JOURNAL
OF
THE CHEMICAL SOCIETY

1. *The Fractionation of Potato Starch by Means of Aluminium Hydroxide.*

By E. J. Bourne, G. H. Donnison, S. Peat, and W. J. Whelan.

Aluminium hydroxide, precipitated *in situ*, will almost completely adsorb potato starch from aqueous solution. When the precipitate is boiled with water the amylose fraction passes into solution but little or none of the amylopectin dissolves. If less aluminium hydroxide is used the amylopectin is preferentially adsorbed, and an amylose fraction of high blue-value remains in the supernatant liquid. Factors influencing the isolation of this amylose fraction have been examined, and a new method based upon these observations is suggested for the fractionation of starch.

The chief methods at present in use for the separation of the branched (amylopectin) and largely unbranched (amylose) components of starch depend on the precipitation of a water-insoluble complex which the amylose component forms with a variety of polar reagents such as butanol (Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2957), pentasol (Schoch, " Advances in Carbohydrate Chemistry," I, 259), *cyclo*hexanol, thymol (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19; Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687), nitroparaffins (Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161), and fatty acids (Schoch and Williams, *J. Amer. Chem. Soc.*, 1944, **66**, 1232). Either the amylopectin component does not form complexes with these reagents or the complexes formed are soluble in water saturated with excess of the reagent.

It is convenient in certain circumstances to be able to reverse the order of fractionation and to precipitate the amylopectin component, leaving the amylose in solution. This we have been able to do by co-precipitation of aluminium hydroxide and the amylopectin component of starch. By adhering to the conditions prescribed below it is possible in this way to remove the branched-chain component while leaving in solution an amylose which, although it is obtained in lower yield, has a higher blue-value than that of the amylose prepared by earlier procedures.

In the course of a study of the adsorption of starch by insoluble metal hydroxides it was observed that in a number of cases the amylopectin was preferentially adsorbed. In particular, the hydroxides of iron, aluminium, and chromium appeared to offer the possibility of a new method for the fractionation of starch. Samec (*Biochem. Z.*, 1929, **205**, 104), following up a suggestion by Stern (*Z. angew. Chem.*, 1928, **41**, 88), showed that a partial separation of amylose and amylopectin was achieved by the treatment of a starch dispersion with barium hydroxide. The method yielded, however, only a part of the amylopectin present, and was unsuitable for the preparation of an amylose free from amylopectin. Accordingly, our investigations were confined to the hydroxides of iron, chromium, and aluminium. Preliminary experiments established that the best separation was afforded by the *in situ* formation of aluminium hydroxide. Iron was discarded because the soluble amylose fraction was contaminated with colloidal ferric hydroxide, and chromium because the amylopectin–chromium hydroxide precipitate was extremely voluminous and difficult to separate.

To aqueous dispersions (3%) of potato starch were added varying amounts of hydrated aluminium sulphate (ranging from 0·3 g./g. of starch to 4·0 g./g. of starch) followed by a slight excess of ammonia. In each case a portion of the starch was not adsorbed and could be recovered from the supernatant liquid by precipitation with alcohol (see Table I*A*). This fraction (*A*), representing 1·2—10·3% of the starch, invariably had a higher blue-value (varying from 1·11 to 1·36 in six experiments, Table I*B*) than that of thymol–amylose (given as 1·10 by Bourne, Donnison, Haworth, and Peat, *loc. cit.*). Exhaustive extraction of the aluminium hydroxide with boiling water gave fractions (*B* to *D*) some of which (*e.g.*, in separation IV.

TABLE I.

Fractionation of potato starch with aluminium hydroxide (precipitated from the sulphate).

A. Percentage yields of fractions.

No.	Starch (g.) (dry basis).	$Al_2(SO_4)_3,18H_2O$ (g./g. of dry starch).	Yields of fractions (%). A.	B.	C.	D.	E.
I	36	0·3	10·2	2·3	1·2	—	—
II	30	0·3	9·8	4·1	7·2	1·6	67·4
III	30	0·3	10·3	4·5	7·0	0·9	—
IV	20	1·5	1·2	11·0	8·1	2·6	30·5
V	30	4·0	1·7	11·3	11·3	—	—
VI	30	4·0	8·4	4·1	5·8	2·2	57·8

B. Blue-values of fractions.

No.	Fraction. A.	B.	C.	D.	E.
I	1·29	1·26	1·24	—	—
II	1·36	0·75	0·39	0·39	0·22
III	1·29	0·65	0·41	0·33	—
IV	1·11	1·17	1·15	1·13	0·18
V	1·34	0·95	0·53	—	—
VI	1·33	1·34	0·75	0·50	0·21

Table I*B*) consisted mainly of amylose, while others (*e.g.*, in separation II) contained a larger proportion of amylopectin. After the precipitate remaining after exhaustive aqueous extraction had been redissolved in dilute alkali, neutralised, and dialysed to remove inorganic material, an amylopectin fraction was recovered by precipitation with alcohol. This fraction stained red-purple with iodine, and had B.V. 0·18—0·22, which was comparable with that of an average amylopectin fraction separated from potato starch by the thymol method (Bourne, Donnison, Haworth, and Peat, *loc. cit.*).

It was clear that although the method of fractionation described yielded amylose and amylopectin it was not so convenient as the thymol method for the routine isolation of the amylopectin of starch. Consequently, attention was directed to its use for the separation of amylose fractions of high blue-value.

In these experiments aluminium nitrate was employed instead of the sulphate, because both aluminium and ammonium nitrates are more soluble in alcohol than are the corresponding sulphates, and any salt remaining after dialysis is therefore less likely to be precipitated with the amylose fraction. The factors examined with regard to their influence on the purity of the amylose were the temperature of precipitation and of " ageing " of the hydroxide, the time of " ageing " of the hydroxide, and the mode of preparation of the starch paste.

TABLE II.

Factors influencing the purity of the amylose fraction.

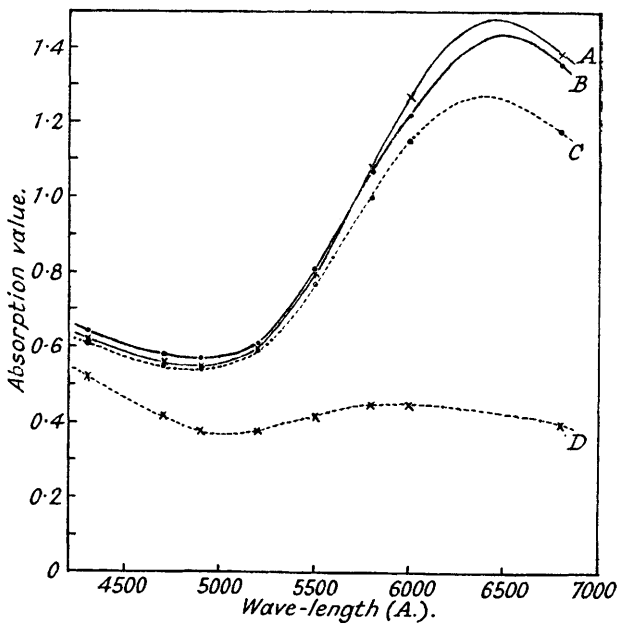
3% Starch paste; $Al(NO_3)_3,9H_2O$, 0·45 g./g. of dry starch.

No.	Time of boiling (hrs.).	Temp. of pptn. of $Al(OH)_3$.	" Ageing " of $Al(OH)_3$. Temp.	Time (days).	Amylose fraction. Yield (%).	B.V.
VII	0·3	14°	14°	3	9·1	0·89
VIII	0·3	30	14	3	9·5	0·96
IX	0·3	30	30	3	9·6	1·03
X	1·0	15	15	0	7·6	1·01
XI	1·0	15	15	1	9·5	1·16
XII	1·0	15	15	2	9·5	1·24
XIII	1·0	15	15	3	10·9	1·27
XIV	1·0	15	15	7	9·5	1·22
XV	2·0	30	30	0	8·0	1·05
XVI	2·0	30	30	3	12·5	1·30
XVII	0·5	30	30	3	13·3	1·23
XVIII	1·0	30	30	3	10·9	1·40
XIX	3·0	30	30	3	12·3	1·22
XX	4·0	30	30	3	12·1	1·16

The first group of separations (VII—IX, Table II), conducted on portions of the same starch paste, showed that when the aluminium hydroxide was both precipitated and " aged " at 30° the amylose fraction had a higher B.V. (1·03) than that obtained when the temperatures of

precipitation and " ageing " were 30° and 14° respectively, or when both operations were carried out at 14°. The time of " ageing " plays an important part in the fractionation, as is shown in separations X—XIV (Table II), in which the starch–aluminium hydroxide suspension was kept at 15° and aliquot portions were removed at intervals for the isolation of the amylose fraction. Whereas the blue-value of the product was 1·01 when no " ageing " occurred, it rose to a maximum of 1·27 after " ageing " of the hydroxide suspension for 3 days, and diminished slightly to 1·22 after 7 days. It was also demonstrated that " ageing " at a higher temperature (30°) for 3 days considerably raised the blue-value of the product (see separations XV and XVI). In another batch of separations (XVI—XX) a 3% paste was stirred at 100° and portions were removed at intervals for co-precipitation with aluminium hydroxide. The amylose fraction with maximum blue-value was isolated from the paste which had been boiled for 1 hour.

Light-absorption curves of polysaccharide–iodine complexes.



A and B: Amylose fractions obtained from potato starch by means of aluminium hydroxide.
C: Potato amylose of relatively high B.V. isolated by the thymol method (Bourne, Donnison, Haworth, and Peat, loc. cit.).
D: Potato starch.

These modifications were incorporated in a new method for the isolation of amylose. Samples of amylose isolated by this standardised technique were consistently of high blue-value (B.V., 1·35—1·40; Table III). The yield was somewhat variable (6—13%) and was always lower than the usual yield (*ca.* 20%) obtained with organic precipitants. Presumably this lower yield was due to the difficulty of completely separating the amylose solution from the aluminium hydroxide gel.

TABLE III.

Amylose fractions obtained by aluminium hydroxide precipitation of starch (final method).

Yield (%)	10·9	12·5	6·0	6·2
B.V.	1·40	1·39	1·37	1·35
Ash (%)	1·10	0·93	1·34	1·34

The light-absorption curves of a selection of " amylose " fractions obtained in the course of this work together with those of thymol–amylose and unfractionated starch are given in the Figure. These curves confirm that adsorption on aluminium hydroxide effects a true fractionation of the starch. This conclusion also finds support in the extent to which the fractions are hydrolysed by the β-amylase of soya bean. For example two amylose fractions (see Table III) having B.V. 1·39 and 1·37 yielded 91% and 89% of maltose respectively.

Experimental.

Procedure for the Analysis of Starch Fractions.—Each starch fraction was dried at 60° in a vacuum over phosphoric oxide before being analysed by the following methods. The results are expressed with reference to the samples thus dried.

(a) *Ash content.* The polysaccharide (20 mg.), contained in a platinum boat, was heated in a small muffle furnace until there was no further change in weight.

(b) *β-Amylolysis.* The limiting percentage conversion into maltose effected by the β-amylase of soya beans was determined by the method of Bourne, Donnison, Haworth, and Peat (*loc. cit.*).

(c) *Nature of the iodine stain.* A solution of the polysaccharide was stained with an iodine–potassium iodide solution under standard conditions, which have already been described by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). These authors also defined the terms "absorption value" and "blue-value" used in this paper. The method of plotting a characteristic "absorption curve" for the polysaccharide–iodine complex from such measurements was outlined by Bourne, Donnison, Haworth, and Peat (*loc. cit.*).

Adsorption of Starch by Aluminium Hydroxide.—Excess of ammonia solution was added to a 3% aqueous dispersion of potato starch containing aluminium sulphate [$Al_2(SO_4)_3,18H_2O$, 3·5 g./g. of dry starch]. After the removal of the precipitated aluminium hydroxide in the centrifuge, the acidified supernatant liquid failed to stain with iodine. The precipitate was exhaustively extracted with boiling water until the extracts, which originally stained intensely blue with iodine, gave only a very faint stain. The residue was dissolved in hot dilute sodium hydroxide solution, cooled, and acidified; the resultant clear solution gave a reddish-purple iodine stain.

Other experiments in which less aluminium sulphate (0·3, 0·7 and 1·0 g./g. of dry starch) was employed gave similar results except that the acidified supernatant liquids stained blue, showing that adsorption of the polysaccharide was not complete.

Fractionation of Potato Starch, using Aluminium Sulphate.—A slight excess of ammonia solution (*d* 0·88) was stirred at room temperature into a 3% aqueous dispersion of potato starch containing hydrated aluminium sulphate. After 5 minutes the precipitated aluminium hydroxide was removed in the centrifuge, and an amylose fraction was precipitated from the supernatant liquid with alcohol (2 vols.). The amylose fraction (*A*) was purified by dialysis against running water for 2 days, re-precipitated with alcohol (2 vols.), triturated with alcohol and then with ether, and dried.

The aluminium hydroxide was exhaustively extracted with water at 100° until no more polysaccharide could be removed. Each extract was dialysed, and the polysaccharide (fractions *B* to *D*) recovered as above. The residual aluminium hydroxide was not readily soluble in dilute mineral acids, and was therefore dissolved in a slight excess of 0·5N-sodium hydroxide at 80°, neutralised with 5N-sulphuric acid at 0°, and a small insoluble impurity removed in the centrifuge. The amylopectin fraction (*E*) was precipitated from the supernatant solution with alcohol (2 vols.), dissolved in water, and re-precipitated with alcohol (2 vols.). After being dialysed for 6 days against running water it was recovered as above.

The results of several experiments of this type, in which varying proportions of starch and aluminium sulphate were used, are recorded in Tables I*A* and I*B*.

Factors influencing the Purity of the Amylose Fraction obtained by using Aluminium Nitrate.—(a) *Temperatures of precipitation and "ageing" of aluminium hydroxide.* A 3% aqueous dispersion of potato starch containing 0·1% of NaCl was stirred at 100° under reflux for 20 minutes, cooled, and divided into several portions (500 c.c. each). To each portion was added a concentrated solution of aluminium nitrate [$Al(NO_3)_3,9H_2O$, 0·45 g./g. of dry starch] and then ammonia solution (*d* 0·88) until the solution was permanently alkaline to phenolphthalein. After 3 days (see below) the precipitated aluminium hydroxide was removed in the centrifuge and the supernatant liquid was dialysed for 2 days against running water. The amylose fraction was precipitated with alcohol (2 vols.), triturated with alcohol and then with ether, and dried. The results of this series of experiments, in which the temperature at which the aluminium hydroxide was precipitated and the temperature at which it was "aged" were both varied, are recorded in Table II (separations VII—IX).

(b) *Temperature and time of "ageing" of aluminium hydroxide.* (i) A solution of hydrated aluminium nitrate (0·45 g./g. of dry starch) was added at 15° to a 3% aqueous dispersion of potato starch, containing 0·1% of NaCl, and ammonia solution (*d* 0·88) was introduced, with stirring, until the mixture was permanently alkaline to phenolphthalein. The suspension was stored at 15°, and at intervals portions (800 c.c.) were removed, the amylose fraction being recovered in each case as in (a).

(ii) This series of experiments was repeated using a second starch paste which had been preheated for 2 hours at 100°. The precipitation and "ageing" process were conducted at 30°. The results of the two series of experiments are given in Table II (separations X—XVI).

(c) *Time of boiling of the starch paste.* A 3% aqueous dispersion of potato starch, containing 0·1% of sodium chloride was stirred at 100° under reflux, and at intervals portions (200 c.c.) were removed and rapidly cooled to 30°. To each was added a concentrated solution of hydrated aluminium nitrate (0·45 g./g. of dry starch), and ammonia solution (*d* 0·88) was stirred in until the mixture was permanently alkaline to phenolphthalein. Each suspension was allowed to "age" for 3 days at 30° before the amylose fraction was recovered as in (a). A summary of the results is given in Table II (separations XVI—XX).

Method Finally adopted for the Isolation of Amylose, using Aluminium Nitrate.—A 3% aqueous dispersion of potato starch, containing 0·1% of sodium chloride, was boiled with stirring for 1 hour and rapidly cooled to 30°. A solution of aluminium nitrate [$Al(NO_3)_3,9H_2O$, 0·45 g./g. of dry starch], was added, and ammonia solution (*d* 0·88) was introduced, with stirring, until the mixture was permanently alkaline to phenolphthalein. The suspension was allowed to "age" for 3 days at 30° before the aluminium hydroxide was removed, and the supernatant liquid was dialysed for 2 days against running water. The amylose fraction was precipitated with alcohol (2 vols.), triturated with alcohol and then with ether, and dried. The results of several fractionations effected by this method are recorded in

Table II. Each of the amylose fractions contained a small amount (*ca.* 1%) of mineral matter in which aluminium and nitrate ions were detected.

Isolation of Amylose, using Aluminium Acetate.—The procedure adopted in this fractionation of potato starch was the same as that used in the final method described above except that aluminium nitrate was replaced by an equivalent amount of the acetate. A solution of aluminium acetate was prepared by precipitating aluminium hydroxide with ammonia from a solution of aluminium nitrate (0·45 g./g. of dry starch) and redissolving the washed precipitate in dilute acetic acid. An experiment in which dialysis was omitted gave an amylose fraction (14·7%), having B.V. 1·28 and ash content 3·32%. A second experiment in which dialysis was included gave an amylose fraction (20·1%), having B.V. 1·23 and ash content 1·51%.

The authors wish to express their thanks to Professor Sir Norman Haworth, F.R.S., for the interest he has taken in this work, and to Mr. P. N. Hobson for assistance in part of the experimental work.

THE A.E. HILLS LABORATORIES,
THE UNIVERSITY, EDGBASTON, BIRMINGHAM 15. [*Received, February 5th*, 1948.]