Alkali Wet-Milling Characteristics of Pearled and Unpearled Amaranth Seed[1]

D. J. MYERS[2] and S. R. FOX[3]

ABSTRACT

Researchers are showing increasing interest in grain amaranth because of its unique microcrystalline starch granules (1–3 μm in diameter). This investigation was conducted to determine the potential of an alkaline wet-milling method to separate the starch from the seed. Pearled and unpearled *Amaranthus cruentus* seed was wet milled using a high-alkaline batch-steeping process and separation methods common to laboratory wet-milling of corn. Starch with a purity of 0.2% protein was obtained from both the pearled and unpearled amaranth. However, more starch was recovered from unpearled amaranth because of the leaching of fine starch granules during steeping. Less germ was recovered using unpearled amaranth.

Cereal Chem. 71(1):96–99

Grain amaranth is a pseudocereal with a seed of relatively small size (1.0–1.5 mm in diameter), which is covered by a seed coat (NRC 1985, Teutonico and Knorr 1985, Tucker 1986). This seed coat is closely associated with the embryo portion of the seed and surrounds the perisperm, which is composed primarily of starch (Irving et al 1981). The grain is composed primarily of starch (48–69%), but it also contains 12–18% protein that is rich in the amino acid lysine, normally limiting in most cereal grains, and 5–8% fat that contains a relatively high level of squalene, a lipid that is an important ingredient in a variety of cosmetics and lubricants (Becker 1981, Bressani 1990, Breene 1991).

The increasing interest in amaranth seed is due to its very small starch granules (1–3 μm in diameter), which may give the biopolymer unique properties in both food and nonfood applications (Goering 1967, Stone and Lorenz 1984, Lehman 1990, Breene 1991). In addition to small size, the starch properties include low gelatinization temperature, good freeze-thaw stability, and resistance to mechanical shear (Yanez et al 1986, Singhal and Kulkarni 1990). Breene (1991) identified several possible food and nonfood uses for the starch including: dusting powders, biodegradable plastics, cosmetics, laundry starch, and food thickeners and stabilizers for products such as gravies, sauces, and soups.

Currently, there is no commercial process for recovering starch from amaranth. A number of studies have used alkaline starch-extraction procedures to isolate amaranth starch for analytical and characterization purposes (Becker et al 1981, Okuno and Sakaguchi 1981, Yanez et al 1986, Paredes-Lopez et al 1989). Betschart et al (1981) used a dry-milling process to recover the starch from the perisperm. None of these efforts has led to a commercial milling process. The objective of this study was to determine whether a wet-milling process with separation procedures similar to those used in the wet milling of corn could be adapted for amaranth. We used a modified laboratory batch-steeping method for wet milling of corn (Fox et al 1992) to recover the starch from amaranth and to characterize the other fractions of the seed that result from the process. The key modification was the use of a high-alkaline steeping process (pH 13.0), compared to the relatively acidic conditions used for steeping corn. Both pearled and unpearled grain were used in this study to determine the effect of seed coat removal on the quantity of starch obtained and the composition of the other fractions. Anticipating that the seed coat removal would cause a physical effect in the recovery of starch, we also milled a 50:50 blend of pearled and unpearled grain to gain insight into how the physical characteristics of the grain can influence the recovery of milling fractions, particularly for starch and germ recovery.

[1]Journal Paper J-15205 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 0161. Research supported by the Center for Crops Utilization Research and the Iowa Agriculture and Home Economics Experiment Station.
[2]Assistant professor, Department of Food Science and Human Nutrition. Iowa State University, Ames.
[3]Senior technician, Center for Crops Utilization Research. Iowa State University, Ames.

©1994 American Association of Cereal Chemists, Inc.

MATERIALS AND METHODS

Milling Procedure

Pearled and unpearled amaranth seed, both containing 62% starch (L. Walters, personal communication 1990), were obtained from New World Amaranth (Naperville, IL). The pearled seed had been prepared using a Strong-Scott barley pearler. Three samples were milled: pearled seed (100 g), unpearled seed (100 g), and a 50:50 blend (50 g of pearled seed + 50 g of unpearled seed). The quantity and composition of the solid and liquid fractions were measured.

In the milling process (Fig. 1), the seeds were steeped in 1 L of a 1% NaOH solution (pH 13.0) for 24 hr under slow agitation. After the steeping, the liquor was decanted and saved for analysis. The remaining amaranth solid was separated manually into two fractions: *germ* and *mill starch*. The use of these terms, as well as *gluten* and *fiber*, is based on nomenclature used in corn wet milling, from which this process was derived. The mill-starch fraction was resuspended with 300 ml of distilled water, stirred at high speed to release any germ trapped in the mill-starch fraction, and allowed to settle to permit the germ to segregate to the top of the mill-starch fraction. Any germ that had separated was manually removed and added to the germ fraction. This washing and separation procedure was repeated.

The germ fraction was then washed with distilled water through a U.S. standard 200 sieve, and the precipitate was dried first in a forced-air oven at 60°C for 48 hr and then in a vacuum oven at 60°C for 16 hr. The wash water (germ liquor) was saved for analysis.

The washed mill-starch fraction was ground in a Waring Blendor at full speed for 2 min. The ground slurry was screened through a 200 sieve on a Ro-Tap sieve shaker for 5 min to remove the fiber fraction. This fraction was washed twice with 500 ml of distilled water, sifted through a 200 sieve, strained through a 52-μm nylon-mesh cloth, and then dried in the same manner as the germ fraction. The wash water was added to the mill-starch fraction.

The remaining mill-starch fraction, with wash-water added, was centrifuged at 6,000 × *g* for 30 min in a Superspeed RC2-B refrigerated centrifuge (Sorvall, Newtown, CT). The supernatant was saved for analysis. The solid fraction was separated (by color) into gluten and starch with a laboratory spoon. Both fractions were dried first in a forced-air oven at 45°C for 48 hr and then in a vacuum oven at 45°C for 16 hr.

The milling procedure (Fig. 1) was replicated six times for each of the three amaranth seed samples.

Proximate Analysis of Wet-Milled Products

The four solid fractions (germ, fiber, gluten, and starch) were analyzed in duplicate for moisture, crude free-fat, protein, and starch contents. Moisture contents were determined by drying 2 g of ground sample in a convection oven at 130°C for 3 hr (AOAC 1984). Percentage yield was calculated on a dry weight basis.

Crude free-fat contents were determined with the Goldfisch

apparatus (AOAC 1984). Protein contents were determined with the Tecator Kjeltec system (Tecator, Hoganas, Sweden), according to the Corn Industries Research Foundation macro-Kjeldahl method A-18 (CRA 1986) using the factor of 6.02 g of protein per gram of nitrogen to calculate protein content (Breene 1990). Starch contents were determined with the Kern Full-Circle polarimeter (Kern, Zurich, Switzerland), according to the Corn Industries Research Foundation polarimetry method A-20 (CRA 1986).

The three liquid fractions (steep liquor, germ liquor, and mill-starch fluid) were analyzed in duplicate for dissolved solids, protein, and starch contents. Dissolved solids were calculated by drying 10 g of sample in a convection oven at 130°C for 16 hr.

Statistical Analysis

The data were analyzed with the Statistical Analysis System program (SAS 1987). Means and least significant difference values were calculated using the general linear models procedure.

TABLE I
Mean Yields[a] (g) of Recovered Wet-Milling Fractions for Pearled, Unpearled, and a 50:50 Blend of Pearled-Unpearled Amaranth Seed

Fractions	Pearled	50:50 Blend	Unpearled	LSD[b]
Solids				
Starch	11.2	16.2	32.6	7.8
Gluten	16.9	10.9	17.3	6.4
Fiber	4.6	6.6	14.1	3.6
Germ	7.3	17.0	1.8	6.1
Total solids	39.9	50.6	65.8	6.5
Liquids				
Steep liquor	20.8	24.9	15.8	4.4
Germ liquor	21.0	13.4	3.2	5.0
Mill-Starch fluid	18.6	11.3	15.2	10.3
Total liquids	60.4	49.7	34.2	6.2
Total recovery	100.2	99.4	100.9	2.9

[a] Based on six replicates for 100 g of milled seed for each sample.
[b] Least significant difference values ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Solid Fraction Yields

The data in Table I show the recovery results for solid milling fractions from pearled and unpearled amaranth seeds. The data show that a greater amount of fiber and starch were recovered from the unpearled seed, while a greater amount of germ was recovered from the pearled seed. The amount of the gluten fraction recovered from each of these samples was comparable. The higher fiber recovery from the unpearled seed was expected because the seed coat was removed from the pearled seed. The higher starch recovery is attributed to the lower amount of leaching present in the liquid fractions. There was a noticeable difference in clarity between the steep solutions of the pearled and unpearled samples. The steep solution of the pearled amaranth was very opaque; the steep solution of the unpearled amaranth was relatively translucent. The steep solution of the pearled sample also had a very gelatinous consistency, which is characteristic of a starch solution. The solids content of the liquid fractions is higher in the pearled sample, particularly in the germ liquor fraction (water used to wash the separated germ). These results suggest that pearling eliminated a major portion of the seed coat, which allowed starch to leach out of the seed and contributed to the lower starch recovery from the pearled seed.

The greater percentage of germ recovered from the pearled seed (Table I) can be explained by physical separation. The seed coat was left on the unpearled sample, so the germ could not be released. Consequently, very little germ was recovered. On the other hand, the pearled sample (with the seed coat removed) allowed some germ to be released and recovered. Conceivably, more of the germ could have been recovered if the steep solution had been less gelatinous. To test this theory, we compared the germ recovery from the sample containing a 50:50 blend of pearled and unpearled amaranth seed with those from the pearled and unpearled samples. The results (Table I) show that a greater amount of germ was recovered from the 50:50 blend than from the other two samples. The 50:50 blend had germ separation because it contained 50% pearled seed. It had less overall leaching

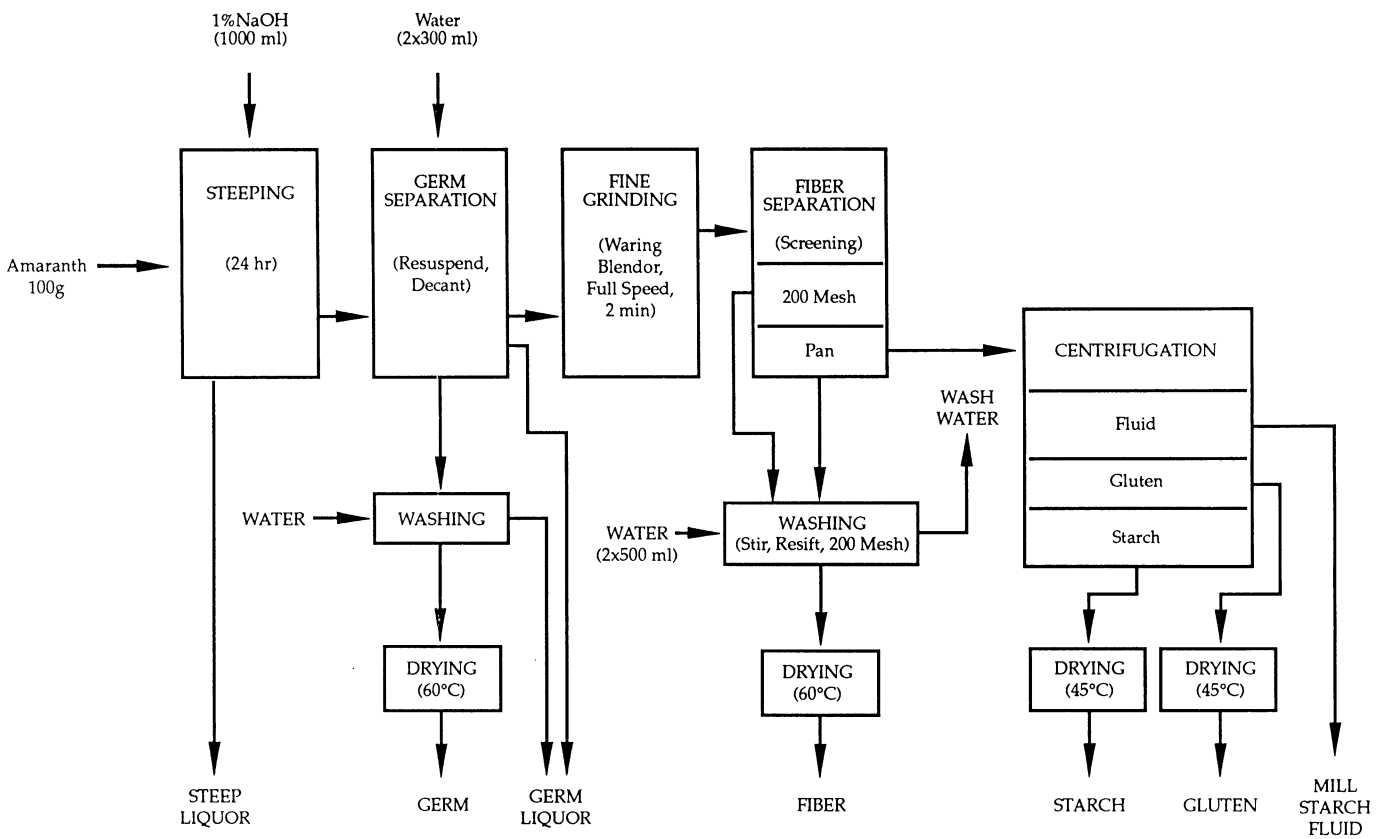

Fig. 1. Laboratory procedure for alkaline wet milling of amaranth seeds.

TABLE II
Mean Percentages[a] for Compositional Properties of Recovered Wet-Milling Liquid Fractions of Pearled, Unpearled, and a 50:50 Blend of Pearled-Unpearled Amaranth Seed

Fraction	Starch	Protein	Fiber[b]	Total[c]
Steep liquor				
Pearled	2.60	0.52	0.30	3.42
50:50 blend	2.90	0.31	0.50	3.71
Unpearled	0.75	0.08	0.98	1.81
Germ liquor				
Pearled	2.00	0.42	0.23	2.65
50:50 blend	1.30	0.22	0.28	1.80
Unpearled	0.22	0.35	0.32	0.89
Mill-starch fluid				
Pearled	0.61	0.15	...	0.76
50:50 blend	0.21	0.20	...	0.41
Unpearled	0.39	0.31	...	0.70

[a] Expressed as percentage of total solution weight ($n = 6$).
[b] Determined as the remaining percentage of solid fraction present after accounting for percent of starch and protein.
[c] Total percentage of dissolved solids in solution.

because it contained 50% unpearled seed, which allows the germ to be recovered.

Unfortunately, we could not use the results from the 50:50 seed blend to explain the amounts recovered for all fractions. Because 50% of each sample was present, one would expect that all results from the 50:50 blend (except germ recovery) would be intermediate compared to the results from pearled and unpearled seeds. However, the amount of gluten, steep liquor, and mill-starch fluid recovered show that this was not the case. These results reveal that factors other the amount of pearled seed that can leach into the steepwater influence the total recovery of solids.

Composition of Liquid Fractions

The composition of liquid fractions is presented in Table II. The steep liquor and germ liquor of the pearled seed and the 50:50 blend had higher percentages of starch, and the higher starch composition results in a higher overall total solids content. This data confirms the results in Table I by showing that the increased solids are composed of starch and contribute to there being less starch recovered using pearled seed. These major differences in starch content are not present in the mill-starch liquid fraction. The lower protein results for the steep liquor from the unpearled sample also illustrates the lower level of leaching from the unpearled sample. Finally, the unpearled sample also shows a higher fiber content in the steep liquor, which indicates the presence of seed coat.

Composition of Solid Fractions

Although the unpearled amaranth gave a significantly greater recovery of starch, all samples yielded starch fractions relatively high in starch purity (>98.0%) (Table III). Data also show, however, that there were significant amounts of starch present in all of the recovered fractions, particularly in the gluten fraction. Based on the amount of those fractions recovered (Table I) and the percentage of starch present in those fractions (Table III), it is clear that there were significant amounts of unrecovered starch in these fractions.

Data for the other fractions (Table III) showed that the highest percentages of crude fat were found in the fiber and germ fractions, and the highest percentages of fiber were found in the germ fractions.

CONCLUSIONS

This study shows that starch can be recovered at a purity of >98% using an alkaline wet-milling procedure. The recovery of starch is greater when the seed is unpearled because leaching from the pearled seed occurs during steeping. The results also show that the recovery of the germ fraction is compromised by this procedure. Based on the amount of the starch recovered from the unpearled sample (Table I) and the starch purity data (Table III), the amount of starch in the unpearled fraction is calculated at 32.2%. Using unpearled seed (based on a seed starch content of 62%), this method is capable of recovering ~52% of the available starch. Further study is needed to develop a method that improves the recovery of amaranth starch.

TABLE III
Mean Percentages[a] for Compositional Properties of Recovered Wet-Milling Solid Fractions of Pearled, Unpearled, and a 50:50 Blend of Pearled-Unpearled Amaranth Seed

Fraction	Starch	Protein	Fat	Fiber[b]
Starch				
Pearled	98.9	0.83	0.26	...
50:50 blend	98.4	1.0	0.56	...
Unpearled	98.8	0.60	0.62	...
LSD[c]	...	1.2	0.47	...
Gluten				
Pearled	66.7	10.7	4.7	17.9
50:50 blend	50.1	8.7	4.7	36.5
Unpearled	50.0	7.5	5.5	37.0
LSD	...	5.5	3.7	...
Fiber				
Pearled	26.5	21.8	9.0	42.7
50:50 blend	35.0	19.3	10.8	34.9
Unpearled	41.5	18.6	9.8	30.1
LSD[3]	...	3.0	5.4	...
Germ				
Pearled	16.1	18.2	6.9	58.8
50:50 blend	24.0	16.7	6.4	52.9
Unpearled	23.7	18.7	8.2	49.4
LSD	...	3.4	4.8	...

[a] Expressed as percentage, dry basis ($n = 6$).
[b] Fiber percent determined as the remaining percentage of solid fraction present after accounting for percentage of starch, protein, and crude free-fat (oil).
[c] Least significant difference ($\alpha = 0.05$).

LITERATURE CITED

AOAC. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists, 14th ed. Methods 14.004, 14.084, and 14.0085. The Association: Washington, DC.

BECKER, R., WHEELER, E. L., LORENZ, K., STAFFORD, A. E., GROSJEAN, O. K., BETSCHART, A. A., SAUNDERS, R. M. 1981. A compositional study of amaranth grain J. Food Sci. 46:1175.

BETSCHART, A. A., IRVING, D. W., SHEPHERD, A. D., and SAUNDERS, R. M. 1981. *Amaranthus cruentus*: Milling characteristics, distribution of nutrients within seed components, and the effects of temperature on nutritional quality. J. Food Sci. 46:1181.

BREENE, W. M. 1990. Contribution of amaranth to consumer products. Pages 75-95 in: Amaranth: Perspectives on Production, Processing and Marketing. Minnesota Extension Service. University of Minnesota: St. Paul, MN.

BREENE, W. M. 1991. Food uses of grain amaranth. Cereal Foods World 36:426.

BRESSANI, R. 1990. Grain amaranth. Its chemical composition and nutritive value. Pages 19-34 in: Amaranth: Perspectives on Production, Processing and Marketing. Minnesota Extension Service. University of Minnesota: Minneapolis, MN.

CRA, 1986. Standard Analytical Methods of the Member Companies of Corn Industry Research Foundation. Method A-18, approved June 1957; Method A-20, approved June 1964. Corn Refiners Association: Washington, DC.

FOX, S. R., JOHNSON, L. A., HURBURGH, C. R., Jr., DORSEY-REDDING, C., and BAILEY, T. B. 1992. Relations of grain proximate composition and physical properties to wet-milling characteristics of maize. Cereal Chem. 69:191.

GOERING, K. J. 1967. New starches. II. The properties of starch chunks from *Amaranthus retroflexus*. Cereal Chem. 44:245.

IRVING, D. W., BETSCHART, A. A., and SAUNDERS, R. M. 1981. Morphological studies on *Amaranthus cruentus*. J. Food Sci. 46:1170.

LEHMAN, J. W. 1990. The potential of grain amaranth in the 1990s and beyond. Pages 1-17 in: Amaranth: Perspectives on Production, Processing and Marketing. Minnesota Extension Service. University

of Minnesota: St. Paul, MN.
NRC. 1985. Amaranth: Modern Prospects for An Ancient Crop. National Research Council. Rodale Press: Emmaus, PA.
OKUNO, K., and SAKAGUCHI, S. 1981. Glutinous and nonglutinous starches in perisperm of grain amaranth. Cereal Res. Commun. 9:305.
PAREDES-LOPEZ, O. P., SCHEVENIN, M. L., HERNANDEZ-LOPEZ, D., and CARABEZ-TREJO A. 1989. Amaranth starch-isolation and partial characterization. Starch/Staerke 41:205.
SAS. 1987. SAS/STAT Guide for Personal Computers. Version 6, ed. SAS Institute: Cary, NC.
SINGHAL, R. S., and KULKARNI, P. R. 1990. Utilisation of *Amaranthus paniculatus* (Rajgeera) starch in salad dressing. Starch/Staerke 42:52.
STONE, L. A., and LORENZ, K. 1984. The starch of *Amaranthus*—Physico-chemical properties and functional characteristics. Starch/Staerke 36:232.
TEUTONICO, R. A., and KNORR, D. 1985. Amaranth: Composition, properties, and applications of a rediscovered food crop. Food Technol. 39:49.
TUCKER, J. B. 1986. Amaranth: The once and future crop. Bioscience 36:9.
YANEZ, G. A., MESSINGER, J. K., WALKER, C. E., and RUPNOW, J. H. 1986. *Amaranthus hypochondriachus* starch isolation and partial characterization. Cereal Chem. 63:273.

[Received January 11, 1993. Accepted October 1, 1993.]