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Trace Analysis of Wine from 6000 BC

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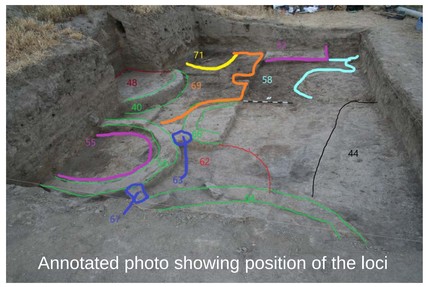
Background

The Neolithic (~10,000-3,500 BC) was the age of achievement and expansion. This period represented a transition where food-collecting cultures shifted to food-producing ones, which allowed people to establish year round settlements. Many plants were domesticated including the Eurasian grape, which is believed to be the first grape used to ferment wine.



Annotated photo showing position of the loci

There is an ongoing archeological dig in the Republic of Georgia to investigate the earliest winemaking and the emergence of wine culture as part of the Gadachrili Gora Regional Archaeological Project Excavations (**G.R.A.P.E.**).

Excavated Pottery Sherds

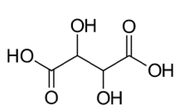

Shulaveris 2018, 4002 base

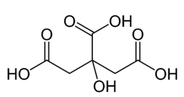

Shulaveris 2018, 4047 base

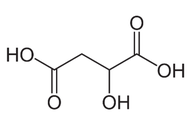

Shulaveris 2018, 4156 base

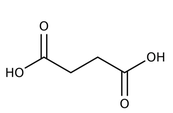
Sherds of pottery jars were excavated from the dig site in the Republic of Georgia in spring 2018. Corresponding soil samples were taken from where the sherds were found. **Sherd 4002 (soil 4003)** comes from Loc 55 (interior of a house, and it appears to have been a purposeful deposit). **Sherd 4047 (soil 4048)** comes from Loc 69 which is a fill layer between three buildings (Loc 40, 54, and 66) and an ashy deposit (Loc 99). **Sherd 4156 (soil 4157)** comes from Loc 80 (not shown in annotated photo but far top right corner of the photo (under loc 58 near loc 30).

Organic Acids as Wine Markers

❖Tartaric Acid 

❖Succinic Acid 

❖Malic Acid 

❖Citric Acid 

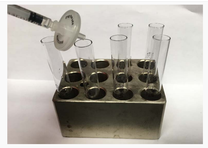
Tartaric acid is the most characteristic, as the Eurasian grape is the only fruit/berry in the caucus region that produces it. **Malic acid** and **citric acid** are commonly found in many fruits and berries, but help to further confirm the presence of the grape. **Succinic acid** is a byproduct of the fermentation process, which helps to confirm that Georgians were fermenting the grape into wine. The sherds and their corresponding soil samples were analyzed for these four characteristic organic acids.

Research Goal

Gadachrili Gora and Shulaveris Gora are Neolithic sites in the Republic of Georgia. Newly excavated pottery (including bases of amphorae) from the 2018 season at Shulaveri is relatively low-fired and highly friable. These characteristics might well reflect an earlier pottery technological phase, close to the beginning of pottery-making in the Near East. **Did these vessels contain ancient wine? When did winemaking start?**

Extraction of Organic Acids

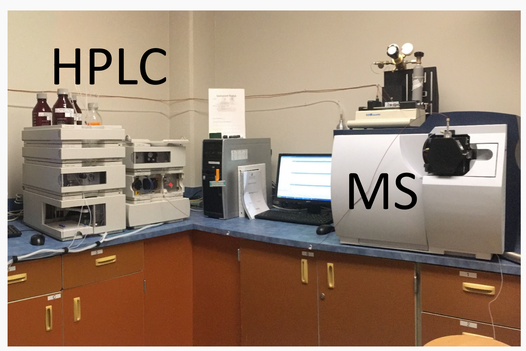

Ampoules


Syringe Filter


Vacuum Centrifuge

~500 mg of the sherd and soil samples were weighted into ampoules, 1.75 mL of 2.8% NH_4OH was added. The ampoules were sealed and placed in an oven at 100 °C. After 24 hours, the ampoules were opened and the top liquid layer was filtered through a syringe filter into test tubes. The test tubes were placed in a vacuum centrifuge to evaporate the solvent. A solid residue was recovered, weighed, and re-constituted for analysis by high performance liquid chromatography-mass spectrometry.

Liquid Chromatography-Mass Spectrometry Analysis



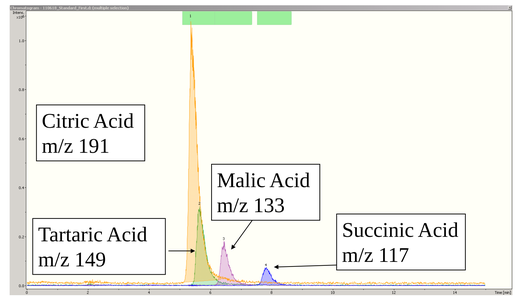
High performance liquid chromatography (HPLC) was used to separate organic acids in sherd/soil extracts. These organic acids were detected using electrospray ionization mass spectrometry (MS) in negative ion mode. LC-MS with its high sensitivity and specificity is well suited for the quantification of organic acids in complex sherd and soil samples.

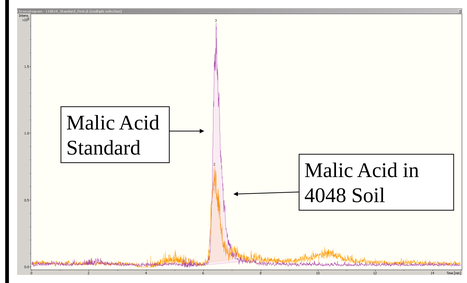
Acknowledgements

- Kim Farrar gratefully acknowledges a Fall 2018 Boise State University HERC Fellowship.
- Stephen Batiuk (University of Toronto) for providing background info on the Georgian archaeological site and excavated samples.
- Israel Shebley (Boise State University) for technical assistance with the Department of Chemistry and Biochemistry's LC-MS instrument.

Data: Extracted Ion Chromatograms

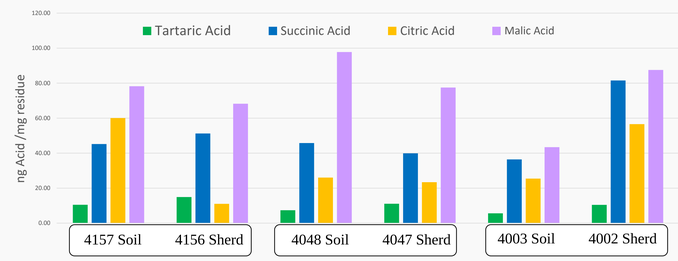
[Right] Four overlaid extracted ion chromatograms (EICs) from the mixed organic acid standard (10 µM each) are shown. Since negative ion mode was used, the mass of the deprotonated acids was followed.





[Left] An example of the detection of malic acid is shown. Retention time of unknown in EIC at m/z 133 matches that of malic acid standard. Quantitation was accomplished using peak areas. In our study, all four acids (tartaric, malic, succinic, and citric) were detected in trace amounts in every sample.

Results



In **4156 Sherd**, the amount of tartaric acid, succinic acid, and malic acid detected are similar to soil concentrations with the exception of citric acid, which was much higher in the soil. The distribution and abundance of organic acids are very similar between **4047 Sherd** and its corresponding soil sample (4048 soil). In **4002 Sherd**, the amount of citric acid, malic acid, and succinic acid are ~2x higher compared to 4003 soil. *All three sherds had slightly higher abundance of tartaric acid compared to their corresponding soil sample; however, these results do not give definitive evidence of grape wine in sherd samples.*

Conclusion

All four organic acids (tartaric, malic, succinic, and citric) were detected in trace amounts in every sample; however, there was no significant difference in the amount of organic acids found in the sherd samples versus the soil samples. **As a result, we could not verify the presence of grape wine in these particular archaeological sherds using our extraction method and LC-MS analyses.**