2. *The Amylose Component of Waxy Maize Starch.*

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Waxy maize starch and the starches from other waxy cereals (rice, barley, and sorghum) are usually regarded as being composed entirely of amylopectin. Although no amylose could be obtained from waxy maize starch by thymol or *cyclo*hexanol precipitation, a fraction with an enriched proportion of amylose, which was similar in properties to potato starch, was isolated after fractionation of the starch with aluminium hydroxide. When thymol (or *cyclo*hexanol) was used in conjunction with sulphuric acid, fractions approximating in properties to the amylose component of potato starch were isolated. The proportion of amylose thus found in waxy maize starch is not more than 2%.

Reference is made to the views of Pacsu and Hiller on the constitution of amylopectin and on the alleged conversion of amylopectin into amylose.

THE ordinary starches stain blue with iodine and are composed of two distinct molecular types, namely amylose which is a linear polyglucose, and amylopectin in which short chains of glucose residues are cross-linked to form a ramified or laminated structure. Certain species of starch, however, are distinguished by the fact that their iodine complexes are reddish-purple. These red-staining starches have been found hitherto only in the waxy varieties of such cereals as maize, rice, barley, and sorghum.

It is now generally accepted that the difference between ordinary starches and the " waxy " varieties is a constitutional one and is due to the absence of the amylose component from the red-staining type. It is obviously of importance, having regard to the recently achieved enzymic synthesis of amylose and amylopectin, to examine in greater detail the question as to whether the power to synthesise the long unbranched chains of amylose is entirely lacking in the waxy cereals. In this communication are given the results of an investigation on a sample of waxy maize starch which support the view that this starch, while being composed mainly of amylopectin, nevertheless contains a separable fraction (less than 2%) which is indubitably an amylose.

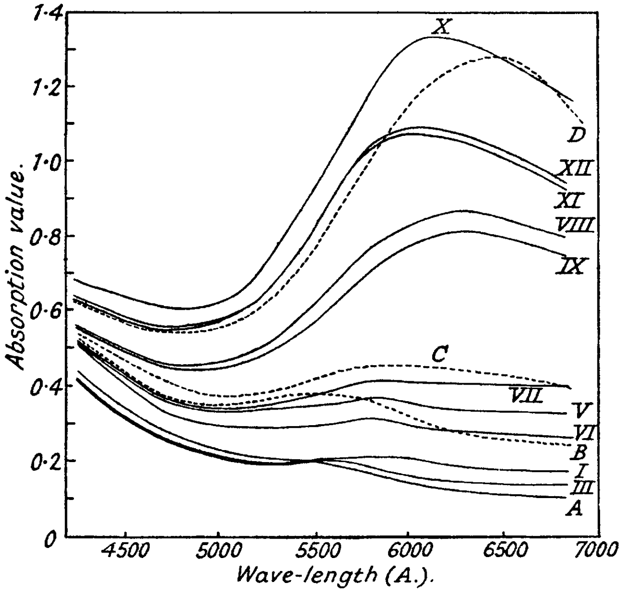
Our sample of waxy maize starch was defatted by treatment with aqueous methyl alcohol and then with dioxan. It is worthy of mention here that aqueous alcohol extracts a substance from waxy maize starch, as it does from potato starch and most cereal starches, which is capable of forming an insoluble precipitate with potato amylose but not with amylopectin. This alcohol-soluble substance appears to be a fatty acid (probably unsaturated), and fatty acids are known to function as amylose-precipitants (Schoch and Williams, *J. Amer. Chem. Soc.*, 1944, **66**, 1232).

When the defatted waxy maize starch, which was free from mineral matter and protein, was submitted to the action of hot aqueous acid it was converted completely into glucose. On the other hand, its hydrolysis by the agency of β-amylase (from soya bean) ceased at a conversion limit (to maltose) of 49%. This figure is to be compared with the reported values of 42 (Caldwell and Hixon, *J. Amer. Chem. Soc.*, 1941, **63**, 2876), 55 (Newton, Farley, and Naylor, *Cereal Chem.*, 1940, **17**, 342), 51 (Montgomery and Hilbert, *J. Amer. Chem. Soc.*, 1946, **68**, 916), and 68% (Meyer and Heinrich, *Helv. Chim. Acta*, 1942, **25**, 1639). The wide variations in the conversion limits quoted may be due to the different origins of the β-amylase as much as to actual differences in the specimens of waxy maize starch employed by the different authors.

The blue-value of the starch was 0·10, compared with the average values of 1·10 and 0·22 respectively for amylose and amylopectin isolated from potato starch by the thymol method

(Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687). The lower staining power of waxy maize starch relative to potato amylopectin is emphasised by the light-absorption curves of their iodine complexes (see Fig.). Nevertheless, the gradual addition of a very dilute iodine–potassium iodide solution to a concentrated solution of the " waxy " starch gives a transient blue-purple coloration which is in contrast with the red-purple colour produced with excess of the iodine solution. This observation suggested the presence of a small proportion of blue-staining amylose which has a greater affinity for iodine than has amylopectin and which would therefore preferentially adsorb the small amount of iodine initially available.

Light-absorption curves of waxy maize starch and its fractions.



A : Waxy maize starch.
B : Potato amylopectin (thymol method)
C : Potato starch } *Bourne, Donnison, Haworth, and Peat* (loc. cit.).
D : Potato amylose (thymol method)
I—XII : Fractions of waxy maize starch.

Fractions from Waxy Maize Starch.

Fraction.	Method of isolation.	Yield (%).	Blue-value.	Conversion (%).*
Waxy maize starch	—	—	0·10	49
I	Thymol	4·8	0·17	51
II	,,	91	0·10	46
III	*cyclo*Hexanol	3·1	0·14	49
IV	,,	96	0·08	46
V	$Al(OH)_3$	0·7	0·33	—
VI	,,	2·5	0·26	—
VII	$Al(OH)_3$	1·2	0·40	59
VIII	Thymol + acid	0·5	0·80	68
IX	,,	0·4	0·75	58
X	*cyclo*Hexanol + acid	1·9	1·18	85
XI	,,	0·6	0·93	81
XII	,,	0·3	0·95	—

* *I.e.*, limiting conversion into maltose by β-amylase.

When attempts were made to fractionate waxy maize starch by the use of thymol or *cyclo*hexanol, which are amylose-precipitants (Bourne, Donnison, Haworth, and Peat, *loc. cit.*; Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19), a small fraction (3—5%) was precipitated in each case. The polysaccharide (Fraction I in the table) precipitated by thymol had B.V. 0·17 and a limiting conversion into maltose of 51%, while the corresponding fraction (III) obtained with *cyclo*hexanol had B.V. 0·14 and gave 49% maltose. Although some indication was thus afforded that these fractions were enriched in amylose, they were still red-staining and actually had lower blue-values than the average value for thymol-separated potato amylopectin (0·220). Moreover, the light-absorption curves for these fractions were of amylopectin character (see Fig.).

Since thymol and *cyclo*hexanol, like butanol (Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2957), did not cause the separation of an amylose component, an alternative method of fractionation was employed. This method was recently described by Bourne, Donnison, Peat, and Whelan (see preceding paper) for the fractionation of potato starch. It involves the addition of an aluminium salt to the starch paste and subsequent precipitation of aluminium hydroxide together with the amylopectin fraction, leaving the amylose component in the supernatant liquid. Three experiments in which this method was applied to waxy maize starch gave "amylose" fractions (V, VI, and VII) in yields of 0·7, 2·5, and 1·2%. These fractions had blue-values of 0·33, 0·26, and 0·40 respectively, and the limit of β-amylase conversion of Fraction VII was 59%. Obviously Fraction VII was similar in composition to potato starch, which has B.V. 0·40 and a conversion limit of 60% (Bourne, Donnison, Haworth, and Peat, *loc. cit.*).

It was recently reported (Bourne, Donnison, Haworth, and Peat, *loc. cit.*) that when a solution of potato amylopectin, containing traces of amylose, is saturated with *cyclo*hexanol and then treated with either α-amylase or a mineral acid, a flocculent precipitate, which contains a higher proportion of amylose than does the original polysaccharide, separates. A modification of this procedure, in which a solution of waxy maize starch in N-sulphuric acid saturated with thymol was kept for three days at 30°, yielded a precipitate (Fraction VIII) having B.V. 0·80 and a β-amylase conversion limit of 68%. A duplicate experiment gave Fraction IX, which had B.V. 0·75 and a conversion limit of 58%. A similar flocculent precipitate was obtained when a solution of waxy maize starch in N-sulphuric acid saturated with *cyclo*hexanol was heated to 95°. Three experiments of this type yielded Fractions X, XI, and XII, having blue-values 1·18, 0·93, and 0·95 respectively. These fractions were thus similar to potato amylose in staining properties and in their maltose-conversion limits (85 and 81% for Fractions X and XI). The figure shows that maximum absorption occurs at slightly shorter wavelength than in the case of potato amylose. This is possibly due to some hydrolytic degradation of the amylose during the acid treatment.

The properties of the fractions isolated from waxy maize starch by the procedures described are consistent with the view that this starch does contain the largely unbranched component, amylose. In particular, it will be observed that in respect of the character of the colour given by its iodine complex and of the extent of its β-amylolysis, Fraction X is scarcely to be distinguished from thymol-separated potato amylose. In this starch, however, the amylose : amylopectin ratio is very small. The amylose content appears to be less than 2%.

Since the completion of this work our attention has been drawn to a communication by Pacsu and Hiller (*Text. Research J.*, 1946, **16**, 243) in which is mentioned the isolation of a small amount of amylose from waxy maize starch after mild hydrolysis with 0·1N-sulphuric acid at 86°. No details are given in this paper, which presents a new conception of the constitution of starch and cellulose. According to this tentative hypothesis, amylopectin is to be regarded as having a ramified structure in which amylose chains are joined together through acyclic glucose (or maltose) units by semi-acetal links. Such links would be extremely susceptible to acid or enzymic hydrolysis, and the authors regard the amylose separated from waxy maize starch as being the product of scission by acid hydrolysis of some of the semi-acetal links of the amylopectin. We feel, however, that there are many facts, some of which have already been quoted by Halsall, Hirst, and Jones (*Nature*, 1947, **159**, 97), which cannot be reconciled with this interesting and novel hypothesis of the constitution of starch. To mention only one objection, semi-acetal links will be resistant to alkaline hydrolysis, and consequently the amylose separated from potato starch under alkaline conditions by the aluminium hydroxide method (Bourne, Donnison, Peat, and Whelan, *loc. cit.*) could not have originated in the partial hydrolysis of amylopectin. We see no reason at present for departing from the view that amylose is a pre-formed component of the starches, including that of waxy maize.

EXPERIMENTAL.

Procedure for the Analysis of Waxy Maize Starch and its Fractions.—The waxy maize starch and the fractions derived from it in the course of these experiments were dried at 60° in a vacuum over phosphoric oxide before they were 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) *Hydrolysis and β-amylolysis.* The amount of glucose produced from the polysaccharide by hydrolysis with sulphuric acid at 100° and the limiting conversion into maltose effected by the β-amylase of soya beans were determined by the methods of Bourne, Donnison, Haworth, and Peat (*loc. cit.*). The results are expressed as percentages of the values calculated for complete conversions into the respective sugars.

(c) *Light absorption by the polysaccharide–iodine solution.* The polysaccharide was stained with iodine in potassium iodide solution under standard conditions, and the absorption values at λ 4300—6800 A. were determined. The precise technique employed, together with definitions of these values, has already been described by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). From these measurements a characteristic " absorption curve " was obtained as outlined by Bourne, Donnison, Haworth, and Peat (*loc. cit.*).

Defatting of Waxy Maize Starch.—Waxy maize starch was extracted in a Soxhlet apparatus for 24 hours with aqueous methanol containing 10% of water (by volume) and then for 48 hours with aqueous dioxan containing 20% of water (by volume). It was thoroughly washed by trituration with alcohol and then with ether, and dried. The product was free from protein since it failed to give the Millon, biuret, and arginine tests, and did not form cyanide when fused with sodium. It contained no mineral matter and was completely converted into glucose by hydrolysis with aqueous acid (Found : conversion, 101%). A solution of the defatted starch stained blue-purple with 1 drop of very dilute iodine–potassium iodide solution, and red on further addition.

The aqueous methanol extract was evaporated to a syrup which was insoluble in water but soluble in methanol or aqueous alkali. When an alkaline solution of the syrup was mixed with an aqueous solution of potato amylose and then neutralised, the amylose was completely precipitated, probably because of the formation of an insoluble fatty acid–amylose complex.

Fractionation with Thymol.—Defatted waxy maize starch (9·44 g., dry weight) was dispersed in 0·1% sodium chloride solution (1000 c.c.). The paste was boiled and stirred for 20 minutes before being cooled and centrifuged, a process which removed only a trace of insoluble material. After addition of powdered thymol (2·0 g.), the paste was kept at 30° for 3 days, and a small precipitate was collected in the centrifuge. This was washed with saturated thymol solution (400 c.c.), triturated with alcohol and then with ether, and dried. The product (Fraction I), which was free from mineral matter, was isolated in 4·8% yield (Found : conversion into glucose by acid, 109%).

The supernatant liquid and aqueous washings were combined, concentrated under reduced pressure to 250 c.c., and mixed with alcohol (750 c.c.). The resultant precipitate (Fraction II), after dehydration with alcohol and then with ether, was dried to a white powder (yield 91%) (Found : ash, 0·45; conversion into glucose by acid, 101%).

Fractionation with cyclo*Hexanol.*—The above fractionation was repeated with a layer of *cyclo*hexanol in place of thymol, and saturated *cyclo*hexanol solution in the washing process. The precipitated component was termed Fraction III (yield, 3·1%), and the soluble component Fraction IV (yield, 96%) [Found : ash, nil (III) and 1·20% (IV); conversion into glucose by acid, 105% (III) and 101% (IV)].

Fractionation with Aluminium Hydroxide.—(a) *Small scale.* A dispersion of defatted waxy maize starch (2·00 g. dry weight) in water (200 c.c.) was boiled and stirred for 20 minutes and cooled to 15°. After addition of an aqueous solution (20 c.c.) of aluminium nitrate [$Al(NO_3)_3,9H_2O$; 1·18 g.], ammonia solution (*d* 0·88) was added until the solution was permanently alkaline towards phenolphthalein. The precipitated aluminium hydroxide was allowed to " age " at 30° for 18 hours before it was removed in the centrifuge and discarded. The supernatant liquid was neutralised with hydrochloric acid and mixed with alcohol (3 vols.) which precipitated a polysaccharide fraction. This was collected in a centrifuge and redissolved in hot water (100 c.c.). A trace of undissolved material was removed before the solution was dialysed for 3 days against running water. The polysaccharide (Fraction V) was recovered by precipitation with alcohol, triturated with alcohol and then with ether, and dried; yield, 0·7%.

In a similar experiment in which less aluminium nitrate (0·80 g.) was employed, Fraction VI was isolated in 2·5% yield.

(b) *Large scale.* The fractionation was repeated on a larger scale, the quantities of defatted waxy maize starch and aluminium nitrate being 20·5 g. (dry weight) and 8·00 g., respectively. Yield of Fraction VII, 1·2% (Found : ash, 0·99; conversion into glucose by acid, 102%).

Fractionation with Thymol and Mineral Acid.—A dispersion of defatted waxy maize starch (12·4 g., dry weight) in 0·1% sodium chloride solution (1300 c.c.) was boiled and stirred for 20 minutes, cooled, and freed from a trace of insoluble matter in the centrifuge. To the supernatant liquid, molten thymol (2·6 g.) was added before it was kept at 30° for 48 hours. Sufficient concentrated sulphuric acid to give a N-solution was introduced, and the mixture was kept at 30° for another 72 hours. A precipitated fraction was removed in the centrifuge, washed with saturated thymol solution (50 c.c.) and thrice with aqueous alcohol (50 c.c.) containing 30% of water, triturated with alcohol and then with ether and dried (Fraction VIII); yield, 0·5% (Found : conversion into glucose by acid, 96%).

In a duplicate experiment, Fraction IX was isolated in 0·4% yield.

Fractionation with cyclo*Hexanol and Mineral Acid.*—A dispersion of defatted waxy maize starch (14·0 g. dry weight) in 0·1% sodium chloride solution (1400 c.c.) was boiled and stirred for 20 minutes, cooled, and freed from a trace of insoluble matter in the centrifuge. To the supernatant liquid, *cyclo*hexanol (80 c.c.) was added, and, after being stirred for 20 minutes, the mixture was kept at 30° for 24 hours. A further quantity (50 c.c.) of *cyclo*hexanol and sufficient sulphuric acid to give a N-solution were introduced, with stirring, and the flask was placed in a cold water-bath, which was heated until a flocculent precipitate settled. At this point, the temperature of the water-bath was 95°. When cold, the precipitate was collected in the centrifuge, washed with saturated *cyclo*hexanol solution (30 c.c.), dissolved in water (20 c.c.), and precipitated with alcohol (80 c.c.). After it had been precipitated a second time with alcohol from aqueous solution, the polysaccharide product (Fraction X) was triturated with alcohol and then with ether and dried; yield 1·9% (Found : ash, nil; conversion into glucose by acid, 100%).

In two similar experiments Fractions XI and XII were obtained in 0·6% and 0·3% yields, respectively [Found : ash, 0·71% (XI) and 0·56% (XII)].

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