Electron Microscopy of Unmodified and Acid-Modified Corn Starches

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ABSTRACT

The morphologies and internal structures of sectioned yellow dent corn, waxy maize, and amylomaize starches were studied by light and electron microscopy. Yellow dent and waxy maize starches were similar, but amylomaize starch was quite different. Although the yellow dent and waxy maize starches differ in amylose content, both have similar well-defined concentric lamellae in which the electron-thin lamellae are preferentially attacked by acid. Amylomaize starch granules are heterogeneous in morphology, internal structure, and resistance to acid. The morphology varies from approximately spherical granules having knobs or protuberances of various sizes and shapes extending from the surface. The protuberances and the filamentous granules do not show a lamellar structure. Spherical amylomaize granules sometimes have a lamellar structure which is never as well formed as that in yellow dent and waxy maize starches. Acid treatment accentuates the structural heterogeneity in amylomaize granules. When lamellae are present, the acid erodes the electron-thin rings. Some granules or parts of granules are acid-resistant. Others disintegrate into fragments of irregular sizes and shapes.

The literature contains several papers on the study of starch structure by electron microscopy. Most of that work was done on unmodified starch, with the emphasis placed on preservation of starch structure in its native state and on the mechanism of granule formation following pollination (1,2). Buttrose (3) studied, by electron microscopy, several acid-modified starches including yellow dent, waxy maize, and amylomaize. However, Buttrose did not include work done on unmodified starches, since he found that the granules must be eroded by enzyme or acid to render the shell structure visible. He also found little variation in appearance between granules in a given preparation.

We have found extreme variations in the morphologies and internal structures of unmodified amylomaize granules. These variations, and the differences between amylomaize and both yellow dent and waxy maize starches, are accentuated by acid-modification. Wolf, Seckinger, and Dimler (4) studied amylomaize starches by light microscopy. They described extreme variations in morphologies, birefringence, and iodine-staining properties among granules. Their results on unmodified amylomaize starches are complemented and extended by our electron-microscopy results on both unmodified and acid-modified starches.

MATERIALS AND METHODS

Unmodified[1] granular starches studied were yellow dent corn starch, which contains about 25% amylose and 75% amylopectin; waxy maize starch, which contains essentially no amylose and 100% amylopectin; and an amylomaize starch which contains a nominal 80% amylose and 20% amylopectin. The same starches were modified with 7% hydrochloric acid at room tem-

[1]The term "unmodified" is used in this paper in the sense in which it is used commercially. It is recognized that starch may undergo certain changes, such as cavity formation, during commercial processing.

perature for 35 days. These conditions of modification are mild enough so that the starches remain granular.

Without fixing or staining, the granular unmodified and acid-modified starches were suspended and washed in water, dehydrated in a gradient ethanol series, infiltered and embedded in a butyl-methyl methacrylate mixture. Polymerization of the methacrylate was activated with UV irradiation. Sections about 2 to 40 μ thick were cut for light microscopy. Ultrathin sections, about 1,000 A thick, were cut with glass knives onto 20% aqueous acetone for electron microscopy. The sections were mounted on collodion-coated grids. The sections were studied as-is, without staining; or the embedding material was dissolved in toluene followed by shadowing and stabilization of the sections with evaporated germanium. The starch sections were studied in an RCA EMU-3F electron microscope operating at 50 kv. Micrographs were studied at magnifications of 12,000 to 25,000 diameters.

A wide range of morphologies is naturally present in any starch species. This heterogeneity is accentuated in modified starches. This, plus the fact that 1 g. of corn starch contains about 10^9 granules, makes it impossible to present all the variations in structure which were studied. Therefore, electron micrographs have been selected for this report to illustrate typical morphologies and structures observed.

RESULTS AND DISCUSSION

Light Microscopy

The appearance of starch granules by light microscopy have been described many times in the literature. Our only objective in studying the granules by light microscopy was to determine if the embedding procedure had any apparent effect on the granules.

Sections, 2 to 40 μ thick, of embedded, unmodified yellow dent corn starch granules stained dark blue with iodine and the granules were birefringent. The acid-modified granules stained blue-violet with iodine and were strongly birefringent. Rare swollen granules and rare granules that failed to stain with iodine were found in both the embedded samples and the original sample.

Sections of waxy maize starch granules stained a typical reddish brown with iodine. Rare granules stained blue. These were present in the original sample. Badenhuizen (5) reported that waxy starch granules sometimes have a blue-staining amylose nucleus. We have confirmed this observation on embedded waxy maize starch. Also, the birefringence was normal for waxy maize.

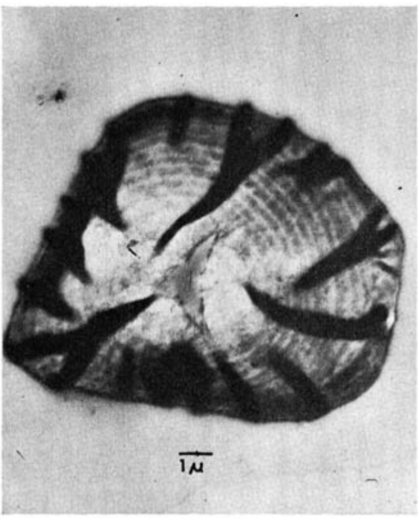
The iodine-staining and birefringence characteristics of embedded amylomaize starch were essentially the same as described by Wolf *et al.* (4). Their observation, confirmed by us in embedded samples, that both birefringent and isotropic spherical granules and only isotropic filamentous bodies are present in amylomaize starch, is especially interesting. This will be discussed later.

A lamellar, or shell structure, could not be detected by light microscopy in any of the unmodified corn starches. The lamellar structure, which can be seen by electron microscopy, is too small to be resolved by light microscopy. Some acid-modified yellow dent corn and waxy maize starch granules exhibit a concentric ring structure by light microscopy. However, fewer rings with larger spacings are seen by light than by electron microscopy. Potato starch exhibits, by light microscopy, a ring structure with a 2- to 7-μ spacing. A spacing of about 0.2 μ was resolved by electron microscopy[2]. Therefore the ring structure seen by electron microscopy is not necessarily the same as that seen by light microscopy.

We concluded that the embedding procedure did not damage the granules, at least to a degree detectable by light microscopy. We therefore proceeded to studies by electron microscopy.

Unmodified Yellow Dent Corn Starch

Essentially all ultrathin sections of unmodified starch granules contain folds and buckles. These artifacts are apparent as extremely electron-dense[3] areas radiating in from the periphery of the section of yellow dent corn starch (Fig. 1).



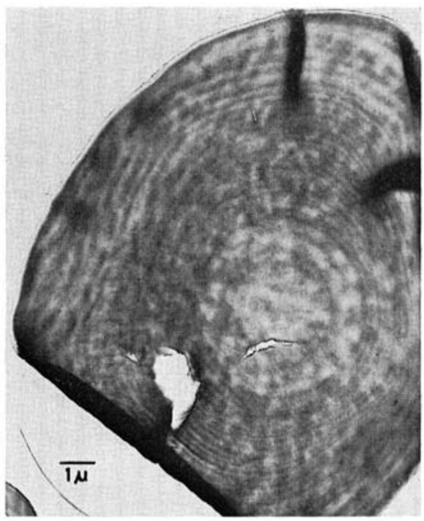


Fig. 1 (left). Unmodified yellow dent corn starch, as-is.
Fig. 2 (right). Unmodified waxy maize starch, as-is.

This granule shows a typical lamellar structure (seen as concentric electron-dense and electron-thin rings in sections) which is present in all mature yellow dent corn starch granules. The lamellar periodicity[4] varies between 0.15 and 0.7 μ but is usually between 0.25 and 0.36 μ. The periodicity in any one granule is essentially constant from the periphery to the hilum.

An apparent broadening of the lamellae as the hilum is approached is a result of sectioning an appreciable distance from the center of the granule. The electron-dense rings are about two to four times as wide as the electron-

[2] Authors, unpublished results.

[3] All electron micrographs are presented as positives, so electron-dense areas are dark and electron-thin areas are light.

[4] Distance from center to center of adjacent electron-dense rings: therefore, the sum of the widths of one electron-dense and one electron-thin ring.

thin rings. Electron density is a product of mass and thickness. Since the thickness is constant across a section of a granule, electron density is a function of mass. Hence, the starch molecules are more densely packed in the electron-dense rings than in the electron-thin rings. This difference in packing may be a difference in crystallinity. That is, the electron-dense and electron-thin rings may be areas of comparatively high and low degrees of crystallinity, respectively.

The three-point star-shaped cavity seen in the center of the yellow dent corn starch section is typical of commercially processed starch. Cavity points sometimes extend almost to the granule periphery. A few granules have no cavity.

The surface of the granule is covered with an electron-dense layer. Thicknesses of this layer have varied between 0.03 and 0.13 μ. The nature of this layer is not known. It may be a highly packed or crystalline starch layer or it may be a thin protein or enzyme layer. This electron-dense layer should be further studied and its role in the swelling, pasting, and enzymolysis of starch determined.

Fissures or pits 0.3 to 0.7 μ deep and about 0.07 μ wide are present in the surface of the lower right quadrant of this corn starch granule (Fig. 1). These were probably formed during processing. Most unmodified starch granules do not contain fissures, pits, or channels.

Unmodified Waxy Maize Starch

Waxy maize starch (Fig. 2) contains structures which are essentially the same as those in yellow dent corn starch. The granule sizes shown (Figs. 1 and 2) of about 12 and 15 μ are normal for both starches. Maximum diameters of about 25 μ were observed in each sample. The lamellar periodicity range in waxy maize starch was the same as that described for yellow dent corn starch. The difference in lamellar contrast (Figs. 1 and 2) is well within the range of contrast seen in both starches.

The lamellar structure in granular starch was assumed by Czaja (6) to consist of alternate layers of amylose and amylopectin. Waxy maize contains essentially 100% amylopectin, but has a lamellar structure as well formed as that in yellow dent corn starch which contains about 25% amylose and 75% amylopectin. Therefore, the lamellar structure, at least in waxy maize, cannot be due to alternating high and low ratios of amylose to amylopectin.

Unmodified Amylomaize Starch

Since amylose and amylopectin have different physical properties, there is a demand for corn starches with different amylose-amylopectin ratios. Waxy maize and yellow dent corn starches are natural variants which have been available for many years. In recent years, corn breeders have successfully produced amylomaize hybrids known as classes V, VII, and VIII, which yield starches containing nominal 50, 70, and 80% amylose respectively. These starches contain granules with morphologies and internal structures more heterogeneous and different from those in yellow dent and waxy maize starches. The percentage of anomalous granules increases as the amylose content increases (4). Only class VIII granules are presented in this report, but similar granules are present in classes V and VII.

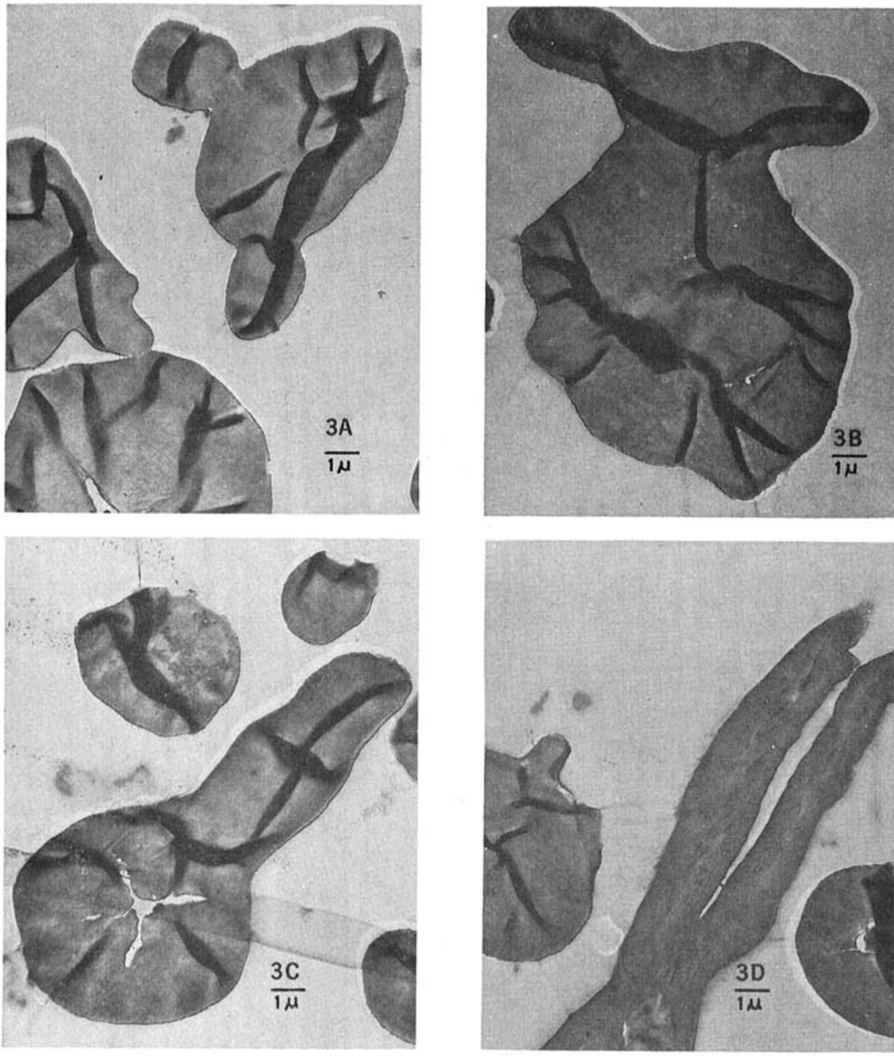


Fig. 3, A, B, C, D. Unmodified amylomaize starch, shadowed.

A lamellar structure has been demonstrated in a few amylomaize starch granules (Fig. 3, A). The main bodies of the upper right and lower left granules exhibit distinct lamellae, but they are poorly defined in comparison with those in yellow dent and waxy maize starches. The granule at left center does not exhibit lamellae. If a well-defined lamellar structure depends on alternating layers of high and low degrees of crystallinity, then this work indicates that the degree of crystallinity is more evenly distributed in amylomaize starch than it is in yellow dent and waxy maize starches.

One of the granules has two budlike protuberances in the plane of sectioning. Many granules in amylomaize starch have definite protuberances which are too small (less than 1 μ) to be resolved by light microscopy. Lamellae in protuberances have never been seen. It is possible that a poorly defined lamellar structure could be destroyed by ultrathin sectioning. However, examination of similar granules with a polarizing microscope reveals anisotropy in the main body of the granule, but the protuberances are isotropic.

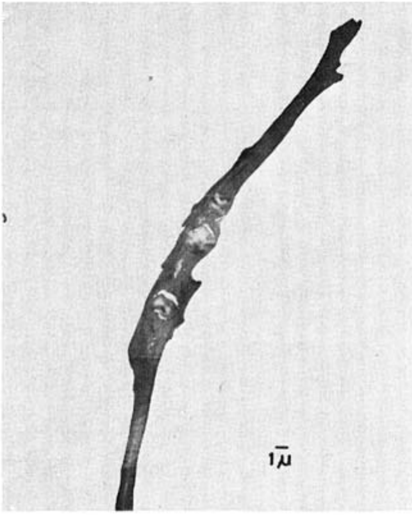
The protuberances in amylomaize starch assume many sizes, shapes, and orientations relative to the main body of the granule (Fig. 3, B and C). The left side of the granule in Fig. 3, B, has a protuberance 0.25 μ across. It also has a large protuberance oriented tangentially to the main body. The large granule in Fig. 3, C, has a protuberance oriented perpendicularly to the main body. The main body of this granule shows only a trace of lamellar structure. Wolf *et al.* (4) classified amylomaize granules into those which are normal without outgrowths or extensions of any kind and those which are irregular. They found, by light microscopy, that class VII amylomaize contains 62 to 72% irregular granules. We have found, by electron microscopy, that the frequency of irregular granules approaches 100%. Some granules have protuberances too small (left side, Fig. 3, B) to be resolved by light microscopy. This, plus the fact that a protuberance could easily be missed in a section which is only about 1% of the total granule, suggests that essentially all the granules in amylomaize starch are irregular.

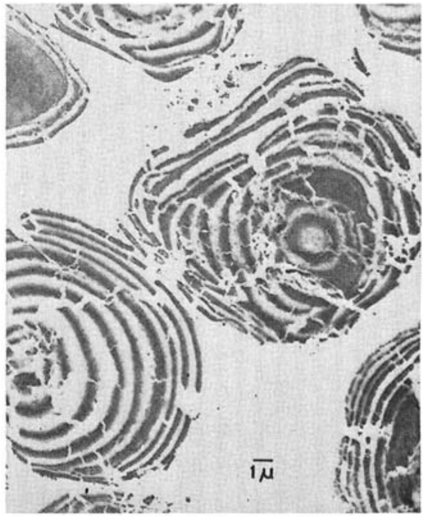
Some amylomaize starch granules do not have a main body. These granules are long, narrow filamentous bodies (Fig. 3, D). They do not have a lamellar structure and do not exhibit birefringence in a polarizing microscope. They do have a fine structure which is oriented longitudinally and conforms with the general contours of the starch body. This finding is compatible with work (4) which indicated that the molecular orientation was in the direction of the long axis of filamentous bodies. Approximately spherical inclusion bodies, about 1 μ in diameter, are often found in the filamentous starch granules. It is assumed that these inclusion bodies are starch, but this has not been confirmed. The effects of amylases on granular starches are being studied. It is hoped that the inclusion bodies will be defined during this work.

The probability of the plane of sectioning being exactly in the plane of a long, filamentous granule is small. This, plus the limited open area in a microscope grid, has made it impossible to observe the entire length of a filamentous granule in the electron microscope. One granule (Fig. 4) was obtained with about 50 μ of length visible. This granule varied in width from about 1 to 4 μ. It contained three well-formed inclusion bodies.

Acid-Modified Yellow Dent Corn Starch

The conditions of acid modification used grossly affected the structure of yellow dent corn starch, but still left the starch in a granular state consisting of concentric rings seen in cross-section in the electron micrograph (Fig. 5). The electron-thin rings have been completely disintegrated and dispersed by the acid-modification and subsequent washing. This has left the electron-dense rings in bold relief. The electron-dense rings are swollen and show some evidence of disintegration into particles about 0.05 μ across. The marked differences in the effect of acid on the rings is evidence that the





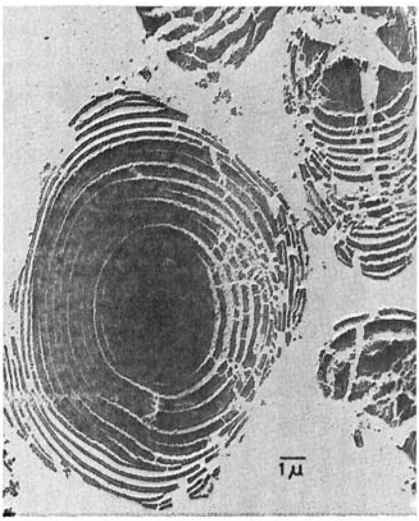


Fig. 4 (upper left). Unmodified amylomaize starch; montage of three electron micrographs; as-is.

Fig. 5 (upper right) and Fig. 6 (lower left). Acid-modified yellow dent corn starch, shadowed.

Fig. 7 (lower right). Acid-modified waxy maize starch, shadowed.

electron-thin rings are relatively amorphous and that the electron-dense rings are crystalline. A few yellow dent corn starch granules, and especially the hilum of these granules (Fig. 6), were more resistant to acid than most of the granules.

Acid-Modified Waxy Maize Starch

The effect of acid on granular waxy maize starch (Fig. 7) was similar to the effect on yellow dent corn starch. The only difference was in degree.

Partial disintegration of the electron-dense rings into particles about 0.05 μ across was more evident in waxy maize than in yellow dent corn starch. It is not known if the 0.05 μ particles seen in acid-modified yellow dent and waxy maize starches were present as such in the unmodified granules and became visible when acid-labile material was eroded, or whether they were in some other shape, such as long rodlets, and contracted to spherical particles during acid-modification. The particles are too large to be individual molecules. However, the uniformity of their size and their universal presence in acid-modified granules suggests that they are a basic part or result of the fine structure of granules. The role of these particles in the fine structure of granules should be further studied. Also, particles this size are small enough to stay in suspension in a starch "solution."

It would be interesting to determine what percentage of the solids in a starch "solution" is actually a suspension of discrete particles, and what effects these have on physical properties such as viscosity and clarity.

The effect of acid-modification indicates that the rings in both waxy maize and yellow dent corn starch are alternating layers of comparatively high and low degrees of crystallinity. This cannot be amylose-dependent, since waxy maize does not contain amylose.

Acid-Modified Amylomaize Starch

Since amylomaize starch is extremely heterogeneous in both morphology and internal structure, it was not surprising to find that acid affected individual granules in different ways.

Amylomaize granules rarely showed a definite lamellar structure (Fig. 8, A). In these granules, the electron-thin rings are preferentially attacked by acid, but this effect is not so pronounced as it is in starch of yellow dent corn and waxy maize. This is compatible with the findings that unmodified amylomaize starch granules never have a well-formed lamellar structure.

There is a marked difference in resistance to acid among amylomaize starch granules. Some granules showed no apparent effect, while others were almost completely disintegrated by acid. A few amylomaize granules (see lower left in Fig. 8, A, and left in Fig. 8, C) disintegrated into particles about 0.05 μ across similar to those seen in modified yellow dent and waxy maize starches. However, the particle distribution appears random and not in definite concentric rings, as was the case in yellow dent and waxy maize starches. No correlation between general morphology and resistance to acid was found. Some approximately spherical granules were extremely acid-resistant (center in Fig. 8, A; right in Fig. 8, B). Some anomalous granules were extremely acid-resistant (Fig. 8, C). Some spherical granules (Fig. 8, B and D) and some anomalous granules (Fig. 8, D) were markedly affected by acid. The fine filamentous material seen outside the resistant granule (Fig. 8, C) is debris from sectioning. The structure is interesting and should be studied to prove that it is starch and to determine its significance.

When amylomaize starch granules are attacked by acid, the effect is different from the effect on yellow dent and waxy maize starches. While yellow dent and waxy maize starches are affected tangentially through the

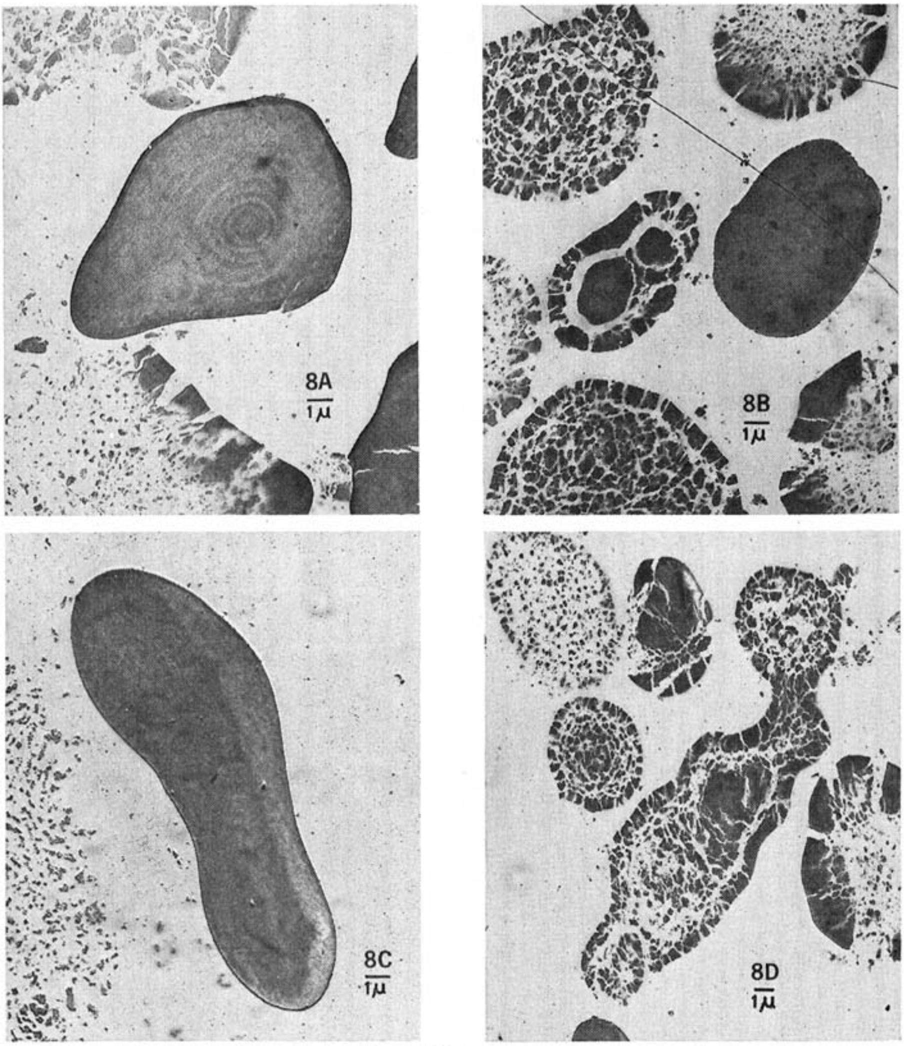


Fig. 8, A, B, C, D. Acid-modified amylomaize starch, shadowed.

electron-thin rings, amylomaize starch, with rare exceptions, disintegrates into fragments of irregular shapes and sizes, usually from 0.1 to 1.0 μ across. There is a tendency toward radial disintegration near the granule surface (Fig. 8, B and D).

If it is true that resistance to acid corrosion is associated with degree of crystallinity, then this work suggests that organization into crystalline areas in amylomaize starch is quite different and variable compared to that in yellow dent and waxy maize starches. The mode of acid attack on amylomaize granules suggests that they be classified as those which have:

1) Alternating rings of comparatively high and low degrees of crystallinity,

the difference in degree being less than that in yellow dent corn and waxy maize starches—these granules have a lamellar or shell structure;

2) A high degree of crystallinity distributed evenly throughout the granule—these are acid-resistant granules of any of the morphologies present in amylomaize starch;

3) High degrees of crystallinity randomly distributed into irregularly shaped areas about 0.1 to 1.0 μ across; and

4) Low degrees of crystallinity distributed evenly throughout the granule—these are the granules which disintegrate into 0.05-μ particles when acid-modified.

Wolf *et al.* (4) have observed a variability in the intensity of the starch-iodine color in amylomaize granules. We have confirmed the observation. This suggests an appreciable variation in amylose content between granules. The anomalous-shaped granules appear to stain most intensely. If the anomalous granules do have the highest amylose content, and since no correlation was found between granule morphology and acid resistance, then it could be concluded that amylose content alone does not determine the granule's resistance to acid.

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