559. *Physicochemical Studies on Starches. Part VI.* Aqueous Leaching and the Fractionation of Potato Starch.*

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A critical study has been made of the effect of aqueous leaching at various temperatures on potato starch granules. The efficiency of this procedure for fractionating starch has been followed by measuring the purity and molecular weight of the resultant components. Water leaches preferentially short-chain linear material, and its action is both inefficient and incomplete compared with the conventional methods involving complete disruption of the granular structure and formation of the amylose–thymol complex. The effect of aqueous leaching on granular structure is discussed. Potato amylose has a number-average degree of polymerization ($\overline{D.P.}$) of about 4000 glucose units. The relations, $\overline{D.P.} = 7{\cdot}4[\eta]$ for potato amylose fractions in M-potassium hydroxide, and $[\eta] = 4{\cdot}30(\overline{D.P.})^{0{\cdot}61}$ for the acetates in chloroform, are suggested.

Although the two components of starch can be relatively easily separated, little is known of how they are incorporated into the granule and how the physical nature of the granule governs the efficiency and ease of fractionation. Methods of fractionation have been recently reviewed;[1] the most satisfactory ones so far involve complete dissolution of the granule followed by the addition of a polar organic molecule to form an insoluble amylose complex. These procedures cannot give much information regarding granular structure, but some can be obtained by aqueous leaching. This method has often been used[2-4] to fractionate starch, although reports of its efficiency are at variance and there are insufficient accurate data regarding the purity of the fractionation products and their molecular weights. In continuance of studies of potato starch—its structure, methods of fractionation, and measurements of the size and shape of its components—we now report the results of a series of aqueous leaching experiments and estimations of the purity and molecular weight of the resultant fractions. Various features regarding the effect of leaching on granular structure are discussed, and its efficiency for fractionation is compared with other methods.

Experimental Methods

Preparation of Starches.—Starches were prepared from several varieties of potato by the method previously described.[5]

Fractionation Procedures.—(a) *Leaching at* 98°. Starch suspension (0·5% in 0·1% sodium chloride) was deaerated at room temperature (stream of oxygen-free nitrogen for 15 min.). The suspension was then placed on a vigorously boiling water-bath and stirred for 5—7 min. under nitrogen. The resultant gelatinized mixture was then cooled and centrifuged at 20,000 g. (preparative rotor of Spinco ultracentrifuge) for 10 min. Supernatant liquors were then removed, saturated with butan-1-ol, and kept at room temperature overnight. The sedimented granules were washed with distilled water (6 times), redispersed in saline, and heated for a further 5—7 min. The solid obtained after centrifugation was washed free from salt and freeze-dried directly, to give the " amylopectin " fraction. (Further experiments indicated

* Part V, *J.*, 1957, 2658.

[1] Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.
[2] Tranet, *Bull. Soc. chim. France*, 1915, **17**, 83; Sherman and Baker, *J. Amer. Chem. Soc.*, 1916, **38**, 1885; Karrer and Krauss, *Helv. Chim. Acta*, 1929, **12**, 1144; Baldwin, *J. Amer. Chem. Soc.*, 1930, **52**, 2907.
[3] Kerr and Severson, *J. Amer. Chem. Soc.*, 1943, **65**, 193; Meyer and Rathgeb, *Helv. Chim. Acta*, 1948, **31**, 1533.
[4] Baum and Gilbert, *Chem. and Ind.*, 1954, 489, 490; Baum, Gilbert, and Wood, *J.*, 1955, 4047.
[5] Cowie and Greenwood, preceding paper.

that the gelatinized granules would not readily sediment in the absence of salt in the above force-field.)

(b) *Complete dispersion of granular structure.* Starch granules (0·5% in water) were stirred vigorously at 100° for ½—2 hr. under nitrogen. After cooling to 60°, thymol (1 g./l.) was added, and the mixture set aside for 3 days at room temperature. The amylose–thymol complex was then removed on the Sharples supercentrifuge and recrystallized three times from hot saturated butan-1-ol solution. The amylopectin-containing supernatant liquor (from the thymol complex) was freeze-dried, refluxed with methanol (3 times), dissolved in water, and freeze-dried again. (Later experiments showed that a more soluble product was obtained if treatment with methanol was avoided.[5]).

(c) *Combination of aqueous leaching at* 70° *and dispersion at* 100°. Starch suspension (0·5% in water) was stirred at 70° for 1 hr. under nitrogen. (Gelatinization occurred within 10 min.) The mixture was allowed to cool and centrifuged. After decantation of the supernatant liquor and filtration through a sintered-glass filter (G3), excess of butan-1-ol was added. Immediate amylose-complex formation was observed, but the mixture was kept for 24 hr. at room temperature for complete precipitation. The sediment of gelatinized granule residues was redispersed in water and boiled for 1½ hr. under nitrogen before fractionation as above.

Characterization of Fractionation Products.—(a) *Amylopectin.* The amount of amylose contaminating the branched component was estimated by potentiometric determinations of the amount of iodine bound.[6] The linear portion of the iodine sorption curve was extrapolated to zero free-iodine concentration; the ratio of the value of the intercept to that for pure potato amylose (19·5%) gave the amount of linear material present.

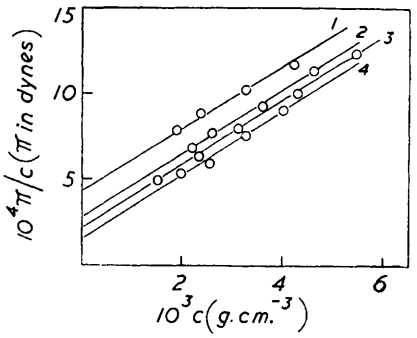


FIG. 1. *Graph of* π/c versus c *for the acetylated amyloses in chloroform solution.*

(1) *Amylose leached at* 70°. (2) *Amylose dispersed for* 1 *hr. at* 98°. (3) *Amylose dispersed for* 2 *hr. at* 98°. (4) *Amylose dispersed at* 98° *after leaching at* 70°.

(b) *Amylose.* Purity was determined by titration as above. Molecular-weight determinations were made by measurements of (i) limiting viscosity number of the free component in M-potassium hydroxide at 22·5°, (ii) rates of sedimentation in 0·2M-potassium hydroxide, and (iii) osmotic pressure of the acetate in chloroform solution (at 22·5°). (For details of methods and procedures see ref. 5. The osmotic-pressure measurements were carried out in collaboration with Dr. W. N. Broatch.) Fig. 1 shows the experimental osmotic-pressure results.

RESULTS

The effect of aqueous leaching and fractionation was found to be identical for the two varieties of potato starch investigated, Redskin and Arran Banner.

Aqueous Leaching at 98°.—The results of typical experiments are shown in Table 1. Small-scale experiments indicated that successive leaching for two periods of about 7 min. yielded the purest amylopectin. The amyloses produced vary from 80% to 90% purity from the first leaching, but, on re-extraction, their purity drops to 60%.

Microscopic observation showed that at the end of 5 min. the granules were all swollen but mainly intact. The residual " sacs " stained mauve with iodine and blue-staining amylose had diffused into the supernatant liquors. Some granules still had accumulations of amylose towards one end, whilst others appeared to have been completely ruptured and to be devoid of any blue-staining material.

[6] Anderson and Greenwood, *J.*, 1955, 3016.

Whilst small-scale experiments proved relatively easy, leaching on a large scale (3 l.) was not successful. The fluid-like consistency of the gelatinized granules made their removal on the Sharples supercentrifuge extremely difficult and, further, on re-extraction, a higher proportion of amylopectin went into solution to yield a cruder amylose.

TABLE 1. *Properties of the products obtained by leaching at* 98°.

		Amylopectin		Amylose		
Expt.[a]	Leaching time (min.)	Purity (%) †	Amylose impurity (%) ‡	Initial purity (%)	$[\eta]$ in M-KOH [b]	$\overline{D.P.}$ calc.[c]
1—5 S	2 × 5—2 × 10	96—98	10—20	80—90	—	—
6 S	7	95	25	90	—	—
7 S	5	93	35	90	340 *	2520
8 S	2 × 5	96	20	90	365 **	2700
1 L	2 × 7·5	97	15	62	340 ***	2500 [d]

† Calc. from iodine uptake. ‡ Calc. from (iodine uptake ÷ 20) (see ref. 6.)
[a] S = small scale (50—100 ml.); L = large scale (3 l.). [b] Limiting viscosity number $[\eta]$ of recrystallized amylose in M-KOH at 22·5°. [c] Calc. from $\overline{D.P.} = 7{\cdot}4[\eta]$. [d] A value of 2500 was obtained from osmotic-pressure measurements on the acetate in chloroform solution; $[\eta]$ of acetate = 520.
* ** *** 1, 2, and 3 recrystallizations, respectively.

TABLE 2. *Properties of amylopectins and recrystallized amylose obtained from dispersions.*

	Amylopectin		Amylose			
Dispersion time (hr.)	Purity (%) [a]	Amylose impurity (%) [a]	$[\eta]$ in M-KOH [a]	$[\eta]$ of acetate [b]	$\overline{M}_n$ [c]	$\overline{D.P.}$ [d]
½	98·8	6	490	—	—	3600
1	99·2	4	520	680	1,125,000	3900
1½	—	—	470	—	—	3500
2	99·5	2·5	450	640	940,000	3260

[a] As for Table 1. [b] Measured in $CHCl_3$. [c] Determined from osmotic-pressure measurements on the acetate in $CHCl_3$ solution. [d] Calc. from previous column, or from $\overline{D.P.} = 7{\cdot}4\,[\eta]$.

TABLE 3. *Properties of components from combined leaching and dispersion.*

Expt.	Fraction	Purity [a]	Amylose impurity (%) [b]	$[\eta]$ in M-KOH	$[\eta]$ of acetate [b]	$\overline{M}_n$ [b]	$\overline{D.P.}$ [b]
70° leach	Amylose	94—98	—	240	480	527,000	1830
	Amylopectin	88	60	—	—	—	—
98° dispersion	Amylose *	100	—	560	770	1,534,000	5300
	Amylopectin	99·8	1	178	—	—	—

[a] Before recrystallization in case of the amylose. [b] As in footnotes to Table 2.
* Recrystallized twice.

Complete Dispersion of Granular Structure.—The results of these experiments are shown in Table 2. Thymol–amyloses were found to be about 75% pure, but recrystallization with butan-1-ol gave products binding 19·5% of iodine. Microscopic observation showed that after 30 minutes' dispersion, a few granules were still gelatinized, but not completely disrupted: after 1 hr. only occasional fragments of disrupted granules were apparent.

Leaching at 70° *followed by Dispersion at* 98°.—The results are shown in Table 3.

DISCUSSION

Aqueous Leaching at 98°.—The qualitative results of previous workers [2,4] were confirmed. Aqueous leaching of potato starch granules at 98° for a short time was shown to result in the extraction from the granule of material which is predominantly amylose, with the consequent formation of a residual granular network which is predominantly amylopectin. However, the extracted amylose (1) is contaminated with amylopectin (the latter being presumably of low molecular weight), and (2) possesses a much lower D.P.

(2500) than that obtained from a conventional dispersive fractionation (cf. Tables 1 and 2). At the same time, 10—20% of the amylose is retained in the swollen granules.

These results suggest that preferential extraction of shorter amylose chains is occurring, owing to incomplete disruption of the granular structure and consequent inability of the larger amylose chains to diffuse out.

Aqueous leaching at 98° is therefore not suitable for the preparation from potato starch of either amylose of a high $\overline{D.P.}$, or amylopectin of high purity.

Dispersion Experiments.—Dispersion for 1 hr. resulted in amylose having a $\overline{D.P.}$ of 3900 glucose units, a value much larger than that from the above leaching experiments. After ½ hour's boiling, disintegration of the granule is incomplete with consequent retention of some material of high molecular weight (see below). Prolonged boiling results in hydrolysis, even under the oxygen-free conditions of the experiment. The amylopectins were considerably purer than those obtained by leaching.

Fractionation involving complete disruption of granular structure results therefore in the simultaneous production of amylose of high $\overline{D.P.}$ and pure amylopectin.

Leaching at 70° followed by Dispersion.—Leaching at 70° removed about 40% of the total amylose from the granule. The leached amylose-product was 97—98% pure (compare leaching at 98°), but was smaller ($\overline{D.P.}$ 1830). By comparison, the amylose subsequently isolated after dispersion of the residual granular structure had a very high $\overline{D.P.}$ of 5300 glucose units. The amylopectin obtained after this dispersion was the purest obtained in this work (see Table 3).

These results suggest, in agreement with Meyer and his co-workers [7] that subfractionation of the amylose has again occurred, short chains being preferentially leached at 70° whilst the larger ones are not able to diffuse out until the granular structure is further disrupted by dispersion. Similar results have been obtained by Schoch,[8] who suggested that leaching methods at 70° were inefficient for fractionation, since 50% of the amylose " retrograded " *in situ* in the granule. This appears unlikely, as *insolubilized* retrograded amylose cannot be redispersed at 98° under the conditions used here, and it is more probable that simple subfractionation occurs.

Aqueous Leaching and Granular Structure.—General comments on granular structure have been made previously.[1,5] Only the question of the nature of the granular " sacs " will be dealt with here. In agreement with Frey-Wyssling [9] and Meyer and Menzi,[10] we have found that potato starch readily forms granular " sacs " on treatment with hot water. Depending on the extraction temperature, these sacs contain from 88 to 98% of amylopectin, and retain (microscopically) the characteristics of swollen, disrupted granules. The persistent, highly swollen remains of granules, after relatively prolonged heating in water, suggest that strong secondary valency forces of the hydrogen-bond type must be present, *i.e.*, close and compact packing of the amylopectin must occur. Dissolution of amylopectin must simply entail disruption of these bonds. This problem is complex, and is being further examined.

Molecular Size of Potato Amylose.—The observed molecular size of any amylose depends on the methods of isolating and fractionating the starch.[1] In this work, where methods were chosen to avoid as far as practicable any hydrolytic degradation, the number-average $\overline{D.P.}$ of potato amylose has been found to be of the order of 4000 glucose residues. This is to be compared with previous values in the literature of 240—1130,[1] and emphasises the importance of oxygen-free conditions for the dispersion. (Preliminary unpublished experiments have shown that the presence of oxygen causes degradation.) In addition, this value for the molecular size was independent of the variety of potato.

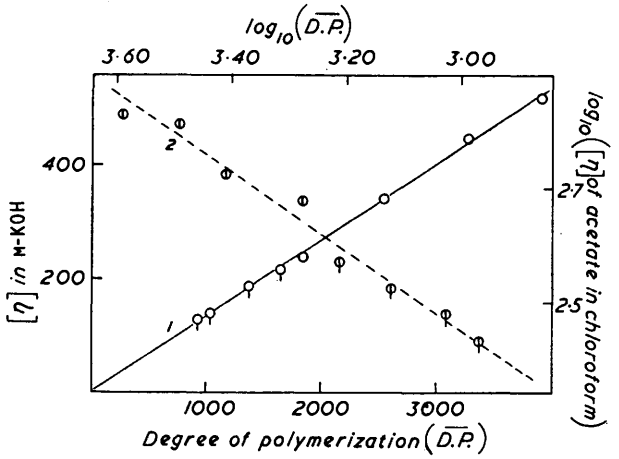
[7] Meyer, Bernfeld, Boissonnas, Gürtler, and Noelting, *J. Phys. Colloid Chem.*, 1949, **53**, 319.
[8] Schoch in Radley's " Starch and its Derivatives," Chapman and Hall, London, 1953, Vol. I, p. 123.
[9] Frey-Wyssling, *Experientia*, 1952, **8**, 101.
[10] Meyer and Menzi, *Helv. Chim. Acta*, 1953, **36**, 702.

A value of about 4000 was found for both the Redskin and Arran Banner potato starches studied here, whilst a similar result has been obtained by Mr. W. A. J. Bryce for Majestic and Golden Wonder potato starches.

This value for the $\overline{D.P.}$ must be a *minimum*, since the possibility of inadvertent degradation in this work still cannot be eliminated.

Relation between $\overline{D.P.}$ and $[\eta]$ for Potato Amylose.—The results in this and previous work [5] enabled values to be calculated for K and α in the relation, $[\eta] = K(\overline{D.P.})^{\alpha}$. Although the distribution of molecular weight in some of the samples may well have been altered, it was found that, within experimental error, there was a linear relation between $[\eta]$ in M-potassium hydroxide and the $\overline{D.P.}$ derived from osmotic-pressure measurements on the corresponding acetate. This relationship is illustrated in Fig. 2 (curve 1) which

FIG. 2. *Graph of $[\eta]$ as a function of $\overline{D.P.}$*



(1) ($[\eta]$ *in* M-KOH) versus ($\overline{D.P.}$ *of acetate from osmotic-pressure measurements*).

○ *this work*; ⚲ *previous results* (*see ref.* 5).

(2) $\log_{10}$ ($[\eta]$ *of acetate in* $CHCl_3$) versus $\log_{10}$ (*D.P. of acetate from osmotic-pressure measurements*).

① *this work*; ⓵ *previous results* (*see ref.* 5).

also includes the previously reported values for acid-degraded samples of amylose.[5] The equation, $\overline{D.P.} = 7{\cdot}4[\eta]$, holds for $\overline{D.P.}$ values up to 4000.

For values of $[\eta]$ for the acetates in chloroform solution, calculation by the method of least squares showed that the results were best represented by the equation, $[\eta] = 4{\cdot}30(\overline{D.P.})^{0{\cdot}61}$ (see Fig. 2, curve 2).

The significance of the above values of α in the modified Staudinger law, together with calculations of molecular dimensions, will be discussed elsewhere.

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