CHEMICAL CHARACTERIZATION OF LIPID RESIDUES IN NEOLITHIC AND CHALCOLITHIC POTTERY FROM ANATOLIA

by

Ayla Türkekul Bıyık

B.S., Chemistry, Yıldız Technical University, 1997

M.S., Chemistry, Boğaziçi University, 2001

Submitted to the Institute for Graduate Studies in
Science and Engineering in partial fulfillment of
the requirement for the degree of
Doctor of Philosophy

Graduate Program in Chemistry

Boğaziçi University

2009

To my son Can,
my parents Meryem and Hasan Türkekul
and my beloved husband Mehmet

ACKNOWLEDGMENTS

I would like to thank my supervisor Professor Hadi Özbal, whose support and guidence over the last six years has been invaluable. I would also like to thank for his confidence in trusting me to undertake this project and for his help and friendship throughout.

I would like to extend my gratitude to the members of my Ph.D. thesis committee: Prof. Belkıs Halfon, Prof. Günhan Danışman, Prof. Gülaçtı Topçu and Assoc. Prof. Neylan Dirilgen for their valuable critisizm, understanding and their comments on the manuscript.

I would also like to thank the following archaeologists who have provided the potsherds which have made this project possible: Dr. Rana Özbal Gerritsen, Dr. Fokke Gerritsen, and Prof. Mehmet Özdoğan. Dr. Rana Özbal Gerritsen and Dr. Fokke Gerritsen are also gratefully acknowledged for archaeological information about Tell Kurdu and Barcın Höyük sites and for classification of the potteries from these sites.

I owe thanks to Prof. Richard Evershed from Bristol University, School of Chemistry who generously opened his laboratory for my research. Also, many thanks go to Dr. David Chivall and Dr. Ian Bull of the same university for undertaking GC-C-IRMS analysis with efficiency.

Many thanks go to Recep Koç, who helped me to solve the technical problems arising from GC instrument.

I would like to thank also to my friends from Chemistry Department Dr. Gökhan Çaylı, Dr. Aylin Ziylan Albayrak, Dr. Funda Oğuz, Rıza Dervişoğlu and Fatma Haksan for their valuable help and friendship.

I would like to express my sincere gratitudes to my dear friends from Advance Technologies R&D Center of Boğaziçi University: Burcu Selen Çağlayan, Ayşe Çetin and Semra Türe for their help, understanding and moral support all the time.

I owe huge thanks to my mother Meryem Türkekul,my father Hasan Türkekul and my brother Mesru Türkekul for their endless love, support and encouragement and above all their belief in me thoughout my many years at university.... I couldn't done this without you.

Finally, my deepest thanks are extended to my husband Mehmet Bıyık who has supported me endlessly and help in every way to make my work easy. Also, I would like to thank to my son Can Bıyık for being with me.

The Boğaziçi University Scientific Research Fund with project number 05B505D is gratefully acknowledged for the financial support.

ÖZET

ANADOLU NEOLİTİK VE KALKOLİTİK ÇANAK ÇÖMLEKLERİNDEKİ LİPİD KALINTILARININ KİMYASAL KARAKTERIZASYONU

Bu çalışmanın başlıca amacı Neolitik ve Kalkolitik dönemlerinde Anadolu'nun farklı bölgelerinde yaşamış olan insan topluluklarının besin tercihlerini kullanmış oldukları hayvansal ve bitkisel ürünler açısından araştırmaktır. Bunu gerçekleştirmek için Neolitik, Kalkolitik ve İlk Tunç Çağ'ına ait toplam 118 çanak çömlekte absorp edilmiş lipid kalıntılarının sistematik kimyasal analizi GC, GC/MS ve GC-C-IRMS kullanarak yapılmıştır.

Analizi gerçekleştirilmiş toplam 118 çanak çömlekten sadece 15'inde (yüzde 12.7) kayda değer miktarda lipid kalıntısına rastlanmıştır. Tüm kalıntıların hayvansal kökenli oldukları belirlenmiştir. Bir çanak çömlekte balmumuna rastlanmıştır. Palmitik ve stearik asitlerin ($C_{16:0}$ ve $C_{18:0}$) $\delta^{13}C$ değerleri söz konusu lipid kalıntılarının kaynaklarını geviş getiren, domuz veya süt kökenli olarak sınıflandırılması için kullanılmıştır. Bu ayırımı yapmak hayvanların farklı metabolizmaları ve farklı sindirim fizyolojilerinden dolayı mümkündür. Barcın Höyük örneklerinde süt kökenli yağların çoğunlukta olduğu gözlenmiştir. Tell Kurdu örneklerinde geviş getiren hayvansal yağlarına rastlanırken İlk Tunç Çağ İkiztepe'den çıkan delikli testide ise süt ve domuz yağsı karışımına rastlanmıştır. Tell Kurdu'da geviş getiren yağlarına rastlanması kazıdan çıkan hayvan kemikleri ile de uyumlu bir sonuç vermiştir.

Barcın Höyük'üğün Neolitik katmanlarında tespit edilmiş olan süt yağları M.Ö. yedinci bin yılda sütün işlenerek kullandğını göstermiştir. Bu tarih Yakın Doğu'da evcileştirilen ilk inek, koyun ve keçiden bin yıl sonrasına tekabül etmektedir. İstanbul yakınlarındaki Pendik ve Fikirtepe, Kırklareli yakınlarındaki Aşağı Pınar ve Bursa

yakınlarındaki Toptepe'de yapılan benzer çalışmalar da göz önünde tutulduğunda sütü erken dönem Kuzeybatı Anadolu'da önemli bir besin kaynağı olduğu anlaşılmaktadır.

Barcın Höyük çanak çömleklerinin analizleri sırasında kısmen bozunmaya uğramış balmumu kalıntısı tespit edilmiştir. Dolayısıyla Barcın Höyük'te arıcılık yapılmış olabileceği sonucuna da varılmıştır. Ayrıca bu örnek Anadolu çanak çömleklerinde şimdiye kadar bulunan ilk balmumu olma özelliğini de taşımaktadır.

ABSTRACT

CHEMICAL CHARACTERIZATION OF LIPID RESIDUES IN NEOLITHIC AND CHALCOLITHIC POTTERY FROM ANATOLIA

The objective of this study was to investigate the dietary preferences such as the exploitation of animal and plant products during the Neolithic and Chalcolithic in Anatolia. This was realized by the systematic analysis of absorbed lipid residues in 118 potsherds from three Neolithic and Chalcolithic and an Early Bronze Age sites by using GC, GC-MS, and GC-C-IRMS. The stable isotope analysis of the lipid residues from the potsherds was undertaken in order to probe their origin which could not be comprehended by simple GC and GC-MS analysis.

Out of 118 potherds anaylized only 15 samples (12.7 per cent) yielded significant levels of lipid residues. All ofthe lipid residues were drived from animal fats. Beeswax was also observed in a potshered. The $\delta^{13}C$ values of the ($C_{16:0}$ and $C_{18:0}$) faty acids from the degrated animal fats were used to assign the ruminant, porcine and dairy origins of the residues. The distinction was possible due to the different metabolisms and digestive physiologies of the animals. Barcın Höyük potsherds contained a higher incidence of dairy fats than ruminats and porcine fats. The Tell Kurdu extracts were assigned to ruminats adipose fats whereas the perforated potsherd from the EBA levels of İkiztepe contains a mixture of dairy and porcine adipose fat. The occurence of ruminat fats from the Tell Kurdu potsherds correlate with the faunal assemblage of this site.

Detection of milk fats from the Neolithic levels of Barcin Höyük demostrated that processed milk was in use by the seventh millennium BC. This is about a millennium after the first domestication of cattle, sheep and goat in the Near East. Based on similar studies from Aşağı Pinar near Kırklareli, Toptepe near Bursa, Pendik and Fikirtepe near İstanbul, it was concluded that milk processing was particulary important at an early period in Northwesren Anatolia.

Partially degrated beeswax was also identified in a Neolithic potsherd from Barcın Höyük. It can be concluded that possible bee keeping was also practiced in the Neolithic Barcın Höyük. The sample constitues the earliest evidence of beeswax associated with pottery in Anatolia.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOL/ ABBREVIATIONS

BSTFA	N,*O-bis*(trimethylsilyl)trifluoroacetamide
CAM	crassulacean acid metabolism
CoA	coenzyme A
DAG	diacylglycerol
EI	electron ionisation
FAME	fatty acid methyl ester
FID	flame ionisation detector
GC	gas chromatography
GC-C-IRMS	gas chromatography-combustion-isotope ratio mass spectrometry
GC-MS	gas chromatography/mass spectrometry
HPLC	high performance liquid chromatography
HTGC	high temperature gas chromatography
IRMS	isotope ratio mass spectrometer
MAG	monoacylglycerol
RuBisCO	ribulose bisphosphate carboxylase
TAG	triacylglycerol
TLE	total lipid extract
TMS	trimethylsilyl
VPDB	Vienna Pee Dee Belemnite

$C_{x:y}$	denotes long-chain fatty acids with x carbon atoms and y degree of unsaturation
$C_{x:y}$br	denotes branched-chain fatty acids with carbon chain length x and y degree of unsaturation
X M	monoacylglycerols of carbon chain length x
X D	diacylglycerols of carbon chain length x
X T	triacylglycerols of carbon chain length x
X K	mid-chain ketones of carbon chain length x
A_x	*n*-alkanes of carbon chain length x
C_x OH	alcohols of carbon chain length x

X WE	wax esters of carbon chain length x
*	phthalate ester plasticisers emanating from the storage of potsherds in plastic bags
IS	internal standard (*n*-tetratriacontane) added at the extraction stage to enable lipid quantification

1. INTRODUCTION

1.1. Organic Residues in Archaeology

Archaeological sites often provide a wide variety of objects and materials which have been collected, processed and used by human beings through time. Due to their prolonged burial in a sedimentary matrix, some of these materials and objects have been partially or totally altered. Objects such as pottery, flint, obsidian and stone artifacts are made out of inorganic materials, as well as bones are preserved for millennia in various environments (Regert *et al.,* 2003). The preservation of pure organic material, however, is still considered as quite unusual in archaeological context.

The recent expansion of analytical organic chemistry within archaeological sciences has been made possible by the application of micro chemical techniques that are capable of processing complex mixtures. This has been stimulated by the idea that organic traces may be preserved in a wider range of microenvironments than previously recognized. Organic substances recovered from archaeological contexts are commonly referred to as residues, implying the presence of also decomposition products.

The major challenge in organic residue analysis in archaeology involves the identification of the origins of constituents in a specific sample and to understand the mechanisms that have allowed the preservation or induced degradation. Two important categories of residue may be distinguished, namely those derived from edible foodstuffs and those from inedible organic substances. Edible organic foodstuffs can be detected by the identification of chemical constituents (fats, proteins and carbohydrates). Identification of foodstuff leads to the study of ancient diet that is one of the most important research areas within archaeology, reflecting the complex nature of human beings developing relationship with the environment.

The second category, inedible organic residues, relates to natural products exploited by human beings produced by or derived from various animals and plants. There is a wide range of substances utilized for an important range of functions. These include resins,

waxes, glues, gums and dyes. In nature many of these substances have protective functions, which provide greater chemical stability on constituent components compared to the more easily degradable foodstuff. For example, some trees exude natural resins when the bark is cut in order to stop the loss of water or prohibit the entrance of disease-producing organisms. Natural products are more likely to survive in favorable conditions such as permanently waterlogged areas, although under normal burial conditions survival of even trace of the substances is rare.

The presence of organic residue is dependent on two survival mechanisms namely survival in an external atmosphere not conductive to decay or survival of organic constituents in a so-called 'transformed' state. Examples for survival in atmosphere not conductive to decay include extreme aridity and freezing. The former environment inhibits bacterial attack as a result of the exclusion of moisture and the latter low temperature depositional state prevents bacterial growth.

An additional microenvironment, which may afford a degree of protection, is the porous microstructure of the fired clay matrix. Traces of liquid, such as oils and resins will migrate into the wall of such vessels on contact and may be retained indefinitely. The lack of free oxygen supply into the clay microstructure will inhibit severe bacterial reworking of the organic constituents.

A major example of the second survival mechanism occurs during the accidental burning of edible animal and plant material during cooking. Charring of food on the walls of the vessels may entrap organic components within a matrix of carbon. Preservation is promoted since carbon is indigestible by microorganisms.

Pottery assemblages are frequently studied by archaeologist in search of information about a variety of different aspects of past societies, such as socio-economic developments, the organization of production and trade, and the mechanism of cultural interaction. Ceramic containers form an optimal source material for archaeological studies because they were used in daily activities, were commonly produced locally, had relatively limited use-life and are generally preserved well in archaeological context.

Pottery vessels serve as a convenient place for an analytical study of organic traces preserved in archaeological contexts. Such an association is not coincidental. The utilitarian functions of pottery in antiquity centre on the packaging, transport, and storage of solid and liquid materials and the preparation and consumption of food and drink' (Jones, 1986). Since the preservation of the original contents of the vessel is a rarity, recent interest has focused upon the observed have decomposed residues, which may suggest a particular function or content.

Ceramics objects, such as pottery vessels, are among the most common class of artifacts found at the archaeological sites. The walls of unglazed archaeological pottery vessels have been shown frequently to contain appreciable quantities of lipid or lipid residues absorbed in the microspores of clay matrix during processing of foodstuff and other organic materials (Charters *et al.,* 1993). The important aspect of the biomarker analysis depends on the property that the fired clay functions as a molecular sieve or trap which can preserve organic biomolecules during burial over many millennia. Deriving functional and dietary evidence from the archaeological pottery is focused on the analysis of organic residues preserved in unglazed pottery, either as charred surface deposits or, more commonly, as absorbed within the ceramic fabric. Absorbed lipids do not always accumulate uniformly throughout a vessel but may be relatively more concentrated in the rim/neck, body, or base (Charters *et al.*, 1993; Evershed *et al.*, 1995). The distribution is influenced by the former contents of the vessels, the manner of use (i.e. food storage versus preparation and cooking) and, for cooking pots, the method of cooking (Charters *et al.*, 1993). Residues produced by boiling foodstuff generally concentrate at the rim/neck, as lipids float to the surface, and gradually decrease towards the base where the residues are least concentrated as a result of thermal degradation during cooking (Charters *et al.*, 1993, Charters *et al.*, 1997). A greater density of lipids at the base of a vessel may suggest that foods were roasted or fired (Charters *et al.*, 1993), while a more evenly distributed residue may indicate the use of fat as a post-firing treatment to seal the vessel and the storage of oil (Charters *et al.*, 1993).

Charred or carbonized residues are brown-black encrustations found on the surfaces of some ceramic vessels. Charred food residues may form on the interior of the rim/neck, body, or base of a vessel. The position of the residue is related to the method of food

preparation, the type of food cooked, and vessel morphology. For example, when cooking mixtures of vegetables and meat, food material caught on the vessel wall of the liquid is sometimes charred in a band (Koyabayashi 1994). The research carried out on the carbonized residues by Rötlander and Schichtherle (1979), Needham and Evans (1987), Gurfinkle and Franklin (1988),) constitute some of the earliest analyses of organic residues associated with pottery vessels

1.2. Archaeological Biomarkers

During the 1970s and 1980s it became evident that lipids are preserved under favorable conditions in association with various classes of archaeological artifacts. Thus, it was recognized that the concept of chemical fossils or biomarkers might be applied to derive information relating to the activities of ancient people (Evershed, 1993). It is been now shown that lipids occur widely at archaeological context.

Investigations of such residues have revealed a wide range of commodities associated with vessel usage in the past, including plant oils, epicuticular leaf waxes, beeswax, and degraded animal fats. Such studies utilize a 'biomarker approach'. Biomarkers, in general, are organic indicator compounds that can be used as tracers for biological, geological and environmental processes. "The carbon skeletons of the natural product precursor compounds are synthesized by biota, and are found either as such or as altered directly or indirectly by diagenetic changes to derivative products" (Simoneit, 2005). In other words, this approach involves the matching of a chemical property of the organic residue, such as presence or absence of a particular characteristic compound or mixture of compounds, to that of a contemporary plant or animal product (reference material) likely to have been exploited in antiquity (Evershed *et al.*, 1991).

The use of biomarkers is essentially based on chemotaxonomic and phylogenetic principles (Evershed, 1993). Figure 1.1. shows the main classes of lipids that may be of value in identifying the origins of organic residues based on their lipid compositions. There is always the possibility that lipid distributions in archaeological materials have been affected by decay. Great care must be taken in drawing conclusions on the basis of

components bearing reactive functional groups e.g. polyunsaturated fatty acids and ester linkages in triacylglycerols.

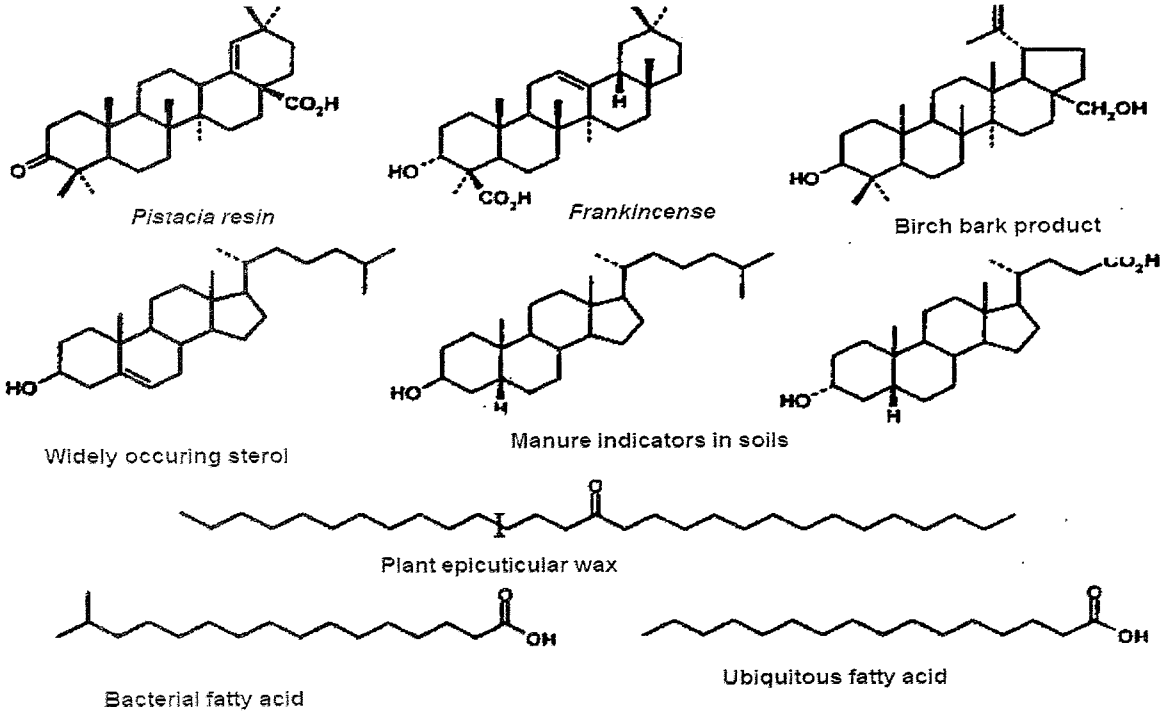


Figure 1.1. Lipid biomarkers

1.3. Objectives

The overall aim of this study is to investigate the animal exploitation and the dietary preferences of indigenous communities to be revealed by detailed compositional analysis of Chalcolithic and Neolithic pottery vessel fragments from Tell Kurdu and Barcın Höyük respectively. Emphasis will be given to undestand the importance of dairy products in these earliest farming communities of prehistoric Anatolia. This is realized through the analysis of absorbed and surface residues from Neolithic and Chalcolithic potsherds. Chemical analysis comprise the study of:

i) Overall lipid distribution

ii) Relative abundances of *n*-alcanoic fatty acids

iii) Distribution of intact triacylglycerols

iv) $\Delta\ ^{13}C$ values of C16:0 and C18:0 fatty acids.

The specific objectives are:

1. The systematic analysis of absorbed and surface lipid residues extracted from more than 100 potsherds from Chalcolithic and Neoloilithic sites of Tell Kurdu and Barcın Höyük. Lipid composition, TAG (triacylglycerol) distributions and $\delta\ ^{13}C$ and $\Delta\ ^{13}C$ values of the major fatty acids are considered and discussed on site-by-site basis.

2. To investigate trends observed within the $\delta\ ^{13}C$ variations of fatty acids which are used to understand the use of dairy products and other animal products in potpotsherds as well as any other plant or animal products processed in the vessels. The following questions are then addressed:

   i) Were the dairy products processed in the Barcın Höyük vessels?
   ii) Were ruminant and non-ruminant animals processed in the vessels?
   iii) Were there any other natural products processed or used in the vessels?
   iv) Were the lipid residues preserved in significant amounts in different burial environments?
   v) Were certain type of poterry vessels associated with processing certain types of products?

3. Apart from the chemical analysis of the lipid residues, microstructural investigation of the pottery fragments with and without residues are carried out in order to find possible relationships between fabric, temper and firing condition of the potsherds with the ability of these potsherds to absorb residues. SEM-EDX and X-ray diffraction techniques are used for this purpose. This investigation will be useful to select potteries with high probability of absorbed residues which will reduce the time and cost of expensive residue anlysis performed on the potsherds.

2. LIPIDS

2.1. Introduction

Lipids are animal fats, plant oils, waxes, resins, etc., of the natural world, occurring ubiquitously in plant and animals. The term lipid covers an extremely diverse range of molecular species. All molecules soluble in non-polar organic solvents and not in water are defined as 'lipids'. They can be classified as neutral or apolar and polar molecules. The apolar lipids comprise hydrocarbons, carotenes, triacylglycerols, wax esters, sterols and fatty acids. The polar lipids contain phospholipids, glycolipids, sulpholipids, sphingolipids, oxygenated carotenoids and chlorophylls (Belitz and Grosch, 1999). Another classification of lipids is based on the well-known availability of some lipids to be hydrolyzed by basic solutions i.e. one glycerol and one, two or three fatty acids. These are called saponifiable lipids. The unsaponifiable are resistant to such treatment. There are several crossing points between the two classifications since both polar and neutral lipids can be saponifiable or not. Depending on their physical properties lipids can be divided into 'fats' and 'oils'. The terms 'fat' and 'oil' refer to lipids that are solid or liquid at room temperature respectively.

Lipids act as storage materials in animal, plant and microbial cells and typically occur in the form of triacylglycerols. They are also responsible for the structure of cell membranes, which are mainly composed of amphiphilic, glyco- and phopholipids. Besides these well-known roles, lipids also carry out many other metabolic or physiological functions (Ratledge and Wilkinson, 1988).

2.2. Major Lipid Types

2.2.1. Fatty Acids

The most widely distributed lipids in living cells are fatty acids. In nature, they are rarely found in free form, but rather are linked to variety of molecules of which glycerol is the most common. They can be divided into groups according to their chain length and the number, position and configuration of the double bonds. In literature, fatty acids are

usually denoted by an abbreviation, which shows the number of the double bonds. For example, linoleic acid is denoted as C18:2 indicating that these fatty acids with 18-carbon chain length contain two double bonds (Table 2.1.). By default, double bonds are considered to have a *cis*-configuration, meaning that the two hydrogen atoms attached to the double bond are located at the same side of the carbon chain. When *trans*- unsaturated bonds are present, an additional '*tr*' is added to the abbreviation. The 18-carbon skeleton of stearic acid is the base structure for the common unsaturated fatty acids such as oleic, linoleic and linolenic acid (Table 2.1).

Table 2.1. Nomenclature and structures of major naturally occurring fatty acids in lipids.

Common name	Abbreviated notation	Strcuture
Capric acid	$C_{10:0}$	$CH_3(CH_2)_6CO_2H$
Lauric acid	$C_{12:0}$	$CH_3(CH_2)_{10}CO_2H$
Myristic acid	$C_{14:0}$	$CH_3(CH_2)_{12}CO_2H$
Palmitic acid	$C_{16:0}$	$CH_3(CH_2)_{14}CO_2H$
Stearic acid	$C_{18:0}$	$CH_3(CH_2)_{16}CO_2H$
Trans vaccenic acid	$C_{18:1(11tr)}$	$CH_3(CH_2)_9CH{=}CH(CH_2)_5CO_2H$
Oleic acid	$C_{18:1(9)}$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$
Linoleic acid	$C_{18:2(9,12)}$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
Linolenic acid	$C_{18:3(9,12,15)}$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
Arachidonic acid	$C_{20:0}$	$CH_3(CH_2)_{16}CO_2H$
Isopentadecanoic acid	*iso*$C_{15:0}$	
Anteisopendecanoic acid	*Anteiso*$C_{15:0}$	

Branched-chain acids, such as *iso* (with an isopropyl group) or *anteiso* (with a secondary butyl terminal group) are rarely found in foodstuff, but are typical constituents in the membranes of bacteria (Table 2.1).

2.2.2. Acyllipids

The most common acyllipids are those in which fatty acids are esterified with an alcohol, usually glycerol.

2.2.2.1. Acylglycerols. Acylglycerols comprise the mono-, di- or triesters of glycerol with fatty acids. Triacylglycerols are important constituents in most fats and oils. The three fatty

acids in a typical natural triacylglycerol are usually not identical and may vary within a given oil or fat. When the acyl residues in position 1 and 3 are different, the secondary carbon atom in glycerol is a chiral center. In the stereospecific numbering of acyl residues (prefix *sn*), the glycerol molecule is shown in its *Fisher* projection with the secondary OH group (*sn*-2) pointing to the left; the top carbon is then denoted sn1 (Figure 2.1.) (Belitz and Grosch, 1999).

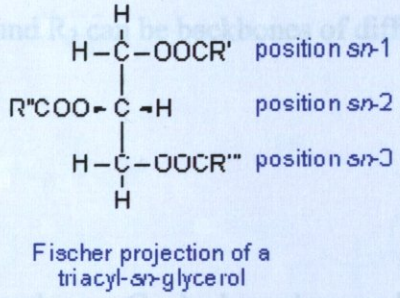


Figure 2.1. Fisher projection of a triacylglycerol molecule

For example the nomenclature for a triacylglycerol that contains palmitic, strearic and oleic acid can be *sn*-1-palmytoyl-2-oleoyl-3-stearoglycerol (*sn*-POS). This designation can only be used when the stereospecific information about the triacylglycerol is available. Especially differentiation among fatty acids in position *sn*-1 and *sn*-3 is complicated. Therefore, no distinction is generally made among, for example, *sn*-POS and *sn*-SOP and the notation *rac*-POS is used. If no stereospecific information is available the notation POS is used.

2.2.2.2.Phospho- and Glycolipids. Phospho- and glycolipids are the building blocks of biological membranes. They occur in all foodstuffs from animal and plant origin. Glycolipids consist of 1,2-diacylglycerols and mono-, di- or less frequently tri-or tetrasaccharide in position 3 of glycerol. Glycolipids are especially found in chloroplast of plants.

Phospholipids contain a phosphate residue at the *sn*-3 position of glycerol. The most common phospholipid is phosphatidyl choline or lecithin. For example, egg yolk is characterized by high amounts of lecithin. Due to their higher polarity phospho- and glycolipids are less stable than the neutral lipids (Fig. 2.2).

acids in a typical natural triacylglycerol are usually not identical and may vary within a given oil or fat. When the acyl residues in position 1 and 3 are different, the secondary carbon atom in glycerol is a chiral center. In the stereospecific numbering of acyl residues (prefix *sn*), the glycerol molecule is shown in its *Fisher* projection with the secondary OH group (*sn*-2) pointing to the left; the top carbon is then denoted sn1 (Figure 2.1.) (Belitz and Grosch, 1999).

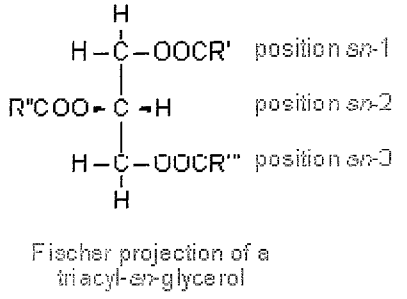


Figure 2.1. Fisher projection of a triacylglycerol molecule

For example the nomenclature for a triacylglycerol that contains palmitic, strearic and oleic acid can be *sn*-1-palmytoyl-2-oleoyl-3-stearoglycerol (*sn*-POS). This designation can only be used when the stereospecific information about the triacylglycerol is available. Especially differentiation among fatty acids in position *sn*-1 and *sn*-3 is complicated. Therefore, no distinction is generally made among, for example, *sn*-POS and *sn*-SOP and the notation *rac*-POS is used. If no stereospecific information is available the notation POS is used.

2.2.2.2.Phospho- and Glycolipids. Phospho- and glycolipids are the building blocks of biological membranes. They occur in all foodstuffs from animal and plant origin. Glycolipids consist of 1,2-diacylglycerols and mono-, di- or less frequently tri-or tetrasaccharide in position 3 of glycerol. Glycolipids are especially found in chloroplast of plants.

Phospholipids contain a phosphate residue at the *sn*-3 position of glycerol. The most common phospholipid is phosphatidyl choline or lecithin. For example, egg yolk is characterized by high amounts of lecithin. Due to their higher polarity phospho- and glycolipids are less stable than the neutral lipids (Fig. 2.2).

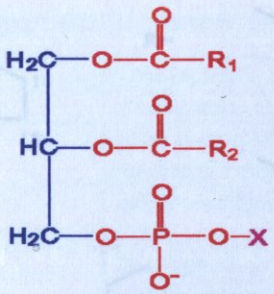


Figure 2.2. Basic composition of phospholipids. X can be a number of different substituents and R_1 and R_2 can be backbones of different fatty acids

2.2.3. Sterols and Stanols

Sterols are derived from squalene, a C_{30} hydrocarbon, and contain a fused four-ring system (Figure 2.3). They are important component of cell membranes.

The major sterols found in plants are sitosterol (stigmast-5en-3β-ol) (70-80 per cent), stigmasterol (stigmasta-5,22-dien-3β-ol) (5-20 per cent) and campesterol (24-methylcholest-5-en-3β-ol) (5-20 per cent), while in animals cholesterol (cholest-5-ene-3β-ol) is the most prevailing sterol (Belitz and Grosch, 1999). The main phytosterols can be distinguished from cholesterol by the nature of the side chain in C-24, which has an additional C_1 or C_2 (Figure 2.3 (a)). In fungi, ergosterol (Figure 2.3 (b)) (ergosta-5,7,22-trien-3β-ol) is the predominant sterol (Harwood and Russell, 1984).

Stanols are formed in nature by the microbial reduction of the double bond between the C-5 and C-6 carbon atom of sterols in the gut of higher animals (Bethell *et al.*, 1993; Bethell *et al.*, 1994) or by soil microorganisms. The most prevailing stanols are coprostanol (Figure 2.3 (c)) and stigmastanol which are the reduction products of cholesterol and sitosterol, respectively.

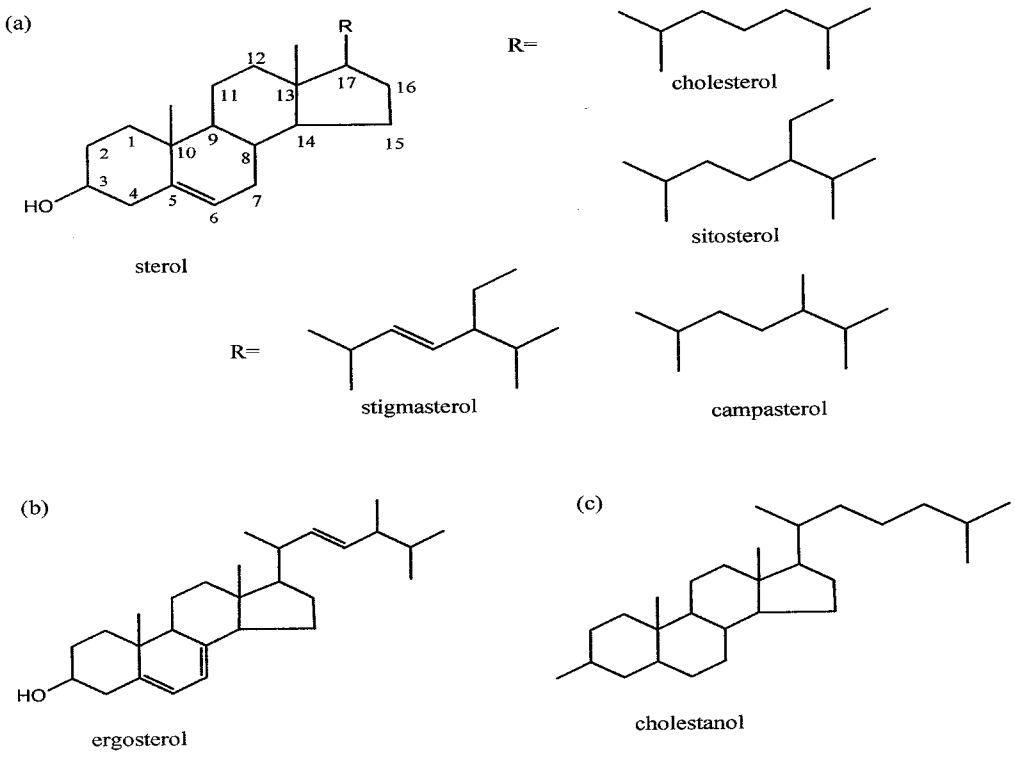


Figure 2.3. Structures of common sterols: a-)cholesterol, sitosterol, stigmasterol, campesterol; b-) ergosterol; c-)cholestanol

2.2.4. Waxes

Plants are unique because the structural component of their surface (cuticle) is made up of hydroxy fatty acid polymer, cutin. In contrast, other organisms utilize polymers of amino acids or carbohydrates. Inner and surface cuts in plants are covered by another type of lipid-derived polymeric material known as suberin. These polymers are associated with or embedded in a complex mixture of less polar lipids called waxes. The major component of plant waxes are shown in Table 2.2. The term 'wax' is not a specific one, but is derived from the similarity of properties of plant waxes to those of beeswax. In general, most of the major wax components are non-polar molecules with long hydrocarbon chains (Kollattukudy, 1980).

Table 2.2. Major components of plant waxes (Kolattukudy, 1980)

Compound	Structure	Occurence
n- Alkanes	$CH_3(CH_2)_nCH_3$	most plants; major components 29C or 31C
Monoesters	R_1COOR_2	most plants; composed of even-chain saturated acids and alcohols
Ketones	R_1COR_2	not as commons as alkanes; usually 29C and 31C
Secondary alcohols	$R_1CH(OH)R_2$	as common as ketones; usually 29C and 31C
β-ketones	$R_1COCH_2COR_2$	usually minor
Primary alcohols	RCH_2OH	most plants; even-chains predominate, usually 26C and 28C
Acids	RCOOH	very common; even-chains predominate, usually 24-28C

Abbreviations:R_1,R_2 are alkyl chains

2.3. Other Lipids

Other lipids comprise carotenoids, tocopherols and tocotrienols. Carotenoids are synthesized from eight isoprene units (tetraterpenes) and have a 40-C skeleton. They provide an intensively yellow, orange or red color to a great number of foodstuffs from plant origin. Only plants synthesize them. Nevertheless due to their presence in plants used as animal food, they can also be stored in animal tissues. A well-known example is chicken egg yolk, which is colored by carotenoids. Most carotenoids are only present in ppm concentration levels in plant or animal tissues.

Tocopherols and tocotrienols constitute a series of related benzopyranols. These lipids are primarily found in cereals, nuts and plant oils. They act as antioxidants and prolong the shelf lives of many foodstuffs containing fat or oil. Also such compounds are present less than one per cent in the lipid fractions of the above-mentioned plants (Belitz and Grosch, 1999).

2.4. The Occurrence of Lipids in Nature

2.4.1. Distribution and Composition of Lipids in Nature

The distribution of lipids in nature is shown in Table 2.3. The fruits from olive or palm tree are known as important sources of lipids. Also nuts and oil-rich seeds such as sesame and safflower seeds contain high amounts of lipids, whereas cereals, vegetables

and most fruits have low lipid content. The fat or oil content of meat and fish is highly variable. Depending on the type of meat or fish it can be either high as seen in pork side cuts and tuna or rather low as seen in chicken breast and carp. The lipid content is influenced not only by the kind of meat or fish, but also by the part of the animal, its maturity, the season and the feeding habits. Likewise the lipid content of milk is dependent on the source, with sheep milk having the highest fat concentration followed by goat and cow.

Not only the lipid content but also the lipid composition is different for most natural products as can be seen from Table 2.4. Triacylglycerols are regarded as the most important lipids in fats and oils. Other constituents are the hydrocarbons, sterols, tocopherols, carotenoids and a number of other acyllipids such as phospho-, and glycolipids, traces of free fatty acids, mono- and diacylglycerols.

Triacylglycerols are the largest class of lipids in pulp-fruit, seed oils and animal fats as well. Fruits and vegetables contain smaller amounts of triacylglycerols whereas sterols, phospho- and glycolipids and waxes are found in higher amounts in plant lipids. In cereals, triacylglycerols are the main lipid types, but also phospho- and glycolipids are also well presented.

Table 2.3. Lipid content of different natural products (weight per cent) (Belitz and Grosch, 1999)

Oil rich fruits	**Weight per cent**	**Meat**	**Weight per cent**
Olive (*Olea europaea*)	38-58	Pork(side cuts)	48.2
Oil palm *(Elais guineensis)*	30-55	Pork(muscle)	2.4
Oil-rich seeds		Beef(beefsteak)	0.7-2.2
Safflower(*Carthamus tinctonus L.*)	45-55	Chicken(breast)	1.2
Sesame(*Sesamum indicum*)	64.4	Chicken(hind leg)	5.5
Nuts		Rabbit	2.4
Walnut(Juglans regia)	64.4	Milk	
Cereals		Cow	3.7
Barley(*Hordenum vulgare*)	2.1	Sheep	6.3
Wheat (*Tricticum aestivum L.)*	2.0	Goat	4.3
Fruits		Fish	
Apple	0.1	Carp	7
Vegetables	0.1-5	Pika	0.9
Broad beans(*Vicia faba*)		Tuna	16
Peas (*Pisum sativum*)	1.6	Herring	18
Lentills(Lens culinaris)	0.8	Microorganisms	1-80

Table 2.4. Lipid composition of different natural products (weight per cent)

Lipid source	Olive oil	Wheat	Apple	Beef	Sheep	Pork	Milk	Carp
Triacylglycerols	98	41	5	81	92	82	94	43.5
Mono-and diacylglycerols		1		6	2	6	1.5	
Free fatty acids				3	3			
Phospholipids		20	47	1	3	<1	1.5	54
Glycolipids		29	17					
Sphingolipids				1				
Sterols	<1	1	15	<1	<1	<1	<1	
Sterol esters	<1	1	2	1				
Others	<1	7	15					

2.4.2. Lipids in Animal Fats

The fatty acids of triacylglycerols have even number of carbon atoms since they are synthesized from acetic acid units. These units come from acetyl CoA, formed by glycolysis or by oxidation of fatty acids, from acetic acid and Coenzyme A.

The depot fats of animals are believed to be in a state of continuous modification by various processes. The modifications are due to:

a) Alterations by partial or complete hydrogenation of dietary fatty acids in rumen (Gunstone *et al.*, 1998),

b) Synthesis *de novo* by rumen bacteria or by oxidation of other dietary components,

c) Modification of absorbed fatty acids by α and β-oxidation,

d) Desaturation or chain elongation (Christie, 1978) and

e) *de novo* synthesis within the adipose tissue itself.

The ruminant fatty acid profiles are less influenced by the fatty acid distribution of their diet because the unsaturated and polyunsaturated fatty acids in their feed are hydrogenated to saturated fatty acids by the rumen microorganisms. On the contrary the fatty acids composition of non-ruminant animals reflect the composition of their diet (Jakobsen, 1999).

Table 2.5. Typical fatty acid composition (weight per cent) of fats from different sources

Fatty acid	Beef	Sheep	Goat	Milk	Lard	Poultry	Eggs
$C_{4:0}$				3			
$C_{6:0}$				2			
$C_{8:0}$				1			
$C_{10:0}$				2			
$C_{12:0}$	-	0.5		3			
$C_{14:0}$	3	3	2	10	1	1	1
$C_{16:0}$	25	23	20	26	25	25	23
$C_{16:1(9)}$	4	2	3	2	3	7	5
$C_{18:0}$	14	26	24	15	14	4	4
$C_{18:1tr}$	1	4	Na	1			
$C_{18:1(9)}$	47	34	38	30	47	43	47
$C_{18:2(9,12)}$	3	2	4	2	8	18	16
$C_{18:3(9,12,15)}$	1		0.5	1			2
$C_{20:0}$					0.5		
Others	1	5	9		2	2	2

Non-ruminant adipose fats such as poultry and pig lard have in general a lower concentration of saturated fatty acids and a higher concentration of linoleic acid than ruminants (Table 2.5). Eggs contain similar fatty acid distributions as poultry fat.

The majority of animal fats have saturated fatty acids that predominate at the *sn*-1-position. Porcine fat, on the other hand, contains 72 mol per cent palmitic acid in the *sn*-2 position. Beef tallow is further characterized by having 17-18 per cent trisaturated triacylglycerols while pig lard and poultry contain only seven mol per cent and less than three per cent trisaturates respectively (Gunstone 1991). Mammalian milk fats comprise butyric ($C_{4:0}$) and caproic ($C_{6:0}$) acids at the *sn*-3 position, with other fatty acids such as $C_{18:0}$ at the *sn*-1 position (Kuksis *et al.*, 1973).

HPLC-APCI/MS (high pressure liquid chromatography atmospheric pressure chemical ionization mass spectrometry) analysis enables the identification of the positional distribution of fatty acids of triacylglycerols from ruminant and non-ruminant animals from both current and archaeological fats. APCI (atmospheric pressure chemical ionization), which is a very sensitive analytical technique causing very little fragmentation of target molecule and which is one of the common means to connect HPLC to MS, typically gives spectra for triacylglycerols that show the protonated molecular ion, $[M+H]^+$, diacylglycerol ions, $[M+RCO_2]^+$, and acylium ions, RCO^+. The positional distributions of the fatty acids can be identified by the abundance of the diacylglycerols

ions, since the least abundant diacylglycerol ion is the one formed by removal of the *sn*-2 position fatty acid since this is energetically less favorable than *sn*-1 or *sn*-3 positions (Mottram and Evershed, 1996).

2.4.3. Fatty Acid Patterns in Animal Fats

Fatty acids are the building blocks of adipose (storage) tissue in the animals. Their type and distribution in the animal body varies upon factors such as type of animal, intake of dietary fatty acid, tissue type and location, age, physiology and metabolism.

The major fatty acids in cow adipose fats are $C_{16:0}$ and $C_{18:1}$, with a lower levels of $C_{18:0}$. The analysis of ruminant fat has also revealed a range of branched-chain fatty acids with odd number carbon chain lengths (Dudd, 1999). Also the positional isomers of the $C_{18:1}$ component in the fats are found to be *cis* configuration occurring at the double bond on the carbon number nine of oleic acids ($C_{18:1}$ $\blacktriangle^{9}$). The $C_{18:1}$ $\blacktriangle^{11}$ is the most abundant trans acid (Dudd, 1999).

Milk fatty acids are numerous and include aliphatic acids from C_2 to C_{28}, mono-methyl branched-chain fatty acids from C_{11}-C_{28} (including numerous positional isomers), multimethyl branched chain fatty acids from C_{16} to C_{28}, *cis*- and *trans*-monoenoic acids from C_{10} to C_{26}, numerous di- and polyenoic fatty acids, keto and hydroxy fatty acids and cyclohexyl fatty acids. Milk fats from herbivores contain diagnostic short-chain fatty acids ranging from C_2 to C_{14}.

The fatty acid composition of some fish oils are presented in Table 2.6. Fish oils are generally more unsaturated than mammalian fats and are characterized by the presence of long chain polyunsaturated fatty acids such as eicosapentaenoic acid ($C_{20:5}$) and docosahexaenoic acid (22:6). Marine fish (herring and mackerel) contain usually more polyunsaturated fatty acids than river fish (carp).

Table 2.6. Fatty acid composition of some fish oils

Fatty acid	Carp	Herring	Mackerel
$C_{14:0}$	2	8	7
$C_{16:0}$	17	18	14
$C_{16:1(9)}$	7	8	5
$C_{18:0}$	5	2	4
$C_{18:1tr}$	32	17	17
$C_{18:2(9,12)}$	9	1.5	2
$C_{18:3(9,12,15)}$	2	0.5	2
$C_{18:4}$	1	3	4
$C_{20:1}$	1	9.5	9
$C_{20:4}$	5	0.5	
$C_{20:5}$	5	9	6
$C_{22:1}$		11	15
$C_{22:5}$	3	1.5	
$C_{22:6}$	5	7.5	15
Others			

2.4.4. Fatty Acid Patterns in Plants and Plant Products

The fatty acid composition of oil rich fruits and seeds are given in Table 2.7. It can be seen that for all oils listed in the Table 2.7. stearic acid content is low and the percentage of unsaturated fatty acids is high, except for palm kernel oils.

Olive oil is characterized by a high percentage of oleic acid. Wild olive oil trees were already brought under cultivation 5000 to 6000 years ago (O'Keefe, 2000). The early use of olive oil was source of lamp fuel, lubricant and body ointment. Later, the olive oil became a major source of edible oil (Firestone *et al.*, 1996).

Palm oil is obtained from the palm trees. The use of palm in human diets can be dated as far back as 3000 years BC. (Berges and Martin, 2000). The fruits provide two different oils, the first from the pulp and the second from the seeds (palm-kernel oil). These vegetable oils are characterized by their high content of saturated fatty acids. The oil from the palm fruit pulp is characterized by a large amount of palmitic acid next to a high content of oleic acid, whereas the oil obtained from the palm kernel is differentiated by its high lauric and myristic acid content (Basiron, 1996).

Their high linoleic acid levels distinguish the other oils obtained from seeds such as sesame and safflower oil. Sesame is probably the most ancient oilseed known and used by humans as a food source (Deshpande *et al.*, 1996). Sesame oil differs from other vegetable oils because it contains the antioxidants sesamol, sesamin and sesamolin, which provide this oil with an unusual stability to oxidative deterioration. Safflower also has a long history of cultivation and was used as source of dye, food coloring, as cosmetics or for medicinal purposes throughout history (Smith, 1996).

As can be seen from Table 2.7 lipid content of cereals, most fruits and vegetables is so low that they cannot be considered as an exploitable source of fats or oils. Only nuts contain higher level of lipids. In Table 2.7 fatty acid compositions of lipids of some cereals, fruits and vegetables are shown. It can be seen that the unsaturated fatty acids such as linoleic and oleic are very well represented. The family of the *Leguminosae* (lentils and broad beans) is characterized by relative higher contents of arachidonoic and docosanoic acids.

2.4.5. Microbial Lipids

Two different groups of microorganisms can be distinguished, which is also the most basic division between all organisms namely the prokaryotes and eukaryotes. The prokaryotes include the bacteria and cyanobacteria (or blue-green algae). All other organisms, including the fungi, are eukaryotes. The major distinction between prokaryotes and eukaryotes is the relative lack of intracellular organelles and absence of a nuclear membrane in prokaryotes. In Table 2.8 major fatty acids of several classes of organisms are shown. A significant difference between bacteria and most other organisms is that the bacteria do not synthesize polyunsaturated fatty acids. Besides even-chain saturated and unsaturated fatty acids, bacteria characteristically contain odd chain and branched fatty acids. Especially bacteria are characterized by *iso* and *anti-iso* branched fatty acids (Table 2.8).

Table 2.7. Fatty acid compositions of fruits, vegetables and cereals (weight per cent)

Fatty acid	Cereals		Vegetables			Fruits
	Wheat	Barley	Lentils	Beans	Pea	Apple
$C_{12:0}$						0.6
$C_{14:0}$		2	0.9	0.6	10.2	0.6
$C_{16:0}$	20	22	23.2	9.3		30
$C_{16:1}$	1.5	<1	0.15		4.1	0.5
$C_{18:0}$	1.5	<1	4.6	4.9	17.5	6.4
$C_{18:1}$	14	11	36	33.8	58.8	18.5
$C_{18:2}$	55	57	20.6	42.1	10.6	42.5
$C_{18:3}$	4	5	1.6	6.4		1
$C_{20:0}$			2.3	0.7		
$C_{20:1}$			1.9	0.7		
$C_{22:0}$			2.7	0.42		
$C_{24:0}$			0.85			

Bacteria do not synthesize or accumulate triacylglycerols. Fungi on the other hand can synthesize or assimilate unsaturated triacylglycerols (Lundberg *et al.*, 2001). Fungi also contain sterols, which can represent up to ten per cent of the cellular dry weight (Harwood and Russell, 1984).

Table 2.8. Major fatty acids in microorganisms (Harwood and Russell, 1984)

Organism	Major fatty acids
Bacteria	
Gram-positive	$C_{15:0}br+C_{17:0}br$
Gram-negative	$C_{16:0}>C_{16:1}=C_{18:1}$
Cyanobacteria	$C_{16:0}=C_{18:3}>C_{16:1}=C_{18:2}$
Yeast	$C_{16:0}=C_{18:1}>C_{16:1}=C_{18:2}$

3. DEGRADATION OF LIPIDS AND TRANSFORMATION OF LIPID RESIDUES IN ARCHAEOLOGICAL CONTEXT

3.1. Degradation of Lipids

Intact acyllipids, although relatively resistant to decay due to their apolar nature, can be degraded by chemical and/or enzymatic hydrolysis and oxidation.

3.1.1. Hydrolysis

Fatty acids of the lipid fraction of foodstuff can be hydrolyzed by chemical processes, by natural lipolytic enzymes (lipases, triacylglycerols acylhydroases) or by lipases produced by contaminating microorganisms that results in the hydrolytic rancidification of foodstuff. The different steps in the hydrolysis of triacylglycerols are shown in Figure 3.1. The resulting mono- and diacylglycerols do not tend to accumulate, because they are more rapidly hydrolyzed than the triacylglycerols.

Lipases, enzymes secreted in the digestive tract that catalyzes the breakdown of fats into individual fatty acids that can be absorbed into the bloodstream, are activated by iron, magnesium or manganese ions. They are particularly active at the lipid-water interface, and hence homogenization and emulsification stimulate the enzyme activity. Hydrolysis of lipids during storage is also dependent on the water content of the product.

Lipases, which are often present in multiple forms, differ in their specificity according to their origin. Many lipases have positional specificities, for example they preferentially hydrolyze the 1- or 2- fatty acids bound in the triacylglycerol molecule (Figure 3.1). The activity of this enzyme sometimes stops at the monoacylglycerol stage. The lack of specificity of some lipases may be explained by the migration of fatty acids between 1 and 2 positions of diacylglycerol molecule (Figure 3.1).

Besides regioselectivity, several lipases also exhibit typoselectivity. It was shown that short chain fatty acids esters (<14C) are easily hydrolyzed, while long chain saturated triacylglycerols are more resistant to such a reaction. The activity of some lipases increases with increasing degree of unsaturation of the triacylglycerols (Eliot *et al.*, 1999). The preferential hydrolysis of short chain fatty acid esters was shown in an experiment of Dudd and Evershed (1998). In this experiment milk fat absorbed in potsherds was incubated in oxidizing circumstances with mushroom compost. It was observed that after 25 days of incubation the amount of low molecular weight triacylglycerols was largely reduced, while this was not the case for the long chain triacylglycerols.

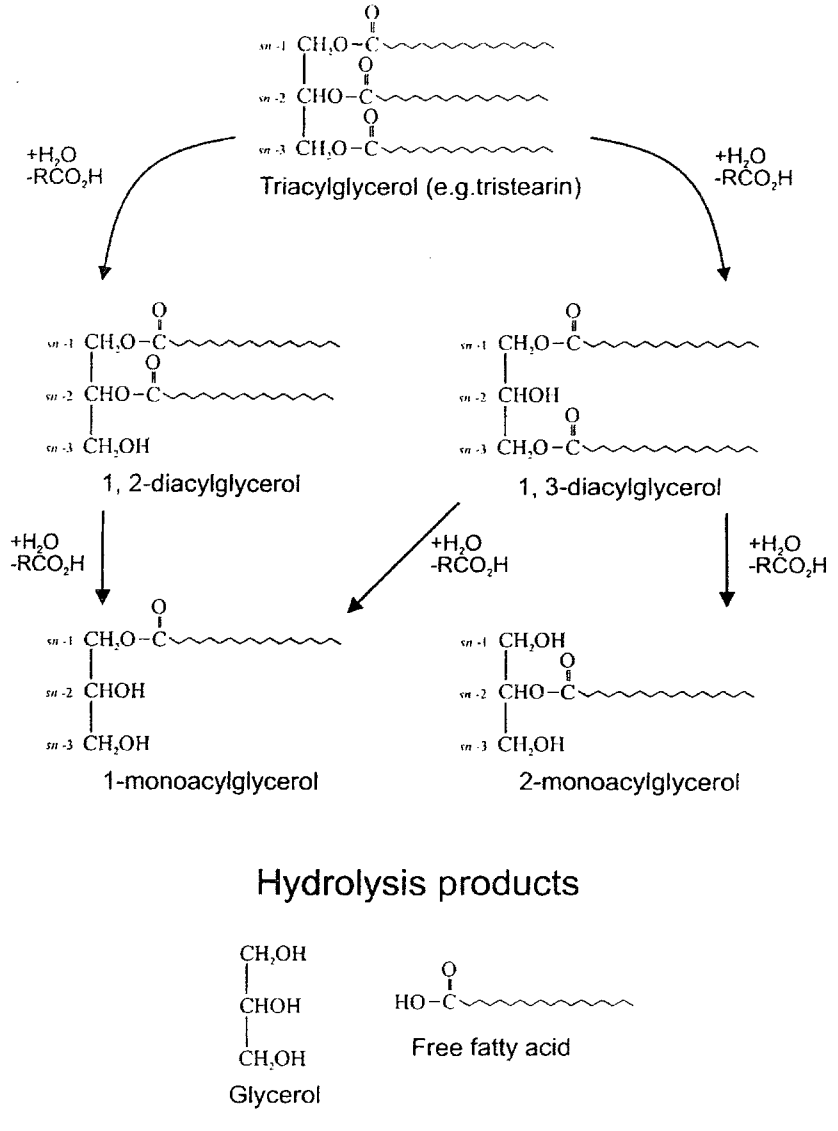


Figure 3.1. The hydrolytic degradation pathways of TAGs to free fatty acids (Evershed *et al.*, 2001).

Acids, bases or metal ions usually catalyze non-enzymatic hydrolysis of lipids. In the absence of water, hydrolysis of lipids is very slow except when heated to boiling temperatures (Davidek, 1990). In abiotic soil processes, acid or base catalyzed hydrolysis is probably responsible for the breakdown of triacylglycerols (Hita *et al.*, 1996).

3.1.2. Oxidation Reactions

Oxidation reactions possibly are the most frequent reactions involved in the decay of foodstuff. Three types of oxidation reactions can be distinguished, namely free radical autoxidation, photosensitized oxidation and enzymatic oxidation. The rate of autoxidation increases with degree of unsaturation of the fatty acids. Linoleate is oxidized ten times faster than oleate and linoleate is oxidized 20 to 30 times faster than oleate (Ho *et al.*, 1996).

3.1.2.1. Free radical autoxidation. The reaction of oxygen with unsaturated lipids is a chain process, which involve initiation, propagation and termination reactions. In the presence of free radical initiators such as light, heat or trace metals, the unsaturated lipids (LipH) can form radicals (Lip·) (Table 3.1 step 1). The generated radicals propagate in the presence of oxygen to form peroxyl radicals (LipOO·) (Table 3.1 step2) and finally by free radical chain mechanism hydroperoxides (LipOOH) form (Table 3.1 step3). The combination of free radicals is one of the termination steps (Frankel, 1984 and 1991).

In an oxidizing atmosphere, the first step in the propagation chain (Table 3.1- step 2) is much more rapid than the second step (Table 3.1- step 3), so that the concentration of peroxyl radicals is much greater than that of lipid radicals alone. At room temperature a radical may initiate the formation of 100 hydroperoxide radicals before chain termination occurs.

The generated lipid hydroperoxides are unstable and decompose to unstable radicals (Tabel 3.1. – step 5 to 6). This reaction is promoted by heavy metal ions. The generated hydroperoxides can also react again with oxygen to form other secondary products. All these secondary products can in turn decompose to form volatile breakdown products such as aldehydes, short chain alkanes, alkenes and furan derivatives (Frankel, 1984 and 1991).

Table 3.1. Basic steps in the autooxidation of lipids

Initiation	*initiator*		
LipH	→	$Lip^{\bullet} + H^{\bullet}$	(1)
Propagation			
$Lip^{\bullet} + O2$	→	$LipOO^{\bullet}$	(2)
$LipOO^{\bullet} + LipH$	→	$LipOOH + Lip^{\bullet}$	(3)
$LipO^{\bullet} + LipH$	→	$LipOH + Lip^{\bullet}$	(4)
Branching			
$LipOOH + M^{n+}$	→	$LipO^{\bullet} + M^{(n+1)+} + OH^{-}$	(5)
$LipOOH + M^{(n+1)+}$	→	$LipOO^{\bullet} + M^{n+} + H^{+}$	(6)
Termination			
$Lip^{\bullet} + Lip^{\bullet}$	→	Lip-Lip	(7)
$Lip^{\bullet} + LipOO^{\bullet}$	→	LipOOLip	(8)
$LipOO^{\bullet} + LipOO^{\bullet}$	→	$LipOOLip + O_2$	(9)

3.1.2.2. Photosensitized oxidation. Another important mechanism by which unsaturated lipids can be oxidized involves exposure to light and a sensitizer such as chlorophyll, pheophytins or riboflavin. In this non-free radical process, oxygen which is a triplet in its ground state, become activated to a singlet state by transfer of energy from the sensitizer. The resulting singlet oxygen is extremely reactive and reacts directly with the unsaturated lipid as can be seen from the reaction scheme in Figure 3.3. The resulting hydroperoxides can be decomposed as described for the hydroperoxides formed by the free radical chain reaction (Frankel, 1984 and 1990).

3.1.2.3. Enzymatic oxidation. Fatty acids can be oxidized by a number enzymes. Lipoxygenase and the enzyme system mediating in β-oxidation are the most important cases.

Lipoxygenases are widely distributed enzymes in many plant materials, in microorganisms and in erythro- and leukocytes. They catalyze the oxidation of some unsaturated fatty acids to their corresponding monohydroperoxides. Unlike autoxidation, the reactions catalyzed by lipoxygenase are characterized by all features of enzyme catalysis. These include substrate specificity, peroxidation selectivity, optimum pH, susceptibility to deactivation by heat treatment and a high reaction rate in the range of 0 to 20°C. Lipoxygenase oxidizes fatty acids which contain only a 1-cis, 4-cis-pentadiene

(CH=CH-CH_2-CH=CH) system. Other enzymes can then degrade the generated hydroperoxides (Davidek, 1990).

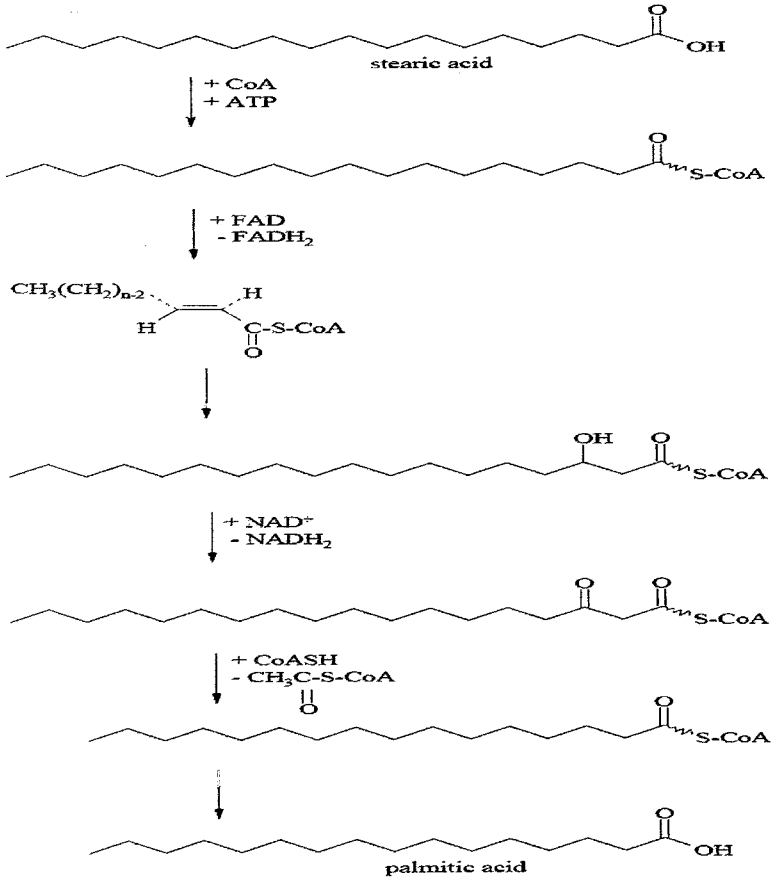


Figure 3.2. β-Oxidation cycle of fatty acid (Belitz and Groasch, 1999)

The pathway of β-oxidation involves a stepwise degradation of fatty acids (Figure 3.2). Each cycle yields a fatty acid that is shortened by two carbon atoms. By this process unsaturated as well as saturated fatty acids can be degraded. This oxidation pathway is catalyzed by several enzymes and is carried out in the mitochondria of animals and higher plants, in the micro bodies of yeasts and in the cytoplasm of bacteria. If one of the enzymes is not capable of transforming its substrate, intermediate products may be released (Harwood and Russel, 1984).

The β-oxidation cycle for oxidation of fatty acids is probably also responsible for the formation of adipocere. Adipocere is a conversion product that is generated during the incomplete decomposition of human and animal remains. In this transformation as the

triacylglycerols disappear and fatty acids begin the predominate. The latter are characterized by the depletion of oleic acid and especially palmitic and 10-hydroxystearic acids. Adipocere formation is considered to be the result of the bacterial action, commonly occurring in warm and anaerobic environments. An example for the formation of adipocere in anaerobic circumstances was shown by analysis of bog body samples (Evershed, 1992).

In an experiment by Den Dooren de Jong (1961) olive oil was incubated in anaerobic circumstances in the presence of soil bacteria. After six weeks decrease in oleic acid while an increase in palmitic and hydroxystearic acid was observed. It was proposed that the main mechanisms in the formation of adipocere, accounting for the abundant presence of 10-hydroxystearic acid, were hydrolysis, hydration and oxidation. Reduction and chain shortening by β-oxidation cycle are possible mechanisms responsible for the high amounts of palmitic acid (Den Dooren de Jong, 1961).

3.1.2.4. Oxidation products in archaeological pottery. It is likely that several of the above mentioned oxidation reactions occurred when foodstuffs were heated or stored in archaeological ceramics, or afterwards during burial. Nevertheless only occasionally evidence has been provided for the presence of these degradation products (Passi, 1981). The reason for this lack of information is probably a consequence of the rapid removal of these components from archaeological ceramics due to their enhanced solubility in water. However Regert *et al.* (1998) were able to release components bound in the matrix of the pottery after alkaline treatment of the residues. In this way a series of short chain α, ω-dicarboxylic acids, hydroxy and dihydroxy acids were identified. It was proposed that these products were oxidation products of the original lipids processed in these pots (Regert *et al.*, 1998). The formation of diacids from unsaturated fatty acids through chemical and biological oxidation was demonstrated by Passi *et al.* (1993). An example of the formation of diacids from linoleic acid by free radical autooxidation is shown in Figure 3.4. The chain length of the formed diacids is dependent on the position of the double bond.

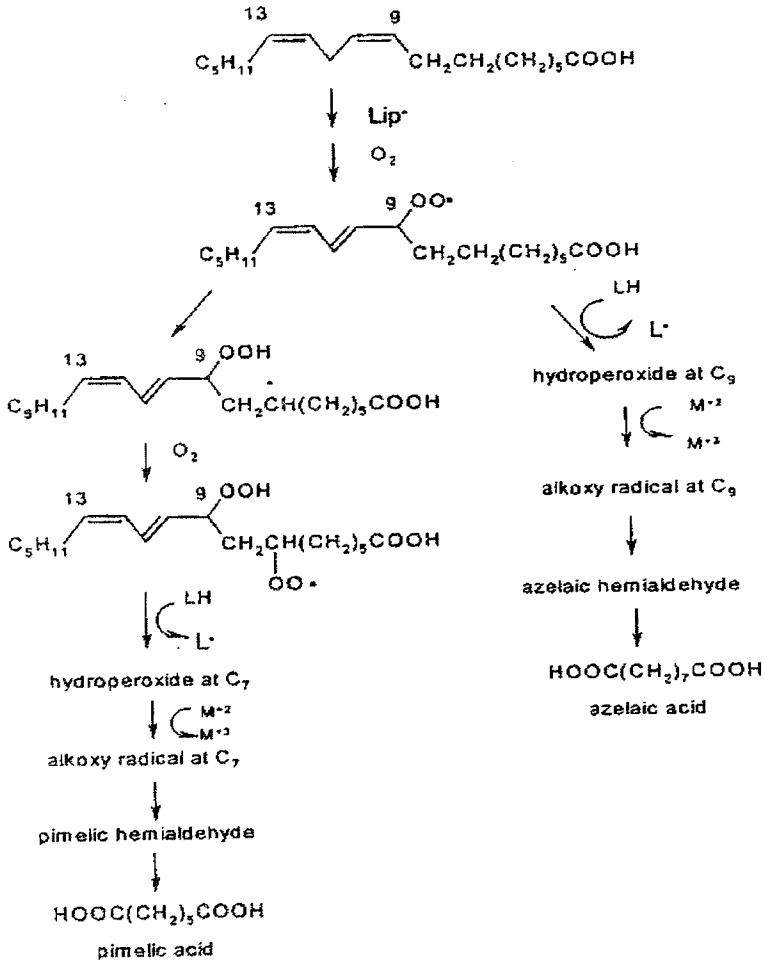


Figure 3.3. Formation of dicarboxylic acids azelaic (C_9) and pimelic acid (C_7) upon peroxidative attack on linoleic acid. LH: linoleic acid, M: trace metals, Lip·: initiator radical (Passi *et al.*, 1993).

3.1.2.5. Oxidation of sterols. Sterols are relatively resistant to autoxidation in the pure form, but they are easily oxidized during peroxidation of fats and oils. Autooxidation of sterols is similar to that of other lipids and is enhanced by contact with atmospheric oxygen at elevated temperature (Maerker, 1987). The oxidation of cholesterol proceeds by hydrogen abstraction, predominantly at the C-7 position and leads to the formation of allylic hydroperoxides. The formed hydroperoxides are unstable and decompose to yield various hydroxylic, oxo and epoxy derivatives.

In contrast with the absence of oxidized products of fatty acids in archaeological lipid extracts, several autooxidation products of cholesterol were found. The presence of these products can indicate that the fat was heated (Evershed et al., 1992).

3.1.3. Biodegradation

Although lipids are highly resistant to biodegradation, even the more resistant lipids will eventually be mineralized (Dunel *et al.*, 1990). Microorganisms can grow on variety of lipids such as alkanes, fatty acids or esters of fatty acids (Ratledge, 1994). The first step in the degradation of esters is their hydrolysis by lipases, which was already discussed in section 2.4.1. The freed fatty acids can then be assimilated into lipids of the cells of microorganisms or mineralized by for example the β-oxidation cycle. Mineralization is an indication of ultimate biodegradation, meaning that organic molecules are transformed to CO_2, inorganic forms of N, P, S or other elements.

The rate of biodegradation of lipids is dependant on several factors which include next to the environmental factor the accessibility of the lipid, the solubility in water and the reactivity of the lipid (Alexander, 1999).

Due to limited accessibility the presence of lipids in small pores will delay or prevent their degradation. Heron *et al.* (1991) suggested that the appreciable amounts of lipids in the pottery wall of ceramics were the result of limited access of microorganisms in the sherd matrix. The fired clay appears to function as a 'molecular sieve' or 'trap' preserving lipids during burial over many years (Evershed *et al.*, 2001).

As a consequence of their greater reactivity and water solubility fatty acids are degraded faster than neutral lipids like alkanes. Whereas, in anaerobic sediments unsaturated and branched fatty acids are more reactive than their saturated or straight counterparts (Grossi *et al.*, 2001), however in oxidizing sediments no difference was observed between the degradation rate of saturated and unsaturated fatty acids (Sun *et al.*, 1996). Also no difference was measured in the rate of degradation of saturated and unsaturated hydrocarbons (Moucawi *et al.*, 1981).

3.2. Residue Transformations in an Archaeological Context

3.2.1. Transformation Processes

The ability to infer the use of particular biomaterials in prehistoric times through organic residue analysis is to large extent determined by a correct understanding of transformation processes. These processes influence the chemical composition of the remaining residues.

The transformation processes include processes in the original prehistoric context C-1 transforms (cultural transforms) (Figure 3.4). The transformation processes also include the processes in post-depositional context or archaeological context so called N-transforms (natural transforms). C2 transforms or cultural transforms take place during and after excavation. Each of the transformation processes creates changes in the chemical composition of the original organic materials in the vessels. Some of these changes cause the degradation of specific chemical characteristics (degradation processes), while other changes will enhance their preservation (preservation processes) (Oudemans,2006).

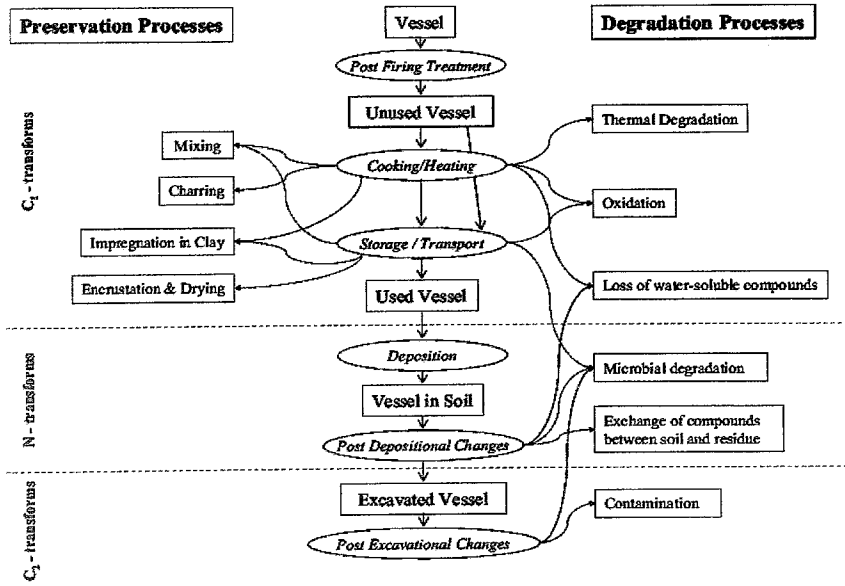


Figure 3.4. Residue transformation processes (Oudemans, 2006)

3.2.2. Changes in Lipid Distributions Post-excavation and Post-deposition

Post-excavation contamination can easily occur in archaeological samples. Both contamination with skin lipids (cholesterol and squalene are the best known) and contamination with various packing materials (phthalate esters most common plasticizers) are likely to occur in the daily practice of post-excavation ceramic treatment and handling. The most common way to prevent such contaminations is to remove about 1 mm layer from the outer layer of the pottery before taking residue samples.

Additional microbial degradation or oxidation of organic materials takes place after excavation. Thus the growth of microorganisms and fungi must be prevented by storing the samples in dry and cold place.

A large number of degradation processes are expected to have altered the chemical composition of the organic residues during the long period of burial. The most significant changes are caused by microbial degradation and the exchange of organic compounds between the residue and soil.

Some water-soluble compounds can be detected in the residues from archaeological potsherds, which would indicate a possible loss of compounds over time. Also, no intact saccharides, water-soluble amino acids or short chain free fatty acids were detected in archaeological residues. Selective loss of short chain fatty acids can occur as a result of hydrolysis of acyl lipids during the use of the vessel and after deposition. Alkaline environment can also enhance the transformation of free fatty acids to leach out of their matrix. It is better if the soil in the excavation site is mildly acidic.

According to Heron *et al.* (1991) no evidence is found for the exchange of any significant quantity of compounds between archaeological residues and soil. Furthermore, no correlation could be found between the residue and the sediment in which they are preserved.

Charred residues are known to be less susceptible to microbial degradation due to partial denaturation of the organic materials during charring and the refractory nature of

the resulting material (Oudemans, 2006). But even in such cases the biodegradation of residues may influence the chemical composition.

3.2.3. Degradation during Vessel Use and Burial

There are two important transformations of organic residues in potsherds i.e. cooking or heating of organic materials and the storage or transport of organic foodstuff in ceramic vessels. Cooking and heating cause impregnation of organic compounds in the clay matrix and may result in charring on the surface or absorption of organics in interior of the vessel. The formation of smoke condensates on the exterior of the vessel is also a secondary result. Storage and transport may also cause impregnation of compounds into the clay (Kimpe *et al.*, 2004) and may result in the formation of dried crusts on the interior vessel surface. Both processes cause mixing of many different biomolecular compounds.

Using ceramic vessels for serving, storage and transport of organic materials (whether it is foods or non-foods) may leave residues behind. Liquids may be absorbed into the ceramic potsherds. Examples of recognizable archaeological residues of such liquids include mostly residues of wines or other fermented beverages (McGovern *et al.* 1996 and 2004) and oils (Condamin *et al.*, 1979). Residues in the storage and transport pottery vessels show no chemical signs of thermal degradation, but show signs of oxidation due to long-term exposure to oxygen.

3.2.4. Post-firing Treatment

Post-firing treatment with a mixture of organic compounds is common among traditional potters and is performed with a variety of materials such as beeswax, bitumen, various resins and other plant materials (Kobayashi, 1994). Most commonly the treatment involves the application of an organic liquid or paste to the pots while they are still hot from firing. Cooking pots tend to seal themselves even after a single cooking phase (Charters *et al.*, 1997), so no need for sealing seems necessary.

4. COOKING POTS: MANIFACTURE, FORM AND FUNCTION

4.1. Producing the Earliest Cooking Pots

Pottery was the first synthetic material humans created. It combines the four basic elements identified by the Greeks: earth, water, fire and air. Clay, the raw material of pottery, is one of the most abundant, cheap, adaptable resources available for human exploitation. The use of clay to make pottery vessels doest not seem to have be originated in any single time and place in human history; rather, the idea seems have been independently invented in an unknown number of centers (Rice, 1987). About 10,000 years ago humans begun to form utilitarian devices out of materials from the earth and harden them with heat (Lambert, 1998).

The clay was appreciated for its ability to be worked when wet. Such a material is said to be plastic. After shaping clay into an object, it hardens on drying and generally holds its form. When clay vessels were used for cooking containers, it must have been recognized that heating such objects improves their utility by reducing their fragility (Rice, 1987).

Wet clay contains two types of water molecules. Those that are not bound chemically to clay are lost through evaporation or heating to the boiling point of water (100°C). At much higher temperatures (about 600°), clay begins to lose water that is bound chemically to clay molecules. The silicon (Si), aluminum (Al), and oxygen (O) atoms (as aluminosilicates) are forced to adjust their structure to the loss of chemically bonded water molecules, and the material loses its plasticity and hardens into rather soft forms of pottery. Above about 850°C, profound changes begin to occur in aluminosilicate structure, a process that has been termed vitrification (from Latin for 'to make glass') (Lambert, 1998).

The most primitive pottery is fired at about 900°C and falls into the category of terra cotta, which is highly porous and can be easily scratched. Heating at such temperatures also affects metal atoms present in the original clay, causing changes in color. Iron (Fe) has a strong coloring effect in the range 900-1100°C, and the material produced, called red

earthenware, is stronger and less porous than terra-cotta. At 1100-1200°C, calcium (Ca), in the form of lime, can bring out the cream color in earthenware. Above 1300°C, highly vitrified material such as porcelain is obtained. Materials produced by heating clay or other nonmetallic minerals in general are called ceramics (Olin and Franklin,1982).

The nature of the end product is determined not only by the firing temperature, but also by the composition of raw material. Three constituents go into the production of pottery. The first is clay. The normal weathering of silicate rocks leads to formation of clay, which is made up not only of aluminum (Al), silicon (Si) and oxygen (O), but also contains alkali and alkaline earth elements such as sodium (Na), potassium (K), magnesium (Mg), and calcium, as well as transition metal such as (Fe). The major clay minerals are kaolinite, illite, and montmorillonite. Raw clay is first crushed and/or ground and then wetted to remove coarse materials such as pebbles or twigs (Rice, 1984).

The second constituent of pottery is water. Dry clay is not workable, where as clay containing too much water does not retain its shape. Within a narrow range of water content, ca. 25 per cent, clay possesses plastic properties that may be worked to a form that retains its shape. After the shape desired is obtained the water of plasticity is lost by simple atmospheric evaporation. During the drying process shrinkage may result and if not properly controlled, can lead into cracking or distortion of the object (Orton *et al.,* 1993)

The third constituent of pottery is temper or filler. Potters in antiquity have used a wide variety of tempering materials, including sand (quartz), limestone, shells, basalt, volcanic ash, mica, straw and even potsherds (always crushed). The addition of such nonplastic tempering materials allows water to evaporate more smoothly, minimize shrinkage and prevent cracking. In some cases these materials may also assist in vitrification. Temper is normally added at the time that the clay is brought to the right consistency by kneading with water. The material called a paste, is then shaped, the surface is smoothed, and the object is allowed to dry to form pottery body or fabric. Sometimes at this stage the object is dipped into a dilute suspension of clay in water to provide a thin layer, or slip, which provides a smoother and less porous surface or it can serve as decoration (Lambert, 1998).

In selecting a clay source for producing cooking pot, one of the primary requirements is to ensure that the clay is sufficiently plastic. It should also have very small change in volume when dried in order to avoid cracking. These requirements are achieved by balancing the clay mineral with non-plastic inclusion known as temper materials. The non-plastic temper materials such as sand, grog (crushed potsherd), organic material (e.g. chaff), crushed flint, seashell or limestone. The size of the temper material is also a critical factor. A delicate balance must be maintained between the clay minerals and temper materials. Alternatively, clays from more than one source are sometimes mixed together.

During firing of the potsherd, depending on the temperature of the oven, the clay minerals first dehydroxylate and then decompose to other minerals by vitrification. Thus it is not normally possible to use X-ray diffraction analysis to determine original clay minerals. However, the use of more refractory clay, rich in kaolinite can be inferred from higher alumina (>20 per cent Al_2O_3) and lower alkali (<3 per cent Na_2O+K_2O) contents. The bulk chemical analysis of the clay matrix can be determined by scanning electron microscope-energy dispersive X-ray analysis (SEM-EDX). On the basis of the lime content of the pottery, it is also possible to distinguish between the use of non-calcareous clays and calcareous clays, which usually contain 15-25 per cent lime (Tite *et al.*,1982). In the latter, crystalline calcium and calcium aluminum silicates are formed during firing.

The non-plastic inclusions in the pottery can be identified and their particle size distributions estimated. Further, it is sometimes possible to distinguish mineral inclusions that are specific to the clay minerals from those that are added as temper on the basis of whether they are rounded or angular. If they are angular it generally infers that temper materials are added (Tite *et al.*,1982).

Considerable effort has been devoted to determine the firing temperatures employed in antiquity (Maniatis and Tite, 1981). The methods used all involve establishing relationship between the firing temperature and the changing in either the mineralogy or the microstructure of the clay fabric. The mineralogical changes can be followed using X-ray diffraction and micro structural changes can be observed by examination of thin section by polarizing microscope or by SEM.

Increasing the firing temperature results in first dehydroxylation of clay minerals. Carbonates that may be present decompose around 800°C. Further increase in temperature will result in the breakdown of clay matrix and depending on the type of clay minerals, feldspars, pyroxenes and anorthide minerals may form.

i-) dehydroxylation of clay mineral
ii-) carbonate decomposition
iii-)formation of Ca-bearing silicates e.g. gehlenite, wollastonite, pyroxene and anorthite

The most primitive pottery probably was heated in an open fire, but enclosed structures called kilns provide higher and better control of temperatures. The two critical conditions important in the kiln are the temperature and the amount of oxygen. A simple wind-aided fire might attain a temperature over 900°C, which is sufficient to produce terra cotta or earthenware. More highly vitrified pottery, however, requires higher temperatures that can be reached by the use of bellows or better designed kilns. When there is excess oxygen in the kiln, oxidizing conditions will prevail and the organic materials will burn off as carbon dioxide which will increase the vessels' porosity. Iron will also oxidize to red hematite (FeO_2) that will give the red color of terra cotta. In the poorly vented kilns, reducing conditions, however, kiln atmosphere will be dominated carbon monoxide or hydrocarbons. This will result in the incomplete combustion of organic materials and result in the dark coloring of pottery. Under such reducing conditions iron will also reduce the black ferrous oxide (FeO) and also contribute to black coloring (Rice, 1987).

The color of pottery thus is a function of several factors, including the original composition of clay, firing temperature, the oxidizing properties of the kiln, and the surface treatment of pottery, such as the use of slip. Blacks and grays are caused by a reducing atmosphere, either by charred organics or reduced iron. When conditions are oxidizing and iron is present colors may vary from pink to red to orange, depending on the firing temperature. Yellow and cream colors require temperatures above 1100°C and the presence of calcium (Orton *et al.*, 1993).

The earliest hand-building techniques for forming pottery included pinching (shaping the ball of clay), slab building (pressing slab of clay together), coiling (using the long coil to build up the overall shape and pressing the coil together), and molding (pressing slabs into a mold) (Lambert, 1998)

4.2. Form and Function

The vessel forms that are of most interest for residue analysis are cooking pots and storage/transport vessels. Organic residues from cooking pots have been carefully investigated in many ethnographic and archaeological cultures (Reber, 2001). The similarities among these studies suggest that only a limited number of technological solutions exist for the problem of producing a vessel capable of surviving the stresses of repeated cooking. Investigation of cooking pots gives the best representation of the cooking and dietary practices of a community. Serving and preparation vessels tend not to absorb enough lipids for residue analysis. Storage and transport vessels contain only one or two important resources, which allows easier interpretation of residue results. However, cooking pots give a complex but wide range of data for the entire foodstuff cooked in a pot.

Cooking pots meant for boiling tend to combine certain traits, including a slightly restricted rim that prevents boiling over, but at the same time allow access to the contents of the pot. Cooking pots tend to be somewhat low-fired and heavily tempered to reduce thermal shock (Rice, 1987). Surface treatment varies among different cultures, even though there seems to be a slight preference for surface roughening on the body of cooking pots (Skibo and Schaffer 1995).

Regular heating and cooling causes thermal stress in pottery. Pores, tempering agents, irregularities in construction, or stresses during drying can all lead to crack initiation (Rice, 1987). If cracks propagate, the pottery vessel crumbles and fails. Pores created by the grains of temper material can stop crack propagation. Low-fired pots tend to have larger pores than high-fired pots, and therefore are more resistant to crack propagation resulting from thermal stress than high-fired pots (Figure 4.1.) (Rice 1987). A

cooking vessel is subject to a large amount of thermal stress, thus low-fired pots serve better as cooking vessels than high-fired pots.

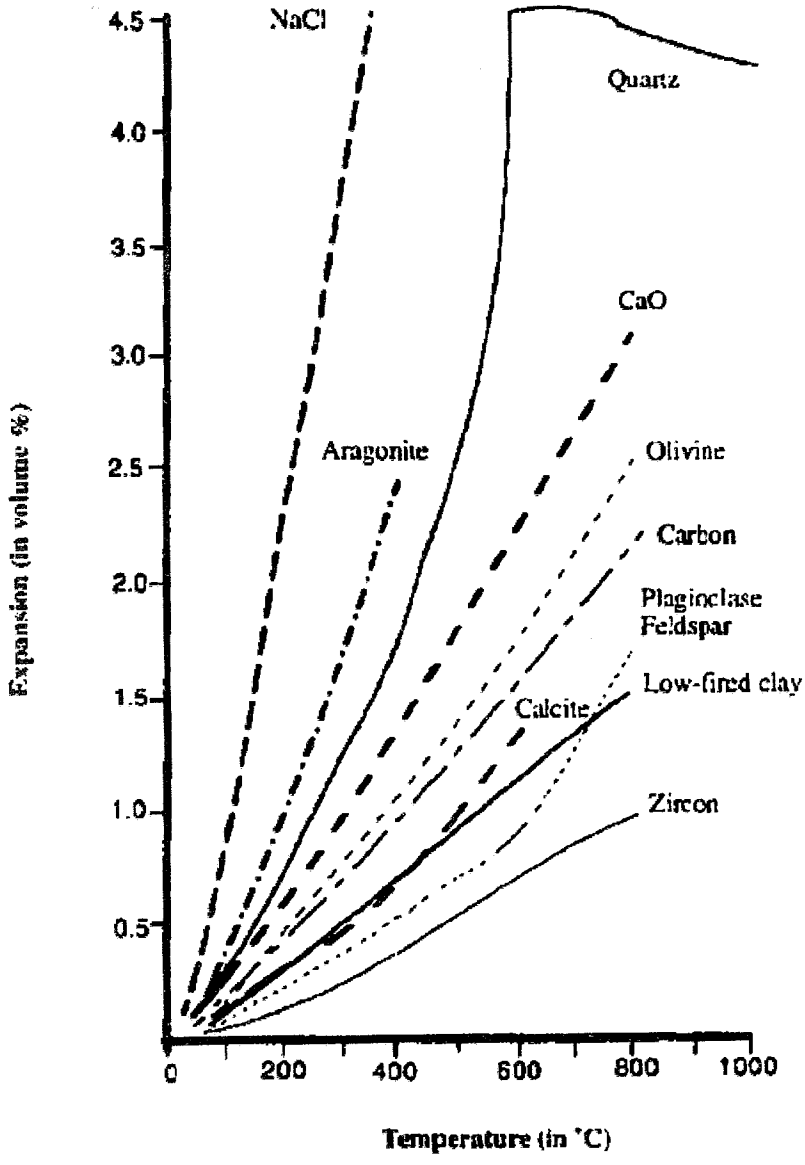


Figure 4.1. Thermal expansion curve of low-fired and common tempering agents (Rice, 1987)

Tempering is a very important issue in pottery production and function. Certain types and quantities of temper material are clearly more appropriate for cooking vessels than others. It is not clear to what extent ancient potters understood and exploited these technological differences. Temper is an aplastic material mixed with clay to balance plasticity and moisture. It also adds strength to the pot while the clay is still wet and increases the resistance to thermal stress of the finished pot. Furthermore, it reduces the

shrinkage of wet clay upon drying (Rice, 1987; Skibo 1994). Almost all materials expand when exposed to heat. If tempering material expands at a faster rate than the clay surrounding it, crack initiation occurs. The temper in a cooking pot should have a coefficient of thermal expansion similar to or less than that of the paste. The most common tempering agents with thermal expansion coefficient similar to or less than clay are crushed pottery, calcite (crystal structure of $CaCO_3$ that is stable at low-firing temperatures and feldspar. Quartz, the primary mineral in sand, on the other hand, expands about twice as fast as clay yet it is one of the most common tempering materials used in the world, (Rice, 1987).

The amount and size of tempering in a vessel has complex functional implications as well. Large amounts of temper tend to decrease the mechanical strength of the pot, or its resistance to crack initiation. Large amounts of temper also tend to increase the crack propagation due to thermal shock, if tempering agent has a low thermal expansion coefficient and high degree of thermal conductivity (Skibo and Schiffer 1995). Thermal conductivity decreases the thermal gradient throughout the pot; a high gradient causes strain because different pots expand at different rates. Temper particles can also stop crack propagation and increase resistance to thermal shock (Skibo and Schiffer 1995). Burnt shell temper seems to improve resistance to thermal shock proportionally to the amount of temper in a vessel (Bronitsky, 1986). Other temper types appear to impart the same amount of resistance to thermal shock regardless of the amount of temper used (Bronitsky 1986).

Large particle size has a similar effect on thermal shock resistance (Rice,1987). Large particles of burnt shell appear to increase resistance to thermal shock (Bronitsky 1986). Large particles of highly conductive inclusions, such as quartz, can reduce the thermal gradient through the pot and increase the rate of heating (Skibo and Schiffer 1995).

Vessel wall thickness is another attribute that can have functional implications. The thinner the pot wall less will be the thermal gradient over the pot. Thin walls also minimize thermal stress and aid in the heat transfer (Rice, 1987). Thin pot walls also have less mechanical strength and are less resistant to breakage than pots with thick walls. The thinner the wall of a vessel, the longer its life as a cooking vessel, but the less resistant it is to everyday accidents and shocks (Rice, 1987).

Slipping and polishing also have benefits for the cooking vessels. The less permeable a vessel, less liquid is lost over the course of use, and less liquid passes through the walls to produce steam and stress on the pot (Rice, 1987). Filling or decreasing the number of pores on the surfaces of a pot decreases vessel permeability. This can be accomplished by burnishing, slipping and treating the interior of the pot with resins or grease (Rice, 1997). Burnishing appears to increase vessel strength by causing a compressed surface on the exterior of the pot. The choice of surface treatment for a cooking vessel is a difficult choice with multiple possibilities for different benefits.

Cooking vessels tend to have globular or conical bases. Strains resulting in crack initiation are concentrated at sharp corners, causing flat-bottomed clay cooking vessels to break sooner than their round-bottom counterparts (Skibo and Sciffer 1995).

Functionally the ideal cooking vessel would be globular with a slightly constricted neck with considerable amounts of calcite temper. It will also have thin walls and surface treatment depending on the specific needs of the potter and pot users.

Form/function relations exist in pottery, but the extent and depth of functional contribution to vessel morphology depends upon a given culture

5. ANIMAL DOMESTICATION AND DAIRYING IN PREHISTORY

5.1. Animal Domestication

In Anatolia, modern faunal research has begun in the late 1960s with the work at Çayönü, Suberde and Can Hasan (French, 1970). Study of the animal remains from these Neolithic sites have shown that transition from hunting to husbandary in Anatolia took place between about 7000 and 6000 BC in the settled agricultural villages. The keeping of domestic animals was not immediately accepted everywhere. At a number of contemporary sites (e.g. Aşıklı and Cafer Höyük), the faunal remains came from wild relatives of the stock that was already being kept and bred (McGovern *et al.*, 1995).

At the sites of later periods where faunal remains are dominated by domestic forms, the husbandry practices and the animal economy became important. Topics like selective breeding, site provisioning, exchange of animal products and animals used by different social groups were investigated. Comparison of the areas in which domesticated animals originated shows that a certain level in the cultural development was a prerequisite for domestication (Reed,1959).

Present evidence indicates that 'food producing revolution' such as the domestication of goat (*Capra hircus)* and sheep (*Ovis aries*) occurred in Anatolia in prehistoric times, probably in the 7^{th} millennium BC. Cattle (*Bos Taurus*) was domesticated somewhat later and pigs (*Sus scrofa*), even later (Reed, 1959).

Early Neolithic sheep had hair, but they did not have wool at this time so that it is assumed that they were kept primarily for milk. Cattle would have provided a source of meat, milk, blood, leather and bone. Neolithic pig husbandry became effective only after the emergence of cultivation because they could be fed on agricultural by-products. It would be another millennium before ducks and chickens were domesticated (Dudd, 1999).

The provision of meat seems to have been an important reason for domestication. As Reed (1959) points out, the domestication begins when growing human population changes over to a more settled and culturally higher form of life. So the view suggests itself that the keeping of domestic animals arises from the necessity of ensuring a regular food supply for larger groups of people. But the actual patterns of exploitation of various natural commodities obtained from animals are more difficult to interpret. The question of resource utilization in prehistory would benefit from identification of animal products themselves in order to obtain direct information relating to animal exploitation, consumption and manufacturing in the past (Dudd, 1999).

5.2. Milk

Milk is a valuable resource as it has high protein, nutrient and fat content. Already in ancient times it was important: "Of all the liquids in our diet, milk is the most nourishing" (Varro, On Agriculture 2.10-11.1). It is characterized by a large concentration of short chain fatty acids ($<C_{14:0}$). Rottlander (1990) and Rottlander and Schlictherle (1979) identified milk in a small number of archaeological vessels using this criterion. However, the high content of low molecular weight triacylglycerols makes milk more susceptible to hydrolysis (see 2.4.1). The liberated short chain fatty acids are more readily lost due to their larger water solubility. Due to degradation reactions, the triacylglycerol pattern of degraded milk cannot be distinguished from animal adipose fats based on triacylglycerol distributions (Dudd and Evershed, 1998). Stable carbon isotope analyses of the abundant $C_{16:0}$ and $C_{18:0}$ fatty acids, however, provide a possibility to discern milk fats from adipose fats. This is due to the *de novo* synthesis of the $C_{16:0}$ fatty acid from acetate, with the $C_{18:0}$ component derived in part directly from bacterial reduction in the rumen of the dietary fatty acids i.e. mainly $C_{18:2}$ and $C_{18:3}$, and in part from acetate derived from dietary carbohydrate. There is enhanced routing of dietary fatty acids during the lactation resulting in more negative $\delta^{13}C$ values for $C_{18:0}$ fatty acid for milk fats compared with the adipose fats of animals fed with the same diet (Evershed *et al.*, 1999). Based on this information milk was identified in several vessels from different time periods (Dudd and Evershed, 1998).

Furthermore, milk fat was also recognized by the identification of protein α-casein in prehistoric ceramics (Craig *et al.*, 2000). Bovine milk is mainly characterized by the latter protein. With the use of an antibody against heat degraded bovine α-casein, it was concentrated and detected using the immunological digestion and capture immune assay method. The captured casein by the antibody is detected by a change in color.

5.3. Dairying in Prehistory

The timing of the exploitation of domesticated ruminant animals for their 'secondary products', such as milk wool, etc., is crucial to our understanding how domesticated animals were utilized in prehistory. It is possible that this occurred during a considerable period of time following their initial domestication (Sherrat, 1981). Dairying was probably older than the first iconographic evidence for the practice, which appears only in Uruk contexts of the fourth millennium BC (Sherrat, 1981)

The organic component of milk primarily comprises fats, proteins and carbohydrates. The majority of the milk carbohydrate consists of lactose which is broken down by the enzyme lactase prior to its absorption in the human gut. Although all humans produce lactase during infancy, only a minority of modern populations is actually able to digest the milk sugar in adulthood. This may suggest that at some point in the past, consumption of milk into adulthood was tolerated, due to the extended production of lactase, perhaps through genetic expression (Sherrat, 1983). However, this does not mean that the milking of ruminant animals in the past was restricted to certain populations, because milk can be converted to butter, cheese or yoghurt which contains little or no lactose (Copley *et al.*, 2005). Therefore, it is possible most cultures in the past must have consumed dairy products only in their fermented form.

Traces of dairy fats absorbed into the walls of ceramic vessels during use may be detected by lipid residue analysis. Therefore, not only can the emergence and dispersal of dairying in antiquity be determined, but also the importance of dairy products, relative to other types of foodstuffs understood.

One of the traditional evidences for dairying includes the reconstruction of age and sex profiles based on preserved animal bones (Payne, 1973). High abundances of mature females, low number of mature female and high abundances of very young animals provide evidence for dairying in ancient farming practices.

Even though special ceramic vessels, described as 'cheese strainers' or 'strainer' may indicate that they were used for the processing of dairy products in certain regions in the world (Bogucki, 1986), it is only through organic residue analysis that direct identification of dairying can be detected. This rests on the lower $\delta\ ^{13}C$ values of $C_{18:0}$ fatty acid in dairy fats which results from the lack of capacity of the mammary gland to biosynthesize $C_{18:0}$ (Christie, 1981). Different carbon isotope values for lipids and carbohydrates in plants results from the fractionation during the decarboxylation of pyruvate in forming acetyl CoA (Deniro and Epstein, 1977) and the routing of significant proportion of dietary $C_{18:2}$ and $C_{18:3}$ fatty acids after biohydrogenation to $C_{18:0}$ in the rumen to milk during lactation (McDonald, 1988). The results consistent with these factors were obtained experimentally (Copley *et al.*, 2003; Dudd and Evershed, 1998), providing the basis for the compound-specific stable carbon isotope approach to the detection of dairy fats in archaeological pottery.

6. IDENTIFICATION OF ORGANIC RESIDUES IN ANCIENT POTTERY VESSELS

6.1. Fatty Acids Ratios

Condamin and coworkers (1976) were the first researchers to show that fatty acids can be preserved in the porous matrix of archaeological potsherds. This and following early studies of organic residue analysis in ceramic vessels were based on the distribution of fatty acids recovered after extracting the lipids and the subsequent analysis with gas chromatography (GC).

By determining the distribution of free fatty acid fraction or the fatty acids constituting the saponified lipid extract, several researchers tried to determine the origin of the lipids by comparing with the distributions of fatty acids in modern reference fats (Rottlander and Schlictherle, 1979; Patrick *et al.*, 1985). However, it was observed that during decomposition the relative percentage of fatty acids change thus direct comparison was not reliable (Skibo, 1992). Malainey and coworkers (1999) tried to overcome this problem by comparing the fatty acid ratios obtained from archaeological vessels with fatty acid ratios of both fresh and decomposed foodstuffs. In a study by these authors (Malainey *et al.*, 1999) the fatty acid composition of 130 native Canadian plants and animals was analyzed. Also several foodstuffs were degraded by thermal simulations and thermal oxidative decompositions (Malainey *et al.*, 1999). It was shown that the amount of unsaturated fatty acids were especially reduced, which made the composition of the lipid residues from berries, roots and several vegetables and seeds appear very similar. Nevertheless, with the use of these fatty acid patterns the origins of the fatty acids from vessels dating from the Late Precontact Period in Western Canada were assigned to different food groups such as large herbivore, corn, fish, plant, marrow and beaver by using hierarchical cluster and principal component analysis. However this approach is difficult to apply in general since it is very time consuming to simulate degradation for all possible foodstuff and impossible to imitate all degradation mechanisms.

6.2. Identification of Animal Fats

6.2.1. Animal Adipose Fat

Animal adipose fats are among the most common class of lipids seen in archaeological pottery (Evershed *et al.*, 1992; Evershed *et al.*, 2001). The high presence of saturated fatty acids increases th possibility of their frequent occurrence in pottery. Degraded animal fats are characterized by a typical pattern with large amounts of saturated free acids, followed by smaller amounts of mono-, di- and triacylglycerols generated by hydrolysis of the present triacylglycerols.

Different criteria can be used for the identification of lipid residues. For example, the types of fatty acids present may indicate possible plant or animal origin when their relative abundances are determined. TAG distributions and structures are also potentially useful indicators of origin (Dudd & Evershed, 1998; Kimpe *et al.*, 2001, 2002). However, caution must be exercised when using these criteria since fatty acid ratios can change over time and TAG's are only present in very low abundances or are completely absent. A more reliable method for the elucidation of lipid origin is to determine the $\delta^{13}C$ values of individual $C_{16:0}$ and $C_{18:0}$ fatty acids (Evershed et al., 1997; Dudd & Evershed, 1998; Mottram *et al.*, 1999).

6.2.1.1. GC and GC-MS Analysis. It is potentially possible to identify even the highly degraded natural products by GC and/or GC-MS. Reliable classification of foodstuff processed in archaeological vessels can be made by comparing chemical structures of individual compounds with those obtained for modern and/or archaeological reference materials. A general knowledge of the degradation processes that occur during vessel use and burial is essential in order to identify the lipid residues preserved within.

A typical degraded animal fat profile obtained by HTGC (high temperature gas chromatography) from a Romano-British pottery from Stanwick, UK is shown in Figure 6.1 (Evershed *et al.*, 2002). In Figure 6.1, FA12, FA14, FA15, etc. correspond to *n*-alkanoic fatty acids with 12, 14 and 15 carbon atoms, respectively; FA17br refers to a branched-chain alkanoic fatty acid with 17 carbon atoms; FA16:1 and FA18:1 refer to

monounsaturated *n*-alkanoic fatty acids containing 16 and 18 carbon atoms, respectively; M16 and M18 refer to MAGs containing 16 and 18 acyl carbon atoms, respectively where the 1-isomer elutes before the 2-isomer. In this chromatogram, K31, K32, etc. refer to mid-chain ketones; D30, D32, etc. refer to DAGs containing 30, 32, etc. acyl carbon atoms, respectively where the 1,2-isomer elutes before the 1,3-isomer; T44, T46, T48, etc. correspond to TAGs bearing 44, 46, 48, etc. acyl carbon atoms, respectively; IS is the internal standard (*n*-tetratriacontane) added at the extraction stage in order to enable quantification of lipid. Low abundances of intact TAGs are observed at retention times over 30 min. However, the majority of the TAGs have been hydrolyzed either chemically or enzymatically during vessel use or during the burial resulting in the formation of DAGs, MAGs and free fatty acids. The free fatty acids present that elute between 10 to 20 min, comprise mainly the $C_{16:0}$ and $C_{18:0}$ components. Generally the high abundance of $C_{18:0}$ fatty acid is indicative of animal fat origin.

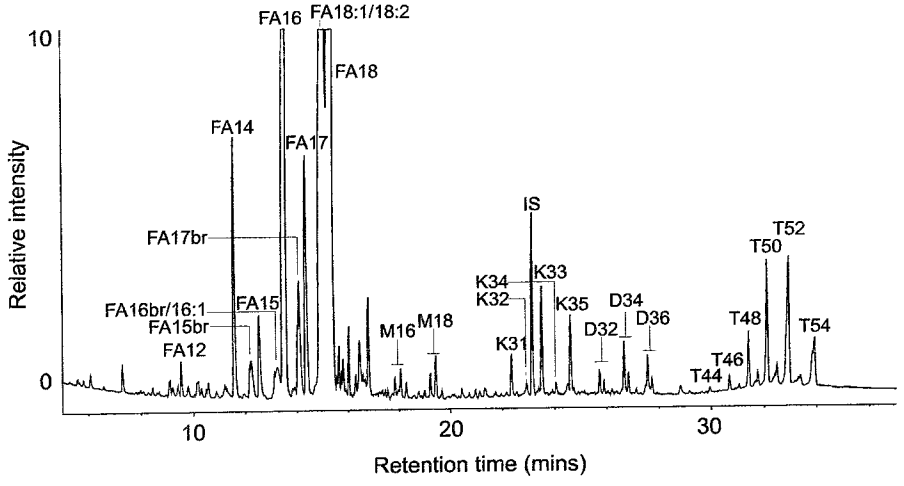


Figure 6.1. Partial HTGC profile of the trimethylsilylated extract from a Romano-British sherd from Stanwick, Northamptonshire (Evershed *et al.*, 2002).

6.2.1.2. Identification by Stable Isotope Analysis:

i-) Sources of natural variation in the $\delta^{13}C$ values of animal fats:

Three different photosynthetic pathways are used by terrestrial plants, namely C_3, C_4 and CAM. The C_3 plants (e.g. wheat, rye, barley, legumes, cotton, tobacco, tubers) are the most abundant and are found mainly in temperate zones. They fix atmospheric CO_2 using the Calvin cycle whereby $^{13}CO_2$ is discriminated against by RuBisCO thus giving relatively low $\delta^{13}C$ values ranging from –32 to –20 ‰ (Boutton, 1991). C_4 plants (sugar cane, maize, sorghum, dryland grasses) fix CO_2 via the Hatch-Slack pathway (Hatch & Slack, 1966) using the enzyme PEP carboxylase to reduce CO_2 to malic acid. The latter pathway discriminates less against $^{13}CO_2$ giving $\delta^{13}C$ values in the range of –17 to –19 ‰ (Boutton, 1991). CAM plants (succulents, pineapple, aloe vera, jade plant) can be either obligate (assimilate CO_2 at night) or facultative (assimilate CO_2 night and day). Fixation of CO_2 at night occurs *via* PEP carboxylase as in C_4 plants resulting in comparable $\delta^{13}C$ values. However, some CAM plants can switch to the C_3 pathway and use RuBisCO to fix CO_2 during the day. $\delta^{13}C$ values for facultative CAM plants span the entire C_3 and C_4 $\delta^{13}C$ range (Farquhar *et al.*, 1989).

Marine plants do not absorb the carbon dioxide from the atmosphere but from dissolved gasses in the surrounding water. Therefore the $\delta^{13}C$ value of marine CO_2 is variable and depends on depth and other localised factors, but it is usually in the region of 0 ‰. Even though the photosynthetic mechanisms are different, the marine phytoplanktons fractionate carbon approximately the same extent as terrestrial C_3 plants (Chisholm *et al.*, 1982). Thus, marine plants have $\delta^{13}C$ values in the range of –11 to –19 ‰. Foreshore plants that are not constantly submerged can be more complicated but still show a 'marine' signature, which is distinguishable from C_3 plants which have $\delta^{13}C$ values around –25 ‰ (Ambers, 1990). The marine signature is carried up the food chain so the animals that eat large amounts of marine fodders, such as seaweed, should be distinguishable from those eating predominantly terrestrial diets. Discriminating between marine and C_4 plant input into the diet of animals is difficult, however, it is not a problem for studies that are carried out in areas where there are no native C_4 plants such as temperate Northern Europe. However it must be taken into account when animal fats from the coastal C_4 environments

are studied. Table 6.1 displays the ranges of bulk $\delta^{13}C$ values for various natural materials that provide a guide to the trends that might be seen in lipid residues.

Table 6.1. Stable carbon isotope ratios for the major components of terrestrial ecosystems (Boutton, 1991)

Material	Bulk $\delta^{13}C$ value (‰)
C_3 plants	-32 to –20
C_4 plants	-17 to –9
CAM plants	-20 to –10
Groundwater	-25 to –10
Atmospheric CO_2	-8
Sea grasses	-15 to –3
Marine vertebrates	-17
Marine carbonates	0

In lipid residue analysis, C_4 contributions to an animal's diet would result in enrichment of $\delta^{13}C$ values in the fatty acids. This may lead to misinterpretation of pure ruminant adipose fat as a mixture of ruminant and porcine adipose fats, or even pure porcine.

The reference animals used to compile the database in the literature were reared on C_3 diets. Therefore, when using the reference animal data to identify animal fats with a possible marine or C_4 diet contribution an offset from the corresponding animal fat ellipse may be observed. This discrepancy may be compensated by comparing the difference in the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids for the reference and archaeological fats ($\Delta^{13}C$) by using Equation 6.1. In this equation $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ are the isotope value for $C_{16:0}$ and $C_{18:0}$ fatty acids respectively.

$$\Delta^{13}C = \delta^{13}C_{18:0} - \delta^{13}C_{16:0} \qquad (6.1)$$

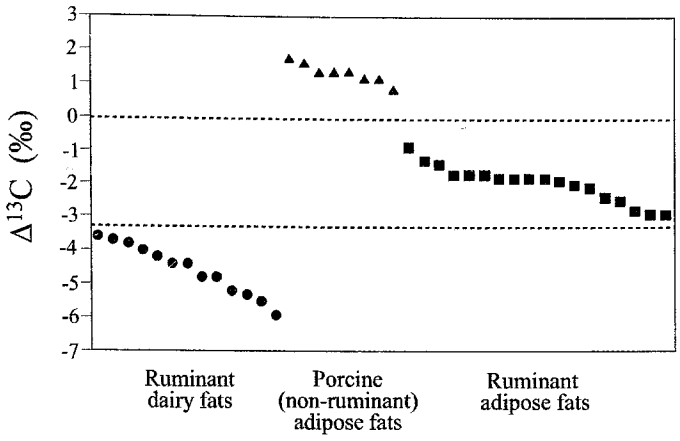


Figure 6.2. Plot of the $\Delta^{13}C$ values obtained from modern reference fats (Copley *et al.*, 2003).

Figure 6.2. display the range of $\Delta^{13}C$ values observed for reference animal fats. The effect of any C_4 and marine contributions can be interpreting only by comparing the $\Delta^{13}C$ values in conjunction with TAG distributions (Copley, 2001).

In dense forests there is a significant deviation in $\delta^{13}C$ distribution compared to the global norms, due to depletion depleted in ^{13}C. The degree of depletion appears to correlate with forest density. A gradient of leaf $\delta^{13}C$ values also exists from the forest floor canopy, with the most negative values occurring near the ground (van der Merwe & Medina, 1991). This phenomenon is known as the 'canopy effect'. The mean bulk $\delta^{13}C$ value for C_3 grasses in open habitats is *ca.* –26.5 ‰ (Vogel *et al.*, 1975), however, $\delta^{13}C$ values of *ca.* –35 ‰ have been recorded for leaves in subtropical monsoon forests (Ehleringer *et al.*, 1987) and values as low as –37 ‰ have been observed in the Amazonian forests (Medina *et al.*, 1980). Ruminant animals, however, are generally raised on open grassland and should not be affected by the canopy effect. Wild pigs, however, are naturally forest dwelling animals. $\delta^{13}C$ collagen values of prehistoric pigs from the Kenyan Rift Valley were found to be several *per mil* more negative than those observed for the contemporary individuals from the same location. These values were thought to indicate a denser and more closed canopy environment of the region prior to 5600 BP (Ambrose & DeNiro, 1989). Since the reference pig samples used in this study were not raised within a forested environment, their fatty acid $\delta^{13}C$ values may be more enriched than those of their ancient forest-dwelling counterparts. Which means that the $\delta^{13}C$ values of the fatty acids in archaeological porcine fats will be more negative than those of the modern counterparts.

Any temperature variations that may occurr as a result of climate change or differences in the values of atmospheric CO_2 over a time period could result in deviations in the $\delta^{13}C$ values from the reference range observed for animals from the archaeological period of interest. Evidence for variations in the $\delta^{13}C$ values with global temperature change has been recognized from the long tree-ring record of Irish Oaks, extending back *ca.* 7000 years and suggests century scale changes up to 1.5 ‰ (McCormac *et al.*, 1994). It is assumed that other terrestrial plants will respond to climate/environmental changes in the same way as trees, but the magnitude of this effect on various species may differ (Heaton, 1999). Differences in isotope composition between modern and ancient fats resulting from such temperature variations in CO_2 $\delta^{13}C$ values can be overcome by comparing the $\Delta^{13}C$ values for reference and archaeological fats.

Early work in the use of stable isotopes in archaeological pottery involved bulk isotopic analysis (Hastdorf & DeNiro, 1985; Morton & Schwarcz, 1988; Sherriff *et al.*, 1995). However, the application of compound-specific stable isotope analysis via GC-C-IRMS to lipid residues in archaeological pottery (Evershed *et al.*, 1994) allows greater specificity to be achieved since the structures of diagnostic (biomarker) components of complex mixtures can be unambiguously linked to their stable isotope values. Thus, compound-specific stable isotope analysis avoids ambiguities arising from contamination, e.g. plasticizers originating from plastic bags in which potsherds are often stored. Such contaminations cannot be resolved from endogenous components in bulk isotope analyses. Compound-specific $\delta^{13}C$ values also afford insights into the biochemical sources of carbon even when chemical structures are identical. $\delta^{13}C$ values of fatty acids provide the basis for distinguishing between ruminant (e.g. sheep/goat and cattle) and porcine (pig) adipose fats (Evershed *et al.*, 1997; Mottram *et al.*, 1999). It is also possible to distinguish between ruminant adipose fats and ruminant dairy fats (Dudd & Evershed, 1998). Such results will provide the means to understand some of the key questions concerning animal husbandry in prehistory, such as the earliest evidence for dairying in prehistory (Copley *et al.*, 2003). Compound-specific $\delta^{13}C$ values can be readily determined for fatty acids (analysed as FAMEs) deriving from archaeological artefacts by GC-C-IRMS. Although the detection of degraded animal fat is straightforward, the identification of the species origin is complicated by diagenetic alteration and inherent similarities between animal fats. However, due to subtle differences in the way that different animals assimilate their diet,

the $\delta^{13}C$ values of the major fatty acids ($C_{16:0}$ and $C_{18:0}$) are sufficiently distinct to allow differentiation between the fats of the major domesticates.

ii-) Modern reference animal fats

Naturally, animals farmed today cannot be directly compared with those raised in antiquity due to the changes in fat composition that have occurred over the last few hundred years. This is due to several factors, including:

- intensive farming which has led animals to be fed by supplements to enhance their diets and to improve the nutritional quality of their meat and milk (e.g. Nürnberg *et al.*, 1998; Chilliard *et al.*, 2001; Lowe *et al.*, 2002);
- selective breeding resulting in changes in the composition of the fat and milk of domestic animals;
- the burning of fossil fuels since the industrial revolution causing changes in the isotopic composition of atmospheric CO_2 (Friedli *et al.*, 1986) resulting in depletion of ^{13}C in the tissues of modern animals compared to their ancient counterpart;
- C_4 plants (e.g. maize) incorporated into animals' diets, again significantly alter the carbon isotopic composition of animal tissues.

The identification of remnant animal fats extracted from archaeological pottery has been aided by a carefully assembled database of modern fats (Dudd & Evershed, 1998; Copley *et al.*, 2003; Evershed *et al.*, 2003) and with reference to published database (e.g. Enser, 1991). The reference animals sampled in order to compile the database were selected having been reared on known diets of C_3 origin and comprise adipose fat from cattle, sheep and pigs, and milk fat from both cattle and sheep. The scatter graph shown in Figure 6.3 displays the $\Delta^{13}C$ values obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids from each of the reference animal fats. It can be seen sheep and cattle data are grouped together as ruminant animals. The $\Delta^{13}C$ values obtained from the modern reference animals were adjusted for post-Industrial Revolution effects of fossil fuel burning by addition of 1.2 ‰ (Friedli *et al.*, 1986). Confidence ellipses corresponding to pig adipose, ruminant adipose and ruminant milk provide the reference $\Delta^{13}C$ ranges, onto which the data for archaeological samples can be overlaid to positively assign lipid origins (Fig. 6.2).

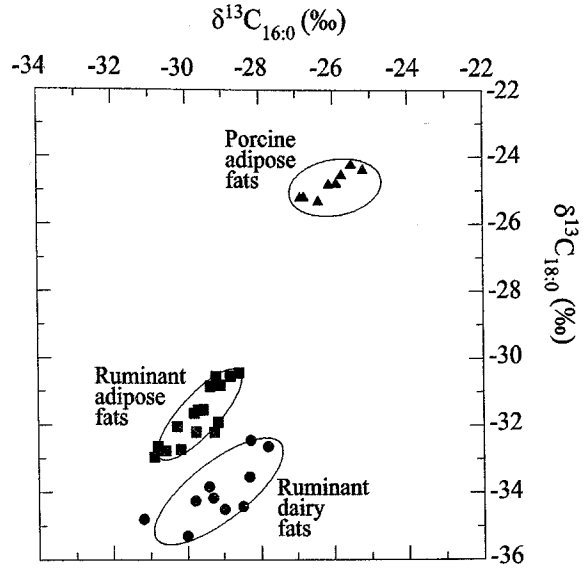


Figure 6.3. Scatter plot of $\Delta^{13}C$ values fot the $C_{16:0}$ and $C_{18:0}$ fatty acids (Dudd & Evershed 1998; Dudd, 1999).

The $\Delta^{13}C$ values exhibited by these animals must reflect their different diets and variations in their metabolisms and physiologies (Evershed *et al.*, 1999). Dairy and adipose fats from ruminant animals can also be distinguished since the $C_{18:0}$ fatty acid in dairy fat is significantly more depleted in ^{13}C by about 2.1 ‰ (Copley *et al.*, 2003). Fatty acids in ruminant adipose are mainly synthesized from acetate as acetyl CoA that originate predominantely from the fermentation of dietary carbohydrate in the rumen. The mammary gland is incapable of synthesizing the $C_{18:0}$ fatty acid, instead, it is obtained *via* the remobilization of adipose fatty acids and directly from the dietary C_{18} fatty acids, after biohydrogenation in the rumen (Moore & Christie, 1981). The difference between the $C_{18:0}$ fatty acids from ruminant adipose and dairy fat can be explained by the fact that lipids are more depleted in ^{13}C than carbohydrates (DeNiro & Epstein, 1977). Approximately 60 % of the $C_{18:0}$ fatty acid in dairy fat is derived *via* biohydrogenation of dietary unsaturated C_{18} fatty acids (i.e. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) in the rumen. The $\Delta^{13}C$ values of the various contributors to the $C_{18:0}$ fatty acid in dairy fats are summarised in Figure 6.4.

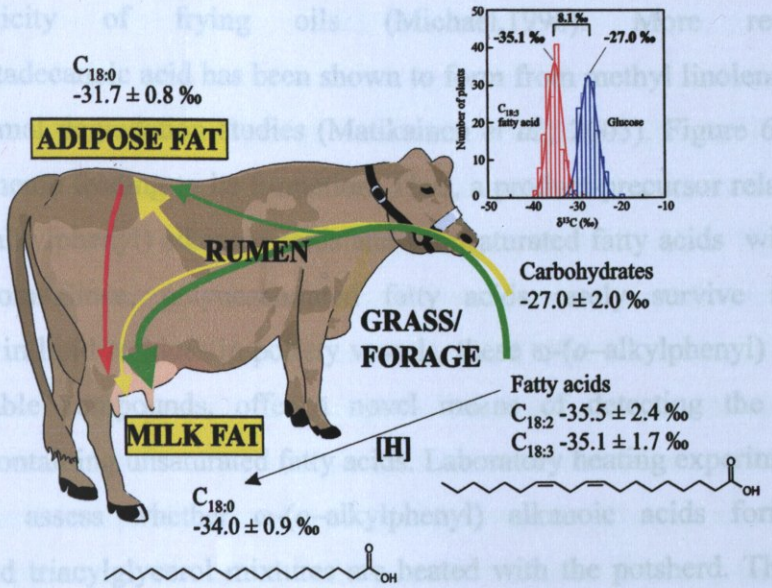


Figure 6.4. Routing of dietary fatty acids and carbohydrates in the rumen, adipose tissue, and mammary gland of the ruminant animal (Copley *et al.*, 2003).

6.2.2. Fish

Fish oil is characterized by high content of long chain polyunsaturated fatty acids (Table 2.7). Since polyunsaturated fatty acids are extremely susceptible to oxidation (Song *et al.*, 1997), they are rapidly lost. Probably due to this loss of diagnostic fatty acids, fish is very difficult to identify in the lipid fraction from archaeological pottery .

The presence of appreciable proportion of long chain C_{20} to C_{24} fatty acids, indicated the presence of fish at the bottom of a Basque whaling shipwreck (Morgan *et al.*, 1992). High amounts of long chain fatty acids were also found in ceramics of the Southwestern Cape where fish was also identified (Patrick *et al.*, 1985). Nevertheless in both cases the interpretation was solely based on the distribution of fatty acids. In a study by Sheriff *et al.* (1995) the fish residues were determined by using $\delta^{13}C$ and the $\delta^{14}N$ values of the organic residues in pottery.

A new types of lipid was recently observed in archaeological pottery namely ω-(*o*-alkylphenyl)alkanoic acids with sixteen to twenty carbon atoms (Hansel *et al.*,2004). These fatty acids contain an unusual benzenyl moiety within the alkyl chain. ω-(*o*-alkylphenyl)octadecanoic acid was first detected during the heating of modern cooking oils containing triunsaturated fatty acyl lipids in experiments employed to determine the

potential toxicity of frying oils (Michael,1996). More recently ω-(*o*-alkylphenyl)octadecanoic acid has been shown to form from methyl linolenic acid through laboratory thermal degradation studies (Matikainen *et al.,* 2003). Figure 6.5 summarizes the reaction scheme leading to its formation. Thus, a product-precursor relationship exists between ω-(*o*-alkylphenyl) alkanoic acids and triunsaturated fatty acids with greater than 18 carbon atoms.Since, polyunsaturated fatty acids rarely survive in appreciable concentrations in lipid residues in pottery vessels, these ω-(*o*–alkylphenyl) alkanoic acids, which are stable compounds, offer a novel means of detecting the processing of commodities containing unsaturated fatty acids. Laboratory heating experiments have been undertaken to assess whether ω-(*o*–alkylphenyl) alkanoic acids form when pure compounds and triacylglycerol mixtures are heated with the potsherd. The results show that these compounds form only when tri-, di- and monounsaturated fatty acids, but not form saturated fatty acids, are heated at 270°C with a potsherd (Evershed *et al.*, 2008). Distributions obtained are consistent with those seen in lipid residues obtained from potsherds from coastal archaeological sites in South Africa and Denmark (Hansel *et al.,* 2004; Copley *et al.*, 2004). Therefore confirming that they can serve as indicators for processing of marine foods, high in marine oils, which contain high abundances of unsaturatedfattyacids.

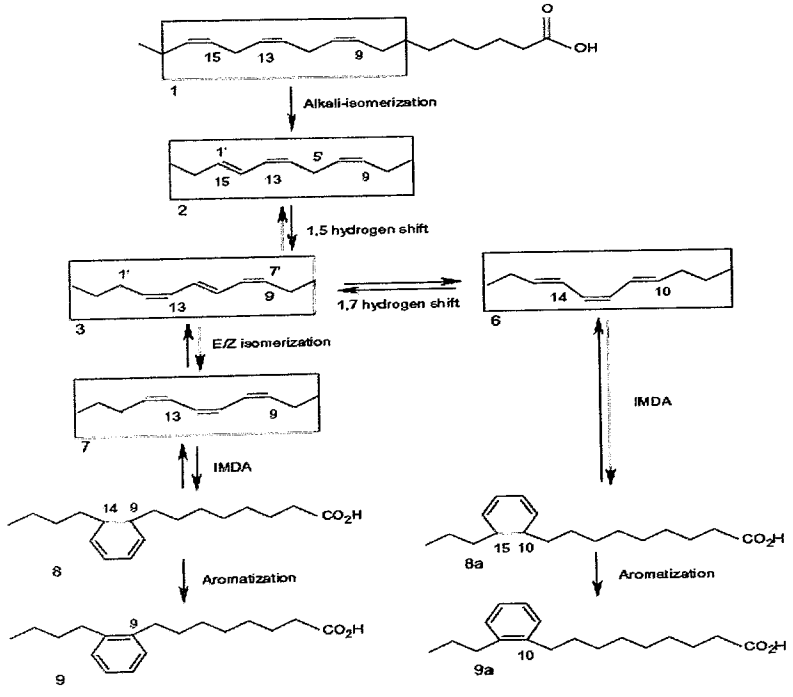


Figure 6.5. Pyrolytic formation of ω-(o-alkylphenyl)alkanoic acids (Hansel *et al.,* 2004)

6.3. Beeswax

Beeswax is characterized by high amounts of *n*-alkanes (C_{23} to C_{33}), long-chain alcohols (C_{22} to C_{34}) and long chain palmitic acid wax esters (C_{40} to C_{52}). Due to the inertness of these molecules for oxidation and hydrolysis, beeswax is highly stable and easily recognized in the archaeological record. Beeswax was identified in conical lamps from a Minoan site in eastern Crete (Evershed *et al.*, 1997). It was taken as a proof that beeswax was used as fuel instead of the expected olive oil. The presence of beeswax was also shown in ceramics from a Neolithic settlement (Heron *et al.*, 1994). Beeswax in addition to animal fat was identified in two medieval vessels (Charters *et al.*, 1994). In the latter cases the presence of beeswax in the ceramic was predominantly used as a sealant. Generally post-firing treatments are applied to pottery in order to decrease permeability by sealing the vessel surface, to increase its strength or simply to improve its appearance. Beeswax is a natural product utilized for this purpose (Rice, 1987). Garnier *et al.* (2002) have investigated archaeological beeswax by GC/MS (as TMS derivatives), and also by electrospray ionization mass spectrometry. The results show that the nonderivatized higher molecular weight biomarkers (such as diesters), with very high molecular weights can not be analyzed by GC/MS.

6.4. Plant Lipids

6.4.1. Plant Oils

Although several plant oils are of great economic importance in the present and past economies, surprisingly few reports on the identification of archaeological plant oils exist. Plant oils are characterized by high levels of unsaturated fatty acids and are highly susceptible to oxidative degradation. After prolonged burial many vegetable oil residues are dominated by palmitic and smaller amounts of stearic acid making the distribution rather undiagnostic (Evershed *et al.*, 2001).

Recently, palm kernel oil was positively identified in several vessels from a Nubian site (Copley *et al.*, 2001). These vessels were characterized by high amounts of the saturated short chain fatty acids such as lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$), which

are characteristic for palm kernel oil (Table 2.8). The arid environment of Nibia is probably responsible for the survival of these short chain fatty acids, since they are more water-soluble than the longer chain fatty acids. $\delta^{13}C$ analysis indicates that the vessel was contaminated with fatty acids from another lipid source.

6.3.2. Plant Epicuticular Wax

The resistance of waxes to degradation was shown through the identification of epicuticular leaf wax of *Brassica oleracea* (cabbage) in several medieval cooking vessels (Evershed *et al.*, 1991). In these pots considerable quantities of *n*-nonacosane, nonacosan-15-one and nonacosan-15-ol were detected. The distribution of these waxen residues was similar to the waxes of present day *Brassica oleracea* species. It is generally very difficult to observe the utilization of leafy vegetables by early societies since their residues are very fragile. Lipid analysis is probably the only technique that enables scientists to identify them.

6.4.3. Terpenoids

The terpenoids are the largest family of natural products, with more than 23 000 identified. They are the secondary products of plants, having very specific functions and are therefore produced in small quantities. In the past they have been used by humans for wide range of purposes including perfumes, phsyoactives, medicines, flavors, adhesives, and waterproofing agents (Pollard and Heron, 1996; Lampert *et al.* 2002). They occur in all parts of plants but are often extruded as the major component of resins. The terpenoids are composed of an integral number of isoprene units (C_5H_8), which is 2-methylbutadiene. Subsequent modifications of the structure by rearrangements and/or functional group alterations, give rise to a wide range of terpenoids.

Compared to many other organic materials found in the archaeological record, the terpenoids generally show good preservation (Eglinton and Logan, 1991). However, degradation products are often present and the lower molecular weight terpenoids (monoterpenoids and sesquiterpenoids) are often lost due to their volatility. Despite these problems, the terpenoids can be extracted easily by solvent, can be separated by GC and

are identified by their characteristic molecular fragmentation using GC/MS (Mill and White,1994; Pollard and Heron, 1996). They can be excellent biomarkers, if their biosynthesis is limited to a few plants.

Plants rarely biosynthesize both diterpenoids and triterpenoids, so by a simple GC analysis and comparison with the authentic samples, it may be possible to identify the type of resin or if mixing has occurred. However, for complete identification of molecular components GC/MS is required. By heating raw plant materials it is possible to process pitches and tars and they both may also be identified. However, degradation processes can change the composition, and identification at species level may not always be possible (Pollard *et al.* 2007).

6.4.3.1. Diterpenoids: amber, pine resins and pitches. Amber is a fossilized resin, and contains polymerized monoterpenoids and diterpenoids. Although some free terpenoids may be solvent extracted and identified by GC/MS, the polymerized component has too high a molecular weight for such analysis. IR spectroscopy has been used to identify provenance amber (Beck, 1995). *Pinaceae* resin is composed of diterpenoids. The trees which produced amber-forming resins are now extinct. *Pinaceae* resins do not polymerize that makes the terpenoids suitable for solvent extraction and GC/MS analysis (Pollard *et al.*, 2007). Trees may produce as much as five kilograms of resin per year, making *Pinaceae* resin useful resource for trade. Pitch, on the other hand is produced by the destructive distillation of the wood and also has been recovered from archaeological contexts. By using GC and GC/MS, it is possible to identify both pitch and resin. These materials have been used to seal porous pottery such as amphorae, probably to facilitate transport (Heron and Pollard, 1988), and also to waterproof ships (Evershed *et al.*, 1985).

6.4.3.2. Triterpenoids: birch bark tar and *Pistacia* resin. Birch bark tar has been reported from the Middle Paleolithic to Modern times in Europe (Heron and Pollard, 1996). The uses of birch bark tar include hafting, waterproofing, caulking, repairing, and even reported as a 'chewing gum' (Aveling and Heron, 1999). Although the process has not been fully understood, the birch bark tar is thought to have been produced by the destructive heating of birch bark in a sealed container (Pollard *et al.*, 2007). The bark of birch trees (*Betula*) contains variety of diagnostic triterpenoid compounds including betulin, betulone,

lupenone and lupeol (Ekman, 1983; Cole *et al.*, 1991 Fig. 6.6). The presence of these and other components will identify residues as birch bark tar. Such materials have been identified using GC and GC/MS by a number of workers (Binder *et al.*, 1990; Charters *et al*, 1993; Regert, 1997; Regert *et al.* 1998). Due to the presence of components not found in modern birch bark tar, mixing of birch bark tar with animal fat (Regert *et al.* 1998; Dudd and Evershed, 1999) has also been reported.

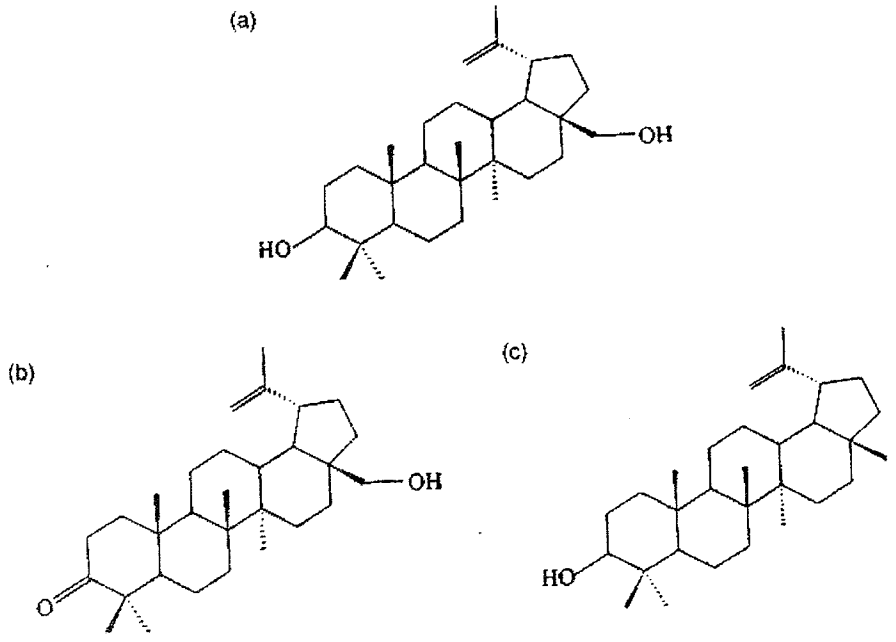


Figure 6.6. Some diagnostic triterpenoid compounds from birch bark tar: a-) betulin ($C_{30}H_{50}O_2$), b-) betulone ($C_{30}H_{49}O_2$), C-) lupeol ($C_{30}H_{50}O$)

Resin from the *Pistachio* species is also known as mastic, Chios terpentine and terebinth (the word terpenoid is derived from this). The genus *Pistachio* has four species (*atlantica*, *khinjuk*, *lentiscus*, and *terebinthus*), which may have been available in the Mediterranean for the production of resin (Pollard *et al.*, 2007). However, it is not possible to distinguish the species based on composition, so evidence for the species used relies on modern evidence, such as amounts of resin produced by each species and their geographical distribution. The resin is largely composed of triterpenoids, the chemical composition of which has been studied by a variety of workers all cited in Stern *et al.*(2003). The major components (Figure 6.7) are moronic, oleanonic, *iso*masticadienonic, and masticadienonic acids (isomers of one another). The characteristic composition and

distribution of triterpenoids, especially in comparison to modern resins, enable the identification of *Pistacia* resin when found as a residue in an archaeological context (Mills and White, 1989). Such analyses allowed the resins in Canaate amphorae from Bronze Age shipwreck at Ulu Burun, of the South coast of Turkey, to be identified as originating from genus *Pistacia* (Mills and White, 1989). Stern *et al.* (2003) have also identified compositional variations between unheated and heated resins- presumed to be the result of incense burning.

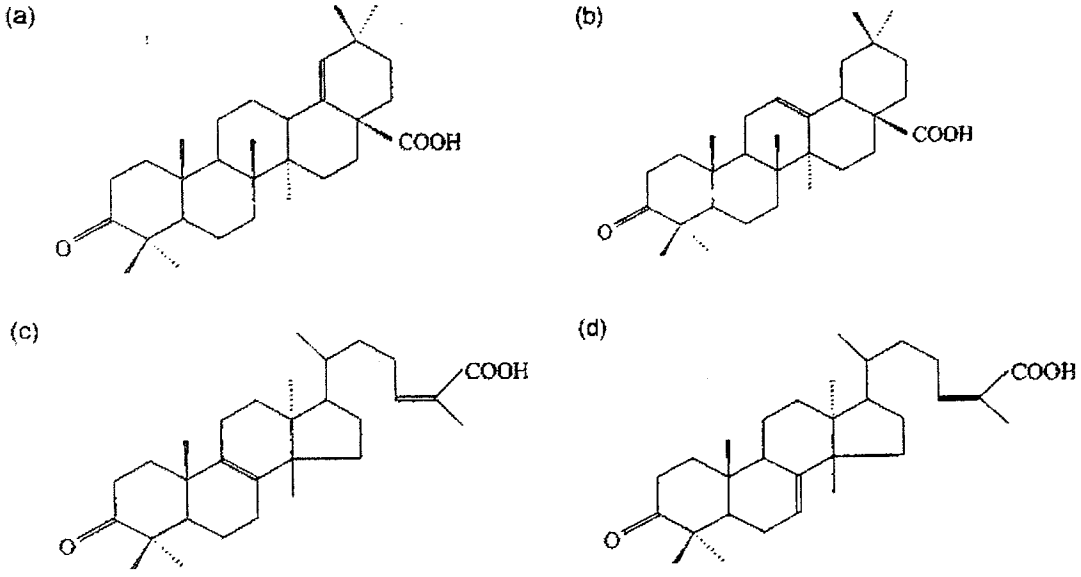


Figure 6.7. Some triterpenoid compounds found in mastic (*Pistacia* resin): a-) moronic acid, b-) oleanonic acid, c-) *iso*masticadienonic acid and d-) masticadienonic acid

6.5. Wine and beer

The lipid content of most alcoholic beverages is very low and as such their former presence in vessels is rarely confirmed by residue or lipid analysis. The presence of wine was shown by the identification of the calcium salt of tartaric acid in a Neolithic vessel by McGovern *et al.*(1996). Tartaric acid, naturally occurring mainly in grapes, was converted to insoluble salts in the calcareous environment of the Neolithic site. Also, analytical techniques such as spot tests, IR spectroscopy (McGovern and Michel, 1996; McGovern, 1997) and chromatography (TLC, GC, HPLC: Badler *et al.* 1990; Formenti and Duthel, 1996) were applied. However, many of these lack selectivity and sensitivity and, without use of separation and identification techniques e.g. GC/MS and HPLC/MS,

misinterpretation of degradation products and contamination leads to false positive results (Murray *et al.*, 2000). More recently the biomarker approach (tartaric acid- a wine marker rarely found in nature other than grapes- and syringic acid derived from malvidin from red wine) has been more convincingly applied with HPLC-MS/MS separation and identification (Gausch-Jane *et al.*, 2004).

The same researchers claimed that also finding of relics of ancient beer via the identification of calcium oxalate in ceramics from Mesopotamia and from the 'tomb of King Midas' in central Turkey (McGovern *et al.*, 1999). As oxalic acid is also present in several vegetables and fruits (Belitz and Grosh, 1999), as such it cannot be accepted as biomarker of beer.

6.6. Thermal Degradation Products of Lipids

During the analyses of lipid extracts of archaeological pottery a range of unusual compounds have been identified that were suspected to be thermal transformation products of lipids formed during vessel use. These observations have required detailed experimentation to determine the conditions required for the various transformations. It is difficult to distinguish between oxidation products (e.g. dihydroxy-, hydroxyl-, and diacids of unsaturated fatty acids since these can form naturally (Regert *et al.*, 1998; Ailaud 2001) though such reactions will occur more rapidly at elevated temperatures (Copley *et al.* 2005). However, the following examples provide evidence for thermal transformations at high temperature during vessel use.

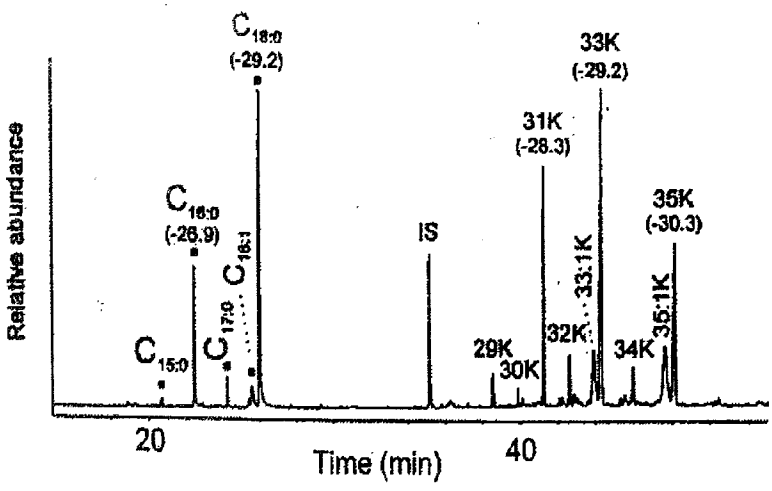


Figure 6.8. Partial gas chromatogram of total lipid extract from Bronze Age cooking vessels. (Evershed *et al.* 2002)

A significant number of lipid extracts of cooking vessels that have been heated display series of ketones eluted in the same region of chromatogram as the leaf wax components. In Figure 6.8 peak identities are as follows: $C_{16:0}$, $C_{18:0}$, etc indicate saturated fatty acids; $C_{18:1}$ indicates monounsaturated fatty acids: 31K etc., are long-chain ketones; IS= 5α-cholestane added as internal standard. The negative numbers on the peaks are the $\delta^{13}C$ values of the individual compounds. Beneath the chromatogram is shown the reaction conditions for the formation of the saturated ketones from the major fatty acids so the obvious interpretation is that these compounds also derive from processing plant products. However, the distribution of ketones of this type has never been seen in nature and so an alternative source had to be considered. Indeed, a reaction involving a free radical induced dehydration and decarboxylation, has been reported to occur for variety of carboxylic acid salts at temperatures generally in excess of 400° C (March 1977). Laboratory experiments have been performed using either triacylglycerols or free fatty acids in the presence of fired clay (Raven *et al.* 1997). Such experiments were performed on a small scale in order to be able to control the experimental conditions. The most effective heating experiments involved the use of capillary reaction tubes filled to circa 4 cm with mixture of ceramic and reagents then topped up with glass beads. Heat was supplied by a micro-burner to the sealed end with thermocouple introduced to monitor the temperature reached. The heating was stopped once condensate appeared on the glass beads. Ketones were formed under a variety of conditions with optimum formation occurring with a ceramic matrix present and high temperatures (350-450° C). These conditions might be provided during the heating of

fats at high temperatures in ancient pottery vessels. These findings show that caution must be exercised in interpreting the origins of long-chain ketones in archaeological pottery (Evershed *et al.*, 1995)

7. ANALYTICAL TECHNIQUES FOR THE ANALYSIS OF ORGANIC RESIDUES

Early investigation of organic residues were limited to the determination of physical properties of organic residues using simple tests such as melting points and solubility measurements since suitable analytical techniques were not available (Lucas and Harris, 1962). Identification of compounds became even more limited as the complexity of the substances under investigation increased. It was further complicated by effect of decay over archaeological time. The advent of modern instrumentations in chemical and biochemical techniques eventually provided the effective recovery, detection and characterization of biomolecules and their decay products in archaeological materials.

Elemental and isotopic characterization of organic samples is obtained by using a destructive technique based on the gas chromatographic analysis of the combustion gases from small amounts of sample. Elementary composition of carbon, hydrogen and nitrogen (CHN) indicates what fraction of the sample is organic and suggests possible chemical composition of the material. The C/N ratio indicates the protein fraction in the material where as the C/H ratio illustrates the degree of saturation and condensation of material. This technique was successfully applied to determine what percentage of total organic material present in ground ceramic material that could be extracted using different preparation steps (Craig *et al.*, 2004).

The bulk stable isotope ratio analysis (SIA) of stable nitrogen and carbon isotopes $^{15}N/^{14}N$ and $^{13}C/^{12}C$ respectively, may yield information about the source of the residue, namely animals, fish or plants (Morton and Schwarcz, 2004). However, multiple use of a ceramic vessel for the processing different foodstuff often limits the applicability of this technique due mixing. This technique, on the other hand, has been shown to be useful in the study of human remains, bones, dental materials and identifiable plant remains. Compounds-specific- isotope analysis of individual lipids or amino acids using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) has been shown to be thre technique for the identification of the origin of particular biomarkers.

Chemical characterization of complete samples can also be obtained through the application of non-destructive spectroscopic techniques such as Fourier Transform Infrared (FTIR) spectroscopy or solid- state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy.

FTIR is based on the light absorption characteristic wavelengths in the infrared region of electromagnetic spectrum by various organic compounds in the extracted residue. The presence or absence of certain absorption peaks indicates the nature of particular bonds and/or functional groups. It is a rapid analytical technique ideal for the initial classification of organic residues (Colombini *et al.*, 2003; Regert *et al.*, 2003). General identification of the components of the samples can be made by comparison with reference materials. FTIR can rarely be used, however, for detailed identification of complex mixtures because increasing the complexity of the analyzed sample results in decreased resolution and the loss of identification potential (Hill and Evans, 1988). In addition, FTIR is insensitive to components present in the residue that are less then five per cent. It is limited in its capacity to distinguish between samples containing different proportions of similar compounds. IR data will be complicated by the effects of decay, which yields a range of compounds of slightly differing structure. IR analysis of such mixtures results in an array of absorption bands with similar absorption maxima. These broad peaks with little fine structure complicate comparisons with modern reference materials. Nonetheless, FTIR can often be used to distinguish between broad classes of highly refractory natural products such as resins, tars, pitches or beeswax which can yield characteristic IR spectra even after several thousand years (Nicholson and Show, 2000).

NMR spectroscopy is among the most powerful techniques available to chemists to elucidate the structure of organic compounds. NMR helps to determine the presence of various types of hydrogen and carbon atoms to provide information on the bonds present and to identify and locate the position of oxygen-based functional groups along hydrocarbon chain. However, NMR is most effective only when the individual compounds can be isolated in reasonable quantities between 1000 micrograms and few milligrams in high purity. Beck *et al.* (1974) discusses the use of NMR in the identification of ancient oils and fat containing mixtures of fatty acids. The attention was drawn to the fact that the technique can reveal the average chain-length, unsaturation of fatty acids and degree of hydrolysis of glyceryl lipids. Generally the interpretation of the NMR spectra of complex

mixtures of compounds is difficult. The resonances of the major potentially diagnostic, constituents may be masked by those of more abundant, but less characteristic components.

Solid-state ^{13}C NMR spectroscopy has recently been applied in the field of organic residue analysis (Oudemans *et al.*, 1992; Sheriff *et al.*, 1995). Solid-state ^{13}C NMR spectroscopy has the analytical advantage because it provides quantitative results, and is less sensitive to samples that are not homogeneous compared to FTIR, however, it requires a much larger sample in the order of 10 to 100 mg. Such sample size is not possible from archaeological pottery.

Ultraviolet/visible (UV/vis) spectroscopy has limited application in organic residue analysis. UV/vis spectroscopy is one of the methods of choice of conservators for the analysis of ancient dyes. Application of this technique to the routine analysis of lipids preserved in potsherds is restricted by the lack of strong chromophores.

In order to identify the individual compounds present in mixed organic materials, a separation or fragmentation is often required. Certain classes of compounds, such as lipids, terpenoids, waxes, hydrocarbons and alcohols can be extracted from complex mixture with organic solvent. These extractable compounds can be separated and identified using gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) after appropriate derivatisation or preparative separation.

Condensed materials such as degraded proteins, using thermal fragmentation may fragment caramelized sugars, cross-linked drying oil and partially carbonized materials. Analytical pyrolysis techniques such as Curie-point pyrolysis mass spectrometry (CuPyMS) or Curie-point pyrolysis gas chromatography/mass spectrometry (CuPyGCMS), and Direct Temperature-resolved Mass Spectrometry (DTMS) use a form of controlled heating in an oxygen free environment. The thermal energy causes the macromolecular compounds to split along the weakest bond in the chain into fragments specific to the original molecule.

The most popular technique for analysis of nonvolatile lipids such as triacylglycerols is mass spectrometry with *electrospray ionization* (ESI). Electrospray ionization is a very sensitive analytical technique by which very little fragmentation occurs with the target

molecule. The sample is dissolved in a polar volatile solvent and pumped though a narrow stainless steel capillary tube. A voltage of a few kV is applied to the tip of the tube, and, as the liquid emerges from the tube, it is converted to an aerosol of charged droplets. The whole process is aided by the flow of an inert gas such as nitrogen. A portion of the inert gas is injected around the nebulazing gas, and some is directed across the stream (drying gas). The inert gas serves to aid the production of the aerosol, and promote the drying process. The solvent evaporates from the droplets to leave a stream of charged particles, which pass through a sampling cone, into regions of increasing vacuum, until they enter the analyzer of the mass spectrometer. Mirabaund *et al.* (2007) applied both GC and GC/MS on series of samples to determine the compositions, followed by ESI MS and ESI MS/MS to obtain precise structural information on TAG (triacylglycerol) biomarkers. It was possible to determine the characteristic TAG distributions of goat milk, cow milk and sheep adipose fat.

Recently attention was focused on techniques based on direct mass spectrometry that can reduce sample manipulation. Direct inlet mass spectrometry, direct exposure mass spectrometry (DE-MS) and direct temperature resolved mass spectrometry (DTMS) have demonstrated their potential for characterizing residues in archaeological samples (Colombini *et al.,* 2005; Regert and Rolando, 2002). DE-MS method provides mass spectral fingerprint of organic material in a few minutes, which highlights the main components in the sample. However, it does not give information on the less abundant components. Due to its high sensitivity, selectivity and minimum sample requirement it is suitable for identifying samples from archaeological sources. Surface residues in the range of 1-3 mg are analyzed directly or after dissolution in dichloromethane (1mg/1mL). Aliquot of 1 μL of dissolved sample is deposited on the rhenium filament wire of the direct exposure probe. After the solvent is evaporated, the sample is introduced to the mass spectrometer (Colombini *et al.,* 2005). Colombini and co-workers (2005) were able to recognize mastic resin from genus *Pistachio* from a Roman censer found in Egypt with the help of DE-MS analysis alone.

The usefulness of HPLC/MS lies in its potential for the analysis of mixtures of components that are too nonvolatile or thermally unstable to be analyzed by GC/MS. HPLC uses tubular metal columns typically 25, 15, or 10 cm in length by 4.6 to 2 mm

internal diameter packed with a chromatographic adsorbent. Most commonly used packing material is silica gel or reversed-phase silica (octadecylsilyted silica). In normal phase HPLC the mobile phase is commonly a mixture of organic solvents. Acetonitrile and water are used in reversed phase analyses. The most common methods of interfacing an HPLC to a MS are the electrospray (ESI) and atmospheric pressure chemical ionization (APCI).

8. INSTRUMENTATION AND ANALYTICAL PROCEDURES

8.1. Analytical Procedures

8.1.1. Sampling Protocol:

i-) Handling: Lipid Analysis is based on survival and recognition of molecules common to many sources around us in the contemporary world and fingerprints will deposit lipid molecules such as fatty acids, cholesterol and wax esters onto the surfaces of samples. Therefore, if at all possible handling of the samples intended for analysis should be excluded. To prevent direct handling of the samples trowel or tweezers were used.

ii-) Washing: Pottery fragments intended for organic analysis should not be washed. Washing could dislodge surface residues, remove more labile constituents and introduce contaminants. Cleaning the potsherds with HCl should not be done as this is likely to bring about the removal of organic components. Also pen and varnish on the inner surface of the sherd should be avoided.

iii-) Destructive sampling: Lipid analysis is destructive and is based upon solvent extraction of powdered ceramic absorbed residues or visible residues. Most analysts will leave a portion of the sample for future analysis. A modeling drill fitted with a tungsten abrasive bit (solvent cleaned between samples) can be used to remove a fine powder of 1 mm. All sherd powder are collected on aluminum foil before transferring to a glass vial. The ceramic sample size of circa 2 grams is advisable for use. Visible residues require a very small amount of organic residue for analysis. If it is a pure organic compound, then a pinhead-sized sample will be enough to carry out a complete analysis. In both cases if more sample is spared it gives greater options for duplicate analysis or isotopic analysis. When possible, it is extremely helpful to have more than one sample extracted from the vessel.

iv-) Archaeological considerations: In order to maximize the potential for archaeological investigation, it is extremely helpful to determine the pottery matrix as well as the exact location of the potsherd with respect to the whole vessel such as rim, body or base. It is also equally important to know the well dated stratigraphicaly contexts of the sample. Practices such as boiling are likely to lead to differential deposition of lipid within the wall of the pot. Some potsherds display visible surface deposits on their interior surface. This is often burned organic matter and matrix. Finally, shooting on the outer surfaces of the potsherds/vessels is useful in understanding the activities associated by the vessels and may assist in interpreting the lipid distribution.

v-) Storage: Storage in plastic should be totally avoided since plasticizers can adhere to samples and tend to swamp the generally low levels of organic residues. Therefore, preferably the sample should be placed into solvent washed glass vials or should be wrapped in aluminum foil with proper labeling.

8.1.2. Preparation of the Samples

All of the potsherds from Barcın Höyük are obtained during the excavation. The samples are not washed and are immediately wrapped in aluminum foil. The samples from the other sites, however, were brought to our laboratory. They were washed and considerable hand contact is expected.

All solvents used in this research are of HPLC grade. All glassware are solvent washed with a mixture of chloroform and methanol (2:1 v/v) and dried in an oven. To prevent contamination, foil leafs and tweezers are used to manipulate samples. Modern animal tissues were extracted using different glassware and stored separately from the archaeological samples.

8.1.3. Solvent Extraction Procedure for Archaeological Samples

Two methods are utilized to extact the lipids from potpotsherds:

i) Ultrasone exctraction: The potsherds are ground in a de-greased pestle and mortar in to a fine powder, accurately weighed and transffered to a 20 ml screw-top glass vial with a Teflon-lined cap followed by addition of 20 μg of internal standard *n*-tetratriacontane. The addition of known amount of internal standard will allow the quantitative assessment of the individual eluting components based on simple comparisons of GC peak areas determined by electronic integration. The amount of internal standard can be varied according to the demands of particular analyses (Evershed *et al.*, 1990). Organic residues are extracted using chloroform:methanol (10 ml, 2:1 v/v) by ultrasone twice for 15 minutes. The extract is then centrifuged at 2500 rpm for 20 minutes. and the supernatant, comprising the TLE (total lipid extract), removed and transferred by using pasteur pipette to a solvent washed screw-top glass vial with with Teflon lined cap. The TLE is then filtered through a column containing 1 gram of silica to remove any particulate matter. The solvent is then evaporated under a gentle stream of nitrogen. The dried total lipid extracts (TLE) are stored in freze at – 20°C until HTGC and GC-MS analysis. Extracts that may contain acylglycerol components are hydrolysed to yield free fatty acids followed by derivatisation before GC and GC-C-IRMS analysis.

ii) Soxhlet extraction: Approximately, 5 grams of sample is weighed and transferred with aid of aluminum foil into a 100 ml solvent washed round bottom flask. Then, 20μl of *n*-tetratriaconatane is added as an internal standard. The lipids are extracted with a soxhlet apparatus with 60 ml of chloroform and methanol (2:1 v/v) for 12 hours. The soxhlet thimbles are pre-extracted before sample extraction in order to avoid contamination that might come from the thimble and the soxhlet system. The solvent is then evaporated in rotary evaporator at 40°C under vacuum until 2 ml solution is left and then transferred with Pasteur pipette to a small pre-weighed 2 ml screw top glass vial with Teflon-lined cap and the remaining solvent is removed under gentle stream of nitrogen. The dried total lipid extracts (TLE) are stored in a in freezer at – 20°C until HTGC and GC-MS analysis.

Sometimes highly carbonized residues were observed on the surface of the potsherds. These residues were scraped from the surfaces of the potsherds using scalpel and powdered using agate mortar. Same extraction procedure was applied.

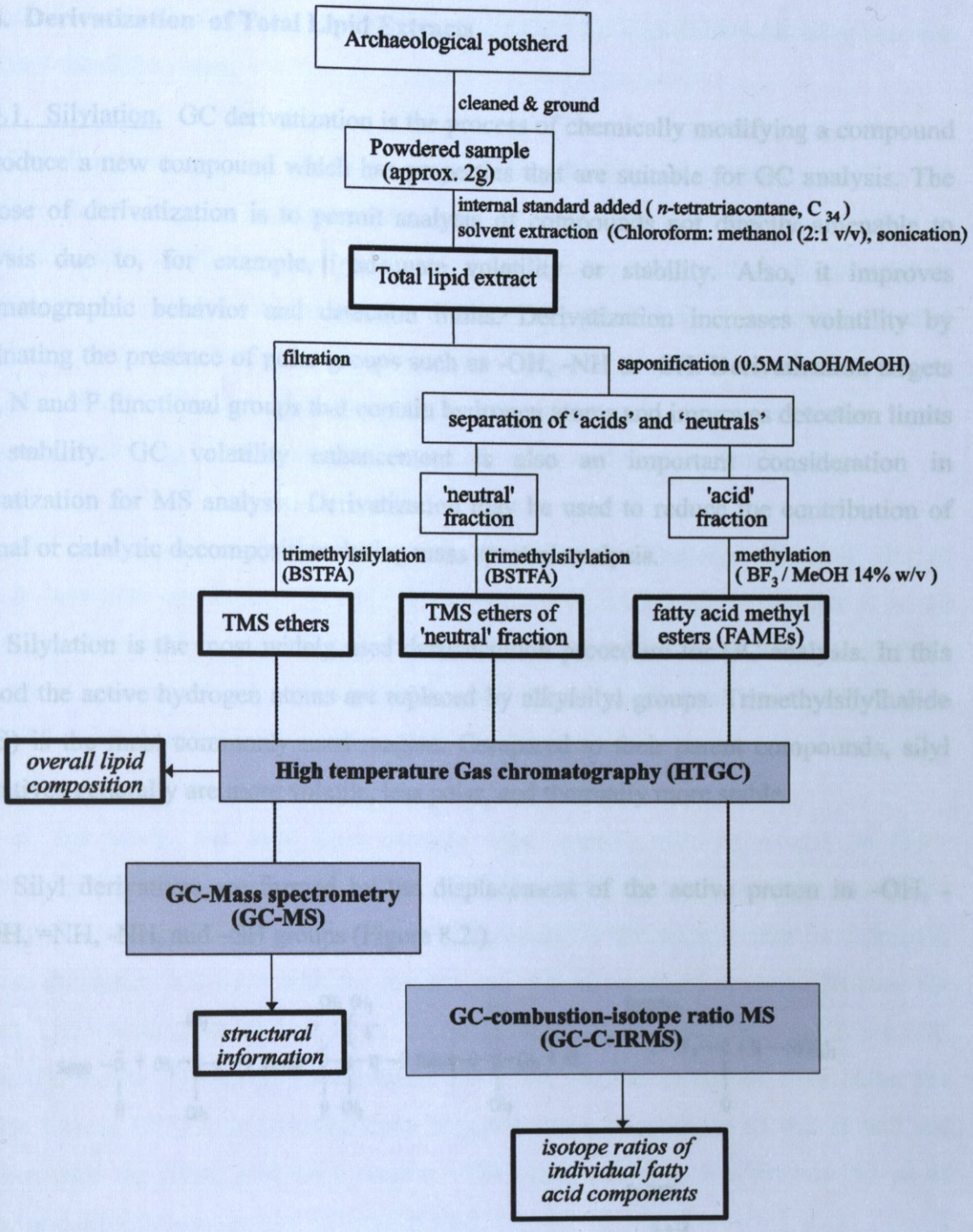


Figure 8.1. Flow diagram for the analytical protocol used in THA lipid extract from archaeological potsherds (Dudd,1999).

8.1.4. Derivatization of Total Lipid Extracts

8.1.4.1. Silylation. GC derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for GC analysis. The purpose of derivatization is to permit analysis of compounds not directly amenable to analysis due to, for example, inadequate volatility or stability. Also, it improves chromatographic behavior and detection limits. Derivatization increases volatility by eliminating the presence of polar groups such as -OH, -NH or -SH. Derivatization targets O, S, N and P functional groups that contain hydrogen atoms and improves detection limits and stability. GC volatility enhancement is also an important consideration in derivatization for MS analysis. Derivatization may be used to reduce the contribution of thermal or catalytic decomposition during mass spectral analysis.

Silylation is the most widely used derivatization procedure for GC analysis. In this method the active hydrogen atoms are replaced by alkylsilyl groups. Trimethylsilylhalide (TMS) is the most commonly used reagent. Compared to their parent compounds, silyl derivatives generally are more volatile, less polar, and thermally more stable.

Silyl derivatives are formed by the displacement of the active proton in –OH, -COOH, =NH, -NH, and –SH groups (Figure 8.2.)

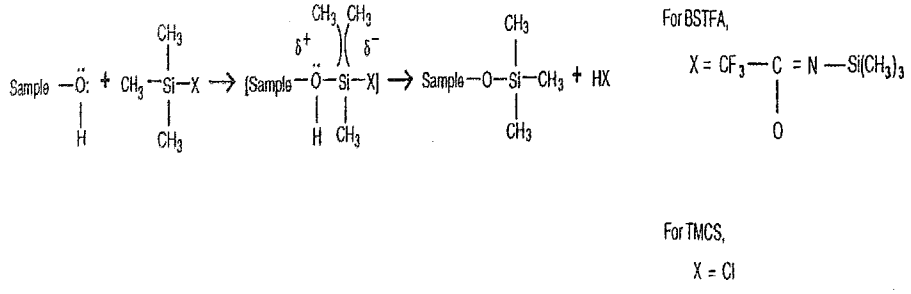


Figure 8.2. Derivatization of organic compounds containing –OH groups with TMS reagent

The reaction proceeds via a nucleophilic attack on the silicon atom of the silyl group by nucleophilic oxygen or nitrogen atom via a biomolecular transition state. The leaving group (X) on the silyl group must posses lower basicity, to stabilize a negative charge in

the transition state, and should have little or no tendency for π (p-d) back bonding between itself and the silicon atom.

The ideal leaving group (X) on the silyl compound must be such that it is readily lost from the transition state during reaction, but possesses sufficient chemical stability in combination with alkyl silyl group to allow long-term storage for the derivatization agent. Since the formation of the transition state is reversible, the derivatization will proceed to completion only if the basicity of leaving group X exceeds that of the group it replaces. The ease of derivatization of various functional groups with a given sylilating agent follows the following order: alcohol>phenol>carboxylic acid>amine>amide.

There are a number of advantages of trimethylsilylation over saponification. First of all it is less time consuming. When a large number of archeological samples is to be derivatized, this method provides definite advantage. Secondly, it retains the integrity of the original lipid extract, which is not always possible in saponification, and finally there is no need for wet chemical treatments by which possible contaminations are prevented.

In this study, the total lipid extracts were treated with an excess of N,O-bis(trimethylsilyl)trifluoroacetamide (Figure 8.2), which contains one per cent v/v trimethylchlorosilane. Very small amount of moisture in the sample can be tolerated, because the water will react with the reagent and will be removed chemically from the system. The reaction is carried out in a clean screw top bottle after the components are well mixed. The bottle is heated in a sand bath at 70° C for approximately one hour. After the reaction excess BSTFA is removed under a gentle steam of nitrogen so that it will not interfere with the flame ionization detector. The sample is then dissolved in 50 μl of hexane and directly analyzed by GC and GC/MS.

8.1.5. Preparation of Fatty Acid Methyl Ester Derivatives (FAME)

Before the preparation of FAME, the TLE must be saponified to liberate any mono-, di- and triacylglycerols that may be present. For saponification 2 ml of five per cent (w/v) methanolic sodium hydroxide is used. The mixture is heated in a sand bath at 70°C for one

hour. The solution is neutralized by 3M HCl and the fatty acids are extracted two times by 3 ml portions of hexane. The solvent is removed by rotary evaporation.

FAMEs are prepared by reaction of fatty acids with BF_3-methanol (14 per cent w/v), at 70°C for one hour. The methyl ester derivatives are extracted with diethyl ether or chloroform and the solvent is removed under nitrogen gas. No kinetic isotope effect is associated with this derivatization because the reaction is rapid and quantitative with regard to carbonyl group (Dudd, 1999). FAMEs are re-dissolved in hexane for analysis by GC and GC-C-IRMS.

8.1.6. Preparation of Analytical Blanks

Analytical blanks are prepared with each sample batch during all lipid extraction and derivatisation procedures to monitor for any possible sources of contamination.

The blanks are prepared following the same procedures used for the extraction of potsherds as described in sec. 8.1.3.

Also a blank containing only internal standard and derivatization reagents as well as blank containing only distilled solvents were prepared in order to check for contaminants that may be present in these reagents.

8.1.7. Solvent Extraction of Modern Reference Fats

About 2 g of adipose tissue is placed in a conical flask and 20 ml mixture of chloroform and methanol (2:1 v/v) is added and kept in ultrasone bath twice for 15 minutes. The extract is filtered through sodium sulphate to remove residual water, and the bulk of the solvent is removed using a rotary evaporator. The remaining solvent is removed under a gentle stream of nitrogen and the TLE is stored at - 20°C until analysis (Dudd, 1999)

8.2. Instrumentation

8.2.1. Gas Chromatography (GC)

Gas chromatography (GC) is the most widely used analytical technique for the separation, identification and quantification of volatile organic compounds. The resolution is sufficient to routinely separate the components of archaeological residue samples, such as homologous series, saturated and unsaturated fatty acids, terpenoids, triacylglycerols and other components. In essence, the GC technique involves the separation of mixtures of volatile or semi volatile organic compounds according to the differences in their partition coefficients between stationary phase and a mobile inert gas phase. Generally nitrogen or helium is used as the mobile phase carrier gas, which passes through a capillary column made of inert and highly flexible fused silica. Modern capillary columns are between 15 to 60 meters long with an internal diameter between 0.2 to 2 millimeters. Such columns allow a high resolution or separation of compounds in complex mixtures. The stationary phase is a thin film of high boiling liquid coated onto the interior surface of the column (Christie, 1989). The stationary liquid phase in a gas-liquid chromatographic column should have the following properties:

a-) should have low volatility such that the boiling point of the liquid phase should be at least 100°C higher than the maximum operating temperature for the column.
b-) Should be thermal stable.
c-) Should be chemically inert.

Compounds must haves some degree of compatibility or solubility with the liquid stationary phase in order to have a reasonable residence time in the column. The principle of 'like dissolves like' applies, where 'like' refers to the polarities of the solute and the stationary liquid phase (Skoog *et al.* 1998).

In the gas chromatographic analysis, the column is heated in an oven. In an isothermal operation the temperature of the oven is fixed. However, if separation of components with a wide range of molecular weights is necessary, the column temperature is programmed to

increase over a time period. This is known as the temperature programming or gradient operation. The sample is dissolved in a small quantity of volatile solvent and about 1 micro liter of this solution is injected by using special syringe through the injector port of the instrument. There are two common types of injector port. In the on-column injector, the fine needle of the syringe penetrates into the end of the column allowing the whole sample to be separated in the column. There is no discrimination between the components. In the split-split less injector system, however, the sample is injected into a lined cylinder where the carrier gas sweeps some of the sample into the column. The rest of the sample is swept out of the instrument. The ratios of splitting of sample can be changed, and this technique is commonly used for unknown or 'dirty' samples. The vaporized sample moves though the column with the carrier gas. Its various components are separated according to differences in volatility, molecular weight, functional groups, number and position of double bonds, and their affinity for the stationary phase. More volatile substances move through the column faster. The separated compounds are than detected and quantified at a detector as they emerge from the column. Flame ionization detector (FID) is generally used for lipid analysis. It has a highly sensitive response to almost all organic compounds, and also has a low signal to noise ratio which can detect even the trace quantities of materials as low as 10^{-12} g/ml. Organic compounds moved by the carrier gas will produce ions at the FID detector causing a current flow which is proportional to the quantity of sample in the eluent. A gas chromatogram is obtained when the current in mV is plotted against time where the peaks observed will reflect their elution order from the column. Generally low molecular weight components with low polarity have shorter retention times and elute first. Fatty acids with short or branched carbon chains will elute before the saturated, straight chain fatty acids with the same number of carbon atoms (Christie, 1989).

GC, however, cannot be used to directly identify the molecular species present in the sample. It is, however, possible to infer their identity by comparison with known standards. One can determine the retention time of the known standard first and then compare it with that of the unknown compound. A more secure method is to analyze the unknown sample, and then spike the sample with a known amount of the standard. If the standard corresponds to one of the components in the mixture, a subsequent analysis will then show a corresponding increase in peak areas.

8.2.1.1. Quantification. The response of the GC detector is proportional to the amount of component eluted and therefore it is possible to quantify the components. In practice, however, quantification is carried out by adding a known amount of a standard to a weighted sample and then compare the peak area of the standard with those of the unknown peaks. The internal standard should be very pure, and should not co-elute with the components of the sample. In addition, it should not be volatile so that it is not preferentially lost before the unknown peaks or insufficiently soluble so that it does not dissolve in the solvent. The most common internal standard used in lipid analysis is C_{34} *n*-alkane. Usually, the standard is added immediately prior to analysis by GC, however, it is also possible to add the standard before extraction step. This method enables one to estimate the efficiency of the extraction protocol. Although the area of the peak is proportional to the quantity of the eluted components, the detector does not have exactly the same response to all functional groups or even to homologous series. Therefore, it is preferable to use an internal standard of similar functionality and molecular weight with that of the unknowns. It is also possible to measure the relative response factors of different molecules, by making test solutions for a variety of standards of known weights and then comparing the peak areas.

The sensitivity factor (SF_e) for compounds (e) such as free fatty acids, mono-, di-, and triacylglycerols is determined with respect to the *n*-tetratriacontane internal standard (i) is calculated by using equation 8.1. In equation 8.1, G is the weight of the compound in grams and A is the area of the chromatographic peak.

$$SF_e = (G_e * A_i) / (G * A_e) \quad (8.1)$$

The amount of the component of interest in the lipid extract is calculated by using the equation 8.2. In this equation m_e is the weight of a compound of interest in the lipid extract and m_i is the weight of internal standard added to the lipid extract.

$$m_e = SF_e * A_i / A_e * m_i \quad (8.2)$$

8.2.1.2. GC parameters. Unicam 610 gas chromatograph equipped with a Flame Ionization Detector (FID) and split/split less injection system is used this study. The analysis is performed on a 30m x 0,32 mm inner diameter fused silica capillary column coated with five per cent dimethyl polysiloxane (CEC-5) stationary phase with 0.25μm film thickness. Samples are dissolved in an appropriate volume of hexane and 1-1.5 μl quantities are used for injection. Samples are introduced into an injector set at 250 °C where they are evaporated before they enter the column. Nitrogen is used as the carrier gas at a column head pressure of 30 psi. Column temperature programming is used. Sample is injected when the initial oven temperature is set at 50°C. After injection, the GC oven temperature is held isothermally for two minutes. It is then programmed to increase at a rate of 10°/min up to 340°C. The run is stopped after waiting for 20 minutes at 340°C. The flame ionization detector is set at 350°C.

Before the analysis the column is conditioned by injecting trimethylsilylation (TMS) reagent until stable baseline is attained. For conditioning the column is maintained at 200 °C and approximately 10-50 μl portions of (TMS) is injected at two to three minutes intervals until the conditioning is completed. After the final injection the column temperature is increased to 350°C maintained at this temperature for 5-10 minutes which is the maximum recommended temperature for this column.

8.2.2. Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS is an instrumental technique, where a gas chromatograph (GC) is coupled to a mass spectrometer (MS). The system allows the separation, identification and quantification of complex mixtures (Figure 8.3). Gas chromatography is the most widely used sample introduction technique for the mass spectrometric analysis of organic materials in archaeology and art. The compounds to be analyzed by GC/MS must be sufficiently volatile and thermally stable. In addition, compounds with certain functional groups may require chemical modification by derivatization prior to analysis in order to eliminate undesirable adsorption effects that would otherwise affect the quality of the data obtained.

The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (Figure 8.3). The components flow though the column and the compounds are separated by virtue of their relative interaction with the stationary phase on the column. The end of the GC column is located at the heated transfer line which is the entrance the to ion source of the mass spectrometer. Here the individual compounds eluted from the column are ionized. The temperature of the transfer line between the GC and MS must be maintained at or slightly above the maximum temperature of the GC column in order to avoid any condensation of components.

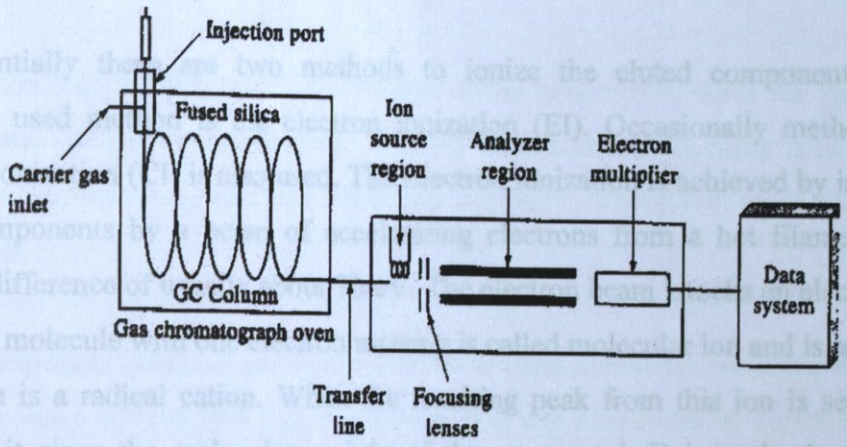


Figure 8.3. Schematic diagram of GC/MS (Skoog *et al.*, 1998)

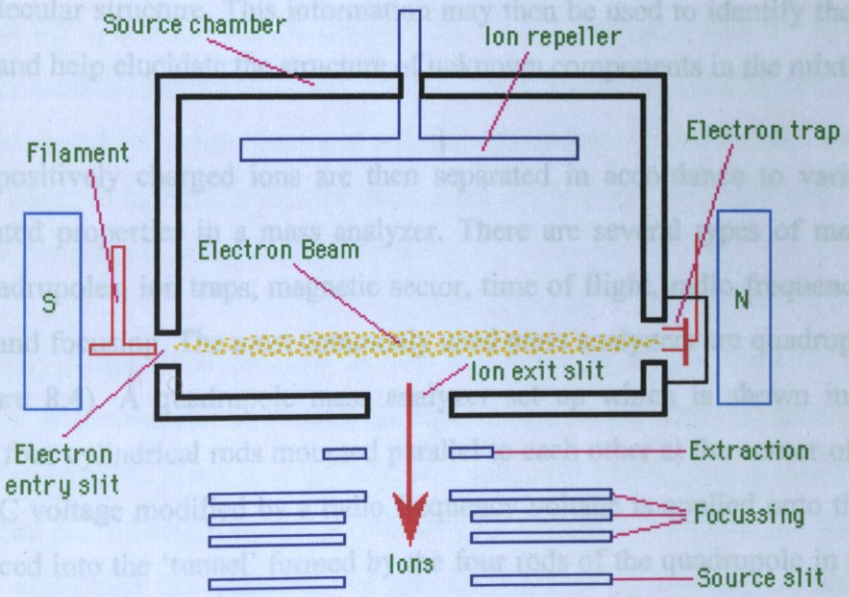


Figure 8.4. Schematic diagram of an ion source

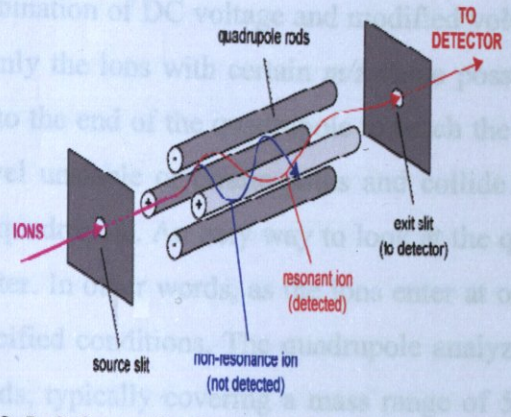


Figure 8.5. Schematic diagram of a quadrupole analyser

Potentially there are two methods to ionize the eluted components. The most frequently used method is the electron ionization (EI). Occasionally method known as chemical ionization (CI) is also used. The Electron ionization is achieved by interacting the eluted components by a beam of accelerating electrons from a hot filament through a potential difference of usually about 70 eV. The electron beam knocks an electron from the sample. A molecule with one electron missing is called molecular ion and is represented by M^+, which is a radical cation. When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Due to the large amount of energy imparted on to the molecular ion it usually fragments producing further smaller ions with characteristic relative masses and abundances that provide a 'fingerprint' of the related molecular structure. This information may then be used to identify the compounds of interest and help elucidate the structure of unknown components in the mixtures.

The positively charged ions are then separated in accordance to various mass to charge related properties in a mass analyzer. There are several types of mass analyzers such as quadrupoles, ion traps, magnetic sector, time of flight, radio frequency, cyclotron resonance and focusing. The most commonly used mass analyzers are quadrupoles and ion traps (Figure 8.4). A quadrupole mass analyzer set up which is shown in Figure 8.5. consists of four cylindrical rods mounted parallel to each other at the corner of a square. A constant DC voltage modified by a radio frequency voltage is applied onto the rods. Ions are introduced into the 'tunnel' formed by the four rods of the quadrupole in the center of the square at one end to the rods, and they travel along the axis.

For any given combination of DC voltage and modified voltage that is applied at the appropriate frequency, only the ions with certain *m/z* value posses a stable trajectory are able to pass all the way to the end of the quadrupole to reach the detector. All atoms with different *m/z* values travel unstable or erratic paths and collide with one of the rods or forced to go outside the quadrupole. An easy way to look at the quadrupole mass analyzer is like a tunable mass filter. In other words, as the ions enter at one end, only one *m/z* ion will pass through at specified conditions. The quadrupole analyzer is generally proffered due to its fast scan speeds, typically covering a mass range of 50-700 am. This range is sufficient for most organic samples that are suitable for GC separation.

The ion trap is considered as a variant of the quadrupole, since the appearance and the operation of the two are related. The ion trap, however, is more sensitive than the quadrupole arrangement, and the ions are routinely configured to carry out tandem experiments.

Ion trap mass analyzer generally consists of three electrodes, one ring electrode with hyperbolic inner surface and two hyperbolic end cap electrodes at either end. The ring electrode is operated with sinusoidal radio frequency field while the end cap electrodes are operated in one of three modes. First, when the ion trap is operated with a fixed RF voltage without any DC bias between the end cap and ring electrodes. In this set up all ions above a certain cut off *m/z* ration will be trapped. As the RF voltage is raised, the cutoff *m/z* is increased in a controlled manner and the ions are sequentially ejected and detected. The result is the standard mass spectrum and this procedure is called the 'mass selective instability' mode of operation. Ions of mass contained beyond the upper limit are removed after the RF potential is brought back to zero.

One major difference between GC/MS and other mass spectrometric techniques is that complex molecules can be ionized. In some instances the resulting molecular ion may be stable, but in most cases it is not and will split into neutral and a positively charged fragment. The neutral fragment will be removed by the vacuum pumps, but the positive ion with lower mass than the molecular ion will be analyzed by the mass spectrometer. The result is a complex spectrum of masses plotted against the intensity of each ion produced by many different fragments of the molecular ion. This yields a reproducible, predictable

and characteristic fingerprint of each component, as they elute from the GC column. Since the mass spectrometer must scans across a mass range many times as the components elute from GC column, it is possible to view the data in a number of different ways. The most common method is to scan the entire mass range and to combine the scans to produce a mass spectrum. This yields a total ion current (TIC), which is a plot of detector response for all masses against retention time and is therefore similar to a GC chromatogram, and can be used to compare samples run on both instruments. Another application is to produce a mass chromatogram, where only selected masses are plotted against the retention time. This can be useful to identify molecules with known ions fragment and homologous series. For components of low abundance, it is possible to set the mass spectrometer to measure only certain masses and therefore increase sensitivity. This is known as selected ion monitoring. The computerized data analysis systems can eventually compare an unknown mass spectrum to a library of previously recorded mass spectra. It is very use for archaeological purposes to assemble a personal library of mass spectra. However, experience of the literature of related molecules and data from known modern samples or standards are also useful.

8.2.2.1. GC/MS parameters. In this study the GC/MS analysis is carrier out by a Finnigan quadrupole mass spectrometer. The GC/MS is operated with the ion source at 200°C where the emission current set at 400μA and the electron energy at 70 eV. The GC/MS interface is maintained at 250°C. The Spectra are recorded over a range of *m/z* 40-760 every 1,5 s. The data are acquired are processed by Finnigan INCOS data system. The GC has a 30 m x 0.25 mm i.d. column with a five per cent polysilarylene and 95 per cent polydimethylsiloxane at 0.25μm film thickness. All the other GC conditions are the same as those described above (SECTION except that helium is used as the carrier gas. Compound identifications are based on GC elution order and comparisons with reference spectra from computerized library.

8.2.3. Gas Chromatography-Combustion- Isotope Mass Spectrometry (GC-C-IRMS)

Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) is a highly specialised instrumental technique used to ascertain the realative ratios of stable isotopes of carbon ($^{13}C/^{12}C$), hydrogen ($^{2}H/^{1}H$), nitrogen ($^{15}N/^{14}N$) or oxygen ($^{18}O/^{16}0$) of

individual compounds that are separated from complex mixtures. The ratio of these isotopes in natural materials varies slightly as a result of isotopic fractionation during physical, chemical and biological processes. Relative isotope ratios of stable elements of specific compounds may be being highly diagnostic in understanding some of the key environmental processes. Furthermore, growth substrates incorporating compounds that are artificially enriched in the heavier isotope can aid in deconvolution of often highly complex and obscure biogeochemical pathways. The primary prerequisite for GC-C-IRMS analysis is that the compounds in the mixture should be amenable to GC, i.e. they should be suitably volatile and thermally stable. Polar compounds may require further chemical modification such as derivatization and in such cases the relative stable isotope ratio of the derivatization agent must also be considered.

Figure 8.7. depicts a schematic of a typical GC-C-IRMS instrument based on ThermoElectron Delta XP model. The sample solution is injected into the GC inlet where it is vaporized and swept through the chromatographic column by the carrier gas which is usually helium. The components in the mixture are seperated as described in section 8.2.1. The individual compounds that are eluted from the chromatographic column are oxidatively combusted when they pass through a combustion reactor composed of an aluminum tube containing Cu, Ni and Pt wires which are maintained at 940 °C. The process is then followed by a reduction reactor with an aluminum tube containing three Cu wires that are maintained at 600 °C in order to reduce any nitrogen oxides to nitrogen (Figure 8.6). For hydrogen and oxygen a high temperature thermal conversion reactor is required which is not shown in the scheme. Water is then removed in a water separator by passing the gas stream through a tube constructed from a water permeable nafion membrane. The sample is then introduced into the ion source of the MS by an open split interface.

Ionization of the analyte gases (CO_2, H_2, N_2 or CO) is achieved by using an electron ionization system as described in section 8.2.2. The ionized gases are separated in a single magnetic sector analyzer by virtue of their momentum and are detected by an array of Faraday cups at the output where the final ratios of the stable isotopes are calculated. The calculations are based relative to a standard of known isotopic composition and are expressed by using the dimensionless 'per mil' (‰) notation.

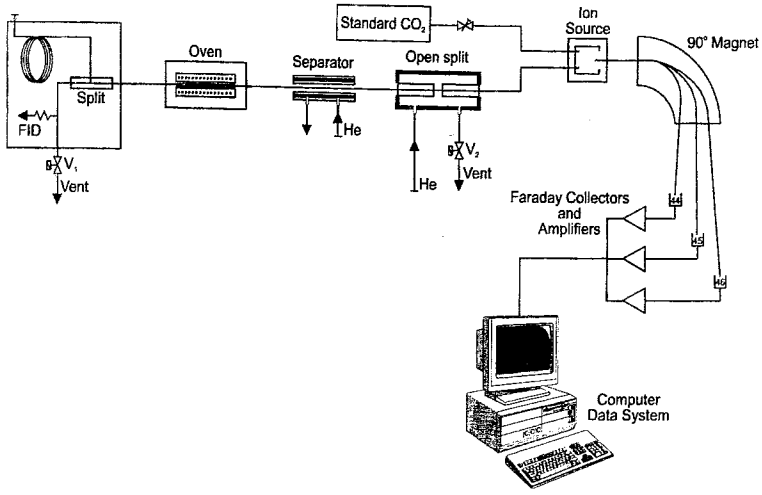


Figure 8.6. Schematic diagram of a GC-C-IRMS (Brand, 1996).

In nature carbon has three isotopes, ^{12}C and ^{13}C, which are both stable and ^{14}C which is radioactive. They account for 98.89 %, 1.11 % and $1x10^{-10}$ % of the global carbon pool, respectively. The stable carbon isotope ratios are measured as the relative differences between the sample and a standard gas. They are expressed by the delta (δ) notation as shown in Eq. 8.3. In equation 8.3. R_{sample} is the molar $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ is the molar $^{13}C/^{12}C$ ratio of the standard

$$\delta^{13}C = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} \right] \times 1000 \qquad (8.3)$$

The $\delta^{13}C$ valus thus represent the difference between the ^{13}C content of the sample and that of the standard in parts per thousand (‰) and is expressed relative to an international standard VPDB (Craig, 1957) which is assigned a value of 0 ‰. Pee Dee Belemnite (PDB) is a belemnite from the Cretaceous Pee Dee formation in South Carolina, USA. It is used as the accepted standard zero point for expression for the carbon and oxygen isotope abundances. The typical precision of compound-specific $\delta^{13}C$ values for fatty acids is ± 0.3 ‰.

8.2.3.1. GC-C-IRMS DELTA-S system. .GC-C-IRMS analyses of fatty acids from potpotsherds in this study are performed by using a Varian 3400 GC coupled to a Finnigan MAT Delta-S IRMS via an extensively modified Finnigan MAT Type I combustion interface. The interface has a 0.1 mm o.d Cu and Pt wires in an aluminum reactor having a

0.5 mm i.d. The reactor temperature is maintained at 860°C and the mass spectrometer source pressure is kept at 6 x 10^{-6} mbar. Faraday cups are used to detect ions with *m/z* = 44 for ($^{12}C^{16}O_2$), 45 for ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 for ($^{12}C^{18}O^{16}O$). The GC column was a silica capillary column (50 m x 0.32 i.d.) coated with a polyethylene glycol (CP Wax-52 CB) stationary phase with 0.25 μm film thickness or equivalent. The temperature programme for FAME determinations started with a one min isothermal period at 50°C, followed by an increase to 150°C at 15°C min^{-1}, followed by an another increase to 220°C at 4°C min^{-1} then a further increase to 240°C at 15 °C min^{-1} with final isothermal period of 10 min at this temperature.

8.2.3.2. Data processing The $^{13}C/^{12}C$ ratios are expressed relative to the VPDB (*Belemnitella americana*) standard (Equation 8.4.) where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the standard.

$$\delta^{13}C\ (‰) = \frac{(R_{sample} - R_{standard})}{R_{standard}} \times 10^3 \qquad (8.4)$$

Each sample is run in duplicate. In case of discrepency the runs are repeated. The three co-injected standards of known isotopic composition of $C_{11:0}$, $C_{13:0}$ and $C_{21:0}$ FAMEs with $\delta^{13}C$ values of –27.6, -32.0 and –28.7, respectively, are used to ensure the integrity of the data. Results are calibrated against a reference CO_2 standard, which is injected directly into the ion source three times at the beginning and three times at the end of each run. Instrumental analytical error is ± 0.3 ‰. The $\delta^{13}C$ values for the individual fatty acids are determined by correcting the values obtained for the corresponding FAMEs by using a simple mass balance calculation to account for the extra carbon added during derivatisation (Equation 8.5.).

$$\delta^{13}C_{FA} = \frac{(no.C_{FAME} \times \delta^{13}C_{FAME}) - \delta^{13}C_{MeOH}}{no.C_{FA}} \qquad (8.5)$$

In Equation 8.5:

$\delta^{13}C_{FA}$ = $\delta^{13}C$ value of the fatty acid

$\delta^{13}C_{FAME}$ = $\delta^{13}C$ value of the FAME

$\delta^{13}C_{MeOH}$ = $\delta^{13}C$ value of the derivatising methanol ($\delta^{13}C$ BF_3/MeOH = -44.04 ± -0.37 ‰)
no.C_{FAME} = total number of carbon atoms in the FAME

no.C_{FA} = total number of carbon atoms in the original fatty acid

9. SITES & MATERIALS

9.1 Neolithic in Turkey

Neolithic Period is defined as the time period of human cultural history when the hunter-gatherer way of life was replaced by a sedentary lifestyle involving the practice of horticulture and keeping of domestic livestock . At the beginning of the Holocene, about 13,000 years ago, human communities first began to settle in Turkey and the neighboring countries of southwestern Asia. They established small villages and progressed step by step to create, over a span of three thousand years, a culture that was termed "Neolithic Revolution" (Melleart, 1975).

The earliest sites with Neolithic culture, Hacılar near Burdur and Çatalhöyük near Çumra, Konya were excavated in the 1950s (Melleart, 1975; Melleart, 1967; Hodder, 1996). Earlier Chalcolithic settlements were excavated at Çayönü near Ergani, Diyarbakır (Çambel and Braidwood 1980; A. Özdoğan, 1995). Other important sites are Suberde and Erbaba (Bordaz, 1973), as well as Can Hasan III and I (French, 1972). New Neolithic sites besides Çayönü in the south and southeast of Anatolia were added to the list in the late 1970s and 1980s when salvage excavations started in the areas to be flooded by the reservoirs of the Karakaya and Atatürk dams. These include Gritille, Adıyaman (Voigt, 1988), Hayaz Höyük, Malatya (Molist and Cauvin, 1991) and Nevali Çori, Şanlıurfa (Hauptmann, 1993). These were followed in the 1990s by new excavations when new dams were planned such as Hallan Çemi, Batman (Rosenberg *et al.*, 1995) and Gürcütepe and Göbekli Tepe, Şanlıurfa (Schmidt, 1995,1997).

Parallel to the more recent work in southeastern Anatolia, excavations in Central Anatolia began in the 80s, with work still underway at Aşıklı Höyük, Aksaray (Esin, 1998) and at Köşk Höyük, Niğde (Silistreli, 1991; Gates, 1997). In southwestern Turkey there have been excavations at Kuruçay and Höyücek in the Lake District near Burdur (Duru, 1999) as well as Bademağacı near Antalya (Duru, 1998). Excavations at Southwestern Anatolia demonstrated the existence of both Aceramic and Ceramic Neolithic cultures distinct from those known in the southeast of Anatolia.

Presently there are few Neolithic settlements under excavation in the Marmara, most notably dating to Ceramic Neolithic. Excavations in the Marmara basin including Ilıpınar and Menteşe near Orhangazi and Yenişehir respectively (Roodenberg, 1998), Hoca Çeşme near Edirne and Aşağı Pınar near Kırklareli (Özdoğan, 1998) have enlarged the cultural mosaic of Turkey. There are now more concrete evidences to suggest that the Marmara Region has served as a bridge between Anatolia and southeastern Europe throughout human history.

In this study, a large number of pottery samples both from the Chalcolithic site of Tell Kurdu in the Amuq plain and from the Neolithic levels Barcın Höyük near Yenişehir Bursa in the Marmara basin are investigated. There were also pottery samples from Mezraa-Teleilat in Southeastern Anatolia, Tell Atchana in the Amuq plain and İkiztepe North Central Anatolia.

9.2. Sites

9.2.1. Chalcolithic Site of Tell Kurdu

Tell Kurdu is located in the central part of the Amuq Plain in Southeastern Turkey and covers an area of about 15 ha. It is one of the largest Chalcolithic sites in the region (Fig 9.1). Ceramics and other artifacts from the site are associated with Ubaid and Halaf-related assemblage dating to 5^{th} and 6^{th} millennia BC that indicates a close relationship with Mesopotamia during the early stages of urbanization in the Near East. The Ubaid and Halaf Periods represent the development of complex state societies.

The Halaf Period at Tell Kurdu (Phase C, c.5700-5200 BC) so far represents the earliest coherent remains from this period to date (Bressy *et. al.* 2005). An extensive neighborhood, consisting of four different types of architectural units, was exposed in the 2001 excavation season (Fig 9.2. and 9.3) (Özbal *et al.* 2003). Its ceramic assemblages contained Halaf-related elements including carinated bowls with bucrania and bowl fragments in a Halaf style, made with well-fired creamy paste with very lustrous red paint decorated with dotted circles (Bressy *et al.* 2005).

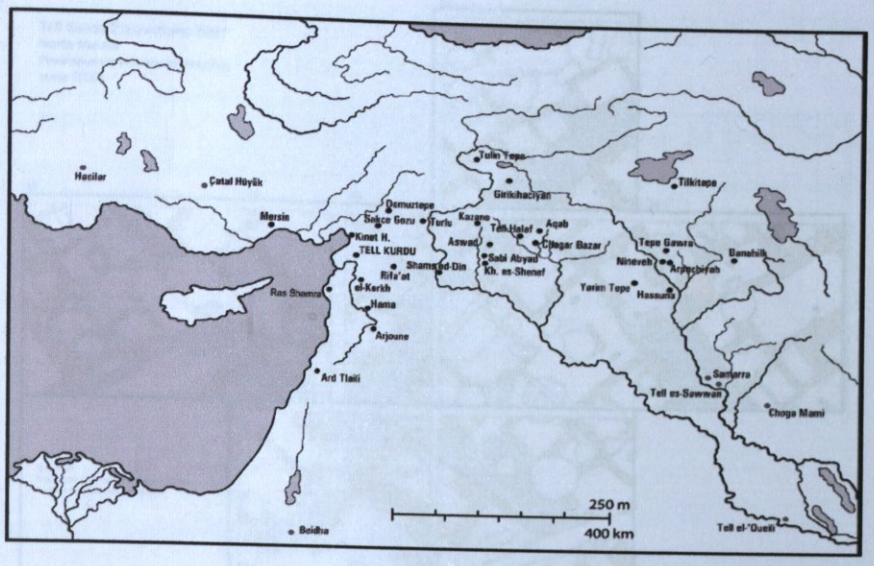


Figure 9.1. Tell Kurdu and the neighboring Halaf sites (Özbal, 2006)

Figure 9.2. Topographic plan of Tell Kurdu. The 2001 trenches are indicated in black (Özbal, 2006)

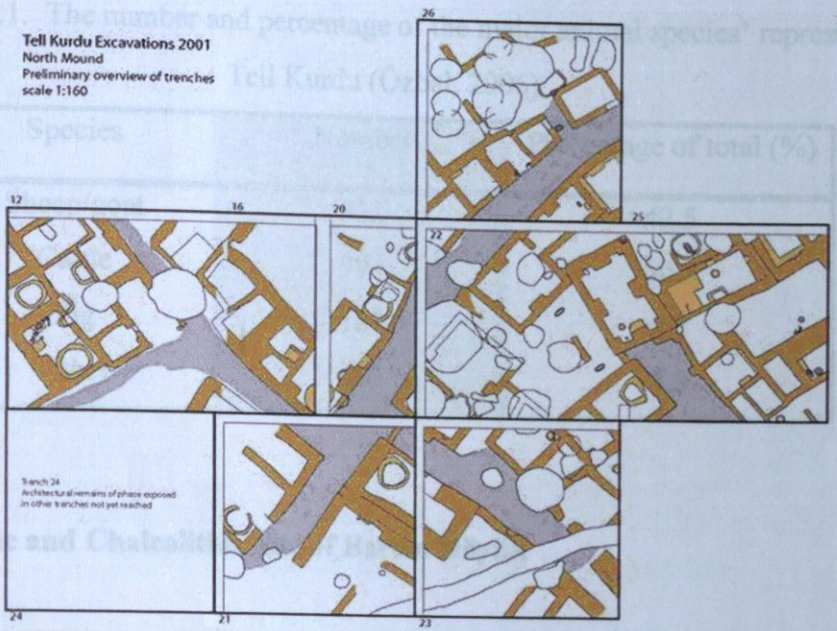


Figure 9.3. General overview of the architecture exposed during the 2001 season at Tell Kurdu (Özbal, 2006)

Tell Kurdu also yielded important information about subsequent level, Amuq D/E Phase (c. 5100-4300 BC), relating to the Ubaid period. It was mostly exposed during the 1996-1999 excavation seasons and it was recognized that the settlement shrank in size after the Halaf period (Yener *et al.* 2000). Nevertheless, a large multi-roomed building with long, narrow storage rooms made of slabs was found set on terrace on the mound summit. Large quantities of small finds such as clay tags, tokens and baling tags, personal ornaments, stamp seals and geometric devices indicate a clear connection between the storage facilities and bureaucratic accounting. During this period the archaeological record reveals the spread of similar material culture from Southern Mesopotamia across Northern Iraq and Syria, and into the Eastern Turkey (Özbal *et al.*2001).

9.2.2. Tell Kurdu Faunal Assemblage

The total assemblage of over 5,500 animal bones excavated at Tell Kurdu was studied. Significant proportion of sheep/goat, cattle and pig domesticates were identified in the Amuq C Phase (Özbal, 2006). Wild animals including gazelle and fish were also recognized. When the bone remains were contextually assessed, it was found that sheep/goat and cattle were distributed relatively evenly across excavated site.

Table 9.1. The number and percentage of the major animal species' represented at Tell Kurdu (Özbal, 2006)

Species	Number	Percentage of total (%)
Sheep/goat	832	42.5
Cattle	794	40.5
Pig	167	8.5
Other	163	7.3

9.2.3. Neolithic and Chalcolithic Site of Barcın Höyük

The mound of Barcın Höyük, also known as Yenişehir II, is located about 5 km west of Yenişehir, very near the Yenişehir Bursa Highway. İznik lake is located about 25 Km North of the mound (Fig. 9.4 and 9.5.). It consists of two joined mounds, one with a 120 m diameter and 4 m high and the second mound has a 50 m diameter and is only 2.5 m high. In the saddle between the two mounds is a Byzantine graveyard, The site has deposits from Roman, Middle and Early Bronze Ages, Chalcolithic and Neolithic Periods. Excavations are part of the Eastern Mediterranean Early Farming Project by the Netherlands Institute in Turkey, under the direction of Dr. Fokke Gerritsen.

Marmara region is the connecting bridge between the Western Asia and Southeastern Europe. This region was therefore thought to be critical for uncovering links between the two continents (Roodenberg, 1995). Unfortunately a systematic surveys and excavations in the region were neglected by the archaeologists for many years. Barcın excavation will not only provide new data about the first sedentary life in the region, but also it will provide evidences for the theory that agriculture and animal husbandry of Southeastern Europe were spread through West Asia (Roodenbeg, 1995).

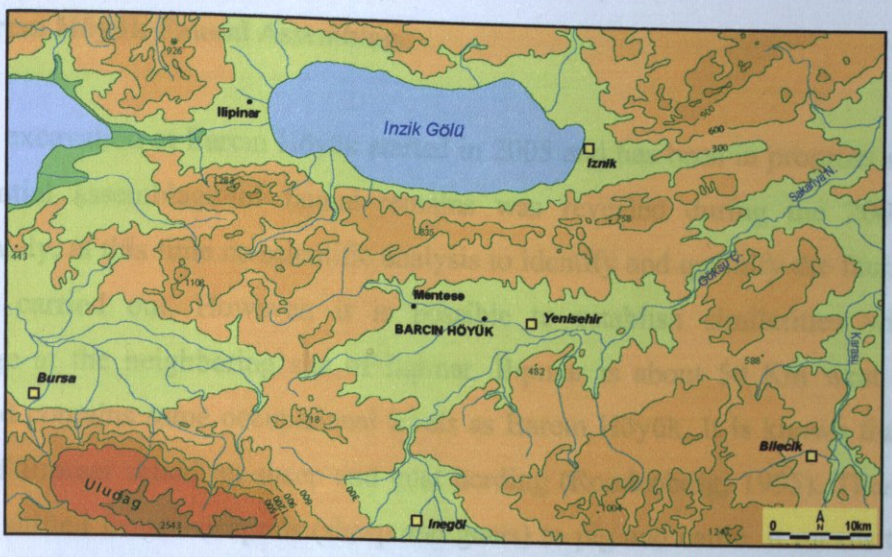


Figure 9.4. Barcın Höyük and the Yenişehir Plain

During the 2007 season excavations were carried out in four trenches at Barcın Höyük namely L11, L12, M10 and M11. L11 trench is dated to 6400-6200 BC (calibrated) by radiocarbon analysis of charcoal samples from this trench which correspond to late Neolithic period of Southern Marmara Region (Gerittsen, unpublished report). T certain places Middle to Late Neolithic layers are about 2.5 m thick. These early levels are similar to those observed at the nearby Menteşe Höyük and Ilıpınar, which are contemporary with Çatalhöyük VI (Roodenbeg, 1995).

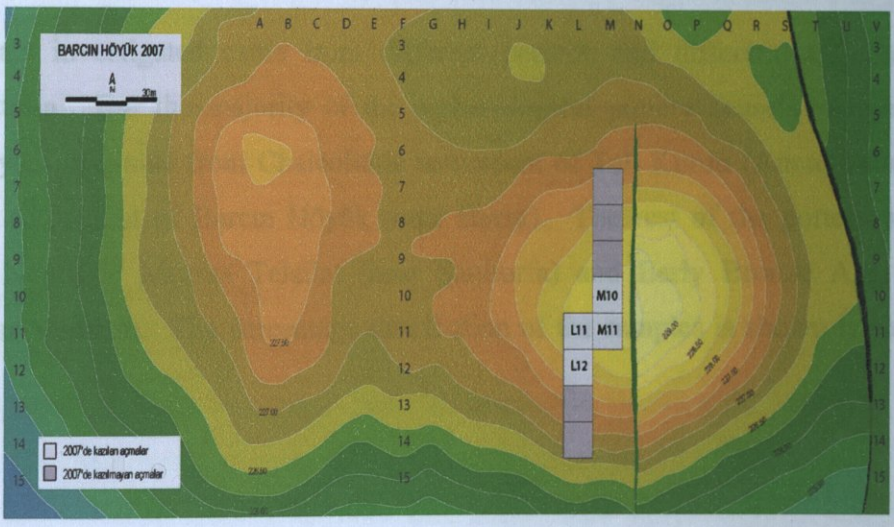


Figure 9.5. Topographic plan of Barcın Höyük after 2007 excavation season (Gerritsen, unpublished report)

9.2.4. Barcın Höyük Faunal Assemblage

The excavation at Barcın Höyük started in 2005 and has been in progress since then. A substantial assemblage of faunal remains was revealed during the 2007 season. Unfortunately, at this time no scientific analysis to identify and quantify the faunal species has been carried out. However, it is possible to establish similarities with faunal assemblage at the neighboring site of Ilıpınar. Ilıpınar is about 50 Km west of Barcın Höyük and contains same occupational levels as Barcın Höyük. It is known that the first farmers of Ilıpınar relied on sheep and goat herding (Roodenberg, 1995). These farmers gradually shifted from ovicaprids (sheep and goats) to pig and cattle. It means that in the course of a few centuries, pig and cattle became dominant in the animal husbandry. This change in economic strategies can be interpreted as an adaptation to the woodland environment of the eastern Marmara region where pigs and cattle had better chance to prosper than ovicaprids (Roodenberg, 1995). Buitenhuis (1995) recognizes that livestock breeding of sheep and goat is more suited to the drier environment of the Central Anatolia plateau rather than to pluvial lowlands of the Marmara region.

9.3. Materials

Materials investigated came from different periods and different settlements of ancient Anatolia. But the majority of the archaeological pottery vessels subjected to residue analyses originate from Chalcolithic settlement of Tell Kurdu (Amuq valley) and Chalcolithic settlement of Barcın Höyük (near Bursa). The rest of the pottery samples came from Neolithic Mezraa Teleilat (near Şanlıurfa) and Early Bronze Age site of İkiztepe (near Samsun). The percentage distribution of the samples is shown is shown in Table 9.2

Table 9.2. A summary of investigated potsherds

Site	Number of samples analyzed	Number of samples with lipid residues	Percentage of samples with lipid residue
Tell Kurdu	40	2	5
Barcın Höyük	60	10	17
Mezraa Teleilat	15	2	13
İkiztepe	3	1	33

The selection of pottery samples for residue analysis was governed by a number of considerations. The main concern was to obtain samples that would reflect the dietary practices of the population in the selected sites. Analysed samples are supposed to have been used as cooking ware, so the samples were selected among coarse ware potteries which are more likely to have been used for preparation of food and processing of the foodstuff. Unfornatenately, most of the pottery samples were in fragmentary form without any diagnostic feature. Thus, it is not possible to predict the complete shape and function of the vessel. However one can still have some idea about the products prepared, processed, stored, served or transported in these vessels. Pottery samples were selected from well-stratified and well-dated contexts.

9.3.1. Tell Kurdu Pottery Assemblage

Pottery samples were recovered from all excavated trenches of Tell Kurdu. The pottery samples span over a period of 1,400 years between 5,700 BC and 4,300 BC and includes substantial assemblages of Halaf and Ubaid type ware. Most of the selected potsherds were supposed to have been used as cooking pots but servicing and storage vessels such as bowls and jars were also studied for lipid analysis. At Tell Kurdu, the cooking pots have thick splayed rims (Fig.9.9). Both burnished and un-burnished vessels are found in the Amuq Valley since the earliest Neolithic assemblages (Özbal, 2006). By

the Amuq C phase around sixth millenium BC, un-burnished and splayed-rimmed vessels became the standard for cooking vessels. Splayed vessels with soot, charing on the exterior and the coarse sand and grit inclusions are good indicators that they were used for cooking (Fig. 9.10.) (Özbal, 2006) .

A contextual distribution of splayed rimmed potsherds across the site suggests that these vessels were located in rooms with small fire pits (Özbal, 2006).

Bowls and jar pots with decorations on the sliped layer applied on the surfaces were usually the fine wares (Fig 9.6 , 9.7 and 9.8).

A number of potsherds have thin white coating identified as calcite deposits. Although this could be formed naturally, it could also be deliberately washed lime as it is present only on the exterior of the potsherds.

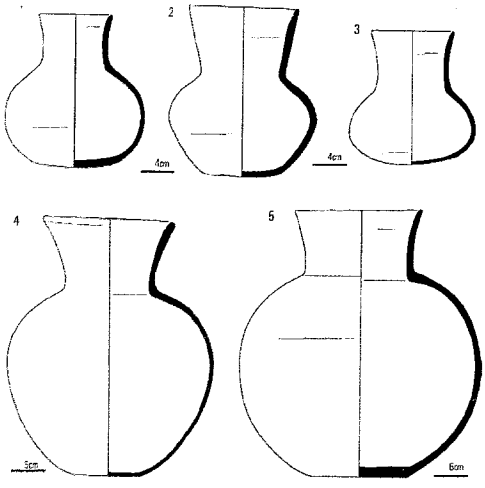


Figure 9.6. Tall-necked jars from Tell Kurdu (Özbal, 2006)

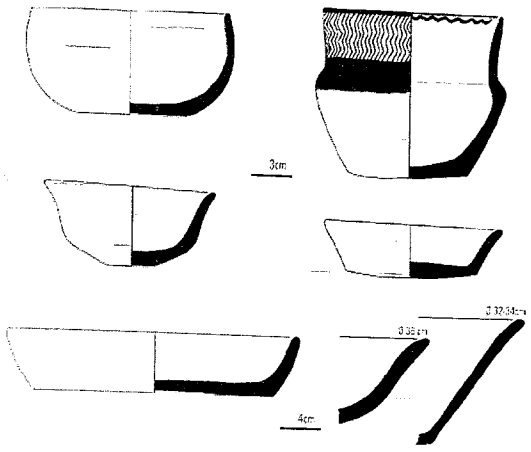


Figure 9.7. Open bowl shape vessels from Tell Kurdu (Özbal, 2006)

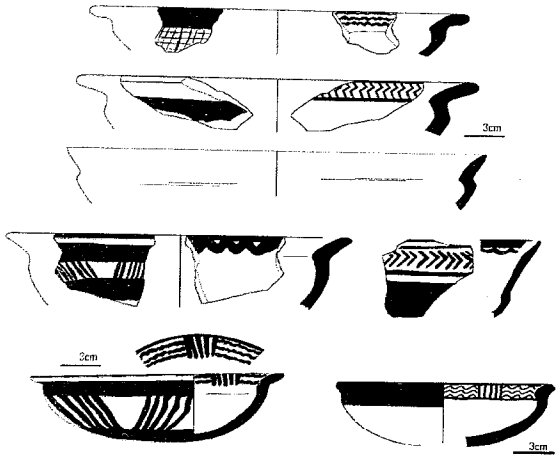


Figure 9.8. Collard rim bowls from Tell Kurdu (Özbal, 2006)

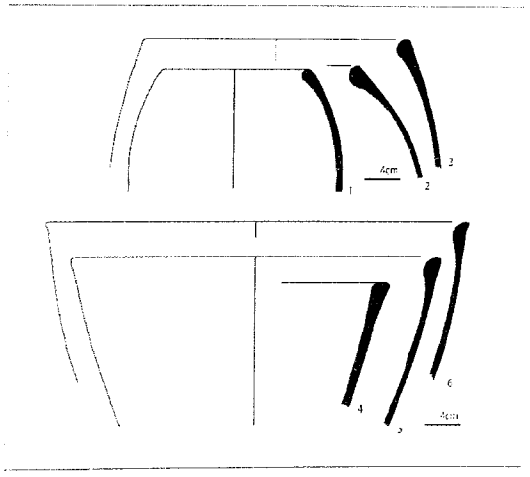


Figure 9.9. Splayed-rim cooking vessels from Tell Kurdu (Özbal, 2006)

Figure 9.10. Example of Tell Kurdu potsherds investigated in this study.

9.3.2. Barcın Höyük Pottery Assemblage

The potpotsherds from Barcın Höyük were recovered from the L11, M10 and M11 trenches corresponding to time span from the Late Neolithic to Chalcolithic periods. Plain pottery fragments dominated the assemblage (Fig. 9.11). Decorated vessels were not present at the site. The plain pottery samples were coarse wares and most of them were heavily tempered. The majority of the potsherds were tempered with one or more non-plastic inclusions including sandstone, quartz, mudstone, calcite and shell. In other words, they were expected to be used as cooking purposes. This assumption was supported by the fact that there were both signs of soot and burning on the interior and exterior of the fragments.

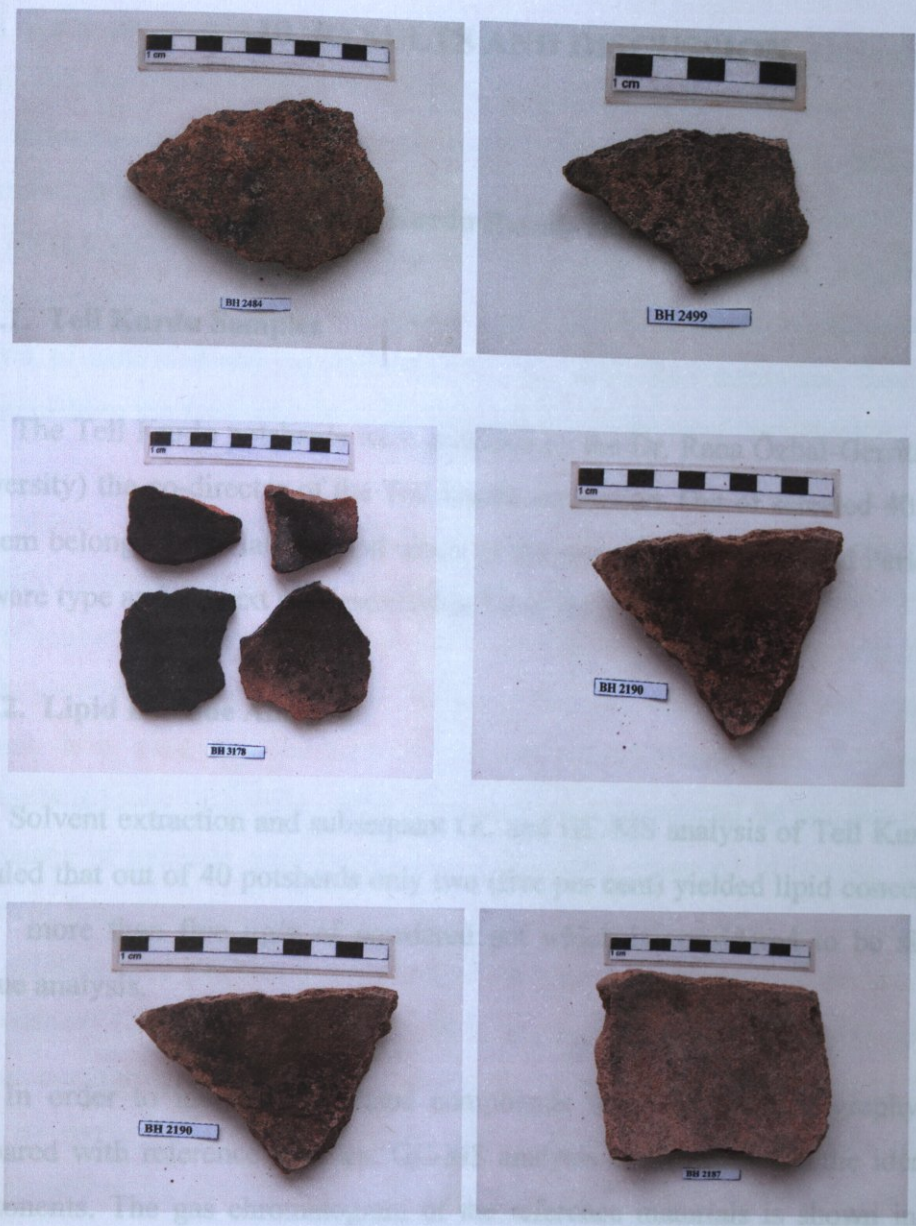


Figure 9.11. Barcın Höyük potsherds selected for this study.

10. RESULTS AND DISCUSSION

10.1. Tell Kurdu Results and Discussion

10.1.1. Tell Kurdu Samples

The Tell Kurdu potsherds were provided by the Dr. Rana Özbal-Gerritsen (Boğaziçi University) the co-director of the Tell Kurdu excavation. Out of selected 40 potsherds 30 of them belong to the Halaf Period where as the rest was from the Ubaid Period. Details of the ware type and context are described in Table A1, Appendix A.

10.1.2. Lipid Residue Analysis

Solvent extraction and subsequent GC and GC-MS analysis of Tell Kurdu potsherds revealed that out of 40 potsherds only two (five per cent) yielded lipid concentrations that were more than five μg/g of powdered pot which is considered to be significant for residue analysis.

In order to identify the eluted compounds with gas chromatography the data is compared with reference samples. GC-MS analysis is also used for the identification of components. The gas chromatogram of the reference materials is shown in Figure B.1, Appendix B. The mass spectra of the most significant peaks are given in Table 10.1. The GC data showed dominant peaks corresponding to saturated free fatty acids with carbon between C14 and C18 (Figures B.2 and B.3 Appendix B) which are the TMS esters that appear between 10 and 20 minutes. The source of these fatty acids is most likely from highly degraded animal fat. The animal fats are degraded most likely by chemical or enzymatic hydrolysis of acylglycerols.

The mass spectrum of TMS derivative of some saturated fatty acids such as myristic, palmitic, margaric and strearic acids are shown in Figures C.3, C.4, C.5, and C.6, respectively, Appendix C. The saturated fatty acids shows very similar fragmentation patterns. For example palmitic acid shows a $M^{+\cdot}$ ion (*m/z* 328) with much more prominent

peak due to the $[M\text{-}CH_3]^+$ ion (*m/z* 313) (Figure C.4. Appendix C). Highly diagnostic $[M\text{-}CH_3]^+$ ion fragment is present in the mass spectra of the TMS derivatives of all saturated and unsaturated fatty acids and is used to assign carbon number and the degree of unsaturation in the case of unknowns. The ions at *m/z* 73 and 75 corresponds to $[(CH_3)_3Si]^+$ and $[(CH_3)_2SiOH]^+$ respectively and are very common in the mass spectra of fatty acid TMS derivatives. The fragment ions at *m/z* 117, 129, 132 and 145 are also prominent in the spectra of saturated and monounsaturated fatty acid TMS esters and they are useful in distinguishing the fatty acid from the TMS ether derivatives of fatty alcohols.

Mono-, di- and triacylglycerols are absent in the Tell Kurdu potsherds. Therefore attention is focused mainly to the distribution of major *n*-alkanoic (fatty) acids. Palmitic acid ($C_{16:0}$) and stearic acid $C_{18:0}$ are the most abundant saturated fatty acid in the two Tell Kurdu samples. On the other hand branched margaric acid ($C_{17:0br}$) was present in lower amount. It is clear that the ratio of palmitic acid to stearic acid (P/S) in Tell Kurdu potsherds is lower than three, which is an indication that animal fat was processed. Greater abundance of palmitic over stearic acid is generally observed in bovine, milk and non-ruminant fats. The sheep and goats fats, however, contain higher amount of stearic acid than palmitic acid and this is not changed during the degradation of the fats in the burial environment (Kimpe *et al.*, 2002). It is known that branched-chain and odd number of carbon containing fatty acids are present in high abundance in ruminant fats, due to bacterial synthesis in the gut (Christie, 1981). Since such branched fatty acids with odd carbon number are observed in the GC chromatograms, it is possible to argue that ruminant fats particularly bovine adipose fats could have been the major component in the sherds. Table 10.1 summarizes the mass spectrometric data for samples TK6760C and TK6226-4. Phthalate ester contaminations are also observed in the residue extracts and they are most probably originate from plastic bags in which the potsherds have been stored since the excavation (Figure B.3, Appendix B). These contaminations are denoted as * in the HTGC chromatograms. They are also easily indentified by the abundant *m/z* 149 peak in the mass spectrum of lipid residue exctracts.

Table 10.1. Mass spectrometric fragmentation data for fatty acids observed in TK6760-C and TK6226-4 (as TMS derivatives)

Compound name	Mass spectral data *m/z*
Myristic acid	285(M^+-15).145,132,117,73
Palmitic acid	313(M^+-15),145,132,117,73
Margaric acid	327(M+-15),145,132,117,73
Stearic acid	341(M^+-15),145,132,117,73

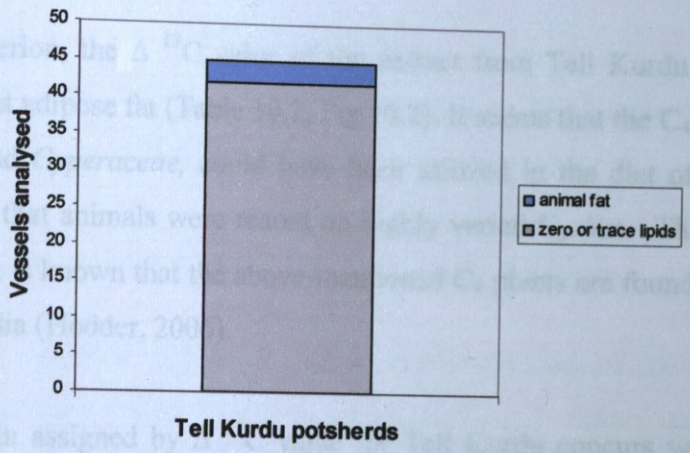


Figure 10. 1. Lipid residues extracted from Tell Kurdu potsherds

The lipid residues extracted from Tell Kurdu, unfortunately, are not well preserved. No intact TAGs are observed in any of the analyzed potsherds. Plant lipid biomarkers such as mid-chain ketones, wax esters, alcohols, alkanes, short chain diacids are also absent in the lipid extracts indicating that plant products were not processed or used in these potsherds.

10.1.3. Compound-Specific Stable Carbon Isotope Analysis Results

Out of two lipid extracts from the Tell Kurdu samples only TK 6760C is subjected to compound specific stable carbon isotope analysis. Stable isotope measurement is made in order to obtain $\delta\ ^{13}C$ values for the FAME derivatives of $C_{16:0}$ and $C_{18:0}$ fatty acids in the extract.

$\Delta\ ^{13}C$, which is the difference between $\delta\ ^{13}C_{16:0}$ and $\delta\ ^{13}C_{18:0}$, is a useful indicator of lipid origins when significant dietary or environmental variations in isotope values occur (Copley *et al.*, 2003). In figure 10.2 the $\Delta\ ^{13}C$ is plotted against the $\delta\ ^{13}C_{16:0}$ data of the TK 6760C sample from Tell Kurdu. The $\Delta\ ^{13}C$ value in the region between 3.3 to 6.3 ‰ corresponds to ruminant dairy fats, between 1.0 to 2.8 ‰ represents ruminant adipose fats while those between -0.7 to – 1.9 ‰ indicates porcine adipose fats (Figure 10.2). Similarly an increase in both the marine diet or C_4 diet have the similar effects on $\Delta\ ^{13}C$ values, because in both cases the stable carbon isotope values are more enriched compared to stable carbon values for predominantly C_3 diet.

Using this criterion, the $\Delta\ ^{13}C$ value of the extract from Tell Kurdu is -1.6 and is identified as ruminant adipose fat (Table 10.2, Fig 10.2). It seems that the C_4 plants such as *Chenopodiacceae* and *Cyperaceae,* could have been utilized in the diet of domesticated animals or similarly that animals were reared on highly varied C_3 diet. This result is not surprising, because it is known that the above-mentioned C_4 plants are found in prehistoric Çatalhöyük in Anatolia (Hodder, 2006).

The lipid origin assigned by $\Delta\ ^{13}C$ value for Tell Kurdu concurs with the results determined from the lipid residue analysis by HTGC. The faunal of assemblages of bones from Tell Kurdu site revealed high percentage of ovine and bovine bones (Özbal, 2006). This fact is consistent with the stable isotope analysis and residue analysis. The result also indicates that GC-C-IRMS analysis of lipid residues can yield definite evidence for exploitation of different animals when faunal evidences are absent from the excavation sites. This result also highlights the importance of using different methods of data analysis in conjunction with each other.

Table 10.2. Stable carbon isotope data of TK6760C

Sample No	$\delta\ ^{13}C_{16:0}$‰	$\delta\ ^{13}C_{18:0}$‰	$\Delta\ ^{13}C$
TK 6760C	-26.2	- 27.8	- 1.6

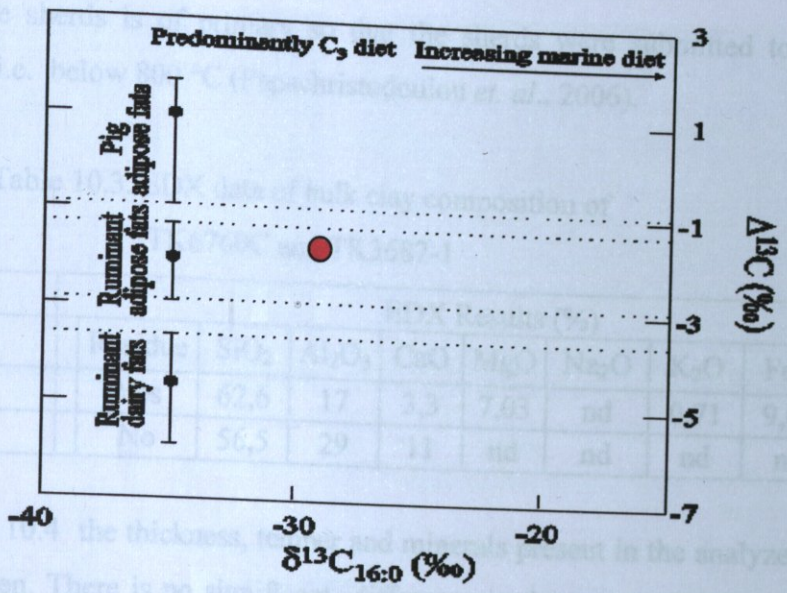


Figure 10.2. Plot of $\Delta\ ^{13}C$ vs. $\delta\ ^{13}C_{16:0}$ TK 6760C from Tell Kurdu. The ranges for modern reference fats are shown on the y-axis.

10.1.4. Micro structural and Mineralogical Investigation of Pottery

Along with the chemical and stable carbon isotope analysis of the lipid residues, micro structural investigation of the pottery fragments with and without residues are also investigated in order to find possible relationship between fabric, temper and firing condition of the potsherds with its ability to absorb and conserve lipid residues. SEM-EDX and X-ray diffraction techniques are used for this purpose.

The chemical composition of the fabric of the two potsherds (TK6760C and TK 3687-1) determined by EDX and the mineral content determined by XRD are shown in Table10.3. The sample TK 6760C was found to contain significant amount of lipid residue where as sample TK 3687-1 did not contain any residue. The high content of Al_2O_3 and the low content of Na_2O+K_2O in clay matrix indicate that refractory kaolinite clay was used in the production of these potsherds. Also the lime (CaO) content (less than 15 per cent) is indicative of usage of non-calcareous clays. The major mineral detected in both sherds was quartz. Quartz is often indigenous inclusion found in the clay deposits and therefore its use as an intentional temper is not easy to determine. It is possible that, when preparing cooking pots, potters preferred clays from particular local deposits for their physical and thermal properties. The absence of diopside ($CaMgSi_2O_6$) in both sherds indicates calcite

present in the sherds is of primary so that the sherds were submitted to a low firing temperature- i.e. below 800 °C (Papachristodoulou *et. al.*, 2006).

Table 10.3. EDX data of bulk clay composition of TK6760C and TK3687-1

	EDX Results (%)								
Sample #	Residue	SiO_2	Al_2O_3	CaO	MgO	Na_2O	K_2O	FeO	TiO_2
TK6760C	Yes	62,6	17	3,3	7,03	nd	0,71	9,01	nd
TK3687-1	No	56,5	29	11	nd	nd	nd	nd	nd

In Table 10.4 the thickness, temper and minerals present in the analyzed Tell Kurdu sherds are given. There is no significant difference in the temper, thickness and mineral content between in the potsherds. The temper in both cases seems to be grog (crushed pottery). The large grog inclusion can be seen in both Figure 10.3 and 10.4. The rounded shape of such inclusions suggests that they are indigenous rather than added.

Table 10.4. XRD data of temper minerals

Sample	Thickness	Temper Type	Mineral
TK6760C	7mm	grog	Anorthite
TK3687-1	8mm	grog	Albite

In the light of micro structural and mineralogical investigation of the two Tell Kurdu potteries it can be stated that they were exposed to low firing temperatures, but their clay composition and microstructure do not differ significantly. The low firing temperatures allow pots to be porous, which in turn make the pots suitable to absorb organic residues. The sample 3687-1, which does not contain residues can used for processing products low in lipid content or it might be crushed at its first use .

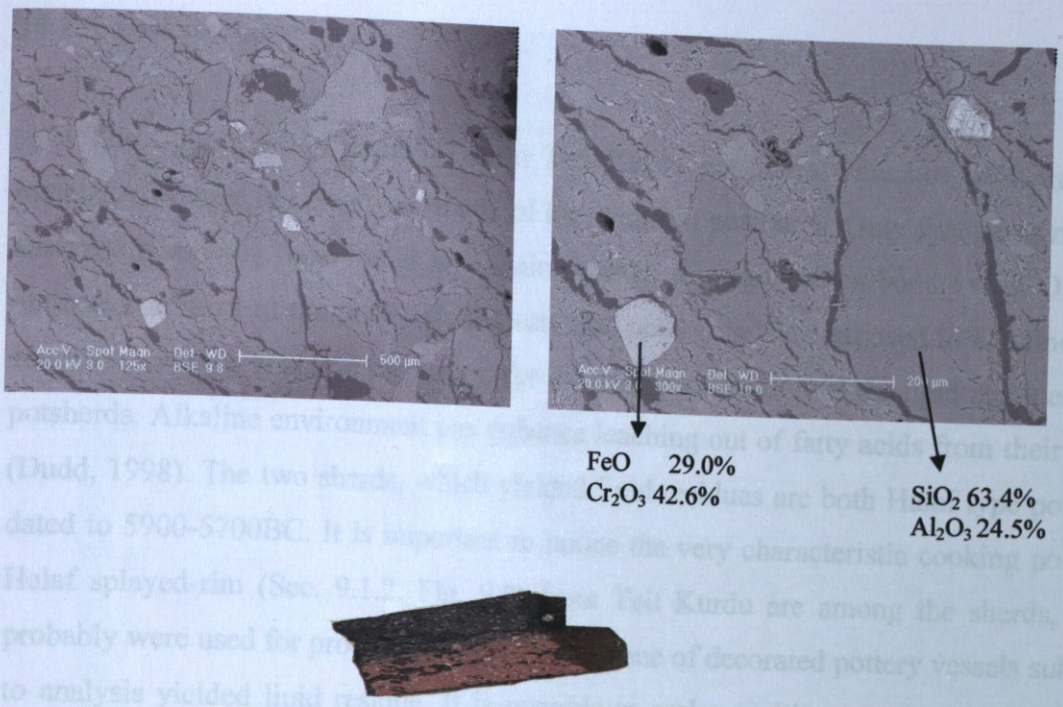


Figure 10.3. Back-scattered electron images of TK 6760 C with residue

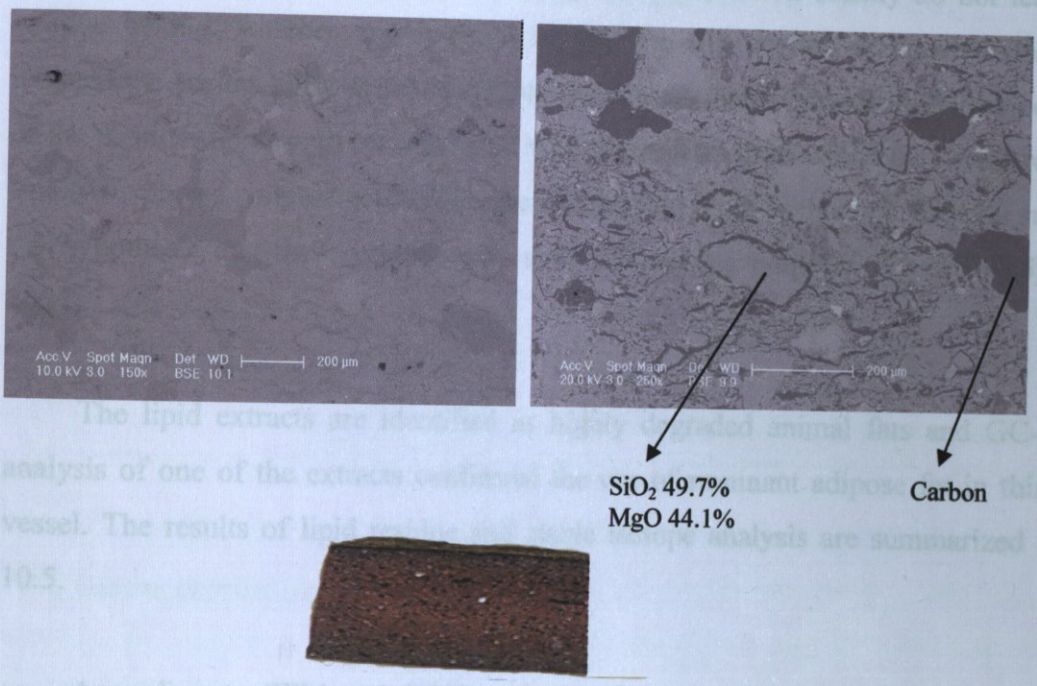


Figure 10.4. Back-scattered electron images of TK 3687-1 without residue

10.1.5. Discussions

The lipid residues extracted from Tell Kurdu potsherds were not well preserved. Intact TAGs were not observed in any of the analyzed potsherds. Only five per cent of the analyzed potsherds were found to contain residue. The calcium carbonate ($CaCO_3$) layer observed on most of the potsherds indicate that potsherds were exposed to alkaline burial conditions, which could be the reason for the poor preservation of the lipid residues in the potsherds. Alkaline environment can enhance leaching out of fatty acids from their matrix (Dudd, 1998). The two sherds, which yielded lipid residues are both Halaf type potsherds dated to 5900-5700BC. It is important to notice the very characteristic cooking pots with Halaf splayed-rim (Sec. 9.1.2, Fig. 9.8) from Tell Kurdu are among the sherds, which probably were used for processing animal fats. None of decorated pottery vessels subjected to analysis yielded lipid residue. It is possible to make positive correlation between the decorated potsherds and the absence of absorbed residues. One possible reason could be that they were used for storing or servicing foodstuff, which usually do not leave much residue behind. Another reason could be that the slip applied to decorate the pottery reduced the permeability of foodstuff into the vessels fabric. Interestingly, the occurrence of the residues appears to correlate well with the context from which they were recovered. The two splayed rimmed potsherds were located in a room with small fire pits supporting the hypothesis that these vessels were used for cooking purposes (Figure 10.5) (Özbal, 2006).

The lipid extracts are identified as highly degraded animal fats and GC-C-IRMS analysis of one of the extracts confirmed the use of ruminant adipose fat in this pottery vessel. The results of lipid residue and stable isotope analysis are summarized in Table 10.5.

According to SEM and XRD analysis of two sherds, one with residue and one without residue, there is no significant difference between the type, fabric, temper and wall thickness used in the production of these cooking pots. Both potsherds were fired at low temperatures allowing them to have highly porous structure.

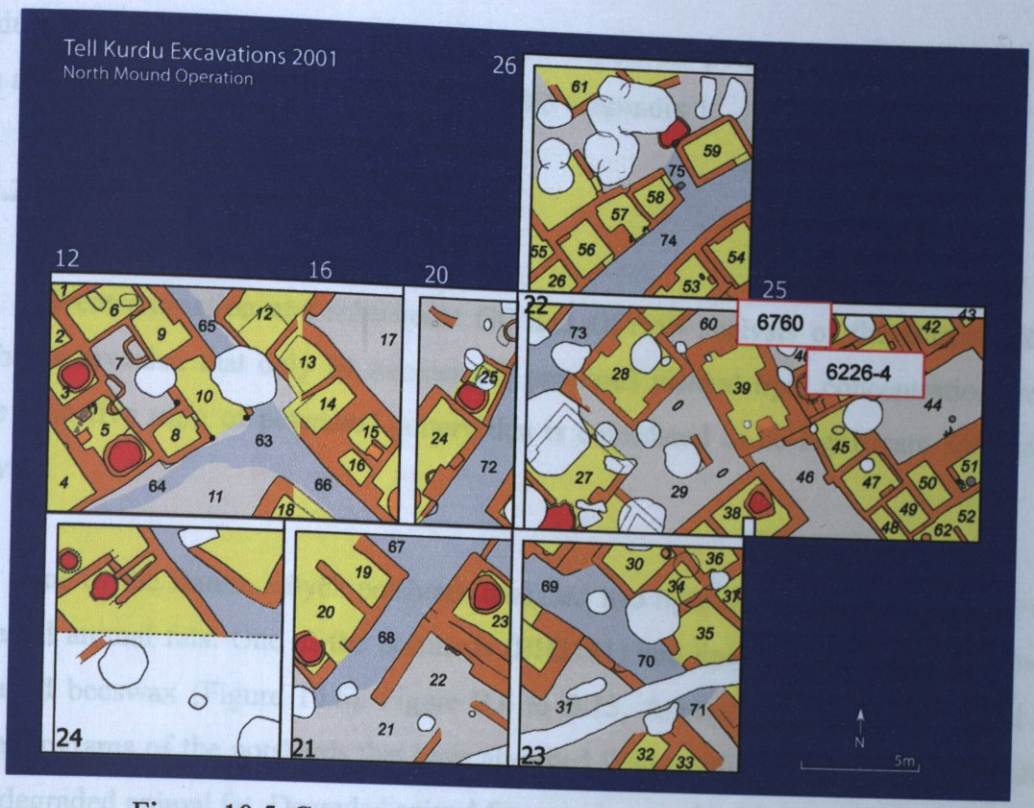


Figure 10.5. Context of TK6760C and TK6226-4 potsherds.

Table 10.5. The lipid assignments from Tell Kurdu pottery as suggested from TLEs, TAG distributions and $\Delta\ ^{13}C$ values

Sample name	TLE	TAG distribution	$\Delta\ ^{13}C$ values	Interpretation
TK 6760C	animal fat	-	rum.adipose	rum.adipose
TK 6226-4	animal fat (ruminant?)	-	-	animal fat (ruminant?)

10.2. Barcın Höyük Results and Discussion

10.2.1. Barcın Höyük Samples

All of the Barcın Höyük pottery samples analyzed in this study are provided by Dr. Fokke Gerritsen, director of the Netherlands Institute in Istanbul and the director of the Barcın Höyük excavation. From Barcın Höyük a total of 60 potsherds are selected for lipid

residue analysis. No surface residues are observed on the potsherds. Details of the ware type and their context are described in Table A2, Appendix 1.

10.2.2. Lipid Residue Analysis Results

Solvent extraction and subsequent GC and GC/MS analysis of the Barcın Höyük potsherds revealed that only 10 samples (17 per cent) yielded lipid concentration that is more than five μg/g of powdered pottery that is considered to be significant for residue analysis.

Nine of the Barcın Höyük potsherds analyzed had lipid distributions consistent with degraded animal fats. One of the potsherds (BH3181) had lipid distribution indicative of degraded beeswax (Figure 10.6). Figure B.4 to B.13, Appendix B show the partial gas chromatograms of the potsherds that have absorbed lipid residues with profiles consistent with degraded animal fat. Degraded animal fats are characterized by the distribution of free fatty acids, mono-, di- and triacylglycerols. The amount of mono- and diacylyglycerols produced by the loss of two or one fatty acyl moieties, respectively; in Barcın Höyük samples are quite low in abundance. Four potsherds (BH2484, BH2499, BH3190 and BH3184) are found to contain predominantly saturated free fatty acids between C14-C18, which result from the extensive chemical or enzymatic hydrolysis of fats. The $C_{16:0}/C_{18:0}$ ratio of the four samples is less than one, which is a further indication of the lipid residues from animal origin. Myristic acid ($C_{14:0}$) was observed in the samples BH2499 and BH3164. This fatty acid is also very characteristic for the presence of animal fat.

It is notable that in some of the Barcın Höyük potsherds (BH2187, BH3168, and BH2304-2) as seen in the figures B.4, B.5 and B.8 respectively, the $C_{16:0}$ and $C_{18:0}$ relative abundances are lower than TAGs. In such case the sublimation phenomenon may cause partial or total disappearance of the free fatty acids in the potsherds, thus reducing their relative abundances in the lipid extracts (Regert *et al.*, 2001). Relatively high occurrence of unhydrolized fats in these potsherds may be due to the non-calcareous soil of the region that results in the low alkalinity around the burial environment and a potential advantage for the survival of TAGs absorbed in the potsherds.

Cholesterol, which is very important biomarker of the animal fats was also identified in samples BH2187, BH3168 and BH2484. It is generally difficult to detect this biomarker since it is found is very trace amount (around one per cent) in the animal fats.

Mid-chain ketones (C_{31}-C_{35}) are not observed in the Barcın Höyük potsherds. These are known, through laboratory degradation experiments, to form by self- and cross-head-to-head condensation of fatty acids during the heating of vessels and their contents in excess of 300°C (Raven *et al.*, 1997). Also, no plant lipids (e.g. *n*-alkanes, alcohols, wax esters) are detected in any of the sherds.

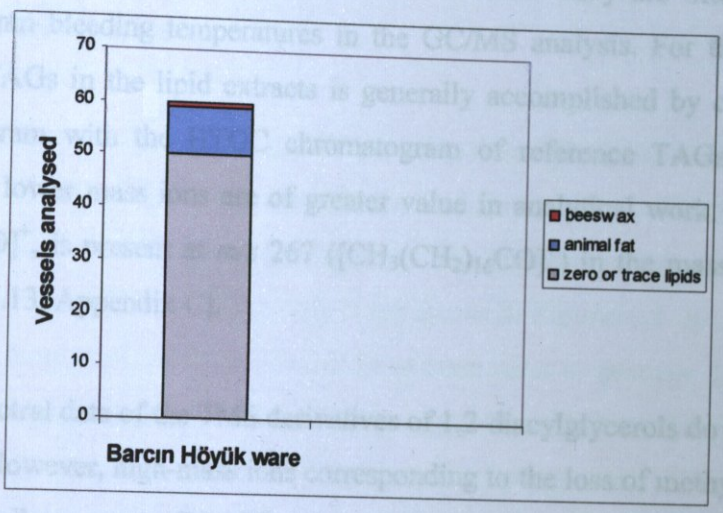


Figure 10.6. Barcın Höyük potsherd extraction results

One of the potsherds (BH3181, Figure 10.17) is found to contain degraded beeswax lipids as indicated by the presence of long chain alkanes, alcohols and wax esters. This sample is discussed in detail in section 10.3.1.

One of the most abundant components seen in the TIC of the total lipid extracts from Barcın Höyük are the intact triacylglycerols (TAG) eluting between 30 to 40 min. The mass spectra data of the TAGs are used to determine the nature of the fatty the acyl moieties present in the individual TAGs. The mass spectra of TAGs are complex due to the presence of a large number of different fatty acids. Therefore, any triacylglycerols analysis will only provide partial compositional information. The GC/MS instrument with the cross linked dimethyl polysiloxane coated capillary GC column could resolve the triacylglycerides only according to the number of carbon atoms present. More polar

stationary phases are available that can resolve triacylglyceride species according to their differing degree of unsaturation (Evershed, 1996).

The mass fragmentation patterns characteristic for free fatty acids were already discussed in section 10.1.2. Since TAGs, DAGs and MAGs were also observed in the Barcın potsherds the mass spectrum interpretation of some of the major TAGs, DAGs and MAGs are given as well. For example the mass spectrum of tristearin is shown in Figure C13, Appendix C. High mass ions, such as $M^{+\cdot}$, or $[M-18]^{+\cdot}$ can be seen in the mass spectra of TAGs, however, they will have low abundance. They are often difficult to detect above column bleeding temperatures in the GC/MS analysis. For this reason the identification of TAGs in the lipid extracts is generally accomplished by comparing the HTGC chromatogram with the HTGC chromatogram of reference TAGs (Figure B1, Appendix 1). The lower mass ions are of greater value in analytical work. An abundant acylium ion, $[RCO]^+$, is present at *m/z* 267 ($[CH_3(CH_2)_{16}CO]^+$) in the mass spectrum of tristearin (Figure C.13, Appendix C).

The mass spectral data of the TMS derivatives of 1,2-diacylglycerols do not generally contain $M^{+\cdot}$ ions. However, high-mass ions corresponding to the loss of methyl group, $[M-15]^+$, and trimethylsiloxy group, $[M-90]^+$ are used in assigning the carbon number of the intact diacylglycerols (Figure C.10., Appendix C). Since these ions are often weak in GC/MS analyses, carbon number is more reliably determined by retention time comparison with authentic compounds.

GC/MS analysis of the TMS ether derivatives of monoacylglycerols can be used for their identification. The mass spectra of their TMS ethers are useful since they often display weak $M^{+\cdot}$ and $[M-CH_3]^+$ ions from which the carbon number is deduced (Figure C.8 and C9, Appendix C). The appearance of a characteristic fragment ions at *m/z* 218 in the mass spectra of the bis-TMS of 2-monoacylglycerols and $[M-103]^+$ ion in the spectra of 1-monoacylglycerols allows isomeric species to be reliably distinguished.

Intact TAGs are observed in seven of the lipid extracts from Barcın Höyük Figure 10.7. Six of the extracts have TAG distributions indicative of ruminant adipose or degraded dairy fat (BH2473, BH2499, BH3164, BH 3168, BH3195 and BH2187). One

extract (BH2304-2) has TAG (C_{48}-C_{54}) distribution consistent with porcine adipose fats (Figure 10.7).

Highly degraded dairy fats may also resemble ruminant adipose fats due to preferential loss of the lower molecular weight TAG components (Copley *et al.*,2005). Therefore, without $\delta\ ^{13}C$ values of fatty acids, determination of the lipid origin based on TAG distributions alone should be approached with caution, as false negative results may arise.

10.2.3. Stable Isotope Analysis

The $\delta\ ^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids of samples that contain abundant residues from the Barcın Höyük are also determined by to GC-C-IRMS analyses to identify their origins. The $\Delta\ ^{13}C$ values for the seven Barcın potsherd samples are given in Table 10.6. The plot of $\Delta\ ^{13}C$ vs. $\delta\ ^{13}C_{16:0}$ values are shown in Figure10.8. It is immediately apparent that high proportion of the potsherds have been used to process dairy products. Using the above stated criterion five of samples were identified as dairy fats, one as ruminant adipose and one extract as porcine or a mixture of porcine and ruminant fat.

In interpreting the values obtained for the Barcın Höyük potsherds, it is important to consider whether the domesticated animals were fed on C_3 or C_4 plants. Based on the stable isotope results from Tell Kurdu (sec 10.1.3.) and stable isotope results from Çatal Höyük (Evershed, 2006), it can be assumed that animals were reared on mix (C_3/C_4) diet or were reared on highly varied C_3 diet.

It is important to notice, that all five samples that indicate processing dairy fats were recovered from Trench L11 i.e. dated to Late Neolithic Period (circa 6400 BC). The pots used for the processing of ruminant adipose and porcine fats, on the other hand, both belong to the Chalcolithic Period and were recovered from Trench M10 and M11. It is not possible to distinguish between the milk fat originating from the different species of ruminant animals.

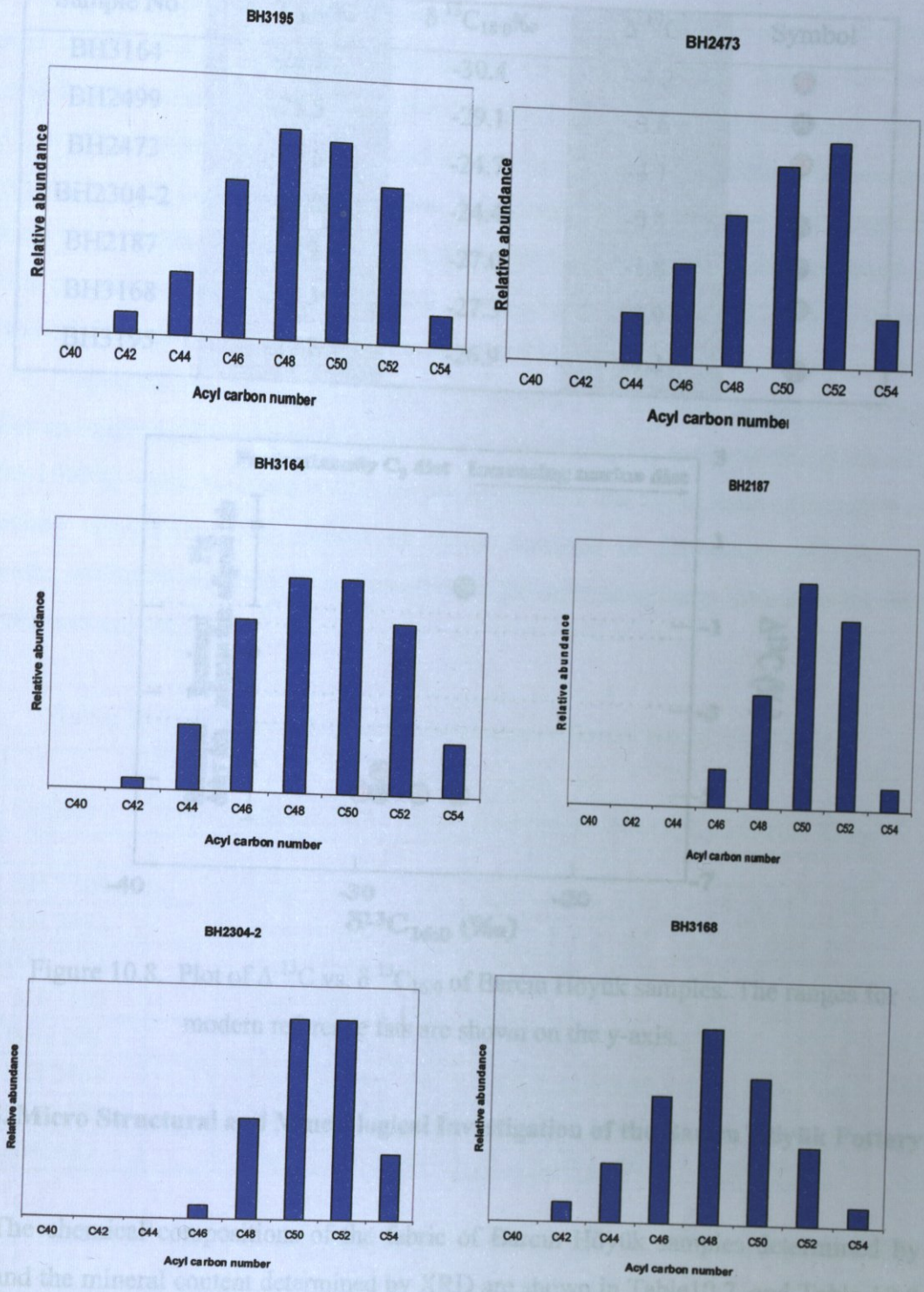


Figure 10.7. Acyl carbon number distributions of TAGs from lipid the extracts of Barcın Höyük

Table 10.6. Stable carbon isotope data of Barcın Höyük

Sample No	$\delta\ ^{13}C_{16:0}$‰	$\delta\ ^{13}C_{18:0}$‰	$\Delta\ ^{13}C$	Symbol
BH3164	-26.2	-30.4	- 4.2	
BH2499	-25.5	-29.1	-3.6	
BH2473	-20.6	-24.7	-4.1	
BH2304-2	-24.0	-24.4	-0.4	
BH2187	-25.2	-27.0	-1.8	
BH3168	-23.4	-27.3	-3.9	
BH3195	-22.7	-26.9	-4.2	

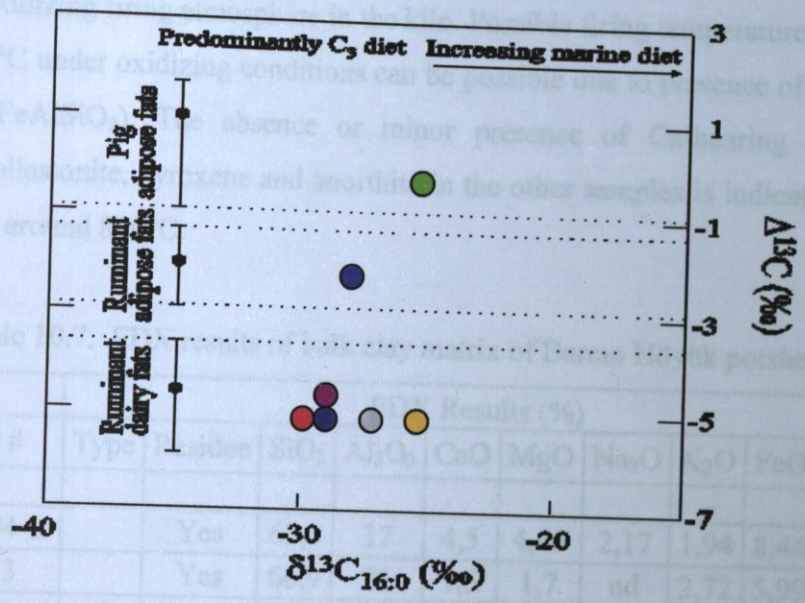


Figure 10.8. Plot of $\Delta\ ^{13}C$ vs. $\delta\ ^{13}C_{16:0}$ of Barcın Höyük samples. The ranges for modern reference fats are shown on the y-axis.

10.2.4. Micro Structural and Mineralogical Investigation of the Barcın Höyük Pottery

The chemical compositions of the fabric of Barcın Höyük samples determined by EDX and the mineral content determined by XRD are shown in Table10.7. and Table 10.8 respectively. Four of the sherds (BH2304-2, BH2473, BH3181 and BH3195) are found to contain significant amount of lipid residue whereas the remaining four samples do not contain any residue. A cursory examination of Table 10.7, shows that the bulk clay matrix of all potsherds are high in Al_2O_3 and low in Na_2O+K_2O. This is a typical composition for

high refractory kaolinite type of clay was used in the production of these potsherds. Also, low lime (CaO) content of the matrix indicates that they are extracted from non-calcareous deposit and are not refined with calcite temper (Broekmans *et al.*, 2004). The major mineral detected in samples BH2304-2, BH2190, BH3195 and BH2454 was quartz. Quartz (Si_2O) is often indigenous inclusion found in the clay deposits and therefore its use as an intentional temper is not easy to determine. It is possible, however, that when preparing cooking pots, potters preferred clays from particular local deposits for their physical and thermal properties. The absence of diopside ($CaMgSi_2O_6$) in sample BH319 may indicate that the calcite present in the potsherd was submitted to a low firing temperature i.e. below 800 °C (Papachristodoulou *et. al.*, 2006). The presence of hematite in sample BH2480 implies an oxidizing firing atmosphere in the kiln. Possible firing temperatures in the range of 900-1000°C under oxidizing conditions can be possible due to presence of hematite and orthoclase ($FeAlSiO_2$). The absence or minor presence of Ca-bearing silicates e.g. gehlenite, wollastonite, pyroxene and anorthite in the other samples is indicative for firing temperatures around 800°C.

Table 10.7. EDX results of bulk clay matrix of Barcın Höyük potsherds

	EDX Results (%)									
Sample #	Type	Residue	SiO_2	Al_2O_3	CaO	MgO	Na_2O	K_2O	FeO	TiO_2
BH 2304-2		Yes	63,4	17	4,5	4,45	2,17	1,94	8,44	1,48
BH 2473		Yes	66,9	21	1,5	1,7	nd	2,72	5,99	nd
BH 3181		Yes	54,9	24	4,3	2,56	0,99	3,45	9,3	0,96
BH 3193		Yes	65,3	18	3,4	3,1	1,6	3,53	7,64	nd
BH2190		No	65,3	18	4,2	2,58	2,23	2,17	5,17	nd
BH 2454		No	66,4	19	1,4	2,52	3,39	2,8	5,38	nd
BH 2480		No	50,4	24	9,4	2,83	nd	1,74	10,4	1,65
BH2487		No	62	21	3	1,29	nd	3,91	8,06	1,5

In Table 10.6 the thickness, temper and minerals present in the analyzed Barcın Höyük potsherds are also given. Scanning electron images (Figure 10.9 to 10.16) off all potsherds, except sample BH2473, contain large quartz inclusions. The angular shape of the inclusion in samples BH3195, BH2454, BH2190 and BH2480 suggest that the inclusions are added rather than indigenous. On the other hand, the inclusions in samples

BH2304-2, BH2487 and BH3181 are rounded, indicative of indigenous character. There were no correlation between inclusions and possible ability of lipid absorption.

Table 10.8. XRD data of the minerals in the temper

Sample	Thickness	Temper Type	Mineral
BH2304-2	10mm	Quartz	Albite-muscovite
BH2473	7mm	-	Anorthite ($CaAl_2SiO_8$)
BH3181	7.8mm	Quartz	Anorthite & Albite
BH3195	4mm	Quartz & Calcite	Calcite & Muscovite
BH2454	10mm	Quartz	Albite-muscovite
BH2480	10mm	Quartz	Anorthite ($CaAl_2SiO_{8)}$
BH2487	9mm	Quartz & Hematite	Orthoclase(KAl_2SiO_8)
BH2190	10mm	Quartz	Albite

In accordance with the micro structural and mineralogical investigation results of Barcın Höyük cooking pots, it can be argued that these pots were made out of non-calcareous clay and with abundant quartz inclusions. All the potsherds, except BH2480, are fired at low temperatures. The main body of the potsherds shows highly uniform chemical composition. Given the limited number of sherds analyzed, however, it is not possible to make a definite conclusion about the structure and firing temperatures of the cooking pots and the relation of these parameters with lipid absorption. There are no distinctive differences with composition and the structure of the group of potsherds with residue contents.

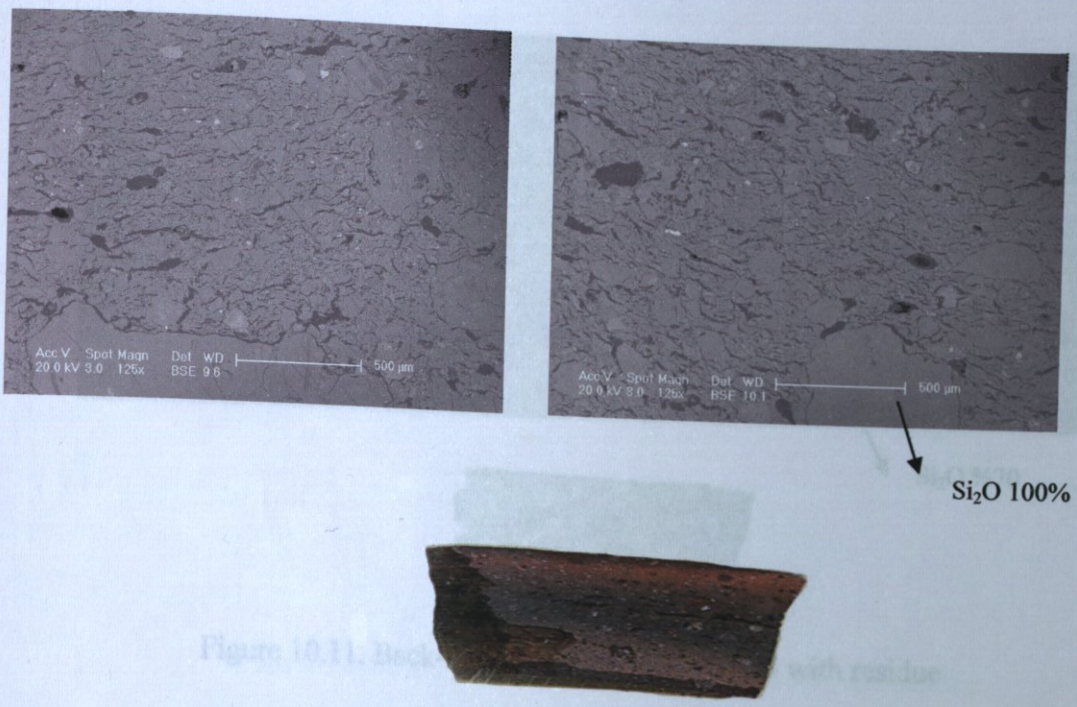


Figure 10.9. Back-scattered image of BH2190 without residue

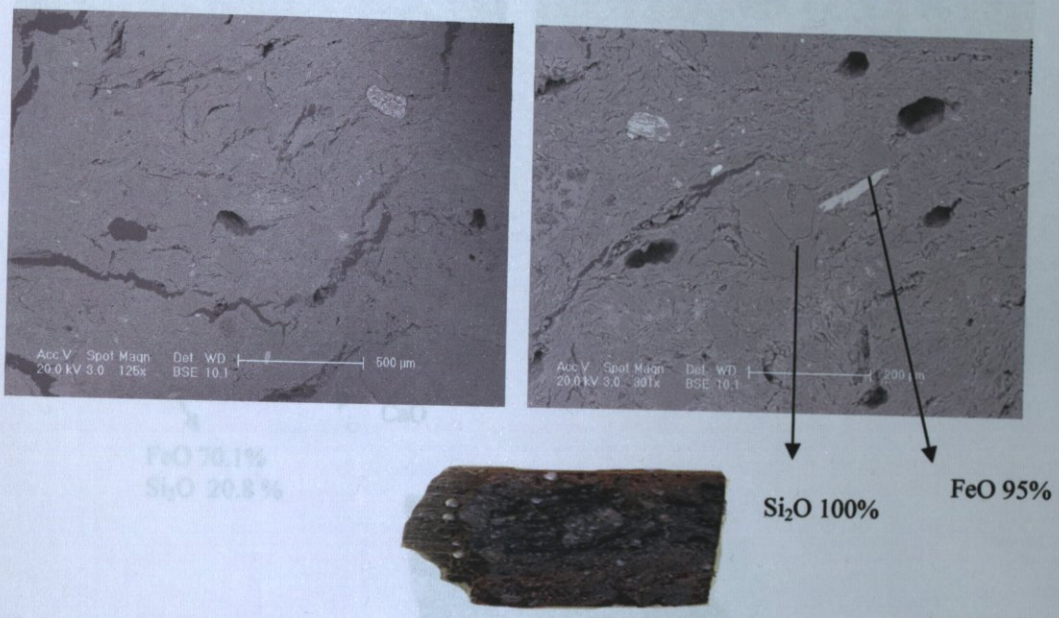


Figure 10.10. Back-scattered image of BH2304-2 with residue

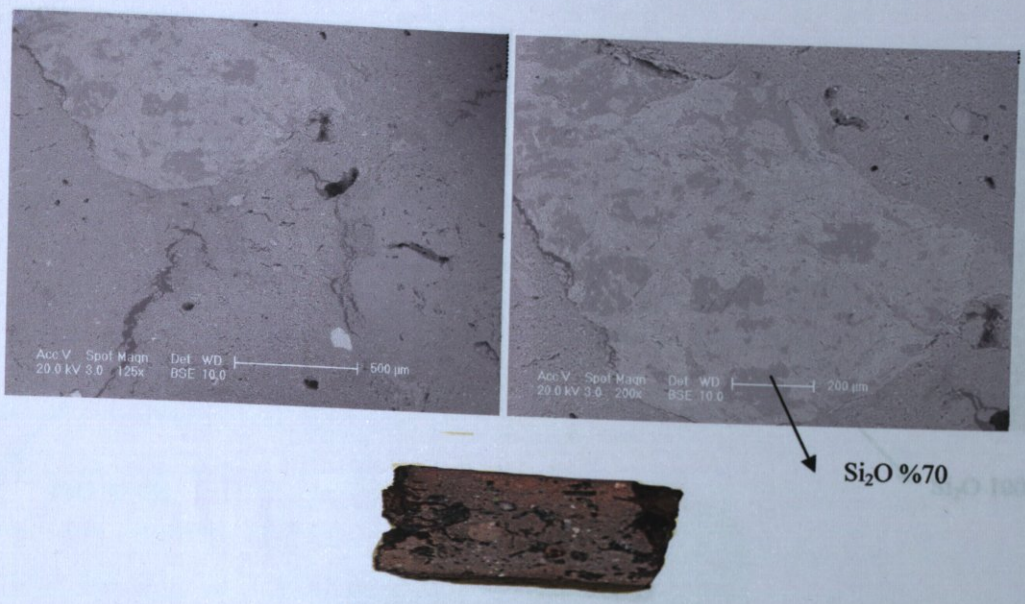


Figure 10.11. Back-scattered image of BH2473 with residue

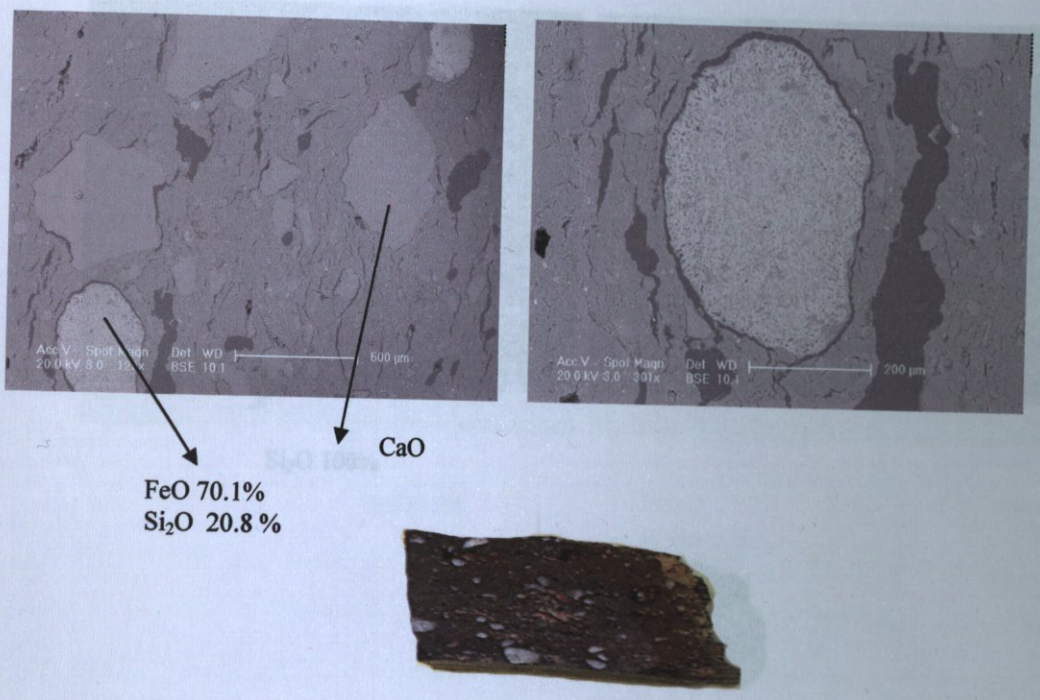


Figure 10.12. Back-scattered image of BH2487 without residue

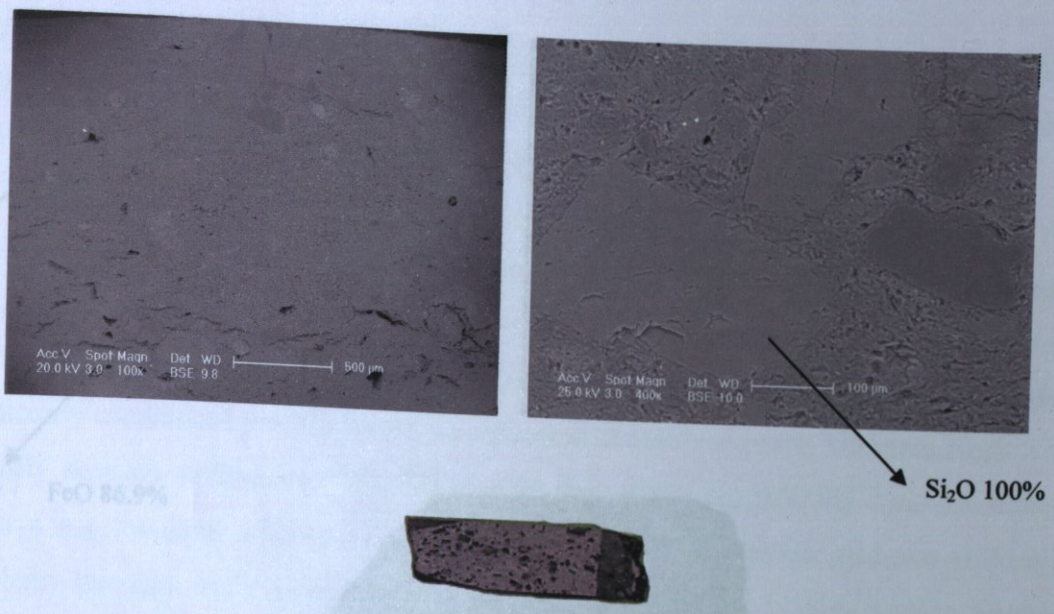


Figure 10.13. Back scattered image of BH3195 with residue

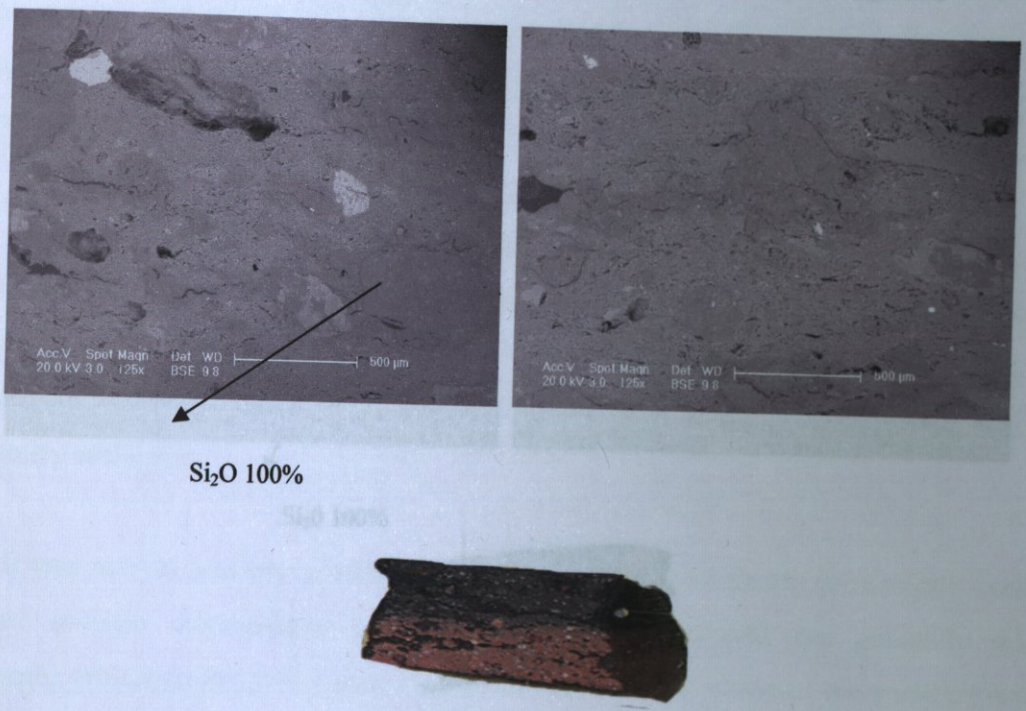


Figure 10.14. Back-scattered image of BH3181 with residue

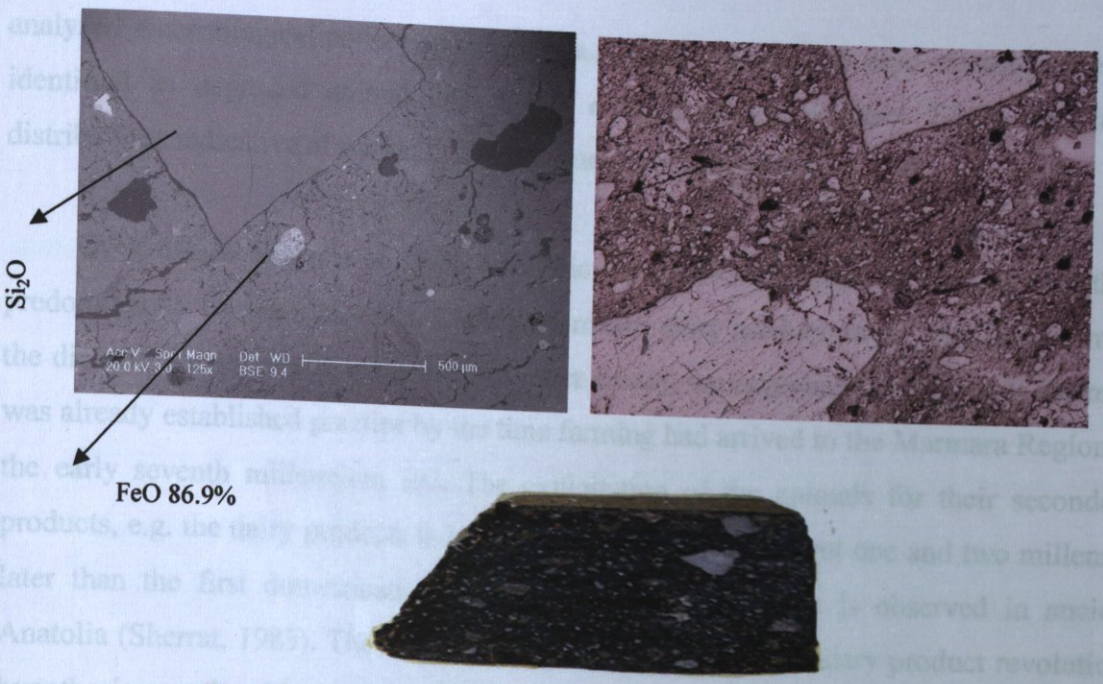


Figure 10.15. Back-scattered image of BH2454 without residue

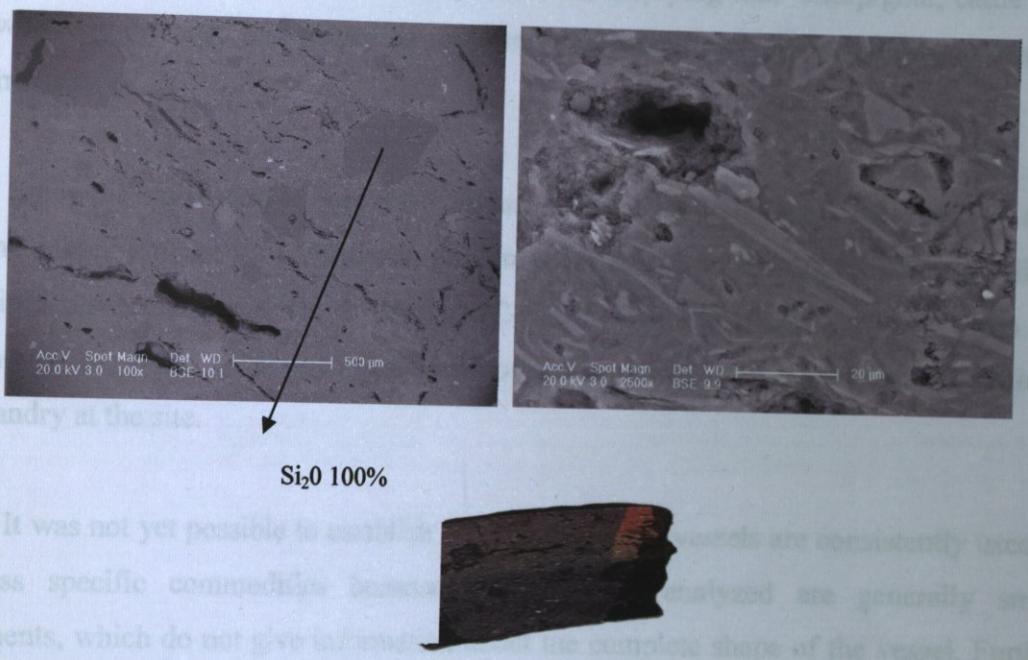


Figure 10.16. Back-scattered image of BH2480 without residue

10.2.5. Discussion

The lipid residues extracted from Barcın Höyük are fairly well preserved with 17 per cent yielding significant concentration. High percentages of TAG components in the lipid residues illustrate a remarkable level of survival for these lipids in some of the oldest

analyzed archaeological pottery samples from Anatolia. Out of 10 lipid extracts, nine are identified as degraded animal fats. Seven extracts exhibited intact TAGs that have distributions indicative of ruminant and non-ruminant animal fats.

GC-C-IRMS analysis of seven Neolithic Barcın Höyük potsherds showed that they predominantly contain dairy fats. Clearly, ruminant dairy products were very important in the diets of Barcın Höyük residence. In other words, the exploitation of animals for milk was already established practice by the time farming had arrived to the Marmara Region in the early seventh millennium BC. The exploitation of the animals for their secondary products, e.g. the dairy products is believed to have occurred about one and two millennia later than the first domestication of sheep/goat and cattle that is observed in ancient Anatolia (Sherrat, 1983). The results shown above fit with 'secondary product revolution' hypothesis postulated by Andrew Sherrat (1983). The two potsherds, dated to Chalcolithic period, contained ruminant adipose and porcine fats implying that sheep/goat, cattle and pig were domesticated and played an important role in the diets of in indigenous Barcın community.

Since the excavation at Barcın Höyük just started over a year ago, there are no data for the animal remains associated with this site in order to compare with the findings from the lipid residue analysis. However, the GC-C-IRMS results of lipid extracts from the potsherds by itself give considerable information about the importance of animal husbandry at the site.

It was not yet possible to establish whether particular vessels are consistently used to process specific commodities because the potsherds analyzed are generally small fragments, which do not give information about the complete shape of the vessel. Further study is needed to establish such relationships.

No evidence of processing foodstuff from plant source is observed in the investigated vessels. This can be explained by the fact that plants generally have lower abundances of lipid compared to animal tissues. It is also due to the easier hydrolysis and oxidation of plant oils. However, there are a number of examples of processing plant products in pottery

vessels from some prehistoric European sites as well as some from highly arid ancient Egypt. (Copley *et al.* 2005)

The results of the lipid residues and stable isotope analysis from Barcın Höyük are summarized in Table 10.9.

Table 10.9. The lipid assignments of Barcın Höyük potsherd derived from TLEs, TAG distributions and $\Delta\ ^{13}C$ values

Sample name	TLE	TAG distribution	$\Delta\ ^{13}C$ values	Interpretation
BH3164	animal fat	rum.adipose/or degraded dairy	rum.dairy	rum.dairy
BH2499	animal fat	-	rum.dairy	rum.dairy
BH2473	animal fat	rum.adipose/or dairy	rum.dairy	rum.dairy
BH2304-2	animal fat	porcine	porcine	porcine
BH2187	animal fat	rum.adipose	rum.adipose	rum.adipose
BH3168	animal fat		dairy	dairy
BH3195	animal fat		dairy	dairy
BH3190	animal fat	-	-	animal fat
BH3184	animal fat	-	-	animal fat
BH3181	beeswax	-	-	beeswax

10.3. Others

10.3.1. Beeswax in Barcın Höyük Pottery

The archaeological sample (BH3181) from Barcın Höyük was recovered in the excavation in 2007. The pottery sample was collected from trench L11 i.e. dated to Late Neolithic period (circa 6.400 BC). The pottery surface was free of visible residue (Figure 10.17). The shape of the vessels to which this sample belong is not known as the sample is just small fragment of a vessel.

Figure 10.17. Neolithic Barcın Höyük potsherd (BH3181)

The presence of beeswax is easily established by HT-GC analysis which enables the separation of a range of wax esters, long chain alcohols and n- alkanes (Evershed, 2001). The example for HTGC of contemporary beeswax can be seen in Figure 10.18. Peak identities in this chromatogram are: FA24-FA34, saturated fatty acids bearing 24-34 carbon atoms, respectively, FA24 = tetracosanoic acid (C_{24}); FA26 = hexacosanoic acid (C_{26}); FA28 = octacosanoic acid (C_{28}); FA30 = triacontanoic acid (C_{30}); FA32 = dotriacontanoic acid (C_{32}); FA34 = tetratriacontanoic acid (C_{34}); AL23-AL31, *n*-alkanes containing 23-31 carbons, respectively, AL23 = triacosane (C_{23}); AL25 = pentacosane (C_{25}); AL27 = heptacosane (C_{27}); AL29 = nonacosane (C_{29}); AL31 = hentriacontane (C_{31}); IS, internal standard, *n*-tetratriacontane (C_{34}); W40-W54, wax esters containing 40-54 carbons, respectively, W40 = tetracosanyl palmitate (C_{40}); W42 = hexacosanyl palmitate (C_{42}); W44 = octacosanyl palmitate (C_{44}); W46 = triacontanyl palmitate (C_{46}); W48 = dotriacontanyl palmitate (C_{48}); W50 = tetratriacontanyl palmitate (C_{50}); W52 = hexatriacontanyl palmitate (C_{52}); W54 = octacontanyl palmitate; HW42-HW50, hydroxy-fatty acid-wax esters containing 42-50 carbons, respectively, HW42 = hexacosanyl hydroxy-palmitate (C_{42}); HW44 = octacosanyl hydroxy-palmitate (C_{44}); HW46 = triacontanyl hydroxy-palmitate (C_{46}); HW48 = dotriacontanyl hydroxy-palmitate (C_{48}); HW50 = tetratriacontanyl hydroxy-palmitate (C_{50}), and HW52 = hexatriacontanyl hydroxy-palmitate (C_{52}). In short fresh beeswax contains hydrocarbons, free fatty acids, wax and hydroxywax esters (Tulloch, 1980). The hydrocarbons range from 25 to 33 in carbon number, C27 being the major one.

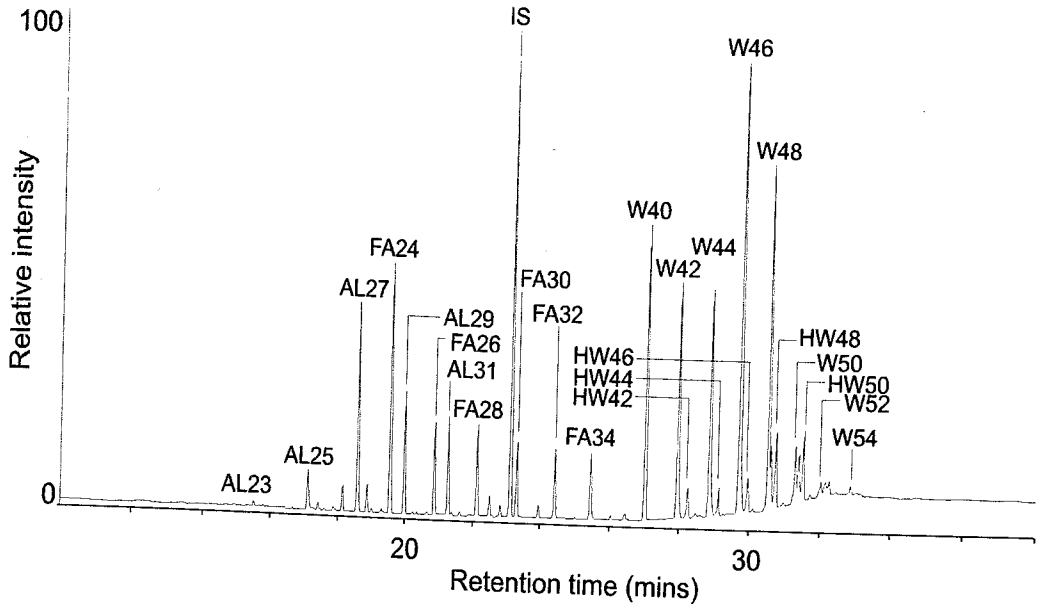


Figure 10.18. Partial HTGC chromatogram of contemporary trimetylsilylated beeswax (Evershed *et al.*, 1997).

The chromatographic profile of ancient beeswax often presents significant differences to that of contemporary beeswax, due to degradation of this material through time (Regert *et al.*,2001). The ester pattern appears to be very stable despite a partial hydrolysis leading to the formation of palmitic acid and long-chain even-numbered alcohols, whereas the alkane pattern is partly modified and depleted (Evans and Heron, 1993; Regert *et al.*, 1999). This is also the case with Barcın Höyük potsherd. This potsherd revealed a particular chromatographic profile as shown in Figure 10.19.

GC analysis of BH3181 potsherd led to identification of trace amount of palmitic acid and four series of homologous compounds, including odd-numbered n-alkanes in the range C_{27}-C_{31}. The high amount of alcohols clearly indicates that the esters have been hydrolysed. The fatty alcohols are present with a carbon number distribution indicating that they originated by the hydrolysis, from long-chain wax esters. The low amount of palmitic acid released by ester hydrolysis, may be explained by a sublimation phenomenon of this compound, similar to that observed on n-alkanes see Figure 10.20.

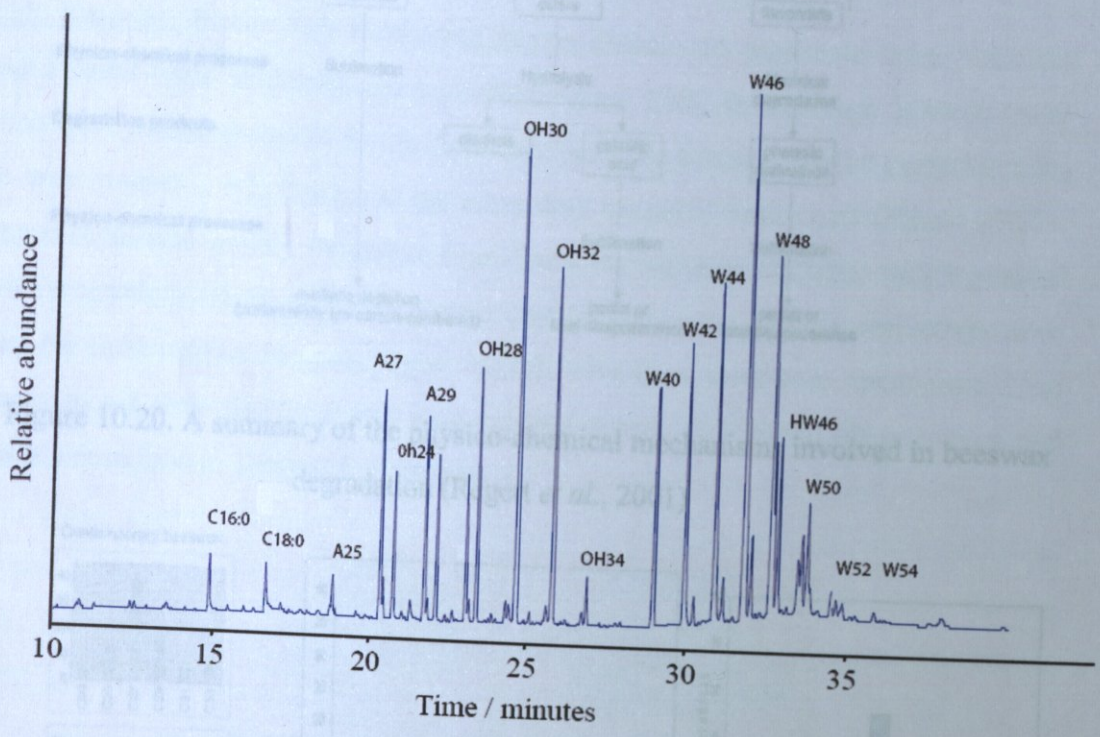


Figure 10.18. Partial HTGC of trimethylsilylated BH3181

If we look at the histograms in Figure 10.21. (were Cx corresponds to n-alkane with x carbon atoms and WE corresponds to wax ester containing y carbon atoms) we can clearly notice that wax ester distribution in the Barcın sample is remarkably close to that of the authentic wax, although slight depletion in the C_{40} wax ester relative to the other ester is evident. The C46 wax ester (triacontyl hexadecanoate) is the most abundant constituent in the ancient and modern samples.

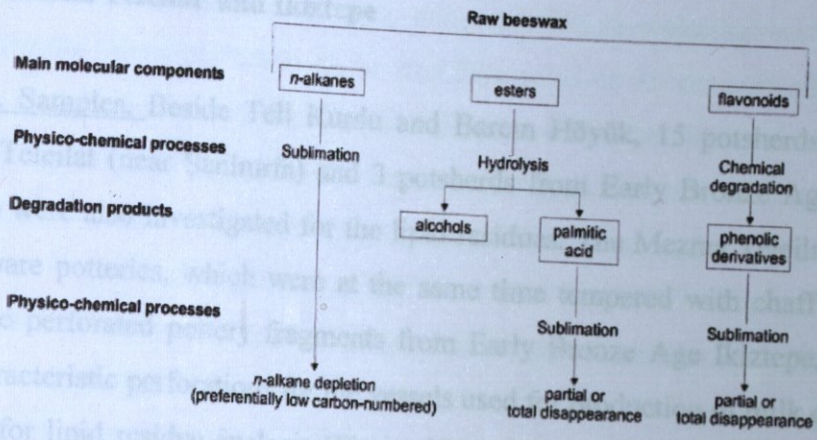
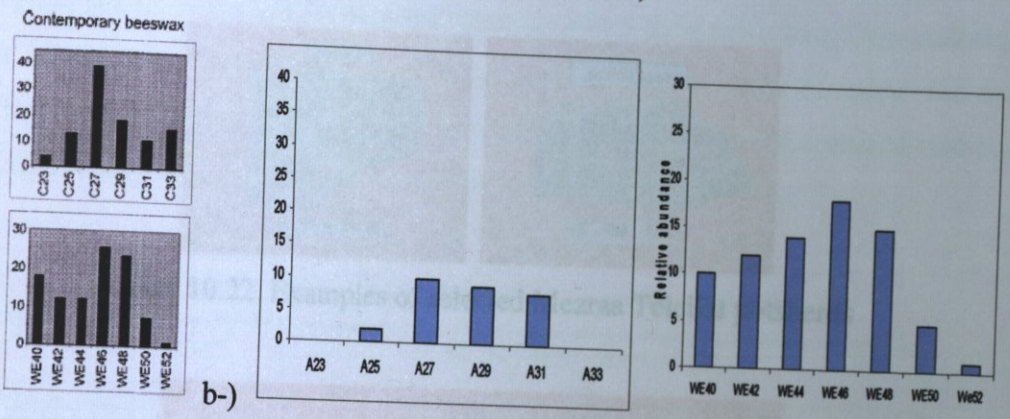


Figure 10.20. A summary of the physico-chemical mechanisms involved in beeswax degradation (Regert *et al.*, 2001)



a-) b-)

Figure 10.21. Wax ester and alkane distributions in a-) contemporary beeswax Regert *et al.*,2001) and b-) Barcın beeswax

The results of the analysis of beeswax in Neolithic Barcın Höyük pottery provided the first evidence for one of the oldest use of beeswax directly attested to Turkey. The remains of beeswax dating to Late Neolithic were also known in Dikili Tash in Greece (Regert et al., 2003), Neolithic Germany (Heron et al.,1994) and Neolithic Bercy in France (Regert et al., 2003). But even the oldest beeswax among them was dated to a few hundred years later than Barcın Höyük sample.

It is difficult to clearly understand the function of this material in ceramic vessels. It may have been used to waterproof the porous matrix of ancient pottery or another possibility is that beeswax was the part of culinary preparations containing honey.

10.3.2. Mezraa Teleilat and İkiztepe

10.3.2.1. Samples. Beside Tell Kurdu and Barcın Höyük, 15 potsherds from Neolithic Mezraa Teleilat (near Şanlıurfa) and 3 potsherds from Early Bronze Age İkiztepe (near Samsun) were also investigated for the lipid residues. The Mezraa Teleilat potteries were coarse ware potteries, which were at the same time tempered with chaff (Figure 10.22). The three perforated pottery fragments from Early Bronze Age İkiztepe, which possess very characteristic perforations for the vessels used for production of milk derivatives were selected for lipid residue analysis (Figure 10.23). Also there were soot traces over these potsherds, which show that the sherds were exposed to heat. Details of the ware type and periods are described in Table A.2., Appendix A.

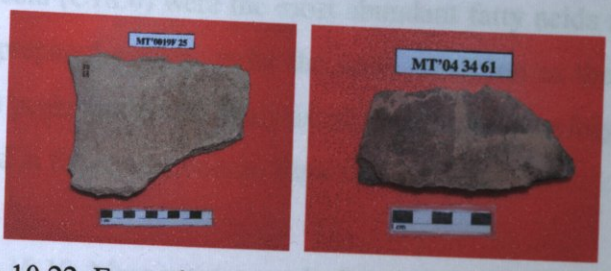


Figure 10.22. Examples of selected Mezraa Teleilat potsherds

Figure 10.23. Perforated potsherds from İkiztepe

10.3.2.2. Results & Disccussion. The organic residues extracted from the pottery vessels both with ultrasonication bath and soxhlet apparatus were screened with GC and GC/MS instruments in order to separate and identify the components present in the residue. Out of totally 15 potsherds from Mezraa Teleilat only two yielded lipid residues. From the three İkiztepe potsherds only one was found to contain lipid residue. GC analyses of total lipid

extracts obtained from soxhlet and ultrasonic extraction gave similar results. According to the GC results, the distribution of the free fatty acids in the chromatograms indicate the presence of degraded animal fat. The identification of the free fatty acids was done on the basis of the previously measured GC chromatograms of reference materials as well as on the basis of mass spectrometry fragmentation patterns given in the Table 10.10. In Table 10.10. the fragmentation patterns of MT'99 19F25 and I-440 samples were given. The trace amounts of mono-, di- and triacylglycerols were detected only in the sample MT'99 19 F 25 and result from extensive chemical or enzymatic hydrolysis of acylglycerol moieties. The sample I-440 from İkiztepe was found to contain free fatty acids and only trace amount of monoacylglycerol which is indicative of highly degraded animal fats (Figure B14 Appendix B). GC and GC/MS analyses also show that especially palmitic acid ($C_{16:0}$) and stearic acid (C18:0) were the most abundant fatty acids in all the samples. The blank which was prepared exactly under the same condition as the samples did not give any free fatty acid peaks in the GC analysis, so that it can be safely assumed that the free fatty acids observed in the residues come from the samples.

Table 10.10. Mass spectroscopic data of I-440 potsherd

Compound name	EI MS spectra *m/z values*
Pelagronic acid ($C_{9:0}$)	215(M+-15),145,132,117,75,73
Capric acid ($C_{10:0}$)	229(M+-15),145,132,117,75,73
Lauric acid ($C_{12:0}$)	257(M+-15),145,132,117,75,73
Myristic acid ($C_{14:0}$)	285(M+-15),145,132,117,73
Palmitic asit ($C_{16:0}$)	313 (M+-15),145, 132,117,73
Stearic asit ($C_{18:0}$)	341(M+-15),145, 132,117,73
Monoolein ($MAG_{18:1}$)	485(M+-15),410,397,339,265,147,129,103,73

In the modern animal fats the ratio between $C_{16:0}/C_{18:0}$ is one to two. But in the plant oils it is around three (Evershed, 2005). The ratio of $C_{16:0}/C_{18:0}$ was found to be less than three in the analysed residues therefore it indicates that they derived from animal fat. Generally sheep fat contains greater amount of stearic acid ($C_{18:0}$) than paltimic ($C_{16:0}$) acid but since this is not observed in any of the samples the origin the sheep was not likely to be the source of these fats. Besides palmitic and stearic acids myristic acid (C14:0) was detected in samples MT'04 34R64 and I-440, which is another indication of the animal origin of the fats (Figure B.14 and B15 Appendix B). Also oleic acid ($C_{18:1}$) was observed in trace amount in some of samples namely MT'04 34R64 (Figure B15 Appendix B).

Unsaturated fatty acids (e.g. oleic acid) react spontenously with atmospheric oxygen and produce water soluble compounds, which leach out of ceramics easily. This fact explains the trace amount of oleic acid present in the samples. On the other hand the cholesterol, one of the most important biomarkers of animal fat, was not detected by GC analysis in any sample.

Distribution of trace amount of TAGs (C_{48}-C_{54}) in the sample MT'9919F25 from Mezraa Teleilat is indicative of porcine fat.

Some contamination products like phthalate esters (plasticizers) were observed during GC and GC/MS analyses. The identification of these contaminants relied on the abundant *m/z* 149 peak found in the mass spectrum. Plasticizers generally originate from the plastic bags which were used for sample storing and also from the vial caps used for sample preparation in laboratory. But peaks due to contaminants are very well-known and they do not interfere with the peaks of interest, so that they can be tolerated to a certain extent.

No *n*-alkane, waxester, ketone, sitosterol etc. were observed in the studied residues, so it can be assumed that plant products were not processed in these pottery vessels.

In light of the above given results, the free fatty acids and the trace amounts of mono-, di- and triacylglycerols found in the two lipid extracts from Neolithic Mezraa Teleilat pottery vessels are among the oldest organic residues ever submitted to analysis from such ancient archaeological context

The three perforated potsherds from Early Bronze Age İkiztepe settlement are of special importance (Figure 10.23). These types of perforated potteries have been proposed to be used for processing dairy products and especially for production of butter (Schoop, 1998). Today, in Central Anatolia people still use similar perforated vessels for butter production.

In the sample I-440 from İkiztepe lauric acid, which is found in milk fats, was observed. Again in the same sample short chain fatty acids specific for the milk fats like

pelagronic acid ($C_{9:0}$) and capric acid ($C_{10:0}$) were detected. Observing these fatty acids after many millennia burial is very rare. It can be explained with relatively arid envoirment and suitable pH of the soil. Besides lauric acid, pelagronic acid and capric acid , palmitic and stearic acids were identified in higher amounts. In order to obtain definite information about the origin of the lipids in this potsherd GC-C-IRMS analysis was applied.

The lipid extract of I-440 potsherd was analysed by the GC-C-IRMS and the $\delta\ ^{13}C$ values are displayed in Table 10.11. The $\Delta\ ^{13}C$ values plotted against the $\delta\ ^{13}C_{16:0}$ are displayed in Figure 10.24. Using this criterion, the İkiztepe sample was found out the value of -2.15 was found corresponding to ruminant adipose fat. But it is known that a mixture of dairy and porcine fats give rise to a $\Delta\ ^{13}C$ value corresponding to that of ruminant fat (Evershed *et. al.*,2003). The mixing of fat is a problem that highlights the importance of using the different methods of data analysis in conjuction with each other and applying cautious interpretation to the observed data.

Table 10.11. Stable carbon isotope values for I-440 potsherd

Sample No	$\delta\ ^{13}C_{16:0}$‰	$\delta\ ^{13}C_{18:0}$‰	$\Delta\ ^{13}C$
I-440	-28.38	- 30.54	- 2.15

Above all, this result indicates these perforated vessels were really used for processing of dairy products even in Early Bronze Age and that some porcine fat was also processed in this particular potsherd. Furthermore, this result also demonstrates that the animals used for milking were reared on C_3 diet. In short, the above stated hypothesis for perforated vessels was confirmed by chemical analysis.

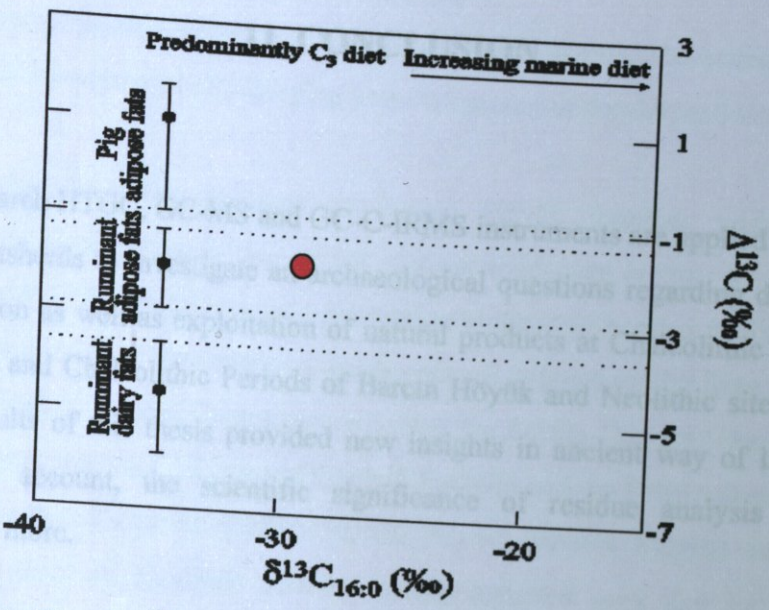


Figure 10.24. Plot of $\Delta\ ^{13}C$ vs. $\delta\ ^{13}C_{16:0}$ I-440 from Ikiztepe. The ranges for modern reference fats are shown on the y-axis.

11. CONCLUSION

In this research HTGC, GC-MS and GC-C-IRMS instruments are applied on the lipid extracts from potsherds to investigate an archaeological questions regarding dairying and animal exploitation as well as exploitation of natural products at Chalcolithic site of Tell Kurdu, Neolithic and Chalcolithic Periods of Barcın Höyük and Neolithic site of Mezraa Teleilat. The results of this thesis provided new insights in ancient way of life. Taking those facts into account, the scientific significance of residue analysis has been demostrated once more.

A total of 118 potsherds were analysed; 40 were excavated from Tell Kurdu, 60 from Barcın Höyük, 15 from Mezraa Teleilat and 3 three from Ikiztepe. Only 15 potsherds yielded appreciable amounts of lipid residues to determine the distribution of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acid. Lipid preservation was found to vary greatly between the sites. Potsherds from the Barçın Höyük yielded the hightest lipid amounts of lipid residues as well as highest concentration of TAGs. TAGs detected in the Barcın Höyük potsherds illustrates the remarkable level of survival of these compounds in some of the oldest archaeological pottery ever submitted to organic residue analysis. The lowest preservation was observed in Tell Kurdu potsherds. All of the potsherds except one from all sites that yielded significant lipid residues were identified as degraded animal fats. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from 10 lipid extracts analysed by GC-C-IRMS showed that five samples were from dairy fats, two contained ruminant adipose fats, one contained porcine adipose fat and one contained a mixture of dairy and porcine adipose fat. No plant lipids (e.g. n-alkanes, wax esters) were detected in any of the potsherds.

One of the most striking outcome of the data obtained in this study is the evidence of extensive processing of dairy products in Barcın Höyük pottery samplas dated to between 6500-6000 BC. Evershed *et al.* (2008) also obtained evidences for processing of dairy products in the potteries from Northwestern Anatolian sites such as Aşağı Pınar (5500-5000BC), Toptepe (5500-5000), Yarımburgaz (6000-5500), Fikirtepe (6000-5500), Hoca Çeşme (6500-5500) and Pendik (6500-6000). Thus, the milking of ruminant animals was clearly practiced during seventh millenium BC in Barcın Höyük as well as in other

Northwestern Anatolian sites mentioned above. In other words, the results yield new information about the emergence of dairying as a component of the domesticated animals.

The detection of dairy products at Barcın Höyük together with the other Northwestern sites cited above is one of the earliest evidences so far, by one to two millenia dating back to the start of ceramic production in the region. Also, it is important to notice that extensive milking of ruminant animals occured at locations far from the original region of domesticaion, namely Central Anatolia and the Fertile Cresent. The analyses of pottery samples from the Neolithic sites of Central Anatolia and the Fertile Cresent such as Çatalhöyük, Domuztepe, Tepecik Çiftlik Çayönü Tepesi, Mezraa Teleilat and Akarçay Tepe as well as some of Neolithic sites in Levant revealed very few indications of processing dairy products (Eversed *et al.* 2008). In addition, Evershed *et al.* (2008) showed that potsherds belonging to Neolithic sites of Central Europe and Northern Greece also have a low indications of processed dairy fats. Therefore, it can be concluded that regional conditions linked with raising cattle, sheep and goats seem to play important role in the intensification of milk usage in different parts of Near East and Southeastern and Central Europe.

In short, our findings support the hypothesis that the early history of processed milk goes back to the seventh millenium BC, very early in the evolution of animal domestication and pottery production and use. Significantly, the results suggests that even around 6500 BC milk was processed, which was a remarkable achievement to store milk products. This could be explanation why, in spite of lactose intolerance, milk use could be adopted quickly.

It was noticebale that two of the potsherds from Tell Kurdu, which were used for processing ruminant adipose fats were extracted from the very charactersitic Halaf type splayed-rim cooking pots. They were found in context including small fire pits, which enable us to assign these vessels as cooking pots by direct chemical analysis.

Analysis of a series of archaeological potsherds from Neolithic and Chalcolithic Barcın Höyük led to the identification of degraded beeswax in one potsherd. To our knowledge the identification of beeswax in a Neolithic ceramic vessel from Barcın Höyük

corresponds to one of the earliest use of beeswax in Anatolia. Most probably beeswax was used as a waterproofing agent of ceramic vessels but it can also be used for culinary purpose as well.

This work also incorporates chemical analyses on few unique archaeological potsherds from İkiztepe. The three perforated potsherds that were dated to Early Bronze Age also showed the presence of milk fat biomarkers. Further stable isotope analysis confirmed the use of dairy fat together with porcine adipose fat in these perforated potherds. Thus, by chemical analyses we were able to demostrate that potsherds with this charcaterictic feature were primarily used for processing dairy fats since Early Bronze Age.

APPENDIX A: LIST OF ANALYZED POTSHERDS

Table A1. List of Tell Kurdu Potshreds

Exc.No	Site	Trench	Locus	Lot	Period	Type
TK 2537-4	Tell Kurdu	13	2	5	Ubaid	Ceramic sieve
TK 3687-1	Tell Kurdu	13	5	33	Ubaid	Rim/neck
TK 3687-2	Tell Kurdu	13	5	33	Ubaid	Body
TK 6237-1	Tell Kurdu	25	1	3	Halaf	Body of a splayed rim cooking pot
TK 6237-2	Tell Kurdu	25	1	3	Halaf	Base
TK 6237-3	Tell Kurdu	25	1	3	Halaf	Body
TK 5377-1	Tell Kurdu	21	1	5	Halaf	Rim of a mini splayed rim cooking vessel
TK 5377-2	Tell Kurdu	21	1	5	Halaf	Body
TK 5377-3	Tell Kurdu	21	1	5	Halaf	Neck/shoulder of a collared rim bowl
TK 5377-4	Tell Kurdu	21	1	5	Halaf	Body
TK 5377-5	Tell Kurdu	21	1	5	Halaf	Body
TK 5377-6	Tell Kurdu	21	1	5	Ubaid	Body
TK 5377-7	Tell Kurdu	21	1	5	Halaf	Body
TK 2825-1	Tell Kurdu	13	5	16	Ubaid	Body
TK 2825-2	Tell Kurdu	13	5	16	Ubaid	Body
TK 2825-3	Tell Kurdu	13	5	16	Ubaid	Body
TK 2825-4	Tell Kurdu	13	5	16	Ubaid	Rim/neck
TK 2565-1	Tell Kurdu	13	3	8	Ubaid	Body
TK 2565-2	Tell Kurdu	13	3	8	Ubaid	Body
TK 2565-3	Tell Kurdu	13	3	8	Ubaid	Body
TK 2565-4	Tell Kurdu	13	3	8	Ubaid	Body
TK 2565-5	Tell Kurdu	13	3	8	Ubaid	Body
TK 6226-1	Tell Kurdu	25	1	1	Halaf	Rim of a splayed rim cooking vessel
TK 6226-2	Tell Kurdu	25	1	1	Halaf	Neck and shoulder of a collared rim bowl
TK 6226-3	Tell Kurdu	25	1	1	Halaf	Body, thick sherd with shell and grot temper
TK 6226-4	Tell Kurdu	25	1	1	Halaf	Rim of a splayed rim cooking vessel
TK 6226-5	Tell Kurdu	25	1	1	Halaf	Rim of a splayed-rimmed vessel
TK6760C	Tell Kurdu	25	36	34	halaf	Rim of a splayed-rimmed vessel
TK 6226-6	Tell Kurdu	25	1	1	Halaf	Flat Base
TK 6226-7	Tell Kurdu	25	1	1	Halaf	Flat Base
TK 6226-8	Tell Kurdu	25	1	1	Halaf	Shoulder of a collared rim bowl
TK 6226-9	Tell Kurdu	25	1	1	Halaf	Body, thick sherd with shell and grot temper
TK 6226-10	Tell Kurdu	25	1	1	Halaf	Rounded but

						partically flat bottomed Base
TK 6226-11	Tell Kurdu	25	1	1	Halaf	Thick body sherd
TK 6226-12	Tell Kurdu	25	1	1	Halaf	Rim
TK 6226-13	Tell Kurdu	25	1	1	Halaf	Shoulder of a collared rim bowl
TK 3657-1	Tell Kurdu	13	5	33	Chalcolithic	Rim
TK 5330-1	Tell Kurdu	21	1	1	Ubaid	Rim of bowl or jar
TK 5330-2	Tell Kurdu	21	1	1	Ubaid	Body
TK 5330-3	Tell Kurdu	21	1	1	Halaf	Body and neck of a collared rim bowl
TK 5330-4	Tell Kurdu	21	1	1	Halaf	Rim of a very very fine ware vessel, could be bowl or jar
TK 5330-5	Tell Kurdu	21	1	1	Halaf	Rim
TK 5330-6	Tell Kurdu	21	1	1	Ubaid	Body
TK 5332-1	Tell Kurdu	21	1	2	Halaf	Rim
TK 5332-2	Tell Kurdu	21	1	2	Halaf	Body
TK 5332-3	Tell Kurdu	21	1	2	Halaf??	Base
TK 5360-1	Tell Kurdu	21	1	4	Halaf	Rim
TK 5360-2	Tell Kurdu	21	1	4	Halaf	Body
TK 5394-1	Tell Kurdu	21	1	7	Halaf	Rim/neck of a collared rim bowl
TK 5394-2	Tell Kurdu	21	1	7	Halaf	Shoulder of a collared rim bowl, no rim preserved
TK 6334-1	Tell Kurdu	25	28	27	Halaf	Body
TK 5338-1	Tell Kurdu	21	1	3	Halaf ?	Base
TK 5338-2	Tell Kurdu	21	1	3	Halaf	Full rim to base profile of a platter
TK 5338-3	Tell Kurdu	21	1	3	Halaf	Body
TK 5338-4	Tell Kurdu	21	1	3	Halaf	Rim
TK 5338-5	Tell Kurdu	21	1	3	Halaf	Rim
TK 5338-6	Tell Kurdu	21	1	3	Halaf	Rim and shoulder of a collared rim bowl

Table A.2. List of Barcın Höyük Potsherds

EXC	SİTE	TRENCH	LOCUS	LOT	PERIOD
BH 2463	Barçın Höyük	M10	64	?	Chalcolithic
BH 2453	Barçın Höyük	M10	60	228	Chalcolithic
BH2480	Barçın Höyük	M11	15	70	Chalcolithic
BH 2500	Barçın Höyük	L11	210	276	Chalcolithic
BH 2479	Barçın Höyük	L11	205	251	Chalcolithic
BH 2495	Barçın Höyük	M11	35	90	Chalcolithic
BH 2458	Barçın Höyük	M10	64	240	Chalcolithic
BH 2477	Barçın Höyük	M11	8	63	Chalcolithic
BH 3194	Barçın Höyük	M10	75	285	Chalcolithic
BH 2459	Barçın Höyük	M11	5	43	Chalcolithic
BH 2474	Barçın Höyük	M10	64	250	Chalcolithic
BH 2496	Barçın Höyük	M11	35	93	Chalcolithic
BH 2462	Barçın Höyük	M11	5	45	Chalcolithic

BH 2451	Barçın Höyük	M11	5	31	Chalcolithic
BH 2471	Barçın Höyük	M10	64	247	Chalcolithic
BH 2454	Barçın Höyük	M10	59	227	Chalcolithic
BH 2484	Barçın Höyük	M11	15	73/72	Chalcolithic
BH 2489	Barçın Höyük	M11	15	82	Chalcolithic
BH 2481	Barçın Höyük	M11	15	71	Chalcolithic
BH 2487	Barçın Höyük	M11	15	74	Chalcolithic
BH 2486	Barçın Höyük	L11	218	258	Chalcolithic
BH 2482	Barçın Höyük	M10	69	257	Chalcolithic
BH 2460	Barçın Höyük	M11	5	43	Chalcolithic
BH 2497	Barçın Höyük	M10	73	270	Chalcolithic
BH 2490	Barçın Höyük	M11	30	80	Chalcolithic
BH 2465	Barçın Höyük	M11	5	48	Chalcolithic
BH 2473	Barçın Höyük	L11	205	244	Chalcolithic
BH 2483	Barçın Höyük	L11	217	252	Chalcolithic
BH 2499	Barçın Höyük	L11	218	274	Chalcolithic
BH 2455	Barçın Höyük	L11	205	231	Chalcolithic
BH 2478	Barçın Höyük	M10	69	254	Chalcolithic
BH 3176	Barçın Höyük	L11	223	292	Chalcolithic
BH 3182	Barçın Höyük	M11	3	47	Early Bronze Age
BH 3189	Barçın Höyük	M10	64	244	Neolithic
BH 2268	Barçın Höyük	L11	208	226	Chalcolithic
BH 3172	Barçın Höyük	M11	35	98	Chalcolithic
BH 3181	Barçın Höyük	L11	205	251	Chalcolithic
BH 3175	Barçın Höyük	L11	218	290	Chalcolithic
BH 2241	Barçın Höyük	L11	205	225	Chalcolithic
BH 3169	Barçın Höyük	L11	210	288	Chalcolithic
BH 3193	Barçın Höyük	L11	218	296	Chalcolithic
BH 2303	Barçın Höyük	M10	59	222	Chalcolithic
BH3168	Barçın Höyük	L11	218	289	Chalcolithic
BH3161	Barçın Höyük	M10	69	271	Chalcolithic
BH 2304	Barçın Höyük	M10	56	223	Chalcolithic
BH 2187	Barçın Höyük	M11	2	23	Chalcolithic
BH3164	Barçın Höyük	L11	218	281	Chalcolithic
BH2190	Barçın Höyük	M11	3	26	Chalcolithic
BH3187	Barçın Höyük	M10	63	234	Chalcolithic
BH2354	Barçın Höyük	M11	5	30	Chalcolithic
BH3173	Barçın Höyük	M11	35	97	Chalcolithic
BH2239	Barçın Höyük	L11	208	223	Chalcolithic
BH3170	Barçın Höyük	M11	35	95	Chalcolithic
BH3166	Barçın Höyük	M11	35	95	Chalcolithic
BH3195	Barçın Höyük	M11	35	104	Chalcolithic
BH3163	Barçın Höyük	M11	35	94	Chalcolithic
BH2191	Barçın Höyük	M11	2	25	Chalcolithic
BH3191	Barçın Höyük	M11	35	100	Chalcolithic

BH3196	Barçın Höyük	L11	218	300	Chalcolithic
BH3184	Barçın Höyük	M10	64	251	Chalcolithic
BH3165	Barçın Höyük	M11	5	28	Chalcolithic
BH3167	Barçın Höyük	M10	69	275	Chalcolithic
BH3174	Barçın Höyük	M10	69	278	Chalcolithic
BH3192	Barçın Höyük	M11	35	101	Chalcolithic
BH3190	Barçın Höyük	M10	64	243	Neolithic
BH3185	Barçın Höyük	M10	58	239	Neolithic
BH3188	Barçın Höyük	M10	64	247	Neolithic
BH3186	Barçın Höyük	M10	64	235	Neolithic
BH3180	Barçın Höyük	L11	205	229	Neolithic
BH3181	Barçın Höyük	L11	205	251	Neolithic
BH3178	Barçın höyük	M10	64	240	Neolithic
BH3183	Barçın Höyük	L11	210	259	Neolithic
BH 3179	Barçın Höyük	L11	218	255	Neolithic

Table A.3. Mezraa Teleilat and Ikiztepe Potsherds

Exc. No	**Type**	**Site**	**Period**
MT'99 19F25	Body	Mezraa Teilat	Neolithic
MT'0434R61	Body	Mezraa Teleilat	Neolithic
MT'99 18H20	Body	Mezraa Teleilat	Neolithic
MT'99 18H11	Base	Mezraa Teilat	Neolithic
MT'99 21HI18	Body	Mezraa Teilat	Neolithic
MT'99 21HI17	Body	Mezraa Teilat	Neolithic
MT'99 18H32	Shoulder	Mezraa Teilat	Neolithic
MT'01 23G14	Base	Mezraa Teilat	Neolithic
MT'01 23H29	Shoulder	Mezraa Teilat	Neolithic
MT'04 34R8	Body	Mezraa Teilat	Neolithic
MT'00 18G70	Shoulder	Mezraa Teilat	Neolithic
MT'04 P3	Body	Mezraaa Teilat	Neolithic
MT'04 34R64	Body	Mezraa Teilat	Neolithic
MT'00 I35	Body	Mezraa Teleilat	Neolithic
MT' 01 23L9	Body	Mezraa Teleilat	Neolithic
İ-440	Perforation	İkiztepe	Early Bronze
İ-389	Perforation	İkiztepe	Early Bronze
İ-80	Perforation	İkiztepe	Early Bronze

APPENDIX B: HTGC CHROMATOGRAMS

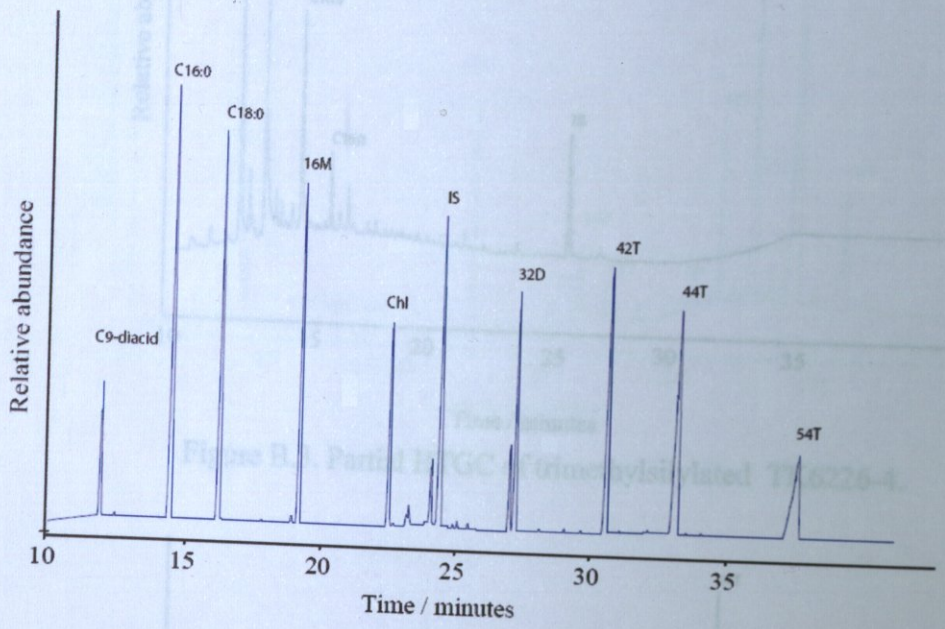


Figure B.1. Partial HTGC of trimethylsilylated reference compounds.

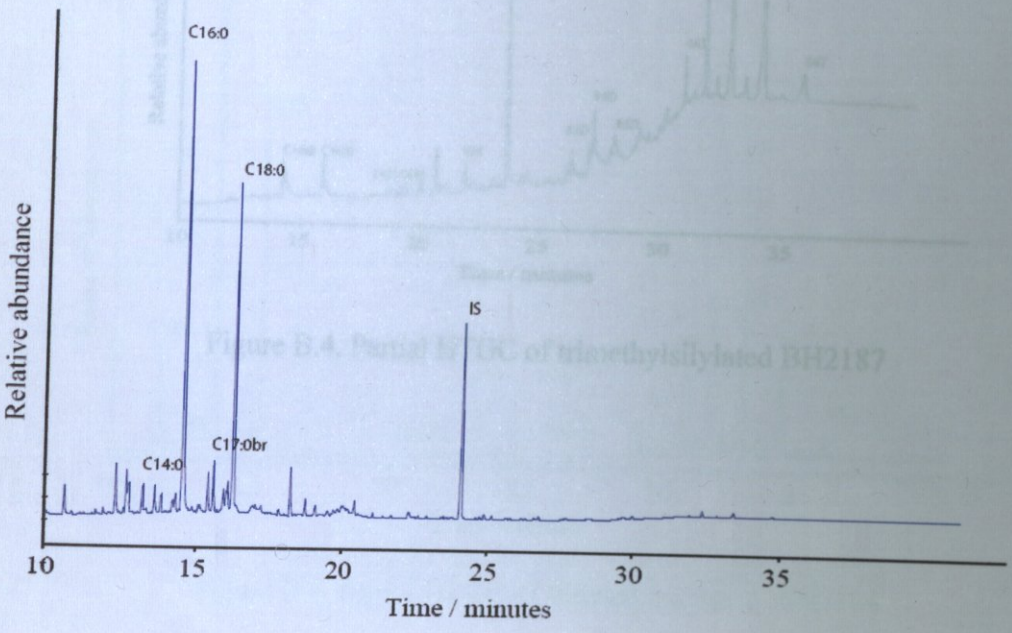


Figure B.2. Partial HTGC of trimethylsilylated TK6760C.

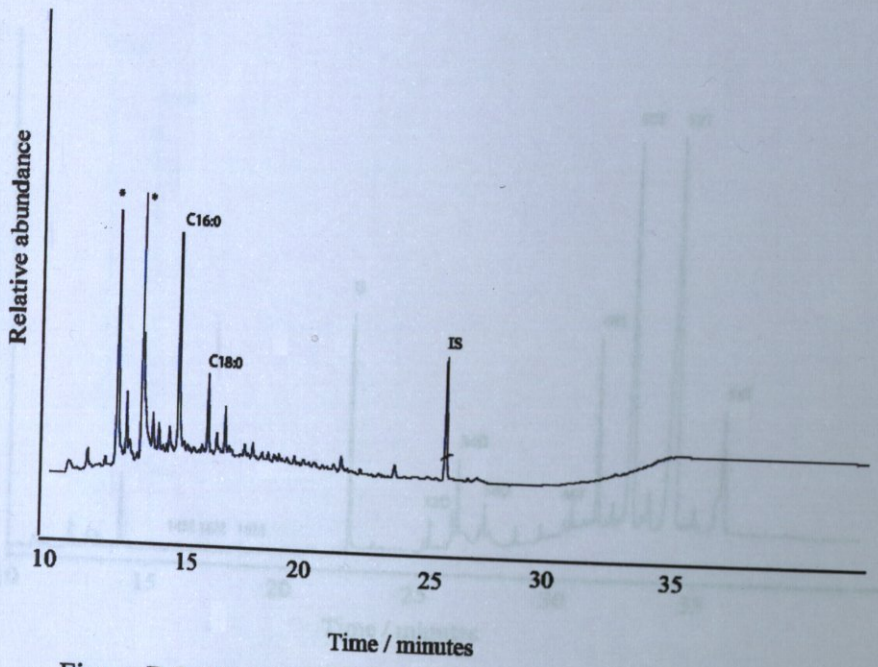


Figure B.3. Partial HTGC of trimethylsilylated TK6226-4.

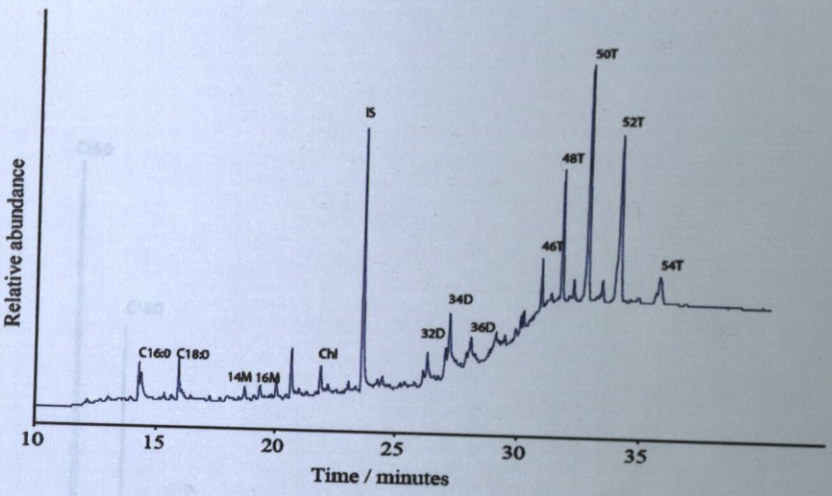


Figure B.4. Partial HTGC of trimethylsilylated BH2187

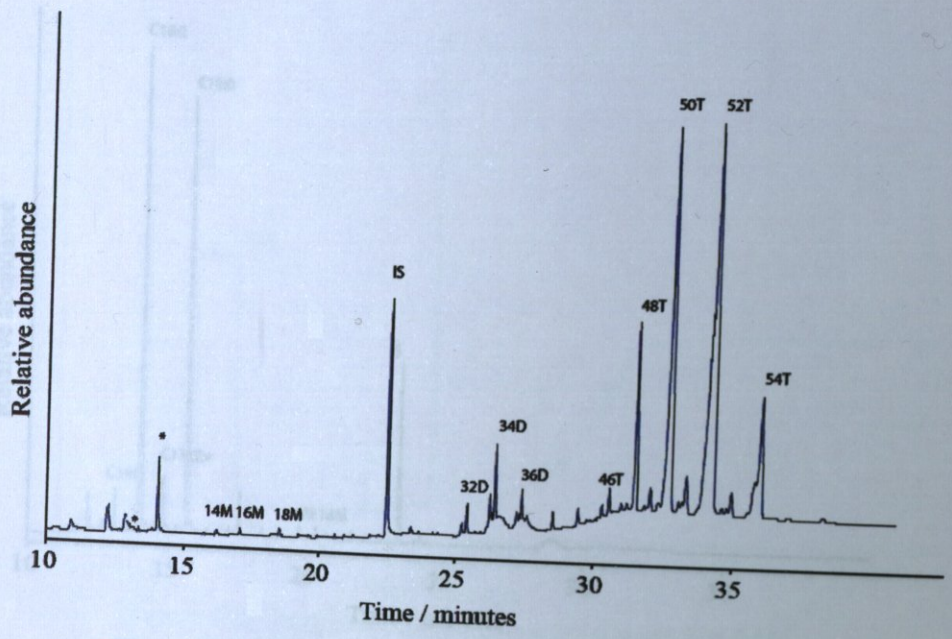


Figure B.5. Partial HTGC of trimethylsilylated BH2304-2.

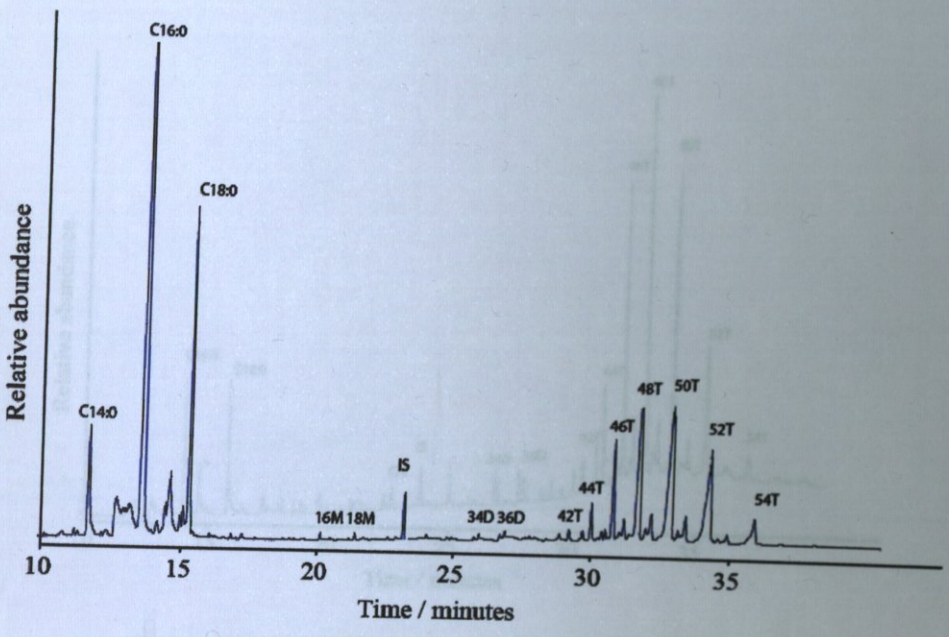


Figure B.6. Partial HTGC of trimethylsilylated BH3164.

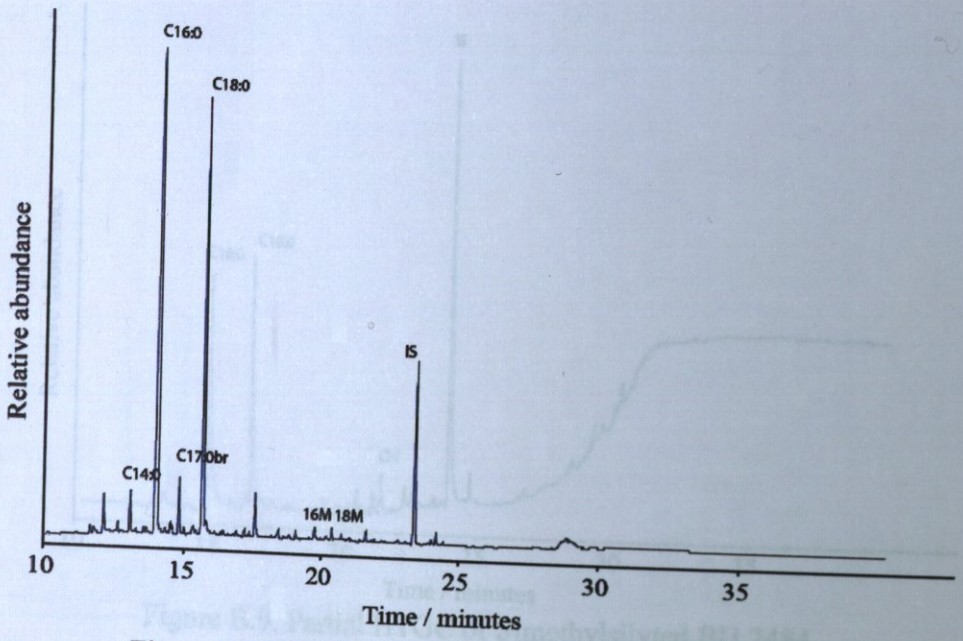


Figure B.7. Partial HTGC of trimethylsilylated BH 2499.

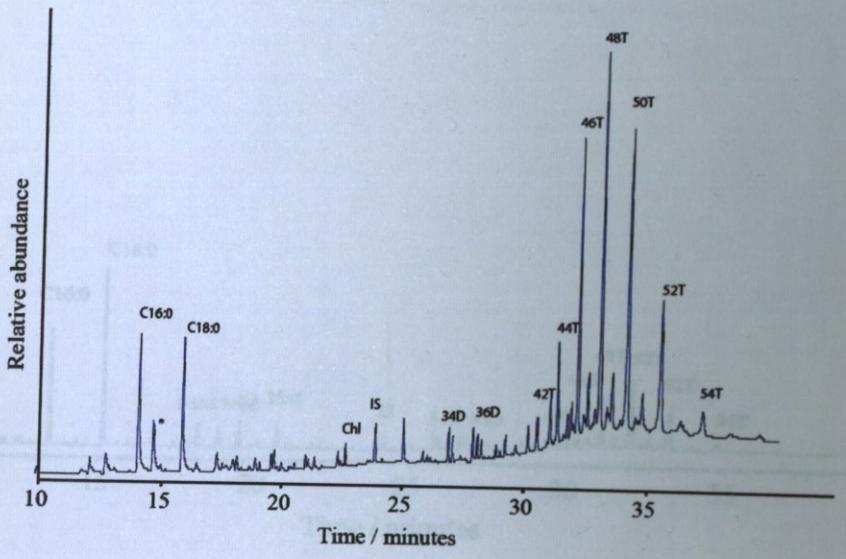


Figure B.8. Partial HTGC of trimethylsilylated BH 3168.

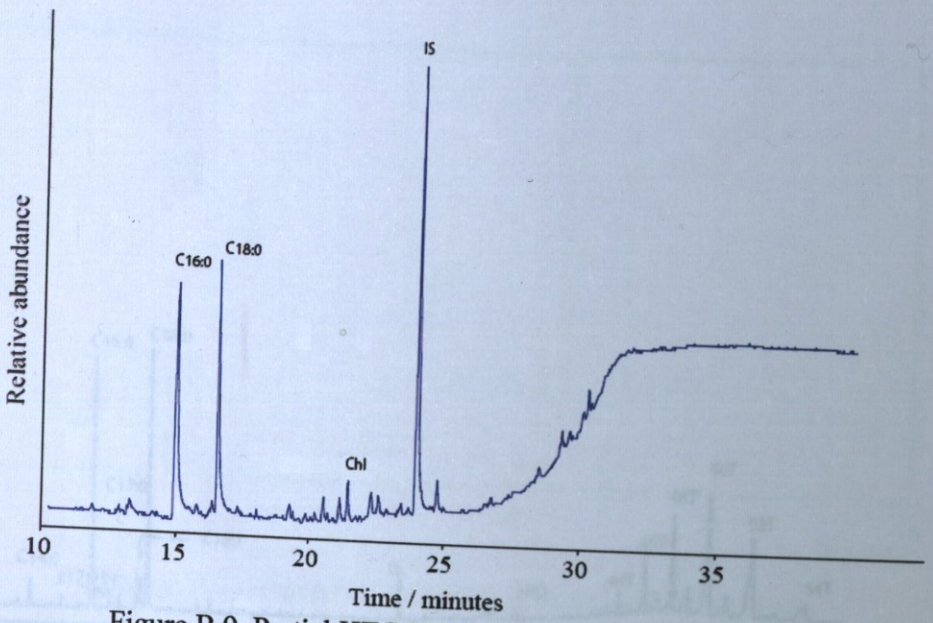


Figure B.9. Partial HTGC of trimethylsilyted BH 2484.

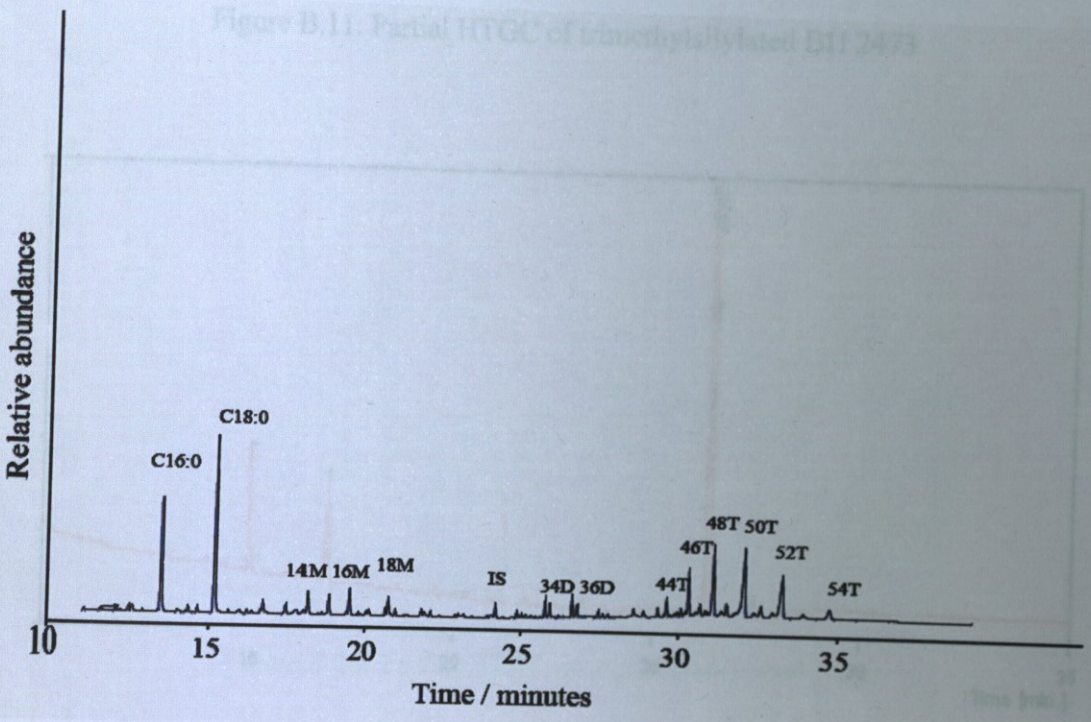


Figure B.10. Partial HTGC of trimethylsilylated BH3195.

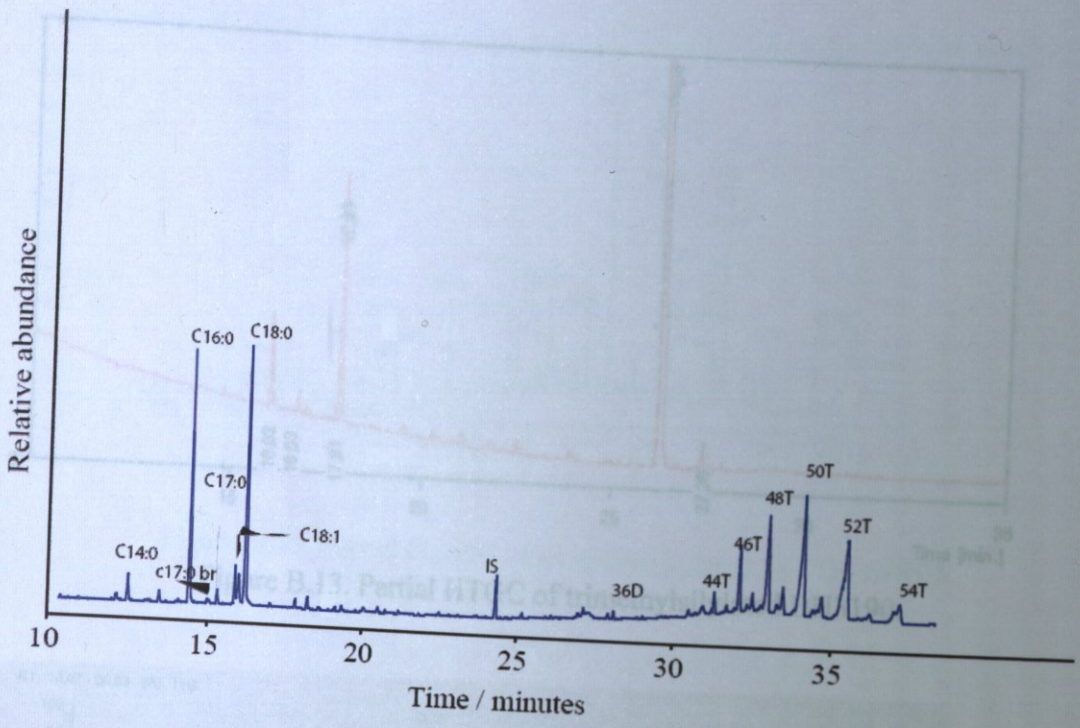


Figure B.11. Partial HTGC of trimethylsilylated BH 2473

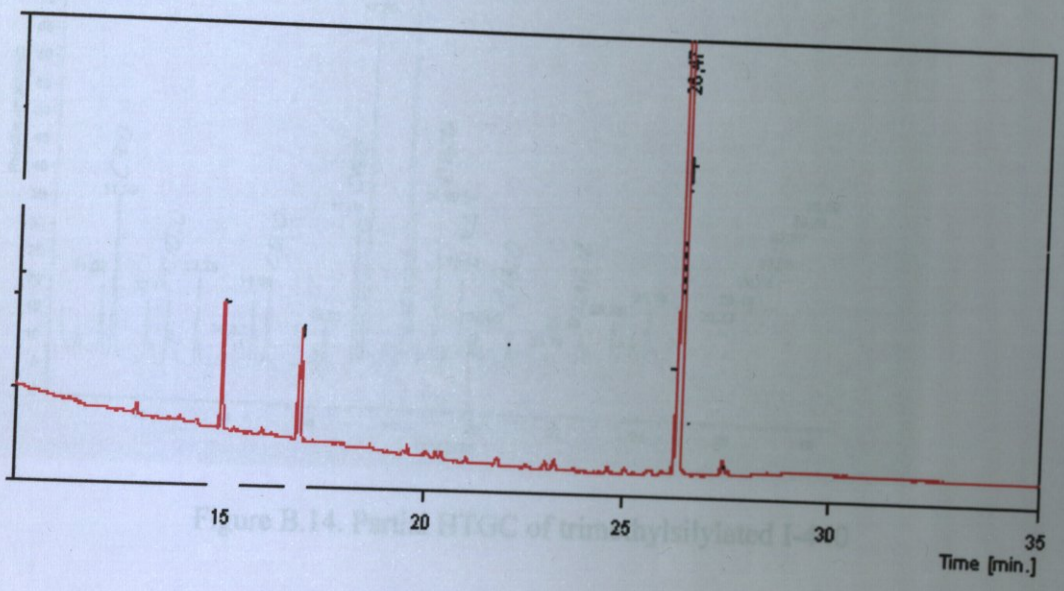


Figure B.12. Partial HTGC of trimethylsilylated BH3184.

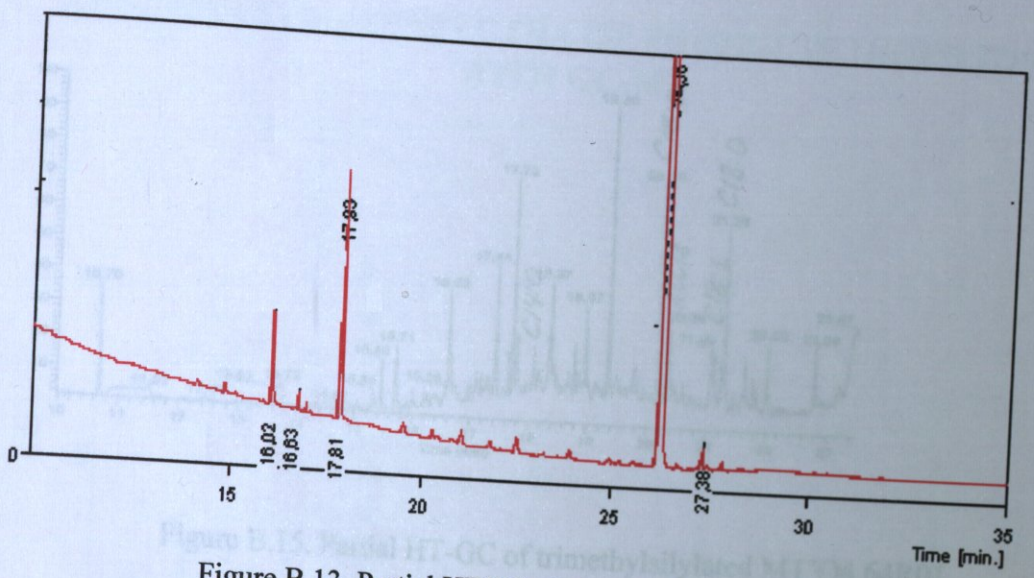


Figure B.13. Partial HTGC of trimethylsilylated BH3190.

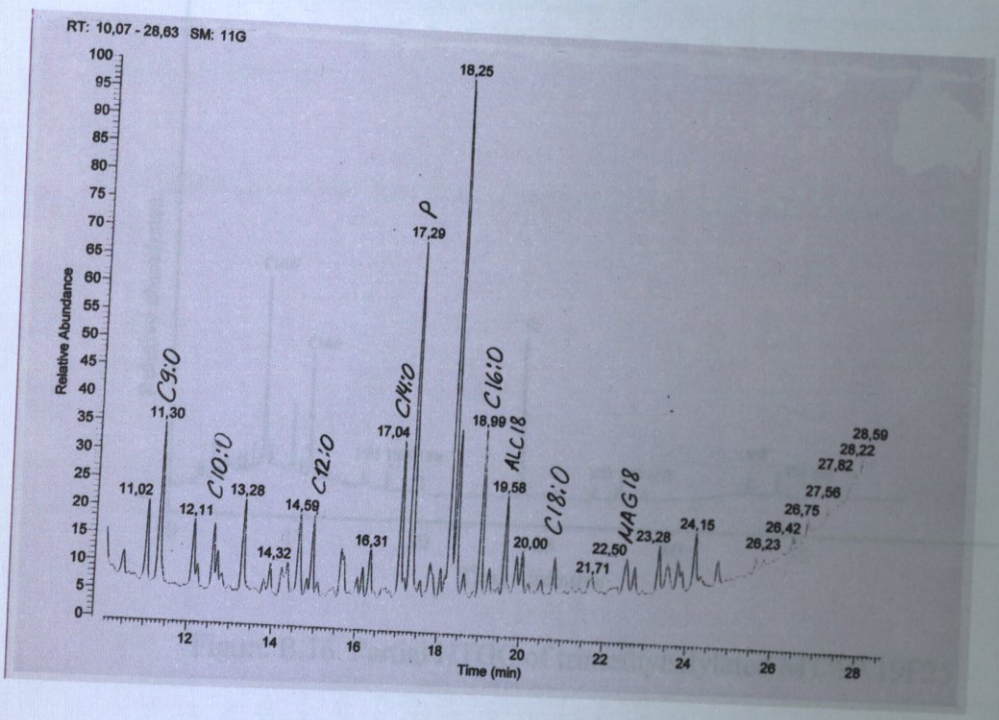


Figure B.14. Partial HTGC of trimethylsilylated I-440

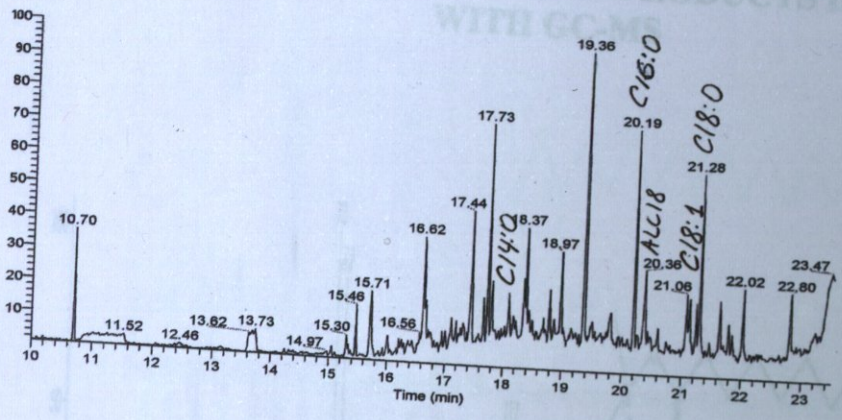


Figure B.15. Partial HT-GC of trimethylsilylated MT'O4 64R01

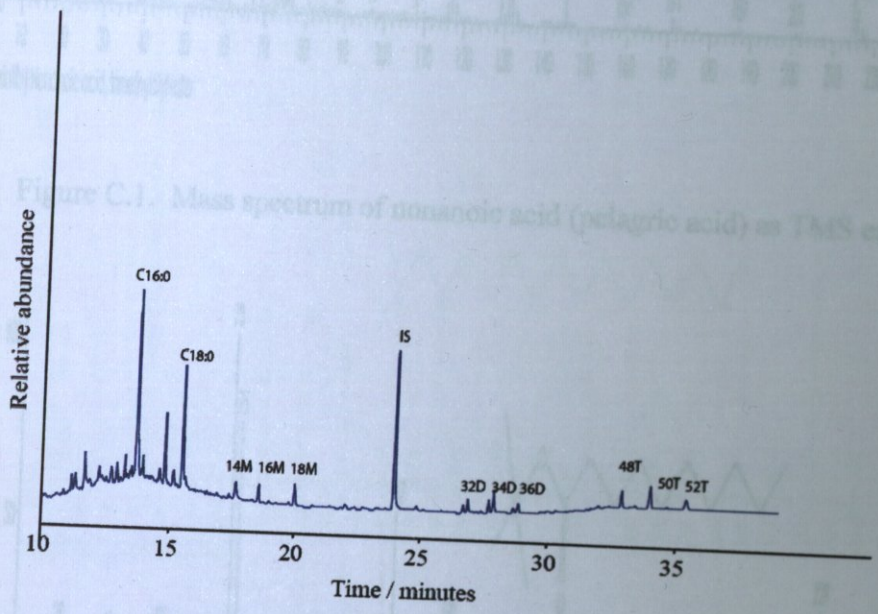


Figure B.16. Partial HTGC of trimethylsilylated MT'99 19F25

APPENDIX C: MASS SPECTRA OF PRODUCTS INDENTIFIED WITH GC-MS

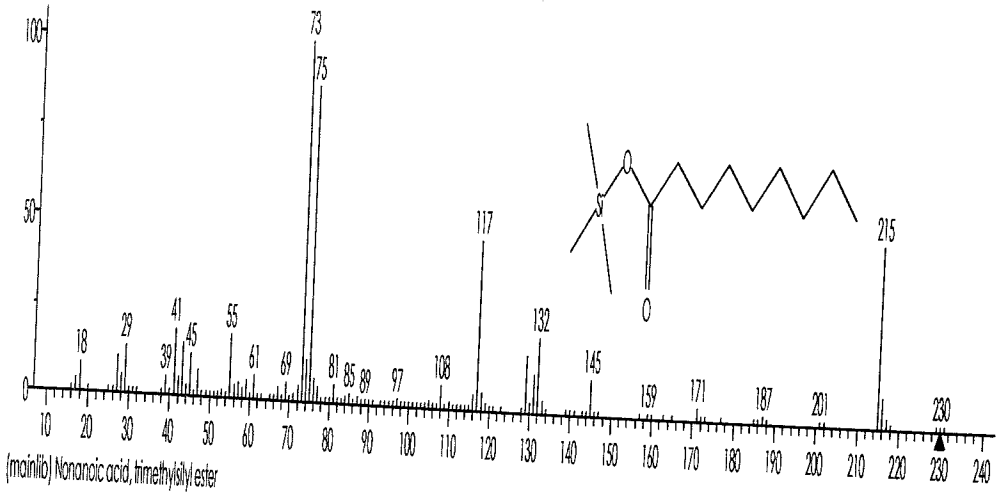


Figure C.1. Mass spectrum of nonanoic acid (pelagric acid) as TMS ester

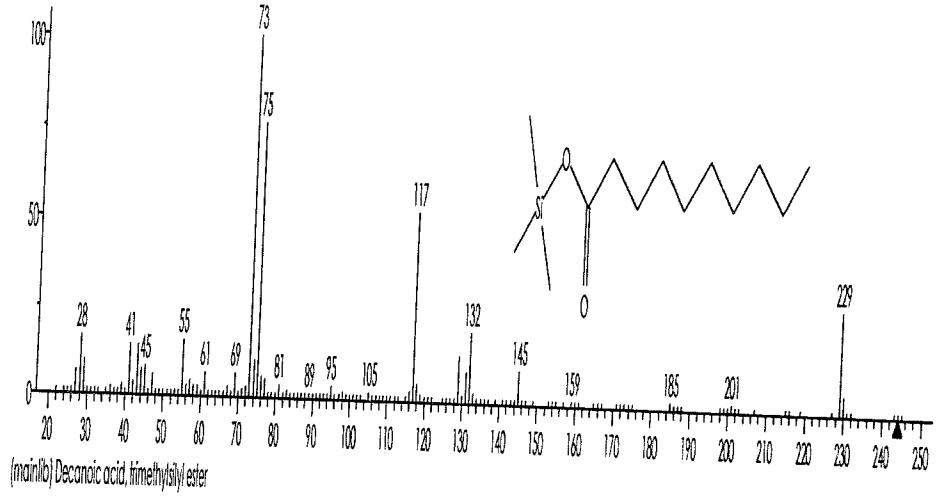


Figure C.2. Mass spectrum of decanoic acid (capric acid) as TMS ester

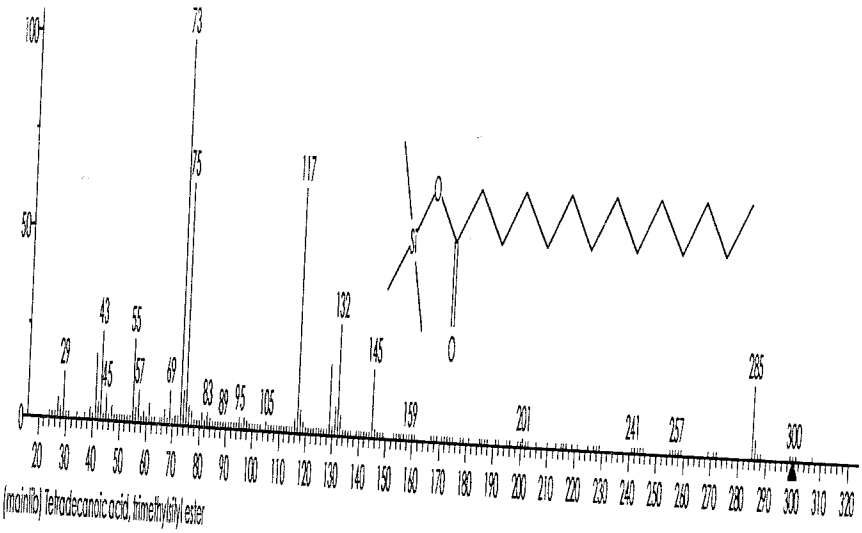


Figure C.3. Mass spectrum of tetradecanoic acid (myristic acid) as TMS ester

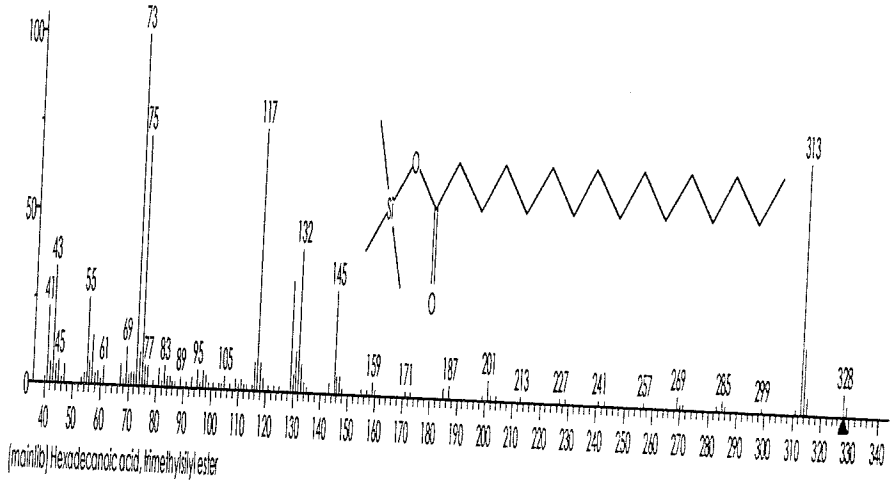


Figure C.4. Mass spectrum of hexadecanoic acid (palmitic acid) as TMS ester

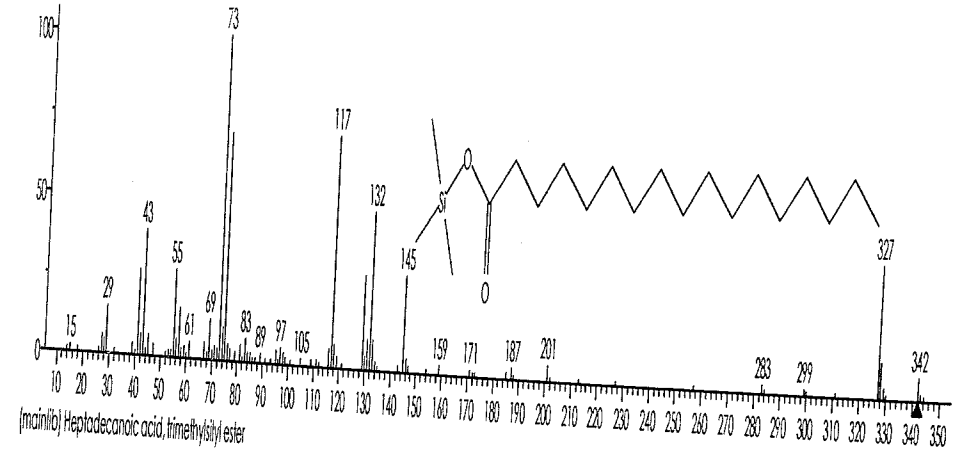


Figure C.5. Mass spectrum of heptadecanoic acid (margaric acid) as TMS ester

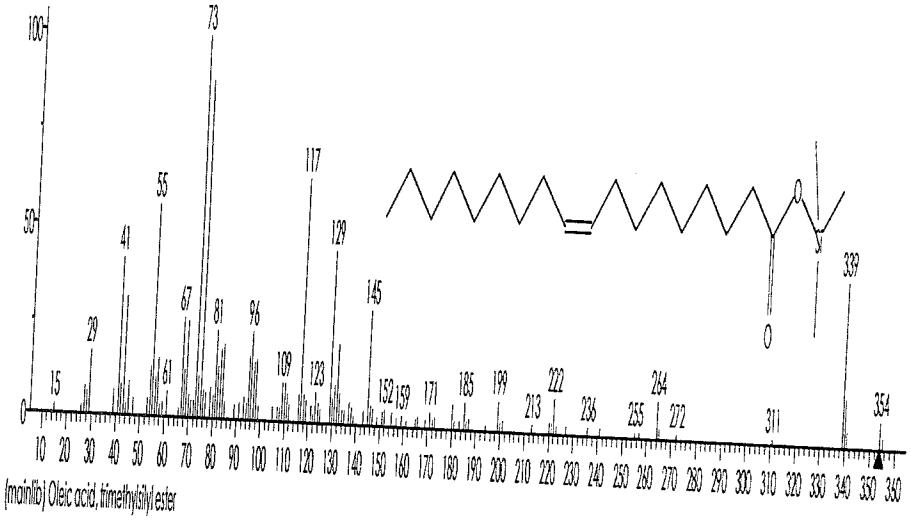


Figure C.6. Mass spectrum of oleic acid as TMS ester

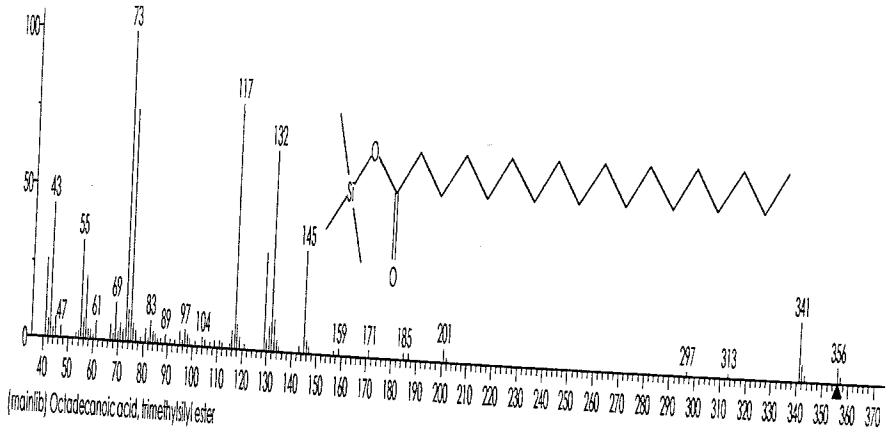


Figure C.7. Mass spectrum of octadecanoic acid (stearic acid) as TMS ester

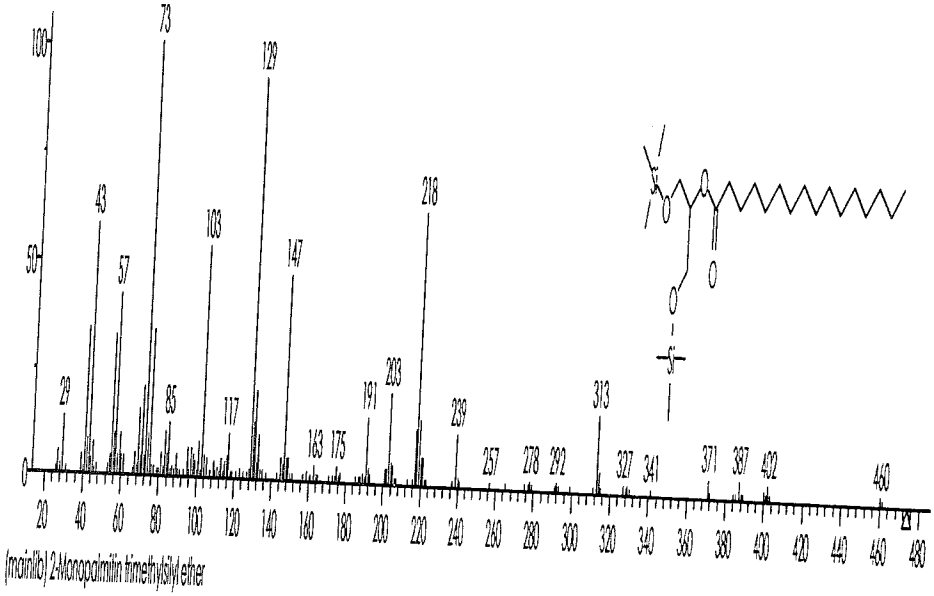


Figure C.8. Mass spectrum of 2-monopalmitin as TMS ether

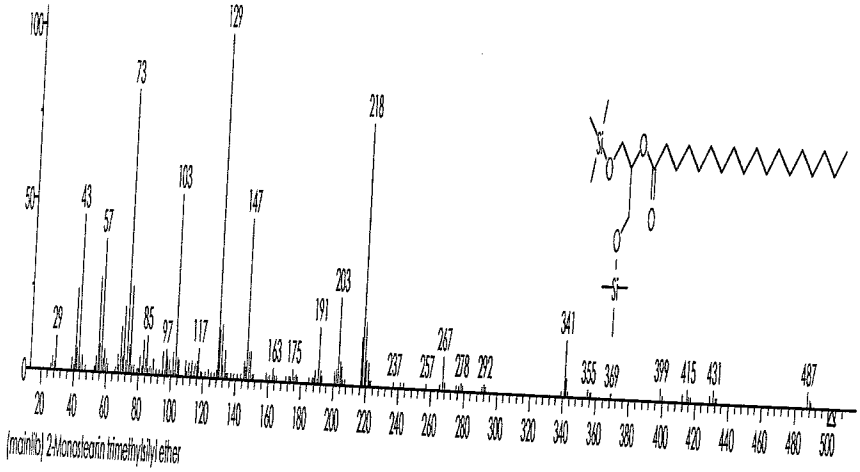


Figure C.9. Mass spectrum of 2-monostearin as TMS ether

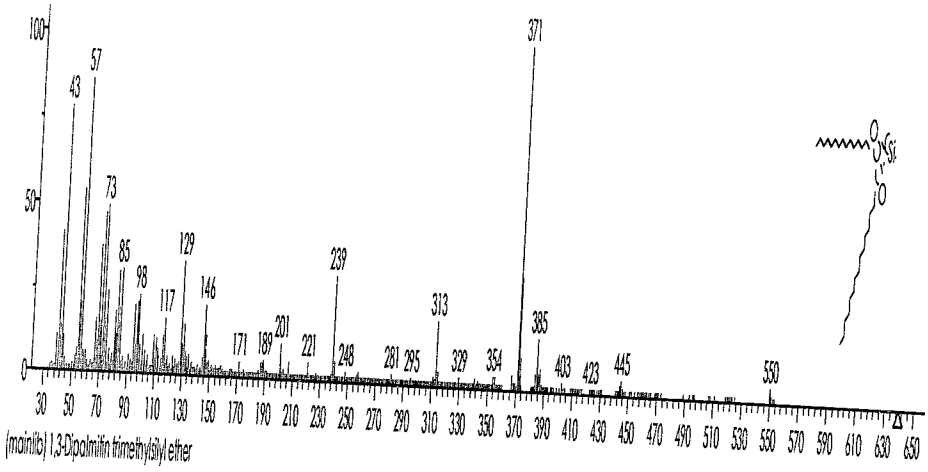


Figure C.10. Mass spectrum of 1,3-dipalmitin as TMS ether

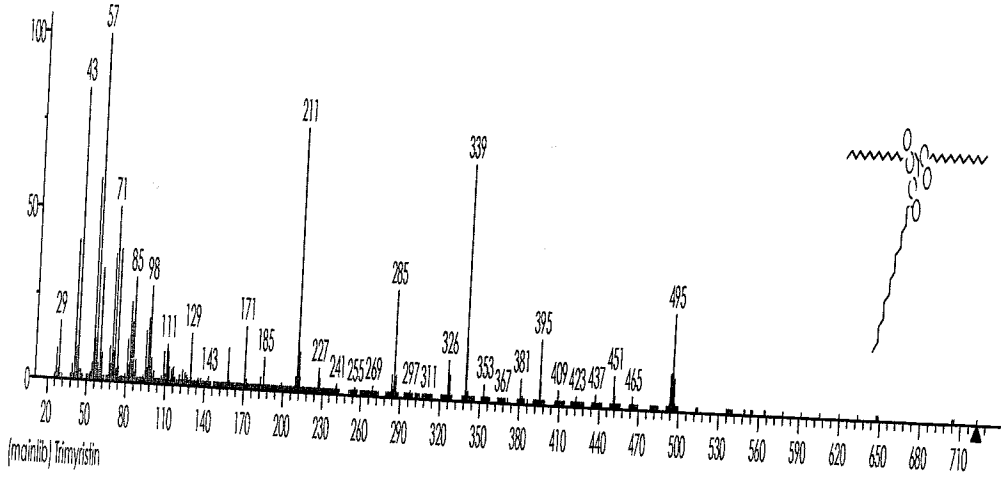


Figure C.11. Mass spectrum of trimyristin

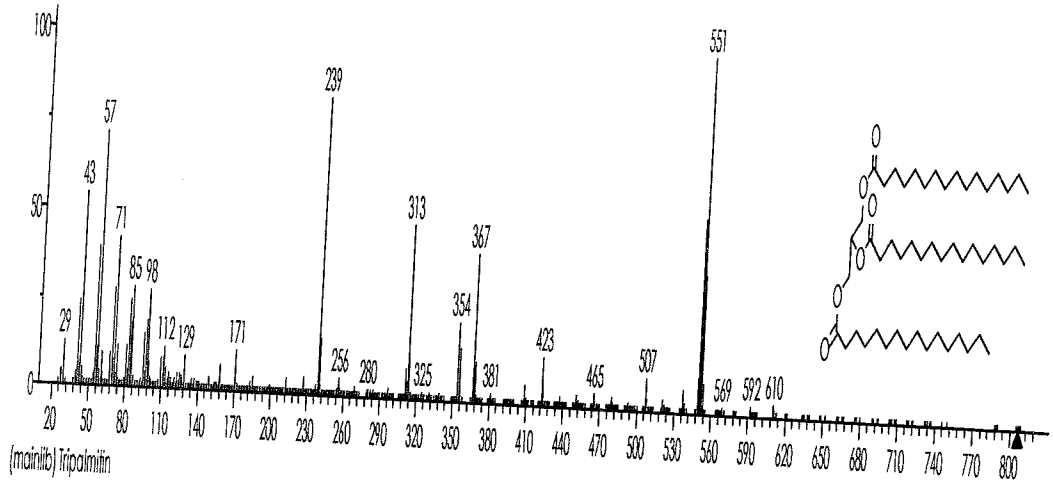


Figure C.12. Mass spectrum of tripalmitin

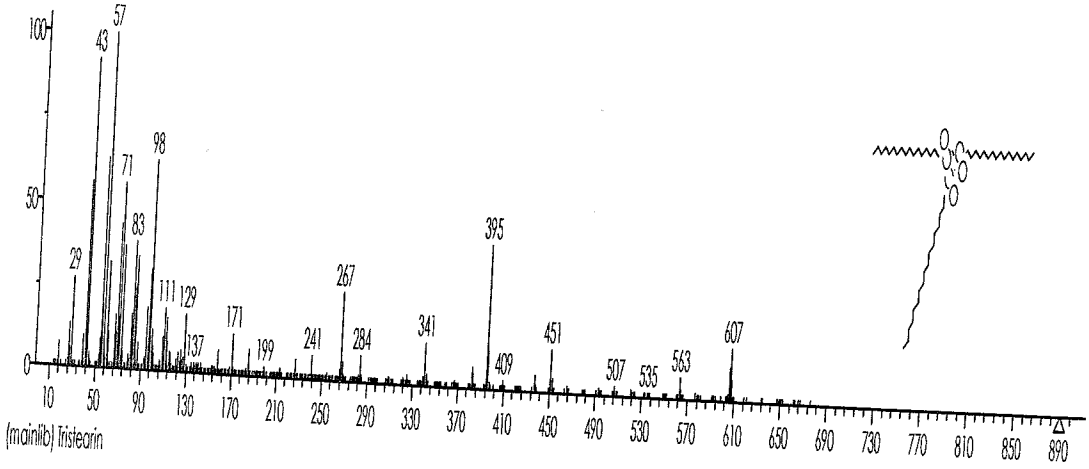


Figure C.13. Mass spectrum of tristearin

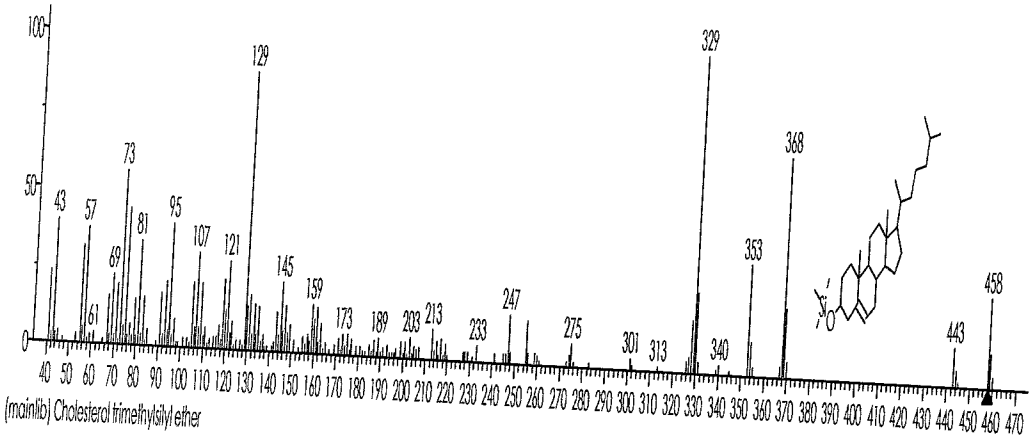


Figure C.14. Mass spectrum cholesterol as TMS ether

REFERENCES

Ambers, J. C. , 1990, " Identification of the use of marine plant material as animal fodder by stable isotope ratios", In *2nd International symposium on ^{14}C and archaeology*, pp. 251-258

Alexander, M., 1999, *Biodegradation and bioremediation*, Academic Press, London

Basiron, Y., 1996, "*Palm oil*", in H.Y. Hui (ed.) *Bailey's Industrial oil and fat products*, pp.271-376,John Wiley and Sons

Beck, C.W., C.J.Smart and D.J. Ossenkop, 1989, " Residues and Linings in Ancient Mediaterranean Transport Amphorae", In R.O.Allen (ed.) *Archaeological Chemistry IV, Advances in Chemistry Series 220*, pp.369-380, ACS, Washington

Belitz, H.D. and W. Grosch, 1999, *Food Chemistry*, Sprinter-Verlag, Berlin

Bethell, P.H., L.J. Goad and R.P. Evershed, 1994, "The Study of Molecular Markers of Human Activity: Use of Coprostanol in the Soil as Indicator of Human Faecal Material", *Journal of Archaeological Science*, Vol.21, pp.619-632

Bethell, P.H., R.P. Evershed and L.J. Goad, 1993, "The Investigation of Lipids in Organic Residues by Gas Chromatography/Mass Spectrometry: Application to Paleadietary Studies", in J.B. Lambert and G. Grupe (eds), *Molecular Archaeology of Prehistoric Human Bone*, pp.229-235, Sprinter Verlag, Berlin

Boutton, T. W., 1991, "Stable carbon isotope ratios of natural materials II: atmospheric , terrestrial, marine and freshwater environments", in Coleman, D. C. and Fry, B (eds) *Carbon Isotope Techniques*, Academic press, New York

Brand, W. A., 1996, "High precision isotope ratio monitoring techniques in mass spectrometry", *Journal of Mass Spectrometry*, Vol.31, pp.225-235.

Calvin, M. and Benson, A. A., 1948, "The path of carbon in photosynthesis", *Science,* Vol. 107, pp.476-480.

Charters, S., Evershed, R. P., Blinkhorn, P. W. and Denham, V., 1995, "Evidence for the mixing of fats and waxes in archaeological ceramics", *Archaeometry,* Vol.37, pp.113-127.

Charters, S., Evershed, R. P., Goad, L. J., Heron, C. and Blinkhorn, P. W., 1993, "Identification of an adhesive used to repair a Roman jar", *Archaeometry,* Vol.35, pp.91-101.

Charters, S., Evershed, R. P., Quye, A., Blinkhorn, P. W. and Reeves, V., 1997, " Simulation experiments for determining the use of ancient pottery vessels: The behaviour of epicuticular leaf wax during boiling of a leafy vegetable" *Journal of Archaeological Science,* Vol. 24, pp.1-7.

Chilliard, Y., Ferlay, A. and Doreau, M., 2001, "Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids", *Livestock Production Science,* Vol.70, pp.31-48.

Chisholm, B. S., Nelson, D. E. and Schwarcz, H. P., 1982, "Sstable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets", *Science,* Vol.216, pp.1131-1132.

Christie, W.W., 1978, ' The composition, structure and function of lipids in the tissues of ruminant animals', *Progress in Lipid Research,* Vol.17, pp.111-205

Christie, W.W., 1981, *Lipid metabolism in ruminant animals*, Pergamon Press, Oxford, UK

Christie, W.W., 1989, *Gas Chromatography and Lipids*, The Oily Pres, Ayr, Scotland

Den Dooren de Jong, L.E, 1961, " On the formation of adipocere from fats: Contribution to microbiology of systems containing two lipid phase", *Journal of Microbiology and Serology*, Vol.27., pp.337-361

Copley, M. S., Rose, P. J., Clapham, A., Edwards, D. N., Horton, M. C. and Evershed, R. P., 2001, "Processing palm fruits in the Nile Valley - biomolecular evidence from Qasr Ibrim", *Antiquity, Vol.* 75, pp.538-542

Copley, M. S., Berstan, R., Dudd, S. N., Docherty, G., Mukherjee, A. J., Straker, V., Payne, S. and Evershed, R. P., 2003, "Direct chemical evidence for widespread dairying in prehistoric Britain", *Proceedings of the National Academy of the United States of America,* Vol.100, pp.1524-1529.

Copley, M. S., Jim, S., Jones, V., Rose, P., Clapham, A., Edwards, D. N., Horton, M., Rowly-Conwy, P. and Evershed, R. P., 2004, "Short- and long-term foraging and foddering strategies of domesticated animals from Qasr Ibrim, Egypt" *Journal of Archaeological Science,* Vol.31, pp.1273-1286.

Craig, O., Mulville, J., Pearson. P., Sokol, R., Gelshope, K., R. Staceyll and M. Collins, 2000, "Detecting Milk Proteins in Ancient Pots", *Nature*, Vol.312

Craig, H., 1957, "Isotopic standards for carbon and oxygen and correlation factors for mass spectrometric analysis of CO_2 " ,*Geochimica et Cosmochimica Acta,* Vol.12, pp.133-149.

Den Dooren de Jong, L.E, 1961, " On the formation of adipocere from fats: Contribution to microbiology of systems containing two lipid phase", *Journal of Microbiology and Serology*, Vol.27. pp.337-361

DeNiro, M. J., 1985, "Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction" *Nature,* Vol.317, pp.806-809

DeNiro, M. J. and Epstein, S., 1977, "Mechanism of carbon isotope fractionation associated with lipid synthesis", *Science,* Vol. 197, pp.261-263.

DeNiro, M. J. and Epstein, S., 1978, "Influence of diet on the distribution of carbon isotopes in animals", *Geochimica et Cosmochimica Acta,* Vol. 42, pp.495-506.

Deshpande, S., Dessphande, U. and D.K. Salunkhe, 1996, Sesame Oil, in H.y. Hui (ed.), Bailey's Industrial Oil and Fat Products, pp.457-470, John Wiley and Sons

Dudd,S.N., 1999, *Molecular and Isotopic Characterisation of Animal Fats in Archaeological Pottery,* PhD thesis, University of Bristol.

Dudd, S. N. and Evershed, R. P., 1998, "Direct demonstration of milk as an element of archaeological economies", *Science,* Vol.282, pp.1478-1481.

Dudd, S. N., Regert, M. and Evershed, R. P.,1998, "Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds" *Organic Geochemistry,* Vol. 29, pp. 1345-1354.

Dudd, S. N., Evershed, R. P. and Gibson, A. M., 1999, "Evidence for varying patterns of exploitation of animal products in different prehistoric pottery traditions based on lipids preserved in surface and absorbed residues", *Journal of Archaeological Science,* Vol.26, pp.1473-1482.

Eglinton, G. and G.A. Logan, 1991, "Molecular Preservation", *Philosophical Transactions of the Royal Society of London,* Vol.B333, pp. 253-265

Ehleringer, J. R., Lin, Z. F., Field, C. B., Sun, G. C. and Kuo, C. Y., 1987, "Leaf carbon isotope ratios of plants from a subtropical monsoon forest ",*Oecologia,* Vol.72, pp.109-114

Enser, M., 1991, "Animal carcass fats and fish oils", In , Rossel, J. B. and Pritchard, J. L. R. (eds.) *Analysis of oilseed, fats and fatty foods* , pp.329-394,Elsevier Applied Science, London and New York

Evershed, R. P., 1993, "Biomolecular Archaeology and Lipids", *World Archaeology,* Vol.25, pp.74-93.

Evershed, R. P., Heron, C. and Goad, L. J., 1991, "Epicuticular Wax Components Preserved in Potsherds As Chemical Indicators of Leafy Vegetables in Ancient Diets ",*Antiquity,* Vol. 65, pp.540-544.

Evershed, R. P., Heron, C., Charters, S. and Goad, L. J., 1992, "The survival of food residues: new methods of analysis, interpretation and application" *Proceedings of the British academy,* Vol.77, pp.187-208.

Evershed, R. P., Arnot, K. I., Collister, J., Eglington, G. and Charters, S., 1994, "Application of isotope ratio monitoring gas chromatography-mass spectrometry to the analysis of organic residues of archaeological origin", *Analyst,* Vol.119, pp.909-914.

Evershed, R. P., Stott, A. W., Raven, A., Dudd, S. N., Charters, S. and Leyden, A.,1995a, "Formation of Long-Chain Ketones in Ancient-Pottery Vessels By Pyrolysis of Acyl Lipids", *Tetrahedron Letters,* Vol.36, pp.8875-8878.

Evershed, R. P., Turner-Walker, G., Hedges, R. E. M., Tuross, N. and Leyden, A., 1995b, "Preliminary results for the analysis of lipids in ancient bone", *Journal of Archaeological Science,* Vol.22, pp. 277-290.

Evershed, R. P., Charters, S. and Quye, A., 1995c, "Interpreting lipid residues in archaeological ceramics: preliminary results from laboratory simulations of vessel use and burial", *Materials Research Society Symposium Proceedings,* Vol.352, pp.85-95.

Evershed, R. P., Vaughan, S. J., Dudd, S. N. and Soles, J. S., 1997a, " Fuel for thought? Beeswax in lamps and conical cups from the late Minoan Crete ", *Antiquity,* Vol.71, pp. 979-985.

Evershed, R. P., Mottram, H. R., Dudd, S. N., Charters, S., Stott, A. W., Gibson, A. M., Conner, A., Blinkhorn, P. W. and Reeves, V., 1997b, "New criteria for the identification of animal fats preserved in archaeological pottery", *Naturwissenschaften,* Vol.84, pp.402-406.

Evershed, R. P., Dudd, S. N., Charters, S., Mottram, H., Stott, A. W., Raven, A., van Bergen, P. F. and Bland, H. A., 1999, "Lipids as carriers of anthropogenic signals from prehistory", *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,*Vol. 354, pp. 19-31.

Evershed, R. P., Dudd, S. N., Lockheart, M. J. and Jim, S., 2001, "Lipids in Archaeology", In Brothwell, D. R. and Pollard, A. M. (eds.) *Handbook of archaeological sciences*

Evershed, R., Dudd, S. N., Copley, M. S., Berstan, R., Stott, A. W., Mottram, H. R., Buckley, S. A. and Crossman, Z., 2002, "Chemistry of archaeological animal fats" *Accounts of Chemical Research,* Vol.35, pp.660-668

Evershed, R. P., Dudd, S. N., Copley, M. S. and Mukherjee, A. J., 2003, "Identification of animal fats via compound specific $d^{13}C$ values of individual fatty acids: assessments of results for reference fats and lipid extracts of archaeological pottery vessels", in M Budja (ed.) *Documenta Praehistorica, XXIX; 9th Neolithic Studies,* pp. 73-96.

Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T., 1989, "Carbon isotope discrimination and photosynthesis", *Annual Reviews in Plant Physiology and Plant Molecular Biology,* Vol.40, pp.503-537.

Frankel, E.N., 1991, "Recent Advances in Lipid Oxidation", Journal of Science of Food and Agriculture, Vol.54, pp.495-511

Frankel, E.N., 2000, *Wine and Oil Production in Antiquity in Israel and Other Mediterranean Countries,* Sheffield Academic Press

Friedli, H., Lotcher, H., Oeschger, H., Siegenthaler, U. and Stauffer, B., 1986, "Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries" *Nature,* Vol.324, pp. 237-238

Goad, L.J., 1977, The Biosyntrhesis of Plant Sterols, in M. Tevisi and Lichtenthaler (eds.), *Lipids and Lipid Polymers in Higher Plants*, pp.146-168, Sprinter-Verlag, Berlin

Gunstone, F.D. J.L. Harwood and F.B. Padley, 1986, *Lipid Handbook,* London:Chapman and Hall

Hansel, F. A., Copley, M. S., Madureira, L. A. S. and Evershed, R. P., 2004,"Thermally produced ω-(*o*-alkylphenyl)alkanoic acids provide evidence for the processing of marine products in archaeological pottery vessels", *Tetrahedron Letters,* Vol.45, pp.2999-3002.

Harfoot, C. G., 1981, "Anatomy, physiology and microbiology of the ruminant digestive tract", in W.W. Christie (ed.) *Progress in lipid research supplement No. 1. Lipid metabolism in ruminant animals*, pp.1-19, Pergamon press, Oxford.

Harwood, L.J and N.J. Russell, 1984, *Lipids in Plants and Microbes*, George Allen and Unwin, London

Hastdorf, C. A. and DeNiro, M. J., 1985, "Reconstruction of prehistoric plant production and cooking practice by a new isotopic method" ,*Nature,* Vol.**315,** pp.489-551.

Hatch, M. D. and Slack, C. R., 1966, "Photosynthesis by sugar cane leaves. A new carboxylation reaction and the pathway of sugar formation", *Journal of Biochemistry,* Vol.101, pp.103-111.

Heron, C. and Evershed, R. P., 1992, "The analysis of organic residues and the study of pottery use", in M. Schiffer (ed.) *Archaeological method and theory* , pp.247-284 University of Arizona Press, Arizona

Heron., C. and A.M. Pollard, 1988, The Analysis of Natural Resinous Materials from Roman Amphorae, in A. Slater and J.Tite (eds.), *Science and Archaeology-Glasgow 1987*, pp.429-446, Bristish Archaeological Reports, Oxford

Heron, C., Nemcek, N., Bonfield, K.M., D. Dixon and B.S. Ottaway, 1994, "The Chemistry of Neolithic Beeswax", *Naturwissenschaften,* Vol.81, pp.266-269

Heron, C., R.P. Evershed and L.J. Goad, 1991, "Effects of Migration of Soil Lipids on Organic Residues Associated with Buried Potsherds", *Journal of Archaeological Science*, Vol.18, pp.641-659

Hita, C., Parlanti, E., Jambu, P., J. Joffre and A. Ambles, 1996, "Triglyceride Degradation in Soil", *Organic Geochemistry*, Vol.25, pp.19-28

Jones, A., 1996, " Food for thought: material culture and the transformation of food use from the Mesolithic to Neolithic", in T.Pollard and Morrison, A.(eds) *The early prehistory of Scotland*, pp.291-300, Edinburgh University Press, Edinburgh

Kimpe, K., Jacobs, P. A. and Waelkens, M., 2001, "Analysis of oil used in late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat", *Journal of Chromatography A,* Vol.937, pp. 87-95.

Kimpe, K., Jacobs, P. A. and Waelkens, M., 2002, "Mass spectrometric methods prove the use of beeswax and ruminant fat in late Roman cooking pots", *Journal of Chromatography A,* Vol.968, pp.151-160.

Kolattukudy, P. E., 1980, *Chemistry and Biochemistry of Natural Waxes,* Elsevier, Amsterdam.

Laakso. P., 1996, "Analysis of Triacyglycerols-approaching the Molecular Composition", *Fod Reviews International*, Vol.12, pp.199-250

Love, J.A., 1996, Animal fats, in H.Y. Hui (ed.) *Bailey's Industrial Oil and Fat Products*, pp. 1-19, John Wiley and Sons

Lowe, T. E., Peachey, B. M. and Devine, C. E., 2002, " The effect of nutritional supplements on growth rate, stress responsiveness, muscle glycogen and meat tenderness in pastoral lambs" ,*Meat Science,* Vol.62, pp. 391-397.

McCormac, F. G., Baillie, G. L., Pilcher, J. R., Brown, D. M. and Hoper, S. T., 1994, "$\delta^{13}C$ measurements from the Irish oak chronology", *Radiocarbon,* Vol.36, pp.27-35.

Malainey, M.E., R. Przybylski and B.L. Sheriff, 1999, "The Effects of Thermal and Oxidative Degradation on the Fatty Acid Composition of Food Plants and Animals of Western Canada: Implication of the Identification of Archaeological Residues", *Journal of Archaeological Science,* Vol.26, pp.95-103

Malainey, M.E., R. Przybylski and B.L. Sheriff, 1999, "The Fatty Acid Composition of Native Food Plants and Animals of Western Canada", *Journal of Archaeological Science,* Vol.26, pp.83-94

Malainey, M.E., R. Przybylski and B.L. Sheriff, 1999, "Identifying the Former Contents of Late Precontact Period Pottery Vessels from Western Canada using Gas Chromatography", *Journal of Archaeological Science*, Vol.26, pp.425-438

McDonald P., R.D. Edwards and J.F.D Greenhalgh, 1988, *Animal Nutrition,* Essex, Pergamon Press

McGovern,P., Glusker, D.L. L.J. Exner and M.M. Voigt, 1996, "Neolithic Resinated Wine", *Nature*, Vol.381, pp.480-481