Laboratory Procedure to Wet-Mill 100 g of Grain Sorghum into Six Fractions[1]

X. J. Xie[2] and P. A. Seib[2,3]

ABSTRACT

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A small-scale (100 g of grain) procedure was developed to wet-mill grain sorghum into six fractions by modifying the procedure of Eckhoff et al (1996). The wet-milling process was repeated five times on commercial grain sorghum, and the mean yield (69.4%) of starch (≤0.3% protein) varied by 0.3%, whereas the yields of fiber, gluten, and germ plus bran fractions varied by 5–6%. The starch fraction accounted for ≈95% of that in the grain, while the total solids recovered was 99.0%. Four other samples of grain sorghum gave 92–95% recoveries of starches and 98.2–99.8% recoveries of total solids. All grain sorghum starches had lightness (*L**) values and pasting curves nearly equal to those of a commercial maize starch.

Grain sorghum is a cereal crop grown chiefly in regions of Asia, Africa, and North America. Its starch and protein are similar to those in maize, but grain sorghum is more tolerant than maize to semi-arid and cool climate conditions. Grain sorghum is used for food (Murty and Kumar 1995) and feed (Bramel-Cox et al 1995), and for a few industrial products such as ethanol, wall board, and loose-fill packaging material.

Wet-milling of grain sorghum is rarely done industrially, mostly because grain sorghum's germ is small and its endosperm contains cross-linked proteins and proteinaceous peripheral cells (Watson et al 1955, Watson 1984, Hamaker et al 1992, El Nour et al 1998). Some grain sorghums have 3–4% of starch encased in the middle layer of the pericarp, some have pigments that discolor the starch, and some contain starch with pastes that undergo excessive shear-thinning (Watson 1970, Subramanian et al 1994a,b). The 3–4% starch in the mesocarp occurs as tiny (4–5 µm) granules that are difficult to recover. Moreover, the starch in the mesocarp may cause some shattering of the pericarp into particles that interfere with separation of protein from starch (Freeman and Watson 1969).

Except for the size of the germ, and possibly shear-thinning, the other negative factors can be circumvented by choice of raw material. When contrasted to wet-milling of maize, several factors favor wet-milling of grain sorghum. Grain sorghum sells at ≈10% less than maize in the United States, and its kernels are small and should hydrate quickly during steeping (Fan et al 1963). Davis et al (1990) presented evidence that the oil in grain sorghum germ may be changed readily to a high oleic type. If accomplished, the high oleic germ would represent a high value added coproduct when wet-milling grain sorghum for starch.

Several investigators have devised laboratory tests to predict the wet-milling quality of grain sorghum starting with 300–1,800 g samples (Zipf et al 1950; Watson and Hirata 1954; Watson et al 1955; Norris and Rooney 1970; Watson 1970; Buffo et al 1997, 1998; Moheno-Perez et al 1999). We chose the 100 g sample size used by Eckhoff et al (1996) for maize, and modified the steeping step and the coarse grinding and sieving conditions to isolate six frations. Our principal interests were the recoveries of both starch and total solids, the repeatability of the wet-milling test, and the purity of the starch as well as its quality compared with commercial maize starch.

MATERIALS AND METHODS

Materials

Five samples of grain sorghum were used: two commercial grain sorghums, one obtained in 1996 and the other in 1998, from ADM Milling Co., Dodge City, KS; a white grain sorghum (Golden Harvest 388 WC) from Golden Harvest, Waterloo, NE; a red grain sorghum (Pioneer 8500) from Pioneer Hybrid International, Johnston, IA; and white food-grade sorghum (ATx631 × RTx436) from Barney Gordon, North Central Experimental Field, Belleville, KS. The grain was cleaned by hand to remove foreign material and broken kernels (1–2%) and then stored at room temperature. All the chemicals were reagent grade.

Coarse grinding of steeped sorghum grain was done on a Waring blender (1L, model 34BL97, Dynamic Corp. of America, New Hartford City, CT) fitted with a tachometer and a stainless steel agitator. The agitator had four blades with a pitch of 40° that were 3.15 cm long, 1 cm wide, and 0.3 cm thick. Fine grinding was done on a plate mill (Quaker City, model No. 4E, Straub Co., Warminster, PA). The new plates (type 4B) were conditioned before use by grinding them down under applied pressure (audible loading of the motor) for ≈105 hr with constant running water to cool the plates (Eckhoff et al 1996). Sieves were No. 16 and 200 wire mesh from Fisher Scientific Company. The starch table was an aluminum I-beam, which, when turned 90°, formed a channel that was 244 cm long, 5.08 cm wide, and 2.54 cm high and had rounded internal corners. An E-series pump (Cat. No. 72-410-014, Manostat Corp.) was used for pumping a starch slurry onto the starch table that was positioned with a slope of 1.0%.

General Methods

Moisture was determined by oven drying at 130°C for 1 hr according to AACC Approved Method 44-15A, and fat and ash were determined by AACC Approved Methods 30-25 and 08-01, respectively (AACC 2000). Protein content was assayed by an protein-nitrogen analyzer (FP 2000, Leco Corporation, St. Joseph, MI), and total starch and damaged starch were determined with kits from Megazyme (Bray, Ireland) by AACC Approved Methods 76-12 and 76-31, respectively. Lightness (*L**) and yellowness (*b**) were measured on a chromameter (Minolta Co., Ltd., Osaka, Japan), and pasting curves were measured on a Rapid Visco Analyser (RVA) (Newport Scientific, Syndey, Australia) at 8.6% starch solids in water. Hardness of grain sorghum was determined by the method of deFrancisco et al (1982) with some modification. Grain (50 g) was ground once through a plate attrition mill at the closest setting (model 275, Hobart Mfg. Co., Troy, OH). The ground material was sieved for 1 min on a Tyler Ro-Tap shaker equipped with a No. 20 wire screen (opening of 841 µm). The higher the percent overs, the harder the grain.

Wet-Milling of Grain Sorghum

Grain sorghum 100 g (dry basis) was added to 200 mL of water containing 0.32% sodium bisulfite (2,000 ppm of SO_2) and 0.55% lactic acid in an Erlenmeyer flask. The flask was stoppered and placed in a water bath at 52°C, and after 24–96 hr (48 hr recommended), the steep liquor was decanted. Sorghum kernels were washed with 350 mL of water, and the washings and steep liquor were combined and concentrated at <60°C under vacuum to a thick

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[1] Contribution 99-490-J from the Kansas Agricultural Experiment Station, Manhattan KS 66506.

[2] Dept. of Grain Science and Industry, Kansas State University, Manhattan KS 66506.

[3] Corresponding author: Fax: 785/532/7010. E-mail: pas@wheat.ksu.edu

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syrup. The syrup was dried to constant weight in a forced-draft oven at 60°C. Water (150 mL) and the steeped sorghum (≈150 mL) were placed in the Waring blender, and the mixture was coarsely ground at <40°C and 9,400–9,500 rpm for 6 min or at 7,500 rpm for 15 min. The slurry was poured onto a No. 16 wire sieve (opening 1,190 μm), allowed to drain for ≈5 min, and the overs washed with a fine stream of water (800 mL). Before complete drainage of the liquid phase from the sieves, the overs were transferred into a beaker, stirred by hand, and resieved. The overs were washed with a fine stream of water (200 mL), drained, and dried at 60°C to give the bran plus germ fraction.

The combined throughs were allowed to settle for 30 min, and the supernatant (≈940 mL) was decanted and saved as supernatant 1. The bottom phase containing ≈30 wt% solids was ground finely by one pass through the plate mill operated with audible loading, and the mill was rinsed with water (250 mL). The ground mixture was filtered through a No. 200 wire sieve (73 μm) to remove fine fiber, and the fiber was washed with supernatant 1 followed by water (750 mL) and then dried at 60°C. The washings of the fine fiber and the throughs from the No. 200 screen were combined, and the mixture was allowed to stand for 1 hr, after which time the clear supernatant (≈1.8L) was decanted and saved as supernatant 2. The specific gravity of the bottom phase was adjusted to 1.04 at 25°C by adding a portion of supernatant 2, and the resultant slurry was stirred constantly while it was pumped onto the starch table at a flow rate of 50 mL/min. The remainder of supernatant 2 was then pumped onto the starch table at the same rate followed by water (150 mL). The overflow from the starch table was centrifuged at 3,000 × *g*, and the sedimented protein fraction was collected and dried at 60°C. The supernatant was dried to produce the process-water solubles. The starch was allowed to dry overnight on the table, and the amount of starch on the table was determined by weighing. The starch was removed with a plastic scraper, and its moisture content determined.

Statistics

Analysis of variance with a *t*-test was performed using a completely randomized design according to the general linear model procedure (SAS Institute, Inc. Cary, NC). Means were compared by least significant difference at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The five samples of grain sorghum all had low or no condensed tannins. The two commercial sorghums had thin pericarps, and the white and red hybrids were medium thick. The food-grade white sorghum had a thick pericarp because of its thick mesocarp layer, which probably contained a low percentage of starch (Serna-Saldivar and Rooney 1995). Protein in the samples varied from 10.3 to 13.9%, starch from 68 to 73%, and grain hardness from 44 to 57% (Table I). The food-grade white sorghum, which was harvested from plants with nonpigmented glumes, contained the hardest kernels.

Wet-Milling

The small kernel size of grain sorghum, compared with maize, suggested the need for changes in three process steps: steeping of the grain, coarse grinding of the steeped grain in water, and separation of the bran and germ fraction from the coarsely ground grain. The remaining steps in the wet-milling test of grain sorghum (Fig. 1) were conducted essentially as reported by Eckhoff et al (1996) for maize.

A series of tests were done on thoroughly steeped (48 hr at 52°C) commercial (1996) grain sorghum to establish the best coarse-grinding conditions in the Waring blender. Eckhoff et al (1996) reported the volume ratio of steeped grain to water should be 1:1 for coarse grinding of steeped maize. We found the same optimum ratio of steeped grain sorghum to water (data not shown). In addition, we varied grinding times from 6 to 15 min, and grinding speeds from 7,500 to 10,000 rpm giving blade tip speeds of 25.1–33.5 ms^{-1} (90.4–121 Km/hr). At a grinding speed of 7,450 ± 50 rpm, intact kernels remained if the grinding time was too short, but after 15 min, no kernels survived and starch recovery leveled off at ≈95% (Fig. 2). The protein level of all four starches was 0.3%. The consistently low level of protein contamination was attributed to excellent fractionation on the starch table. After coarse grinding for 15 min at 7,450 ± 50 rpm, the temperature of the slurry reached ≈40°C, which is one variable accounting for low damage in the isolated starch.

If the time of coarse grinding was set at 6.0 min, a speed of >9,500 rpm was required to release 95% of the starch (Fig. 3), again with all isolated starches contaminated with 0.3% protein.

TABLE I
Composition of Grain Sorghums, Starch Recovery, and Starch Protein Content and Color

	Grain					Starch				
Grain	Pericarp Color	Hardness[a] (%)	Protein (%)	Fat (%)	Ash (%)	Starch Recovery (%)	Protein (%)	Damaged (%)[b]	Lightness[c] (*L**)	Yellowness[c] (*b**)
Mix (1996)	44	10.3	3.3	1.6	73.0	95.0	0.3	0.3	94.7	2.4
Mix (1998)	45	10.0	3.0	1.4	71.9	95.0	0.3	0.3	94.9	2.3
Red	50	13.9	3.4	1.5	69.6	92.5	0.3	0.3	94.5	2.4
White	50	13.4	3.4	1.7	69.2	93.8	0.3	0.5	94.9	2.1
White (food grade)	57	10.4	2.8	1.1	68.2	91.9	0.4	0.4	94.a	1.8

[a] Hardness least significant difference ($P < 0.05$).($\alpha = 0.05$) = 1.6
[b] Damaged starch of commercial corn starch 0.64%/
[c] Lightness of commercial corn starch 95.2, yellowness 4.2/

TABLE II
Yields of Fractions, and Protein, Starch, and Solids Recovery for Wet-Milling of Commercial (1996) Grain Sorghum (100g dry solids)

				Recovered in Fractions			
				Protein[b]		Starch[b]	
Fraction	Solids Isolated[a] (g)	Protein Level[a] (%)	Starch Level[a] (%)	g	(%)	g	(%)
Germ and bran	8.3 ± 0.4	15.2 ± 1.1	10.0 ± 0.7	1.3	12.6	0.8	1.4
Fine fiber	4.8 ± 0.3	28.1 ± 1.4	26.3 ± 1.0	1.3	12.6	1.3	1.8
Gluten	8.5 ± 0.4	58.2 ± 1.4	17.9 ± 1.1	4.9	47.5	1.5	2.1
Starch	69.4 ± 0.2	0.3 ± 0	· · ·	0.3	0.1	69.1	94.7
Process water solubles	2.0 ± 0.4	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
Steep liquor solids	5.6 ± 0.1	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
Total	98.9 ± 0.7 g			7.8 g	(75.7%)	72.7 g	(99.5%)

[a] Means and standard deviations of five replicates. Data on dry solids basis.
[b] Means of duplicate assays.

After 6 min of grinding at 9,450 ± 50 rpm, the temperature of the slurry reached ≈35°C.

Starch recovery increased from 90 to 95% when the steep time for one part of grain sorghum in two parts of steep liquid was increased from 24 to 48 hr. No further increase was observed when steep time was increased from 48 to 96 hr. Yang and Seib (1996) found that grain sorghum gained weight during steeping almost at the same rate as dent maize, in spite of the small size of the sorghum kernels. However, the distribution of water in the kernels was not determined. Apparently water moves quickly through the tip caps of kernels in both grains, and then throughout the cross and tube cells in the endocarp. Fan et al (1963) reported that the rate of diffusion of water in grain sorghum (one sample tested) within the first 2 hr of steeping at 0–100°C, was ≈60% of the rate in dent maize ($D = 0.06\ cm^2s^{-1}$ vs. $0.11\ cm^2s^{-1}$), but that the volume of a single kernel of grain sorghum was ≈6% of a volume of a single maize kernel. Presumably then, the endosperm in the small (25–35 mg) kernels of grain sorghum hydrates in a shorter time than in the larger (170–367 mg) kernels of dent maize, even though the difference in kernel sphericities would reduce the advantage.

Repeatability of Wet-Milling Test on Commercial Grain Sorghum

The wet-milling test was repeated five times on commercial grain sorghum, and the average yields of the six fractions are given in Table II. Recoveries were ≈99% for total solids, ≈99.5% for total starch, and ≈76% for total protein. The coefficient of variation on starch recovery was 0.3%, which was the same as that reported by Eckhoff et al (1996) for recovery of maize starch in their process. The coefficients of variation on the recoveries of other fractions were ≤6%, except for process-water solubles. Protein recovered in the gluten fraction was 48% of grain sorghum protein, whereas starch in the gluten fraction accounted for 2% of that in the grain. The repeatabilities of the yields of the protein fraction and the germ and fiber fraction from maize reported by Eckhoff et al (1996) were somewhat better than those that we found for grain sorghum.

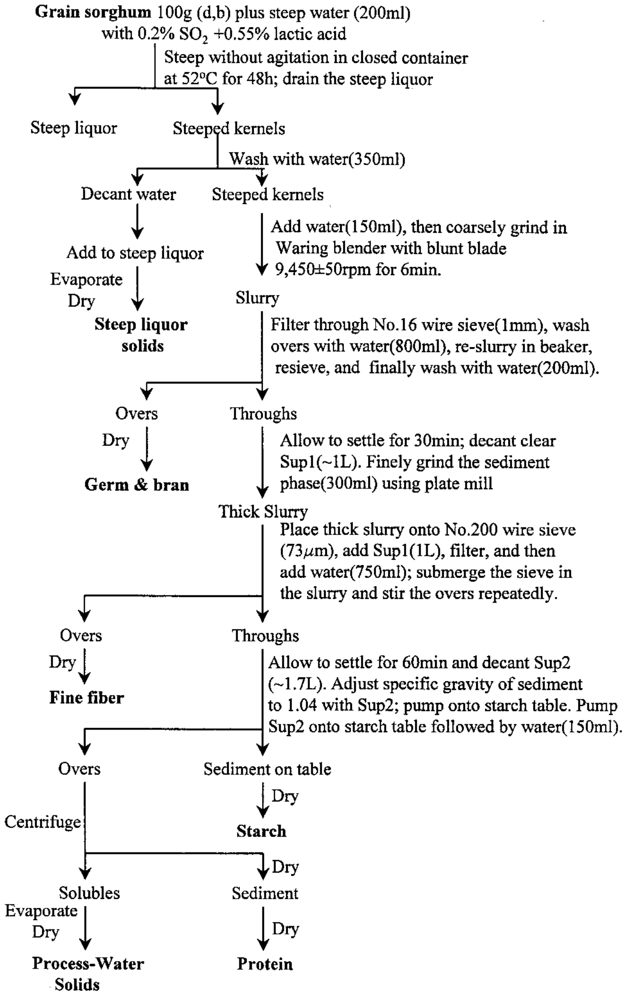

Fig. 1. Exhaustive wet-milling of grain sorghum to give six fractions.

Wet-Milling of Other Grain Sorghums and Properties of Sorghum Starch

Using the steeping and coarse-grinding conditions derived for the 1996 commercial grain sorghum sample, three other grain sorghums and a 1998 commercial grain sorghum sample were wet-

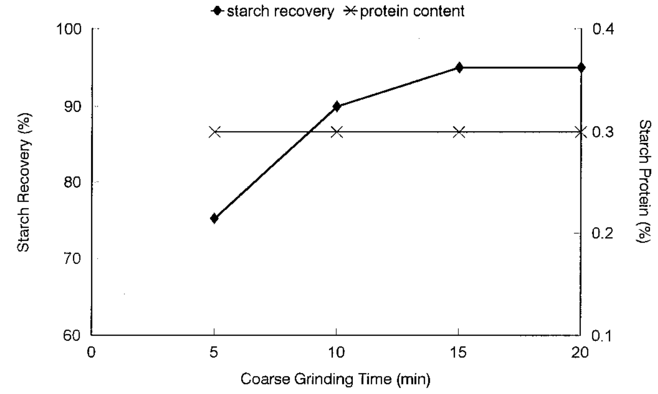

Fig. 2. Starch recovery and protein in starch from steeped (0.2% SO_2, 0.6% lactic acid, 52°C, 48 hr) commercial (1996) grain sorghum (100 g of dry solid) ground at 7,500 rpm with 150 mL of water for different times.

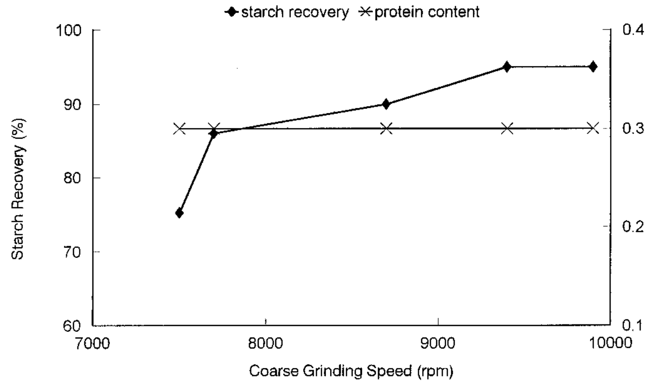

Fig. 3. Starch recovery and protein in starch from steeped (0.2% SO_2, 0.6% lactic acid, 52°C, 48 hr) commercial (1996) grain sorghum (100 g of dry solid) ground for 6 min with 150 mL water at different speeds.

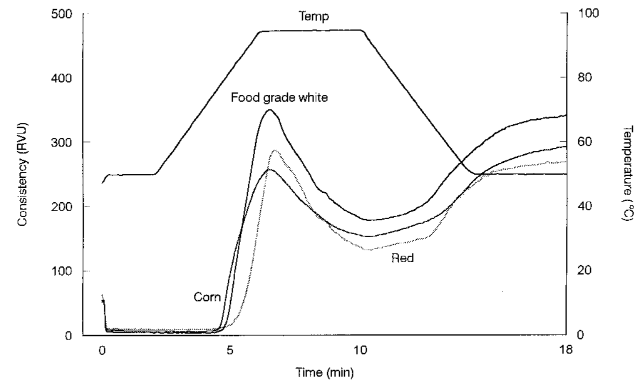

Fig. 4. Pasting curves (8.6% starch solid) of commercial corn starch and sorghum starches.

milled by the process outlined in Fig. 1. Recoveries of starches and some properties of the starches are shown in Table I. The 1996 and 1998 commercial grain sorghums had almost the same composition and hardness, and the starch recoveries, protein levels, and properties were the same for both. The commercial grain sorghum had the softest kernels and gave the highest starch recovery (95%), whereas the food-grade white sorghum with the hardest kernels and probably starch in its mesocarp gave the lowest recovery (92%). The elevated (0.4%) protein in the starch from the food-grade sorghum also supports the suggestion of starch in its mesocarp. A starchy mesocarp would likely fragment to give tiny pieces of pericarp that interfere with starch purification (Freeman and Watson 1969). The level of damaged starch in every sample was ≤0.5%,which was lower than the 0.6% found in commercial corn starch, and lightness (*L**) was high and similar to commercial corn starch. Thus, pericarp pigments in the red and yellow kernels did not discolor the starch during wet-milling, and the grinding steps did not create excessive starch damage.

The 92–95% recovery of pure starch from grain sorghum found in this work was higher than the 70–90% reported by others (Zipf et al 1950, Watson and Hirata 1954, Watson et al 1955, Norris and Rooney 1970, Buffo et al 1998), and the contamination of starch by protein (≈0.3%) was usually below the levels previously reported (0.2–0.9%). Contamination by lipid also was low as indicated by negligible ether extractables. The recovery of solids after wet-milling the five grain sorghum samples was 98–99% which was about equal to the 97–98% recoveries reported by Watson (1970) and Zipf et al (1950). The excellent recoveries of starch and grain solids were due to care in steeping, coarse grinding, and sieving.

Upon setting up the small-scale wet-milling system, and before wet-milling of grain sorghum, a commercial sample (U.S. No. 2 grade) of yellow dent corn (100 g) was wet-milled five times on the equipment using the procedure of Eckhoff et al (1996), except for manually sieving and washing the coarse ground material rather than by machine. Our results showed 95 ± 0.3% recovery of starch with 0.3% protein and 99.8 ± 0.1% recovery of total solids, comparable to the results of Eckhoff et al (1996).

The RVA pasting curves of three of the five sorghum starches at 8.6% solids in water were similar to that of commercial corn starch, whereas the other two were somewhat deviant (Fig. 4). The starch from the red grain sorghum showed a delayed pasting temperature, whereas that from the food-grade grain sorghum gave a higher pasting consistency. The pasting curves for the white and two commercial grain sorghum starches were omitted from Fig. 4 for clarity.

CONCLUSIONS

A wet-processing procedure was devised for 100 g of grain sorghum that gave six fractions with practically quantitative recovery of grain solids. The starch fraction contained a low level of protein (≤0.3%), and 92–95% of the starch in the grain was recovered. Starches from four U.S. hybrids of grain sorghum and that from commercial grain sorghum had lightness in color, and pasting curves almost equal to commercial corn starch. The laboratory wet-milling procedure can be used to determine starch yield and purity for different steeping conditions on grain sorghum.

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Erratum

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On page 393, Table I should be:

TABLE I
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	Grain					**Starch**				
Pericarp Color	**Hardness[a] (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Starch (%)**	**Recovery (%)**	**Protein (%)**	**Damaged[b] (%)**	**Lightness[c] (L^*)**	**Yellowness[c] (b^*)**
Mix (1996)	44	10.3	3.3	1.6	73.0	95.0	0.3	0.3	94.7	2.4
Mix (1998)	45	10.0	3.0	1.4	71.9	95.0	0.3	0.3	94.9	2.3
Red	50	13.9	3.4	1.5	69.6	92.5	0.3	0.3	94.5	2.4
White	50	13.4	3.4	1.7	69.2	93.8	0.3	0.5	94.9	2.1
White (food grade)	57	10.4	2.8	1.1	68.2	91.9	0.4	0.4	94.a	1.8

[a] Hardness least significant difference ($P < 0.05$) • ($\alpha = 0.05$) = 1.6.
[b] Damaged starch of commercial corn starch 0.64%.
[c] Lightness of commercial corn starch 95.2, yellowness 4.2.