

LETTERS TO THE EDITORS

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Activity of the Phytase in Different Cereals and its Resistance to Dry Heat

WHEAT, oats, barley and rye all contain between 0.18 and 0.26 per cent of phytic acid phosphorus. Wheatmeals and flours also contain an active phytase, and considerable interaction between this enzyme and its substrate may accompany the preparation of wheat for the table^{1,2}.

It was observed by Mellanby³ many years ago that oatmeal is more rachitogenic than wheatmeal, and, later, that hydrolysis with acid or germination and malting⁴ can remove the anticarcinogenic properties of oatmeal. Recent work has helped to explain these last two pioneering observations, for it has tended to incriminate phytic acid as the rachitogenic agent in cereals. In trying to account for the difference between oats and wheat in terms of phytic acid, it was found that oatmeal as purchased has a negligible phytase activity. It is accordingly suggested that this lack of phytase may explain Mellanby's results³. It is certain that only the merest fraction of the phytic acid in oatmeal could have been destroyed in preparing his puppies' food, whereas much of the phytic acid in the wheatmeals might have gone over to inositol and inorganic phosphorus before the puppies ate it. The lack of phytase in oatmeal explains why little or no hydrolysis of the phytic acid in oatmeal takes place in the making of porridge, even if the meal is left to soak overnight and then brought slowly to the boil.

The lack of phytase in oatmeal as purchased was at first tentatively attributed to the kilning process to which 'green' oats are subjected before being milled. Samples of kilned oats and of the green oats from which they had been prepared were, however, obtained from four well-known Scottish firms. These were ground up and incubated with ten times their weight of water at 50° C. and pH 4.5 for varying lengths of time. Two observations were at once made. The first was that the phytase activity even of 'green' oats was never more than a fraction of that of either English or Manitoba wheat. This led to an examination of rye and barley, and the results of all these tests are given in Table 1. It will be seen that the rye phytase was the most active. The inclusion of rye such as this in a wheaten grist would, therefore, tend to increase the amount of phytic acid destroyed in baking the bread, and the destruction in a rye bread must sometimes be very high. Dr. Kent-Jones kindly baked some rye bread from sample 2 according to the German technique⁵, and 48 per cent of the phytic acid in this whole rye flour was found to have been destroyed in spite of the short time allowed for the bread to 'rise'.

It might be supposed that the apparent lack of activity of oatmeal phytase is due to this cereal containing a different and perhaps more resistant phytic acid; but this is unlikely, for when oats and wheat were boiled with hydrochloric acid the rates of hydrolysis of the phytic acids of the two cereals were found to be exactly the same (Table 1). Nor can the slow rate of enzymic hydrolysis in oatmeal be attributed to its phytic acid being present in a particularly insoluble form for, although the various samples of oats contained 44-72 mgm. of

TABLE 1. RATE OF HYDROLYSIS OF PHYTIC ACID IN DIFFERENT CEREALS.

Nature of cereal	Phytic acid P. in cereal mgm. per 100 gm.	Hydrolysing agent	Time required for hydrolysis of 50% of the phytic acid
Rye, Sample 1	242	Phytase in cereal itself at 50° C. and pH 4.5	5 min.
" " 2	217	" "	8 min.
Wheat, English	242	" "	12 min.
" " Manitoba	233	" "	14 min.
Barley	260	" "	43 min.
Oats, Green (N.S.)	233	" "	11 hr.
" " (J.J.)	210	" "	11 hr.
" " (J.I.)	182	" "	13.5 hr.
" " (W.E.)	198	" "	15 hr.
Oats, Kilned (N.S.)	220	" "	30 hr.
" " (J.J.)	218	" "	46 hr.
" " (J.I.)	223	" "	46 hr.
" " (W.E.)	206	" "	40 hr.
Wheat, English	242	Boiling 2N HCl	7.1 hr.
Oats, Green (J.S.)	182	" "	7.1 hr.

TABLE 2. THE PHYTASE ACTIVITY OF BRAN (AS MEASURED BY THE DESTRUCTION OF PHYTIC ACID) AFTER THE BRAN HAD BEEN HEATED DRY AND IN WATER AT 90° C. All samples were incubated for 50 min. at 50° C. and at pH 4.5. The bran originally contained 10.3 per cent of moisture and 0.92 per cent of phytic acid phosphorus.

Time of heating at 90° C. (hr.)	% Destruction of phytic acid during incubation	
	After dry heat	After wet heat
0	87	87
1/2	87	0
1	85	0
2	82	0
5	78	0

calcium per 100 gm. as against 29-41 mgm. for the other cereals, at least 75 per cent of the phytic acid in the oats was present as the magnesium and potassium salts, both of which must have been in solution at pH 4.5.

The second observation was that kilning, even at temperatures of 260-360° F. for two and a half hours, did not destroy all the phytase. It was thought possible that this might be due to the enzyme being resistant to heat once it was dry. This was subjected to test. Some wheat bran was dried over phosphorus pentoxide in a serum-drying plant and afterwards heated in the dry state at 90° C. for 1/2, 1, 2 and 5 hours. Other samples were dropped into water at 90° C. and maintained there for the same times. The activity of the phytase was then studied by measuring the rate of destruction of the phytic acid in the bran on incubating it at 50° and pH 4.5. The results are given in Table 2, and it will be seen that whereas, as expected, the enzyme was completely and instantaneously destroyed in water at 90° C., it suffered no measurable destruction in 30 minutes at this temperature when it was heated dry, and very little destruction even after five hours. Further observations are in progress on the resistance of other enzymes to dry heat.

Our thanks are due to the millers who supplied the oats, to Dr. Greaves for drying the bran, to Drs. Moran and Kent for grinding the cereal grains for us, and to Dr. Kent-Jones for baking the rye bread. One of us (E. M. W.) is in the whole-time service of the Medical Research Council.

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¹ Widdowson, E. M., *NATURE*, 148, 219 (1941).² Pringle, W. J. S., and Moran, T., *J. Soc. Chem. Ind.*, 61, 108 (1942).³ Mellanby, E., *Spec. Rep. Ser. Med. Research Coun., Lond.*, No. 93 (1925).⁴ Mellanby, M., *Spec. Rep. Ser. Med. Research Coun., Lond.*, No. 140 (1929).⁵ Kent-Jones, D. W., "Modern Cereal Chemistry" (Liverpool: The Northern Publishing Co., 1939).