932. *Physicochemical Studies on Starches. Part VIII.* Further Observations on the Fractionation of Potato Starch.*

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The fractionation conditions necessary to achieve effective separation and minimise degradation of the components of potato starch have been critically examined. Laboratory-prepared and *commercial* starch samples have been compared. The amylose from commercial starch is much smaller ($\overline{D.P.}$ ~2500 glucose residues) than that from the laboratory-prepared sample ($\overline{D.P.}$ ~4000), and the effect of aqueous leaching at 70° and 98° also differs. The uniformity of structure of amylose in the granule is discussed. " Sub-fractions " obtained during the recrystallisation of the fractionation products of the laboratory-prepared starch have also been studied.

In Part VI of this Series,[1] we reported the results of aqueous leaching at various temperatures on laboratory-prepared potato-starch granules. The problem of fractionation and the fine structure of the starch granule are further considered here. It is essential to obtain the separated components in an unmodified form, and artefacts or degradation products must be avoided. Amylose is susceptible to oxidative degradation at elevated temperatures.[2] The extent to which this occurs during fractionation has now been examined in detail, as have methods suggested [3] for minimising this effect. Commercial potato starch has also been fractionated. In addition, the " sub-fractions " obtained during recrystallisation of the amylose component of laboratory-prepared starch have been studied in an attempt to establish the presence of material intermediate in structure between amylose and amylopectin.

Experimental

Preparation of Starch.—Starch was extracted from potatoes (var. Golden Wonder) by the method outlined previously.[4] A sample of commercial potato starch (" Superfine Farina ") was kindly supplied by Messrs. Brown & Polson, Ltd.

Fractionation Methods.—The methods of fractionation were as shown in Table 1.

Characterisation of Fractionation Products.—Measurements of (*a*) iodine affinity (I.A.),[5] (*b*) limiting viscosity number $[\eta]$ in M-potassium hydroxide,[4] and (*c*) sedimentation velocity in

* Part VII, *J. Polymer Sci.*, 1957, in the press.

[1] Cowie and Greenwood, *J.*, 1957, 2862.
[2] Bottle, Gilbert, Greenwood, and Saad, *Chem. and Ind.*, 1953, 541.
[3] Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.
[4] Cowie and Greenwood, *J.*, 1957, 2658.
[5] Anderson and Greenwood, *J.*, 1955, 3016.

0·2M-potassium hydroxide [6] were carried out on the separated components as detailed previously.

Results and Discussion

Our previous results [1] have shown that amylose isolated from laboratory-prepared potato starch possesses a number-average degree of polymerisation ($\overline{D.P.}$) of the order of 4000 glucose units. This value was therefore taken as a standard to judge the effect of modifications in fractionation techniques.

Oxidative Degradation.—Many investigators have suggested that starch, particularly the linear amylose component, may be degraded during fractionation procedures.[3] By measuring the molecular weight of the separated components, we have shown that the presence of oxygen during fractionation is, in fact, a serious source of degradation. The results in Table 1 (cf. F1 and F2) show that the $\overline{D.P.}$ of the amylose produced by passage

Table 1. *Fractionation methods and the properties of the separated components.*

Expt.	Fractionation method: Precipitant	Atm.	Ref.	Component	[η]	I.A. (%)	Purity [a] (%)	$\overline{D.P.}$ [b]
F 1	Thymol, then Bu^{n}OH	N_2	1	Initial-complex	—	14·2	74	—
				Amylose †	500	19·5	100	3700
				Amylose ‡	440	19·5	100	3200
				Amylopectin	180	0·16	99·2	—
F 2	Thymol, then Bu^{n}OH	O_2	1	Amylose §	272	—	—	2000
				Amylose ‖	220	—	—	1600
				Amylopectin	138	—	—	—
F 3	Thymol, then Bu^{n}OH	Air	1	Amylose	350	—	—	2600
				Amylopectin	176	—	—	—
F 4 *	Thymol, then Bu^{n}OH	N_2	1	Initial-complex	—	15·5	79·5	—
				Amylose	340	—	—	2500
				Amylopectin	170	0·3	98·5	
F 5 *	Aq. leaching at 70°	N_2	1	Amylose	330	—	—	2400
				Amylopectin	—	2·3	88·2	—
F 6 *	Fractionation after leaching at 70°	N_2	1	Amylose	330	—	—	2400
				Amylopectin	170	—	—	—
F 7	Aq. leaching at 70°	N_2	1	Amylose	240	19·5	100	1800
				Amylopectin	—	2·5	88	—
F 8	Fractionation after leaching at 70°	N_2	1	Amylose	560	19·5	100	5300
				Amylopectin	—	0·04	99·8	—
F 9	Pyridine	N_2	8	Initial complex	—	17·2	90·5	—
				Amylose	360	—	—	2700
				Amylopectin	165	0·7	96·4	—
F 10	Pentanol	N_2	9	Initial complex	—	14·0	72·0	—
				Amylose	405	—	—	3000
				Amylopectin	180	0·1	99·5	—
F 11	KOH-dispersion, then pentanol	N_2	10	Amylose	395	—	—	2900
				Amylopectin	180	—	—	—
F 12	Thymol and BuOH + *N*-phenyl-2-naphthylamine	Air	3	Amylose	330	—	—	2400
F 13	Thymol and BuOH + quinol	Air	3	Amylose	100	—	—	740

[a] Purity calc. as in Table of ref. 1. [b] D.P. calc.[1] from $\overline{D.P.} = 7{\cdot}4[\eta]$ for amylose.
* Fractionation using commercial starch. † Small-scale (500 ml.) fractionation. ‡ Large-scale (3 l.) fractionation. § Recrystalln. under N_2. ‖ Recrystalln. under O_2.

of oxygen through the fractionation and recrystallisation media is reduced by one half (equivalent to *ca.* 1·2 bonds broken/initial amylose molecule). Even when the recrystallisations are carried out under nitrogen, degradation is still appreciable. Amylopectin is also depolymerised under these exaggerated conditions.

Fractionation in the presence of air (F3) results in a less drastic but still appreciable decrease in $\overline{D.P.}$; the amylose undergoes the scission of 0·2—0·5 bond/molecule, whilst viscometrically the amylopectin is virtually unchanged.

Oxidative degradation can also occur during isolation of the amylose complex. A

[6] Bryce, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.

Sharples supercentrifuge was used originally.[7] It has since been shown that oxygen-free conditions cannot be maintained during centrifugation, and excessive use of this instrument causes some degradation. (For example, after ½, 1, and 1½ hours' centrifugation, the limiting viscosity number of a given amylose sample was 400, 365, and 375.) The Sharples centrifuge is now only used to remove the initial complex, and thereafter the preparative ultracentrifuge or M.S.E. centrifuge is utilised. The resultant products, although less densely packed, are cleaner and more easily re-dispersed.

Oxygen-free conditions are therefore essential to avoid degradation of amylose during fractionation. This can be achieved by passing nitrogen (or hydrogen [7a]) through the

TABLE 2. *β-Amylolysis limits of amylose samples.*

	Laboratory-prepared starch				Commercial starch			
Prep. of sample in N_2	Sample †	% of total amylose	D.P.	β-Amylolysis limit *	Sample †	% of total amylose	D.P.	β-Amylolysis limit *
Aq. leaching at 70°	F 7 ‡	40	1800	100	F 5	40	2400	100
Aq. leaching at 98°	—	80	2700	86	—	—	—	—
Dispersion of granule	F 1 ‡	100	3200	77	F 4	100	2500	95

* Expressed as % conversion into maltose. † Sample number in Table 1 where appropriate. ‡ Further enzymic experiments on these fractions have been described by Cowie, Fleming, Greenwood, and Manners (*J.*, in the press).

TABLE 3. *Analysis of fractionation products.*

Product	% of total weight [a]	Iodine affinity	% of amylose	[η] in M-KOH	$10^{13}S_0$ [b]
Whole starch	100	4·03	20·7	—	—
(a) *Precipitates*					
Thymol complex	41	14·2	74	—	—
Butanol complex 1	—	17·5	90·3	—	—
,, 2	—	19·0	97·5	—	—
,, 3	15 *	19·5 †	100	440	12·5
(b) *Materials in supernatant liquors from*:					
Thymol complex	50	0·16	0·82	180	200
Butanol complex 1	10	0·4	2·05	76	11·3
,, 2	5	7·8	40	—	—
,, 3	2	2·0	10·25	—	—

[a] Estimated for (a) by hydrolysis of aliquot parts and estimation of liberated glucose and (b) by direct weighing of freeze-dried product. Losses are mechanical. [b] Sedimentation constant in c.g.s. units at infinite dilution obtained by graphical extrapolation from $S = f(c)$.
* A further 5% was used in analysis of butanol complexes 1 and 2.
† Value constant on further recrystallisation.

fractionation medium, which should preferably be on a small scale (*i.e.*, <500 ml.). (The results for small- and large-scale preparations in Table 1 indicate that oxygen-free conditions are more easily maintained in small-scale preparations. Also the length of time needed to centrifuge the thymol-complex is short.)

Anti-oxidants (*e.g.*, *N*-phenyl-2-naphthylamine and quinol), added to the fractionation medium in the presence of air, did not minimise oxidative degradation (cf. F2, F12, and F13; Table 1).

Other Precipitants and Fractionation Methods.—As already reported,[1] the most effective method of fractionating potato starch involved complete disruption of the granular structure, followed by precipitation of the amylose component by a polar organic molecule. Many complex-forming agents have been used, but thymol followed by butan-1-ol is a very satisfactory combination.

Higginbotham and Morrison [8] studied the use of butan-1-ol, pyridine, and *iso*pentyl

[7] Greenwood and Robertson, *J.*, 1954, 3769.
[7a] Baum and Gilbert, *Chem. and Ind.*, 1954, 489.
[8] Higginbotham and Morrison, *Shirley Inst. Mem.*, 1948, **22**, 141.

alcohol, and concluded that pyridine and butanol were comparable but *iso*pentyl alcohol was not so efficient. Results showing the effect of pyridine [8] and pentan-1-ol [9] as initial precipitants (F9 and F10; Table 1) suggest that, for potato starch, pyridine is an inefficient precipitant, yielding an amylopectin only 96·4% pure. Pentan-1-ol, however, is comparable to thymol, and a good initial separation of the components is obtained.

Starch subjected to pre-treatment with potassium hydroxide (a method suggested [10] for fractionating starches which are difficult to disperse) yielded only slightly degraded amylose. The fact that potato starch is relatively easy to swell and disperse may account for this. However, unpublished results [11] indicate that the method is also satisfactory for cereal starches.

Fractionation of Commercial Potato Starch.—Values reported for the $\overline{\text{D.P.}}$ of potato amylose have varied considerably.[3] With the exception of Husemann and Bartle's results,[12] none has been as large as our own values.[4] This could be attributed to degradation during isolation, or fractionation, or during the formation of derivatives. In our work, the latter possibility has been eliminated by studying the free component. However, previous investigators have often used commercial starch samples, so we examined such a starch.

When best-quality commercial potato starch was fractionated under *oxygen-free* conditions, the resultant amylose had a $\overline{\text{D.P.}}$ of only 2500 (F4 in Table 1). Thus commercial extraction of starch appears to degrade the amylose. This fact, together with oxidative degradation, probably accounts for the lower reported values of $\overline{\text{D.P.}}$ (A fractionation in the presence of air might be expected to result in an amylose of $\overline{\text{D.P.}}$ ~1700 before preparation of derivatives.)

Aqueous leaching of the commercial starch at 70° also gave amylose of $\overline{\text{D.P.}}$ 2400, and this value was unchanged when the granule was subsequently dispersed at 98° (see Table 1). This behaviour is completely different from that of laboratory-prepared samples,[1] and suggests that in the commercial samples all the amylose is equally accessible.

Uniformity of Structure of Amylose.—Our aqueous-leaching experiments [1] suggest that in potato starch there may be two amylose fractions, (1) easily accessible material of relatively low $\overline{\text{D.P.}}$ and (2) a fraction of higher $\overline{\text{D.P.}}$ requiring disruption of the granule before isolation. There is the possibility that these fractions may also differ in *structure*.[3] Although physical evidence shows that their iodine affinities are identical, and the same ln M *versus* ln $[\eta]$ relationship holds within experimental error, the $\overline{\text{D.P.}}$'s are so large that chemical methods are not satisfactory. However, β-amylase will degrade a linear amylose molecule completely to maltose, whilst its hydrolytic action stops at any branch-points or other anomaly.[3] Experiments carried out in collaboration with Mr. I. D. Fleming showed that the β-amylolysis limits of the different amylose fractions varied considerably (Table 2).

For laboratory-prepared starches, the complete degradation of the amylose leached at 70° suggests that this short-chain material is completely linear. Extraction at higher temperatures gives amylose which is incompletely hydrolysed, the amount of resistant material increasing with increase in temperature and consequent disruption of the granule. The probable nature of this barrier to β-amylolysis will be discussed elsewhere, but it appears to be associated with disruption of the granule and hence some branching is not improbable.[3]

The leaching and β-amylolysis results for the commercial starch differ greatly. All the amylose products were of virtually the same size, and, whilst that leached at 70° was again linear, the product of a conventional dispersion was also nearly completely hydrolysed.

[9] Schoch in Radley, " Starch and its Derivatives," Chapman and Hall, London, 1953, Vol. I, p. 123.
[10] Potter, Silveira, McCready, and Owens, *J. Amer. Chem. Soc.*, 1953, **75**, 1335.
[11] Arbuckle and Greenwood, unpublished experiments.
[12] Husemann and Bartle, *Makromol. Chem.*, 1956, **18—19**, 342.

Analysis of the "Sub-fractions" obtained during Fractionation.—The granule may contain fractions with properties intermediate between those of amylose and amylopectin.[3] In an attempt to identify such material, a careful analysis was made of all the products from a fractionation of a laboratory-prepared starch, especially the solids from the mother-liquors obtained from recrystallisation of amylose. The results are shown in Table 3. The weight of material in the supernatant liquors decreased regularly and the iodine affinity increased generally. With the exception of the solid from the supernatant liquor from butanol complex 2, the iodine-binding curves were identical in character with those previously reported for a similar analysis of *Hevea brasiliensis* starch.[7] Examination of the thymol complex in the ultracentrifuge revealed an apparently *homogeneous* fraction, although it contained about one-third of amylopectin. Amylopectin normally has a sedimentation constant about twenty times larger than amylose, but the amylopectin product isolated on reprecipitation of the thymol complex had a value equivalent to that for amylose itself. Without further evidence, it is impossible to decide whether there are also two amylopectin fractions of widely differing $\overline{D.P.}$, or whether this "thymol-amylopectin" has a different structure, although it has similar iodine-binding properties to amylopectin. Experiments are in progress to investigate this. Recrystallisation of the amylose apparently results in the elimination of branched material and presumably also short-chain amyloses, although the latter are difficult to detect by potentiometric iodine titration. With the possible exception of the "thymol-amylopectin," no major component has been detected which might be an intermediate between amylose and amylopectin.

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