234. *The Enzymic Synthesis and Degradation of Starch. Part II. The Amylolytic Function of the Q-Enzyme of the Potato.*

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The Q-enzyme isolated from potato juice, described in Part I, is shown to possess an amylolytic function in addition to its capacity to catalyse the synthesis of amylopectin. Evidence is presented which indicates that the mode of action of Q-enzyme on amylose distinguishes it sharply from amylases of the known α- and β-types. The Q-enzyme has not yet been obtained entirely free from amylases of the ordinary type, but it has been obtained sufficiently pure to justify the view that the enzyme attacks amylose in such a way that the latter is converted into a red-staining polysaccharide without the concomitant liberation of reducing groups.

The hypothesis is advanced that the action of Q-enzyme on amylose is twofold, namely, a cleavage of the amylose chain into the short unbranched chains of pseudo-amylose and the lateral combination of the latter to form amylopectin:

Amylose ⟶ Pseudoamylose ⟶ Amylopectin.

In Part I (preceding paper) is described the isolation from potato juice of an enzyme—Q-enzyme—which catalyses the synthesis of amylopectin. In this communication an account is given of the hydrolytic function of Q-enzyme.

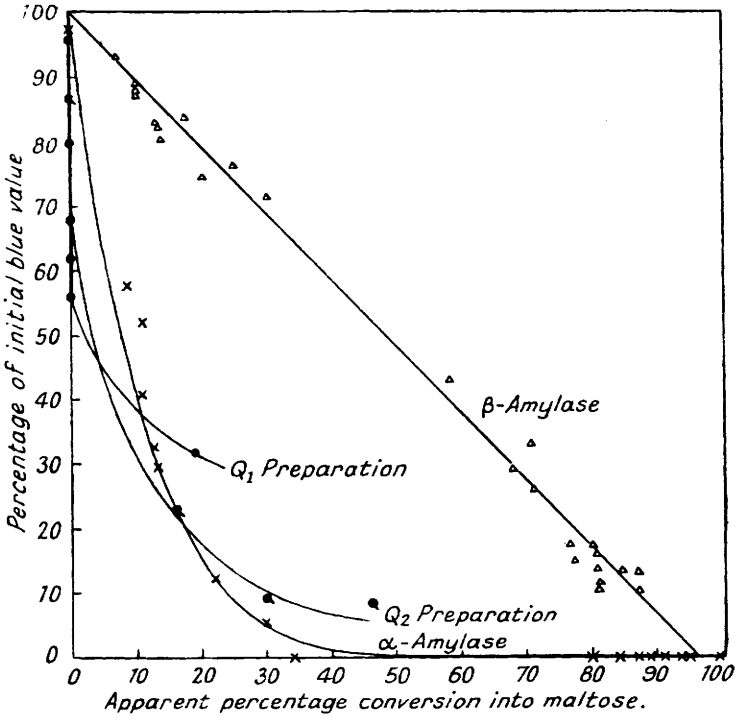
It is generally assumed that starch-hydrolysing enzymes belong to one or other of two classes and are described as either α-amylases or β-amylases. A third class of amylases may exist, namely, the liquefying enzymes, so called because of their power of diminishing the viscosity of a starch solution. This liquefying property is associated always with preparations of the amylases proper and it is still a matter of controversy as to whether the liquefying effect is due to a separate enzyme or is merely a manifestation of the initial stages

of hydrolysis of starch by α- or β-amylases (see the review by Hanes, *New Phytol.*, 1937, **36**, 101). The only case reported so far of the isolation of a liquefying enzyme is that of the amylophosphatase of Waldschmidt-Leitz and Mayer (*Z. physiol. Chem.*, 1935, **236**, 168). It was alleged that amylophosphatase brought about the diminution of the viscosity of a starch paste without much accompanying hydrolysis to maltose (β-amylase action) or to dextrins which did not stain with iodine (α-amylase action). Unfortunately, these authors disregarded the profound depression of viscosity of potato starch solutions that is produced by ions and it is not possible to say whether the observed viscosity fall was due to enzymic action or to the ions of the buffered amylophosphatase solution.

In a search for an enzyme whose specific function was the liquefaction of starch paste, we had occasion to use potato juice as a possible source and found that superimposed upon the instantaneous ionic effect there was a true enzymic effect on the viscosity of a starch solution. This liquefying action was observed with juice that had been dialysed to remove ions but was not observed with juice that had been boiled or precipitated with alcohol. A preparation of purified phosphorylase (P-enzyme) was without this liquefying action on starch.

FIG. 1.

The hydrolysis of amylose by amylases and Q-enzyme.



The liquefying enzyme is found in the Q-fraction of juice precipitated with ammonium sulphate and there are therefore three possibilities to be envisaged. (1) The liquefaction of starch paste and the synthesis of amylopectin are but different functions of the same enzyme, Q-enzyme; (2) the liquefaction represents but the initial stages of amylolysis brought about by amylases of either the α- or the β-type or both; (3) potato juice contains a specific liquefying enzyme which is not identical with either Q-enzyme or amylase. The important issue to be decided, however, is whether the liquefying effect represents another aspect of the activity of Q-enzyme or is due to amylases of the ordinary types present in potato juice.

ing the time of digestion as a factor, because it eliminates variations due to differences of effective concentration of the enzymes and more clearly demonstrates differences of type, rather than of rate, of reaction.

Two preparations of Q-enzyme were used in these tests: Q1-preparation from comparatively young potatoes, Q2-preparation from potatoes at a later growth stage when sprouting had begun. Reference to Fig. 1 shows that the Q2-preparation is much nearer in type to α-amylase than is Q1, although both are very different from β-amylase.

The shape of the β-amylase curve supports the accepted view of the course of action of this enzyme as being the progressive shortening of the amylose chain by the splitting off of maltose units until the whole of the amylose is converted into this sugar. In consequence, reducing groups appear in the earliest stages of the reaction and as the reducing power of the digest augments, the blue value diminishes proportionately. The action of α-amylase is different in two particulars: the blue-staining amylose diminishes and ultimately disappears but there is not a proportionate increase in reducing power; instead, when the achroic point is reached, the copper number corresponds to an apparent conversion to maltose of less than 40%. Nevertheless, the liberation of reducing groups by α-amylase does not cease at the attainment of the achroic point but rather continues until higher reducing values are obtained. In the case illustrated, for instance, in Fig. 1 reducing groups are set free corresponding to an apparent 99% conversion of amylose to maltose.* The current explanation of this phenomenon is that the action of α-amylase involves the fragmentation of the amylose primarily into dextrins which are feebly reducing and do not stain with iodine. During the earlier stages of α-amylase action on amylose there is much evidence that no maltose is liberated but at later stages the main reaction is the saccharification of the achroic dextrins. With the Q-preparations an achroic point, such as is observed with both α- and β-amylase, never appears to be reached. The eye-observed blue stain of amylose changes to a red-purple and thereafter to a red colour which, with some Q-preparations, persists even after 9 days' incubation. Although in the initial stages of α-amylase action the liberation of reducing groups is slight it is nevertheless quite definite and detectable. With the Q1-preparation (from "resting" potatoes), however, the major part of the degradation of the amylose, as represented by the diminution of blue value, occurs without the appearance of any reducing groups at all. The Q2-preparation (from sprouting potatoes) does not show this effect to such an extent. The curves for Q2 and α-amylase almost coincide at first, although they ultimately diverge, the amylase digest becoming colourless while the Q2 digest still shows a small "blue value."

It is almost axiomatic that a starch-bearing plant will develop amylases at some stage in its life cycle and there is no reason to except the potato from this rule. It is to be expected, therefore, that α- or β-amylase will be found in potato juice and we are inclined to the view that the differences between the two preparations, Q1 and Q2, may be ascribed to the possibility that Q2 contains a higher proportion of α-amylase (and perhaps β-amylase) than does Q1. The major amylolytic effect of Q-preparations is due, however, to an enzyme which is neither an α- nor a β-amylase. Until evidence to the contrary is found, it will be convenient to ascribe this amylolytic function and also the amylopectin-synthesising function to the same Q-enzyme.

Further evidence that the amylolytic activity of Q-preparations is mainly due to an enzyme other than α- or β-amylase is supplied by a comparison of the effect on the three enzymes of (i) heat treatment and (ii) precipitation with alcohol. It is seen from Fig. 2 that if Q-enzyme is warmed to 55° for 15 mins. or if it is pre-treated by alcohol precipitation, its action on amylose is almost completely eliminated. It is otherwise with the amylases. Similar heat treatment of soya-bean amylase does not affect its activity and only slightly diminishes the activity of salivary amylase. Alcohol precipitation has no inhibitory action on the α-enzyme although it has a pronounced action on β-amylase.

It would therefore seem to be justifiable to regard Q-preparations of potato juice as containing an enzyme which exerts an amylolytic function (at least, in so far as the amylose component of starch is concerned). This amylolytic action is characterised by the conversion of blue-staining amylose into a red-staining polysaccharide without the concomitant liberation of reducing groups. This view assumes that the small reducing power which eventually develops with Q1- and Q2-preparations (Fig. 1) is due to a residuum of amylase not removed by the preliminary treatment with kaolin to which the potato juice is submitted.

If neither sugars nor reducing dextrins are produced by the action of pure preparations of Q-enzyme on amylose and if a red-staining polysaccharide is the only product, the phenomenon finds a reasonable interpretation in the assumption that the apparent amylolytic action of this enzyme is the conversion of the long, unbranched chain of amylose into the branched structure of amylopectin. This view received strong support when the properties of the polysaccharide formed by the action of the purest preparations of Q-enzyme on potato starch were examined. This polysaccharide, RS1, was soluble in water, stained reddish-purple with iodine and was non-reducing to Fehling's solution. It was free from ash and was completely converted to glucose by acid hydrolysis. It was significant that the polysaccharide was not resistant to the action of β-amylase; on the contrary, it was converted into maltose to the extent of 42% by this enzyme. This demonstrated that the Q-preparation was substantially free from β-amylase, the effect of which on whole starch would have been to convert the amylopectin component into dextrin-A and maltose. Dextrin-A, unlike RS1, is not attacked by β-amylase (Haworth, Kitchen, and Peat, J., 1943, 619). Furthermore, had the Q-preparation contained α-amylase or had its action been of the α-amylase type then, during its prolonged action on

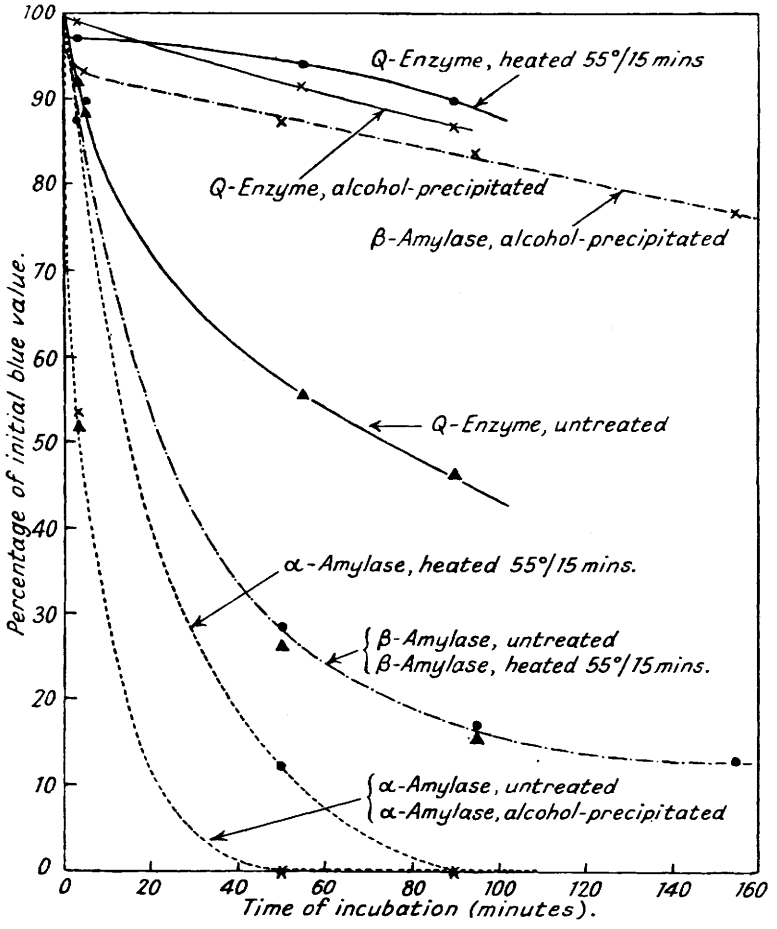
* This figure does not necessarily imply complete saccharification inasmuch as the amylase may liberate glucose as well as maltose in the later stages of digestion.

starch, any dextrins in the products would have been non-staining and in small amount. Actually RS1 was obtained in a yield of 70% of the original starch. The " blue value " of the polysaccharide RS1 measured in a Spekker absorptiometer was 0·117, corresponding to the value given by a natural amylopectin containing 3% of amylose.

It has not yet been proved that the polysaccharide RS1 is amylopectin; such proof will have to wait upon end-group assay and molecular weight determination. Nevertheless, all the evidence detailed above points to the conclusion that Q-enzyme has in fact converted the amylose component of whole starch into amylopectin and that it has not effected the hydrolysis of the amylopectin component. Expressed in other words, Q-enzyme has effected the conversion of potato starch into a red-staining starch of the waxy maize type.

FIG. 2.

The effect of heat and alcohol treatment on Q-enzyme and amylases.



The last-named starch is reported to contain no amylose component (see, for example, Schoch, *J. Amer Chem. Soc.*, 1942, **64**, 2957; Bates, French, and Rundle, *ibid.*, 1943, **65**, 142).

EXPERIMENTAL.

Liquefaction of Potato Starch Solutions.—Preparation of starch sols. The starch (5 g.) suspended in cold water (50 c.c.) was vigorously stirred into boiling water (400 c.c.), the solution made up to 500 c.c. and stirred at 90° for one hour. It was then cooled to room temperature before use.

The effect of ions. To a 1% potato starch solution (10 c.c.) was added water (1 c.c.) and the relative viscosity of the solution was measured at 38° in an Ostwald viscometer; found: η_r 12·2, constant for 1 hour. To a second equal volume of the same starch solution was added 4·6M NaCl (1 c.c.); found: η_r 2·5, constant for at least 20 mins. The sodium chloride instantaneously reduced the η_r of 1% potato starch from 12·2 to 2·5.

The effect of dialysed potato juice. The potato juice, expressed under the conditions described in Part I, was dialysed for 48 hours against running tap water (cellophane membrane). The protein precipitate which separated was removed on the centrifuge. The supernatant liquid was divided into two parts: (A) control; (B) the juice was boiled for 10 minutes and filtered. Viscosity measurements made at 38°.

1% potato starch (10 c.c.) solution + 1 c.c. water: η_r 26·0 (constant)
1% ,, ,, ,, ,, + 1 c.c. boiled juice: η_r 6·8 (constant)

When unboiled juice was used, the viscosity of the starch solution was not constant (see Table I).

TABLE I.

Unboiled Juice (1 *c.c.*) + 1% *Potato Starch* (10 *c.c.*)

Viscosity measured at 38°.

Time after mixing (mins.).	2.	6.	12.	32.	52.	66.	120.	168.	198.
η_r	6·65	6·06	5·78	5·35	4·78	4·50	3·71	3·29	3·14

Dialysis had not completely removed the ions from the juice since instantaneous liquefaction occurred with both the boiled and the untreated juice. With the latter there occurred a slow and progressive diminution of η_r which was not produced by the boiled juice. Clearly potato juice contains an enzyme which catalyses the liquefaction of starch solutions.

The action of ammonium sulphate precipitated juice. P- and Q-Fractions of potato were prepared as described in Part I. The liquefying effect was determined with the results shown in Table II.

P-Enzyme solution (1 c.c.) + potato starch solution (10 c.c.); η_r 3·58, constant for one hour.

TABLE II.

Q-Enzyme Solution (1 *c.c.*) + 1% *Potato Starch* (10 *c.c.*).

Viscosity measured at 38°.

Time after mixing (mins.).	5.	15.	30.	40.	65.
η_r	2·03	1·60	1·43	1·37	1·26

Alcohol precipitated Q-enzyme. The Q-enzyme solution was precipitated by the addition of 3 vol. of ethanol. The precipitate was washed and redissolved in water. This enzyme solution (1 c.c.) + 1% potato starch (10 c.c.) were incubated at 38°; η_r was constant at 3·1 for 1 hour.

Heat-treated juice. Potato juice was maintained at 65° for 15 mins. The precipitate which separated was removed by being centrifuged. The supernatant liquid had no liquefying property: 1 c.c. of supernate + 1% potato starch (10 c.c.) at 38° showed η_r 3·1, constant for 1 hour.

Conclusions. The liquefying enzyme is found in the Q-fraction but not in the P-fraction of potato juice. It is destroyed by contact with 75% alcohol and by being heated to 65° for 15 mins.

The Enzymic Hydrolysis of Amylose.—Q-enzyme was prepared from potato juice by the fractionation procedure with ammonium sulphate, described in Part I. The Q1-preparation (Part I, p. 880) was from new King Edward potatoes, Q2-preparation from sprouting potatoes.

β-Amylase from soya bean. Claims had been made that a β-amylase free from α-amylase was obtainable from soya bean, but no details of the extraction were available. The following method was found to give a satisfactory product. Soya bean flour (75 g.) was shaken for 18 hours with 20% aqueous alcohol (380 c.c.). The residue was removed by the centrifuge and the alcohol concentration of the extract was increased to 65%. β-Amylase was thus precipitated. It was separated by the centrifuge, washed with alcohol and ether and dried under reduced pressure over P_2O_5; weight 2·7 g. Maltase was not present in this preparation, for when it was incubated with maltose at pH 4·8, there was no increase in reducing power in 24 hours. The β-amylase from ungerminated wheat has been found almost invariably to contain maltase.

α-Amylase from saliva. Saliva (5 c.c.) was diluted with water (5 c.c.) and the precipitate of mucin removed by the centrifuge. The supernatant liquid was usually diluted 100 times before use. Maltase was shown to be absent by incubation with maltose, no increase in reducing power occurring.

"Thymol" Amylose.—The amylose used as substrate in these experiments was separated from whole potato starch by precipitation with thymol. Details of this method will be published elsewhere. The "blue values" (B.V.) of the specimens of amylose used, when measured with a Spekker absorptiometer by the method of McCready and Hassid (*J. Amer. Chem. Soc.*, 1943, **65**, 1154), were 0·95, 1·10, and 1·14. The "percentage conversion to maltose" was measured by the copper-reduction micro-method of Shaffer and Hartmann (*J. Biol. Chem.*, 1921, **45**, 377).

Method of Determining the Blue Value/Copper Number Relation.—(a) *For Q-enzyme.* A fresh sample of Q-enzyme was prepared each day. The enzyme from potato juice (300 c.c.) was dissolved in water (180 c.c.) and was used as such in the following digests:

Digest A. Amylose (100 mg.) dissolved in 5N NaOH (5 c.c.) + water (15 c.c.). The solution was exactly neutralised before use with 5N HCl. To this neutral solution were added citrate buffer (pH 6·0) (20 c.c.) and Q-enzyme solution (30 c.c.) and the solution was made up to 80 c.c. with water. A layer of toluene maintained aseptic conditions.

Digest B. As (A) except that amylose was not present.

Digest C. As (A), but using a boiled and filtered Q-preparation.

The digests were incubated at 35·5°. At intervals an aliquot portion (2—5 c.c.) of each digest was removed and the reducing power estimated by the Shaffer–Hartmann method. The difference in reducing power between digests A and B represented reducing groups liberated by the action of the enzyme on amylose. Any difference between digests (B) and (C) would represent reducing groups liberated by the boiled Q-preparation. The increase in reducing power during digestion is recorded as percentage apparent conversion to maltose. At the same time, 4 c.c. (≡ 5 mg. of initial amylose) of each digest were taken and the blue value estimated under the standard conditions of McCready and Hassid (*loc. cit.*). The blue value observed was recorded as a percentage of the initial blue value of the amylose used.

TABLE III.

Action of Q1*-preparation on amylose* (*B.V.* 1·10).

	With Q-enzyme.			With boiled Q-enzyme.		
Time.	Apparent % conversion to maltose.	Iodine stain.	Spekker reading.	Apparent % conversion to maltose.	Iodine stain.	Spekker reading.
2 mins.	0	BP	1·06	0	B	1·10
40 ,,	0	P	0·88	0	B	1·11
80 ,,	0	P	0·75	0	B	1·10
120 ,,	0	P	0·68	0	B	1·10
160 ,,	0	RP	0·61	0	B	1·10
22 hrs.	19	—	0·35	0	B	1·06

B = blue; R = red; P = purple.

A typical result for a Q-preparation (Q1) from non-sprouting King Edward potatoes is given in Table III and for a preparation (Q2) from sprouting potatoes in Table IV.

TABLE IV.

Action of Q2-preparation on amylose (B.V. 1·10).

	On amylose.		
Time.	Apparent % conversion to maltose.	Iodine stain.	Spekker reading.
3 mins.	0	B	0·950
40 ,,	16	RP	0·252
80 ,,	30	C	0·103
135 ,,	46	C	0·086
21 hrs.	55	C	—

C = no stain.

(b) *For β-amylase.* Typical results for soya bean β-amylase are given in Table V (at pH 4·8) and Table IX (at pH 6·0). Estimations of reducing power and blue value were made as for Q-enzyme.

TABLE V.

Amylose, B.V. 1·10.

Digest A. Amylose (100 mg.) dissolved in alkali and neutralised, acetate buffer (pH 4·8) (12 c.c.), 0·04% β-amylase solution (20 c.c.), water to 100 c.c., toluene preservative.
Digest B. Amylose (60 mg.), acetate buffer (pH 4·8) (12 c.c.), 0·01% β-amylase (20 c.c.), water to 100 c.c., toluene.

	Digest A.			Digest B.		
Time (mins.).	% conversion to maltose.	Iodine stain.	Spekker reading.	% conversion to maltose.	Iodine stain.	Spekker reading.
2	9	B	1·00	—	B	1·03
35	70	B	0·360	30	B	0·79
65	77	B(F)	0·190	53	B	0·64
125	87	C	0·110	58	B	0·47

(F) = faint.

(c) *For α-amylase.* Typical results for salivary amylase, diluted as described above, are shown in Table VI. Estimations of reducing power and blue value were made as for Q-enzyme.

TABLE VI.

Amylose, B.V. 0·95.

Digest A. Amylose (100 mg.) dissolved in alkali and neutralised, acetate buffer (pH 4·8) (12 c.c.), α-amylase (20 c.c.), water to 100 c.c., toluene.
Digest B. Amylose (100 mg.), citrate buffer (pH 6·0) (20 c.c.), α-amylase (30 c.c.), water to 80 c.c., toluene.
Estimations made on volumes equivalent to 5 mg. of amylose in each case.

	Digest A.			Digest B.		
Time.	% conversion to maltose.	Iodine stain.	Spekker reading.	% conversion to maltose.	Iodine stain.	Spekker reading.
3 mins.	—	B	0·93	14·4	P	0·51
30 ,,	8·5	RP	0·55	—	—	—
60 ,,	10·1	RP	0·385	—	C	0·0
90 ,,	12·2	RP	0·310	—	—	—
105 ,,	—	—	—	87·0	C	0·0
120 ,,	12·9	RP	0·280	—	—	—
24 hrs.	29·7	R	0·051	95·0	C	0·0

The Effect of Heat and Alcohol-precipitation on the Enzymes.—(a) *On Q-enzyme.* The Q-preparation from potato juice (300 c.c.) was dissolved in water (180 c.c.). The enzyme solution was divided into three equal parts, the first of which was not treated; the second was heated at 55° for 15 mins., rapidly cooled and filtered. To the third was added ethanol (3 vols.) with rapid stirring at 0°. The mixture was kept at 0° for 10 mins., after which it was centrifuged and the precipitate redissolved in water (1 vol.). The results are given in Table VII.

TABLE VII.

Amylose (B.V. 0·95). Q-Enzyme.

Digest A. Amylose (100 mg.) dissolved as before, citrate buffer (pH 6·0) (20 c.c.), Q-enzyme solution untreated (30 c.c.), water to 80 c.c., toluene.
Digest B. As digest (A), using heat-treated Q-enzyme.
Digest C. As digest (A), using alcohol-precipitated Q-enzyme.

	Untreated enzyme.		Heated enzyme (55° for 15 mins.).		Alcohol-precipitated enzyme.	
Time.	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading
3 mins.	BP	0·870	B	0·920	B	0·940
55 ,,	RP	0·530	B	0·890	B	0·870
90 ,,	RP	0·440	B	0·850	B	0·820
20 hrs.	C	0·118	P	0·460	P	0·370

(b) *On α-amylase of saliva.* The salivary amylase, prepared as described, was shown to be free from maltase.

TABLE VIII.

Amylose (B.V. 0·95). α-Amylase.

Digests A, B, and C, made up as for Q-enzyme (Table VII).

	Untreated enzyme.		Heated enzyme (55° for 15 mins.).		Alcohol-precipitated enzyme.	
Time.	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading.
3 mins.	RP	0·490	B	0·830	RP	0·510
50 ,,	C	0·000	R	0·116	C	0·000
90 ,,	C	0·000	R	0·000	C	0·000
20 hrs.	C	0·000	C	0·000	C	0·000

(c) *On β-amylase of soya bean.* A 0·015% solution of the enzyme was used in the following digests, at pH 6·0.

TABLE IX.

Amylose (B.V. 1·14). β-Amylase.

Digests A, B, and C, made up as for Q-enzyme.

	Untreated enzyme.		Heated enzyme (55° for 15 mins.).		Alcohol-precipitated enzyme.	
Time (mins.).	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading.	Iodine stain.	Spekker reading.
5	B	1·00	B	1·02	B	1·06
50	B(F)	0·296	B(F)	0·327	B	0·99
95	B(F)	0·180	B(F)	0·195	B	0·95
155	B(F)	0·148	B(F)	0·148	B	0·87

The effect of heat and alcohol treatment on Q-enzyme, α-amylase, and β-amylase is illustrated in Fig. 2.

The Action of Q-Enzyme on Whole Starch.—Isolation of a red-staining polysaccharide (RS1). Dry potato starch (41·7 g.) was dissolved in water (2 l.) in the usual way. The solution, covered with a layer of toluene, was cooled to room temperature and Q-enzyme solutions from young potatoes were added daily until the digest stained reddish-purple with iodine. At this stage only very slight reducing power had developed.

The digest was boiled, centrifuged, and concentrated at 50°/15 mm. to half volume. The polysaccharide was then precipitated by the addition of methylated spirits (3 l.). It was separated by centrifuging, redissolved in water (1200 c.c.) and dialysed against running tap water for 3 days to remove inorganic material. A further small quantity of protein separated when the solution was boiled. The polysaccharide was reprecipitated by the addition of methylated spirits (4 l.), washed with acetone and dried under reduced pressure at 40°; yield: 68% of the starch.

Acid hydrolysis of polysaccharide RS1. The dried polysaccharide (20—30 mg.) was heated under reflux with 7% sulphuric acid (25 c.c.) for 12 hours. The cooled solution was neutralised with 5N NaOH and diluted to 100 c.c. The glucose in this solution was estimated by removal of 5 c.c. portions for analysis by the Shaffer–Hartmann method; found: conversion to glucose, 101%.

Hydrolysis of polysaccharide RS1 by β-amylase:

Digest. Polysaccharide (30 mg.), water (34 c.c.), acetate buffer (pH 4·8) (6 c.c.), 0·2% β-amylase (10 c.c.), toluene. Each digest at 35·5° was carried out in duplicate. Results are given in Table X.

TABLE X.

		Time of digestion.						
Polysaccharide.		5 mins.	30 mins.	1½ hrs.	2½ hrs.	3½ hrs.	4½ hrs.	5½ hrs.
RS1	1	18·4	46·5	52·2	—	57·0	57·7	58·7
	2	14·5	38·8	48·2	51·5	53·3	52·6	55·2
"Butanol"-amylopectin	1	—	26·0	41·5	47·6	—	49·4	50·4
	2	—	24·1	43·2	47·6	—	—	51·7
		2 hrs.	4 hrs.	6 hrs.	8 hrs.	11 hrs.	22½ hrs.	28 hrs.
"Butanol"-amylose	1	35·6	52·7	63·5	73·5	79·5	90·5	99·6
	2	39·2	—	63·4	72·7	79·7	91·0	98·0

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