PARTICLE SIZE IN AQUEOUS STARCH DISPERSIONS[1]

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ABSTRACT

Particle size in starch dispersions prepared by commonly used methods or by high-shear treatments was examined by ultracentrifugal and light-scattering methods. Heating at 98° or 120°C. in water left nearly 70% of the corn starch as relatively large, incompletely dispersed granule fragments which had sedimentation coefficients S_{20} greater than 1,500S (Svedberg units). About one-half the dispersed starch was free amylose, which sedimented as a separate component with $S_{20} = 5-6S$. Remaining dispersed particles had sedimentation coefficients up to 600S. Most marked effect of sodium hydroxide (1.0N) on autoclaved solutions was to free all the amylose component with little change in the degree of dispersion of the amylopectin residue. Ammonia pregelatinization weakened the granule structure of corn starch and doubled the extent to which it was dispersed in water at 98°. High rate of mechanical shear was effective in dispersing granule fragments in starch pastes. Average particle size could be reduced readily to a narrow distribution with $S_{20} = 80-150S$ and particle weights of 80-150 million. Little difference was found in the extent of dispersion of corn and wheat starch pastes under shear. Amount of free amylose in solution was relatively unaffected by reduction in particle size and comprised about 60 to 75% of the total amylose content of the starch.

The swelling behavior of starch granules on heating in water is well known; but the extent of dispersion or disaggregation of the gelatinized starch has received relatively little study. The granules swell rapidly when the temperature of the water reaches about 60°C.; the extent of swelling generally increases with a further rise in temperature. When fully swollen or gelatinized by heating for a few minutes at 100°C., granules of corn starch increase manyfold in volume and appear as gelatinous particles. At least part of the starch

is dispersed sufficiently so that particles or molecules can diffuse through the swollen gel particle and enter the solution.

Although the swollen granules of some starches spontaneously disintegrate on continued heating near 100°C., corn starch granules are relatively resistant and suffer little damage unless subjected to higher temperatures or to mechanical shearing forces. Complete rupture of granules occurs, for example, if a corn starch suspension is vigorously stirred while pasted at 100°C. Undispersed fragments of granules still remain in the starch solution and can be observed with an ordinary microscope. The use of a phase microscope enabled Bechtel (1) to detect particulate matter as well as remnants of granule fragments in starch solutions prepared by dispersion in alkali, by autoclaving at 125°C., or by treatment in a Waring Blendor[2] after pasting at 96°C. No estimates have been made of the proportion of such incompletely dispersed starch and no data have been obtained on the size of particles below the limit of resolution of the microscope.

An investigation was undertaken to obtain more information on the range of particle sizes present in corn starch dispersions prepared by conventional methods and to study particularly the effect of shearing forces on particle size. The analytical ultracentrifuge is well adapted for this purpose since, by adjusting the rotor speed, sedimentation over an extremely wide range of particle weights can be observed. The data on sedimentation coefficients of constituent particles was supplemented by light-scattering measurements on some of the starch dispersions to establish an approximate relationship between sedimentation coefficient and particle weight.

Experimental

Starch Samples. Commercial corn starch samples were obtained through the courtesy of the Corn Products Company, Argo, Illinois; A. E. Staley Manufacturing Company, Decatur, Illinois; and the Clinton Corn Processing Company, Clinton, Iowa. Laboratory-prepared corn starch was isolated from No. 1 Schwenk hybrid corn by wet-milling subsequent to water-steeping at 37°C. for 48 hours. Wheat starch was prepared in the laboratory by a wet-milling process after steeping of the grain at 37°C. Commercial wheat starch was supplied by the Huron Milling Division, Hercules Powder Co., Harbor Beach, Michigan.

Preparation of Starch Dispersions. Starch dispersions were prepared by pasting 1% suspensions for 0.5 to 2 hours at 98°C. with mechanical

stirring. Solutions were stirred with a magnetic- or propeller-type stirrer at speeds under 250 r.p.m. Prolonged stirring at these low speeds did not affect the size distribution of the pasted dispersions.

Several additional treatments were given to starches after preliminary dispersion in water at 98°C. for 30 minutes. These treatments included autoclaving at 120°C. for 0.5 to 1.5 hours, stirring in a No. 700 Waring Blendor for 15 and 45 minutes, and shearing by forcing through a rayon-type spinnerette. Holes in the spinnerette were 0.003 in. in diameter. Rate of flow and shear gradient were controlled by regulating the pressure of the compressed nitrogen applied to the dispersion. Action of a Gaulin laboratory homogenizer on 1% pastes of gelatinized starch was also studied. In this apparatus the starch dispersion was forced through the small clearance between a valve and seat under high pressure. Pastes were passed through the homogenizer at pressures of 1,000, 2,000, and 3,000 p.s.i. All dispersions were diluted to 0.5% concentration before sedimentation measurements were made.

Laboratory-prepared corn starch was dispersed directly in 0.5N sodium hydroxide solution which had been sparged thoroughly with nitrogen. Aliquots of this solution were sparged with oxygen, and sedimentation measurements were made on both sets of solutions over a period of 30 days.

Sedimentation patterns of the starch dispersions were obtained with a Spinco Model E ultracentrifuge using a 30-mm. double-sector epon-resin centerpiece with the starch dispersion in one sector and the solvent in the other. The resulting baseline on each photograph facilitated area measurements. The centrifuge was operated first at 12,590 r.p.m. (11,500 × G), after which the speed was raised to 35,600 r.p.m. (96,200 × G). Three classes of starch particles were differentiated in this manner: (a) incompletely dispersed particles that reached the bottom of the cell by the time the first pattern was photographed, 8 minutes after the centrifuge reached full initial operating speed (estimated sedimentation coefficient 1,500 Svedberg units and larger); (b) rapidly sedimenting particles having sedimentation coefficients up to 600S (Svedberg units) and having particle weights in the multi-millions (primarily amylopectin); and (c) slowly sedimenting material requiring the higher rotor speed and having sedimentation coefficient in the region of 5 to 6S (primarily amylose).

Sedimentation coefficients were measured by a method of Svedberg (9). Areas of patterns were calculated by a formula of Pickels (7) using 0.00151 as the specific refractive increment of starch in water dispersions and 0.00160 for alkali dispersions. The specific refractive increment of starch in alkali was determined from a sedimentation

pattern of known concentration of amylose. To facilitate comparison of samples, the sedimentation constant-concentration relationship of isolated amylose and amylopectin was determined, and sedimentation coefficients were adjusted to a common concentration. In most cases this correction was small.

Concentration of starch in the dispersions was determined by the anthrone method (8). The observed area under a peak of the sedimentation pattern divided by the area calculated as equivalent to the total concentration of starch in the starch dispersion gave the fraction of the carbohydrate observed in that peak. The dispersed starch was considered to be the sum of the rapidly sedimenting particles (amylopectin) and the slowly sedimenting particles (amylose). The remainder was considered to be the incompletely dispersed starch, the particles of which reached the bottom of the cell too quickly to be included in the pattern.

Results and Discussion

Starch Dispersed by Heating and Low-Speed Stirring. The sedimentation pattern represented by the solid line in Fig. 1 is the type
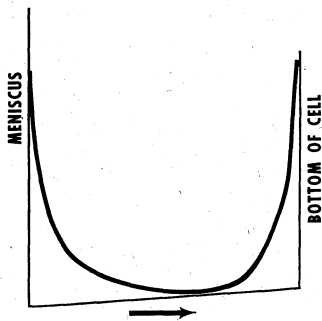

Fig. 1. Sedimentation pattern of corn starch dispersed by autoclaving (centrifuge rotor speed 12,590 r.p.m.).

obtained at the lower rotor speed (12,590 r.p.m.) when laboratory-prepared corn starch was dispersed by pasting at 98°C. for 30 minutes with low-speed mechanical stirring, followed by autoclaving at 120°C. for 3 hours. The left portion of Fig. 1 results from rapidly sedimenting particles which have not yet all moved away from the meniscus. These have a rather broad weight distribution as shown by the extension of the curve past the center of the cell. The sedimentation coefficients of the fast component range up to 600S. The fast-sedimenting particles comprise only about 13% of the total starch

in the dispersion with another 18% of the starch in a slow-sediment-ing component which has not moved away appreciably from the men-iscus in Fig. 1. The slow component has a sedimentation coefficient of 5 to 6S and was identified as amylose, on the basis of iodine sorp-tion in separate experiments. The remaining 69% of the starch in the dispersion must be accounted for as incompletely dispersed material which sedimented to the bottom of the ultracentrifuge cell before the rotor attained full operating speed. This material gives rise to the concentration gradient at the extreme right of Fig. 1. These extreme-ly large particles are referred to hereafter as incompletely dispersed starch and must include those granule fragments observed with the optical microscope as well as swollen gelatinous fragments which dif-fer too little in refractive index from the solvent to be seen with the microscope.

The use of milder conditions (2 hours at 98°C. instead of 3 hours at 120°C.) made very little difference in the state of dispersion of the starch (Table I). The amount of incompletely dispersed starch was increased slightly (71% compared with 69%), the amount of fast component being unchanged at 13%, and that of the slow component (amylose) having dropped from 18 to 16% of the total starch. Pre-treatment of the starch by gelatinization in liquid ammonia (5) prior to pasting at 98°C. doubled the amount of starch dispersed (Table I). The quantity of slow-sedimenting or amylose component increased

TABLE I

EFFECT OF PASTING CONDITIONS ON EXTENT OF DISPERSION OF
CORN STARCH IN AQUEOUS SOLUTIONS

STARCH PRETREATMENT	DISPERSION CONDITIONS	DISPERSED STARCH [a]	
		Total	Amylose
		%	%
None	2 hours at 98°	29	16
None	3 hours at 120°	31	18
NH$_3$-gelatinized	2 hours at 98°	62	20
[b]	1N NaOH at 25°	41	29

[a] "Dispersed starch" is defined as the fraction that has sufficiently small particle size to sediment at an observable rate at 11,500 × G under conditions of operation of the ultracentrifuge.
[b] Pasted at 120° for 3 hours prior to addition of alkali.

relatively little (20 vs. 16%), the major change having occurred in the amount of fast-sedimenting component (42% instead of 13% of the total starch). Incompletely dispersed starch in this case was only 38% compared with 71% when untreated starch was used. The pre-treatment with liquid ammonia improved greatly the dispersibility of the starch, yet nearly one-fourth of the amylose in the starch failed to be released as a slow-sedimenting component.

Dispersion in Alkali. An increased degree of dispersion was obtained when the starch was dispersed with $1N$ sodium hydroxide solution at 25°C. as compared with autoclaving in water alone (Table I). In contrast to pretreatment with liquid ammonia, alkali increased considerably the amount of slow-sedimenting, or amylose component, while leaving the amount of fast-sedimenting component relatively unchanged. This result indicates a preferential solubility of the amylose component in alkali; the amount liberated from the starch was essentially that predicted from iodine sorption measurements of amylose content of starch. Baum and Gilbert (3) recently used this preferential solubility of amylose in alkali as a basis for fractionating potato starch.

Since oxidative degradation of starch is considered to occur in the presence of alkali, a comparison was made between an alkaline starch dispersion exposed to oxygen and one exposed to nitrogen. Sedimentation patterns showed no significant difference between the portion of the alkaline dispersion sparged with nitrogen and that sparged with oxygen and allowed to stand at room temperature up to 30 days (Table II). In both cases the amount of slow-sedimenting

TABLE II
EFFFECT OF $0.5N$ SODIUM HYDROXIDE ON DISPERSION OF CORN STARCH IN PRESENCE OF OXYGEN AND OF NITROGEN

TIME STANDING	TREATMENT	S_{20} SLOW COMPONENT	DISPERSED STARCH	
			Slow Component	Total
days			%	%
1	Nitrogen	6.02	30	55
2	Oxygen	6.21	28	71
3	Nitrogen	6.42	28	72
7	Oxygen	5.50	29	87
13	Nitrogen	5.48	30	90
13	Oxygen	4.97	30	91
30	Nitrogen	4.46	40	93
30	Oxygen	4.81	39	94

component remained essentially constant at 30% of the total starch during the first 13 days of standing. The samples analyzed after 30 days showed an increase in amount of slow-sedimenting component to a value of 40% and a spread in the distribution of particle weights, probably as a result of breakdown of larger particles. A gradual decrease in particle size of the slow-sedimenting component is indicated by the lower sedimentation constants for samples which stood more than 3 days. The total amount of starch dispersed increased with time from 55% after 1 day to 93–94% after 30 days, indicating a pro-

gressive breakdown of the large granule fragments. The resulting rapidly sedimenting particles had too broad a distribution of particle size to permit reliable measurements; however, the average sedimentation coefficient at 0.5% concentration dropped from 100S after 1 day's standing to 35S after 30 days' standing, again indicating the occurrence of chemical degradation. However, this degradation appeared to be independent of the presence of large quantities of oxygen.

Dispersion of Pasted Starch by High-Speed Stirring. In contrast to the relatively incomplete disruption of swollen granule fragments in starch pasted at 98°C. by low-speed mechanical stirring, the shear developed in a high-speed laboratory blender effected a marked reduction in particle size of the gelatinized starch (Table III). The area under the amylose peak, together with that under the main peak

TABLE III

SEDIMENTATION COEFFICIENTS AND PARTICLE WEIGHTS OF STARCH DISPERSED IN A
BLENDER AFTER GELATINIZATION AT 98°C.

STARCH SAMPLE	TREAT-MENT	DISPERSED STARCH			S_{20} FAST COMPONENT [a]	PARTICLE WEIGHT [b]
		Slow Component	Fast Component	Total		
	minutes	%	%	%		
Commercial corn B	15	17	66	83	106	115×10^6
Commercial corn B	45	18	75	93	80	80×10^6
Laboratory-prepared corn	15	20	67	87	110	145×10^6
Laboratory-prepared corn	45	20	70	90	91	....
Commercial wheat	15	19	70	89	112	....
Commercial wheat	45	19	70	89	84	....

[a] Corrected to $C = 0.3\%$.
[b] Measured by light-scattering method.

of the pattern, accounted for up to 93% of the starch in the dispersion. There appeared to be no marked difference in the dispersibility of the commercial starches compared to the laboratory-prepared corn starch. Extent of dispersion as well as the sedimentation coefficients of the fast-moving peak was about the same for the two types of starches. If commercial corn starch was kept in the blender 45 minutes instead of 15, the average particle size dropped from 115 million to 80 million. Despite this reduction in particle size, the free amylose in solution only increased from 17 to 18%. Since corn starch contains about 27% amylose, the remaining amylose must be firmly bound to the amylopectin in the fast-sedimenting particles.

Shearing by Forcing Pastes through Small Orifices. A more extensive study of the effect of shear on the particle size of corn starch

was carried out by forcing starch pastes through the capillary openings of a rayon-type spinnerette. Rate of shear was controlled by the pressure on the solution in the spinnerette, and the amount of shearing was varied by the number of times the solution was passed through the spinnerette.

Ammonia-pregelatinized starch was used to study the effect of shear on the dispersed starch because of the greater ease with which it is brought into essentially complete dispersion (30 passages through the spinnerette at only 10 p.s.i.). The sedimentation coefficient of the main component decreased to a limiting value, as shown in Table IV, on

TABLE IV
EFFECT OF SHEARING ON SEDIMENTATION COEFFICIENTS OF AMMONIA-
PREGELATINIZED STARCH IN AQUEOUS DISPERSIONS

SHEARING CONDITIONS		S_{20} [b]
Passes [a]	Pressure	
	p s i	
20	10	385
30	10	277
40	10	282
50	10	285
5	25	212
10	25	203
15	25	205
10	50	182
20	50	175
30	50	162
40	50	165

[a] Number of times through 0.003-in. spinnerette.
[b] Measured at $C = 0.3\%$.

repeated passage of the paste through the spinnerette at a given pressure. This limiting value varied with the pressure or rate of shear, being lower at higher rates of shear. The number of passes of the solution through the spinnerette required to reach the constant sedimentation coefficient also varied with the pressure and ranged from about 10 to 30 passes.

Untreated corn starches were much more difficult to disperse than was ammonia-pregelatinized starch. Thus, untreated starch after pasting for 30 minutes at 98°C. was dispersed to an average of only 80% of theoretical after 60 passages through the spinnerette at 50 p.s.i., compared with essentially complete dispersion of ammonia-pregelatinized starch by 30 passages at only 10 p.s.i. The changes in the sedimentation pattern of the fast sedimenting component from untreated starch on repeated passage of the dispersion through the spinnerette are shown in Fig 2. A marked reduction in the breadth of the distribution of particle sizes occurs, as evidenced by the decreasing width
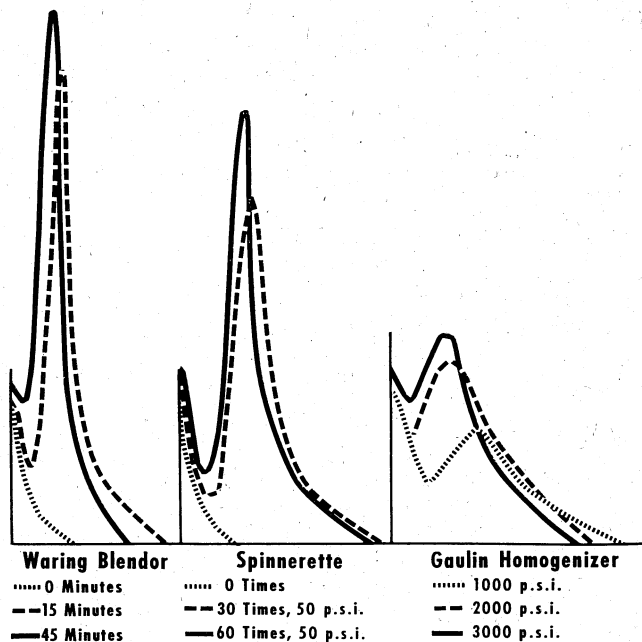
Fig. 2. Comparison of sedimentation patterns of starch pastes after shear treatment in a Waring Blendor, rayon-type spinnerette, and a Gaulin homogenizer. Patterns taken after 24 minutes at 12,950 r.p.m., 40° bar angle, C = 0.5%. Comparable to Fig. 1 but after a longer period of sedimentation and omitting the region at the bottom of the cell.

of the peak in the sedimentation patterns. A similar sharpening of the fast-sedimenting peak also occurred in starch dispersions subjected to the action of a Waring Blendor and, to a lesser degree, with a Gaulin homogenizer (Fig. 2).

High-shear gradients, developed in high-speed stirring or by passing starch pastes through narrow orifices, not only effectively reduce the particle size of the granule fragments but also reduce the breadth of the particle size distribution. Similar results have been reported for other high-polymeric substances when subjected to high-shear gradients (2). Particle size reduction was attributed to breakage of primary bonds and this probably occurs to some extent with the starch. Computation of the shearing stress in the spinnerette orifice gives a value comparable to one Bestul and Belcher (2) found sufficient to break primary bonds in high-molecular-weight polyisobutylenes. The results do not exclude, however, disaggregation through breakage of secondary bonds. In most of the cases listed in Tables III, IV, and V, the particle weight as given by the light-scattering

determination, or, as indicated by the sedimentation coefficients, was about equal to or greater than that determined for isolated amylopectin. Thus Stacy and Foster (10) report a molecular weight of 80 million for corn amylopectin in $1N$ potassium hydroxide solution, and Erlander and French (4) recently found a value of 168 million for immature dent and 45 million for mature dent amylopectin. Although the large particles in solution after shear treatment contained about 10% amylose, this factor would not contribute significantly to their molecular weight; it is apparent that most of the reduction in particle size could have come from secondary bond breakage.

Although the shear forces were sufficient to disaggregate or degrade the amylopectin appreciably, the amount of amylose in aqueous dispersion appearing in a sedimentation pattern never approached the approximate 28% in the original corn starch. Little if any increase of amylose occurred after shear treatment. The same incomplete liberation of amylose occurred with wheat starch preparations, despite their greater extent of dispersion after shear treatment (Table V).

TABLE V

EFFECT OF SPINNERETTE TREATMENT[a] ON PASTED STARCHES

| STARCH SAMPLE | DISPERSED STARCH | | | S_{20}[b] |
	Fast Component	Slow Component	Total	Fast Component
	%	%	%	
Commercial corn A	21	61	82	120
Commercial corn B	20	57	77	138
Commercial corn C	19	63	82	154
Laboratory-prepared corn	16	63	79	149
Laboratory-prepared wheat	19	77	96	156
Commercial wheat	19	71	90	139

[a] Pasted for 30 minutes at 98°C. with slow-mechanical stirring followed by 60 passes at 50 p. s. i. through a spinnerette having 0.003-in.-diameter holes.
[b] Corrected to $C = 0.3\%$.

About 25 to 40% of the amylose in both corn starch and wheat starch appears to remain attached to the amylopectin in aqueous dispersion after pasting and shear degradation by any of the three methods used—capillary spinnerette, high-speed propeller, or colloid mill. In contrast, aqueous alkali appears to liberate amylose completely from corn starch (Table I).

To confirm the identity of the slow-moving component as amylose and to check the analytical ultracentrifugal analysis of the concentration of free amylose, solutions were analyzed for amylose by the spectrophotometric iodine color method (6) after removal of the large par-

ticle components by centrifugation. After spinnerette treatment the dispersions were centrifuged at 105,000 × G for 15 minutes, a time calculated as sufficient to sediment most of the large particle components to the bottom of the tube and leave amylose in solution. Results show (Table VI) that the concentration of free amylose

TABLE VI

EFFECT OF SHEAR ON FREE AMYLOSE IN STARCH DISPERSIONS AS
DETERMINED BY IODINE COLOR OF CENTRIFUGAL SUPERNATANTS

	PASSES THROUGH SPINNERETTE [a]	FREE AMYLOSE, PERCENT	
		Iodine Color	Centrifuge Pattern
Granular Corn Starch	0	13.0	16.0
	5	16.4	15.8
	15	16.0	16.3
Ammonia-Pregelatinized Corn Starch	0	22.5	20.0
	1	23.5	20.9
	10	21.1	20.7

[a] Number of times starch paste was forced through 0.003-in. spinnerette at 25 p. s. i.

determined colorimetrically is in good agreement with that determined from the sedimentation patterns. Waxy maize starch dispersed at 97°C. failed to show a comparable slow-sedimenting component, thereby further substantiating identification of this component as amylose.

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