New Starches. II. The Properties of the Starch Chunks from *Amaranthus retroflexus*[1]

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ABSTRACT

Starch has been produced from *Amaranthus retroflexus* (pigweed) by a modified wet-milling technique. The starch in this seed is composed of two distinct entities, namely, a small amount of very small spherical granules and a large amount of massive starch chunks. These starch chunks were found to have unusual pasting characteristics. The Brabender viscosity curve for this starch does not show a cooking maximum; therefore, it is different from any other natural starch. The low solubility and swelling power of chunk pigweed starch suggests it has very strong, uniform, and extensive forces holding the mass together. The ease of attack by the amylases, in direct conflict with its solubility in dimethyl sulfoxide, suggests that the starch mass is very homogeneous.

The unusual properties of the starch from *Saponaria vaccaria* (1) suggested that a search should be made for other small-granule starches to determine if these unusual properties were associated with small granule size rather than with different plant species. To obtain sufficient starch for this study, it was necessary to select a seed which was available in quantity. Preliminary work indicated that *Amaranthus retroflexus* (pigweed) might be a good selection. In the process of separating this fine-granule starch, the details of which will be reported in a later paper, it was observed that a large amount of a white coarse material separated with the protein screenings regardless of the procedure used to separate this starch.

The present study was initiated to isolate, purify, and study the properties of this unusual material.

MATERIALS AND METHODS

Preparation of Starch

A modified wet-milling process was used for the preparation of fine-granule pigweed starch such as that described for cow-cockle (1). The residue remaining after the screening operation was suspended in distilled water at 50°C., placed in a Waring Blendor, and agitated for 5 min. The contents of the blender were placed in a large beaker and allowed to stand for 15–20 min., after which a dark oily residue came to the top of the beaker where it could be floated off. The suspension was stirred up again and the process repeated many times. When no additional material could be floated off, the entire mass was returned to the blender for another treatment. After many such treatments, a white, heavy, granular mass remained at the bottom of the beaker. This material was washed many times by suspension in warm water and was screened on an 80-mesh screen which allowed the impurities to pass through. The white residue was then suspended in cold 0.2% NaOH (pH 11.6) and allowed to stand overnight in the cold room. The alkali was then removed by screening out the solid, and the process was repeated three more times. One sample was treated with the

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enzyme pronase to see if additional protein could be removed. After thorough washing in distilled water, the starch was dried at room temperature with a stream of warm dry air passed over the samples.

The corn starch used as a control for this work was supplied by Corn Products Co. through the courtesy of T. J. Schoch.

Physical Properties and Phosphorus Content

Protein, Ash, and Fat. Protein content was determined by a modified Kjeldahl method (2, p. 12) (conversion factor, 6.25). The samples were ashed according to the usual procedure (2, p. 284). Total free fat was determined by ether extraction (2, p. 287).

Phosphorus. This was determined colorimetrically, after digestion with nitric and perchloric acid, by a slight modification of the method of Allen (3).

Swelling Power and Solubility. Swelling power and solubility were determined by the procedure described by Leach *et al.* (4).

Brabender Viscosity. The Brabender viscosity was determined by the procedure described by Mazurs *et al.* (5), except that a maximum temperature of 92.5°C. was used because of the elevation of our laboratory.

Brabender Pasting Temperature. The pasting temperature range was obtained by amylograms modified by carboxymethyl cellulose (CMC) as described by Crossland and Favor (6) and as modified by Sandstedt and Abbott (7).

Viscosity Reduction with Alpha-Amylase. The effect of alpha-amylase action was determined by the use of the Brabender Amylograph as described by Goering and Brelsford (8).

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

Fermentability. Fermentation tests were run by heating a 5% starch suspension to 80°C. in the presence of 0.05% bacterial amylase (Miles HT-1000), cooking for 0.5 hr. at 15 p.s.i., cooling to 55°, adding 10% distillers' malt, holding at 55° for 0.5 hr., cooling to 30°, and inoculating with yeast. The progress of the fermentation was followed daily by checking CO_2 loss gravimetrically. Final yields were determined at 72 hr., after correction for fermentables in enzymes and yeast used. Since 100% yields are not encountered, a control using corn starch was run with the unknown. The percent fermentability is the sum of alcohol and CO_2 produced, divided by dry weight of the sample $\times$ 100.

Iodine Affinity. The iodine affinity was determined by the procedure of Schoch (10).

RESULTS AND DISCUSSION

Starch Particles

Photomicrographs were taken under various conditions and the starch particles were compared to those of granular pigweed starch. These photomicrographs are shown in Fig. 1.

It is apparent that pigweed contains two vastly different starch particles, namely, the small spherical granules which show crosses under polarized light

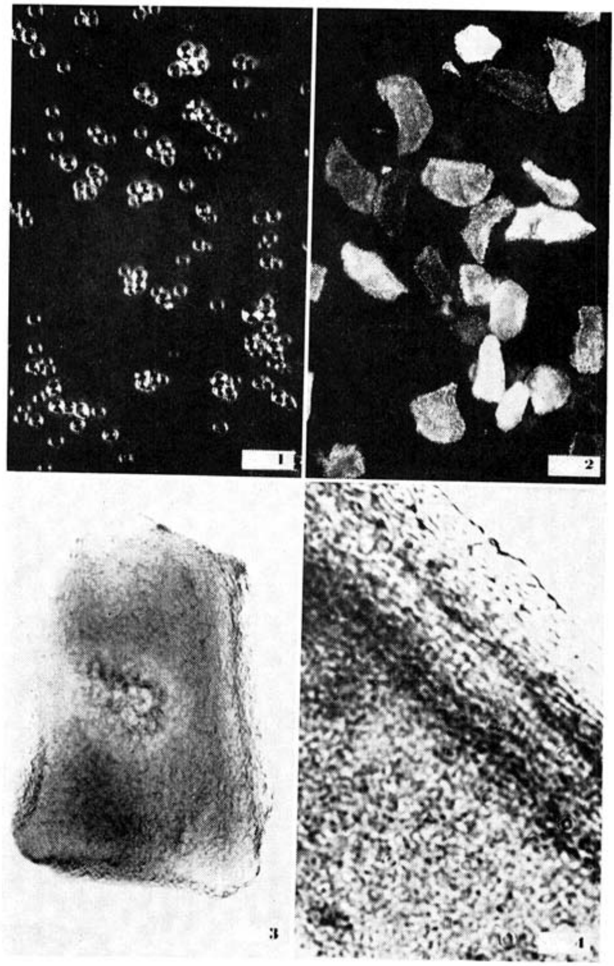

Fig. 1. Starch from the seed of *Amaranthus retroflexus:* 1, small-granule starch under crossed polars (× about 1,000); 2, chunk under crossed polars with yellow filter (× about 100); 3, chunk under crossed polars (× about 650); 4, same granule as in 3 at maximum magnification with light microscope.

and the large pieces which are not affected by polarized light; this suggests that if any crystalline regions are present they have random orientation. These large pieces stain well and uniformly with iodine, and at the highest magnification possible with our equipment no fissures or discontinuities were observed. The irregular shapes suggest the possible jigsaw-puzzle fit shown by Badenhuizen (11) for starch granules in the cell of *Iris* rhizome or amorphous pieces of nongranular starch. Although direct microscopic observations fail to indicate these chunks to be compound granules, this concept cannot be completely ruled out. All the common proteolytic enzymes that were tried failed to disrupt these chunks, but limited observations on the use of purified bacterial amylase suggest some disintegration of the starch masses with increasing numbers of small granules. This problem is now under investigation and will be reported on at a later date.

Chemical Composition

Analysis on chunk pigweed starch indicated that it contained 0.05% ash, less than 0.01% ether-extractable fat, and 1.18% protein. The phosphorus content was 0.0069%, indicating that no bound phosphorus was present. The ash and ether-extractable fat are extremely low in this material. The protein content was not lowered by treatments with the enzyme pronase, which suggests that this protein is buried in the starch mass. This might be the enzyme protein associated with starch synthesis, or it might be in the cementing material if we accept the concept that this starch is in compound granules. This possibility is now being investigated in our laboratory along with the structure of the amylose and amylopectin. The results will be reported in a later paper. Measurement of iodine affinity was somewhat difficult, owing to the failure of this material to make a clear solution in KOH. The average of the values obtained, 4.9%, indicated an amylose content slightly higher than the corn starch control.

Pasting Characteristics

The large size of the starch chunks and the fact that these chunks were not birefringent precluded direct determination of gelatinization temperature. Hence, the amylograph with CMC was used to obtain information on pasting characteristics. Preliminary microscopic observations suggested that this curve would be somewhat unusual. The results of this amylograph study, along with results for a corn starch control, are shown in Fig. 2.

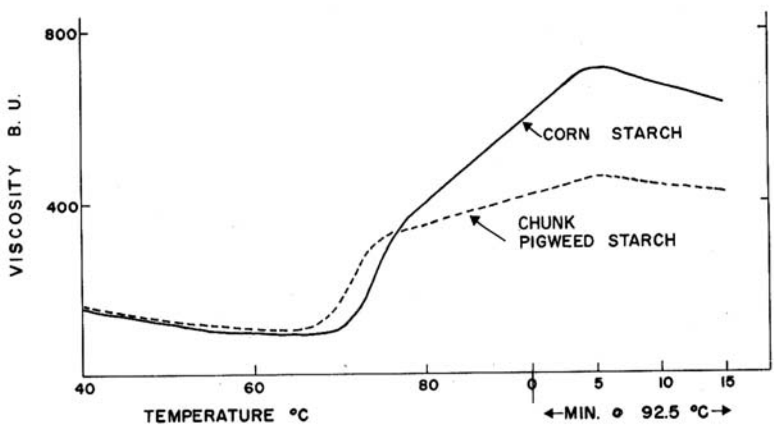

Fig. 2. Chunk pigweed starch pasting: comparison of curve obtained from corn starch; 5.5% starch + 0.8% CMC.

The curve for the chunk pigweed starch is most unusual in that the viscosity is much less than that of any other starch run by the author. The general shape of the curve resembles that of the corn starch control, except that the viscosity increases very little after the temperature of 76°C. is attained. Figure 2 indicates that chunk pigweed starch undergoes single-stage swelling, with the maximum swelling in the range of 66°–76°C. The curve suggests that very strong bonding forces occur in this starch mass.

Solubility and Swelling Power

Because the amount of material available was limited, the solubility and swelling power were determined at 85°C., at which temperature the values were 6.3% and 10.1, respectively. The fact that chunk pigweed starch has only 60% of the solubility and 80% of the swelling power of the corn starch control suggests again that either very strong forces must be present to stabilize the swollen starch mass, or, if this chunk starch is a compound granule, the cementing material might resist water penetration.

Paste Viscosity

In general, it is agreed that probably the most satisfactory method for this determination of starch viscosity is the use of the Brabender Amylograph. Figure 3 is a graphical representation of the Brabender curves for corn and chunk pigweed starch.

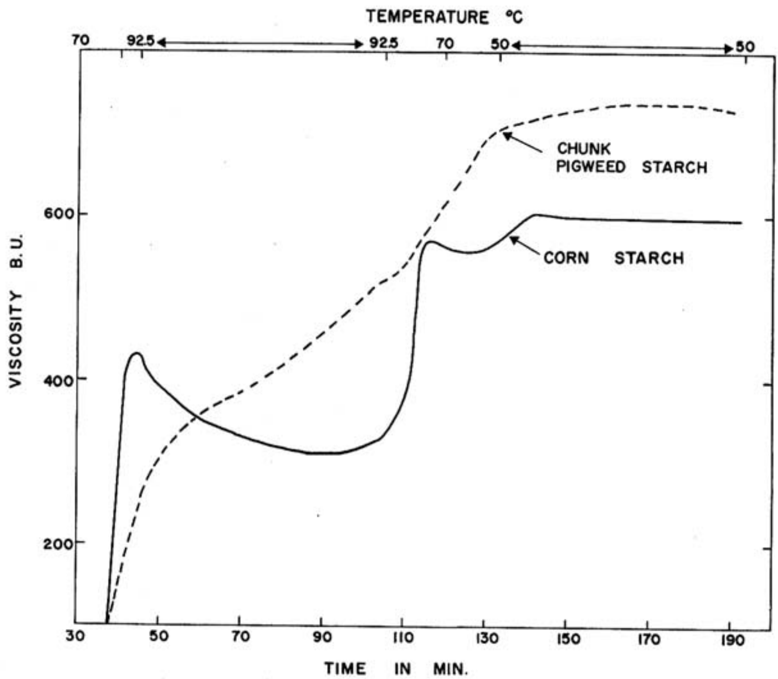

Fig. 3. Brabender amylograms: a comparison of corn and chunk pigweed starch; concentration 8 g./100 ml.

The curve for chunk pigweed starch is highly unusual in that it shows no cooking maximum and no break when the starch is cooling from 92.5° to 50°C. This highly unusual curve is what one would expect from cross-bonded waxy starch. It suggests restricted granule swelling, which could be due to internal cross-linkages; or it might be due to the failure of water to penetrate into the center of the large starch masses. The amylose content, slightly above normal, would not account for the lack of swelling in this starch, unless this amylose has unusually long chains.

Viscosity Reduction with Alpha-Amylase

The viscosity reduction by treatment with alpha-amylase was followed with the amylograph. These results are shown in Fig. 4.

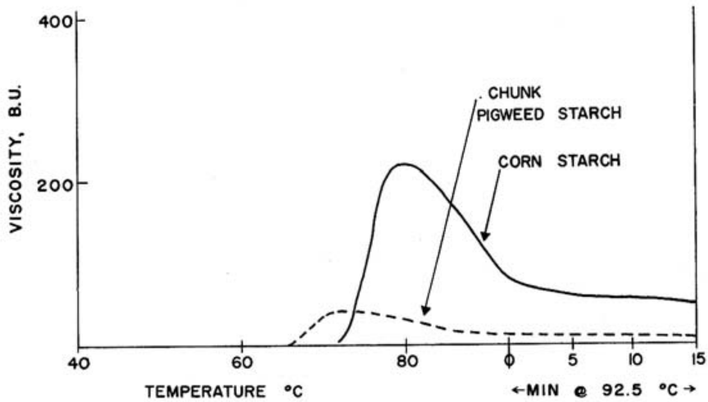

Fig. 4. Starch liquefaction: a comparison of the effect of alpha-amylase on chunk pigweed starch with corn starch as a control; 7.6% starch and 0.006% HT-1000.

The curve for chunk pigweed starch resembles that obtained for cow-cockle starch (1). It suggests that this starch is much more readily converted to the small-dextrin stage than is corn starch. This would argue against both explanations given for the unusual viscosity curve, as both delayed water penetration and cross-linking should retard the action of alpha-amylase. However, if bacterial enzymes can disrupt these large chunks over an extended period of time, it is possible that the same reaction is occurring in the amylograph and that we are measuring the enzyme susceptibility of the breakdown product, namely, the small-granule pigweed starch. Withdrawal of samples from the amylograph for microscopic examination gave inconclusive results, and lack of adequate amounts of sample prevented additional experiments of this type.

Fermentability

To check the chunk starch for the presence of nonstarch glucans, it was decided to run fermentation tests on this starch along with a corn starch control. Not only was the chunk pigweed starch fermented more rapidly, but the final yields for the pigweed and corn starch respectively were 94.7 and 90.0%. This further demonstrates the purity of chunk pigweed starch and again illustrates its extreme susceptibility to enzyme action.

Solubility in Dimethyl Sulfoxide

Because Leach and Schoch (9) reported that the solubility of granular starches in anhydrous dimethyl sulfoxide can be used as a measure of susceptibility to amylase action and since this proved to be true with cow-cockle starch (1), the results obtained on chunk pigweed starch (Fig. 5) were somewhat surprising.

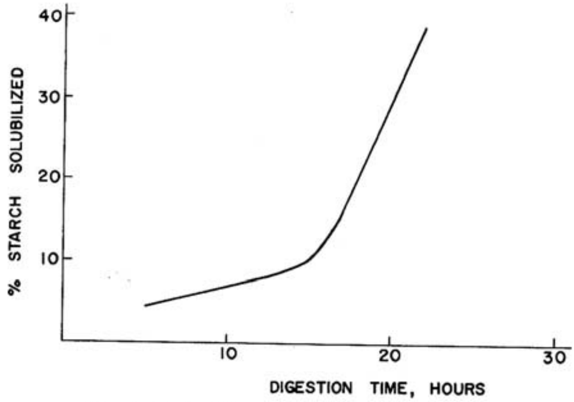

Fig. 5. Solubility in dimethyl sulfoxide.

Not only was this starch very resistant to dissolution in dimethyl sulfoxide, but after 24 hr. there was a large deposit of swollen, very viscous, sedimented residue, so clear that it could hardly be differentiated from the soluble material. However, when the tube was tilted, this clear viscous mass remained in the centrifuge tube. This starch is unusual in that it is insoluble in dimethyl sulfoxide although readily attacked by bacterial amylase. This might be explained on the basis that the enzyme destroys the cementing material, releasing small granules which may be very susceptible to amylolytic action. The resistance of this starch to solubilization in dimethyl sulfoxide might be due to the insolubility of the cementing material.

CONCLUSIONS

Pigweed contains two different starch entities, namely, a fine-granule starch found in very small amounts, and large amounts of large, irregular starch chunks. The physical properties of chunk pigweed starch suggest that it has very large, strong, uniform, and extensive forces holding the starch mass intact. The viscosity curve suggests either poor water penetration or cross-bonding in the starch masses. However, its extreme susceptibility to the action of enzymes is in direct opposition to this and suggests that something very unusual is responsible for these properties. The ease of attack by enzymes is also in conflict with the solubility in dimethyl sulfoxide. On the basis of information acquired in this investigation, we conclude that chunk pigweed starch is a homogeneous mass very strongly bound together but very susceptible to the attack of amylases. The possibility that this chunk starch might be made up of small granules cemented together by an unknown cementing material can not be overlooked. The existence of this starch should be of extreme interest to those investigators interested in the mechanism of starch granule formation. The unusual cooking curve for this starch might suggest its use in certain food applications. Further investigation of this material is currently in progress in our laboratories.

Acknowledgments

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