LC-MS/MS-MRM of Organic Residues in Archaeological Ceramics to Corroborate the Presence of Wine

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SUMMARY: *sophisticated wine producing technologies had developed in the Fertile Crescent of the Near East before 4000 BCE (6000 years ago)*

Research Question

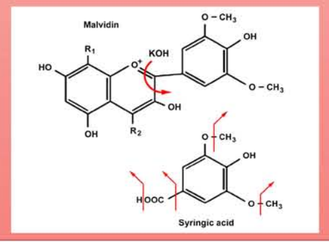
SAMPLES: three potsherds from what appeared to be a wine producing facility, radio-carbon dated to around 4000 BCE, were collected.

COMPOUNDS: instead of L-tartaric acid or resins (terpenoids), which can be less specific, malvidin—the anthocyanin that gives wine its red color—was chosen as a marker for wine residues.

Plants species containing malvidin
possibly present in the *Fertile Crescent* during Neolithic times

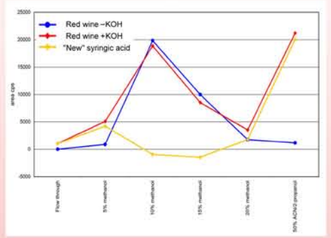
Species	Part	Common name	Remarks
Malva sylvestris	Plant	High mallow	Medicinal use, dye
Punica granatum	Fruit	Pomegranate	Native to Afghanistan, Pakistan and Iran Early domesticate
Trifolium pratense	Flower	Red clover	Animal fodder, medicinal use
Vaccinium myrtillus	Fruit	Bilberry, wortleberry	Native to northern Europe
Vitis vinifera	Fruit	Grape	Native to the Mediterranean region Early domesticate

REACTIONS: over time malvidin polymerizes, which aids its preservation in archaeological contexts. In a strong alkaline environment it releases syringic acid.

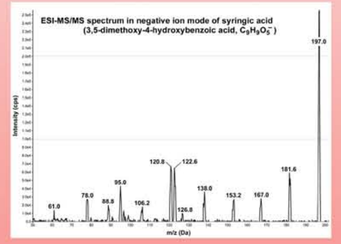


Material and Methods

SPE: free syringic acid was removed using Agilent AccuBond ODS-C18 SPE cartridges; syringic acid eluted with the 10%, malvidin with the 50 % organic fraction.



MS: during negative ion ESI-MS/MS syringic acid produces fragments with calculated average masses of 197.2, 182.1 and 123.1 m/z.



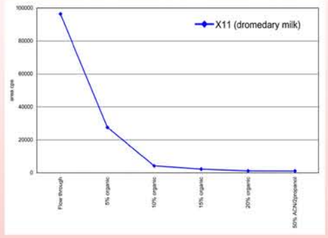
LC: newly formed syringic acid was identified following the 197=>182 and 197=>123 m/z transitions using an AB-MDS SCIEX 4000QTrap, with a Waters Nova-Pak C18 column in an Agilent 1200 LC system.

Solvent A: water with 2% acetic acid
Solvent B: methanol with 2% acetic acid

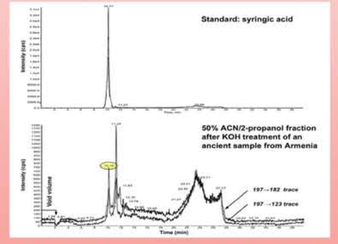
Time (min)	Flow (µL/min) 4000QTrap	Flow (µL/min) 6460 QqQ	%A	%B
0	900	100	99	1
5	900	100	99	1
20	900	100	5	95
24	900	100	5	95
25	900	100	99	1
40	900	100	99	1

Results

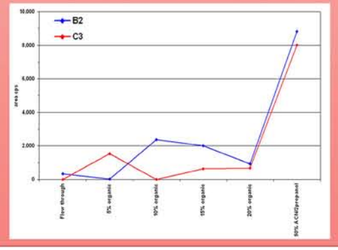
CONTROLS: Charles Shaw cabernet sauvignon and authentic syringic acid (Sigma-Aldrich S6881) were used as positive controls; a new vessel in which dromedary milk was cooked as the negative control.



SAMPLES: small amounts of newly formed syringic acid were identified in the residues in two of the three ancient potsherds.

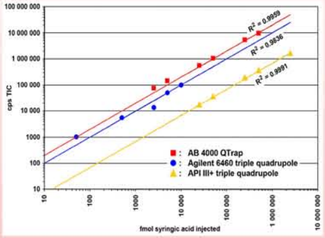


QUANTIFICATION: the amounts of syringic acid in all KOH-treated SPE fractions produced a curve similar to that of red wine for these two samples.



Discussion

SENSITIVITY: additional research was done on a PerkinElmer-SCIEX API III+ QqQ, with a Waters Nova-Pak C18 column in a HP 1090 LC system; and on an Agilent 6460 QqQ, with an Agilent ZORBAX Rapid Resolution High Definition Eclipse Plus C18 column in an Agilent 1290 Infinity LC system.



CONCLUSIONS: the results for samples B2 and C3 are interpreted as the presence of malvidin (corresponding to >30 nL red wine) in the ancient vessels.

This in turn is interpreted as the former presence of a grape or pomegranate product.

If the malvidin is indeed from grapes, it could be associated with red wine, grape juice, raisins or *defrutum (pekmez).*

Given the archaeological context, the cave near Areni was likely a wine production site; our analysis corroborates this hypothesis.

Bibliography

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