New Starches. III. The Properties of the Starch from *Phalaris canariensis*[1]

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ABSTRACT

Starch has been prepared from seed of *Phalaris canariensis* (canary grass) by a modified alkali process. This starch is composed of polygonal granules with an average size of 2.5–5.0 μ. The extremely low solubility and swelling power and very high pasting temperature would not be expected from a starch with normal iodine affinity and high content of esterified phosphate. It is suggested that either these properties may be the result of covalent links between starch chains through a phosphate, or the starch fractions in canary grass are not normal.

The unusual properties of the very small-granule starch from *Saponaria vaccaria* (1) and the very large chunks from *Amaranthus retroflexus* (2) suggest that our present knowledge of starch chemistry, which is based on the starch found in a small number of plants cultivated for food purposes, may not be valid for starches in general. Although Reichert (3) mentions the starch from *Phalaris canariensis* (a source of bird seed commonly referred to as canary-grass seed), he neither shows photomicrographs nor indicates anything about its properties other than the size and shape of the granules. Since the granule size of this starch is larger than that of cow cockle but smaller than that of rice, this material was considered a good substrate to examine in our study of whether or not granule size has any significant influence on the properties of the starch.

MATERIALS AND METHODS

Preparation of Starch

A modified alkali process was used for the preparation of starch from canary grass seed. The seed was first ground to a fine meal, pasted with warm water, and screened through a 115-mesh screen to remove the major part of the fiber and protein. The material going through the screen was centrifuged in a solid basket and dried overnight in a convection oven at 50°C. It was then resuspended in water, screened through a 400-mesh screen, and centrifuged again, and the starch was suspended in cold alkali at pH 11. After standing several hours under gentle agitation, it was removed by centrifugation and the process was repeated several times. Then the starch was washed with water and again resuspended in water; the pH was adjusted to 7.0 with HCl and the material was centrifuged. The final starch was dried in the convection oven as before.

The corn starch used for a control was supplied by Corn Products Co. by courtesy of T. J. Schoch.

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Determinations

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).

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 carboxymethyl cellulose (CMC) as described by Crossland and Favor (9) and 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 (11).

Solubility in Dimethyl Sulfoxide. The solubility in dimethyl sulfoxide was determined by the procedure described by Leach and Schoch (12).

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

Determination of Residual Fat in Starch Used for Iodine Affinity. Determination of total fat was made on the sample used for iodine affinity, by the method of Schoch (14).

Ionic Character. The ionic character of starch from canary grass seed was examined by the technique described by Schoch and Maywald (15).

RESULTS AND DISCUSSION

Starch Granules

Starch granules of canary grass seed are polygonal, resembling rice starch, with an average size of 2.5 to 5.0μ as shown in Fig. 1. The largest starch granules of canary grass seed (CGS) are approximately the same size as the smallest granules of rice starch. The granules have birefringent structures, as shown in Fig. 1.

Chemical Composition

Analysis of the CGS starch indicated that it contained 0.45% ash, 0.08% fat, and 0.48% protein. The phosphorus content of the original starch was 0.15%. After extraction for 48 hr. with 80% dioxane, the phosphorus content dropped to 0.09% and additional extraction for 48 hr. did not change this value; this suggests that an appreciable amount of esterified phosphate was present. However, as pointed out by T. J. Schoch (personal communication), this phosphate might be inorganic and therefore not

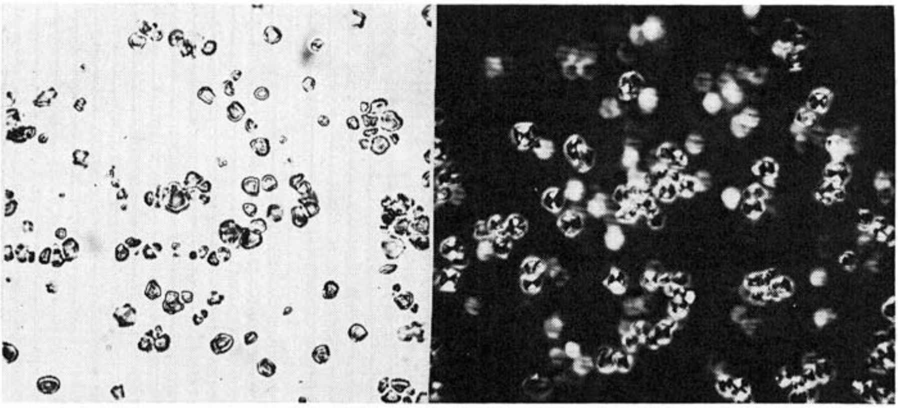

Fig. 1. Left, starch from seed of *Phalaris canariensis;* right, under polarized light.

extracted by the above procedure. The CGS starch granules did not stain with methylene blue (15), but, owing to the unusual character of the granules and the possibility that nonionic phosphate was present, this test could not be accepted as proving the absence of bound phosphate. The phosphorus content observed in CGS starch is even higher than that reported for potato starch (16). Measurement of the iodine affinity gave a value of 4.7%, indicating a normal amylose content. Since this low value for iodine affinity was not consistent with other physical properties, the sample of defatted starch used for amylose determinations was examined for residual fat by the acid hydrolysis method of Schoch and Maywald (15). This determination gave a value of less than 0.0004%, indicating the measurement of iodine affinity was probably reliable.

Pasting Characteristics

Since a Kofler electrically heated microscopic hot stage and a polarizing microscope were not available and since this instrument is barely usable

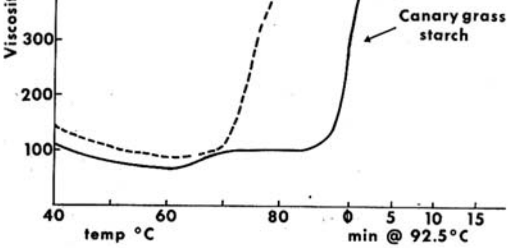

Fig. 2. Pasting of starch from canary grass seed: comparison of curves from corn starch; 5.5% starch + 0.8% CMC.

on rice starch, the pasting characteristics were determined with the amylograph in the presence of CMC. The results of this study are shown in Fig. 2.

Figure 2 indicates that CGS starch undergoes two-stage gelatinization. The slight change of slope of the curve between 60° and 70°C. is real; this was checked, with higher amounts of CMC, which exaggerated this effect. The amount of pasting taking place at this lower temperature is insignificant, as shown in the solubility and swelling measurements. The principal pasting occurs between 85° and 92.5°C. This unusually high pasting temperature suggests very strong bonding forces in the granules. This is typical of high-amylose starch, and has been explained in other starches as the result of extensive hydrogen-bonding caused by the large number of amylose molecules present. The fact that CGS starch has a normal amylose content calls for some other explanation for the strong internal bonding of these granules. It has also been suggested (17) that the weak bonding which permits gelatinization of potato starch at low temperatures is partly due to the presence of ionizable esterified phosphate groups which assist in the swelling by mutual electrical repulsion. Since CGS starch contains considerable esterified phosphate, from this reasoning one would expect its pasting temperature to be low instead of high. It is apparent that the above-mentioned reasons for the resistance of high-amylose starches and the readiness of potato starch to gelatinize do not apply to the behavior of CGS starch and that some new concept must be involved.

Solubility and Swelling Power

The solubility and swelling power for CGS starch are shown in Fig. 3. The extremely low values obtained for both again indicate that very strong forces must be present to stabilize the starch granule under these conditions. Although the swelling power of CGS starch is somewhat higher than that of high-amylose starch, its solubility is much less. As pointed out in the previous section, this is difficult to explain in a starch containing a normal amount of amylose and an appreciable amount of esterified phosphate.

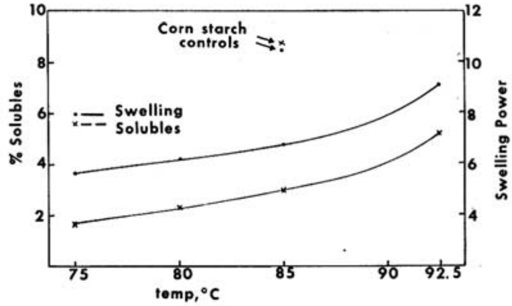

Fig. 3. Solubility and swelling power.

Paste Viscosity

The paste viscosity as measured by the Brabender Amylograph is shown in Fig. 4. The peak pasting is about the same as that for corn starch. The cooking stability of CGS starch is very good, in that the peak viscosity and

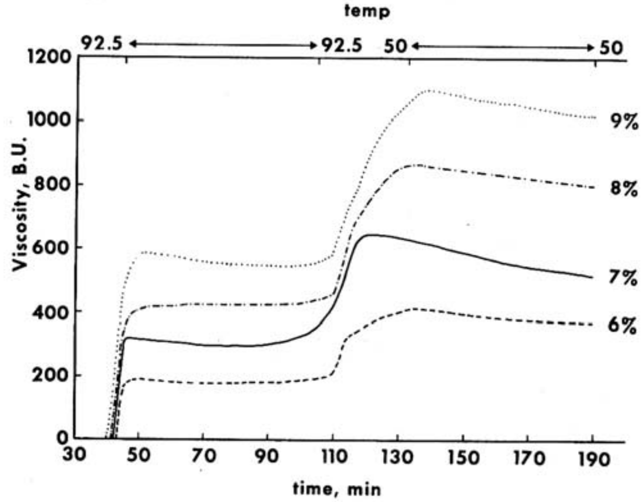

Fig. 4. Brabender amylograms on starch from canary grass seed, at different concentrations.

the viscosity after 1 hr. of cooking are approximately the same. This suggests extreme stability in the starch granule, which would be expected from the previous tests. Curves as shown in Fig. 4 are somewhat different from those of high-amylose starch; they resemble the curves obtained with cross-bonded waxy starch and, to the authors' knowledge, do not resemble curves of any other natural starch.

Some forces must be present, such as internal cross-linkages, which retard and restrict granule swelling.

Viscosity Reduction with Alpha-Amylase

The viscosity reduction by treatment with bacterial alpha-amylase was followed by means of the amylograph and the results were compared with those from corn starch. This information is presented in Fig. 5.

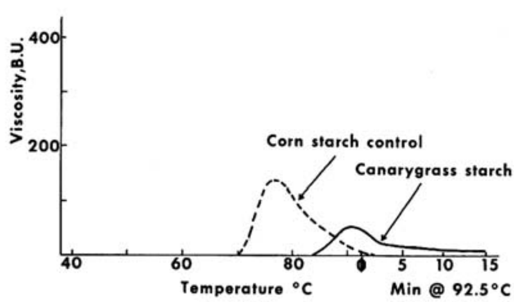

Fig. 5. Starch liquefaction: a comparison of the effect of alpha-amylase on starch from canary grass seed, with corn starch as a control; 7.6% starch and 0.006% HT-1000.

These curves substantiate the high gelatinization point of CGS starch and suggest that the major portion of this starch is more readily converted into dextrins of small molecular weight than is corn starch. They also indicate that an alpha-amylase-resistant fraction is present which is not found in corn starch. This appears to be similar to that found in barley starch and could be explained by covalent linkage between starch chains through phosphodiesters. However, it is possible that this resistance to amylase action may be an artifact resulting from the extreme difficulty of getting all the granules to swell sufficiently.

Solubility in Dimethyl Sulfoxide

Since the solubility of granular starches in anhydrous dimethyl sulfoxide has been suggested as a measure of susceptibility to amylase action (12), the solubility of CGS starch was determined in this solvent. Results are shown in Fig. 6.

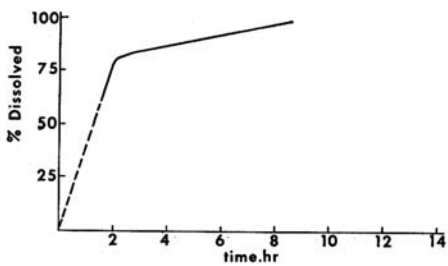

Fig. 6. Solubility in dimethyl sulfoxide.

The extreme solubility suggests that these starch granules should have a markedly heterogeneous structure and should be extremely susceptible to alpha-amylase action. This would not explain the resistance of this starch to complete liquefaction as shown in Fig. 5. This fact, along with the results reported on pigweed starch (2), suggests that the use of solubility in dimethyl sulfoxide as a measure of susceptibility to alpha-amylase action should be used with reservations.

CONCLUSIONS

Starch from canary grass seed is a very unusual starch in that, although it has very low solubility and swelling power and a very high pasting temperature, it apparently has a normal amylose content as determined by iodine affinity. The extreme stability of the granule suggests strong binding forces of some kind which must be different from those in common starches. The presence of appreciable amounts of esterified phosphate, which should increase swelling, apparently does not do so. Either these properties are the result of phosphodiester bonds between starch chains, or they must be due to an unusual structure in the amylose and amylopectin fractions of this starch. Although the phosphodiester bond has not been reported in natural carbohydrates to the authors' knowledge, there seems to be no sound reason for excluding this possibility. This thesis should not be too difficult to prove or disprove, and work is in progress in our laboratories to study the problem.

From the rather limited number of starches examined, namely those from cow cockle, pigweed, and canary grass, we fail to find any correlation between granule size and starch properties.

The small size of this starch and its ease of production suggest that it might be valuable as an industrial dusting starch and in cosmetics, although it does not have nearly the spreading power of cow-cockle starch.

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