An Evaluation of Methods using Stearic Acid and Cellulose for the Purification of Amylopectin.

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Removal of residual amylose from amylopectin by selective precipitation with stearic acid has been claimed by Meyer and Gibbons (*Helv. Chim. Acta*, 1950, **33**, 210). In the present work, tests have been made of this method on amylopectin and a mixture of amylopectin and amylose. No selective precipitation has been found. It is shown that the apparent purification obtained by Meyer and Gibbons was due to a retention of stearic acid by amylose. In addition, the preferential adsorption of amylose by cellulose was found to be inefficient, and ineffective for the removal of the residual amylose in preparations of potato amylopectin.

Traces of contaminating amylose in the amylopectin product of a starch fractionation are easily detected and estimated by their preferential uptake of iodine (see, *e.g.*, Bates, French, and Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 142; Watson and Whistler, *Analyt. Chem.*, 1946, **18**, 75; Nussenbaum, *ibid.*, 1951, **23**, 1478). Removal of this amylose is difficult. Tanret (*Compt. rend.*, 1914, **158**, 1353) believed that it could be removed by selective adsorption on cotton wool, and later work supported this (Baldwin, *J. Amer. Chem. Soc.*, 1930, **52**, 2907; Samec, *Ber.*, 1940, **73**, *A*, 85; Mullen and Pacsu, *J. Amer. Chem. Soc.*, 1941, **63**, 1168; Bates, French, and Rundle, *loc. cit.*). Schoch, Williams, and Schink (Schoch, *Adv. Carbohydrate Chem.*, 1945, **1**, 247; see p. 260) showed, however, that Tanret's method had not removed the residual amylose and suggested that fatty acids extracted from the cotton wool had suppressed the iodine colour by which it was detected. Higginbotham and Morrison (*J. Textile Inst.*, 1949, **40**, T 208) agreed with this conclusion. Meyer and Gibbons (*Helv. Chim. Acta*, 1950, **33**, 210) have claimed that complete purification of amylopectin can be achieved by removing the contaminating amylose as an insoluble fatty acid complex obtained by shaking the crude amylopectin in aqueous solution with a suspension of stearic acid. These authors detected amylose, not by the colour of its iodine stain, but by quantitative potentiometric titration with iodine (Bates, French, and Rundle, *loc. cit.*). In the work described below, it is shown that the results of Meyer and Gibbons can be repeated but that amylopectin is not purified by their treatment; their extraction procedure fails to remove all the stearic acid, thus preventing detection of the amylose by iodine.

The amylose content of the amylopectin samples used was measured by the differential potentiometric method of Gilbert and Marriott (*Trans. Faraday Soc.*, 1948, **44**, 84). Calibration experiments with mixtures of amylopectin and amylose showed that amylose is saturated at 20° with iodine at a concentration of free iodine ($I_3^- + I_2$) of *ca.* 2×10^{-6}M-I in 10^{-3}M-potassium iodide, at which concentration the adsorption of iodine by amylopectin is only just beginning. To increase the accuracy of the titration, especially at low concentrations of iodine, a procedure was adopted which had been developed for a study of the dissociation constants of the amylopectin–iodine complexes (Gilbert and Hybart, unpublished work).

In this procedure, before the actual differential titration, excess of iodine was added to the solutions to be titrated in order to eliminate any impurities which would react irreversibly with iodine. Excess of thiosulphate was next added, and then further iodine until the electrodes were just depolarized, corresponding to the removal of thiosulphate. The titration was then carried out as in the original method. Repetition of any titration was possible by adding excess of thiosulphate and then iodine to the depolarization point before beginning the titration again.

FIG 1. FIG 2.

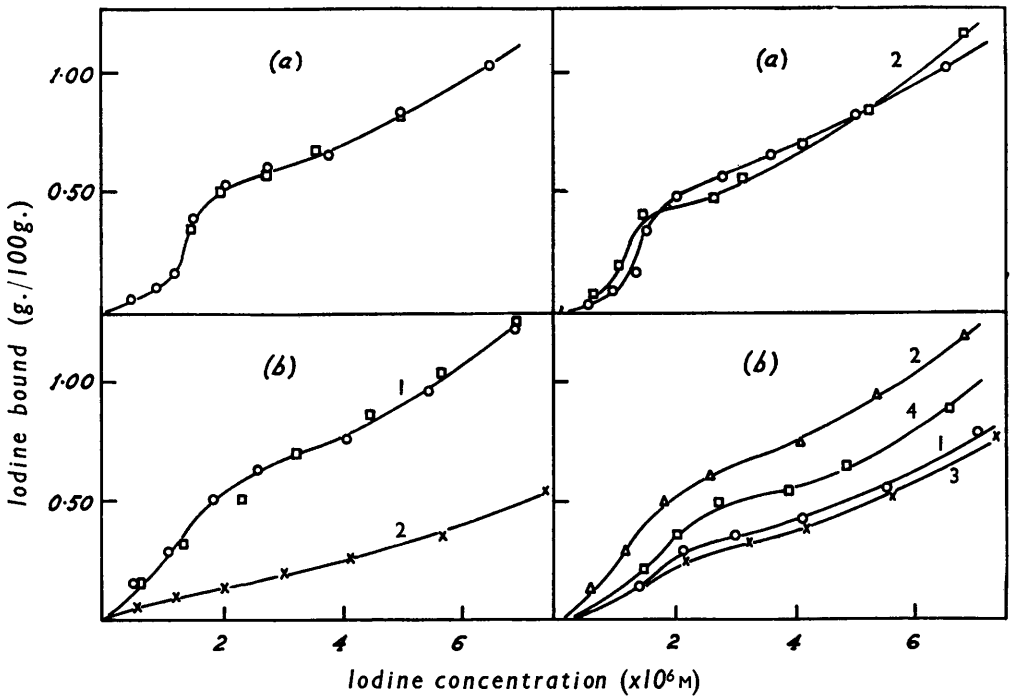

FIG. 1. *Effect of treatment with stearic acid on amylopectin containing traces of amylose.*

(*a*) *Titration curves of amylopectin :* ——○—— *before treatment,* ——⊡—— *after treatment and defatting with methanol.*
(*b*) *Titration curves of amylopectin–amylose :* (1) ——○—— *before treatment,* ——⊡—— *after treatment and defatting with methanol;* (2) *after treatment but without defatting.*

FIG. 2. *Effect of treatment with cellulose on amylopectin containing traces of amylose.*

(*a*) *Titration curves of amylopectin :* (1) *before and* (2) *after treatment.*
(*b*) *Titration curves of amylopectin–amylose :* (1) *original amylopectin;* (2) *amylopectin–amylose;* (3) *treated mixture before defatting with methanol;* (4) *treated mixture after defatting with methanol.*

Meyer and Gibbons (*loc. cit.*) found that the step in the amylopectin titration curve which corresponds to residual amylose (see Fig. 1*a*) was no longer present after amylopectin had been treated with stearic acid. Amylopectin prepared from fresh undried potato starch from which amylose had been precipitated as a complex with thymol (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19) was therefore treated by their method. To ensure complete removal of fatty acid, the treated amylopectin was extracted with hot methanol before titration. Fig. 1*a* shows that the stearic acid-treated amylopectin had the same content of amylose as the original sample, and that there had been no purification. This could have meant either that the residual amylose of this amylopectin was an integral part of the amylopectin and chemically bound, or that in Meyer and Gibbons's measurements stearic acid was interfering with the adsorption of iodine by amylose. Treatment

with stearic acid was therefore repeated on a sample of amylopectin to which 2·75% of pure amylose had been added. The titration curve of the mixture before (curve 1) and after (curve 2) stearic acid treatment is shown in Fig. 1*b*. This time the result of Meyer and Gibbons was confirmed. The treated mixture was then subjected to extraction with hot methanol and titrated again, whereupon the original titration curve was once more obtained (Fig. 1*b*). It can therefore be said that the only effect of treatment with stearic acid in accordance with the method of Meyer and Gibbons is to prevent the formation of the amylose–iodine complex, and that no amylose is removed even when it has been added to amylopectin, and is therefore certainly in an uncombined state.

The use of defatted cotton wool for the purification of amylopectin was also investigated. A solution of amylopectin was passed through a column of cotton wool, and then extracted with methanol to remove any fatty acids. The titration curve of the product (Fig. 2*a*) was not significantly different from that of the original. The experiment was then repeated on the amylopectin–amylose mixture (2·75% of free amylose). Comparison of curves 2 and 4 of Fig. 2*b* shows that cotton wool removed a small proportion of amylose selectively from the mixture. Curve 3 shows the difficulty of defatting cotton wool completely, and the avidity of starch for fatty materials. The experiments thus do not rule out the use of cellulose for the preferential adsorption of amylose, but show it to be inefficient (it could not have been saturated as the amylose removed was only 0·008% of the weight of cellulose; cf. Higginbotham and Morrison, *loc. cit.*), and useless for the removal of amylose remaining in amylopectin prepared by the " thymol method " of fractionating potato starch. They also give no indication of whether the residual amylose is an inherent part of the amylopectin molecule.

Experimental

Isolation of Amylopectin from Potato Starch.—Potatoes were pulped in M/100-sodium chloride in an " Atomix " blender at 0°, and the pulp was filtered through muslin. The resultant starch was washed by repeated sedimentation in M/100-sodium chloride at 0°, and then defatted with methanol–chloroform (1 : 2 v/v; 10 ml./1 g. of starch) (Folch, *Fed. Proc.*, March 1950, p. 171). A dispersion of the starch (*ca.* 1%) was prepared by boiling the undried defatted granules in 0·1% sodium chloride for 25 min. with vigorous stirring. The solution was then cooled to 60° and excess of powdered thymol (4 g./2500 ml.) stirred in. After being kept at 30° for 84 hr., the amylose–thymol complex was removed on the centrifuge, and the resulting supernatant liquid filtered (Grade 4, sinter). To half of the filtrate were added ethanol (1 vol.) and a trace of sodium chloride; the resulting precipitate, after being refluxed with methanol, was redispersed in water and freeze-dried to give amylopectin (sample 1). The rest of the filtrate was kept at 0° for 9 weeks, filtered (Grade 4, sinter), and treated as above to give amylopectin (sample 2).

Treatment of Amylopectin with Stearic Acid.—A solution of amylopectin in water (sample 1; 75 mg./10 ml.) was adjusted to pH 5 with acetic acid and shaken with a fine suspension of stearic acid (50 mg./0·2 ml. of ethanol). After filtration (Grade 4, sinter), the amylopectin was precipitated with ethanol (8 vol.; 0° for 18 hr.), and the resulting solid extracted with hot methanol (3 × 50 ml.) to remove stearic acid. After the product had been redissolved and freeze-dried, its iodine adsorption isotherm was determined differentially as described (Gilbert and Marriott, *loc. cit.*; Gilbert and Hybart, *loc. cit.*).

Treatment of a Mixture of Amylopectin and Amylose with Stearic Acid.—The mixture was prepared by freeze-drying an aqueous solution of amylopectin (sample 2; 200 mg.) and amylose (5·5 mg.). A portion was titrated with iodine. The mixture was then treated as above with stearic acid (80 mg./200 mg. of mixture) and filtered and the filtrate was freeze-dried. One portion of the freeze-dried solid was titrated directly, and another extracted three times with refluxing methanol before titration.

Treatment of Amylopectin with Cotton Wool.—Cotton wool was defatted by Soxhlet extraction (12 hr.; methanol–chloroform, 1 : 1, v/v). After drying, the cotton (20 g.) was packed into a glass tube (1 cm. diam.) and wetted with water under vacuum. A solution of amylopectin (sample 1; 50 ml.; 1%) was passed slowly through the column (15 drops per min.) and then freeze-dried. After extraction by refluxing methanol, the product was titrated with iodine.

Treatment of the Amylopectin–Amylose Mixture with Cotton Wool.—The above experiment was

repeated with the prepared mixture of amylopectin and amylose. Solution (0·2%; 85 ml.) was passed through a column (48 × 1 cm.) of cotton wool (12 g.) at a rate of 17 drops per min. The product was freeze-dried and titrated with iodine before and after extraction with boiling methanol.

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