科技資料

FRONTIERS IN CARBOHYDRATE RESEARCH-2

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Edited by

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Preface

An objective of the Whistler Center for Carbohydrate Research is " to sponsor workshops and conferences for updates on practical applications of carbohydrates and pertinent research results ". The *Frontiers in Carbohydrate Research* conferences are a principal means of accomplishing this objective.

The second Frontiers conference was held at Purdue University, 1-3 May 1990. Feedback assured us that conference attendees benefited from it, that is, that it was for them a successful learning experience that was intellectually enriching and contributed to their professional development. Through publication of these proceedings, we are now able to present the conference to a wider audience.

The 1990 conference covered biochemistry, molecular biology and physiology; chemistry and chemical structures; molecular structures; physical methods and properties; and applications. All but one lecture involved polysaccharides. We were fortunate to have five speakers from overseas.

The conference started where all carbohydrates start, with molecular biology and biochemistry (of starch biosynthesis) (J. Preiss). Also covered was fructan synthesis in grasses (N. Carpita) and some industrial starch hydrolyzing enzymes (J. Robyt).

It continued with a discussion of the use of sucrose as a chemical raw material (G. Descotes), a description of the characterization of complex carbohydrates by the reductive cleavage method (G. Gray), application of structural analysis to the cell wall structure of grasses (N. Carpita), and cell wall material as dietary fiber and its effect on serum cholesterol levels (J. Story).

The bulk of the conference dealt with structure-functional property relations of polysaccharides. This still-evolving field has come a long way in the past few decades. Much of the attention to date has been given to bacterial polysaccharides. It is necessary to examine polysaccharides with regular repeating unit structures in order to develop concepts and principals that can be applied to polysaccharides with less well-defined structures. In this section, good cases were built for the value of kinetic analysis for study of polysaccharide conformations (D. Goodall) and use of calorimetry and polarimetry to study relations of conformation and solution properties (V. Crescenzi). We also learned of a thermodynamic approach to solution conformations of charged polysaccharides (A. Cesaro) and received a short course on what NMR can contribute to an understanding of conformations and interactions in complex solid systems (T. Eads). Three speakers addressed the general topic of the effects of side-chains on the shapes and functionalities of

essentially linear polysaccharides (R. Chandrasekaran, R. Millane, W. Winter). The gross structure of starch gels/pastes (and granules) (J.Fannon), how a variety of techniques from x-ray diffraction to viscosity profiles can be used to relate polymer shape to rheological and gel properties (V. Morris), and a method for correlation of sensory and mechanical analysis of gel properties that allows comparison of gels formed by different gelling agents (R. Clark) were also described and discussed.

The conference closed with a look to future biotechnological applications of chitosan and alginate preparations (P. Sandford).

All but two of these 4 lectures are presented in this book.

On behalf of myself and all contributors, I thank Professor R. Chandrasekaran for the long hours he contributed to the editing, formatting and otherwise preparing this volume for publication. He and I thank Deborah D. Zerth and Rebecca S. Atkinson-Hitt for their skillful typing and formatting.

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Contents

USE OF LOW-TEMPERATURE SCANNING ELECTRON MICROSCOPY TO EXAMINE STARCH GRANULE STRUCTURE AND BEHAVIOR

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ABSTRACT

Examination of starch granules from a variety of sources *via* scanning electron microscopy (SEM) revealed that some have pores randomly distributed over their surfaces. These pores were found not to be artifacts of the isolation, preparation, or observation techniques and characteristic only of granules of corn, sorghum, and millet starches (entire granule surface) and wheat, rye, and barley starches (along equatorial groove only).

Cryopreparation and low temperature-SEM was used to investigate starch granule "ghosts" and pastes. A significant difference in the appearance of the ghosts of surface crosslinked starch granules, as compared to those of the underivatized granules, was found. Ghosts of both crosslinked normal yellow dent corn starch and crosslinked waxy maize starch were thicker walled and remained as open hollow spheres, while the ghosts of the parent starches were flimsy and collapsed.

Starch pastes appeared to be composed of a complex network surrounding the insoluble remains of starch granules. Paste structure varied; in some samples, ghosts were the major component of the pastes, while in others they constituted a small portion of the overall structure. Pastes made from crosslinked starches were composed of expanded hollow spheres in a matrix formed from a molecular dispersion of the internal granule contents. If the starch was not surface crosslinked, the hollow spheres collapsed.

Although we recognize that the cryopreparation and observation technique can induce ice crystal artifacts, we believe that the observed paste structures are related to both the actual structures and the properties, and therefore, that they can be useful in establishing gross structure-functional property relationships.

INTRODUCTION

Native and derivatized starch granules have been examined in the dry state, after hydration, and after the cooking of slurries using scanning electron microscopy following several specimen preparation techniques. Examination of granule remnants isolated from

pastes has also been done, all as part of a series of investigations of the morphological features of starch granules and their pastes.

While all common cereal grain, root, and tuber starches were examined, the investigation concentrated on starch granules from maize cultivars because of the commercial importance of corn starches and products derived from them. The significance of investigating the chemical reactivity, enzyme-catalyzed degradation, and pasting of corn starch is that processes based on them play important roles in the preparation of starch-based products and their use. The relationship between paste/gel structures and their rheologic and organoleptic properties are of special interest to the food industry.

The objectives of these research projects were (a) to increase our understanding of the surface morphology of uncooked starch granules, (b) to relate the surface morphology to the enzymic and chemical reactivity of granules, (c) to produce a representative view of the granule remnant or ghost (the insoluble remains of granules after pasting), (d) to produce a representation of the structure of starch pastes/gels, and (e) to determine if the functional properties of native and modified starches from different corn cultivars could be related to the structure of the granule ghost and/or to the structure of the paste.

SURFACE MORPHOLOGY

While the morphology, internal structure, and surface characteristics of starch granules have been examined extensively [1-7], a number of questions about the nature of starch granule surfaces and their relationship to the chemical and enzymic reactivity of the granule remain unanswered. In practice, modification reactions of starch will not proceed unless the granules are pretreated to "activate" them [8]. Then, reactions occur predominately on the outer surface, on the surfaces of cracks in the granule, and in the area of the hilum [9].

Native starch granules exhibit different resistances to enzyme-catalyzed digestion. When corn starch granules are treated with amylases, a characteristic pattern of holes caused by digestion is seen on the surface, indicating that some surface areas are more susceptible to attack than are others [10].

This examination of the surface morphology of starch granules concentrated on determination of the origin of very small openings (which we observed serendipitously and call pores) found randomly distributed over the surface of starch granules isolated from members of the subfamily Panicoideae (family Gramineae) and their relationship to granule reactivity. Although these features were first reported by Hall and Sayre [11], who referred to them as pinholes, no investigation into their origin, function, or effect on granule reactivity had been undertaken.

Commercial, native, yellow dent corn starch granules (10% moisture) were dry mounted by sprinkling them onto two-sided cellophane tape stuck to aluminum specimen stubs, then sputter coated with about 300 Å of gold-palladium and examined in a JEOL JSM-840 Scanning Electron Microscope (SEM) at accelerating volatages of 10 to 15 KeV.

A large variety of shapes, sizes, and surface characteristics were observed (Figure 1). Groups of granules with similarities became evident; these groupings were spherical or oval, angular, irregular, and dimpled (Figure 2). With higher magnification and better resolution, small openings randomly distributed over the surface of some granules were noticed (Figure 3). These pores were most often present on the smoother, more round granules. Some granules had many pores, while none were seen on others (Figure 4). Several sources of yellow dent corn starch were examined to see if all preparations contained granules with pores. They did (Figure 5). Next, the surface features of numerous other cultivars of corn were examined, and again, pores were found in several of them, waxy maize and dull waxy being examples (Figures 6 and 7).

Then, the origin of these pores was investigated. First, it was hypothesized that they could have been produced by drying in the field, after harvesting, or in the wet-milling process. To test this hypothesis, we isolated starch from dough-stage yellow dent corn and dried it by lyophilization. Pores were present, ruling out their formation as a result of either drying in the field or in commercial processes (Figure 8).

Second, we reasoned that they could be formed by the action of enzymes during the wet-milling process or during the laboratory isolation procedures used on the early dough-stage corn. To determine if enzyme action was the source of the pores, granules from three sources were examined: granules scraped directly from field-dried kernels (Figure 9), starch from dough-stage dent corn isolated in the presence of enzyme inhibitors (a, mercuric chloride; b, SDS plus mercaptoethanol) then dried by solvent exchange (Figure 10), and granules in sections of a dough-stage corn kernel that were dehydrated by a combination of solvent exchange and critical point drying (Figure 11). Granules from each of these three sources had pores, ruling out the possibility of formation by enzyme action during either the wet-milling or the laboratory process used for isolation.

Third, we speculated that it was possible that the pores were formed by the drying resulting from the high vacuum of the SEM specimen chamber. To account for this possibility, early dough-stage corn starch, isolated using enzyme inhibitors, was examined by a cryopreparation technique (explained later) and low-temperature SEM (LT-SEM), in which hydrated samples are frozen rapidly by plunging them into a liquid cryogen, followed by sublimation of excess water in the microscope specimen chamber. The value of this technique is that samples are not dried before observation. The pores were still present (Figure 12), so the pores are not formed by drying.

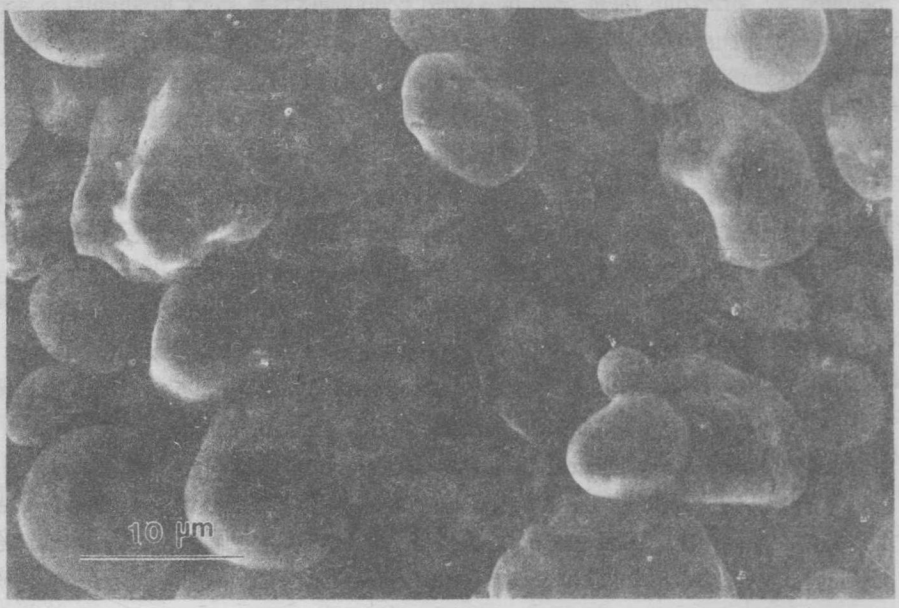

Figure 1. Commercially isolated yellow dent corn starch granules, 10% moisture (1900X).

Figure 2. Commercially isolated yellow dent corn starch, 10% moisture (1500X), showing spherical, angular, irregular, and dimpled granules.

Figure 3. Clear view of pores found randomly distributed on granule surfaces of commercially isolated yellow dent corn starch (6000X).

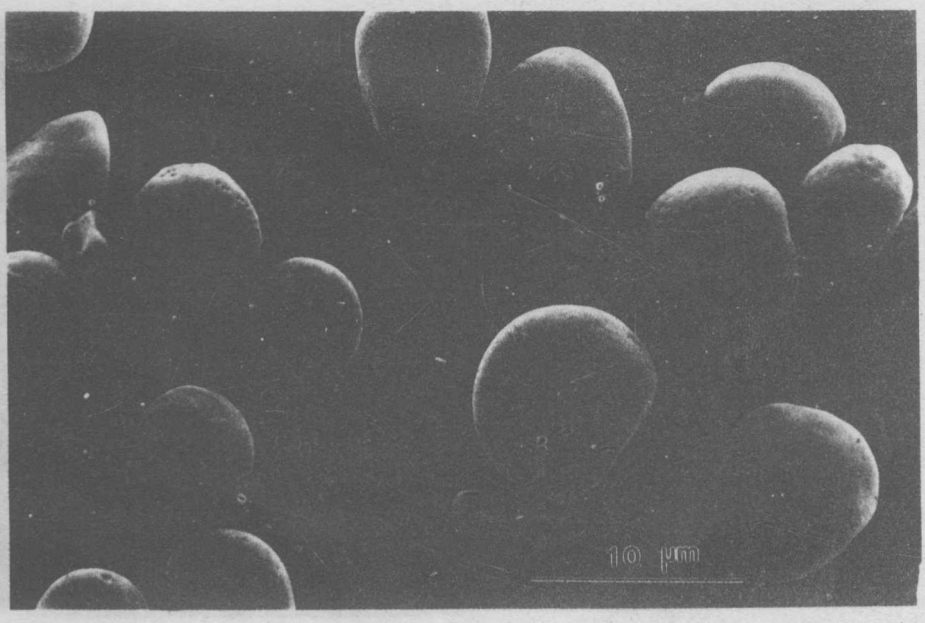

Figure 4. Commercially isolated yellow dent corn starch, 10% moisture (2500X). Arrows indicate granules with pores.

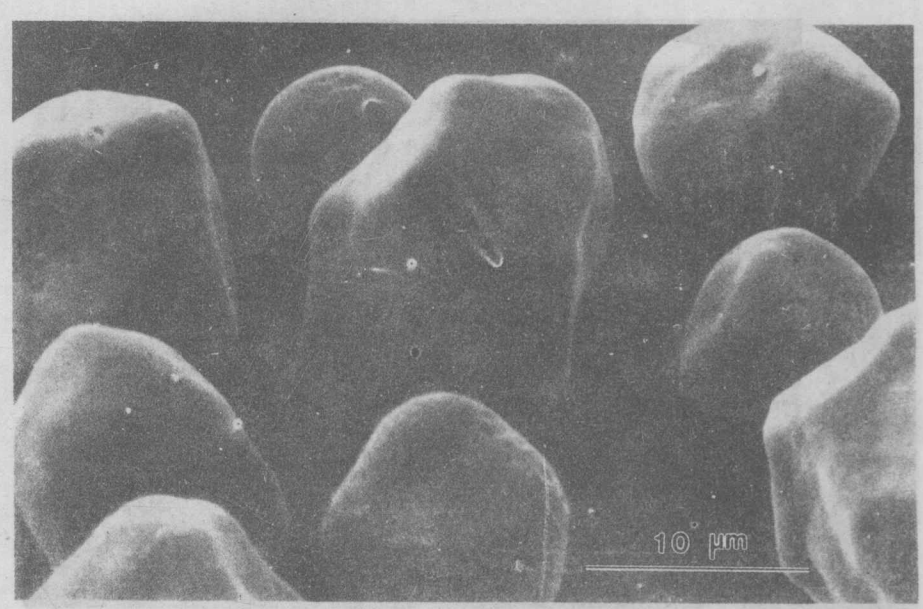

Figure 5. Commercially isolated yellow dent corn starch granules (2700X).

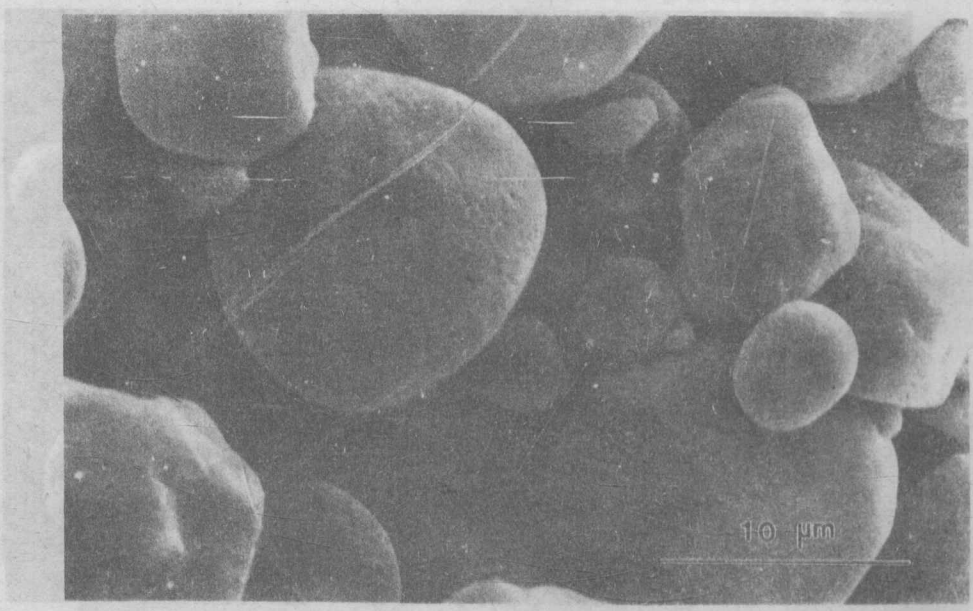

Figure 6. Commercially isolated waxy maize starch granules (3000X).

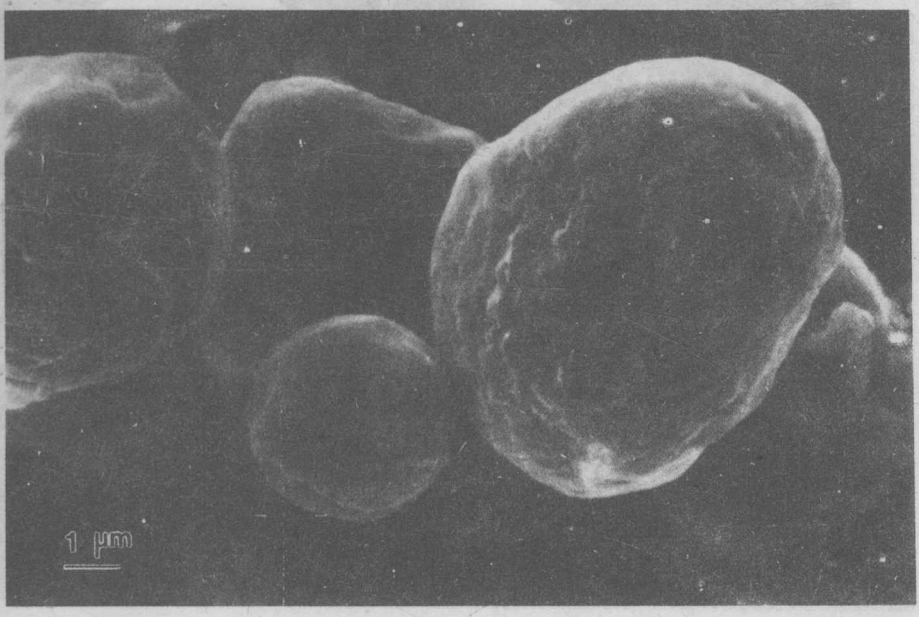

Figure 7. Commercially isolated dull waxy corn (maize) starch granules (7000X).

Figure 8. Laboratory-isolated, freeze-dried, dough-stage yellow dent corn starch granules (4000X).

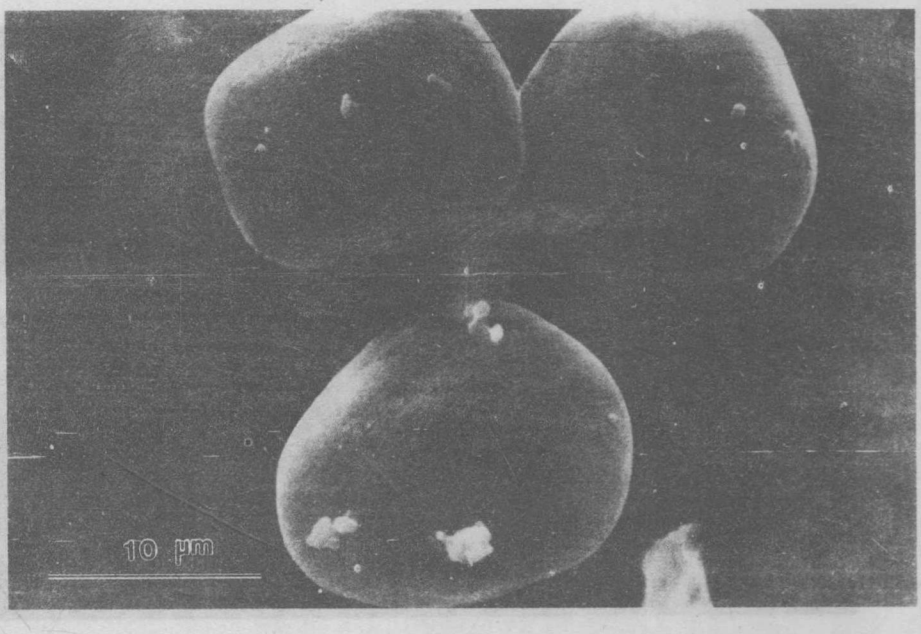

Figure 9. Starch granules scraped directly from kernels of field-dried yellow dent corn exhibiting pores (2500X).

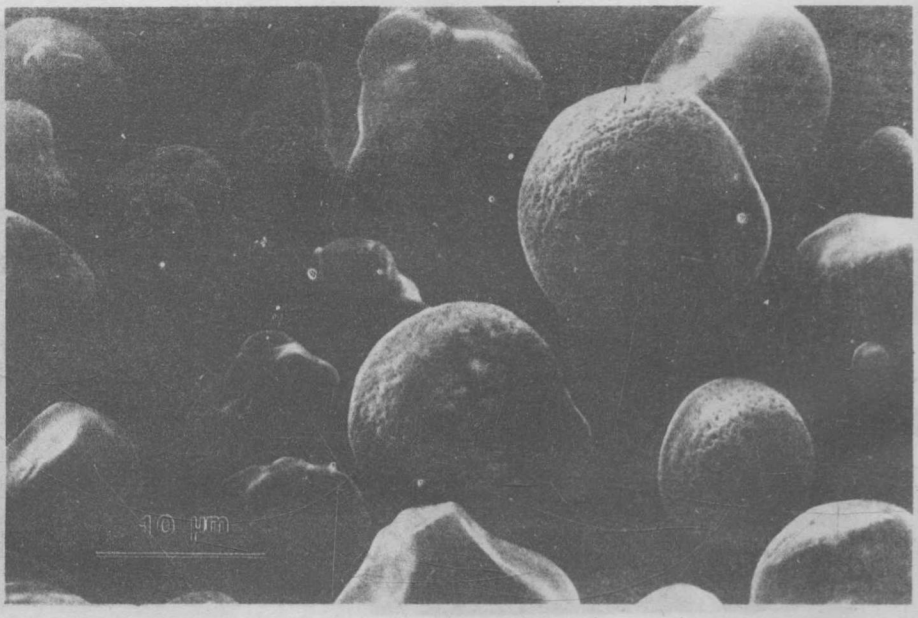

Figure 10. Laboratory-isolated granules from dough-stage yellow dent corn in the presence of enzyme inhibitors (SDS + mercaptoethanol) (2000X).

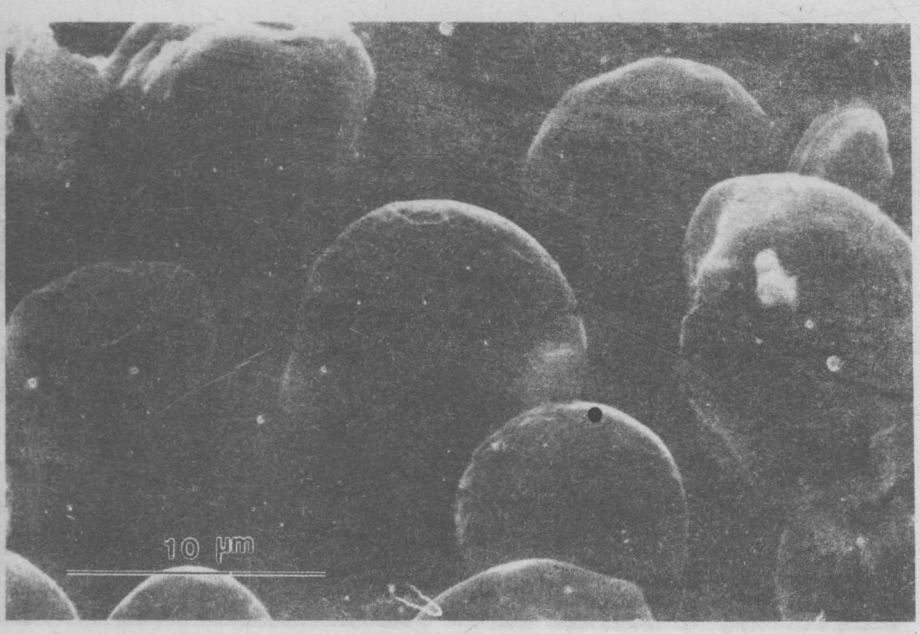

Figure 11. Granules scraped from a section of solvent exchange-dried, dough-stage yellow dent corn (3000X).

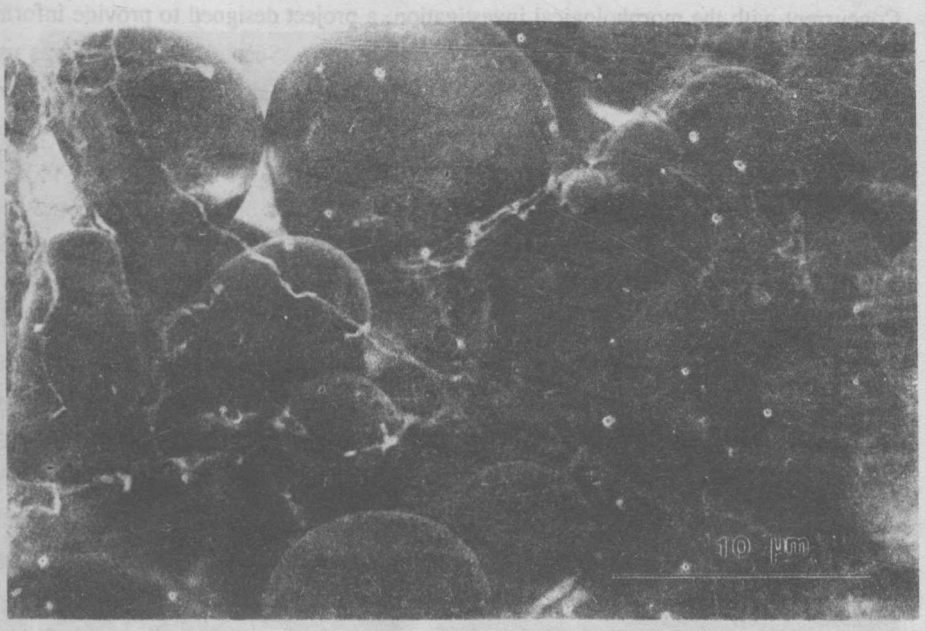

Figure 12. Dough-stage yellow dent corn starch granules isolated in the presence of enzyme inhibitors. A drop of slurry was frozen rapidly by plunging it into liquid nitrogen slush, fractured, sublimed, and coated prior to viewing at -160° C (3000X).