PEARL MILLET. I. CHARACTERIZATION BY SEM, AMINO ACID ANALYSIS, LIPID COMPOSITION, AND PROLAMINE SOLUBILITY[1]

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ABSTRACT

Cereal Chemistry 53(4): 478-487

Pearl millet (random mating population from 'Serere 17' × 'Tift 239') grain endosperm is composed of both hard (translucent) and soft (opaque) parts. The hard part has tightly packed, polygonal-shaped starch granules and a matrix protein containing relatively large, embedded protein bodies. The soft endosperm has loosely packed, spherical starch granules covered with a thin sheet of protein. The soft endosperm contains many voids, or air spaces, and no protein bodies. Dry milling of millet and sorghum grain gave similar flour extractions: 58% for millet, and 53% for sorghum grain. Total grain protein recovered in the flour was approximately constant at 45% for both grains. Millet had higher values for lysine, arginine, aspartic acid, threonine, serine, glycine, valine, and methionine, and lower values for glutamic acid, proline, alanine, and leucine than did sorghum grain. Lysine content of millet was 3.6 g/100 g protein, comparable to that in high-lysine corn. The protein bodies in pearl millet were soluble in 70% ethanol at 60° C (30.7% of total protein), and at least part (27.1% of total protein) of the matrix protein was soluble in 100% t-butyl alcohol at room temperature. Two successive extractions under more vigorous conditions (blender) gave about 55% of the total millet protein soluble in either 60° C, 70% ethanol or 60° C, 60% t-butyl alcohol.

Pearl millet (*Pennisetum americanum* (L.) K. Schum.) is locally known as bulrush or Dukhn in Africa, and as bajra, cumbo, or Sajja in India (1,2). Millet in this paper refers to pearl millet unless specified otherwise.

Pearl millet is one of the important millets of the tropical and subtropical regions of Asia and Africa (3). It is grown extensively for human food in Eastern Europe, Africa, India, and the Far East (1,4,5). Worldwide hectarage of all types of millets nearly equals that of grain sorghum (*Sorghum bicolor* (L.) Moench). However, generally they yield only half that of grain sorghum per hectare (6). In areas of greatest production, the yields of millet and grain sorghum are nearly equal, but both disappointingly low.

Pearl millet is thought to have originated in Egypt or Arabia (1,5). The wide diversity of areas that produce millet and grain sorghum indicates that millet may be as feasible as grain sorghum for any area of the world. Pearl millet has not been grown in the United States for grain because of low yields and because plants are too tall for mechanical harvesting. The development of hybrid (7) and dwarf types (3) may help pearl millet become an attractive grain crop for arid parts of the United States and may improve yields of grain in areas now producing millet.

[1]Cooperative investigations, Agricultural Research Service, U.S. Department of Agriculture, and the Depts. of Grain Science and Industry and Agronomy, Kansas State University; Contribution No. 876, Dept. of Grain Science and Industry, and Cont. No. 1460, Dept. of Agronomy, Kansas Agricultural Experiment Station, Manhattan 66506.

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Millet and sorghum grain milling has usually been done with roller mills developed for wheat milling (8–10). Sorghum grain's tough bran and millet's small kernels usually result in dark flours. Flour color has been improved by modifying the flow sheet, using proper tempering, or decorticating the kernels (9,11,12).

This study was undertaken to characterize, at least in part, the structure, and to obtain information on the amino acid, lipid, and prolamine composition of pearl millet.

MATERIALS AND METHODS

Grain Samples and Dry Milling

Pearl millet (F_3 and F_4 seed from random mating populations of combine-height plants) derived from the cross 'Serere 17' × 'Tift 239' was used in this study. For comparison, we used two hybrid grain sorghums, a bulk, red-seeded commercial sample, and a yellow-seeded (C-42Y) sample obtained from DeKalb.

The samples were milled on a Quadrumat junior experimental mill to yield four fractions: bran, low-grade flour (dark-colored), +9XX flour, and −9XX flour. These fractions were analyzed for protein, moisture, and amino acid distribution.

Scanning Electron Photomicrographs

Millet kernels were cross-sectioned at the top of the germ with a razor blade,

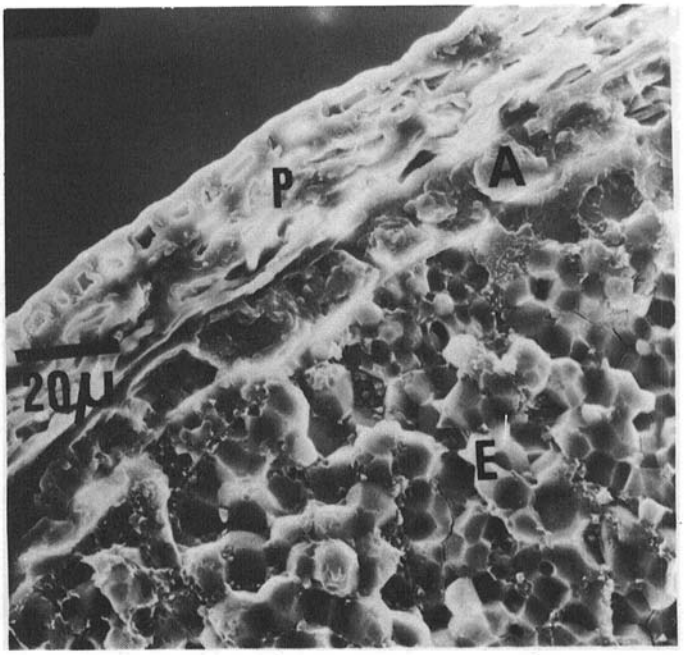

Fig. 1. Scanning electron photomicrograph (SEM) of the outer edge of millet grain showing the pericarp (P), aleurone (A), and endosperm (E).

which produced a fracture rather than a smooth cut. The half kernels were mounted on aluminum stubs, coated with a 150-Å-thick gold-palladium layer, viewed, and photographed in an ETEC Autoscan scanning electron microscope at an accelerating voltage of 20 kV. Numerous kernels representing various kernel sizes and shapes and genetic variations were examined. The photomicrographs presented are representative of the sample used; however, with the wide diversity of the many lines of pearl millet grown in the world, they may not represent all those lines.

Amino Acid Analysis

Amino acid compositions of grain and milled fractions were determined in a Beckman Model 120B amino acid autoanalyzer. Samples were hydrolyzed for 22 hr with 6*N* HCl at 110° C in sealed tubes.

Lipid Samples

Free lipids were extracted with petroleum ether (bp 35° to 60° C) in a Soxhlet apparatus. Bound lipids were extracted from the petroleum ether extracted grain with water-saturated butanol and purified by evaporating the solvent and redissolving in petroleum ether. All lipid fractions were recovered by evaporation under vacuum at less than 40° C. The lipid fractions were characterized by thin-layer chromatography (tlc) on plates coated with 50 mm of Silica gel G. One plate was developed for nonpolar lipids with chloroform. A second plate was developed with chloroform, methanol, and water (65:25:4) for

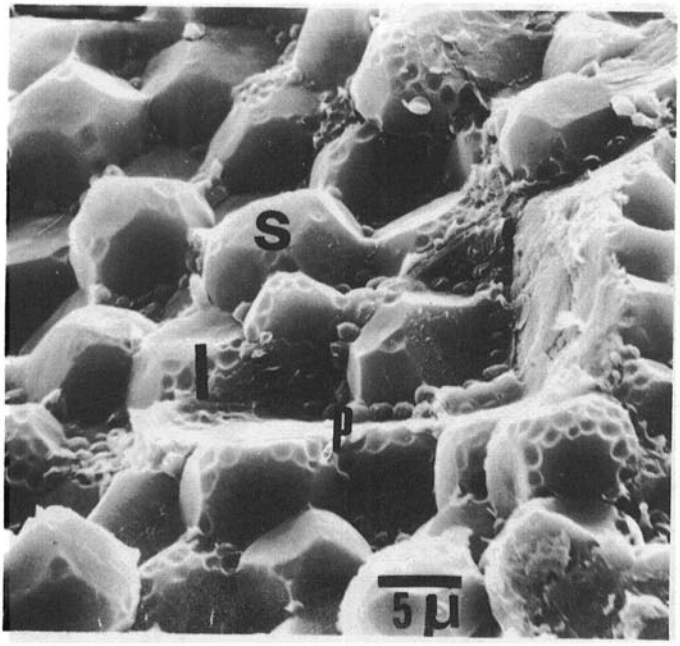

Fig. 2. SEM of hard endosperm portion of millet grain showing protein bodies (P), indentations (I), and starch (S).

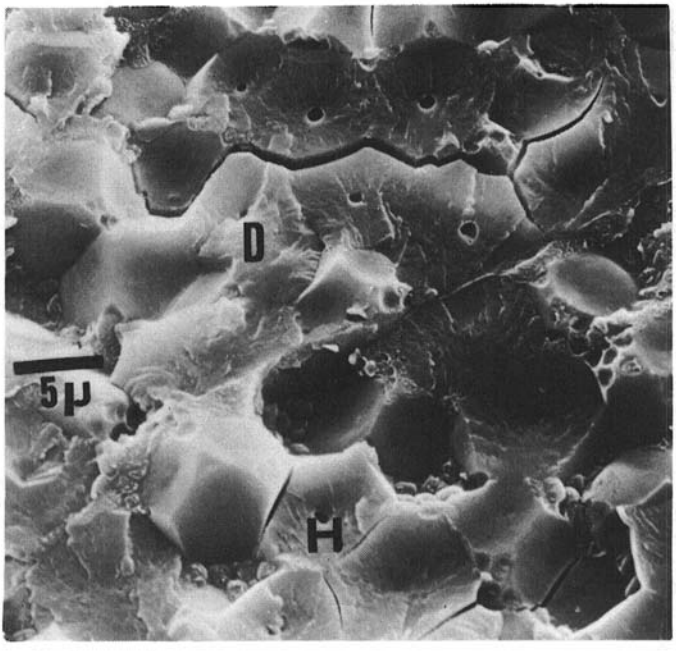

Fig. 3. SEM of hard endosperm part of millet grain, showing damaged starch granules (D) and starch hila (H).

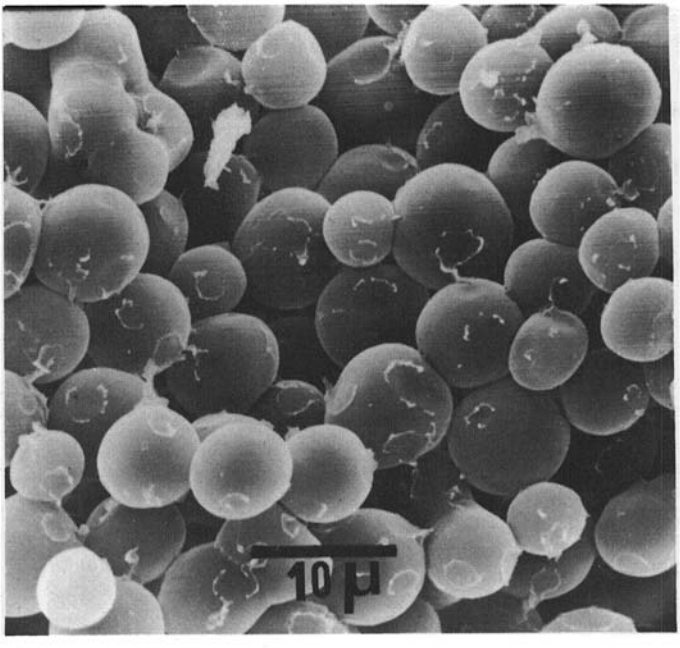

Fig. 4. SEM of soft endosperm part of millet grain.

polar lipids. Plates were sprayed with a saturated solution of potassium dichromate in 55% aqueous sulfuric acid, charred at 150°C for 30 min, and photographed under ultraviolet light.

RESULTS AND DISCUSSION

Pearl millet kernels are about one-third as large as grain sorghum. The pericarp of pearl millet (Fig. 1) appears similar to that of grain sorghum, except that no starch granules are found in millet's mesocarp. The aleurone layer is a single cell thick. The endosperm has both a hard (translucent) and a soft (opaque) part, as do corn and grain sorghum. The hard or translucent part (Fig. 2) has a

TABLE I
Yield, Protein (N × 6.25), and Moisture for Quadrumat-Milled Sorghum and Millet

Fractions	Sorghum			Millet		
	Yield %	Moisture %	Protein %	Yield %	Moisture %	Protein %
Whole grain	...	10.0	9.6	...	10.6	12.3
Bran	46.9	11.5	11.5	42.0	11.2	16.4
Low-grade flour	14.8	12.2	9.3	16.6	12.2	11.3
+9XX flour	33.1	12.5	10.0	31.1	12.6	9.2
−9XX flour	6.3	12.1	5.9	10.3	12.4	7.2
Total extraction	54.2	...	...	58.0	...	...

TABLE II
Amino Acid Composition (g AA/100 g Protein) for Two Sorghums and a Millet[a]

Amino Acid	Bulk Sorghum	Sorghum C-42Y	Millet
Lysine	2.2	2.5	3.6
Histidine	1.7	2.3	2.6
Ammonia	3.0	2.6	2.5
Arginine	3.2	4.0	6.0
Aspartic acid	6.9	6.6	8.2
Threonine	3.6	3.0	4.1
Serine	4.7	4.3	4.9
Glutamic acid	22.3	20.8	19.0
Proline	7.2	8.8	5.9
Glycine	3.4	3.0	3.7
Alanine	9.1	8.6	7.8
Half-cystine	1.7	2.4	2.5
Valine	4.5	4.5	5.2
Methionine	1.2	1.4	1.9
Isoleucine	3.8	3.7	3.9
Leucine	13.1	13.1	9.8
Tyrosine	3.4	3.6	3.5
Phenylalanine	4.9	4.8	5.0

[a]Recovery Kjeldahl protein basis: bulk sorghum, 93.44; sorghum C-42Y, 101.63; and millet, 100.00.

tightly packed structure which contains no air spaces. The starch granules are polygonal and somewhat smaller (10 μ) than those of grain sorghum or corn starch. Rather large (1.5 μ) protein bodies are embedded in the protein matrix that covers the starch. Those protein bodies leave large indentations at the edges of the starch granules.

Strength of the protein-starch bond in the hard endosperm is shown by many fractured starch granules (Fig. 3) when the kernel is fractured. This structure is similar to that in corn (13) and grain sorghum (14). The hila (0.5 μ) are clearly evident in many fractured starch granules and appear to be somewhat smaller in diameter than those in grain sorghum.

The soft endosperm part (Fig. 4) has relatively large air spaces and loosely packed, spherical starch granules. In contrast to the soft endosperm of grain sorghum (14), millet soft endosperm appears to contain no protein bodies.

Dry Milling

Flour yields from the Quadrumat mill for both millet (58%) and sorghum grain (53%) were low, and both flours were gray in color. Higher flour extractions and lighter-colored products were obtained for millet (63%) and grain sorghum (60%), using a Buhler mill for millet and an experimental mill for sorghum grain (15). The improved flour yield and color probably occurred because those mills have greater flexibility than does the fixed-flow Quadrumat.

Yield, protein (N $\times$ 6.25), and moisture values for the Quadrumat-milled samples are given in Table I. Although the protein content of the bran fraction from millet appears high, the percentage of the total grain protein recovered in the flour was approximately constant at 45% for both millet and sorghum grain.

TABLE III
Amino Acid Composition (g AA/100 g Protein) of Millet Fractions[a]

Amino Acid	Fractions			
	Bran	Low-grade flour	+9XX Flour	−9XX Flour
Lysine	4.1	2.6	2.4	2.7
Histidine	2.6	2.3	2.2	2.3
Ammonia	1.7	1.8	1.9	2.0
Arginine	7.0	4.9	4.2	4.5
Aspartic acid	8.7	7.6	7.6	7.9
Threonine	4.1	3.9	3.9	3.9
Serine	4.9	4.7	4.7	4.6
Glutamic acid	17.8	20.5	20.9	20.5
Proline	5.6	6.6	6.6	6.5
Glycine	4.1	3.0	2.8	2.9
Alanine	7.8	8.0	8.1	7.8
Half-cystine	2.9	3.3	3.5	2.8
Valine	5.4	5.3	5.1	5.5
Methionine	2.1	2.3	2.5	2.8
Isoleucine	4.0	4.1	4.2	4.2
Leucine	9.1	10.5	10.5	10.4
Tyrosine	3.4	3.4	3.5	3.6
Phenylalanine	4.9	5.3	5.4	5.3

[a]Recovery Kjeldahl protein basis: bran, 94.97; low-grade flour, 101.43; +9XX flour, 100.28; and −9XX flour, 96.65.

Amino Acid Analysis

The amino acid analyses of millet and sorghum grain (Table II) showed millet to contain more of the essential amino acids. Millet had higher values for lysine, arginine, aspartic acid, threonine, serine, glycine, valine, and methionine, and lower values for glutamic acid, proline, alanine, and leucine than did sorghum grain.

The lysine content of millet used in this study was 3.6 g/100 g protein, comparable to that in high-lysine corn. Analysis of other millet samples of various genetic backgrounds has shown as little as 2.1 g lysine/100 g protein, indicating wide genetic variation for lysine content of pearl millet. The amino acid contents of the three flour fractions from millet were similar (Table III).

Lipids

Free (extractable in petroleum ether) and bound (extractable in water-saturated butanol after extraction with petroleum ether) lipids were extracted from millet, sorghum grain, and wheat. A tlc comparison of the polar and nonpolar lipids in each fraction from the three grains is given in Fig. 5.

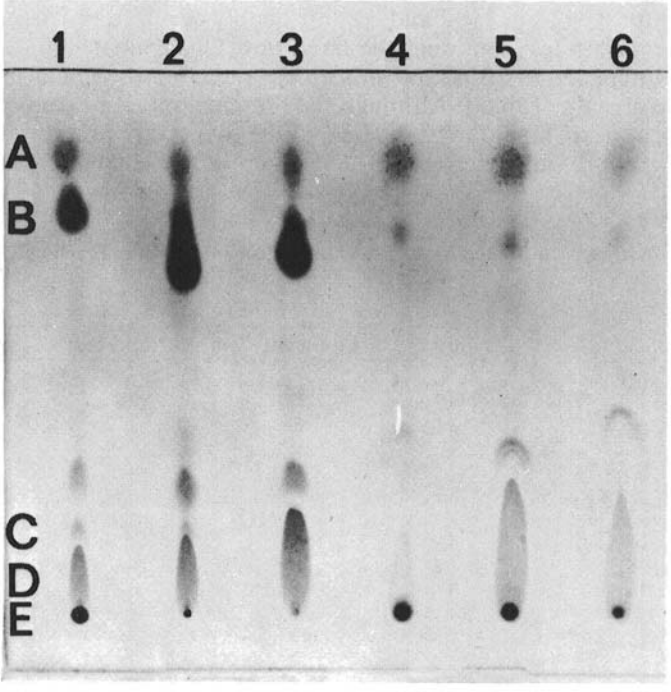

Fig. 5. Thin-layer chromatograms of nonpolar free and bound lipids from wheat, sorghum, and millet. Pattern 1, free wheat lipids; pattern 2, free sorghum lipids; pattern 3, free millet lipids; pattern 4, bound wheat lipids; pattern 5, bound sorghum lipids; and pattern 6, bound millet lipids. The spots are tentatively identified as A, hydrocarbons and steryl esters; B, triglycerides; C, diglycerides; D, free fatty acids; and E, unresolved polar lipids.

The major nonpolar components of the free lipids of all three grains were triglycerides. The bound nonpolar lipids contained the same components in different ratios as did the free nonpolar lipids. The pattern for polar-free lipids (Fig. 6) from wheat differed widely from those for millet and sorghum grain, contrary to a report (16) that millet and wheat contain the same lipids. Neither millet- nor sorghum grain-free lipids contained phosphatidyl ethanolamine, digalactosyl diglycerides, or phosphatidyl choline. The patterns for bound polar lipids were similar for all three grains. The major difference in wheat lipids and those from the other two grains appeared to be in the polar-free lipid fraction.

Solubility of Millet Prolamines

Proso millet prolamines were reportedly soluble in t-butyl alcohol (4), and sorghum prolamines (Karfrin) in 60° C, 70% ethanol or 60% t-butyl alcohol (8,17). Therefore, half-kernels of millet were soaked in 70% ethanol for 3 hr at 60° C, air-dried, mounted, and viewed with the SEM (Fig. 7). Clearly the protein bodies were soluble under those conditions and thus similar to the protein bodies in grain sorghum (14). Half-kernels of pearl millet were also soaked in 100% t-

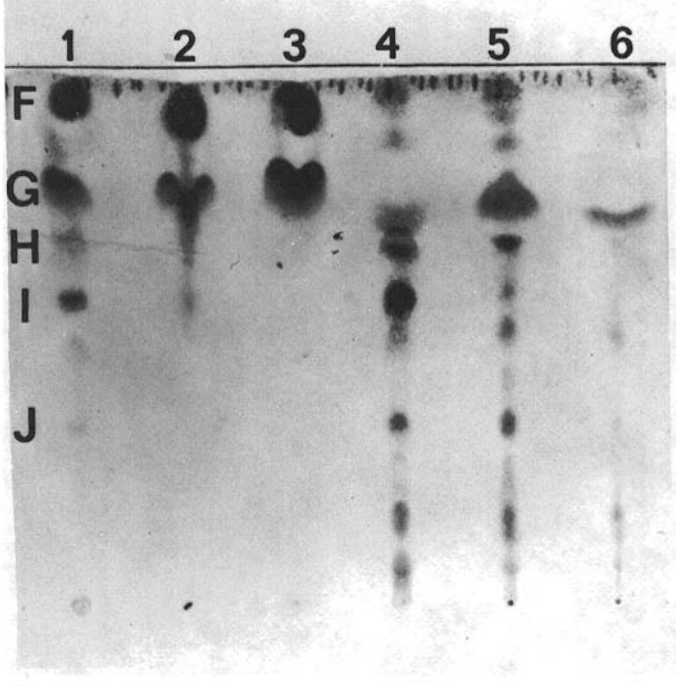

Fig. 6. Thin-layer chromatograms of polar free and bound lipids from wheat, sorghum, and millet. Pattern 1, free wheat lipids; pattern 2, free sorghum lipids; pattern 3, free millet lipids; pattern 4, bound wheat lipids; pattern 5, bound sorghum lipids; and pattern 6, bound millet lipids. The spots are tentatively identified as F, unresolved nonpolar lipids; G. monogalactosyl diglyceride; H, phosphatidyl ethanolamine; I, digalactosyl diglyceride; and J, phosphatidyl choline.

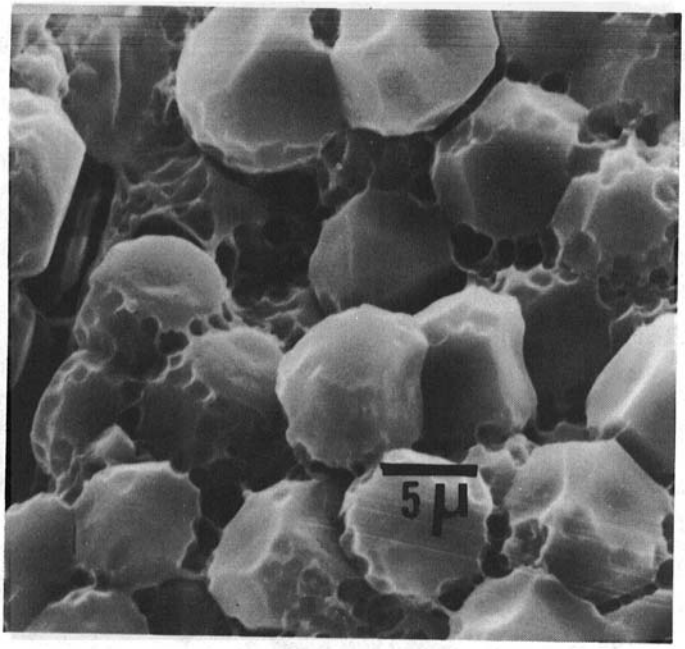

Fig. 7. SEM of millet endosperm treated with 60°C, 70% ethanol.

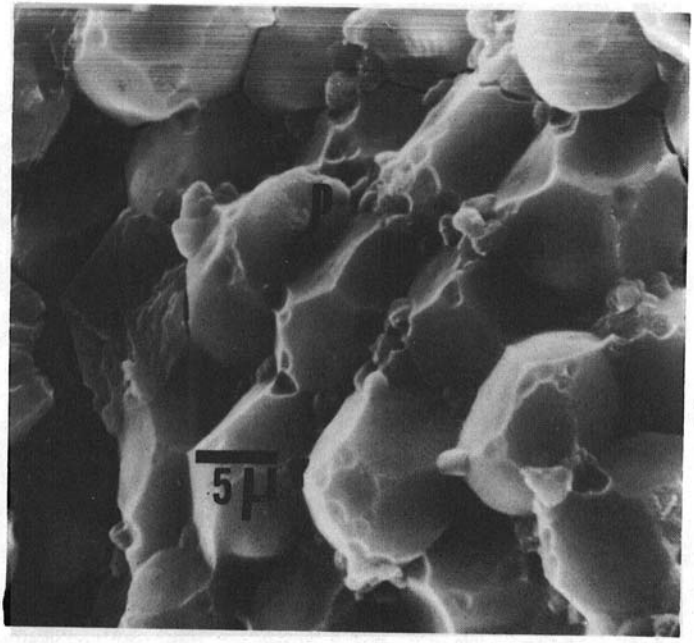

Fig. 8. SEM of millet endosperm treated with t-butyl alcohol, showing protein bodies (P) remaining.

butyl alcohol overnight at room temperature. The protein bodies (Fig. 8) seemed to be unaffected by this treatment; however, the protein matrix was removed. Treatment of ground millet with those two solvent systems showed that 30.7% of the total millet protein was soluble in ethanol and 27.1% in t-butyl alcohol. Thus, there appear to be two alcohol-soluble proteins present in millet.

More vigorous extraction of ground millet in a Waring Blendor gave 44% of the total protein soluble in 60% t-butyl alcohol (60° C) and 42% of the protein soluble in 70% ethanol (60° C). A second extraction with the same solvent (butanol after butanol and ethanol after ethanol) or switched solvents (ethanol after butanol or butanol after ethanol) solubilized an additional 13% of the total protein in all cases. The values for the single extraction are similar to those Freeman and Bocan (2) reported.

Thus, the differences noted by SEM in protein solubility were not apparent when a vigorous extraction and aqueous alcohol mixtures at 60° C were used. More work is needed to clarify the type and solubility of millet proteins.

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[Received January 3, 1975. Accepted September 15, 1975]