ARCHAEOLOGICAL METHODS FOR ASSESSING PREHISTORIC FERMENTATION

A Thesis

by

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ABSTRACT

This thesis reviews and expands established methodologies for recognizing fermentation in the archaeological record. Ethnographic correlates, organic residue / lipid analyses, ancient DNA, palynology, and starch analysis have been used to detect evidence of brewing. Also presented are results of two experiments in fermentation microfossil research. The first indicates that pollen profiles are unchanged through the brewing process. The second illustrates that malted maize starch gelatinizes rapidly during *chicha* production. This study concludes with suggestions for a research strategy for extracting maximum information about the possibility of fermentation from residue adhering to ceramics.

DEDICATION

This work is dedicated to my best friend and favorite person, Joseph Dozier.

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NOMENCLATURE

ABV	Alcohol by Volume (%)
BCE	Before the Common Era
BP	Before Present
CE	Common Era
DNA	Deoxyribonucleic Acid
LC-MS	Liquid Chromatography – Mass Spectrometry
SG	Standard Gravity

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1. INTRODUCTION

Humans have long been enamored with alcohol and alcohol production; genetic studies indicate that primates developed the ability to digest ethanol over 10 million years ago (Carrigan et al., 2014). Modern primates are attuned to recognize fermented foods, primarily as overripe fruits (Dominy, 2004; Dudley, 2004; Stephens and Dudley, 2004). Fermentation allows for better digestion of complex carbohydrates by transforming them into simple sugars, including ethanol, and therefore increasing caloric intake and decreasing digestion costs. Ethanol also has psychotropic properties, which humans regularly exploit (Dietler, 2006; Fillmore and Weafer, 2004; Heath, 1987; Keller, 1975). Fermentation technology was likely regularly exploited in the Levant Late Epipaleolithic, ca 10-12,000 BP (Hayden et al., 2013). In their impressive overview of fermentation correlates with the pre-pottery Neolithic, Hayden et al. (2013, p. 103) contend that fermentation technology is only developed within 'complex' foraging societies and alcohol may play an important role within feasting traditions that predate and/or coincide with sedentism and agrarian subsistence patterns (Arnold et al., 2015). The domestication of many cereal grains can be tied to the establishment of brewing technologies (Braidwood et al., 1953; Hayden, 2014a, 2009, 2003; Hornsey, 2003; Iltis, 2000; Smalley and Blake, 2003) and have deep historical importance to many cultural processes (Arnold, 1999; Guerra-Doce, 2014; Moore, 1989). The introduction and spread of this techno-complex clearly has important implications for food processing regimes, social interactions such as feasting, and plant domestication, along with all that those themes imply (Biwer and

VanDerwarker, 2015; Tamara L. Bray, 2003; Dietler, 2006; Gero, 1990; Guerra-Doce, 2014; Hayden, 2014b).

2. FERMENTATION BIOCHEMISTRY AND TECHNOLOGY

For purposes here, I define fermentation simply as a microbial process in which sugars are transformed into ethanol and carbon dioxide. I contrast the biological process of fermentation with the process of producing alcoholic beverages; I use the term fermentation technology in reference to the complex of behaviors and requirements necessary to produce the desired alcoholic product. Biological fermentation is also used to create non-alcoholic foodstuffs, such as cheese, yogurt, pickled vegetables, and various vinegars/sauces, but the technologies therein are outside the scope of this paper (Steinkraus, 1994; Tamang and Samuel, 2010).

2.1 Biochemistry of Fermentation

Select microorganisms (i.e. some bacteria, mold, and fungi) use metabolic pathways to convert sugars to alcohols, which are then excreted as waste products. Common waste products to interests here include acetone, ethanol, and lactic acid. The production of these compounds is dependent on several factors, most importantly host microorganism, but also media pH, nutrient composition, and temperature (Wilkins and Atiyeh, 2012). In an anaerobic condition, all of the nutrients within the substrate will be digested by the microorganisms, resulting in stasis and eventual death. The temporal regime of fermentation relies on the growth curve for the alcohol-producing microorganism. Most of the valuable fermentation products are manufactured during the exponential growth phase in the lifecycle of an organism. Fermentation processes are considered completed after stasis.

Saccharomyces cerevisiae is the most common brewing organism, commonly referred to as brewer's (or baker's) yeast; the yeast occurs naturally worldwide (Koufopanou et al., 2006). This yeast is particularly useful in brewing due to its survival in relatively lower pHs; more acidic substrates do not support the growth of more harmful bacterial. While *S. cerevisiae* requires small amounts of oxygen, along with other nutrients, to reproduce, oxygen is often considered the bane of brewers. Ubiquitous, naturally occurring, acetic acid bacteria, such as *Acetobacter*, metabolize ethanol into acetic acid in the presence of oxygen—a process called secondary fermentation that is used to produce vinegars worldwide (Raspor and Goranovič, 2008).

2.2 Fermentation Technology

Fermentation technology, in terms of human production of an appropriate food or beverage, requires several stages of processing in order to achieve the desired product. Primarily, an appropriate, sugar-rich substrate for the fermenting microorganism must be produced. One of the most basic fermented products, mead, requires just honey and water. The honey contains enough accessible sugar for microorganisms that no additional processing is required. Fruit-based substrates, either taken directly as juice, juice with water, or water and fruit pulp, require basic mechanical processing to break up the pulp and release as much sugar into a liquid medium as possible. Complex carbohydrates, such as found in cereals and grains, require enzymatic processing in order to break down saccharides into glucose.

The initial step in brewing techniques for grains, such as barley wheat, and maize, requires the enzymatic conversion of carbohydrates into sugars, a process known as

saccharification. After unwanted hulls and shells are removed from the desired grain, there are several techniques to simulate enzymatic production within the grain, called *malting*. Controlled germination, in which the grains are soaked in water until they germinate, stimulates the β-amylase enzyme that occurs naturally in raw, ungerminated grains and produces the α-amylase enzyme. Malted grains can then be dried and stored until needed for further processing and are at this stage called *malt*. Raw grains can also be inundated with amylase enzymes through human saliva, and grain mastication has been recorded worldwide, although it may not produce beverages with as high alcohol content as malting (Driver and Massey, 1957, p. 268; Hayden et al., 2013, p. 120; Jennings, 2004). Grains can be selectively harvested in the late summer when the sprouts have sprung and have naturally started the enzymatic process of malting.

Medieval (Dineley, 2004) and modern brewers sometimes toast grain after sprouting and drying; while drying is necessary to keep the malt stable and halt sprouting, roasting grains at higher temperature also converts more of the starches to sugar and can crystallize the sugars already present in the grains. Increased simple, available sugars produces a more alcoholic final project and different toasting levels allows for customization of the beverage's completed flavor profile (Burger and LaBerge, 1985).

To speed up the enzymatic saccharification, the substrate must then be heated and held (depending on the circumstances) between 50 and 70 °C for anywhere from 30 minutes to 4 hours; this process is called *mashing*. In order to facilitate the enzymes, the malted or masticated grains are usually ground or crushed before mixing with water. The smaller particle size allows enzymes to better penetrate the full extent of the substrate. The

liquefaction of the wort (pre-innoculated substrate) is caused by the gelatinization of the starches. Pre-pottery brewers probably employed stone boiling in order to manage the appropriate temperature (Cutler and Cardenas, 1947; Hayden et al., 2013, p. 106). After mashing, the spent grains are no longer required; these cereals may be reused as animal feed or within gruel/bread. Recall, however, that only grains require this step in processing, though it is possible to produce some low-alcohol cereal beer without mashing as seen in some chicha (maize beer) traditions (Bruman, 2000; Cutler and Cardenas, 1947). Alcoholic beverages made from sweet saps and fruit juice do not require mashing, as the simple sugars are already accessible.

After an appropriate substrate has been produced, fermentation is not a laborious project. The substrate must be inoculated with an appropriate microorganism, and left to let the microorganism multiply. Yeast occurs naturally in many environments, including on the skin of fruits, on *Quercus* trees, and are transmitted through many insects (Hornsey, 2003). Hayden et al (2013, p. 109) suggest that a possible first employment of yeast in beer production could have happened when an acorn storage vessel was repurposed for storage of grain and inoculated the grain with an appropriate yeast for brewing; archaeologists will never actually know if this hypothesis is true.

Depending on the sugar and nutrient content of the substrate, the activity of the microorganisms, and the temperature, fermentation can be completed in four days to four weeks. Indigenous fermentation processes produce beer/meads anywhere from 1-10% ABV (alcohol by volume). Without bottling techniques, the produced alcoholic beverage must be consumed fairly soon after fermentation to prevent inoculation of unwanted

microorganisms. Distillation, the process of concentrating ethanol produced during fermentation, is an Old World tradition and not appear to be represented within indigenous brewing practices of the Americas.

3. FERMENTATION IN THE ARCHAEOLOGICAL RECORD

As illustrated above, the technological requirements for fermentation are fairly simple. Much of what is interpreted about ancient fermentation technologies stems from ethnographic analogies, as is practice for understanding most archaeological technologies (Binford, 2001, 1967; Fogelin, 2007; Seetah, 2008). Biological fermentation does not require domesticated cereals; many fermentation techniques rely on wild foods. The creation of an alcoholic beverage from a fruit or honey base only requires mechanical processing and a vessel large enough to hold the desired volume. Ground stone implements are the first requirement for most fermentation processes; sugary saps, such as palm, can begin fermenting from naturally occurring yeast almost immediately after collection and do not require any additional processing. Alcoholic beverages produced from cereal grains or complex carbohydrates require more steps, the most crucial being the mashing. Stone boiling is therefore a requirement for the production of beer, which is by definition made by fermenting grains. For example, Buskirk (1986, p. 230) describes the malting technique of the Western Apache in the production of *tiswin*, a variant of chicha (maize beer).

> …the White Mountain soaked their corn overnight, put it in a hole in the ground blanketed with yucca leaves, covered it with a gunnysack, and sprinkled it once a day for about a week. By the end of this time the corn had sprouted about two inches. It was then spread on a blanket for one day to dry somewhat, and the next day it was ground, mixed, and kneaded like dough. About ten pounds of corn dough was mixed in an earthen pot with about four gallons of water. This was stirred and boiled until reduced to half its original quantity. While the liquid was cooking, roots of Datura metaloides were added. After the first boiling, water was added to replace that lost through evaporation; the new liquid was again boiled and reduced by half. It was then strained through a perforated can, cooled until

> lukewarm, and poured into a pot that was never washed and was used for no other purpose.

Fermentation vessels can be made of stone, wood, or skin, as long as some kind of lid could be fit in order to limit the introduction of oxygen into the fermentation process. Hayden et al. (2013) implicate the use of ground stone vessels. Among the Mescalero Apache, ethnographic research reveals one indigenous method of fermenting that requires only the most basic technological correlates, without ceramics, and could have been used deep into the distant past (Castetter and Opler, 1936, p. 52).

> …sotol crowns (Dasylirion wheeleri) formerly were pit-baked for one night only, removed, peeled, crushed, mixed with a small amount of water in a rawhide container, and allowed to ferment underground about a day, or until fermentation had practically ceased, when the drink was ready for use. The concoction was also placed in pitch covered water jars or in wooden jugs cut from trees. Informants reported that the beverage might be allowed to stand for a month before being used.

The beverage could be consumed directly from the fermentation vessel, perhaps aided by straws (Maeir and Garfinkel, 1992), or the produced beverage could be consumed from a secondary vessel of any water resistant fabric. Many specialized ceramic forms are recognized in the archaeological record as having a direct correlation with the consumption of alcohol, with most of those forms having a narrow spouted form in order to reduce contact with outside microbes and minimize spillage (McGovern et al., 2003, p. 55).

Given the biological and technological requirements for fermentation, it follows that it was possible for non-agricultural groups without ceramics to utilize fermentation technology in North America, as has already been argued for the Old World (Hayden et

al., 2013). Of course, the very real likelihood that such technology existed does not translate into archaeological or ethnographic visibility. Archaeological evidence of fermentation technology is not lacking, however. In the following sections, I outline several different lines of evidence that archaeologists have employed in the study of fermentation-based cooking technologies of the past.

3.1 Ethnohistoric Correlates

Archaeologists use known historical or ethnographic brewing techniques to interpret architectural remains, especially in Europe and Western Asia. Due to Dr. Patrick McGovern's influence from the Biomolecular Archaeology Project at University of Pennsylvania, quite a bit of research on wine production has been published. Early Israeli wine presses have been identified due to their similarity to historically known presses (Hirschfeld, 1983). Ancient Egyptian brewing technologies are fairly well known due to documentary and artefactual evidence, which includes diagrams of wine and beer found in tombs (McGovern et al., 2009; Samuel, 1996). Mesopotamian brewing practices are known from tablet inscriptions, remains of fermentation equipment, and experimental work (Homan, 2004; Hornsey, 2003; Katz and Voigt, 1986; Maeir and Garfinkel, 1992; Sallaberger, 2015; Sicard and Legras, 2011). Similarly, malting floors have been identified in British historic contexts (Patrick, 1996) and archaeological interest in malting technologies have been inferred into prehistoric times (Dineley, 2004; Dineley and Dineley, 2000).

Alcoholic beverage production and consumption has also been recorded through artistic rendering in prehistory (Guerra-Doce, 2014; Immerwahr, 1992; M. Á. Rojo-Guerra

et al., 2006; Sheehy, 2001). This source can have valuable information for archaeologists regarding the contexts of ethanol production and consumption.

The most common form by which archaeologists have inferred brewing is through the use of specialized ceramics. *Chicha*¸ corn beer indigenous to South America, has been identified through the use of specialized drinking cups (Bray, 2009; Tamara L Bray, 2003; Goodman-Elgar, 2009; Jennings et al., 2009; Lantos et al., 2015; Weismantel, 2009). Roman amphorae are known to have been used to transport wine, among other things (Arobba et al., 2014). Recent excavations in Bulgaria have revealed ceramic tools for the manufacture of *raiki*, a fortified wine (Saraceni, 2015). The earliest evidence of wine in China, at 7,000 BCE, (McGovern et al., 2004) was suspected from the similarity of drinking vessels known from dynastic periods. The proclivity of identifying fermentation through ceramic vessels is useful because those hypotheses can sometimes be tested through different forms of organic residue analysis.

3.2 Organic Residue Analysis

Fermentation is an organically-based technology, and as such, the remains of such are rarely found in archaeological contexts. However, ceramic vessels which have retained residue of their prior contents have been subjected to several different kinds of molecular analysis.

Patrick McGovern and his colleagues have established the majority of the research concerning ancient fermented beverages (Cavalieri et al., 2003; Henderson et al., 2007; McGovern et al., 2013, 2009, 2004, 2003, 1996). Chemical and molecular analyses use a variety of equipment, such as gas/liquid chromatographs, mass spectrometers, and DNA

sequencers in order to determine the profile of a residue. In order to extract the most residue from ceramics, molecular analyses often require the pulverizing of the sample, making the process destructive and costly. Other spectrometry methods, such as Raman or X-ray fluorescence, may provide non-destructive insights (Short et al., 2013).

The earliest analyses focused on the identification of tartaric acid, chosen for its close association with grapes (Guasch-Jané et al., 2004; McGovern et al., 2003). McGovern (2003, pp. 56–58) outlines five logical steps in the identification of ancient wine:

1) Consideration of the vessel to be the fabricated to hold liquid.
2) Liquid chromatography- mass spectrometry (LC-MS) determines if tartaric salts are present in residue.
3) If tartaric salts are found in a vessel meant to hold liquid, it can be assumed that the liquid underwent fermentation.
4) Unless the liquid is prohibited from exposure to oxygen, eventually the wine would undergo a secondary fermentation by acetic acid bacteria into vinegar.
5) If there is evidence of deliberate action to prevent oxygen from entering the container, either through evidence for a lid/stopper for the container or through evidence for the addition of an anti-microbial, such as tree resins.

This basic, logical methodology has been used to assess the presence of wine in several studies (Arobba et al., 2014; Barnard et al., 2011; Cavalieri et al., 2003; Guasch-Jané et al., 2004; McGovern et al., 1996; Sicard and Legras, 2011). The methodology rests on the

identification of tartaric salts to identify grape as *Vitis vinifera vinifera* is the only known edible plant in the Near East with such high levels of tartaric acid.

Syringic acid is also associated with the enological process, and has been used to infer winemaking within Armenia (Barnard et al., 2011). Unfortunately, both of these acids are present within the skins of grapes naturally, and fermentation technology is not necessarily implicated. To adapt this methodology to situations where the suspected fermented substrate is not known, however, other tests are needed. Similarly, residue analysis has come under scrutiny as little experimental work has been conducted to ask questions about whether these residues are the result of single use, compounded use, last time use, or any of those combinations (Barnard and Eerkens, 2007; McGovern et al., 2013).

Amino acid and ancient protein analysis have not been readily applied in fermentation studies, primarily because there are few expectations for fatty acids within fermented residues. Lipid analysis, however, has been able to find byproducts of fermentation in both the New and Old World. Lipid analysis has been most utilized in analysis of amphora protective resins (McGovern et al., 1996; Stern et al., 2008) A recent paper, however, reveals the possibility of using a lipid biomarker associated with the ethanol-producing bacterium *Zymomonas mobilis* as a way to reveal fermented beverages (Correa-Ascencio et al., 2014). This technique is exciting as it is one of the few methodologies developed directly for application in the New World and it is applied to the production of *pulque*, which is made from agave sap. Pine resins sealants of ollas may provide a similar insights (Correa-Ascencio et al., 2014, p. 14227).

Raman spectrometry may help recognize carbohydrate residues (Short et al., 2013), but recognition of carbohydrates alone does not implicate fermentation as the cooking technique. Molecular analysis is limited by a predetermination hypothesis; one can only assess if the sample meets the expectations of the researchers.

3.3 DNA

Ancient DNA analyses provide an additional avenue for studying prehistoric foods and cooking methods. The proliferation of different yeast strains has been attributed to the specialization of different cooking technologies of human, whereas humans are responsible for the production of so many variety of yeast strains (Sicard and Legras, 2011). Using 651 strains from 56 geographical origins Legras et al (2007) attribute up to 28% of genetic diversity between strains of *S. cerevisiae*, brewer's yeast, as associated with geographical differences, indicating that local domestication is an important and ubiquitous factor. They suggest that *S. cerevisiae* originated in the Middle East, with a post-Pleistocene divergence. The domestication processes for yeast are complicated and little known despite their incredible importance in modern subsistence patterns. Even yeast strains developed relatively recently in the Middle Ages, such as the common cold-temperature lager strain of yeast, can only be well studied with the use of comparative genomics (Libkind et al., 2011). *S. cereviseae* has been identified in Peruvian *chicherias*, albeit with many genetic differences from European varieties (Vallejo et al., 2013). Other researchers have identified a new yeast, *Candida theae*, associated with chicha production (Chang et al., 2012).

New ancient DNA techniques are shedding light on the domestication and adaptations of many foods. There is growing consensus that initial domestication of maize from wild teosinte occurred in Mexican lowlands before 9000 BCE (Bryant, 2007; Doebley and Stec, 1993, 1991; Matsuoka et al., 2002; Piperno et al., 2009). Smalley and Blake (2003) argue that exploitation of sugary pith for fermentation was the primary driver for the domestication and spread of early maize; this beer-before-bread argument is not an uncommon argument for the domestication of cereals around the world (Braidwood et al., 1953; Hayden et al., 2013; Hornsey, 2003; Iltis, 2000). Archaeological DNA evidence from wine grape pips can help understand how these species have adapted by human use, even in terms of skin color to develop white wine from an originally red fruit (Runge, 2015).

3.4 Oldest Evidence of Fermentation

3.4.1 *Old World Archaeological Evidence*

The earliest circumstantial evidence for fermentation technology exists in the Pre-Pottery Neolithic A (PPNA) in the Near East circa 9500 BCE at sites such as Jerf el Ahmar and Tell ‘Abr 3, which would place fermentation technology as precedent to domestication of cereals seen in the Pre-Pottery Neolithic B (Hayden et al., 2013). Hayden et al. (2013) argue that the technological requirements for brewing are met in the PPN-A, with archaeological correlates such as stone grinding, large stone vessels, and stone bowls presumed to be used for the personal consumption of liquids (ibid). Stone boiling is recognized within the PPN-A through fire cracked rock and the presence of grease bones (Hayden et al., 2013, p. 115). Evidence for fermentation is recognized more widely

coincident with domestication practices in a global context. Chemical identification of tartaric acid residues indicate grape wine production in China circa 7000 BCE (McGovern et al., 2004), in Iran circa 5000 BCE (McGovern et al., 1996), and within Armenia circa 4000 BCE (Barnard et al., 2011). Fermentation in southern Europe is seen as early as the fifth millennium BCE, becoming increasingly common, especially in ritual contexts, after 3000 BCE, which coincides with the technology's (and the Roman Empire's) spread into northern Europe (Arnold, 1999; Dickson, 1978; Dietler, 1990; Dietler and Hayden, 2010; Guerra-Doce, 2014; Lagerås, 2000).

3.4.2 *New World Ethnoarchaeological Evidence*

Smalley and Blake (2003) argue for the domestication of maize as specifically to accommodate fermentation requirements through the exploitation of sweet stalk juice. Microfossils indicate that maize was likely domesticated 7000 BCE in the Mexican lowlands (Bryant, 2007; Piperno et al., 2009), though there is no textual or chemical evidence for alcohol production until fully agricultural regimes of the Maya (Bruman, 2000; Correa-Ascencio et al., 2014; Sheehy, 2001). If teosinte quids represent the extraction of fermented sugars from the stalks (Smalley and Blake, 2003, p. 682), however, then the exploitation of biological fermentation may be seen as early as 3000 BCE in Mexico.

Fermentation production in the New World has not been acknowledged in archaeological works until after the development of pottery, even though ethnographic observations indicate that non-pottery groups can and do produce alcoholic beverages (Buskirk, 1986, pp. 230–232). The earliest evidence of chicha from archaeological

remains date to the Early Horizon, 900-200 BCE (Jennings, 2004, p. 244); chemical analyses have indicated that fermentation of cacao beans extends at least to 1000 BCE (Henderson et al., 2007). All chemical analyses, as expounded upon below, have been conducted on residues found on ceramics, so the earliest examples of fermentation are from ceramic producing cultures.

Studies of complex foragers are just beginning to illustrate the important developments in political organization, especially in terms of labor exploitation and hierarchies, in the ethnographic and archaeological record (Arnold et al., 2015). Sedentism seems to be a better indicator of fermentation than agricultural production (Abbott, 1996); this correlates well with theories concerning competitive feasting and the role of specialized food in the creation of hierarchical power regimes (Hayden, 2014a, 2014b, 2003; Hayden and Villeneuve, 2011).

Ethnographic reports provide an insight into the some of the brewing practices within North America before European colonization. (Bruman, 2000; Buskirk, 1986, pp. 230–232; Castetter and Bell, 1942, p. 223; Castetter and Opler, 1936; Waugh, 1916, pp. 145–147). The ethnographic record for North American indigenous peoples is understandably incomplete and skewed. Brewing, in these contexts often taking a ceremonial or specialized focus, may not have been shown to ethnographers; brewing is often a woman's task (Castetter and Opler, 1936, p. 50; Hayashida, 2008; Jennings, 2004) and therefore may have been less visible to male ethnographers. Fermentation takes on a political agenda in light of issues of alcoholism among indigenous peoples currently under the reservation system (Beauvais, 1998; Frank et al., 2000; Spillane and Smith, 2007;

Teret and Michaelis, 2005). There are many reasons why the ethnographic record may not reflect the full diversity of techniques, especially for mobile, non-agricultural societies that were particularly susceptible to complete obliteration during colonization.

4. EXPERIMENTS IN MICROFOSSIL ALTERTIONS OF FERMENTATION*

4.1 Pollen Evidence of Fermentation

4.1.1 *Prior Studies*

Honey is a prized commodity in many cultures and is a common source of sugar in a variety of fermented beverages, such as beer, wine, and mead. Humans have been exploiting the byproducts of honeybees (*Apis mellifera*) for at least the past 8,500 years (Roffet-Salque et al., 2015). Both beeswax and honey are incredibly useful bee products; honey is particularly important as one of the few sources of simple sugars available without processing. Hayden et al. (2013:108–109) convincingly argue that the yeasts required for early fermentation probably originated either in honey or on acorn shells. Many early fermented beverages included at least some honey to increase the available sugars for the yeast (and thereby increase alcohol content), perhaps to inoculate (introduce the yeast or fermenting microorganism) the beverage, and undoubtedly for taste (McGovern et al., 2003).

Archaeological pollen has been recovered from ceramic containers as residue and within coprolites and interpreted as consumption of alcoholic beverages through several interpretive frameworks, usually with the use of a distinctive species marker as an identifier (Moe and Oeggl, 2014). *Vitis vinifera* (grape) pollen has been used as a marker to distinguish wine amphorae from other storage vessels in Mediterranean shipwrecks

(Arobba et al., 2014; Gorham and Bryant, 2001; Jacobsen and Bryant, 1998). High concentrations of *Filipendula ulmaria* (meadowsweet) pollen in coprolites has been interpreted as alcohol consumption as the plant is historically known to have been used as a flavoring agent in mead production (Moe and Oeggl, 2014). The presence of cereal pollen has also been used to interpret ale and mead production (Lagerås, 2000). This interpretive framework of looking for an indicator species only allows for confirmation of previously understood fermentation processes. Honey contains an extremely high concentration (and often diversity) of pollen grains, some of which are only commensal species-pollinated taxa. Diversity of commensal species-pollinated pollen has been used to identify honey and mead (Kvavadze et al., 2006; Rösch, 1999).

Extensive review by Guerra-Doce (2014) indicates that a number of researchers in Europe have exploited palynology, the study of pollen grains, to identify potentially fermented beverages. She highlights studies that utilize palynology to identify mead and beer, as indicated in Table 1. No studies in North or South America have taken this approach; *Apix* honey bees were only introduced after the Columbian exchange (Whitfield et al., 2006), although stingless bees (meliponines) were exploited by the Maya for honey in the creation of a mead called *balché* (Bruman, 2000, pp. 91–93).

Table 1. Fermented Beverages Identified through Palynology from Guerra-Doce (2014).

Identification of mead	(Bartel et al., 1997; Dickson, 1978; Körber-Grohne, 1985; Nielsen, 1988; Prieto Martínez et al., 2003; Ramírez et al., 2005; Rösch, 2005, 1999)
Identification of beer	(Barclay, 1983; Dineley and Dineley, 2000; Lagerås, 2000; Thomsen, 1929; Wickham-Jones et al., 1990)

Melissopalynology describes pollen found within honey (Jones and Bryant, 1996, 1992; Low et al., 1989; Todd and Vansell, 1942). Pollen is the reproductive agent of flowering plants; pollen grains have a wall containing sporopollenin that protect gametophytes during fertilization in spermatophytes. Angiosperms and gymnosperms use pollen to distribute genetic material between individuals, which aids in genetic diversity and the ability for a species to adapt to changing conditions. Luckily for archaeologists, sporopollenin is very resistant to destruction over time and pollen grains can often be identified to species with use of light microscopy and computer-aided microscopy, such as SEM. Pollen is sequestered in/on plant reproductive organs (including fruits), sometimes released airborne, and carried by commensal species. Pollen does seem to provide some kind of nutritional value to brewing yeasts (Roldán et al., 2011).

4.1.2 *Mead Experiment*

A recent food science paper (Roldán et al., 2011) found that the addition of pollen while brewing mead resulted in higher alcohol content, as well as an improvement in taste. The researchers conclude that pollen acts a nutrient for *Saccharomyces cerevisiae*, the

most common brewing yeast. While palynologists have long understood that ethanol does not alter the structure of pollen grains (pollen is often stored in ethanol), no direct study has looked at how *S. cerevisiae* metabolism affects pollen grains. A variety of bacteria and fungi are known to obliterate pollen from soil samples (Goldstein, 1960; Havinga, 1967). If brewing yeast consumes or alters the pollen in fermented beverages, prior arguments built on palynological assessments of fermented beverages may be unfounded. The research presented here observed pollen grains through the fermentation process to see if yeast metabolism altered the sporopollenin of individual grains or the pollen profile of honey as fermented into mead, beer, or wine.

4.1.2.1 Materials and Methods

In order assess the effect that fermentation had on a pollen profile, I brewed a simple mead (honey wine) and tracked the pollen through the fermentation process.

As shown in Table 2, the only ingredients used in this mead were water, honey, yeast, and pollen pellets. Industrial honeys, such as was used in this experiment, are commonly micro-filtered to remove pollen and other particles—the misconception in the industry maintains that the pollen can precipitate crystallization (Bryant, 2014a). Therefore, pollen pellets were added in proportions close to Roldán et al. (2011)'s methods to ensure pollen in the mead. The pollen pellets contained a variety of common North American taxa with a variety of sizes and morphological traits.

Table 2. Mead Materials.

Water	10 l	Fort Worth, TX Municipal Water
Honey	2512 g	Fermentap, Concord CA
Pollen Pellets	192 g	The Wealth Savings Center, Valley Stream NY
S. cerevisiae Dry Yeast	2 g	Lalvin Bourgovin RC212, Denmark

The honey and water were brought to a sanitizing threshold of 71 °C to create the must (the pre-inoculated substrate). The must was then cooled and the pollen pellets added and mixed thoroughly. The must was inoculated with *S. cerevisiae* yeast and let to ferment in a sealed, food grade plastic tub for seven days at 72-83 °F (22-28 °C). The specific gravity of the mead dropped from original gravity of 1.081 to a final gravity of 1.007; therefore, the resulting mead obtained approximately 9.71-10.5% alcohol by volume, consistent with mead characteristics. The mead finished with a Standard Reference Method (SRM) color of 15, a rich golden light brown.

Samples were analyzed adopting standard melissopalynological procedures (Jones and Bryant, 1996) at the Texas A&M Palynological Research Laboratory. Ten-ml subsamples were taken from each of the mead/must samples, to which one lycopodium tablet (18583 spores/tab) was added. The water source for the mead/must was not tested for pollen as pollen was intentionally added to the must; however, all equipment within the Texas A&M Palynological Research Laboratory is regularly tested for pollen contamination and the Laboratory distilled water is pollen-free, so no pollen was added subsequent to fermentation. The samples were then centrifuged at 3,500 rpm for two

minutes, the same time and speed for all of the subsequent washes. The supernatant liquid was decanted and the samples were washed with 7 ml of glacial acetic acid. A standard acetolysis regime was followed whereas 7 ml of a 1:9 ratio of sulfuric acid to acetic anhydride was added to each sample and heated for 10 minutes at 80 °C. The samples were stirred occasionally before being washed with 7 ml of glacial acetic acid. The samples were then washed in water and ethanol (Jones and Bryant, 2004) before seven drops glycerin were added per sample and the samples were mounted to a slide.

One slide from each sample was analyzed under light microscopy. At least 200 grains were analyzed from each sample, as is standard practice and statistically secure (Barkley, 1934). Broad identifications were made to assign pollen grains to taxonomic family or genera, but exact identifications were irrelevant. Rather, the relative frequency of different pollen types was the most important facet to understand if and how the yeast used the pollen as a nutrient.

4.1.2.2 Results

Pollen preservation in the must and mead samples was phenomenal and no damage to pollen walls was observed under light microscopy. Figure 1 indicates the diversity of pollen taxa recovered. Figure 2 illustrates the relative frequencies of the different pollen types; no significant differences in the pollen profiles of any of the samples was observed following fermentation. Raw counts of the pollen can be obtained as a supplementary file of Dozier (2016).

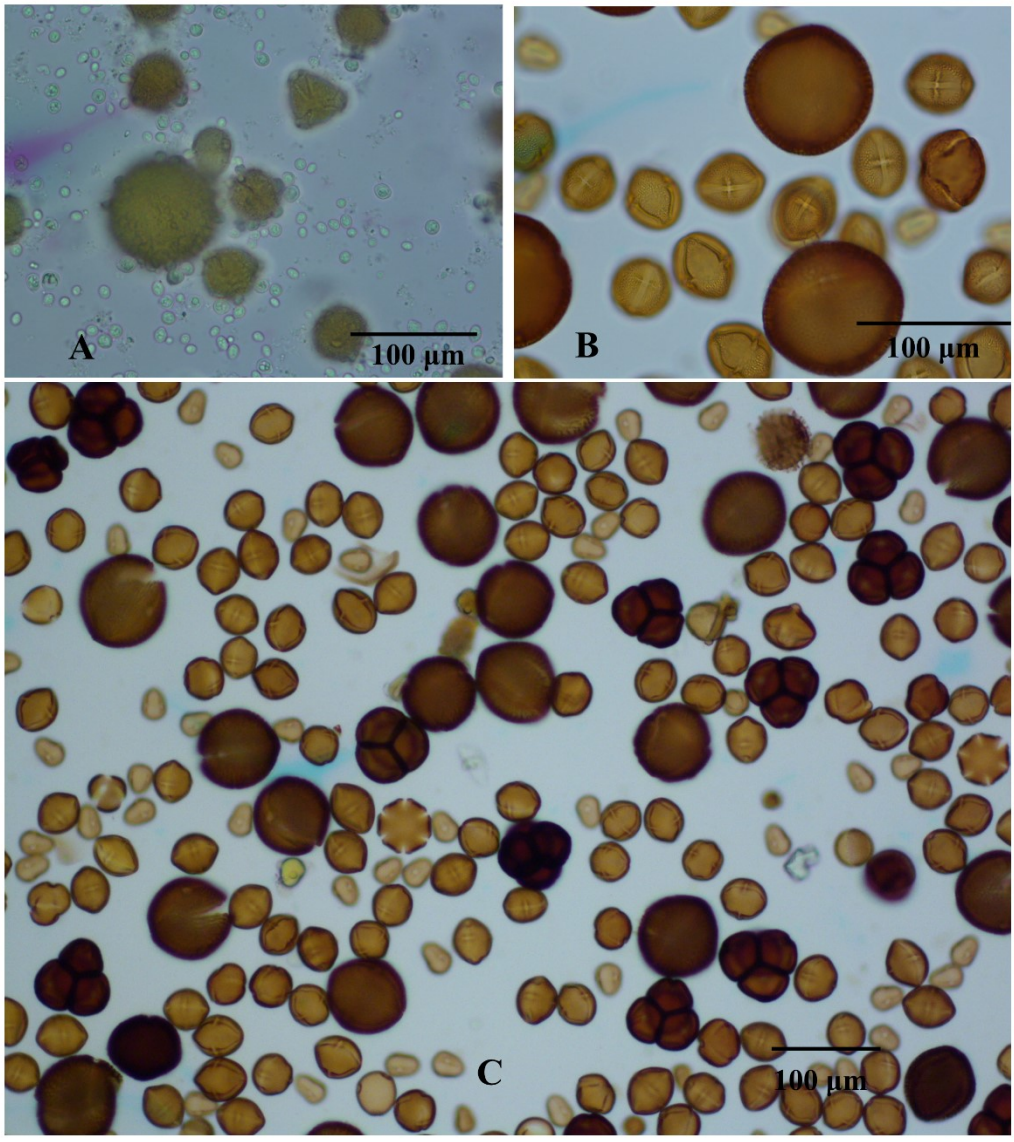


Figure 1. A: Mead sample prior to processing. Note presence of yeast cells around pollen grains. B and C: Mead sample after acetolysis. No damage to pollen exines. Note pollen and spore diversity.

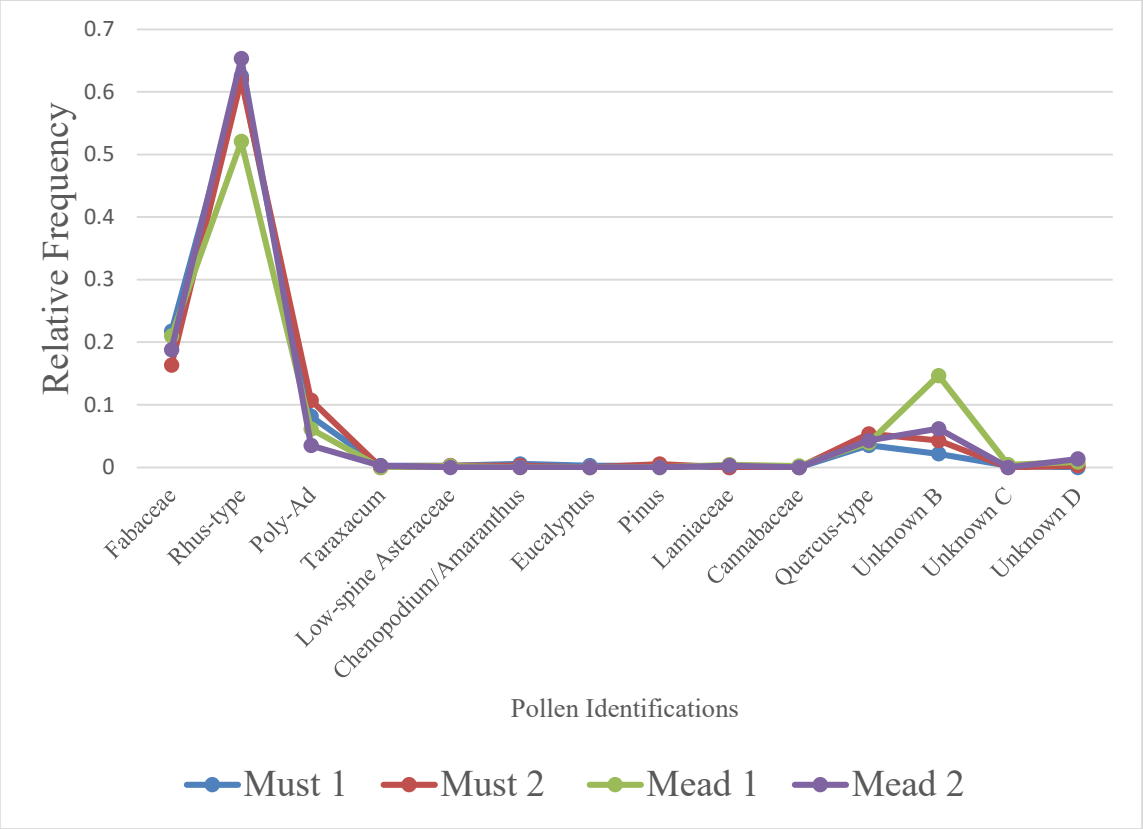


Figure 2. Pollen profiles of must and mead samples. No significant differences are observed between samples.

4.1.2.3 Conclusions

While *S. cerevisiae* likely metabolize lipids on the exterior of pollen gains (Roldán et al., 2011), the sporopollenin and pollen profile of honey remained unchanged under light microscopy through fermentation. Any nutrition that the yeast garners from the pollen therefore leaves sporopollenin intact; differentiation between unaltered and fermented food residues based on palynological analysis alone is unlikely. It is possible that minor damage to sporopollenin may be visible under scanning electron microscopy (SEM). This

study did not have the resources to pursue this possibility, which may be an avenue for future research.

The research presented here supports interpretations of honey in archaeological residues through palynological analysis (Kvavadze et al., 2006; Lagerås, 2000; Moe and Oeggl, 2014; Rösch, 1999). As always, though, archaeologists and paleo(ethno)botanists need to remain cognizant of other taphonomic processes (Bryant and Hall, 1993; Cushing, 1967; Havinga, 1967) that may affect the palynological record. For example, wetting and drying cycles are known to destroy pollen grains (Campbell and Campbell, 1994). With secure context and proper control sampling, however, palynological analysis of possibly fermented residues have the potential of greatly informing the archaeological record. Because pollen in honey profiles can reflect some aspects of the local environments (Bryant, 2014b), palynological assessments may provide additional paleoenvironmental insights beyond identifying honey utilization (Kvavadze et al., 2006; Rösch, 1999).

4.2 Malted Starch Evidence

Starch granules are semi-crystalline structures produced in plants that can be of particular utility in discovering ancient plant exploitation, as they may resist degradation for thousands of years (Loy et al., 1992; Piperno et al., 2004). Starch granules expand in layers from a central point, called a hilum. The layers alternative a crystalline amylopectin structure with amorphous amylose layers; different plants produce different ratios of amylopectin to amylose. Two types of starch granules are produced in plants, transportation and storage (also called reserve) starch. Storage starch is of great use to archaeologists, due to the crystalline nature of the granule (Gott et al., 2006; Henry, 2015).

Starch granules can be identified to varying levels of taxonomy according to its morphological characteristics, through there may be variability in starch production from plant to plant and storage organ to storage organ, dependent on the development of the granules. Due to its crystalline structure, starch can be analyzed microscopically using regular light, polarized light, and cross-polarized light. In polarized light, many undamaged starch granules produce an extinction cross that can be distinctive to species. Starch granules also have birefringent properties that can be used in identification (Torrence, 2006).

Henry et al. (2009), among others (Crowther, 2012; Gong et al., 2011; Messner and Schindler, 2010; Raviele, 2011; Thoms et al., 2015), have found that starch granules altered through different cooking processes exhibit distinctive morphologies. The mechanical and chemical pressures of cooking—grinding, parching, boiling, and fermenting—break down the crystalline structure in predictable ways. The gelatinization process breaks down the layers of amylopectin and amorphous amylose molecules starting at the hilum (Jenkins and Donald, 1998). Gelatinization processes are dependent on contextual variables such as time, temperature, pressure, starch density, and nixtamalization (Bryant and Hamaker, 1997; Cabrera et al., 1984; Crowther, 2012; Jenkins and Donald, 1998; Li et al., 2015; Lund and Lorenz, 1984; Ratnayake et al., 2007; Robles et al., 1988). While iodine stain is normally used to identify starch, cooking damage makes the extinction crosses and other identifiable markers less distinct; altered granules, however, may be more susceptible to Congo Red dye (Lamb and Loy, 2005). Tracking

changes in starch morphology through the cooking processes, especially malting, may allow for a greater understanding of plant use in the archaeological record (Samuel, 2006).

Spanish archaeologists have used malted cereal starch granules to argue for the presence of malting floors in beer production (M. Á. Rojo-Guerra et al., 2006; M. A. R. Rojo-Guerra et al., 2006). Macrofloral remains of malted wheat have been preserved in Germany ca 2400-2600 BCE after a catastrophic fire (Stika, 1996). *Zea mays* can be readily distinguished from other starch granules (Piperno and Holst, 1998), however, to present, few experiments have looked at maize starch alteration through cooking processes (Raviele, 2011). One of the uses of malted maize is in the production of *chicha*, a corn beer made in South America of great caloric and symbolic value.

4.2.1 *Prior Studies*

Chicha is the best-studied Native American brewing tradition (Bruman, 2000; Cutler and Cardenas, 1947; Hayashida, 2008; Jennings, 2004; Moore, 1989; Vallejo et al., 2013). There is a wide diversity in brewing technologies for chicha, and the term is sometimes used to fermented beverages made from starches other than maize (Weismantel, 1988, p. 96) or to describe sweetened beverages which may not be alcoholic (Cutler and Cardenas, 1947; Smalley and Blake, 2003, p. 695). I employ the term chicha to characterize alcoholic maize beer because it is concise and familiar to a larger archaeological audience, although there are other indigenous terms, such as *tiswin* (Buskirk, 1986), *aswa* (Weismantel, 1988), or *tesgüino* (Bruman, 2000), that could be used to talk about more localized traditions.

While understanding the development of this technology is important to study feasting traditions among maize-intensifying cultures (Bray, 2009; Tamara L Bray, 2003; Hayashida, 2008; Jennings, 2004; Jennings and Bowser, 2009), archaeological manifestations of the underlying processes remain understudied. Chicha has been implicated by contextual evidence in the Titicaca Basin as early as the Formative Period, 800-250 BCE (Logan et al., 2012). Bray (2003) identifies three ceramic traditions associated with chicha production, which has been used to infer chicha production in Inca traditions. Unlike brewing traditions within the Old World (Henry et al., 2009; M. Á. Rojo-Guerra et al., 2006; M. A. R. Rojo-Guerra et al., 2006), no work has been published to identify the microfossil transformations that occur to maize during fermentation. Bio-technological requirements (such as enzymatic activation, malting, mashing, and fermentation) of cereal brewing are crucial to understand the production stream of chicha and what may be recoverable in the archaeological record. The experiment presented here assesses the morphological changes and availability of starch through experimental chicha production.

4.2.2 *Chicha Experiment*

Chicha in Peru is fermented in large ceramic vessels that are only used for chicha; small amounts of chicha are left from the prior batch to encourage yeast inoculation (Cutler and Cardenas, 1947; Hayashida, 2008). Fermentation requires 24 hours to 8 days to reach completion, which yields 3-6% alcohol by volume (ABV). Chicha is usually served in small, individual serving cups. Batches much be consumed fairly soon after they are produced; chicha has a higher pH than other fermented beverages such as wine and

therefore is more susceptible to unwanted bacterial contamination (Jennings, 2004; Wilkins and Atiyeh, 2012). Ethnographic reports indicate that chicha is usually consumed within a week of production and can be consumed before fermentation is complete (Bruman, 2000; Cutler and Cardenas, 1947; Hayashida, 2008; Nicholson, 1960). Of course, ethnographic and historical analogues cannot be assumed to perfectly model pre-Hispanic chicha production and consumption regimes (Hayashida, 2009).

Because the maize starches undergo distinctive digestive processes through chicha production, starch may provide insights into archaeological residues of brewing technology (Henry et al., 2009; Lantos et al., 2015). This study explores how maize starch is transformed through the brewing process.

4.2.2.1 Materials and Methods

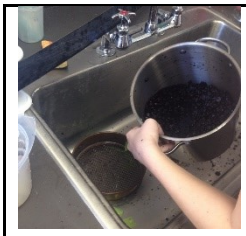
To have the widest applicability for archaeological situations, I replicated a fermentation regime that approximated the basic steps (malting, mashing, and fermentation) available throughout pre-Columbian history. The maize utilized in these experiments was a purple Peruvian variety, grown by Barry Hill Farm in Ohio. Five pounds (2.3 kg) of dried kernels were rinsed, then soaked with tap water for two days in plastic tubs to initiate the malting process. The rinse water was analyzed under light microscopy and contained a few starch granules (both maize and non-maize) and no pollen. The soaking grains were rinsed and refreshed with new water every 12 hours. The soaked kernels were then rinsed and drained, and kept with a moist paper towel covering them. The sprouting kernels were kept by a window and rinsed 1-2 times a day to prohibit fungal growth. Sprouting was halted after 5 days, when a majority of the aospires (rootlets)

reached twice the length of the maize kernels, a common rule of thumb for both modern and ethnographically recorded chicha producers. Sprouting rate exceeded 85 percent; both sprouted and non-sprouted kernels were kept in the experiment. The sprouted kernels were then completely drained, and spread on a single layer to dry on paper towels. Kernels were dried for 10 days before being ground with a stone mano and slab metate. Figure 3 illustrates the malting sequence; Figure 4 illustrates the grinding equipment. Grinding took place both indoors and outdoors, with water traps set up to explore how far airborne starch travels from stone grinding; results of that experiment can be found in Dozier (under review).

This experiment sought to understand the taphonomic changes that the starch granules underwent during the boiling and fermentation process, methodologically inspired by Henry et al. (2009). The *jora* was split into two chicha samples with different boiling regimes to capture variation in maize starch morphological changes. Each chicha sample contained 914 grams of *jora* and 24 cups (5.6 l) of water and was boiled over an electric stove for consistency in heat distribution. Chicha 1 boiled for an hour; Chicha 2 was heated just below boiling, but above the sanitation threshold of 160 degrees F (71 °C). Figure 5 displays wort temperatures of both chichas and indicates when starch samples were taken through the boiling (mashing) procedure. After mashing, the worts had a specific gravity of 1.045 and 1.036, respectively.

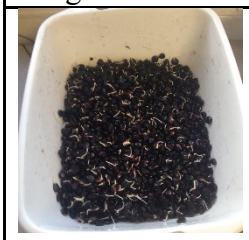
After heating, each wort was poured into food-grade, 5 gallon plastic fermenters and inoculated with 5.5 grams of commercial *Saccharomyces cereviseae* (Dawnstar, Nottingham brewing yeast). Since no South American commercial yeasts are available, it

was suggested that I use the Nottingham yeast strain as other chicha homebrewers (Gordon Spect, personal communication 2015). The chicha samples fermented for six days before starch sampling. Final specific gravities were 1.024 and 1.014 for boiled (Chicha 1) and heated (Chicha 2) samples, respectively; the chichas therefore achieved 2.75-2.89%. An additional sample was taken from the final fermented chichas.



Day 1: Rinsing the grain

Day 1-2: Soaking



Day 2-7: Germination start

Day 7: Germination complete

Day 7-17: Drying of sprouting grain

Figure 3. Maize sprouting sequence.

Figure 4. Outdoor grinding procedure.

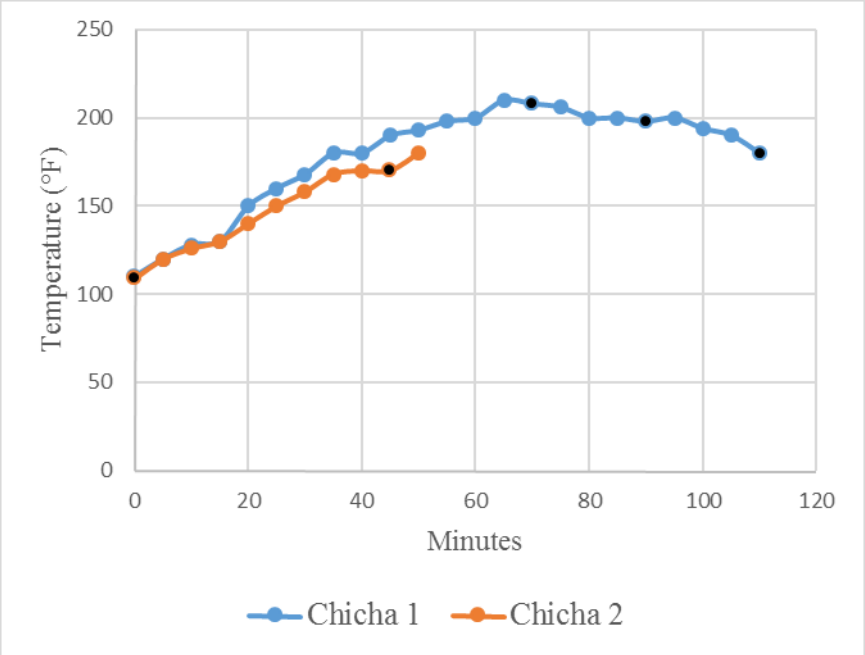


Figure 5. Chicha mashing regime. Each point is a temperature check; black dots indicate where a starch sample was taken. Boiling was observed after 55 minutes in Chicha 2.

Each chicha sample was 11 ml, which was then centrifuged at 1000 rpm for two minutes. The concentrated samples were then applied directly to a slide without further processing. Each sample was examined under brightfield, polarized, and cross-polarized light under x200 power on a N200 Nikon Optiphot. Figure 6 illustrates examples of changes in maize starch granule morphology through the course of chicha heating and fermentation.

4.2.2.2 Results

As illustrated in Figure 6, maize starch granules underwent extensive morphological changes through the chicha production stream. Maize granules from the *jora* exhibit distinct birefringence and slightly fuzzy extinction crosses, probably due to

the slight crushing damage at to hilum. Starch granules became gelatinized very early in the mashing process, swelling and losing distinctive structures (extinction cross and birefringence) at 170 degrees F (77 °C), after just 45 minutes of heating. For unmalted cereal starch granules of 10 domesticated Old World species, Henry et al. found that it took anywhere from 5-30 minutes of boiling for complete gelatinization to occur when whole; gelatinization happened within 1-5 minutes of boiling for ground samples (2009, pp. 917–919). In industrial situations, Jenkins and Donald (1998) report that maize starch gelatinizes between 144-183 degrees F (62-84 °C). The exact temperature for boiling is not noted in their methods; partial boiling was identified in this experiment by persistent bubbles from the wort, which occurred at 198 degrees F (92 °C) for Chicha Sample 1. Chicha Sample 2 was removed from heat at 180 degrees F (82 °C)—after which gelatinization processes obscured any distinctive features of the starch granules. No starch granules were recovered from any fermented Chicha samples I analyzed. It is possible that a few stray starch granules could survive the sprouting, mashing, and fermentation process in some conditions; maize gelatinization becomes less complete the more dense the starch concentration becomes (Li et al., 2015).

Special attention was paid to reduce airborne starch contamination (Crowther et al., 2014; Laurence et al., 2011); although the facilities employed in the study were found to contain latent starch by Laurene et al. (2011), no non-maize starch granules were observed during analysis.

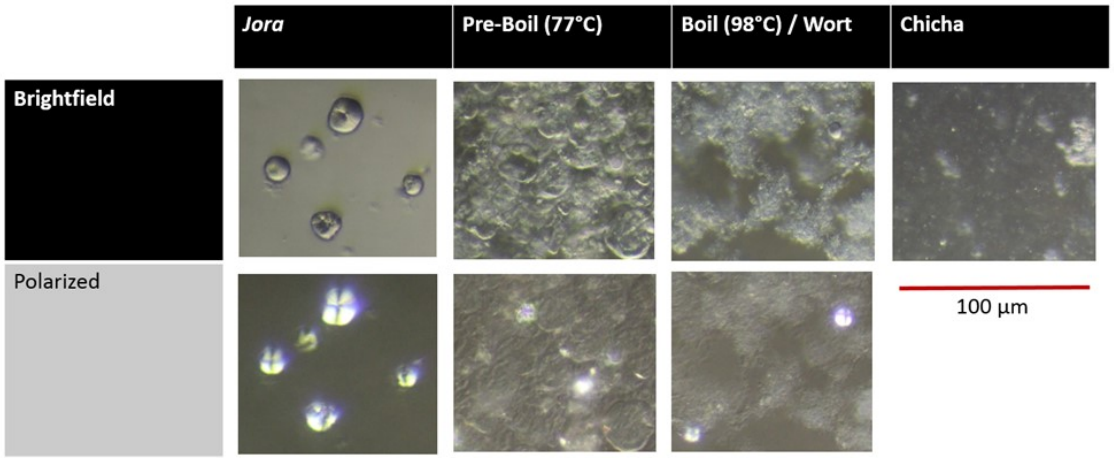


Figure 6. Gelatinization of *jora* starch through mashing and fermentations for chicha.

4.2.2.3 Conclusions

This fermentation experiment suggests that starch is unlikely to be recovered from chicha boiling/mashing, fermentation, or consumption vessels in archaeological contexts. While Jennings and Bowser (2009, p. 9) valiantly called for residue analysis on *kero* drinking cups associated with chicha consumption, archaeological starch research should look to more utilitarian artifacts to sample. Archaeologists will need to consider other chemical and microbotanical regimes of residue analysis for those containers (Barnard and Eerkens, 2007); finding concentrations of undamaged maize starch in containers could be indicative of storing rather than wet cooking activity, though some starches can survive undamaged through certain wet cooking regimes (Thoms et al., 2015). The damage from malting the maize, as shown in this experiment, may be indicative and could remain on processing surfaces such as *manos*, *metates*, drying mats, or within storage vessels, complementary to Moore's (1989) assessment for wheat. Spanish archaeologists have

used malted cereal starch granules to argue for the presence of malting floors in beer production (M. Á. Rojo-Guerra et al., 2006; M. A. R. Rojo-Guerra et al., 2006). Maize starch has been recovered from a variety of archaeological artifacts (Boyd et al., 2008, 2006; Louderback et al., 2015; Mickleburgh and Pagán-Jiménez, 2012; Zarrillo et al., 2008) though none have identified malting as a possible cultural modification to the granules.

The chicha production stream indicates that malted maize may be found in a variety of contexts, including on grinding equipment, storage equipment, and perhaps even in dental calculus. The experiments presented here could allow for more direct evidence of chicha production, particularly for sites which have been assumed or implicated in chicha production but where no direct evidence exists, such as at Marayniyoq in Peru (Valdez et al., 2010). Recent work indicates that previously excavated, and even curated collections, may still retain economic starch (Louderback et al., 2015).

Non-ceramic artifacts should also be considered for starch analysis. Cutler and Cardenas (1947) report that *jora* was often masticated into small flour ball packages, *muko*, which could then be stored for extended periods of time. *Muko* had higher value than *jora* alone; women were responsible for the creation of *muko*, as they produced the majority (if not all) of the *chicha* in Peruvian contexts (Cutler and Cardenas, 1947; Hayashida, 2008; Jennings and Chatfield, 2009; Weismantel, 2009). These high-starch packages may leave traces within dental calculus, from which starch has been recovered in a variety of archaeological contexts (Dudgeon and Tromp, 2014; Hardy et al., 2009; Henry et al., 2012, 2011; Henry and Piperno, 2008; Li et al., 2010; Piperno and Dillehay,

2008). Recent work has indicated that the long boiling regimes, such as associated with chicha production, can lead to enrichment in heavy stable oxygen isotopes (Brettell et al., 2012; Gagnon et al., 2015), complicating analyses that look to parse immigration/migration patterns from diet, though opening a door to exploring differential fermented/boiled food consumption (Hastorf, 1991).

The experiments explored here showcased archaeological methodologies to better understand brewing technologies, though there remain many research questions yet unanswered. A more detailed sampling of the boiling process may more accurately describe the gelatinization sequence for malted, non-malted, nixtamalized, and green corn variables. Future work should evaluate how diagnostic the crushing at the hilum of maize grains during malting is. Unfortunately, traditional *chicherias* are disappearing across South America as indigenous production is increasingly replaced by commercial beverages; researchers of chicha desperately need to record production and yeast diversity and to directly test for microfossils and chemical regimes that may be indicative of chicha within the archaeological record.

5. DISCUSSION

Inspired by McGovern's (2003, pp. 56–58) methodology for identifying wine residue, I propose a generalized workflow to make decisions about how food residues found in ceramic containers may be analyzed, especially within a North American context. Multi-evidenced approaches best evaluate the different aspects of food preparation (Biwer and VanDerwarker, 2015) which is especially important for studies of fermented foods; this basic logical assessment, which includes experimental confirmation, has just started to be employed in residue analysis (Lantos et al., 2015). There are several important distinctions when thinking about New World brewing technologies, however.

Firstly, there is little evidence that fermented beverages were stored for any length of time, rather, immediate consumption seems to be the pattern. Therefore, evidence for keeping the liquid from oxygen is not necessary to withhold the conversion of wine/beer into vinegar. Secondly, there are many fermentable items that could have been used as a substrate. While some *Vitis* varieties are indigenous to North America, little ethnographic evidence has been accumulated to indicate that grape was preferred over other berries. In warmer climates, indigenous peoples utilized maize and CAM plants in the production of alcoholic beverages (Abbott, 1996); therefore, it is important the archaeologists are able to properly identify the source of the residue before assessing if fermentation was the cooking process employed. Thirdly, it is important to remember that honey made by now-ubiquitious honey bee (*Apis mellifera*) was not available in North America until after the Columbian exchange in the 1500s (Whitfield et al., 2006); nonetheless, the experiment presented here supports the utility in testing for Mayan *balche* (Bruman, 2000, pp. 91–93)

through pollen analysis. Fourthly, it is important to remember that ceramics are not a necessary technology for fermentation, as shown above, but rather that archaeological samples are simply more easily identified and isolated from pottery. Figure 7 illustrates a logical flow to assess what kinds of methods may be most appropriate for testing hypotheses about alcoholic fermentation from archaeological residues.

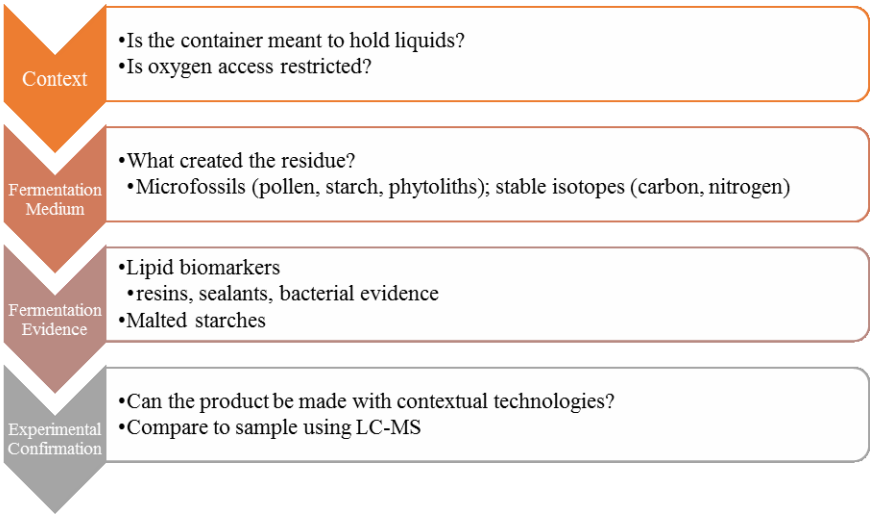


Figure 7. Workflow for assessing fermentation on ceramic residues.

5.1 Importance of Studying Fermentation

As presented above, the development of fermentation technology is not well understood in the New World. Ethnographers have consistently disregarded indigenous brewing technology as a primarily European tradition, a view exposed even by those researching Native peoples fermenting wild New World products (Buskirk, 1986; Honigmann, 1949; Waugh, 1916). If hypotheses concerning the importance of fermentation and feasting to domestication processes are to be tested, more archaeological and ethnographical research need to better address the basic, culture-historical trends in

the adoption of fermentation technology. Research presented here indicates that pre-pottery, non-sedentary brewing is technologically viable and ethnographically salient, however, the archaeological methods for recognizing such behavior are extremely limited by the transient and organic nature of those techniques. Microbotanical and chemical analyses look to be promising avenues for understanding the development of fermentation traditions in ceramic cultures, though experimental work is needed to ensure the accuracy and viability of those techniques. With increased interest in the development of feasting traditions worldwide (Tamara L. Bray, 2003; Dietler and Hayden, 2010; Hayden and Villeneuve, 2011) and their relationship to domestication (Hayden, 2014a, 2009), North American archaeologists have some catching up to do to understand the complexity of indigenous brewing technologies. Multi-evidenced approaches (Biwer and VanDerwarker, 2015) will be crucial in expanding our knowledge of cooking technologies in the past.

6. CONCLUSIONS

Alcoholic beverages have been developed independently worldwide, yet archaeological science is just now developing tools to recognize these cooking technologies. The ethnographic and archaeological record indicates that brewing technologies are not reliant on ceramic production nor agricultural subsistence strategies, but rather seem to correlate with the development of hierarchical political mechanisms (Arnold, 1999; Arnold et al., 2015; Dietler, 2006, 1990; Dietler and Hayden, 2010; Guerra-Doce, 2014; Hayden, 2014a, 2014b). In addition to ethnohistoric correlates, organic residue analysis, and DNA analysis, palynology and starch analysis are contributing to the study of ancient fermented foods. A careful review of archaeological methodologies to detect prehistoric fermentation indicates that multi-evidenced approaches are most convincing (Biwer and VanDerwarker, 2015).

In advancement of scientific methodologies to assess fermentation in the archaeological record, I presented two experiments in microfossil alteration through actualistic fermentation regimes. In the first experiment, I found that pollen profiles and cell walls are unaltered through fermentation processes, at least under light microscopy. While this implies that identification of fermentation rather than unaltered honey usage will be difficult, environmental reconstructions from honey/mead residues are secure. In the second experiment, I tracked the morphology of maize starch through chicha fermentation. I found that while malting may leave a distinctive scar on the hilum of maize starch granules, the starch is quickly gelatinized through the fermentation process. I suggest that starch-based evidence for brewing will be well supported through the

identification of malted grain. In conclusion, I present a general workflow to assess what kind of analyses may be appropriate for testing for alcoholic beverage production in ceramics in Figure 7.

Archaeological studies of indigenous brewing traditions are important to understand fermentation technologies, to identify specialized food and labor's impact on complex, hierarchical societies, and to combat problematic and hurtful stereotypes of indigenous alcohol usage. the understanding of fermentation technologies (Steinkraus, 1994), to the role that specialized food and labor motivated the development of complex, hierarchical societies (Arnold et al., 2015; Hayden, 2014b), as well as to combat problematic and hurtful stereotypes of indigenous alcohol usage (Cunningham et al., 2016).

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