Modified Opaque-2 Corn Endosperms. II. Structure Viewed with a Scanning Electron Microscope [1]

J. L. ROBUTTI[2], R. C. HOSENEY[3], and C. E. WASSOM[3]

ABSTRACT

Scanning electron micrographs of soft endosperms from normal, *opaque-2*, or modified *opaque-2* corn showed loosely packed, nearly round starch granules associated with thin sheets of protein and many intergranular air spaces. The hard endosperms had tightly packed, polygonal starch granules associated with a continuous protein matrix, and no intergranular air spaces. Normal hard endosperms had zein bodies embedded in the protein matrix; modified hard endosperms did not. Starch damage was greater in the hard endosperm than in soft because of a stronger adhesion between starch and protein. The low density and opaqueness of soft endosperm were attributed to the intergranular air spaces. Interaction between protein matrix and starch granules during drying explains the shape of starch granules.

To explain the difference between soft and hard corn endosperms, Duvick (1) hypothesized that the cytoplasmic protein ruptures upon drying, thus forming voids. He attributed the opaqueness of soft endosperm to light refraction resulting from air in those voids. In translucent endosperm, he reasoned, granular inclusions—absent in soft endosperm protein matrix—that fill the voids prevent light refraction. Histochemical tests produced some evidence that the granular bodies were zein. Christianson et al. (2) isolated the protein bodies and characterized them as zein by their solubility in 70% EtOH, amino acid composition, and starch-gel electrophoretic mobilities. Transmission electron micrographs (3) showed the presence of zein bodies (about one-tenth the size of those in normal corn hard endosperm) in *opaque-2* endosperms.

Lambert et al. (4) reported that *opaque-2* kernels are less dense than their normal counterpart and that the softer endosperm makes *opaque-2* kernels more susceptible to cracking by mechanical action. Scanning electron micrographs (5) of soft and hard portions of sorghum endosperm have shown that the starch granules of soft endosperm are loosely associated with papery sheets of protein materials and that hard endosperm is tightly packed in a rigid protein matrix.

In this study we characterized the structure of normal, *opaque-2*, and hard and soft portions of modified *opaque-2* endosperms of corn.

MATERIALS AND METHODS

Kernels from some samples previously described (6) were cut transversely with a razor blade, which produced a fracture rather than a clean cut. The transverse sections were not smooth and planar but irregular. The cut kernels were mounted on aluminum stubs with Delco No. 93 colloidal silver, coated with a 150-Å-thick gold-palladium layer, and viewed and photographed in an ETEC Autoscan scanning electron microscope.

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[1]Contribution No. 819, Department of Grain Science and Industry, No. 1347, Dept. of Agronomy, Kansas Agricultural Experiment Station, Manhattan 66506.

[2]Graduate Student, Department of Grain Science and Industry, Kansas State University. Present address: Estacion Experimental Agropecuaria Pergamino, C. C. 31. Peramino (B.A.) Argentina.

[3]Respectively: Associate Professors, Departments of Grain Science and Industry and Agronomy.

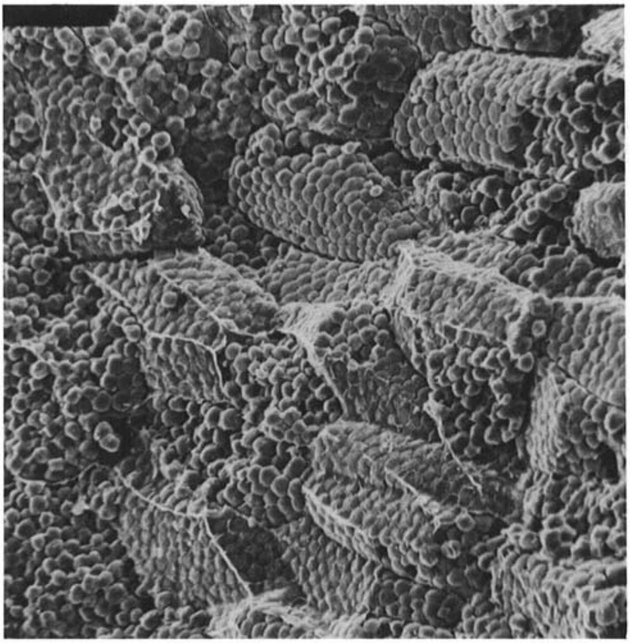

Fig. 1. Scanning electron photomicrograph (140X) of corn endosperm showing the cellular structure.

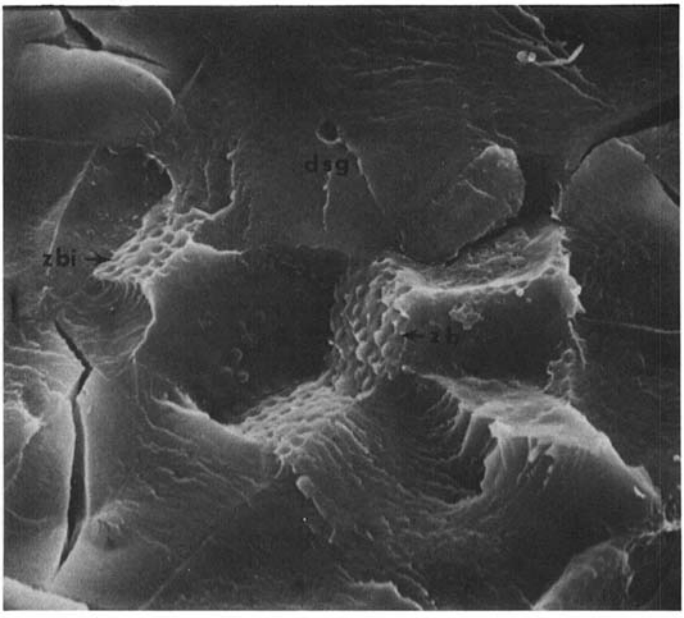

Fig. 2. Scanning electron photomicrograph (2300X) of normal V181 hard endosperm, showing damaged starch granules (dsg), zein bodies (zb), and indentations made by zein bodies (zbi).

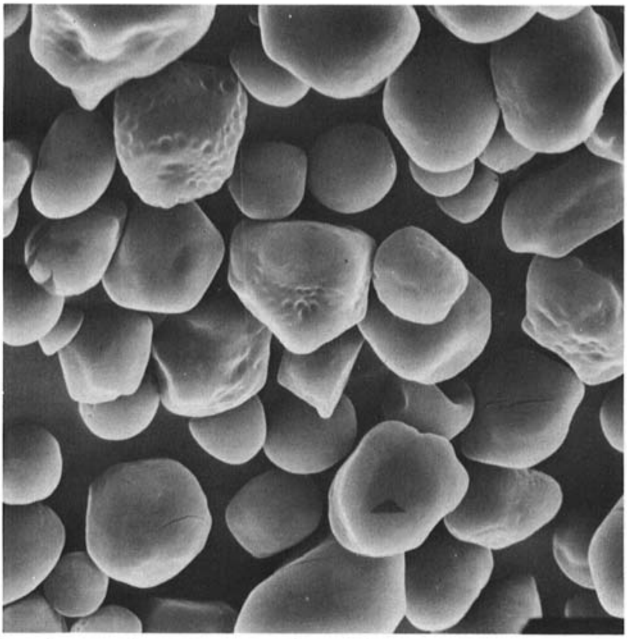

Fig. 3. Scanning electron photomicrograph (1600X) of commercial corn starch showing the zein bodies' indentations.

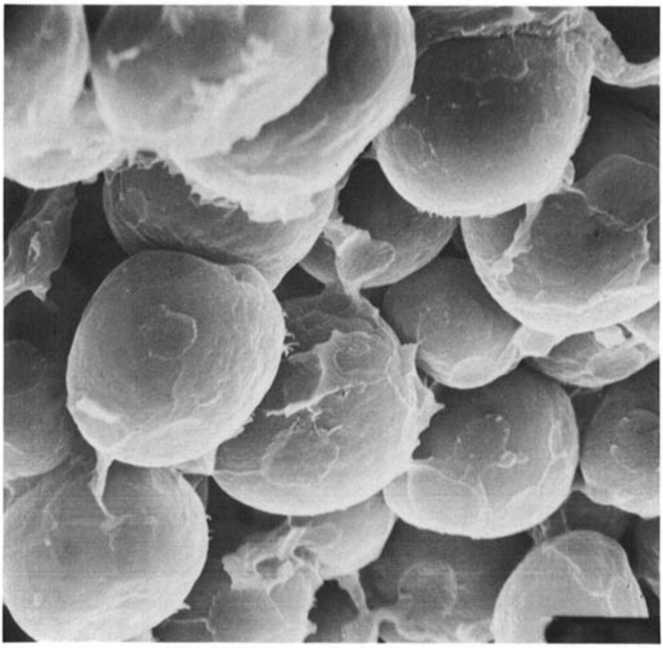

Fig. 4. Scanning electron photomicrograph (2900X) of an *opaque-2* endosperm.

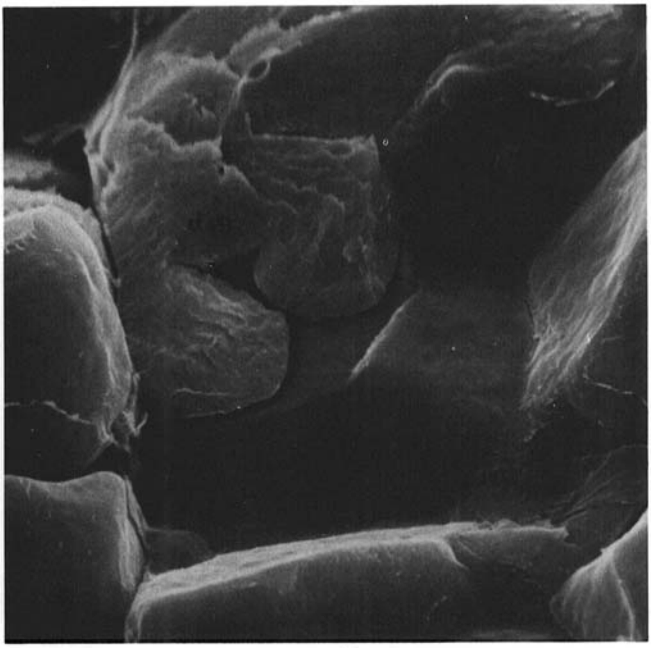

Fig. 5. Scanning electron photomicrograph (2900X) of modified V181 hard endosperm showing damaged starch granules (dsg) and no zein bodies.

RESULTS AND DISCUSSION

Scanning Electron Micrographs

The cellular structure of corn endosperm is shown in Fig. 1. Several cells can be seen distinctly: some, cut open by the razor blade, expose the starch granules and protein matrix; others have their walls intact and can be observed in their entirety. The picture is of opaque corn; similar structures, however, have been noted in normal corn.

The starch granules in hard, normal endosperm are polygonal (Fig. 2), tightly packed with no air spaces, and apparently enclosed in a thin continuous layer of protein. Embedded in that protein matrix are small spheroidal bodies known to be zein (1,2). Figure 2 shows both zein bodies (zb) and indentations (zbi) left in the starch surface by bodies ripped off by the razor blade; also evident are starch granules damaged (dsg) by the cutting (fracture). A sample of commercial corn starch (Fig. 3) also shows indentations left by zein bodies.

A photomicrograph of *opaque-2* endosperm (Fig. 4), in contrast with that of normal endosperm (Fig. 2), shows nearly round starch granules. The protein matrix, however, is continuous (except where torn by the cutting) and appears to enclose the starch granules, which are not tightly packed; the result is many intergranular air spaces. That kind of difference between soft and hard endosperm also has been found for sorghum (5).

A photomicrograph of the hard endosperm of modified V181 (Fig. 5) shows

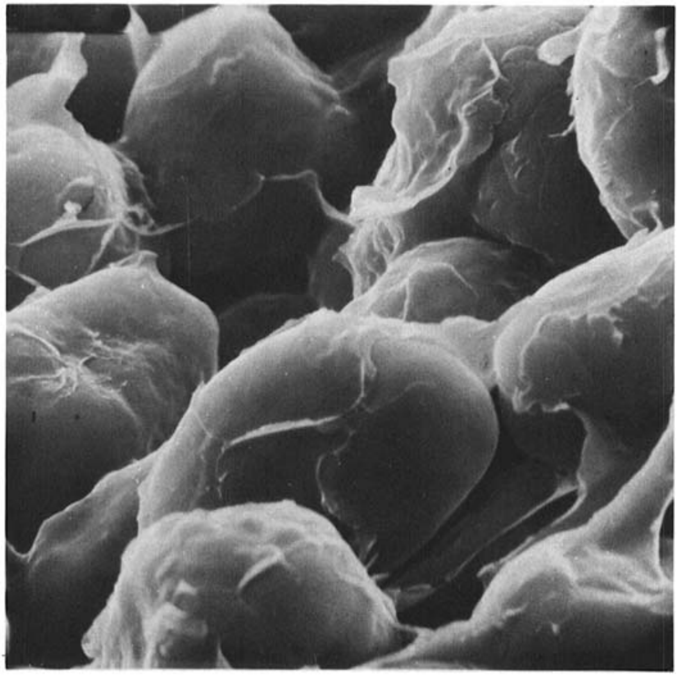

Fig. 6. Scanning electron photomicrograph (2900X) of modified V181 soft endosperm.

angular, tightly packed starch granules similar in appearance to those of a normal hard endosperm (Fig. 2). However, there is one distinctive difference: the modified protein matrix does not have zein bodies. The photomicrograph of the soft endosperm of modified V181 (Fig. 6) reveals granules similar to those of opaque corn endosperm (Fig. 4).

The scanning electron photomicrographs supported the protein and amino acid data (6). They showed zein bodies embedded in the protein matrix of hard normal endosperms (which had a high alcohol-soluble protein fraction) but none in hard modified endosperms, even though both had the same general endosperm structure (polygonal, tightly packed starch granules in a continuous protein matrix). Modified hard endosperms were found to be much lower in alcohol-soluble protein (about 14%) than were normal corn endosperms (about 40%).

Physical Properties

Based on the scanning electron photomicrographs and the chemical data, several conclusions on the structure of corn seem warranted. The soft endosperm's loose structure and high proportion of intergranular air spaces should explain the lower density reported for opaque materials. Those air spaces would cause the opaqueness, by refracting light, as Duvick (1) pointed out. By allowing the endosperm to give under stress, the air spaces, loosely packed starch granules, and fine protein matrix would be responsible for its softness.

Duvick (1) had hypothesized that the hard endosperm was transulucent because the intergranular spaces were filled with zein bodies. Based on the scanning electron

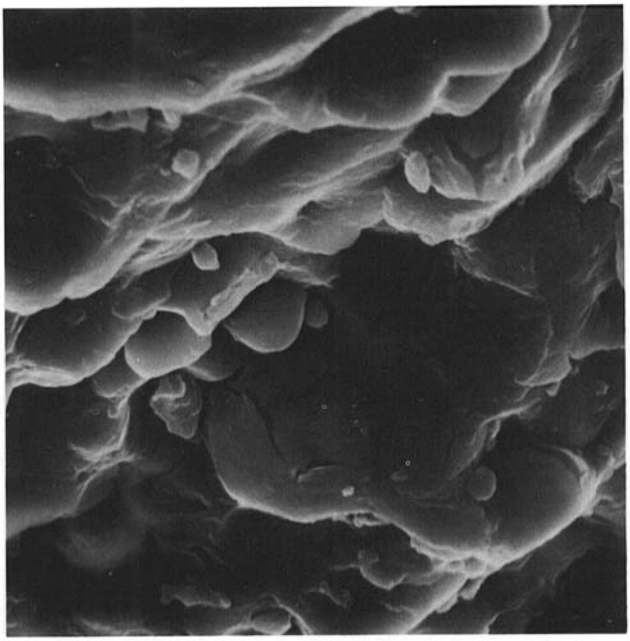

Fig. 7. Scanning electron photomicrograph (1600X) of commercial corn grits.

micrographs, we found very few intergranular spaces in the hard endosperm; those that were present were filled with the protein matrix, thus leaving no intergranular air space and therefore giving the translucent appearance. We suggest that the tight packing of the starch granules–rather than the amount or type of the protein in the matrix–is responsible for the translucency. That would account for our finding modified hard endosperms that are translucent and contain few or no zein bodies. The absence of zein bodies confirms the findings of other authors (3,7,8). However, even though the protein bodies were found only in normal hard endosperms, the proportion of zein in the other types of endosperms was not negligible (6). Christianson et al. (2) suggested that zein was deposited only in zein bodies; if true, the zein bodies in soft endosperms or modified hard endosperms are so small they are not visible under the scanning electron microscope. Transmission electron micrographs (3) showed that *opaque-2* endosperms contained zein bodies, which were about one tenth the size of those in normal hard endosperm.

The hard endosperm's tightly packed starch with the tightly adhering protein matrix would not be expected to give under external stress, and thus would be responsible for its hardness.

Shape of the Starch Granules

That starch granules from the floury and from the horny endosperms differ in shape has been noted previously (9). One possible explanation of why a single kernel of grain should have those two starch types is that during the natural drying process the protein loses water and shrinks. If the adhesion within the protein is strong enough, the starch granules are pulled closer and closer together. If the starch granules still contain enough water to be somewhat flexible at this stage, the result would be polygonal, tightly packed granules. Evidence for the flexibility of starch during the drying process is the indentation made by zein bodies in the starch of the hard endosperm (Fig. 2). In the soft endosperm (6) protein distribution and amino acid composition are quite different. Should that distribution give a weaker adhesion of the protein-protein bonds which rupture during drying, the result would be intergranular air spaces and round starch granules.

Wrigley (10), working with wheat, reported that the amount of water-soluble protein surrounding starch granules in hard wheat was triple the amount in the soft isogenic line in two varieties studied. For that to apply to corn, most water-soluble protein in hard endosperms would have to be attached to the surface of starch granules, because we found that in corn hard endosperms are lower in the water-soluble fraction that are soft endosperms.

Starch Damage

If sufficient force is applied, the corn kernel fractures. If the strength of adhesion between protein and starch is less than that within the starch granule, the fracture should leave intact starch granules and exposed protein surfaces. We found that to be the case for soft endosperms (either *opaque-2* or modified) as shown by the photomicrographs (Figs. 4 and 6). Photomicrographs of hard endosperms (Figs. 2 and 5) reveal a predominance of fractured starch granules, indicating stronger adhesion between protein and starch than within the starch granules.

The degree of such starch damage might raise questions about the amount of starch damage in dry-mill corn products. Corn grits, examined under the scanning electron microscope (Fig. 7), revealed the same fracture patterns, indicating the degree of starch damage increases tremendously as particle size decreases. Corn flour has a limited market, perhaps at least in part because of its high degree of starch damage. In the wet-milling process, corn is soaked in acid solution, which weakens the protein-starch bond and allows the starch to be separated.

Acknowledgments

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