

Pottery use at the transition to agriculture in the western Mediterranean.

Evidence from biomolecular and isotopic characterisation of organic residues in Impressed/Cardial Ware vessels.

Cynthianne Debono Spiteri

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Abstract

Recent research has attributed the introduction of agriculture in the western Mediterranean to several rapid waves of ‘maritime pioneer colonisation’, followed by indigenous adoption. Impressed/Cardial Wares are thought to have spread simultaneously with domesticates through this region, and are hypothesised to have been used to process domestic plant and animal products. To test this hypothesis, organic residue analysis (ORA) has been applied to 301 Impressed/Cardial Ware vessels recovered from 14 Early and Middle Neolithic sites in the western Mediterranean, to determine their content and function. ORA is a well established technique that can provide direct and sometimes specific evidence of an artefact’s function by analysing lipid residues trapped within its matrix. Characterisation of these fatty residues was carried out using Gas Chromatography (GC), GC-Mass Spectrometry (GC-MS), and GC-combustion-Isotope Ratio MS (GC-c-IRMS). The latter is especially useful, since it allows the $\delta^{13}\text{C}$ values of two particular fatty acids, $\text{C}_{16:0}$ and $\text{C}_{18:0}$, to be measured. Because of variations in the way these two fatty acids are biosynthesised and routed in different organisms, the difference between their $\delta^{13}\text{C}$ measurements (i.e. $\Delta^{13}\text{C}$ values) allow distinction between various types of fat, namely between ruminant and non-ruminant adipose, and ruminant adipose and ruminant dairy products. This research presents the first extensive application of ORA to Early Neolithic pottery recovered from Mediterranean contexts, and was aimed at better understanding the origins of pottery in the Mediterranean and testing their implied association with the transmission of farming from south-west Asia. However, as most ORA studies have been carried out on ceramic assemblages excavated from northern European contexts, the suitability of the method to the Mediterranean region needed to be rigorously tested, in particular since: i) lipid preservation in warmer climates was observed to be considerably lower; ii) shifts in the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ isotopic values were identified, compared to UK reference data sets. In view of this, two experiments were set up to: i) determine whether the low chemical fingerprint of plant lipid residues may be a significant contributor to the low lipid yields obtained, which was investigated by re-creating plant residues in ceramic vessels through a series of cooking experiments, and analysing the degraded profile obtained after burial; ii) quantify the shift in the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ isotopic signals, by setting up a controlled feeding experiment in the Mediterranean, and comparing the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements obtained to northern European values. The results obtained from the experimental work carried out were used to interpret archaeological residues extracted from Impressed/Cardial Ware vessels. Shifts in the $\delta^{13}\text{C}$ isotopic signals of modern Mediterranean reference fats were identified, and also in the $\Delta^{13}\text{C}$ values that demarcate the different fat categories. The degraded plant lipid profiles obtained from the cooking experiment were used to identify plant contributions in archaeological residues, and also showed that low quantities of absorbed lipid in archaeological potsherds could potentially indicate a plant input. ORA analysis confirmed the processing of animal and plant products in Impressed/Cardial Wares, and more importantly, unequivocally identified evidence for the use of dairy products in the Mediterranean, dating to the late 7th millennium BC.

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Author's declaration

I hereby certify that the work described in this thesis is my own, except where otherwise acknowledged, and has not been submitted previously for a degree at this, or any other, university.

Cynthianne Debono Spiteri

Chapter 1

Introduction

1.1 Introduction

The onset of agriculture is one of the most important milestones to be reached by humans, as far as demographic and economic development is concerned. It allowed communities to sustain an increased population, and ultimately revolutionised the way humans use their environment and live. How agriculture came about and what triggered the shift from food procurement to food production is still a much debated topic, which continues to generate a lot of research. Many theories have been proposed, including climate change, population growth, as well as changes in social and cultural values; yet the answer is still elusive. This shift in subsistence eventually spread worldwide, and decades of research have proposed various models to explain the mechanism by which it spread. Developments in the application of scientific analysis to archaeological research, together with data generated from archaeological investigations, provide a dynamic duo to study the origin and spread of agriculture, as will be described, debated and discussed in this research.

The earliest dates for the first attestations of agrarian practices and domestication are found in the Levant, and are supported by genetic studies (e.g. Bruford and Townsend, 2006; Luikart *et al.*, 2006). As in other regions, theories and models which attempt to explain the introduction and spread of farming in the Mediterranean have been widely published (e.g. Higgs and Jarman 1969; Ammerman and Cavalli-Sforza 1984; Barker 1985; Zilhão 1993; Zeder 2008; Rowley-Conwy 2011). The 20th century saw Childe's idea of an 'agricultural revolution' and the diffusion of agrarian practices from east to west (*ex oriente lux*), arguments for an indigenous origin of agriculture which were later dismissed by genetic studies, and debates over whether the spread of agriculture occurred through colonisation by farming communities or adoption of agrarian practices by Mesolithic people who came into contact with these mobile farming communities (e.g. Higgs and Jarman 1969; Ammerman and Cavalli-Sforza 1984; Barker 1985). Subsequent research suggested that indeed, the spread of domesticates, agriculture and other distinguishing characteristics of the Neolithic are far more complex than previously thought. Genetic and zooarchaeological studies have nowadays shown that the shift to food production was not, in fact a 'revolution', and that the process through which wild animals and plants became domesticated is much more protracted than previously thought (e.g. Allaby, 2008; Zeder, 2008). The

terminology used to address research on the origin and spread of agriculture has also been heavily scrutinized, in particular, the term 'Neolithic', which is synonymous with a wide variety of meanings, attributed since the term was first coined. The criteria used to identify farming settlements have also been heavily critiqued, especially the concept of a 'Neolithic Package', in particular the inclusion of pottery as an indicator of Neolithic communities. Research into the origin and spread of agriculture has gradually taken on a more multidisciplinary approach, and is revealing a multifaceted series of events which took place at the transitory period.

The current model proposed to explain the expansion of farming in the western Mediterranean suggests that this was a punctuated event, brought about by seafaring farming communities (Zilhão 2001). This view is supported by radiocarbon dates obtained from domestic plants and animals found along coastal sites from Italy to Portugal, which are statistically indistinguishable and cluster around 5500 cal. BC (Zilhão, 2001). At this time, characteristic Impressed/Cardial pottery and domesticates appear contemporaneously on Mediterranean coastal sites. As described above, pottery has for a long time been perceived as an indicator of agrarian settlements. In fact, its association with farming communities was widely accepted until evidence for the production of ceramic vessels was identified in hunter-gatherer communities in Asia and the Russian Far East, dating back to the Pleistocene (e.g. Jordan and Zvelebil, 2010a and references therein). This therefore questions whether there is in fact a direct association between Impressed/Cardial Wares and domesticates, despite their contemporaneous chronological attestations at the onset of the Neolithic in the western Mediterranean, as it is also plausible to hypothesise that Impressed/Cardial Wares could have been spread by highly mobile hunter-gatherer-fishing communities. The key to understanding the link between Impressed/Cardial Wares and farming in the Mediterranean, and therefore also how these ceramics were spread, is to identify the contents, hence function, of these vessels.

This research aims to apply organic residue analysis (ORA) to Impressed/Cardial Wares vessels recovered from key Early Neolithic sites in Italy, Malta, Croatia and Catalonia, to identify the contents and function of these ceramic wares. If a direct link for the use of Impressed/Cardial Wares in agrarian/pastoral activities can be established, it can then be suggested that these ceramics spread simultaneously with domesticates by farming communities at the start of the Neolithic. Conversely, if a direct link between Impressed/Cardial Wares and farming activities cannot be determined, it can then be suggested that the spread of these ceramics could have been facilitated by highly mobile hunter-gatherer-fishing communities, and their function (e.g. to process wild food products) could have been more varied and regionally defined. Identifying the

contents of Impressed/Cardial Wares is therefore crucial to understanding the link between these ceramics and early farming communities, and how they were spread across the western Mediterranean basin. This is the main research question being addressed in this study.

1.2 The research method

ORA was selected as the main analytical technique. ORA is a well-established technique, which has been routinely used over the past two decades to characterise a wide range of animal and plant products present in archaeological artefacts (Evershed *et al.*, 1999). The premise for using ORA is that when animal and plant products are processed in unglazed ceramics, the heat generated will cause the fatty components in these commodities to become absorbed within the ceramic walls (Evershed, 1993; Heron and Evershed, 1993). These absorbed lipid residues can be extracted and characterised, hence the contents of individual vessels can be identified. This in turn, establishes a direct link to vessel use (Evershed *et al.*, 1999). Residues from charred visible crusts, which are sometimes found adhered to the surface of ceramic vessels, can also be similarly extracted and characterised. Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS), are the main analytical instruments used to characterise lipid residues. GC analysis is used to separate out and quantify the lipid constituents present in the extracted residue, while GC-MS provides structural information on these lipid constituents, which allows a preliminary identification of the source material to be made. By measuring the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values of two particular fatty acids, palmitic ($\text{C}_{16:0}$) and stearic ($\text{C}_{18:0}$) acids, GC-c-IRMS analysis allows further distinction between ruminant and non-ruminant adipose, and ruminant adipose and dairy fats, whose lipid profiles are too similar to permit a secure characterisation using GC and GC-MS analysis (Evershed *et al.* 2002).

To date, ORA has mostly been used to characterise residues from archaeological artefacts recovered from burial contexts situated in northern latitudes, in particular, the United Kingdom (Evershed *et al.*, 1994, 1997; Dudd and Evershed, 1998; Dudd, 1999; Copley *et al.*, 2003, 2005a, 2005b, 2005c, 2005d; Craig *et al.*, 2005b; Mukherjee *et al.*, 2007, 2008; Evershed, 2008a, 2008b), and the Baltic (Craig *et al.* 2007, 2011). It was only more recently that ORA started to be applied to artefacts excavated from warmer climatic regions, namely in the Near East and southeast Europe. These studies, carried out by Evershed *et al.* (2008b), and Gregg *et al.* (2009), highlighted two important issues: i) that lipid recovery from ceramics excavated in warm climatic regions is generally poor, and ii) that shifts to the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values may occur, which affect the

established categories for identifying animal fats based on the $\delta^{13}\text{C}$ measurements of their $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids. The climatic conditions in the Mediterranean region are very similar, hence the two issues highlighted by Evershed *et al.* (2008b) and Gregg *et al.* (2009) were likely to impact on the analysis of lipid residues extracted from Impressed/Cardial Wares recovered from Mediterranean burial contexts.

Low lipid yields pose two major problems: i) they preclude a secure characterisation of lipid residues because of heightened signals from background contamination, and ii) negligible residues do not necessarily represent advanced phases of lipid degradation, but could also be produced as a result of the function assigned to individual vessels, which need not necessarily have been conducive to the formation of an absorbed residue. On the other hand, Gregg *et al.*'s (2009) cautionary note pertaining to possible shifts in the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements needs to be validated before residues extracted from ceramics buried in warmer climatic conditions can be characterised using GC-c-IRMS analysis. This is important because shifts in the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements may cause changes to the established categories which distinguish between the different types of animal fats. It was therefore necessary to compile a reference collection of $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values from modern animal fats of Mediterranean origin, and observe any changes to the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements when compared to northern European modern reference animal fats. Experimental work was therefore required to address these two issues before ORA could be applied to Impressed/Cardial Wares.

1.3 Research aims and objectives

The main aim of this research is to identify the function of Impressed/Cardial Wares recovered from stratigraphic deposits associated with the earliest attestations of the Neolithic in the western Mediterranean, and therefore attempt to determine whether a direct connection exists between Impressed/Cardial Wares and the earliest farming communities present in this region. The simultaneous appearance of domesticates and Impressed/Cardial Wares in Early Neolithic deposits indirectly infers that these ceramic wares were used to process agricultural products including milk, which further implies a close association between the spread of Impressed/Cardial Wares and of agriculture and pastoralism. A counter-hypothesis is presented here, which suggests that this need not necessarily have been the case, since Impressed/Cardial pottery could also have been used to process wild products by highly mobile fishing and/or foraging communities, which is supported by the presence of ceramic vessels in non-agrarian communities (e.g. in Africa and the Far East). In this scenario, the presence of Impressed/Cardial pottery need not attest to

an agrarian society, and the usage patterns of these ceramics could have been less consistent and more regionally defined. Identifying the function of Impressed/Cardial Wares, possibly unique to agrarian/pastoral communities, is therefore crucial to understanding the link between the spread of these ceramic wares and farming in the western Mediterranean.

The following objectives were therefore set:

1. To collect Impressed/Cardial Wares from key Early Neolithic sites situated in the western Mediterranean and analyse these using ORA to determine past vessel use. This will be carried out by extracting lipid residues absorbed within the walls of the ceramic vessels tested and charred visible residue (when available), which will then be characterised using GC, GC-MS and GC-c-IRMS to identify the original source material processed. Quantification of the lipid residues recovered will establish whether ORA can be successfully used to study the function of Impressed/Cardial Wares. The following observations will then be investigated using the data obtained:
 - a. Observe any trends between the shape, fabric and decoration of the Impressed/Cardial Ware vessels tested. In particular, to try to identify whether the different vessel shapes, ceramic fabrics and decorative motifs are indicative of a specific function, and whether the type of commodity processed within Impressed/Cardial wares was particular to a vessel's shape, fabric and/or surface treatment.
 - b. Observe any changes in the use of Impressed/Cardial Wares over time, by analysing Impressed/Cardial Ware vessels excavated from Early and Middle Neolithic stratigraphic deposits, and comparing the quantity and type of lipid residue extracted.
 - c. Observe any intra-site variation in the function of Impressed/Cardial Wares collected from 14 sites situated in Italy, Malta, Croatia and Catalonia, by comparing the quantity and type of lipid residue extracted. Similarly, to assess the degree of regional variability between sites located within coastal areas and those located further inland, as well as between sites situated in different countries within the Mediterranean basin.
2. In view of the cautionary note issued by Gregg *et al.* (2009) on possible shifts, caused by warmer climatic conditions, to the established $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements used to distinguish between different categories of animal fats, namely non-ruminant and ruminant adipose, and ruminant adipose and ruminant milk fats, it is necessary to:

- a. Use GC-c-IRMS analysis to create a reference dataset of $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ measurements extracted from modern terrestrial animal and marine fats of Mediterranean origin.
 - b. Use this dataset to observe any changes to the $\delta^{13}\text{C}$ values, and the established $\Delta^{13}\text{C}$ parameters by comparing the Mediterranean dataset to their northern European and Near Eastern counterparts using published literature (Dudd, 1999; Craig *et al.*, 2007; Gregg *et al.*, 2009).
 - c. Establish the $\Delta^{13}\text{C}$ parameters of modern reference animal and marine fats of Mediterranean origin, to be used when interpreting GC-c-IRMS data of residues extracted from Impressed/Cardial Wares.
3. To investigate the presence of plant products in pottery using ORA. Archaeobotanical and palaeodietary evidence both suggest a strong reliance on plant material in the diet of the Early Neolithic settlements investigated, and Impressed/Cardial Wares could therefore have been used to store/process plant products. However, plant lipids leave a low chemical fingerprint which is difficult to distinguish from low lipid yields resulting from poor lipid preservation. This problem needed to be addressed by:
- a. Carrying out a series of cooking experiments using different plant materials which were likely to have been processed in the Impressed/Cardial Wares investigated, and assessing the lipid profiles obtained after further degradation of the absorbed plant lipid residues through a prolonged burial period, which was required to better simulate plant lipid degradation over archaeological timescales.
 - b. Using GC to quantify the amount of plant lipid residue retained after burial, and hence attempt to determine whether the quantity of lipid absorbed in ceramic vessels used to process plant material can be distinguished from low lipid yields resulting from advanced phases of lipid degradation.
 - c. Using GC-MS analysis to carry out an in-depth study of the lipid profile obtained from the degraded plant oils, to attempt to identify specific biomarkers which could potentially be used to indicate the presence of plant products, and perhaps identify different plant varieties to species level.

1.4 Overview of the thesis structure

Chapter 1 has outlined the main aim and objectives investigated in this research. An overview of the archaeological context which holds the framework for the hypothesis and counter-hypothesis put forward was provided. The analytical method chosen, ORA, was also briefly described to

justify its application to meet the research objectives set, and ultimately answer the research question. This chapter has also introduced two important analytical issues highlighted by previous research, which applied ORA to study lipid residues extracted from ceramic vessels buried in warm climatic regions. These issues are pertinent to the present study, which proposes to apply ORA to Impressed/Cardial Wares from Mediterranean archaeological contexts. In view of this, two experimental procedures have been outlined, targeted to address these issues, and ensure an accurate characterisation of lipid residues extracted from the Impressed/Cardial Wares tested.

Chapter 2 provides a more detailed overview of the archaeological context. It includes a critique of the terms 'Neolithic' and the so-called 'Neolithic Package', and attempts to provide a comprehensive review of the models and theories put forward to explain the origin and spread of agriculture. The main focus of this section is directed towards the Mediterranean region, and includes an overview of current research pertaining to the spread of agriculture in this area, which is thought to have occurred through maritime colonisation by pioneer, seafaring farmers. The role of pottery in agrarian and non-agrarian communities is discussed in some detail, with a particular focus on the 'conditions' required for pottery production and the 'social aspect' of pottery. However, the central theme of this chapter is a detailed examination of the main archaeological evidence related to the earliest appearance of domesticates and Impressed/Cardial Wares in the western Mediterranean region. The evidence available to date is reviewed in chronological order, and includes a description of settlement patterns and evidence for contact between the different settlements, a description of the floral and faunal remains recovered and a detailed overview of the physical and stylistic characteristics of Impressed/Cardial Wares across the Mediterranean basin. This data is synthesised towards the end of the chapter and reviewed in terms of the archaeological evidence available to explain how farming spread across the western Mediterranean, hence providing the framework for the hypothesis being tested in this research.

Chapter 3 provides an overview of ORA. It details important developments in methodology and describes the various instruments used to analyse archaeological residues, in particular, GC, GC-MS and GC-c-IRMS. The molecular and isotopic criteria used to distinguish between different types of animal fats and plant oils extracted from archaeological residues are provided, including a critique of fatty acid ratios and an overview of the known biomarkers. Various applications of ORA are described in terms of the wide repertoire of animal and plant commodities which have been identified using this technique so far. A brief overview of lipid chemistry is provided, while lipid preservation and degradation are discussed in some detail to provide the background

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required for the plant degradation experiment. Possible issues with contamination are also outlined.

Chapter 4 reports the results obtained from the controlled feeding experiment. A step-wise description of the experimental set up is provided, and the analytical procedure followed is recorded. The rationale behind the experimental set up is outlined, which includes a detailed analysis of the biosynthesis and routing of the $C_{16:0}$ and $C_{18:0}$ fatty acids, that are key to understanding how the different categories of animal fat can be distinguished using GC-c-IRMS analysis. The data obtained from this experiment are presented and compared to published GC-c-IRMS data obtained from modern reference animal fats sourced from northern Europe and the Near East. The more positive values observed in the $\delta^{13}C$ measurements of both the $C_{16:0}$ and $C_{18:0}$ fatty acids from modern Mediterranean non-ruminant and ruminant blood, and ruminant dairy products is explained in terms of changes in the $\delta^{13}C$ values of dietary plant material with latitude, which are then transmitted along the food chain. Shifts in the $\Delta^{13}C$ were also observed and found to be statistically significant, which therefore called for a revision of the $\Delta^{13}C$ values used to differentiate between the different categories of animal fat sourced from the Mediterranean region. Interestingly Mediterranean marine fish $\delta^{13}C$ values for both $C_{16:0}$ and $C_{18:0}$ were found to be more negative compared to the values obtained for fish caught in the Baltic. This as yet remains unexplained. The chapter concludes with the resulting archaeological implications and recommended further work.

Chapter 5 describes the degradation experiment carried out on three plant varieties, namely, acorns, nettles and einkorn. The experimental rationale and the main objectives set for this experiment are described in the introduction. In addition to the objectives listed in Section 1.3 above, an additional experiment was carried out to test whether the lipid profile obtained for degraded einkorn can still be identified when boiled with a fattier substance, in this case milk. The lipid profiles and quantified values of the total lipid extracts obtained prior to and after burial, for each of the cooking experiments, are reported and discussed separately. Charred visible residues which formed during cooking were also analysed. Potential biomarkers for the degraded plant residues analysed using GC-MS are also reported. Finally, the archaeological implications resulting from this experiment are outlined, and recommendations of further work conclude the chapter.

Chapter 6 describes the methodological and analytical protocol followed to prepare and analyse archaeological residues extracted from Impressed/Cardial Wares. This chapter describes the criteria followed to select the sites included in this research, and to sample the ceramics analysed.

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It describes the protocol followed to extract the lipid residues and prepare samples for analysis. The instrument parameters used for GC, GC-MS and GC-c-IRMS analysis are also described. This chapter also provides a summary of the fragmentation patterns pertaining to the main classes of lipids identified in archaeological residues using GC-MS.

Chapter 7 describes the results obtained following ORA of the Impressed/Cardial Ware samples selected for analysis. The results are presented by region, and include a brief description of each site investigated, the pottery assemblage analysed and the floral and faunal assemblage recovered. These were used to interpret the results obtained following GC, GC-MS and GC-c-IRMS analysis. The results obtained from the controlled feeding experiment were used to interpret the GC-c-IRMS results obtained from Impressed/Cardial Ware residues. The chapter concludes with an overview of the results obtained.

Chapter 8 provides a synthesis of the experimental and archaeological results obtained from this research. As expected, a large percentage of the vessels analysed produced negligible lipid yields. The significance of the negligible residues obtained are discussed in light of the results obtained from the plant degradation experiment conducted, and experimental analysis carried out at the University of Durham, which showed that the number of times a vessel is used will also influence whether or not a residue is likely to form. The preservation potential of the burial context is also considered, and a comparative review of quantified lipid values extracted from ceramic vessels buried in cooler and warmer climatic conditions is provided. Subsequent sections provide a critical synthesis of the results obtained. Tentative identifications of plant lipid residues in Impressed/Cardial Wares are supported by the results obtained from the plant burial experiment conducted, available archaeobotanical evidence and published palaeodietary data carried out at a number of sites included in this research. The animal products identified using ORA, including ruminant and non-ruminant fats, and dairy products are discussed separately, and trends in the function of Impressed/Cardial Wares relative to vessel shape, ceramic fabric and decorative motifs are described. Dairy products are discussed in more detail, and the data obtained from Impressed/Cardial Wares are tied in with the earliest evidence for pastoral activities in the Levant, Europe, and Africa obtained using ORA in other published research. The absence of marine products in Impressed/Cardial Wares is argued in light of the data produced by stable carbon and nitrogen analysis carried out on Early Neolithic osteological assemblages in Europe and the Mediterranean. The chapter concludes with recommendations for further work, and an overall conclusion of the data produced by this research.

Supplementary data obtained for both the experimental and archaeological analyses carried out in this study are reported in the appendices, which can be accessed from the cd included. Appendices A and B contain additional information relevant to the controlled feeding experiment and the plant degradation experiment, respectively. A sample catalogue containing detailed descriptions of the Impressed/Cardial Ware samples analysed are recorded in Appendix C. Appendix D records the quantified values obtained for the total lipid extracts extracted from the Impressed/Cardial Ware samples analysed, and includes a record of the different classes of lipids identified using GC-MS, as well as the isotopic measurements obtained for selected samples using GC-c-IRMS. Appendix E is a catalogue of the gas chromatograms obtained after analysis of the lipid residues extracted from Impressed/Cardial Ware vessels included in this research.

Chapter 2

Pottery and agriculture: a Mediterranean perspective

2.1 Introduction

The aim of this chapter is to provide a synthesis of archaeological research on the spread of the Neolithic in the Mediterranean. It does not purport to be exhaustive of the current available data, which is considerable and spans a wide geographical region. The key issue addressed will be the dynamics of the spread of ceramics and domesticates in the Mediterranean, and whether archaeological evidence supports the idea of a diffusion of the so called 'Neolithic Package' in this region.

Pottery has long been considered to be a fundamental part of this 'Neolithic Package', so much so that Childe (1951:76) labelled it as a universal characteristic of the Neolithic. Three decades later Rice (1987:9) still considered pottery to be part of the Neolithic Technocomplex, which included among other things, tools and containers for food storage and preparation. However, as more sites were excavated, evidence came to light which questioned the relevance of pottery as an indicator of the onset of the Neolithic, as well as the seemingly straightforward interpretation of the spread of agricultural paraphernalia. In particular, this is evident in the varying chronological attestations for pottery and agriculture in the stratigraphic deposits of the Levant, Africa and Asia. In Japan, China and the Russian Far East, pottery vessels appear to have been used quite a few millennia before the onset of plant and animal husbandry (Aikens, 1995; Jordan and Zvebil, 2010b; Kaner, 2010; Zhushchikhovskaya, 2010), and similarly in North Africa, where agriculture appears two or three thousand years after the beginning of pottery production (Close, 1995). On the other hand, evidence for agrarian practices in the Levant occurs well before the earliest indications for pottery production (Moore, 1995). The Mediterranean makes an interesting comparison. Current data shows that during the transition to agriculture in the eastern Mediterranean, aceramic agrarian communities did exist (e.g. in Thessaly; Pessina and Tiné, 2008:26-27), while pottery has also been retrieved from contexts associated with purely wild taxa (e.g. at Odmut; Forenbaher and Miracle, 2005 and reference therein). However, excavations in Italy consistently show a simultaneous introduction of domesticates and pottery (Muntoni, 2009),

which appear to have spread rapidly westwards as a package (Zilhão, 2001; Rowley-Conwy, 2011). There is as yet no evidence to suggest interaction between Mesolithic and Neolithic groups, except at Sidari in Greece (Perlès, 2001:49-50; Pessina and Tiné, 2008:27-28), and in Iberia (Zilhão, 2000; Arias, 2007) (discussed below).

The role of pottery at the transition to agriculture in the Mediterranean is therefore the major theme of this chapter. The terminology used to describe the onset of farming, namely the terms 'Neolithic' and 'Neolithic Package' will first be discussed, followed by a brief overview of the models and theories used to describe how and why agriculture came about. The next section will provide a general discussion on the much debated conditions required for pottery production, followed by a description of Impressed/Cardial Wares, which are the earliest pottery type present in the western Mediterranean, and the key artefacts studied in this research. Theories on the spread of the Neolithic in the Mediterranean will then be explored, the main focus being the apparent simultaneous occurrence of pottery and domesticates in association with the earliest Mediterranean Neolithic stratigraphies. Therefore chronology also plays a key role in this chapter; hence the dates cited were obtained from the most recent publications available. This chapter will conclude with a brief discussion on the role of Impressed/Cardial Wares in relation to agrarian practices during the Mediterranean Neolithic.

2.2 Terminology

When first coined by Sir John Lubbock (1865), the term *Neolithic* was used to describe a technological rather than an economic phenomenon. Since then, it has been used to signify a time period, a cultural phase, an economic change, and more recently, a social and cultural transformation (Whittle, 1996:4-9; Thomas, 1999:13; Price, 2000:4; Lewis-Williams and Pearce, 2005:17). However, there is no one standard form for the manifestation of the Neolithic worldwide, which has led to much debate over its terminological significance. Chronologically speaking, the widespread geographical appearance of the Neolithic did not occur simultaneously, but over several thousand years, while different aspects of its economic (e.g. domesticated plants) and social (e.g. sedentism) characteristics, are not necessarily concurrently attested (Simmons, 2007:4). Pluciennik (1998) notes that archaeological evidence for the Neolithic is so variable that 'there were probably many different Neolithics'. Zvelebil and Lille (2000) further note that if the term *Neolithisation* is to be retained, it needs to have a common denominator, which they suggest, can only be evidence for the development of agro-pastoral farming. Çilingiroğlu (2005) provides a comprehensive perspective of what the term came to represent,

namely a change in the procurement of food, accompanied by technological and economic developments, that were socially constructed and which eventually led to their spread.

The Neolithic eventually became equated with a defined set of materials, collectively labelled as the '*Neolithic Package*'. The meaning of the term, which was initially intended to differentiate between Palaeolithic and Neolithic assemblages, gradually came to comprise different components of the Neolithic way of life, including, domesticated animals and plants, permanent villages with rectangular structures, religious artefacts, pottery, ground stone tools, as well as the knowledge and concepts required for agrarian practice (Price, 2000:5). However, establishing a list of universal criteria to define agrarian groups is impossible, because not all the items making up the package will necessarily appear simultaneously (Simmons, 2007:5), and the retrieval of parts of the package cannot confidently account for an agrarian community (Thomas, 1999:13; Price, 2000:5). In fact, Thomas (1999:13-14) points out that attempts to identify the Neolithic through a set of attributes 'will be arbitrary in the extreme', and 'at no point did a homogeneous Neolithic 'package' of economic practice and material culture ever [exist]'. Apart from lacking a clear definition of what the package constitutes, over time and space the contents may become more diverse, and subject to changes in appearance, function and meaning (Çilingiroğlu, 2005). Identifying the different constituents making up the package is not always straightforward; for example, one of the most diagnostic classes of artefacts pertaining to the Neolithic in the Near East is projectile points, which are more likely to be associated with hunting rather than domestic practices (Simmons, 2007:4). Moreover, different communities will not perceive material culture (albeit similar) in the same way; hence the Neolithic Package cannot point towards 'identical cultural formations' (Çilingiroğlu, 2005). However, despite not being 'a 'magical' term that guarantees an explanation of everything, ...it does have important methodological implications for future research in terms of integrating all the find groups in order to achieve a synthetic approach' (Çilingiroğlu, 2005).

2.3 The 'how' and 'why' of the origin and spread of agriculture

Despite decades of research into how and why agriculture came to be, answers are still elusive. Evidence comes in the form of snapshots, defined by set time-frames and demarcated by the space used by their inhabitants, which are revealed through systematic excavation. Most interpretations are nonetheless still speculative due to a lack of direct evidence, which because of the nature of archaeological data, is not always available. However, although the question being asked may be a long-standing one, development in excavation methodology and scientific

application support a dynamic field of research, which slowly but surely, is shedding light on the Neolithic transition.

Change probably qualifies as the keyword word in studying the transition to agriculture, for it is essentially what researchers are aiming to better understand and interpret. The change which occurred during this timeframe was the way humans acquired food, from hunting and foraging to food production. However, the phenomenon surrounding this change is not the shift itself, but the way it spread worldwide, to become, over several generations, the main form of subsistence. What is striking is the acceptance of this change by an overwhelming majority of existing cultures. Change is not easy to cope with as it takes on board a cascade effect of possible consequences, which are not always beneficial. Yet, so many different cultures recognised the potential of food production, and eventually shifted from a relatively secure way of procuring food, to the initially, much riskier option of producing it. Change in food production meant change to the technological repertoire, along with economic, social and cultural implications. What changed first is still very much debated. Were people forced to change their subsistence strategy because of a climatic change which stressed their resource base? Was it because of population growth, which required a more consistent availability of food? Was it a change in social and cultural values? Some of the theories and models suggested to explain what triggered the origin of agriculture are listed in Table 2.1. This table is by no means exhaustive, but it gives a general idea of how the focus of research and underlying concepts changed over time.

Food is a basic requirement for subsistence, and no person would risk jeopardising such a vital need, if not for something which is strongly believed to bring abundance and security. Change occurs due to innovation, or as a reaction to other transformations. The latter is exemplified by environmental shifts, especially those occurring during the retreat of the Pleistocene ice sheets around 14,000 years ago, which altered the geographic distribution of species, forming new ecological niches (Childe, 1936; Sauer, 1952; Bender, 1975:65-88). Another reason could have been knowledge, acquired through an intimate understanding of the surrounding environment; hence familiarity with the nature of plants, the technology required to exploit resources, and methods of storage to ensure enough produce during times of strife (Braidwood, 1960; Harris, 1969; Hayden, 1981; Rindos, 1984). Sedentism could also have led to the onset of food production (Whittle, 1996). Ethnographic studies have challenged the renowned assumption of foragers leading mobile lives and farmers being more sedentary; permanent and semi-permanent hunter-gatherer settlements are in fact known (Bender, 1975:1-16; Whittle, 1996). Hence, it is possible that in becoming more sedentary, cultivation and herding could have been more easily adopted

(Binford, 1968; Whittle, 1996). Sedentism has also been inherently linked to population growth, which in turn, encourages more reliable subsistence practices, hence the need for agriculture (Smith, 1976; Cohen, 1977; Hassan, 1981; Rosenberg, 1998). Early theories on the origin of agriculture have therefore focused on culture-historic interpretations, with a strong reliance on climatic change and the interaction of humans with their surrounding environment (see Table 2.1). Most of these theories were criticised, mainly because they were too simplistic and could not be corroborated by archaeological evidence (e.g. see Mangelsdorf, 1953 for a criticism of Sauer 1952). Furthermore, the spread of agrarian practices was thought to have occurred by diffusion from the Levant, the notion of *ex oriente lux*, through migration and colonization, without questioning how innovations spread and why they were adopted or rejected (Harris, 2003:46).

A preoccupation with the processes of cultural change followed, and the need to investigate the various hypotheses being put forward by building explanatory models and testing them against real world data (Harris, 2003:48; Simmons, 2007:15). Hence, pioneers of the 'New'/Processual archaeology, argued for a more scientific approach (see Binford and Binford, 1968; Flannery, 1968; Clarke, 1972; Renfrew, 1973). The basic premise which characterised research at this time was that larger populations led to the need for agriculture and not the other way round (Binford, 1968; Cohen, 1977; Redding, 1988; Rosenberg, 1998), and comprised theories on demographics, broad-spectrum subsistence, resource stress, and climatic change, or models based on different combinations of these themes (Simmons, 2007:15). The research focus eventually turned to reconstructing the spread of agriculture and domesticates from selected point(s) of origin, and attempting to test hypothetical routes against models, which were later deemed too general to be tested, especially against archaeological data (Harris, 2003). The debate centred on two main theories: i) that the transition to agriculture had spread through the adoption of the 'Neolithic package' by Mesolithic communities (e.g. the idea of an agricultural frontier proposed by Zvelebil, 1986; see also Zvelebil and Renfrew, 1986; Renfrew, 1987; Zvelebil and Zvelebil, 1988), and ii) demic diffusion (Ammerman and Cavalli-Sforza, 1979, 1984). Zvelebil and Lille (2000) provide a good summary on the shortcomings of the two view points, and stress that although these 'indigenist' and 'colonist' models appear to be contradictory, a combination of the two (population movement and local adoption) was in fact possible.

Phase	Author	Model / Theory	
Early models	Vavilov (1926)	Mapped out the distribution and degree of genetic diversity of various crops, and suggested that regions exhibiting the greatest diversity are likely to have been the earliest to be domesticated.	
	Childe (1936)	Oasis-Propinquity Theory: Major climatic change at the end of the Pleistocene caused large areas to become dry, forcing animals and plants to cluster close to water sources. Humans offered protection for specific animals and were forced to experiment with new plant foods, which eventually led to domestication and the 'Neolithic Revolution'.	
	Sauer (1952)	Focused on the ecology of food production. Transition occurred because of changes in the way culture and ecology interacted, not because of a lack of food. Areas of ecological diversity are likely to be hearths of domestication.	
	Braidwood (1960)	Hilly Flanks Theory: Domestication occurred due to increasing cultural specialisation and to a lesser extent the environment. People living on hilly flanks were the first to begin domestication of animals and plants, due to their familiarity with their natural habitat. Domestication later diffused from the nuclear zones.	
Processual / 'New' Archaeology	Resource Stress	Binford (1968)	Sedentism leads to population growth, and an increase in the need for locally available plant foods (e.g. cereals). Intensification of use and the technology required for processing leads to a regular cycle of planting and harvesting, and eventually domestication.
		Flannery (1968)	'Broad spectrum subsistence' at the end of the Epipalaeolithic led to sedentism and population growth. People were forced to adopt agriculture to ensure an adequate food supply. First village societies based on communal sharing (round buildings), followed by evolution of nuclear families (rectangular structures).
		Hayden (1981)	Resource availability stressed due to climatic conditions resulting in an: i) attempt to maintain population at an acceptable level; ii) attempt to lessen resource stress by increasing the reliability of the exploited resources through a) utilisation of a wider range of food products in resource poor zones, and b) by targeting particular resources in richer areas. This resulted in technological innovations.
		Rindos (1984)	Long term coevolution of plants and animals resulted in domestication. Thus, the transition was unintentional.
	Population Stress	Cohen (1977)	Alternative subsistence strategies required due to population growth, ultimately resulting in agriculture.
		Redding (1988)	Growth in hunter-gatherer populations stressed their resource basis forcing groups to react by: i) regulating population growth by emigration and mobility; ii) diversifying their resource base; iii) storage; iv) domesticating plants and animals.
		Rosenberg (1998)	Imbalances in population size and resource availability are due to the inherent tendency of populations to grow. However, in the context of escalating competition, sedentism provides the advantage of increased access to the most productive areas of a territory.
	Social Motifs	Bender (1978)	The success of food production was the ability of selected individuals to accumulate food surpluses and transform these into valued items. Social changes acted independent of technology and economy to create pressures on production.
		Hayden (1995a)	Proposed feasting as a driver for the origins of food production. Food is viewed as power.
	Post-processual	Hodder (1992)	Focused on the concept of the 'domestication of society', and 'taming of the wild'. Symbolised the role of the <i>domus</i> (the house/domestic, which apart from referring to practical activities, also symbolises social and economic strategies and relations of power), and the <i>agrius</i> (the wild).
Tilley (1996)		Suggested that food domestication did not come about purely due to economic reasons, but rather due to its social and ideological significance.	
Cauvin (2000)		Viewed the 'Neolithic Revolution' as one of symbols and not economics; a religious experience.	

Table 2.1: Table listing proposed models/theories on the origins of agriculture. Based on Simmons (2007).

However, the persistent perception of the Neolithic as an economic phenomenon introduced by Childe's 1920s label of a 'Neolithic Revolution', and later emphasised by the notion of a 'secondary products revolution', has detracted from a closer consideration of the social context of food production (Whittle, 1996). The individuality and cultural distinction of the different communities appears to have been lost in an effort to fit these societies to models. The social context of the transition did eventually gain importance in the debate. Earlier references to the importance of social changes as independent motives for the adoption of farming (e.g. Bender, 1978), and emphasis of 'the human agency' in allowing the transition to happen, was brought forward by the 'Post-Processual' paradigm (Simmons, 2007:19). For example, Gosden (1999) highlighted an important factor which played a major role at the transition, *choice*. He observed that farming, hunting and gathering are not polar opposites, and that the complexity of techniques and processes involved, as well as the opportunity for separate groups and communities to decide how to subsist, needed to be acknowledged (Gosden, 1999). Hodder (1992:241-244) pointed out another important transformation required by farmers: the acceptance of a delayed return for labour and long-term social commitments, as opposed to the immediate return for labour and fewer social commitments required by a hunter-gatherer subsistence. This acceptance involved a 'domestication' of people, who chose to lose their personal authority and be constrained to a larger group in order to enjoy the advantages of an increased production (e.g. social dominance, better access to goods, feasts and prestige; see also Hayden, 1995a), and to benefit from the protection of the house (*domus*), against the wild (*agrrios*) (Hodder, 1992:241-244). Cauvin (2000) went as far as to argue that changes in subsistence (infrastructure) were preceded by a change in religion and symbolism (*thought* - the superstructure). The cultivation of cereals in the Levant during the Pre-Pottery Neolithic A (PPNA) seems to have coincided with the appearance of the first ceramic female figurines, which prompted Cauvin (2000) to suggest that it was the inception of both religion and the Venus figurines which encouraged the development of agriculture, and its subsequent diffusion. This theory has not been widely accepted, however it has already been recognised that what we interpret as evidence for domestication has been a long time in the making (e.g. Allaby *et al.*, 2008; Zeder, 2008). Hence, as Whittle (1996) very aptly put it, change cannot be confined to the Neolithic period, for there was no uniform process or single history, nor the spread of something which was already formed, but 'a series of becomings'.

Archaeological evidence for food, including artefacts required for food processing (e.g. tools, grindstones, and cooking pots), and food wasters (e.g. faunal and floral remains), is rarely interpreted on a cultural basis, rather, it is the economic aspect which is generally singled out

(Gosden, 1999). Yet, 'human diets are culturally patterned and many items that are known to be edible may not be considered to be food' (van der Merwe, 1992). Hence, apart from understanding the ecological and genetic matters related to cultigens and domesticates, it is also necessary to understand how these people approached the landscape they lived and worked on, how they divided their food between edible and inedible, how they impressed their culture onto their surroundings, and tried to change it for their benefit (Gosden, 1999). Eventually, the ability to store food and create a surplus, gave it a whole new meaning. Food could be used as a medium for exchange, to secure allegiance, and purchase labour (Hole, 1992). Hence, without detracting from the importance of an economic interpretation of food, a wealth of information can be obtained by focusing on its cultural aspect.

The establishment of agro-pastoralism eventually came to be considered as more complex and regionally varied, and previously proposed models of a demic diffusion (*cf.* the Wave of Advance model put forward by Ammerman and Cavalli-Sforza, 1979) lost influence in favour of a piecemeal adoption of the Neolithic, or its acceptance as a package (Harris, 2003). To date, the two theories which appear to dominate the transition to agriculture debate (migration and acculturation), have not much varied from those put forward by the Processual archaeologists of the 1980s. Robb and Miracle (2007) proposed that the key to understanding the transition is to use social logic; hence the focus should be on how the different groups of foragers, farmers and communities practising both farming and foraging economies, used cultural differences among themselves and their neighbours to 'recreate their way of life'. Smith (2001) built on Bogucki's (1995) challenge of the hunter-gatherer and agriculture duality, stating that by focussing on the beginning (hunter-gatherers) and end point (farmers), we are missing what comes in between; hence, a sounder conceptual framework of the 'middle-ground' is required. In light of this, Smith (2001) proposed that terms such as 'cultivation', 'domestication' and 'husbandry' do not really work, and suggested instead a lower-level, three-part division of food production. Therefore, the 'middle ground' between hunter-gatherer and agrarian communities is defined by groups of people whose annual caloric intake from domesticates amounts to less than 30-50% of their dietary requirements, and their faunal record may or may not provide evidence for the presence of domesticates (Smith, 2001, Fig. 7). Both Robb and Miracle (2007), and Smith (2001) recognised the need to move on from the 'migrationist/acculturation' view of the transition, and re-focus archaeological research on the cultural changes taking place in the different communities present during the transition to food production.

Scientific analyses have, over the years, come to play an important role in understanding the origin and spread of agriculture. DNA analyses have been instrumental in establishing a point of origin for domesticates (e.g. Bradley, 2006; Bradley and Magee, 2006; Luikart *et al.*, 2006; Larson *et al.*, 2007; Allaby *et al.*, 2008), and have also been used to map the spread of agrarian communities (e.g. Diamond and Bellwood, 2003; Pinhasi, 2003; Budja, 2005; Bramanti *et al.*, 2009), while research projects using stable isotope (e.g. Le Bras-Gaude and Claustre, 2009; Lightfoot *et al.*, 2011; Lelli *et al.*, in press), and organic residue analysis (Evershed *et al.*, 2008b), have been used to focus on the dietary practices and patterns at the transition. Hence, perhaps the future of the research into the origins and spread of agriculture lies in combining archaeological and scientific analysis, which will hopefully enhance our understanding of the *choices* that were made in reaction to the *change* in human subsistence, and *why* food production was eventually accepted by almost all existing communities.

2.4 'Conditions' for pottery production

The earliest archaeological evidence for the use of clay in central and western Europe dates back to the Upper Palaeolithic, and can be seen in several examples of artistic sculpting (e.g. clay bison at Tuc d'Audoubert cave, in France), and the rich array of Venus figurines recovered from several sites, including Dolní Věstonice, Pavlov and Kostenki (Zimmerman and Huxtable, 1971; Budja, 2007:18-19). Clay-lined hearths dating to this early period have also been documented (Koumouzelis *et al.*, 2001; Karkanas *et al.*, 2004). Hence, as Rice (1987:8) pointed out, a working knowledge of the plasticity of moist clay and the fact that it retains its shape when dry had already been discovered, along with the realisation that clay hardens upon firing, and that the addition of various substances to clay will improve its properties and usefulness. Various theories have been put forward as to how pottery vessels came about. Childe (1951:76) referred to pot making as the earliest, conscious use by man of a chemical change, and suggested that perhaps the first attempts at pot making were as skeuomorphs of basketry containers. Rice (1987:8-9) suggested that the first attempts might have involved modelling clay containers which were then dried and hardened in the sun, and which would have served well for storing dry products, such as grains, seeds and herbs; the one drawback being that such items would very rarely survive in the archaeological record. Clay could also have been used to line baskets (Childe, 1951:76) or pits (Rice, 1987:9), and the (perhaps accidental) exposure to heat might have quickly led to recognising the potential of fired clay in creating portable, impermeable containers, as well as important implements in cooking, which would allow a wider range of products to be consumed (Arnold, 1985:128). The role of pottery also expanded to comprise a social function, as items of

prestige, to be used in ritual displays (Hayden, 1995b), and symbols of ethnicity and group identity, perhaps reinforced through the application of particular decorative motifs (Armit and Finlayson, 1995; Hoopes and Barnett, 1995).

The potential for pottery studies to reveal information about the social aspect of the society they belonged to was highlighted in a major publication edited by Hoopes and Barnett (1995), which comprised a worldwide coverage of contributions on innovative concepts relating to the emergence of pottery and possible interpretations for their role in society. Vitelli (1995) suggested that the emergence of pottery in Neolithic Europe was a symbolic process associated with the use of pottery in social practices, such as consumption, which are often linked to prestige and communality. Hayden (1995b) was in fact one of several authors to suggest that pottery stemmed as a prestige item for the storage and preparation of prestige foods used in ceremonies and feasting; the latter being important in both the creation of communities and in ascertaining power. He further stated that, in this scenario, the first pottery to appear would be the food-serving containers, and perhaps vessels for the processing of prestige foods through boiling, straining or brewing. This would then be followed by a rapid evolution towards a labour-intensive, specialized production of highly decorated forms, showing a good control of the medium and expertise (Hayden, 1995b). Pottery studies are therefore an integral part of archaeological research, mainly due to their excellent preservation over archaeological timescales and the wealth of information they can potentially provide about different aspects of a community's way of life.

It has long been assumed, that the earliest ceramic vessels were produced and used by early farmers, thus implying that the spread of pottery is linked to that of agrarian practices (Jordan and Zvelebil, 2010b). However, there is no real pattern between the onset of agriculture and the inception of pottery, and neither appears to be dependent on the other (Barnett, 1995). Several authors in fact agree that pottery did not originate in a single time and place, but rather, that the idea was independently brought about in different cultural contexts and in an unknown number of centres (Rice, 1987:8; Hoopes and Barnett, 1995). More recently, archaeological evidence has shown that the use of clay to make pottery containers does indeed date back to before the beginning of the Holocene, therefore dismissing current theories for a later production (e.g. Hoopes and Barnett, 1995). Work carried out in the Far East has demonstrated that the first ceramic vessels appeared during the Upper Palaeolithic, in Pleistocene hunter-gatherer societies, therefore long before the transition to agriculture in the early Holocene (Jordan and Zvelebil, 2010b; Kaner, 2010; Zhushchikhovskaya, 2010; Zhao, 2011). As Jordan and Zvelebil (2010) point

out, these findings will hopefully begin to dispel the long standing concept that pottery using hunter-gatherer societies are anomalous, and that the presence of pottery can only be explained through contact with Neolithic farmers (Jordan and Zvelebil, 2010b). These findings also reinforce Barnett's (1995:79) proposal, that new technologies (in this case pottery) can be the driving force behind a change in subsistence, and vice versa, that is, pottery production is needed to store an accumulation of products brought about by agriculture. However, ultimately, the adoption of both pottery making and agrarian practices are subject to the distinctive requirements and lifestyles of the different communities.

Sedentism was also strongly associated with the onset of pottery production, so much so that it too formed part of the 'Neolithic Package', along with ceramics and agricultural technology (Armit and Finlayson, 1995:267). Given the nature of the work involved in food production and pot making, it was perhaps only natural to assume that the communities who produced these were required to be sedentary. Rice (1987:9) acknowledged that there is no necessary causal relationship between agrarian practices and pot production, however she did make a case for pottery production being more popular among present day sedentary communities rather than nomadic societies. The initial error was therefore to assume that the three (pottery production, agriculture and sedentism) were necessarily linked (Hoopes and Barnett, 1995). Archaeological evidence has shown that complex hunter-gatherer societies utilized many of the practices labelled as Neolithic innovations; for example pottery technology, ground stone axes, metallurgy, monumental architecture, long distance trade, complex social organization, and sedentary village life (Hoopes and Barnett, 1995). The presence of aceramic Neolithic communities are in fact well documented in the Near East (Moore, 1995), and evidence for an independent spread of pottery and food production is evident in Africa (Close, 1995), and eastern Asia (including China, Japan and the Russian Far East) (Jordan and Zvelebil, 2010b; Kaner, 2010; Zhushchikhovskaya, 2010; Zhao, 2011). Furthermore, evidence for seasonal occupation of sites by food-producing communities has also been recorded (Bellwood, 2005:23), showing that all three elements can exist separately. The use of pottery vessels may therefore complement rather than follow from agricultural processes, hence, pottery production needs to be disassociated from both sedentism and agriculture (Hoopes and Barnett, 1995; Jordan and Zvelebil, 2010a and references therein).

2.5 Impressed/Cardial Wares

Impressed/Cardial Wares are among the earliest types of pottery to appear in the Mediterranean region. The type-ware describes their distinctive decorative motifs, comprising a wide array of

impressions created using fingers, fingernails and other small instruments (*Impressa/Impresso Wares*), and/or impressions made by using the edges of the *Cerastoderma edule* L. (*Cardium*) and *Glycymeris insubricus* Broc. shells (*Cardial Wares*), in the soft, unfired clay (Spataro, 2009a:64) (Figure 2.1). *Impressa* decorations are generally associated with the eastern and central Mediterranean, up to the Ligurian coast of Italy; although, cardial impressions are well documented in Italy. Similarly, Cardial Wares tend to dominate in the western Mediterranean, though *Impressa* decorations were also used (Barnett, 2000).

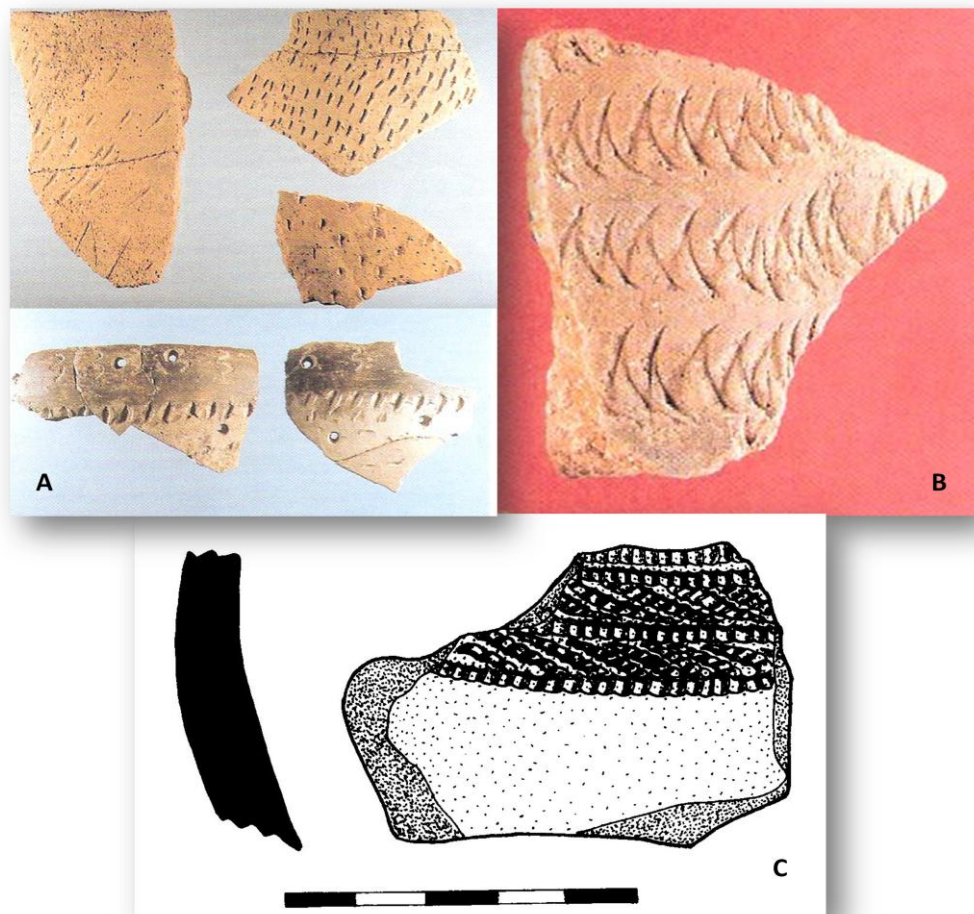


Figure 2.1: Impressed and Cardial Ware decorations. A: Impressed decorative motifs from Balsignano (Apulia) (Muntoni, 2002a); B: Rocker decoration, created by the continuous zig-zag motion of a shell along a set trajectory, from Balsignano (Apulia) (Muntoni, 2002a); C: Cardial decoration, executed using a *Cardium* shell, from Can Sadurní (Catalonia) (Courtesy of M. Edo).

Early pottery was divided into two main categories, coarse and fine wares, the former possibly used for cooking, while the latter appears to have been used in the consumption of specific foods and drinks (Tiné, 2002:139). The differences between the two categories were not simply aesthetic (e.g. different styles of surface finishing) but sometimes structural (e.g. the type of

temper used, which may have played a significant role in the functional properties of the vessels produced) (Pessina, 2002:120). Impressed/Cardial Wares had rather simple shapes, comprising hemispherical and conical bowls, large deep vessels, cups and more rarely, bi-conical vessels and necked flasks (Spataro, 2009a:64). These wares were influenced by local customs, but they also spread very rapidly across the Mediterranean area (Gheorghiu, 2008:164). In fact, pottery is one of the best known aspects of the Impressed Ware culture (Spataro, 2011), and it is still considered an indicator of these farming communities and the major means of investigating their way of life (Muntoni, 2002b:209; Spataro, 2009a:63).

Section 2.6 will provide a chronological synthesis of the earliest attestations of Impressed/Cardial Wares and domesticates in the Mediterranean. The onset of farming in this region will be discussed in terms of the archaeological evidence related to subsistence patterns, similarity in pottery assemblages, settlement size and location, trade and seafaring.

2.6 The Mediterranean Neolithic

The Mediterranean is characterised by an intricate combination of very diverse topographies (Allen, 2001), yet the Mediterranean sea links these different landscape communities, creating an area of high connectivity (Horden and Purcell, 2000). It is also the sea which defines this region as a coherent spatial, cultural and archaeological entity, unifying the Mediterranean conceptually as well as topographically, hence avoiding a simple geographic identification as the southern part of Europe, or the northern tip of Africa (Horden and Purcell, 2000; Knapp and Blake, 2005). The Mediterranean ecosystem is very vulnerable to human impact due to its characteristic topography, climate and vegetation (Knapp and Blake, 2005). Hence, the historical unity and the character of the Mediterranean region arises from the perception of constraints and opportunities offered by the landscape, and people's reaction to them (Horden and Purcell, 2000). The ancient landscapes modified since prehistoric times, are therefore the result of this complex and dynamic interaction between environmental and cultural factors (Knapp and Blake, 2005). Although not consistently accessible due to changing seasonal currents and conditions, and intermittently treacherous, the Mediterranean sea, which is however virtually tide-less and relatively calm, did not act as a barrier to communication, but encouraged maritime trade and migration, which compared to travel over land, is relatively quick and unobstructed (Horden and Purcell, 2000; Knapp and Blake, 2005; Robb and Farr, 2005). Contacts could also be extended from the sea into the land through numerous navigable rivers and streams, and through coastal wetlands (Horden and Purcell, 2000). The Mediterranean sea has had unpredictable

consequences on human societies, facilitating the movement of people, ideas, ideologies, technologies and objects, and generally rewarding settlers with the benefits of the region's rich and varied natural resources which permitted economic development, complex social systems, artistic and cultural achievements (Knapp and Blake, 2005).

The spread of agriculture in the Mediterranean has been widely disputed. Several authors, such as Higgs and Jarman (1969), and Barker (1985) rejected Ammerman and Cavalli-Sforza's (1984) 'wave of advance model' (described in Section 2.3), since it portrayed indigenous Mesolithic people as passive; hence an alternative theory was put forward, suggesting that domestication of crops and livestock in the Mediterranean was indigenous in origin. This theory was later over-ruled by genetics, which showed that domestic wheat, barley and pulses did originate in the Near East (Zohary, 1996; Zohary and Hopf, 2000). Furthermore, genetic studies have shown that the progenitors of the 'wild' sheep and goat found in the Mediterranean were not the direct ancestors of local domestic caprids, but were in fact, their feral Near Eastern descendants (Bruford and Townsend, 2006; Luikart *et al.*, 2006). Despite this, researchers did not accept the idea of a diffused colonist expansion model which was then proposed, and it was argued that cultural, not demic diffusion was the primary drive behind the transition to agriculture; hence the Neolithic was introduced through trade and technology transfer alone (Lewthwaite, 1986; Zvelebil, 1989).

More recent publications based on archaeological evidence, attribute the transition to agriculture in the Mediterranean to several waves of maritime pioneer colonisation, and the subsequent adoption of these new techniques by indigenous populations (Zilhão, 2001; Zeder, 2008; Rowley-Conwy, 2011). Given the growing accumulation of evidence for sea travel associated with forager societies in the early Holocene prior to the advent of farming (Fugazzola Delpino, 2002a; Barker, 2005), it is not unexpected that the sea would feature prominently in the transition to agriculture in this region. New data show that the first maritime pioneers arrived on Cyprus from the Levant around 10,500 BP (Peltenburg, 2004; Steel, 2004:16), and two thousand years later a leap-frog colonisation of the Aegean took place (Perlès, 2001; Gkiasta *et al.*, 2003). The Apulian region was colonised some four hundred years later, spreading to Sicily by 7600 BP and northern Italy by 7800 BP (Biagi, 2003; Skeates, 2003). In the next five hundred years, the Neolithic spread through southern France and the Mediterranean coast of Spain, to the Atlantic coast of Portugal (Binder, 2000; Zilhão, 2001; Guilaine, 2003; Tressert and Vigne, 2007) (Figure 2.2). The late Mesolithic in the Mediterranean seems to have been a period of decline and relocation, with the sudden appearance of fully agro-pastoral Neolithic settlements on coastal areas (Zeder, 2008). The spread

to the interior is thought to have proceeded through a combination of colonist expansion, selective local adoption of Neolithic technologies and the integration of colonist and indigenous populations (Zeder, 2008).

The idea of a punctuated transition from east to west has however been challenged, and it is thought that this model might not hold true for the entire Mediterranean region. In discussing botanical evidence retrieved from Franchthi Cave (Greece) and Grotta dell'Uzzo (Sicily), Barker (2005) states that several cultivars, which had previously been thought endemic to the Near East, were actually native to the Mediterranean, and were recognised and used by indigenous foragers from the beginning of the Holocene. Barker (2005) also points out that, although several prior identifications of domestic sheep in pre-agricultural layers have been dismissed, there is indisputable evidence of Barbary sheep being corralled and fed by Mesolithic foragers in the Libyan Sahara one thousand years before the introduction of domestic sheep in this area. This study was published by Cremaschi and di Lernia (1998), and later by di Lernia (2001). Hence, although several publications firmly attribute Mediterranean cultigens and domesticates to the Near East, it is possible that complex sets of 'microecological' histories characterised the Mediterranean at the transition, rather than an east to west colonization (Barker, 2005). Another important consideration is the cautionary note published by Zilhão (2001) on erroneous radiocarbon dates obtained by selecting inappropriate material for dating, most notoriously charcoal. This calls for a revision of published dates, to establish more confidently the sequence of events occurring at the transition to agriculture in the Mediterranean.

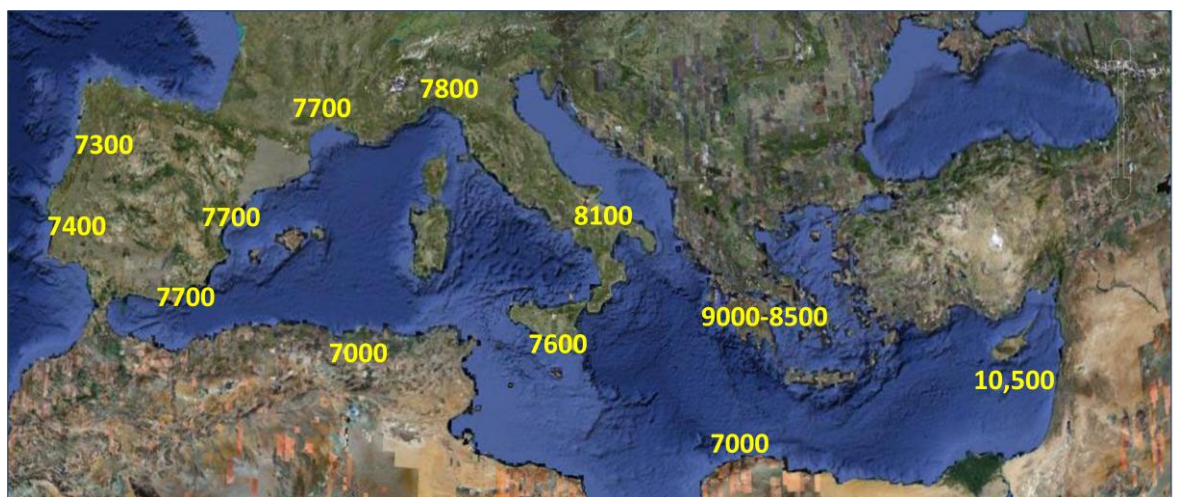


Figure 2.2: Map showing the earliest attestations of domestication in the Mediterranean [Dates reported in cal. BP] (After Zeder, 2008 and references within).

The following sub-sections will take a closer look at the earliest chronological attestation of Impressed/Cardial Wares and domesticates in Early Neolithic settlements across the Mediterranean, and explore in more depth the technology, form and decoration of the pottery produced. Particular attention will be given to the location of the settlements across the Mediterranean Neolithic, and similarities observed between the pottery assemblages excavated across this wide geographical location, which, it will be argued, provide a good platform for the 'punctuated maritime pioneer farming' model to explain the spread of farming in this region. The location of the main sites mentioned in the text is shown in Figure 2.3.

2.6.1 Cyprus and Greece

Until recently, Cyprus was thought to have been occupied by about 8500 BP by fully Neolithic mainland cultures; however more recent research at the rock shelter of Akrotiri-*Aetokremnos*, has shown that the first signs of human activities (in the form of stone tools and hearths) date to 10,665±25 BP (Steel, 2004:16). By the late 10th millennium BP, the earliest farming communities appeared at the sites of Parekklisha-*Shillourokambos* and Kissonerga-*Mylothkia* (Steel, 2004:45). Evidence for this comes from the retrieval of domesticated cultigens including einkorn and emmer wheat from deep wells constructed on early sites, neither of which was native to Cyprus (Peltenburg, 2004). The faunal record also shows the presence of the major livestock species, including sheep, goat, cattle and pig, which were introduced to the island, while hunting and fishing were still practised (Steel, 2004:59-62). These early seafaring colonists are thought to have originated from the northern Levant, and to have established the first aceramic Neolithic settlements (Peltenburg, 2004).

Ceramics are thought to have been introduced in Cyprus about 500-1000 years after the introduction of domesticates (Steel, 2004:63); however, impressed decorations appear to be missing from the decorative repertoire (Steel, 2004:65-66). This is curious given the close proximity of the Levantine sites, which featured pottery with impressed decorations from the beginning of the 7th millennium BC (Balossi and Frangipane, 2002:12). Impressed and incised decorations also appear in Anatolia (e.g. at Yarimburgaz and Ilipinar) towards the end of the 7th and beginning of the 6th millennium BC, and similarities between the Levantine and Anatolian decorative sequences prompted Balossi and Frangipane (2002:11) to suggest a possible westwards spread of impressed motifs. However, although evidence points towards the introduction of a fully formed ceramic technology in Cyprus, and a Levantine origin, impressed motifs do not feature on the pottery they produced (Steel, 2004:63-66). This absence is

particularly interesting since it appears to mirror the gap in impressed decorations between the Anatolian and Levantine areas (see Figure 2.3); in fact, no sites bearing impressed decorations have been located in the area separating the two regions (Balossi and Frangipane, 2002:12). Balossi and Frangipane (2002:7) proposed that these decorated vessels may have served as a way of expressing and representing an affiliation to a particular cultural group during episodes of demic movement and the exchange of items, even over long distances, between the first sedentary agricultural communities of the Near East. They further suggested that the regions to the north and west of the Sea of Marmara, namely Thrace and the Balkans, could have played an important role in the diffusion and circulation of socio-economic and cultural models, thus creating a circular route for the transmission of these models, rather than a simple east-west transmission (Balossi and Frangipane, 2002:12).

The arrival of domesticated plants and animals in Greece was, at one point, strongly argued to have occurred as a result of exchange and natural spread. This hypothesis rejected theories on demic diffusion, in particular since botanical evidence from Franchthi appeared to suggest a local domestication (Perlès, 2001:38-40). This theory was eventually rejected by Hansen (1991:163, 1992:235-238, 241), who observed that the last occurrence of wild barley, and the first remains of domestic barley at Franchthi were actually separated by one metre of deposit. A Near Eastern origin for the introduction of domestic plants (Zohary and Hopf, 2000) and animals (Hemler, 1992) was eventually conceded, as well as the immigration of farming communities into Greece (Hansen, 1991:163, 1992:235-238, 241). Similarities in the cultural systems between these early prospectors in Greece during the Initial Neolithic phase (c.7000-6500 BC; characterised by the full 'Neolithic package' though still aceramic), and the late Pre-ceramic Neolithic B (PPNB) communities in the Levant, also suggest a Levantine origin, perhaps connected to the 'great exodus' that signalled the end of the PPNB and the transition to the Pottery Neolithic A (PNA) in the Levant (Pessina and Tiné, 2008:26-27). Furthermore, Perlès (2001:43-44) noted that approximately 15 new domesticated species would have been introduced simultaneously to increase the chances of survival and development, and the lack of a 'pre-adaptive' phase strongly suggests an 'active participation of the original farmers', because of the practical and technological skills and concepts needed to tend to all these different species.

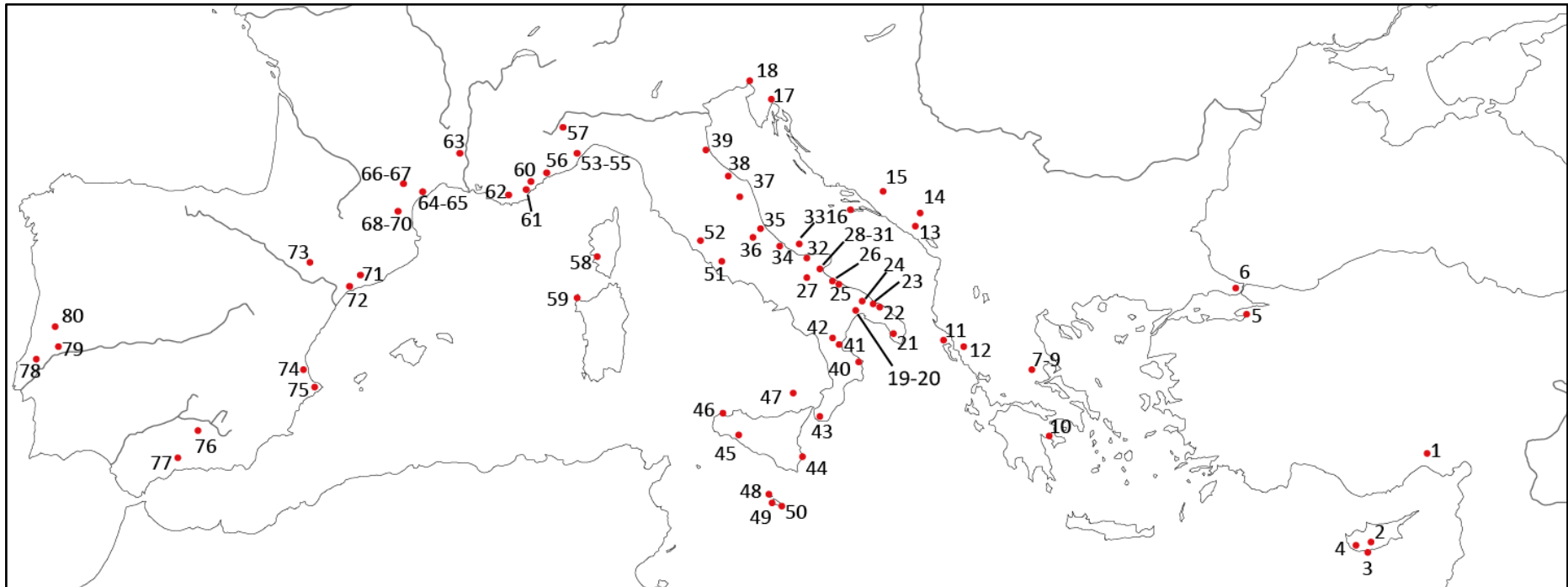


Figure 2.3: Map showing the location of the main sites mentioned in the text. 1: Mersin; 2: Parekklisha-*Shillourokambos*; 3: Akrotiri-*Aetokremnos*; 4: Kissonerga-*Mylouthkia*; 5: Ilipinar; 6: Yarımburgaz; 7: Nessonis; 8: Achilleion; 9: Theopetra; 10: Franchthi; 11: Sidari; 12: Konispol Cave; 13: Crvena Stijena; 14: Odmut; 15: Obre; 16: Vela Špila; 17: Pupičina; 18: Edera; 19-20: Neolithic villages at Matera including Trasano; 21: Torre Sabea; 22: Torre Canne; 23: Ostuni; 24: Grotta del Guardiano; 25: Balsignano; 26: Pulo di Molfetta; 27: Rendina; 28: Lagnano da Piede; 29: Masseria La Quercia; 30: Coppa Navigata; 31: Masseria Candelaro; 32: Guadone; 33: Prato Don Michele; 34: Torre Sinello; 35: Fontanelle; 36: Tricalle; 37: Maddalena di Muccia; 38: Ripabianca di Monterado; 39: Fornace Cappuccini; 40: Capo Alfiere; 41: Favella; 42: Grotta San Michele di Saracena; 43: Umbro; 44: Stentinello; 45: Grotta del Kronio; 46: Grotta dell’Uzzo; 47: Castellaro Vecchio; 48: Ghajn Abdul; 49: Skorba; 50: Ghar Dalam; 51: Le Caprine di Montecelio; 52: La Marmotta; 53: Arma dello Stefanin; 54: Grotta Pollera; 55: Arene Candide; 56: Riparo Mochi; 57: Alba; 58: Basi; 59: Grotta Verde; 60: Pendimoun; 61: Caucade; 62: Chateaneuf; 63: Grotta l’Aigle; 64: Peiro Signado; 65: Pont-de-Roque-Haute; 66: Camprafaud; 67: Grotte Gazel; 68: Jean Cros; 69: Dourgne; 70: Leucate; 71: Les Guixeres de Vilobi; 72: L’Esquerda Roques del Pany ; 73: Chaves; 74: Cova de l’Or ; 75: Cova de les Cendres ; 76: Cariguela; 77: Los Castillejos de Montefrió; 78: Almonda; 79: Calderiã; 80: Eira Pedrinha.

In the next phase, the Early Neolithic, a new wave of Neolithic colonies is thought to have arrived via terrestrial routes from Anatolia, who proceeded to Neolithise all of northern Greece during the second part of the 7th millennium BC (Pessina and Tiné, 2008:26-27). All fundamental aspects of the Neolithic in Greece appear simultaneously, without direct precursors, and they diffuse very rapidly, as evidenced by the densely occupied settlements, particularly on the plain of Thessaly (Perlès, 2001:40, 43-44; Pessina and Tiné, 2008:26). With the exception of Franchthi, Sidari and Theopetra, all Neolithic sites appear to have been located in areas devoid of Mesolithic settlements (Perlès, 2001:38-40). It has been suggested that these maritime pioneers followed a leapfrog pattern to establish farming communities in favourable environments in coastal Greece and other Aegean Islands (Zeder, 2008).

Around the same time (end of the 7th, beginning of the 6th millennium BC), impressed-incised decorations appear in Thessaly, and are to be found mainly in the northern and western parts of Greece (Benvenuti and Metallinou, 2002:17). This pottery typology was preceded by burnished, monochrome pottery (Perlès, 2001:210-213), and it is thought to have originated in the north-western areas of the Balkan Peninsula (Macedonia and Adriatic), since it has never been found in southern and eastern Greece, and in terms of technology, it lacks any of the features attributed to Anatolian pottery, but may have had parallels with the Starčevo-Criş-Körös culture (Benvenuti and Metallinou, 2002:17). Impressed and incised decorations are thought to have been present in Thessaly (and perhaps other parts of Greece), even before the appearance of the *Barbotin-Cardium* Ware (term coined for impressed pottery) (Benvenuti and Metallinou, 2002:17). Decorations on *Barbotin-Cardium* Wares comprised deep, impressed decorations made using fingernails and utensils; the characteristic of this decoration being the absence of any syntax to the decoration, thus contrasting with the later Cardial phase (Benvenuti and Metallinou, 2002:20-21). The simultaneous appearance of both impressed and incised decorative motifs in Greece, as recorded at Yarimburgaz and Ilipinar also raised the question as to whether or not impressed decoration had actually spread from the Levant, more so since ceramic fragments dating to the final stages of the Protosesklo in Nessonis I (Greece), were found to be very similar to Hassuna Ib-c and Achilleion, and similarities were also observed with ceramics from Mersin XXVIII-XXVII (Benvenuti and Metallinou, 2002:19). Compared to the 'simple' Early Neolithic pottery (Perlès, 2001:216-217), which Vitelli (1995) described as coarse, but nonetheless fine products considering the inexperienced potters producing them, the later impressed pottery in Thessaly were deemed to be technologically inferior, although aesthetically-speaking, it is still in-keeping with the spirit of the earliest ceramics produced in Thessaly (Benvenuti and Metallinou, 2002:20).

Possible contacts between Mesolithic groups and early farmers may have occurred at Sidari and Franchthi. At Franchthi, very few pottery sherds have been retrieved from the Initial Pottery Phase (which may be intrusive), but there is an abrupt increase in the quantity and variety of pottery in the Franchthi Ceramic Phase 1, which corresponds to the Early Neolithic (Vitelli, 1993). At Sidari, a Mesolithic stratum (Level D) dating to 6700 BC, containing monochrome ceramics following the Anatolian-Balkan pottery traditions and evidence for the exploitation of sea resources, was uncovered beneath a first Neolithic phase (Level C Base) containing incised pottery and caprid remains, and an Early Neolithic layer (Level C Top) containing Impressed Ware pottery dating to 6300 BC (Perlès, 2001:49-50; Pessina and Tiné, 2008:27-28). The two pottery phases were separated by a thick sterile layer, but there appears to be a sedimentological and stratigraphic continuity between the Mesolithic and the Neolithic (Perlès, 2001:49-50). Furthermore, the Mesolithic and Early Neolithic layers at Sidari are dated to the mid-7th millennium BC, and appear to be contemporaneous with the Early Neolithic of Thessaly, Macedonia and the Argolid; while the Initial Neolithic at Franchthi dates to the early 7th millennium BC and is thought to correspond to the aceramic Neolithic Phase (Perlès, 2001:49-50). Perlès (2001:49-50) therefore suggested that because the Pindus Range cut off the inhabitants of Sidari from the main Early Neolithic settlements of eastern Greece, they retained their foraging tradition for longer. It is at Sidari that pottery bearing impressed decorations comes to define the Impressed/Cardial Ware culture.

2.6.2 The Adriatic region

The Impressed Ware culture is attested in around 42 localities down the length of the Balkan coast (from Istria, the Dalmatian coast, Herzegovina, Montenegro and Albania, to the island of Corfu), and similarly, along the east coast of Italy (Starnini, 2002:31, 33). The stratigraphy at Sidari is crucial in interpreting the first appearance of farming and Impressed Wares. The incised pottery found in Level C Base appears to be unique to the region, and at this stage, evidence points towards parts of the 'Neolithic Package' being adopted within an existing Mesolithic site (Forenbaher and Miracle, 2005). Forenbaher and Miracle (2005) observed that at this stage, the new 'Neolithic' technology does not travel far, as they are not present in the Mesolithic Levels at Konispol Cave, dated to 6500-6200 BC, which is only 35km away. However, after the appearance of Impressed Wares, the pottery and domesticates spread rapidly up the Adriatic; the distribution of these Impressed Ware sites along the coast of the eastern Adriatic islands indicating that seafaring played an important role in the spread (Forenbaher and Miracle, 2005). The date obtained from Level C Top (the Impressed Ware Layer) at Sidari, was found to roughly coincide

with the earliest dates obtained for the Neolithic on both shores of the Adriatic (Pessina and Tiné, 2008:26-27). From Sidari, Impressed Wares spread rapidly into the Balkan hinterland (Albania), up towards the Dalmatian coast, reaching northern Dalmatia and southern Istria around 5900 BC and 5750 BC, respectively (Forenbaher and Miracle, 2005). By 6100 BC, Impressed Wares are present across the Otranto Straits, where the first settlements occur along the coastal planes and valleys of south-eastern Italy (Pessina and Tiné, 2008:28, 190-200).

The decoration and production of the Impressed pottery produced on both sides of the Adriatic is very similar, implying a close interaction between the two (Starnini, 2002:31). Evidence exists for the co-existence of the Impressed Ware Culture and the Starčevo-Criş-Körös in Albania, and in central Bosnia at Obre I, where Impressed Ware motifs were found together with elements of the Starčevo culture (Spataro, 2009a:73, 78). This evidence has led to the hypothetical theory that both cultures may have originated in this area, later developing their particular characteristic traits which spread at different speeds into the northern regions of the Balkan Peninsula (Biagi *et al.*, 2005; cited in Spataro, 2009a:78). Why this divergence, which saw the emergence of two different pottery techniques occurred is unknown, but it is thought to have taken place by the end of the 7th millennium BC (Spataro, 2009a:79). Spataro (2009a:79) further pointed out, that due to the evidence obtained, one might assume a certain level of interaction between both cultures; however with regard to pottery technology, archaeometric analysis showed no signs of exchange of ideas on pottery manufacture.

Two principal classes of ceramics have been identified: Coarse Wares (*Classe Grossolana*), whose fabric is characterised by a considerable amount of inclusions and smoothed surfaces (Figure 2.4); and Fine Wares (*Classe Fine*), which are generally better levigated, and comprised a homogeneous clay paste and smoothed surfaces that were at times burnished (Figure 2.5) (Pessina, 2002:120; Tiné, 2002:136-137; Spataro, 2009a:64). The shapes of the vessels produced depended on the function they were required to perform; hence the occurrence of open and closed forms, vessels with handles or no handles, with complex or more simple forms (Pessina and Tiné, 2008:66-68). Table 2.2 provides a brief description of the different decorative motifs applied to Impressed Wares.

The technique applied to pottery production in south-eastern Italy is remarkable, and it quickly diversified in terms of the wares, shapes and decorative styles produced, so much so as to seem exclusive to particular regions (Muntoni, 2009:89). For example, rocker decorations applied to Coarse Wares during the Archaic Phase were not present at all sites; they were absent at Favella,

Prato Don Michele, Fontanelle, and Rendina I, but present at Trasano, Coppa Nevigata, Torre Canne, Torre Sabea, Masseria Candelaro and Grotta del Guardiano (Tin , 2002:136-137). Finely impressed, scratched, red and brown painted, and stamped impressed fine wares, typified at the sites of Guadone, Matera/Ostuni, Masseria La Quercia/Lagnano da Piede and Stentinello respectively, were very quickly adopted and produced in the Early Neolithic settlements (Muntoni, 2009:89). However despite this spread, the distinct pot forms, surface treatment and decorative styles developed in the separate communities, which suggests that pottery assemblages in the south-east of Italy also varied considerably between the different regions, and may have been particular to the society that produced them (Muntoni, 2009:89-90).

<i>Decoration</i>	<i>Description</i>
<i>Impressed</i>	This is the oldest form of decorating pottery vessels, and is typical in the Early Neolithic of peninsular Italy and the Tyrrhenian islands. Impressions were created using fingers, shells, splinters of flint, bone or wood, and also using punches made from wood or ceramic.
<i>Incised</i>	Incisions were applied to the vessels at the leather hard stage with a pointed instrument. Incised decorations occur together with impressed motifs during the Early Neolithic, but incisions become the characteristic decorative motif during the advanced stages of the Early and Middle Neolithic in central and northern Italy.
<i>Excised</i>	Also applied at the leather hard stage. This type of decoration is particular and rare, but appears quite frequently in Stentinellian Wares, particularly in western Sicily and in the Vasi a Bocca Quadrata (VBQ) culture in the north of Italy.
<i>Graffito</i>	Differs from incisions because it is applied after drying, thus producing narrow irregular grooves with chipped edges. It is a particular technique, which characterises certain <i>facies</i> in southern Italy and Liguria at the transition between Early and Middle Neolithic, and reappears at the transition to the Chalcolithic.
<i>Painted</i>	Typical during the Middle Neolithic in southern Italy, and particularly well documented in a variety of styles in the Tavoliere region, but it is also present along the Adriatic coast of central Italy.
<i>Applied</i>	Application of ‘pastiglie’, ‘bugne’, ‘listelli’, ‘cordoni’ and other elements. This type of decoration was used during all the ceramic phases, but acquired a distinct relevance during the Late Neolithic, when the other decorative motifs diminished drastically.

Table 2.2: Description of the decorative motifs applied to Impressed Wares (From Pessina and Tin , 2008:64-65).

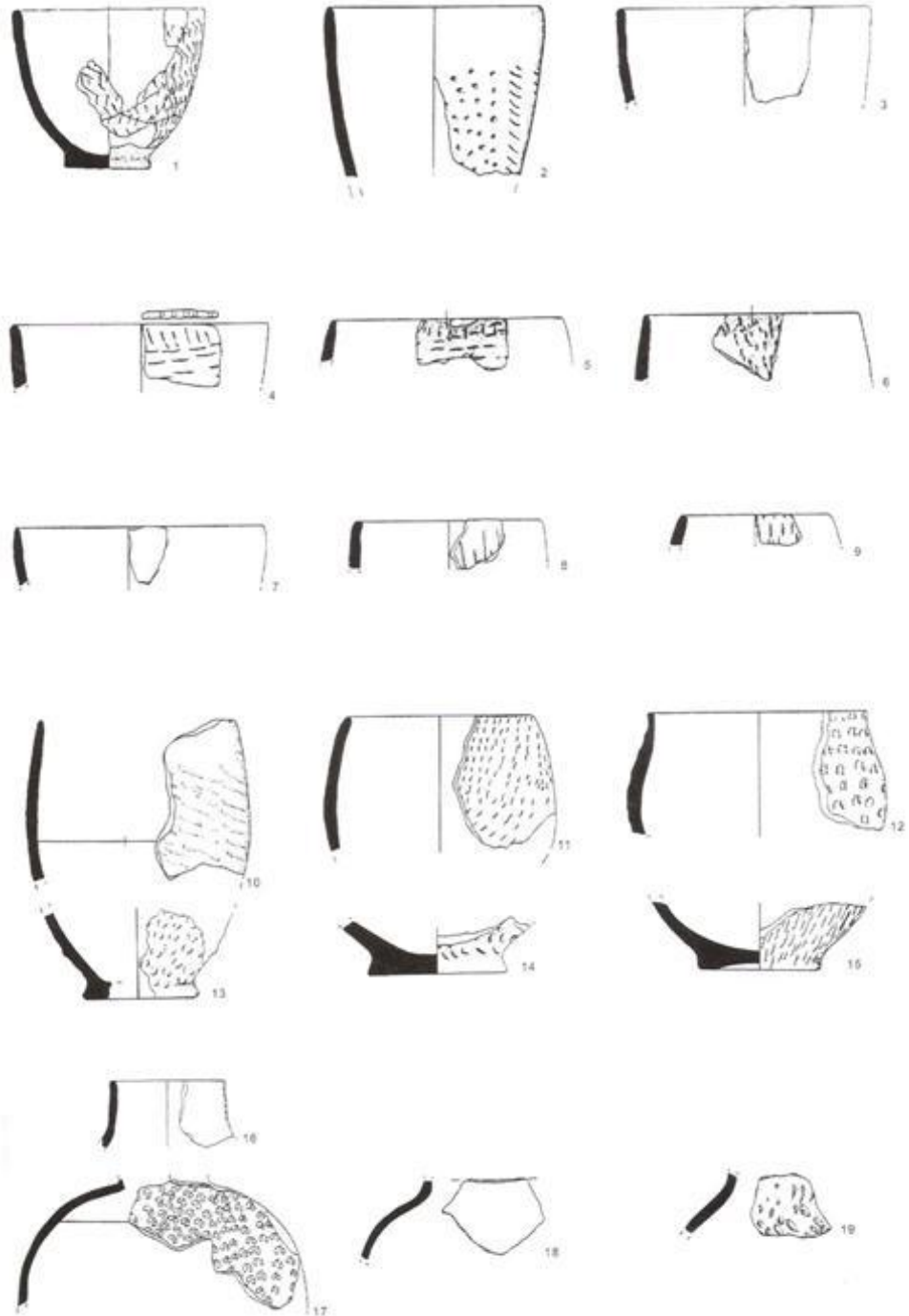


Figure 2.4: Early Impressed Wares: Coarse Wares (From Tiné, 2002:135). [1: Defensola; 2, 10-17: Favella; 3, 4, 7-9, 18, 19: Lago del Rendina; 5,6: Coppa Navigata]

Coarse Wares comprise vases with a pedestal base and an ovoid, cylindrical or tronco-conic body, at times with a restricted mouth or an inverted rim. Dimensions range from moderate to large; rarer forms include globular bodies and narrow mouths. They are mainly coil-made. Their surface is usually fully decorated with impressed and incised decorations in a regular or disorganised manner. The bands just below the rim and above the base are generally devoid of decorations, which comprise deep fingernail impressions as well as pinched and dragged motifs. Instrumental decorations include deep and simple punches, circular impressions carried out using a blade of straw, triangular motifs and small notches. Decorations are at times organised in triangular patterns or oblique irregular horizons (After Pessina, 2002:120; and Tiné, 2002:134-137).

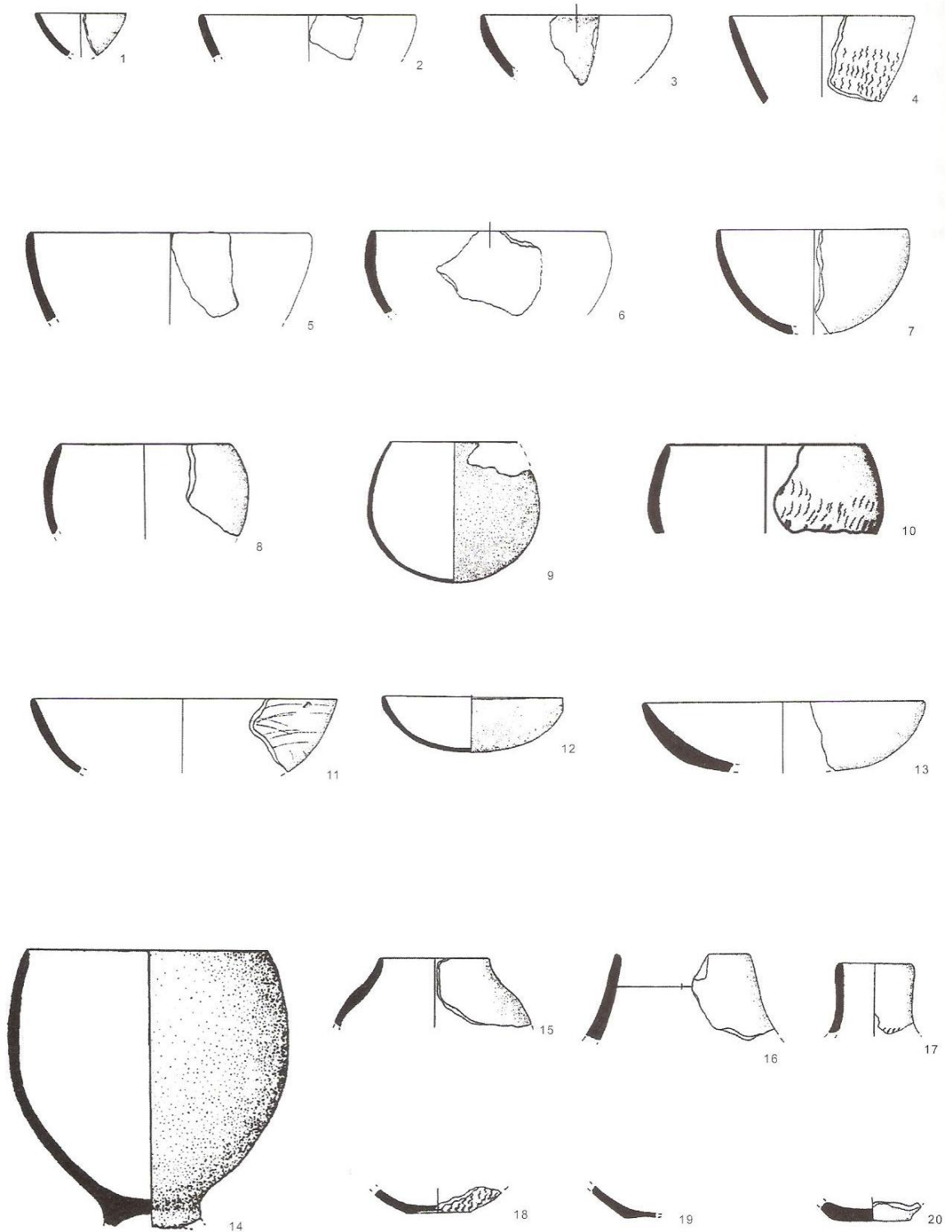


Figure 2.5: Early Impressed Wares: Fine Wares (From Tiné, 2002:134). [1,4,7-8, 10, 11, 13, 15-18: Favella; 2, 5, 19, 20: Lago del Rendina; 3, 6: Coppa Nevigata; 9, 12, 14: Defensola]

Fine Wares include flasks, deep and hemispherical bowls, and cups. They were mainly produced by pinching or moulding, and are usually moderate to small in size. Most are undecorated, however at Favella, decorations were applied using the serrated edges of the *Cardium* shell, while at Torre Canne and Torre Sebea, micro-rocker decorations were applied. Other minute impressions were applied at sites including Trasano I and Rendina I (After Pessina, 2002:120; and Tiné, 2002:134-137).

Evidence for the spread of Impressed Wares originating from the Apulia and Basilicata regions of southern Italy, up the Adriatic coast of Italy (towards Abruzzo and Marche), is based on the type of decoration found on the pottery at various sites (e.g. Tricalle, Fontanelle, and Torre Sinello), which are not typical of the region (Pessina, 2002:118-119). These include impressions made with instruments and fingers, incised tracts, rocker decoration and sequences, impressions made with shells and scratched motifs, as well as decorations organised in bands and geometric figures or in strips following a zigzag trajectory (Pessina, 2002:118-119). These decorations are particularly similar to those produced during the later Impressed Ware phases in south-eastern Italy (Evolved or Recent Phases), at the sites of Guadone, in Apulia and in Phases II and III at Rendina, in Basilicata (Pessina, 2002:118-119). Further north, in Romagna, decorations are also similar, but the Coarse Wares exhibit different combinations of impressed decorations, which are usually restricted to the upper parts of the vessels (Pessina, 2002:121). Radiocarbon dates show that Impressed Wares reached the regions of Molise, Abruzzo and the south of Marche around 5500 cal. BC, and by 5250 cal. BC had spread westwards across the Apennines towards Umbria and north west to the province of Ancona (Skeates, 2003). When considering the spread of the Impressed Ware culture in the north-eastern regions of Italy, such as Friuli, Pessina (2002:126-127) states that although it is possible that it diffused from the south along the Adriatic coast, it is also possible that the spread may have occurred due to the diffusion of Impressed Ceramic cultures in Istria. In the Padano-Alpini territory (situated north of Romagna), evidence for Impressed Ware cultures is very rare, and it is thought that this area was never colonised by this culture (Pessina, 2002:127). This lack of infiltration could be due to different ecological and environmental factors which differ from those on the Adriatic coast (Pessina, 2002:128).

The economy of Impressed Ware cultures along the Adriatic coast of the Balkans and Italy was mainly based on farming, with the cultivation of domestic species including cereals (e.g. *T. monococcum*, and *T. dicoccum*), barley (*H. vulgare*) and pulses (*Lens culinaris*, and *Pisum* sp.) (Pessina and Tiné, 2008:190-200; Spataro, 2009a:65). In the Adriatic, despite the use of floatation methods to recover plant material, these appear only sporadically (Forenbaher and Miracle, 2005), although their presence has been attested in organic temper included in the ceramic paste, as chaff, straw and grains (Starnini 2002: 33). In Italy, palaeobotanical evidence is also scarce, but it indicates that agriculture was introduced in open areas, characterised by the sparse forests of deciduous oak, with the progressive appearance of taxa resistant to the hot Mediterranean climate, which could be more readily cultivated (Pessina and Tiné, 2008:190-200). In central Italy, the agricultural repertoire appears to be more limited, with no evidence for the cultivation of legumes (Pessina and Tiné, 2008:190-200). On the other hand, in northern Italy, theories for a late

introduction of agriculture in the Padano-Alpina region are contradicted by various settlements showing a full agricultural economy. Cereals and numerous legumes were already present at the middle of the VI millennium BC (Pessina and Tiné, 2008:190-200). Faunal remains showed a dominance of domesticated species, mainly ovicaprids, on both the Balkan and Italian Adriatic coasts, with marginal evidence for hunting and fishing present (Wilkins, 2002; Spataro, 2009a:65), although evidence for the use of marine products have been identified at Favella (Natali and Tiné, 2002). Deer and wild boar remains have been found along the coast of Dalmatia, and at some sites, for example Crvena Stijena and Vela Špila, only wild fauna were collected (Starnini, 2002:33). In Italy, evidence for hunting was found in various sites, including Balsignano and Pulo di Molfetta in Apulia (Radina, 2002e, 2002a), and Ripabianca di Monterado (Pignocchi and Silvestrini, 2002:477) and Maddalena di Muccia (Silvestrini and Pignocchi, 2002:484) in the Marche region.

While in cave sites along the eastern Adriatic, pottery sherds have been found in association with wild taxa (e.g. at Odmuť), in layers with evenly represented wild and domestic taxa (e.g. Edera 3/3a) and in levels where domestic animals dominated the faunal assemblage (e.g. Pupićina) (Forenbaier and Miracle, 2005 and reference therein), in Italy, Impressed Wares appear to have been always associated with a predominantly domestic faunal assemblage. In fact, excavations in Italy have so far shown that when found, the Neolithic is always fully attested comprising Impressed pottery and domesticated botanical and faunal evidence, as well as other utensils associated with this change in economy (Muntoni, 2009:87); thus supporting the hypothesis for a diffusion of the Neolithic into the western Mediterranean as a process which is highly unlikely to have developed independently (Leighton, 1999:52).

2.6.3 Sicily, Calabria and Malta

Between 5700 and 5200 BC, the Impressed Ware culture moved rapidly southwards towards Calabria, Sicily, Malta and the small islands, where it is commonly known as Stentinello Ware, after the type-site of Stentinello (near Syracuse in Sicily), where this characteristic pottery was first found in large quantities (Leighton, 1999:61-62; Pessina and Tiné, 2008:46-47). By the second half of the 6th millennium BC, Impressed Ware villages were widespread in coastal regions of southern Italy and Sicily, and by around the same time, Malta and the surrounding islands had been settled (Leighton, 1999:54). The first use of clay was attested at Grotta dell'Uzzo (Sicily) during the Mesolithic period, where a fire-hardened clay platform (around 50cm long and 2-3cm thick), set into the floor was located. It is reminiscent of the clay cooking bases found in later

prehistoric contexts, and indicates that at this early stage, people were already familiar with making use of clay, although not for building pottery vessels (Leighton, 1999:32). A Pre-Stentinello pottery phase has been recorded in Sicily (at Grotta del Kronio and Grotta dell'Uzzo; dating to 6000-5750 BC) and Calabria (Grotta San Michele di Saracena in Calabria) (Pessina and Tiné, 2008:46). The pottery produced is very similar to the early Impressed Wares described above, except for one decorative motif executed using bands of cardial impressions reminiscent of Tyrrhenian cardial designs (Pessina and Tiné, 2008:79).

The Stentinello Phase eventually spread all over Sicily, down to Malta, and also up towards Calabria, where this culture has been attested in sites in the Vibo, Crotona, Catanzaro and Reggio Provinces (Tiné, 2002:160). Fine Wares dominate among the Stentinello Wares typified at Grotta dell'Uzzo and Kronio, on the island of Sicily (Tiné, 2002:156, 160) (Figure 2.6). Decorations are structured and complex and are executed using incisions and excisions, as well as stamped and impressed motifs, including cardial impressions (Tiné, 2002:156, 160; Tusa, 2002:757-759; Pessina and Tiné, 2008:80). This type of decoration is considered to be the most advanced in the Impressed Ware sequence (Tiné, 2002:156, 160). Vessel forms also become more elegant, and include carinated bowls, and jars with long, 'reversed' tronco-conic necks (Tusa, 2002:759). Different styles allow a regional identification of groups, for example the surrounding regions of Mount Etna in eastern Sicily are characterised by decorations comprising stylised eyes and lozenges, while rhomboids, diamonds and 'V' impressions characterise the Tyrrhenian side of Calabria (Tiné, 2002:156, 160; see also Pessina and Tiné, 2008:79-81). Even the organisation of the decorations follows particular characteristics. Along both the eastern and western parts of Calabria, vases tend to be decorated with only one or more parallel bands beneath the rim, which descend in vertical bands up until the widest part of the vessel is reached (Tiné, 2002:156, 161). White, red and yellow inlay was also used to emphasise the impressed decorations. White and red were usually used in the Tyrrhenian and Ionic parts of Calabria; however at Umbro and Capo Alfieri all colours are used, whereas at Castellaro Vecchio, there was a predilection for red (Tiné, 2002:156, 160).

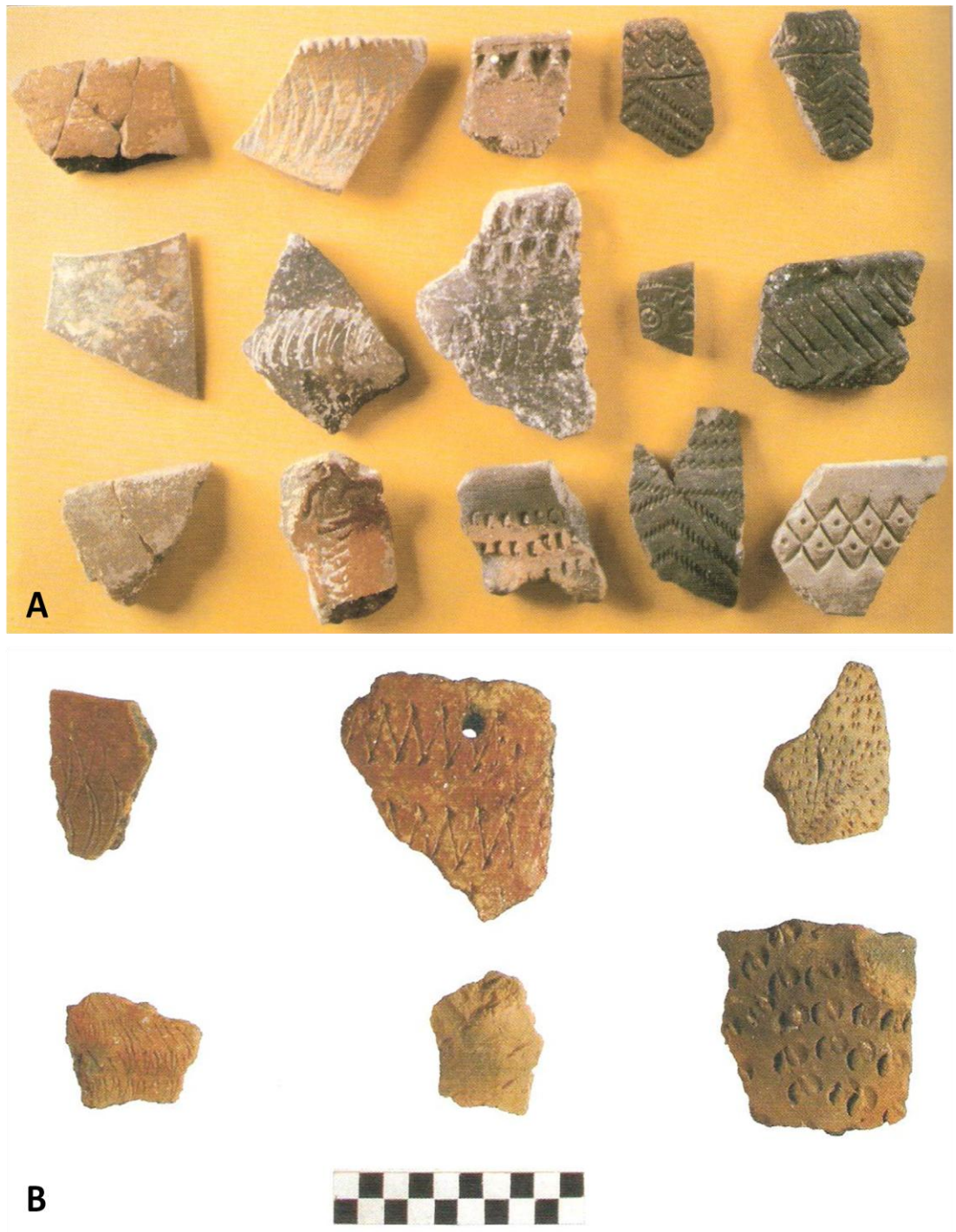


Figure 2.6: Examples of Stentinello Wares from **A:** Capo Alfiere (Calabria) (From Morter, 2002:732); **B:** Grotta dell’Uzzo (Sicily) (From Tusa, 2002:758).

In Malta, the first archaeological evidence for human settlement dates to around 5000 BC, when Sicilian farmers are thought to have crossed the channel bringing with them various aspects of the Neolithic, including pottery and domesticates (Trump, 2002:23-24). Good weather permitting, both islands can be easily sighted, with better visual contact being made from the island of Gozo, in the Maltese archipelago (Pace 2004:24). More recently, calibrated radiocarbon dating has

shown that the Neolithic took over a millennium to develop, and is subdivided into three phases: Għar Dalam and Grey Skorba which overlap (5500-4100 cal. BC), and Red Skorba (4350-3650 cal. BC) (Fenech, 2007:35). Archaeological remains dating to this period have been retrieved from the caves of Għar Dalam (Malta) and Għajn Abdul (Gozo), and the Neolithic village of Skorba (Mgarr, Malta), which is the only Neolithic village uncovered on the islands to date. The remains of a wall were found, indicating a ditched enclosure; however extensive investigations have been limited due to the subsequent building of a megalithic temple structure above the village. Pace (2004:22) suggested that perhaps other such scenarios might have existed in Malta, hence by the Għar Dalam phase, the islands may have been populated by other such settlements, which in later centuries may or may not have been developed into megalithic monuments.

The first pottery in Malta, attested at the cave of Għar Dalam and the village of Skorba, is clearly of Stentinellian origin (Malone, 2003). This pottery is of good quality and characterised by impressed decorations which are frequently present around the lip and base of the neck (Trump, 2002:46) (Figure 2.7). Multiple cut-out lines forming horizontal bands or chevrons were also commonly used, and were usually filled with a white paste that contrasted with the polished grey surfaces (Trump, 2004:251). Linear decorations on yellower coloured vessels were usually carried out using soft incision or finger-tip pinching (Trump, 2004:251). The shapes built were mostly simple, including small bowls and globular jars; round bases were more common, but a few vessels were set on foot-rings. Handles ranged from small pierced lugs to prominent strap handles (Trump, 2004:251). The later Grey Skorba Phase shows a decline in the use of decoration, which eventually vanishes altogether (Trump, 2004:251). Continuity from the Għar Dalam phase is shown in the retention of the open bowl forms and in two of the handle forms, although the horizontally pierced lugs now display splayed ends and the vertical strap handles were formed to rise much higher above the rim but reducing the dimensions of the loop (Trump, 2002:47). Vertical and recessed 'tunnel' handles also appear at this time (Trump, 2004:251). The Red Skorba Phase corresponds to the Late Neolithic on the islands, and is reminiscent of the Sicilian Diana Ware (Trump, 2004:251). The fabric is similar to the Grey Skorba phase, and although some changes occur to the form repertoire (e.g. larger and more sharply carinated bowls with flat or pedestal bases), the main change is the application of a bright red slip (Trump, 2002:48). Decorations include deeply scratched-'C's and 'S's, loops and occasionally spirals, which appear on or below the carination (Trump, 2002:48).

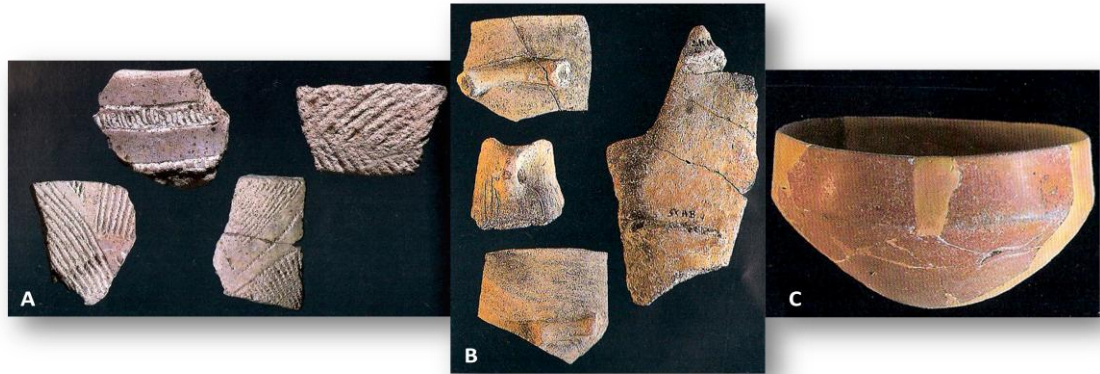


Figure 2.7: Examples of: A: Għar Dalam, B: Grey Skorba and C: Red Skorba wares excavated from Skorba, Malta (From Trump, 2002:46-48).

In Malta, faunal and botanical remains retrieved from Skorba are still in the process of being studied. However, faunal remains show evidence for domestic animals, primarily ovicaprids, but remains of pigs and cows were also retrieved (Trump, 1966, Appendix III; Borg, 2008). Of interest, Borg (2008) noted the absence of wild species (e.g. deer), despite their earlier attestations in the archaeological record. He suggests that the number of wild species present in Malta during the Neolithic could have seriously declined, or they could possibly have already been extinct. Scant botanical remains attest to the presence of barley, wheat and lentils (Trump, 1966, Appendix IV).

The long stratigraphic sequence present at Grotta dell'Uzzo, which comprises Mesolithic and Neolithic levels of occupation (Tusa, 2002:757), is ideal for understanding the change in economy, as evidenced by the archaeobotanical and faunal assemblages retrieved. During the Mesolithic, hunting, fishing and gathering of marine molluscs predominated, the main species targeted being deer, the grouper and *Monodonta*, respectively (Pessina and Tiné, 2008:213). At the transitory period, reliance on marine products increases and hunting becomes focused on male deer and wild boar. Eventually, during the Pre-Stentinello phase, domesticates are gradually introduced, including the ox, sheep and goat, which were not local in origin (Tusa, 2002:761-762; Pessina and Tiné, 2008:213). A meat-producing economy was probably in place at this stage, while hunting, fishing and shell fish gathering were still being carried out (Pessina and Tiné, 2008:213). Domesticated pig is also present, and is thought to have been introduced as a result of local domestication due to the loose management of wild boar during the previous transitory period (Tusa, 2002:761-762; Pessina and Tiné, 2008:213). During the Stentinello phase, hunting is abandoned, and the focus turns to animal husbandry (mainly of ovicaprids), probably together with the exploitation of secondary products (Pessina and Tiné, 2008:213). Fishing however, is still

an important activity, and during this phase, it appears that the community at Grotta dell'Uzzo developed an economy based on pastoralism and fishing (Pessina and Tiné, 2008:213). Plants appear to have played only a marginal role during both the Mesolithic and Neolithic periods at Grotta dell'Uzzo. They are not widely attested, however cereals (*T. monococcum*, *T. dicoccum* and *T. aestivum*), as well as barley (*Hordeum*) were found in Early Neolithic stratigraphic contexts (Tusa, 2002:761-762).

2.6.4 The Tyrrhenian coast of Italy

Impressed Ware communities dot the coastal strip between Liguria and Provence, and interestingly, they all occur in areas which do not show signs of previous Mesolithic habitation (Maggi, 2002:92); as similarly attested in Portugal (Zilhão, 2003) and Greece (van Andel and Runnels, 1995). The spread appears to have been carried out in a leap-frog pattern, and the Neolithic communities are thought to have travelled by sea (Maggi, 2002:92). Given the large distances which were at times covered, Maggi (2002:92) suggests that these communities might actually have been targeting areas not in use by Mesolithic communities. Seafaring knowledge at this time has been evidenced by the retrieval of two boats found at the submerged Neolithic village of La Marmotta (Fugazzola Delpino, 2002a), as well as the spread of obsidian collected from Mesolithic layers in Lazio (Circeo) and Liguria (Riparo Mochi, Arma dello Stefanin), which show a familiarity with seafaring routes between the Italian Tyrrhenian coast and the major islands in the Tyrrhenian Sea (Fugazzola Delpino, 2002b:97). Hence Fugazzola Delpino's (2002b:97-98) hypothesis, that the western coast of Sicily could have acted as a point of respite during long seafaring voyages undertaken by Neolithic seafarers is quite conceivable. She supports her claim using evidence retrieved from Grotta del Kronio and Uzzo, where ceramics with cardial impressions, very similar in style to pottery in the Tyrrhenian area, were found. Dates obtained for stratigraphic layers containing these Impressed Wares were contemporary to the dates obtained in Sardinia, Lazio and Spain, which appear to support her theory that Neolithic mariners were breaking their journeys in Sicily while travelling to Sardinia, the Tyrrhenian coast of Italy and the Iberian Peninsula.

Bernabò Brea (1946:259) stated that Impressed Wares characterise the first phase of the new (Neolithic) communities along the Tyrrhenian coast of Italy, and distinguishes them from subsequent cultures during which cardial, and subsequently incised decorations dominated. Excavations at Arene Candide showed that the Ligurian Impressed Wares were divided into two phases: a first phase dating to 5800-5400 BC, characterised by decorations carried out 'in

sequence' using instruments or pinched motifs, and a second phase characterised by Cardial decorations, which occurred between 5400-5100 BC (Maggi, 2002:94). Typical forms include cups with curved walls, cylindrical cups with one handle, hemispherical bowls, deep bowls, large plates, vases, jars and large jugs, which vary in sizes from quite small to very large (Fugazzola Delpino, 2002b:102) (Figure 2.8). The types of fabrics produced vary from coarse wares used for very large vessels and cooking wares which are usually not well fired, to carefully levigated clay pastes used to build medium-sized vessels; while fine wares become thinner and more refined (Fugazzola Delpino, 2002b:102). Surfaces are smooth, and sometimes burnished, and are usually dark grey or brown in colour (Fugazzola Delpino, 2002b:102). The majority of the decorations were executed using instruments or the *Cardium* shell. Applied decorations are rare, as are incisions and grooves. Impressed decorations are varied, ranging from small to large punches, circular motifs, commas and 'S' motifs, notches and finger work, while instruments sometimes have more than one point with which to decorate (Fugazzola Delpino, 2002b:103). Cardial decorations were carried out using the *Cardium*, *Pectunculus* or *Tellina* shells, and other molluscs, e.g. *Patella ferruginea* (Fugazzola Delpino, 2002b:103). Decorations were usually limited to the upper part of vessels; the handles were usually decorated as well as the rims, while decorations which covered the entire exterior surface were limited to some bowls and globular vases (Fugazzola Delpino, 2002b:103; Pessina and Tiné, 2008:81). Decorations followed simple and complex trajectories, including linear, horizontal and sometimes oblique or zigzag paths, and usually occurred in bands, alternating between spans of decorated and non-decorated areas (Fugazzola Delpino, 2002b:103; Pessina and Tiné, 2008:81). Triangular and rectangular motifs were at times present, as well as dotted linear horizons and zoomorphic motifs (Fugazzola Delpino, 2002b:103). Some decorative styles are reminiscent of those found in Emilia-Romagna, in particular at Fornace Cappuccini (Fugazzola Delpino, 2002b:103-104). Painted wares are rare, however instances of impressed and painted decoration have been found in Corsica in the settlement of Basi, in Sardinia at Grotta Verde, and in Lazio at Le Caprine di Montecelio (Fugazzola Delpino, 2002b:103-104). Impressed decorations in Italy generally precede painted wares, however this is reversed at the site of La Marmotta, where a previous horizon of painted wares was detected before the appearance of Impressed Wares (Fugazzola Delpino, 2002b:104). This is the only instance where it occurs in the central Tyrrhenian area, which Fugazzola Delpino (2002b:104) considers to be a centre of mixing with other western Mediterranean communities.



Figure 2.8: Tyrrhenian Cardial Wares: an example from the underwater site of La Marmotta showing a bowl with an impressed decoration reminiscent of a zoomorphic figure (From Fugazzola Delpino, 2002b:103).

The subsistence economy centred on domestic animals and cultigens; however, there is also evidence for the continued hunting and gathering of 'wild' species. At the cave of Arene Candide (Liguria), evidence for animal herding was collected in the form of teeth from domestic animals and coprolites, and this site showed how caves made excellent locations for controlling and sheltering animals, with people requiring only to bring in forage (Maggi, 2002:93). However, wild species still formed the predominant assemblage, with 27 out of the 48 species being wild (Traverso, 2002:299-300). Domesticated goats and sheep were the main species, followed by wild and domesticated pig, while botanical data showed evidence for a decrease of the forested area during the Early Neolithic period (Traverso, 2002:299-300). Human involvement was also attested at Grotta Pollera (Liguria), where botanical remains showed the presence of a canopy cover of oak, plums and maples, which are associated with human presence (Odetti, 2002:309-310). In Piemonte at the site of Alba, evidence has been collected for an economy based on agriculture and herding, but also for nut and berry picking, as well as the gathering of firewood from hedges which was used to fence off small enclosures to protect the herds (Venturino, 2002:327). In the central Tyrrhenian region, the site of La Marmotta, located underwater in Lago di Bracciano, has provided an exceptionally well preserved palaeobotanical record. Volcanic activity in the area produced very fertile soils, which are a characteristic feature of the area. Palaeobotanical evidence identified the use of cereals, barley, and legumes, as well as grapes, and evidence for the collection of fruit is also abundant (e.g. *Prunus domestica*, *Malus mas*, *Fragaria carica*, *Rubus fruticosus*, *Corylus avellana*, and *Quercus* sp.) (Pessina and Tiné, 2008:200-201).

Maggi (2002:94) commented on the rather long initial phase of Impressed Wares in the Early Neolithic in Liguria, during which no significant changes were observed over the course of eight or nine centuries. He suggested that these ceramics were being produced by small intrusive groups, who through seafaring managed to occupy several small territorial enclaves between Liguria and l'Herault, including Finalese, Pendimoun, Caucade, Peiro Signado, Pont-de-Roque-Haute, as well as other sites along the Tyrrhenian coast (Maggi, 2002:96). Maggi's theory appears to be supported by an archaeometric study on pottery sampled from several Ligurian sites, which allowed the reconstruction of a possible route taken by these pioneering farmers. The data obtained showed a possible departure point from Lazio or southern Tuscany, with the journey proceeding by coasting towards central Tuscany or Corsica, then on to eastern and central Liguria, to Albenga (Capelli *et al.*, 2011).

In Sardinia, the earliest evidence for the presence of Cardial Wares were retrieved from shelters, caves and inland sites on the north-western part of the island, and date to between 6710±75 BP/6690±80 to 6400±40 BP (Fugazzola Delpino, 2002b:113). The onset of the Neolithic in Corsica has been heavily debated due to variations in the radiocarbon dates obtained; hence it can only be hypothesised that the majority of the Neolithic sites date to around the second part of the 6th millennium BC (6430±140-6320±140 BP), and that these might have become inhabited after a previous period of scouting during the Mesolithic, with the first landings occurring around middle of the 6th millennium BC (6670±130-6650±150 BP) (Fugazzola Delpino, 2002b:110-111).

2.6.5 The Mediterranean coast of France

The first Neolithic settlements occur in southern France during the first half of the 6th millennium BC, and appear to be directly connected to the Italian Impressed Ware culture; while it is only later, towards the end of the 6th millennium, that Franco-Iberian Cardial societies develop (Guilaine and Manen, 2002:37). Although little is known about previous Mesolithic settlements in this area, the current chronology does not indicate an overlap between the Late Mesolithic (6600-6000 BC) and subsequent Neolithic communities (5800-5000 BC) (Guilaine and Manen, 2007). Hence the earliest presence of the Neolithic in southern France is attributed to small groups of 'settlers' of Italic origin, distinguished by their characteristic small settlements of limited duration, and other elements of the 'Neolithic Package', including agrarian practise, pottery, and polished axes (Guilaine and Manen, 2007).

Three sites indicate the presence of Italian Impressed Ware cultures prior to the Franco-Iberian Cardial settlements in southern France; the settlement of Peiro Signado (Portiragnes, Hérault), where ceramics recalling those excavated at Arene Candide were termed 'Ligurien', in Provence where several sites showed a similar connection with Italy, and at Pendimoun (Guilaine and Manen, 2002:39). Pottery at Pendimoun I was observed to show similarities with ceramics found in Apulia, Marche and Abruzzo, thus corresponding to the later phases of Impressed Wares which originated in the south-eastern Italy (Guilaine and Manen, 2002:42). Forms included bottles with narrow necks, small pots shaped like flattened domes, and spheroid or truncated conical open pots, while decorations comprised, pinched patterns, nail impressions and discontinuous impressions made with shells (e.g. cardium and patella) (Guilaine and Manen, 2007). Similarly, the pottery assemblage at Peiro Signado is comparable to ceramics found in the Gulf of Genoa, whereas at Pont de Roque-Haute, similarities have been observed with the central-southern regions of Italy, in particular, on l'Isola del Giglio (Guilaine and Manen, 2002:42). Most of the decorations at the site of Peiro Signado are made 'à sillon d'impressions', and organised in linear formations, or geometric schemes, including vertical or horizontal chevrons or zigzags aligned in bands (van Willigen, 2006), and are very similar to the decorations identified at Arene Candide as 'pseudo-cordicella' and 'pseudo-rotella' (Guilaine and Manen, 2002:40; Guilaine, 2003). The quality of the pottery is generally good, made with a homogeneous clay paste and a smooth surface finish (van Willigen, 2006). At this early phase, less than 10% of the other forms of decorations were made using cardial impressions, short, vertical or arched incisions, impressions executed using digits and pinched motifs, and more rarely, the application of grooves (Guilaine and Manen, 2002:40). Decorations are similar at Pont de Roque-Haute, where these tend to cover the whole vessel, albeit occasionally, decorations were organised into geometrical motifs (triangles or other angled motifs) and applied to just the upper part of a vessel (Guilaine and Manen, 2002:40). The forms at Pont de Roque-Haute and Peiro Signado are also similar, and include bowls, bottles, cooking pots, and flat-based basin type vessels, with very little use of handles (Guilaine and Manen, 2007) (Figure 2.9).

These settlements suggest either an early habitation of the area, or occasional settlements by mariners departing from Italy. Guilaine and Manen (2002:43) support the latter view, citing the occupation levels at both sites in Portiragnes which date to 5750-5500 BC. Furthermore, the structures found at Portiragnes, and their dimensions do not favour a long term occupation, which is mirrored at the site of Peiro Signado, whose one habitation unit suggests a small number of people inhabiting the community, while the lowest layer at Pendimoun (5800-5600 BC) shows isolated episodes of occupation (Guilaine and Manen, 2002:43). Even in the later Cardial Phase,

sedentism appears to have been relative, and a lasting territorial attachment does not appear to have been an important driving force (Guilaine and Manen, 2007).

The subsequent Cardial Phase in France has recently been identified as a secondary process, and its origin is still widely debated. It is thought that the earlier Italic communities, the Tyrrhenian Cardial group and perhaps a native influence, could have contributed towards its occurrence (Guilaine and Manen, 2007). The Cardial Ware phase along the southern coast of France was subdivided into two stages according to the excavations carried out at Chateauneuf; the earliest phase dating to 5600-5400/5300 BC, and a later second Cardial Phase dating to 5400/5300-5000 BC (Guilaine and Manen, 2002:44, 46). Cardial pottery is well attested here, and evidence exists for their presence further inland, in particular near river terraces; however, little is as yet known about such settlements, which makes it increasingly difficult to attempt to identify locations that might have been inhabited by Cardial Ware communities (Guilaine and Manen, 2002:43). The pottery during these two phases is morphologically identical, the main difference being that in the second phase, the geometric decorative motifs organised in bands are filled in with simple lines of impressions, while the typical geometric motifs used in the earlier phase are rarely represented (Guilaine and Manen, 2002:46; van Willigen, 2006). The pottery was built using local clay, and, especially in the earlier phase, chamotte particles were often added (Guilaine and Manen, 2007). The vessels ranged from small to medium sized, and comprised cooking pots, bowls, basins and globular pots (Guilaine and Manen, 2007). There is a distinct similarity to the decorative motifs produced on the Tyrrhenian Cardial pottery, in particular the banded decorations, but cultural distinctions between the two are evident (Guilaine and Manen, 2002:43, 2007). At the submerged site of Leucate the decorations bear more resemblance to the Catalan Wares, such as those produced at Les Guixeres de Vilobi or L'Esquerda Roques del Pany (Guilaine and Manen, 2002:46). Cultural frontiers cannot however be distinguished, despite the evident similarities between the Provence-Languedoc sites on the one hand, and western Languedoc-Catalonia on the other, since Cardial Wares found in the caves around Montserrat were found to bear a striking resemblance in decorations with the pottery found in the Provence area (Guilaine and Manen, 2002:46).

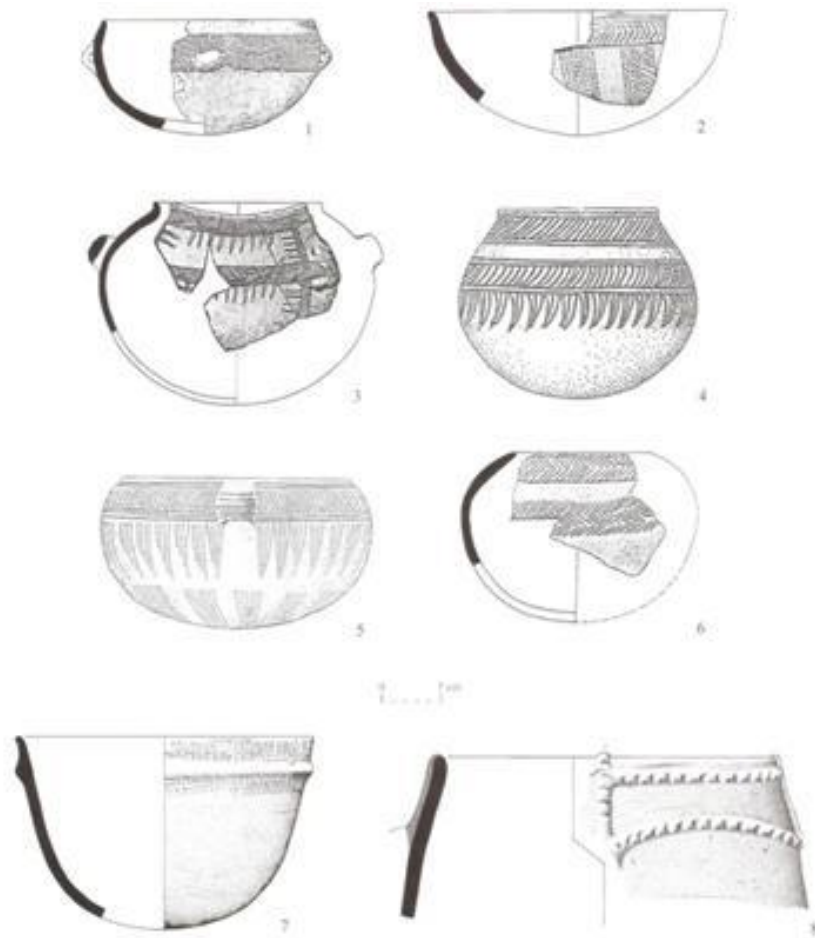


Figure 2.9: Characteristic early Cardial Wares from several sites in southern France (From Guilaine and Manen, 2002:45). [1,3 & 6: Fontrégoua; 2: La Corrèze; 4: Baume d'Oullins; 5: Grotte de l'Aigle; 7 & 8: Grotte Gazel]

Faunal assemblages from early Neolithic Impressed site of Pont de Roque-Haute showed that the first Italian pioneers brought with them a rather specialised subsistence pattern, dominated by sheep, but without hunting and poor in fishing and shellfish collection (Tressert and Vigne, 2007). Hunting appears to have increased in importance through time, as attested by the faunal record present at Grotte Gazel, Dougné and Camprafaud (Tressert and Vigne, 2007). The faunal assemblage at Pont de Roque-Haute suggests a skilled animal husbandry practice, which points towards the hypothesis that the occupants had already acquired a long experience in animal herding elsewhere (Guilaine and Manen, 2002:41). Among the cultivated plants were compact wheat, naked barley and legumes, and numerous millstones were also found (Guilaine and Manen, 2002:47). During the Cardial phase, the economy shows a broad diversification, with different communities attesting to a particular specialisation with regard to their subsistence, some focusing on land cultivation and/or herding (e.g. at Baratin and Petites Baites), while others

hunted (e.g. at Jean Cros, Grotte Lombard, and Grotte l'Aigle) (Guilaine and Manen, 2002:48, 2007).

2.6.6 The Iberian Peninsula

The earliest Neolithic in the Iberian Peninsula is also identified by the presence of Cardial Wares, which appear around 5600 BC in deep stratigraphies within cave sequences (e.g. at Chaves and Cendres), together with the remains of domestic plants (e.g. wheat and barley) and animals (mainly ovicaprids) (Zilhão, 2000:148, 2001, 2003:217). In Iberia, pioneering Italic culture establishments have not yet been identified (Guilaine and Manen, 2007). An interesting observation was made by Fugazzola Delpino (2002b:98-99), who noted that despite the material evidence for seafaring crossings that exist on Corsica and Sardinia (e.g. obsidian from Sardinia has been found in Early Neolithic deposits at Pont de Roque-Haute in southern France; Guilaine and Manen, 2007), they are noticeably absent on the Balearic Islands, which would have presumably played an important stepping stone in westward voyages towards the Iberian Peninsula. It has been suggested, that the Cardial Ware culture spread from Provence and Languedoc, and may have introduced agriculture and other elements of the 'Neolithic Package' in Iberia, with the first settlements appearing in Catalonia and Valencia (Guilaine and Manen, 2007). Also, in the Iberian Peninsula, Cardial pottery is well developed, as in the rest of the Mediterranean, which prompts Martí-Oliver (2002:57) to support a foreign origin. However, this topic is still open to debate (see Zilhão, 2000:166-180), and more so in view of the more recent identification of Boquique pottery, which appears to be technologically and decoratively distinct from the Cardial Wares, but occurs contemporaneously, although radiocarbon dating tentatively suggests earlier dates for the first appearance of Boquique pottery (Alday Ruiz and Moral del Hoyo, 2011).

The earliest pottery dates to the Cardial Early Neolithic which is dominated by ceramics decorated with cardial impressions covering the whole external surface of the vessel, while a decrease in the use of cardial decoration signifies the next phase, termed the Early Neolithic Epicardial (Martí-Oliver, 2002:52). In central Catalonia, several cave sites have been found containing Cardial Ware pottery, which show that during the Early Neolithic, the pre-littoral and adjacent mountains of Catalonia, an area which was previously unoccupied by hunter-gatherers, were indeed colonised (Martí-Oliver, 2002:53). In Portugal, the first Neolithic assemblages appear in the Estremadura and Algarve around 5400 BC (Zilhão, 2001, 2003:217). Cardial settlements along the Atlantic coast of Portugal appear to be contemporaneous to Neolithic settlements along the Mediterranean coast of Spain, because of the remarkable similarities in pottery decorative motifs at sites in the

Estremadura (e.g. Almonda and Eira Pedrinha) to the early Cardial wares in Spain, such as at Cova de l'Or (Zilhão, 2000:150). By the end of the 6th millennium BC, these communities were flourishing, populations grew and a continuous occupation in caves became evident, with the latter being used, in many cases, as pens to shelter their herds (Martí-Oliver, 2002:55). The sites occupied were usually vacant from other inhabitants or situated in between hunter-gatherer niches, for example around the Caldeirão in the northern part of Estremadura or Figueira da Foz, which continued to be exploited by non-agrarian communities (Martí-Oliver, 2002:55). Hence, for around 500 years, Early Neolithic communities and Late Mesolithic hunter-gatherers thrived in their respective territories, following a different way of life in terms of their economy, subsistence and mortuary practices (Zilhão, 1993, 2000:148-163; Arias, 2007). In light of this, the presence of pottery in archaeological deposits dated to the 6th millennium cal. BC located at the foot of the Pyrenees, which however show no evidence of agriculture or animal husbandry is interesting, as it suggests possible acquisition of pottery through exchange, or evidence for pottery producing foragers (Arias, 2007:53).

Botanical remains show a well developed agrarian community, attested by the presence of carbonised seeds of wheat and barley, as well as leguminous remains of peas (particularly abundant at Cova de les Cendres), lentils and beans (Zilhão, 2000:148; Martí-Oliver, 2002:56). Evidence for the use of wild fruits and honey were also found (Martí-Oliver, 2002:56). The faunal remains recovered show the importance of domesticated animals, in particular ovicaprids (predominantly sheep), as well as ox, pig and dog (Zilhão, 2000:148; Martí-Oliver, 2002:56). Hunting, gathering and fishing complemented the agrarian practices and comprised deer, rabbit, roe deer, wild goat and boar, horse, and some birds (Martí-Oliver, 2002:56). Stable isotope analysis carried out on human bone from the Neolithic site of Calderião however showed a terrestrial diet, which contrasts with the heavy reliance on marine resources (at least 50% of the diet) reported for individuals analysed from the contemporaneous cluster of Mesolithic shell midden sites located in the Tagus estuary (Zilhão, 2000:161). However, fishing appears to have been important at other sites, for example at Cendres, where remains of fish (e.g. grouper, sea bream and sea bass) were collected, as well as evidence for the exploitation of marine molluscs, in particular, *Patella* and *Monodonta* (Martí-Oliver, 2002:56).

Pottery analysis has been amply carried out, especially in the regions of Valencia (Cova del' Or and Cendres) and Andalusia (Carigüela and Los Castillejos de Montefrió). Small bits of calcite, quartz or mica, were used as temper when preparing the clay paste, and organic temper was also used to moderate its plasticity. Once a homogenous paste was obtained, the vessels were formed using

the pressure or coiling techniques (Martí-Oliver, 2002:57). Analysis at Cova de l'Or show that Neolithic pottery developed in connection with decoration, typology and function. At the beginning of the Neolithic, ceramic vessels were constructed using raw materials which were particularly well levigated, high in clay minerals and low in crystalline inclusions, using organic material as temper (Martí-Oliver, 2002:57). The surface was particularly well refined, perfectly burnished and richly decorated, mostly with cardial impressions (Figure 2.10). At Cova de l'Or, these vessels do not appear to have been used directly over a fire as they would otherwise have been broken, and it is thought that their function was more likely connected with storage (Martí-Oliver, 2002:57). Another type of fabric existed, which was coarse, and contained crystalline calcite inclusions characteristic of pots likely to be placed in fire and capable of withstanding the tension produced without risking breakage (Martí-Oliver, 2002:57). Forms included typical hemispherical and globular bowls, flat-based vases, jars and pitchers, as well as large vessels thought to have been used for storage, small bottles, and spoons (Martí-Oliver, 2002:57-58). Spoons were produced using femurs from oxen, and interestingly, their use-wear patterns, produced as a consequence of their rubbing against the interior surfaces of the ceramic vessels, was identified; this also indirectly indicated that a change in diet was occurring (Martí-Oliver, 2002:56).



Figure 2.10: Examples of Iberian Cardial Wares from Cova del'Or (From Martí-Oliver, 2002:58).

Martí-Oliver (2002:57-58) speculated on the possible function of these ceramics, and observed that domestic wares and containers used for liquids and seeds were generally the most minutely decorated. Other vessels do not appear to have an everyday use, for example, small recipients or bottles with asymmetric looped handles which often contained the remnants of ochre, and which were perhaps hung from the shoulder or waist, could have been used to contain paints used to cover their bodies (Martí-Oliver, 2002:57-58). Decorative techniques applied during the Early

Neolithic focus on cardial decorations, using geometrical, symbolic and figurative motifs, as well as impressions obtained using instruments, combs, punches, spatula and fingernails (Martí-Oliver, 2002:57-58). Most of these impressed decorations appear to have been inlaid with ochre, remains of which has often been found, and relief decorations including indentations and cord impressions were also generally combined with other decorative techniques, while grooves and incisions begin to be applied only at the end of the first phase of the Neolithic (Martí-Oliver, 2002:57-58).

2.7 Africa

Impressed ceramics in Sahara and Sub-Saharan Africa are among the oldest in the world, dating to about 9500 BP and found in several sites stretching from the Niger to the Nile Valley (Caneva, 2002:63). This pottery was produced by hunter-gatherers thousands of years before agrarian production (Close, 1995), and are noticeable in their abundance and good quality both in the preparation of the clay and firing (Caneva, 2002:63). The density and variation of the impressed and incised decorations contrast with the simplicity of their forms (globular, having distinctive necks but no bases, and lacking any form of handles), which lead researchers to define this culture based on these decorative motifs (Caneva, 2002:63). These earliest ceramics in North Africa are therefore distinct from those produced along the Mediterranean coast typologically, in their economic and social contexts, as well as in form and technology. Being older by more than a thousand years, their origin is independent and not associated with the Neolithic economy (in particular agriculture), but with hunter-gatherers and they are found away from the coast, with the rare exception being the Nile Delta (Caneva, 2002:63, 68). At this time, the interior of Africa was characterised by sedentary, ceramic producing hunter-gatherer communities living near water sources, and mobile pastoral communities (Caneva, 2002:64). The absence of agriculture can be attributed to a lack of the right species, which therefore induced nomadism among animal herders, and it is only in the northern parts of the Nile Valley (Egypt) that this situation is reversed, probably due to the introduction of domesticated wheat, barley, sheep and goats through the Suez isthmus (Caneva, 2002:64).

Archaeologically, there is no evidence for contact between the Saharo-Sudanese Impressed Ware cultures, and those in the Mediterranean (Caneva, 2002:68), however, excavations in North Africa have uncovered evidence which suggests the presence of northern Mediterranean Impressed Ware cultures. Fugazzola Delpino (2002b:98) hypothesised that during the westward voyages, seafaring Neolithic mariners were likely to have stopped in North Africa. The retrieval of obsidian

from Pantelleria may point to an existing southern route, whereby Neolithic people reached the western part of Sicily from the south (Fugazzola Delpino, 2002b:98). It is known that obsidian from Lipari and Pantelleria was transported to the coasts of Algeria and Tunisia, for example, at the settlement of Kef Hamda, where the presence of obsidian originating from Pantelleria was dated to 5560±125 BP (Fugazzola Delpino, 2002b:98). It is not yet possible to state during which period of the Neolithic, contact and exchange between the southern Mediterranean and North Africa took place, and unfortunately, little research has yet been conducted on the coastal regions of Tunisia, Algeria and Morocco. An important site is Gar Cahal, in northern Morocco, which is situated in the straits of Gibraltar. Three ceramic fragments decorated with cordial impressions were retrieved from Level III/b dating to the Neolithic, which occur slightly after the introduction of painted ceramics (Fugazzola Delpino, 2002b:98). This decorative sequence, where painted ware is subsequently abandoned for impressed decoration, has so far occurred only in one other site in Italy, at La Marmotta, situated on the Tyrrhenian coast of Italy (Fugazzola Delpino, 2002b:98).

Four decorative techniques have been identified in the Impressed pottery of Africa: the rocker stamp, the alternating pivoting stamp, simple impressions and incisions (Caneva, 2002:65). These techniques are never combined in the same pattern, except at the rims, which are independently decorated from the rest of the vessel (Caneva, 2002:65). Different instruments were used to produce different designs within each technique, and each combination appears to have defined a particular cultural entity, such that this visual code appears to have had an important social relevance (Caneva, 2002:65). Long combs with regularly spaced teeth were used in the Mesolithic, whereas short combs with irregular teeth were used in the Early Neolithic, and smooth bordered and curved combs in the later Neolithic (Caneva, 2002:68). Impressed decorations on African prehistoric ceramics went through some significant changes, in particular, in the utilization of the different techniques and the type of instruments used. This sequence was reconstructed by correlating microstratigraphies, especially at Shaqadud in the Sudanese side of the Nile Valley, where it was observed that a substantial amount of characteristics were present throughout all the territory (Caneva, 2002:68-69).

2.8 Pottery and agriculture: a Mediterranean perspective

Apart from presenting a synthesis of the archaeological work carried out pertaining to the earliest attestation of the Neolithic in the Mediterranean, one of the major themes of this chapter was to assess the role of pottery as part of the Mediterranean 'Neolithic Package'. Section 2.6 attempted to provide a brief chronological overview of the first appearance of cultigens and domestic

animals in the archaeological record across the Mediterranean, together with the simultaneous introduction of Impressed/Cardial Wares (summarised in Figure 2.11). Hence, while acknowledging the fact that pottery production is not dependent on agriculture, and vice versa, archaeological evidence and radiocarbon dates suggest that in the Mediterranean, the two spread contemporaneously. Zilhão's (2001) revision of the Iberian radiocarbon dates further proved that hypotheses suggesting a local domestication of animals during the Late Mesolithic (e.g. at Grotte Gazel) were erroneous, and showed that none of the material culture associated with the 'Neolithic Package', including polished stone axes, village dwelling, ceramics and domesticates could be ascribed to undisturbed Mesolithic contexts. On the other hand, the evidence available to date points towards the appearance of the agro-pastoral 'package' along with the first attestations of Impressed/Cardial Ware cultures in the western Mediterranean (Zilhão, 2001).



Figure 2.11: Map showing the earliest dates for the presence for domesticates and Impressed/Cardial Wares. Dates reported in BC. (After Zilhão, 2001; Balossi and Frangipane, 2002; Benvenuti and Metallinou, 2002; Maggi, 2002; Trump, 2002; Skeates, 2003; Zilhão, 2003; Pace, 2004; Forenbaher and Miracle, 2005; Fenech, 2007; Guilaine and Manen, 2007; Pessina and Tiné, 2008:27).

A summary of the evidence supporting a 'punctuated maritime pioneer' model for the spread of agriculture in the Mediterranean is presented below:

1. **Chronology:** Radiocarbon dates become progressively younger from the first appearance of impressed decorations in the agrarian communities of the Levant, to the various attestations of Impressed/Cardial Ware communities across the central and western Mediterranean (Figure 2.11).

2. **Impressed/Cardial Wares:** The rapid appearance of ceramic vessels decorated with cardial and instrumental impressions following a similar decorative syntax, along with agrarian practices, also suggests a seafaring route, particularly because:
 - a. The decorative repertoire is strikingly similar over such a wide geographical area, which suggests that perhaps the communities that produced them shared a common origin. In fact, it has been suggested that impressed and cardial decorative motifs identified Impressed/Cardial Ware communities, and were a symbol of their ideology and mentality, which differed from the local hunter-gathering communities (Martí-Oliver, 2002:60).
 - b. The pottery retrieved from the earliest levels shows a good quality production, therefore suggesting that the vessels were being produced by experienced potters. Furthermore, there is a lack of evidence for the trade of Impressed/Cardial Wares (Robb and Farr, 2005:29-30), which could perhaps indicate that it was the know-how of pottery production, rather than the ceramic vessels themselves, which were being spread along with the techniques for food production. Evidence for the use of local clays has been obtained through archaeometric analyses carried out in Greece (Benvenuti and Metallinou, 2002:18), Italy (Muntoni, 2002c, 2003), and the Iberian Peninsula (Martí-Oliver, 2002:57), while Spataro's (2009a:68) studies on Impressed Wares produced along both shores of the Adriatic indicated that there was no exchange between the two coastlines. It is only in southern France that raw materials appear to show a strong element of mobility, in particular in the Languedoc and Provence areas (Guilaine and Manen, 2002:48).

3. Settlement patterns

- a. As described in Section 2.6, coastal settlement patterns dominate during the Early Neolithic. This suggests a link to the sea , which does not appear to have been connected to dietary requirements , given the low representation of marine remains in the faunal assemblages , and the isotopic study carried out on human remains at Calderião , in Portugal (described above). In light of this, the coastal location was probably associated with their means of transport, seafaring.
- b. The location of Early Neolithic settlements, away from areas inhabited by local Mesolithic communities appears to have been intentionally targeted, and indicates that the onset of the Neolithic was intrusive.
- c. In southern France, evidence suggests that these early settlers had no particular territorial attachment, and settlements appear to have been occupied only

briefly, therefore suggesting that the communities inhabiting them were highly mobile.

4. Evidence for seafaring and trade:

- a. Archaeological evidence for seafaring knowledge dates to around 11,000 BC, attested by the retrieval of obsidian originating from Melos in pre-Neolithic levels at Franchthi Cave (Robb and Farr, 2005:26-27). Hence, it is therefore plausible to assume maritime activity during the Neolithic (see also Robb and Farr, 2005; Broodbank, 2006).
- b. Although remains of boats dating to the Early Neolithic are scarce, two boats have been found at the site of La Marmotta (Fugazzola Delpino, 2002a), and experimental reconstructions have shown that these vessels could indeed have been used to transport people, goods and livestock (e.g. Broodbank and Strasser, 1991).
- c. Evidence for seafaring along the Tyrrhenian coast of Italy has been obtained through archaeometric analysis of the pottery retrieved (Capelli *et al.*, 2011).
- d. Evidence for known sea routes can be attested through evidence of trading. For example, flint, chert and obsidian are known to have been circulated at three distinct geographical scales, while the distribution system ranged to over 100km in radius. Examples include flint from the Gargano area in south-east Italy, which was traded westwards towards the Apennines and eastwards towards the central Adriatic islands and Croatia. Flint from Monti Iblei in Sicily was found in Malta, eastern Sicily, Lipari and southern Calabria, while obsidian and polished stone tools were also traded widely during the Early Neolithic in the Mediterranean (Fugazzola Delpino, 2002b; Robb and Farr, 2005:28-29; Guilaine and Manen, 2007).
- e. Barnett (1995:81) and Gheorghiu (2008:168) observed an apparent connection between the spread of pottery technology and agriculture, and water, since in Europe and the Mediterranean this appeared to have taken place along rivers and their tributaries, as well as the Mediterranean coast. Gheorghiu (2008:169) also commented on the importance of the symbolism of water in the Impressed/Cardial communities and suggested that this was perhaps emphasised by the use of the marine *Cardium* shell to carry out the impressed, 'wave'-like decorations.

2.9 Conclusion

There are several lines of evidence which point towards a ‘punctuated maritime pioneer’ model for the spread of agriculture in the Mediterranean. Archaeological research is however dynamic and future investigations can add support to or dismiss this hypothesis. Apart from the already mentioned revision of radiocarbon dates, as suggested by Zilhão (2001), further archaeological excavations (both on land and underwater) will hopefully shed more light on this field of study. Changes in sea-water levels are known to have led to coastal sites, dating to the Neolithic period, to become submerged. For example, between 7000 and 5500 cal. BC, the sea level along the Dalmatian coast rose from around -27m to -13.5m (Benjamin *et al.*, 2011:201-204). Investigating submerged sites has the added advantage of potentially producing evidence for well preserved organic material, which may yet answer many questions related to daily life and subsistence patterns.

Most of the work carried out on Impressed/Cardial Wares has focussed on their chronological appearance and spread, their form, decorative styles and manufacture. Function has however, only tentatively been discussed, and has been attributed mainly in terms of the size, form and level of decoration of the vessels studied. Pottery played a fundamental role in the development of cuisine and the transformation of food. It facilitated the adoption of new foodstuffs, such as cereals, and enabled a wider diversification of food combinations, while certain types of food could also be more intensely used, perhaps over a greater part of the year (Ingold, 1983; Manson, 1995). Hence, identifying the contents of Impressed/Cardial Wares could potentially allow a better understanding of their role at the transition to agriculture.

The close association between domesticates and Impressed/Cardial Wares in the earliest Neolithic stratigraphies of the western Mediterranean suggests that perhaps these vessels were used to process agrarian products. This in turn, implies that the spread of Impressed/Cardial Wares was very closely associated with that of agriculture and pastoralism. However, archaeological investigations have shown that the initial production of ceramic vessels had no direct connection to agrarian practices or contact with farming communities, since evidence was obtained for pottery production among foraging communities dating to the Pleistocene (e.g. Jordan and Zvelebil, 2010a and references therein). A similar situation has been described in Section 2.7, when discussing African Impressed Wares, and their occurrence in the archaeological record around two to three thousand years prior to the first attestations of agriculture in the area (Close, 1995). Hence, the spread of Impressed/Cardial Wares in the western Mediterranean could

have been chronologically related to, but not directly associated with the spread of agrarian and pastoral practices. This counter-hypothesis therefore proposes that, if this was the case, pottery vessels would have been used to process wild resources, including marine fish, and their function would have been more varied, and perhaps regionally dependent. Identifying the contents of Impressed/Cardial Wares is therefore crucial to understanding the link between pottery and farming in the western Mediterranean. As described in Chapter 1, this research will focus on trying to determine the function of Impressed/Cardial wares by analysing the residual lipid content trapped within the ceramic fabric. Chapter 3 provides a detailed overview of the method used, while the results obtained are presented and discussed in Chapters 7 and 8, respectively.

Chapter 3

Methods for characterising lipid residues from archaeological ceramics¹

3.1 Introduction

Just over thirty-five years ago, Colin Renfrew encouraged archaeologists to develop the study of pottery beyond typology and dating (Renfrew, 1977). Renfrew, with foresight, included the potential for studying the contents and uses of pottery through the analysis of the organic residues of original food materials. Two references were deployed in support of his argument - the classic work of Alfred Lucas on often well-preserved and substantive organic substances from Egyptian tombs and similar contexts (Lucas and Harris, 1962), and the pioneering investigation of Roman transport amphorae by Condamin and co-workers (1976), which was reproduced in the 50th anniversary virtual volume of *Archaeometry*. Over 30 years on, lipid analysis in archaeology has advanced dramatically. The potential of lipid analysis to characterise organic residues and provide assessments of food and non-food products in pottery vessels has emerged as a powerful tool in recent years. For the past 20 years, this field has been led by a research group headed by Richard Evershed with numerous applications and technical developments across a wide front. By the early 1990s, Heron and Evershed (1993) were able to document an increasing range of applications and techniques in the intervening period since Renfrew's review. In addition, Evershed (1993) presented an important overview of the chemistry of lipids of relevance to archaeology. Evershed *et al.* (1992) focused on lipid degradation, building on more general reviews of molecular preservation (e.g. Eglinton and Logan, 1991) that were appearing at the time. More recently, in comprehensive reviews of lipid investigations in archaeology, Regert (2011) and Evershed (2008a, 2008b), as well as Evershed *et al.* (1999, 2001, 2002a) provided important updates on the progress in the field by documenting the range of organic substances associated with pottery vessels that different research groups have identified to date. The list

¹Updated from the following publication: Debono Spiteri, C., Heron, C. and Craig, O. (2011). Characterising surviving residues from archaeological ceramics: a biomolecular approach. In Sarcella, S. (ed.), *Archaeological ceramics: a review of current research*. BAR International Series S2193. Oxford, Archaeopress. C. Debono Spiteri wrote the article. C. Heron and O. Craig read the manuscript and added contributions. Revisions added by C. Debono Spiteri.

includes plant epicuticular waxes, beeswax, animal adipose fats, dairy fats, plant oils, marine oils, bitumen, conifer resin/tar, birch bark tar and *Boswellia* resin (frankincense).

Fatty residues, derived from animal and plant products, are absorbed into the walls of unglazed ceramics through the action of heat (Heron and Evershed, 1993). Using organic residue analysis (ORA), the surviving lipid is extracted from within the ceramic matrix. Characterisation of these residues allows the vessel content to be identified, hence establishing a direct link to vessel use (Evershed *et al.*, 1999). The success of this work rests upon biomarker recognition and discrete isotopic signatures determined on specific molecules present in the residue. Charred deposits which are occasionally found on the surface of ceramics can also be analysed (Oudemans and Boon, 1991; Campbell *et al.*, 2004). ORA has therefore been very useful in providing information on diet and economy in past societies.

Research into ORA has primarily focused on biomarker identification, and, since the residues extracted are essentially decayed products of the original lipid compounds, studies have also been carried out to investigate the degradation patterns of various products extracted from archaeological artefacts. This chapter will review these studies, and highlight the strengths and limitations of ORA. It will focus mainly on method development and explain the rationale for applying this technique to analyse the contents of Early Neolithic Impressed/Cardial Wares from the western Mediterranean investigated in this research. In view of the simulation degradation experiment carried out on plant material described in Chapter 5, this chapter includes a detailed discussion on the previous research carried out on lipid decay and preservation in archaeological residues.

3.2 Applications of ORA

ORA has been extensively applied to archaeological studies: to determine the original contents of vessels (Condamin *et al.*, 1976; Patrick *et al.*, 1985; Evershed *et al.*, 1991; Malainey *et al.*, 1999a; Urem-Kotsou *et al.*, 2002; Roumpou *et al.*, 2003; Stern *et al.*, 2003; Solazzo and Erhardt, 2007; Evershed, 2008b; Stern *et al.*, 2008), to determine vessel use (Charters *et al.*, 1993, 1995, 1997; Evershed *et al.*, 1995b; Raven *et al.*, 1997; Mottram *et al.*, 1999), and occasionally, to disprove erroneous theories regarding ancient diet and economy (Evershed *et al.*, 1997c; Copley *et al.*, 2004). Another successful application of ORA is in detecting dairying, a key secondary product which confirms the practice of pastoralism (Craig *et al.*, 2005a; Evershed *et al.*, 2008b), as well as marine fats and oils (Copley *et al.*, 2004; Hansel *et al.*, 2004; Craig *et al.*, 2007, 2011).

Lipid analysis has been carried out on a wide variety of materials including 'bog butter' (e.g. Berstan *et al.*, 2005), burned rocks (e.g. Buonasera, 2005) and the contents of metal (e.g. Evershed *et al.* 2004), enamel (e.g. Dudd and Evershed, 1999), and glass vessels (e.g. Ribechini *et al.*, 2008). The identification of lipids in soils and sediments as anthropogenic markers of manuring, cess deposition and so on (e.g. Knights *et al.*, 1983; Bethell *et al.*, 1994; Evershed *et al.*, 1997a; Bull *et al.*, 1999, 2001, 2003; Simpson *et al.*, 1999; Kedrowski *et al.*, 2009), have similarly been achieved. Analysis and identification of plant resins and other exudates together with products such as tar and pitch produced by heating these materials have been occasionally reviewed, for example the European evidence is included in Pollard and Heron (2008:235-269) and Regert (2004), while Serpico (2000) reviewed Egyptian finds. Beeswax is another extensively researched product which has been found associated with a very wide range of pottery vessels and other artefacts in Europe and the Near East (e.g. Heron *et al.*, 1994; Evershed *et al.*, 1997c; Regert *et al.*, 2001a; Evershed *et al.*, 2003; Roumpou *et al.*, 2003). It has also been identified in pottery vessels in a mixture with animal fats prompting discussion about the precise use(s) the vessel was put to (Charters *et al.*, 1995). Simulation experiments have been undertaken to explore alteration processes (Regert *et al.*, 2001b) and the carbon isotope ratios of the *n*-alkanes have also been reported (Evershed *et al.*, 2003).

Lipid molecules associated with human remains pertaining to endogenous (Evershed *et al.*, 1995c; Stott and Evershed, 1996; Stott *et al.*, 1999; Jim *et al.* 2003, 2004) and microbial lipids (Gernaey and Minnikin, 2000; Gernaey *et al.*, 2001; Redman *et al.*, 2002) in bone, bog body tissues (Evershed, 1990, 1991, 1992a; Evershed and Connolly, 1994), permafrost preserved (Bereuter *et al.*, 1996; Mayer *et al.*, 1997; Makristathis *et al.*, 2002; Dickson *et al.*, 2004; Varmuza *et al.*, 2005) and mummified tissues (Gulaçar *et al.*, 1990), have also been studied.

The recovery of amino acids and proteins from archaeological ceramics has been attempted (e.g. Evershed and Tuross, 1996; Craig *et al.*, 2000a, 2005a; Craig and Collins, 2000, 2002), and a review of such investigations was published in Barnard *et al.* (2007). The potential for DNA extraction and amplification in complementing and extending residue analysis based on molecular lipid signatures is largely untested, although small-scale investigations have appeared (e.g. Cavalieri *et al.*, 2003; Hansson and Foley, 2008).

3.3 Lipids

Lipids occur ubiquitously, and are required by all living organisms (animals, plants and microorganisms) in several biological functions, including structural and metabolic processes, and as energy sources and stores (Gurr *et al.*, 2002). Lipids are generally defined on the basis of their solubility, being more soluble in organic than aqueous solvents (Voet and Voet, 2004), and include different classes of compounds, namely fatty acids and their derivatives, steroids, terpenes, carotenoids and bile acids (Christie, 2003). Available literature on the structure and metabolism of lipids is extensive (see Gunstone, 1967, 1992, 2004; Perkins, 1993; Gurr *et al.*, 2002; Christie, 2003).

The chemical properties of lipids are determined by their structure (Mills and White, 1994). Animal fats and plant oils comprise mainly triacylglycerols (TAGs), which consist of three fatty acids joined to a glycerol molecule through esterification (Mills and White, 1994). Mono- and diacylglycerols have one and two fatty acids respectively (Gunstone, 2004) (Figure 3.1). The fatty carboxylic acids are long unbranched chains, usually with an even number of carbon atoms, although branched and cyclic acids occur, as do fatty acids with an odd number of carbon atoms (Gunstone, 1992). They can be saturated or unsaturated, having one (monounsaturated acids) or more (polyunsaturated acids) double bonds, the *cis* configuration generally being more common than the *trans* (Gunstone, 1992; Mills and White, 1994) (Figure 3.2). Fats have a higher proportion of saturated fatty acids as opposed to oils, which consist mainly of unsaturated acids (Gunstone, 1992). The unsaturated nature of oils lowers their melting point; hence at room temperature oils occur as liquids, and fats as solids (Gunstone, 1992).

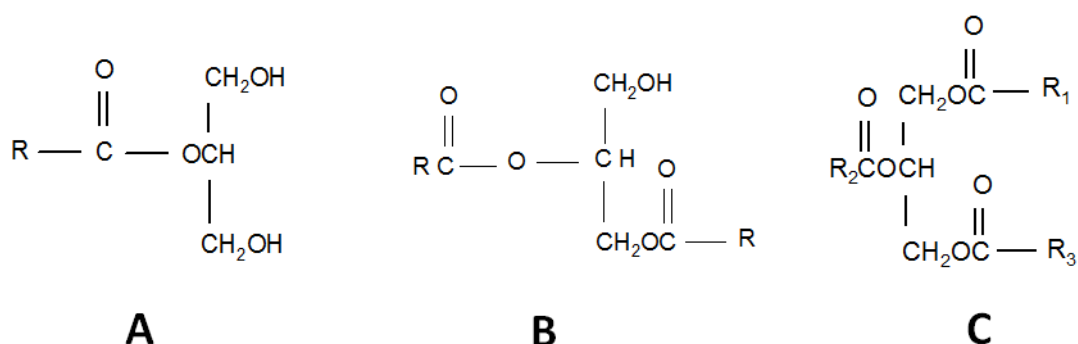


Figure 3.1: Acylglycerols: A: Monoacylglycerol; B: Diacylglycerol; C: Triacylglycerol.

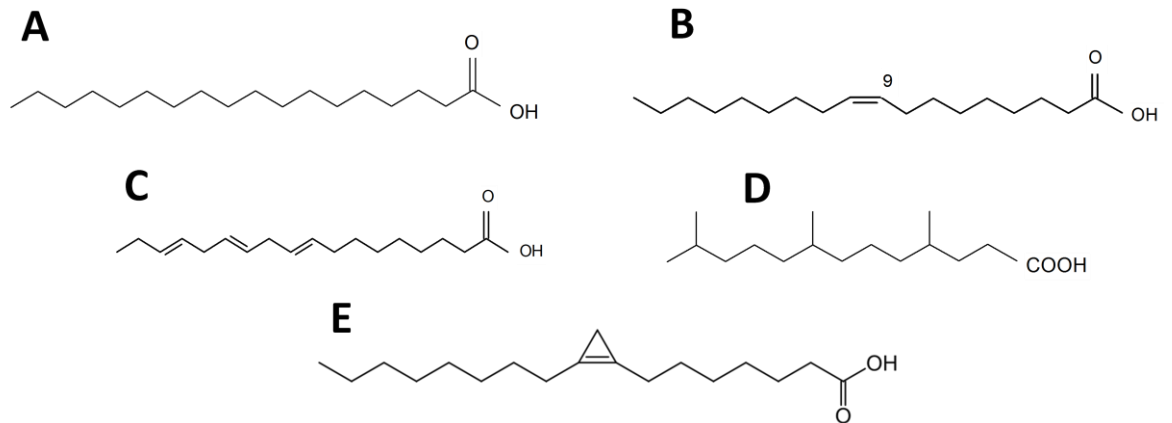


Figure 3.2: Fatty acids: A: Octadecanoic acid (saturated); B: cis-9-octadecenoic acid (monounsaturated); C: trans-9,12,15-octadecatrienoic acid (polyunsaturated); D: 4,8,12-trimethyltridecanoic acid (branched chain); E: Malvalic acid (cyclic).

Sterols are minor constituents of animal and plant lipids (Evershed, 1993). Cholesterol is the main sterol in animal fats, fish and marine oils and fats. Steryl esters, comprising a molecule of cholesterol esterified to a long-chain fatty acid may also be present. Animal fats may also contain minor amounts of other sterols, such as lanosterol. Plants comprise mostly phytosterols, including β -sitosterol, campesterol and stigmasterol, with only trace amounts of cholesterol (deMan, 1999). Another important sterol is ergosterol, which occurs mainly in yeasts and fungi (Gunstone *et al.*, 1994) (Figure 3.3).

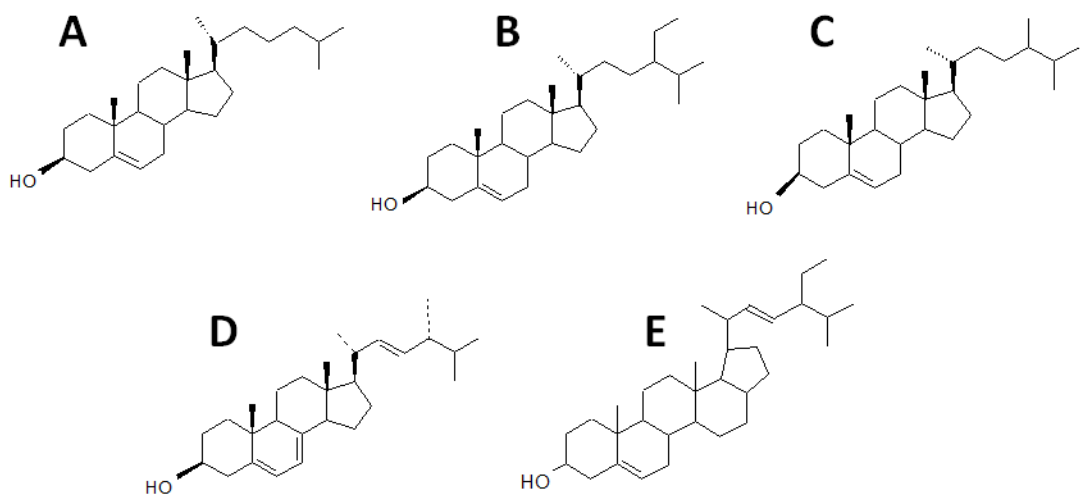


Figure 3.3: Major sterols found in animal and plant fats and oils: A: Cholesterol; B: Sitosterol; C: Campesterol; D: Ergosterol; E: Stigmasterol.

Fatty acids are the most frequently encountered compounds when undertaking ORA. Their abundance and distribution is particular to a fatty product, hence the different lipid profiles obtained help identify the original material. Fatty acid ratios have also been used to identify source materials (Eerkens, 2005), but their reliability is still debated. Other important biomarkers have been identified and are discussed below.

3.4 Sampling and extraction

Technical developments, which increase confidence in results, and better integrated (in archaeological terms) programmes of analysis, have enabled a much greater number of samples to be processed than could have been envisaged a decade ago. Perhaps the most comprehensive studies to date are the analyses of more than 950 pottery sherds from 14 British sites undertaken by Copley *et al.* (2005b) and the study of 2225 ceramic samples from 23 sites in the Near East and southeast Europe carried out by Evershed *et al.* (2008b). Building on the work of Dudd and Evershed (1998), residue analysis was used to trace dairy products in a significant number of Neolithic vessels, the former suggesting that dairying was widespread by the Early Neolithic in northern Europe, while the latter provided direct evidence for the use of dairy products in the Near East by the seventh millennium BC.

The amount of powdered ceramic required for analysis is usually about 1 or 2g, but larger (100g; Condamin *et al.*, 1976), and smaller (0.1g; Stern *et al.*, 2000) sample sizes have been used. Several methods for sample preparation have been developed. Lipids are extracted using an adequate solvent, generally a mixture of chloroform or dichloromethane and methanol, in which the lipids dissolve. Several lipid extraction techniques are available, including, soxhlet (Condamin *et al.*, 1976), sonication and saponification (e.g. Mukherjee *et al.*, 2008), accelerated solvent extraction (ASE) (Hughen *et al.*, 2004; Jansen *et al.*, 2006), and microwave accelerated reaction system (MARS) (Gregg *et al.*, 2009; Gregg and Slater, 2010). A non-destructive extraction method was proposed by Gerhardt *et al.* (1990), which involved successive extractions with chloroform then methanol of a complete ceramic vessel. This method has not been widely used, as research has generally been carried out using established methods, but given its non-destructive advantage, further investigations into its effectiveness may prove beneficial.

3.5 Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

GC and GC-MS are the two preferred analytical techniques for ORA (Evershed *et al.*, 1999; Pollard *et al.*, 2007:142). Identification of compounds by GC is carried out by comparing retention times with known standards (Evershed, 1992b), whereas structural information on individual molecules can be obtained by combining the GC with a MS detector, which is useful for the identification of components in complex samples. GC and GC-MS allow the analysis of intact lipids with a wide range of molecular weights (Evershed, 1992b, 1992c). However, although samples can be derivatized to produce volatile components, not all compounds are amenable to this type of analysis. GC and GC-MS provide the following general categories of information:

- (i) Comparing the lipid abundance and profile obtained from the total lipid extracts: This enables fatty acid and acylglycerols to be identified in the same run (Evershed *et al.*, 1990), and is helpful to provide an indication of lipid preservation. In a recent article, Mukherjee *et al.* (2008) were able to conclude that 43 extracted lipid residues out of a total of 222 Grooved Ware sherds from 11 British Neolithic sites yielded intact triacylglycerols in addition to free fatty acids. Despite being subjected to hydrolytic reactions, the carbon-number distribution of triacylglycerols can complement other criteria used to characterise ancient fats (Dudd and Evershed, 1998). Mirabaud *et al.* (2007) carried out extensive analysis, and proved that by analysing the fatty acid distribution of residual triacylglycerols, distinctions can start to be made between cow, goat and sheep adipose and milk fat. Their findings are further discussed in Section 3.7.

A number of researchers have based the identification of the original source of the lipid residue through determining the ratio of palmitic (C_{16:0}) and stearic (C_{18:0}) acids in particular, or the abundance of a range of fatty acids present. It is difficult to generalise but where the abundance of C_{16:0} is less than that of C_{18:0} then it suggests the presence of an animal fat. Where the abundance of C_{16:0} is 3-4 times greater than C_{18:0} then it suggests a plant oil (Copley *et al.*, 2005e). Fatty acid ratios have featured as key markers in the characterisation of residues in several publications (e.g. Malainey *et al.*, 1999a, 1999b, 1999c, 2001; Eerkens, 2005; Malainey, 2007). By way of example, Malainey *et al.* (2001) have explored the idea of fish avoidance by hunter-gatherers in western Canada from 1800 BP onwards. The archaeological hypothesis was tested by applying lipid analysis to potsherds from a range of site types. Residues comprising freshwater fish were identified by comparison to a reference

collection subjected to experimental cooking and consequent lipid alteration. Fish was identified on the basis of the relative abundance of medium chain ($C_{12:0}$, $C_{14:0}$ and $C_{15:0}$) and long chain fatty acids ($C_{18:0}$ and $C_{18:1}$ isomers). The absence of fish residues from the archaeological sites of big-game-dependent hunter-gatherers was interpreted as deliberate fish avoidance by those with a high lean meat diet. Consequently the effects of lipid malabsorption arising from a sudden switch to spring-spawning fish were avoided. The challenges of identifying organic substances based solely on distributions of fatty acids are, however, well documented (e.g. Heron and Evershed, 1993:268) and result from their widespread distribution in many food sources, variations in composition according to diet, climate and other factors such as the preferential loss over time of unsaturated fatty acids and diagnostic short-chain fatty acids (see Section 3.9 for further detail). Table 3.1 lists the $C_{16:0}:C_{18:0}$ fatty acid ratios for fresh and degraded modern fats in various food commodities. Wide $C_{16:0}:C_{18:0}$ ranges can be observed to occur in the fresh food types analysed, which increase considerably when degradation is taken into consideration, causing significant overlap in the $C_{16:0}:C_{18:0}$ between the different food products. This suggests that degraded lipid residues cannot be confidently interpreted based solely on their $C_{16:0}:C_{18:0}$, since it is difficult to discriminate between the wide ranges of potential sources. They can however, still provide supporting evidence to the more reliable biomarker and isotopic data (discussed below).

	Terrestrial Mammals	Fish	Roots	Greens	Seeds & Nuts	Berries
Fresh	<3.5	4–6	3-12	5-12	0-9	2-6
Degraded	<7	8–12	6-24	10-24	0-18	4-12

Table 3.1: Table showing the $C_{16:0}:C_{18:0}$ from various sources of fresh and degraded animal and plant products. (Compiled from Eerkens, 2005)

- (ii) *Biomarkers*: The identification of particular biomolecules pertaining to specific fat sources have led to some impressive identifications, based in part on the presence of unusual fatty acids that are restricted to limited biological sources (e.g. Copley *et al.*, 2005e) or on unusually high abundances of single fatty acids in plant oils (e.g. Copley *et al.*, 2001a, 2001b). Other examples include the identification of specific biomarkers present in epicuticular waxes of *Brassica* sp. (Evershed *et al.*, 1991, and further discussed in Chapter 5), ω -(*o*-alkylphenyl)alkanoic acids, isoprenoid fatty acids and dihydroxy acids in marine and freshwater organisms (Section 8.5; Copley *et al.*, 2004; Hansel *et al.*, 2004; Craig *et al.*, 2007; Evershed *et al.*, 2008a; Hansel and Evershed, 2009), hydroxy fatty acids and α,ω -dicarboxylic acids from oils and fats burned in lamps (Copley *et al.*, 2005e) and plant and animal sterols

(Stacey, 1999) amongst others. Biomarker analysis can also help identify different fat sources in mixtures of food products (see Chapter 7). Table 3.2 summarises some of the more commonly encountered fatty residues extracted from archaeological ceramics.

3.6 Gas Chromatography-combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS)

By the mid 1990s, GC-c-IRMS was being used to determine the $\delta^{13}\text{C}$ values of individual components in extracts, providing information on the source of the lipid (Evershed *et al.*, 1994; Evershed *et al.*, 1999). In GC-c-IRMS, isotopic ratios of individual compounds within a mixture can be determined. Compounds are first separated by GC, then combusted in an online reactor to carbon dioxide (CO_2). The relative abundance ratio of the $^{13}\text{C}/^{12}\text{C}$ as CO_2 is then determined by the IRMS (Evershed *et al.*, 1994). Meier-Augenstein (2002) provides an interesting review of the technique and the various applications of GC-c-IRMS in several fields of study.

$\text{C}_{16:0}$ and $\text{C}_{18:0}$ are the most common fatty acids present in organic residues, which are readily extracted from archaeological samples and whose stable carbon isotope composition appears to be unaffected by diagenetic alterations during burial (Craig *et al.*, 2003; Spangenberg *et al.*, 2006; Evershed, 2008a). The latter has been demonstrated in a small number of studies (Dudd, 1999; Aillaud, 2001; Spangenberg *et al.*, 2006), while experiments carried out by Craig *et al.* (2004) showed no significant bias in the stable carbon isotopic signatures of free and covalently-bound lipid compounds in archaeological ceramics when subjected to solvent extraction, alkaline hydrolysis and catalytic hydrolysis. Differences in the carbon isotope values of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ arise from variations in their biosynthesis and routing in different organisms (Evershed *et al.*, 2002b). These variations allow different sources of fat to be separated by calculating the $\Delta^{13}\text{C}$, which is the difference between the $\delta^{13}\text{C}$ values of the $\text{C}_{18:0}$ and $\text{C}_{16:0}$ fatty acids (Evershed *et al.*, 1999, 2002b) (Figure 3.4 and see Table 3.2). Therefore, $\delta^{13}\text{C}$ values obtained for $\text{C}_{16:0}$ and $\text{C}_{18:0}$ of modern terrestrial and aquatic animal tissues can be used to construct fields to serve as reference points against which to compare similar isotopic measurements obtained from archaeological residues (Figure 3.4). The carbon isotopic measurements obtained from modern reference fats first need to be corrected for discrepancies with the isotopically heavier (1.6‰), preindustrial atmospheric CO_2 (Spangenberg *et al.*, 2006). A more detailed description of the rationale behind using GC-c-IRMS analysis to characterise archaeological lipid residues is provided in Chapter 4.

Fat type		Triacylglycerols	Fatty acids	Other constituents	$\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ signature
Non-ruminant animals	Porcine adipose fats	<ul style="list-style-type: none"> × Narrow distribution C_{44} to C_{54} with low abundance of C_{44}, C_{46} and C_{54} × Rich in tripalmitin × Palmitic acid preferentially located in the 2-position (P:S ratio in <i>sn</i>-2 position is of $\approx 95:5$) 	<ul style="list-style-type: none"> × $\text{C}_{16:0}$ more abundant than $\text{C}_{18:0}$ × Absence of minor odd carbon number fatty acids × Monounsaturated fatty acids: only a single isomer Z-9-octadecenoic acid 	<ul style="list-style-type: none"> × Odd-numbered ketones ranging from C_{29} to C_{35}, maximising at C_{33} × Monounsaturated ketones with 33 and 35 carbon atoms, resulting from condensation of fatty acids during heating of animal fats × Free or bound oxidised fatty acids (diacides, hydroxy-acids) 	<ul style="list-style-type: none"> × $\text{C}_{16:0}$ and $\text{C}_{18:0}$ enriched in ^{13}C compared to ruminant fats × $\Delta^{13}\text{C} > -1\text{‰}$
	Cattle adipose fats	<ul style="list-style-type: none"> × Distribution from C_{42} to C_{54} × P:S ratio in <i>sn</i>-2 is of $\approx 60:40$ 	<ul style="list-style-type: none"> × $\text{C}_{16:0}$ less abundant than $\text{C}_{18:0}$ × Low amount of straight carbon chain with odd carbon number, specifically $\text{C}_{15:0}$ and $\text{C}_{17:0}$ × Low amount of branched-chain alkanic acid ($\text{C}_{15:0}$ and $\text{C}_{17:0}$) × Mixture of isomers of octadecenoic acid (double bond at 9, 11, 13, 14, 15 and 16 – positions) 	<ul style="list-style-type: none"> × Same ketones as for porcine adipose fats 	<ul style="list-style-type: none"> × $\Delta^{13}\text{C}$ from -3 to -1‰
	Goat adipose fats	<ul style="list-style-type: none"> × Distribution from C_{44} (trace) to C_{54} × P:S ratio in <i>sn</i>-2 is of $\approx 60:40$ 			
Sheep adipose fats	<ul style="list-style-type: none"> × Distribution from C_{44} (trace) to C_{54} × P:S ratio in <i>sn</i>-2 is of $\approx 60:40$ 				
Ruminant dairy fats	Cow milk	<ul style="list-style-type: none"> × Large distribution from C_{40} to C_{54} × In all TAGs, $\text{C}_{10:0}$ is present at lower abundance than in goat milk 	<ul style="list-style-type: none"> × Same fatty acids as for adipose fats of ruminant animals 	<ul style="list-style-type: none"> × Same ketones as for porcine adipose fats 	<ul style="list-style-type: none"> × $\text{C}_{18:0}$ depleted in ^{13}C in comparison with adipose animal fats × $\Delta^{13}\text{C} < -3.3\text{‰}$
	Goat milk	<ul style="list-style-type: none"> × Large distribution from C_{40} to C_{54} × In all TAGs, $\text{C}_{10:0}$ is present at lower abundance than in cow milk 			
	Sheep milk	<ul style="list-style-type: none"> × Large distribution from C_{40} to C_{54} 			
Fats from aquatic resources	Marine fish	Not preserved	<ul style="list-style-type: none"> × $\text{C}_{16:0}$ more abundant than $\text{C}_{18:0}$ × Long chain fatty acids with more than 18 carbon atoms × Triunsaturated fatty acids (usually not preserved in archaeological contexts) 	<ul style="list-style-type: none"> × Presence of isoprenoid acids (Phytanic acid: 3,7,11,15-tetramethylhexacosanoic acid, and 4,8,12-trimethyltridecanoic acid: 4,8,12-TMTD) at low abundance × Series of isomers containing 16, 18 and 20 carbon atoms of ω-(<i>o</i>-alkylphenyl)alkanoic acids with a wide range of positional isomers formed by degradation of triunsaturated fatty acids × Vicinal dihydroxy fatty acids with ranging from C_{16} to C_{22} 	<ul style="list-style-type: none"> × $\text{C}_{16:0}$ and $\text{C}_{18:0}$ are isotopically enriched in ^{13}C compared to those of terrestrial animals, even though they plot not far from adipose fats of domestic pigs
	Freshwater fish	Not preserved	<ul style="list-style-type: none"> × Same acids as for marine fish 	<ul style="list-style-type: none"> × Same constituents as for marine fish 	<ul style="list-style-type: none"> × Freshwater fish are depleted in ^{13}C for both $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids compared to marine resources

Table 3.2: Table summarising the molecular and isotopic criteria used to distinguish between different types of animal fats extracted from archaeological ceramics. The instruments used to affect identification include HT-GC, nano-ESI-MS/MS, HPLC-APCI-MS and Gc-c-IRMS. (Reproduced from Regert, 2011, with additions)

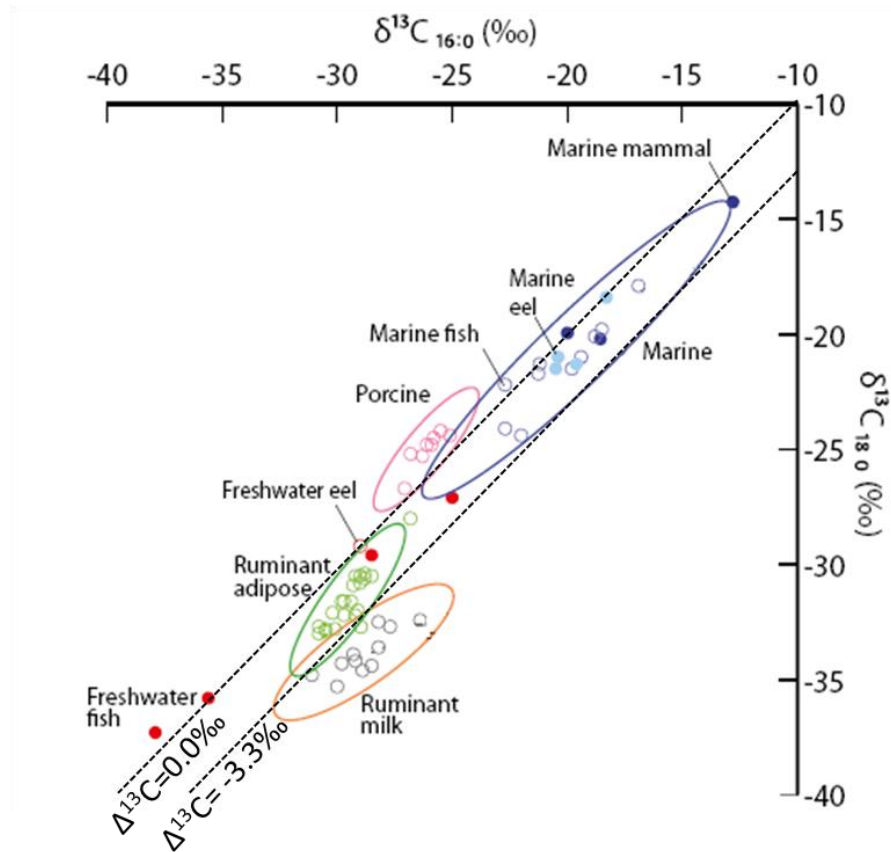


Figure 3.4: Plot of $\delta^{13}\text{C}$ of palmitic ($\text{C}_{16:0}$) and stearic ($\text{C}_{18:0}$) fatty acids obtained from modern animal tissues, following GC-c-IRMS analysis. The separation of fats into different fields allows a better identification of archaeological residues. (From Craig *et al.*, 2011 with changes)

GC-c-IRMS has proved an efficient and effective tool in archaeological research. It was first used in an archaeological context by Evershed *et al.* (1994), who showed that it could be used to derive stable isotope ratios for individual lipid species, hence complementing and extending GC-MS data in assigning origins to archaeological organic residues. Subsequent research focused on the distinction between ruminant and non-ruminant adipose fats (e.g. Evershed *et al.*, 1997b), the detection of dairy products (e.g. Dudd and Evershed, 1998; Copley *et al.*, 2003) and other animal fats, such as horse (Dudd *et al.*, 2003). The detection of dairy products is perhaps the most well known application (Copley *et al.*, 2003, 2005a, 2005b, 2005c, 2005d; Spangenberg *et al.*, 2006; Evershed *et al.*, 2008b; Outram, 2009). More recently the distinction between marine and freshwater organisms has been proposed (Craig *et al.*, 2007). Differentiating the various components within mixtures of fats has also been attempted (e.g. Mottram *et al.*, 1999; Evershed *et al.*, 2002b; Mukherjee *et al.*, 2008). Reber and Evershed (2004a) have shown how carbon isotope measurements determined on a long-chain alcohol constituent of plant waxes (*n*-dotriacontanol) or on $\text{C}_{16:0}$ and $\text{C}_{18:0}$ can be used to trace the C_4 plant contribution to lipid residues

absorbed in potsherds from sites in the Mississippi Valley, USA dating from the Middle Woodland to the Mississippian periods. The recognition of lipids with a C₄ photosynthetic pathway is strong evidence of the preparation and consumption of maize in these vessels. Although the proportion of sherds comprising maize lipids was low (8 out of 81 sherds analysed by GC-c-IRMS), these new data complement the well-established tracing of C₄ plant foods (maize) in human bone collagen from contemporary sites in North America and extend opportunities for assessing food preparation and the social contexts of maize preparation and consumption. In addition to the discrimination between different lipid sources, GC-c-IRMS has also been used to confirm changes (ketonic decarboxylation) to lipids as a result of heating, plausibly during food preparation and other activities involving the heating of lipid in pottery vessels (Evershed *et al.*, 1995b; Raven *et al.*, 1997).

3.7 Other Approaches

In addition to the use of GC, GC-MS and GC-c-IRMS, other mass spectrometric techniques are opening up complementary areas of research, which not only increase the instruments' ability to detect targeted compounds, but also the ability to discriminate between different fat categories. Using nanoelectrospray QqTOF mass spectrometry, Mirabaud *et al.* (2007) have increased the sensitivity of studying the fatty acyl moieties of TAGs preserved in pottery sherds from the Neolithic site of Clairvaux XIV - a waterlogged site yielding exceptional preservation of the lipid fraction. This work suggests that it is possible to discriminate between cow and goat's milk and cow and sheep adipose, which extend the differentiation achieved from compound-specific carbon isotope signatures alone.

Kimpe *et al.* (2001) have explored the potential of liquid chromatography-mass spectrometry using atmospheric pressure chemical ionization (LC-APCI-MS) to identify TAGs comprising specific fatty acyl moieties in Roman to early Byzantine ceramic oil lamps from Sagalassos, southwest Turkey (see also Mottram *et al.*, 1997 and 2001 for further consideration of this technique). The presence of TAGs dominated by monounsaturated fatty acids (octadecenoic acid) and the recognition of phytosterols led the authors to conclude that the lamps were fuelled predominantly using olive oil. LC-APCI-MS has been shown to be more sensitive to the detection of TAGs compared with GC-MS (Kimpe *et al.*, 2004). In this study, saturated and unsaturated moieties esterified to the TAGs were determined and classified according to vessel type. Romanus *et al.* (2007) have compared ratios of palmitic to stearic acid (P/S), single compound carbon isotope measurements on the same fatty acids using GC-c-IRMS and TAG composition using LC-

APCI-MS on the same lipid extracts from 26 sherds from the site of Sagalassos, Turkey. The authors tentatively assigned the lipid residues to a ruminant or non-ruminant origin based on the P/S ratios. There was good agreement between the GC-c-IRMS and HPLC-MS analysis, including isotopic and corresponding TAG evidence of dairy lipid in one vessel.

Macromolecular polymers formed during the heating of food associated with pottery vessels are not amenable to GC analysis, but have been analysed using Curie-Point Pyrolysis Mass Spectrometry (Oudemans and Boon, 1991, 1996), Nuclear Magnetic Resonance Spectroscopy and Fourier Transform IR Spectroscopy (Evans and Biek, 1980; Shearer, 1988; Edwards *et al.*, 1996; Ghisalberti and Godfrey, 1998; Lambert *et al.*, 2000; Oudemans *et al.*, 2007a), and Direct Temperature-Resolved Mass Spectrometry (Oudemans *et al.*, 2007b). These techniques have proved useful for detecting a wide range of markers for lipids, polyaromatic hydrocarbons, residual proteins and polysaccharides and newly formed complex condensed polymers. Craig *et al.* (2004) reported the application of catalytic hydrolysis, devised for the recovery of lipids which are inaccessible to both solvent extraction and saponification, which moreover preserves important structural and stereo chemical features in hydrocarbon products.

3.8 Lipid preservation

Biomolecular preservation is dependent on molecular structure, the depositional environment and diagenetic history (Eglinton and Logan, 1991). Compared to other biomolecules, the structure and hydrophobic nature of lipids impart a higher preservation potential in relation to other biomolecules such as DNA, proteins and carbohydrates (Eglinton and Logan, 1991; Evershed, 1993). Lipid preservation is further enhanced by their incorporation into the ceramic matrix (Evershed, 1993), charring (Oudemans and Boon, 1991; Oudemans and Erhardt, 1996), and 'sacrificial decomposition,' that is the preferential decay of other biological organic matter (Eglinton and Logan, 1991). The physico-chemical properties of the burial environment will however influence the rate and extent of degradation, as well as the decay mechanisms (Evershed, 1993; Aillaud, 2001). The local geology influences the pH, soil aeration and water movement, while the local climate determines the burial temperature and soil water content. These factors will in turn influence the level of microbial activity in the burial environment, which will determine the survival potential of the absorbed lipid residues (Moucawi *et al.*, 1981; Eglinton and Logan, 1991; Evershed *et al.*, 1992; Shimoyama *et al.*, 1995; Oudemans and Erhardt, 1996; Aillaud, 2001).

The concentration of lipid residue absorbed within ceramic vessels and their preservation over archaeological timescales depends on several factors, including the intensity and mode of use, the permeability of the vessel, the initial deposition, and alterations to the lipid composition and concentration due to diagenetic processes during burial (Charters *et al.*, 1993; Copley *et al.*, 2005b). Experimental investigations on lipid yields extracted from pottery have shown that the estimated lipid capacity of a potsherd is around 10mg g⁻¹ of lipid, of which only around 100µg g⁻¹ of lipid will survive in archaeological contexts (Evershed, 2008a). Hence, although lipids may not decompose completely (Sherriff *et al.*, 1995:109), they are still susceptible to diagenetic alteration prior to and during burial. In particular, changes occur within the lipid structure, namely hydrogenation of double bonds and aromatization of rings, while functional groups, especially ester groups and double bonds, may also be lost (Eglinton and Logan, 1991; Logan *et al.*, 1991; Aillaud, 2001). These will be discussed in more detail in the following section.

3.9 Lipid degradation

The reactions that fats and oils undergo during food processing and storage are less varied than those involving carbohydrates and proteins, since there are fewer reactive sites on lipid molecules. Ester groups between fatty acids and glycerol or various alcohols hydrolyse to produce free fatty acids, while oxidation of double bonds present on the hydrocarbon chains of fatty acids occurs frequently and is an important reaction (Davídek *et al.*, 1990). Sterols are relatively more resistant to degradation than fatty acids, but are subject to oxidation and reduction (Evershed, 1993). External conditions also play a major role in lipid degradation, as discussed in Section 3.8 above.

By 1962, Lucas and Harris had already drawn attention to the difficulties involved in identifying the original source of extracted residues, pointing out that the surviving fats, oils and waxes were not necessarily a representative proportion of the original source (Lucas and Harris, 1962). Since degradation processes alter the lipid profile, the relationship between the degraded and original fatty material becomes obscured, thus requiring experimentation to study the decay patterns and improve the identification of the source material. However, although researchers had been aware of lipid degradation since the 1960s, few studies were targeted at investigating degradation processes until the mid-1990s, when the number of simulation experiments being carried out gradually increased. Published articles dutifully mentioned the need to take lipid degradation into account during data interpretation, and cautioned against more definite assignments of data to specific plant and animal fats (Gerhardt *et al.*, 1990; Heron *et al.*, 1991b; Evershed *et al.*, 1992;

Shimoyama *et al.*, 1995). Evershed *et al.* (1992) point out that few earlier investigations were able to confidently identify food constituents due to a lack of analytical techniques or oversimplification of the analytical approach, particularly in light of pre- and post-depositional changes.

3.9.1 Fatty acid ratios

Early work focused on the transformation of fatty material, particularly the formation of adipocere. Den Dooren de Jong (1961) carried out a series of experiments on olive oil, and observed its change to adipocere, a substance which was commonly known to form through microbial action on human fat. Morgan *et al.* (1973) showed that adipocere also resulted from the decay of mutton fat and butter, and confirmed that the composition of fatty material alters during burial due to microbial action. Preliminary results for the decomposition of mutton fat showed a loss of unsaturated fatty acids especially oleic acid ($C_{18:1}$), and its replacement by $C_{16:0}$ (Morgan *et al.*, 1973). This conversion was also observed by Den Dooren de Jong (1961), who suggested that replacement occurred after the hydrolysis of acylglycerols, and could be the result of β -oxidation and reduction. However Dudd *et al.* (1998) later proposed that microbial action was responsible for this conversion since myristic acid ($C_{14:0}$), a known bacterial component, was found to accumulate during degradation experiments, and the halting of β -oxidation after one cycle was unlikely to lead to this accumulation.

Patrick *et al.* (1985) attempted to identify the original source of absorbed residues from archaeological ceramics by comparing their $C_{16:0}$ to $C_{18:0}$ fatty acid ratio to that obtained from reference material. This ratio was eventually found to be unreliable, since it often incorporates the degradation products of other acids (Den Dooren De Jong, 1961; Morgan *et al.*, 1973, 1984; Heron and Evershed, 1993). Depletion of labile unsaturated acids with the concomitant increase in saturated fatty acids was observed to be a general trend in decomposition (Malainey *et al.*, 1999a). Moreover, following hydrolysis, fatty acids were found to be more susceptible to oxidation and other diagenetic alteration, hence making fatty acid ratios unreliable in determining food type (Reber and Evershed, 2004b). To further test their hypothesis, Patrick *et al.* (1985) carried out a simulation experiment on the degradation of Cape fur seal tissue. Results showed that the oleic ($C_{18:1\omega9}$) to vaccenic ($C_{18:1\omega7}$) acid ratio did not alter considerably with age, and this was used to partially confirm their conclusions. Subsequently, Malainey *et al.* (1999a, 1999b, 1999c) carried out principal component analysis on fatty acid compositions obtained from fresh and degraded modern reference plant oils and animal fats, as well as archaeological residues, and

confirmed the reliability of using $C_{18:0}$ to combined $C_{18:1}$ isomers ($C_{18:1\omega9}$ and $C_{18:1\omega11}$) fatty acid ratios to identify the original food source. However, as stated above, unsaturated fatty acids are unlikely to survive over archaeological timescales, thus limiting their use in determining food sources based on fatty acid ratios.

3.9.2 The burial context

By the mid-1990s, simulation experiments became targeted to answer specific research questions. Experiments were conducted using pre-set variables and constants to monitor degradation processes under controlled conditions, hence shedding light on particular decay factors and processes. Evershed *et al.* (1995a) conducted experiments using lamb fat to study the degradation of absorbed lipids during vessel use and after burial. The lipid distribution obtained after thermal degradation was observed to consist mainly of TAGs and differed from the lipid profiles of archaeological samples. Conversely, the lipid profile of buried sherds was found to be consistent with that obtained from archaeological material. This showed that further chemical and microbial decay occurred during burial (Evershed *et al.*, 1995a). Ethnographic studies also showed that ester hydrolysis and the formation of long-chain ketones are indicative of degradation during vessel use (Evershed *et al.*, 1995b; Raven *et al.*, 1997; Dudd *et al.*, 1998).

Degradation processes in varying archaeological contexts were still poorly understood, and led to two simulation experiments carried out by Evershed and Charters (1995) and Reber and Evershed (2004b). Evershed and Charters (1995) observed the degradation of animal fat incubated under aerobic and anaerobic conditions. Different chromatograms were obtained for the two variables, indicating variation in the rate and degree of degradation, and decay in aerobic conditions was observed to proceed quicker (Evershed and Charters, 1995). Reber and Evershed (2004b) investigated the degradation of maize oils in various contexts. After six months, cooked and buried samples retained no identifiable lipid profile, possibly due to the small amount of lipid originally present in maize (Reber and Evershed, 2004b). This study allowed a better understanding of the differential decomposition of TAGs and sterols in maize under different depositional environments. Sherds left exposed to the ground surface were subjected to scavenging, and other samples showed extensive oxidation of fatty acids (Reber and Evershed, 2004b). Varying the depositional environment affected the relative abundance of saturated and unsaturated fatty acids, caused differential preservation of saturated fatty acids with different carbon numbers, and variation in the amounts of total and free fatty acids retained (Reber and Evershed, 2004b). More recently, Evershed *et al.* (2008b) and Gregg *et al.* (2009) observed that

seasonal temperature variations in the warmer climatic regions of the Near East and southeast Europe led to a very poor lipid recovery. Indeed, in Evershed *et al.*'s (2008b) study, only 12% of 2225 sherds analysed contained enough lipid residue for a reliable identification of the source product, as opposed to the much higher lipid yields obtained from sites situated in northern latitudes (*cf.* Copley *et al.*, 2003; Craig *et al.*, 2011). Furthermore, during food preparation, oxidative lipid degradation is expected to occur, but the resulting products, such as diacids and hydroxy acids, are reactive and water soluble, hence easily lost to groundwater leaching (Regert *et al.*, 1998a). To monitor this, Regert *et al.* (1998a) analysed samples obtained from lacustrine and arid deposits. The former confirmed the loss of oxidised degradation products, while the latter retained these products.

3.9.3 Plant lipids

Bimolecular evidence for plant residues is still difficult to identify using residue analysis. When cooked, only small quantities of plant lipids are transferred into ceramics vessels (Evershed *et al.*, 1995a), decreasing their survival potential over archaeological timescales. Certain classes of plant lipids can be identified (Evershed, 2008b), in particular plants comprising epicuticular leaf wax (Evershed *et al.*, 1991; Charters *et al.*, 1997). However, evidence for starchy grains such as barley, wheat, maize and rice, which have a lower lipid content, is often masked by oilier/fattier foodstuffs, making their identification extremely difficult (Reber and Evershed, 2004b). Reliable immunological detection of plant proteins are similarly problematic (Leach, 1998). Steele *et al.* (2010) used GC-c-IRMS to determine the distribution of modern plant oils, including almond, argan, olive, moringa, sesame and walnut oil. When the $\Delta^{13}\text{C}$ vs. $\delta^{13}\text{C}_{16:0}$ plot was used, the isotopic signatures produced by these plant oils could not be distinguished from porcine and ruminant adipose fats; however, they were found to form a tight cluster (except the moringa oil, which plotted separately) when the standard $\delta^{13}\text{C}_{18:0}$ vs. $\delta^{13}\text{C}_{16:0}$ plot was used. Unfortunately in the latter, plant oils plotted within the area which is generally considered to represent mixtures of ruminant and porcine adipose fats, thus introducing complexity when interpreting archaeological data (Steele *et al.*, 2010). Further work is required in this area, especially when considering that biomolecular evidence for starchy cereal grains in pots and other artefacts would be very useful for studying the emergence of agriculture and pre-agricultural plant exploitation.

3.9.4 Microbial input and the decay of dairy products

Experiments were also carried out to determine the effects of degradation on different fatty acyl lipid species, as suggested in Evershed and Charters (1995), and to investigate the microbial contribution to degradation processes. This was investigated by Dudd *et al.* (1998), using olive oil and milk. Bacterial action alters the original distribution of lipid components, and is identified through the detection of branched-chain fatty acids of the *iso*- and *anteiso*- series (Dudd *et al.*, 1998). Results showed that the relative proportion of fatty acids from olive oil degradation was not significantly altered. Microbial lipids, namely ergosterol and branched fatty acids C_{15:0} and C_{17:0} were incorporated during degradation, and the lipid content was observed to decrease as incubation time increased, due to lipids being consumed by microorganisms (Dudd *et al.*, 1998). Initial hydrolysis was attributed to microbial and abiological action, but identifying the input of each process was difficult (Dudd *et al.* 1998), although Hita *et al.* (1996) showed that during the first week of incubation, lipases caused the hydrolysis of tristearin. Microbial activity in milk was detected by the presence of ergosterol, which unlike branched fatty acids, does not occur naturally in milk (Dudd *et al.*, 1998). Dudd *et al.* (1998) concluded that similar degradative patterns occur for acyl lipids of varying origin, and that microbial contribution to the overall lipid profile was minimal.

Similar results on milk degradation were obtained by Aillaud (2001), who observed the decay of butter, milk and heat-treated milk buried in garden soil. Aillaud (2001) further showed that decay was largely caused by soil microorganisms, since the results obtained for fresh and heat-treated milk had similar rates of decay. This was confirmed by Copley *et al.* (2005b), who further showed that dairy residues were more likely to originate from butter rather than milk. Apart from having a lower concentration of fat, milk comprises a higher carbohydrate and protein content than butter, which therefore encourages the proliferation of soil microbes in milk rather than butter, increasing the survival potential of the latter in archaeological deposits (Copley *et al.*, 2005b). Subsequently, the reduced steric effects at ester linkages in triacylglycerols cause preferential hydrolysis of short-chain fatty acids, which are water-soluble, hence easily leached (Dudd and Evershed, 1998). After 25 days, the characteristic short-chain free fatty acid fraction in milk fats was largely undetectable (Dudd *et al.*, 1998), altering its lipid profile so that it resembled adipose fat (Dudd and Evershed, 1998). This made the detection and distinction of dairy fats from adipose fats difficult prior to GC-c-IRMS.

3.9.5 Visible residues

In visible residues, lipids are susceptible to oxidation, hydrolysis, pyrolysis and bacterial degradation due to exposure to high temperatures during vessel use, and close contact with the burial environment (Campbell *et al.*, 2004). Organic preservation of charred material is therefore subject to differential effects of exposure to temperature, the degree of charring a sample has undergone and conditions specific to individual burial locations (Campbell *et al.*, 2004). It is however, still possible to evaluate and quantify the effects brought about by these factors by looking at the samples in terms of their relative sample size, the ratio of carbon to clay content and the presence of organic compounds (Campbell *et al.*, 2004). Detailed comparisons of lipid preservation in surface deposits and residues absorbed into the ceramic matrix of the same vessel are few in number. In one study (Mukherjee *et al.*, 2008), lipids were less preserved in the surface deposits, which provides support to the long-held view that lipids are better preserved as absorbed residues (Rottländer, 1990). Furthermore, the $\delta^{13}\text{C}$ values and the abundances of different lipid components varied between the surface and absorbed extracts, perhaps representing different accumulations of lipid in the sequence of use (Mukherjee *et al.*, 2008). It is however, still unclear whether lipid residues are better preserved in charred or absorbed residues (see Rottländer, 1990; Deal *et al.*, 1991; Oudemans and Evershed, 1991; Mukherjee *et al.*, 2008).

3.10 Contamination issues in ORA

The estimated fraction of absorbed lipid residues which survive in an archaeological assemblage has been calculated, using thousands of sherds analysed by ORA, to be around 1%, and the average amount of lipid extracted from a sherd was found to be less than $100\mu\text{g g}^{-1}$ (Evershed, 2008a). Contamination in ORA is therefore a serious issue, since the already small amount of residue recovered can become masked by contaminant peaks, which may co-elute with GC and GC-MS peaks of interest hindering identification (Oudemans and Boon, 1991; Evershed *et al.*, 1995a). Due to underlying problems with contamination, Evershed (2008a) suggested that the minimum amount of total lipid extract (TLE) recovered, which can be used for a reliable identification, should not be less than $5\mu\text{g}$ of lipid per gram of sherd.

Since many of the compounds identified through ORA are common in nature, handling the ceramic samples should be avoided to prevent contamination with lipids shed from the hands (Evershed *et al.*, 1992). Another modern source of contaminants are phthalate plasticizers, which are very easily introduced through contact with plastic (Pollard *et al.*, 2007). Post-firing processes

can also be a possible source of contamination in ORA, since foodstuffs (e.g. milk) used as sealants will hinder the identification of the actual vessel content (Heron and Evershed, 1993). Furthermore, experiments conducted by Romanus *et al.* (2009) showed that the impermeability of sealants may not be universal to all commodities. Their results showed that while pitch lined amphorae were impermeable to olive oil, wine polyphenols easily infiltrated into the ceramic fabric. Moreover, the simultaneous exposure of pitched vessels to wine and olive oil showed that while the oil appeared to retard the infiltration of wine, the presence of the latter was found to render the pitch layer permeable to oil infiltration, which is significant when considering multiple uses of amphorae (Romanus *et al.*, 2009). Re-utilization of vessels (Charters *et al.*, 1995; Evershed *et al.*, 1995a) is difficult to interpret using ORA. Although GC-c-IRMS has proved very efficient in identifying different food sources, interpreting mixing and sequential cooking episodes is still problematic (e.g. Steele *et al.*, 2010: as discussed above).

Contamination from the external environment is another possible concern (*cf.* Condamin *et al.*, 1976). Although migration of lipids during burial was found to be negligible (Rottländer, 1990; Heron *et al.*, 1991a), Forster (2006) highlighted the possibility of ceramic vessels becoming contaminated during burial through contact with soft animal tissue buried in close proximity. To counteract this possibility, it is good practise to analyse the exterior as well as the interior surfaces of ceramic sherds, to detect any possible contamination (Stern *et al.*, 2000).

3.11 Conclusion

How pottery vessels were actually used in the past presents a surprising gap in archaeological knowledge. Nevertheless, since Renfrew's publication in the late 1970s recent advances in lipid biomarker detection and single-compound carbon isotope measurements have enabled greater opportunities to tackle questions of resource introduction and use at specific times and places in the past. The analysis of lipid residues is not simply a record of technical advances in analytical instrumentation. In general, the sample size in terms of the numbers of vessels investigated has increased. Similarly, sampling strategy, relating to the identification of explicit research designs and their integration with wider archaeological enquiries, is ensuring that the results of such work are mainstreamed and considered in relation to other evidential sources. Nevertheless, documenting vessel usage patterns is not straightforward, for example, some foods without a prominent lipid fraction may be under-represented in the surviving residue. Where mixtures of foods can be determined (for example, in cases where both animal and plant sterols are present albeit in very low abundance), it is unclear as to whether these represent the combination of

foods in a single cooking event (such as a stew) or the sequential use of the vessel for different purposes. Degradation and contamination add to the complexity of the overall signal. Nevertheless the potential of lipid analysis to characterise 'organic residues' and provide assessments of food and non-food products in pottery vessels has emerged as a powerful tool and the recent studies reviewed here testify to a robust and dynamic field of enquiry.

This chapter has outlined the development of ORA, both in terms of sample preparation and the instrumentation used, and has justified the use of ORA as the main analytical technique to identify the contents of Early Neolithic Impressed/Cardial Wares (described in Chapters 7 and 8). Sections 1.5 and 1.6 provided the rationale for interpreting lipid residues extracted from archaeological ceramics using GC, GC-MS and GC-c-IRMS. The discussion on lipid degradation in Section 3.9 gave the necessary background to report the results obtained from the plant degradation experiment (Chapter 5), and to interpret the archaeological residues extracted from Impressed/Cardial Wares (Chapter 7). Finally, Section 1.6, which introduced the application and instrument mechanism of GC-c-IRMS, was required to describe the results obtained from the controlled feeding experiment in Chapter 4.

Chapter 4

Investigating shifts in hexadecanoic and octadecanoic $\delta^{13}\text{C}$ isotopic values in the Mediterranean region

4.1 Introduction

Chapter 3 discussed in some detail the principles and challenges of using lipid analysis to identify archaeological fats and oils, particularly in the interpretation of degraded archaeological lipid profiles. Diagenetic processes lead to the loss of distinctive characteristics in the lipid composition of different organic products, such that the degraded profiles obtained often comprise mainly *n*-alkanoic acids, which are not particularly diagnostic (Evershed, 2008b). Thus, although biomarker analysis is fundamental for the identification of archaeological residues (especially marine oils), it is not sufficient to differentiate between ruminant and non-ruminant fats, or ruminant adipose fats and dairy products; although in the case of the latter, the two fats can be distinguished by identifying the fatty acid distribution of residual triacylglycerols (Mirabaud *et al.*, 2007). Thus the significance of Gas Chromatography-combustion-Isotope Ratio Mass Spectrometry (GC-c-IRMS) lies in its capacity to separate the different animal and marine products based on the biosynthetic origin of their *n*-hexadecanoic ($\text{C}_{16:0}$; palmitic) and *n*-octadecanoic ($\text{C}_{18:0}$; stearic) fatty acids, which as highlighted in Chapter 3, is obtained by measuring their individual $\delta^{13}\text{C}$ isotopic values.

For the most part, GC-c-IRMS analysis has been conducted on modern fats (used as reference datasets) and archaeological residues obtained from the United Kingdom (Evershed *et al.*, 1994, 1997b; Dudd and Evershed, 1998; Dudd, 1999; Aillaud, 2001; Copley *et al.*, 2003, 2005a, 2005b, 2005c, 2005d; Craig *et al.*, 2005b; Mukherjee *et al.*, 2007, 2008; Evershed, 2008a, 2008b). GC-c-IRMS has gradually been applied to archaeological material from other locations. In northern Europe, for example, compound specific isotopic analysis has been conducted by Craig *et al.* (2007, 2011) to investigate the use of aquatic resources in Denmark, while the identification of dairy products has been a major research topic in central Europe (Craig *et al.*, 2005a; Spangenberg *et al.*, 2006), the Near East (Evershed *et al.*, 2008b), as well as the Far East (Outram, 2009). Compound specific isotope analysis has more recently been applied to archaeological material

originating from the Near East (Gregg *et al.*, 2009), while the ceramics included in this research are all Mediterranean in origin.

GC-c-IRMS isotopic values from modern British animal species have generally been used as reference data points for comparison against isotopic measurements obtained from archaeological residues, regardless of where the archaeological material originates. However, the validity of using this dataset as a universal reference collection has been recently challenged, primarily by Gregg *et al.* (2009). Gregg *et al.* (2009) observed a shift in the $\delta^{13}\text{C}$ of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids in modern ruminant adipose and dairy samples obtained from the Near East, which plotted within the porcine and ruminant adipose offsets respectively (Figure 4.1). In view of these results, Gregg *et al.* (2009) urged researchers to compare ancient residues with modern reference fats obtained from regions having a similar climate and vegetation to the catchment area of the archaeological site under study.

For this research, it was therefore important to compare GC-c-IRMS values obtained for $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids of Mediterranean animal fats and marine oils with published datasets acquired from northern Europe and the Near East, to assess any variation in isotopic values from different geographical contexts. The major difficulty in building a Mediterranean reference collection was sourcing herds of animals whose diet comprised solely C_3 vegetation, which would have been the diet of herds in Neolithic Europe and the Mediterranean (Hunt *et al.*, 2008). As will be discussed in Section 4.3.6, the dietary intake of the animals is reflected in the $\delta^{13}\text{C}$ isotopic values of their lipid composition; hence it was imperative that their modern correlates were animals eating a purely C_3 diet. Nowadays, the arid climatic conditions of several Mediterranean countries, including Apulia (where this study is mainly based) and Malta, do not support sufficient rainfall for grasslands to flourish and for herds to obtain all their dietary requirements through grazing. Farmers in these regions are therefore required to supplement at least 50% of the dietary intake of their herds with concentrates, which for the most part, include C_4 plant material, in particular maize. It was therefore necessary to conduct a controlled feeding experiment which ensured that all animals and dietary components were Mediterranean in origin, and that the latter comprised solely C_3 plant material.

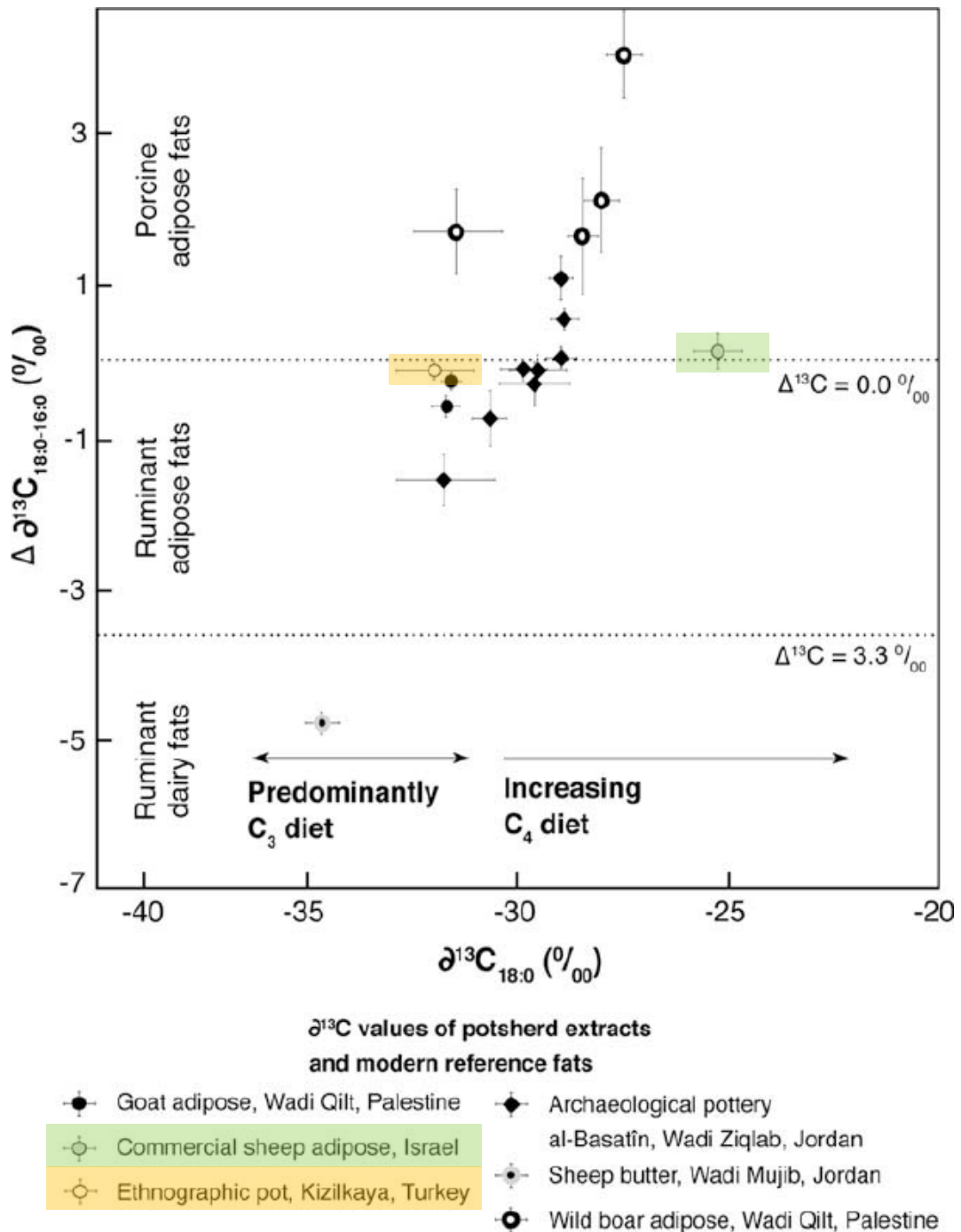


Figure 4.1: Gregg *et al.*'s (2009) plot showing the $\Delta^{13}\text{C}$ ($\delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) against the $\delta^{13}\text{C}_{18:0}$ of modern reference fats obtained from the Near East and the ethnographic milk pot from central Turkey. The ruminant animals were fed on an exclusively C_3 diet, while the pigs were fed on a combination of C_3 and C_4 foods. Plotting the $\Delta^{13}\text{C}$ values shows the relative contribution of C_3 and C_4 plants in the animals' diet. Note how the sheep adipose sample plots within the porcine $\Delta^{13}\text{C}$ values, and how the ethnographic milk pot residue plots within the ruminant adipose offset.

The first part of this chapter will discuss the criteria established for the controlled feeding experiment and the rationale behind the methodology adopted. The laboratory preparation and instrumental parameters are recorded in Section 4.4. The results obtained are presented in

Section 4.6, which is followed by a detailed analysis of the data acquired from the controlled feeding experiment carried out in relation to published GC-c-IRMS data on modern animal and marine oils. Archaeological implications and possibilities for further research conclude the chapter.

4.2 Controlled feeding experiment: methodology

The main aim of the experiment was to establish whether the $\delta^{13}\text{C}$ values obtained from the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids in ruminant and non-ruminant adipose, dairy products and marine oils from Mediterranean species were consistent with northern European $\delta^{13}\text{C}$ values. The measurements obtained would also provide a reference dataset of Mediterranean $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values, against which to compare similar isotopic data obtained from archaeological residues. The experiment was therefore required to re-create, as closely as possible, the conditions experienced by domesticated animals living during the Neolithic period in the Mediterranean region. As discussed above, it was therefore necessary to impose an exclusively C_3 diet for all of the animals selected for the controlled feeding experiment.

The experiment was carried out at the Government Farm (Għammieri), in Malta. Since cows (bovines), goats (caprines), sheep (ovines) and pigs are the dominant domestic animal species found in most Early Neolithic archaeological sites, two of each species were selected for the study. Choosing pairs of animals also provided an opportunity to replicate the results obtained and test for consistency. Another factor that had to be considered in the experimental set up was the latitudinal variation in $\delta^{13}\text{C}$ values that has been documented by previous studies (e.g. van Klinken *et al.*, 1994). Their research showed a difference of almost 3‰ in $\delta^{13}\text{C}$ values between tree wood charcoal in northern Europe and the Mediterranean, and established a significant trend in plant samples from north-western to southern Europe, which they attributed to climatic differences influencing the $^{13}\text{C}/^{12}\text{C}$ ratios during carbon fixation. This variation is mostly due to the process of photosynthesis in plants, which is then transmitted through the food chain with minimal overall modification (Schwarcz and Schoeninger, 1991) (see Section 4.7.1 for further discussion). To ensure that the $\delta^{13}\text{C}$ values reflect a Mediterranean origin, the livestock selected for the study were raised in Malta, while the raw plant materials comprised in the C_3 diet were acquired from companies that supply Mediterranean grown crops (Table 4.1).

Common Name	Latin Name	Origin
Alfalfa	<i>Medicago sativa</i>	West Catalonia, Spain
Barley	<i>Hordeum vulgare</i>	Ragusa, Sicily
Beans	<i>Vicia faba minor</i>	Ragusa, Sicily
Sunflower	<i>Helianthus annuus</i>	Languedoc Roussilo, France
Wheat	<i>Triticum aestivum</i>	Provence, France

Table 4.1: List of the C₃ plant material and respective country of origin included in the concentrates prepared for the controlled feeding experiment. The full diet comprised minerals and a premix which ensured that the animals were being supplied with the required nutrients (Appendix A).

Table 4.2 shows a list of the animals selected for the experiment. The animals were isolated from their herd for the duration of the experiment and housed in separate pens. To re-create the Neolithic scenario as closely as possible, at least 60% of the ruminant animals' daily food intake was obtained by grazing on nearby alfalfa fields, while pigs required a 100% intake from concentrates. The experiment was conducted during the spring, while the occasional spells of rain ensured the availability of fresh alfalfa. The remainder of the ruminant animals' dietary requirements were supplied through concentrates, which required the formulation of three separate dietary plans for cows, sheep/goats and pigs. The diets were compiled at Andrews Feeds (Malta), who specialise in animal feed. A detailed record of the composition of each diet and complementary nutrient analysis is reproduced in Appendix A. The proportion of foraging and concentrate in each animal's diet is reported in Table 4.2.

Species / TAG	Breed	Age	History	Diet	
				Forage	Concentrate
Cow [0262814]	Red, Holstein Freizen, Holland	4 yrs	Lactating; parturition in January 2010	60%	40%
Cow [0212823]	Black, Holstein Freizen, Holland	5 yrs	Lactating; parturition in December 2009.	60%	40%
Sheep [0340957]	Maltese	2 yrs	Lactating; parturition in January 2010	60%	40%
Sheep [0340972]	Maltese	2 yrs	Lactating; parturition in February 2010	60%	40%
Goat [0267402]	Maltese	3 yrs	Lactating	75%	25%
Goat [0266923]	Maltese	3 yrs	Lactating	75%	25%
Pig [A98/9]	Large White (Sire Line)	7 mts	-	0%	100%
Pig [084/3]	F1 cross (Large White & Landrace)	5 mts	-	0%	100%

Table 4.2: List of the livestock selected for the controlled feeding experiment. All the animals were female adults, raised in Malta.

This special diet was introduced in 25% increments one week before the beginning of the experiment. This was necessary to avoid any digestive disturbances which could have potentially harmed the animals.

4.2.1 Sampling

The controlled feeding experiment was carried out over a five week period during which blood and milk samples were obtained from each animal on a weekly basis (Section 4.3 explains the rationale behind the choice of samples and the duration of the experiment). The first samples were taken prior to the introduction of the new diet. Farmers were responsible for collecting milk samples (approximately 30mL) from the bovines, ovines and caprines in sterilised, glass scintillation vials, whereas blood samples were taken from all eight animals by veterinary surgeons using vacutainers with EDTA anticoagulant. Samples were stored at -16°C pending analysis.

Blood was collected from the jugular vein in ovines and caprines and the ventral median coccygeal vein in the bovines. Extracting blood from pigs was slightly more taxing, mainly due to the excessive thickness of their skin which makes it very difficult to locate blood vessels and extract blood without harming the pigs. It was therefore decided to sample blood from the auricular vein. To do this, it was necessary to inject each pig with a mild sedative (Stresnil) prior to the blood sampling, to calm the animals and allow the vet to complete the sampling. Between 1mL and 8mL of blood were generally extracted.

Around 200g of each plant material listed in Table 4.1 were collected for bulk stable carbon isotope measurements. The samples were prepared as described in Section 4.4.7. The measurements acquired through this analysis were compared with the results obtained from a similar study carried out by Stott *et al.* (1997) (Section 4.5).

For comparative purposes, ruminant adipose samples from animals whose diet included a concentrate containing maize (hence a C₄ dietary contribution), were collected from the abattoir in Malta. Milk samples were obtained from a herd of sheep and goats whose bulk dietary intake is from foraging on the cliffs at Dingli, in Malta, but whose diet was also at times supplemented by small amounts of concentrate containing maize. Samples of ricotta cheese and milk were obtained from markets in Italy and Germany respectively, but the animals' diet in both cases is unknown (Table 4.3). Since wild boar and deer remains were found in the faunal records of the archaeological sites studied in this research, samples of wild boar adipose from Italy and Germany as well as deer adipose from Germany were also analysed (Table 4.3). Unfortunately, it was not possible to sample Mediterranean deer adipose.

Sample	Origin	Diet	N
Cow adipose	Abattoir, Malta	Concentrates contained maize (C4)	1
Sheep adipose	Abattoir, Malta	Concentrates contained maize (C4)	1
Goat adipose	Abattoir, Malta	Concentrates contained maize (C4)	1
Sheep milk	Dingli, Malta	Dingli herd fed primarily on forage but the diet was supplemented with smaller amounts of concentrate	1
Goat milk	Dingli, Malta	containing maize.	1
Sheep ricotta	Trani, Italy	Unknown	1
Sheep ricotta	Matera, Italy	Unknown	1
Cow milk	Germany	Unknown	1
Roe deer	Germany	Unknown	1
Red deer	Germany	Unknown	1
Wild boar	Germany	Unknown	1
Wild boar	Italy	Unknown	1

Table 4.3: List of the additional samples of Mediterranean and northern European origin analysed. Samples originating from the Mediterranean were obtained from animals which were fed concentrates containing maize (C₄). The adipose/flesh tissue was sampled from the shoulder area of each animal. [N: number of samples]

Marine fish were bought from fish markets in Malta and Apulia, Italy. They were included in this study since fish bones were documented in some of the archaeological sites included in this research. Table 4.4 lists the different species chosen for GC-c-IRMS analysis. To ensure a Mediterranean marine $\delta^{13}\text{C}$ isotopic value, the fish selected are known to occur commonly in the Mediterranean Sea, and to inhabit relatively shallow waters close to the coast (in particular the *Diplodus* species). They therefore tend to remain within Mediterranean waters (Fa, 2010, pers. comm.).

Common Name	Latin Name	Origin	N
White seabream	<i>Diplodus sargus sargus</i>	Malta	1
Bluespotted seabream	<i>Pagrus caeruleostictus</i>	Malta	1
Large-scaled gurnard	<i>Lepidotrigla cavillone</i>	Malta	1
Red mullet	<i>Mullus barbatus barbatus</i>	Between Malta and Libya	1
Anchovies	<i>Engraulis encrasicolus</i>	Trani, Italy	1

Table 4.4: List of fish obtained from local fish markets in Malta and Apulia denoting the territorial waters where each fish was caught.

4.3 Experimental rationale

Due to the nature of the experiment, namely the time constraints imposed by the dietary upkeep for the loaned animals and to avoid perturbing the animals over a prolonged period, the experiment was carried out for 35 days. During this time-frame, the carbon in the adipose tissue of the selected animals would not undergo a complete turnover to reflect the C₃ diet (a study by Tieszen *et al.* (1983) showed that 208 days are required for 99.99% carbon turnover in the adipose tissue of gerbils); hence the decision to analyse the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ in blood lipids. This section explores the rationale behind the sampling strategy and the timeframe selected for

the duration of the controlled feeding experiment. Lipid digestion and fatty acid turnover rates in both ruminants and non-ruminants are described, which demonstrate that the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ in the blood samples analysed do indeed reflect the C_3 diet compiled for this experiment.

4.3.1 Lipid digestion in the stomach and small intestine

In monogastric animals, such as pigs, digestion and absorption begin when the dietary fats reach the small intestine. However in ruminants (e.g. cows, sheep and goats), the pre-gastric microbial fermentation of cellulose and other plant polymers, which are not normally hydrolysed by digestive enzymes, have a significant effect on the chemical and physical nature of the lipids which subsequently enter the small intestine (Noble, 1981:57). Considerable chemical degradative and synthetic changes occur in the rumen due to the microbiological population present, in particular, lipolysis of the dietary lipids and the hydrogenation of their unsaturated fatty acid constituents (Katz and Keeney, 1966). The final outcome is the production of unesterified fatty acids, in particular stearic acid, which generally makes up a very minor proportion of the dietary lipid intake that is conversely rich in linoleic ($\text{C}_{18:2}$) and linolenic ($\text{C}_{18:3}$) acids, but which become the predominant lipid fraction in the digesta (Masson and Phillipson, 1951; Garton, 1960; Noble, 1981:58-61). Bacteria and protozoa in the rumen can also synthesise fatty acids with 15 to 18 carbon atoms *de novo*, which comprise a considerable contribution of microbial lipids to the digesta of the host animal (Noble, 1981:61).

In ruminant animals, studies have shown that by the time the digesta leaves the abomasum and enters the small intestine, the concentrations of the short-chain fatty acids are extremely low (Huber and Moore, 1964). In fact, about 70% of the short-chain fatty acids produced in the rumen (mainly acetic, propionic and butyric), are absorbed into the bloodstream through the epithelial cells which line the rumen, during which they undergo extensive metabolism and are transported as acetate, glucose and β -hydroxybutyrate, which can be readily metabolised by most tissues (Noble, 1981:62-63). Evidence also exists for the absorption of long-chain fatty acids into the bloodstream before they reach the small intestine; however the quantitative significance relative to the overall absorption in the gastrointestinal tract is limited (Noble, 1981:65). Analysis of the digesta that passes through the pyloric sphincter into the duodenum has shown that the fatty acid composition of the lipid component is virtually identical to that in the rumen, hence confirming that during their passage through the omasum and abomasum, the fatty acid composition of the lipid components of the digesta remain unaltered (Noble, 1981:68).

In non-ruminants, dietary triglycerides enter the small intestine from the stomach and are mixed with secretions of bile and pancreatic juice in the duodenum. Bile salts allow the emulsification of lipid droplets by lowering the surface tension, thus increasing their surface area; while pancreatic lipase (in the presence of co-lipase), hydrolyses triglycerides to free fatty acids and 2-monoglyceride (Harrison and Leat, 1975). The micelle that form allow the hydrolysed compounds to dissolve in the intestinal contents, as well as other water-insoluble compounds, such as fat-soluble vitamins and cholesterol. These can then be absorbed into the mucosal cells, where fatty acids are re-synthesised to triglycerides before passing into the lymph (Harrison and Leat, 1975). Figure 4.2 shows a simplified schematic representation of the digestion, re-synthesis and absorption of fat in non-ruminants.

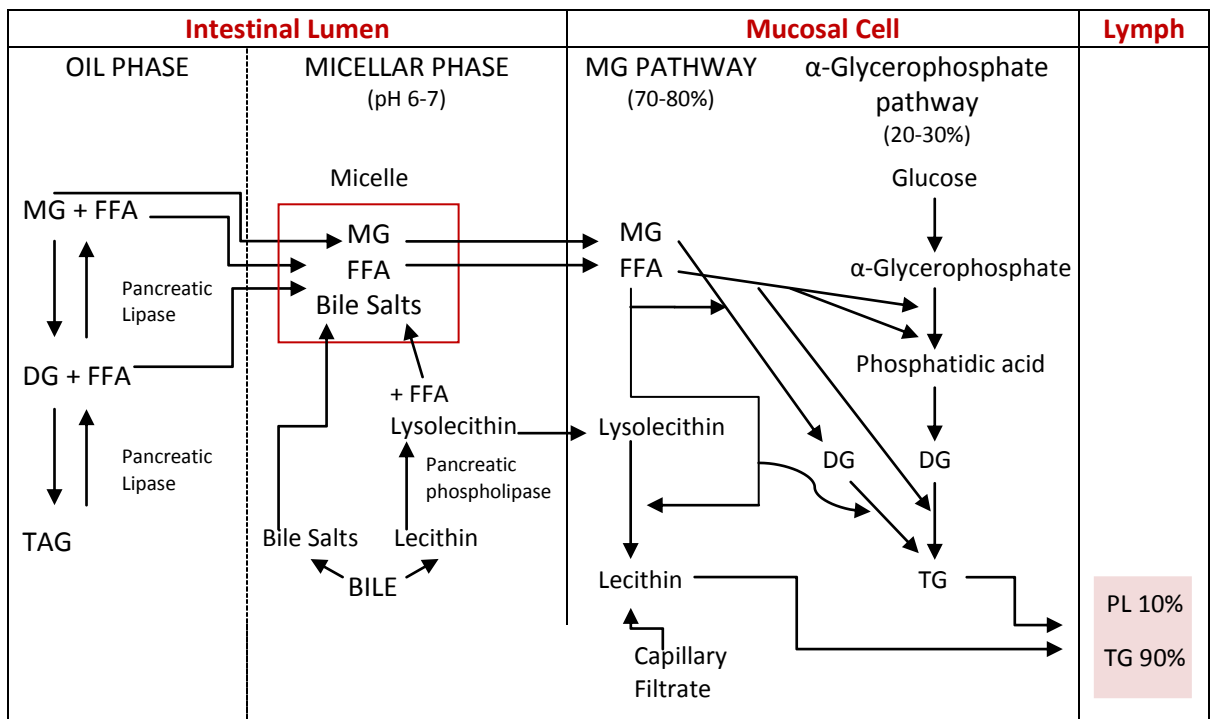


Figure 4.2: Digestion and absorption in non-ruminant animals (After Harrison and Leat, 1975). [FFA: Free fatty acid; MG: Monoglycerides; DG: Diglycerides; TG: Triglycerides; PL: Phospholipids]

In ruminants, the free fatty acid fraction, bound as an insoluble complex with food particles and other minor amounts of unhydrolysed dietary and microbial lipids reach the small intestine, where they are transferred from an insoluble, particulate phase to a soluble micellar phase (Harrison and Leat, 1975) (Figure 4.3). Experimental analysis showed that most of the fatty acid are absorbed before reaching the lower ileum (Noble, 1981:78). Although the pathway of re-synthesis is different in ruminants and non-ruminants, there is no evidence for any morphological difference in the absorption of lipids between the two (Harrison and Leat, 1975).

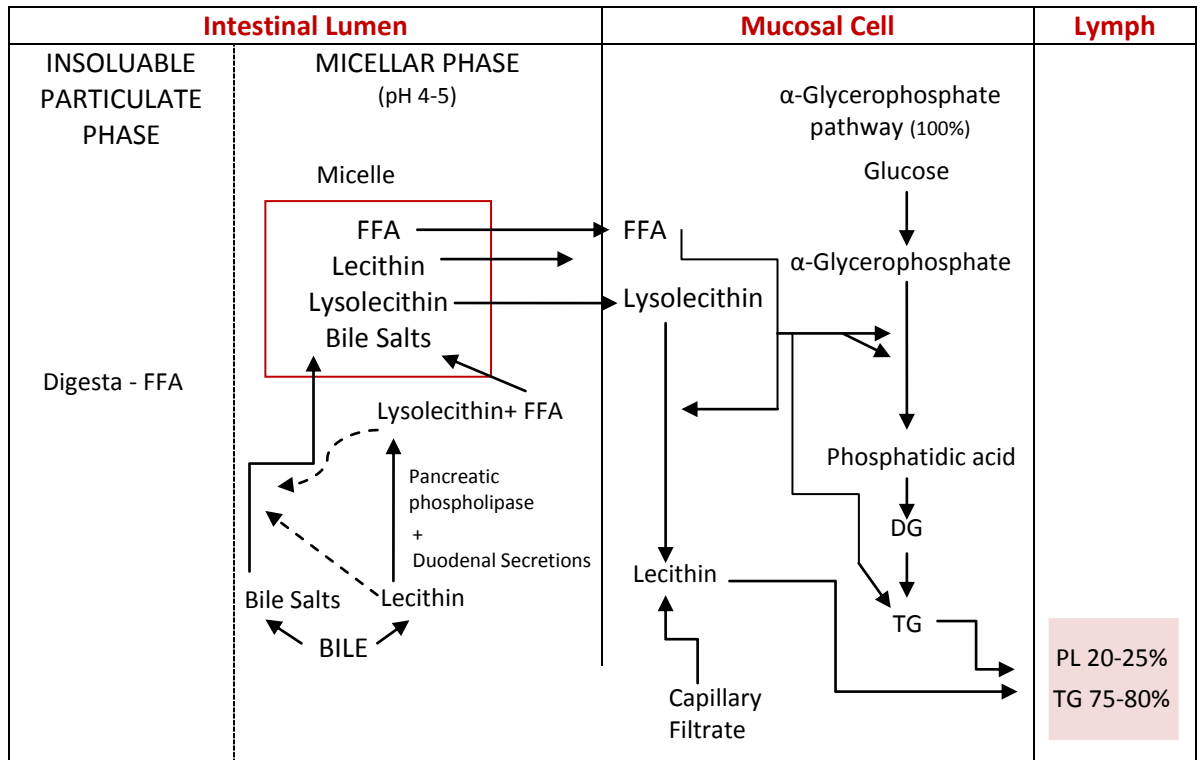


Figure 4.3: Digestion and absorption in ruminant animals (After Harrison and Leat, 1975). [FFA: Free fatty acid; MG: Monoglycerides; DG: Diglycerides; TG: Triglycerides; PL: Phospholipids]

4.3.2 The transport of lipids in the lymph

In both ruminants and non-ruminants, the re-synthesised lipid components in the intestinal mucosal cells are followed by packaging into lipoprotein particles, in particular chylomicrons and very low density lipoproteins (VLDL). Once in the intestinal lymphatic system, the lipid aggregates drain into the thoracic duct before being delivered to the plasma (Noble, 1981:84). Triglycerols account for 85 to 90% of lymph lipids in non-ruminants, with phospholipids, cholesterol and cholesteryl esters making up the remaining 10%; ruminants have a lower triglyceride content (75%) and a larger amount of phospholipids (20 to 25%) (Harrison and Leat, 1975). Fluctuations occur in the flow and lipid concentration in non-ruminant lymph, whereas under normal dietary conditions, lipid absorption and lymphatic transport in ruminants are essentially continuous (Noble, 1981:84). Labelled fatty acid experiments carried out on ruminants have established a rapid turnover of lipid in the lymph. After one hour, fatty acids administered in the duodenum appeared in the lymph, and uptake was complete in six hours (Noble, 1981:85).

4.3.3 Lipids in the blood plasma

The principal components in ruminant blood plasma are cholesteryl esters and phospholipids, together with smaller quantities of triglycerides, unesterified fatty acids, free cholesterol and hydrocarbons (Christie, 1981a:125). The fatty acids which are absorbed by the intestinal mucosa are normally saturated; however, polyunsaturated fatty acids which escape biohydrogenation become preferentially esterified to the plasma cholesteryl esters and phospholipids at a comparatively slow turnover rate when compared to the triglyceride and free fatty acid fractions, which are turned over rapidly and supply fatty acids to other tissues, such as adipose tissue and the mammary gland (Noble, 1981:86). The lipid composition in blood plasma depends on the diet, time since the animal was fed, variations in the rumen microflora and factors such as age, breed, sex, pregnancy or stage of lactation (Christie, 1981a:125). With the animals involved in this research, consistence in the blood plasma composition was controlled for by selecting female adults of identical breeds from each species, and in the case of the ruminant animals, at similar stages of lactation (Table 4.2). Moreover, each pair of animals fed on the same diet, and blood samples from each pair were taken at similar time intervals, thus taking into consideration the buffering effect in the rumen which helps to diminish the diurnal variation in blood lipid (Christie, 1981a:125). Table 4.5 lists the fatty acid composition of interest for analysis, including the main simple lipids and total phospholipids in ruminant plasma. In mature cattle, sheep and goats, triglycerides contain a high proportion of saturated fatty acids (e.g. C_{16:0} and C_{18:0}) and very small amounts of polyunsaturated fatty acids (Christie, 1981a:126). The fatty acid composition of plasma cholesteryl esters and phospholipids in pigs is not appreciably different from that of ruminants, the major difference being the plasma triglycerides, which although similar in fatty acid composition, tend to be more saturated in ruminants (Leat, 1966). Ruminant triglycerides contain more C_{18:0}, which probably reflect the large amounts of this acid produced in the rumen by microbial action (Leat, 1966).

FA	Cattle			Sheep			Goat		
	TG	CE	PL	TG	CE	PL	TG	CE	PL
16:0	32.1	5.5	19.4	27.8	10.3	20.2	20.2	10.9	15.9
18:0	32.2	1.5	27.6	22.2	1.6	25.5	42.0	5.6	29.5
18:1	18.1	5.6	25.3	31.5	27.4	20.3	22.7	36.2	20.1

Table 4.5: The fatty acid composition in plasma lipids presented as wt% of the total of triglycerides (TG), cholesteryl esters (CE) and total phospholipids (PL). The figures reported herein pertain to mature animals. Only fatty acids of interest for the controlled feeding experiment are presented. The values were obtained from Christie (1981a:127).

Free fatty acids in ruminant plasma contribute considerably towards the caloric requirement of the animal, even though this is primarily met through the metabolism of short-chain fatty acids (Noble, 1981:86-87). Free fatty acids can function as readily available energy sources because of their extremely rapid turnover rate, which in ruminant plasma is about two minutes, with similar rates being observed in non-ruminants (Noble, 1981:87). This quick turnover rate therefore ensured that the lipid content in the blood plasma of the animals included in the experiment reflected the new dietary intake at an early stage of the experiment.

4.3.4 The mammary gland

Experimental procedures have shown that in lactating ruminant animals, acetate and β -hydroxybutyrate are taken up by the mammary gland through the blood (Moore and Christie, 1981:246). Acetate and β -hydroxybutyrate are the two most important substrates and under most conditions, the only sources of carbon for *de novo* fatty acid synthesis in the mammary gland (Moore and Christie, 1981:246). However, while $C_{16:0}$ can be biosynthesised in the mammary gland, $C_{18:0}$ is incorporated from dietary fatty acids. Figure 4.4 shows a schematic representation of the routing of dietary $C_{18:0}$ to the mammary gland. Bickerstaffe *et al.* (1972) studied the uptake of individual fatty acids from the plasma lipids by the mammary gland and their subsequent incorporation into milk fat in four goats by examining arterio-venous differences, which appeared significant only in triglycerides. Their experiment demonstrated that during uptake, triglycerides become hydrolysed and the liberated fatty acids equilibrate with the plasma free fatty acid fraction during passage through the mammary gland (Bickerstaffe *et al.*, 1972). This was investigated through an intravenous infusion of a mixture of [9,10- 3 H]-oleate and [1- 14 C]elaidate. The fall in the specific radioactivity of the [9,10- 3 H]-oleate and [1- 14 C]elaidate across the gland was consistent with the intravascular hydrolysis of circulating triglycerides and equilibration of the released fatty acids with the plasma free fatty acids (Bickerstaffe *et al.*, 1972). The proportions of octadecanoate isomers in milk fat were also closely similar to those in arterial and mammary venous blood (Bickerstaffe *et al.*, 1972).

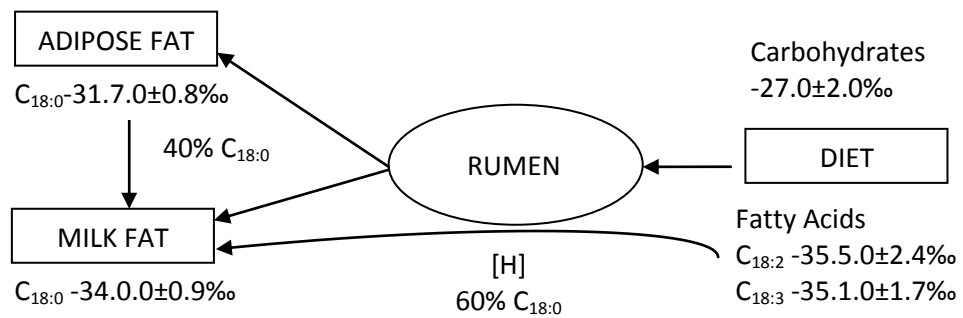


Figure 4.4: Diagram showing the routing of $C_{18:0}$ fatty acid to the adipose tissue and mammary gland in ruminant animals after digestion in the rumen (After Copley *et al.*, 2003).

However, recent studies depict a more complex routing of both $C_{16:0}$ and $C_{18:0}$ to the mammary gland. With regard to the $C_{16:0}$ component, it has been established that while around 50% of the $C_{16:0}$ is biosynthesised in the mammary gland, the remaining 50% of the $C_{16:0}$ present results from direct or indirect incorporation from the diet (Moate *et al.*, 2008). On the other hand, and as previously determined, the $C_{18:0}$ that is taken up by the mammary gland is fully derived from the diet. It is absorbed in triglyceride form through the intestines and travels via the bloodstream to the udder and the adipose tissue in approximately equal proportions (Figure 4.4). However, in early lactation (up to week 10), lactating animals cannot consume enough food to supply them with the energy they need, hence triglycerides stored in the adipose tissue are mobilised and travel to the udder to provide additional $C_{18:0}$. The proportion of milk $C_{18:0}$ derived directly from the diet in a modern cow will therefore vary throughout lactation (Moate, 2011, pers. comm.). With regard to the mobilization of $C_{18:0}$ from the stored adipose, this is of particular concern for the feeding experiment, as it could introduce a C_4 signal to the milk samples. However, this was avoided by only selecting animals that had been lactating for over ten weeks. Interestingly, unless under dietary stress, mobilization of $C_{18:0}$ from adipose tissue may not have been required in ancient times, as milk yields were not very high. For example, if cows suckled only one calf and needed to provide only small amounts of milk for human consumption, extreme body fat mobilization during lactation would have been prevented (Moate, 2011, pers. comm.).

4.3.5 Lipid changes in blood plasma during fasting

Experimental data show that during fasting there is a rapid increase in the concentration of free fatty acids in the plasma of non-pregnant, pregnant and lactating sheep and cattle, which is approximately four-fold after 24 hours and ten-fold after 9 days (Christie, 1981b:202-203). Researchers attributed this to the mobilization of fats by lipolysis of the adipose tissue, which is necessary to supply the animals' energy requirements. The proportions of $C_{16:0}$ and $C_{18:0}$ in adipose tissue lipids were found to decrease over the first 9 days, with a parallel increase in $C_{18:1}$, though

this trend was later found to be reversed. Interestingly, although the amounts of the principal fatty acids in the free fatty acid fraction of the plasma (except C_{18:2}) increased, there was no increase in the uptake of free fatty acids. The rate of production of volatile fatty acids, particularly acetate, was also reduced, and is reflected in the diminished acetate concentrations in the plasma and the rate of *de novo* fatty acid synthesis in the tissues (Christie, 1981b:202-203).

The experiment conducted by Tieszen *et al.* (1983), referred to in the introduction to Section 4.3, showed that the adipose tissue of gerbils had a relatively short half-life of 15.6 days. In Ingle *et al.*'s (1972) publication, variation in the rate of fatty acid synthesis was observed in the different deposits of adipose tissue within the same organism, possibly due to differences in the adipocytes per unit weight of tissue. Tieszen *et al.*'s (1983) study also tested the hypothesis that the higher the metabolic activity in particular tissues in the body (e.g. liver, brain, muscle, hair and fat), the more rapid the carbon turnover, and their investigation showed a significant relationship between the two. From these experimental results it can be speculated that different species will have different rates of carbon turnover in their adipose tissues, and, by extrapolating Tieszen *et al.*'s (1983) correlation between carbon turnover rates and high metabolic activity, the half-life of carbon in the adipose tissue of larger animals will likely be appreciably longer.

In view of the mobilization of fats by lipolysis in the adipose tissue during fasting, and the considerably long period of time (compared to the duration of the experiment) for a complete turnover of carbon in the adipose tissue of the animals included in the experiment, an adequate supply of forage and concentrates was provided to promote continuous feeding during the experiment, and hence avoid any possible contributions of fatty acids through lipolysis brought about by fasting.

4.3.6 Biosynthesis and routing of C_{16:0} and C_{18:0} fatty acids in plants and animals

The carbon isotopic composition of the C_{16:0} and C_{18:0} fatty acids of modern animal and plant fats and oils are distinct from each other, primarily due to the different biosynthetic reactions involved in their synthesis (Evershed *et al.*, 2002b). The reactions which lead to fatty acid biosynthesis in plants are described in Figure 4.5, and are essentially unchanged in all plant species (Post-Beittenmiller *et al.*, 1992; Bowsher *et al.*, 2008:307-333). Stable isotope analysis has shown that in plants, the $\delta^{13}\text{C}$ values vary according to the photosynthetic pathway they follow. Trees, shrubs and grasses growing in temperate areas which follow the C₃ (Calvin-Benson) cycle

generally have $\delta^{13}\text{C}$ values around -27‰, while some grasses native to hot, arid environments which follow the C_4 (Hatch-Slack) pathway have $\delta^{13}\text{C}$ values around -13‰ (Sealy, 2001). The stable isotopic composition of an organism's diet will strongly influence that of its biosynthesised tissue, since the carbon isotope values of the food ingested is not substantially altered by the animals (DeNiro and Epstein, 1978). Contributions from C_3 and C_4 dietary plant materials in the food chain will therefore affect an organism's $\delta^{13}\text{C}$ isotopic values (DeNiro and Epstein, 1978). This highlights the importance of avoiding any contamination from C_4 plant material in the diets compiled for the controlled feeding experiment, so that the $\delta^{13}\text{C}$ values obtained can truly reflect the environment present during the Mediterranean Neolithic, which was devoid of C_4 plants.

The precise relationship between the isotopic composition of the ingested material and a particular tissue or molecular component is quite complex, and responds to variations in the nutritional status, biosynthetic pathways, as well as the type and turnover rate of the tissue analysed (Tieszen *et al.*, 1983; Stott *et al.*, 1997; Evershed *et al.*, 2002b). Research carried out by Tieszen *et al.* (1983) showed that adipose tissue is 3‰ more depleted in ^{13}C than the diet due to the discrimination against ^{13}C , which occurs during lipid synthesis (DeNiro and Epstein, 1977). Figure 4.6 shows a schematic representation of lipid synthesis, compiled after an experimental procedure which identified the biochemical step in the synthesis of lipids that leads to a lower $\delta^{13}\text{C}$ value of the original source of carbon (DeNiro and Epstein, 1977). The depletion was observed in the ^{13}C of the hydroxyethyl groups relative to the pyruvate during the enzymatic decarboxylation of pyruvate to acetyl co-enzyme A (CoA), which occurs due to a kinetic isotope effect associated with the initial stages of the pyruvate dehydrogenase reaction (DeNiro and Epstein, 1977). The ^{13}C depletion of acetyl CoA primarily affects the carbonyl carbon atom, while the methyl group retains the $\delta^{13}\text{C}$ value of the glucose from which it is derived (DeNiro and Epstein, 1977). The depleted ^{13}C acetyl units are then incorporated into lipid components, and result in a ^{13}C depletion of the lipid fraction (DeNiro and Epstein, 1977). DeNiro and Epstein (1977) note that the basic design of lipid synthesis is the same in all organisms.

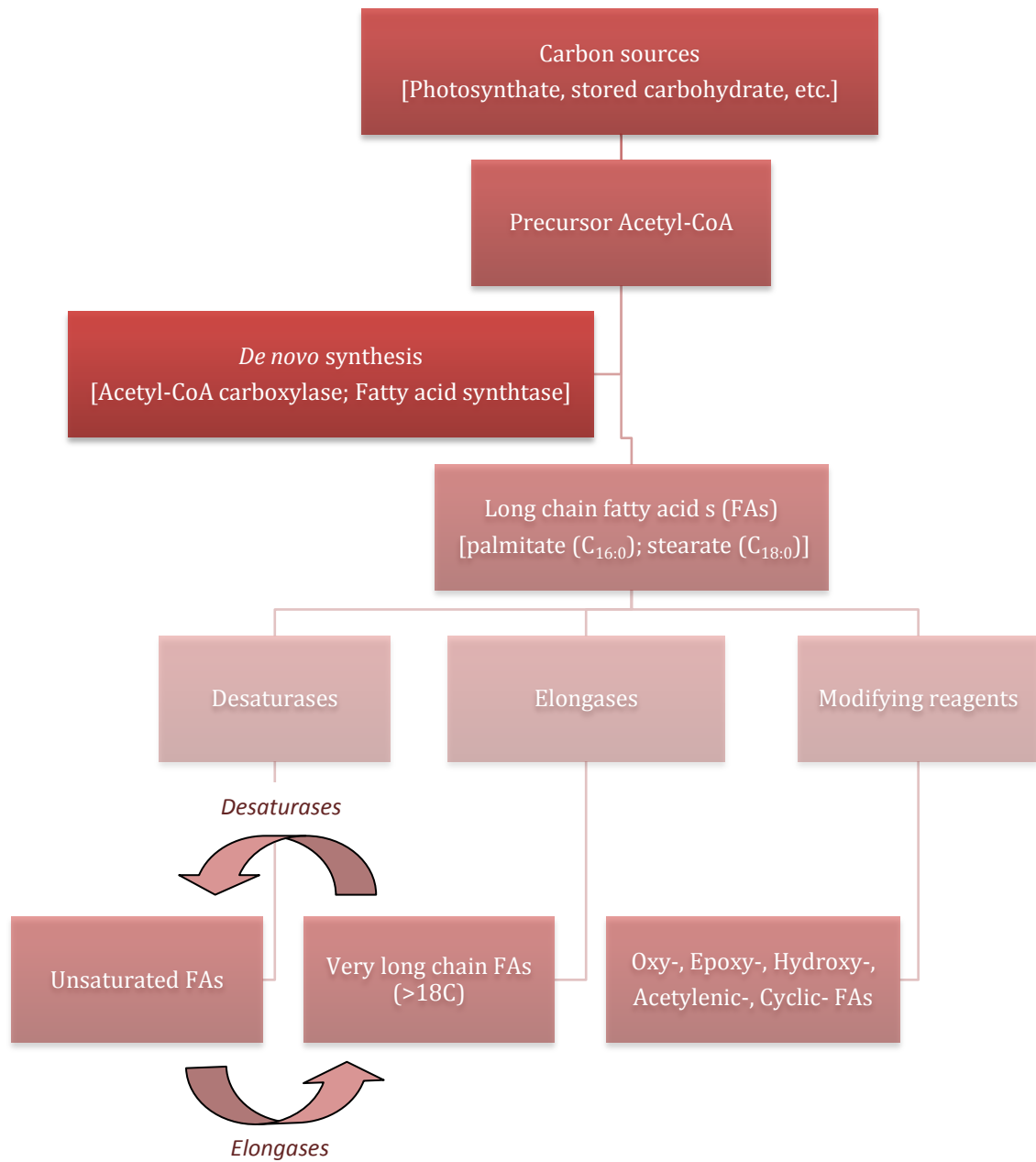


Figure 4.5: Schematic representation of fatty acid synthesis in plants (After Harwood, 2010).

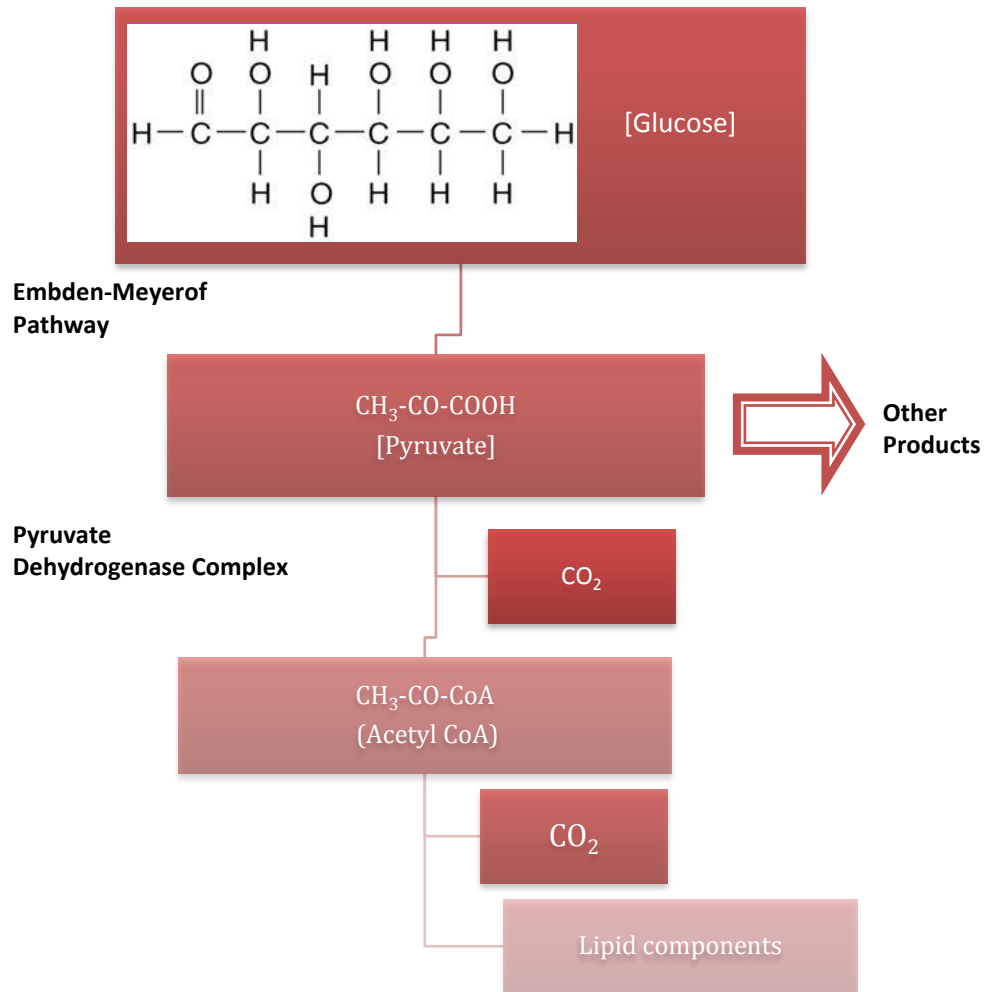


Figure 4.6: Simplified schematic representation of lipid synthesis in microorganisms growing on glucose (After DeNiro and Epstein, 1977).

In organic residue analysis (ORA), different animal species (ruminants, non-ruminants and marine fish) and dairy products can be identified by using GC-c-IRMS analysis to calculate the $\Delta^{13}\text{C}$, that is the difference between the $\delta^{13}\text{C}$ values of their $\text{C}_{18:0}$ and $\text{C}_{16:0}$ fatty acids, which differs due to variation in the biosynthesis and routing of the two fatty acids (Evershed *et al.*, 2002b). Isotopic distribution in animals depends on the relative contribution of *de novo* synthesis and assimilation of precursors from the diet (Dudd, 1999:34), and changes to the $\delta^{13}\text{C}$ values are also known to occur as a result of various metabolic pathways which allow the conversion of carbon from one biochemical fraction to another (Mahler and Cordes, 1971). In ORA, $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids are the principle molecular complexes targeted because they are present in all living organisms, their $\delta^{13}\text{C}$ values are not affected by digenesis over archaeological timescales (Evershed *et al.*, 1999), and they are readily extractable from prehistoric pottery.

Figure 4.7 plots the $\delta^{13}\text{C}$ values of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids from ruminant and non-ruminant adipose fats, dairy products and marine fish, and shows how the different types of fat separate out distinctly based on the $\delta^{13}\text{C}$ measurements. Non-ruminant adipose fats were found to be isotopically heavier than ruminant adipose fats, with the variation between the $\delta^{13}\text{C}$ values of their $\text{C}_{18:0}$ and $\text{C}_{16:0}$ fatty acids being approximately 4‰ and 7‰ respectively (Evershed *et al.*, 2002b). Koch (1994) suggested that this variation could be caused by differences in metabolic factors and dietary preferences between the two species. Unlike most animal species (e.g. humans and birds), where *de novo* fatty acid synthesis occurs primarily in the liver, in non-lactating ruminants and also in pigs, the major site for fatty acid synthesis is the adipose tissue; in pigs, the liver has a negligible capacity to synthesise fatty acids (Vernon, 1981:282). However, while ruminants utilise acetate as their main carbon precursor for *de novo* fatty acid synthesis, (Vernon, 1981:282-283), which they use to synthesise $\text{C}_{16:0}$, $\text{C}_{18:0}$ and $\text{C}_{18:1}$ (oleic) acids (Pothoven *et al.*, 1974; Deeth and Christie, 1979), pigs incorporate carbon from both acetate and glucose (O'Hea and Leveille, 1969). This is important because the $\delta^{13}\text{C}$ of acetate is smaller than the $\delta^{13}\text{C}$ of glucose (Spangenberg *et al.*, 2006), which contributes towards lower $\delta^{13}\text{C}$ values of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids in ruminant adipose compared to non-ruminant adipose $\delta^{13}\text{C}$ measurements.

Figure 4.7 also shows that the $\delta^{13}\text{C}_{18:0}$ measurements of ruminant dairy products are lower compared to ruminant adipose fats, which can be explained by the distinct metabolic pathway of milk fatty acids described in Section 4.3.4. Since the mammary gland cannot synthesise *de novo* fatty acids with more than 16 carbon atoms, the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ in milk are essentially derived from different carbon sources: carbohydrates (for the biosynthesis of the $\text{C}_{16:0}$ in the mammary gland) and fatty acids (routing of $\text{C}_{18:0}$ fatty acids directly from the diet) (Copley *et al.*, 2003). This explains the lower $\delta^{13}\text{C}$ measurements of the $\text{C}_{18:0}$ compared to the $\text{C}_{16:0}$ in dairy products, since the $\delta^{13}\text{C}$ of lipids is isotopically lighter than in carbohydrates in plants (DeNiro and Epstein, 1978). Therefore, since the $\text{C}_{18:0}$ fatty acids in ruminant adipose fats derive mainly from the *de novo* biosynthesis of acetate, as described above, the higher $\delta^{13}\text{C}$ values of the $\text{C}_{18:0}$ fatty acid in adipose allow ruminant adipose fats to be distinctly identified from dairy products. The difference in $\delta^{13}\text{C}$ values between the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids in dairy products is around -3.3‰ (Evershed *et al.*, 2002b)(Figure 4.8).

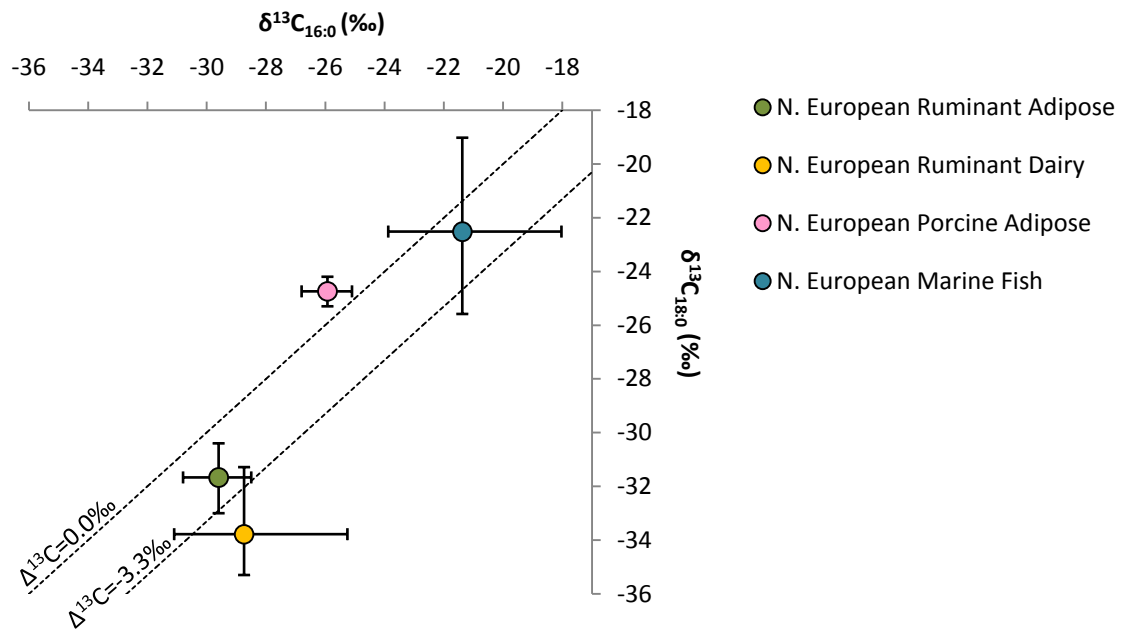


Figure 4.7: Plot showing the $\delta^{13}\text{C}$ values of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids obtained from northern European modern animal, marine and dairy fats/oils. The values were obtained from Dudd (1999) and Craig *et al.* (2007), and reflect a dietary C_3 origin. The error bars denote the range of values obtained for the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$. [Green: Ruminant adipose; Yellow: Ruminant milk; Pink: Porcine adipose; Blue: Marine fish]

Marine $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ isotopic values also reflect different admixtures of fatty acids from different origins, possibly related to different metabolic processes (Spangenberg *et al.*, 2006) and the isotopic fractionation associated with the carbon from dissolved CO_2 , which is in constant exchange with bicarbonate and carbonate ions (Sealy, 2001). Although Figure 4.8 shows that the $\Delta^{13}\text{C}$ values between terrestrial and marine fats cannot confidently distinguish between the two, the $\delta^{13}\text{C}$ values for both the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids in marine fish are higher when compared to the terrestrial animal values, and can be seen to plot separately from, but close to, the pig adipose (Figure 4.7). Combining GC-c-IRMS and biomarker analysis (Copley *et al.*, 2004; Hansel *et al.*, 2004; Evershed *et al.*, 2008a), ensures a confident identification of marine products.

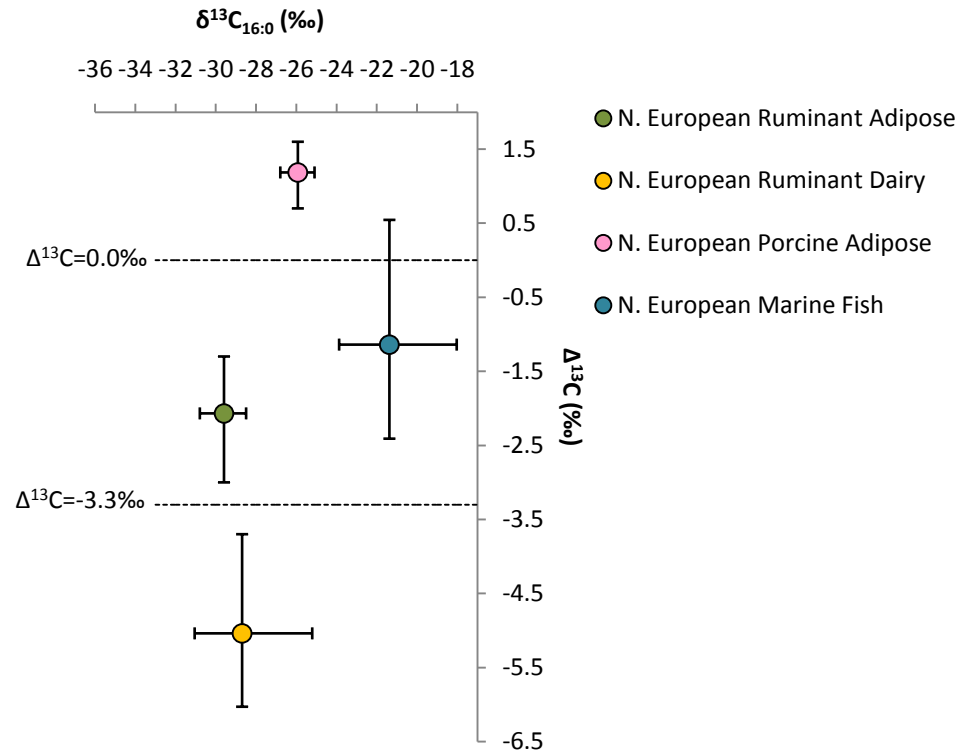


Figure 4.8: Plot of the $\Delta^{13}\text{C}$ ($=\text{C}_{18:0}-\text{C}_{16:0}$) against the $\text{C}_{16:0}$ values for northern European marine, animal and dairy fats/oils. This type of plot emphasises the biosynthetic and metabolic characteristics of the lipid source (Evershed *et al.*, 2008b), and allows any isotopic variation in dietary carbon (C_3 and C_4 plant contributions) to be detected (Copley *et al.*, 2003). The values were obtained from Dudd (1999) and Craig *et al.* (2007), and reflect a dietary C_3 origin. The error bars denote the range of values obtained for the $\delta^{13}\text{C}_{16:0}$ and $\Delta^{13}\text{C}$. [Green: Ruminant adipose; Yellow: Ruminant milk; Pink: Porcine adipose; Blue: Marine fish]

4.4 Laboratory sample preparation

To avoid contamination, all glassware was heated at 450°C for eight hours and nitrile gloves were worn at all times. Adipose tissue and marine fish were sub-sampled, and the tissue homogenised prior to extraction. About 2g of the tissue were collected in sterilised scintillation vials. Between 1 and 2mL of milk were transferred to sterilised screw-capped test tubes in preparation for extraction. When possible, 2mL of blood was sub-sampled from the vacutainers, however in the case of pigs, blood samples were between 0.5 and 1mL due to the difficulties encountered during blood extraction, previously discussed in Section 4.2.

$\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acid standards (Sigma, c.99% pure) were prepared at a concentration of 1 $\mu\text{g}/\mu\text{L}$. The $\delta^{13}\text{C}$ isotopic signature of both standards was measured using bulk stable isotope analysis at the University of Bradford. Mass spectrometry was carried out on an Europa 20-20 instrument with Roboprep combustion system, following Richards and Hedges (1999). The

measurements obtained were later used to correct the GC-c-IRMS $\delta^{13}\text{C}$ values for the carbon atom added during methylation. Control blanks were run with every batch of samples prepared.

The samples collected during the controlled feeding experiment were not all analysed. Samples taken prior to the start of the experiment, and during the last two weeks (weeks four and five) were extracted. However, only the samples obtained during the final sampling were sent for GC-c-IRMS analysis, as they best represented the change in diet.

4.4.1 Extraction

Extraction was carried out using an adaptation of the Folch Method (Folch *et al.*, 1957). To each of the samples, 5mL of dichloromethane (Scharlou; Reagent Grade) and methanol (Sigma-Aldrich; HPLC Grade) solution made up as 2:1 (v:v) were added. The samples were sonicated for 15 minutes, and then centrifuged at room temperature and at 3000rpm for 10 minutes. The liquid phase was decanted into sterilised glassware and 2mL of 0.9% sodium chloride solution were added. Each vial was shaken well and allowed to settle, then the top layer (containing methanol, water and polar molecules) was siphoned with a glass Pasteur pipette and discarded. The lower phase containing dichloromethane and the extracted lipids was retained, and was further washed using 2mL of methanol (Fischer; HPLC Grade) and ultrapure water (1:1; v:v) solution. The upper layer was again removed with a glass pipette and discarded. The samples were partitioned and 50% of the total lipid extract (TLE) was stored at -20°C . Both partitions were evaporated to dryness under a gentle stream of nitrogen and mild heating before storing.

4.4.2 Saponification

Saponification was carried out on one half of the TLE to release the esterified fatty acids. 4mL of 1.25M methanolic sodium hydroxide solution were added to each sample. The vials were then heated at 70°C for 2 hours on a pre-heated thermal block. The samples were allowed to cool before the neutral fraction was extracted. This was carried out three times using 2mL of hexane (Fischer; HPLC Grade). The solvent was evaporated to dryness using mild heating and a gentle stream of nitrogen, and the neutral fraction was stored at -20°C . The remaining fraction was acidified to a pH 3 using between 0.8 to 1mL of 6M hydrochloric acid (Aristar; sp. Gr. 1.18). The acid fraction was extracted into sterilised screw-capped test tubes three times using 2mL of hexane. The solvent was once again evaporated under a gentle stream of nitrogen and mild heating. The acid fraction was stored at -20°C pending derivatisation.

4.4.3 Derivatisation

1mL of boron trifluoride methanol solution (14%; Sigma Life Science) was added to the dry acid fraction. The samples were then heated for 1 hour at 70°C on a pre-heated thermal block. The reaction was quenched with two drops of ultrapure water, and the samples were allowed to cool before extracting the methylated lipids. Extraction was carried out three times using 2mL of hexane into sterilised screw-capped test tubes. The hexane was evaporated under very mild heating (30°C) on a pre-heated thermal block. The reaction was quenched with two drops of ultrapure w and a gentle stream of nitrogen. C_{16:0} and C_{18:0} fatty acid standards of known $\delta^{13}\text{C}$ values were methylated alongside the samples.

4.4.4 Gas Chromatography (GC)

Samples were analysed on an Agilent 7890 series Gas Chromatogram (GC) using a 30m, HP-5 (5%-Phenyl)-methylpolysiloxane (J&W Scientific) column, with a 0.32mm internal diameter and a film thickness of 0.25 μm . The inlet (in splitless mode) and flame ionisation detector (FID) were set at 300°C, and the flow rate was 2mL/min. The oven was programmed at 50°C for 2 minutes, then ramped at 10°C per minute to 325°C and held for 15 minutes. Hydrogen was used as the carrier gas. All blood and milk samples obtained during the controlled feeding experiments were analysed on the same instrument, but the GC was fitted with a 20m, ZB-5HT 5% Phenyl 95% Dimethylpolysiloxane (Zebron) column, with a 0.18mm internal diameter and a film thickness of 0.18 μm . The inlet (in splitless mode) and flame ionisation detector (FID) were set at 300°C and 400°C respectively, and the flow rate was 0.79mL min⁻¹. The oven was programmed to 50°C for 2 minutes, then increased to 150°C at 20°C minute⁻¹, then raised again to 200°C at 4°C minute⁻¹, and finally ramped to 350°C at 20°C minute⁻¹ and held for 15 minutes. Hydrogen was used as the carrier gas.

GC analysis was required to ensure that all the esterified fatty acids had been released and to ensure that an adequate amount of C_{16:0} and C_{18:0} fatty acids were present for GC-c-IRMS analysis. The chromatograms obtained for all the modern samples analysed are reproduced in Appendix A. Data acquisition and analyses was carried out on a ChemStation Rev. B.04.02 SP1.

4.4.5 Gas Chromatography-combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS)

GC-c-IRMS analysis was carried out at the Natural Environment Research Council (NERC) Life Science Mass Spectrometry Facility in Bristol, UK. Analyses were carried out on a Finnigan MAT Delta S, with Septum Equipped Programmable Injector (SPI). The GC was fitted with a VF-23ms (60m x 0.32mm x 0.15DF) column. The injector temperature was set at 50°C. The oven was initially held at 50°C for 1 minute, then ramped at 12°C minute⁻¹ to 120°C, and finally increased to 250°C at 6°C minute⁻¹ and held for 20 minutes. Helium was used as the carrier gas. The combustion reactor was set at 940°C. The samples obtained during the controlled feeding experiments were analysed on the same instrument, but the GC was fitted with an HP-1 (50m x 0.32mm x 0.17µm) column with a 100% Dimethylpolysiloxane stationary phase. The injector temperature was initially set at 70°C then immediately increased to 250°C at 10°C minute⁻¹ and held for 10 minutes. The oven was programmed at 50°C for 1 minute, then ramped to 250°C at 10°C minute⁻¹ and held for 10 minutes. The carrier gas was helium.

Data was acquired and analysed using ISODAT 3.0 software. Analytical accuracy was confirmed by running fatty acid methyl ester (FAME) standards of known isotopic values prior to each batch of analysis. During each run, six pulses of carbon dioxide of known isotopic composition were fed into the ion source from the reference gas injector. These measures ensured that the instrument and combustion furnace were functioning correctly. Instrument precision was ±0.3‰.

4.4.6 Methylation and post-industrial carbon (PIC) corrections

The correction factor for the isotopic shift caused by the carbon introduced in the fatty acids during derivatisation by methylation was corrected by measuring the difference between the bulk $\delta^{13}\text{C}$ value obtained for the C_{16:0} and C_{18:0} fatty acid standards (described at the beginning of this section), and the $\delta^{13}\text{C}$ measurement obtained after GC-c-IRMS analysis of the same standards. The correction was applied to all $\delta^{13}\text{C}$ measurements of the modern samples analysed.

The $\delta^{13}\text{C}$ measurements for C_{16:0} and C_{18:0} of the modern samples were also corrected for the post-Industrial Revolution effects of fossil fuel burning, which were found to have decreased the $\delta^{13}\text{C}$ of atmospheric CO₂ by 1.14‰ (Friedli *et al.*, 1986). To correct for PIC, 1.14‰ was added to the $\delta^{13}\text{C}$ values of the samples measured.

4.4.7 Bulk carbon isotope analysis

Bulk stable carbon isotope analysis was carried out on sub-samples of the three diets compiled for the pigs, cows and ovicaprids (sheep and goats). Since pigs were fed solely on concentrates a sub-sample of their diet was taken from the mixture provided by Andrews Feeds, however it was necessary to prepare a representative sample of the diet fed to cattle, goats and sheep since their diet comprised both forage and concentrate at a ratio of 3:2. The quantities used are listed in Tables 4.6 and 4.7. After measuring the various dietary components, the plant material was placed in a freeze drier for 2 days to remove all moisture and allow it to become brittle in preparation for milling. Milling was carried out on a Retsch MM301 mill, for approximately 55 minutes at a frequency of 18.9 revolutions per second (1/s). Each stainless steel holder and ball bearings were cleaned with ultrapure water and ethanol prior to milling, to eliminate contamination. The powdered plant material was then vortexed for 1 minute to ensure a homogenous mixture.

<i>Plant material</i>		<i>% Weight</i>
<i>Ground barley</i>	Concentrate	18.9
<i>Ground beans</i>		11.8
<i>Sunflower meal</i>		5.5
<i>Alfalfa meal</i>		2.4
<i>Beef premix</i>		1.6
<i>Fresh alfalfa</i>	Forage	29.9
<i>Straw</i>		29.9

Table 4.6: List of the amounts required to prepare a representative sample of the cattle diet in proportion to the whole dietary intake.

<i>Plant material</i>		<i>% Weight</i>
<i>Whole barley</i>	Concentrate	10.0
<i>Whole beans</i>		10.0
<i>Sunflower pellets</i>		7.1
<i>Whole wheat</i>		7.1
<i>Whole alfalfa</i>		1.6
<i>Sheep premix</i>		4.0
<i>Fresh alfalfa</i>	Forage	30.1
<i>Straw</i>		30.1

Table 4.7: List of the amounts required to prepare a representative sample of the sheep and goat diet in proportion to the whole dietary intake.

Around 0.7mg of each of the three diets was weighed into sterilised tin capsules using a Sartorius MZP microbalance. These were then sent to the Department of Archaeological Sciences at the University of Bradford, UK for analysis. The tin capsules were combusted to release carbon dioxide and nitrogen on a Thermo Flash EA 1112 coupled to a Delta plus XL mass spectrometer.

4.5 Bulk stable carbon isotope results

Table 4.8 shows the results obtained after bulk carbon stable isotope analysis of the three animal feeds. Only the carbon isotope results need to be considered for the purpose of this analysis.

Sample	Weight	$\delta^{15}\text{N}$	Amt % N	$\delta^{13}\text{C}$	Amt % C
Pig feed	0.7	2.3	3.3	-25.9	40.5
Cattle feed	0.7	3.2	2.8	-27.2	40.6
Sheep/goat feed	0.7	2.1	3.4	-27.3	40.5
Wheat standard (C3)	1.1	2.8	1.5	-27.3	39.9
Sorghum standard (C4)	1.2	1.1	1.7	-13.5	41.6

Table 4.8: Table showing the bulk stable isotope results for nitrogen and carbon obtained from the animal feed used during the controlled feeding experiment. Wheat and sorghum (unknown geographical location) standards were run and their ranges fell within the generally accepted values [Wheat: $\text{N}=2.85\pm 0.17$; $\text{C}=-27.21\pm 0.13$, Sorghum: $\text{N}=1.58\pm 0.15$; $\text{C}=-13.68\pm 0.19$].

The results show that all three diets fall within the range of values attributed to C_3 vegetation, with a mean value of -26.8‰ . Stott *et al.* (1997) obtained a mean value of -25.3‰ for bulk carbon isotope analysis of the C_3 diet used in the experiment they carried out. The plant material included in the diet used by Stott *et al.* (1997) is similar to that used in the C_3 Mediterranean diet, apart from the use of soybean which was not included in the latter. The difference between the mean values of Stott *et al.*'s (1997) and the present experimental diet is 1.5‰ . The range of Mediterranean bulk carbon isotope values are also comparable to the range of values obtained by Dudd (1999), which fall between -23.9‰ and -28.6‰ , and include values for barely, wheat and silage as well as C_3 concentrate mixes. The values obtained for bulk carbon isotope analysis therefore show that the diet used for the controlled feeding experiment reflect an exclusively C_3 environment.

4.6 Results

Table 4.9 shows the $\delta^{13}\text{C}$ values and standard deviations obtained for the experimental blood and milk samples, as well as the marine, adipose and milk samples acquired from the Mediterranean and northern Europe (described in Section 4.2). The $\delta^{13}\text{C}$ isotope values for the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids obtained from the controlled feeding experiment and Mediterranean marine fish are plotted in Figure 4.9.

It can immediately be observed that not all of the experimental data points plot within the accepted $\Delta^{13}\text{C}$ ranges which differentiate between dairy products, ruminant and non-ruminant adipose (Figure 4.9). One of the cattle blood samples plots within the porcine $\Delta^{13}\text{C}$ range, while a

porcine outlier falls into the range of $\Delta^{13}\text{C}$ values denoting ruminant adipose. Dairy products show a larger $\Delta^{13}\text{C}$ variation than previously reported, with only the sheep milk plotting within the accepted range for dairy products, while the cow and goat milk samples plot with the ruminant adipose $\Delta^{13}\text{C}$ values. Mediterranean marine fish fall within the expected $\Delta^{13}\text{C}$ range (Figure 4.9). Of major concern are the $\Delta^{13}\text{C}$ values obtained for the dairy products in the experimental samples, since both cow and goat milk had $\Delta^{13}\text{C}$ values larger than -3.3‰ .

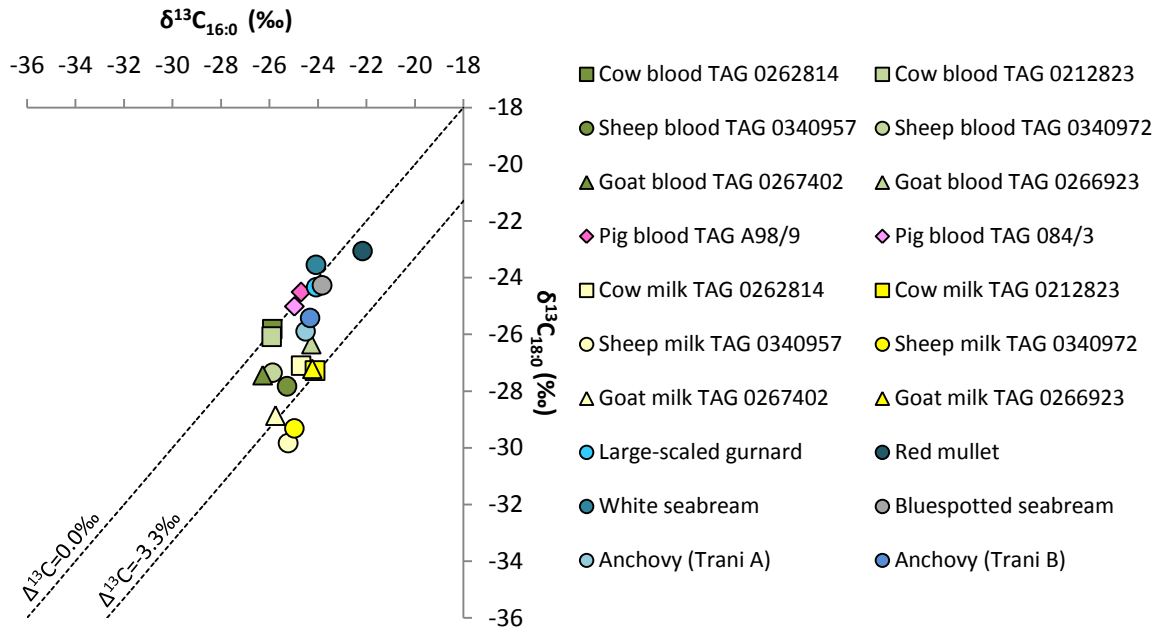


Figure 4.9: GC-c-IRMS results of the $\delta^{13}\text{C}$ isotope ratios for the measured fatty acids ($\text{C}_{16:0}$ and $\text{C}_{18:0}$) obtained from the controlled feeding experiment and Mediterranean marine fish.

The main aim of this experiment was to compare the values obtained from the Mediterranean region to their northern European and Near Eastern counterparts. The $\delta^{13}\text{C}$ measurements obtained were therefore plotted together with published values from Britain (Dudd, 1999; Craig *et al.*, 2005a), Denmark (Craig *et al.*, 2007) and the Near East (Gregg *et al.*, 2009) (Figure 4.10). Figure 4.11 shows the average $\delta^{13}\text{C}$ values obtained for northern European and Mediterranean fats, with the error bars denoting the range of the isotopic measurements obtained for the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids. The different geographic locations were classified as northern European (comprising $\delta^{13}\text{C}$ values from Germany, Britain and Denmark), Near Eastern (including samples originating from Israel, Palestine and Jordan) and Mediterranean (namely Italy and Malta). A list of all the samples included and their individual $\delta^{13}\text{C}$ values can be found in Appendix A. Table 4.10 lists the average $\delta^{13}\text{C}$ values of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ obtained per classification, together with their range, standard deviation, mean $\Delta^{13}\text{C}$ values and the number of samples which comprised each category.

Origin	Sample	C _{16:0}		C _{18:0}	
		δ ¹³ C value	SD	δ ¹³ C value	SD
Malta Controlled feeding experiment	Cow blood TAG 0262814	-25.9	0.1	-25.8	0.2
	Cow blood TAG 0212823	-25.9	0.1	-26.1	0.0
	Sheep blood TAG 0340957	-25.3	0.0	-27.8	0.4
	Sheep blood TAG 0340972	-25.9	0.0	-27.3	0.2
	Goat blood TAG 0267402	-26.3	0.5	-27.4	0.9
	Goat blood TAG 0266923	-24.3	0.4	-26.3	0.1
	Pig blood TAG A98/9	-24.7	0.2	-24.5	0.1
	Pig blood TAG 084/3	-25.0	0.1	-25.0	0.1
	Cow milk TAG 0262814	-24.7	0.4	-27.1	0.5
	Cow milk TAG 0212823	-24.1	0.2	-27.3	0.1
	Sheep milk TAG 0340957	-25.2	0.1	-29.8	0.1
	Sheep milk TAG 0340972	-25.0	0.1	-29.3	0.2
	Goat milk TAG 0267402	-25.7	0.0	-28.9	0.1
	Goat milk TAG 0266923	-24.2	0.3	-27.2	0.1
Malta	Large-scaled gurnard	-24.1	0.2	-24.3	0.0
	Red Mullet	-22.2	0.5	-23.1	0.6
	White seabream	-24.1	0.0	-23.5	0.6
	Bluespotted seabream	-23.8	0.1	-24.3	0.4
Italy	Anchovy (A)	-24.5	0.1	-25.9	0.1
	Anchovy (B)	-24.3	0.2	-25.4	0.8
Malta (C₄contribution)	Cow adipose (C ₄)	-22.8	0.1	-23.1	0.1
	Sheep adipose (C ₄)	-22.0	0.6	-22.8	0.1
	Goat adipose (C ₄)	-18.7	0.1	-19.3	0.2
	Sheep milk (C ₄)	-22.4	0.1	-26.7	0.1
	Goat milk (C ₄)	-20.1	0.1	-27.2	0.0
Italy (C₄contribution)	Sheep ricotta (Matera) (C ₄)	-23.3	0.2	-28.4	0.1
	Sheep ricotta (Trani) (C ₄)	-22.1	0.4	-27.5	0.1
Germany	Cow milk	-25.3	0.0	-31.3	0.0
	Roe deer adipose	-27.8	0.1	-31.6	0.0
	Red deer adipose	-25.7	0.3	-26.9	0.0
	Wild boar adipose	-25.9	0.2	-25.6	0.1
Italy	Wild boar adipose	-28.9	0.4	-28.8	0.1

Table 4.9: δ¹³C and standard deviation values of C_{16:0} and C_{18:0} fatty acids extracted from Mediterranean and northern European reference samples listed in Section 4.2. δ¹³C_{16:0} and δ¹³C_{18:0} values were corrected for post industrial carbon according to Friedli *et al.*(1986). [SD: Standard deviation ±1; (C₄): denotes a C₄ plant contribution to the diet]

As expected, the $C_{18:0}$ component of the northern European and Mediterranean dairy measurements had more negative ^{13}C values when compared to ruminant adipose/blood (as described in Section 4.3.4). The ruminant dairy and adipose/blood samples also appear to cluster separately, according to geographic location (Figure 4.11). Mediterranean dairy $^{13}C_{16:0}$ and $^{13}C_{18:0}$ values were more positive compared to the northern European values recorded, with a mean difference of 3.9‰ and 5.5‰ respectively (Table 4.10). Figure 4.10 shows that the four milk samples of Mediterranean origin obtained from animals having a C_4 contribution in their diet plot within the expected $\Delta^{13}C$ range. The sheep butter sample from Jordan falls well within the northern European dairy range, however Gregg *et al.* (2009) reported an ethnographic pot from Turkey containing dairy residues with a $\Delta^{13}C$ greater than 0.0‰, hence plotting within the porcine adipose range. The small sample size ($n=2$), however, prevents a confident interpretation with regard to a possible significant shift in $\Delta^{13}C$ values of Near Eastern dairy products. Mediterranean dairy samples appear to have a more positive range of $\Delta^{13}C$ values (mean: -3.4‰; max: 2.4‰; min: -4.6‰) than their northern European counterparts, which resulted in four out of six milk samples (from cows and goats) plotting within the northern European ruminant adipose range (Figure 4.9). Only Mediterranean sheep milk plotted within the expected range for dairy products ($\Delta^{13}C < -3.3‰$).

The mean $\delta^{13}C$ for ruminant adipose tissue of northern European origin shows lower $\delta^{13}C$ values in both $C_{16:0}$ and $C_{18:0}$ when compared to the $\delta^{13}C$ values of the blood samples taken from Mediterranean ruminant animals. The mean difference between the two means was 4.0‰ and 4.9‰ for the $C_{16:0}$ and $C_{18:0}$ respectively (Table 4.10). The outlier in this case was a cow blood sample (TAG 0262814), which plots just beyond the threshold denoting porcine adipose values, with a $\Delta^{13}C$ of 0.1‰ (Figure 4.9). Compared to northern European ruminant adipose, Mediterranean ruminant blood exhibited a more positive $\Delta^{13}C$ range of values (mean: -1.2‰; max: 0.1‰; min: -2.6‰), which overlaps with the established ranges for northern European non-ruminant adipose and Mediterranean dairy products. The adipose tissue collected from ruminants bred in the Mediterranean which included a C_4 plant component in their diet plot separately as expected, and are much more positive in both their $C_{16:0}$ and $C_{18:0}$ isotopic values compared to the C_3 fed Mediterranean ruminants, but fall within the expected ruminant adipose $\Delta^{13}C$ values (Figure 4.12). Two of the three ruminant adipose samples of Near Eastern origin plot within the ruminant adipose range obtained for northern Europe, however, the third sample shows a considerable shift, and plots within the porcine adipose range (Figure 4.10). Again, more samples are required to test the significance of this unexpected Near Eastern result.

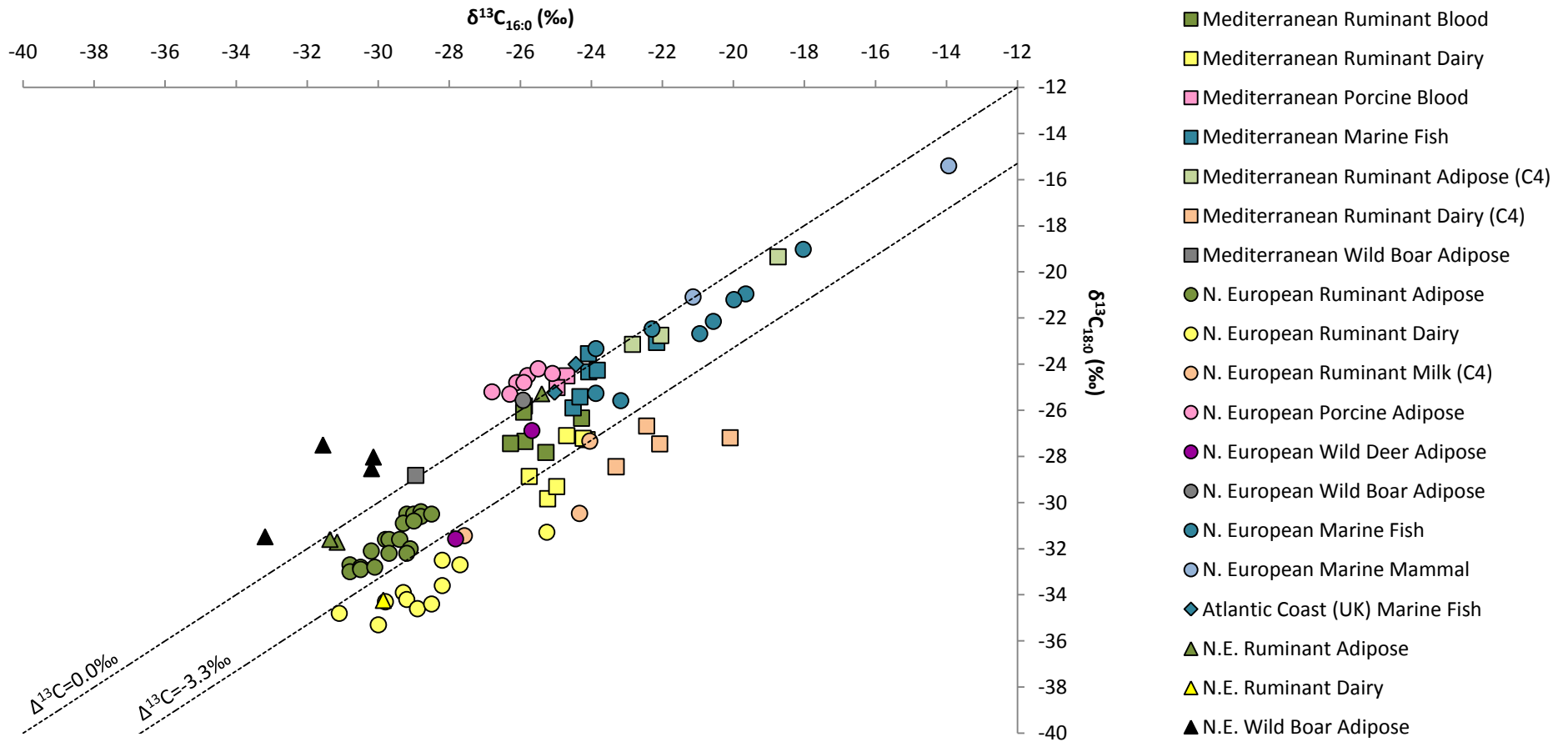


Figure 4.10: Plot of the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ isotope ratios for modern reference fats. Data includes modern reference fats from northern Europe (Dudd, 1999; Craig *et al.*, 2005a, 2007), the Near East (Gregg *et al.*, 2009), as well as the results obtained from the controlled feeding experiment carried out in Malta and the Mediterranean caught marine fish. [(C4) denotes a C_4 plant contribution to the diet]

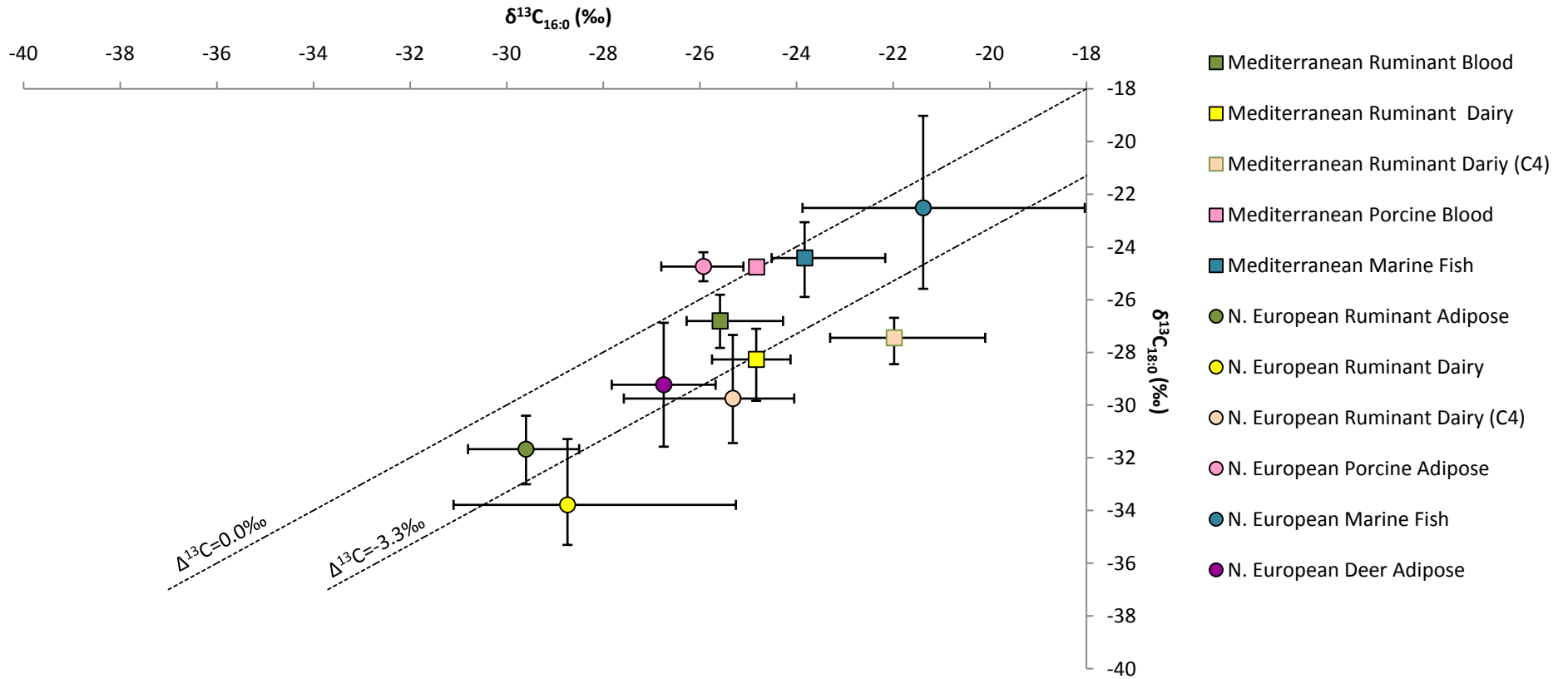


Figure 4.11: Plot of the average $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ values for modern reference fats. The error bars encompass the range of $\delta^{13}C$ measurements obtained for each category. Data includes modern reference fats from northern Europe (Dudd, 1999; Craig *et al.*, 2005a, 2007), as well as the results obtained from the controlled feeding experiment carried out in Malta and the Mediterranean caught marine fish. [(C4) denotes a C₄ plant contribution to the diet]

Country	Species	Type	Mean	SD	Mean	SD	Max		Min		Mean $\Delta^{13}\text{C}$	N
			$\text{C}_{16:0}$	$\text{C}_{16:0}$	$\text{C}_{18:0}$	$\text{C}_{18:0}$	$\text{C}_{16:0}$	$\text{C}_{18:0}$	$\text{C}_{16:0}$	$\text{C}_{18:0}$		
Atlantic coast UK	Marine Fish	Flesh	-24.7	0.4	-24.6	0.9	-24.4	-24.0	-25.0	-25.2	0.1	2
Mediterranean	Marine Fish	Flesh	-23.8	0.9	-24.4	0.6	-22.2	-23.1	-24.5	-25.9	-0.6	4
N. European	Marine Fish	Flesh	-21.4	2.0	-22.5	2.1	-18.0	-19.0	-23.9	-25.6	-1.1	9
Mediterranean	Porcine	Blood	-24.8	0.2	-24.8	0.4	-24.7	-24.5	-25.0	-25.0	0.1	2
N. European	Porcine	Adipose	-25.9	0.5	-24.7	0.4	-25.1	-24.2	-26.8	-25.3	1.2	7
Mediterranean	Wild Boar	Adipose	-28.9	0.0	-28.8	0.0	-28.9	-28.8	-28.9	-28.8	0.1	1
Near Eastern	Wild Boar	Adipose	-31.3	1.4	-28.9	1.8	-30.1	-27.5	-33.2	-31.5	2.4	4
N. European	Wild Boar	Adipose	-25.9	0.0	-25.6	0.0	-25.9	-25.6	-25.9	-25.6	0.4	1
Mediterranean	Ruminant	Blood	-25.6	0.7	-26.8	0.8	-24.3	-25.8	-26.3	-27.8	-1.2	6
Mediterranean	Ruminant	Adipose (C ₄)	-21.2	2.2	-21.7	2.1	-18.7	-19.3	-22.8	-23.1	-0.5	3
Near Eastern	Ruminant	Adipose	-29.3	3.4	-29.5	3.7	-25.4	-25.3	-31.4	-31.7	-0.2	3
N. European	Ruminant	Adipose	-29.6	0.7	-31.7	0.9	-28.5	-30.4	-30.8	-33.0	-2.1	19
N. European	Wild Deer	Adipose	-26.8	1.5	-29.2	3.3	-25.7	-26.9	-27.8	-31.6	-2.5	2
Mediterranean	Ruminant	Dairy	-24.8	0.6	-28.3	1.2	-24.1	-27.1	-25.7	-29.8	-3.4	6
Mediterranean	Ruminant	Dairy (C ₄)	-22.0	1.4	-27.4	0.7	-20.1	-26.7	-23.3	-28.4	-5.5	4
Near Eastern	Ruminant	Dairy	-29.9	0.0	-34.2	0.0	-29.9	-34.2	-29.9	-34.2	-4.4	1
N. European	Ruminant	Dairy	-28.7	1.5	-33.8	1.2	-25.3	-31.3	-31.1	-35.3	-5.0	11
N. European	Ruminant	Dairy (C ₄)	-25.3	2.0	-29.7	2.1	-24.0	-27.3	-27.6	-31.4	-4.4	3

Table 4.10: Table showing the mean, maximum, minimum and SD values of the $\delta^{13}\text{C}$ measurements for the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids, and mean $\Delta^{13}\text{C}$ values. The northern European dataset was obtained from Dudd (1999), Craig *et al.* (2005a) and Craig *et al.* (2007), while the Near Eastern values were taken from Gregg *et al.* (2009). [N: number of samples; SD: Standard deviation ± 1 ; (C₄) denotes a C₄ plant contribution to the diet]

Mean $\delta^{13}\text{C}_{16:0}$ values for the Mediterranean pig blood were 1.1‰ higher when compared to the $\delta^{13}\text{C}_{16:0}$ values obtained for the northern European porcine adipose, but there was no difference between their mean $\delta^{13}\text{C}_{18:0}$ values (Figure 4.10; Table 4.10). Whereas Evershed *et al.* (2002b) reported that the expected $\Delta^{13}\text{C}$ values between the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ of ruminants and non-ruminants is about 4‰ and 7‰ respectively, the difference shown here between Mediterranean ruminant and porcine blood was 0.8‰ and 2.0‰, for their $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$, respectively. Mediterranean porcine blood $\Delta^{13}\text{C}$ values are consistent with non-ruminant adipose fat ($\Delta^{13}\text{C} > 0.0\text{‰}$; mean: 0.1‰; max: 0.2‰; min: 0.0‰). As expected, Mediterranean porcine $\delta^{13}\text{C}$ values are higher compared to the Mediterranean ruminant blood, although their $\Delta^{13}\text{C}$ ranges do slightly overlap (Figure 4.12).

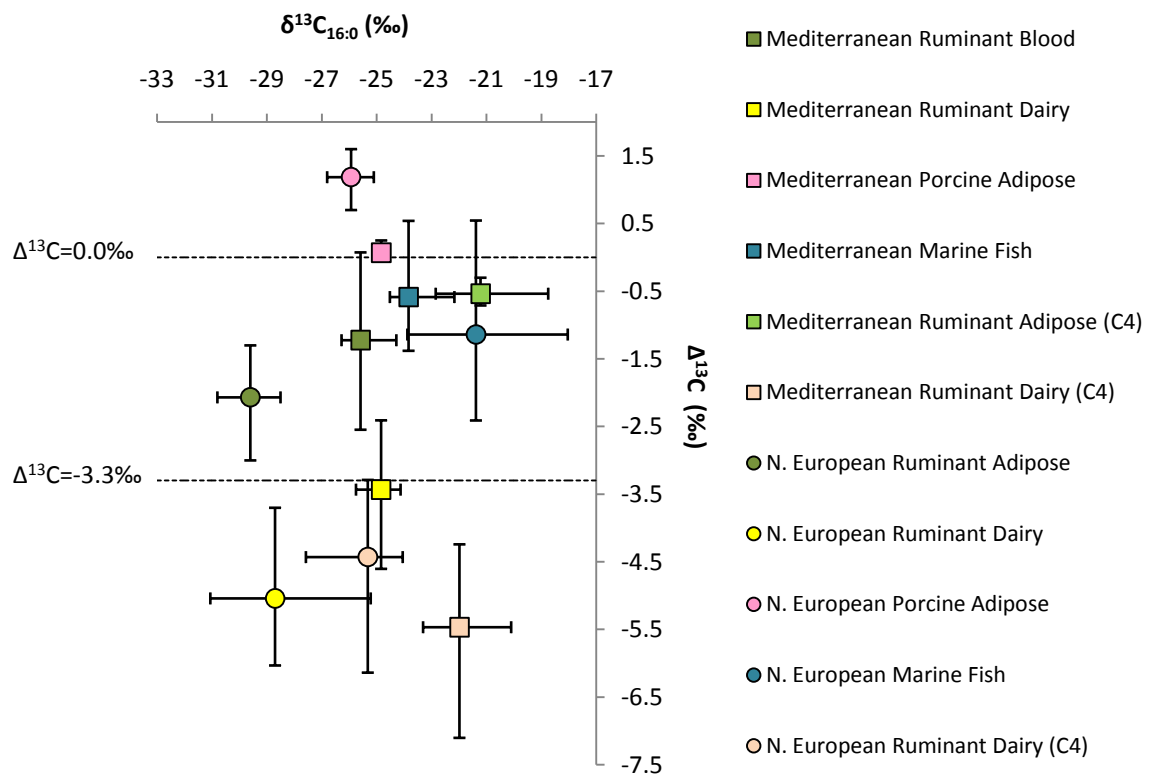


Figure 4.12: Plot of mean $\Delta^{13}\text{C}$ value (*i.e.* the difference in $\delta^{13}\text{C}$ values between the $\text{C}_{18:0}$ and $\text{C}_{16:0}$ fatty acids) against mean $\delta^{13}\text{C}_{16:0}$ of modern reference fats. Data includes modern reference fats from northern Europe (Dudd, 1999; Craig *et al.*, 2005a, 2007), and the results obtained from the controlled feeding experiment carried out in Malta as well as the Mediterranean caught marine fish. The error bars encompass the range of $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements obtained for each category. [(C4) denotes a C4 plant contribution to the diet]

The pairs of animals which were included in the controlled feeding experiment allowed the GC-c-IRMS results obtained to be replicated. The measured $C_{16:0}$ and $C_{18:0}$ fatty acid isotopic values in the blood and milk obtained from all pairs of animals included in the experiment showed only a small intra-species variation, except in the goats (Figure 4.13). The $\delta^{13}C_{16:0}$ values of the blood samples taken from the two goats differed by 2.0‰, while their $\delta^{13}C_{18:0}$ results showed a difference of 1.1‰. Goat milk samples differed by 1.5‰ between their $\delta^{13}C_{16:0}$ values and 1.7‰ in the $\delta^{13}C_{18:0}$ values. This variation is surprising, since both animals selected were identical in sex, age, breed and stages of lactation, and were exposed to the same conditions during the feeding experiment. A possible explanation could be ‘ecological variability’, which is the 0.2 to 2.0‰ standard deviation in animals of the same species raised on similar diets and environments, which is attributed to heterogeneity in a single food source, selectivity, the biochemical state of the animal, intra-organism variation and seasonal differences (Tieszen *et al.*, 1983; Dudd, 1999:32). All but the latter may be considered as contributing factors to the variation in the goat samples.

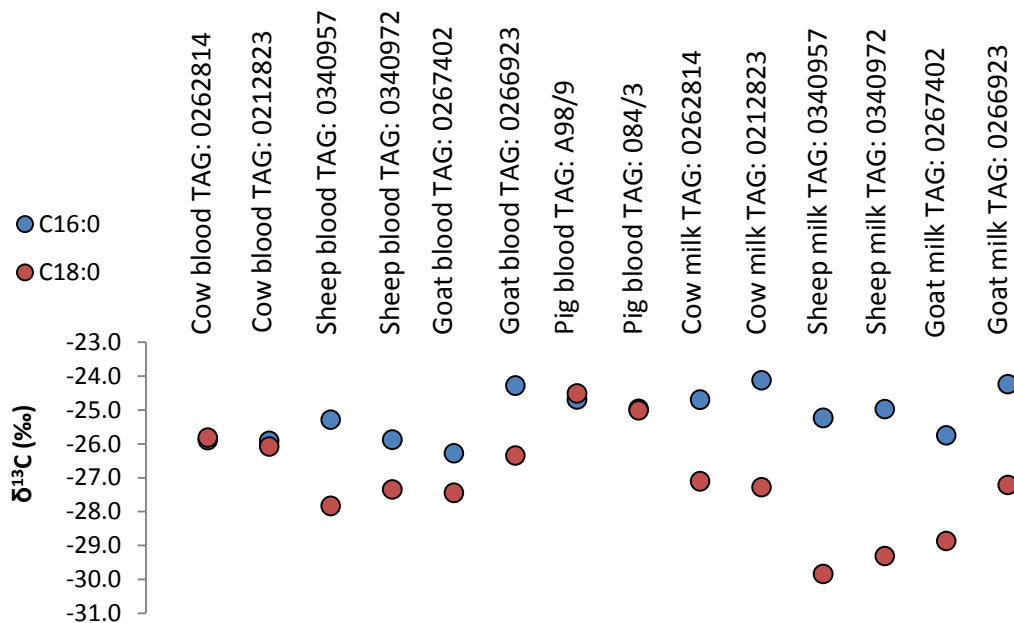


Figure 4.13: Plot showing the difference between the $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ of the blood and milk samples obtained from the controlled feeding experiment.

The difference between the mean isotopic values obtained for Mediterranean and northern European marine fish showed lower values in the former, with a mean regional difference in the $\delta^{13}C_{16:0}$ of 2.5‰ and 1.9‰ for the $\delta^{13}C_{18:0}$ values (Table 4.10). Mediterranean marine fish therefore plot closely to the Mediterranean and northern European pig adipose values, and it is difficult to differentiate between the two groups (Figure 4.10). The $\Delta^{13}C$ values between the

$\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ of pigs and marine fish are greater in northern European samples ($\text{C}_{16:0}$: 4.5‰; $\text{C}_{18:0}$: 2.2‰, Table 4.10) than in the Mediterranean ($\text{C}_{16:0}$: 1.0‰; $\text{C}_{18:0}$: 0.3‰, Table 4.10), which leads to a better separation of the two species in the former. The Atlantic marine fish are also depleted compared to the Danish values (Table 4.10). Possible reasons for the shift registered between the different geographic locations are discussed in Section 4.7.2.

Figures 4.10 and 4.11 also include $\delta^{13}\text{C}$ measurements for deer adipose, which show a very wide variation such that their $\Delta^{13}\text{C}$ values overlap with both the ruminant adipose and ruminant dairy fat categories. Wild boar values plot within the porcine adipose range, and show lower $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values when compared to the domesticated pig adipose. Mediterranean and Near Eastern wild boar samples are also more depleted compared to the sample obtained from Germany. For the purposes of this chapter, deer and wild boar adipose shall not be discussed further, although they will be considered when interpreting the use of the archaeological pottery as these species were available in the Neolithic of southern Italy.

4.6.1 Overview of the results

A comparative analysis of the $\delta^{13}\text{C}$ results obtained from northern Europe, the Near East and the Mediterranean shows that:

1. **Ruminant adipose:** northern European and Near Eastern ruminant adipose data points plot within a $\delta^{13}\text{C}_{16:0}$ range of -25.4‰ to -31.4‰ and a $\delta^{13}\text{C}_{18:0}$ range of -25.3‰ to -33.0‰, with the exception of one Near Eastern sheep adipose sample which had considerably higher $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values. Compared to northern European ruminant adipose, the average $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ fatty acid values for the Mediterranean ruminant blood samples are increased by 4.0‰ and 4.9‰ respectively. The $\Delta^{13}\text{C}$ values obtained for the experimental ruminant blood samples ranged between 0.1‰ and -2.6‰ (mean: -1.2‰). Ruminant adipose values overlapped with the porcine offset at a $\Delta^{13}\text{C}$ of 0.1‰.
2. **Ruminant dairy:** northern European and Near Eastern dairy fats plot within a $\delta^{13}\text{C}_{16:0}$ range of between -25.3‰ to -31.1‰ and a $\delta^{13}\text{C}_{18:0}$ range of -32.3‰ to -35.3‰, the exception being an ethnographic sample (not plotted) which Gregg *et al.* (2009) observed to fall within the porcine $\Delta^{13}\text{C}$ range. $\delta^{13}\text{C}$ values of Mediterranean milk samples were more positive than northern European milk samples by 3.9‰ and 5.5‰ for their $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values respectively, and had a more positive range of $\Delta^{13}\text{C}$ values, between -2.4‰ and -4.6‰. Mediterranean milk samples from the two cows and goats plotted above the northern

European dairy $\Delta^{13}\text{C}$ range for dairy products, while sheep milk sample fell within the northern European range denoting dairy products (i.e. $\Delta^{13}\text{C} < -3.3\text{‰}$).

3. Mediterranean ruminant adipose and dairy $\delta^{13}\text{C}$ values in animals having a C_4 contribution to their diet plot within the expected $\Delta^{13}\text{C}$ ranges, but exhibit more positive values reflecting the C_4 origin of their dietary intake.
4. **Porcine adipose:** northern European $\delta^{13}\text{C}$ values cluster between a $\delta^{13}\text{C}_{16:0}$ range of -25.1‰ to -26.8‰ and a $\delta^{13}\text{C}_{18:0}$ range of -24.2‰ to -25.3‰ . $\delta^{13}\text{C}_{16:0}$ values for Mediterranean porcine blood was 1.1‰ higher than the northern European values, while there was no difference between the $\delta^{13}\text{C}_{18:0}$ measurements. Mediterranean porcine values overlapped with the ruminant adipose $\Delta^{13}\text{C}$ values at 0.1‰ .
5. **Marine fish:** $\delta^{13}\text{C}_{16:0}$ values for northern European marine fish (including fish caught off the Atlantic coast of the UK and the Baltic) range between -18.0‰ and -25.0‰ , while their $\delta^{13}\text{C}_{18:0}$ measurements range between -19.0‰ and -25.6‰ . The Mediterranean red mullet measurement was found to cluster within the northern European values, however the five remaining samples had more negative measurements, that were 2.5‰ and 1.9‰ lower in their $^{13}\text{C}_{16:0}$ and $^{13}\text{C}_{18:0}$ values respectively. $\Delta^{13}\text{C}$ values ranged between 0.5‰ and -1.4‰ (mean: 0.6‰).
6. Wild boar samples fell within their expected $\Delta^{13}\text{C}$ values, which were however observed to be relatively wide.
7. $\Delta^{13}\text{C}$ values of the deer adipose fat samples were considerably wide, with the roe deer $\Delta^{13}\text{C}$ value plotting within the dairy fat category, with a mean $\Delta^{13}\text{C}$ of -3.8‰ .
8. The most indicative $\Delta^{13}\text{C}$ values obtained from the controlled feeding experiment were those which showed $\Delta^{13}\text{C}$ values lower than -3.3‰ , which identifies dairy products. All but one of the samples with a $\Delta^{13}\text{C}$ value lower than -3.3‰ were in fact known to have a dairy origin. Unfortunately, the roe deer outlier value is a point of concern, and further investigation is required.
9. There is a difference of 1.5‰ between the bulk stable carbon analysis of the C_3 animal feed used by Stott *et al.* (1997) and the Mediterranean sourced diet. This indicates variation in the isotopic values of plant material at different latitudes.

4.7 Discussion²

One of the issues in carrying out the controlled feeding experiment was sampling blood plasma instead of adipose tissue for GC-c-IRMS analysis. The rationale behind the controlled feeding experiment described in Section 4.3 argued that in sampling blood plasma lipids, the $\delta^{13}\text{C}$ of the fatty acids analysed would indeed reflect the isotopic signature of the C_3 diet fed to the animals. This was confirmed by the $\delta^{13}\text{C}$ values obtained, where experimental blood and milk samples of animals fed on a wholly C_3 diet were always lower than the $\delta^{13}\text{C}$ measurements obtained from the Mediterranean adipose and milk samples taken from animals who had a known C_4 contribution to their diet (Figures 4.10, 4.11 and 4.12). Compared to blood $\delta^{13}\text{C}$ measurements, adipose values of C_4 fed ruminants were found to be more positive by 4.4‰ and 5.1‰ in their $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ measurements respectively. $\delta^{13}\text{C}$ values of Mediterranean dairy products sampled from ruminants feeding on a C_3 and C_4 diet show a similar trend, with milk $\delta^{13}\text{C}$ values from C_4 fed ruminants showing higher $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values (by 2.9‰ and 0.8‰ respectively). However, it is well known that $\delta^{13}\text{C}$ values within the tissue of the same organisms do vary. Tieszen *et al.* (1983) showed that the $\delta^{13}\text{C}$ values obtained from different tissues in gerbils fed on a diet with a constant carbon isotopic composition did indeed differ as follows: $\delta^{13}\text{C}$ hair > $\delta^{13}\text{C}$ brain > $\delta^{13}\text{C}$ muscle > $\delta^{13}\text{C}$ liver > $\delta^{13}\text{C}$ fat. Hence, the validity of comparing $\delta^{13}\text{C}$ values for fatty acids in blood plasma from the controlled feeding experiment to adipose $\delta^{13}\text{C}$ measurements from published research was questioned. Unfortunately, blood plasma lipids from northern European animals feeding on a known C_3 diet were not available for a comparative study; hence the following explanation is put forward to justify the blood and adipose comparison.

Christie (1981a:203) states that in ruminant adipose tissue, a large proportion of the total fatty acids is of dietary origin, derived from the triglycerides and unesterified fatty acid fractions of the plasma, which is brought about by the action of the enzyme lipoprotein lipase. This appears to contrast Emery (1980), who suggested that since the quantity of lipid in the diet of ruminant animals is relatively low (<5%), the major proportion of the lipid deposited as adipose fat is not directly routed from the dietary intake, but biosynthesised by the animal itself. However, it is also known that 'the fatty acids present in any tissue will consist of fatty acids synthesised *de novo* in the tissue and those synthesised elsewhere in the animal or obtained from the diet and transported to the tissue in the plasma' (Christie, 1981a:195). Plasma lipids are therefore known

²Near Eastern $\delta^{13}\text{C}$ values will not be discussed, since insufficient information is provided relevant to the origin of the different fats analysed.

to influence the composition of the tissue lipids, since these were found to change in response to the content and composition of the dietary components, pregnancy, lactation, season and climate (Christie, 1981a:195). These factors were kept constant for the duration of the feeding experiment. The $\delta^{13}\text{C}$ in blood plasma lipids therefore appears to represent the isotopic values of both the dietary carbon, and that of *de novo* synthesised fatty acids in the adipose tissue. The $\delta^{13}\text{C}$ values of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ of the experimental ruminant milk and blood do in fact appear to follow the northern European trend. The $\delta^{13}\text{C}_{18:0}$ values in the milk were always more negative than in blood, supporting routing from biohydrogenated plant $\text{C}_{18:2}$ and $\text{C}_{18:3}$ in the rumen (Harrison and Leat, 1975), while the $\delta^{13}\text{C}_{16:0}$ in milk was on average 0.7‰ more positive than in blood due to its biosynthesis from carbohydrates in the mammary gland, which is comparable to the 0.9‰ difference between northern European $\delta^{13}\text{C}_{16:0}$ in ruminant milk and adipose (Figure 4.11). It is therefore probable that the blood plasma values are not significantly different to the adipose values.

Another concern was Dudd's (1999:180) observation that a 2.0‰ increase in the $\delta^{13}\text{C}$ measurements occurred in the adipose tissue of cows fed on concentrate diets as opposed to grass fed cows, reflecting the isotopically heavier carbohydrate component of higher plants (e.g. cereals) included in the concentrates (Tieszen *et al.*, 1983). An increase in the $\Delta^{13}\text{C}$ values would also be observed if the concentrates fed to the animals contain mixtures of C_3 and C_4 plant material, with different carbohydrate to lipid ratios. The ruminant adipose samples plotted in Figures 4.10 and 4.12 include Dudd's (1999:180) values for cows fed on C_3 forage and the concentrate-fed cows, but as can be observed in these plots, Mediterranean ruminant adipose and dairy $\delta^{13}\text{C}$ values are still higher than those obtained from northern Europe. Dudd (1999:181) also observed that the $\delta^{13}\text{C}$ values of sheep fed on supplements were found to be similar to grass fed sheep, since the bulk of their diet was composed of grass. During the controlled feeding experiment, 60% of the ruminants' dietary intake was by foraging on C_3 alfalfa, which following Dudd's observation, suggests that the supplements included in the controlled feeding experiment do not significantly affect the isotopic values obtained.

Mediterranean terrestrial blood and milk samples exhibited higher $\delta^{13}\text{C}$ values. On average, the $\delta^{13}\text{C}_{16:0}$ values for Mediterranean blood and milk are 4.0‰ more positive than in northern Europe, while the corresponding mean $\delta^{13}\text{C}_{18:0}$ values are 5.2‰ more positive than northern European measurements. These higher $\delta^{13}\text{C}$ values are attributed to climatic variations between the two geographical locations, and will be further discussed in Section 4.7.1. Changes to the established $\Delta^{13}\text{C}$ value ranges between the different terrestrial and marine fats/oils, which are not influenced

by latitudinal variations, present more of an issue. Figure 4.14 shows the average $\Delta^{13}\text{C}$ values plotted against the mean $\delta^{13}\text{C}_{16:0}$ values for northern European and Mediterranean fats. As already indicated in Section 4.6, there is a degree of overlap between the ranges of Mediterranean $\Delta^{13}\text{C}$ values, which do not fall within the northern European $\Delta^{13}\text{C}$ ranges. Statistics have been used to test whether changes to the offsets observed are significant³.

Comparing northern European ruminant adipose $\delta^{13}\text{C}$ values for the fatty acids of interest ($\text{C}_{16:0}$ and $\text{C}_{18:0}$) to the isotopic measurements obtained for the same fatty acids in Mediterranean ruminant blood, a statistically significant difference was found to exist between the different geographical contexts (Kruskal-Wallis Test; $p < 0.05$), but there was no significant difference between their $\Delta^{13}\text{C}$ values (Independent sample T-Test; $p = 0.105$). Similarly, $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values for northern European and Mediterranean ruminant milk were also found to be statistically different (Kruskal-Wallis Test; $p < 0.05$), however this time, the $\Delta^{13}\text{C}$ values between the milk products from the two geographical locations was in fact also found to be statistically different (Independent sample T-Test; $p = 0.001$). $\Delta^{13}\text{C}$ values between Mediterranean porcine blood and northern European porcine adipose were also statistically different (Independent sample T-Test; $p = 0.002$). The more positive $^{13}\text{C}_{16:0}$ values in Mediterranean porcine blood compared to northern European porcine adipose, with no corresponding increase in the $^{13}\text{C}_{18:0}$ values is interesting, and may be due to different dietary plans fed to the pigs in both experiments. Mean $\Delta^{13}\text{C}$ values for Mediterranean ruminant blood and Mediterranean milk were found to be statistically different (Independent sample T-Test; $p = 0.002$), but no statistical variation exists between mean $\Delta^{13}\text{C}$ values for Mediterranean ruminant and Mediterranean porcine blood (Independent sample T-Test; $p = 0.133$). However, although the $\Delta^{13}\text{C}$ values of ruminant and porcine blood are not sufficiently resolved to allow a confident interpretation, Figure 4.11 shows that both $^{13}\text{C}_{16:0}$ and $^{13}\text{C}_{18:0}$ values in porcine blood are more positive compared to the ruminant blood, and plot separately, and can therefore be differentiated. The archaeological implications for these results are discussed in Section 4.8 below.

³The Kolomogrov-Smirnov statistical test was used to investigate if the distribution of the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ could be approximated by a normal distribution. The test produced p -values < 0.05 , rejecting the assumption of normality. The Shaprio-Wilks test however produced p -values > 0.05 for the $\Delta^{13}\text{C}$ values, hence showing that these variables were normally distributed. For variables for which the normality assumption has been accepted ($\Delta^{13}\text{C}$ values), a parametric test, the two-tailed Independent sample T-Test was used, while a non-parametric test, Kruskal-Wallis, was applied to the $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ measurements. Note that the Kruskal-Wallis and two-tailed Independent sample T-Test are testing for differences between the average $\Delta^{13}\text{C}$, and $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values (respectively), of the different fats. The hypothesis is defined as follows: H_0 : there is no significant difference between the average $\Delta^{13}\text{C}/\delta^{13}\text{C}_{16:0}/\delta^{13}\text{C}_{18:0}$ values; H_1 : There is a significant difference between the average $\Delta^{13}\text{C}/\delta^{13}\text{C}_{16:0}/\delta^{13}\text{C}_{18:0}$ values.

Mediterranean marine fish do not appear to follow the trend. Their $\delta^{13}\text{C}$ measurements were in fact lower in their $\delta^{13}\text{C}_{16:0}$ by 2.5‰, and by 1.9‰ in their $\delta^{13}\text{C}_{18:0}$ values when compared to northern European values. The lower $\delta^{13}\text{C}$ values resulted in the Mediterranean marine fish isotopic measurements plotting closer to the Mediterranean porcine blood values, such that there is no statistical variation between their $\delta^{13}\text{C}_{18:0}$ values (Kruskal-Wallis Test; $p>0.05$), while the variation between their $\delta^{13}\text{C}_{16:0}$ values does not confidently separate the two fats (Kruskal-Wallis Test; $p=0.046$). Mediterranean porcine blood values and marine fish are therefore difficult to distinguish based on GC-c-IRMS analysis. The Mediterranean marine fish ^{13}C values will be discussed separately in Section 4.7.2.

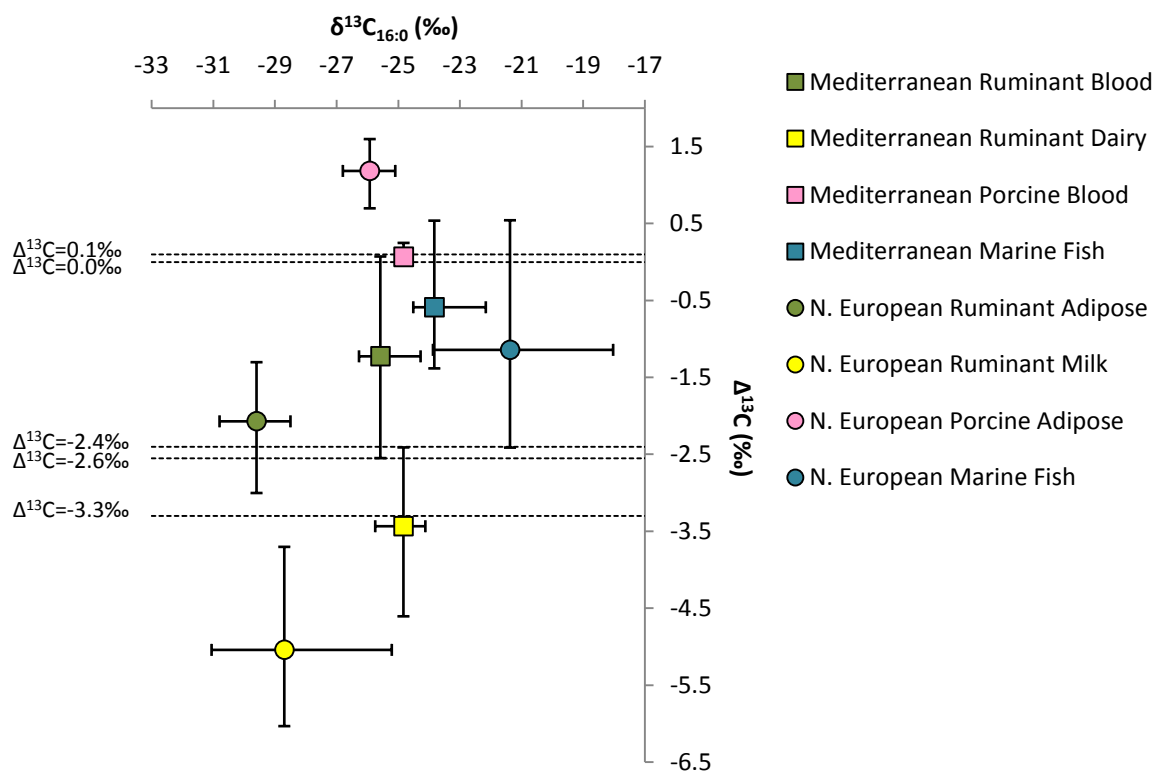


Figure 4.14: Plot of mean $\Delta^{13}\text{C}$ values ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) against mean $\delta^{13}\text{C}_{16:0}$ of modern reference fats, showing the $\Delta^{13}\text{C}$ range of values between ruminant and non-ruminant adipose/blood, ruminant milk and marine oils of Mediterranean and northern European origin. Data includes modern reference fats from northern Europe (Dudd, 1999; Craig *et al.*, 2005a, 2007), as well as the results obtained from the controlled feeding experiment carried out in Malta and the Mediterranean caught marine fish. The error bars encompass the range of $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements obtained for each category.

4.7.1 Variations due to latitude

In this Section, it will be argued that the shift towards heavier isotopic values in Mediterranean fats may be occurring due to the different climatic and environmental conditions experienced in the Mediterranean region as opposed to northern Europe. C_3 and C_4 photosynthetic pathways are

the most important atmospheric CO₂ fixing reactions (Spangenberg *et al.*, 2006). For the purpose of this analysis, only C₃ photosynthetic pathways will be considered. During photosynthesis, atmospheric CO₂, which has a δ¹³C value of about -8‰, diffuses into the pores on plant leaves, and because the lighter isotope (¹²C) diffuses slightly quicker, plant tissues are always enriched in ¹²C compared to ¹³C (Sealy, 2001) (values reported in Section 4.3.6). An additional isotope effect occurs due to selection for ¹²C by the enzymes involved in photosynthesis; hence plants contain less ¹³C and have lower ¹³C/¹²C ratios than air (Sealy, 2001). The magnitude of the fractionation caused by enzymatic action (c.28‰) is larger than that occurring during diffusion (4.4‰) (Tieszen, 1991). It is the isotopic effect associated with photosynthesis which is primarily responsible for the isotopic variability in plant carbon, and is mainly due to exposure to different climatic conditions (van Klinken *et al.*, 1994). Table 4.11 provides a list of the environmental factors which influence the discrimination of carbon in C₃ plants and their affect on plant δ¹³C values.

Temperature and/or relative humidity affect the δ¹³C values of plants by influencing the diffusion of gases into and from the plant leaves through the stomata, and also the biochemical (enzymatic) step that brings about the fixation of carbon which is affected by temperature and the partial pressure of CO₂ (*p*CO₂) (van Klinken *et al.*, 2000:42). The δ¹³C of plants is partly dependant on the ratio of the internal (interstitial; *i*) to external (air; *a*) CO₂ concentrations (*C_i/C_a*), which is influenced by the rate of carbon fixation and stomatal conductance (van Klinken *et al.*, 2000:42). Environmental factors of temperature, relative humidity and water stress act upon the *C_i/C_a* ratio, which in turn results in higher or lower plant δ¹³C values (van Klinken *et al.*, 2000:42). For example, high *C_i/C_a* ratios resulting from low temperatures, high stomatal conductance and low rates of photosynthesis will bring about more negative plant δ¹³C values, while during high temperatures and increasing water stress (when the stomata are closed to prevent water loss, hence low stomatal conductance) there is a decrease in the interstitial CO₂ concentrations (low *C_i/C_a*) and proportionately more ¹³CO₂ is fixed, therefore less negative plant δ¹³C values are obtained (Tieszen, 1991; van Klinken *et al.*, 2000:42). A regional patterning can in fact be observed when comparing plant δ¹³C values resulting from exposure to different climatic conditions. Van Klinken *et al.* (1994) observed a variation in the ¹³C/¹²C ratio in archaeological wood, charcoal and bone obtained from a number of European countries. All three materials appeared to follow a similar trend. Studies on charcoal showed a shift from -25.8‰ in the Netherlands to -22.9‰ in Libya, while analysis conducted on wood showed a similar shift in the ¹³C/¹²C ratio from -27.5‰ in the United Kingdom to -23.8‰ in Israel/Jordan (van Klinken *et al.*, 1994).

Environmental parameter	Environment plant coupling	Effect on C_i/C_a	Expected range of $\delta^{13}C_{plant}$ and the range direction		Most likely ecological occurrence
Recycled, respired CO_2	N.A.	Small	8	Negative	Very dense and closed tree canopies
Declining irradiance	E	↑	5-6	Negative	Dense and closed tree canopies
Increased water stress	S	↓	3-6	Positive	Open environments, arid to semi-arid
Increasing osmotic stress	S	↓	5-10	Positive	Localized osmotically impacted areas
Low nutrient content	E	↑	3-5	Negative	N or P limited natural areas or depleted soils
Low temperature	E	↑	3	Negative	High polar latitudes
Reduced CO_2 partial pressure (increase altitude)	'S'	↓	3-7	Positive	Steep elevation gradients
Chilling injury	E	↑	?	Negative	
Photoinhibition	E	↑	?	Negative	

Table 4.11: Table showing the environmental factors which affect carbon isotope fractionation in C_3 plants (After Tieszen, 1991:Table 1). [E: Carboxylase enzyme; S: Stomatal conductance]

$\delta^{13}C$ values of plants will affect the $\delta^{13}C$ values of the animals that ingest them. A 3‰ shift in the isotopic value of dietary carbon compared to adipose tissue was in fact found to occur as a result of the metabolic processes involved in the conversion of food to body tissue (Sealy, 2001) (Section 4.3.6). Hence, when comparing the data reported by van Klinken *et al.* (1994) to GC-c-IRMS results of animal products raised under different climatic conditions, it is not surprising to observe a similar trend. Van Klinken *et al.* (1994) show a shift of 3.5‰ between values obtained for charcoal between the United Kingdom (-27.5‰) and Spain (-24‰), and a 2.5‰ increase in $\delta^{13}C$ between charcoal samples in the United Kingdom (-25.7‰) and Libya (-23.2‰)⁴ (van Klinken *et al.*, 2000:43, Fig. 3.1). This trend was also noted by Richards and van Klinken (1997), who observed that humans with a wholly land-based diet from warm European regions have $\delta^{13}C$ values which are more positive by about 1‰ and 2‰ than those from colder European countries. In another publication, Richards *et al.* (1998) were able to identify possible migrants from the Mediterranean to Poundbury in Dorset, UK, by using $\delta^{13}C$ values taken from the ends and middle of the femur of a child, which represent the latest and earliest dietary input respectively. Values obtained from the middle of the femur were found to be enriched by 1‰ when compared to the measured $\delta^{13}C$ at the ends, and showed that the child had spent the first part of its life in a warmer (possibly Mediterranean) climate prior to migrating to Poundbury. Table 4.12 shows the mean $\delta^{13}C$

⁴Van Klinken *et al.* (2000:43; Fig. 3.1) reports a -24.3‰ value for central Italy, which would result in a 1.4‰ shift between United Kingdom and Italian $\delta^{13}C$ values. However, northern African climatic conditions mirror more accurately those experienced in the countries where the plant material included in the animal diet for the controlled feeding experiment was grown. Furthermore, other articles, namely Sealy (2001) and Richards *et al.* (1998) all reported an approximate 3‰ variation between the UK and the Mediterranean.

measurements obtained from bone collagen extracted from caprid remains which were collected from various sites in the Mediterranean and the UK. The mean $\delta^{13}\text{C}$ value obtained for the Mediterranean sites (excluding Cugnaux, which unlike the Languedoc region, is subject to oceanic rather than Mediterranean climatic influences; Herrscher and Le Bras-Goude, 2010) is -19.8‰ , while the values obtained from the northern sites is -21.5‰ . This shows that Mediterranean bone collagen is also enriched in $\delta^{13}\text{C}$ by 1.7‰ relative to UK values.

Region		Period	N	\bar{x} bone collagen $\delta^{13}\text{C}$ (‰)	Reference
Vela Spilja-Vela Luka	Dalmatia	Mesolithic	4	-18.6 ± 0.6	(Lightfoot <i>et al.</i> , 2011)
Vela Spilja Losinj	Dalmatia	EN	5	-20.4 ± 0.9	(Lightfoot <i>et al.</i> , 2011)
Karagadur	Istria	EN	5	-19.9 ± 1.8	(Lightfoot <i>et al.</i> , 2011)
Pupićina	Istria	MN	5	-20.2 ± 0.1	(Lightfoot <i>et al.</i> , 2011)
Apulia	S.E. Italy	EN	7	-18.9 ± 0.6	(Lelli <i>et al.</i> , in press)
Murge	S.E. Italy	EN	5	-19.2 ± 0.5	(Lelli <i>et al.</i> , in press)
Tavoliere	S.E. Italy	EN	4	-20.0 ± 0.1	(Lelli <i>et al.</i> , in press)
Montou	Languedoc, France	Neolithic	4	-19.8 ± 1.2	(Le Bras-Goude and Claustre, 2009)
Les Crès	Languedoc, France	Neolithic	3	-19.9 ± 0.2	(Herrscher and Le Bras-Goude)
Ancona	Central-East Italy	EN	4	-20.5 ± 0.1	(Lelli <i>et al.</i> , in press)
Cugnaux	Garonne, France	Neolithic	4	-21.1 ± 0.2	(Herrscher and Le Bras-Goude)
Arene Candide	Liguria, N. Italy	EN	5	-20.7 ± 0.5	(Le Bras-Goude <i>et al.</i> , 2006)
Brean Down	UK	BA	11	-21.3 ± 0.8	(Britton <i>et al.</i> , 2008)
Newark Bay	Orkney, Scotland	IA-Medieval	5	-21.8 ± 0.8	(Richards <i>et al.</i> , 2006)
St. Giles	UK	Medieval	9	-21.7 ± 0.3	(Müldner and Richards, 2005)
Wharram Percy	UK	Medieval	5	-21.8 ± 0.3	(Müldner and Richards, 2005)

Table 4.12: Mean bone collagen $\delta^{13}\text{C}$ (‰) extracted from archaeological caprid bones obtained from different geographic regions. [EN: Early Neolithic; MN: Middle Neolithic; BA: Bronze Age; IA: Iron Age]

Hence, the enrichment in ^{13}C observed in Mediterranean ruminant and non-ruminant blood and milk samples is consistent with the trend observed by van Klinken *et al.* (1994, 2000), Richards *et al.* (1998) and caprid bone collagen. Furthermore, a shift towards more positive values was also observed between the bulk carbon isotope measurements of the C_3 diet compiled by Stott *et al.* (1997) and the Mediterranean sourced C_3 diet, with the latter measuring on average 1.5‰ heavier than the former. However, since the geographical sourcing of Stott *et al.*'s (1997) diet is not known, further comparative analysis with the Mediterranean values cannot be confidently reported. Steele *et al.* (2010) tested the hypothesis that fractionation might occur during the saponification process while preparing samples in the laboratory, but found no significant isotopic

effects. The ^{13}C enrichment observed in Mediterranean reference fats therefore appears to be consistent with the variation observed in the $\delta^{13}\text{C}$ of plants grown in warmer climatic conditions.

4.7.2 Shift in the Mediterranean marine fish $\delta^{13}\text{C}$ data

Plankton is the primary producer in marine environments, whose $\delta^{13}\text{C}$ values enter the marine food web when it is fed upon by aquatic organisms. A similar transmission of $\delta^{13}\text{C}$ values can therefore be considered for the marine environment, to explain the shift obtained between the Mediterranean and northern European $\delta^{13}\text{C}$ values of marine fish.

No apparent difference was found between the stable carbon isotope content of zoo and phytoplankton living in the same environment. Phytoplankton fixes carbon during photosynthesis, which zooplankton merely utilizes without changing its own overall carbon isotope composition; hence it makes no difference whether the values for zoo or phytoplankton are considered, or even a mixture of the two (Deuser *et al.*, 1968). However, a change was observed in the biogeochemistry of carbon and the associated isotopic fractionation with varying temperature and latitude (Rau *et al.*, 1989). Deuser *et al.* (1968) ascribed variations in the $\delta^{13}\text{C}$ of marine plankton to environmental differences in temperature and pH (which control the amount of CO_2 available for photosynthesis), and carbon availability. Studies have shown that phytoplankton utilises dissolved CO_2 rather than bicarbonate for photosynthesis, and furthermore, the $\delta^{13}\text{C}$ value of dissolved CO_2 was found to be depleted compared to the bicarbonate (Deuser *et al.*, 1968). Thus, the greater the CO_2 availability (which increases at lower temperatures; Rau *et al.*, 1989), the more depleted the $\delta^{13}\text{C}$ of the marine plankton. Increasing temperatures diminish carbon availability, and more carbon is present as bicarbonate and carbonate. As starvation conditions approach, there is less fractionation between the CO_2 and the cells, resulting in higher $\delta^{13}\text{C}$ values (Deuser *et al.*, 1968).

The results obtained for the fish samples caught off the coasts of Malta and Italy in the Mediterranean showed more negative $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values when compared to marine fish caught in the Baltic Sea. The results obtained therefore do not appear to follow the predictions made by Deuser *et al.* (1968) and Rau *et al.* (1989), since it is more likely that the warmer Mediterranean waters would have produced more positive $\delta^{13}\text{C}$ fatty acid values. It is possible that the results obtained reflect more complex scenarios in place in the Mediterranean Sea. The Mediterranean is an enclosed sea with a rapid response to changes in climatic conditions, and high anthropogenic pressures (Luchetta *et al.*, n.d.). Moreover, its size, location, morphology and

external influences result in rich and complex physical dynamics (Siokou-Frangou *et al.*, 2010). One point to consider is whether this depletion is being caused by a natural variation in the dissolved carbon in the Mediterranean compared to the Baltic, or whether the variation is due to more recent anthropogenic activity. Input from geological carbonate also needs to be considered, as well as the influx of the numerous rivers flowing into the Mediterranean, which may be influencing the biogeochemistry of the carbon present. Therefore as yet, no explanation can be provided with regard to the depleted $\delta^{13}\text{C}$ values obtained for the Mediterranean marine fish. No $\delta^{13}\text{C}$ values of Mediterranean fish lipid residues are currently published which prevents a comparative analysis. A trend can however be observed when comparing stable carbon isotope values of fish bone collagen collected from warmer and cooler geographical locations. Stable carbon isotope analysis of four fish bone collagen samples retrieved from the multi-period site at Newark Bay (Orkney) gave values of $-11.8 \pm 1.2\text{‰}$ (Richards *et al.*, 2006), while Schulting and Richards (2002b) confirmed a value of $-12.0 \pm 1\text{‰}$ for stable carbon isotope measures of marine bone collagen in Britain. On the other hand, a mean value of -14.6‰ was obtained in the Mediterranean (Le Bras-Gaude *et al.*, 2006; Lightfoot *et al.*, 2011; Lelli *et al.*, in press), thus showing approximately a 3‰ depletion in the Mediterranean values. $\delta^{13}\text{C}$ of modern lipid fish residues therefore appear to follow this same trend.

4.8 Archaeological Implications

$\Delta^{13}\text{C}$ values of Mediterranean ruminant dairy and non-ruminant fats were shown to be statistically different (Independent sample T-Test; $p < 0.05$) from their northern European counterparts, while no statistical difference was observed between the $\Delta^{13}\text{C}$ values of ruminant adipose tissues of Mediterranean and northern European origin (Independent sample T-Test; $p > 0.05$) (see Section 4.7). Figure 4.15 shows the mean $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ values for Mediterranean modern reference fats, with error bars denoting the standard deviation at $\pm 1\text{‰}$. This plot suggests that residues of Mediterranean origin with $\Delta^{13}\text{C}$ values less than -2.6‰ can be attributed to dairy products, while $\Delta^{13}\text{C}$ values ranging between -0.2‰ and -2.3‰ can be identified as ruminant adipose. Non-ruminant fats of Mediterranean origin have $\Delta^{13}\text{C}$ values of between 0.3‰ and 0.0‰ , while marine oil $\Delta^{13}\text{C}$ values overlap considerably with non-ruminant and ruminant adipose $\Delta^{13}\text{C}$ values, and range between 0.1‰ and -1.3‰ . These can, however be distinguished by their $\delta^{13}\text{C}_{16:0}$ values.

Figure 4.15 also shows the mean $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ values with error bars denoting standard deviation at $\pm 1\text{‰}$, which are obtained when the isotopic measurements for northern European

and Mediterranean modern reference fats are combined. In view of the small number of Mediterranean modern reference fat isotopic measurements which could be obtained within the constraints of the controlled feeding experiment carried out, combining both northern European and Mediterranean datasets provides a more comprehensive range of $\Delta^{13}\text{C}$ modern reference fat values, which adds to previously published reference fat ranges, and takes into consideration the Mediterranean $\Delta^{13}\text{C}$ values. Figure 4.15 shows that the combined northern European and Mediterranean ranges of $\Delta^{13}\text{C}$ values denoting porcine and ruminant adipose are very well defined, but may however introduce uncertainty when interpreting values which fall in between the two ranges. A similar situation can be observed between ruminant adipose and ruminant dairy fats; however, in this case, residues can still be identified as ruminant fats. Hence, in Chapter 7, when discussing the GC-c-IRMS data obtained from archaeological residues extracted from Impressed/Cardial Wares, GC-MS data and archaeological evidence (mainly archaeozoological and archaeobotanical data), will be used to further support and qualify $\Delta^{13}\text{C}$ measurements.

One point of concern mentioned in Section 4.6 was the wide range of $\Delta^{13}\text{C}$ values obtained for deer adipose, which has been identified in Craig *et al.* (in press) as ranging between -1.2‰ to -4.3‰ , with a mean value of -3.5‰ . These values need to be taken into consideration since deer bones were attested in Early Neolithic settlements in the Mediterranean, and deer adipose could therefore be present in the pottery vessels analysed. In Chapter 7, the $\Delta^{13}\text{C}$ values of both wild (deer) and domestic ruminant adipose were combined to ensure that the modern ruminant adipose reference range of $\Delta^{13}\text{C}$ values takes into consideration both wild and domestic ruminant adipose isotopic measurements. However, ruminant adipose and ruminant milk fat categories partly overlap, and considerable variation still exists within the datasets which leads to uncertainty. This is further discussed in Section 7.2.1.

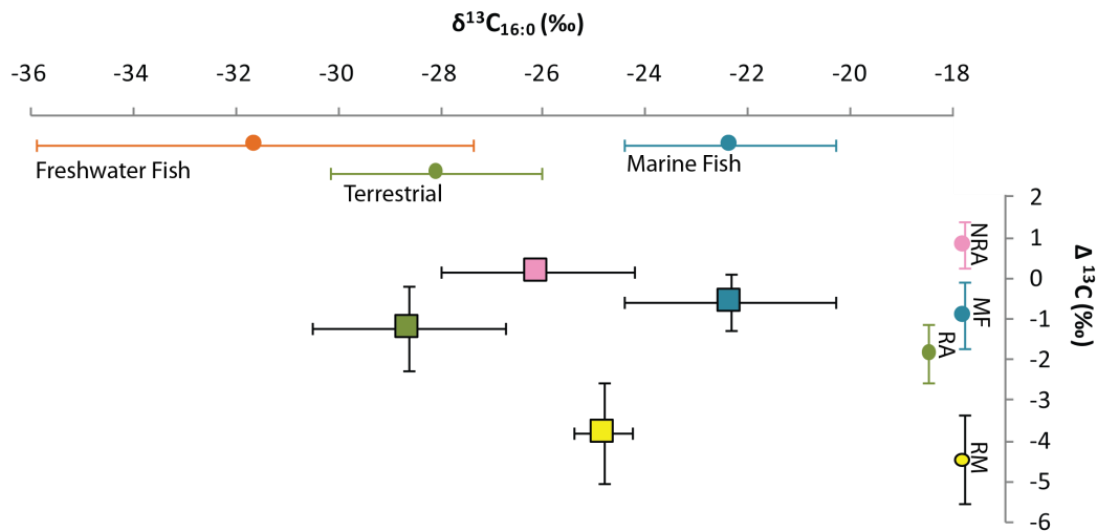


Figure 4.15: Plot of mean $\Delta^{13}\text{C}$ values ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) and mean $\delta^{13}\text{C}_{16:0}$ of modern Mediterranean reference fats [squares; Pink: Porcine blood; Blue: Marine fish; Green: Ruminant blood; Yellow: Ruminant milk]; against the combined mean $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements obtained from northern Europe (Dudd, 1999 and Craig *et al*, 2005a, 2007), and the Mediterranean values [circles]. The error bars denote $\pm 1\%$ standard deviation. [NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish]

Finally, further investigation is required to explain the outlier values obtained for Near Eastern ruminant adipose and milk values observed by Gregg *et al.* (2009), in particular since these two points show the greatest departure from the range of $\delta^{13}\text{C}$ values published so far. As discussed in Section 4.7.1, the more positive $\delta^{13}\text{C}$ values of the terrestrial fats appears to be caused by different climatic conditions, however, the range of $\Delta^{13}\text{C}$ values for some of the fats considered was also affected. In light of these results, it is advisable to be cautious in interpreting $\delta^{13}\text{C}$ values of archaeological residues originating from different countries. The results of this feeding experiment have highlighted the necessity of analysing modern animal and plant products that have been raised/grown locally to the region being studied. Further cautionary notes in interpreting archaeological $\delta^{13}\text{C}$ have been highlighted in previous published work, and were important in supporting the results described in this Section, and are summarised below:

- *Correction for post industrial carbon:* Since the carbon isotopic composition of plants (as primary producers) and animal fats (as consumers) depend on the isotopic composition of the atmospheric CO_2 fixed into organic compounds by photosynthesis, it is important to correct modern samples for the fossil fuel effect when comparing them to archaeological samples (Spangenberg *et al.*, 2006).
- *Isotopic values of carbon in urban centres:* In a study conducted by Smith and Epstein (1971) it was observed that although atmospheric CO_2 does not change isotopically with geography or

topography, isotopic variations do occur in urban air due to fossil fuel combustion increasing the ^{12}C content, which is reflected in plant tissues during carbon fixation in photosynthesis. A systematic difference was observed in plants collected from the Los Angeles area and those sampled from more rural areas in Utah and Texas, with the former consistently registering 0.4‰ to 1.2‰ lighter carbon isotopic values. Smith and Epstein's (1971) observations need to be taken into account when sampling.

- *The canopy effect*: This is caused by the re-use of plant fractionated respired CO_2 in areas of dense vegetation, which will change the local $\delta^{13}\text{C}$ of atmospheric CO_2 to values between -21‰ and -26‰ (Keeling, 1961). Van Klinken *et al.* (2000:41-42) point out that this can cause a bias when analysing animal and plant species living in forest floors and open environments. Canopy effects are therefore likely to influence the carbon isotope ratios of forest-dwelling communities and those living in more open landscapes, since variations will be expected between Mesolithic hunter-gatherers who consumed food from forest environments and Neolithic agrarian communities who obtained their food from more open landscapes (van Klinken *et al.*, 2000:41-42).
- *Seasonality*: In Copley *et al.* (2003) seasonal changes to the $\delta^{13}\text{C}$ values of plant biochemical components were accounted for by collecting plant samples at various times of the year, and it was concluded that factors such as latitude, altitude and atmospheric CO_2 concentrations were unlikely to have been sources of major variation in the $\delta^{13}\text{C}$ values of prehistoric animal fats from southern British archaeological sites. However, these results cannot be extrapolated to include other geographical locations, especially those which have considerable climatic differences. When considering the changes in $\delta^{13}\text{C}$ values of plant material (which then enter the food chain) with the fluctuations in temperature discussed above, seasonality might yet be found to play a major role in the $\delta^{13}\text{C}$ shifts, especially in countries that experience significant variations in temperature and water availability. Furthermore, archaeological scenarios which comprise domestic animals feeding on plant material obtained during different seasons have been recorded, for example during the winter months, animals might be feeding on available fresh produce but their diet may be further supplemented by fodder stored by farmers during the summer months. What if any, would be the effect on the $\delta^{13}\text{C}$ values of the plant material, and how would this be reflected in the animals' carbon isotopic values?
- *Food processing*: Differences in the $\delta^{13}\text{C}$ values of modern and archaeological lipids may result from alteration during food processing. Spangenberg *et al.* (2006) observed higher values (around 4‰) in the main fatty acids of sheep and goat cheese compared to raw milk samples, which they attributed to bacterial degradation of the long chain fatty acids during cheese

making and storage. In experiments conducted by Evershed *et al.* (1999), it has been established that the isotopic composition of the C_{16:0} and C_{18:0} fatty acids does not alter during burial. Spangenberg *et al.* (2006), however, observed more positive values for the $\delta^{13}\text{C}$ of bulk cow milk and the main fatty acids during heating, which was associated with the preferential release of isotopically light, more volatile and thermally and chemically less stable compounds (including carbohydrates, lipids, proteins and vitamins) during heating. The higher $\delta^{13}\text{C}$ values obtained for the C_{18:0} and C_{18:1} fatty acids was explained as being due to the preferential loss of ¹²C-rich moieties (Spangenberg *et al.*, 2006). A similar isotopic shift was observed during the thermal treatment of olive oil (Spangenberg *et al.*, 1998; Spangenberg and Ogrinc, 2001).

4.9 Conclusion and further work

The results of the controlled feeding experiment carried out on Mediterranean animal products complement the cautionary note issued by Gregg *et al.* (2009), who also observed shifts in the $\delta^{13}\text{C}$ measurements of Near Eastern terrestrial fats when compared to northern European measurements. As discussed above, this shift is probably caused by changes to the $\delta^{13}\text{C}$ of plants which occurs due to varying temperature and humidity levels during carbon fixation in different geographical regions, which are then transmitted along the food chain and thus produce a shift in the $\delta^{13}\text{C}$ values of animal products. Although the nature of the experiment did not allow a large number of replicated samples to be analysed, ruminant dairy products showed more positive $\delta^{13}\text{C}$ values with respect to northern European values, which was also observed in ruminant and non-ruminant blood samples. The lower $\delta^{13}\text{C}$ values observed in Mediterranean marine fish needs further investigation. Of greater importance, $\Delta^{13}\text{C}$ values of Mediterranean ruminant and non-ruminant blood/adipose, and ruminant milk differed from those obtained from modern reference fats of northern European origin.

The next step is to conduct broader bulk and compound specific carbon isotope analyses on a variety of modern plant samples within the Mediterranean and northern European regions, to better assess the extent of the $\delta^{13}\text{C}$ shifts at different geographical latitudes. Carrying out a controlled feeding experiment with the aim of analysing, using bulk and compound specific isotope analyses, both the plant material included in the diet, and adipose and milk samples obtained from the animals feeding on the specified diet (as carried out Dudd, 1999), may help better identify the relationship between dietary, adipose and milk $\delta^{13}\text{C}$ values. The $\delta^{13}\text{C}$ values of carbohydrates, fats and proteins are different; hence varying the proportion of each in the diet is likely to influence the $\delta^{13}\text{C}$ measurements of the animals that consume them. Monitoring changes

to the $\delta^{13}\text{C}$ values by varying the proportion of each could be carried out to investigate possible shifts in the $\delta^{13}\text{C}$ values, which can be attributed to variations in the diet. Extending the controlled feeding experiment to include different climatic regions would allow a more comprehensive comparative analysis to be made on the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values obtained.

The controlled feeding experiment described in this chapter produced the first set of modern reference $\delta^{13}\text{C}$ values for Mediterranean animal and marine fats. The results obtained here will be used to interpret lipid residues extracted from Impressed/Cardial Wares, described in Chapter 7.

Chapter 5

Plant lipid biomarkers and multiple cooking episodes: archaeological implications

5.1 Introduction

Archaeobotany has contributed considerably to our understanding of crop plant evolution and palaeodiet, through meticulous archaeological and scientific studies of botanical evidence recovered as charred, desiccated or waterlogged remains from various stratigraphic deposits. Plants have been attested as impressions on pottery, daub and bricks, digested or partly digested in coprolites, and occasionally recovered *in situ* from ceramic vessels, for example at the sites of Can Sadurní (Antolín and Buxó, 2011) and La Draga (Tarrús, 2008), in Spain. Indirect evidence for plant cultivation has been obtained by studying tool assemblages associated with cultivation, harvesting and the processing of crops, the presence of irrigation canals, terraces, plough marks and cultivation boundaries, palynology, weeds, and also artistic attestations of cultivated plants (Zohary and Hopf, 2000:1-7). Multidisciplinary high resolution scientific analyses have also been applied to investigate the presence of plant remains in the past, for example investigation of plant microfossils such as starch and phytoliths (Hardy *et al.*, 2009; Piperno *et al.*, 2009; Henry *et al.*, 2011), stable isotope analyses of bulk plant remains (Hastorf and DeNiro, 1985; Tieszen, 1991), organic residue analysis (ORA) (Evershed *et al.*, 1991; Reber and Evershed, 2004b; Copley *et al.*, 2005e; Steele *et al.*, 2010), and genetics (Salamini *et al.*, 2002; Doebley *et al.*, 2006; Allaby, 2008; Allaby *et al.*, 2008).

ORA has already proved successful in identifying specific biomarkers for epicuticular waxes of leafy vegetables from the *Brassica* family, dating to the 9th-13th centuries AD (Evershed *et al.*, 1991). These were found to include a complex pattern of preserved acyl lipids, as well as nonacosane-15-one, nonacosan-15-ol and nonacosane (Evershed *et al.*, 1991). As already described in Chapter 3, the quantity of plant lipids which become absorbed within the ceramic matrix of cooking vessels is very low (Evershed *et al.*, 1995a), which is not conducive to their survival over archaeological timescales. Moreover, because of the low quantities of lipids in starchy grains (e.g. cereals), their chemical signature is easily masked if products containing higher quantities of fat are processed within the same vessel, especially animal products (Reber and

Evershed, 2004b). Experimental work has shown that the fat deposited in a vessel's wall after five boiling of meat was around 150 times greater than that absorbed after ten boiling episodes of *Brassica* vegetable leaves (Evershed, 2008a). Plant products have been widely attested in the archaeobotanical evidence recovered from the Early Neolithic sites included in this study, though at times the evidence is quite fragmentary (see Chapter 2). However, combined with the palaeodietary evidence discussed in Chapter 8, there is strong circumstantial evidence to support a heavy reliance on plant material, in particular during the Early Neolithic on the Murge Plateau in Apulia, Italy.

This chapter describes a series of cooking experiments carried out using three plant varieties, acorns (*Quercus* spp.), nettles (*Urtica dioica*) and einkorn (*Triticum* spp.), whose remains were identified through botanical studies as being part of the Early Neolithic subsistence in both Europe and the Mediterranean (Price, 2000:6; Pessina and Tiné, 2008:203). Based on previous experiments (e.g. Reber and Evershed, 2004b), degraded plant residues extracted from ceramic vessels are likely to produce chromatograms that are very similar and perhaps indistinguishable from lipid residues at an advanced stage of degradation. This required further investigation, which therefore led to the experimental procedure described in this chapter. The main aims of this experiment were:

- To establish the impact of burial on the survival of lipid residues absorbed within ceramic vessels after boiling acorns, nettles and einkorn, by examining the quantity of lipid residue extracted after a seven month burial period.
- To investigate whether specific biomarkers survive which would allow a more confident identification to species level of acorns, nettles and einkorn, in both the absorbed and visible residues, using Gas-Chromatography Mass Spectrometry (GC-MS).
- To investigate whether the lipid profile obtained for einkorn can still be determined when boiled with milk, or whether the fat content of the latter would completely mask its presence, by comparing the degraded lipid residues obtained after boiling einkorn in water and milk.
- To assesses the affect of pH on the survival potential of the lipid residues by burying sub-samples of the pottery vessels used during the cooking experiments in acidic and more alkaline burial contexts, the latter being consistent with the burial deposits at the sites investigated.

In this chapter, lipid residues are considered significant if they contain more than $5\mu\text{g g}^{-1}$ of lipid. Residues containing less than the recommended quantity of lipid cannot be confidently characterised because they cannot be securely distinguished from background contamination (Evershed, 2008a).

5.2 Experimental methodology

The cooking experiments were carried out in Schleswig-Holstein, Germany in July 2009 as part of the *Pottery Use among Late Foragers and Early Farmers in the Baltic* project. Replica pointed-base (Ertebølle) vessels and funnel beaker pots (1-2L capacity) were built using modelling clay with equal proportions of granite temper. The vessels were constructed using the coiling technique, and then kiln fired (Figure 5.1). Since the main aim of the experiment was to attempt to better understand the bimolecular imprint and preservation of plant lipids absorbed within ceramic vessels, the form of the vessels, which is not consistent with Mediterranean Early Neolithic pottery, was considered irrelevant to the wider objectives. The cooking episodes were carried out over a direct fire by perching the cooking vessel onto three pointed stones used to construct the hearths (Figure 5.1). Once the cooking experiments were completed, the vessels were wrapped in foil and stored at -20°C pending analysis.

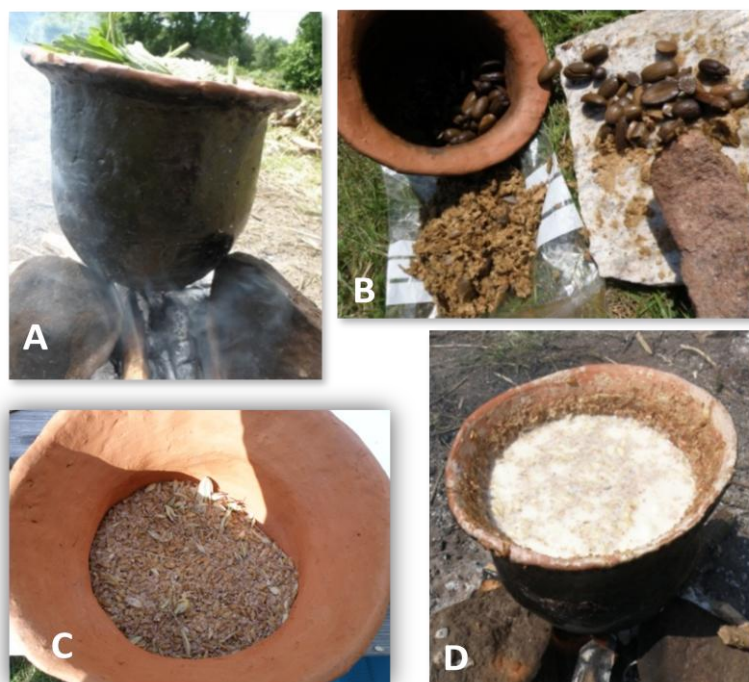


Figure 5.1: Replica Ertebølle and funnel beaker pots used to boil A: Nettles; B: Acorns; C: Einkorn, and D: Einkorn and milk. [Photos courtesy of Hayley Saul]

5.2.1 Acorns (*Quercus* spp.)

The acorns used for this experiment were collected from Northamptonshire (UK) in October 2008. Three 1 hour boiling episodes were carried out, each comprising 117g of acorns and 750mL of water (Figure 5.1). Acorn remnants from each cooking episode were removed prior to the next boiling. At the end of the last boiling, the remaining acorns were crushed then boiled in water for a further two hours.

5.2.2 Nettles (*Urtica dioica*)

Nettles were collected from the area surrounding the site where the cooking experiments were being conducted. Around 120g of nettles were boiled for 1 hour using 1 litre of water, with an additional 500mL of water being added 30 minutes into the first boiling episode to replace water lost through evaporation (Figure 5.1). Six boiling episodes were carried out. Fresh nettles were used at the start of each cooking episode.

5.2.3 Einkorn (*Triticum* spp.)

This experiment included shop bought einkorn and wheat bran, as well as wheat chaff collected during harvest time in Yorkshire (UK). One hour boiling episodes were repeated 6 times, and were carried out using 250g of einkorn, 44g of wheat bran and 6g of wheat chaff in 750mL of water (Figure 5.1). Leftover plant material was cleaned out from the funnel beaker vessel prior to each boiling episode.

5.2.4 Einkorn and milk

This experiment comprised the ingredients and conditions listed in Section 5.2.3, however 750mL of unpasteurised cow's milk was used instead of water. Six cooking episodes were carried out, each lasting 1 hour. The unpasteurised milk was purchased locally from Schleswig-Holstein (Figure 5.1).

5.2.5 Sampling and burial conditions

A crust was observed to form on the surface of each of the four vessels during cooking. Between 10 and 20mg of this visible residue was collected for analysis prior to burial. Samples of the thermally degraded lipid absorbed within the ceramic walls were also required. These were

obtained by removing a section of each vessel before burial, then sub-sampling around 2g of ceramic powder from the inner surface using a Dremmel drill with an abrasive tungsten bit, discarding the first layer to remove any possible contaminants.

The cooking vessels were then prepared for burial in North Yorkshire, UK. Each vessel was further sub-divided and one part of each of the pots was buried to a depth of 0.5m in two locations chosen for the acidic and alkaline nature of their soil, Star Carr and Thixendale respectively (Figure 5.2). Clean ceramic sherds were buried as controls at each location. The sherds remained buried for seven months, between February and September 2010, before being unearthed. Sub-samples comprising around 2g of powdered ceramic were obtained for analysis using a Dremmel drill as described above. Soil samples were collected from both locations for pH testing. A complete list of the samples analysed are recorded in Table 5.1.

<i>Samples</i>	<i>Description</i>	<i>Burial conditions</i>
Acorns	Acorn crust	Visible residue from cooking vessel
	ACE-02	Absorbed lipid residue
	SAC-02	Absorbed lipid residue
	WOD-02	Absorbed lipid residue
Nettles	Nettle crust	Visible residue from cooking vessel
	NCE-01	Absorbed lipid residue
	SAC-01	Absorbed lipid residue
	WOD-1	Absorbed lipid residue
Einkorn	Einkorn crust	Visible residue from cooking vessel
	ECE-06	Absorbed lipid residue
	SAC-06	Absorbed lipid residue
	WOD-06	Absorbed lipid residue
Einkorn & milk	Einkorn & milk crust	Visible residue from cooking vessel
	EMC-08	Absorbed lipid residue
	SAC-08	Absorbed lipid residue
	WOD-08	Absorbed lipid residue
Controls & soils	SAC Control	Control blank from Star Carr
	WOD Control	Control blank from Thixendale
	SAC Soil*	Soil sample from Star Carr
	WOD Soil*	Soil sample from Thixendale

Table 5.1: Complete list of the samples analysed. [*Samples prepared and quantified by Hancox (2011)]



Figure 5.2: Map of the United Kingdom showing the locations of Star Carr and Thixendale, in North Yorkshire where the pot sherds were buried.

5.3 Laboratory sample preparation

Sample preparation and analysis followed the same protocol described in Chapter 6, although 2 μ g of tetratricontane (C34 *n*-alkane) internal standard (IS) were added to each sample for quantification purposes prior to extraction. All samples were run as trimethylsilyl (TMS) derivatives. Prior to analysis, 1 μ g of hexatricontane (C36 *n*-alkane) internal standard was added, and the samples were vortexed for 30s to ensure a homogenised solution.

Samples were analysed in duplicate by High Temperature-Gas Chromatography (HT-GC), to promote the elution of higher molecular weight, hence less volatile biomolecules, in particular triacylglycerols. An Agilent 7890 series GC was used, fitted with a 15m, DB-1HT (100% Dimethylpolysiloxane) (J&W Scientific) column with a 0.32mm internal diameter and a film thickness of 0.1 μ m. The peaks were identified by comparing retention times to known standards (detailed in Chapter 6), which were run using the same chromatographic conditions as the samples.

The samples were also analysed using GC-Mass Spectrometry (GC-MS), using the instrumental details outlined in Chapter 6. Identification of the mass spectra was carried out using extensive NIST library searches and published data, mainly Evershed (1992c), Stacey (1999) and Christie (2011a), as well as knowledge of fragmentation patterns.

5.3.1 Quantification and error calculation

Peak integration was carried out using ChemStation Rev. B.04.02 SP1. Automated integration was selected over manual integration to eliminate inconsistencies; hence the error introduced at this point was constant in all samples and can therefore be considered insignificant. Good chromatographic resolution supported accurate integration. The variable response of the FID to different functional groups was taken into consideration. Correction factors can be applied to offset such discrepancies, but only slight variations to the overall value have been reported (Christie, 2003:59) and consequently, they were not applied in the present study. Moreover, not all the peaks analysed by GC and GC-MS could be confidently identified, hence correcting the response factor solely for the known components would have biased the quantification value of the TLE against the unidentified components. The lipid content in each sample was quantified using the following formula: $(\text{Area}_{(\text{Sample})}/\text{Area}_{(\text{IS})}) * (\text{Weight}_{(\text{IS})}/\text{Weight}_{(\text{Ceramic sample})})$, omitting contaminant peaks such as plasticisers.

Accurate quantification measures were required to meet the objectives of the present study. Samples were therefore run in duplicate, and an average calculated for the quantified residue. To assess the precision of both internal standards, the standard error was calculated for the FID response (area) obtained for each internal standard. The average percentage standard error for the C34 and C36 *n*-alkanes was found to be 2.2% and 1.9%, respectively. Chromatographic reproducibility was consistent. Two internal standards were used to calculate the percentage recovery of the total lipid extract (TLE). C34 *n*-alkane was introduced before extraction and C36 *n*-alkane was added just prior to sample injection, as detailed above. Using 2µg of C34 *n*-alkane prior to extraction was however not conducive to obtaining realistic results. Extractions were therefore repeated for six of the samples using 40µg of C34 *n*-alkane before solvent extracting. The average percentage recovery for the six samples was 66.8% (±4.6%). Since the extraction method was the same as that described above, and was consistently applied to all the samples, this value is considered to be representative of the whole. The quantified results reported below were calculated against the C36 *n*-alkane, which was considered to be the more reliable and consistent of the two internal standards.

5.4 Results and discussion

5.4.1 Control blanks

Control blanks were buried at Star Carr and Thixendale to test for possible infiltrations of organic material from the burial environment. Trace amounts of C_{16:0} and C_{18:0} fatty acids were identified in the chromatograms obtained, along with phytosterols β -sitosterol and stigmasterol, the latter being observed only at Thixendale (Appendix B). The compounds observed have already been identified by Heron *et al.* (1991a) as characteristic components of soil lipid composition, and will be discussed further in the following section. Quantification of the absorbed residue in both control blanks was less than 0.3 $\mu\text{g g}^{-1}$. Soil lipid migration can therefore be considered negligible. Laboratory blank controls also contained negligible quantities of residue.

5.4.2 Soil Samples: pH and lipid residue

Soil pH analysis was carried out at the University of Bradford following established protocols (Foth, 1990; Powell, 1994), and was reported by Hancox (2011:49) as being mildly acidic at Star Carr (pH 6) and moderately alkaline at Thixendale (pH 8). Soil composition is remarkably complex and comprises a wide range of components (e.g. lipids, carbohydrates, peptides, cellulose, lignin and humic material) primarily contributed from localised animal and plant detritus, which is subject to microbial activity (Simoneit *et al.*, 2004). Table 5.2 shows the lipid composition identified by GC-MS analysis at both sites, while Figure 5.3 shows their respective chromatograms.

Trace amounts of odd and even chain fatty acids were detected at both sites as TMS derivatives. They were identified by the major fragment ion present at $[M-15]^+$ and their respective molecular ion (M^+) which was also generally present. Chain lengths were mostly consistent with those reported by Heron *et al.* (1991a) (comprising 16 to 33 carbon atoms), although shorter chain fatty acids (C_{10:0}, C_{12:0}, C_{14:0} and C_{15:0}) were identified at both sites. Unlike Heron *et al.*'s (1991a) observations, pentacosanoic acid (C_{25:0}), which was identified as a major soil component in the 1991 study, was detected as a minor peak in the present study. Hexacosanoic (C_{26:0}) and tetracosanoic (C_{24:0}) fatty acids were the major fatty acids identified at Star Carr, while none of the fatty acids were observed to dominate the chromatogram obtained for the Thixendale soil sample (Figure 5.3). The presence of even-chain, saturated fatty acids are most likely to have been introduced by plants and fungi, as observed in previous studies (Stacey, 1999:101). Branched-

chain fatty acids and fatty acids with odd and even chain lengths in excess of 20 carbon atoms (often unsaturated) are generally associated with bacterial input (Stacey, 1999:101). Saturated, straight chain fatty acids with over 20 carbon atoms were observed in both soil samples, however C_{15:0} and C_{17:0}, which are more commonly associated with bacterial activity (Dudd *et al.*, 1998), were identified only at Thixendale. Fatty acids in the soils are also thought to arise from the terminal oxidation of plant alcohols and alkanes by soil microorganisms (Amblès *et al.*, 1994).

Both odd and even numbered alkanes comprising 20 to 31 carbon atoms were identified at Star Carr, while the range of alkanes present at Thixendale was more limited (Table 5.2). Chain lengths of 29 and 31 carbons were dominant in the homologous series at both sites. Identification was based on the characteristic fragmentation pattern produced by mass fragments of diminishing intensity corresponding to lost alkyl groups, and to the presence of a weak M⁺. The occurrence of even numbered alkanes does not tally with Heron *et al.*'s (1991a) results, who reported only odd numbered alkanes with 21 to 33 carbon atoms. Traces of alkenes were also identified at Star Carr, but their chain length could not be confidently determined.

Sample	Quantification (µg g ⁻¹)	Lipid compounds identified
Soil sample Star Carr	7	FAs: C _{10:0} , C _{12:0} , C _{14:0} , C _{16:0} , C _{18:0} , C _{20:0} , C _{21:0} , C _{22:0} , C _{23:0} , C _{24:0} , C _{25:0} , C _{26:0} ; Alkanes: C20, C23, C25, C26, C29, C31; Alcohols: C16, C22, C24, C26, C28, C31; Alkenes: Unidentified; Glycerol TMS ether; Sterols: β-sitosterol; Sugars: Glucose, Mycoses; WEs: C42, C44
Soil sample Thixendale	23	FAs: C _{12:0} , C _{14:0} , C _{15:0br} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} , C _{22:0} , C _{24:0} , C _{25:0} , C _{26:0} , C _{30:0} ; Alkanes: C29, C31; Alcohols: C20, C22, C24, C25, C26, C27, C28, C32; Sterols: β-sitosterol, Stigmastan-3,5-dien, Campesterol

Table 5.2: GC-MS identification of the soil lipid composition at Star Carr and Thixendale. [FA/C_{x,y}: Fatty acids where x is the carbon number and y is the degree of unsaturation; Br: Branched fatty acid; WE: Wax ester]

Phytosterols, important constituents of higher plants comprising mainly β-sitosterol, campesterol and stigmasterol (deMan, 1999:51, 54), were identified in both soil samples (Table 5.2). Identification was based on the presence of a base peak at *m/z* 129 and the M⁺, as well as ions present at [M-90]⁺ and [M-129]⁺ which identify each sterol. A more detailed explanation of the fragmentation patterns of sterols can be obtained from Evershed (1992c:293-295). Incorporation of phytosterols as soil constituents is likely to be due to input from plant detritus. β-sitosterol was the dominant phytosterol in both samples, which is not surprising since it has already been identified as the major contributor of the sterol fraction in soils (Stacey, 1999:99).

Primary long chain alcohols also occur in plants, as free components or esterified to fatty acids to form wax esters (Stacey, 1999:98). A wide range of alcohols comprising chain lengths between 16 and 32 carbon atoms (Table 5.2), were identified in the soil samples collected at both locations. Both even and odd chain alcohols were observed, the latter probably resulting from terminal bio-oxidation of soil alkanes by microorganisms (Jambu *et al.*, 1993). Identification of alcohols as TMS derivatives was based on the presence of the strong fragment ions produced at $[M-15]^+$ and weaker M^+ . In addition to the distinctive spectrum produced, the ion at m/z 103 representing the $CH_2OSi(CH_3)_3$ fragment, is a good indicator of *n*-alcohols. Both samples were dominated by hexacosanol (C26) alcohol, which was also identified as a component of the palmitate and stearate wax esters detected at Star Carr. These were identified by their distinctive base peaks forming at m/z 257 ($C_{16}H_{33}O_2$)⁺ and m/z 285 ($C_{18}H_{35}O_2$)⁺ for the palmitate and stearate moieties respectively, and also from the presence of their corresponding M^+ at m/z 648 and 620. Wax esters with carbon lengths of between 42 and 56 have already been documented as soil constituents (Heron *et al.*, 1991a), and probably originate from the surrounding vegetation (Simoneit *et al.*, 2004).

Acylglycerols have also been reported as constituents of soil lipids (Heron *et al.*, 1991a). Glycerol TMS ether was identified at Star Carr, however no acylglycerols were identified in either soil sample. The presence of glycerol at Star Carr might indicate hydrolysis of acylglycerols (Hita *et al.*, 1996). Lastly, Figure 5.3 shows that several peaks remained unidentified in both soil samples. The fragment ion patterns observed in the TIC of these peaks was not present in the absorbed residue extracted from the sherds and visible residue, prior to or after burial. Hence, these unknown constituents appear to be particular to the soil samples and are unlikely to have influenced the composition of the residues obtained during the cooking experiments.

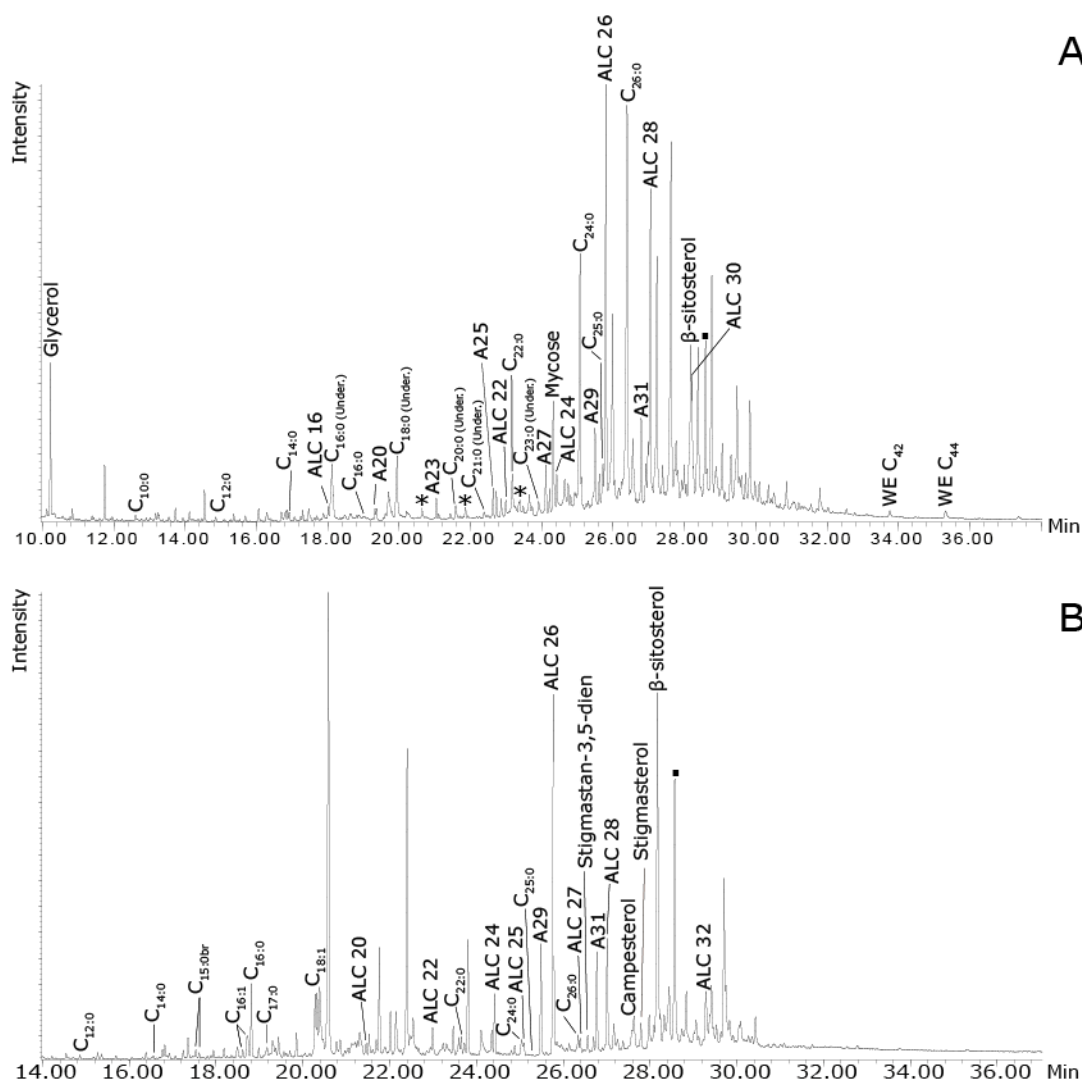


Figure 5.3: Partial Total Ion Chromatograms (TIC) of the soil samples obtained from A: Star Carr and B: Thixendale. [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; Br: branched; Under.: Underivatised; A: Alkane; *: Alkene; ALC: Alcohol; WE: Wax Ester; ■: C34 Internal standard]

5.4.3 Acorns

Acorns characteristically contain low amounts of protein but are rich in fat and starch (León-Camacho *et al.*, 2004b), which is conducive to lipid absorption within the walls of ceramic vessels during cooking. However, does the absorbed lipid survive over archaeological timescales, and are particular biomarkers present which would identify the residue as acorn? León-Camacho *et al.*'s (2004b) publication, which investigated the lipid composition of three species of acorn in Spain, has been used as the main reference in the following discussion. Table 5.3 lists the compounds identified in the visible and absorbed residue obtained after thermal degradation (boiling) and subsequent burial.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
Acorn Crust (Unburied visible residue)	722	FAs: C _{14:0} , C _{16:1} , C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien; MAGs: 1-Monopalmitin, 1-Monolinoleate; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C42, C50, C52, C54; Gamma-Tocopherol TMS Ether; Sugars: unidentified
ACE-02 (Absorbed residue; Pre-burial)	17	FAs: C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol; Alkenes: unidentified; Alcohols: C26, Inositol; MAGs: 1-Monopalmitin; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D32, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C44, C46, C48, C50, C52, C54; Gamma-Tocopherol TMS Ether; Sugars: unidentified
SAC-02 (Absorbed residue; Star Carr Burial)	2	FAs: C _{14:0} , C _{16:0} , C _{18:1} , C _{18:0} ; Alkanes: C25, C27, C29, C31; Alcohols: C24, C26, C28; Sterols: β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol, Cholestan-3,5-dien; MAGs: 1-Monopalmitin, 2-Monopalmitin; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C50, C52, C54; Sugars: unidentified
WOD-02 (Absorbed residue; Thixendale Burial)	3	FAs: C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Alkanes: 25, 27, 29, 31; Alkenes: unidentified; Alcohols: C24, C26, C28, C30; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol, Ergosterol; MAGs: 1-Monopalmitin, 2-Monopalmitin, 1-Monooleate; DAG: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C50, C52, C54; Sugars: unidentified

Table 5.3: GC-MS and HT-GC identification of the lipid composition of boiled acorn analysed before and after burial. [FA/C_{x,y}: Fatty acids where x is the carbon number and y is the degree of unsaturation; MAG: Monoacylglycerol; D & DAG: Diacylglycerol; TAG: Triacylglycerol]

5.4.3.1 Fatty acids

León-Camacho *et al.* (2004b) identified C_{14:0}, C_{16:0}, C_{17:0}, C_{18:0} and C_{20:0} with up to three unsaturations in the C_{18:0} and one unsaturation in C_{16:0}, C_{17:0} and C_{20:0} fatty acids in fresh acorn. The fatty acid profile in the degraded residues showed a similar distribution (Table 5.3) comprising C_{14:0}, C_{16:0} and C_{18:0}, with up to one unsaturation in the C_{16:0} and two unsaturations in the C_{18:0}; although as expected, the relative percentage composition of the individual fatty acids varied considerably when compared to the non-degraded profile. Table 5.4 records the percentage composition of free fatty acids in the TLE of degraded acorn. The most abundant fatty acid observed in the degraded residues was C_{16:0}, which comprised 6% of the TLE content of the unburied absorbed residue, and on average, 10% of the TLE of samples further degraded by burial (Table 5.4).

FA	Acorn Crust (%) (Pre-burial)	Acorn Absorbed (%) (Pre-burial)	SAC-02 (%) (Post-burial)	WOD-02 (%) (Post-burial)
16:0	3.2	6.4	10.3	9.6
18:2	0.3	3.6	-	1.2
18:1	1.1	7.6	6.2	2.7
18:0	0.9	4.1	9.1	9.1

Table 5.4: Percentage composition of individual free fatty acids in the TLE of degraded acorn. [FA: Fatty acid; SAC: Star Carr burial; WOD: Thixendale burial]

Unsaturated fatty acids are unlikely to survive over archaeological timescales due to the susceptibility of their double bonds to oxidation reactions (Evershed *et al.*, 1992; Regert *et al.*, 1998a, 2001b). They were however detected in both the thermally degraded and buried samples, although the ratio of unsaturated:saturated fatty acids decreased from 5 in the fresh samples (León-Camacho *et al.*, 2004b) to between 0.2 and 1 in the degraded acorn residues. $C_{18:1}$ decreased by over threefold when compared to the amount present in fresh acorns. Depletion in $C_{18:1}$ and the concomitant increase in $C_{16:0}$ has been observed in degradation experiments carried out by Morgan *et al.* (1973) and Den Dooren de Jong (1961), who attributed this to β -oxidation and reduction reactions leading to the replacement of $C_{18:1}$ by $C_{16:0}$ after the hydrolysis of acylglycerols. Dudd *et al.* (1998) later showed that this conversion was more likely to be caused by microbial action, as already mentioned in Chapter 3. This is supported by the percentage compositions obtained for the buried acorn samples (SAC-02 and WOD-02), both of which showed an increase in $C_{16:0}$ with respect to $C_{18:1}$. Furthermore, in the pre-burial absorbed residue (ACE-02), the percentage composition of $C_{18:1}$ was still higher than $C_{16:0}$, showing less advanced degradation. The presence of unsaturated free fatty acids in the degraded acorn residues could also result from hydrolysis of triacylglycerols, since both mono- and diacylglycerols were identified (Table 5.3).

The visible crust showed low levels of free fatty acids when compared to the absorbed residues (Figure 5.4 and Table 5.5). $C_{16:0}$ comprised 3.2% of the total free fatty acid content in the TLE, with levels of $C_{18:1}$ being even lower than those obtained for the absorbed residue buried at Star Carr (SAC-02) (Table 5.4), hence showing a considerable loss of $C_{18:1}$. However, as shown in Figure 5.4 and Table 5.5, triacylglycerol preservation appears to be considerably better in the visible crust than in the corresponding unburied absorbed residue (ACE-02), which indicates low rates of triacylglycerol hydrolysis (discussed below). It therefore appears that crusts are more susceptible to oxidative degradation, than to hydrolysis. These seemingly opposing results suggest that residues absorbed within the ceramic matrix are better protected against the onset of oxidation than visible residues, but the absence/or reduced quantities of metal ions in the latter, which are known to act as catalysts for hydrolysis (Aillaud, 2001:5), would considerably slow down this degradative process.

5.4.3.2 Acylglycerols

Tri-, di- and monoacylglycerols comprise three, two or one fatty acid moieties attached to a glycerol backbone respectively (see Chapter 3). Mills and White (1994:31-34) define fats and oils as 'mixtures of mixed triglycerides' (TAG), containing a broad range of possible combinations of long chain fatty acids, which can be saturated or unsaturated. As already described in Chapter 3 and in the previous section, TAGs are subject to hydrolysis, which essentially involves cleavage of the ester bonds, thus releasing the individual constituents (Evershed *et al.*, 1992). Hence, depending on the stage of the reaction, TAGs are broken down to diacylglycerols (DAGs), monoacylglycerols (MAGs) and finally, glycerol and free fatty acids. Hydrolysis was found to accelerate after the first fatty acid has been removed (Dudd *et al.*, 1998), and this was found to occur during cooking, storage and burial (Evershed *et al.*, 1992).

<i>Compound</i>	<i>Acorn Crust (%) (Pre-burial)</i>	<i>Acorn Absorbed (%) (Pre-burial)</i>	<i>SAC-02 (%) (Post-burial)</i>	<i>WOD-02 (%) (Post-burial)</i>
<i>FAs</i>	5.1	21.7	25.7	22.7
<i>MAGs</i>	5.2	1.1	3.3	7.5
<i>DAGs</i>	16.1	15.5	3.8	6.5
<i>TAGs</i>	42.4	29.1	10.0	14.8

Table 5.5: Percentage composition of fatty acids (FAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and triacylglycerols (TAGs) in the TLE of degraded acorn. [SAC: Star Carr burial; WOD: Thixendale burial]

Chapter 5

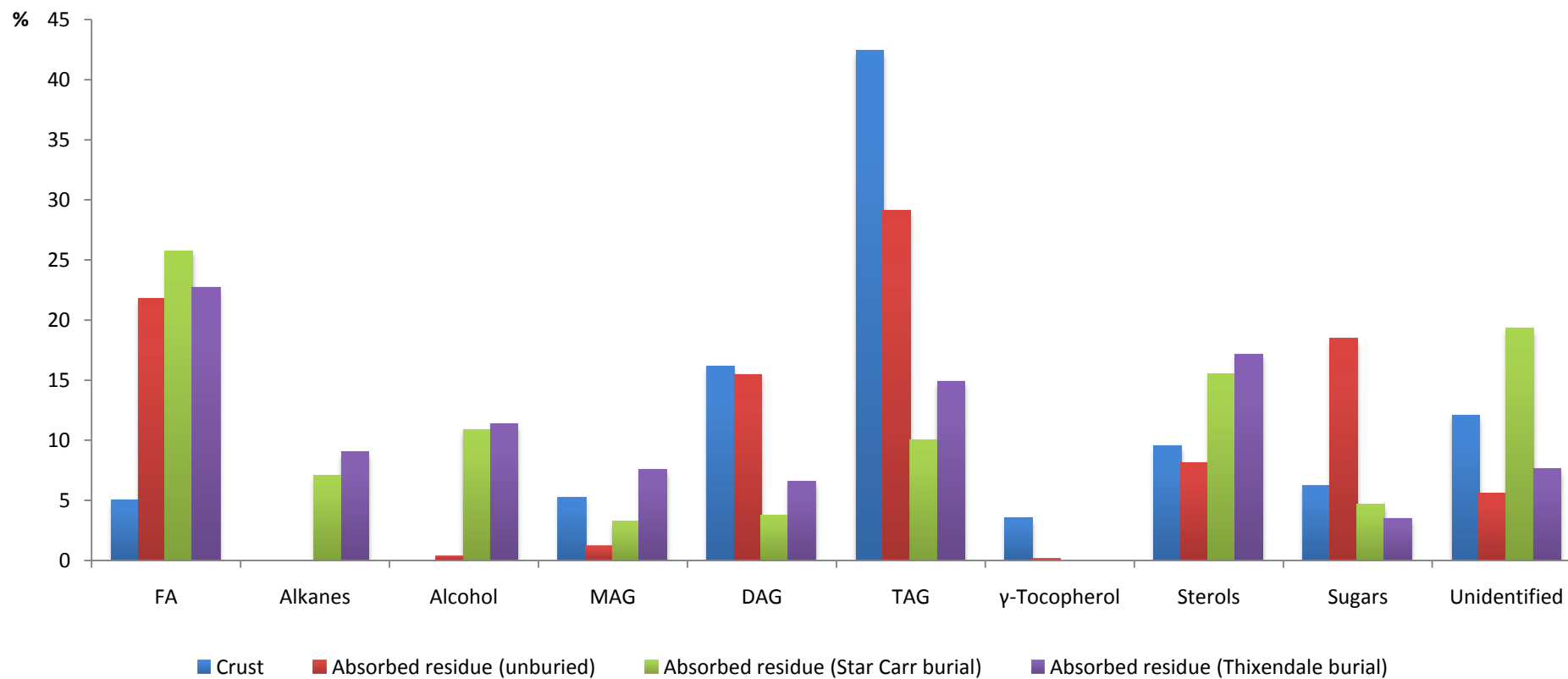


Figure 5.4: TLE profile of degraded acorn as percentages of the absolute quantified residue. [FA: Fatty Acid; MAG: Monoacylglycerol; DAG: Diacylglycerol; TAG: Triacylglycerol]

TAGs and DAGs were identified by comparing retention times to known standards. DAGs were further characterised by the fragmentation patterns obtained through GC-MS analysis following Evershed (1992c) and Stacey (1999). Evidence for the occurrence of hydrolysis in degraded acorn can be observed by the presence of DAGs and MAGs (Figures 5.6 and 5.7). In the pre-burial samples, TAGs comprised 42% and 29% of the TLE for the crust and absorbed residue respectively (see Figure 5.4 and Table 5.5). Upon further degradation by burial, TAG hydrolysis increased as expected, thus there is a considerable reduction in the percentage TAG composition of the TLE, particularly in the acidic environment of Star Carr where the TLE comprises only 10% TAGs. The low quantities of DAGs and MAGs in the buried samples, whose quantified values were found to be consistently lower than for TAGs, shows the rapidity with which hydrolysis reactions reach completion. This is further highlighted by the inversely proportional relationship between the percentage composition of TAGs and fatty acids in the TLE of the degraded residues (Figure 5.4 and Table 5.5).

León-Camacho *et al.* (2004b) observed a range of TAGs of between 40 and 54 carbon atoms (C₄₀-C₅₄), comprising different combinations of the following fatty acids: palmitic (C_{16:0}), stearic (C_{18:0}), oleic (C_{18:1}), linoleic (C_{18:2}) and linolenic (C_{18:3}). In the present study, the widest range of TAGs survived in the unburied absorbed residue (ACE-02), in which all the TAGs mentioned in León-Camacho *et al.* (2004b) were identified and eluted between 32 and 42 minutes (Figure 5.6). TAGs with 50, 52 and 54 carbon atoms were preserved in both buried samples as well as the crust, with C₄₂ also being preserved in the latter. C₅₂ was the most prominent TAG in all the samples. DAGs and MAGs with up to two unsaturations in the C_{16:0} and C_{18:0} fatty acid moieties were also identified (Table 5.3), and reflect the unsaturated fatty acid constituents identified by León-Camacho *et al.* (2004b). Figure 5.5 shows the mass spectrum obtained for a DAG containing a C_{16:0} and C_{18:2} fatty acid moieties. Characteristic ions at *m/z* 129 and *m/z* 145 were present, together with fragments denoting [M-15] (*m/z* 649) and [M-90] (*m/z* 574), showing the loss of a methyl group and loss of a TMS group respectively. The ions at *m/z* 313 and 395 were identified as [M-RCOOCH₂] fragments indicating C_{16:0} and C_{18:2} respectively. DAGs and MAGs listed in Table 5.3 were similarly identified. The TAG pattern in acorns is therefore well known and could perhaps be used to indicate acorn residues; more so since the unsaturated fatty acid moieties in the TAGs can still be observed in the DAGs and MAGs as products of hydrolysis. However, the unsaturated nature of the fatty acid moieties is subject to oxidation, and hydrolysis will further affect TAG preservation. Hence over archaeological timescales, the onset of degradation is unlikely to allow the assignation of an acorn origin based on TAG distribution.

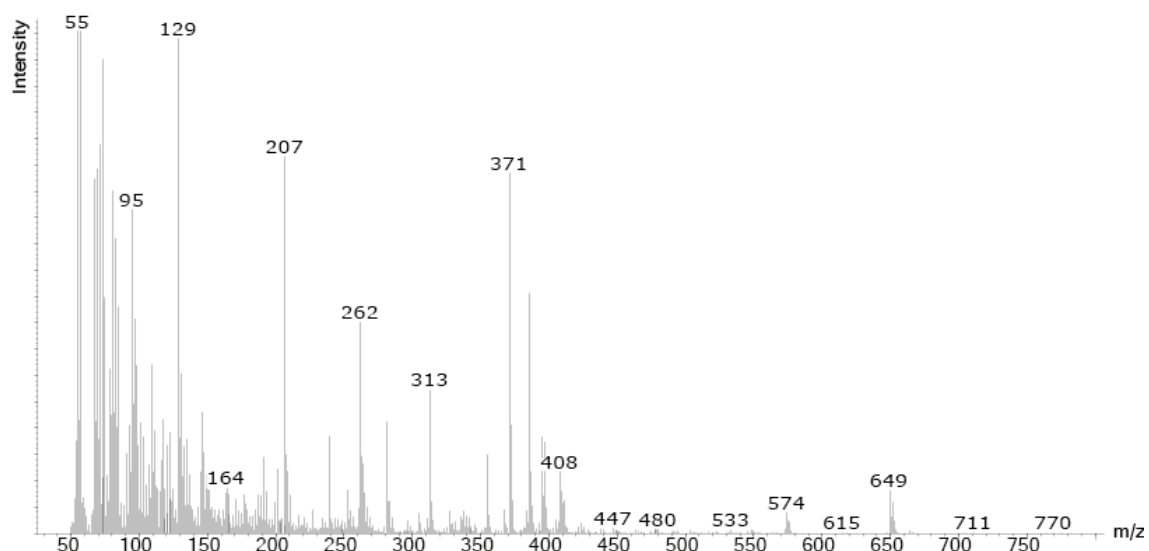


Figure 5.5: Mass spectrum produced at 33.88 minutes in the TIC of the unburied acorn absorbed residue (ACE-02) identified as a DAG comprising $C_{16:0}$ and $C_{18:2}$ fatty acid moieties represented by the following fragments: $[CH_2CHCHOTMS]^+$ at m/z 129; $[CH(O)CHCH_2OTMS]^+$ at m/z 145; $[M-15]$ at m/z 649; $[M-90]$ at m/z 574; $[M-RCOOCH_2]$ at m/z 313 (indicating $C_{16:0}$) and $[M-RCOOCH_2]$ at m/z 395 (indicating $C_{18:2}$).

5.4.3.3 Sterols and phytosterols

Acorn oil comprises a wide range of phytosterols, including β -sitosterol, which constitutes 80% of the total sterol fraction, campesterol, stigmasterol, Δ^5 -avenasterol and Δ^7 -stigmastenol, in addition to some minor components (León-Camacho *et al.*, 2004b). β -sitosterol comprised on average 78% of the TLE in the samples prior to burial, but this decreased to 47% after burial, although it was still the dominant sterol present in the extracted residues. Campesterol and stigmasterol were the next most abundant sterols, although the former was absent in the absorbed residue buried at Star Carr (SAC-02). Cholesterol was also identified in very low quantities in the unburied absorbed residue (ACE-02), and the sherds buried at Star Carr and Thixendale (SAC-02 and WOD-02), and comprised 5, 24 and 6% of the sterol fraction respectively. Trace amounts of cholesta-3,5-diene and stigmastan-3,5-dien, formed by dehydration of cholesterol and β -sitosterol respectively (Lercker and Rodrigue-Estrada, 2002:6; León-Camacho *et al.*, 2004a), were also observed. This was initially attributed to soil migration, however none was detected in the soil and control samples analysed. Its presence in the unburied absorbed residue (ACE-02) suggests that cholesterol must be present in the residue, which is supported by findings that 2% of the sterol composition of acorn oil was indeed found to comprise cholesterol (León-Camacho *et al.*, 2004b). However, it was not identified in the visible crust, which suggests that the cholesterol identified in the unburied absorbed residue (ACE-02), and the buried samples (SAC-02 and WOD-02) was introduced when handling the pots during the cooking experiment. The

presence of squalene would have strengthened this hypothesis (see Archer *et al.* 2005), however in this case, it was not identified in the mass spectra. Interestingly, ergosterol, which is a component of yeast and fungi (Christie, 2011b), was only present in WOD-02, which was buried at the more alkaline environment at Thixendale.

5.4.3.4 Alkanes and alkenes

León-Camacho *et al.* (2004b) identified medium chain alkanes (C20-C31) in fresh acorn oils, which were dominated by C29 and C27, and made up around 80% of the total hydrocarbon fraction. Interestingly, no alkanes were identified in the crust and unburied absorbed residue (ACE-02), while only odd numbered alkanes (C25-C31) were identified in the buried samples with none dominating this fraction. Alkenes were only observed in the crust and in the absorbed residue buried at Thixendale (WOD-02); however they could not be more specifically identified other than through their characteristic fragmentation pattern which shows prominent mass fragments at m/z 97 and 111. Alkenes with 20, 27, 29, 30 and 31 carbon atoms have been identified as constituents of fresh acorn oils (León-Camacho *et al.*, 2004b). The presence of a carbon-carbon double bond in alkenes means that they are subject to oxidation reactions, which results in the formation of the corresponding alkanes, and might explain the origin of some of the alkanes identified in the degraded samples. The alkane distribution in the buried samples (SAC-02 and WOD-02) is consistent with that observed by Heron *et al.* (1991a) as soil constituents, and furthermore, alkanes were not present in the unburied residues. It is therefore likely that the alkanes present in the buried absorbed residues were introduced from the burial environment, although their origin in the acorn residue cannot be completely discounted since the range of alkanes present falls within that identified in the fresh acorns (León-Camacho *et al.*, 2004b).

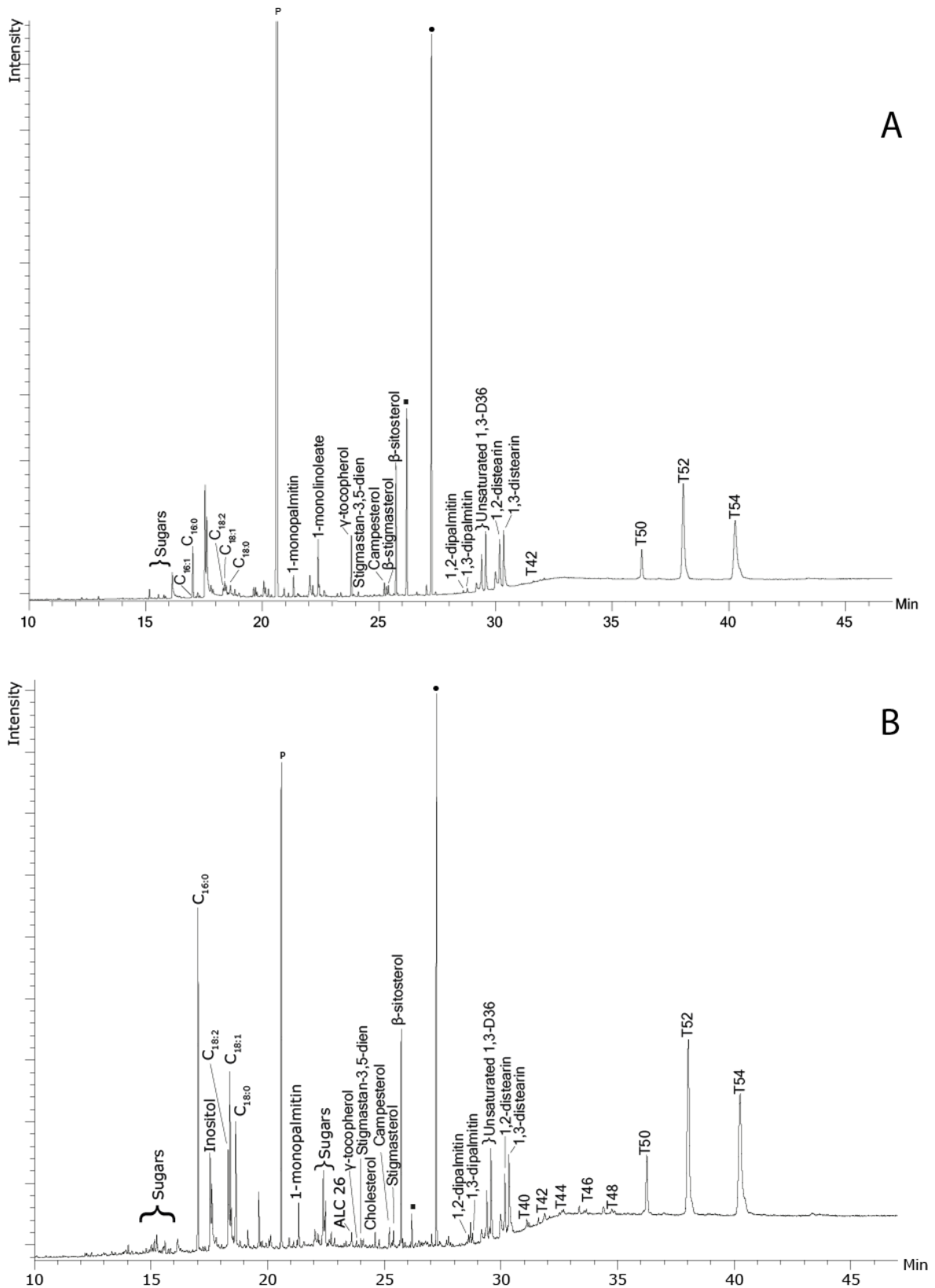


Figure 5.6: Partial gas chromatograms showing A: Acorn Crust prior to burial; B: Absorbed acorn residue prior to burial (ACE-02). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; ALC: Alcohol; D: DAG; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

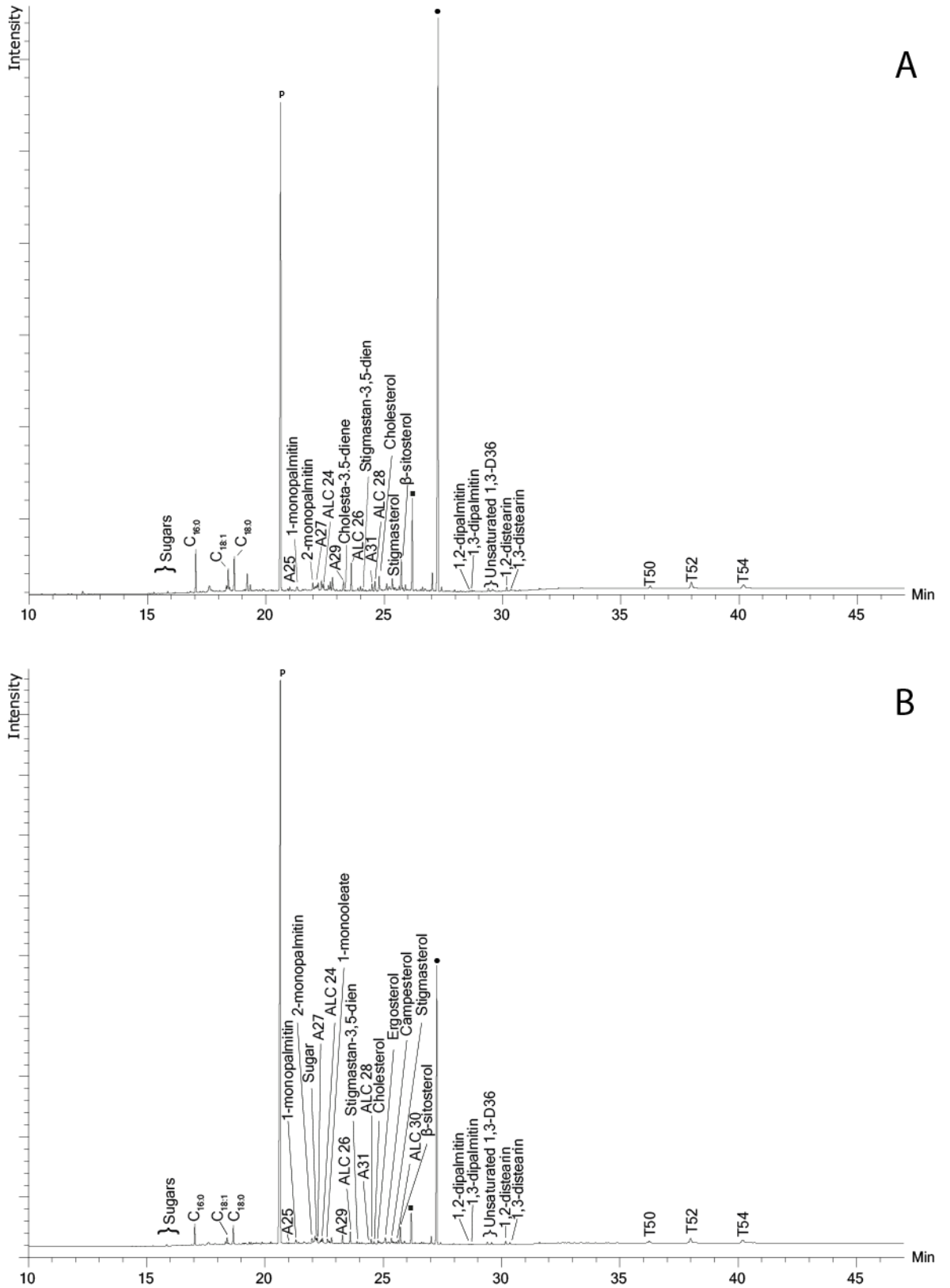


Figure 5.7: Partial gas chromatograms showing A: Absorbed acorn residue buried at Star Carr (SAC-02); B: Absorbed acorn residue buried at Thixendale (WOD-02). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Alkane; ALC: Alcohol; D: DAG; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

5.4.3.5 Tocopherols (vitamin E isomers)

Tocopherols are natural antioxidants, whose occurrence is often correlated with a relatively high abundance of unsaturated fatty acids. The major tocopherol identified in fresh acorn oil was γ -tocopherol, which comprised about 90% of the total tocopherol content, although minor quantities of α - and δ -tocopherols were also detected (León-Camacho *et al.*, 2004b). γ -tocopherol was identified in the acorn crust and unburied absorbed residue (ACE-02) (Figure 5.6), based on its characteristic spectrum (Figure 5.8). In the latter it survived only in trace amounts ($0.03\mu\text{g g}^{-1}$), with $27.23\mu\text{g g}^{-1}$ of γ -tocopherol quantified in the crust. However, none survived after burial, thus compromising its use as a biomarker for acorns.

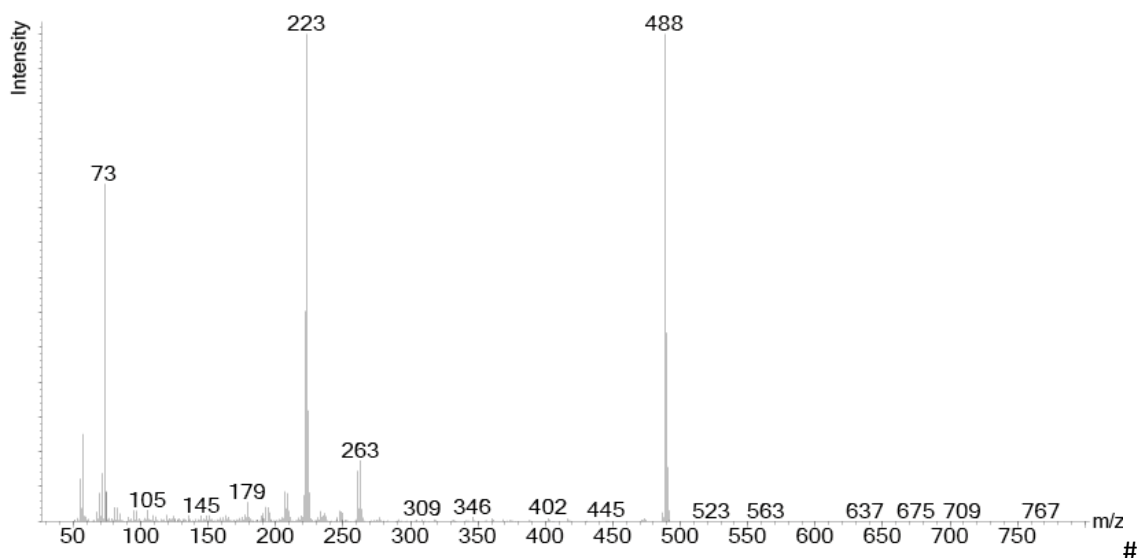


Figure 5.8: Mass Spectrum obtained at 26.59 minutes from the TIC of the acorn crust showing γ -tocopherol TMS ether.

5.4.3.6 Alcohols

Aliphatic and esterified alcohols with 22, 24, 26 and 28 carbon atoms were identified in fresh acorn oil, the major alcohol being tetracosanol (C24) (León-Camacho *et al.*, 2004b). In the present study, alcohols were not detected in the visible residue and only trace amounts of hexacosanol (C26) were identified in the unburied absorbed residue (ACE-02). In the buried samples, alcohols with 24, 26 and 28 carbon atoms were identified, hexacosanol being slightly more abundant in both samples. Since the presence of hexacosanol was dominant in the soil samples, migration from the soil was initially considered possible; however, no alcohols were detected in the control blanks buried at both sites. The alcohols in the residue can therefore be attributed to the

degraded acorn residue. León-Camacho *et al.* (2004b) further identified terpenic alcohols in fresh acorn, in particular β -amyrin and dammaradienol. These were not present in the samples, although inositol (Figure 5.9), a hexahydroxy alcohol (Devlin, 1991:182), was identified in the unburied absorbed residue (ACE-02). *Myo*-inositol is one of nine stereoisomers identified for inositol, which was found to occur in fresh acorns at quantities of 0.22mg g^{-1} (Clements Jr. and Darnell, 1980). The occurrence of inositol can perhaps be indicative of acorn processing, but it was not identified in either of the buried samples.

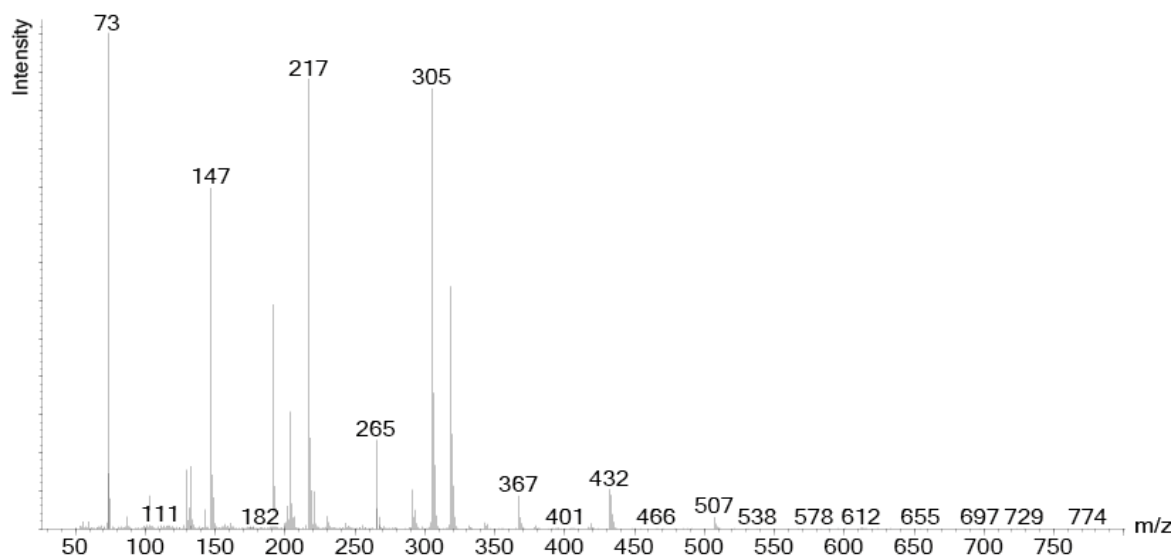


Figure 5.9: Mass Spectrum of inositol identified in the unburied acorn absorbed residue (ACE-02) at 19.42 minutes.

5.4.3.7 Sugars

A series of peaks were identified between 12.00 and 18.55 minutes, and again between 24.00 and 25.50 minutes in the TIC of acorns, particular in the unburied absorbed residue (ACE-02). They have been tentatively identified as sugars due to the presence of ions at m/z 73, 147, 191, 217, 204, 361 (Figure 5.10), which are present in the spectra produced by saccharides and disaccharides (Simoneit *et al.*, 2004), although the spectra did not show the characteristic distribution. A confident interpretation cannot be made at this point, and further research is required to interpret the fragmentation patterns.

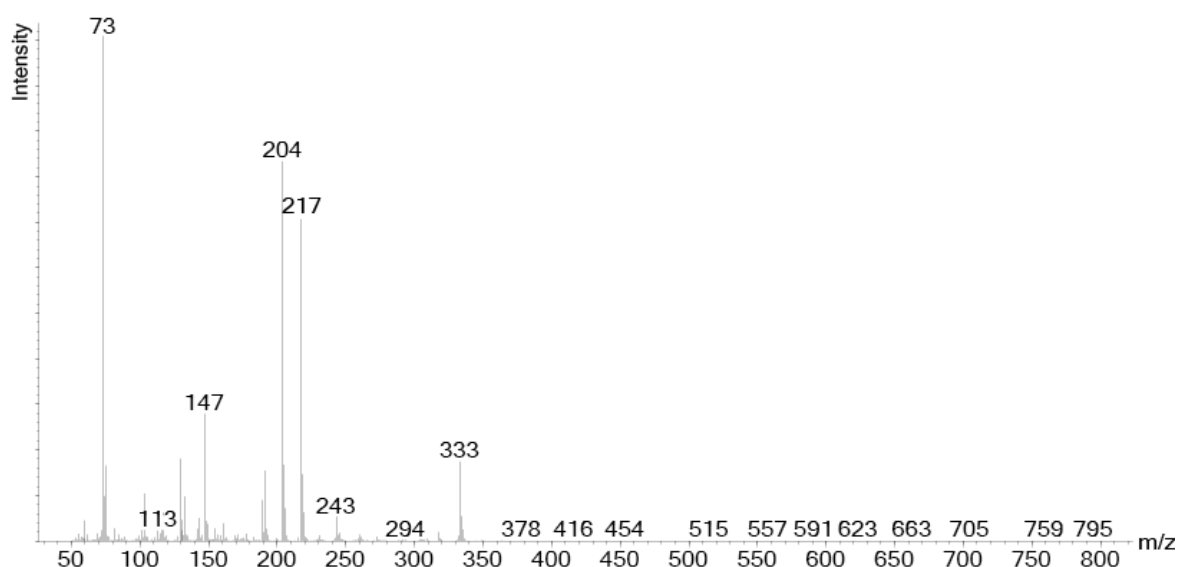


Figure 5.10: Mass Spectrum at 15.50 minutes obtained from the TIC of the acorn crust and tentatively identified as a sugar.

5.4.3.8 Quantification

Figure 5.11 shows a graphical representation of the quantified TLE obtained for the acorn cooking experiment. It can immediately be observed that prior to burial, the crust appears to be more conducive to lipid preservation than absorbed residues, while there was no particular differentiation between the preservation conditions at Star Carr and Thixendale, though the latter did yield an additional $1\mu\text{g g}^{-1}$ of residue. However, more detailed analysis showed that differential preservation did take place, with crusts being more subject to oxidation reactions but less affected by hydrolysis when compared to the unburied absorbed residue (ACE-02). In the buried samples, Table 5.4 shows a better preservation of unsaturated fatty acids at Thixendale, although more $\text{C}_{18:1}$ was retrieved at Star Carr. This however could be due to contributions of unsaturated fatty acid moieties released during TAG hydrolysis, which appears to have been more advanced at Star Carr. Sterol preservation during burial was remarkable, and was found to comprise 15% and 17% of the TLE at Star Carr and Thixendale, respectively. The tentatively identified sugars were best preserved in the unburied residue, as expected, since they are known to be more susceptible to degradation than lipids (Eglinton and Logan, 1991). Finally, γ -tocopherol and inositol were only identified in the unburied residues. In view of their identification in fresh acorn oil, alkanes and alcohols were expected to be present in the unburied residues, and other than perhaps leaching, no explanation can be put forward to explain their absence.

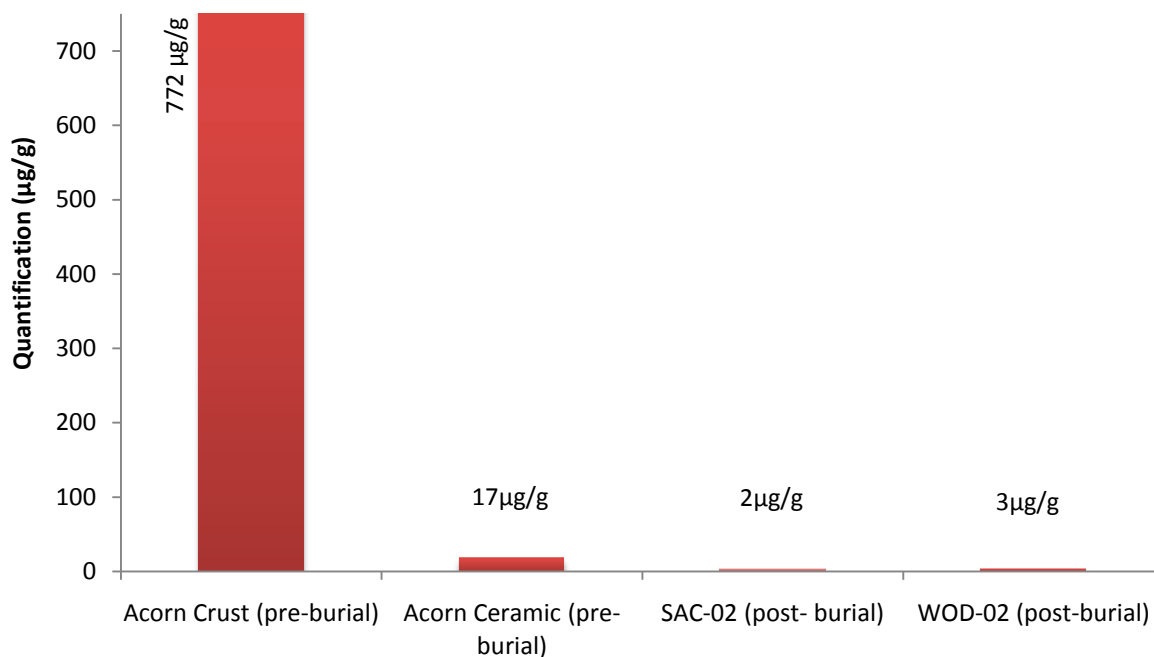


Figure 5.11: Quantified values for the TLE obtained from the absorbed and visible residues produced after repeated boiling of acorns, expressed as $\mu\text{g g}^{-1}$. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.3.9 Archaeological implications

After six boiling of acorns and a seven month burial period, the amount of lipid quantified was below the recommended $5\mu\text{g g}^{-1}$ which would be considered significant. With regard to possible biomarkers, the TAG distribution with the different combinations of unsaturated fatty acid moieties might be considered distinctive; however, these are not likely to survive over archaeological timescales due to hydrolysis and oxidation. Phytosterols are perhaps the most likely to survive, but are unlikely to distinguish a plant residue to species level.

5.4.4 Nettles

Stinging nettles have long been used in herbal remedies, as healthy additions to the diet, and in textiles (Barber, 1991; Guil-Guerrero *et al.*, 2003). They are a good source of amino acids, ascorbic acid, available and unavailable carbohydrates and comprise several mineral elements (Guil-Guerrero *et al.*, 2003). Other constituents include flavonoids, lectins, chlorophylls, carotenoids, vitamins, triterpenes, sterols and carboxylic acids (Lam, 2001). Nettles also have a very high phytolith count (Tsartsidou *et al.*, 2007), which is very useful for assessing microfossil preservation of nettles in archaeological contexts, but this is outside the remit of this thesis.

5.4.4.1 Fatty acids

Not considering seeds, the fatty acid content in fresh nettles have been reported to be highest in young leaves, with $C_{16:0}$ and $C_{18:0}$ being observed to occur in all the different parts of the plant. $C_{16:0}$ made up 18% of the total saponifiable lipid in mature leaves and 25% in the seeds. On the other hand, low quantities of $C_{18:0}$ were generally present (Guil-Guerrero *et al.*, 2003). Monounsaturated fatty acids occurred in low quantities, namely $C_{16:1}$ comprised 0.5% (in stems) to 3% (in roots) of the saponifiable oil, the highest quantities of $C_{18:1}$ was found in the roots (9%), while $C_{20:1}$ and $C_{22:1}$ were found to occur most abundantly in the roots and seeds respectively, where they each made up 1% of the saponifiable oil (Guil-Guerrero *et al.*, 2003). Polyunsaturated fatty acids were the most abundant, with $C_{18:2}$ ranging from 12% (in mature leaves) to 34% (in roots) of the saponifiable oil, and $C_{18:3}$ comprising 2% to 41% of the saponifiable oil in roots and mature leaves respectively (Guil-Guerrero *et al.*, 2003).

As already discussed in Section 5.4.3.1 above, the onset of oxidation acting on the mono- and polyunsaturated fatty acids will alter their distribution within the fatty acid profile obtained. $C_{18:3}$, which was the most abundant fatty acid observed in Guil-Guerrero *et al.*'s (2003) study, was in fact absent in both buried and unburied nettle residues. Unsaturated fatty acids were only observed in the unburied absorbed residue (NCE-01) and the absorbed residue buried at Thixendale (WOD-01), both of which retained $C_{18:2}$ and $C_{18:1}$ (Table 5.6). Surprisingly, unsaturated fatty acids appear to have been better preserved at Thixendale rather than in the unburied absorbed residue (NCE-01), which was not subjected to further degradation by burial. NCE-01 also contained comparatively low quantities of $C_{16:1}$.

In the unburied absorbed residue (NCE-01) and the visible crust, $C_{16:0}$ was the most abundant fatty acid present followed by $C_{18:0}$. The considerably higher percentage composition of $C_{18:0}$ in the fatty acid fraction of the thermally degraded samples as opposed to its lower occurrence in fresh nettles attests to progressive oxidation of the unsaturated fatty acids containing chain lengths of 18 carbon atoms, thus leading to an increase in $C_{18:0}$ (see Table 5.6). In the unburied samples, $C_{18:0}$ was the most abundant fatty acid present. The visible crust contained the highest quantities of fatty acids ($96\mu\text{g g}^{-1}$) which comprised 40% of its TLE, while the unburied absorbed residue (NCE-01) contained $2\mu\text{g g}^{-1}$ making up 27% of its TLE (Figure 5.12). Comparatively, the lipid fraction in the buried samples was very small, with trace amounts retrieved from the residues buried at Star Carr (SAC-01) and $1\mu\text{g g}^{-1}$ quantified at Thixendale (WOD-01). Trace amounts of short and medium chain fatty acids were observed in the crust, which were not present in the absorbed residues,

both buried and unburied (Table 5.6). The unburied absorbed residue (NCE-01) was observed to comprise very long chain fatty acids with 20, 24 and 26 carbon atoms. The fatty acid distribution varied considerably between the crust and the unburied absorbed residue (NCE-01), and between the unburied and buried samples. The degraded residue was quite consistent in composition, although the preservation at Thixendale was marginally better.

<i>FA</i>	<i>Nettle Crust (%) (Pre-burial)</i>	<i>Nettle Absorbed (%) (Pre-burial)</i>	<i>SAC-01 (%) (Post-burial)</i>	<i>WOD-01 (%) (Post-burial)</i>
<i>10:0</i>	Tr	-	-	-
<i>12:0</i>	Tr	-	-	-
<i>14:1</i>	Tr	-	-	-
<i>14:0</i>	1.1	1.3	0.5	0.3
<i>15:0</i>	Tr	-	-	-
<i>16:1</i>	Tr	Tr	-	-
<i>16:0</i>	16.2	12.7	4.8	5.7
<i>17:0</i>	-	-	-	-
<i>18:2</i>	-	1.3	-	17.7
<i>18:1</i>	Tr	2.5	-	2.9
<i>18:0</i>	22.9	8.9	5.6	7.0
<i>20:0</i>	-	1.0	-	-
<i>24:0</i>	-	3.8	-	-
<i>26:0</i>	-	1.1	-	-

Table 5.6: Percentage composition of individual free fatty acids in the TLE of degraded nettles. [FA: Fatty Acid; Tr: Trace amounts; SAC: Star Carr burial; WOD: Thixendale burial]

5.4.4.2 Acylglycerols

Figure 5.12 and Table 5.7 show that percentage composition of TAGs in the TLE of nettle residues is quite low, with trace amounts present in the unburied absorbed residue (NCE-01), and in both buried residues and none present in the nettle crust. DAGs were observed only in the unburied absorbed residue (NCE-01) and the absorbed residue buried at Star Carr (SAC-01) (Table 5.8). This suggests that TAG hydrolysis occurred very rapidly in the nettle residues. The widest range of TAGs, containing between 40 and 54 carbon atoms, was obtained in the unburied absorbed nettle residue (NCE-01), with C52 being marginally more abundant. At Star Carr, the range comprised C40 to C46, while at Thixendale only C40, C42 and C44 were present.

<i>Compound</i>	<i>Nettle Crust (%) (Pre-burial)</i>	<i>Nettle Absorbed (%) (Pre-burial)</i>	<i>SAC-01 (%) (Post-burial)</i>	<i>WOD-01 (%) (Post-burial)</i>
<i>FAs</i>	40.2	27.4	10.8	17.6
<i>MAGs</i>	2.4	0.0	0.0	0.0
<i>DAGs</i>	0.0	2.8	2.0	0.0
<i>TAGs</i>	0.0	2.2	4.4	0.7

Table 5.7: Percentage composition of fatty acids (FAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and triacylglycerols (TAGs) in the TLE of degraded nettles. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.4.3 Sterols and phytosterols

A study carried out by Kovacheva *et al.* (1990) determined the sterol composition of nettles as comprising β -sitosterol as the major phytosterol, campesterol, stigmasterol, Δ^7 -avenasterol and trace amounts of Δ^7 -stigmastenol. Table 5.8 lists the sterol fraction identified in the degraded nettle samples. Sterols in nettles were identified in quite low quantities, and comprised between 4 and 8% of the TLE (Figure 5.12). β -sitosterol was the main phytosterol identified in all samples, however cholesterol was also detected in near equal quantities and was found to be the major sterol in the unburied absorbed residue (NCE-01) and the sherd buried at Star Carr (SAC-01) (Figures 5.13 and 5.14). It was not detected in the soil samples or the control blanks buried on site. Cholesterol has been reported as trace constituent in plants, as a component of plant membranes and has been recorded as the major sterol in the surface lipids of leaves (Christie, 2003; Behrman and Gopalan, 2005:13). In Kovacheva *et al.*'s (1990), cholesterol was used as an internal standard, which may have precluded its identification as a constituent of nettle; however no publications could be found to support cholesterol as a sterol component of nettle. As already suggested in Section 5.4.3.3, the cholesterol identified in this case was probably introduced when the pots were handled during the cooking experiment. Stigmasta-3,5-dien and cholesta-3,5-diene were present in samples WOD-01 and SAC-01 respectively. Their formation as dehydration products of β -sitosterol and cholesterol have already been described in Section 5.4.3.3.

5.4.4.4 Alkanes and alkenes

Minor quantities of alkanes were identified in all the degraded nettle samples except the crust. Only odd numbered chain lengths were identified containing between 25 and 33 carbon atoms. Odd numbered, long-chain alkanes comprising between 27 and 33 carbon atoms are commonly found in plants (Baker, 1982). In general, they occur as minor constituents in the plant cuticle and in epicuticular wax, which is essential to protect the plant from, among many things, bacterial and fungal pathogens, to limit non-stomatal water loss and mediate plant-insect interactions (Kunst *et al.*, 2005:270). Hence, the presence of alkanes in the degraded nettle samples can be attributed to migration from the soil (Heron *et al.*, 1991a), introduced as common laboratory contaminants, or as constituents of nettles. Since alkanes were not identified in either of the buried control samples and the laboratory blanks, but were present in the unburied absorbed residue (NCE-01), their origin, possibly from nettle leaves, cannot be dismissed. Trace amounts of alkenes were detected in the buried samples, but their chain lengths could not be confidently identified.

5.4.4.5 Alcohols and wax esters

In plants, alcohols have been observed to occur as free constituents or esterified to fatty acids to form wax esters (Stacey, 1999:98). Alcohols comprising 24 to 30 carbon atoms were identified in the unburied absorbed residue (NCE-01), and the sherds buried at Star Carr (SAC-01) and Thixendale (WOD-01) (Table 5.8), with hexacosanol (C26) being marginally dominant. No alcohols were detected in the visible crusts, but alcohols comprised over 30% of the TLE in the unburied absorbed residue (NCE-01) and in the sherd buried at Star Carr (SAC-01), and around 10% of the TLE in the sample buried at Thixendale (WOD-01) (Figure 5.12). The widest range of alcohols (C24 to C30) was identified in the unburied absorbed residue (NCE-01), which was also the only sample in which wax esters were identified. These included tetracosanoyl myristate (C38), tetracosanoyl palmitate (C40), hexacosanoyl palmitate (C42), and hexacosanoyl stearate (C48), based on their corresponding M^+ at m/z 564, 592, 620 and 648, as well as their characteristic base peak at m/z 229, 257 and 285 for the myristate, palmitate and stearate waxes respectively. Wax esters did not survive in the buried residue and the crust, which is interesting since the ester bond in wax esters is known to be more resistant to hydrolysis than triacylglycerols (Pollard *et al.*, 2007:156), which were however retrieved at both Star Carr and Thixendale.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
Nettle Crust (Unburied visible residue)	239	FAs: C _{10:0} , C _{12:0} , C _{14:1} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} ; Sterols: Cholesterol, β -sitosterol; MAGs: 1-Monopalmitin; DAGs: 1,2-D30; 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34; Sugars: unidentified
Nettle Ceramic (Absorbed residue; Pre-burial)	6	FAs: C _{14:0} , C _{16:1} , C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} , C _{20:0} , C _{24:0} , C _{26:0} ; Alkanes: C ₂₉ , C ₃₁ , C ₃₃ ; Alcohols: C ₂₄ , C ₂₆ , C ₂₈ , C ₃₀ , β -amyrin; Sterols: β -sitosterol, Cholesterol; DAGs: 1,2-Dipalmitin, 1,2-D34; TAGs: C ₄₀ , C ₄₂ , C ₄₄ , C ₄₆ , C ₄₈ , C ₅₀ , C ₅₂ , C ₅₄ ; Sugars: unidentified; WEs: C ₃₈ , C ₄₀ , C ₄₂ , C ₄₄
SAC-01 (Absorbed residue; Star Carr Burial)	1	FAs: C _{14:0} , C _{16:0} , C _{18:0} ; Alkanes: C ₂₅ , C ₂₇ , C ₂₉ , C ₃₁ ; Alkenes: unidentified; Alcohols: C ₂₄ , C ₂₆ , 28; Sterols: β -sitosterol, Cholesta-3,5-diene, Cholesterol; DAGs: 1,3-Dipalmitin; TAGs: C ₄₀ , C ₄₂ , C ₄₄ , C ₄₆
WOD-01 (Absorbed residue; Thixendale Burial)	4	FAs: C _{14:0} , C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Alkanes: C ₂₉ , C ₃₁ ; Alkenes: unidentified; Alcohols: C ₂₆ ; Sterols: β -sitosterol, Stigmastan-3,5-dien, Cholesterol; TAGs: C ₄₀ , C ₄₂ , C ₄₄ ; Ketones: C ₃₃

Table 5.8: GC-MS and HT-GC identification of the lipid composition of boiled nettles analysed before and after burial. [FA/C_{x,y}: Fatty acids where x is the carbon number and y is the degree of unsaturation; MAG: Monoacylglycerol; D & DAG: Diacylglycerol; TAG: Triacylglycerol; WE: Wax esters]

Chapter 5

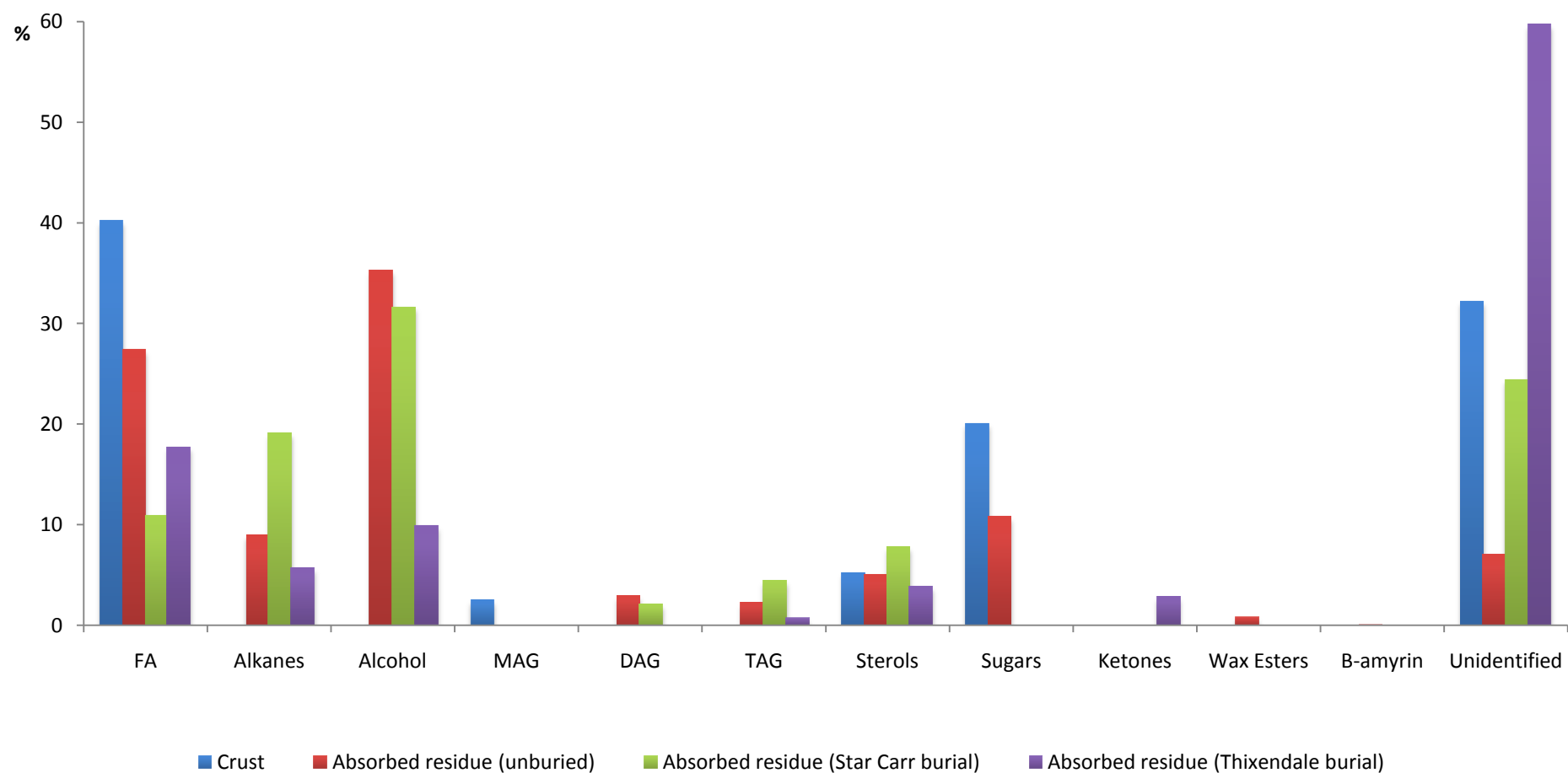
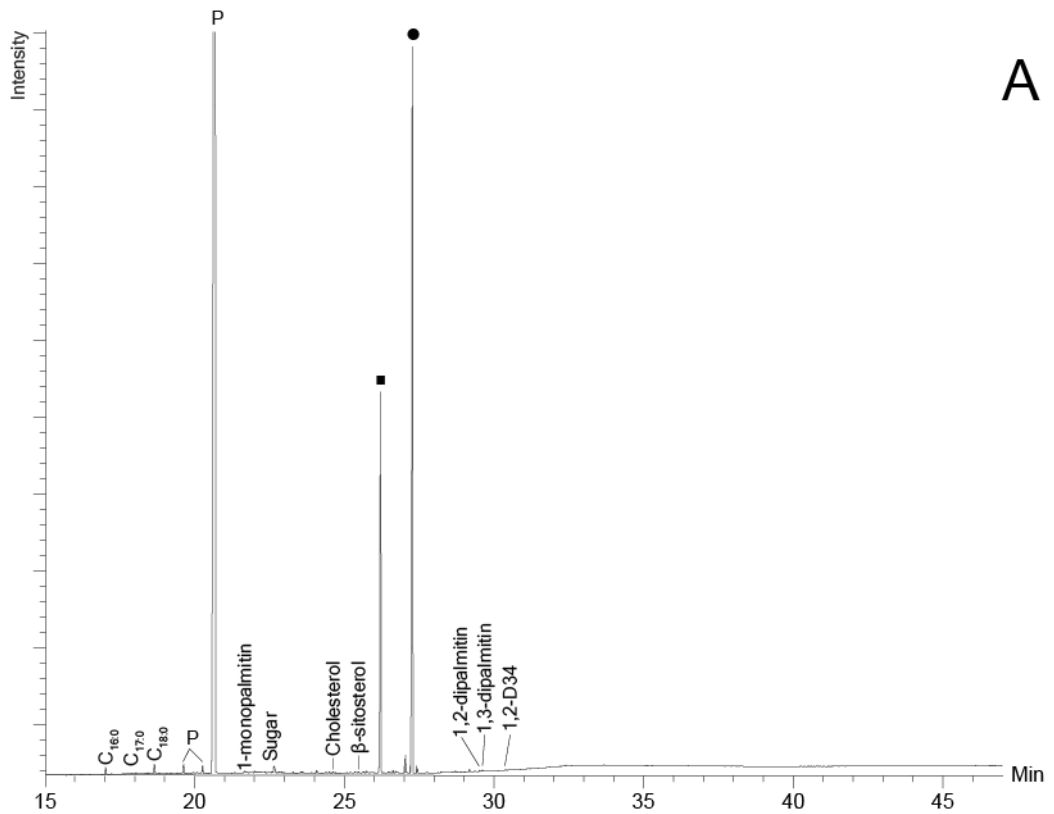
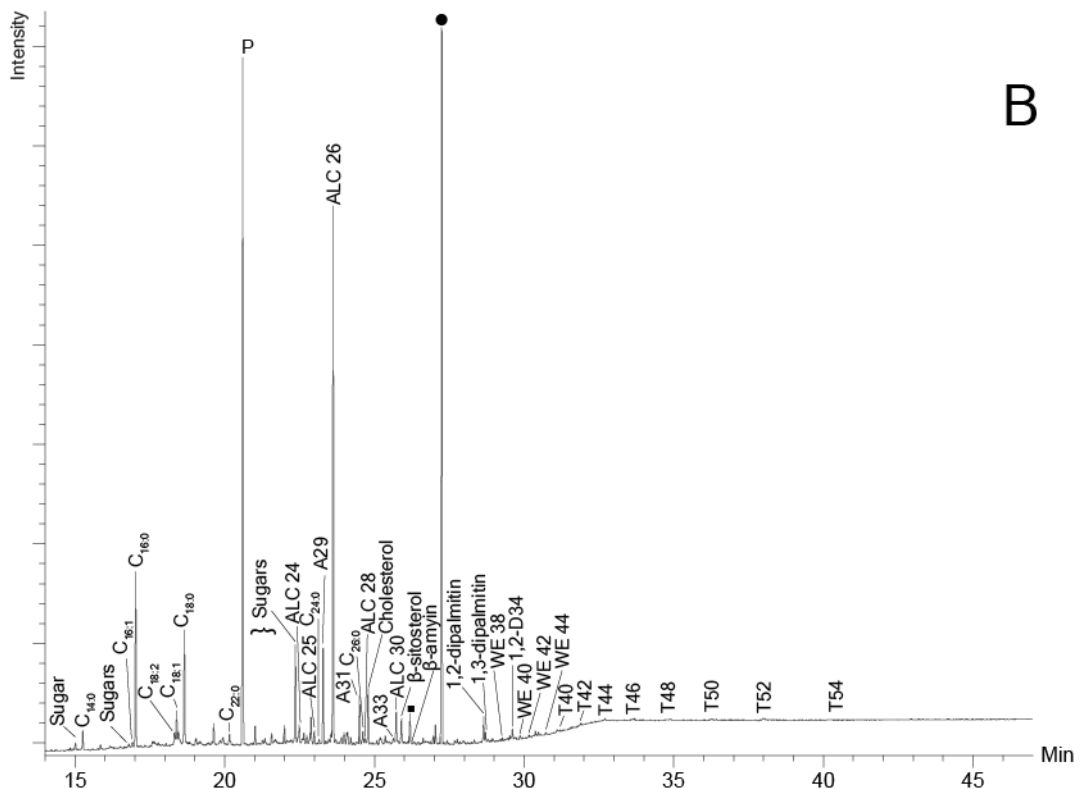


Figure 5.12: TLE profile of degraded nettle as percentages of the absolute quantified residue. [FA: Fatty Acid; MAG: Monoacylglycerol; DAG: Diacylglycerol; TAG: Triacylglycerol]

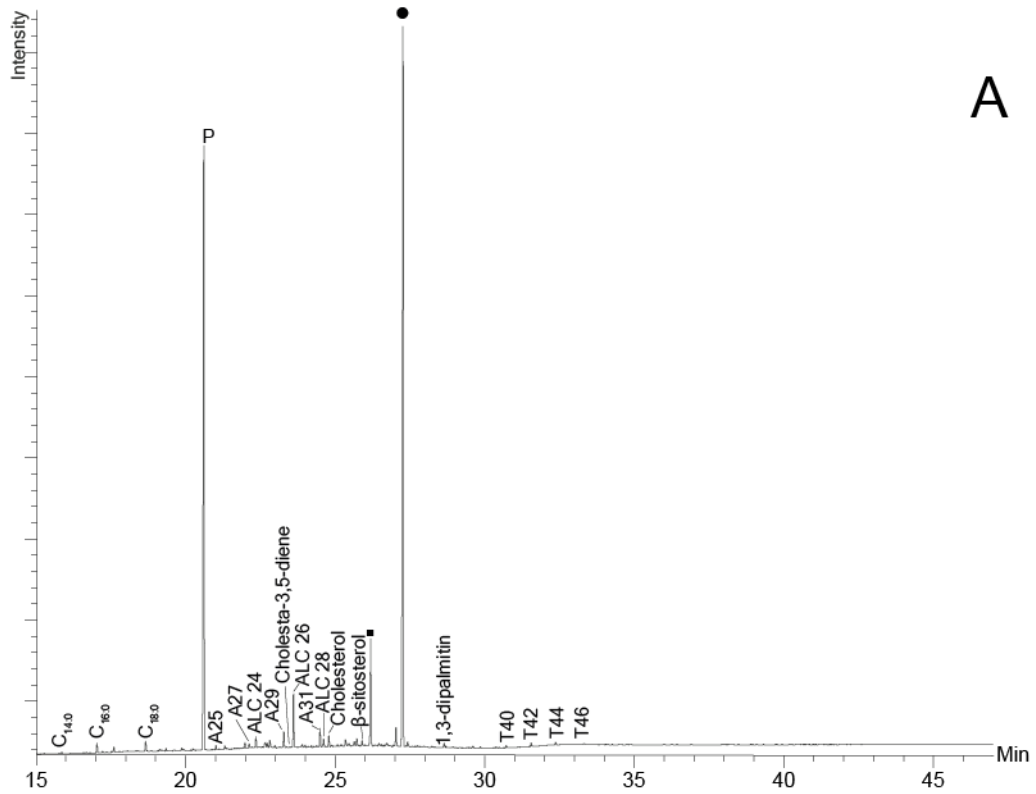


A

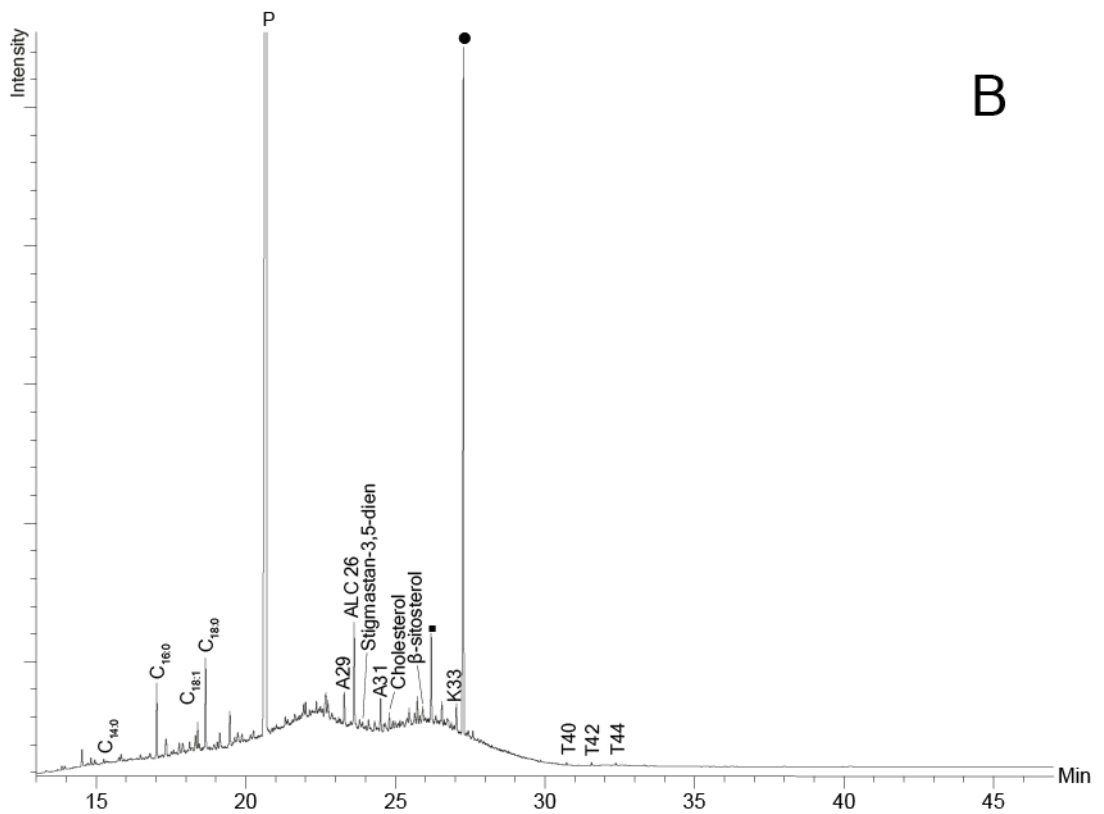


B

Figure 5.13: Partial gas chromatograms showing A: Nettle Crust prior to burial; B: Unburied absorbed nettle residue (NCE-01). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Alkane; ALC: Alcohol; D: DAG; T: TAG; WE: Wax Ester; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]



A



B

Figure 5.14: Partial gas chromatograms showing A: Absorbed nettle residue buried at Star Carr (SAC-01); B: Absorbed nettle residue buried at Thixendale (WOD-01). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Alkane; ALC: Alcohol; K: Ketone; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

5.4.4.6 Terpenoids

Terpenoids are the largest family of natural compounds and secondary plant products; the latter due to their not being involved in primary metabolism. Consequently, only small amounts of terpenoids are produced, and occur in all the different parts making up a plant (Pollard *et al.*, 2007:153). Terpenoids are complex six carbon ring systems with attached hydrogen and oxygen atoms, with triterpenoids comprising 30 carbon atoms (Serpico and White, 2000:444). The latter have already been identified as nettle constituents (Lam, 2001). The triterpenoid β -amyrin was present only in the unburied absorbed residue, sample NCE-01. Identification of β -amyrin as a TMS derivative was based on the fragmentation pattern obtained through GC-MS analysis (Regert *et al.*, 1998b) and confirmed using the NIST library (Figure 5.15).

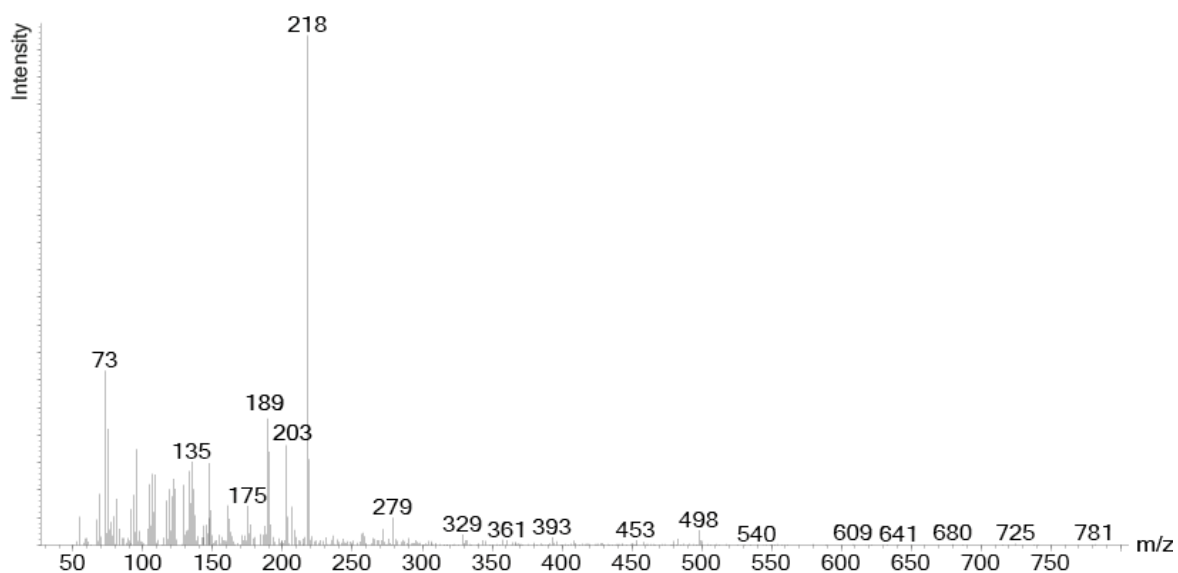


Figure 5.15: Mass Spectrum of β -amyrin (TMS) obtained at 28.64 minutes in the unburied absorbed nettle residue (NCE-01). The fragment ions present include the M⁺. at m/z 498, as well as ions at m/z 218, 203, 189, and 73.

5.4.4.7 Ketones

Tritriacontan-16-one was identified in the absorbed residue of the sherd buried at Thixendale (WOD-01) (Table 5.8). Identification followed the fragmentation pattern in Stacey (1999:210). Ions present at m/z 239 and 267 showed fragmentation α to the carbonyl group, while ions occurring at m/z 254 and 282 showed cleavage of the carbon-carbon bond β to the carbonyl group, with the transfer of a γ -hydrogen atom. β -cleavage also produced ions at m/z 255 and 283. The weak M⁺. ion at m/z 478 was present.

Long-chain ketones are constituents of higher plant leaf waxes, but were also observed to form as a result of ketonic decarboxylation, which will be discussed below (Evershed *et al.*, 1995b). The C33 ketone observed in WOD-01 is likely to have formed as a result of the latter. Following Raven *et al.*'s (1997) criteria, tritriacontan-16-one might be considered a constituent of nettle leaf wax since it is the only homologue present and it falls within the observed range of C24-C33. However, it is an asymmetrical ketone, therefore unlikely to have originated in the epicuticular wax which usually comprises symmetrical ketones. Furthermore, the absence of an alkane or alcohol with a corresponding chain length of 33 carbon atoms precludes its biosynthesis in the plant (Raven *et al.*, 1997). Hence, tritriacontan-16-one cannot be considered as a biomarker denoting nettles, but simply attests to processing at high temperature. Its survival in just one sample is interesting, and may be explained in terms of different temperature gradients within the ceramic vessel during cooking, which may not have reached the required 300°C for the formation of ketones.

5.4.4.8 Sugars

A series of peaks were identified between 14.00 and 18.55 minutes, and again between 23.00 and 25.50 minutes in the TIC of the unburied nettle residues, particular in the crust. None survived in the buried samples. The fragment ions observed were similar to those described in Section 5.4.3.7 above, comprising ions at m/z 73, 147, 191, 217, 204, 361. They have similarly been tentatively identified as saccharides (Simoneit *et al.*, 2004).

5.4.4.9 Quantification

Figure 5.16 shows the quantified values obtained for the TLE in the nettle cooking experiment. It can immediately be observed that while the visible crust contained a significant quantity of residue ($>5\mu\text{g g}^{-1}$), that retained by the absorbed residues is markedly lower. The unburied sherd, NCE-01, contained $6\mu\text{g g}^{-1}$ of lipid, which is close to the minimum value considered significant, and suggests that perhaps not enough lipid residue is absorbed within the walls of a ceramic vessel to make it conducive to survive over archaeological timescales. This is confirmed by the quantified lipid calculated for the unburied samples, in particular at Star Carr which comprised only around $1\mu\text{g g}^{-1}$.

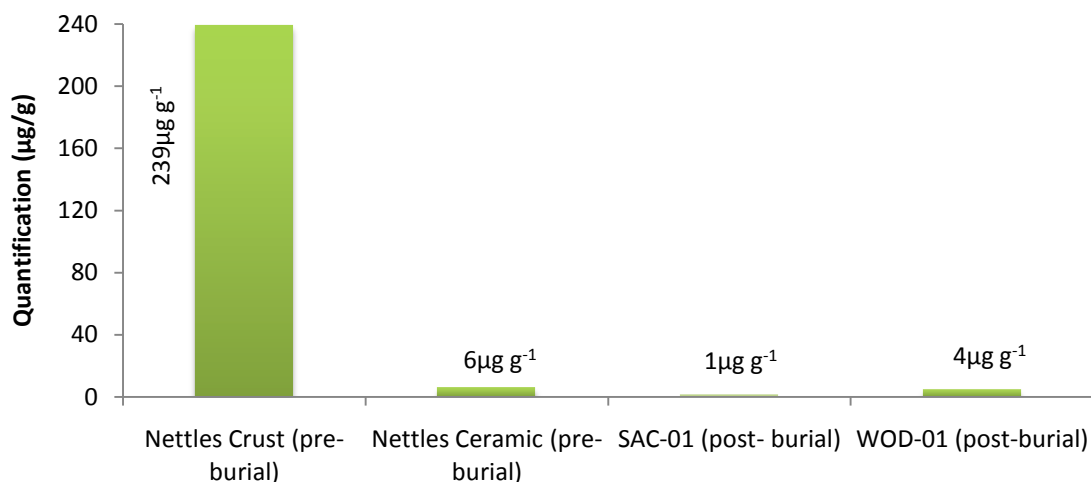


Figure 5.16: Quantified values for the TLE obtained from the absorbed and visible residues produced after repeated boiling of nettles, expressed as $\mu\text{g g}^{-1}$. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.4.10 Archaeological Implications

The fatty acid profile obtained for the buried residues comprised $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$, with $C_{18:2}$ and $C_{18:1}$ also surviving at Thixendale. The palmitic ($C_{16:0}$) to stearic ($C_{18:0}$) (P/S) fatty acid ratio obtained for the buried samples is however inconsistent with a plant origin (Copley *et al.*, 2005e), since moderately higher quantities of $C_{18:0}$ over $C_{16:0}$ were obtained, which may however revert with more advanced degradation. No characteristic distribution was observed in the acylglycerols and alkanes present. The marginal dominance of hexacosanol in the alcohol series cannot be considered unique to nettles as it was also observed to occur in degraded acorn residues, and is perhaps likely to similarly occur in other plant types. The sterol and wax ester fraction identified is perhaps more likely to survive, and although useful in denoting a plant origin, it is not particular to nettles. The tentatively identified saccharide series was also present in acorns, and moreover was already lost after a seven month burial period. Although terpenoids can potentially be quite distinctive of the plant variety they originate from (Pollard *et al.*, 2007:154), β -amyrin was also identified in fresh acorns (León-Camacho *et al.*, 2004b), and moreover, did not survive after burial. Hence at this stage, nettle biomarkers could not be identified. Re-analyses of the unidentified peaks might contribute new information. Altering the derivatisation method of the extract and analysis on reversed-phase High Performance Liquid Chromatography (HPLC) following Guil-Guerrero *et al.*'s (2003) study, to better examine the carotenoid fraction may prove helpful. However, the low absorption of residue observed in the unburied absorbed residue (NCE-01) and the quantification values obtained for the buried TLE denotes its low preservation potential; hence it is unlikely for nettle residues to survive in archaeological residues.

5.4.5 Einkorn

Einkorn (*T. monococcum*) is one of the primary crops attested in Near Eastern Neolithic agriculture and also one of the earliest crops to spread into Europe and the Mediterranean (Zohary and Hopf, 2000:34-35). It is composed of 62% starch, 3% sugars, 35% protein, 2% ash and 2% crude fat. Other constituents include polar lipids, phytate and oligosaccharides. Einkorn is also rich in β -carotene, retinol equivalent and phosphorus (Pomeranz *et al.*, 1966; Abdel-Aal *et al.*, 1995). Table 5.11 shows the distribution of the different lipid fractions identified after six boiling episodes and subsequent burial.

5.4.5.1 Fatty Acids

Fatty acids are the major lipid components in cereals, and in fresh samples, occur mostly as acyl esters of glycerols and bound to other cellular components such as long-chain alcohol esters to form wax esters (Becker, 2007:303). $C_{18:2}$ was the predominant unsaturated fatty acid in fresh wheat, followed by $C_{18:1}$ and $C_{16:0}$. Lower quantities (in decreasing order) of $C_{18:3}$, $C_{18:0}$, $C_{16:1}$ and $C_{14:0}$ were also present (Price and Parsons, 1975). Trace amounts of other fatty acids were also observed, although highly unsaturated fatty acids and hydroxyl acids were not typically detected (Becker, 2007:304).

A range of saturated and unsaturated fatty acids with up to 20 carbon atoms and two unsaturations were identified in all the degraded einkorn samples. Fatty acids made up between 90 and 97% of the TLE in the buried samples, but were a considerably smaller fraction in the unburied samples, comprising 25% of the crust and only 15% of ECE-06. The degraded fatty acid profile in einkorn was found to be similar in composition to that reported by Becker (2007), Morrison (1988) and Price and Parsons (1975), however variations were observed in the quantification of the individual lipids. The most abundant fatty acid in the unburied samples was $C_{16:0}$, followed by $C_{18:1}$, $C_{18:2}$ and $C_{18:0}$ (Table 5.9). In the buried samples, $C_{18:0}$ was the major fatty acid, followed closely by $C_{16:0}$ (Table 5.9), with a marked reduction in the retrieval of $C_{18:1}$ and $C_{18:2}$. The resulting profile is due to the onset of degradation, and mainly due to oxidation and hydrolysis as described in Section 5.4.3.1. Degradation can be observed to be more advanced in the buried samples, where unsaturated fatty acids make up a smaller percentage of the total fatty acid fraction. Interestingly, no $C_{16:1}$ was observed in the unburied samples, whereas it made up circa 1% of the TLE of the buried samples. Similarly, $C_{14:0}$ was found only in the buried samples. $C_{14:0}$ is commonly associated with bacterial lipid (Dudd *et al.*, 1998), however, it has been

observed in wheat (Price and Parsons, 1975; Beare-Rogers *et al.*, 2001), which supports its presence at Star Carr. Margaric acid (C_{17:0}) was observed in the crust and the unburied absorbed residue (ECE-06), but not in the buried absorbed residues. It comprised quite a high percentage of the TLE in the crust (6%), and its occurrence in wheat has not been reported. Its presence is usually associated with ruminant fat and milk products (Dudd *et al.*, 1998, 1999). A similar situation was observed for C_{20:1}. Becker (2007:304) records that trace amounts of other fatty acids were observed, but does not specify which ones; C_{17:0} and C_{20:1} may have been observed.

Plant oils were observed to contain considerably greater proportions of C_{16:0} than C_{18:0} (Copley *et al.*, 2005e) (see Section 5.4.4.10). The P/S fatty acid ratio in the unburied samples was 4, supporting Copley *et al.*'s (2005e) observation. In the buried samples the P/S ratio was closer to 1, which raises concern as to the confidence attributed to the assignment of origin based on fatty acid ratios. However, as already discussed in Section 5.4.3.1, it is likely that over time, degradation reactions will see an increase in the C_{16:0} of the buried samples, with a concomitant decrease in C_{18:0} as a result of the processes detailed by Morgan *et al.* (1973) and den Dooren de Jong (1961). However, the C_{16:0} is more soluble than C_{18:0}, and will be more easily lost through leaching during burial, which could also affect the reliability of using P/S ratios.

FA	Einkorn Crust (%) (Pre-burial)	Einkorn Absorbed (%) (Pre-burial)	SAC-06 (%) (Post-burial)	WOD-06 (%) (Post-burial)
14:0	-	-	2.8	1.0
16:1	-	-	0.9	0.7
16:0	8.6	7.0	43.8	39.8
17:0	6.1	1.0	-	-
18:2	2.8	2.9	0.7	1.2
18:1	4.5	3.7	3.2	4.3
18:0	2.3	1.2	46.0	43.5
20:1	0.7	Tr	-	-

Table 5.9: Percentage composition of individual free fatty acids in the TLE of degraded einkorn. [FA: Fatty Acid; Tr: Trace amounts; SAC: Star Carr burial; WOD: Thixendale burial]

5.4.5.2 Acylglycerols

TAGs were found to be the major constituents of neutral lipids in fresh wheat, together with MAGs and DAGs (Price and Parsons, 1975; Morrison, 1988). The TAG and fatty acid fractions in the present study are once more in inverse relation (Table 5.10), hence indicating the onset of TAG hydrolysis. This appears to have been complete at Star Carr since TAGs were not retained in the residue, but fatty acids made up 97% of the TLE. No DAGs were identified, and only trace amounts of MAGs were detected. The sample buried at Thixendale showed a similar pattern in TAG degradation, however hydrolysis was not complete. A wide range of TAGs (C40 to C54) was

identified in the unburied absorbed residue (ECE-06) and the sample buried at Thixendale (WOD-06), while the greatest abundance of TAGs by far was found in the crust. This enhanced preservation of TAGs in the visible residue has already been discussed in Section 5.4.3.1. C54 was the most abundant TAG in both unburied samples, while C48 was marginally more abundant in the degraded einkorn residue buried at Thixendale (WOD-06).

<i>Compound</i>	<i>Einkorn Crust (%) (Pre-burial)</i>	<i>Einkorn Absorbed (%) (Pre-burial)</i>	<i>SAC-06 (%) (Post-burial)</i>	<i>WOD-06 (%) (Post-burial)</i>
FAs	17.1	15.5	85.4	82.2
MAGs	0.0	0.0	2.2	0.9
DAGs	15.4	2.3	0.0	0.0
TAGs	32.4	4.1	0.0	2.6

Table 5.10: Percentage composition of fatty acids (FAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and triacylglycerols (TAGs) in the TLE of degraded einkorn. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.5.3 Sterols and phytosterols

A study of the phytosterol content of several varieties of wheat, including einkorn, determined that there is indeed considerable variation in the phytosterol constituents of the different wheat types. Campesterol, campestanol and sitostanol were observed in all the wheat types analysed, with sitosterol, which made up 40-61% of the total sterol content, being the most abundant sterol in all the wheat varieties tested. Minor sterol constituents comprised brassicasterol, stigmasterol, Δ^5 -avenasterol, Δ^7 -avenasterol, stigmasta-5,24(25)-dienol, and Δ^7 -stigmastenol (Nurmi *et al.*, 2008).

Sterols made up between 1% and 2% of the unburied TLE residue in the present study, and between 5% and 6% in the buried residues (Figure 5.17). A range of phytosterols comprising campesterol, β -sitosterol, stigmasterol and stigmastan-3,5-dien were identified. Supporting the findings of Nurmi *et al.* (2008), β -sitosterol was observed to be the most abundant sterol in all samples except in the sample buried at Thixendale (WOD-06). In the latter, stigmastan-3,5-dien was dominant, showing extensive dehydration of β -sitosterol which is supported by the concomitant decrease in the latter. In fresh samples, an average of $500\mu\text{g g}^{-1}$ of β -sitosterol were recovered (Nurmi *et al.*, 2008), which was considerably higher than that present in the degraded residues which ranged between trace amounts in the absorbed residue buried at Thixendale (WOD-06) and $12\mu\text{g g}^{-1}$ in the crust. Phytostanols were not observed.

Cholesterol was identified, in trace amounts, in the buried absorbed residues (WOD-06 and SAC-06). As indicated above, its presence is attributed to handling of the ceramic vessels during the cooking experiment.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
Einkorn Crust (Unburied visible residue)	388	FAs: C _{14:0} , C _{16:0} , C _{17:0} , C _{18:2} , C _{18:1} , C _{18:0} , C _{20:1} ; Alcohols: C24; Sterols: Campesterol, β -sitosterol, Stigmastan-3,5-dien; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C50, C52, C54; Sugars: unidentified; ω-(<i>o</i>-alkylphenyl)octadecanoic acids; Unidentified peaks: including a series of four peaks with a base peak at <i>m/z</i> 268
ECE-06 (Absorbed residue; Pre-burial)	56	FAs: C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} , C _{20:1} ; Alcohols: C24; Sterols: Campesterol, β -sitosterol; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C46, C50, C52, C54; Sugars: unidentified; ω-(<i>o</i>-alkylphenyl)octadecanoic acids; Unidentified peaks: including a series of four peaks with a base peak at <i>m/z</i> 268
SAC-06I (Absorbed residue; Star Carr Burial)	9	FAs: C _{14:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Alkanes: C25, C27, C29; Alcohols: C24, C26; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol; MAGs: 2-Monopalmitin, 1-Monooleate; Dehydroabietic acid; Sugars: unidentified; Unidentified peaks: including a series of four peaks with a base peak at <i>m/z</i> 268
WOD-06I (Absorbed residue; Thixendale Burial)	16	FAs: C _{14:0} , C _{16:1} , C _{16:0} , C _{18:2} , C _{18:1} , C _{18:0} ; Alkanes: C25, C27, C29; Alkenes: unidentified; Alcohols: C24, C26, C28, C30; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol; MAGs: 2-Monopalmitin; TAGs: C40, C42, C44, C46, C48, C50, C52, C54; Sugars: unidentified

Table 5.11: GC-MS and HT-GC identification of the lipid composition of boiled einkorn analysed before and after burial. [FA/C_{x,y}: Fatty acids where x is the carbon number and y is the degree of unsaturation; MAG: Monoacylglycerol; D & DAG: Diacylglycerol; TAG: Triacylglycerol]

5.4.5.4 Alkanes and alkenes

Hydrocarbons are commonly found in plants (Baker, 1982), and are also known to be constituents of einkorn (Morrison, 1988). Odd numbered alkanes with 25, 27 and 29 carbon atoms were detected only in the buried residues. The range of alkanes identified is also consistent with soil lipid composition (Heron *et al.*, 1991a). In view of the fact that alkanes were only detected in the buried samples, and comprised only trace amounts, migration from the soil is likely, in particular, since none were detected in the unburied residues. Alkenes with unspecified chain lengths were identified only at Thixendale (Table 5.11).

Chapter 5

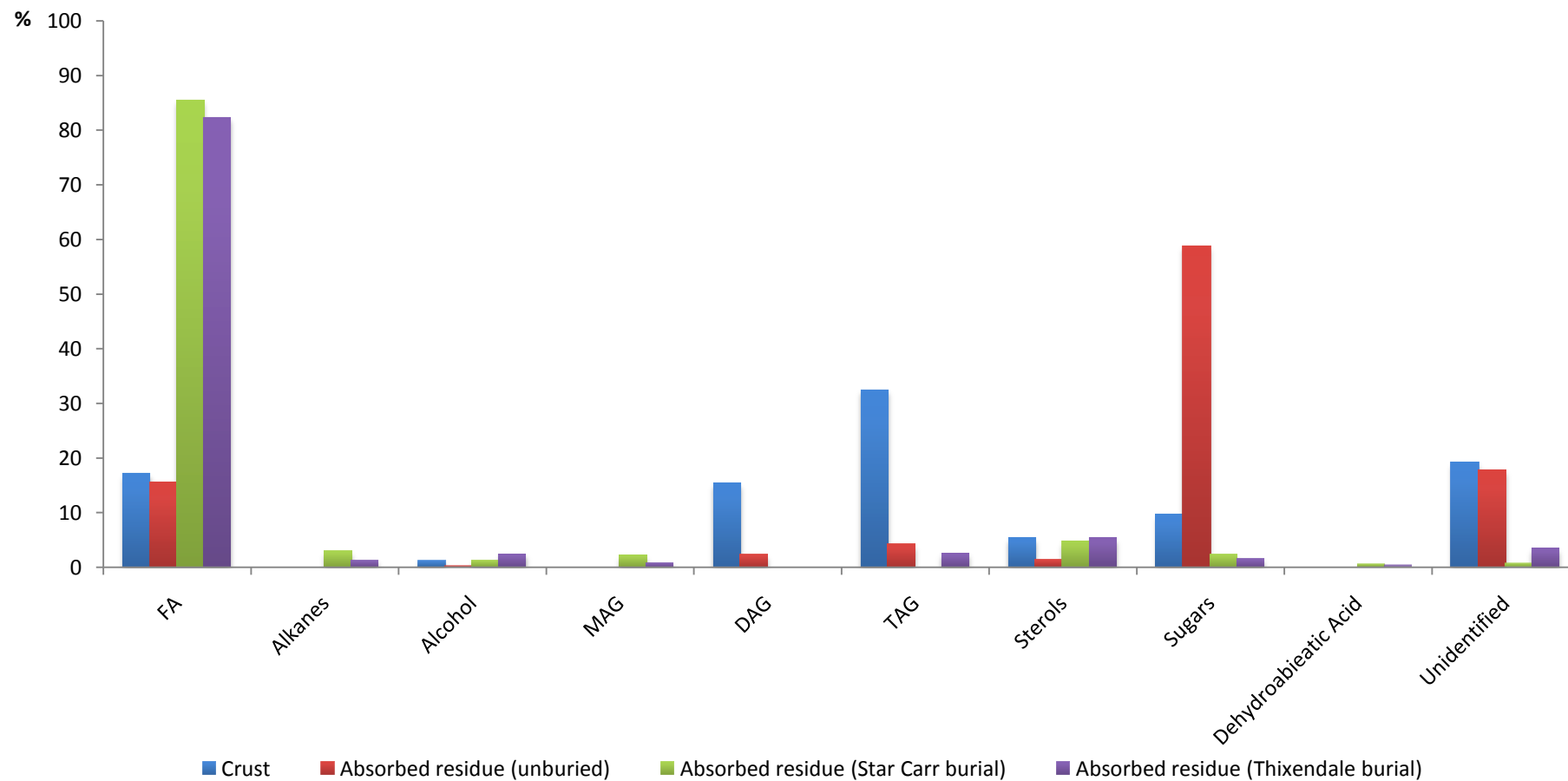


Figure 5.17: TLE profile of degraded einkorn as percentages of the absolute quantified residue. [FA: Fatty Acid; MAG: Monoacylglycerol; DAG: Diacylglycerol; TAG: Triacylglycerol]

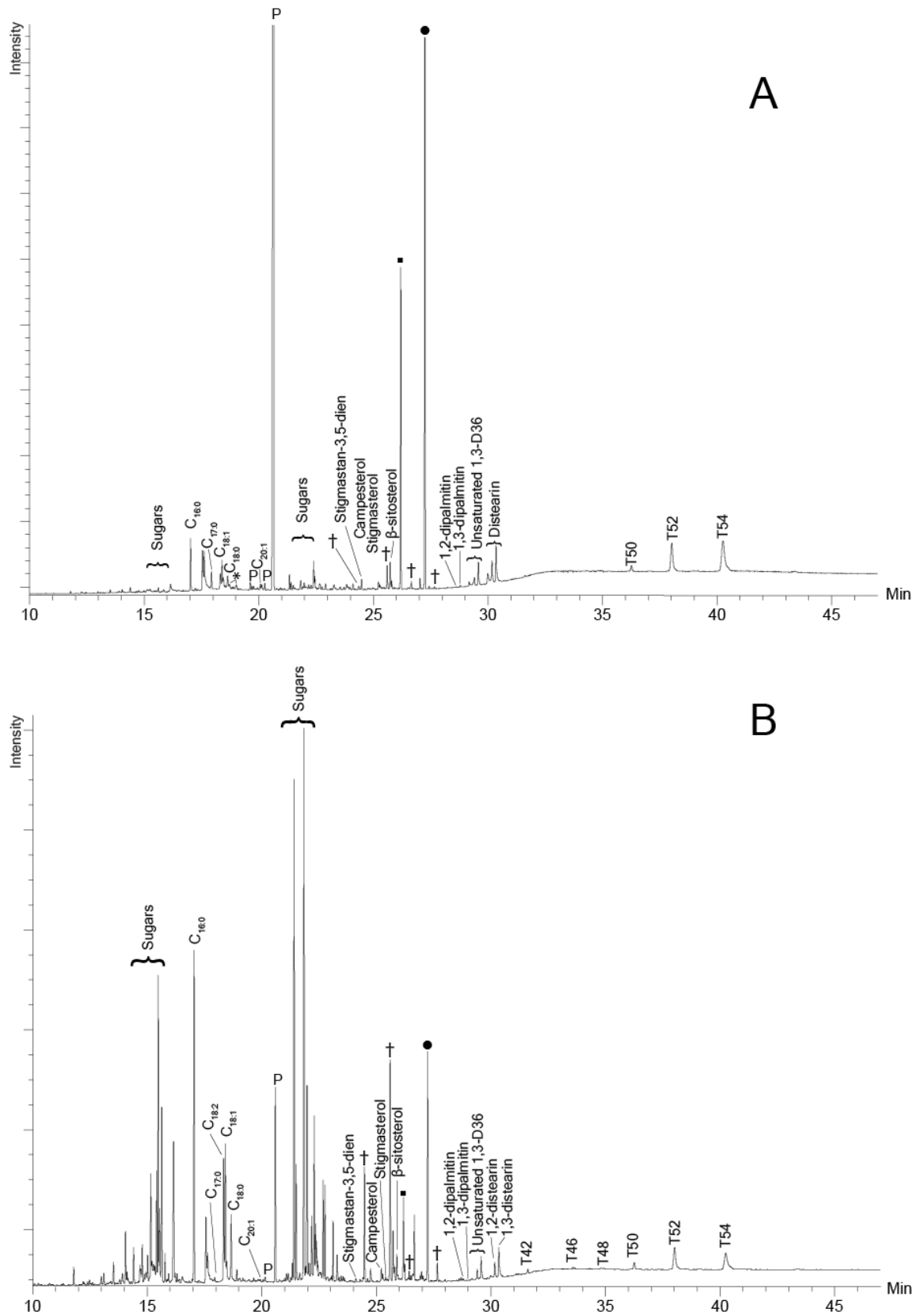


Figure 5.18: Partial gas chromatograms showing A: Einkorn crust prior to burial; B: Unburied absorbed einkorn residue (ECE-06). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; *: ω-(o-alkylphenyl)octadecanoic acid; †: Unidentified peaks; D: DAG; T: TAG; P: Phthalate Plasticser; ■: C34 Internal standard; ●: C36 Internal standard]

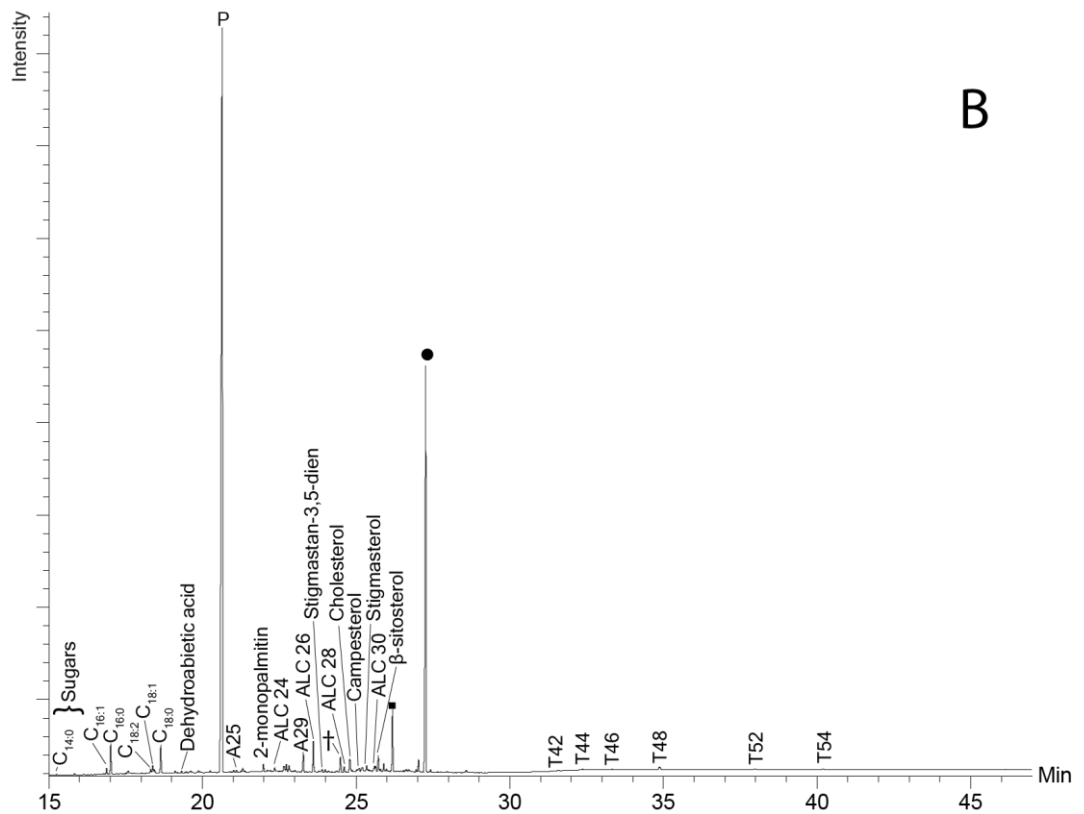
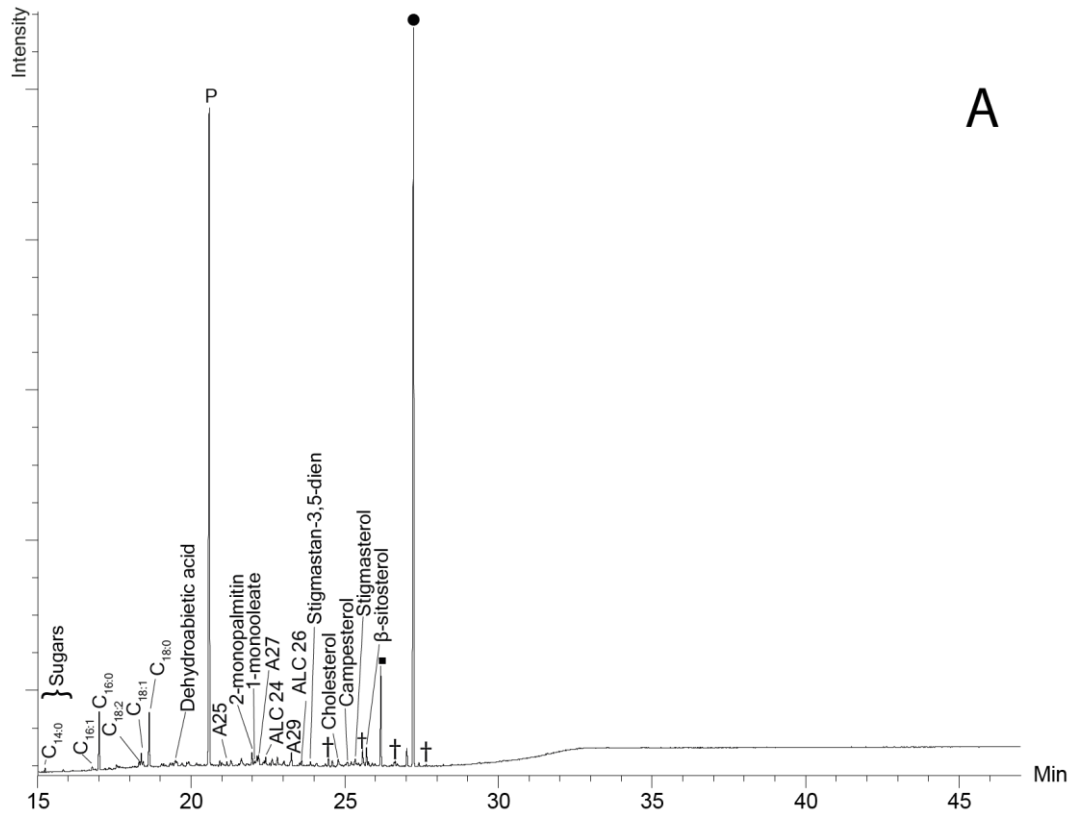


Figure 5.19: Partial gas chromatograms showing A: Einkorn residue buried at Star Carr (SAC-06); B: Einkorn residue buried at Thixendale (WOD-06). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Alkane; ALC: Alcohol; †: Unidentified peaks; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

5.4.5.5 Alcohols

Alcohols were identified in all samples, although they comprised a low percentage (between 0.3% and 3%) of the TLE (Figure 5.17). Fatty alcohols are present in cereal grains; also esterified to fatty acids to form waxes (Becker, 2007), which were however not identified in any of the degraded einkorn samples. Only tetracosanol (C₂₄) was identified in the unburied samples. In the absorbed residue buried at Star Carr (SAC-06), hexacosanol was also identified, but the widest range of alcohols was obtained from the sherd buried at Thixendale (WOD-06) (Table 5.11). No alcohol appears to dominate the series (Figure 5.18 and Figure 5.19). Alcohols are common soil lipid constituents, as stated in Section 5.4.2, and some migration is possible since the alcohols reported in the einkorn residues were also present in the burial contexts. However, none migrated into the control samples buried at both sites, and tetracosanol was identified in the unburied crust and in the unburied absorbed residue (ECE-06).

5.4.5.6 ω -(*o*-alkylphenyl)octadecanoic acids

Laboratory experiments have shown a product-precursor relationship exists between ω -(*o*-alkylphenyl)alkanoic acids and tri-unsaturated fatty acids, when the latter are subjected to prolonged heating (270°C) within a pottery matrix. The process is described in Evershed *et al.* (2008a), but briefly, it involves an alkali isomerisation of the tri-unsaturated fatty acid, followed by a 1,5-hydrogen shift. A *trans/cis* isomerisation then occurs which yields a fully conjugated fatty acid. Cyclic products are produced through intramolecular Diels-Alder (IMDA) reactions, and once the cyclohexenyl ester is formed, aromatisation becomes energetically favourable and will occur quickly at cooking temperatures. 1,7-hydrogen shifts were also found to occur, and these are responsible for the presence of a wider distribution of ω -(*o*-alkylphenyl)alkanoic acid isomers (Evershed *et al.*, 2008a).

Two ω -(*o*-alkylphenyl)octadecanoic acids were observed to form in the unburied einkorn residue (ECE-06) and the visible crust (Figure 5.18). These were identified as TMS derivatives through the characteristic fragment ions comprising the M⁺ at *m/z* 348, [M-15] at *m/z* 333 and the dialkybenzene fragment ion which occurs at *m/z* 105. Einkorn comprises considerable quantities of C_{18:3}, which upon the application of heat and the presence of ceramic, will lead to the formation of ω -(*o*-alkylphenyl)octadecanoic acids. Prolonged heating of C_{18:2} and C_{18:1}, also significantly present in einkorn, will lead to the formation of the corresponding ω -(*o*-alkylphenyl)alkanoic acid through desaturation (Evershed *et al.*, 2008a). The presence of ω -(*o*-

alkylphenyl)octadecanoic acids in the einkorn crust is interesting, since they formed in the absence of a catalyst, namely metal ions found in the ceramic fabric. Furthermore, the stability of ω -(*o*-alkylphenyl)alkanoic acids increases their potential to survive in the archaeological record, however, none were observed in the buried samples. This could be due to the relatively low proportions formed, which would reduce their survival potential.

5.4.5.7 Dehydroabietic acid

Dehydroabietic acid is an abietane-type diterpene resin acid, and is more commonly known as a degradation marker of *Pinaceae* resin (Regert *et al.*, 2005; Tisser, 2008). It can be however biosynthesised by plants, and is generally associated with the Coniferae and Legumionae families (Mills and White, 1994; Tisser, 2008:99). Dehydroabietic acid was identified in the TIC of the einkorn residue buried at Star Carr at 21.74 minutes. Identification was based on its characteristic base peak at m/z 239, as well as key ions present at m/z 250, 357 and 372 as detailed in Regert *et al.* (2005). Its presence has not been reported in relation to einkorn in the publications mentioned herein, and it was observed in only one of the samples obtained after the einkorn cooking experiment. On the other hand, it was not observed in either the control or soil samples collected at both sites. Since dehydroabietic acid was not detected in the crust and unburied absorbed residue, it is an unlikely constituent in einkorn, and its presence must be attributed to the burial context at Star Carr, albeit not present in the soil sample collected from this site.

5.4.5.8 Sugars

As already reported for the acorn and nettle residues, the presence of a series of peaks tentatively attributed to saccharides, were identified in all the einkorn samples (Figures 5.17, 5.18 and 5.19). They were found to comprise the fragment ions recorded by Simoneit *et al.* (2004) as pertaining to this group, and which have already been detailed in Sections 5.4.3.7 and 5.4.4.8 above. Soluble sugars were observed as constituents of einkorn, and were found to occur in low concentrations and in inverse proportion to the recorded levels of starch (Abdel-Aal *et al.*, 1995).

Two groups of sugars were detected prior to 18.50 and after 22.00 minutes in the TIC of the crust and the unburied absorbed residue (ECE-06), whereas in the buried samples, sugars were only detected prior to 18.50 minutes. There was a significant decrease in the quantity of sugars retained after burial. On average prior to burial, residues contained $35\mu\text{g g}^{-1}$ sugars, which decreased to trace amounts after burial. This is consistent with the low survival potential

attributed this class of biomolecules (Eglinton and Logan, 1991), making sugars unlikely to survive in archaeological residues.

5.4.5.9 Unidentified peaks

A series of four peaks were identified in the TIC of the unburied samples at 27.75, 27.96, 29.08, and 30.22 minutes, which have a similar base peak at m/z 268 and ion fragments at m/z 57, 73 and 281. The M^+ and $[M-15]$ ions are also present at m/z 520/505, 548/533, 576/561, and 604/589, in order of elution. Figure 5.20 shows a typical spectrum obtained. These unidentified peaks were also observed in the buried samples, however, while all four peaks occurred in the sample buried at Star Carr (SAC-6), only the peak present at 27.96 minutes survived at Thixendale (WOD-06). Carotenoids (lutein, α -carotene and β -carotene) and tocots (α -tocopherol, α -tocotrienol, β -tocopherol and β -tocotrienol), both lipid-soluble antioxidants were identified as important constituents in einkorn (Hidalgo *et al.*, 2006). However the ion fragmentation patterns obtained for these compounds are not consistent with those obtained in the as yet unidentified spectra. Further analysis is required to identify these compounds.

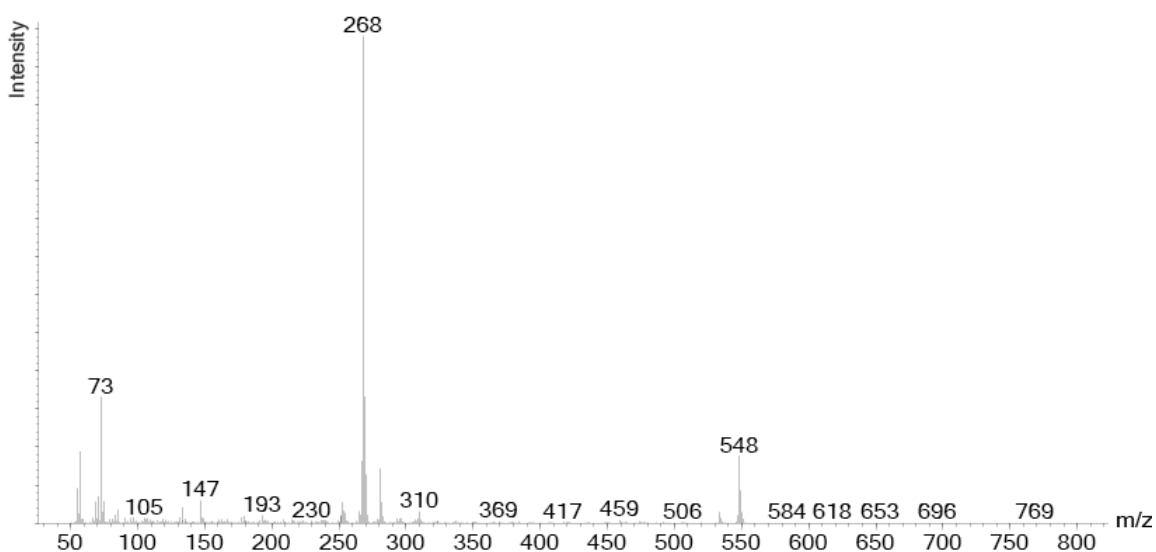


Figure 5.20: Mass Spectrum obtained at 27.94 minutes from the einkorn crust spectrum, showing the characteristic ion fragmentation pattern with a base peak at m/z 268, observed in the series of unidentified compounds.

5.4.5.10 Quantification

As already observed in nettle and acorn, the crust contained the most abundant levels of lipid in einkorn, however, significant amounts were also seen to become absorbed within the wall of the

unburied ceramic vessel (ECE-06), thus increasing its survival potential over archaeological timescales (Figure 5.21). After a seven month burial period, appreciable quantities of einkorn residue were still detected at Thixendale ($16\mu\text{g g}^{-1}$), while considerably lower, but still significant amounts were retained at Star Carr ($9\mu\text{g g}^{-1}$).

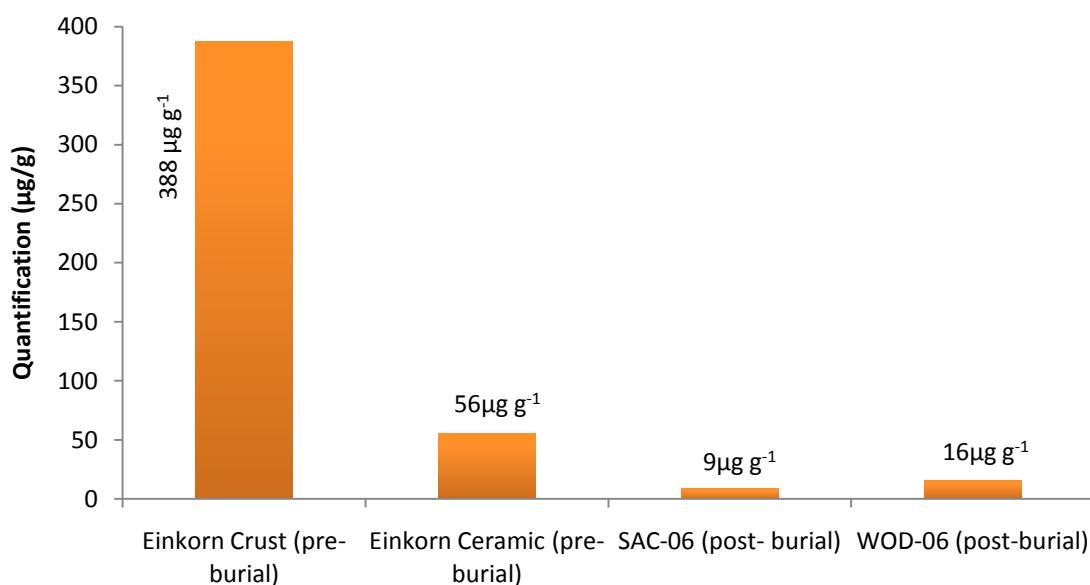


Figure 5.21: Quantified values for the TLE obtained from the absorbed and visible residues produced after repeated boiling of einkorn, expressed as $\mu\text{g g}^{-1}$. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.5.11 Archaeological Implications

Once again, no particular compound or unique distribution was observed to qualify as a biomarker denoting the presence of einkorn. The fatty acid, acylglycerol, alcohol and alkane distribution denote the presence of a residue, but are not distinctive to einkorn. The presence of ω -(*o*-alkylphenyl)alkanoic acids, although not present in either acorn or nettle, cannot be used as a biomarker, since polyunsaturated fatty acids are common to plant oils and the onset of cooking in ceramic vessel may lead to their formation in other plants. The range of sterols observed in the degraded residue is also not particular to einkorn, and is typical of plant degraded residues. However, under better preservation conditions, survival of the wider distribution of sterols detailed in Nurmi *et al.* (2008) may perhaps suggest an einkorn origin. The series of as yet unidentified peaks, with a base peak at m/z 268, may however provide specific and durable markers for einkorn; however, further work is required to identify them. Finally, under moderately alkaline conditions, it appears that significant quantities of einkorn residue are still

retained, although under more acidic conditions preservation appears to be diminished. Einkorn therefore appears to retain the best potential to survive over archaeological timescales.

5.4.6 Einkorn and milk

The last in the series of cooking experiments carried out describes the residue obtained after the simultaneous boiling of einkorn and milk. The quantification results obtained are by far the highest recorded in the present study (Figure 5.22), both prior to and after burial, thus more conducive to survival over long burial periods. Degradation studies on dairy products have already been published (Dudd *et al.*, 1998), and have often been recorded from archaeological contexts (Dudd and Evershed, 1998; Copley *et al.*, 2003; Craig *et al.*, 2005a; Evershed *et al.*, 2008b). Hence the aim of this experiment was to determine whether traces of the lipid distribution obtained for the einkorn experiment can still be detected in the presence of dairy fatty residues.

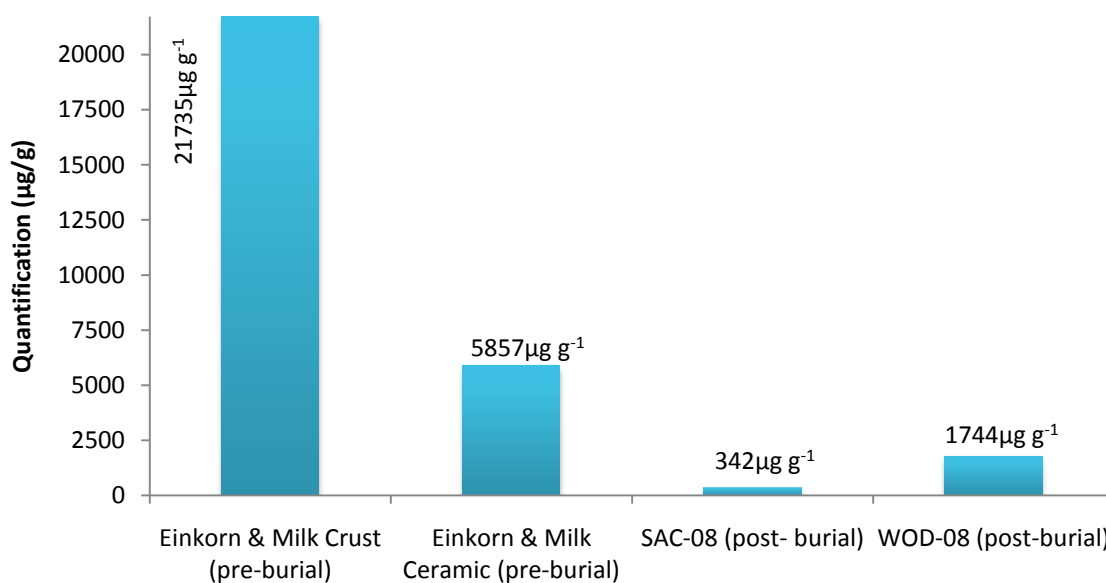


Figure 5.22: Quantified values for the TLE obtained from the absorbed and visible residues produced after repeated boiling of einkorn and milk, expressed as $\mu\text{g g}^{-1}$. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.6.1 Fatty acids

The wider range of fatty acids obtained in the einkorn and milk residues, in particular short-chain fatty acids, can immediately be discerned. Short chain fatty acids ranging from $\text{C}_{4:0}$ to $\text{C}_{10:0}$ are diagnostic of a dairy source, with $\text{C}_{16:0}$, $\text{C}_{18:0}$ and $\text{C}_{18:1}$ making up more than half the fatty acid composition (Pollard and Heron, 2008:390). This fatty acid distribution was observed in the four samples comprising degraded milk and einkorn residues (Table 5.12). Dudd *et al.* (1998) reported

that over the course of the degradation experiment, no significant changes were observed with regard to the relative proportions of C_{16:0} and C_{18:0} fatty acids, however residues tested after a 25 day burial period showed that the characteristic short chain fatty acids (C_{4:0} to C_{12:0}) were largely undetected. The distribution of the major fatty acids (C_{16:0}, C_{18:0} and C_{18:1}) obtained for the buried einkorn and milk samples was consistent; C_{16:0} was always more abundant than C_{18:0}, with C_{18:1} being prominent in all samples, in particularly, in WOD-08 where it was the most abundant fatty acid (Figures 5.23 and 5.24, and Table 5.12).

FA	Einkorn & Milk Crust (%) (Pre-burial)	Einkorn & Milk Absorbed (%) (Pre-burial)	SAC-08 (%) (Post-burial)	WOD-08 (%) (Post-burial)
8:0	0.7	0.3	Tr	Tr
9:0	-	Tr	Tr	Tr
10:1	-	0.1	-	-
10:0	1.0	0.6	0.1	0.8
11:0	-	Tr	0.1	Tr
12:0	1.9	1.1	1.9	3.5
13:0	0.4	0.2	0.4	0.1
14:1	0.7	0.4	8.1	1.1
14:0	6.7	3.6	0.3	13.3
15:0	0.7	0.6	14.0	1.5
16:1	0.4	0.5	-	2.1
16:0	12.2	10.2	12.3	29.5
17:0	4.1	0.4	0.2	0.1
18:1	5.6	10.0	12.8	40.5
18:0	3.2	4.0	7.4	9.5
19:1	0.4	0.2	-	0.1
20:0	0.2	0.1	-	-
22:0	-	-	0.4	-

Table 5.12: Percentage composition of individual free fatty acids in the TLE of the degraded einkorn and milk mixture. [FA: Fatty Acid; Tr: Trace amounts; SAC: Star Carr burial; WOD: Thixendale burial]

It was interesting to note that after a seven month burial period, small quantities of short-chain fatty acids (C_{8:0} to C_{12:0}) were still detected. Considerable quantities of branched C_{15:0} and C_{17:0} fatty acids were also present. These fatty acids occur as a result of microbial action and are found naturally in ruminant milk (Dudd *et al.*, 1998). Although trace amounts of C_{17:0} were observed in the nettle crust and moderate quantities occurred also in the einkorn crust and unburied einkorn absorbed residue (ECE-06), the quantities observed in the einkorn and milk residues by far exceed that observed in the previous experiments, indicating their introduction from the milk component. Furthermore, C_{15:0} was only observed in the einkorn and milk residues. The fatty acid profile obtained therefore primarily indicates the presence of a dairy product, except for the persistent occurrence of C_{18:2} in the buried samples (SAC-08 and WOD-08). Although present in dairy products (Lindmark Måsson, 2008), C_{18:2} comprises a smaller percentage of the total fatty acids, while it is the predominant fatty acid in einkorn (Price and Parsons, 1975). However, despite

this, C_{18:2} is unlikely to survive over archaeological timescale due to its susceptibility to oxidation, hence not a likely indicator for plant material within the mixture.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
Einkorn & Milk Crust (Unburied visible residue)	21735	FAs: C _{8:0} , C _{9:0} , C _{10:1} , C _{10:0} , C _{11:0} , C _{12:0} , C _{14:1} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} , C _{19:1} , C _{20:0} ; Alcohols: C26; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol; MAGs: 2-Monomyrstin, 1-Monomyrstin, 2-Monopalmitin, 1-Monopalmitin, 1-Monooleate, 2-Monostearin, 1-Monostearin; DAGs: 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C44, C46, C48, C50, C52, C54; Sugars: unidentified
EMC-08 (Absorbed residue; Pre-burial)	5857	FAs: C _{8:0} , C _{9:0} , C _{10:1} , C _{10:0} , C _{11:0} , C _{12:0} , C _{13:0} , C _{14:1} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} ; Alcohols: C26; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol; MAGs: 1-Monomyrstin, 2-Monopalmitin, 1-Monopalmitin, 2-Monostearin, 1-Monostearin; DAGs: 1,2-D26, 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C44, C46, C48, C50, C52, C54; Sugars: unidentified
SAC-08 (Absorbed residue; Star Carr Burial)	342	FAs: C _{8:0} , C _{9:0} , C _{10:1} , C _{10:0} , C _{11:0} , C _{12:0} , C _{13:0} , C _{14:1} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} , C _{22:0} ; Alkenes: unidentified; Alcohols: C26; Sterols: Campesterol, β -sitosterol, Stigmasterol, Stigmastan-3,5-dien, Cholesterol, Cholesta-3,5-diene; Ketones: C29, C31, C35; MAGs: 1-Monomyrstin, 1-Monopalmitin, 1-Monooleate; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C46, C48, C50, C52; ω-(<i>o</i>-alkylphenyl)octadecanoic acids; Sugars: unidentified
WOD-08 (Absorbed residue; Thixendale Burial)	1744	FAs: C _{8:0} , C _{9:0} , C _{10:1} , C _{10:0} , C _{11:0} , C _{12:0} , C _{13:0} , C _{14:1} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} , C _{19:1} ; Alcohols: C24, C26; Sterols: Campesterol, Stigmasterol, Cholesterol; MAGs: 2-Monomyrstin, 1-Monomyrstin, 2-Monopalmitin, 1-Monopalmitin, 2-Monostearin, 1-Monooleate, 1-Monostearin; DAGs: 1,2-D26, 1,3-D28, 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, Unsaturated 1,3-D36, 1,2-D34, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C46, C48, C50, C52

Table 5.13: GC-MS and HT-GC identification of the lipid composition of boiled einkorn and milk analysed before and after burial. [FA/C_{x:y}: Fatty acids where x is the carbon number and y is the degree of unsaturation; MAG: Monoacylglycerol; D & DAG: Diacylglycerol; TAG: Triacylglycerol]

5.4.6.2 Acylglycerols

Milk mainly consists of water containing globules of milk fat and solids, the latter comprising protein, sugar, minerals and vitamins, while the former is dominated by TAGs, which make up 98% of milk fat (Pollard and Heron, 2008:390). In fresh milk, TAGs comprise between C26 to C54 acyl carbon atoms, and their fatty acid composition ranges between C_{4:0} and C_{20:0} (Dudd *et al.*, 1998).

The onset of hydrolysis in the einkorn and milk experiments is evident from the proportions of TAGs, DAGs, MAGs and free fatty acids obtained, with the more degraded residues containing less TAGs, but more free fatty acids (Table 5.14). Figure 5.25 also shows that TAGs in the degraded samples comprise a much lower percentage of the TLE than in the unburied samples (between 2 and 22%), with a corresponding increase in the fatty acid composition and the presence of DAGs and MAGs in the lipid profile (Table 5.14). Hydrolysis was found to proceed more rapidly after the cleavage of the first fatty acid (Dudd *et al.*, 1998), which explains the lower accumulation of DAGs and MAGs as the reaction quickly proceeds to completion. The highest quantities of TAGs survived in the crust ($3457\mu\text{g g}^{-1}$), followed by the unburied einkorn and milk residue (EMC-08) ($1289.81\mu\text{g g}^{-1}$), the sample buried at Star Carr (SAC-08) ($58\mu\text{g g}^{-1}$) and the sample buried at Thixendale (WOD-08) ($29\mu\text{g g}^{-1}$), with TAGs comprising C40 to C54 acyl carbon atoms in all four samples. The loss of the lower molecular weight TAGs was expected, and was already observed by Dudd *et al.* (1998). Reduced steric hindrance between short-chain fatty acids moieties making up the TAG increases their susceptibility to hydrolysis during processing and burial; moreover, these short chain fatty acids are more water-soluble, hence liable to be lost by leaching from the burial environment (Pollard and Heron, 2008:391).

In boiled einkorn, the amount of TAGs which occurred in the crust ($126\mu\text{g g}^{-1}$) and the unburied absorbed einkorn residue (ECE-06) ($2\mu\text{g g}^{-1}$) was significantly less than in the einkorn and milk residues, and preservation in the buried einkorn samples was very poor, with trace amounts in the sample buried at Thixendale (WOD-06) and none detected in the sample buried at Star Carr (SAC-06). C54 was the most abundant TAG in the unburied einkorn samples, but no particular TAG dominated in the buried einkorn sample. In the einkorn and milk residues C40 was observed to dominate the TAG fraction in the crust and the absorbed residue buried at Thixendale (WOD-08), C50 was dominant in the sherd buried at Star Carr (SAC-08), while C52 was the most abundant TAG in unburied einkorn and milk absorbed residue (EMC-08). This variation, in particular between the unburied and buried residues, does not lend a distinctive TAG profile to the mixture of einkorn and milk. C54 was consistently the most abundant TAG in einkorn, however over archaeological timescales this characteristic might not survive. Using NanoESI MS and MS/MS techniques, already applied by Mirabaud *et al.* (2007), may lead to a better distinction of the TAGs present in einkorn based on their fatty acid composition. This might enable its distinction from the milk TAG profile already identified in Mirabaud *et al.* (2007).

<i>Compound</i>	<i>Einkorn & Milk Crust (%) (Pre-burial)</i>	<i>Einkorn & Milk Absorbed (%) (Pre-burial)</i>	<i>SAC-08 (%) (Post-burial)</i>	<i>WOD-08 (%) (Post-burial)</i>
FAs	38.0	31.9	57.1	71.0
MAGs	3.1	2.3	1.3	4.0
DAGs	13.9	13.5	9.3	7.8
TAGs	15.9	22.0	17.0	1.7

Table 5.14: Percentage composition of fatty acids (FAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and triacylglycerols (TAGs) in the TLE of the degraded einkorn and milk mixture. [SAC: Star Carr burial; WOD: Thixendale burial]

5.4.6.3 Sterols and phytosterols

The sterol fraction comprised a small proportion of the TLE, making up to 3% of the unburied and 1% of the buried residue (Figure 5.25). Cholesterol was the dominant sterol in the unburied residue, which was expected since cholesterol is a constituent in milk (Pollard and Heron, 2008:390). However, in both the crust and the unburied absorbed residue (EMC-08), the phytosterol content was consistently high, and comprised 48 and 38% of the total sterol content respectively. β -sitosterol was the dominant phytosterol in the unburied residues.

In the buried einkorn and milk residues, phytosterols consistently made up the higher percentage of the total sterol content. In the absorbed residue buried at Star Carr (SAC-08), phytosterols dominate the sterol fraction, comprising 78% of the total sterol content. Cholesterol and cholesta-3,5-diene made up the remaining 22%, with the former being slightly more abundant. Stigmastan-3,5-dien dominated the phytosterol fraction, which also comprised campesterol, stigmasterol and β -sitosterol. In the absorbed residue buried at Thixendale (WOD-08), the phytosterol fraction, which included stigmasterol and campesterol, was also found to dominate, comprising 54% of the total sterol fraction. Cholesterol made up the remaining 46% of the sterol fraction. Only trace amounts of phytosterols were detected in the control blanks buried at each site, hence the phytosterol content present in the einkorn and milk residues can be securely identified as originating from the einkorn. Phytosterols appear to persist in degraded buried residues, and although they cannot be used to identify a plant to species level, they are good indicators of plant residues.

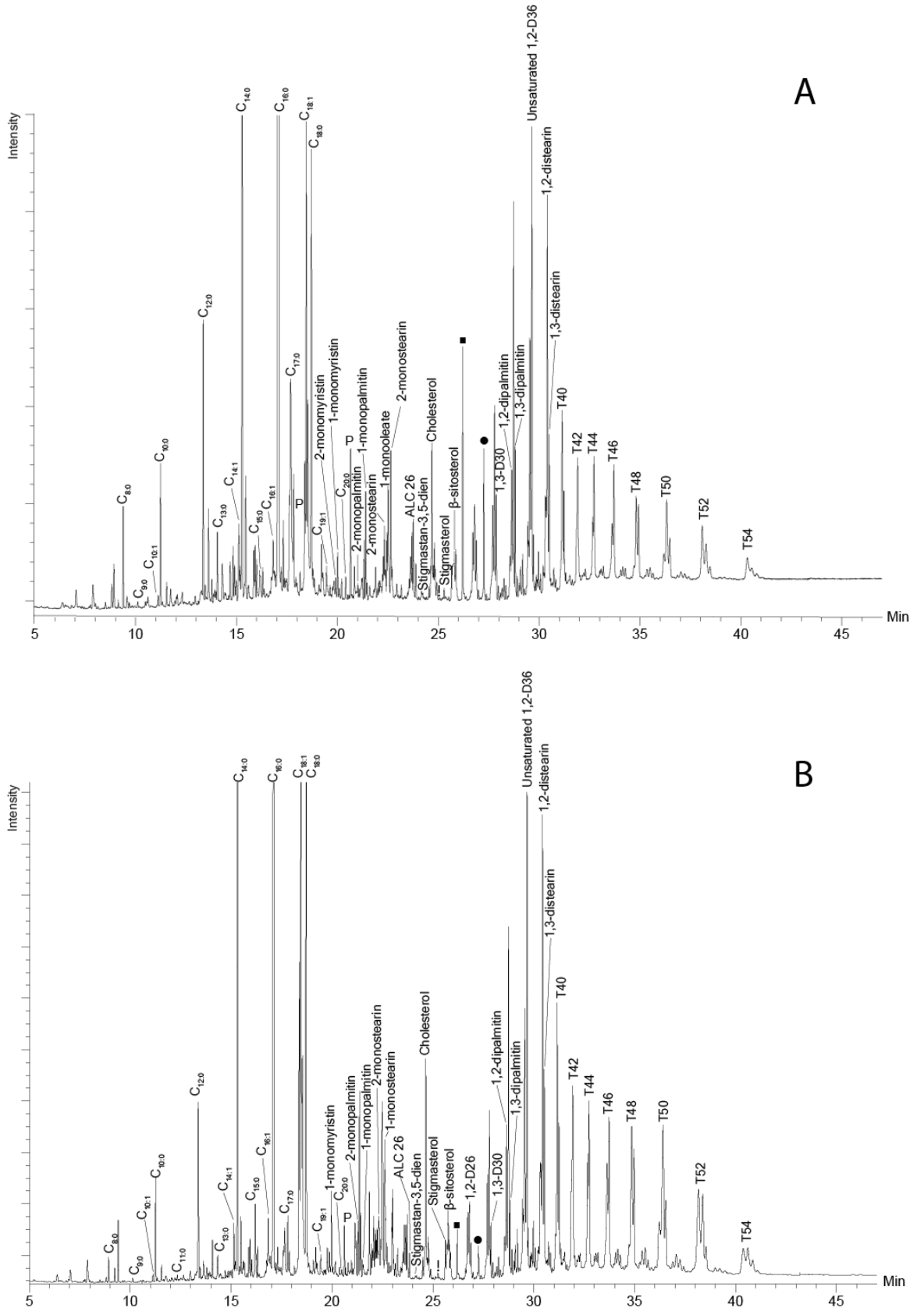


Figure 5.23: Partial gas chromatograms showing A: Einkorn & milk crust prior to burial; B: Unburied absorbed einkorn & milk residue (EMC-08). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; !: Campesterol; ALC: Alcohol; D: DAG; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

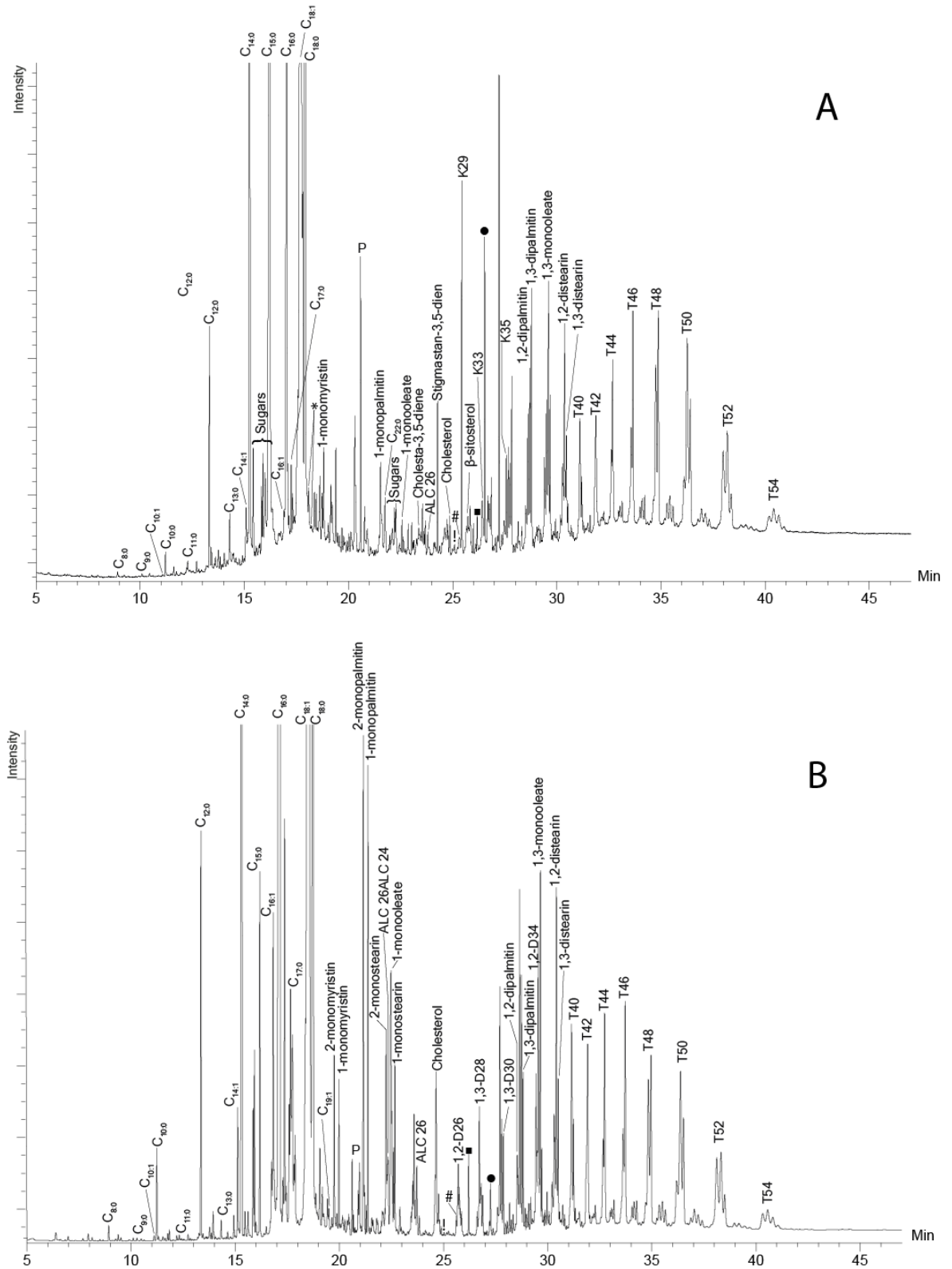


Figure 5.24: Partial gas chromatograms showing A: Absorbed einkorn & milk residue buried at Star Carr (SAC-08); B: Absorbed einkorn & milk residue buried at Thixendale (WOD-08). [Cx:y: Fatty acid where x is the carbon number and y is the degree of unsaturation; ALC: Alcohol; !: Campesterol; #: Stigmaterol; K: Ketone; D: DAG; T: TAG; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

Chapter 5

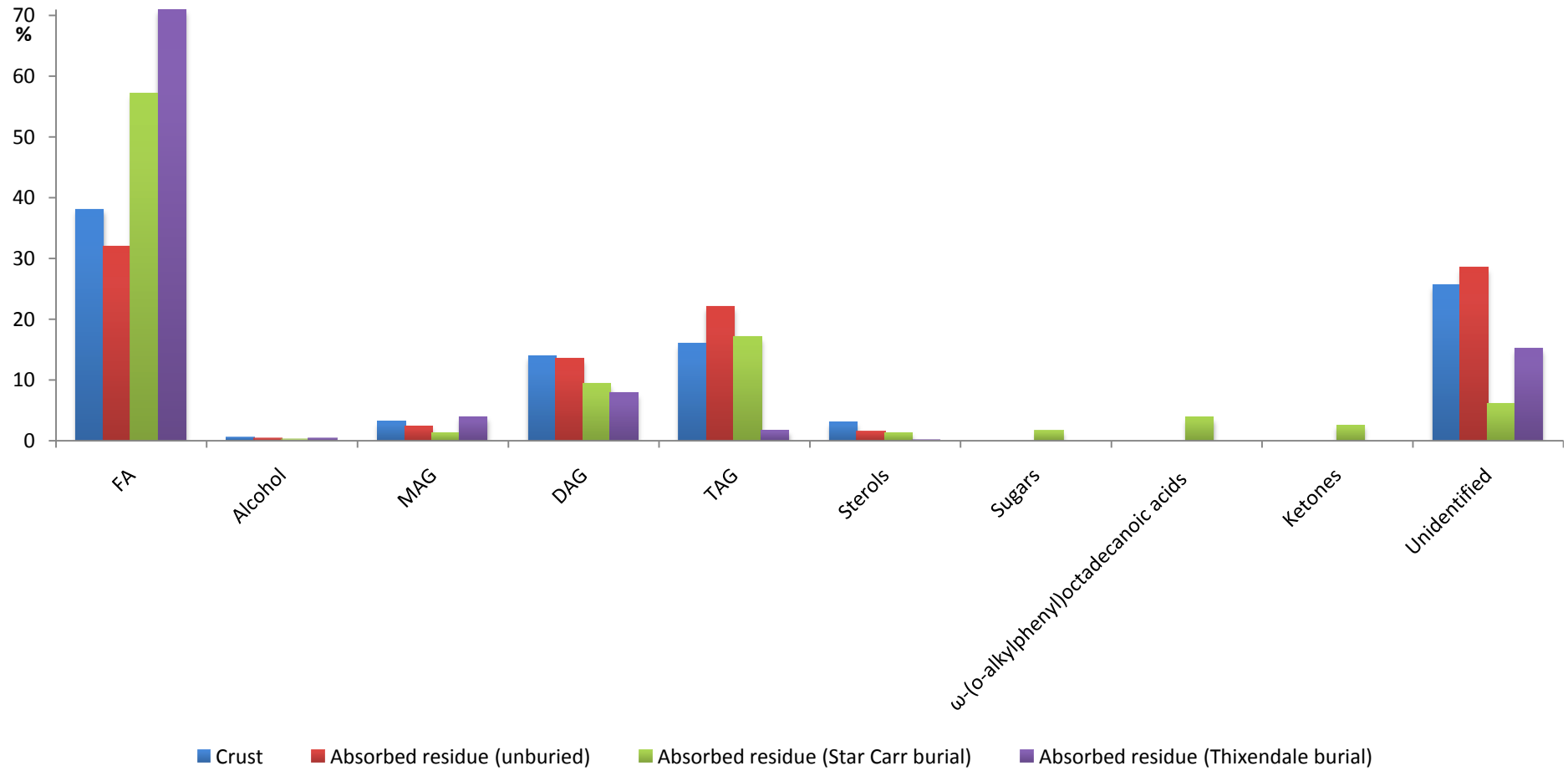


Figure 5.25: TLE profile of degraded einkorn and milk as percentages of the absolute quantified residue. [FA: Fatty Acid; MAG: Monoacylglycerol; DAG: Diacylglycerol; TAG: Triacylglycerol]

5.4.6.4 Alkanes, alkenes and alcohols

No alkanes were detected in the einkorn and milk residues, while only trace amounts of alkenes were identified in sample buried at Star Carr (SAC-08). Similar results were obtained when einkorn was boiled with water.

Alcohols were similarly poorly attested, with only hexacosanol (C26) being identified in the unburied crust and absorbed unburied residue (EMC-08), and the absorbed residue buried at Star Carr (SAC-08), while both hexacosanol and tetracosanol (C24) were present in the sample buried at Thixendale (WOD-08). Although alcohols made up a relatively low percentage of the TLE in all degraded residues (Figure 5.25), they amounted to 113, 20, 1 and 6 $\mu\text{g g}^{-1}$ in the crust, EMC-08, SAC-08 and WOD-08 residues respectively. This is considerably more than observed in degraded einkorn residues, with 5 $\mu\text{g g}^{-1}$ identified in the crust and trace amounts present in the absorbed residues. Since alcohol is not a constituent of milk, it must therefore originate from einkorn. However, no explanation can be put forward at this point with regard to the increased quantities retrieved in the einkorn and milk residue as opposed to einkorn.

5.4.6.5 Ketones

Ketones were identified only in the absorbed residue buried at Star Carr (SAC-08) and comprised C29, C31, and C35. The presence of C35 which is rarely found in plants, and the absence of alkanes and alcohols with the same carbon number indicate that the long chain ketones identified in SAC-08 are degradation products, and not plant constituents. Moreover, ketones were not detected in the boiled einkorn residue. Hence their presence in the absorbed residue buried at Star Carr (SAC-08) is attributed to ketonic decarboxylation, which occurs during cooking and is indicative of vessel use (Evershed *et al.*, 1995b; Raven *et al.*, 1997).

5.4.6.6 ω -(*o*-alkylphenyl)octadecanoic acids

Two ω -(*o*-alkylphenyl)octadecanoic acid isomers were identified in the absorbed residue buried at Star Carr (SAC-08), but none were detected in the sample buried at Thixendale (WOD-08), in the unburied absorbed residue (EMC-08) and crust. The formation of ω -(*o*-alkylphenyl)octadecanoic acids has already been described in Section 5.4.5.6, in relation to their presence in the unburied einkorn residue (ECE-06) and the einkorn crust. The presence of ω -(*o*-alkylphenyl)octadecanoic acid isomers in SAC-08, can therefore be attributed to the einkorn fraction in the einkorn and milk mixture.

5.4.6.7 Sugars

Saccharide peaks were tentatively identified at 17.50 minutes and between 23.00 and 25.00 minutes in the TIC of the buried absorbed residue at Star Carr (SAC-08). The basis for their identification has already been discussed in Sections 5.4.3.7 and 5.4.5.8, above. These peaks were not detected at in the sample buried at Thixendale and in the unburied einkorn and milk residues. Although sugars were present in the boiled einkorn residues (Section 5.4.5.8), they are also known constituents of milk. However, none have so far been reported in archaeological dairy residues, which is not surprising given their low survival potential.

5.4.6.8 Archaeological implications

The degraded lipid profiles obtained for the einkorn and milk mixtures are mainly dominated by the dairy component, comprising $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids, cholesterol, and a wide TAG distribution. In addition, alcohols, ω -(*o*-alkylphenyl)octadecanoic acids and phytosterols were also identified, which were found to occur in the einkorn lipid profile, and therefore must originate from the einkorn component. Their presence, in particular phytosterols, which appear to survive after prolonged burial, and dominate the sterol fraction, provide a good indication that the residue in question is a mixture and comprises a plant input. Interestingly, the unidentified peaks which appeared consistently in the boiled einkorn residue (Section 5.4.5.9), were not present in the einkorn and milk mixture.

The persistence of unsaturated fatty acids, even after burial, was observed in all three plant species included in the cooking experiments, however by far the greatest proportion was observed in the einkorn and milk mixture (Table 5.15). This may be explained in terms of the formation of colloidal-scale crystalline aggregates, which arise from the ability of acylglycerols and fatty acids to self-sort, namely through polar and hydrogen bonding between the head groups and aliphatic chains, and π - π interactions between double bonds and the methyl end groups. Organisation within a lipid crystal depends on the structure of the component lipids and their exposure to physical conditions (polymorphism) and can range from relatively disordered to highly structured forms. Lipid crystals will affect the rate of lipid degradation (Craig, 2008:11-12). Experimental results showed that the high quantities of mono- and polyunsaturated fatty acids and TAGs present in plant oils are not amenable to the formation of lipid crystals or colloidal-level aggregates, whereas the low proportion of polyunsaturated and high proportion of saturated lipids allow animal derived residues to form lipid crystals, which shield unsaturated lipids (Craig,

2008:119-120). This may perhaps explain why over archaeological timescales, unsaturated fatty acids are still present and usually associated with animal products; while plant lipid residues do not tend to survive. Crystalline aggregates form between molecules that are structurally and chemically identical (Craig, 2008:11-12); hence, their formation perhaps from the interaction between molecules originating from different lipid sources (e.g. plant and animal lipid) within the same vessel, may perhaps be conducive to the preservation of plant lipids in mixtures.

Sample		Unsaturated Fatty Acids ($\mu\text{g g}^{-1}$)				
		C _{14:1}	C _{16:1}	C _{18:2}	C _{18:1}	C _{19:1}
Acorn	SAC-02	-	-	-	0.1	-
	WOD-02	-	-	Tr	0.1	-
Nettle	SAC-01	-	-	-	-	-
	WOD-01	-	-	0.1	0.1	-
Einkorn	SAC-06	-	0.1	0.2	-	-
	WOD-06	-	0.1	0.2	0.6	-
Einkorn & milk	SAC-08	-	-	-	43.8	-
	WOD-08	13.1	25.2	-	491.6	0.8

Table 5.15: Table showing the quantified values in $\mu\text{g g}^{-1}$ of unsaturated free fatty acids identified in the buried acorn, nettle, einkorn and einkorn & milk residues. [SAC: Star Carr burial; WOD: Thixendal burial; Tr: Trace amount]

Craig (2008:44-45) also conducted experiments into the re-use of ceramic vessels. Beef, sesame oil and linseed oil were boiled sequentially in this order, each for two hours. It was observed that the residue obtained mostly resembled beef, with the next highest contribution made by sesame oil, and only traces of linseed oil being retained (Craig, 2008:113). It therefore appears that the dominant residue is produced after the first cooking episode, which can be explained by the increased availability of ceramic voids in the cooking vessels, which will allow a more favourable absorption of the residue. As the voids fill up, absorbed lipid from subsequent cooking episodes becomes less well represented, although it is possible that thermal degradation within the ceramic fabric may continuously produce a series of small voids leading to further absorption (Craig, 2008:113). This suggests that variation in the composition of the residue is likely to occur across the ceramic profile, as was indeed observed by Craig (2008:114-115). It follows, that an equal representation of mixtures of residues would be introduced into ceramic fabrics upon first using a ceramic vessel. This however was not the case in the einkorn and milk experiment, which was mainly dominated by the dairy profile, with only traces of the einkorn component retained (Figure 5.26 and Table 5.13). This can be explained in terms of the lipid content that comprises

animal and plant products. As already observed by Reber and Evershed (2004b), the higher lipid component in animal fats leads to masking of the plant oil profile.

Figure 5.21 and Figure 5.22 highlight the considerable increase in quantified lipid when einkorn was boiled with milk, compared to boiling einkorn in water, while Figure 5.26 further emphasises the apparent masking of the einkorn component.

Simultaneous boiling of einkorn and milk therefore showed a dominance of dairy lipids in the profile of the residue; however, the presence of a mixture was still detected in particular, by the retention of considerable quantities of phytosterols, even after burial.

5.5 Conclusion

The importance of plant identification in archaeological studies has already been discussed in the introduction. In the context of this research, finding unequivocal evidence for the processing of plant material in Early Neolithic Impressed/Cardial Wares would provide direct proof for their use, in particular, in view of the strong circumstantial evidence obtained from botanical and palaeodietary studies, which indicate a heavy reliance on plants from the earliest Neolithic phases in the Mediterranean. Hence, the main aim for conducting the experiment described herein was to test whether plant lipid residues absorbed within the walls of ceramic vessels could still be identified after a prolonged burial period, hence attest to their direct anthropological use in ceramics, for both culinary and non-culinary processes.

However, characteristic plant biomarkers, in particular unsaturated fatty acids and phytosterols, are prone to alteration and degradation during burial, and rarely survive in archaeological residues. The cooking experiments described above all show a remarkably low absorption of plant lipids in the ceramic fabric, which was, for the most part, not conducive to survival after being subject to further degradation during burial. The quantity of plant lipid absorbed during cooking of acorn, nettle and einkorn was relatively low when compared to the amount of lipid absorbed from the einkorn and milk mixture (Figure 5.27). This low absorption is reflected in their preservation after burial, which was negligible in both acorns and nettles, and low in einkorn. On the other hand, $342\mu\text{g g}^{-1}$ and $1744\mu\text{g g}^{-1}$ of lipid were retained in the einkorn and milk residues buried at Star Carr and Thixendale respectively. More importantly, this study has shown that the chromatograms obtained from degraded plant lipid residues cannot be distinguished from those showing advanced stages of decayed lipid. Consequently, ORA will under-represent the use of

ceramics to processes plant products, which highlights the need to interpret ORA results in light of the archaeological data available, and integrate multiple lines of evidence (e.g. plant microfossils and stable isotope analysis). This will be further discussed in Chapter 8.

No specific biomarker, whether a particular biomolecule or a characteristic distribution were identified, which could be directly associated with the plant materials included in this experiment. Although γ -tocopherol and β -amyrin were identified in acorn and nettle respectively, they did not survive in the buried residue, which makes them unlikely to be useful as biomarkers. Similarly, ω -(*o*-alkylphenyl)alkanoic acid isomers were only present in the unburied einkorn residues, and moreover, because of their formation from polyunsaturated fatty acids which are commonly found in most plant oils, they cannot be considered unique to einkorn. Phytosterols were consistently the best preserved plant biomarkers which occurred in varying quantities and distributions in all plant residues included in this experiment. Although these are also known soil constituents and could potentially be introduced by migration from the burial environment, only trace amounts of phytosterols were present in the control samples buried at Star Carr and Thixendale. The quantity of phytosterols present in all the buried samples was consistently higher than that obtained from the control samples, and their origin can therefore be securely attributed to the plant material processed within the vessel analysed. However, although good indicators of plant products, phytosterols could not be used to differentiate between the different plant types.

The third aim of the experiment was to determine whether the einkorn lipid profile could still be detected when boiled with milk. The results supported Reber and Evershed's (2004b) observations, that the low quantities of plant lipids which become transferred into the ceramic matrix are likely to be overprinted by fattier animal products, in this case, milk. However, although the lipid profile obtained showed a predominantly dairy origin, the presence of phytosterols, and to a lesser extent alcohols, indicated the presence of a plant input. As described above, the formation of colloidal-scale crystalline aggregates, particularly in the presence of saturated animal fats, could potentially protect plant lipid signatures over archaeological timescales, and therefore allow their identification in archaeological residues.

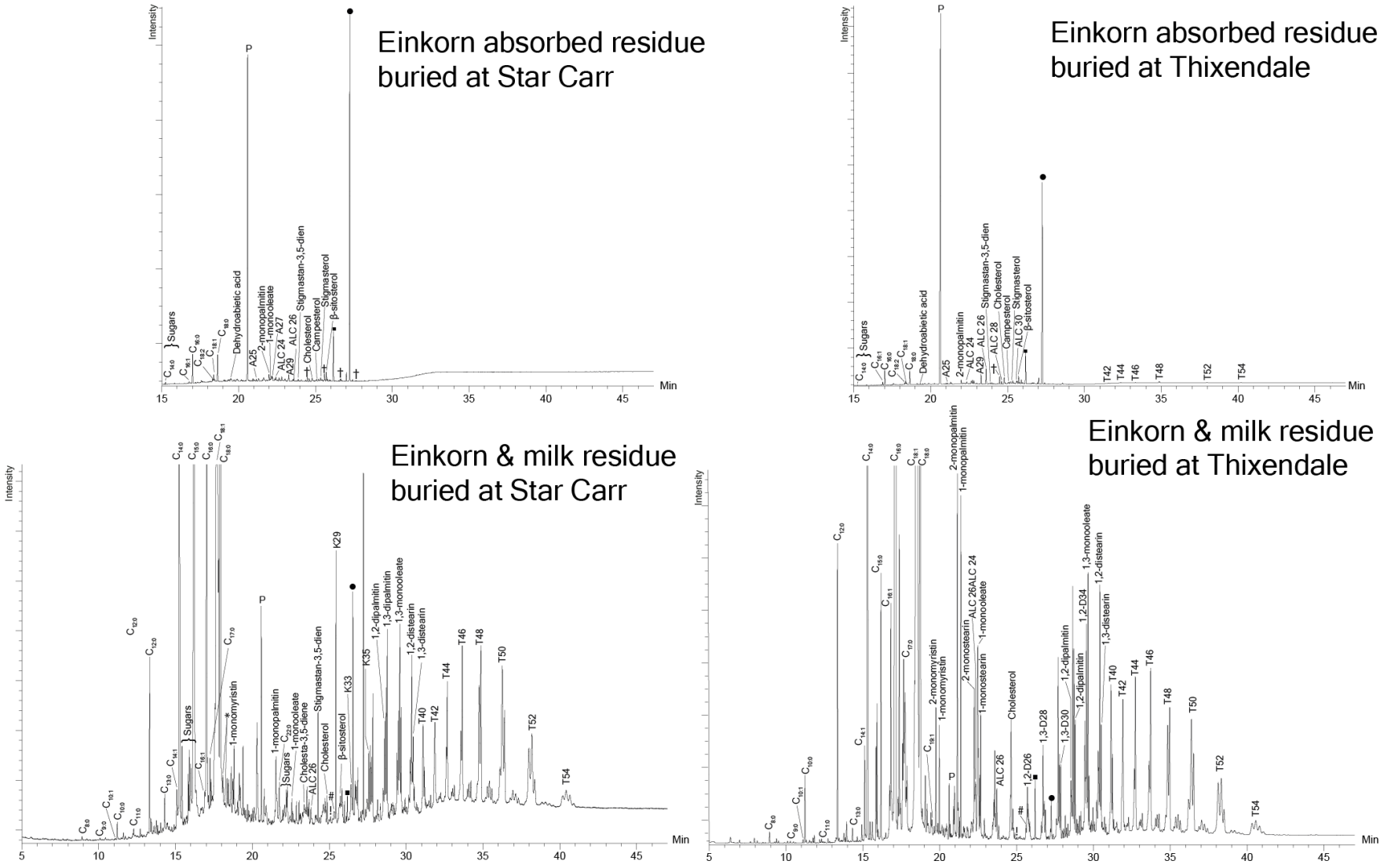


Figure 5.1: Comparative partial gas chromatograms showing the buried einkorn, residues, and buried einkorn and milk residues. [Cxy: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Ketone; ALC: Alkane; DAG: Diacylglycerol; TAG: Triacylglycerol; P: Phthalate Plasticiser; ■: C34 Internal standard; ●: C36 Internal standard]

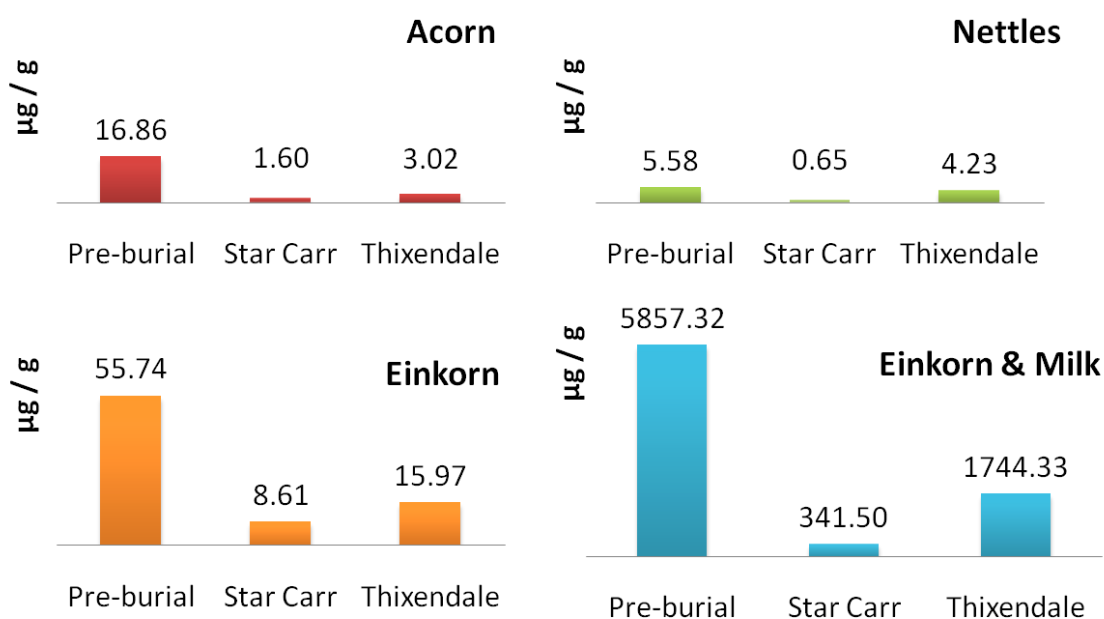


Figure 5.27: Charts showing the quantification in $\mu\text{g g}^{-1}$ of lipid, of the different plant residues absorbed within the ceramic matrix of their respective ceramic containers, prior to and after burial.

Finally, the experiment also tested differences in plant lipid preservation in moderately acidic (pH 6) and alkaline (pH 8) burial contexts. In general, the lipid constituents making up the plant material survived in both burial contexts and none of the different lipid classes was observed to survive preferentially at a specific pH. Quantification of the TLE however showed that lipid residues were consistently more abundant at Thixendale, thus suggesting that lipid preservation appears to be moderately enhanced in basic conditions. These results appear to conflict with previous research, in which alkaline conditions were observed to be less conducive to the survival of lipid residues during burial, while acidic conditions appeared to inhibit free radical oxidation and auto-oxidation, and promote a degree of preservation (Craig, 2008:70). This can however be attributed to the fact that at Star Carr, the pH of the burial context was only mildly acidic, and a much lower pH (around pH 4) is required to obtain good preservation conditions (Moucawi *et al.*, 1981).

The experimental results described in this chapter showed that because of the low quantities of plant lipids which become absorbed within ceramic vessel walls, plant lipid residues are unlikely to survive over archaeological timescales, and therefore will not allow a secure identification using ORA. Expanding the repertoire of plant types analysed so far and modifying the extraction and analytical protocol, could potentially lead to the identification of specific biomarkers that are

likely to survive over archaeological timescales, and which could be targeted during analysis. Pyrolysis techniques have already been successfully applied to the identification of visible crusts (Oudemans and Boon, 1991), however these rarely survive in archaeological contexts. GC-combustion-Isotope Ratio Mass Spectrometry (GC-c-IRMS) has also been used to analyse modern plant lipid residues, however these were found to cluster between ruminant and non-ruminant animal fats, which usually denotes mixtures of the two animal fats (Steele *et al.*, 2010). Furthermore, as shown in the plant burial experiment conducted in this research, it is unlikely that sufficient C_{16:0} and C_{18:0} originating from plant oils will survive in archaeological residues to allow analysis using GC-c-IRMS. Perhaps targeting the carbohydrate and protein fractions, which are also major plant constituents, using analytical techniques amenable to the identification of high molecular weight compounds, (e.g. Pyrolysis-GC/MS and Liquid Chromatography-MS), in conjunction with soft ionisation techniques (e.g. Electrospray Ionisation) to avoid unnecessary molecular fragmentation, could increase sensitivity to the detection of plant biomolecules. Conversely, experimenting with different types of detectors (e.g. Tandem MS), could also provide further information on the structural composition of the residues analysed. As already discussed in the introduction to this chapter, various other analytical techniques have already been applied to identify plant products, including stable isotope analysis, genetics, and the identification of plant microfossils obtained from various contexts (e.g. ceramic vessels, lithics and dental calculus). There is therefore much scope and need to improve the current methods used to detect plant material in archaeological contexts, which could potentially provide more direct evidence for the anthropological utilization of plant products.

Chapter 6

Method and sample preparation

6.1 Introduction

This chapter outlines the sampling criteria and laboratory procedures followed to extract and analyse organic residues from Early and Middle Neolithic pottery. These comprised 363 sherds, 9 carbonised surface deposits and 10 soil samples obtained from 301 ceramic vessels excavated from Mediterranean Neolithic contexts including sites in Italy, Malta, Croatia and Catalonia. Appendix C provides a detailed catalogue of the ceramics sampled.

6.2 Site selection and sampling criteria

Eight Early Neolithic sites were selected from both coastal and inland settlements in Apulia (Italy), which contained some of the earliest Impressed Ware ceramics attested in Italy and provided an excellent opportunity to study culinary practices at the transition to agriculture. The archaeology of this region has been widely studied and more recently, an extensive petrographic analyses of several pottery assemblages was undertaken (Muntoni, 2003), generating important data for a more in-depth interpretation of the function and provenance of Impressed Wares. Sampling pottery from inland and coastal zones could potentially allow any variability in the exploitation of resources to be identified, in particular between terrestrial and marine products. The research area was broadened by including Neolithic sites from Calabria in southern Italy and an underwater settlement in Lazio (central Italy) to allow an inter-regional analysis, as well as another three sites located in the Adriatic, Malta and Catalonia (Figure 6.1, Table 6.1). Samples were obtained primarily from Early Neolithic deposits since the main research question addresses the earliest ceramics occurring at the transition to agriculture. However sherds from Middle Neolithic contexts were also sampled when possible, in particular in sites comprising continuous occupation layers. This allowed any changes in culinary preferences over time to be observed.

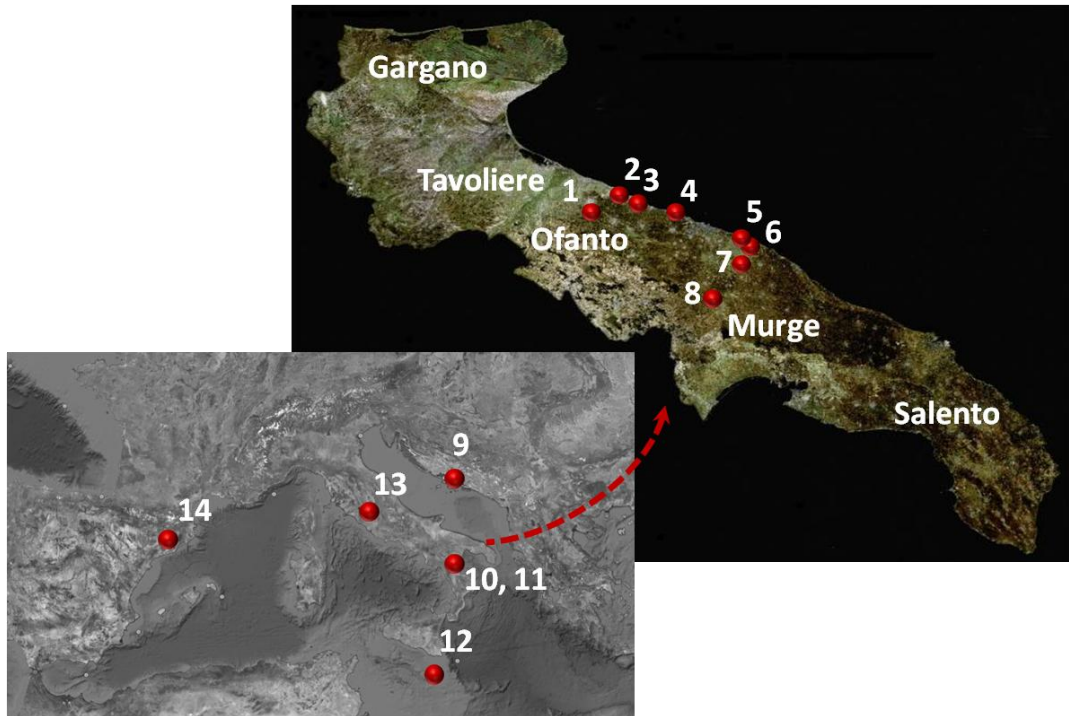


Figure 6.1: Map showing the location of the sites investigated. [1: Palata 1; 2: Canne Setteponti; 3: Seconda Spiaggia di Colonna; 4: Fondo Azzollini; 5: Serri-San Gabriele, Bari San Paolo; 6: Masseria Maselli; 7: Balsignano; 8: Ciccotto; 9: Nakovana Cave; 10: Favella della Corte; 11: Grotta San Michele, Saracena; 12: Skorba; 13: La Marmotta; 14: Can Sadurní]

Samples were selected from domestic contexts and comprised a selection of rims, bases and body fragments from jugs, bowls, flasks, plates and pots which may have been used as cooking vessels. Detailed records of the fabric and decoration were kept (Appendix C), and the shape of the vessel was noted, although the latter was not always possible due to the fragmented nature of the sherds. When whole pots were available, samples were taken along the vessel's profile with the purpose of attempting to establish the mode of cooking as described in Charters *et al.* (1993). Sampling was carried out using a Dremmel modelling drill with a tungsten bit. About 2g of ceramic powder was drilled from the internal surface of each sherd to a depth of around 4mm, discarding the first layer to remove any possible contamination due to handling and contact with plastic. External surfaces were also sampled to assess possible contamination introduced from the burial context. The ceramic powder was collected on foil, then weighed and transferred to sterilised scintillation vials. Charred surface deposits were sampled using clean scalpels.

<i>Site</i>	<i>Code</i>	<i>Country</i>	<i>Period</i>	<i>N</i>
<i>Fondo Azzollini, Pulo di Molfetta</i>	AZZ	Apulia, Italy	EN	25
<i>Ciccotto, Gravina in Puglia</i>	CIC	Apulia, Italy	EN-MN	25
<i>Balsignano, Modugno</i>	BAL	Apulia, Italy	EN	35
<i>Palata 1, Canosa di Puglia</i>	PAL	Apulia, Italy	EN	39
<i>Serri-San Gabriele, Bari San Paolo</i>	BSP	Apulia, Italy	EN	15
<i>Masseria Maselli, Bari</i>	MAS	Apulia, Italy	EN	12
<i>Canne Setteponti, Barletta</i>	SET	Apulia, Italy	MN	12
<i>Seconda Spiaggia di Colonna, Trani</i>	TRA	Apulia, Italy	MN	26
<i>Favella della Corte, Corigliano Calabro</i>	FAV	Calabria, Italy	EN	27
<i>Grotta San Michele, Saracena</i>	SAR	Calabria, Italy	EN-SW	15
<i>La Marmotta, Anguillara Sabazia</i>	MAR	Lazio, Italy	EN	6
<i>Skorba, Mgarr</i>	SKR	Malta	EN-TP	16
<i>Nakovana Cave</i>	NAK	Croatia	EN-MN	17
<i>Can Sadurní, Begues</i>	CNS	Barcelona	Cardial-PC	31

Table 6.1: List of Neolithic Mediterranean sites included in this study. [EN: Early Neolithic; MN: Middle Neolithic; SW: Stamped Ware; TP: Temple Period; PC: Post-cardial; N: Number of vessels analysed]

6.3 Laboratory sample preparation

All glassware was sterilised by heating at 450°C for eight hours prior to use, and nitrile gloves were worn at all times to avoid contamination. Most of the ceramic samples had previously been stored in plastic bags after being excavated; hence the introduction of phthalate plasticisers was inevitable. Blanks were run with every batch of samples to assess laboratory contamination. Figure 6.2 shows the analytical procedure followed.

6.3.1 Standards

The following reference standards were prepared at concentration of 1µg/µL: Palmitic (C_{16:0}) and Stearic (C_{18:0}) fatty acid standards (Sigma, c.99% pure), azelaic acid (Sigma, c.98% pure), 1-monopalmitin (Sigma, c.99% pure), 1,2-dipalmitin (Sigma, c.99% pure), glycerol trimyristate (Sigma, c.99% pure), glycerol tripalmitate (Sigma, c.99% pure), glycerol tristearate (Sigma, c.99% pure), and cholesterol (Sigma, c.99% pure). Internal standards *n*-tetratriacontane (Sigma, c.99% pure) and *n*-hexatriacontane (Supelco, c.99.5% pure) were prepared at concentrations of 0.2µg/µL.

The δ¹³C isotopic signature of the C_{16:0} and C_{18:0} fatty acid standards was measured using bulk stable isotope analysis at the University of Bradford. Mass spectrometry was carried out on an Europa 20-20 instrument with Roboprep combustion system. The measurements obtained were later used to correct the GC-c-IRMS δ¹³C values for the carbon atom added during methylation.

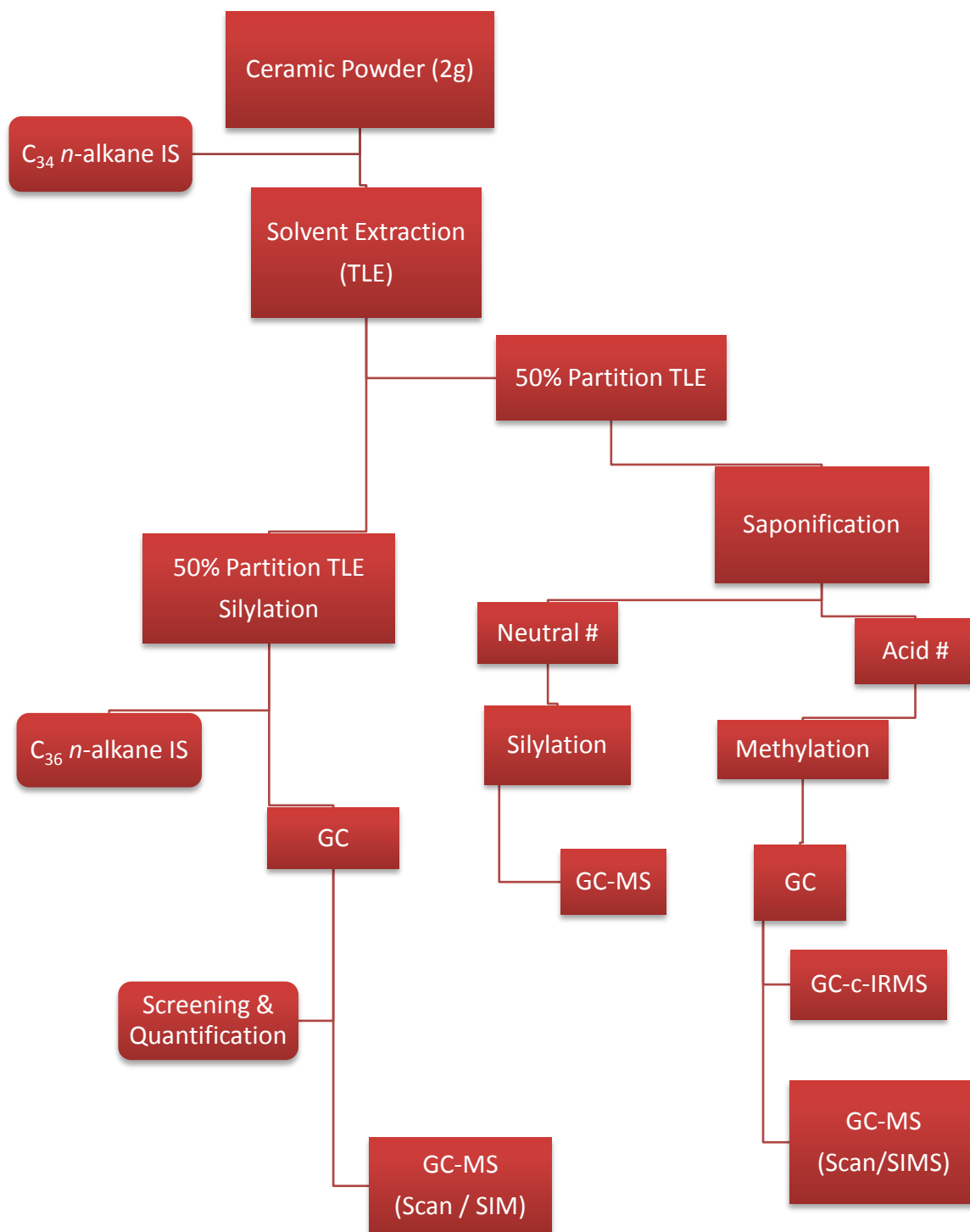


Figure 6.2: Chart showing the analytical methodology followed for the preparation of the archaeological samples. [TLE: Total Lipid Extract; GC: Gas Chromatography; GC-MS: GC-Mass Spectrometry; GC-c-IRMS: GC-combustion-Isotope Ratio MS; SIM: Selected Ion Monitoring; #: fraction]

6.3.2 Solvent extraction

Prior to extraction, 1µg of tetratricosane (C_{34} *n*-alkane) internal standard (IS) was added to each of the powdered ceramic samples for quantification purposes. Lipids were extracted by adding 5mL of dichloromethane:methanol (2:1; *v:v*) (HPLC grade; Fischer) to the ceramic powder, and sonicating for 15 minutes. Following separation from the powdered ceramic by centrifuging at 2000rpm for 10 minutes at room temperature, the solvent was decanted into clean screw capped vials. This process was repeated three times. The solvent was removed under a gentle stream of nitrogen and mild heating (40°C) to obtain the total lipid extract (TLE). The TLE was partitioned (50%).

6.3.3 Derivatisation: silylation

50% of the TLE was derivatised before analysis using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). 50µL of hexane (HPLC grade; Fischer) were added to the dry TLEs, followed by 4 drops of the derivatising agent. The vials were then heated on a pre-heated thermal block at 70°C for 1 hour, and allowed to cool prior to evaporation to dryness using nitrogen gas and mild heating. 1µg of hexatricosane (C_{36} *n*-alkane) internal standard was added and each sample was re-hydrated in hexane. The samples were vortexed for 30s to ensure a homogenised solution.

6.3.4 Saponification

Saponification was carried out on one half of the partitioned TLE of selected samples to release esterified fatty acids. 3mL of 0.5M methanolic sodium hydroxide solution made up in methanol:water (9:1, *v:v*) were added to each sample. The vials were heated at 70°C for 1 hour on a pre-heated thermal block, and then allowed to cool before extracting the neutral fraction three times with 2mL of hexane (Fischer; HPLC Grade). The solvent was evaporated to dryness using mild heating (30°C) and a gentle stream of nitrogen. The neutral fraction was stored at -20°C prior to silylation and analysis. The remaining fraction was acidified to a pH 3 using between 1 and 2mL of 1M hydrochloric acid (Aristar; sp. Gr. 1.18). The acid fraction was extracted into sterilised screw-capped test tubes three times using 2mL of hexane. The solvent was again evaporated under a gentle stream of nitrogen and mild heating (30°C). The acid fraction was stored at -20°C pending methylation.

Saponification was also carried out on 30 previously extracted ceramic powder samples, to analyse the 'bound' lipid fraction not released by solvent extraction. The procedure was essentially identical to that described above, however 4mL of 0.5M methanolic sodium hydroxide solution made up in methanol:water (9:1, v:v) were added to each sample, and heated at 70°C for 90 minutes. The supernatant was then removed prior to extraction of the neutral and acid fractions using hexane, as described above.

6.3.5 Derivatisation: methylation

200µL of boron trifluoride methanol solution (14%; Sigma Life Science) were added to the dry acid fraction obtained after saponification. The samples were heated for 1 hour at 70°C on a pre-heated thermal block. The reaction was quenched with two drops of ultrapure water, and the samples were allowed to cool before extracting the methylated lipids. Extraction was carried out three times using 2mL of hexane into sterilised screw-capped test tubes. The hexane was evaporated under very mild heating (30°C) and a gentle stream of nitrogen. C_{16:0} and C_{18:0} fatty acid standards of known carbon isotopic composition (-29.7‰ and -29.5‰ respectively), were methylated alongside the samples

6.4 Gas Chromatography (GC)

All samples were initially screened as trimethylsilylated (TMS) derivatives using GC before proceeding with further analysis. Different columns and instrumental parameters were used during the course of this research, which are described below. Appendix C records the instrument details used for the individual samples, whereas the gas chromatograms obtained for all archaeological samples are presented in Appendix E.

Initial screening of the TMS derivatives was carried out on an Agilent 7890 series GC using a 30m, HP-5 (5%-Phenyl)-methylpolysiloxane (J&W Scientific) column, with a 0.32mm internal diameter and a film thickness of 0.25µm. The inlet (in splitless mode) and flame ionisation detector (FID) were set at 300°C, and the flow rate was 2mL/min. The oven was programmed at 50°C for 2 minutes, then ramped at 10°C per minute to 325°C and held for 15 minutes. Hydrogen was used as the carrier gas. Data acquisition and analyses was carried out on a ChemStation Rev. B.04.02 SP1. A Thermo Trace GC Ultra with a Triplus autosampler using a 60m, DB1 column, with a 0.32mm internal diameter and a film thickness of 1µm, was also used to screen TMS derivatives. The inlet (in splitless mode) and flame ionisation detector (FID) were set at 310°C and 330°C

respectively, and the flow rate was 1mL/min. The oven was programmed at 50°C for 5 minutes, then ramped at 4°C per minute to 320°C and held for 20 minutes. Hydrogen was used as the carrier gas.

Prior to submitting for GC-c-IRMS analysis, the selected samples prepared as fatty acid methyl esters (FAMES) were first run on the GC to ensure that all the esterified fatty acids had been released.

6.5 High Temperature-Gas Chromatography (HT-GC)

HT-GC was carried out on selected samples to ensure elution of the less volatile compounds. The samples were run as TMS derivatives. An Agilent 7890 series GC was used. It was initially fitted with a ZB-5HT 5% Phenyl 95% Dimethylpolysiloxane (Zebron) 20m column, with an internal diameter of 0.18mm and a film thickness of 0.18µm. This was eventually replaced by a 15m DB-1HT (100% Dimethylpolysiloxane) (J&W Scientific) column, with a 0.32mm internal diameter and a film thickness of 0.1µm due to normal use wear. The inlet (in splitless mode) and flame ionisation detector (FID) were set at 300°C and 400°C respectively, while the flow rate was set at 0.79mL/min. The oven was programmed at 50°C for 2 minutes, then ramped at 10°C per minute to 400°C and 350°C for the ZB-5HT and DB-1HT columns respectively, and held for 15 minutes. Hydrogen was used as the carrier gas. Data acquisition and analyses was carried out on a ChemStation Rev. B.04.02 SP1.

6.6 GC-Mass Spectrometry (GC-MS)

An Agilent 7890A Series GC connected to a 5975C Inert XL mass selective detector was used. The splitless injector and interface were maintained at 300°C and 280°C respectively. The oven temperature was programmed at 50°C for 2 minutes, and ramped to 340°C at 10°C/minute and held for 10 minutes. Helium was used as the carrier gas, and was kept at constant inlet pressure. The GC was fitted with a 30m, Agilent HP-5ms column, with an internal diameter of 0.32mm and film thickness of 0.25µm. The column was directly inserted into the ion source where electron impact (EI) spectra were obtained at 70eV with full scan from m/z 50 to 800. Data was acquired using an MSD ChemStation E.02.00.493.

6.7 GC-MS fragmentation analysis

Mass spectra fragmentation patterns were analysed using NIST library searches and published data, mainly Evershed (1992c), Stacey (1999) and Christie (2011a), as well as knowledge of fragmentation patterns. Table 6.2 shows the main fragmentation patterns identified.

Compound	Fragmentation patterns
Fatty acids (TMS esters)	Major fragment ion at $[M-15]^+$ representing loss of a methyl group Molecular ion $[M^+]$ is generally present Characteristic peaks at m/z : 73, 75, 117, 129, 132 and 145
Fatty acids (Methyl esters)	Weak molecular ion $[M^+]$ $[M-32]^+$ and $[M-31]^+$ Characteristic peaks at m/z : 74 and 87
Long-chain alcohols (TMS ethers)	Weak molecular ion $[M^+]$ Strong $[M-15]^+$ The ion at m/z 103 representing the $\text{CH}_2\text{OSi}(\text{CH}_3)_3$ distinguishes <i>n</i> -alcohols from TMS esters of long-chain fatty acids
Alkanes	Weak molecular ion $[M^+]$ $[M-29]^+$ present Characteristic fragmentation pattern produced by mass fragments of diminishing intensity corresponding to lost alkyl groups (m/z : 51, 71, 85, 99, etc.)
Sterols/Phytosterols (TMS ethers)	Prominent $[M^+]$, $[M-15]^+$ and $[M-90]^+$ Base peak at m/z 129 $[M-129]^+$ present
Wax esters	Weak molecular ion $[M^+]$ Base peaks: $[\text{C}_{14}\text{H}_{31}\text{O}_2]^+$ at m/z 229, $[\text{C}_{16}\text{H}_{33}\text{O}_2]^+$ at m/z 257 and $[\text{C}_{18}\text{H}_{35}\text{O}_2]^+$ at m/z 285
Monoacylglycerols (MAG) (TMS ethers)	Weak $[M^+]$ and $[M-15]^+$ $[M-90]^+$ $[\text{M}-\text{CH}_2\text{OSi}(\text{CH}_3)_3]^+$ denotes 1-MAG isomer $[(\text{CH}_3)_3\text{SiOCH}=\text{CHCH}_2\text{OSi}(\text{CH}_3)_3]^+$ (m/z 218) denotes 2-MAG isomer
Diacylglycerols (DAG) (TMS ethers)	$[M^+]$ not generally present Weak $[M-15]^+$ and $[M-90]^+$ Characteristic ions at m/z 129 and 145 Individual acyl moieties identified from loss of $[\text{M}-\text{RCO}_2]^+$ or $[\text{M}-\text{RCO}_2\text{H}]^+$ for unsaturated acyl moieties Prominent peaks at $[\text{RCO}]^+$, $[\text{RCO}+74]^+$ and $[\text{RCO}+90]^+$ $[\text{M}-\text{RCOOCH}_2]^+$ denotes 1,3-DAG
Triacylglycerols	Minor peaks: $[M^+]$ and $[M-158]^+$ $[\text{RCO}]^+$ and $[\text{RCO}+74]^+$ indicate the molecular mass $[\text{M}-\text{RCOOH}]^+$ occurs from the rearrangement stabilising the $[\text{M}-\text{RCOO}]^+$ Abundant ion: $[\text{RCO}]^+$ shows the carbon number and degree of unsaturation
Long-chain ketones	Weak $[M^+]$ Prominent peaks result from fragmentation α and β to the carbonyl group. The latter occurs less readily and in conjunction with the transfer of a γ -hydrogen atom (McLafferty rearrangement). Prominent ions also occur one mass unit higher than those arising from β cleavage

Table 6.2: MS fragmentation patterns of the most common lipid compounds present in archaeological residues (following Evershed, 1992c; Stacey, 1999; Pollard *et al.*, 2007:177).

6.8 GC-MS: Select Ion Monitoring (SIM)

A selected number of samples were run on GC-MS in SIM mode, which heightens the sensitivity of the MS to selected fragment ions and is therefore particularly useful for identifying components with a typically low abundance (Pollard *et al.*, 2007:175). This was carried out primarily to check for marine biomarkers. Marine oils and fats are rich in mono- ($C_{16:1}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$) and polyunsaturated ($C_{20:5}$ and $C_{22:6}$) fatty acids, which however rarely survive in archaeological residues due to their susceptibility to oxidation reactions. Dihydroxy acids (C_{16} - C_{22}) have been found to be direct degradation products of Z-monounsaturated alkenoic acids formed via secondary reactions with hydroperoxides, and their occurrence together with isoprenoids and ω -(*o*-alkylphenyl)alkanoic acids (C_{16} - C_{22}) were found to constitute clear evidence of marine products (Copley *et al.*, 2004; Hansel *et al.*, 2004; Hansel and Evershed, 2009). A series of α,ω -dicarboxylic acids (C_8 - C_{11}) were also detected in appreciable abundances, with a predominance of C_9 (azelaic acid) obtained from the thermal degradation of α - $C_{18:3}$ (Evershed *et al.*, 2008a). Table 6.3 lists the fragment ions selected for SIM.

Compound	Derivatisation	Fragment ions (m/z)
9,10-Dihydroxyhexadecanoic acid	TMS derivative	73, 187, 317, 489, 504
9,10-Dihydroxyoctadecanoic acid	TMS derivative	73, 215, 317, 517, 532
11,12-Dihydroxyeicosanoic acid	TMS derivative	73, 215, 345, 545, 560
11,12-Dihydroxydocosanoic acid	TMS derivative	73, 315, 373, 573, 588
α,ω-nonanedioic acid	TMS derivative	188, 201, 317, 332
ω-(<i>o</i>-alkylphenyl)hexadecanoic acid	Methyl ester	91, 105, 262
ω-(<i>o</i>-alkylphenyl)octadecanoic acid	Methyl ester	91, 105, 290
ω-(<i>o</i>-alkylphenyl)eicosanoic acid	Methyl ester	91, 105, 318
ω-(<i>o</i>-alkylphenyl)docosanoic acid	Methyl ester	91, 105, 346
4,8,12-trimethyltridecanoate (4,8,12-TMTD)	Methyl ester	87, 213, 157, 270
2,6,10,14-tetramethylpentadecanoate (Pristanate)	Methyl ester	88, 101, 157, 222, 312
3,7,11,15-tetramethylhexadecanoate (Phytane)	Methyl ester	101, 171, 326
5,8,11,14- Eicosatetraenoic acid (Arachidonic Acid)	Methyl ester	79, 150, 318

Table 6.3: Table listing the fragment ions selected for SIM (following Evershed *et al.*, 2008a; Heron *et al.*, 2010; Hansel *et al.*, 2011).

6.9 GC-combustion-Isotope Ratio MS (GC-c-IRMS)

GC-c-IRMS analysis on residues extracted from Impressed/Cardial Wares was carried out at facilities at the Universities of Newcastle and Liverpool. The instrumental details used are described below.

At the University of Newcastle, GC-c-IRMS analysis was carried out on a Thermo Trace Ultra GC using a splitless injector (280°C) via a Combustion III Interface linked to a Thermo Delta V+ IR-MS

(HT voltage 3-5kV, Trap current 0.75mA, Box current 0.7mA). The acquisition was controlled by a Dell computer using Isodat software in Carbon mode monitoring the Carbon Dioxide (CO_2) $^{44/45}\delta\text{C}^{12/13}$ ratio. The sample (1 μl) in hexane was injected by a CTC auto sampler and the split opened after 1 minute. The GC was temperature programmed from 40-140°C at 10°C per minute and then to 300°C at 4°C per minute, and held at final temperature for 1 minute. Helium was used as the carrier gas (flow 1mL/min, initial pressure of 50kPa, split at 20mLs/min). The solvent peak was diverted to the FID and CO_2 Reference Gas was pulsed into the MS. After 7 minutes the back flush valve directed the split sample via the Combustion Furnace (940°C) and Reduction Furnace (650°C) into the MS and the isotope ratio was measured. Chromatographic separation was performed on a fused silica capillary column (60m x 0.25mm internal diameter (i.d.)) coated with 0.25 μm Di-methyl Poly-siloxane (HP-1) phase. The acquired data was processed using the Isodat dynamic background integration Workspace software to give the peak retention times and isotope ratios as $\delta^{13}\text{C}$ values. Instrument precision was $\pm 0.5\%$.

At the facility in Liverpool, stable carbon isotopic compositions of individual lipids were determined using a Delta V Advantage mass spectrometer (Thermo Fisher, Bremen) linked to a Trace Ultra GC with a ConFlo IV interface. Samples re-hydrated in hexane were loaded on to a TriPlus autosampler and 1 μl was injected in splitless mode on a J&W Scientific DB5 fused silica column (30m, 0.25mm i.d., and 0.25 μm film thickness). The effluent from the GC passed from the GC column immediately into and through a combustion reactor consisting of a NiO tube with CuO/NiO wires which was held at 1030°C. The effluent then passed through a water separator consisting of a Nafion tube prior to entering the MS. The GC programme was ramped from 45°C (1 minute) to 280°C at 4°C/minute to a 20 minute hold time. The injector was held at 300°C. Ultra high purity grade helium was used as the carrier gas at a constant flow of 1.4mL/min. The effluent from the GC was diverted away from the combustion reactor during the initial period of solvent elution and out of a divert valve to the atmosphere (backflush mode), while helium was passed backwards through the combustion reactor. During the solvent-divert period, CO_2 reference gas was automatically introduced into the isotope ratio mass spectrometer in a series of pulses and its $^{13}\text{C}/^{12}\text{C}$ ratios measured. After the solvent-divert period, the effluent from the GC was allowed to enter the combustion reactor and IRMS. The IRMS automatically measured the ion intensities of m/z 44, 45, 46 in its three Faraday cups corresponding to $^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{CO}_2$, and $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ respectively. The Isodat 3 software automatically computed the $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of each sample peak, referenced to the standard CO_2 gas and its known $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ content. Carbon isotopic compositions represent averaged values of duplicate or triplicate analyses. The CO_2 reference gas was externally calibrated relative to Vienna Pee Dee Belemnite (VPDB) on SIRA.

The results were presented in per mil (‰) relative to VPDB standard. External standards containing FAMEs (Indiana University standard F8 and FAME Standard supplied with the submitted samples) with accurately known $\delta^{13}\text{C}$ values were analysed with every batch of FAME samples. The accelerating voltage was 3KV and the trap and box currents were set at 0.84mA and 0.66V respectively; the electron energy was set at 124V. The MS vacuum was 1.9×10^{-6} mbar. Instrument precision was $\pm 0.33\%$.

The isotopic shift caused by the addition of a carbon atom to the fatty acids during methylation was corrected by measuring the difference between the bulk $\delta^{13}\text{C}$ value obtained for the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acid standards (described Section 6.3.1), and the $\delta^{13}\text{C}$ measurement obtained after GC-c-IRMS analysis of the same standards. The correction factor was applied to all $\delta^{13}\text{C}$ measurements of the archaeological samples analysed.

6.10 Integration and quantification of lipid residues following GC analysis

Peak integration was carried out using ChemStation Rev. B.04.02 SP1. Automated integration was selected to eliminate inconsistencies; ensuring that the error introduced at this stage was constant in all samples and can therefore be considered insignificant. The lipid content in each sample was quantified using the following formula: $(\text{Area}_{(\text{Sample})}/\text{Area}_{(\text{IS})}) * (\text{Weight}_{(\text{IS})}/\text{Weight}_{(\text{Ceramic sample})})$, omitting contaminant peaks and plasticisers.

Chapter 7

Characterising lipid residues from Impressed/Cardial Wares in the western Mediterranean

7.1 Introduction

Although Impressed/Cardial Wares have been extensively studied, research has mostly focussed on their chronological appearance, intra- and inter-regional variations in form, fabric and decorative motifs, their provenance and circulation. Chapter 2 described in some detail the spread of these early ceramics throughout the western Mediterranean, and their close stratigraphic association with domesticates from the earliest Neolithic phases, which, together with other lines of evidence discussed in Chapter 2, appears to support the proposed model of a ‘punctuated maritime pioneer colonisation’ (Zilhão, 2001; Zeder, 2008; Rowley-Conwy, 2011). Hence, the function of Impressed/Cardial Wares has always been assumed to be closely associated with agrarian products, therefore implying that the spread of pottery was linked to the spread of agriculture and pastoralism. The counter-hypothesis presented in Chapter 2 however does not accept this assumption, and questions whether the spread of pottery and farming in the Mediterranean could be chronologically related to, but not directly associated with the spread of farming. Research carried out in Africa and the Far East has proved that foraging communities did produce ceramic vessels; hence the presence of pottery does not necessarily indicate agrarian/pastoral practice or the association of non-agrarian communities with farmers (Close, 1995; Jordan and Zvelebil, 2010a and references therein). In this scenario, pottery vessels would have been used to process wild food resources, including marine fish, and their function would be more varied, and perhaps regionally dependent.

This main aim of the present research is to investigate the function of Impressed/Cardial Wares, which has so far been inferred by analysing the physical properties of the ceramic assemblages (e.g. form, size, and fabric). Direct evidence for a vessel’s contents can be obtained by analysing the lipid profile of residues absorbed within the ceramic walls, which therefore provides a stronger interpretation for its use. An added advantage of using organic residue analysis (ORA) is

that it allows the identification of products which are very often under-represented or missing in the archaeological record because of poor preservation, such as dairy products and evidence for the use of marine fish. In most of the sites analysed in the present study, the faunal assemblage was too fragmented to allow a secure interpretation of the mortality profile, hence the economy practised. Fish bones were rarely attested, perhaps due to differential preservation. Using ORA, dairy and marine residues can be securely identified, and therefore provide direct evidence for their use by Early Neolithic communities.

For this purpose, 363 pottery samples have been analysed from 301 vessels, which were obtained from a number of key Early Neolithic sites in Italy, Malta, Croatia and Catalonia (Tables 7.1 and 7.2). A detailed record of the samples analysed is available in Appendix C. Sample preparation and the instrumental parameters are described in Chapter 6, and Appendix E provides a complete catalogue of the gas chromatograms obtained. Gas Chromatography (GC), High Temperature-GC (HT-GC) and GC-Mass Spectrometry (GC-MS) identification of the lipid residues run as trimethylsilyl (TMS) derivatives was carried out using NIST library searches and published data (see Chapter 6), and by comparing retention times with known standards. All samples which contained sufficient C_{16:0} and C_{18:0} were submitted for GC-c-IRMS analysis. Appendix D provides a list of the GC-combustion-Isotope Ratio MS (GC-c-IRMS) measurements obtained and a full record of the identified lipid constituents of the residues extracted.

<i>Sites</i>	<i>Code</i>	<i>Country</i>	<i>Site Type</i>	<i>Period</i>	<i>Date (cal. BC)</i>
<i>Fondo Azzollini, Pulo di Molfetta</i>	AZZ	Apulia, Italy	Open air	EN	6100-5880 ^{†*}
<i>Ciccotto, Gravina in Puglia</i>	CIC	Apulia, Italy	Open air	EN-MN	n.d.
<i>Balsignano, Modugno</i>	BAL	Apulia, Italy	Open air	EN	5600-5450 ^{†*}
<i>Palata 1, Canosa di Puglia</i>	PAL	Apulia, Italy	Open air	EN	5616-5476 ^{†*}
<i>Serri-San Gabriele, Bari San Paolo</i>	BSP	Apulia, Italy	Open air	EN	n.d.
<i>Masseria Maselli, Bari</i>	MAS	Apulia, Italy	Open air	EN	5620-5380 ^{†*}
<i>Canne Setteponti, Barletta</i>	SET	Apulia, Italy	Open air	MN	n.d.
<i>Seconda Spiaggia di Colonna, Trani</i>	TRA	Apulia, Italy	Open air	MN	5051-4849 [*]
<i>Favella della Corte, Corigliano Calabro</i>	FAV	Calabria, Italy	Open air	EN	6000-5700 [*]
<i>Grotta San Michele, Saracena</i>	SAR	Calabria, Italy	Cave	EN-SW	6100-5600 ^{†*}
<i>La Marmotta, Anguillara Sabazia</i>	MAR	Lazio, Italy	Submerged	EN	n.d.
<i>Skorba, Mġarr</i>	SKR	Malta	Open air	EN-TP	5500-3050 [†]
<i>Nakovana Cave</i>	NAK	Croatia	Cave	EN-MN	5850-4900 [†]
<i>Can Sadurní, Begues</i>	CNS	Barcelona	Cave	Cardial-PC	5476-4037 ^{†*}

Table 7.1: Table listing the sites included in the study. [EN: Early Neolithic; MN: Middle Neolithic; SW: Stamped Ware; TP: Temple Period; PC: Post-cardial; n.d.: No date; *: AMS radiometric dating; †: Conventional radiocarbon dating]

Lipid residues were considered significant if more than 5µg of lipid per gram of sherd (µg g⁻¹) were obtained, as suggested by Evershed (2008a). Residues containing less than 5µg g⁻¹ of lipid are difficult to interpret because they cannot be securely distinguished from background

contamination. The archaeological $\delta^{13}\text{C}$ measurements obtained are interpreted according to the modern reference fat $\Delta^{13}\text{C}$ values reported in Section 4.8. The results obtained from the plant burial experiment described in Chapter 5 have also been used to discuss the low lipid yields obtained from some of the sites included in this study (Table 7.2).

<i>Site</i>	<i>Site Type</i>	<i>Whole vessels (N)</i>	<i>Sherds analysed (N)</i>	<i>Soil samples (N)</i>	<i>Visible residues (N)</i>	<i>Quantification (>5$\mu\text{g g}^{-1}$)</i>
AZZ	Open air	25	26	-	-	20
CIC	Open air	25	27	-	-	10
BAL	Open air	35	42	-	-	2
PAL	Open air	39	44	3	-	5
BSP	Open air	15	17	-	-	-
MAS	Open air	12	19	-	3	-
SET	Open air	12	13	-	-	-
TRA	Open air	26	31	6	1	1
FAV	Open air	27	33	-	-	4
SAR	Cave	15	26	-	-	11
MAR	Submerged	6	6	-	5	-
SKR	Open air	16	22	-	-	1
NAK	Cave	17	21	-	-	16
CNS	Cave	31	38	1	-	13

Table 7.2: Table listing the number of pottery vessels, visible residues and soil samples analysed from each site, as well as the number of vessels which retained a significant quantity of lipid. The number of sherds analysed includes multiple sampling along the profile of some of the vessels, and sampling of the external surfaces. [N: denotes the sample size]

The following sections present the results obtained from each site. A brief overview of the site and a description of the floral and faunal remains are provided, which were key to interpreting the extracted lipid residues. Most of the pottery assemblages studied were highly fragmented, however when possible, the type of residue identified in the various ceramic vessels was compared to the pots' form, fabric and decoration, and any trends noted. All residues showed evidence for contamination from phthalate plasticizers, which were introduced due to the packaging of the pot sherds in plastic bags. External surfaces and soil samples were also analysed when possible, to test for contamination from the burial environment. When available, visible residues were sampled for ORA (Table 7.2). The following abbreviations have been used to denote the type of sample analysed: 'I' denotes absorbed residues extracted from the interior surface of a vessel, while the external surface absorbed residues are qualified by 'E'. Soil samples and residues from visible crusts are denoted by 'S' and 'V' respectively. 'C' denotes the carbon number identified in the alkanes, alcohols and fatty acids present in the residues analysed. The site codes used are listed in Table 7.1. This chapter concludes with an overview of the results obtained. A synthesis and discussion of the results generated by ORA and corresponding archaeological data will be presented in Chapter 8.

7.2 Italy: Apulia

The ditched or dry-stone wall enclosures on the Ofanto river valley and the Murge Plateau date to the earliest attestations of the Neolithic in Italy. Pottery samples from eight sites excavated in this region have been selected for analysis, which date from the earliest Neolithic phases to the Middle Neolithic period (Table 7.1). The sites are located on the Murge Plateau, a Mesozoic limestone formation that extends from the Ofanto valley to the Salento hills (Muntoni, 2009:97) (Figure 7.1). The Murge Plateau is divided into three formations running parallel to the coast. The highest (elevated to 500-600m) and most internally located formation is the Murgia Alta, which slopes towards the Adriatic Sea in a series of terraces, defined as the Murgia Bassa and the Murgia Costiera (Fiorentino *et al.*, 2000:382). Permanent watercourses are nowadays missing, but a dense fluvial network etched into the calcareous rock (known as the 'lame') was originally present. These originated in the Murgia Alta, and flowed into the sea, thus connecting the coastal and inland zones (Fiorentino *et al.*, 2000:382-383; Muntoni, 2003:35). The environment on the Murge is thought to have been sparsely covered by forests, with open spaces and good arable soil (Radina, 2002d:4), characterised by evergreens, holm, oak, olive trees and more sporadically buckthorn and carob trees (Fiorentino *et al.*, 2000). Between the 7th and 5th millennia BC, the Murge area became rapidly and densely populated by agrarian communities, who exploited the fertile soil and favourable climate (Muntoni, 2009:89).



Figure 7.1: Map showing the location of the Apulian sites included in the research. [1: Palata 1; 2: Canne Setteponti; 3: Seconda Spiaggia di Colonna; 4: Fondo Azzolini; 5: Serri-San Gabriele, Bari San Paolo; 6: Masseria Maselli; 7: Balsignano; 8: Ciccotto]

Most of the faunal and botanical remains from the sites investigated in this research are still being studied, and most assemblages are too fragmented to allow an in-depth analysis, to establish the economic basis for subsistence. However, the majority of the sites on the Murge show a well established agrarian economy (Radina, 2002d:5). Around 50% of the faunal assemblages on the Murge pertain to sheep remains, which denote a strong reliance on sheep herding (Wilkins, 2002:217). Sheep were generally killed when they reached early adulthood, and could therefore provide good quality meat, while the presence of very young (infant) sheep at Balsignano have been interpreted as evidence for dairying (Wilkins, 2002:217). Hunting appears to have been mainly ignored, and wild animals were only occasionally killed, possibly to defend the cultivated land rather than for dietary purposes (Wilkins, 2002:215). Extensive cereal cultivation is evidenced by the botanical remains, which include wheat (*T. monococcum*, *T. dicocum*, *T. aestivum* and *T. durum*), barley (*Humdrum* sp.) and legumes (e.g. *Pisum* sp., *Vicia* sp. and *Vicia faba*) (Fiorentino, 2002a:222). The cultivation techniques used appear to have been well developed, showing good management of the fields and storage of the harvest (Fiorentino, 2002a:222). The wide range of species identified has been interpreted as a preoccupation with the need to know which cultigens could best support a good harvest, and there also appears to be

a distinction in the choice of species grown in the coastal and hilly regions located further inland. This is probably due to the different characteristics of the soil and the availability of water (Fiorentino *et al.*, 2000).

The Neolithic ceramic traditions of south-eastern Italy have already been described in Chapter 2. Figure 7.2 illustrates some of the main vessel forms present in the Italian ceramic assemblages included in the present analysis.

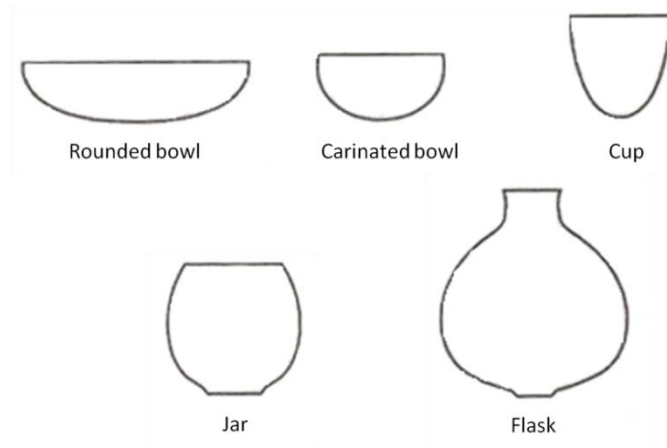


Figure 7.2: The main pottery shapes identified for the Italian Neolithic ceramics (After Pessina and Tiné, 2008:64)

7.2.1 Fondo Azzollini, Pulo di Molfetta

The site is located 47m above sea level, along the Adriatic coast of the Bassa Murgia and about 2km south-west of Molfetta (Bari province) (Radina, 2002e). The settlement occupied a strip of land of around 2 acres on a wide plateau located around an ovoid-shaped, karst sinkhole, which is around 30m deep and has a perimeter of around 600m (Radina, 2002c). The sinkhole is characterised by the presence of caves along its vertical walls, which are particularly dense towards the north-west, and provided shelter for both cultic and funerary purposes (Radina, 2002c). This represented an ideal settlement location during the Neolithic; the plateau was not densely forested, hence ideal for cereal cultivation, while the microclimate and water sources present at the base of the sinkhole supported cultivation (Fiorentino, 2002a, 2002b).

Excavations at Fondo Azzollini have been carried out by the *Soprintendenza per i Beni Archeologici della Puglia*, and comprised several campaigns since their onset in 1997 (Radina, 2007). An Early Neolithic settlement enclosed within a wide dry-stone wall dated to around 6100-5800 cal. BC

was uncovered (Muntoni, 2003:83, 2009). The pottery assemblage retrieved comprised mainly large, ovoid-shaped, coarse ware jars and collared jars, with smoothed surfaces often covered with impressed/cardinal decorations, and small, rounded, plain bowls with smoothed or carefully burnished undecorated surfaces (Muntoni, 2003, 2009). All the samples analysed in this investigation were excavated from Area 3, which corresponds to the wide dry-stone wall structure (Figure 7.3). In close proximity to the wall, the remains of what was probably a hut were uncovered. Both the wall and the hut are dated to the Early Neolithic phase (Muntoni, 2009-2012, pers. comm.).



Figure 7.3: Site plan of the Early Neolithic settlement at Fondo Azzollini, Pulo di Molfetta, showing the earlier (blue) and more recent (red) excavation campaigns (Radina, 2002e:618). ●: denotes the location of the dry-stone wall (inset, courtesy of Dr. Italo Muntoni), corresponding to Area 3.

Ovicaprids dominated the faunal assemblage at Fond Azzollini, comprising 71% of the whole, with a limited presence of cattle and pigs (Wilkens, 2002:218). Hunting targeted mainly red deer (11%), but roe deer and aurochs were also hunted (Wilkens, 2002:218). This site is also characterized by a high percentage of tortoise remains, some of which showed signs of burning from cooking (Wilkens, 2002:218). Although the animal bone assemblage was highly fragmented, the subsistence economy at Fondo Azzollini, based on the analysis of faunal remains, was tentatively identified as being oriented towards meat production, and to a lesser extent dairying (Masala, n.d. b).

ORA carried out on Impressed Wares from Fondo Azzollini showed remarkably good lipid preservation. Out of the 25 ceramic vessels analysed, 20 showed a significant residue, that is 80% of the samples analysed (Table 7.2). Contamination from the external environment was found to be negligible, since the external surface of pot AZZ-11E comprised low lipid levels ($<3\mu\text{g g}^{-1}$), and the lipid profile contained traces of fatty acids and a series of alkanes (C23-C31), consistent with a soil lipid profile (*cf.* Heron *et al.*, 1991a).

Table 7.3 lists the molecular compounds identified by GC-MS analysis for selected sherds from Fondo Azzollini. Most of the pots contained short to medium chained fatty acids, with C_{16:0} and C_{18:0} being the most abundant in all pot sherds containing a significant residue, and C_{18:0} being generally more abundant than C_{16:0}. Long chain and odd-numbered chain fatty acids (in particular C_{15:0} and C_{17:0}) were also identified in most of the sherds. Cholesterol and its dehydration product cholesta-3,5-diene, were also detected along with mono- and diacylglycerols. Trimyristate was also identified in four pot sherds by HT-GC (AZZ-13I, AZZ-18I, AZZ-23I and AZZ-26I). The lipid profiles described are consistent with animal fats, while the occurrence of C_{15:0} and C_{17:0}, which are known to be produced by microorganisms in the rumen, suggest a ruminant origin (Figure 7.4).

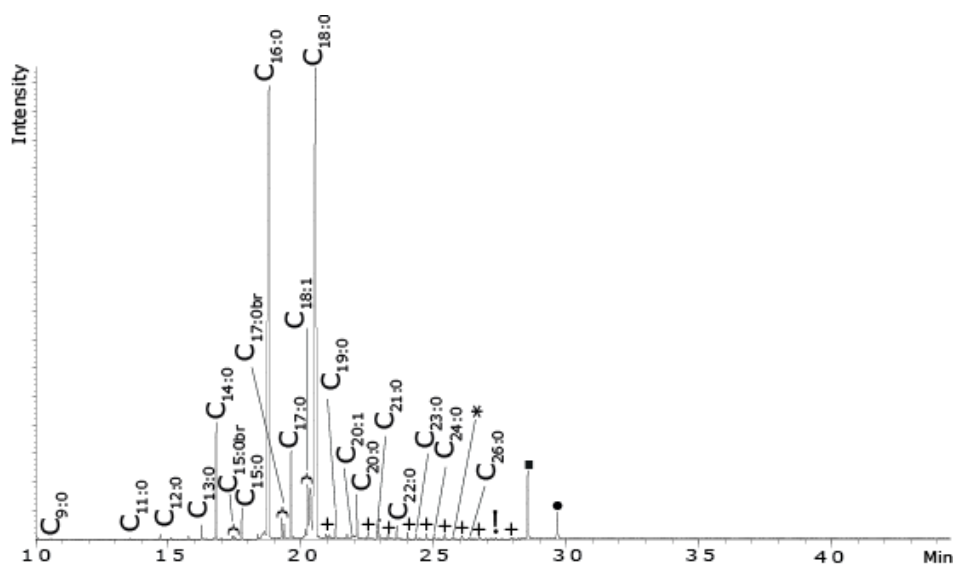


Figure 7.4: Total Ion Chromatogram (TIC) of pot number AZZ-13I, a coarse ware bowl identified by GC-c-IRMS as containing a ruminant dairy residue. [C_{x,y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C23-C33); *: Alcohols (C26); !: Cholesterol; ■: Internal standard (C34); ●: Internal standard (C36)]

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
AZZ-11I	27	FAs: C _{14:0} , C _{16:0} , C _{18:0} ; Alkanes: C24, C26, C27, C28; Phthalates
AZZ-13I	130	FAs: C _{9:0} -C _{17:0} , C _{18:1} , C _{18:0} -C _{19:0} , C _{20:1} , C _{20:0} -C _{26:0} ; Alkanes: C23-C33; Alkenes: unidentified; Alcohols: C12, C18, C26; Sterols: Cholesterol; TAG: C42; Phthalates
AZZ-14I	17	FAs: C _{9:0} , C _{10:0} , C _{12:0} , C _{14:0} -C _{18:0} ; Alkanes: C17, C18; Alcohols: C12, C14, C16, C18; Ketones: C31, C33, C35; DAGs: 1,2-Dipalmitin; Sterols: Cholesterol
AZZ-18I	293	FAs: C _{16:0} , C _{18:0} ; Alkanes: C16, C18, C23-C27, C29, C31; Alcohols: C12, C14, C18, C26, C28; MAGs: 2-Monomyrstin, 1-Monomyrstin, 2-Monopalmitin, 1-Monopalmitin, 2-Monostearin; DAGs: 1,2-Dipalmitin; TAGs: C42; Sterols: Cholesterol; Phthalates
AZZ-19I	92	FAs: C _{14:0} , C _{16:0} , C _{18:0} ; Alkanes: C25, C27, C28; Alcohols: C12, C14, C18; TAGs: C42; Sterols: Cholesterol; Phthalates
AZZ-20I	63	FAs: C _{14:0} -C _{18:0} , C _{20:0} , C _{22:0} , C _{24:0} , C _{26:0} , C _{18:1} ; Alkanes: C23, C-C29, C31; Alcohols: C18, C26; MAGs: 2-Monostearin, 1-Monostearin; TAGs: C42; Sterols: Cholesterol; Phthalates
AZZ-21I	5	FAs: C _{16:0} -C _{18:0} , C _{22:0} , C _{25:0} ; Alkanes: C23-C31; Sterols: Cholesterol; Phthalates
AZZ-22I	71	FAs: C _{9:0} , C _{11:0} , C _{14:0} -C _{18:0} ; Alkanes: C23-C31; Alcohols: C14, C18, C26; Phthalates
AZZ-23I	1550	FAs: C _{9:0} , C _{10:0} , C _{13:0} , C _{14:0} -C _{18:0} ; Alkanes: C29, C31; Alcohols: C12; Ketones: C33, C35; TAGs: C42; Phthalates
AZZ-24I	43	FAs: C _{16:0} , C _{18:0} ; Alkanes: C22-C29, C31; Sterols: Cholesta-3,5-diene; Phthalates
AZZ-25I	343	FAs: C _{9:0} -C _{12:0} , C _{14:0} , C _{16:0} -C _{20:0} , C _{22:0} -C _{24:0} ; Alkanes: C23-C29; Alcohols: C18; Phthalates
AZZ-26I	3663	FAs: C _{8:0} -C _{24:0} ; Alkanes: C29, C31; Alcohols: C13, C14, C16, C20, C24, C26-C28, C30; Ketones: C32, C33, C35; MAGs: 1-Monopalmitin; TAGs: C42; WEs: C32, C34; Sterols: Cholesterol; Phthalates
AZZ-27I	35	FAs: C _{14:0} , C _{16:0} -C _{19:0} , C _{22:0} -C _{24:0} , C _{26:0} ; Alkanes: C23-C31; Alcohols: C26; Phthalates
AZZ-28I	25	FAs: C _{14:0} , C _{16:0} -C _{20:0} , C _{23:0} , C _{24:0} ; Alkanes: C24, C26, C28; Phthalates
AZZ-29I	17	FAs: C _{11:0} -C _{20:0} ; Alkanes: C23-C30; MAGs: 2-Monomyrstin, 2-Monopalmitin, 1-Monopalmitin; Phthalates
AZZ-30I	6	FAs: C _{14:0} -C _{18:0} ; Alkanes: C24, C26-C30, C32; Alcohols: C18; Phthalates
AZZ-31I	36	FAs: C _{9:0} -C _{12:0} , C _{14:0} -C _{20:0} , C _{22:0} -C _{24:0} , C _{26:0} , C _{18:1} ; Alkanes: C23, C25-C30; Alcohols: C26; MAGs: 2-Monomyrstin, 1-Monopalmitin, 2-Monostearin, 1-Monostearin; DAGs: 1,2-Dipalmitin; Sterols: Cholesterol; Phthalates
AZZ-33I	8	FAs: C _{16:0} -C _{20:0} , C _{24:0} , C _{26:0} ; Alkanes: C20, C24-C33; Phthalates
AZZ-34I	5	FAs: C _{9:0} , C _{10:0} , C _{13:0} -C _{20:0} , C _{22:0} -C _{24:0} ; Alkanes: C23-C33; Alcohols: C18; MAGs: 1-Monomyrstin; Phthalates
AZZ-35I	11	FAs: C _{13:0} -C _{18:0} , C _{20:0} , C _{22:0} , C _{24:0} , C _{26:0} ; Alkanes: C23-C29, C31, C33; Alcohols: C18, C26; Phthalates

Table 7.3: GC-MS and HT-GC identification of lipid residues extracted from Fondo Azzollini, Pulo di Molfetta (Apulia, Italy) and run as TMS derivatives.[FAs: Fatty acids; MAGs: Monoacylglycerols; DAGs: Diacylglycerols; TAGs: Triacylglycerols; WEs: Wax Esters]

Nine samples were submitted for GC-c-IRMS analysis. Figure 7.5 shows an example of the GC-c-IRMS traces obtained for fatty acids run as methyl ester derivatives. The GC-c-IRMS results obtained from Fondo Azzollini are plotted in Figure 7.6, which also shows the mean $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements obtained from modern reference fats (as plotted in Figure 4.15), with one exception. Ruminant adipose reference fats in Figure 7.6 and the GC-c-IRMS plots presented in

this chapter and Chapter 8, comprise values obtained for both domestic and wild ruminants (i.e. deer adipose). This is an important consideration, since deer bones have been found in several of the sites investigated in this study. As already discussed in Section 4.8, $\delta^{13}\text{C}$ values for deer adipose were observed to be quite wide, and overlapped with $\Delta^{13}\text{C}$ value ranges of domestic ruminant adipose and ruminant dairy fats (Craig *et al.* in press). In fact, $\Delta^{13}\text{C}$ measurements for deer adipose at $\pm 1\text{‰}$ standard deviation were -1.9‰ to -4.3‰ (mean -3.1‰) (see Figure 7.6). By combining both wild (deer) and domestic ruminant adipose $\delta^{13}\text{C}$ values, a more comprehensive range for ruminant adipose is obtained. However, uncertainty still exists, because the datasets contain considerable variations, and the ruminant adipose and ruminant dairy reference categories partly overlap.

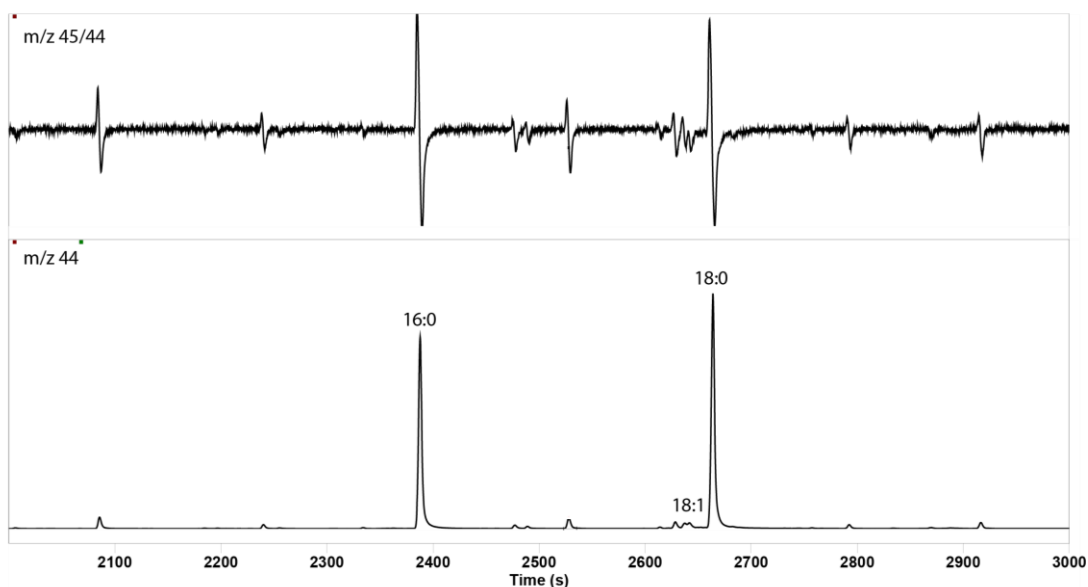


Figure 7.5: Partial m/z 45/44 and m/z 44 ion chromatogram obtained by GC-c-IRMS analysis of fatty acids as their methyl ester derivatives, of sample AZZ-13I. The lower chromatogram shows the baseline resolution, while the upper trace is the ratio of m/z 45/44 ions detected by the GC-c-IRMS.

All samples, except AZZ-13I, fall within the terrestrial $\delta^{13}\text{C}_{16:0}$ range. Only one sample, AZZ-26I, plotted within the $\Delta^{13}\text{C}$ range denoting ruminant adipose, while samples, AZZ-11I, AZZ-14I, AZZ-18I and AZZ-31I, plotted between the ruminant and non-ruminant adipose categories. Of these, the $\Delta^{13}\text{C}$ values of AZZ-31I, a coarse ware jar, was found to be 0.2‰ , which tentatively identifies it as a non-ruminant adipose. AZZ-31I plotted very close to a modern adipose fat residue obtained from a wild boar of Mediterranean origin. However, wild boar was not attested in the faunal record at Fondo Azzollini, although domestic pig remains made up 1% of the bone assemblage recovered on site, which support its identification as a non-ruminant adipose residue (Masala, n.d. b). Since GC-MS data cannot be reliably used to further distinguish between a ruminant

and non-ruminant adipose origin for samples AZZ-11I, AZZ-14I and AZZ-18I, they can only be identified as terrestrial animal adipose residues. Samples AZZ-13I, AZZ-23I, AZZ-25I and AZZ-35I had $\Delta^{13}\text{C}$ values less than -3.4‰ , which identified them as dairy products. However, as can be observed in Figure 7.6, sample AZZ-35I plots within the area of overlap between ruminant adipose and ruminant dairy products. A domestic ruminant adipose origin can be tentatively excluded since modern reference values for domestic ruminant adipose show $\Delta^{13}\text{C}$ values of more than -2.6‰ , but this does not exclude a deer adipose origin. The presence of short-chain fatty acids, which are more consistent with dairy products (*cf.* Dudd *et al.*, 1998), and the low incidence of deer bone at Fondo Azzollini (2% of the bone assemblage studied, compared to the ovicaprid remains which comprised 71% of the faunal assemblage) (Masala, n.d. b), suggest that pot AZZ-35I is more likely to have contained dairy products. Furthermore, the $\delta^{13}\text{C}$ values for the $\text{C}_{16:0}$ fatty acid of sample AZZ-13I is indicative of a marine origin, although its $\Delta^{13}\text{C}$ values ($=-4.1\text{‰}$) are more consistent with a dairy origin. Hence, a possible interpretation for AZZ-13I could be as a mixture between marine oils and dairy fats. However, this characterisation is not supported by the presence of fish bones on site, or the presence of marine biomarkers, which could however, have been lost due to degradation. Hence, although a marine input cannot be excluded, it cannot be securely suggested.

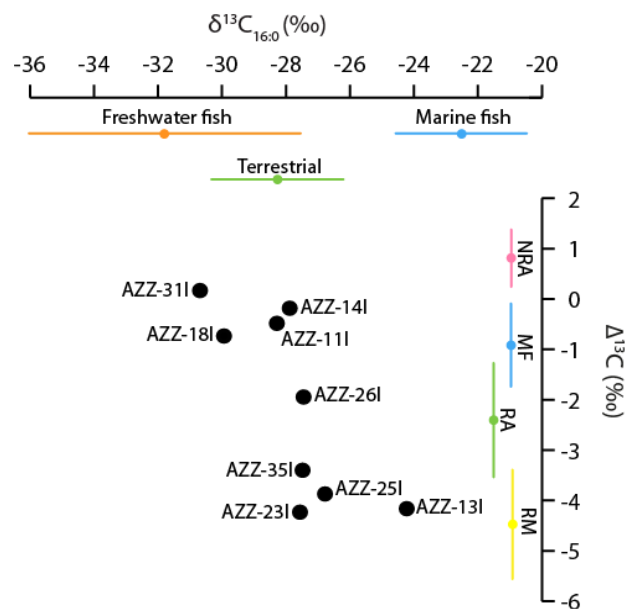


Figure 7.6: GC-c-IRMS results: Fondo Azzollini, Pulo di Molfetta (Apulia, Italy). [$\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\text{‰}$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

Evidence for the heating of animal fats in the ceramic vessels was also identified through the presence of long-chain, asymmetric ketones in vessels AZZ-14I, AZZ-23I and AZZ-26I. Following Raven *et al.*'s (1997) criteria, a plant origin for the ketones identified (namely C31, C33 and C35) was dismissed, since i) their alkane and alkanol precursors were not present, which is inconsistent with their biosynthesis in plants, ii) C35 is rarely found in plants and iii) ketones in epicuticular waxes are generally symmetrical. Their presence is therefore associated with the condensation reactions of fatty acids moieties absorbed within the ceramic vessel during heating; hence indicative of cooking (Evershed *et al.*, 1995b; Raven *et al.*, 1997).

Palmitate wax esters were identified in the residue extracted from the interior surface of AZZ-26I, a coarse ware bowl, which also comprised a wide range of alkanes and alcohols. Wax esters are known components of the plant cuticle (Pollard *et al.*, 2007:156), but they are also known to occur in soil (Heron *et al.*, 1991a). However, in this case, they were not identified in the external surface analysed. AZZ-26I was also identified by GC-c-IRMS as a ruminant adipose fat. Combining GC-c-IRMS and GC-MS data, a ruminant and plant input can be suggested for the residue extracted from pot AZZ-26I. However, it is not possible to state whether the two were cooked simultaneously (as a mixture e.g. a stew), or whether the two residues became absorbed within the ceramic walls as a result of subsequent cooking episodes.

The pottery assemblage analysed comprised mainly jars and bowls, and there does not appear to be any particular association between form and the food product identified from the absorbed residues. Most of the vessels were coarse wares, however significant residues were also extracted from two out of the three fine wares analysed (see Appendix D).

GC-c-IRMS analysis has provided direct evidence for the processing of ruminant and non-ruminant adipose, and dairy products in ceramic vessels from Fondo Azzollini. The botanical remains (described above) show a heavy reliance on cereals and legumes, and a possible plant contribution to the residues analysed is suggested, mainly due to the presence of wax esters in the interior surface of AZZ-26I. Evidence for a marine input was tentatively associated with vessel AZZ-13I, but in the absence of more secure biomarker evidence, this interpretation cannot be securely held.

7.2.2 Ciccotto, Gravina in Puglia

Ciccotto is situated around 442m above sea level, about 2km east of Gravina in Puglia and 60km from the present coastline (Figure 7.1), and extends for about 5 hectares (Muntoni, 2003:93). The site was excavated between 1984 and 1985 by the *Soprintendenza per i Beni Archeologici della Puglia* (Muntoni, 2003:93). No radiocarbon dates are yet available, but the stratigraphy is associated with Early and Middle Neolithic deposits (Muntoni, 2003:93). A ditch was uncovered during the 1984 excavations, which dated to the Early Neolithic phase, and appeared to run in a straight line for around 200m (Muntoni, 2003:94). The pottery assemblage included in this analysis comprised collared jars, bowls, jugs and plates, and decorations included impressed motifs (particularly pottery dated to the Early Neolithic), red painted decorations, as well as burnished and smoothed surfaces. The Early Neolithic pottery sampled was retrieved from the ditch and Layer 17L, while the Middle Neolithic pottery sampled was recovered from Layer 16N. The faunal and floral remains at Ciccotto are still under study.

Ten out of the 25 vessels, that is 40% of the samples analysed using ORA, yielded a significant lipid residue (Table 7.2). The external surfaces of two vessels (CIC-11E and CIC-25E) were analysed to test for contamination from the burial environment. CIC-11E and CIC-25E were found to contain negligible quantities of lipid ($<4\mu\text{g g}^{-1}$), and their lipid profile comprised trace amounts of medium-chained fatty acids and alkanes (C23-C33), consistent with soil lipid extracts (*cf.* Heron *et al.*, 1991a). Table 7.4 lists the lipid composition of the absorbed residues extracted from the Ciccotto ceramic assemblage. They comprised mainly a range of medium-chain, saturated fatty acids, with prominent C_{16:0} and C_{18:0} peaks, the latter generally more abundant than C_{16:0}. Cholesterol was identified in two pots (CIC-05I and CIC-06I). Trimyristate and 1,2-dipalmitin were present in one sample (CIC-18I). The lipid profiles obtained are therefore consistent with an animal fat, with the presence of C_{15:0} and C_{17:0} suggesting a ruminant fat. Long-chain asymmetric ketones were also identified in vessels CIC-14I, CIC-15I and CIC-18I, and following Raven *et al.*'s (1997) criteria (described above), the ketones present are indicative of cooking.

Three coarse ware jars contained sufficient C_{16:0} and C_{18:0} for GC-c-IRMS analysis. The results identified one sample as a dairy product (CIC-14I; $\Delta^{13}\text{C}=-4.7\text{‰}$), one sample as a ruminant adipose (CIC-12I; $\Delta^{13}\text{C}=-2.8\text{‰}$, therefore possibly a deer adipose as described above), and one sample has been tentatively identified as a porcine fat, possibly wild boar because the $\delta^{13}\text{C}$ values obtained were lower than those for domestic porcine fats, and sample CIC-13I plotted closer to the modern wild boar adipose sample analysed (CIC-13I; $\Delta^{13}\text{C}=0.2\text{‰}$) (Figure 7.8). The gas chromatogram and

$\Delta^{13}\text{C}$ value obtained for CIC-13I is very similar to sample AZZ-31I, the coarse ware jar from Fondo Azzollini described above, which was also tentatively identified as containing porcine adipose (Figure 7.7). $\delta^{13}\text{C}$ values for the $\text{C}_{16:0}$ fatty acid of vessel CIC-31I plots within the freshwater fish range, however, again, no fish biomarkers were identified in the sample, and its TIC, obtained by GC-MS, is more consistent with a terrestrial fat. The faunal assemblage at Ciccotto is still being studied, however, the work carried out by Wilkens (2002) on faunal remains recovered from several sites in the Murge region also identified domestic pig remains.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
CIC-02I	6	FAs: $\text{C}_{14:0}$ - $\text{C}_{16:0}$, $\text{C}_{18:0}$, $\text{C}_{20:0}$; Alkanes: C25-C33; Phthalates
CIC-04I	6	FAs: $\text{C}_{14:0}$, $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C24, C26-C33; Ketones: C33; Phthalates
CIC-05I	12	FAs: $\text{C}_{14:0}$ - $\text{C}_{20:0}$, $\text{C}_{22:0}$ - $\text{C}_{26:0}$, $\text{C}_{16:1}$, $\text{C}_{18:1}$; Alkanes: C22, C24, C25, C26, C27, C28, C29, C30, C31; Sterols: Cholesterol; Phthalates
CIC-11I	5	FAs: $\text{C}_{14:0}$ - $\text{C}_{18:0}$; Alkanes: C23-C33; Phthalates
CIC-12I	53	FAs: $\text{C}_{14:0}$ - $\text{C}_{18:0}$, $\text{C}_{20:0}$; Alkanes: C27, C28, C29, C31; Phthalates
CIC-13I	18	FAs: $\text{C}_{7:0}$ - $\text{C}_{9:0}$, $\text{C}_{11:0}$ - $\text{C}_{20:0}$, $\text{C}_{18:1}$; Alkanes: C20, C22, C25, C29, C31; Alcohols: C14, C16; Ketones: C33, C35; Phthalates
CIC-14I	221	FAs: $\text{C}_{7:0}$ - $\text{C}_{9:0}$, $\text{C}_{11:0}$ - $\text{C}_{18:0}$, $\text{C}_{20:0}$; Alkanes: C23-C33; Alkenes: unidentified; Ketones: C31, C33, C35; Phthalates
CIC-16I	9	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C25-C27, C29, C30; Alcohols: C18; Phthalates
CIC-18I	1	FAs: $\text{C}_{9:0}$, $\text{C}_{10:0}$, $\text{C}_{12:0}$, $\text{C}_{14:0}$ - $\text{C}_{18:0}$, $\text{C}_{20:0}$, $\text{C}_{18:1}$; Alkanes: C22-C33; Alcohols: C14, C18, C20, C21; Ketones: C35; DAG: 1,2-Dipalmitin; TAG: C42; Phthalates
CIC-24I	5	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C23-C30; Phthalates
CIC-25I	7	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C26, C27, C29, C30, C33; Phthalates

Table 7.4: GC-MS and HT-GC identification of lipid residues extracted from Ciccotto, Gravina in Puglia (Apulia, Italy) and run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols; DAGs: Diacylglycerols; TAGs: Triacylglycerols; WEs: Wax Esters]

The stratigraphic sequence at Ciccotto offered the opportunity to observe any variation in the use of pots through time, from the Early to the Middle Neolithic period. Out of 15 Early Neolithic vessels, seven gave a significant residue, while three out of ten Middle Neolithic vessels contained $>5\mu\text{g g}^{-1}$ lipid. The lipid profiles obtained for the Early Neolithic residues were more consistent with animal fats, while the three significant residues obtained from the Middle Neolithic ceramics (CIC-16I, CIC-24I and CIC-25I) comprised low quantities of $\text{C}_{16:0}$ and $\text{C}_{18:0}$, and a wide range of alkanes (C23-C33), reminiscent of a plant residue (Table 7.4). As described in Chapter 5, low quantities of lipid residue containing a homologous series of alkanes and alcohols are characteristic of degraded plant residues. A similar lipid profile was obtained in most of the Middle Neolithic Figulina Wares at Ciccotto (see Appendix D Table D.2). Figulina Wares appear in the ceramic repertoire from the Middle Neolithic (Spataro, 2009b:56), and are discussed in further detail in Section 7.2.6. Their function has been interpreted as containers for prestige items, and they are often found in funerary contexts (Barfield, 1981; Bagolini, 1990; Spataro,

2009a:66). At Ciccotto, the predominant occurrence of negligible residues in Figulina Wares, and the tentative plant product identification in three of the vessels analysed (also Figulina Wares) may possibly indicate their use to process or store plant material, although uptake from the burial environment is not excluded. However, the botanical evidence present in the Murge area suggests the presence of a flourishing agrarian community based on cereal cultivation (Fiorentino, 2002a), while other palaeodietary research (discussed in Chapter 8) also appears to support a heavy reliance on plant material during the Neolithic in the Murge region. This suggests, that the low quantities of lipid residue extracted from the Figulina Wares collected from Middle Neolithic deposits at Ciccotto, comprising mainly jars and bowls, could result from i) advanced degradation of absorbed lipid residues, ii) vessel function, including storage and processing of plant material. Chapter 8 provides a more detailed discussion on the interpretation of negligible residues. No distinctive patterns were identified between the type of residue extracted from Early Neolithic Impressed Wares at Ciccotto, with their form and fabric. Ruminant and non-ruminant adipose and ruminant dairy products were only present in coarse ware jars, with only one of two fine ware small jars analysed containing a ruminant fat. One Early Neolithic carinated bowl was analysed, which produced a negligible lipid residue (see Appendix D).

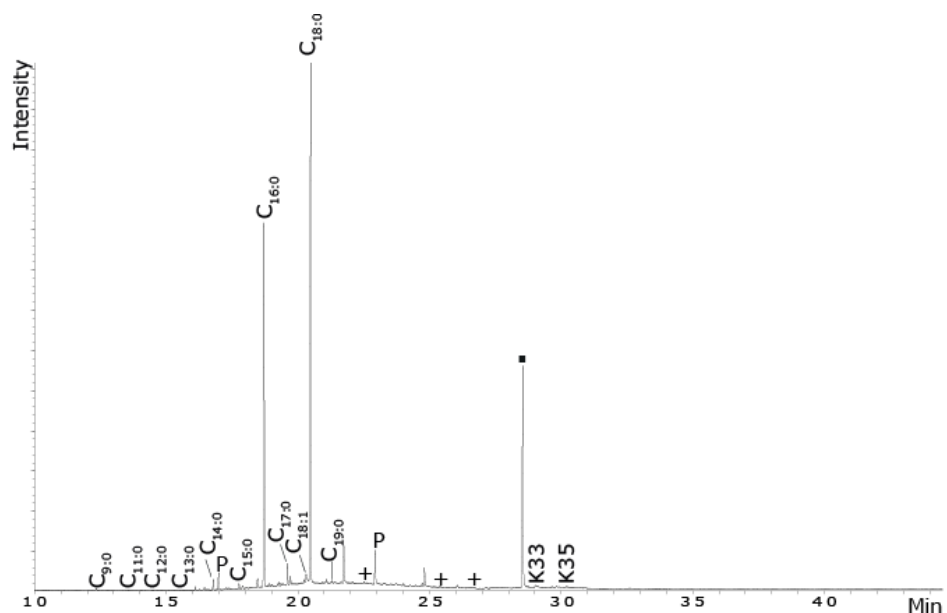


Figure 7.7: TIC of a coarse ware jar CIC-131, identified by GC-c-IRMS as containing a non-ruminant residue. [C_{x,y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C25-C31); K; Ketones; P: Phthalates; ■: Internal standard (C34)]

In the absence of a faunal analysis report, GC-c-IRMS has identified the processing of ruminant and non-ruminant adipose fats (possibly wild boar), as well as dairy products during the Early Neolithic phase at Ciccotto. A plant contribution has been tentatively suggested for the Middle

Neolithic Figulina Wares, while no evidence of a marine dietary input was observed in either phase.

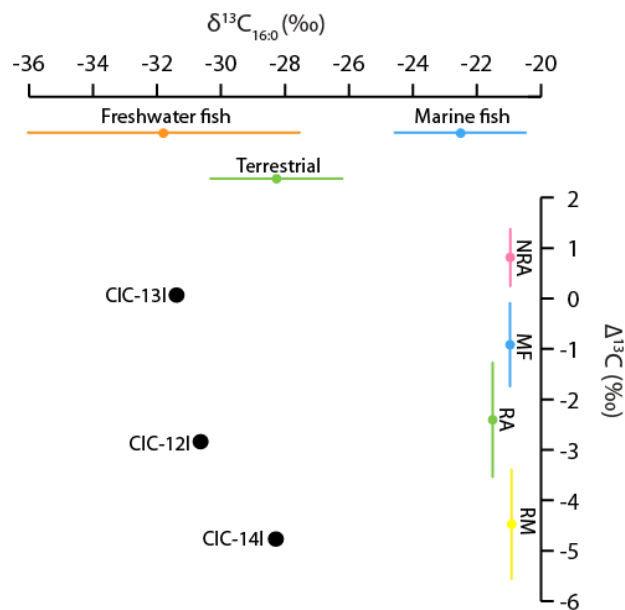


Figure 7.8: GC-c-IRMS results: Ciccotto, Gravina in Puglia (Apulia, Italy). [$\Delta^{13}\text{C}$ values ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\%$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

7.2.3 Balsignano, Modugno

The settlement of Balsignano (Modugno), is located about 8km from the Adriatic coast, and has revealed substantial structural remains. It is a typical inland site, occupying around 2 hectares on the Calcare di Bari, located 82m above sea level on a marine terrace in between two branches of the Lama Lamasinata (Radina, 2002b:143). The *Soprintendenza per i Beni Archeologici della Puglia* has been conducting archaeological research at Balsignano since 1993, and has so far uncovered two burned rectangular domestic structures (Hut 1 and Hut 2), with stone footings and a wooden frame with a daub covering (Radina, 2002b). Burials and outside functional areas, including structured firing places and post holes, were also identified, and were all dated to an advanced phase of the Early Neolithic (5600-5450 BC). The pottery repertory was characterised by coarse ware vessels, which were decorated with impressed/cardial motifs or left undecorated, as well as fine ware pots with smoothed or carefully burnished surfaces, sometimes decorated with finely impressed or incised motives and/or brown-painted bands (Muntoni, 2003, 2009). Most of the samples analysed were excavated from Hut 2, which is a little bigger than Hut 1 and lies about

30m away from it (Muntoni, 2003:90). Three burial deposits were identified, one in a pit near Hut 1, which contained a male adult and indications of burning, another skeleton was uncovered in Hut 2, and a third burial was located about 20m north-east of Hut 2 (Radina, 2002a).

Thirty-five ceramic vessels were analysed from the open-air settlement of Balsignano; however, only 2 vessels (BAL-04I and BAL-08I) contained a significant lipid residue (Tables 7.2 and 7.5). Analysis of the external surfaces of four pot sherds (BAL-06E, BAL-08E, BAL-24E and BAL-31E), showed negligible lipid quantities ($<1\mu\text{g g}^{-1}$), and their lipid profile comprised traces of fatty acids and alkanes. Samples BAL-06E and BAL-08E also contained small peaks identified as cholesterol and dehydroabiatic acid. Low quantities of these two compounds were also identified in several of the internal surfaces, which are therefore likely to have originated from the burial environment. Dehydroabiatic acid is a known biomarker for *Pinaceae* resin (Regert *et al.*, 2005), and pine trees are present along the littoral zones in the Murge region (Fiorentino *et al.*, 2000). Hence the pine biomarker identified could have been introduced by migration from pine tree debris in the soil. Its presence as an artefact arising from instrumental contamination has also been taken into consideration.

Nearly all the samples comprised trace amounts of medium-chain fatty acids (mainly $\text{C}_{16:0}$ and $\text{C}_{18:0}$), and wide ranges of alkanes and alcohols. Long-chain ketones (C_{33} and C_{35}) were identified in one sample (BAL-02I) and are suggestive of cooking (*cf.* Raven *et al.*, 1997), but the quantity of lipid extracted from this sherd was negligible, hence the residue cannot be securely identified. Similarly, a range of monoacylglycerols (Table 7.5) were identified in sample BAL-03I, but the sherd comprised $<5\mu\text{g g}^{-1}$ lipid for a confident interpretation. In general, a predominance of $\text{C}_{16:0}$ over $\text{C}_{18:0}$ was observed, with the palmitic ($\text{C}_{16:0}$) to stearic ($\text{C}_{18:0}$) acid ratio (P/S) being greater than 4 in most cases. Copley *et al.* (2001b) suggested that when $\text{C}_{16:0}$ is 3 to 4 times more abundant than $\text{C}_{18:0}$, this could be indicative of a plant origin. As already described in Chapter 5 and elsewhere (Reber and Evershed, 2004b; Evershed, 2008a), absorbed residues originating from plant oils tend to be very low and often masked by fattier products, and are therefore difficult to identify securely. BAL-04I and BAL-08I did retain a significant lipid yield. The low quantities of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ present in BAL-08I, but clear predominance of $\text{C}_{16:0}$ over $\text{C}_{18:0}$ in BAL-04I (P/S $>$ 4), the odd over even preference in the alkanes identified in BAL-08I and the presence of alcohols in both residues are reminiscent of a plant input (Figure 7.9). As already discussed above, it is likely that the very low levels of cholesterol (and similarly, dehydroabiatic acid) identified were introduced by migration from the burial environment, hence precluding an animal origin. BAL-04I and BAL-08I have therefore been tentatively identified as plant residues.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
BAL-02I	4	FAs: C _{16:0} , C _{18:1} , C _{18:0} , C _{23:0} , C _{25:0} ; Alkanes: unidentified; Alcohols: C18, C24; Ketones: C33, C35; Sterols: Cholesterol; Dehydroabiatic acid; Phthalates
BAL-03I	4	FAs: C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{18:1} , C _{18:0} ; Alkanes: C20, C23, C24, C25, C26, C27, C28; Alcohols: C16, C18, C20, C22, C24, C26; MAGs: 1-Monopalmitin, 1-Monooleate, 2-Monostearin; Sterols: Cholesterol; Phthalates
BAL-04I	13	FAs: C _{14:0} , C _{16:0} , C _{18:0} ; Alkanes: unidentified; Alcohols: C18, C20; Sterols: Cholesterol; Dehydroabiatic acid; Phthalates
BAL-08I	10	FAs: C _{16:0} , C _{18:0} ; Alkanes: C23, C25, C27-C30; Alcohols: C24, C26; Sterols: Cholesterol; Phthalates
BAL-09I	2	FAs: C _{14:0} , C _{15:0} , C _{16:0} , C _{18:0} ; Alkanes: C22, C23, C24, C25, C26, C27, C28, C29; Alkenes: unidentified; Alcohols: C18, C20, C22, C24; Sterols: Cholesterol; Dehydroabiatic acid; Phthalates
BAL-12I	1	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Alcohols: unidentified; Phthalates
BAL-13I	1	Alkanes: unidentified; Alcohols: unidentified; Phthalates
BAL-14I	1	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Phthalates
BAL-31I	1	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Alcohols: unidentified; Phthalates

Table 7.5: Mass spectral and HT-GC identification of lipid residues extracted from Balsignano, Modugno (Apulia, Italy) and run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols]

The absence of evidence for the processing of animal products in the Balsignano ceramics analysed is intriguing, given that osteological remains from ovicaprids, oxen and pigs were found on site (Masala, n.d. a), and a dairying economy was also suggested (Wilkins, 2002:217). However, animal products were not represented in any of the 35 vessels analysed using ORA. Analysis of the skeletal remains at Balsignano (Scattarella and Sublimi Saponetti, 2002) and more recent stable isotope analysis (Lelli *et al.*, in press) do however indicate a strong plant component in the Neolithic diet. As discussed in Section 7.2.1, this is supported by the archaeobotanical remains present in the Murge region, which have identified a strong reliance on the cultivation of cereals (Fiorentino, 2002a). Circumstantial evidence at Balsignano strongly suggests a plant input as the function of Impressed Wares, which will be discussed in more detail in Chapter 8.

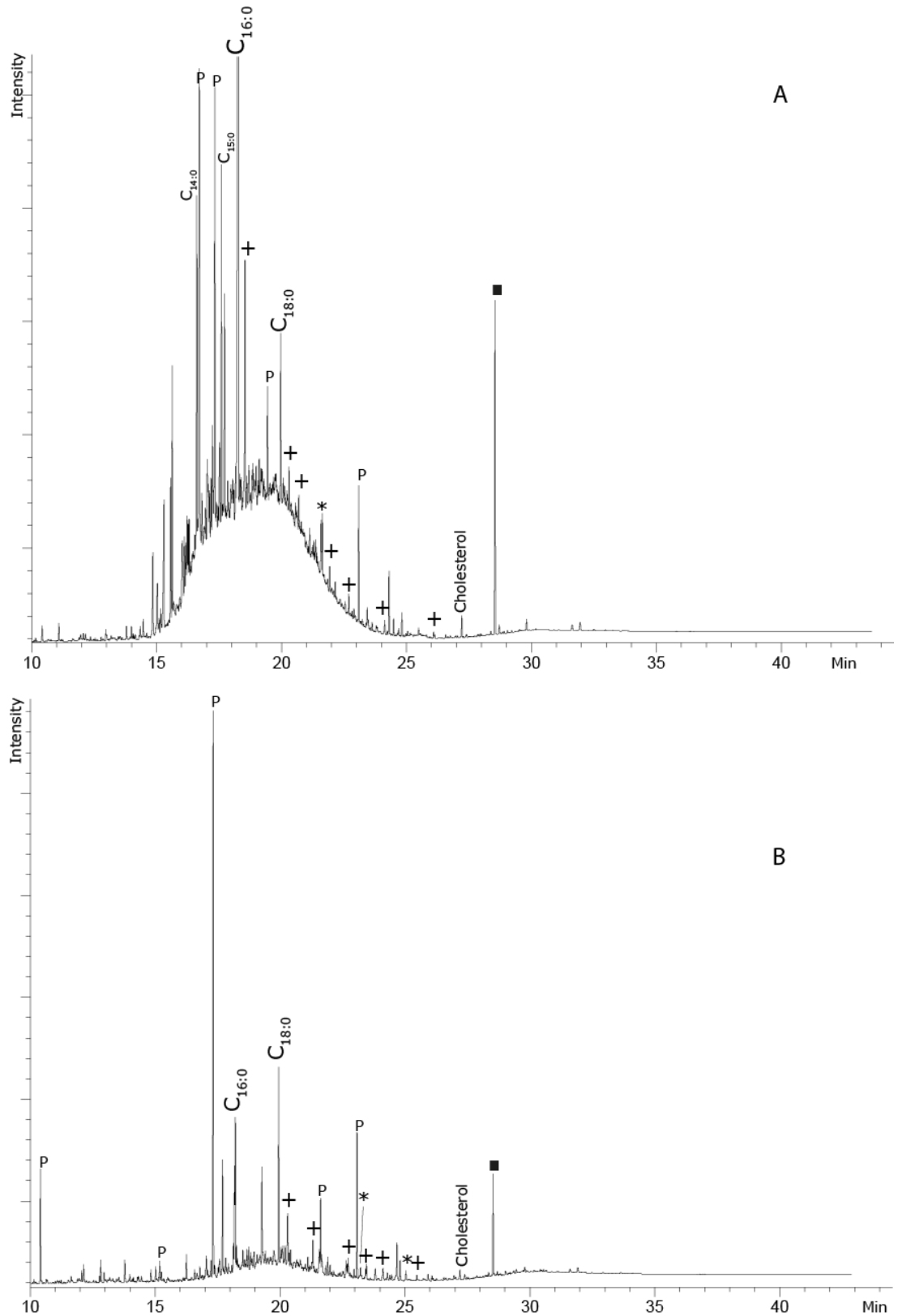


Figure 7.9: A: Partial Gas Chromatograms of BAL-041, an undecorated coarse ware jar; B: Partial Gas Chromatogram of BAL-081, an undecorated coarse ware jar. [C_{x:y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C₂₁-C₃₀); *: Alcohols (C₂₄-C₂₆); P: Phthalates; ■: Internal standard (C₃₄)]

7.2.4 Palata 1, Canosa di Puglia

The Early Neolithic settlement of Palata 1 is located on an alluvial terrace on the Ofanto river valley, and lies around 73m above sea level (Radina *et al.*, 2011). It comprises a small-sized terraced settlement, enclosed by a circular ditch (Radina *et al.*, 2011). The *Soprintendenza per i Beni Archeologici della Puglia* carried out rescue archaeological research in 2008, and uncovered a dwelling structure, pits and one grave containing an adult female individual, dated to an advanced phase of the Early Neolithic (5800-5500 BC) (Spiteri *et al.*, in press). The pottery excavated at Palata 1 was characterised by coarse vessels with impressed/cardial decorative motifs and smoothed or carefully burnished fine ware pots, which were sometimes decorated with finely impressed or incised motives and/or brown-painted bands. The pot forms are consistent with serving bowls and jars, and the latter could have been used for storage or cooking purposes.

Five vessels out of the 39 analysed contained a significant residue, however none of the samples contained enough C_{16:0} and C_{18:0} for GC-c-IRMS analysis, and none of the residues could be securely identified (Table 7.2). PAL-35I, an ovoid, fine ware jar with impressed decorations contained dehydroabietic acid, a pine resin biomarker (Regert *et al.*, 2005), and low quantities of cholesterol, which were not identified in the soil samples or the external surfaces analysed (Table 7.6). Despite the occurrence of cholesterol, the low quantities of C_{16:0} and C_{18:0} recovered preclude the secure identification of the residue as being of animal origin. Another tronco-conic fine ware bowl (PAL-32I) comprised low levels of medium to long-chain saturated fatty acids, and was dominated by one peak identified as dehydroabietic acid (*Pinaceae* resin biomarker), long-chain ketones resulting from ketonic decarboxylation (the C31:C33:C35 ratio present is 1:2:1 typically found in archaeological residues, but not in modern plants, see Evershed *et al.*, 1995b; Raven *et al.*, 1997), and phytosterols, which clearly indicate a plant input (Table 7.6). Phytosterols were not observed in the four external sherds and two soil samples analysed, and therefore must originate from the absorbed residue. A plant input can therefore be strongly suggested for the residue in pot PAL-32I, while the presence of ketones indicates heating/cooking.

Since dehydroabietic acid was not found in the external surface and soil residues, its presence in the absorbed lipids extracted from the internal surfaces of PAL-32I and PAL-35I cannot be attributed to uptake from the burial environment, and this conclusion is further strengthened by the fact that dehydroabietic acid was a dominant peak in PAL-32I. Pine resin is a known sealant, which is applied to ceramic vessels after firing to enhance their impermeability to liquids (Heron

and Evershed, 1993). This could perhaps explain the presence of *Pinaceae* resin in PAL-32I and PAL-35I.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
PAL-05I	5	FAs: C _{14:0} -C _{16:0} , C _{18:0} ; Alkanes: C21, C23, C25-C33; Alcohols: C18, C22, C24, C26, C28, C30, C32, C34; DAGs: 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin; TAGs: C48; WE: C40, C42, C44; Phthalates
PAL-06I	8	FAs: C _{14:0} -C _{16:0} , C _{18:0} ; Alkanes: C21-C33; Alcohols: C18, C22, C24, C26, C28, C30, C32, C34; DAGs: 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34; TAGs: C48; WE: C40, C42, C44; Phthalates
PAL-15I	5	FAs: C _{14:0} -C _{16:0} , C _{18:0} ; Alkanes: C21-C23, C25-C33; Alcohols: C26, C28, C30, C32, C34; DAGs: 1,3-D30, 1,2-D32; WE: C44; Phthalates
PAL-24I	4	Alkanes: C12, C14, C18, C23-C33; Phthalates
PAL-32I	8	FAs: C _{16:0} , C _{18:0} , C _{20:0} , C _{22:0} -C _{24:0} ; Alkanes: unidentified; Alcohols: C24; MAGs: 1-Monooleate; Ketones: C31, C33, C35; Dehydroabiatic acid; Sterols: Stigmastan-3,5-diene, β -Sitosterol; Phthalates
PAL-35I	8	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Sterols: Cholesterol; Dehydroabiatic acid; Phthalates

Table 7.6: GC-MS and HT-GC identification of lipid residues extracted from Palata 1, Canosa di Puglia (Apulia, Italy), run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols; DAGs: Diacylglycerols; TAGs: Triacylglycerols; WEs: Wax Esters; D_x: Number of carbon atoms in the DAG]

The alkane distribution identified in most of the Palata 1 samples (e.g. PAL-15I and PAL-24I, see Appendix E), is similar to that produced by paraffin wax (Regert *et al.*, 2005). A similar alkane distribution was also observed at Trani (discussed below). Instrumental contamination is excluded since the alkane profile was not present in all the samples, which were run under the same conditions, and must therefore originate from the burial environment. A paraffin wax profile was in fact observed in both soil samples analysed (Appendix E).

PAL-05I and PAL-06I bore strikingly similar chromatograms. Both were identified as coarse ware jars, and their chromatograms were dominated by C_{16:0} and C_{18:0} fatty acids, alkanes and alcohols. Diacylglycerols, tripalmitate and palmitate wax esters were also identified, suggesting the presence of a plant wax (Table 7.6 and Figure 7.10). Both sherds were obtained from the same stratigraphic layer. The external surface of PAL-05E was analysed, and the lipid profile obtained was found to be very similar to that obtained for the interior surfaces of PAL-05I and PAL-06I, although low quantities of C_{16:0} and C_{18:0} were present. Quantification of the lipid extracted showed that the interior surfaces contained over five times as much lipid as the external surface, suggesting that migration of lipid components from the burial context was negligible. The C_{16:0} was less abundant than the C_{18:0}, and C_{15:0} was identified, which is consistent with a ruminant fat. In the absence of GC-c-IRMS analysis, the origin of the residue cannot be securely identified, however it might indicate a mixture comprising a ruminant fat and plant material.

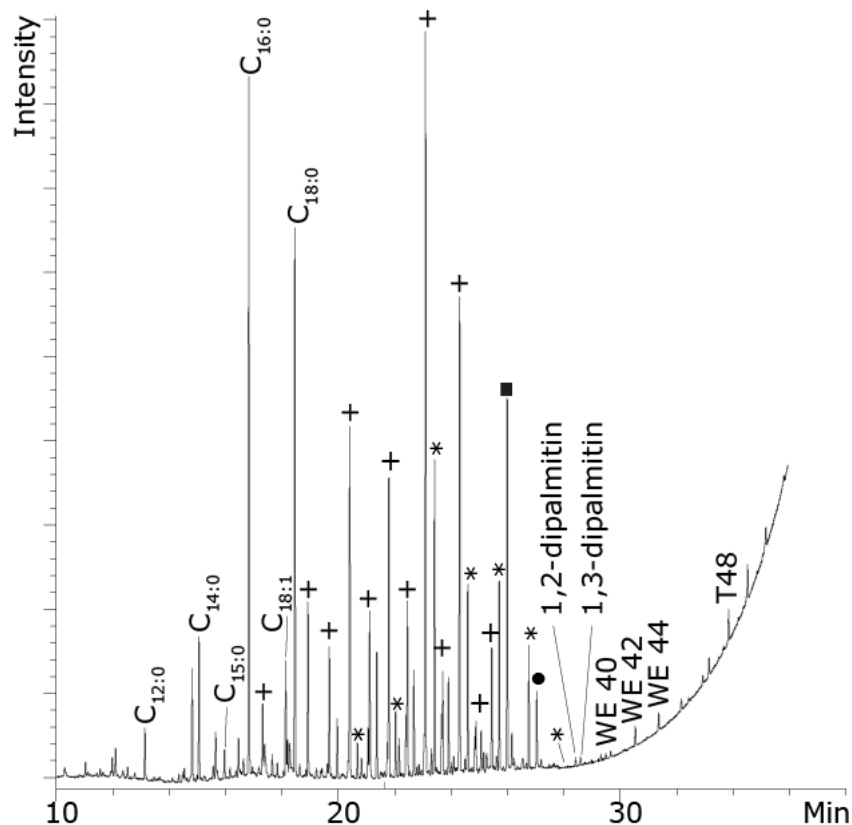


Figure 7.10: Partial HT-Gas Chromatogram from a coarse ware jar with impressed decorations, PAL-06I. [C_{x:y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C21-A33); *: Alcohols (C22-C34); WE: Wax ester; T: Triacylglycerols; P: Phtalates; ■: Internal standard (C34); ●: Internal standard (C36)]

Only 13% of the absorbed residues extracted from Impressed Wares at Palata 1 yielded a significant residue (Table 7.2), which however, could not be securely identified. The faunal remains retrieved from this site are currently under study, but so far, a total of 97 fragments including bone and teeth have been identified as ox, pig, ovicaprids, aurochs, and dog. The ovicaprids are thought to have been bred also for their secondary products (Radina *et al.*, 2011). Whether evidence for the processing of animal products in ceramic vessels at Palata 1 did not survive because of poor lipid preservation, or simply because they were not processed in pots in the first place, is unknown. The lipid profiles of PAL-05I, PAL-06I, PAL-32I, and PAL-35I could perhaps indicate a highly degraded animal fat, particularly when considering the presence of ketones in PAL-32I, which are strongly suggestive of cooking. The botanical assemblage analysed at Palata 1 indicates a heavy reliance on cereals, mainly *Hordeum* sp. and *Triticum* sp., whose remains make up 87% of the floral assemblage, and legumes, which make up the remaining 3% (Radina *et al.*, 2011). A plant contribution (tentatively suggested for PAL-05I and PAL-06I) can also

be considered, and may perhaps be present in the large proportion of negligible residues identified in the Palata 1 ceramics analysed.

7.2.5 Masseria Maselli, Bari, and Serri-San Gabriele, Bari San Paolo

Both sites are located on the outskirts of Bari, around 2km away from the coast, and situated on opposite sides of the Lama Balice (Figure 7.1). Masseria Maselli was excavated by the *Soprintendenza per i Beni Archeologici della Puglia* in 2005. The remains of quadrangular-shaped huts with evidence for a daub covering and post holes were uncovered, similar to those found at Balsignano, located only a few kilometres away (Radina, 2009). One grave, containing an adult female individual was discovered, which together with the stone structures uncovered, was dated to an advanced phase of the Early Neolithic (5570-5460 cal. BC) (Radina, 2009). Serri-San Gabriele, Bari San Paolo was excavated by the *Soprintendenza per i Beni Archeologici della Puglia* between 2007 and 2008. The site is located on a calcareous terrace and extended for about 2 hectares. It is thought to have been occupied for a short period towards the late VI millennium. Rudimentary dry-stone walls and post holes were uncovered, which were identified as quadrangular structures similar to those identified at Masseria Maselli and Balsignano (Radina, 2009). A series of well-aligned pits were also found, which must have served a utilitarian purpose since one was found to contain the base of a large ceramic container, decorated with impressed motifs (Radina, 2009). Hearths still containing soil and ash deposits were uncovered, and one of them contained a broken ceramic anthropomorphic female figurine, perhaps attesting to the cult of the mother goddess (Radina, 2009). A man-made canal, interpreted as a water channel which was re-filled, perhaps during a phase of abandonment, was also uncovered (Radina, 2009).

Fifteen ceramic vessels were analysed from the Early Neolithic assemblage at Serri-San Gabriele, Bari San Paolo, which included a variety of jars in different sizes, and one bowl. None of the pots studied contained enough lipids for a secure characterisation of the residue extracted; the lipid yield was less than $2\mu\text{g g}^{-1}$ (Table 7.2). Trace amounts of alkanes (C₂₄-C₃₃) were found in all the samples, while BSP-04I, BSP-05I and BSP-15I contained very low quantities of C_{16:0} and C_{18:0}. The external surface of two sherds were analysed (BSP-05E and BSP-14E), and were found to contain only traces of alkanes (Table 7.7).

A similar situation was observed for the pottery from Masseria Maselli (Table 7.2). The assemblage studied included 12 different-sized jars, and three visible crusts, which were sampled from the internal surfaces of vessels (MAS-01VI, MAS-02VI and MAS-05VI). The lipid residue

extracted from both the absorbed and visible residues amounted to $<0.4\mu\text{g g}^{-1}$. The lipid profile and quantity of lipid obtained from the exterior surfaces were very similar to the absorbed residues extracted from the interior of the potsherds, and are therefore likely to denote uptake from the burial environment (Table 7.7). MAS-01VI and MAS-05VI also contained trace amounts of cholesterol, which generally suggests an animal fat, however this cannot be securely determined.

<i>Sample</i>	<i>Quantification</i> ($\mu\text{g g}^{-1}$)	<i>Lipid compounds identified</i>
BSP-04I	2	FAs: C_{16:0}, C_{18:0}; Alkanes: unidentified; Phthalates.
MAS-06I	0.4	FAs: C_{16:0}, C_{18:0}; Alkanes: C16-C33; Alcohols: C18; Phthalates
MAS-07I	0.3	FAs: C_{13:0}-C_{16:0}, C_{18:0}; Alkanes: C20, C25-C28; Alcohols: C14, C18, C20, C24; Phthalates

Table 7.7: GC-MS and HT-GC identification of lipid residues extracted from Serri-San Gabriele, Bari San Paolo and Masseria Maselli (Apulia, Italy), run as TMS derivatives. [FAs: Fatty acids]

The faunal remains at Serri-San Gabriele, Bari San Paolo and Masseria Maselli are very scarce, and are still under study. However, preliminary investigations indicate the presence of an economy based on the exploitation of ovicaprids; remains of wild deer were also found (Muntoni, 2009-2012, pers. comm.), which however, are not attested in the ceramic assemblage studied. The absence of any residues in the pots is intriguing, given the considerably good yields extracted from the Fondo Azzollini ceramics, which is located only about 100km away, and pre-dates the Masseria Maselli pottery analysed (no radiocarbon dates are available for Serri-San Gabriele, Bari San Paolo).

7.2.6 Seconda Spiaggia di Colonna, Trani

Rescue excavation carried out by the *Soprintendenza per i Beni Archeologici della Puglia* between 2006 and 2008, uncovered the settlement of Seconda Spiaggia di Colonna. The site is located on the Adriatic coast, south-east of Trani (Figure 7.1), and was originally constructed near a small coastal basin, now submerged and eroded by wave action (Spiteri *et al.*, in press). The stratigraphic sequence spans from an advanced phase of the Middle Neolithic (5000-4500 BC, Serra d'Alto *facies*) to the 1st/2nd century AD, the Roman Imperial Age (Spiteri *et al.*, in press). The structures pertaining to the Middle Neolithic phase are insubstantial, and are represented by a large pit, which contained ceramics and domestic debris (Spiteri *et al.*, in press). The pottery assemblage consisted mainly of fine depurated pottery (Figulina) with ribbon handles, often surmounted by plastic appendices. Decorations included complex brown painted motifs, including meanders, lattices, and spirals (Muntoni and Laviano, 2008). The ceramic assemblage submitted

for ORA comprised a variety of coarse wares and Figulina pottery, and include mainly jars, collared jars, and bowls.

Only one small, coarse ware jar (TRA-16I), out of 26 vessels analysed from Trani contained a significant residue ($7.33\mu\text{g g}^{-1}$) (Table 7.2). GC-MS analysis identified a wide range of saturated and unsaturated fatty acids, ranging from medium to long-chain, as well as odd-numbered carbon chain lengths. $\text{C}_{16:0}$ and $\text{C}_{18:0}$ are the dominant fatty acids, with $\text{C}_{18:0}$ being the more abundant. Mono- and diacylglycerols are also present, and a wide range of triacylglycerols (C42-C54) were identified using HT-GC (Table 7.8). The lipid profile obtained, in particular the triacylglycerol distribution, the presence of $\text{C}_{15:0}$ and $\text{C}_{17:0}$, and the greater abundance of $\text{C}_{18:0}$ over $\text{C}_{16:0}$, suggest a ruminant fat. TRA-16I was submitted for GC-c-IRMS analysis, and was identified as a ruminant dairy fat ($\Delta^{13}\text{C}=-4.2\text{‰}$) (Figure 7.11).

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
TRA-03S	14	FAs: $\text{C}_{16:0}$, $\text{C}_{18:1}$, $\text{C}_{18:0}$; Alkanes: C23-C29, C31; Alcohols: C22, C24, C25, C26, C27, C28, C29, C30, C32, C34; Phthalates.
TRA-04I	3	FAs: $\text{C}_{14:0}$, $\text{C}_{15:0}$, $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C20-C30; Alcohols: C18, C20, C24, C25, C26, C27, C28, C29, C30, C32; Phthalates
TRA-16I	7	FAs: $\text{C}_{10:0}$, $\text{C}_{12:0}$, $\text{C}_{14:0}$, $\text{C}_{15:0}$, $\text{C}_{16:1}$, $\text{C}_{16:0}$, $\text{C}_{17:0}$, $\text{C}_{18:1}$, $\text{C}_{18:0}$, $\text{C}_{19:0}$, $\text{C}_{20:0}$, $\text{C}_{21:0}$, $\text{C}_{22:0}$, $\text{C}_{23:0}$, $\text{C}_{25:0}$, $\text{C}_{26:0}$; Alkanes: unidentified; Alcohols: C24, C26, C28, C30, C32; MAGs: 1-Monostearin, 2-Monostearin; DAGs: 1,2-Dipalmitin; TAGs: C42-C54; Phthalates
TRA-22I	2	FAs: $\text{C}_{8:0}$, $\text{C}_{9:0}$, $\text{C}_{11:0}$, $\text{C}_{13:0}$, $\text{C}_{14:0}$, $\text{C}_{15:0}$, $\text{C}_{16:0}$, $\text{C}_{17:0}$, $\text{C}_{18:0}$; Alkanes: C25-C27, C29, C31, C33, C36; Alcohols: C26; Sterols: Cholesta-3,5-diene; Phthalates
TRA-25VI	105	FAs: $\text{C}_{14:0}$ - $\text{C}_{22:0}$, $\text{C}_{16:1}$, $\text{C}_{18:1}$; Alkanes: C27, C29, C31; Alcohols: C18, C28, C30, C32; Ketones: C33, C35; Sterols: Cholesterol; Dehydroabietic acid; Phthalates
TRA-25VE	22	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C29, C31; Alkenes: unidentified; Alcohols: C18, C24, C26, C28, C30, C32; Sterols: Cholesterol; Phthalates

Table 7.8: GC-MS and HT-GC identification of lipid residues extracted from Seconda Spiaggia di Colonna, Trani (Apulia, Italy), run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols; DAGs: Diacylglycerols; TAGs: Triacylglycerols; WEs: Wax Esters; D_x : Number of carbon atoms in the DAG; S: Soil sample; V: Visible residue]

Vessel TRA-25I, an undecorated coarse ware jar, had some encrustations on its inner and outer surfaces. These were sub-sampled and analysed using GC-MS (Table 7.8). The crusts sampled from both surfaces were found to have a similar lipid profile, suggesting a common origin, however the lipid obtained from the crust on the interior surface of the pot was quantitatively more abundant than the deposit on the exterior surface. The crust sampled from the inner surface (TRA-25VI) also contained long-chain ketones, consistent with the onset of ketonic decarboxylation (Evershed *et al.*, 1995b; Raven *et al.*, 1997), indicating that this particular residue was formed as a direct action

of heating. It also contained dehydroabietic acid, which was not found in any of the exterior or soil samples analysed. This suggests that the *Pinaceae* resin biomarker did not migrate from the burial environment but originates in the visible residue, although its presence as an artefact from instrumental contamination was also considered. The cholesterol identified, together with the predominance of C_{16:0} and C_{18:0} fatty acids (C_{18:0} being the more abundant), suggest an animal fat, and the presence of C_{15:0} and C_{17:0} indicate a ruminant origin. However, in the absence of GC-c-IRMS analysis the residue cannot be securely identified. The absorbed residue sampled from pot TRA-25I however showed a negligible residue, and comprised only traces of medium-chain fatty acids and alkanes, while cholesterol and dehydroabietic acid are absent. It is still unclear whether lipid residues are better preserved in charred or absorbed residues (see Rottländer, 1990; Deal *et al.*, 1991; Oudemans and Evershed, 1991; Mukherjee *et al.*, 2008), while in Chapter 5, it was suggested that differential preservation of lipid constituents may occur, with crusts being more susceptible to oxidative degradation than hydrolysis, and vice versa for absorbed residues. This is important because the quantified results obtained from the absorbed and visible residues sampled from the interior surface of pot TRA-25I, could perhaps better indicate an advanced degradation of absorbed residues, as opposed to their absence being related to the function of these ceramics.

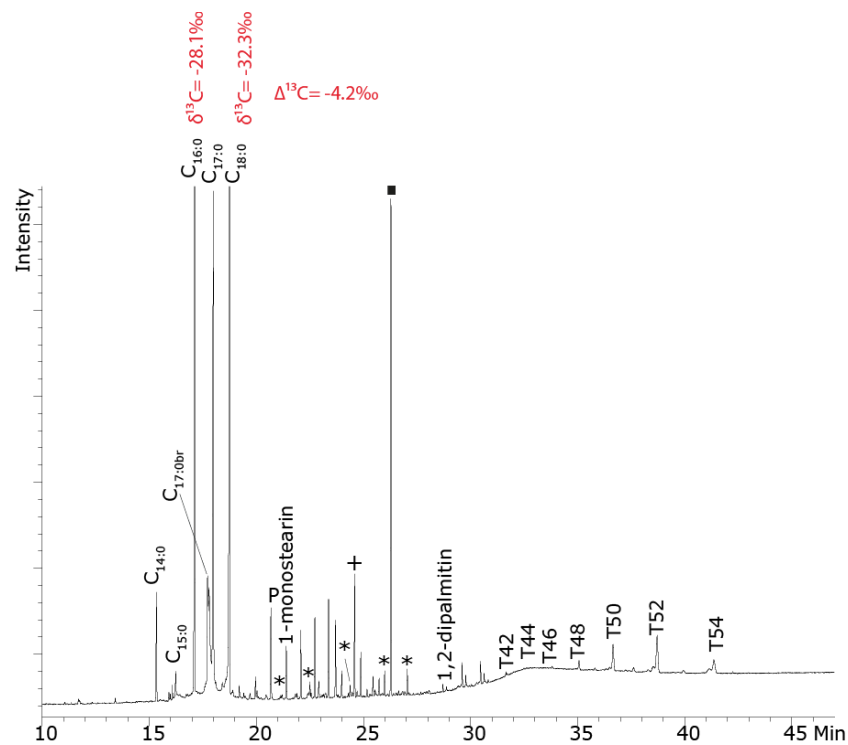


Figure 7.11: Partial HT-Gas Chromatogram from an undecorated coarse ware small jar TRA-16I. [C_{x:y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C30); *: Alcohols (C24-C32); T: Triacylglycerols; P: Phtalates; ■: Internal standard (C34)]

As already reported when discussing the results obtained from Palata 1, most of the samples from Trani have an alkane distribution consistent with a paraffin wax. This characteristic distribution has been identified in the soil samples analysed and the external surfaces tested (Appendix E), and is therefore attributed to uptake from the burial context.

Information on the faunal remains at Trani are unavailable as the bone assemblage is currently under study, however GC-c-IRMS has provided direct evidence for the processing of dairy products at Trani. The high proportion of negligible residues is intriguing, especially when considering that 26 vessels were analysed. Eleven of the samples analysed were identified as Figulina Wares, and included both bowls and jars, but none contained a significant residue. As already described above, Figulina Wares appeared during the Middle Neolithic period, around the middle of the 6th millennium cal. BC, and disappeared during the late 5th millennium cal. BC (Spataro, 2009b:56). They are present along the Adriatic coast of Italy, mainly between Apulia and Abruzzo, and along the Dalmatian coast in the Danilo and Hvar cultures (Malone, 2003; Spataro, 2009a:66). Their production is characterised by high-quality clay and skilled technical ability in their modelling, firing and decoration (painting when present) (Malone, 2003; Spataro, 2009b:59). The fabric is generally powdery in texture and ranges from pinkish, yellowish to greyish colours (Spataro, 2009a:66). Archaeometric studies have been carried out on Figulina Wares from sites located along both the Italian and Dalmatian coastlines by Spataro (2009a) and Muntoni (2002c). Interestingly, both studies showed that while the production of Impressed Wares generally comprised the utilization of local clays with minimal exchange of whole pots at an inter-site level, Figulina Wares appear to have been specialised productions, perhaps for regional (or wider) consumption, and may therefore have been circulated (Muntoni, 2002c; Malone, 2003; Spataro, 2009b). Their suggested function, as containers for special goods (e.g. wine), and their presence as grave goods in funerary contexts (hence perhaps not used for processing food products) (Barfield, 1981; Bagolini, 1990; Spataro, 2009a:66), may explain the reason for the negligible residues obtained by ORA. Analytical techniques, namely Liquid Chromatography (LC) combined with Tandem Mass-Spectrometry (LC-MS/MS) (Guasch-Jané *et al.*, 2004; Stern *et al.*, 2008; Barnard *et al.*, 2010), are more sensitive to the identification of wine biomarkers (tartaric acid, syringic acid and malvidin), but were not utilised in the present study. Finally, the residues extracted from Figulina Wares at Ciccotto were tentatively associated with a plant input, which may well be the case at Trani. However, as yet, it is not clear whether the absence of absorbed residues in the Figulina Wares are related to their function or poor preservation conditions leading to the degradation of absorbed lipid residues. Further investigation may help identify specific biomarkers, which may shed light on the vessel contents and use.

7.2.7 Canne Setteponti, Barletta

The site is situated in the locality of Setteponti, a few kilometres north-east of Canne and 6km away from the coast (Figure 7.1). It dates to the Middle Neolithic period, *facies* Serra d'Alto (Radina, 2003). A 1.8m-deep, bell-shaped pit was uncovered, and tentatively identified as a grain silo, similar to the ones excavated at Trasano and Catignano (Radina, 2003). Ceramics were the most abundant find, and included open vessels with strap handles, globular jars and jars with tronco-conic necks. Decorations rarely included cardial impressions, but were mostly painted (red and brown), and zoomorphic decorations were also common (Radina, 2003). Querns, obsidian, flint and bone tools were also present (Radina, 2003). The faunal record comprised 438 remains, including mainly ox, sheep and goat bones, and to a lesser extent, dog, deer and tortoise (Alhaique and Cerilli, 2003). The mortality profile indicated that oxen were used for meat production, whereas ovicaprids were bred for their secondary products (Alhaique and Cerilli, 2003).

Twelve different-sized jars were analysed from this site, which date to the Middle Neolithic period and are associated with the Serra d'Alto phase. As observed with the other Middle Neolithic ceramics analysed in this study (Ciccotto and Trani), none of the sherds contained a significant residue (the maximum lipid yield observed was $3\mu\text{g g}^{-1}$) (Table 7.2). The lipid profiles obtained for the external and internal surfaces analysed, as well as the quantity of lipid extracted were very similar, suggesting low levels of lipid migration from the burial environment.

Residues comprised mainly low levels of medium-chain length fatty acids, alcohols, and a wide series of alkanes (C14-C33) (Table 7.9). SET-011, also contained traces of cholesterol, but the levels of C_{16:0} and C_{18:0} are too low to attempt an interpretation. The faunal assemblage described above suggests a dependence on animal products, hence that none of the sherds should retain any evidence for the processing of these products is interesting. The site has essentially been interpreted as a bell-shaped pit, possibly used as a grain silo. This may perhaps suggest that the function of these Serra d'Alto ceramics (including two Figulina Wares) may have been associated with storage or processing of plant material, which is also consistent with the low lipid yields identified, and the lipid profiles obtained.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
SET-O11	3	FAs: C _{16:0} , C _{18:0} ; Alkanes: C14, C16-C18, C20, C24, C26-C33; Alcohols: C12, C14, C16, C18; Sterols: Cholesterol; Phthalates.

Table 7.9: GC-MS and HT-GC identification of lipid residues extracted from a Figulina Ware jar from Canne Setteponti (Apulia, Italy), run as TMS derivatives. [FAs: Fatty acids]

7.3 Favella della Corte, Corigliano Calabro (Calabria, Italy)

The site is situated in the middle of the Sybaris Plain (near Corigliano Calabro, Cosenza), around 6km from the present coastline to the east, 1.5km south of the river Crati, and 3.5km away from the confluence of Crati into the river Coscile to the northwest (Tin , 2009a:581) (Figure 7.12). The site is backed by hills, characterised by a well-drained arable terrain (Natali and Tin , 2002:708-709). Aerial photography showed that, during the Neolithic, the river enveloped the northern side of the settlement, suggesting that the site was arranged to benefit both from the hilly interior, as well as the marshy coastal environment (Natali and Tin , 2002:722). Two villages were uncovered whose dates partly overlapped; the earliest attributed to the Early Neolithic phase (6000-5700 BC; Ceramiche Impresse Arcaiche), and the other to the Late Neolithic (Capanna Gravela di Serra d'Alto/Diana-Bellavista) (Tin , 2009a:582). The two phases are separated by a gap of more than a millennium (Tin , 2009a:582).

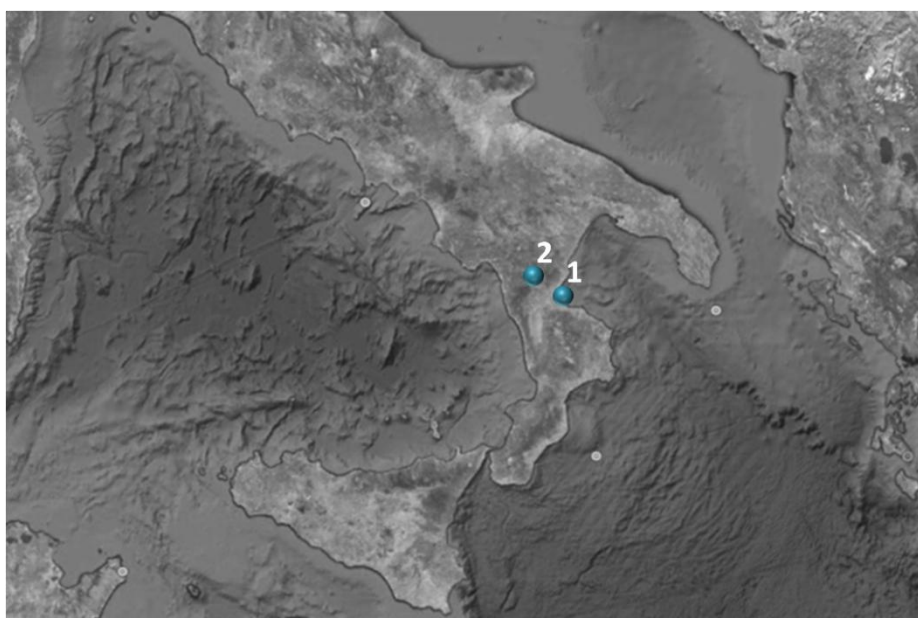


Figure 7.12: Map showing the location of the Calabrian sites investigated. [1: Favella della Corte; 2: Grotta San Michele di Saracena]

Several pit structures were uncovered in the Early Neolithic deposits, which contained pottery, lithics, daub and bones (Tin , 2009a:582). Most of these structures consist of 2 or 4 large pits linked together, and appear to have a similar stratigraphic pattern (Figure 7.13): Layer 3 comprising structural remains (e.g. daub and timber fragments), and Layer 4, containing everyday refuse, including pottery and stone, organic remains and animal bones (Tin , 2009a:584-585). These were interpreted as quarries, as they appear to be located adjacent to wattle and daub huts with a timber frame construction, which experimental and archaeometric evidence suggested had been burnt down, perhaps on purpose (Tin , 2009a:585-586, 2009b). The pottery selected for ORA was obtained from the Early Neolithic structures D (Layer 4) and F (Layers 3, 7 and 8).

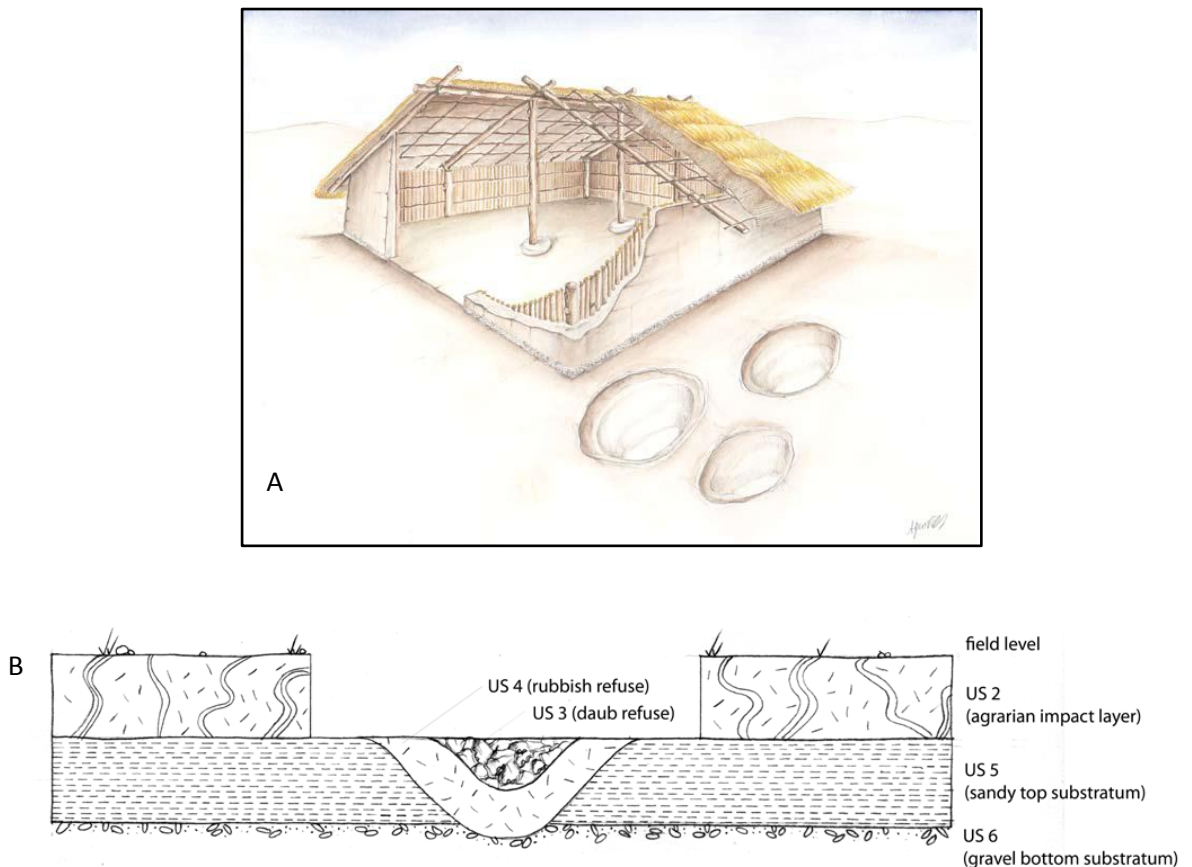


Figure 7.13: A: Hypothetical reconstruction of one of the huts at Favella, with the associated pits; B: The stratigraphic pattern uncovered at Favella (Tin , 2009a:585-586, drawings by M. Agrostelli).

The botanical evidence retrieved from Favella included evidence for cereal cultivation (*Hordeum* sp., *H. vulgare*, *H. sativum*, *Triticum* sp., and *T. dicoccum*), legumes (e.g. *Lens*), and weeds (e.g. *Lolium temolentum/remotum*) (Natali and Tin , 2002:722). Despite the use of floatation techniques, the botanical remains retrieved were fragmented and scarce; however, the available

data suggest a dry agriculture, based on a few taxa of resistant cereals and legumes (Coubray, 2009:599; Tiné, 2009b). The faunal assemblage comprised mainly domestic animals, including ovicaprids (69%), cattle and pigs, while wild species (red deer, roe deer, wild boar, aurochs, fox and tortoise) made up 3% of the assemblage (Tiné, 2009a:598). The mortality profile obtained from the analysis of cattle, sheep and goat remains, suggests that the animals were bred for meat as well as for dairy products (Tiné, 2009a:598). Fish, particularly the grouper, are also well attested, and made up around 20% of the faunal remains in Structure D. Freshwater species are absent, and marine species dominate (Albertini, 2009; Tiné, 2009a:599).

The ceramic assemblage analysed from Favella comprised 27 vessels, which included a variety of different-sized jars, bowls and one dish. Only four fine ware bowls (FAV-24I, FAV-25I, FAV-26I and FAV-27I) contained a significant quantity of lipids, while all the coarse wares analysed produced a negligible residue (Table 7.2). This is perhaps significant, since coarse wares were principally associated with storage and transport functions, whereas fine wares are thought to have been used as serving and display vases, and perhaps for cooking (Natali, 2009; Tiné, 2009a:593). GC-MS analysis identified a series of medium-chain fatty acids, alcohols and alkanes, with cholesterol present in only one sample, FAV-26I, and absent from the exterior surface residues analysed (Table 7.10). However, very low quantities of $C_{16:0}$ and $C_{18:0}$ were identified in pots FAV-24I, FAV-25I, and FAV-26I, which precluded a secure interpretation using GC-c-IRMS analysis. In FAV-27I, the $C_{16:0}$ was observed to be slightly more abundant than the $C_{18:0}$, while odd-numbered chain fatty acids ($C_{15:0}$ and $C_{17:0}$) were present (Figure 7.14). However, the quantities of $C_{16:0}$ and $C_{18:0}$ present in FAV-27I were again not sufficient for GC-c-IRMS analysis. The absorbed residue in FAV-27I, and possibly FAV-26I due to the presence of cholesterol, have been tentatively identified as ruminant and animal fat respectively (odd-numbered fatty acids were not present in FAV-26I). The chromatograms obtained for FAV-24I and FAV-25I (Appendix E) are more typical of a plant residue.

<i>Sample</i>	<i>Quantification ($\mu\text{g g}^{-1}$)</i>	<i>Lipid compounds identified</i>
FAV-14I	2	FAs: $C_{16:0}$, $C_{18:0}$; Alkanes: unidentified; Phthalates
FAV-24I	9	FAs: $C_{14:0}$ - $C_{16:0}$, $C_{18:0}$; Alkanes: unidentified; Phthalates
FAV-25I	6	FAs: $C_{16:0}$, $C_{18:0}$; Alkanes: unidentified; Phthalates
FAV-26I	5	FAs: $C_{16:0}$, $C_{18:0}$; Alkanes: C24-C33; Alcohols: C14,C18; Sterols: Cholesterol; Phthalates
FAV-27I	8	FAs: $C_{14:0}$ - $C_{20:0}$, $C_{18:1}$; Alkanes: C25, C26, C27, C29, C30; Alcohols: C14,C24; Phthalates

Table 7.10: GC-MS and HT-GC identification of lipid residues extracted from Favella della Corte, Corigliano Calabro (Calabria, Italy), run as TMS derivatives. [FAs: Fatty acids]

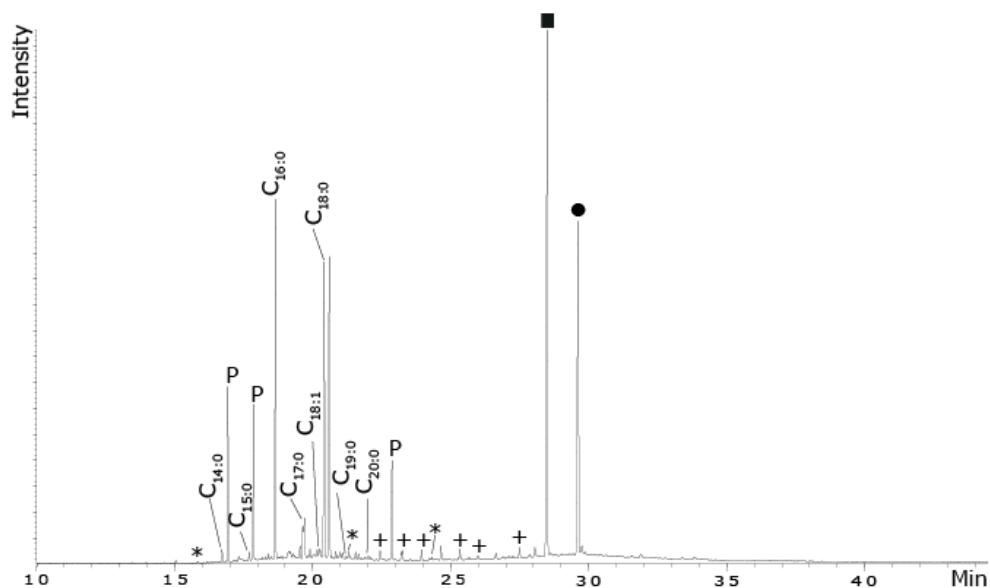


Figure 7.14: TIC of a fine ware bowl with impressed decorations, FAV-271, tentatively identified as a ruminant fat. [$C_{x,y}$: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C25-C33); *: Alcohols (C14-C24); ■: Internal standard (C34); ●: Internal standard (C36)]

Low lipid yields were once again retrieved from the Favella ceramics analysed, despite the faunal record studied, which indicated a thriving herding economy, with a major input from fishing, and hunting as a less important, but nonetheless practiced activity (Natali and Tiné, 2002:721). Structure D, from which most of the pottery analysed was obtained, was found to contain considerable quantities of marine fish remains, but no marine biomarkers were present in the residues analysed.

7.4 Grotta San Michele di Saracena (Calabria, Italy)

Grotta San Michele di Saracena, located almost opposite the village of Saracena on the right bank of the river Garga (Figure 7.12), is a large karst cavity which revealed an uninterrupted depositional sequence dating from the Early Neolithic to the Late Bronze Age. Excavations were carried out in 1998, 2000 and 2003 by the *Associazione Sexito (Soprintendenza Archaeologica della Calabria)* and the *Soprintendenza Special al Museo 'Luigi Pigorini'*. Two areas were excavated: *alpha* located towards the back of the cave, in which the depositional sequence was preserved in its entirety till the Bronze Age, and *beta*, situated towards the front of the cave (Figure 7.15). The upper layers of Area *beta* have been disturbed in modern times; hence investigations focused on Late to the Early Neolithic periods (Tiné and Natali, 2004).

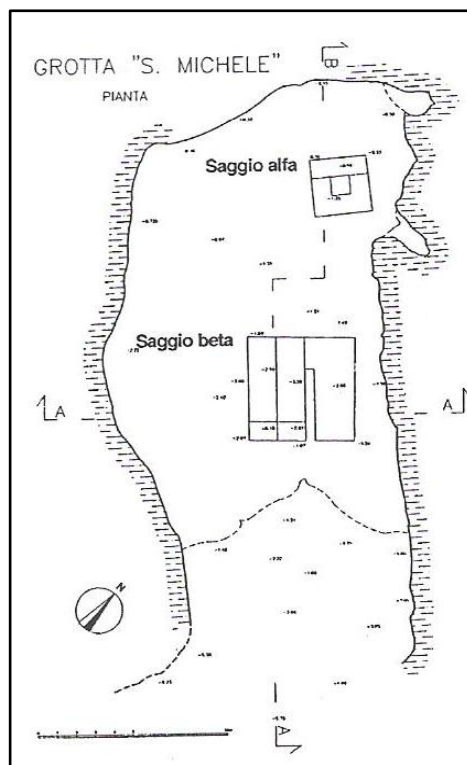


Figure 7.15: Plan showing areas alpha and beta excavated at Grotta San Michele di Saracena (After Tiné and Natali, 2004:Fig. 1).

At Grotta San Michele, 11 of the 15 vessels analysed contained a significant lipid yield (between 5 and $24\mu\text{g g}^{-1}$); hence 73% of the assemblage studied provided evidence for processing of animal/plant products in ceramic pots (Table 7.2). Most of the lipid residues comprised saturated and unsaturated fatty acids, with short, medium and long carbon chain lengths (Table 7.11). $\text{C}_{16:0}$ and $\text{C}_{18:0}$ dominated the fatty acid profiles, with $\text{C}_{18:0}$ being generally more abundant. Most of the samples contained low levels of mono- and diacylglycerols, while HT-GC also detected the presence of a wide range of triacylglycerols (C_{44} - C_{54}) in four of the vessels sampled (SAR-01IA, SAR-07IA, SAR-08IA and SAR-09IA and B). Cholesterol and its bi-products were also consistently present. The lipid profiles obtained are therefore indicative of an animal fat, with the identification of $\text{C}_{15:0}$ and $\text{C}_{17:0}$ suggesting a ruminant origin. Palmitate wax esters were identified in pots SAR-02I, SAR-11I, SAR-12I, SAR-13I and SAR-14I, which, as already previously discussed, are consistent with a plant contribution. Wax esters were not identified in the external surfaces of pots SAR-02E and SAR-11E, and hence must originate from the absorbed residue. Alcohols and fatty acids with corresponding chain lengths of the wax esters identified were also present, and suggest the onset of degradation of the wax esters into their constituents (Figure 7.16).

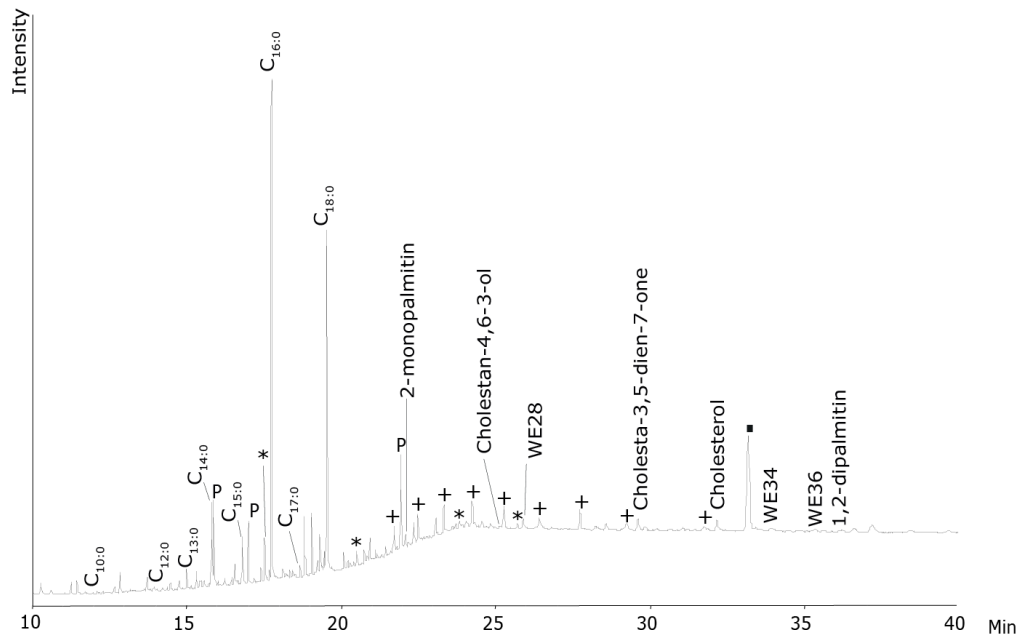


Figure 7.16: Partial GC of a coarse ware collared jar, SAR-12I with impressed decorations, identified as ruminant fat but the palmitate wax esters present suggest a possible mixture also comprising plant material. [C_{x:y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C25-C33); *: Alcohols (C17-26); WE: Wax esters; ■: Internal standard (C34)]

Three samples were analysed using GC-c-IRMS analysis, which are consistent with the processing of ruminant adipose in pot SAR-02IA⁵, and ruminant dairy products in vessels SAR-09IA and SAR-11IA (Figure 7.17). Palmitate wax esters from pots SAR-02IA and SAR-11IA suggest a mixture of plant and animal products, which could have been processed simultaneously or in sequential cooking episodes. A similar lipid profile was obtained for the residues extracted from pots SAR-12I and SAR-13I, and could also represent a mixture between a ruminant fat and plant material (Figure 7.16). The faunal assemblage recovered at Grotta Saracena is still in the initial phases of analysis. However a detailed archaeobotanical study has been carried out, which has shown that a good knowledge of agrarian practises existed from the earliest Neolithic phases, and has provided evidence for the cultivation of cereals (including *T. monococcum*, *T. dicocum*, *T. durum*, *T. aestivum* and *H. vulgare* L.), and legumes (e.g. *Vicia* sp. and *Lathyrus* sp.) (Agrostelli, 2010), which is consistent with the interpretation of a plant input in the residues extracted.

⁵ The $\delta^{13}\text{C}_{16:0}$ values obtained for sample SAR-02IA identify it as a terrestrial fat (no fish biomarkers were found using GC-MS), while the $\Delta^{13}\text{C}$ measurement of -1.1‰ precludes its identification as a non-ruminant fat, and suggests that it originates from a domestic ruminant animal rather than deer, since the $\Delta^{13}\text{C}$ measurements at $\pm 1\text{‰}$ standard deviation obtained for the latter ranged from -1.9‰ to -4.3‰ .

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
SAR-011A	7	FAs: C _{12:0} , C _{14:0} -C _{18:0} , C _{20:0} , C _{18:1} ; Alkanes: unidentified; Alkenes: unidentified; Alcohols: C20, C26; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34; TAGs: C42, C44, C48, C50, C52; Phthalates
SAR-011B	3	FAs: C _{8:0} , C _{9:0} , C _{12:0} , C _{14:0} -C _{18:0} , C _{18:1} ; Alkanes: C20, C22, C24-C28, C31; Alcohols: C12, C18, C26, C28; Sterols: Cholesterol; Phthalates
SAR-021A	13	FAs: C _{8:0} -C _{10:0} , C _{12:0} -C _{14:0} , C _{16:0} -C _{18:0} ; Alkanes: C22-C29; Alkenes: unidentified; Alcohols: C14, C16, C18, C20, C24, C26; Sterols: Cholestan-3,5-dien-7-one, Cholesterol; WEs: C30, 32, C34; Phthalates
SAR-02B	24	FAs: C _{8:0} , C _{9:0} , C _{10:0} , C _{12:0} , C _{14:0} , C _{15:0} , C _{16:0} , C _{17:0} , C _{18:1} , C _{18:0} , C _{19:0} , C _{20:0} , C _{22:0} , C _{24:0} , C _{26:0} ; Alkanes: C14, C16, C18, C20, C22, C24, C26, C28, C30, C32; Alcohols: C12, C18, C20, C26; DAGs: 1,3-Dipalmitin; Phthalates
SAR-031	2	FAs: C _{14:0} -C _{16:0} , C _{18:0} , C _{25:0} ; Alkanes: C19-C22, C25-C33; Alcohols: C16, C18, C20, C24, C26, C28, C30; Phthalates
SAR-061	6	FAs: C _{8:0} -C _{10:0} , C _{14:0} -C _{19:0} ; Alkanes: C23-C25, C27, C29, C30, C31; DAGs: 1,2-D34, 1,2-Distearin; Phthalates
SAR-071A	17	FAs: C _{8:0} , C _{9:0} , C _{12:0} , C _{14:0} -C _{16:0} , C _{18:0} ; Alkanes: unidentified; MAGs: 1-Monooleate; DAGs: 1,2-D34, 1,3-Dioleate; 1.3-Dioleate; TAGs: C42, C48, C50, C52, C54; Phthalates
SAR-071B	6	FAs: C _{14:0} -C _{16:0} , C _{18:0} , C _{18:1} ; Alkanes: unidentified; Phthalates
SAR-081A	3	FAs: C _{8:0} , C _{11:0} , C _{12:0} , C _{14:0} -C _{20:0} , C _{22:0} , C _{18:1} ; Alkanes: C25, C29, C31; Alcohols: C18, C24, C26, C28, C30; MAGs: 2-Monooleate, 1-Monostearate; DAGs: 1,2-D30, 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-Distearin; 1.3-Distearin; TAGs: C42, C44, C46, C48, C50, C52, C54; Phthalates
SAR-081B	5	FAs: C _{14:0} -C _{18:0} , C _{24:0} , C _{26:0} , C _{16:1} , C _{18:1} ; Alkanes: C25, C29; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34; TAGs: C46, C48, C50, C52; Sterols: Cholesterol; Phthalates
SAR-091A	8	FAs: C _{8:0} -C _{12:0} , C _{14:0} -C _{24:0} , C _{26:0} , C _{18:1} ; Alkanes: unidentified; Alcohols: C16; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34, 1,2-Distearin; TAGs: C44, C48, C50, C52; Phthalates
SAR-091B	10	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Phthalates
SAR-101	12	FAs: C _{8:0} -C _{24:0} , C _{26:0} , C _{16:1} , C _{18:1} ; Alkanes: unidentified; Alcohols: C12, C14, C16, C18, C20; Sterols: Cholesterol; Phthalates
SAR-111A	15	FAs: C _{10:0} , C _{12:0} -C _{18:0} , C _{18:1} ; Alkanes: C16, C20, C23-C33; Alkenes: unidentified; Alcohols: C12, C14, C16, C17, C24, C26; Sterols: Cholesta-4,6-dien-3-ol (3-beta); Cholesta-3,5-dien; WEs: C32, C34; Phthalates
SAR-121	5	FA: C _{10:0} , C _{12:0} , C _{14:0} -C _{18:0} ; Alkanes: C14, C16, C18, C20, C22, C24, C25-C33; Alcohols: C12, C14, C15, C16, C17, C18, C19, C20, C22; Sterols: Cholesta-4,6-dien-3-ol (3-beta); Cholesta-3,5-dien-7-one; MAGs: 2-Monpalmitin; DAGs: 1,2-Dipalmitin; WEs: C28, C34, C36; Phthalates
SAR-131	7	FAs: C _{8:0} -C _{12:0} , C _{14:0} -C _{18:0} , C _{24:0} , C _{26:0} ; Alkanes: C25-C33; Alcohols: C16, C18; WE: C32, C34; Phthalates
SAR-141	2	FAs: C _{9:0} , C _{10:0} , C _{12:0} -C _{18:0} , C _{20:0} ; Alkanes: C12-C15, C18, C20, C22, C24, C26, C30, C32; Alcohols: C14, C17, C18, C19; Sterols: Cholesterol; WEs: C32; Phthalates
SAR-151	7	FAs: C _{8:0} , C _{9:0} , C _{14:0} -C _{18:0} , C _{18:1} ; Alkanes: unidentified; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34; Phthalates

Table 7.11: GC-MS and HT-GC identification of lipid residues extracted from Grotta San Michele, Saracena (Calabria, Italy), run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols; DAGs: Diacylglycerols; TAGs: Triacylglycerols; WEs: Wax Esters; D_x: Number of carbon atoms in the DAG]

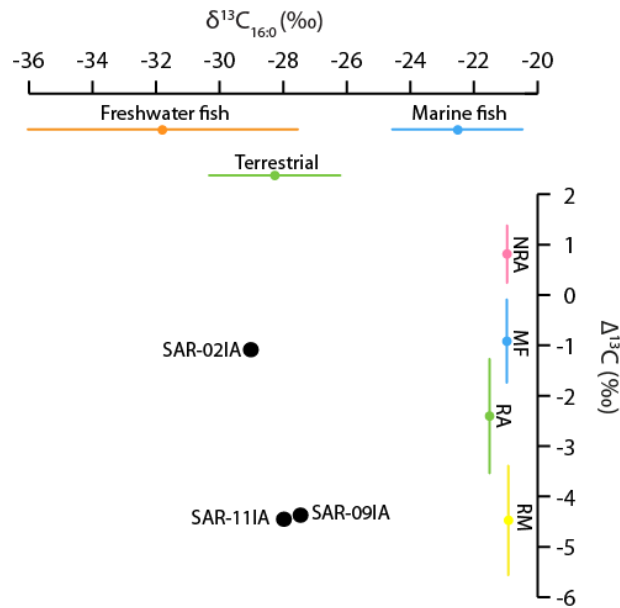


Figure 7.17: GC-c-IRMS Results: Grotta San Michele, Saracena (Calabria, Italy). [$\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\%$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

No trend was observed in the accumulation of lipid residues in coarse wares as opposed to fine wares, and between decorated and undecorated vessels. However, while acknowledging that because of the small sample size, observations related to changes in pottery use over time cannot be confidently made, 2 out of 4 vessels dated to the Early Neolithic phase were found to contain a significant amount of lipid, whereas 9 out of 11 vessels dated to the subsequent Stamped Ware phase contained more than the recommended $5\mu\text{g g}^{-1}$. This might suggest an increase in the utilization of ceramic vessels for culinary or non-culinary activities in the later stages of the Early Neolithic. However the sample size is too small for further discussion.

Previous studies have shown that analysing the lipid accumulation at the rim, body and base of pots can help identify vessel use, in particular the cooking method (Charters *et al.*, 1993). This was attempted on the Grotta Saracena assemblage, since some of the bigger and less fragmented vessels allowed sampling along the pot profiles. These included pots SAR-01, SAR-02, SAR-05, SAR-07, SAR-08, SAR-09 and SAR-11. All these vessels are coarse wares, except SAR-09I, and all but SAR-09I and SAR-11 are jars. SAR-09I is a bowl decorated with impressed and incised decorations, while SAR-11I is a collared jar decorated with incised motifs (Figure 7.18). The lipid yield from both samples taken from pot SAR-05I was negligible, and has therefore been excluded from this discussion. Vessels SAR-07 and SAR-11 showed a clear accumulation of lipid at the rim (17 and

15 $\mu\text{g g}^{-1}$, respectively), when compared to the base (6 and 1 $\mu\text{g g}^{-1}$, respectively). This pattern of lipid distribution is consistent with boiling, since the hydrophobic lipid compounds tend to rise to the surface and become absorbed at the level of the water mark, while the greater heat intensity at the base is thought to promote the breakdown of lipids and other organic compounds (e.g. carbohydrates and proteins), therefore resulting in low lipid yields (Charters *et al.* 1993). The lipid accumulation pattern in pots SAR-01, SAR-02, SAR-08 and SAR-09 appears to be more consistent throughout the whole vessel (see Appendices C and D for quantification details). This accumulation pattern is consistent with i) the application of a sealant (e.g. resins, waxes, milk and other foodstuffs) after firing, which makes the vessel more impermeable to liquids (Rice, 1987; Heron and Evershed, 1993:163-164), ii) prolonged use of the vessel, which may lead to a more homogeneous lipid accumulation, for example during roasting, iii) the type of heat applied to the vessel (e.g. temperature, cooking over a direct fire, or warming next to the fire), iv) covering the pot with a lid as opposed open boiling (Charters *et al.* 1993). Any of these explanations could possibly apply to the vessels in question, however SAR-09I (the decorated bowl), is more likely to have been used as a serving vessel, which is consistent with the roughly homogenous distribution of this residue, identified by GC-c-IRMS as a dairy product.

The absorbed residues extracted from the Grotta Saracena pottery assemblage analysed were generally consistent with ruminant fats, while a plant input has also been tentatively suggested, particularly due to the presence of palmitate wax esters which were not detected in any of the external surface residues analysed. GC-c-IRMS has also securely identified processing of ruminant dairy and ruminant adipose fats.

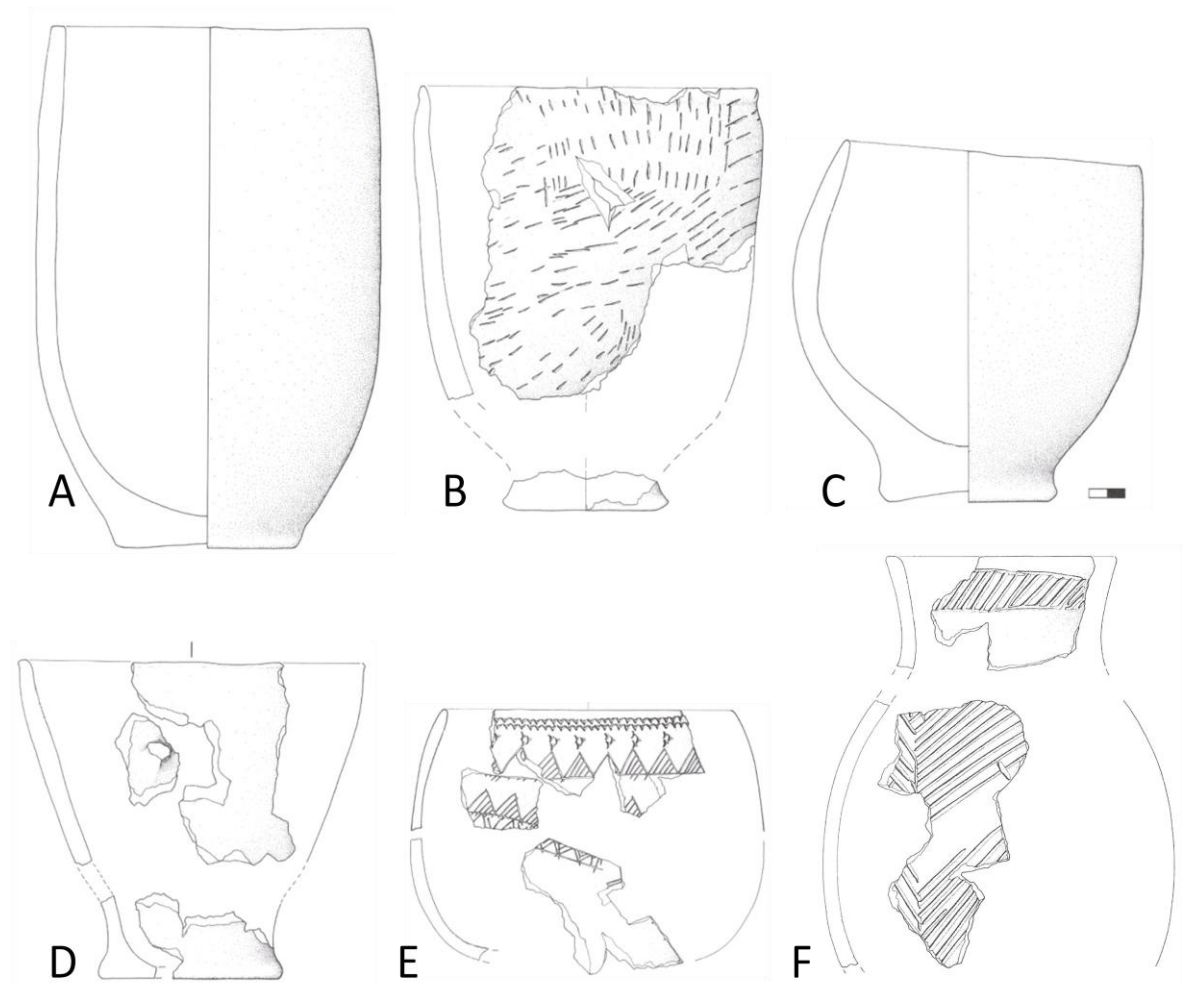


Figure 7.18: A: SAR-01: undecorated jar, coarse ware; B: SAR-02: jar with impressed decorations, coarse ware; C: SAR-07; undecorated jar, coarse ware; D: SAR-08: undecorated jar, coarse ware; E: SAR-09: bowl with impressed and incised decorations; fine ware; F: SAR-11: collared jar with incised decorations, coarse ware. [Drawings courtesy of E. Natali]

7.5 La Marmotta, Anguillara Sabazia, (Lazio, Italy)

The Early Neolithic village of La Marmotta is located on the south-east side of the Lago di Bracciano, in Anguillara Sabazia (Lazio) (Figure 7.19). It is now submerged to a depth of around 6 to 8m, and lies about 300m away from the shore. The site has a perimeter of 31.5km, and an irregular elliptic form (Fugazzola Delpino, 2002a:373-374). The area was ideal for human settlement, with a temperate climate and the presence of volcanic lakes, arable fields, vines and olive trees, pastures good for herding, as well as underground and above-ground streams (Fugazzola Delpino, 2002a:373-374).



Figure 7.19: Map showing the location of the Early Neolithic village of La Marmotta.

Large structures appear to have been constructed along what appear to be major roads oriented from north to south, and minor streets were also discernible, extending from east to west. The structures uncovered varied in size, hearths were located centrally within the buildings, and numerous other constructions were identified, including quadrangular-shaped buildings annexed to the dwelling structures, bell-shaped silos and pits (Fugazzola Delpino, 2002a:376-377). The village is thought to have flourished uninterruptedly for about 450 years, between 5600-5150 BC (Fugazzola Delpino, 2002a:389). The importance of La Marmotta for reconstructing the spread of the Mediterranean Neolithic, particularly the attestation of seafaring demonstrated by the retrieval of two boats from this site, has already been highlighted in Chapter 2.

Approximately 15,000 faunal remains have been collected from La Marmotta, the majority belonging to domesticated species (ovicaprids, cattle and pigs), although wild animal remains (e.g. red deer, roe deer, wild boar, aurochs, otters and badgers) were also documented. The culling pattern is consistent with that of a domesticated herd; pigs and ovicaprids were culled before reaching the age of two, and three to four respectively. Evidence for dairying was observed in the culling of cattle, with animals surviving to sub-adult and adult stages (Fugazzola Delpino, 2002a:386-387). Fish remains were rare, and comprised pike, tench and other species belonging to the *cyprinidae* family (Fugazzola Delpino, 2002a:387). Cereals, including spelt, einkorn, barley, and wheat were the main cultivars, and they appear to have been grown separately on the land surrounding the village. Legumes (e.g. lentils, peas, vetch and bitter vetch) were also grown, and

fruit (e.g. plums, prunes, cherries, pears, apples, acorns and figs) was widely available (Fugazzola Delpino, 2002a:388).

The pottery assemblage recovered at La Marmotta has already been described in some detail in Chapter 2. Coarse wares, which were included in the vessels selected for ORA, comprised mainly large containers, which were presumably used to store food and liquids (Fugazzola Delpino, 2002a:377). These large jars could contain up to 72kg of cereals or 85lt of liquid, while the large flasks could contain up to 102lt of liquids (Fugazzola Delpino, 2002a:377). Ceramic boat models were also found (Fugazzola Delpino, 2002a:378), and permission was granted to sample one of these models for ORA.

Six ceramic vessels were analysed from this site, including a 2 large jars, 3 vases and one of the ceramic boat models. None of the absorbed residues provided significant quantities of lipid ($<4\mu\text{g g}^{-1}$), and their lipid profiles comprised mainly traces of medium chain length fatty acids, alcohols, and a wide series of alkanes (C22-C31) (Table 7.12). This was however not surprising given the exceptionally hard ceramic fabric, which was very difficult to drill during sampling, and therefore lacked the porosity required to encourage lipid absorption.

<i>Sample</i>	<i>Quantification ($\mu\text{g g}^{-1}$)</i>	<i>Lipid compounds identified</i>
MAR-01V	381	FAs: C _{14:0} -C _{18:0} , C _{22:0} , C _{16:1} , C _{18:1} ; Alkanes: unidentified; Alcohols: C18; Sterols: Cholesterol; Phthalates
MAR-01I	4	FAs: C _{14:0} -C _{18:0} ; Alkanes: C22, C23, C25-C29, C31; Alcohols: C18; Sterols: Cholesterol; Phthalates
MAR-02V	107	FAs: C _{16:0} -C _{18:0} , C _{22:0} -C _{24:0} , C ₂₆ , C _{18:1} ; Alkanes: C25, C27, C29, C31; Alcohols: C24, C26, C28, C30; Sterols: Campesterol, β -Sitosterol; ω-(<i>o</i>-alkylphenol)-octadecanoic acids (2 isomers); Phthalates
MAR-02I	2	FAs: C _{16:0} -C _{18:0} ; Alkanes: C22-C31; Phthalates
MAR-04V	2887	FAs: C _{14:0} , C _{15:0} , C _{16:0} , C _{18:1} , C _{18:0} , C _{20:0} , C _{22:0} -C _{24:0} , C _{26:0} ; Alkanes: unidentified; Alcohols: C32; MAGs: 1-Monopalmitin, 1-Monostearin; Sterols: Cholesterol; ω-(<i>o</i>-alkylphenol)-octadecanoic acids (2 isomers); Phthalates
MAR-04I	2	FAs: C _{16:0} , C _{18:0} ; Alkanes: C25-C31; Phthalates
MAR-05VA	799	FAs: C _{14:0} -C _{20:0} , C _{22:0} , C _{23:0} , C _{24:0} , C _{26:0} , C _{18:1} ; Alkanes: unidentified; ω-(<i>o</i>-alkylphenol)-octadecanoic acids (2 isomers); Phthalates

Table 7.12: GC-MS and HT-GC identification of lipid residues extracted from La Marmotta, Anguillara Sabazia (Lazio, Italy), run as TMS derivatives. [FAs: Fatty acids; MAGs: Monoacylglycerols]

Higher quantities of lipid were obtained from the visible residues sampled from the internal surfaces of pots MAR-01, MAR-02, MAR-04 and MAR-05 ($>106\mu\text{g g}^{-1}$; Table 7.12). These showed a wide range of saturated and unsaturated fatty acids, with medium to long carbon chain lengths. Cholesterol was identified in the visible residue obtained from the clay boat model (MAR-01V),

and traces of cholesterol were also present in its corresponding absorbed residue (MAR-01I), which could tentatively be identified as a ruminant fat given the presence of C_{15:0} and C_{17:0} fatty acids. The visible residue obtained from a large jar (MAR-02V), contained low levels of campesterol and β -sitosterol, which are indicative of a plant material. MAR-02V, MAR-04V, and MAR-05V also contained isomers of ω -(*o*-alkylphenol)-octadecanoic acids run as TMS derivatives, which are known to form as a result of prolonged heating of polyunsaturated fatty acids (Evershed *et al.*, 2008a)(Figure 7.20). In the absence of other biomarkers (e.g. isoprenoids and dihydroxy fatty acids) supporting an otherwise fish origin, the ω -(*o*-alkylphenol)-octadecanoic acids can perhaps indicate a plant origin. Plants are rich in polyunsaturated fatty acids, for example einkorn comprises considerable quantities of C_{18:3} (Price and Parsons, 1975), and could therefore be a precursor in the formation of ω -(*o*-alkylphenol)-alkanoic acids. This has already been suggested in Chapter 5, when discussing modern einkorn residues. A secure interpretation of the visible residues is not possible, but the presence of alcohols, alkanes, and phytosterols in particular, suggests a plant origin. The P/S ratio for samples MAR-04V and MAR-05V is greater than 6, also indicating a plant input, as suggested by Copley *et al.* (2001b), while the botanical remains at La Marmotta provide evidence for ample use of plant material, further supporting a plant origin. MAR-04V however also contained low amounts of cholesterol, which could be indicative of an animal fat. This may therefore, possibly indicate of a mixture comprising both animal fats and plant material.

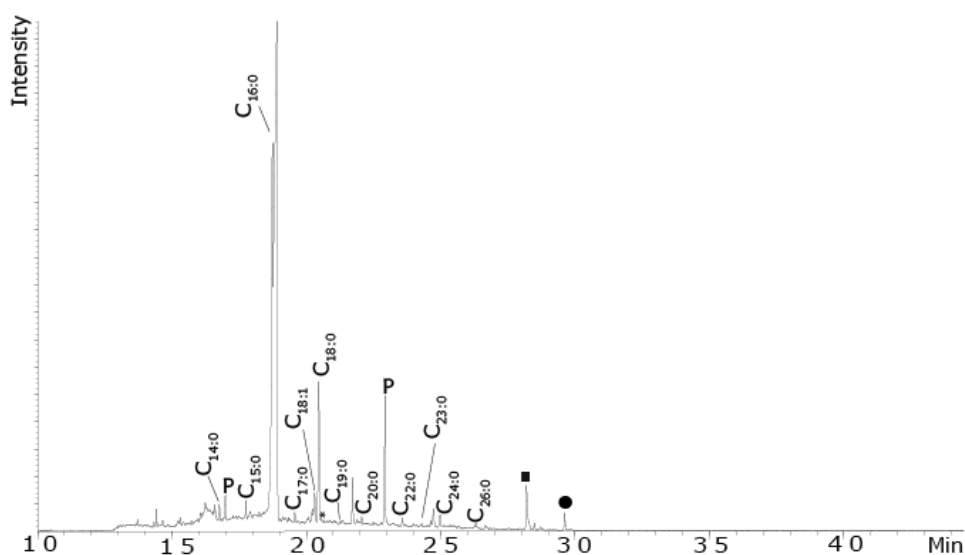


Figure 7.20: TIC of the visible residue sampled from a ceramic vase, sample MAR-05IV. [C_{x,y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; *: ω -(*o*-alkylphenol)-octadecanoic acid isomers; P: Phthalates; ■: Internal standard (C34); ●: Internal standard (C36)]

7.6 Skorba, Mġarr (Malta)

The Neolithic village of Skorba, situated on a hill overlooking Żebbiegħ in the north-west of Malta (Figure 7.21) is a rare occurrence on the Maltese islands, since only two other villages dating to this period have been uncovered to date, both of which are located on the sister island of Gozo. The valleys to the north and south of the site provided good arable soil, with a freshwater spring in the former and easy access to the coast. The village lies beneath a later three-apsed temple, which was subsequently modified and extended. Unfortunately, this structure impedes a full excavation of the Neolithic village extending beneath it, while ploughing over the years, has led to further destruction of the archaeological remains. However, what remains provides evidence for a flourishing Neolithic village. The site was excavated between 1961 and 1963.

The village is thought to have been established by 5000 BC, but the first settlers may have arrived earlier, as their first settlement is likely to have been on the shore, rather than 3km inland (Trump, 2008:34). The earliest structure identified on site is an 11m wall founded firmly in the rock, which was dated to the first level of occupation on the islands, the Għar Dalam (GħD) phase. The thickness of the wall varied between 60 and 80cm, and comprised two faces of stones filled with rubble in between (Trump, 1966:10). Domestic finds found along its northern side included fragments of daub, charcoal and carbonised grain. The daub fragments, some of which had been accidentally baked, could perhaps be interpreted as parts of the wall's own superstructure, or of huts enclosed by the wall, of which no other evidence has survived (Trump, 1966:10-11). The remains of a hut, also dated to the GħD phase by the pottery found in association, were also uncovered (Figure 7.21). Only the western walls survived making interpretation difficult, however it appears to be oval in shape, with relatively thin walls (0.7m), which could not have supported a superstructure, not even in mud-brick. At its centre was an inverted quern, possibly used as the base of a wooden column. Three human jaw fragments belonging to two children aged around 4.5 and 7, and fragments of a human skull were uncovered, and are thought to be associated with this hut (Trump, 1966:10-11). The only feature which could be attributed to the next phase, Grey Skorba (GSK), was a series of irregular largish stones set on sterile natural red clay, which were interpreted as the surviving walls of a ruined structure. This wall was found lying beneath the remnants of huts dated to the Żebbuġ (Żb) and Mġarr (Mġ) phases in the subsequent Temple Period (Trump, 1966:11, 14-15) (Figure 7.21). Of importance are two structures, one oval and the other D-shaped, dated to the Red Skorba (RSK) phase (Figure 7.21). Above the surviving stone, the building is thought to have been continued in mud brick. No hearth was found in either room, the walls were all built differently, and the bedrock appears to have been the only flooring. However,

large quantities of RSk pottery were found, and fragments of stylized female figurines were discovered in the North Room. Domestic animal bones were found in similar abundance to pottery, but bone-working was observed only on cow tarsals. Six goat skulls were also uncovered. Their cranium was complete, with long and fairly straight horns, but the facial bones from the upper edge of the orbits had been knocked away. No human bones were found, which eliminates a funerary function of the two rooms, while the irregularity of the floor and the absence of hearths makes domestic use unlikely. The most plausible explanation so far, is that these buildings served as shrines for votive offerings (Trump, 1966:11-14). Around 3500 BC, a standard early three-apsed temple was built on the site, and the extent to which it affected the village (whether all or just the part adjacent to it) is still unknown (Trump, 2008:34).

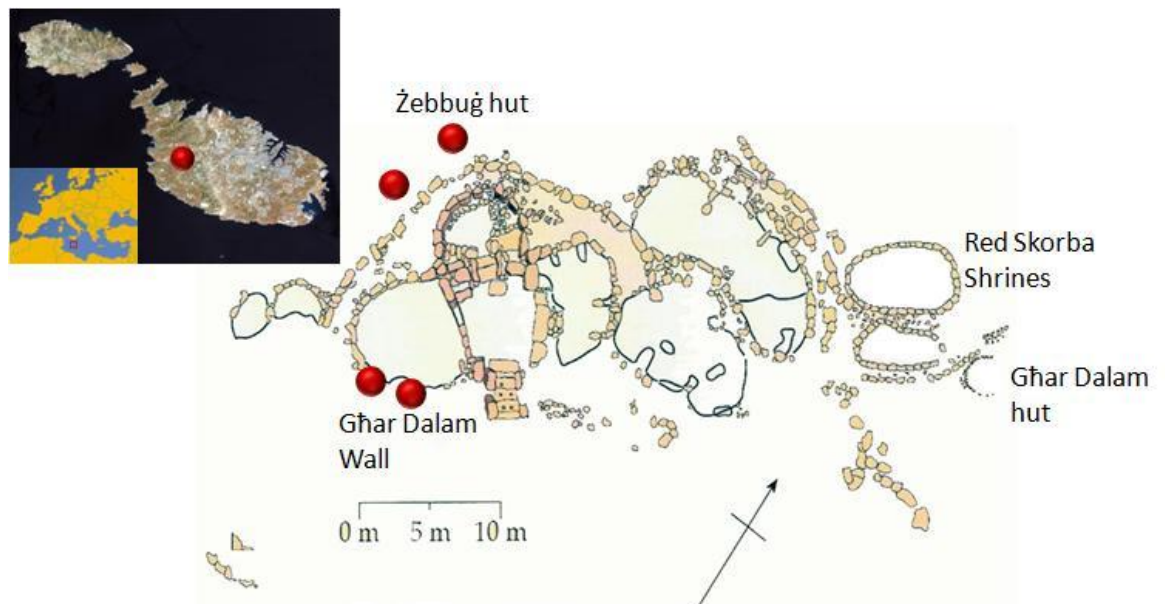


Figure 7.21: Plan of Skorba showing the main features pertaining to the Neolithic village, and the later apsed temple (Trump, 2002:157). [●: denote the location of the pottery vessels sampled]

A selection of pottery sherds were sampled from various locations within the site (Figure 7.21), dating to the four phases associated with the Early to the Middle Neolithic on the island (dates reported in Appendix C), with the aim of assessing changes in use over time. Unfortunately, only one sample (SKR-16I) out of 16 vessels analysed provided a significant residue, which therefore precludes further discussion (Table 7.2). MS analysis is consistent with a ruminant fat, and comprised abundant $C_{16:0}$ and $C_{18:0}$ fatty acids ($C_{18:0}$ being more abundant than $C_{16:0}$), including $C_{15:0}$ and $C_{17:0}$, indicating origin from microorganisms in the rumen, and short chain fatty acids. A series of diacylglycerols were also identified, although no trace of triacylglycerols were apparent when the sample was run on HT-GC. GC-c-IRMS showed an offset of -3.1‰, which is consistent with a

ruminant adipose residue (Figure 7.22). Similar to sample AZZ-13I, the C_{16:0} extracted from sample SKR-16I shows high $\delta^{13}\text{C}$ values, which, in the absence of C₄ vegetation, is indicative of a marine origin (Figure 8.6). This residue may potentially represent a mixture comprising ruminant adipose and marine oils, possibly introduced during sequential cooking episodes. However, in the absence of marine fish biomarkers and fish bones in the archaeological deposit, a marine input cannot be securely suggested. Two long-chain ketones, consistent with the condensation of fatty acids during heating were also present, and indicative of cooking (Evershed *et al.*, 1995b; Raven *et al.*, 1997)

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
SKR-16I	5.44	FAs: C _{10:0} -C _{16:0} , C _{18:0} ; Alkanes: C15, C17-C20, C23, C29, C30, C31; Alcohols: C12, C13, 14; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34, 1,2-Distearin, 1,3-Distearin; Ketones: C31, C33; Phthalates

Table 7.13: GC-MS and HT-GC identification of lipid residues extracted from Skorba (Malta), run as TMS derivatives. [FA: Fatty acids; DAG: Diacylglycerols; D_x: Number of carbon atoms in the DAG]

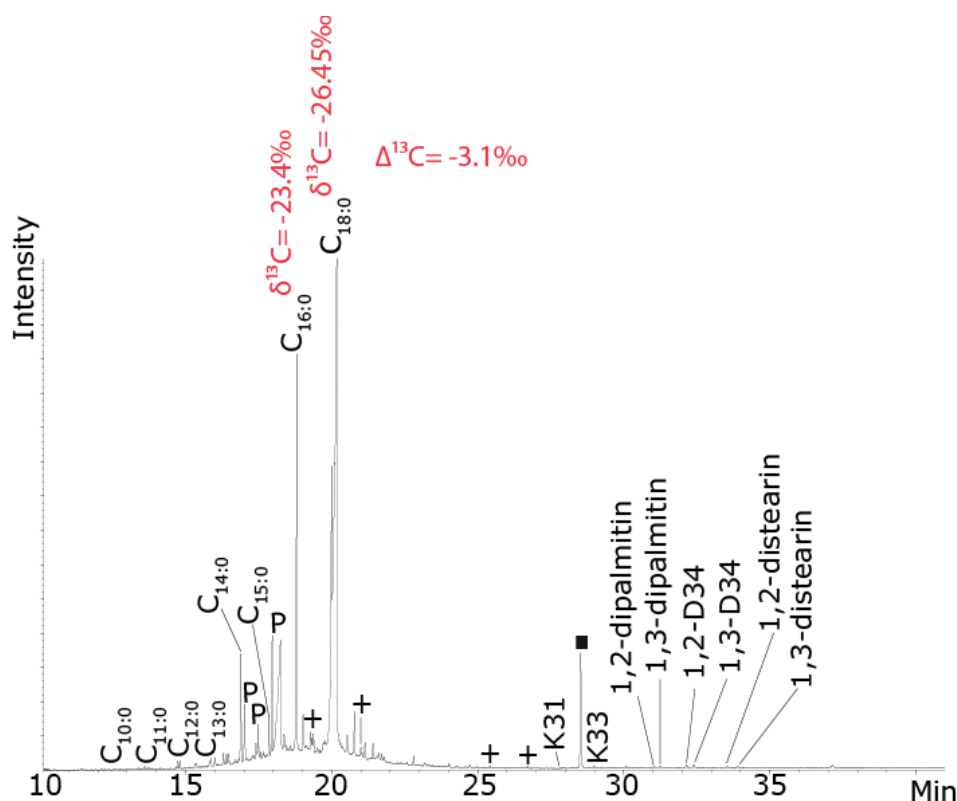


Figure 7.22: TIC of sample SKR-16I. The $\delta^{13}\text{C}$ measurements of the C_{16:0} and C_{18:0} fatty acids are indicated ($\Delta^{13}\text{C} = -3.1\text{‰}$). [C_{x,y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; A: Alkanes; K: Ketones; P: Phthalates; ■: Internal standard (C34)]

The rest of the samples analysed showed negligible amounts of residue absorbed within the ceramic walls, and comprised mainly traces of fatty acids and alkanes, probably introduced from the burial environment, as they are consistent in lipid profile and quantification with the five external sherds analysed (Appendix D). The pottery sherds analysed were too fragmented to allow a clear identification of the pot shape, which could be indicative of function. Trump (1966:21-35) provides a detailed description of the ceramics uncovered at Skorba (briefly discussed in Chapter 2), which could have been used for a variety of purposes, including cooking, storage and serving. The floral and faunal assemblages at Skorba have already been discussed in Chapter 2. Briefly, ovicaprids, pigs and cow bones were retrieved (Trump, 1966, Appendix III; Borġ, 2008), as well as scant botanical remains of barley, wheat and lentils (Trump, 1966, Appendix IV). Interpreting the function of Impressed Wares at Skorba is therefore not straightforward, since although loss of lipid residue due to degradation is likely, this could also result from the function of the pots (e.g. storage), or processing of plant material. Although very few plant remains have been documented from this site, this could be due to the excavation technique applied, since although the sediment was sieved, floatation was not used, and plant fragments (and equally fish bones) could therefore have been lost.

7.7 Nakovana Cave (Dalmatian Coast, Croatia)

Nakovana Cave is situated around 370m above sea level, and can be reached from the coast. It overlooks the Adriatic Sea, and is situated on a high ridge on the Peliješac peninsula on Croatia's Dalmatian coast (Figure 7.23). The entrance chamber resembles a deep rock shelter, with a terrace on the outside (Forenbaher and Kaiser, 2006) (Figure 7.23). Between 1999 and 2003, five excavation campaigns were carried out at Nakovana cave, as part of the Nakovana Project. Figure 7.23 shows the plan and section drawing of the cave, and the sectors excavated are outlined. Excavations uncovered a long stratigraphic sequence, comprising 11 occupation phases dating from the Early Neolithic (6000 BC) to the Illyrian Iron Age (1 AD) (Forenbaher and Kaiser, 2006). There are no obvious long breaks in the stratigraphy, but perhaps many short to medium-term phases of abandonment (Forenbaher, 2010, pers. comm.).

The cave is perhaps more widely known as a Hellenistic cave sanctuary (Forenbaher and Kaiser, 2006), however it is thought to have been occupied by pastoral communities from the Early Neolithic, which appears to have been the case at other similar contemporary sites in the area (Forenbaher, 2010, pers. comm.). Osteological remains of sheep and goat dominate the faunal assemblage. The faunal remains are still under study, but a dairying economy has been tentatively

suggested, in particular given the evidence for pastoral activities carried out at Pupičina, in Istria (Miracle and Forenbaher, 2005). Botanical remains are scarce, and despite the coastal location of the cave, few marine shells have been identified but no fish bones were retrieved. Floatation techniques were rigorously applied and 5L of sediment from each context were floated using 6mm sieves, which would have picked up any small, fragmented botanical and marine remains (Forenbaher, 2010, pers. comm.). The paucity of the botanical evidence retrieved can be explained in terms of the rocky and mountainous surrounding environment, which provides some areas for pasture, but is unsuitable for cultivation (Forenbaher, 2010, pers. comm.). A similar scenario was identified during the Early Neolithic at Grapčeva Cave, on the neighbouring island of Hvar, (Borojević *et al.*, 2008). The presence of open air, Neolithic settlements is tentatively identified through lithic scatters located a few kilometres below the cave, which comprise flat areas covered with arable soils. Hence, it is likely that these Neolithic communities cultivated crops and herded animals, using the cave, which is big enough to hold a herd of several dozen animals, as a shelter (Forenbaher, 2010, pers. comm.).

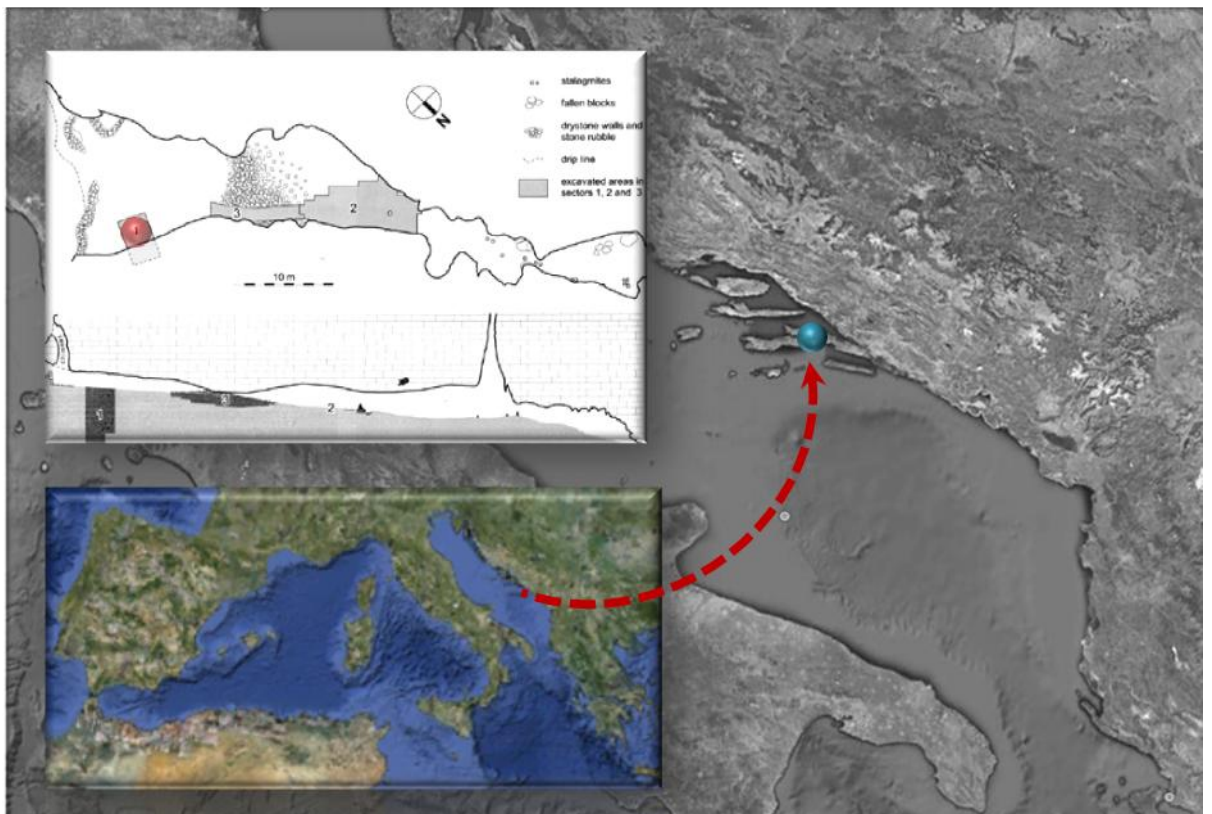


Figure 7.23: Plan and section of the areas excavated at Nakovana Cave (Plan of site from Forenbaher and Kaiser, 2006). [●: denotes the location of the pottery vessels sampled]

The pottery retrieved from Nakovana was generally very fragmented. The most common shapes present were jars and globular bowls (Figure 7.24) and given their fragmented state, they can only be tentatively identified through their dimensions. The sherds selected for ORA were retrieved from deep sounding deposits from Sector 1, situated at the cave entrance (Figure 7.23). They are fairly coarse and are likely to have been produced at household level. Most of the vessels are plain, with simple rounded bases, and small to medium in size (the diameter at the mouth is generally around 20cm). The assemblage is dominated by plain, coarse domestic pottery, which points towards a utilitarian purpose, primarily for cooking (sooting is sometimes present), storage, or serving (Forenbaher, 2010, pers. comm.). The samples analysed were obtained from Early to Middle Neolithic layers, comprising Impressed Wares, Plain Wares, Danilo Wares and Vela Luka Polychrome Wares (see Appendix C).

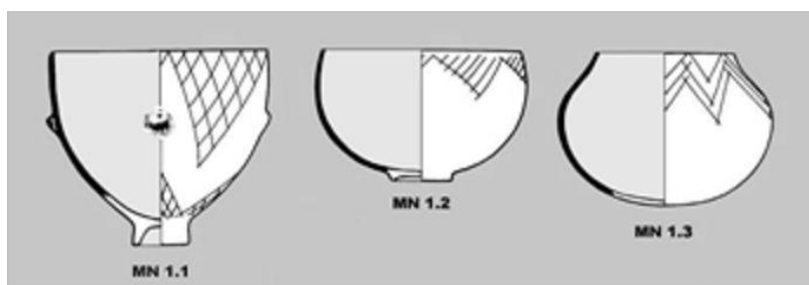


Figure 7.24: Illustrations of the most common Neolithic pot shapes (Courtesy of S. Forenbaher).

Lipid preservation at Nakovana cave was remarkably good. A significant amount of lipid was extracted from 15 of the 17 vessels analysed (on average $26\mu\text{g g}^{-1}$) (Table 7.2). Table 7.14 shows the range of lipid components identified in the sherds analysed after trimethylsilylation. Most residues contained medium to long chain, saturated and unsaturated fatty acids. Odd-numbered fatty acids were also present in some of the samples, particularly, $\text{C}_{15:0}$ and $\text{C}_{17:0}$. This fatty acid profile, together with the presence of cholesterol and its degradation product cholesta-3,5-diene, as well as mono- and diacylglycerols indicative of triacylglycerol hydrolysis, strongly suggest a ruminant fat. HT-GC identified a wide range of triacylglycerols in only one vessel, NAK-04I (Figure 7.25). Long-chain ketones were also identified in eight vessels, including NAK-07I, NAK-08I and NAK-12I (Table 7.14), which are consistent with the condensation of absorbed fatty acid moieties during heating (Evershed *et al.*, 1995b; Raven *et al.*, 1997), hence good indicators of cooking events.

Using GC-c-IRMS analysis, pot NAK-04I was tentatively identified as a ruminant adipose fat (Figure 7.26). This interpretation was based on its $\delta^{13}\text{C}_{16:0}$ value which falls within the range denoting

terrestrial fats, while its $\Delta^{13}\text{C}$ measurement, -0.9‰ precludes its identification as a non-ruminant fat. The $\Delta^{13}\text{C}$ value for NAK-04I also tentatively suggests its origin from domestic ruminant animals rather than deer, since the $\Delta^{13}\text{C}$ measurements at $\pm 1\text{‰}$ standard deviation obtained for deer adipose were much smaller, ranging from -1.9‰ to -4.3‰ . NAK-07I, NAK-08I and NAK-12I had $\Delta^{13}\text{C}$ values less than -4.6‰ , and were identified as dairy residues, thus providing direct evidence for the use of pastoral products at Nakovana. Evidence for dairy products was not identified in pottery dating to the first occupation levels at Nakovana (Impressed Wares), however the sample size for this phase was relatively small, and none of the early Impressed Wares analysed contained sufficient $\text{C}_{16:0}$ and $\text{C}_{18:0}$ for GC-c-IRMS analysis. The earliest presence of dairying goes back to the Plain Ware Phase (NAK-07I), while NAK-08I and NAK-09I belong to the following Danilo Ware Phase. More samples are required to test for the presence of dairying in the earliest layers.

Sample	Quantification ($\mu\text{g g}^{-1}$)	Lipid compounds identified
NAK-02I	15	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C17, C25-C29, C31, C33; Alcohols: 14, 18, 28; Sterols: Cholesterol, β -sitosterol; Phthalates
NAK-04I	16	FAs: $\text{C}_{14:0}$ - $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C29, C31; Alcohols: C14, C18, C24, C26, C28, C30; MAGs: 2-Monopalmitin, 1-Monopalmitin, 2-Monostearin, 1-Monostearin; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34, 1,2-Distearin, 1,3-Distearin; TAGs: C40, C42, C46, C48, C50, C52; Sterols: Cholesterol; Phthalates
NAK-05I	8	FAs: $\text{C}_{14:0}$, $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C20, C23, C25, C29, C31; Alcohols: C18, C20; MAGs: 1-Monomyrstin; 1-Monopalmitin, 1-Monostearin; Ketones: C29, C31, C33, C35; Phthalates
NAK-07I	19	FAs: $\text{C}_{14:0}$ - $\text{C}_{18:0}$; Ketones: C31; Phthalates
NAK-08I	247	FAs: $\text{C}_{12:0}$ - $\text{C}_{22:0}$, $\text{C}_{24:0}$, $\text{C}_{26:0}$, $\text{C}_{18:1}$; Alkanes: C25-C29; Alcohols: C14, C16, C18, C24, C26, C28, C30; Ketones: C29, C31, C33, C35; Phthalates
NAK-09I	33	FAs: $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C18, C19; Alkenes: unidentified; Alcohols: C14, C18, C24, C28, C30, C32; Ketones: C29, C31, C33, C35; Sterols: β -sitosterol; Phthalates
NAK-10I	14	FAs: $\text{C}_{14:0}$ - $\text{C}_{16:0}$, $\text{C}_{18:0}$; Alkanes: C18, C22-C31, C33; Alcohols: C18; Phthalates
NAK-12I	67	FAs: $\text{C}_{12:0}$ - $\text{C}_{16:0}$, $\text{C}_{18:0}$ - $\text{C}_{24:0}$, $\text{C}_{16:1}$, $\text{C}_{18:1}$; Alkanes: C19-C23, C25-C27, C29, C32; Alcohols: C12, C14, C18; Ketones: C29, C31, C32, C33, C35, C37; Sterols: Squalene, Cholesta-3,5-diene, Cholesterol; Dehydroabietic acid; Phthalates

Table 7.14: GC-MS and HT-GC identification of lipid residues extracted from Nakovana Cave (Croatia), run as TMS derivatives. [FA: Fatty acids; MAG: Monoacylglycerols; DAG: Diacylglycerols; TAG: Triacylglycerols]

A wide range of alkanes and alcohols were identified in most residues, which together with the presence of β -sitosterol in vessels NAK-02I, NAK-09I, and NAK-14I, a phytosterol which is a major constituent of plant residues (and was consistently identified in the plant experiment described in Chapter 5), strongly suggests the presence of plant material. Phytosterols were not identified in the four external surface residues analysed (see below), suggesting that it originated within the

absorbed residues. The chromatograms obtained for NAK-02I, NAK-09I and NAK-14I are consistent with a ruminant fat identification, but the presence of β -sitosterol indicates that perhaps these vessels contained a mixture of animal and plant products (e.g. a stew), or perhaps, were used in sequential cooking episodes comprising these two products. Although not abundant, plant remains have been identified at Nakovana, and the use of the cave site as an animal shelter does not preclude the use of plants. NAK-08I, in particular, contains doublets of long-chain alcohols and long-chain fatty acids, which may indicate the presence of a degraded wax ester (Figure 7.27).

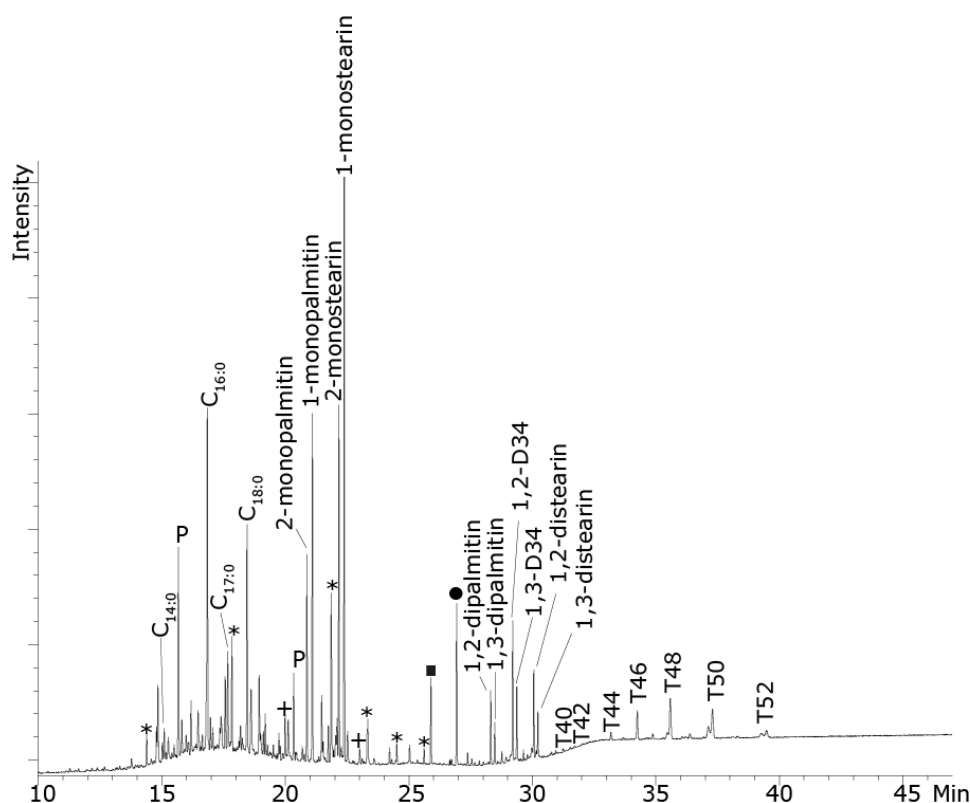


Figure 7.25: Partial HT-GC of NAK-04I, of a coarse ware globular jar/bowl dating to the Early Neolithic Plain Wares Phase. It was identified by GC-c-IRMS as a ruminant adipose residue. [C_{x,y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C23-C29); *: Alcohols (C14-C30); T: Triacylglycerols; P: Phthalates; ■: Internal standard (C34)]

The external surfaces of four vessels (NAK-03E, NAK-07E, NAK-11E and NAK-15E) were analysed to test for possible migration of contaminants from the burial environment. The low quantities of lipid, and the profiles obtained, which comprised mainly a series of alkanes, alcohols and medium-chain length fatty acids, are typically found in soil (Heron *et al.*, 1991a). However, the external surface residues of samples NAK-03E and NAK-15E were found to contain a significant quantity of lipid (9 and 18 $\mu\text{g g}^{-1}$, respectively). Their lipid profiles were very similar in quantification and

content to their corresponding interior surface residues, which were tentatively identified as ruminant fats. This suggests that perhaps the pot contents had over spilled during processing, or that the porous nature of the fabric encouraged the absorption of lipids across the ceramic wall, which is known to occur prior to saturation of the ceramic fabric, in the absence of sealants. Low quantities of dehydroabietic acid (*Pinaceae* resin biomarker) were identified in one vessel (NAK-12I). As already suggested above, *Pinaceae* resin is known to have been used as a sealant, thus increasing the impermeability of pots to liquids (e.g. Romanus *et al.*, 2009). Although this biomarker was not present in any of the external surface residues, hence suggesting that it is indeed a component of the residue extracted from the internal surface of vessel NAK-12, it was found in just one sample, and pine groves are located a few kilometres away from the site (Forenbaer, 2011, pers. comm.). Therefore its presence can signify remnants of a *Pinaceae* resin sealant, processing of *Pinaceae* resin within the ceramic vessel (although perhaps this would have contributed higher quantities of dehydroabietic acid), migration from the burial environment, or it could also have been introduced as an artefact from instrumental contamination.

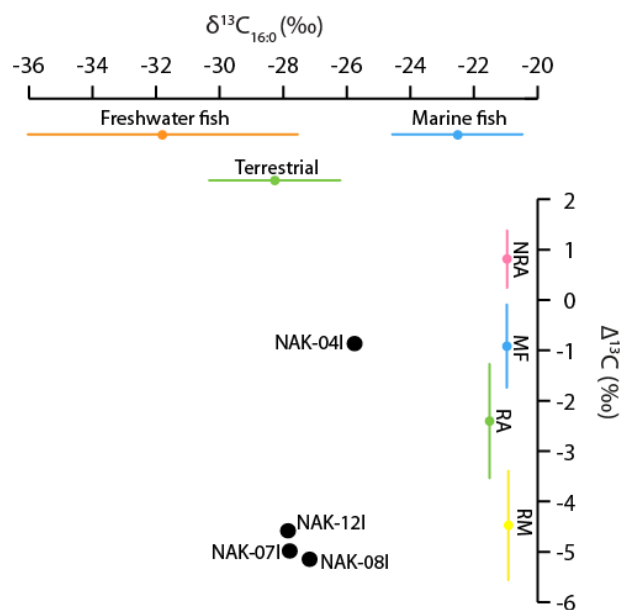


Figure 7.26: GC-c-IRMS results: Nakovana Cave (Croatia). [$\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\%$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

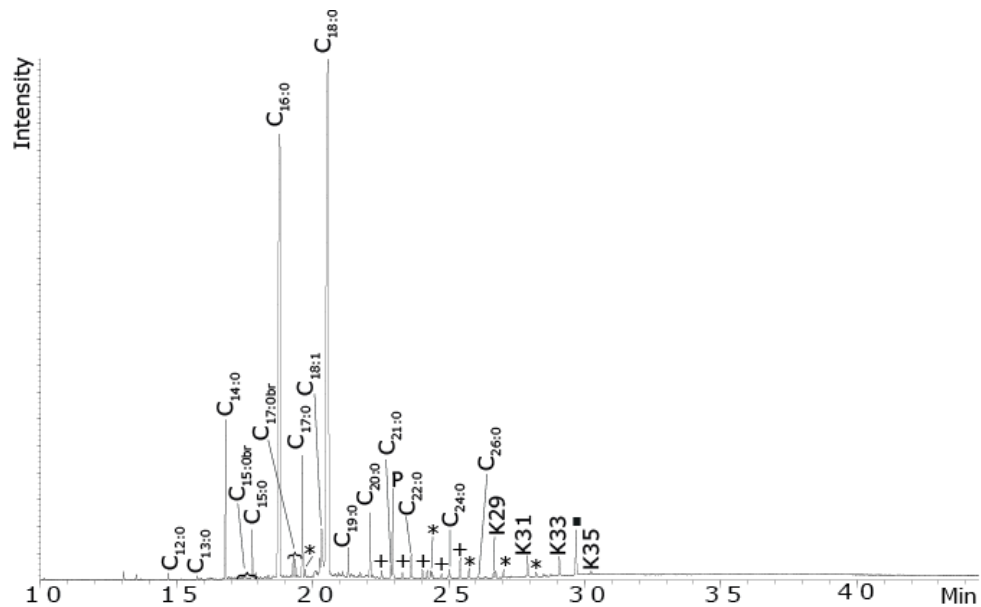


Figure 7.27: TIC of sample NAK-081, identified by GC-c-IRMS as a dairy residue, however, the long series of alkanes, long-chain alcohols and fatty acids also suggest a plant input. [C_{x:y}: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C25-C29); *: Alcohols (C18-C30); K: Ketones; P: Phthalates; ■: Internal standard (C34)]

The ceramic repertoire was too fragmented to allow any further observations related to pot content, form and fabric. All the vessels analysed were classified as coarse wares, but their shape could not be securely identified, and were catalogued as being either globular jars or bowls. They were utilitarian in function, with the presence of soot and the identification of long-chain ketones providing evidence that they were used for cooking. ORA results have identified the presence of ruminant adipose and dairy products, with a possible input of plant commodities, attesting to mixtures of food products, or sequential cooking episodes.

7.8 Can Sadurní, Begues (Barcelona)

Pottery sherds dating to the earliest Neolithic phases in Catalonia were obtained from the cave site of Can Sadurní. The cave is located at the side of a small hill, about 420m above sea level, on the calcareous Garraf Massif overlooking the plain of Begues, in Barcelona (Antolín and Buxó, 2011; Saña *et al.*, submitted) (Figure 7.28). It is oriented towards the south-east, and comprises an outdoor terrace which offers good protection from the wind (Saña *et al.*, submitted). Excavations over the past 30 years have uncovered 21 stratigraphic layers, which chronologically span from around 11,000 cal. BC to the last century (Antolín and Buxó, 2011). The Early Neolithic phases are

attested in layers 10 to 18, and ORA analysis has focussed mainly on ceramic vessels obtained from strata 17 and 18. Details for the pottery samples analysed are provided in Appendix C. Figure 7.28 shows the stratigraphy uncovered at Can Sadurní.

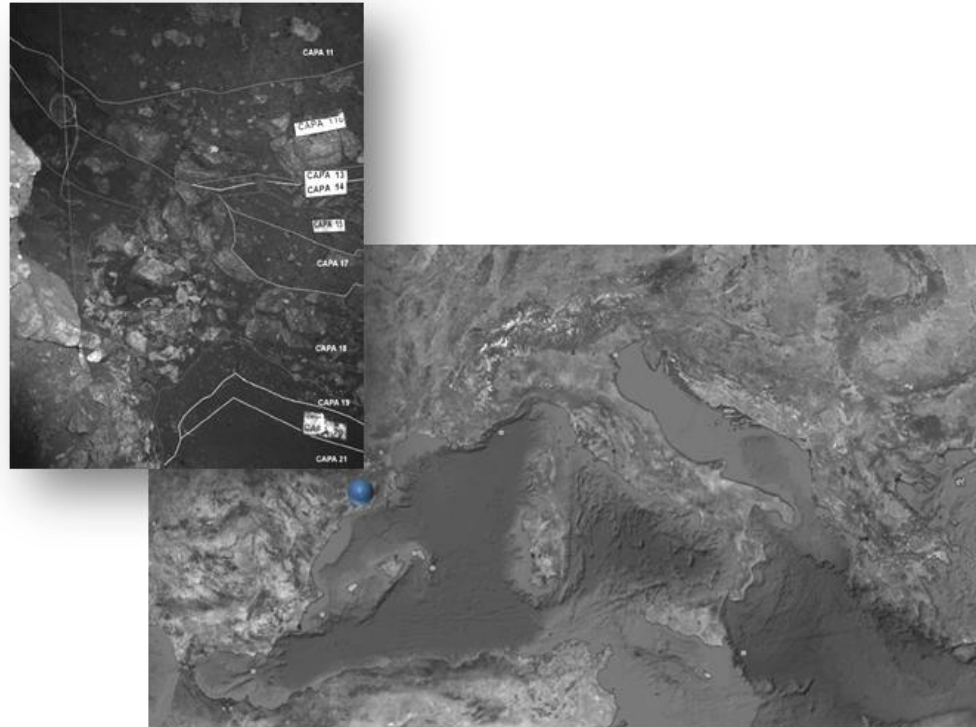


Figure 7.28: The stratigraphy uncovered at Can Sadurní. Layers 10 to 18 represent the early Neolithic phase (From Edo *et al.*, 2011).

The earliest Neolithic phase at Can Sadurní is represented in Layer 18, which also provided one of the earliest dates for the appearance of the Neolithic in north-eastern Iberia (5475-5305 cal. BC) (Blasco *et al.*, 2005). DNA results have shown that between 7 and 11 individuals had been buried here, making Layer 18 unique as an Early Neolithic funerary deposit (Antolín and Buxó, 2011). The skeletal remains were accompanied by large ceramic vessels filled with carbonised grains, unused mill stones, sheep extremities, and plaques made of sea shells (Blasco *et al.*, 2005; Edo *et al.* 2011). More than half of the ceramic vessels recovered were decorated, predominantly using cardial impressions (80%), but impressions with cordons (15%) and incised motifs (5%) were also executed (Blasco *et al.*, 2005), and comprised vessel forms which ranged from shallow, deep to very deep vessels (Edo *et al.*, 2011). Furthermore, it appears that the pottery vessels had been broken *in situ*, and signs of fire were also observed on most of the archaeological material retrieved, making this assemblage highly significant in terms of its social aspect (Antolín and Buxó, 2011). Around 60,000 remains from cereal grains were collected by floatation from Layer 18,

which comprised 14 different taxa. Domestic cereals included barley and wheat (e.g. *H. vulgare*, *T. aestivum*, *T. dicoccum*, and *T. monococcum*), five synanthropic plants were also present, and four wild species, including *Arbutus unedo*, *Pinus* sp., *Quercus* sp., and *Rubus idaeus* (Antolín and Buxó, 2011). Layer 17 corresponds to the Epicardial phase dating to 5227-4709 cal. BC, and the material retrieved included seeds, grinders, lithics, ceramics (mainly decorated with cardial impressions) and faunal remains (Edo *et al.*, 2011). The latter make up 70% of the material excavated from Layer 17, dominated by ovicaprids, and, in order of importance, pig and cattle. Wild boar and rabbit were also identified (Edo *et al.*, 2011).

A detailed investigation carried out by Saña *et al.* (submitted) on 5870 faunal remains recovered from Can Sadurní showed changes in animal management from the Epipalaeolithic to the Middle Neolithic. While Epipalaeolithic assemblages were dominated by rabbit (98%) and remains of aurochs (0.3%), deer (0.5%), boar (0.1%), ibex (0.7%) and roe deer (0.01%), Neolithic layers show a relatively high overall percentage of domestic animal remains (71%), the majority being ovicaprids (34%) (Saña *et al.*, submitted). The number of domestic animals increases during the Epicardial (89%) and Middle Neolithic (92%), during which there is a relative increase in the consumption of cattle and pigs, and a significant decrease in the hunting of rabbits, but deer hunting was observed to increase (Saña *et al.*, submitted). The culling profile obtained from this study suggests an initial focus on dairying, with meat production becoming increasingly important during the Post Cardial phases, and the cave-site being more permanently used to house animals. It has also been suggested, that apart from human consumption, the vast quantities of plant remains retrieved from the cave deposits may have been used as fodder for the animals (Saña *et al.*, submitted). Interestingly, very few fish remains were found, despite the floatation method used to screen the sediment (Antolín, 2011, pers. comm.), and the location of the site being within 15km of the Mediterranean Sea.

Out of the 31 ceramic vessels analysed, significant residues were obtained from 13 pots, that is 42% of the vessels sampled. Of these, eight dated to the Epicardial phase (Layer 17; 15 vessels analysed), and only three were associated with the earlier Cardial deposits (Layer 18; 12 vessels analysed) (Table 7.2). The remaining two vessels displaying a significant lipid residue date to the Post Cardial phase (Layers 10 and 13). One soil sample from Layer 17 was available for analysis. GC analysis showed trace amounts of fatty acids with chain lengths of between 16 and 20 carbon atoms, alkanes with 25 to 33 carbon chain lengths, and alcohols (namely C₂₆, C₂₈ and C₃₀), which are generally found in soils (Heron *et al.*, 1991a; Jambu *et al.*, 1993; Ambliès *et al.*, 1994). The external surfaces of two vessels (CNS-02E and CNS-10E) were analysed, and their gas

chromatograms were found to be consistent with uptake from the depositional environment. Their lipid yield was very low ($<3\mu\text{g g}^{-1}$), showing negligible contamination of the interior surfaces during burial. Another external residue (CNS-01E) analysed produced anomalous results, which are discussed below.

Table 7.15 shows the different classes of lipids identified in the ceramic vessels following trimethylsilylation. Most residues contained saturated and unsaturated fatty acids, with $\text{C}_{16:0}$ and $\text{C}_{18:0}$ being the most common and abundant, while chain lengths varied from short to long chains and odd-numbered fatty acids were also present. A wide range of alkanes and alcohols were identified in most of the residues, which when coupled with the presence of palmitate wax esters identified in 4 of the samples (Table 7.15), suggest perhaps a plant contribution to the residue. However, phytosterols are absent, and the only sterol detected was cholesterol and its dehydration product cholesta-3,5-diene, which is indicative of an animal fat. The hydrolysis products of triacylglycerols, mono- and diacylglycerols were frequently identified, and using HT-GC, traces of triacylglycerols were identified in two samples (CNS-10I and CNS-11I). Long-chain ketones were also identified in five vessels (CNS-01, CNS-03, CNS-10, CNS-11 and CNS-13), and following Raven *et al.*'s (1997) criteria, they are all associated with the condensation reactions of absorbed fatty acids moieties during heating, and therefore indicative of cooking.

Figure 7.29 plots the results obtained for six residues submitted for GC-c-IRMS analysis. Samples CNS-01E and CNS-11I were identified as ruminant adipose residues, sample CNS-03I plots securely within the dairy products category (at $\pm 1\%$ standard deviation), while samples CNS-06I, CNS-10I and CNS-13 plot within the area of overlap between ruminant adipose and ruminant milk fats. GC-MS analysis for these three residues identified short chain fatty acids, which are indicative of a dairy residue, as well as fatty acids with an odd number of carbon atoms (namely $\text{C}_{15:0}$ and $\text{C}_{17:0}$), indicative of a ruminant fat (Dudd *et al.* 1998). Their interpretation as originating from cow, sheep or goat adipose can be tentatively excluded, since the $\Delta^{13}\text{C}$ value ranges for the latter were observed to plot between -1.1% and -2.6% , while the $\Delta^{13}\text{C}$ values obtained for CNS-06I, CNS-10I and CNS-13 are less than -3.3% . This therefore suggests a ruminant dairy or deer adipose origin. A deer origin cannot be excluded since deer bones are present throughout the whole of the Neolithic at Can Sadurní (Saña *et al.*, submitted). However, the presence of short chain fatty acids, and the low occurrence of deer bones, which comprised less than 3% of the Early Neolithic faunal assemblage analysed (Saña *et al.*, submitted), tentatively suggest that samples CNS-06I, CNS-10I and CNS-13, are perhaps more likely to be dairy residues. The $\text{C}_{16:0}$ fatty acid in sample CNS-06I has a high $\delta^{13}\text{C}$ value, which in the absence of C_4 plants, is indicative of a marine input. As already

suggested for sample AZZ-13I, this residue may represent a mixture between a ruminant dairy fat and marine oil; however, marine biomarkers were not securely identified (see below), which precludes a confident indication of a marine input. Samples CNS-11I and CNS-01E had $\Delta^{13}\text{C}$ values of -2.4‰ and -1.9‰ , respectively; hence while they are confidently identified as ruminant adipose residues, it is not possible to suggest whether they originated from wild deer or domestic ruminants.

Ketones were identified in pots CNS-03I, CNS-10I and CNS-13, which are indicative of heating. The identification of ω -(*o*-alkylphenol)-octadecanoic acid isomers in pot CNS-06I is more consistent with a plant input, in view of the absence of other marine biomarkers (isoprenoids and dihydroxy fatty acids), as suggested for the similar results obtained from La Marmotta. Furthermore, wax esters have been identified in pots CNS-10I and CNS-11I (Figure 7.30) and in the former, doublets of fatty acids and alcohols also occurred, which indicate the degradation of wax esters into its constituents. These are also associated with plant material. Together with the evidence for wax esters and alcohols, it could therefore be suggested that samples CNS-06I, CNS-11I and CNS-10I may be indicative of a mixture of ruminant adipose/dairy and plant material.

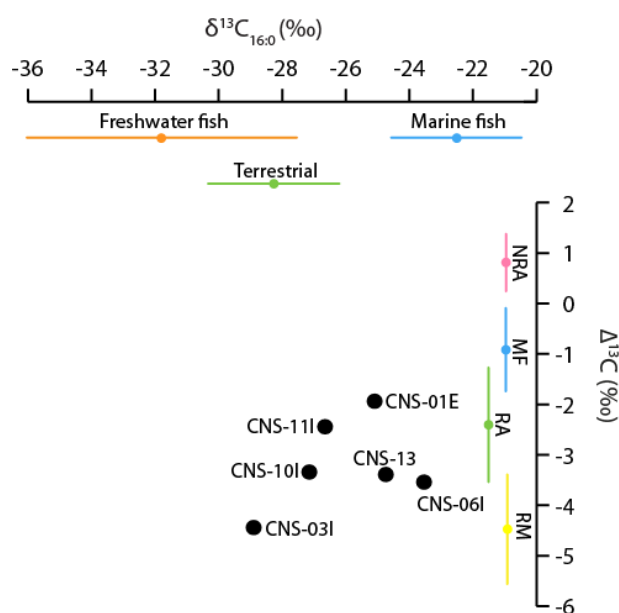


Figure 7.29: GC-c-IRMS Results: Can Sadurní (Barcelona). [$\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\text{‰}$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

<i>Sample</i>	<i>Quantification ($\mu\text{g g}^{-1}$)</i>	<i>Lipid compounds identified</i>
<i>CNS-01II</i>	Trace	FAs: C _{14:0} , C _{16:0} , C _{18:0} ; Alkanes: C18-C24, C27, C29, C30-C36; Alcohols: C18; Ketones: C31, C33; Sterol: Cholesterol; Phthalates
<i>CNS-01E</i>	33	FAs: C _{9:0} -C _{20:0} , C _{24:0} -C _{28:0} ; Alkanes: C16-C21, C24-C34; Alcohols: C12, C14, C24, C26, C28, C30, C32, C34; Ketones: C31, C32, C33, C35, C37; WE: C40; Phthalates
<i>CNS-02I</i>	80	FAs: C _{12:0} , C _{13:0} , C _{14:0} , C _{15:0} , C _{16:0} , C _{17:0} , C _{18:0} ; Alkanes: C29, C31; Alkenes: unidentified; Ketones: C33; Sterols: Cholesterol; Phthalates
<i>CNS-03I</i>	9	FAs: C _{12:0} , C _{14:0} , C _{15:0} , C _{16:0} , C _{17:0} , C _{18:0} ; Alkanes: C16-C33; Alcohols: C12; Ketones: C33, C35; DAGs: 1,3-Dipalmitin; Phthalates
<i>CNS-05I</i>	7	FAs: C _{16:0} , C _{18:0} ; Alkanes: C24-C33; Phthalates
<i>CNS-06I</i>	460	FAs: C _{12:0} , C _{14:0} , C _{15:0} , C _{16:1} , C _{16:0} , C _{18:1} , C _{18:0} , C _{19:0} , C _{20:0} , C _{22:0} , C _{23:0} , C _{24:0} , C _{25:0} , C _{26:0} ; Alkanes: C16, C23-C33; ω-(<i>o</i>-alkylphenol)-octadecanoic acid; Phthalates
<i>CNS-07I</i>	Trace	FAs: C _{16:0} , C _{18:0} ; Alkanes: C18-C30, C32, C33; Ketones: C31, C33, C35; Phthalates
<i>CNS-10I</i>	137	FAs: C _{8:0} -C _{18:0} , C _{22:0} , C _{14:1} , C _{16:1} , C _{18:1} ; Alkanes: C19, C22, C24, C25, C27-C31, C36, C38, C39; Alcohols: C14, C20; Ketones: C31; MAGs: 1-Monomyrstin, 1-Monopalmitin, 1-Monostearin; DAGs: 1,2-D30, 1,2-Dipalmitin, 1,3-D34, 1,2-Distearin, 1,3-Distearin; TAGs: C42, C48; WE: C34, C36; Sterols: Cholesterol, Cholesta-3,5-diene; Phthalates
<i>CNS-11I</i>	414	FAs: C _{12:0} -C _{17:0} , C _{18:0} ; Alkanes: C17, C18, C22-C33, C36; Alcohols: C18; Ketones: C29, C31, C33; DAGs: 1,3-D30, 1,3-Dipalmitin, 1,3-D34; TAGs: C42, C44, C46, C48, C50; Sterols: Cholesta-3,5-diene; WE: C34, C36; Phthalates
<i>CNS-13</i>	11	FAs: C _{11:0} -C _{18:0} ; Alkanes: C18, C23-C25, C27-C33; Ketones: C31, C33, C35; DAGs: 1,3-D34; WE: C34, C36; Phthalates
<i>CNS-14I</i>	Trace	FAs: C _{13:0} -C _{16:0} , C _{18:0} ; Alkanes: C17-C33, C36, C40; Ketones: C33, C35; Phthalates
<i>CNS-23IA</i>	389	FAs: C _{12:0} -C _{24:0} , C _{16:1} , C _{18:1} , C _{26:0} ; Alkanes: C32; Alcohols: C14, C16, C18, C26; MAGs: 1-Monopalmitin, 2-Monostearin, 1-Monostearin; DAGs: 1,2-D30; Sterols: Cholesterol
<i>CNS-23IB</i>	93	FAs: C _{12:0} -C _{32:0} , C _{14:1} , C _{16:1} , C _{18:1} ; Alkanes: unidentified; Alcohols: C14, C16, C18, C20, C24, C26, C30, C32, C34; MAGs: 2-Monostearin; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,3-D34, 1,2-Distearin; Phthalates
<i>CNS-24I</i>	20	FAs: C _{12:0} -C _{20:0} , C _{22:0} -C _{32:0} , C _{14:1} , C _{16:1} , C _{18:1} ; Alkanes: unidentified; Alcohols: C14, C18, C28; Ketones: C33, C35; DAGs: 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34; Sterols: Cholesterol; Phthalates
<i>CNS-29I</i>	88	FAs: C _{14:0} , C _{16:0} , C _{18:1} , C _{18:0} , C _{28:1} , C _{30:1} , C _{32:0} ; Alkanes: unidentified; Alcohols: C14, C18, C28; Ketones: C29, C31, C33; MAGs: 2-Monopalmitin, 2-Monostearin; DAGs: 1,2-D30, 1,3-D30, 1,2-Dipalmitin, 1,3-Dipalmitin, 1,2-D34, 1,3-D34; Sterols: Cholesterol; Phthalates
<i>CNS-30IA</i>	66	FAs: C _{14:0} -C _{20:0} , C _{22:0} -C _{26:0} , C _{28:0} -C _{33:0} , C _{16:1} , C _{17:1} , C _{18:1} ; Alkanes: C29, C31; Alcohols: C28, C30; Ketones: C29, C31, C33, C35; Sterols: Cholesterol; Phthalates
<i>CNS-30IB</i>	Trace	FAs: C _{14:0} -C _{20:0} , C _{22:0} -C _{24:0} , C _{26:0} , C _{28:0} , C _{29:0} , C _{16:1} , C _{17:1} , C _{18:1} ; Alkanes: unidentified; Alcohols: C24; Sterols: Cholesterol; Dehydroabiestic acid; Phthalates
<i>CNS-31I</i>	151	FAs: C _{14:0} -C _{20:0} , C _{22:0} , C _{16:1} , C _{18:1} ; Alkanes: C27, C29, C31; Alcohols: C18, C24, C26, C28; Sterols: Cholesterol; Phthalates
<i>CNS-32IA</i>	Trace	FAs: C _{16:0} , C _{18:0} ; Alkanes: unidentified; Alcohols: C18, C20; Sterols: Cholesterol; Phthalates

Table 7.15: GC-MS and HT-GC identification of lipid residues extracted from ceramic vessels excavated from Can Sadurní (Barcelona), run as TMS derivatives. [FA: Fatty acids; MAG: Monoacylglycerols; DAG: Diacylglycerols; TAG: Triacylglycerols; WE: Wax esters]

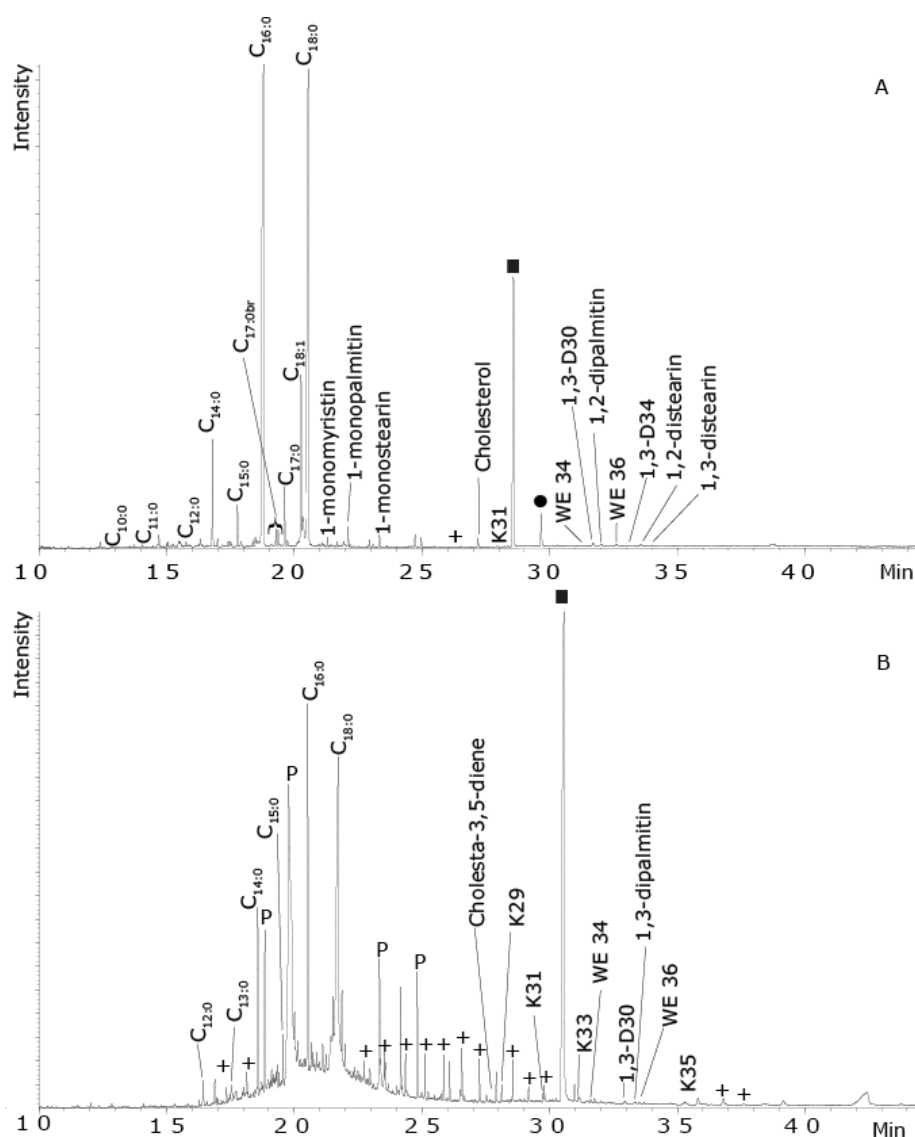


Figure 7.30: TIC of A: CNS-10I and B: CNS-11I. [$C_{x,y}$: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C17-C33); K: Ketones; WE: Wax esters; P: Phthalates; ■: Internal standard (C34); ●: Internal standard (C36)]

Pot CNS-01 produced an unusual result, in that its exterior surface (CNS-01E) was found to contain considerable quantities of lipid ($33\mu\text{g g}^{-1}$), whereas trace amounts of lipid were extracted from the interior surface (Figure 7.31). Both surfaces had a similar lipid profile, and contamination from the burial environment is doubtful since the external surface of pot CNS-02E, which was similarly retrieved from Layer 18, showed negligible residues. GC-c-IRMS analysis of CNS-01E identified the residue as ruminant adipose, while the ketones identified indicate that the residue had been heated. This result is difficult to interpret, since the negligible lipid quantities present on the interior surface preclude the possibility of an overspill. No further explanations can be put forward at this stage.

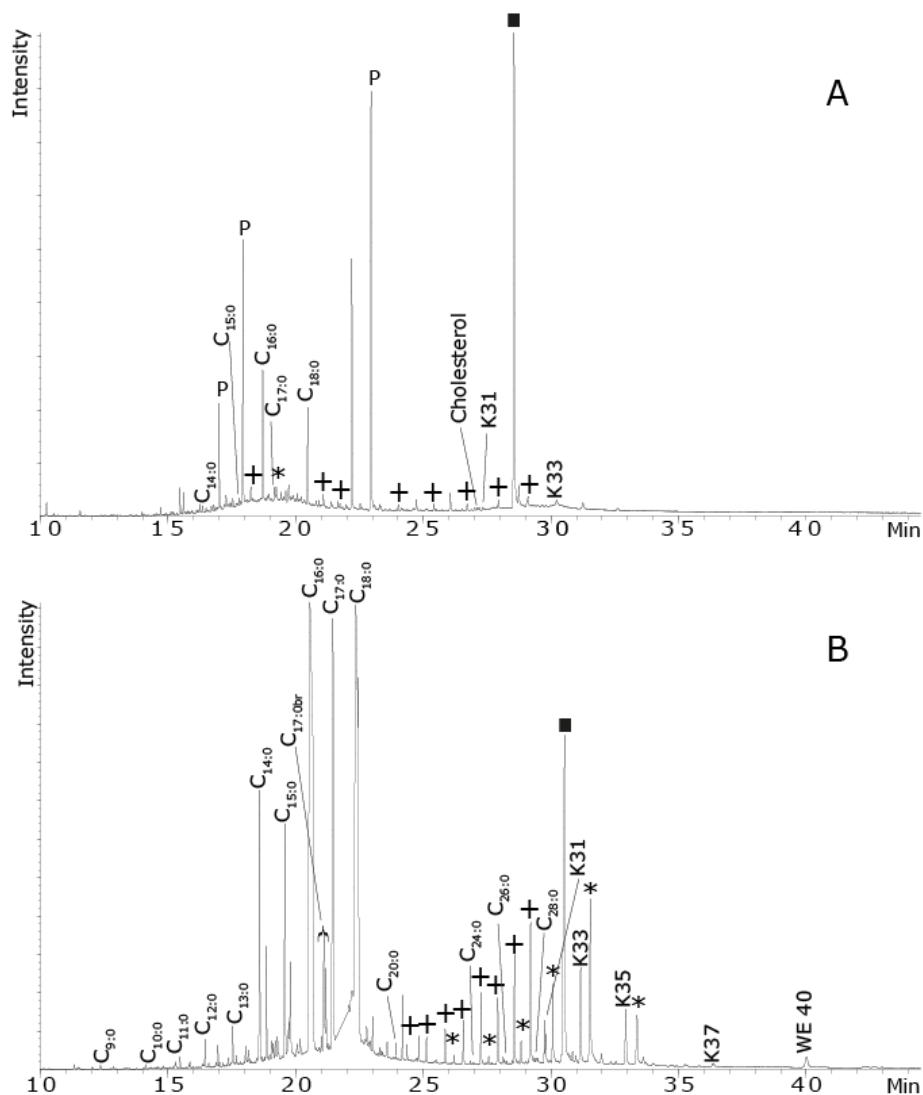


Figure 7.31: TIC of A: CNS-01I (interior surface); B: CNS-01E (external surface). [$C_{x,y}$: Fatty acid where x is the carbon number and y is the degree of unsaturation; +: Alkanes (C20-C33); *: Alcohols (C18-C34); K: Ketones; WE: Wax esters; P: Phthalates; ■: Internal standard (C34)]

It was also possible to take multiple samples from the rim and body of two globular vessels, pots CNS-23 and CNS-30 (Figure 7.32). GC-MS data identified both residues as animal fats (Table 7.15). The fatty acid profile showed dominant $C_{16:0}$ and $C_{18:0}$ fatty acids, with odd-numbered chain fatty acids $C_{15:0}$ and $C_{17:0}$ present in CNS-30, suggesting a ruminant origin. Mono- and diacylglycerols were identified in CNS-29I, and cholesterol was identified in both vessels, also suggesting an animal fat. Ketones were present in both pots, indicating heating of the absorbed fats. In both cases, lipid accumulation was greatest at the rim; in CNS-29I, the quantified lipid at the rim was four times the amount obtained from the body, while in CNS-30, negligible quantities were obtained from the body, but the rim contained $66\mu\text{g g}^{-1}$. This profile is strongly indicative of

boiling episodes (Charters *et al.* 1993). Traces of dehydroabietic acid were identified only in the residue extracted from the body of CNS-30 (CNS-30IB). Dehydroabietic acid was not identified in any of the external residues, which suggests that this *Pinaceae* resin biomarker must originate from the residue. However pine remains were retrieved during floatation (Antolín, 2011, pers. comm.), and in view of the low lipid quantities obtained from this sherd, it is likely to have been introduced from the burial context, or as an artefact arising from instrumental contamination.

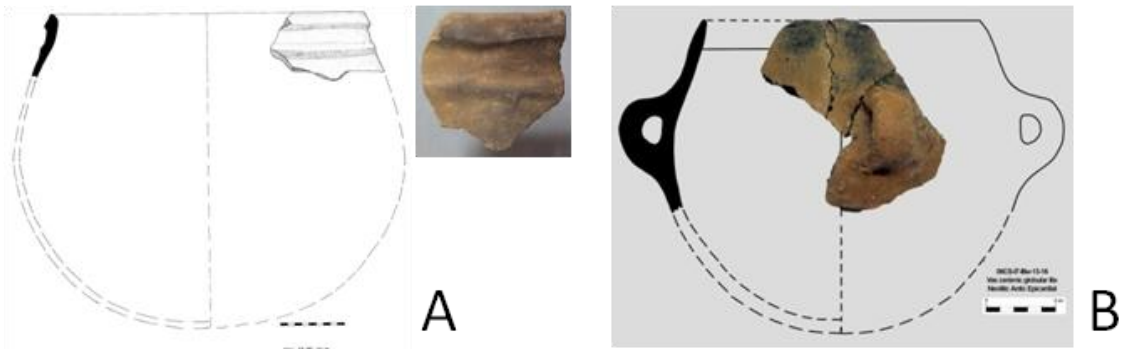


Figure 7.32: A: Vessel CNS-23; B: Vessel CNS-30 [Coutesy of M. Edo].

ORA results appear to mirror the faunal and floral remains retrieved from Can Sadurní. ORA results for the funerary context, Layer 18, were predominantly negligible, with only 3 out of the 12 vessels analysed retaining a significant residues attributed to ruminant adipose/dairy fats. Layer 18 was however characterised by ceramic vessels containing charred remains of cereals, of which over 60,000 remains were attested (Antolín and Buxó, 2011). The archaeological evidence in this case provides excellent supporting evidence for an interpretation of the negligible residues as indicative of the storage or processing of plant material. The wide ranges of alkanes, alcohols and wax esters observed in the residues obtained from Layer 18 further support a plant input. On the other hand, Layer 17 was interpreted as a habitation context (Antolín, 2011, pers. comm.), and was dominated by faunal remains (Edo *et al.*, 2011). ORA results mirror this, in that 8 out of the 15 vessels analysed from this layer contained a significant residue, and the lipid profiles obtained, as well as the GC-c-IRMS results, identified ruminant adipose and dairy fats. A plant input was still observed in at least 4 of the vessels analysed, three of which (CNS-06I, CNS-10I and CNS-11I) showed indications of containing both plant and ruminant products, whereas the lipid profile obtained for pot CNS-05I was consistent with a plant residue, further supported by a P/S ratio of 5 (see Copley *et al.*, 2001b). GC-c-IRMS analysis also confirmed Saña *et al.*'s (submitted) interpretation of the mortality profile recorded for the Can Sadurní faunal assemblage, as

indicating an economy based not only on meat production, but also on pastoral activities. As at Fondo Azzollini and Skorba, a marine input could not be securely identified at Can Sadurní.

7.9 Results overview

Out of the 301 ceramic vessels analysed, only 81 yielded a significant residue, while 220 contained $<5\mu\text{g g}^{-1}$ lipid (Table 7.16). The highest percentage of significant lipid yields were obtained from the cave sites, Can Sadurní (42%), Nakovana Cave (94%) and Grotta Saracena (73%), but two open air settlements, Fondo Azzollini and Ciccotto, also yielded a good proportion of significant residues (80% and 40%, respectively). All the sites are located on calcareous formations, and previous research has shown that this is not conducive to the survival of lipids (Moucawi *et al.*, 1981). However, as already suggested above, the low levels of absorbed lipids obtained from most of the sites investigated may not necessarily be the result of advanced degradative processes associated with the burial environment. The vessels' function may also be a contributing factor. This hypothesis will be further discussed in Chapter 8.

Site	Site Type	Whole vessels (N)	No. of vessels containing $>5\mu\text{g g}^{-1}$ lipid	Percentage of vessels containing $>5\mu\text{g g}^{-1}$ (%)	Mean quantity lipid extracted ($\mu\text{g g}^{-1}$)	Max. quantity of lipid extracted ($\mu\text{g g}^{-1}$)	Min. quantity of lipid extracted ($\mu\text{g g}^{-1}$)
AZZ	Open air	25	20	80	247.9	3662.8	0.0
CIC	Open air	25	10	40	13.9	221.4	0.2
PAL	Open air	39	5	13	2.2	11.7	0.1
BAL	Open air	35	2	6	1.9	13.0	0.0
BSP	Open air	15	-	0	0.3	1.9	0.0
MAS	Open air	12	-	0	0.2	0.4	0.0
TRA	Open air	26	1	4	0.9	7.3	0.0
SET	Open air	12	-	0	0.7	2.3	0.0
FAV	Open air	27	4	15	4.4	77.5	0.4
SAR	Cave	15	11	73	7.2	25.7	0.1
MAR	Submerged	6	-	0	2.9	4.2	1.8
SKR	Open air	16	1	6	1.3	5.4	0.0
NAK	Cave	17	15	88	25.5	247.1	1.0
CNS	Cave	31	13	42	50.7	460.0	0.0

Table 7.16: Table listing the number of ceramic vessels analysed from each site, the number of vessels which retained a significant quantity of absorbed lipid, and the corresponding percentage of vessels yielding significant levels of absorbed lipids. The mean, maximum and minimum quantities of lipid extracted from each site are also reported in $\mu\text{g g}^{-1}$. [N: denotes the sample size; AZZ: Fondo Azzollini, Pulo di Molfetta; CIC: Ciccotto, Gravina in Puglia; PAL: Palata 1, Canosa di Puglia; BAL: Balsignano, Modugno; BSP: Serri-San Gabriele, Bari San Paolo; MAS: Masseria Maselli, Bari; TRA: Seconda Spiaggia di Colonna, Trani; SET: Canne Setteponti, Barletta; FAV: Favella della Corte, Corigliano Calabro; SAR: Grotta San Michele, Saracena; MAR: La Marmotta, Anguillara Sabazia; SKR: Skorba, Mgarr; NAK: Nakovana Cave; CNS: Can Sadurní, Begues]

The residues identified included ruminant and non-ruminant adipose fats, ruminant dairy fats, plant oils and mixtures comprising animal fats and plant oils, hence providing direct evidence for the association of Impressed/Cardial Wares with agrarian produce and pastoral activities (Figure 7.33). Fish oils could not be securely identified. As already mentioned above, a considerable number of vessels contained a significant residue, but lacked sufficient quantities of C_{16:0} and C_{18:0} for GC-c-IRMS analysis. Consequently, 36 residues have been classified as ruminant fats, since they could not be securely identified as adipose or dairy fats. Mixtures of animal and plant products were identified in 13 vessels. Two of the animal fat inputs in these mixtures were identified by GC-c-IRMS as dairy fats, and three were similarly identified as ruminant adipose. Of the remaining eight animal fats in the mixtures, two could only be identified as animal fats, while the remaining six residues were classed as ruminant fats.

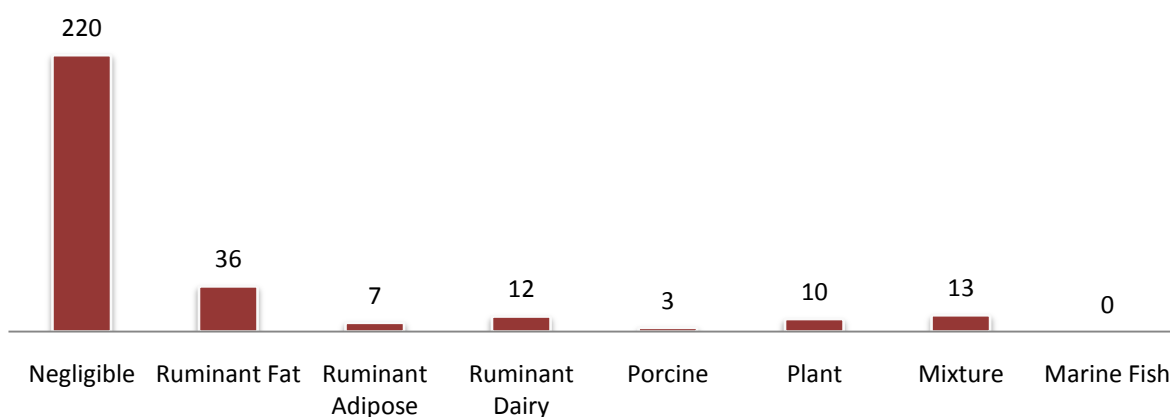


Figure 7.33: Chart showing the number of vessels containing $>5\mu\text{g g}^{-1}$ of residue, whose origin has been identified by GC-MS and GC-c-IRMS. Since marine fish contributions have not been securely identified, the three tentative identifications made (AZZ-13I, SKR-16I and CNS-06I) were not included in this chart. [Mixture: includes residues with a combined animal (ruminant adipose, dairy and perhaps non-ruminant adipose) and plant contribution; Ruminant Fats: includes residues which could not be securely assigned as dairy or adipose fats]

Several mechanisms involving chemical bonding, possibly through ester linkages, are responsible for interactions between organics and clay minerals, which may lead to lipids becoming trapped within an insoluble polymeric matrix (Regert *et al.*, 1998a; Stacey, 1999:146-151). This is known as the 'bound fraction', and offers added protection to lipid biomolecules, which are otherwise easily lost during vessel use and upon burial (e.g. dicarboxylic and hydroxy carboxylic acids). The 'bound' lipid fraction cannot be released using solvent extraction, but lipid residues trapped within this protective polymeric matrix can be analysed following a base extraction. This was carried out on 30 Impressed/Cardial Ware samples (see Chapter 6 for a description of the method followed). However, the gas chromatograms obtained from the samples that were base extracted were very

similar to those produced when the same samples were solvent extracted, therefore precluding further analysis.

Figure 7.34 shows the percentage composition of the different categories of residues absorbed within the walls of Impressed/Cardial Wares. The greatest identified contribution is from ruminant fats, which are present in 19% of all significant residues, 4% of which are dairy in origin. Non-ruminant fats were identified in 1% of the residues, while 3% of the residues were tentatively identified as plant oils. These figures however do not include the combinations of animal and plant fats and oils making up the mixtures identified in 13 of the vessels analysed. Hence, a more accurate measure of the contributions of the different food products is presented in Figure 7.35, which shows the number of attestations of the different food products identified. A considerable contribution of plant material can be better appreciated, as well as the dominance of ruminant fats and, to a lesser extent, dairy products. The five visible residues analysed (two from Masseria Maselli identified as ruminant fats and three from La Marmotta comprising one ruminant fat, two plant residues and one animal fat and plant mixture), were not included in this chart, but they show a similar range of food products. Fish oils were also excluded since they were not securely identified.

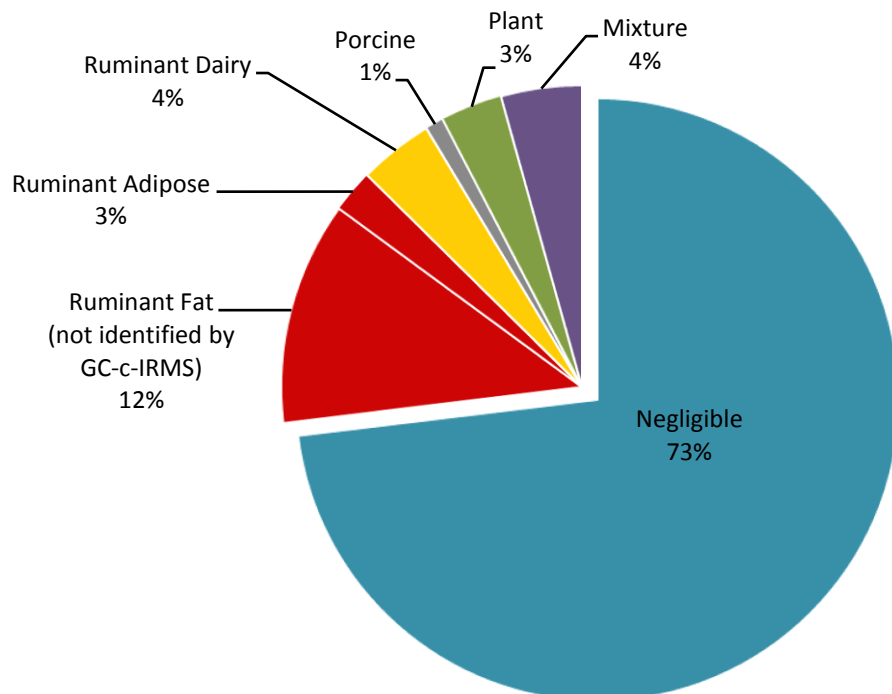


Figure 7.34: Pie-chart showing the percentage composition of the different categories of residues identified in the absorbed residues of Impressed/Cardial Wares. [Mixture: includes residues with a combined animal (ruminant adipose, dairy and perhaps non-ruminant adipose) and plant contribution; Ruminant Fats: includes residues which could not be securely assigned as dairy or adipose fats]

As already discussed above, marine biomarkers are absent in the ceramic vessels analysed, although indications of a possible marine input was identified in three vessels. This is consistent with the available data on the faunal remains at the various sites investigated, except at Favella della Corte and La Marmotta. To further test for the presence of marine biomarkers, 15 residues selected from the sites of Favella, Grotta Saracena, Fondo Azzollini, Can Sadurní and Nakovana Cave were prepared as methyl esters and run on the GC-MS in combined SIM/SCAN mode (see Chapter 6 for methodological details). This enhances the sensitivity of the MS detector to selected ions, in this case marine biomarkers including ω -(*o*-alkylphenol)alkanoic acids, isoprenoids, and dihydroxy fatty acids (Copley *et al.*, 2004; Hansel *et al.*, 2004; Craig *et al.*, 2007; Evershed *et al.*, 2008a; Hansel and Evershed, 2009). However none of these were detected. A possible way forward would have been to analyse the 'bound' fraction, which is more likely to release dihydroxy fatty acids than solvent extraction, as suggested by Hansel *et al.* (2011). However, in the absence of ω -(*o*-alkylphenol)-alkanoic acids and isoprenoids, a secure marine identification would not have been possible. Although ceramic vessels are not necessary to cook fish, the paucity of fish bones attested on site (except at Favella and La Marmotta, although the fish remains in the latter were not substantial), does not support a significant marine component to the Neolithic diet. Given the coastal location of most of the sites, and the strong seafaring element indicated by the archaeological data, at least during the earliest phases of the Neolithic in the Mediterranean, which were targeted in this investigation, this low attestation of a marine component is intriguing, and will be discussed further in Chapter 8.

As discussed above, the interpretation of some of the GC-c-IRMS measurements from Fondo Azzollini and Can Sadurní was complicated by the wide $\Delta^{13}\text{C}$ values observed for modern deer adipose. Deer bones were present at both sites, hence increasing the likelihood that venison may have been processed in Impressed/Cardial Wares. Hence a deer adipose origin needed to be taken into consideration. Since deer are also ruminant animals, it was possible to combine the $\Delta^{13}\text{C}$ values for both domestic and wild (i.e. deer) modern ruminant adipose, which therefore provided a more comprehensive range of values denoting reference ruminant adipose. By interpreting the $\Delta^{13}\text{C}$ values obtained from archaeological residues in light of GC-MS data and known $\Delta^{13}\text{C}$ values of domestic ruminant adipose (see Section 4.8), a tentative indication of the animal product processed could be made in most cases.

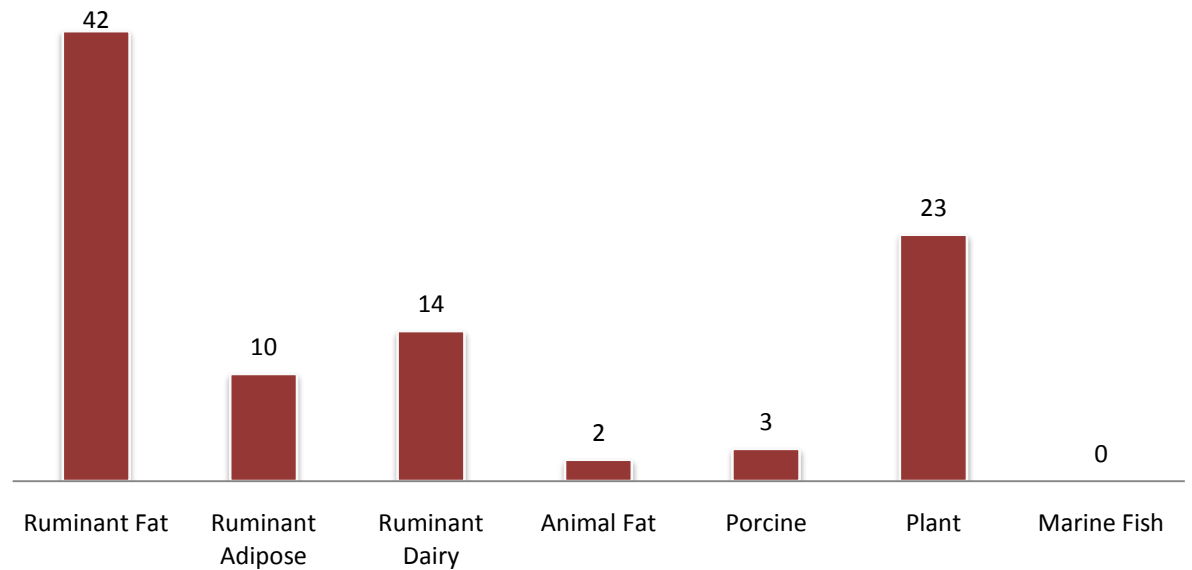


Figure 7.35: Chart showing the number of times the different categories of absorbed residues were identified. [Ruminant Fats: includes residues which could not be securely assigned as dairy or adipose fats; Animal Fats: includes residues which could not be securely assigned a ruminant or non-ruminant origin]

No particular association was observed between the type of residue identified and the form, fabric and decorative motifs of the ceramic vessels analysed. This is perhaps primarily due to the fragmented state of the ceramic vessels analysed, which in many cases were not sufficient to identify vessel form. Coarse wares and fine wares were both utilised to process food products, and the type of decoration applied to the vessel does not appear to have any influence on the function of the vessel (see Chapter 8 for further discussion). Ceramics dated to the Middle Neolithic were found to be consistently poorer in lipid residues compared to the Early Neolithic vessels, as perhaps best attested at Ciccotto. Two sites, Trani and Canne Setteponti, both dated to the Middle Neolithic period, produced very low lipid yields, with only one sample from Trani retaining a significant residue. If the negligible residues (especially at Canne Setteponti) can be securely interpreted as plant deposits, this may perhaps suggest an increasingly heavier dependence on cultivated vegetative material during the later Neolithic phases on the Murge Plateau. Finally, no variations were observed in the use of Impressed/Cardial Wares between the coastal and more inland settlements included in this study (see Chapter 8 for further discussion).

7.10 Conclusion

The application of ORA to Impressed/Cardial Wares has shown that their function was indeed associated with domesticates. In particular, this is attested by the identification of dairy products in pots retrieved from the earliest Neolithic Phases at Fondo Azzollini, Ciccotto, and Can Sadurní. Porcine residues and the possible attestation of wild deer adipose have been suggested, both by

ORA and the faunal remains, while marine residues could not be securely identified using ORA. This study has also shown, that in the absence of faunal remains, ORA is instrumental in providing direct evidence for vessel use. Nevertheless, faunal and floral remains are also important in supporting ORA identifications, in particular plant residues, as will be further discussed in Chapter 8. The following chapter will also put forward possible interpretations for the high proportion of negligible results obtained from the Mediterranean assemblage studied.

Chapter 8

Organic residue analysis: a Mediterranean perspective

8.1 Introduction

The aim of this chapter is to provide a synthesis of the experimental and archaeological work carried out in this research, which has presented the first extensive application of organic residue analysis (ORA) in the Mediterranean region. ORA has only recently started to be applied to warmer climatic areas (Evershed *et al.*, 2008b; Gregg *et al.*, 2009; Martí-Oliver *et al.*, 2009), and the research carried out so far has highlighted two important issues: i) the preservation of lipid residues in warmer climatic zones is generally poor, ii) shifts to the $\delta^{13}\text{C}$ measurements of the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids occur, resulting in possible changes to the $\Delta^{13}\text{C}$ values which define the different animal fat categories (Gregg *et al.*, 2009). Both issues needed to be taken into consideration to enable an accurate interpretation of the archaeological residues extracted from Mediterranean Impressed/Cardial Wares, and hence attempt a better understanding of the function of these ceramic vessels at the transition to agriculture. These two issues will be discussed in terms of the Impressed/Cardial Ware data obtained, followed by general recommendations for further work and an overall conclusion of the study.

8.2 The significance of negligible residues

ORA of Mediterranean Impressed/Cardial Wares produced a high percentage of negligible residues, with only 27% of the pottery vessels yielding a significant lipid residue ($>5\mu\text{g g}^{-1}$). Although the hydrophobic properties of lipids, as well as their absorption within the ceramic matrix (or charred residues) do considerably increase their survival potential over archaeological timescales, factors such as soil pH, microbial activity, as well as chemical reactions, in particular hydrolysis and oxidation reactions, will lead to lipid degradation, and the loss of the more labile components (Eglinton and Logan, 1991; Heron *et al.*, 1991b; Evershed *et al.*, 1992; Aillaud, 2001). This has already been discussed in detail in Chapter 3.

The burial environment plays an important role in defining the rate of degradation, and whether or not lipid residues survive over archaeological timescales. Figure 8.1 shows the percentage lipid recovery reported in published research from various geographical areas with differing climatic conditions, including northern Europe (Copley *et al.*, 2005a, 2005c, 2005d; Mukherjee *et al.*, 2008; Craig *et al.*, 2011), central and eastern Europe (Craig *et al.*, 2003, 2005a; Spangenberg *et al.*, 2006; Evershed *et al.*, 2008b), the Levant (Evershed *et al.*, 2008b; Gregg *et al.*, 2009), and the western Mediterranean data presented in this research. The lowest percentage lipid recovery was obtained in the Levant area, with only around 4% (30 out of 679 sherds) providing a significant lipid yield. The percentage recovery in the western Mediterranean is slightly better than the eastern Mediterranean and central Europe, however by far the best preservation appears to be in the northern latitudes.

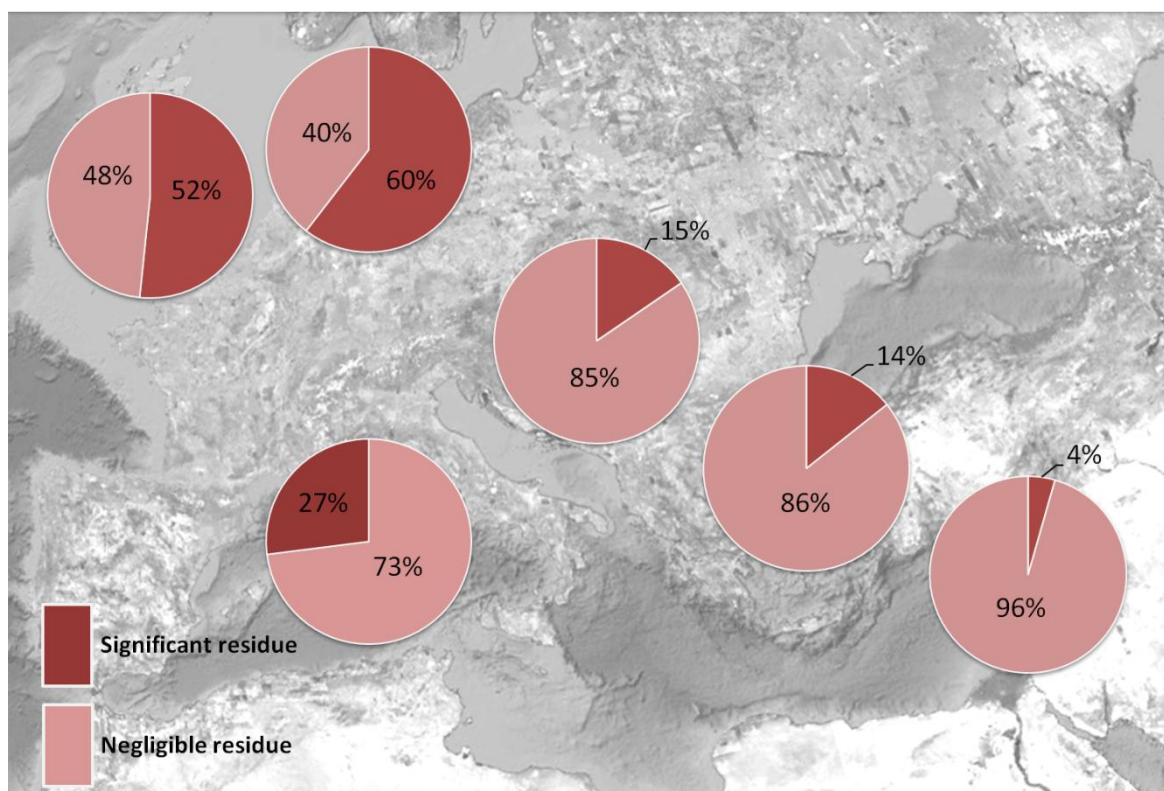


Figure 8.1: Map showing the percentage composition of significant and negligible residues obtained from ORA carried out in different geographical contexts with differing climatic conditions. The western Mediterranean data was obtained from the present research, while published literature was used to compile the data for the Levant, central and northern Europe, and the eastern Mediterranean (Craig *et al.*, 2003, 2005, 2011; Copley *et al.*, 2005a, 2005c, 2005d; Spangenberg *et al.*, 2006; Evershed *et al.*, 2008b; Mukherjee *et al.*, 2008; Gregg *et al.*, 2009).

However, the regions showing the lowest lipid yields are also the regions of greatest antiquity, which could also be a determining factor in the survival of lipid residues. Not enough data was

available to allow an analysis of the percentage lipid recovery through time, however using Copley *et al.*'s (2005a, 2005c, 2005d) work it was possible to observe changes to lipid recovery over a 4000 year span, from Neolithic to Iron Age contexts in the UK. Figure 8.2 shows, that for the first 3400 years, there appears to be no difference in lipid yields, with around a 16% increase in lipid recovery obtained from pottery vessels in Iron Age deposits. This is perhaps more likely to be associated with an increase in the intensity of use of ceramics during the Iron Age, given the similar lipid recovery rates over the first 3400 years, which suggested that the burial context and climatic conditions play a more crucial role in the preservation of lipids than time.

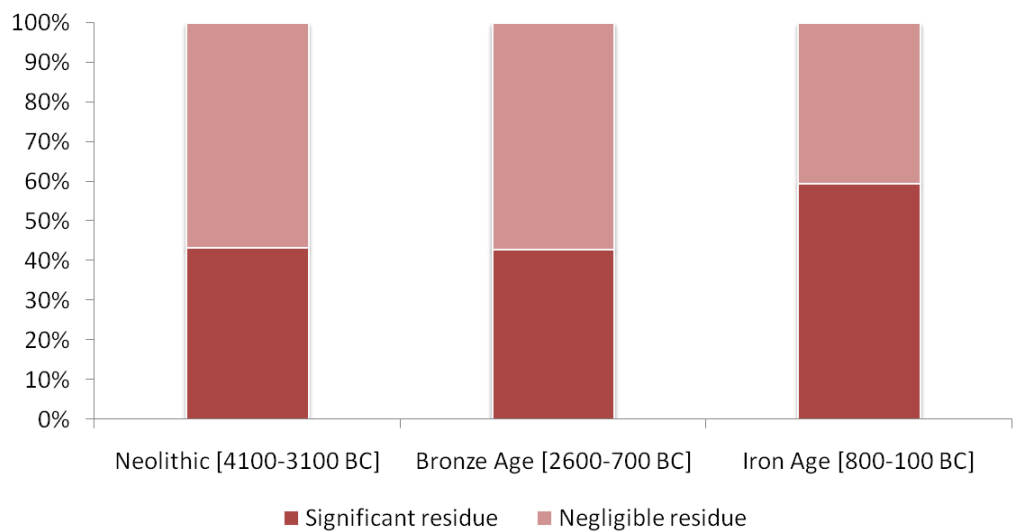


Figure 8.2: Chart showing the percentage lipid recovery obtained from prehistoric contexts in the UK, which spans a period of about 4000 years (From Copley *et al.*, 2005a, 2005c, 2005d).

Organic residues are more likely to survive in waterlogged and desiccated environments (Regert *et al.* 1998, Copley *et al.* 2005), rather than in areas where seasonal variations alternate between heavy rainfall and hot dry spells (Evershed 2008b). The climate in the Mediterranean is more consistent with the latter, and moreover, all the sites investigated lie on calcareous deposits, which are not conducive to the survival of lipid residues mainly because they support a richer microbial population than acidic environments, therefore enhancing lipid degradation (Moucawi *et al.*, 1981). Hence the climatic conditions and burial contexts could be the main reason behind the low lipid yields retrieved from Mediterranean Impressed/Cardial Wares. This appears to be supported by the high percentage of lipid recovered from cave sites (Grotta Saracena, Nakovana and Can Sadurní), which are more sheltered from the seasonal cycles, and lessen leaching of the more soluble lipid components. However, the ceramic assemblage analysed from the open air sites at Fondo Azzollini and Ciccotto in Apulia did show a good lipid yield, in particular at Fondo

Azzollini, where 80% of the ceramics analysed retained a significant quantity of residue (Figure 8.3). This suggests that perhaps the burial context is not the only factor leading to a low lipid yields.

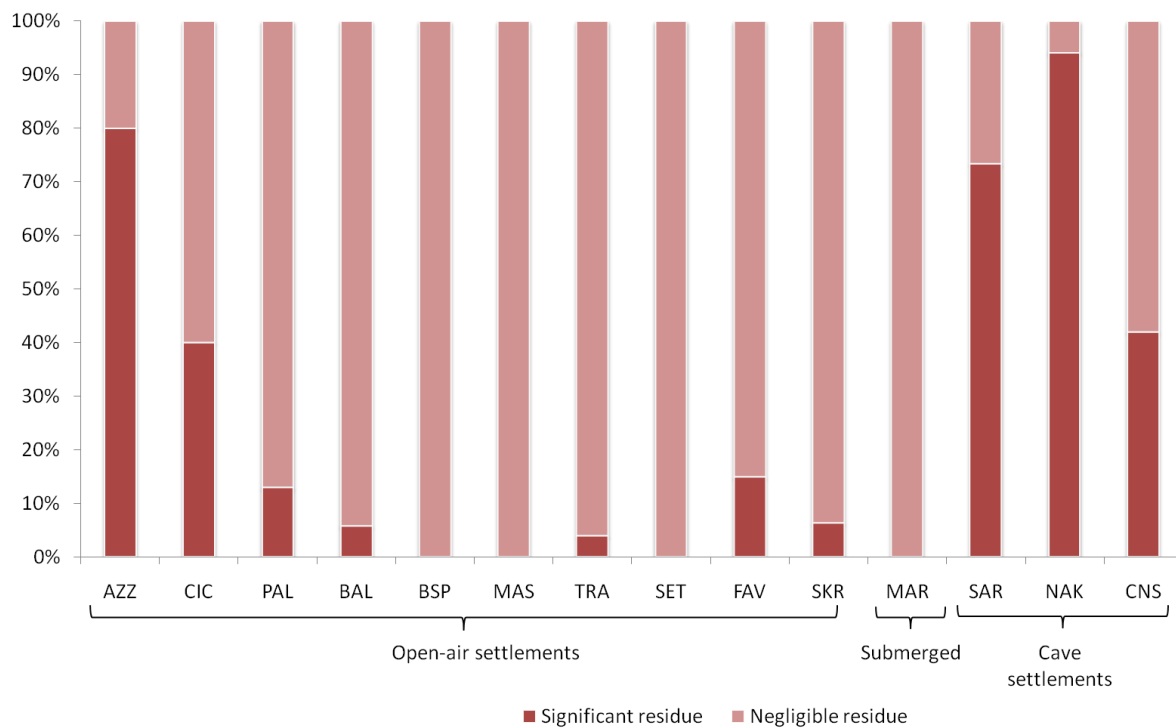


Figure 8.3: Chart showing the percentage of vessels containing negligible and significant residues following ORA of Mediterranean Impressed/Cardial Wares. [AZZ: Fondo Azzollini, Pulo di Molfetta; CIC: Ciccotto, Gravina in Puglia; PAL: Palata 1, Canosa di Puglia; BAL: Balsignano, Modugno; BSP: Serri-San Gabriele, Bari San Paolo; MAS: Masseria Maselli, Bari; TRA: Seconda Spiaggia di Colonna, Trani; SET: Canne Setteponti, Barletta; FAV: Favella della Corte, Corigliano Calabro; SKR: Skorba, Mġarr; SAR: Grotta San Michele, Saracena; NAK: Nakovana Cave; CNS: Can Sadurní, Begues; MAR: La Marmotta, Anguillara Sabazia]

In this scenario, the reason behind the high percentage of negligible residue is not securely identified. Impressed/Cardial Wares could well have been used to process animal and plant commodities, which however did not survive because the burial environment and climatic conditions were not conducive to the preservation of absorbed residue, but the function assigned to the ceramic vessels could equally be a contributing factor. For example, serving vessels such as bowls, cups and plates may not yield significant lipid quantities, unless their contents comprised products which were sufficiently fatty/oily to allow a significant amount of residue to become absorbed. Ceramic vessels may also have been used for storage, for example of cereals and vegetative material. Absorption of lipids within vessel walls is highly improbable in this case, and ORA will therefore yield negligible lipid residues.

Another important point to consider in terms of vessel use, is how many times a pot was used and how many cooking episodes are required to produce a significant residue. More recently, a series of cooking experiments were carried out using replica Grooved Ware pots which were then analysed using ORA. In one of the experiments, three separate vessels were used to cook beef once, three times and thirteen times (Millson, 2012). Figure 8.4 shows the partial gas chromatograms obtained after analysis using HT-GC, and the quantities of lipids extracted from the three pots. After one cooking episode, $3.92\mu\text{g g}^{-1}$ of sherd were recovered, while after three and thirteen boiling episodes 34.97 and $147.17\mu\text{g g}^{-1}$ were obtained respectively (Millson, 2012). When considering that the estimated mean capacity of lipid absorbed within the ceramic walls of a pot is around 10mg g^{-1} of sherd, and that on average an archaeological residue will yield about $100\mu\text{g g}^{-1}$ of sherd (Evershed, 2008a) the amount of lipid obtained, even after thirteen boiling episodes is very small and unlikely to survive over archaeological timescales. This suggests that negligible residues can also be produced if a pot is not sufficiently utilised, such that not enough lipid is absorbed to produce a detectable residues after a long burial period.

The commodity processed in a ceramic vessel will also affect whether or not a residue is likely to form, and its potential to survive over archaeological timescales. Plants are a case in point, as already discussed in Chapter 5. Experiments have already shown that very low quantities of plant oils become absorbed within the walls of ceramic vessels during processing of plant material (Evershed *et al.*, 1995a), and plant residues are easily masked by fattier products if these are cooked simultaneously with plant products or during previous or subsequent cooking episodes (as observed in the einkorn and milk cooking experiment discussed in Chapter 5, and see also Reber and Evershed, 2004b; Evershed, 2008a). While specific biomarkers for particular plant groups have been identified (such as nonacosane-15-one, nonacosan-15-ol and nonacosane identified in leaf wax of the Brassica family; see Evershed *et al.*, 1991), the degraded profiles of most plant residues, for example cereals which are widely attested in the archaeological botanical evidence, generally comprise low levels of $\text{C}_{16:0}$ and $\text{C}_{18:0}$, and a series of alcohols and alkanes. Phytosterols and wax esters may also survive, in particular the former (see Chapter 5), which are highly suggestive of a plant residue. However, as also observed in Chapter 5, most of the degraded plant residues contain less than the required $5\mu\text{g g}^{-1}$ lipid, and the chromatograms obtained are so similar to the soil profiles attributed to migration from the burial environment, that it is difficult to distinguish between the two. Plant residues in pots are therefore very often under-represented.

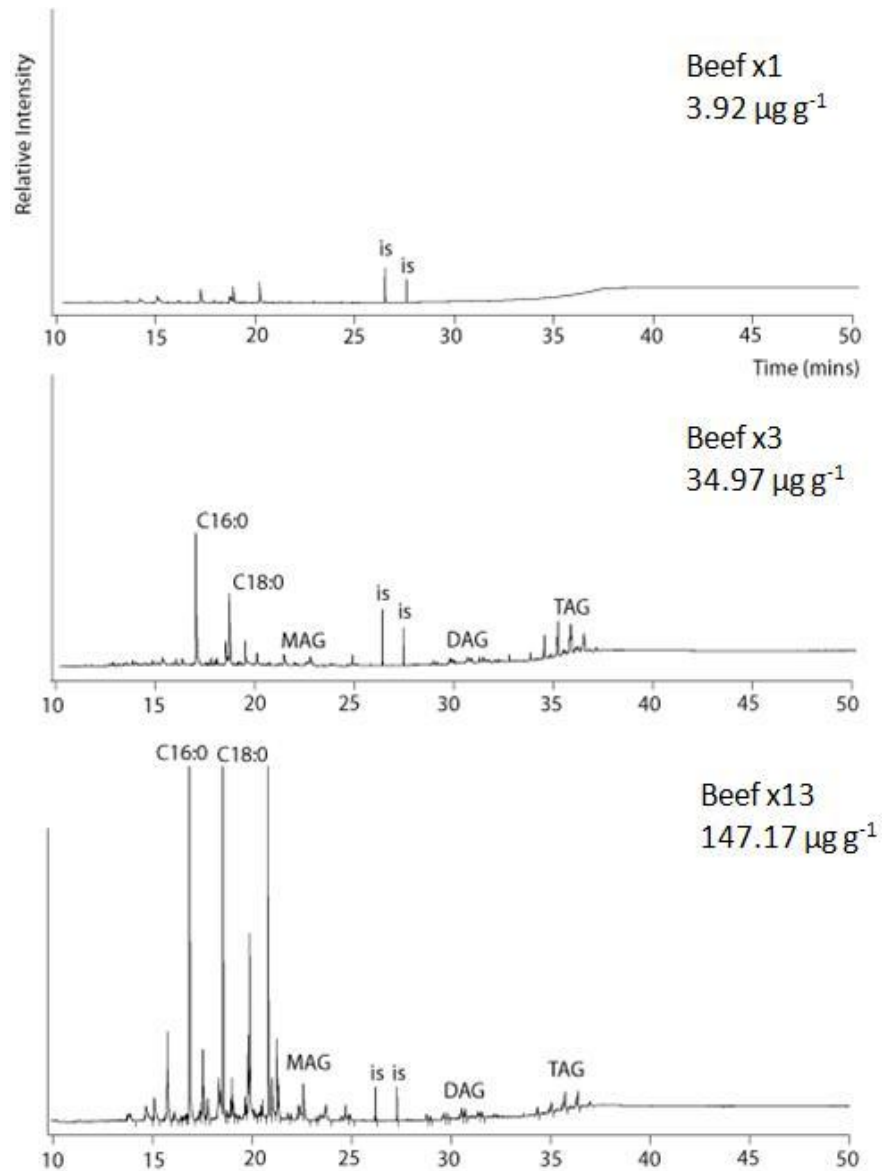


Figure 8.4: Partial HT-GC obtained after 1, 3 and 13 boiling episodes with beef (After Millson, 2012). [C_x: denotes the number of carbon in the fatty acids; MAG: Monoacylglycerols; DAG: Diacylglycerols; TAG: Triacylglycerols; IS: Internal standard]

Negligible residues can occur both as a result of advanced stages of degradation during burial, or equally as a result of vessel function during the life time of a pot. Hence, low lipid yields cannot be simply attributed to taphonomic factors, which therefore highlight the need to interpret ORA results in light of the archaeological evidence pertaining to the palaeoeconomy of a site, and the features (e.g. fabric and form) of the ceramic assemblage analysed.

8.3 Plant input in the Mediterranean Neolithic diet

Plant remains have been heavily attested in most of the sites analysed. Archaeobotanical remains on the Murge Plateau provide evidence for extensive cereal cultivation during the Neolithic (Fiorentino, 2002a:222), while evidence for the use of cultigens have been preserved in the archaeological record at La Marmotta, Favella, Grotta Saracena, and in particular, Layer 18 at Can Sadurní, where ceramic vessels containing cereal grains were found. Only two sites, Nakovana Cave and Skorba showed limited evidence for plant remains. As already discussed in Chapter 7, Nakovana Cave is thought to have been used by pastoralists to shelter herds, and the surrounding environment is not suitable for cultivation. However evidence for plant use was obtained using ORA, which identified mixtures of plant and animal contributions in six of the vessels analysed. It was suggested that perhaps ceramic vessels containing food products were transported to the cave from nearby hamlets, where the surrounding arable land could have been used to grow cultigens. At Skorba, botanical evidence is supported by only a few charred grains, however, this does not preclude a thriving cultivation practice, since flotation was not used during excavations, and plant remains are likely to be under-represented. Out of the 81 significant residues extracted from the Impressed/Cardial Ware assemblage submitted for ORA analysis, 10 were tentatively attributed to plant contributions based on the quantity of lipid extracted (low but $>5\mu\text{g g}^{-1}$) and the lipid profiles obtained, which generally comprised low levels of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ (with a P/S ratio >4), a wide series of alkanes and alcohols, as well as the occasional presence of phytosterols and wax esters. A plant contribution was further identified in another 13 vessels, as mixtures with animal products (Figure 8.5). Whether the high percentage of negligible residues pertains to a plant contribution is not known. However, the archaeobotanical evidence retrieved from the various sites and palaeodietary data obtained from skeletons found in several Apulian sites appear to support a heavy reliance on plant material during the Neolithic.

Bartoli (1996) used trace element analysis (zinc and strontium) to analyse the skeletal remains of 27 individuals dated to the Neolithic period from various sites in southern Italy. The results he obtained suggest a diet rich in protein and fibre derived from plant material. Similar results were observed when the same analysis was carried out by Scattarella and Sublimi Saponetti (2002) on 38 individuals excavated from seven sites in Apulia, dating from the Early to the Late Neolithic, including a male individual from Balsignano dated to the Early Neolithic (Scattarella et al. 2002). Both studies suggest that with the onset of agriculture, the proportion of meat in the diet appears to have been drastically reduced, while the consumption of cultivated plant material was found to comprise 90% of the diet. However, the validity of the results obtained is questioned, primarily

due to an as yet poor understanding of how trace elements are incorporated in the bone and diagenesis, which therefore preclude a reliable interpretation using this technique (Sealy, 2001).

Scattarella and Sublimi Saponetti (2002) also reported the results obtained from macroscopic analysis of human teeth from the Apulian skeletons, which showed that 65% of the individuals suffered from enamel hypoplasia. This condition is frequently identified in prehistoric populations whose diet is based on the consumption of cereals with small quantities of animal protein (Scattarella and Sublimi Saponetti, 2002). Enamel hypoplasia was observed to first appear in children aged two to six, with an average age of four years (Scattarella and Sublimi Saponetti, 2002). Despite this, a very low incidence of dental wear was observed in the Apulian individuals studied, with only three instances of caries observed out of the 275 teeth examined (Scattarella and Sublimi Saponetti, 2002). Diets rich in carbohydrates and cereals usually produce greater instances of wear and tear on the teeth, in particular due to their coarse nature which causes abrasion of the enamel and flattening of the dental cusps, hence increased attestations of dental caries. These results are seemingly conflicting, since the high incidence of enamel hypoplasia suggests a diet rich in plant material, but the low levels of dental wear and caries imply the opposite. An interesting interpretation has been put forward by the authors, which appears to explain the results obtained, namely cooking the plant material prior to consumption. This softens the tough plant matter hence lessening abrasion on the teeth, and therefore also dental wear (Scattarella and Sublimi Saponetti, 2002; see also Larsen, 2006). Furthermore, Lelli *et al.* (in press) carried out stable isotope analysis on human and animal bones from several Early Neolithic sites in southern Italy, including Balsignano. The $\delta^{15}\text{N}$ obtained from human bone collagen extracted from individuals buried in the Murge area was found to be very low, in fact, only a 1‰ variation separated them from the ovicaprid $\delta^{15}\text{N}$, suggesting that a large proportion of their dietary protein (c. 60-100%) was obtained from plants (Lelli *et al.*, in press). Similar results were also obtained at Alepotrypa Cave in Greece, which was occupied by Early Neolithic agrarian communities between 5000 and 3200 BC. The remains of 20 individuals studied showed a high frequency of porotic hyperostosis and cribra orbitalia, suggesting a chronic iron deficiency anaemia which could result from a diet based on iron-deficient cereals (Papathanasiou *et al.*, 2000). This interpretation is supported by stable isotope analysis, which showed a diet rich in C_3 plants, and dental analysis, which identified a high instance of caries (Papathanasiou *et al.*, 2000).

It is not surprising that plants should be found to play an important role in the Early Neolithic Mediterranean cuisine. Their use as a key dietary component is widespread, and pre-dates both farming and the production of pottery vessels, which are not essential when processing plant

material (e.g. the use of domestic plants is well documented during the PPNA phase in the Levant). The key issue put forward in this section pertains to the interpretation of negligible residues, which has already been discussed in some detail in Section 8.2. The various scientific investigations carried out at Balsignano provide an excellent platform to argue that negligible residues need not be summarily dismissed as resulting from advanced phases of lipid degradation. Negligible lipid residues were extracted from Impressed Wares recovered from Balsignano, with only two vessels containing a significant residue, tentatively identified as originating from plant material (see Section 7.2.3). However, the strong circumstantial evidence for a heavy reliance on plant products obtained from botanical, stable isotope and palaeopathological analysis (discussed above) suggest that perhaps storage and/or processing of plant material played a major role in the function of Impressed Ware vessels at this site. This is also supported by the lipid profiles analysed at Balsignano, which are consistent with those obtained for the experimentally degraded plant residues described in Chapter 5. Furthermore, direct archaeological evidence for the processing/storage of plant material in Cardial Wares was obtained from Layer 18 at Can Sadurní, where ceramic vessels containing cereal grains were recovered *in situ*.

As discussed in Section 8.2 above, interpreting negligible residues is not straightforward, and it cannot be assumed that these always originate as a result of degradation. This is a major concern in this research, since ORA of Early to Middle Neolithic Impressed/Cardial Wares produced a high percentage (73%) of negligible residues. Although the calcareous burial environment and the Mediterranean climatic conditions were not conducive to lipid preservation, as observed by the better survival of lipid in ceramics recovered from cave sites, lipid recovery from Impressed Wares collected from two open air settlements (Fondo Azzollini and Ciccotto) was good (see Figure 8.3), suggesting that the burial context was not the only factor contributing to the low lipid yields extracted. The evidence obtained from the various scientific investigations carried out at Balsignano, and studies carried out on the botanical remains which indicate that the Murge region was highly cultivated from the Early Neolithic (Fiorentino *et al.*, 2000; Fiorentino, 2002a, 2002b), suggest that plant material could have been processed in Impressed Wares, which is reflected in the poor lipid recovery obtained from the ceramic vessels analysed. Mixtures of plant and animal products were also identified in absorbed residues extracted from Impressed/Cardial Wares at Fondo Azzollini, Palata 1, Grotta San Michele di Saracena, Nakovana Cave and Can Sadurní, which further confirms that plant material was indeed processed in Early Neolithic ceramic vessels, and justifies caution when interpreting low lipid yields, which, as demonstrated above, could equally result from how a pot was used, and what it contained.

8.4 Animal products in the Mediterranean Neolithic diet

Animal products were identified in 24% of the ceramic vessels analysed, and in nine of the 14 sites investigated. As reported in Chapter 7, these comprised ruminant and non-ruminant adipose, and ruminant dairy products. Ruminant and non-ruminant adipose and dairy fats were also identified as mixtures with plant oils in 13 of the vessels analysed, suggesting simultaneous cooking of animal and plant products (e.g. in stews or broths), or re-utilisation of Impressed/Cardial Ware vessels. Marine oils were not securely identified, and are further discussed in Section 8.5.

Figure 8.5 illustrates the percentage of Impressed/Cardial Ware vessels collected from the 14 sites investigated, containing negligible, animal (ruminant and non-ruminant adipose and dairy fats) and/or plant absorbed residues. GC-c-IRMS results obtained from 27 lipid extracts, which contained sufficient $C_{16:0}$ and $C_{18:0}$ to allow the $\delta^{13}C$ of these two fatty acids to be measured are plotted in Figure 8.6. The $\Delta^{13}C$ values used to distinguish between the different fat categories (non-ruminant adipose, ruminant adipose, ruminant dairy products and marine oils) were determined after carrying out a controlled feeding experiment (except the marine fish which were bought from local markets), which included Mediterranean born and bred animals, feeding on a wholly C_3 plant, Mediterranean grown diet (reported in Chapter 4).

Ruminant fats are the most widely represented in the lipid rich sites, with at least 4% of the total number of ceramic vessels analysed being securely found to contain dairy fats (discussed in Section 8.4.1). As already detailed in Chapter 7, most of the faunal assemblages recovered from the sites investigated were either too fragmented to allow an in depth analysis, or are still in the process of being studied. However, when available, a predominance of ruminant animal remains was found, dominated by ovicaprids, which consistently made up over 69% of the faunal remains recovered at the different sites investigated (e.g. as identified in various sites located on the Murge Plateau in Apulia, Italy and at Can Sadurní in Catalonia). Cattle remains were generally less abundant, as were pig bones. Research carried out by Mirabaud *et al.* (2007) has shown that using ORA, it is possible to distinguish between cow and goat dairy products, and sheep and cow adipose fats, by analysing the fatty acid distribution of residual triacylglycerols. Unfortunately, when present, triacylglycerols were identified in trace amounts, which precluded further analysis. However, as illustrated in Figure 7.33, ruminant fats comprised the highest percentage of animal residues identified in Impressed/Cardial Ware vessels, which is consistent with the faunal records studied to date. Non-ruminant fats were also identified following GC-c-IRMS analysis in two Impressed Ware jars from Fondo Azzollini and Ciccotto, in Apulia (Italy), which is also consistent

with the retrieval of domestic pig bone in several Early Neolithic sites in the Murge region (Wilkins, 2002). Hunting, especially of roe and red deer, is known to have continued during the Early Neolithic, as recorded in the faunal assemblages recovered from the Murge area (Wilkins, 2002) and at Can Sadurní (Saña *et al.*, submitted). As already discussed in Sections 4.8 and 7.2.1, the $\Delta^{13}\text{C}$ values obtained for modern deer adipose overlap with both the ruminant adipose and ruminant dairy fat categories, and therefore introduce complexity when interpreting residues plotting within this area of overlap. This affected the interpretation of absorbed residues extracted from vessels recovered at the sites of Can Sadurní and Fondo Azzollini. However, in most cases, using the criteria outlined in Section 7.2.1, it was possible to provide a strong indication of the animal product present.

There appears to be no distinct regional patterning in the type of residues identified from Impressed/Cardial Wares (Figure 8.5), although porcine fats were not identified at Can Sadurní (but pig remains were present in the faunal record), and only ruminant adipose fats were identified in Malta. Lipid degradation is however thought to be a major contributing factor in interpreting the results obtained from Skorba (Malta), as only one of the 16 sherds analysed retained a significant residue. Negligible lipid yields were also identified in ceramic vessels analysed from the Early Neolithic site of La Marmotta (Lazio, Italy) and Seconda Spiaggia di Colonna, Trani (Apulia, Italy) which dates to the Middle Neolithic. The low lipid yield at these sites could potentially be associated with advanced lipid degradation, since visible crusts found attached to the interior surfaces of pots from La Marmotta and Trani were tentatively identified as ruminant fats, suggesting that these pots had been used to process animal products. However, plant products were also identified in the visible residues analysed at La Marmotta (samples MAR-02V and MAR-05VA and B, discussed in Section 7.5), and furthermore, the low porosity of the ceramic fabric of the pots analysed from this site could also have contributed to the negligible quantities of absorbed lipid present. Similar results were also obtained from other sites investigated (Figure 8.5); however, as discussed in Sections 8.2 and 8.3 above, pot function as well as lipid degradation needs to be considered. Distinguishing between the two is difficult, and interpretations can only be tentatively suggested. It is however interesting to note that a higher percentage of ceramics obtained from Early Neolithic deposits in the Apulian region were found to contain significant residues (22%), when compared to pots recovered from Middle Neolithic deposits (8%) in this area (namely Ciccotto, Trani and Canne Setteponti) (Figure 8.5). In Chapter 7 (Sections 7.2.2, 7.2.6, and 7.2.7), it was suggested that perhaps this could have resulted from an increased use of ceramics to store/process plant material, especially in the case of Figulina Wares

which are introduced during this period. This is however only tentatively suggested, as lipid degradation or limited use of the vessels could in fact, produce similar results.

There also appears to be no particular variation in the use of Impressed/Cardial Wares between sites located close to the Mediterranean coast, and settlements located further inland (Figure 8.7). Similar percentages of vessels were found to contain ruminant fats, although ruminant dairy fats were more frequently identified in ceramics recovered further inland, whereas plant residues and mixtures of plant and animal residues were more common on coastal sites. Fatty residues pertaining to ruminant and porcine adipose, ruminant milk fats and plant products were identified in a variety of vessel shapes, including cooking (e.g. jars) and serving vessels (e.g. bowls) (Figure 8.8), and there appears to be no apparent association between vessel shape and the type of products processed within. No distinctive trends were identified when comparing the ceramic fabric to the type of residue absorbed within, although Figulina Wares appear to be associated only with plant remains (Figure 8.9), as indicated above. As described in Chapter 2, Impressed/Cardial Wares were highly decorated, so much so, that it has been suggested that the decorations applied could in fact have had a social significance. Decorative motifs could however, similarly have been used to identify the contents, hence function of particular vessels. Figure 8.10 illustrates the different types of fatty residues absorbed within sherds bearing different decorative motifs. Significant quantities of absorbed fatty residues were identified in sherds bearing impressed decorations, and pots whose surface had been burnished, smoothed or left undecorated. Mixtures comprising plant and animal residues were also identified in sherds bearing incised and scratched decorations, while none of the sherds bearing cardial and cordon decorations, as well as the red painted and red-slipped sherds contained significant quantities of absorbed residue. However, most of the sherds analysed were sampled from highly fragmented assemblages, and therefore not all the sherds classified as 'undecorated' necessarily originated from undecorated vessels, and similarly, different decorative techniques could have been applied to the same vessel, which however, could not be identified in the present research. Hence, interpretations pertaining to associations between food product and decorative motifs are only tentative, and based on the data available at the time of analysis. In light of the results obtained, there appears to be no particular association between decorative motif, and the fatty absorbed residues identified. Impressed/Cardial Ware vessels seem to have been used indiscriminately to process animal and plant products, and their function appears to have been consistent in the different regional contexts investigated within the Mediterranean.

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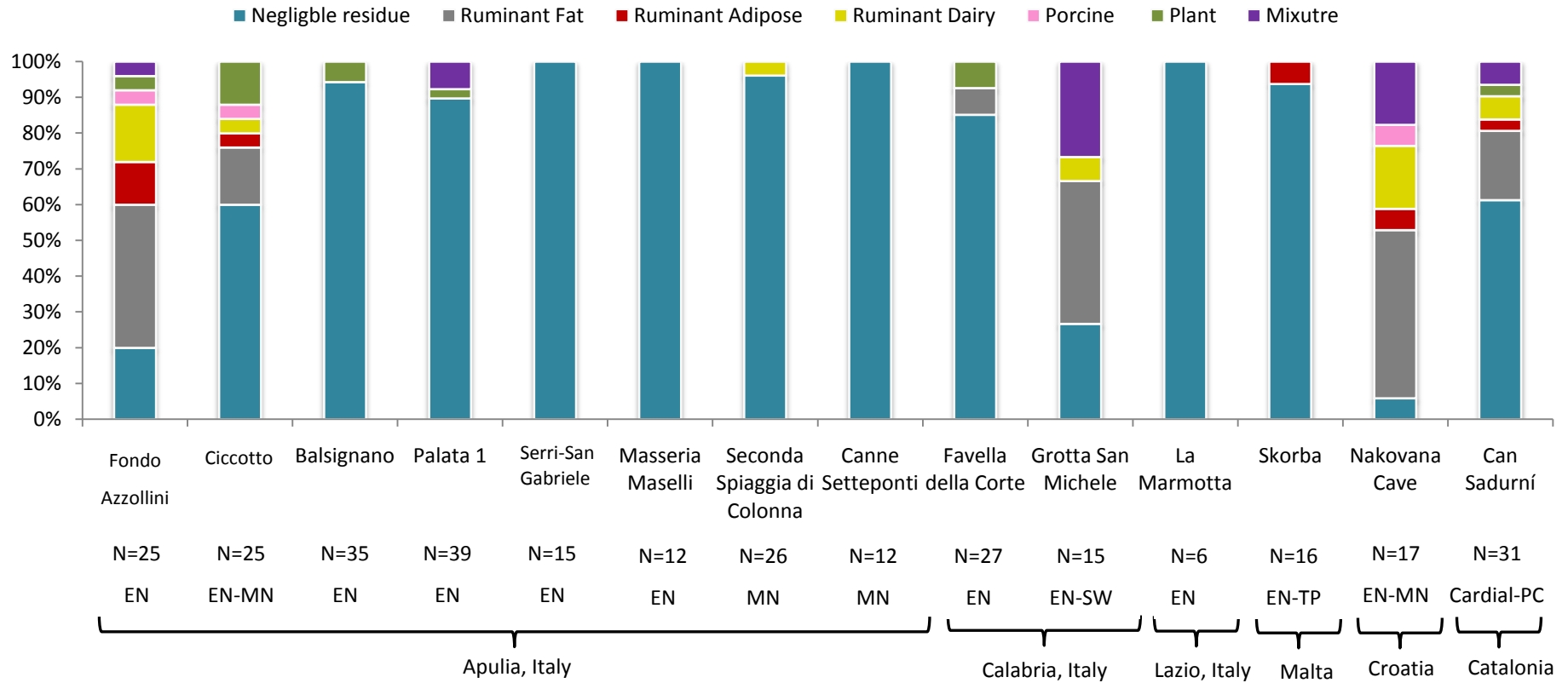


Figure 8.5: Chart showing the percentage of Impressed/Cardinal Ware vessels containing negligible, animal and/or plant absorbed residues, recovered from the 14 sites investigated. Residues were considered significant if $>5\mu\text{g g}^{-1}$ of lipid was present. The residues were identified using GC-MS and GC-c-IRMS. [Mixture: includes residues with a combined animal (ruminant adipose, dairy and perhaps non-ruminant adipose) and plant contribution; Ruminant Fats: include residues which could not be securely assigned as dairy or adipose fats; N: Number of vessels analysed; EN: Early Neolithic; MN: Middle Neolithic; SW: Stamped Ware; TP: Temple Period; PC: Post-cardial]

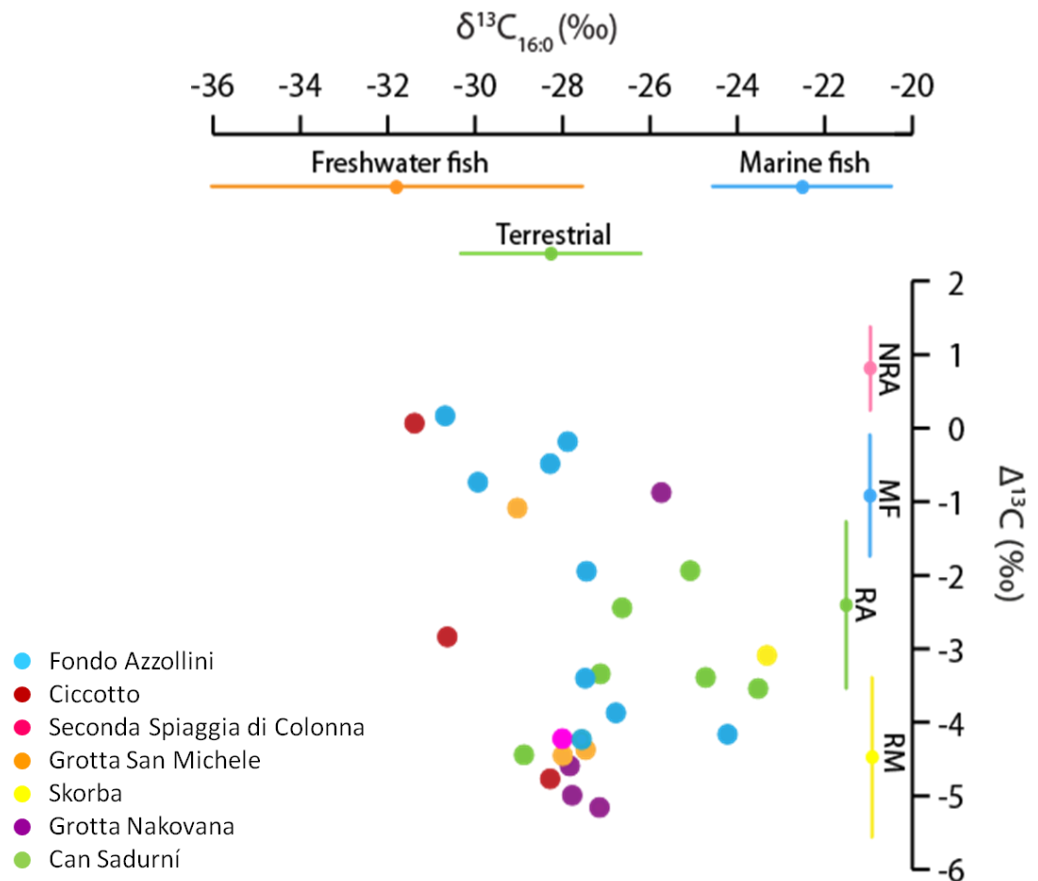


Figure 8.6: Plot of mean $\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$) against mean $\delta^{13}\text{C}_{16:0}$ of the archaeological residues obtained from the various sites investigated. The distribution of archaeological residues plotting within the different fat categories are quite wide, in particular the dairy samples. This is attributed to intra-site variation, which was observed in bulk stable carbon analysis carried out by Lelli *et al.* (in press), who noted up to a 2.1‰ variation in the $\delta^{13}\text{C}$ of the terrestrial fauna analysed. Evershed *et al.* (2008b) also observed a wide $\delta^{13}\text{C}$ range for the $\text{C}_{16:0}$ fatty acids, and attributed this to the inclusion in the diet of water stressed plants, which are known to affect the $\delta^{13}\text{C}$ measurements of the organisms feeding on them (Tieszen, 1991). This represents a likely scenario in the Mediterranean, which could also cause the wide distribution of the measurements obtained. [$\Delta^{13}\text{C}$ values ($=\delta^{13}\text{C}_{18:0}-\delta^{13}\text{C}_{16:0}$); the $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}_{16:0}$ measurements denoting the different modern reference fat categories show the mean values obtained from Dudd (1999), Craig *et al.* (2005a, 2007, in press) and the Mediterranean measurements; the error bars denote $\pm 1\%$ standard deviation; NRA: Non-ruminant adipose; RA: Ruminant adipose; RM: Ruminant milk; MF: Marine fish].

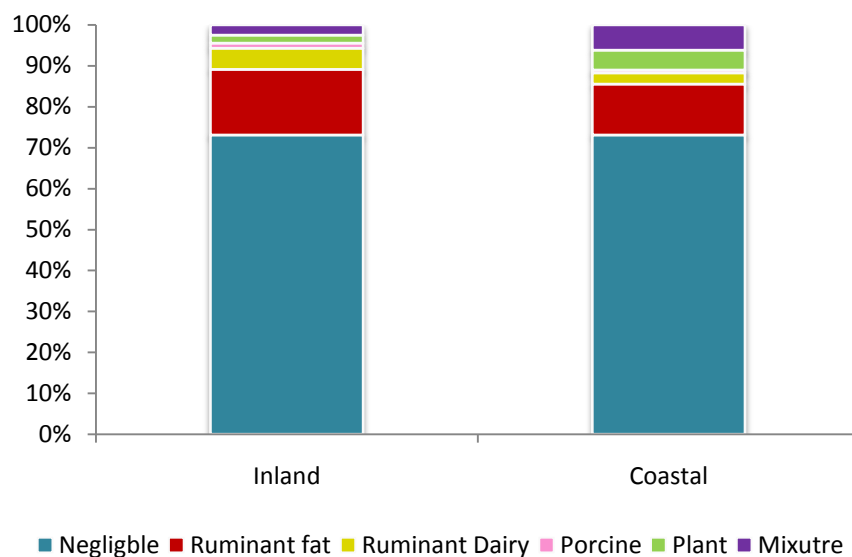


Figure 8.7: Chart showing the percentage of Impressed/Cardinal Ware vessels containing negligible, animal and/or plant absorbed residues, recovered from coastal and more inland sites investigated (defined in Table 8.1 and Table 8.2). [Mixture: includes residues with a combined animal (ruminant adipose, dairy and perhaps non-ruminant adipose) and plant contribution; Ruminant Fats: include residues which could not be securely assigned as dairy or adipose fats]

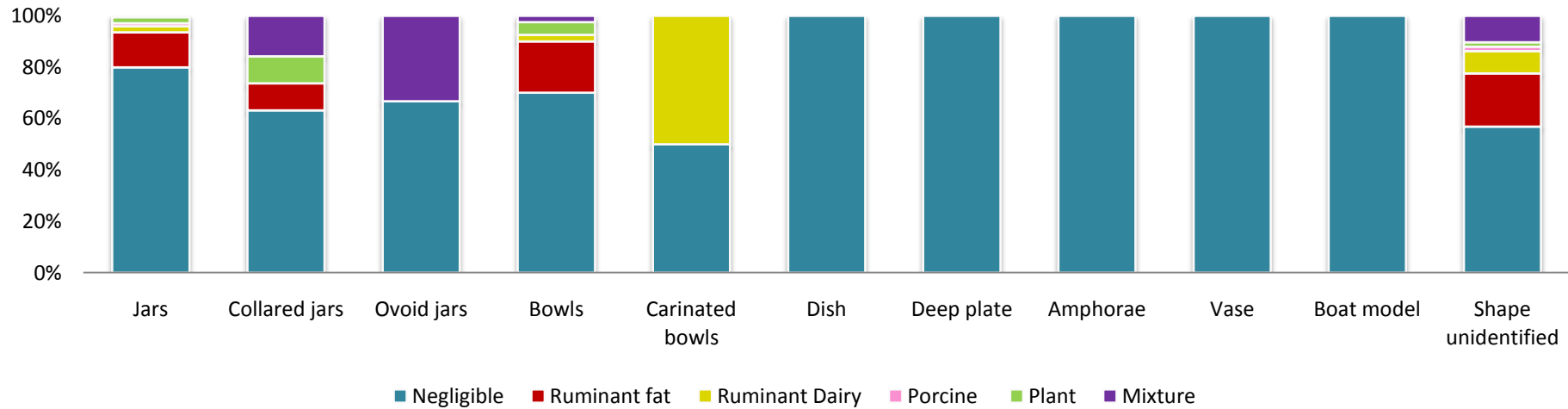
COASTAL SITES	Apulia, Italy					Calabria, Italy	Lazio, Italy	Malta	Croatia
	AZZ	BSP	MAS	SET	TRA	FAV	MAR	SKR	NAK
<6km from the coast									
Negligible	5	15	12	12	25	23	6	15	1
Ruminant fat	13	0	0	0	0	2	0	1	9
Ruminant Dairy	4	0	0	0	1	0	0	0	3
Porcine	1	0	0	0	0	0	0	0	1
Plant	1	0	0	0	0	2	0	0	0
Mixture	1	0	0	0	0	0	0	0	3

Table 8.1: Table listing the number of vessels recovered from the coastal sites investigated, containing negligible, animal and/or plant absorbed residues.

INLAND SITES	Apulia, Italy		Calabria, Italy		Catalonia
	CIC	BAL	PAL	SAR	CNS
>8km from the coast					
Negligible	15	33	35	4	19
Ruminant fat	5	0	0	6	7
Ruminant Dairy	1	0	0	1	2
Porcine	1	0	0	0	0
Plant	3	2	1	0	1
Mixture	0	0	3	4	2

Table 8.2: Table listing the number of vessels recovered from the more inland sites investigated, containing negligible, animal and/or plant absorbed residues.

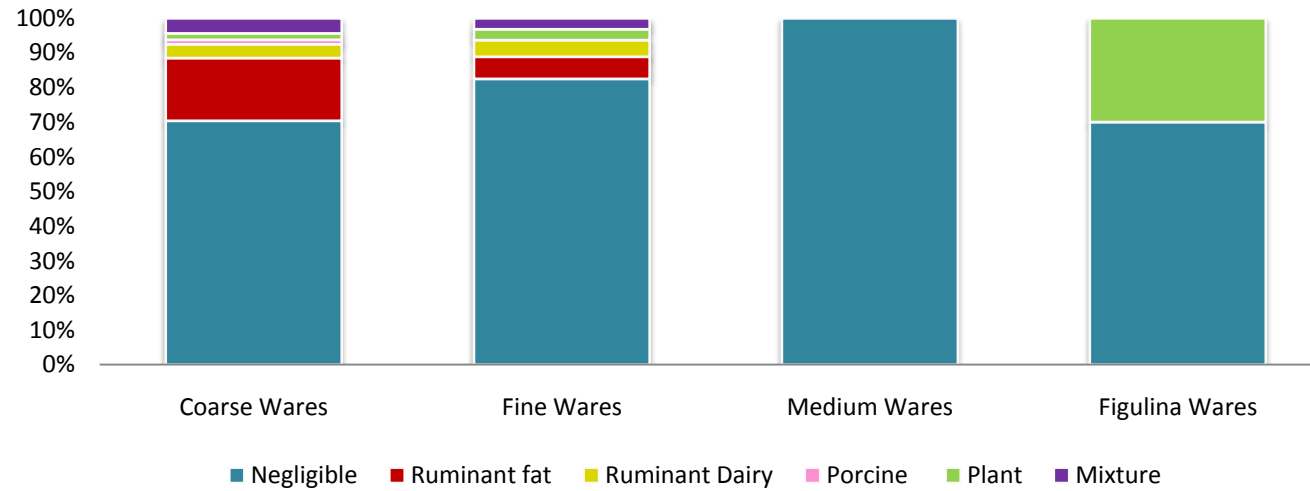
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Residue type	Jars (N)	Collared jars (N)	Ovoid jars (N)	Bowls (N)	Carinated bowls (N)	Dishes (N)	Deep plates (N)	Amphorae (N)	Vases (N)	Boat models (N)	Shape unidentified (N)
Negligible	135	12	2	28	2	1	1	2	3	1	33
Ruminant fat	23	2	0	8	0	0	0	0	0	0	12
Ruminant Dairy	4	0	0	1	2	0	0	0	0	0	5
Porcine	2	0	0	0	0	0	0	0	0	0	1
Plant	4	2	0	2	0	0	0	0	0	0	1
Mixture	1	3	1	1	0	0	0	0	0	0	6

Figure 8.8: Chart showing the different categories of fatty residues identified (if any) in the different vessel forms analysed, expressed as percentages. [N: Number of vessels]

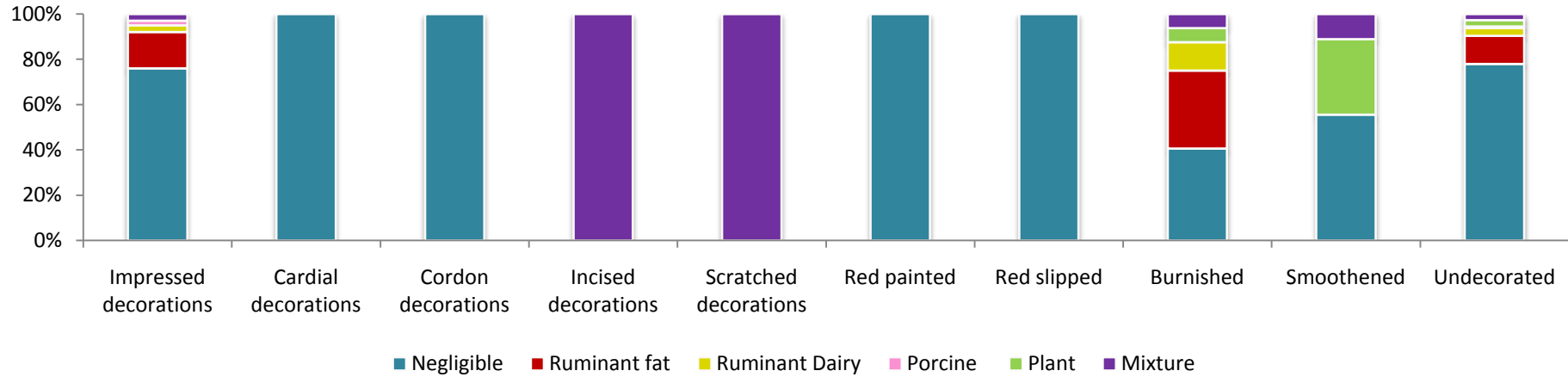
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Residue type	Coarse Wares (N)	Fine Wares (N)	Medium Wares (N)	Figulina Wares (N)
Negligible	159	52	2	7
Ruminant fat	41	4	0	0
Ruminant Dairy	9	3	0	0
Porcine	3	0	0	0
Plant	4	2	0	3
Mixture	10	2	0	0

Figure 8.9: Chart showing the different categories of fatty residues identified (if any) associated with the different ceramic fabrics analysed, expressed as percentages. [N: Number of vessels]

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Residue type	Impressed decorations (N)	Cardial decorations (N)	Cordon decorations (N)	Incised decorations (N)	Scratched decorations (N)	Red painted (N)	Red slipped (N)	Burnished (N)	Smoothened (N)	Undecorated (N)
Negligible	76	7	1	0	0	3	2	13	5	113
Ruminant fat	16	0	0	0	0	0	0	11	0	18
Ruminant Dairy	3	0	0	0	0	0	0	4	0	5
Porcine	2	0	0	0	0	0	0	0	0	1
Plant	0	0	0	0	0	0	0	2	3	4
Mixture	3	0	0	1	1	0	0	2	1	4

Figure 8.10: Chart showing the different categories of fatty residues identified (if any) associated with the decorative motifs, expressed as percentages. [N: Number of vessels]

8.4.1 Dairying in the Early Mediterranean Neolithic

In a recent publication, Rowley-Conwy (2011) suggested that dairy products may have played a crucial role in the survival of 'maritime pioneer farming' communities spreading throughout the western Mediterranean, especially during the first year of settlement, before reaping their first harvest. The nutritional value of ruminant milk is well known, and could potentially tide struggling communities over seasons of low productivity. Furthermore, the ability to process milk provides the added advantage of storing surplus dairy products (e.g. as cheese, yoghurt and butter) and make them available all year round (Evershed *et al.*, 2008b). A gene-culture coevolution between humans and cattle in Europe was in fact identified by Beja-Pereira *et al.* (2003). However studies conducted by Burger *et al.* (2007) suggest that prior to 8000 years ago, milk consumption in Europe could not have been widespread, since the allele responsible for digesting lactose, 13.910*T was absent. On the other hand, processed dairy products contain little or no lactose, hence lactose-intolerant people could still have consumed dairy products (Pollard and Heron, 2008:400).

The origins of dairying in Europe have been subject to much debate. The earliest artefactual evidence for milking dates to the Early Dynastic period of southern Mesopotamia (around 2600 BC), where pictorial representations of milking scenes were discovered on an inlaid limestone frieze in the Ninhursag Temple at Tell al-Ubaid (Greenfield, 2010), and a cylinder seal from the Uruk period dating to around 3500 BC, which shows herds of cattle on its upper register, and cattle tethered outside huts with calves and large ceramic vessels on the lower register (Sherratt, 1981). Particular ceramic vessel forms have also been associated with processing of dairy products, such as the spouted vessels from Kasteelberg D east in South Africa (Copley *et al.*, 2004), 'milk jugs' from central Europe (Craig *et al.*, 2003), and ceramic sieves from the Linear Pottery settlements in central Europe which are thought to have been used to separate curds and whey (Bogucki, 1984, 1993). Pastoral activities have also been inferred from the analysis of faunal remains recovered from archaeological sites, which are used to construct mortality profiles that can indicate the economic basis of a settlement. Hence, mortality profiles showing the slaughtering of very young animals (infants and juveniles) and adult females, have been associated with a dairying economy (Vigne and Hemler, 2007; Greenfield, 2010). However, although this technique has developed considerably over the last years, in particular in standardising the methodology applied and in being better able to identify the sex of the animal bones studied (Vigne and Hemler, 2007), kill-off patterns are never perfectly predictable, and faunal remains are subject to problems associated with bias, introduced by differential

preservation and recovery of the animal bones, and equifinality, that is, the possibility that different production strategies may still produce very similar mortality profiles (Halstead, 1988). The advantage of applying ORA is that it provides unequivocal, direct evidence for the use of dairy products, and can therefore securely identify pastoral activities. Immunological techniques have also been successfully used to analyse milk proteins in ceramics (targeting mainly α -casein) (Craig *et al.*, 2000b), however the advantage of using lipid residues is their higher survival potential over archaeological timescales.

The onset of dairying was until more recently, thought to have occurred towards the end of the Neolithic. Sherratt's (1981, 1983) concept of a 'secondary products revolution' suggested that during the Early Neolithic, sheep, goat and cattle were mainly utilised for their primary products (meat, bone and hide), with a limited use of their secondary products (milk, traction and wool) (Sherratt, 1997), which however intensified towards the end of the Neolithic, around the 4th millennium BC in the Near East and the 3rd millennium BC in Europe. Zooarchaeological analysis carried out by Vigne and Hemler (2007) on faunal remains recovered from several sites in the Near East and the Mediterranean (including southern France, Italy and the Balkans), suggest that dairying was already practiced at the earliest stages of the Neolithic, starting from the early 8th millennium BC (mid-PPNB) in the Near East, and the mid 6th millennium BC in Mediterranean Europe (Vigne and Hemler, 2007). They observed that out of 36 mortality profiles analysed, 80% showed a mixed meat and milk production strategy, while 19% were pure milk. In view of issues outlined above on the use of faunal remains to determine economic subsistence, particularly equifinality, more direct methods are required to further support these results, which can be obtained by the application of ORA. ORA was in fact carried out by Evershed *et al.* (2008b) on pottery vessels dated to the 7th millennium BC in the Near East and Anatolia, and the results obtained directly confirmed the presence of dairy residues. Other applications of ORA have also established the presence of dairy residues in Early Neolithic ceramics excavated from sites located in central Europe (Craig *et al.*, 2005a; Evershed *et al.*, 2008b) and the UK (Copley *et al.*, 2003, 2005b, 2005d). Direct evidence for the processing of dairy products in ceramic pots has been more recently obtained from the Libyan Sahara, through the application of ORA to pottery vessels recovered from the rock shelter of Takarkori, located in the Tadrart Acacus Mountains (Dunne *et al.*, 2012). This study showed that pastoral activities were already being carried out by the Middle Pastoral period, dating to 5200-3800 BC (Dunne *et al.*, 2012). An early date for the use of dairy products in the Mediterranean region can also be confirmed through the present study, which identified dairy residues in Impressed/Cardial Wares dating to the late 7th millennium BC. This early attestation of pastoral practises in the Mediterranean was confirmed by the presence of

dairy residues in Impressed Wares recovered from the site of Fondo Azzollini, Pulo di Molfetta (Apulia, Italy), dated to 6100-5880 cal. BC. Dairy residues were also present in Early Neolithic Impressed/Cardial Wares recovered from Croatia, Catalonia, and other sites in the Apulian and Calabrian regions of Italy, which suggest a widespread use of dairy products from the onset of the Neolithic in the Mediterranean.

Figure 8.11 maps the earliest identification of dairy residues using ORA, from the Levant westwards. The earliest dates associated with direct evidence for the use of dairy products were obtained in Anatolia and the Near East, and can be observed to become progressively younger when moving westwards, mirroring the earliest chronological attestations for the onset of farming (*cf.* Figure 8.12). Dairying can therefore be traced back to the early stages of the Neolithic, and probably spread together with agrarian practices, across Europe and the Mediterranean. This can perhaps be supported by the identification of dairy residues in ceramic vessels obtained from Layer 18 at Can Sadurní (Catalonia), which produced one of the earliest dates for the first attestation of the Neolithic on the Iberian Peninsula (Layer 18 dated to 5475-5305 cal. BC; Blasco *et al.*, 2005), and therefore proves that pastoral activities were indeed present at the onset of the Neolithic at this site. In a study conducted by Martí-Oliver *et al.* (2009) at Cova de l'Or (Alicante, Spain), GC-c-IRMS analysis also attested to the use of dairy products in early Cardial Wares, which support the results obtained at Can Sadurní. Dunne *et al.* (2012) suggest that the direct attestation of dairy products in ceramics dated to the Middle Pastoral period in the Libyan Sahara indicate 'a local process of pastoral development'. However, the dates obtained for the earliest direct attestation of dairying at the Takarkori rock shelter in the Libyan Sahara are later than those obtained in Anatolia, the Levant and the Mediterranean (*cf.* Figure 8.11). While this does not preclude an independent onset of pastoralism, this hypothesis requires evidence for a local domestication of cattle in Africa, to prove that the onset of dairying was not influenced by the spread of exogenous pastoral activities. However as yet, there is no consensus on the origin of cattle domestication in Africa (see Gifford-Gonzalez, 2011 for an overview, Loftus *et al.*, 1994; Mikko and Anderson, 1995; Bradley *et al.*, 1996; Troy *et al.*, 2001; Beja-Pereira *et al.*, 2006).

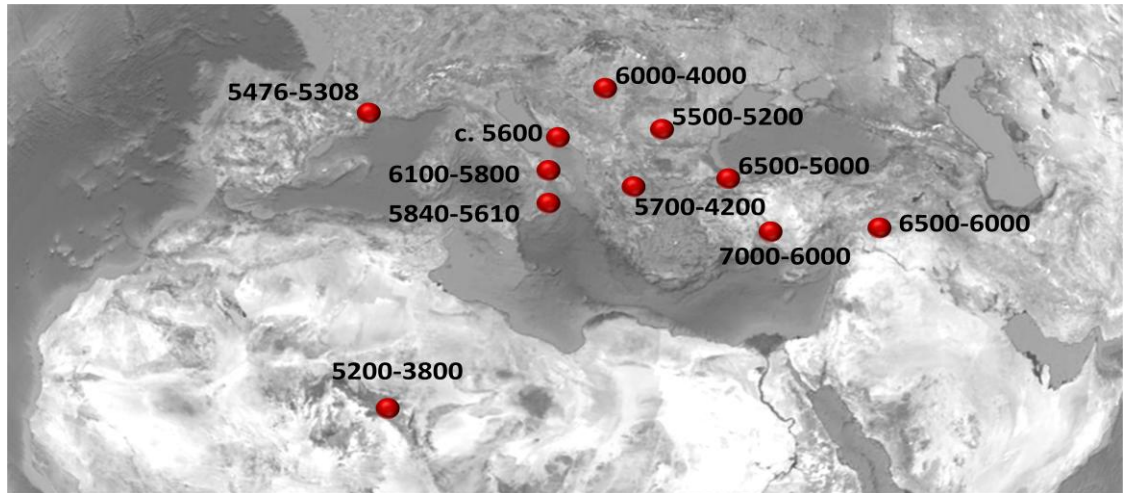


Figure 8.11: Map showing the earliest direct evidence for dairy products obtained through the application of Organic Residue Analysis (ORA) to ceramic vessels. The dates are reported as BC. The map was compiled using data from the present research and published literature (Craig *et al.*, 2005a; Evershed *et al.*, 2008b; Dunne *et al.*, 2012).

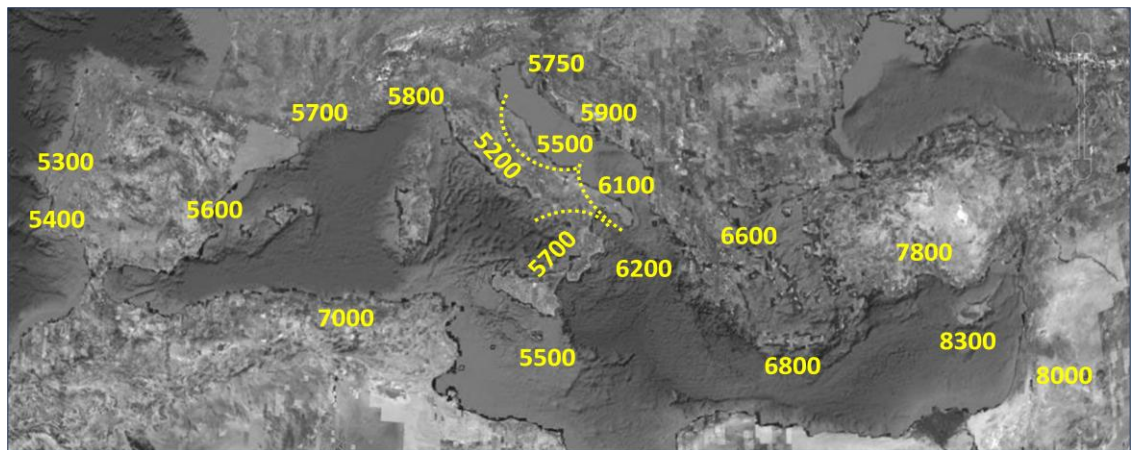


Figure 8.12: Map showing the earliest dates for the presence of domesticates and Impressed/Cardial Wares. Dates are reported in BC. (After Zilhão, 2001; Balossi and Frangipane, 2002; Benvenuti and Metallinou, 2002; Maggi, 2002; Trump, 2002; Skeates, 2003; Zilhão, 2003; Pace, 2004; Forenbaier and Miracle, 2005; Fenech, 2007; Guilaine and Manen, 2007; Pessina and Tiné, 2008:27).

As already stated above, it was not possible to analyse the fatty acid distribution of the triacylglycerols identified (following the method described in Mirabaud *et al.*, 2007), to attempt to distinguish between ruminant dairy fats originating from goats and cattle, since only trace amounts of triacylglycerols survived. However, the faunal remains recovered from the sites investigated were generally dominated by ovicaprids, with fewer cattle bones, suggesting perhaps a greater exploitation of the former. Vigne and Hemler's (2007) study described above, also suggested that goats were the first to be exploited for their milk, followed by sheep, and despite the fewer cattle bone remains, post-lactation kill off patterns were identified, suggesting that cattle were also exploited for dairying during the Early Neolithic, both in the Near East and the

Mediterranean. Vigne and Hemler's (2007) results are therefore comparable to the remains attested at the sites investigated in the present research, which could perhaps indicate a similar pattern of exploitation. Of interest, cattle bones appear to be the dominant species exploited for their dairy products in north-western Anatolia (Evershed *et al.*, 2008b) and the Libyan Sahara (Dunne *et al.*, 2012).

ORA on ceramics dated to 6500-5000 BC in north-western Anatolia showed that 10% of the ceramic assemblage analysed contained dairy residues (Evershed *et al.*, 2008b), while 14% of the ceramics analysed from the Neolithic sites of Schela Cladovei and Escegfalva 23 in central Europe were dairy in origin (Craig *et al.*, 2005a). At the Early Neolithic site Fondo Azzollini (Apulia, Italy), 16% of the assemblage studied yielded dairy residues, while at Ciccotto (Apulia, Italy) and Grotta San Michele, Saracena (Calabria, Italy), dairy fats were identified in only one or two Early Neolithic vessels. At Nakovana Cave (Croatia), which was a particularly lipid rich site, dairy products were identified in 18% of the ceramics analysed, while at Can Sadurní, 18% of the vessels dated to the Epicardial Phase comprised dairy residues, as opposed to 8% of the vessels dated to the Cardial phase. However, the earlier Cardial Phase was associated with a funerary deposit, whereas the Epicardial Phase comprised a habitation context, which may explain the higher dairy attestations in the latter. Using ORA to assess the scale of pastoral activities is therefore difficult, and cannot be interpreted by comparing the number of times dairy residues are attested. The type of deposition context from which ceramic vessels are analysed needs to be carefully considered, as in the case of Can Sadurní, while factors such as vessel re-utilization and lipid preservation will also introduce bias. For example, there is a considerable increase in the percentage of Early Neolithic ceramic vessels containing dairy residues recovered from the UK (22%) (Copley *et al.*, 2005b), when compared to those present in north-west Anatolia, central Europe and the Mediterranean. This is however, not necessarily indicative of a more intense use of dairying in the UK, which could be related to cultural practices or dietary requirements. It could also simply reflect the better lipid preservation conditions associated with cooler, northern latitudes, as discussed in Section 8.2. Hence, while ORA can be securely used to provide direct evidence for the presence of ruminant dairy residues, and can provide indications relevant to the intensity of use of dairy products, it cannot be used to provide confident assessments pertaining to the scale of production.

In conclusion, dairy products were identified in Impressed/Cardial Wares dating to the late 7th millennium BC, in six out of the 14 sites investigated in the Mediterranean situated in Croatia, Italy and Catalonia. This confirms that pastoral activities were carried out from the onset of the

Neolithic in this region, and that the use of dairy products was already widespread across the Mediterranean basin. The identification of dairy products also ties the function of Impressed/Cardial Wares more securely with agrarian practices, and it is therefore highly likely that both domestic animals and pottery spread simultaneously throughout the Mediterranean. Finally, experimental re-constructions have shown that domesticates could indeed have been transported in boats (Broodbank and Strasser, 1991), evidence for which has been found at La Marmotta in Italy (Fugazzola Delpino, 2002a). The identification of dairy products in Early Neolithic Impressed/Cardial Wares therefore supports Rowley-Conwy's (2011) hypotheses, that dairy products could have been crucial to the survival of early settlers, and by extrapolation, appears to sustain the 'maritime pioneer colonization' model proposed to explain the spread of farming in the western Mediterranean.

8.5 Marine products, or rather, the lack of...

The extent of human dependence on marine products, in particular at the transition to agriculture, has been widely researched and debated. Stable carbon and nitrogen isotope analysis has consistently shown a dietary shift, from a predominantly marine to a terrestrial diet during the Mesolithic-Neolithic transition in the United Kingdom and Scandinavia (Schulting and Richards, 2002a; Richards *et al.*, 2003; Lidén *et al.*, 2004). This approach measures the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of human bone collagen, and can differentiate between contributions from marine and terrestrial animal foods because both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are more positive in marine sources compared to terrestrial animals (Sealy, 2001). Stable carbon and nitrogen analysis applied to Mediterranean Neolithic contexts at Arene Candide (Liguria, Italy) and Pendimoun (southern France) (Le Bras-Gaude *et al.*, 2006), Fontbrégoua, also in southern France (Le Bras-Gaude *et al.*, 2010), Montou in the Pyrenees (Le Bras-Gaude and Claustre, 2009), and in several other Early Neolithic sites along the Croatian coast (including Pupićina, Grapčeva and Crono Vrlo) (Lightfoot *et al.*, 2011), also appear to suggest a departure from the inclusion of marine food sources during the Neolithic. Moreover, little or no evidence for fish bones have been found in the archaeological deposits at these sites, which could however be potentially due to preservation issues. Stable isotope analysis carried out on eight skeletons excavated from the Brochtorff Circle in Gozo (Malta) showed no evidence for a marine input (Richards *et al.*, 2001), as at Alepotrypa Cave, Franchthi and Kephala in Greece (Papathanasiou *et al.*, 2000; Papathanasiou, 2003). All these sites are located within easy reach of the Mediterranean Sea, except Fontbrégoua which lies about 100km inland. Recent stable isotope analyses carried out on skeletons recovered from various sites in the Marche, Tavoliere and Murge regions of Italy (Lelli *et al.*, in press), some of which

(Balsignano, Masseria Maselli and Palata 1) were also investigated in this research, showed a small but significant marine input in humans buried along the Apulian and Marche coastal areas, whereas limited or no evidence was obtained for a marine contribution to the dietary requirements of individuals buried further inland (Lelli *et al.*, in press).

Using Hawkes and O'Connell's (1992) and Winterhalder's (1993) discussion on optimal foraging theory, Richards and Schulting (2006) suggest that compared to agrarian practice, fishing is more time consuming and does not produce high yield returns. Hence, although the sea was an available resource, Neolithic communities were more likely to subsist on the more efficient and productive activities of terrestrial produce (Richards and Schulting, 2006). While acknowledging the logic behind this argument, Craig *et al.*'s (2011) research showed that at the transition to agriculture in the Baltic, marine resources were still an important dietary component, which therefore reopens the issue as to whether this apparent under-use of an available resource during the Neolithic is in fact a cultural choice, or whether the research methods applied to date are perhaps not sensitive enough to detect a marine signal. This issue has already been widely debated in terms of bias in the zooarchaeological record and in the number of human bone collagen samples analysed which are then used to infer the dietary composition of a population, as well as the efficiency of the stable isotope method used (Hedges, 2004; Milner *et al.*, 2004, 2006; Richards and Schulting, 2006). The main issue in the latter, is the low isotopic visibility of marine contributions, despite non-isotopic evidence for the consumption of marine products (Hedges, 2004). For example, if marine products are consumed seasonally or only on special occasions during adulthood, it is unlikely that a marine contribution will be registered in the bone collagen, which is laid down during adolescence, with subsequent remodeling occurring during an individual's lifetime (Hedges, 2004). Hedges (2004) suggests that a minimum of 20% marine protein needs to be included in the diet for it to be detected isotopically. However, changes to the $\delta^{13}\text{C}$ values of bone collagen can occur, which may mask a marine contribution and are dependent on the amount of protein included in the diet (Richards and Schulting, 2006). In diets rich in protein, the carbon in bone collagen is likely to originate from dietary protein, hence reflect its $\delta^{13}\text{C}$ measurements. However, in diets which are low in protein, the carbon in bone collagen is more likely to originate from the total dietary carbon, therefore include contributions from carbohydrates and lipids which are depleted in ^{13}C by 1‰ and 5‰ with respect to protein, respectively (Hedges, 2004). This may lead to masking, hence underrepresentation of a marine component (Hedges, 2004). This could in fact, be a major contributing factor to the absence of a marine signature in the diet of Early Neolithic individuals in Apulia (Italy) studied by Lelli *et al.* (in

press), since their research and other palaeodietary evidence (see Section 8.3) provide strong evidence for a heavy reliance on plant material in the Early Neolithic diet in this area.

Three of the samples extracted from Impressed/Cardial Wares (AZZ-13I, SKR-16I and CNS-06I), did show higher $\delta^{13}\text{C}$ values for their $\text{C}_{16:0}$ fatty acids, which in the absence of C_4 vegetation, could be indicative of a fish origin. However, fish biomarkers, which would have securely identified the processing of fish products, were not present, and it could only be tentatively suggested that perhaps these residues originated from mixed fish and terrestrial products. ORA results therefore suggest a limited use of marine products during the Early Neolithic in the Mediterranean. Of course, pottery vessels are not always used to cook fish, however despite floatation methods used at most sites, fish bones were remarkably scarce, with none being identified in most of the sites included in this research, except at Favella della Corte (Calabria, Italy) and La Marmotta (Lazio, Italy). Biomolecular identification of marine products in archaeological residues using ORA has a relatively long history of research (Morgan *et al.*, 1983, 1984, 1992; Patrick *et al.*, 1985; Brown, 2001; Brown and Heron, 2003; Copley *et al.*, 2004; Hansel *et al.*, 2004; Craig *et al.*, 2007; Evershed *et al.*, 2008a; Olsson and Isaksson, 2008; Hansel and Evershed, 2009; Heron *et al.*, 2010; Craig *et al.*, 2011; Hansel *et al.*, 2011). Fish have a very complex fatty acid composition, comprising relatively little saturated fatty acids ($\text{C}_{14:0}$, $\text{C}_{16:0}$ and $\text{C}_{18:0}$), and consisting mainly of long chain unsaturated fatty acids containing 20 to 22 carbon atoms, with up to six double bonds (deMan, 1999). These unsaturated fatty acids are highly susceptible to degradation, especially by oxidation (Aillaud, 2001), which hindered early attempts to detect them in pottery and sediments. Recent experiments have successfully related various degradation products of marine fats and oils to their precursor compounds, including ω -(*o*-alkylphenyl)alkanoic acids of carbon length C18, C20 and C22, isoprenoid fatty acids (phytanic, pristanic and 4,8,12-tetramethyltridecanoic acid), and dihydroxy acids (Hansel *et al.*, 2004; Evershed *et al.*, 2008a; Hansel and Evershed, 2009; Heron *et al.*, 2010; Hansel *et al.*, 2011). The increased stability of these compounds make them excellent biomarkers for marine oils, and they have been detected in archaeological ceramics dating to around 4000 cal. BC (e.g. Hansel *et al.*, 2004; Craig *et al.*, 2007, 2011; Heron *et al.*, 2010). It was in fact, the application of ORA on ceramics dating to the Mesolithic and Neolithic periods in the Baltic that identified, for the first time, a continued exploitation of aquatic resources during the transition to agriculture (Craig *et al.*, 2011).

It is difficult to perceive why people would 'turn their backs' on a freely available resource, especially when considering that most of the sites investigated in this research are located within 6km or less of the Mediterranean coast. Yet only three out of the 301 ceramic vessels analysed

tentatively suggest a marine input, while a secure characterization for fish oils could not be made. It must however be noted that the poor lipid yield obtained from the ceramics investigated may have resulted in the marine biomarkers being too depleted to be detected. However, the absence of fish bones in most of the sites included in this study provides no indication that perhaps other cooking/preparation methods had been utilized. When considering that current models for the transition to agriculture in the Mediterranean suggest that these early farmers were seafarers, and hence had a close connection to the sea, the absence of a marine component perhaps indicates a conscious decision to avoid marine food products, in favour of terrestrial produce.

8.6 Recommendations for further work

The following recommendations are proposed to build on the experimental work carried out in this research, and expand the analytical work carried out on identifying the function of Early Neolithic Impressed/Cardial Wares using ORA:

- A. Chapter 4 reported shifts in the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ measurements of modern reference animal fats of Mediterranean origin compared to their northern European counterparts, which were observed after carrying out a controlled feeding experiment. The following recommendations for further work propose to validate the results obtained from experiment carried out:
1. Extend the evidence obtained for regional patterning of $\delta^{13}\text{C}$ values by analysing a wider variety of plants obtained from different environmental contexts, to monitor changes to the $\delta^{13}\text{C}$ measurements.
 2. Compare $\delta^{13}\text{C}$ values of plant material used as fodder/forage to the $\delta^{13}\text{C}$ values obtained from the adipose tissue/blood and milk, to better understand the relationship between the dietary and adipose/blood and milk $\delta^{13}\text{C}$ values.
 3. Assess changes to the $\delta^{13}\text{C}$ in adipose/blood and milk by varying the contribution of carbohydrate, protein and lipid components in the diet. This could perhaps explain why only the $\delta^{13}\text{C}$ measurement of the $\text{C}_{16:0}$ was observed to be higher in Mediterranean non-ruminants, while no changes were observed to the $\delta^{13}\text{C}$ values of the $\text{C}_{18:0}$.
 4. Attempt to better understand the depleted $\delta^{13}\text{C}$ values obtained for the Mediterranean marine fish, when compared to the $\delta^{13}\text{C}$ values obtained for fish caught in the Baltic Sea.
 5. Further investigation is required to define the as yet wide $\Delta^{13}\text{C}$ values produced by deer adipose, which overlap considerably with the fields denoting ruminant adipose and dairy fats, and add complexity to the interpretation of archaeological residues plotting within the areas of overlap.

- B. Plant material is a major dietary component, and much work has been directed towards identifying different plant varieties consumed in the past. As discussed in Chapter 5 and Section 8.3 above, identification of plant residues using ORA is difficult and indications for the processing of plant material in ceramic vessels are usually tentative at best. This leads to under-representation of plant residues in ceramics analysed using ORA, which justifies the necessity for further work to improve detection of plant residues. The following are recommended as further work:
1. Expand the range of plant types analysed, targeting potential biomarkers which are likely to securely confirm the presence of a plant residue, and perhaps identify plant species present in archaeological residues.
 2. Modify the extraction and analytical protocol to target specific plant lipid constituents known to occur in different plant types, which are likely to survive over archaeological timescales.
 3. Target the carbohydrate and protein fractions, which are also major constituents in plants (e.g. einkorn and acorn). These higher molecular weight compounds can be identified using several analytical techniques, including HT-GC/MS, Liquid Chromatography-MS (LC-MS) and Pyrolysis-GC/MS (Py-GC/MS). Soft ionization techniques, e.g. Electrospray Ionisation (ESI) or Desportion Elecrospray Ionisation (DESI) coupled to a MS detector could also help identify higher molecular weight compounds without the need for further fractionation of the compounds. Carrying out comparative analysis using different types of detectors (e.g. Tandem MS), could also provide further information on the structural composition of the residues analysed.
 4. Much work has already been carried out on developing accurate databases which allow different plant varieties to be identified through the morphological characteristics of their microfossils (e.g. starches and phytoliths), which survive on lithics, in ceramics vessels and in dental calculus. Further investigation at biomolecular level might also help improve species identification.
- C. One of the major difficulties encountered in applying ORA to Impressed/Cardial Wares was the large percentage of vessels containing very low lipid yields, which could not be securely interpreted as resulting from poor lipid preservation conditions during burial, or as reflecting the function assigned to the individual vessels. This problem could potentially be addressed by:
1. Increasing the number of vessels analysed from each site, and expanding the number of Early Neolithic sites included in the study, which have a similar burial depositional environment.

2. Further experimentation using different lipid extraction techniques could also potentially enhance lipid yields, in particular, Accelerated Solvent Extraction (ASE) and Microwave Accelerated Reaction System (MARS). The latter has already proved considerably efficient at enhancing the extraction of $C_{16:0}$ and $C_{18:0}$ from samples obtained from the Near East, which could then be characterised using GC-c-IRMS (Gregg *et al.*, 2009; Gregg and Slater, 2010). The drawback in using MARS is that i) further investigation is required to ensure that the $C_{16:0}$ and $C_{18:0}$ extracted using MARS originate solely from the absorbed residues; ii) GC-c-IRMS analysis is still quite expensive.
- D. Another difficulty related to the large percentage of vessels showing negligible residues was that this limited further analysis which attempted to observe variation over time (by analysing vessels dating from Early and Middle Neolithic deposits), and space (by including ceramic vessels recovered from inland and coastal sites, and sites located in different Mediterranean countries). The assemblages studied were also highly fragmented, which precluded a more in depth analysis on possible trends associated with vessel shape and the type of residue absorbed within the ceramic walls. The following are therefore recommended:
1. Expand the area of research, which could potentially identify lipid rich sites that would allow a further comparative analysis over time and space, and test the observations presented in this research.
 2. Target vessels with a known shape. This however is often difficult, since ORA is a destructive technique, which therefore requires careful consideration by museum curators before allowing sampling.
- E. As discussed in the previous Section, the absence of a marine input in the diet of Early Neolithic Mediterranean individuals is an intriguing issue, which requires further investigation. While ORA is a powerful tool for characterising residues of food commodities processed in ceramic vessels, it cannot offer a more encompassing view of human palaeodiet, since not all food preparation requires processing in ceramic pots. Perhaps increasing the sensitivity of carbon and nitrogen stable isotope analysis to marine signals, or targeting other isotopes (e.g. sulfur), which is a known detector of marine signals, could perhaps shed some light on the issue.

8.7 Conclusion

This research provided the first extensive application of ORA in the Mediterranean region. The controlled feeding experiment carried out produced the first reference database of $\delta^{13}C$ and $\Delta^{13}C$ measurements from modern terrestrial animal fats and marine oils of Mediterranean origin. Shifts

to the $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ values of Mediterranean modern reference fats were identified when compared to northern European counterparts, and were used to produce the $\Delta^{13}\text{C}$ ranges of modern reference fats used to interpret archaeological residues extracted from Impressed/Cardial Wares.

Lipid preservation absorbed within the walls of ceramic vessels recovered from Mediterranean archaeological contexts was found to be low, which is consistent with previous research carried out in areas with similar climatic conditions (e.g. the Near East). However, in view of further experimental work carried out in this research, the high proportion of negligible residues identified could not be solely attributed to poor preservation conditions during burial. Experimental investigation into the degradation of plant residues showed that ceramic vessels used to process plant products will produce negligible residues after a prolonged burial period. This showed that the function assigned to individual vessels also determines whether or not lipids are likely to become absorbed within ceramic walls, hence retain a significant residue over archaeological timescales. As discussed in detail in Section 8.2 above, negligible residues can be produced by i) poor preservation conditions in the burial context, ii) the fat/oil content of the commodity processed within the ceramic vessel, which is likely to lead to a lipid residue being absorbed within the ceramic wall; iii) the number of times a pot was re-utilised and iv) the function assigned to a particular vessel, that is whether it was used for cooking, storage or as a serving vessel. This introduces a cautionary note in interpreting negligible residues, which has been emphasized throughout this research, and applied when interpreting archaeological residues extracted from the Impressed/Cardial Ware vessels analysed.

Finally, 301 lipid residues extracted from Impressed/Cardial Ware vessels recovered from 14 Early Neolithic sites located in Italy, Croatia, Malta and Catalonia were characterised using ORA. GC, GC-MS and GC-c-IRMS analysis confirmed that 15% of the vessels submitted for analysis had been used to process ruminant fats, while 4% of the pots were securely identified as having been used to process dairy products. Non-ruminant fats and plant oils were also identified, as well as residues containing mixtures of animal fats (including ruminant and porcine adipose and dairy fats) and plant products. Of particular interest was the absence of marine biomarkers from all the residues extracted, which perhaps suggest a conscious avoidance of marine products in the Early Neolithic diet at the sites investigated. No distinctive trends were observed between the type of absorbed residue identified, and the different vessel forms and ceramic fabrics. Similarly, the type of decoration applied to the vessels was not particular to the different food commodities processed with these wares. The function of Impressed/Cardial Wares was also observed to have

been consistent in sites located close to the Mediterranean coast, and those situated further inland. No variations were noted in the utilization of Impressed/Cardial ceramics in sites located in different countries within the Mediterranean basin. Hence, the function of Impressed/Cardial Wares during the Early Neolithic appears to have been quite homogenous over such a widespread geographical context.

Finally, the identification of dairy residues in Impressed/Cardial Wares using ORA produced the first unequivocal direct evidence for the widespread use of dairying from the earliest phases of the Neolithic in the western Mediterranean, dating to the late 7th millennium BC. Dairy products were identified in Impressed/Cardial Ware vessels recovered from Early Neolithic sites situated in Croatia, Italy and Catalonia, hence providing secure evidence that the nourishing qualities of dairy products were widely recognised and included in the Early Neolithic diet. The identification of dairy residues in Impressed/Cardial Wares also allowed a more direct connection to be made, between these ceramic wares and the first agrarian/pastoral communities in this region. This finding addresses the main research question set for this study, which questioned the association between Impressed/Cardial Wares and domesticates, despite their close chronological appearance in Early Neolithic stratigraphies. Identifying evidence for the use of Impressed/Cardial Wares in pastoral activities directly ties their use to agrarian/herding communities, and suggests that the spread of Impressed/Cardial Wares occurred together with domesticates, by farming communities who arrived in the western Mediterranean by the late 7th millennium BC.

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