

New investigations into the Uluburun resin cargo

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Received 6 November 2007; received in revised form 4 February 2008; accepted 5 February 2008

Abstract

Resin found within Canaanite amphorae from the Late Bronze Age shipwreck discovered off the coast of southwest Turkey at Uluburun has previously been identified as *Pistacia* sp. Although evidence from Egypt suggests that this resin was in high demand and typically transported in such amphorae, it has also been proposed that the amphorae contained wine, with the resin used to seal the interior surfaces and to flavour and/or preserve the wine. To attempt to resolve this question, we have analysed five samples of pistacia resin found in amphorae from the shipwreck using a range of analytical techniques which have been used in the past for the analysis of wine residues: spot tests, FT-IR, and HPLC-MS-MS. As well as the archaeological samples, we have analysed modern samples of pistacia resin, leaves and fruit to determine the effectiveness of each technique and to exclude the possibility of false positive results. In addition to the analyses for wine we also detail analysis (GC-MS) of the terpenoids for the purpose of further molecular characterisation of the resin. Bulk stable isotope analysis was used in comparison with similar resins to attempt to identify the geographical origin of the resin.

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Keywords: Uluburun; Amarna; Pistacia resin; Wine; IR; HPLC-MS-MS; GC-MS; Tartaric acid; Syringic acid

1. Introduction

The Late Bronze Age shipwreck discovered off the coast of southwest Turkey at Uluburun contained, amongst other goods, nearly 150 Canaanite amphorae (Bass, 1986; Bass et al., 1989; Pulak, 1988, 1994, 1998, 2001). Approximately 120 of these coarseware ceramic transport jars were 1/4–1/2 full of resin, and given their average estimated capacity of about 9 kilos, this suggests a total resin cargo of about a ton (Haldane, 1993: 352–353). Mills and White (1989) used gas chromatography-mass spectrometry (GC-MS) to identify the botanical source of the resin as the genus *Pistacia* sp. based on the characteristic triterpenoid biomarkers. They also speculated that the resin was *Pistacia atlantica* based on the amounts of resin produced by modern *terebinthus* and *atlantica* species. Hairfield and Hairfield (1990) also performed

analysis by GC-MS and reported that this technique cannot be used to distinguish beyond genus (*Pistacia*). They however attempted to identify the species as *Pistacia terebinthus* based on unidentified components observed by thin layer chromatography. We believe that analysis of the resin cannot be used to distinguish the species due to degradation, confusion between species and attributing contemporary modern varieties (Serpico and White, 2000a).

The resin of *Pistacia* sp. was a highly valued commodity in ancient Egypt, widely used as incense (Serpico and White, 2000b; Stern et al., 2003) and as varnish on New Kingdom funerary equipment (Serpico and White, 2001). Pistacia resin has also been identified from a Temple pedestal from Ismant el-Kharab (Ross and Stern, 2004) and the resin has also been reported in association with Ptolemaic (Kaup et al., 1994; Buckley and Evershed, 2001), 7th century BC mummies (Colombini et al., 2000a) and a Graeco-Roman decorated sarcophagal coffin-lid (Edwards et al., 2007). Further evidence to support the theory that resin had significant economic value in its own right includes Canaanite amphorae containing *Pistacia*

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sp. imported into Egypt from the Levant (Bourriau et al., 2001; Serpico, 2003; Serpico et al., 2003), the large quantities of resin recovered at Uluburun (approximately one ton in total) and quantification of the resin trade in the Late Bronze Age Eastern Mediterranean (Serpico, 2003).

Despite the evidence presented above, analysis of samples of resin from the Uluburun shipwreck (of several of the same samples as analysed in this study) led to the conclusion that the samples contained a mixture of resin and wine: ‘...since we discovered that the resin from the amphoras tested positive for tartaric acid and tartrate. The best explanation for this result is that the amphoras had originally been topped up with wine. Only a finite amount of resin is dissolved into wine, and, as a space-saving measure, it makes sense to ship jars of wine containing extra resin’ (McGovern, 2003; 130). Unfortunately, the ‘test’ for tartaric acid is not specified in this case, although analysis by the ‘Feigl spot test’, IR (infra red spectroscopy) and HPLC (high performance liquid chromatography, using UV detection) have been used to assert that the amphorae were filled with wine and that the resin was used in wine processing (perhaps to inhibit the growth of bacteria) or to preserve the pistacia fruit (McGovern, 1997) although the latter is unlikely given the low occurrence of the fruit relative to the volume of resin. The use of such techniques for the identification of wine has wider implications since they have been used at a number of other sites (e.g. McGovern, 1996; McGovern et al., 1996, 1997, 1999, 2001, 2004). It should also be emphasized that the identification of resin should not necessarily lead to the assumption of the presence of wine as the resin could have been used for other purposes (Boulton and Heron, 2000).

The biomarkers for the presence of ancient wine are considered to be tartaric acid, its salts, and syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) (Singleton, 1996). Tartaric acid is specific to grape-derived wine (red or white), and is present in quantities averaging four grams per litre in modern samples. No other fruit has been reported as having significant amounts although it can occur in plant material at levels <100 mg/L (Singleton and Esau, 1969: 9–10, Singleton, 1996). Although tartaric acid has three stereoisomeric forms, and all three forms occur naturally, the L(+) isomer is the most abundant (McGovern and Michel, 1996). Despite tartaric acid being water soluble, its salts are far less so (Singleton, 1996), and in addition it is suggested that the presence of strong hydrogen bonds enables the binding of tartaric acid to archaeological ceramics (McGovern and Michel, 1996). Syringic acid is a derivative of malvidin, an anthocyanidin, responsible for the colouring of red wine (Guasch-Jané et al., 2004), and is found in modern samples in quantities 200 mg/L, but it can occur in older wines in amounts as much as five times that. No other fruit has significant amounts of syringic acid (Singleton and Esau, 1969: 43; Singleton, 1996). Syringic acid can polymerize, potentially aiding its preservation and retention in ancient samples (Singleton, 1996).

In addition to the resin, other storage jars from the shipwreck contained a variety of commodities including olive pits, glass beads and orpiment. Haldane (1993) suggested

that substantial mixing of these diverse contents occurred within the hull and into the amphorae, further complicating the interpretation of the original vessel contents. Interestingly, pistacia fruit was commonly found embedded in the resin in the Canaanite amphorae and raising the possibility that the tartaric acid found in the earlier study might derive from the fruit.

The aims of our current work were to: i) Sample a wider range of resin samples from the Uluburun cargo to compare the variation in molecular composition of *Pistacia* sp. of archaeological date. ii) To test a range of existing methodologies for the detection of biomarkers of wine in archaeological and modern samples of *Pistacia* sp. iii) To determine the light stable isotope composition of the resin samples to explore the geographical origin of the trees from which the resin was sourced.

2. Methodological considerations

2.1. Triterpenoid composition of the resins

Very few resin samples from the cargo have been subject to combined GC-MS analysis (Mills and White, 1989; Hairfield and Hairfield, 1990). More recently, analysis of pistacia resins found associated with imported Canaanite amphorae and locally-made bowls from Amarna, Egypt has served to demonstrate the presence of biomarkers of heating and to document a wider range of trace constituents in the resins (Stern et al., 2003).

2.2. Analytical tests for wine

2.2.1. Chemical spot tests

Fritz Feigl first developed and published the ‘Feigl spot test’ in *Spot Tests in Organic Analysis* in the 1930’s (Feigl et al., 1966). He describes spot tests as the ‘chemistry of specific, selective and sensitive reactions’ (Feigl et al., 1966: 4). However, in some cases the presence of other compounds or functional groups can produce false positive results. In the case of the L-tartaric acid spot test, these are glucolic, glucuronic, dihydroxytartaric, and malic acids (Feigl et al., 1966: 470). In grape juice, both malic and tartaric are the predominant acids accounting for 0.1–0.5% and 0.2–0.8% of total acidity respectively (Austin, 1968: 127). These amounts are variable according to geographical regions and degree of ripeness of the fruits. In cooler climates, malic acid will dominate in lieu of tartaric which is dominant in warmer Mediterranean climates (Austin, 1968: 127–129). The accuracy of the Feigl spot test is limited to concentrations of tartaric acid above 5–10 µg/mL (Feigl et al., 1966; Jungreis, 2000). The advantage of the procedure lies in that it is relatively rapid and allows for many samples to be processed simultaneously. McGovern (1997) has employed wet chemistry techniques as a means of ‘confirming’ the results of infrared spectroscopy. Samples KW144 and KW181 analysed here (Table 1) have been reported by McGovern (1997) as testing positive for tartaric acid/tartrate. Unfortunately the test is unspecified, although it is likely to be the Feigl spot test described above,

Table 1
The samples analysed in this study, including the archaeological Uluburun resins, modern pistacia leaves and berries, and modern analogues of the archaeological ceramics

Sample description	Sample type	Feigl spot test	FT-IR	GC-MS		HPLC-MS	
		L-tartaric acid detected	L-tartaric acid detected	L-tartaric acid detected	Syringic acid detected	L-tartaric acid detected	Syringic acid detected
KW102	Ulu burun resin	N	N	N	N	N	N
KW144	Ulu burun resin	N	N	N	N	N	N
KW165	Ulu burun resin	N	N	N	N	N	N
KW181	Ulu burun resin	N	N	N	N	N	N
KW48	Ulu burun resin	N	N	N	N	N	N
Modern sherd, no treatment	Modern residue	N	N	N	N	N	N
Modern sherd, Red Wine	Modern residue	N	N	Y	Y	Y	Y
Modern sherd, <i>Pine Resin</i> /Red Wine	Modern residue	N	—	—	—	Y	Y
Modern sherd, <i>Pistacia Resin</i> /Red Wine	Modern residue	N	—	—	—	Y	Y
Modern sherd, White Wine	Modern residue	N	N	Y	N	Y	N
Modern sherd, <i>Pine Resin</i> /White Wine	Modern residue	N	—	—	—	Y	N
Modern sherd, <i>Pistacia Resin</i> /White Wine	Modern residue	N	—	Y	Y*	Y	N
Modern <i>Pistacia terebinthus</i> , Crete	Fruit	N	N	N	N	N	Y
Modern <i>Pistacia lentiscus</i> , Cap d'Antibes, France	Leaves and Fruit	N	N	—	—	N	N
Modern <i>Pistacia</i> , Gourdon, France	Leaves and Fruit	N	N	N	N	N	Y
Modern <i>Pistacia</i> , Gourdon, France	Leaves and Fruit	N	N (fruit only)	N	N	N	Y
Modern <i>Pistacia atlantica</i> , Bodrum, Turkey	Leaves and Fruit	N	N	N	N	N	Y
Modern <i>Pistacia atlantica</i> , Bodrum, Turkey	Fruit	N	N	—	—	N	N
Modern <i>Pistacia atlantica</i> , Bodrum, Turkey	Fruit	N	—	N	N	N	Y
Modern <i>Pistacia lentiscus</i> , Bodrum, Turkey	Leaves and Fruit	N	N	N	N	N	Y
Modern <i>Pistacia terebinthus</i> , Bodrum, Turkey	Fruit	N	N	N	N	N	Y
Modern <i>Pistacia terebinthus</i> , Bodrum, Turkey	Leaves and Fruit	N	—	—	—	N	Y
Modern <i>Pistacia terebinthus</i> , Bodrum, Turkey	Leaves and Fruit	N	N	—	—	N	N

Y = positive analysis, N = no detection, — = analysis not carried out. * = likely instrumental contamination for this sample.

as this was used in their identification of wine in samples from three jars found at Abydos in Egypt (McGovern et al., 2001: 400).

2.2.2. Infrared spectroscopy (IR)

McGovern and Michel (1996) and McGovern (1997) state that infrared absorptions at around (slightly different wave

numbers are given in different publications) 1710–1725 cm⁻¹, 1450–1460 cm⁻¹ and 1240–1255 cm⁻¹ are definitive for carbonyl and carboxylic acid groups of tartaric acid. Tartrate (probably as the calcium salt) is indicated by absorption at 1550–1650 cm⁻¹, whilst an absorption band at 3350 cm⁻¹ indicates the hydroxyl groups in tartaric acid and water of hydration. In addition, absorptions at 2900 cm⁻¹

and $1550\text{--}800\text{ cm}^{-1}$ are due to C-H stretching and bonding vibrations respectively from either the tartaric acid/tartrate or (equally likely) other organics in the residues. Samples KW144 and KW181 were analysed by IR by McGovern (1997), who states that the carbonyl peaks (from the resin) at $1720\text{--}1740\text{ cm}^{-1}$ overlap with those from L-tartaric acid (the stereoisomers all have similar IR spectra (McGovern and Michel, 1996)) and peaks at 1460, 1380, 1250 cm^{-1} and from $1550\text{--}800\text{ cm}^{-1}$ confirm the presence of a tree resin. It is therefore highly likely that the presence of the resin precludes the use of IR for the trace identification of tartaric acid and tartrate salts in samples. McGovern himself states that IR does not unambiguously identify the compound (McGovern and Michel, 1996) and they therefore used the Feigl test to 'confirm' the IR data for the presence of wine.

Boulton and Heron (2000) state that although spot tests and IR are relatively cheap and simple to use, these approaches are not the most appropriate due to the nature of degraded, contaminated mixtures often encountered in archaeological samples. They also highlight that combined separation and identification techniques would be preferred for detailed analysis.

2.2.3. High performance liquid chromatography (HPLC)

Perhaps the strongest evidence for the presence of wine provided by McGovern (1997) is analysis using HPLC separation with UV detection for sample KW144, where a peak was observed at a similar retention time to an L-tartaric acid standard. However, given the complex mixture of components in this sample it is possible the peak is coincidental and the UV detector cannot be used to further identify the separated compounds with any degree of confidence. In contrast, Guasch-Jané et al. (2004) established a method for extraction/sample preparation and analysis of ancient ceramic-absorbed wine that served as a basis for developing a method for this project. The technique of high performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) can identify both tartaric and syringic acids, and is based on both chromatographic separation of the underivatised molecules by HPLC and sensitive detection by mass spectrometry. The MS-MS technique has significant advantages in that, in addition to retention times from the chromatographic separation, identification relies on characteristic mass fragmentation patterns, providing absolute confirmation of the biomarkers present. In addition to the analysis of tartaric acid the detection of syringic acid relies on an extraction procedure known as 'alkaline fusion' which extracts a greater percentage of syringic acid (Guasch-Jané et al., 2004).

This method was used to identify tartaric acid in 4 of 5 ancient Egyptian wine jars, one of which was further tested for syringic acid, revealing the presence of red wine (Guasch-Jané et al., 2004). Further analysis, applied to residues from six amphorae from Tutankhamun's burial chamber, distinguished one red and five white wines (Guasch-Jané et al., 2006a) and a grape product specifically made from red grapes (Guasch-Jané et al., 2006b). However, the interpretation of the presence of white as opposed to red wine is based on the

absence of syringic acid. Such assignments should be treated with caution as very little is known about the fate of this component relative to tartaric acid.

Alternative approaches to the study of wine residues have also been proposed (e.g., Garnier et al., 2002). In this paper, a method employing thermally assisted hydrolysis and methylation followed by GC-MS to detect polyphenols from modern and archaeological vine products was tested. Pyrolysis of polyphenolic residues enabled characteristic markers to be identified.

2.3. Isotopic analysis of resins

In contrast to the isotopic study of bone collagen, the analysis of other bulk organic materials by light stable isotopes (δD , $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) is in its infancy. However, the analysis of birch bark tars of archaeological date (Stern et al., 2006) and *Pistacia* sp. resin from Canaanite amphorae found at Amarna, Egypt (Stern et al., 2008) has illustrated that modern resins show differences between geographical areas, that there are separate groupings between modern and archaeological resins and some archaeological samples are distinct from others. However, although study of the clays used to manufacture the Canaanite amphorae used to transport *Pistacia* sp. resin has shown that two different fabrics were used, coming from two different geographical areas (Serpico et al., 2003), there are no clear patterns corresponding to the different Canaanite amphorae fabrics. And it is not yet clear if isotopic signatures can assist in pinpointing the geographical origin of the resin. Nevertheless the availability of new samples from the Uluburun shipwreck provides a further opportunity to evaluate bulk isotope analysis of resins.

3. Materials and methods

3.1. Samples

Five resin samples from different amphorae from the Uluburun shipwreck were kindly provided by Cemal Pulak, director of the Uluburun expedition (Table 1). In addition, the clay fabrics used to manufacture nearly all of the jars containing resin were studied with a $10\times$ handlens and in some cases with a binocular microscope. This included four of the five jars sampled here (with the exception of KW181). The results indicated an overall similarity in the clay fabric and also that this fabric closely resembled one of the two groups (Group 1) used to transport *Pistacia* sp. resin at Amarna (Serpico and White, 2000b). Petrographic and chemical analysis of Group 1 Amarna samples indicated that the likely source of the clay was in northern Israel, in the area of the Haifa Bay to the north of the Carmel Ridge, roughly between Haifa and Akko (Bourriau et al., 2001; Smith et al., 2004). Although the Uluburun amphorae with resin do seem to fall within Group 1, slight differences in the clay were noted in some examples, and thus it should be cautioned that, despite the overall similarity, the geographic provenance for the clay may not fall within the exact same perimeters.

The resin samples ranged in colour from light yellow hue (KW102) to a deeper reddish brown (KW144). Moreover, there were surface patinas and accumulations of whitish-grey material with a consistency of chalk. This was described by Mills and White (1989) as a weathering crust. Resin, leaf and fruit from modern *P. atlantica*, *P. terebinthus* and *P. lentiscus* were also subjected to the same methodology (Table 1). Pistacia fruit is commonly embedded in the resin in the Cannaanite amphorae and there is a question as to whether tartaric or syringic acids are present in the fruit. Two of the samples (KW102 and KW165) are from amphorae which contained other organic debris. Typically, these are mixtures of grass, leaves, and seeds believed to have come from the original mud jar sealings which disintegrated in the water and collapsed into the jars, collecting at the bottom (Pulak, 1988: 11). However, the samples tested were all discrete lumps of resin which contained no visible traces of this organic conglomerate. For IR analysis separate analyses were carried out on the modern fruit and leaves. Due to the small quantity of sample the fruits and leaves were combined for the destructive analysis by GC-MS and HPLC-MS-MS.

To provide references for testing the analytical techniques pieces of a modern unused standard flower pot were first Soxhlet extracted using dichloromethane:methanol (DCM:MeOH; 93:7 v/v for 24 h) to remove any residual lipids. One sample was not treated any further and therefore served as a method control. Selected sherds were coated with modern pistacia or pine resin. The coated sherds were then immersed for a period of three months along with uncoated controls in modern red and white wines to simulate the conditions of storage.

In addition to the samples described above, L-tartaric (99.5%) and syringic acid (98%) standards (Sigma-Aldrich) were obtained to act as positive controls and to confirm analytical results.

3.2. Fiegl spot test

Table 1 details the number and type of samples analysed by the Fiegl spot test. For each, the pulverized sample (2–3 mg) was combined with 1–2 mg of 2,2'-dihydroxyl-1,1-dinaphthyl. To this was then added approximately 3–4 mL of concentrated sulfuric acid. The mixture was then heated to a constant temperature of 85 °C for 25 min. Upon removal from the heat source, the vials were placed beneath a UV light source for evaluation. According to Feigl et al. (1966), a positive test will display a green fluorescence. Both syringic and tartaric acid standards were prepared to enable comparison with the samples listed in Table 1. For the tartaric acid standard, several concentrations were prepared, the limits of detection (LOD) indicated by our experiments was 6.25 µg/mL. This is within the range of the values of 5 and 10 µg/mL suggested by Jungreis (2000) and Feigl et al. (1966) respectively.

3.3. Fourier transform/infrared spectroscopy (FT/IR)

Table 1 presents the number and type of samples analysed by FT/IR. For the modern samples both fruit and leaves were

analysed separately (with the exception of one sample of modern pistacia (Gourdon, France) where there was only enough sample for analysis of the fruit only). A Digilab UMA 400 FT/IR equipped with an attenuated total reflection (ATR) germanium crystal and microscope was used for analysis of the surface of the samples at the point of contact with the crystal. A total of 256 scans were used to obtain the IR spectra within the range of 700–4000 cm⁻¹. All results were obtained using Digilab Win-IR Pro 3.4 software. For the majority of the samples, it was possible to place them beneath the germanium crystal. However, there was a great deal of difficulty in obtaining good-quality spectra from the ceramic sherds (both modern and archaeological) due to their size as well as risk of damage to the germanium crystal. To solve this problem, these samples were powdered prior to analysis.

3.4. Gas chromatography-mass spectrometry (GC-MS)

Table 1 lists the number and type of samples analysed by GC-MS. Two sub-samples of each Uluburun resin were taken for GC-MS analysis. Each were extracted with three aliquots of ~3 mL DCM:MeOH (dichloromethane:methanol 2:1, v/v), with ultrasonication for 5 min. The solvent extracts were then transferred to a clean glass vial and the solvent removed under a stream of nitrogen. To produce the silyl esters, one sub-sample was derivatised using BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) (Pierce). BSTFA was added to derivatise the sample which was warmed overnight. Excess derivatising agent was removed under a stream of nitrogen. The other sub-sample was derivatised with diazomethane (to produce the methyl esters) after the method of Fales et al. (1973). The derivatised samples were then diluted in DCM for analysis.

A portion of the fresh resin, leaf and fruit was lightly crushed, solvent extracted and derivatised as described above. Sub-samples of the modern wine soaked sherds (0.1 g) were removed to a depth of 2 mm with a Dremel electric drill fitted with a tungsten abrasive bit. These samples were solvent extracted and derivatised as described above.

Combined gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett Packard 5890 series II GC connected to a 5972 series mass selective detector. The splitless injector and interface were maintained at 300 °C and 340 °C respectively. Helium was the carrier gas at constant inlet pressure. The temperature of the oven was programmed from 50 °C (2 min) to 340 °C (10 min) at 10 °C/min. The GC was fitted with a 15 m × 0.25 mm, 0.1 µm OV1 phase fused silica column (MEGA). The column was directly inserted into the ion source where electron impact (EI) spectra were obtained at 70 eV with full scan from *m/z* 50 to 700.

The GC-MS was able to detect L-tartaric acid in quantities of 0.18 µg/mL and the syringic acid at 0.23 µg/mL. These trace amounts were established by calculating the amount of a compound within a sample detected when compared with a known amount of an *n*-alkane internal standard (C₃₄).

3.5. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS)

Table 1 details the number and type of samples analysed by HPLC-MS-MS. The extraction and analytical methods used here are adapted from those of Guasch-Jané et al. (2004). For the analysis of tartaric acid (present in both red and white wines), ~2 mg of the pulverized ceramic sample is added to 5 mL 0.1% formic acid in methanol and water (20:80 v/v ratio) (the HPLC mobile phase). This is then sonicated for 3 min to ensure dissolution of the tartaric acid, followed by centrifugation (2000 RPM for 5 min) to separate any suspended solids. The solvent volume is separated from the solid and reduced to 1/5 volume under a stream of nitrogen gas.

For the analysis of syringic acid (present in red wines), alkaline fusion is required (Singleton, 1996). The pulverized sample (100 mg) is added to 500 mg potassium hydroxide (KOH). This is heated until the KOH is melted and heating is continued (avoiding charring) for a further 5 min. The sample is then cooled and minimal water added to dissolve the melt and the solution acidified with formic acid. This was then sonicated for 3 min, followed by centrifugation (2000 RPM for 5 min) to separate any suspended solids. A total of 2–3 mL of ethyl acetate was added to each sample, which were sonicated and centrifuged again. The upper layer of ethyl acetate was removed and placed into a solvent cleaned glass vial. These solvent extraction steps were repeated two additional times. These combined solvent extracts were then reduced to 1/3 volume under a stream of nitrogen gas.

HPLC-MS-MS analyses were performed using a Waters Alliance 2695 HPLC Pump coupled with a Micromass Quattro Ultima mass spectrometer unit operating with electrospray ionization in the negative mode. Optimization of the MS was performed using repeated injections of the standard solutions at a concentration of 1 µg/mL. The Waters alliance 2695 HPLC pump/auto sampler conditions were as follows; flow rate 0.2 mL/min, run time 30 min, and a total injection volume of 15 µL. The column used was a Phenomenex Synergi 4u Fusion-RP 80, 150 × 2 mm. Solvent A is defined as 0.1% formic acid in deionized water and solvent B is acetonitrile. The gradient was isocratic for a period of five minutes with solvent A running at 100%, for the next ten minutes the solvents are run A/B combined at a mix of 80:20, finally the mix is altered a final time at 15 min when solvents A and B were run at a ratio of 50:50, this continued until the end of the run at 30 min. Micromass Quattro Ultima conditions for both tartaric and syringic acids were as follows: capillary voltage 2.44–2.52 kV, collision gas (Ar), collision energy 15 V, source temperature 100 °C, desolvation temperature 360 °C, and cone voltage 99 V. A total of 3458 scans over the course of 30 min, scanning two channels: L-tartaric acid (149/87 *m/z*) and syringic acid (197/182 *m/z*) in MRM mode.

The LOD were established as 0.01 µg/mL for both tartaric and syringic acids. This was accomplished by running varied concentrations of standards directly through the quadrupole mass spectrometer at an injection volume of 15 µL over the

course of the thirty minutes, confirming peak shape and intensity as well as consistency of elution times.

3.6. Light stable isotopic (δD , $\delta^{13}C$ and $\delta^{18}O$) values for bulk resins

Samples for isotopic analysis were prepared by dissolving a portion of each archaeological resin in ~2 mL of dichloromethane:methanol (2:1, v:v). Any solvent insoluble material was removed by filtration through solvent extracted glass wool in a Pasteur pipette. The solvent was then completely removed from the solvent soluble fraction by nitrogen blow-down and the resultant fractions kept in a desiccator prior to analysis. Isotopic measurements were carried out according to the procedure discussed elsewhere (Stern et al., 2008).

4. Results and discussion

4.1. Triterpenoid composition of the resins

Fig. 1 shows the partial TIC (total ion current) chromatograms of the five resins in the retention time region of the triterpenoids derivatised as the methyl esters. The mass spectra are described by Stern et al. (2003, Table 2). These samples contained only triterpenoids with no evidence of any other resin, oil or fat. Additional triterpenoid components have been identified in this study than in previous publications of the Uluburun resins (Mills and White, 1989; Hairfield and Hairfield, 1990). These samples are confirmed as *Pistacia* spp. resin due to their characteristic composition and distribution of triterpenoids (notably O (methyl moronate), Q (methyl oleanonate), U (methyl isomasticadienonate) and W (methyl masticadienonate)). In addition there are a number of triterpenoids characteristic of aged pistacia resin: 28-norolean-17-en-3-one (J) formed from oleanonic acid (Pastorova, 1997: 104), P (unknown *m/z* 143) and R (unknown *m/z* 143) (van der Does et al., 1998; Colombini et al., 2000b), present in significant abundance in these samples. The components methyl 18 α -oleanonate (S), methyl 3 α -acetoxy-isomasticadienolate (X) and methyl 3 α -acetoxy-masticadienolate (Y) observed in some of these resins have only previously been observed in modern resin and notably not in the pistacia resins from Amarna, Egypt (Stern et al., 2003). In addition, the component 20-hydroxy-24-dammaren-3-one was not observed by Stern et al. (2003) in the Amarna resins nor by Mills and White (1989) in their study of the samples. A number of unidentified components with a mass spectral base peak at *m/z* 453 have previously been associated with the heating (>250 °C) of modern pistacia resin (Stern et al., 2003). These components were not observed in any of these samples and therefore indicate that these samples have not been subject to significant heating, although gentle heating to melt the solid resin and make it flow may have been used at the time the resin was transferred to the amphorae.

The components methyl dihydroisomasticadienonate (*m/z* M⁺470, 455 bp) and methyl dihydromasticadienonate (identical mass spectra but later retention time) were found in four of

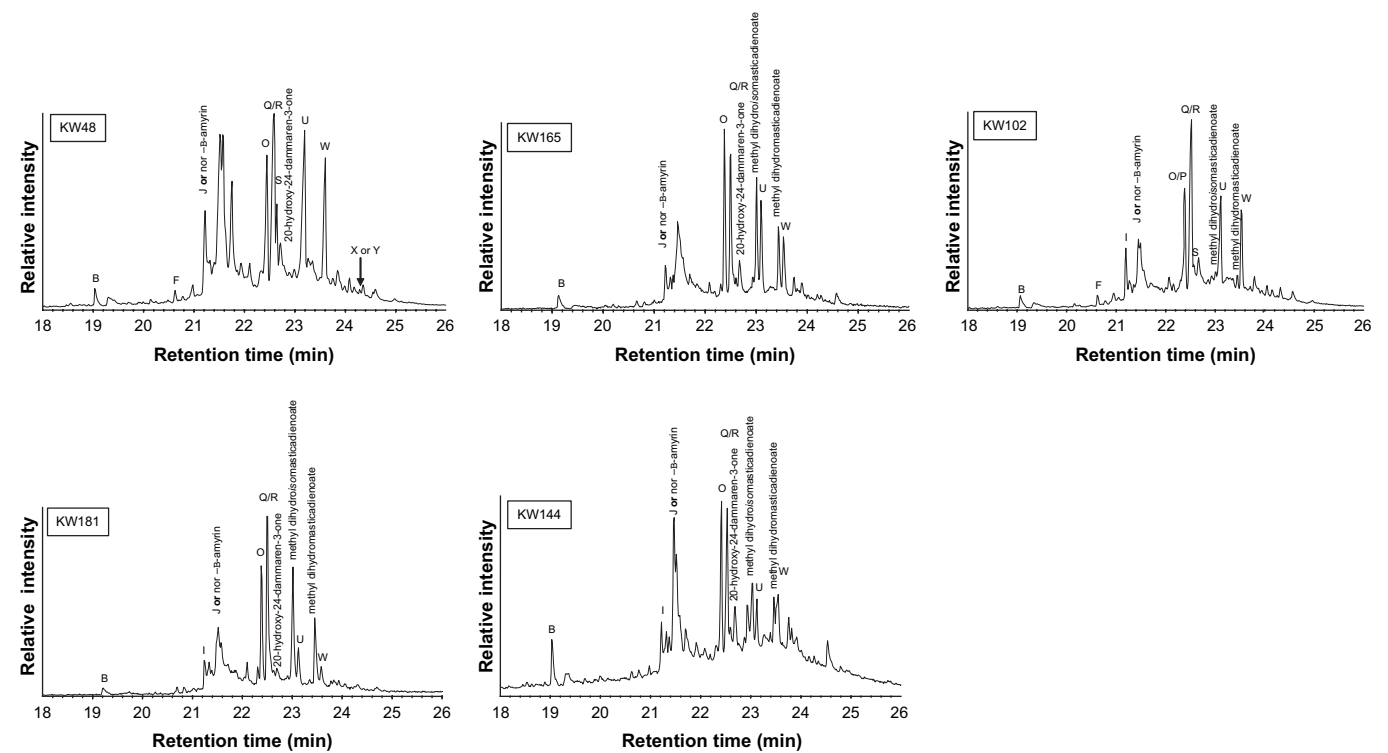


Fig. 1. Partial total ion chromatograms (TIC) of the methyl derivatives of the five Uluburun resin samples. Letter codes used for peak labels are previously described components used in Stern et al. (2003): B (unknown), F (28-norolean-12,17-dien-3-one), I (28-norolean-en-3-one), J (28-norolean-17-en-3-one) or nor-β-amyryn, O (methyl moronate), P (unknown *m/z* 143, co-elutes with O), Q (methyl oleanonate), R (unknown *m/z* 143, co-elutes with Q), S (methyl 18α-oleanonate), methyl dihydroisomasticadienoate, U (methyl isomasticadienonate), methyl dihydromasticadienonate, W (methyl masticadienonate), 20-hydroxy-24-dammaren-3-one and X (methyl 3α-acetoxy-isomasticadienolate) or Y (methyl 3α-acetoxy-masticadienolate).

the five samples examined in this study (all except KW48, Fig. 5). These components were also reported in one of the two Uluburun resin samples examined by Mills and White (1989: e and g Fig. 1). These components were not observed by Stern et al. (2003) in any of the 40 Canaanite amphorae or bowl samples containing pistacia resin from Amarna, or from modern samples of pistacia resin investigated as part of this study. Of related interest is that a previously analysed sample MSSA1, pistacia resin from an unprovenanced Canaanite amphorae excavated in Israel and now in the Rockefeller Museum (V.783), does contain these components (Stern et al. unpublished results). Mills and White (1989) comment on these dihydro-compounds and state that they could

represent natural variability in the ancient composition of the resin, or they may have differentially formed under the near anaerobic reducing conditions of the seabed. It is intriguing that sample KW48 is most similar to the Amarna pistacia resins, whilst MSSA1 is similar to the remaining four samples.

4.2. Analytical tests for wine

4.2.1. Feigl spot test

The pure tartaric acid standard produced distinctive positive results using the Feigl spot test. There is a notable difference in colour observed under ultraviolet light between the tartaric (green = positive) and syringic (blue/violet = negative)

Table 2
Light stable isotopic (δD , $\delta^{13}C$ and $\delta^{18}O$) values and percentage element composition for bulk resins

	$\delta^{13}C$ (‰)	Carbon (%)	$\delta^{18}O_{V-SMOW}$ (‰)	Oxygen (%)	δ^2H_{V-SMOW} (‰)	Hydrogen (%)
KW102	−25.6	70	15.6	11	−167	10
KW144	−24.2	70	24.8	22	−136	8
KW165	−24.4	57	20.2	13	−158	9
KW181	−25.0	83	17.2	10	−168	10
KW48	−24.9	75	16.9	13	−156	9
<i>Pistacia atlantica</i>	−27.1	79	13.4	13	−170	10
<i>Pistacia terebinthus</i>	−29.1	64	14.1	12	−172	9
<i>Pistacia terebinthus</i>	−29.0	76	12.0	14	−174	10
<i>Pistacia terebinthus</i>	−25.3	73	12.7	12	−170	10

The modern pistacia resin samples were recently collected from Bodrum Turkey.

standards. Moreover, variation in the amount of standard used did not have an effect on the intensity of colour produced. However, the green fluorescence did tend to diminish over a period of several hours.

No green fluorescence was observed in any of the modern wine soaked samples. In addition, the contemporary botanical samples (pistacia leaves and fruit) and the Uluburun resins showed no discernable fluorescence (Table 1). It was noted however that when the ceramic blank was analysed a consistent blue-violet fluorescence was observed.

The consistent positive results (i.e. green fluorescence) given by the tartaric acid standards worked well to establish the method for the spot test. Feigl et al. (1966) discuss the possibility of other colours that can result from the test. For example, a violet colour is indicative of the reagent, β,β -dinaphthol, and will fade over time. Furthermore, a grey colour is associated with glyceric and mesoxalic acids (Feigl et al., 1966: 470). While both of these are possible interpretations for the blue/violet colour produced by the samples and the ceramic blank, the original published method failed to provide visual references for colour variation. The statement that spot tests are the ‘chemistry of specific, selective and sensitive reactions’ (Feigl et al., 1966: 4) is not supported.

The botanical samples, including both the archaeological resins and the modern leaves and berries of the *Pistacia* sp, also produced negative results showing no fluorescence at all. While a negative result is acceptable for the purposes of analysis, it is possible that the limit of detection ($6.25\text{ }\mu\text{g/}$

mL) was not low enough to account for trace amounts of tartaric acid. However, the lack of positive results from any of the samples (apart from the standards) supports the conclusion that resin aboard the Uluburun was traded for its own economic value. Consequently, the suggestion proposed by McGovern regarding the transport of wine and resin in Canaanite amphorae is not supported by these analyses.

4.2.2. Infrared spectroscopy (IR)

The FT/IR spectra of the L-tartaric and syringic acid standards are shown in Fig. 2. Table 1 lists the modern and archaeological samples analysed. The detection of L-tartaric acid in archaeological samples depends on the presence of moderately intense carboxylic acid groups at $1470\text{--}1385$ and $1250\text{--}1240\text{ cm}^{-1}$ (COOH, Fig. 2), and doublet carbonyl peaks at $1720\text{--}1740\text{ cm}^{-1}$ (C=O, Fig. 2) (McGovern and Michel, 1996; McGovern et al., 1997). Not addressed by these studies, but nonetheless important to the study of ancient wine is calcium tartrate. According to McGovern (1997), this results from the ingress of calcium from the burial environment and its reaction with tartaric acid. The absorption bands for these salts are identified between 1550 and 1650 cm^{-1} which is related to the CO_2 symmetrical stretching (McGovern et al., 1997). The IR spectrum of syringic acid has similarities to L-tartaric acid which underlines the difficulty of discriminating biomarkers based on IR spectra alone (Fig. 2).

Fig. 3 shows the FT/IR spectra of a modern sherd soaked in red wine in comparison to selected examples of modern

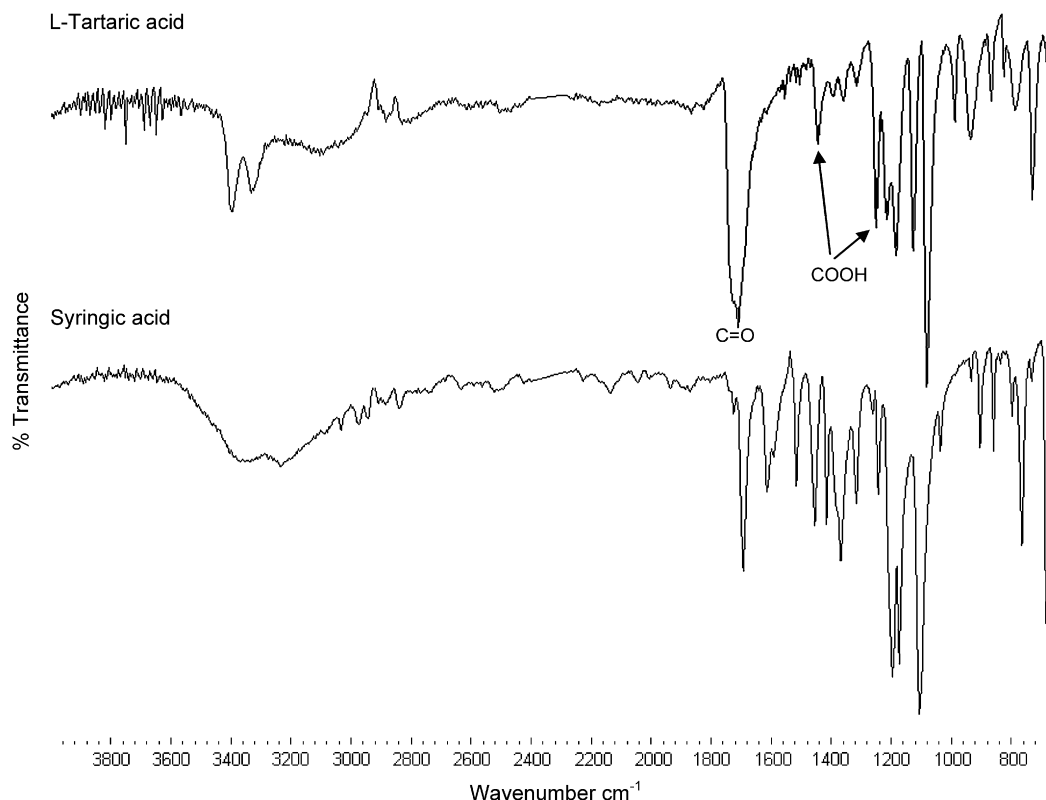


Fig. 2. FT/IR spectra for L-tartaric and syringic acid standards.

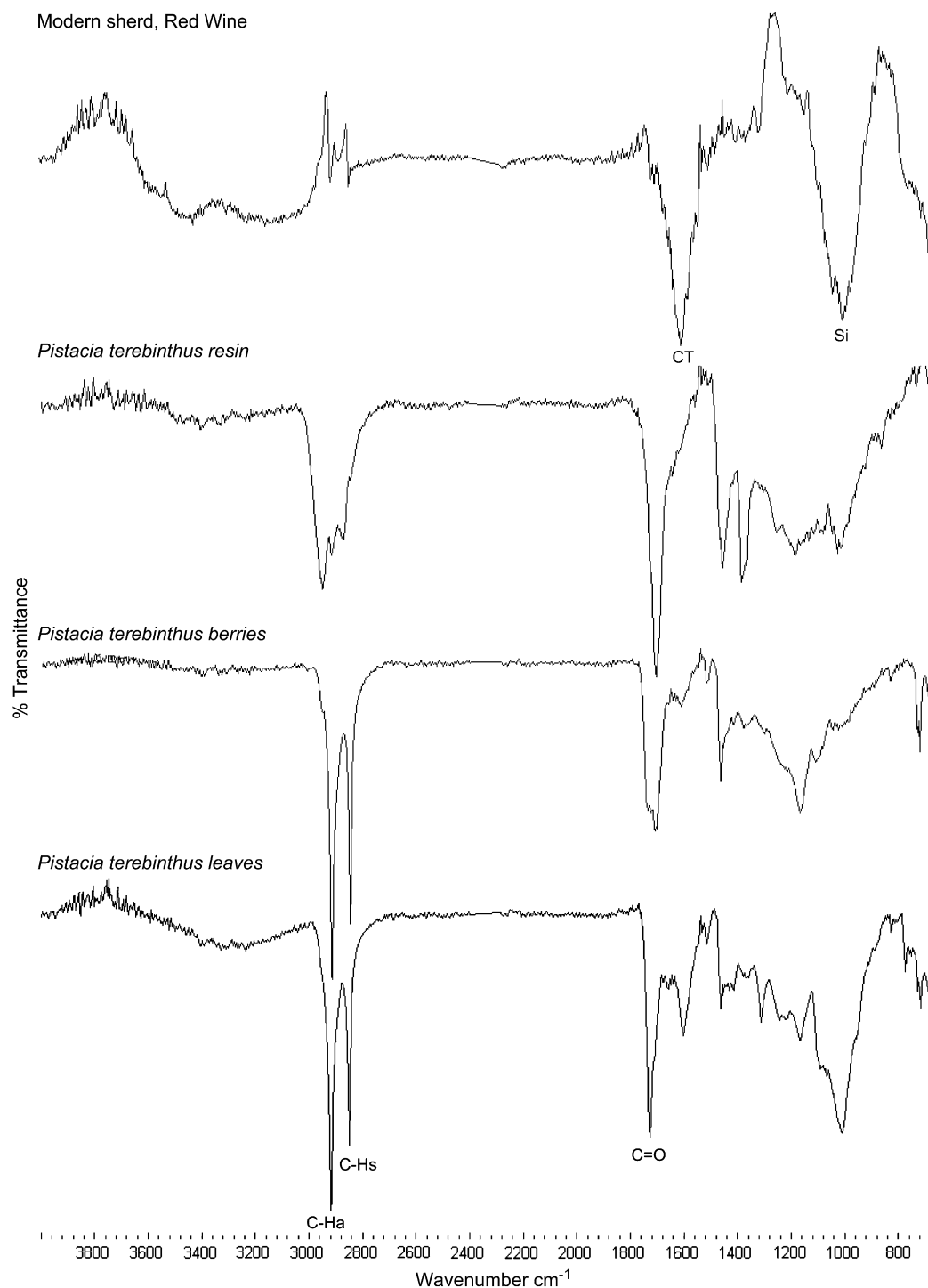


Fig. 3. Characteristic FT/IR (ATR) spectra of modern sherd soaked in red wine, *Pistacia terebinthus* resin, berries and leaves. C–Hs = C–H symmetric stretch $2900\text{--}2865\text{ cm}^{-1}$, C–Ha = C–H asymmetric stretch $2995\text{--}2955\text{ cm}^{-1}$, CT = calcium tartrate, Si = approximately 1100 cm^{-1} from silicates/clay minerals.

pistacia leaf and fruit. The modern sherd shows an absorption at approximately 1100 cm^{-1} caused by silicates/clay minerals. McGovern and Michel (1996) avoided these overlapping silicate absorption bands by solvent extracting the visible residue, then removing the solvent prior to analysis. The modern, wine treated potsherd displays moderately intense absorption bands more like those given by calcium tartrate between 1613 and

1531 cm^{-1} , (labelled CT Fig. 2) as opposed to bands related to L-tartaric acid (Fig. 2). McGovern (1997) attributed this absence of tartaric acid and presence of calcium tartrate to the geological conditions of the burial environment. However, in our study the samples were not buried, but merely soaked to simulate storage of wine within a vessel. A better explanation may be the processes of aging; related to rates of oxidation,

temperature and the precipitation of phenolic substances present in grape derivatives (Singleton and Esau, 1969: 175–176). As the samples were maintained at constant room temperature and exposed to light during the period of soaking the accumulation of tartrate salts could be expected. Therefore, in order to adequately determine the presence of ancient wine, it is necessary to look for calcium tartrate in addition to the L-tartaric acid.

The IR spectra of the leaves and fruit of the *Pistacia atlantica*, *Pistacia lentiscus* and *Pistacia terebinthus* species are similar and display strong, intense absorption bands in the 1720–1740 cm^{-1} region associated with the carbonyl region of the spectrum. Absorptions at 2900 cm^{-1} due to C–H bonds are also present. However, these absorption bands are shared by a huge variety of organic substances and are clearly not

restricted to tartaric acid/tartrate or terebinth resin (McGovern, 1997).

The FT/IR spectra of the Uluburun resins (Fig. 4) were characterized by intense absorption bands between 2800 and 3000 cm^{-1} (C–H stretches), as well as one situated at 1720 cm^{-1} (C=O, saturated aliphatic carbonyl). When compared to the spectra of modern pistacia resin (Fig. 3), the primary differences are diminished peaks and overlapping absorption bands. Moreover, all of the archaeological resins displayed distinctive dual absorption bands at 1440 and 1380 cm^{-1} indicating the presence of carboxylic acid salts. It is not known whether this is natural or due to the ingress of salts from the marine burial environment.

There is great resemblance between the absorption peaks defined between 1720 and 1740 cm^{-1} for samples of the

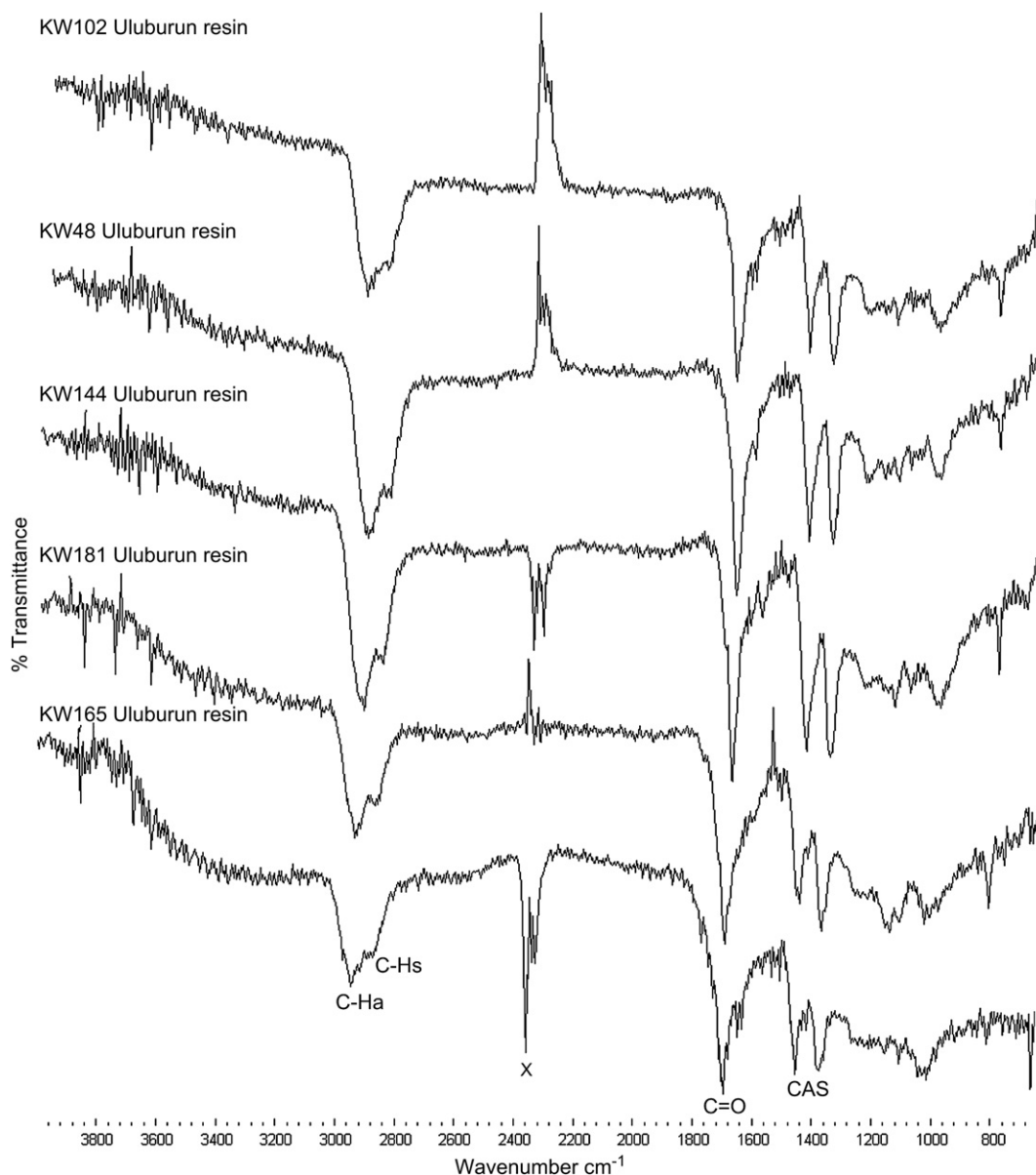


Fig. 4. FT/IR (ATR) spectra overlay of all Uluburun resin samples. CAS = carboxylic acid salts 1440–1335 cm^{-1} , X = analytical artifact due to atmospheric CO_2 (approximately 2350 cm^{-1}).

Uluburun resin, modern pistacia berry, leaf and resin and the L-tartaric acid. Therefore this technique lacks the ability to distinguish subtle differences within the carboxylic acid and carbonyl absorption regions and cannot be used to identify the presence of wine. Such spectroscopic interferences have also been observed in the analysis of modern wines when strong absorption bands from different components do not differ significantly (Moreira and Santos, 2004).

4.2.3. Gas chromatography-mass spectrometry (GC-MS)

Fig. 5 shows the trimethylsilyl (TMS) derivatives of the L-tartaric acid and syringic acid standards analysed by GC-MS. The mass spectrum for L-tartaric acid is characterized by a $M-15$ ($M-CH_3^+$) ion at m/z 423. Moreover, there are prominent fragment ions at m/z 292, 147, and 73 that are representative of TMS esters and associated fragment ions. The molecular ion (M^+) at m/z 438 was not present. The elution time for L-tartaric acid is between 10 and 12 min. Syringic acid is identified by a molecular ion (M^+) at m/z 342 and several consecutive ion fragments representing the successive loss of CH_3 groups; at m/z 327, 312, 297, and 283. Several other ion fragments can be found at m/z 73 ($TMSO^+$) as well as m/z 141, 223, and 253. The elution time for syringic acid is between 12 and 13 min. It is therefore possible to obtain chromatographic separation and distinctive mass spectra using GC-MS for L-tartaric and syringic acids. The GC-MS was able to detect the L-tartaric acid in quantities of 0.18 $\mu\text{g/mL}$ and syringic acid at 0.23 $\mu\text{g/mL}$.

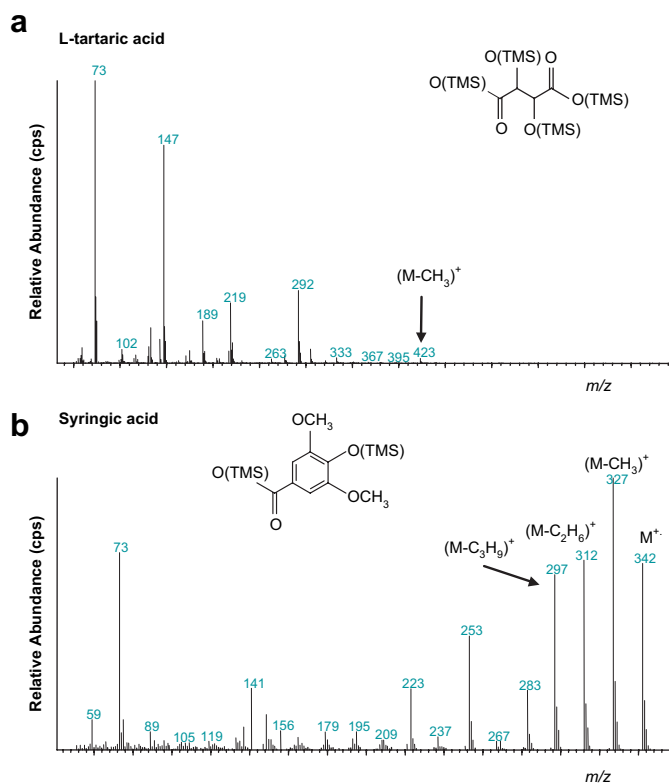


Fig. 5. Reference mass spectra and molecular structures for a) L-tartaric acid and b) syringic acid as their trimethylsilyl ester derivatives.

Modern, treated potsherds served as a means by which we could determine if the sensitivity of the instrument was adequate to detect trace amounts of the biomarkers for detection of ancient wine. Two modern, red and white wine-soaked potsherd samples were analysed (Table 1). L-tartaric acid was identified in both samples, confirming this molecule as a biomarker for grape-derived wine. Syringic acid (responsible for the colouring of red wine) was absent in the white wine soaked sherd (as expected) but identified in the red wine soaked sherd. Syringic acid was however found in one modern pistacia resin white wine soaked coated sample (Table 1). This sample did however follow an overloaded syringic acid standard and the presence of syringic acid for this sample is likely to be due to instrument contamination. This highlights the necessary caution involved in the analysis and interpretation of novel biomarkers.

A total of seven modern botanical samples were analysed for the purpose of determining the presence of false-positives in archaeological samples where pistacia resin and wine might be in the same context (i.e. blended wine residue). The samples available included *Pistacia atlantica*, *P. lentiscus*, and *P. terebinthus*. While the wine biomarkers (tartaric and syringic acids) were not detected using this method, analyses did succeed in identifying other major constituents of the botanical samples. These included significant abundances of $C_{16:0}$ (palmitic) and $C_{18:1}$ (oleic) long chain fatty acids including their methyl esters. This corresponds well to the results of a previous study which confirmed the major fatty acids of terebinth fruit oil as oleic (52%), palmitic (21%), and linoleic (20%) (Ozcan, 2004).

While the method for the detection of ancient wine was established by the analysis of standards and identification of these biomarkers within the residues of modern, treated potsherd samples, the analysis of the archaeological material failed to show evidence for the presence of wine derived organic residues. In the case of the Uluburun resins, conclusions once again support a hypothesis attributing to the trade in resin alone.

4.2.4. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS)

All samples were prepared using two separate methods as outlined by Guasch-Jané et al. (2004); solvent extraction for L-tartaric acid and alkaline fusion for syringic acid. As the instrumental conditions for the analysis of both wine markers were identical, it was possible to use the same instrumental method for simultaneous detection with the quadrupole MS detector operating in Multiple Reaction Monitoring (MRM) mode. For all analyses, L-tartaric acid (149, 87 m/z) eluted first with a mean elution time of 2.16 min, followed by syringic acid (197, 182 m/z) at 2.73 min (Fig. 6). For syringic acid we achieved earlier retention times than those previously reported (Guasch-Jané et al., 2004). The significant advantages of using the instrument in this way are the speed, sensitivity, selectivity, low LOD achieved and no need for sample derivatisation. In addition, identification relies on retention times and

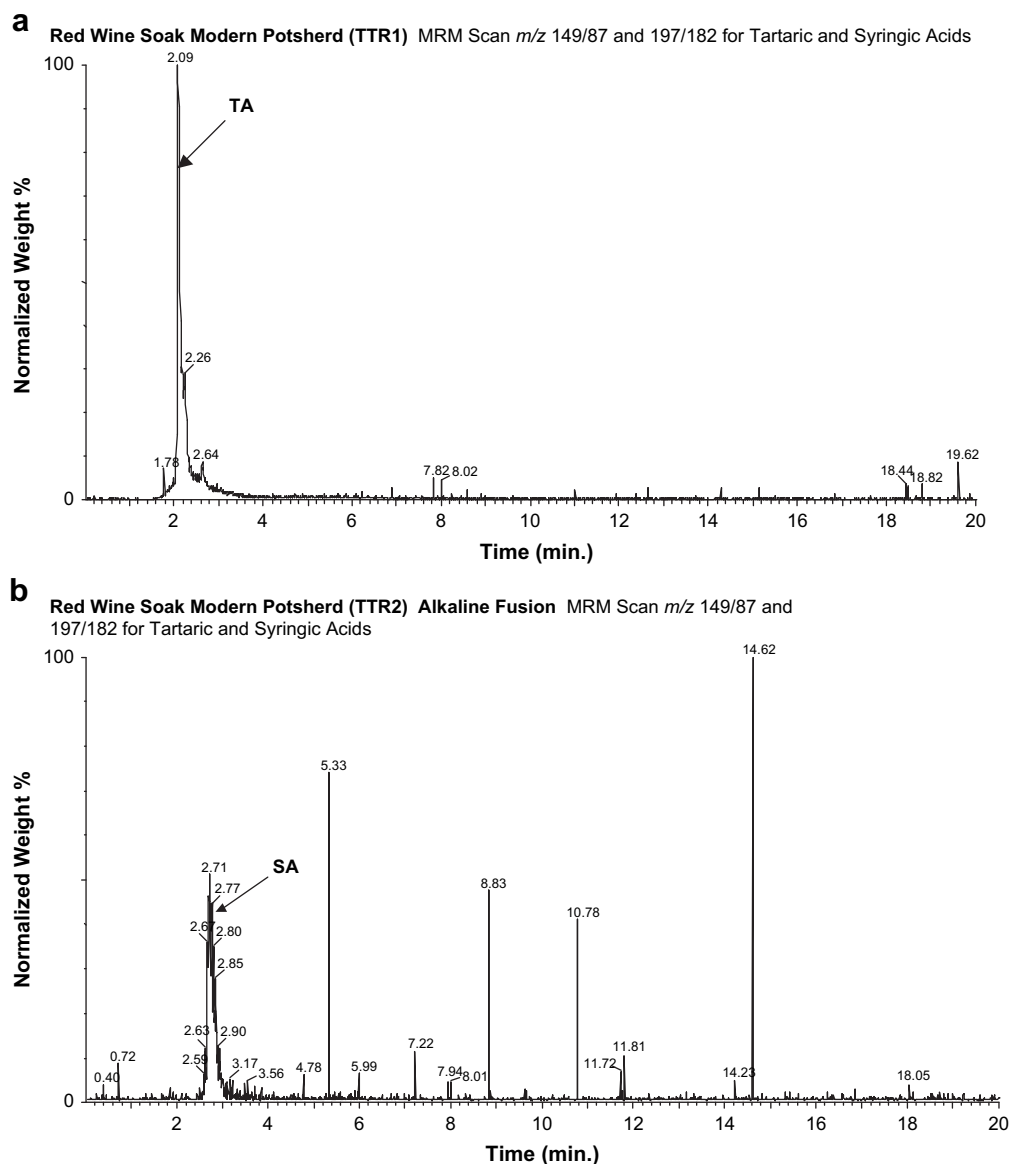


Fig. 6. HPLC-MS-MS m/z 149/87 and 197/182 ion chromatograms. (TA = L-tartaric acid, SA = syringic acid) a) modern, red wine soaked potsherd using L-tartaric specific sample preparation, b) modern, red wine soaked potsherd after alkaline fusion preparation of sample displaying greater potential for detection of syringic acid, c) modern, white wine soaked potsherd using L-tartaric specific sample preparation, d) modern, white wine soaked potsherd after alkaline fusion preparation of sample displaying no evidence for the presence of syringic acid with the application of alkaline fusion.

mass fragmentation patterns, providing absolute confirmation of any biomarkers present.

For the modern samples soaked in red wine, L-tartaric acid was easily detected (Fig. 6a), and upon the application of the alkaline fusion technique, a greater abundance of syringic acid was observed (Fig. 6b). Modern samples soaked in white wine displayed evidence of L-tartaric acid (Fig. 6c), but (as expected) lacked the presence of syringic acid regardless of the preparation method applied (Fig. 6d) (summarized in Table 1). The modern, treated potsherds confirmed the ability of this method to detect both syringic and tartaric acid. Significantly, the application of resins to the surfaces of the modern sherds did not appear to have an adverse effect on the detection of either syringic or tartaric acids absorbed into the ceramic/resin surface, therefore the resins did not act as a totally

impermeable barrier, preventing the detection of actual vessel contents (Table 1).

All eleven of the modern botanical sources were analysed by HPLC-MS-MS (Table 1). L-tartaric acid was not detected in any of these samples (e.g. Fig. 7a). However, following alkaline fusion, syringic acid was identified in eight of the eleven samples (e.g. Fig. 7b, Table 1). Previous studies on the composition of oils derived from the *Pistacia atlantica* fruits utilizing HPLC did not report the presence of syringic acid (Yousfi et al., 2005). The use of alkaline fusion in this work undoubtedly led to the recovery of this compound, not identified by other techniques. Furthermore, with the identification of syringic acid, there is the potential for false positives to be identified with respect to archaeological samples containing the remains of pistacia fruits. On the other hand,

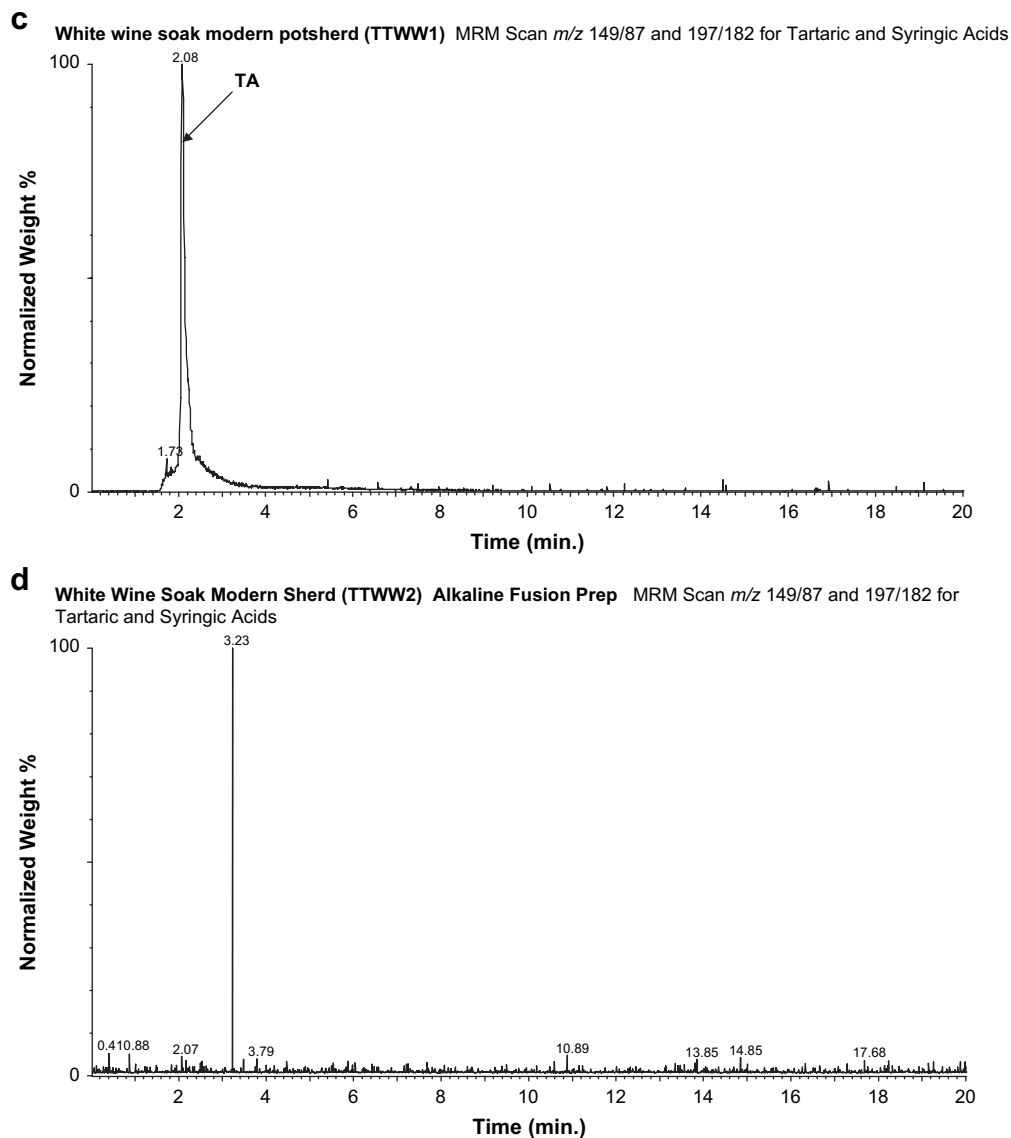


Fig. 6 (continued).

positive identification for the presence of ancient wine, in the past, has relied primarily on the presence of tartaric acid because it is specific to grapes and it is present in both red and white varieties of wine. Only in recent years have phenolic compounds been sought to determine the colouring of wine derived residues (Guasch-Jané et al., 2004).

No biomarkers for wine were detected in the resin samples by HPLC-MS-MS using both sample preparation methods (Table 1).

4.2.5. Light stable isotopic (δD , $\delta^{13}C$ and $\delta^{18}O$) values for bulk resins

Figs. 8a,b show the δD , $\delta^{13}C$ and $\delta^{18}O$ values of the Uluburun resins (open triangles) in comparison to modern pistacia resins (filled circles) and archaeological pistacia resin from Amarna (open circles) reported in Stern et al. (2008). The Uluburun resins are isotopically different to those from Amarna and the modern resins, although they are similar to sample

MSSA1 the unprovenanced sample from Israel. Sample KW144 is a possible outlier being more similar to the Amarna resins. This sample is however not the same outlier (KW48) as discussed above for the molecular compositions of the resins.

Although it is tempting to argue for a different isotopic provenance for the Uluburun and Amarna resins, Fig. 8d shows that the percentage oxygen for the Uluburun samples is similar to the modern resins, whilst the Amarna resins and KW144 have a much higher percentage oxygen composition. This indicates that the Amarna resins and sample KW144 have gained oxygen and therefore the measured $\delta^{18}O$ values do not reflect those of the original resin. Similarly, Fig. 8e shows that the percentage hydrogen for the Uluburun samples is similar to the modern resins, whilst the Amarna resins have a lower percentage hydrogen composition. It therefore seems likely that four of the five Uluburun resins have not undergone significant isotopic changes, but the measured values cannot be further interpreted due to the possibility of diagenetic changes.

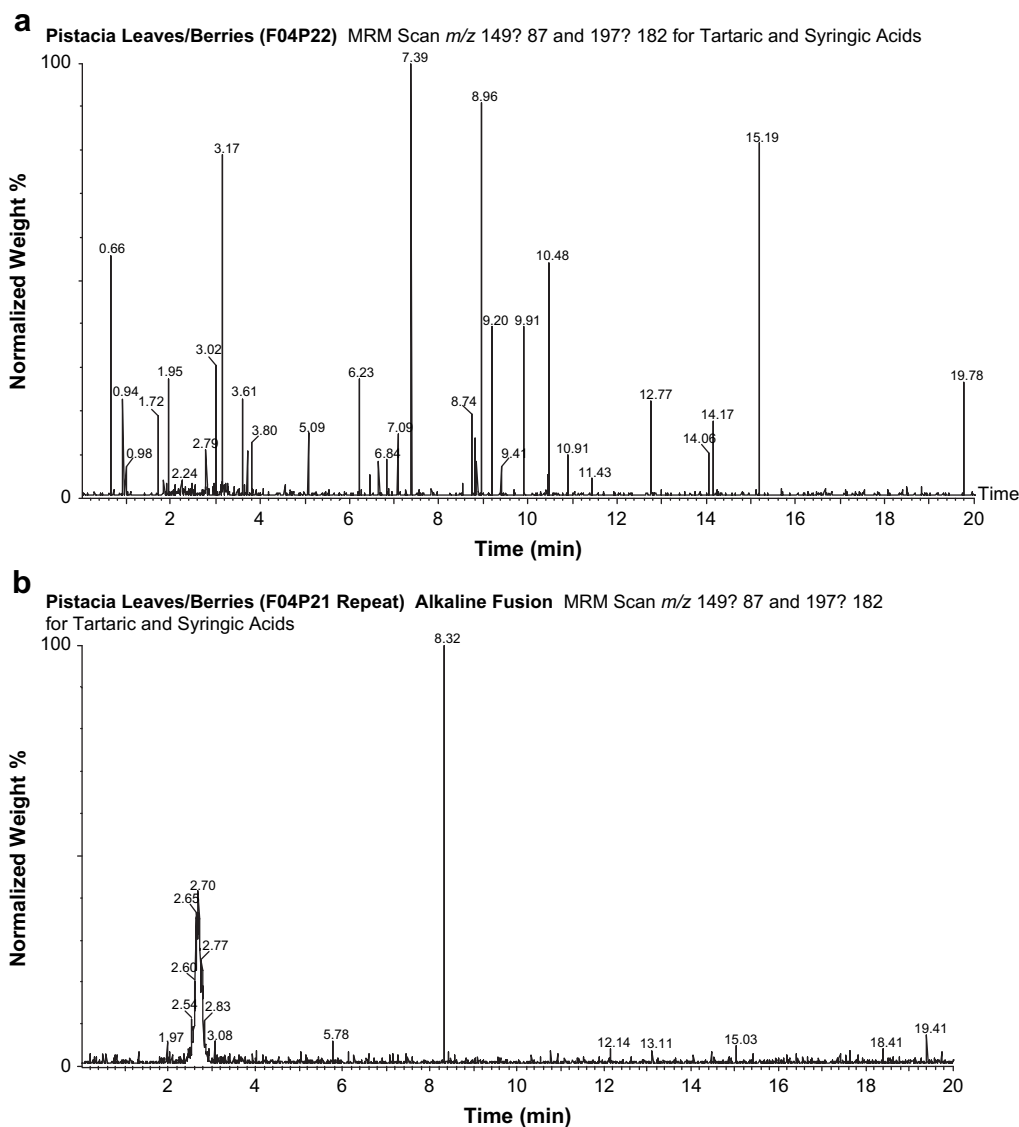


Fig. 7. Examples of HPLC-MSMS ion chromatograph for a) pistacia leaves and berries displaying no detection of L-tartaric or syringic acids, b) pistacia leaves and berries displaying elution of syringic acid with the application of alkaline fusion.

5. Conclusions

- (i) GC-MS analysis of five resin samples from the Ulu-burun shipwreck confirms resins of *Pistacia* sp. in each case. No other lipid component was detected. There are molecular differences between the five samples and the resins are degraded, but show no indication of having been significantly heated.
- (ii) Isotopic and molecular analysis of the pistacia resins allows comparison with samples found in Egypt and Israel but some diagenetic effects are apparent.
- (iii) Application of the Feigl spot test, FT-IR, GC-MS and HPLC-MS-MS sought to determine the effectiveness of each technique for the detection of tartaric and syringic acids and to determine the possible presence of wine in archaeological samples. The analysis of modern samples of pistacia resin, leaves, fruit and wine residues revealed that although successful for pure standards, in complex samples the spot test, FT-IR and GC-MS were unable to unambiguously confirm the presence of tartaric or syringic acids. The application of resins (pistacia and pine) to the surfaces of modern sherds then dosed with modern wine did not appear to have an adverse effect on the detection of either compound. In some of the modern leaves and fruit we did detect syringic acid (but not tartaric) which could result in the false identification of red wine.
- (iv) Whilst previous analytical investigations have stimulated interest in the detection of wine, chemical 'spot tests' are not advised in archaeological investigations as suggested by Boulton and Heron (2000). The presence of trace organic compounds (such as tartaric and syringic acids) in complex degraded mixtures is best resolved through selective and sensitive analytical techniques with unambiguous compound identification by the use of chromatography combined with mass spectrometry as Guasch-Jané et al. (2004) have demonstrated.

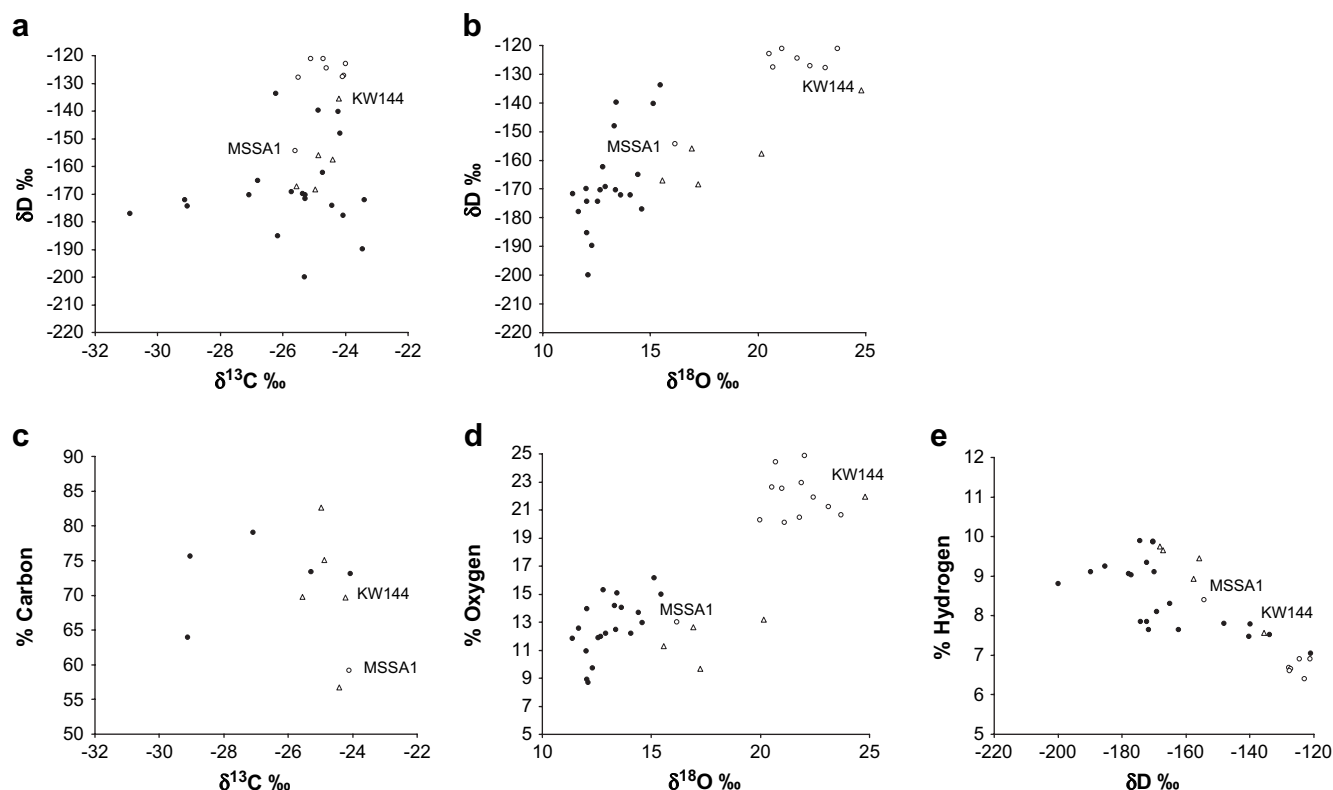


Fig. 8. Light stable isotopic (δD , $\delta^{13}C$ and $\delta^{18}O$) and percentage values for bulk resins. Filled circles are modern pistacia resins and open circles are archaeological pistacia from Amarna, with unprovenanced sample MSSA1 labeled (previously published in Stern et al., 2008) with additional data from modern pistacia resin as shown in Table 2. Open triangles are the Uluburun resins.

- (v) Neither syringic nor tartaric acid was detected in the Uluburun resin samples, including samples KW144 and KW181 analysed here which were reported by McGovern (1997) as testing positive for tartaric acid/tartrate. Given that we have applied more sophisticated techniques of analysis with an order of magnitude increase in sensitivity over the Feigl spot test it is concluded that the Canaanite amphorae on the Uluburun shipwreck transported the resin itself as a commodity and did not contain wine.

Acknowledgements

We thank Cemal Pulak, director of the Uluburun expedition, for providing the Uluburun resin samples. Anna Nicolaou and Andrew Healey for assistance with HPLC-MS-MS, Dennis Farwell with FT-IR. Andrew Gledhill for $\delta^{13}C$ and Iso-Analytical laboratories for δD and $\delta^{18}O$ determinations.

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