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Destruction of Phytic Acid During Home Breadmaking

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ABSTRACT

The loss of phytate in home-made proprietary yeast bread mixes was less in brown (22–58%) than in white (67–90%) mixes. Since the initial phytate content was higher (0·36–0·52 g phytic acid/100 g DM), the brown loaves contained more phytate in the final product than did the white loaves (0·22–0·52 g compared with 0·01–0·06 g phytic acid/100 g DM). Bread made from conventional ingredients and 100% wholemeal flour lost 30–48% phytate during making and contained 0·52–0·71 g phytic acid/100 g DM. White bread made from conventional ingredients lost all of the initially low amount of phytate present. Phytate was lost at all stages of making (mixing, kneading, proving and baking). Very little phytate was lost in unleavened wholemeal breads made from a proprietary mix or from conventional ingredients.

INTRODUCTION

Bread is an important foodstuff in western-type diets, although it does not assume the same importance as in some countries in the Middle East and Asia. In rural Iran, for example, unleavened bread made from wheaten wholemeal provides about half of the daily energy and protein intakes (Reinhold *et al.*, 1974). Cereals (including wheat) are one of the main dietary sources of phytate (*myo*-inositol hexaphosphate) which has an

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inhibitory influence on mineral availability in the gut, notably of calcium (Harrison & Mellanby, 1939), magnesium (Roberts & Yudkin, 1960), zinc (O'Dell, 1969, 1984) and perhaps iron (Sharpe *et al.*, 1950; Morris & Ellis, 1975). The amount of phytate in the diet is therefore of practical importance, as was demonstrated initially by McCance & Widdowson (1942*a*, *b*) in relation to calcium nutrition on diets based on brown or white bread. Recent suggestions that dietary zinc intake might be marginal in America (Sandstead, 1973) and in Scotland (Lyon *et al.*, 1979) and the recognition that availability of zinc is an important consideration in determining dietary requirements (WHO, 1973; National Academy of Sciences, 1980) imply that dietary phytate is still an important consideration. The loss of phytate during normal food preparation therefore also deserves investigation.

Previous studies on the loss of phytate during breadmaking have identified factors influencing the extent of destruction, usually in reference to a commercial process. The present study reports the loss of phytate at each stage of preparing home-made bread using both proprietary bread mixes and conventional basic ingredients for yeast breads and for soda breads.

MATERIALS AND METHODS

Breadmaking

All loaves were made by one person in as standard a manner as possible to ensure comparability of results. Proving was carried out in an egg incubator with a closely regulated temperature; loaves were baked in a standard laboratory oven.

Proprietary yeast bread mixes

Three brands of proprietary bread mixes ('GS', 'MD' and 'L') were purchased from retail outlets and made according to instructions. Four loaves of each brand and each type of bread were prepared. Tap water at 40°C was used for mixing the dry ingredients; kneading time was exactly 10 min; proving temperature was 37°C. Proving time was initially standardised at 35–40 min but had to be increased to up to 80 min to attain an acceptable loaf volume before baking, especially with the 'GS' brown loaves (see Table 1 for proving times). All loaves were baked in a non-stick pan at 220°C for 35 min. Five samples were taken for analysis during the making of each loaf: (1) before adding the liquid; (2) after mixing; (3) after kneading; (4) after proving; (5) after baking and cooling.

TABLE 1
Phytic Acid Content and Loss in Proprietary Yeast Bread Mixes

Type of bread	*Loaf*	*Av. proving time*[b] *(min)*	*Phytic acid*[a] *(g/100 g DM) Sample No.*[c] *1*	*2*	*3*	*4*	*5*	*% loss*
Brown	'GS'	80	0·52	0·47	0·43	0·32	0·22	58
			±0·04	±0·03	±0·05	±0·08	±0·05	±6
	'MD'	40	0·43	0·41	0·34	0·27	0·25	43
			±0·07	±0·06	±0·07	±0·08	±0·06	±6
	'L'	35	0·36	0·37	0·31	0·30	0·28	22
			±0·08	±0·10	±0·08	±0·08	±0·07	±8
White	'GS'	40	0·10	0·10	0·10	0·03	0·01	90
			±0·008	±0·008	±0·007	±0·008	±0·001	±10
	'MD'	60	0·17	0·13	0·11	0·06	0·03	84
			±0·03	±0·03	±0·03	±0·03	±0·02	±9
	'L'	55	0·18	0·16	0·15	0·09	0·06	67

[a] Values are the mean ±SE of four loaves except for white 'L' (mean of three loaves).
[b] Proving time was increased as necessary to attain a sufficient loaf volume before baking.
[c] Samples were numbered according to the stage of sampling: (1) before adding liquid; (2) after mixing; (3) after kneading; (4) after proving; (5) after baking and cooling.

Yeast breads using conventional ingredients

Wholemeal stone-ground flour (100% extraction) and strong white bread flour were purchased from retail outlets, as well as fresh yeast and dried yeast (Allinson's 'active' yeast). Wholemeal bread loaves were made with the following quantities of ingredients: 230 g flour, 7·5 g dried yeast or 12·5 g fresh yeast, 18·5 g salt, 7·5 g butter, 15·0 g brown sugar, approximately 220 ml water at 40°C. Four loaves, each with fresh yeast or dried yeast, were prepared. In addition, four loaves with fresh yeast and $CaCO_3$ added to the flour to the same level as in white flour, and four loaves with dried yeast and 12·5 g vitamin C were prepared.

White bread with fresh yeast was made with the same quantities of ingredients as for wholemeal bread except that sugar was not added. Except for those with vitamin C, all loaves were kneaded for 10 min, proved for 35–40 min at 37°C, knocked back, shaped and proved again at 37°C for 30 min, and baked at 220°C for 35 min. The loaves with vitamin C were rested for 5 min at room temperature in place of the first proving; the shaped loaf was then proved at 37°C for 40 min before baking.

Some additional loaves of both wholemeal and white bread were made with fresh yeast which had been stored for various lengths of time since purchasing to determine the influence of age of the yeast on phytate hydrolysis. The yeast was stored in tinfoil at 5°C.

Samples from all loaves were taken for analysis (1) before adding the liquid; (2) after mixing; (3) after kneading; (4) after the first proving (or rest); (5) after the second proving; (6) after baking and cooling.

Soda breads

Four loaves each of a proprietary mix ('H') were made with water at 40°C according to the instructions. As well, four loaves of wholemeal soda bread were made with the following quantities of ingredients (Paul & Southgate, 1978): 250 g wholemeal flour, 1·5 g bicarbonate of soda, 1·5 g cream of tartar, 6·0 g salt, approximately 150 ml milk. The wholemeal flour was from the same source as for the wholemeal yeast breads. All loaves were baked for 40 min at 220°C. Samples were taken for analysis: (1) before adding liquid; (2) after mixing; (3) after baking and cooling.

Methods

Samples taken during breadmaking were dropped immediately into liquid nitrogen; the final sample was a slice from the middle of the loaf and included both the crust and the crumb. All samples were stored at −40°C and then lyophilised and ground finely. Phytate analyses were done in triplicate on 0·1–0·5 g samples according to the method of Davies & Reid (1979).

RESULTS

Proprietary yeast bread mixes

The initial phytate concentration in the brown bread mixes (Table 1) indicated that none was composed solely of flour of 100% extraction. Loaves from all three brands had about the same final phytate concentration (0·22–0·28 g/100 g) but since the 'L' mixes had a lower initial phytate content the loss was less (22%) than for the other brands (58% and 43%). The white bread mixes had a low initial phytate content (0·10–0·18 g/100 g) and for some loaves for all brands there was no measureable phytate in the final sample. Overall, the loss of phytate was high in the white loaves (67–90%).

Although the highest rate of phytate loss occurred more often than not during mixing or kneading (Table 2), the greatest amount of destruction appeared to occur during proving (sample 4) with up to two-thirds of the loss occurring then in some white loaves (Table 1). Proving time did not have a consistent influence on phytate loss. For example, in one 'GS' brown loaf, proved for 120 min, the proving stage accounted for the loss

TABLE 2
Rate of Loss of Phytic Acid in Proprietary Yeast Bread Mixes

Type of bread	*Loaf*	*Rate of loss*[a] *(g/100 g DM/min)*			
		During mixing	*During kneading*	*During proving*	*During baking*
Brown	'GS'	0·018	0·004	0·008	0·003
		±0·006	±0·002	±0·004	±0·001
	'MD'	0·007	0·006	0·001	<0·001
		±0·005	±0·002	± <0·001	± <0·001
	'L'	0·005	0·006	0·002	<0·001
		±0·003	±0·002	±0·001	± <0·001
White	'GS'	<0·001	<0·001	0·002	<0·001
		± <0·001	± <0·001	± <0·001	± <0·001
	'MD'	0·015	0·002	<0·001	<0·001
		±0·008	± <0·001	± <0·001	± <0·001
	'L'	0·008	0·002	<0·001	0·001
		±0·005	±0·001	± <0·001	± <0·001

[a] Values are the mean ±SE of four loaves except for White 'L' (three loaves); see text and Table 1 for times for each step.

of 62% of the initial phytate content (total loss, 66%) whereas, in another 'GS' brown loaf, proved for 130 min, only 9% of the initial phytate content was lost during proving (total loss, 61%). In a third 'GS' brown loaf, proved for 40 min, 13% of the initial phytate content was lost during that time. In most loaves (brown and white) there was some loss of phytate at all stages during making; the slowest rate of loss often occurred during baking (Table 2).

Yeast breads using conventional ingredients

Standardization of proving times was possible (Table 3) because there was no variability in loaf volumes after proving with either the fresh or the dried yeast. Dried yeast appeared to promote a greater loss of phytate in the wholemeal loaves compared with fresh yeast (48% versus 30%) mainly because of a more rapid loss during mixing (Table 4). Addition of calcium to the flour did not affect the total amount of phytate destroyed, although there was no loss of phytate during mixing with calcium present. The use of vitamin C appeared to result in less phytate being destroyed compared to the use of dried yeast without vitamin C present. It seemed that the reduced total loss was because the first proving time was replaced with a 5-min rest period when vitamin C was used, i.e. there was insufficient time for any measureable phytate loss to occur.

TABLE 3
Phytic Acid Content and Loss in Homemade Yeast Breads

Type of bread	*Type of yeast*	*Other additions*	*Proving time (min)*		*Phytic acid[a] (g/100 g DM) Sample No.[b]*					*% loss*
			a	*b*	*1*	*2*	*3*	*4*	*5*	*6*	
Wholemeal	Fresh		40	30	1·01	0·94 ±0·01	0·88 ±0·01	0·83 ±0·01	0·77 ±0·03	0·71 ±0·01	30 ±1
	Fresh	Ca	35	30	1·01	1·06 ±0·03	0·93 ±0·03	0·80 ±0·02	0·78 ±0·01	0·71 ±0·01	30 ±1
	Dried		35	30	1·01	0·87 ±0·01	0·79 ±0·01	0·66 ±0·02	0·58 ±0·03	0·52 ±0·05	48 ±5
	Dried	Vit. C	5	40	1·01	0·88 ±0·03	0·79 ±0·06	0·79[c] ±0·04	0·64 ±0·05	0·60 ±0·02	40 ±2
White	Fresh		40	30	0·19	0·16 ±0·03	0·12 ±0·04	0·05 ±0·02	0	0	100

[a] Values are the mean ±SE of four loaves.
[b] Sample numbers correspond to the stage of sampling: (1) before adding liquid; (2) after mixing; (3) after kneading; (4) after first proving; (5) after second proving; (6) after baking and cooling.
[c] 5-min rest in place of first proving.

TABLE 4
Rate of Loss of Phytic Acid in Homemade Yeast Bread

Type of bread	*Type of yeast*	*Other additions*	*Rate of loss[a] (g/100 g DM min)*				
			During mixing	*During kneading*	*During first proving*	*During second proving*	*During baking*
Wholemeal	Fresh		0·020 ±0·004	0·006 ±0·001	<0·001 ± <0·001	0·002 ±0·001	0·002 ± <0·001
	Fresh	Ca	0	0·008 ±0·002	0·003 ± <0·001	<0·001 ± <0·001	0·002 ± <0·001
	Dried		0·047 ±0·005	0·008 ±0·001	0·004 ± <0·001	0·003 ± <0·001	0·001 ± <0·001
	Dried	Vit. C	0·045 ±0·008	0·006 ± <0·001	0[b]	0·003 ± <0·001	0·002 ± <0·001
White	Fresh		0·017 ±0·008	0·003 ±0·001	0·001 ± <0·001	0·002 ±0·001	0

[a] Values are the mean ±SE of four loaves; see text and Table 3 for times for each step.
[b] 5-min rest in place of first proving.

TABLE 5
Phytic Acid Loss in White and Wholemeal Breads According to the Age of the Fresh Yeast

Time since purchase of the yeast (days)	*% Phytate loss during making*	
	White bread	*Wholemeal bread*
2		29
3		31
5	100	
8		30
13	100	
17		66
17		55
21		48
23	100	
28		33
32	53	
35		31
39	74	
45	37	

White bread, starting with a much lower content (0·19 g/100 g), had no measureable phytate remaining in the loaf by the end of the second proving. However, when the yeast was stored for more than 30 days, not all of the phytate was destroyed (Table 5) even though there was still sufficient leavening action to raise the loaf adequately during a lengthened proving period, with the exception of the 45-day old yeast. In the wholemeal loaves, made with yeast stored for 17–21 days, there was an increased phytate loss (48–66%), i.e. the rate of loss was accelerated at all stages except during kneading. Phytate loss then returned to what it had been (about 30%) with the newer yeast although, as for the white loaves, a longer proving time was required. Since the loss was already 100% in the white loaves it was not possible to observe whether there had been a similar phenomenon in them with the intermediate-aged yeast.

Soda breads

The proprietary mix did not have as high an initial phytate content (0·65 g/100 g) as did the 100% extraction flour (1·01 g/100 g; Table 6) even though the list of ingredients included wheat bran and wheat germ. There was no loss of phytate in the loaf made from the mix, and only a small loss (9%), mainly during baking, with the conventional ingredients. In those loaves the rate of loss during mixing (0·007 $\pm$ 0·02 g/100 g DM/min) was slower than in most of the wholemeal homemade yeast breads, while during baking it was similar (0·001 $\pm$ $<$0·001 g/100 g DM/min).

TABLE 6
Phytic Acid Content and Loss in Unleavened Wholemeal Breads

Type of mix	*Loaf*	*Phytic acid[a] (g/100 g DM) Sample No.[b]*			*% loss*
		1	*2*	*3*	
Proprietary (bran bread)	'H'	0·65 ±0·008	0·64 ±0·006	0·65 ±0·007	0
Homemade (wholemeal)		1·01	0·99 ±0·02	0·92 ±0·04	9 ±3

[a] Values are the mean ±SE of four loaves.
[b] Sample numbers correspond to the stage of sampling: (1) before adding liquid; (2) after mixing; (3) after baking and cooling.

DISCUSSION

There is not good agreement in the literature on the extent of phytate destruction during breadmaking, nor on the source of the phytase activity in a fermented dough. Widdowson (1941) reported 85% destruction in a 70% extraction white flour bread, 69% destruction in an 85% extraction bread, and only 31% destruction in a 92% extraction bread; she pointed out that the naturally occurring phytase in wheat flour could destroy phytic acid during 'accepted cooking practices'. Pringle & Moran (1942) then reported that, normally, 50–60% of phytate is destroyed during breadmaking using 85% extraction flour, and they also attributed most of the loss to the action of phytase in the flour.. Mellanby (1944) considered that yeast was rich in phytase and reported that, in a mixture of 2·1 g yeast per 100 g flour (85% extraction) proved for 2 h, the yeast phytase and the naturally occurring wheat phytase appeared to be equally active in producing a total loss of 71% of the phytate originally present. Reinhold *et al.* (1974) reported that only 15–25% of phytate was hydrolysed in wholemeal bread and also stated that the phytate of wholemeal flour of high extraction was resistant to destruction by yeast phytase unless the yeast concentration was increased to 4–5% and fermentation time was increased. Ranhotra *et al.* (1974) have observed 100% loss of phytate in white bread. Tangkongchitr *et al.* (1981) speculated that the change in pH of the dough was the main mechanism whereby yeast fermentation accelerated loss of phytate and subsequently concluded that, contrary to some previous proposals (Reinhold *et al.*, 1974; Harland & Harland, 1980), no phytase enzyme is associated with yeast cells (Tangkongchitr *et al.*, 1982). After 3 h of fermentation of a whole wheat dough containing 2%

yeast, approximately 34% of phytate had been lost; after 6 h 43% had been lost.

The results from the present study confirm that in white bread most, or all, of what little phytate is present is destroyed, and that, very generally, as the extraction rate of the flour increases, the phytate content increases but the percentage destruction of phytate decreases, resulting in a higher final concentration of phytate in bread made from 100% extraction flour. There was about twice as much phytate in the yeast breads made with 100% extraction flour than in the brown yeast breads made from the proprietary mixes.

The factors reported to affect the extent of the phytate destruction during breadmaking are the species and variety of wheat (Peers, 1953), the extraction rate of the flour (Widdowson, 1941; Reinhold *et al.*, 1974), freshness of the flour (Mollgaard *et al.*, 1946), the amount of yeast present (Reinhold *et al.*, 1974; Ranhotra *et al.*, 1974; Harland & Harland, 1980), leavening time (Widdowson, 1941; Pringle & Moran, 1942; Harland & Harland, 1980) and temperature (Mollgaard *et al.*, 1946; Peers, 1953), pH of the dough (Widdowson, 1941; Pringle & Moran, 1942; Mollgaard *et al.*, 1946; Peers, 1953; Tangkongchitr *et al.*, 1982), water content of the dough (Mollgaard *et al.*, 1946), presence and type of calcium salt added (Pringle & Moran, 1942; Ranhotra, 1972), presence and concentration of oxy acids (Mollgaard *et al.*, 1946), permitted additives such as vitamin C (Ranhotra, 1972) and the solubility of phytate, especially of its magnesium salt (Tangkongchitr *et al.*, 1982). The fermentation of both yeasts and other sour dough organisms has been assumed to cause the destruction of phytate (Reinhold *et al.*, 1974; Harland & Harland, 1980). Mollgaard *et al.* (1946) had earlier observed the loss of 39–49% of the phytate in bread made from 92% extraction rye flour and fermented with a lactic acid bacillus. Conversely, Tangkongchitr *et al.* (1982) found no phytase enzyme activity associated with yeast cells.

A comparison between fresh yeast and dried yeast in their effect on phytate destruction does not appear to have been made previously. The greater loss of phytate with dried yeast was diminished when vitamin C was used as well. Ranhotra (1972) has reported that 20 mg vitamin C per 100 g flour stimulated phytate hydrolysis in a dough (albeit slightly) while 100 mg vitamin C per 100 g flour inhibited phytate hydrolysis. The proportion of vitamin C used in this study (5·4 mg vitamin C per 100 g flour) was similar to that recommended in recipe books for homemade bread. However, the difference in the final concentrations of phytate in the loaves with or without vitamin C was not great, so that there was still less phytate in those loaves than in the ones made with fresh yeast.

Calcium carbonate has previously been reported (Pringle & Moran,

1942) to have no appreciable effect on phytate hydrolysis although Mollgaard *et al.* (1946) considered that $CaHPO_4$ had an advantage over $CaCO_3$ because the presence of $CaCO_3$ inhibited phytate hydrolysis. Ranhotra (1972) found that all four calcium salts tested (carbonate, acetate, sulphate, lactate) inhibited phytate hydrolysis. Tangkongchitr *et al.* (1982) concluded that the native levels of calcium in a dough did not cause phytate to precipitate and thus be unavailable to hydrolysis; in more concentrated systems, some phytate phosphorus was insoluble. In the present study the added calcium carbonate in the wholemeal flour had no effect on phytate destruction.

Contrary to expectation, proving time did not seem to affect the amount of phytate lost in the 'GS' brown loaves. Although there were no obvious identifications on the packet to indicate packaging date or batch number, perhaps the age of the ingredients was an explanation since it has been reported (Mollgaard *et al.*, 1946) that, after flour had been kept at room temperature for a month, the phytase content—and hence the phytase activity—in the subsequent dough, was diminished. The leavening activity of the (dried) yeast might also be diminished in an older mix.

The greater loss of phytate in the wholemeal bread made with fresh yeast stored for about 20 days compared with just a few days after purchase is difficult to interpret. However, for both the white and wholemeal loaves the decreased leavening activity in the very old yeast was, not surprisingly, associated with a lesser destruction of phytate compared with the intermediate-aged yeast.

In the present study phytate was lost at all stages of making bread in most of the loaves; a loss occurred even in the few minutes required to mix the liquid with the dry ingredients. The greatest proportion was lost usually during proving and the hydrolysis continued during baking, at least presumably in the earlier part of baking, since at pH 6 (the pH of a whole wheat dough, Tangkongchitr *et al.*, 1982) phytase is relatively thermostable and is not destroyed until the temperature reaches 70–100° (Mollgaard *et al.*, 1946; Peers, 1953). Little destruction was expected as a result of baking itself since autoclaving a moist slurry of wheat at 115°C for 2 h resulted in only 5% loss of phytate (de Boland *et al.*, 1975).

It is clear that the destruction of phytate, whether by enzymatic or by chemical hydrolysis, is largely prevented in the making of soda bread, so that the high phytate concentration remains. Widdowson (1941) likewise reported only 5% destruction of phytic acid in a baking powder bread made with 92% extraction flour.

Zinc is the trace element of most concern when considering mineral availability from foods high in phytate. Davies & Olpin (1979) showed that the molar ratio of phytate and zinc in a diet gave the most satisfactory

indication of zinc availability and that, for the rat, phytate:Zn ratios exceeding 15–20 resulted in low hair and plasma zinc concentrations and low growth rates. Wholemeal bread in the United Kingdom has a phytate: zinc ratio of about 20:1 (Paul & Southgate, 1978) so that zinc might not be as available from wholemeal bread as from other foods containing less phytate. Any suggestion that the zinc content of a diet could be increased, for example, by substituting white flour with wholemeal flour (Lyon *et al.*, 1979) in a population which might have a marginally low zinc intake, therefore needs careful evaluation.

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