New Starches. V. Properties of the Small Starch Granules from Amaranthus retroflexus[1]

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ABSTRACT

Starch with a granule size of 0.75 to 1.25 μ has been prepared from chunk starch of *Amaranthus retroflexus* by three different procedures. Physical properties of small-granule starch are different in some respects from those of chunk starch. In general, these differences might be explained by resistance of chunks to penetration of solvents. Viscosity curves resemble those of cow-cockle starch, suggesting that low pasting temperature, granule stability, and lack of retrogradation are associated with small-granule starches.

In our search for unusual starches we isolated and studied the properties of starch chunks from *Amaranthus retroflexus* (pigweed) (1). At that time it was reported that, although common proteolytic enzymes failed to disrupt these chunks, there was some evidence that purified alpha-amylase caused considerable disintegration of starch chunks, releasing substantial amounts of small granules. The fact that these chunks failed to show the presence of individual small granules on the surface at the highest magnification obtainable with a light microscope suggested that the chunks were made up of small granules cemented together with amorphous starch.

Since the enzyme-released granules might be modified by the treatment, it was decided to attempt to recover small granules by mechanical and mild acid treatment so that their properties could be compared with those of enzyme-released granules.

The present study was conducted to compare physical properties of small granules released by these procedures with each other and with the original chunk starch.

MATERIALS AND METHODS

Preparation of Starch

Chunk starch was prepared as described by Goering (1). The small granules were prepared by digestion of chunks with purified bacterial amylase, by mechanical treatment, and by mild treatment with hydrochloric acid.

Corn starch used as control was purchased from Matheson, Coleman & Bell.

Enzyme-Released Granules

The small granules were released as follows: 250 g. of chunks were digested with 0.5% of Wallerstein's analytical-grade bacterial amylase in 2 liters of water buffered to pH 6.5, the digest was saturated with toluene and maintained with

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agitation at 50°C. for 8 hr. The suspension was screened on a 400-mesh screen; the overs were returned for further digestion, and small granules passing through the screen were collected by centrifugation. This digestion process was repeated four times. Previous results indicated that the alpha-amylase was retained on the granules in an active form even after treatment at pH 3.0 for 15 min., since this starch was completely liquefied, showing almost no peak viscosity in the Brabender instrument. Therefore, the granules were extracted for 3 hr. by a solution adjusted to pH 11.0 with NaOH, washed, and then given the inactivation treatment. After extensive washing, the granules were recovered from a suspension adjusted to pH 6.9 and carefully dried in a forced-air convection oven held at 50°C. A 57% yield of small granules was obtained.

Mechanically Released Granules

A 10% suspension of chunk starch was steeped for 24 hr. in 0.4% sodium bisulfite solution at 50°C. The suspension was then agitated for 2 min. in a Waring Blendor and screened on a 400-mesh screen. The material remaining on the screen was soaked for an additional 3-hr. period under the same conditions described above, and the process was repeated. After five treatments the combined granules passing through the screen were recovered and treated in the same manner as enzyme-released granules, except that the enzyme-inactivation step was eliminated. Microscopic examination indicated that more than 95% of the product obtained was small-granule starch with occasional small pieces of chunk starch. The yield was approximately 20%.

Acid-Released Granules

A 10% suspension of chunk starch was soaked in 0.2N HCl with gentle agitation for 24 hr. at 23°C. The starch suspension was agitated for 2 min. in a Waring Blendor at low speed and screened on a 400-mesh screen. The screen residue was soaked for 3 hr. with fresh acid, and the process was repeated. After four treatments the small-granule starch was recovered, washed, treated with alkali, and finally dried; the same technique was used as described in the preparation of enzyme-released granules. The yield of small granules was 10%. A separate study on acid corrosion indicated that the chunks were more readily dissolved than were the mechanically released small granules, suggesting that these granules were not just the result of mechanical agitation of the chunks.

Determinations

Protein content was determined by a modified Kjeldahl method (2, p. 12) (conversion factor, 6.25). Samples were ashed according to the usual procedure (2, p. 287). Total free fat was determined by ether extraction (2, p. 287).

Iodine affinity was determined by the procedure of Schoch (3).

Brabender pasting temperature range was determined by amylograms modified by CMC as described by Crossland and Favor (4) and as modified by Sandstedt and Abbott (5).

Brabender viscosity curves were determined by the procedure described by Mazurs et al. (6), except that our maximum temperature was 92.5°C. because the altitude of our laboratory prevented heating to 95°C.

Viscosity reduction with alpha-amylase: the effect of alpha-amylase was determined with the Brabender Amylograph as described by Goering and Brelsford (7).

Solubility and swelling power were determined by the procedure described by Leach et al. (8).

Solubility in dimethyl sulfoxide was determined by the procedure described by Leach and Schoch (9).

RESULTS AND DISCUSSION

Starch Granules

Photomicrographs of small starch granules released from pigweed chunk starch by three different methods are shown in Fig. 1.

Measurements on these granules gave values of 0.75 to 1.25 μ, approximately the same as those in cow-cockle starch. It is apparent that the granules are identical and not artifacts of the treatment, and that the small granules are more resistant to enzyme action than is the cementing starch which holds them in the chunk. A detailed study on the release of these granules from the chunk will be the subject of a later report.

Chemical Composition

Average values for the composition of the small-granule starch were: ash, 0.18%; fat, 0.14%; and protein, 0.45%. Results reported for chunk starch indicated less ash and fat, but more protein (1). This would suggest that the cementing starch contained more protein and less ash than the small granules. The small difference in extractable fat might be an artifact resulting from inability of the solvent to completely extract the fat. The iodine affinity of 4.8% for the small granules is nearly identical with that reported for the large chunks (1), and therefore it would appear as if there is no difference in amylose content of granules and chunks.

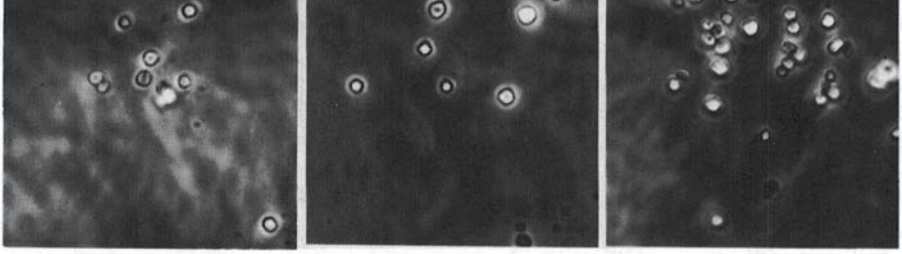

Fig. 1. Small-granule starch obtained from chunk pigweed starch at 1,600×: a, mechanically released; b, acid-released; c, enzyme-released.

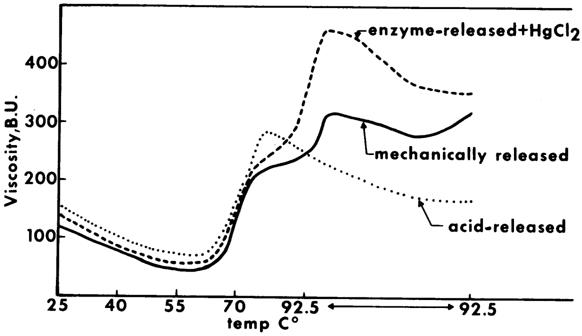

Fig. 2. Pasting of small-granule starch from pigweed: comparison of curves from acid-released, mechanically and enzyme-released granules, 5.5% starch plus 0.8% CMC.

Pasting Characteristics

Because of the small granule size, pasting characteristics were determined with the amylograph. Results are shown in Fig. 2.

Examination of pasting curves indicates that, regardless of how the granules are prepared, their pasting temperatures are nearly identical. Interestingly, the pasting temperature is approximately the same as that reported for cow cockle (10). The initial pasting temperature of small granules is somewhat less than that observed for chunk starch (1). This may be due to slow penetration of water into large chunks. These results and those reported for cow cockle seem to substantiate the nonvalidity of the reasons reported for low gelatinization temperature of potato starch (10). Furthermore, it is interesting that, although enzyme-released and mechanically released granules show two-stage gelatinization curves, this characteristic is not present in the acid-released granules. This would suggest that two-stage gelatinization curves may be artifacts and should be more thoroughly investigated.

Paste Viscosity

Paste viscosity curves are shown in Fig. 3. Acid-released and mechanically released granules give curves which resemble those reported for cow cockle (11), except that they have a slightly lower maximum. The granules are very stable during cooking and show very little setback on cooling. This suggested a very strong granule and great stability of the linear molecules. It is our opinion, based on unpublished data, that the linear molecules are very strongly bound to the amylopectin and this may be responsible for the lack of extensive retrogradation in this starch. The enzyme-released granules absorb alpha-amylase so strongly that it can neither be extracted by alkali nor inactivated by the usual acid treatment. This observation is substantiated by one of the curves in Fig. 3. The reason for this unusual effect is being investigated in our laboratory. It was observed that the addition of 200 mg. of $HgCl_2$ to 420 ml. of 8% starch inactivated the enzyme, but caused a slightly higher peak viscosity on the corn-starch control. We think the slightly different shape of the curves observed for enzyme-released granules is due

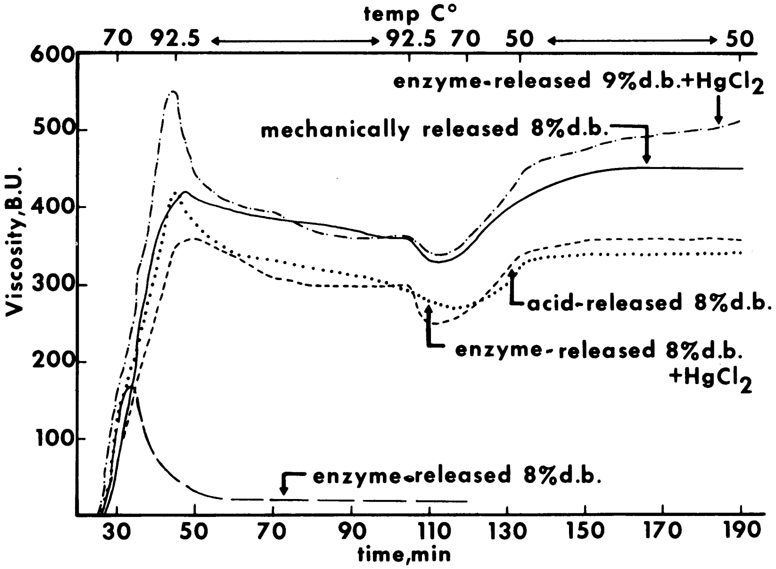

Fig. 3. Brabender amylograms on small-granule pigweed starch prepared by various treatments.

to this salt effect. The acid- and enzyme-released granules both have somewhat less viscosity than the mechanically released granules, suggesting that they might have been slightly modified in their production. A comparison of these curves with that of the chunk starch (1)suggests that much more retrogradation is taking place in the chunk starch. This would suggest that amylose is more readily extracted from the chunk starch than from the small granules. The unusual shape of the curves for the chunk starch is probably the result of slow penetration of water into the chunk.

Viscosity Reduction with Alpha-Amylase

Since there appeared to be some damage in acid-released granules and since enzyme-released granules could not be used, mechanically released granules were used for this study. Although the general shape of the curve was similar to that reported for chunk starch, it did not show as low viscosity after treatment as was observed for the chunk starch (1) (Fig. 4). This result would be expected, since the method of preparation indicated small granules to be more resistant to enzyme action than chunk starch.

Solubility and Swelling Power

Results for solubility and swelling power (Table I) suggest that there was no significant difference in small granules prepared by the different procedures. Values obtained for chunk starch suggest that it is less soluble and has slightly lower swelling power than isolated granules. This may be due to lack of water penetration.

Solubility in Dimethyl Sulfoxide

Solubility in dimethyl sulfoxide is shown in Fig. 5. Although pigweed chunk

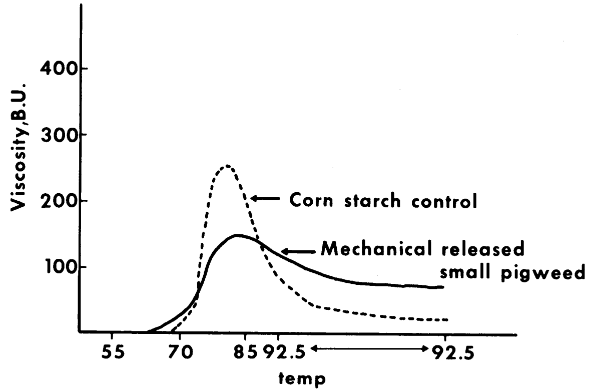

Fig. 4. Starch liquefaction: a comparison of the effect of alpha-amylase on the small-granule starch from pigweed with corn starch as a control, 7.6% starch plus 0.006% HT-100.

TABLE I. SOLUBILITY AND SWELLING POWER AT 85°C.

	Corn-Starch Control	Pigweed Granules Released by Treatment			Chunk Pigweed[a]
		Mechanical	Acid	Enzyme	
Percent Solubility	9.7	7.4	7.2	7.3	6.3
Swelling power	12.9	11.2	10.6	11.0	10.1

[a] Reported in reference 1.

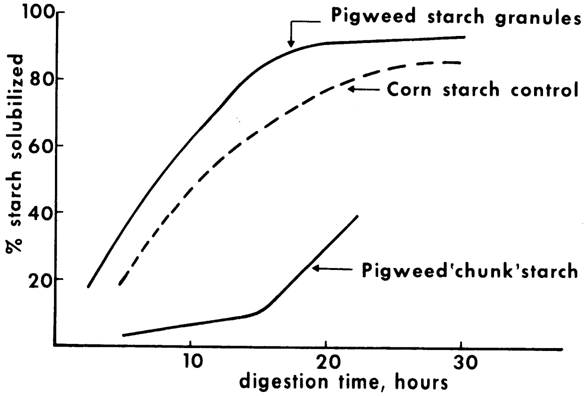

Fig. 5. Solubility in dimethyl sulfoxide: a comparison of small-granule pigweed starch with corn starch and chunk pigweed starch.

starch was very resistant to solution in this solvent, small-granule starch appeared to be slightly more soluble than corn starch. The reduced solubility of the chunk starch may have been due to failure of the solvent to rapidly penetrate massive chunks. The chunk starch curve shows a definite lag, followed by rapid solution. Although pigweed-starch granules are fairly soluble, they do not show the rapid solution observed in cow-cockle starch. This suggests that granule size alone is not responsible for rapid solution of cow-cockle starch.

CONCLUSIONS

Physical properties of the small-granule starch from pigweed are different from those of chunk starch. In general, these differences might be explained by resistance of chunks to penetration of solvents. Viscosity curves of small-granule pigweed starch suggest extreme granule stability and resistance to retrogradation. Since the same observations were made on cow-cockle starch, it would appear that this behavior is associated with small-granule starches. The tendency of chunk-pigweed starch to retrograde would suggest that amylose is more readily removed from chunks than from granules. The low pasting temperature of small-granule pigweed starch is further evidence that the reasons usually given for the low gelatinization temperature of potato starch are not valid. The observation that the granules prepared by acid treatment did not show the two-stage pasting curve suggests that two-stage gelatinization may be an artifact and that the general assumption that these curves indicate two sets of bonding forces in the granule may not be valid.

Acknowledgments

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