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Examination of prehistoric artifacts via fatty acid methyl ester (FAME) techniques using modern environmental stewardship

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ABSTRACT

Elucidating the specific use and purpose of archaeological specimens such as stone axes and grinding stones can improve the understanding of an area's ecosystem and civilization. Chemical analysis of residues preserved on these tools may provide key information in identifying how the tool was implemented. The arid US Great Basin provides an ideal environment for the preservation of fatty acid residues.

We have successfully optimized methods for extraction, methylation, and analysis of fatty acids for use on archaeological specimens. These analyses implement techniques that do not alter artifact integrity, facilitate analyte modification through transesterification to enhance analyte volatility for identification by direct injection GC–MS methods. Method development focused on the use of fatty acids found within the Great Basin of the USA, and has been designed using chemical stewardship to avoid environmental contamination and to protect the health of the analyst. In evaluating analysis methods, five traditional methylation techniques were examined only to prove inadequate for this study. By combining attributes of these methods with extraction goals, a modified single step extraction derivatization method was developed. Using this method we have demonstrated solid relationships between fatty acid ratios and plant/animal types. We have focused on method development and optimization for the detection of these fatty acids. In addition to optimization of instrumental variables we have compared various methylation methods to achieve optimal yields.

1. Introduction

1.1. Fatty acids Introduction

Fatty acids are so named as they were originally found to be the constituents of animals and vegetables fats and fatty oils. Originally, the term was applied to the saturated fatty acids and especially to the long chain acids (Christie, 1989). At present, the term fatty acid is more generic in the sense that it encompasses saturated and unsaturated monobasic carboxylic acids and several series of substituted acids having carbon skeletons identical with normal saturated acids.

For the past two decades archaeological residue analysis investigations have gained importance over traditional form-function relationships in studying artifacts. This change is evident by a marked significant increase in the number of papers using archaeological residue analysis (e.g., Evershed et al., 1994, 1997, 2003; Charters et al., 1997; Malainey et al., 1999c; Mottram et al., 1999; Stott et al., 1999; Eerkens, 2002, 2005; Maniatis and Tsirtsoni, 2002; Rafferty, 2002; Stauffer et al., 2005; Stauffer 2006). In spite of numerous complications associated with extraction and identification, these studies have demonstrated that a variety of compounds, including fatty acids, waxes, sterols, resins, tars, pitches and amino acids, are aptly preserved in prehistoric shreds and can be used to indicate the source of stone tools or ports (Christie et al., 1993; Eerkens, 2005). These researchers have demonstrated numerous advantages of fatty acid analysis over the analysis of other moieties, like; DNA, carbohydrate, protein analysis, etc. Even though various types of biomolecules are preserved in prehistoric tools and shreds, in particular the environments fatty acids and lipids demonstrate the highest stability (Evershed et al., 1994; Christie, 1989; Eerkens, 2001, 2002, 2004, 2005).

The residue analysis approach of archaeological samples takes advantage of the fact that different plants and animals produce different types and quantities of organic compounds. This well-known fact has been demonstrated by numerous papers and books on this topic (Malainey et al., 1999a; Frankel, 1998; Chow, 1992). Some plants and animals show different fatty acid compositions even between different sub species or species of the same family thus providing an opportunity to differentiate the source readily. Our project decided to take advantage of exactly this chemical

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uniqueness that nature has provided us to establish a relationship between fatty acid ratio and the source and/or use of artifacts.

Organic materials from foods effectively lodge in the porous spaces within the structure of stone tools, grinding stones, hunting tools, and other artifacts under examination. Fats and oils are no different. In fact, they often adhere more effectively than other organic sources present due to their ability to bond to the surfaces they come in contact with. After these organic residues clog the porous spaces in the artifact they are efficiently sequestered and preserved by nature. Residue profiles are not subsequently contaminated by the influx of materials from the surrounding soil (Deal and Silk, 1988; Eerkens et al., 1999). Thus, it is commonly understood that preserved residues characterize the first few uses of the stone tools or grinding stones under investigation (Bright et al., 2002; Eerkens et al., 2002).

Residue studies of archeological samples have commonly been centered around the recovery of lipids, since they are relatively stable and do not degrade as quickly as other compounds like carbohydrates and DNA (Christie, 1989; Evershed, 1993; Eerkens, 2005). In this paper our group as well as others demonstrate solid relationships between fatty acid ratios and plant/animal types. By evaluating fatty acid ratios rather than individual fatty acids one can mitigate the effect of environmental degradation on the results. Fatty acid profiles are distinct between different plants and animals and can often be considered as a fingerprint of a source. The extent of degradation in a shred depends on the depositional context, how well lipids are sealed and the length of time since the artifact/tool was used. Hydrolysis is unlikely to be significant in our study area, the desert environment, yet oxidation is a potential problem (Wandsnider, 1997). Still, even here some researchers suggest that the oxidative effects are minimal (e.g., Hill and Evans, 1989; Malainey, 1997, p. 109).

Oxidation results in the breakdown of lipids into various by-products (Frankel, 1980, 1987, 1998; Porter et al., 1981). The most common way to deal with oxidation in the archaeological contexts is to examine ratios of lipids to one another (Eerkens, 2005; Kedrowski et al., 2008), rather than absolute values – an approach our group adopted. Not all lipids oxidize at the same rate. For example, unsaturated fats oxidize more quickly than saturated ones, the rate increasing over 10 times for each double bond present. Furthermore, it is estimated that the rate of oxidation between C18:0, C18:1, C18:2 and C18:3 at 100 °C is 1:100:1200:2500 (deMan, 1992). In addition, longer-chained compounds oxidize more quickly than shorter-chained compounds. Thus, when using ratios of lipids to identify foods, a goal should be to examine the ratios of compounds that oxidize at similar rates.

Unfortunately, compounds that degrade at similar rates tend to be related and serve similar biological functions in plants and animals, and consequently tend to be produced in similar amounts (either high or low) in different species. As a result, the ratios of these compounds will not differ dramatically between species. The confusion, then, is deciding between using ratios of compounds that are more discriminatory between modern foods types but may degrade at different rates, verses using ratios of compounds that have less discriminatory power but degrade at similar rates. The latter approach is more relevant in ancient contexts, and our studies have focused mainly on fatty acids with similar degradation rates. Lastly, Malainey et al. (1999b), suggest that simulated long-term decomposition greatly affects fatty acid composition, but their data suggests that the ratios of similar compounds are relatively constant even after long-term decomposition.

1.2. Residue analysis

Residue analysis has the potential to be more precise than the traditional form-function relationships in identifying artifact use.

Table 1
Dielectric constants of various fatty acids at 20 °C and 40 °C.

Dielectric constants	20 °C	40 °C
Methyl		
Acetate	7.11	6.59
Propionate	5.75	6.12
Butyrate	5.43	5.10
Undecanoate	3.67	3.53
Laurate or dodecanoate	3.54	3.41
Tridecanoate	3.42	3.32
Myristate or teradecanoate	3.35	3.24
Pentadecanoate	3.30	3.20
Palmitate or hexadecanoate		3.12
Heptadecanoate		3.07
Stearate or octadecanoate		3.02
Nonadecanoate		2.98

Common fatty acids of animal and plant origin have even-numbered chains of 16–22 carbon atoms with zero to six double bonds in the *cis* configuration. However, nature provides countless exceptions; and odd- and even-numbered fatty acids with up to nearly a hundred carbon atoms exist. In addition, double bonds are not always *cis* and can also be found in the *trans* configuration. Furthermore, there can be numerous other structural identification features, such as branching points, rings, and oxygenated functional groups.

With such a compelling power of identifying artifact sources we find it essential to develop simple, quick, and precise methods for determination of fatty acid structures. Many different methods have been employed to investigate fatty acids. In particular, new methods involving gas chromatography–mass spectrometry (GC–MS) (Gohkle and McLafferty, 1993), GC linked to Fourier-transform infrared spectroscopy (FTIR), and reversed-phase high-performance liquid chromatography (HPLC) have been extensively investigated, amongst others (Alexander and Justice, 1985; Mottram et al., 1999). Of these methods, GC–MS analysis has shown significant promise. Simple derivatization procedures are required that utilize readily available reagents and have minimized glassware requirements. One of the major advantages with GC–MS analysis is that it is not necessary to isolate components in a pure form. Spectroscopic methods including NMR spectroscopy require lengthy purification procedures to elucidate a single chemical compound. In fact, GC–MS is used in separation of different fatty acids present in a sample to determine the ratios of different fatty acids present in the sample (Ulberth and Henninger, 1992).

Due to low volatility of fatty acids, gas chromatography (GC) usually analyzes fatty acids as methyl ester derivatives known as fatty acid methyl esters (FAMEs). A mass spectrum identifies molecular fragment ions indicative of structural features, including the positions of double bonds in the aliphatic chain. Molecular weight and retention times are useful analytical parameters, some limited structural information may be available, and indeed definitive spectra can be obtained often with branched-chain fatty acids or those with additional oxygenated functional groups (Barry and Grob, 2004).

Table 2
Dielectric constants of various non-polar organic solvents.

Solvents	20°C	40°C
Benzene	2.28	2.24
Cyclohexane	2.02	1.99
Dibutyl ether	3.08	2.94
Toluene	2.26	2.40
Hexane	1.89	2.02