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Field Starch Extraction from Ground Stone: Experiment and Protocol Recommendations
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Introduction and Research Question:

This report presents an experiment in which four starch sampling methods are compared in order to identify the most successful and effective technique for starch extraction from large groundstone and milling features in the field. Testing archaeological specimens in situ is necessary when the artifact is too large or cumbersome to bring back to a laboratory (Pearsall 2015). In addition, preliminary testing in the field allows researchers to obtain quick results that provides guidance for ongoing excavation and sampling methods.

Agitation is needed to release both post-depositional sediment and adhered starch from a specimen, which then is collected as an aqueous accumulation with a pipette. This project tested the hypothesis: *if* ultrasonic vibrations from electric hand instruments (electric toothbrush and sonicating facial scrubber) are more effective at dislodging starch from groundstone, *then* more starch grains should be observed in prepared slides when compared to those using non-electric instruments (plastic pipette and regular toothbrush). The vibrations of sonicating instruments have the power to deeply clean uneven or porous surfaces, which may be necessary when dealing with pitted ground stone. Multiple heads for modern electric toothbrushes can be purchased quite cheaply, so this seemed to be an economical tool to test for the field.

The sampling methods used here included two sonicators and two manual tools. The procedures for each were similar: a water wash was used along with a tool to agitate the wetted sample (either manually or through sonication), the liquid and tool were each retained, the tools were washed, and the presumably starch filled liquid from each was then mounted on slides and viewed under a polarizing light microscope. The materials tested were pieces of sandstone (imitation metates and manos) taken from Putah Creek Wildlife Area, Solano County, California, which were washed and used to grind wheat, maize, and manioc. The first three pairs of stones were used for one taxon each, while the fourth received all three taxa processed on it in turn before sampling. These four collections were completed so that the differences in starch deposition due to plant taxa would not compromise the results.

The impetus for this experiment was Lamb and Loy's (2004) article in which the authors extracted starch from a replicated grinding stone. They poured water onto the ground stone, and agitated it with a pipette for over two minutes before pipetting the material onto a slide for analysis (Lamb and Loy, 2004). This protocol was also used by Barton (2007), and Pearsall (2015). A plain or electric toothbrush could also be effectively used (Pearsall 2015:360). Plain toothbrushes have traditionally been used to brush phytoliths from dental calculus and ground stone in the field (Boyadjian, Eggers, and Reinhard 2007; Logan et al. 2012; Middleton and Rovner 1994). Shanti Morell-Hart agreed with this assertion and reported successfully using a sonicating facial spatula to extract starch from pottery and ground stone (Morell-Hart 2018, personal communication). Due to the variety of instruments that could be used to extract starch

in the field, it was deemed necessary to test each of them to discover which was the most efficacious for the recovery of starch in the field.

Background – The Application of Starches in Archaeology:

Starch Biology

The main function of starch is to store energy in the form of glucose for the plant. Photosynthetic processes in chloroplasts use sunlight to convert water and carbon dioxide molecules into chain-like carbohydrates that are transported throughout the plant and stored long-term as energy reserves in the form of starch (Pearsall 2015). Starch, also known as amylose, is produced in amyloplasts (organelles) in all parts of the plant: leaves, seeds, roots, fruits, pollen, stems, corms, bulbs, rhizomes, burls, piths, and inflorescences. However, starch is not equally distributed amongst plant parts, as underground storage organs can range between 65-90% starch, compared to leaf, stem, and seed counter-parts whose starch concentrations are variable depending on taxa (Gott et al. 2006:37-39).

Within an amyloplast, the hilum is the point of origin in which starches are formed by overlapping lamellar and crystalline structures ranging between 120 to 400 nm thick (Gott et al. 2006). Within a given population of starches, no two granules will be identical. Starch morphology is incumbent on the genetic disposition of the taxon, and is plastic to internal conditions and external environments (Gott et al. 2006). Like phytoliths, starches can take several distinct forms and types in one plant. Granules can be simple, compound, and semi-compound. Simple starches are single granules produced within an amyloplast, while compound starches, like those found within manioc, occur as a series of granules, or *granula*, formed within a single amyloplast.

Starches are observed in a suite of shapes and sizes as well: oval, sphere, discoid, elongate, round, kidney-shaped, polyhedral, and irregular to name a few (Gott et al. 2006:40). Shape depends on amylose content within each taxon. Starches can range between 1 - 100 microns in length, depending on the amount of water present. Several shapes of starches will take place within the organs of the plant, depending on the location and position of tissue which is producing the starch (e.g. root tips, the center of a root, between bark, at the tip of a leaf). More importantly, the age of starch directly correlates with size: young starches are small while mature starches are larger. However, based on experiments, external factors, such as heat and drought stress also produce smaller starch granules (Gott et al. 2006:42).

The Archaeology of Starches

Starches are one of the most important sources of metabolic fuels in the evolution of the human species (Pearsall 2015). Evidence for non-dietary starch has also been found in association with ancient papyrus and cosmetic products (Gott et al. 2006). Archaeologists encounter two kinds of starches on artifacts: native and modified. Native starches are essentially unchanged and are readily observed when extracted from sediments and cordage manufacturing toolkits. Cultural practices produce modified starches from grinding, pounding, milling, heating, fermenting, and gelatinization that may be extracted from dental calculus, groundstone, ceramic vessels, and other culinary tools.

Archaeologists focus on a series of unique characteristics of starch for microscopic study. Under a polarizing microscope, *anisotropic* [measurable value is directionally-dependent, like strength properties in wood] starches reveal strong birefringence due to light travelling at differential speeds through the lamellar structures of the granule (Gott et al. 2006). It has been found that chemical extraction and dehydration of starches negatively affects the birefringence of starches, while higher hydration levels can increase birefringence. Polarizing lenses can reveal extinction crosses that intersect at the center of the hilum. Starches will also intake iodine-potassium-iodine stains at differential rates to provide a blue-purple gradient across starch taxa (Gott et al. 2006). In other words, the amounts of amylose in a starch granule (generally depending on taxa) will determine how much of the stain is absorbed and thus a chronometric approach can be used to discriminate between taxa based on the variability in amylose content. Under heating, the hydrogen bonds in starch structures break down and starches swell until the irreversible process of gelatinization occurs. See Gott et al. (2006:4) for a table of taxa and their gelatinization temperature ranges.

Experimental Procedure:

To test the range of collection procedures, we collected and prepared four stones for use as manos and four for metates, to grind wheat (*Triticum* sp.), maize (*Zea mays*), manioc (*Manihot* sp.), and a combination of the three taxa. With these, we tested 4 methods for starch extraction on each stone:

1. direct pipetting after agitation with the pipette tip,
2. pipetting and sonicating toothbrush bristles after agitation with regular toothbrushes,
3. pipetting and sonicating toothbrush bristles after agitation with sonicating toothbrushes,
4. pipetting after agitation with a sonicating facial tool.

Materials:

- 4 sandstone metates, 4 sandstone manos, and 1 extra stone for environmental background control test, all scrubbed clean of algae and dirt, and allowed to dry fully
- 5, 40 ml glass beakers
- New plastic zip lock bags
- Philips Sonicare Essence Rechargable Sonic Toothbrush with 9 detachable heads
- KINGA Fashion Light Skin Scrubber Ultrasonic facial spatula
- 4 regular toothbrushes
- Pipettes (plastic and glass)
- Sealable test tubes
- Microscope slides and covers
- Clear nail polish (Sally Hansen)
- Ultrapure water
- Olympus BX51 light microscope with polarizing light
- 200g dried wheat
- 200g dried maize
- 300g dried manioc

- Non-powdered plastic gloves, to be worn during entirety of procedure

Methods:

1. Four large and four small (metates and manos, respectively) pieces of sandstone and one extra (control) stone were collected from the local Putah Creek, located in Solano County (Figure 1), on October 7, 2018.

2. *Metate and Mano Preparation:* Once brought into the Soils Laboratory of the Archaeological Research Facility, the stones were washed thoroughly with tap water and allowed to dry.

Figure 1: *Local sandstone, collected from the Putah Creek Wildlife Area, Solano County California was used for our experiment. All stones were collected from the riverbed.*

2. *Environmental Contaminant Check:* In order to check for background starch contamination (from algae, etc.), we performed our 'starch extraction' protocol on our control stone, which

was washed like the others but had nothing ground on it. We poured 10 ml of ultrapure water on the center of our environmental control stone. Using a sonicating toothbrush with a new, clean head, we sonicated the wet surface for 30 seconds by rubbing the vibrating toothbrush in a circular motion. After 30 seconds, we turned off the toothbrush and carefully detached the removable head into a clean plastic bag. The remaining water was pipetted with a plastic pipette onto a labelled slide, covered, and sealed with nail polish. No starch was observed under cross-polarization at 20x, indicating that our samples were free of natural starch contaminants from the river stones. Given that this test did not yield a single starch grain during analysis, we are confident that the rest of our samples are free of natural starch contaminants from the river environment.

3. *Plant Preparation*: Dry wheat and maize were stored by Hastorf for two years having been purchased from a farm in Iowa in 2016. Manioc was purchased from the supermarket. The wheat and maize were ground dry and whole on separate stones. Manioc was segmented, grated, and then dried in a desiccator for 10 days before grinding (Figure 2).

Figure 2: *Manioc after being grated and expelled of water, before being placed in desiccator.*

4. *Grinding:* The four metates were labelled 1-4 (Figure 3). On metate 1, we placed 81.8g of dried wheat grains. We placed 68.33g of dried maize kernels on metate 2, and 100g of grated manioc on metate 3 (Figure 2). On metate 4, we put another 125.15g of dried wheat grains. We ground each by pushing and pulling the manos across the metates repeatedly (Figure 3). After 15 minutes of grinding we stopped and removed the ground flour. On metate 4, we repeated the same process with 150g of maize kernels, and then with 100g of manioc. *Note: Although we intended to keep a constant 100g of each ground taxa, we varied our amounts in order to keep the grinding time constant.*

Figure 3: *Grinding stations staged with pre-weighed plant samples (left). Timed grinding of samples with pushing and pulling motion (right).*

5. Sampling and Pipette Extraction After Grinding:

a. Sonicating Toothbrush: on each of the four metates, we poured 10 ml of ultrapure water. Using a sonicating toothbrush with a new, clean head, we gently sonicated the wet surface of the metate for 30 seconds by gently rubbing the now vibrating toothbrush in a circular motion. After 30 seconds, we turned off each toothbrush and carefully detached the removable head and placed it into an individual, clean, new, plastic bag. This was sealed and labelled. We then pipetted the remaining water onto a slide with a new plastic pipette, covered, and sealed it with nail polish. We repeated this for each of the four metates and each of the manos.

b. Regular Toothbrush: On each of the four metates, we poured 10 ml of ultrapure water onto a location that was not previously sampled but where grinding had occurred. Using a non-electric toothbrush, we brushed in a circular motion on one point for 30 seconds (Figure 4). After 30 seconds, we put each toothbrush into an individual, clean, plastic bag. This was sealed and labelled. We pipetted the remaining water onto a slide with a new plastic pipette, covered, and sealed it with nail polish. We repeated this for each metate.

Figure 4: *Using regular toothbrushes in circular motion before pipette extraction.*

c. Sonicating Facial Spatula: On each of the four metates, we poured 10 ml of ultrapure water over a spot on the grinding area. Using a clean ultrasonic facial spatula, we gently sonicated the wet surface of the metate for 30 seconds by gently rubbing the vibrating ultrasonic facial spatula in a circular motion (Figure 5). After 30 seconds, we turned off the ultrasonic facial spatula, pipetted the water onto a slide with a new plastic pipette, covered, and sealed it with nail polish. We repeated this for each metate, cleaning the tool with ultrapure water between samples.

Figure 5: *Using sonicating facial spatula in a circular motion and extracting the material with pipette.*

d. Pipette Only: On each of the four metates, we poured 10 ml of ultrapure water onto a location that was not previously sampled. With a plastic pipette, we agitated the surface for 30 seconds. We withdrew the suspended residue with a pipette and ejected it onto a labelled slide. Then, we covered the slide and sealed it with nail polish.

6. *Extraction from Toothbrushes:* In the wet laboratory of the Archaeological Research Facility, we placed 14 ml of ultrapure water into the Ziploc bags with the toothbrushes (regular and sonicating heads) and resealed the bags. We held the bagged brushes in a sonication bath for 1 minute (Figure 6A). Next, we removed the toothbrush heads from the bags, and poured water from the bags into sealable, labelled test tubes (to pour smoothly, we held water in one bottom corner of each Ziploc while carefully cutting the other (Figure 6B). We poured from this corner).

Figure 6: A. *Holding scrubbing instruments in sonic bath (left).* **B.** *Cutting bottom corner to carefully pour contents into test tube.*

7. We next allowed these samples to rest for 1 week in test tubes, so that the starch could settle to the bottom of the test tubes without centrifuging, in order to replicate field conditions (Figure 7).

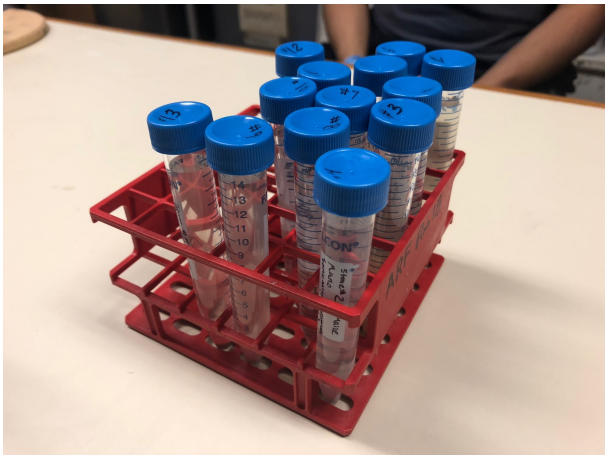


Figure 7: *Labeled test tubes with extracted starches.*

8. After one week, we decanted the supernatant liquid with a glass pipette, allowing for 1-2 cm of liquid to remain. With the same pipette, we removed a drop of water from the lowest point of the test tube and mounted it onto a labelled microscope slide. We carefully covered the slide and sealed it with clear nail polish. This process was repeated for all remaining samples.

9. *Analysis:* We counted starches on one transect of each slide, using a polarized light microscope, in order to compare the densities produced by each method. Transects were counted at 20x magnification (Figure 8). On slides that contained multiple taxa, the total starch grains were counted (individual species were not separated and identified). See Appendix A for descriptions of the 34 slides and their respective counts.

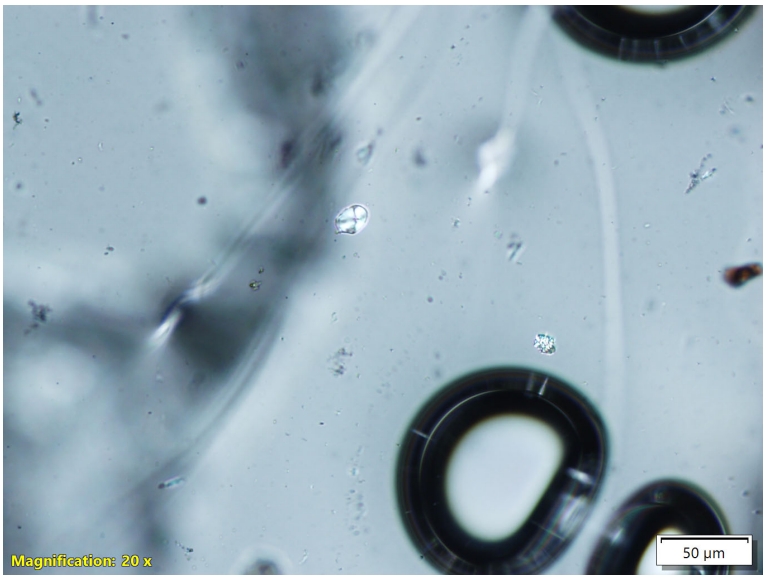


Figure 8: *Starch granule with extinction cross observed under cross-polarization (20X).*

Results:

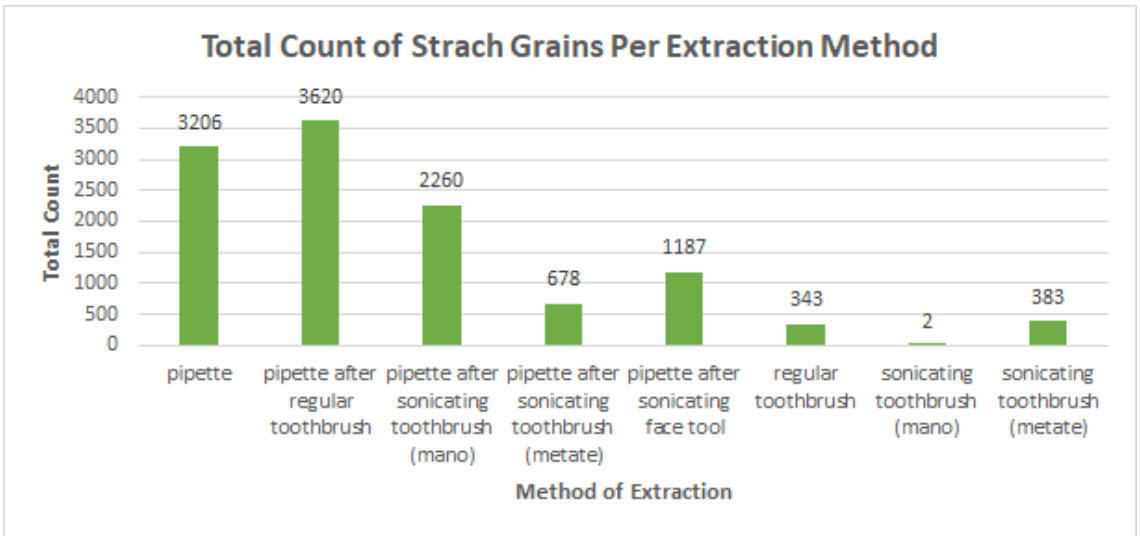


Figure 9*: Total count (1 transect counted per slide) of starch grains per extraction method*

Methods that Extracted Material Using a Pipette

Both the method of agitation and the source of extracted starch (from the stone itself or from the agitating tool) impacted the starch count observed on our slides. As noted in Figure 9, samples that were extracted using a pipette, from the stone surface, yielded a higher count of starch grains than those that were extracted from the agitation tools. This suggests that for starch grain analysis, it is best to extract material using a pipette, given a large artifact that cannot fit into a sonicator. This result bodes well for field extraction—simply pipetting water onto a slide is entirely feasible and sufficient in the field for gathering what was ground on the stone.

Methods of Extraction that Involved Sonication

Any method of extraction that involved sonication, whether pipetting the liquid off after sonication or extracting the material directly off of the brushes, yielded lower numbers of starch grains in comparison to other extraction methods. Sonication therefore did not improve our results, contrary to popular practices that incorporate sonication (Lamb and Loy 2005; Pearsall 2015). Our results suggest that it is more productive to simply agitate the artifacts with a pipette or a normal toothbrush than to incorporate any sort of sonication into the method of extraction. It may still be useful to clean an artifact using sonication if it is substantially covered in sediment. In terms of extracting the microbotanical remains, however, the agitation of sonication may be too much. This agitation may have actually expelled microbotanical materials off of the artifact and scattered them into the air during our procedures.

Sonication of the toothbrushes that agitated Metate Surfaces:

The tooth brushes that were submerged in a sonication bath did not have abundant starch grains attached to their bristles. These samples revealed the lowest quantities of starch grains compared to any other methodologies incorporated into this experiment.

Manos vs. Metate: Which artifact yields more starch grains?

Our group's hypothesis was that the methods of extraction that involved sonication would be the most effective. For this reason, along with time constraints, we only tested the manos with one method of extraction. This involved the use of sonicating toothbrushes to agitate the stone surface. Comparing this specific method on metates versus manos, our results suggest that more starch grains can be recovered from a mano compared to a metate. This may be due to the mano having more constant contact with the plant material during the grinding process. There is a limited area of contact on the mano, creating a more concentrated area for starches to be extracted from. For metates, on the other hand, grinding occurred over a much larger surface area so the concentration of starch grains was likely more dispersed.

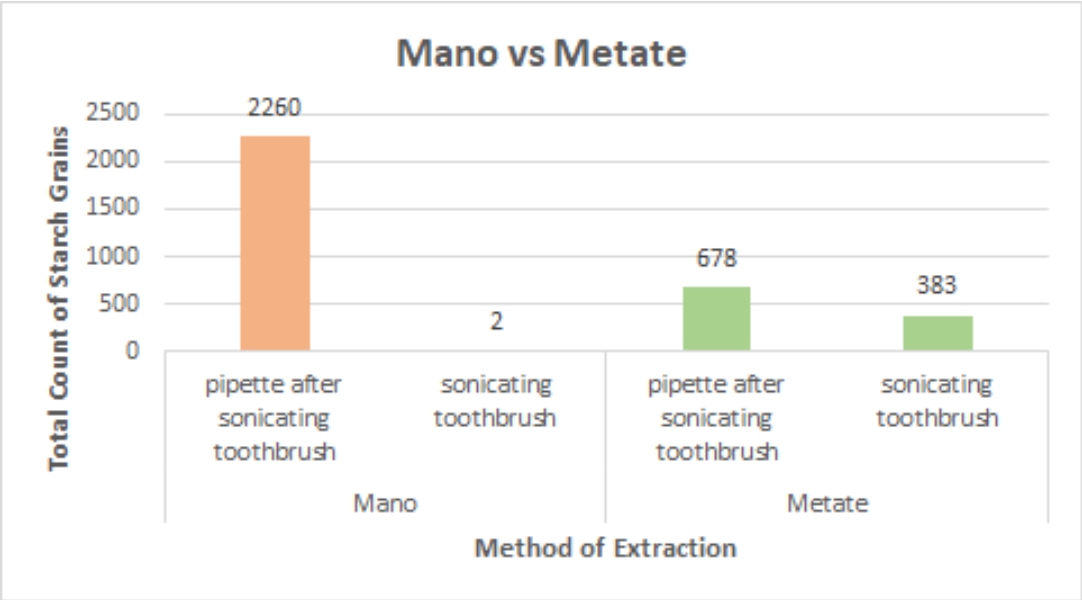


Figure 10: *Total count of starch grains by manos and metates comparing sonicating toothbrush vs. simple collection after sonication.*

Combined Taxa on Metate 4

Our experiment tested three different taxa that were ground on metates: wheat, maize, and manioc. Each species was ground on a separate metate, with a fourth metate devoted to a combination of all three taxa. This fourth metate yielded much higher counts of starch grains than any of the metates that only had one plant species ground on the stone. This could be a result of additional grinding time and plant matter, since the metates with only one species each were used for 15 minutes, whereas the stone that combined the species had 15 minutes of use for each individual species in succession (a total of 45 minutes of grinding activity). This also meant that the combination stone had significantly more plant material ground on its surface (375.15g) in comparison to the others which had 100g or less of plant material each when grinding. Future research should make the grinding on each metate more comparable by using the same amount of plant material and the same amount of grinding time on each stone.

Issues Encountered:

Different Conditions of Plants Before Processing

Our experiment shows that samples with manioc yielded more starch grains than wheat or maize (Figure 11). Underground storage organs have more starches, per weight, than seeds (see "Starch Biology" section above, Gott et al. 2006), which could explain why samples that incorporated manioc storage units had a much higher starch count than the other starch samples from seeds. Manioc and cassava roots generally have a higher starch content than other plants. Manioc produces compound starches that occur as a series of granules, or *granula*, formed within a single amyloplast (Gott et al. 2006). Interestingly, Gott et al. (2006) introduces a study by Sivak and Preiss (1998:3) showing differential patterns and occurrences of starch in cassava storage organs, adding further variation into our study.

Cultivars store starch in their seeds, but far less than is stored in any tuber, rhizome, or corm. Gott et. al. (2006) suggest that there is no 1:1 ratio of starch between plant taxa: season of harvest, location on plant, evolutionary traits, endosperm composition, heat and drought stress can influence starch production. In order to make the results of this experiment more comparable, it would have been more productive to test just one taxon. These differences can be seen in more detail within Figure 12.

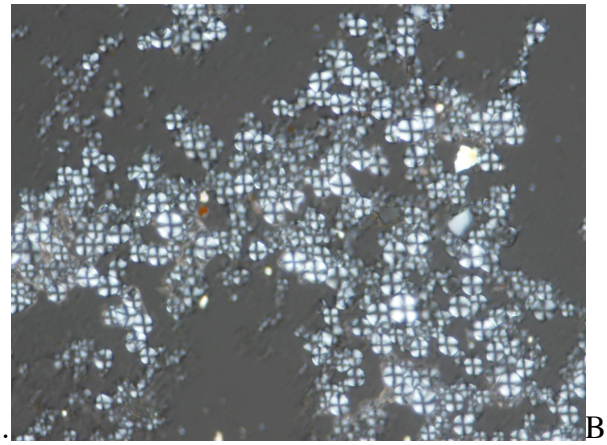
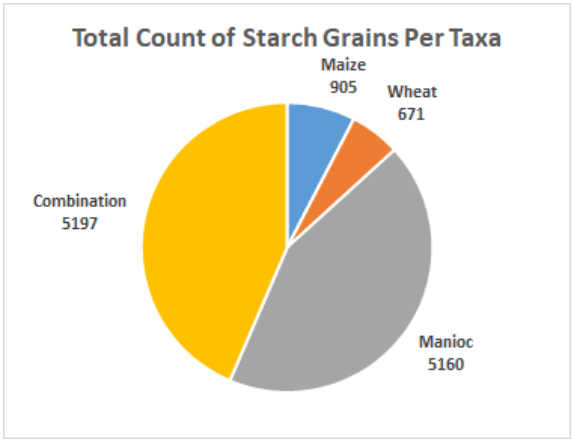
A. 
B. 

Figure 11: *A: Example photograph demonstrating the high density of manioc starch grains encountered on a slide (20x magnification). B: Total count of starch grains per taxon as studied in this experiment.*

The manioc was already partially processed (and wet) when ground, which may have also aided the release of the starch grains. The maize and wheat (both ground dry) did not produce as much starch. The maize could have been soaked or pounded beforehand, as is done on occasion traditionally.

The manioc starch was very densely packed, and stayed that way on the slide, interfering with count accuracy. This is likely because starch was extracted immediately after metate use. The wet manioc 'stuck' to the metates and was thus very dense on the slides. In the future we would a) allow the manioc to dry thoroughly before grinding, and b) let the metates dry completely and brush them off before sampling.

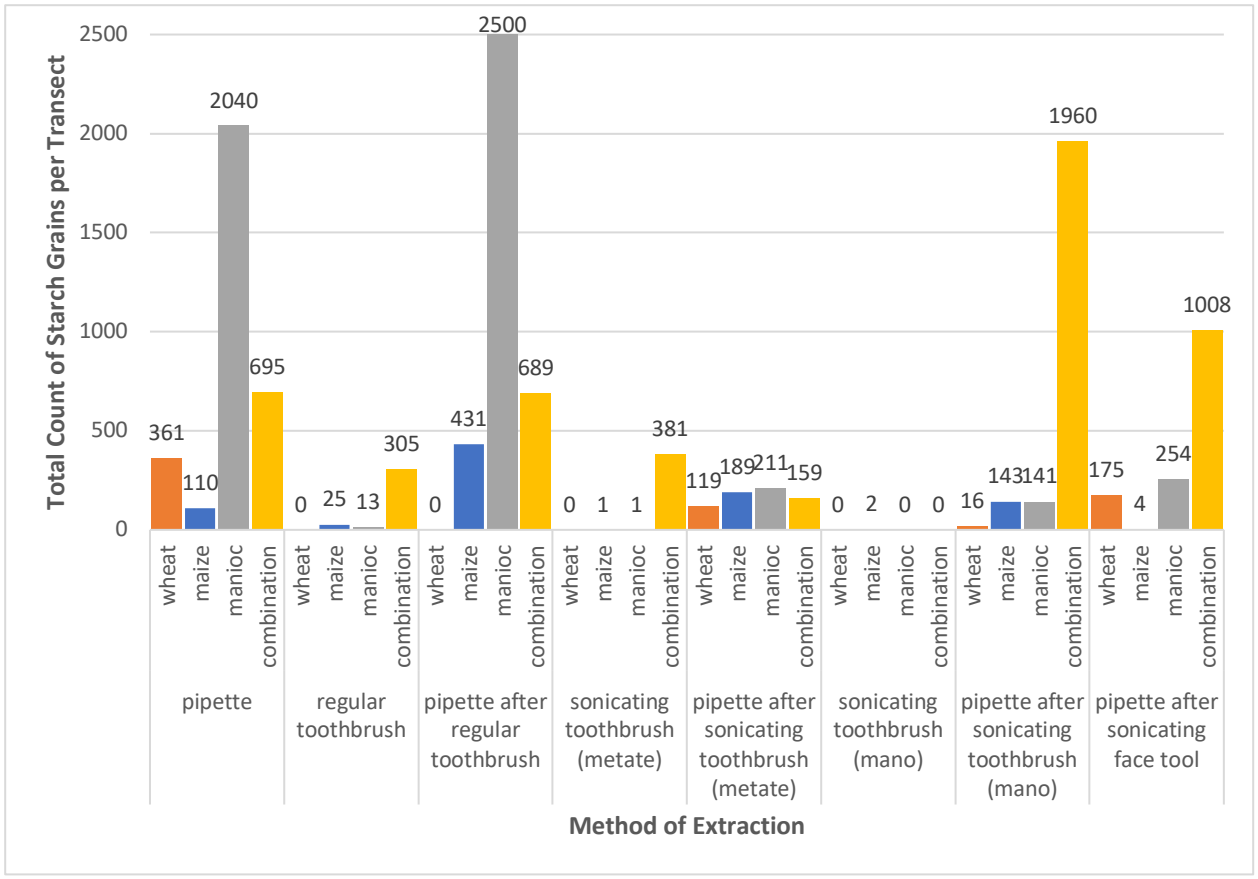


Figure 12: *Comparison of each taxon within each method of agitation and extraction from the metates and manos.*

Contamination from Nail Polish

The nail polish seems to have been contaminated while sealing the slides —starches from water that escaped the coverslips were introduced into the nail polish via the re-used brush (Figure 13), created mixed, dense, areas of starch in the slide borders, which were not counted. Going forward, we might use the nail polish in a dropper container and use disposable implements (i.e. plastic toothpicks) to drag the polish and seal the slide. In order to pursue this, plastic toothpicks (or other implements) should be tested for potentially contaminating starches.

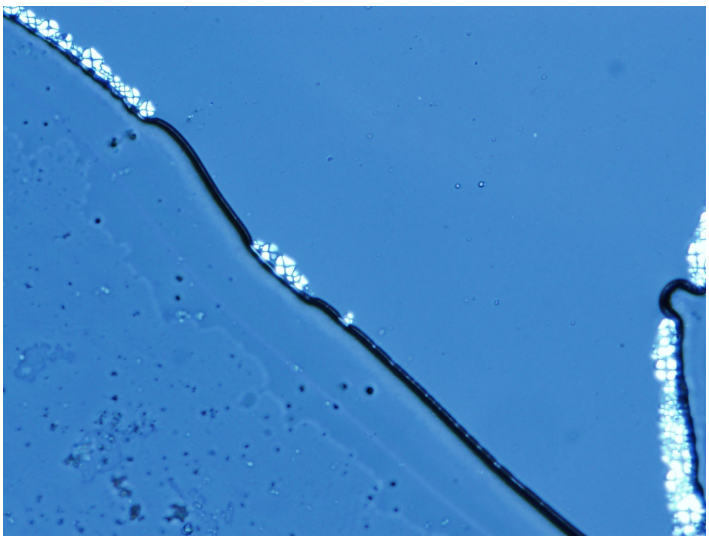


Figure 13: *Cross-polarization showing end of cover slip and possible starch contamination from nail polish application (20x).*

Metate Selection

The surfaces of our metates were sometimes uneven or sloping in a way that did not allow water to pool on them. This made pipette extraction more difficult. Additionally, sediment ground off during grinding, producing an accumulation of grit on our slides that impacted our view of starch grains.

The stone gathered was thought to be Greywacke Sandstone, which has been used in the region for metates and manos (e.g. Buonasera 2013). It is present at the location site, Putah Creek Wildlife Area. Unfortunately, due to the nature of wet rock and untrained eyes, a more coarse-grained sandstone was collected. Greywacke might result in fewer instances of stone breakage and excess sediment on slides.

Microbotanical Slide Preparation

We believe that differential mounting experience between the six analysts who completed the mounting may have affected our slide preparation. Applying too much pressure on the slide appears to have moved most of the starch material to the edges, which potentially put it in contact with the nail polish. Barton (2007) mentioned that they had to slightly modify their protocol based on where the starch migrated on the slide due to the mounting process. He also talked about having an approximate weight of material added to each slide to counter this. However, to try to avoid contamination and potentially throwing off counts, any material in contact with the polish we did not count.

In addition, because of varying experience, some slides were mounted without a consistent number of drops of material. This could have affected the counts. In future experiments the volume of material should be consistent.

Lastly, while counting, we encountered that some of the slides had dry patches where starches were not visible. We believe this had to do with the fact that they may not have been completely sealed when mounting. This could have been averted by mounting the slides with glycerol.

Starch Identification

We counted the total amount of starch grains per transect, but did not spend time separating them by taxon. Results would be more meaningful if we identified the taxon of each starch grain that was counted, especially for the combination slides. Future research should address this.

Conclusions and Future Research:

Our results indicate that directly pipetting the sample from the stone to the slide, after either pipette or regular toothbrush agitation, yielded the best results. This method is also the simplest for collection in the field: it requires minimal equipment, and only slides need be taken back to a laboratory for analysis.

The extraction procedures that included some kind of sonication yielded lower counts. However, the lowest came from the samples that were extracted directly from the toothbrush washes. Nonetheless, it should be mentioned that allowing samples to settle over time instead of using a

centrifuge still yielded results on the latter. We also found that manos had increased evidence of starches in comparison to the metates.

As was mentioned, we believe the nail polish proved to be a source of contamination. We propose that future research could use a new toothpick to disperse the nail polish when mounting, instead of the nail polish brush, thereby impeding that any source of contamination is passed from one slide to another.

Although we used plastic pipettes for extracting water from the stones and glass for extracting from the toothbrush washes, future research should compare both types of pipettes in all extraction procedures to see if they yield different results.

Overall, this study has yielded valuable results regarding the efficiency of different starch extraction methods from grinding stones. Moreover, according to these results, the most efficient method requires minimal equipment and is of relatively low cost. This will be especially useful for archaeobotanists undertaking processing and analysis in the field. Furthermore, this this study has implications for hunter-gatherer archaeologists who seek to integrate starch grain analysis as part of the survey and non-destructive analysis of bedrock milling features.

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Appendix A. Table of Results.

Sample #	Stone	Taxa	Method of Extraction	Artifact Type	Total Count (20x)
1	1	wheat	sonicating toothbrush	mano	0
2	1	wheat	sonicating toothbrush	metate	0
3	1	wheat	regular toothbrush	metate	0
4	2	maize	sonicating toothbrush	mano	2
5	2	maize	sonicating toothbrush	metate	1
6	2	maize	regular toothbrush	metate	25
7	3	manioc	sonicating toothbrush	mano	0
8	3	manioc	sonicating toothbrush	metate	1
9	3	manioc	regular toothbrush	metate	13
10	4	combination	sonicating toothbrush	mano	0
11	4	combination	sonicating toothbrush	metate	381
12	4	combination	regular toothbrush	metate	305
13	5	environmental	pipette after sonicating face tool	n/a	0
14	1	wheat	pipette after sonicating face tool	metate	175
15	1	wheat	pipette	metate	361
16	1	wheat	pipette after regular toothbrush	metate	0

Sample #	Stone	Plant	Method of Extraction	Artifact Type	Total Count (20x)
17	1	wheat	pipette after sonicating toothbrush	metate	119
18	1	wheat	pipette after sonicating toothbrush	mano	16
19	2	maize	pipette after regular toothbrush	metate	431
20	2	maize	pipette after sonicating toothbrush	metate	189
21	2	maize	pipette after sonicating toothbrush	mano	143
22	2	maize	pipette after sonicating face tool	metate	4
23	2	maize	pipette	metate	110
24	3	manioc	pipette after regular toothbrush	metate	2500
25	3	manioc	pipette	metate	2040
26	3	manioc	pipette after sonicating toothbrush	mano	141
27	3	manioc	pipette after sonicating toothbrush	metate	211
28	3	manioc	pipette after sonicating face tool	metate	254
29	4	combination	pipette after regular toothbrush	metate	689
30	4	combination	pipette	metate	695
31	4	combination	pipette after sonicating toothbrush	metate	159
32	4	combination	pipette after sonicating face tool	metate	1008
33	4	combination	pipette after sonicating toothbrush	mano	1960
34	5	environmental	pipette after sonicating toothbrush	n/a	0