pH-ACTIVITY RELATIONSHIP OF THE α- AND β-AMYLASES OF RESTING AND GERMINATED BARLEY AND RAGI (*ELEUSINE CORACANA*)

By (Miss) K. Saroja and K. V. Giri

(*Department of Biochemistry, Indian Institute of Science, Bangalore-3*)

Summary

1. The presence of an α-amylase with optimum activity at pH 7·0 has been demonstrated in resting barley and ragi.

2. Resting ragi contains mainly α-amylase and a very low β-amylolytic activity.

3. The activity of the α-amylase (optimum pH 7·0) of resting barley is low, and is extracted by phosphate buffer pH 7·0 better than by water alone.

4. The α-amylases of resting barley and ragi have optimum activity at pH 7·0, whereas the respective α-amylases of germinated grains show maximum activity at pH 4·5 to 5·0.

The investigations of various workers (Nordh and Ohlsson, 1932; Lüers and Rümmler, 1933) on the starch degrading enzymes of barley and other cereals have led to the belief that ungerminated grains of barley, rye and oats are almost devoid of α-amylase, whereas, during germination, particularly in the sprouting stage, this enzyme becomes active. Giri and Srinivasan (1936, 1937) have shown the presence of an α-amylase of optimum pH 7·0 in resting rice. Hills and Bailey (1938) suggest the possibility of the presence of a measurable α-amylase activity in resting barley and attribute the increase in activity during germination entirely to α-amylase. Waldschmidt-Leitz, Reichel and Purr (1932) have shown that resting barley contains both α- and β-amylases, the former in a completely inactive condition. Our investigations on the amylolytic activity of barley and ragi (*Eleusine coracana*) have shown the presence of an α-amylase in the resting grains which is different from the α-amylase produced during the germination of cereals in its pH-activity relationship.

Experimental

Preparation of enzyme extract.—The enzyme extracts from the resting grains were prepared by extracting the powdered grains for 24 hours in presence of toluene with phosphate buffer (twice the weight of M/15 phosphate buffer pH 7·0 + 6 times the weight of water) or with 8 times the

weight of water alone, as the case may be, and filtering. In the case of germinated seeds, the fresh seedlings were taken and extracted with 8 times the weight of water on dry weight basis.

The seeds were germinated by steeping healthy grains obtained from the local market for 24 hours in flowing water and spreading them on germinating trays. The trays were kept in the dark for 48 hours for ragi, and 36 hours for barley, by which time, the sprouts were about 1 cm. and 2 cm. in length respectively.

Preparation of the substrate.—Amylose was used as substrate for the determination of the activity of the enzyme extracts; it was prepared from sweet potato starch by the thymol precipitation method (Haworth, Peat and Sagrott 1946; Bourne, *et al.*, 1948; Lakshmanan, Sri Ram and Giri, 1948).

Measurement of activity

(i) *Saccharifying power.*—The saccharifying power of the extracts was determined by estimating the reducing sugars produced in a suitable digest after definite intervals by the micromethod described by Somogyi (1952). The saccharifying power was expressed as the number of milligrams of maltose formed in 100 c.c. of the digest, after a known interval of time, irrespective of the volume of reaction mixture taken. The time allowed for hydrolysis was 4 hours for resting grain extracts and 1 hour for germinated seed extracts.

(ii) *Dextrinising power.*—The dextrinising power was determined by measuring the intensity of the colour obtained with iodine solution under strictly parallel conditions in a Klett-Summerson photoelectric colorimeter using the 64 red filter supplied with the instrument. The dextrinising power was expressed as the per cent. decrease in absorption value after a known interval of time (60 minutes for resting grain extracts and 10 minutes for germinated grain extracts).

Characterisation of the amylases.—α- and β-amylases were differentiated by plotting the percentage maltose formed in a digest against the percentage decrease in absorption value at the same time and comparing the curve with standard curves prepared for pure sweet potato β-amylase and α-amylase from human saliva (Sri Ram and Giri, 1952).

The reaction mixtures always contained the following in the proportions given:

12 mgm. amylose
1 c.c. buffer
1 c.c. enzyme extract in a total volume of 6 c.c.

The mixtures were incubated at 37° C., 0·5 c.c. of reaction mixture being diluted to 5 c.c. for sugar determination and 1 c.c. of reaction mixture was treated with 1 c.c. of iodine solution (0·2 per cent. iodine in 2 per cent. potassium iodide) and diluted to 100 c.c. for colorimetric estimation.

M/5 acetate buffers were used for the pH range 3·0 to 6·0 and M/15 phosphate buffers for the range 6·0 to 8·0.

In Table I are presented the results obtained for the saccharifying power of resting and germinated barley and ragi extracts in relation to pH.

TABLE I

Saccharifying Power of Resting and Germinated Barley and Ragi Extracts in Relation to pH

pH	3·0	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0
	(mg. of maltose)									
esting barley - phosphate buffer extract	108	148	177	157	130	117	65	248	186	125
esting barley-water extract	77	113	115	127	128	108	123	130	140	133
; hours germinated barley-water extract	0	233	235	238	232	195	192	190	177	163
esting ragi-phosphate buffer extract	44	58	60	76	61	49	68	78	66	50
3 hours germinated ragi-water extract	0	34	78	128	122	112	83	65	55	50

The results are represented graphically in Figs. 1 and 2.

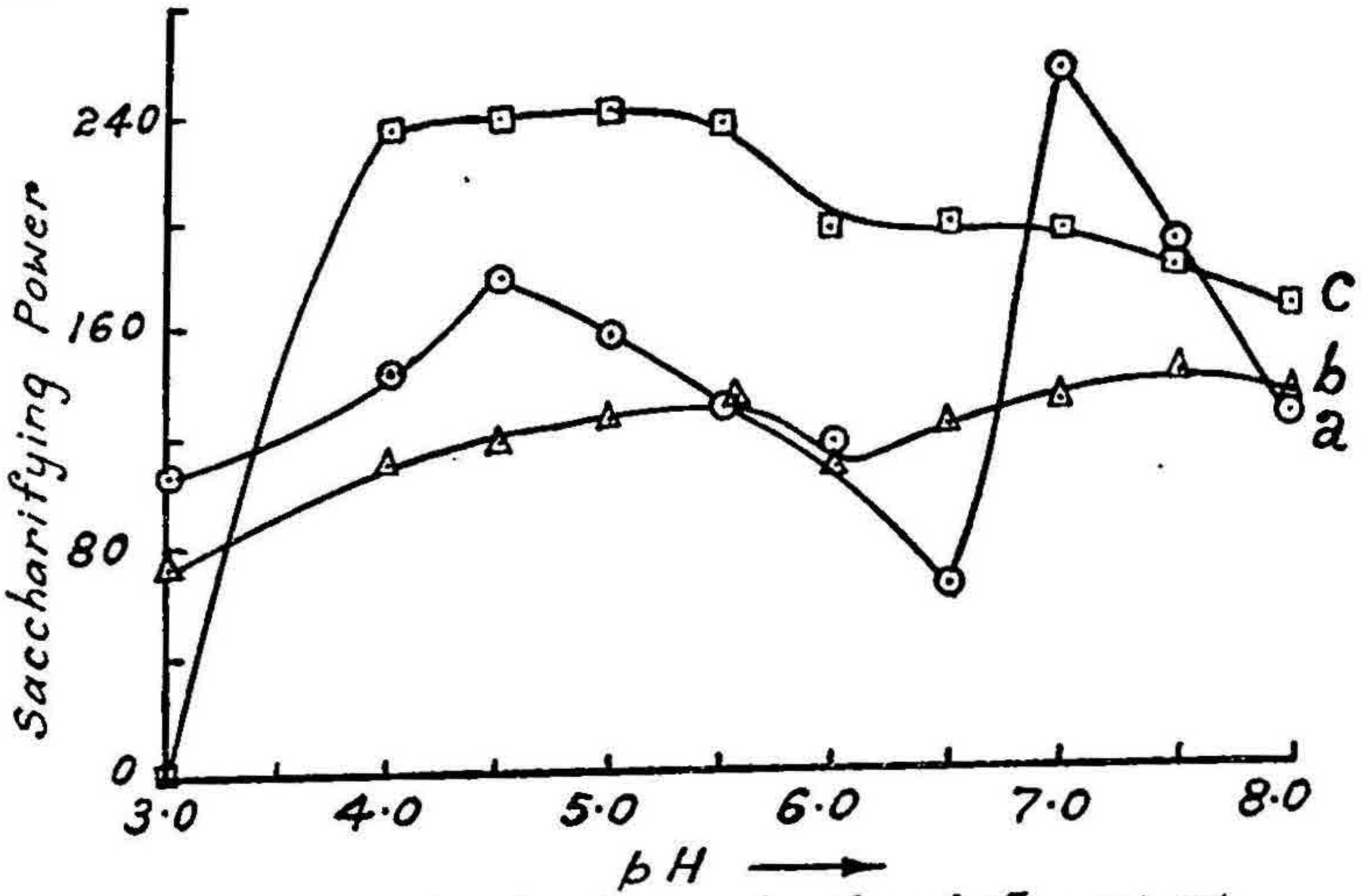


FIG. 1. Saccharifying power of resting and germinated barley extracts in relation to pH.

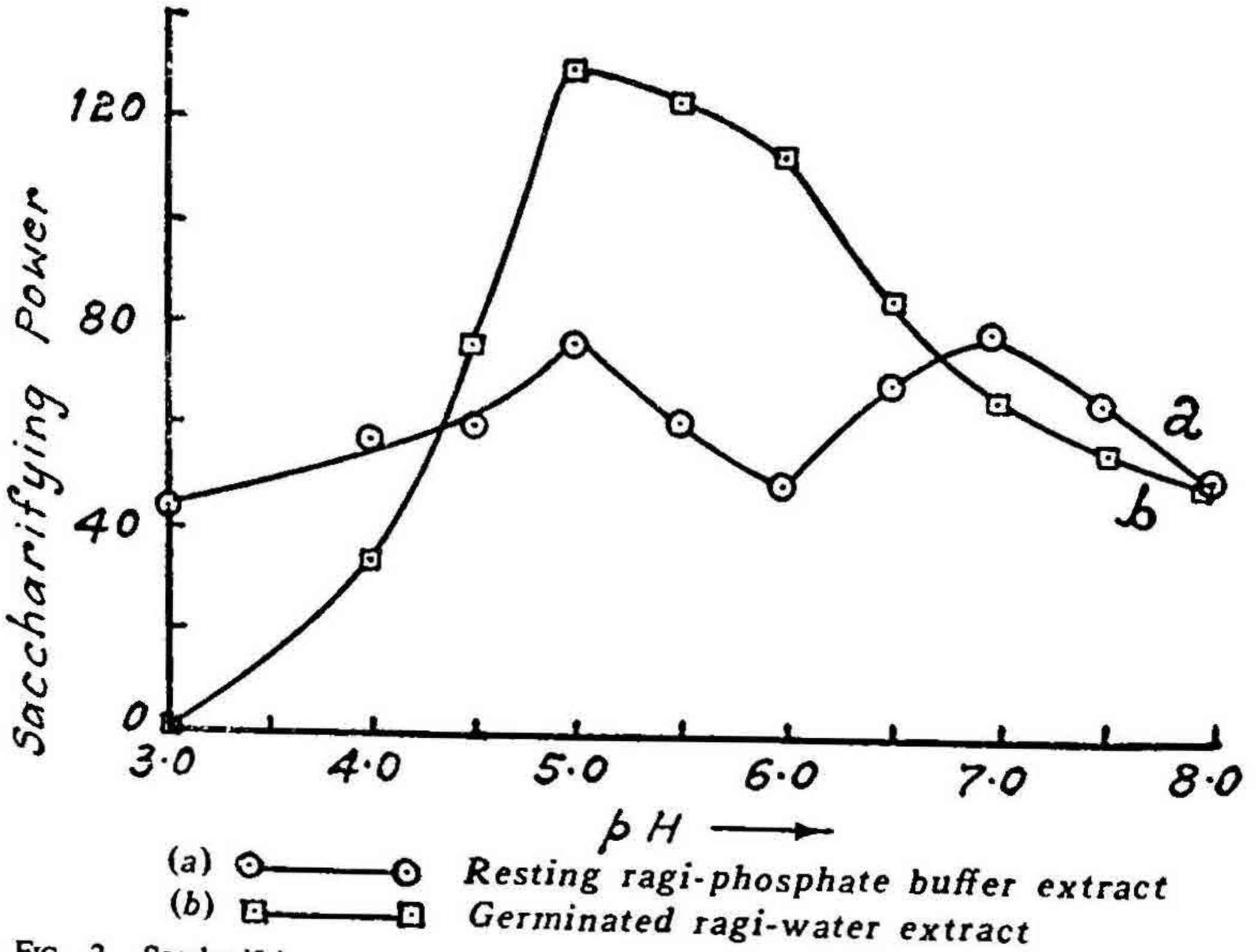


FIG. 2. Saccharifying power of resting and germinated ragi extracts in relation to pH.

In Table II are presented the dextrinising powers of resting and germinated barley and ragi extracts in relation to pH.

TABLE II

Dextrinising Power of Resting and Germinated Barley and Ragi Extracts in Relation to pH

pH	3·0	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0
	(per cent. decrease in absorption value)									
Resting barley-phosphate buffer extract	58	76	81	82	80	76	85	94	89	88
Resting barley-water extract	71	84	93	90	93	90	91	93	90	82
36 hours germinated barley-water extract	9	68	97	98	97	71	73	74	62	56
Resting ragi-phosphate buffer extract	0·5	7	8	9	19	26	39	44	40	36
48 hours germinated ragi-water extract	0	32	39	31	27	24	17	13	7	5

The results are represented graphically in Figs. 3 and 4.

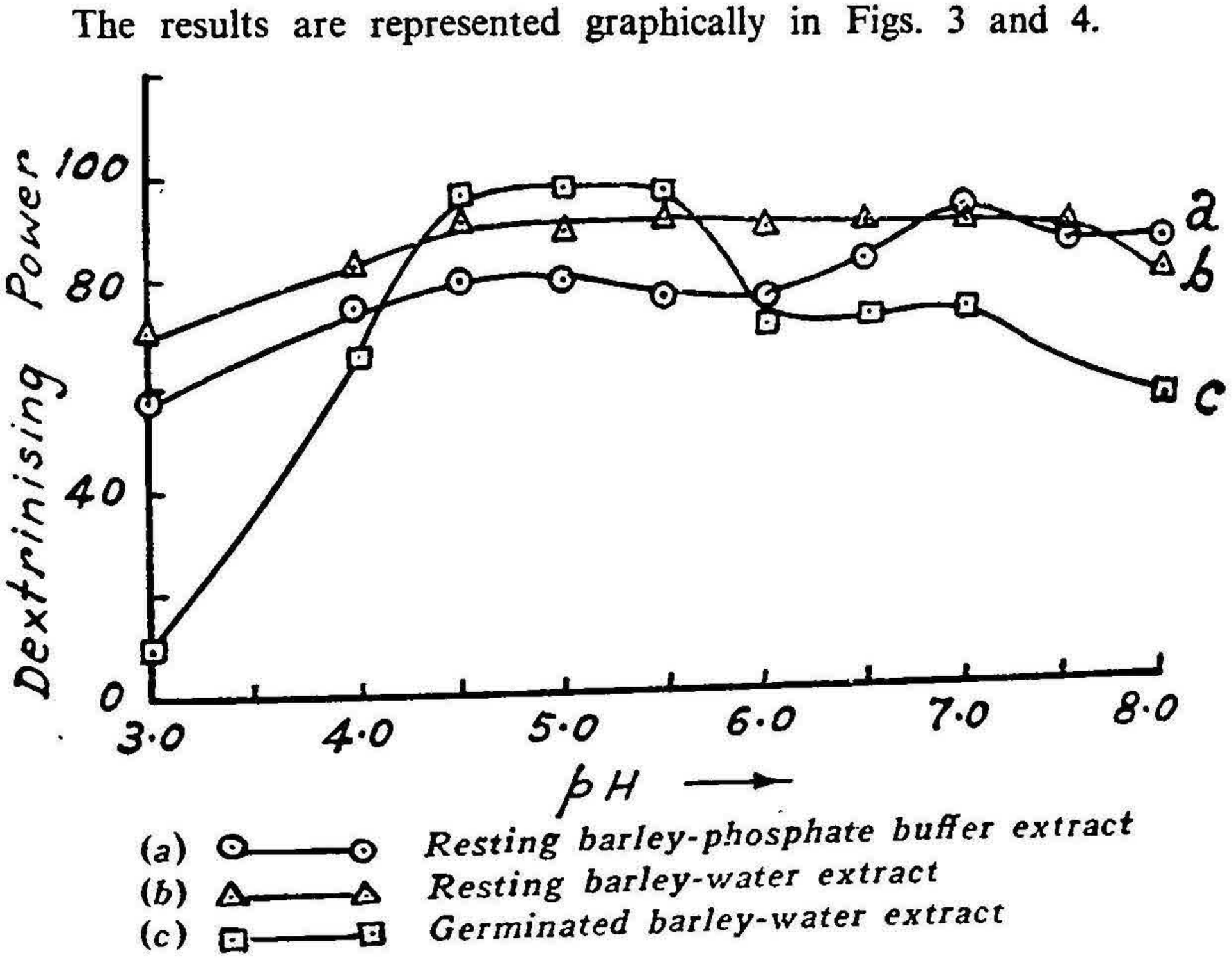
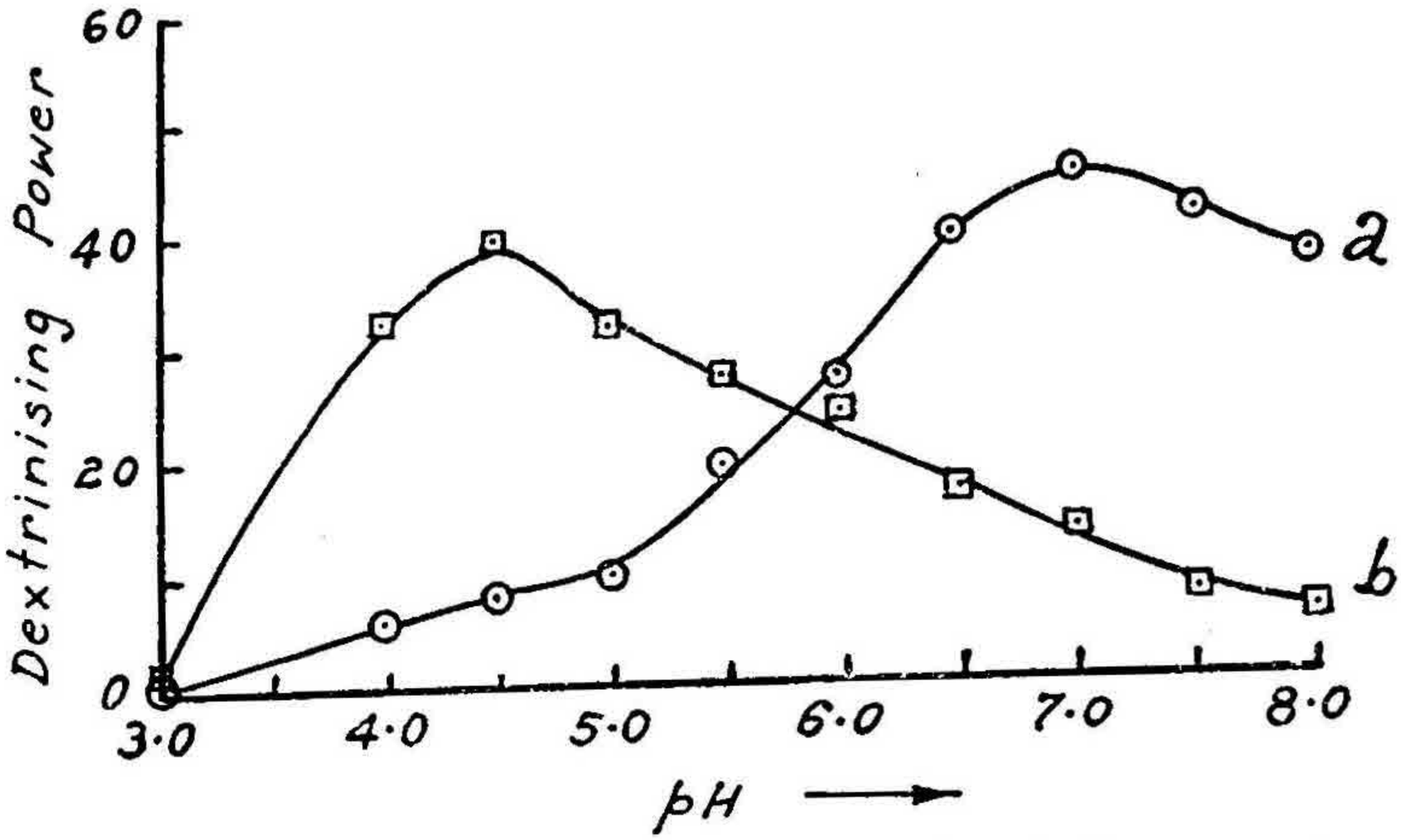


FIG. 3. Dextrinising power of resting and germinated barley extracts in relation to pH.



(a) ⊙——⊙ *Resting ragi-phosphate buffer extract*
(b) ⊡——⊡ *Germinated ragi-water extract*

FIG. 4. Dextrinising power of resting and germinated ragi extracts in relation to pH.

DISCUSSION

From Figs. 1 and 2 it is evident that there are two pH optima of saccharification for the resting grain-phosphate buffer extracts, pointing to two different enzymes active at pH 4·5 to 5·0 and 7·0 to 7·5 respectively. Further, from Figs. 3 and 4, it is seen that for dextrinisation, there is a single optimum for resting grain extracts at pH 7·0 showing that the dextrinising function is mainly due to the enzyme of optimum pH 7·0. On germination, however, the extracts show an almost uniform activity over the pH range 4·5 to 6·0. The enzyme of optimum pH 4·5 to 5·0 present in the resting grains was shown in the case of ragi, to be a β-amylase. This is the predominant type in barley, but from the results obtained on the distribution of the amylases in ragi, it was found that the β-enzyme was not so active compared to the activity of the α-amylase present in the resting grain.

Attempts were made to isolate the enzyme of optimum pH 7·0 from ragi, and we could demonstrate that it is an α-amylase liberating about 25 per cent. reducing sugars as maltose at 90 per cent. reduction in absorption value (*i.e.*, very near the achromic point), whereas, for the β-enzyme of ragi, the reduction in absorption value was only 23 per cent. for the same quantity of sugar formed.

It is seen from Fig. 1 that the two pH optima are not sharply defined in the case of resting barley-water extract, whereas in the case of the phosphate buffer extract of resting barley, a clear-cut differentiation is possible. The observation of Giri and Srinivasan (1936) in connection with the amylases of resting rice grain, that α-amylase is in an insoluble or adsorbed condition and is extracted by phosphate buffer better than by water alone is thus applicable to resting ragi and barley α-amylases also.

The phosphate buffer extract of resting barley was freed from β-amylase by a combination of the procedures of Van Klinkenberg (1932) and Nordh and Ohlsson (1932) and the resulting extract was found to dextrinise amylose with 19·2 per cent. maltose production near the achromic point. The optimum pH for activity was 7·0 which is different from the optimum pH 4·5 to 5·0 of the α-amylase present in germinated barley and ragi. Chandrasekhara and Swaminathan (private communication) have also observed that the optimum pH for ragi malt α-amylase is 5·0 at 30° C. That the enzyme is an α-amylase was further confirmed by the method described by Schwimmer (1945) for the detection of traces of α-amylase in β-amylase preparations.

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