137. *Physicochemical Studies on Starches. Part XI.* The Granular Starch of Sweet Corn,* Zea mays.

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The starch present in sweet corn, *Z. mays*, has been isolated in granular form and obtained free from protein. It has been fractionated into its component amylose and amylopectin, and some physical properties of these fractions, including estimations of molecular weight, are described.

In view of recent interest [1] in the structure of the water-soluble glucosans of sweet corn (*Zea mays*), we have examined the *granular* starch which co-exists in the cereal grain. No detailed study of this starch appears to have been made previously.

Experimental

Isolation of Starch.—Mature *Zea mays* (var. Golden Bantam; 300 g.) was ground to a coarse powder (Found: H_2O, 12·3; ash, 1·81; N, 1·86%) and exhaustively extracted with boiling benzene–methanol (2 : 1 v/v; 2 l.) (Found: loss in wt., 12·3%). Defatted grain was then shaken vigorously with 0·1M-sodium chloride (1·5 l.; 5 × 6 hr.) under toluene, the aqueous suspensions filtered through muslin, and crude starch (A) allowed to settle (Found: N, 1·38%).

Removal of Protein from Starch A.—The crude starch was suspended in 1M-sodium chloride, then treated with toluene as previously described.[2] The process was repeated to yield starch B, which was stored in saline under toluene at 0° (yield, 5% of original dry wt. of grain) (Found: N, 0·13%).

Properties of Starch B.—On hydrolysis with 2% sulphuric acid, the starch yielded 96% of the theoretical amount of glucose (quantitative chromatography), and no other sugar was detected on the paper chromatogram. This material had $[\alpha]_D^{17}$ +152° (*c* 0·77% in N-NaOH).

* Part X, preceding paper.

[1] Peat, Whelan, and Turvey, *J.*, 1956, 2317, and references therein.
[2] Greenwood and Robertson, *J.*, 1954, 3769.

The optical density of the colour developed when starch (1 mg.) was stained with iodine (2 mg.) and potassium iodide (20 mg.) in distilled water (100 ml.) and measured at 6800 Å in cells of 2 cm. length (against the same iodine solution by means of a Unicam spectrophotometer) was 0·23. The iodine affinity of the starch (see below) was 5·5%, corresponding to 28% of amylose, whilst the average length of unit chain of the amylopectin was shown by calculation from the results of periodate oxidation [3] of the whole starch, and of the isolated amylopectin, to be 23 glucose residues.

Starch B was used in all further investigations.

Fractionation Methods.—Attempts were made to fractionate the *Z. mays* starch by the methods previously described in this Series: (i) dispersion in water at 98° followed by the addition of thymol as precipitant and butan-1-ol as the recrystallisation agent,[4] (ii) aqueous leaching at 70° followed by dispersion of the residue at 98° and addition of butan-1-ol,[4] and (iii) pretreatment of the granules with M-potassium hydroxide at 0°, before neutralization and dispersion and the addition of pentyl alcohol.[5, 6]

Characterisation of the Components.—These were characterized by (i) potentiometric titrations to determine iodine affinity,[7] (ii) measurements of limiting viscosity number [η] in M-potassium hydroxide,[8] and (iii) measurements of sedimentation velocity in 0·2M-potassium hydroxide.

Measurement of Sedimentation Velocity.—Rates of sedimentation of the two components in 0·2M-potassium hydroxide were determined by using a " Spinco " electrically driven ultracentrifuge (Spinco Division, Beckman Instruments Corporation, Belmont, California). Measurements were made in a 12 mm. cell incorporating a Kel-F centrepiece. The initial experiments with alkali as a solvent for the components [9] showed that the optimum speed for solutions of amylose of concentration greater than 0·1 g./100 ml. was 60,000 r.p.m., whilst for more dilute solutions, 30,000 r.p.m. was more suitable. Amylopectin solutions were spun at either 15,000 r.p.m. or 12,600 r.p.m. depending on the concentration. The pressure in the vacuum-chamber was less than 1 μ Hg, and the temperature rise in the rotor was about 0·6°/hr. at 60,000 r.p.m. and correspondingly less for lower speeds. (The refrigerating system was not utilized.) Runs were normally complete within 30 min. The temperature of the rotor was measured before and after the completion of each run, and the temperature at any time during the run obtained by linear interpolation. A correction was applied to allow for the adiabatic cooling of the rotor during acceleration.[10, 11] A modified Philpot–Svensson optical system enabled movement of the boundaries to be followed directly. The magnification of this lens system was shown to be constant over the whole field. The distance from the reference line of the optical system to the axis of rotation was 5·730 cm. Measurements of the rates of sedimentation were made by measuring the position of the boundary to 0·01 mm. directly from the photographic plates, a two-dimensional travelling microscope being used. Sedimentation constants (S) were evaluated from the equation: $\mathrm{d}\,(\log_{10} X/\omega^2)\mathrm{d}t = S/2{\cdot}303$, X being in cm. from the centre of rotation, t the time from the start of the acceleration in sec., and ω the angular velocity in radians. In this manner, linear graphs of $\mathrm{d}\log_{10} X$ against t were obtained: the point where the lines cut the time-axis at the value of $\mathrm{d}\log_{10} X$ for the meniscus, representing the time required before sedimentation started (*i.e.*, approx. two-thirds of the acceleration period). Results were corrected [12] to water at 20°.

Diffusion Measurements.—The method is outlined in Part X.[13] The solvent was 0·2M-potassium hydroxide, and values of the diffusion constant (D_m) were calculated by the moment method.

Results and Discussion

Zea mays starch has been isolated in granular form, reagents likely to cause degradation being avoided. Contamination with protein was reduced to 0·8% under the necessary

[3] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.
[4] Cowie and Greenwood, *J.*, 1957, 2862.
[5] Cowie and Greenwood, *J.*, 1957, 4640.
[6] Potter, Silveira, McCready, and Owens, *J. Amer. Chem. Soc.*, 1953, **75**, 1335.
[7] Anderson and Greenwood, *J.*, 1956, 3016.
[8] Cowie and Greenwood, *J.*, 1957, 2658.
[9] Brice, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.
[10] Waugh and Yphantis, *Rev. Sci. Instr.*, 1952, **23**, 609.
[11] Biancheria and Kegeles, *J. Amer. Chem. Soc.*, 1954, **76**, 3737.
[12] Svedberg and Pedersen, " The Ultracentrifuge," Oxford Univ. Press, 1940.
[13] Greenwood and Das Gupta, preceding paper.

mild conditions by the technique previously described.[2] However, purely physical methods did not further reduce the protein content. This phenomenon has been encountered [14] during the isolation of a large number of starches. The significance of the protein is doubtful, although it may well be incorporated into the granular structure.

The starch proved difficult to disperse in hot water. This is a feature which has been found in this laboratory to be common to a large number of cereal starches. Its significance with regard to granular structure is not yet known. The apparent resistance of the granule made its fractionation difficult. Conventional aqueous dispersive and leaching methods of fractionation were, in fact, unsuccessful, and the method involving preliminary swelling in M-potassium hydroxide [5,6] had to be used. The properties of the components from the most effective separation achieved by this method were as shown in the Table.

Properties of Z. mays *starch components.*

	Iodine affinity [a]	Purity (%)	$[\eta]$ in M-KOH	$10^{13}(S_{20})_0$ [b]	$\overline{M}_{S,D}$ [c]	$\overline{M}_n$
Amylose	18·8 *	100	150	5·2	317,000	180,000
Amylopectin	0·3	98	100	275	—	—

[a] See ref. 7. [b] Values obtained by graphical extrapolation, see text. [c] Calc. from $\overline{M}_{S,D} = RT(S_{20})_0/(1 - \overline{V}\rho)D_m$.
* Constant on further recrystallization.

The iodine affinity of the amylose is slightly lower than that for potato amylose, but similar to that found for other cereal starches in this laboratory. Barriers to β-amylolysis are present. Enzymic experiments carried out by Mr. A. W. Arbuckle showed that treatment with pure β-amylase resulted in only 78% conversion into maltose, a value very similar to the 77% limit reported for potato amylose.[15] The concurrent action of β-amylase and Z-enzyme resulted in 100% conversion, thus giving a further check that the amount of amylopectin impurity was negligible.

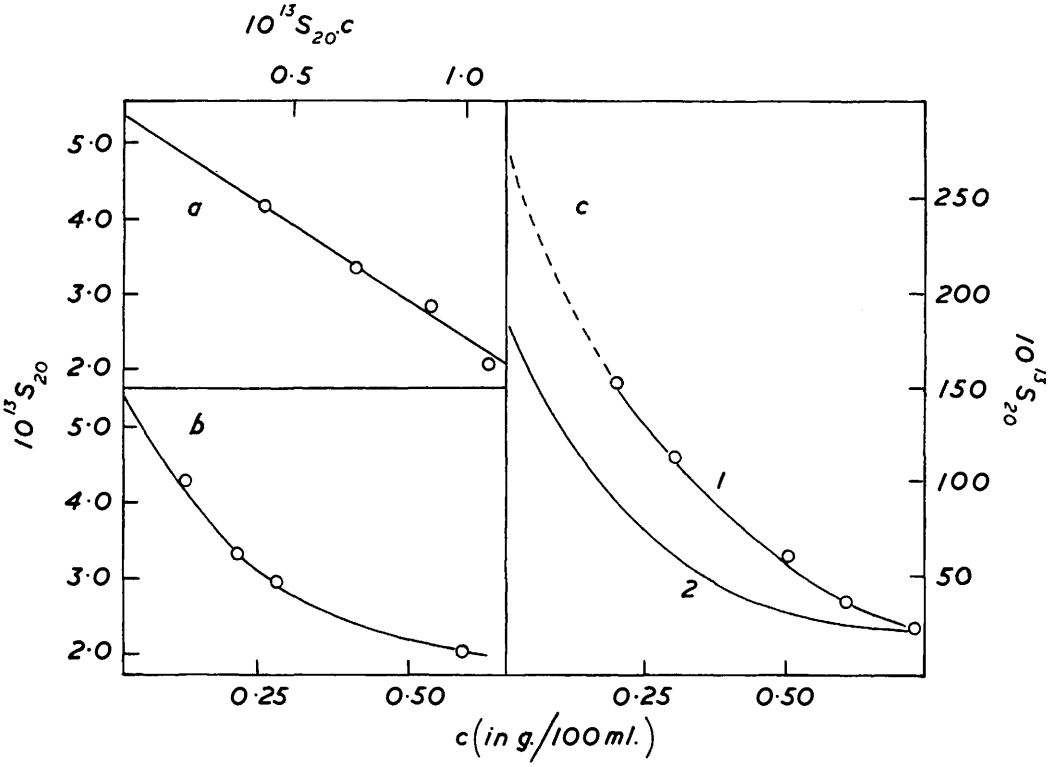
As reported previously,[9] the sedimentation constant (S_{20}) for both components is strongly dependent on the concentration c (see Figure). For amylose, the extrapolation to infinite dilution was simplified by plotting S_{20} against $S_{20}.c$ as well as against c. The resultant value $(S_{20})_0$ was $5{\cdot}2 \times 10^{-13}$ c.g.s. units. The diffusion coefficient (D_m) at $c = 0{\cdot}2$ g./100 ml. was measured by Mr. W. A. J. Bryce who found $D_m = 1{\cdot}0 \times 10^{-7}$, and hence, when the partial specific volume ($\overline{V}$) of amylose in this solvent [16] is taken to be 0·60, the molecular weight of the amylose ($\overline{M}_{S,D}$) is 317,000 and its D.P. = 1950. Calculation of the number-average molecular weight ($\overline{M}_n$) from the viscosity data by the relation previously obtained for potato amylose [4] gave $\overline{M}_n$ 180,000 and D.P. 1100. These values are lower than those for potato amylose.

Whilst the sedimentation diagrams for the amylose were well defined at low dilutions, those for the amylopectin showed a great tendency to spread and so make measurement of the mean position of the peak difficult. In view of the extreme concentration dependence, no attempt was made to extrapolate S_{20} accurately to infinite dilution, but for comparison our previous results [9] for potato amylopectin are also shown in the Figure. Although without knowledge of the diffusion coefficient the molecular weight cannot be estimated, this would appear to be greater than for potato amylopectin for which values of 36×10^6 have been found.[17] This extreme size might well cause the difficulties in dispersion and fractionation of the starch. Ultracentrifugal examination of some of the more impure amylopectins showed two peaks, in agreement with Stacy and Foster's recent results.[18] However, any appreciable amylose impurity (greater than about 5%) is

[14] Anderson and Greenwood, unpublished results.
[15] Cowie, Fleming, Greenwood, and Manners, *J.*, 1958, 697.
[16] Bryce and Greenwood, unpublished results.
[17] Witnauer, Senti, and Stein, *J. Polymer. Sci.*, 1955, **16**, 1.
[18] Stacy and Foster, *ibid.*, 1957, **25**, 39.

sufficient to distort completely the sedimentation diagram at low concentrations of amylopectin. This phenomenon, together with boundary anomaly effects,[19] makes reliable estimates of S_{20} and its consequent extrapolation to infinite dilution impossible. Experience in this laboratory has shown that ultracentrifugal analysis of impure amylopectins is most unsatisfactory (cf. ref. 18).

The variation of sedimentation constant (S_{20}) with concentration (c) for (a) amylose, S_{20} versus $S_{20}.c$, (b) amylose, S_{20} versus c, (c) amylopectin, S_{20} versus c for (1) Z. mays and (2) potato.



The granular starch in *Z. mays* is obviously a typical cereal starch, and its properties are completely unaltered by the co-existence in the cereal grain of the glycogen-like water-soluble glucosans.[1,13]

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Department of Chemistry,
The University, Edinburgh, 9. [*Received, August 12th*, 1957.]

[19] See, *e.g.*, Trautman, Schumaker, Harrington, and Schachman, *J. Chem. Phys.*, 1954, **22**, 555.