Surface Pores of Starch Granules[1]

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ABSTRACT

Some but not all granules of corn (all cultivars and all developmental stages examined), sorghum, and millet starches have small openings (pores) randomly distributed over their surfaces, often in clusters and to different degrees. The pores are normal, real, anatomical features of the native granule structure and are not artifacts produced by the isolation, specimen preparation, or observation techniques used. Pores also were found along the equatorial groove of large granules of wheat, rye, and barley starches but not on other starches (rice, oat, potato, tapioca, arrowroot, canna). It is proposed that the pores affect the pattern of attack by amylases and by at least some chemical reagents.

Although the morphology, internal structure, and surface characteristics of starch granules have been extensively examined (Sandstedt 1965, Greenwood 1976, Hood and Liboff 1983, French 1984, Guilbot and Mercier 1985, Gallant and Bouchet 1986, Zobel 1988), a number of questions about the nature of the outer surface and its relationship to the chemical and enzymatic reactivity of granules remain unanswered. In general, starch granules are practically inert toward chemical reactions unless they are pretreated to "activate" them (Lohmar and Rist 1950, Lohmar et al 1950). Native starch granules also exhibit resistance to enzyme-catalyzed digestion, and each type of granule is attacked in a characteristic pattern (Leach and Shoch 1961, Gallant et al 1973). The pattern of digestion that develops when native corn and wheat starch granules are treated with amylases indicates that some areas of their surface are much more susceptible to attack than are others (Evers and McDermott 1970, Evers et al 1971, Dronzek et al 1972, Gallant et al 1973, Fuwa et al 1977, Hood and Liboff 1983). The surface of potato starch granules is eroded much more evenly (Gallant et al 1973).

In this work, two types of scanning electron microscopes, the JEOL (Tokyo, Japan) JSM-840 scanning electron microscope (SEM) and the ElectroScan (model II, Wilmington, MA) environmental scanning electron microscope (ESEM), were used to investigate the general morphology and specific surface features of starch granules with a long-term goal of examining and controlling their chemical and enzymatic reactivity. In this work, we concentrated on determining the origin of very small openings (which we rediscovered serendipitously and call pores) that are found randomly distributed over the surface of starch granules of members of the subfamily Panicoideae. Although these features were first reported by Hall and Sayre (1970), who referred to them as "pin holes" and as "microscopic pores or holes," the pores seem to have been forgotten, and their origin, function, and effect on granule reactivity have not been investigated. Although all common cereal grain, root, and tuber starches were examined, this study concentrated on starch granules from a variety of maize cultivars.

After observing pores, we decided it was important to determine whether they were formed during granule formation or during some process after granule formation. Several possible origins of the pores were considered: 1) they are caused by drying in the kernel or after isolation; 2) they are produced by in situ amylases or by amylases produced during wet milling; 3) they are artifacts of preparation techniques; 4) they are a natural feature integral to the granule structure. Various techniques then were used to determine their origin.

MATERIALS AND METHODS

We examined commercially prepared yellow dent (common) corn, waxy maize, dull waxy maize, rice, wheat, canna, arrowroot, tapioca, and potato starches; laboratory-prepared yellow dent (common) corn, barley, rye, oat, sorghum, and millet starches; fresh dough-stage and field-dried yellow dent corn starches; and field-dried millet and sorghum starches.

Sample Preparations

Granules were isolated from dough-stage, mature, yellow dent (common) corn kernels by a laboratory version of the method generally employed by commercial corn starch refiners (Watson 1964). Isolated and purified granules in a water slurry were dehydrated by one of two methods. Some samples were dried successively by solvent exchange successively with 70% ethanol, acetone, and diethyl ether, and the samples then were placed in an evacuated desiccator until used for electron microscopy. Other slurries were freeze-dried.

Granules also were isolated in the presence of enzyme inhibitors. In one case, the method of Badenhuizen (1964), which uses a cold 0.01*M* mercuric chloride solution, was used with two modifications. The time of agitation with toluene was reduced from one period of several hours to five periods of 3 min each, with removal of the protein-toluene interface between agitations as described by Banks et al (1973), and the defatting step was eliminated. These samples were then dehydrated by the solvent exchange procedure described above. To test for the inhibitor's effectiveness, controls containing added α-amylase and amyloglucosidase were used in slurries either with or without the inhibitors. Thin-layer chromatography was performed on silica gel 60 using 3:2:1 (v/v) ethyl acetate-glacial acetic acid-water as the developer; a detection spray of 10% H_2SO_4 in 95% ethanol (followed by heating) was used to examine for glucose.

Granules also were isolated with another enzyme inhibitor system. The general procedure (Watson 1964) was followed, except that the first step (soaking in 0.10% sulfur dioxide solution) was eliminated and replaced by rapid grinding (using a Waring Blendor, New Hartford, CT) of the kernels in a solution of 10% sodium dodecyl sulfate and 1.0% 2-mercaptoethanol, which inhibited α-amylase without affecting the starch granules. These samples were either dried by solvent exchange as described above or kept hydrated in the enzyme-inhibited solution. Again, samples were processed either with or without the added enzymes as a test for inhibitor efficiency.

Dough-stage corn kernels were sectioned with a razor blade. Sections were dehydrated in a graded ethanol series and then dried by carbon dioxide in a critical-point drier.

The enzymes used were crystalline porcine pancreatic α-amylase and lyophilized *Aspergillus niger* amyloglucosidase, both from Sigma Chemical Co., St. Louis, MO.

Electron Microscopy

For the JEOL JSM-840 SEM, dry samples (from field-dried kernels, commercial samples, solvent-exchanged samples, freeze-dried samples, and critical point-dried samples) were sprinkled

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onto double-sided cellophane tape attached to aluminum stubs. These samples were then coated in a Technics Hummer I sputter coater (distributed by Anatech, Alexandria, VA) with 300 Å of gold-palladium alloy and examined at 10.0 kV. Wet samples (from dough-stage corn with or without enzyme inhibitors) were quick-frozen in liquid nitrogen slush (−160°C) and sublimed in the microscope at −90°C with a Hexland cryopreparation control unit (distributed in the U.S. by Oxford Instruments North America, Concord, MA). Then they were coated at −160°C with 150 Å of gold and examined at −160°C at 8.0 kV (Sargent 1988) (low-temperature SEM).

For examination with the ElectroScan model II ESEM, dry samples were mounted on glue tabs attached either directly to aluminum stubs or to a carbon surface on an aluminum stub. Wet samples were put on the stub as a drop of suspended granules. Both wet and dry samples were examined in a water vapor environment of 2–10 torr at an accelerating voltage of 20.0 kV.

RESULTS AND DISCUSSION

When examined by SEM, dry granules of commercial, native, common corn starch can be grouped by general morphology into the following categories: generally spherical, generally angular, dimpled, and irregular (Fig. 1). Some but not all granules have pores distributed over their surfaces. These pores are usually found on the smoother, more spherical granules from the floury endosperm and often occur in clusters. Some granules have many pores, some have only a few, and others appear to have none (Fig. 1). Each field of common corn starch from several sources contained granules with pores. Starch granules of other corn cultivars, such as waxy and dull waxy maize, also contained pores (Fig. 2).

Once the surface pores of corn starch granules were observed, it was realized that they may be a factor in granule reactivity, for they could be openings that penetrate into the granule. From their size (approximately 1,000 Å in diameter), they could easily allow very large molecules, including enzymes, direct access to the granule interior. Therefore, it seemed important to determine their origin.

The pores might have been formed during granule formation. They might have been formed during drying of the granules. They might have been formed by action of amylase(s) on granules after formation. Or they might be artifacts of electron microscopy preparation techniques and thus have no relationship to granule structure or reactivity.

Drying has been reported to form fissures breaching the surface and penetrating to the hilum of corn starch granules (Whistler and Turner 1955; Whistler et al 1955, 1958; Whistler and Thornburg 1957). Whistler et al (1959) and Whistler and Spencer (1960) further reported that chemical reactivity of corn starch granules was greatest at the granule surface and at the surface of cavities, indicating that reagents have access to the granule interior and that the cavities are not artifacts of the electron microscopy technique. Others have also observed fissures and cavities in corn starch granules (Gallant et al 1972). To our knowledge, however, there are no published reports of granule fissures being observed on the granule surface by SEM, even though they appear by light and transmission electron microscopy to extend to the surface (see also Hall and Sayre 1970). The only surface openings that have been found on dry granules are these pores, which also were present on granules of dough-stage yellow dent corn. Whistler et al (1959) reported that the percentage of cavitated granules was consistent, regardless of the drying method used, including freeze-drying, which indicates that pores and channels into the granule are not an artifact of drying, although they could be altered or enlarged by drying.

It has been reported that enzymatic digestion of corn starch granules initiates within the hilum of either predried or dough-stage granules (Leach and Schoch 1961, Nikuni 1978, Hood and Liboff 1983). The question to be asked then is how the enzyme gains access to the hilum and begins digesting from the inside out. Leach and Schoch (1961) and others (Gallant et al 1973, Fuwa et al 1977, Kanenaga et al 1990) also reported that corn starch, which has pores, was more susceptible to enzymatic digestion than potato starch, on which we found no pores, and which Leach and Schoch (1961) found was digested in a rather different pattern. Leach and Schoch (1961) also reported that wheat starch, which we found to possess pores only along the equatorial groove, was intermediate in digestibility. Badenhuizen (1959) suggested that more susceptible granules may have pores of a size sufficient to admit enzyme molecules. Leach and Schoch (1961) suggested that these pores must be characteristic of the particular species of starch and not produced by drying.

After examining photomicrographs of common and waxy sorghum starch, Hall and Sayre (1970) suggested that "it may be that the small holes or pores in the granules are 'real' and are of sufficient size that enzymes can gain entry into the granule interior, thereby increasing the rate of reaction" and that "evidently, the more numerous small pores have a greater long-term effect on enzyme entry and diffusion of soluble reaction products than a smaller number of larger holes." We agree with both suppositions and further suggest that not only is the number

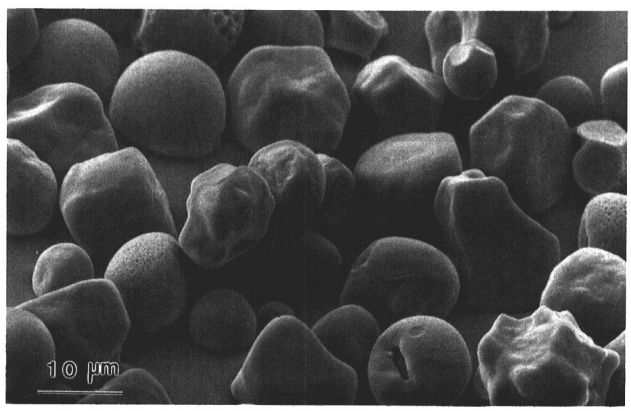

Fig. 1. The variety of sizes and shapes of common corn starch granules.

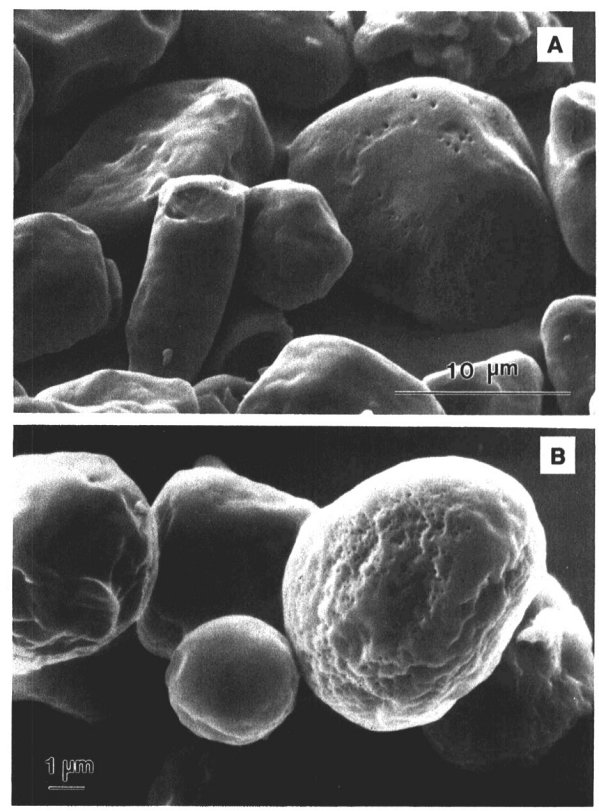

Fig. 2. Starch granules with surface pores from waxy maize (**A**) and dull waxy corn (**B**).

of pores per granule correlated with the rate of digestion by amylases, but the variation in the number of pores per granule may be important in providing a controlled and steady breakdown of starch during seed germination.

Amyloglucosidase (in 500 m*M* citrate buffer, pH 5.0, 37°C, 10 U/ml) attacks corn starch granules in surface patterns resembling those of pore distribution (Fig. 3A) (see also Gallant et al 1973, Fuwa et al 1977, Hood and Liboff 1983). No small pores are observed; therefore, the areas around the pores could be areas of lesser molecular association where enzyme susceptibility might be greatest. These same enzymes do not produce recognizable patterns when degrading root or tuber starches (potato, arrowroot, etc.) (Fig. 3B), on which we found no surface pores. The pattern of activity on wheat, rye, and barley starches shows that the region of the equatorial groove, which is found on large granules and is the region where we observed surface pores, is most susceptible to enzymatic digestion (Evers and McDermott 1970, Evers et al 1971, Dronzek et al 1972, Hood and Liboff 1983).

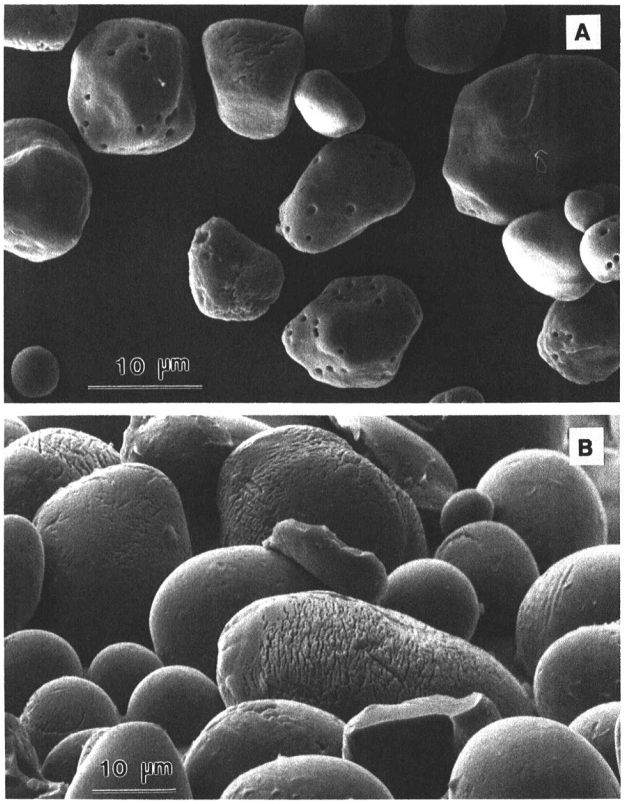

Fig. 3. Enzyme-treated common corn starch granules (**A**) and potato starch granules (**B**).

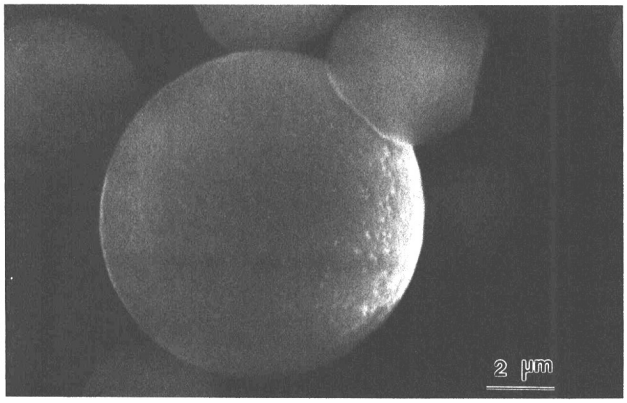

Fig. 4. Environmental scanning electron micrograph of an uncoated dough-stage common corn starch granule taken directly from the kernel and examined without dehydration.

To determine whether the pores could have been produced by drying in the field, postharvest drying, or industrial drying after the wet-milling process, starch was isolated from dough-stage yellow dent corn, dried by freeze-drying or solvent exchange, and examined by SEM. These samples also were examined without prior dehydration by ESEM (Fig. 4). Pores were present, seemingly ruling out their formation as a result of natural or commercial drying.

To determine whether the pores could have been formed by the action of enzymes during the wet-milling process or during the isolation procedure used on the dough-stage corn, three experiments were performed. Granules scraped directly from field-dried yellow dent corn kernels, granules from dough-stage kernels isolated in the presence of enzyme inhibitors (mercuric chloride or sodium dodecyl sulfate plus 2-mercaptoethanol), and granules from slices of dough-stage kernels dehydrated by a combination of solvent exchange and critical point-drying were examined by SEM. Granules from all three preparations had pores, ruling out the possibility of formation by enzymes during wet milling. No glucose was detected in the isolation slurries by thin-layer chromatography, either with or without added enzymes, when inhibitors were present; glucose was detected if amylases were added in the absence of an inhibitor system, confirming the conclusion that the pores are not introduced by enzymes after granule formation.

Finding larger holes in granules of a "refined, premium, common corn starch," Hall and Sayre (1970) concluded that numerous holes or pores are produced during starch purification. We think that our results provide strong evidence against this interpretation, although we acknowledge that exposure to certain enzymes and/or reagents may enlarge the pores.

The pores also could have been formed by the drying associated with the high vacuum of the sputter coater or the SEM specimen chamber, so dough-stage corn starch isolated in the presence of enzyme inhibitors was examined by a cryopreparation technique and low-temperature SEM. Pores were still present in specimens dehydrated by sublimation after quick-freezing. These same preparations were also examined by ESEM, without prior drying or sputter coating, to eliminate the possibility that pores were formed during sputter coating, including exposure to the high vacuum. Pores were present.

Corn starch granules that had been commercially derivatized in an alkaline salt solution, which involves limited swelling, reaction, washing, and drying, retained their pores.

Convinced that the pores are real features of at least certain corn starch granules and are somehow related to granule formation, i.e., that they are not artifacts of the isolation, preparation, or observation techniques, we decided to determine whether they are characteristic of all starches.

We did not find pores in tuber (potato) or root (tapioca) starches by either SEM or ESEM. These results differ from those of Hall and Sayre (1969), who reported pores in tapioca starch granules;

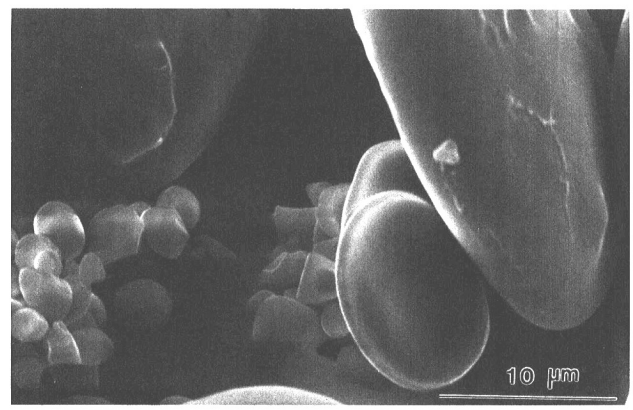

Fig. 5. Rye starch granules.

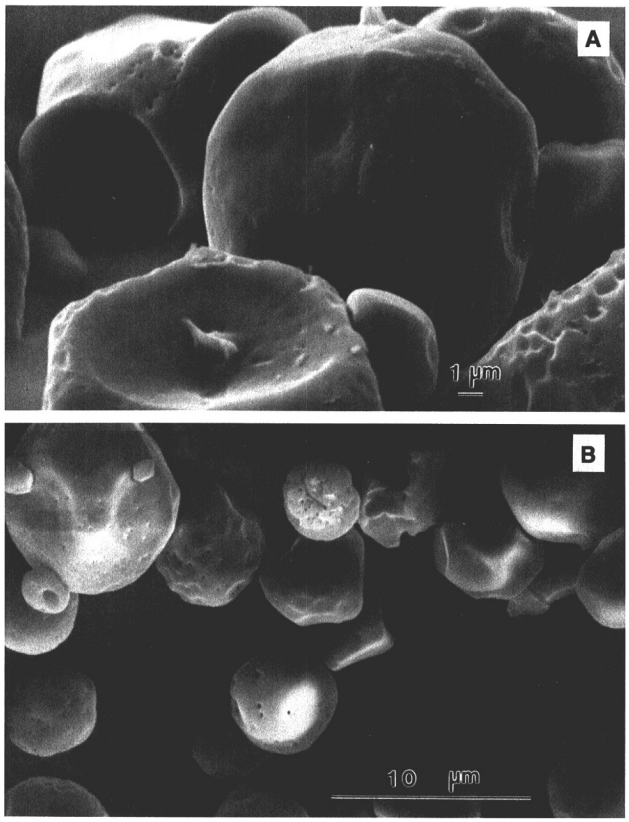

Fig. 6. A, Sorghum starch granules; **B,** millet starch granules.

also, Leach and Schoch (1961) reported that tapioca starch was second only to waxy maize starch with respect to enzyme-catalyzed digestibility. With the cereal starches, no pores were found in the two starches that contain compound granules, i.e., rice and oat starches. And in wheat, rye, and barley starches, which occur in bimodal distributions, pores were found on some large granules along the equatorial groove but never distributed over the surface (Fig. 5).

Pores were present over the entire granule surface in only three kinds of starches: corn, sorghum, and millet (Figs. 1 and 6). These three cereal grains are closely related species in the same subfamily (Panicoideae), and corn and sorghum have similar proteins and digestion patterns (Leach and Schoch 1961, Schull et al 1991). Pores also could be seen in a published photomicrograph of sorghum starch, although its accompanying article did not mention them (Craig and Stark 1984).

In summary, the pores found on the surface of granules of corn, sorghum, and millet starches and along the equatorial groove of large granules of wheat, rye, and barley starches, but not on other starch granules, appear to be real rather than artifactual. These pores may be the site of initial enzyme attack, openings that allow enzyme molecules direct access to the granule interior (hilum), or both; therefore, they may be related to control of starch conversion during germination. Additional investigations are needed to determine their depth of penetration into the granule and any relationship to granule reactivity.

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