STUDIES IN ENZYME ACTION. PART III. AMYLASE FROM CUMBU (*Pennisetum typhoideum*).

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Effront (*Compt. rend.*, 1922, **174**, 18) has shown that the ratio $\frac{\text{saccharification power}}{\text{liquefaction power}}$ can vary from 0·1 to 2,400 depending on the source from which the diastase has been prepared. The experiments of Windisch, Dietrich and Beyer (*Woch. Brau.*, 1923, Nos. 40, 49, 55, 61, 67) also indicate that the ratio is not constant for all malts, thus suggesting that diastase consists of two components and that these occur in different proportions in the various grains. The experiments of Narayanamurti and Norris (*J. Ind. Inst. Sc.*, 1928, **11 A**, 134) produced strong evidence in favour of the two enzyme theory. A detailed investigation of diastase from different sources has therefore been undertaken, and this part is concerned with the optimum P_H of the enzyme prepared from malted cumbu, under varying conditions of time, temperature and age.

EXPERIMENTAL.

The enzyme was extracted from about 200 gms. of malt with 600 c.c. of distilled water in presence of toluene, dialysing the filtered extract in collodion bags in flowing distilled water for a week. Hydrolysis took place in an electrically controlled thermostat and the sugar was estimated according to the iodometric method of Schaffer and Hartman (*J. Biol. Chem.*, 1920–1921, **45**, 365).

Optimum P_H and period of hydrolysis.—Four periods 15, 30, 60 and 140 minutes have been studied. The results are given in Table I and Figure I.

TABLE I.

The reaction mixture contained 5 c.c. starch solution (0·5 per cent.), 5 c.c. enzyme solution, 10 c.c. Walpole's acetate buffer and a few crystals of thymol as antiseptic. The temperature was 37°.

P_H	Maltose in c.c. thiosulphate			
	15 mins.	30 mins.	60 mins.	140 mins.
6·5	1·8	2·9	6·7	11·0
6·2	2·4	5·0	**8·9**	**12·6**
6·0	2·7	5·5	8·7	12·5
5·9	2·9	...	8·5	11·9
5·6	3·3	6·0	9·6	13·2
5·4	3·5	6·4	9·9	13·2
5·2	3·6	6·4	...	13·5
5·1	**3·9**	6·5	...	13·8
5·0	...	6·6	10·2	13·8
4·8	3·7	**6·7**	...	**14·2**
4·6	3·6	6·2	...	14·1
4·5	3·5	6·1	10·0	13·6
4·3	3·4	6·0	9·4	...
4·0	...	5·0	8·7	12·5
3·7	1·1	3·8	6·9	10·9
3·3	0·7	1·2	4·2	6·0

It is evident that the optimum P_H for 15 minutes hydrolysis lies at about 5·1 and is slightly diverted to the acid side for longer periods. The occurrence of maltase in malt has recently been confirmed by Leibowitz (*Z. Physiol. Chem.*, 1925, **149**, 184; 1926, **154**, 64) who showed that maltase acts best at P_H 4·0–4·6. This suggested that the slight deviation observed is due to the occurrence of maltase in cumbu malt, and on testing the enzyme solution with maltose the presence of maltase was confirmed; this was found to act best at a P_H of 4·6 (see Table II).

TABLE II.

The reaction mixture contained 5 c.c. maltose (0·2 per cent.), 5 c.c. enzyme, 10 c.c. buffer and a few crystals of thymol as antiseptic. The temperature was 37°.

P_H	...	6·2	5·9	5·2	5·1	4·8	4·6	4·5	4·3	4·0
Glucose (c.c. thiosulphate)	...	0·2	0·3	1·2	1·5	1·6	1·7	1·3	1·2	0·8

For periods over 30 minutes there are two optima, at about 4·8 and 6·2. A similar observation has been made with regard to emulsion by Brunswik (*Osterr. botan. Z.*, 1923, No. 1–5, 58), and was explained

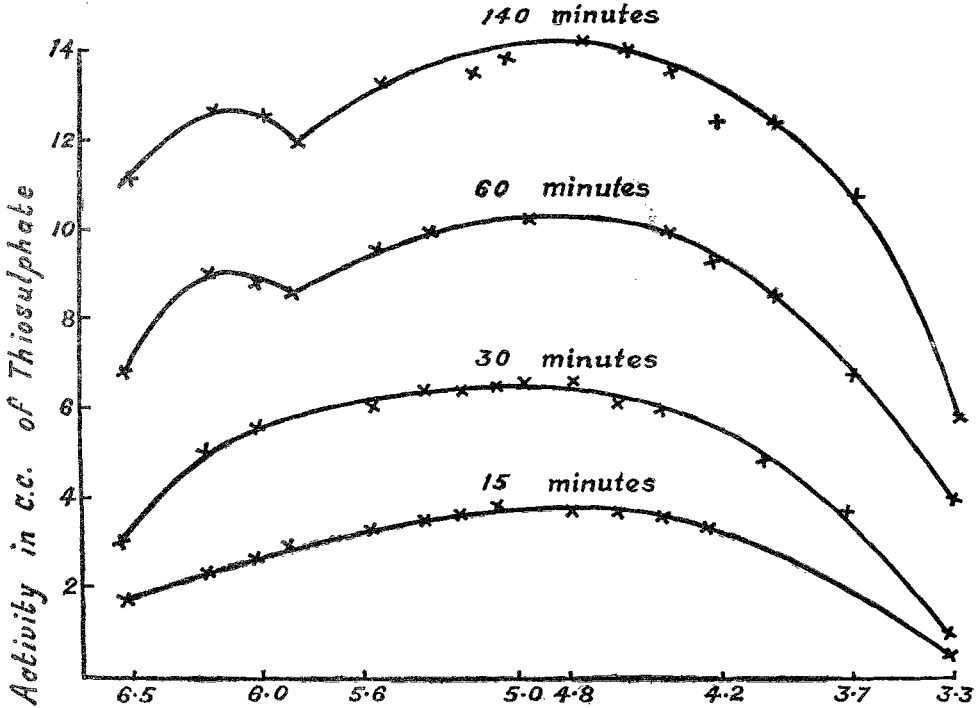

140 minutes
60 minutes
30 minutes
15 minutes
Activity in c.c. of Thiosulphate
14
12
10
8
6
4
2
6·5
6·0
5·6
5·0
4·8
4·2
3·7
3·3
PH

Fig.I

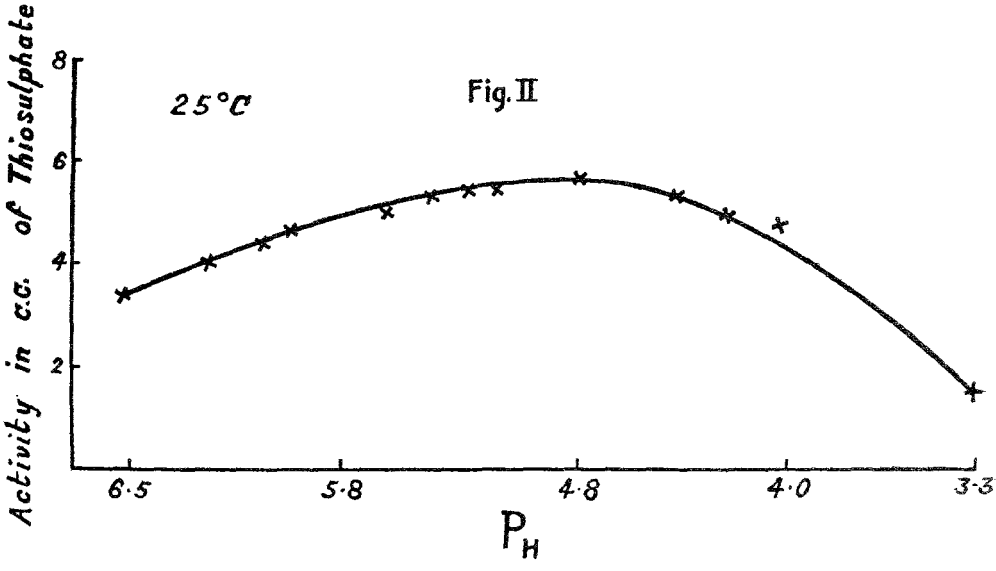

Activity in c.c. of Thiosulphate
8
6
4
2
25°C
Fig. II
6·5
5·8
4·8
4·0
3·3
PH

Fig. III

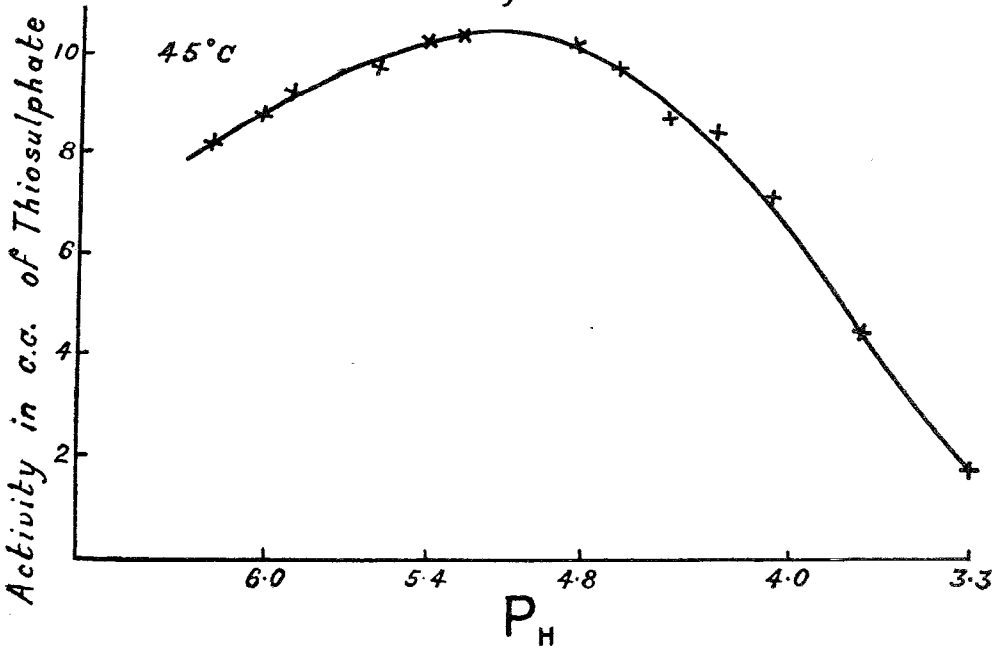
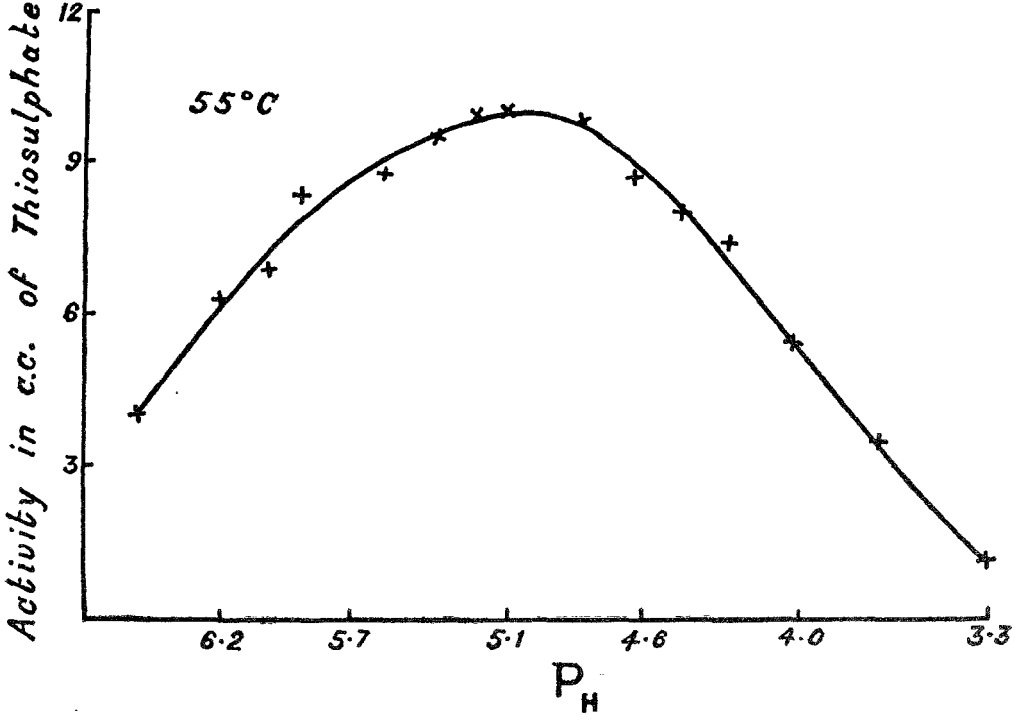


Fig. IV



as due to the presence of amygdalase and prunase in the enzyme preparation. The point is being carefully investigated to see whether in diastase also it is due to two components.

The effect of temperature is recorded in Table III and Figures II, III and IV.

TABLE III.

The reaction mixture contained 5 c. c. starch (0·5 per cent.), 5 c. c. enzyme solution, 10 c. c. Walpole's acetate buffer and a few crystals of thymol as antiseptic. The period of hydrolysis was 30 minutes.

P_H	Maltose in c. c. thiosulphate		
	Temperature		
	25°	45°	55°
6·5	3·4	...	3·9
6·2	4·0	8·2	6·2
6·0	4·0	8·8	6·8
5·9	4·6	9·2	8·3
5·6	5·0	9·7	8·7
5·4	5·4	10·2	9·4
5 2	5·5	10·3	9·9
5·1	5 5	...	9·9
4·8	5·7	10·1	9·7
4·6	...	9·6	9·6
4·5	5·3	8·6	7·9
4·3	5·0	8·4	7·2
4·0	4·8	7·0	5·2
3·7	3·3	4·3	3·3
3·3	1·6	1·7	1·1

With rise in temperature we find a slight deviation to less acid hydrogen-ion concentration. Probably the enzyme is more stable in these hydrogen-ion concentrations. We have to distinguish two effects of temperature on the enzyme, acceleration of action and destruction of

the enzyme. Ernstrom (*Z. Physiol. Chem.*, 1922, **119**, 190; 1923, **141**, 40) and Sjoberg (*Biochem Z.*, 1922, **133**, 298) have shown that with rise in temperature the stability optimum is diverted to less acid hydrogen-ion concentrations, agreeing with the above results. Another point to be mentioned is that the zone of optimum P_H becomes narrowed with rise in temperature. The results for 55° are of special interest, for, according to several investigations the temperature of inactivation lies at about this point.

Effect of age.—Preliminary experiments indicate that the P_H optimum is diverted to less acid solutions. Experiments are in progress to follow the changes taking place with age by measuring the conductivity, viscosity, surface tension and other physical constants of the enzyme solution. We are also studying the effect of the nature of the buffer, salt-content and other factors.

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