Sorghum Protein Ultrastructure as it Relates to Composition[1]

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ABSTRACT

The ultrastructure of endosperm protein from seven hybrids and eight experimental lines was studied with both transmission and scanning electron microscopes. Vitreous endosperm shows a well-developed two-component structure consisting of protein bodies embedded in a matrix protein. The aqueous alcohol-soluble fraction (prolamine) proved to be the major component of globular protein bodies. The surrounding matrix protein consisted mostly of glutelin. Globular protein bodies have a nucleus that is insoluble in aqueous alcohol. Protein bodies of almost all grain sorghums were 2 to 3 μm. in diameter. One experimental line with above average lysine content had smaller protein bodies, a condition which verifies the negative correlation between prolamine and lysine. Distribution of protein within the sorghum kernel is similar to that of other cereal grains. The peripheral vitreous area of the kernel is rich in protein; interior areas have smaller amounts of protein. Microscopic observations show that protein bodies make up the major part of sorghum endosperm protein.

In many respects, sorghum endosperm protein is similar to that of corn. Both proteins contain a large percentage of prolamine (aqueous alcohol-soluble fraction) which is deficient in the essential amino acid lysine (1,2). Attempts to find improved lysine types in existing stocks of sorghum have not been rewarding (3,4).

In contrast, the nutritional value of maize was improved by the discovery that the *opaque-2* mutant had a much higher level of lysine and tryptophan and a greatly reduced zein content compared to normal varieties of corn (5). Microscopically, the altered endosperm protein composition in *opaque-2* was reflected in a marked reduction in size of protein bodies (6), a major site of zein in maize (7). Protein bodies of normal corn are easily resolved in a light microscope, but an electron microscope is required to reveal protein bodies of *opaque-2*.

Sorghum endosperm protein also contains microscopic granules, which could be deposits of prolamine (8). Information, however, is lacking on the microscopic structure of the subcellular protein. In our work, endosperm protein from a number of sorghum varieties was studied by optical and electron microscopy. Protein structure, distribution, solubility, and enzyme digestion were examined.

MATERIALS AND METHODS

Sorghum hybrid varieties were: RS 626, OK 612, TE 77, G 766, C 42y, TX 09, and Cody waxy.

Eight experimental lines of sorghum were supplied by the Department of Agronomy, Purdue University, along with analysis for protein and lysine content.

Optical Microscopy

Observations of endosperm protein were made on destarched sections as described in the glass knife sectioning technique (9). Sections were stained with either dilute I-KI solution or 0.1% acid orange-12 dye C.I. 15970 (AO-12) stabilized in a pH 2 buffer (10).

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Electron Microscopy

Sections of horny areas were prepared by the same technique as for optical microscopy, except 0.2- to 0.5-μm. thick sections were cut on a Porter Blum ultramicrotome. Floury material was embedded in epoxy resin before sectioning. Unfixed sections were floated on distilled water and picked up on collodion-covered copper grids. Protein solubility tests were made by submerging entire grids with sections in various solvents–60% ethanol, 60% *tert*-butanol, or 1% NaCl–at room temperature. Protein was digested with 0.1% Pronase in a pH 7.4 acetate buffer at room temperature. Sections were stained with 1% uranyl acetate and examined in an RCA EMU3F transmission electron microscope at 50 and 100 kv.

Specimens for the scanning electron microscope were coated with gold-palladium and examined in a Cambridge Stereoscan Mark VI microscope.

RESULTS AND DISCUSSION

Normal sorghum endosperm contains about 10% protein, which is unevenly distributed among three main types of cells: subaleurone, vitreous, and floury.

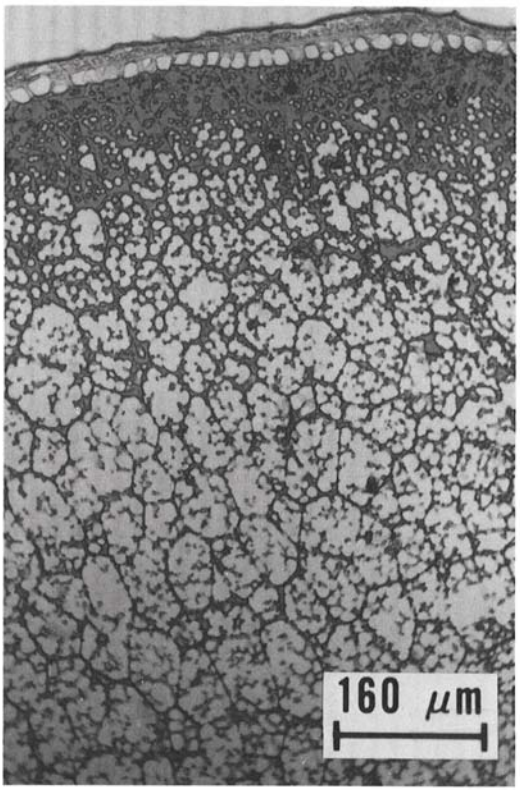


Fig. 1. Optical photomicrograph of destarched cross section of sorghum endosperm showing cell walls and protein network; 7-μm. thich section stained with AO-12 (125X).

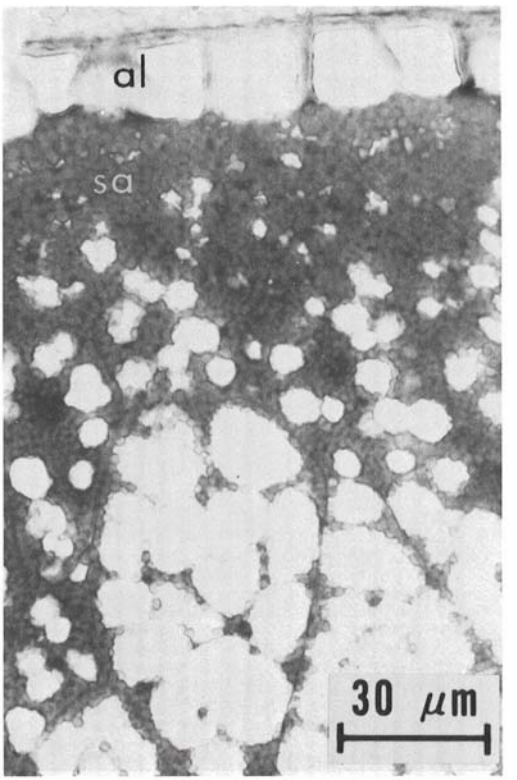


Fig. 2. Optical photomicrograph of destarched section of peripheral area of the kernel showing subaleurone (sa) proteinaceous cells, protein granules, and the aleurone layer (al); 7-μm. thick section stained with AO-12 (650X).

Subaleurone cells are atypical because they contain mostly protein with little or no starch. Vitreous and floury cells are typical starchy endosperm cells, but they differ in density, texture, and protein content. Several facts concerning sorghum endosperm and protein quality can be summarized:

1) Vitreous cells contain about twice as much protein as floury cells (11).
2) The increase in protein content is accompanied by an increase in the prolamine fraction (4).
3) A high prolamine content in protein is undesirable because prolamine has the lowest lysine value of all the protein fractions (albumin, globulin, prolamine, and glutelin) (2).
4) Nutritional studies on milled sorghum products revealed that vitreous endosperm is inferior to floury endosperm because of a deficiency in lysine (11).

Light microscopy can display general structures and protein patterns over large areas of the kernel (Fig. 1). Endosperm cell walls and the protein network are clearly defined after starch has been removed by amylase digestion. One area of

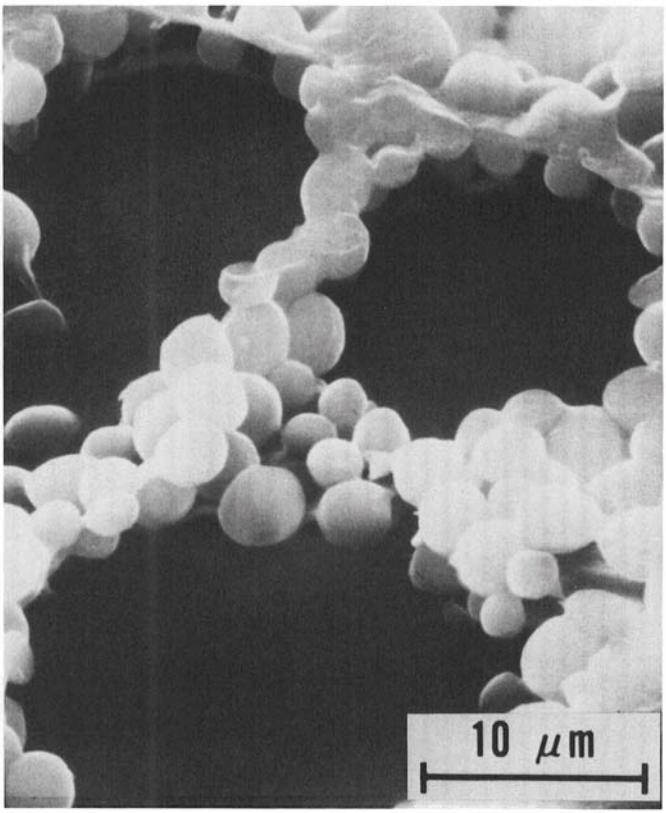


Fig. 3. Scanning electron micrograph of destarched section showing protein granules (3,000×).

particular interest is the subaleurone layer, which has cells almost entirely filled with protein. The thickness of the subaleurone layer is a variety characteristic and is related to total endosperm protein content. The remaining endosperm cells gradually decrease in protein content toward the center of the kernel.

Closer inspection at higher magnification reveals that the protein network has a beaded appearance (Fig. 2). Large areas of subaleurone protein appear as a mass of microscopic granules held together by a protein matrix. Since protein granules absorb much less stain than matrix protein, they are easily observed in the light microscope, providing the granules are more than 1 μm. in diameter.

Increased resolution and large depth of focus of the scanning electron microscope give unmistakable evidence that protein granules exist (Fig. 3). Some granules appear free while others are coated with matrix protein. The nearly spherical granules have a smooth outer surface.

Internal structure of protein is illustrated in Fig. 4, a transmission electron micrograph of a section through the peripheral part of the kernel that has numerous protein granules. Inside almost all the granules is a darkly stained core or nucleus. Around the nucleus some granules also exhibit heavily stained concentric

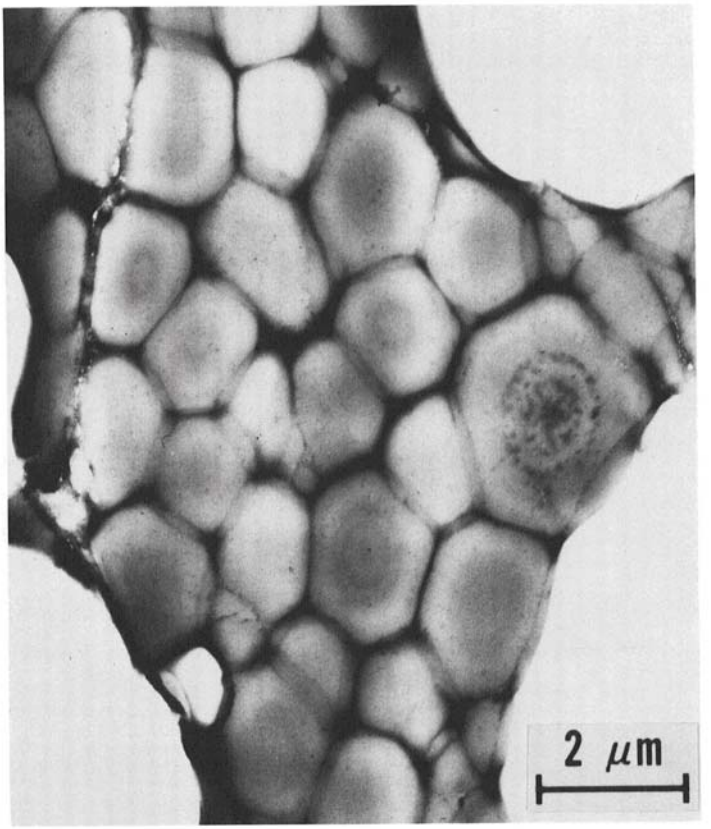
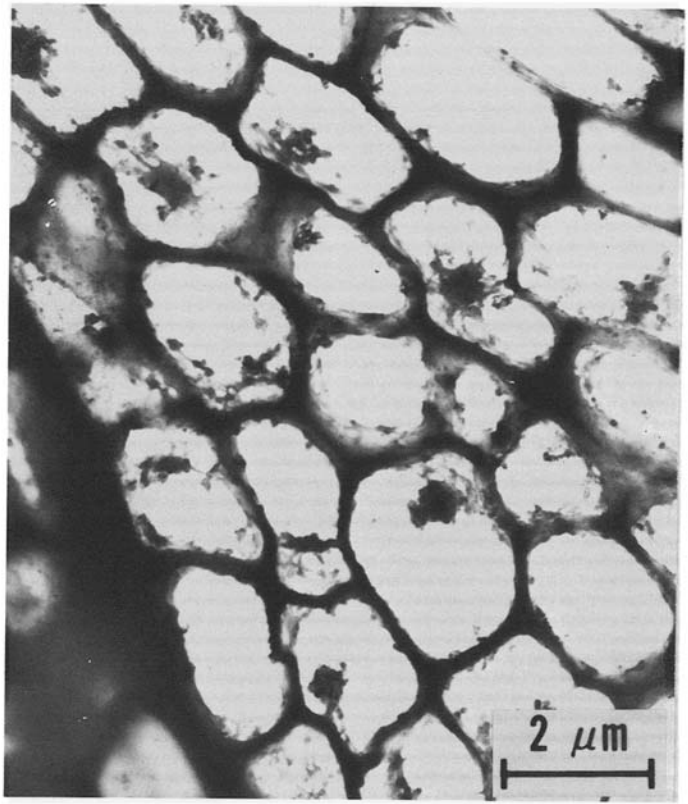


Fig. 4. Transmission electron micrograph of protein granules and matrix stained with uranyl acetate. Granules have darkly stained centers and concentric rings (10,000×).

rings which indicate a spherical laminar structure caused by differences in either densities or composition. The matrix protein shows no internal variation in staining properties. From these pictures, the protein granules appear to constitute the major portion of the endosperm protein.

To establish their nature, protein bodies were subjected to common protein solvents and examined by electron microscopy. Extraction of sections for 16 hr. with water or 1% NaCl had no visible effect on the microscopic structure of the protein. Aqueous 60% ethanol, a common solvent for prolamine, also failed to change protein structure. Treating sections for 1 hr. with 60% *tert*-butanol, however, dissolved the protein bodies without visibly affecting the matrix protein (Fig. 5). The same effect can be achieved by using hot aqueous ethanol. Both *tert*-butanol and hot ethanol have been reported to be good solvents for extracting prolamine from sorghum (2). Thus solubility confirms the idea that protein granules are the major site of the prolamine fraction in sorghum and that the remaining protein contains the glutelin fraction.



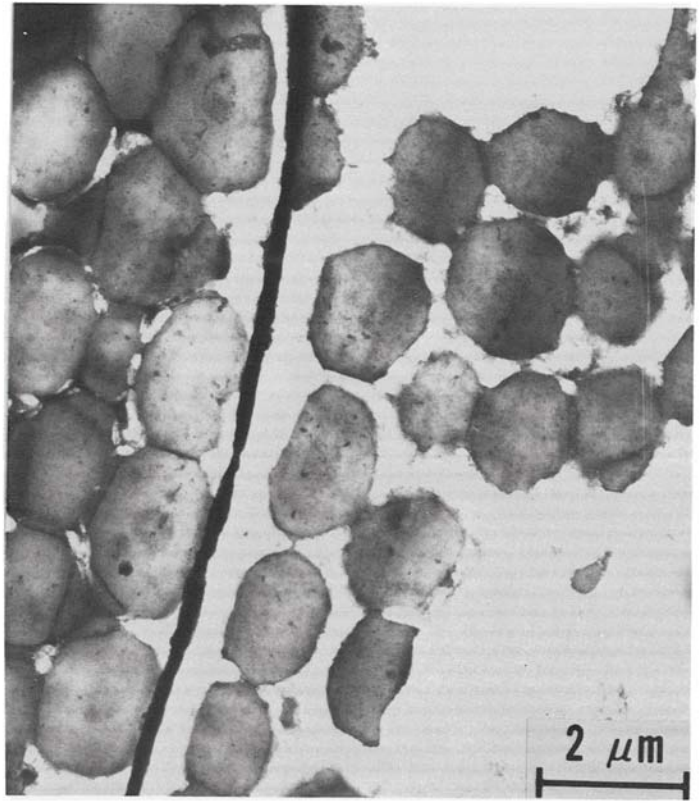
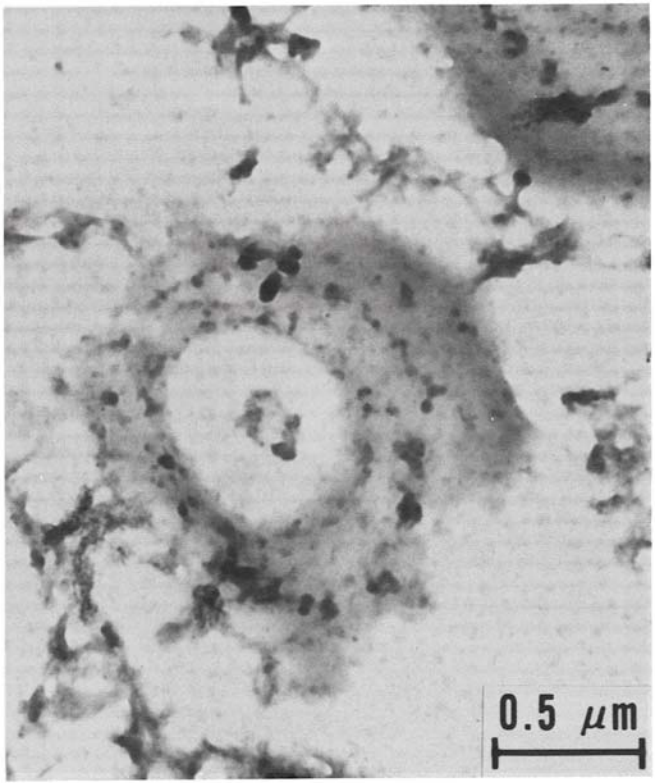


Fig. 5 (left). Transmission electron micrograph of protein after 60% *tert*-butanol extraction, stained with uranyl acetate. Protein granules dissolved, leaving matrix protein and core of granules (10,000×).
Fig. 6 (right). Transmission electron micrograph of protein after 2 hr. Pronase digestion. Matrix protein digested, protein granules intact (10,000×).



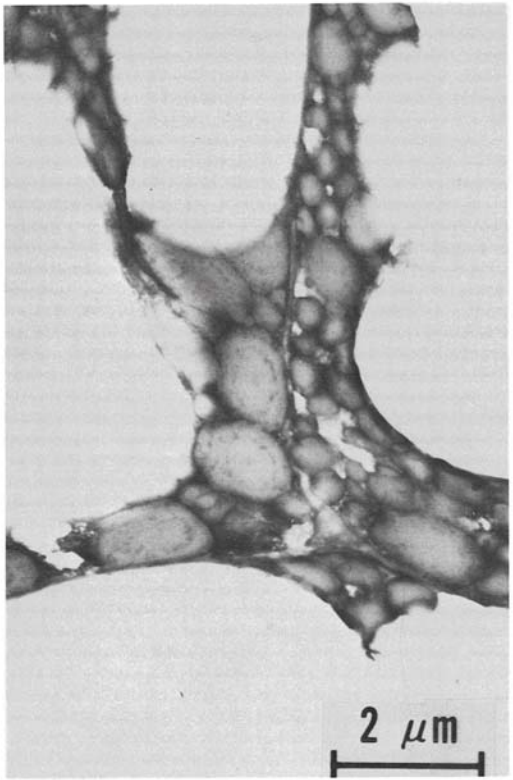


Fig. 7 (left). Transmission electron micrograph of protein granule after 5 hr. of Pronase digestion. Central core is degraded. Ring structure still present (40,000×).

Fig. 8 (right). Transmission electron micrograph of protein from inner portion of kernel showing variation in protein granules (10,000×). Compare with Fig. 4.

Closer examination of extracted sections shows that whereas most of the protein in the bodies has dissolved, frequently a residue remains which was unaffected by the solvent (Fig. 5). This residue is the central core of protein granules. Untreated sections reveal this nucleus as the darkly stained center in protein granules (Fig. 4). On the basis of these solubility properties, protein granules are composed of prolamine and a protein nucleus similar in dye binding and solubility to the matrix protein.

Digestion of the protein with Pronase resulted in a differential degradation that was quite evident after 2 hr. (Fig. 6). The matrix protein was degraded leaving only the protein bodies intact. Judging from the space between granules, some protein had been digested from the outer portion of the granules. Besides providing evidence for the proteinaceous character of the network, digestion also points to a significant difference in digestibility. This difference might provide a method for separating protein granules from other cell protein.

Continued digestion for 5 hr. caused partial degradation of protein granules.

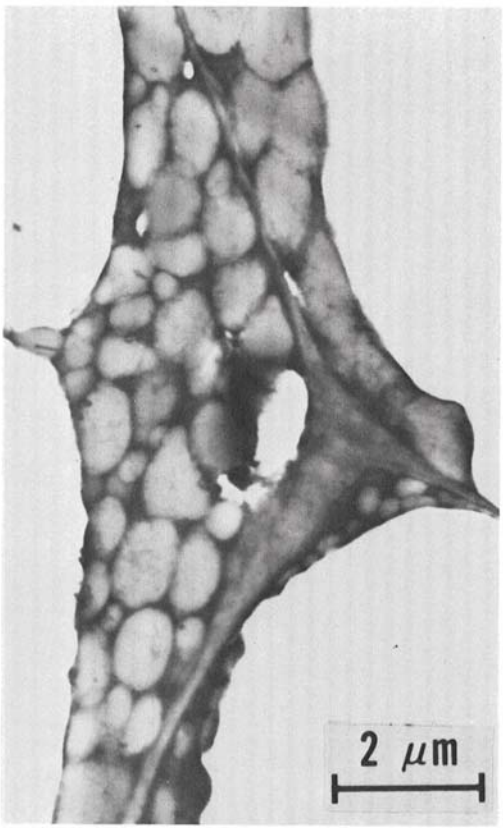


Fig. 9. Transmission electron micrograph of protein of higher lysine sorghum showing numerous small protein granules (10,000×).

Figure 7 shows a granule with the central core completely digested. The remainder of the granule still shows heavily stained material that forms concentric rings. This material is believed to contain a lipoprotein. The way the protein separates from the outer portions of the granule suggests a laminar structure.

The above description and figures illustrate the protein structure which exists in subaleurone and horny areas of the kernel. Protein granules have an average size of about 2 μm. in diameter and are tightly packed within a network of matrix protein. They are easily seen in the light microscope.

The floury endosperm is somewhat different (Fig. 8). Protein granules are not so tightly packed as in horny areas and are much smaller in size, ranging from 0.3 to 1.5 μm. in diameter. Almost all these granules are undetected under a light microscope. The reduction in average diameter of protein granules corresponds to a change in the ratio of protein fractions. The ratio of prolamine to glutelin is higher in vitreous areas than in floury areas (4).

To check the influence of protein granules on composition, selected samples of

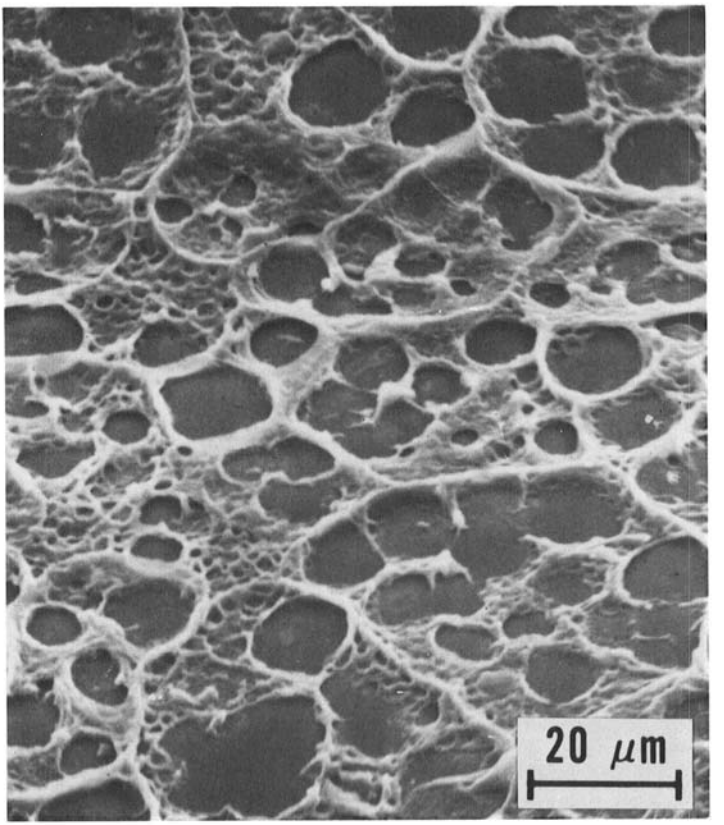


Fig. 10. Scanning electron micrograph of protein matrix of higher lysine sorghum after 60% *tert*-butanol extraction (1,000×).

experimental breeding lines of sorghum, which varied in protein and lysine content, were examined microscopically. Endosperm sections were treated and examined in the same manner as described previously.

Samples with a normal lysine content of 1.7 to 1.9 mg. per 100 g. protein had large protein granules similar in size to those of normal sorghum hybrids.

One sample that had a lysine content of 2.6 mg. per 100 g. protein had much smaller protein granules, which were not resolved in the light microscope. The protein structure, however, was clearly resolved with the electron microscope (Fig. 9). Granules are loosely packed and have an average size below 1 μm. in diameter.

A comparison between electron micrographs (Figs. 8 and 9) shows that the submicroscopic protein structure of the high-lysine sorghum horny cells is similar to that of floury cells in ordinary sorghum. Therefore, only the outer peripheral parts of the kernel should be examined in determining size of protein granules in relation to prolamine content.

Extraction of a thick section from high-lysine sorghum with 60% *tert*-butanol for 3 hr. dissolved the protein granules leaving the matrix protein intact (Fig. 10). Note the small holes that were the former sites of protein granules.

CONCLUSIONS

Microscopic observations show that the concentration of protein within the endosperm of grain sorghum varies with texture. Vitreous cells of the subaleurone layer on the one hand contain mostly protein with little or no starch. Floury endosperm cells, on the other hand, contain mostly loose starch with a small percentage of protein. Normal vitreous areas have cells tightly packed with starch granules that are held together with a protein network. This network consists of microscopic protein granules embedded in a protein matrix. The protein granules are soluble in aqueous alcohol solutions, a property that defines the prolamine fraction. The remaining matrix protein contains the glutelin fraction.

Protein granules have a nucleus that has solubility properties similar to those of matrix protein but is different in other respects. A laminar structure is suggested for the protein granules. Matrix protein shows no signs of an internal structure.

Correlating size of protein bodies with prolamine content can provide an indirect measure of the lysine content and suggests that an evaluation of protein quality can be made by microscopic examination.

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