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Appendix 1: Analytical Procedures and Protocols

FT-IR

The FT-IR data were obtained on Thermo Scientific Nicolet spectrometers, both by diffuse-reflectance on samples mixed with KBr and on neat (unmixed) samples run using a diamond cell.

FT-IR spectra were searched for "matches" against large databases of relevant natural products and processed organic materials, and synthetic compounds.

GC-MS

For the analyses of solvent extracts by GC-MS, the residues from the extracted samples were taken up in a 1:1 mixture of chloroform and methanol, heated for 1 hr at 60°C, centrifuged, concentrated down, and derivatized with Alltech II Me-Prep to the methyl esters. One microliter samples were injected splitless onto a 30m x 250µ x 0.25µ film thickness HP-5MS column (5% phenyl methyl siloxane) of an Agilent HP-6890 gas chromatograph, run at a 1.5 mL/min flow rate. A 5973mass selective detector was used with the injector port at 325°C. The oven temperature was held at 50°C for 2 min, then programmed to increase at 10°C/min to 325°C where it was held for 10.5 min for a total run time of 40 min. The transfer line to the mass spectrometer was held at 300°C. Compound identification was made by retention time and comparison of unknown mass spectra to the spectra in the NIST 08 mass spectral library using NIST Search program version 2.0.

To check for contaminants, two control blanks of high-purity solvent were extracted and prepared like the modern replica and ancient pottery and soil samples. These samples were derivatized and analyzed by GC-MS under identical conditions. One blank was processed and run at the same time as all the other samples. One sample (no. 6B) was later re-extracted and run a second time with another control blank, using new glassware and a reduced volume for the solvent, to minimize contamination.

As discussed in the text, these control blanks enabled us to spot contaminants, including docos-9-enedioic acid, amides, phthalates, and behenic alcohol. Excessive contaminants have the disadvantage of swamping the signals of ancient compounds of interest and cause them to be "lost" in the baseline noise.

SPME-GC-MS

Solid-phase microextraction (SPME) is a proprietary technology (Supelco Inc., Bellefonte PA) that employs a thin fused-silica fiber coated with an adsorbent. The materials used for collection of all VOCs were the 2 cm, 50/30µm Divinylbenzene/Carboxen/polydimethylsiloxan (DVB/Carboxen/PDMS) "Stableflex" fibers (Supelco Corp.). VOCs evolving from a solid or liquid surface are exposed to the coated fiber and dissolve or absorb in the coating.

SPME is of great utility in biomolecular archaeological studies (Bonaduce et al. 2016). It requires only milligram quantities of valuable archaeological samples, and analyses can be performed rapidly, at lower detection limits, in solution without prior extraction in an organic solvent.

Using freshly powdered samples, the headspace SPME analyses of replica and ancient Colima samples were done using sample vials which were tightly capped with a white silicone/TFE septum-containing screw cap. To enable maximum release of VOCs from the samples, we determined by experimentation that the sample should be added to distilled water and stirred in the vial while heating to 60°C in a water bath. Although some researchers add salt to release more volatiles into the headspace, our results showed no difference between saline solutions and pure water, so we report results with only water added. The optimal sample size to yield a significant amount of volatile organic compounds (VOCs) was 50-75mg.

The headspace VOCs were collected on the SPME fiber for 30 min. The fiber was then inserted into the injection port of a GC-MS and the VOCs were desorbed for 1 min at 230°C.

A Thermo Scientific ISQ single quadrupole GC-MS with Xcalibur software (ThermoElectron Corp.) was used for separation and analysis of the desorbed VOCs. The GC/MS was equipped with a polar "Stabilwax" column, 30m x 0.32mm with 1.0µ film thickness (Restek Corp.). The injection port was set at 230°C. The oven temperature was held at 60°C for 4 min, raised to 230°C at 6°C $min^{-1,}$ and maintained at 230°C for 40 min. Helium carrier gas constantly flowed at 2.5 mL min^{-1}. The mass spectrometer was operated at an ionizing energy of 70 eV with a rate of 2 scans per second over a scan range of m/z 40-400 and an ion source temperature of 200°C.

To control for contaminants in laboratory air and small amounts of additives to plastic, moisturizers, and other consumer products (e.g., 2-ethylhexanol, dodecanol, and various phthalates), we ran a water blank at the start of each day's runs (usually 3 or 4 samples). If a peak were present in the blank as well as in the sample, it was judged a contaminant.

To assess background contaminants in the environment of the El Diezmo-Adonaí (Colima) cemetery, we also ran two soil samples and pottery fabrics from the exteriors of two double-chambered jars.

With age, the SPME fiber also begins to break down, and it was necessary to change it periodically to avoid silicone peaks and an imine-containing compound in the blank water and sample chromatograms. When a new fiber is used, it is first conditioned at 230°C (the analysis temperature) for 2hr before each run. In addition, to ensure that no compounds are remaining on the fiber from the previous sample, the fiber is conditioned at 230°C for 30 min before a new sample is run.

The compounds in the SPME-GC-MS VOC chromatograms were identified using two different calibration libraries. The first was developed specifically for the polar Monell column using a retention time/retention index file for a range of normal hydrocarbons from C_{12} –C_{30} which had been run on this column. Following conversion of Xcalibur into AMDIS (Automatic Mass Spectral Deconvolution and Identification System) data, additional compounds were then identified by searching the NIST/EPA/NIH Mass Spectral Library (NIST 08) of more than 160,000 compounds with the NIST Mass Spectral Search Program (Version 2.71), to identify the best matches. This Monell calibration library comprised 37 compounds.

A second calibration library, developed by the Getty Conservation Institute and including many conservation-related compounds not in the NIST library, contained 498 compounds. Since these compounds had been run on a non-polar column, retention times and indices differed from those run on the Monell polar column. By locating the expected retention times of the compounds on a polar column by Chem Spider and matching their NIST mass spectra, we were able to identify them to a high level of probability.

Both the Monell and Getty calibrated libraries were incorporated into our NIST-AMDIS program, thus facilitating the analysis of numerous samples with many peaks.