Abstract A large volume-headspace apparatus that permits the heating of pottery fragments for direct analysis by gas chromatography/mass spectrometry is described here. A series of fermented-corn beverages were produced in modern clay pots and the pots were analyzed to develop organic-species profiles for comparison with fragments of ancient pottery. Brewing pots from the Tarahumara of northern Mexico, a tribe that produces a corn-based fermented beverage, were also examined for volatile residues and the organic-species profiles were generated. Finally, organic species were generated from ancient potsherds from an archeological site and compared with the modern spectra. The datasets yielded similar organic species, many of which were identified by computer matching of the resulting mass spectra with the NIST mass spectral library. Additional analyses are now underway to highlight patterns of organic species common to all the spectra. This presentation demonstrates the utility of thermal desorption coupled with GC/MS for detecting fermentation residues in the fabric of unglazed archaeological ceramics after centuries of burial.

Introduction This work was instigated by an inquiry to perform a spot analysis for furfural on pottery sherds.[1] Since the spot test involves the use of hydrochloric acid, and we did not want use this potentially destructive test on historical specimens, an alternative analytical scheme that utilized dynamic large-volume headspace sampling was proposed.

Dynamic large-volume headspace sampling with gas chromatographic separation with mass spectrometric identification, as used in our laboratory, is a technique that permits the collection of trace organic species from materials that are undergoing thermal treatment in an inert atmosphere. We have developed this technique because:

1. It does not utilize solvents; there is no need to be concerned with selectivity, dilution, or loss of analytes.

2. Ultimate temperature may be selected to minimize thermal stress on articles tested.

3. If necessary, further testing on the same article may be performed without too much concern for this method altering nonvolatile residues.

4. It is nondestructive to the article examined.

A test specimen is placed in a heating apparatus that has been previously demonstrated to be free of organic species. The test specimen is then slowly heated in a flowing stream of ultra-high purity nitrogen, and the offgases collected using a cryogenically cooled, 3-trap environmental air sampling system (Entech Instruments, Simi Valley, CA). This system removes most of the water and carbon dioxide from the analytical stream, then concentrates the organic species, permitting part per billion detection limits. The system is represented schematically in Figure 1. This scheme can be reduced to 1) heating to evolve volatile organic species, 2) concentration of organics, 3) separation of organics, and 4) identification of species by mass spectrometry.

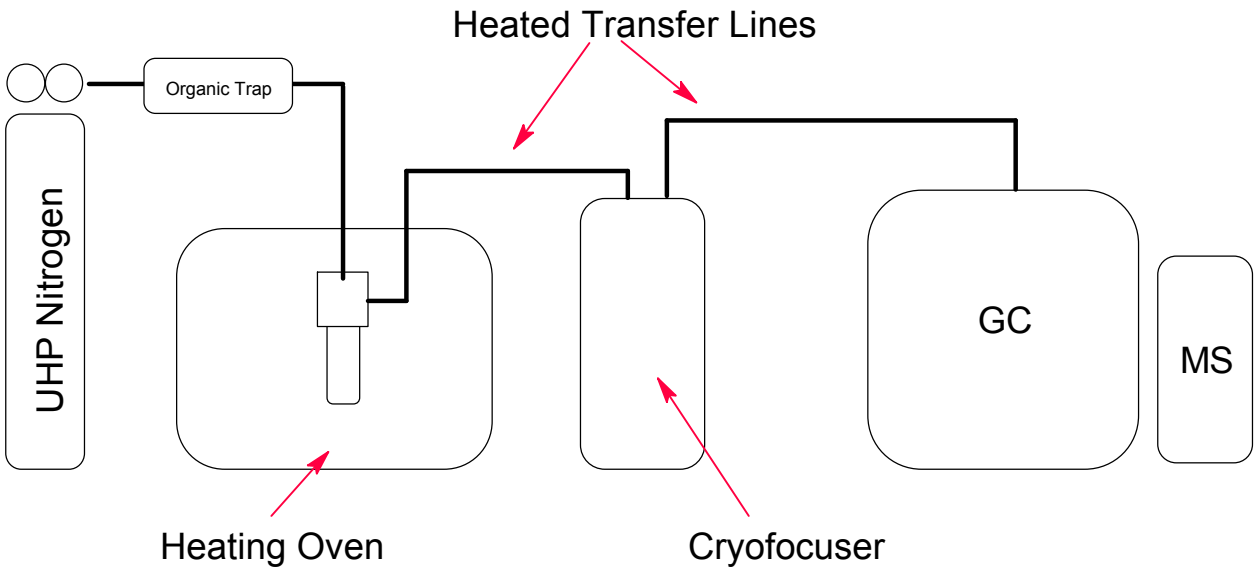

Figure 1. Analytical System for Pot Sherd Volatile Analysis

Separation of the focused organic species occurs in the gas chromatograph. Each separated species is then analyzed by mass spectrometry; this mass spectrometric information may then be computer-matched to a database, and a tentative identification of the organic species may be made.

To determine if the analytical system would work as proposed and provide a baseline set of organic species response, intentional fermentation of a corn-based liquid was conducted. This intentional fermentation was carried out in clay pots were produced by Archeobotanical Services; the liquid was produced using standard fermentation practices. Corn was purchased at a local feed store, allowed to germinate in warm water, and then chopped. This chopped material strained, and the liquid placed into the clay pot and either allowed to ferment using wild yeast or was intentionally inoculated with Brewer's yeast. A typical fermentation is shown in Figure 2.

Figure 2. Corn liquid fermentation in modern clay pot

After the fermentation was judged to be complete, the liquid was drained from the pot, the pot allowed to air-dry, the pot was sectioned for analysis as shown in Figure 3. Samples for analysis could be taken from anywhere along the section profile.

Figure 3. Modern pot preparation for volatile analysis

We also obtained brewing vessels from the Tarahumara Nation in the Republic of Mexico. The Tarahumara brew ‘tiswin’ or ‘tiswino’ from corn ceremonial purposes. Two brewing vessels were obtained in order to search for chemical markers of corn-based brewing for comparison to archaeological samples. One vessel is shown in Figure 4.

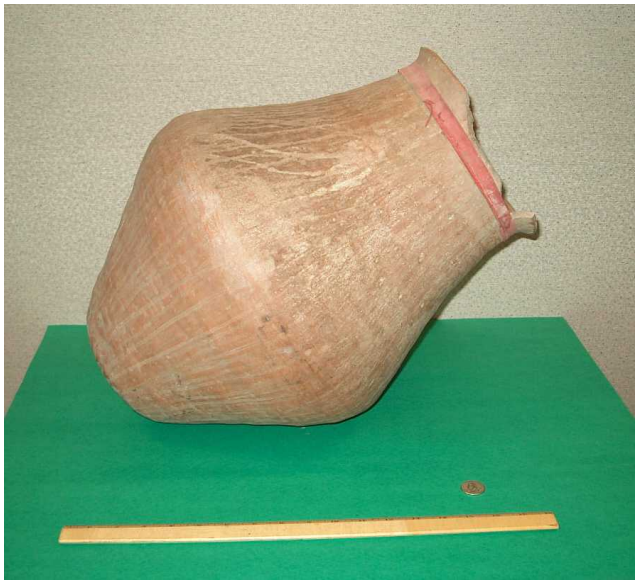

Figure 4. Tarahumara Brew pot

Finally, a collection of recently excavated sherds were obtained from an archeological dig conducted in New Mexico. These field samples had not been pre-treated in any manner prior to analysis.

3 Black/white sherds Type A

3 Black/white sherds Type B

3 Bowl sherds

Experimental For each analysis, a piece of sherd no larger than 20mm by 30mm was placed in a 25mm diameter by 150mm long glass test tube and this tube was attached to the headspace gas sampling apparatus as shown in Figure 5. This fixture was then placed in a heating oven for thermal treatment of the sample.

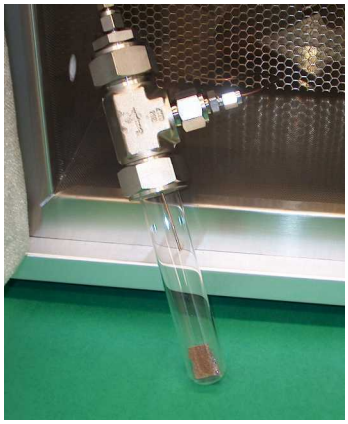

Figure 5. Pot Sherd Heating Fixture

Ultra high purity nitrogen, conditioned by an organic gas trap, was passed through this fixture at 40cc/min. The heating oven warmed the samples from 50°C to 190°C over a 27 minute period using the profile shown in Figure 6.

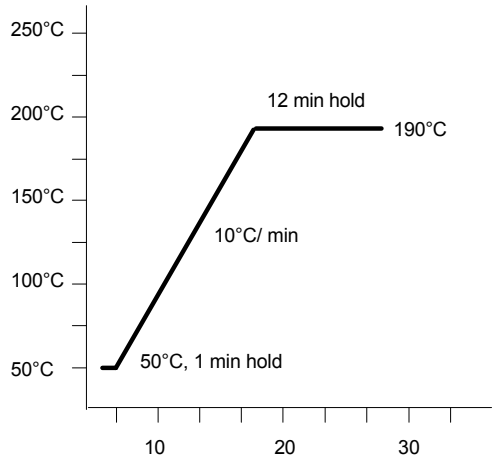

Figure 6. Heating oven thermal profile

Organic gases evolved from the sample were concentrated in a commercial cryofocusing inlet system. The cryofocused gases were then separated using gas chromatography, using an Agilent DB-624 column, 60meter x 0.25mm x 1.4µm film thickness; GC heating conditions were 35°C for 8 minutes, ramp at 7°C/min to 150°C, ramp at 12°C/min to 255°C, and hold at 255°C for 10 minutes. The transfer line to the mass spectrometer temperature was maintained at 260°C. The GC column flow was set to 1.4mL/min at 35°C; the column was maintained at a constant pressure of 21.5 psig. The mass spectrometer was operated in the full scan mode, 33 to 340 amu, 2.4 scans/sec, peak threshold at 150 counts, and a 3 minute solvent delay. The mass

spectrometer source was heated to 230°C; the quadrupole to 150°C. Organic gases such as methane, ethane, ethylene, acetylene, propane, and methanol are not be observed under the conditions used for this study.

Discussion A variety of chemical species was detected in most samples. In some samples it was clear that the technique was limited by the sample size. These species ranged from simple hydrocarbons (pentane, octane) to aldehydes (acetaldehyde, decanal), chlorinated hydrocarbons (chlorobenzene), and oxygenated compounds (furfural, furan, 2-butenol).

A typical result for a modern pot used for fermentation is shown in Figure 7. Each peak is a distinct chemical species.

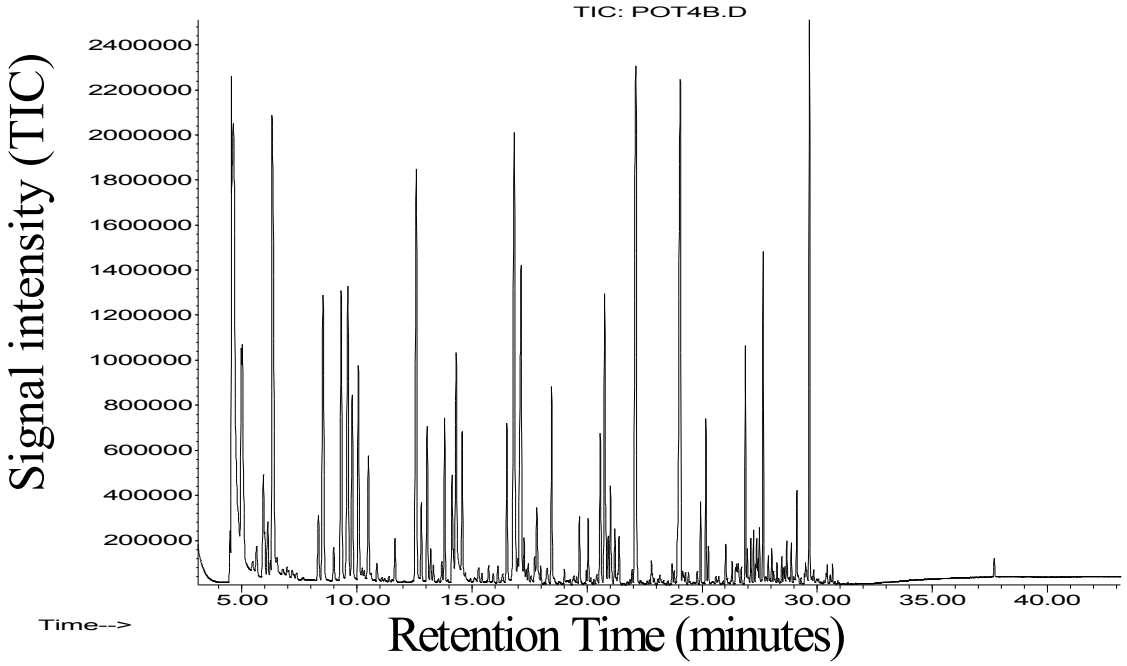

Figure 7: Modern pot sherd used for fermentation, signal intensity versus time

A closer examination of this chromatogram with some of the peaks identified is shown in Figure 8. In addition to furfural, which was the original species of interest, many other species are observed and identified.

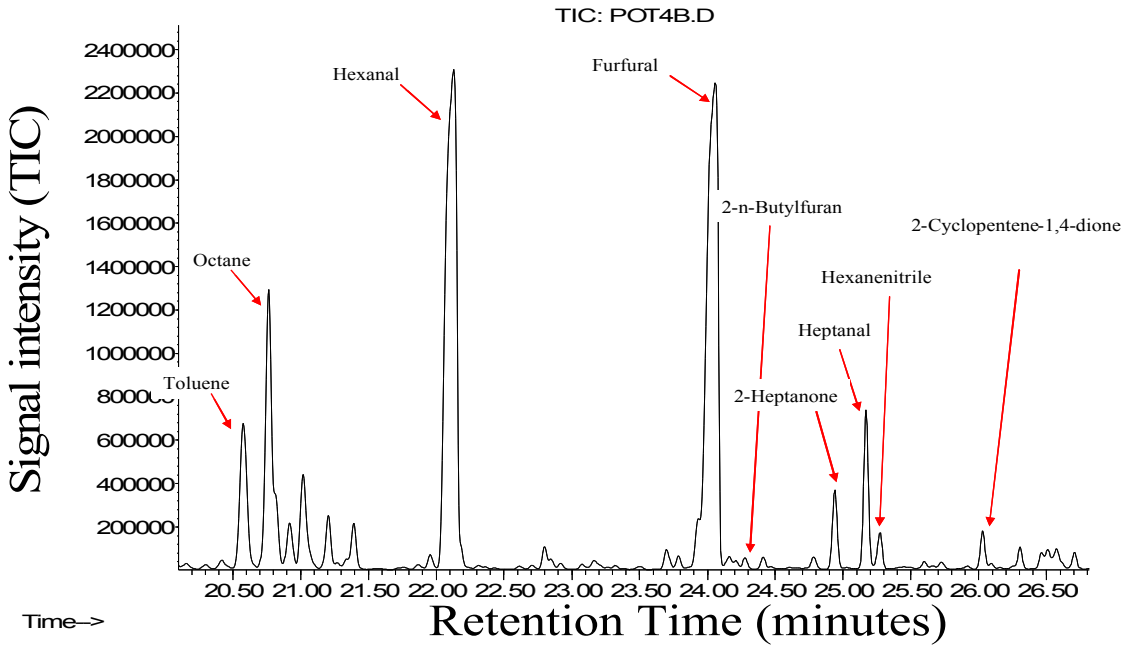

Figure 8: Closer examination of previous chromatogram with identification of several peaks.

The analysis of a portion of a Tarahumara brew pot is shown in Figure 9. In addition to furfural, which is indicated on the figure, many other organic species are observed.

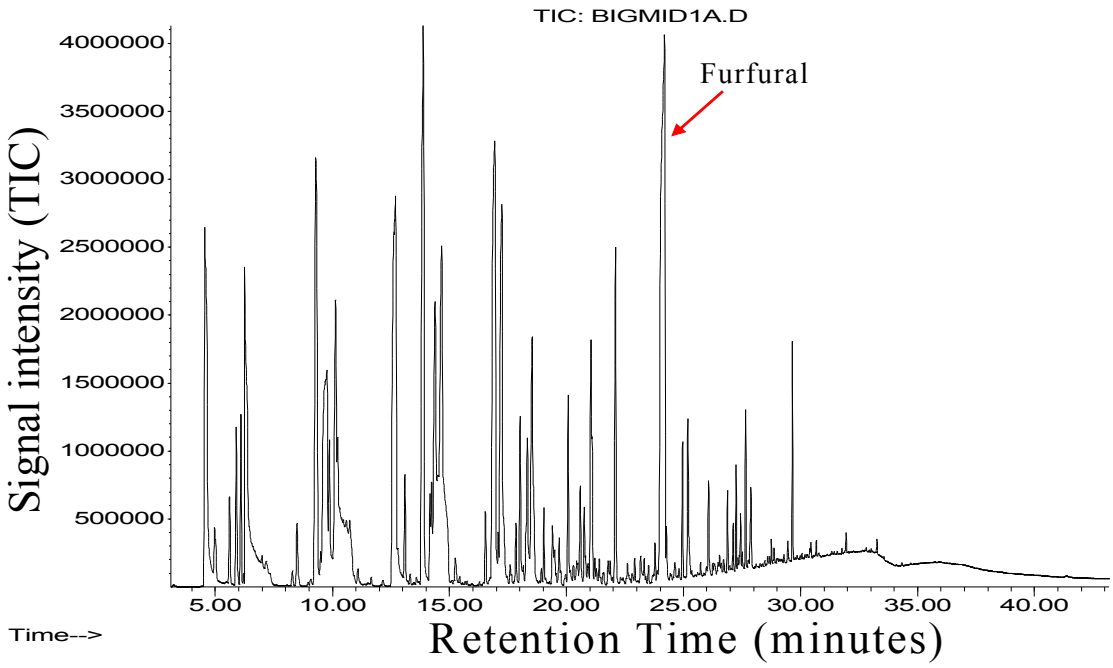

Figure 9: Tarahumara brew pot sherd, signal intensity versus retention time

The analysis of one of the ancient sherds is shown in Figure 10. This example shows just a small portion of the chromatographic result. The peak intensities for this sample are much less than those observed in the modern samples, but are nevertheless present in the analysis. The species observed are indicated on the figure.

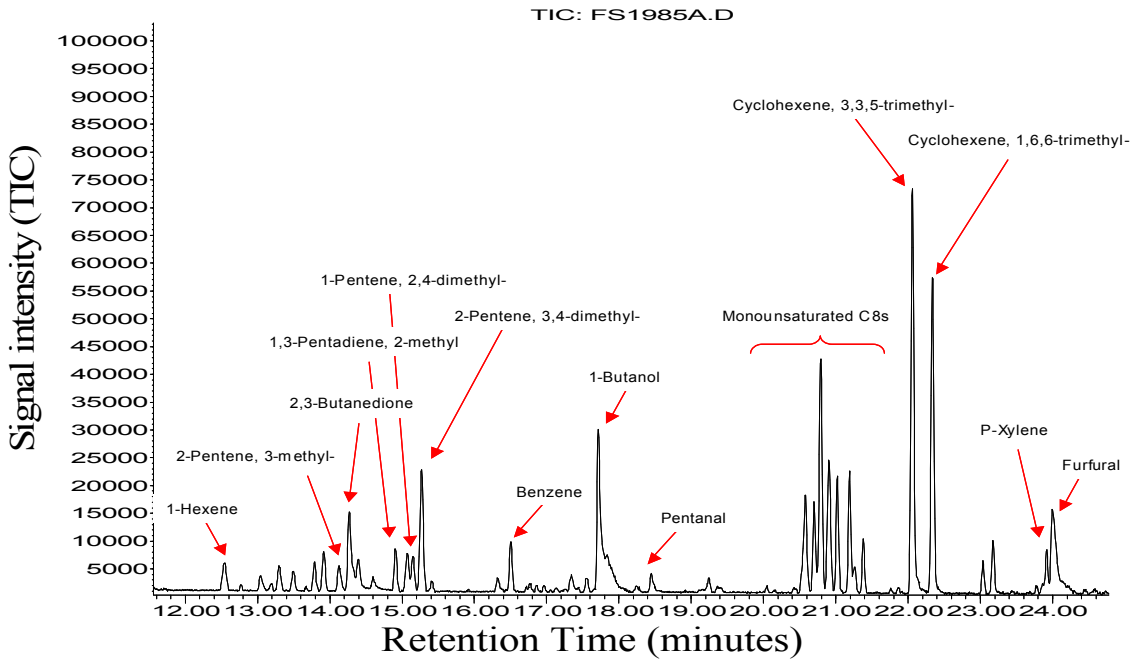

Figure 10: Black/white sherd type B analysis

Further evaluation of the data was then performed; it is necessary to confirm that species with similar retention times have the same mass spectral signature, and then which species may be found across some or the entire sample set. While chromatograms can be compared strictly as x,y data, it is important to remember that with complex samples such as these the mass spectral data must be taken into account as well.

For example, an overlay of the total ion chromatogram plots from several analyses is shown in Figure 11. Almost all of the chromatograms have a peak detected at 22.0 minutes, and the species present has a mass fragment of mass to charge (m/z) of 109 amu. Upon closer examination of the mass spectral data, however, it is revealed that the there are 2 distinct chemical species desorbing from the archeological and Tarahumara Nation samples.

Additional differences can be observed in the selected ion plot, such as the peaks at 22.4 and 22.9 minutes, only observed in Tarahumara Nation pots. Additional data analysis is aimed at finding such differences and similarities that may indicate the type of usage for the archaeological sherds.

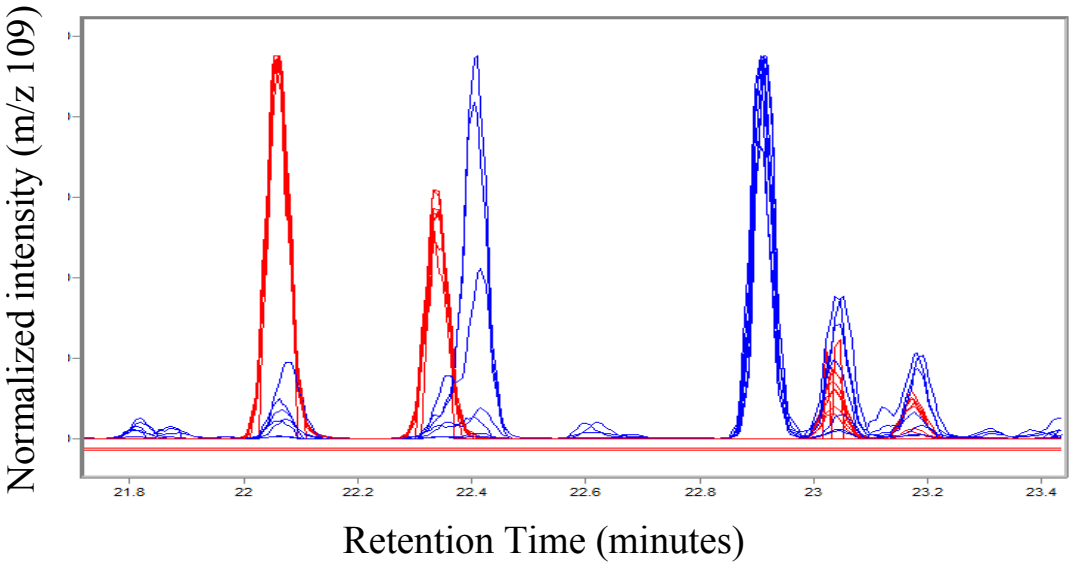

Figure 11: Comparison of results: Archeological samples (red) and Tarahumara (blue) overlaid chromatograms region from 21.5 to 23.4 minutes retention time

References:

1. V. Anger and S. Ofri, *Fresenius Z. Anal Chem*; 1964; v.203, no.6, p.422-430