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CHAPTER EIGHT
Organic Residue Analysis and the Decomposition of Fatty Acids in Ancient Potsherds

J.W. Eerkens

Jelmer Eerkens, Associate Professor; Department of Anthropology, University of California, Davis; One Shields Avenue; Davis, CA 95616; USA; <jweerkens@ucdavis.edu>.

Determining the function of prehistoric artifacts has long been an important avenue of archaeological inquiry (for instance Bennett 1943, 1944; Clark 1939; Linton 1944; Smith 1910 or Steward and Setzler 1938). Although archaeology has moved beyond the 'functionalist' theoretical paradigm, so influential in the 1930's through 1950's (Trigger 1989; Willey and Sabloff 1980), where every artifact was understood to confer some adaptive advantage to the people who used it, determining the use of artifacts continues to play an important role in reconstructing prehistoric behavior. In archaeology today, determining the function of an object is rarely an end product. Instead, the function of an artifact is usually used as one data set to help inform on other behavior, such as the organization of technology, the division of labor, gender relations and issues concerning diet, among other topics.

Organic residue analysis reflects one line of investigation that archaeologists have employed to attempt to deduce function of artifacts, especially pots (Charters et al. 1997; Copley et al. 2005; Deal and Silk 1988; Eerkens 2002, 2005; Evershed et al. 1997, 2003; Heron et al. 1991; Malainey 1999c; Morton and Schwarcz 2004; Mottram et al. 1999; Reber and Evershed 2004; Skibo 1992; Stott et al. 1999) which are the subject of this chapter, though pipes (Rafferty 2002), hunting weapons (Craig and Collins 2002; Fullager and Jones 2004; Pearsall et al. 2004; Rots and Williamson 2004; Wadley et al. 2004) and cooking stones (Quigg et al. 2001, Buonasera 2005) have also been examined. There are other approaches to help reconstruct the function of ancient pots, including engineering analyses (Arnold 1985; Bronitsky and Hamer 1986; Brown 1989; Feathers 1989; Juhl 1995; Linton 1944; Rice 1987; Rye 1976; Skibo et al. 1989; Smith 1985), use wear studies (Beck et al. 2002; Halley 1983; Rice 1987; Shiffer 1989; Skibo 1992) and ethnographic analogy (Costin 2000; Hegmon 2000; Henrickson and McDonald 1983), but these methods are not often definitive. They usually provide only hypotheses about the types of foods that may have been cooked or stored in a pot. Organic residue analysis has the potential to be more precise about the foods that were prepared or stored within a pot, hence the intense interest by archaeologists in developing this method over the last decades. Indeed, if the rapidly expanding literature is any measure, the expected future payoffs from this field are high. However, as several of the chapters in this volume attest, residue analysis is still in its infancy and there is much to be learned and fine-tuned, particularly on the methodology of the final interpretation of the biochemical findings.

Organic Residue Analysis

Although a range of organic compounds have been recovered from archaeological potsherds including amino acids, waxes, and cholesterol, fatty acids have been the principal class of compounds targeted for analysis in archaeological studies. This is due in part to their ease of extraction from potsherds and the widespread availability of the instruments needed to detect and quantify their presence. However, the main reason fatty acids have been targeted is undoubtedly the stability of these biomolecules over long periods of time (Christie 1989; Evershed 1993). Relative to DNA, proteins or carbohydrates, lipids (including fatty acids) are relatively resistant to decomposition and degradation.

That fatty acids are often present in ancient sherds, occasionally in very high quantities, has been amply demonstrated by archaeologists and chemists. Fatty acids are particularly prevalent in the interior walls of pots, especially near the neck and rim and occupy small vugs or open spaces within the ceramic fabric. What is less clear is that such residues actually represent the unmodified, or little modified, byproducts of ancient foods cooked or stored in the vessels as several alternatives exist. First, fatty acids are produced by nearly every organism, from bacteria to mammals, and are therefore present in virtually every environment on earth and can simply be native to the clays that people use to make pots. Second, fatty acids could represent post-depositional contamination by, for example, bacteria that are consuming other food residues such as proteins within the sherds, or free fatty acids leaching into sherds from the surrounding soil. Third, the fatty acids that we find may only represent a small fraction of what remains after decomposition due to processes such as oxidation and hydrolysis. Finally, fatty acids may be entirely the product of laboratory contamination.

We can probably rule out several of these possibilities. The first possibility, native contamination, is unlikely. The exposure of fatty acids to high temperatures, which promotes oxidation, leads to decomposition. Fatty acids

are quite stable in temperatures below 200°C, but rapidly oxidize between 200-250°C (De Souza et al. 2004; Frankel 1980, 1987, 2005; Santos et al. 2002), with polyunsaturated and monounsaturated fats degrading at slightly lower temperatures than saturated fats. This is much lower than the minimum temperature required to fire a pot, 500-800°C. Thus, any native fatty acids in a clay are extremely unlikely to survive firing. As well, it is higher than the temperature achieved in most pre-industrial ceramic cooking methods. Thus, we can be fairly confident that pots start out clean of fatty acid s and that the act of cooking will not degrade them. Indeed, experiments by Johnson et al. (1988) suggest that firing of clay tiles to 400-600°C in both oxidizing and non-oxidizing environments essentially removes all hydrocarbons and fatty acids, though some carbon remains in the form of inorganic compounds and pure carbon (such as coal).

Similarly, the fourth possibility, that fatty acids are merely the product of laboratory contamination, is also dismissible. Most laboratories run blanks and other controls to evaluate the influence of such contamination. Low levels of fatty acid contamination are in most cases unavoidable due to their ubiquity in the environment. However, archaeological sherds often contain concentrations of fatty acids that are a level of magnitude or greater than the blanks, indicating that most are native to the sherds themselves. In any case, these are the sherds that archaeologists should be including in their interpretations.

As well, it has been demonstrated that sherds buried in archaeological sediments are not contaminated by the influx of fatty acids from nearby soils. Tests examining the fatty acid profiles of sherds and the immediately surrounding soil show that the two are quite different (Deal and Silk 1988; Heron et al. 1991). This is likely due to the fact that fatty acids, like all lipids, are water insoluble which would also help keep water out of the walls of pots infused with lipids or coated with residues or carbonized remains. Many ethnographic studies suggest people either coat cooking pots with, or simply soak them in, lipid-rich products (Arnold 1985, 140). These activities help to prevent water from the interior from leaking through the pot. As well, experimental studies suggest that water rich in organic matter penetrates the walls of porous pots and deposit residues there (Skibo 1992, 151), presumably as the water evaporates leaving behind organic materials. Such residues are not removed by washing. These findings suggest that the residues in the interior walls most likely represent the application of such lipid-rich mixtures, or the primarily remains of the first several uses of a pot. After a pot has become infused with organic residues, water no longer leaks through and there is little room for the accumulation of additional residues.

I am unaware of studies examining the potential role of bacteria in contributing to the pool of fatty acids recovered from archaeological sherds. Bacteria produce the same types of fatty acids as most plants and animals, especially the more common saturated and monounsaturated fats typically found in potsherds. This is a concern that needs to be addressed by future research beyond the scope of this chapter. Instead, I will here examine the final possibility mentioned above, that is, the potential effects of decomposition of fatty acids in ancient sherds. Specifically, I aim to examine how differential decomposition between organic compounds affects our interpretation of fatty acid profiles.

Decomposition: Food Sciences Perspective

While fatty acids are relatively resistant to decomposition when compared to many other biomolecules, they still degrade when exposed to oxygen and water, processes that will be accelerated by higher temperatures.[1] The processes of decomposition have been of much interest to food scientists. Many of the foul tastes and smells associated with spoiled food are the byproducts of fatty acid degradation. As long-chained compounds break down, they form short-chained and often volatile (airborne) aromatic ones. Evolution has predisposed humans to recognize these compounds as foul, representing foods to be avoided, because various toxic-compound-producing bacteria live on rancid foods. These processes, then, have attracted much research by food chemists to understand what exactly happens when foods, and the fatty acids within them, decompose.

Research in the food sciences has demonstrated that fatty acid decomposition is an extremely complex process that can produce a diverse range of organic compounds depending on environment (Frankel 2005; Fritsch and Deatherage 1956; Hudlicky 1990). For example, saturated fatty acids can oxidize to produce several shorter derivative compounds, each of which may further decompose into other unstable and short-lived compounds, which may again decompose into yet other compounds. Indeed, the decomposition process for many isolated fatty acids is still not completely understood by food scientists, much less for whole foods.

In any case, the relevance of such decomposition research to archaeology is unclear. Food scientists are interested primarily in the short-term (months) decomposition products while archaeologists are, of course, interested in the long-term outcomes (centuries to millennia). In most cases, archaeologists will not be

[1] In fact, a prominent food chemist at the University of California, Davis expressed amazement when I told him that I was studying fatty acids over 500 years old. He was surprised that any fatty acids could survive that long.

interested in all the intermediary biomolecules, only the final and relatively stable endproducts. To date, this does not seem to be an arena that has attracted much attention by archaeologists.

Decomposition: Archaeological Perspective

In linking organic residues to particular types of foods, there are basically two methods by which residue studies in archaeology have progressed. The first involves the presence of distinct biomarkers (Evershed et al. 1999). Biomarkers are hypothesized to be synthesized only by particular species or genera, hence their presence in organic residues is indicative of the cooking or storage of that plant or animal in the past. Claims for biomarkers include certain ω-3 fatty acids for fish and erucic acid (C22:1) for plants in the mustard family. Unfortunately, such biomarker lipids are rare in nature, and in fact many plants and animals are producing small amounts of compounds that are considered biomarkers for other species. Thus, although plants in the mustard family produce significantly higher densities of erucic acid, other plants also produce this molecule in small amounts.

The second method is more generalized and assumes that different plants and animals produce different quantities of fatty acids. For example, certain plant families might produce higher relative quantities of long-chained fatty acids than others, or certain categories of food, such as nuts, have higher quantities of saturated relative to unsaturated fatty acids. By this logic, the ratios of different compounds will be different for different plant and animal groups, rather than only the presence or absence of specific organic compounds as above.

Much work has been carried out in both archaeology (Eerkens 2005; Evershed et al. 1997; Malainey et al. 1999a; Mottram et al. 1999; Skibo 1992) and food science to show that modern fresh foods do indeed have different and recognizable fatty acid profiles. This is hardly surprising, for different plants and animals eat different foods and metabolize and store energy in different ways. Similarly, different parts of a plant (root, leaves, seeds) serve different biological functions and will be made up of varying quantities of organic compounds that help to serve those functions. For archaeologists, the assumption is that these profiles remain relatively unchanged over time. To determine what types of foods were processed or stored within a pot, the assumption is that one need only extract the organic residues, determine the relative amounts of different fatty acids, and then compare this profile to some fatty acid database of different foods.[2]

Dealing with Decomposition

Decomposition of fatty acids will have differing effects on these two methods. In the first method, decomposition of biomarkers below the detection threshold of whatever equipment is being used (GC/MS, HPLC) will simply lead to the inability to identify ancient foods. Either the biomarkers are there, and the pot function can be identified, or not. In the second method, decomposition is potentially more problematic. If all fatty acids decompose at the same rate, their relative percentages will stay the same, and it is a simple matter to calculate the percentage of each fatty acid which will stay constant over time. If, on the other hand, fatty acids decompose at different rates, the relative percentages will constantly change over time.

Unfortunately, it is the latter case that is the rule. Unsaturated fats oxidize faster than saturated ones,. As well, longer- and shorter-chained compounds (those greater than 18 and less than 14 carbon atoms) oxidize more quickly than medium-chained compounds (with 14 to 18 carbon atoms). Unfortunately, the precise relative rate at which fats decompose depends on a number of factors including temperature, the availability of oxygen and water, and the original relative densities of different compounds. and the rate increasing over ten times for each double bond present. For example, deMan (1992) estimated that the rate of oxidation between C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) at 100°C is 1:100:1200:2500, but these ratios are likely to change under different conditions such as higher or lower temperatures. As well, longer-chain compounds oxidize more quickly than shorter-chained compounds. Reconstructing all these parameters for archaeological pot sherds is extremely difficult, if not impossible. It also suggests that the approximation of long-term decomposition using artificial means, such as high temperature or exposure to oxygen, may not necessarily replicate natural decomposition. Additional research is necessary to determine the accuracy of such methods.

[2] In essence, this process is similar to provenance analysis of lithic and ceramic artifacts using techniques such as X-ray fluorescence (XRF) or instrumental neutron activation analysis (INAA). In the organic residue case, the relative quantity of different fatty acids are the analytical analogue for parts-per-million quantities of elements, such as rubidium, strontium, etc.

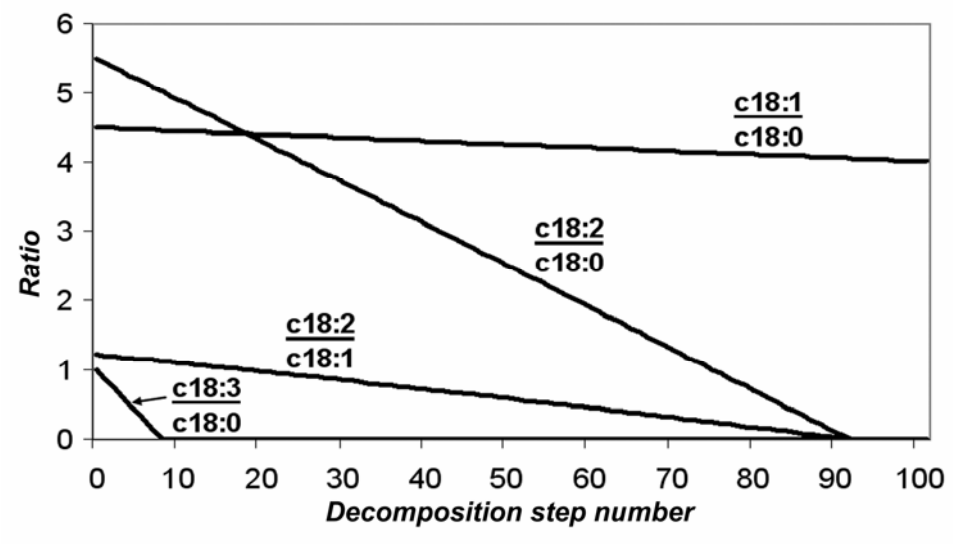

Figure 1: Simulated decomposition for four fatty acid ratios over 100 decomposition steps (based on deMan 1992).

The upshot is that the identification of any archaeological residues relying on the relative percentage of different fatty acids to one another is potentially problematic, unless those fatty acids decompose at similar rates or we can estimate the precise age of a pot, know the relative rate of decomposition between different fatty acids, and determine how much decomposition has taken place. The latter is unlikely given the complex nature of decomposition (Frankel 2005). In other words, if some food resource, such as maize, has high quantities of C18:1, it is not enough to simply detect a high quantity of C18:1 in an archaeological sherd to identify the reside as deriving from maize. A high quantity of C18:1 could mean that C18:1 was present in large amounts in the original residue (potentially maize), but it could also mean that C18:1 was originally present in low quantities, but that several other high-quantity fatty acids have decomposed, leaving mainly C18:1 after such long-term decomposition.

To demonstrate this problem, Figure 1 shows the simulated ratios of several fatty acids relative to one another over time, using the relative decomposition rate provided by DeMan (1992). The starting values represent the approximate concentration of the four different fatty acids in rabbit meat. Each unit along the X-axis (time) represents a 'decomposition step.' This is an arbitrary unit that is proportional to real time, but will accelerate or decelerate depending on environmental factors such as temperature, humidity and the presence of oxygen. As seen, the ratios have different slopes indicating quicker or slower change, and none of the four ratios is constant over time. In particular, ratios involving polyunsaturated fatty acids quickly approach zero as these molecules degrade.

Not surprisingly, the recovery of polyunsaturated fatty acids is rare in archaeological sherds. All of this demonstrates that the use of fatty acid ratios to identify ancient residues is problematic (Skibo 1992), again, unless those fatty acids decompose at similar rates. The use of advanced statistical methods such as principal components analysis (PCA) or cluster analysis appears not to resolve this issue (Malainey 1999c).

Fatty Acid Ratios

To deal with this decomposition issue, it is necessary for archaeologists to develop fatty acid ratios that are relatively constant despite decomposition, in other words, to use the ratios of fatty acids that decompose at similar rates. This would include isomers of the same fatty acid, for example, C18:1ω9 and C18:1ω7, two monounsaturated fats with the double bond located at different positions along the carbon chain, or fatty acids with identical number of double bonds and of similar length, for example, C18:0 and C16:0 or C18:1 and C16:1. Unfortunately, compounds that degrade at similar rates tend to be related and serve similar biological functions in plants and animals. As a result, they tend to be produced in similar amounts within a plant or animal, and the ratios of these compounds may be similar across different species. In other words, the use of fatty acids that decompose at similar rates may not provide great discriminatory power for determining pot function. I have shown elsewhere that such fatty acid ratios are very similar across different plant species, but also that they do vary systematically by food product (Eerkens 2005). Thus, the leaves and greens of plants have ratios other than seeds and nuts, which are again different than roots

and bulbs. Similarly, terrestrial mammals are different than fish, which are again different than plant products.

Experimental data generated by Malainey et al. (1999b) serve to show that such ratios remain relatively stable in spite of overall fatty acid degradation. They boiled various food products in small earthenware pots, broke the pots and separated the sherds into three groups. They extracted residues from the first group immediately, subjected the second group to 100°C temperatures for four days, before extracting residues, and subjected the last group to 100°C temperatures for approximately 30 days. Figure 2 plots two fatty acid ratios, C16:0/C18:0 and C16:1/C18:1 for three different food products (bison, catfish and greens) across these three time frames. As seen, although there is some variability, the fatty acid ratios stay relatively constant, particularly the latter ratio. The major exception is C16:0/C18:0 for greens which shows marked change in its ratio over time.

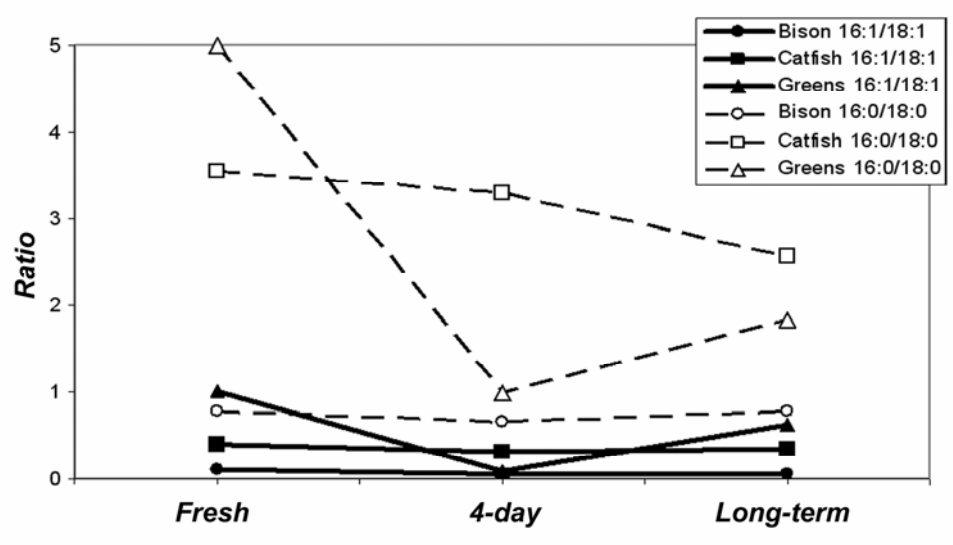

Figure 2: Induced decomposition for three foods and two fatty acid ratios (based on Malainey et al. 1999b)

Although this approach using fatty acid ratios cannot make fine-scale distinctions between different specific foods in degraded residues, it can make some general divisions for different food classes. Using data on boiled foods generated by Malainey et al. (1999a), I was able to derive four ratios that were useful in discriminating five different food classes (Eerkens 2005), including terrestrial mammals, fish, seeds and berries, roots, and greens. These four ratios are C12:0/C14:0, C16:0/C18:0, C16:1/C18:1 and (C15:0 + C17:0)/C18:0, consisting of eight different fatty acids that are commonly encountered in archaeological residues.

Figures 3 and 4 plot these ratios and show the separation between the general food classes discussed above. Ellipses represent subjective estimations of the range of values for each food group, not mathematically-defined confidence intervals. As shown in these figures, there is some overlap in the ellipses. This makes it difficult to assign a definitive function to individual sherds. I would argue that the best use of such classification schemes is to analyze multiple sherds from a site or region and evaluate which food groups seem to be best represented among the samples. Correlating these assignments by pot shape, burial context and decoration motifs may assist further in the reconstruction of pot function.

Conclusions

Two approaches have characterized most archaeological residue studies involving fatty acids. The first consists of locating specific biomarkers distinctive of particular species, genera, or families of plants or animals. While useful in some cases, fatty acid biomarkers are unfortunately rare in nature. As a result this approach has seen only limited application. The second approach has been to use the ratios of more common fatty acids to define particular classes of foods. The problem with this approach is that different fatty acids decompose at different rates over due to oxidation and hydrolysis. As a result such ratios are not stable over time, unless care is taken to only rely on ratios of fatty acids that decompose at similar rates.

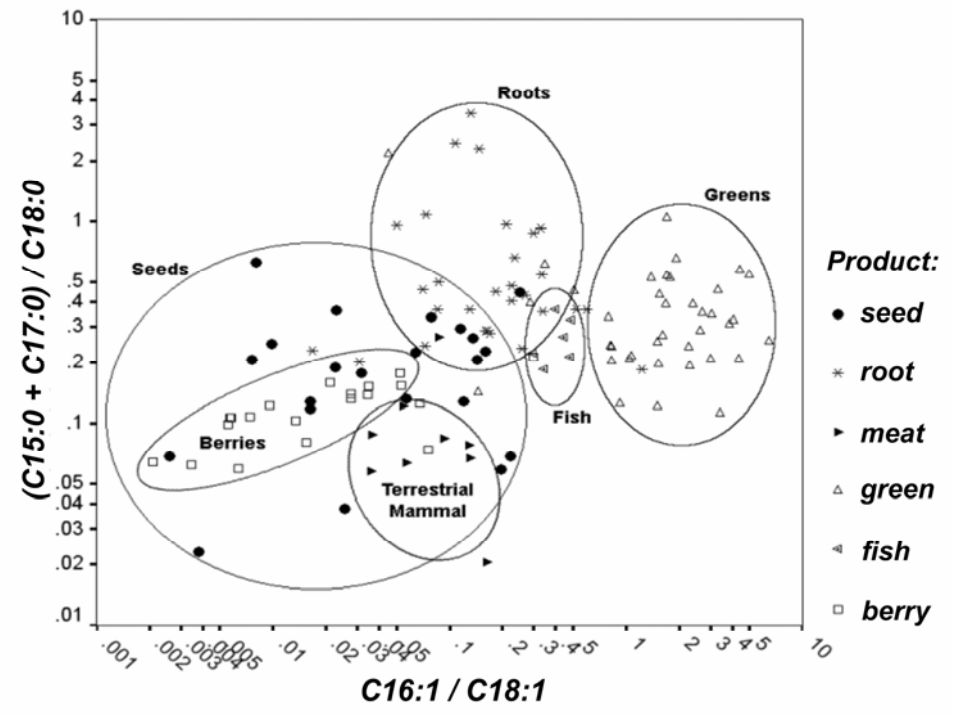

Figure 3: Biplot of two conservative fatty acid ratios for modern food products (ellipses represent subjective estimations of the range of values for each food group).

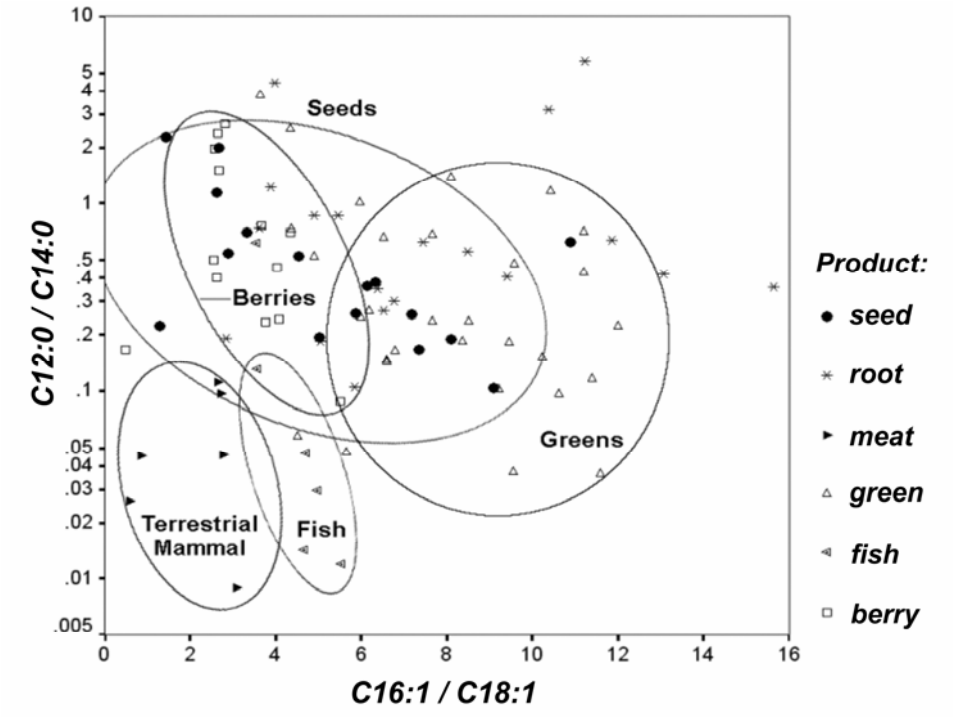

Figure 4: Biplot of two additional conservative fatty acid ratios for modern foods. Ellipses represent subjective estimations of the range of values for each food group (after Eerkens 2005).

In this chapter I argue that a small set of four ratios comprising eight common fatty acids regularly recovered in archaeological studies are relatively stable over time. Preliminary studies suggest that these four ratios can be used to separate fatty acid residues into five to six very general food classes which can help classify archaeological samples (Eerkens 2005). While this level of detail may not allow archaeologists to answer all of the questions they have about pots, or other residue containing artifacts, they can be useful as a starting point for generating basic data about the function of artifacts from archaeological settings. Further, I argue that it is not possible to assign individual sherds specific functions, but that groups of sherds can be evaluated based on their overall fatty acid makeup. Thus, despite the promise of such residue analyses to identify specific functions of artifacts, as discussed in the opening paragraphs, decomposition and other confounding problems probably relegate such analyses more to the role of producing hypotheses or supporting information about the function of artifacts.

In this respect, it would be worthwhile to augment fatty acid analysis with the extraction and evaluation of other organic residues, such as longer-chained lipids, waxes, carbohydrates or amino acids, and to use a range of analytical and derivatization techniques, such as stable isotope analysis and the isolation of isomers of the same fatty acid. Some in the field perform such multi-pronged approaches, but the majority (myself included) do not, favoring only one method for various reasons. The identification of additional organic compounds and new analytical techniques could serve to further subdivide some of the ellipses plotted in Figures 3 and 4 into smaller and more specific food categories.

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