I.—AMYLASE FROM RICE.

By Dattatreya Vishnu Karmarkar and Vinayak Narayan Patwardhan.

The present communication forms a part of a series on amylases from cereals, of which some have already been published (*Journal Indian Inst. Sci.*, 1928, **11A**, 121; 1929, **12A**, 185; 1930, **13A**, 31, 159). The rice-malt amylase possesses properties similar to those of other cereal amylases examined in this laboratory. Ungerminated paddy does not contain any active enzyme.

EXPERIMENTAL.

Preparation of the enzyme.—Paddy requires steeping for four days at room temperature (24–26°) before it can be spread out to germinate. After four days, when the plumules are about half an inch high, the seedlings are collected, sun-dried and treated as usual for preparation of dry malt.

Since rice contains only a small quantity of water-soluble proteins, it was very difficult to obtain an active precipitate of the enzyme by the method of Ling and Baker (*J. C. S.*, 1895, **67**, 702). An active preparation was, however, obtained on extracting the malt powder with a 0·6 per cent. solution of sodium bicarbonate and precipitating with 95 per cent. alcohol. It was also observed that if malted rice is allowed to stand overnight in contact with water and 95 per cent. alcohol then added to the extract, the precipitate is as active as that obtained from the alkaline extract of the malt. The reaction of the water extract after twenty-four hours was P_H 4·8, that of the fresh extract being P_H 5.9. Active enzyme was also obtained adjusting the reaction of the fresh extract to P_H 4·8 by adding dilute acid followed by precipitation with alcohol. The enzymes obtained by the three methods mentioned above were found to be equally active.

HYDROLYSIS OF STARCH BY RICE AMYLASE.

(*a*) *Saccharification.*—To 100 c.c. of 2 per cent. starch solution, 20 c.c. enzyme solution (0·02 per cent.) was added with 1 c.c. of toluene. The mixture was maintained at 38° and sugar estimated at

intervals. The results are compared with the corresponding figures for wheat-malt in Table I.

TABLE I.

Time in hours	Maltose in terms of c.c. N/20 thiosulphate	
	Rice	Wheat
½	1·8	8·2
1	3·8	17·1
2	10·1	23·2
4	15·0	24·6
8	21·8	24·8

It may be noted that in the earlier stages the wheat-malt amylase is about five times as active as the one from rice-malt; in estimating liquefaction of potato-starch the enzymes were therefore taken in the ratio 1 : 5.

(*b*) *Liquefaction.*—Potato-starch paste (25 c.c. of 2 per cent.) was mixed with 25 c.c. of rice amylase (0·1 per cent.) at 30° and 10 c.c. of the mixture transferred to a viscometer maintained at 30°. The readings taken at intervals were compared with those in a similar experiment with a 0·02 per cent. solution of wheat-malt amylase (Table II).

TABLE II.

Time in minutes	t in seconds	
	Rice	Wheat
0	201	176
15	117	112
30	107	109
60	107	109
90	106	108
120	105	107

Fig. I

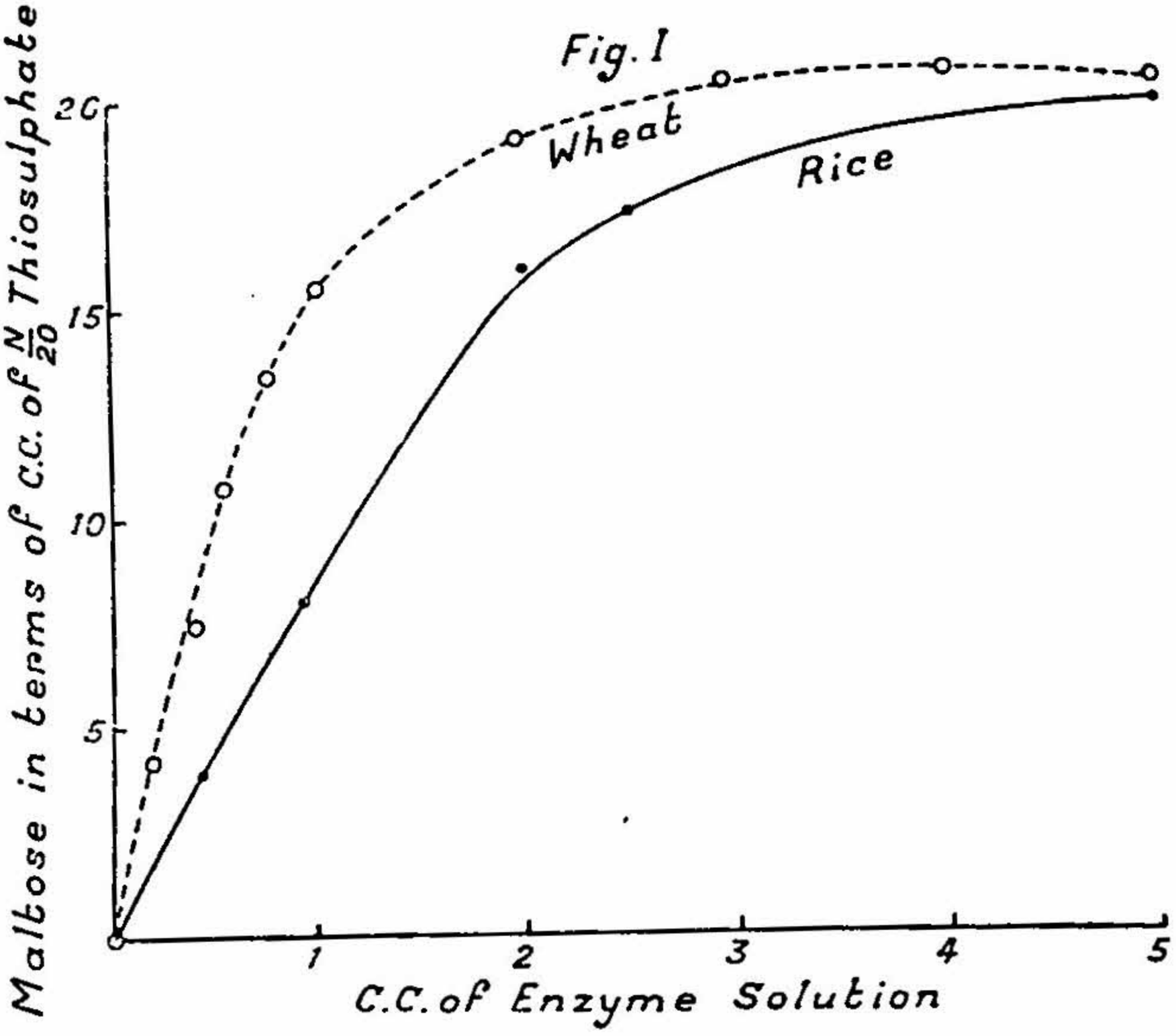
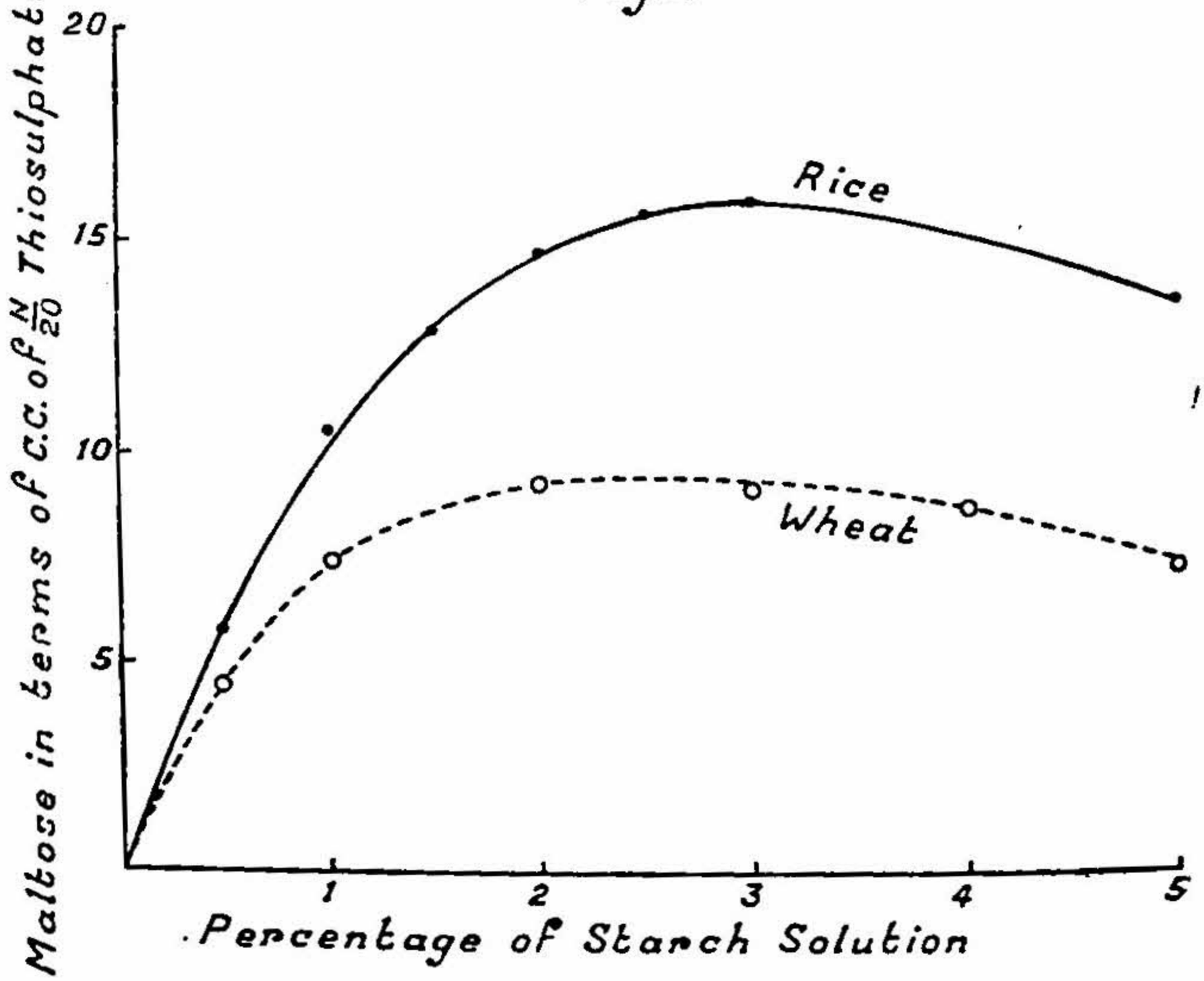

Wheat
Rice
Maltose in terms of c.c. of N/20 Thiosulphate
20
15
10
5
1
2
3
4
5
C.C. of Enzyme Solution

Fig. II


Rice
Wheat
Maltose in terms of c.c. of N/20 Thiosulphate
20
15
10
5
1
2
3
4
5
Percentage of Starch Solution

The observations show that rice amylase liquefies starch to the same extent as wheat amylase if the rates of saccharification are adjusted so as to be equal in both the cases.

Effect of temperature on rice amylase.—To each of a series of flasks containing 45 c.c. of starch solution (2 per cent.) 5 c.c. of malt extract (8 per cent.) was added after bringing the former to the desired temperature. The reaction was allowed to proceed for 30 minutes and then arrested by 5 drops of 30 per cent. sodium hydroxide. The mixtures were cooled and their respective sugar contents determined with the necessary correction for sugar content of malt.

TABLE III.

Temperature...	25	35	40	45	50	55	60	65	75
Maltose (c.c. of N/20 thiosulphate) ...	9·1	10·6	11·6	14·5	15·1	15·5	15·8	14·1	3·7

Effect of reaction.—To each of a series of flasks containing 40 c.c. of starch solution (2 per cent.) and 10 c.c. McIlvaine's buffer solution adjusted to a particular reaction, 10 c.c. of enzyme solution (0·10 per cent.) were added and the mixtures incubated at 38° for 1 hour.

TABLE IV.

P_H ...	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·	5·6	5·8	6·0	6·4
Maltose (c.c. of N/20 thiosulphate) ...	8·8	9·6	10·2	11·61	1·7	11·4	11·2	11·0	· ·	9·5	8·6	8·0	7·2

Effect of concentration:—(*a*) *Substrate.*—To seven flasks each containing 50 c.c. of starch solution of a particular strength 10 c.c. of rice amylase solution (0·08 per cent.) was added, and sugar estimated after incubation for one hour at 38°. The results are presented in Fig. I.

(*b*) *Enzyme.*—A series of nine flasks each containing 50 c.c. of starch solution (2 per cent.) and a particular quantity of water were arranged. The total volume was made up to 60 c.c. in each case by adding the required volume of enzyme solution (0·40 per cent.). The flasks were incubated for 1 hour at 38° and sugar determined as usual; the results are given in Fig. II.

Effect of electrolytes and amino-acids on the activity of rice amylase.—The experiments were conducted with electrolyte-free enzymes under controlled conditions of temperature and reaction, with and without addition of buffer solutions. The details of the experiments were the same as those described in the previous communications by Patwardhan and his co-workers (*loc. cit.*).

The electrolytes tested were the chlorides of potassium, sodium and calcium, and the sulphate, oxalate, malate and tartrate of potassium, in 0·0025, 0·005 and 0·010 molar concentrations. In no case could any effect on the activity of amylase be observed as the result of the adding of any of the electrolytes. The amino-acids, glycine, leucine, *l*-alanine, aspartic acid and hippuric acid in concentrations similar to those described above, also led to negative results.

Effect of prolonged dialysis.—The enzyme loses most of its activity on dialysis in collodion bags (6 per cent., 2 layers) prolonged for about fifteen days. The results of the above and other similar experiments are discussed in the following paper.

SUMMARY.

1. An active preparation of rice amylase can be obtained by extracting the malt (*a*) with dilute (0·6 per cent.) alkali or (*b*) after adjusting the reaction to P_H 4·8 followed by precipitation with 95 per cent. alcohol and subsequent dehydration with absolute alcohol and ether.

2. The activity of rice amylase is less than one quarter that of the corresponding wheat enzyme.

3. The optimum temperature of the rice-malt enzyme is 60° and optimum reaction, P_H 4·6. The enzyme does not respond to addition of electrolytes or amino-acids, and on continued dialysis it loses its activity in fifteen days.

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