AMYLASE FROM WHEAT.

By Dattatreya Vishnu Karmarkar and Vinayak Narayan Patwardhan.

Lintner (*J. pr. Chem.*, 1890, **41**, 91) first described the amylase from ungerminated wheat; he found it similar to that from barley malt, but without liquefying power. Chrzaszcz (*Biochem. Z.*, 1923, **142**, 147) observed that the optimum temperature for the amylase from wheat malt lay between 49° and 55°. Naylor, Spencer and House (*J. Amer. Chem. Soc.*, 1925, **47**, 3037) noted that the amylase from germinated wheat was highly active and that its optimum reaction lay between P_H 4.5 and P_H 5.1. We found the amylase from germinated wheat to be about four times as active as that from barley malt: the optimum temperature zone was between 49° and 58°, and the optimum reaction, P_H 4·6.

That dry malt-amylase does not suffer loss of activity if heated to 100°, but is completely inactivated at 170° was first observed by Hueppe (*Pharm. Zentr.*, 1887, **22**, 488). We found that heating dry wheat-amylase for 1 hour at 160° was sufficient to inactivate it. Temperatures up to 110° did not much affect the activity, but with further rise in temperature, the activity diminished steadily and was completely lost at 160°.

In the course of their attempts to purify malt-amylase, Sherman and Schlessinger (*J. Amer. Chem. Soc.*, 1915, **37**, 643) observed that loss of activity during dialysis was unavoidable. Patwardhan (*J. Indian Inst. Sci.*, 1929, **12A**, 185) observed that maize-malt lost its activity on prolonged dialysis in collodion bags. Patwardhan and Narayana (*J. Indian Inst. Sci.*, 1930, **13A**, 38) found that ragi-amylase behaved in a similar manner. We found that germinated wheat-amylase also lost nearly all activity when subjected to continued dialysis in collodion bags; the activity could not be restored by addition of salts, or buffer solutions, or amino-acids.

Sherman and Walker (*J. Amer. Chem. Soc.*, 1919, **41**, 1867; 1921, **43**, 2461) found that aspartic acid, asparagine, glycine, alanine, phenylalanine and tyrosine caused marked increase in the rates of hydrolysis of starch by purified pancreatic amylase, commercial pancreatin, saliva, and purified malt-amylase. Malt extract, however, remained unaffected by the addition of amino-acids. The above authors suggested that amino-acids act as protectors against the destruction of the enzyme in aqueous solutions, but did not show how the destruction actually took place.

Wroblewski, (*Z. physiol. Chem.*, 1898, **24**, 173) showed that in weak alkaline solutions trypsin did not inactivate diastase, but that pepsin destroyed it in weak acid solutions. Biedermann (*Biochem. Z.*, 1922, **127**, 38) found that salivary amylase was inactivated by pepsin though it resisted the action of trypsin. We found that preparations of amylase from germinated and ungerminated wheat were rendered inactive by pepsin, the rate of inactivation increasing with the quantity of pepsin added. Under similar conditions, however, pepsin failed to inactivate amylases from barley, cholam, maize and ragi : trypsin and papain were also without any action.

The influence of salts on amylases has lately led to some controversy. Sherman and Thomas (*J. Amer. Chem. Soc.*, 1915, **37**, 623) found that the chlorides, sulphates, nitrates and phosphates of sodium and potassium greatly accelerates the hydrolysis of starch by amylases. Koga (*Biochem. Z.*, 1923, **142**, 159) observed no activation and used the absence of response by plant amylases to stimulation by sodium chloride as a criterion for distinguishing them from animal amylases. Eadie (*Biochem. J.*, 1926, **20**, 1016) observed that high concentrations of salts only partially restored the activity lost by malt and pancreatic amylases during dialysis. One of us (Patwardhan, *J. Indian Inst. Sci.*, 1929, **12A**, 185) found that sodium chloride was without any regenerating action on inactivated amylase from maize. Narayanamurti (*J. Indian Inst. Sci.*, 1930, **13A**, 63) found the same salt inhibitive to purified cholam-malt amylase when its concentration exceeded 0·0000166 *N*. Our experiments with salts of different inorganic and organic acids show that under no condition could any of them activate wheat-malt amylase.

EXPERIMENTAL.

Malting and Preparation of the Enzyme.—In view of the observations of Prokopenko (*Deut. landw. Rundschau*, 1928, **1**, 824) that the amylase content of wheat-malt is independent of the temperature of germination, no special precautions were adopted. The details with regard to steeping and germination of the grain, drying and storage of the malt, and precipitation and preservation of the enzyme were the same as those described in an earlier communication (Patwardhan, *loc. cit.*).

Kahlbaum's pure soluble starch was used after repeated washing with water and decantation until the reducing action was constant; it was then filtered and dried. Sugar was estimated by the method of Willstätter and Schudel (*Ber.*, 1918, **51**, 780).

Comparative activity of barley and wheat-malt extracts.—The extracts were prepared by shaking the respective malts (2 gms.) with

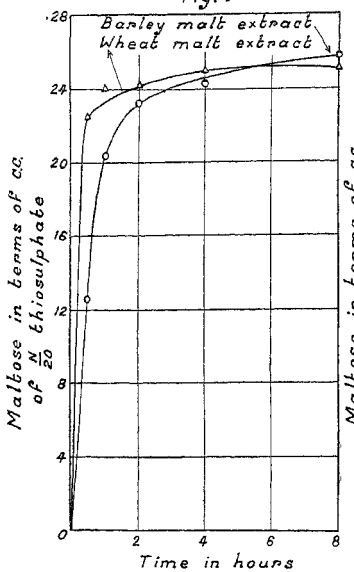
Fig. I
Barley malt extract
Wheat malt extract
Maltose in terms of c.c. of N/20 thiosulphate
Time in hours

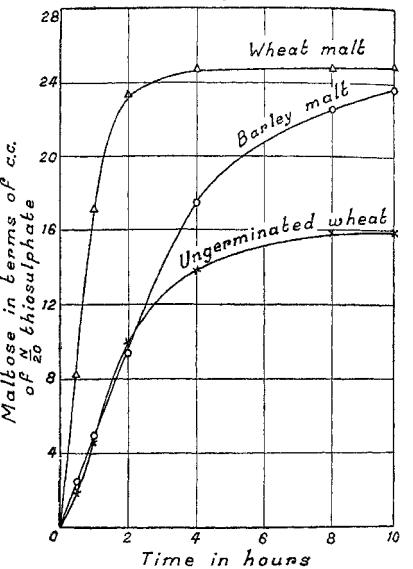
Fig. II
Wheat malt
Barley malt
Ungerminated wheat
Maltose in terms of c.c. of N/20 thiosulphate
Time in hours

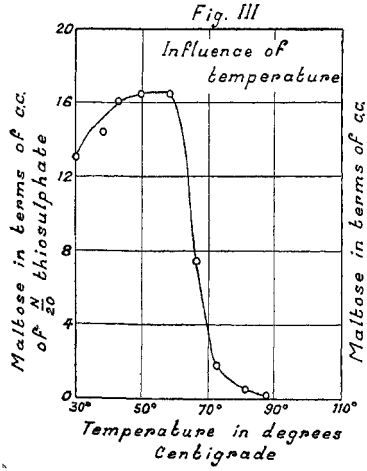
Fig. III
Influence of temperature
Maltose in terms of c.c. of N/20 thiosulphate
Temperature in degrees Centigrade

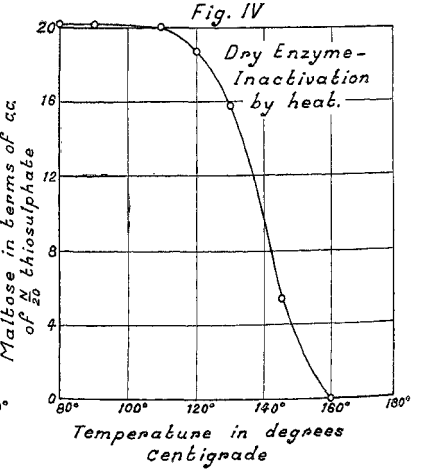
Fig. IV
Dry Enzyme-Inactivation by heat.
Maltose in terms of c.c. of N/20 thiosulphate
Temperature in degrees Centigrade

water for about one hour, filtering and bulking to 100 c.c. Starch solution (2 per cent.) was distributed in quantities of 100 c.c., one group receiving 20 c.c. of wheat-malt extract each, and the other 20 c.c. of barley malt extract each; toluene was added and the mixtures incubated at 38°. At regular intervals, aliquot parts were removed and their sugar contents determined. The results have been plotted in Fig. I.

Comparative activity of amylases from barley malt, germinated wheat and ungerminated wheat.—Experimental details were as above, except that solutions (0·02 per cent.) of the precipitated enzymes were added in place of malt extracts. The results have been plotted in Fig. II, showing that germinated wheat-amylase is more active than the corresponding barley-enzyme during the first four hours; in the later stages the activities are very nearly equal.

Effect of Temperature on Wheat-malt Amylase.

Enzyme in aqueous solution.—A 2 per cent. starch solution (45 c.c.) at nine different temperatures was treated with 5 c.c. of enzyme solution (0·0375 per cent.). Sugar was estimated after hydrolysis for 30 minutes. The results plotted in Fig. III show that the optimum zone lies between 49° and 58°; above this the enzyme is steadily weakened until completely inactivated at 88°.

Dry enzyme.—Specimens of the dry enzyme in weighing bottles were kept at different temperatures for one hour. They were then cooled to the room temperature and their respective activities determined in the manner described in the last experiment with the difference that the concentration of the enzyme was 0·05 per cent. and the time of incubation 1 hour. The results (Fig. IV) show that above 110° the enzyme weakens rapidly and is finally killed at 160°.

Response to Change in Hydrogen-ion Concentration.

To each of twelve flasks containing 40 c.c. of starch solution (2 per cent.) and 20 c.c. McIlvaine's phosphate and citric acid buffer of different reactions, 20 c.c. of enzyme solution (0·01674 per cent.) were added and the mixtures incubated at 38° for one hour. Sugar was then determined in aliquot portions. The results (Table I) show the optimum reaction at P_H 4.6.

TABLE I.

P_H	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·6	5·8	6·0	6·8
Maltose (c.c. *N*/20 thiosulphate).	8·8	9·6	10·2	11·6	11·7	11·45	11·25	11·0	9·5	8·6	8·0	7·2

Effect of Concentration.

Enzyme.—To a number of flasks containing 50 c.c. of starch solution (2 per cent.), varying quantities of the enzyme solution (0·1674 per cent.) were added, and the volume made up to 60 c.c. by distilled water; after incubation for one hour at 38°, sugar was estimated, Fig. V showing that the rate of hydrolysis increased with concentration of the enzyme up to a maximum and then remained constant.

Substrate.—Seven flasks each containing starch solution (50 c.c.) with concentration between 0·5 and 6·0 per cent. were incubated with 10 c.c. of enzyme solution (0·01 per cent.) at 38°. Sugar was estimated after one hour and Fig. VI shows that the range of optimum substrate concentration is 2–4 per cent.

Inactivation of the Enzyme during Dialysis.

Bags were prepared from 6 per cent. collodion, and into each 10 c.c. of enzyme solution (0·10 per cent.) was pipetted and dialysed against distilled water saturated with toluene, for several days at room temperature, along with control tubes containing 10 c.c. of enzyme and toluene. At each stage of observation, two collodion bags and one control tube were taken out, their respective contents made up to 50 c.c. and activities tested against 2 per cent. starch solution.

The results (Fig. VII) show that dialysis diminishes the activity to a negligible figure in a fortnight, while the undialysed enzyme remains unaffected.

Influence of Pepsin, Trypsin and Papain on the Activity.

To determine whether the inactivation observed on prolonged dialysis was due to hydrolysis of the enzyme protein, the following experiments were conducted.

Two specimens (50 c.c.) of 0·02 per cent. amylase were treated with 10 c.c. of trypsin solution (0·5 per cent.); to a third, 10 c.c. of boiled trypsin was added. The flasks were incubated at 38° after addition of toluene. Similar trials were also carried out with amylase solutions to which fresh and boiled solutions of pepsin and papain, respectively, had been added. After 28 hours, aliquot parts from the different experiments were transferred to nine other flasks each containing 50 c.c. of starch solution (2 per cent.) and the respective amylase activities determined after incubation for 1 hour at 38°.

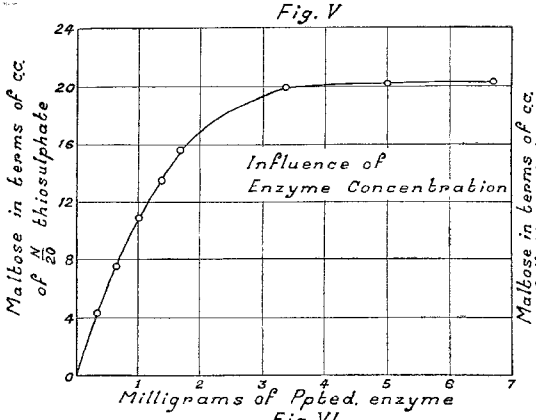
Fig. V
Influence of
Enzyme Concentration
Maltose in terms of cc.
of N/20 thiosulphate
Milligrams of Ppted. enzyme

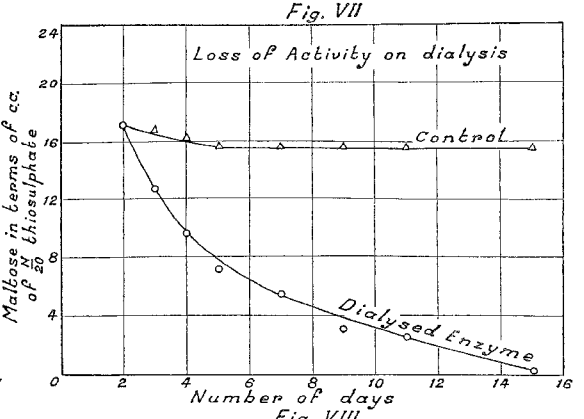
Fig. VII
Loss of Activity on dialysis
Control
Dialysed Enzyme
Maltose in terms of cc.
of N/20 thiosulphate
Number of days

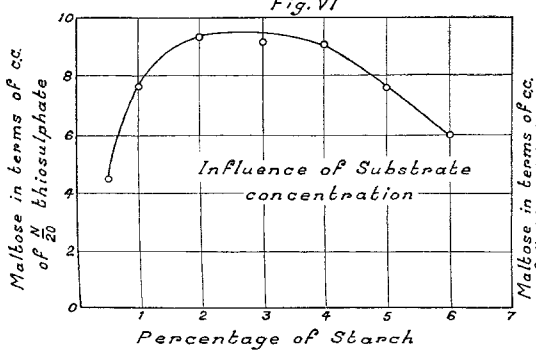
Fig. VI
Influence of Substrate
concentration
Maltose in terms of cc.
of N/20 thiosulphate
Percentage of Starch

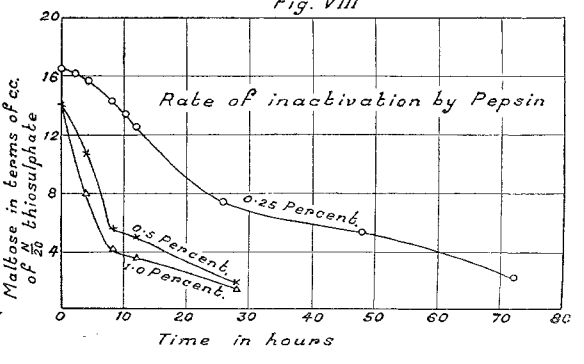
Fig. VIII
Rate of inactivation by Pepsin
0.25 Percent.
0.5 Percent.
1.0 Percent.
Maltose in terms of cc.
of N/20 thiosulphate
Time in hours

The results (Table II) show that there was marked inactivation in presence of pepsin while trypsin and papain had no effect.

TABLE II.

Proteolytic enzyme	Mgms. of maltose in terms of c.c. thiosulphate		
	Unboiled		Boiled (control)
	I	II	
Pepsin	4·1	2·1	17·9
Papain	19·0	19·2	20·7
Trypsin	17·4	17·8	20·1

Rate of inactivation by pepsin.—To two specimens (25 c.c.) of the enzyme solution (0·2 per cent.), 25 c.c. of 1·0 and 2·0 per cent. solutions respectively of pepsin was added with 1 c.c. of toluene. At stated intervals, 5 c.c. was removed and diluted to 25 c.c., 10 c.c. of this being mixed with 40 c.c. of starch solution (2 per cent.) and incubated at 38° for one hour after addition of toluene. The results (Fig. VIII) show that the inactivation was most rapid in the first 10 hours; it then slackened and was nearly complete at the end of 28 hours.

INFLUENCE OF SALTS ON ACTIVE AND INACTIVE ENZYME.

Active enzyme.—Several mixtures containing 40 c.c. of starch solution (2 per cent.), 20 c.c. of McIlvaine's buffer of P_H 4·6, 10 c.c. of the enzyme solution, 10 c.c. of a salt solution of a particular concentration and toluene, were arranged. The salts used were the chlorides of sodium, potassium and calcium and the sulphate, oxalate, malate and tartrate of potassium. They were added in such quantities as to give final concentrations of 0·0025, 0·005 and 0·01 molar. Adequate controls were also maintained. After incubation at 38° for 1 hour the sugar contents were determined.

No differences being observed, it is inferred that, under the experimental conditions, salts were without any influence on the activity of wheat-amylase.

Inactive enzyme.—The details were the same as those of the previous experiment except that amylase inactivated by prolonged dialysis was substituted for the active enzyme. In no case could any activity be observed.

INFLUENCE OF AMINO-ACIDS.

The procedure was the same as that adopted in the previous experiment. Sodium salts of glycine, alanine, aspartic acid, hippuric acid and leucine were added in such quantities as to make the final concentrations 0·0025, 0·005 and 0·01 molar. It was observed that neither the active enzyme nor the one inactivated by prolonged dialysis was appreciably influenced by the amino-acids.

SUMMARY.

1. Amylase from germinated wheat is more active than the corresponding barley enzyme.

2. The optimum temperature for the action of wheat-amylase lies between 49° and 58°. If heated for 1 hour at 160° the dry enzyme is completely inactivated. The optimum reaction is P_H 4·6.

3. The enzyme is inactivated on prolonged dialysis in collodion bags. The activity is not regenerated by adding various salts and amino-acids.

4. Neutral salts and amino-acids are without any influence on the activity of wheat-amylase. Pepsin inactivates the enzyme, but trypsin and papain do not.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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