemical stages of lipid hydrolysis showing the conversion of triastearin to di- and monoacylglycerols and then to its component glycerol and free fatty acids (Evershed et. al. 2001).

2.2.2.2 Oxidation and autoxidation

Oxidative alteration is the most significant chemical process for lipid products (Frankel 2005). Oxidation and autoxidation affect unsaturated hydrocarbon bonds by replacing a hydrogen atom at the double bond with an oxygen atom or atoms. Consequently, oxidative alterations apply only to unsaturated fatty acids. When this process occurs in mild conditions in the presence of oxygen it is described as autoxidation. Oxidation requires additional environmental or chemical factors to initiate, such as free radicals.

Free radical reactions begin with the removal of a hydrogen radical (H˙) from an unsaturated lipid (LH) at an unsaturated bond by an initiator molecule (I), forming a lipid free radical (L˙) (LH + I → L˙ + IH). For autoxidation, singlet oxygen ($^1O_2$) possessing two unpaired electrons (˙O-O˙) initiates the reaction. Dissociated hydroperoxides (LOOH → LO˙ + ˙OH) or certain metals initiate free radical reactions. Initiator molecules are themselves free radicals that are stabilised by taking hydrogen radicals from lipid double bonds.

Radical unsaturated lipids react rapidly with molecular oxygen, forming peroxyl radicals ($L˙+O_2$ → LOO˙). Peroxyl radicals then abstract hydrogen from unsaturated lipids forming hydroperoxides (LOO˙ + LH → LOOH + L˙), perpetuating the reaction. Alternatively, LO˙ radicals can interact with lipids to form alcohols (LO˙ + LH → LOH + L˙) and unsaturated aldehydes (LO˙ → RCHO + L˙). Other archaeologically important hydroperoxide derivatives include ketogenated fatty acids (RCOOHO) (Frankel 2005:89), dicarboxylic acids (COOH-R-COOH) (Passi et al. 1993) and short-chain fatty acids (Velasco et al. 2004).

The oxidization process may be terminated in several ways, producing a variety of products. Two peroxyl radicals can interact to form non-radical products and oxygen (LOO˙ + LOO˙ → non-radical products + O_2). At low temperatures, peroxyl radicals condense to form lipid dimers (2 LOO˙ → LOOL +O_2).

The structure of unsaturated lipids in part defines their liability to oxidation and autoxidation (Frankel 2005:21). Autoxidative change in the C18 unsaturated fatty acids C18:1, C18:2, and C18:3 increases at a relative rate of 1:41:98 with C18:3 being 98 times more liable to autoxidation than C18:1. The organisation of fatty acids into larger structural units also influences the rate of oxidative change. For example,

linoleic acids (C18:2) bound in trilinolein triacylglycerols autoxidate at 84% of the rate of simple linolenic methyl esters (Larsson et al. 2006).

2.2.2.3 Colloidal lipid proprieties

Acylglycerols and fatty acids possess a remarkable ability to self-sort and organise into highly structured colloidal-scale crystalline aggregates (Larsson et al. 2006). Lipid crystals form through polar and hydrogen bonding between the head groups, aliphatic chains, Π-Π interactions between double bonds, and methyl end groups (Figure 2.5).

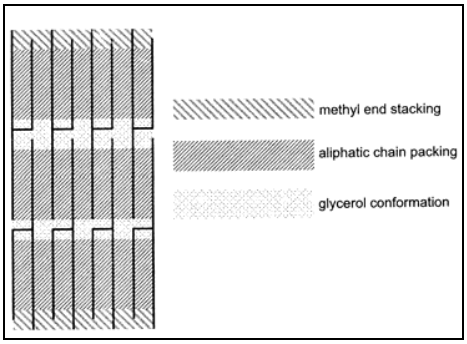

Figure 2.5: Schematic representation of the crystalline conformation of triacylglycerols showing the interactions between methylene functions, glycerol conformation and interactions along the aliphatic chains (Larsson et. al. 2006:40).

Lipid crystals can organise in several ways, depending on the structure of the component lipids and their exposure to physical conditions (polymorphism). Polymorphs range from relatively disordered α forms through γ, β, β' forms to highly structured β_1 and β_2 forms (Figure 2.6).

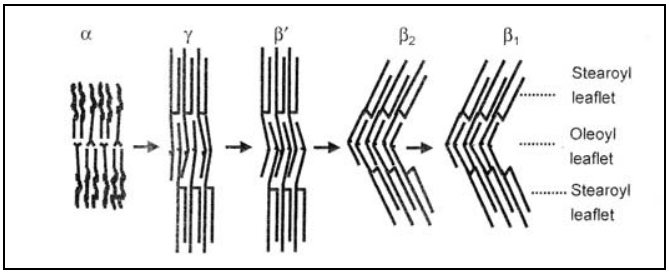

Figure 2.6: Common lipid crystal polymorphs of SOS ranging from the relatively disordered α polymorphs to highly ordered β polymorphs (Larsson et. al. 2006:32).

To form crystalline structures component molecules generally need to be chemically and structurally identical. Enantiomeric pairs and very similar lipid

species may also form relatively disordered crystals (Larsson et al. 2006). For example, SLS and SOS can form α and γ crystals, but are unable to organise into more compact β forms (Figure 2.7). Crystal form affects the physical properties of the component lipids. For example, the relatively disordered α form of tristearin has a melting point of 55°C while the highly ordered β form melts at 73°C (Larsson et al. 2006).

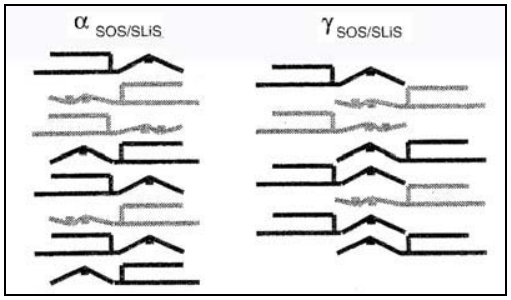

Figure 2.7: α and γ polymorphs of SOS and SLS (Larsson et. al. 2006:47).

Lipid degradation rates are altered by crystalline aggregates. The solid state of the crystals limits the degree of direct exposure of individual molecules to environmental factors, such as oxygen and free radicals and inhibits free radical chain reactions. The example of lower rates of autoxidation in trilinolein relative to neat methyl linolenate in Section 2.2.2.2 is partly due to the formation of α-form crystals at the exposure temperature (37°C), while methyl linolenate is a liquid. At lower temperatures and higher states of order, differences are even more pronounced.

Interactions between lipid crystals also influence alteration. Aggregates of lipid crystals can form networks that effectively encase pockets of liquid lipid, reducing their environmental exposure (Larsson et al. 2006). In appropriate proportions and environmental conditions more unsaturated liquid lipids will be encased in a matrix of relatively unreactive lipid crystals lowering their liability to oxidative change.

2.3 Molecular Archaeology

Molecular archaeology is concerned with the molecular composition of archaeological organic remains preserved in a range of environments. Molecular archaeology is capable of analysing a wide range of archaeological remains, from

large artefacts such as corpses (Evershed 1990) and bone (Baron et al. 1996; Evershed et al. 1995; Stott and Evershed 1996; Yang et al. 2004) to less dramatic but far more frequent amorphous residues preserved on and within artefacts (e.g. lithics and ceramics) (Buonasera 2005; Buonasera 2007; Condamin et al. 1976; Copley et al. 2005a; Del Pilar Babot and Apella 2003; Evershed et al. 2001; Evershed et al. 1985; Fox et al. 1995; Quigg et al. 2001; Shanks et al. 2001; Shanks et al. 1999). Most molecular archaeological studies have focused on ceramic residues due to their relatively frequent recovery and the economic and social importance of ceramics in ancient societies.

In two seminal papers in the early 1990s R. P. Evershed and coworkers (Evershed 1993; Evershed et al. 1992) defined the methodological and theoretical agenda for residue research as 'a problem of biochemical phylogeny or chemotaxonomy' aiming to 'match a specific (archaeological) compound or mixture of compounds to that of a contemporary plant or animal natural product likely to have been exploited in antiquity' (Evershed et al. 1992). This chemotaxonomic approach focuses on identifying diagnostic features and patterns in modern organic analogues and linking these to similar features in archaeological remains (Evershed 1993).

While a broad range of organic molecules can be preserved, in most archaeological remains complex and unstable molecules are preferentially degraded. Evershed (1993) broadly classifies the susceptibility of organic molecules to alteration as follows: Lignin and other plant polymers < Lipids (fats, oils and resins) < Carbohydrates < Proteins < Nucleotides (RNA and DNA).

The chemotaxonomic utility of molecular class is generally inversely proportional to its potential for archaeological survival. Where DNA survives it can provide very specific information, such as sex, species and disease (Arndt et al. 2003; Baron et al. 1996; Boscato et al. 2008; Faerman and Kahila Bar-Gal 1998; Hasson and Foley 2008; Loreille et al. 1997; Pääbo 1985; Schlumbaum et al. 1998; Shanks et al. 2001; Speller 2005; Taylor et al. 1996; Yang et al. 2004). Similarly, proteins are archaeologically uncommon but highly diagnostic, providing specific chemotaxonomic information (Craig et al. 2005; Shanks et al. 2001; Zeven et al. 1975). Lipids are much more commonly preserved but are less diagnostic, often only capable of broad discriminations, such as plant or animal, or the identification of a group of related sources, such as the ruminants (Evershed et al. 2001). Plant polymers are generally uncharacteristic and have not been studied archaeologically (Evershed

1993). Lipids, most importantly fatty acids, are the most widely analysed class residue molecule due to their chemotaxonomic properties, analytic simplicity and frequent archaeological recovery. Because of the predominance of lipid analysis in residue archaeology, I focus on the analysis of these molecules in this study.

Applications of the chemotaxonomic approach utilise a relatively standardised methodology. Studies first identify molecular features of modern reference products that appear diagnostic (Craig 2004; Eerkens 2005; Evershed et al. 2002; Malainey et al. 1999a; Marchbanks 1989; Skibo 1992). Reference samples are then altered, simulating cultural or post-depositional changes to determine the stability of chemotaxonomic features. Cultural alteration is simulated via heating and boiling (Charters and Evershed 1997; Craig 2004; Malainey et al. 1999b; Patrick et al. 1985) and post-depositional alterations by exposure to slightly elevated heat over several months (Patrick et al. 1985; Malainey et al. 1999b). Stable chemotaxonomic features are then identified in archaeological residues and related to organic sources.

2.3.1 Analytic techniques

Chromatographic and Mass Spectrometric instrumentation are most frequently used to study archaeological residues as they require small sample volumes (less than 1μg) and can identify a wide range of lipids (Evershed et al. 2001). Gas Chromatography (GC) and coupled Gas Chromatography-Mass Spectrometry (GC-MS) are most commonly utilised by archaeological researchers (Copley et al. 2004; Evershed et al. 1992; Fox et al. 1995; Quigg et al. 2001). Gas Chromatography separates lipid species by their evaporative point while Mass Spectrometry measures the masses of molecules molecular fragments of lipid species (Abian 1999). Modern high-precision GC-MS systems are simple to use, can rapidly analyse a large number of samples, and identify and quantify a broad range of lipids (Evershed et al. 2001).

While versatile, GC is poorly suited to separating heavy non-volatile lipids (triacylglycerols) (Abian 1999). Triacylglycerols dominate modern fats and oils (~99% of lipids) (Holcapek et al. 2003), have been observed archaeologically (Craig 2004; Dudd et al. 1999; Evershed et al. 2002), and can be diagnostic (Craig 2004:79). High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) is better suited to the analysis of triacylglycerols (Abian 1999; Buchgraber et al. 2004; Byrdwell 2001; Christie 1987:172-85; Christie 2003:309-15; Holcapek et al. 2003;

Mottram et al. 2001; Mottram and Evershed 1996) and researchers have recently begun to explore the chemotaxonomic utility of these molecules (Charrié-Duhaut et al. 2007; Craig 2004; Evershed et al. 2002; Kimpe et al. 2004). HPLC separates molecules based on their polarity and solubility in a mobile solvent phase (Abian 1999). Using both GC-MS and HPLC-MS it is possible to analyse a broader range of lipid species.

A wide range of instrumentation is used less frequently including Direct Temperature-resolved Mass Spectrometry (DTMS) (Oudemans et al. 2007b), Infrared Spectroscopy (IR) (Beck et al. 1978), Nuclear Magnetic Resonance Spectroscopy (NMR) (Oudemans et al. 2007a), FT-Raman Spectroscopy (FTR) (Edwards et al. 2007) and Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (GC C IRMS) (Copley et al. 2005a; Copley et al. 2005c; Copley et al. 2005b; Craig et al. 2007; Craig et al. 2005; Reber and Evershed 2004). These instruments are capable of providing detailed but more specialised information for some organic remains. IR has been used to differentiate European amber sources (Beck et al. 1978) and GC C IRMS has been principally used to identify ruminant milk residues (Copley et al. 2005a; Copley et al. 2005b; Copley, 2005c; Craig et al. 2007; Craig et al. 2005; Dudd et al. 1999; Evershed et al. 1997) and C4 plants (Reber and Evershed 2004). NMR and DTMS have not yet been widely applied to archaeological remains but appear to be capable of differentiating a range of plant and animal sources (Oudemans et al. 2007a; Oudemans et al. 2007b). These specialised instruments are beyond the scope of the present study.

2.4 Chemotaxonomic Approaches and Interpretive Techniques

In this section I detail the chemotaxonomic methods and assumptions used in previous research. First I consider the causes of molecular variability both within and between species to explain why chemotaxonomic features exist. Next I review previous experimental simulations and archaeological observations used to establish the robusticity and validity of chemotaxonomic features. Finally I detail specific chemotaxonomic techniques developed in previous research.

2.4.1 Molecular variability

Chemotaxonomy is based on the wide molecular variability observed in different organic products (Eerkens 2007; Evershed et al. 1992). Differences in the proportions of lipid molecules between botanical and biological species will generally be greater than those within species, allowing the differentiation of lipid sources. While many of the causes of this variability remain poorly understood, and beyond the scope of this study, organic products can be differentiated into three broad categories: plants, marine animals and non-marine animals. Plant products characteristically contain high proportions of unsaturated fatty acids, such as oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and palmitoleic (C16:1). Marine animals are characterised by high proportions of higher polyunsaturated fatty acids (e.g. C16:2, C18:4, C20:5, C20:6, C22:6), and non-marine animals by relatively high levels of saturated and monounsaturated fatty acids (C16:0, C18:0, C18:1) (Hilditch and Williams 1964). While these features readily differentiate modern products, most are considered too unstable to persist archaeologically. Archaeological analyses most frequently rely on more stable saturated lipids for their interpretations.

Lipid composition also varies between individuals within species. Causes of intra-species variability include nutrition (Biong et al. 2006; Göttsche and Straarup 2006), sex (Nuernberg et al. 2006), genetic modification (Byrdwell and Neff 1996), environmental conditions (e.g. water, soil and temperature) (Bada et al. 2004; Roche et al. 2006) and seasonality (Sieiro et al. 2006). Variation can sometimes be significant. For example, the linolenic (C18:3) component of linseed oil can vary between 3% and 63% (Krist et al. 2006). Variability within species primarily affects unsaturated lipid molecules that are usually not used for chemotaxonomy.

2.4.2 Experimental and archaeological foundations of chemotaxonomy

Experimental residue simulation has focused on determining the stability of chemotaxonomic features (i.e. diagnostic marker molecules and proportional relationships between molecules) through cultural processes and over archaeological timescales (Charters and Evershed 1997; Craig 2004; Malainey et al. 1999b; Patrick et al. 1985). Cultural alterations are usually studied by exposing food products to heat

or experimentally boiling them in ceramic pots. The key differences between studies relate to variations of organic product, exposure type, conditions, and exposure time.

Patrick and coworkers (1985) first explored the stability of chemotaxonomic fatty acid relationships in Cape Fur Seal. Similarly, Charters and coworkers (1997) considered characteristic molecules and relationships of *Brassica* leaf waxes the effects of boiling in water on the penetration of *Brassica* leaf waxes into ceramic fabrics. T. J. Craig (2004) examined the stability of chemotaxonomic features observed in fatty acids and triacylglycerols from a suite of modern food products based on remains recovered from Turkish archaeological deposits. Malainey and coworkers (1999a, 1999b) considered the stability of taxonomic properties of a very wide range of west Canadian native foods as part of a study of late prehistoric ceramics from the region. The emphasis on boiling reflects the nature of archaeological residue research that has placed a strongly emphasis on ancient food technologies (Copley et al. 2005a; Copley et al. 2005c; Copley et al. 2005b; Eerkens 2005; Evershed et al. 2008; Malainey et al. 1999c). Most cultural simulations have concluded that chemotaxonomic features based on relatively stable saturated and monounsaturated lipids will survive cultural processing.

The effects of post-depositional environments have been explored through simulating 'archaeological time' by exposing fats and oils to slightly elevated heat for periods of several months (Evershed et al. 2002; Malainey et al. 1999b; Patrick et al. 1985). Exposure principally affects unsaturated lipids, accelerating oxidation, autoxidation and hydrolysis. Simulated archaeological time is capable of producing molecular profiles that closely resemble archaeological residues. Patrick and coworkers (1985) were able to transform modern seal fats into products that closely resembled their archaeological sample. Similarly, Evershed has highlighted the strong similarities between beef fats exposed to simulated archaeological time and archaeological residues characterised as ruminant fats (Evershed et al. 2002). The close similarities between experimental and archaeological lipids have led to the widespread acceptance of long-term accelerated oxidization as an adequate simulation of post-depositional alteration of archaeological residues.

Another substantive attempt to simulate archaeological conditions is Berstan and coworkers' (2004) simulation of the chemical processes that create 'bog butter'. By exposing fats to anaerobic conditions similar to peat bogs Berstan and coworkers were able to produce a substance that closely resembled archaeologically recovered

bog butters. While significant, bog butters are substantially different to ceramic residues which are usually recovered from aerobic archaeological contexts.

An assumption of chemotaxonomy is that the ceramic fabric forms an essentially enclosed environment, relatively impervious to the penetration of endogenous material. Heron and coworkers (Heron et al. 1991) considered the effects of the migration of soil lipids into archaeological ceramics while examining Late Saxon and Early Medieval ceramic residues from West Cotton, Raunds, Northamptonshire, UK. Stern and coworkers (Stern et al. 2000) observed similar features in their sectional analysis of Canaanite amphorae. These studies indicate that contamination from soil microbes was minor, and could be effectively eliminated by removing the outer surfaces of ceramics and establishing minimum residue recovery thresholds for analytic viability. Abrading the outer surfaces of a ceramic has been widely accepted as a general method of removing potential contaminants, although the reliability of this method for controlling microbial alteration in a wider range of archaeological environments is unclear.

Two recent studies have highlighted the pitfalls of accepting a narrow range of experimental results as representative of the range of potential archaeological variability. The first was conducted by sending a 'blind sample' of modern ceramic impregnated with camel milk to several researchers for analysis (Barnard et al. 2007). The source identifications of these analyses differ not only from the actual residue source, but also from each other. Most lipid-based interpretations of the residue broadly identified its source (e.g. 'veal, egg, or goat milk', 'animal or egg'). However, one technique interpreted the residue as a plant product. This experiment demonstrates the limited interpretive power of current chemotaxonomic techniques.

The second study was conducted by Reber and Evershed (2004) who examined the effects of real environments on corn residues by burying corn-impregnated ceramics in a range of different depositional environments. After a period of six months the residues were substantially altered both relative to their original composition and to the other environmentally-exposed samples (Reber and Evershed 2004). Most significantly, molecular relationships supposed to be stable over archaeological timeframes were altered after just six months.

Reber and Evershed suggested that the processes that define archaeological residues may be more complex than 'simulated archaeological time', with relatively large environmentally-induced changes over relatively short timeframes. The study

highlights the problems of underestimating residue complexity through archaeological time simulations. Archaeological time does not alter the composition of artefacts. Rather, artefacts are altered by specific processes that operate over archaeological timeframes (Schiffer 1983). Local environmental variables produce very different processes between sites that cannot be simplified into a universal concept of 'archaeological time'. To achieve a more robust understanding of residue alteration, Reber and Evershed (2004) suggested enhancing archaeological time simulations through addition of soil matrices and other variables designed to more accurately reflect post-depositional conditions.

2.4.3 Lipid interpretation

Three main chemotaxonomic methodologies have been developed to identify the sources of archaeological residues: diagnostic biomarkers, characteristic ratios of molecules, and multivariate mathematical analyses.

2.4.3.1 Biomarkers

Identifying chemotaxonomic marker molecules is the simplest method of characterising archaeological residues. Biomarkers are compounds that reflect taxonomic differences between species, classes, or, more commonly, general differences between plants and animals. To be archaeologically useful potential biomarkers must either: a) survive archaeologically and be unable to form as digenesis products of other molecules (Evershed 1993), or b) be only capable of arising from the digenesis of a diagnostic but archaeological unstable molecule (forming a proxy for the unstable molecule) (Evershed et al. 2008; Hansel et al. 2004).

Biomarker techniques are sometimes capable of producing highly specific source information. Terpenoids from resinous remains are capable of determining both botanical sources of archaeological remains and the processing technologies to which they were exposed (Charrié-Duhaut et al. 2007; Connan and Nissenbaum 2003; Edwards et al. 2007; Eerkens 2002; Evershed et al. 1997; Mudugno et al. 2006; Pyatt et al. 2005; Regert 2004; Shackley 1982; Stacey et al. 2006; Stern et al. 2003; Stern et al. 2008). Evershed and coworkers (1997) have identified archaeological examples of

frankincense terpenoids in amorphous, resinous remains. Eerkens (2002) and Fox and coworkers (1995) identified terpenoids typical of *Piñon* resins, and Edwards and coworkers (2007) identified *Pistacia* resins on Egyptian Sarcophagi. Evidence of the conversion of terpenoid resins to tars through high-temperature heating has also been reported archaeologically (Charters et al. 1993; Dudd and Evershed 1999; Regert 2004; Urem-Kotsou et al. 2002). The molecular composition of bitumen has also been used to identify material sources and trade (Gregg et al. 2007; Harrell and Lewdan 2002; Stern et al. 2008).

O.E. Craig and coworkers (2005) utilised Digestion and Capture Immunoassays (DACIA) to identify bovine $\acute{\alpha}_{s1}$-casein protein, diagnostic of bovine milk, in charred encrustations on ceramics from the Scottish Western Isles. The identification of beeswax from its characteristic combination of long-chain hydrocarbons and palmitic wax esters is well established, and has been identified in a wide range of archaeological ceramics (Charters et al. 1995; Evershed et al. 2003; Evershed et al. 1997; Gariner et al. 2002; Knappett et al. 2005). Evershed (2001) has also suggested that high concentrations of ricinoleic acid could be characteristic of castor oil, but this molecule can arise from the oxidation of oleic acid and has not been suggested to identify archaeological residues to date.

More frequently, biomarkers are capable of producing broader, more generalised chemotaxonomic identifications. Charters and Evershed (1997) identified nonacosane, nonacosan-15-one, and nonacosan-15-ol as indicative of *Brassica*, but were unable to resolve these to individual species. The presence of relatively high volumes of branched and odd-chained fatty acids (C15:0, C15:0br, C16:0br, C17:0, C17:0br, C18:0br) has been cited as indicative of ruminant products (Dudd et al. 1999; Evershed et al. 2002). Fatty acids with multiple branched chains (e.g. 4,8,12-trimethyltetradecanoic and 3,7,11,15 tetramethylhexadecanoic) have been suggested as biomarkers for marine animals (Hansel et al. 2004; Olsson and Isaksson 2008). T. J. Craig (2004) has suggested that certain triacylglycerol species containing odd-chained fatty acids such as MaSS, MaPP and MaPS are also indicative of ruminant products. The sterols are capable of differentiating plant and animal products. Cholesterol is diagnostic of animal residues while other sterols (campesterol, sitosterol, stigmasterol) are indicative of plant products (Evershed et al. 1997; Evershed and Connolly 1994; Kimpe et al. 2004).

A recent adaptation of biomarker chemotaxonomy is the identification of the degradation products of diagnostic but unstable molecules. The most significant of these has been the identification of ω-(*o*-alkylphenyl)alkanoic acids that arise from the degradation of highly unsaturated fatty acids typically found in marine animals (Craig et al. 2007; Evershed et al. 2008; Hansel et al. 2004). Both Hansel and coworkers (2004) and O. E. Craig and coworkers (2007) have used these molecules to identify marine residues. Another potential digenetic biomarker is the presence of large volumes of dicarboxylic fatty acids and lipid hydroperoxides as potential indicators of plant oils but the reliability of these molecules has yet to be tested (Knappett et al. 2005).

Biomarker techniques are limited by their relative scarcity in archaeological remains. While residue biomarkers are capable of producing highly specific identifications of source products, they are poorly suited to the interpretation of the vast bulk of archaeological residues that do not contain recognised biomarker molecules.

2.4.3.2 Ratio analysis

Identifying characteristic relationships between otherwise undiagnostic molecules is sometimes used to elucidate the source products that contribute to archaeological residues. Evaluating the relative proportions of commonly encountered archaeological molecules allows researchers to characterise a much broader range of archaeological remains than is possible using biomarkers. In many instances, the molecules used to construct diagnostic proportions are ubiquitous in modern organic products (Hilditch and Williams 1964). Chemotaxonomic relationships are usually identified in modern reference products and their archaeological stability assessed through alteration simulations (usually boiling or simulated archaeological time). Modern lipid extracts contain many chemotaxonomic features that can differentiate source products, but relatively few of these molecules are observed archaeologically (Evershed et al. 2001). Generally, chemotaxonomic relationships have been restricted to proportional relationships between chemically similar species of fatty acids that should possess a similar liability to alteration in archaeological conditions (Eerkens 2005; Kimpe et al. 2004; Patrick et al. 1985).

Patrick and coworkers (1985) suggested two proportional relationships to interpret an archaeological residue from the South Cape of South Africa: the ratios of C16:0/C18:0 and C18:1ω9/C18:1ω7. The former was suggested as a tool to differentiate plant/animal and the latter marine/terrestrial. The stability of the relationships was tested by both experimentally boiling seal in a replica ceramic pot and exposing residues to simulated archaeological time. The two experiments form the earliest experimental residue simulations. Patrick and coworkers concluded that their archaeological sample was a marine animal residue, most likely Cape Fur Seal. This correlated well with other evidence from the site.

Marchbanks (1989:68) and Skibo (1992) have also proposed proportional chemotaxonomic features suitable for residue interpretation. Marchbanks suggested the relative proportions of (C12:0+C14:0)/(C12:0+C14:0+C18:2+C18:3) expressed as a percentage could discriminate plants (<20%), fish (20%-45%) and animals (>45%). However, Marchbanks did not test the robusticity of his chemotaxonomic proportions through experimental simulations. Skibo (1992) attempted to utilise Marchbanks' ratios, but found them impractical as many modern food products lack C12:0 and C14:0. Later experimental and archaeological research has also indicated that proportional relationships rely on polyunsaturated fatty acid species that are not archaeologically robust (Eerkens 2005; Malainey et al. 1999b). Skibo (1992) proposed the proportions of C18:0/C16:0 and C18:1/C16:0 as diagnostic of plant and animal products in his ethnoarchaeological study of Kalinga cooking pot ware. These chemotaxonomic proportions survived ethnographic cooking events where only one product was cooked, but were unsuitable when more than one product was cooked in the same pot and could not make reliable identifications of archaeological residues (Skibo 1992).

Malainey and coworkers (1999b, 1999c) proposed that the proportions of medium-chain saturated fatty acids (C12:0+C14:0+C15:0) to oleic acid isomers (C18:1) to stearic acid (C18:0) can discriminate a range of west Canadian plant and animal products as well as mixtures of these products. Malainey's chemotaxonomic proportions are based on the experimental cooking of a wide range West Canadian native foods and mixtures and simulated archaeological time (Malainey et al. 1999a; Malainey et al. 1999b). The proportional relationships identified by Malainey and coworkers are detailed in Table 2.2. When applied to late prehistoric ceramic residues

Malainey's proportions appear to differentiate residues into logical classes (Malainey et al. 1999c).

Identification	Medium Chain	C18:0	C18:1 isomers
Large Herbivore	≤ 15%	≥ 27.5%	≤ 15%
Large herbivore with plant OR bone marrow	Low	≥ 25%	15% ≤ X ≤ 25%
Plant with large herbivore	≥ 15%	≥ 25%	no data
Beaver	Low	low	≥ 25%
Fish or corn	Low	≤ 25%	15% ≤ X ≤ 27.5%
Fish or corn with plant	≥ 15%	≤ 25%	15% ≤ X ≤ 27.5%
Plant (except corn)	≥ 10%	≤ 27.5%	≤ 15%

Table 2.2: The diagnostic proportions of fatty acids in found in experimentally cooked and altered west Canadian food products developed by Malainey (1999b).

Eerkens (2005) utilised Malainey's extensive food product and residue data to develop an alternative set of chemotaxonomic proportions. Eerkens suggested that biplots of values for (C15:0+C17:0)/C18:0 to C16:1/C18:1 and C12:0/C14:0 to C16:0/C18:0 could differentiate terrestrial mammals, roots, greens, fish and seeds/berries in both Malainey's foods and experimental residues. When the proportions were applied to archaeological remains, Eerkens found that it was necessary to adjust the C16:1/C18:1 ratio by a factor of 10 to account for a 'shift' in the relationship between C16:1 and C18:1, with the preferential removal of C16:1 but the cause of such a large degree of differential alteration between two very similar molecular species is unclear. Both Malainey's and Eerkens' proportional methodologies are significant in their potential ability to differentiate a relatively broad range of organic residues and the extensive experimental datasets used in their construction.

2.4.3.3 Multivariate analysis

Both biomarker and ratio chemotaxonomic methodologies utilise small subsets of residue molecules for diagnostic purposes. A potential disadvantage of these reductionist methods is that significant additional diagnostic information may be present in the remaining unanalysed molecular data. An alternative approach is to

mathematically transform high-dimensional datasets typically produced by residue analysis to extract the significant structural information present. In mathematical transformations such as Principal Components Analysis, diagnostic relationships between residues and residue molecules are maintained at the same time as reducing the dataset into a manageable number of components (Baxter 1991; Baxter 1992; Craig 2004; Grave et al. 2005; Grave et al. 2008; Malainey et al. 1999c). By examining structural relationships and the molecules that define these relationships diagnostic information can be gleaned.

Both Principal Components Analysis (PCA) (Craig 2004; Malainey et al. 1999c) and Hierarchical Cluster Analysis (HCA) (Malainey et al. 1999c; Marchbanks 1989:81) have been used to analyse experimental and archaeological residues. PCA transformations usually account for most of the variability in high-dimensional datasets in their first few components. This allows a readily interpretable three dimensional graphical representation of the most structured features and groups present in the data (Craig 2004; Grave et al. 2008). Samples that cluster together will be structurally similar to each other and, conversely, structurally different samples will be spatially separated. HCA transforms datasets into a two dimensional dendrogram, separating groups and subgroups of samples based on their structural similarities and differences (Malainey et al. 1999c). However, the relationship between samples and component molecules is not evident and HCA results are highly dependant on the choice of algorithm (e.g. Ward's method, average linkage).

Marchbanks (1989:81) used HCA as a method of differentiating organic products and experimentally produced residues, finding that HCA was capable differentiating groups of residues based on their molecular similarities and differences. Malainey applied HCA to archaeological datasets, concluding that structural differences in residues related to different biological residue sources (Malainey et al. 1999c).

Principal Components Analysis has been used less frequently in residue research, but where applied the results have been encouraging. Malainey explored PCA finding that it could logically differentiate both modern food products and their experimental derivatives (Malainey et al. 1999a; Malainey et al. 1999b). When applied to archaeological samples, interpretations were less clear but structural differences in the data were apparent (Malainey et al. 1999c). As part of an earlier research project I also explored PCA as a diagnostic tool for archaeological residues

(Craig 2004). These results indicated that PCA clearly differentiated modern food products as well as their experimental derivatives. When applied to archaeological samples, PCA appeared suited to the differentiation of plant, ruminant animal and non-ruminant animal residues.

Multivariate analyses of residues produce groups of related samples, but do not assign these to biological or botanical sources. Residue sources can be diagnosed by examining the molecular species (variables) that define structural groups (e.g. a structural group largely defined by odd- and branched-chain fatty acids is likely to be derived from ruminants) (Craig 2004:51-6). In this way, multivariate residue analyses resemble, or are partially dependent on, biomarker techniques. However, by first producing structurally significant residue groups multivariate techniques are capable of characterising a larger proportion of ambiguous residue datasets than is possible with biomarker analyses.

2.5 Archaeological Taphonomy

Modern molecular analyses of archaeological ceramic residues have largely developed in parallel to molecular geology (Evershed 1993). During the 1980s, geologists became aware that organic molecules could be preserved in a wide range of fossil material (Eglinton et al. 1991; Golenberg et al. 1990; Goth et al. 1988; Mills et al. 1984). This produced a substantial body of research that explored the factors that define the preservation of organic molecules over geological timescales (i.e. their molecular taphonomy) (Eglinton et al. 1991). Researchers interested in archaeological molecules have based many aspects of their research on these geologically-based studies (Evershed 1993; Hedges and Sykes 1992). Among these, the most important is the geologic observation that highly compacted, stable sediments are more suited to organic preservation than less structured sediments (Eglinton et al. 1991). Compacted sediments limit environmental impacts on organic molecules, most importantly the consumption and alteration of molecules through the actions of microbes (Eglinton et al. 1991).

Archaeological residue researchers have accepted these geological taphonomic features as suited to the analysis of ceramic residues. The fabric of earthenware ceramics has similar properties to those likely to preserve geological organic

molecules (i.e. a stable, relatively compact matrix). These features, along with the apparent preservation of ancient molecules within ceramic matrices, has led to the widespread acceptance that archaeological ceramic fabrics effectively insulate archaeological molecules from most environmental affects, forming the basis for chemotaxonomic analyses. This assumption, coupled with a limited range of experimental simulations (Charters and Evershed 1997; Craig 2004; Eerkens 2007; Evershed et al. 2008; Evershed et al. 2002; Heron et al. 1991; Malainey et al. 1999a; Malainey et al. 1999b; Patrick et al. 1985; Reber and Evershed 2004; Stern et al. 2000), may have led to an underestimation of the complex permutations involved in taphonomic/ceramic/residue interactions. This can at least partly be understood as a consequence of the lack of clear theoretical structures to account for how archaeological residues are created through human behaviour and altered in archaeological contexts. This study applies a systematic archaeologically-based taphonomic approach to begin modelling these processes in relation to ceramic residues. In this section I detail the taphonomic framework that I adapt in Chapter 3 to systematise consideration of the molecular taphonomy of archaeological residues. First, I detail Schiffer's (1972) cultural taphonomic flow model. I discuss post-depositional taphonomic processes, how these have been dealt with in previous residue studies, and the potentially significant taphonomic factors that have not been explored in previous research.

2.5.1 Cultural taphonomy

Before archaeological remains of any kind can be interpreted, the *formation processes* responsible for their emplacement and preservation must be understood. Without this understanding, it is impossible to conclude whether archaeological remains reflect aspects of the human society under study or the work of later cultural and natural phenomena (Schiffer 1995). Archaeological taphonomic frameworks are explicitly designed to identify and evaluate these formation processes, and to enable a systematic separation of cultural and post-depositional contributions to the archaeological record (Child 1995; Dennell 2005; Schiffer 1995; Wilson and Pollard 2002).

The spatial and temporal interactions between formation processes make the identification of neat conceptual categories of taphonomic process a “stubbornly difficult” task (Schiffer 1972), but this is required to construct conceptually useful models. One of the more practical solutions is to divide the formation process into those which occur within a cultural system, and those that occur once an item becomes part of the archaeological record. Schiffer (1972) uses this approach to define a generalised model of *systemic context*, referring to the formation processes that artefacts are exposed to within a cultural system. This conceptual framework forms the basis for the exploration of the cultural taphonomy of residues used in this study.

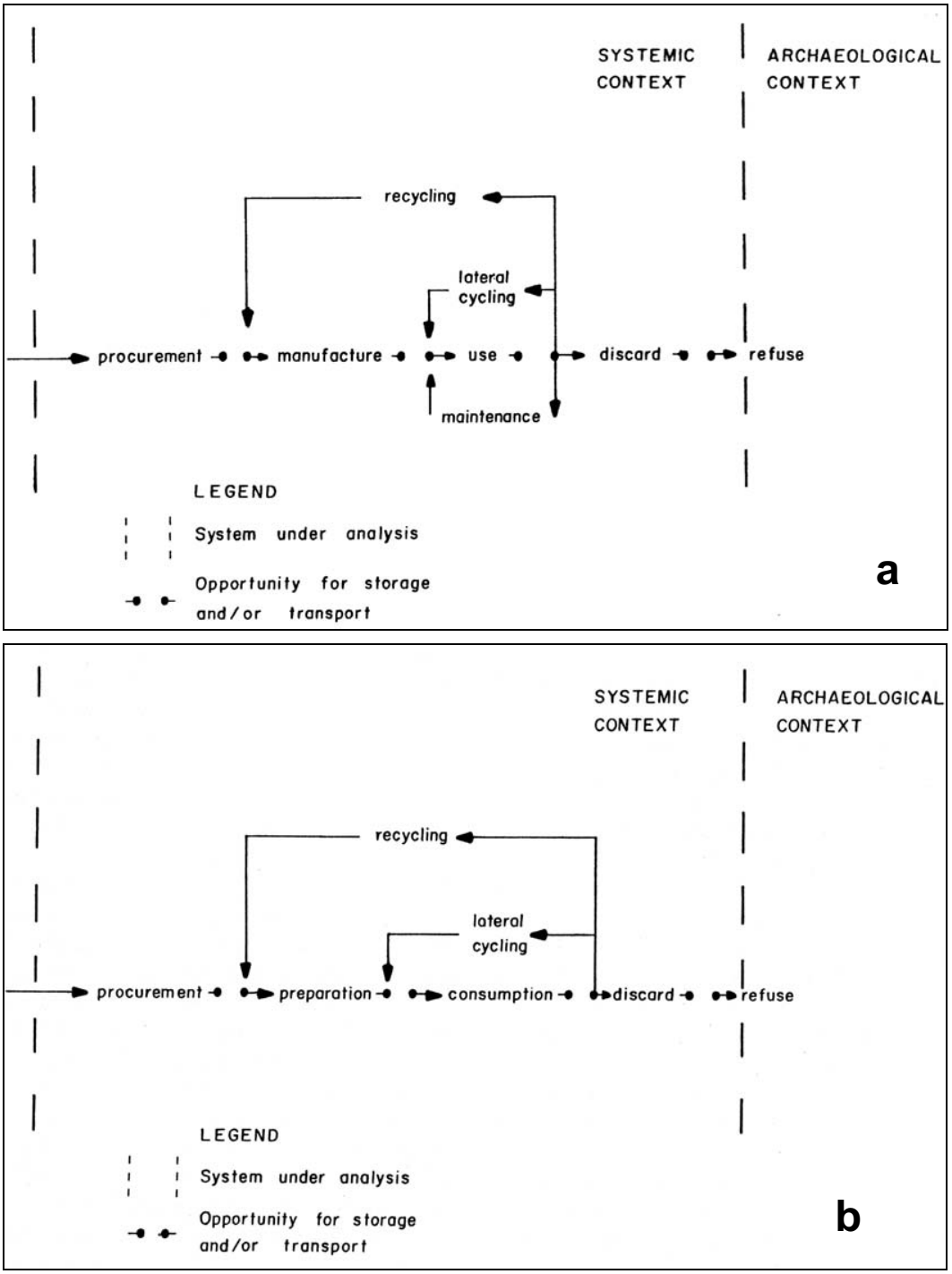

Figure 2.8: a) Schiffer's cultural flow model for durable products showing the movement of materials through cultural contexts and the activities that may affect these materials; b) Schiffer's cultural flow model for consumable products showing the movement of materials through cultural contexts and the activities that may affect these materials (Schiffer 1972).

The systemic context describes a cultural system as a structured series of *activities* that use energy to transform *elements* into different forms. Elements are either *durables* or *consumables* (Schiffer 1972). Durables are transformers and preservers of energy, such as tools, and consumables are elements that liberate energy on consumption, such as food and fuels. The systemic context of an element within a cultural system is its position relative to other elements and activities and its eventual transition to the archaeological record.

Schiffer (1972) provides a generalised map of the systemic context composed of five key *processes* (Figure 2.8a, 2.8b). For durable products, these processes are *procurement, manufacture, use, maintenance,* and *discard*. Processes for consumables are broadly equivalent, but with varied terminology to more accurately reflect the nature of consumable elements (*procurement, preparation, consumption,* and *discard*). Consumable elements are not generally maintained; therefore this process is removed from the systemic model for consumables. In addition to these five processes, Schiffer (1972) defines *storage* and *transport* as events that occur between processes, spatially and temporally displacing elements.

Elements may not flow in a straight-line chain through a cultural system, but may follow several paths before entering the archaeological context. Schiffer defines two forms of reuse, *recycling* and *lateral cycling.* Recycling is defined as the routing of an element at the completion of use to the manufacture of another element, such as the conversion of coins into jewellery or new coins. *Lateral cycling* describes the termination of an element's use in one set of activities, and its resumption in another, with only storage, transport and maintenance between activities (Schiffer 1972).

As an artefact leaves the systemic context it enters the *archaeological context* as *refuse*. While the flow-model shows artefacts entering the archaeological context through *discard* after use, artefacts may move to the archaeological context at or between any processes in the systemic context.

Schiffer (1972) concedes that the models are "only a simplification of a stubbornly complex reality" and that they are "not likely to fit neatly the sequences of activities in which elements of all cultural systems participate within their systemic contexts". However, the models provide a starting point for more detailed consideration of cultural formation processes that affect specific classes of artefacts and sites. Constructing models of systemic contexts that account for the peculiarities of individual classes of cultural remains provides a framework for the identification

and evaluation of how these cultural processes contribute to the formation of the archaeological record.

2.5.2 Post-depositional taphonomy

After leaving the systemic context, cultural material enters the archaeological context. Post-depositional taphonomic processes are responsible for the destruction, preservation and alteration of remains in archaeological contexts. Conceptual frameworks for post-depositional artefact alterations differ significantly to cultural alterations. Artefacts in systemic contexts move through a logical sequence of events from procurement to discard, with different processes affecting artefacts through the sequence (Schiffer 1972). In contrast, artefacts in archaeological contexts are exposed to numerous taphonomic processes over periods of centuries or millennia. The types of processes that affect organic remains (including residues) are broadly similar regardless of exact composition of the artefact. These can be organised within three broad categories of taphonomic process (biological, chemical and physical).

2.5.2.1 Biological processes

Biological processes are caused by plants and animals, such as the displacement of architecture by tree roots (Nawrocki 1991) or the consumption of organic remains by animals (Chase et al. 1994; L'Abbé 2005) or microbacteria (Child 1995; Eglinton et al. 1991; Jans et al. 2004). Microbial alteration is considered the principal taphonomic effect that influences the survival and composition of archaeological survival of organic remains (Hedges and Sykes 1992). While the effects of microbial action are usually considered to be controlled by removing the outer 1-2mm of a ceramic surface prior to extraction (Heron et al. 1991; Stern et al. 2000), the scale of microbial affects on other organic remains suggests that a wider program of analysis is needed to determine the extent and effects of microbial processes.

2.5.2.2 Chemical processes

Chemical processes affect the composition and survivability of archaeological remains through chemical reactions with the molecules that comprise these remains. Chemical processes include the oxidization of metals (Wilson and Pollard 2002), the dissolution of bone in acidic conditions (Mays 1998:17), and the preservation of organic remains in waterlogged and acidic conditions (Coles and Lawson 1987; Evershed 1990; Evershed and Connolly 1994). For archaeological residues, the most widely considered chemical process has been the oxidation and autoxidation of lipid products through simulated 'archaeological time' (Malainey et al. 1999b; Patrick et al. 1985). Other oxidative effects have also been observed archaeologically including short-chain dicarboxylic hydroxyl fatty acids (Knappett et al. 2005).

The effects of environmental pH conditions are perhaps the most significant unexplored chemical process relevant to residue archaeology. The pH conditions of a depositional context can alter the survivability of organic remains both on the macroscopic and molecular level. Whether these taphonomic biases also extend to molecules within ceramic fabrics is unclear.

2.5.2.3 Physical processes

Physical processes are non-chemical, non-biogenic processes, such as wind erosion, sediment compaction (Andrews 2006), leaching (Evershed 1993) and displacement of artefacts (e.g. transportation by rivers) (Bollong 1994; Coard 1999; Dennell 2005). Physical processes are also in part the conditions in which other taphonomic processes operate (e.g. temperature, aerobic/anaerobic environment) (Evershed 1993). Temperature-related processes significantly enhance the survivability of organic remains through freezing (Hart Hansen et al. 1991; Rudenko 1970). These effects are sometimes observed in archaeological residues with unusually high degrees of molecular preservation in residues from artefacts in cold climates (Craig et al. 2007; Craig et al. 2005; Morgan et al. 1992; Morgan et al. 1984). Evershed (1993) has suggested that the movement of groundwater through archaeological contexts (leaching) may explain the general absence of short-chain fatty acids in most archaeological ceramics. This process is likely to be the most

significant physical process affecting ceramic residues. However, the precise effects of groundwater leaching have yet to be systematically assessed and should form an important research focus.

2.6 Summary

In this chapter I presented a review of previous research relevant to the three major areas of this study. First I described the chemical and physical properties of lipids both to provide a context for discussing previous archaeological residue research and to highlighting the importance of oxidation and autoxidation for archaeological residues and the potential effects of colloidal lipid properties on the rate of lipid alteration. I considered previous archaeological lipid analyses by: a) discussing the objectives and methodological foundations of the research area, and b) describing the instrumentation and interpretive techniques typically used by residue researchers. Next, I discussed the limitations of conventional interpretive techniques for identifying ancient sources of archaeological residue lipids. Finally, I presented a description of archaeological taphonomic frameworks and discussed the potential advantages of using systematic approaches to understanding the complex formation processes of archaeological residues.

Chapter 3 - Analytic Rationale

3.1 Introduction

In this chapter I present a model for residue taphonomy that combines Schiffer's *systemic context* with key post-depositional formation processes and chemical lipid properties. Its purpose is a) to summarise the key cultural, post-depositional and chemical variables involved in residue formation, and b) to highlight the wide range of permutations possible between these variables. It is designed to guide a systematic and comprehensive exploration of archaeological residue formation. Out of this model a series of queries are generated that cover the three main areas of investigation (cultural, post-depositional and chemical/mechanical). These are used to define the experiments described in Chapter 4.

3.2 The Taphonomic Residue Framework

The taphonomic residue framework combines an adaptation of Schiffer's (1972) general model of systemic context with key post-depositional formation processes. I first present a flow model adapted from Schiffer's systemic context to explore cultural residue formation pathways. Through this systemic flow model I identify the most critical cultural processes likely to contribute to residue formation.

Next, based on the review in Chapter 2, I identify the most critical natural processes likely to affect residues on "entering archaeological contexts" (Schiffer 1972). Finally, I consider how molecular properties of different residues may affect the rate and nature of their taphonomic transformation.

3.2.1 The cultural residue taphonomic model

Schiffer's (1972) systemic context flow model describes the intentional conversion of raw materials to useful products. His model ends at the point where cultural material enters the archaeological record. A principal difference between many archaeological products and residues is that ceramic residues, with few exceptions (i.e. linings and sealants), have to be treated as unintentional by-products

of the storage or preparation of organic products in ceramics. Therefore, Schiffer's flow model cannot be directly applied to residues but requires modification (i.e. addition of residue specific processes; redefinition of key terms such as storage to reflect an active process role).

The cultural (systemic) context of residues is interwoven with the systemic context of host ceramics. Ceramics (and their bound residues) usually remain in systemic contexts for extended periods of time until entry into the archaeological context. To represent this relationship the general model of systemic context must be reformulated as a synthetic residue/ceramic systemic context (Figure 3.1).

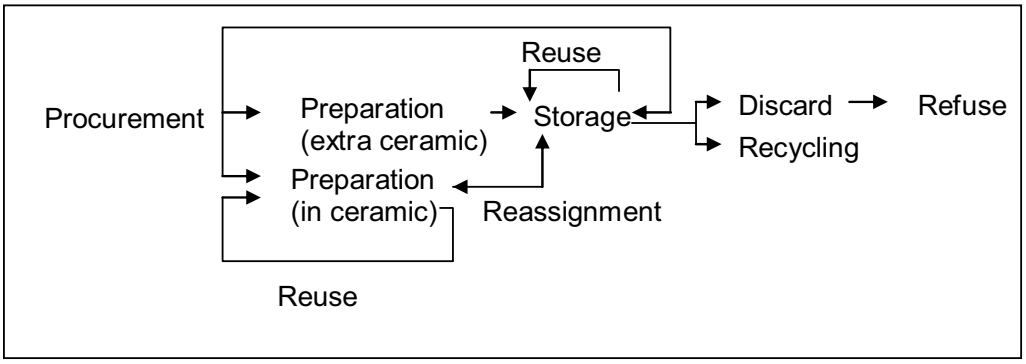

Figure 3.1: Hybrid ceramic/residue flow model showing the movement of residues through cultural systems and the processes that influence their formation.

The systemic context of archaeological residue formation differs from Schiffer's general model in several important ways. 'Use' in Schiffer's flow-model is highly generalised. An artefact in a systemic context is used after manufacture and only leaves that process temporarily through maintenance, lateral cycling, transport or storage, or permanently through discard and recycling. The model does not indicate different types of use a cultural element may be exposed to, the frequency or intensity of use, or the temporal period of use. It is impossible to represent these aspects of the systemic context in a generalised model encompassing a wide range of artefact types and cultural contexts. However, it is necessary to consider these factors when examining a specific artefact class such as ceramic residues. In the residue/ceramic hybrid systemic context all use processes refer the deliberately-used ceramic, not the incidental residue by-products. The model of residue/ceramic systemic context expands Schiffer's 'use' process in two ways. First, the types of 'use' that ceramics are exposed to are expanded to capture their two key functions: preparation and

storage. The archaeological residue flow model contains two new use processes, *reuse* and *reassignment*. *Reuse* describes the repetition of a sequence of events designed to produce similar products, such as repeated boiling of foods in a cooking pot. *Reassignment* describes a change in utilisation, such as a change in preparation function of a ceramic (e.g. conversion from boiling to microbial processing such as brewing and curdling or a change from preparation to storage process). Conversely, a number of terms in Schiffer's generalised model have been removed as irrelevant for the modified model (i.e. lateral cycling and transport).

Procurement of raw materials forms the first activity in the systemic residue/ceramic flow-model. After procurement, three processes may occur that are relevant to residue formation. Raw materials may be *prepared* or *stored* in a ceramic (*in ceramic*), producing a residue, or prepared without ceramics (*extra ceramic*), producing no residue. After preparation, transformed products may be consumed directly, transferred to a non-ceramic storage medium, or *stored* in another ceramic vessel. *Storage* may vary in duration (e.g. long-term storage of oils in amphorae; serving foodstuffs in ceramics immediately prior to consumption). In the modified model *storage* does not distinguish between long or short term duration but rather reflects the *state of interaction between organic products and ceramic fabrics without additional transformation processes* (work).

Ceramics may be *reused* through both preparation and storage processes. This affects residue composition in two principal ways: through the penetration of new organic material into the ceramic matrix, and through exposure of pre-existing residues to additional transformation processes (work). *Reassignment* may also occur, altering the type of work pre-existing residues are exposed to and the types of interactions between pre-existing residues and later organic materials.

As residues are not intentionally produced or used artefacts, they cannot be recycled into new products. However, the ceramics in which residues are contained may be recycled. Recycling ceramics either destroys residues (e.g. crushing old ceramics as temper for new ceramics), or preserves them (e.g. ceramics incorporated as architectural decoration or in construction fill). The recycling process removes residues from the systemic context. Recycling closely resembles the discard process, and therefore occupies a similar space within the flow model.

3.2.2 Residue types

The residue/ceramic systemic context involves three principal residue formation pathways: *pristine*, *reused* and *reassigned*. *Pristine residues* result from a 'straight-through' flow of residues and ceramics through the model. Organic products are procured, prepared within or without ceramics, stored or consumed, and the ceramic discarded after a single use. Residues may result from either preparation or storage but each stage contains a single event where residues are formed from the interaction between an organic source and a single cultural process. Pristine residues can be expressed symbolically as $R = S \; x \; E$ where R is the residue, S is the organic source and E is the processing/storage exposure. Most experimental simulations of cultural processes have produced pristine residues.

Reuse - the repetitive use of ceramics to perform an identical or similar task (e.g. boiling of foodstuffs in cooking pots or refilling storage wares) - results in a residue that on entry into the archaeological context is a composite of all systemic use-events. Two interacting factors define the formation processes during reuse: interactions between the pre-existing residue and new organic materials, and exposure of pre-existing residues to additional work. This can be expressed as $R = [S_1 \; x \; nE] + [S_2 \; x \; (n\text{-}1)E] + [S_3 \; x \; (n\text{-}2)E] + [S_n \; x \; E]$ where n is the number of use-events.

Reassignment - when the mode of ceramic use changes (e.g. conversion of a cooking pot to storage or a change in cooking mode from boiling to dry-roasting) - is likely to produce the most complex and ambiguous residues as it comprises of one or more organic sources exposed to multiple technological processes. Even in a simple example of reuse a vessel used twice in two technological processes is expressible as $R = [S_1 \; x \; (2E_1 + 2E_2)] + [S_2 \; x \; (E_1 + 2E_2)] + [S_3 \; x \; 2E_2] + [S_4 \; x \; E_2]$ where E_1 is the first type of work and E_2 is the second.

Symbolic reduction of complex processes provides a conceptual means to express key factors and relationships that produce different types of residues. However, other factors can variably impact residue formation in the systemic context and exponentially increase the level of modelling complexity. These can best be considered as a series of propositions:

1. Values for E (work) will not be equal between use events. E-values will vary by exposure time, temperature, atmospheric and microbial conditions.
2. Different modes of work will produce different residues and E-values.

3. Organic products have different liabilities to alteration and residue formation based on their molecular composition and volume.
4. Pre-existing residues will not respond linearly to additional work; as molecules are altered so is their response to work.
5. Pre-existing residues alter the ability of additional residues to be absorbed into the ceramic fabric.
6. Different sites on a ceramic vessel may have different liabilities to residue uptake depending on the cultural processes that produce residues.
7. The porosity of a ceramic will influence the uptake and composition of residues.

In the following sections I outline a range of post-depositional taphonomic and chemical issues that, while critical for this study, remain either wholly or largely undeveloped in the archaeological residue literature.

3.2.3 Post-depositional processes

In Chapter 2 I outlined post-depositional taphonomy in three classes of processes: biological, chemical and physical. This section identifies the key processes likely to affect ceramic residues in archaeological contexts within these three categories. These identifications form the basis for the post-depositional experimental component of this study.

3.2.3.1 Biological processes

The key biological processes relevant to residue research are the effects of microbes. Microbial action is treated as a contaminant that researchers attempt to remove (Heron et al. 1991). How microbes affect archaeological residues has yet to be systematically assessed. The scale of microbial alterations in other classes of organic remain indicate that a more systematic understanding of microbe/residue interactions is required.

3.2.3.2 Chemical processes

Chemical interactions between residues and the environment have received some consideration by residue researchers. The main focus has been the simulation of archaeological time through accelerated oxidative and autoxidative interactions between residue lipids and the environment (Malainey et al. 1999b; Patrick et al. 1985). This type of simulation captures only one aspect of post-depositional chemical taphonomy. A chemical process that may significantly influence residues composition is the exposure different soil pHs. This is especially important when comparing residues from different contexts within a site, between sites and time periods. All archaeological residues have been recovered from contexts that possess specific pH characteristics, either alkaline or acidic. The effect of environmental pH on residue molecules and whether pH interactions are significant for archaeological residue formation remains to be systematically studied.

3.2.3.3 Physical processes

Evershed (1993) has suggested that the physical process of groundwater percolation can explain the general absence of short-chain fatty acids in archaeological residues. Short-chain fatty acids are less hydrophobic, therefore more likely to be mobilised by groundwater passing through archaeological contexts. This hypothesis has not yet been systematically tested, but may substantially impact on reliability of chemotaxonomic techniques that use the proportions of shorter-chain fatty acids for diagnosis.

3.2.4 Physical residue properties

In Chapter 2 I detailed the physical and chemical properties of lipids, discussing two forms of molecular interactions that are key to understanding physical residue properties: acylglycerols and colloidal lipid crystals.

Colloidal lipid chemistry directly affects the liability of molecules to alteration. It is a complex field that remains only partly understood despite concerted efforts by a number of researchers. Colloidal effects in archaeological residues have not been proven or even reported in previous research. In this study I focus on

identification of the possible effects of colloidal interactions rather than attempting to account for the complexities of colloidal lipid chemistry.

The role of acylglycerols in residue taphonomy is also only partly understood. Yet acylglycerols are the primary source of archaeological fatty acids and are regularly observed in archaeological residues. This has not translated into an attempt to understand how they affect residue formation. Understanding the influence of acylglycerols has been complicated by a lack of instrumentation suited to the detailed analysis of triacylglycerols and the inherent complexity of identifying chemical effects that relate specifically to the organisation of fatty acids into acylglycerols.

3.2.5 Issues

In the preceding sections I detailed key processes and interactions likely to be involved in archaeological residue formation. At this stage, broad considerations relevant to the three main areas of taphonomic investigation (cultural, post-depositional, and chemical/mechanical) can be posited as a series of queries. These will define the experimental program as detailed in Chapter 4.

Cultural:

1. How do different E-values (work) affect the composition of residues? I address three interrelated aspects of residue taphonomy in this question: Different *types*, *intensities*, and *durations* of work.
2. How are reused residues composed? This question considers the cumulative effects and interactions of repeated use of a ceramic vessel on residue formation.
3. To what extent does the site of residue penetration on a ceramic vessel affect residue composition?

Post Depositional:

1. How does groundwater percolation affect residue composition?
2. What effect does environmental pH have on residue composition?
3. To what extent does microbial action affect residue composition?
4. How are residues altered by long term storage?

Chemical/Mechanical:

1. How do the colloidal properties of different lipid species affect the interactions between residues and taphonomic processes?
2. How does the organisation of fatty acids into acylglycerols affect their interactions with taphonomic processes?
3. What role does the ceramic fabric play in residue formation?

Archaeological Application:

1. Can taphonomic processes be observed in archaeological residues and do these observations assist in the interpretation of the archaeological record?

3.3 Summary

This chapter presented a rationale for developing a model of residue taphonomy. This model combined a hybrid ceramic/residue adaptation of Schiffer's general systemic context model with key post-depositional formation processes, summaring the key cultural and post-depositional variables into a single framework. From the hybrid ceramic/residue model of systemic context three key pathways of cultural residue formation were identified: *pristine, reused* and *reassigned* residues, enabling a more systematic conceptualisation of the cultural processes that define archaeological residues. The cultural and post-depositional formation processes identified highlight the complex range of interactions that can occur form archaeological residues. I then extended this complexity by highlighting the potential chemical and physical properties of lipid molecules likely to condition these interactions. The systematic conceptualisation of cultural, post-depositional and chemical variables raised a series of key taphonomic issues as a series of queries that define the controlled experiments developed in Chapter 4.

Chapter 4 - Experimental Methodology and the Archaeological Sample

4.1 Introduction

This chapter is divided into three sections (analytical methods, experimental design and archaeological sample). In the first section I present the extraction procedures, instrumentation and data processing protocols adopted for this study. As these techniques are well established in the literature (High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) and Gas Chromatography – Mass Spectrometry (GC-MS)) the majority of this section concentrates on validation procedures for the measurement and extraction protocols used.

In the second section I describe the experimental simulations conducted in this study (i.e. heating, boiling, vessel reuse, groundwater percolation, pH, microbial exposure, alteration over time) and how these relate to the key issues identified in the previous chapter.

In the third section I present the archaeological sample assembled for this study. These reflect a range of vessel types (i.e. cooking pots, storage jars, and "precious oil" containers) from widely separated archaeological sites in Central and Western Turkey. The selection is designed to represent a broad range of archaeological residues from a wide range of depositional contexts.

4.2 Analytic Methods

4.2.1 Lipid extraction and preparation

Residue extraction follows a standard procedure of ultrasonicating powdered ceramic in a mixture of methanol and chloroform (Ways and Hanahan 1964). After ultrasonication, the mixture was centrifuged, supernatant decanted and the process repeated once. For a complete description of extraction procedures see appendix 1a.

Extracts and residues were first analysed with HPLC-MS then derivatised to Fatty Acid Methyl Esters (FAMEs) for GC-MS analysis. The process involves heating

50mg (or total available volume if <50mg) of material in a sealed vial in a mixture of methanolic hydrochloric acid and anhydrous ether. After cooling, the reaction was stopped by adding water and FAMEs extracted by washing with hexane. For a complete description of extraction procedures see appendix 1b.

4.2.2 Mass spectrometry

Mass Spectrometry was conducted on a Varian 1200L Quadrupole Mass Spectrometer. HPLC-MS was used to examine non-volatile di- and triacylglycerols. For HPLC-MS analysis, an Atmospheric Pressure Chemical Ionisation module (APCI) was used. Samples were mixed to 5mg/1ml in Dichloromethane and 10µl injected using a Varian Prostar 430 autosampler and were chromatographed on two in series Waters C-18A columns (150mm x 3.9mm i.d., 4µm packing), fitted with a 4µm pre-column guard. A solvent gradient of Acetonitrile (MeCN) and Dichloromethane (DCM) used as a mobile phase. All solvents were HPLC grade, periodically degassed manually and automatically degassed before introduction to solvent pumps. For complete operating conditions see appendix 1c. Figure 4.1 shows an example of the separation typically achieved using technique.

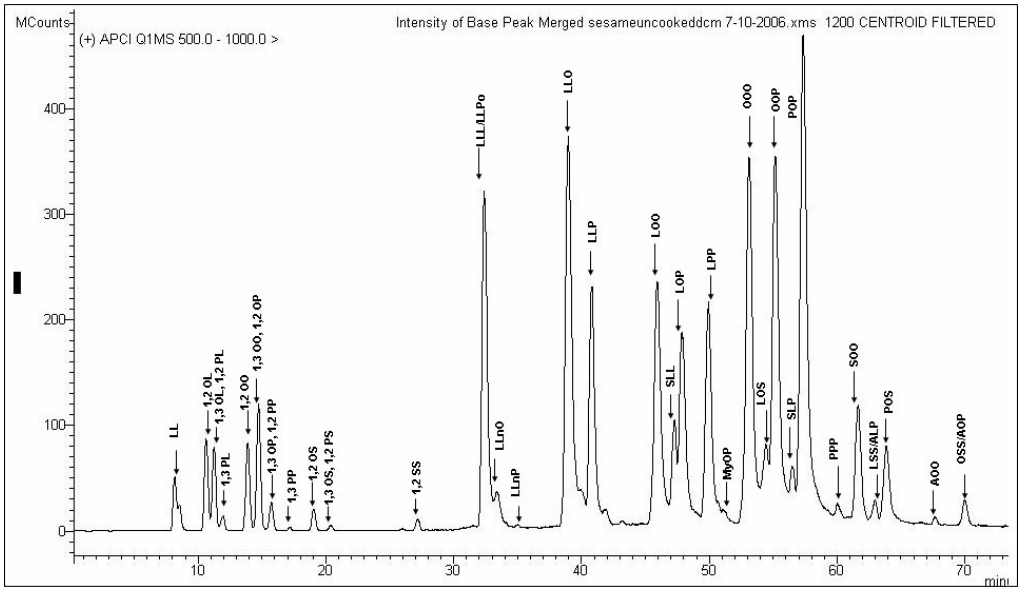

Figure 4.1: High Performance Liquid Chromatograph of unaltered sesame oil with major di- and triacylglycerol peaks labelled.

Gas Chromatography was primarily used to analyse fatty acid methyl esters (FAMEs). A range of other volatile molecules were also observed during these analyses (e.g. hydrocarbons, alcohols, terpenoids, sterols). Gas Chromatography was conducted using a Varian CP 3800 Gas Chromatograph fitted with a VF-5ms Varian Factor Four Capillary Column (30m x 0.25mm i.d.). Samples mixed to 5mg/1ml in hexane and were manually injected using a 1µl glass syringe. Molecules were ionised using Electron Ionisation (EI), with helium as the carrier gas. For complete operating conditions see appendix 1d. Figure 4.2 shows an example of the separation typically achieved using technique.

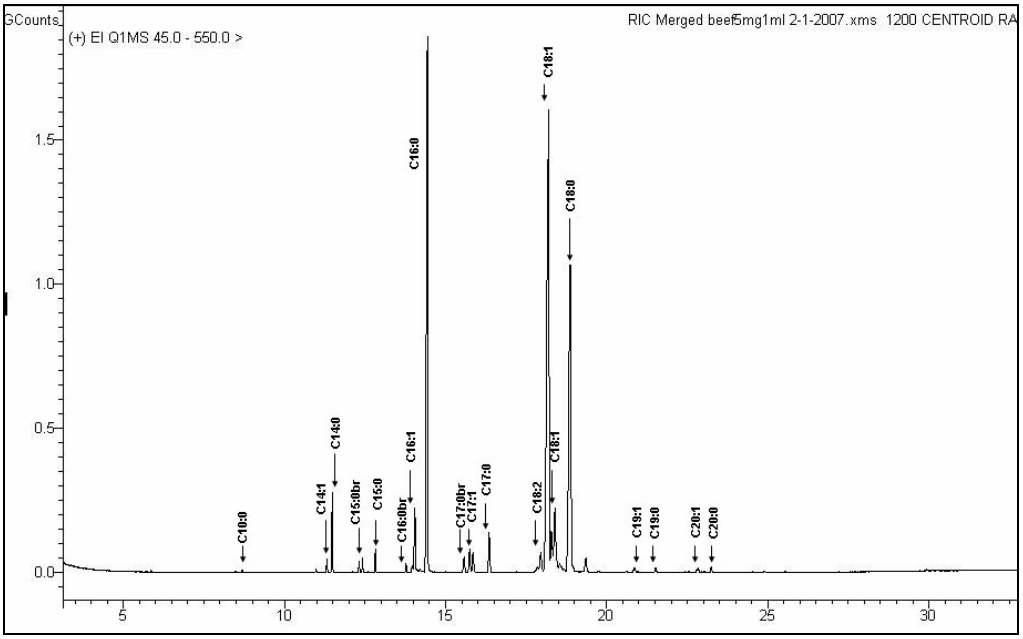

Figure 4.2: Gas Chromatograph of unaltered beef Fatty Acid Methyl Esters with major peaks labelled.

4.2.3 Identification and quantification

Mass spectra are interpreted and quantified using MS Data Review 6.41 (Varian 2004) and NIST MS Search 2.0d (NIST 2005). Acylglycerols were identified by their relative retention time, diagnostic molecular and diacylglycerol ions (Mottram and Evershed 1996). HPLC results were quantified by calculating the sum of the integrated area of the molecular ion $[M+H]^+$, the molecule plus an acetonitrile ion $[M+41]^+$, and the one to three relevant diacylglycerol ions $[M+H\text{-}HO_2CR]^+$ from extracted ion chromatograms. Figure 4.3 shows the $[M+41]^+$ ion (922.9), the

molecular ion (881.9), the two relevant diacylglycerol ions (599.5, 601.5) and the integrated area of their sum for OLL. These values are expressed as a percentage of all relevant molecules within the chromatogram.

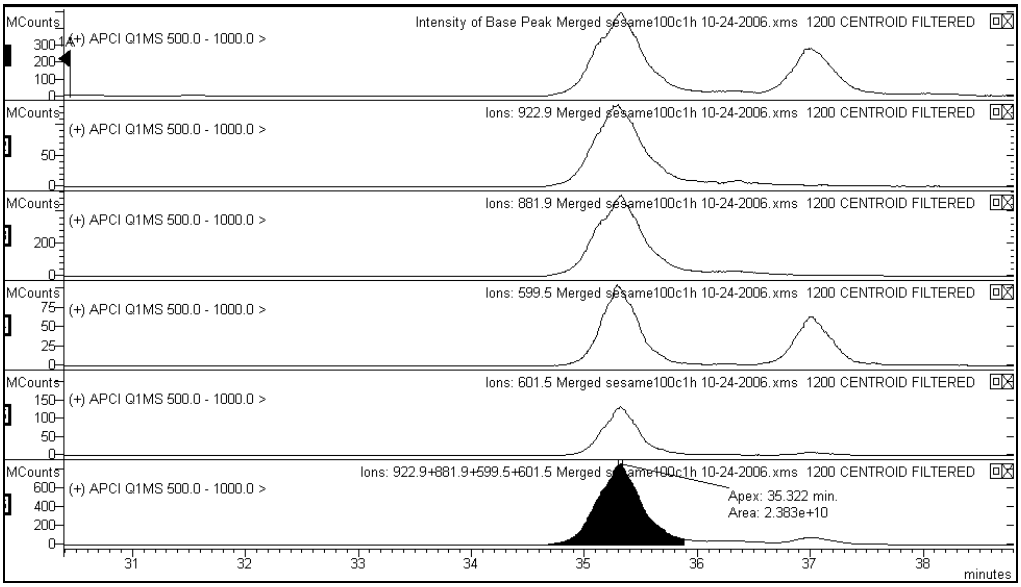

Figure 4.3: Partial HPLC MS chromatogram showing the quantification of OLL by the sum of its molecule plus an acetonitrile ion (922.9), its molecular ion (881.9) two relevant diacylglycerol ions, OL (601.5) and LL (599.5).

Molecular species separated by GC were identified using a combination of relative retention time and identifications using the NIST MS Search 2.0d reference database (NIST 2005). Gas Chromatography results were quantified by calculating the integrated area of all ions within a given peak.

4.2.4 Benchmarking and background correction

The instrumentation used in this study is applied to a wide range of organic molecules. The linear relationship between analyte concentration and detector response is well established (Jennison and Jennison n.d.; Schmidt 2004; Yang 2003) and confirmed in this study through a series of certified lipid standards. For full details of certified lipid standard results see appendix 1e. Certified lipid standards were also periodically run to verify instrument stability over time.

Potential laboratory contamination was controlled through the use of analytic blanks throughout all extraction and derivatisation procedures. These revealed that while very low levels of lipid contamination can occur during extraction these do not

interfere with the reliability of results. For examples of lipid contamination see appendix 1f.

4.3 Experimental Methods

4.3.1 Experimental approach

In Chapter 3 I detailed the formation processes that define archaeological residues through theoretical modelling, observational information from previous archaeological analyses, the untested hypotheses of other researchers, and observations of the chemical properties of lipid molecules. From this I highlighted a series of key issues in residue taphonomy. These queries form the basis for experimental designs presented in this section. The results of these experiments are used to develop expectations for the interpretation of archaeological ceramic residues analysed in this study.

4.3.2 Experimental datasets

For the experiments I use a suite of commercial fats and oils (RFOs: beef fat, linseed oil, sesame oil and fish oil). While most archaeological residues are likely to derive from whole organic products rather than pure fats and oils, analysing pure fats and oils has the advantage of simplifying the interpretation of experimental simulations. As detailed in Chapter 2, residue analyses focus on lipids as these molecules are most frequently preserved in archaeological ceramics. Understanding the taphonomy of archaeological residues requires an understanding of how specific taphonomic processes affect lipid molecules. Experimental simulations using pure fats and oils allow direct examination of the effects of taphonomic processes on lipid molecules, removing potential interpretive ambiguities that more molecularly diverse whole products may introduce.

RFOs were selected to represent a broad range of fatty acid and acylglycerol species found in plant and animal products. Linseed oil was selected for its high proportion of linolenic acid (C18:3), sesame for high concentrations of linolenic and oleic acids (C18:2, C18:1), beef tallow for high levels of saturated (C16:0, C18:0),

odd- and branched-chain fatty acids (C15:0, C17:0, C15:0br, C16:0br, C17:0br, C18:0br) and fish oil for higher polyunsaturates (C18:4, C20:4, C20:5, C22:6). The differences between reference samples allows the assessment of a wide range of lipid chemical behaviours.

A second dataset consists of a series of experimental residues produced as part of an earlier study in 2004. Samples were prepared by boiling a range of food products (beef, sheep, pork, freshwater fish (rainbow trout), chicken, duck, linseed oil, sesame oil, olive oil, peas, chickpeas, barley, and wheat) in ceramic vessels and extracting resulting residues. These ceramics were stored in sealed plastic bags in controlled conditions for three years prior to reanalysis. For full experimental conditions see appendix 1g.

4.3.3 Direct heating

The heating experiments are designed to address queries on the effects of E-values (work) on the composition of residues (i.e. different *types, intensities* and *durations* of work. The direct heating experiment explores intensity and duration variables by incrementally heating the RFOs (100°C, 200°C, 300°C) in a thermostat controlled oven (H. B. Selby). Each temperature level is maintained for five hours with samples collected every hour. Temperatures are selected to represent the range of heats that organic products are likely to have been exposed to in antiquity. Three hundred degrees Celsius represents the upper temperature limit to which food residues can be realistically heated (i.e. the 'smoking point' of many lipid products). Modern fry oils are usually heated to 140-200°C (Kochhar and Gertz 2004; Li et al. 2005; Velasco et al. 2004). No 300°C samples were collected for fish oil at this temperature fish oil became combustible.

4.3.4 Boiling

The boiling experiment addresses three queries posed in Chapter 3. First, the effects of different *types* of work can be evaluated by comparing boiled samples with the results of the direct heating experiment. The boiling experiment involves heating 150ml of RFO in 600ml of distilled water for 5 hours in a glass laboratory beaker on a thermostat controlled electric hotplate (Thermoline model # 34531). Samples are

collected every hour. No fish oil samples were collected for this experiment. When heated in water, fish oil has a tendency to 'explode' rather than boil, making sample collection unsafe.

4.3.5 Ceramic reuse ($R = [S_1 \times nE] + [S_2 \times (n-1)E] + [S_3 \times (n-2)E] + [S_n \times E]$)

The vessel reuse experiment is designed to provide information for two cultural taphonomic questions: how are *reused* residues formed and the relationship between residue penetration site and residue composition (cultural taphonomy query 2, Chapter 3). In this experiment, 200ml of an RFO and 1000ml of hot water is placed in a modern earthenware pot. The ceramic is heated to 150-160°C for two hours after which its contents are poured out. The contents are poured over the same section of the rim each time to examine residue formation in ceramic rims. The inner surface is then wiped clean with a wet cloth and the cooking process repeated with a different RFO to simulate *reuse* residue formation.

The experiment utilises three RFOs: beef fat, sesame and linseed oil. Fish oil is excluded due to safety concerns as flagged in the boiling experiment. The order of RFOs was designed to ensure that the most resistant to heat was exposed to the longest duration (i.e. saturated beef fat, followed by sesame oil then linseed oil). Residue samples are collected along the profile by dividing the ceramic into 2cm vertical sections, producing eight samples from base to rim.

4.3.6 Leaching

The leaching experiment is designed to provide data for the effects of groundwater movement on archaeological ceramic residues (post-depositional query 3, Chapter 3). The effects of leaching on residue composition are simulated by ultrasonicating residue impregnated ceramics in a glass beaker of distilled water. For the leaching experiment, base sections from the 2004 experimental ceramics were used. Base sections contain lower concentrations of residues that are more consistent with reported recoveries for archaeological ceramics. The 2004 ceramics were prepared in an oven rather than heating over a direct flame (appendix 1g). Consequently, thermal degradation of base section residues reported in previous

experimental simulations (Charters et. al. 1993) is not relevant to these experimental samples. Of the total range of the experimental ceramics, only the equivalents to the RFOs were selected. Ultrasonication agitates molecules to increase their solubility in solvent (water), allowing the accelerated simulation of leaching. Ultrasonication was performed in a Thermoline D150H ultrasonic bath. Two residues were extracted from each sample using the protocols outline in Section 4.2.1. One extract was from the non-ultrasonicated half of the sample used as a control, and the other from the ultrasonicated half.

4.3.7 Soil pH

The soil pH experiment provides data to assess the effects of different pH levels on residue composition (post-depositional query 2, Chapter 3). Fifty milligrams each of the four RFOs was placed in sealed vials with deionised water solutions prepared at pH 4, pH 7, and pH 8. These stored for 21 days at 35°C. Samples are vigorously agitated twice a day. The pH conditions are selected to reflect a normal range of soil pH, with pH 4 representing very acidic soils and pH 8 very basic (Leeper and Uren 1993:18). A pH 7 solution is included as a neutral control. The relatively small volume of RFO used in this experiment is designed to maximise the surface area exposed to the different pH conditions. The slightly elevated temperature of 35°C was also designed to enhance pH solution – RFO interactions.

4.3.8 Microbial alteration

The microbial alteration experiment is designed to evaluate the effects of soil microbes on archaeological residues (post-depositional query 3, Chapter 3). It involves inoculating a piece of filter paper already impregnated with one of the RFOs with a commercial microbial solution. The microbial solution is designed to promote the rapid decomposition of organic materials. The impregnated filter paper and microbial solutions are stored in closed Petri dishes for 30 days at room temperature. The resulting product was dehydrated in a drying oven and lipids extracted using the standard procedures detailed in Section 4.2.1.

4.3.9 Reanalysis of experimental ceramics

The reanalysis experiment assesses the changes in residue composition of experimentally generated residues after three years of storage. This experiment compares the results of analyses of residues extracted in 2004 with those extracted in 2007 from the same set of experimental ceramics. This comparison provides a direct measure of how residues are altered over time (post-depositional question 4, Chapter 3). The experiment also addresses the two chemical/mechanical questions (the effects of colloidal lipid aggregates and the action of triacylglycerols).

The experimental ceramics were produced by a commercial potter in white raku clay. The ceramics are capable of adsorbing approximately 12% of their mass in residues (based on gravimetric comparisons of ceramic dry mass to the same ceramic after it had been saturated in water (i.e. the pore spaces filled)). Residues were prepared by cooking 500g or 500ml of foodstuff in one litre of distilled water in an oven at 160°C for five hours. Complete preparation procedures for these ceramics are detailed in appendix 1g.

Between 2004 and 2007 the ceramics were stored in sealed plastic bags in controlled conditions (c. 15-20°C). For this comparison both the 2004 and 2007 samples were collected from the part of the ceramic likely to contain the highest volume of lipid residues (i.e. the waterline) (Charters et al. 1993).

4.4 Residue Characterisation and Interpretation

To measure the effects of taphonomic simulations on lipid profiles, this study employs four types of measurement to characterise residues and alteration products. The degree of alteration in experimental samples is first examined with an evaluation of their divergence – a rapid screening indexing method to estimate the degree of experimentally induced variation in relation to an unaltered RFO. Following the divergence screening test all experimental samples are submitted to three different well established chemotaxonomic methods: biomarker, ratio and mathematical analysis. The use of these three chemotaxonomic techniques serves two interrelated purposes, one substantive and the other methodological: a) to assess the extent of experimental alteration, and b) to assess the robusticity of these methods.

4.4.1 Divergence indices

Divergence indices (DI) are calculated by subtracting the relative percentages of lipids in an experimentally altered dataset from those of its unaltered RFO equivalent. As an initial screening technique the DI provides a measure of the total divergence of an experimental sample. The DI is the sum of differences between an altered dataset and its RFO, irrespective of whether these differences are expressed as negative or positive values (all negative values are converted to positive avoid a zero sum outcome, and the total divided by two to give the DI). Table 4.1 provides a worked example of the DI calculation.

As DIs sum the variation between all measurable lipid species in samples, they can also reflect minor Mass Spectrometric signal instabilities and derivatisation methods. These background levels of divergence are largely dependent on the complexity of the sample under analysis, with more complex samples having higher background levels of divergence than simpler samples. Background divergences are calculated by comparing the differences between analyses of unaltered RFO samples. The relatively simple fatty acid components of beef, sesame and linseed samples have a maximum background divergence of <4%. More complex fish oil fatty acids have a higher background divergence of ~7%. For acylglycerols, the background divergence for the simpler plant oils is <4%, while the more complex acylglycerol composition of beef generates a background of ~6%.

	C14:0	C16:0	C18:1	C18:0	C20:0	Σ
RFO	6.67	26.67	53.33	10.00	3.33	100.00
Altered	15.38	38.46	15.38	23.08	7.69	100.00
Raw divergence	-8.72	-11.79	37.95	-13.08	-4.36	0.00
Converted	8.72	11.79	37.95	13.08	4.36	75.90
Net DI						37.95

Table 4.1: Example of DI calculation. Hypothetical values for an RFO and an experimentally altered product over five fatty acids calculated as percentages summing to 100. The difference between the two is expressed as the raw divergence which if left unconverted. Conversion of the negatives to positives corrects the zero sum effect. This value is then divided by two to give the final DI value.

4.4.2 Biomarkers

Part of the experimental objective of this study is to assess the survival potential of biomarkers in archaeological conditions. To achieve this I use fatty acid

and acylglycerol molecules with biomarker-like properties (comparatively resistant to alteration and occurring in relatively low abundances (<3%)). These 'pseudo-biomarkers' provide an empirical measure of survivability that can be applied to the archaeological datasets. Pseudo-biomarkers are required because actual biomarkers are comparatively rare. By measuring their survival in experimental samples, I develop a proxy of resilience for comparable biomarker molecules (e.g. if PPS occurs in a RFO in concentrations of <3% it can be used as a proxy for the behaviour of the biomarker MaPS, which has similar chemical and physical properties). This measure is then directly applicable to the archaeological samples where I anticipate the preservation a wider range of biomarker molecules including fatty acids, acylglycerols and sterols.

4.4.3 Ratio analyses

I evaluate the ratio methodologies proposed in two recent studies. Both these methods claim to be able to differentiate a wide range of products, and have been widely applied (Barnard et al. 2007; Buonasera 2005; Eerkens 2005; Eerkens 2007; Malainey et al. 1999b; Malainey et al. 1999c; Quigg et al. 2001; Rosen et al. 2001). The Eerkens' (2005) ratio methodology is designed to identify a range of modern and archaeological sources. It involves a combination of C12:0/C14:0, C16:1/C18:1, C16:0/C18:0 and (C15:0+C17:0)/C18:0). This method is claimed to be capable of differentiating berries, seeds and nuts, fish, greens, roots, and terrestrial mammal products. Malainey's ratio methodology is more specifically developed to account for archaeological products. It uses the proportions of short-chain fatty acids (C12:0+C14:0+C15:0) to C18:1 to C18:0 to characterise plant, large herbivore, herbivore/plant/marrow mixtures, plant with large herbivore, beaver, fish or corn, fish or corn with plants, and plants except for corn.

Both methodologies have been designed to evaluate North American remains, but may have wider diagnostic applicability. I evaluate Eerkens ratio methodology against both the experimental and archaeological datasets of this study and restrict evaluation of Malainey's ratio methodology to the archaeological samples.

4.4.4 Mathematical techniques

In this study I use Principal Components Analysis (PCA) of the relative percentages of fatty acid methyl esters to interpret experimental and archaeological samples. PCA enables graphic summaries of compositional relationships (i.e. group compactness, that can be used to indicate the homogeneity or heterogeneity of a group; and relative group orientation, that is defined by the relationships between group-specific variables).

To facilitate group identification I adopt the procedures of Grave et al. (2008). In this approach, the first three components of a PCA dataset are projected in three dimensions using a software package designed for interactive group classification (SAS 2006). Dynamic rotation of the first three components enables the structural characteristics of compositional groups to be readily identified. In highly structured datasets, this procedure will identify a relatively small number of groups that are composed of highly compositionally distinct outer groups and more compositionally similar, closely packed inner groups. Iterative removal of outer groups and reanalysis of the remaining dataset by PCA enables the elucidation of inner core groups. This process is continued until no further groups can be distinguished.

4.5 The Archaeological Sample

Sixty one archaeological ceramics were sampled this study from a number of Late Bronze and Early Iron Age sites in Central and Western Turkey (Figure 4.4). Samples include perfume containers (*Lydions*) from Sardis and Gordion (Figure 4.5), storage and transport pithoi from Troy, putative oil containers (*Red Lustrous wheel-made wares*) from Boğazkoy (Figure 4.5) and cooking wares from Gordion and Boğazkoy. Fourteen environmental soils and archaeological sediments from Sardis and Gordion were included in this sample to provide a measure of environmental contributions to archaeological residues.

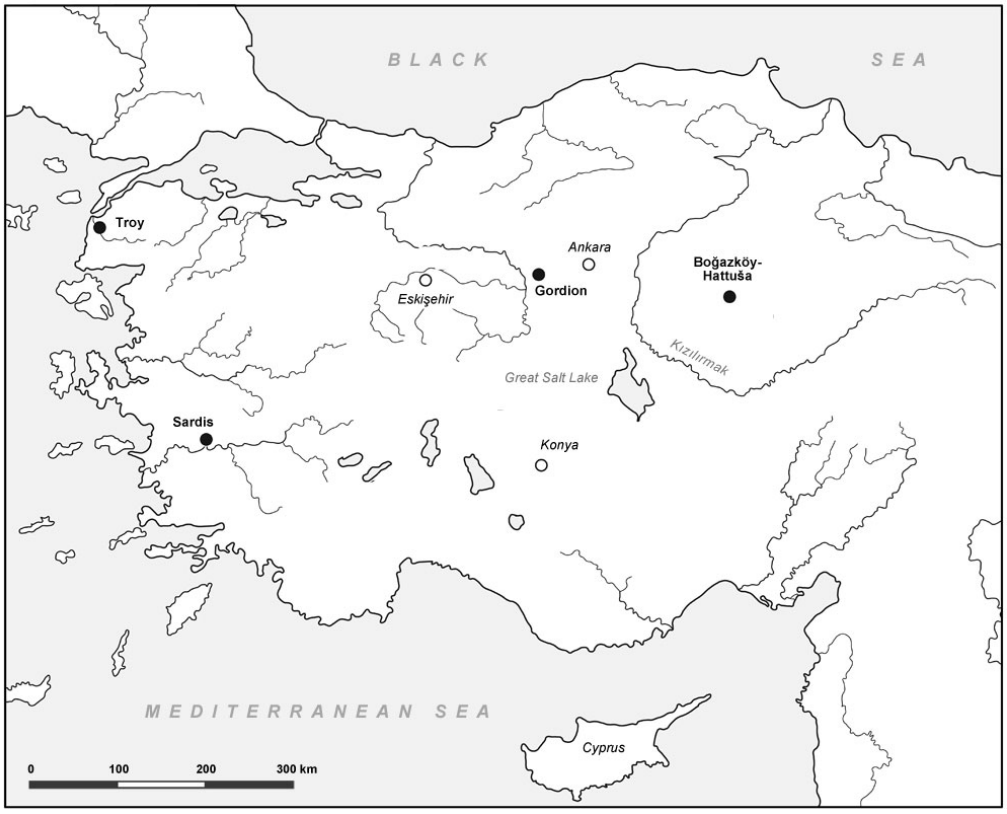

Figure 4.4: Map of Turkey showing the location of archaeological sites (black dots) that provided the archaeological samples used in this study.

Residues were extracted from eight grams of powdered ceramic. In the taphonomic framework adopted in this study, the volume of residue recovered forms an important piece of taphonomic information. This study therefore considers all residues, regardless of the masses of recovered. This contrasts with most other residue research where the focus is on analytic viability.

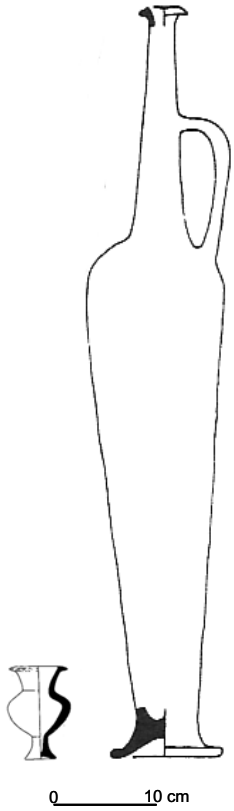

Figure 4.5: Profiles of a typical Iron Age Lydion from Sardis (left) and a Late Bronze Age spindle-bottle form of Red Lustrous Wheel-made ware typical of the Red Lustrous samples from Boğazkoy (Eriksson 1991; Greenewalt Jr. 1966).

4.5.2 Pithoi

The role of bulk storage and transport of organic products in ancient societies is a substantial line of archaeological enquiry. Often, these goods were stored and transported in large ceramic vessels such as pithoi, which are most commonly associated with the bulk movement or storage of liquid commodities such as oil and wine. A common feature of Iron Age palatial complexes is very large storage and smaller transportation pithoi (Sherratt and Sherratt 1993). These ceramics are key pieces of evidence for theories about the accumulation and distribution of wealth from Iron Age palatial complexes in the Mediterranean and the Near East (Chase-Dunn and Hall 1991; Sherratt and Sherratt 1993). While the ceramics have been considered an important social and economic indicator, less attention has been focused on identifying the commodities stored within these ceramics (Condamin et al. 1976; Hasson and Foley 2008; Stern et al. 2000).

This study considers eleven residues from large storage pithoi at Iron Age Troy. A second class of Trojan pithoi is also examined in this study. These ceramics are transport pithoi that may be imported from the Greek island of Kythera. These residues provide an insight into the storage function of the residue/ceramic systemic context model.

4.5.3 Red Lustrous wheel-made ware

Red Lustrous wares are an enigmatic class of fine ceramics principally produced in Cyprus during the Late Bronze Age (Eriksson 1991). The ceramics are widely distributed across Turkey, the Levant, and Egypt (Eriksson 1991). Red Lustrous wares appear to sometimes have a ceremonial function, based on the unusual and elaborate form of some examples (e.g. 'libation arm' vessels) and the unusually high concentrations of these ceramics found at religious sites and palaces (Knappett et al. 2005).

Knappett and coworkers (2005) examined residues extracted from a range of Red Lustrous wares from several sites across Turkey and the Near East, including Boğazkoy and Kilise Tepe in Turkey, Kazaphani (Ayois Andronikos) in Cyprus and Memphis-Saqqara in Egypt. A range of residue products were identified in ceramics including beeswax, plant oils and bitumen. Samples from Boğazkoy were notable for their frequent preservation of beeswax-like molecules.

Samples for this study are also sourced from Boğazkoy. These samples (n=11) are from the Southern Ponds (Schoop 2006) and consist of spindle bottles body sections.

4.5.4 Lydions

Lydions are a class of value-added ceramic, characterised by a spherical body, a wide, straight neck, horizontal rim and narrow, conical foot (Greenewalt Jr. 1966). These ceramics are highly variable, both in size and degree of decoration. Ceramics may be plain, have horizontal bands, figurative works or decorated in 'Lydianised' marbling style.

Lydion production was focused around the city of Sardis in Lydia. The ceramics were primarily produced between the mid c. 600 to 530 BCE, with latter examples of 'spindly' Lydions continuing until the 4th century BCE. Between 600-500 BCE, Lydions, or the products they contained, were an important international trade good.

Lydion contents are unknown, although Greenewalt (1966) hypothesised a link between Lydions and the ancient Lydian product *Bakkaris*. Bakkaris is mentioned by Greek sources as a scented unguent that was smeared across the face and head. Bakkaris and Lydions are the only two manufactured Lydian products for which there is evidence of significant export, leading Greenewalt to link the two products. The fine, value-added nature of Lydions makes them likely candidates for containers of a high-prestige product like Bakkaris.

The association between Lydions and some form of high-value scented product has been enhanced by the recent discovery of Lydions *in situ* with a toiletry set in a destruction level (c. 547 BCE) at Sardis (Cahill 2000). Whether or not Lydions were associated with Bakkaris, it seems clear that Lydions were containers for some form of high-value scented product. Part of this study considers the identification of the former contents of Lydions.

Perfumes and other scented products are often created through complex production sequences involving many ingredients and technological processes (Kennett 1975). Perfume containers are often only storage vessel for products prepared *extra ceramic* (Kennett 1975:59-61). Lydions examined in this study are sourced from two sites, five samples from Sardis and four from Gordion. Sardis Lydions were recovered from a destruction level associated with the Persian conquest of Lydia in 547 BCE. The level has significant evidence for burning, which correlates with historical accounts of the city's destruction (Cahill 2000). The known destruction level presents an opportunity to investigate known archaeological taphonomic processes on ceramic residues. By comparing Sardis residues to those of Gordion the effects of the destruction event on the residues may be partially measured.

4.5.5 Cooking pot wares

Cooking pot residues are the most frequently analysed class of archaeological residue due to their archaeological frequency, relatively high residue recoveries and a strong interest among residue researchers in food technologies. Food technologies are sensitive markers of economic and social organisation as well as demographic change (Craig et al. 2005; de Garine 1976; Zeder and Arter 1994). However, only a vanishingly small proportion of the material debris of food technologies survives archaeologically, complicating attempts to understand how ancient societies exploited food resources. Ceramic residues can produce substantive new insights into past societies through the analysis of the residues of ancient food technology.

In Chapter 3, I suggested that the repetitive reuse and recycling of ceramics can produce very complex residues. Cooking pots are prime candidates for these kinds of processes as they may have complex use-lives, involving frequent reuse and reassignment, and potentially processing a wide range of organic products. This complexity is a significant problem for developing diagnostic techniques to identify archaeological residue contributors. A category of archaeological cooking pot residues is included to explore the cultural taphonomic model. Two sets of cooking pot ware are analysed in this study, the first seven Late Bronze Age cooking pots from Boğazkoy; the second eighteen cooking pot and utilitarian wares from Late Bronze Age-Early Iron Age Gordion. Residues from Gordion were previously analysed in 2004 as part of a study examining the analytic potential of HPLC-MS for archaeological residues. After this study, residues were stored at -18°C under nitrogen.

4.6 Summary

This chapter presented the extraction procedures, instrumentation and data processing protocols adopted for this study. I detailed the instrumentation and methodology used to analyse the key classes of lipids examined (i.e. HPLC-MS for acylglycerols and GC-MS for fatty acids). Next, I discussed the interpretive

techniques applied to experimental and archaeological results (i.e. divergence indices, biomarkers, ratios and Principal Components Analysis).

Following this, I detailed the experimental simulations conducted in this study (i.e. heating, boiling, vessel reuse, groundwater percolation, pH exposure, microbial exposure, and alteration over time) and how these relate to the key taphonomic issues identified in Chapter 3. Finally, I described the archaeological sample analysed in this study, discussing both the archaeological significance of these wares and their relevance to the taphonomic approach developed in this study.

Chapter 5 - Results

5.1 Introduction

In this chapter I present the outcomes of the experimental and archaeological analyses. First, I detail the results of the experimental simulations, using divergence indices (DI) as described in Chapter 4, followed by a range of conventional chemotaxonomic methods. This process is designed to: a) provide controls on alteration products, and b) evaluate the extent to which chemotaxonomic identifications are obscured by experimental alterations of reference fats and oils (RFOs).

Following the presentation of the experimental results, I present the analysis of the archaeological dataset. Archaeological residue recoveries and their molecular compositions are described before the application of chemotaxonomic techniques as discussed in Chapter 4. The significance of the experimental and archaeological results are discussed in Chapter 6.

5.2 Experimental Simulations

In the following sections I describe the results of seven alteration experiments designed to simulate the major processes argued to contribute to the composition of archaeological residues. Complete tabulated results of these experiments are presented in appendix 2.

5.2.1 Direct heating

In this section I describe the effects of heating fats and oils at different temperatures and over different time periods to simulate cooking-induced alteration. I examine the results of three separate experimental conditions where fats and oils are heated for five hours at 100°C, 200°C and 300°C with aliquots collected every hour.

I first apply the DI detailed in Chapter 4 to assess whether the sample aliquots are significantly altered (i.e. above baseline variations) by each heating experiment.

This involves examining the molecular composition of key lipid species within the fat or oil.

5.2.1.1 Direct heating 100°C

For the 100°C experiment, negligible effects are evident in fatty acids and acylglycerol composition of the four RFOs (i.e. beef, fish, linseed, sesame). Fatty acid compositions generally diverge from the RFOs by less than 4% and are within the background variation (Figure 5.1a-d). The higher level of background variation in fish oil inhibits the observation of potential experiment induced alterations.

Regression data shows that there is little correlation between degree of divergence and time. In many instances divergence trends downwards over exposure time even suggest that later samples more closely resemble their unheated baseline equivalents. In only one instance (linseed oil) is there a weak positive correlation (R^2=0.26).

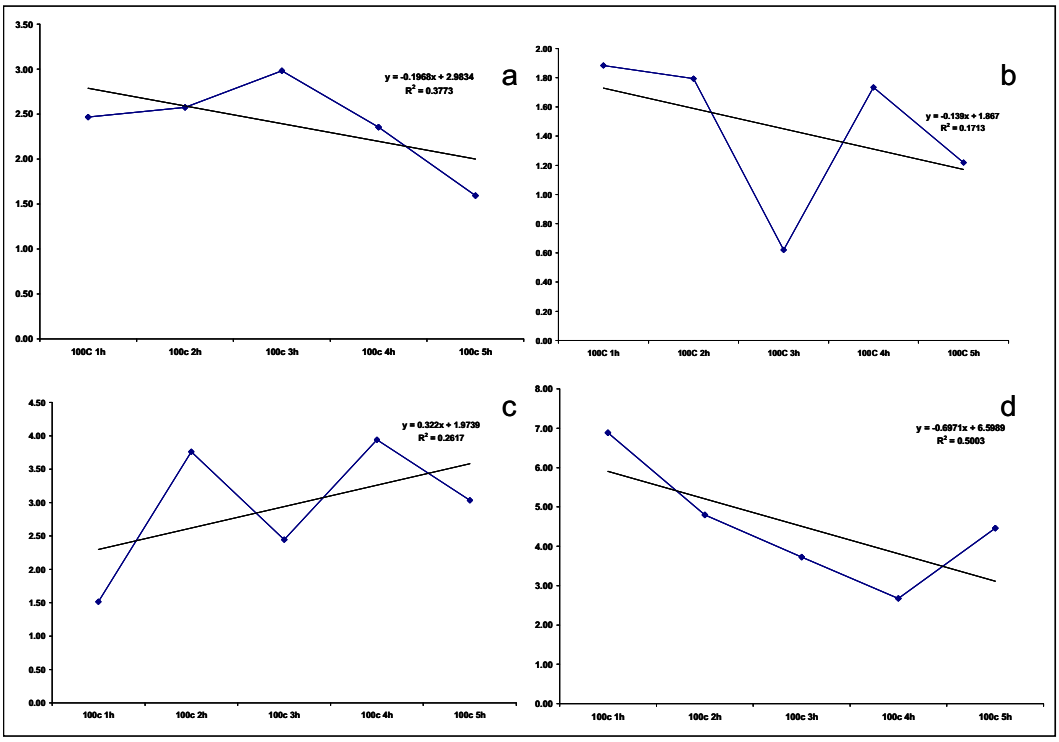

Figure 5.1: Time series divergence index (DI) for fatty acid compositions of a) beef fat, b) sesame oil, c) linseed oil and d) fish oil exposed to 100°C over a period of five hours.

Acylglycerols are also unaffected by the 100°C five hour experiment. Divergence values were 5.9% for beef, 3.2% for sesame and 4.1% for linseed. The low levels of divergence can be attributed to background variability.

5.2.1.2 Direct heating 200°C

Experimentally heating fats and oils to 200°C produced relatively minor but systematic alteration in two of the four samples. Divergence measurements indicate that beef and fish oils remain unaltered by the 200°C experiment (Figure 5.2a, 5.2d). Maximum divergences were 3.6% for beef (2 hours) and 5.7% for fish (3 hours), within the range for background variation.

However, linseed and sesame oils show compositional changes over exposure time (Figure 5.2b, Figure 5.2c). This is most pronounced for linseed oil, with divergences increasing from 2.1% at one hour to 6.2% at five hours. Later divergences are well above background variability. Sesame oil follows a similar trend.

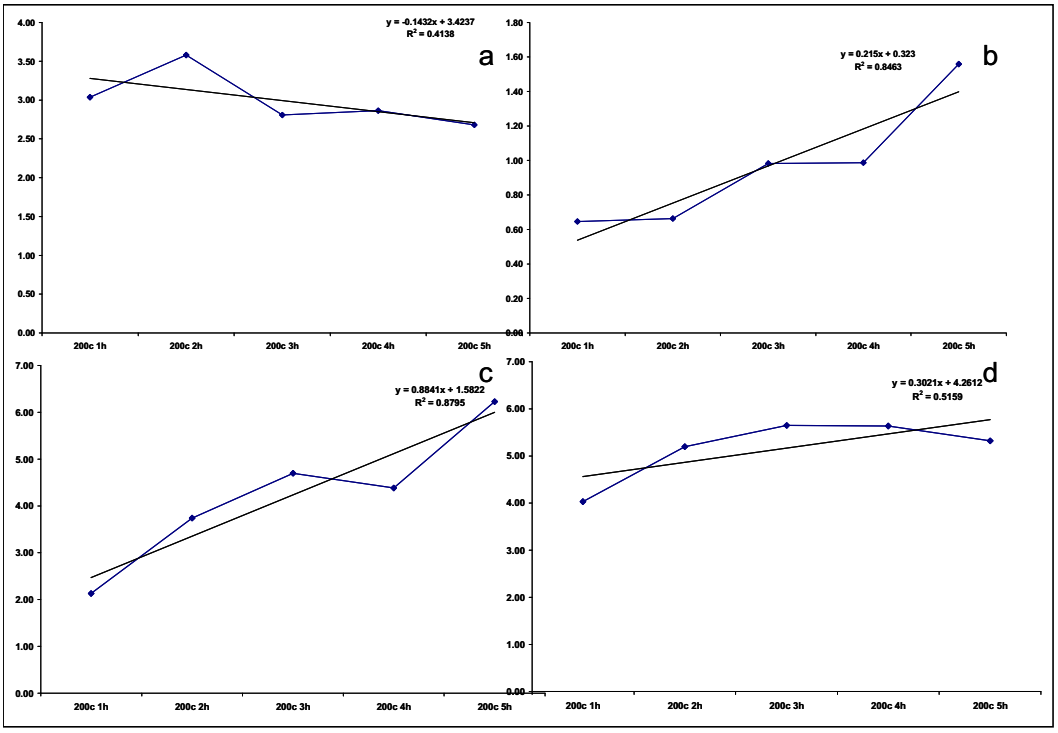

Figure 5.2: Time series DI for fatty acid compositions of a) beef fat, b) sesame oil, c) linseed oil and d) fish oil exposed to 100°C over a period of five hours.

The time series for linseed oil follows a pronounced trend, with the relative decline of C18:2 and C18:3 (Figure 5.3a, 5.3b) species and an increase in C18:1 (Figure 5.3c). The saturated fatty acids C16:0 and C18:0 also appear to increase, but

their relatively low concentrations (~6% and ~8%) mean that common but less stable C18:1 species increase more consistently as less stable C18:2 and C18:3 species decline. Sesame oil exhibits a similar shift towards an increasing proportion of C16:0 fatty acids (Figure 5.4a) and a decrease in C18:2 (Figure 5.4b). These two fatty acid species comprise approximately 50% of sesame oil fatty acids by volume, contributing most significantly to the divergence index. Declining proportions of C18:2 and increasing proportions of C16:0 are most likely caused by the preferential degradation of highly unsaturated fatty acid species during heating, consequently increasing the relative importance of more stable saturated fatty acids.

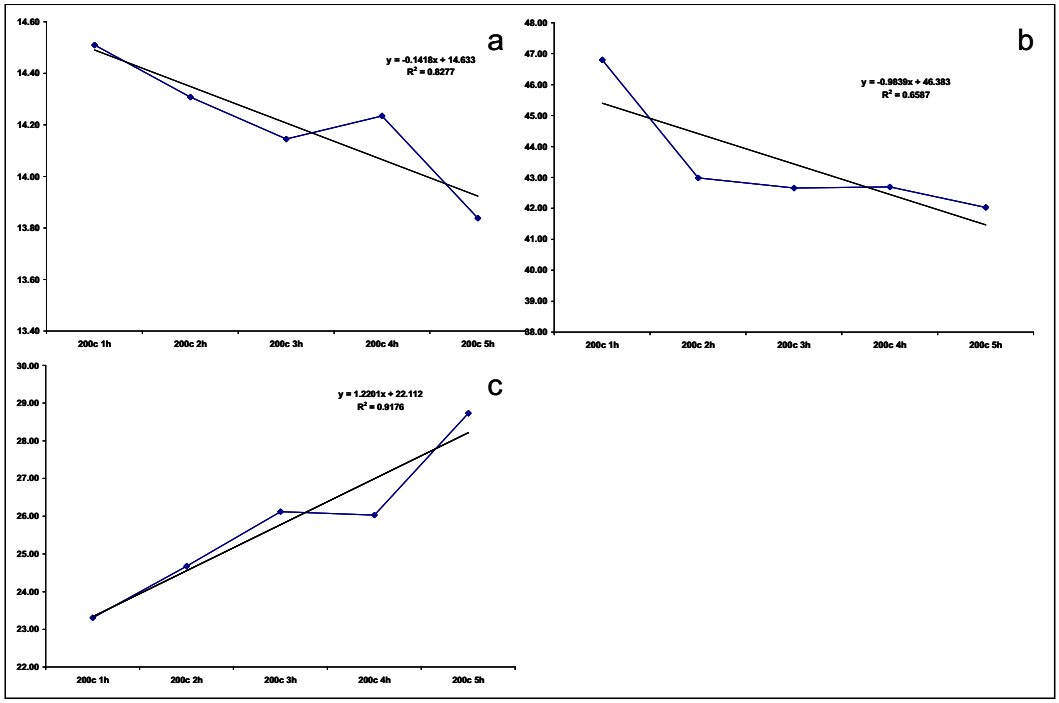

Figure 5.3: Time series of the individual fatty acids a) C18:2, b) C18:3 and c) C18:1 in linseed oil heated to 200°C for five hours.

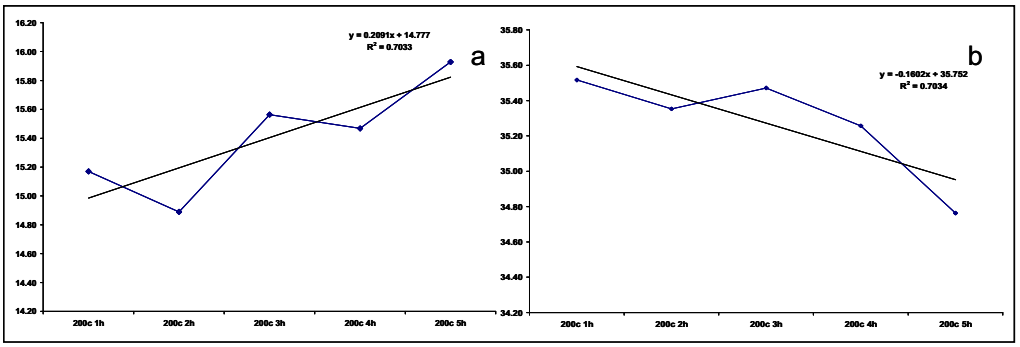

Figure 5.4: Time series of the individual fatty acids a) C16:0, b) C18:2 in sesame oil heated to 200°C for five hours.

As acylglycerol molecules are more complex than their component fatty acids, divergence indices for acylglycerols are predictably higher. After heating to 200°C for five hours beef divergence was 7.8%, linseed 6.0% and sesame 9.6%. The increased beef divergence is largely explained by a decline in the relative proportions of triacylglycerols containing two or more oleic acid moieties (e.g. OOO, OOP, SOO), and an increase in the relative proportions of more saturated triacylglycerols such as POP, SOP and MaOS. Sesame and linseed follow a similar pattern, with declining proportions of the most highly unsaturated triacylglycerol species (e.g. LnLnLn, LLL, OLL, LLnLn) and an increased proportion of more saturated triacylglycerols (e.g. LOP, PLP, OOP).

Higher divergence indices for acylglycerols are a result of the wider range of chemical alterations that are possible for this complex molecular class. At 200°C, fatty acids are primarily altered by oxidation and autoxidation. Acylglycerols are subject to these chemical reactions but can also be altered by hydrolysis, which breaks acylglycerols into simpler forms (i.e triacylglyerols to di- and monoacyglycerols and free fatty acids).

5.2.1.3 Direct heating 300°C

The 300°C experiment produces substantial alterations in all the reference fats and oils. Divergence indices show a strong linear trend over time (R^2 values between 0.94 and 0.997 (Figures 5.5a-c). The linear alteration behaviour of fatty acid species

can be best summarised by grouping fatty acids with similar degrees of unsaturation (i.e. saturated, monounsaturated and polyunsaturated fatty acids) (Table 5.1).

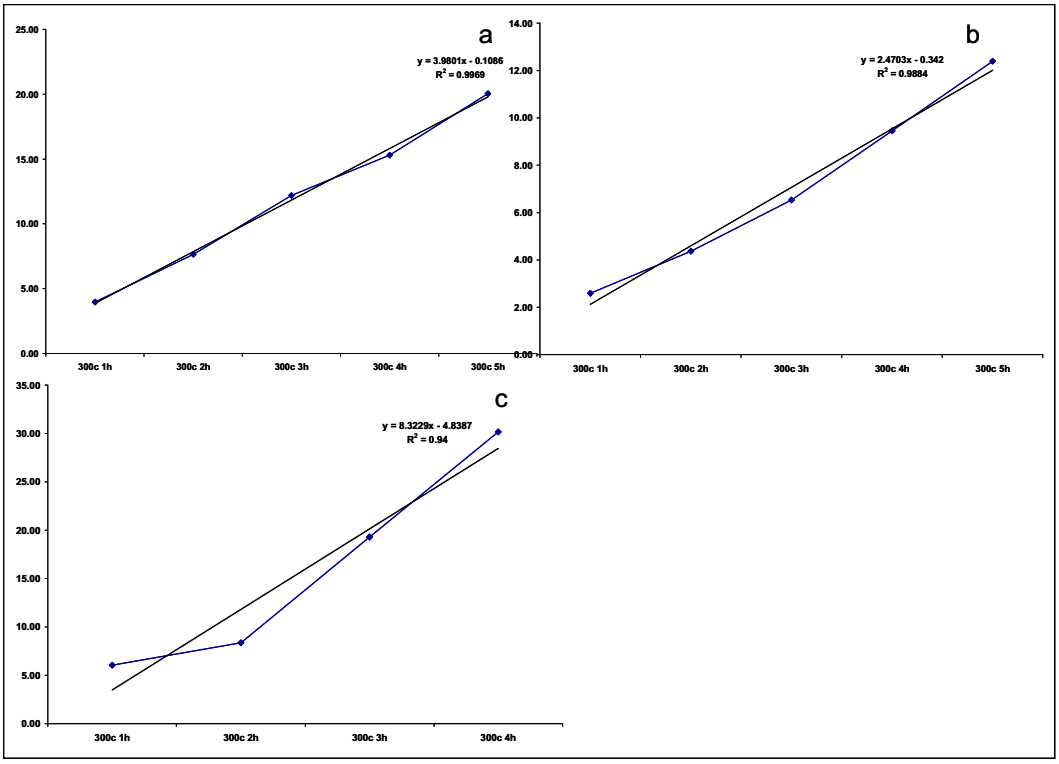

Figure 5.5: Time series DI for fatty acid compositions of a) beef fat, and b) sesame oil 300°C; anc c) linseed oil exposed to 300°C over a period of four hours.

After 5 hours of exposure, beef fatty acids are altered 20% relative to their original composition (Figure 5.5a). The divergence is the result of the preferential removal of polyunsaturated and monounsaturated fatty acids. The small volumes of polyunsaturated fats were rapidly removed from beef at 300°C, with C18:3 (~0.4% of the beef RFO) disappearing entirely within the first hour. Monounsaturated fatty acids decreased linearly ($y = -3.5874x + 50.05$, $R^2 = 0.9914$), while saturated fatty acids increased linearly ($y = 3.9495x + 48.042$, $R^2 = 0.9953$). The increase in saturates mirrors the declining proportion of monounsaturates (Table 5.1).

Sample	CX:0 %	CX:1 %	CX:2+ %
Beef RFO	51.86	45.76	2.37
300°C 1h	55.57	43.76	0.66
300°C 2h	60.68	39.04	0.29
300°C 3h	64.04	35.79	0.17
300°C 4h	67.13	32.65	0.22
300°C 5h	71.91	27.96	0.13

Table 5.1: Relative concentrations of saturated (CX:0), monounsaturated (CX:1) and polyunsaturated (CX:2+) fatty acids in beef fat heated to 300°C over five hours.

For sesame oil, the higher proportions of unsaturated fatty acids, predominantly C18:2, produced slightly different results to beef (Figure 5.5b). Polyunsaturated fatty acids decreased significantly during heating, declining linearly ($y = -2.9762x + 41.777$, $R^2 = 0.9992$) from a relative proportion of 36% in baseline products to 24% after five hours of exposure. In contrast to beef fat, the high proportion of more degradable polyunsaturated fatty acids produced an increasing proportion of monounsaturates over exposure time, from 42% in the RFO to 50% after five hours ($y = 1.7224x + 36.202$, $R^2 = 0.9932$). Saturated fatty acids also increase linearly ($y = 1.2537x + 22.022$, $R^2 = 0.973$) (Table 5.2).

Sample	CX:0 %	CX:1 %	CX:2+ %
Sesame RFO	22.01	41.62	36.36
300°C 1h	24.14	39.87	35.99
300°C 2h	26.13	41.23	32.64
300°C 3h	27.20	42.96	29.84
300°C 4h	28.49	44.60	26.92
300°C 5h	29.23	46.80	23.97

Table 5.2: Relative concentrations of saturated (CX:0), monounsaturated (CX:1) and polyunsaturated (CX:2+) fatty acids in sesame oil heated to 300°C over five hours.

Linseed oil behaves similarly to sesame oil (Figure 5.5c). The relative proportions of polyunsaturated fatty acids declines linearly ($y = -7.4157x + 70.529$, $R^2 = 0.9467$), while the relative proportion of saturated ($y = 3.1656x + 11.641$, $R^2 = 0.9393$) and monounsaturated fatty acids ($y = 4.2501x + 17.83$, $R^2 = 0.9291$) increases linearly (Table 5.3).

Heating linseed oil to 300°C produces several linolenic acid isomers. These molecules are most likely products of the structural reconfiguration of *n*-linolenic acid during heating. While the result indicates a relatively high proportion of polyunsaturated fatty acids in linseed, *n*-linolenic, the major component of the reference oil, is only a relatively minor feature after four hours of heating.

Sample	CX:0 %	CX:1 %	CX:2+ %
Linseed RFO	16.12	22.95	60.94
300°C 1h	17.25	27.27	55.48
300°C 2h	20.11	27.32	52.57
300°C 3h	23.29	35.07	41.65
300°C 4h	28.93	40.30	30.77

Table 5.3: Relative concentrations of saturated (CX:0), monounsaturated (CX:1) and polyunsaturated (CX:2+) fatty acids in sesame oil heated to 300°C over four hours.

For all RFOs the 300°C experiment produces significant volumes of short-chain saturated fatty acids (C8:0-C12:0). The increase may be partially explained in

terms of the relative loss of more highly unsaturated lipid species. However, short-chain fatty acids are also produced by oxidative scission of longer-chain unsaturated fatty acids. Most unsaturated lipids in plants have double bonds at the eighth and ninth carbon atom along the aliphatic chain. Oxidative scission would primarily produce C8:0 and C9:0 fatty acids, which are observed in the experimental samples (Table 5.4). Beef fats contain a wide range of oleic acid isomer with double bond positions from the eighth to the thirteenth hydrocarbon (Evershed et al. 2002). Beef fat scission consequently produces a much broader range of short-chain fatty acids (C8:0-C12:0).

Sample	C8:0 %	C9:0 %	C10:0 %	C11:0 %	C12:0 %
Beef RFO	0.00	0.00	0.04	0.00	0.06
300°C 1h	0.03	0.00	0.04	0.00	0.06
300°C 2h	0.10	0.03	0.05	0.02	0.07
300°C 3h	0.15	0.04	0.11	0.03	0.12
300°C 4h	0.38	0.12	0.17	0.09	0.19
300°C 5h	0.46	0.16	0.19	0.11	0.22
Sesame RFO	0.00	0.00	0.00	0.00	0.04
300°C 1h	0.05	0.00	0.00	0.00	0.06
300°C 2h	0.10	0.00	0.00	0.00	0.06
300°C 3h	0.15	0.00	0.00	0.00	0.06
300°C 4h	0.21	0.01	0.01	0.00	0.06
300°C 5h	0.17	0.02	0.00	0.00	0.05
Linseed RFO	0.00	0.00	0.00	0.00	0.00
300°C 1h	0.05	0.00	0.00	0.00	0.00
300°C 2h	0.17	0.00	0.00	0.00	0.00
300°C 3h	0.13	0.02	0.00	0.03	0.00
300°C 4h	0.54	0.07	0.03	0.00	0.03

Table 5.4: Time series data showing the percent concentrations of short-chain fatty acids in beef, sesame, and linseed RFOs and their alteration at 300°C over 5 hours for beef and sesame and 4 hours for linseed.

Acylglycerols are also significantly affected in the 300°C experiment, with divergences of 33% and 26% for beef and sesame respectively after five hours and 65% for linseed after four hours (at five hours linseed had polymerised to become an insoluble semi-solid). Like the 200°C experiments, acylglycerol divergence is significantly higher than fatty acid divergence, highlighting the liability of acylglycerols to alteration.

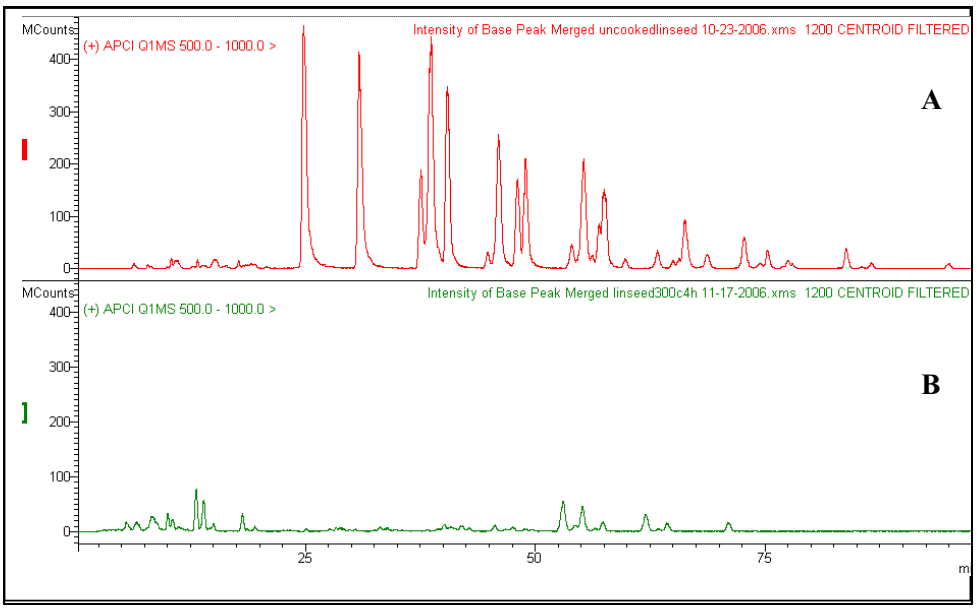

Figure 5.6: Chromatographic traces showing the difference between a) a linseed RFO and b) linseed heated to 300°C for four hours.

The 300°C experiment affected both the relative proportions of acylglycerols in fats and oils and their absolute concentrations. Figure 5.6 shows the relative intensities of HPLC chromatographs for unaltered linseed oil acylglycerols (A) and oil exposed to 300°C for four hours (B). In unaltered fats and oils, acylglycerols compose ~99% of total lipids. After 4-5 hours at 300°C, this proportion is greatly reduced.

As indicated by DIs, linseed oil is most affected by high temperature exposure. Most triacylglycerol species found in linseed oil are either missing or present as minor components. Thirty-four triacylglycerol species were found in unaltered linseed oil, while only 12 were recorded after four hours of exposure. The triacylglycerols LnLnLn, LLnLn, OLnLn, LnLnP, and OLnL, which account for 35%+ of acylglycerols in unaltered linseed oil, are absent after 4 hours as are numerous other minor, highly unsaturated triacylglycerol species. Two triacylglycerol species containing linolenic acid were found in the heated oil, OOLn and OLnP. Two interrelated factors explain their presence: a) high concentrations in the reference oil (6.6% and 4.9% respectively), and b) the triacylglycerol configuration of a single linolenic acid moiety with two saturated or monounsaturated moieties. Other triacylglycerol species with similar configurational properties are found in the linseed RFO (e.g. SLnS, SLnP and SOLn), but these species were only present in low concentrations (<1.5%). While equally or less liable to degradation than OOLn and

OLnP, their low concentrations mean that they are entirely altered before the more prevalent species. A similar pattern can also be observed in linoleic acid containing triacylglycerol species.

The preservation of triacylglycerol species in the heated sesame oil samples was much more frequent than in linseed but their proportions were significantly altered. Highly unsaturated triacylglycerols principally composed of linoleic acids (e.g. LLL, OLL, LOP, OOL) are significantly reduced while those composed of monounsaturated and saturated fatty acid species (e.g. OOP, POP) increase in relative importance. Several minor triacylglycerol species (<1%) such as LiOP, LiOO, and ALL were absent after five hours. Like linseed oils, the minor sesame oil lipids, while being less liable to degradation than highly unsaturated linoleic containing triacylglycerols, are more difficult to detect after heating.

The effects of the 300°C experiment on beef fat triacylglycerols differ slightly from the plant oils due to the absence of large volumes of polyunsaturated lipids. Alteration of beef triacylglycerols in this experiment can be summarised as a declining proportion of triacylglycerols containing monounsaturated moieties, coupled with an increasing proportion of triacylglycerols predominantly composed of saturated fatty acid species. In contrast, the proportion of monounsaturated-containing triacylglycerols increased in plant oils during exposure. This difference can only be partially explained by the inflation of monounsaturates through the preferential removal of polyunsaturated triacylglycerols.

Triolein (OOO) is common to linseed, sesame and beef in similar proportions (3.3%, 7.3% and 4.0% respectively). Triolein remains in linseed oil after 4 hours and sesame oil after 5 hours, but is absent from beef fat after 5 hours. This difference is best explained by sacrificial preservation (Eglinton et al. 1991). For plant oils, hydrolysis, oxidation and autoxidation preferentially affect the more reactive polyunsaturated triacylglycerol species, reducing alteration of triolein. In beef fat, the absence of large volumes of polyunsaturated lipids exposes triolein to more hydrolytic and oxidative factors, preferentially removing it and other monounsaturate-containing triacylglycerols from the fat.

A similar pattern occurs in fatty acid data. By comparing the proportion of monounsaturated C18:1 to saturated C18:0 the relative degradation of C18:1 can be estimated (Table 5.5). C18:0 is much less liable to alteration than C18:1 as it lacks a double bond. As lipid products are altered, the ratio of C18:1/C18:0 should decrease.

In beef the C18:1/C18:0 declines by approximately half, from 1.97 to 0.94. In contrast, the relative proportions C18:1/C18:0 in sesame and linseed oils are largely unchanged indicating a much lower degree of C18:1 alteration in plant oils.

Sample	C18:1 %	C18:0 %	C18:1/C18:0
beef RFO	41.32	21.01	1.97
300°C 5h	25.33	26.96	0.94
Sesame RFO	41.38	6.17	6.70
300°C 5h	46.46	6.84	6.80
linseed RFO	22.63	7.83	2.89
300°C 4h	39.66	13.56	2.93

Table 5.5: Relative percentages of oleic (C18:1) and stearic (C18:0) and their ratio in RFOs and their alteration products when heated to 300°C for five hours for beef and sesame and four hours for linseed.

The effects of heating to 300°C on minor triacylglycerols vary within beef fat. Minor unsaturated triacylglycerols behave similarly to those in plant oils, and are not found after five hours. Minor saturated components increased in relative importance through exposure. For example, the proportion of MaSS increased from 0.7% to 1.2% and MaPS from 1.9% to 3.6%, resulting from the declining proportion of unsaturated lipid species.

5.2.2 Boiling

Boiling experiments produced relatively minor alterations to fatty acid composition. DIs for all three boiled products (fish oil was excluded for safety concerns as detailed in Chapter 4) were generally less than 5%, and within the range of background variability (Figures 5.5a-d).

All divergence indices for triacylglycerols after five hours were also low. Beef divergence is 5.1%, sesame 6.3%, and linseed 4.2%. Overall, boiled samples closely resemble those heated to 100°C, indicating that boiling process produces an effect similar to direct heating.

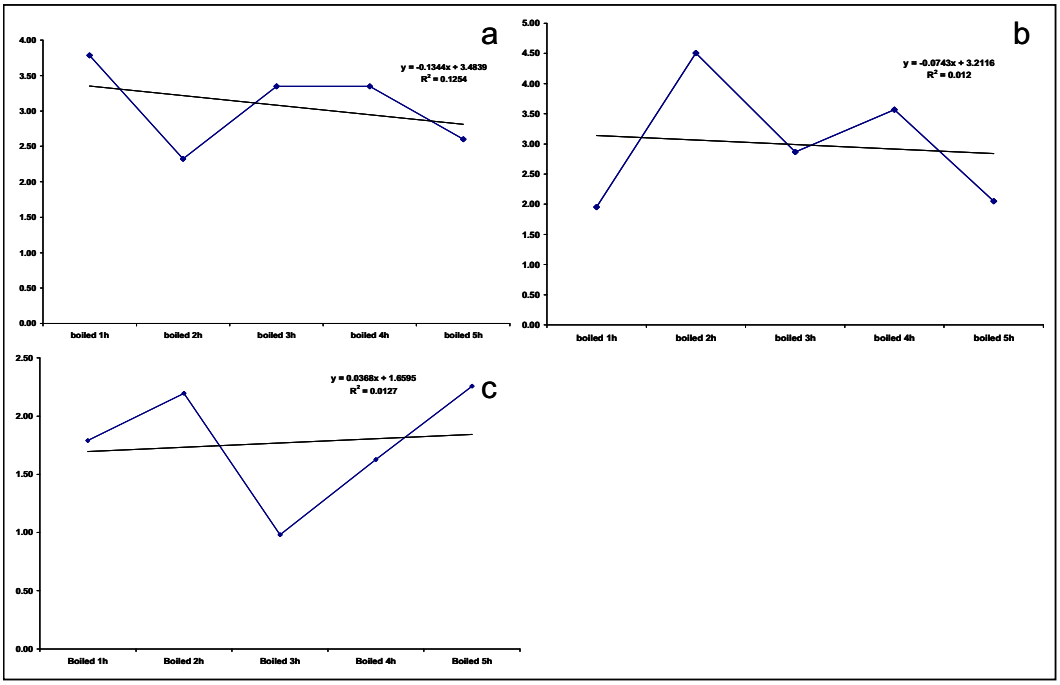

Figure 5.7: Time series DI for fatty acid compositions of a) beef fat, b) sesame oil, c) linseed oil and d) fish oil exposed to boiling over five hours.

5.2.3 Ceramic reuse ($R = [S_1 \times nE] + [S_2 \times (n-1)E] + [S_3 \times (n-2)E] + [S_n \times E]$)

In the ceramic reuse experiment, beef, sesame and linseed oil were sequentially boiled in the same vessel (each boiled for two hours before being replaced by the next). Predictably, total lipid recoveries vary considerably along the profile of the test ceramic (Figure 5.8). Recoveries are lowest in the bottom sections of the ceramic (RC S1, RC S2, RC S3), increase around the 'waterline' (RC S4, RC S5, RC S6), and decline towards the rim (RC S7, RC S8). The pattern reflects the hydrophobic nature of lipids with lower concentrations in ceramic sections mostly in contact with water and highest concentrations in sections around the ceramic 'waterline', where hydrophobic fats and oils float on the water surface. Rim sections are also exposed to significant volumes of lipid, but only briefly through pouring off. This brief exposure seems sufficient to deposit a substantial volume of residue in the ceramic matrix.

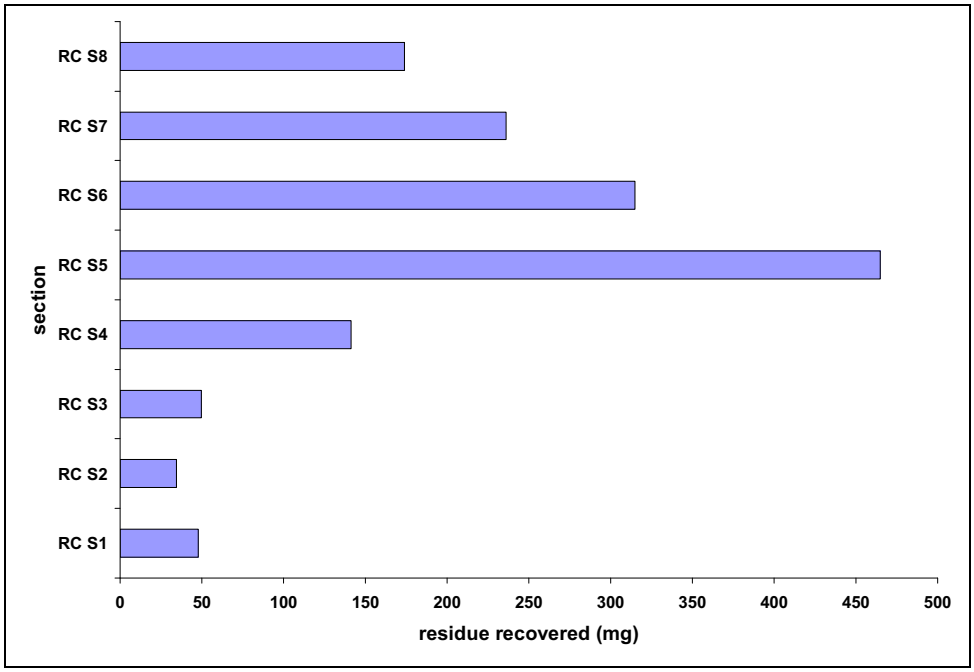

Figure 5.8: Mass (mg) of total lipid extract recovered from the eight grams of powdered ceramic from the eight sections taken along the profile of the experimentally reused ceramic (RC S1 is the base, RC S8 is the rim).

The relative proportions of lipid species also vary considerably across the ceramic fabric. The three base sections of the ceramic closely resemble unaltered beef fat, with DIs of 10.5-13.1% relative to the reference fat. Most of the divergence (>6%) is caused by higher volumes of oleic acid, likely derived from sesame oil. Linolenic and linoleic acid proportions do not differ significantly from beef, indicating that linseed is a very minor component of these three sections.

RC S4 and RC S5 residues are more complex, with evidence for the contribution of all three lipid products. The presence of beef is evident by odd-numbered (C15:0, C17:0) and branched-chain fatty acids (C15:0br, C16:0br, C17:0br, C18:0br) in these sections, although their relative concentration is about half that of the reference beef fat. Higher volumes of linoleic (9.1 and 15.4%) and linolenic acids (5.3% and 14%) are contributed by sesame and linseed oils. The lower volume of linolenic acid in RC S4 suggests linseed is a lesser contribution to this section than RC S5. RC S6 is more complex. In contrast to the other waterline sections, RC S6 closely resembles reference beef fat, diverging from it by only 5.5%. This suggests that either the beef fat experiment had a slightly higher waterline, or that beef fats boiled more freely, moving further up the ceramic profile than later use events (during

the boiling experiment the beef fat/water emulsion boiled more vigorously than plant oils). The high concentration of beef lipids in this section of the test ceramic appears to have prevented additional lipids penetrating the ceramic when they were poured out.

The three part reuse experiment was designed to measure interactions between different fats and oils. These are most evident in the water line sections (RC S4 and RC S5) and to a lesser extent in the two rim sections (RC S7 and RC S8). The samples predominantly resemble beef fat (i.e. the first fat boiled in the test ceramic) but contain an elevated linoleic acid component (7.6% and 8.8%), most likely contributed by sesame oil. A minor contribution from linseed oil is also observed in slightly elevated proportions of linolenic acid (2.8% in each section).

5.2.4 Leaching

The leaching experiment aims to evaluate the mobility of residues in a ceramic matrix by simulating groundwater leaching conditions. A set of three year old experimental ceramics, boiled with the four reference materials, was used. Comparable base sections were removed from each experimental ceramic. A baseline for this experiment is established by the extraction and analysis of the residue component of a non-ultrasonicated, powdered, 8 gram sample from each ceramic base. The remaining unpowdered fragment is ultrasonicated in a beaker of distilled water for 8 hours. Following ultrasonication, samples were dried, powdered and residues extracted and analysed.

Ultrasonicating residue-impregnated ceramics significantly alters residue recoveries (Table 5.6). While beef was relatively unaffected, sesame and linseed residues were reduced by 34% and 44% respectively. Recoveries of fish residue are too low for reliable measurement and are therefore excluded.

	Base (mg)	Ultrasonicated (mg)
Beef	8.5	8.6
Sesame	105.3	69.5
Linseed	115.3	64.9
Fish	0.9	0.5

Table 5.6: Mass of residues in milligrams recovered from untreated and ultrasonicated sections of experimental ceramics boiled with beef, sesame, linseed and fish (these were boiled for five hours).

Changes in residue recoveries do not readily translate into alterations of lipid compositions. DIs between ultrasonicated and unultrasonicated residues are relatively low, 1.8% for linseed, 2.4% for sesame and 6% for beef. Divergence in beef is largely the result of an increased proportion of oleic and palmitic acids a decline in linoleic acid. The low DIs suggest that the large differences in residue recoveries reflect a molecular fraction not analysed in this study.

While divergence indices indicate that ultrasonication produces relatively low levels of compositional change there are important changes in the relative proportions of short-chain and medium chain fatty acids (≤C12:0). Ultrasonication in water appears to reduce the relative abundance of short-chain fatty acids within ceramic fabrics (Table 5.7).

	C8:0 %	C9:0 %	C10:0 %	C12:0 %
Beef non-ultra.	0.00	0.07	0.08	0.43
Beef ultra.	0.00	0.00	0.00	0.12
Linseed non-ulta.	0.29	0.96	0.16	0.00
Linseed ultra.	0.20	0.93	0.12	0.00
Sesame non-ultra.	1.51	2.11	0.08	0.00
Sesame ultra	1.22	1.77	0.06	0.00

Table 5.7: Percentage proportions of short-chain fatty acids in unultrasonicated beef, linseed and sesame residues from the experimental ceramics compared to their ultrasonicated samples from the experimental ceramics (these were boiled for five hours).

5.2.5 Soil pH

The pH experiment simulated the effects of environmental pH on residues by exposing RFOs to acidic, neutral, and alkaline conditions detailed in Chapter 4. In practice, archaeological conditions are unlikely to be as severe as those used in this experiment. While most archaeological deposits have milder conditions, these operate over much longer time scales than this experiment. The more extreme pH levels simulated in this experiment are an attempt to replicate these time frames in an experimental environment. In sum, oxidation under alkaline conditions substantially alters the relative proportions of unsaturated fatty acids relative to the reference fats and oils. Acidic conditions appear to inhibit oxidation and provide a degree of preservation (Table 5.8).

RFO	pH4	pH7	pH8
Beef	1.81	2.17	11.21
Linseed	5.46	59.00	57.96
Sesame	2.67	4.42	24.47
Fish	43.92	46.28	46.24

Table 5.8: DIs for beef, linseed, sesame and fish oils exposed to acidic (pH4), neutral (pH7), and basic (pH8) solutions for 21 days at 35°C.

RFOs exposed to pH4 conditions had generally minor compositional changes. Beef diverges 1.81% from its RFO, linseed 5.46% and sesame 2.67%. Beef and sesame divergences are within the range expected for instrument variability and linseed is only slightly above this level. Fish oil shows comparably high levels of alteration across the range of pH conditions. The similarity and high values of the fish oil divergence index suggests that the unsaturated lipids dominant in fish oil are very susceptible to oxidation regardless of pH.

For the remaining fats and oils, neutral (pH7) exposure produces varying degrees of compositional alteration. The degree of variation reflects the physical properties of lipids within the reference samples. Beef fat, principally composed of monounsaturated and saturated lipids, was not significantly affected by exposure to neutral conditions (2.17% divergence). Sesame oil, dominated by oleic (C18:1) and linoleic acid (C18:2) also had relatively minor changes in composition (4.42%). Samples with higher volumes of higher polyunsaturates are significantly affected. Linseed oil, dominated by linolenic acid (C18:3), has a divergence value of 59% from the reference linseed oil. Linseed divergence results from the loss of linolenic and most linoleic acids from the lipid mixture.

Alkaline conditions produce the most significant alterations in beef and sesame lipids. Beef fat and sesame oils diverge by 11.21% and 24.47% respectively as a result of declining relative proportions of linoleic acid in sesame and oleic acid in beef.

Comparison of fatty acid species in linseed and sesame oils enables the identification of the chemical processes that altered lipids in this experiment. In both products, linoleic acid (C18:2) is a significant component (sesame=35.95%, linseed=15.48%). In the neutral control, sesame oil is unaffected but linoleic acid is almost entirely removed from linseed oil. Linseed oil also contains large amounts of more reactive linolenic acids (45.46%), which are not found in sesame oil. Free radical chain reactions explain preservation differences between linseed and sesame

oils. Linolenic acid molecules rapidly form peroxyl radicals and other oxidation products (see Chapter 2), which then accelerate the conversion of other lipids to free radical derivatives. The abundance of free radicals in linseed oil results in the alteration of both linolenic and linoleic acid. Sesame oil, lacking large volumes of reactive higher polyunsaturates, does not produce free radicals in the same abundance, and hence shows a lesser degree of alteration. Acidic conditions appear to inhibit the development and/or action of free radicals and other oxidative agents, and basic conditions promote their formation and/or accelerate their functions.

5.2.6 Microbial alteration

In the microbial alteration experiment, RFOs were inoculated with a microbial solution, exposed to oxidising conditions for 30 days at room temperature. For this a piece of filter paper was impregnated with one of the four RFOs. The impregnated filter paper was then placed in a Petri dish with a small volume of water and a commercial microbial culture added. After 30 days of exposure, lipids were recovered from the petri dishes and analysed. The microbial exposure experiment produced only minor alterations in lipid composition. DIs for beef (4.36%), sesame (2.76%) and fish (5.73%) were low, within the background range of variation. Linseed oil was slightly more altered with a divergence index of (7.08%). The divergence is entirely a result of a reduced proportion of linolenic acid (C18:3). The alteration of linseed oil is more likely to result from oxidation and autoxidation caused by other environmental factors rather than a microbial effect.

The result indicates that the microbial species utilised in this experiment do not significantly affect residue compositions, either through the addition of endogenous lipids or the alteration and removal of existing lipids. The relatively large volume of lipid product may have obscured microbial changes in lipid composition.

5.2.7 Reanalysis of experimental residues

This experiment measures the alterations in ceramic residues using the same three year old experimental ceramics as in the leaching experiment above. In the

reanalysis of the 2004 residues experiment the volumes of residue recovered varied. In some residues recovered in 2007 the recovered volume was above the 2004 recovery while the opposite was observed in other residues (Table 5.9). For both extractions care was taken to ensure comparable ceramic sections were sampled. However, recovery differences between 2004 and 2007 residues are most likely caused by samples being taken from slightly different places along the ceramic profile in each extraction. A residue produced by boiling fish (rainbow trout) was analytically viable in 2004, but by 2007 residue recoveries were too small for analysis.

Residue	2007 yield (mg)	2004 yield (mg)
Barley	3.2	8.1
Beef	9.7	6.7
Chicken	58	31.7
Chickpea	7	13.3
Duck	41.7	33.1
Linseed	421.5	473.8
Olive	282.4	245.4
Pea	5.3	6.4
Pork	16	27.1
Sesame	464.6	297.9
Sheep	12.7	23.9
Wheat	1.9	4.8

Table 5.9: Comparison of the residues recovered in 2004 and 2007 from the ceramics used in the cooking experiment.

The analytic technique used to examine fatty acid composition in 2004 (HPLC-MS) was less suited to the identification of a broad range of fatty acids than that used in this study (GC-MS). Because of the analytic differences between the two datasets, the precision of divergence indices is likely to be lower than in other sections of this study. To improve the comparability of the datasets, I modify the 2007 data to include only lipid species observed using 2004 analytic protocols (appendix 2).

Three groups of residues can be identified by comparing the divergence indices and total lipid recoveries (Table 5.9, 5.10): a) a group of animal fats that have relatively low divergence (<13%) (sheep, beef, chicken, pork, duck); b) a group of plant oils with total lipid recoveries (>35mg/1g) and large divergences (>23%) (linseed, sesame, olive); and c) a group of plant products with low total lipid recoveries (<1mg/1g) and variable degrees of divergence (9-28%) (barley, pea, chickpea, wheat).

Residue	DI
Sheep	9.46
Barley	9.73
Beef	10.83
Chicken	11.76
Pork	13.41
Duck	13.46
Pea	21.92
Chickpea	22.99
Linseed	23.48
Wheat	27.22
Sesame	47.61
Olive	78.14

Table 5.10: DI comparing the difference between the molecular compositions of residues recovered from experimental ceramics 2004 and 2007.

Transformations during storage appear to be largely the result of preferential alteration of unsaturated fatty acid species. The degree and nature of these transformations varies considerably between samples. For example, in 2004 olive oil residues contained more than 75% oleic acid and beef residues contained 44.5% oleic acid. By 2007 olive oil residues contained only 3% oleic acid while the proportion in beef remained virtually unchanged (45.3%). Similar characteristics are seen in all animal and plant oil residues, with the animal residues retaining much higher proportions of their monounsaturated and polyunsaturated fatty acids than plant oils (Table 5.11).

2004	CX:0 %	CX:1 %	CX:2+ %	2007	CX:0 %	CX:1 %	CX:2+ %
Beef	46.70	46.08	3.47	**Beef**	49.76	46.58	0.93
Chicken	37.75	46.73	4.35	**Chicken**	45.27	49.24	0.29
Duck	38.98	46.09	4.03	**Duck**	51.92	44.60	0.00
Pork	44.59	41.03	5.43	**Pork**	54.15	42.22	0.33
Sheep	53.19	38.92	3.78	**Lamb**	58.21	39.45	0.40
Linseed	78.00	20.36	1.31	**Linseed**	100.00	0.00	0.00
Olive	18.77	76.89	2.53	**Olive**	96.91	3.09	0.00
Sesame	52.40	47.15	0.10	**Sesame**	100.00	0.00	0.00
Barley	84.31	11.93	1.74	**Barley**	80.56	17.80	1.64
Chickpea	62.29	21.99	12.93	**Chickpea**	76.98	20.80	2.23
Pea	65.60	22.65	7.01	**Pea**	78.31	19.29	2.40
Wheat	58.85	19.04	19.75	**Wheat**	72.06	24.93	3.01

Table 5.11: Relative concentrations of saturated (CX:0), monounsaturated (CX:1) and polyunsaturated (CX:2+) fatty acids in residues extracted from the experimental ceramics in 2004 and 2007.

With one exception (barley), the relative importance of unsaturated fatty acids (CX:1 + CX:2) declined, reflecting the higher liability of unsaturated fatty acids to alteration (Table 5.11). In some instances, the relative proportion of monounsaturated fatty acids increased over time as a result of the preferential loss of polyunsaturated

lipid species. The exception to this trend - barley residue - had a relatively similar proportion of polyunsaturated fatty acids in 2004 (1.7%) and 2007 (1.6%) but had higher proportions of monounsaturated fatty acids in 2007 (12% in 2004, 17.8% in 2007).

The high proportions of oleic acid in low-lipid plant residues relative to plant oil residues may be the result of contamination, most likely microbial, during storage. The low residue recoveries from the barley ceramic support this interpretation. Low volumes of source-related residue within the ceramic would increase the relative contribution of microbial action. This may also explain the wide divergences and presence of unsaturated fatty acids in other low recovery plant residues. However, a microbial origin for oleic acid in low-lipid residues should also produce an increased proportion of other unsaturated fatty acids, most importantly C18:2 and C18:3, as well as unsaturated acylglycerols. These molecular species are either reduced relative to their 2004 proportions or entirely absent.

An alternative, and more plausible, explanation for the presence of unsaturated fatty acids in low-lipid recovery residues is sacrificial decomposition. The residues of experimentally cooked plant oils are composed almost entirely of lipids. On the other hand, plant residues with low total lipid recoveries are prepared from whole foods, producing a more molecularly complex residue, including molecular species more liable to alteration than oleic acid (e.g. proteins, nucleonic acids, complex carbohydrates). The more complex and fragile molecules in whole food residues are liable to preferential degradation providing a buffer to lipid alteration. In plant oil residues, no such buffer exists, resulting in a more rapid alteration of unsaturated lipid species (similar to that observed in Section 5.2.1.3).

Alterations in the triacylglycerol fractions of the residues resemble the fatty acid data. In 2004, all residues except wheat contained analytically viable volumes of triacylglycerols. Some residues contained only a few triacylglycerol species in relatively low concentrations in 2004, such as barley, chickpeas and linseed oil (Table 5.12). By 2007, olive oil was with only plant residue with analytically viable volumes of triacylglycerols, and these were so minor that they were almost undetectable. Animal residue triacylglycerols were much more resistant to alteration. Some compositional changes during storage were observed, with the absence of some minor, unsaturated triacylglycerols in 2007. Improved chromatographic techniques

used in 2007 resulted in the identification of additional triacylglycerol species, resulting in a higher number of identified triacylglycerols in beef residues.

Residue	2004	2007
Barley	3	0
Beef	20	23
Chicken	20	15
Chickpeas	5	0
Duck	21	15
Linseed Oil	5	0
Olive Oil	19	3
Pea	8	0
Pork	30	16
Sesame Oil	12	0
Sheep	28	23
Wheat	0	0

Table 5.12: Comparison of the number of triacylglycerol species identified between residues extracted from the experimental ceramics in 2004 and 2007.

Compositionally, animal residue acylglycerols resemble the fatty acid data with acylglycerol species predominantly composed of saturated and monounsaturated moieties (e.g. POP, OOP, OOO, SOO, SOS, SSS).

5.3 Chemotaxonomic Application

In this section I explore the applicability of current chemotaxonomic methodologies to the experimentally produced datasets. On one hand, chemotaxonomic methodologies allow a more detailed and targeted exploration of experimental datasets. On the other, their application to the controlled experimental datasets of this study allows an evaluation of the relative robusticity of different chemotaxonomic methods. This section considers three main chemotaxonomic methods: biomarkers, ratios and multivariate mathematical analysis.

5.3.1 Biomarkers

Several established residue biomarkers can be identified within the fatty acid and acylglycerol fractions of reference fats and oils. Beef fat contains branched chain fatty acids and triacylglycerols containing odd-numbered fatty acid moieties (C15:0, C17:0, C17:0br, C18:0br), which have been used to diagnose ruminant products.

Similarly, fish oil contains fatty acids with multiple branched chain fatty acids (C20:0br4), which have been proposed as markers of fish products. The acylglycerol and fatty acid fractions of linseed and sesame oils contain no recognised biomarker lipids.

In addition to recognised biomarkers a number of biomarker-like lipids (pseudo biomarkers) can also be identified. These are identified based on their similarity in terms of structure and abundance (<3%) to recognised biomarkers. A lower limit of 0.1% was utilised to filter lipid selections as below this level lipids were not always identifiable. The biomarker and pseudo biomarker molecules used in this analysis are (pseudo-biomarkers are in italics):

Beef: C15:0, C17:0br, C17:0, C18:0br

Fish: *C15:0br, C17:0br*, C20:0br4, *C20:0*

Linseed: *C20:0, C22:0, C24:0*

Sesame: *C14:0, C20:0, C22:0*

The fatty acid biomarkers selected are robust to all simulated cultural and post-depositional experiments conducted in this study. As lipid products become more altered, the proportion of fatty acid biomarkers generally increases. Residues from the vessel reuse experiment contained fatty acid biomarkers and pseudo-biomarkers of all three products cooked within them, although in some cases mixing reduced their relative proportions. Sesame, linseed and beef residues from the reanalysed 2004 ceramics contained all biomarker and pseudo biomarker molecules listed in this analysis.

The analysis of acylglycerol biomarkers uses two classes of biomarker and pseudo-biomarker: fully saturated and partially unsaturated triacylglycerols. The former are composed entirely of saturated fatty acid moieties while the latter contain one monounsaturated fatty acid moiety. Partially saturated triacylglycerols, sometimes observed in archaeological residues, can be diagnostically important biomarkers. The following triacylglycerol biomarkers and pseudo-biomarkers were identified for this analysis (pseudo-biomarkers in italics):

Beef: MaPP, MaPS, MaSS , MaOP, MaOS

Linseed: *POP, SOP, SOS*

Sesame: *PPP, SOP*

Most samples analysed for acylglycerols in this study contain the biomarker and pseudo-biomarker triacylglycerols identified for this analysis. As with fatty acids,

the relative proportions of these molecules tend to increase as the lipid products are altered. The exceptions to this are the reanalysed experimental ceramic residues for linseed and sesame, which do not contain analytically viable volumes of acylglycerols.

5.3.2 Ratio analysis

In this section I apply Eerkens' ratio technique to the reference and experimentally altered fats and oils to determine the reliability and robusticity of residue source identifications. As detailed in Chapter 3, I use Eerkens' ratio method because of its reported capacity to differentiate a wide range of organic products. For the experimental data, I apply Eerkens' 'fresh' ratio values as these have smaller ranges and more likely to reveal significant alterations.

Application of Eerken's ratios to all reference and experimental samples correctly identified terrestrial mammal (beef fat), seed and nut (linseed and sesame oils), and fish (fish oil) categories (Figures 5.9, 5.10). Some samples lacked the fatty acids required to perform all ratio tests. Linseed oil contained only traces of C12:0, which were often below the limits of detection. When detected, C12:0 formed only a very minor trace component (<0.01%), but produces ratio values in the range expected for seeds or nuts. The ratio of C12:0/C14:0 primarily discriminates plant (greens, seeds or nuts, and berries) from animal products (fish and terrestrial mammals). While less conclusive, the remaining three ratios categorise linseed as a seed or nut product.

Several samples also lack C16:1, complicating analysis by C16:1/C18:1 ratios. These samples are residues extracted from base sections of the 2004 experimental ceramics (linseed and sesame residues), and their ultrasonicated derivatives. C16:1/C18:1 principally discriminates 'greens', and to a lesser extent fish, from other plant and animal products. In this instance, the absence of this ratio does not interfere with the identification of residue sources. The lack of C16:1 in some residues after 3 years highlights the problems of using unsaturated fatty acids to interpret archaeological remains.

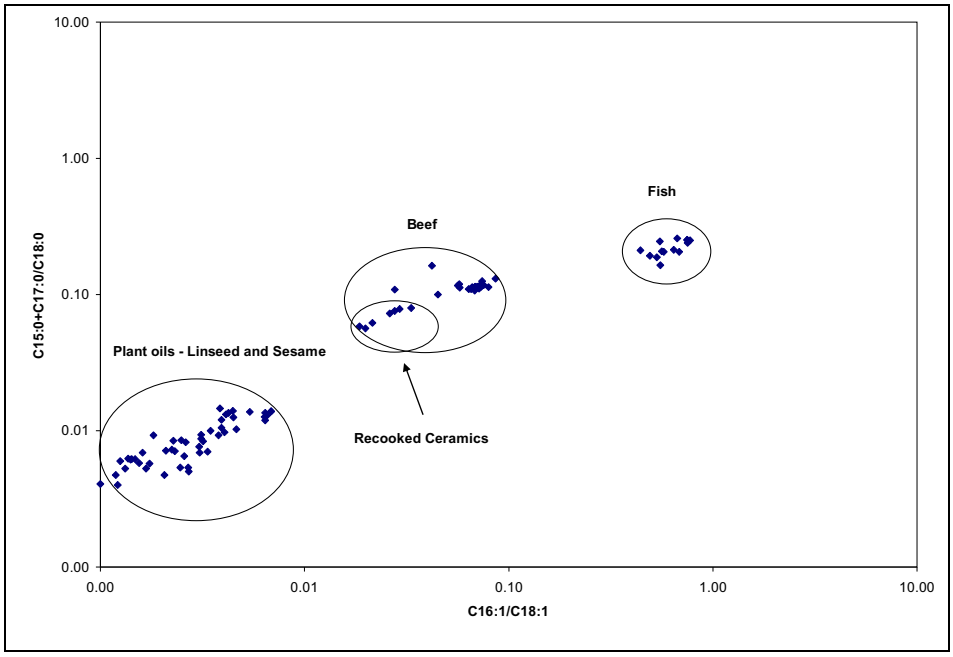

Figure 5.9: Logarithmic biplot using Eerkens' ratios of C16:1/C18:1 and C15:0+C17:0/C18:0 for the experimentally altered samples and RFOs. Recooked ceramics refers to the experimental reuse residues.

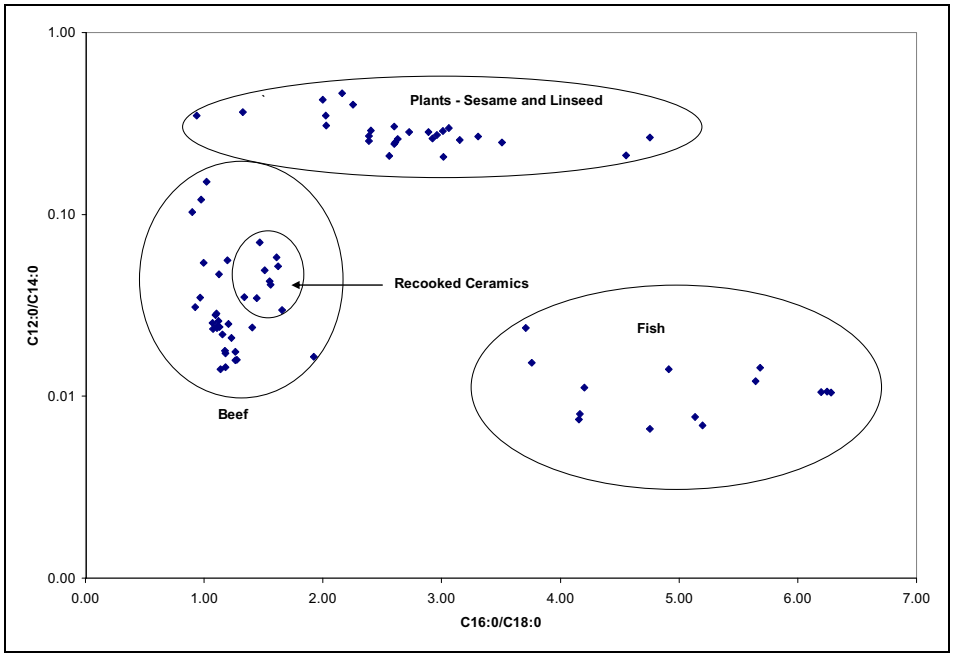

Figure 5.10: Biplot using Eerkens' ratios of C16:0/C18:0 and C12:0/C14:0 for the experimentally altered samples and RFOs. Recooked ceramics refers to the experimental reuse residues.

All plant residues extracted from the waterline sections of 2004 experimental ceramics are seeds or seed-derived (wheat, barley, chickpea, green pea, sesame, olive and linseed), and are characterised as 'seeds or nuts' by Eerkens' ratios (Figure 5.11). Animal products are composed of terrestrial mammals (beef, pork and sheep) and avians (chicken and duck) (Figure 5.11, 5.12). Eerkens did not discriminate avian products in his analysis, probably due to Malainey's database (the basis for Eerkens' analysis) containing only one avian sample (grouse). In this analysis, the ratios for avians and terrestrial mammals are virtually identical. If Eerkens' 'terrestrial mammal' group is widened to include avians, ratios successfully identify all residue samples except olive, which can only be characterised as 'animal or seed/nut' due to a lack of C12:0. The absence of C12:0 is a feature of olive oil and not a result of degradation. No plant residues retained both C16:1 and C18:1. C16:1/C18:1 principally discriminates greens from other plant products, which are not a feature of this dataset.

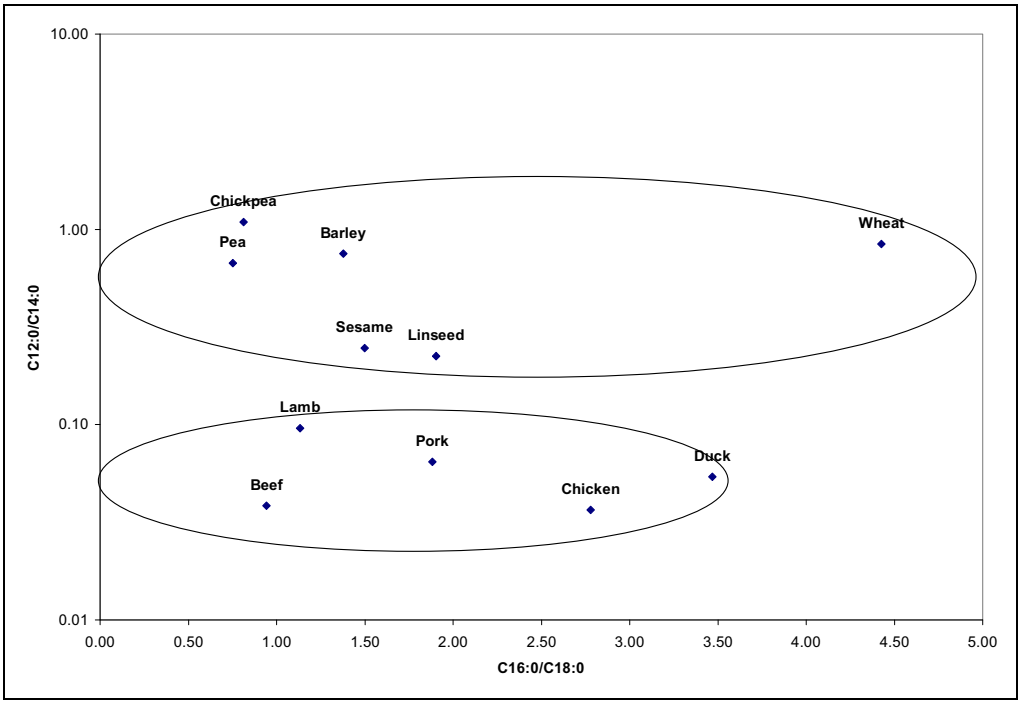

Figure 5.11: Biplot using Eerkens' ratios of C16:0/C18:0 and C12:0/C14:0 for residues 2007 extraction of the 2004 experimental samples.

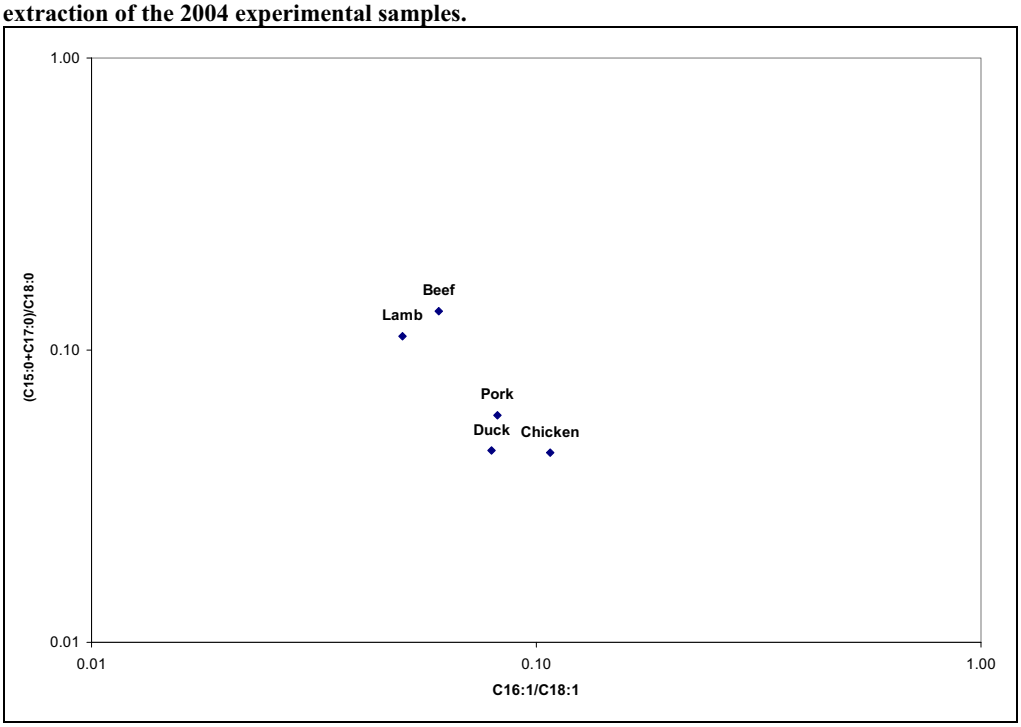

Figure 5.12: Logarithmic biplot using Eerkens' ratios of C16:1/C18:1 and C15:0+C17:0/C18:0 for residues 2007 extraction of the 2004 experimental samples (note: lack of plant residues reflects the absence of required unsaturated fatty acids for C16:1/C18:1 ratio).

5.3.3 Principal Components Analysis

Reference and experimentally altered fatty acid data generally produce compact groups that relate to biological sources in Principal Components Analysis (PCA) projections (Figure 5.11). With few exceptions, beef, linseed, sesame and fish form single distinct groups. Sesame and linseed cluster more closely together than other samples reflecting their similar compositions and botanical sources. Fish is very spatially distinct, reflecting the high proportion of unique molecules found in fish oil. Residues recovered from vessel reuse experiment occur in a clearly defined but intermediate group that forms between beef, linseed and sesame, reflecting the composite nature of these residues. The relative proximity of individual vessel reuse residues to beef, linseed or sesame groups reflects their compositional similarity to these products.

Nine compositional groups can be identified within the experimental PCA (Figure 5.14). Groups are generally inner cores of relatively unaltered products and outer groups that follow a trajectory towards higher degrees of taphonomic transformation. For brevity, core groups are not exhaustively described but contain all samples not mentioned in outer groups.

Groups 1, 2, and 3 contain the beef samples. Group 2 falls closest to the core beef group (Group 1) and contains beef lipids heated to 300°C for two and three hours. Group 3 is the greatest distance from the core and contains beef lipids heated to 300°C for four and five hours. Group 4 is the linseed core group, while Group 5 contains linseed lipids exposed to neutral (pH7) and basic (pH8) conditions, as well as linseed oil heated to 300°C for four hours.

Somewhat unexpectedly, structural groups cannot be differentiated from sesame data, which form Group 6. Fish oil form two groups, 7 and 8. Group 7 contains most fish oil samples, while Group 8 contains those samples exposed to acidic, neutral and basic conditions. No fish oil samples were heated to 300°C. Fish oil does not follow the general trend for samples to form a trajectory based on their degree of alteration. The highly altered (divergence indices >43%) pH exposure samples form a unique group which does not relate to the core fish oil group. The dissociation between the two fish oil groups reflects their large compositional

differences. Group 8 almost entirely lacks the very highly unsaturated fatty acids that define Group 7. Group 9 contains residues from the ceramic reuse experiment.

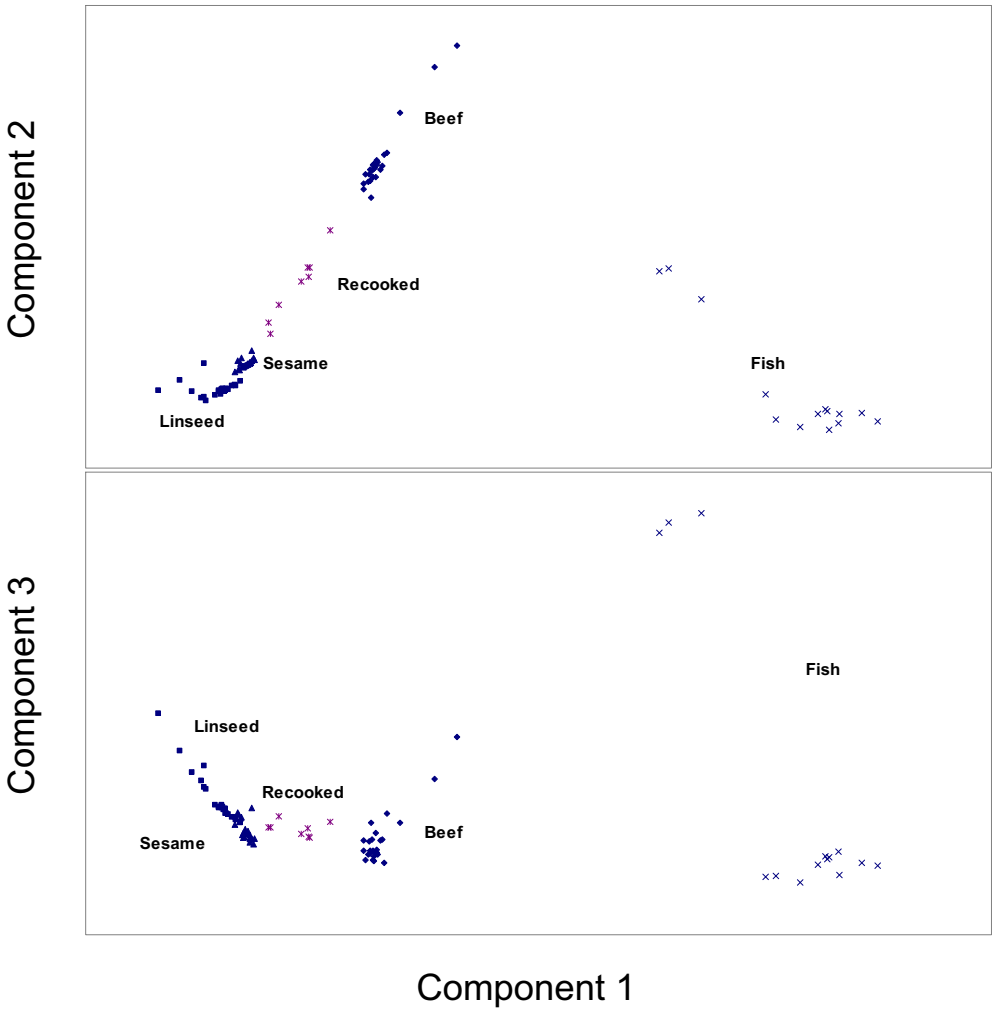

Figure 5.13: Principal Components Analysis of RFOs, experimentally altered samples and reuse residues. Biplots of first against second (upper) and first against third components (lower) showing five key lipid classes (beef, linseed, sesame, fish and recooked ceramic residues).

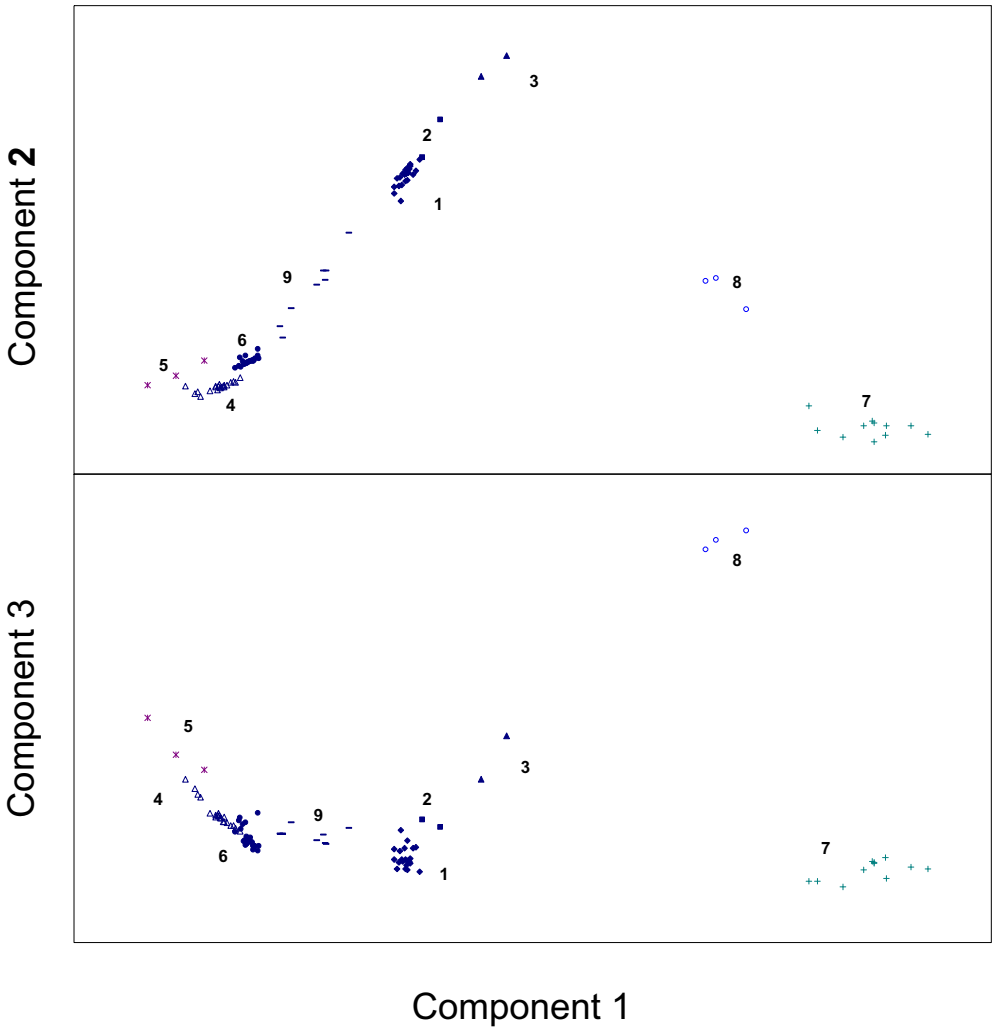

Figure 5.14: Principal Components Analysis of RFOs, experimentally altered samples and reuse residues. Biplots of first against second (upper) and first against third components (lower) for experimental samples showing structural groups labelled 1-9: 1 (diamonds), 2 (squares), 3 (filled triangles), 4 (hollow triangles), 5 (asterisks), 6 (filled circles), 7 (crosses), 8 (hollow circles), 9 (bars).

5.4 Archaeological Residues

In the following sections I describe the results of the archaeological component of this study. I first describe residue recoveries and consider general molecular features of the archaeological data. Next, I interpret the archaeological residues by the three chemotaxonomic methodologies used in this study (biomarkers, ratios and Principal Components Analysis). Complete tabulated archaeological results are presented in appendix 2.

5.4.1 Residue recoveries

Residue recoveries vary considerably both between samples and between classes of residue (i.e. between 0.5mg and 8.3mg for eight grams of powdered ceramic) (Table 5.13). To minimize the distortion of average recoveries of ware types caused by high but infrequent recoveries from certain ceramics (e.g. AIA 1780, AIA 2277 and YHP 106) I use median rather than mean values.

The Troy pithoi are characterised by low residue recoveries. Large, local storage pithoi have a median recovery of 0.6mg, while imported wares have a median recovery of 0.7mg (range = 0.5 – 5.5mg). One local pithos sample had a much higher residue recovery of 5.5mg (AIA 1780).

Red Lustrous Wheel-made wares have relatively high residue recoveries (median = 2.1mg, range = 0.7 – 3.7 mg). The median residue recovery varies between the two sets of Lydion residues. Residues recovered from Sardis Lydions have an average recovery 1.5mg (range = 1.2 – 1.7mg). In contrast, Gordian Lydions have substantially higher residue recoveries with a median of 2.4mg (range = 1.7 – 2.6mg).

Cooking pot and other utilitarian wares are relatively rich in residues, with a median recovery of 2.1mg (range = 0.6 – 5.1mg) for cooking pots from Boğazkoy. The Boğazkoy cooking pot residues include one sample (AIA 2277) with a recovery of 5.1mg. The sampling strategy used for residues from Gordion differs slightly from other samples in this study. While other classes of residue represent all extracts from archaeological ceramics, the Gordion residues are a subset of a larger group of residues analysed in an earlier study. These samples were partly selected for their relatively high residue recoveries. The sampling difference causes the relatively high median recovery of 2.6mg (range = 1.5 – 8.3mg) in Gordion cooking and utilitarian ware residues. Two Gordian residues are unusually high, YHP 106 with 8.3mg of recovered residues and YHP 514 with 7.2mg.

Soil and sediment extracts also have substantially different residue recoveries. While soils collected from environmental contexts have median recoveries of 1.4mg per 8 grams of soil (range = 1.1 - 2.2mg), those associated with Lydions have median recoveries of 2.4mg (range = 2.0 – 4.2). There is little difference between recoveries

from environmental soil extracts from Gordion and those from Sardis. The former has a median recovery of 1.45mg while the latter a median of 1.3mg.

Sample	Ware	Site	recovery (mg)
AIA 1861	Lydion	Gordion	2.6
AIA 1857	Lydion	Gordion	2.5
AIA 1858	Lydion	Gordion	2.4
AIA 1863	Lydion	Gordion	2.3
AIA 1870	Lydion	Gordion	1.7
AIA 1743	Lydion	Sardis	1.7
AIA 1724	Lydion	Sardis	1.6
AIA 978	Lydion	Sardis	1.5
AIA 982	Lydion	Sardis	1.5
AIA 1725	Lydion	Sardis	1.2
AIA 1776	Lydion Sediment	Sardis	4.2
AIA 1769	Lydion Sediment	Sardis	2.4
AIA 1771	Lydion Sediment	Sardis	2.4
AIA 1777	Lydion Sediment	Sardis	2.4
AIA 1772	Lydion Sediment	Sardis	2.0
AIA 1773	Lydion Sediment	Sardis	2.0
AIA 1780	Pithos	Troy	5.5
AIA 1787	Pithos	Troy	1.7
AIA 1785	Pithos	Troy	1.2
AIA 2224	Pithos	Troy	1.0
AIA 1782	Pithos	Troy	0.8
AIA 2226	Pithos	Troy	0.7
AIA 1784	Pithos	Troy	0.6
AIA 1781	Pithos	Troy	0.6
AIA 2228	Pithos	Troy	0.6
AIA 1778	Pithos	Troy	0.5
AIA 1786	Pithos	Troy	0.5
AIA 2225	Pithos	Troy	0.5
AIA 2227	Pithos	Troy	0.5
AIA 1788	Pithos (imported)	Troy	0.8
AIA 1791	Pithos (imported)	Troy	0.7
AIA 1789	Pithos (imported)	Troy	0.7
AIA 1790	Pithos (imported)	Troy	0.6
AIA 1792	Pithos (imported)	Troy	0.5
AIA 2233	Red Lustrous	Bogazkoy	3.7
AIA 2232	Red Lustrous	Bogazkoy	3.0
AIA 2231	Red Lustrous	Bogazkoy	2.9
AIA 2242	Red Lustrous	Bogazkoy	2.8
AIA 2235	Red Lustrous	Bogazkoy	2.1
AIA 2237	Red Lustrous	Bogazkoy	1.9
AIA 2243	Red Lustrous	Bogazkoy	1.9
AIA 2238	Red Lustrous	Bogazkoy	1.8
AIA 2236	Red Lustrous	Bogazkoy	1.4
AIA 2240	Red Lustrous	Bogazkoy	1.1
AIA 2241	Red Lustrous	Bogazkoy	0.7
AIA 2277	Cooking Pot	Bogazkoy	5.1
AIA 2280	Cooking Pot	Bogazkoy	2.2
AIA 2278	Cooking Pot	Bogazkoy	1.9
AIA 2279	Cooking Pot	Bogazkoy	1.9
AIA 2276	Cooking Pot	Bogazkoy	1.5
AIA 2282	Cooking Pot	Bogazkoy	1.3
AIA 2274	Cooking Pot	Bogazkoy	0.6
YHP 106	Cooking Pot	Gordion	8.3
YHP 514	Cooking Pot	Gordion	7.2
YHP 503	Cooking Pot	Gordion	3.5
YHP 156	Cooking Pot	Gordion	2.8
YHP 529	Cooking Pot	Gordion	2.3
YHP 242	Cooking Pot	Gordion	2.2
YHP 050	Cooking Pot	Gordion	2.1
YHP 342	Cooking Pot	Gordion	2.1
YHP 160	Cooking Pot	Gordion	1.9
YHP 105	Cooking Pot	Gordion	1.8
YHP 074	Cooking Pot	Gordion	1.8
YHP 153	Cooking Pot	Gordion	1.8
YHP 249	Cooking Pot	Gordion	1.8
YHP 161	Cooking Pot	Gordion	1.6
YHP 341	Cooking Pot	Gordion	1.6
YHP 508	Cooking Pot	Gordion	1.6
YHP 053	Cooking Pot	Gordion	1.5
YHP 501	Cooking Pot	Gordion	1.5
AIA 2291	Soil	Gordion	1.5
AIA 2289	Soil	Gordion	1.4
AIA 2262	Soil	Sardis	2.2
AIA 2260	Soil	Sardis	1.3
AIA 2264	Soil	Sardis	1.3
AIA 2261	Soil	Sardis	1.1

Table 5.13: Mass of residue recovered from eight grams of powdered archaeological ceramic sorted by site, ware and recovery.

5.4.2 General molecular features of archaeological residues

All archaeological residues contain oleic acid and linoleic acid in proportions of up to 50% and 20% respectively. Most samples also contain palmitoleic acid (C16:1), in proportions of up to 40%. The experimental data and previous archaeological observations indicate that the archaeological survival of such high proportions of unsaturated fatty acids is unlikely. The high proportions of unsaturated fatty acids in some residues may reflect the sampling strategy employed in this study. Generally, residue analyses exclude residues with recoveries below a certain threshold (e.g. 0.2mg/g). This study examined all residues regardless of lipid recoveries, and may include a number of samples with little or no culturally-derived molecules. In these instances, microbes or other environmental factors are the only possible source for recovered lipid residues, resulting in relatively high proportions of unsaturated lipids.

The proportion of linolenic and oleic acid is broadly related to the intensity of chromatographic signals in GC-MS analyses. These molecules tend to form a higher proportion of total fatty acids in samples that have low lipid recoveries and weak chromatographic responses. The intensity of chromatographic signals is caused by the relative concentrations of molecules within the analyte and can be used to estimate the overall lipid volume within archaeological residues. Therefore, high proportions of oleic and linoleic acids occur in archaeological residues that contain relatively low concentrations of lipid. Figure 5.15 contrasts the signal intensity and relative proportions of C16:0, C18:1 and C18:0 for AIA 1789 (Figure 5.15A) and AIA 2277 (Figure 5.15B). AIA 1789 is characterised by a weak signal and high proportions of C18:1 (38.9%) and C18:2 (5.3%). In contrast, AIA 2277 is characterised by a strong chromatographic trace and relatively low volumes of C18:1 (7.5%) and C18:2 (0.03%). Similarly, total lipid recoveries from the two ceramics vary considerably (AIA 1789 = 0.7mg, AIA 2277 = 5.1mg). Most oleic acid in AIA 1789 most occurs in the C18:1(*cis*-9) configuration. In contrast, C18:1(*cis*-9) in AIA 2277 forms only one of at least five oleic acid isomers and is less than half of the observed oleic acid. These oleic acid isomers are not observed in residues with low signal intensities, and have been previously associated with archaeological ruminant fats.

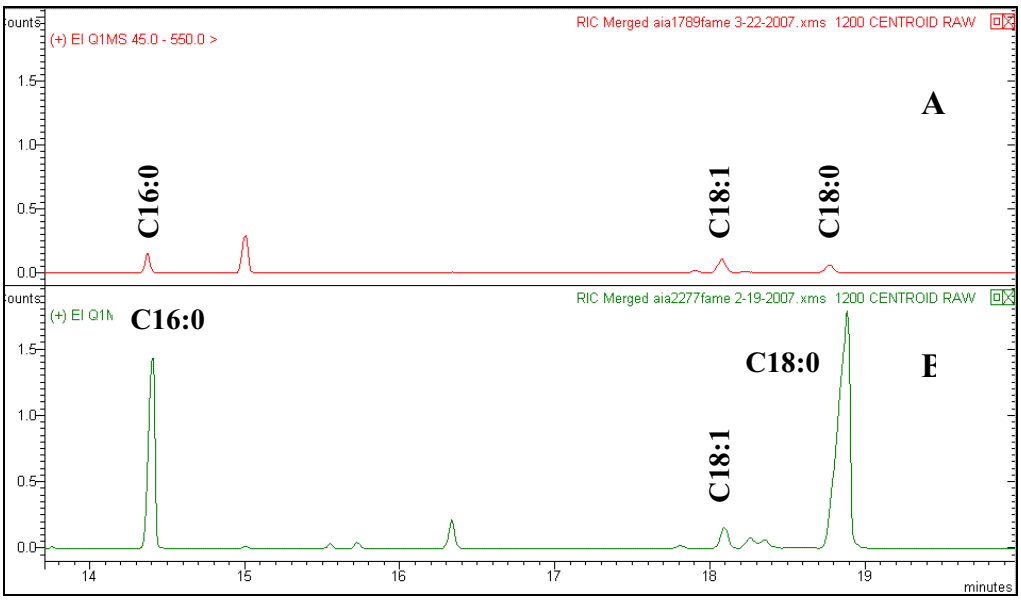

Figure 5.15: Partial gas chromatograms for the archaeological residues from a a) Troy pithos (AIA 1789), and b) a Bogazkoy cooking pot (AIA 2277) showing differences in relative intensity of the signals for C16:0, C18:1 and C18:0.

Oleic and linoleic acids observed in archaeological residues are not likely to be an artefact of extraction and preparation procedures. Blanks prepared during residue extraction and preparation did contain some fatty acid species including C16:0, C18:0 C18:1 and C18:2 (appendix 1f), but these were minimal and cannot account for the volume of lipid in archaeological residues (Figure 5.16). No traces of squalene or cholesterol, typical markers of recent handling, were observed in extraction blanks.

All archaeological residues contain plasticizers and small volumes of hydrocarbons. These molecules may in part result from storage techniques (i.e. plastic bags), but plasticizer contamination is a common problem in all lipid analyses. The low volumes of lipids recovered from archaeological residues increase the relative proportions of plasticizers and hydrocarbons. These often form significant peaks in GC-MS chromatograms of archaeological residues, but these do not interfere with fatty acid analysis.

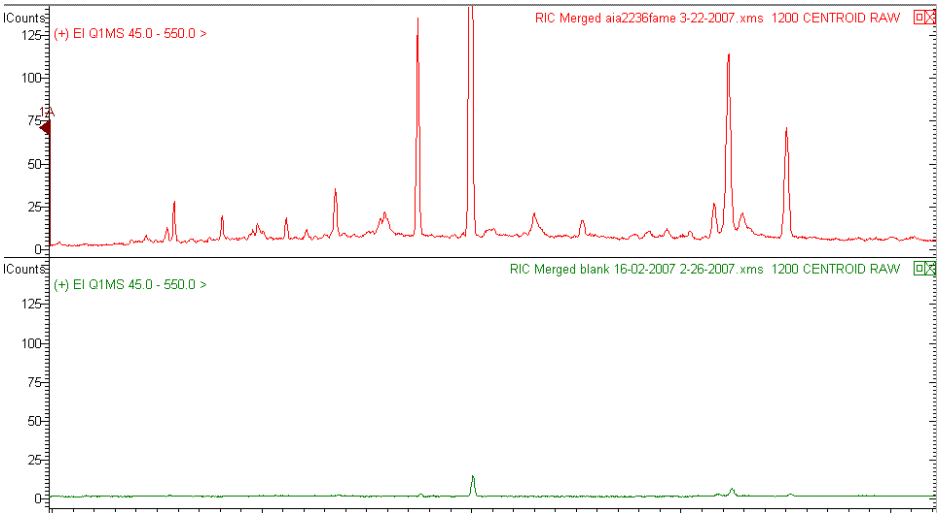

Figure 5.16: Partial gas chromatograms for a typical archaeological residue (AIA 2236) with an extraction blank low levels of contamination present.

5.4.3 Archaeological residue biomarkers

5.4.3.1 Pithoi

Residues extracted from several of large storage pithoi (AIA 1781, 2226 and 2228) contain significant volumes of palmitoleic acid and palmitoleic acid-based acylglycerols (e.g. PoPoPo, PoPoP, PoPo, PoP). Palmitoleic acid occurs in proportions of up to 40% and occurs as C16:1(*cis*-11) rather than the more typical C16:1(*cis*-9) (Figure 5.17). The relative proportions and structural organisation of palmitoleic acid is typical of soil yeasts and suggests the addition of a microbial component to these residues (Bradley et al. 2006; Drijber et al. 2000).

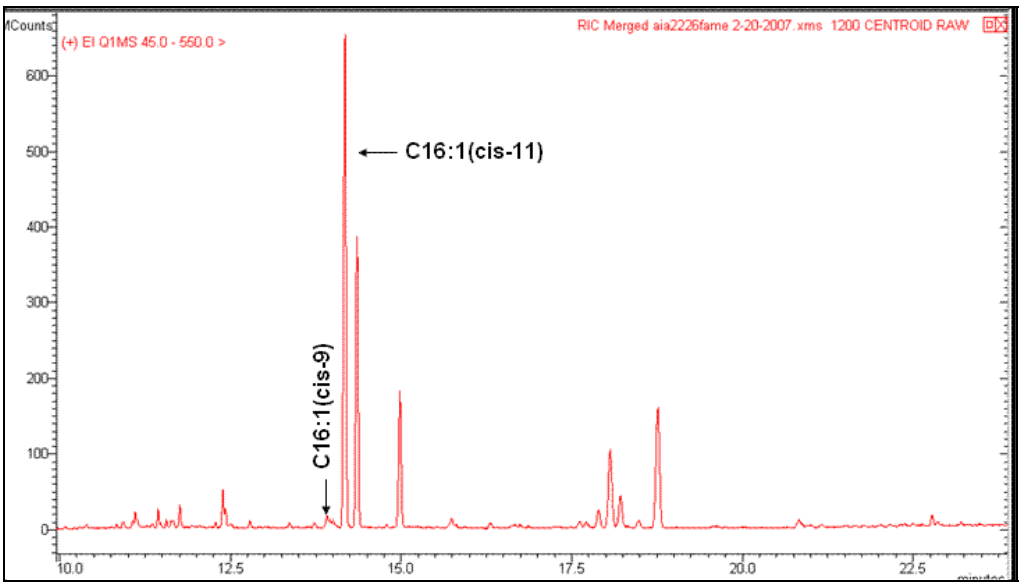

Figure 5.17: Gas Chromatograph of the Trojan Pithos AIA 2226 showing the large relative concentration of C16:1(*cis*-11) relative to C16:1(*cis*-9).

Terpenoids are also observed in one pithos residue (AIA 1780, Figure 5.18) This sample contains both diterpenes and triterpenes. Mixtures of di- and triterpenes do no occur in natural products (Evershed et al. 2001), and may reflect ancient mixing of resinous products.

Because this study is primarily focused on the analysis of acylglycerol and fatty acid fractions of residues, terpenoid molecules are not exhaustively explored. Comparing terpenoid mass spectra to the NIST database (NIST 2005) and Adam's essential oil database (Adams 2001) produced no conclusive molecular identifications. This may indicate that molecules may have been altered from their original forms. Pyrolytically altered terpenoid resins (pitch) have been observed in other archaeological analyses (Evershed et al. 1985; Regert 2004; Urem-Kotsou et al. 2002) and may account for this residue.

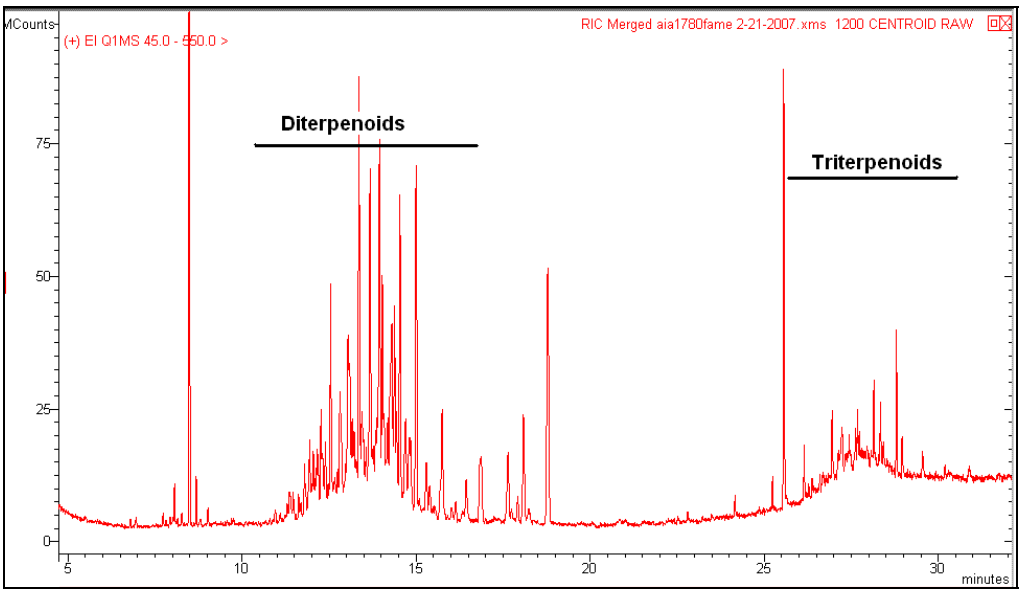

Figure 5.18: Gas Chromatogram of the Troy pithos AIA 1780 showing the high proportions of diterpenoids and triterpenoids present.

Two pithoi (AIA 1782 and 1785) contained triacylglycerols typical of ruminant fats. AIA 1782 contained small volumes of MaOO and MaOP, but also contained triacylglycerols with linoleic acid moieties that may be indicative of microbes. The composition of the ruminant-type triacylglycerols in AIA 1782 (i.e. containing one or more unsaturated moieties), as well as the presence of linoleic-acid containing triacylglycerols, suggest that the molecules may be of microbial origin.. AIA 1785 contained MaPS and MaSS, both indicative of ruminant fats.

5.4.3.2 Red Lustrous wheel-made ware

Five Red Lustrous wheel-made wares (AIA 2231, 2232, 2233, 2242, and 2243) contain large volumes of palmitic acid, long-chain hydrocarbons, and alcohols typically associated with beeswax (Figure 5.19). These molecules are the derivatives of palmitic wax esters, converted to their component molecules during methyl ester generation (appendix 1b).

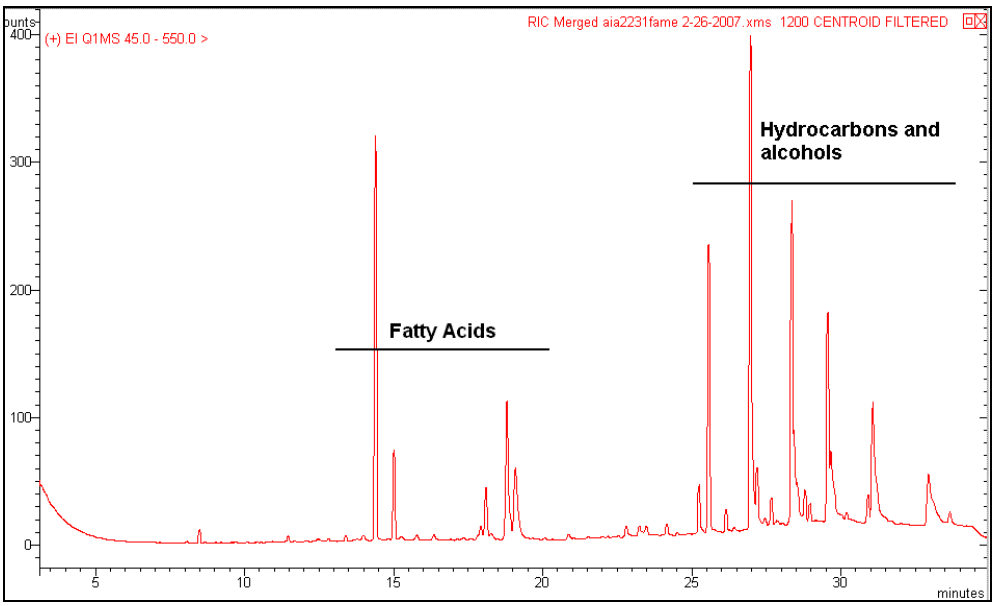

Figure 5.19: Gas Chromatogram of a Boğazkoy Red Lustrous Wheel-made sample (AIA 2231) showing hydrocarbons and fatty acids typical of beeswax.

5.4.3.3 Lydions

All Lydion samples contain branched-chain fatty acids, most frequently C15:0br (found in all Lydions), C16:0br (AIA 978, 1724, 1725, 1858, 1863 and 1870) and C17:0br (AIA 1724, 1725, 1857, 1858, 1861 and 1863). The Lydion from Gordion with the lowest residue recovery (AIA 1863) contains high proportions of C22:1 (11%). Branched chain fatty acids have been used to diagnose ruminant residues and C22:1 to identify plant products. However, these molecules may also also arise from soil microbes (Section 5.5.3.5).

Sterols were only observed in one sample, AIA 1857, which also contained significant volumes of triterpenoids (Figure 5.20). Among these were stigmastanol species, which are diagnostic biomarkers of plant products.

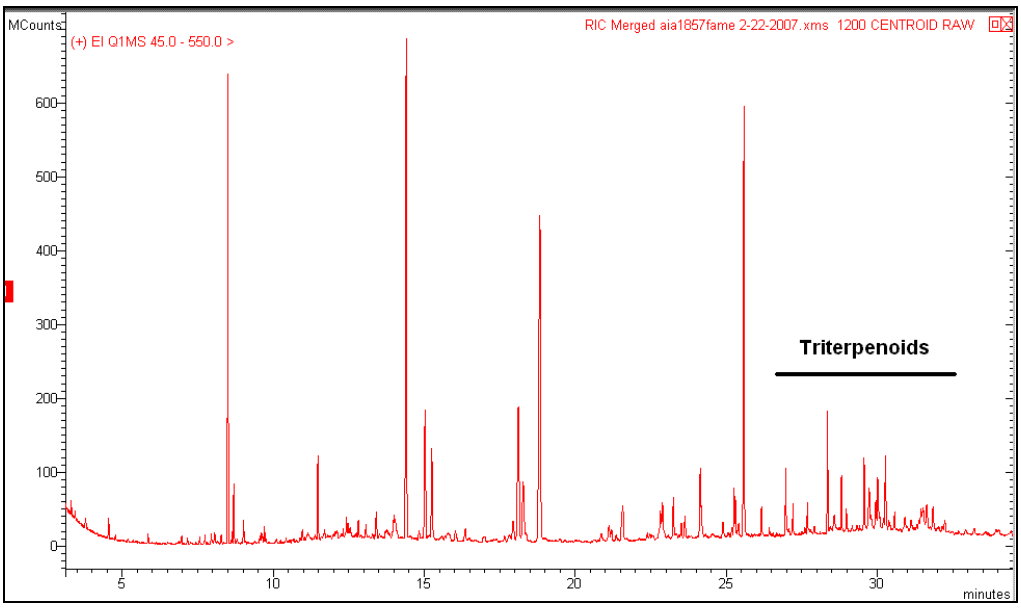

Figure 5.20: Gas Chromatograph of a Lydion from Gordion (AIA 1857) showing triterpenoid peaks.

Acylglycerol fractions also contain biomarkers indicative of ruminant products. All Lydions except AIA 1870 contain diacylglycerols consistent with the degradation of ruminant fats. All residues except AIA 1863 and 1870 contain MaS and AIA 1858 and 1861 contain MaP. These diacylglycerols can arise from the degradation of ruminant biomarkers such as MaOS, MaPS and MaOP (Chapter 2).

The biomarker evidence suggests that most Lydion residues are composed of ruminant animal fats with some evidence mixing with plant products. The sterol component of AIA 1857 provides strong evidence of plant products.

5.4.3.4 Cooking pot wares

Many cooking pot ware residues contain branched chain fatty acids typical of ruminant fats. Four of seven cooking pot residues from Boğazkoy (AIA 2276, 2277, 2279, 2280) contain branched-chain fatty acids (C15:0br, C16:0br, C17:0br, C18:0br) consistent with ruminant fats. Six residues from Gordion (YHP 161, 242, 503, 508, 514 and 529) also contained these branched-chain fatty acids.

AIA 2277 and 2279 contained triacylglycerols consistent with ruminant fat (MaPP, MaPS, MaSS). Other Boğazkoy cooking pots contained no acylglycerols.

YHP 242, 503, 508, 514 and 529 also contained triacylglycerol biomarkers consistent with ruminant fats.

5.4.3.5 Soils and sediments

Soils and sediments contain numerous lipid species usually considered diagnostic of key classes of organic products. Branched chain and odd chain fatty acids, usually considered diagnostic of ruminant fats, are a common feature of soil and sediment extracts. Eerkens (2005) has suggested that C22:1 can be used to identify plant products in archaeological residues, but this molecule is a major contributor to most soil extracts. Similarly, high volumes of long-chain saturated fatty acids, suggested as markers of plant products, are also a feature of soil extracts. A large proportion of soil microbes are plant products, therefore their resemblance to other plant products should be expected. The presence of these biomarkers in depositional contexts highlights the importance of clearly understanding and identifying potential environmental contributions to archaeological residues.

5.4.3.6 Summary of chemotaxonomic biomarkers in archaeological samples

Table 5.14 summarises the chemotaxonomic identifications made by biomarker analysis. The soil samples analysed in this study indicate that several established chemotaxonomic markers may arise from microbial additions to archaeological residues (e.g. branched chain fatty acids). Where these are identified in archaeological ceramic residues, they are given their usual chemotaxonomic identifications (e.g. ruminant fats for branched chain fatty acids). Where unique markers of microbes are identified, samples are identified as ‘microbial’.

Sample	Type	Source	Biomarkers
AIA 978	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 982	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1743	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1724	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1725	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1857	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs, Sterols
AIA 1858	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1861	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1863	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1870	Lydion	Ruminants, plants	BFAs, VLCSs, DAGs
AIA 1778	Pithos (local)	unidentified	
AIA 1782	Pithos (local)	Ruminants	TAGs
AIA 1784	Pithos (local)	unidentified	
AIA 1785	Pithos (local)	Ruminants	TAGs
AIA 1786	Pithos (local)	unidentified	
AIA 1787	Pithos (local)	unidentified	
AIA 1780	Pithos (local)	unidentified	
AIA 1781	Pithos (local)	Yeast/microbes	C16:1ω7 , TAGs
AIA 1792	Pithos (imported)	unidentified	
AIA 1788	Pithos (imported)	unidentified	
AIA 1789	Pithos (imported)	unidentified	
AIA 1790	Pithos (imported)	unidentified	
AIA 1791	Pithos (imported)	unidentified	
AIA 2224	Pithos (local)	unidentified	
AIA 2225	Pithos (local)	unidentified	
AIA 2226	Pithos (local)	Yeast/microbes	C16:1ω7, TAGs
AIA 2227	Pithos (local)	unidentified	
AIA 2228	Pithos (local)	Yeast/microbes	C16:1ω7, TAGs
AIA 2231	Red Lustrous	Beeswax	VLCS, C16:0, hydrocarbons
AIA 2232	Red Lustrous	Beeswax	VLCS, C16:0, hydrocarbons
AIA 2233	Red Lustrous	Beeswax	VLCS, C16:0, hydrocarbons
AIA 2235	Red Lustrous	unidentified	
AIA 2236	Red Lustrous	unidentified	
AIA 2237	Red Lustrous	unidentified	
AIA 2240	Red Lustrous	unidentified	
AIA 2241	Red Lustrous	unidentified	
AIA 2242	Red Lustrous	Beeswax	VLCS, C16:0, hydrocarbons
AIA 2238	Red Lustrous	unidentified	
AIA 2243	Red Lustrous	Beeswax	VLCS, C16:0, hydrocarbons
AIA 2274	Cooking pot	unidentified	
AIA 2276	Cooking pot	Ruminant	BFAs
AIA 2277	Cooking pot	Ruminant	BFAs, TAGs
AIA 2279	Cooking pot	Ruminant	BFAs, TAGs
AIA 2280	Cooking pot	Ruminant	BFAs
AIA 2282	Cooking pot	unidentified	
AIA 2278	Cooking pot	unidentified	
YHP 105	Cooking pot	unidentified	
YHP 106	Cooking pot	unidentified	
YHP 050	Cooking pot	unidentified	
YHP 053	Cooking pot	unidentified	
YHP 074	Cooking pot	unidentified	
YHP 153	Cooking pot	unidentified	
YHP 156	Cooking pot	unidentified	
YHP 106	Cooking pot	unidentified	
YHP 161	Cooking pot	Ruminant	BFAs
YHP 242	Cooking pot	Ruminant	BFAs, TAGs
YHP 249	Cooking pot	unidentified	
YHP 341	Cooking pot	unidentified	
YHP 342	Cooking pot	unidentified	
YHP 501	Cooking pot	unidentified	
YHP 503	Cooking pot	Ruminant	BFAs, TAGs
YHP 508	Cooking pot	Ruminant	BFAs, TAGs
YHP 514	Cooking pot	Ruminant	BFAs, TAGs
YHP 529	Cooking pot	Ruminant	TAGs

Table 5.14: Summary of biomarker source characterisation for archaeological residues sorted by ware type. BFAs=Branched Chain Fatty Acids, DAGs=Diacylglycerols, TAGS=Triacylglycerols, VLCS=Very Long Chain Saturated Fatty Acids

5.4.4 Ratio analyses

I: Eerkens' ratios

Eerkens' ratio methodology distinguishes two classes of residue source from the archaeological data: terrestrial mammal, and seeds and nuts (Table 5.15).

Two issues complicate the application of this ratio method. First, sixteen of the 76 residues and soil extracts lack all fatty acids required for the four ratio analyses. Fatty acids required for C12:0/C14:0 were missing from eleven samples, C16:1/C18:1 from seven and C15:0+C17:0/C18:0 from two. C12:0/C14:0 is primarily used to discriminate plant and animal products. The absence of this ratio in an archaeological dataset that appears to be largely composed of plant and animal products limits the interpretive ability of this test. A second problem is a degree of equifinality in several samples with two combinations of three ratios indicating different sources (i.e. C15:0+C17:0/C18:0, C16:0/C18:0 and C12:0/C14:0 indicating terrestrial mammal products while C15:0+C17:0/C18:0, C16:0/C18:0 and C16:1/C18:1 indicating seeds and nuts).

Eerkens' ratios largely identify Lydions as seed and nut products. Two samples are ambiguous (AIA 1861, AIA 982). AIA 982 lacks C12:0 and AIA 1861 is equifinal with three of four ratios indicating terrestrial mammal and three of four ratios indicating seeds and nuts. Both samples are classified as terrestrial mammal or seeds and nuts.

The absence of requisite fatty acids complicates the interpretation of Trojan pithoi residues. Of the five imported pithoi, four lack the fatty acids required for at least one ratio, all four are missing C12:0/C14:0 and two C16:1/C18:1. The fifth residue has all required fatty acids but is equifinal. Eerkens' ratios classify two pithoi as seed and nut (AIA 1790, AIA 1792), and the remaining samples as terrestrial mammal or seed and nut.

Local pithoi contain more fatty acids required for ratio analysis. Of the thirteen residues, three lack fatty acids for one ratio test (AIA 1784, AIA 1785 - C16:1/C18:1, AIA 2227 - C15:0+C17:0/C18:0) and one for two ratios (AIA 1780 – C15:0+C17:0/C18:0, C16:1/C18:1). All local pithoi samples are characterised as seed and nut products, except AIA 1781 which is classed as a terrestrial mammal residue.

Four of the seven Boğazkoy cooking pots are classified as seeds or nuts (AIA 2274, 2276, 2278 and 2282). The remaining three cooking pots (AIA 2277, 2279, 2280) are equifinal, and are categorised as terrestrial mammal or seed and nut (AIA 2277, 2279, 2280). Limited diagnosis in AIA 2279 is caused by the lack of C16:1/C18:1 and C12:0/C14:0, but other residues (AIA 2277 and AIA 2280) contain all fatty species required for ratio analysis.

Twelve of the eighteen residues from Gordion are categorised as seed or nut products (YHP 106, 050, 053, 156, 161, 242, 341, 501, 503, 508, 514 and 529) and four classified as animal products (YHP 105, 074, 160, 342). YHP 153 and YHP 249 are classified as either terrestrial mammal or seed and nut and lack the fatty acids required for the C12:0/C14:0 ratios.

Nine of the eleven Red Lustrous Wheel-made wares are seed or nut products by Eerkens' ratios. One sample is classified as a terrestrial mammal (AIA 2243) and one (AIA 2236) is classified as terrestrial mammal or seed and nut.

Most soil and sediment extracts are classified as 'seeds or nuts'. Two samples (AIA 1769, AIA 2289) are classified as 'terrestrial animal'. This broad classification of soil extracts as plant products most likely reflects the contribution of soil microflora to the lipid profile.

Sample	Type	Category	Missing Ratios
AIA 978	Lydion	terrestrial mammal or seeds and nuts	C12:0/C14:0
AIA 982	Lydion	seeds and nuts	
AIA 1743	Lydion	seeds and nuts	
AIA 1724	Lydion	seeds and nuts	
AIA 1725	Lydion	seeds and nuts	
AIA 1857	Lydion	seeds and nuts	
AIA 1858	Lydion	seeds and nuts	
AIA 1861	Lydion	terrestrial mammal or seeds and nuts	
AIA 1863	Lydion	seeds and nuts	
AIA 1870	Lydion	seeds and nuts	
AIA 1769	Sediment	terrestrial mammal or seeds and nuts	C12:0/C14:0
AIA 1771	Sediment	seeds and nuts	
AIA 1772	Sediment	seeds and nuts	
AIA 1773	Sediment	seeds and nuts	
AIA 1776	Sediment	seeds and nuts	
AIA 1777	Sediment	seeds and nuts	
AIA 2289	Soil	terrestrial mammal	
AIA 2291	Soil	seeds and nuts	
AIA 2260	Soil	seeds and nuts	
AIA 2261	Soil	seeds and nuts	
AIA 2262	Soil	seeds and nuts	
AIA 2264	Soil	seeds and nuts	
AIA 1792	Pithos (imported)	seeds and nuts	C12:0/C14:0
AIA 1788	Pithos (imported)	terrestrial mammal or seeds and nuts	
AIA 1789	Pithos (imported)	terrestrial mammal or seeds and nuts	C12:0/C14:0, C16:1/C18:1
AIA 1790	Pithos (imported)	seeds and nuts	C12:0/C14:0
AIA 1791	Pithos (imported)	terrestrial mammal or seeds and nuts	C12:0/C14:0, C16:1/C18:1
AIA 2224	Pithos (local)	seeds and nuts	
AIA 2225	Pithos (local)	seeds and nuts	
AIA 2226	Pithos (local)	seeds and nuts	
AIA 2227	Pithos (local)	seeds and nuts	C15:0+C17:0/C18:0
AIA 2228	Pithos (local)	seeds and nuts	
AIA 1778	Pithos (local)	seeds and nuts	
AIA 1782	Pithos (local)	seeds and nuts	
AIA 1784	Pithos (local)	seeds and nuts	C16:1/C18:1
AIA 1785	Pithos (local)	seeds and nuts	C16:1/C18:1
AIA 1786	Pithos (local)	seeds and nuts	
AIA 1787	Pithos (local)	seeds and nuts	
AIA 1780	Pithos (local)	seeds and nuts	C15:0+C17:0/C18:0, C16:1/C18:1
AIA 1781	Pithos (local)	terrestrial mammal	
AIA 2231	Red Lustrous	seeds and nuts	
AIA 2232	Red Lustrous	seeds and nuts	
AIA 2233	Red Lustrous	seeds and nuts	
AIA 2235	Red Lustrous	seeds and nuts	
AIA 2236	Red Lustrous	terrestrial mammal or seeds and nuts	
AIA 2237	Red Lustrous	seeds and nuts	
AIA 2240	Red Lustrous	seeds and nuts	C12:0/C14:0
AIA 2241	Red Lustrous	seeds and nuts	C12:0/C14:0
AIA 2242	Red Lustrous	seeds and nuts	
AIA 2238	Red Lustrous	seeds and nuts	
AIA 2243	Red Lustrous	terrestrial mammal	
AIA 2274	Cooking pot	seeds and nuts	
AIA 2276	Cooking pot	seeds and nuts	
AIA 2277	Cooking pot	terrestrial mammal or seeds and nuts	
AIA 2279	Cooking pot	terrestrial mammal or seeds and nuts	C12:0/C14:0, C16:1/C18:1
AIA 2280	Cooking pot	terrestrial mammal or seeds and nuts	
AIA 2282	Cooking pot	seeds and nuts	
AIA 2278	Cooking pot	seeds and nuts	C16:1/C18:1
YHP 105	Cooking pot	terrestrial mammal	
YHP 106	Cooking pot	seeds and nuts	
YHP 050	Cooking pot	seeds and nuts	
YHP 053	Cooking pot	seeds and nuts	
YHP 074	Cooking pot	terrestrial mammal	
YHP 153	Cooking pot	terrestrial mammal or seeds and nuts	C12:0/C14:0
YHP 156	Cooking pot	seeds and nuts	
YHP 160	Cooking pot	terrestrial mammal	
YHP 161	Cooking pot	seeds and nuts	
YHP 242	Cooking pot	seeds and nuts	
YHP 249	Cooking pot	terrestrial mammal or seeds and nuts	C12:0/C14:0
YHP 341	Cooking pot	seeds and nuts	
YHP 342	Cooking pot	terrrestrial mammal	
YHP 501	Cooking pot	seeds and nuts	
YHP 503	Cooking pot	seeds and nuts	
YHP 508	Cooking pot	seeds and nuts	
YHP 514	Cooking pot	seeds and nuts	
YHP 529	Cooking pot	seeds and nuts	

Table 5.15: Diagnoses of archaeological residues using Eerkens' degraded-value ratios. Missing ratios list the ratios that were not applied due to a lack of required fatty acid species.

II: Malainey's Ratios

Malainey's ratio methodology largely identifies residue sources as either large herbivore products or mixtures of large herbivore products and plant/bone marrow (LH w. p/m) (Table 5.16).

Most pithoi residues are characterised as LH w. p/m, with the exceptions of AIA 1785, which is classed as large herbivore as a result of lower levels of oleic acid, and AIA 1785, 1789, 1790, 2224 and 2226 which could not be characterised using this method. Similarly, seven of the eleven Red Lustrous Wheel-made ware residues could not be characterised. The remaining samples are characterised as either large herbivore (AIA 2242, AIA 2243) or LH w. p/m (AIA 2235, AIA 2238).

Lydions are classified as large herbivore residues, with the exception of AIA 1857, 1861 and 1870 which are categorised as LH w. p/m. The three samples are differentiated on the basis of slightly higher levels of oleic acid.

All cooking pot wares from Boğazkoy except AIA 2278 were characterised by Malainey's proportions. Of these, all are classified as large herbivore except AIA 2282 which is classified as LH w. p/m. Gordion cooking pot wares are similarly dominated by large herbivore and LH w. p/m classifications. Exceptions to this include five samples that cannot be diagnosed and two samples that are classified as fish/corn (maize) with plant (YHP 053) and plant with large herbivore (YHP 156). As a New World plant, corn residues can be discounted from the interpretation of YHP 053, giving a fish and plant mixture. The difference between YHP 156 and LH w. p/m residues relates to the relative contributions of each product. In LH w. p/m herbivore lipids are dominant with a minor plant or bone marrow component. Plant with large herbivore residues are principally composed of plant molecules with some contributions by herbivores.

Soil and sediment extracts are not easily categorised by Malainey's ratios. Of the twelve extracts, ten cannot be identified and the remaining two are identified as fish or corn. This low number of source identifications could be anticipated from the methodology used to develop Malainey's ratios. The method was developed to interpret residues that had undergone substantial changes over archaeological timescales, and should not easily or accurately characterise 'fresh' extracts from recently collected soils and sediments.

Sample	Type	Med Chain %	C18:0 %	C18:1 %	Category
AIA 978	Lydion	2.65	51.22	12.70	Large Herbivore
AIA 982	Lydion	8.10	38.85	13.44	Large Herbivore
AIA 1743	Lydion	8.46	41.48	12.70	Large Herbivore
AIA 1724	Lydion	9.09	39.61	11.22	Large Herbivore
AIA 1725	Lydion	7.65	38.82	13.46	Large Herbivore
AIA 1857	Lydion	6.15	29.30	18.26	Large Herbivore with plant/marrow
AIA 1858	Lydion	5.96	39.19	10.15	Large Herbivore
AIA 1861	Lydion	3.51	39.16	15.10	Large Herbivore with plant/marrow
AIA 1863	Lydion	8.83	32.78	12.83	Large Herbivore
AIA 1870	Lydion	9.33	33.41	17.90	Large Herbivore with plant/marrow
AIA 1769	Sediment	1.20	13.53	11.25	none
AIA 1771	Sediment	2.22	10.61	9.12	none
AIA 1772	Sediment	2.80	22.23	6.72	none
AIA 1773	Sediment	4.17	18.81	9.49	none
AIA 1776	Sediment	3.62	11.86	7.16	none
AIA 1777	Sediment	6.65	15.01	13.64	none
AIA 2289	Soil	2.60	20.77	7.30	none
AIA 2291	Soil	4.35	20.12	6.27	none
AIA 2260	Soil	3.91	13.66	15.50	Fish/corn
AIA 2261	Soil	5.20	15.24	15.82	Fish/corn
AIA 2262	Soil	5.80	17.63	9.09	none
AIA 2264	Soil	4.60	21.90	8.21	none
AIA 1778	Pithos (local)	4.11	42.65	20.32	Large Herbivore with plant/marrow
AIA 1782	Pithos (local)	6.96	40.76	18.82	Large Herbivore with plant/marrow
AIA 1784	Pithos (local)	9.32	46.22	20.15	Large Herbivore with plant/marrow
AIA 1785	Pithos (local)	2.00	57.92	7.68	Large Herbivore
AIA 1786	Pithos (local)	8.37	49.24	17.71	Large Herbivore with plant/marrow
AIA 1787	Pithos (local)	6.66	51.29	16.60	Large Herbivore with plant/marrow
AIA 1780	Pithos (local)	8.16	51.40	21.84	Large Herbivore with plant/marrow
AIA 1781	Pithos (local)	2.18	38.55	16.81	Large Herbivore with plant/marrow
AIA 1792	Pithos (imported)	0.30	52.08	24.11	Large Herbivore with plant/marrow
AIA 1788	Pithos (imported)	2.97	46.06	25.84	none
AIA 1789	Pithos (imported)	1.36	20.25	38.84	none
AIA 1790	Pithos (imported)	1.86	39.80	25.50	none
AIA 1791	Pithos (imported)	1.65	46.74	24.36	Large Herbivore with plant/marrow
AIA 2224	Pithos (local)	3.01	25.87	46.79	none
AIA 2225	Pithos (local)	8.23	51.20	18.35	Large Herbivore with plant/marrow
AIA 2226	Pithos (local)	2.70	14.38	13.62	none
AIA 2227	Pithos (local)	6.76	48.49	22.11	Large Herbivore with plant/marrow
AIA 2228	Pithos (local)	5.40	37.71	20.06	Large Herbivore with plant/marrow
AIA 2231	Red Lustrous	1.19	20.38	8.04	none
AIA 2232	Red Lustrous	2.54	20.67	13.08	none
AIA 2233	Red Lustrous	1.89	11.15	9.56	none
AIA 2235	Red Lustrous	5.86	38.73	24.37	Large Herbivore with plant/marrow
AIA 2236	Red Lustrous	5.31	18.87	39.27	none
AIA 2237	Red Lustrous	7.75	37.17	26.81	none
AIA 2240	Red Lustrous	2.30	20.13	37.77	none
AIA 2241	Red Lustrous	1.71	29.08	27.74	none
AIA 2242	Red Lustrous	1.58	28.81	13.03	Large Herbivore
AIA 2238	Red Lustrous	7.29	37.98	15.85	Large Herbivore with plant/marrow
AIA 2243	Red Lustrous	2.53	35.29	10.64	Large Herbivore
AIA 2274	Cooking pot	4.96	56.87	8.95	Large Herbivore
AIA 2276	Cooking pot	3.26	49.15	13.94	Large Herbivore
AIA 2277	Cooking pot	2.51	55.58	7.54	Large Herbivore
AIA 2279	Cooking pot	2.41	46.85	7.64	Large Herbivore
AIA 2280	Cooking pot	4.57	47.93	9.00	Large Herbivore
AIA 2282	Cooking pot	6.24	33.20	21.73	Large Herbivore with plant/marrow
AIA 2278	Cooking pot	4.75	44.82	26.08	none
YHP 105	Cooking pot	5.11	28.14	25.21	none
YHP 106	Cooking pot	8.47	35.49	6.02	Large Herbivore
YHP 050	Cooking pot	11.98	22.91	22.56	Large Herbivore with plant/marrow
YHP 053	Cooking pot	17.73	23.76	20.74	Fish/corn with plant
YHP 074	Cooking pot	4.91	30.02	27.32	none
YHP 153	Cooking pot	3.19	32.26	31.70	none
YHP 156	Cooking pot	16.15	26.29	22.87	Plant with large herbivore
YHP 160	Cooking pot	4.55	21.42	41.75	none
YHP 161	Cooking pot	5.55	40.67	14.93	Large Herbivore
YHP 242	Cooking pot	5.62	45.04	9.15	Large Herbivore
YHP 249	Cooking pot	2.95	31.19	28.14	none
YHP 341	Cooking pot	11.66	28.19	19.76	Large Herbivore with plant/marrow
YHP 342	Cooking pot	3.90	29.47	24.96	Large Herbivore with plant/marrow
YHP 501	Cooking pot	11.98	28.74	20.89	Large Herbivore with plant/marrow
YHP 503	Cooking pot	2.88	53.88	5.29	Large Herbivore
YHP 508	Cooking pot	4.27	46.64	6.31	Large Herbivore
YHP 514	Cooking pot	4.60	43.81	17.77	Large Herbivore with plant/marrow
YHP 529	Cooking pot	5.19	44.09	8.58	Large Herbivore

Table 5.16: Diagnoses of archaeological residues using Malainey's ratio technique showing the relative proportions of medium chain fatty acids C18:1 and C18:0.

5.4.5 Multivariate interpretation

Iterative PCA of the fatty acid dataset from the archaeological sample allows identification of a total of 11 major compositional groups and several outliers (Figures 5.21-5.23). In the first PCA, projection of the total archaeological dataset is highly compressed by the presence of four outliers (Figure 5.21). The four outliers are lipids extracted from two environmental soils at Gordion (AIA 2289, AIA 2291), one from Sardis (AIA 2262) and one sediment associated with a Lydion from Sardis (AIA 1769). Outliers are differentiated by high proportions of very long chain saturated fatty acids (C20:0-C32:0), as well as branched-chain fatty acids (e.g. C15:0br, C16:0br, C19:0br). These features are typical of soil microbes and consistent with a soil origin.

Removing the outliers from the dataset for a second PCA enables identification of several compositional groups, as well as a central, more tightly packed core of samples (Figure 5.22). In this analysis Group 11 contains three Red Lustrous samples (AIA 2231, 2232, 2242) characterised by high levels of very long chain saturated fatty acids similar to the soil outliers but in lower quantities. Group 10 is composed of four contextual Lydion sediments (AIA 1771, 1772, 1773, 1776) and one environmental soil from Sardis (AIA 2264). This group is differentiated by high levels of very long chain monounsaturated fatty acids, most importantly C22:1, composing an average of 48% of total lipids for the group. Group 8 is also composed of environmental soil extracts from Sardis (AIA 2260, AIA 2261) and largely resembles Group 10 but with lower concentrations of C22:1 (36%).

While readily distinguished as a separate cluster, Group 9 residues also form the most variable group within the dataset. It contains eleven samples from several archaeological classes. Most samples are residues extracted from cooking pot ware at Gordion (YHP 050, 053, 105, 106, 156, 341, 501), but also includes one Sardis Lydion (AIA 1725), two Gordion Lydions (AIA 1857, AIA 1863) and one sediment (AIA 1777). The group is largely defined by relatively high volumes of short and medium-chain fatty acids (≤ C15:0). Group 5 predominantly contains cooking pot residues from both Gordion (YHP 161, 242, 503, 508, 514, 529) and Boğazkoy (AIA 2274, 2276, 2277, 2279, 2280) as well as one pithos from Troy (AIA 1785). The

group is defined by odd- and even-numbered saturated and branched-chain fatty acids, most importantly C16:0, C17:0, C18:0, C19:0, C17:0br, C18:0br.

By removing Groups 5, 8, 9, 10 and 11 for a third PCA six inner core groups can be differentiated (Figure 5.23). Group 7 contains two samples, a Lydion from Gordion (AIA 1870) and a pithos from Troy (AIA 1782). Group 6 also contains two samples one Red Lustrous ware (AIA 2243) and one pithos (AIA 2226), differentiated largely by high levels of C16:1. Group 4 contains three samples, two Lydions from Gordion (AIA 982, AIA 1858) and one Red Lustrous ware sample (AIA 2233). Group 3 contains three pithoi from Troy, AIA 1778, 1787 and 2228.

Groups 1 and 2 are close to the multivariate centroid and comprise the bulk of the archaeological sample. They contain 21 and 8 samples respectively. Group 2 is composed of a mixture of Sardis Lydions (AIA 1743, AIA 1724), Trojan pithoi (AIA 1780, 1784, 1786, 2225, 2227) and one Red Lustrous ware sample (AIA 2237). Group 1 contains a diverse mixture of residues including Lydions (AIA 978, AIA 1861) two local Trojan pithos (AIA 1781, AIA 2224), five imported pithoi from Troy (AIA 1788, 1789, 1790, 1791, 1792), five Red Lustrous ware samples (AIA 2235, 2236, 2238, 2240, 2241), and a range of cooking pot residues from Boğazkoy (AIA 2278, AIA 2282) and Gordion (YHP 074, 153, 160, 249, 342).

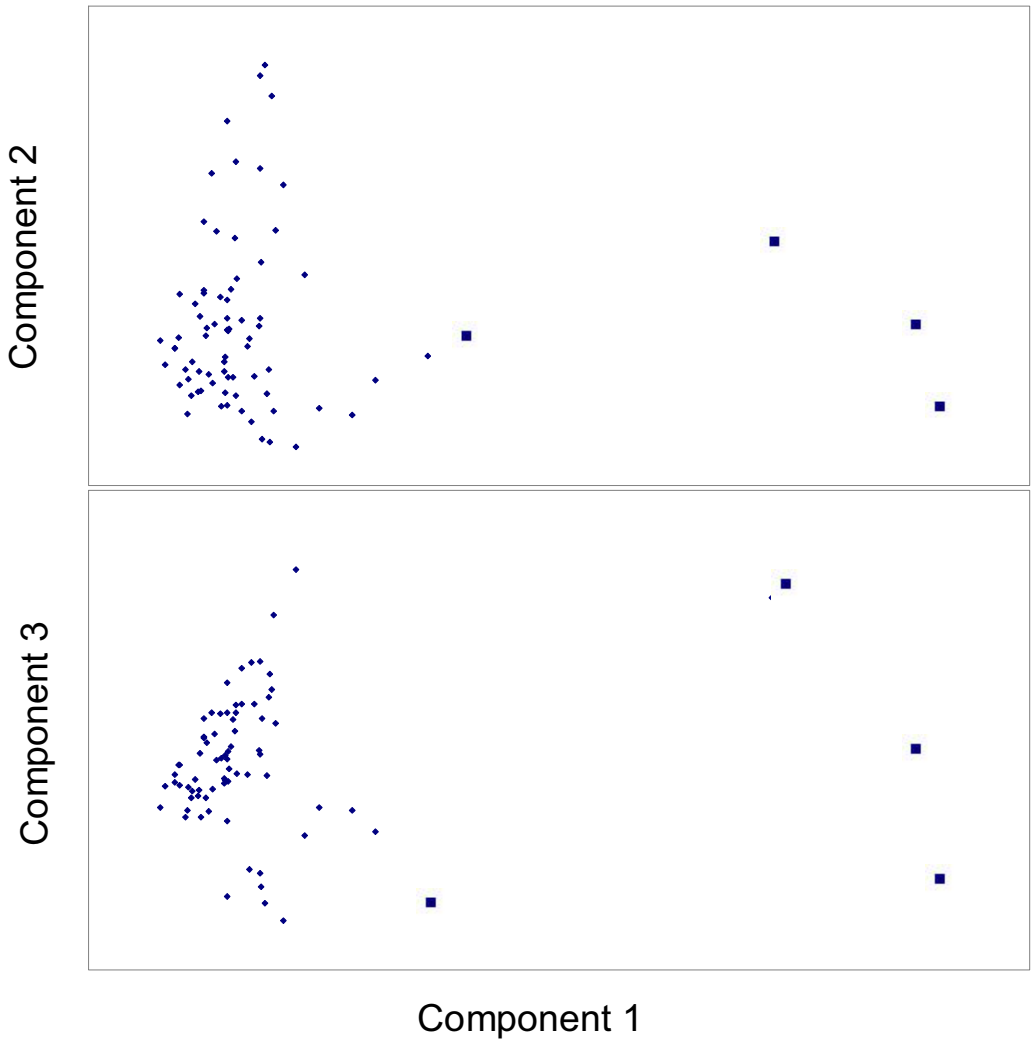

Figure 5.21: Principal Components Analysis of all archaeological fatty acid data. Biplots of first against second (upper) and first against third components (lower). Squares represent four soil outliers.

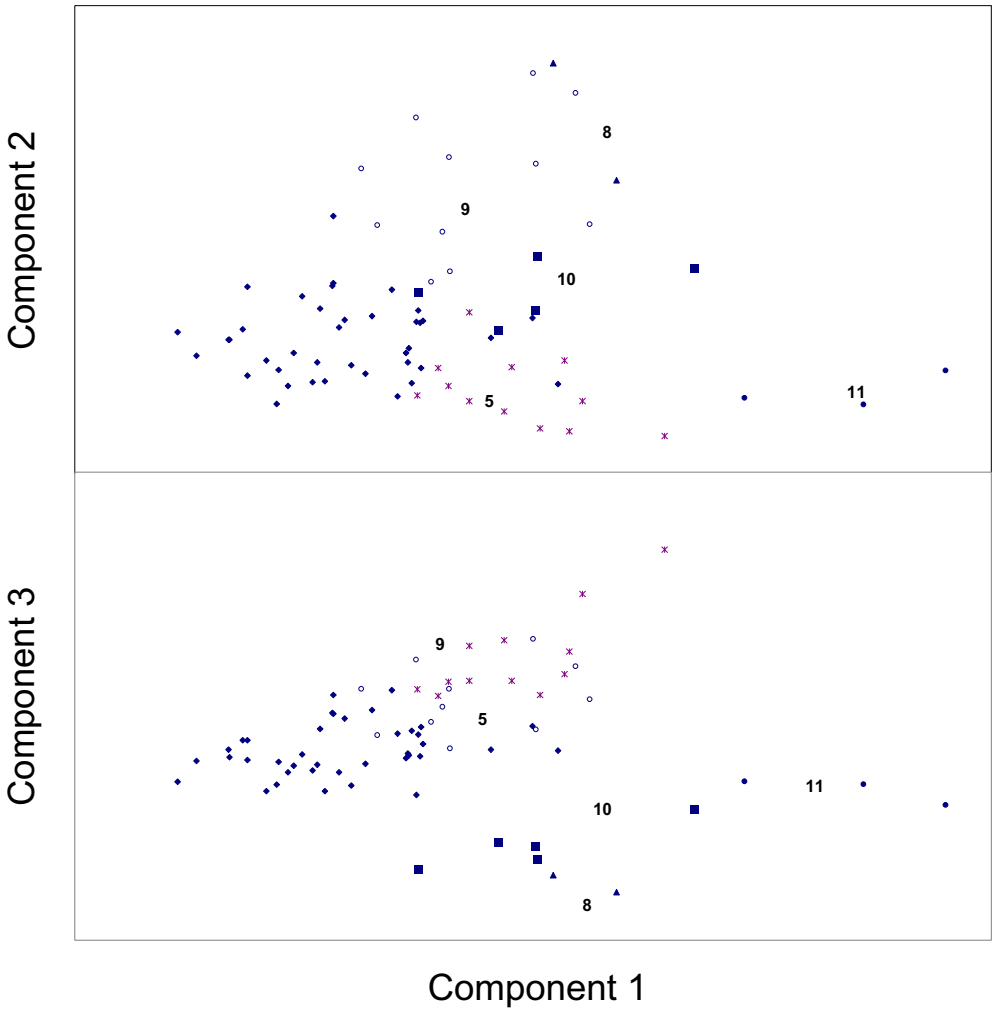

Figure 5.22: Principal Components Analysis of archaeological data with soil outliers removed Biplots of first against second (upper) and first against third components (lower) for archaeological residue data showing structural groups 11 (filled circles) 10 (squares), 9 (hollow circles), 8 (triangles) , 5 (asterisks) and undefined (diamonds).

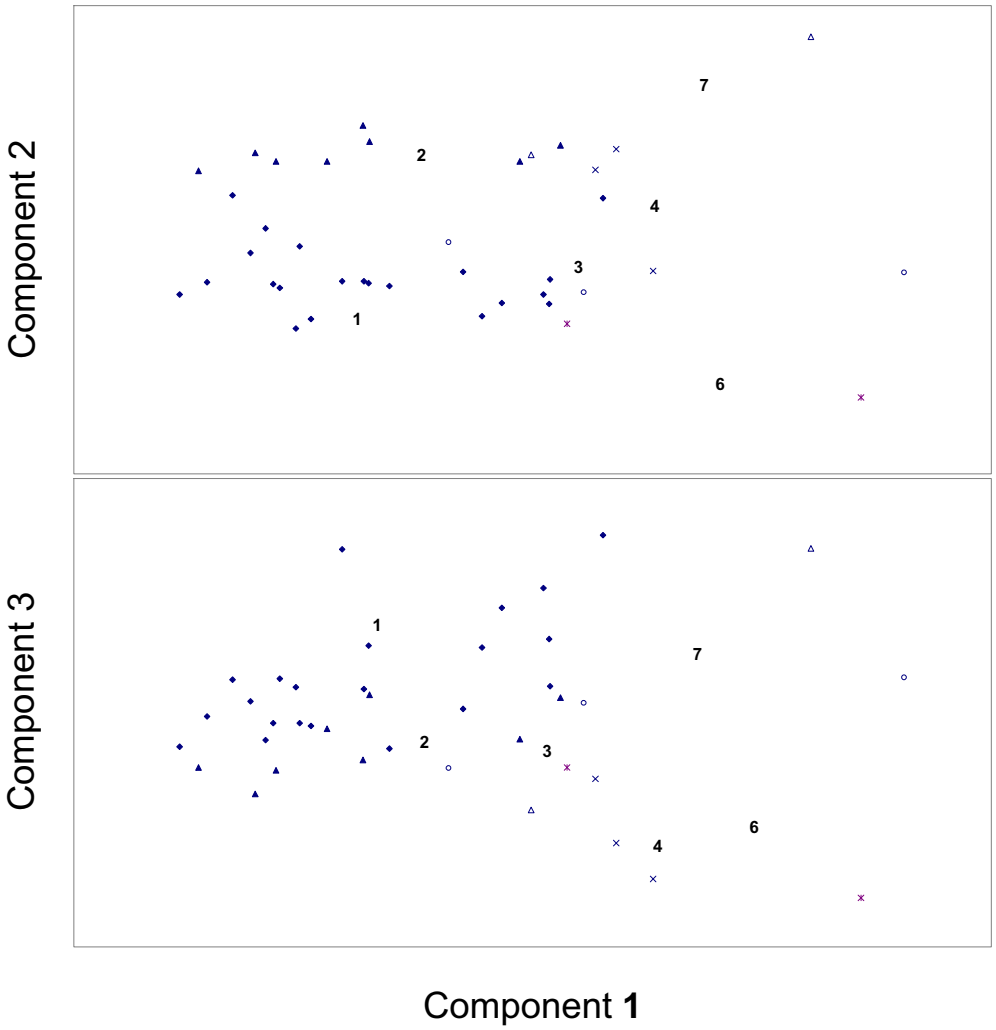

Figure 5.23: Principal Components Analysis of archaeological data with groups 5, 8-11 removed. Biplots of first against second (upper) and first against third components (lower) for archaeological residue data showing structural groups 1 (diamonds), 2 (filled triangles), 3 (hollow circles), 4 (crosses), 6 (asterisks) and 7 (hollow triangles).

Group no.	1 *n*=21	2 *n*=8	3 *n*=3	4 *n*=3	5 *n*=12	6 *n*=2	7 *n*=2	8 *n*=2	9 *n*=11	10 *n*=5	11 *n*=3	outliers *n*=4
C8:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
C9:0	0.00	0.12	0.04	0.21	0.00	0.00	0.39	0.17	0.16	0.01	0.09	0.02
C10:0	0.02	0.18	0.05	0.19	0.01	0.05	0.28	0.08	0.15	0.02	0.05	0.00
C11:0	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00
C12:0	1.07	4.56	2.28	2.60	0.78	0.79	4.07	1.09	3.41	1.20	0.51	0.35
c13:0br	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00
C13:0	0.04	0.02	0.09	0.00	0.05	0.00	0.10	0.14	0.17	0.10	0.02	0.06
C14:0br	0.03	0.00	0.00	0.00	0.04	0.00	0.00	0.18	0.09	0.04	0.00	0.14
C14:1	0.00	0.00	0.00	0.00	0.02	0.00	0.08	0.25	0.15	0.05	0.00	0.00
C14:0	2.22	3.20	2.28	2.23	2.46	1.37	3.08	2.43	4.27	1.82	1.10	2.06
c15:0br	0.25	0.46	0.73	0.21	0.33	0.28	1.21	0.81	0.82	0.42	0.13	2.08
C15:1	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.59	0.11	0.09	0.00	0.00
C15:0	0.78	0.51	0.82	0.48	0.74	0.45	1.00	1.03	1.35	0.46	0.17	1.07
C16:0br	0.09	0.02	0.08	0.05	0.18	0.28	0.42	0.32	0.22	0.11	0.00	1.14
C16:1	2.47	1.02	2.46	0.76	0.82	20.84	2.13	4.94	4.35	1.06	1.74	1.42
C16:0	22.80	20.40	19.51	46.87	28.23	31.89	24.09	13.92	25.30	14.95	48.20	20.62
C17:0br	0.09	0.01	0.36	0.14	0.86	0.07	0.28	0.31	0.17	0.03	0.00	1.10
C17:1	0.00	0.00	0.00	0.03	0.00	0.00	0.16	0.00	0.12	0.00	0.00	0.00
C17:0	0.60	0.38	0.55	0.68	2.22	0.54	0.83	0.22	0.86	0.31	0.27	1.05
C18:0br	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.14
C18:2	5.62	3.70	3.77	2.76	0.97	2.17	5.03	1.44	4.13	1.87	1.78	2.30
C18:1	27.04	18.86	18.99	11.05	9.73	12.13	18.36	15.66	18.64	8.14	11.38	8.48
C18:0	34.33	45.60	43.88	29.73	49.03	24.84	37.08	14.45	28.67	17.08	23.29	18.01
C19:0br	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.17
C19:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
C19:0	0.03	0.00	0.00	0.02	0.47	0.00	0.12	0.00	0.04	0.00	0.00	0.00
C20:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00	0.27	0.05	0.00
C20:0	0.91	0.47	1.03	1.08	1.09	0.79	0.72	0.72	1.08	0.88	1.61	2.74
c21:0	0.00	0.00	0.11	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.22	0.96
C22:1	1.14	0.39	0.18	0.06	0.15	0.56	0.00	36.27	3.83	48.13	0.36	15.20
C22:0	0.08	0.02	0.62	0.17	0.34	0.36	0.00	1.19	0.38	1.41	1.22	4.60
C23:0	0.01	0.00	0.78	0.00	0.21	0.12	0.00	0.15	0.07	0.00	0.34	1.61
C24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.24	0.00	0.06
C24:0	0.39	0.08	1.35	0.44	0.53	1.98	0.58	0.76	0.90	0.75	4.03	4.49
C25:0	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.07	0.07	0.00	0.81
C26:0	0.00	0.00	0.00	0.18	0.16	0.33	0.00	0.30	0.26	0.29	1.32	3.16
C27:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44
C28:0	0.00	0.00	0.00	0.00	0.02	0.15	0.00	0.15	0.00	0.19	0.94	2.97
C29:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35
C30:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.80	2.08
C32:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.32

Table 5.17: Principal Components defined groups. Mean fatty acid compositions for groups 1-11 and soil outliers.

The structure of the archaeological PCA plots is readily interpretable in terms of molecular abundance with the most unusual samples forming groups most distant from the centroid and least molecularly distinctive groups clustering around the

centroid. While identification of structural groups in multivariate space forms an important initial step, characterising residue sources is less straightforward. As detailed in Chapter 2, PCA does not provide direct diagnoses of residue sources but rather defines structural groups and the molecular species that define those groups. Residue sources are assigned by relating group-defining molecules to organic sources.

Groups composed of soil and sediment extracts can be readily identified as their sources are known. The groupings of soil and sediment extracts are an important control for archaeological residues. With the exception AIA 1777, all soil extracts are either outliers or part of Groups 8 and 10 and are distinct from archaeological residue samples. AIA 1777 falls on the edge of Group 9, which is a poorly defined group.

Group 11 was largely defined by very long chain fatty acids, but unlike the soil samples, did not contain high levels of C22:1 (3.8%, Table 5.17). This is consistent with strong biomarker evidence that Group 11 Red Lustrous samples contained beeswax. Group 9 residues were recovered primarily from Gordion, with only one Lydion sample from Sardis. This group contains relatively high proportions of short and medium chain fatty acids, which have been suggested as indicative of plant and milk products.

The correlation between Group 5 residues and odd-chain and branched-chain fatty acids is consistent with animal fats, particularly ruminants. Identifying residue sources within inner core of the archaeological PCA is more complicated. The structural differences within the core groups may be largely related to their taphonomy. Group 1 is a diverse range of residues derived from cooking pots, pithoi and Lydions. The group is characterised by low mean residue recovery (Table 5.18), weak Mass Spectra signal responses and high volumes of C18:1 and C18:2. These factors are indicative of either low abundances of initial residues or residues that have been significantly denatured by taphonomic processes. Group 2 residues are similar, composed of pithoi, two Sardis Lydions and a Red Lustrous residue that are principally differentiated from Group 1 by their higher abundances of short and medium chain fatty acids. Group 2 also has a slightly higher mean residue recovery. This may reflect differences in residue source or slightly different taphonomic histories. The composition of the remaining groups is unclear and complicated by their low group populations. Previous PCA analysis of similar archaeological datasets has suggested that ‘plant’ residues tend to cluster around the centroid (Craig 2004). Groups 1, 2, 3, 4, 6, and 7 are therefore cautiously classified as plant residues.

Group	Avg. residue recovery (mg)
1	1.34
2	1.60
5	2.58
9	2.55

Table 5.18: Principal Components defined groups; mean residue recoveries groups that contain more than 3 samples excluding soil extracts.

5.4.6 Comparison of experimental and archaeological PCA

Conventional PCA employs an orthogonal linear transformation of the dataset such that the first component accounts for the most variation, with each successive component accounting for a diminishing proportion of the variation not otherwise accounted for. As a measure of this transformation, eigenvalues summarise how much of the total variance has been accounted for in each successive component of the PCA. Eigenvalues can also be used as a measure of how structured the original dataset is in terms of the variance explained. Typically, in highly structured datasets (where there a strong correlation between variables either alone or in groups) the first four components account for more than 70% of total variation. In less well structured ("noisy") datasets more components will be required to explain the same amount of variability. In low dimensional (fewer variables) datasets more of the variation will be explained in each eigenvalue than higher dimensional datasets.

By comparing the eigenvalues for experimental and archaeological PCAs, the degree of structure in the archaeological dataset can be evaluated. Eigenvalues for the PCAs of the archaeological data show moderate structure in comparison to the experimental data. The first four eigenvalues account for 53% and 81% of the total variability for archaeological and experimental datasets respectively (Table 5.19). The first two eigenvalues explain 65% of experimental variation while the first two archaeological components explain only 35%. Subsequent eigenvalues for archaeological data are more "noisy" by 1% and 0.8% than experimental data.

Eigenvalue	Experimental FAs	Archaeological FAs
1	35.43	24.11
2	29.63	11.25
3	8.61	9.66
4	7.55	8.36
% total variance	81.21	53.38

Table 5.19: Principal Components comparison between experimental and archaeological fatty acid data. Eigenvalues for the first four principal components giving the percent of variation account for by each component.

The lesser degree of structure in archaeological data could be anticipated. The experimental dataset is composed of four RFOs. PCA of the experimental dataset produced tight clusters reflecting both molecular compositional differences between RFOs and the fairly limited alterations of most of the experimental samples (e.g. lipids exposed to 100°C heat and boiling). In contrast, archaeological lipids are derived from a much broader range of organic sources exposed to a much more complex and undefined suite of processes over archaeological timescales. The lower degree of structure in the archaeological data of this study parallels similar findings of previous studies using PCA for archaeological residue interpretation (Craig 2004).

5.5 Summary

In this chapter I presented the experimental and archaeological results of this study. The experimental simulation of taphonomic processes demonstrates that substantial alterations in the molecular composition of fats and oils may occur through cultural and post-depositional processes. However, these changes were usually not sufficient to disrupt the identifications of established chemotaxonomic techniques.

The results for the archaeological residues are substantially different from the experimental results, indicating a higher degree of transformation than could be produced experimentally. Archaeological residue interpretations are also more complex, with experimentally robust chemotaxonomic techniques producing sometimes contradictory residue source identifications. In the following chapter I discuss the implications of these results.

Chapter 6 - Discussion

6.1 Introduction

This chapter is divided into two sections to present the methodological and substantive implications of this study. In the first section I discuss the experimental results within the framework of the cultural, post-depositional and chemical questions posed in Chapter 3. I consider the reliability and applicability of chemotaxonomic interpretations within the context of the experimental dataset and highlight potential interpretive problems. I then compare the chemotaxonomic identifications of archaeological residues using the four techniques detailed in Chapter 5 (biomarkers, Eerkens' ratios, Malainey's ratios and Principal Components Analysis), highlighting the poor fit between each technique. I conclude by discussing the implications of the experimental chemotaxonomy of this study for archaeological chemotaxonomic interpretation.

In the second section I consider how archaeological taphonomic frameworks can provide a more systematic alternative for archaeological residue interpretations than the broad generalisations dominant in the literature. Finally, I attempt to draw substantive conclusions from archaeological data within a taphonomically-informed framework, considering how the results of this study contribute to current understanding of ware types and sites.

6.2 Experimental Implications

6.2.1 Cultural taphonomy

6.2.1.1 Cultural "work" and residue formation: E-value effects

This study posed three questions about the effects of 'work' in the formation of archaeological residues. The questions relate to the effects of different *types, intensities* and *lengths* of work. In the symbolic representations of residue formation these are the *E-values*, agents that actively transform molecules and generate residues.

The effects of different types of work can be established by comparing the effects of boiling RFOs and heating RFOs to 100°C. Both experiments are characterised by low levels of alteration. In both experiments the divergence indices of fatty acids from the reference fats and oils (RFOs) is generally less than 5%. Acylglycerol variation mirrors this, with low degrees of compositional change in both datasets (<6%). These values are below the levels of background variation and the precise changes in molecular proportions are not systematic. There is no tendency for certain lipids to be under- or overrepresented in samples collected over exposure time.

Taphonomically, the two experiments indicate that, in this case, the two different *types* of work do not produce significantly different lipid products. Further interpretation is complicated by the minimal alteration observed in the two datasets. However, these two experiments examine a very narrow range of potential types of work. It is probable that other forms of work will produce significantly different residue compositions and should be a focus of future research.

Exposures of lipid products to 100°C, 200°C, and 300°C demonstrate that the *intensity* of work can substantially affects the composition of lipid products. As detailed above, 100°C exposures do not affect lipid profiles in RFOs. At 200°C some molecular changes in fats and oils are observed in later samples. These changes occur in plant oils and largely relate to the loss of polyunsaturated fatty acids, resulting in an increasing proportion of saturated and monounsaturated fatty acids. Triacylglycerols follow a similar pattern, with slightly higher divergence indices than fatty acid products. All lipid products follow a similar pattern when heated to 300°C, although the effects are much more pronounced.

Chemical alterations through exposure to different intensities of work (heat) preferentially affect unsaturated lipid species. In some instances, the degree of alteration can be significant. Several 300°C samples were altered by more than 20% relative to their fatty acid RFO and more than 60% for triacylglycerol RFO. Most of the alteration is accounted for by the oxidative and autoxidative destruction of unsaturated fatty acids. One other important feature observed in samples exposed to high temperatures is the production of short and medium chain fatty acids. These occur through scission of longer-chain monounsaturated fatty acids, predominately C18:1. In plant products this is reflected in increased volumes of C8:0 and C9:0. The numerous oleic acid isomers in beef fat produce a more complex set of breakdown products, including short-chain fatty acids up to C12:0.

Different *lengths* of work also affect the nature of lipid molecule transformation. These effects are largely bound together with the effects of different *intensities* of work, the degree of molecular alteration (divergence indices) increasing over exposure time for plant oils exposed to 200°C, and all fats and oils exposed to 300°C.

These experiments correlate well with results of food science research and previous archaeological simulations of cultural processes. Food science studies of fry oils typically heated to 140-200°C require exposure times much longer than the five hour exposures in this study to be significantly altered (Kochhar and Gertz 2004; Li et al. 2005; Velasco et al. 2004). The negligible changes in 100°C samples and minor compositional alterations in 200°C samples are consistent with the studies of fry oils. The alterations also agree with most previous experimental simulations of ancient cultural practices (Craig 2004; Malainey et al. 1999b; Patrick et al. 1985). The preferential degradation of unsaturated lipid species and the relative stability of saturated lipids are commonly observed in the simulation of ancient culinary practices.

Increasing proportions of short- and medium-chain fatty acids in fats and oils exposed to 300°C temperatures are an important feature of the heating experiment. These molecules most likely result from the oxidative scission of oleic acid. Short-chain fatty acids have been used as indicators of plant residues and C12:0 forms an important component of both Malainey's and Eerkens' ratio techniques. However, the experimental data shows that the proportion of short and medium chain fatty acids can be altered by cultural processes and their relative proportions need not reflect a specific source product. In the case of archaeological samples, oxidative scission could be expected to substantially impact on the accuracy of chemotaxonomic diagnostics.

6.2.1.2 Ceramic reuse effects

The reuse of vessels creates a complex suite of issues for residue analysis that remain inadequately characterised. In Chapter 3, I proposed a way to reduce the complexity of potential interactions through symbolic representation of the sequence of events and interactions that occur during vessel reuse:

$R = [S_1 \times nE] + [S_2 \times (n-1)E] + [S_3 \times (n-2)E] + [S_n \times E]$

where R is the residue; S_n is the organic source; n is the number of use events; E is the total work that occurs during a use event.

In this experiment, the formula of reuse can be expressed as:

R = [beef x 3boiling] + [sesame oil x 2boiling] + [linseed oil x boiling].

Residues recovered from reuse experiment ceramic demonstrate that vessel reuse produces a composite residue of all use events. Within this, the first use event (beef) forms the dominant feature of the residue, and each subsequent use (sesame, linseed) contributes a smaller proportion of molecules to the final residue. Molecular species and concentrations within the eight ceramic sections analysed most closely resemble beef fats. The next most significant compositional contributor was sesame oil, with only slight evidence for linseed oil contributions.

It is possible that ceramic fabrics may eventually become saturated by residues, effectively stopping further penetration of molecules. Based on experimental observations, saturation would require a substantial number of use-events. In the reuse experiment only pure fats and oils were used that are well suited to infiltration of ceramic fabrics and residue formation. For cooking pot wares, the preparation of whole foods produces a much lower concentration of lipids. Consequently, a much larger number of use-events would be required to seal ceramic fabrics from further penetration. In the reuse experiment, the highest concentrations of lipid (58mg/gram) had only filled half the capacity of ceramic fabric (c. 12% of total ceramic mass). In a cooking pot-like environment, it is possible that thermal degradation within the ceramic fabric could produce a continuous series of small voids for further residue penetration. While beyond the scope of the present study, these considerations are likely to be a productive area for future research.

Chemotaxonomic analyses show evidence for both the composite nature of residues and the predominance of beef within residues. The former can be seen in Principal Components space, with reuse residues occurring between beef, sesame and linseed samples. Eerkens' ratios diagnosed all eight sections as terrestrial mammal residues, demonstrating the dominance of beef fat.

The reuse experiment resolves a substantial problem in the conceptualisation of archaeological residues. While several forms of residue uptake through reuse have been proposed, they have not been supported by experimental testing. Prior to this experiment it was unclear whether ceramic residues are the product of the first few

cooking events, the last few, or a composite of all use-events. Perhaps unsurprisingly, the reuse experiment indicates that while residues are composites of all use events, earlier use-events contribute more than later use events.

The composite nature of reuse residues has major implications for the interpretation of archaeological residues. Archaeological residues combine two levels of uncertainty: a) source products and mixes; and b) potentially highly complex taphonomic processes. The potential composite complexity of many archaeological residues will tend to confound chemotaxonomic techniques developed to diagnose single organic sources or relatively simple mixtures of sources.

6.2.1.3 Residue penetration site

The results of the reuse experiment also provide information to address the question of how the penetration site of a residue affects its molecular composition. Data for this experiment was collected by sampling along the ceramic profile in 2cm sections to produce a total of eight residue extracts. The results not only confirm that residue compositions can vary considerably across ceramic profiles, but also allow specific mechanisms of penetration to be proposed.

While all sections contain molecular contributions from all three fats and oils, three types of residues were identified that relate to their position on the ceramic profile. First, base sections were predominately composed of beef fats. Secondly, three ‘waterline’ samples were more composite with clear contributions from each of the fats and oils. Rim sections were also composites, predominately composed of molecules from earlier cooking use-events (beef and sesame).

The volumes of residue recovered from ceramic sections vary depending on penetration site. Base sections have relatively low volumes of residues; waterline sections very high recoveries (up to 58mg/g); and rim sections intermediate recoveries.

In the previous section the reuse experiment demonstrated that reuse residues are composites of all products prepared in the ceramic. The sectional analysis of the ceramic profile adds additional complexity to this picture. The molecular species preserved in each section demonstrated that the degree of residue penetration during a cooking event varied depending on the penetration site along the ceramic profile. The

base sections most closely resemble beef, while waterline sections contain more molecules from the plant oils. Rim sections resemble base sections but have a higher plant oil component.

Differential residue recoveries along ceramic profiles have been noted in previous boiling simulations (Charters and Evershed 1997) and are occasionally observed in archaeological ceramics (Charters et al. 1993). The tendency for ceramics to contain relatively high concentrations of residue at or near rims has led to some researchers focusing on the analysis of residues from these sections (Charters et al. 1993).

The large differences in residue composition across the ceramic profile reveal a substantial problem in archaeological ceramic residue interpretation. The selection of sampling site therefore is a significant problem, with the potential for different sections of the same ceramic to produce different chemotaxonomic interpretations of residue source.

6.2.2 Post-depositional taphonomy

6.2.2.1 Microbial effects

The exposure of fats and oils to microbes produced relatively minor alterations to experimental samples. This result is most likely explained by the microbial solution used being unsuited to the decomposition of lipid molecules. Divergence indices between reference fats and oils and microbially exposed fats and oils were low. Linseed oil was the only product significantly transformed during the experiment. The slight decline in linolenic acid is more likely a result of oxidation or autoxidation during exposure time than a microbial effect.

Microbes are widely recognised as a substantial influence on the survival and composition of organic remains (Child 1995; Jans et al. 2004), including lipids (Ciafardini et al. 2005; Sugimori et al. 2002). The minor alteration observed in this experiment indicates that these particular microbes do not affect lipids, rather than that microbial affects are not taphonomically significant.

6.2.2.2 Soil pH

The pH experiment exposed RFOs to acidic (pH4), neutral (pH 7), and alkaline (pH 8) conditions. While the more extreme pH values of this experiment attempted to mimic the longer time frames archaeological residues are subjected to, the results (e.g. high acidity environments as alteration inhibitors) are more artificial than intended. However, two interrelated chemical processes were identified in the pH experiment. First, alkaline conditions promote the alteration of lipid products. Second, acidic conditions inhibit the alteration of lipid products. Neutral exposures provide a comparative baseline for the analysis. In these samples, the degree of alteration is a function of overall unsaturation. Fish and linseed oils are highly affected, while only minor compositional changes are observed in sesame and beef. While linseed diverges by 59% in neutral conditions, its alteration is relatively minor in acidic conditions (5%). In contrast, beef and sesame samples that are relatively stable in acidic and neutral conditions are significantly altered in basic conditions (11% and 24%) indicating that this environment accelerates the rate of compositional change within lipid products.

Compositional alterations of RFOs exposed to different pH conditions are not substantially different from RFOs altered in cultural simulation experiments, but the pH alterations are sometimes more pronounced. These changes are largely the result of oxidization of unsaturated fatty acids, with acidic conditions inhibiting free radical oxidation and autoxidation, and basic conditions accelerating the process.

Chemotaxonomic analyses of lipid samples from pH exposures are largely robust. Saturated fatty acid biomarkers are present after pH exposure. Eerkens' ratios also correctly characterise highly altered lipid products. PCA enables greater refinement of these groups, especially notable in fish oils. The pH experimental lipid products group closely with other samples from the same source with the exception of fish oils. Fish oils form a unique characteristic group that occurs from the removal of higher polyunsaturated fatty acids that largely define the other fish oil samples.

The pH experiment highlights a problem with ratio and proportional methodologies that examine the relationships between unsaturated and saturated fatty acids. The rate of unsaturated decomposition is influenced by the pH of the depositional environment. Disentangling the proportion of unsaturated lipids that

occur from source products and the proportion that occurs as a taphonomic survival bias requires careful evaluation, especially in studies examining residues from multiple depositional contexts and sites. In light of this, it would appear that current residue research does not include adequate controls to quantify the effects of depositional pH.

6.2.2.3 Leaching

The ultrasonication of experimentally-impregnated ceramics largely confirms Evershed's (1993) suppositions regarding the effects of groundwater movement on archaeological residues. Ultrasonicated samples contain lower proportions of less hydrophobic, short and medium chain fatty acids than non-ultrasonicated examples. Over archaeological timescales, this process may account for the absence of short-chain fatty acids in most ceramic residues.

The most significant of these short and medium chained fatty acids is C12:0. Ultrasonication significantly reduced the proportions of this fatty acid species in ceramic residues. As this molecule forms an integral component of many proportional tests, the loss of C12:0 could be significant. While the declined proportion of C12:0 did not impede chemotaxonomic identification in the leaching experiment, this process may complicate interpretations of archaeological residues exposed to much longer periods of leaching than simulated in this experiment. As with environmental pH, understanding this issue is vital to the interpretation of archaeological remains, especially for inter-context and inter-site studies. To achieve this, residue researchers must carefully consider the hydrology of depositional contexts before attempting to interpret archaeological remains.

6.2.2.4 Residue storage effects

The reanalysis of ceramic residues produced in 2004 provides several important insights into the taphonomic transformation of residues over time during laboratory storage under controlled conditions.

Three classes of residue were identified from the experimental ceramic residues extracted in 2007: low concentration plant residues, high concentration plant

residues and animal residues. The first group contains barley, wheat, pea and chickpea residues and is characterised by low (<1mg/g) residue recoveries, the presence of relatively high proportions of oleic and linoleic acids, and the absence of triacylglycerols. Olive, sesame and linseed oils form the second group and are characterised by high residue recoveries (>35mg/g), low proportions or absence of unsaturated lipids, and the absence of triacylglycerols. The final group contains the animal fats (beef, chicken, duck, pork and sheep), which have relatively high lipid recoveries (1-7mg/g), and contain relatively high volumes of unsaturated lipids, including triacylglycerols.

The overall trend between the two analyses was of a declining proportion of unsaturated lipid species. Within this trend, several taphonomically important features were observed. The most important of these is the behaviour of unsaturated fatty acids within plant residues. In plant oil residues (Group 2) unsaturated lipids are almost entirely absent. Only olive oil retains some oleic acid, ~3% compared to 75% in 2004. In contrast, Group 1 residues contain relatively high proportions of monounsaturated (17-25%) and polyunsaturated fatty acids (1-3%). This contrasts with residue compositions in 2004, in which Group 2 residues frequently contained higher proportions of unsaturated fatty acids than Group 1.

The different densities of residue between the two groups should not substantially affect the rate at which unsaturated fatty acids are altered. The most likely explanation is sacrificial preservation in low-lipid plant residues. While plant oil residues are composed almost entirely of lipids, low-lipid residues contain a much broader range of molecular species (as a consequence of being prepared from whole foods) that are much more liable to degradation than C18:1 and C18:2. The degradation of less stable molecules within a residue effectively buffers lipids from alteration. Plant oil residues had no such buffer, and were consequently rapidly altered. A similar phenomenon was observed in RFOs heated to 300°C, where C18:2 and C18:3 molecules effectively buffered C18:1 from oxidation in plant oils (Section 5.2.1.3).

Sacrificial preservation presents a challenge to understanding residue taphonomy. In most instances, non-lipid residue fractions are not analysed, and are likely to survive only infrequency. It is difficult to assess which aspects of archaeological residues relate to the original source product, and which are a consequence of buffering caused by other residue fractions. This problem is especially

relevant for reused and reassigned residues which may contain lipids and other molecules that have no biological or botanical connection. Alternately, sacrificial preservation may be a relatively short-lived phenomenon. The residues analysed here were three years old at reanalysis. Over longer timespans, the less stable non-lipid fractions of a residue may be entirely consumed, leaving lipids more liable to alteration. In archaeological contexts, this may largely negate any sacrificial preservation related effects. This line of enquiry should form an important focus for future taphonomic research.

6.2.3 Chemical and mechanical effects

6.2.3.1 Lipid organisation and colloidal properties

The re-examination of ceramic residues prepared in 2004 also provides the data required to explore how the colloidal properties of residue lipids affect the nature of their alteration. Experimental ceramic Groups 2 (plant oils) and 3 (animal products) provide an insight into the effects of colloidal lipid properties. After initial residue generation, plant oil residues were characterised by their high volumes of monounsaturated and polyunsaturated fatty acids and triacylglycerols. These molecules are relatively liable to oxidation and autoxidation and do not form lipid crystals or colloidal-level aggregates of crystals (flocs) at storage temperatures (Larsson et al. 2006). Animal residues contained relatively high volumes of monounsaturated fatty acids, principally oleic acid isomers (C18:1), saturated fatty acids and triacylglycerols composed of these molecules. The low proportions of polyunsaturates and high proportion of saturated lipids allows animal residues to form lipid crystals at storage temperatures.

In simple, non-colloidal terms, the molecular species within residues should be altered and removed at similar rates. The higher proportions of monounsaturates in plant products should lead to higher relative proportions of these molecules in later residues. For example, an initial residue that contains 80% oleic acid should have a higher proportion of oleic acid at a later date than one that initially contains 20% oleic acid. In this instance, plant oil residues generated in 2004 should contain significantly higher proportions of unsaturated lipids in 2007 than animal products. Residues

extracted in 2007 produce a substantially different result with the preservation of most unsaturated lipids in animal products and their virtual absence from plant oil residues.

Differences in residue colloidal properties explain the gap between formation processes in plant and animal residues. The unsaturated lipids in animal products were shielded through colloidal lipid properties in two ways: a) as unsaturated fatty acid moieties within crystal forming acylglycerols, and b) through the entrapment of pockets of liquid lipids between crystalline aggregates.

The extent that colloidal crystals within animal residues shield unsaturated lipids is unclear. Over three years, the proportions of C18:2 and C18:3 in animal fats reduced considerably, from 3.5-5.5% to less than 1%. Monounsaturated fatty acids were not substantially affected over the 3 years. In many instances, the overall proportion of monounsaturates had increased by 2007 as a result of the declining importance of polyunsaturated species. Previous archaeological analyses have indicated that oleic acid can persist in animal fat residues over archaeological timeframes (Evershed et al. 2002). Residues identified as ruminant products sometimes contain several species of oleic acid isomers. This range of oleic acid isomers are not common in soil microbes and are unlikely to arise through the microbial additions in archaeological contexts. These molecules therefore may be part of the original, culturally formed residue that has been preserved over archaeological time partly as a function of colloidal lipid crystals.

Differential alteration through lipid properties has significant consequences for the interpretation of archaeological remains. Colloidal lipid properties suggest that animal residues may be better long-term preservers of unsaturated lipids than plant residues, potentially complicating ratio methods that rely on unsaturated lipids for diagnosis. Both Malainey's and Eerkens' ratio methods utilise unsaturated fatty acids as diagnostic features. Malainey (1999c) uses the proportions of C18:1 isomers to discriminate large herbivores, plants, and mixtures of plants, large herbivores and marrow, while Eerkens (2005) uses ratios of C16:1/C18:1. .

DIs for experimental fatty acids and acylglycerols show that acylglycerols have systematically higher divergence indices than fatty acids. This may be explained in part by the greater chemical complexity of acylglycerol fractions but it may also reflect the higher liability of acylglycerols to chemical alteration through hydrolysis of the ester linkages between fatty acids moieties and glycerol backbones. The likely

complexity of these relationships would require a concerted and systematic study beyond the scope of the present work.

6.2.3.2 Residue/ceramic interactions

Comparison of the lipid residues prepared in 2004 and the products of experimental boiling of RFOs allows an evaluation of the role of the ceramic matrix in residue formation. The key difference between the residues generated in 2004 and the experimental samples generated by boiling in beakers is the interaction between lipid molecules and the ceramic fabric. Comparison of the two samples provides an insight into the role of ceramic fabrics in residue formation. While boiling RFOs in beakers for five hours produces no noticeable changes in lipid compositions, residues extracted from experimental ceramics in 2004 were highly altered relative to their original products.

The mechanism by which ceramic fabrics alter the liability of lipid molecules to alteration is unclear. One possibility is the exposure of lipid products to higher heats once they penetrate the ceramic fabric. In 2004, residues were generated by boiling foods in water within an oven heated to 160°C. While the boiling environment within the experimental ceramics could only reach 100°C due to the presence of water (comparable to boiling in beakers), hydrophobic lipid residues within the ceramic fabric may have effectively excluded water. This may have created an environment with temperatures of >100°C, producing a more altered residue product. However, this is unlikely as samples heated to 200°C for five hours experienced only slight changes in molecular composition.

Another possibility is that residues cover a large surface area within the ceramic fabric. Earthenware ceramic consists of a complex structure of voids between ceramic particles (pores). These voids form a substantial fraction of the total volume of a ceramic. For the ceramics used in this experiment, the void space was approximately 12% of ceramic volume. This void space forms a large surface area over which residues can spread in a thin layer. In 2004, the highest concentrations of residue (linseed oil) filled less than half the 12% potential within the ceramic. Within a thin layer, residue molecules may be more liable to alteration as they have more interaction with the environment (e.g. free radicals, oxygen and hydrolysis) then when they are part of a more compact mass.

A third potential influence is the direct influence of the ceramic on organic molecules. Metal ions, such as iron (Fe^{+}), are catalysts in unsaturated lipid oxidation (Frankel 2005). Iron is a common component of the elemental profile of earthenware ceramics, and ceramics are likely to contain some free iron ions. Metal ion catalysis would not only be restricted to the cooking process, but continue during storage. Given the degree of change over the five hours of cooking, all unsaturated molecules should be rapidly oxidized through metal ion catalysis within weeks. However, unsaturated lipid products are found in most experimental ceramics after several years, indicating that metal catalysis may not be a significant factor.

It is likely that all three processes contribute to the alteration of lipid molecules within the ceramic fabric to varying degrees. Disentangling this complex issue is beyond the scope of this study, but it presents an opportunity for future taphonomic research.

6.3 Chemotaxonomic Diagnostics

Experimental taphonomic simulations produce a wide range of alterations in the four RFOs used in this study. Overall, alterations are relatively predictable, with a tendency towards the preferential oxidization and removal of lipids based on their degree of unsaturation. While the chemical changes in experimental samples rarely impact on their chemotaxonomic identifications, several important features were observed in the experimental data that have implications for chemotaxonomic analyses of archaeological residues.

6.3.1 Biomarkers

Biomarker and pseudo-biomarker molecules are found in all experimentally altered samples. Where lipid products are significantly altered, the relative proportion of biomarkers tends to increase as a result of the more chemically stable composition (saturated fatty acids and triacylglycerols) of biomarkers and pseudo-biomarkers. In this study, this resulted in the selection of robust saturated fatty acids and triacylglycerols that are less liable to alteration than unsaturated lipids. In the reanalysed 2004 ceramic residues, most biomarkers and pseudo-biomarkers are also

identified. An exception to this is the absence of monounsaturated triacylglycerol biomarkers from the plant oils, indicating that these may not be robust enough to be applied archaeologically.

6.3.2 Ratio analyses

Eerkens' ratio analyses are largely robust for the experimental taphonomic simulations performed in this study. The technique is able to accurately diagnose the sources of most experimental samples and residues. However, several exceptions to this pattern occur as a result of the absence of one or more fatty acid species required to construct the ratios. These occur in two forms: a) samples that have a naturally low abundance of required fatty acids (i.e. C12:0 in linseed and olive oil), and b) samples in which alteration has removed required molecules from the experimental sample (i.e. plant residues from the reanalysed 2004 ceramics and fish oil from the pH experiment). The diagnostic robusticity of Eerkens ratios for a dataset of unaltered and experimentally altered Old World plant and animal products suggests that Eerkens ratios may be applicable to a wider range of archaeological residues than previously considered (i.e. non-North American ceramic residues).

The production of short- and medium-chain fatty acids by exposing fats and oils to 300°C has significant implications for ratio analyses. The most important of these is C12:0, which forms an important part of several ratio methods. The most likely source of these molecules is the breakdown of monounsaturated fatty acids, such as oleic acid (C18:1). While in the experimental samples the degree of alteration was not sufficient to influence chemotaxonomic interpretations, the trend observed indicates that sufficient changes may occur to influence source identifications in archaeological residues.

Another experimentally observed complication of ratio analyses is the preferential stripping of short-chain fatty acids observed in the leaching experiment. In this experiment the relative proportions of short chain fatty acids including C12:0 were substantially reduced after a relatively short period of ultrasonication (8 hours). The leaching of C12:0 presents a similar problem for chemotaxonomic ratios as the previous experimental observation. That is, the alteration of the relative proportions of important ratio molecules.

6.3.3 Principal Components Analysis

Principal Components Analysis was able to provide clear groupings of experimental samples that were related to their organic sources. Experimental samples are largely composed of compact core groups, that are only slightly altered, and outer core groups, that follow trajectories towards greater alteration. The exception to this trend is fish oils, which form two distinct groups. The first of these is a core group, which contains most fish oil samples. The second is composed of highly altered fish oil samples from the pH experiment that lack the highly unsaturated fatty acid species typical of fish oil. The oxidation of polyunsaturated fats in fish oil as a result of prolonged mildly elevated temperature (rather than pH) contributes to the outlying position of this fish oil subgroup.

The diagnostic capacity of PCA is evident in analysis of the reuse residue experimental dataset. The reuse residues are composed of a mixture of beef fat, linseed oil and sesame oil and these form a group in the PCA that occupies a space between these samples.

In archaeological residues the diagnostic utility of PCA is more limited because of: a) a lower number of fatty acids species are available to differentiate residues; b) the lack of appropriate controls to identify sources materials; and c) the likely highly complex combination of cultural and post depositional taphonomic processes involved.

The application of chemotaxonomic interpretive techniques to experimentally produced samples indicates that biomarker, ratio and multivariate methods are suited to the identification of the sources of altered fats and oils. However, applying these to an archaeological dataset involves significant challenges.

6.3.4 Comparison of archaeological chemotaxonomic identifications

The chemotaxonomic techniques used in this study correctly diagnose most experimentally altered sample sources, but their applicability to, and reliability for, the characterisation of unknown and uncontrolled archaeological residues is more difficult to establish. For the experimental section, there was strong correlation

between the identifications made by different chemotaxonomic techniques. In a hypothetical situation where source of an experimental sample was unknown, the strong agreement between these techniques would provide a means to cross-validate reliability. By applying this approach to archaeological samples the overall reliability of chemotaxonomic interpretations of the archaeological dataset can be assessed.

To achieve this, I calculate the degree of correlation in residue source identifications between two chemotaxonomic techniques (e.g. biomarker identification of ruminant residue and a ratio characterisation of large herbivore residue) (Table 6.1). For a correlation, the different methods must produce a sufficiently specific identification to be useful (e.g. Eerken's identification of 'terrestrial mammal or seed and nuts' is too broad to be archaeologically useful). Correlation is only measured on residues where both chemotaxonomic methods provide source identification (i.e. no biomarker identification and a PCA identification of plant residue is not included).

	Eerkens	Malainey	PCA
Biomarkers	16.00%	84.62%	46.67%
Eerkens	-	57.50%	76.92%
Malainey	-	-	67.39%

Table 6.1: Correlation of source product identifications between the four established chemotaxonomic techniques used in this study for archaeological residues.

The source classifications in biomarker analysis and Eerkens' ratios correlate very poorly. Of the 25 samples that are meaningfully identified by both tests, three had similar classifications (16%). AIA 1857 is classified as a seed or nut (plant) product by Eerkens' ratios and a plant component was identified by sterol biomarkers. AIA 2226 and AIA 2228 are classified as soil yeasts by biomarkers and as seed or nut products by ratio analysis. Eerkens' analysis is not developed to classify microbes such as yeast but the correlation between yeast (microflora) and plant products is sufficient.

Biomarkers correlate more strongly with Malainey's proportional characterisations. Of the 26 suitable samples, 22 have similar identifications. Most of these were correlations of 'ruminants' identified by biomarkers and 'large herbivore' or 'large herbivore with plant or bone marrow' by Malainey's proportions. The strong correlation reflects the nature of the two tests. Biomarkers are limited by the availability of suitable diagnostic molecules. Archaeologically robust animal biomarkers are much more common than those suitable for characterising plants,

skewing biomarker identifications towards high proportions of animal products. Malainey's ratios show a strong tendency to classify all archaeological residues examined as large herbivores products. Of the 45 residues categorised by Malainey's proportions, 44 of are identified as large herbivore products, or mixtures that include large herbivore products.

Thirty samples are characterised by both biomarker analysis and Principal Components Analysis. Fourteen samples have correlating identifications, primarily beeswax products and animal fat residues. The correlation is partly a result of the identification of characteristic molecules as a tool for interpreting PCA structural groups. Of the 30 residues, 16 are part of the relatively uncharacteristic inner core groups which were tentatively identified as plant residues. When these are removed ten of the remaining 14 residue identifications correlate (71%).

The comparison of Eerkens' and Malainey's ratios is particularly interesting. The two techniques not only use a similar approach to interpret archaeological residues (i.e. ratios) but are also both based on the experimental dataset produced by Malainey. The two methodologies both characterised 40 archaeological residues with 23 residues correlations. Most correlations are a result of Malainey's attempts to identify product mixtures. Nineteen of the correlations are matches between Eerkens' 'seed and nut' category and Malainey's 'terrestrial mammal with plant or bone marrow' category. For Malainey's analysis, animal products are meant to be the dominant feature and plant or bone marrow form a more minor compositional input. This should also be reflected in other chemotaxonomic tests such as Eerkens' ratios. If these correlations are removed four of the remaining 20 samples correlate (20%), with two correlations between terrestrial mammal and large herbivore with plant or bone marrow, one terrestrial mammal/large herbivore and one seeds and nuts/fish or corn with plant.

The causes of the poor correlation between the two ratio techniques are unclear. It is possible that the chemotaxonomic techniques, developed using and to interpret North American pre-contact food products may be unsuited to diagnosing Late Bronze and Iron Age Turkish ceramics. However, Eerkens' ratios were capable of differentiating the suite of unaltered and experimentally altered products and residues examined in this study. All of these products are represented in Turkish archaeological deposits (Craig 2004:32-3). Eerkens' chemotaxonomic identifications of the archaeological dataset seem broadly compatible with what would be expected

from most classes of ceramic examined (i.e. plant products in pithoi, Lydions, Red Lustrous wheel-made wares, and a sizable proportion of the cooking pot wares). On the other hand, no assessment of the reliability of Malainey's ratios was possible as the experimental datasets were unsuited to this analysis. Clearly, at least one of these diagnostic techniques is poorly suited to identifying the sources of the archaeological sample. However, determining which of these two methodologies more accurately interprets archaeological residue sources is more difficult to establish.

PCA and ratio analyses have relatively high degrees of correlation. Fifty two samples are diagnosed by both Eerkens' methodology and PCA, with 40 correlations. Many of these are a result of the poorly defined core groups of the PCA that are tentatively identified as plant products. If these samples are removed, only eleven of the 24 remaining samples correlate (42%). Malainey's ratios appear more robust. Of the 46 residue characterisations identified by both methods, 31 correlate. Many of the residue samples are also from the core PCA groups. When removed, 14 of the remaining 19 residue identifications correlate (74%).

The degree of source product correlation between chemotaxonomic tests is low. Three main classes of residue source are identified by the chemotaxonomic tests: beeswax, animal products, and plant products (including microbes). If data was entirely random, a correlation of 33% would be expected for these three groups. Beeswax identifications form a relatively minor component of the dataset, being contained to a few biomarker and PCA identifications. Removing beeswax leaves two classes of residue source, which would have a 50% correlation in a random dataset. Correlations between chemotaxonomic techniques are therefore low; in many instances less than what would be expected from random data.

The poor fit between chemotaxonomic methods highlights the problems of extrapolating diagnostic features of modern unaltered and experimentally altered products to unknown and highly transformed archaeological residues. Clearly, a more systematic understanding of the processes that determine the final composition of archaeological residues is required before these can be accurately interpreted. Considering residue formation through taphonomic frameworks provides one way of producing a more systematic understanding of archaeological residue formation.

6.4 Archaeological Taphonomy

6.4.1 Taphonomic features of archaeological residues

The most significant and widespread taphonomic feature observed is the augmentation of ancient molecules with more modern lipid molecules, evidenced by the high proportions of unsaturated fatty acids. The preservation of such large proportions of unsaturated molecules from ancient source products is unlikely, and may result from a relatively recent formation process.

The most likely source of these molecules is microbial. Clear examples of post-depositional taphonomic augmentation can be seen in several archaeological samples. Several pithoi contain high levels of palmitoleic acid (C16:1) and acylglycerols containing palmitoleic acid moieties. These molecules are typical of soil microbes such as yeast, and were also observed in lipids extracted from environmental soils and contextual sediments. Another feature of several archaeological residues was elevated levels of docosenoic acid (C22:1), which is also an important feature of soil extracts. Many archaeological residues also contain relatively high volumes of odd- and branched-chain fatty acids, especially Lydion residues. Relatively high concentrations of these fatty acids are also found in soil and sediment extracts. It is unclear whether these odd- and branched chain fatty acids are of microbial origin as they are also a feature of ruminant products.

Gross microbial contamination has not been reported in previous archaeological analyses. Several factors may explain their observation in this study, and their absence in previous residue analyses. First, in an attempt to systematically understand residue formation processes, this study did not follow the usual practice of establishing a threshold of residue recovery for analytic viability. If such a threshold had been established many microbially-altered residues would have been excluded from analysis. However, employing this strategy would have substantially reduced the archaeological sample and impaired taphonomic observations. For example, applying a threshold of analytic viability of 0.2mg/g would have removed 36 of the 60 ceramic residues from analysis. The composition of the archaeological ceramics analysed also contributes to the presence of microbial contaminants. The large Trojan storage pithoi analysed were very coarse and porous, and therefore more liable to microbial

infiltration than many other classes of earthenware ceramic. Third, some archaeological ceramics were derived from contexts recognised as poor preservers of archaeological residues (i.e. Saridan Lydions for burnt contexts). Culturally-produced residues associated with these ceramics were most likely destroyed during the creation of the archaeological context. Consequently, all residues recovered from these ceramics are likely to derive from non-cultural sources.

Other taphonomic features are more difficult to elucidate. Evidence for cultural taphonomic processes is rare, but sometimes observed. Almost half of the Red Lustrous ware samples contain beeswax or beeswax like residues. These are likely to have been deliberately impregnated into the ceramic fabrics as a sealant. Another example of sealant production is the pithos AIA 1780, which contains a complex mixture of diterpenoids and triterpenoids. These molecules do not occur together naturally (Evershed et al. 2001) and their presence in this residue is most likely the result of deliberate mixing and then applied as a sealant in antiquity.

6.4.2 Taphonomic implications of residue recovery

The recovered volumes of archaeological residues from different classes of ceramic may reflect the processes that contributed to their cultural generation and archaeological transformation. While the interpretation of these contains a degree of equifinality (i.e. many features can be attributed to either cultural or post-depositional processes), the differences suggest that that different residue classes have undergone different taphonomic processes.

The Trojan ceramics are characterised by low residue recoveries (local pithoi median=0.6mg, imported pithoi median=0.7mg). In many instances, microbial addition forms a part of the residue mass. The unusually low recoveries may reflect either local taphonomic conditions that are unsuited to residue preservation, or a functional aspect of the ceramics. The low recoveries may reflect the use of pithoi for the storage of products unlikely to form significant residues, such as water or grain. This is supported by the identification of a resin or pitch in a locally-produced storage pithos (AIA 1780).

Red Lustrous wares have relatively high residue recoveries (median=2.1mg). This is largely a result of the presence of beeswax-type products that are highly resistant to molecular alteration.

Lydions from Sardis and Gordion have significantly different residue recoveries (Sardis median=1.5mg, Gordion median=2.4mg). The Sardis Lydions were recovered from a destruction level (Cahill 2000), and residues are likely to have been altered or destroyed during this process (reflected by low concentrations). Lydions from Gordion were not burnt, and have higher residue concentrations. An alternative explanation for these differences is environmental. Gordion is located in a cooler and seasonally drier highland environment than Sardis that is better suited to organic preservation.

Cooking pots and other functional wares have relatively high residue recoveries, (Boğazkoy median=2.1mg, Gordion median=2.6mg). The higher median residue recoveries for Gordion ceramics is an artefact of sample selection. The Gordion cooking pot residues are a subset of a larger body of residues partly selected for their relatively high recoveries. Median recovery for all residues from this Gordion dataset was 1.5mg. The relatively high residue volumes in cooking pot wares may result from the apparently high proportions of animal residues (identified on the basis of triacylglycerol biomarkers). Animal products produce relatively high initial concentrations of residues and these molecules are more resistant to alteration than more unsaturated plant-derived lipids.

Sediments and soils may also reflect taphonomic differences. Lipids extracted from sediments associated with Lydions in grave contexts (median=2.4mg) had higher median recoveries than extracts from environmental soils (median=1.4mg). The differences most likely reflect an anthropogenic contribution to grave sediments, either via the contextual association between Lydions and sediment or deliberately adding organics to the context (e.g. bodies, grave goods).

6.5 Implications of Chemotaxonomic and Taphonomic Application

The taphonomic approach adopted in this study to archaeological residue analysis demonstrates the complexity of the cultural and post-depositional processes that form the ceramic residues recovered by archaeological researchers. The complexity of these interactions substantially impacts on the capacity of chemotaxonomic techniques to identify the ancient sources of archaeological remains. While many chemotaxonomic techniques rely on the stability of relationships between molecules over archaeological time (ratio and multivariate analyses), the experimental and archaeological results of this study demonstrate that these initially-diagnostic proportions can be transformed through a complex series of cultural (e.g. mixing and vessel reuse), post-depositional (e.g. microbial addition) and physical (e.g. colloidal lipid structures) processes.

Understanding the taphonomic processes that form archaeological residues is complex task, but one that needs to be undertaken before the capacity of ratio and multivariate techniques can be reliably established. Biomarkers, as a simple presence/absence analysis, provide a more direct approach to understanding archaeological residues that is not as liable to alteration by taphonomic processes. However, care must be taken to ensure that biomarkers cannot arise from soil microbes or other environmental processes.

Biomarker analyses are limited by their relatively infrequent archaeological preservation. To characterise a broad range of residues, methods like ratio and multivariate analyses are required. These proportional techniques need to be reconsidered within an archaeologically-based taphonomic framework to determine which conditions are suited to ratio and multivariate analyses. This reconsideration may require the development of more specific techniques which are tailored to the taphonomies of individual wares, depositional contexts or sites.

6.6 Archaeological Conclusions

The poor fit between techniques used to interpret archaeological residues presents a significant challenge for deriving substantive conclusions about residue

source and how the products within these ceramics relate to archaeological questions. In this interpretation, I primarily rely on biomarker evidence as the most direct diagnostic tool.

6.6.1 Pithoi

The pithoi residues highlight the potential risks of microbial contamination of ceramic residues in archaeological contexts, with clear evidence for microbial lipids in several residues. Overall, lipid recoveries from Trojan pithoi were very low, usually lower than those recovered from environmental soil samples. While these low recoveries may reflect local taphonomic processes that are not suited to residue preservation, they may also reflect the nature of pithos utilisation.

The utilisation of pithoi in the storage and transportation of products with low concentrations of lipids would produce very sparse residues. In one local pithos (AIA 1780) there is evidence that the vessel may have been sealed with a resin or pitch. These types of products are typically used as sealants for liquid products, and the storage of liquids in this ceramic seems probable. For the imported pithoi, it is likely that they were used to transport products with low concentrations of lipids, such as wine.

6.6.2 Red Lustrous wheel-made wares

Approximately half the Red Lustrous ware samples contain strong evidence for the presence of beeswax in high proportions of palmitic acid, long chain hydrocarbons and alcohols. The remaining samples do not contain many diagnostic features, but may be plant oil products. This correlates well with previous studies of Red Lustrous wares from Boğazkoy. Knappett and coworkers (2005) identified three sources of Red Lustrous ware residues at Boğazkoy: beeswax, mixtures of plant oils and beeswax, and plant oils.

The confirmation of Knappett and coworkers' results is, in itself, significant. The re-examination of classes of archaeological ceramics has not been a common feature of residue analyses to date. The very similar identifications in the two studies demonstrate the robusticity of residue analysis methodologies.

It is more difficult to draw substantive conclusions from the Red Lustrous ware data. Beeswax may have been deliberately added to vessels as a sealant which inhibited the penetration of later residues. However, both this study and Knappett and coworkers' (2005) found that Red Lustrous residues were consistent with mixtures of plant oils and beeswax. This inter-study validation confirms that Red Lustrous wares at Boğazkoy were at least partially used for the storage or dispensing of plant oils.

6.6.3 Lydions

Lydion residues appear to contain a mixture of ruminant fats and plant molecules. Ruminant fats are identified on the basis of fatty acids and acylglycerols typical of ruminant products. One sample contained substantial evidence for plant products, with the presence of a range of triterpenoids and plant sterols. Triterpenoids are typical features of scented resins (e.g. frankincense, myrrh). It is unclear whether this sample is atypical of ancient Lydion contents or represents an unusually well preserved example. However, a mixture of a ruminant fat and triterpenoid resins fits well with the supposed function of Lydions as containers for a scented product known as *Bakkaris*. This product was a scented oil or unguent that was applied by smearing across the face and head. A mixture of ruminant fats and resin would produce a product with suitable properties to be used like Bakkaris, although a more definite link between the two products most likely impossible.

6.6.4 Cooking pot wares

Within the cooking pot and utilitarian wares there is substantial evidence for the presence of ruminant products. Almost half of the cooking pot samples contain substantial volumes of branched chain fatty acids. These have been used to characterise ruminants, but are also a feature of soil microbes. Acylglycerols provide clearer evidence of ruminant products with substantial proportions of triacylglycerols that contain odd-numbered fatty acid moieties (C17:0).

The high proportion of ruminant residues indicates that these products were an important feature of Late Bronze and Early Iron Age economies at Boğazkoy and

Gordion. This correlates well with animal bone data from both Gordion and other sites across Turkey from these periods (Zeder and Arter 1994).

6.7 Summary

This chapter discussed the methodological and substantive conclusions of this study. By exploring the experimental results through the taphonomic questions posed in Chapter 3, I demonstrated that archaeological residues are the products of a complex range of taphonomic interactions. Through the experimental results I highlighted the importance of understanding both cultural and post-depositional taphonomic processes before archaeological residues can be meaningfully interpreted. This taphonomic complexity tends to confound current chemotaxonomic interpretations of archaeological residue sources. This complexity was highlighted by comparing the source identifications of a range of established chemotaxonomic techniques on the archaeological sample, revealing a poor fit between residue source identifications at even the broadest plant/animal level.

Chapter 7 - Summary and Conclusions

7.1 Introduction

In Chapter 1 I highlighted how the historical development of archaeological residue research has been largely driven by a focus on assessing the analytic capacities of subsequent generations of instrumentation. As a result, residue research has been largely decoupled from archaeological research agendas. One consequence of this decoupling has been an underestimation of the complexities of cultural alterations and the effects of archaeological depositional environments. Understanding these taphonomic effects has been the primary focus of this study.

The objectives of this chapter are: a) to summarise the methodological and substantive conclusions of this study; b) to summarise the areas identified during the course of this study that would be productive for future research; and c) to consider the likely future development of archaeological residue studies both in terms of technological innovations and more programmatic archaeological agendas.

The archaeological taphonomic framework of this study was developed to specifically redress a lack of systematic approaches to understanding archaeological residue formation processes. The taphonomic framework was used to identify key taphonomic processes that then formed the basis of a series of experimental simulations. These experiments provided the controlled conditions essential to explore and evaluate the effects of these processes. Both the taphonomic model and experimental program developed in this study provide substantive new insights into the complex nature of archaeological ceramic residues.

New avenues of research identified in the course of this study also highlight the large gaps that still exist in our understanding of archaeological residue formation processes. I conclude by considering future directions for archaeological residue research, the likely advances that technological innovations will facilitate, and the importance of an archaeologically driven research agenda in harnessing these innovations.

7.2 Archaeological Taphonomic Frameworks in Residue Analysis

This study applied two taphonomic approaches to evaluate cultural and post-depositional processes. A systematic approach to cultural taphonomy was developed by adapting Schiffer's (1972) general taphonomic model to residue formation by creating a hybrid ceramic/residue flow model of residue formation. Symbolic representations of *pristine*, *reused* and *recycled* residues were constructed from the residue flow model to provide a clear conceptualisation of the processes that define different types of ceramic residues. The second taphonomic approach applied in this study evaluated the post-depositional processes that contribute to archaeological residues. This approach identified key variables that affect organic archaeological remains within the three broad types of post-depositional taphonomic processes (chemical, physical and biological). It was used to provide a clear framework for: a) the identification of key processes likely to affect the composition of archaeological residues, and b) the exploration and evaluation of these processes through a systematic program of experimental taphonomic simulations.

Adopting a systematic approach to evaluating residue formation processes generally confirmed the conclusions of previous research (e.g. preferential degradation of unsaturated lipid species; increased proportions of saturated lipid species in archaeological residues; variable penetration of lipids across a ceramic profile). However, beyond these general confirmatory results, the systematic approach adopted by this study identified previously unconsidered and untested effects and developed new interpretive innovations. These include high temperature production of short chain fatty acids; confirmed assumptions about short chain fatty acid leaching; lipid preservation in acidic conditions; the role of colloidal residue properties in differential alteration; the effects of sacrificial preservation; demonstration of ceramic potential for residue absorption; demonstration of the role of the ceramic matrix on defining residue composition; highlighted the potential for microbes to contribute to the formation of archaeological residues and the necessity for thresholds of analytic viability for residue recovery; flagged the highly problematic character of established chemotaxonomic techniques for accurate residue identification; and demonstrated the utility of divergence indices as a rapid means to assess the degree of residue alteration.

The controlled simulation of a suite of taphonomic effects has enabled the identification of the complex cultural and post-depositional taphonomic interactions involved in the formation of archaeological residues. This complexity has been underestimated or ignored by previous studies. Comparison of established chemotaxonomic techniques for archaeological residues showed that these often produced contradictory results, primarily because these techniques are predicated on diagnosing simple residues.

7.3 Future Directions

The key taphonomic processes identified in this study contained molecular and biogenic components that can interact in highly complex pathways. The scope of this study was restricted to an initial evaluation of these processes through a program of experimental simulations. However, it was not feasible to cover all the permutations possible. In the archaeological dataset, a wide range of potential cultural processes may have been involved in the production of residues that were not considered in this study (e.g. brewing, curdling, roasting and mixing). More detailed understanding of the implications of molecular and biogenic components of archaeological residue formation will require ongoing systematic effort. This involves two key areas: a) the development of an archaeological basis for residue research, part of which must include an archaeologically-based taphonomic understanding of how residues are formed, and b) the continued exploration of complex taphonomic processes that form archaeological residues to produce a more complete understanding of how these remains can be used to address important archaeological problems.

A productive area of future residue taphonomic research is likely to involve examination of post-depositional taphonomic processes on residue artefacts. Groundwater movement and pH are prime candidates for this type of research as the scale and intensity of these processes can be estimated within depositional contexts. Conducting this type of post-depositional taphonomic research closely resembles the approaches used to assess post-depositional taphonomic effects on other artefact classes (Bollong 1994; Wilson and Pollard 2002).

Future research needs to systematically evaluate a wider range of taphonomic variables than were possible here (e.g. longer exposure periods, more complex forms

of vessel reuse) and the interactions between these variables (e.g. the interaction between groundwater leaching and soil pH) on residue composition. While complex, understanding these interactions will be essential for increasing the relevance of residue analysis to a broader range of archaeological artefact classes.

A final direction for future research should involve greater scales of molecular complexity than those explored in the experimental alteration of a set of reference fats and oils presented in this study. Most archaeological residues are likely to result from the processing or storage of whole foods and other organic products that contain a much more complex range of molecules, including nucleonic acids, proteins, hydrocarbons and polymers in addition to lipids. While this study simplified the experimental design by using only lipid molecules, the effects of these molecular classes on archaeological residue composition deserves closer attention. Clearly, an important focus of future research will involve understanding the microbial alteration of archaeological residues.

7.4 Conclusion

Technological developments will continue to greatly influence the field of archaeological residue research. Recently developed and emerging instrumentation (e.g. Ultra Performance Liquid Chromatography – Mass Spectrometry) have already simplified the analysis of archaeological residues and other molecular mixtures, both in the technical skill required to perform analyses and interpret archaeological results, in higher sample throughputs, and in the level of automation these instruments are capable of providing (Waters 2004). These innovations should make the instrumental technology required (and hence results) more accessible to archaeologists who are not necessarily specialists in chemical analysis.

By providing a more archaeologically relevant framework to situate residue analysis in this study presents an opportunity to: a) allow archaeologists to be more directly involved in residue research, and b) to explore more complex problems in archaeological residue analysis. These two factors should allow archaeological residue analysis to develop from the technical and method-driven approaches that

currently dominate the field into a more integrated component of broader archaeological research agendas.

The taphonomic approach to residue formation developed in this study enabled a more systematic means of defining, organising and exploring residue formation processes. This enabled a clear and focused set of experiments to be developed that addressed some of the key factors identified in this framework. However, a number of outstanding permutations as described above still remain untested. Clearly, an ongoing program of systematic experimentation will be needed to enable residue analysis to reliably identify a wider range of complex residues.

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Appendices

Appendix 1: Experimental Procedures

1a: Residue extraction procedures

1. Samples were reduced to appropriate size either by breaking or cutting.
2. Samples were cleaned by removal of the outer surface (1-2mm) using a dental drill equipped with a carbide bur to remove potential soil contaminants and plasticisers resulting from ceramic storage (Christie 1987:74, Heron et. al. 1991).
3. The cleaned sample was ground to a fine powder with a porcelain mortar and pestle.
4. 8 grams of ceramic was placed in a four disposable glass test tubes. 9ml of HPLC grade methanol/chloroform (1:2) per test tube was added and the mixture ultrasonicated for 15 minutes. Test tubes were cleaned by ultrasonicating in an AR grade methanol/chloroform mixture (1:2) for 20 minutes prior to use.
5. The mixture was then centrifuged (3500rpm for 15 mins). After centrifugation, the supernatant was decanted. Steps 4 and 5 were repeated once.
6. Solvent was removed by evaporation, yielding the residue.
7. Residue extracts were stored in deep-freeze (-18°C) until required for analysis.

1b: Fatty acid methyl ester (FAME) preparation

1. 50mg of lipid or residue (or total available amount if >50mg) was placed in a glass vial with 3ml of 0.5M methanolic Sodium Hydroxide (NaOH) and 6ml of anhydrous ether.
2. The vial was sealed and the mixture heated to 35°C for 48 hours.
3. 3ml of HPLC grade water was added to stop the reaction.
4. FAMEs were collected by washing the mixture in 3x3ml of HPLC grade Hexane.
5. Following collection, hexane was evaporated and the samples stored in deep-freeze (-18°C) until required for analysis.

1c: HPLC-MS operating conditions

Ionisation module: APCI
Ion Mode: Positive.
Drying gas: N_2 at 150ºC, 12psi
Corona current: 10µA, 1400v
Shield: 600v
Capillary CID: 50v
Spectra range: 500 to 1000 *m/z*
Columns: Two in series Waters C18-A columns (150mm x 3.9mm i.d., 4µm packing), fitted with a 4µm pre-column guard.
Scan method: Centroid
Sim Width 0.7 amu
Scan time: 1 sec
Mobile Phase: Acetonitrile (MeCN), Dichloromethane (DCM) at 1 ml/min
Injection Volume: Samples were concentrated 5mg/1ml and filtered prior to HPLC column injection. Samples (10µl) were injected into the column via a Prostar 430 autosampler.

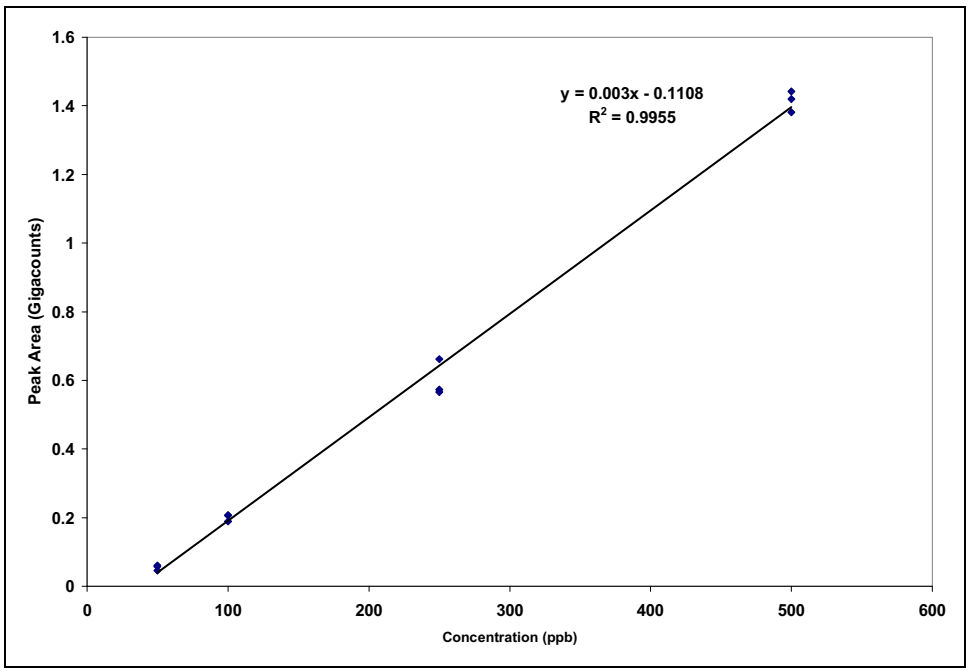
Solvent Gradient:
0 min 80% MeCN 20% DCM
90 min 50% MeCN 50% DCM
110 min 50% MeCN 50% DCM
115 min 80% MeCN 20% DCM

1d. GC-MS operating conditions

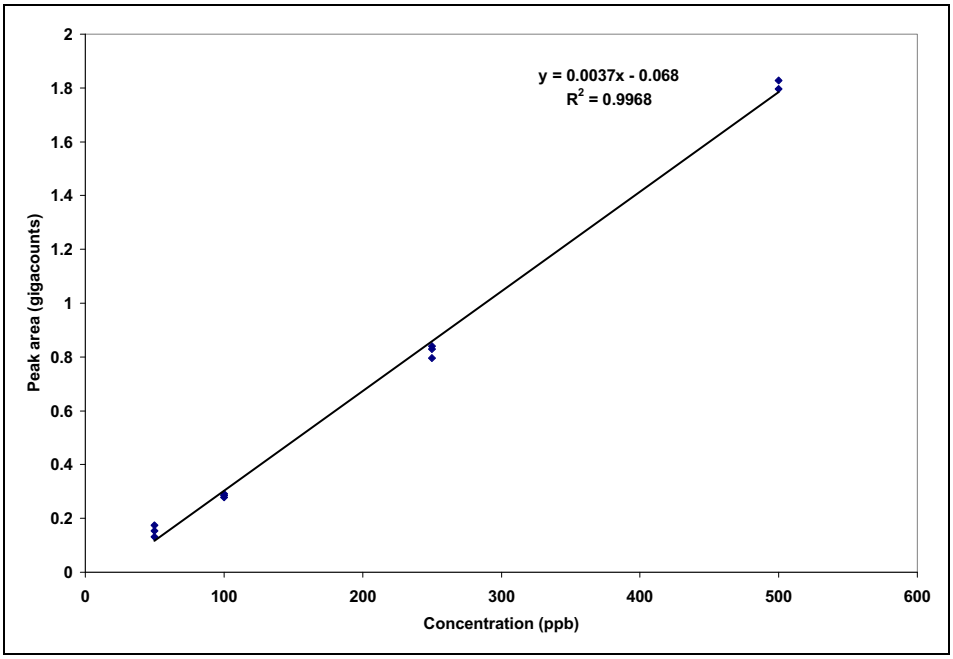
Ionisation module: EI
Ion Mode: Negative
Carrier Gas: Helium
Electron Energy: -70 eV
Detector Voltage: 940v
Ion Source Temperature: 200°C
Transfer Line Temperature: 250°C
Spectra range: 45 to 550 *m/z*
Column: VF-5ms Varian Factor Four Capillary Column (30m x 0.25mm i.d.)
Scan method: Centroid
Sim Width 0.7 amu
Scan time: 0.5 sec
Injection Volume: 0.1µl Mobile Phase: Acetonitrile (MeCN), Dichloromethane (DCM) at 1 ml/min
Injection Volume: Samples were concentrated to 5mg/1ml and filtered prior to GC column injection. Samples (0.1µl) were manually injected using a 1µl glass syringe with 0.1µl gradations.

Temperature Program:
0 min 100°C
12.5 min 200°C @ 8°C/min
20.5 min 208°C @ 1°C/min
34.42 min 315°C @12°C/min

1e. Detector response graphs

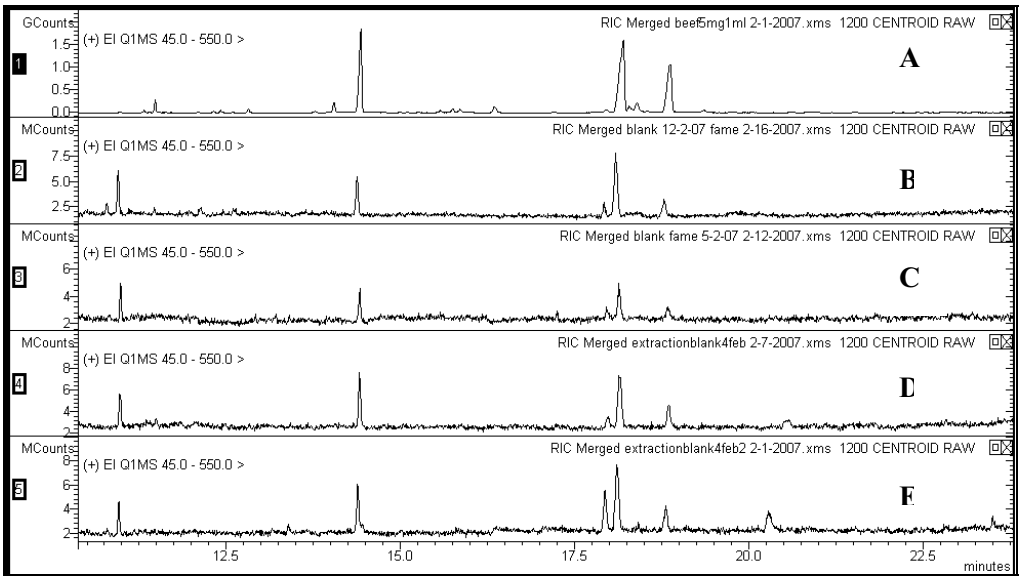


Appendix Figure 0.1: Calibration curve for authentic stearic acid methyl ester (C18:0) between 50ppb and 500ppb (sample concentrations are 50ppb, 100ppb, 250ppb, and 500ppb).



Appendix Figure 0.2: Calibration curve for authentic oleic acid methyl ester (C18:0) between 50ppb and 500ppb (sample concentrations are 50ppb, 100ppb, 250ppb, and 500ppb).

1f. Extraction contamination



Appendix Figure 0.3: Partial Gas Chromotagrams showing the relative intensity of a) a beef RFO used for comparison, and b-e) a sample of typical blanks generated during residue extraction and derivatisation. Note the large difference in signal intensity between the beef RFO (1.5 gigacounts) and the extraction blanks (>10 megacounts).

1g. Generation of the 2004 experimental ceramics

1. 500 grams, or 500ml of material and 1000ml of distilled water was placed in an earthenware pot.
2. The pot was placed in a plastic ‘oven bag’ to avoid sample cross contamination, and cooked in a domestic oven at a moderate heat (150°C) for 5 hours.
3. Cooked material and liquid removed and disposed. The ceramic pot was washed with distilled water to remove foodstuffs adhering to the ceramic surface.
4. The pot was dehydrated at 50°C for 24 hours to remove water from the ceramic fabric.
5. After cooling, the sealed in plastic wrap until required (typically less than four days).

Appendix 2: GC-MS and HPLC-MS Data

The data generated in this study can be found on a compact disk included with this thesis. Data tables were prepared in Microsoft Excel 2003 and are in .xls format. Specific data can be found in the following files:

GC Experimental Data Part 1: Contains the GC MS results of all experimental data based on RFO samples. Includes RFO samples, boiling experiment samples, heating experiment (100°C, 200°C, 300°C) samples, pH experiment samples, microbial alteration experiment samples, reused ceramic experiment samples and leaching experiment samples.

GC Experimental Data Part 2: Contains the fatty acid results for the experimental residues generated in 2004. Includes the results of 2004 unaltered lipid extracts, results of the 2004 residue extractions and GC-MS data for residues extracted in 2007.

GC Archaeological Data: Contains all GC-MS results for the archaeological section of this study (Red Lustrous Wheel-made ware, Lydions, Troy Pithoi and Cooking pot wares) and soil/sediment extracts.

HPLC Experimental Data Part 1: Contains all HPLC-MS experimental data based on RFO samples. Includes Beef, Sesame and Linseed RFOs, boiling experiment samples and heating experiment (100°C, 200°C, 300°C) samples. Where TAG species could not be separated, their ions were summed and are expressed as TAG1/TAG2 (e.g. PPP/MyPS).

HPLC Experimental Data Part 2: Contains HPLC-MS results for the experimental residues generated in 2004. Includes results of 2004 unaltered lipid extract, 2004 residue extractions and 2007 residue extractions. Where TAG species could not be separated, their ions were summed and are expressed as TAG1/TAG2 (e.g. PPP/MyPS).

HPLC Archaeological Data: Contains all HPLC-MS results for the archaeological section of this study (Red Lustrous Wheel-made ware, Lydions, Troy Pithoi and Cooking pot wares) and soil/sediment extracts. Note that the Gordion cooking pot samples (YHP series) were analysed using a different HPLC-MS

technique in 2004. Where TAG species could not be separated, their ions were summed and are expressed as TAG1/TAG2 (e.g. PPP/MyPS).

Appendix 3: Catalogue of Archaeological Samples

The archaeological samples used in this study were from two sources. The first was the Anatolian Iron Ages Ceramics Project. These samples are prefixed with AIA. The second set of ceramics used in this study was collected by Robert Henrickson, the ceramist during Mary M. Voigt's excavation of Gordion. These samples are prefixed with YHP (Yassi Hoyuk Polatı).

Sample	Site	Period	Type	Sample Description
AIA 1778	Troy		earthenware	Pithos
AIA 1780	Troy		earthenware	Pithos
AIA 1781	Troy		earthenware	Pithos
AIA 1782	Troy		earthenware	Pithos
AIA 1784	Troy		earthenware	Pithos
AIA 1785	Troy		earthenware	Pithos
AIA 1786	Troy		earthenware	Pithos
AIA 1787	Troy		earthenware	Pithos
AIA 1788	Troy	VI?	earthenware	Type 9973 Imported Pithos - Kythera? Micaceous
AIA 1789	Troy	VI?	earthenware	Type 9973 Imported Pithos - Kythera? Micaceous early? LBA
AIA 1790	Troy	VI?	earthenware	Type 9973 Imported Pithos - Kythera? Micaceous early? LBA
AIA 1791	Troy	VI?	earthenware	Type 9973 Imported Pithos - Kythera? Micaceous early? LBA
AIA 1792	Troy	VI?	earthenware	Imported pithos - Kythera? LBA (early?) micaceous
AIA 2224	Troy		earthenware	Pithos
AIA 2225	Troy		earthenware	Pithos
AIA 2226	Troy		earthenware	Pithos
AIA 2227	Troy		earthenware	Pithos
AIA 2228	Troy		earthenware	Pithos
AIA 978	Sardis	Iron Age	earthenware	Lydion base - streaky
AIA 982	Sardis	Iron Age	earthenware	Lydion black lines
AIA 1724	Sardis	IA	earthenware	Buff; Lydion Body; banded, streaky
AIA 1725	Sardis	IA	earthenware	Buff; Lydion body; orange slip
AIA 1743	Sardis	IA	earthenware	Buff; Lydion body; red and white lines on shoulder , red body, streaky brown base
AIA 1857	Gordion	LP; 3rd Qtr 6th Cent	earthenware	Buff; Lydion; No decoration
AIA 1858	Gordion	LP; 3rd qtr 6th cent	earthenware	Buff; Lydion; red slip
AIA 1861	Gordion	LP; 3rd Qtr 6th Cent	earthenware	Buff; Lydion; mottled black glaze (2 pcs)
AIA 1863	Gordion	LP; 3rd Qtr 6th Cent	earthenware	Buff; Lydion; black with white lines (burned)
AIA 1870	Gordion		earthenware	Buff; Lydion; streaky red/orange
AIA 2231	Bogazköy	Hittite		pottery cache
AIA 2232	Bogazköy	Hittite		pottery cache
AIA 2233	Bogazköy	Hittite		pottery cache
AIA 2235	Bogazköy	Hittite		pottery cache
AIA 2236	Bogazköy	Hittite		pottery cache
AIA 2237	Bogazköy	Hittite		pottery cache
AIA 2238	Bogazköy	Hittite		pottery cache
AIA 2240	Bogazköy	Hittite		
AIA 2241	Bogazköy	Hittite		
AIA 2242	Bogazköy	Hittite		
AIA 2243	Bogazköy	Hittite		
AIA 2274	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2276	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2277	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2278	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2279	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2280	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 2282	Bogazköy	Hittite	cooking pot	coarse, burned
AIA 1769	Sardis		Sediment	Sediment from Lydion 98
AIA 1771	Sardis		Sediment	Sediment from Lydion 45
AIA 1772	Sardis		Sediment	Sediment from Lydion 84
AIA 1773	Sardis		Sediment	Sediment from Lydion 69
AIA 1776	Sardis		Sediment	Sediment from Lydion 118
AIA 1777	Sardis		Sediment	Sediment from lydion

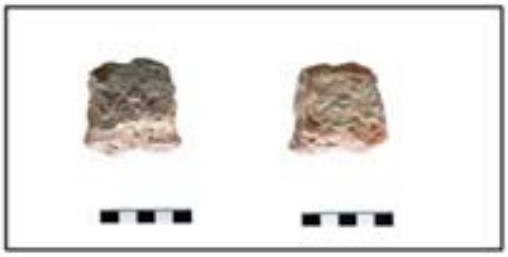
Sample ID	Source Institution	Accession	Source	Context	Site	Operation	Excavation Data	Period	Phase	Traditional Name	Ceramic Type	Form	Form Part	Surface Treatment	Temper	Texture	Early Date	Late Date	Comment
YHP050	Univ Museum (Penn)	YH 22790	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22790 Loc 40 Lot 90	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Buff Common		Bodysherd	Smoothed	Grit	Medium	1500 BC	1200 BC	
YHP053	Univ Museum (Penn)	YH 23169	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	23169 Loc 40 Lot 95	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Buff Common		Bodysherd	Smoothed	Grit	Medium	1500 BC	1200 BC	
YHP074	Univ Museum (Penn)	YH 27297	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	9	27297 Loc 12 Lot 58	YHSS 7A (Early Iron Age)	705.01	Early Iron Age	Buff Common		Bodysherd	Smoothed	Grit	Medium	1000 BC	950 BC	
YHP105	Univ Museum (Penn)	YH 22494	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22494 Loc 45 Lot 81	YHSS 7A (Early Iron Age)	730.02	Early Iron Age	Buff Common		Bodysherd	Smoothed	Grit	Medium	1000 BC	950 BC	
YHP106	Univ Museum (Penn)	YH 22494	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22494 Loc 45 Lot 81	YHSS 7A (Early Iron Age)	730.02	Early Iron Age	Buff Common		Bodysherd	Smoothed	Grit	Medium	1000 BC	950 BC	
YHP153	Univ Museum (Penn)	YH 22794	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22794 Loc 40 Lot 91	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Cooking		Bodysherd	Smoothed	Grit	Medium	1400 BC	1200 BC	
YHP156	Univ Museum (Penn)	YH 22794	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22794 Loc 40 Lot 91	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Cooking		Bodysherd	Smoothed	Grit	Medium	1400 BC	1200 BC	
YHP160	Univ Museum (Penn)	YH 23179	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	23179 Loc 40 Lot 97	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Cooking		Bodysherd	Smoothed	Grit	Medium	1400 BC	1200 BC	
YHP161	Univ Museum (Penn)	YH 22790	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22790 Loc 40 Lot 90	YHSS 8 (Late Bronze Age)	850.00	Late Bronze Age	Cooking		Bodysherd	Smoothed	Grit	Medium	1400 BC	1200 BC	
YHP242	Univ Museum (Penn)	YH 22784	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22784 Loc 42 Lot 88	YHSS 7A (Early Iron Age)	730.06	Early Iron Age	Early Iron Age Handmade		Bodysherd	Smoothed, partial burnish	Grit	Medium	1000 BC	950 BC	Soft when crushed
YHP249	Univ Museum (Penn)	YH 31489	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	8	31489 Loc 30 Lot 154	YHSS 7A (Early Iron Age)	705.12	Early Iron Age	Early Iron Age Handmade		Bodysherd	Smoothed, partial burnish	Grit	Medium	1000 BC	950 BC	Soft when crushed
YHP341	Univ Museum (Penn)	YH 25760	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	14	25760 Loc 4 Lot 4	YHSS 6B (Early Phrygian)	660.00	Early Iron Age Handmade	Early Iron Age Handmade		Bodysherd	Smoothed; burnished	Grit	Medium	950 BC	700 BC	Medium-soft when crushed
YHP342	Univ Museum (Penn)	YH 20337	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	20337 Loc 9 Lot 16	YHSS 6B (Early Phrygian)	660.00	Early Iron Age Handmade	Early Iron Age Handmade		Bodysherd	Smoothed; burnished	Grit	Medium	950 BC	700 BC	Medium-soft when crushed
YHP501	Univ Museum (Penn)	YH 26671	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	14	26671 Loc 11 Lot 27	YHSS 7 (Early Iron Age)	730.00	Early Handmade	Early Handmade (brown-tan)	Large pot	Bodysherd	'Slipped'	Grit	Medium	1100 BC	950 BC	Soft and friable when crushed
YHP503	Univ Museum (Penn)	YH 20596	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	20596 Loc 18 Lot 29	YHSS 7 (Early Iron Age)	735.00	Early Handmade	Early Handmade (grey)	Large pot	Bodysherd	'Slipped'	Grit	Medium	1100 BC	950 BC	Soft and friable when crushed
YHP508	Univ Museum (Penn)	YH 20992.10	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	20992 Loc 23 Lot 30	YHSS 7 (Early Iron Age)	735.00	Early Handmade	Early Handmade (grey)	Bowl	Rim	'Slipped'/burnished	Grit	Medium	1100 BC	950 BC	Soft and friable when crushed
YHP514	Univ Museum (Penn)	YH 21331.1	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	5	21331 Loc 27 Lot 31	YHSS 7 (Early Iron Age)	700.00	Early Handmade	Early Handmade (grey)	Large pot	Bodysherd	'Slipped'/burnished	Grit	Medium	1100 BC	950 BC	Soft and friable when crushed
YHP529	Univ Museum (Penn)	YH 22460.18	M. M. Voigt	Excavated	Gordion (Yassihoyuk)	3	22460 Loc 23 Lot 72	YHSS 7 (Early Iron Age)	735.00	Early Handmade	Early Handmade (grey)	Pot	Bodysherd	'Slipped'/burnished	Grit	Medium	1100 BC	950 BC	Soft and friable when crushed

Troy

Local Pithoi (n=13), Imported Pithoi (n=5).

AIA 1778

AIA 1780



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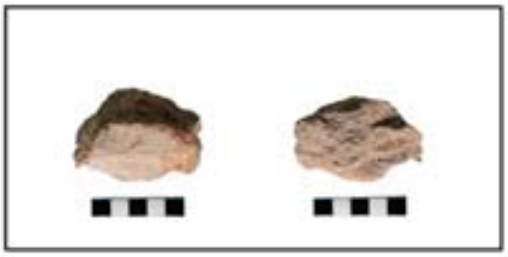
AIA 1782



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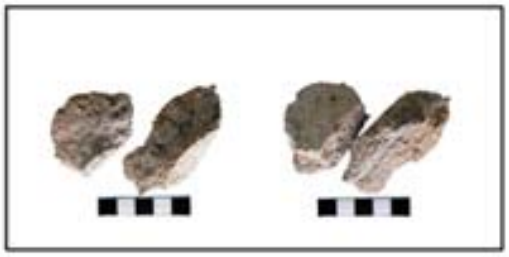

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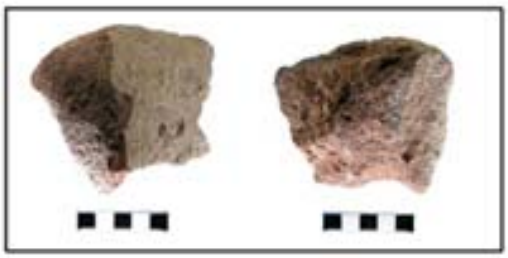

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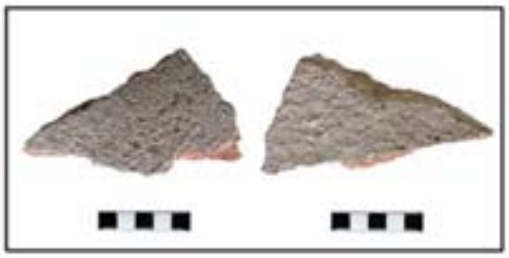

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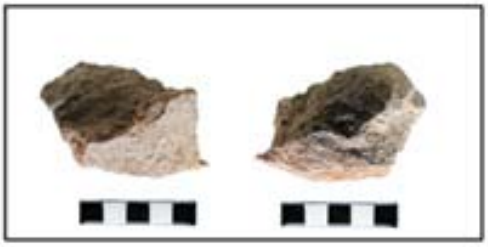
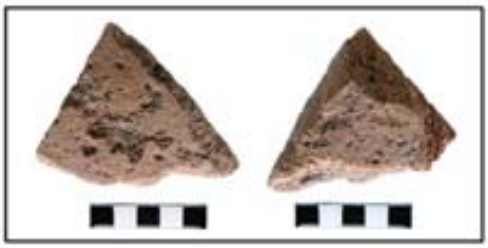


AIA 2224

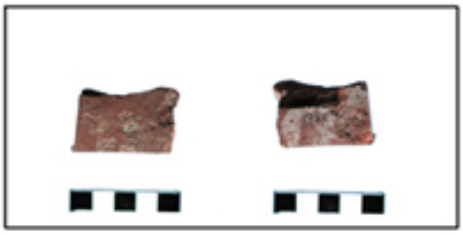
AIA 2225

AIA 2226

AIA 2227



AIA 2228



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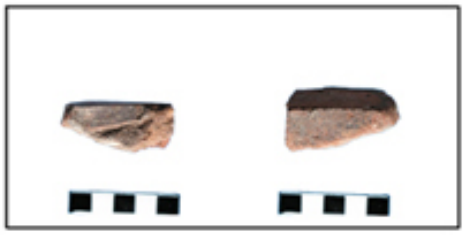

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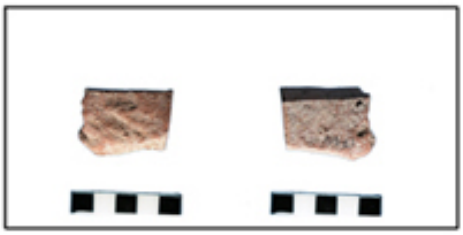

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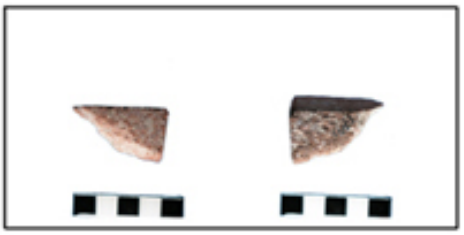

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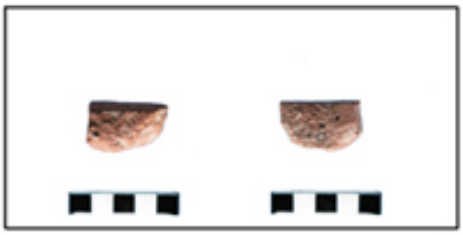
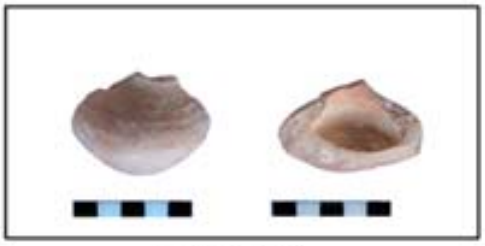


AIA 1792

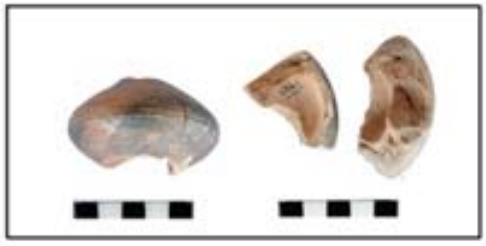
Gordion

Lydions (n=5).

AIA 1857



AIA 1858



AIA 1861

AIA 1863

AIA 1870

Sardis

Lydions (n=5).

AIA 978

AIA 982

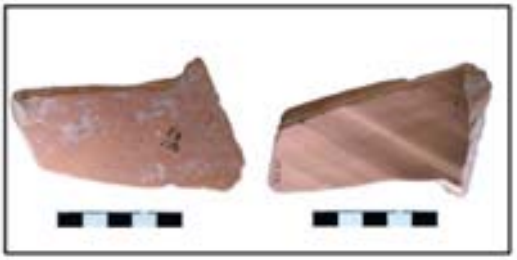
AIA 1724

AIA 1725

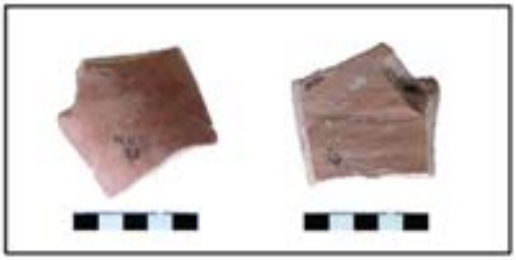
AIA 1743

Boğazkoy

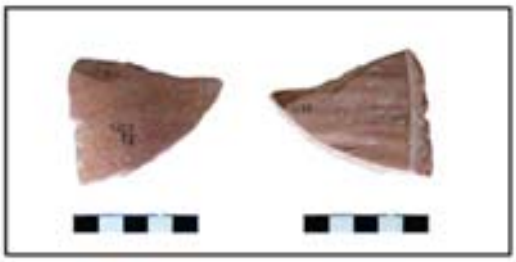
Red Lustrous wheel-made ware (n=11), Cooking Pot wares (n=7).



AIA 2231



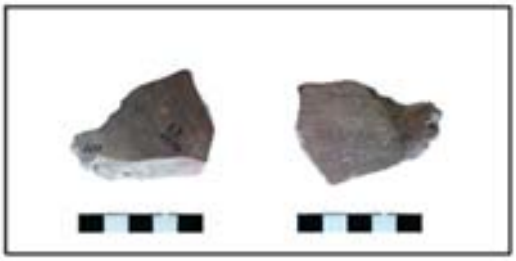
AIA 2232



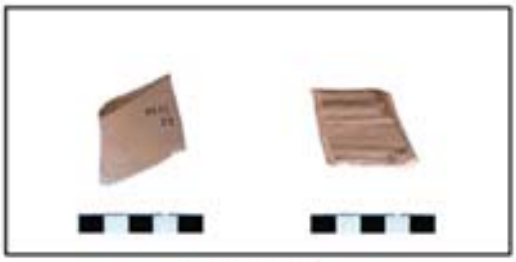
AIA 2233

AIA 2235

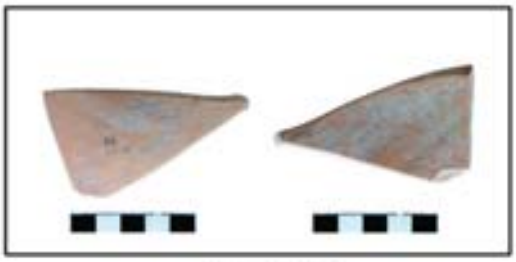
AIA 2236



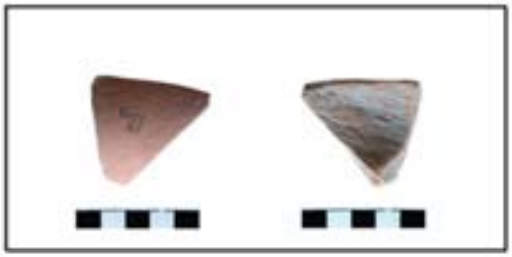
AIA 2237



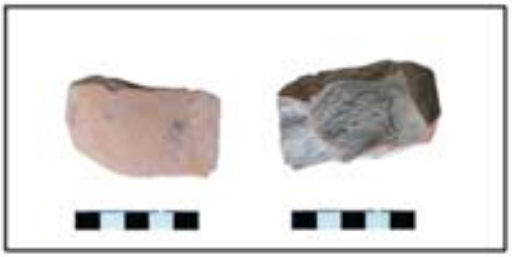
AIA 2238



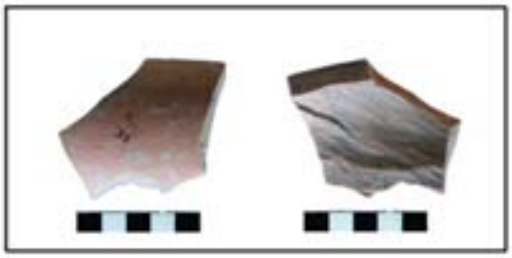
AIA 2240



AIA 2241



AIA 2242



AIA 2243

AIA 2274

AIA 2276

AIA 2277

AIA 2278

AIA 2279

AIA 2280

AIA 2282