

# Scanning Electron Microscopy of Starch from Sprouted Wheat<sup>1</sup>

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## ABSTRACT

The scanning electron and light microscopes were used to study the changes that occur in starch granules during the sprouting of wheat. Enzymatically degraded starch granules were visible near the aleurone layer in grains sprouted for two days. Most of the attack was confined to the larger A-type of granules. Differences between the mode of enzymatic attack on the larger A-type granules than on the smaller B-type granules suggested that these granules differ in physical structure.

The scanning electron microscope (SEM) has been used to study the shape and surface details of various cereal starches (1). Microscopic studies of the wheat kernel with the SEM showed that starch granules of different shapes and sizes are embedded in a protein matrix (2). In 1955, Sandstedt (3) investigated the effect of amylase action on damaged and undamaged cereal starches with the light microscope and cinemicrography. Evers et al. (4,5), with the SEM, examined wheat starch granules after subjection to wheat  $\alpha$ -amylase and fungal glucoamylase attack. They reported that the granules treated with glucoamylase of fungal origin showed a pattern of erosion on the surface which was quite different from that resulting from the action of wheat  $\alpha$ -amylase. The detrimental effect of sprouting on breadmaking quality has been generally attributed to the abnormally high  $\alpha$ -amylase activity that develops during premature germination (6). The changes in other endosperm components are not considered important.

In the present work, electron microscopy and light microscopy were used to study the changes that occur in starch granules during sprouting of wheat. In addition,  $\alpha$ -amylase activity and the formation of free sugars in the same sprouted samples were determined in an attempt to establish a correlation between the physical changes in the granules and elaboration of  $\alpha$ -amylases during sprouting.

## MATERIALS AND METHODS

One variety of Canadian hard red spring wheat (Manitou) was used for this study. The grain was grown in Manitoba during the 1967 crop year, which was normal in temperature and moisture.

### Preparation and Milling of Sprouted-Wheat Samples

Sprouted samples were produced by germinating wheat that was first soaked in distilled water for 48 hr. The water was changed twice during the soaking period. The wheat was then germinated and sprouted at 20°C. for specific periods of time. After 0, 2, 4, and 8 days, samples of wheat were removed, frozen, and freeze-dried. The rootlets and coleoptiles were removed from the sprouted wheat by moderate

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shaking on a coarse wire sieve. All five samples (control, soaked, and three germinated) were milled into a straight-grade flour on a Buhler experimental mill after tempering to 15.5% moisture.

#### Light Microscopy

Starch granules, washed out by hand from the five flour samples, were examined by ordinary light microscopy, using the iodine staining technique described by Williams (7). In each case, the same field was also examined by ordinary microscopy with polarized light. Photographs were taken on a 35-mm. Kodachrome II film.

#### Scanning Electron Microscopy

Three types of specimens were prepared for viewing in the SEM: flour; starch (washed from flour); and cracked (into halves) whole seeds. The starch and flour were sprinkled onto double-backed Scotch tape attached to the specimen stubs. The cracked seeds were attached to the specimen stubs by a spot of silver dag so that the cracked surface was exposed. All specimens were coated in a vacuum evaporator first with carbon and then with approximately 200 to 250A of gold. The coated specimens were viewed in a Cambridge "Stereoscan" MKIIa scanning electron microscope at 5-kv. accelerating potential and photographed on a 35-mm. Kodak Tri X film.

#### Analytical Methods

The percentage of damaged starch in the flour was determined by the enzymatic procedure of Farrand (8) and the iodine-staining method of Williams and Fegol (9). Alpha-amylase activity was measured on 0.2 g. of flour by the method of MacGregor et al. (10). Free sugars in the flours were extracted as recommended by Shannon (11). The sugar content of the extract was then determined by the phenol-sulfuric acid method (12) and expressed as glucose per unit weight of flour.

## RESULTS AND DISCUSSION

#### Analytical Data

Table I summarizes the analytical data obtained in this study. The amount of free sugars and the  $\alpha$ -amylase activity increased exponentially with time during

TABLE I. STARCH DAMAGE, FREE SUGARS, AND  $\alpha$ -AMYLASE ACTIVITY OF FLOURS FROM DORMANT AND SPROUTED WHEAT

	Starch Damage, %		Free Sugars $\gamma$ glucose/g. flour	$\alpha$ -Amylase Activity (relative)
	Method "A" <sup>a</sup>	Method "B" <sup>b</sup>		
Control	22.5	25.7	3,120	1
Soaked	0.0	5.9	3,600	750
Germinated				
2 days	14.4	20.3	3,960	2,060
4 days	34.8	30.3	8,460	11,400
8 days	44.4	59.7	21,600	27,000

<sup>a</sup>Method of Farrand (8).

<sup>b</sup>Method of Williams and Fegol (9).

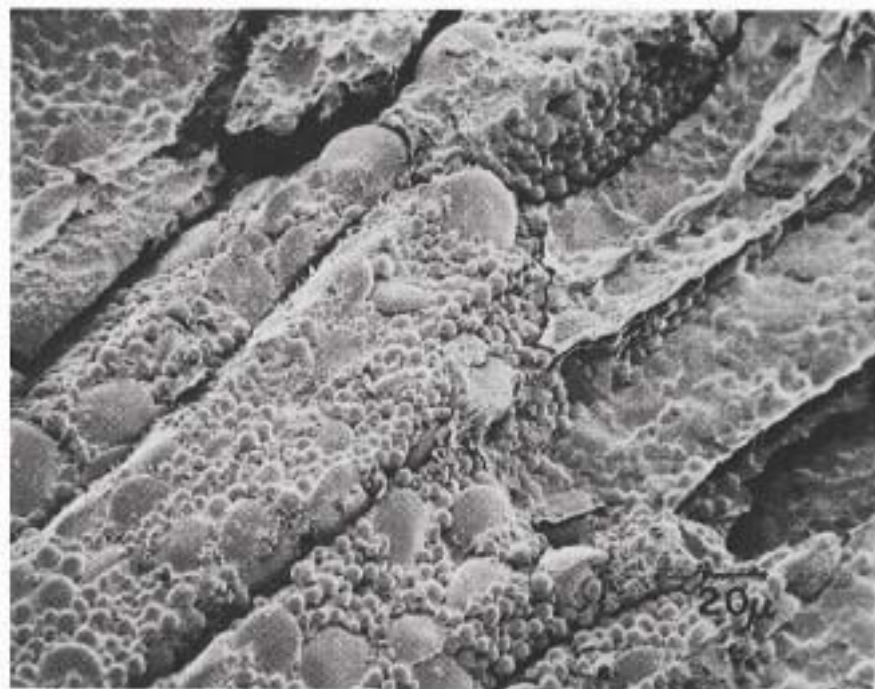


Fig. 1. Endosperm cells of the soaked wheat sample.

which the wheat was permitted to sprout. Concomitantly there was a drop in the amount of starch damage in the milled flour from 22.5 to 0% when the wheat was soaked prior to sprouting. However, with subsequent sprouting the amount of damaged starch increased rapidly. Analogous results were obtained by both the Farrand (8) and Williams and Fegol (9) methods. It is inferred from these data that the starch in the kernel was gradually degraded as sprouting progressed. Furthermore, it was suspected that the nature of the damaged starch in the flours from the control and sprouted wheats was quite different. Microscopic evidence of this difference is presented below.

#### SEM Microscopy

Figure 1 shows SEM micrographs of the endosperm cells of soaked wheat kernels exposed by fracturing. There was no evidence of any type of damage of the granules in these specimens. The variety of shapes and sizes of starch granules and the lamellar membrane surrounding the granules are readily apparent. Similar micrographs were obtained by Aranyi and Hawrylewicz (2).

Figure 2 shows a lump of flour from the control wheat sample with large lenticular-shaped or A-type, and smaller spherical or B-type granules—as previously described by Kent (13). These are embedded in an amorphous matrix. Most of the starch granules are covered with this amorphous film, which could well be the adhering or haft protein described by Hess (14).

Some enzymatic erosion of the starch granules was visible in the cracked grain of the sample germinated for 2 and 4 days. At the early stage of sprouting, most of the

enzyme attack was confined to the larger A-type granules. However, after 8 days of sprouting, both the large and small starch granules were severely eroded in the seed (Fig. 3). Examination of a large number of affected granules revealed that the enzymatic attack of the A-type granules usually started at the equatorial groove. This type of enzymatic erosion was also observed by Evers et al. (4), who studied degradation of raw wheat starch by adding wheat  $\alpha$ -amylase in a water suspension. The erosion of small spherical granules was in small circular spots randomly distributed over the surface. In the sprouted samples, the starch granules near the aleurone layer of the kernel were attacked more severely than starch granules in the inner endosperm. This suggests that in sprouted wheat the amylase activity is higher in the aleurone than in the inner endosperm. Even after 8 days of sprouting, not all of the granules were eroded by the amylases. There are still a large number of intact granules. In general, the A-type granules were more severely eroded than the smaller B-type of granule.

The difference in the mode of enzymatic attack on the large A-type and the smaller B-type granules suggests that the physical structure of these granules is probably different. The large granules were attacked at the groove and at localized sites on the surface. Once the surface is eroded, the degradation seems to move

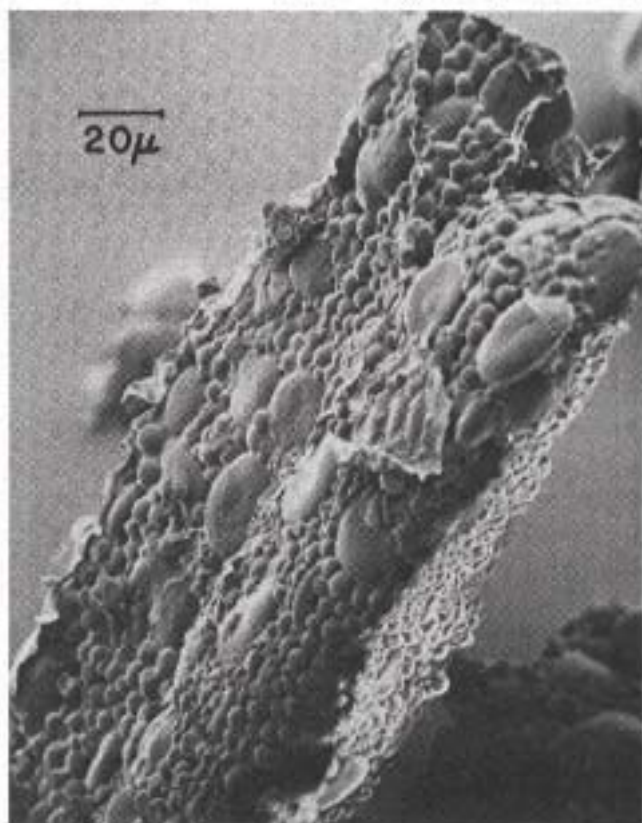


Fig. 2. Flour from the control Manitou wheat sample.

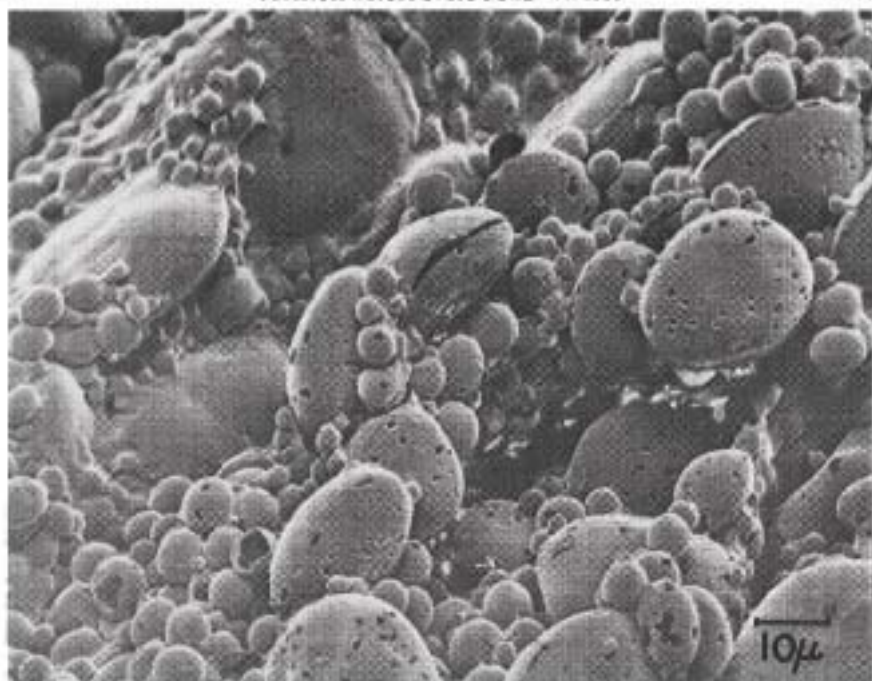


Fig. 3. Severely eroded starch granules in the seed of 8-day sprouted wheat.

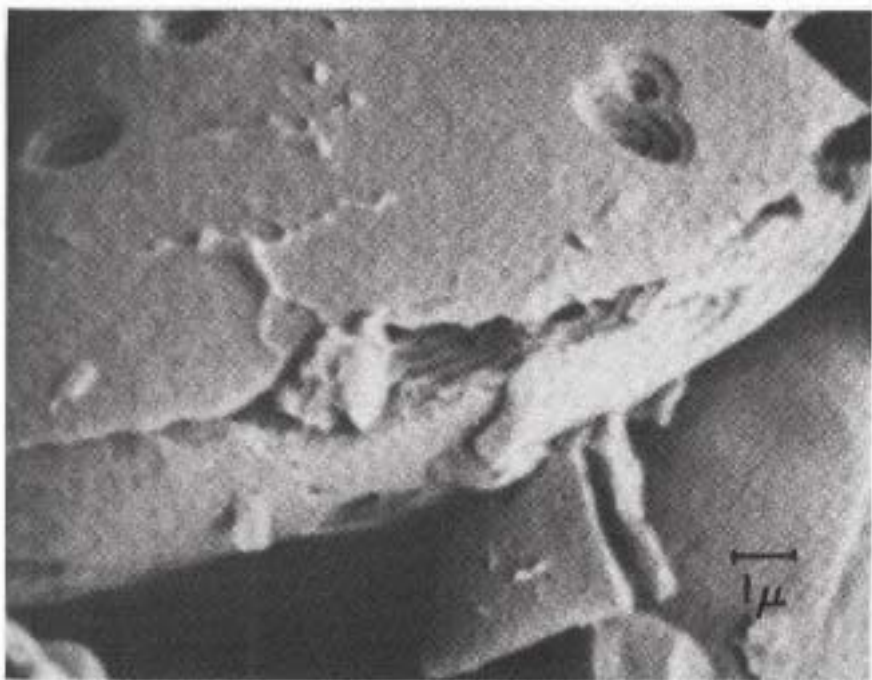


Fig. 4. Exterior of an eroded A-type starch granules from 8-day sprouted wheat.



Fig. 5. Interior cross-section of an eroded A-type starch granule from 8-day sprouted wheat.

through the layers of the granule towards the center (Fig. 4). The interior cross-section of a starch granule from the 8-day sprouted wheat is shown in Fig. 5. The center was completely digested, whereas only portions of the radial starch layers were broken down. Figure 6 shows a few attacked and broken small starch granules. In these granules the enzyme appears to have entered the granule at one or two sites and then completely digested the interior core of the granule.

#### Light Microscopy

The starch from the control sample, examined in the ordinary light microscope, showed a considerable proportion of mechanically damaged granules. All three types of damage reported by Williams (7) were observed. However, only a few damaged granules were observed in starch prepared from the soaked-wheat sample. This is in agreement with the analytical data presented above. A peculiar type of starch damage, not reported previously, was found in all three germinated samples. The appearance in the ordinary light microscope, after iodine staining, of a starch granule which has suffered this type of damage is shown in Fig. 7. The damaged granule showed randomly distributed lighter and darker (stained) areas, suggesting a

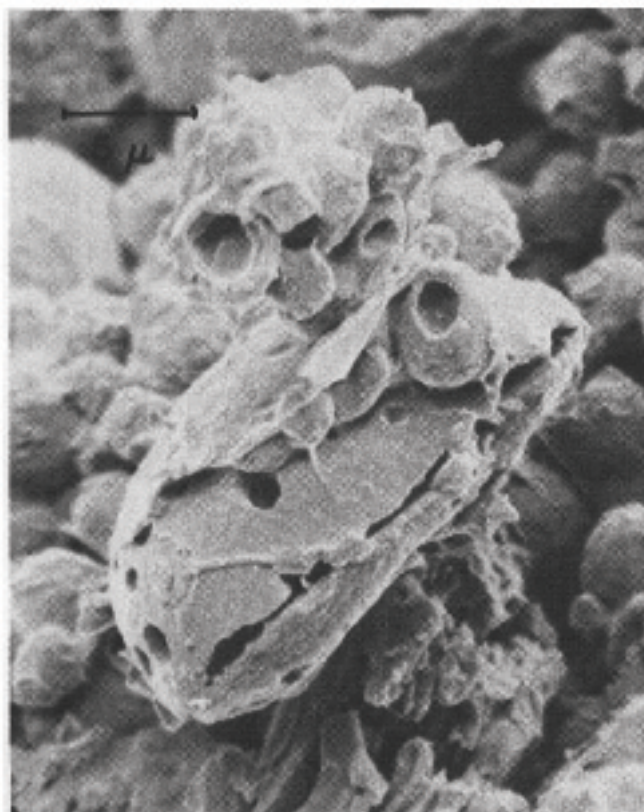


Fig. 6. Severely attacked large and small starch granules in the seed of an 8-day sprouted-wheat sample.

random distribution of damaged portions of its surface. This granule and others like it, however, still retained most of their crystalline structure when viewed under polarized light.

#### SUMMARY

During sprouting of wheat, the starch granules are gradually degraded by the action of  $\alpha$ -amylase in the kernel. This is degradation accompanied by a production of free sugars. Granules near the aleurone layer were attacked at an earlier stage of sprouting than granules in the inner endosperm. Large, A-type granules were eroded differently than the small, spherical, B-type granules. This enzymatic degradation contributes to the starch-damage values of the flours milled from sprouted wheat, and could affect the breadmaking quality, in addition to the well-known effect of  $\alpha$ -amylase.

#### Literature Cited

1. HALL, D. M., and SAYRE, J. G. A scanning electron-microscope study of starches. Part II. Cereal starches. *Text. Res. J.* 40: 256 (1970).
2. ARANYI, CATHERINE, and HAWRYLEWICZ, E. J. Application of scanning electron microscopy to cereal specimens. *Cereal Sci. Today* 14: 230 (1969).

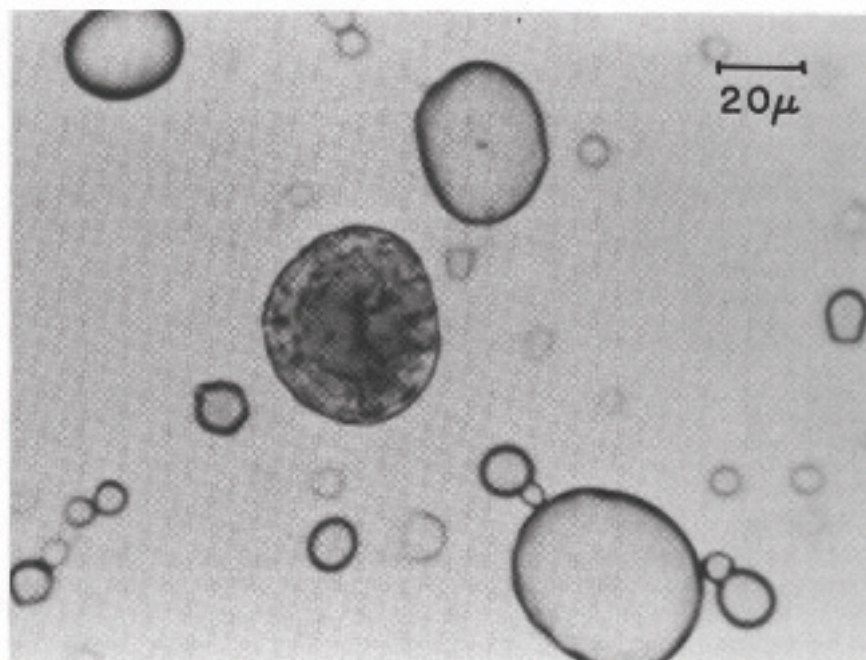


Fig. 7. Pattern of enzymatic digestion of 8-day sprouted-wheat starch as seen by ordinary light.

3. SANDSTEDT, R. M. Photomicrographic studies of wheat starch, III. Enzymatic digestion and granule structure. *Cereal Chem.* 31 (Suppl.): 17 (1954).
4. EVERS, A. D., and McDERMOTT, E. E. Scanning electron microscopy of wheat starch, II. Structure of granules modified by alpha-amylolysis - preliminary report. *Stärke* 22: 23 (1970).
5. EVERS, A. D., GOUGH, B. M., and PYBUS, J. N. Scanning electron microscopy of wheat starch. IV. Digestion of large granules by glucoamylase of fungal (*Aspergillus niger*) origin. *Stärke* 23: 16 (1971).
6. TIPPLES, K. H., KILBORN, R. H., and BUSHUK, W. Effect of malt and sprouted wheat. *Cereal Sci. Today* 11: 362 (1966).
7. WILLIAMS, P. C. Nature of mechanically damaged starch, its production in flour. Part I. *Northwest. Miller* 276: 8 (1969).
8. FARRAND, E. A. Flour properties in relation to the modern bread processes in the United Kingdom, with special reference to alpha-amylase and starch damage. *Cereal Chem.* 41: 98 (1964).
9. WILLIAMS, P. C., and FEGOL, K. S. W. Colorimetric determination of damaged starch in flour. *Cereal Chem.* 47: 56 (1969).
10. MacGREGOR, A. W., LaBERGE, D. E., and MEREDITH, W. O. S. Changes in barley kernels during growth and maturation. *Cereal Chem.* 48: 255 (1971).
11. SHANNON, J. C. A procedure for the extraction and fractionation of carbohydrates from immature *Zea mays* kernels. *Purdue Univ., Agr. Exp. Sta., Res. Bull. No. 842* (1968).
12. SIMPSON, F. J., PERLIN, A. S., and SIEBEN, A. S. Erythrose 4-phosphate. In: *Methods in enzymology*, IX, ed. by W. A. Wood. Academic Press: New York (1966).
13. KENT, N. L. Technology of cereals. Pergamon Press: Oxford (1966).
14. HESS, K. Protein, Kleber, und Lipoid im Weizenkorn und Mehl. *Kolloid Z.* 136: 84 (1954).

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