NEW STARCHES

I. The Unusual Properties of the Starch from Saponaria vaccaria[1]

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ABSTRACT

Starch has been produced from *Saponaria vaccaria* by a modified wet-milling technique. This starch is made up of extremely small granules of uniform size measuring 0.5 to 1.6 μ. Although its pasting characteristics resemble those of potato starch, the swelling power and solubility indicate that the granule has both strong and extensive bonding forces. Brabender curves indicate that very little retrogradation of the linear fraction takes place on cooling. This is verified by freeze-thaw data. Its extreme solubility in dimethyl sulfoxide suggests that cow-cockle starch has a very heterogeneous structure in the granule. This starch does not seem to have properties which follow those of any common starch.

Several years ago a program was established to search for new Montana sources of raw material for chemical utilization. All plants producing substantial amounts of seed were examined from the chemical standpoint. Once a promising variety was found, other selections from the same family were tested on one or more of the six branch stations of the Montana Experiment Station. One of these selections, *Saponaria vaccaria* (cow cockle, cow soapwort, or cow fat) was found to have very favorable agronomic characteristics, and the chemical analysis suggested it should be a good source of starch (1). Preliminary examination indicated that this starch consists of small, essentially uniform spherical granules of approximately the same size as those reported for the starch of *Amaranthus cruentus* by MacMasters *et al.* (2).

The present study was initiated to isolate substantial amounts of cow-cockle starch so that it might be characterized for possible commercial use.

Materials and Methods

Preparation of Starches. A modified wet-milling process was used for the preparation of several pounds of cow-cockle starch. The cow-cockle seed was steeped for 48 hr. at 48°–50°C. with 0.15*N* lactic acid. After steeping, it was run through a Quaker City Drug Mill and screened over 80-mesh screen, and the residue retained on the screen was reground. The material passing through the 80-mesh screen was rescreened on a 400-mesh screen and the starch slurry was allowed to

[1]Manuscript received June 7, 1965. Contribution from Montana Agricultural Experiment Station, paper No. 691, Journal Series.

stand for a short period of time. During this time the heavy, small, black pieces of hull tend to settle; the starch suspension decanted, and the starch separated by centrifugation. Because of the small size of the starch granules, the impurities settled more rapidly than the starch. The final starch was resuspended several times in distilled water and screened again through 400-mesh screen before the final centrifugation. The starch was dried at room temperature by passing a stream of warm dry air over the samples.

The corn starch was obtained from Corn Products Company. The wheat and rice starches were obtained from Matheson-Coleman and Bell. The potato starch was a sample of very pure starch which had been prepared in connection with a project on blue-fleshed potatoes. This potato starch was substituted for the supposedly pure potato starch supplied as a laboratory research grade material when the latter produced Brabender curves indicating severe degradation. The barley starch was prepared in the laboratory as described by Goering and Brelsford (3).

Protein, Ash, and Fat. The protein content was determined by a modified Kjeldahl method (4, p. 12) (conversion factor, 6.25). The samples were ashed according to the usual procedure (4, p. 284). The total free fat was determined by ether extraction (4, p. 287).

Phosphorus. This was determined colorimetrically after digestion with nitric and perchloric acid, by a slight modification of the method of Allen (5).

Esterified Phosphate. Prior to the determination of esterified phosphate, a 48-hr. extraction with 80% dioxane was used as described by Schoch (6).

Swelling Power and Solubility. Swelling power and solubility were determined by a modification of the procedure described by Leach *et al.* (7). The small size of cow-cockle starch granules made it necessary to double the speed of the centrifuge.

Brabender Viscosity Curves. Brabender curves were determined and analyzed by the procedure described by Mazurs *et al.* (8), except that maximum temperature was 92.5°C., because the altitude of our laboratory would not permit heating to 95°C. without boiling.

Brabender Pasting Temperatures. The pasting temperature range was determined by amylograms modified by CMC as described by Crossland and Favor (9) as modified by Sandstedt and Abbott (10).

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 (3).

Solubility in Dimethyl Sulfoxide. The solubility in dimethyl sulfoxide was determined by the procedure described by Leach and Schoch (11).

Freezing Stability. Freezing stability was determined by a modification of the method of Kite *et al.* (12) which only involved observation because of the difficulty of making any satisfactory measurements on amount of water separated.

Iodine Affinity. The iodine affinity was determined by the procedure of Schoch (13).

Results and Discussion

Starch Granules. Preliminary work on the starch obtained from cow-cockle indicated this starch to be very small indeed. Photomicrographs were taken on cow-cockle, rice, and barley starch at the same magnification. For comparison, these photomicrographs are shown in Fig. 1. Measurements made on the diameter of individual granules

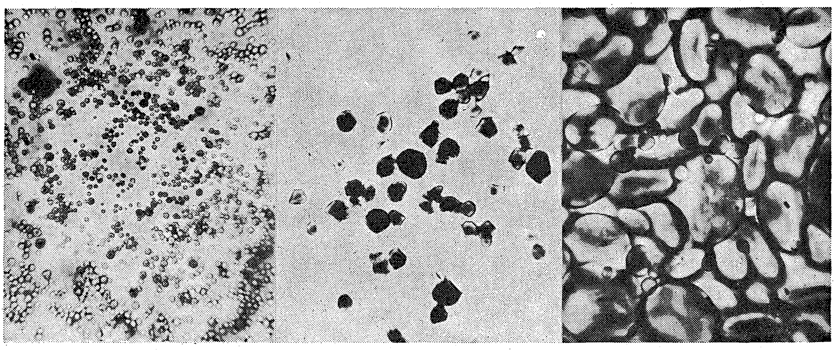

Fig. 1. Left, starch from seed of *Saponaria vaccaria*; center, rice starch; right, barley starch. (× about 900.)

gave values of from 0.5 to 1.6 μ. This starch is even smaller than that of *Amaranthus cruentus* reported by MacMasters *et al.* (2).

Chemical Composition. Analysis of cow-cockle starch indicated that it contained 0.30% ash, 0.20% fat, and 0.38% protein. The phosphorus content of the original starch was 0.0126%. After extraction for 48 hr. with 80% dioxane, the phosphorus content dropped to 0.0008, and after an additional period of 48 hr., the phosphorus was below the limit of detection of the method used. From this information it was concluded that cow-cockle starch has the normal amount of ash, fat, and protein. It was also apparent that it does not contain any esterified phosphate. Measurement of the iodine affinity gave a value of 4.3, indicating normal amylose content.

Pasting Characteristics. Because of small granule size, gelatinization temperature probably cannot be determined by the polarizing microscope. Hence, the amylograph with CMC provides a practical alternative. Pasting characteristics were determined, with wheat, rice, and potato starch as controls. The results of this study are shown in Fig. 2.

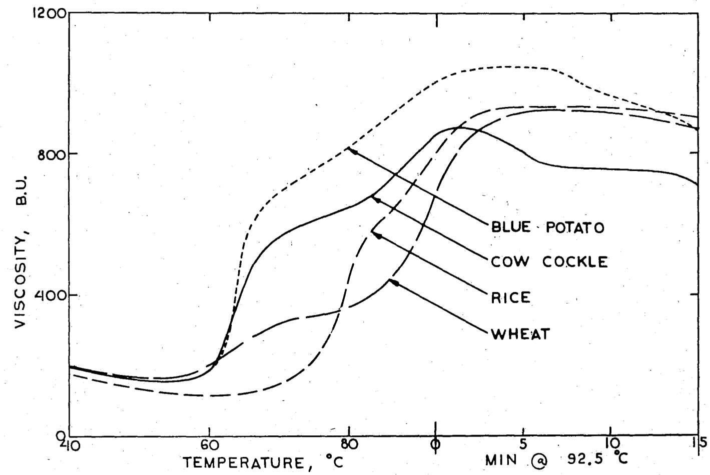

Fig. 2. Cow-cockle starch pasting: comparison of curves obtained from potato, rice, and wheat starch; 5.5% starch + 0.8% CMC (potato 4.12% starch + 0.8% CMC).

Examination of Fig. 2 shows that most of cow-cockle starch granules are gelatinized in the range of 60°–65°C., and in this respect it resembles potato starch. Low-temperature gelatinization of this type has been attributed to weak bonding forces of approximately uniform strength (7). From Fig. 2 it is apparent that the pasting characteristics of cow-cockle starch resemble those of potato starch more than those of rice or wheat starch. This would suggest that cow-cockle starch has weak bonding forces of uniform strength. The above data also suggest that granule size has no apparent effect on pasting temperature.

Solubility and Swelling Power. The solubility and swelling power are shown in Fig. 3.

The swelling-power curve for cow-cockle starch is much flatter than for any reported starch, but it does resemble that of rice starch. The low values obtained suggest moderately strong uniform forces in the granule, and in this respect cow-cockle starch is very different from potato starch, which shows rapid swelling at very low temperatures.

The solubility curve is likewise an unusual one, and resembles that of rice starch except that at the higher temperature the solubility does not increase so rapidly. Cow-cockle starch does not show the two-

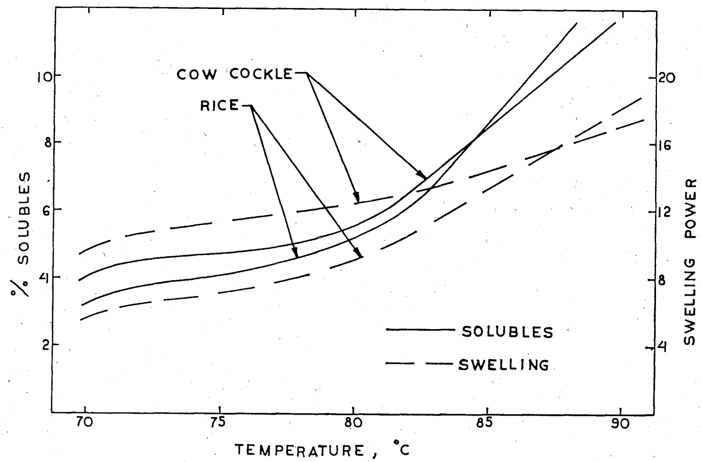

Fig. 3. Solubility and swelling power: comparison of curves obtained from cow-cockle and rice starch.

stage solubilization of corn starch, but only a gradual increase in solubility from 75° to 90°C. Certain strong forces must be present which stabilize the swollen starch granule. The solubility is far less than for potato starch, which shows a solubility of approximately 40% at 85°C. (7).

Paste Viscosity. In general, it is agreed that probably the most satisfactory method of determining starch viscosity is with the Brabender Amylograph. The graphical representation of the Brabender curves for corn, rice, and cow-cockle starch under our conditions are shown in Fig. 4.

It is apparent that cow-cockle starch differs from the controls. It has better cooking stability and higher initial viscosities. In contrast to corn, cow-cockle shows very little setback on cooling, and the cooled paste seems to be more stable than that of rice. This behavior suggests a very strong granule which is quite resistant to rupture. It also suggests a great stability of the linear molecules.

A graphical analysis of the Brabender curves for different concentration using the semilog plot recommended by Mazurs *et al.* (8) is shown in Fig. 5. The curves indicate that cow-cockle starch is distinctly different in pasting characteristics from any previously reported starch in that its values for peak viscosity, viscosity after 1 hr. of cooking, and viscosity after holding for 1 hr. at 50° remain remarkably close together over a rather wide range of concentrations.

Viscosity Reduction with Alpha-Amylase. Preliminary fermentation tests indicated that cow-cockle starch is readily converted into fer-

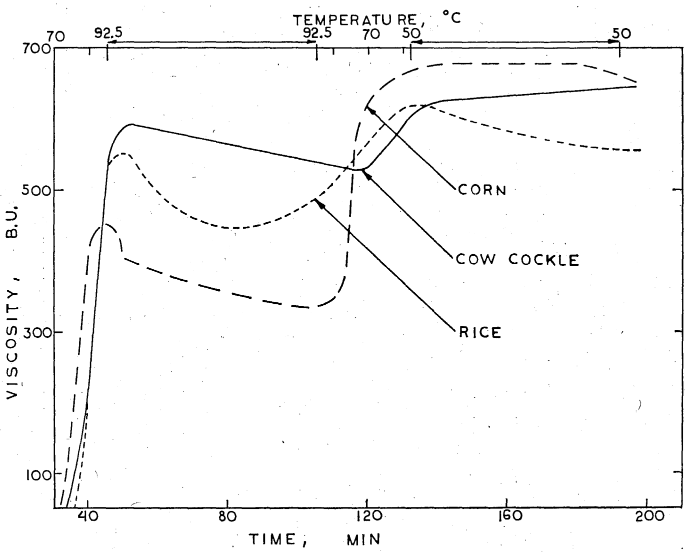

Fig. 4. Brabender amylograms: a comparison of rice, corn, and cow-cockle starch; concentration 8 g./100 ml.

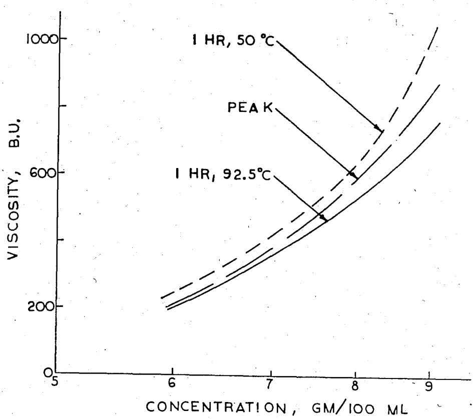

Fig. 5. Graphical analysis of Brabender viscosity curves: comparison of viscosities at peak pasting, cooking, and after holding for 1 hr. at 50°C. for cow-cockle starch.

mentable sugars. The viscosity reduction treatment with alpha-amylase was followed by means of the Brabender Amylograph and compared to corn and barley starch. The resulting information is illustrated in Fig. 6. The curves indicate that cow-cockle starch is much more readily attacked by alpha-amylase than either corn or barley.

Solubility in Dimethyl Sulfoxide. Because Leach and Schoch (11)

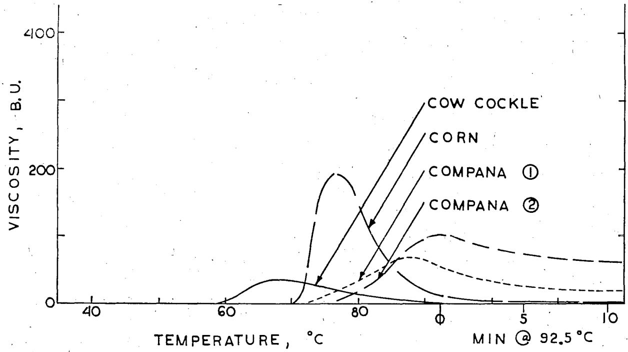

Fig. 6. Starch liquefaction: a comparison of the effect of alpha-amylase on cow-cockle starch with corn and barley starch as controls; 7.6% starch and 0.006% HT-1000. Compana (1) is barley starch prepared by batter process and Compana (2) by the alkali process.

reported that the solubility of granular starches in anhydrous dimethyl sulfoxide can be used as a measure of susceptibility to amylase action, it was decided to test this thesis. From the above results, it would be expected that the solubility would be somewhat more, but the nearly complete solubility of cow-cockle starch in this solvent, as shown in Fig. 7, was completely unexpected.

Rice starch was used as a control and the solubilities for this starch agree very well with the results reported by Leach and Schoch (11). The solubility of cow-cockle, nearly complete in 4 hr., is greater than that of any previously reported starch. Cow-cockle starch is much more

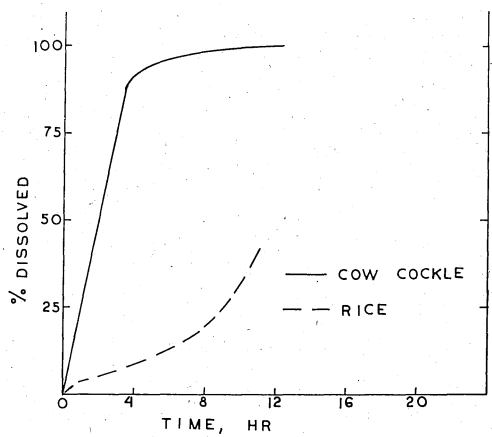

Fig. 7. Solubility in dimethyl sulfoxide: a comparison of cow-cockle and rice starches.

soluble than waxy sorghum, which was the most soluble of the starches reported previously (11). This would suggest that cow-cockle starch has a more heterogeneous structure than any previously reported starch. Since starches showing good solubility in dimethyl sulfoxide have an A-type X-ray pattern, whereas those less soluble, such as canna starch, show a B-type pattern, it would be of extreme interest to examine the X-ray pattern of cow-cockle starch. This point is presently under investigation in our laboratories.

Freezing Stability. In the studies on starch viscosity it was observed that cow-cockle starch appeared very stable on cooling and that it failed to show the usual setback observed with corn and barley starches. This suggested that cow-cockle amylose was less readily retrograded than is the amylose of normal cereal starches. To show changes in retrogradation, an experiment was made to study freeze-thaw stability. Although we were unable to get satisfactory measurements on the percentage of separated water, the experiment gave results as shown in Fig. 8.

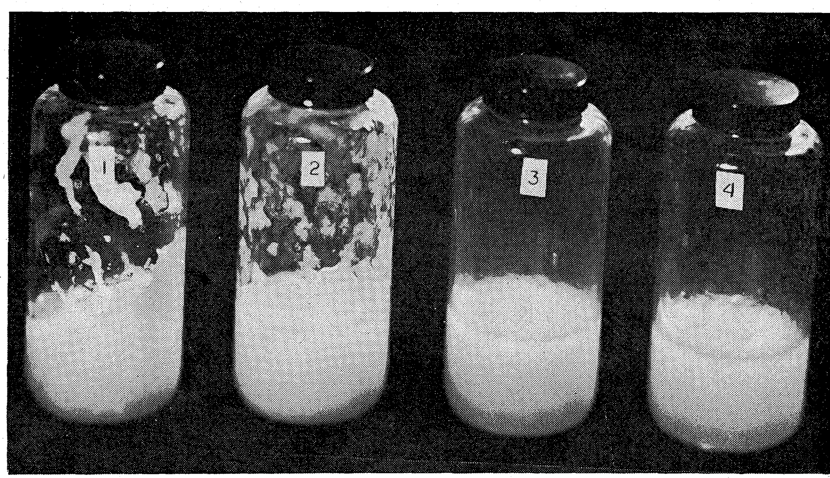

Fig. 8. Freeze-thaw stability of cow-cockle starch: 1) corn starch, 2) rice starch, and 3) cow-cockle after one freeze-thaw cycle; 4) cow-cockle after three cycles. Concentration 5%, samples shaken immediately before picture was taken, to show curd-like structure.

The corn and rice starches exhibit a flocculent nature, with considerable water separating after one cycle. The cow-cockle starch failed to show any appreciable change after three cycles. This behavior is similar to that observed for waxy sorghum, and would not be anticipated for a starch containing the normal amount of amylose.

Conclusions

Cow-cockle starch has the smallest granule of any starch reported to date. It has pasting characteristics resembling those of potato starch, which suggests weak bonding forces of uniform strength. In direct contrast to this, the swelling and solubility data indicate strong, uniform, and extensive forces in the granule. The paste viscosity and freezing stability of cow-cockle starch suggest that it has an unusually stable amylose. Its susceptibility to alpha-amylase action correlates with its solubility in dimethyl sulfoxide. The extremely high solubility in dimethyl sulfoxide suggests an extremely heterogeneous structure in the granule.

From the above data, one must conclude that the granule of cow-cockle starch has a low degree of association in the amorphous area, but strong and extensive bonding forces across micelles. This could occur either if it has exceptionally long amylose chains, or if the amylopectin had long unbranched areas in its structure. To the authors, the latter assumption seems more likely, as it would explain the reluctance of cow-cockle amylose to retrograde. A study of molecular structure of cow-cockle amylose and amylopectin might provide answers to these questions. There are two areas of substantial interest for cow-cockle starch. One is as a new starch of commercial usefulness specifically based on small granule size, which adapts this starch to numerous applications such as dusting starch in cosmetics and industrial dusting starch. The second area is as a starch of unusual paste properties for fundamental studies.

Acknowledgments

The authors wish to express their thanks to Robert Eslick for supplying cow-cockle seed, to Donald Fritts for the photomicrographs, and to the laboratory personnel of the Agricultural Experiment Station for analysis of ash, fat, protein, and phosphorus.

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