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Charting a Future Course for Organic Residue Analysis in Archaeology

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Abstract Working hypotheses, which draw upon as many relevant disciplines as possible to derive the maximum information from a very limited database, are key to the highly interdisciplinary field of organic residue analysis in archaeology, a branch of biomolecular archaeology. Archaeology and chemistry are most important for effectively developing and testing such hypotheses, but botany, zoology, geology, etc. also need to be taken into account. Archaeologically, the goal is to obtain as many relevant samples as possible from the best preserved and dated contexts, which have been subjected to the least degradation and disturbance by later natural processes and human handling, including washing and conservation treatment. Chemically, molecular biomarkers of natural products need to be defined and identified by the best and most appropriate techniques, together with bioinformatics searches and assessment of degradation. With ever-improving techniques and new data, previously analyzed samples need to be retested and hypotheses possibly reformulated. Consideration of three case studies illustrates this holistic approach to inductive hypothesis generation and deductive testing: (1) new chemical findings that attest to grape wine in amphoras on board the 14th c. B.C. Uluburun ship, the earliest recorded Mediterranean wreck; (2) recently published research on beeswax/mead in Chalcolithic Israel and Neolithic China and Poland; and (3) recent articles on milk products from 2nd millennium B.C. Central Asia and Neolithic Poland. Potential pitfalls leading to weak hypotheses and mistaken conclusions are described, and a more productive approach is proposed.

Keywords Biomolecular archaeology · Uluburun shipwreck · Wine · Tartaric acid · *Pistacia* sp. (terebinth) resin · Beeswax · Honey · Mead · Milk · Cheese

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Because of its highly interdisciplinary nature, ancient organic residue analysis needs to critically assess not just the chemical data, but also the relevant data from archaeology and other disciplines as much as possible. Working hypotheses, which seek to integrate the fullest range of available data, can then be formulated and tested.

In what follows, the reader should consult McGovern *et al.* (1995) for a more detailed treatment of the relationship between the sciences and humanities in general—the seemingly irreconcilable "two cultures" of C. P. Snow—and state-of-the-art discussions of natural science-based archaeology of that period. So-called middle-range theory in anthropological archaeology, which seeks to bridge the practical and theoretical divide between natural scientists and archaeologists, also follows many of the same principles and procedures as those advocated here. A recent review article (Nigra *et al.* 2015) argues for a similar methodology and provides an extensive bibliography of both theoretical approaches and practical applications.

Three case studies are presented to buttress the argumentation. They involve different time periods (ranging from the Neolithic to the Bronze Age), different archaeological contexts (underwater and on land in tombs and settlements), different pottery types (amphoras, jars, "cornets," and strainers), different areas of the world (the Mediterranean Sea, Levant, Central Asia, central China, and Poland), and different foods and beverages derived from different natural products (including wine made from the Eurasian grape, honey mead, and other products associated with beeswax; cheeses and beverages processed from ruminant milk).

Together, these case studies highlight the necessity of applying a rigorous methodological and theoretical approach to obtain the best results from an organic residue investigation in archaeology. The proposed approach is generally applicable to archaeological sites, artifacts and ecofacts, and residues worldwide for any time period.

What are the Essential Criteria in Developing and Testing Working Hypotheses in Organic Residue Analysis?

In charting a future course for organic residue analysis in archaeology, primary emphasis needs to be placed on the following criteria:

1. As with any historical science (including geology and astronomy) which has time dimensions, biomolecular archaeology *per force* bases its hypotheses and verification procedures on extremely limited databases. For ancient organic residue analysis, archaeological, geological, and environmental factors, together with textual, artistic, and recent ethnographic considerations—each of which must be evaluated on its own terms—can variously come into play in developing a working hypothesis to understand best the chemical results.

   For example, documentary sources and artistic depictions, especially those which are contemporaneous with the mute, nontextual archaeological data, can help to "flesh out" what is otherwise equivocal from the archaeological, chemical, and other scientific data alone. In developing an amphora wine hypothesis (Case Study 1, below), very detailed ancient Egyptian frescos and reliefs of winemaking, along with wine inscriptions, provided invaluable resources for possible interpretations of the archaeological and chemical data. But, every

human text or artistic production is inherently limited by the personal experiences and current knowledge, sometimes prejudicial, of its maker(s). Specific canons of historical and artistic criticism must be applied to ferret out "truth" from "falsehood." Similarly, oral traditions in nonliterate societies, which can also shed important light on ancient practices, need to be carefully evaluated by specific criteria.

2. Unlike the hard physical sciences, past archaeological events cannot be fully replicated by experiments in the present. So-called "experimental archaeology," a branch of ethnoarchaeology, in which various possible ancient scenarios are tested for their viability, is of some explanatory value, but is usually quite limited.
3. A corollary of a highly probabilistic discipline is that *absolute* certainty or refutation of a posited working hypothesis of archaeological significance—at least one that is at a higher level of abstraction, such as a social activity, technology, or ideology, as opposed to the chemical or physical identification of a specific material or chemical compound—is unattainable.
4. Since organic residues are obtained from artifacts and ecofacts, alteration by natural or human interference since their original deposition is of special concern. Any contamination by organics of more recent date than the artifacts/ecofacts of interest should be assessed by analyzing soil background samples and the geological context, since more recent organics might have been deposited by groundwater percolation through the soil at any time between the original deposition and the present.

   The probability of changes of an artifact or ecofact residue due to chemical (e.g., hydrolysis and oxidation) and physical processes (e.g., disturbance by animals and geological activity) also needs to be assessed, since any unexplained changes may well compromise the working hypothesis.

   In general, archaeological contexts under low-oxygen and/or low-humidity conditions provide the best conditions for ancient organic preservation. The best conditions can be found deep underwater, in bogs, at high elevations or northern latitudes where materials are encased in ice and snow, in deserts, and in well-sealed and/or specially improvised contexts of human construction or processing, such as tombs and mummification. Clearly, the goal is to obtain and analyze the best-preserved and least contaminated samples, which bear most directly on the working hypothesis that is formulated and tested.
5. The artifacts/ecofacts and their residues should be as precisely dated as possible.
6. Larger databases are better than smaller ones for statistical purposes, but not if the integrity (archaeological context, preservation, dating, etc.) of the artifacts/ecofacts has been compromised in some way.
7. A "working hypothesis" in biomolecular archaeology is continually redefining itself as more sensitive archaeological, chemical, botanical, and other techniques become available and are applied. As these methods improve and new data and hypotheses are generated, archaeologists, chemists, and other researchers should test previous results and cull out any "false positives." Any deduced consequences of such modified hypotheses then need to be tested further.
8. Method development of a specific approach or technique is essential. For example, in the course of applying LC-MS-MS and GC-MS to the detection of tartaric acid/tartrate (see Case Study 1 and Appendix 1, below), we developed new

methods for their better recovery. For the first time, we monitored two transitions for tartaric acid by LC-MS-MS, rather than one, and subsequently identified the compound at 10 parts per billion using an Orbitrap detector (McGovern *et al.* 2013b), thus providing a stronger case for the presence/absence of tartaric acid/tartrate in an ancient sample.

9. Discovering unique, well-preserved biomarkers of natural products are essential to any successful ancient organic residue investigation. This methodological concern, broached by Evershed (2008), deserves special attention.

   Many compounds are not unique to a given natural product of biomolecular archaeological interest, principally because metabolic pathways and biological processes are too closely shared by related organisms. Finding and chemically testing for unique biomarkers, however, are essential for successfully pursuing ancient residue analysis. In the biological sciences, bioinformatics as a separate discipline has arisen to meet the need of searching through massive databases (whether DNA sequences or the compositions of modern natural products) for relevant chemical data on biomarkers. Search engines, based on statistical algorithms, are employed, which can be as readily available and easy to use as Google or Yahoo. For more intensive literature searches, SciFinder Scholar, PubMed, the Institute for Scientific Information (ISI), the Kirk-Othmer Encyclopedia of Chemical Technology (2004), Dr. Duke's Phytochemical and Ethnobotanical Databases (2015), and the Amber Research Laboratory's chemical database (2015) are essential resources.

   Even after assembling the available literature and reliably translating non-English articles, the biomolecular archaeologist is faced with critically evaluating the evidence. Extraction methods and analytical methods vary from laboratory to laboratory, with corresponding differences in the concentrations of reported compounds. For example, steam distillation and HPLC-UV/visible analyses are still common, but more precise techniques are now available.

   Moreover, only a small percentage of the natural products worldwide have been analyzed. Given such qualifications, one must be prepared for some "biomarkers" to be demoted because they are not sufficiently discriminating, while others are elevated as more information becomes available.

   Some compounds are better for use as biomarkers than others. For example, terpenoids are very water-insoluble and resistant to oxidation. Lipids, on the other hand, can be easily attacked and altered by hydrolytic and oxidative processes, making chemical and archaeological interpretation of the original natural products more difficult. When archaeological contexts are not carefully evaluated, the results of lipid analyses can be suspect (see Case Study 3). The better the preservation of the lipids is, the greater the potential for identifying distinctive biomarkers. For example, a series of unsaturated triacylglycerols were recovered by HPLC-MS from food residues inside pottery jars deposited in the well-preserved burial chamber of the Midas tumulus at Gordion in Turkey (McGovern *et al*. 1999). These specific lipids were biomarkers for pulses, probably lentil, which was further corroborated archaeobotanically by masses of the legume filling large jars in the kitchen storeroom of the palace.

10. As the foregoing Midas tumulus example illustrates, the establishment of natural product biomarkers will ideally be borne out by ancillary archaeological and other

scientific data. Biomarkers should also be specific to a given geographic area, and the possibility of importation of another natural product containing the same biomarker needs to be addressed and ruled out if possible.

11. Control samples—both ancient and modern—should be used as extensively as possible for assessing degradation processes and identifying the original compounds. Ideally, they should be geographically isolated to the area of interest. If studying a Chinese food or beverage, the controls should be obtained from China or nearby regions (as done in Case Study 2, below); if a putative ancient corn *chicha* from Peru is being analyzed, then samples of the beverage, made from native natural products and according to traditional methods, should be included as references; if studying ruminant milk products in ancient Polish sites, then modern samples from that country should be tested (as was not done in Case Study 3, below).
12. The nascent discipline of ancient organic residue analysis, as a sub-discipline of biomolecular archaeology, will be impeded in its development if a single chemical technique is over-stressed, as illustrated by Case Study 3.

    Large databases of well-defined modern controls and ancient "reference" and unknown samples, which can be statistically searched, are essential.

Case Study 1: Formulating an "Amphora Wine Hypothesis": Was Wine on Board the Uluburun Ship?

The 14th c. B.C. Uluburun ship (Fig. 1), excavated off the southern coast of Turkey, is the earliest shipwreck that has been discovered thus far in the Mediterranean Sea (Bass *et al*. 1989; Pulak 1988, 1998, 2008). The ship carried a rich cargo of international goods and raw materials belonging to the Late Bronze Age (LBA; ca. 1550–1200 B.C.), including 11 tons of copper and tin ingots, faience spouted "drinking horns" (Gk. *rhyta*), Egyptian scarabs and Near Eastern cylinder seals, Mycenaean drinking-cups, (Gk. *kylikes*), etc. Altogether, Pulak (2005) estimates that nine or ten cultures—extending from the western Mediterranean to Mesopotamia and from northern Europe to sub-Saharan Africa—are represented by the ship's artifacts.

The ship is likely a Canaanite merchantman for two reasons (Pulak 2005: 43–44): (1) it was constructed of cedar of Lebanon (*Cedrus libani*) according to eastern Mediterranean technology and (2) items for personal adornment, religious practice, and utilitarian use, including Syro-Palestinian gold jewelry and oil lamps, a Canaanite goddess figurine covered in gold leaf, and sets of Levantine animal-shaped stone weights—marked its crew, officers, and traders as probably hailing from the eastern Mediterranean littoral.

We might then ask: if the Uluburun ship dates to a period of intense international commerce and was built and operated by Canaanites, was it also carrying wine, as many later Mediterranean ships did, and if so, how should a working hypothesis to answer this question be developed and tested?

First, multiple lines of evidence—archaeological, archaeobotanical, textual, art historical, etc.—have to be assessed in their broader cultural context. For example, Canaanite social, religious, and economic life is generally thought to have been centered on grape wine, to such an extent that one can describe their culture as a "wine

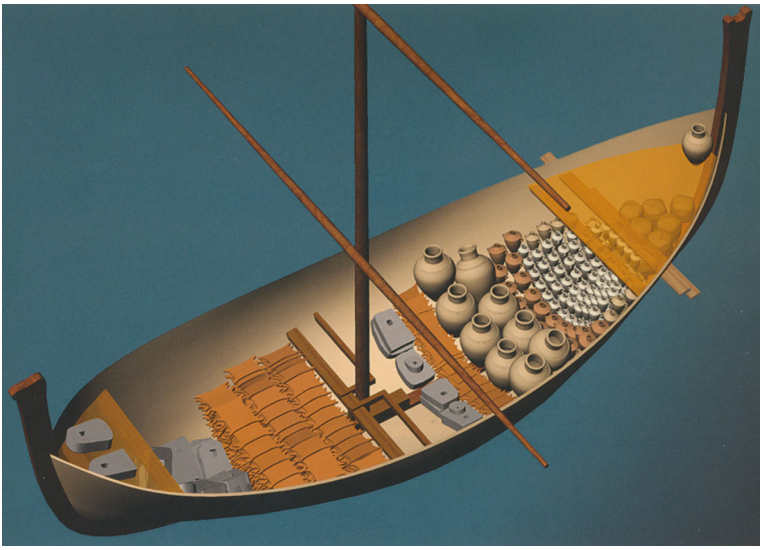

Fig. 1 Reconstruction of original arrangement of cargo on the Uluburun ship. The amphoras, grouped by size, are shown in the aft part of the ship (after Pulak 2008: Fig. 94; drawing courtesy of Shih-han Samuel Lin)

culture" (McGovern 2003/2007: chapter 9; McGovern 2009/2010: chapter 6, with additional references). If we accept this proposition and acknowledge that amphoras (or "Canaanite Jars," which are designated as amphoras from the LBA onwards; Fig. 2) were the principal means of transporting Canaanite and later Phoenician wine by ship, an "amphora wine hypothesis" for the Uluburun ship would constitute a reasonable working hypothesis, even apart from direct chemical evidence (detailed below). This hypothesis posits that some amphoras on the ship originally contained wine.

Second, in pursuing the "amphora wine hypothesis," we need to examine the more narrowly defined archaeological contexts of the ship and the amphoras and their contents. Among some of the many questions to be asked are the following. Were the artifacts/ecofacts relevant to wine recovered from their original locations (e.g., cargo hold or cabin) and to what extent were they protected from secondary disturbances by humans, other animals, and geological processes? If the artifacts/ecofacts are from primary loci, were they subjected to physical or microbial degradation or were they from protected (sealed) contexts? Do the spatial associations of the artifacts/ecofacts and/or any special features (typology, material composition, inscriptions, iconograpy, etc.) point to their function(s)? Were they contaminated by any materials external to the ship before or after excavation? Ideally, the goal is to obtain the best-provenienced, best-dated, best-preserved, least contaminated, and most significant artifacts/ecofacts that bear on the working hypothesis.

Archaeological Specifics of the Uluburun Ship and the Amphoras and Their Contents

The general archaeological context of the Uluburun ship itself, as well as its cargo, were far from ideal. After the ship went down, it came to rest on a steep slope averaging 30° see (Pulak 2008: fig. 91–93). Unlike other Mediterranean shipwrecks (e.g., the 4th c.

B.C. Kyrenia ship, excavated off the northern coast of Cyprus—Katzev 2007), the Uluburun ship did not sink in its entirely into a soft, protective layer on the sea floor. With the exception of some loci which were covered by sand, planks, or other debris, artifacts/ecofacts have traveled downhill over the past nearly three and a half millennia.

A group of approximately 150 amphoras were recovered from the ship, of which 84 were intact and 60 or more were broken and had lost their contents. None of the amphoras had stoppers in place, although 10 possibly retained stopper remnants (below). Our organic residue analyses (detailed in sections C and D, below) focus on resin of *Pistacia* sp. (probably terebinth) inside five intact amphoras. Before addressing our chemical findings, it is important to assess the bearing of the archaeological contexts and other evidence, such as the archaeobotanical, on the "amphora wine hypothesis."

The 150 amphoras fall into three general types by size (Pulak (2005: 38): small (ca. 7 L volume), medium (ca. 15 L), and large (ca. 30 L). Although they are presumed to have originally been grouped by size in the ship's hull (Fig. 1), they were found spread out along the sea floor (Fig. 3). Publication of the shipwreck is ongoing and should provide more details of the original locations of the amphoras and their subsequent dispersion, along with their contents.

The 84 intact amphoras all contained nodules and chunks of *Pistacia* sp. (most likely terebinth) resin in varying amounts. Several jars were a quarter to half filled with the resin, weighing as much as a kilogram, but most of the jars had less than 100 g (C. Pulak, pers. comm., Sept. 22, 2008). Pulak (2005: 38) has reported that about 500 kg (half ton) of resin was carried on the ship, basing this calculation on 84 intact amphoras,

Fig. 2 "Canaanite jar" or amphora of small type (Photograph slide# KW-4176 courtesy of the International Nautical Institute)

Fig. 3 C. Pulak excavating a gold chalice, beside an amphora, on the seabed (Photograph slide# KW-1127 courtesy of the International Nautical Institute)

each with a capacity of at least 6 L, having been originally full of resin. The amount of resin actually recovered by excavation, however, was much less and likely in the 10–30-kg range, pending a final tally (C. Pulak, pers. comm., April 19, 2012).

Clusters of grape seeds were also recovered from inside some amphoras (the final tabulation is to be published by the expedition's archaeobotanist, Cheryl Haldane Ward). Indeed, grape seeds were the most prolific, preserved botanical remains on the ship and were scattered everywhere, "throughout the site and also, but in lesser amounts, in some of the jars" C. (Pulak, pers. comm., Sept. 22, 2008).

The clustering of materials inside some amphoras implies that, although they might eventually have lost their stoppers and much of their contents, some of their original contents might have been retained. For example, one amphora with more than 2500 olive pits was probably originally filled with whole olives, and other very large jars (Gk., *pithoi*) contained numerous glass beads, specialty pottery, and likely pomegranate fruit (based on 1000 seeds and skin fragments).

Ten intact amphoras also contained the remains of what could have been their original stoppers (Pulak 1988: 10–11), which would have minimized loss of their contents or intermixing of contents between amphoras after the stoppers were lost or disintegrated. In the bases ("toes") of these amphoras was a raw clay conglomerate, mixed with terebinth resin and fruit, carbonized grape seeds, weed and other plant seeds, grass and twigs, and other materials, covered by a pottery sherd.

Clay stoppers made of mixed plant remains and an overlying sherd are well-attested for wine amphoras of contemporaneous New Kingdom Egypt (Hope 1978; McGovern 1997). If the Uluburun amphoras were stoppered by comparable closures, they might have imploded and partly dissolved under pressure in the sea water, causing heavier materials to sink to the amphora bottoms. Moreover, if the jars originally contained wine, which was produced in the same region as the stoppers, then it might be expected that other natural products available there, especially terebinth resin and grape seeds, might have been incorporated into the stoppers' clay matrix. The carbonization and

segregation of the grape seeds in the clay conglomerates of the 10 amphoras also suggest that they are to be explained differently than the numerous uncarbonized specimens found elsewhere.

Other explanations for the conglomerates in the 10 jar bases are possible: they might have served to strengthen the bases, which are often the weakest part of a pottery vessel, or less likely, the jars might have originally been filled with the mixed material, which then was mostly lost.

On balance, the stopper hypothesis appears to best explain the carbonized grape seeds and other botanical debris, including terebinth resin, and the pottery sherds in the conglomerates, which accords with the known contemporaneous composition of Egyptian stoppers.

Even if some amphoras were less liable to cross-contamination, Cheryl Haldane (1993) cautions that "as a shipwreck disintegrates, residues of spilled cargoes, both organic and inorganic, get into shipping jars" and cause "substantial mixing of these diverse contents within the hull…further complicating the interpretation of the original vessel contents." Mixing can also occur when plant dunnage materials, which might originally have cushioned the amphoras, subsequently found their way into the jars when their stoppers were lost. Contamination by natural sea vegetation and the possible intrusion of nearby modern garbage are additional possibilities. The Byzantine Yassiada shipwreck (Ward, C. Plant remains from the old wine jars on the Byzantine ship at Yassiada. In F. H. van Doorninck, Jr.(Ed.), *50 years of a Byzantine voyage: Yassiada II*, forthcoming) and the 11th c. A.D. Serçe Limani shipwreck (Ward 2004) provide examples of such interpretative difficulties.

Cemal Pulak (pers. comm., Sept. 22, 2008) concurs in Ward's assessment when he writes that "none of the Uluburun jars were found with their sealings intact during excavation....I suspect that much of the spilled contents of the jars, as well [as] most of the ship's festering organic cargo would have mixed together in the hull like a bathtub filled with fruit punch!"

The "Amphora Wine Hypothesis" Vis-à-Vis Terebinth Resin

Since all 84 of the intact amphoras recovered from the Uluburun shipwreck contained some terebinth resin, how does the latter enter into our working hypothesis?

While terebinth resin was renowned in antiquity for its use as a medicinal and mummification agent and for incense, it was also highly regarded as a preservative for wine (Pliny the Elder, the 1st c. A.D. Roman encyclopedist, in his *Historia naturalis*, book 14, describes it as the "queen of resins" for this purpose; also see McGovern 2003/2007: 70–72, McGovern 2009/2010: 75–76, and McGovern *et al*. 2013a, b). Tree resin as a wine preservative can be considered as the ancient equivalent of adding extra hops in making India pale ale or fortifying sherry and port with alcohol in more recent times. In classical Greece, about 100–200 g of pine resin was the standard amount for resinating 25 L of wine (Hostetter, *et al.* 1994), comparable to the amount in most of the intact Uluburun amphoras. Today, generally half or as little as a sixth of this amount of resin (usually Aleppo pine = *Pinus halepensis*) is added to Greek *retsina* wine.

Based on this information, a corollary to the "amphora wine hypothesis" can then be proposed, viz., a small amount of resin (less than 100 g) in an amphora might represent either undissolved resin of a resinated wine and/or extra resin added to the wine as a preservative for the journey. Adding more rather than less resin to an amphora could also have been more deliberate, since the additional resin would have no negative

effects on the wine (which can solubilize only so much resin) and after the wine was decanted from an amphora, the resin could have been dried and used for other purposes.

Therefore, most of the intact amphoras, which had less than 100 g of resin, and an unknown number of the broken "empties" might originally have contained wine for trade and gifting in the eastern Mediterranean (McGovern 1997: 86, McGovern 2003/2007: 130; McGovern 2009/2010: 174). Additionally, some wine might well have been set aside in amphoras for the crew and officers on an extended voyage, probably lasting months.

This working hypothesis allows for the possibility that some amphoras, particularly the several jars which were a quarter to half filled with resin, were filled exclusively with terebinth resin. A stock of resin might be needed for emergency repairs to the ship or to preserve cargo goods, such as fresh fruit. Amphoras full of resin have been documented for other Mediterranean shipwrecks, viz., the 5th c. B.C. Tektaš Burnu ship (Carlson 2003), which is believed to have been carrying over 1500 amphoras when it went down off the western coast of Turkey. While the vast majority of the amphoras on this ship are interpreted as containing wine for which the region was famous and as corroborated by grape pips inside the vessels and stamped impressions on the exteriors of the amphoras, a group of nine amphoras of different origin were 40 % full of a pine resin tar according to chemical analyses by Curt Beck of Vassar College's Amber Research Laboratory. Large globules of the tar found nearby likely spilled out from these jars. Similarly, it can be hypothesized that while the majority of the Uluburun amphoras held resinated wine, some contained only terebinth resin.

Preliminary Testing of the "Amphora Wine Hypothesis" by Organic Residue Analysis

The "amphora wine hypothesis" for the Uluburun ship might be variously tested. For example, the proposed sea route of the Uluburun ship might be surveyed to locate better preserved contemporaneous ships, whose amphoras are still stoppered. Amphoras with wine inscriptions might even be discovered. The likelihood of finding another such LBA ship, however, is remote.

More realistically, an organic residue analysis of the Uluburun amphoras, many of which might have contained wine based on multiple lines of evidence (above), might be carried out to obtain chemical evidence for their original contents. We chose this approach and report here on our preliminary and definitive results for the presence/absence of tartaric acid/tartrate (the biomarker for grape/wine, below) inside five of the 84 intact amphoras. Because these jars had narrow mouths, albeit lacking stoppers, and contained relatively large amounts of resin, they were less likely to have been contaminated.

The ideal approach would have been to analyze a representative group of amphoras by solvent extracting pottery sherds and/or interior residues of broken "empties" and intact amphoras containing less than 100 g of resin, in addition to only resin inside five amphoras. Additional samples, however, have thus far been unavailable. The poor archaeological contexts of the five amphoras were an additional deterrent, but because of the ship's exceptional nature, a limited study appeared justified.

Our initial archaeological and organic residue study of the Uluburun amphoras was carried out in 1996, using small fragments of the terebinth resin chunks (ranging from

0.2 to 1.7 g in weight and up to 5×5×10 mm in size) from inside five intact amphoras, which were unusual in containing a larger amount of resin, viz., ca. 200–300 g as contrasted with the usual less than 100-g amount.

Our principal goal initially was to obtain a reference Fourier transform infrared spectrometric (FT-IR) spectrum for ancient terebinth resin, to be included in our database. All the Uluburun samples (KW 102, KW 144, KW 165, KW 181, and KW 215—see Table 1) showed only the characteristic absorptions of this tree resin.

Our FT-IR database, which is crucial to our ancient residue analysis program, also includes other relevant natural products and processed organic materials, synthetic compounds, modern wine samples, and "ancient wine reference samples," i.e., ancient vessels which likely originally contained wine as based on strong archaeological criteria or exterior inscriptions which recorded their contents. Among the latter is the especially relevant group of inscribed wine amphoras from the Palace of Amenhotep III at Malkata (Egypt), which dated to the same century as the Uluburun ship (McGovern 1997: 80–83, spectrum for KW 144 on fig. 3). Because the latter were extracted with methanol to concentrate any tartaric acid/tartrate present, FT-IR absorptions for both tartaric acid/tartrate and terebinth resin were obtained.

Subsequently, and in light of other findings that resinated wine was common through the ancient world for millennia, two samples (KW 144 and KW 181) were tested for the principal biomarker or fingerprint compound of the Eurasian grape species, *Vitis vinifera*, in the Middle East and Mediterranean area: tartaric acid and its derived tartrate salts (tartaric acid/tartrate). Only the Eurasian grape in this region has very high amounts of this acid (about 4 g/L: Singleton 1995: 67). Other natural sources with high amounts of the acid—e.g., Chinese hawthorn fruit and New World yellow plum—were too far away to have been traded during the 14th c. B.C. (see McGovern *et al*. 2013a, b), and activity by microorganisms to produce more than trace amounts of tartaric acid/tartrate were unlikely under water.

We employed a very specific Feigl spot test for tartaric acid/tartrate, and the results were illuminating, if not definitive. KW 181 gave a strong green fluorescence, a positive for tartaric acid/tartrate, whereas KW 144's fluorescence was relatively weak

Table 1 Comparison of preliminary and LC-MS-MS Penn Museum laboratory analyses of Uluburun resin samples vs University of Bradford laboratory results

Ulu Burun resin sample (amphora no.)	Penn Museum laboratory preliminary results	Univ. Bradford laboratory results	Penn/TTB[a] laboratories LC-MS-MS results
KW 48	Sample unavailable	Negative	Unavailable
KW 102	Inconclusive FT-IR	Negative	Borderline positive
KW 144	Borderline positive Feigl test and inconclusive FT-IR	Negative	Negative
KW 165	Inconclusive FT-IR	Negative	Negative
KW 181	Positive Feigl test and inconclusive FT-IR	Negative	Positive
KW 215	Inconclusive FT-IR	Not run	Positive

[a] *TTB* Tax and Trade Bureau, Beverage Alcohol Laboratory

and assigned as borderline positive. We obtained these results without extracting the resin samples with methanol.

The reason that these results were inconclusive was because we had not followed our usual protocol at that time by employing three independent, complementary chemical techniques—viz., FT-IR, high-performance liquid chromatography (HPLC), and the Feigl spot test—to test for tartaric acid/tartrate. Our pioneering approach (Badler *et al*. 1990; McGovern *et al*. 1996, 2001, 2007) stressed the need to do all three tests. If any were negative, that would raise questions about the identification of tartaric acid/tartrate using the other methods. Moreover, any positive identification of tartaric acid/tartrate needed to be followed up by applying important archaeological and other scientific criteria, together with deductive testing, to make the case that the ancient grape product was wine and not another liquid grape product such as vinegar or a concentrate (see McGovern *et al*. 2013a and b).

In summary, our preliminary results for two resin samples were positive (KW 181) and borderline positive (KW 144) for tartaric acid/tartrate by the Feigl spot test (Table 1). The FT-IR results were inconclusive for all five resin samples, most likely because we did not extract the samples using methanol and any tartaric acid/tartrate absorptions were buried beneath the intense terebinth resin peaks. We did not analyze any sample by HPLC.

Since this study was not in keeping with our recommended approach, we did not publish the results in detail and only tentatively alluded to them and the proposed "amphora wine hypothesis" in scholarly and popular publications as a stimulus for future research.

More Definitive Organic Residue Evidence for the "Amphora Wine Hypothesis"

In 2007, we began to use a much more sensitive chemical method for detecting tartaric acid/tartrate: liquid chromatography tandem mass spectrometry (LC-MS-MS). Researchers at the University of Barcelona (Guasch-Jané *et al*. 2004), prompted by our earlier work on ancient wine, unequivocally identified this marker compound in well-provenienced and well-preserved amphoras from the tomb of Pharaoh Tutankhamun, which coincidentally dated to the same century as the Uluburun and Malkata amphoras (above). The Tutankhamun amphoras also bore inscriptions identifying them as wine amphoras.

In our ongoing endeavor to develop more precise chemical techniques, we collaborated with the Beverage Alcohol Laboratory of the US Treasury's Tax and Trade Bureau (TTB) and the University of Barcelona group to reanalyze by LC-MS-MS the two resin samples, for which we had preliminarily obtained positive results for tartaric acid/tartrate, in addition to three other samples.

Before carrying out the Uluburun analyses, we first reanalyzed one of the earliest "wine jars" from Egypt, which had been recovered from King Scorpion I's tomb at Abydos, dated to ca. 3150 B.C. (McGovern *et al*. 2009). Using a new extraction procedure, it was shown to be positive for tartaric acid/tartrate by LC-MS-MS (see Appendix 1, for details). Doubt about whether these jars contained wine, for which there was considerable supporting archaeological and archaeobotanical evidence, had been raised by a group in the Archaeological Sciences Department at the University of

Bradford (Boulton and Heron 2000, as part of Murray 2000). This group was even more skeptical about any of the Uluburun jars containing wine (Stern *et al*. 2008).

Our new LC-MS-MS results, however, were *conclusive* in showing that tartaric acid/tartrate was present in both the Abydos jar and several of the terebinth resin samples from the Uluburun amphoras (Table 1). How could these findings be reconciled with those of the Bradford group? The answer to that question has significant implications for the current practice of ancient organic residue analysis in developing and testing working hypotheses.

Figures 4 and 5 show the chromatograms of the five Uluburun samples that we analyzed by LC-MS-MS. KW 181 and KW 215 are clearly positive for tartaric acid/tartrate, whereas KW 102 is borderline positive and KW 144 and KW 165 negative. The borderline Feigl positive for KW 144 (above) is possibly explained as due to sampling one part of a heterogeneous chunk which had come in contact with tartaric acid/tartrate and another part, analyzed by LC-MS-MS, which had not.

KW 215 was also analyzed for tartaric acid/tartrate using gas chromatography–mass spectrometry (GC-MS) and gave a borderline positive result near the limit of detection (see Discussion and Appendix 1, below).

Discussion

Our analysis of terebinth resin samples from five intact Uluburun amphoras, using state-of-the-art LC-MS-MS and new extraction protocols (Appendix 1), conclusively identified tartaric acid/tartrate, the biomarker of grape and wine, in two samples and possibly a third. These results did not agree with those of Stern *et al*. (2008), who argued that our earlier, admittedly less precise, chemical methodology was flawed. In trying to prove a negative, however, they opened themselves to similar scrutiny. Their investigation underscores the potential pitfalls into which an ancient organic residue investigation can fall, as follows:

1. Stern *et al*. do not sufficiently appraise the *archaeological* contexts of the terebinth resin samples, which is crucial in obtaining relevant data to back up their contrary hypothesis. They state (p. 2188) that "120 of these coarse-ware ceramic transport jars [amphoras] were 1/4-1/2 full of resin," but the excavator (Pulak, pers. comm., Sept. 22, 2008) reports that the number was much less and that generally less than 100 g were recovered from the 84 intact amphoras with resin. The case for the mass shipment of resin (Stern *et al*. 2008: 2202) is correspondingly much reduced, leaving open the possibility that some of the jars with lesser amounts of resin might have originally been filled with resinated wine with undissolved resin and/or had extra resin added as a preservative.

   The terebinth resin in the amphoras could even have come in contact with a grape product prior to shipment, as implied by the carbonized grape seeds mixed together with the resin in the raw clay conglomerates in the bases of 10 better-preserved amphoras (above).
2. Stern *et al*. do not mention that all the Uluburun amphoras, as found, lacked intact stoppers or other closures. They do not discuss the extensive mixing of the contents within the hull of the ship. Wine or another grape product—fresh grapes, raisins, vinegar, etc.—could have spilled from one amphora into another when the ship

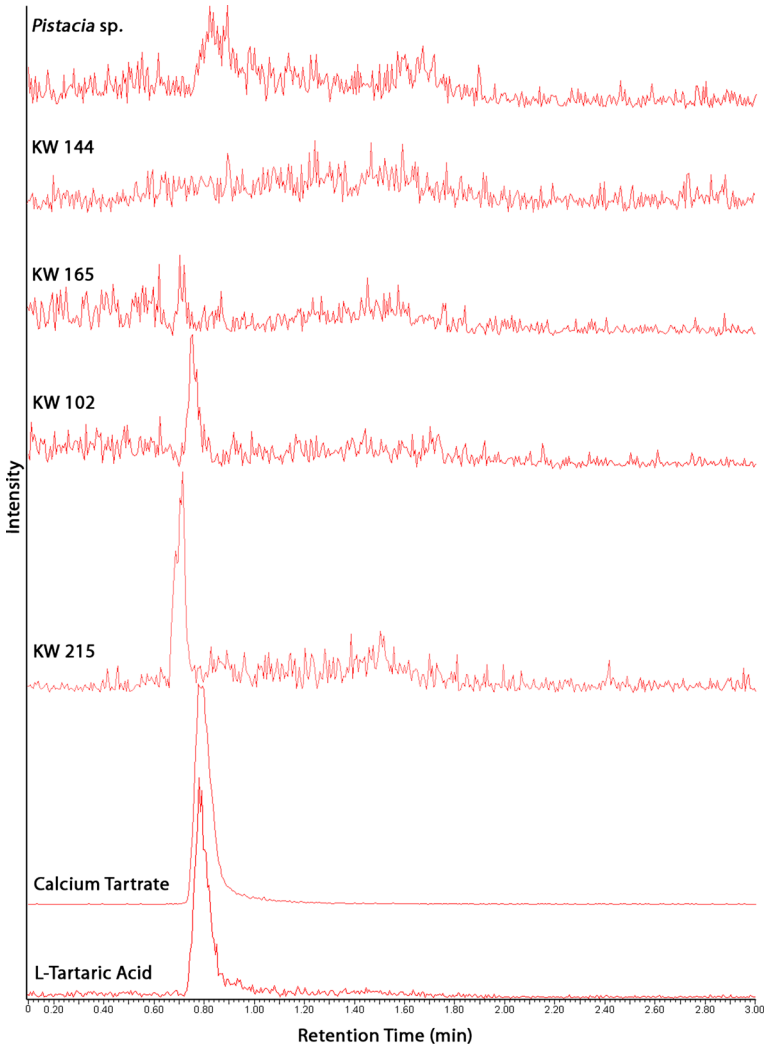

Fig. 4 LC-MS-MS MRM traces for the 149→87.1 transition of modern standards and ancient Uluburun *Pistacia* sp. samples. Although not shown, the 149→73 transitions confirmed these results. The tartaric acid standard (2 ppm) has a peak intensity of 25,500 cps, and the other chromatograms have a maximum of 4800 cps. Note that KW 215 and KW 102 are, respectively, positive and borderline positive for tartaric acid/tartrate; KW 165 and KW 144 are negative. The estimated concentration of tartaric acid/tartrate for KW 215 is 0.25 ppm. The *Pistacia* sp. samples were extracted with an ammonium hydroxide/methylene chloride mixture, according to the protocol described in the text

went down and during some 3400 years thereafter. More recent materials might also have contaminated the amphoras' contents.

3. The Bradford group discounts the Feigl spot test for tartaric acid/tartrate, because they were unable to obtain "positives" for any of their ancient samples. Since their modern wine sample controls (Stern *et al.* 2008: Table 1) were also "negative" but should have been positive, several possibilities singly or in combination may account for the discrepancies: (a) they might have directly tested the resin, rather

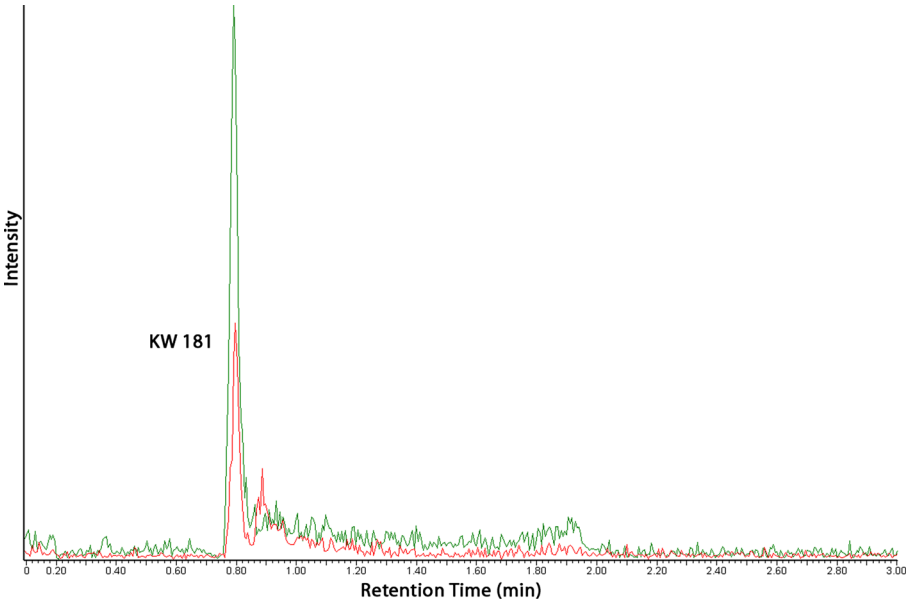

Fig. 5 Tartaric acid/tartrate was absorbed into and recovered from Uluburun *Pistacia* sp. sample KW 181, as conclusively shown by the two LC-MS-MS MRM transitions of tartaric acid: 149□87.1 (*upper trace*) and 149→73 (*lower*). The estimated concentration of tartaric acid/tartrate is 1 ppm, and the maximum of the 149→87.1 trace corresponds to 21,400 cps. The sample was extracted with an ammonium hydroxide/methylene chloride mixture, according to the protocol described in the text

than extracting it first, which is needed to concentrate any tartaric acid/tartrate present (see above); (b) the heterogeneity of a resin chunk might cause one sample to test positive and another negative; and/or (c) their experimental methodology is questionable.

4. Stern *et al.* (2008: 2198) state that infrared spectrometry cannot identify tartaric acid/tartrate, because "this technique lacks the ability to distinguish subtle differences within the carboxylic acid and carbonyl absorption regions and *cannot* [emphasis added] be used to identify the presence of wine." Yet, the Bradford group has failed to see not just the subtle differences in these regions in their own spectra of L-tartaric acid, syringic acid, and terebinth resin (Stern *et al.* 2008: Figs. 2–4), but have overlooked quite considerable differences that distinguish them while also contradicting themselves (see Appendix 2).

   Since mixtures of compounds can be equivocal for FT-IR spectra, they need to be deconvoluted by statistical methods and scrutinized for the presence/absence of key absorptions. If a known absorption for a compound is lacking, that compound can be excluded as a possibility. The IR spectra also should be searched for "matches" against large databases of relevant natural products and processed organic materials, synthetic compounds, modern wine samples, and "ancient wine reference samples" (above). It is also essential to extract samples which are dominated by another material, such as terebinth resin, before running the FT-IR analysis. It is unclear whether the Bradford group followed such procedures, thus contributing to equivocal results.

5. Stern *et al.* (2008: 2198) state that they identified the trimethylsilyl derivative of tartaric acid by GC-MS but do not provide details for the ancient samples tested or any modern wine reference standards. Their methodology is unclear, since they also describe (p. 2192) converting the acid to the methyl ester, which is too polar to elute through the column. We were only able to tentatively identify the key silylated tartaric acid ion in sample KW 215 (Appendix 1).
6. In general, as already touched upon above, the extraction procedures of Stern *et al.* are unclear. If their terebinth resins were sampled directly, tartaric acid/tartrate concentrations would have been greatly diluted. Tartaric acid/tartrate is also sometimes tied up in polar matrices, together with polyphenolic complexes (e.g., tannins and pigments), and is only released by acid or base pretreatment (i.e., a stronger agent than the 0.1 % formic acid of the Guasch-Jané *et al.* 2004 protocol–see Appendix 1). Similarly, the less polar terebinth resin requires special extraction methods to release and solubilize the tartaric acid/tartrate. For GC-MS, Stern *et al.* used a 5-min sonication of the ancient and modern sherds at room temperature, but a 20–40-min boiling or overnight immersion (as is used in our refined LC-MS-MS extraction protocol—Appendix 1) is better able to obtain sufficient ancient organics for analysis.

   In short, arguing for absolutely no wine on board the Uluburun ship, in light of our new LC-MS-MS evidence and the flawed argumentation of Stern *et al.* (2008), is like having a Bavaria without beer or a Newcastle without coal.

Case Study 2: Chalcolithic Beeswax, Honey, and/or Mead?

The second case study focuses on the interpretation of a sequence of C_{23} to C_{33} hydrocarbons, which were obtained from extracts of pottery "cornets" (cone-shaped vessels) from occupational and burial contexts in Israel (Namdar *et al.* 2009), dated to the Chalcolithic period (ca. 4700–3700 B.C.), and of pottery jars from occupational and possible ceremonial contexts at the site of Jiahu in the Yellow River valley of central China (McGovern *et al.* 2004), dated to Neolithic period (ca. 7000–5500 B.C.). In both instances, the well-known series of only odd C-number hydrocarbons typical of beeswax had intervening, unexplained even-numbered compounds, albeit at lower concentrations than the odd-numbered compounds. Evershed (2008: 899) questioned the working hypothesis that beeswax could account for the odd/even sequence, concluding that the latter was a "textbook *n*-alkane distribution characteristic of petroleum, wherein both odd and even carbon number homologues are present at similar abundance" in the soil. He concluded that "the null hypothesis must be accepted—these do not unambiguously derive from beeswax."

Namdar *et al.*, however, were able to test the soil matrix surrounding the cornets and found no traces of petroleum. Follow-up experiments by Namdar *et al.* showed that the distribution could be produced by heating beeswax in the presence of other beehive constituents (probably bee cuticles) or by aging processes. An ancient pottery beehive from Tel Rehov (Israel), dated to Iron Age IIA, gave a similar odd/even distribution of hydrocarbons.

Although no background soil samples could be run for the Chinese samples to rule out contamination by a petroleum product, similar explanations as proposed by Namdar

et al. might well apply to the alkane distribution there. McGovern *et al*. (2004: 17,596) also discuss the possibility that plant epicuticular wax, which occurs on the surfaces of leaves and fruits of many plants, might explain the distribution, especially for senescent or fossilized plants and, by extension, archaeological samples derived from degraded plant materials in which n-alkanes represent a small percentage of the total sample.

While Namdar *et al*. argue that the cornets were likely filled with beeswax for illumination, another possibility is that the horn- or goblet-like vessels, six of which came from a "cult building" at En Gedi, might have originally contained honey or a fermented beverage made from it. In processing honey, which often includes heating it, some beeswax is always left behind after filtering. The honey would probably have fermented if it were diluted down, because osmophilic *S. cerevisiae* in honey, which can tolerate high sugar concentrations, would then become active.

In brief, the entire n-alkane series, which are not at "similar abundance" (Evershed 2008: 899), was examined, probed, and tested for possible biomarkers, and working hypotheses were proposed in accord with the available archaeological and other evidence. Evershed's null hypothesis fails, because it demands that the chemical composition of ancient beeswax be identical to that of modern beeswax, without taking other factors into account.

Besides the issue of whether the degradation could have an effect on the n-alkane distribution, archaeological and stylistic considerations also come into play in developing, testing, and refining working hypotheses. The form and arrangement of pottery beehives in Israel, for instance, are well-attested on ancient Egyptian tomb reliefs. Because of close ties between Egypt and the southern Levant in the Bronze and Iron Ages, the technology might well have been transferred to the northern Jordan Valley. If a strong archaeological case can be made for these jars being beehives, then remnants of beeswax should be present and identifiable. Similarly, the Chinese jars that were analyzed were narrow-mouthed and most likely used for storing and/or serving a liquid. Mead, a fermented beverage made from diluted honey (approximately 80 % water to 20 % honey), is an ideal candidate to account for the combined archaeological and chemical evidence.

Case Study 3: Milk, Cheese, Fermented Kefir, or Another Natural Product?

The third case study involves biomarkers for ancient milk and cheese associated with mummies from Central Asian tombs in 2nd millennium B.C. China (Yang *et al*. 2014) and from residues inside strainers and jars in occupational contexts of Neolithic Poland (Salque *et al*. 2013), dated to ca. 5200–4800 B.C. The Yang *et al*. article employs a state-of-the-art, extremely sensitive method—proteomics characterization by LC-MS-MS, together with FT-IR and elemental analyses—to *directly* identify specific protein biomarkers of fermented Asian cattle milk products, viz., kefir cheese and beverage. Kefir is one of numerous traditional fermented milk beverages found throughout Eurasia, including Central Asia (*koumiss*), Finland (*viili*), and Tibet (*tara*) (Katz 2012). In Poland today, kefir is unknown, but sour milk is drunk ((Ł. Łukasz, pers. comm., March 13, 2014).

Some background on kefir is helpful before discussing the basis and validity of the ancient Chinese study. Kefir is particularly associated with Transcaucasia and is made by inoculating milk with a unique symbiotic bacterial-fungal colony (so-called SCOBY=Symbiotic Colony of Bacteria and Yeast), to produce variously sized "grain" agglomerations and a thick, sour, effervescent, and mildly alcoholic liquid, sometimes referred to as the "champagne of milks." With extended fermentation, grain size, sourness, and the alcohol content of the beverage (ca. 0.5–3 %) increase. Both solid and liquid kefirs are flavorful, highly proteinaceous, and probiotic. Solid kefir cheese is low in lactose, because the whey has been removed. The kefir drink, which retains more whey, still has less lactose than milk, because some of lactose is fermented to alcohol and lactic acid. Both products keep well and can be consumed by lactose-intolerant populations, which are now known to have prevailed in ancient Eurasia and still predominate in Central Asia (Itan *et al.* 2009).

The working hypothesis of the Yang *et al.* article combines archaeological, chemical, and other lines of evidence to make a very persuasive and powerful case for detecting milk biomarkers, which are best interpreted as kefir. Biomarkers for SCOBY fermentation are well-attested, including lactic acid bacteria, *Saccharomycetaceae* yeasts, and molds primarily of the *Aspergillus* genus. Their association with the cattle milk proteins (caseins) leaves little doubt that the cheese that was analyzed was kefir cheese. The latter was recovered intact, remarkably well-preserved, in well-defined, well-dated 2nd millennium B.C. archaeological contexts, viz., near the necks or chests of mummies, which were wrapped in bear skins and inside wooden coffins. Zooarchaeological evidence provides corroborating evidence that the people were cattle herders.

The Yang *et al.* article provides new insights into the processing of ancient milk products, which remain to be tested further and, if confirmed, will strengthen their subsidiary hypotheses that the ancient kefir cheese and kefir-like beverage were generally made from skim milk by physically removing milk fats, that the kefir was likely not brined, and that rennet (a complex of enzymes found in ruminant stomachs) was not used to curdle the casein proteins.

It should be noted that Yang *et al.*'s methodology and direct detection of milk proteins have been most recently borne out by a comprehensive study of Old World milk products, using β-lactoglobulin which is well-preserved in dental calculus as the biomarker (Warinner *et al.* 2014).

By contrast, the archaeology and chemistry underlying the Salque *et al.* paper are less rigorous, and consequently, their working hypothesis is more open to question.

Salque *et al.* employ only one chemical technique—GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to identify primarily lipids. Two fatty acids that are widespread in nature—palmitic and stearic—are targeted and argued to have specific $\delta^{13}C$ isotope values for specific natural products.

This methodology is underlain by several crucial, debatable assumptions:

1. That the proxy correction factor ($\Delta^{13}C$) for "exogenous factors linked to the environment is correct. This factor assumes that the depletion of ^{13}C is due to specific C3 and C4 metabolic pathways in plants and microorganisms and that the same lipid synthesis pathways via acetyl coenzyme A are common to all animals (DeNiro and Epstein 1977: 262–263). But, other pathways exist and have been

shown *in vitro* to cause $\delta^{13}C$ values to vary by 5–10 %, implying that at least two sources of acetyl coenzyme A with different metabolic pathways are involved (Hayes 2001: 266).

2. That palmitic and stearic acids do not move appreciably in the groundwater of a well-watered region, such as Poland, and might cause admixture of ancient and modern carbon sources, including plants and microorganisms. Such amphiphilic, small compounds, even though relatively insoluble in water, might still be transported, especially since many of the samples were recovered from pits and trenches rather than dry, well-provenienced (ideally, *in situ*) archaeological contexts.
3. That the modern reference samples from Britain, North Africa, and Central Asia can be combined together for comparison to the ancient Polish samples. No reference materials for Poland are reported.
4. That the added precaution of removing the outer surface of the pottery lowers the risk of contamination. Neolithic earthenware fabric was quite porous and absorbent (P. Bogucki, pers. comm., March 10, 2014), so that groundwater would probably have penetrated through the entire cross section of a strainer (about 1.5 cm thick for the examples shown in Fig. 1 of Salque *et al.*).

Salque *et al.* do not provide any additional chemical confirmation for their lipid biomarkers by complementary, independent techniques (e.g., FT-IR, HPLC, and LC-MS-MS). *Direct* detection by LC-MS-MS of milk proteins and those characteristic of specific fermentation microorganisms, as was done by Yang *et al.*, would be most definitive. If their interpretation of the GC-C-IRMS isotopic evidence is correct, then primarily dairy fats are attested. In that case, the detection of cholesterol would be anticipated, but it is not reported and its absence not explained. Nor were biomarkers for other possible natural products (fruits, cereals, herbs, etc.) sought, with the exception of those detected by GC-MS, including beeswax.

The working hypothesis of the Salque *et al.* paper is that Neolithic strainers from Poland were used to separate the liquid whey from the solid casein curds of cattle milk to make cheese. Their chemical evidence, however, goes only part way in support of the cheese-making hypothesis. No cheese per se was recovered from any of the strainers or elsewhere on the sites, such as that reported in the Chinese study (above). Possibly, some remnants were retained in the material shown filling the small holes of Fig. 1d (ca. 2–3 mm in diameter) or other examples, but no mention is made of collecting and analyzing this material.

Salque *et al.* cite zooarchaeological evidence for ruminants, primarily cattle, at the Polish sites. Yet, because of the poor preservation of the bones in the acidic loess soil, distributions of old/young and male/female animals, which might shed light on whether the cattle were used primarily for dairying is uncertain, and other purposes, such as traction, transport, and/or a source of meat and hides, cannot be ruled out (Bogucki 1984).

Salque *et al.* also compare the ancient strainers to modern plastic cheese strainers from Poland and 19th-20th c. traditional types from France and Vermont. But, the modern Polish examples are more similar to colanders (with solid bases), rather than their ancient "counterparts" which combine a strainer and funnel (see Fig. 1a; confirmed by P. Bogucki, pers. comm., March 10, 2014). Since most of the ancient strainer sherds were from the bodies of vessels, with very few rims and bases represented, only

one other strainer type could be stylistically defined: a perforated bowl (P. Bogucki, pers. comm., March 10, 2014).

The funnel of the ancient strainer-funnel type suggests that the liquid collected during straining was as important as the solid, since it assured that liquid was directed into a lower container. To prevent solids with dimensions less than the diameter of the funnel holes (about 1.5 cm for Salque *et al.*'s Fig. 1a example) from passing through, the funnel hole would need to have been filled or covered with a porous material with smaller diameter openings, such as grass, raw wool, or a textile.

If the zooarchaeological results and ethnographic parallels are arguable, it becomes all the more essential to provide definitive chemical evidence by other techniques, such as those employed by Yang *et al*. Such corroborative data might well shed light on whether any cheese made in the strainer-funnels was kefir-like or one made by first coagulating the milk caseins and then ripening them with a different fermentation culture. The latter scenario presupposes that Neolithic peoples of northern Europe already had the technology to curdle milk, using a complex of enzymes (rennet) taken from ruminant stomachs or by other means (e.g., elevated acid by bacterial fermentation or a vegetable coagulant). An advantage of a kefir-like liquid is that it is lower in lactose and mildly alcoholic (as much as 3 %), as compared to whey.

Even if the cheese hypothesis is borne out, other possible natural products, which might have been strained through the strainer-funnels, need to be considered and tested for. As Salque *et al*. point out, strainers are common industrial tools for separating all kinds of materials, both liquid and solid.

Perhaps significantly, beeswax compounds were detected in three strainer-funnels. Since these strainers lacked a distinct layering of this material on their interiors (P. Bogucki, pers. comm., March 10, 2014), beeswax was probably not used as a waterproofer. Any beeswax then must derive from whatever was strained. Honey itself is unlikely: as a very viscous liquid, any solid debris from comb or hive would be difficult to separate through the small holes of the strainers. On the other hand, when honey is diluted to about 30 % honey/70 % water, natural osmophilic yeast in the honey become active and readily convert it to mead. Moreover, mead has long been a beverage of choice in Poland, whose forests are still exploited for wild beehives (Crane 1999). The combined chemical, archaeobotanical, and palynological evidence attest to its popularity as early as the 4th millennium B.C. in Scotland and Scandinavia (Dickson 1978; McGovern *et al*. 2013a). This possibility, however, is not addressed by Salque *et al.*

A large bronze strainer from Kostræde in Denmark (McGovern *et al*. 2013a: Fig. 2b) is particularly relevant to whether mead might have been strained, because of its overall pattern of small holes and similarity in shape to the Neolithic Polish strainers. Since its base was missing, it is uncertain whether it was a combination strainer and funnel. The strainer is thus far the only example of LBA date (Periods IV-VI, ca. 1100–500 B.C.) found in the country and was probably imported as part of a drinking set from the continent, based on eastern Hungarian stylistic parallels (McGovern *et al*. 2013a: 2, 13). Our multiple, complementary analyses of residue from one of its perforations showed that beeswax was unquestionably present. Specifically, GC-MS identified odd-numbered C_{25}-C_{31} n-alkanes, even-numbered C_{24}-C_{30} fatty acids with C_{24} dominant, and wax esters of the C_{16} fatty acid (palmitic) with C_{24}, C_{26}, and C_{28} alcohols (Fig. 5 and Tables 1 and 2). This beeswax was associated with other Scandinavian natural products (juniper berries, birch and pine resins, bog myrtle, and yarrow), which were

well-attested chemically and archaeobotanically. In addition, wine or another grape product, likely imported from central Europe, had come in contact with the strainer. Together with mead, these ingredients are hallmarks of "Nordic grog" (see further, below).

Similarly, the beeswax detected in 80 % of the collared flasks from Neolithic Poland might derive from mead. Their narrow mouths would be more appropriate for storing and serving mead than thick honey. Again, no distinct layering of beeswax was observed on their interiors (P. Bogucki, pers. comm., March 10, 2014).

Beer is another candidate for straining, which is briefly noted by Salque *et al*. This fermented beverage, too, was an important beverage of ancient Europe generally (Nelson 2005; Unger 2007; Dalby 2011; McGovern 2009/2010: Ch. 5; McGovern *et al*. 2013a, b). Ancient beer needs not have been strained and was often drunk from the same vessel in which it was made (thus retaining flavors, aromas, and valuable nutrients), using drinking tubes to filter out solids (for ancient Near Eastern and Egyptian examples and African ethnographic analogies, see McGovern 2009/2010: 67–71, 241–50). But, many beers were strained before consumption, as most probably illustrated in ancient Egyptian tomb scenes as early as the Old Kingdom's Tomb of Ti at Saqqara, ca. 2450 B.C. (Wild 1966). Straining helped to remove large quantities of spent grains and husks from the liquid wort, following the mashing operation in which malt carbohydrates were broken down into sugars. The wort might also have been strained of any added bittering agents or herbs before or after fermentation. Such traditional beer making practices have been practiced around the world, including Poland, from ancient times up to the present (Hornsey 2003).

The combination strainer-funnel might also have been used to process native fruit or pseudo-fruit such as whortleberry/bilberry (*Vaccinium myrtillus*), wild strawberry (*Fragaria vesca*), European raspberry (*Rubus idaeus*), blackcurrant (*Ribes nigrum* L.), May lily (*Maianthemum bifolium*), and juniper (*Juniperus communis* L.) berries, as well as birch (*Betula pendula Roth*) and Norway maple (*Acer platanoides*) resins, which have been traditional ingredients in Polish fermented beverages ((Ł. Łukasz, pers. comm., March 21, 2014; Madej *et al*. 2014; Łuczaj and Szymański 2007). Unfortunately, biomarkers for these other possible natural products were not detected by GC-C-IRMS.

Thus, it can be hypothesized that an array of Polish natural products might have been filtered through the strainer-funnels, with the primary goal being to make a mixed fermented beverage. Neolithic peoples around the world were great experimenters, not just in domesticating plants and animals, but in developing new beverages, cuisines, and technologies (such as pottery making). By culling as much sugar as they could from available plants and resins in a region, they could make higher alcoholic beverages with its attendant benefits: special flavors and aromas; enhanced energy production and nutritional content; antibacterial, anticholesterol, and anticancer medicinal effects; "social lubrication"; economic value; religious significance due to the mind-altering effects, etc. (for a fuller discussion, with references, see McGovern 2009/2010). An alcoholic beverage is also ideal for dissolving herbal and tree resin compounds, which can be readily administered by drinking or applying to the skin. Not surprisingly, such recipes predominate in the pharmacopeias of ancient China, India, Egypt, Greece, and Rome (McGovern *et al*. 2010).

Such a mixed fermented beverage was attested by the combined archaeological, chemical, and archaeobotanical evidence from sites in Scandinavia (McGovern *et al*. 2013a), dating between ca. 1500 B.C. and the 1st c. A.D. and including the Kostræde strainer (above). In general, Nordic peoples preferred a hybrid beverage or "grog," in which many ingredients

were fermented together, including locally available honey, fruit (e.g., bog cranberry and lingonberry) and cereals (wheat, rye and/or barley), and sometimes grape wine imported from farther south in Europe. Local herbs/spices, such as bog myrtle, yarrow and juniper, and birch and pine resins rounded out the concoction and provide the earliest chemical attestations for their use in Nordic fermented beverages. Similar beverages were made throughout medieval times and up to the present. This tradition probably has earlier roots, perhaps extending back to the Neolithic period. Why should not a similar type of mixed fermented beverage have been made in Neolithic Poland, which, like Scandinavia, borders the Baltic Sea and likely had contacts with its more westerly and northerly neighbors?

A milk whey or kefir-like liquid might also have gone into such a grog. The famous *kykeon* of the Homeric epics and the later Eleusinian mystery religion, which combined Pramnian wine, mead, and barley, was finished off by grating cheese on top of it (Ridgway 1997; McGovern *et al*. 2007).

Clearly, more exacting chemical analyses of the Neolithic Polish strainers are needed to substantiate Salque *et al*.'s working hypothesis that only cheese was the intended product of the strainer-funnels. The liquids of other native natural products—minimally, honey as mead—might have been strained and collected in a lower container. A beverage might have been made by mixing these liquids together, some of which had undergone alcoholic fermentation due to associated yeast and/or bacteria. Whey or a kefir-like liquid might have been one of those ingredients.

Other lines of evidence, besides the chemical, have their own contributions to make in determining what working hypothesis is most plausible and best supported. For example, associated archaeobotanical evidence might help in identifying and further corroborating the strained liquids. Further archaeological investigation might be able to identify a lower container type for collecting the liquid that passed through the strainer-funnel, which could then be chemically analyzed. Workshops might be defined in older excavations or targeted and found in new excavations. Such interdisciplinary, deductive reasoning and practice are the *sine qua non* of ancient organic residue analysis and the biomolecular archaeology of the future.

Conclusions

In the interests of articulating a more fruitful approach to organic residue analysis in archaeology, which is applicable around the world for any time period, we recommend that the discipline's essential nature and limitations need to be better understood. Our main point, which has not been adequately addressed in the literature, is that ancient organic residue analysis and biomolecular archaeology generally are very interdisciplinary. Much more attention needs to be given to *all* the available evidence—not just the chemical, but also that from archaeology and other pertinent fields—to induce the best working hypotheses for an archaeologically important issue.

Unlike the hard sciences, a historical ("soft") science like biomolecular archaeology cannot repeat the same experiment, as is customary for the scientific method, but must assume certain regularities in physical, biological, and cultural development over time, and then deduce what inferences necessarily follow and can be observed in the archaeological, chemical, or other scientific record. Such "tests" are best if they demand a tangible outcome, such as specific chemical compounds, associated findings

from ancillary fields, or artifact/ecofact spatial distributions. Chemical and microscopic data, which are unintentional and contemporaneous with what is to be explained, are especially powerful as confirmatory evidence for a hypothesis. When corroborative evidence is not forthcoming, then further testing is needed before the hypothesis is accepted, partially modified, or abandoned.

Such a transparent approach will be much less likely to fall into the methodological, theoretical, and experimental failings as do some of the studies documented above, which are by no means unique in the literature.

This approach is further illustrated by how our studies and working hypotheses on ancient viniculture—taking a larger perspective than the Uluburun shipwreck (Case Study 1)—have evolved over the years. We have necessarily had to stay abreast of the ever-growing archaeological and scientific databases and ever-improving archaeological, chemical, and other scientific techniques.

Thus, while we have always stressed the need to analyze the best archaeological material possible, especially from contexts that provide other relevant artifacts/ecofacts bearing on a hypothesis, the rapid development of more sensitive chemical techniques in the past 20 years led to the refinement of our chemical protocol. In the 1990s, our laboratory made the most of the analytical instruments then available, together with fruitful collaborations with local industry (specifically, The Dupont Co. and the Rohm and Haas Corp.) and other university laboratories. Yet, our pioneering approach did not include a chemical technique that subsequently has become essential: mass spectrometry, whether GC-MS or LC-MS.

When Curt Beck, our collaborator, expressed doubt about whether the contents of the inscribed Malkata amphoras was a wine to which a tree resin had been added, we provided him with samples. Using GC-MS, he confirmed our results (pers. comm., June 6, 2002) and went on to show that Middle-LBA amphoras, jars, and other vessels from Greece (Beck *et al.* 2007) and a 5th c. B.C. Italian bronze *situla* or bucket (Hostetter *et al.* 1994) likely contained wine which had been preserved by adding pine or terebinth resin. We subsequently applied LC-MS-MS in detecting tartaric acid/tartrate to a very high level of probability in both vessels already shown to have contained wine by our earlier methodology and new samples. Other researchers have advocated and used a similar, multistep methodology for identifying pine and birch resin, beeswax, terpenes, and other materials and compounds (Colombini *et al.* 2005; Regert 2007; Ribechini *et al.* 2009a, b).

More broadly and at a higher level of abstraction, our "working hypothesis" of viniculture having begun in the mountainous Near East, and from there and later having spread out to the rest of the world (the so-called "Noah Hypothesis"), has gained increasing credibility as deductions made from this hypothesis have been tested by ever more sophisticated and independent approaches of adjunct disciplines. The latter include archaeobotany (see McGovern *et al.* 2013a, b), DNA analysis of the Eurasian grape (Vouillamoz *et al.* 2006; also compare Myles *et al.* 2011) and of the principle fermentation yeast, *Saccharomyces cerevisiae* (Cavalieri *et al.* 2003), oenolomics (McGovern *et al.* 2009; Gougeon *et al.* 2009), etc. Significantly, our organic residue studies on ancient wine led to the Eurasian grapevine being chosen as the first fruit to have its complete genome sequenced (Jaillon *et al.* 2007).

The subsequent discovery of what has been called the earliest winery in the world in the Areni cave of Armenia (Barnard *et al.* 2011)—with plastered treading floors and

large underground jars to collect the juice and vinify and age it—fits well with our hypothesized Near Eastern mountainous "wine culture." This finding constitutes, as it were, deductive corroboration of our hypothesis. The Areni winery, dated to ca. 4100 B.C. (some argue for a later date of ca. 3500–3000 B.C.), is approximately a millennium later than our earliest chemical evidence for wine at Hajji Firuz (McGovern *et al.* 1996). The two sites are separated by only about 300 km; thus, knowledge of viniculture might well have traveled from one area to the other, thus consolidating the "wine culture" in the uplands from whence it traveled southwards to Egypt and Shiraz in the southern Zagros Mountains in later times.

Most recently (McGovern *et al.* 2013b), we have shown that viniculture was likely transferred by merchant ships to southern France by the Etruscans of central Italy, who in turn had probably received the domesticated Eurasian grapevine and winemaking from the Phoenicians in their colonization of the western Mediterranean by sea. The Phoenician shipbuilders and explorers were carrying on in the tradition of their ancestors, the Canaanites, who probably built and manned the Uluburun ship.

Our vinicultural hypotheses, albeit modified and expanded over the years, have largely stood the test of time, because they were originally based on multidisciplinary lines of evidence, as well as a methodological, theoretical, and experimental approach appropriate to any historical science.

Such a strategy can result in more than reconstructing and following the historical course of an ancient technology such as viniculture up to the present day, as interesting, even exciting, as that history might have been. As we pointed out in our article on the importation of Etruscan wine into southern France around 600 B.C. and the ensuing transplantation of the domesticated grapevine and emergence of native Celtic winemaking by at least 425 B.C. (McGovern *et al.* 2013b), where wine went, many other cultural elements, including related technologies (e.g., pottery making and technology), religious and social customs, medicinal recipes, the alphabet, etc., soon followed.

Biomolecular archaeological findings can go beyond these historical reconstruction in providing, as one example, detailed information on the grape cultivars and their genetic makeups in ecologically specific regions, so that germ plasm adapted to those environments can be preserved and added to the *Vitis* gene pool. This endeavor is still in the beginning stages. Ancient horticulture and winemaking, which might have been lost but have distinct advantages for the modern industry, can also sometimes be recovered. For example, vinification and aging in pottery jars, which was widespread in the ancient world, yield distinctly different tastes and aromas compared to processing in oak barrels or metal containers. Pottery belongs to a group of ionic solids—zeolites—which promote oxidation/reduction, enzymatic, polymerization, and other chemical reactions. By contrast, oak, especially when it has been toasted, is much more intrusive than pottery to the final flavor and aroma profile of the wine. Oak is not a zeolitic material, and heating it produces carmelization products and breaks down lignins into phenolic aldehydes, such as vanillin. Oak's lipids have a "woody" flavor and aroma and its tannins readily hydrolyze to form acetal, which has a "vegetal" character that is alien to grape wine.

Reconstructing ancient viniculture's history and technology is a single example of how ancient organic residue analysis, when buttressed by as solid a methodological, theoretical, and experimental foundation as possible, promises to shed new light on a host of biocultural developments that have made humans the organisms and cultures that we are

today. With that knowledge, we can move more confidently into the future. Most of what we are as humans is organic—our houses, our clothes, our bodies, etc.—and we now have the analytical tools to recover and identify and interpret ancient organic remains.

We stand at the beginning of a process of discovery in which we can find out much more about ourselves and our past world, including our bodies and brains, the microbiomes that surround and inhabit us, our languages and social systems, diets and economies, trade routes which brought peoples and their organic commodities and ideologies together, how we domesticated plants and animals, developed medicines, innovated in music, dance, theater, and the arts generally—the possibilities are endless. This knowledge may well lead to new taste sensations, alternative medicines, a better understanding of our shared biological and cultural heritages, and much else. But, such advances will require chemistry, archaeology, and ancillary historical and social sciences working together in a joint appreciation and application of rigorous theory, methodology, and data collection. Optimistically, we might envision a "new history of humankind" eventually being written.

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Figures 1, 4, 5, and 6 were prepared by Shih-han Samuel Lin, Jeffrey Hoyt, Anne Bomalaski, and Kimberly Leaman.

Conflict of Interest The authors declare no conflicts of interest.

Appendix 1: LC-MS-MS Analyses

In reanalyzing the ancient Egyptian sample from Scorpion I's tomb at Abydos, we followed the general methodology of Guasch-Jané *et al.* (2004), with modification of the extraction procedure to detect tartaric acid/tartrate in the negative mode (McGovern *et al.* 2009).

An even more refined extraction procedure was found to enhance the detection of tartaric acid/tartrate by LC-MS-MS for the Uluburun terebinth resin samples. Whole pieces of resin were stirred overnight in 1.5 mL of ammonium hydroxide

(2.8 % by vol) and 1.5 mL methylene chloride. The mixture was centrifuged for at least 10 min at 4400 rpm to clarify the layers and cause any remaining materials and emulsions to precipitate. The upper basic aqueous layer was then removed, reduced in volume, filtered through a 0.45-μm membrane, placed into 200 μL vial inserts, and analyzed.

Two MRM transitions (149→87.1 and 149→73), not just one as we had previously done, at a specific retention time (0.80 min) were monitored for tartaric acid, as can be seen on Fig. 5. Together, these transitions and the resulting daughter ions are definitive for the acid's presence/absence. Monitoring both transitions has not been applied to ancient samples before and provides very strong evidence for tartaric acid/tartrate in KW 181, KW 215, and probably KW 102.

The LC mobile phase conditions were also modified to achieve better peak shapes, as follows: A (water, 0.1 % *v*/*v* formic acid) and B (acetonitrile, 0.1 % *v*/*v* formic acid) had an initial composition of 98 % phase A and 2 % B, which was changed to 50 % A/50 % B over a 2-min period, and then equilibrated back to 98 % A/2 % B.

Accentuating the detection of a tartrate salt by this new method takes advantage of the much lower solubilities of potassium bitartrate and calcium tartrate, about 4 and 0.3 g/L in cold water, respectively, compared to the acid's solubility of about 1400 g/L at 20 °C (Singleton 1995: 68). The salts readily form in wine and precipitate out in the lees. Calcium can interchange with potassium in highly calcareous pottery fabrics, which are characteristic of Eastern Mediterranean amphoras.

GC-MS was employed to further test KW 215, which had given a positive tartaric acid/tartrate by LC-MS-MS. The resin sample was completely dissolved in methylene chloride and treated with a small amount of formic acid to acidify any tartrate present to tartaric acid. Silylation was carried out with BSTFA (N,O-bis(trimethyl-silyl)trifluoro acetamide). The sample was injected splitless onto an HP-5MS column (5 % phenyl methyl siloxane) of an Agilent HP-6890 gas chromatograph equipped with a Hewlett-Packard 5973 mass selective detector. The key silylated tartaric acid ion at 219^{+} was tentatively identified by selected ion monitoring which enhances sensitivity.

Both LC-MS-MS and GC-MS yielded an approximately 0.25-ppm concentration of the acid/salt for sample KW 215, which was close to the GC-MS detection limit and only achievable in the selected ion mode.

Appendix 2: FT-IR Analyses

Although potentially many absorptions in the standard FT-IR region of 4000–400 cm^{-1} can be equivocal, careful observation, combined with rigorous methodology, enables the critical peaks for individual compounds in mixtures to be sorted out. Moreover, fine spectral details can be distinguished by extracting an ancient sample separately with methanol and other organic solvents (e.g., chloroform). While chloroform selectively extracts and accentuates the FT-IR spectra of low-polarity compounds, methanol highlights those of high-polarity compounds.

Figure 6 and Table 2 illustrate how powerful a combined solvent approach can be in distinguishing the carbonyl and acid hydroxyl absorptions of synthetic L-tartaric acid from those of ancient and modern *Pistacia* sp. resin. The higher-polarity tartaric acid, which was extracted by methanol, has a distinctive doublet in the 1740–1720 cm^{-1} carbonyl region, with a less intense shoulder at the lower wave number (frequency). Its hydroxyl absorption occurs in the 1450–1430 cm^{-1} region. By contrast, the carbonyl of the lower polarity terebinth resinous acids, which were extracted by chloroform, have a single intense absorption at 1710–1700 cm^{-1}, and their hydroxyl absorption is in the 1470–1445 cm^{-1} region.

In light of their marked spectral differences, the discussion of the FT-IR spectra of tartaric acid and *Pistacia* sp. resin by Stern *et al*. (2008) (pp. 2197–2198) is confusing.

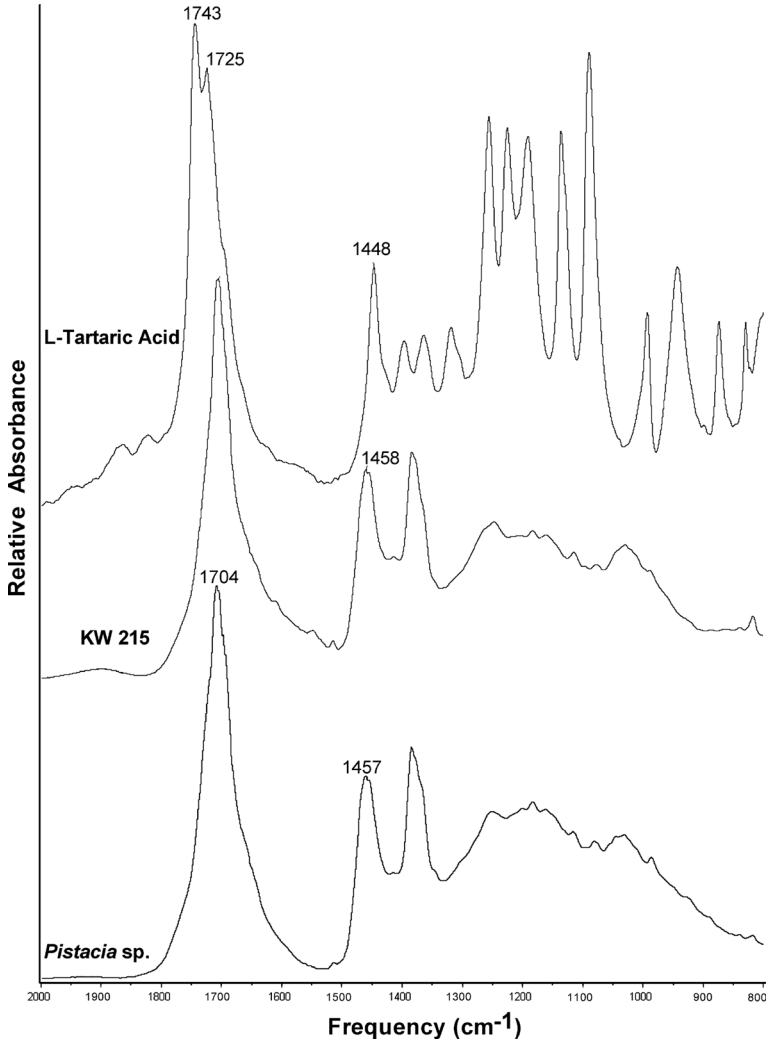

Fig. 6 The FT-IR analysis of L-tartaric acid is clearly different at key carbonyl stretch and hydroxyl bend absorptions from those of ancient (KW 215) and modern *Pistacia* sp

Table 2 Comparison of key FT-IR absorption of methanol and chloroform extracts of L-tartaric acid, tartrate, and *Pistacia* sp. resin

Band (cm^{-1})	Methanol	Chloroform	Group assignment	Compound/natural product
Broad 3500	Strong	Weak	Hydroxyl stretch	Tartaric acid
2950–2800	Moderate	Very strong	Hydrocarbon stretch	*Pistacia* sp. resin
1740–1720	Moderate	Moderate	Carbonyl stretch	Tartaric acid
1710–1700	Weak	Strong	Carbonyl stretch	*Pistacia* sp. resin
1695–1670	Weak	Moderate	Carbonyl stretch	*Pistacia* sp. resin
1635–1600	Strong	Weak	Carbonyl stretch	Tartrate
1470–1455	Weak	Strong	Hydroxyl bend	*Pistacia* sp. resin
1450–1430	Strong	Weak	Hydroxyl bend	Tartaric acid

They state that their Uluburun resin samples all have absorptions in the 1740–1720 cm^{-1} region, similar to those of tartaric acid. They then dismiss the FT-IR evidence. Yet, their own Fig. 4 shows that the carbonyl peak (viz., 1710–1700 cm^{-1}) of the Uluburun resin samples is significantly lower and does not overlap with that of tartaric acid.

The FT-IR spectrum for syringic acid also differs from that of tartaric acid and *Pistacia* sp. resin, as Stern *et al*. (2008) show in their Fig. 2. Its carbonyl stretch is a single intense absorption at 1690 cm^{-1}, and its OH bend is at 1450 cm^{-1}.

The blanket statement by Stern *et al*. (2008) (p. 2197) that "these absorption bands are shared by a huge variety of organic substances and are *clearly not restricted* [added emphasis] to tartaric acid/tartrate or terebinth resin" is far from being clearly established and undermines the value of infrared spectrometry. For the spectrometrist, including Moreira and Santos 2004 which Stern *et al*. cite in support of their contention, the hydroxyl and carbonyl absorption bands are important discriminators of organic compounds. Certainly, Moreira and Santos would not confuse the spectrum of syringic acid with that of tartaric acid, as implied by Stern *et al*. (2008) (p. 2195). Stern *et al*. also assert that interferences from ethanol and acetic acid pose a problem in interpreting the FT-IR spectra of wine, again citing Moreira and Santos 2004. Since such compounds have volatilized and disappeared in ancient wine samples, their point is not relevant.

In summary, it is very important to carefully scrutinize and statistically deconvolute an IR spectrum, and also to run searches of unknown archaeological samples against the large databases which are now available. Stern *et al*. do not appear to have exercised such care in their analyses, and thus they write off the FT-IR data too quickly.

Three subsidiary points should also be noted:

1. Even if it were possible to confuse the IR spectra of L-tartaric acid and syringic acid (Stern *et al*. 2008: 2195), this is irrelevant to the analysis of an ancient wine sample. Syringic acid is not a "derivative" of malvidin (p. 2189), one of the red pigments in wine, but can only be produced as a breakdown product of the latter by alkaline fusion, which was beyond the expertise of ancient peoples. In fact, it is

malvidin, not syringic acid, which is present in modern red wine at 200 mg/L, according to Singleton (1995: 70) (see below).

2. It is very puzzling that the modern red wine, analyzed by Stern *et al*. (2008) (p. 2196, Fig. 3), is dominated by calcium tartrate. Tartaric acid, at a concentration of about 1400 g/L at 20 °C, is the principal acid in wine and should be detected. Moreover, any tartrate that has precipitated out should be in the form of potassium bitartrate, not calcium tartrate. Again, Stern *et al*. do not detail their extraction procedure, if any, which might explain these anomalies. Their explanation for the origin of calcium tartarte in ancient samples is equally suspect.
3. Stern *et al*. (2008) do not appear to have included "ancient reference samples" in their databases, comparable to the inscribed Malkata amphoras of our studies. If they had, they might have been able to detect differences in the FT-IR spectra of "aged" and modern wine samples.

Together and apart from the lack of care in the interpretation of the FT-IR spectra, the subsidiary points highlight the confusing, ill-founded argumentation of Stern *et al*. (2008).

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