

Rapid Isolation of Sorghum and Other Cereal Starches Using Sonication

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ABSTRACT

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High-intensity ultrasound (sonication) was investigated as a method to rapidly purify starch from sorghum and other cereal grains. To improve the process, buffers were optimized to solubilize sorghum proteins in combination with the sonication. Protein content and starch color were determined to evaluate the efficiency of the extraction process. Sonication times, SDS concentration, different types and concentrations of reducing agents (sodium metabisulfite, dithiothreitol, and β -mercaptoethanol), and centrifugation speeds of the starch washing procedure were tested. Protein content of isolated sorghum starch was reduced to 0–0.14% (db) after 2 min of sonication (using any of the reducing agents tested). Sodium metabisulfite was chosen as the preferred reducing agent because of its lower toxicity and odor compared with other reducing agents tested. The optimum conditions for producing high-purity sorghum starches (0.06%

protein) were obtained using the following conditions: 2 min of sonication time with 12.5 mM sodium borate buffer, pH 10, containing 0.5% SDS (w/v) and 0.5% sodium metabisulfite (w/v) using 1,500 rpm centrifugation speed during starch washing. Starches separated by this method showed significantly less protein content and *b* values (yellowness) compared with starches separated by enzymatic methods or methods using NaCl solutions and protein extraction buffers with multiple washing steps, both of which take several hours to complete. Differential scanning calorimetry thermogram values for starches isolated by three different methods showed similar patterns, except that starches obtained with the enzymatic method had slightly higher values of T_o , T_p , and ΔH . Other cereal starches from whole wheat meal, wheat flour, corn, rice, and barley were also obtained rapidly using sonication.

Sorghum (*Sorghum bicolor* L. Moench) has been consumed as a major food staple in Asia and Africa for centuries. However, in the United States, sorghum has been used mainly for livestock feed with only a small percentage used for food and industrial purposes (Rooney and Waniska 2000). Sorghum is the third major cereal crop in the United States with annual production of \approx 13 million metric tonne (mmt) harvested from \approx 4 million ha (Smith 2000).

Because sorghum is currently used mainly as animal feed, significant opportunities exist for increased utilization of sorghum. Sorghum is increasingly being used to produce fuel ethanol, especially in the sorghum growing regions. Sorghum also has potential for increased human consumption due to its high level of phytochemical components (Taylor and Belton 2002; Awika and Rooney 2004). Sorghum is also being used in the production of wheat-free food products suitable for consumption by people with celiac disease.

Sorghum starch plays an important role in both the production of food products and the fermentation of sorghum to produce products such as fuel ethanol. During fermentation, it is the starch that is broken down into sugars, later to be converted into ethanol. Starch content has been positively correlated to ethanol yields in sorghum (Zhan et al 2003). Sorghum starch plays an important role in the production of many sorghum-based food products, including bread (Schober et al 2005).

For better application of sorghum starch in foods, feeds, and industrial utilization, the study of physicochemical properties of sorghum starch is needed. Often the characterization of starches requires a purified starch. Currently, there is no specific method for isolating sorghum starch. Starch can be produced by wet milling sorghum, though the procedures typically require long time periods to complete because of the steeping process needed to loosen up the kernel requires 24–96 hr (Wang et al 2000). Several different wheat starch isolation/purifying methods have been used such as

the dough hand-washing method (Park et al 2004), the enzymatic method (Bechtel and Wilson 2000), and the chemical buffer method (Zhao and Sharp 1996). While the above methods are effective, they are time-consuming, though typically less so than wet milling. Therefore, a rapid, reproducible technique for isolating starch would benefit research aimed at studying the physicochemical properties of sorghum starches and for screening new sorghum lines for their starch properties. A rapid method may also be of benefit industrially if scale-up of the process was possible.

Sonication has been used to solubilize and disperse starch from cooked maize (Jackson et al 1988) as well as modify starch from heated samples of mung bean, potato, and rice starches (Chung et al 2002). These authors reported that starch isolated by sonication had increased paste clarity and decreased alkaline viscosity after treatment by sonication. Sonication was thus recommended as a method to produce modified starches with improved properties. Wang and Wang (2004) used sonication in combination with surfactants to purify starch from rice. This method used 5-sec cycles of on and off sonication for periods of 30–120 min. These reports show that sonication can be used to isolate starch from plant sources. When sonication energy transferred to sample through cavitation cycle, depolymerization of high molecular weight protein or breakdown of the protein polypeptide could occur (Hamer 2003). Reducing agents open up the protein structure, and detergents bind to protein. Therefore, the combination of sonication and chemical reagents would make the starch isolation process more efficient. Previously, we reported an optimum sorghum protein extraction condition of pH, detergent type, reducing agent type, and sample-to-solvent ratio (Park and Bean 2003). By using buffers optimized for extraction of sorghum proteins, we hypothesized that sonication time could be drastically shortened compared with previous methods that utilize sonication. Thus, the objectives of this study were to develop a rapid and high-purity sorghum starch isolation method and to compare the starch properties with those of starches isolated by other methods. Also, this proposed isolation method for sorghum starch was tested to purify starch from other cereals.

MATERIALS AND METHODS

Sample Preparation

Whole sorghum kernels were decorticated to 20% (by weight) removed using a tangential abrasive dehulling device (Venables Machine Works, Saskatoon, SK, Canada) equipped with an 80-grit abrasive disk and then ground using a Udy mill (Udy Corp., Fort

¹ USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Collins, CO) through a 0.25-mm screen. For isolating starch from whole meal, undecorticated sorghum kernels were milled to different particle sizes using a Falling Number mill (type KT-30, Stockholm, Sweden) and a Udy mill. The Falling Number mill was adjusted to levels 6, 2, and 0 (6 coarsest, 0 finest), and the Udy mill was used with 1- and 0.25-mm screens.

Sorghum Starch Isolation

Sorghum flour (2.5 g) was mixed with 50 mL of protein extraction buffer in a ≈300 mL glass jar, and the jar was gently shaken to disperse the flour. For initial tests, the protein extraction buffer was 12.5 mM sodium borate buffer, pH 10, containing 2% SDS and 2% β-mercaptoethanol (β-ME) (Park and Bean 2003). During sonication, the sample jar was placed in ice water to prevent starch gelatinization caused by heat build-up during the process. For sonication, an ultrasonic processor (VCF-1500, Sonic & Materials, Newtown, CT) was used with a 1-in. (25.4 mm) diameter probe. The sonication probe was placed within ≈5 mm from the bottom of the jar. The sonication amplitude was set at an instrument setting of 75% and this setting was used for all procedures. Sonication times were varied in different experiments (1, 2, 4, 6, 8, and 10 min). Different reducing agents (β-ME, dithiothreitol, and sodium metabisulfite), SDS concentrations (0, 0.5, 1, and 2%), centrifugation speeds (1,000, 1,500, and 2,000 rpm; 201, 453, and 805 × *g*, respectively), and solvent-to-flour ratios of 10:1 and 20:1 were tested. After sonication, the slurry was collected in a 50-mL plastic bottle, centrifuged at 2,500 rpm (1,258 × *g*) for 5 min, and the supernatant was decanted. Approximately 40 mL of distilled water was poured into the bottle to wash out precipitates. The slurry was then vortexed for 10 sec and centrifuged for 2 min at various speeds (1,000, 1,500, or 2,000 rpm). During the first washing step, the slurry (≈40 mL) was passed through a screen (62 μm) to remove residual bran before centrifugation. The starch fraction was washed one additional time (for a total of three times) and freeze-dried. The dry starch was ground lightly with a mortar and pestle for further analysis of physicochemical properties.

For comparison to the sonication procedure, two other starch isolation methods were used. Starch was isolated with an enzymatic method using pepsin A (P7012, Sigma, St. Louis, MO), hemicellulase 90 (90,000 U/g activity, Amano Enzyme U.S.A., Lombard, IL), and a detergent mix (5% SDS, 5% Triton X-100, 5% Tween 40, and 5% Triton X-15) according to Bechtel and Wilson (2000). Starch was also isolated using a modified method of Zhao and Sharp (1996), which utilizes several washes with different buffers. For this method, we used the protein extraction buffer optimized for sorghum proteins (Park and Bean 2003) instead of the washing buffer used by Zhao and Sharp (1996) (55 mM Tris-Cl pH 6.8, 2.3% SDS, 5% β-ME) and substituted NaCl

for CsCl. Starch was washed after isolation by these methods as described for the sonication method.

Physicochemical Measurement

Protein (N × 6.25) content of the isolated starches was determined by combustion (Approved Method 46-30, AACC International 2000) using a nitrogen determinator (Leco Corp., St. Joseph, MI). Moisture content was measured using Approved Method 44-15A. Starch damage was measured using a colorimetric assay kit from Megazyme International, Bray, Ireland (Approved Method 76-31). Starch color where *L* was lightness; *a* was redness-greenness; and *b* was yellowness-blueness was determined using a chromameter (Minolta model CR-300). Thermogram values from differential scanning calorimetry (Diamond DSC, PerkinElmer, Wellesley, MA) were determined using ≈5 mg of starch with 20 μL of water with heating regime from 5 to 130°C at 10.0°C/min. Particle size distributions of isolated starches were determined using a laser diffraction particle size analyzer (Beckman/Coulter, Miami, FL).

Statistical Analysis

The study was conducted with a completely randomized design and samples were analyzed at least in duplicate. Least significant difference (LSD) was determined using statistical software (v. 8.0, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Effects of Different Reducing Agents and Sonication Times on Protein Content, Yield, and Color of Isolated Starch

Sorghum starches were isolated using different reducing agents and sonication times (Table I). Protein content, yield, and color of the starches were determined to evaluate the effectiveness of the isolation process. As a control to investigate the effects of sonication only, decorticated sorghum flour was sonicated for 2 min in water. As expected, after sonication, this starch fraction contained significant levels of protein (6.6%) and a low starch yield (61.7%). Considering the protein content (10.2%, data not shown) of the original flour, more than half of the protein still remained in the starch fraction. Starch yield was significantly low compared with other treatments, probably due to difficulties involved during the washing step that sieved out some aggregates of bran, starch, and protein that were not effectively separated during sonication.

To optimize the effectiveness of the sonication, a 12.5 mM sodium borate buffer, pH 10, and 2% SDS was used to enhance the removal of protein during processing. This buffer has been widely used to extract sorghum proteins and has been very effective (Hamaker et al 1995; Park and Bean 2003). Starch from this process had fair level of purity (0.68% protein), yield (69.5%),

TABLE I
Protein Content, Yield, and Color of Starches Isolated by Sonication with Different Reducing Agents and Reaction Times^a

Treatment ^c	Protein (% db)	Starch Yield (% flour, db)	Color ^b		
			<i>L</i>	<i>a</i>	<i>b</i>
Water, 2 min	6.6a	61.7c	88.0h	-0.67a	5.7a
12.5 mM sodium borate, pH 10					
2% SDS, 2 min	0.68b	69.5b	91.0f	-0.81b	3.8b
2% SDS + 2% β-ME, 2 min	nd	72.0ab	93.0d	-0.89c	2.9c
2% SDS + 2% DTT, 2 min	0.14c	72.9ab	93.1cd	-0.89c	2.9c
2% SDS + 2% Na ₂ S ₂ O ₅ , 1 min	0.70b	62.2c	93.9a	-0.84	3.7b
2% SDS + 2% Na ₂ S ₂ O ₅ , 2 min	0.09c	72.6ab	93.9a	-0.92cd	2.9c
2% SDS + 2% Na ₂ S ₂ O ₅ , 4 min	nd	73.6a	93.5b	-0.95d	2.5d
2% SDS + 2% Na ₂ S ₂ O ₅ , 6 min	nd	72.5ab	93.2c	-0.92cd	2.1e
2% SDS + 2% Na ₂ S ₂ O ₅ , 8 min	nd	72.0ab	92.4e	-0.93cd	2.0e
2% SDS + 2% Na ₂ S ₂ O ₅ , 10 min	nd	72.6ab	90.7g	-0.94d	1.8e

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$); nd, not detectable.

^b *L*, lightness; *a*, redness; and *b*, yellowness.

^c β-ME, β-mercaptoethanol; DTT, dithiothreitol.

and color values (91.0, -0.81, and 3.8 for *L*, *a*, and *b*, respectively) (Table I).

While the above procedure was effective using SDS alone with no reducing agent, it is widely known that sorghum storage proteins are highly cross-linked and need reducing agents to be effectively solubilized (Wall and Paulis 1978). Three reducing agents were evaluated: β -ME, dithiothreitol, and sodium metabisulfite at a concentration of 2% (w/v). All reducing agents were effective and produced good protein extraction, high yield, and starch with a bright color when used with sonication times of ≥ 2 min (Table I). The shortest sonication time tested (1 min) still produced starch with fairly low protein content and a good *L* value but had low starch yield (Table I). Among the three reducing agents tested, sodium metabisulfite was selected for further study as it was as effective as β -ME and dithiothreitol but had lower toxicity and less odor.

Various sonication times were tested up to 10 min. As sonication times increased, the temperature of the starch slurry gradually increased. At sonication times of ≤ 4 min, temperature in the slurry was $< 50^\circ\text{C}$. However, when the sonication time was extended to 10 min, the temperature was close to 60°C . After only 2 min of sonication, starch protein content was already close to 0%. After 4 min, protein content was undetectable but starch yield was not improved. On the contrary, starch color was darker after sonication times of ≥ 4 min. Very fine dark particles on top of the starch layer were observed during washing steps after the longer sonication times (4–10 min) and may be responsible for the darker starch color. The identity of this dark particulate layer is currently unknown. Based on these results, a 2-min sonication time was selected as optimum for obtaining starch with high purity, yield, and bright color.

Effects of Different Centrifugation Speed and SDS Concentration on Protein Content, Yield, and Color of Isolated Starch

Previous studies have found that SDS is effective at extracting sorghum proteins. As SDS concentration increases, the amount of protein extracted increases, up to a level of 2% SDS (Park and Bean 2003). In this study, lower concentrations of SDS were tested to determine whether lower SDS levels would still effectively extract sorghum proteins in combination with ultrasound. Lower SDS levels would decrease costs associated with isolating starch with this method.

In conjunction with testing different concentrations of SDS, different centrifugation speeds were tested during the starch washing process. During preliminary studies, we found that after starch

washing there was a dark layer on top of the prime starch, probably containing tailings and cell wall materials (this was different from the dark layer found on top of the centrifugate when using long sonication times). Separation of this layer from the prime starch was difficult and tedious, and reproducibility of the results was low. Therefore, various centrifugation speeds were tested that would give a good separation of prime starch from tailings without the need for scraping of the starch residue after sonication.

With the aid of ultrasound, high purity starch (0.06% protein), with good brightness (*L* value of 93.6) and yield (72.0%) could be produced using lower concentrations of SDS (0.5%) (Table II). When using 1 or 2% SDS, the protein content in starches was not detectable.

The effect of centrifugation speed on the starch purification process can be found in Table II. A centrifugation speed of 1,000 rpm did not produce good starch yields (60.5–67.7%), probably because the low speed was not effective in gathering some small starch granules from the supernatant. However, because most of tailings starch (dark color) remained in solution, the colors of starch washed at 1,000 rpm were brighter than color of starches obtained from higher centrifugation speeds. Protein contents were also slightly higher in the starch centrifuged at lower speeds compared with starches obtained from higher speeds. This is possibly due to higher speeds being more effective at packing the starch and reducing the residual buffer, which would be expected to contain solubilized protein, left in the starch. Thus, a moderate centrifuge speed of 1,500 rpm was selected as optimum.

Effects of Different Sodium Metabisulfite Concentration and Solvent-to-Flour Ratio on Protein Content, Yield, and Color of Isolated Starch

As described earlier, initial tests with different reducing agents were conducted and sodium metabisulfite was chosen as the reducing agent. For the same reasons that attempts to lower the SDS concentration were made, different concentrations of sodium metabisulfite were evaluated. Results showed that concentration of sodium metabisulfite could be reduced to 0.5% without loss of quality in starch purity (0.06% protein), yield (70.6%), and color (93.3, -0.85, and 3.1 for *L*, *a*, and *b*, respectively) (Table III).

For initial research, a solvent-to-flour ratio of 20:1 was used in this study based on previous research on the extraction of sorghum proteins (Park and Bean 2003). Starch yield and color were not significantly different among the different ratios investigated. However, the starch protein content was significantly higher for low solvent-to-flour ratio (10:1), regardless of concentration of sodium metabisulfite used.

TABLE II
Protein Content, Yield, and Color of Starches Isolated by Sonication with Different Centrifugation Speeds and SDS Concentrations^a

Treatment	Protein (% db)	Starch Yield (% flour, db)	Color ^b		
			<i>L</i>	<i>a</i>	<i>b</i>
2% Na ₂ S ₂ O ₅ 12.5 mM sodium borate, pH 10, 2 min					
1,000 rpm (201 × g)					
0% SDS	8.0a	67.7cd	91.8fg	-1.55h	7.2a
0.5% SDS	0.71c	60.5e	93.9ab	-1.05e	3.8d
1% SDS	0.12d	62.1de	94.1a	-1.00d	3.3e
2% SDS	0.12d	64.0cd	94.0a	-0.99dc	3.2ef
1,500 rpm (453 × g)					
0% SDS	6.6b	71.9a-c	92.1f	-1.19f	5.4c
0.5% SDS	0.06d	72.0ab	93.6d	-0.92a	2.9fg
1% SDS	nd	70.1a-c	93.3e	-0.94ab	2.9fg
2% SDS	nd	72.8ab	93.4de	-0.96bc	2.8g
2,000 rpm (805 × g)					
0% SDS	8.2a	73.7a	91.5g	-1.40g	6.4b
0.5% SDS	0.08d	70.4a-c	93.6cd	-0.94ab	3.0e-g
1% SDS	nd	71.5a-c	93.7b-d	-0.94ab	2.8g
2% SDS	nd	70.2a-c	93.9ab	-0.94ab	2.8g

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$); nd, not detectable.

^b *L*, lightness; *a*, redness; and *b*, yellowness.

Therefore, the optimum conditions for sorghum starch isolation using sonication were a 12.5 mM sodium borate buffer, pH 10, containing 0.5% SDS (w/v) and 0.5% sodium metabisulfite (w/v), with 20:1 solvent-to-flour ratio, sonicated for 2 min, and then washed three times with water at a centrifugation speed of 1,500 rpm for 2 min.

Comparison of Three Different Starch Isolation Methods on Protein Content, Yield, Damaged Starch, Color, and DSC Thermogram Values

Sorghum starch was isolated with two more different methods, an enzymatic method (Bechtel and Wilson 2000) and a chemical buffer method (Zhao and Sharp 1996). Results were compared with starch isolated using the optimum sonication method described above (Table IV). Protein content of starch isolated with the sonication method was significantly lower (0.09%) than the starch isolated by the enzymatic method (0.68%) and the chemical buffer method (0.42%). Starch yield for the sonication method was also higher than that by obtained with the chemical buffer method. Starch damage was lower for the enzymatic method compared with the other two methods. As explained below (Fig. 1), it is speculated that, slight swelling of starch granules probably caused by sonication and long steeping process (chemical buffer method) provides more surface area for enzymatic hydrolysis, resulting in higher starch damage level. Regarding starch color, *L* values were generally similar for all starches, whereas the *b* value of starch prepared by sonication was significantly lower than the other two methods, thus giving a whiter or lighter appearance to the starch.

The DSC thermogram values including onset temperature (T_o), peak temperature (T_p), and energy required for gelatinization (ΔH) showed that all starches had similar thermogram properties. Starch isolated by sonication had slightly lower T_o and T_p and higher ΔH compared with the other starches but were not significantly different (Table IV). It appears that 2 min of sonication did not alter the crystalline structure of starch. It has been reported that residual protein content (Lim et al 1999; Wang and Wang 2001) and SDS (Zhang and Hamaker 1999) in starch may affect the thermal properties of starch granules. This may infer that the three starches had similar levels of residual protein and SDS but did not have different thermal behavior.

Starch granule size distributions of starches isolated by the three different methods were analyzed and compared (Fig. 1). The starch granule number distribution of sizes showed almost identical curves among the different starches. Thus, the experimental processes used including washing steps (centrifugation and decanting) were reproducible and, most importantly, sonication did not alter the size distribution by disrupting granules, otherwise the number of small granules would have increased.

On the other hand, starch granule volume distributions isolated by sonication and chemical buffer (long steeping process) methods showed a small shift to the right compared with starches isolated from the enzymatic method. It is speculated that even though 2 min of sonication under 50°C did not disrupt the granules, the sonication process seemed to make the starch granules slightly swell. Long steeping process also seem to make starch granules slightly swell.

TABLE III
Protein Content, Yield, and Color of Starches Isolated by Sonication with Different Sodium Metabisulfite Concentrations and Solvent-to-Flour Ratios^a

Treatment	Protein (% db)	Starch Yield (% flour, db)	Color ^b		
			<i>L</i>	<i>a</i>	<i>b</i>
0.5% SDS 12.5 mM sodium borate, pH 10, 1,500 rpm, 2 min					
20:1 solvent-to-flour ratio					
0% Na ₂ S ₂ O ₅	0.32c	63.2b	92.4c	-0.83a	4.0a
0.5% Na ₂ S ₂ O ₅	0.06d	70.6a	93.3b	-0.85ab	3.1bc
1% Na ₂ S ₂ O ₅	nd	70.1a	93.4ab	-0.87ab	3.0c
2% Na ₂ S ₂ O ₅	nd	70.4a	93.6a	-0.88b	3.2c
10:1 solvent-to-flour ratio					
0.5% Na ₂ S ₂ O ₅	0.86a	69.7a	93.4ab	-0.85ab	3.5b
1% Na ₂ S ₂ O ₅	0.75ab	69.6a	93.2b	-0.84a	3.4b
2% Na ₂ S ₂ O ₅	0.66b	70.1a	93.5a	-0.86ab	3.3bc

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$); nd, not detectable.

^b *L*, lightness; *a*, redness; and *b*, yellowness.

TABLE IV
Protein Content, Starch Yield, Starch Damage, Starch Color, and Differential Scanning Colorimetry (DSC) Thermogram Values of Starches Separated by Three Different Methods^a

Property	Sonication + Extraction Buffer A ^b	Protease + Detergent Mix ^c	NaCl Solution + Extraction Buffer B ^d
Protein (% db)	0.09c	0.68a	0.42b
Starch yield (% flour, db)	72.6a	70.1ab	68.8b
Starch damage (% as is)	8.4a	6.3b	8.2a
Starch color ^e			
<i>L</i>	93.9ab	93.7b	94.1a
<i>a</i>	-0.92a	-0.79b	-0.91a
<i>b</i>	2.9b	4.2a	4.4a
DSC thermogram values ^f			
T_o (°C)	59.0a	61.3a	61.7a
T_p (°C)	70.9a	71.5a	72.0a
ΔH (J/g)	12.4a	10.7a	10.7a

^a Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

^b 0.5% SDS + 0.5% Na₂S₂O₅ + 12.5 mM sodium borate, pH 10.

^c 5% SDS + 5% Triton X-100 + 5% Tween 40 + 5% Triton X-15.

^d 2% SDS + 2% β-mercaptoethanol + 12.5 mM sodium borate, pH 10.

^e *L*, lightness; *a*, redness; and *b*, yellowness.

^f T_o temperature of onset; T_p temperature of peak; and ΔH amount of energy required for gelatinization.

Overall, the optimized sonication method produced starch that had significantly higher purity with slightly better yields and color values, and with comparable starch damage, DSC thermogram, and starch granule size distribution in only a fraction of the time compared with other starch isolation methods which required 5–24 hr+ (Zhao and Sharp 1996; Bechtel and Wilson 2000) and wet milling, which requires 24–96 hr (Wang et al 2000; Higiro et al 2003). The current methodology was faster than the previous reports that utilized sonication to isolate starch from rice (Wang and Wang 2004).

Expansion of the Method for Whole Meal Sorghum

Initial work to isolate starch from sorghum was conducted using decorticated sorghum flour, the equivalent of wheat flour. However, the decortication process requires additional steps, as does milling of wheat. The use of whole meal sorghum as a starting material would further reduce the time required to obtain pure starch. However, preliminary experiments with very fine whole meal from undecorticated red sorghum, ground using a Udy mill equipped with a 0.25-mm screen, showed that the sonication method for starch isolation produced brownish starch due to the higher amounts of fine bran particles remaining in the starch. An additional study was conducted on the effect of particle size of the starting material on the properties of isolated starch from whole meal sorghum. The optimized sonication method was used to isolate starch from whole grain sorghum that was ground to different particle sizes (Table V). Protein ($N \times 6.25$) and ash in the starches had ranges of 0.4–0.6% (db, data not shown) and showed no trend for the different samples. Undecorticated sorghum milled on a Udy mill with 0.25-mm screen resulted in unacceptable starch. This starch had a visibly brown color that was reflected in the low *L* value and high *a* and *b* values. Samples coarsely milled produced fairly low starch yields (only 45%, flour basis, data not shown) most likely because the starch could not be removed from the very large particles by ultrasound. Whole meal samples with intermediate particle size produced starch with properties between these

two extremes. Milling whole grains with a Udy mill with a 1-mm screen appears to be a good compromise for isolating starch from whole meal flours. The starch yield ($\approx 70\%$, flour basis, data not shown), was acceptable and, at the same time, the color was sufficiently white. Note that for the whole meal test, a sorghum with a red pericarp was intentionally selected for testing as this represents the an extreme case for producing white starch. Pigments from red sorghums tainted color of the starch produced from sorghum (Subramanian et al 1994; Xie and Seib 2000).

When using sorghum decorticated to a level where most of the bran is removed, the pericarp is removed before processing, thus the pigments in the outer layer of the grain have little effect on the final starch color. This, of course, depends on the degree of decortication. For highest purity starch with the brightest color, the use of decorticated flour is recommended.

Application of Process to Different Cereals

The optimized method for isolation of sorghum starch was applied to different cereal grains including wheat, corn, rice, and barley to test the general effectiveness of the method. Starch from wheat meal showed a low residual protein and bright color, and starch isolated from wheat flour had high purity and bright color (Table VI). Starch was also effectively isolated from corn whole meal with a low residual protein and bright color. On the other hand, starch from paddy rice contained relatively high residual protein and less bright color. Ground hulls, which covered the outside of the paddy rice, appeared to hinder the isolation process. Starch from dehulled or polished rice would be expected to be of high purity and brightness, similar to that of wheat. Barley starch contained a low residual protein, but the color was darker and yellowish. Overall, the optimized process worked well on the ground cereals, suggesting that this process could be applied to starch isolation from a broad range of cereals without much modification. Buffers optimized for extracting proteins specific to the other cereals may produce better results when used with the sonication method.

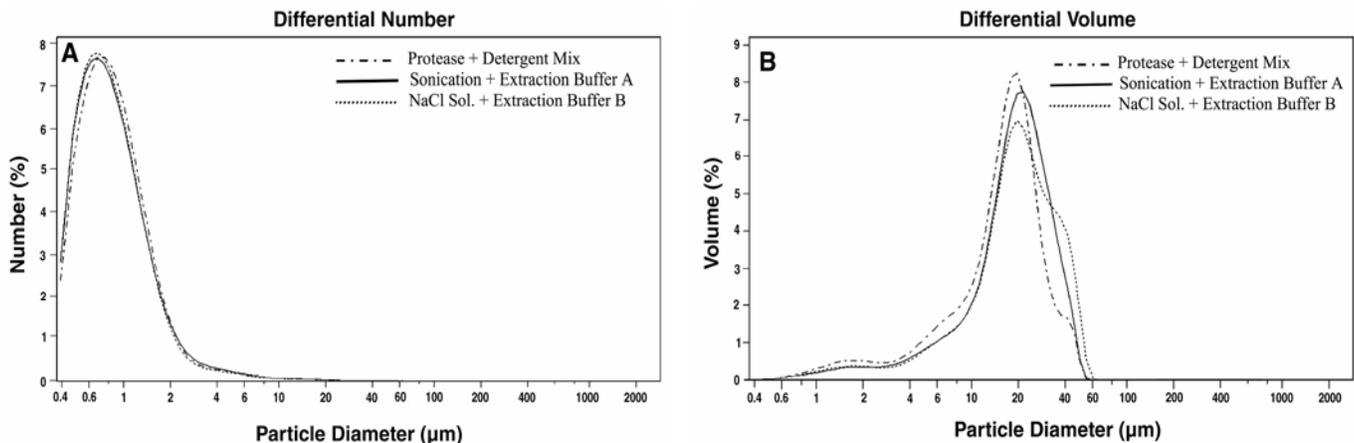


Fig. 1. Starch particle size (A, number and B, volume) distribution by three different isolation methods.

TABLE V
Particles Sizes of Whole Meals from Undecorticated Sorghum and Color of Isolated Starches^a

Milling Procedure	Particle Size Distribution (vol %)			Color ^b		
	<100 µm	100–1,000 µm	>1,000 µm	<i>L</i>	<i>a</i>	<i>b</i>
Coarse, Falling No. level 6	3.6e	43.6d	52.8a	91.0a	0.03bc	2.57c
Middle, Falling No. level 2	7.2d	88.8a	4.0b	91.3a	0.00c	2.70c
Fine, Falling No. level 0	14.5c	84.2b	1.3c	90.0b	0.11b	3.33b
Udy, 1-mm screen	23.2b	73.6c	3.3b	89.7b	0.11b	3.54b
Udy, 0.25-mm screen	58.6a	41.4e	0.0d	85.4c	0.93a	5.67a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^b *L*, lightness; *a*, redness; and *b*, yellowness.

TABLE VI
Protein Content and Color of Starches of Different Cereals Isolated by Sonication with Protein Extraction Buffer A^{a,b}

Cereals	Protein (% db)	Color ^c		
		<i>L</i>	<i>a</i>	<i>b</i>
Wheat	0.12c	92.3c	-0.35a	3.90b
Wheat flour	nd	94.8a	-0.71b	0.38d
Corn	0.35b	94.3b	-1.35c	2.37c
Paddy rice	0.80a	91.2d	-0.86b	3.18b
Barley	0.15c	89.3e	-0.32a	5.52a

^a 0.5% SDS + 0.5% Na₂S₂O₅ + 12.5 mM sodium borate pH 10.

^b Values followed by the same letter in the same row are not significantly different ($P < 0.05$); nd, not detectable.

^c *L*, lightness; *a*, redness; and *b*, yellowness.

CONCLUSIONS

Using ultrasound in combination with buffers optimized to extract sorghum proteins was an effective method for rapidly isolating sorghum starch. With a 2-min sonication treatment followed by washing in water, starches could be isolated with almost no detectable protein and good brightness values. Starch isolated by the sonication method was similar in its physicochemical properties to starch isolated using enzymatic and chemical buffer methods of isolation. The use of sonication to rapidly isolate starch should prove useful for laboratories characterizing starch chemistry as well as for industries interested in scaling up such a process.

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