

Optimum Steeping Process for Wet Milling of Sorghum¹

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ABSTRACT

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Pioneer 8500, a red hard sorghum hybrid, was steeped batchwise using three steeping solutions at 50°C: SO₂ solution; SO₂ solution containing 1.25% (w/w) of a commercial multiple-enzyme preparation (Novo SP249); and SO₂ solution with the addition of 0.5% (w/w) lactic acid. Novo SP249 contained pectolytic, cellulolytic, hemicellulolytic, and proteolytic activities and small amounts of saccharolytic activities. Three SO₂ concentrations (0.1, 0.2, and 0.3% w/v) prepared by dissolving sodium bisulfite in distilled water and three steeping times (24, 36, and 48 hr) were used. Incorporation of multiple enzymes into the SO₂ resulted in an increase in starch yield with reduced protein content compared with the SO₂ solution alone. The best wet-milling performance for sorghum resulted from the SO₂ solution containing 0.5% lactic acid; it produced the whitest

starch with the highest yield and the lowest protein content. Both higher SO₂ concentration of the steeping solution and longer steeping time led to higher starch yield, lower protein content in starch, and whiter starch. However, no significant differences in starch yield, protein content in starch, and starch color occurred between SO₂ concentrations of 0.2 and 0.3% for all three steeping solutions. The optimum steeping process for wet milling of sorghum was using a 0.2% SO₂ solution with 0.5% lactic acid for 36 hr at 50°C. Under these conditions, the starch yield, protein content in starch, and *L* value of starch color were 60.2% (db), 0.49% (db), and 92.7, respectively, which were not significantly different from the best values from the 48-hr steeping using the solution with 0.3% SO₂ and 0.5% lactic acid.

Sorghum ranks fifth among cereal crops, with 59 million metric tons of total world production in 1987 (FAO 1988). The major producing centers are in Asia and Africa (47% of total production), although ≈32% of the world sorghum production is grown in the United States. The structure and composition of sorghum are generally similar to those of corn. Approximately 82% endosperm, 10% germ, and 8% bran with 73.8% starch, 12.3% protein, 3.6% fat, and 1.65% ash on a dry basis (Rooney and Clark 1968). Sorghum is the major food staple in many developing countries. In the United States, sorghum is used mainly as livestock feed, with only a small quantity grown for industrial and food purposes (Hoseney et al 1974). A plentiful supply of sorghum at low cost compared with that of corn is necessary for economical wet milling of sorghum (Rooney and Serna-Saldivar 1991).

Industrial wet milling of sorghum for starch production was developed in the United States during World War II as an alternative to corn starch production (Watson 1970). In 1948, Corn Products Co. built a modern sorghum wet-milling plant in Texas (20,000 bu of grain per day) that ran until the 1970's on an enlarged capacity as the only one in the United States. This wet-milling factory closed down in 1975, when sorghum prices increased to the same levels as corn prices (Rooney and Serna-Saldivar 1991). Caransa and Bakker (1987) reported the establishment of a sorghum starch plant in Sudan (capacity 150 t/day).

Sorghum is wet-milled in a manner similar to corn (Watson 1984). Basically, the process includes steeping of the grain, separation of germ and fiber, and separation of starch from protein. Compared with corn, the recovery of starch from sorghum is more difficult because of the structure of the sorghum kernel (Watson 1984). The sorghum pericarp is more fragile than the pericarp of corn, thus small pericarp particles impede the separation of the starch and protein and cause off-colored starch. Also, sorghum contains a larger proportion of horny endosperm than corn.

Several laboratory-scale studies have been done to determine the optimum parameters for wet milling of sorghum steeped with SO₂ solution. Zipf et al (1950) found the optimum SO₂ concen-

tration to be 0.25%, and maximum recovery of starch occurred at 43°C after steeping for 65 hr. However, a steeping time of 24 hr was found to be adequate. Anderson (1963) confirmed that the optimum conditions for steeping sorghum were 24 hr at 43°C, with a SO₂ concentration of 0.25%. In another study of wet milling of sorghum, Watson and Hirata (1954) steeped the sorghum in a solution of 0.5% lactic acid and 0.15% SO₂ adjusted initially to pH 3 with potassium hydroxide. The temperature was 49–50°C, and the steeping water was circulated with a pump for 10 min every hour for 48 hr.

The economics of using sorghum for wet milling could be improved if new cultivars with improved properties are developed. Yellow endosperm sorghum hybrids with significantly improved wet-milling properties have been identified (Norris and Rooney 1970). Recently, Yang and Seib (1996) used a short process to wet-mill sorghum that involved steeping for 2–4 hr at 58°C with a 0.25% SO₂ solution. With this method, the sorghum released only about half its starch. A typical pilot trial (Watson 1970) was done to compare the wet milling of the red, waxy white, and yellow endosperm sorghums.

The first and most important step in wet milling is steeping of grain under controlled processing conditions of temperature, time, and steeping medium concentration. These are necessary to promote diffusion of the water into the germ, endosperm, and cellular components. SO₂ commonly is added to the steep water. The most extensive study on the function of SO₂ as a steeping agent for corn was done by Cox et al (1944). They indicated that both high SO₂ concentration and high steeping temperature led to increased protein disintegration and dispersion. In commercial wet milling, lactic acid builds up in the steep water from microbial glycolysis. Therefore, Watson and Hirata (1954) and Watson et al (1951) recommended addition of lactic acid to the steeping solution in the laboratory. Roushdi et al (1981) and Eckhoff and Tso (1991a) reported decreased starch yields of 4–6% in batch steeping corn when lactic acid was not used.

The addition of enzymes to enhance starch-protein separation in wet milling of corn also has been studied. Spanheimer et al (1972) found that a variety of proteolytic enzymes can increase protein solubility of corn grits. They also noted that a combination of bromelin and SO₂ performs better than either treatment alone at the same pH level. Eckhoff and Tso (1991b) reported that the addition of protease had a significant effect on starch recovery from corn. Steinke and Johnson (1991) investigated the feasibility of adding multiple enzymes to steep water containing typical levels of SO₂ to enhance corn starch separation and reduce steeping time. Steeping for 24 hr in a solution of multiple enzymes and SO₂ produced

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milling results equivalent to those obtained by steeping for 48 hr in a 0.20% SO₂ solution alone.

The major objective of this study was to develop an optimum steeping process for wet-milling sorghum. Specifically, the objectives were to: evaluate steeping solutions with different SO₂ concentrations and different steeping times; determine the feasibility of adding multiple enzymes to the SO₂ steeping solution to reduce steeping time and enhance starch separation; and determine the appropriate SO₂ level and steeping time for wet-milling sorghum steeped using SO₂ solution with the addition of lactic acid.

MATERIALS AND METHODS

Sample Preparation

Pioneer 8500 (Sorghum Research Station, Pioneer Hi-Bred International, Inc., Manhattan, KS) red-pericarp hard sorghum hybrid with a bright unmolded surface was used. The grain was cleaned with a mini cleaner and grader (Labofix, MCK Maschinenbau, Germany) to remove foreign materials and broken kernels. Fifty-four representative 500-g sorghum samples were prepared using a precision divider (Gamet Mfg. Co., Minneapolis, MA), placed into polyethylene bags, and stored at 4°C until used.

The moisture content (12.6%, wb) of the sorghum was determined in triplicate by using Approved Method 44-15A (AACC 2000). Standard laboratory analytical procedures were followed to determine starch (71.3%, db), crude protein (10.9%, db), fat (3.9%, db), and ash (1.2%, db) contents in duplicate.

Steeping Treatments

Sorghum was steeped batchwise using three different steeping solutions at 50°C: SO₂ solution (SDS); SO₂ solution containing 1.25% (w/w) of a commercial multiple-enzyme preparation (Novo SP249) (SDE); and SO₂ solution with the addition of 0.5% (w/w) lactic acid (SDL). For all steeping solutions, three different steeping times, (24, 36, and 48 hr) and three concentrations of SO₂ (0.1, 0.2, and 0.3% w/v) were tested.

The 0.1, 0.2, and 0.3% (w/v) SDS were prepared by dissolving sodium bisulfite in distilled water. SDL were prepared by diluting an 85% lactic acid in SDS. The commercial multiple-enzyme preparation Novo SP249 (Novo Industries, Wilton, CT) was added to SDS. It contained pectolytic, cellulolytic, hemicellulolytic, and proteolytic activities, and small amounts of saccharolytic activities and had optimum pH and temperature ranges of 3.5–5.5 and 40–50°C, respectively.

Wet-Milling Procedure

A 500-g test sample of sorghum was steeped in 1,000 mL of the steeping solution in a 2,000-mL beaker. The beaker was immersed in a water bath at 50°C. After steeping, the steeping solution was drained off and the grain sorghum was wet-milled immediately as shown in Fig. 1. The steeped sorghum kernels first were ground coarsely in a plate mill (Quaker City model 4E, The Straub Co., Hatboro, PA). This mill had one stationary and one rotating plate with an external plate gap adjustment. The corrugated plates on this mill contact each other and must mate well to give consistent particle size. Because the corrugated plates usually were not perfectly flat when new, they had to be broken-in before use in this milling procedure by running them in the mill for ≈10 hr or until properly mated. The plates were kept cool by wetting them periodically during the break-in period. The mill was set to tear and shred the grain without damaging the germ, and no whole kernels were left after the first grinding, which was used as a qualitative measure in establishing first-grinding parameters for this procedure. The necessary fineness of the grind was achieved by adjusting the plate-to-plate gap, thereby increasing the plate-to-plate pressure in the mill until the motor began to load noticeably when the plates were wet. The plates did not heat appreciably during the grinding process because of the large amount of liquid passing between them. Distilled water (500

mL) was added into the mill to cool and wash it and to enhance the coarse grinding process. The coarsely ground material was diluted with 3,000 mL of water under continuous mixing (D300 mixer, Hobart Corp., Troy, OH) to yield a slurry.

The slurry was pumped into a vibration sieve (Vorti-Siv RBF-15, MM Industries, Inc., Salem, OH) on which was mounted a stack of four sieves with openings of 1,000, 500, 250, and 50 μm, respectively, from top to bottom. The sieve was shaken for 10 min, while ≈1,000 mL of distilled water was sprinkled over the top. The mechanical action of the vigorous vibrating sieve and the rinse water effectively removed starch and protein from fiber. The slurry was separated into five fractions. The fractions retained on the top two sieves were considered the coarse fiber and germ, and the fractions retained on the bottom two sieves were considered the fine fiber plus fine endosperm. The materials that passed through all four sieves were considered mill starch.

The fine fiber (with endosperm) fractions were ground again using the plate mill at closer settings. This fine grinding released the endosperm from the bran and helped release the starch from the protein matrix in the endosperm. The slurry was stirred as it was being poured into the mill, and 200 mL of distilled water was used to wash the mill when the fine grind was finished. The ground slurry from the mill was mixed with 1,000 mL of water before screening again. The materials retained on the four sieves were considered the final fine fiber.

The starch and protein slurry (mill starch) that passed through the 50-μm sieve was then transferred to 750-mL centrifuge bottles and centrifuged for 20 min at 5,200 rpm in a programmable centrifuge (IEC PR-7000M, International Equipment Co., Needham Heights, MA). After centrifugation, the water was decanted, and the protein layer was scraped off carefully from the lower starch layer with a spatula. Between the two layers was an off-white layer composed of protein-bound starch. This layer was scraped off and collected as “inseparables”. The inseparable layer was resuspended, centrifuged, and separated from protein as already described. Two centrifugations were required to achieve clean starch-protein separations for each treatment. Sorghum from all steeping treatments was wet-milled in the same manner. Each treatment was replicated twice.

The starch, protein, and fiber fraction were put on separate trays and dried for 24 hr in a laboratory forced-air oven (Blue M Electric Co., Blue Island, IL) at 50°C. All fractions were weighed for yield before sampling. The yield determination was calculated on the basis of raw grain dry substance. The moisture contents of these products were determined by using Approved Method 44-15A (AACC 2000). Representative samples were taken for analysis of protein content (N × 6.25) by Kjeldahl nitrogen (Approved Method 46-13). Starch color was measured with a chromameter (CR-310, Minolta, Osaka, Japan). The Hunter *L*, *a*, *b* opponent color scale was used to study the starch color. The *L* (lightness), *a* (redness), and *b* (yellowness) values were recorded.

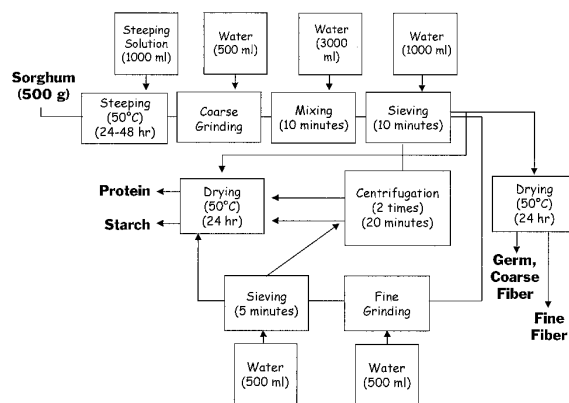


Fig. 1. Flow sheet for wet milling of sorghum.

Statistical Analysis

A three-factor factorial experimental design was used. The three factors were steeping solutions (SOLU), SO₂ concentrations (CONC) of steeping solutions, and steeping times (TIME), and three levels of each factor were used. This experiment design had 27 treatment combinations with two replicates of each treatment. Analysis of variance and Duncan's multiple range tests were used for data analysis (SAS Institute, Cary, NC). All statistical tests were done at the 5% significant level.

RESULTS AND DISCUSSION

Starch Fraction Yield

A significant difference in average starch fraction yields (Table I) occurred among the three steeping solutions: SDL (60.1%, db) > SDE (57.6%, db) > SDS (56.1%, db). This indicates that the SDE and SDL steeping treatments had significantly higher capabilities to release the starch compared with SDS treatment.

No significant differences in average starch fraction yields of sorghum occurred between SO₂ concentrations of 0.2 and 0.3% in all three steeping solutions. Also, no significant difference was ob-

served in average starch yield for the SDS and SDE treatments when the SO₂ concentrations of steeping media increased from 0.1 to 0.2%, but a significant increase was observed for the SDL treatment. These results indicate that SO₂ concentrations of the steeping media can be reduced from 0.3 to 0.2% without significantly affecting starch yield.

For all three steeping solutions, a significant increase in average starch yield resulted when steeping time increased from 24 to 36 hr. However, no significant change in starch yield was observed when steeping time increased from 36 to 48 hr for the SDL treatment. These results show that steeping time can be reduced from 48 to 36 hr without significantly affecting starch yield for the SDL treatment but not for SDS and SDE treatments.

The highest starch fraction yield (62.2%, db) and recovery (87.2%) were obtained from sorghum steeped in the 0.3% SDL solution for 48 hr.

No significant differences in starch yield occurred when SO₂ concentration was reduced from 0.3 to 0.2% and the steeping time was reduced from 48 to 36 hr for the SDL treatment. The lowest starch yield (53.1%, db) was obtained for SDS with 0.1% SO₂ and 24 hr of steeping.

TABLE I
Yields (% db) of Wet-Milling Fractions Obtained from Sorghum Steeped Under Different Conditions at 50°C^a

Steeping Time (hr)	SDS ^b (% SO ₂)				SDE ^b (% SO ₂)				SDL ^b (% SO ₂)			
	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c
Starch fraction												
24	53.1a	54.0ab	54.4a-c	53.8I	54.6a-d	56.3c-f	55.9b-e	55.6J	57.4e-i	58.9ij	58.8ij	58.4KL
36	55.5b-e	56.4d-g	57.5e-i	56.5J	56.9e-I	57.4e-i	58.7ij	57.7K	59.5jk	61.2k-m	61.9lm	60.9M
48	56.7e-h	58.2f-j	58.6h-j	57.8K	58.4g-j	59.7jk	60.1j-l	59.4L	60.1j-l	61.2k-m	62.2m	60.2M
Mean ^d	55.1A	56.2AB	56.8BC	56.1X ^e	56.7BC	57.8CD	58.2DE	57.6Y ^e	59.0E	60.4F	61.0F	60.1Z ^e
Protein fraction												
24	16.1a	15.7ab	15.2a-d	15.7I	15.7ab	15.2a-d	15.5a-c	15.5I	15.2a-d	15.2a-d	14.9b-d	15.1I-K
36	16.1a	15.2a-d	15.4a-d	15.6I	15.3a-d	15.1a-d	15.1b-d	15.2IJ	14.9b-d	14.6cd	14.8b-d	14.7JK
48	15.5a-c	15.1b-d	15.3a-d	15.3IJ	15.1b-d	14.8b-d	14.9b-d	14.9I-K	14.7cd	14.5d	14.5d	14.6K
Mean ^d	15.9A	15.3B	15.3BC	15.5X ^e	15.4AB	15.1B-D	15.2B-D	15.2X ^e	14.9B-D	14.8CD	14.7D	14.8Y ^e
Fiber fraction												
24	23.9a	22.4a-c	21.9b-d	22.7I	22.9ab	21.5b-e	21.2b-f	21.9I	19.0h-l	18.1k-n	18.4j-m	18.5L
36	20.7c-g	19.6f-k	18.7h-m	19.7JK	20.7c-g	20.3d-h	19.2g-l	20.1J	18.5i-m	16.5no	16.0o	17.0M
48	20.2e-i	18.5i-m	17.9l-n	18.8KL	20.1e-j	18.7h-m	17.0m-o	18.6L	18.4j-m	16.0o	15.5o	16.6M
Mean ^d	21.6A	20.2B	19.5BC	20.4X ^e	21.2A	20.2B	19.1C	20.2X ^e	18.7C	16.9D	16.6D	17.4Y ^e

^a Values represent mean of two replicates; mean comparisons followed by the same letters (a-o) are not significantly different ($P < 0.05$) for any specific fraction.

^b SDS = SO₂ solution alone; SDE = SO₂ solution containing 1.25% (w/w) of a commercial multiple-enzyme preparation (Novo SP249); and SDL = SO₂ solution with the addition of 0.5% (w/w) lactic acid.

^c Values represent the average of three means from different SO₂ concentrations with any specific steeping time for different solutions; values followed by the same letter (I-M) are not significantly different ($P < 0.05$) for any specific fraction.

^d Values represent the average of three means from different steeping times at any specific SO₂ concentration for different steeping solutions; values followed by the same letter (A-F) are not significantly different ($P < 0.05$) for any specific fraction.

^e Values represent the average of nine means from all steeping conditions for any specific steeping solution; values followed by the same letter (X-Z) are not significantly different ($P < 0.05$) for any specific fraction.

TABLE II
Protein Content (% db) of Starch Obtained from Wet Milling of Sorghum Steeped Under Different Conditions at 50°C^a

Steeping Time (hr)	SDS ^b (% SO ₂)				SDE ^b (% SO ₂)				SDL ^b (% SO ₂)			
	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c
24	0.69a	0.67ab	0.65a-c	0.67I	0.62b-e	0.59e-i	0.54j-l	0.58JK	0.62b-e	0.59e-h	0.56f-j	0.59JK
36	0.64b-d	0.60d-f	0.55h-k	0.60J	0.60d-g	0.51k-n	0.52j-n	0.54L	0.53j-m	0.49l-o	0.49m-o	0.50MN
48	0.62c-e	0.55h-k	0.54i-k	0.57K	0.55g-k	0.51k-n	0.49l-o	0.52LM	0.51j-n	0.48no	0.45o	0.48N
Mean ^d	0.65A	0.60B	0.58B	0.61X ^e	0.59B	0.53CD	0.51DE	0.54Y ^e	0.55C	0.52DE	0.50DE	0.52Z ^e

^a Values represent mean of two replicates; mean comparisons followed by the same letters (a-o) are not significantly different ($P < 0.05$) for any specific fraction.

^b SDS = SO₂ solution alone; SDE = SO₂ solution containing 1.25% (w/w) of a commercial multiple-enzyme preparation (Novo SP249); and SDL = SO₂ solution with the addition of 0.5% (w/w) lactic acid.

^c Values represent the average of three means from different SO₂ concentrations with any specific steeping time for different solutions; values followed by the same letter (I-M) are not significantly different ($P < 0.05$) for any specific fraction.

^d Values represent the average of three means from different steeping times at any specific SO₂ concentration for different steeping solutions; values followed by the same letter (A-F) are not significantly different ($P < 0.05$) for any specific fraction.

^e Values represent the average of nine means from all steeping conditions for any specific steeping solution; values followed by the same letter (X-Z) are not significantly different ($P < 0.05$) for any specific fraction.

Protein Fraction Yield

The lowest average protein fraction yield was observed from the SDL steeping treatment (Table I). The SDS and SDE treatments did not differ significantly, but had significantly higher protein yields than the SDL treatment.

No significant change was observed in mean protein yields between the SDE and SDL treatments when the SO₂ concentrations of the steeping media increased from 0.1 to 0.3%. Only in the SDS steeping solution did the mean protein yield decrease significantly when the SO₂ concentrations were increased from 0.1 to 0.2%. For each of the three steeping treatments, a slight decrease in protein yield was observed when the steeping times increased from 24 to 48 hr. Comparison of means showed no significant difference in protein yield across steeping times.

Fiber Fraction Yield

The lower the fiber fraction yield is, the higher the starch fraction yield will be. The lowest fiber yield (15.5%, db) was recorded in the SDL treatment when SO₂ concentration and steeping time were 0.3% and 48 hr, respectively (Table I), indicating lower residual starch in the fiber. However, the difference in fiber yield was not significant for the SDL at all the SO₂ concentrations with

steeping times of 36 and 48 hr. The highest fiber yield (23.9%, db) was observed in the SDS with 0.1% SO₂ and 24 hr of steeping. The mean values were in the order SDS > SDE > SDL (significantly lower).

A significant decrease occurred in mean fiber yields in each of the three treatments when the SO₂ concentrations of steeping solutions increased from 0.1 to 0.3%. For each of the three steeping treatments, a significant decrease in mean fiber yield was observed when the times increased from 24 to 48 hr.

Protein Content of Starch Fraction

Protein content in the starch fraction is considered an indication of the degree of separation. The protein contents in starch ranged from 0.54 to 0.69% (db) for SDS, from 0.49 to 0.62% (db) for SDE, and from 0.45 to 0.62% (db) for SDL steeping treatments (Table II), all of which are somewhat higher than the 0.3–0.4% protein in commercial corn starch. The differences in mean protein content among the three steeping treatments were statistically significant. The lowest protein content (0.45%, db) was in the starch obtained from the SDL treatment at 48 hr of steeping with 0.3% SO₂ concentration. However, no significant differences in protein content of starch occurred when SO₂ concentration was reduced

TABLE III
Color of Wet-Milling Fractions Obtained from Sorghum Steeped Under Different Conditions at 50°C^a

Steeping Time (hr)	SDS ^b (% SO ₂)				SDE ^b (% SO ₂)				SDL ^b (% SO ₂)			
	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c	0.1	0.2	0.3	Mean ^c
<i>L</i> value												
24	91.5a	91.7ab	91.8ab	91.7I	91.7IJ	92.1a-g	92.2a-g	92.0IJ	92.2a-g	92.9fg	92.8e-g	92.6KL
36	91.8a-c	91.9a-d	92.4b-g	92.0IJ	91.8I-K	92.0a-f	92.3a-g	92.0IJ	92.3a-g	92.7d-g	92.8d-g	92.6KL
48	92.0a-f	92.1a-g	92.6c-g	92.2JK	92.0I-M	92.3a-g	92.8b-g	92.4J-L	92.5b-g	92.9g	93.0g	92.8L
Mean ^d	91.8A	91.9AB	92.3BC	92.0X ^e	91.8AB	92.2A-C	92.5CD	92.1X ^e	92.3BC	92.8D	92.9D	92.7Y ^e
<i>a</i> value												
24	-0.83a-c	-0.82ab	-1.01b-g	-0.89I	-0.75a	-1.05b-h	-1.05b-h	-0.95IJ	-0.88a-d	-1.13e-i	-1.27h-k	-1.09K
36	-0.97a-f	-1.07c-h	-1.10d-i	-1.05JK	-0.94a-e	-1.23g-j	-1.20g-j	-1.12KL	-1.21f-j	-1.24g-j	-1.34i-k	-1.26M
48	-1.22g-j	-1.32i-k	-1.20f-j	-1.25LM	-1.28h-k	-1.26h-k	-1.48jk	-1.34M	-1.34i-k	-1.40jk	-1.42jk	-1.38M
Mean ^d	-1.01AB	-1.07A-C	-1.10A-D	-1.06X ^e	-0.99A	-1.18C-E	-1.24D-F	-1.13X ^e	-1.14B-E	-1.25EF	-1.34EF	-1.25Y ^e
<i>b</i> value												
24	2.85a	2.80a-c	2.70c-f	2.78I	2.85a	2.74b-e	2.68c-g	2.75I	2.80a-c	2.77a-d	2.58c-I	2.71I
36	2.66c-g	2.52c-j	2.43f-k	2.54JK	2.82ab	2.59c-h	2.51c-j	2.64IJ	2.68c-g	2.46e-k	2.48d-k	2.54JK
48	2.57c-i	2.44f-k	2.27jk	2.43KL	2.46e-k	2.23jk	2.29i-k	2.33L	2.39h-k	2.33i-k	2.19k	2.30L
Mean ^d	2.69A	2.59A-C	2.46BC	2.58X ^e	2.71A	2.52BC	2.49BC	2.57X ^e	2.62AB	2.52BC	2.42C	2.52X ^e

^a Values represent mean of two replicates; mean comparisons followed by the same letters (a-o) are not significantly different ($P < 0.05$) for any specific fraction.

^b SDS = SO₂ solution alone; SDE = SO₂ solution containing 1.25% (w/w) of a commercial multiple-enzyme preparation (Novo SP249); and SDL = SO₂ solution with the addition of 0.5% (w/w) lactic acid.

^c Values represent the average of three means from different SO₂ concentrations with any specific steeping time for different solutions; values followed by the same letter (I-M) are not significantly different ($P < 0.05$) for any specific fraction.

^d Values represent the average of three means from different steeping times at any specific SO₂ concentration for different steeping solutions; values followed by the same letter (A-F) are not significantly different ($P < 0.05$) for any specific fraction.

^e Values represent the average of nine means from all steeping conditions for any specific steeping solution; values followed by the same letter (X-Z) are not significantly different ($P < 0.05$) for any specific fraction.

TABLE IV
Comparisons of Two Sorghum Wet-Milling Methods in the Present Study^a with Previous Studies

	Present Study 1	Present Study 2	Zipf et al (1950)	Yang and Seib (1996)	Norris and Rooney (1970) ^b
Steeping condition					
Medium	SO ₂	SO ₂ + lactic acid	SO ₂	SO ₂	SO ₂ + lactic acid
Concentration, %	0.30	0.20 + 0.50	0.25	0.25	0.05 + 1.5 (I) & 0.1 + 0.5 (II)
Temperature, °C	50	50	43.3	58	52 (I & II)
Time, hr	48	36	65	4	40 (I) & 8 (II)
Equipment					
Mill type	Disk attrition	Disk attrition	Disk attrition	Blender	Blender
Grinding	2	2	2	1	1
Separation (fiber)	Screen	Screen	Screen	Screen	Screen
Separation (starch)	Centrifuge	Centrifuge	Starch table	Centrifuge	Starch table
Results					
Starch recovery, %	82.2	85.9	78.4	50.5	67.7–80.8
Protein in starch, %	0.54	0.49	0.78	0.79	0.8–1.9

^a Present study (1) steeping solution with SO₂; (2) steeping solution with SO₂ and lactic acid.

^b Two steeping phases (I and II) at different concentrations and steeping times.

from 0.3 to 0.2% and the steeping time was reduced from 48 to 36 hr for the SDL treatment. The highest protein content (0.69%, db) was in the starch obtained from the SDS treatment at 24 hr of steeping with 0.1% SO₂.

A significant decrease in mean protein content of starch occurred for all three steeping treatments when the SO₂ concentration was increased from 0.1 to 0.2%. However, no significant difference in mean protein content occurred with the increase from 0.2 to 0.3% for all three treatments. Zipf et al (1950) found that there was no significant effect on the protein content in starch between 0.1 and 0.25% SO₂ concentration when corn was used. For all three steeping solutions, a significant decrease in mean protein content of starch was observed when steeping time increased from 24 to 36 hr. However, no significant change in mean protein content of starch was observed when steeping time increased from 36 to 48 hr, except for the SDS treatment.

Starch Color

The color of finished starch is an important quality characteristic for wet milling of sorghum, where pure white is the ultimate objective. All *L* values (Table III) were >90, indicating that the degree of lightness of each sorghum starch sample was satisfactory. The average values for starch color were in the order SDL > SDE > SDS for *L*; SDL < SDE < SDS for *a*; and SDL < SDE < SDS for *b*. The average *L* value for the SDL steeping treatment was significantly higher (lighter colored) than values for the SDE and SDS treatments. Correspondingly, the average *a* values for the SDL treatment were the lowest, indicating less intense red color. No significant differences in mean *L*, *a*, and *b* values were found between the SDE and SDS treatments. The higher mean *L* values and lower *a* and *b* values for the SDL treatment are indications of lower levels of contaminating protein and associated pigments.

As the SO₂ concentrations of the steeping media increased from 0.1 to 0.3%, mean *L* values showed a significant increase, whereas *a* and *b* values decreased significantly. This indicates that the whiteness of starch increased with increasing SO₂ concentration of the steeping solution.

L values showed no significant change when steeping time changed from 24 to 48 hr, except for the SDS treatment. The mean *a* and *b* values for all three treatments decreased as steeping time increased. This indicates that a longer steeping time could improve the color of sorghum starch.

Comparison with Previous Studies

The results from the present study with 0.3% SO₂ and the optimal steeping condition (0.2% SO₂ with 0.5% lactic acid) were compared with the procedures and results from several previous studies (Zipf et al 1950, Norris and Rooney 1970, Yang and Seib 1996) (Table IV). The most important criteria for judging wet-milling processing are starch recovery and purity of starch. Norris and Rooney (1970) found that starch recovery from the wet milling of sorghum was significantly dependent on cultivars. Because of differences in raw materials and procedures, it is not suitable to rank one procedure over another. However, a significant difference in procedures might have some effect on starch recovery as well. Two different types of mills, a disk attrition mill and a blender, were used in the studies shown in Table IV. The disk mill was used with two grinding stages, but the blender was used in a single stage. The disk attrition mill has a mechanism to control the gap between the disks, which determines the degree of grinding. In the blender, the degree of grinding is controlled by the speed of the blades and grinding time. Yang and Seib (1996) showed that increasing the grinding time in the blender dramatically improved the yield of starch.

Another major difference among the procedures in this study was the equipment for separating starch and protein (centrifuge vs. starch table). Both units use density difference between starch and protein to separate them. Watson (1970) stated that the only suitable

method for separating starch from protein in the laboratory is with a starch table. However, separation of starch and protein using a centrifuge in this investigation produced comparable starch recovery with equivalent purity when compared with that of the starch table (Table IV).

Many laboratory procedures have been developed for sorghum wet milling. As Watson (1984) said that even though the complete laboratory fractionation method is slow and cumbersome, there is no other substitute for adequate evaluation of wet-milling properties of grain. All the commercial mills need to do is choose the one that is right for them.

CONCLUSIONS

Incorporation of multiple enzymes (1.25% commercial Novo SP249 preparation) into the SO₂ for the batchwise steeping of sorghum resulted in an increase in starch yield with reduced protein content in the starch compared with the SO₂ solution alone. However, the level of multiple enzymes used probably exceeded commercially practical levels. The best wet-milling performance for sorghum resulted from the steeping solution containing 0.5% lactic acid, which produced the whitest starch with the highest yield and the lowest protein content. The steeping solution with both SO₂ and lactic acid also gave the lowest average yields of protein and fiber.

Both higher SO₂ concentration of steeping media and longer steeping time led to higher starch yield, lower protein content in starch, and whiter starch. However, no significant differences in starch yield, protein content in starch, and starch color occurred between SO₂ concentrations 0.2 and 0.3% for all three steeping media. Neither SO₂ concentration of steeping media nor steeping time had a significant effect on protein yield for all three treatments. Both high SO₂ concentration of steeping media and long steeping time led to a decrease in the fiber yield for each of the treatments.

The optimum steeping process found for wet milling of sorghum in this study is using 0.2% SO₂ solution with 0.5% lactic acid for 36 hr at 50°C. Under these conditions, the starch yield, protein content in starch, and *L* value of starch color were 60.2% (db), 0.49% (db), and 92.7, respectively, which were not significantly different from the best values from the 48-hr steeping at 0.3% SO₂ solution with the addition of 0.5% lactic acid.

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