Journal of Archaeological Science 38 (2011) 977–984

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Chemical evidence for wine production around 4000 BCE in the Late Chalcolithic Near Eastern highlands☆

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ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form
5 November 2010
Accepted 21 November 2010

Keywords:
Armenia
Ceramic analysis
Chromatography
Grape
Malvidin
Mass spectrometry
Pomegranate
Residue analysis
Solid phase extraction
Syria
Syringic acid
Wine

ABSTRACT

Archaeological excavations in the Areni-1 cave complex in southeastern Armenia revealed installations and artifacts dating to around 4000 cal. BCE that are strongly indicative of wine production. Chemical evidence for this hypothesis is presented here using a new method to detect the anthocyanin malvidin that gives grapes and pomegranates their red color. Using solid phase extraction (SPE) and alkaline treatment of the samples, followed by combined liquid chromatography-tandem mass spectrometry (LC-MS/MS), this method was applied to authentic standards and four ancient potsherds from Armenia and Syria. A positive result was observed for two of the samples from the Areni-1 cave complex, adding evidence supporting the hypothesis that wine was produced in the Near Eastern highlands in the Late Chalcolithic Period.

1. Introduction

The deliberate fermentation of carbohydrates into alcohol (ethanol) has long been recognized as one of the many innovations marking the transition from Paleolithic to Neolithic societies, believed by some to be at least one of the factors that prompted the domestication of wild plants and the development of ceramic technology (Vitelli, 1989; McGovern, 2009). Many foodstuffs can be fermented into alcoholic beverages, and most have been and still are, but none are as widely used for this purpose as cereals (for making beer) and grape juice (from *Vitus vinifera*, for making wine). Apart from direct consumption, often in a specific social setting, the resulting organic solutions were also widely used for dissolving and dispensing medicinal compounds (Cruse, 2004: pp. 66–74; McGovern et al., 2009). The historical evidence for the early spread of wine technology throughout the Old World is plentiful (Zohary and Hopf, 1993; Estriecher, 2006; McGovern, 2007), and supporting archaeological evidence is accumulating (Zohary and Hopf, 1993; Manen et al., 2003; Margaritis and Jones, 2006; Sadori et al., 2006; Miller, 2008; Morris, 2008; Schlumbaum et al., 2008; Djamali et al., 2009).

Chemical evidence supporting the assumed presence of wine includes the detection of compounds that can be indicative of wine in the preserved contents of ancient ceramic vessels or in the residues attached to the ceramic matrix of unglazed pottery sherds. Such compounds include L-tartaric acid, a molecule abundant in wine (Guasch-Jané et al., 2004, 2006; McGovern, 2009), and resins deliberately added to wine for its preservation or flavor enhancement (McGovern et al., 1996, 2004;

☆ The main conclusions in this article were previously presented at the 75th Annual Meeting of the Society of American Archaeology, St. Louis, 15th April 2010.

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doi:10.1016/j.jas.2010.11.012

McGovern, 1997; McGovern, 2007). These compounds, however, do not conclusively prove the former presence of wine. Tartaric acid is present in many vegetables and fruits (Winter and Herrmann, 1986; DeBolt et al., 2006), albeit at approximately 4000 parts per million (ppm) in a relatively high concentration in grapes (Peña-Neira et al., 2000; Soyer et al., 2003). This concentration varies with the ripeness and variety of the grape, as well as the geographical latitude (Stern et al., 2008); in temperate regions most of the acidity of grapes is due to malic rather than tartaric acid. The fruits of the hawthorn tree (*Crataegus monogyna*) contain about 16,000 ppm of tartaric acid (McGovern et al., 2004). In the Near Eastern highlands of Anatolia this plant was used as early as the second millennium BCE (Collins, 1990; Ertuğ, 2000), and probably much earlier. Other fruits, not native to the Near East, with amounts of tartaric acid much higher than grapes include, for instance, tamarind (*Tamarindus indica*, 180,000 ppm), star fruit (*Averrhoa carambola*, 25,000 ppm) and yellow plum (*Spondia mombin*, 15,000 ppm). The amounts of tartaric acid have only been reliably quantified for a limited number of plant species; plants that are not yet studied in detail may prove to contain high amounts of tartaric acid as well (Stern et al., 2008). From an archaeological perspective it is furthermore important to note that tartaric acid is easily soluble in water; it will thus readily leach out of buried vessels and potentially into adjacent ones, making the presence and absence of tartaric acid in archaeological samples difficult to interpret. This is less so for its potassium and calcium salts (Fig. 1), although the solubility of these in water is still sufficiently high to result in percolation in and out of archaeological contexts (Singleton, 2000). Tartaric acid can thus only serve as a reliable indicator for any of the food-plants mentioned above in dry contexts and with favorable conditions for the preservation of organic materials. Furthermore, tartaric acid from grapes needs not necessarily be associated with wine, but can instead indicate the former presence of grape juice, raisins or concentrated grape syrup used as a sweetener (Miller, 2008), such as *defrutum* in Classical times (Feldman, 2005), or modern *pekmez* (Karababa and Isikli, 2005).

Resins found in archaeological contexts could be associated with a number of products (Boulton and Heron, 2000; Stern et al., 2008), or have been used to seal unglazed vessels (Serpico and White, 2000), rather than to preserve or enhance wine. Wine does not naturally contain such resins and only becomes resinated when it is produced, stored or transported in resinated containers, probably so treated to make them less porous. Resins could also have been added at some time during the wine production process to preserve the product or change its taste, both likely resulting from experience with contaminated wine from resinated containers. The practice of deliberately resinating wines with pine resins is first mentioned in the historical record in the first century CE (Columella, *De re rustica 12.20–14* and Pliny the Elder, *Naturalis Historia 14.124*, *16.60*, Rackham, 1945; Forster and Heffner, 1958), but probably started much earlier. At present the practice is mostly limited to Greece and few outsiders prefer the taste of its resinated wines, generically referred to as *retsina*, over the non-resinated product. In ancient times most wine was probably not kept very long nor transported very far and it is likely that most wine was thus not resinated. Traces of resins in archaeological samples as early as the Neolithic (McGovern et al., 1996) and as far from the Mediterranean as China (McGovern et al., 2004) therefore in themselves provide insufficient evidence for the former presence of (resinated) wine.

A better chemical indicator for the former presence of red wine is malvidin, the anthocyanin that gives grapes and wines their red color (Cheynier et al., 2006). Compared to tartaric acid, malvidin is present in few other plants, most importantly here in pomegranate (*Punica granatum*, Alighourchi et al., 2008), a popular fruit in the Near East from ancient times onward, the juice of which was sometimes added to wine (Ward, 2003). Other Old World plants that produce significant amounts of malvidin are whortleberry (*Vaccinium myrtillus*), red clover (*Trifolium pratense*) and high mallow (*Malva sylvestris*), all of which are used by humans and may be part of medicinal concoctions. As with tartaric acid, the amounts of malvidin have only been reliably quantified for a limited number of plant species, but given the nature of the compound it is expected to be present in fewer species than tartaric acid. Malvidin is a stable compound

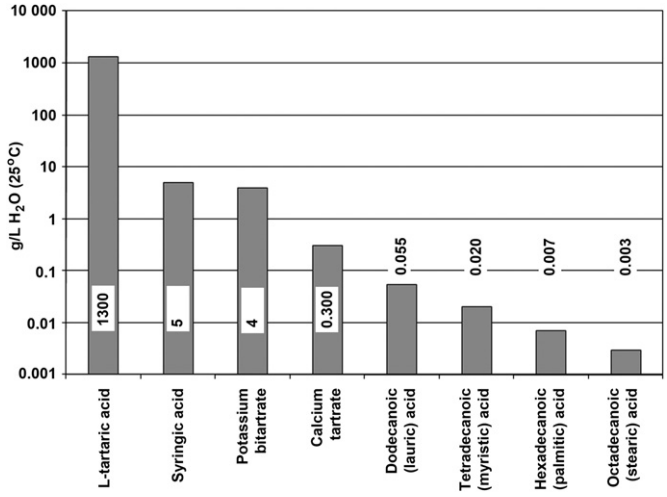

Fig. 1. Solubility in water (g/L at 20 °C) of L-tartaric acid, syringic acid, selected salts of tartaric acid, and some of the fatty acids that are routinely found in ancient organic residues. Note the logarithmic scale (data from Material Safety Data Sheets and the Cyberlipid Center, http://www.cyberlipid.org/).

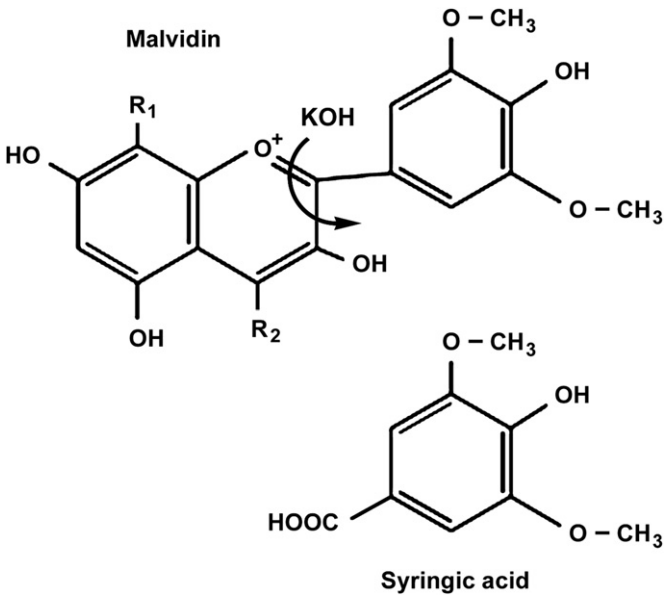

Fig. 2. Alkaline treatment of malvidin results in the release of syringic acid (KOH = potassium hydroxide). Syringic acid = 3,5-dimethoxy-4-hydroxybenzoic acid ($C_9H_{10}O_5$); mass = 198.173 Da (average), 198.053 Da (monoisotopic); melting point = 206–209 °C (experimental); solubility in H_2O = 3.71–5.78 mg/mL (predicted); pKa_1: 3.86 (experimental); pKa_2 = 7.76 (experimental).

(McDougall et al., 2005), which polymerizes over time with a corresponding color change from bright red to the darker dusky red of mature wines (Asenstorfer et al., 2003), although the actual color also depends on the pH of the solution. The solubility of malvidin in water decreases with an increased degree of polymerization and it is clear from house-hold experience that stains left by red wine are extraordinarily persistent and may well be preserved in archaeological contexts. Due to their inherent heterogeneous nature, polymers are difficult to study, but when placed in a strong alkaline environment malvidin releases syringic acid (Fig. 2, after Zsuga and Kiss, 1987; Singleton, 2000; Guasch-Jané et al., 2004; Guasch-Jané, 2005), a small and relatively little studied molecule (Erdemgil et al., 2007). Free syringic acid is naturally present in many plant products, including barley (*Hordeum vulgare*, Yu et al., 2001), wheat (*Triticum aestivum*, Kim et al., 2005) and wine (Peña-Neira et al., 2000; Salas et al., 2003; Monagas et al., 2005; Cheynier et al., 2006), but also in soil and other organic environments due to microbiological activity (Mukherjee et al., 2006). The presence of syringic acid after alkaline treatment of an archaeological residue can therefore not by itself prove the former presence of red wine (Guasch-Jané et al., 2004), nor can its absence prove the former presence of white wine (Guasch-Jané et al., 2006; Stern et al., 2008). We describe here an improved analytical method to show the presence of malvidin that may support or falsify the anthropological and historical considerations regarding the presence of wine in the archaeological record using four ancient potsherds from Armenia and Syria as an archaeological case study.

2. Materials and instrumentation

The archaeological samples used in this study included three potsherds from the cave complex Areni-1, located near the village of Areni in the Vayots Dzor province of Armenia. A platform inside the cave preserved raised edges made of packed clay slanting towards the mouth of a large jar inserted into the lower edge of the platform. The design of this installation suggests that it was meant to direct a liquid originating on the platform to flow into the jar, interpreted as a *pithos* or *karas*. The installation was surrounded by other large jars (Fig. 3), interpreted as storage jars, and thus strongly resembles historical grape pressing installations. The discovery in close proximity to this installation of desiccated grapes, grape seeds, grape rachises, and grape skins still attached to pedicels corroborates the interpretation that it was used for grape crushing and pressing. The installation and associated artifacts were unearthed by controlled stratigraphic excavation from the well-preserved second Chalcolithic level in the central gallery of the cave complex, which has been dated to 4223–3790 cal. BCE by radio-carbon analysis of three samples (OxA-18197, UCIAMS-40182 and UCIAMS-48413). One of the ceramic samples for this study was taken from the surface of the pressing platform and two others from inside one of the adjacent vessels (Fig. 3). Without any further

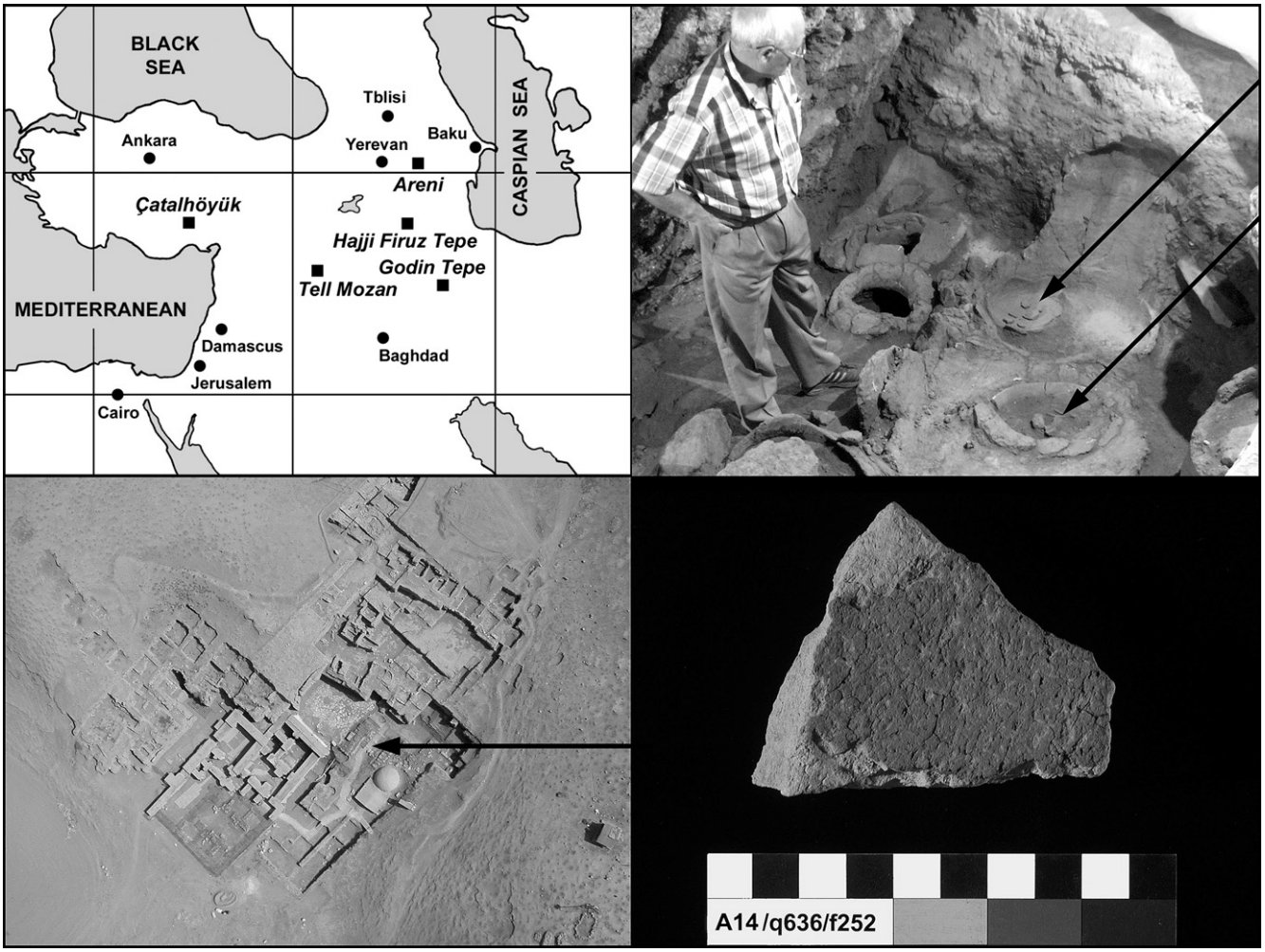

Fig. 3. Map of the Near East (top-left), showing archaeological sites where early evidence for the production of wine has been described; the provenance of the ancient samples from Areni (Armenia, top-right) and Tell Mozan (Syria, bottom-left); the ancient sample from Syria (bottom-right), with red-stained interior.

cleaning or treatment the sherds were crushed into a fine powder, using an aluminum-oxide mortar and pestle, stored in a clean glass vial, and transported to the Pasarow Mass Spectrometry Laboratory (UCLA) for analysis. A fourth archaeological sample comprised the inner layer of about 2 mm of a potsherd from Late Akkadian deposits (around 2200 BCE) associated with the palace of King Tupkish in Tell Mozan (ancient Urkesh), in northeastern Syria (Buccellati and Kelly-Buccellati, 1996, 2000). This sherd displayed a bright red interior surface, obviously not the result of the original treatment of the vessel, but rather the remains of its former contents. Given that it was found in the royal palace it was suggested that the vessel had possibly contained red wine, known to reflect the status of the consumer in the period (beer being the more common beverage). The inner surface was scraped off the sherd, which had been washed with water before being recorded and stored as is common practice at most excavations. The resulting powder was transported to the laboratory in a clean glass vial. To prevent contamination latex gloves were worn at all times while handling the sherds and the pottery powder.

Given the strong archaeological indications for an association with ancient wine production, the three sherds from Armenia were used to test and further develop analytical methods to show the presence of wine residues based on published research and the considerations discussed above. The sherd from Syria was entered into the study to further test any improved or newly developed method. The powder of a ground sherd from a new vessel in which dromedary milk was cooked was included as a known negative (Barnard et al., 2007); known positive standards included commercially available red wine (Charles Shaw cabernet sauvignon) and authentic syringic acid (Sigma–Aldrich part number S6881). Measurements were performed on a PerkinElmer-Sciex API III$^+$ triple quadrupole tandem mass spectrometer (MS/MS) with an attached Hewlett Packard 1090 liquid chromatograph (LC), for the development of the method, and on an Applied Biosystems-MDS Sciex 4000QTrap Hybrid Triple Quadrupole Linear Ion Trap MS/MS instrument with an attached Agilent 1200 LC system, for the analysis of the ancient samples because of its much greater sensitivity (Fig. 4). These LC-MS/MS instruments separate a complex sample into its components by high pressure (or high performance) liquid chromatography (HPLC). The molecules are subsequently ionized, in our case by negative ion electrospray ionization (ESI$^-$). When the instrument is operated in the tandem mode only molecules with a predetermined mass (the precursor ions) are allowed

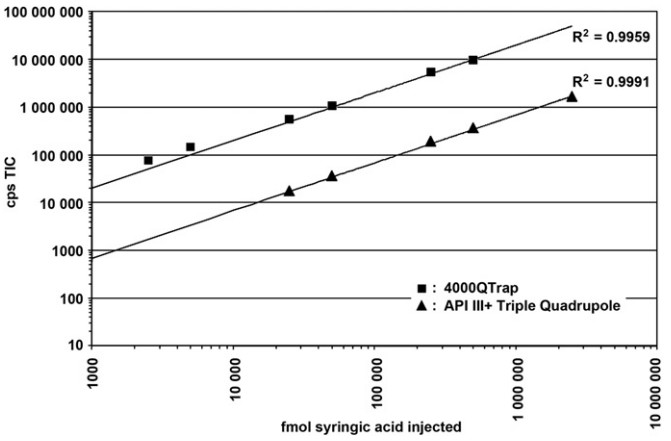

Fig. 4. Sensitivity for syringic acid detection by the PerkinElmer-Sciex API III$^+$ triple quadrupole and the Applied Biosystems-MDS Sciex 4000QTrap hybrid triple quadrupole linear ion trap LC-MS/MS instruments used in this study. Note logarithmic scales of both axes (cps TIC: counts per second of the total ion current).

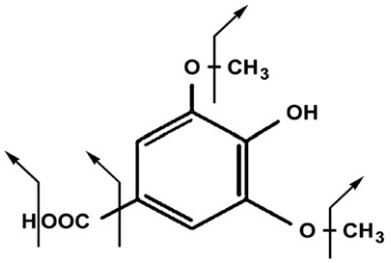

Fig. 5. Fragmentation of syringic acid during collision activated dissociation (CAD). The calculated average masses of the fragments are m/z 197.2 for $C_9H_9O_5$ $(M-H)^-$, 182.1 for $C_8H_6O_5$ $(M-H-CH_3)^-$, and 123.1 for $C_6H_3O_3$ $(M-H-CO_2-CH_3-CH_3)^-$.

into a collision chamber filled with an inert gas (Ar or N_2). The resulting fragments (the product ions), formed by collisionally activated dissociation (CAD), are then separated in a second mass analyzer. In the multiple reaction monitoring (MRM) mode the detector records the intensity for the various precursor ion → product ion (I_{Pre} → I_{Pro}) transitions over time. MRM allows specific and sensitive quantitative measurements to be made on the compound of interest (Faull et al., 2009). A positive identification is made if the sample gives a significant signal for the selected I_{Pre} → I_{Pro} transitions at a specific time after the injection (the retention time of the molecule on the LC-column). In addition to sensitivity and specificity for trace level identification and quantification of many compounds, the advantages of LC-MS/MS technology include compatibility with aqueous samples, indifference to the thermal stability of the analytes (thus obviating the need for chemical derivatization), and the ability to inject a relatively large portion of the sample.

3. Methods

Initially a slightly adapted version of a published analytical method was used (Guasch-Jané et al., 2004), but we were unable to satisfactory duplicate the reported results. We were thus motivated to make improvements in both the chromatography and the sample preparation. These improvements are partly based on original research and partly on research published by others (Fernández de Simón et al., 1990; Alighourchi et al., 2008). Similar to tartaric acid, syringic acid is poorly retained during reverse phase HPLC, although retention can be increased by lowering the pH (Erdemgil et al., 2007), and increasing the polarity of the eluting solvent (Shui and Leong, 2002). The negative ion ESI mass spectrum of syringic acid revealed, as others have also reported (Guasch-Jané et al., 2004, 2006), an ion at m/z 197 corresponding to the carboxylate anion (Fig. 5). Using the published chromatography while following the m/z 197 → 182 and 197 → 123 I_{Pre} → I_{Pro} transitions on the 4000QTrap, short and poorly reproducible retention times ranging between 1.73 and 2.82 min were obtained. Longer and more

Table 1
The liquid chromatography gradient used in this study.

Solvent A: water with 2% acetic acid			
Solvent B: methanol with 2% acetic acid			
Time (min)	Flow (μL/min)	%A	%B
0	900	99	1
5	900	99	1
20	900	5	95
24	900	5	95
25	900	99	1
40	900	99	1

reproducible retention of syringic acid was achieved with a different liquid chromatograph gradient using water and methanol, both with 2% acetic acid (Table 1).

Next, the method of sample preparation was adapted to remove as much free syringic acid from the sample as possible before liberating any new syringic acid from malvidin polymers, by the addition of potassium hydroxide (KOH), and at the same time minimizing the amount of salts resulting from the alkaline treatment and the subsequent acidification of the sample. After a series of experiments using wine and syringic acid standards, analyzed on the API III$^+$ triple quadrupole instrument, solid phase extraction (SPE) of the sample was selected as the method of choice. An extraction solution was prepared consisting of H_2O/methanol/HCl (150/1.5/0.1, v/v/v, ~6.7 mM HCl, pH < 2.5), and a reagent solution of 5.9 M aqueous KOH, freshly prepared by dissolving 12 pellets (1.1–1.3 g) of 85% pure KOH in 3 mL H_2O (pH > 14). The sample preparation began with the combination of 3 mL extraction solution with 2 g sample (crushed potsherd) in a test tube. This was mixed (30 s), sonicated (30 min) and centrifuged (10 min, 1500 × *g*), after which the supernatant was decanted into a second test tube. The insoluble residue was then re-extracted as above with an additional 2 mL of the extraction solution. The two supernatants were pooled and loaded under low speed centrifugation (30 s, 100 × *g*) onto a disposable reverse phase SPE cartridge (Agilent AccuBond ODS-C18), previously activated with 1 mL methanol followed by three rinses of 1 mL H_2O each. The flow-though was retained and loaded again onto the same cartridge. After the second loading, the cartridge was eluted with 1 mL each of 5%, 10%, 15%, 20% methanol in H_2O, respectively, and finally with three 1 mL aliquots of acetonitrile/2-propanol/H_2O (25/25/50, v/v/v). All eluants were retained separately. They were dried in a vacuum centrifuge and then treated with 0.5 mL reagent solution at 50 °C for 5 min. Next, 1 mL H_2O was added and the solutions were acidified with hydrochloric acid (HCl) to pH < 2.5 (about 250 μL). The samples were cooled on ice and extracted with 2 × 2 mL ice-cold ethyl-acetate using centrifugation (10 min, 1500 × *g*) to separate the phases. The pooled ethyl-acetate supernatants were dried in a vacuum centrifuge and the samples redissolved in 2 × 90 μL H_2O/methanol (95/5, v/v) for analysis by LC-MS/MS-MRM. This method uses HCl rather than formic and trifluoracetic acid, as well as less KOH and acid compared to methods published elsewhere.

Using a custom-made loop, 90 μL of standard solutions, appropriate blanks or the redissolved extracts were injected onto a reverse phase HPLC column (Waters Nova-Pak C18, 300 × 3.9 mm, 4 μm particle size) equilibrated in solvent A (2% acetic acid in water) and eluted (900 μL/min at 40 °C) with an increasing concentration of solvent B (2% acetic acid in methanol, Table 1). The effluent from the column was directed to the ESI source attached to the 4000QTrap instrument operating in negative ion MRM mode. The mass spectrometer ion source parameters were optimized for maximal intensity of the selected transitions from syringic acid: curtain gas: 25 psi; ion spray voltage: −4500 V; ion spray temperature: 550 °C; gas one (N_2): 45 psi; gas two (N_2): 55 si, CAD

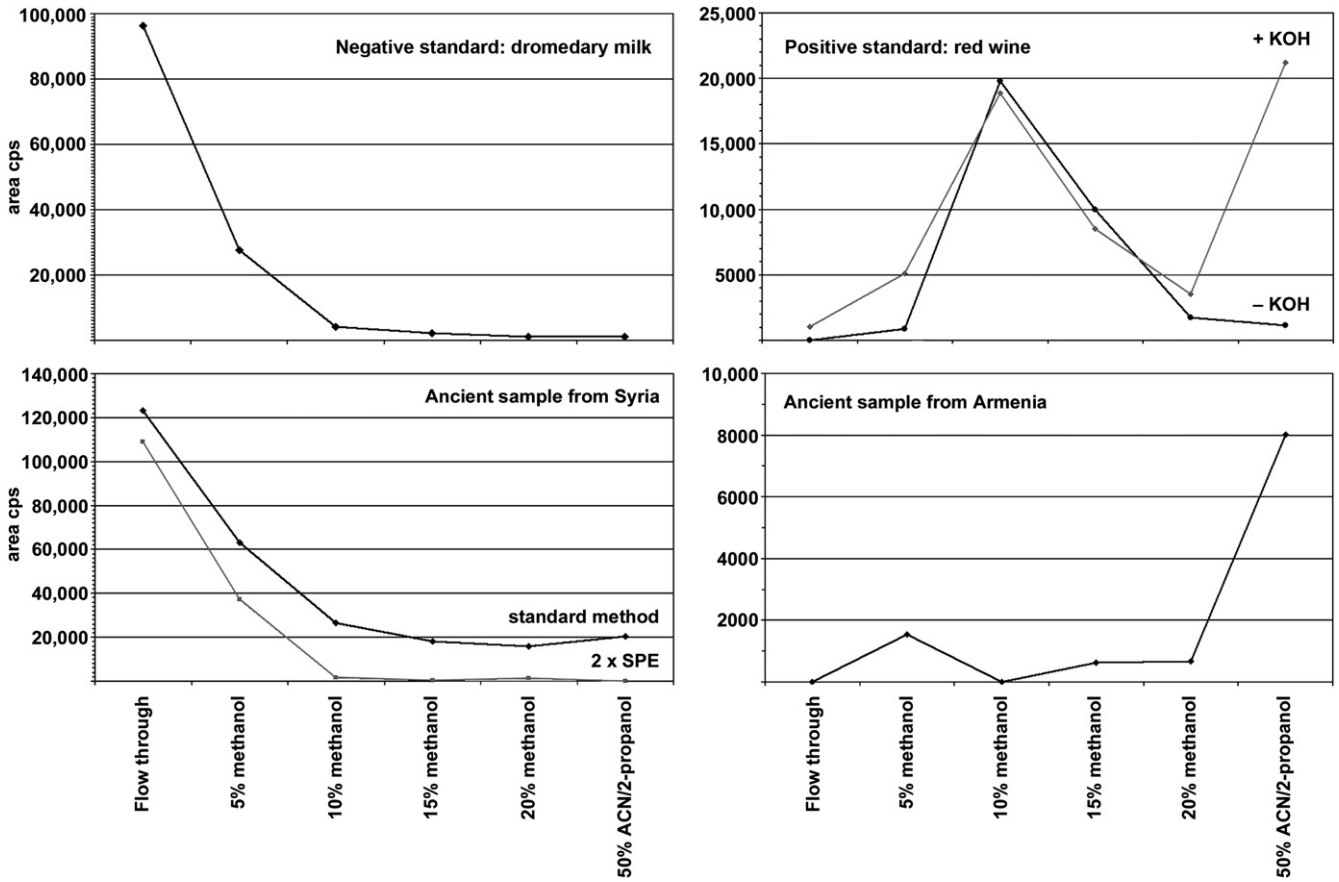

Fig. 6. Negative (top-left) and positive (top-right) curves indicating the absence or presence of malvidin, after SPE and KOH treatment, respectively. Of the sample from Syria (bottom-left) the flow-through of the SPE column was treated as another sample (2 × SPE) in case molecules in the original sample had preferentially taken up the available binding places for malvidin. The ancient sample from Armenia (bottom-right) clearly displays a positive response with a distinctly higher amount of syringic acid in the 50% organic fraction compared to the 20% organic fraction, similar to the +KOH-trace in the positive standard (red wine).

gas (N_2) setting: medium. The MRM transitions for syringic acid were: 197 → 182 *m*/*z* and 197 → 123 *m*/*z* (I_{Pre} → I_{Pro}), with a declustering potential of −55 V, collision energy of −20 V, entrance potential of −10 V, and collision cell exit potential of −7 V.

4. Results

In developing this method particular attention was given to separating free syringic acid from syringic acid resulting from the alkaline treatment of malvidin polymers, and to the reliability of the chromatography of syringic acid. Most of the free syringic acid appeared to elute from the SPE cartridge with the 10% organic fraction and most of the malvidin, if present, with the 50% organic fraction. Using syringic acid, wine or the powder of a crushed vessel in which dromedary milk was cooked, positive and negative curves indicating the presence and absence of malvidin, respectively, were established based on the presence of syringic acid after alkaline treatment (Fig. 6, top). The sensitivity of the API III$^+$ triple quadrupole instrument for modern red wine, containing a mix of different malvidin polymers, was calculated to be better than 300 nL wine in the sample. This is independent of the amount of pottery powder used once this is enough to contain at least the equivalent of 300 nL wine. As the 4000QTrap instrument is at least ten times more sensitive (Fig. 4), this instrument should be able to detect as little as 30 nL of red wine. Obviously, over extended periods of time the volatile components in wine will evaporate and the soluble components leach out, while malvidin will both polymerize and oxidize. Nevertheless, we present these numbers here as an approximation of the limit of detection of our method with the confidence of having developed an assay that can determine malvidin content as either positive or negative (below the limit of detection).

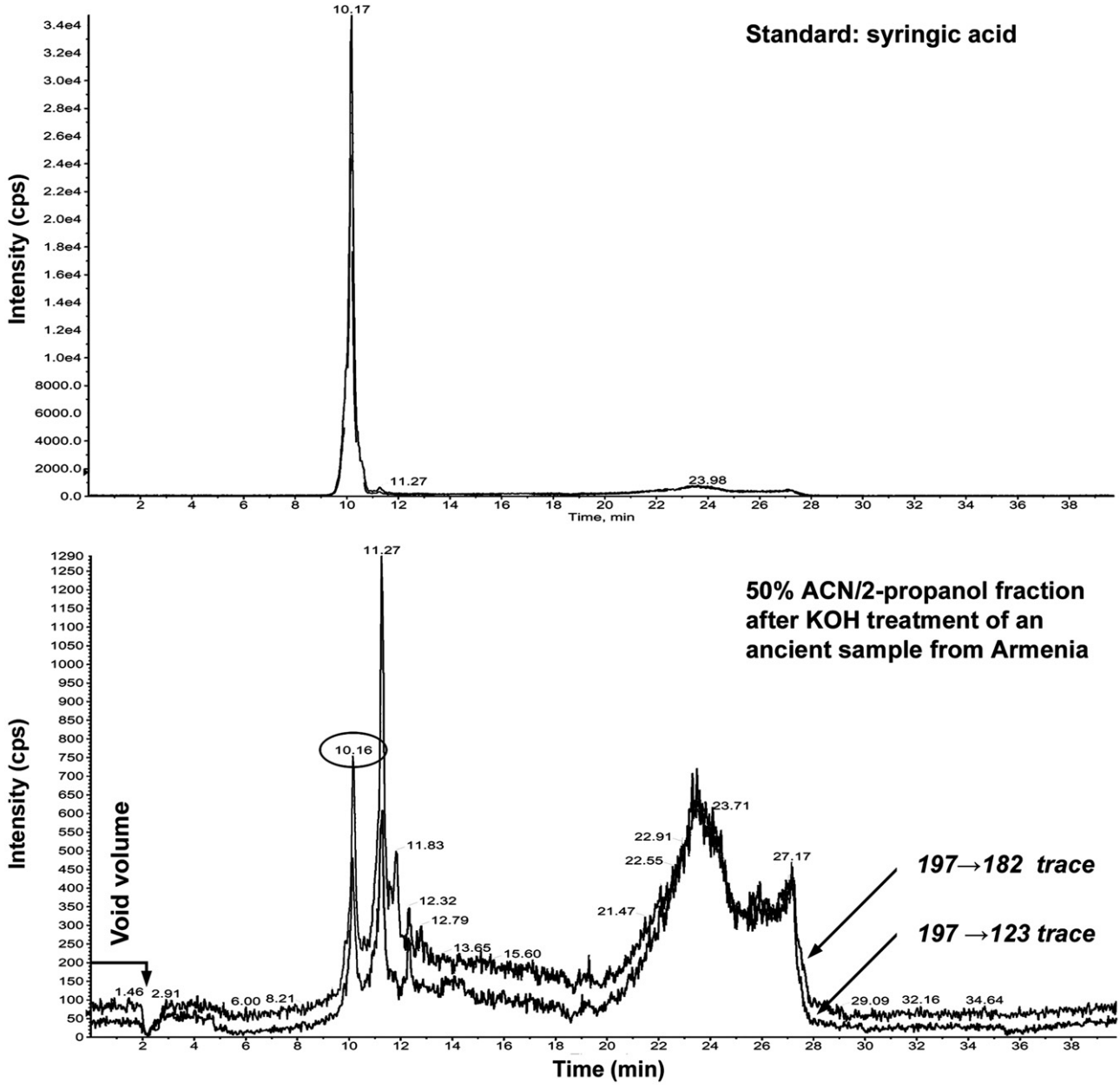

Fig. 7. LC-MS/MS-MRM chromatograms of authentic syringic acid (top, 2 nmol injected), and showing the presence of syringic acid (10.16 min after injection) in the 50% organic fraction of ancient sample B-2 from Armenia after SPE and KOH treatment (bottom). Note that syringic acid elutes from the column well after the discharge of the void volume.

The ancient sample from Syria produced a negative curve (Fig. 6, bottom), also for the flow-through that was re-loaded onto a new cartridge to investigate whether the binding capacity of the first cartridge had been exhausted by other molecules in the sample, causing malvidin to be lost. Further research is necessary to indicate what this vessel may have contained and what caused the red discoloration of its inside surface. Sample B2 from Armenia, found inside one of the large storage jars, produced a positive curve (Fig. 6, bottom), with a small but clearly detectable amount of syringic acid in the 50% organic fraction (Fig. 7). The other peaks in the chromatogram represent other unknown molecules which happen to produce a response in the selected MRM transitions (Guasch-Jané et al., 2006: Fig. 2B; Stern et al., 2008: Fig. 6B), illustrating the importance of reliable chromatography for the identification of compounds by this method (Stern et al., 2008). The second potsherd from inside the large storage jar in the Areni-1 cave complex also returned a positive curve (not shown), virtually identical to the one illustrated here. These findings are concurrent with the archaeological evidence concerning the context of the potsherds from Armenia. Vessels such as those found partly buried inside the central gallery of the Areni-1 cave complex appear well suited to receive grape juice, or a combination of grape juice and other ingredients (for instance hawthorn or pomegranate juice, or resins), and store it during its fermentation into wine. This obviously does not constitute irrefutable evidence, but chemical data pointing in the same direction can now be added to the archaeological argument.

5. Discussion

Small polar compounds, such as tartaric and syringic acid, are generally poorly retained on reverse phase chromatography columns. Published retention times for tartaric acid during chromatographic separation of assumed wine residues are remarkably short (Guasch-Jané et al., 2004: Fig. 1C; Stern et al., 2008: Fig. 6C), garnering concern that part or all of the observed response in the midst of the non-retained fraction may be a non-specific effect and that the supposed detection may be an artifact of the experimental method. Such short retention times create three related issues with the identification of the analyte. First is that a positive identification of a compound by LC-MS/MS is based on a specific precursor ion creating specific product ions, in a particular ratio, at a determined retention time. With little or no retention of the compound of interest on the column an important part of this equation remains absent rendering a positive identification much less certain. Second is that with many different compounds eluting directly after the void volume of the column has been discharged, as is the case with complex archaeological samples, chances are that any response appearing to be positive is instead due to some other combination of compounds, rather than the analyte in question. Third is the issue of ion suppression, a phenomenon that can occur when using electrospray ionization. If too many compounds in relatively high abundance enter such an ion source at the same time some compounds will get ionized preferentially at the expense of others. During LC-MS/MS experiments this will happen early in the chromatogram, when the non- and poorly-retained compounds (including the salts resulting from the sample preparation) elute as a slug shortly after the injection of the sample, and often also towards the end of a gradient when non-polar compounds elute *en masse*. The power of LC-MS/MS is thus only realized when tandem mass spectrometry is used in combination with high fidelity chromatography (Stern et al., 2008; Faull et al., 2009), as presented above for syringic acid. A similar assay should be developed in the near future for tartaric acid in archaeological samples. Another issue that needs attention is a more complete database of the chemical composition of domesticated plant species, such as the average amounts of tartaric and syringic acid in various parts of such plants.

With an improved method to determine the presence of malvidin we obtained positive results, indicating the possible former presence of grape products, for two Late Chalcolithic (around 4000 BCE) potsherds found in the cave complex Areni-1 in present-day Armenia. It is important to note again that the presence of malvidin, the anthocyanin that gives pomegranates, grapes and wine their red color, is not necessarily associated with the former presence of wine, but only indicates the remains of grapes, pomegranates, or both. Fermentation, although likely, can only be assumed and other products (such as *defrutum*) should not be excluded. The fact that in Armenia the ceramic samples were collected from a context resembling a grape pressing installation with the preserved remains (seeds, stems, skins) of crushed or pressed grapes supports the interpretation that this part of the cave was a site where wine was produced. Another potsherd from Late Akkadian (around 2200 BCE) deposits in an elite context in Tell Mozan in Syria preserved a red interior, initially interpreted as the remnants of red wine, but proved negative for malvidin. Our research thus produced an improved method to identify malvidin in archaeological materials that can, however, only provide supplementary arguments for or against the presence of wine in specific vessels. Like any other scientific technique, biochemical research alone can never create conclusive evidence concerning anthropological issues (Barnard et al., 2007), much like archaeological research alone cannot irrefutably prove wine production. Instead, both should be part of a larger research program, aimed at addressing a specific anthropological or archaeological research question (McGovern, 1995). As the interests, sample materials and experience of analytical chemists and other scientists will always be different from those of archaeologists, a substantial amount of method development should be expected before a viable protocol will be available. We hope to have illustrated this and to have at the same time added to the discussion regarding the presence or absence of wine in the archaeological record.

Acknowledgements

The Applied Biosystems-MDS Sciex 4000QTrap Hybrid Triple Quadrupole Linear Ion Trap LC-MS/MS instrument in the Molecular Instrumentation Center at UCLA was obtained through National Center for Research Resources (NCRR) grant S10RR024605. The content of this article is the sole responsibility of the authors and does not represent the views of the National Science Foundation, the NCRR or the National Institutes of Health. Thanks are due to Thuc Le and John (Mac) Maston, for their help; and to two anonymous reviewers, for their comments on an earlier version of this article. Sincere thanks are also due to Pavel Avetisyan, Giorgio Buccellati, Marilyn Kelly-Buccellati and Frederico Buccellati for allowing access to the samples, as well as to the Armenian and Syrian authorities for allowing us to export and to study them.

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