

Effects of Extended Sonication and Deflocculation on Starch Recovery from Stone Tools

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Introduction

This experiment was designed to determine whether extended sonication time and exposure to sodium hexametaphosphate solution improves starch extraction through sonication. Three sets of artifacts were processed (Sets #1-3, artifact #s SE-01 through SE-12). A control sample was processed with the final set of artifacts (CN-01).

Set #1 – Four chipped stone igneous choppers from site CA-SMA-113 (contexts ca. 1000-1300 CE, excavated 2007-2009; **SE-01 through SE-04**).

Set #2 – Four unprovenienced ground stone tools in the California Archaeology Lab teaching collections. These tools were likely excavated in the late 19th to early 20th centuries and have been curated in banker's boxes for the last several decades (**SE-05 through SE-08**).

Set #3 – Two chipped stone choppers, a pestle fragment, and a handstone from site CA-SMA-113 (contexts ca. 1000-1750 C.E., excavated 2007-2009, **SE-09 through SE-12**).

From each set of artifacts, materials were removed using four methods applied in succession:

Wash 1 (**W1**) – Removal of external sediments with toothbrush. Sediments retained but not analyzed.

Sonication 1 (**S1**) – Five minute sonication treatment.

Sonication 2 (**S2**) – Thirty minute sonication treatment.

Sonication 3 (**S3**) – Artifact exposed to 1% sodium hexametaphosphate solution for 60 (\pm 3) hours, then sonicated for 30 minutes.

After treatment, extract was concentrated, mounted on a slide, stained with iodine, and starches were counted at 200x magnification. Estimates of total number of starches recovered per treatment were made based on per-transect starch counts.

Materials

-Baxter 200 watt, 11 liter ultrasonic bath (Model #C6450-11). Estimated ultrasonic intensity = 15 milliwatts/cm³. Any ultrasonic bath should be fine, as long as the ultrasonic intensity in the system is not too much higher than this one. The method for measuring ultrasonic intensity is outlined below.

- Centra CL2 and CL3 centrifuges (centrifuge with bucket rotor required; tubes must be held horizontally during centrifugation)
- Analytical balance capable of 0.0001 g precision
- Temperature probe capable of 0.1 °C precision
- Hot plate and container for boiling water
- Beakers large enough to contain artifacts selected for analysis
- 50 ml polypropylene centrifuge tubes
- 15 ml polypropylene centrifuge tubes
- 1.5 ml polypropylene centrifuge tubes (or 2.0 ml tubes)
- 20 ml syringes
- disposable glass pipets
- Powder-free gloves
- Aluminum foil
- Tongs
- Squeeze bottles for water
- Microscope slides (Fisher Brand, “Superfrost”)
- 24x30 mm cover slips
- Sally Hansen Hard as Nails Extreme Wear clear acrylic nail polish. Note: This brand is available at CVS/Walgreens. Use of cheaper (less viscous?) nail polish can cause the acrylic to become drawn under the cover slip during drying, creating an irregular distribution of starches and severely limiting the total analyzable area. This brand is preferred though it is more expensive than others.

- Filtered or distilled water
- Sodium polytungstate or other heavy liquid @ 2.0 g/ml
- Sodium hexametaphosphate
- Iodine
- Glycerol

Methods

Important Note: While processing Set #1, we realized that the method used to concentrate extract may have been causing loss of extracted starches. We used “Extract Concentration Method A” to concentrate extract for Set #1, S1 and S2, and “Extract Concentration Method B” to concentrate extract for Set #1, S3. “Extract Concentration Method B” was used to concentrate all extracts in Sets #2-3 and the control sample. Results of analysis of Set #3 suggest that “Extract Concentration Method A” results in loss of the majority of starches and should not be used.

Calorimetry Method for Estimating Ultrasonic Intensity

The method used for estimating ultrasonic intensity was based on:

Kikuchi, T. and T. Uchida

2010 Calorimetric method for measuring high ultrasonic power using water as a heating material. *Advanced Metrology for Ultrasound in Medicine (AMUM 2010); Journal of Physics: Conference Series* 279. doi: 10.1088/1742-6596/279/1/012012

Note: Because we did not create an ideal system such as the authors used, our calorimetry measurements should be considered inexact estimates of ultrasonic intensity.

1. Place a known quantity of water **at room temperature** into the ultrasonic bath. Record the volume of water and the temperature of water before sonication.
2. Turn on the ultrasonic bath for five minutes. Turn off the sonicator and record the temperature immediately after turning the system off (sonication can interfere with temperature probe readings).
3. Calculate total ultrasonic intensity of the system using the equation below:

$$W = [(X_2 - X_1) / T] * C * M$$

Where W = system power (watts); X₂ = final water temperature (°C); X₁ = beginning water temperature (°C); T = sonication time (seconds); C = the thermal constant of water (4.18 J g⁻¹ °C⁻¹); and M = the mass of water (g).

4. Calculate the ultrasonic intensity per cubic centimeter by dividing total system power (W above) by the mass of the water (g).

Note: In the Baxter 200 W system, 6000 g of water was sonicated for 300 seconds, raising the temperature by 1.0 °C. Estimated total system power = 83.6 watts; estimated ultrasonic intensity = 0.0139 mW/cm³.

Methods for Starch Extraction Experiments

Before beginning microbotanical extractions from artifacts, the laboratory was thoroughly cleaned. All floors, walls, surfaces, and equipment was wiped down. Working surfaces and the fume hood were cleaned with bleach and ethanol. All laboratory supplies that were to come into contact with artifacts (e.g., beakers, toothbrushes, tongs) were boiled for several minutes prior to use, except for expendable supplies that were originally sealed in factory packaging.

Note: All artifacts used in this experiment had previously been washed, removing the majority of adhering sediments. All water used in experiments was ultrapure filtered water. All centrifugations were at 3000 rpm for three minutes.

Wash 1 (W1)

1. Artifacts were removed from bags and rinsed with water to remove dust (not retained).
2. Artifacts were placed inside a beaker and brushed with a clean toothbrush, washing occasionally with water from a squeeze bottle. Artifacts were cleaned for ca. 3-5 minutes until all surfaces had been brushed. Artifacts were then rinsed with distilled water, removed from the beaker, and placed in the beaker to be used for Sonication 1 treatment.
3. A new pair of powder-free gloves and a clean (boiled) toothbrush was used for handling each artifact. After each sample was cleaned, the area was wiped down before processing the next artifact. Materials in W1 beakers were concentrated into 50 ml tubes through repeated centrifugation. These materials were not analyzed further.

Sonication 1 (S1)

1. Artifacts in beakers were immersed in water OR, in cases where artifacts were too large to immerse in water, a portion of the artifact was immersed in water (for each artifact, beakers were filled to a consistent level between treatments S1, S2, and S3).
2. Beakers containing artifacts were covered with aluminum foil to prevent airborne starch contamination.
3. Beakers were placed in the ultrasonic bath and the water level of the bath was adjusted to ca. 1cm above the water line in the beakers.
4. S1 beakers were sonicated for five minutes.
5. S1 beakers were removed from the ultrasonic bath and artifacts were removed into S2 beakers using tongs. Tongs were boiled between each use.
6. S1 beaker material was concentrated for mounting using Extract Concentration Method A (for Set #1) or Extract Concentration Method B (for Sets #2-3 and Control).

Sonication 2 (S2)

1. Artifacts in beakers were immersed in water OR, in cases where artifacts were too large to immerse in water, a portion of the artifact was immersed in water (for each artifact, beakers were filled to a consistent level between treatments S1, S2, and S3).
2. Beakers containing artifacts were covered with aluminum foil to prevent airborne starch contamination.
3. Beakers were placed in the ultrasonic bath and the water level of the bath was adjusted to ca. 1cm above the water line in the beakers.
4. S2 beakers were sonicated for thirty minutes.

5. S2 beakers were removed from the ultrasonic bath and artifacts were removed into S3 beakers using tongs. Tongs were boiled between each use.

6. S2 beaker material was concentrated for mounting using Extract Concentration Method A (for Set #1) or Extract Concentration Method B (for Sets #2-3 and Control).

Sonication 3 (S3)

1. Artifacts in beakers were immersed in a **1% solution of sodium hexametaphosphate in water** OR, in cases where artifacts were too large to immerse in solution, a portion of the artifact was immersed in the solution (for each artifact, beakers were filled to a consistent level between treatments S1, S2, and S3).

2. Beakers containing artifacts were covered with aluminum foil to prevent airborne starch contamination.

3. Artifacts in beakers were exposed to the deflocculant solution for 60 (\pm 3) hours.

4. Beakers were placed in the ultrasonic bath and the water level of the bath was adjusted to ca. 1 cm above the water line in the beakers.

5. S3 beakers were sonicated for thirty minutes.

6. S3 beakers were removed from the ultrasonic bath and artifacts were removed onto aluminum foil to dry. Tongs were boiled between each use.

7. S3 beaker material was concentrated for mounting using Extract Concentration Method B.

(Note on Control samples: The control sample was processed in the same way as all other samples, except beakers did not contain an artifact. All steps followed for other samples were followed for the control sample, e.g. concentrating extract into 50 ml tubes, transferring into 15 ml tubes, etc.)

Extract Concentration Method A

This method likely results in loss of the majority of starches and should not be used.

Note: Extract Concentration Methods outline the process of creating a preparation of extract to be mounted from the beaker of material extracted during sonication.

1. Transfer 50 ml of material from beaker (S1, S2, or S3) into 50 ml polypropylene centrifuge tube and centrifuge.

2. Remove supernatant with 20 ml syringe, placing syringe into tube and removing supernatant from water surface. Leave ca. 5 ml of water in the bottom of the tube each time, to ensure pellet

is not disturbed. Supernatant removal was carried out with a syringe rather than through decantation to ensure non-disturbance of the pellet.

3. Transfer material from beaker into 50 ml tube, filling to 50 ml mark, and repeat steps 1-2 until the beaker is empty. When the beaker is empty, invert and use a squeeze bottle to rinse material from base of beaker into 50 ml tube. Fill each tube to 50 ml in each centrifugation to ensure equal weight distribution.

4. After final centrifugation, remove supernatant to 5 ml with syringe. Using a pipet, remove supernatant to ca. 1.5 ml.

Note: A different pipet must be used for each sample. The same pipet can be used for each sample through each of the remaining steps.

5. Using pipet, impel and expel supernatant repeatedly to mix the supernatant and pellet. Use pipet to transfer the remaining 1.5 ml of material into a 2.0 ml polypropylene centrifuge tube.

6. Use squeeze bottle to add <0.5 ml of water into 50 ml tube. Use pipet to impel and expel water repeatedly to rinse tube and pipet. Transfer remaining rinsed material into 2.0 ml tube.

7. Place 2.0 ml tubes into microtube rotor **which holds tubes at a ca. 45° angle** and centrifuge.

8. Use pipet to remove supernatant, leaving pellet and <0.1 ml water. Add 0.5 ml sodium polytungstate at 2.0 g/ml to each 2 ml tube. Using pipet, impel and expel solution in 2 ml tube to thoroughly mix.

Note: With 0.1 ml water in tube, adding 0.5 ml sodium polytungstate produces a solution with final density ca. 1.8-1.9 g/ml.

9. Label and weigh the set of 2.0 ml tubes to be used for the light fraction.

10. Centrifuge 2 ml tubes to separate light and heavy fractions. Using a pipet, gently mix the material floating on the surface of the supernatant (and material adhering to sides of tube) into the supernatant. Use pipet to remove supernatant leaving ca. <0.1 ml supernatant and pellet (“heavy fraction”) undisturbed in bottom of tube. Transfer supernatant (“light fraction”) into a separate 2 ml tube (“light fraction tube”).

11. Add water to light fraction tube to fill to 2.0 ml and vortex to mix. Centrifuge to concentrate starches into pellet.

Note: About 0.4 ml of supernatant at ca. 1.8-1.9 g/ml will be transferred into light fraction tube. Adding ca. 1.6 ml of water and mixing creates a solution with final density ca. 1.2 g/ml.

12. Use pipet to remove supernatant, leaving ca. <0.1 ml solution in tube.

13. Add 1.0 ml of 0.2% iodine solution in water to each 2 ml tube. Vortex to mix and centrifuge. Use pipet to remove supernatant leaving ca. <0.1 ml solution in tube. This stains the starches a dark purple color.

14. Judgmentally add 10-20 drops of glycerol to each 2 ml tube. Use less glycerol when pellet is not visible or only a thin layer on the bottom of the tube. Use more glycerol when pellet is larger.

Note: The purpose of using more glycerol with a larger pellet is to prevent overcrowding of slides with non-starch particles.

15. Re-weigh 2 ml light fraction tubes. Subtract original tube weight from the “tube + light fraction + glycerol” weight to determine the total weight of mountable extract.

Extract Concentration Method B

Note: Extract Concentration Method B was created to simplify the process of creating the mountable extract and to prevent loss of starches during concentration.

1. Transfer 50 ml of material from beaker (S1, S2, or S3) into 50 ml polypropylene centrifuge tube and centrifuge **in a bucket rotor which holds tubes horizontally during centrifugation.**
2. Remove supernatant with 20 ml syringe, placing syringe into tube and removing supernatant from water surface. Leave ca. 5 ml of water in the bottom of the tube each time, to ensure pellet is not disturbed. Supernatant removal was carried out with a syringe rather than through decantation to ensure non-disturbance of the pellet.
3. Transfer material from beaker into 50 ml tube, filling to 50 ml mark, and repeat steps 1-2 until the beaker is empty. When the beaker is empty, invert and use a squeeze bottle to rinse material from base of beaker into 50 ml tube. Fill each tube to 50 ml in each centrifugation to ensure equal weight distribution.
4. After final centrifugation, remove supernatant to 5 ml with 20 ml syringe. Using a pipet, impel and expel supernatant and pellet repeatedly to mix. Transfer material into 15 ml polypropylene centrifuge tube.
5. Add ca. 1 ml water to 50 ml tube and impel/expel into pipet to rinse remaining material from 50 ml tube and pipet. Transfer material into 15 ml tube. Fill each 15 ml centrifuge tube to 10 ml and centrifuge **in a bucket rotor which holds tubes horizontally during centrifugation** to concentrate pellet.
6. Carefully decant 15 ml tubes leaving ca. >2ml of material in bottom of tube. Centrifuge 15 ml tubes to concentrate pellet once again.

Note: To avoid disturbing the pellet, 15 ml tubes are not decanted fully in this step.

7. Using a pipet, remove the remaining ca. 2 ml of supernatant leaving ca. <0.25 ml of water in tubes.

8. Add 2.0 ml of sodium polytungstate at 2.0 g/ml. Impel/expel sodium polytungstate and pellet repeatedly with pipet to mix thoroughly. Centrifuge to separate light and heavy fractions.

Note: Adding 2.0 ml sodium polytungstate at 2.0 g/ml to 0.25 ml water produces a solution with final density ca. 1.9 g/ml. Material is mixed with pipet instead of vortexing to avoid spreading extract around the sides and the top of the tube, where it could stick during centrifugation.

9. Using a pipet, gently mix the material floating on the surface of the supernatant (and material adhering to sides of tube) into the supernatant. Use pipet to remove supernatant leaving ca. <0.1 ml supernatant and pellet (“heavy fraction”) undisturbed in bottom of tube. Transfer supernatant (“light fraction”) into a separate 15 ml tube (“light fraction tube”).

10. Fill the 15 ml light fraction tube (containing ca. 2 ml of light fraction material) to 15 ml with water. Vortex and centrifuge to concentrate pellet.

Note: The final density of solution in the 15 ml tube prior to centrifugation will be ca. 1.13 g/ml.

11. Carefully decant supernatant for recycling, leaving ca. >2.0 ml of supernatant in 15 ml tube. Fill each 15 ml tube to 15 ml with water and vortex to rinse remainder of sodium polytungstate into solution. Centrifuge to concentrate pellet.

Note: To avoid disturbing the pellet, 15 ml tubes are not decanted fully in this step.

12. Carefully decant supernatant, leaving ca. >2.0 ml of supernatant in 15 ml tube. Centrifuge once more to concentrate pellet again, and remove remainder of supernatant with pipet, leaving ca. <0.1 ml of water in tube.

13. Based on the amount of pellet in the 15 ml tube, add 10-20 drops of a solution of: 50% glycerol, 49.95% water, and 0.05% iodine. Add 10 drops if pellet is not visible or only a thin layer on the bottom of the tube. Add 20 drops if pellet is clearly visible.

Note: The purpose of using more mounting medium with a larger pellet is to prevent overcrowding of slides with non-starch particles. The solution of water, glycerol, and iodine acts as both the stain and mounting medium.

14. Label and weigh a set of 1.5 ml polypropylene centrifuge tubes. Using a pipet, impel/expel mounting medium and pellet in 15 ml tube repeatedly to mix. Also use pipet to stir the pellet and mounting medium. Mix thoroughly to create a homogenous solution and transfer solution into 1.5 ml tubes.

Note: A small amount of solution may remain in the bottom of the 15 ml tube or in the pipet. Take care to transfer as much solution as possible out of 15 ml tube and pipet into 1.5 ml tube.

Total estimated number of starches extracted from the artifact will be underestimated in proportion to the amount of solution remaining in the 15 ml tube and pipet!

15. Reweigh the 1.5 ml light fraction tube. Subtract original tube weight from the weight of the “tube + mounting medium + extract” to determine the total weight of mountable extract.

Mounting Method

Note: Begin with a 1.5 or 2.0 ml centrifuge tube containing mountable extract.

1. Lay out a fresh sheet of aluminum foil to create a clean surface. The following materials should be at hand: microscope slides, cover slips, acrylic seal, marker, powder-free gloves.

Note: Microscope slide and cover slip boxes should be closed when they are not in use. A fresh box of slides and cover slips should be used for ancient starch work – do not use a box that was previously opened and used by yourself or someone else.

2. Label a microscope slide, place it on the scale, and tare the scale.

3. Using a pipet, impel and expel the mounting medium and stir repeatedly to thoroughly homogenize the sample. Samples should be mixed for at least ca. 1 minute.

4. Expel material from pipet and collect the equivalent of ca. 2-3 drops of material from the center of the mounting medium volume. Place 1 drop of mounting medium + extract onto the center of the microscope slide.

5. Immediately weigh the microscope slide (previously tared) with mounted extract and record the extract weight.

6. Carefully place the cover slip over the drop of extract. The extract should immediately expand to fill the entire space under the cover slip without expanding outside of the cover slip.

7. Seal the edges of the cover slip completely using acrylic. The brush should never be pressed down onto the edges of the cover slip. Instead of “painting on” the acrylic, the acrylic should be allowed to “run off” of the end of the brush and onto the edge of the cover slip so that the position of the cover slip is disturbed as little as possible. After the acrylic has dried, check for leaks and use acrylic to seal any leaks. (If sealed properly, slides should never leak.)

8. Slides must be stored horizontally (i.e., in the same position as they would be viewed under the microscope). Starches and other materials on slides stored vertically will drift toward the downward edge of the slide.

Analysis Method

1. An Olympus BX-51 stereoscopic microscope was used for analysis. Slides were scanned at 200x magnification. In order to see polarization crosses on stained starches clearly, the **condenser aperture was fully opened** (this is different from the light source aperture). The polarizer was set to partially cross-polarize during slide scanning and was manipulated during scanning to show each view under different cross-polarization settings.
2. Begin at the top edge of the slide and scan one transect. Record the number of starches observed. Be sure to adjust focus while scanning to observe starches at different positions in the mounting medium.
3. Move the stage downward to scan a new transect, leaving ca. $\frac{1}{4}$ of a field-of-view height between this transect and the last one. This ensures transects do not overlap.
4. Continue scanning and recording data from transects until the bottom edge of the slide is reached. This will probably require 12-16 transects.

To calculate the proportion of each slide represented by each transect, determine how many fields-of-view high the slide is. For example, if there are 10 fields of view between the bottom and top of the slide, each transect represents 10% of the total slide area. We found that at 200x magnification, there were 21 fields-of-view between the top and the bottom of the slide, such that each transect represents 4.76% of total slide area.

To calculate the total number of recovered starches (from the artifact) represented by each transect, use the following equation:

$$T = S * (1/X) * (1/Y)$$

Where T = the estimated total number of starches; S = the number of starches recorded in a transect; X = the proportion of the slide area represented by a transect; and Y = the proportion of total mounting medium on the slide.

For example, if I am scanning a slide that contains 10% ($Y = 0.10$) of the total mounting medium from a sample at 200x magnification (transect = 4.76% [$X = 0.0476$]) and I observe 5 ($S = 5$) starches, the estimated total number of starches based on that transect would be 1050.

Calculate the estimated total number of starches based on each transect in an Excel table. Use the distribution of these values in statistical analysis.

Note: Because starches tend to cluster in the center of the slide where the drop of mounting medium is originally placed, the distribution of estimated total starch values will likely be non-normal. Nonparametric statistical techniques may be best for summarizing and comparing data.

Results

Figure 1 presents the results of experimental extractions from artifact Set #1. In this set, “Extract Concentration Method A” was used for treatments S1 and S2, and “Extract Concentration Method B” was used for treatment S3. We modified the extract concentration method after noting visible loss of material using Method A (see Discussion section).

In Set #1, fewer starches were recovered during treatment S2 than in treatment S1, suggesting the majority of starches recoverable through sonication alone were recovered during the first 5-minute treatment (S1). In three of four cases, the S3 treatment increased starch recovery by greater than an order of magnitude over S1 and S2 treatments combined. However, the magnitude of increase likely reflects the different starch extract concentration method used, rather than actual numbers of starches extracted using each method.

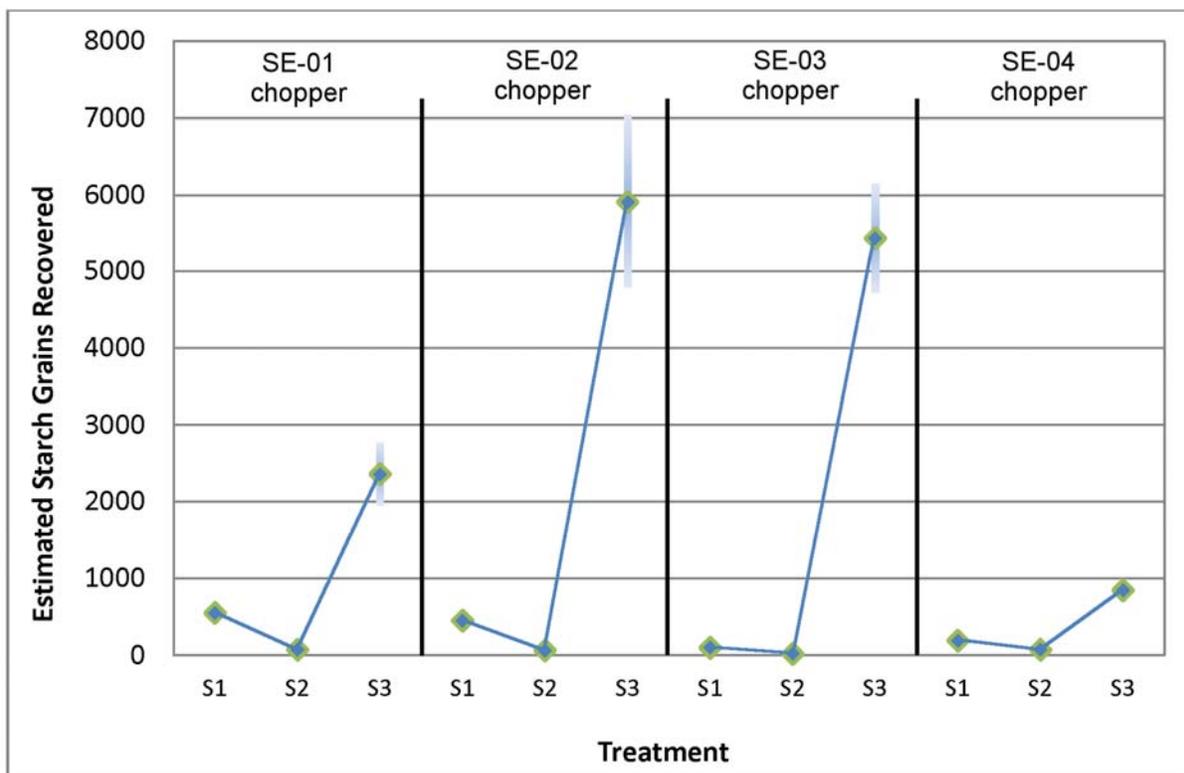


Figure 1. Results of experimental starch extraction treatments on artifact Set #1, samples SE-01 through SE-04. These are four igneous chipped stone choppers from site CA-SMA-113, contexts dating ca. 1000-1300 CE, excavated 2007-2009. Vertical bars indicate one standard error range. **Note: Extract from S1 and S2 treatments was concentrated using “Extraction Concentration Method A,” while extract from S3 treatment was concentrated using “Extraction Concentration Method B.”**

Figure 2 presents the results of experimental extractions from artifact Set #2, comprised of four unprovenienced artifacts in the collections of the California Archaeology Laboratory at UC Berkeley. In three of four cases, the majority of total starches recovered were extracted during the first 5-minute sonication treatment (S1). The standard error range of total recovered starches in the S3 treatment overlaps with that of the control sample for three of four cases, indicating that the starches recovered from S3 treatment could represent primarily modern contaminants. In one case, for artifact SE-06, extended sonication for 30 minutes (S2) recovered several times as much starch as the original 5-minute treatment (S1). Overall starch recovery from this set of artifacts was poor relative to other sets, with an estimated maximum of ca. <800 starches recovered from each artifact from all treatments combined.

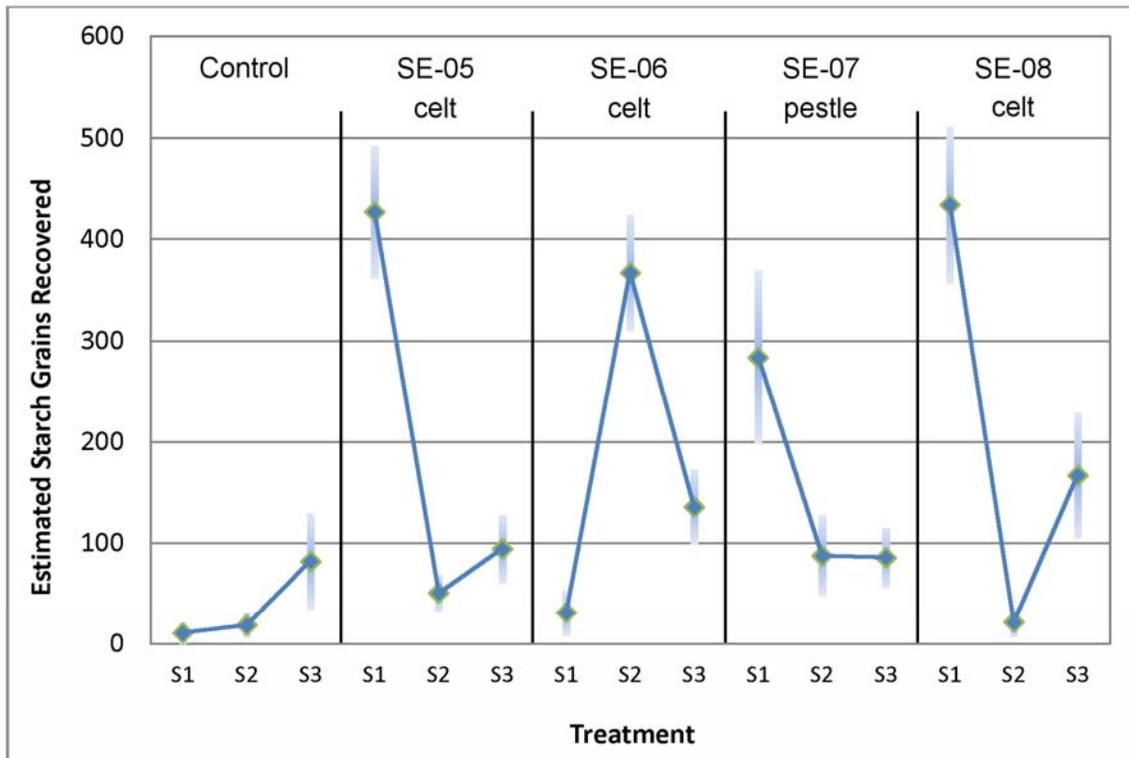


Figure 2. Results of experimental starch extraction treatments on artifact Set #2, samples SE-05 through SE-08, four unprovenienced ground stone tools in the collections of the California Archaeology Laboratory at UC Berkeley. Vertical bars indicate one standard error range. Starch recovery on these artifacts was poor compared to artifacts in Sets #1 and #3.

Figure 3 presents the results of experimental extractions from artifact Set #3. In three of four cases, extended sonication treatment for 30 minutes (S2) recovered about twice as much starch as the first 5-minute treatment (S1). In three of four cases, deflocculation followed by sonication (S3) recovered ca. 100%-500% as much starch as the extended sonication treatment. Overall starch recovery was very high, with total starches recovered from each tool from all treatments ranging from ca. 7,000-14,000 specimens.

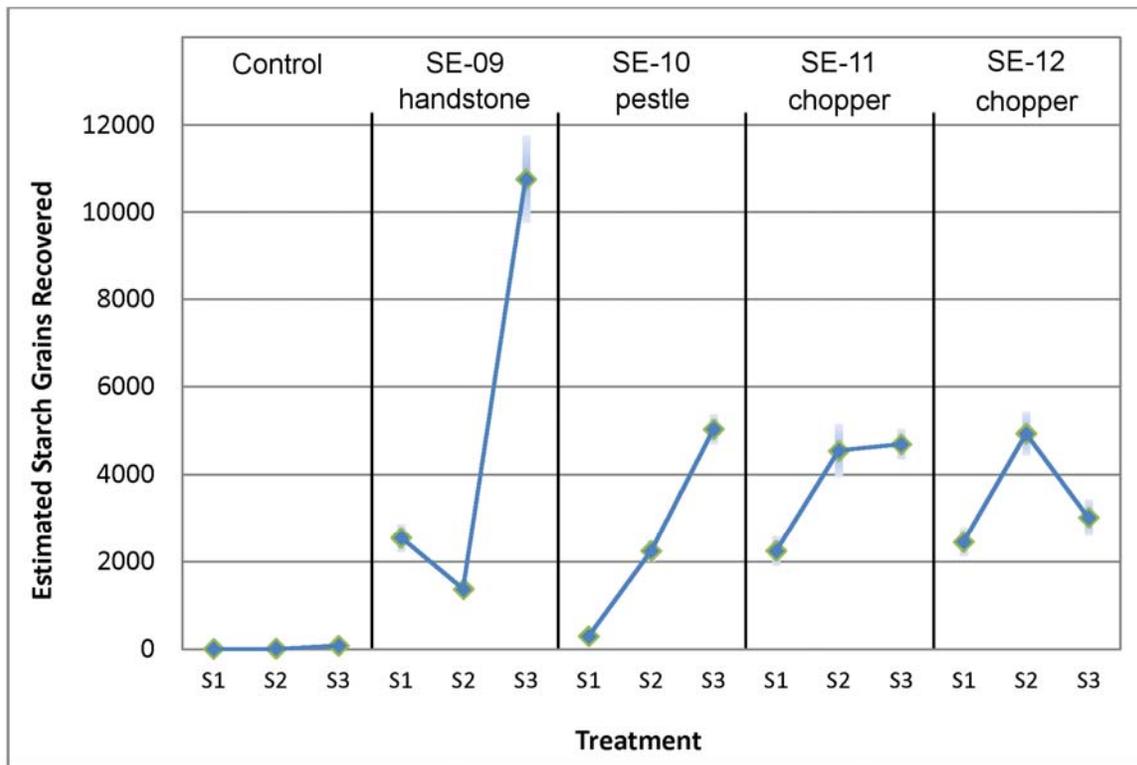


Figure 3. Results of experimental starch extraction treatments on artifact Set #3, samples SE-09 through SE-12. These are four chipped and ground stone artifacts from CA-SMA-113, contexts dating ca. 1000-1750 CE, excavated 2007-2009. Vertical bars indicate one standard error range.

Discussion

Comparison of the results of S1 and S2 treatments from Set #1 (Figure 1) and Set #3 (Figure 3) suggests that “Extract Concentration Method A” results in the loss of the majority of starches and should not be used in future studies. In Set #1 (comprised of choppers from CA-SMA-113), mean estimated total starches recovered during S1 and S2 treatments ranged from ca. 20-550 specimens. In Set #3, means of total starches recovered during S1 and S2 treatments for the two choppers included in the set (SE-11 and SE-12) ranged from ca. 2200-5000. If these two choppers had similar amounts of actual starch residue as those in Set #1, it suggests that using “Extract Concentration Method A” probably results in loss of >90% of starches.

We think there may be two possible reasons for loss of starches using “Extract Concentration Method A.” The first is related to centrifugation. In this method, microcentrifuge tubes were centrifuged using a rotor that held tubes at a ca. 45° degree angle from vertical. When centrifugation was complete, a substantial amount of the pellet adhered to the sides of the tubes rather than resting in the bottom of the tube, and this portion of the pellet was obviously mobile

when removing supernatant with the pipet. The result is that each time extract was centrifuged and supernatant was removed, a portion of the pellet was lost.

A second reason starches may be lost using this method is related to the density of sodium polytungstate solution after dilution. In this method, the post-dilution density of the liquid is ca. 1.2 g/ml, which could be high enough to cause some starches to float. However, it is unlikely that the majority of starches would be lost in this way, since the reported density for starch is reportedly 1.5 g/cm³ (Torrence and Barton 2006:161).

We think it is much more likely that the reason for loss of starches using “Extract Concentration Method A” is related to the angle at which microcentrifuge tubes were centrifuged. **We recommend that only centrifuge rotors which hold tubes horizontally during centrifugation should be used when concentrating microbotanical extracts.**

Despite the incomparability of results between Set #1 and Set #3 S1 and S2 treatments, the observation that the deflocculation treatment (S3) among Set #1 artifacts resulted in recovery of ca. 1,000-6,000 starches that were not recovered during 35 minutes of sonication indicates that these artifacts contained a large number of starches bound in chemically bonded matrices that could not be disaggregated through sonication alone. The results of deflocculation treatment (S3) among artifacts in Set #3 confirms this, and indicates that the amount of starches liberated through deflocculation treatment may be several times higher than the amount recovered from 35 minutes of sonication treatment (see results of SE-09, SE-10).

Starch recovery results from Set #2 of unprovenanced artifacts are difficult to interpret because the history of post-excavation artifact treatment is unknown. The pattern of starch recovery among this set of artifacts is strikingly different from that of all other artifacts examined, showing little benefit from either extended sonication time (except in one case) or from deflocculation treatment. These artifacts may have been treated with chemicals or stored in conditions that caused the destruction of the majority of archaeological starch residues on the artifact, such that the only starches present on the artifact were surficial modern contaminants recovered through the first 5-minute sonication treatment (S1). If this is the case, it suggests that it may be difficult to differentiate modern starch contaminants from ancient starch residue on curated artifacts with unknown histories. Another possible explanation is that these tools may have originated from deposits that were poor for starch preservation (e.g., highly acidic soils).

References

Torrence, Robin and Huw Barton
2006 *Ancient Starch Research*. Left Coast Press, Walnut Creek, California.