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Dephytinisation of wheat bran and the consequences for fibre matrix non-starch polysaccharides

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Wheat bran is a rich source of dietary fibre. Modified milling technologies can yield fibre-enriched bran fractions but the processing also leads to a concentration of phytate, which has a perceived detrimental effect on mineral metabolism. Endogenous phytase activity in wheat grain can be used to reduce phytate levels, and methods to optimise phytase activity have been investigated using aleurone-enriched and pericarp-enriched bran fractions. It was found that dephytinisation of wheat bran could be achieved at a relatively low moisture content for each fraction. The extent of dephytinisation could be controlled either through control of moisture content or through time of incubation. By using yeast, the rate of dephytinisation at 35°C was similar to that achieved by endogenous phytase alone at 55°C. The endogenous phytase activity resulted in the destruction of phytate without accumulation of inositol phosphate intermediates, as monitored using ^{31}P-NMR spectroscopy. Also, at low moisture levels there was no apparent modification of fibre components through polysaccharidase activities which may be present in the bran. Therefore bran fractions can be readily dephytinised at low moisture contents and without modification of the fibre components. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Wheat bran, whilst a rich source a dietary fibre and with a widespread use as a supplement in many food products, is also a source of dietary phytate (McCance & Widdowson, 1943; Mellanby, 1950; Cheryan, 1980; Ryden & Selvendran, 1992). The strong association between phytate (*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) and important dietary cations and the perceived consequences for mineral metabolism (Morris, 1986; Hallberg *et al.*, 1987; Reddy *et al.*, 1989; Persson *et al.*, 1991) has led to caution in the use of wheat bran as a fibre supplement in foods. With innovation in milling techniques, it is now possible to produce 'fibre-enriched' wheat bran, but if the enrichment includes aleurone layer tissue and contents then such products are also enriched in phytate. Thus, benefits resulting from fibre concentration through milling may be lost unless phytate levels can be controlled.

Phytate is susceptibe to degradation during the fermentation of bread dough (Harland & Harland, 1980; Giovanelli & Polo, 1994) and this involves phytase activity (*myo*-inositol hexaphosphate phosphorylase) from yeast as well as endogenous phytase in flour (Peers, 1953; Fretzdorff & Brummer, 1992). Hence mechanisms exist to achieve a reduction in phytate concentration, although such mechanisms operate under relatively low phytate concentrations compared to concentrations that can be present in bran supplements. Whilst yeast activity should not affect non-starch polysaccharides (NSP), the activation of endogenous phytase is associated with germination activity in the grain, phytase activity showing around a six-fold increase after wheat grain hydration and the onset of germination (Peers, 1953; Bartnik & Szafranska, 1987). During germination, or malting, phytase activity increases with germination time and several days are required to achieve high activity (Larsson & Sandberg, 1992). The activation is related to hydration of the grain as well as germination activity in the seed. The activation of endogenous phytase could effect phytate breakdown in cereal bran but the feasibility of this approach to dephytinise bran for food use will depend on the rate and extent of dephytinisation that can be achieved.

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Also, there is the possibility that enzymes in bran which are involved in NSP breakdown could be activated along with phytase.

When investigating endogenous phytase activity, fully hydrated systems have been used, but with high hydration levels there is also the potential for both degrading the fibre matrix and generating waste waters. This would limit the attraction of using endogenous phytase for large-scale preparation of phytate-depleted wheat bran.

The objective of this study was to determine whether endogenous phytase activity could be optimised to reduce phytate concentration at low levels of hydration and also whether activation of phytase was associated with an activation of polysaccharide-degrading enzymes which could lead to a significant modification of the fibre matrix.

MATERIALS AND METHODS

Materials

Wheat bran fractions were supplied by Sofalia, Ennezat, France, as fibre-enriched products. Samples of wheat bran had been seived prior to attrition milling to 80 μm mean particle size and air-classification to enrich in either aleurone tissue or pericarp tissue. Recovery of the original bran in the air-classified fractions was 60% aleurone-enriched and 40% pericarp-enriched (Table 1). Samples were stored in sealed polythene bags at 4°C until required for analysis.

Methods

Bran samples were analysed for moisture, protein, starch, NSP, lignin and phytate. Moisture (6%) was determined after drying to constant weight in a forced draught oven (105°C) overnight. Protein was determined as Kjeldhal nitrogen (N×6.25).

Starch was estimated from samples washed in 80% (v/v) ethanol to remove low molecular weight materials followed by hydrolysis in 1 M H_2SO_4 and quantification of glucose released using gas–liquid chromatography (GLC; see below). A 1 M hydrolysis of corresponding samples after isolation of NSP was used to allow for non-starch glucose released by 1 M H_2SO_4. Net glucose values were multiplied by 0.9 to give a value for starch: i.e. starch = 0.9 × [(total GLC-released) – (GLC-released starch-free sample)]. Fibre analysis was as non-starch polysaccharides (NSP) in samples before and after dephytinisation treatments. The standard Faulks & Timms (1985) method was used for NSP isolation, except that, after amyloglucosidase treatment, the digest was centrifuged (2500*g*, 10 min) to separate and recover soluble NSP in the supernatant. The supernatant was precipitated in 80% (v/v) ethanol to recover soluble NSP. The water-insoluble pellet and ethanol-insoluble fraction were each dialysed (12000 MW cut-off; 3×5 litres distilled water) then freeze-dried prior to analysis for neutral sugars and uronic acid.

Neutral sugars were analysed as their alditol acetates following Saeman hydrolysis, i.e. 72% (w/w) H_2SO_4 for 3 h followed by dilution to 1 M and hydrolysis for 2.5 h at 100°C (Selvendran *et al.*, 1979), and also using 1 M H_2SO_4 to distinguish cellulosic and non-cellulosic glucose. Uronic acids were determined colorimetrically as total uronic acid using a 1 h 1 M H_2SO_4 sample hydrolysate (Blumenkrantz & Absoe-Hansen, 1973).

Lignin was determined using Saeman hydrolysis of a 50 mg sample digested with 2 ml of 72% H_2SO_4, followed by the addition of 22 ml of deionised water and hydrolysis at 100°C for 2.5 h. The residue remaining was recovered by filtration (sinter crucible No. 3), washed to neutrality and dried to constant weight (106°C, 16 h).

Phytate concentration was determined from the extraction of total phosphate in the sample and from the difference between total phosphate and inorganic phosphate by anion-exchange chromatography (Harland & Oberleas, 1986):

$$P_{phy} = P_t - P_i$$

where P_{phy} is phytate phosphate, P_t is total phosphate and P_i is inorganic phosphate. Preliminary experiments confirmed that phytate was the major source of phosphate; in bran (≈90%) sugar phosphates were less than 3% and inorganic phosphate represented less than 5%

Table 1. Recovery of protein, starch, fibre and phytate from bran samples before dephytinisation treatments

Sample	Recovery after milling (%)	Composition[a] (g per 100 g bran)					Phytate (mg per 100 g)	Sample recovery (%)
		Protein[b]	Starch[c]	NSP	Lignin	Total fibre		
Aleurone	60	18.6	16.4	35.2	6.0	41.0	4.1	76.2
Pericarp	40	13.5	7.1	53.9	9.7	63.6	3.7	84.2

[a]Moisture 6%.
[b]N×6.25.
[c]Measured indirectly through acid hydrolysis.

of total phosphate. Dephytinisation was monitored from the loss of total phosphate from bran or the relative increase in inorganic phosphate after experimental treatments. Phytate was determined as the proportion of phosphate from phytate, as the inositol hexaphosphate sodium salt, i.e. phytate = $P_{phy} \times 3.55$.

Dephytinisation treatments involved either leaching bran in excess water or incubating with limited moisture present.

Leaching

Bran samples (1 g) were incubated in 20 volumes of water, acidified to pH 5.1 with HCl, for 30 min at room temperature with stirring. The bran was recovered by centrifugation (1250*g*) and the pellet washed twice with distilled water before freeze-drying. The total volume of leachate was recorded and a sample of the combined supernatant was frozen (−20°C) and retained for phytate analysis.

Limited moisture

Incubation under limited moisture conditions involved mixing the bran with known ratios of acidified water (as above) and incubating the mixture at 55°C, to optimse phytase activity, (Peers, 1953) for set time periods. After incubation each sample was frozen and freeze-dried prior to phytate analysis.

Effects of exogenous phytase activity, under limited moisture conditions, were tested using an actively fermenting bakers yeast culture incubated with bran (35°C). Allinson dried yeast (Westmill Foods Ltd., UK) was activated as a 0.5% suspension in a 2% sucrose solution containing 0.05% ammonium sulphate and incubated for 2 h at 35°C, with further sucrose, 1 g per 100 ml, then added to the actively fermenting culture to provide an energy source for the yeast during incubation with the bran. To encourage yeast phytase activity during incubation with the bran, no mineral supplement was provided. After incubation with the yeast, bran samples were frozen and freeze-dried prior to analysis for phytate.

NMR spectroscopy

The progression of dephytinisation was monitored using ^{31}P-NMR spectroscopy (Frolich *et al.*, 1988), modified to obtain spectra at pH 5 rather than at pH 13. Samples of bran recovered after incubation at a moisture:bran ratio of 2:1 were extracted in HCl and the spectrum for phytate was compared to an extract of the original bran and to a phytate/phytase standard. The phytate standard was a 1% solution of sodium phytate at pH 5.1 and the phytase standard was wheat phytase (Sigma). The standards were incubated at 55°C at a phytate: phytase ratio of 5:1. Samples (10 ml) were removed at intervals during the incubation, with 2 M HCl (10 ml) added to stop enzymic activity. Samples tested for endogenous phytase activity had also been incubated at 55°C, for either 6 h or 18 h. The samples were extracted in 20 volumes of 2.4% HCl for 2 h with stirring followed by centrifugation (2000*g*, 20 min) to recover the supernatant.

Aliquots of standards and sample extracts (5 ml) were combined with EDTA–NaOH (Harland & Oberleas, 1986) used for phytate analysis (8 ml) and the pH was adjusted to 5 prior to dilution to 25 ml for NMR spectroscopy. The NMR spectra were recorded at 161.7 MHz on a JEOL GX-400 spectrometer, without lock, and 85% H_3PO_4 as external reference.

RESULTS AND DISCUSSION

Composition of the enriched fractions

Analysis of the aleurone-enriched and pericarp-enriched fractions (Table 1) confirmed that an enrichment for fibre had occurred but also that there remained a cross-contamination of pericarp and aleurone tissues in each fraction. Similarly, the persistence of starch in the fractions indicated that residual endosperm materials were present and, from the relative proportions of starch, that the aleurone-enriched fraction was the fraction most heavily contaminated by endosperm. Whilst there was a 1.5-fold concentration of NSP in the pericarp-enriched samples, at 53.9% NSP compared to 35.2% for the aleurone-enriched sample, the pericarp-enriched fraction had a phytate content of 3.7 mg per 100 g, similar to that of the aleurone-enriched fraction (4.1 mg per 100 g) (Table 1). Phytase in wheat is located mainly in the scutellum layer and endosperm (Peers, 1953; Frolich, 1994) whilst phytate is concentrated in the aleurone tissues (Pomeranz, 1988). Therefore it was expected that with the apparent greater contribution of endosperm to the aleurone-enriched fraction this fraction would have a higher phytase activity, despite the similarity in phytate content between fractions. The protein content of the pericarp-enriched fraction (13.5%), however, was higher than expected. This may indicate that during fractionation there was a redistribution of cell contents resulting from cell disruption during milling. The overall sample recovery (76% aleurone, 84% pericarp) indicated that low molecular weight cell contents were present in each fraction.

The aleurone-enriched fraction contained 6% lignin, which derives from the pericarp layers of the bran and not aleurone tissues (Selvendran *et al.*, 1987) compared to 9.7% lignin for the pericarp-enriched fraction. Together, the lignin and NSP gave the aleurone-enriched fraction and the pericarp-enriched fraction a fibre content of 41% and 63.6%, respectively. This corresponds to the fibre content of standard brans of around 40% (Holland *et al.*, 1991) and 64% NSP for beeswing bran (Selvendran *et al.*, 1987). Thus, there was a significant enrichment of fibre but each fraction remained rich in phytate.

Dephytinisation treatments: preliminary experiments

The leaching treatment removed the bulk (>90%) of the phytate from both the aleurone-enriched and the pericarp-enriched fractions (Fig. 1). However, the treatment also resulted in a significant loss of the original bran (recovery dry weight of 66% and 78% for aleurone and pericarp fractions, respectively). The loss in weight was ascribed to the solubilisation of low molecular weight compounds, starch and protein but there was also some NSP degradation in the relatively short incubation period used for leaching (Table 3). Whilst leaching may result in a concentration of fibre in each fraction, the treatment will result in the loss of nutrients and also the generation of large volumes of waste waters, requiring disposal. From the analysis of the leached supernatant the increase in P_i and decrease in P_{phy} also indicated a significant degradation of phytate during the treatment period, P_i increasing from around 6% of P_t in the untreated sample to around 21% of P_t in the leachate. This was consistent with the presence of endogenous phytase and its activation after sample hydration (Peers, 1953; Bartnik & Szafranska, 1987; Sandberg & Svandberg, 1991) and also with the rapid dephytinisation noted for germinated wheat grains (Larsson & Sandberg, 1992).

Endogenous phytase treatment, for 6 h at a moisture: bran ratio of 1:1 (Fig. 1), resulted in a reduction in phytate concentration of each fraction of around 11 mg g^{-1} bran, equivalent to between 30% and 35% dephytinisation. Although this was less than the extent of dephytinisation achieved by leaching, there was little generation of waste water and samples could be dried to avoid loss of nutrients in waste water. Hence the stimulation of endogenous phytase activity could provide a convenient mechanism for phytase depletion in bran but without significantly affecting other components of the bran.

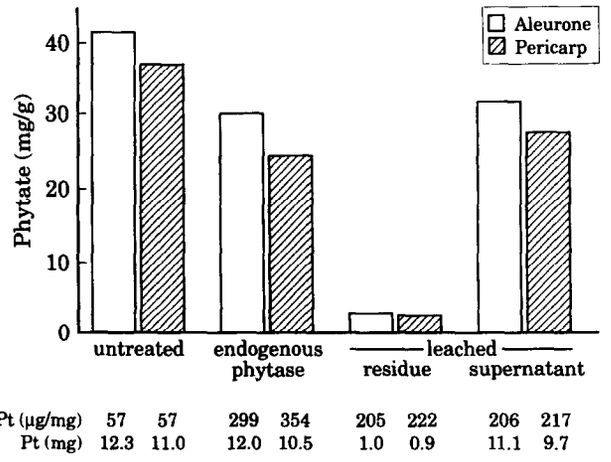

Fig. 1. Effect of dephytinisation procedures on phytate distribution in wheat bran. Dephytinisation was measured from the decrease in P_{phy} and increase in the proportion of P_i after different treatments.

Optimisation of dephytinisation

To optimise the conditions for dephytinisation, the effects of time and moisture content were investigated (Figs 2 and 3). From the measurement of water-holding capacity for bran (Robertson, 1988), saturation of each fraction was found to be between 4 and 5 ml g^{-1} bran. For both fractions, at a subsaturation moisture ratio of 2:1, the P_i:P_t ratio was >50% after a 6 h incubation. This ratio represented around 45% dephytinisation after allowance for P_i in untreated samples and gave an apparent rate of dephytinisation of $\approx$7.5% h^{-1}. This compared to around 35% dephytinisation at a moisture ratio of 1:1 (Fig. 2). Below a moisture ratio of 1:1 the extent of dephytinisation was greatly reduced in both fractions. Thus, hydration level is important for activating the phytase but significant activity can be achieved even at subsaturation levels. Above saturation levels (with leaching) the apparent rate of dephytinisation was increased ($\approx$30% h^{-1}) and this agreed with the rapid and extensive degradation of phytate in germinated wheat grains (Larsson & Sandberg, 1992).

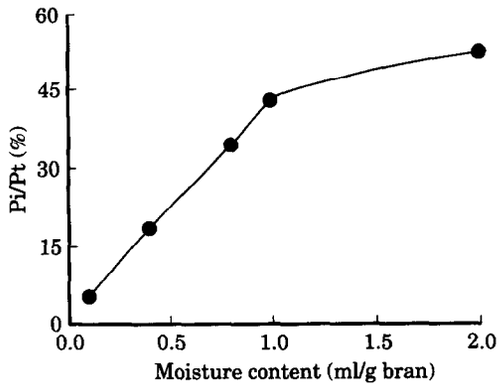

Fig. 2. Effect of moisture content on endogenous phytase activity. Samples were incubated for 6 h at known ratios of moisture:bran and extent of dephytinisation was estimated from the increased proportion of P_i in the samples.

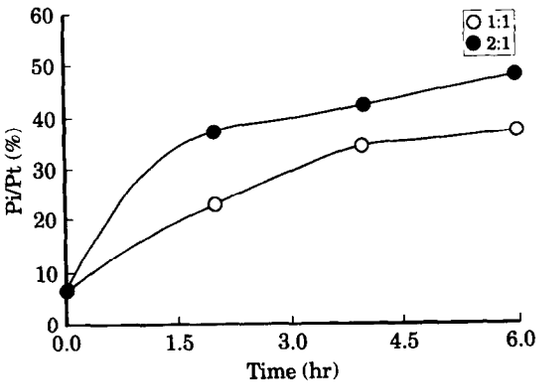

Fig. 3. Effect of time on endogenous phytase activity. Samples were incubated for set time periods at a moisture ratio of either 1:1 or 2:1 and extent of dephytinisation was estimated from the increased proportion of P_i in the samples.

The time course for dephytinisation (Fig. 3) showed that, at a moisture ratio of 2:1, dephytinisation progressed more rapidly in the initial 2 h of incubation. At a moisture ratio of 1:1 the rate of dephytinisation was slower, with around 4.5 h required to achieve a dephytinisation level corresponding to 1.5 h at a moisture ratio of 2:1. Thus, to achieve an equivalent extent of dephytinisation at a moisture ratio of 1:1, a much longer incubation period would be required. This could lead to an increased probability of microbial contamination becoming established in the bran. Extending the incubation period from 6 h to 18 h for a bran at a moisture ratio of 2:1 resulted in an 85% dephytinisation of the bran, similar to that achievable by leaching.

To determine whether increasing the amount of phytase could increase the rate of dephytinisation at reduced moisture levels, the bran fractions were incubated at 35°C with an actively fermenting yeast culture to supply exogenous phytase. A moisture ratio of 2:1 was used as being similar to the flour:liquid ratio used during breadmaking. Addition of the yeast to the bran fractions had no effect on the apparent rate or extent of dephytinisation, but when account is taken of the lower temperature of incubation using yeast and the reduced activity of endogenous activity at lower tempertatures (Bartnik & Szafranska, 1987) then the yeast phytase must have been contributing to the dephytinisation. It remains unclear whether the non- optimum temperature and pH used for the incubation with yeast (Nayini & Markakis, 1984), or subsaturation of the bran, limited the extent of dephytinisation by yeast phytase. Above a concentration of around 1 mM an extract of yeast phytase was inhibited by phytate and the enzyme was shown to have a higher relative activity against the lower inositol phosphates than against phytate (Nayini & Markakis, 1984). In the bran fractions, assuming a homogeneous distribution of phytate then, during incubation at a moisture ratio of 2:1, phytate concentration was around 250 μM. The inoculation with yeast/phytase was estimated to be around two-fold that reported when characterising phytase activity (Nayini & Markakis, 1984), which should be sufficient to prevent inhibition of the yeast phytase. Therefore, although endogenous phytase is an effective agent for dephytinising bran, with yeast phyase and lower incubation temperature a relatively rapid dephytinisation can be achieved. This may have advantages for the preparation of high-bran bakery products.

Characterisation of dephytinisation

Whilst it has been demonstrated that there is a dephytinisation of the bran fractions, it is also important to establish how the dephytinistion proceeds through the

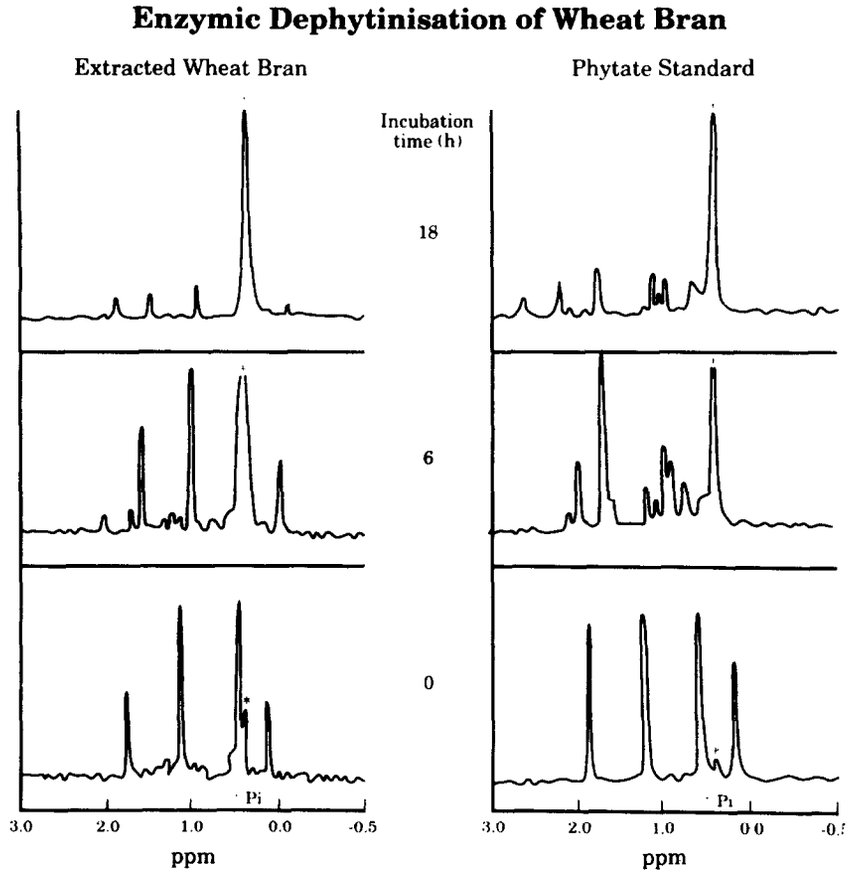

Fig. 4. Enzymic dephytinisation of wheat bran. ^{31}P-NMR spectra obtained for wheat bran and sodium phytate treated by phytase for 0–18 h periods of incubation at 55°C. *Pi indicates inorganic phosphate relative to phytate phosphate spectral peaks.

inositol phosphates, IP_6–IP_1. This was attempted using ^{31}P-NMR spectroscopy to monitor degradation of a standard phytate with a standard wheat phytase and in comparison to the degradation of phytate by endogenous phytase in the bran fractions, at a moisture ratio of 2:1. Dephytinisation profiles were obtained for samples incubated 0, 6 and 18 h to correspond with the experimental dephytinisation treatments. The spectrum for standard phytate (Fig. 4) had four major peaks, with resonances of intensity of approx. 1:2:2:1 as expected from the symmetry of inositol hexaphosphate (Frolich *et al.*, 1988). A minor peak, corresponding to P_i, was also present in the standard but was more noticeable in the bran sample. Otherwise, the spectra for bran and standard phytate showed good correspondence and confirmed that phosphate was present mainly as inositol hexaphosphate in the samples. As dephytinisation progressed, there was a marked increase in P_i in each sample and novel peaks were observed through the generation of lower inositol phosphates, IP_5–IP_1. In the bran samples, novel resonances were less noticeable as dephytinisation progressed. Thus the endogenous phytase treatments had resulted in a degradation of phytate from IP_6 without accumulation of inositol phosphate intermediates.

Effects on NSP

To determine whether the prolonged incubation conditions used for dephytinisation resulted in a modification to the fibre matrix, each fraction before and after treatment was analysed for fibre as NSP (Table 2). The untreated bran fractions were similar in composition, except for an increased proportion of xylose in the pericarp-enriched fraction, representing the increased presence of slightly branched arabinoxylans in the pericarp tissues. The major contribution to the NSP was from arabinose, xylose and glucose, representative of mainly arabinoxylans and cellulose and mixed-linkage β-glucans, respectively, in wheat bran (Du Pont & Selvendran, 1987). Neither the endogenous phytase treatment nor leaching affected the composition of the NSP from either fraction. The apparently increased recovery of NSP residues after leaching treatment was partly accounted for through the loss of protein and some soluble NSP during leaching. The lower recovery of NSP per gram of original bran (Table 3), however, indicated there was also some degradation of 'insoluble' NSP during leaching. Similarly, recovery of starch from the pericarp-enriched fraction was 7.0% after endogenous dephytinisation treatment and 6.3% from the corresponding leached sample. Compared to the 7.1% estimate for starch in the untreated sample, the treatments have also resulted in little if any stimulation of amylase activity.

The recovery of NSP per gram of original bran (from non-leached) was $\approx$34% for aleurone-enriched fraction and $\approx$51% for the pericarp-enriched fraction, compared to 36–43% reported for wheat bran (Holland *et al.*, 1991; Selvendran *et al.*, 1987), and recoveries were consistent with the bran being enriched in fibre. The NSP was recovered mainly as insoluble NSP (>93%) and the dephytinisation treatments had no effect on NSP solubility. Similarly, the proportion of 'cellulosics'

Table 2. Recovery of NSP from bran samples after dephytinisation treatments

	Recovery (g per 100 g bran)	Composition (mg g^{-1})							
		Rha + Fuc	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronics	Sugars
Aleurone									
Untreated									
Insoluble	51.5	Tracc	162	240	4	17	193	20	636
Soluble	19.9	Trace	23	41	4	8	29	14	119
Dephytinised									
Insoluble	48	Trace	181	268	4	17	193	22	685
Soluble	17.4	Trace	22	47	4	7	20	12	112
Leached									
Insoluble	62.7	Trace	156	231	4	16	192	27	626
Soluble	22	Trace	17	39	4	4	26	13	103
Pericarp									
Untreated									
Insoluble	74.7	Trace	148	323	3	13	186	23	696
Soluble	18	1	20	40	6	6	18	13	103
Dephytinised									
Insoluble	72.2	Trace	167	348	3	14	184	21	737
Soluble	19.9	Trace	18	35	5	6	26	12	102
Leached									
Insoluble	80	Trace	145	331	4	13	194	25	712
Soluble	18.5	Trace	16	39	5	4	17	14	95

Table 3. Distribution of NSP in bran samples before and after dephytinisation treatments

Sample	NSP (g per 100 g bran)			'Cellulosics' (% NSP)	X:A
	Soluble	Insoluble	Total		
Aleurone					
Untreated	2.4	32.8	35.2	20.9	1.5
Dephytinised	1.9	32.9	34.8	20.4	1.5
Leached	1.5	25.9	27.4	20.8	1.5
Pericarp					
Untreated	1.9	52.0	53.9	18.9	2.2
Dephytinised	2.0	53.2	55.2	19.2	2.1
Leached	1.4	44.4	45.8	19.0	2.3

X:A, xylose:arabinose.

and the ratio xylose:arabinose was unaffected by dephytinisation. The increased xylose:arabinose ratio for pericarp-enriched fractions reflected the increased presence of glucurono-arabinoxylans, and the increased proportion of 'cellulosics' in the aleurone fraction reflected the increased presence of mixed-linkage β-glucans from the aleurone.

Thus a dephytinisation of wheat bran can be achieved at relatively low moisture content, through activation of endogenous phytase. The endogenous phytase apparently becomes evenly distributed through different bran fractions during their isolation such that pericarp-enriched and aleurone-enriched fractions have a similar phytase activity. The extent of dephytinisation can be controlled, either through control of moisture content or through time of incubation. Endogenous phytase is important in reducing the phytate concentration in bran. But exogenous phytase can also make a significant contribution. The endogenous phytase activity results in the destruction of phytate without accumulation of inositol phosphate intermediates and, at low moisture levels, without resulting in modification to fibre components through polysaccharidase activities which may be present in the bran.

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REFERENCES

Bartnik, M. & Szafranska, I. (1987). Changes in phytate content and phytase activity during the germination of some cereals. *J. Cereal Sci.*, **5**, 23–28.

Blumenkrantz, N. & Absoe-Hansen, G. (1973). A new method for quantitative determination of uronic acids. *Anal. Biochem.*, **54**, 484–489.

Cheryan, M. (1980). Phytic acid interactions in food systems. *CRC Crit. Rev. Food Sci. Technol.*, **13**, 297–335.

Du Pont, M. S. & Selvendran, R. R. (1987). Hemicellulosic polymers from the cell walls of beeswing wheat bran: Part 1. Polymers solubilised by alkali at 2°. *Carbohydr. Res.*, **163**, 99–113.

Faulks, R. M. & Timms, S. B. (1985). A rapid method for determining the carbohydrate component of dietary fibre. *Food Chem.*, **17**, 273–287.

Fretzdorff, B. & Brummer, J.-M. (1992). Reduction of phytic acid during breadmaking of whole-meal breads. *Cereal Chem.*, **69**, 266–270.

Frolich, W., Wahlgren, M. & Drakenberg, T. (1988). Studies on phytase activity in oats and wheat using ^{31}P-NMR spectroscopy. *J. Cereal Sci.*, **8**, 47–53.

Frolich, W. (1994). Phytic acid as dietary constituent associated to dietary fiber. In *Physicochemical Properties of Dietary Fibre and Effect of Processing on Micronutrients Availability*, eds R. Amado, J.-L. Barry & W. Frolich. Proceedings COST 92 Action, Marseilles, October 1993. Commission of European Communities, Luxembourg, pp. 33–37.

Giovanelli, G. & Polo, R. (1994). Formation of fermentation products and reduction in phytic acid in wheat and rye flour breadmaking. *Ital. J. Food Sci.*, **1**, 71–83.

Hallberg, L., Rossander, L. & Skanberg, A.-B. (1987). Phytates and the inhibitory effect of bran on iron absorption in man. *Am. J. Clin. Nutr.*, **45**, 988–996.

Harland, B. F. & Harland, J. (1980). Fermentation reduction of phytate in rye, white and whole wheat breads. *Cereal Chem.*, **58**, 226.

Harland, B. F. & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: collaborative study. *J. Assoc. Off. Anal. Chem.*, **69**, 667–670.

Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A. A. & Southgate, D. A. T. (1991). In *McCance and Widdowson's The Composition of Foods*, 5th edn. Royal Society of Chemistry and MAFF, London.

Larsson, M. & Sandberg, A.-S. (1992). Phytate reduction in oats during malting. *J. Food Sci*, **57**, 994–997.

McCance, R. A. & Widdowson, E. M. (1943). Phytic acid and iron absorption. *Lancet*, **2**, 126–128.

Mellanby, E. (1950). *A Story of Nutritional Research*. Williams and Wilkins, Baltimore, Ch. 8.

Morris, E. R. (1986). Phytate and dietary mineral bioavailability. In *Phytic acid Chemistry and Application*, ed. E. Graf. Pilatus, Minneapolis, pp. 57–76.

Nayini, N. R. & Markakis, P. (1984). The phytase of yeast. *Lebensm. Wiss. Technol.*, **17**, 24–26.

Peers, F. G. (1953). The phytase of wheat. *Biochem. J.*, **53**, 102–110.

Persson, H., Nyman, M., Liljeberg, H., Onning, G. & Frolich, W. (1991). Binding of mineral elements by dietary fibre components in cereals—*in vitro* (III). *Food Chem.*, **40**, 169–183.

Pomeranz, Y. (1988). *Wheat Chemistry and Technology*, Vol. 1, ed. Y. Pomeranz. American Association of Cereal Chemists Inc., St. Paul, MN, p. 97.

Reddy, N. R., Pierson, M. D., Sathe, S. K. & Salunke, D. K. (ed) (1989). *Phytates in Cereals and Legumes*. CRC Press, Boca Raton.

Robertson, J. A. (1988). Physicochemical characteristics of food and the digestion of starch and dietary fibre during gut transit. *Proc. Nutr. Soc.*, **47**, 143–152.

Ryden, P. & Selvendran, R. R. (1992). Phytic acid: properties and determination. In *Encyclopedia of Food Science, Food Technology and Nutrition*, eds R. Macrae, R. K. Robinson & M. J. Sadler. Academic Press, London, pp. 3582–3587.

Sandberg, A. S. & Svandberg, U. (1991). Phytate hydrolysis by phytase in cereals: effects on *in vitro* estimation of iron availability. *J. Food Sci.*, **56**, 1330–1333.

Selvendran, R. R., March, J. F. & Ring, S. G. (1979). Determination of aldoses and uronic acid content of vegetable fibre. *Anal. Biochem.*, **96**, 282–292.

Selvendran, R. R., Stevens, B. J. H. & DuPont, M. S. (1987). Dietary fiber: chemistry, analysis and properties. *Adv. Food Res.*, **31**, 117–209.