USING RAMAN SPECTROSCOPY TO ANALYZE FIRE-CRACKED ROCK FROM EARTH

OVENS IN SOUTH-CENTRAL NORTH AMERICA


A Dissertation

by

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# ABSTRACT

Earth ovens are complex cooking features that have been important worldwide, throughout human history. Knowledge of what was cooked in an individual earth oven is only available if food was charred, so other lines of evidence are being sought by archaeologists. The purpose of this dissertation is develop a method using Raman spectral analysis of biochemical residue found on fire cracked rock (FCR), to assess what was being cooked in archaeological earth ovens. Specifically, the carbohydrate inulin is being pursued, because it is important in earth oven cooking but is not associated with any diagnostic microfossils. A reference collection was created, including modern and archaeological macrobotanicals, and raw and cooked samples. FCR from Fort Hood and Lower Pecos, both in Texas, were analyzed and compared to control samples.

This study demonstrated that is possible that food residues identifiable by Raman spectroscopy are persevered on archaeological FCR from earth ovens – while cooking and diagenetic processes do affect the spectra of food samples, they do not render them unidentifiable. While it is not possible to identify precisely what plants were cooked in an earth oven, there was a tentative identification of carbohydrates on 3 FCR samples from a total of 16 samples. These finds are in line with other research on residue from archaeological FCR. The archaeological samples were different from the non-diagnostic control samples, indicating that it is unlikely that the residue is from the environment.

There is potential for the use of Raman spectroscopy to study earth oven residue; however, it requires substantial continued study before conclusive analysis is consistently achieved. Of

primary concern is separating the signal from the target carbohydrate spectra from background

and environmental spectra, as well as identification of residue-rich FCR for sampling.

# DEDICATION

In memory of

Stephanie

and my grandparents

who started me on this journey

but weren't able to see the finish.

Love you always.

# ACKNOWLEDGEMENTS

Finally I would like to thank my friends and family for their love and patience in supporting me through this progress. There are more people than I can possibly thank, but know that you are all appreciated.

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# CHAPTER I

# INTRODUCTION

While the nature of the archaeological record prevents us from ever knowing exactly when the

first humans cooked their food, archaeological evidence points to the start of cooking at least

250,000 years ago (Wrangham and Carmody 2010). Initially cooking occurred over unprepared

hearths, using only burning fuel, though over millennia more complex cooking features were

developed.  Earth ovens in the archaeological record appear 35,000-31,000 years ago in the Old

World including Europe (Movius 1966; Straus 2006), Japan (Dogome 2000), Australia (Gillespie

1997), and the Bismarck Archipelago (Torrence et al. 2004), and 10,000 year ago in the

Americas, including central and southwest Texas (Black et al. 1998:82–84; Black and Thoms

2014).  Earth ovens (Figure 1) are multi-component cooking features that layer food and packing

material over heated stones or hot coals to bake food (Black and Thoms 2014). While analysis of

earth oven cookery affords important insights into diet and culinary practices of past populations,

current analytical techniques are limited to largely physical (i.e., structural) remains found

charred in the oven. Recent advances in analysis of microscopic remains such as starch,

phytoliths, and raphides – known collectively as microbotanicals or microfossils have expanded

the potential for earth ovens to provide data (Thoms, Laurence, et al. 2014a; Laurence et al.

2011).  Importantly, however, one of the most common types of plant foods processed in earth

ovens, inulin-rich geophytes (plants with underground storage origins) do not produce diagnostic

microbotanical remains. For this dissertation, Raman spectroscopy is explored as a potential

method to identify inulin in food residues on samples of fire-cracked rock (FCR) that severed as

heating elements in pre-Columbian earth ovens in south-central North America.
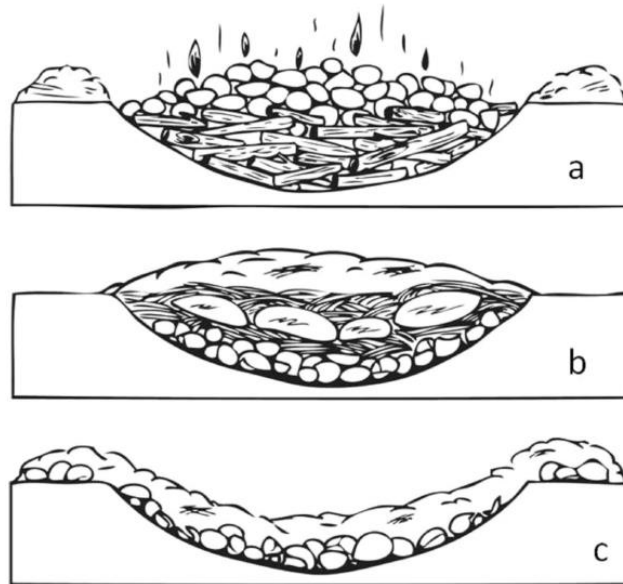


**Figure 1: Schematic of generic earth oven. A: heating rocks with wood fuel. B: cooking food packets in green-vegetation packing material. C: abandoned oven after food removal and decomposition of packing material. Reprinted from Thoms et al. (2014)**

ENVIRONMENTAL CONTEXT

Analyses for this study were undertaken in conjunction with a long-term cultural resources

management research project that focused on recovery of archaeobotanical remains from earth

ovens found at pre-Columbian open-air sites at Fort Hood, a U.S. Army installation in central

Texas.  Fort Hood encompasses 64,226 hectares within the ecotone between the Blackland

Prairie and the Edwards Plateau (Figure 2).  The modern climate is subtropical, characterized by

hot, humid summers and relatively short, dry winters (Kibler 2004). Many of the wild food

plants found in this area are geophytes known to have been cooked in earth ovens. These include

various members of the Liliaceae family such as wild onion (Allium sp.), the Asparagaceae

family that includes camas (*Camassia scilloides*), agave (*Agave* sp.), and sotol (*Dasylirion* sp.),

as well as tuberous plants including scurfpea (*Pediomelum* sp.), groundnut (*Apios americana*),

and flatsedge (*Cyperus* sp.) (Boyd, Mehalchick, et al. 2004). On Fort Hood, there are a number

of localized environmental niches, including the Paluxy sands, which are associated with earth

ovens. These loose sandy deposits eroded from a sandstone and shale bed known as the Paluxy

formation (Abbott et al. 1995; Hayward et al. 1996). They are well drained and easier to dig than

surrounding clayey soils, which contributed to them being a favored location for earth oven

construction  (Boyd, Mehalchick, et al. 2004).



**Figure 2: Site locations. Map data ©2018 Google**

Botanical remains and artifacts from earth ovens inside pre-Columbian dry rockshelters in Texas' arid Lower Pecos region were also included in the analysis for comparative purposes in assessing residue preservation issues in diverse environmental settings. The Lower Pecos region lies in west Texas, along the southwest border of the Edwards Plateau (Figure 2). The modern climate is semiarid, with hot summers and dry winters (Koenig 2012). Plant foods in the area likely to have been processed by earth ovens include prominently desert succulents such as sotol (*Dasylirion* sp.) and agave (*Agave* sp.), though some Liliaceae family members, including wild onion (*Allium* sp.) (Riley 2010; Basham 2015). The region's deep, steep-walled canyons, incised into limestone bedrock, are dotted with rock shelters that contain the remains of earth ovens dated throughout the last 10,000 years. The soils in this area are very thin, predominantly gravelly and silty loams (Golden et al. 1982).

ARCHAEOLOGICAL CONTEXT

Human occupation of Texas was under way by 13,200 to 15,500 BP, with ephemeral sites from before the Paleoindian period (Waters et al. 2011). While there is evidence of earlier earth ovens, they start to appreciably appear during the Early Archaic period (8800-6000 BP). These are generally smaller ovens, not the larger burned rock middens (BRM) seen in the later periods (Boyd, Kibler, et al. 2004). BRMs are the accumulated remains of dozens to hundreds of earth oven built in the same location over decades to several millennia (Thoms, Boyd, et al. 2014). During the Middle Archaic period (6000-4000 BP), drier conditions may have been associated with an expansion of xeric plants, including the common food resources like sotol and yucca, and the apparent greater reliance on BRMs (Johnson and Goode 1994). An increase in population density is seen around 5000-4500 BP, possibly with macrobands visiting some larger

sites seasonally, or several small groups may have used the same sites for longer periods (Boyd, Kibler, et al. 2004). Earth ovens continued to be a major constituent of the Late Archaic subsistence strategies (Prewitt 1981). The late period (4000-13/1200 BP) has increasing population size, and the establishment of cemeteries implying strong territorial ties. The use of burned rock may have reached zenith at some point during this period, but there is some evidence indicating high intensity use continued into early Late Prehistoric (Collins 1995; Black et al. 1997; Kleinbach et al. 1999:795). Horticulture was never an important part of the Texas subsistence system, not appearing until relatively late (Collins 1995). With Indians being forced onto reservations during the 19th century, intensive earth oven use in Texas ceased.

As a population increases, and all surrounding areas are occupied such that territorial expansion is no longer a viable option the population density will reach a critical mass. At that point the society will need to change how it is feeding people—it will need to extract more food from the same area of land. This process is known as intensification, and requires increasing energy spent on food production, in order to increase the amount of food extracted from a single unit of land. Intensification processes include (but are not limited to) increased hunting and gathering efforts on lower caloric yield foods, domestication of plants and animals, and cooking (Binford 2001:188; Morgan 2014). It has been suggested that earth ovens, as opposed to horticulture, was a major form of intensification in Texas, and was part of the reason that horticulture never gained significance in central Texas' pre-Columbian history (Johnson and Hard 2008). When cooking is an intensification method, as population density increases, cooking technology becomes less efficient as more costly foods are used – more heat energy is expender per unit calorie gained from the food item. Thus, the tendency through time is that direct cooking on

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coals gives way to rockless earth ovens, to cook-stone grills, to earth ovens and other forms of cook stone technology, and later to ceramic and metal vessels (Thoms 2009). This is reflected in the archaeological record, where radiocarbon dating indicates that the presence of BRMs increased as population density increased through the Middle Archaic, and peaked during the Late Archaic, when population densities were highest (Black and Creel 1997:280–282). Given that earth ovens indicate significant time and labor investment in the processing of plants, these are an excellent indicator of intensification (Johnson and Hard 2008).

RELATIONSHIP BETWEEN EARTH OVENS AND INULIN

As noted previously, earth ovens bake or steam food in below-ground pits, layering food and packing material over heated stones or hot coals. Most earth ovens are reused multiple times, which requires that the central pit be cleaned and any spent cooking stones be discarded as FCR, older features may be dug into, or the pit may be filled with debris (Black and Thoms 2014). Through time, most components weather away, but the last used heating element typically remains most intact but almost always subjected to some form of pedoturbation that disarticulates heating element rocks to some extent. Other archaeological features indicative of earth-oven cookery include: pits infilled with carbon–stained sediment; FCR concentrations, perhaps resulting from cleaning previous ovens; and linear barrow pits zone representing sources of sediment to cap ovens. Given the amount of digging and transporting sediment associated with earth-oven construction and use, the fill in earth ovens and BRMs often contain incidentally introduce artifacts and ecofacts not necessarily functionally related the ovens. In short, earth oven use creates complex features, often palimpsest in nature with mixed matrix; foods cooked

therein are only preserved when charred (Black and Thoms 2014). Due to their complex nature, multiple lines of evidence are required to accurately interpret patterns of earth oven use.

Earth ovens cook food at relatively low temperatures in a moist environment, over a few hours up to several days. In an oven where food is cooked and not burned, while the rock temperature may reach over 500 °C, the food itself tends to remain at 100 °C or lower (Thoms, Laurence, et al. 2014b). Ovens are well suited to cook tough and fatty cuts of meat and plants rich in complex carbohydrates, since the cooking environment allows for the breakdown of large molecules in carbohydrates, proteins and lipids, as well as preserve food and destroy toxins (Wandsnider 1997). Inulin is a complex carbohydrate, a type of polysaccharide known as a fructan and source of soluble dietary fiber. It is a prebiotic, in that in its raw form, it does not directly provide nutrients for humans, but it is fuel for bacteria in human's lower intestinal tract (Leach 2008). However, when exposed to water and heat, complex carbohydrates, including inulin, break down into easily digestible sugars (Wandsnider 1997). Caramelizing onions is a good example of the process that may be familiar to many people (Leach 2009).

While a variety of foods were cooked in earth ovens, plant foods rich in inulin, including onion, camas, sotol, and agave, are associated with earth ovens in the study area (Thoms 2009; Black and Thoms 2014). Evidence for this includes historic records, ethnographic reports, and charred plants found in archaeological earth ovens (Thoms 2008b, 2009). In the central Texas and Trans Pecos study area, there is a reasonable probability that any particular oven cooked inulin rich foods; it is less certain as to what specific oven cooked a specific inulin-rich food. Charred plant foods are generally the best evidence for what was cooked in an oven, however they are relatively rare in the archaeological record. The presence of microbotanicals, such starch,

raphides, and phytoliths, can also indicate what plant foods were processed in earth ovens (Thoms, Laurence, et al. 2014a).Starch grains, for example, act as direct evidence of the presence of starch rich foods, even if the precise plant cannot be identified (Torrence and Barton 2006). Other diagnostic microfossils include calcium oxalate for cacti, or phytoliths for maize, however, there are no diagnostic microfossils for inulin-rich foods (Jones and Bryant 1992; Piperno 2006).

RESIDUE ANALYSIS AND RAMAN SPECTROSCOPY

During the use-cycle of an earth oven, stones are intensely heated and slowly cool as they cook the food, which causes the rocks to crack, change colors, and minerals to break down (Pagoulatos 2005). This can cause microcracks in the stones, that may help preserve food residues from the cooking process that would otherwise deteriorate (Shanks et al. 2001; Thoms, Boyd, et al. 2014; Thoms, Laurence, et al. 2014a).   The molecular structure of those preserved residues may be identifiable using analytical chemistry, which can then be linked to the potential source of these residues using the archaeological biomarker concept (Evershed 2008b). The biomarker concept states that in some cases particular molecular components of the complex mixtures that comprise all biological materials are unique to certain flora or faunal species. If the particular component is preserved in an identifiable way through the archaeological record, it can be diagnostic for identifying the presence of the flora or fauna it is associated with. For the present study, the flora "species" is inulin-rich plants as a class, using inulin as their biomarker. Whether or not the molecular signature of inulin is present in an identifiable way through cooking and diagenesis is the question addressed by this dissertation.

A variety of techniques can be used for biochemical or organic residue analysis, such as gas chromatography–mass spectrometry (GCMS), liquid chromatography–mass spectrometry (LCMS), Raman spectroscopy, Fourier Transform Infrared (FTIR) absorption spectroscopy, and many others.  Most analyses using biochemical analysis of food residues focus on lipids absorbed in pottery. There are some that analyze residues on FCR from earth ovens (Buonasera 2005; Quigg et al. 2001), and some examining the residues in soils in earth ovens (Isaksson 1996), though these all use GCMS. There are also several biochemical analyses of earth ovens amongst CRM monographs that include both work with GCMS and FTIR (see Quigg et al. 2010 which uses both). These studies show that lipids are preserved on FCR from earth ovens, but that the source(s) of those lipids is up for debate.

Since ethnographic and historic evidence in Texas indicates that mostly plants were cooked in earth ovens, and the biomarker in question would be a carbohydrate, lipid focused methods are not appropriate. Carbohydrates can be characterized by mass spectroscopy and similar methods, but they result in complex signatures, and would likely rely on the same kind of fingerprinting method that is more commonly associated with vibrational spectroscopy. With GCMS, ratios of fatty acids are used sometimes to determine potential source species for archaeological lipids (Malainey et al. 1999b; Skibo 1992; Buonasera 2007). This is similar to the kind of fingerprinting done with Raman spectroscopy and FTIR. While rare, carbohydrates have been identified in archaeological record, though determining their source has proven difficult (Dhakal and Armitage 2013; Oudemans and Kubiak-Martens 2012). In order to identify carbohydrates the fingerprinting method would still be used even if the analytical technique was something like

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GCMS. Raman spectroscopy offers additional benefit in that it requires minimal sample processing, so Raman was chosen as the analytical method.

Raman spectroscopy has been used to analyze a variety of materials and residues including pigments, binders, and resins. Aside from a preliminary study by Short et al. (2014), however, it has not been used to study food residues. This is likely due to problems that arise specifically when attempting to analyze organics; however, recent advances have improved the ability of Raman to characterize organic residues (Schrader et al. 1999; Edwards 2009). Raman spectroscopy characterizes materials based on how light interacts with its molecular structure. When light hits a molecule, it changes the molecule's energy level and causes it vibrate, which in turn changes the frequency of the light reflected from the molecule. The change in the light's frequency is determined by the molecular bond, which is measured by Raman spectroscopy. The raw data is transformed into a spectra which can be interpreted; the relative strength of each wavelength detected indicates the molecular structure of the residue (Malainey 2011a). Certain materials, including organic materials that an archaeologist might study, can be overwhelmed by fluorescence. Fluorescence can show up in Raman spectra, and overwhelm the target signals. Recent advances, especially the use of long-wavelength laser light source, have vastly improved its ability to characterize organic residues by reducing this fluorescence. Thus, Raman is potentially a relatively rapid method for determining presence of inulin in archaeological samples.

RESEARCH OBJECTIVES

The overarching research goal of this dissertation is to determine what was being cooked in earth

ovens via Raman spectral analysis of biochemical residue found on FCR.  To that end, three core

questions are addressed: (1) Are vibrational-spectroscopically identifiable food residues

preserved on archaeological FCR from earth ovens; (2) If they are, can they be reliably assigned

to an ancient baking event(s); (3) If so, can they be used to characterize what was baked, and to

what degree of precision? There are a few ancillary issues within the first question, including

how cooking and the passage of time effects residue spectra, and whether or not different

depositional environments effect the preservation of residues on FCR.

STRUCTURE OF DISSERTATION

Chapter 2 reviews pertinent literature regarding the application biochemical techniques to

identify archaeological food residues. The focus includes separation and analysis techniques

(such as Gas-Chromatography/Mass-Spectroscopy [GCMS]) and vibrational spectroscopy

(including both Raman spectroscopy and Fourier Transform Infrared [FTIR] absorption

spectroscopy).  This section begins with a technical and historical overview of biochemical

residues of archaeological food residues, followed by a discussion of general characteristics of

quantitative and qualitative investigations and current trends. It concludes with a set of best

practices for sample collection and analysis based on issues and analytical difficulties reported in

the literature.

Chapter 3 presents a pilot study demonstrating that a handheld Raman spectrometer can detect

inulin on experimentally produced FCR. For this study spectral signatures were obtained from

sotol (*Dasylirion* spp.) experimentally baked in an earth oven as well as sotol residue on an experimentally used processing tool. Inulin was present in the resulting spectra. The portable handheld Raman spectrometer also detected traces of inulin on experimental boiling stones used to boil commercially obtained inulin. Additional analysis of archaeological FCR from Fort Hood, TX revealed the presence of residues whose further identification required improvement of current optical methods.

Chapter 4 is a proof-of-concept study that develops a reference collection of both modern and archaeological botanical samples, as well as residues on FCR generated by actualistic and laboratory cooking experiments. It demonstrates that inulin is distinguishable from other carbohydrates and identifiable in botanical samples. It also confirms spectra differences between archaeological and modern botanical samples as well as among raw, cooked, and charred food samples. Three of the sixteen FCR samples from earth ovens in the Fort Hood and Lower Pecos region showed tentative evidence for the presence of carbohydrates. While promising, this study confirmed the need for improvement of the optical methods.

CHAPTER II

THIRTY YEARS OF BIOCHEMICAL ANALYSIS OF ARCHAEOLOGICAL

FOOD RESIDUES

INTRODUCTION

Biochemical or organic residue analysis of food stuffs is increasingly important in the

archaeological literature. It provides information about diet, culinary practices, subsistence

patterns, and artifact function. This article is a systematic and critical review of roughly the past

thirty years of biochemical analysis of archaeological food residues. Diagnostic techniques of

interest include separation-analytical techniques (such as Gas-Chromatography/Mass-

Spectroscopy [GCMS]) and vibrational spectroscopy (including both Raman spectroscopy and

Fourier Transform Infrared [FTIR] absorption spectroscopy). Several earlier reviews focused on

the application of these techniques to archaeology in general, but none focus specifically on how

they are applied to food residues (see Evershed 2008b; McGovern and Hall 2015; Regert 2011;

Roffet-Salque et al. 2016; Steele 2013; Vandenabeele et al. 2007).

With rapid growth comes a potential for uncritical application and over-interpretation of results.

While a few articles have suggested best practices for sample collection, analysis, or both, these

assessments tend to be based on anecdotal experiences rather than a systematic review of the

current state of research (see Mazow et al. 2014; McGovern and Hall 2015). This article provides

a set of best practices for residue specialists to follow during sample collection and analysis

based on issues and difficulties others have reported in their analyses. The current review begins

with a technical and historical overview of biochemical residues of archaeological food residues,

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followed by a brief discussion of the methods used for this review. The general characteristics, patterns, and trends of the current body of literature are described both quantitatively and qualitatively. Then, from challenges and recommendations described in the literature a list of best practices is proposed, and directions in further research suggested.

BACKGROUND

Reliable subsistence data for archaeological sites traditionally comes from analysis of macrobotanicals and faunal remains. Many of the tools used in food processing, however, are not directly associated with the foods they process. In most cases it is not immediately obvious what foods were stored in a pot, ground with a mano and metate, or cooked with a stone heating element. Various methods have been developed, most using microscopy such as microwear and microfossil analyses, to identify how tools were used or what substances may have been in or on them. Organic or biochemical residue analysis adds to these techniques by describing what was directly in contact with the artifact. This can then be linked to diet and artifact function, which can indicate culinary practices or be generalized to subsistence practices and other social behavior.

Archaeological food residues are unique among substances normally submitted to analytical chemistry analysis. Most analytical chemistry techniques are geared towards characterizing materials that tend to be relatively pure, materials that are known to the analyst, or both. Substances with simple molecular structures are the easiest to identify; however, if the researcher knows what they are looking for, they can pinpoint their analytical techniques to identify a single substance in otherwise very complex mixtures. This principle is used frequently in the food

industry, where various analytical techniques are used to determine if a food product has had other substances added to it (Ellis et al. 2012).

Food residues are neither simple nor known materials; they are abstruse mixtures of complicated substances. The plants and animals that contribute to the human diet are mixtures of complex molecules: fats, proteins, and carbohydrates. These foods are then combined in various ways. This complexity is further increased by culinary practices that break down some molecules and create new ones (Wandsnider 1997). These residues adhere to artifacts which then enter the archaeological record and undergo taphonomic processes. These processes can affect the molecular structures through biological and physical means, such as water moving through the soil washing away water soluble residue, microbes consuming residues and depositing byproducts, or soil chemistry affecting residues (Oudemans 2007; Hillman et al. 1993). While other types of archaeological residue analysis deal with complex mixtures of substances that have undergone their own taphonomic processes, many, such as resins or pigments, only have a limited number of constituent materials which are likely to be present. The substances which one might find in archaeological food residues are comparatively limitless (Wandsnider 1997).

Fortunately, instrumental analyses have improved to the point that researchers are able to characterize trace amounts of these complex molecules, and the results of these analyses can in turn be interpreted to identify the source of the archaeological residue. The following sub-sections briefly review the historical progress of the analyses of archaeological food residues and give an overview of how the technologies in question work.

History

Archaeological applications closely followed the developments of technology, as can be seen in Figure 3. Initial work with all these techniques tends to focus on inorganic materials, move to organics, and then foodstuffs. As technology improved small amounts of sample could be analyzed with increasing precision. The first written records for chemical analysis are from Egypt during the second millennium BC, documenting the determination of the purity of gold (Szabadváry 1966). The earliest interpretation of social relations based on such scientific investigations comes from Fabroni in 1810, who notes class differences in grave goods from an Etruscan tomb based on metal composition (Fabroni 1810). Though the 19th century was still focused on traditional wet chemistry analytical methods, it is during this period chemists develop processes that will eventually lead to the introduction of instrumental analysis in the latter half of the 20th century. Likewise, in the development of archaeological methods, the late 19th and early 20th century was associated with a switch from amateur collecting for private curios and museums to more systematic professional methodology, and this is when some of the first applications of wet chemistry to archaeology appear. The interest in scientific approaches to archaeology, including analytical chemistry, was so strong that a new term, archaeometry, was coined in the 1950s by Christopher Hawkes to describe it. Since then, there has been a rapid development of organic residue analysis.
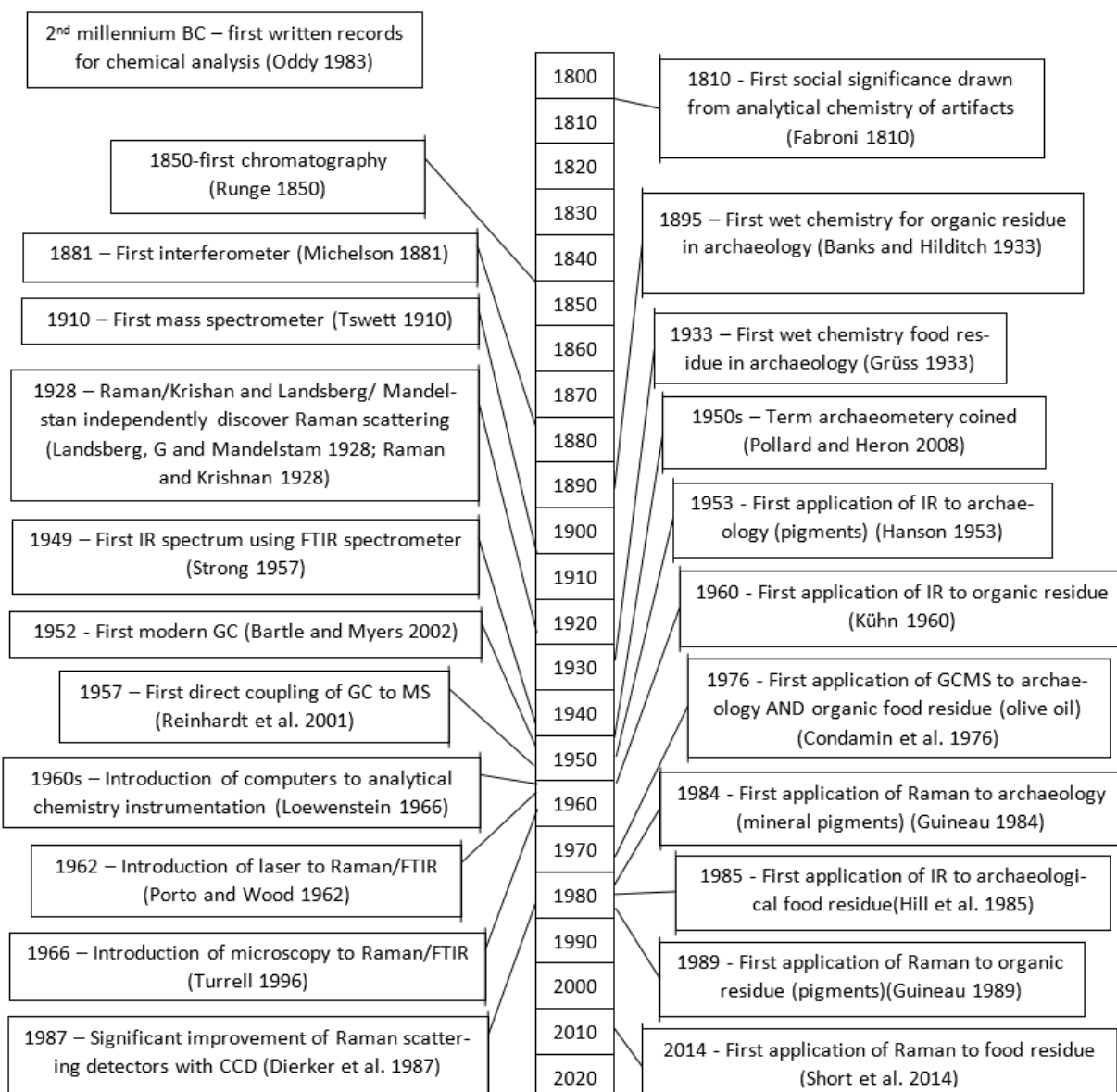
**Figure 3: Timeline of the development of GCMS, Raman spectroscopy, FTIR spectroscopy, and their applications to archaeology**

Analytical Methods

The two techniques of focus in this review are separation-analytical methods and vibrational

spectroscopy. Both techniques produce spectra, which are visualized as graphs with peaks at

certain locations that relate to the components being studied; Figure 4 illustrates an example of

GCMS, FTIR, and Raman spectra of olive oil. These are interpreted by trained specialists:

location along the x axis determines the identification of the molecular component, while the

height of the peak up the y axis is the intensity of the measurement of each component.  Detailed

descriptions and methodological discussions are provided in several books written for

archaeologists (Price and Burton 2011; Malainey 2011b; Castillo and Strivay* 2012).
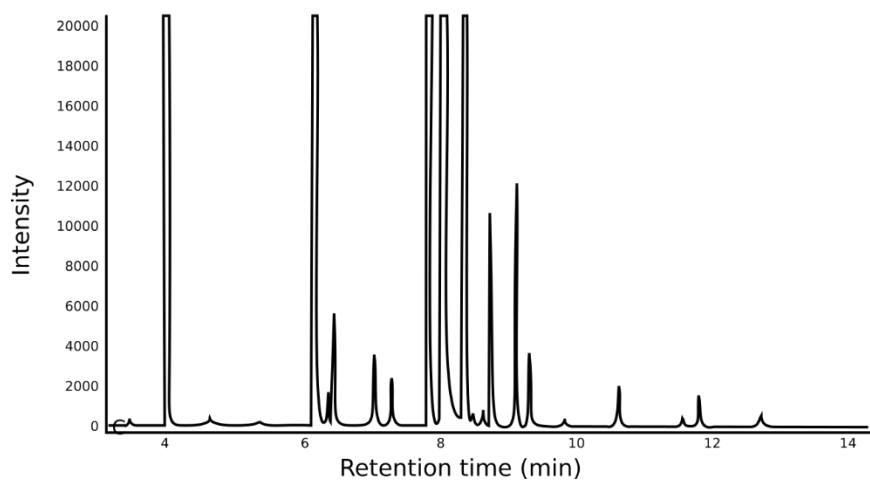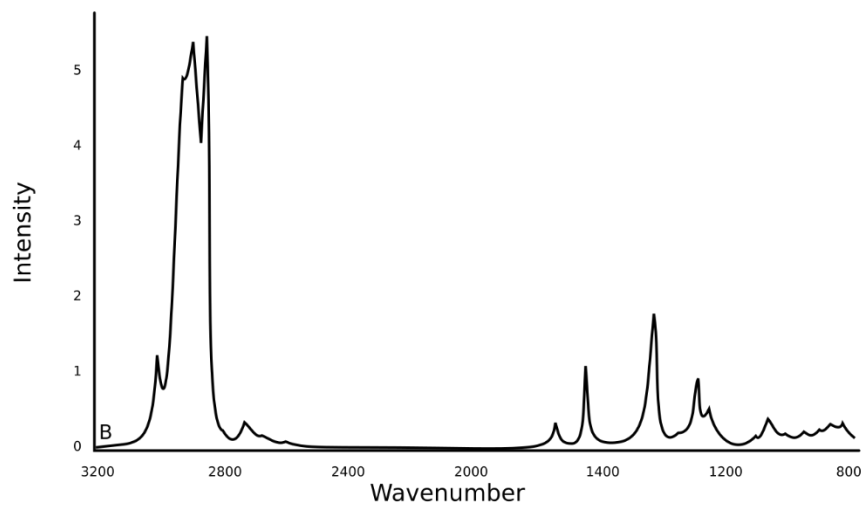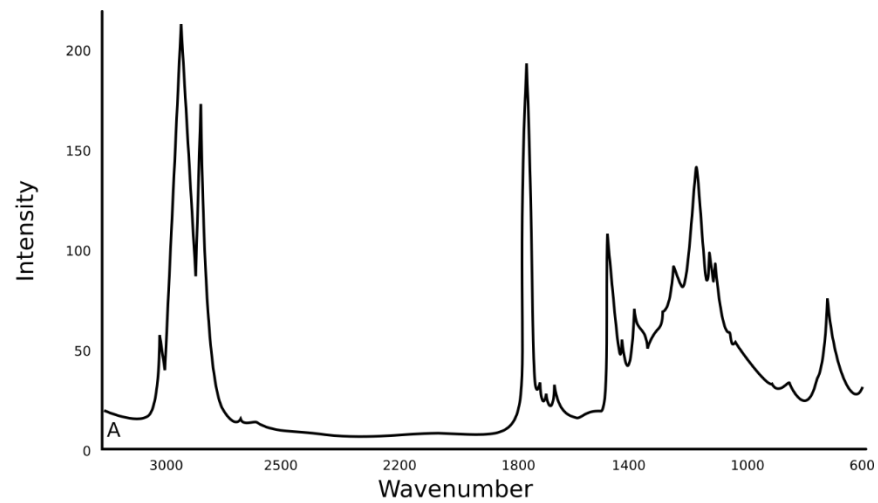
**Figure 4 Spectra of Olive Oil from A) FTIR, B) Raman, and C) GCMS analysis. Adapted from Yang and Irudayaraj** (2001) **and Yang et al.** (2013)

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Separation-analytical methods refers to methods that couple a technique to separate components

and a technique to analyze the separated components. Each component can be used on its own

but the combination allows for a more precise analysis. There are numerous separation-analytical

techniques, but the best known in archaeology is probably GCMS. In the case of GCMS, the gas

chromatograph fragments the sample using heated gas while the mass-spectrometer measures the

mass to charge ratio, as can be seen in Figure 5. There are some limitations, as some molecules

may be too large, too polar, or too thermally unstable to pass through the gas chromatograph.

Others may not accept the charge or they may be destroyed by the ionization process in the

mass-spectrometer. Thus, while GCMS can analyze many materials, not all can be analyzed.

Also different technologies can be used for each instrumental component, so only the analyses

that that used the same or demonstratively comparable methods can be compared.
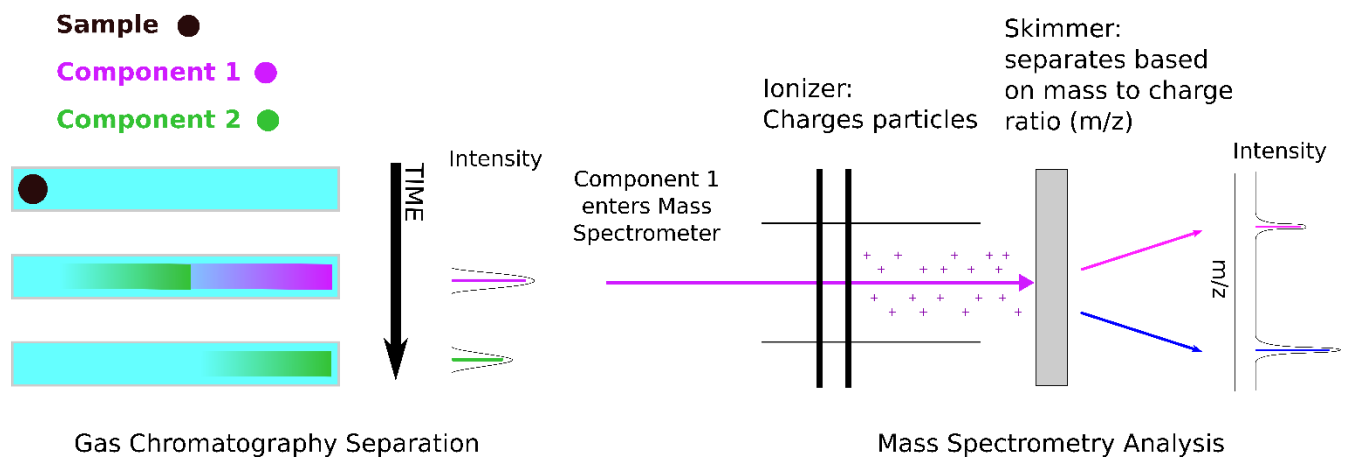


**Figure 5 Diagram of how GCMS works**

Vibrational spectroscopy uses light to vibrate molecular bonds (thus it is also known as optical spectroscopy). The molecular bonds vibrate in consistent ways, such that changes in light frequency can be used to determine what kinds of bonds are present in the molecule, which indicates what molecules are present in a compound. While there are several vibrational spectroscopy techniques, the most common among archaeological biochemical residue studies is FTIR, with Raman a distant second. They are related in that they use the same basic method but are measuring different effects. FTIR measures the change in the light that is transmitted through the sample, while Raman measures the change in the light that is scattered, as can be seen in Figure 6. Vibrational spectroscopy also has limitations. Depending on the technique, not all bonds vibrate. Water, for example, creates a large band in FTIR that obscure the peaks around it, while it does not show up in a Raman spectra at all. Other variations in instrumentation can also affect the resulting spectra; for example, Raman analysis of organic materials is best achieved using longer (1064nm) wavelength lasers for excitation, as shorter wavelengths over-excite the molecules and cause florescence, which overwhelms the Raman signal. These limitations must be kept in mind when comparing multiple studies.
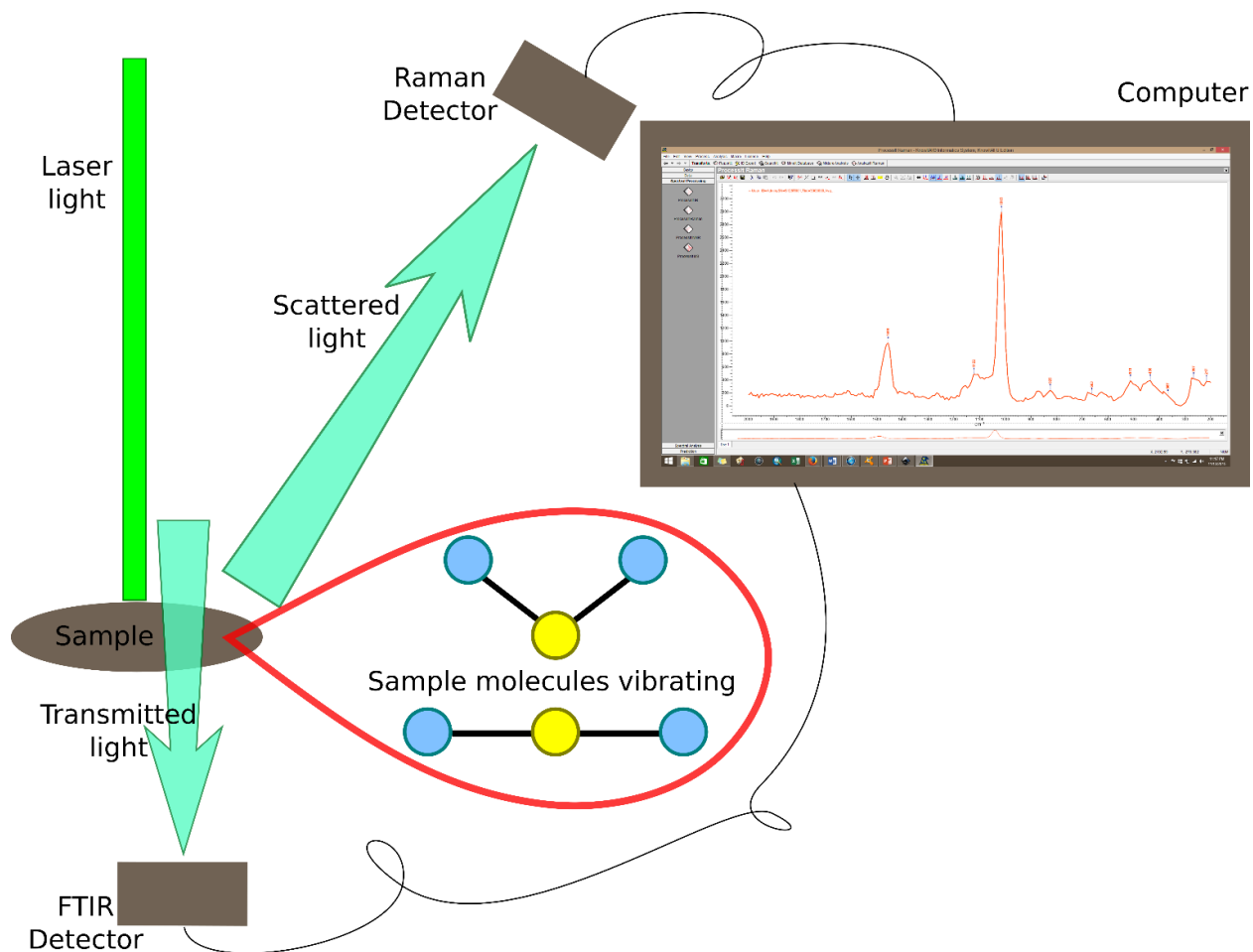
**Figure 6 Diagram of how Raman/FTIR works**

METHODOLOGY

This article reviews 100 English-language peer-reviewed articles, dissertation, and theses published over the past 30 years, representing work by specialists. Works were chosen for their relevance to the theme of biochemical residue studies of archaeological foodstuffs, focused on separation techniques such as GCMS and vibrational spectroscopy such as FTIR and Raman. While protein and blood residue work falls under biochemical residues and may be relevant to subsistence studies, the techniques are fairly specialized and thus excluded (Evershed 2008b).

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Additionally, the review focuses on application of techniques rather than the development of methods or theory. Therefore, articles not focusing on analysis of archaeological materials such as experimental work and blind tests were excluded from the formal review, though they will come into play during the discussion. While this review aims to be comprehensive, it is not exhaustive - in order to prevent weighting the results, some particularly prolific authors' contributions are limited.

The present review identifies and describes qualitative and quantitative patterns in the literature. A deeper meta-analysis was no possible, however, as these articles tended to be inconsistent in what kind of information they provide. This is likely due to the wide range of journals in which these articles were published, across a variety of disciplines, each with different publishing standards; this is discussed further in the quantitative analysis. The current challenges in biochemical residue analysis are identified and best practices standards are proposed to address these issues. While a number of articles have also suggested best practices for sample collection, analysis, or both, these tend to be based on anecdotal experiences rather than a systematic review of the current state of research (see Manzano et al. 2015; Mazow et al. 2014). Their relative proliferation is indicative that such standards are needed. The set of best practices suggested here is based on the systematic review of current body of literature.

QUANTITATIVE PATTERNS IN LITERATURE

The quantitative patterns portion of the review flows from broad questions such as who is doing the studies and where they looking, to what kinds of artifacts are being analyzed and how they handled, to the actual analysis of the residues themselves. Then issues that need to be considered

through the whole of the analysis process are discussed, specifically contamination and degradation.

Number of Articles Published Over Time
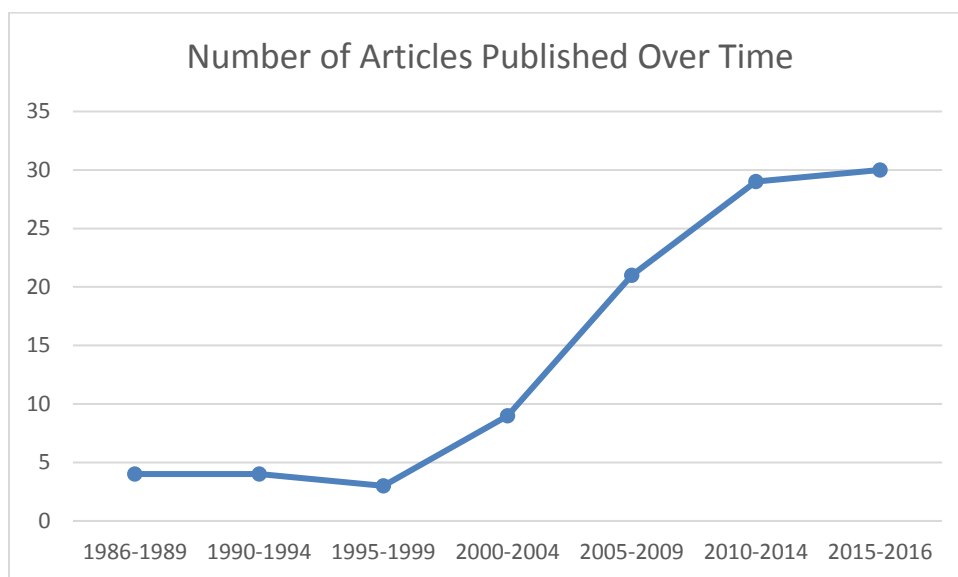


**Figure 7 Number of articles published over time**

Over the past 30 years there has been a significant increase in published articles using separation spectrometry and vibrational spectroscopy to assess foodstuffs and related residues. While these techniques were regularly applied to other archaeological artifacts and non-food residues, food residues did not gain traction until the early 2000s, as seen in Figure 7. It is not clear if this is related to technological development or influence of researchers dedicated to residue studies. Short of a fully comprehensive review, this is probably a good estimate of the actual frequency of these studies. The biggest limitation to a fully comprehensive review is that each article

needed to be skimmed to determine if actually addressed food residue, rather than non-food

residues associated with food processing tools, such as hafting adhesive or pigments that were

ground using ground stone. Secondly the diversity of journals that they were published in—

discussed below—means even if one limited the search to a specific journal the results would be

limited in relation to the field as a whole. To check how well this 100 article review reflects the

actual pattern in publication frequency, two journals, *Journal of Archaeological Science* and

*Archaeometry,* were searched for the co-occurrence of food and residue with each of the

techniques: GCMS, FTIR, and Raman. The totals for both journals show similar patterns, with 6

articles published before 2000, 11 from 2000-2004, jumping dramatically to 48 from 2005-2009,

46 from 2010-2014, and 33 in 2015 and 2016.

**Table I: Area of interest for publications**

| Archaeology/anthropology | 69 |
|---|---|
| General science | 15 |
| Analytical techniques | 6 |
| Other disciplines | 6 |
| Thesis | 4 |

The reviewed articles were published in a variety of journals. Table I shows the general area of

focus for the journals. The majority of them (69) were in archaeology or anthropology journals,

15 were general science, 6 were focused on analytical techniques, 6 were from other scientific

disciplines, and 4 were dissertations or theses. This can be seen in Table II, which shows the

journals with three or more articles. Three of these journals are focused on archaeological

science, and even the one area-based journal on this list has a significant scientific focus. The others were major general science and major general archaeology journals. The specific journals they were published in attests to the interdisciplinary nature of this field of study.

**Table II: Journals**

| | |
|---|---|
| *Journal of Archaeological Science* | 22 |
| *Archaeometry* | 11 |
| *Proceedings of the National Academy of Sciences* | 8 |
| *Antiquity* | 3 |
| *Documenta Praehistorica* | 3 |
| *Mediterranean Archaeology and Archaeometry* | 3 |
| *Nature* | 3 |

To explore who is doing these studies and where, the location of the researchers' institute the study site was determined per paper. Thus, if five researchers were all from different institutions within Europe, that paper's origin was listed as once for the Europe. Relatedly, even if a single European institution produced five papers, since each of those papers was from Europe, that one institution accounts for five instances of Europe. Finally, since there is significant collaboration in these studies, many papers have multiple institution origins; thus, there are somewhat more data points than 100 papers. This is true of many variables examined through this section.

**Table III: Regional location of Researcher's institution and Sites**

| Region | Researcher's institution location | Site location |
|---|---|---|
| Africa | 1 | 5 |
| Americas | 34 | 20 |
| Asia | 11 | 27 |
| Europe | 67 | 54 |
| Oceania | 1 | 3 |

Looking at Table III: Regional location of Researcher's institution and Sites , there is a probable

English language bias: the bulk of the institutions are from Europe and the Americas, and when

broken down by country 37 are from the UK and 30 are from the USA. Institution locations by

country indicate a fair amount of internationally collaborative work. While the majority papers

(62) only represent one country, 26 have authors from two countries, 6 from three countries, and

5 papers have authors from four to six countries. These international collaborations are further

reflected in the site locations. While European sites are the most common; more sites from Asia

are studied than from the Americas.

**Table IV: Depositional context of sites**

| | |
|---|---|
| Curated | 23 |
| Water adjacent | 18 |
| Underwater | 5 |
| Cave | 5 |

Depositional environment and the level of potential preservation plays a role in what sites researchers choose to study. This researcher had hoped to be able to discuss environmental factors in depth; however, most articles only reported minimal information on the sediment, climate, or other environmental factors that affect preservation. Only in rare cases did authors provide detailed information on about the depositional environment, while others provided none at all. Some of the articles were parts of larger projects and this information may have been available in other articles or reports that were not reviewed. Other cases were likely influenced by the journal standards, such as journals focusing on analytical chemistry techniques, and so the importance of these details were overlooked. As a result, this section reflects the limited reported data. As can be seen in Table IV, several of the sites were water adjacent, meaning that the site was near a body of water, including ocean, stream or lake. A few were from underwater or otherwise waterlogged sites; others from dry cave deposits. While it was not always clear what came from collections (if the site information citations were ten years older than the publication of the residue article, for example), but some of the articles indicated that at least some of their samples came from a museum or other storage facility, stored for anywhere between a few years to decades.
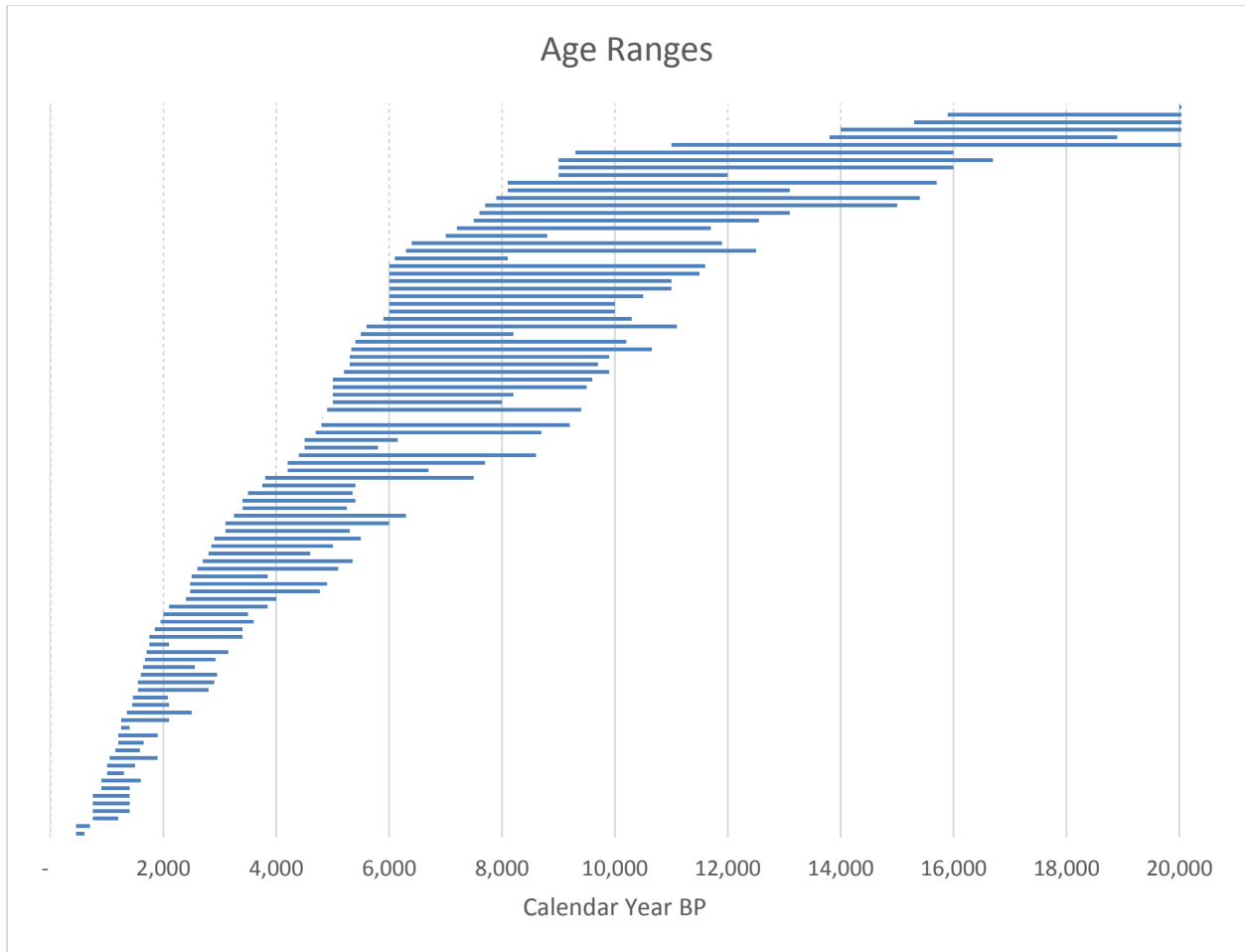
**Figure 8 Age Range Represented by Articles**

Site age also affects preservation. Figure 8 shows how frequent any given date is, in terms of the length of time covered by all sites used by each paper. Age range for each paper was determined by taking the oldest and youngest date reported for all samples in a particular study. It does not necessarily represent site age. The longer periods represented here are either due to large scale studies incorporating many sites or are the result of approximate calendar dates based on reported periods. Figure 9 shows how frequently any millennium appeared in the literature. This

was determined by breaking the whole period up into thousand year intervals, and if the age

range for a paper fell within that interval it was counted.
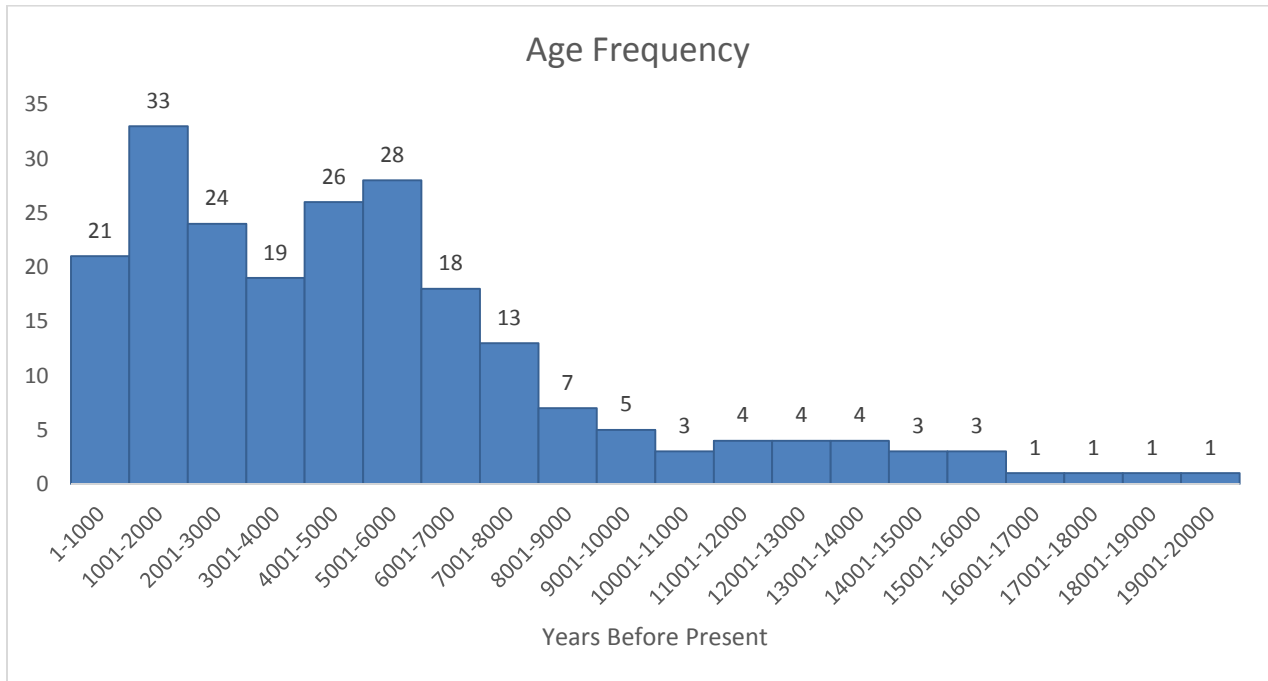


**Figure 9 Frequency of time periods appearing in articles**

Dates recorded here should be considered tentative in large part because dates are reported

inconsistently: a) many authors only gave period, not calendar dates, b) since it was not always

indicated, all "BP" dates were assumed to be calibrated, c) all dates were converted to years

before present then rounded to the closest 50 years. It seems many authors assumed that readers

would be familiar with the local chronology or have access to site reports with detailed date

information. In two cases no dates were given at all. While having period dates is better than

nothing, relative dates based on material culture vary across a region in relation to their associated calendar dates. Thus, the dates used here for the site age are at best an educated guess.

Unsurprisingly the bulk of the dates are relatively recent, dropping off after 6000 years and very few papers touch on dates after 10,000 years ago. Figure 8 shows that these more recent articles tend to focus on very short periods of time, while articles with older sites represent longer periods of time. That residues were still found on these very old artifacts is impressive, and appear to provide opportunity study broad factors in degradation.

**Table V: Artifact type for analysis**

| Pottery | 84 |
|---|---|
| Ground stone | 5 |
| FCR | 5 |
| Lithics | 2 |
| Soil/sediment | 4 |
| Organics | 4 |
| Floor plaster | 1 |

Further narrowing the focus from site description to artifact analysis, Table V summarizes the types of artifacts chosen for analysis. These are predominantly pottery, though various stone artifacts were also examined including ground stone, fire cracked rock (FCR), and chipped lithics. Notable are the more unique cases, including where soil and plaster flooring was examined for food residues from food processing (rather than being used as a baseline control), as well as organic materials. The residues that came from these artifacts were predominantly absorbed into the matrix (88), though 18 articles described visible residue, while in two cases

31

actual foodstuffs (seeds and bread) were analyzed. Pottery is most popular for a variety of

reasons: it is porous and easily absorbs and preserves residues within its matrix, while also being

relatively easy to extract said residues from. It also has the longest history with food residue

analysis and thus has been the most studied with regard to absorption, contamination,

degradation, and related studies. Still, most artifacts related to food production including pottery,

ground stone, and fire cracked rock are not directly associated with either faunal or

macrobotanical materials. Given that it has been demonstrated that other artifacts also have

preserved residues, it is worthwhile to not limit oneself to pottery samples when looking food

residues.
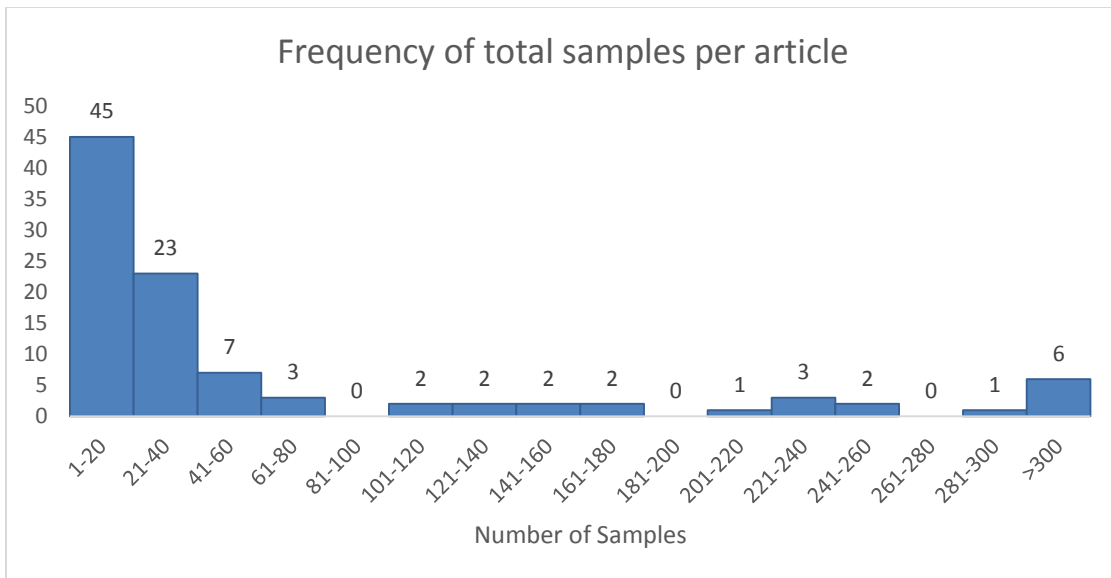


**Figure 10: Frequency of total number of samples per article**

The number of samples a researcher decides to analyze depends on a variety of factors: what is available to analyze, money and time limitations, and research questions being asked. Number of total samples analyzed per article has a large range, from a minimum of 1 to a maximum of 2225. The average is 94 samples, but the median is 23, with a standard deviation of 253, reflecting a significant skew to the lower end of the range. As can be seen from Figure 10, the bulk of the articles have less than 40 samples (n=68). Breaking it down a bit further, 29 articles have 10 or fewer samples, 16 have 11-20 samples, 17 have 21-30 samples, and 6 articles have 31-40 articles. The rest of the articles are distributed in low numbers across the range. Thus, while it is not unusual to have a larger number of samples, almost 70% analyze 40 or fewer, with 30% analyzing 10 or fewer.

**Table VI: Residue extraction technique**

| | |
|---|---|
| Chloroform/methanol | 66 |
| In situ | 14 |
| Hexane sequence | 6 |
| Acidified methanol | 5 |
| Chloroform | 5 |
| Water | 4 |
| Other | 12 |

How residue samples were extracted from the artifacts can be seen in Table VI. The focus is not the full sample preparation, just on how the samples were removed from their matrix. A variety of residue extraction methods utilize a combination of chloroform and methanol in different volumes. The majority are based on either the Folch (1957) or Bligh and Dyer (1959) methods.

One of the most common variations was described by Charters et al. (1993). Another common variation is to substitute chloroform for dichloromethane, an extremely similar but slightly less toxic solvent – those variations are considered chloroform for these purposes. Chloroform-methanol based methods were used by the majority of the articles. The next most common was in situ – i.e. samples studied without extraction – examination, and was applied to whole samples and visible residue using vibrational spectroscopy techniques, as it is not possible for separation techniques. Hexane sequence refers to the extraction method described by Hill and Evans (1987) involving increasingly polar solvents: hexane, chloroform, propanol, and water. Acidified methanol method was recently developed by Correa-Ascencio and Evershed (2014). The various other methods included biomarker specific extractions, significant variation on other techniques, or entirely independent that have not been widely adopted.

**Table VII: Analytical method**

| | |
|---|---|
| Separation-analysis GCMS | 76 |
| Separation-analysis other | 45 |
| Vibrational Spectroscopy - IR absorption | 19 |
| Vibrational Spectroscopy - other | 2 |
| isotopes - GC-C-IRMS | 32 |
| isotopes - bulk | 5 |
| Microscopy | 5 |
| wet chemistry spot tests | 6 |
| XRF | 2 |

Once food residues are removed, they need to be analyze. How they were analyzed has been summarized in Table VII. The bulk of the analytical techniques used were separation-analysis types– most instances were GC-MS, though other types were common. It should be noted that GC-C-IRMS (or Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry, a type of separation-analysis technique that includes isotope analysis) was counted as an isotopic technique, not a separation-analysis. FTIR absorption techniques was the most common the vibrational spectroscopy techniques, while 1 used UV absorption (which functions the same as FTIR except the excitation laser is a different wavelength) and another used Raman. There were a number of techniques that were used to supplement separation-analysis and vibrational spectroscopy. Isotope analysis was common as it is used to help get a detailed characterization of lipids – many used GC-C-IRM though a few used bulk isotopic techniques. Other techniques included microscopy, wet chemistry spot tests, and XRF. Many researchers used more than one technique-the average number of analytical techniques used per article is just under 2 (1.85). While many (47) used one, 28 used two, 20 used three, 4 used four and 1 used five.
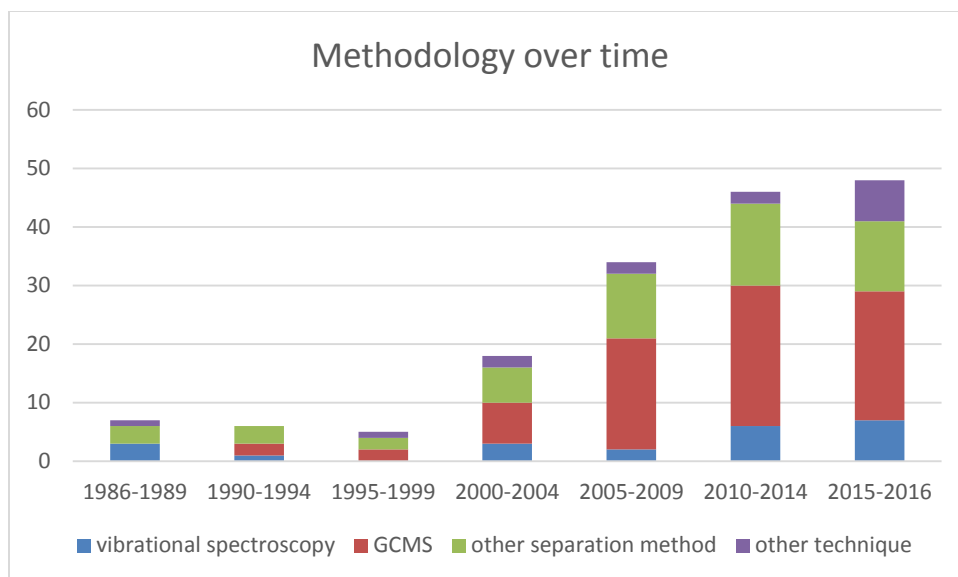
**Figure 11: Methodologies used over time**

The analytical methods that researchers used changed over time, as can be seen in Figure 11. General improvement of technology and increasing interest over time has resulted in diversification in how residues are being studied. Prior to 2000 there appears to a fair amount of variation in the kinds of analyses being performed, probably due to the relatively low numbers of articles during this period. After that, the proportions remain relatively constant with the bulk of analyses using some sort of separation method, though after 2015 there is an increase in relative use of other techniques, and slight relative increase in vibrational spectroscopy.

**Table VIII: Characterization method**

| | |
|---|---|
| Component identification | 91 |
| Biomarker | 65 |
| Finger printing | 56 |
| Not described | 2 |

Once the residues have been analyzed, the raw data needs to be characterized, and when possible, identified. There are three broad ways that a residue can be characterized: component identification, using biomarkers, and using fingerprinting. Most researchers use more than one. Briefly, component identification identifies the molecule or molecular structure associated with each peak. Archaeological biomarkers are compounds, unique to a particular substance or class of substances, that can be linked back to human behavior (Evershed 2008b; Regert 2011; Hillman et al. 1993) Using a biomarker technique first involves identifying components, then using prior knowledge of a substance and how it breaks down (i.e. through reference building including experimental work), connects those components to substances that may have been used in the past. This differs somewhat from fingerprinting, which does not require that components be identified. As used here, fingerprinting includes both matching spectra and using ratios of components (such as fatty acids or isotopes) to identify substances.

The characterization methods are summarized in Table VIII. Almost all of the articles identified components, which is to be expected given it is the first step of the biomarker process. Almost as many articles used a fingerprinting method as used biomarkers, with the grand majority (86) used two or more methods. This is another case in which researchers frequently did not describe the process used – in those cases it was inferred based on what literature they were citing. In two cases the characterization process was not described at all; these were from archaeobotanical publications (one pre-1990 and one post-2010) where the result of the chemical residue analysis were simply indicated. Relatedly, raw data was frequently not reported nor available as supplemental information, making meta-analyses and comparisons between different characterization methods difficult.

**Table IX: Reference library source**

| | |
|---|---|
| Literature | 76 |
| Experimental | 26 |
| Modern references | 27 |
| Ancient references | 4 |
| Not described | 2 |

All of these characterization techniques require prior analysis of potential residues, that is, a reference library. Building reference libraries is key to residue work; they should include not just potential foodstuffs, but those foodstuffs processed and aged, as well as potential contaminants. As no author can do it all, it is expected that they rely on the literature to an extent, but it is important to be sure that the literature is relevant to the area one is studying. Aside from issues with differential degradation across different depositional environments, studies indicate that there is worldwide spatial variation in various residue signatures (see Gregg et al. 2009). A summary of the sources of reference libraries can be seen in Table IX. Most of the authors relied on references libraries published in the literature. Of those that created their own reference library, most tested modern references though a few did look at ancient samples. It was not always clear when authors were referencing their own (or their lab's) reference library as opposed to literature. In some cases an in-house reference collection was mentioned but not described; it was assumed to contain modern references. Additionally, several authors did experimental work, either testing anthropogenic changes due to cooking and processing methods or degradation due to aging. In the two cases where reference library source was not given, information on biomarkers was treated as common knowledge and uncited. This is inappropriate, as even component identifications can be cited within the literature.

**Table X: Food characterization**

| | | |
|---|---|---|
| **Carbohydrates** | | 5 |
| **Proteins** | | 3 |
| **Lipids (all)** | | 80 |
| | Unspecified | 6 |
| | Beeswax | 11 |
| | Plant | 43 |
| | Animal (all animal lipids) | 64 |
| | General Adipose | 28 |
| | Ruminant Adipose | 20 |
| | Non-ruminant Adipose | 17 |
| | Dairy | 23 |
| | Unspecified fish | 12 |
| | Marine | 9 |
| | Freshwater | 3 |
| **Resins** | | 16 |
| **Minerals** | | 3 |
| **Other compounds** | | 18 |
| **Specific resources** | | 5 |
| **Not specified** | | 1 |

The results of these residue characterizations are shown in Table X. Most of the articles

identified lipids, which have been separated out further. In a few of these studies, the lipids were

not further specified, either due to research goals of the paper or because of the limitations of the

analytical technique chosen. The rest were either beeswax, plant, animal lipids. Some of the

studies that found animal lipids– due to research goals, analytical techniques, or both – were

broken down further. Of these, most were adipose fats not further specified, though ruminant

(large herbivores including cattle, goats, sheep, deer) and non-ruminant (sometimes identified

more specifically as porcine) were identified, along with were dairy fats. In most cases when

water resources were found, freshwater and marine were not differentiated. Freshwater fish were

only identified alongside marine resources, when the research goal was to determine what kind of aquatic resources people were using.

There were significantly fewer articles that identified non-lipids. A few articles identified carbohydrates and proteins. Only in one case were the results not specified – in this case, while the goal was to identify resources, the results were overwhelmed by contamination. Resins were identified, usually associated with wine. Minerals were also identified, either background noise or indicative of the consumption of bone. Other non-macronutrient compounds were identified in a number of articles, in Table X as "other compounds". These were usually specific biomarkers being sought out to detect either wine or cacao. In very few cases specific food resources were identified, all by fingerprinting methods using vibrational spectroscopy.

**Table XI: Use of controls**

| none indicated | 65 |
| lab blanks | 23 |
| field controls: soil | 12 |
| field controls: comparable substances | 9 |
| curation control | 0 |

The researcher needs to know that the residues they characterized accurately represent ancient foodstuffs. A control allows for the researcher to account for contamination and degradation (McGovern and Hall 2015). As can be seen in Table XI, despite its documented importance the majority articles did not mention the use of a control. Use of laboratory blanks, a standard

technique to test for contamination during the analytical process, was only mentioned in about a

quarter – given that it is routine procedure, it is possible that this was omitted from the methods

discussion and was under-reported. A few cases soils were collected to test if residues transferred

between soil and the matrix (as opposed to collection for residue testing). In even fewer cases,

controls were taken from like substances – such as the unused portion of pottery or ground stone,

or in the case of FCR a non-cultural rock – to provide a baseline for environmental

contamination, sometimes considered background noise. In no cases were control samples taken

from curation facilities, though Washburn et al. (2014) demonstrated this to be as important for

samples for curated artifacts as freshly excavated ones.

**Table XII: Contamination**

| Not mentioned | 21 |
|---|---|
| Discussed only | 15 |
| Plasticizer noted | 13 |
| Other potential contaminants noted | 14 |
| Removed outer portion | 38 |
| Field controls | 21 |
| Lab blanks | 24 |

Use of controls to evaluate contamination is addressed in Table XII. Contamination may be from

environmental processes or may occur in the lab, thus controls should be used and residues

screened for known potential contaminants. Even if it is concluded that contamination is not an

issue for those residues, how that was determined needs to be documented in the resulting publication.

In a number of the articles contamination was not mentioned at all or it was mentioned but there was no indication that they attempted to control for it. In some cases either plasticizer – contamination from contact with plastics – or other potential contaminants were noted, indicating contaminants from the environment, handling, or both. Contamination can also be controlled via methodological considerations. In many cases the outermost portion of the sample matrix – either pottery or stone – was removed as this is the section most likely to have contamination. Controls were also used – the articles mentioned using either field controls or lab blanks. Field controls provide a baseline for environmental contamination, and lab blanks are helpful to identify contamination during analysis.

**Table XIII: Degradation**

| Not mentioned | 21 |
|---|---|
| Discussed only | 6 |
| Noted during interpretation | 69 |
| Experimental work | 6 |

Degradation is a normal occurrence in the archaeological record that changes the original residue profile. It should be assumed to have occurred, and thus—like contamination—should be accounted for during analysis and reported in the publication. A lot of work has been done on the degradation pathways of fatty acids (Eerkens 1989), though a smaller body of work has also indicated other macronutrients such as proteins and carbohydrates survive (Bland et al. 1998).

Given that the water soluble biomarkers of cacao survive, it may be worthwhile to expand the

non-lipid based work. As can be seen in Table XIII, the grand majority of the articles directly

dealt with degradation: the majority during their interpretation though a few articles did

experimental work specifically related to degradation. However, in some cases it was not

mentioned at all, and in few cases it was discussed but not apparent that it was considered during

the interpretation.
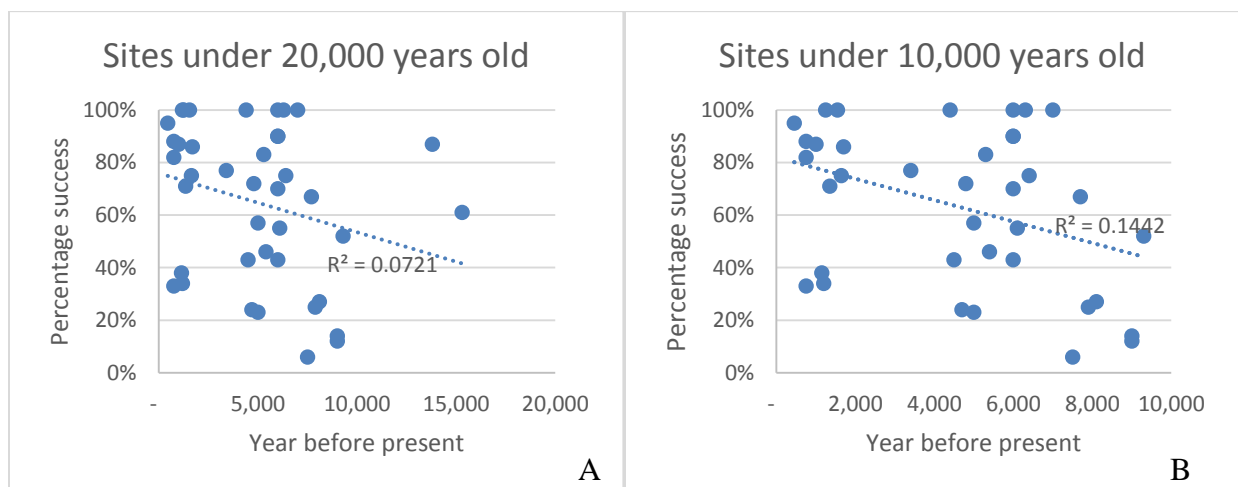


**Figure 12: Relationship between site age and percentage of artifacts with residue as a proxy for preservation**

The reported percentage of samples with identifiable, non-contaminated residues (i.e. successful

results) was compared to broadly reported ages for the sites. Only 40 papers reported the number

or percentage of successful results. Figure 12 displays the relationship between percentage of

successful samples and the age of the sites – a) for all sites, b) for all under 10,000 years. While

there appears to possibly be a weak relationship ($R^2$=0.144, p =0.12) between sites under 10,000

years of age (based on the start date) and successful results, the author has concluded these results are not valid. This is due in part to the way dates were recorded, and in part because different researchers used different criteria to discard samples. Some rejected only those that had insufficient residue to be detected or were obviously contaminated, others were more stringent, rejecting samples that did not meet a minimum amount of lipids (to indicate that they were not the result of background noise/contamination). A more thorough meta-analytical study of degradation is warranted, but may not be possible.
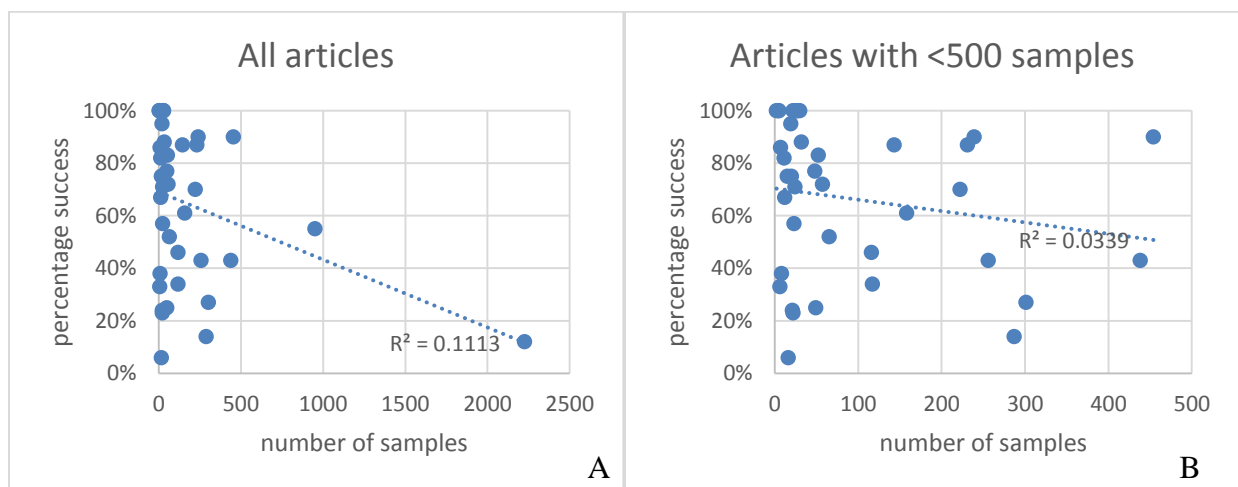


**Figure 13: Relationship between number of samples in an article and the percentage of artifacts with residue**

In order to test for publishing bias, the number of samples in a study were compared to its reported positive results. The papers with the three oldest sites were excluded from the initial regression analysis because despite being between 11,000 and 200,000 years old, they all had over 60% successful results, which is remarkably good preservation. It is possible that there is a

push-pull motivation to publish only positive results-not just refusing to publishing studies where no residue was found or identified, but declining to mention that larger number of samples were tested than reported on. Take two researchers both testing 12 samples, 8 of which provide enough non-contaminated sample to be analyzed. If one researcher reports all 12 samples while the other only reports on the 8 successful samples, then the first will have a 67% success rate while the second will have 100%. If researchers are dropping non-successful results from their reporting, the overall number of samples reported is lower, thus the expectation is that larger studies will have lower success rates. This is a good strategy for the researcher to improve the probability of being published; however, it would be good if publishers advertise their willingness to publish results that were not across the board successful.

Figure 13 shows an initial regression comparing the number of samples in a study and the reported percentage successful results showed a weak but moderately significant relationship ($R^2$=0.111, p-value=0.0354). Given the weak connection, it was prudent to more closely examine the cloud on the left side of the graph, which showed non-significant non-correlation ($R^2$=0.034, p-value=0.269). This indicates that, of those who provide success rates, publishing bias may not be influencing reporting overall. Certainly, differences in preservation and sampling methods plays a significant role in percentage of successful samples. That said, some may still question if preservation in several millennia old sites is good enough that, even with careful sampling decisions, two samples can be tested and both result in identifiable residue. Thus, it can improve confidence to report on all samples examined, even if they are not all identifiable.

QUALITATIVE PATTERNS IN LITERATURE

While quantitative trend data describes the body of literature as a whole, qualitative information is needed to get a full picture. The qualitative patterns described in this section focus on broad research goals including what kind of information was sought in addition to archaeological data.

Several different types of research goals have been addressed by the articles, ranging from basic to higher order anthropological issues. The lowest order is simply characterizing the residues without connecting it to any anthropological considerations. The next order involves inferences about behavior based on those residue characterizations. Some second order studies focused on subsistence, such that residue information was linked to what food sources people were consuming. Other second order studies were focused on what the artifacts were used for, rather than subsistence. Frequently these were pottery studies, wherein the function of the vessel – determined by the residue – was compared to the form of the vessel. The final order is when those behavioral inferences are extrapolated to greater social networks, such as feasting, ritual, and diffusion of knowledge such as dairy or agriculture. These broad research goals were approached in a variety of ways.

Regardless of where they fell within the order of anthropological issues, in some cases researchers were not seeking to generally characterize residues, they were looking for evidence of specific resources, such as dairy (Craig et al. 2005), wine (Guasch-Jané et al. 2004), cacao (Henderson et al. 2007), certain oils (Koh and Betancourt 2010), aquatic resources (Olsson and Isaksson 2008). This is reflected by the use of specific extraction techniques or the focus on particular biomarkers. Studies that focused on just characterizing residues without looking to

higher order questions tended to be 'proof of concept'--they were either demonstrating that identifiable residues were present or were more concerned with methodological issues. While many of the earlier works certainly fall into that category (see Hill et al. 1985), many recent articles are still producing proof of concept type articles (see Bianco et al. 2015) indicating that this field still has significant growth potential.

Among those articles that did look at higher order questions, a number of them were seeking independent testing of specific hypotheses. Most of the form/function studies could be characterized as hypothesis testing. General subsistence patterns were also subject to testing, such as whether or not residues reflected patterns seen in other data sources such as faunal, macro- or microbotanical, or ethnographic. In some cases hypotheses bore out, sometimes they did not. Also among the higher order question articles, a number of these studies looked at variations in subsistence, vessel function, or both across multiple sites or through time. These studies were generally very large involving hundreds of samples, though some were smaller, containing as few as a two dozen. Copley et al. (2003) provides a good example of both. The relationship between pastoralism, farming and the use of dairy was examined by analyzing 950 samples from 14 sites from across Britain from the early Neolithic to late Iron Age. The results of the analysis supported the hypothesis that in Britain animals were being utilized for dairy before farming was established.

A majority of studies generated new information that could not have been obtained without residue studies. Some residues do not have other direct evidence in the archaeological record, especially liquids like dairy, fermented beverages, or oil (McGovern et al. 2013; see Evershed et al. 2008; Pecci and D'Andria 2014). In other instances the site does not have good preservation

of more traditional sources of subsistence information such as faunal or botanical studies (Kwak and Marwick 2015; see Buonasera et al. 2015). Sometimes direct subsistence information is available, but does not answer the research question, such as the case for form/function studies, or when residue data is being used as independent source for hypothesis testing. In very few cases, while residue studies were performed, the actual characterization relied more on other information from the site.

Numerous articles reported on either the first analysis of residues from an area, or the residues were from earliest/oldest dated sites. For example: Bianco et al. (2015) identified the first grape products from Torre di Satriano site, Crown et al. (2012) the earliest known use of black drink at Cahokia, Isaksson and Hallgren (2012) the earliest evidence of dairying in Sweden. Meanwhile, Reber et al. (2015) performed the first published study of lipid distribution from a whole vessel in North America; Tarquini et al. (2014) did the first FTIR microspectroscopy analysis of 3rd century A.D. roman amphorae from Monte Testaccio; and Buonasera (2016) analyzed the first samples of bedrock features from dry caves for lipid content.

In addition to answering questions about human behavior, many of the articles contributed to the field by performing experimental analyses and testing methodologies. Experiments allow researchers to better connect what is found in the archaeological record to actual human behavior, part of middle range research. A number of these experiments were tied into building reference libraries, testing the effects of cooking, aging, or both. These included both lab-based experiments (Oudemans et al. 2007; see Eerkens 2005), and actualistic experiments based on processing methods similar to the methods people originally used to create the archaeological materials (see Eusebio 2015; Heron et al. 2010; Kedrowski et al. 2009). These kinds of cooking

and aging experiments may end up taking years (see Pecci et al. 2013). Though not included in the quantitative analysis, there also a number of papers that focus solely on these kinds of experiments (Fankhauser 1997; Evershed 2008a; see Charters et al. 1997).

In addition to the effects of cooking or aging, several researchers looked at how residues behaved in their matrix. Condamin et al. (1976) established that pottery absorbed food residue, so did work by Buonasera (2005, 2007) and Quigg (2001) establish that burned rock and ground stone absorb food residue as well. There are significantly fewer studies of the residue absorption by stone than by pottery, however. Pecci et al. (2015) studied glazed and unglazed pottery, testing the idea that glazed pots prevent residue from being absorbed, and actually found that residues appeared to be more abundant in the glazed vessels. Romanus et al. (2009) examined the effects of pitch, oil, and wine on their relative absorption. While there are interactions between the substances, all are absorbed into the fabric of the pottery up to 2.5-3 mm. Dimc (2011) studied how deeply contamination may be absorbed by pottery, and while most of the contaminated lipids were in the outer millimeter, some did absorb as far as the 3 mm, the same distance as shown by Romanus. These studies are not exactly comparable, however, as Dimc reports on amount lipids absorbed, while Romanus reports on the biomarkers for pitch/oil/wine absorbed. Of more concern, however, is that while some of residues from the outer were clearly contamination by residues associated with handling and plastics, some of the residue from inner portions would have been interpreted as archaeological foods, specifically aquatic animals or vegetables. Notably, it does not appear that soils or sediments contribute significantly to these kinds of contamination (Heron et al. 1991; Dudd et al. 1998), though Reber and Kerr (2012) do observe that there can be soil-pottery interactions.

Other questions relate to sampling choice, such as where in the matrix to sample and what kinds of residues to focus on. Charters et al. (1993) examined how residues were distributed in vessels, and established that these distributions may vary based on the type of vessel. Though many researchers cite this when justifying their choice of where within a vessel to sample, and some have used the concept when interpreting vessel function (see Pecci et al. 2015; Soberl et al. 2014), it does not appear that others have tested it beyond the later experimental work done by Charter et al. (1997). Generally, researchers have some choice about where in a vessel to take samples, but usually do not have many options regarding what kind of residues (i.e. absorbed residues, visible residues, chars) to analyze. Oudemans and Boon (2007) had the opportunity to compare charred and non-charred residues, and found that chars had higher yields of extractable lipids per gram samples compared to non-charred residues, which may have been related either vessel use or effects of degradation on those types of residues. Their findings illustrate that choices regarding sampling can affect residue analysis.

More technical methodological investigations involved the testing of sample preparation and analytical instrumentation. A number of these tested the applicability of various analytical instrumentation (Bianco et al. 2015; Mirabaud et al. 2007; Garnier and Valamoti 2015). Goals generally include increasing precision and sensitivity of the analysis. Notable among these is Romanus et al. (2007), who compared different instrumental set ups to determine the precision and comparability of these methods. Sample preparation techniques may also improve issues around instrument sensitivity, since if residues are better extracted from their matrix, there is more material to analyze. Most people use established techniques, thus the bulk of these kinds of studies appeared earlier in the literature (Charters et al. 1993; Hill et al. 1985). There have been

some more recent developments, however, such as the microwave assisted method developed by Gregg and Slater (2010) and the single step extraction derivatization method developed by Koirala and Rosentreter (2009). Regardless of the methods used, however, it is consistently noted throughout the literature that while useful information can come for individual analytical techniques, the most complete picture comes when multiple complementary techniques are used.

DISCUSSION

There are a number of relevant observations and suggestions from the literature regarding how biochemical residue analysis is best performed. When developing their own analytical procedure, it may be worthwhile for the emerging specialist to look at how related fields of research address issues related to reference libraries, controls, contamination, and degradation. These include other subfields within archaeology such as microbotany analysis, as well as residue-related analysis in art history, forensics, and food science. That said, what follows are suggestions based on the review of the literature on biochemical analysis of archaeological food residues.

Foremost of the specialist's concerns should be assurance that the archaeological residues are actually being interpreted. Thus, contamination needs to be controlled and degradation accounted for. In addition to work by Washburn et al. (2014) and Buonasera (2005) stressing the importance of controls, Mazow et al. (2014) and related work by McCandless (2012) present a compelling story on the necessity of testing everything, including equipment, for potential contaminants. Additionally, new or unfamiliar procedures should be test run before using them on archaeological materials. An excellent opportunity to test methodology while also expanding the reference library is through the analysis of experimental work or other reference materials.

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Contributions to residues from the environment or natural contaminants cannot be eliminated, but several ways exist to control for it. One common practice being to remove the surface, though there is some doubt if this is necessary (see Evershed et al. 1990; Henderson et al. 2007; McGovern et al. 2013), or one can discard any samples whose total lipids fall below a minimum amount—Evershed (2008a) suggests discarding samples with less than 5µg of lipid per gram of sherd. Many researchers also test soils as a control method, to be sure the residues from the artifact differ from those in the soil. If soils are not available, the exterior portion of a pottery vessel can act as a good proxy (see Stern et al. 2000). Furthermore, while there have been studies establishing a basic understanding of how food residues interact with pottery, there are no similar studies on any types of stone artifacts. In addition the experimental work discussed earlier, such as determining the depth of residue absorption (Dmic 2011; Pecci et al. 2015) and testing interactions between the pottery and soil (Dudd et al. 1998; Heron et al. 1991; Reber and Kerr 2012), Johnson et al. (1988) established that firing pottery destroyed any residues that may be present in raw materials for pottery. Buonasera (2005) similar studies would be useful for cooking stone in particular: establishing that the heating process for cook stone destroys previous environmental residues; depth of residue absorption into rocks; and baseline minimum amount of lipids present in natural rocks. Since environmental residues are present on non-cultural rocks, off-site natural control rocks should be used for each site as this can help establish what an environmental signature may look like and the background environmental lipid levels (Buonasera 2005).

Before interpreting the implications of residues for human behavior, they must be identified or otherwise characterized. To do that, one needs a reference library, and building a reference

library with quality fingerprints, biomarkers, or both is a difficult task. One needs to be sure that

the biomarkers actually represent what they are supposed to. Though Evershed (2008) and

Hillman et al. (1993) focus on biomarkers, the same basic framework applies to fingerprinting.

First, differences component signatures between classes of source material need to be identified.

Second, variations of the signatures within these classes need to be understood. Third, the effects

of cooking and taphonomy need to be accounted for. The disparate effects of processing,

environment and age are especially problematic in the case of fingerprinting, where relative

proportions of components--whether fatty acids, isotopes, or molecular bonds--is key (rather than

presence/absence in a classical biomarker design). Many have noted that food mixing,

degradation, and environmental contaminants all affect these proportions, and that classes may

not be fully differentiated (Buonasera 2005; Barnard et al. 2007; Regert 2011). These three

components are all necessary, and without a strong understanding of each, they are all potential

sources of error in the identification and characterization of residues.

The first point, identifying spectra associated with the material class in question, is the most

straightforward step. Care must be taken that the spectra is only association with a particular

class. Barnard et al. (2007) has discussed how biomarkers may be associated with two or more

unrelated classes. Several articles note that residues need be interpreted in light of existing

archaeological and environmental data, and geographical considerations may be one way to rule

out biomarker confusion(see Koh and Betancourt 2010). Crown et al. (2012, 2015) address this

with regards to using caffeine as a biomarker for the use of certain plants, usually cacoa

(*Theobroma cacoa*) and holly (*Ilex vomitoria* and *I. cassine*). Caffeine has been found in vessels

from regions where there are multiple plants that are caffeine sources and from regions where

there are no caffeine sources. In this case, geography is not sufficient to determine the source of the caffeine, and a more precise biomarker or fingerprint is needed to strengthen the identification. Thus Crown et al. (2012, 2015) uses theobromine, theophylline, and sometimes ursolic acid in addition to caffeine to differentiate between cocoa and holly plants.

The second point, understanding variation in spectra within material classes, is more difficult. It requires testing as many variations with in a class as possible or practical, as a component may be misidentified if there is greater natural variation in the class's spectra than is present in the reference library. Stable carbon isotope values re used to differentiate different types of fates. Researchers often rely on the published data, as library building is costly and the data is relatively well documented. It is clear, however, that there is spatial variation in stable carbon isotope values, as two researchers have demonstrated that their location variation did not match the variation in the published data. Gregg (2009), found that $\delta13C$ values of sheep adipose from Israel and dairy fats from Turkey do not match those from northern Europe. Likewise, Spiteri (2012) found that the $\delta13C$ and $\Delta13C$ isotopic values of ruminant dairy and non-ruminant adipose fats were shifted in comparison to the UK reference data. Even with relatively well established reference literature, it is worthwhile to be sure local variation matches what is anticipated in the literature.

The third point, accounting for the effects of cooking and taphonomy, further complicates the reference library building. It is not enough to know components of modern species, but to be sure they survive through the cooking and taphonomic processes. Again, dealing with these issues take time and funding. Furthermore, fingerprinting may be particularly susceptible as multiple factors may interact in unanticipated ways. Many researchers, as previously noted have

conducted cooking and charring experiments to produce references for cooked residues. Here ethnographic and historic information is valuable for indicating how foods may have been processed. Survival through the archaeological record can be tested by either analyzing identifiable archaeological floral or faunal remains (Isaksson 1999; see Heron et al. 2015), through artificial aging of modern references (see Malainey et al. 1999a), or letting materials age through time (see Pecci et al. 2013).

Once the residue has been characterized, the human behavior implications can be interpreted. While prior knowledge of the archaeological and environmental data can contribute to these interpretations, there is also the danger that they may cause unintentional bias and circular reasoning. Faunal and botanical analyses define relatively narrow classes, usually down to genus or species level. Biochemical analysis of organic residue tends to use more broad classes, such as ruminant versus non-ruminant animal. Thus, if non-ruminant animal fat is found in a vessel, it does not prove that deer fat was cooked in that vessel, even if deer bones were found at the site. Circular logic is a pitfall to be aware of and avoided; however, different aspects of the archaeological record may be combined with biochemical analyses to more fully understand culinary practices. There are a number of good examples of this in the literature. Baeten et al. (2013) note the burn patterns on the vessels they were studying, as well as the texture of associated bones suggest that meat and vegetable cooked in the pots were stewed together. Poulain et al. (2016) also interpreted their results as a stew from a single meal, as the visible residues had been scraped off the outside of glazed vessels, which they argue would have been easily cleaned thus the residues represented a single meal. Koh and Betancourt (2010) found that a vessel with particularly weak peaks for the presence of wine had been repaired in such a way

that could not hold liquids – thus it may have held wine initially but was reused for dry storage. This ties in with an observation by Hill and Evans (1987) regarding the relationship between form and function, that a vessel may have been created for one purpose but used for another.

How results are reported is as important as accurate and precise characterization and interpretation of the residues. Standardization in reporting could improve comparisons of results from sites across time period and regions, allowing for meta analyses. While there were different research goals and different technologies used for the papers, this is not sufficient reason not to clearly report dates or environmental factors related to the actual site. As the field ages, the need to fully describe analytical technology fades, especially as this information is readily available from general reference sources. This pattern reflected in the literature: early papers tend to be focused on the mechanics of residue analysis, including how it works, details of methodology, and concerns related to contamination and degradation, while later papers tend to gloss over this information. However, many researchers are declining to report important information such as whether or not they used a control, or whether or not they noticed degradation/contamination products, which would improve confidence in the characterization. Likewise, negative findings are as important as positive – not only does the literature support that not finding residue on all samples is normal, the number of samples with residues can give information regarding the preservation at the site. Researchers are also declining to give details on how they interpret the source of the residues, such as not giving information about their reference collection. The characterization process is complicated by many variables, and is still developing as an area of study. Thus it remains important to report this information, and when possible provide raw data.

CONCLUSIONS

To summarize the current literature on using separation-analysis and vibrational spectroscopy: most articles reported on GCMS analyses of lipids in pottery. This is unsurprising: A) lipids are the best preserved macronutrients, and thus are most likely to be retrieved B) pottery is highly absorbent and provides a known reservoir for food residues C) vibrational spectroscopy has only recently improved it's sensitivity and ease of use. There appears to be a European/American dominance in who is doing these studies, though it may be the result of the English-language bias of this review. That said, there is a significant amount of collaboration between countries: 38 of the papers have authors from two or more countries. There is potential for a variety of meta-analyses, especially related to degradation but it would require tracking down site reports to get accurate information about the depositional environment. A preliminary examination of the data indicates that (as expected) there is a relationship between age of the site and amount of identifiable residues; however, while statistically significant, it does not account for a majority of the variation. Fortunately an equally preliminary examination does not indicate the appearance of reporting bias.

There is considerable variation in research goals for residue studies. Some of these studies focus on performing proof-of-concept tests, showing that a particular site or time period produced identifiable residues. Others were interpreting residues in the light of subsistence patterns, social behavior, or vessel function. Many of these were testing hypotheses derived from archaeological or ethnohistoric data. The majority of these studies, especially those related vessel function, had research questions that could not be answered without residue analysis. In addition to answering questions about cultural processes, many studies had research goals focused on technical aspects

of residue analysis. Experimental studies included the effects of cooking and aging, and how residues move through their matrix. Technical questions also included sampling choice: if given the option, where to sample from a vessel and what kinds of residues to analyze. Finally, improvements in technology were tested, both methods of sample preparation and what kinds of instrumentation to use. Based on this review and other detailed discussions of archaeological practices, standards for best practices have been developed, outlined below.

Best Practices Standards

In preparation for their study, the specialist, if not doing the sampling themselves, should work closely with the persons collecting the samples from the sites. This includes making sure that they fully understand proper sampling procedure and what kinds of samples to take. While there has not been work done to this end for other potential residue sources such as grinding stone or fire cracked rock, a number of studies have been performed on ceramics. The sampling supplies should be non-reactive – when taking whole samples this often means wrapping with aluminum foil (Lewis and Christensen 2015), or if taking chemical samples in the field that all gauze, filters, and the like are binder-free (Mazow et al. 2014). Additionally, the specialist should stress the importance of control samples; at the very least should ask potential research projects to budget for them.

When sampling, the most important part is making sure the procedures reduces the chances of contamination. The most basic methods involve generally limiting contact with samples: a) No food, aerosols near open pits (and pay attention to wind); b) Powder free gloves, pull back long hair; c) Clean sampling supplies between takes (Thoms 2014). Then they need to appropriately

contained: avoid plastics and sharpies; paper bags, foil, and pencil are best (Heron and Evershed 1993). These should bagged separately from other artifacts (Barton and Torrence 2015). They need to be moved to a cool, dry place as soon possible – if possible, even frozen. Samples should not be washed in the field. Not all contamination can avoided, but it can be controlled through sampling. Non-cultural controls can provide information on 'background noise' or 'environmental influences', for stone samples, this would be a non-cultural rock, from ceramics, the external side of the vessel may be a good option. Potential contamination sources can also be sampled – in archaeological sites, this can be from the soil; from curated samples this can include the dust that collects on the shelving. If performing archaeological excavation, this may also be a good opportunity to take samples for the reference library.

Best practices in the lab also entail controlling for contamination. There are a number of steps in addition to basic good lab practices, such as cleaning and sterilizing equipment (i.e. heating to over 500°C), using powder free gloves, and using a clean bench (Crowther et al. 2014; Hart 2008; Kwak and Marwick 2015; Mayyas and Douglas 2015). This includes using lab blanks to test for contamination during the processing, as well having signatures for potential contaminants, such as solvents, in the lab. Test the equipment to make sure it's non-reactive; this includes stoppers, plungers, and other pieces that may but do not necessarily come into contact with solvents (Mazow et al. 2014).

Best practices do not end in the lab, they extend into analysis and interpretation. The reference library should be relevant to the area, and include non-cultural sources (i.e. possible environmental contaminants) as well. Experimental work relevant to the cooking processes being studied should be done, if possible. If software and literature libraries are being used, they need

to be evaluated to ensure that the methods of data collection are consistent with those used in the study. Potential contamination and degradation should be noted and reported. To maintain confidence in the results, everything should be reported, included negative results and possible sources of error.

Future work

The literature points to several directions for future research. First, experimental studies can elucidate many important aspects of residue analysis including contamination, degradation through cooking and aging, how residues transfer to their matrix. In particular, experimental studies of residues on stone artifacts need to be expanded, including establishing environmental signatures and whether there are residues presents on stones before they absorb cultural residues. Second, reference studies need to be expanded and criteria for identification refined. The variation between and within material classes is not fully understood. Making reference libraries readily available to other researchers may not reduce the amount of reference building any particular researcher needs to do, but gives the opportunity to study this variation. Further, including less economically important ad non-food resources in reference libraries more fully accounts for what materials an artifact may have come in contact with. For example, most residue studies focus on large mammals, but small rodents may have been important to incipient have been important to incipient horticultural societies (c.f. Malainey et al. 1999). Third, with improving instrument sensitivity, it may be worthwhile to generally increase the focus of these studies to included non-lipid based food stuffs. While they are less likely to be preserved, the literature supports that some non-lipids are surviving. This could add several additional steps to lipid-based studies; however, given that it is established that multiple lines of evidence are best,

it is a worthwhile effort. Biochemical residue analysis of archaeological food residues is a growing subfield with many opportunities for future research.

CHAPTER III

FACILE RESIDUE ANALYSIS OF RECENT AND PREHISTORIC COOK-

STONES USING HANDHELD RAMAN SPECTROMETRY[1]

INTRODUCTION

The first analysis of archaeological food residue occurred in the 1930s, when Johannes Grüss

used basic chemical tests to identify black residue on a ceramic vessel as overcooked milk (Craig

2002). Since then, residue analysis has been conducted on a wide variety of substances

including perfumes, cosmetics, beeswax, resins, tar, pitches, proteins and lipids in soils,

pigments, ink, and paint (Evershed 2008b; Ciliberto and Spoto 2000; Edwards and Chalmers

2005; Glascock et al. 2007). Food residue studies generally analyze lipids, proteins, DNA, and

other characteristic compounds of residues absorbed by pottery. A wide range of techniques are

used including chromatography, gas spectrometry, elemental analysis, optical and resonance

spectroscopy, stable isotope analysis, X-ray diffraction and immunological techniques (Malainey

2011b).

Studies of food residue have been most successful with pottery, likely because the porous nature

of the pottery enables substances to become easily absorbed and trapped. There also have been

successful protein and lipid analyses of residues on the surface of grinding implements and

flaked tools (Malainey 2011b). In both cases, blind tests using modern laboratory-created

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[1] Reprinted with permission from: "Facile residue analysis of recent and prehistoric cook-stones using handheld Raman spectrometry" by Laura Short, Alston V. Thoms, Bin Cao, Alexander M Sinyukov, Amitabh Joshi, Virgil Sanders, and Dmitri V Voronine, 2014. *Journal of Raman Spectroscopy,* 1–17, Copyright 2014 John Wiley & Sons, Ltd.

artifacts have shown that these methods are in need of further development and utilization of multiple lines of evidence (Barnard et al. 2007; Colombini et al. 2011b; Leach et al. 1998).

Raman spectroscopy for archaeological analysis has focused on paints and pigments, resins and pitch, and plaster-like materials (Edwards and Chalmers 2005; Malainey 2011b).   It can be used to identify both organic and inorganic substances and has gained popularity due to its non-destructive nature.  However, fluorescence background may limit the sensitivity and archaeological materials may undergo taphonomic processes that make matches to modern reference samples difficult(Smith and Clark 2004). Additionally, until recently, Raman analysis has been laboratory oriented.

Various types of Raman instruments have been developed and optimized for different purposes. A class of miniaturized portable Raman spectrometers is now available for rapid *in situ* experiments such as airport screening, forensics, art authenticity verification, etc. Handheld Raman spectrometers can be used by a single operator in diverse challenging environments and may be particularly useful in archaeology, especially in situations when artifacts cannot be easily moved to the laboratory or when objects are too large for a microscope. Several applications of portable spectrometers to examine the composition of compounds in art such as canvas and rock paintings have been recently reported (Vandenabeele, Castro, et al. 2007; Olivares et al. 2013; Maguregui et al. 2012).

In this paper, we use a handheld Raman spectrometry to perform trace analysis of food residue from limestone rocks (i.e., cook-stones) used experimentally as heating elements in

actualistically constructed and used earth ovens. We also analyzed cook-stones recovered from prehistoric earth ovens at archaeological sites in Fort Hood, TX.

BACKGROUND: HOT-ROCK COOKING TECHNIQUES

Cook-stone technology, the use of heated rocks for cooking, is roughly 30,000 years old, and has occurred worldwide.  Techniques include using heated stones as griddles in open hearths, as heating elements in closed earth ovens and steaming pits, and as the heating element for boiling. Its appearance in the archaeological record has been related to population packing that required people to put more effort into procuring more food from the same area of land. This technology requires more energy input than hot-coal cooking, because stones and green-plant packing material have to be collected in addition to the firewood; however, it is more fuel efficient because the stones retain heat long after the coals cool (Thoms 2008b, 2009).

In their most essential form, earth ovens consist of a pit in which heated stones are used to cook food.  Generally speaking, food may or may not be wrapped into packages, but is always insulated from the stones with green plant material.  Earth ovens are ideal for cooking foods that require a long cooking time.  Ethnographic evidence shows that many groups around the world cooked meat, fish and shellfish in earth ovens. Most archaeological evidence indicates that pre-Columbian (i.e. prehistoric) North Americans living in temperate environs most commonly cooked plants in earth ovens. In the eastern half of Texas, wild root foods, especially bulbs of eastern camas (*Camassia scilloides*), wild onion (*Allium* spp.), and false garlic (*Nothoscordum bivalve*) were baked in earth ovens as early as 8-9,000 years ago. In the western half of Texas desert succulents were commonly baked in ovens, including lechuguilla (*Agave lechuguilla*), sotol (*Dasylirion* spp.), and prickly pear (*Opuntia* spp.) (Thoms 2008b, 2009). For the most part,

knowledge about what was baked in earth ovens comes from ethnographic evidence and, less commonly, carbonized plant remains from archaeological remains of earth ovens.

Cook-stones were also used to boil water, in a process known as stone boiling (Thoms 2008b, 2009). In this case, stones heated in an open fire to about 500 ℃ were removed using tongs, quickly rinsed in water, and dropped into a vessel containing liquid and food. As the stones cooled, they were removed and hot ones were added until the food was adequately boiled. This method boils liquids in bark, wooden, or hide containers more quickly than direct heating methods, and it does not require heat-resistant materials (e.g., ceramic and metal) as do direct heating methods. Stone boiling was used for a wide variety of cooking applications, creating soups, stews, porridge, and rendering fat. Many foods were cooked by stone boiling - nuts and seeds, geophytes, meat, and fish. Nuts and animal parts were both used to render fat. Since stone boiling does not usually result in charred materials, at this point most knowledge of what was cooked by this method is based on ethnographic evidence (Thoms 2009).

Starch granule and other residue analyses are now being used to identify plant-food microfossils in cooking stones, albeit with mixed results(Laurence et al. 2011). Raman spectroscopy also provides the potential to identify what was in direct contact with the cook-stones used in boiling as well as minute food remains adhering to rocks used as heating elements in earth ovens. Handheld Raman methodology could be used at archeological sites to provide additional information about cook stone methods. In principle it could be used to distinguish between the earth-oven heating stones and the boiling stones by analyzing the amount of charred residues. Raman spectra could be used to distinguish between lipid residues and carbohydrates and between plant and animal residues. It is also sensitive to the nature of carbohydrates and could

distinguish between starch, cellulose and inulin and provide specific information on the morphology and chemical composition of the cooked foods.

BACKGROUND: PLANT CARBOHYDRATES

Plant carbohydrates include simple sugars and alcohols, storage polysaccharides and structural polysaccharides. Simple sugars such as glucose and fructose make up the sweetness we taste in fresh fruits and vegetables. Storage polysaccharides such as starch and fructans are used to store energy. Structural polysaccharides such as cellulose and pectin are the components of cell walls known as dietary fiber (Wandsnider 1997).

A specific storage carbohydrate, inulin, is associated with earth-oven baking (Thoms 2009). Inulin is concentrated in the edible underground storage organs (bulbs, tubers, etc.) of some geophytes including many plants in the lily family, such as onion and garlic (*Allium* spp.) and camas (*Camassia* spp.), and many plants in the aster family, including chicory (*Cichorium intybus*), jerusalem artichoke (*Helianthus tuberosus*), and dandelion (*Taraxacum* spp.), as well in the pulpy central stems (i.e. hearts) of succulents such as sotol (*Dasylirion* spp.) and agave (*Agave* spp.). The simpler the carbohydrate, the easier it is for humans to digest and utilize the sugar – complex carbohydrates such as starch and inulin must undergo hydrolysis to be readily digestible. Raw inulin provides energy via digestion by gut flora (which is why it is known as a prebiotic), but inulin breaks down into simpler sugars fructose and glucose when cooked over a long period of time. Earth ovens, which are capable of generating and maintaining sufficient heat for 72 hours, are ideal for the kind of extended cooking required to break down inulin and thereby render it more readily digestible (Wandsnider 1997).
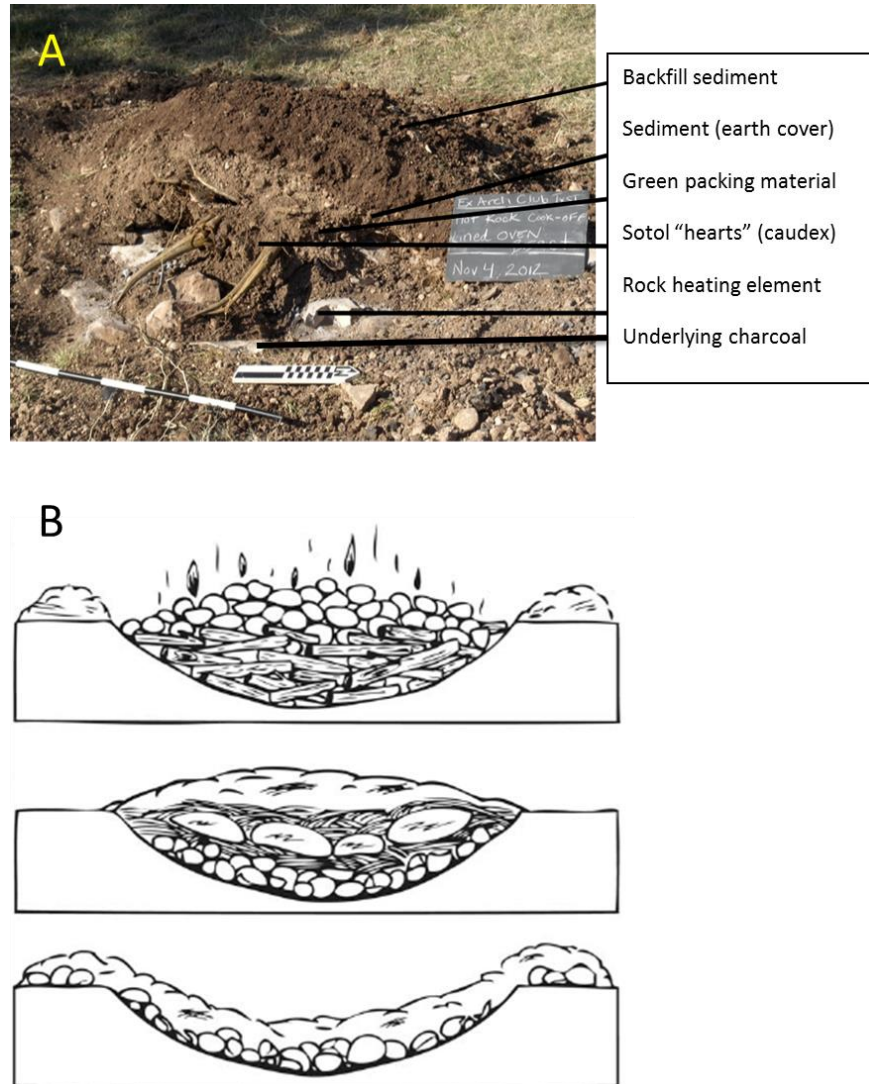
**Figure 14 (A) Photograph of a partially uncovered 48-hour earth oven from the HRCO field experiments in San Marcos, TX that was used to bake sotol. (B) Schematic illustration of construction and use of a typical earth oven (adapted from Thoms, A. V. J. Anthropol. Archaeology 27, 443 (2008)): (B, top) fire is built in a pit overlain by a layer of rocks; (B, middle) when the fire burns completely, red-hot rocks are covered with green packing material, food packs, more packing material, and covered with earth; and (B, bottom) remains of the oven after the food is removed and the oven is abandoned.**

The Hot-Rock Cook Off (HRCO) is an actualistic experimental archaeological cooking event, where cooking methods utilizing cook-stone (the "hot rock") are recreated based on archaeological and ethnographic data. Earth-oven cooking is the focus of the event, though stone boiling and grilling are included. Predominantly an academic venture by anthropology students at Texas A&M and Texas State Universities, it is open to the public and includes other educational activities and information. Each year representatives of Native American groups from the region attend and participate in the event. These experiments are an attempt to replicate archaeological signatures of earth ovens found throughout Texas and elsewhere around the world. Figure 14 depicts earth ovens used during the HRCO event in San Marcos, TX in November 2012. To replicate prehistoric cooking techniques, sotol was baked for approximately 48 hours using heated limestone rocks (Figure 15A and B). We measured the Raman spectra using the 'First Guard' handheld Raman spectrometer from the Rigaku Corporation, which has a 1064 nm laser, a spectral resolution of ~20 cm$^{-1}$, and a detection range from 200 to 2000 cm$^{-1}$. The focal spot size was ~ 1 mm. The 1064 nm wavelength provides advantages of *in situ* investigation and a significant suppression of fluorescence background. This push-button device is most convenient for field experiments that do not require sample preparation. It is therefore especially suitable for non-destructive efficient exploration of prehistoric archaeological sites.
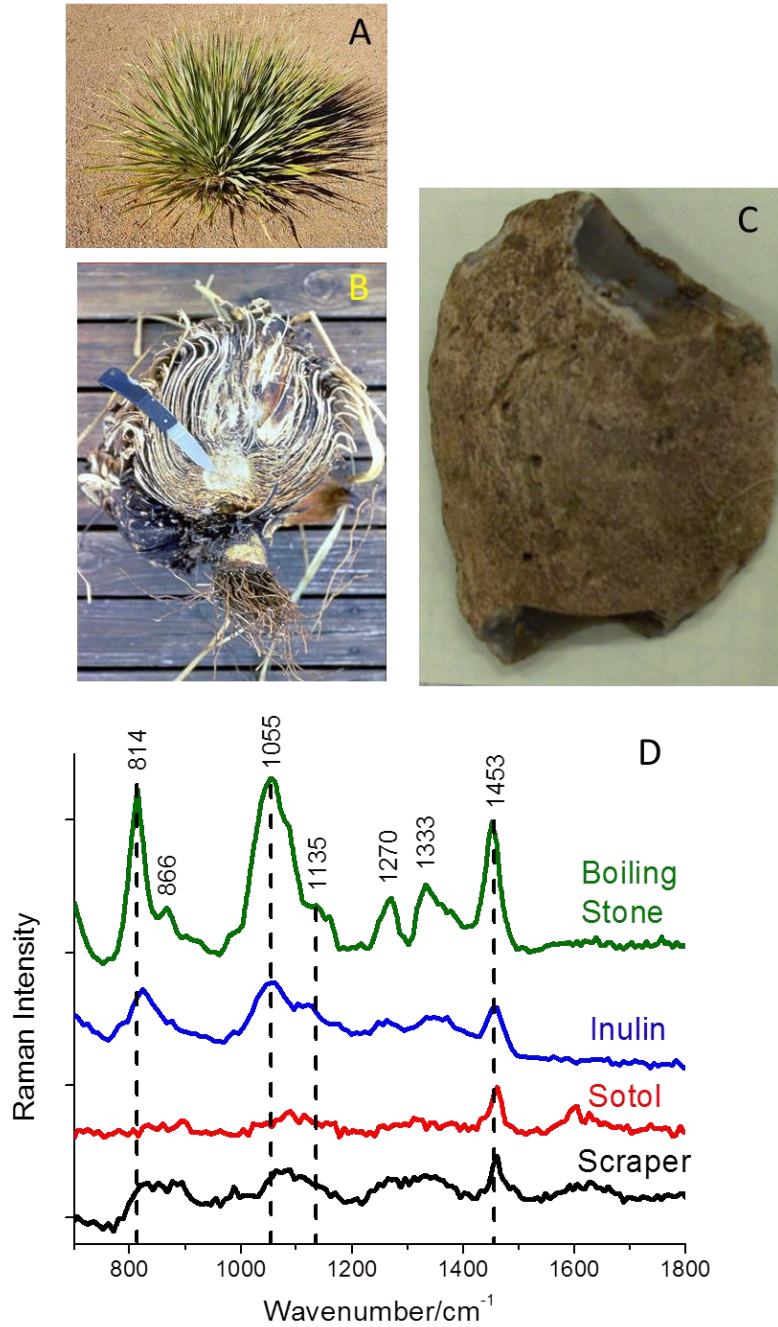
**Figure 15 (A) Sotol. (B) A desiccated sotol "heart" sliced with a saw to show the internal structure of the plant. The knife points to the edible central stem from which the leaves grow, something like an artichoke. (Courtesy of Phil Dering) (C) Stone tool used to scrape cooked sotol at the HRCO site. (D) Raman spectra of cooked sotol (red) and sotol residue on the scraper (black), compared with uncooked inulin (blue) and cooked inulin on boiling stones (green). Similar spectral signatures were found in all samples.**

At the HRCO, a stone tool was used to scrape the baked sotol and make it into cakes more suitable for eating, as is documented ethnographically and likely occurred in the distant past as well (Figure 15C) (Sobolik 1996). The handheld Raman spectrometer was used to examine the visible residue that remained on the scraper. The laser beam was focused on the stone surface at 400 mW laser power with 3 second exposure time and an average of three shots. These conditions were optimized to obtain the maximum S/N. The results are shown in Figure III.2D. Both the surface of the scraper (black) and the spectra from fresh sotol (red) have a well-resolved peak at 1453 cm$^{-1}$ which is absent in a clean stone washed with tap water. There are also other peaks around 800 and 1100 cm$^{-1}$ which are weak. These peaks confirm the presence of sotol on the surface of the scraper. The signal intensity varied depending on the position on the scraper. The results imply that the key issue in detecting residues on artifacts is to find a hotspot where some residue adheres to the surface or in cracks and crevices. That several places on a given artifact can be sampled in a short timeframe indicates the practicality of handheld Raman spectrometry in field and laboratory archaeology.

We compared the spectra of the raw sotol and baked sotol on the surface of the scraper to the spectra of inulin. We also used handheld Raman spectrometry for residue analysis of limestone fragments used to boil chicory root inulin powder purchased from a local grocery store. The limestone was purchased from a local garden center. About 5 grams of inulin were boiled with several stones for an hour.

Figure 15D shows a comparison of Raman spectra of raw inulin (blue) with cooked inulin on the surface of boiling stones (green), and with raw sotol (red) and baked sotol on the surface of the scraper (black). The obtained spectra of inulin are in agreement with previous reports (Manno et

al. 2009; Sigma-Aldrich 2018). Spectra of cooked inulin on boiling stones reveal clear signatures of inulin. Sotol and inulin have similar spectra. Therefore inulin is a major component in Raman spectra of sotol. This confirms the potential of handheld Raman spectrometry for archaeological food residue analysis on boiling stones.

PREHISTORIC COOK-STONES:  METHODS AND ANALYSIS

We examined two cook-stones, commonly known as fire-cracked rocks (FCR) from two ancient earth ovens.  These FCR were among many such cook-stones constituting the heating element of earth ovens excavated at Ft. Hood, TX. Figure 16 B-D and F-H show photographs of different sides of *stones 1* and *2*, respectively. *Stone 1,* from site 41CV1553, dates to approximately 350-650 AD. *Stone 2,* from the site 41CV594, dates to approximately 2,500-500 BC. Raman spectra from the surface of *stones 1* and *2* are shown in Figure 16  A and E, respectively. As described above, the spectrometer was put against the surface of the cook-stones to obtain the spectra, and different spots were selected. A small piece cut from *stone 1* was thoroughly cleaned for comparison (Figure 16 J). The corresponding Raman spectrum is shown in Figure 16 I. The Raman spectra in Figure 16  A, E and I show similar patterns. Both stones showed Raman peaks around 988, 1085, and 1170 cm$^{-1}$. The same peaks were also found on the piece of *stone 1* that was rinsed with tap water (Figure 16 I). Therefore, they were assigned to the stone itself. The strongest Raman peak of calcite at 1087 cm$^{-1}$ matches well with the observed strongest peak at 1085 cm$^{-1}$ (Burgio and Clark 2001). However, the spectra of several spots on the uncleaned cook-stones showed broadening of the 1085 cm$^{-1}$ peak. This broadening was not observed on the cleaned cook stone and is attributed to the presence of residues.
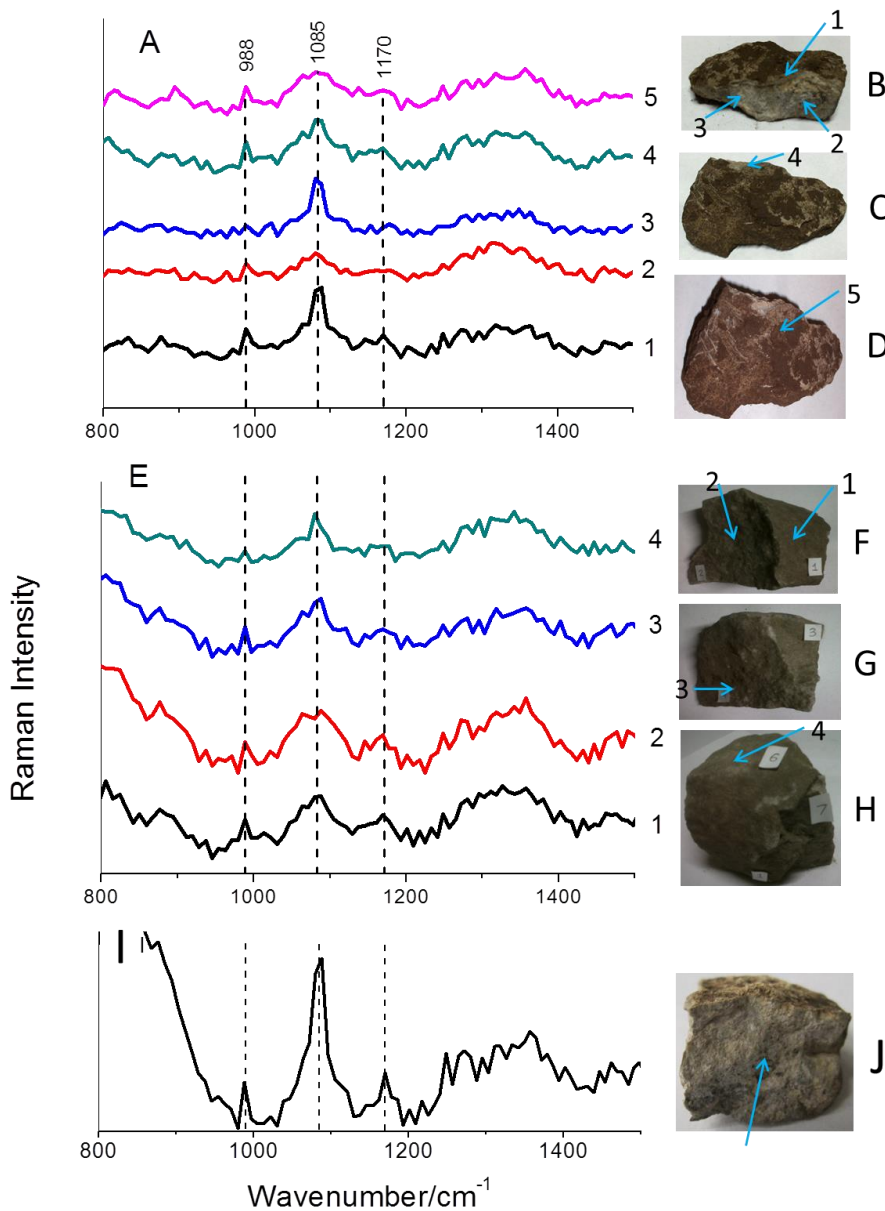
**Figure 16 (A)** and **(E)** are Raman spectra of two different stones from the prehistoric archaeological sites in Ft. Hood, labeled stone 1 and stone 2, respectively. **(B)** - **(D)** and **(F)** - **(H)** are photographs of different sides of stones 1 and 2, respectively. **(B)** - **(D)** and **(F)** - **(H)** are photographs of different sides of *stones 1* and *2,* respectively. **(B)** is a split cross-section of *stone 1* with the corresponding spectra *1 - 3* in **(A)**. **(I)** Raman spectrum of a cracked piece of *stone 1 (J)* after rinsing with tap water. Arrows indicate spatial positions on the cook-stones that correspond to the spectra. The cook-stone sizes vary in the range 3 – 15 cm.

Figure 16 shows that the spectra 1 and 3 in (A) and the spectrum (I) of the section of the cook-stone cleaned by tap water have a narrower width at 1085 cm$^{-1}$ compared to the spectra from the surface of the stones. The broadening is shown more clearly in normalized Raman spectra in Figure 17. It is possible that the observed broadening of the peak at 1085 cm$^{-1}$ is due to organic food residues such as carbohydrates (inulin, cellulose or others). Inulin is present in many wild plants found in the vicinity of the sites, especially onion and camas, both of which have been recovered as charred macrobotanical fragments from remains of ancient oven at Fort Hood (Mehalchick et al. 2004). However, other inulin spectral peaks such as the 1453 cm$^{-1}$ peak were not resolved due to low signal-to-noise ratio. This finding suggests the possibility of identifying organics, including residue of food eaten a thousand or more years ago, using handheld Raman spectrometry. Assessment of this working hypothesis—broadening of the peak at 1085 cm$^{-1}$ is due to organic food residues — requires improvement of the signal-to-noise, spectral resolution and extension of the detection spectral range.
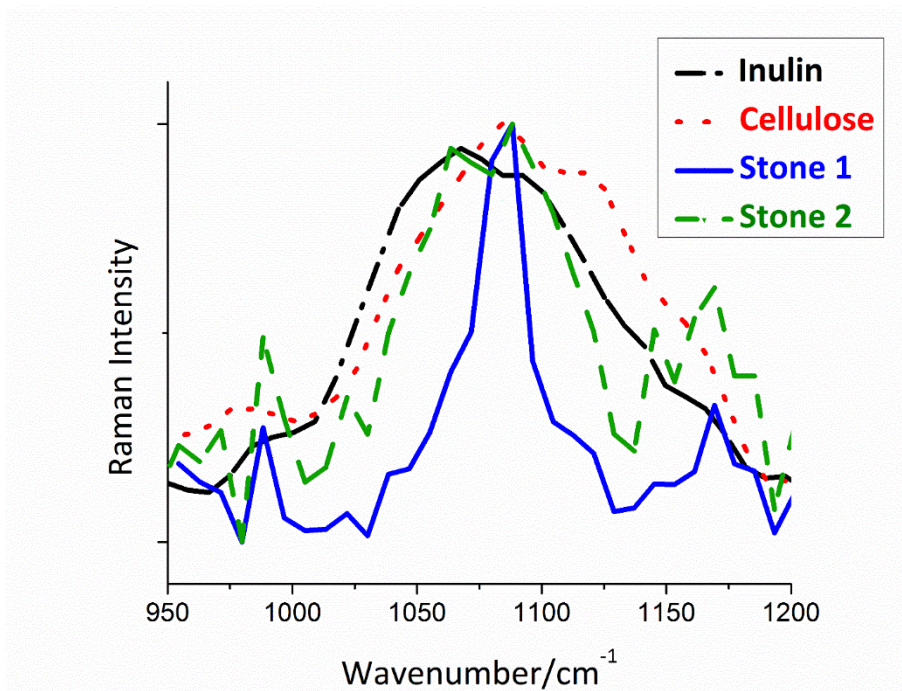
**Figure 17 Normalized Raman spectra of inulin (dash-dotted black), cellulose (dotted red), and two different stones from the prehistoric archaeological sites in Ft. Hood, labeled stone 1 (solid blue) and stone 2 (dash-dotted green). The two stones correspond to Figure III.3E (spot2) and I, respectively. The cracked piece of *stone 1* was cleaned with tap water. The spectra of carbohydrates and uncleaned *stone 2* show a significant broadening of the 1085 cm$^{-1}$ peak.**

COMPARISON OF THE PORTABLE AND LAB-BASED RAMAN INSTRUMENTS

We compared the performance of the portable handheld Raman spectrometer with the state-of-the-art lab-based Raman microscope. The latter was a confocal Raman microscope (Nanonics Imaging, Ltd) with an electric-cooled CCD detector (-70 °C) and iHR550 spectrometer (Horiba), and 180° backscattering detection. The excitation source was a 785 nm CW laser with up to 30 mW power at the sample with a 10x objective. The typical spectral resolution was better than 0.7 cm$^{-1}$. To perform the comparison of the two instruments we purchased two reference materials,

inulin from chicory root and cellulose acetate, from Sigma-Aldrich, Inc. Both of these materials may be present as food residues at archeological sites. Cellulose is the most abundant natural organic polymer on Earth. The ability to distinguish inulin from cellulose using portable Raman spectroscopy will be useful in archeology.

The Raman spectra of inulin and cellulose are shown in Figure 18A and B, respectively. The comparison of the spectra measured using the portable (red) and lab-based (blue) instruments shows that both instruments provide essentially the same information. The lab-based instrument shows an additional feature in the region of $1600 - 1700$ cm$^{-1}$ which is most probably an artifact of fluorescent background subtraction. The 785 nm wavelength of the lab-based instrument can lead to a larger amount of fluorescence than the 1064 nm wavelength of the portable instrument. The portable instrument has lower spectral resolution but is still able to detect most of the spectral lines. For example, both the portable and the handheld instruments measure similar line shapes of the 1270, 1333 and 1453 cm$^{-1}$ transitions in Figure 18A. These transitions have similar line widths and are less congested. However, the portable instrument cannot resolve the transitions in the more congested region around 1059 cm$^{-1}$. It does not affect the detection of inulin and cellulose but can be important in other cases. Then the sample can be analyzed using the lab-based instrument and the portable Raman spectrometer can be used to obtain the preliminary information. This demonstrates that the portable Raman instrument may be used for residue analysis in field experiments.
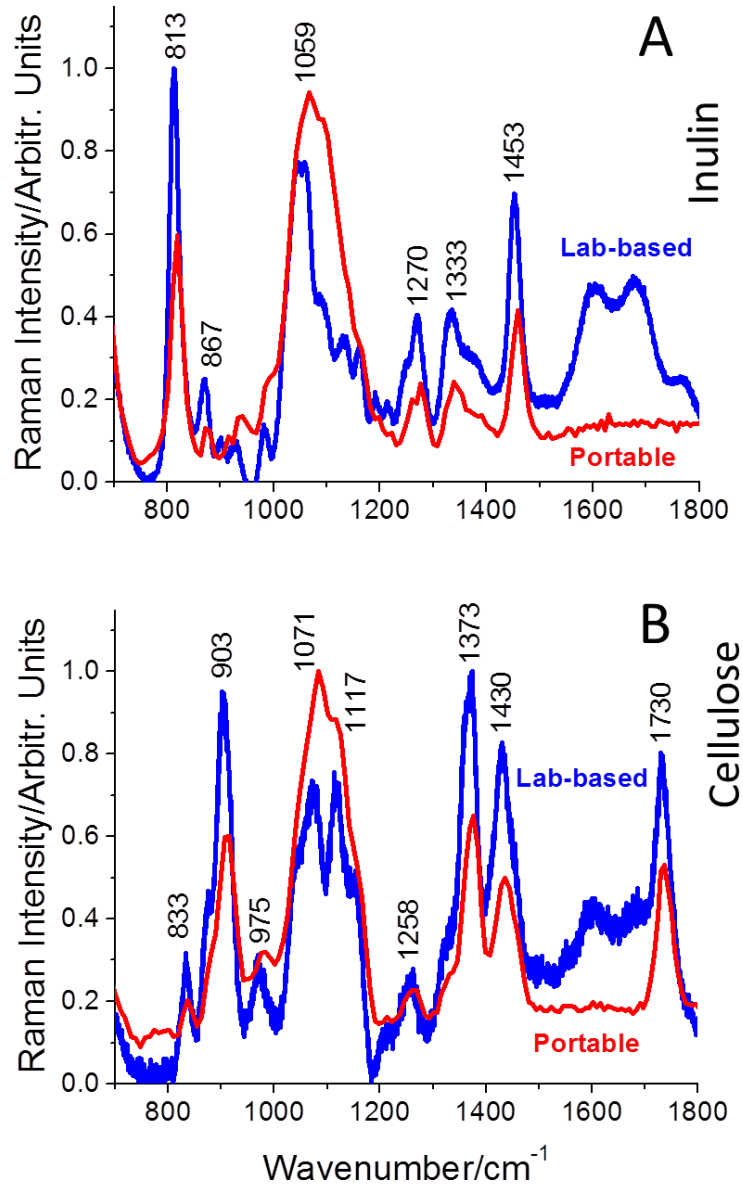
**Figure 18 Raman spectra of inulin (A) and cellulose (B) purchased from Sigma-Aldrich, Inc measured with a lab-based (blue) and portable (red) instruments. Similar spectral signatures obtained with both devices demonstrate that a portable instrument can be used in archeological field experiments.**

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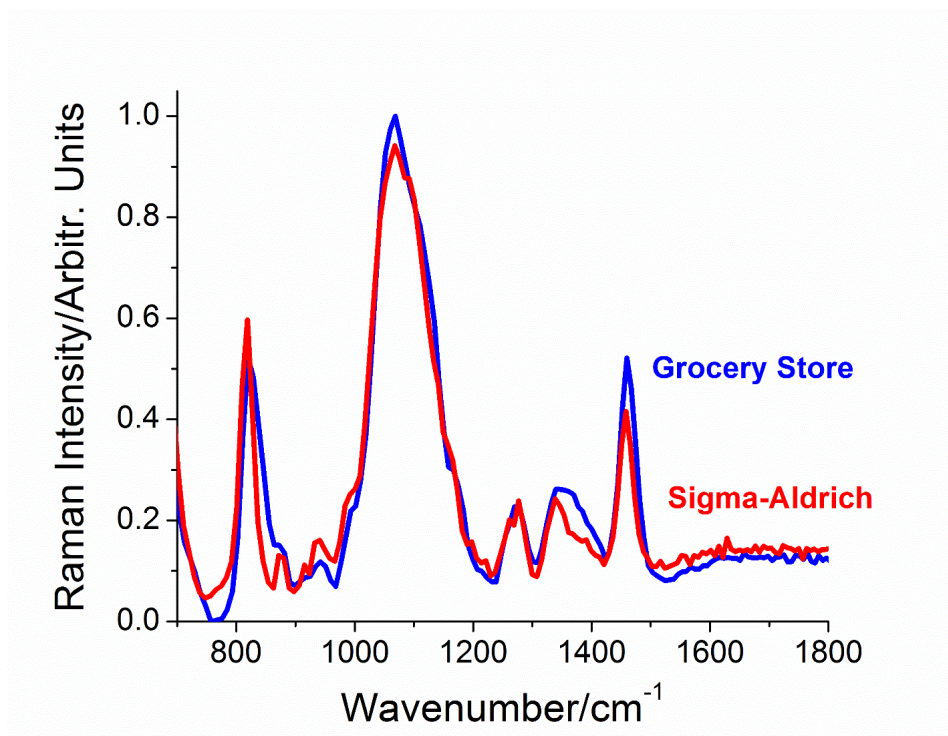
**Figure 19 Comparison of the Raman spectra of inulin from Sigma-Aldrich, Inc (red) and from a grocery store (blue) obtained using a handheld spectrometer. Similar spectral signatures in both cases are observed.**

Figure 19 shows a comparison of the Raman spectra of inulin from a grocery store (blue) to the chemical grade inulin from Sigma-Aldrich (red) measured using the handheld Raman spectrometer. Similar results are obtained. This shows that the portable Raman spectrometer can detect inulin from various sources.

Band assignment was performed based on previous Raman studies of inulin (Manno et al. 2009; Beirão-da-Costa et al. 2013) and cellulose (Barrett 1981; Szymańska-Chargot et al. 2011). These two chemicals are both naturally occurring carbohydrate polymers. Inulin is a fructan mostly

made of fructose units, whereas cellulose consists of a chain of glucose units. Comparison of the bands of inulin and cellulose in Table XVI (see Appendix A) shows that these two different carbohydrates can be distinguished using portable Raman spectroscopy.

Previous literature reports on carbohydrates confirm the ability of Raman spectroscopy to distinguish different chemicals. Raman spectroscopy was shown to be a valuable tool for the studies of carbohydrates (Goral 1992; Brandenburg and Seydel 2002; Vasko et al. 1971; Choi et al. 2010; Mathlouthi and Koenig 1987; Parker 1983). For example, distinct Raman spectra were measured for thirteen different sugars including glucose, fructose, starch and cellulose (Barrett 1981). Pectin and starch were distinguished *in situ* in living potato cells and in carrot roots (Thygesen et al. 2003; Baranski et al. 2005). Raman spectroscopy was used to distinguish starches from potato and maize due to their different structural properties (Bulkin et al. 1987). Our results and previous literature suggest that Raman spectroscopy is able to provide chemically specific signatures of carbohydrates, including inulin.

The ability of Raman spectroscopy to distinguish various carbohydrates is based on the sensitivity of vibrational signatures on molecular structure and conformation. Branched vs linear structures, crystalline vs amorphous, various degrees of hydrogen bonding, and spatial arrangement of substituents relative to the backbone lead to distinguishable Raman shifts. For example, the $CH_2$-OH bending and deformation bands at 1333 and 1453 $cm^{-1}$ in inulin are suppressed and shifted in cellulose. The COC stretching modes in these chemicals are also different due to the different structure of unit cells. These peaks provide unique spectral signatures of inulin as an organic residue on archeological samples. For example, the 1453 $cm^{-1}$ peak is clearly resolved on a scraper stone in Figure 15D and the 1333 band appears as a weak

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shoulder. The handheld Raman instrument may be used as a fast tool to detect the organic residue which can be later more carefully analyzed using other lab-based techniques.

Carbohydrates also have a broad band around ~2900 cm$^{-1}$ (not shown), which lies outside of the available range of the handheld Raman spectrometer (from 200 to 2000 cm$^{-1}$). This band, however, cannot be used for inulin identification because it is present in all carbohydrates. The available spectral range is sufficient to identify inulin at archeological sites using portable measurements (Figure 18). Further analysis in a broader range can be later performed using laboratory-based instruments.

CONCLUSIONS

We demonstrated the use of handheld Raman spectrometry for facile trace analysis of inulin in actualistic experiments and its potential application at prehistoric archaeological sites. We detected spectroscopic features of inulin in the Raman spectra of sotol, which is a potential residue source in prehistoric earth ovens. Future exploration of archaeological samples using handheld Raman spectrometers is anticipated. Given that food residue is most likely to be preserved in the cracks and crevices of ancient, well weathered cook-stones and tools (Laurence et al. 2011; Namdar et al. 2009), we conclude that portable handheld Raman microscopy should focus on these places on a given stone(Smith and Clark 2004).

Coherent anti-Stokes Raman scattering (CARS) spectroscopy has been recently used for the investigation of the molecular composition of gas residues in cracks of translucent materials (Smart 2012; Burruss et al. 2012). CARS can be also used for the archaeological food residue analysis. The CARS signal is (N-1) times stronger than the spontaneous Raman signal used in

this work, where N is the number of molecules. Therefore, CARS can enhance the signal from traces of organic residues which have microscopic amounts of material. Another possible future direction of improving handheld Raman spectrometry is by increasing the sensitivity via surface enhancement (Kneipp et al. 2006; Le Ru and Etchegoin 2009). Surface-enhanced Raman scattering (SERS) micro-spectroscopy has been used for the detection of nucleotide traces in pyroxene rocks as imitation of *in situ* search for life traces on Mars (Muniz-Miranda et al. 2010). SERS will require a special sample preparation to provide a contact between the residue and the enhancing surface. It may also be possible to adapt combinations of these techniques to the *in situ* food residue analysis and to develop portable surface-enhanced CARS (SECARS) and FAST-CARS spectrometers (Liang et al. 1994; Voronine et al. 2012; Scully et al. 2002; Pestov et al. 2007). Sample enrichment procedures could also be used to enhance weak signals. Tighter focusing and higher laser power may burn the sample. Developing portable handheld CARS, SERS and SECARS spectrometers may decrease the signal collection time and will bring many future advantages in the field.

CHAPTER IV

RAMAN SPECTROSCOPY OF BIOCHEMICAL RESIDUES FROM EARTH

OVENS IN SOUTH-CENTRAL NORTH AMERICA

INTRODUCTION

Cooking is one of the most important human activities. Even with abundant evidence of cooking in the archaeological record, it is rarely clear what particular food(s) people were preparing in a given feature. Earth oven baking is a prime example – while ethnographic information provides a general sense of what one might expect, without the preservation of botanical or faunal remains, archaeologists cannot be sure what was processed in any particular oven. Presented here is a proof of concept study developing a method to characterize biochemical residues, aimed at identifying what plant foods were baked in given earth oven or other cooking feature.

Earth ovens first appear in the archaeological record approximately 32,000 years ago, and show up in the Americas by 10,500 years ago (Thoms 2009; Movius 1966; Pearson 1999). In Texas, the location of this study, earth ovens were prevalent starting in the Early Archaic (8800 BP) where ethnographic and macrobotanical evidence indicates they were used primarily to process plant foods (Thoms 2008a).  Earth oven cooking is part of the intensification process. By increasing digestibility through increased input of time and labor, people attempt to maintain an increasingly dense population. This increased resource (including time, labor, technology) exploitation extracts more food energy per unit area of land  (Thoms 2009; Johnson and Hard 2008; Thoms 1989).  It has been suggested that earth ovens, rather than horticulture, were a major form of intensification in Texas, and was part of the reason that horticulture never gained significance in central Texas' pre-Columbian history (Johnson and Hard 2008).

Earth oven cooking usually uses heated stones in below-ground pits to bake or steam food. This serves a number of purposes: to break down complex carbohydrates, proteins and lipids; for preservation; and to destroy toxins (Wandsnider 1997). This makes them well suited to cook tough cuts of meat and plants rich in complex carbohydrates. Inulin is one such complex carbohydrate. Foods rich in it, including onion, camas, sotol, and agave, are frequently associated with earth oven cooking (Thoms 2009; Black and Thoms 2014). Generally speaking, inulin has been shown to be an important resource world-wide, spanning, potentially, back to the dawning of modern humans (Leach 2008). It is a prebiotic, in that in its raw form, it does not directly provide nutrients for humans, but it is fuel for bacteria in humans' lower intestinal tract (Leach 2008). However, when exposed to water and heat, the complex carbohydrate breaks down into easily digestible sugars (Wandsnider 1997). Caramelizing onions is a good example of the process that may be familiar to many people (Leach 2009).

Evidence for the association of earth ovens and inulin rich plant foods comes from historic records, ethnographic reports, and charred plants found in archaeological earth ovens (Thoms 2008b). While charred plant foods provide good evidence of what foods were processed, they are relatively rare in the archaeological record. Other sources of direct evidence include microbotanicals, such starch, raphides, and phytoliths (Thoms, Laurence, et al. 2014a). They are excellent at identifying the processing of starchy foods, for example, since starch grains act as direct evidence of their presence. Other diagnostic microfossils include raphides for cacti, or phytoliths for maize. To date, there have been no correlations made between diagnostic microfossils and inulin-rich foods, however.

Rocks heated in excess of 500 °C by burning wood or other fuel function as heating elements in the closed oven and they cool slowly as they cook the food. This produces a number of changes in the rocks – for example they crack, change color, and some minerals break down, and they are known as fire cracked rocks (FCR) (Pagoulatos 2005). The resulting microcracks help preserve food residues that would otherwise deteriorate (Shanks et al. 2001; Thoms, Boyd, et al. 2014; Thoms, Laurence, et al. 2014a). If those organic residues are preserved, their molecular structure may be identifiable using analytical chemistry.

A variety of techniques are used for biochemical or organic residue analysis, including those that combine separation and analysis such as gas chromatography–mass spectrometry (GCMS) and liquid chromatography–mass spectrometry (LCMS), and vibration spectroscopy such as Raman spectroscopy and Fourier Transform Infrared (FTIR) absorption spectroscopy(McGovern and Hall 2015; Ribechini et al. 2011). These molecular structures can indicate the potential source of the residues using the archaeological biomarker concept to link the structures found in the chemical fingerprint to substances known to have existed in organisms that humans exploited (Evershed 2008b). The biomarker is an identifier for that is unique to a particular organism or class of organisms, and it needs to survive in an identifiable way through the archaeological record. This study is looking for an inulin signal, to act as the biomarker for inulin rich foods.

For this study, Raman spectroscopy was chosen as the analytical method. Raman characterizes the molecular structure of substances by measuring chemical bonds' changes in energy level when hit with light, which are represented in a spectra that analysists interpret (Malainey 2011a). Recent advances, especially the use of long-wavelength laser light source, have vastly improved its ability to characterize organic residues (Schrader et al. 1999; Edwards 2009). The technique

has been used to analyze a wide range of archaeological materials: dyes, pigments and binders; resins, pitches and adhesives; evidence of firing and burning; minerals and their provenance; food and non-food residues in pottery and ceramics; as well as other organic archaeological materials (Malainey 2011b). Aside from a prior pilot study identifying potential organic material on FCR, Raman spectroscopy has not been used to study food residues on archaeological FCR.

The present study is part of a larger project focusing on the use of earth ovens on Fort Hood in central Texas, in an ecotone between the Blackland Prairie and the Edwards Plateau. The modern climate is subtropical, characterized by hot, humid summers and relatively short, dry winters (Kibler 2004). There a number of localized environments niches in the study area, including the Paluxy sands, which are associated with earth ovens in part because they because they are well drained and easier to dig than other soils in the area (Boyd, Mehalchick, et al. 2004). They are pockets of loose sandy deposits eroded from outcrops of the Paluxy formation, a sandstone and shale bed that are the remains of an ancient shoreline (Abbott et al. 1995; Hayward et al. 1996). In order to compare earth ovens from open air sites to ovens from sheltered sites, the study was expanded to include sites from the Lower Pecos. The Lower Pecos is located in west Texas, along the southwest border of the Edwards Plateau. The modern climate is semiarid, with hot summers and dry winters (Koenig 2012). The soils in this area are very thin, predominantly gravelly and silty loams (Golden et al. 1982).

METHODS:

Earth oven cooking features are complicated, and in turn create complicated biomolecular residue signatures. The sources of biochemical food residues start as complex mixtures, which then undergo cooking and taphonomic processes (Oudemans 2007). Earth ovens add further

complexity due to the green vegetation acting as packing material and wood fuel in proximity to the FCR. This makes the residues difficult to interpret. Ideally, reference collections should control for location, the effects of cooking and diagenesis, as well as taking in account possible contaminant and environmental factors. Thus multiple type of samples were analyzed: modern botanicals, archaeological botanicals, FCR from modern cooking experiments, and FCR from archaeological earth ovens. Within this section the samples are described first, followed by sample preparation, and then the details of the Raman spectrometer.

Samples

In order to use inulin as the biomarker for inulin rich plants, the spectra of inulin needs to be first identified. Thus four carbohydrates that are commonly found in plants were analyzed: inulin, starch, cellulose, and pectin. Chemical grade inulin and cellulose were purchased from Sigma-Aldrich, while corn starch and pectin purchased from a local grocery store were used. Previous work as shown that grocery store inulin was comparable to inulin purchased from Sigma-Aldrich, and spectra of the grocery store samples was comparable to what was found in other studies (Short et al. 2014; Kizil et al. 2002; Synytsya 2003). How the spectra is interpreted is detailed in the results section.

**Table XIV: Modern Botanical Samples Analyzed**

| Common Name | Scientific name | Part | Inulin rich | Starch present | Evidence of being cooked in ovens |
|---|---|---|---|---|---|
| Onion (domestic) | *Allium cepa* | bulb | Yes | No | For wild onion: 9, 12 |
| Sotol | *Dasylirion spp.* | blade | Yes | No | 1 |
| Agave | *Agave spp.* | blade/heart | Yes | No | 8, 10 |
| Jerusalem artichoke | *Helianthus tuberosus* | tuber | Yes | Yes | 7, 8, 9, 10, 11, 12, 13, 16 |
| Camas | *Camassia scilloides* | bulb | Yes | No | 1, 3 |
| Crow poison/False Garlic | *Nothoscordum bivalve* | bulb | Yes, Yes | | 17 |
| Copper Lily | *Habranthus tubispathus* | bulb | Yes, Yes | | For Liliaceae sp.: 1 |
| Rain Lily | *Cooperia drummondii* | bulb | Yes, Yes | | 17 |
| Gay feather | *Liatris spp.* | corm | Yes | Yes | 4 |
| Potato (domestic) | *Solanum tuberosum* | tuber | No | Yes | n/a |
| Asian water lily (domestic) | *Nelumbo nucifera* | rhizome | No | Yes | for American Lotus: 5, 6, 7, 10, 11, 12, 13 |
| Cattail | *Typha latifolia* | rhizome | Yes | Yes | 2, 7, 10, 13, 14, 15 |

1: Dering 1997, 2: Bailey 2001, 3: Bean and Saubel 1972, 4: Bolton 1914, 5: Bourke 1895, 6: Driver and Massey 1957, 7: Foster and McCollough 2001, 8: La Vere 2004, 9: McCormick 1973, 10: Newcomb 1961, 11: Opler 1983a, 12: Opler 1983b, 13: Opler 2001, 14: Prikrly 1990, p. 13, 15: Reid 1977, 16: Ricklis 1996, 17: Mehalchick 2004

Modern botanicals, both food resources common to the Texas area and some potential contaminants, were analyzed. See Table XIV for the list of modern botanical samples analyzed in this study. When they could not be found in the wild, they were purchased. They were analyzed in both raw and cooked states to determine the effects of cooking on the Raman spectra of the residue. Samples were cooked in an oven at approximately 180 °C for half an hour to an hour – until visible browning but not burning occurred. Meat samples (venison, turkey, and

bison) were also analyzed in order to compare to the plant foods. Additionally, to assess the

effects of charring, domestic onion was cooked at 180 °C in half hour intervals for four hours.

Archaeological botanicals were analyzed in order to determine the effects of diagenesis on the

samples. They were obtained from Hinds Cave (41VV456), a dry cave site in the Lower Pecos

region that had excellent botanical preservation (Dering 1979). See Table XV for the

archaeological botanical samples analyzed in this study. Identifiable uncharred archaeological

botanical samples were not available from central Texas. These samples were chosen because

they were food resources or found in conjunction with earth ovens (Dering 1999). Prickly pear

(*Opuntia sp.*) was likely used as a packing material, while little walnut (*Juglans microcarpa*) was

likely incorporated in the fuel source or packing material. These were analyzed by Raman both

directly (with no preparation) and by the extraction used for modern samples. When directly

analyzing the intact samples, some problems were encountered with burning. The laser light can

heat up the sample, especially if the sample is dry and dark colored, which interferes with getting

a good spectra. A limited number of samples were extracted because it is a somewhat

destructive. While crushing the sample is not required, some needed to be cut down to fit in the

beaker and some tended to disintegrate when remoistened.

**Table XV: Archaeological Botanical Samples Analyzed**

| Common Name | Scientific name | part | sample |
|---|---|---|---|
| Prickly Pear | *Opuntia sp.* | pad, seed | Extract, intact |
| Sotol | *Dasylirion spp.* | blade | Extract, intact |
| Wild Onion | *Allium sp.* | bulb | intact |
| Mesquite | *Prosopis sp.* | pod | intact |
| Little Walnut | *Juglans microcarpa* | nut | Extract, intact |

Residue samples were taken from FCR from actualistic cooking experiments intended to replicate ethnographic cooking conditions. The actualistic experiments included complicating factors such as additional components of the earth ovens as well as longer cooking times. A number of experimental ovens were built over the larger project period. Of these, samples from three ovens were analyzed. Two were from ovens built in 2010, one that cooked starch-rich domestic potatoes (*Solanum tuberosum)*, and one that cooked inulin-rich domestic onion (*Allium cepa*) and camas (*Camassia scilloides*). These were relatively large ovens that cooked for approximately 72 hours (Thoms, Laurence, et al. 2014b). In 2017 a meat based oven was done for comparison. This was a smaller oven that cooked for only 3-4 hours, done as part of an archaeology program for girl scouts. Cooked in this oven were domestic carrots (*Daucus carota*), potatoes, onions, and beef (*Bos Taurus*).

Archaeological FCR residue samples came from 9 sites at Fort Hood, and 7 sites in the Lower Pecos (Thoms, Boyd, et al. 2014; Basham 2015; Rodriguez 2015). As discussed previously, these were chosen to compare a variety of different site types and potential preservation. The dry, sheltered sites are likely to have been exposed to less weathering than the open air sites. From Fort Hood, samples were predominantly from sites that included macrobotanicals, as it indicate a likelihood of plant foods being cooked. A control sample off site was taken at Fort Hood, and sediment samples were also tested as a control measure.

**Table XVI: Sample descriptions. Sources** (Thoms, Boyd, et al. 2014; Rodriguez 2015; Basham 2015)

| Fort Hood Samples | Site | Feature Number | Notes | Matrix | Age | Associated Macrobotanicals |
|---|---|---|---|---|---|---|
| 1 | 41CV1553 | 6 | Flat Bottomed, Slab lined | gravely, fine sandy loam | 180-45 BC | *Carya illinoinesis, Camassia* sp. |
| 2 | 41CV1553 | 8E | Basin Shaped, Unlined | gravely, fine sandy loam | n/a | *Camassia* sp. |
| 3 & 4 | 41CV984 | 4 | Basin Shaped, Slab lined | sandy loam, increasingly clayey with depth | 760-400 BC and AD 10-210 | indeterminable tuber, *Allium* sp. *Camassia* sp. |
| 5 & 6 | 41CV947 | 5 | Basin Shaped, Slab lined | fine sandy loam | AD 780-1020 | Indeterminable tuber, *Carya illinoinensis* |
| 7 | 41CV594 | 2G | Basin Shaped, Unlined | fine sandy loam | n/a | Indeterminable tuber |
| 8 | 41CV594 | 2C | Disturbed, Large slab lining, basin shape with flat bottom | fine sandy loam | 760–680 and 670–410 BC | *Camassia* sp. |
| 9 | 41CV1657 | 3 | Basin Shaped, Slab lined | gravelly clay loam | AD 890–1030 | Indeterminable botanical |
| Lower Pecos Samples | Site | Feature Number | Notes | Matrix | Age | Associated Macrobotanicals |
| 1 & 5 | 41VV890 | F13 | open air site (basin unlined) | not described | AD 1667-1948 | Agave |
| 2 | 41VV164 | F1 | Disturbed, ash lens | loam/ash | 4356 -4260 BC | Agave, indeterminable bulb |
| 3 & 6 | 41VV890 | F7 | Disturbed, open air (flat unlined) | not described | AD 1317 – 1418 | n/a |
| 4 | 41VV164 | F2 | Disturbed, basin | loam | n/a | Sotol |
| 7 | 41VV165 | F1 | Disturbed, FCR midden deposits | fine sandy loam | AD 1200-1450 | n/a |

Sample preparation and extraction

Botanical samples were rinsed in distilled water to remove any sediment. They were cut to fit in

the beakers, and cut or gently crushed to increase surface area. Approximately 1.5 grams of outer

material removed from a 5x5 cm2 area of the FCR samples, which amounted to  the outer 1 or 2

mm of a given rock and is the  portion most likely to have post-excavation environmental

contaminants (Dimc 2011).  Analyzed samples consisted of about 3 grams, or 2-4 mm of

material was removed from the inner portion. Samples of adhering sediment and inner and outer

powdered samples were reserved from each piece of FCR.  Several samples of sediment and

outer portions were analyzed but the present paper focuses entirely to the inner portion.

Initially an extraction sequence of hexane, chloroform, propanol, and water was used, following

the protocol used by Hill and Evans (1989). However, it was determined this multi-step process

did not result in significant difference as compared to extracting with the simpler combination

chloroform/methanol method, similar to a conclusion reached by Hill and Evans (1989). Thus, a

one-step extraction protocol using 2:1 Choroform:Methanol was used, based on lipid extractions

techniques such as the Folch method (1957). Unlike lipid extraction techniques, however, the

methanol portion was retained, as this is used to extract carbohydrate molecules (Meier and Reid

1982). Additionally, simple soaking versus using a soxhlet extractor apparatus was also

compared. Samples were soaked in solvent for at least 12 hours (up to 24; no appreciable

difference was observed in extraction beyond 12 hours). Solvent was filtered, and then the

residue precipitated into a small vial under a nitrogen stream. Alternatively samples were

processed in a soxhlet for about 12 hours, and again dried under a nitrogen stream. As beakers

and filters were easier and less expensive to set up, more samples could be processed by soaking

in a single run, and thus those were favored for the plant samples. However, soxhlets provide for a more complete extraction. While it did not make a particularly strong difference (in part because of the poor quality of the spectra) the soxhlet extraction resulted in consistently better spectra, while in no cases were the spectra from the filtered extraction better. For the FCR, then, soxhlet extractions were used.

Raman Analysis

Analysis was conducted on the 'First Guard' hand-held Raman spectrometer from the Rigaku Corporation.  It has a 1064-nm laser, a spectral resolution of approximately $20cm^{-1}$, a focal spot size of approximately 1mm,  and a detection range from 200 to $2000cm^{-1}$. The 1064-nm wavelength provides advantages of in situ investigation and a significant suppression of fluorescence background.

RESULTS

When interpreting a spectra, it is common to identify the molecular bonds that cause individual peaks; however, this can be difficult to do when the spectra has many peaks or many components. Since the purpose here is to identify inulin in the archaeological residues, a fingerprint method will be used instead. With fingerprinting, the important peaks and their relative strength are identified in a reference material, which is then compared to the sample to be identified. The whole spectra is affected by the molecular structure, therefore it is not enough to have one or two peaks be the same between the reference and sample spectra – all the peaks must be present for a definitive match. However, mixtures of molecules, as would be expected in complicated substances like plants, can 'move' or 'hide' peaks so it is difficult to precisely match the sample spectra to a reference spectra. These difficulties will become apparent below.

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Carbohydrate samples

The spectra of four carbohydrates – cellulose, pectin, starch, and inulin – were analyzed so that the unique fingerprint of inulin could be identified. Cellulose and pectin are structural carbohydrates, giving the cell walls of many plants their shape. It is therefore reasonable to expect both to show up in the spectra of most plants. Starch and inulin are both storage carbohydrates, meaning they are where the plants store their energy for later use. Many plants mix types of storage carbohydrate, but wild plants are not normally subjected to the kind of macronutrient analysis that would indicate how much inulin or starch we could expect from any particular plant (Ernst and Bufler 1994). The Raman spectra for these carbohydrates is shown in Figure 20.

In seeking to determine which peaks to use as a fingerprint for carbohydrates, the spectrum is divided up in three sections, corresponding to different molecular structures. There are several regions associated with functional groups in carbohydrates. From 800-100 cm^{-1} is associated with CCO deformations, from 1200-800 cm^{-1} is associated with stretching modes of C-O/C-C, and 1500-1200 cm^{-1} is associated with deformations of CH/CH2 (Wiercigroch et al. 2017). The region 1200-800 cm^{-1} can be further subdivided at roughly 1000 cm^{-1}, since the area from 1160-970 cm^{-1} is also the region associated with the carbohydrate backbone. Small changes here can indicate not just which carbohydrate is present, but also details about the structure of the specific carbohydrate. A broad peak here, though, will mask that level of identification (Séné et al. 1994).
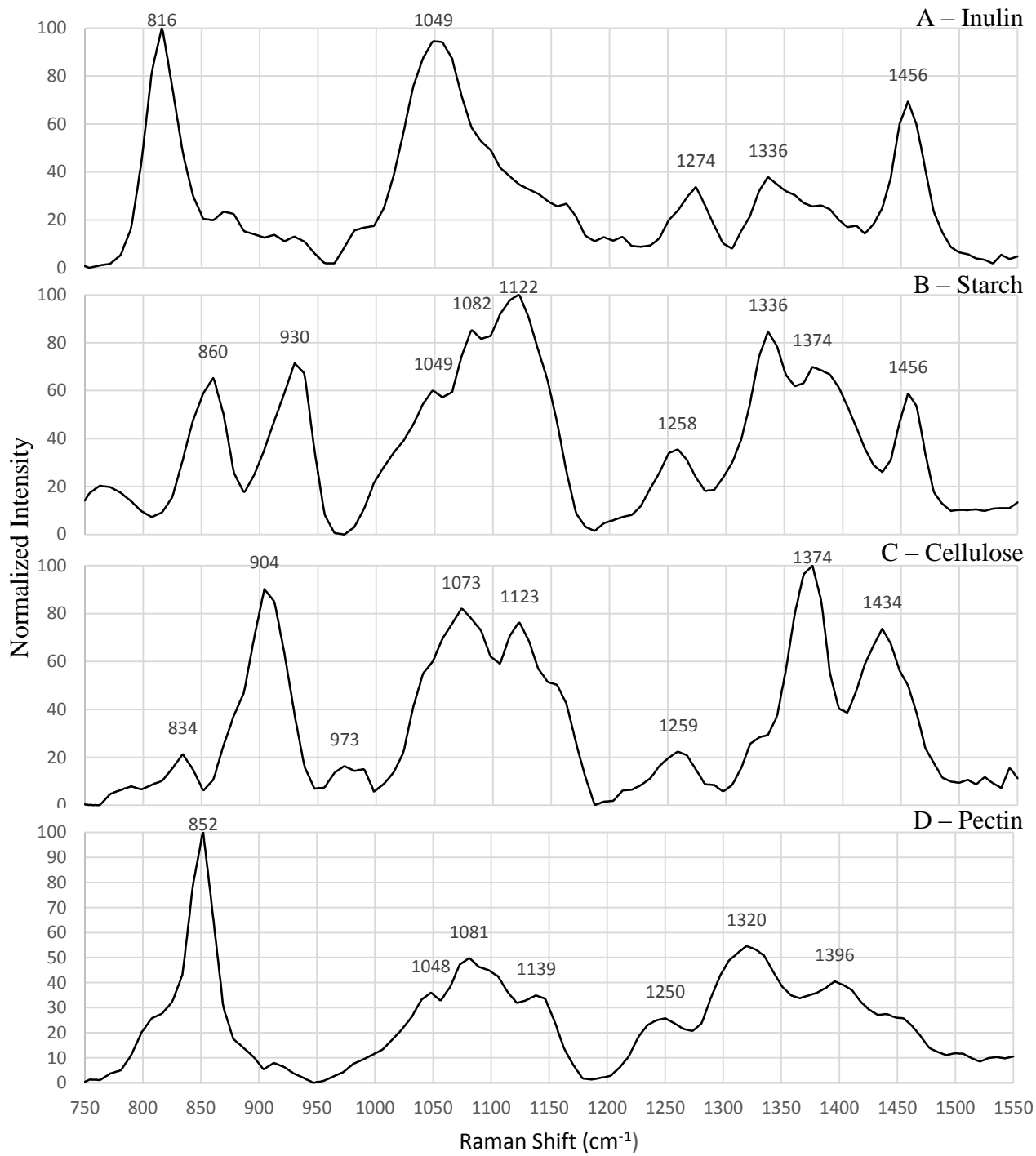
**Figure 20: Raman spectra of carbohydrate samples**

Inulin has a unique peak around 815 cm$^{-1}$, so a peak here and weak or no other peaks between 800-1000 cm$^{-1}$ is strongly indicative of inulin. Inulin, like the rest of the carbohydrates, has broad/overlapping peaks through the 1000-1200 cm$^{-1}$ region, but only has one strong peak around 1050 cm$^{-1}$. In the final region, 1200-1500 cm$^{-1}$, there are a number of weak or moderate peaks, but only one strong peak around 1450 cm$^{-1}$. Strong peaks at these locations (especially around 815) indicate probable inulin.

Starch has one unique peak around 930 cm$^{-1}$. In the 800-1000 cm$^{-1}$ region it has a second strong peak around 860 cm$^{-1}$. In the 1000-1200 cm$^{-1}$ region it has a strong peak around 1120 cm$^{-1}$, with a relatively weaker one around 1080 cm$^{-1}$. In the 1200-1500 cm$^{-1}$ region it has three strong peaks: 1330 cm$^{-1}$, 1375 cm$^{-1}$, and 1450 cm$^{-1}$. Strong peaks at these location indicate possible starch.

Cellulose has a unique peak around 900 cm$^{-1}$. It is the only strong peak present in the 800-1000 cm$^{-1}$ region for cellulose. In the 1000-1200 cm$^{-1}$ region it has two strong peaks around 1120 cm$^{-1}$ and 1070 cm$^{-1}$. In the 1200-1500 cm$^{-1}$ region it has strong peaks around 1375 cm$^{-1}$ and 1430 cm$^{-1}$. There are other weak and moderate peaks through the entire 800-1500 cm$^{-1}$ spectrum.

Pectin's spectra is predominantly broad moderate and weak peaks, with no unique peaks. Its strongest peak is a strong peak around 850-860 cm$^{-1}$. In the 1000-1200 cm$^{-1}$ region it does not have any particularly strong peaks, though the strongest peak in this region is a moderate peak around 1080 cm$^{-1}$. Again, in the 1200-1500 cm$^{-1}$ there are no strong peaks, but there is a moderate peak around 1320 cm$^{-1}$.

Modern reference samples -- Botanicals

Modern botanicals were analyzed to confirm that inulin was identifiable in inulin-rich plants, as well as determine the degree of differentiation in spectra between plant species. Modern botanicals representative of plant foods known or suspected to have been cooked in earth ovens and analyzed for this paper are  divided into two groups: inulin-rich plants without diagnostic starch, and those plants, both inulin- and starch- rich, with diagnostic starch (Laurence 2014). How much of any particular storage carbohydrate a given plant species contains is understudied, especially for the wild food plants, but family level research indicates that inulin is strongly associated with the *Liliaceae* and *Agavaceae*[2] families, among others (Meier and Reid 1982). While many plants contain only one type of storage carbohydrate, some contain a mixture (Kandler and Hopf 1982). In any case, all plants also contain cellulose since it is a structural carbohydrate, but some root foods contain pectin as well (Robert et al. 2008). Plants, therefore, are mixtures of the different carbohydrates, as well as other components. Thus it is anticipated that there will be a certain amount of overlap between peaks from different components.

Modern botanical samples have been evaluated for the presence of cellulose, pectin, starch, and inulin based the criteria laid out in the previous section. See Appendix A for spectra of all the plants examined here. Those showing the characteristic peaks of inulin (approximately 820, 1050, and 1450) are onion (*Allium cepa)*, sotol (*Dasylirion spp.)*, agave (*Agave spp.)*, Jerusalem artichoke (*Helianthus tuberosus)*, camas (*Camassia scilloides)*, and copper lily (*Cooperia*

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[2] Until recently camas (*Camassia sp.)* was in the *Lilliacea* family, but has since been moved to the *Agavaceae* by the Angiosperm Phylogeny Group, though the USDA PLANTS website has not yet changed their classification  (APG 2003; USDA NRCS 2018).
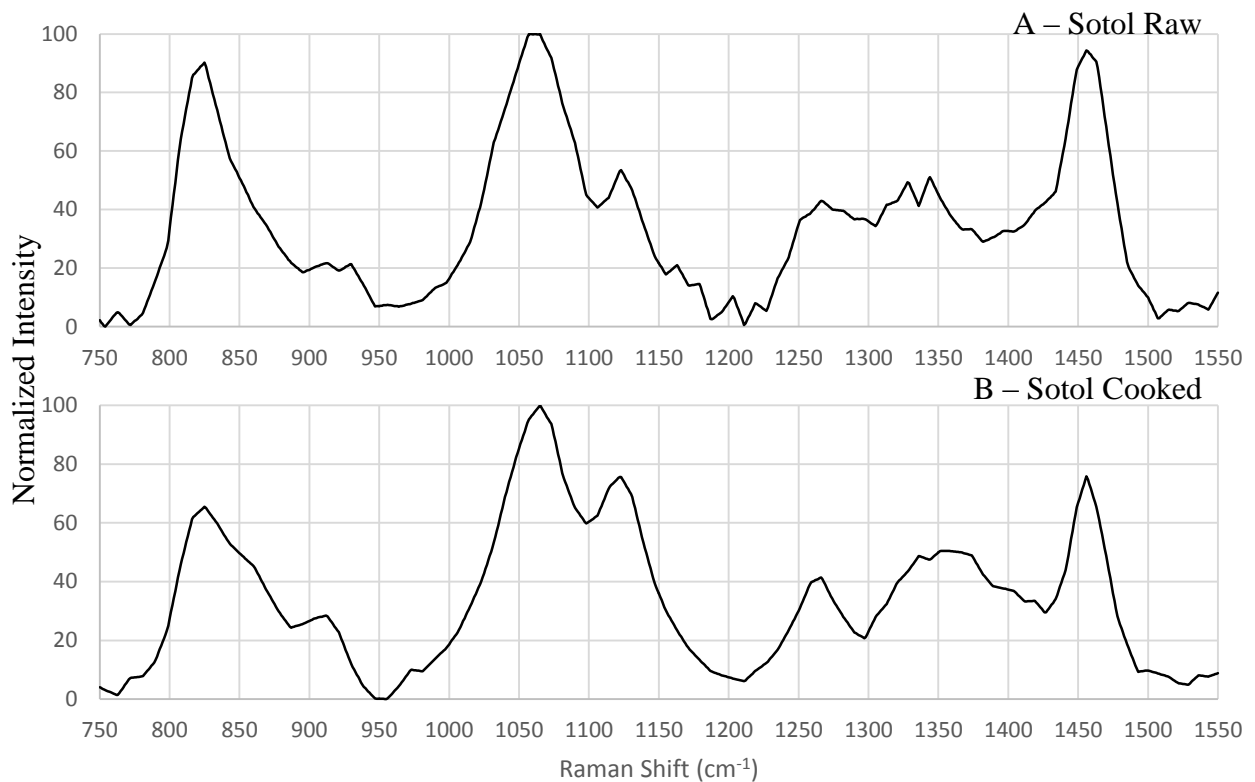
**Figure 21: Raman spectra of modern sotol**

*drummondii).* Figure 21 depicts the Raman spectra of sotol, and Figure 22 depicts the spectra of

camas. False garlic (*Nothoscordum bivalve)*, gayfeather (*Liatris spp.*), and cattail (*Typha*

*latifolia)* show some of the peaks, notably the unique peak around 820, as well as the peak

around 1450 which is also present for starch, as illustrated for false garlic in Figure 23. This is all

as expected, as inulin is known to be present in plants from the order Liliales, which includes the

lily family (Liliaceae) and agave subfamily (Agavoideae, formerly family Agavaceae); the order

Asterales, which includes composite flowers such as gayfeather and Jerusalem artichoke; and the

order Poales, which includes cattail (Meier and Reid 1982). While there has not been much

analysis of inulin-rich plants with Raman spectroscopy, the spectra seen here are in agreement
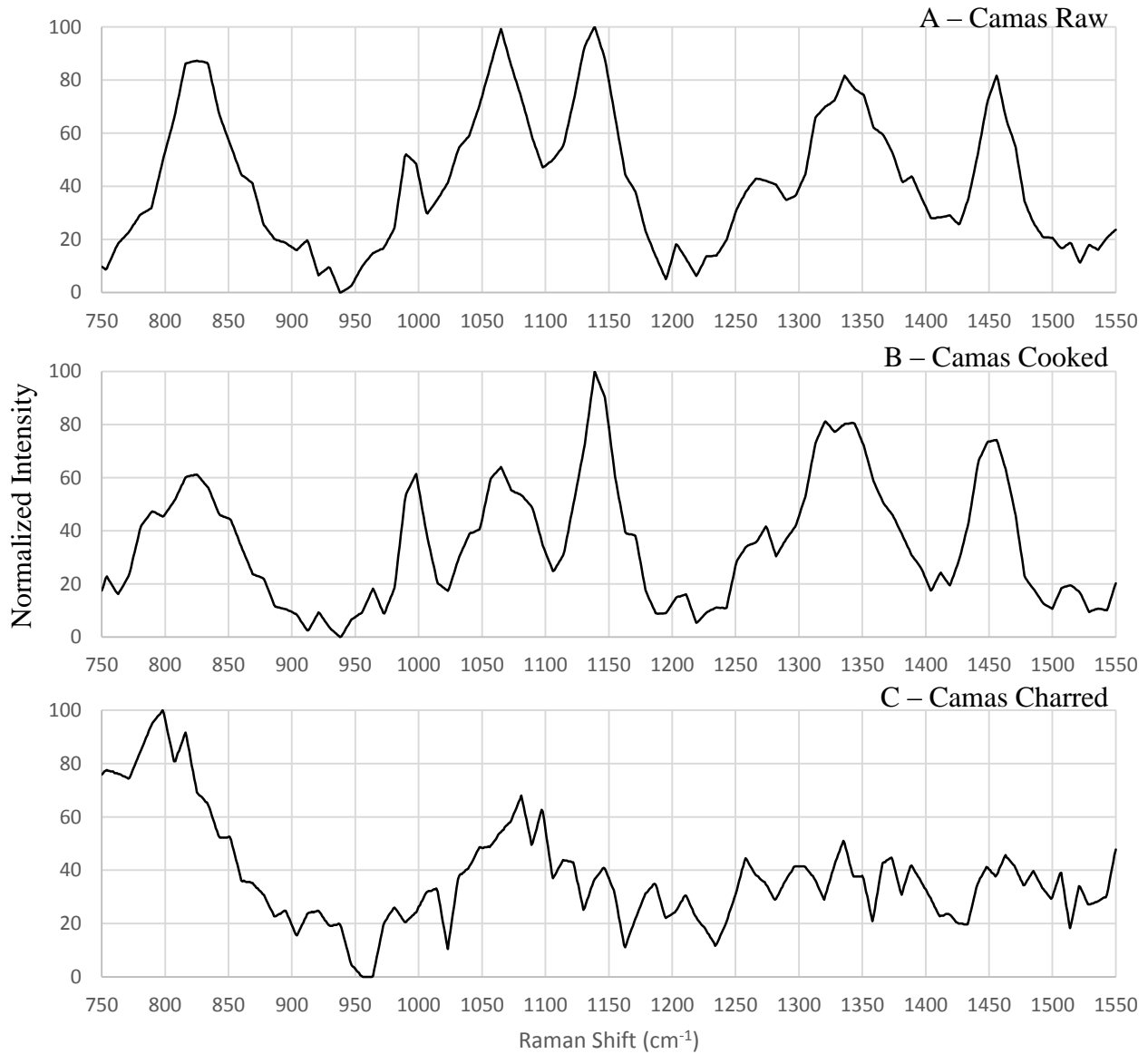
**Figure 22: Raman spectra of modern camas**

with the spectra of other inulin rich plants such as Dahlia tubers (Ciobanu et al. 2016). The

presence of the other carbohydrates in the plants was less conclusive. Only Asian water lily had

all the peaks associated with starch – see Figure 24.
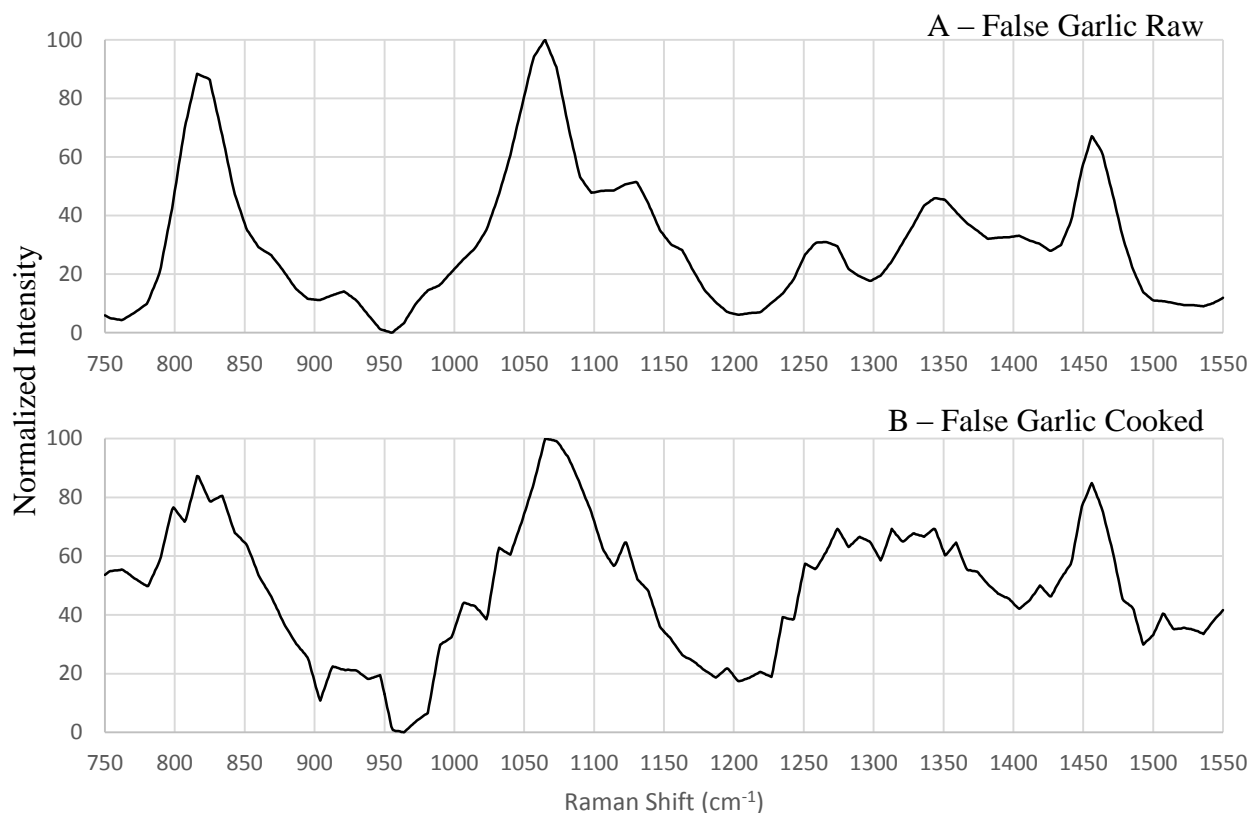
**Figure 23: Raman spectra of false garlic**

Most of the samples were also cooked to determine if there was a change during normal cooking process. For the most part, as can be seen in figures 21-24, while there is change in the intensity of some peaks they are all still recognizable as they go from raw to cooked state. Potato and false garlic Figure 4b, however, lost definitive peaks. Additionally charred camas bulbs (Figure 3c) were available for analysis from an actualistic cooking experiment failure (Thoms, Laurence, et al. 2014b). These had no identifiable signature, indicating that complete carbonization prevents identification
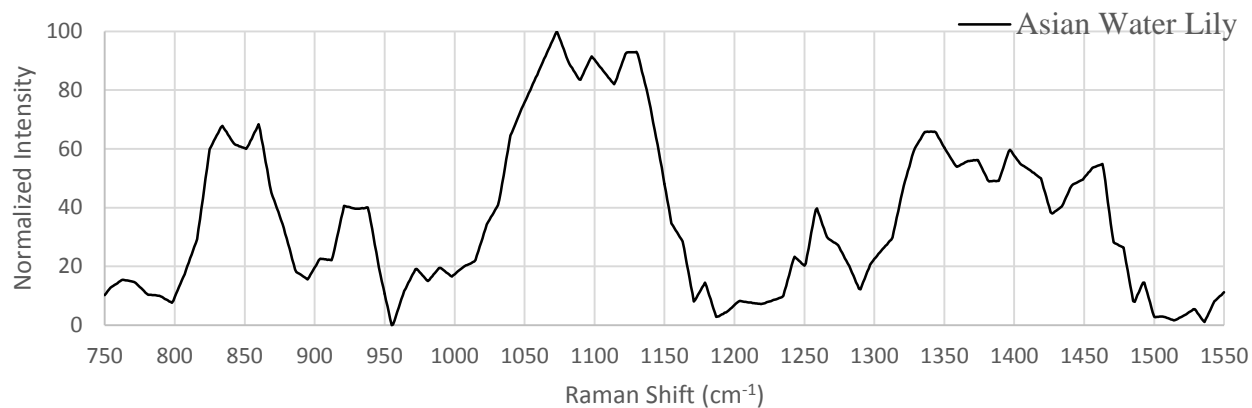
**Figure 24: Raman spectra of Asian water lily**

Modern reference samples -- Faunal

Three meat sources – turkey, venison, and buffalo – were also analyzed for comparison with the plant food sources. While physical archaeological evidence of meat cooking is not common in the study area, there is some ethnohistoric evidence for it, and meat is commonly cooked in earth ovens elsewhere (Wandsnider 1997; Thoms 2007). Thus they were included as a possible residue that might show up, as well as to show how different meat spectra are from plants. In Figure 25, buffalo is shown as a representative of the meat samples. There is a broad peak around 870 cm$^{-1}$, and another at 1065 cm$^{-1}$. The two strongest peaks are at 1298 cm$^{-1}$ and 1441 cm$^{-1}$, which allow for differentiation from carbohydrates. As with the plant samples, the meat spectra are still identifiable after cooking.
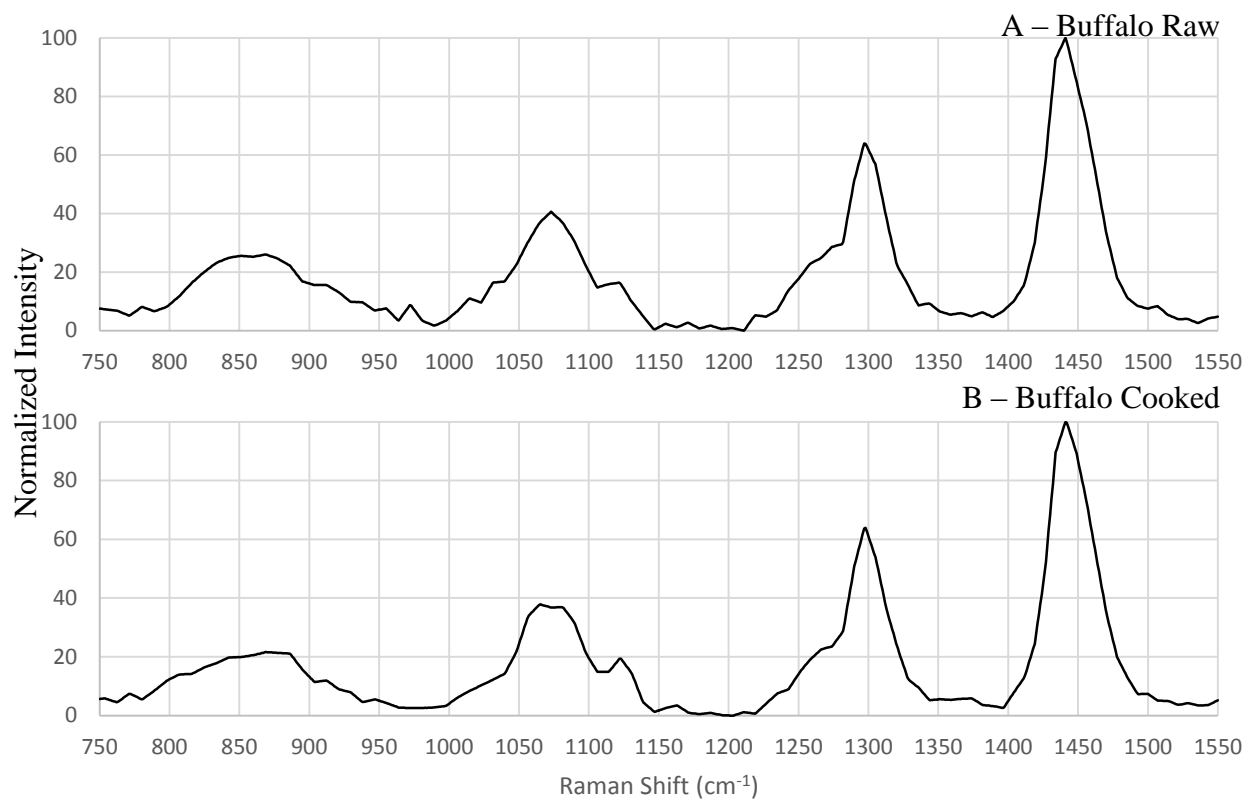
**Figure 25: Raman spectra of modern buffalo meat**

Archaeological botanicals - intact

Intact archaeological botanical samples (i.e., macrobotanical specimens) were analyzed to

demonstrate the effects of taphonomic processes. Figure 26 shows the Raman spectra of little

walnut, prickly pear, sotol, and onion. The samples were difficult to analyze and the resulting

spectra were fairly noisy. Very dark samples can burn when hit with the laser, so the power of

the laser needs to be reduced, which in turn reduces the strength of the signal. That said, the

approximate locations of the peaks spectra were still apparent. Most had a similar pattern: low

broad peak centered at 800 cm$^{-1}$, a somewhat sharper speak centered 1080-1090 cm$^{-1}$, another

broad low peak centered at 1310 cm$^{-1}$, a weaker peak around 1460 cm$^{-1}$.
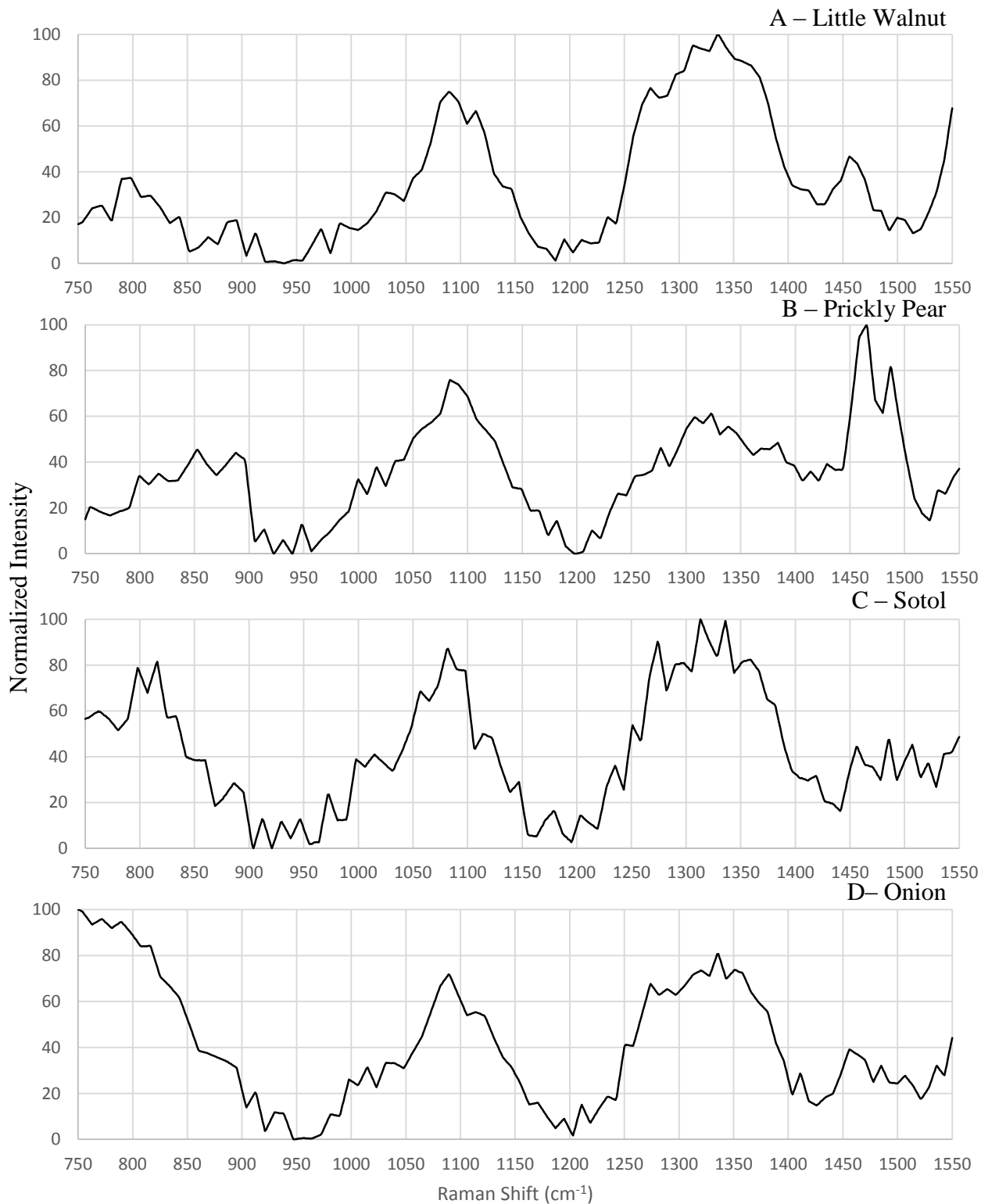
**Figure 26: Raman spectra of intact archaeological botanicals**

Most of the prickly pear pad, cactus seed, and sotol samples had an additional peak around 1485 cm$^{-1}$, and a few had a peak near 900 cm$^{-1}$. Calcium oxalate has peaks around 900 cm$^{-1}$, 1470 cm$^{-1}$, and 1490 cm$^{-1}$ (Frausto-Reyes et al. 2014). Calcium oxalate is well known to come from cacti like prickly pear, and Dering (2008) found it in the sotol species (*Dasylirion texanum*) common to Texas. While the peak around 900 cm$^{-1}$ is not consistently present throughout these species, it seems likely that the source of these peaks is from calcium oxalate. Aside from calcium oxalate it is difficult to assign origins to many of the other peaks. There is a possible peak around 815 cm$^{-1}$ for two of the sotol samples, but there is no associated peak at 1050 cm$^{-1}$, which is insufficient for a confirmation of inulin. None of the other carbohydrate peaks were recognizable in the archaeological macrobotanical sample.

Archaeological botanicals – extracted

Three botanicals were available for comparison between the archaeological intact and extracted samples – sotol, prickly pear, and little walnut (Figure 27). In sotol, all the peaks above 1100 cm$^{-1}$ in the extracted samples were present in the intact samples, though the reverse was not true. The peak around 800 cm$^{-1}$ in the intact sample was shifted to 780 cm$^{-1}$ in the extract, and the peak between 1080 cm$^{-1}$ and 1090 cm$^{-1}$ was shifted slightly to 1075 cm$^{-1}$. The peak at 870 cm$^{-1}$ in the extract is also possibly the 900 cm$^{-1}$ peak shifted. In prickly pear, there was variation within the different spectra of both the intact and extracted samples, so it is not clear if the differences were the result of natural variation or shifts due to extraction. For example, some of the extracted samples have peaks at 800 cm$^{-1}$, 880 cm$^{-1}$ or 930 cm$^{-1}$, while some the intact samples have peak at 800 cm$^{-1}$, 850 cm$^{-1}$ or 888 cm$^{-1}$. Most notably the two peaks associated with calcium oxalate, 1465 cm$^{-1}$ and 1480 cm$^{-1}$, were not present in extract. Little walnut was the

only sample to have more peaks in the extract than the intact sample. In addition to new peaks, there was a dramatic shift between the 1090 cm$^{-1}$ peak in the intact sample and the 1050-1065 cm$^{-1}$ in the extracts, while the peaks at 1330 cm$^{-1}$ and 1450 cm$^{-1}$ were consistent. While the reasons for these changes are unclear, it demonstrates the importance of obtaining a reference sample using the same extraction technique used in the study.
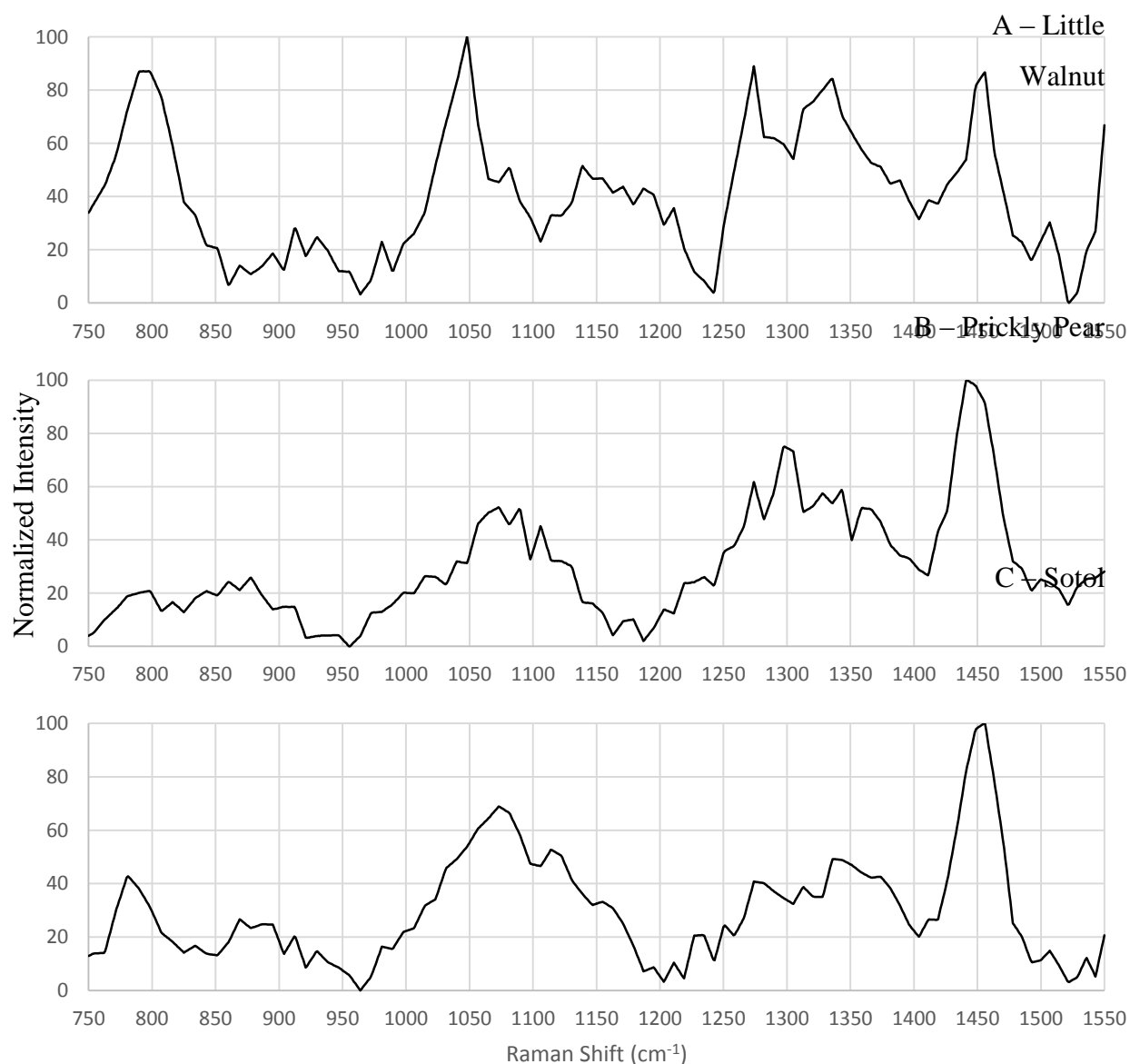


**Figure 27: Raman spectra of extracted samples from archaeological botanicals**

There are also differences between modern and archaeological samples of sotol and onion. While the archaeological sotol peaks are shifted in the same direction between intact and extracted samples, between the archaeological and modern extractions, they shift in different directions. For example, the archaeological sample peak at 1275 cm$^{-1}$ shifts down to 1266 cm$^{-1}$ in the modern sample, while the peak at 1336 cm$^{-1}$ shifts up to 1345 cm$^{-1}$. Notably, the 1450 cm$^{-1}$ peak is consistent across both the archaeological and modern samples. The other inulin peaks, however, appear to be shifted: 825 cm$^{-1}$ in the modern samples becomes 800 cm$^{-1}$ in the archaeological, while 1060 cm$^{-1}$ shifts to 1075 cm$^{-1}$. For onion, only the intact archaeological sample was available for comparison with the modern extract. Again, peaks appear to be shifted, but not always in the same direction. Focusing on the peaks relevant to inulin, the 825 cm$^{-1}$ peak in the modern sample shifts to 800 cm$^{-1}$ in the archaeological, while the modern 1065 cm$^{-1}$ peak shifts to 1090 cm$^{-1}$ in the archaeological sample, and 1450 cm$^{-1}$ remains the same. Assuming that the inulin remains in the archaeological sample, it appears that the 815-825 cm$^{-1}$ peak decreases to around 800 cm$^{-1}$, while the approximately 1050-1065 cm$^{-1}$ peak shifts as far as 1090 cm$^{-1}$, while the 1450 cm$^{-1}$ peak is consistent. While the 800 cm$^{-1}$ peak could be related to inulin, it must be noted that this peak is present in little walnut, which does not have inulin (Kandler and Hopf 1982), indicating the possibility of false positives.

Experimental – charring

In order to determine the effects of extended cooking on the spectra of plants, a charring experiment was performed, though the plants did not reach full carbonization. Raman spectroscopy does not directly give information about the quantity of a substance present in a sample, though it can be calculated (Pelletier 2003). Generally, the relative intensity of a set of peaks is just as important as peak location in determining a spectral signature. Examining the spectra representative of the onion charring experiment in Figure 28, we see that the relative intensity of the peaks stays fairly consistent, and that the peaks associated with inulin (approximately 815, 1050, and 1450 cm^{-1}). Baked in a conventional oven, the moisture loss shown here was approximately 5%, 20%, and 40%. Forty percent moisture loss is consistent with studies of moisture loss in earth ovens (Thoms, Laurence, et al. 2014a). This indicates that it is possible for the spectra of onion to be identifiable after 40% moisture loss.
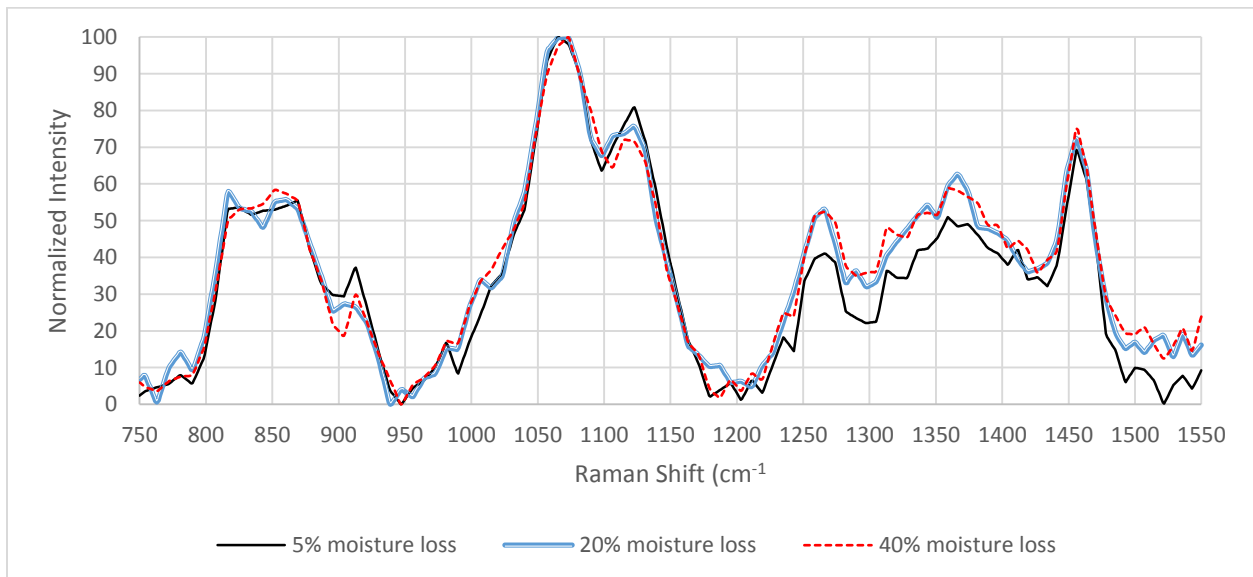


**Figure 28: Raman spectra of Onion charring experiment**

Experimental – earth ovens

The spectra from the experimental oven FCR samples generally had low intensity and low signal to noise ratio, and so most were rejected as non-diagnostic. They can be seen in appendix A. Sample 1 from the inulin-rich oven, seen in Figure 29, has two strong peaks at 799 cm$^{-1}$ and 1066 cm$^{-1}$, and a weaker peak at 1444 cm$^{-1}$.  While these peaks are somewhat offset from the anticipated peaks for inulin, they are within the 20 cm$^{-1}$ resolution of the handheld spectrometer. Thus inulin is provisionally identified in this spectra. No other strong peaks are present in the 800-1000 cm$^{-1}$ region, precluding the presence of other carbohydrates.  This is only a provisional identification because of the similarities to the spectra from sample 1 from the starch-rich oven (Figure 30), the only other sample with spectra intense enough to be analyzed. The lack of a peak at 1450 cm$^{-1}$ renders it non-diagnostic; however, there is a strong peak at 800 cm$^{-1}$, which would not be anticipated for non-inulin carbohydrates.  The source of this spectra is currently unknown.
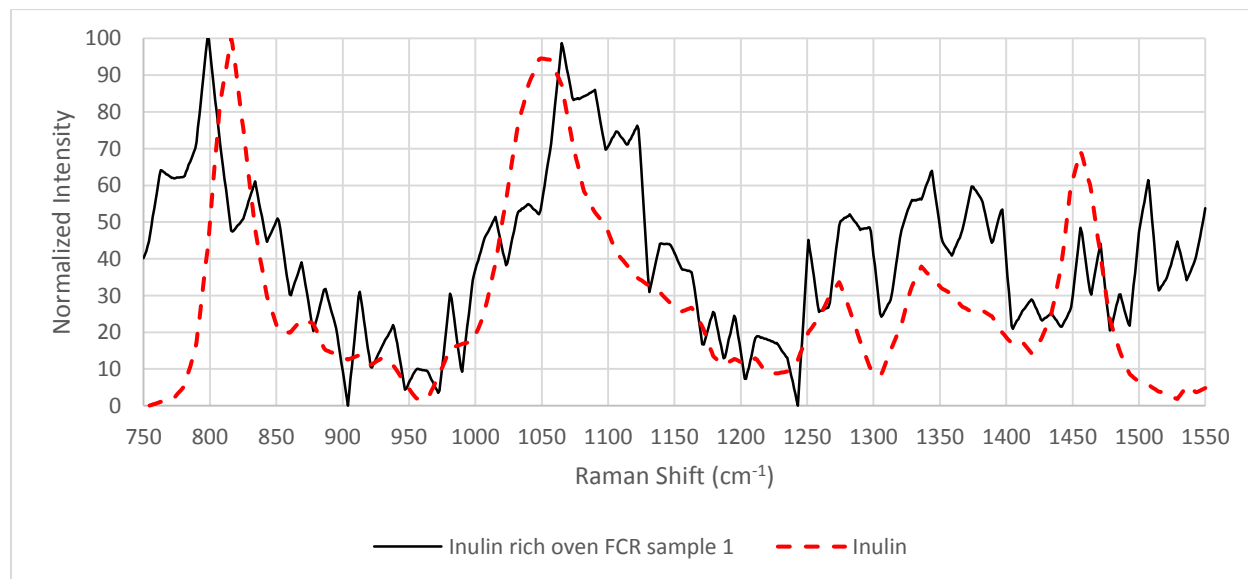


**Figure 29: Raman spectra of inulin-rich oven FCR sample 1**
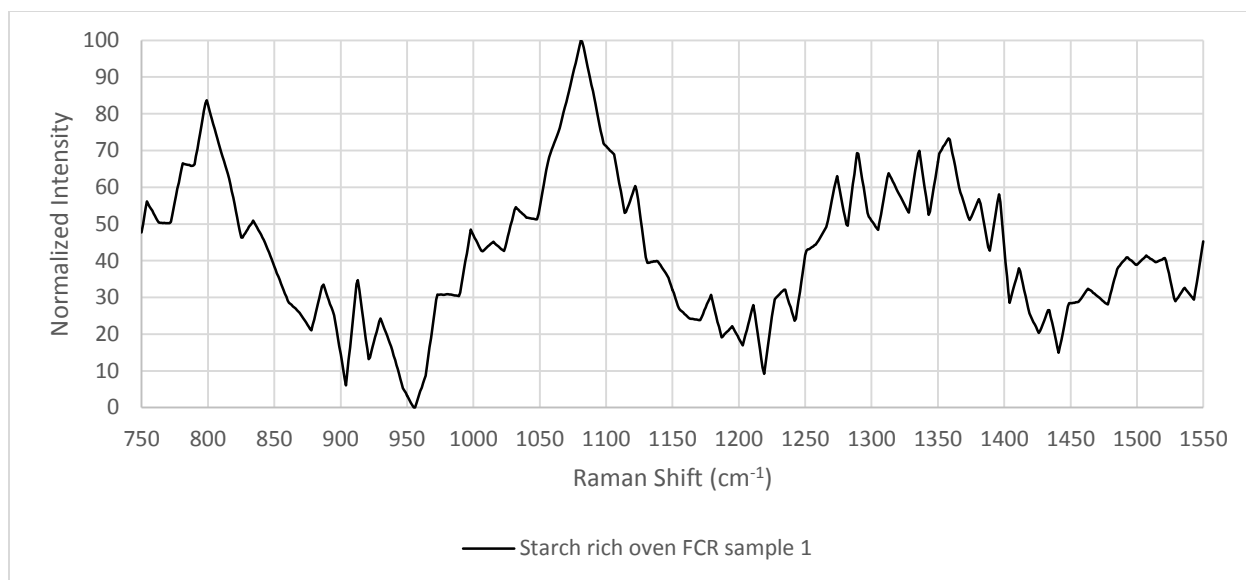
**Figure 30: Raman spectra of starch-rich oven FCR sample 1**

Archaeological ovens

The spectra from the Fort Hood FCR samples tend to show low intensity and to have a low signal to noise ratio such that most were rejected as non-diagnostic. They can be seen in appendix A. Fort Hood sample 1 (FH1), from 41CV1553 feature 6, is a borderline case. Figure 31 shows FH1 overlain with the spectra of inulin. While there is noise interrupting the spectra, there are two relatively strong peaks at 798 cm$^{-1}$ and 1114 cm$^{-1}$. While raw inulin peak appears around 815 cm$^{-1}$ and 1050 cm$^{-1}$, the archaeological inulin rich plants had those peaks shifted closer to 800 and 1100, so these peaks are within expectations the presence of archaeological inulin. To confirm an inulin identification, a peak should be present at 1450 cm$^{-1}$, which we do see. The region from about 1400 to 1500 cm$^{-1}$, however, is very noisy. The lack of peaks in the 850-950 cm$^{-1}$ range rules out the presence of other carbohydrates. Thus there is a very tentative identification of inulin in this sample.

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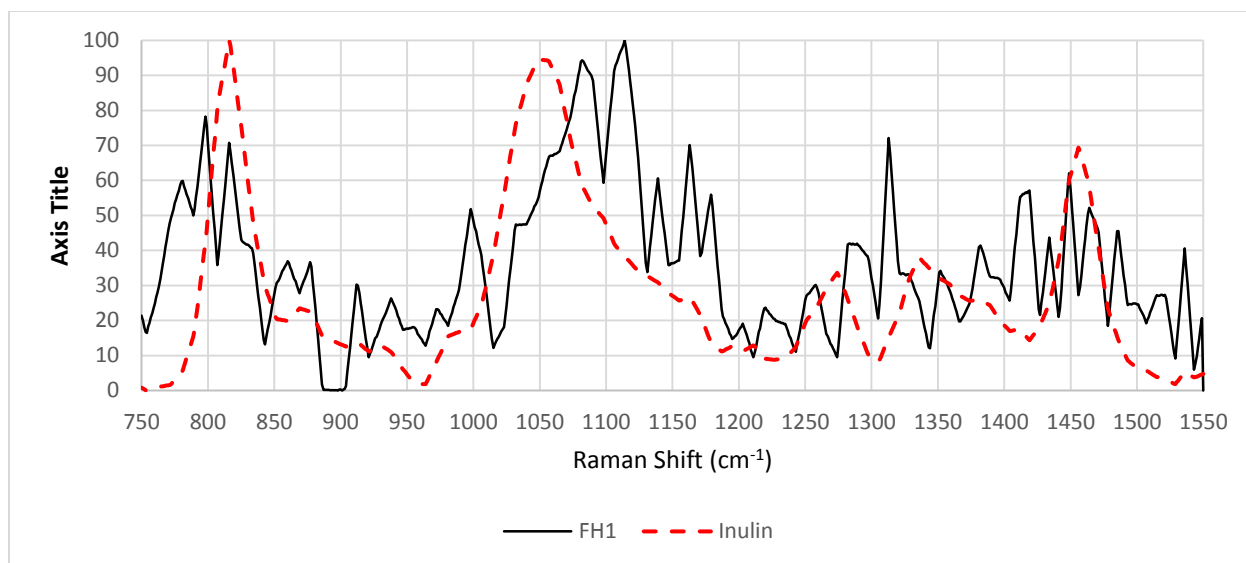
**Figure 31: Raman spectra of FCR sample from 41CV1553 feature 6 (sample FH1), overlain with spectra of inulin**

Fort Hood sample 9 (FH9), from 41CV1657 feature 3, has a higher signal to noise ratio, with more prominent peaks (Figure 32). The peaks at 797 cm$^{-1}$ and 1083 cm$^{-1}$ are strong, but the peak at 1457 cm$^{-1}$ is very weak. This is a tentative identification of inulin in the sample. There is a strong peak at 930 which is also present in starch. In the 1000-1200 cm$^{-1}$ starch has its strongest peak at 1122 cm$^{-1}$. If FH9 has a mixture of starch and inulin present, this may account for the relatively wide peak in this region, even if those peaks cannot be resolved in the spectra. Again the region from 1200-1500 cm$^{-1}$ is fairly noisy, but a broad peak around 1350 cm$^{-1}$ is apparent and could account for the starch peaks at 1336 cm$^{-1}$ and 1375 cm$^{-1}$. Again the weakness of the peak at 1450 cm$^{-1}$, as well as the lack of a distinct peak at 860 cm$^{-1}$ weaken the argument for the presence of starch. A cautious assignment of inulin and starch are made for FH9. Figure 13
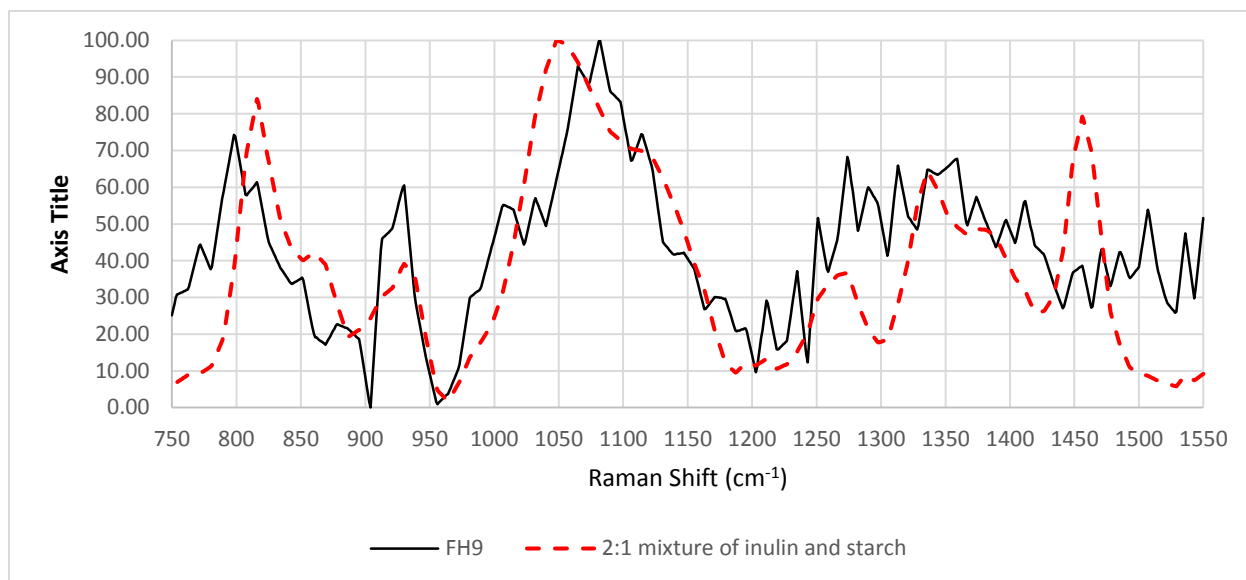
**Figure 32: Raman spectra of FCR sample from 41CV1657 feature 3 (sample FH9), overlain with a 2:1 mixture of inulin and starch**

shows FH9 with overlain with the spectra of a 2:1 mixture of inulin and starch.

FCR samples from the Lower Pecos also have low intensity and low signal to noise ratios. Again, most of the samples are non-diagnostic, as is shown in Appendix A. Lower Pecos sample 9 (LP7), from 41VV165, however, has a stronger signal to noise ratio. The peaks at 800 cm^{-1} and 1081 cm^{-1} are strong, but there is not clear peak at 1450 cm^{-1}. The strong clear peak at 922 cm^{-1} indicates starch could be present. There are peaks at 1080 cm^{-1} and 1135 cm^{-1}, in alignment with starch. The peak at 1350 cm^{-1} could be the 1336 cm^{-1} peak of starch, and the weak peak at 850 cm^{-1} could be the 860 cm^{-1} peak of starch. A cautious assignment of inulin and starch is made for LP7 Again, however, there is no clear peak at 1450 cm^{-1}. Figure 33 shows LP7 overlain by a mixture of starch and inulin.
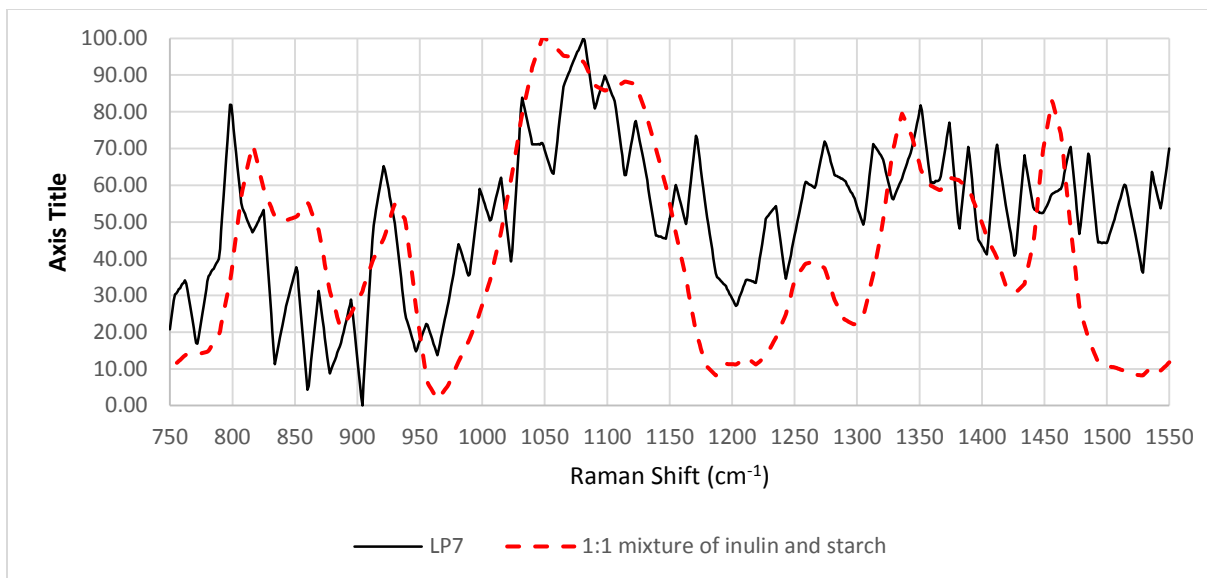
**Figure 33: Raman spectra of FCR sample from 41VV165 (sample LP7), overlain with a 1:1 mixture of inulin and starch**

Control samples provide information about the background environmental signal that may interfere with interpreting food signatures. If the off-site rock has an identifiable signature, it may be mistaken for food. The samples of the associated sediment and external portion of the FCR from the stones sampled from 41CV1553, as well as from the off-site control sample are shown in Figure 34. These show similar spectra in that their strongest peak is between 1050 and 1100 cm$^{-1}$, and a peak around 800 cm$^{-1}$. The off-site control rock has a distinct peak at 932 cm$^{-1}$. They do not have clear peaks in the 1200-1500 area, and are thus non-diagnostic. This indicates that the signals found on the interior portion of the FCR are unlikely to come from the environment.
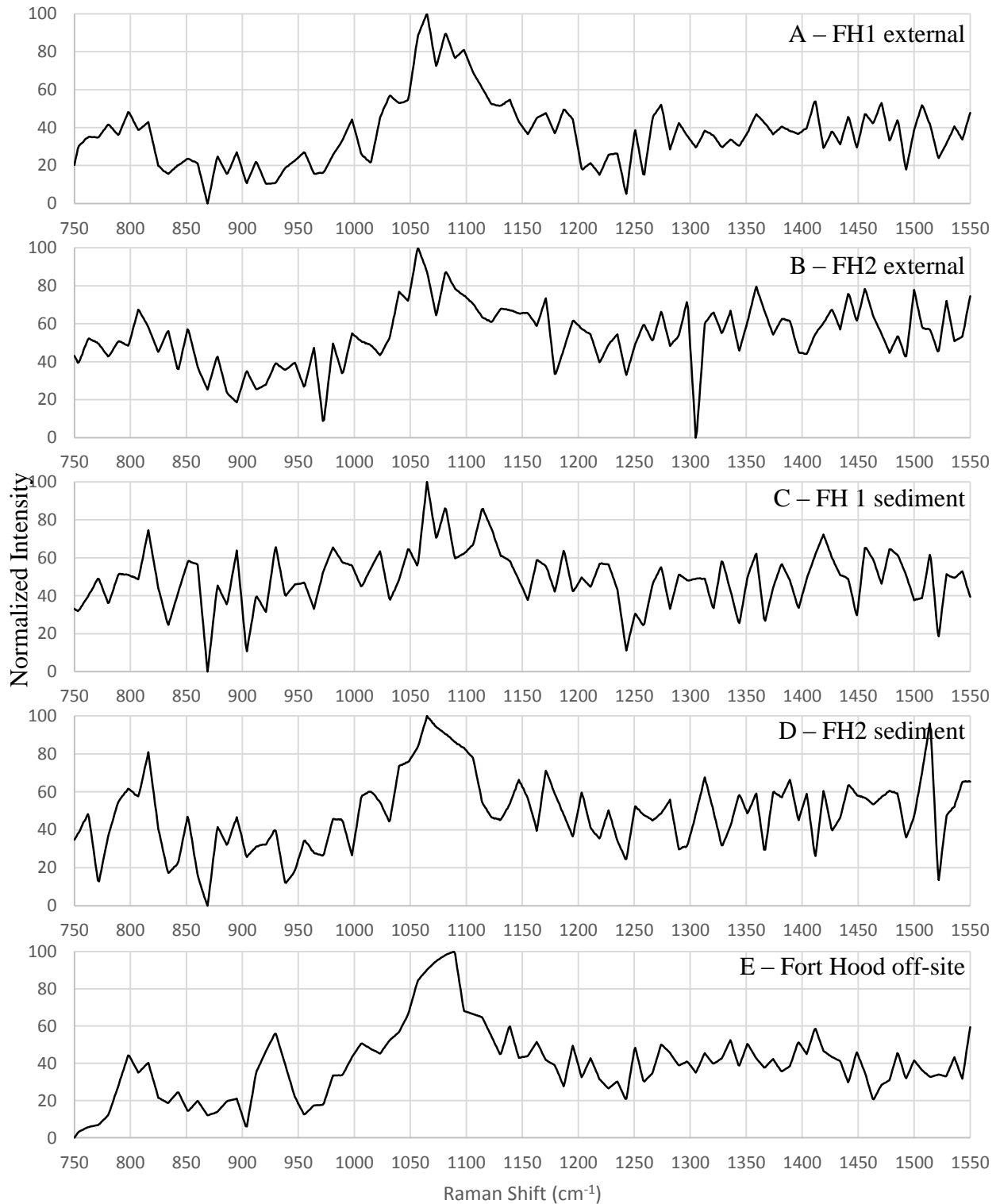
**Figure 34: Raman spectra of control samples including the outer 1-2mm of FCR from Fort Hood sample 1 (A) and 2 (B), the sediment associated with Fort Hood samples 1 (C) and 2 (D), and an off-site stone from Fort Hood (E)**

DISCUSSION AND CONCLUSIONS

Earth ovens are complicated features, potentially reused multiple times, repurposed, or both (Black and Thoms 2014). Even in an idealized setting where a given oven was used once, abandoned, and preserved, the central heating feature is exposed to the elements and potentially disturbed when the food is removed. Interpretations of organic residue of archaeological materials are inherently difficult, given taphonomic processes. Studying cooking residues even more so, since food stuffs start as complex mixtures, which are further modified by heat and moisture. Earth oven cooking has further complicating factors to discerning the signature of food residues, with green packing material and the fuel source likely adding to the 'background' of the spectra. Thus while biochemical analysis has the potential to address what was cooked in individual ovens, it should not be assumed that the answer to such questions will come easily. With multiple layers of complexity, developing a reliable and reproducible technique to identify the chemical structures of food residues from earth ovens and other cooking features will take time and many false starts.

Several different aspects of the reference collection were assessed. It was demonstrated that inulin is discernable from other carbohydrates, both in its isolated form and in plant material. The signals between different carbohydrates are similar, however, so good signal to noise ratio, intensity and resolution are necessary for a conclusive identification. Though the number of archaeological botanicals was limited, comparing archaeological to modern botanical references demonstrates that the spectra changes over time. Similarly, there are some shifts of the peak locations in the spectra between the intact and extracted archaeological botanical samples. This

indicates that the reference collection materials and their processing should be as similar as possible to the samples that are going to be studied.

Of the 16 total archaeological FCR samples from 12 ovens, three were cautiously identified as possibly having the remains of carbohydrates. One appeared to be just inulin, while two could contain a mixture of inulin and starch. Moreover, the control rock sample had a non-diagnostic spectra similar to the archaeological sample, indicating that environmental signatures could potentially be identified as food-related signatures. While not a panacea for those of us intent on determining what plant food(s) were cooked in a given oven, this is in line with other FCR residue studies. For example, Buonasera (2005) found that only 3 of the 9 FCR samples had more lipids than the control samples, which yielded signatures similar to what one might expect for food. Thus as a preliminary study demonstrating proof of concept, this study found potential signatures on FCR from both open and rockshelter sites.

Surprisingly, there does not appear to be a difference in preservation between the different depositional environments. It was anticipated that the dry rockshelters, having better preservation conditions, would have better preserved organic residue. The similarity in the FCR residue signatures between the different environments may also be of concern. It is unlikely to be background from the FCR itself, as limestone has an overwhelming peak at 1093 cm$^{-1}$ and a strong peak at 872 cm$^{-1}$, and it would be apparent in the spectra (Gunasekaran et al. 2006). It is also unlikely to be background from charred fuel, as that creates a broad peak centered around 1350 cm$^{-1}$, not where the diagnostic peaks are (Inoue et al. 2017). Similarities between either fuel source or packing materials are not anticipated between central Texas and the Lower Pecos.

There is ample room to improve the results of future studies. Firstly, there may have been methodological issues. Procedures used here are a modification of extraction methods used in lipid residue analysis. While the methanol fraction should retain the more polar carbohydrate molecules it is possible that another solvent might work better. Initial tests reported here, however, did not show a difference between the chloroform/methanol mixture and the hexane-chloroform-propanol-water series. Given that hot water is used to extract inulin from plants, there is no straightforward solvent alternative (Meier and Reid 1982). Secondly, technological limitations of traditional Raman spectroscopy may have contributed to the noisy findings. Low intensity and low signal to noise ratio may be overcome with more advanced techniques such as Surface- enhanced Raman scattering (SERS), which uses special sample preparation to improve the instrument sensitivity.

While Raman technology has vastly improved in recent decades, readily available spectrometers – such as what was used in this study – may not be a good option for detailed studies of cooking residues from earth oven features. Improved analysis using advanced Raman techniques like SERS may be a good option, but non-Raman techniques chromatography, spectrometry, or both – such as GCMS – have been more extensively used. Importantly, Raman remains a good addition to a GCMS study, as it can be used to screen for the presence of organics after the initial extraction (though a range extending into the 3500 region is recommended).

There remain many directions for further study using either Raman or another analytical technique. Knowledge of biochemical signatures of wild food sources needs to be expanded. In addition to adding more wild foods to a given reference collection, nutrient analysis are needed to identify components of the food that can be identified with spectrometry. Further experimental

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work with residues from earth ovens is needed to pinpoint signature changes resulting for

cooking and charring. We also need to learn more about where within a given heating element

residue-rich FCR are likely to be found. Toward that end, detailed studies need to be undertaken

of potentially single-event archaeological ovens and single-event earth oven experiments

wherein FCR samples are selected from across an oven.

# CHAPTER V

# CONCLUSIONS

Currently the best way to understand earth oven use is to take a "big picture" view, focusing the spatial patterning over a landscape, in conjunction with environmental and ethnographic information. This is because individual ovens are deconstructed as part of their use cycle, frequently reused, repurposed or both. Often ovens are reused through the years, creating massive accumulations of FCR known as bedrock middens. This palimpsest nature makes it difficult to parse the evidence of what was cooked in a particular oven (Black and Thoms 2014). Focusing on food residues on individual stones, which is the subject of this dissertation, affords a means of determining what a single oven was cooking, in much the same way that carbonized macrobotanicals currently do, but without the requisite cooking failure. This is, of course, easier said than done.

INHERENT COMPLEXITIES

Analyzing archaeological biochemical residues is an inherently difficult process. Blind testing of multiple laboratories has shown that while many analytical techniques are able to provide some information, none are able to accurately pinpoint the precise source of the residue (Colombini et al. 2011a; Barnard et al. 2007). This is partially due to the nature of archaeological residues. First, they are often complex mixtures which are more difficult to interpret than single simple substances. Second, the artifact may have come in contact with multiple substances at various points during its use cycle. Third, post-depositional taphonomic processes, such as microbial activity and water washing through the soil, can cause the transfiguration and leeching of components of the residues. Fourth, there are limitations inherent to every analytical technique

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which is why using a variety of complementary methods is recommended. Food residues can be particularly complicated, as the cooking process adds more complexity through the breakdown of food molecules.

With multiple layers of complexity, developing a reliable and reproducible technique to identify the chemical structures of food residues from earth ovens and other cooking features takes time and many false starts. Every developing field has growing pains. In pollen studies of coprolites, the coprolites themselves needed to be demonstrated to be of human origin. Over the decades debates were waged about how to interpret the remains – whether immunological studies were effective, how to interpret phytolith and pollen counts, and how to identify contamination (Bryant and Dean 2006). Starch analysis has seen similar trial and tribulations, including accounting for the effects of cooking and diagenesis, as well as sources of starch transport and contamination (Laurence 2013; Henry et al. 2009, 2016). Patience is necessary, and while disappointment should be expected it should not be disheartening.

There remain numerous challenges for biochemical analysis of food residue from earth ovens. Packing materials and unburnt fuel may contribute to the spectral signature. This will need to be qualified and quantified to effectively identify target spectra. Starch residue analysis of earth ovens has similar issues (Thoms, Laurence, et al. 2014a). Also, while the FCR that was closest to the food seems most likely to have food residue on it, this hypothesis has not been tested. If, as Thoms et al. (2014a) contend, that plant microfossils are deposited on FCR via water vapor and subsequently as organic materials decompose and water percolates through the feature, the same might be possible for the chemical residues. Thus, where exactly residue collects within an earth oven is open to more exploration. Alternatively, it would be helpful to have a way to identify

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residue rich FCR amongst an earth oven during the Raman analysis, like ability of GCMS to quantify lipid amounts (Buonasera 2005). Finally, in south-central North America plant-cooking ovens are more common and the biomarker of interest is a carbohydrate. While carbohydrates have been found on artifacts, they are less likely to be preserved than lipids (Forte et al. 2018).

PRIMARY FINDINGS

As shown in the literature review of chapter 2 a majority of current literature on archaeological food residues focuses on GCMS analysis of lipids in pottery. This is because lipids are relatively well preserved and more likely to be detected than carbohydrates and pottery is highly absorbent and a known reservoir for food residue.  The field is still in its infancy though, given that many articles perform proof-of-concept tests or present preliminary data, as well as performing experimental archaeology and addressing technical issues. There are a number of articles, however, that present analysis of large, long running or otherwise longitudinal studies.

The literature review also provided insight into how to develop a good analytical procedure. First is the necessity of a reliable reference collection, so that the researcher knows that the results actually represent the substance of interest. Foremost for archaeologists is diagenetic processes, to ensure that the signal remains recognizable in the archaeological record.  It is important that either identifiable archaeological reference samples are studied or artificial aging studies are done. Important for studies of food residue is the effects of cooking, since that changes the molecular structure of food. Ideally taphonomic studies of cooked food residues would combine both process. Additionally, site location can affect the residue signature, depending on the type of analysis done.

Second is issues related to contamination. There are a number of steps a researcher can take to prevent contamination in the field and the lab. These include basics such as wearing gloves and being sure not to spray chemicals near artifacts of interest. However, it is not possible to prevent contamination from the environment. Thus it is vital that environmental control samples are taken from off-site contexts. Taking references of non-food plants would also help characterize what kind of signals may show up from the environment, or in the case of earth ovens, the packing material. Knowing the nature of background signatures will allow the signature of the food to be more accurately recognized.

The pilot study presented in chapter 3 demonstrated that the handheld Raman spectrometer has potential for analysis of archaeological food residues. This study focused on visible residues. A scraper used to process sotol during an actualistic oven experiment had a spectra characteristic of inulin. Two pieces of FCR from Fort Hood (from 41CV1553 and 41CV594) showed differences between the stone surface and a cleaned interior portion, indicating the presence of potential organic residue. Inulin was unable to be confirmed due to a low signal to noise ratio preventing the resolution of any peak in the 1200-1500 range. It was concluded that Raman spectroscopy holds potential for a good analytical technique of food residues. While handheld machines like the one the used in this study provide relatively cursory information about the spectra, better optical methods should provide better analytical results. These would include lab-based spectroscopes with higher resolution or better sensitivity, or related techniques that improve upon those, such as SERS or CARS.

In chapter 4 a reference collection was created and used to interpret signatures from archaeological FCR samples. Inulin can be distinguished from other carbohydrates in its isolated

form and in plant materials. However, the signals of various carbohydrates are somewhat similar and there are other compounds that may obscure the spectra of interest. Therefore, definitive identification of a particular carbohydrate requires good signal to noise ratio, intensity and resolution. In the modern reference samples, cooking did not seem to obscure the signal. Short of full carbonization, peaks were still identifiable with moisture loss comparable to what was observed in earth ovens. Comparing modern botanicals to the archaeological botanical references revealed that peak locations shift slightly, possibly a result of weathering processes. Spectra of samples extracted from archaeological macrobotanicals exhibited differences as compared to the spectra of those same intact macrobotanicals. This indicates that the reference materials, their preparation, and analytical set up should be as similar as possible to the samples that are going to be studied.

Of the 16 archaeological FCR samples analyzed, three were cautiously identified as possibly having signature peaks indicative of carbohydrates. One spectra appeared to have peaks associated with inulin alone, while two may represent a mixture of inulin and starch. Samples with possible carbohydrate signatures came from earth ovens in both humid open air sites and arid rock shelters. Of concern is that while the limestone off-site control sample from Fort Hood was deemed non-diagnostic, its spectra did have some similarities to the spectra of the archaeological sample. This, however, is in line with previous residue studies of FCR, including Buonasera (2005) who found that off-site control rocks had diagnostic ratios of lipids comparable to FCR from earth ovens, and Laurence (2013) found starch grains on off-site control rocks in comparable number and types to FCR from earth ovens.

Dry rockshelters are ideal preservation conditions, thus it was anticipated that the Lower Pecos samples would have stronger or more clear spectra; however, there does not appear to be a difference in preservation between the different depositional environments. The similarity in the FCR residue signatures between the different environments is also interesting. It is unlikely to be background from the FCR itself, as limestone has an overwhelming peak at 1093 cm$^{-1}$ and a strong peak at 872 cm$^{-1}$, and it would be apparent in the spectra (Gunasekaran et al. 2006). In figure 4 of the preliminary study, the sharpness of the peak at 1085 cm$^{-1}$ is indicative of what one would expect from a background of limestone spectra. It is also unlikely to be background from charred fuel, as that creates a broad peak centered around 1350 cm$^{-1}$, not where the diagnostic peaks are (Inoue et al. 2017). Similarities in the fuel source or packing materials are not anticipated between central Texas and the Lower Pecos. Further work needs to be done to confirm that the similarities in spectra are due to the fact that both locales cook inulin-rich foods in earth ovens, and not some other cause.

The overarching research goal of this dissertation was to assess what was being cooked in earth ovens, via Raman spectral analysis of biochemical residue found on FCR. The first research question was whether vibrational-spectroscopically identifiable food residues was preserved on archaeological FCR from earth ovens. This study indicates that it is possible. The reference collection showed that while cooking and taphonomic processes affect the spectra, they do not render it unidentifiable. Additionally it was demonstrated that food residues are preserved in open air and rockshelter environments. The second question was whether identified residue could be reliably assigned to an ancient baking event(s). Given that the off-site control sample was non-diagnostic, this is a cautious yes; however there was a spectra that had some similar

peaks. This indicates that in future studies it is vital to account for environmental signatures. The third and final question was whether the residue spectra can be used to characterize what was baked, and to what degree of precision? This is another tentative affirmative. It was not possible to identify a particular plant or plants that may have been cooked in an earth oven. However, the carbohydrate could be (cautiously) identified, which was sufficient for our purposes. This indicates that there is certainly potential for the use of Raman spectroscopy to study earth oven residue; however, it requires significant continued study before conclusive analysis is consistently achieved. Of primary concern is addressing background (such as from packing materials) and environmental noise to better recognize the obscured carbohydrate targets.

FURTHER RESEARCH

First, there may be room for methodological improvement. Two extraction methods were tested as part of this study. One was based on the work of Hill and Evans (1989) and entails use of increasingly polar solvents (hexane, chloroform, propanol, and water) to extract a range of molecules. The other method, is based on Folch (1957), which is used for extraction of lipids, but the methanol fraction is not discarded, which should retain the more polar carbohydrate molecules. Similar to the conclusion reached by Hill and Evans, the present study revealed that there was not a significant difference between the much longer series extraction and the shorter Choroform:Methanol extraction. Nonetheless, it is possible that a different solvent or extraction procedure would have been better for carbohydrates. Secondly, technological limitations of traditional Raman spectroscopy may have contributed. This is partially due to the handheld Raman spectrometer that was used, which is not a precision instrument. While a spectrometer similar to the one used I this study may be good for initial screening of artifacts, for detailed

analysis a lab based machine with better resolution is recommended. Low intensity and low signal to noise ratio can be overcome with more advanced techniques. Two such methods are Coherent anti-Stokes Raman scattering (CARS) and Surface- enhanced Raman scattering (SERS) micro-spectroscopy. Within the past decade both have been used to analyze residues on rock surfaces (Burruss et al. 2012; Muniz-Miranda et al. 2010). These would require that the archaeologists work closely with a physics lab.

There are many potential further directions for the biochemical analysis of earth ovens but all of them should begin with building a regionally specific reference collection. Beyond simply adding more food resources, knowledge of the nutritional components of wild plants would be useful. Wild food resources are understudied, and nutrient analysis enable the researcher to anticipate what kind of spectra to expect from a sample. Modern reference collections should be supplemented with cooked and archaeological samples when possible. In addition to laboratory cooked samples, actualistic earth oven samples allows for not only the more complex spectra anticipated from such an oven, but also allow for idealized analysis of oven components, for example, where one might anticipate residue-rich FCR. Longer term experiments could asses the effects of weathering on ovens that cooked known foods. Hypothetically FCR from the center of ovens with intact heating elements should produce the best spectra, as they have been the least disturbed. Additionally, intact heating elements will provide additional information about the physical structure of oven, as well as possibly including associated macro- and microbotanicals, to provide a more complete a picture of a single cooking event. Along those lines, a detailed analysis of individual ovens would be worthwhile. A systematic sampling system across an oven

may allow for the location of residue-rich FCR to be mapped out, which would facilitate

expedient sampling in other studies.

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# APPENDIX A: 100 ARTICLES ANALYZED

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# APPENDIX B: FACILE RESIDUE ANALYSIS OF RECENT AND PREHISTORIC COOK-STONES USING HANDHELD RAMAN SPECTROMETRY: SUPPLEMENTARY MATERIAL

| Inulin (cm$^{-1}$) | Cellulose (cm$^{-1}$) | Band Assignment |
|---|---|---|
| 813 s | - | CC stretching |
| - | 833 w | CCC, COC, OCC, OCO skeletal bending |
| 867 w | - | COC bending |
| - | 903 s | HCC, HCO bending |
| - | 975 w | HCH bending |
| 1059 s | - | COC stretching and ring deformations |
| - | 1071 s | COC stretching symmetric |
| - | 1117 s | |
| - | 1258 w | HCH (twisting), HCC, HOC, COH (rocking) bending |
| 1270 s | - | CH bending |
| 1333 s | - | CH$_2$-OH bending and deformations symmetric |
| - | 1373 s | HCH, HCC, HOC, COH bending |
| - | 1430 s | HCH asymmetric |
| 1453 s | - | CH$_2$-OH bending and deformations asymmetric |
| - | 1730 s | C=O stretching |

**Figure 35 Summary of Raman shifts and band assignments of inulin and cellulose from Sigma Aldrich (s – strong, w - weak). The band assignment was based on previously reported Raman spectra of inulin (Manno et al. 2009; Beirão-da-Costa et al. 2013) and cellulose** (Barrett 1981; Szymańska-Chargot et al. 2011)**.**

# APPENDIX C: RAMAN SPECTRA

CARBOHYDRATE SAMPLES
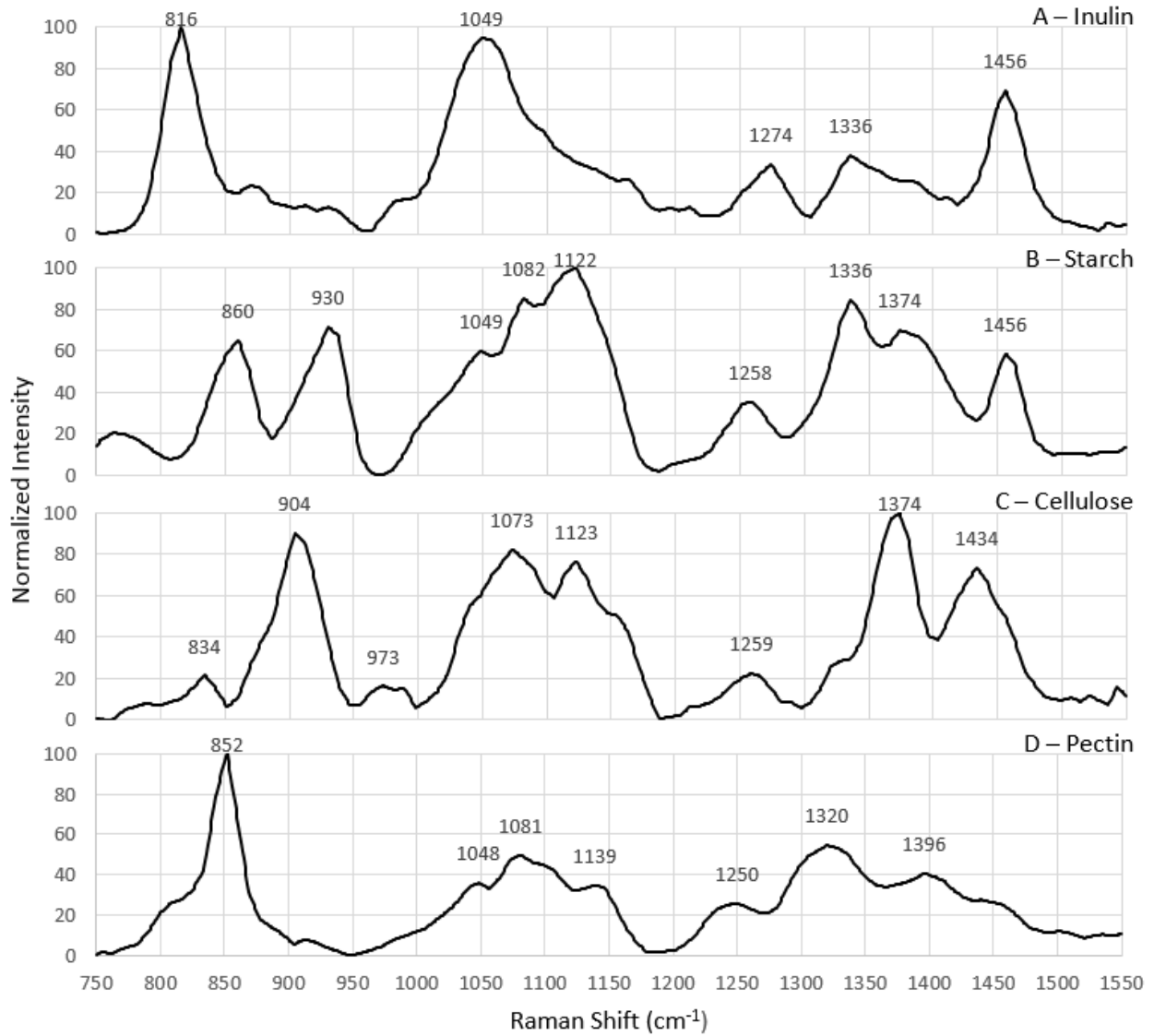


**Figure 36 Raman spectra of carbohydrate samples. A: inulin; B: starch; C: cellulose; D: pectin**

MODERN BOTANICAL REFERENCE SAMPLES
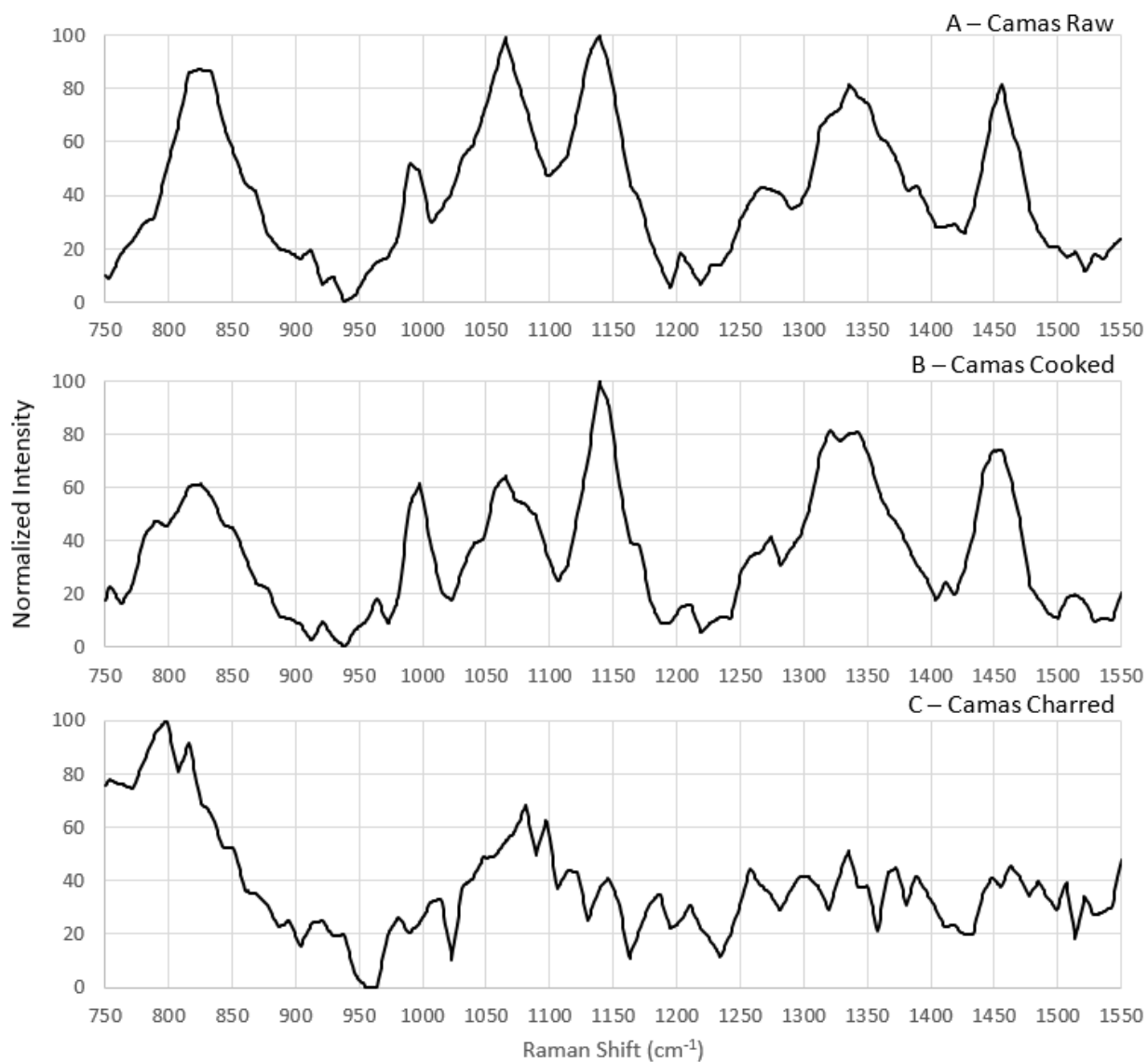


**Figure 37 Raman spectra of modern camas. A: Raw; B: Cooked; C: Charred**
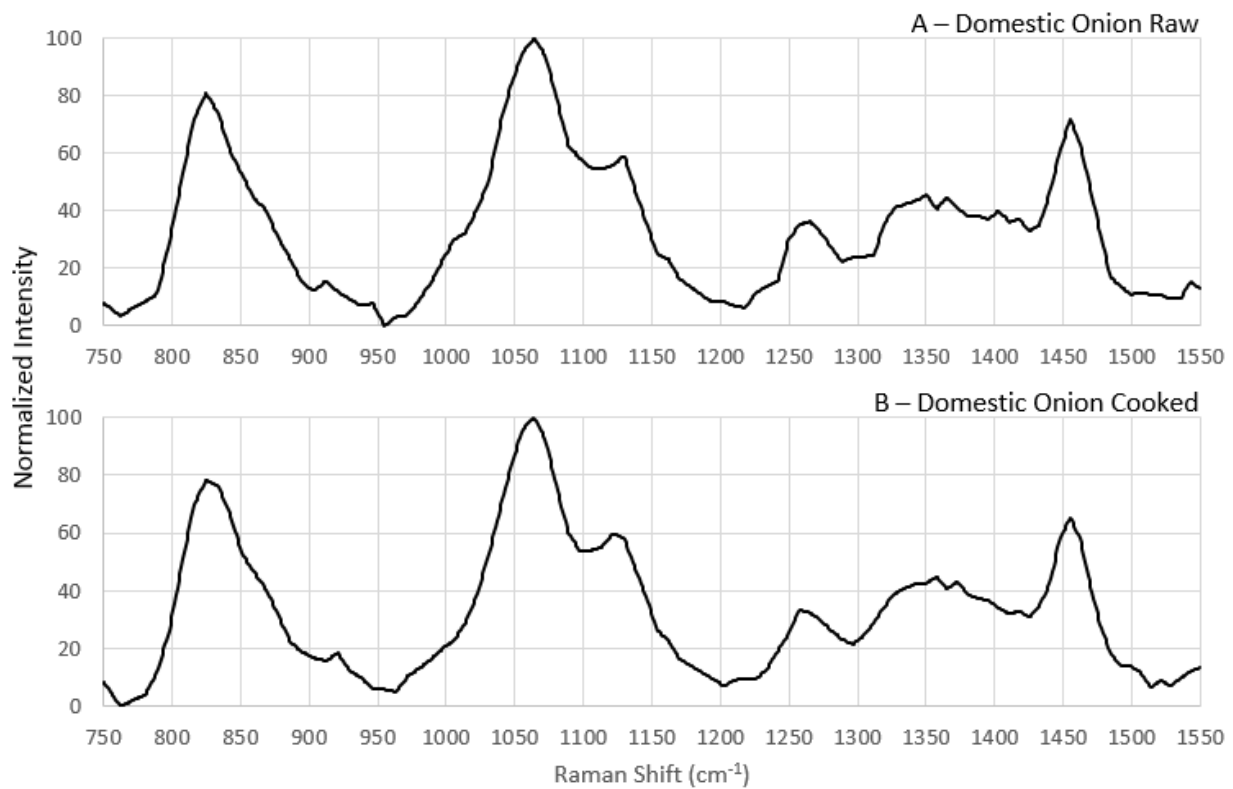
**Figure 38 Raman spectra of modern domestic onion. A: Raw; B: Cooked**
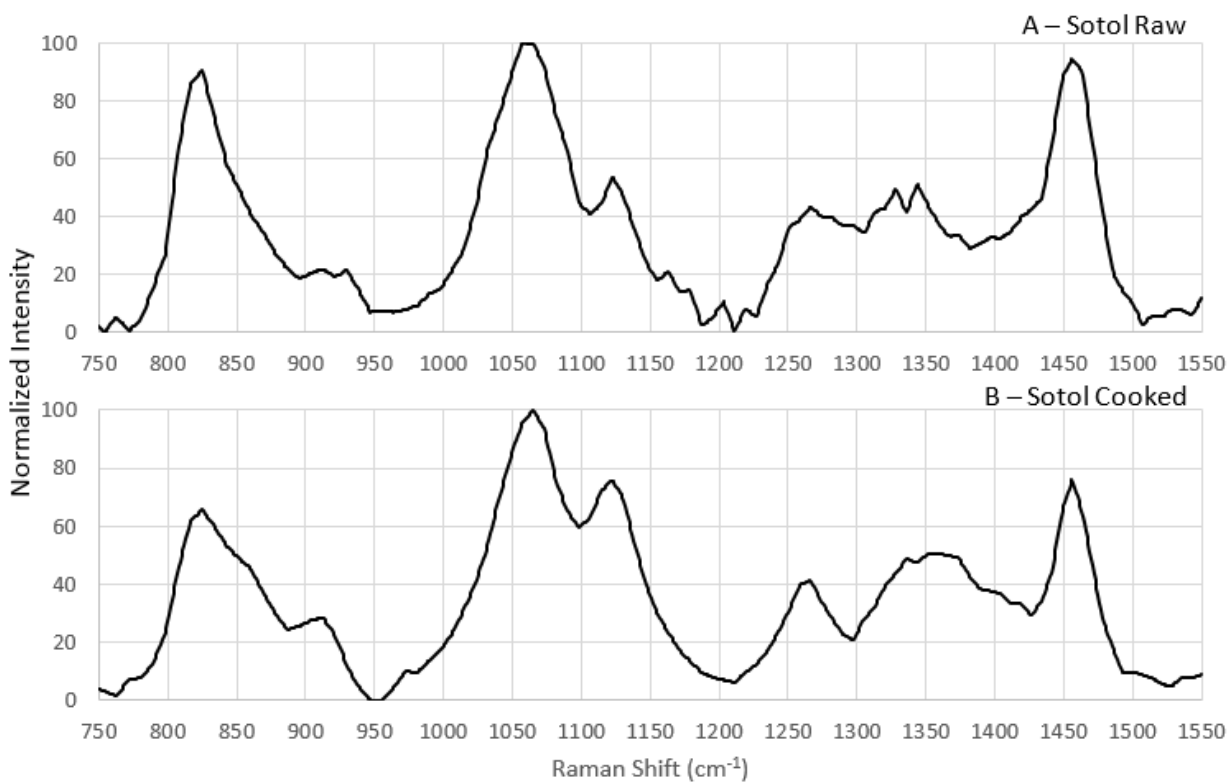
**Figure 39 Raman spectra of modern sotol. A: Raw; B: Cooked**
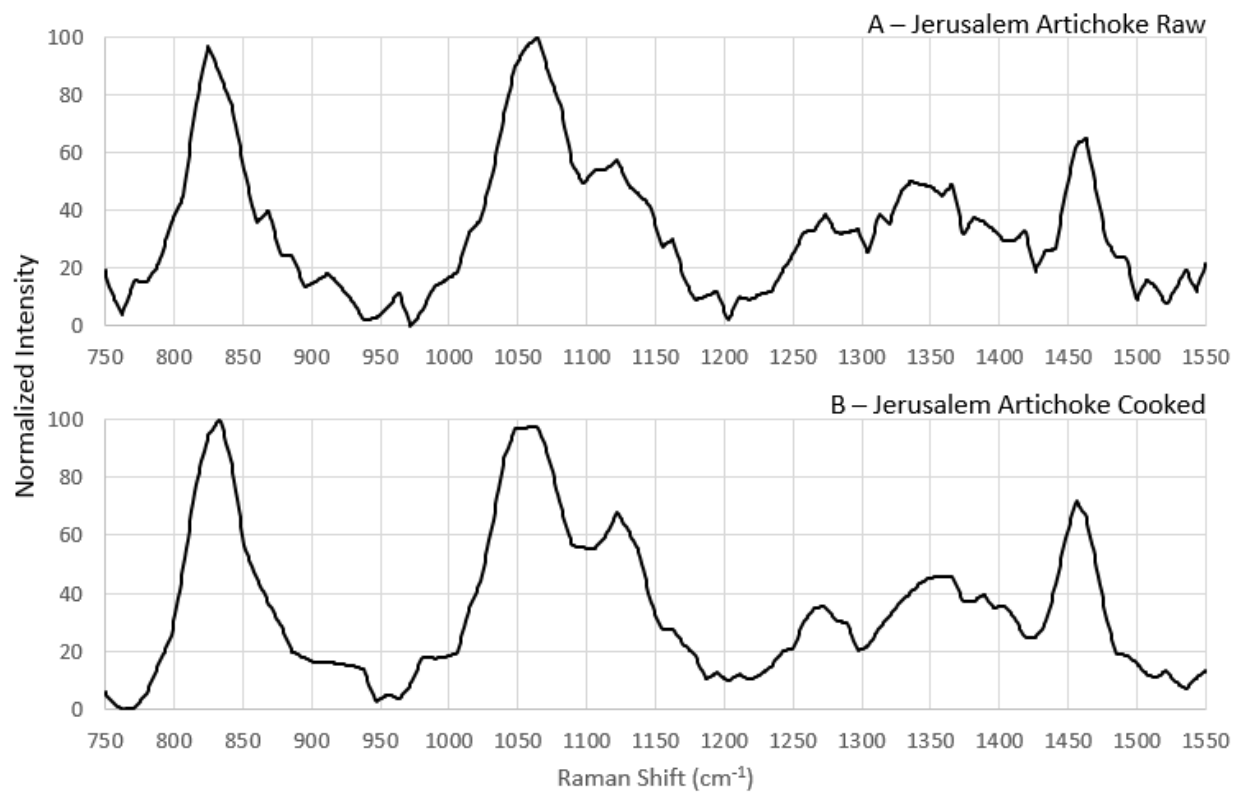
**Figure 40 Raman spectra of modern jerusalem artichoke. A: Raw; B: Cooked**
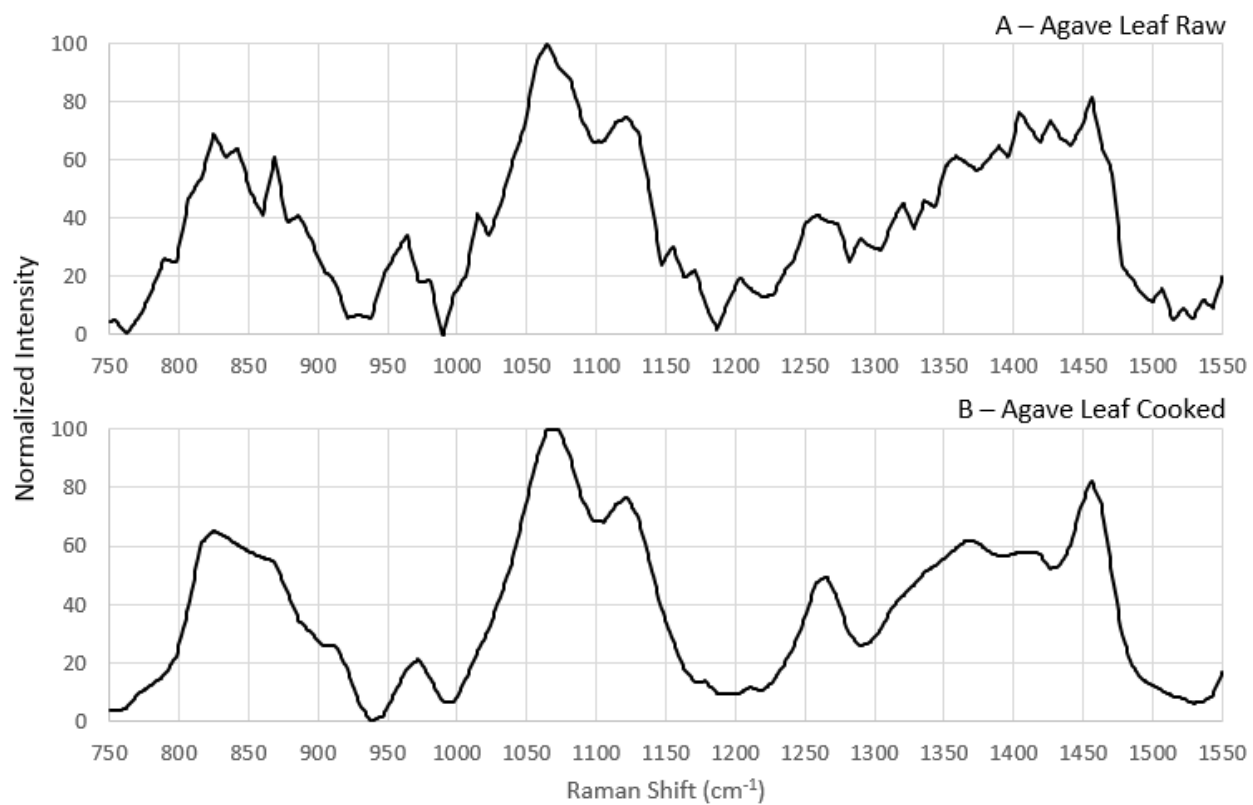
**Figure 41 Raman spectra of modern agave leaf. A: Raw; B: Cooked**
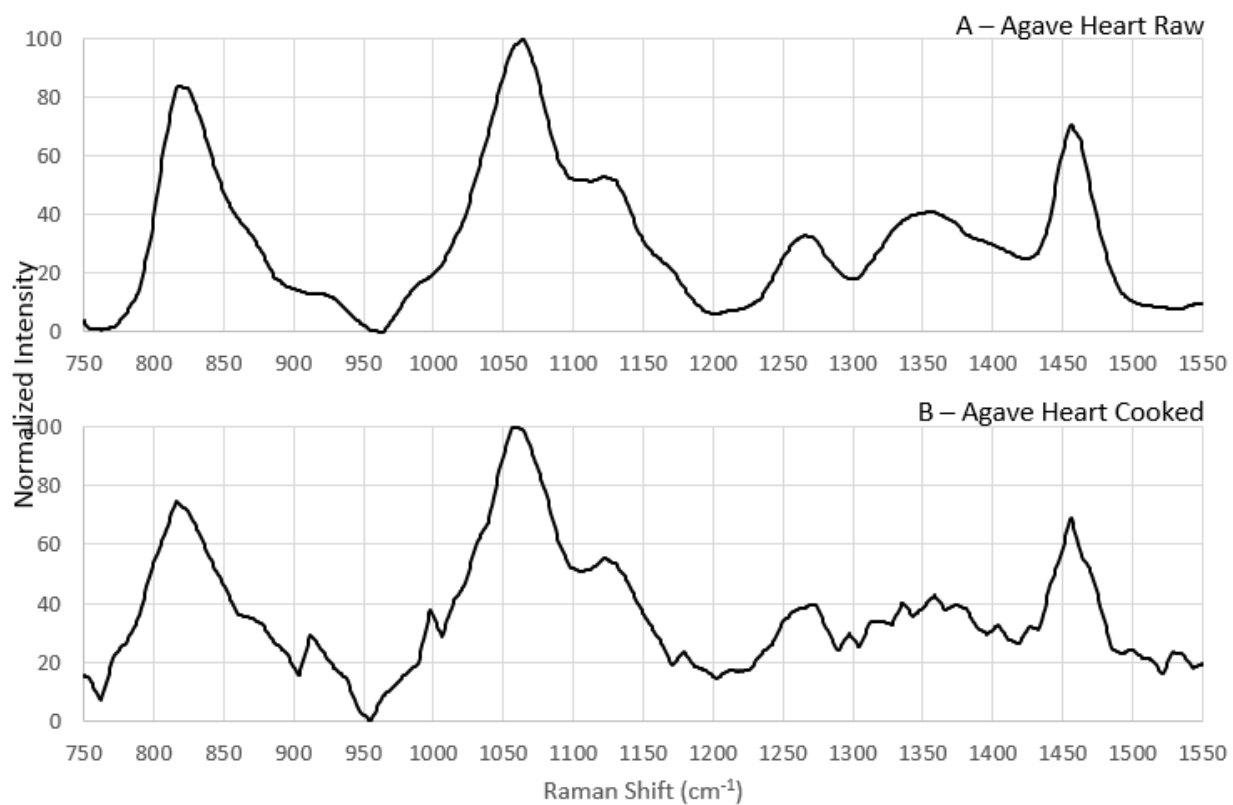
**Figure 42 Raman spectra of modern agave heart. A: Raw; B: Cooked**
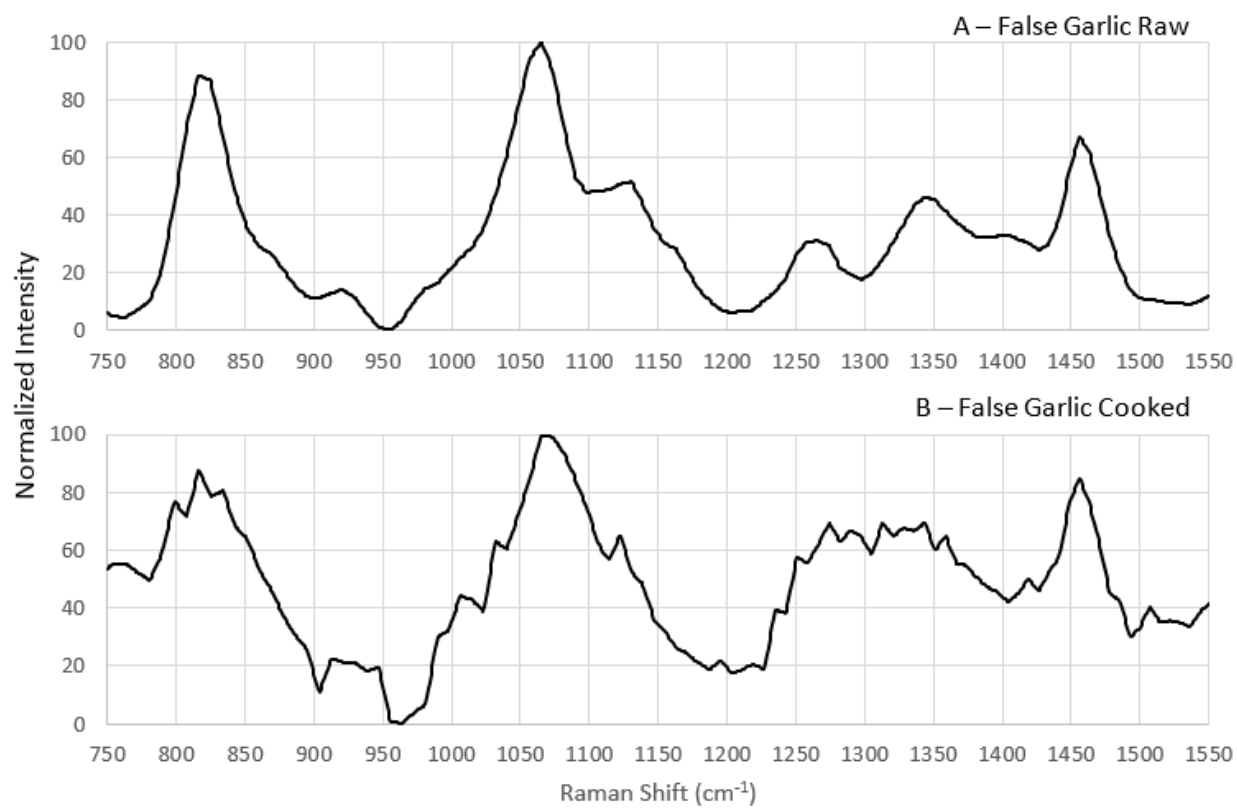
**Figure 43 Raman spectra of modern false garlic. A: Raw; B: Cooked**
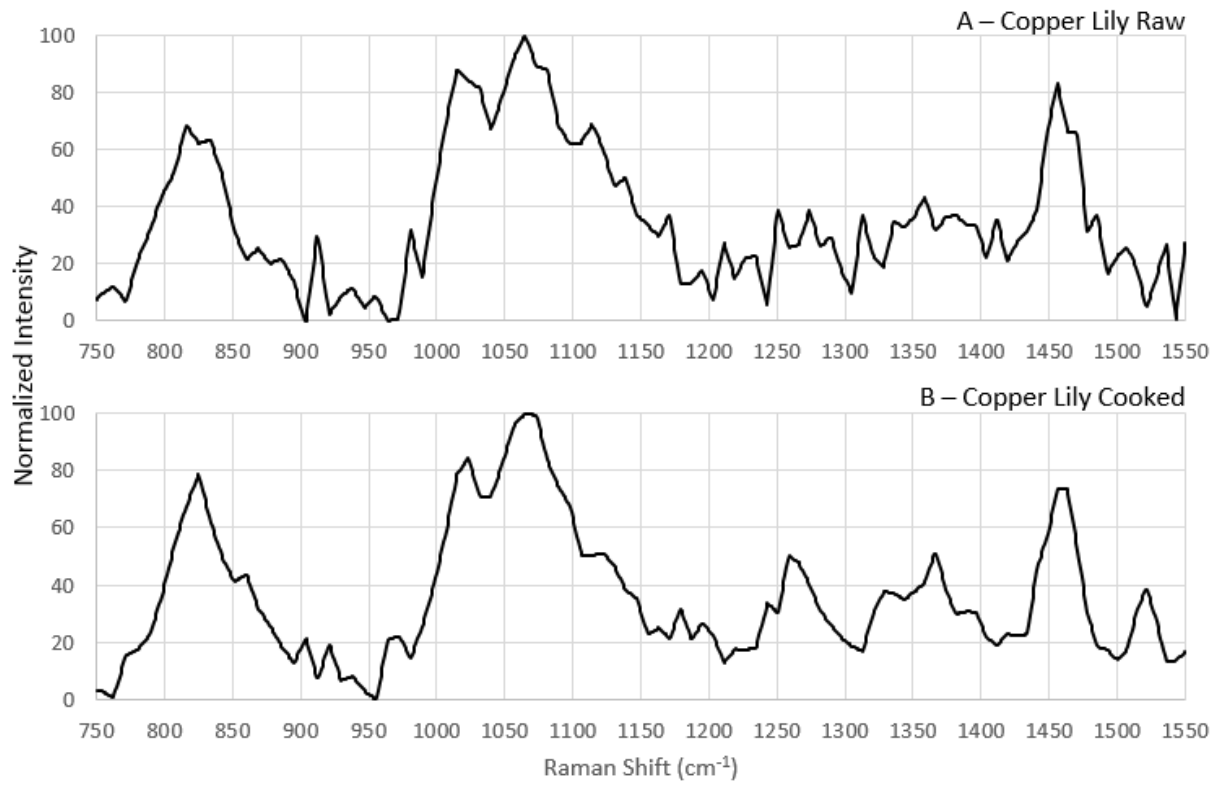
**Figure 44 Raman spectra of modern copper lily. A: Raw; B: Cooked**
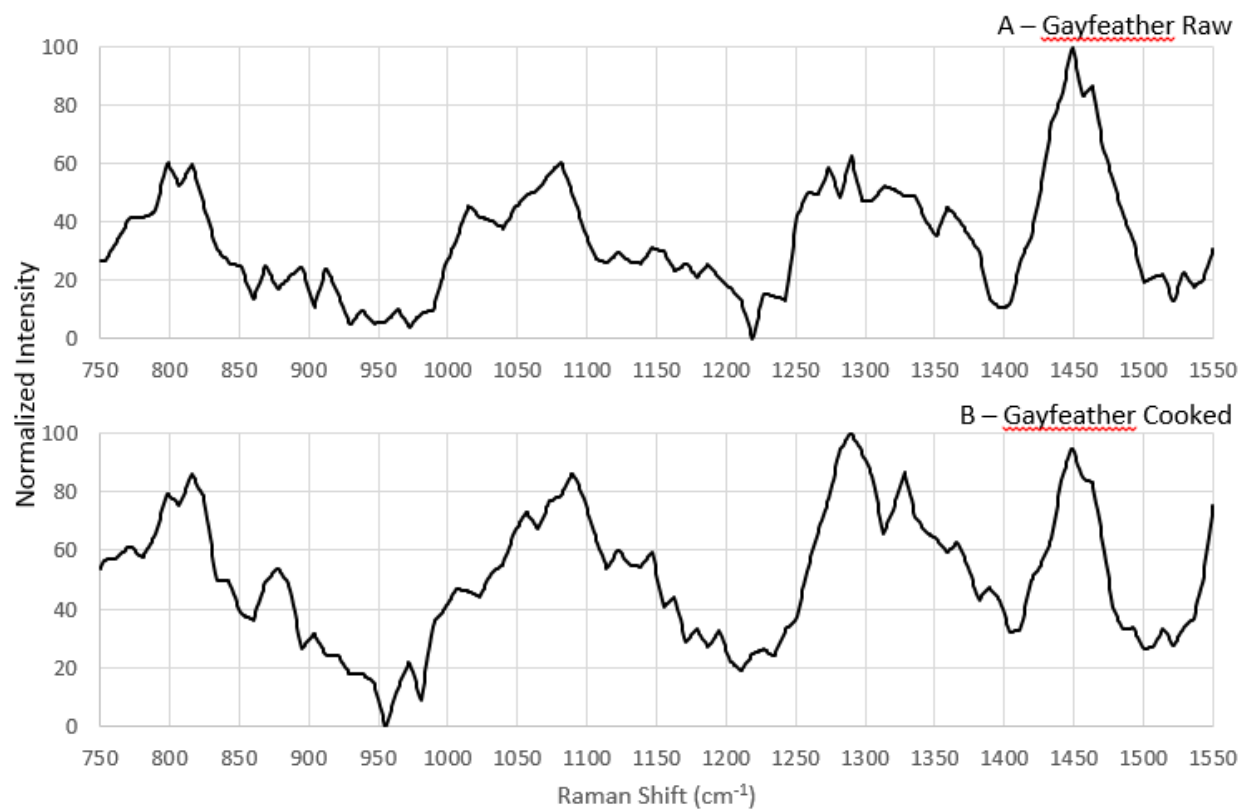
**Figure 45 Raman spectra of modern gayfeather. A: Raw; B: Cooked**
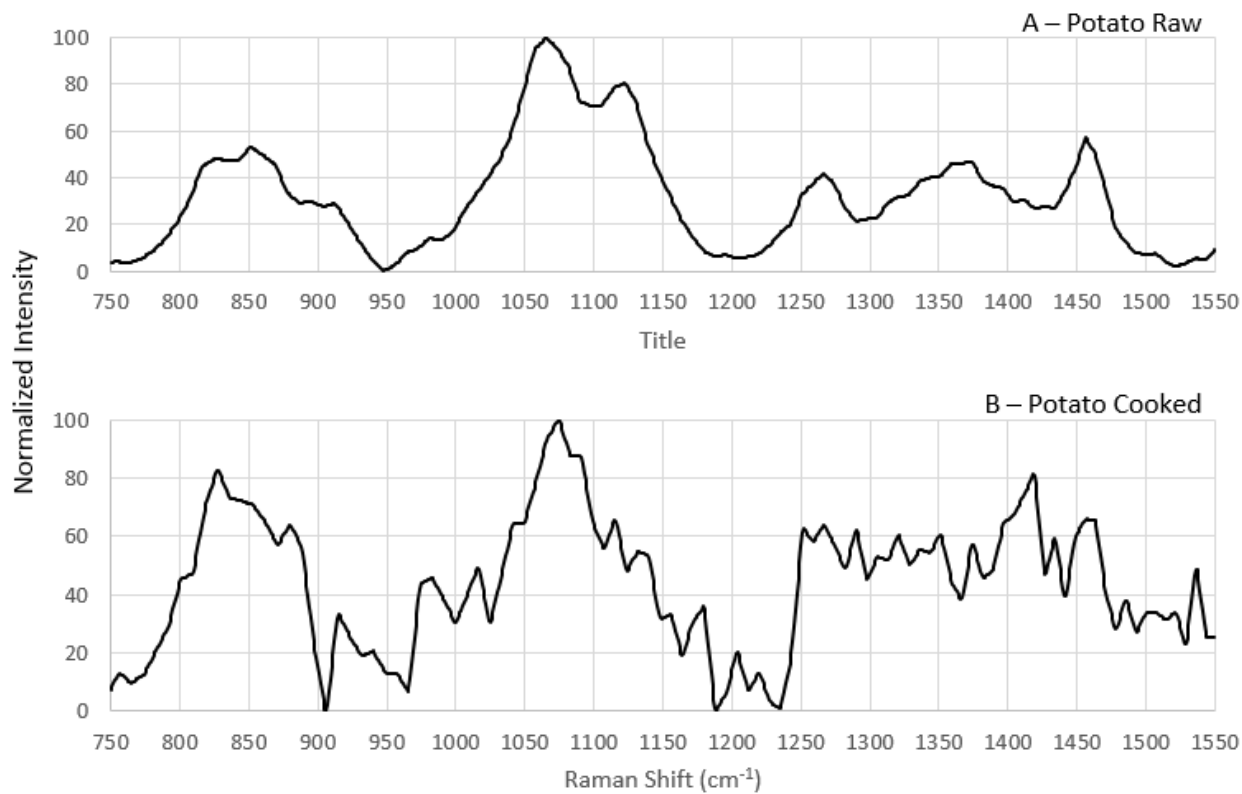
**Figure 46 Raman spectra of modern domestic potato. A: Raw; B: Cooked**
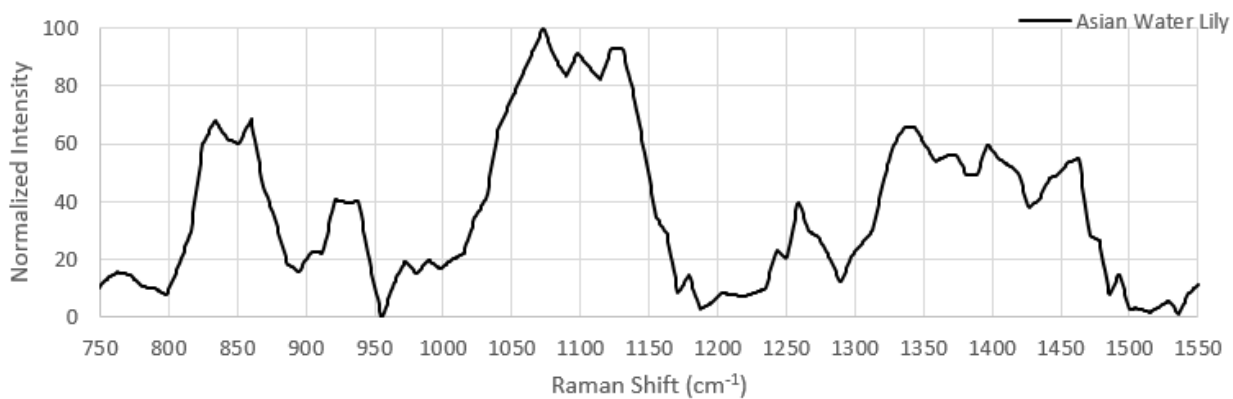


**Figure 47 Raman spectra of modern Asian water lily. A: Raw; B: Cooked**
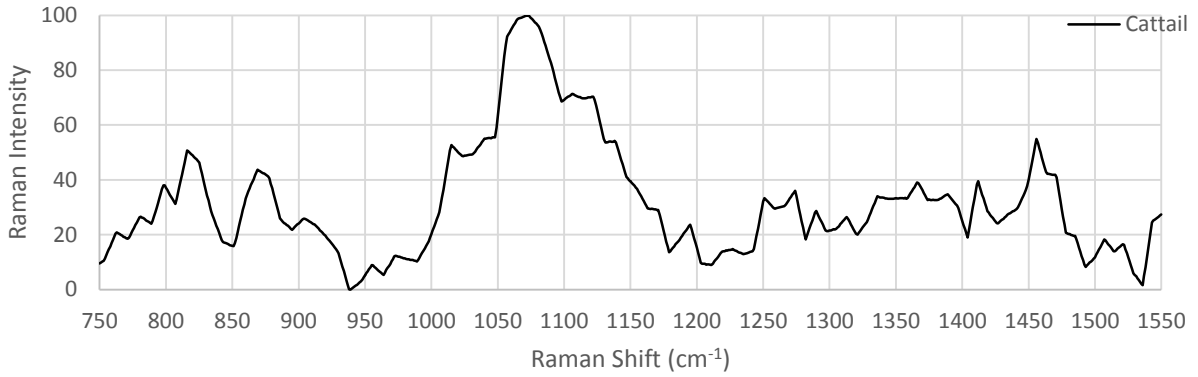
**Figure 48 Raman spectra of modern cattail. A: Raw; B: Cooked**

MODERN MEAT REFERENCE SAMPLES
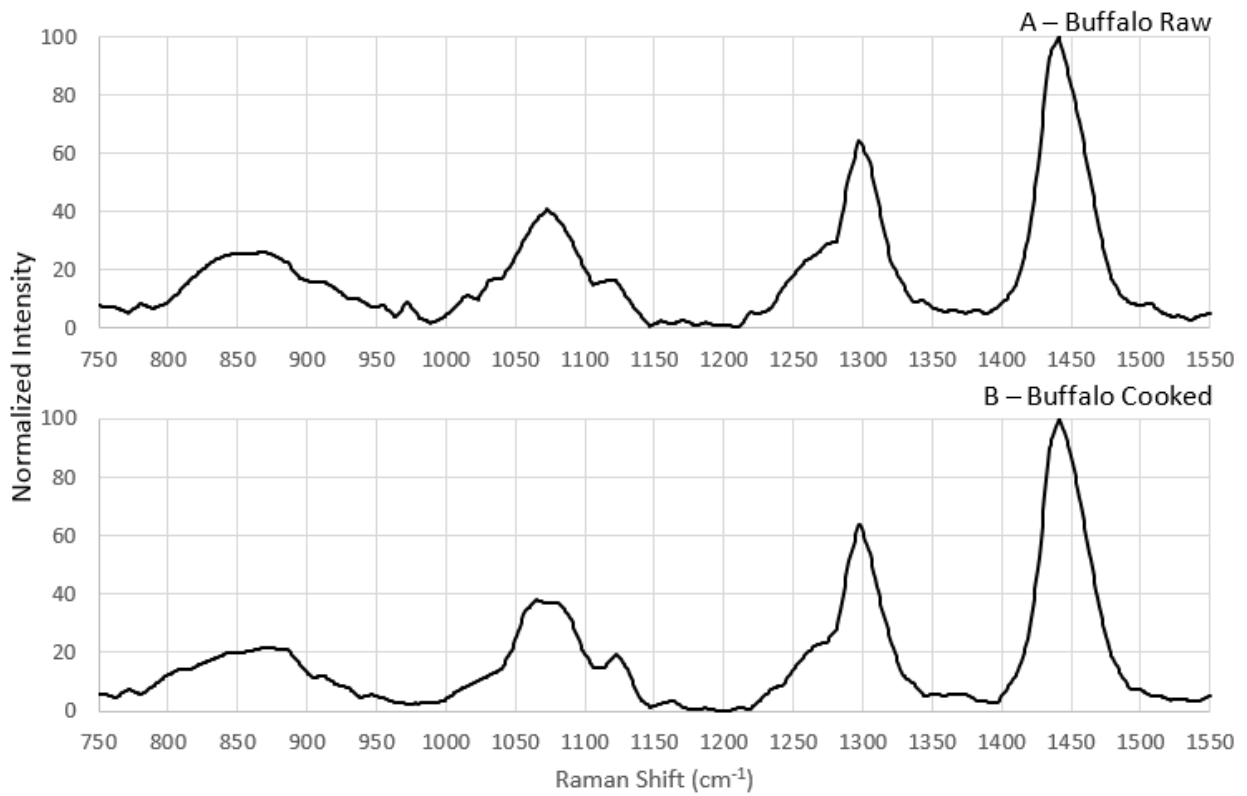


**Figure 49 Raman spectra of modern buffalo. A: Raw; B: Cooked**
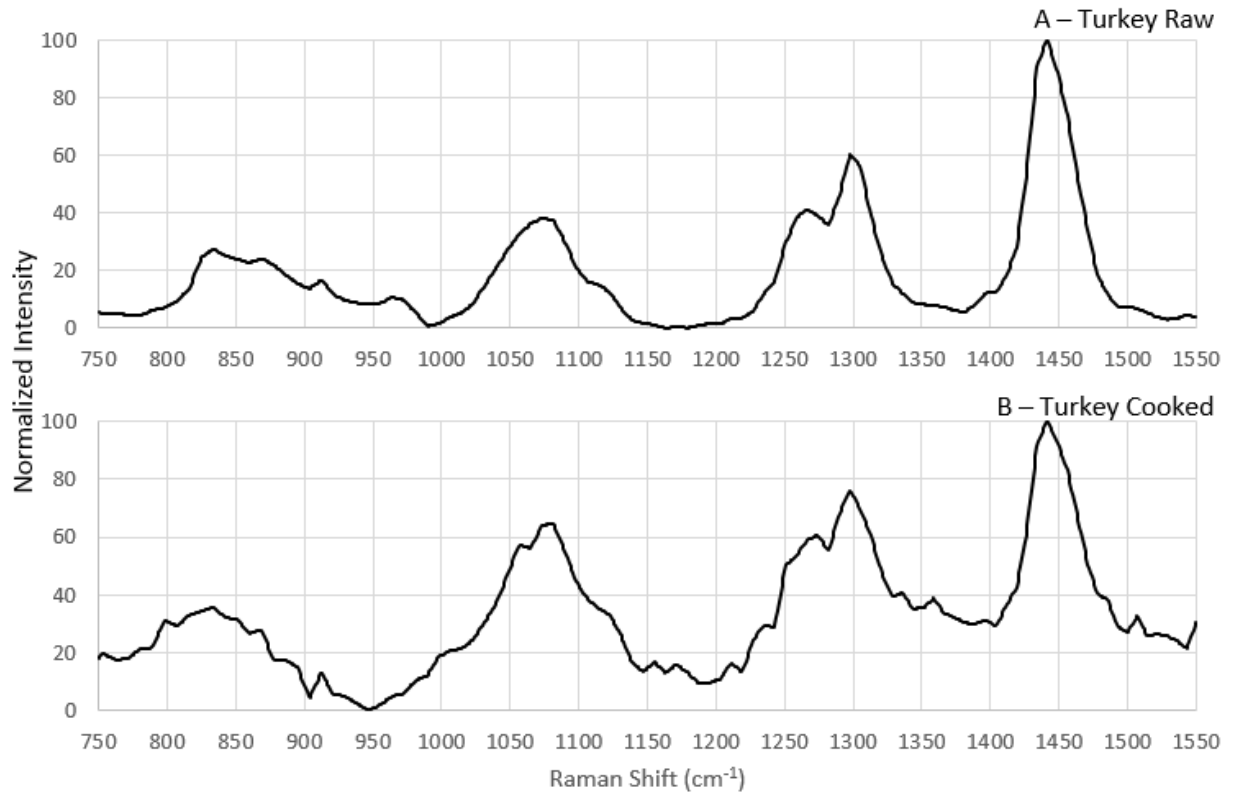
**Figure 50 Raman spectra of modern turkey. A: Raw; B: Cooked**

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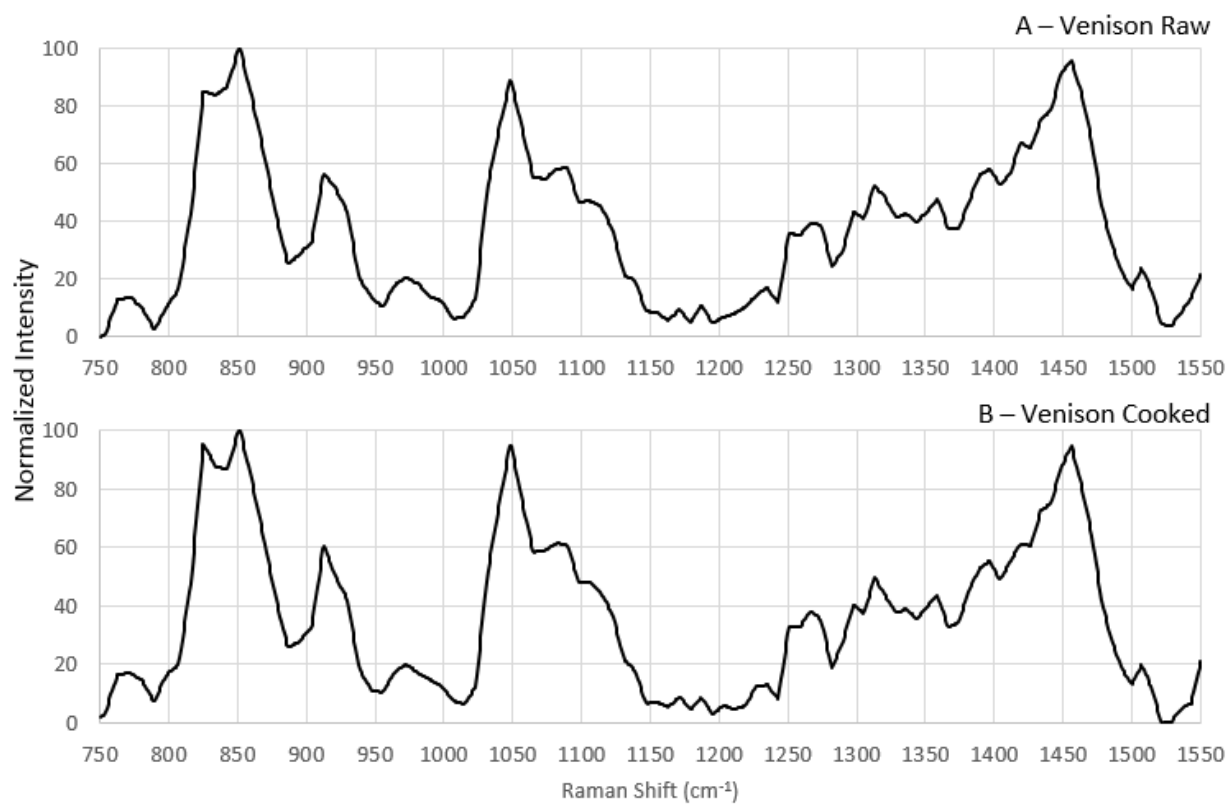
**Figure 51 Raman spectra of modern venison. A: Raw; B: Cooked**

ARCHAEOLOGICAL INTACT BOTANICAL (MACROBOTANICAL) REFERENCE
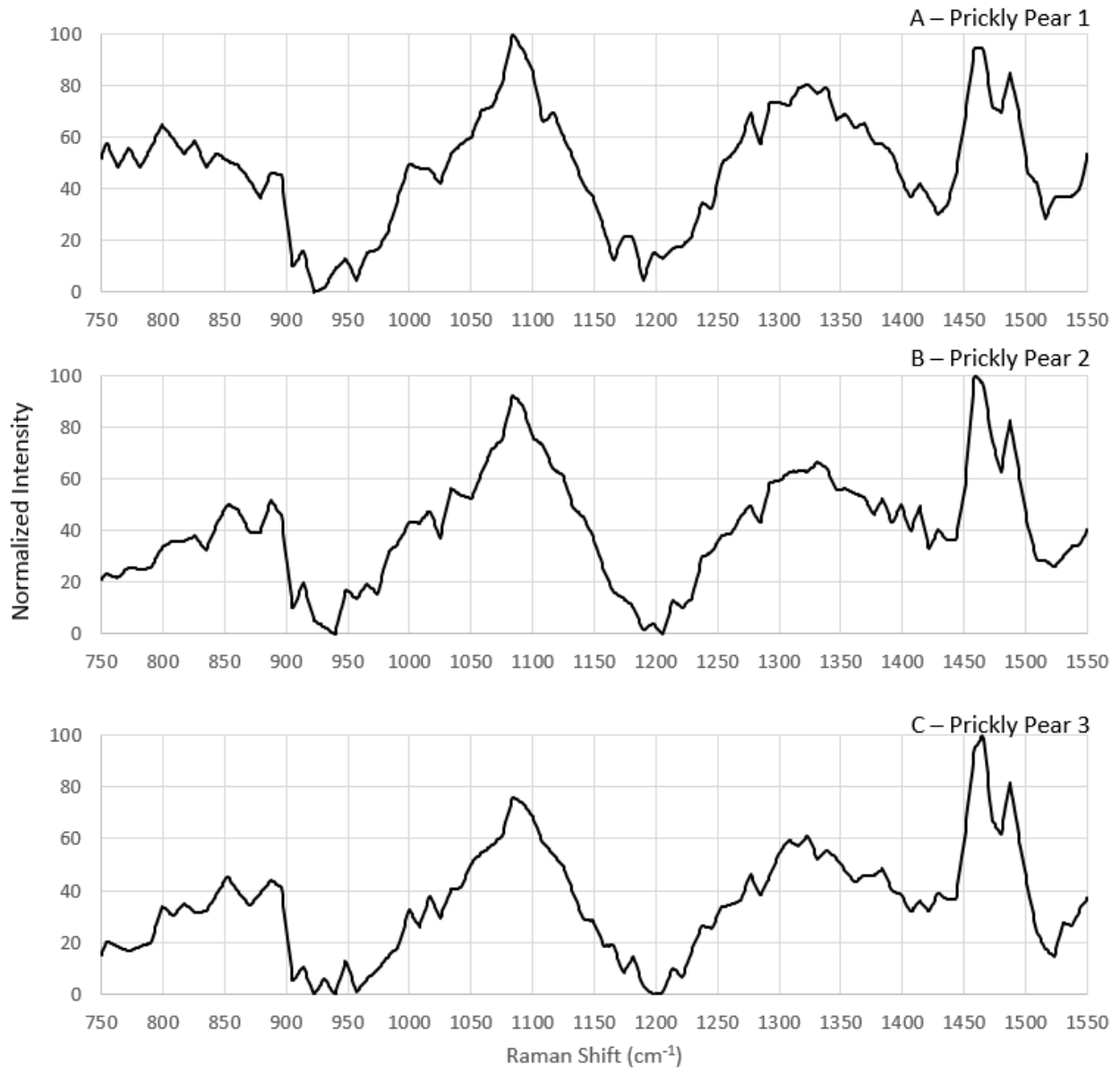
SAMPLES



**Figure 52: Raman spectra from archaeological macrobotanical samples of prickly pear pad from Hinds Cave**
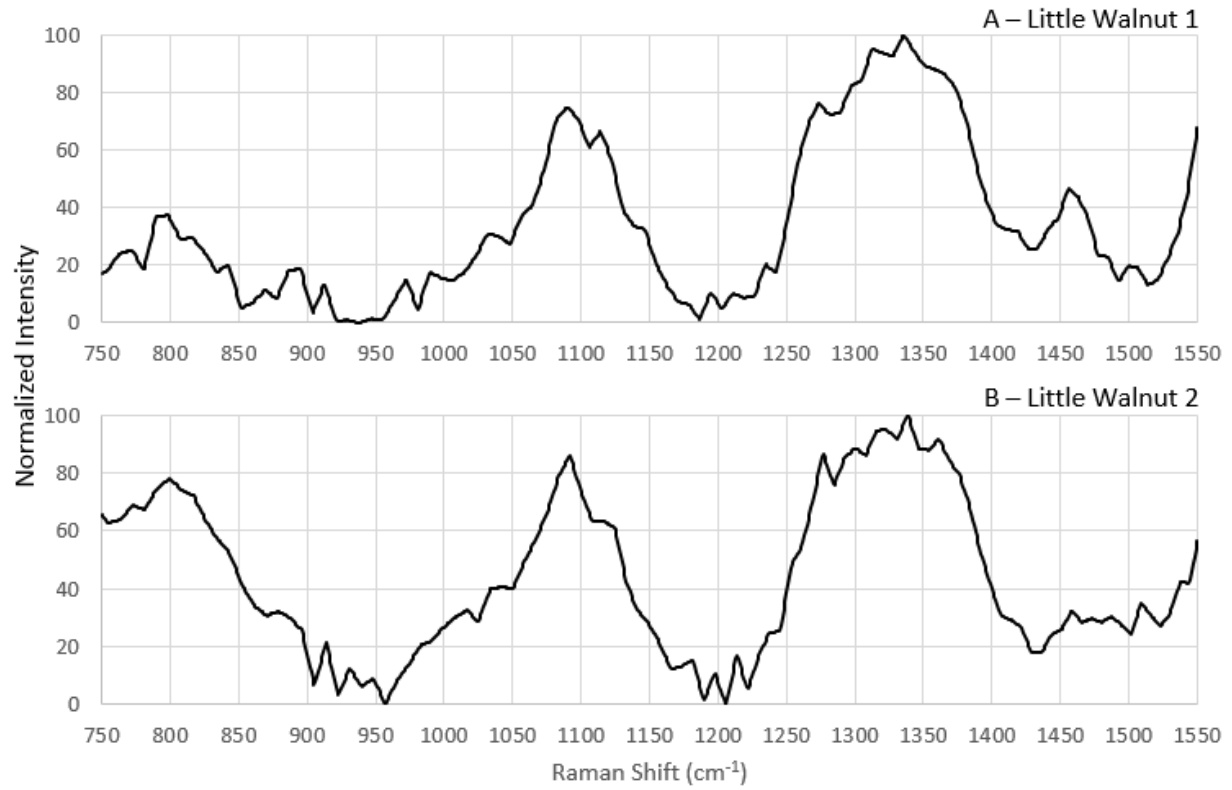
**Figure 53: Raman spectra from archaeological macrobotanical samples of little walnut shell from Hinds Cave**
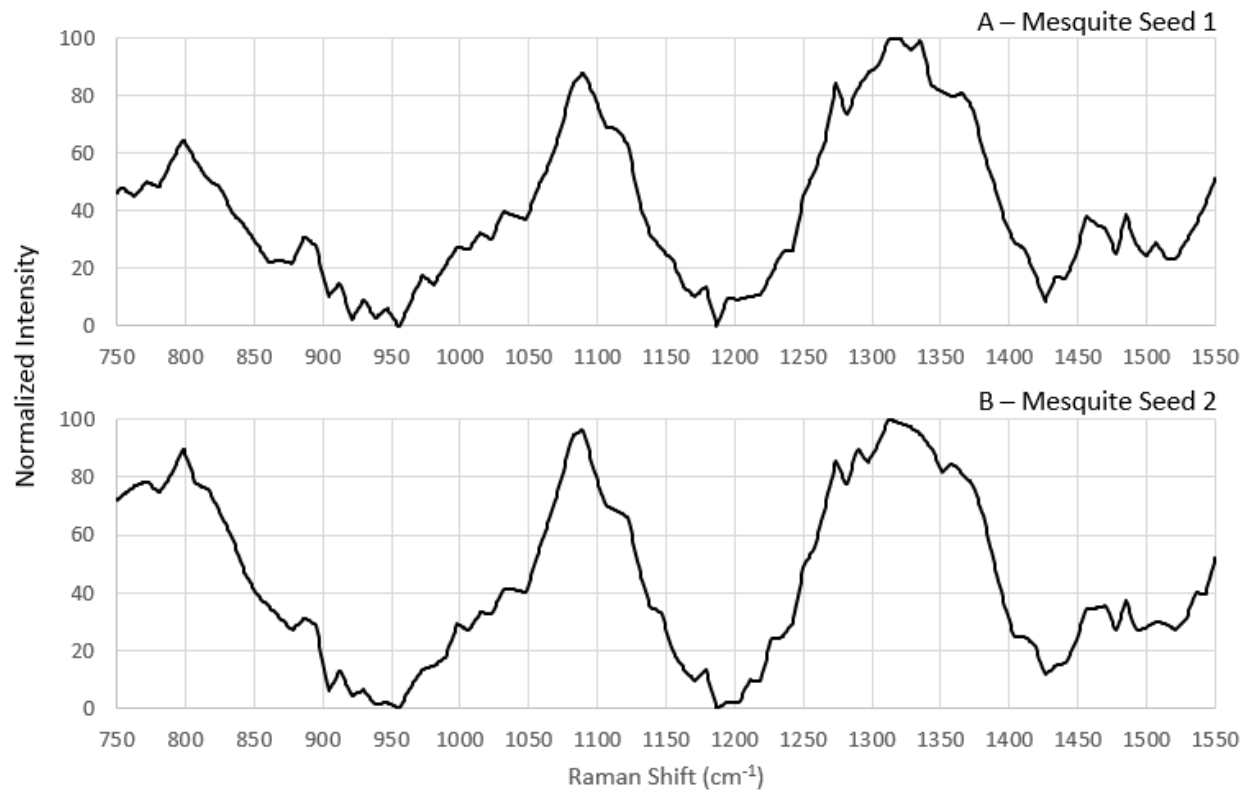
**Figure 54: Raman spectra from archaeological macrobotanical samples of mesquite seed from Hinds Cave**
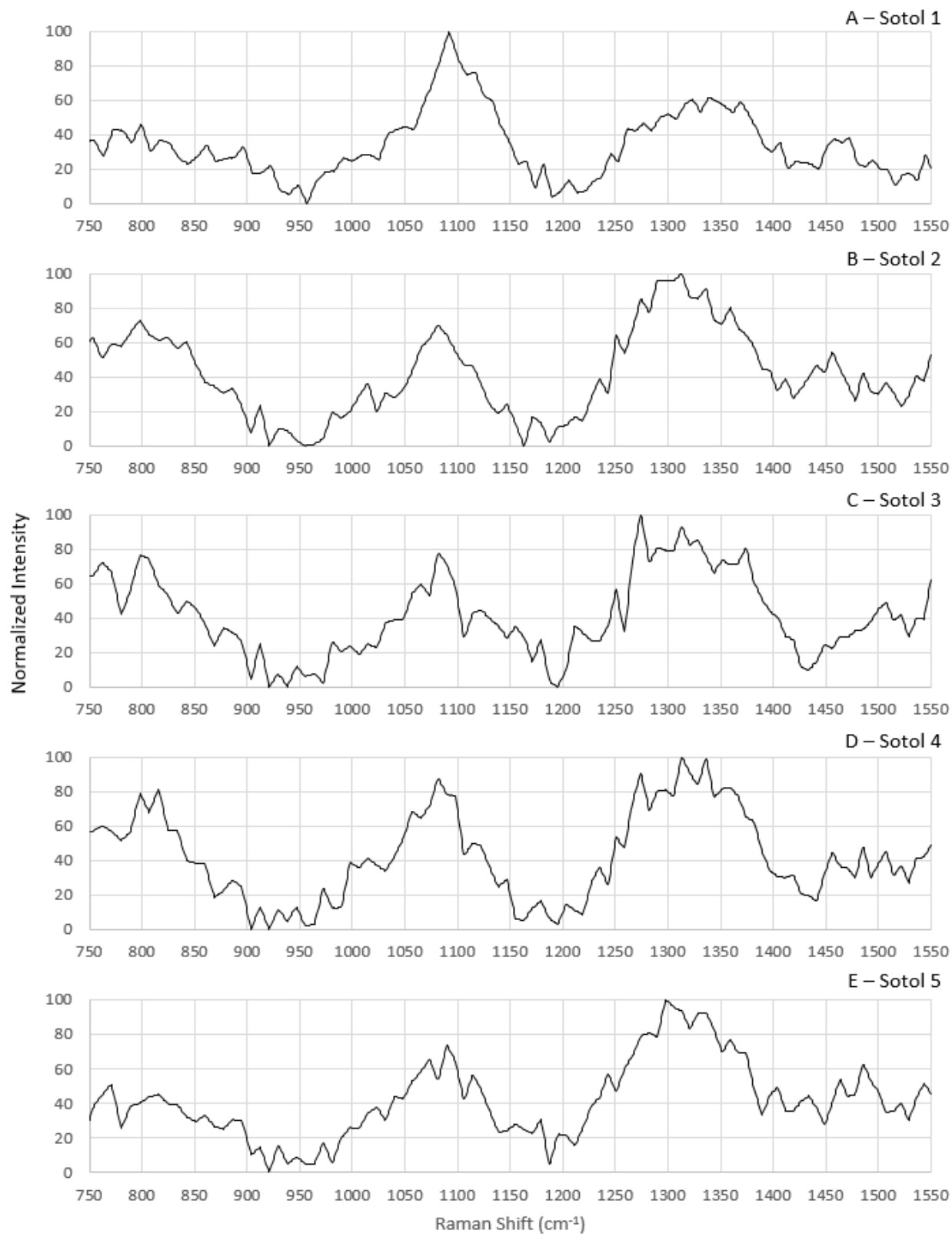
**Figure 55: Raman spectra from archaeological macrobotanical samples of sotol from Hinds Cave**
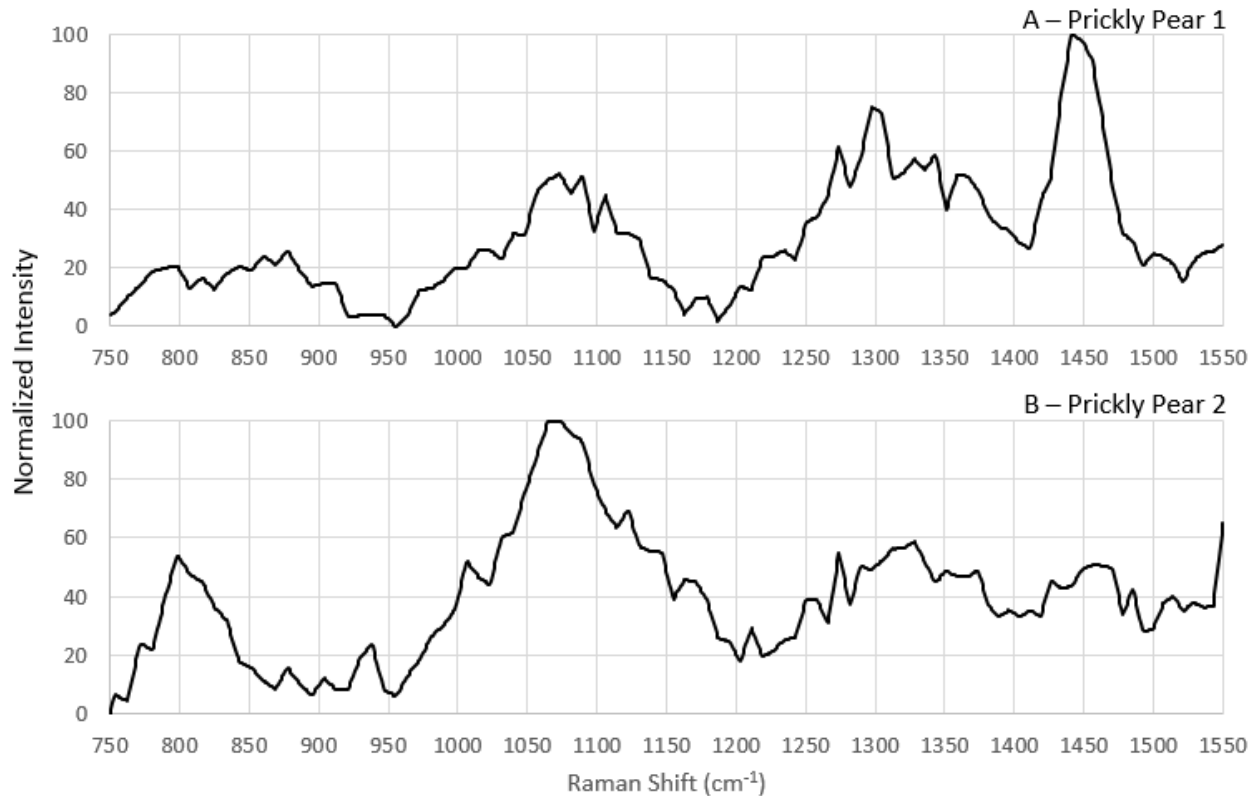
**Figure 56: Raman spectra from extractions of prickly pear from Hinds Cave**
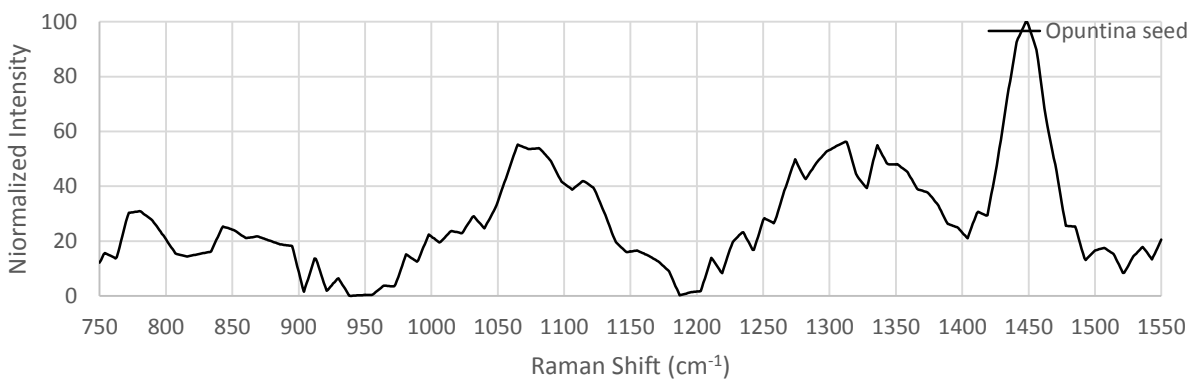


**Figure 57: Raman spectra from extractions of Opuntina seed from Hinds Cave**
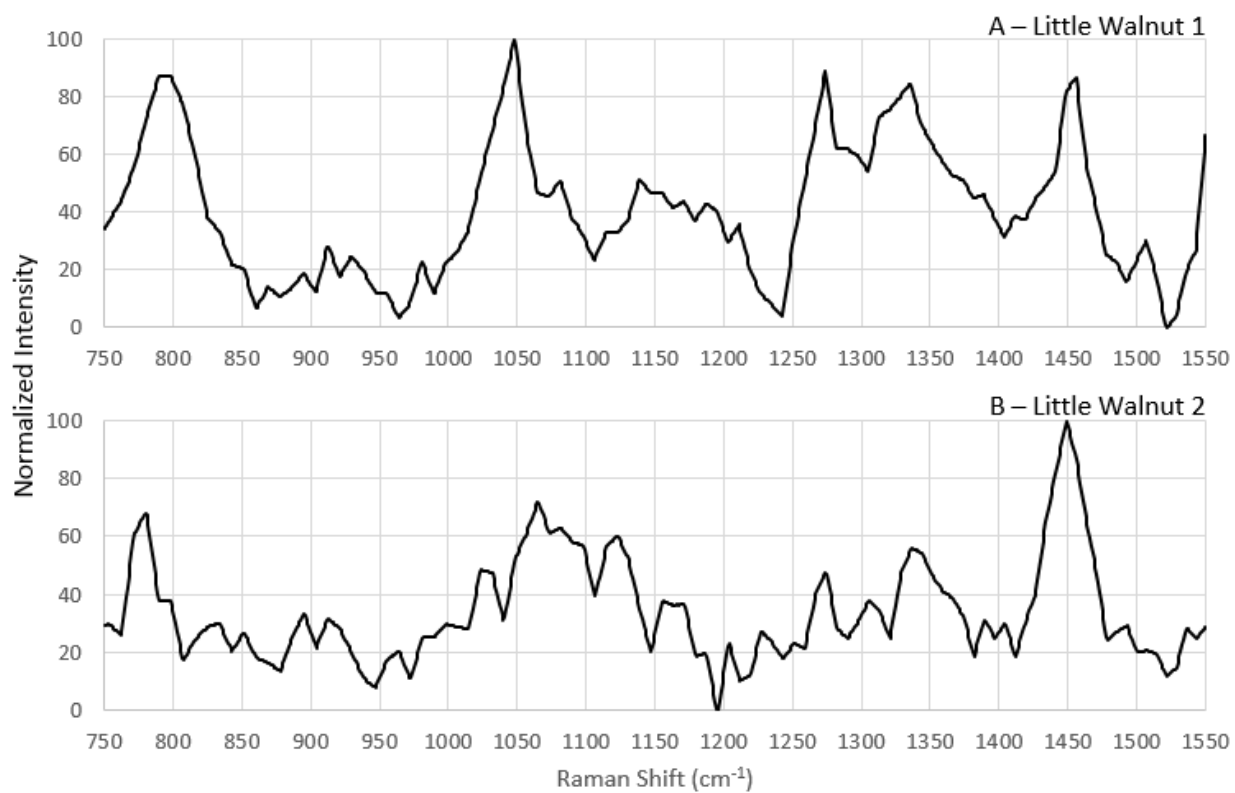
**Figure 58: Raman spectra from extractions of little walnut from Hinds Cave**
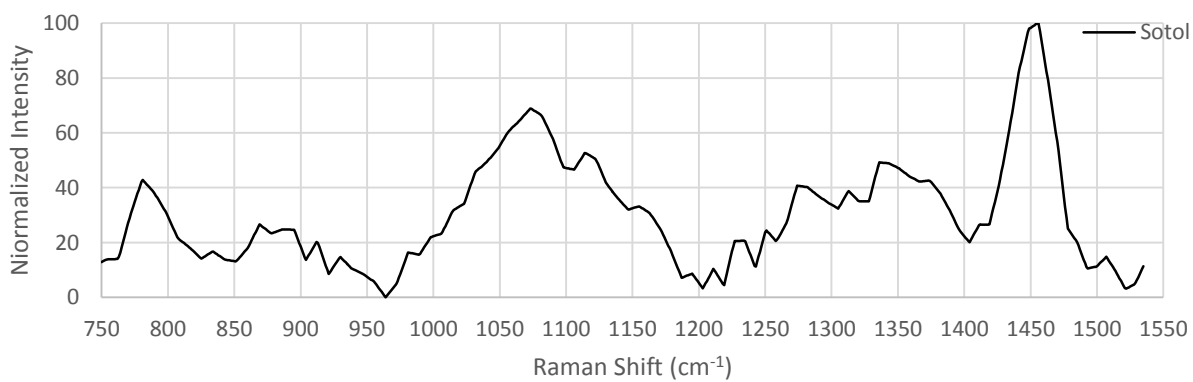


**Figure 59: Raman spectra from extractions of sotol from Hinds Cave**
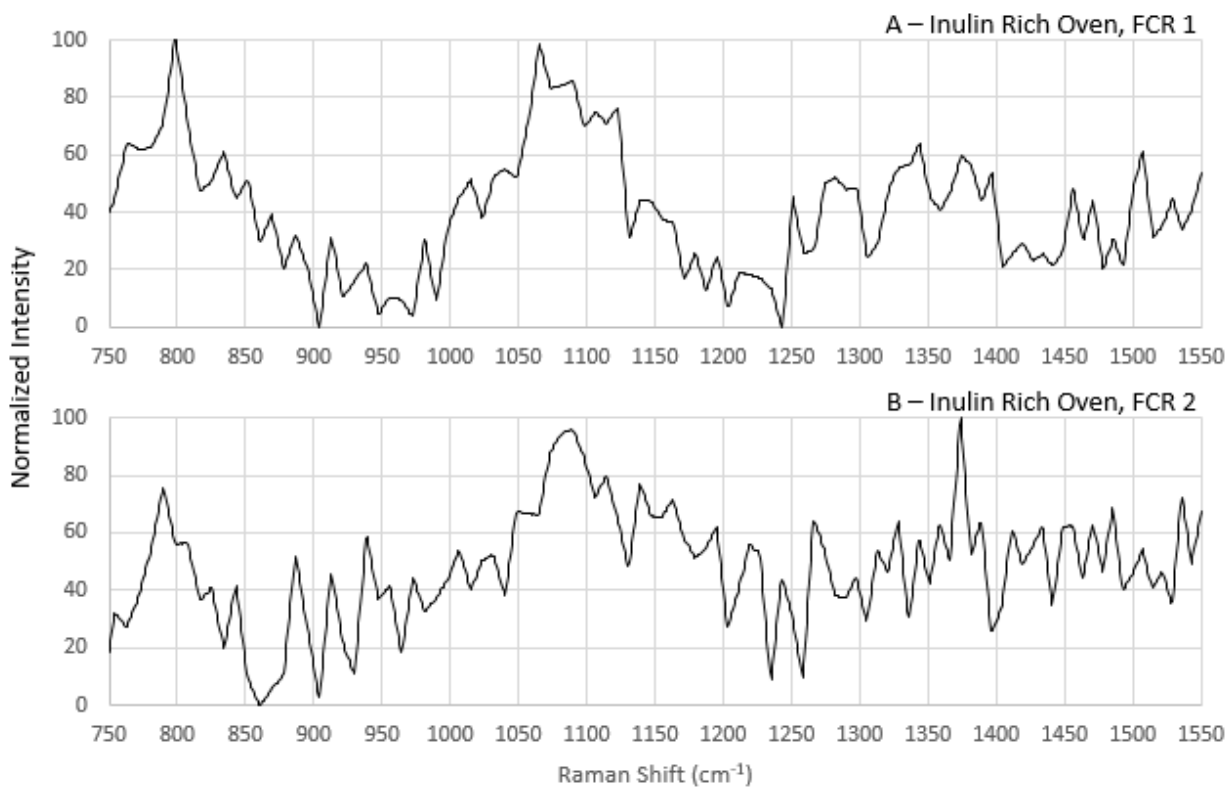
**Figure 60: Raman spectra of FCR samples from inulin-rich actualistic experimental oven**
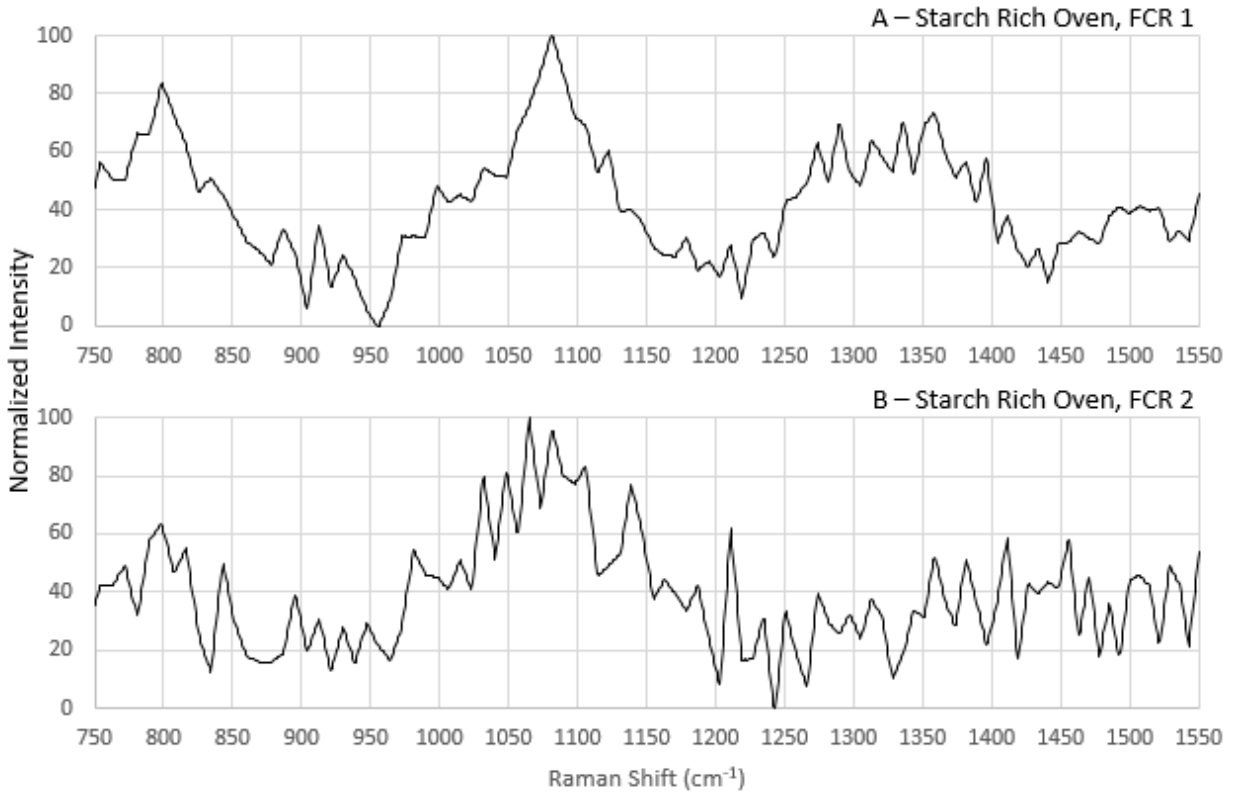
**Figure 61: Raman spectra of FCR samples from starch-rich actualistic experimental oven**
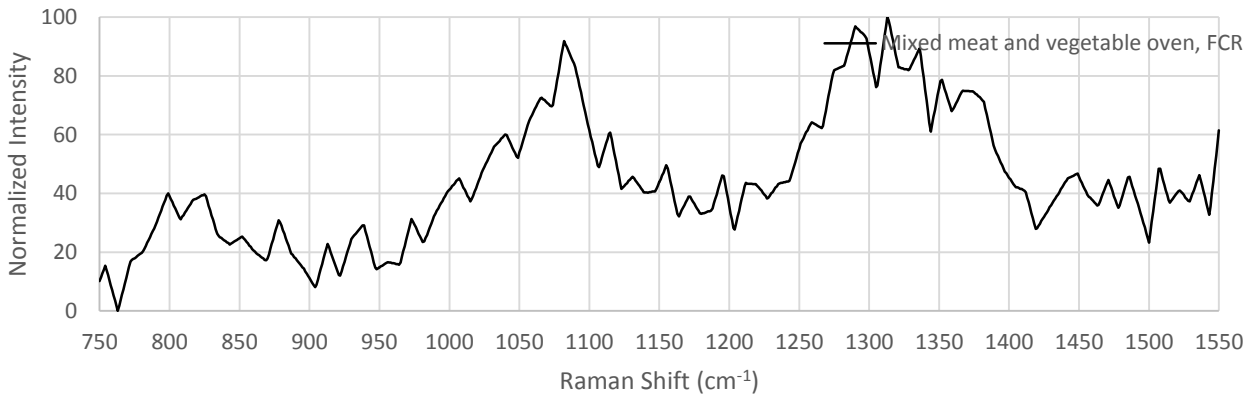


**Figure 62: Raman spectra of FCR samples from mixed meat and vegetable actualistic experimental oven**

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FORT HOOD FCR RESIDUE SAMPLES
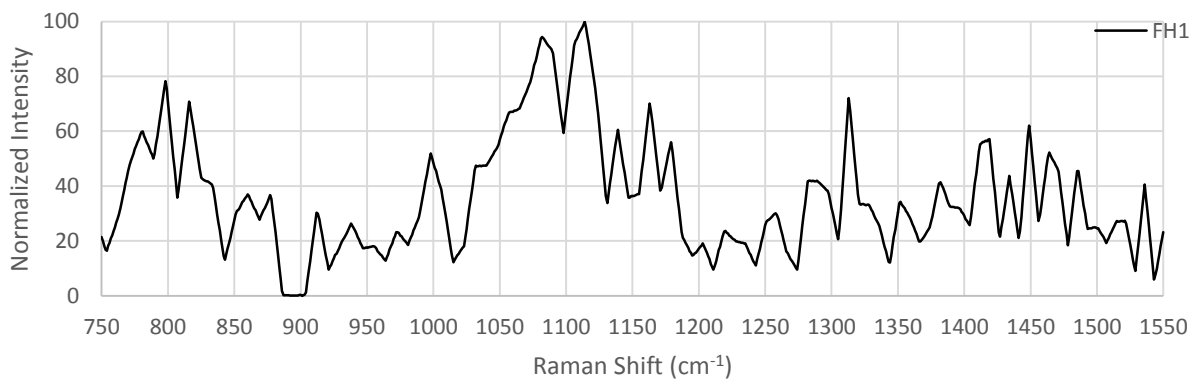


**Figure 63: Raman spectra of FCR from Fort Hood, sample 1**
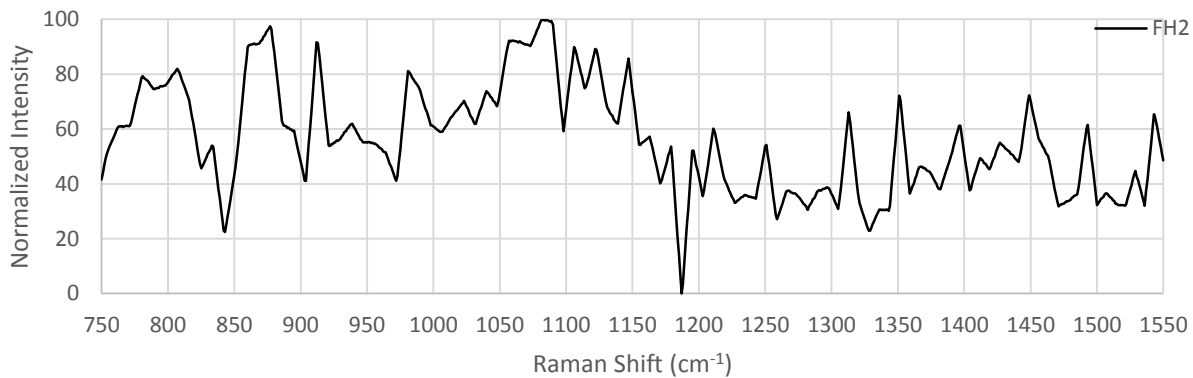


**Figure 64: Raman spectra of FCR from Fort Hood, sample 2**
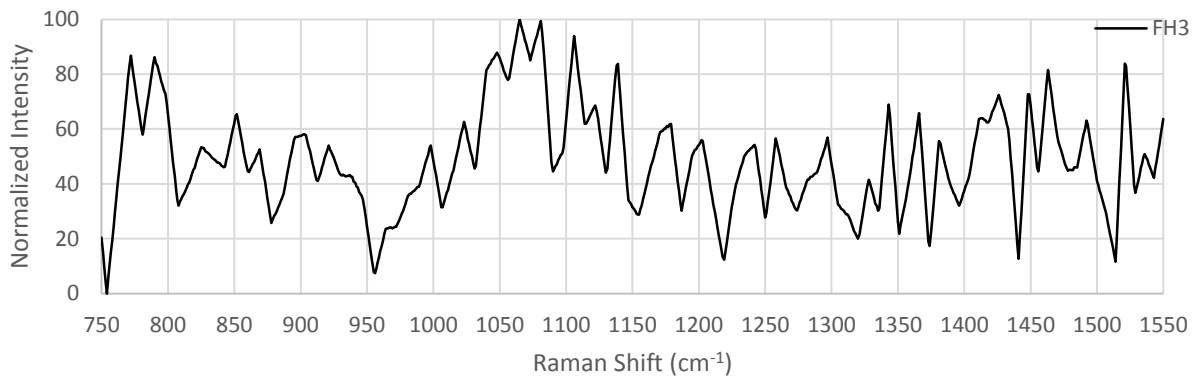
**Figure 65: Raman spectra of FCR from Fort Hood, sample 3**
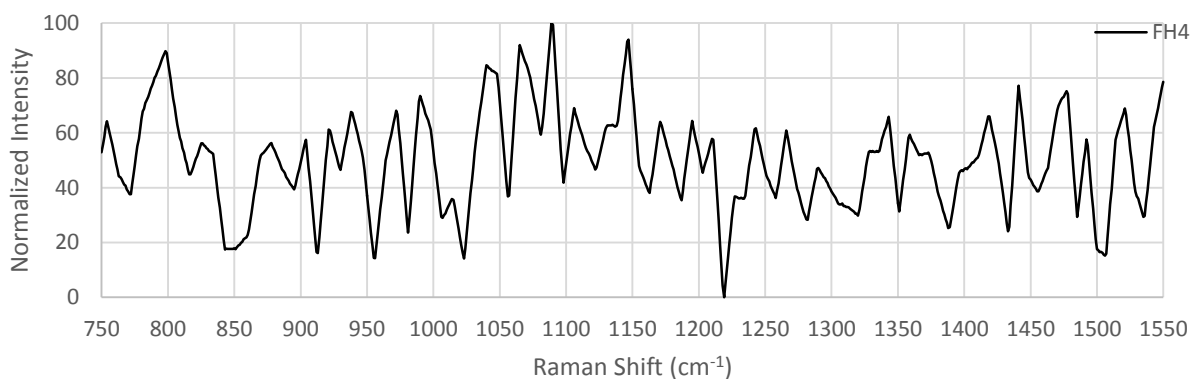


**Figure 66: Raman spectra of FCR from Fort Hood, sample 4**
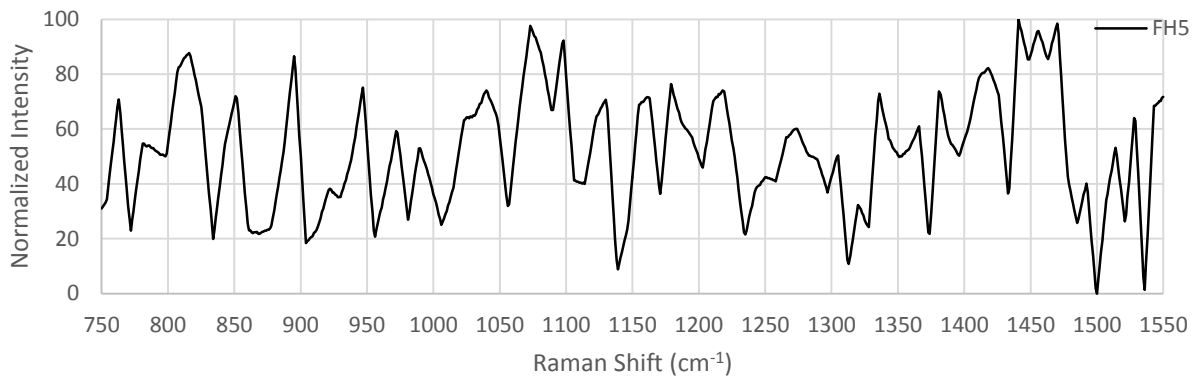
**Figure 67: Raman spectra of FCR from Fort Hood, sample 5**
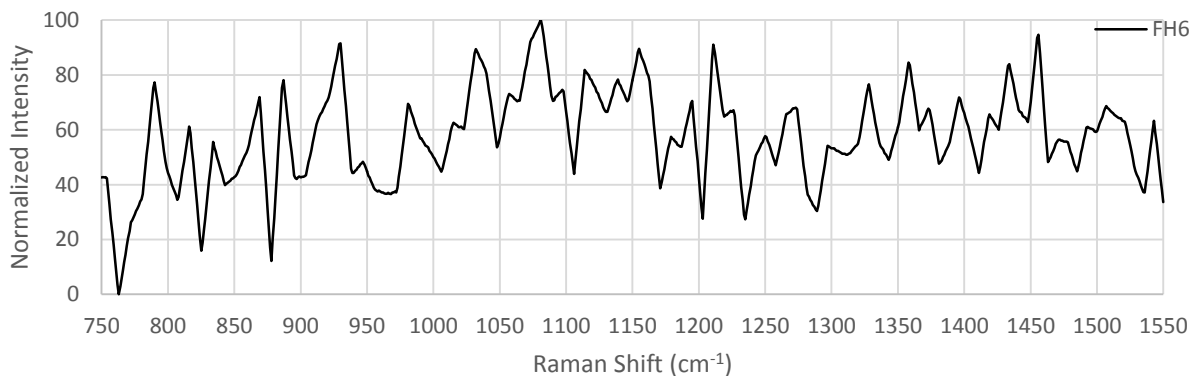


**Figure 68: Raman spectra of FCR from Fort Hood, sample 6**
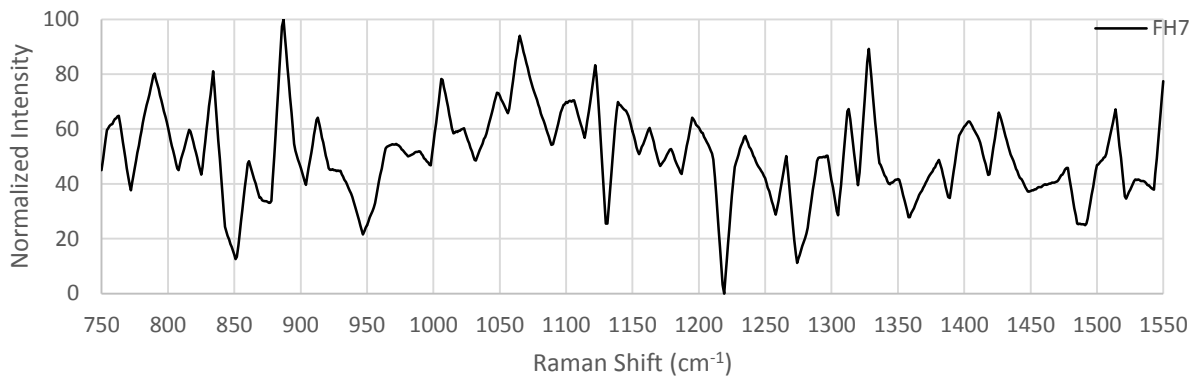
**Figure 69: Raman spectra of FCR from Fort Hood, sample 7**
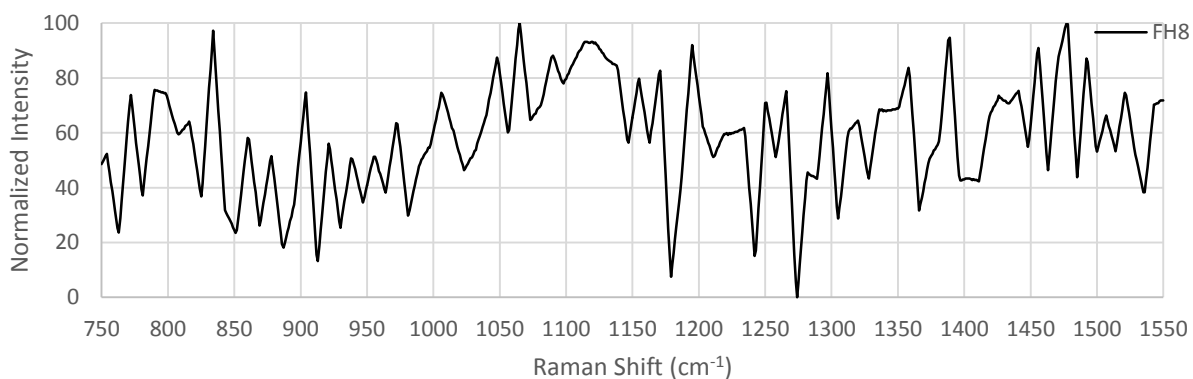


**Figure 70: Raman spectra of FCR from Fort Hood, sample 8**
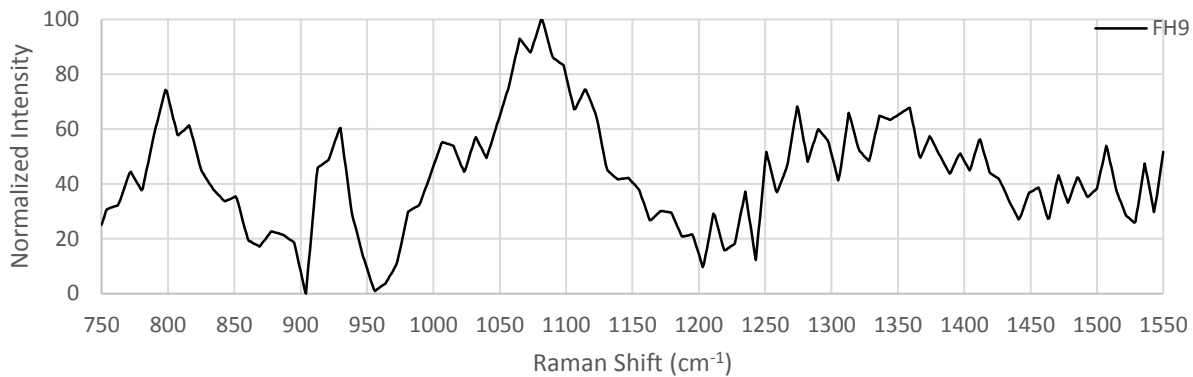
**Figure 71: Raman spectra of FCR from Fort Hood, sample 9**

LOWER PECOS FCR RESIDUE SAMPLES
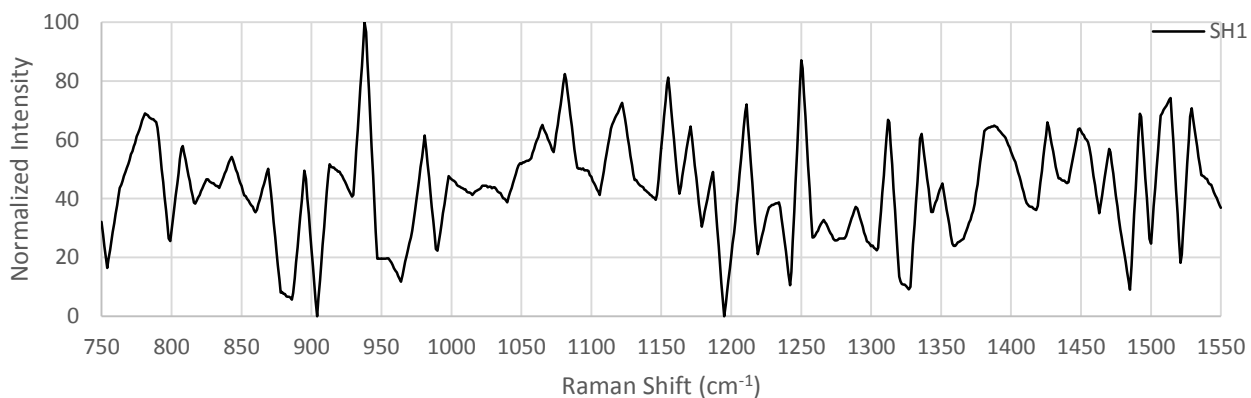


**Figure 72: Raman spectra of FCR from Lower Pecos, sample 1**
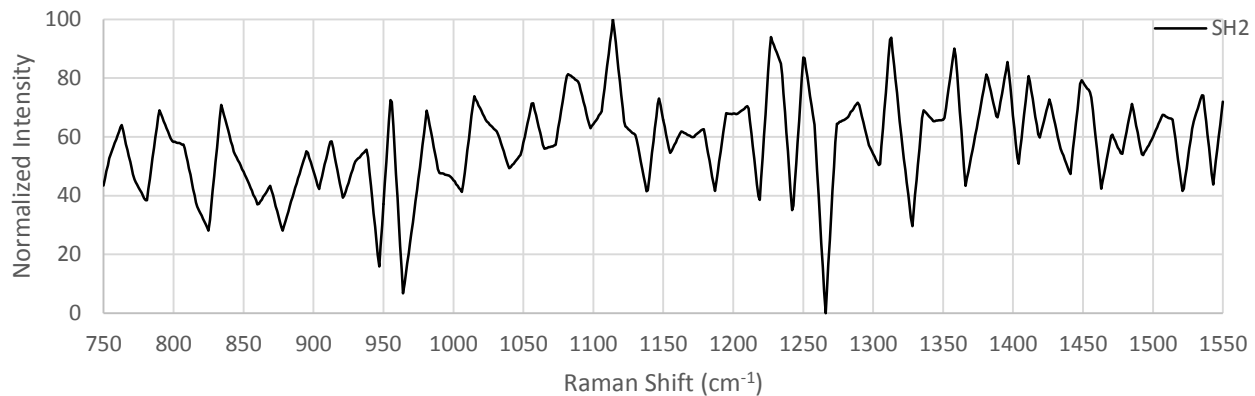
**Figure 73: Raman spectra of FCR from Lower Pecos, sample 2**
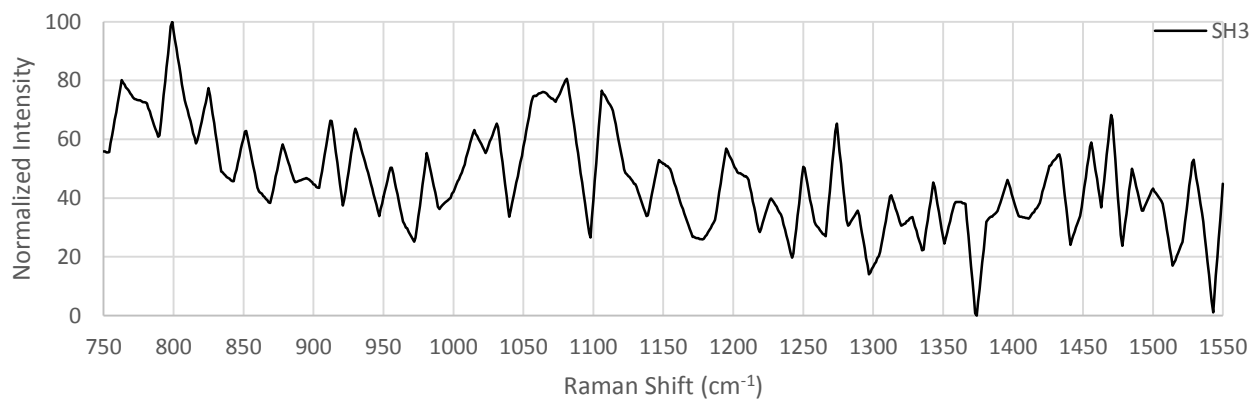


**Figure 74: Raman spectra of FCR from Lower Pecos, sample 3**
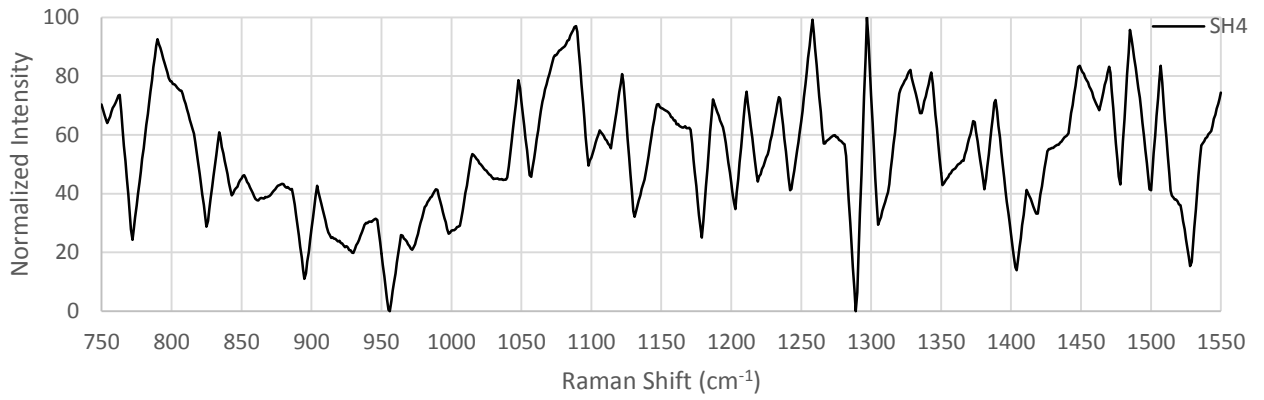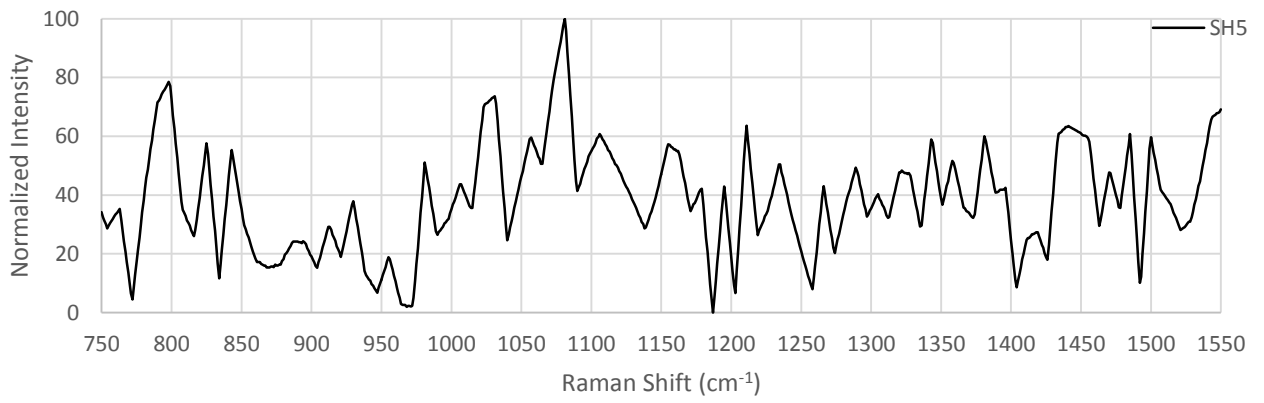
**Figure 75: Raman spectra of FCR from Lower Pecos, sample 4**
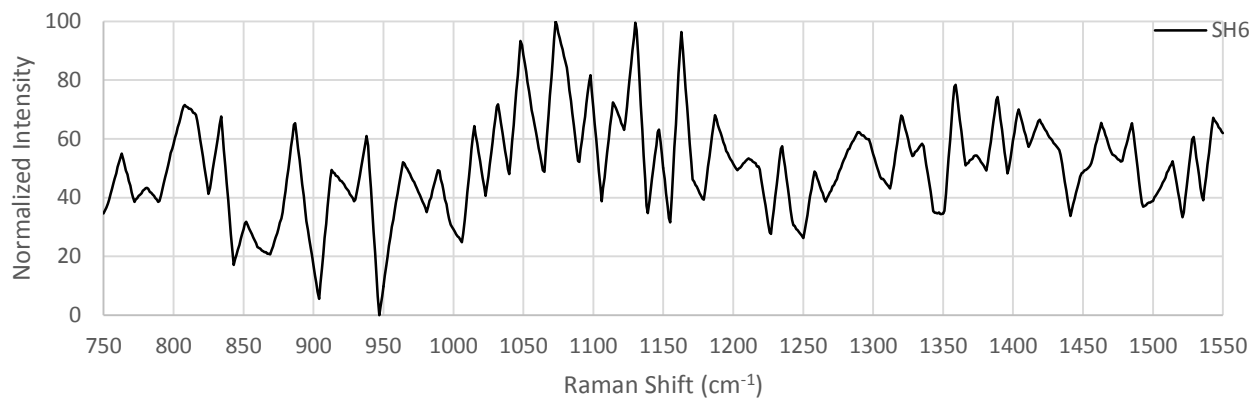


**Figure 76: Raman spectra of FCR from Lower Pecos, sample 5**

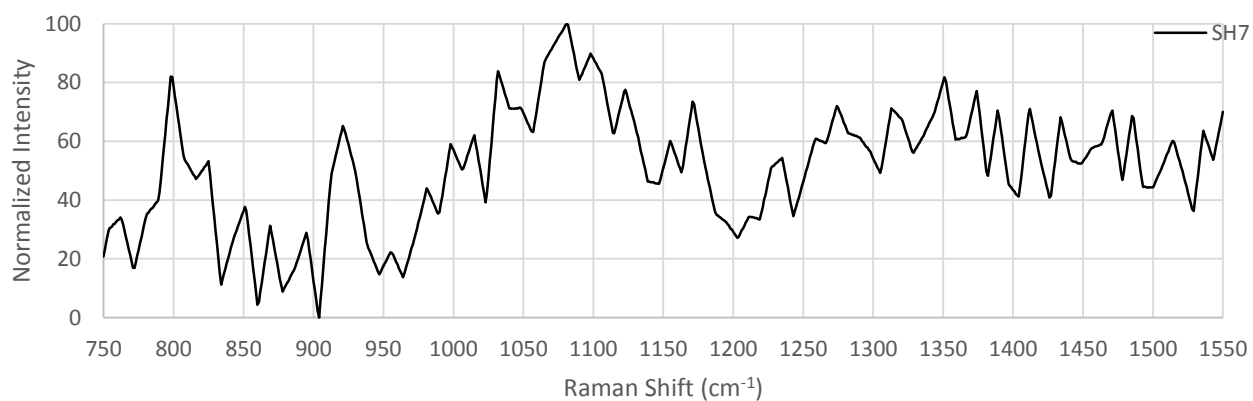**Figure 77: Raman spectra of FCR from Lower Pecos, sample 6**



**Figure 78: Raman spectra of FCR from Lower Pecos, sample 7**