Hydrolysis of the phytate of wheat flour during breadmaking

By D. G. H. DANIELS and N. FISHER

(*Received 8 August 1980 – Accepted 12 January 1981*)

1. Differences in the extent of breakdown of phytate in wholemeal and white flours prepared from three wheats when the flours were made into bread using the three main UK commercial breadmaking processes were investigated.

2. The extent of breakdown (31–46% for wholemeal breads, 88–99% for white breads) was not proportional to the relative processing times involved (1–4 h). The importance of destruction of phytate in the oven is stressed.

3. The phytase (*myo*-inositol hexaphosphate phosphohydrolase, *EC* 3.1.3.8) activities of the wholemeal flours and of the yeast were determined. Re-examination of some information in the literature enabled the relative importance of these activities, and of the various stages of breadmaking, in determining the extent of hydrolysis of phytate to be assessed.

4. Average values for the molar ratio, phytate:zinc, of 22:1 and 0·8:1 were calculated for wholemeal and white breads respectively. The nutritional significance of these results is discussed.

Much of the phosphorus of cereals occurs in the form of phytin, the mixed calcium–magnesium salt of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)). This substance is concentrated in the aleurone and other outer layers of the grain, which contain much dietary fibre. Thus relatively high levels of phytate are present in fibre-rich foods based on cereals, e.g. brown and wholemeal breads and wholewheat breakfast cereals. A reduction in the bioavailability of dietary minerals, especially zinc, in the presence of high levels of phytate or dietary fibre or both is currently of some concern to nutritionists (Cummings, 1978; Anonymous, 1979; Davies, 1979*a*, *b*). However, part of the phytate in wheat flours is normally destroyed during breadmaking through the hydrolytic action of the enzyme phytase (*myo*-inositol hexaphosphate phosphohydrolase, *EC* 3.1.3.8) (Widdowson, 1941; Pringle & Moran, 1942; de Lange *et al.* 1961), which is present in all parts of the grain (Peers, 1953) and also in bakers' yeast (Harland & Harland, 1980). Enzymic destruction during fermentation is widely assumed to be the major cause of phytate losses during breadmaking.

In the foregoing studies bread was made by the traditional long fermentation process (LFP), using flours of 75–100% extraction rates. Most of the bread consumed in the UK is presently made by newer, shorter breadmaking processes such as the Chorleywood Bread Process (CBP) and the Activated Dough Development process (ADD). Whereas the LFP involves prolonged fermentation of the bulk dough, often for 2–3 h, followed by dividing and rounding, further fermentation ('proof') for approximately 45 min and then baking, the other two processes avoid the initial prolonged fermentation of the bulk dough. In the CBP this is accomplished by intense but brief mechanical working of a dough containing extra yeast as well as fat and oxidizing agents, in ADD by use of a mixture of oxidizing and reducing agents. Fermentation is confined to the final proof stage. Processing times for the CBP and ADD are approximately one hour from mixing to oven compared with 3–4 h for the LFP.

The hypothesis that phytate hydrolysis would be less extensive in these newer breadmaking methods has now been tested. Factors influencing the breakdown of phytate in breadmaking and the nutritional significance of the residual phytate are discussed.

MATERIALS AND METHODS

Flours

The wheats used were English (mixed varieties, protein content 112 g/kg, at moisture content 140 g/kg), Canadian Western Red Spring (CWRS, protein 138 g/kg) and a 1:1 mixture of the two (protein 125 g/kg). Wholemeal flours were prepared with a Christy-Norris hammer mill. White flours of approximately 72% extraction rate were milled in a Bühler laboratory mill Type MLU 202. Statutory additions of vitamins and chalk were made to the white flours.

Baking procedures

White bread was made by the LFP, CBP and ADD as previously described (Bell *et al.* 1979) except that sucrose (20 g/kg flour) was included in the LFP recipe to ensure adequate gas production, and doughs were mixed from 1680 g batches of flour to give two 800 g loaves, representing the most common commercial size of loaf. For the wholemeal bread, ascorbic acid was used as the only oxidant in the LFP (15 mg/kg with the English flour, 20 mg/kg with the mixed-grist and Canadian flours), and in the CBP (75 mg/kg); sucrose (20 g/kg) was again used in the LFP. This conforms to the Food Standards Committee's recommendation (Food Standards Committee, 1974) that the use of ascorbic acid should be permitted in making wholemeal bread when the Regulations are revised, even though it is not allowed in such bread at present. For technical reasons, ADD bread could not be made without potassium bromate, so the usual recipe was used. Wholemeal bread can be made in this way for research purposes only.

Two mixings were made with each flour, and one loaf from each mixing was sliced, dried overnight at 50° in a Mitchell drier to a moisture content of approximately 60 g/kg, crumbled and stored at 4° until required. Samples were ground in a laboratory Glen–Creston mill before analysis.

Chemical analyses

Total P was determined by wet-ashing in sulphuric and nitric acids followed by colour development with a molybdo–vanadate reagent (Ministry of Agriculture, Fisheries & Food, 1976). Phytate-P was estimated according to Pringle & Moran (1942), but extracts were not neutralized before precipitating ferric phytate (Anderson, 1963) and P in the precipitate was determined as described previously. Phytase activity was assayed according to Peers (1953) except that inorganic-P in the reaction mixtures was determined as described previously. Protein (nitrogen × 5·7) was determined by the Kjeldahl method.

RESULTS

Total-P and phytate-P in wholemeal flours and bread

The wholemeal bread had the normal appearance and loaf volume for its type. The results of chemical analyses are shown in Table 1. A two-way analysis of variance showed that the breadmaking processes and flour types were both major sources of variance in phytate-P values ($P < 0{\cdot}001$) while the interaction (process × flour type) was also significant ($P < 0{\cdot}05$). Differences between loaves from replicate mixings of the same flour were not significant. The decrease in phytate-P during breadmaking was calculated, ignoring dry weight changes e.g. addition of other ingredients, fermentation losses, which balanced each other to within 2%. The average loss (g phytate-P/kg dry matter) for each process was: LFP 1·24, CBP 0·90, ADD 0·84. Averaging the results from the three processes, the losses for the flours were (g/kg): English 1·21, mixed-grist 0·96, Canadian 0·82. The percentage loss with the LFP (46) was significantly greater ($P < 0{\cdot}01$) than the losses with the CBP

Table 1. *Total- and phytate-phosphorus (g P/kg dry matter) in wholemeal flours and bread*

(Each value is the mean of four determinations done on separate days; the results on breads are derived from duplicate determinations on one loaf from each of two mixings)

Grist	Total-P Flour	Phytate-P Flour	Phytate-P Bread Breadmaking process LFP	CBP	ADD	Means (bread)
English	3·62	2·80	1·36	1·67	1·75	1·59
Mixed-grist	3·57	2·64	1·48	1·78	1·80	1·69
Canadian	3·53	2·62	1·50	1·91	1·98	1·80
Means (grists)	3·57	2·69	1·45	1·79	1·84	—

Standard errors, due to replication, of values for flours were ±0·04 (9 df) and for breads were ±0·025 (27 df).
LFP, Long Fermentation Process; CBP, Chorleywood Bread Process; ADD, Activated Dough Development process.

(33) or the ADD (31): the latter processes did not differ from each other significantly. Similarly, there was a significantly greater loss of phytate with the English flour than with the other flours ($P < 0·01$) and the difference between the losses with the latter was not significant.

Total-P and phytate-P in white flours and bread

The white bread was also normal in appearance and loaf volume. The results of chemical analyses are shown in Table 2. A two-way analysis of variance showed that while differences between breadmaking processes were the largest source of variance ($P < 0·01$), the types of flour and the interaction (process × flour type) were also important in determining phytate contents in white bread, differences due to these sources reaching the same level of significance. The average loss (g phytate-P/kg dry matter) in each process was: LFP 0·33, CBP 0·30, ADD 0·31, and those for the flours were (g/kg): English 0·27, mixed-grist 0·21, Canadian 0·35. The percentage loss with the LFP (99) was significantly greater ($P < 0·01$) than with the CBP (88); differences from the ADD loss (92) were not significant owing to the greater scatter in the ADD results.

Table 2. *Total- and phytate-phosphorus (g P/kg dry matter) in white flours and bread*

(Each value is the mean for four determinations done on separate days; the results on breads are derived from duplicate determinations on one loaf from each of two mixings)

Grist	Total-P Flour	Phytate-P Flour	Phytate-P Bread Breadmaking process LFP	CBP	ADD	Means (bread)
English	1·05	0·294	0·006	0·035	0·030	0·025
Mixed-grist	1·12	0·325	0·003	0·036	0·010	0·016
Canadian	1·12	0·387	0·003	0·050	0·048	0·034
Means (grists)	1·097	0·335	0·004	0·040	0·029	—

Standard errors, due to replication, of values for flours were ±0·004 (9 df) and for breads, ±0·005 (27 df).
LFP, Long Fermentation Process; CBP, Chorleywood Bread Process; ADD, Activated Dough Development process.

Phytase determinations

Activity measurements at 55° according to Peers (1953) gave the following mean (±SE) values with the wholemeal flours (μg inorganic-P released/h per mg dry matter; no. of determinations in parentheses): English 8·2±0·35 (2), 7·1±0·22 (2), Canadian 8·6±0·21 (2). With yeast suspensions in acetate buffer (pH 5·15) at 55° the reaction rate decreased rapidly, suggesting that yeast phytase is more heat-labile than wheat phytase but at 40° this did not occur and the activity of yeast phytase was 7·7±0·32 (2). The phytase activity of the mixed-grist wholemeal flour was 6·0±0·17 (4) at this temperature.

DISCUSSION

The laboratory-scale breadmaking methods used in this work followed commercial practices as closely as possible, particularly with respect to processing temperatures and size of dough-piece. The latter influences the rate of heat transfer to the interior of the loaves, on which the speed and extent of the enzymic degradation of phytate may largely depend.

The results of phytate determinations on the flour and bread samples show that its hydrolysis during breadmaking by the modern rapid processes is indeed less extensive than in the traditional long fermentation process. Nevertheless, the differences were smaller than would be anticipated from the processing times, e.g. with wholemeals, nearly one third of the phytate was lost in the CBP and ADD (approximately 1 h from dough-mixing to oven) compared with nearly half in the LFP (approximately 4 h to oven). The suggestion by Peers (1953), that the high optimum temperature and thermal stability of wheat phytase allows the destruction of phytate to continue at an accelerated rate in the oven, may explain this lack of proportionality, since this stage is common to all the processes. The higher mixing temperature and increased level of yeast used in the CBP and ADD may contribute to this effect.

The relative importance in breadmaking of the phytases of wheat and bakers' yeast is uncertain (Pringle & Moran, 1942; Harland & Prosky, 1979; Harland & Harland, 1980). The present finding that yeast has much the same phytase activity as wholemeal flour at 40° and pH 5·15 suggests that yeast (which is used at a level of 10–25 g/kg flour) does not contribute much to the destruction of phytate in breadmaking (cf. Pringle & Moran, 1942). However, wheat phytase has a lower activity at the pH of dough, 5·5–6·5 than at pH 5·15 (Peers, 1953), whereas yeast phytase may well be nearer to its optimum and therefore in some conditions may be the more active enzyme. Harland & Prosky (1979) and Harland & Harland (1980) emphasized the importance of yeast phytase, and concluded that high-fibre bread with a lower phytate content than usual could best be obtained by increasing yeast levels and fermentation times (rising times) in the LFP method employed. Some of their results have been recalculated (1% phytate is equivalent to 2·82 g phytate-P/kg), enabling the relative contributions of wheat and yeast phytases to the destruction of phytate in various stages of breadmaking to be estimated (Table 3). The apparent inactivity of yeast phytase in the oven is consistent with its heat-lability at 55°. The quantitative results of Harland & Harland (1980) thus confirm that phytate destruction is greater in the oven than in the fermentation stage of the LFP, and therefore support the qualitative interpretation of the results obtained with other breadmaking processes, discussed previously.

Phytate is known to affect the bioavailability of P (Taylor, 1965), calcium (McCance & Widdowson, 1942) and iron (Anonymous, 1967), although the Fe in ferric monophytate, the major Fe compound in wheat, is available to the rat (Morris & Ellis, 1976). Disorders associated with Zn deficiency have been found to be common in populations consuming diets high in phytate (Reinhold, 1975), though other factors may be involved (Davies, 1978). Zn deficiency is unlikely to occur in the UK, where the average consumption of

Table 3. *Loss of phytate-phosphorus in the preparation of wholemeal bread, calculated from the values of Harland & Harland* (1980)

Yeast level (g/kg flour)... Sample/cause of loss	Phytate-P (g/kg, dry basis; value for flour, 2·70) 0	10	20
Bread, 0 h fermentation	1·80	1·80	1·69
Loss in oven	0·90 (33%)	0·90 (33%)	1·01 (37%)
Bread, 4 h fermentation	1·66	1·35	1·32
Loss during fermentation	0·14 (5%)	0·45 (17%)	0·37 (14%)
Loss due to wheat phytase (*myo*-inositol hexaphosphate phosphohydrolase, *EC* 3.1.3.8)	0·14 (5%)	0·14 (5%)	0·14 (5%)
Loss due to yeast phytase	0 (0%)	0·31 (12%)	0·23 (9%)
Total loss	1·04 (38%)	1·35 (50%)	1·38 (51%)

11·2 mg Zn/d is close to the daily requirement estimated by a World Health Organization committee (Anonymous, 1973) assuming 20% availability of Zn in the diet, and is mostly in highly assimilable form in animal-based foods (Davies, 1979*a*). Concern has been expressed about the replacement of such foods by those of plant origin which may have high contents of phytate (Davies, 1979*a*; Anonymous, 1979). In the rat, a critical factor governing the bioavailability of Zn is the molar ratio, phytate:Zn (Davies *et al.* 1977; Davies & Olpin, 1979). Zn deficiency occurs in growing rats receiving diets in which this value exceeds 15:1–25:1.

Calculation of the phytate:Zn molar ratio in bread from the phytate contents reported here and those for Zn content (mg Zn/kg dry matter) reported by Zook *et al.* (1970; wholemeal bread 27, white bread 10) gave values of 19:1 for LFP wholemeal, 24:1 for CBP and ADD wholemeals (average 22:1), 0·1:1 for LFP white bread and 1·2:1 for CBP and ADD white bread (average 0·8:1). These results suggest that the bioavailability of the Zn in wholemeal bread is low while that in white bread, made by any of the three processes, is very satisfactory. The values of phytate content on which this conclusion is based agree with others in the literature (Widdowson, 1941; Pringle & Moran, 1942; Harland & Harland, 1980) but a higher estimate of 0·24 g phytate-P/kg dry matter, for white bread, can be derived from the data in Paul and Southgate (1978), leading to a molar ratio phytate:Zn of 6·5:1. The value of 15:1 in white bread reported by Davies (1979*a*) is doubtful since it was based on an unusually high phytate content of 0·09% 'as is', equivalent to 0·42 g phytate-P/kg dry matter. White bread appears to be a useful source of biologically available Zn presently supplying on average approximately 7% of the daily adult requirement in the UK.

This work was commissioned by the Ministry of Agriculture, Fisheries and Food, and the results are the property of the Ministry and Crown Copyright. Thanks are due to numerous colleagues for help with specialized techniques, and to P. G. Burgess for technical assistance.

REFERENCES

Anderson, G. (1963). *J. Sci. Fd Agric.* **14**, 352.
Anonymous, (1967). *Nutr. Rev.* **25**, 218.
Anonymous, (1973). *Wld Hlth Org. tech. Rep. Ser.* **532**, 13.

Anonymous, (1979). *Nutr. Rev.* **37**, 365.
Bell, B. M., Chamberlain, N., Collins, T. H., Daniels, D. G. H. & Fisher, N. (1979). *J. Sci. Fd Agric.* **30**, 1111.
Cummings, J. H. (1978). *Am. J. clin. Nutr.* **31**, 521.
Davies, N. T. (1978). *J. Plant Foods*, **3**, 113.
Davies, N. T. (1979*a*). *Bull. Br. Nutr. Fdn.* **5**, 34.
Davies, N. T. (1979*b*). *Proc. Nutr. Soc.* **38**, 121.
Davies, N. T., Hristic, V. & Flett, A. A. (1977). *Nutr. Rep. int.* **15**, 207.
Davies, N. T. & Olpin, S. E. (1979). *Br. J. Nutr.* **41**, 591.
de Lange, D. J., Joubert, C. P. & du Preez, S. F. M. (1961). *Proc. Nutr. Soc. Southern Africa*, **2**, 69.
Food Standards Committee (1974). *Second Report on Bread and Flour*. London: HM Stationery Office.
Harland, B. F. & Harland, J. (1980). *Cereal Chem.* **57**, 226.
Harland, B. F. & Prosky, L. (1979). *Cereal Foods World*, **24**, 387.
McCance, R. A. & Widdowson, E. M. (1942). *J. Physiol., Lond.* **101**, 44.
Ministry of Agriculture, Fisheries & Food (1976). *Fertilisers and Feedingstuffs* (*Amendment*) *Regulations 1976*. London: HM Stationery Office, Schedule 5, Method 7.
Morris, E. R. & Ellis, R. (1976). *J. Nutr.* **106**, 753.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's 'The Composition of Foods'* 4th revised ed. London: HM Stationery Office.
Peers, F. G. (1953). *Biochem. J.* **53**, 102.
Pringle, W. J. S. & Moran, T. (1942). *J. Soc. chem. Ind.* **61**, 108.
Reinhold, J. G. (1975). *J. Am. Diet. Ass.* **66**, 38.
Taylor, T. G. (1965). *Proc. Nutr. Soc.* **24**, 105.
Widdowson, E. M. (1941). *Nature, Lond.* **148**, 219.
Zook, E. G., Greene, F. E. & Morris, E. R. (1970). *Cereal Chem.* **47**, 720.

Printed in Great Britain