SEPARATION AND DETERMINATION OF THE AMYLOSE AND AMYLOPECTIN FRACTIONS OF STARCH

By K. G. KRISHNASWAMY and A. SREENIVASAN

(From the Department of Chemical Technology, Bombay University, Bombay, India)

(Received for publication, July 14, 1948)

Most of the earlier procedures used for separating starch into amylose, the linear unbranched component, and amylopectin, the branched chain component (1, 2), have involved degradation and hydrolysis of the starch molecules. During the last few years, however, a number of methods have been proposed which fulfil, more or less, the necessary requirement of protecting the starch constituents from degradation. The more important of these methods for the separation of amylose from starch is based on its selective diffusibility in water at 60° or 80° (1, 3), precipitability with butanol (4), thymol (5), or nitroparaffins (6), and adsorbability on cellulose (7). It is shown in this communication that these methods fail to effect clear-cut as well as quantitative separation of the two starch fractions, while the purity of the products obtained is also variable. It has been possible, by suitable combination of certain of these procedures, to prepare amylose and amylopectin, judged for their purity by the intensity of their iodine colorations under standard conditions, and to determine their exact percentages in any starch preparation by reference to a calibrated curve for intensity of iodine coloration with known mixtures of the pure fractions (3).

EXPERIMENTAL

Preparation of Starch—The major part of the studies reported here was carried out with a sample of starch prepared from a local variety of peas (*Pisum sativum*). The seeds, softened by soaking overnight in water, were ground to a not too fine consistency and the mash was extruded through a cloth bag into a sufficient volume of distilled water. The residual pulp was mashed and pressed out a second time. The combined extract was let stand and the sludge which separated was purified of proteinaceous material by repeated agitation and settling. The starch suspension was finally kneaded through muslin into water and centrifuged to separate the starch, which was washed successively with 20 and 80 per cent ethanol and allowed to dry at room temperature (28°). Analysis of the product gave 0.88 per cent protein, 0.25 per cent ether extractive, and 12.75 per cent moisture.

Determination of Iodine Coloration—The intensity of color developed in a 2 mg. per cent solution of starch or of the various starch fractions, on addition of a solution of iodine in potassium iodide to a final concentration of 4 mg. per cent of iodine, was measured in a 10 mm. cell by a Klett-Summerson photoelectric colorimeter with Filter K_{66} in position (3). The colorimeter was initially adjusted so that the blank, which had a light yellow color due to the iodine in solution, gave a zero reading; the color measurements recorded are in terms of scale readings in the instrument.

Fractionation of Starch by Selective Extraction of Amylose with Hot Water—5 gm. of air-dry starch, mixed with water to avoid lump formation, were treated with about 300 ml. of water at 60° and the suspension maintained at this temperature for 4 hours with slow stirring. It was then centrifuged at 3000 R.P.M. and the supernatant passed through a sintered glass No. 4

TABLE I

Extraction of Starch with Water at 60°

	On dry basis			Intensity of iodine color (scale readings)*		
	Yield 1	Yield 2	Yield 3	Yield 1	Yield 2	Yield 3
	per cent	*per cent*	*per cent*			
Original starch				143	149	148
Fraction I	14.4	14.7	15.8	279	271	269
" II	0.9	0.6	Trace	181	180	
" III	84.5	85.3	83.5	101	103	100

* Klett-Summerson colorimeter.

filter, which was found more convenient to use than filter paper coated with Hyflo Super-Cel, as recommended in the original procedure (3). The clear filtrate, after addition of methanol to a concentration of 20 per cent volume per volume, was let stand for 48 hours. At the end of this period, the precipitated amylose (Fraction I) was filtered through a sintered glass No. 4 crucible, washed with 95 per cent ethanol, and finally with absolute alcohol before drying in a vacuum oven. The filtrate was further treated with methanol to 50 per cent volume per volume strength and allowed to settle as before. The precipitate (Fraction II) was filtered, washed, and dried to constant weight.

The gelatinous residue remaining after the centrifuging of the aqueous starch suspension was ground well, dehydrated by repeated additions of alcohol, filtered, and dried *in vacuo* (Fraction III). Table I gives a set of typical results obtained together with the iodine colorations of the starch and of the different fractions.

In the set of experiments given in Table II, the temperature of fractiona-

tion was kept at 80°, as recommended by Meyer (1); the procedure was otherwise the same as that described above.

Fractionations of pea starch and of the crude amylopectin (Fraction III, Table I) were also attempted by treatment for 48 hours at room temperature with 1:2 chloral hydrate solution in water, as recommended by Meyer. The products obtained gave iodine coloration averaging 74 and 67 respectively; by using chloral hydrate solution at 80°, the corresponding color readings were 54 and 56.

TABLE II

Fractionation of Starch with Water at 80°

	Yield 1	Yield 2	Intensity of iodine color*	
			Yield 1	Yield 2
	per cent	*per cent*		
Fraction I	16.0	16.3	220	224
" II	Trace	Trace		
" III	82.9	79.6	114	104

* See Table I.

TABLE III

Fractionation by Butanol Extraction

Fraction	Yield 1	Yield 2	Iodine coloration*	
			Yield 1	Yield 2
	per cent	*per cent*		
Butanol-pptd. by autoclaving	38.6	39.2	243	241
" " Waring blendor	39.5	39.4	234	230
Butanol-non-pptd. by autoclaving	60.7	59.9	52	49
" " Waring blendor	59.4	60.7	47	50

* See Table I.

Fractionation by Selective Precipitation of Amylose with Butanol—Schoch's butanol precipitation method (4) was closely followed, except for the purification of the separated amylose by recrystallization from the boiling water-butanol mixture. Since it was thought that some hydrolytic degradation of starch was likely to occur as a result of the high temperature treatment under pressure, an attempt was made to eliminate autoclaving in Schoch's procedure by securing dispersion of starch with high speed stirring. A properly gelatinized paste of 5 gm. of starch in about 500 ml. of boiling water was treated in a Waring blendor in two lots for 5 minutes each. Subsequent separation of the starch fractions was effected as described by Schoch. Typical results, by both procedures, are given in Table III.

Fractional Precipitation of Amylose with Thymol—The high speed stirring recommended by Haworth *et al.* (5) did not result in sufficient dispersion and it was found more effective to use a Waring blendor for aiding dispersion. The results obtained by this method are shown in Table IV.

Fractionation by Preferential Adsorption of Amylose on Cotton—With a 1 per cent starch paste, gelatinized and dispersed in a Waring blendor according to the procedure of Pacsu and Mullen (7), not more than 0.3 per cent of amylose (iodine coloration, average 223) was obtainable, therefore suggesting that preferential adsorption of amylose on the cotton used was far from satisfactory; somewhat similar results were obtained by using filter paper pulp as an adsorbent.

TABLE IV

Fractionation by Thymol Extraction

Fraction	Yield 1	Yield 2	Yield 3	Iodine coloration*		
				Yield 1	Yield 2	Yield 3
	per cent	*per cent*	*per cent*			
Thymol-pptd	39.7	39.9	40.7	222	220	208
Thymol-non-pptd	60.1	60.2	59.1	50	52	45

* See Table I.

DISCUSSION OF RESULTS

The pronounced difference in the affinity of amylose and amylopectin for iodine has formed the basis for the determination of their relative proportions in starches potentiometrically (8), absorptiometrically (3), or spectrophotometrically (9, 10). On the assumption that the intensity of iodine coloration with amylose or amylopectin fractions will be determined by their respective freedom from each other, it becomes apparent that the higher the scale reading, the purer will be the fraction in respect to its amylose content, and that, conversely, purity with regard to amylopectin will be connoted by the lowest scale reading. Based on this criterion, it would follow from the data presented here (Tables I to IV) that no single method effects simultaneously efficient and quantitative separation of the two starch fractions. The procedure of McCready and Hassid (Table I) gives, in one step, the purest amylose fraction, judging from its iodine-staining property; its solubility is, however, only of the order of 15 per cent, which is very low. That the amylopectin fraction obtained here is admixed with a high amount of amylose is evident from the values for iodine coloration as compared to the amylopectin fraction obtained by the procedures of Schoch (Table III) and of Haworth *et al.* (Table IV). The amylose fraction yielded by Meyer's extraction procedure (Table II) is only about 80 per cent as

pure as the corresponding fraction obtained according to the method of McCready and Hassid; this is no doubt due to the fractionation temperature being near the gelatinization point of the starch and consequent con-

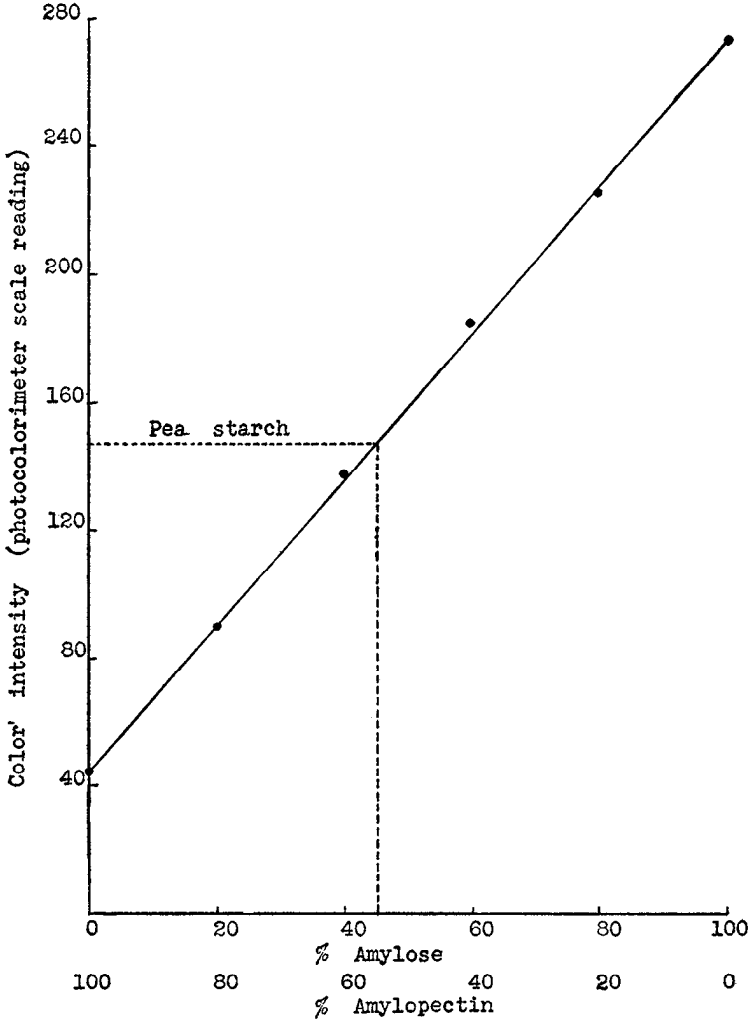

FIG. 1. Color intensities of mixtures of amylose and amylopectin from pea starch with iodine.

tamination with amylopectin by the disintegration and rupture of the granules. The use of chloral hydrate solution to purify amylopectin from admixed amylose resulted in a product still containing about 6 to 10 per cent of amylose.

Both butanol and thymol undoubtedly effect very much better fractionation of the starch components than does extraction with hot water, and, indeed, the yields of amylose and amylopectin correspond more nearly to the correct values deduced below than do those obtained by hot water extraction. However, it is clear from a comparison of the iodine coloration of amylose fractions (Tables III and IV) that they are respectively only about 85 and 77 per cent as pure as that obtained by the McCready and Hassid method. It has been possible to obtain pure amylose by successive recrystallizations from boiling water-butanol mixtures as recommended by Schoch (4, 11), but, the yields being no longer quantitative, it appeared preferable to do so in a single extraction with hot water at 60°.

Although the amylopectin fractions obtained by selective precipitation with butanol or thymol have given the lowest intensity of iodine coloration

TABLE V

Fractionation of Mung Starch

Starch analysis	
Moisture, %	13.50
Proteins, %	0.69
Ether extractives, %	0.23
Iodine coloration of starch*	127
" " " amylose fraction*	287
" " " amylopectin fraction*	34
Amylose (from Fig. 2), %	36.8
Amylopectin (from Fig. 2), %	63.2

* Scale readings, Klett-Summerson colorimeter.

of all the methods studied, it was felt that, since it is always the residue in the mother liquor after the amylose had been precipitated, amylopectin may not be easily obtainable in as pure a form as the amylose component. We therefore attempted to ascertain whether by butanol fractionation of the residue from the hot water treatment of starch at 60° (Fraction III, Table I) a purer preparation of amylopectin could be obtained than by Schoch's method from the original starch. By this procedure, a product was secured which gave an iodine coloration of only 43 or 44 units. This was the purest amylopectin obtainable; a product with similar purity could also be prepared by thymol fractionation of crude amylopectin.

Pure preparations of amylose and amylopectin can thus be obtained in one and two operations, respectively, by a combination of the features of McCready and Hassid's method for amylose and that of Schoch or of Haworth *et al.* for amylopectin. By using various proportions of the starch components prepared as above, the color intensities of the mixtures in

solution (2 mg. per 100 ml.) with iodine can be plotted against per cent concentration of the two fractions when a linear relationship similar to that reported by McCready and Hassid (3) is obtained (Fig. 1), and from which,

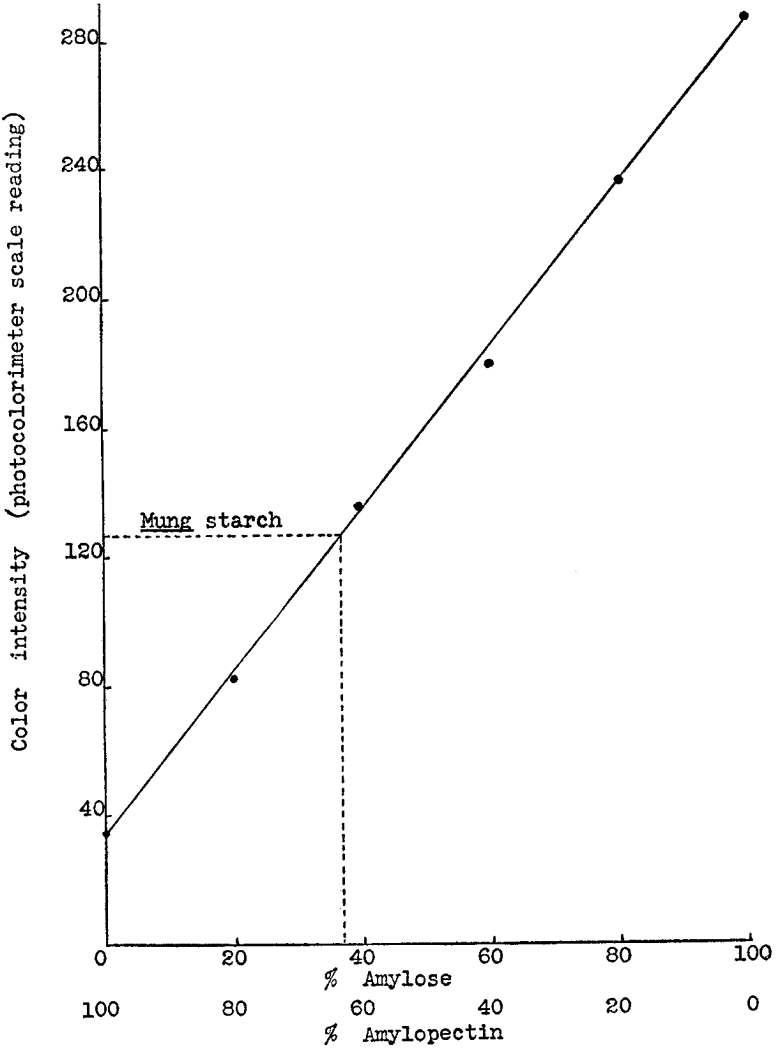

FIG. 2. Color intensities of mixtures of amylose and amylopectin from mung starch with iodine.

after ascertaining the color intensity of the original starch with iodine under identical conditions, its proportions of the two constituents can be read; the latter can also be deduced by simple extrapolation, as there is strict

proportionality between color intensity with iodine and amylose or amylopectin content. In this way, the preparation of pure starch used in these studies, with its iodine coloration of 147 (Table I), can be observed to consist of 45.0 per cent amylose and 55.0 per cent amylopectin.

By the foregoing procedures for the preparation of pure amylose and amylopectin fractions, and by using a preparation of mung (*Phaseolus radiatus*) starch, the resulting observations are given in Table V and in Fig. 2.

Differences such as are recorded here in intensities of iodine coloration with pure amylose or amylopectin preparations from natural starches are bound to exist because of possible heterogeneity as to molecular size as well as, with amylopectin, to variations in the degree of branching (*cf.* (8)).

Although the various methods for the fractionation of starch examined here do not effect a clear-cut and quantitative separation of the unbranched and branched components in their pure state, fractionation by selective precipitation of amylose with butanol or with thymol, as recommended by Schoch (4) and by Haworth *et al.* (5), gives an approximate idea of the relative proportions of the two constituents. However, to obtain them in a pure state for examination of their individual properties or for a precise evaluation of their percentages in any starch sample by reference to a calibrated curve or by extrapolation, as described here, it would appear necessary to resort to a combination of procedures involving the properties of amylose for selective diffusibility in water at 60° and precipitability with butanol- or thymol-saturated water.

SUMMARY

1. A comparative study has been made of the procedures for the fractionation of starch based on the differential solubilities of amylose and amylopectin in hot water and in butanol- or thymol-saturated water.

2. It is shown that the method of extraction with hot water at 60° yields an amylose fraction which is the purest obtainable, judged from the intensity of its coloration with iodine; amylose separation is not, however, quantitative.

3. Fractionation of starch by selective precipitation of amylose with butanol or thymol gives only a rough indication of the relative proportions of the linear and branched components; besides, separation, as judged by the iodine-staining properties of the products obtained, is not clear-cut.

4. A procedure is outlined for obtaining highly pure preparations of amylose and amylopectin from a starch sample. By quantitatively determining the color intensities of the starch and of known mixtures of its amylose and amylopectin fractions with iodine, their proportions in the former can be precisely estimated.

BIBLIOGRAPHY

1. Meyer, K. H., in Kraemer, E. O., Advances in colloid science, New York **1**, 143 (1942).
2. Peat, S., in Annual review of biochemistry, Stanford University, **15**, 75 (1946).
3. McCready, R. M., and Hassid, W. Z., *J. Am. Chem. Soc.*, **65**, 1154 (1943).
4. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2957 (1942).
5. Haworth, W. N., Peat, S., and Sagrott, P. E., *Nature*, **157**, 19 (1946).
6. Whistler, R. L., and Hilbert, G. E., *J. Am. Chem. Soc.*, **67**, 1161 (1945).
7. Pacsu, E., and Mullen, J. W., 2nd, *J. Am. Chem. Soc.*, **63**, 1168 (1941).
8. Bates, F. L., French, D., and Rundle, R. E., *J. Am. Chem. Soc.*, **65**, 142 (1943).
9. Kerr, R. W., and Trubell, O. R., *Paper Trade J.*, **117**, No. 15, 25 (1940).
10. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.*, **66**, 111 (1944).
11. Wilson, E. J., Jr., Schoch, T. J., and Hudson, C. S., *J. Am. Chem. Soc.*, **65**, 1380 (1943).