

STRUCTURE OF THE STARCH GRANULE

III. Solubilities of Granular Starches in Dimethyl Sulfoxide¹

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ABSTRACT

Granular starches dissolve without swelling in cold anhydrous dimethyl sulfoxide. Various starch species show different rates of solution, reflecting differences in molecular bonding within the granules. Corn and sorghum starches and their waxy counterparts undergo rapid fragmentation and dissolution in this solvent, suggesting a porous or heterogeneous structure. Arrowroot and potato starches dissolve much more slowly without granule fragmentation, indicating a more homogeneous and impermeable structure. These results agree with previously reported digestibilities of granular starches by alpha-amylases. Solubilization is decreased by chemical cross-bonding, and increased by hydroxyethylation. In contrast, aqueous dimethyl sulfoxide (80–95%) dissolves corn and potato starches completely and much more rapidly. Viscometric studies show no molecular degradation of corn starch solutions in 90% dimethyl sulfoxide on prolonged standing, but potato starch undergoes a slow breakdown.

A recent publication (7) from this laboratory presented evidence concerning the internal structure of various granular starches, based on the rate and extent of solubilization by massive concentrations of different amylases. However, such evidence is open to certain inherent doubts: 1) whether enzyme action may be hindered by the presence of natural fatty acid or esterified phosphate in the starch, and 2) whether the rate of solubilization is decreased by enzyme inactivation or by the inhibiting effects of end-products. To resolve these doubts, a chemical solvent was sought which would slowly dissolve granular starches without appreciable swelling and without molecular degradation of the starch substance. The high solvent action of dimethyl sulfoxide on various polymeric substances suggested its use in this connection. In particular, Foster and his co-workers have used this medium for isolation (4) and subfractionation (1) of the linear component of starch.

Materials and Methods

The dimethyl sulfoxide was a commercial anhydrous product from Crown Zellerbach Corporation. Moisture content by a modified Karl Fischer test was 0.13%, within the maximum of 0.15% specified by the manufacturer. For reasons to be discussed, it is essential that anhydrous

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solvent be used.

The following unmodified granular starches were studied: corn, sorghum, and waxy sorghum (Corn Products Co.); waxy maize and high-amylose corn (American Maize-Products Co.); rice and tapioca (Stein, Hall & Co.); wheat (Huron Milling Division, Hercules Chemical Co.); St. Vincent arrowroot (Morningstar-Paisley, Inc.); potato (Aroostook Potato Products); canna (authentic Australian "*tous-les-mois*"). Heat-moisture-treated potato starch was included, prepared by refluxing in 70% diacetone alcohol (6). Several commercial modified starches were also tested: thick-boiling hydroxyethyl sorghum starch of 0.1 degree of substitution; phosphate cross-bonded waxy sorghum starch; 80-fluidity acid-modified corn starch.

To determine the rate of solubilization, 0.5 g. of the starch (on dry basis) was suspended in exactly 100 ml. of anhydrous dimethyl sulfoxide in a 250-ml. centrifuge bottle. The bottle was closed with a rubber stopper and placed horizontally in an Eberbach reciprocating shaker. The latter was operated at 133 strokes per minute with a stroke length of 1.5 inches; this gentle rate of agitation was just sufficient to keep the starch continually suspended. After the specified period of shaking, the bottle was removed and centrifuged for 15 minutes at 2,200 r.p.m. In all cases, there was a well-defined sedimentation of undissolved starch substance (either granular or swollen), leaving a perfectly clear supernate. A 50-ml. aliquot of the latter was removed by pipet and added to 150 ml. of vigorously stirred methanol. The mixture was digested on the steam bath for 1 hour and then allowed to stand overnight. If a hazy appearance persisted during this hot digestion (as usually occurred with potato starch), a small pinch of sodium chloride was added to induce complete flocculation. After standing overnight, the clear supernate was removed and discarded, a second 150-ml. portion of methanol added, and the mixture again digested for 1 hour on the steam bath. The precipitated starch was then filtered on a tared fritted Pyrex crucible of medium porosity, thoroughly washed with methanol, and dried in the vacuum oven for 4 hours at 120°C. Separate replicate runs were made for various shaking periods of 3 to 48 hours. Precision of results represented an average deviation of ± 4 parts per hundred in the percentage solubility.

Results and Discussion

Solubilization of Unmodified Starches. Microscopic examination showed two general modes of granule dissolution. Corn and sorghum

starches and their waxy counterparts rapidly developed darkened hilums and showed progressive fragmentation of the granules into three, four, or five segments (Fig. 1). Each of these segments retained

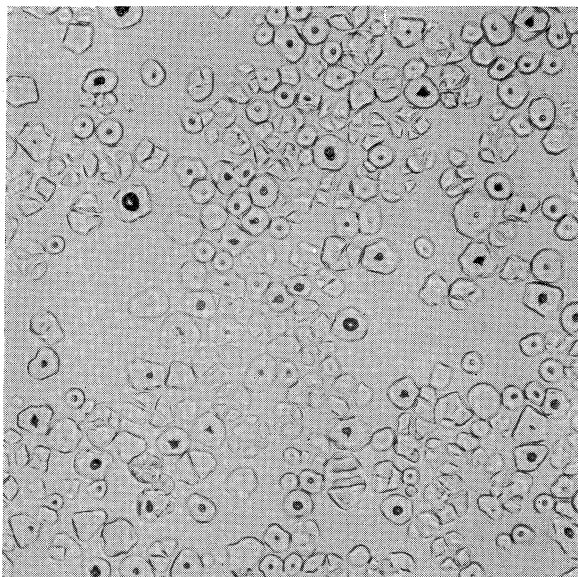


Fig. 1. Granular corn starch after 45 minutes in anhydrous dimethyl sulfoxide.

its original birefringence under polarized light. In contrast, the other starches showed a much slower selective granule attack, with no fragmentation of the granules. Arrowroot offers the best example of this second type of attack (Fig. 2). After 50 hours' shaking, this starch showed no evidence of any fragmentation. Most of the granules remained intact and optically birefringent, though the smoothly rounded contours were obviously eroded and faceted. A minor proportion of the granules became somewhat ghostlike, apparently undergoing dissolution but without appreciable concurrent swelling.

Rates of solution of the various unmodified starches are shown in Figs. 3 and 4. Three general patterns can be distinguished: 1) the rapid dissolution of corn and sorghum starches and their waxy counterparts, 2) the pronounced initial retardation of solution with tapioca and particularly arrowroot starches, and 3) the slow progressive solubilization of the other intermediate starches. Even after substantially complete solution had been obtained by 6 days' shaking in dimethyl sulfoxide, each of the native starches gave a slight trace of sediment

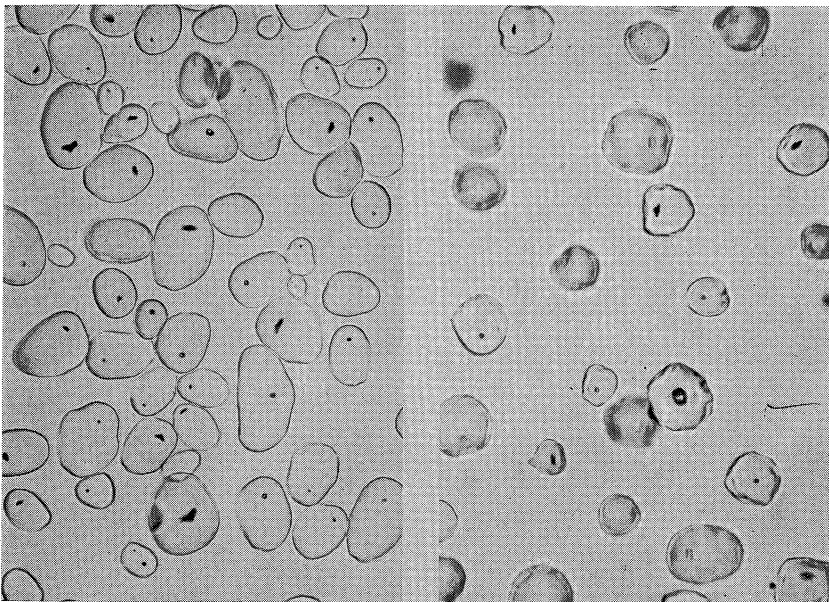


Fig. 2. Arrowroot starch in anhydrous dimethyl sulfoxide: left, immediately after suspension; right, after 50 hours.

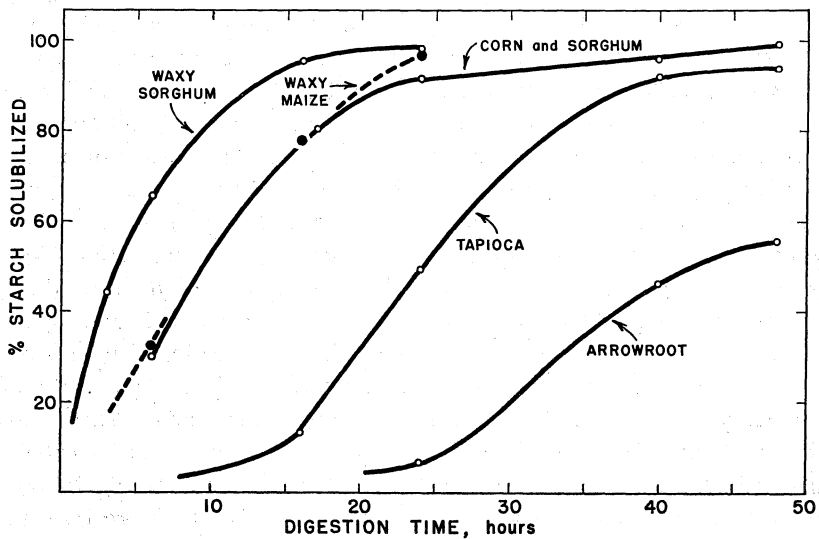


Fig. 3. Rates of solubilization of various granular starches in anhydrous dimethyl sulfoxide.

on centrifuging, and a few intact and birefringent granules could still be detected therein by microscopic examination. Obviously, the individual granules of a single starch species must differ widely in their

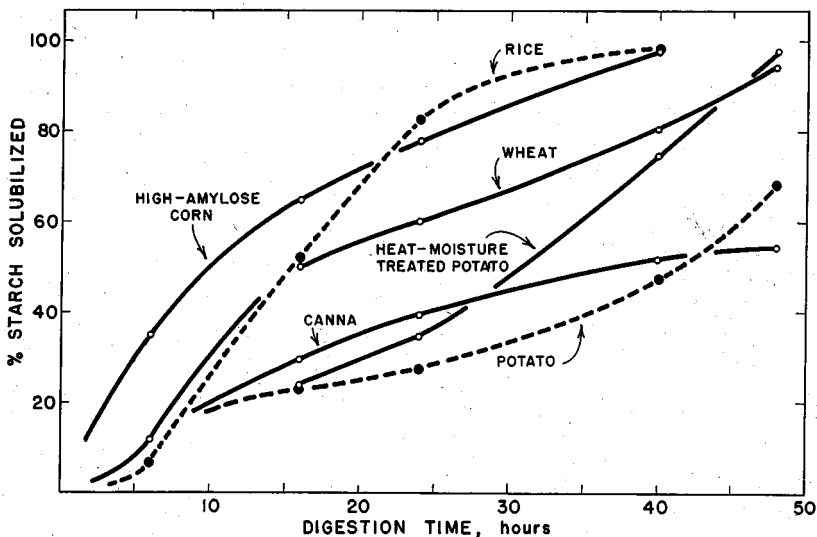


Fig. 4. Rates of solubilization of various granular starches in anhydrous dimethyl sulfoxide.

physical organization and resistance to solution. However, the trace of viscous sedimented residue from heat-moisture-treated potato starch showed no intact granules after 2 days' shaking in dimethyl sulfoxide.

The relative solubilities of these starches in dimethyl sulfoxide are remarkably similar to their previously reported enzyme susceptibilities (7). The relationship between solubility and X-ray spectra becomes more clearly defined, particularly with the inclusion of canna starch which reputedly has a B-type X-ray pattern. The A-spectrum cereal starches are the most readily attacked, either by dimethyl sulfoxide or by enzymes. The granule fragmentation by either treatment suggests some sort of structural heterogeneity within the granule, with resistant regions separated by more open and accessible areas. This could be plausibly explained either by a submicroscopic porous structure, or by the micellar concept of K. H. Meyer (i.e., strongly associated crystalline micelles separated by amorphous intermicellar regions). In contrast, those starches with a B or C X-ray spectrum are more resistant toward enzymes and toward dimethyl sulfoxide, suggesting a more homogeneous granule structure with respect to distribution of internal

bonding forces. These observations are in accord with the theory previously proposed (6) that the corn starch granule has a "black-and-white" structure of very strongly and very weakly bonded molecules, while potato starch has a "gray" structure of moderate but more uniform association. Heat-moisture treatment presumably disrupts this latter structure, increasing the strength of bonding in certain regions, and decreasing it in others.

During the course of solution, the linear fraction is dissolved somewhat more rapidly than the branched fraction. Thus corn and potato starches were partially dissolved by shaking for an appropriate time in dimethyl sulfoxide, and the soluble and insoluble fractions separated by centrifuging followed by alcohol precipitation. Iodine affinities of the soluble fractions were higher than those of the parent starches, whereas the insoluble fractions were correspondingly lower:

Starch	Percent Solubilized	Percent Iodine Affinity		
		Original Starch	Solubles	Insolubles
Corn	52	5.13	5.85	4.60
Potato	28	4.40	6.55	3.20

Solubilization of Modified Starches. Solubilization patterns of the three modified starches are shown in Fig. 5. Chemical cross-bonding

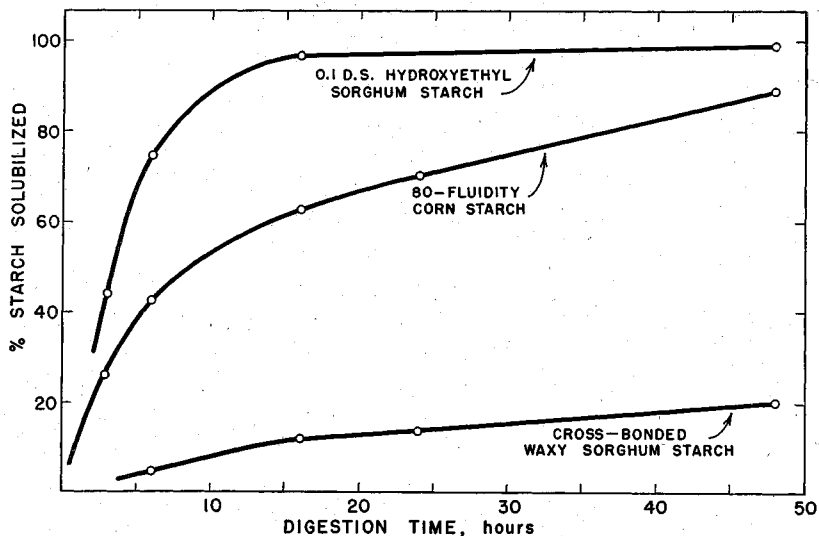


Fig. 5. Rates of solubilization of chemically modified starches in anhydrous dimethyl sulfoxide.

drastically reduced the solubilization of waxy sorghum starch, which is in accord with the greatly reduced swelling and solubilization of this starch when cooked in water (5). Similarly, more rapid solubilization of hydroxyethyl sorghum starch agrees with the theory that the introduction of ether groups extensively weakens the associative bonding within the granule. For this same reason, increasing the level of hydroxyethylation progressively lowers the gelatinization temperature of the starch, and increases both the swelling power and solubility when cooked in water.

The 80-fluidity corn starch provides a particularly interesting case. This starch has been treated with dilute mineral acid at a temperature under the gelatinization point, as is typical of commercial thin-boiling starch processing (3). It is presumed that the acid enters the more open intermicellar areas, hydrolyzing glucosidic linkages at random and thus weakening the intermicellar network, but without solubilizing any significant amount of the starch substance. Hence, when gelatinized in hot water, the granules of such acid-modified starches tend to disintegrate and dissolve to a much greater extent than those of unmodified starch, thus giving pastes of reduced viscosity. However, in the present studies, granular 80-fluidity corn starch was substantially more resistant to solubilization by dimethyl sulfoxide than unmodified corn starch.

Several other observations are pertinent here. First, the Kofler gelatinization temperature range of 80-fluidity corn starch is 69°–79°C., considerably higher than the 62°–72°C. for unmodified corn starch. This cannot be attributed to heat-moisture treatment during acid modification, since corn starch is not subject to such reorganization. Second, 80-fluidity corn starch is more resistant toward gelatinization in cold dilute alkali than is unmodified corn starch. Thus it must adsorb sodium hydroxide in excess of 0.48 meq. per g. of starch before swelling commences, as compared with a critical adsorption level of 0.40 meq. for unmodified corn starch (8). Hence, despite its greater granule fragility and higher solubility in hot water, the total micellar organization of 80-fluidity corn starch is probably higher than that of the unmodified starch. It is presumed that the acid hydrolyzes molecular chains which randomly wander through the more amorphous and unoriented intermicellar regions of the granule. The ends of these chains then become free to associate in micellar organization, by what is virtually a retrogradation within the granular starch. However, the integrity of the molecular network has been badly damaged, and hence the granules fragment and dissolve in hot water, rather

than swell.

Solubilization in Aqueous Dimethyl Sulfoxide. Solubilization of granular starches in dimethyl sulfoxide is greatly increased by the presence of small amounts of water (e.g., less than 2%). For this reason, it is imperative that the moisture content of the solvent be accurately known. Figure 6 shows the percentages of corn and potato starches dissolved in various mixtures of water and dimethyl sulfoxide after 20 hours' shaking at room temperature. These two liquids evolve heat

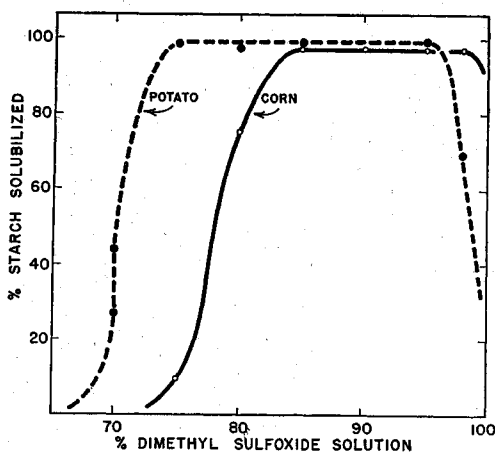


Fig. 6. Solubilization of corn and potato starches by 20 hours' shaking in various mixtures of water and dimethyl sulfoxide.

and undergo pronounced shrinkage in volume on mixing. Hence the various solvent mixtures were prepared by pipetting the requisite amount of water into a volumetric flask, adding and mixing most of the dimethyl sulfoxide, equilibrating to 25°C., and adjusting to mark with dimethyl sulfoxide.

There is a need for a solvent which will dissolve starch in the true sense without causing oxidative or hydrolytic degradation. In particular, such a solvent would be highly useful for the measurement of molecular viscosity free from any influence by granule organization. The high solvent action of 90% dimethyl sulfoxide prompted a study of its use in this connection. For this work, 0.3% solutions of corn and potato starches were prepared by shaking the starch for 20 hours in 90% dimethyl sulfoxide. This low starch concentration was chosen because it appears to represent the "molecular domain" of the starch molecules; i.e., the dilated molecules occupy all of the available sol-

vent volume. Specific viscosities were determined at 30°C. with a Cannon-Fenske No. 100 pipet.

If judged only by tests on corn starch, 90% dimethyl sulfoxide would indeed seem to be the desired "perfect solvent." As shown in Table I, a 0.3% solution showed virtually no change on standing at

TABLE I
EFFECT OF VARIOUS TREATMENTS ON THE SPECIFIC VISCOSITY OF 0.3%
STARCH SOLUTIONS IN 90% DIMETHYL SULFOXIDE

TREATMENT	CORN	POTATO
Allowed to stand for indicated days:		
0	0.618	1.95
1	0.618	1.91
2	0.618	1.86
6	0.619	1.79
9	0.622	...
23	0.614	1.52
31	0.614	1.43
55	0.616	1.32
69	0.611	1.26
Heated at 100°C. for indicated minutes:		
0	0.618	1.95
30	0.607	1.44
60	0.604	1.40
Shaken for indicated days ^a :		
0	0.613	1.74
3	0.580	1.28
6	0.568	1.17
Centrifuging:		
Original solution	0.613	1.74
Centrifuged supernate	0.609	1.70

^aNot including 20 hours' shaking for original solution.

room temperature for 69 days. Heating the solution for 1 hour in the boiling-water bath produced a slight but almost negligible decrease in viscosity. As a sensitive test for the presence of swollen but undissolved residues, the solution was centrifuged for 15 minutes at 2,200 r.p.m., and the viscosity was then determined on the upper portion of solution in the centrifuge bottle; the viscosity differed very little from the original solution, indicating the absence of any undissolved aggregates.

The viscosity of the 0.3% corn starch solution was lowered significantly by shaking for 3 and 6 days. This represents molecular degradation by shear; such breakdown is typical of any very high polymer and does not represent degradation caused by the solvent itself. However, the situation is quite different with potato starch. The viscosity was progressively and substantially reduced during 69 days' standing at room temperature, and likewise by short periods of heating in the boiling-water bath. This cannot be attributed to further dissolution of swollen aggregates, since centrifuging did not significantly reduce

the viscosity. Nor is the action due to aerobic oxidation, since tests under nitrogen showed essentially the same decrease in viscosity. According to Everett and Foster (2), solutions of potato linear fraction in dimethyl sulfoxide showed no change in viscosity during 5 months' standing. Hence it seems plausible that the branched fraction of potato starch may contain some type of anomalous primary-valence linkage which undergoes slow scission in dimethyl sulfoxide.

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