Structure of Corn Starch Paste and Granule Remnants Revealed by Low-Temperature Scanning Electron Microscopy after Cryopreparation[1]

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ABSTRACT

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To determine any correlation between the ultrastructure of corn starch pastes and the functional properties on which their applications are based, starch pastes and granule remnants were plunge-frozen in melting nitrogen and examined by low-temperature scanning electron microscopy. In all cases, pastes were composed of a continuous phase of bundles of molecules surrounding a discontinuous phase of swollen granule remnants commonly referred to as ghosts. Differences between starches were seen in the amount and size of the continuous phase and in the strength and appearance of the ghosts. Because ghost structure is important to starch paste prop-

erties, isolated ghosts were also examined to determine a relationship between ghost structures and functional properties of starches. The ghosts, like the pastes, showed structural differences that correlated strongly with the rheological behavior of each starch type. Low-temperature scanning electron microscopy should be useful as a rapid method for screening starch hybrids with unknown properties because the ultrastructures observed, although they may be somewhat artifactual, correlated with the functional properties of the starch.

Corn starch is used by the food and beverage, paper, textile, cosmetic, mining, petroleum, and other industries. The key to any starch use resides primarily in the behavior of its pastes. Much of the corn starch used in the food industry to control viscosity, texture, and mouthfeel and for other functionalities is chemically modified to enhance its rheological behavior. Rheological behavior depends on the base starch, i.e., the cultivar of corn, and the type of modification used. The corn wet-milling industry is now emphasizing the development of hybrids that produce native starches that behave like chemically modified starches. A rapid method to determine the functional properties of small amounts of starch from new genetic backgrounds would be useful.

An underlying factor controlling the rheological properties of starch pastes and gels is the ratio of the constituent polymers, amylose and amylopectin. The relative amounts of these two polymers in corn starches can be manipulated genetically. Linear amylose molecules can easily associate and form large junction zones and a gel structure. Upon cooling, these junction zones continue to grow and lead to syneresis. Amylopectin is less prone to gel and retrograde and its gels are less prone to syneresis because of its branched structure.

Another genetically controlled factor affecting rheological behavior is the chemical structure of the amylose and amylopectin molecules themselves. For example, a change in the number of branches or the length of side chains in amylopectin creates significant changes in the functional properties of starches containing 100% amylopectin molecules (Wienen et al, *unpublished data*).

Corn starch occurs in granules that are mostly polygonal, but generally spheroid, and average about 10 μm in diameter. The surface of the granule remains as a remnant after cooking under light to moderate shear. The remnant, or ghost as it is commonly called, is important to paste and gel structure and properties.

The ghost can be structurally strengthened by cross-linking, which strongly affects the functional properties of starches by imparting increased stability of the granules to heat, shear, and acid. Unmodified granules of some corn cultivars also produce pronounced ghosts when cooked, indicating a naturally stronger shell.

Gelatinization of starch is a "phase transition from order to disorder" (Ghiasi et al 1982). It is characterized by uptake of heat, loss of crystallinity and birefringence, hydration of starch

molecules, granule swelling, increased suspension viscosity, and decreased relaxation time of water molecules. Starch granules in water at room temperature hydrate, swell slightly, and lose some amylose via leaching (Miller et al 1973). Upon heating, granules continue to imbibe water, lose amylose, and swell, and the suspension becomes more and more viscous. Viscosity at this point is usually ascribed to granule swelling. With continued heating, the granules eventually burst and collapse, producing a ghost (Hoseney et al 1977), a process termed pasting. As the granules collapse, viscosity decreases rapidly. Upon cooling, the dispersed starch molecules go through a reassociation process called retrogradation, and the viscosity rebuilds.

In a starch paste or gel, granule ghosts are embedded within the molecular network produced by, at least, partial molecular association. According to Rockland et al (1977), ghosts are the only identifiable structures that characterize completely gelatinized starch granules. They are flexible and diaphanous, lack their original contents, and are probably filled with the same solution that constitutes the continuous phase (Bowler et al 1980). They are isotropic. While at least some of their constituent molecules have not been molecularly dispersed as have those in the rest of the granule, ghost structures are not the original structures of the outer shells. Rather, ghosts remain only partially intact (to a degree that depends on the shear). So surface composition and structure appear to differ from the composition and structure of the interior of the granule; how is unknown.

The current model of paste and gel structure is that of a three-dimensional matrix of dispersed molecules surrounding the penetrating swollen, collapsed granule ghosts (Morris 1990). According to this model, the major elements influencing functional properties are the rigidity and extent of the continuous phase of dispersed molecules, the rigidity of ghosts, the interaction of ghosts with the dispersed molecules, and the volume occupied by the ghosts (Morris 1990). Changes in molecular or granular structures that affect any of these four elements of the model produce changes in the functional properties of the paste or gel.

In earlier studies of gel ultrastructure, samples were dehydrated by either a combination of solvent exchange and critical point drying or by freeze-drying alone (Hill and Dronzek 1973, Hood et al 1974, Chabot et al 1976, Colombo and Spaeth 1981, Christianson et al 1982, Williams and Bowler 1982). However, dehydration by these methods introduces structural artifacts (Chabot 1979, Varriano-Marston et al 1985, Bowler et al 1987, Sargent 1988). A major shortcoming of freeze-drying is that artifacts can arise from both the freezing and the drying processes.

The objective of this research was to develop a rapid method for revealing starch paste and gel structures that would be useful in determining the functional properties of the starch and to correlate structures with the functional properties of known starch types to develop an index of information.

MATERIALS AND METHODS

The following sources of corn starch were used: 1) common (yellow dent) corn starch (A. E. Staley Manufacturing Co., Decatur, IL), 2) waxy maize starch (A. E. Staley Manufacturing Co.), 3) waxy maize starch (Polar Gel 15, American Maize Products Co., Hammond, IN) "lightly" cross-linked (with phosphate) and stabilized (hydroxypropylated, 3.0–4.5%), 4) common corn starch cross-linked with succinate (prepared in our laboratory), 5) common corn starch (Penford Gum 290, Penford Products Co., Cedar Rapids, IA) hydroxyethylated (~2% ethylene oxide) and acid-thinned (~700 mPa·sec^{-1} at 30% solids and 65°C), 6) commercial dull waxy corn starch (American Maize Products Co.), and 7) commercial high-amylose (50%) corn starch (American Maize Products Co.).

To prepare ghosts, a 0.2% slurry of each starch was heated to boiling (100°C) on a hot plate with moderate stirring for 15 min. The ghosts were separated from the gel network by centrifugation in an Eppendorf centrifuge (Brinkmann Instruments, Westbury, NY) for 1 min, and the pellet was resuspended in hot water (100°C) and centrifuged again. This washing process was repeated three times. Specimens were maintained at a temperature close to 100°C during this procedure and while they were held in a concentrated suspension before the cryopreparation described below.

To prepare pastes, 4% slurries of the starch were heated in a boiling (100°C) water bath for 15 min with only moderate stirring. The hot pastes were immediately cryoprepared, as described below, and examined.

For low-temperature scanning electron microscopy (LT-SEM), a small drop of the hot ghost suspension or hot paste was placed on perforated copper foil attached to a brass Hexland specimen holder (Oxford Instruments North America, Bedford, MA), which was then plunged into nitrogen slush (approximately −210°C). The frozen specimens were transferred, under vacuum, to a Hexland CT-1000 cold stage installed on a JEOL-JSM 840 scanning electron microscope (JEOL U.S.A., Peabody, MA), where they were fractured. Specimens were then transferred to the microscope specimen chamber and sublimed at −90°C for 15 min. They were then returned to the Hexland cold stage for sputter coating with ~15Å of gold. Finally, they were returned to the specimen chamber and examined at an accelerating voltage of 8.0 kV at a temperature less than −160°C.

RESULTS

Unmodified starches produced ghosts that appeared as flimsy networks of aggregated polymers. The expanded surface collapsed and showed signs of deterioration, for example, rips and tears, both while in the gel network and after isolation (Figs. 1A and 2A). Appearances of common corn (Fig. 1A) and waxy maize (Fig. 2A) starch ghosts differed. Pastes from waxy maize starch (Fig. 2B) had much less open structure than those from common corn starch (Fig. 1B).

Ghosts isolated from the modified starches differed significantly from the ghosts of native starches. Walls of the ghosts produced from cross-linked starches were much thicker and more substantial (Figs. 3A and 4A) and did not show as much deterioration or collapse. They remained as extended balloonlike structures in the gel and when isolated. The greater the degree of cross-linking, the more pronounced were these structures. In micrographs of the stabilized and acid-thinned starch, ghosts appeared to be composed of a network similar to that of unmodified common corn starch ghosts (Fig. 5A).
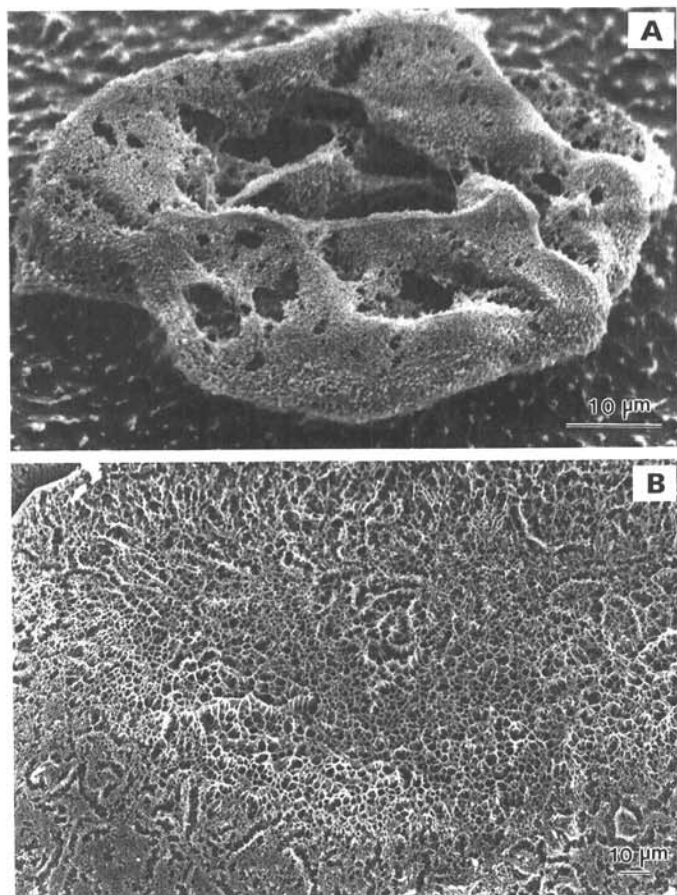

Fig. 1. Scanning electron micrographs of cryoprepared components of a common corn starch paste viewed by low-temperature scanning electron microscopy. A, granule remnant isolated from a 0.2% starch paste (×1,500); B, 4% starch paste (×500).
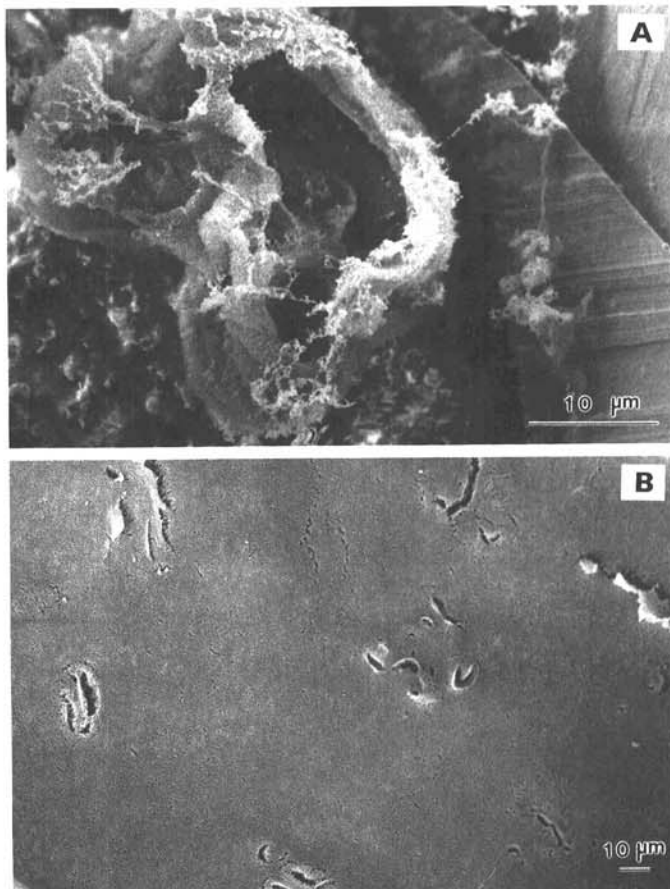

Fig. 2. Scanning electron micrographs of cryoprepared components of a waxy maize starch paste viewed by low-temperature scanning electron microscopy. A, granule remnant isolated from a 0.2% starch paste (×2,000); B, 4% starch paste (×500).

Different cultivars of corn also produced different granule ghosts, indicating a genetic control of ghost structure when starch pastes were prepared and components visualized in this manner. Ghosts from dull waxy corn starch, while appearing similar to those of waxy maize starch, were finer in structure and did not collapse (compare Fig. 6A to Fig. 2A), appearing more like a cross-linked starch in that respect (Figs. 3A, lightly cross-linked, and 4A, heavily cross-linked). Ghosts of high-amylose starches (Fig. 7A) were thick-walled, substantial, extended hollow spheres, similar to those of heavily succinate cross-linked (surface cross-linked) common corn starch (Fig. 4A).

Paste structures appeared much like the model description of a gel. In general, they were composed of netlike structures, presumably formed by molecular associations of the polymers, perhaps as a result of the cryopreparation, that intertwined in a three-dimensional fashion throughout the continuous phase surrounding the granule ghosts. Common corn starch with about 25% amylose produced a coarse, open network (Fig. 1B). Waxy maize corn starch, containing 100% amylopectin, produced a fine network (Fig. 2B).

Pastes of the cross-linked starches consisted mostly of granule ghosts with varying amounts of interspersed network. If the starch was lightly cross-linked, as with the commercial waxy maize starch, the ghosts were enhanced, but a network of associated dispersed molecules was still visible (Fig. 3B). If the starch was highly cross-linked, as with the laboratory-prepared cross-linked common corn starch, there was very little visible network of leached molecules, and the walls of the ghosts were thick (Fig. 4B). The acid-thinned, hydroxyethylated (stabilized) common corn starch formed a paste structure between that formed from common corn starch and that formed from waxy maize starch. Although it has the same amylose content, the network showed

finer detail than that for unmodified corn starch (Fig. 5B), in keeping with its much lesser tendency to undergo retrogradation.

Although ghosts from the dull waxy corn starch had characteristics of those of a cross-linked starch, pastes produced from this starch differed in appearance from those of cross-linked common corn starch (compare Fig. 6B to Figs. 3B and 4B); in dull waxy corn starch pastes, ghosts were not the principal structural component, as they were in the pastes of the lightly (Fig. 3B) and heavily (Fig. 4B) cross-linked starches. Instead, the continuous phase was more prominent. The structure of the continuous phase appeared to fall between that of the coarser, more open structure of common corn starch paste and the finer textured structure of pastes of waxy maize starch and the hydroxyethylated and acid-thinned common corn starch, and it was closer in appearance to that of the finer-textured pastes.

When the starches were cooked in the manner described, pastes from high-amylose corn starch (50% amylose, Fig. 7B) looked very much like the highly succinate cross-linked common corn starch pastes (Fig. 4B). As with heavily cross-linked starches, swelling of and leaching from high-amylose corn starch granules is severely limited, and the extent of the continuous matrix is reduced.

DISCUSSION

These electron micrographs confirm the current model of a starch paste or gel, i.e., they all show, to varying extents, a continuous phase of dispersed molecules surrounding a discontinuous phase of granule ghosts. Factors that affect the functional properties of the gels and pastes are the 1) size of the openings in the continuous phase (matrix), 2) volume fractions of the continuous phase and granule remnants, 3) rigidity of the continuous
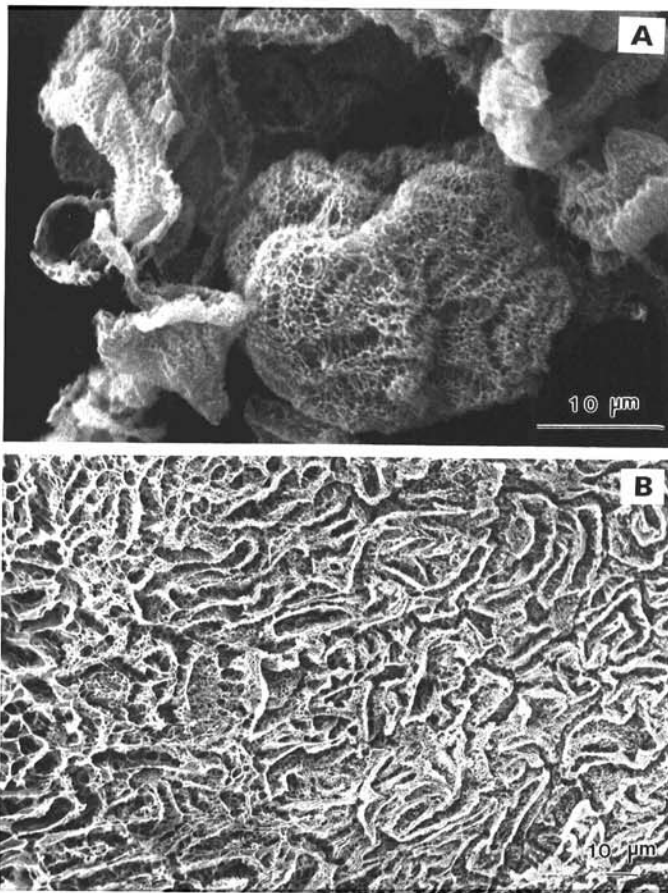

Fig. 3. Scanning electron micrographs of cryoprepared components of a cross-linked and stabilized waxy maize starch (Polar Gel 15) paste viewed by low-temperature scanning electron microscopy. A, granule remnant isolated from a 0.2% starch paste (×2,000); B, 4% starch paste (×500).
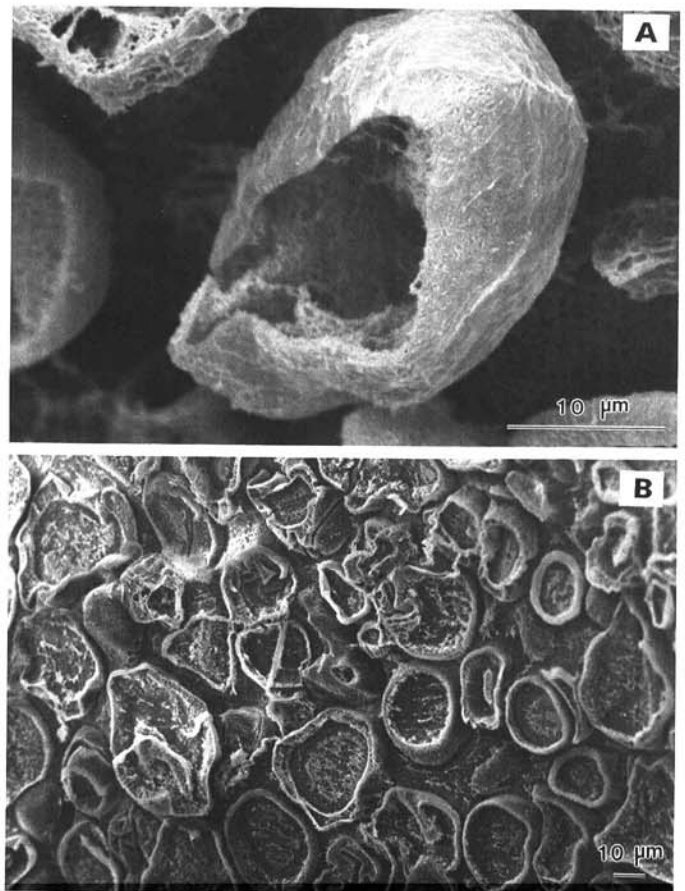

Fig. 4. Scanning electron micrographs of cryoprepared components of a succinate-cross-linked common corn starch paste viewed by low-temperature scanning electron microscopy. A, granule remnant isolated from a 0.2% starch paste (×2,500); B, 4% starch paste (×500).

phase, 4) shape and rigidity of the granule ghosts, and 5) interaction between the matrix and the granule ghosts (see Morris 1990). These micrographs also confirm that these parameters can be evaluated by this method and varied by chemical modification or genetic manipulation of starch. In particular, this technique reveals the size of the openings in the continuous phase produced by rapid freezing, i.e., the tendency for the polymers to associate upon rapid freezing.

Ice crystal formation during the freezing process affects the coarseness of the network. However, the molecular composition of the network may also affect the rate of ice crystal formation because of the water-holding and cryoprotectant capacities of the polymers (Langton and Hermansson 1989). This means that the molecular composition of the starch has a significant effect on the observed structure because of differences in both the inherent tendency of the polymers to associate and the effect they have on ice crystal formation. We recognize that the structures seen are likely artifacts from ice crystal formation. However, this technique is believed to provide a representative view of the structures that will form eventually via molecular associations. Freezing by means of the cryopreparation technique used only accelerates the process. Because of the strong, direct correlation between the observed structures and known functional properties, the structures revealed by this method can be used to compare the functional properties of the pastes of the various starches, and the method can be used as a screening test for starch paste and gel characteristics.

Highly branched polymers resist association, whereas linear polymers associate quite easily. This is borne out by the micrographs of pastes of waxy maize starch and common corn starch (Figs. 1B and 2B). Substitutions along the polymer chains also

increase the stability of a starch, as shown in the micrograph of an acid-thinned, hydroxyethylated common corn starch (Fig. 5B; compare Fig. 1B).

Cross-linked starches are traditionally used to maintain high hot-paste viscosities. Typical cross-linked granules swell but retain much of their contents and do not collapse. Food starches are often also derivatized with monofunctional reagents to minimize molecular reassociations (retrogradation). The ultrastructural basis for these properties can be observed in the micrograph of the paste from the cross-linked and stabilized starch (Fig. 3B). Granules of the derivatized starch are swollen (Fig. 3B) but not completely disintegrated (compare Fig. 2B).

Starches from corn mutants, such as the newly developed dull waxy or the older high-amylose, demonstrate that molecular composition strongly affects paste structure and, therefore, its functionality. Dull waxy corn starch pastes are more stable than are those of either waxy maize or common corn starch (Friedman et al 1989). The micrographs of the dull waxy corn starch paste (Fig. 6B) revealed a continuous network intermediate between that produced by common corn starch and that of waxy maize starch (perhaps more like the latter). Figure 6B, therefore, suggests a stable paste that is resistant to retrogradation.

Micrographs of the high-amylose starch pastes confirmed that granule integrity can also be manipulated genetically (Fig. 7A and B), and those of a starch chemically cross-linked in the laboratory confirmed that greater amounts of cross-linking produce inhibited granules (Fig. 4A and B).

In summary, we believe that this method, which requires only milligram quantities of starch, provides a rapid procedure for screening new starches for desirable properties because the structures revealed by LT-SEM, although they probably contain freezing artifacts, are correlated with the starch functionality and
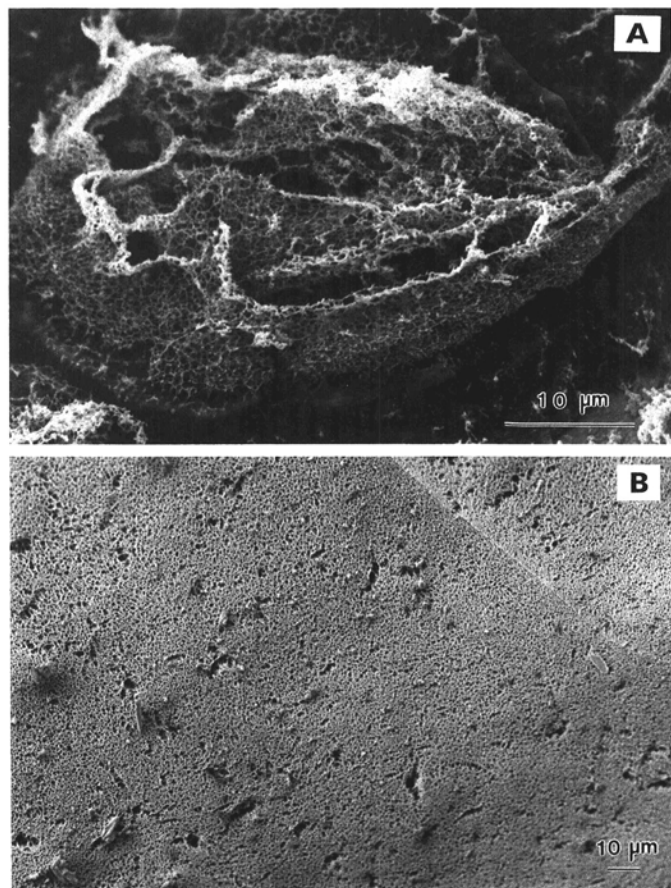

Fig. 5. Scanning electron micrographs of cryoprepared components of a hydroxyethylated and acid-thinned common corn starch (Penford Gum 290) paste viewed by low-temperature scanning electron microscopy. **A**, granule remnant isolated from a 0.2% starch paste ($\times$2,000); **B**, 4% starch paste ($\times$500).
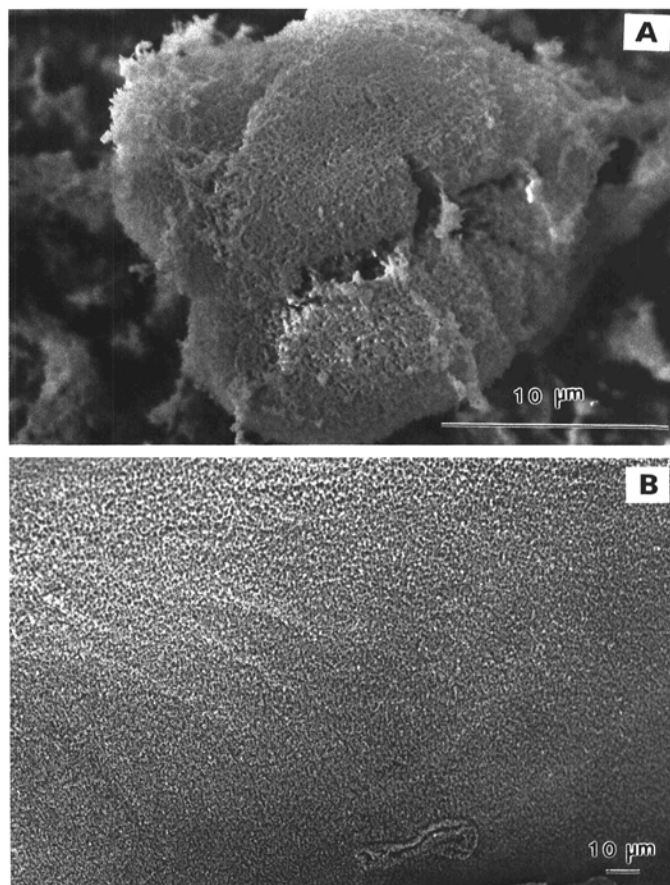

Fig. 6. Scanning electron micrographs of cryoprepared components of a dull waxy corn starch paste viewed by low-temperature scanning electron microscopy. **A**, granule remnant isolated from a 0.2% starch paste ($\times$3,500); **B**, 4% starch paste ($\times$500).
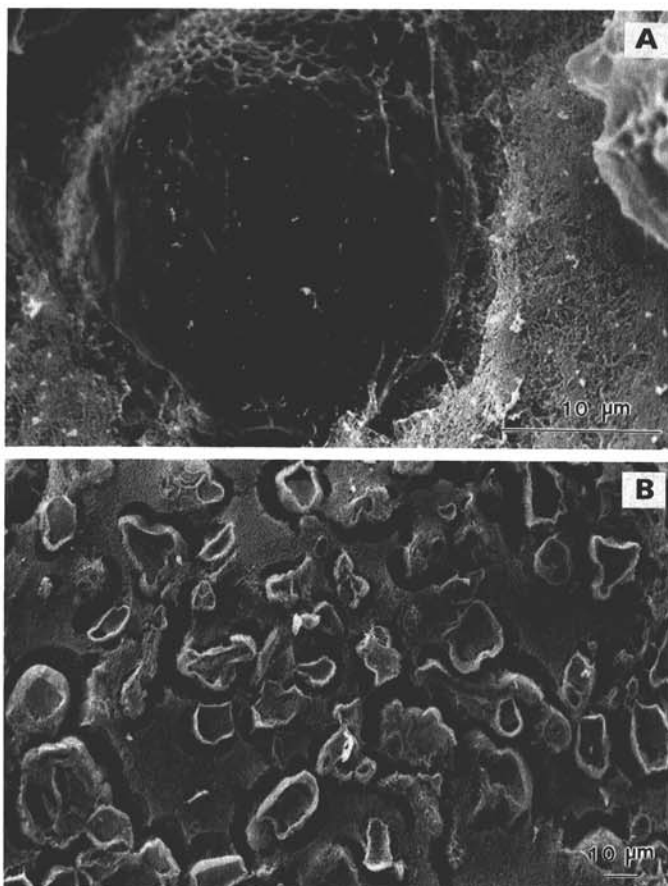
Fig. 7. Scanning electron micrographs of cryoprepared components of a high-amylose (55%) corn starch paste viewed by low-temperature scanning electron microscopy. **A**, granule remnant isolated from a 0.2% starch paste (×2,500); **B**, 4% starch paste (×500).

therefore can be used to predict paste properties and behavior. Work by us not described here suggests to us that starch pastes cryoprepared for LT-SEM by an extremely rapid freezing technique, while they may be free of freezing artifacts, are not as useful for comparison of different starches as are pastes cryoprepared as described here, as this technique appears to magnify behavioral differences.

ACKNOWLEDGMENTS

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