II.—AMYLASE FROM RAGI (*ELEUSINE CORACANA*).

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The abnormal results obtained as a result of prolonged dialysis of the amylase from *Zea mais* (Patwardhan, *J. Indian Inst. Sci.*, 1929, **12A**, 185) interested the author to extend the investigation to similar enzymes from other seeds. The present investigation deals with the enzyme from ragi.

That the activity of an amylase is diminished after dialysis and that the loss is more pronounced in case of an animal amylase than in one of vegetable origin is well known (Guyenot, *Compt. rend. Soc. Biol.*, 1907, **63**, 768; Brunacci, *Chem. Absts.*, 1910, **4**, 2324; Lisbonne, *Compt. rend. Soc. Biol.*, 1911, **70**, 62; Biery, *Compt. rend. Soc. Biol.*, 1922, **87**, 1111). The activity thus lost by an animal amylase can, however, be restored by the addition of neutral salts and certain amino-acids (Sherman and Walker, *J. Amer. Chem. Soc.*, 1923, **45**, 1960; Haehn, *Biochem. Zeit.*, 1923, **143**, 516). The behaviour of amylases from vegetable sources is not however, so well established. Contradictory evidence is forthcoming regarding loss of activity by barley malt amylase on dialysis, and the possible reactivation of vegetable amylases in general is still an open question (Eadie, *Biochem. J.*, 1926, **20**, 1016). Like the maize amylase, the enzyme from ragi lost its activity on prolonged dialysis and was not reactivated on addition of neutral salts, amino-acids or buffer solutions. The cause of such behaviour might have been the same in both cases, but it was felt that an intensive study of the subject was necessary before any conclusions could be drawn.

The ragi enzyme had greater saccharifying power than cholam malt and maize malt enzymes, but less than that of barley malt amylase. The optimum temperature lay between 55° and 60°. It was less liable to destruction at higher temperatures, which were observed to cause rapid inactivation of the cholam enzyme. The optimum reaction was between P_H 4·86 and 5·07. There was no evidence to show that a thermostable amylase complement occurred with the enzyme.

EXPERIMENTAL.

Malting and preparation of the enzyme.—The methods were identical with those described in the earlier communications (Patwardhan, *loc. cit.*)

Hydrolysis of potato starch by malted ragi amylase.—(a) A flask containing 40 c.c. of 2 per cent. starch paste, 30 c.c. water and 2 c.c.

toluene was brought to 30° and 10 c. c. of enzyme solution (0·502 per cent.) added. Sugar was determined at intervals.

TABLE I A.

Time in mins.	Mgms. maltose in 10 c.c.	Colour with iodine
0	0	Deep blue
15	4·8	Deep purple
30	13·4	Purple
60	26·8	Brown
100	38·9	Brown
1430	84·7	Nil

The reaction reached the equilibrium point in 24 hours, when about 80 per cent. of the starch had been saccharified to maltose. Comparative figures for maltose production in 100 minutes from the start of the hydrolysis as observed for different enzymes are given below.

	Amylases			
	Barley	Ragi	Maize	Cholam
Mgms. maltose ...	76·8	38·9	29·0	18·4

(*b*) To 8 c.c. of starch paste (2 per cent.) and 6 c.c. of water at 30° 2 c.c. enzyme solution (0·502 per cent.) was added in a viscosimeter and the readings taken at intervals.

TABLE I B.

Change of Viscosity in terms of time of flow (t) *in secs.*

Time of reading in minutes	1	15	30	45	60	102
t	211·0	125·5	120·5	114·0	114·0	110·0

Effect of change of temperature.

Eight flasks containing 15 c.c. each of starch paste (2 per cent.) were brought to the desired temperature and 15 c.c. of 2 per cent. ragi malt extract added. Readings were taken after hydrolysis for 30 minutes in each case.

TABLE II.

Temperature Centigrade	Mgms. maltose in 10 c.c.	Mgms. ppt. (dry) in 10 c.c.
30	18·9	85·6
40	19·2	83·5
45	22·7	67·9
50	24·2	...
55	35·8	59·3
60	38·2	52·3
65	27·9	77·4
70	25·6	81·5

Effect of Change of hydrogen-ion concentration.

A series of flasks containing 15 c.c. of starch paste and 10 c.c. of McIlvaine's buffer solution were brought to 30° and 5 c.c. ragi malt extract (6 per cent.) added to each. Readings were taken after hydrolysis for 30 minutes.

TABLE III.

P_H	Mgms. maltose in 10 c.c.	Mgms. dry ppt. in 10 c.c.
7·0	19·5	175·3
6·6	27·4	190·5
6·15	28·5	175·0
5·96	28·5	173·0
5·8	29·4	171·4
5·6	29·4	163·8
5·26	31·7	164·8
5·07	32·3	159·4
4·86	32·3	155·2
4·67	29·9	163·0
4·50	29·0	160·5
4·34	29·0	164·4

Effect of dialysis on ragi malt enzyme.

Tubes prepared from 6 per cent. collodion were used for dialysis. All dialyses were carried out in a cold chamber between 0° and 3° with toluenated water in presence of toluene.

(*a*) 0·502 per cent. enzyme when dialysed lost its activity as shown below:—

No. of days ...	0	2	5	9	9
c.c. $KMnO_4$ for 10 c.c.	4·70	3·50	3·05	1·05	1·10 (Salt added to the last one)

(*b*) Ragi malt extract (22·5 per cent.) lost its activity practically completely in sixteen days. Extract in a glass tube kept in the same dialyser as control had lost only a little of its activity even after 28 days.

No. of days	0	3	16	17	28
c.c. $KMnO_4$ required for 10 c.c. (corrected) ...	9·30	5·30	0·05	0·05	...
Control—$KMnO_4$ required for 10 c.c. (corrected)	9·30	...	...	...	8·00

The activity could not be regenerated by addition of salt, buffer solutions of known P_H or asparagine. The details of the trials were the same as those described in the paper on maize amylase.

(*c*) Experiment to test the regenerating action of boiled enzyme.

Three flasks, A, B and C each containing 12·5 c.c. of dialysed enzyme and 12·5 c.c. of 2 per cent. starch paste were treated as follows:—To A, 7·5 c.c. of boiled, undialysed enzyme was added, to B, 7·5 c.c. of undialysed, unboiled enzyme, and to C, 7.5 c.c. of distilled water. The volumes of permanganate required after 1 hour at 30° were, A, 0·05; B, 4·85; and C, 0·40 c.c. respectively. The results show clearly that the boiled and dialysed enzymes were inactive, singly and together.

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