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Phytate Reduction in Whole Grains of Wheat, Rye, Barley and Oats after Hydrothermal Treatment

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ABSTRACT

Whole grains of different cereals have traditionally been prepared with water and heat prior to dehulling, but knowledge of the effect on nutritional properties is limited. The aim of the present study was to investigate if phytate reduction occurred during hydrothermal treatment of whole grains. Wheat, rye, hulled and dehulled barley, hulled oats and naked oats were incubated with either water or acetate buffer (pH 4·8) at 55 °C for 24 h with the exception of oats, which were incubated at 37 °C. Phytate in wheat, rye and barley was reduced by 46–77% when water was used and by 84–99% when acetate buffer was used. The phytate reduction in oats was considerably less, 8–26%, but, after grinding and soaking, phytate was reduced by 72–77% in dehulled oats and by 88–94% in naked oats. Citric acid and citrate buffer was used for pH adjustment in some experiments, and their use resulted in less phytate reduction than when acetate and lactic acid were used. Wet-steeping of naked oats and naked barley in water at 53–57 °C for 20–30 min reduced the bacterial counts by 99·97%, and the addition of acid prevented bacterial growth during the incubations. It was concluded that cereals with reduced phytate content and good hygienic quality can be developed and produced using hydrothermal treatment of whole grains.

Keywords: whole grains, phytate reduction, hydrothermal treatment.

INTRODUCTION

Cereals have been a basic food for the past 10 000 years. Until the industrial period, cereals, such as barley and oats, were treated by hydrothermal techniques prior to dehulling[1–3]. The grains of cereals, such as barley, rice, millet and dinkel, were dehulled by pounding in a mortar after sufficient water had been added to prevent disintegration of the grains. The grains were pounded over many hours and became warm through the procedure. Hulled oats were steeped in hot water, kept in a warm place over night and dried in an oven. After processing, oats were easily dehulled in a grain pound. The hulls were separated from the kernels by the winnowing, or were sifted out after milling. Hydrothermal techniques are still practised in some countries.

Phytate is present in all cereals and contains 50–85% of the total content of phosphorus[4,5]. Phytate negatively affects the bioavailability of many essential elements, such as calcium, iron and zinc[6–12].

Phytase, a naturally occurring enzyme in cereals, hydrolyses phytate (*myo*-inositol hexaphosphate) to *myo*-inositol and inorganic phosphates via intermediate *myo*-inositol phosphates (penta-

ABBREVIATIONS USED: IP_3–IP_6 = Inositol tri-, tetra-, penta- and hexaphosphate; c.v. = cultivar; cfu = colony forming units; pHd = pH measured directly; pHh = pH measured after homogenising; d.m. = dry matter.

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to mono-phosphates). Phytase is inactive in dry cereals, but is activated when the moisture level is increased. Optimal conditions for wheat phytase are 55 °C and pH 5·15[13]. Phytase in rye and barley have high activity under the same conditions as wheat[4,14,15]. In oats, phytase activity is often reduced by heat treatment, but the phytase activity is lower, even in unheated oats, than in wheat, rye and barley[14–16]. Phytate reduction in ground, malted oats was greater after soaking at 37–40 °C than at 55 °C[15].

Hydrothermal treatment of whole grains, historically practised to facilitate dehulling, may have had a positive influence on the nutritional value of cereals by reducing hexa- and pentainositol phosphates to inorganic phosphates and lower inositol phosphates.

The aim of the present study was to investigate the reduction in phytate levels in whole grains after hydrothermal treatment at conditions considered optimal for phytase activity.

MATERIALS AND METHODS

Experimental

Materials

Whole grains of wheat (c.v. Kosack), rye (c.v. Danko), hulled and dehulled barley (c.v. Salka), hulled oats (c.v. Magne) and naked oats (c.v. Rhiannon) were used in Experiments 1–3 and 6–8. Whole grains of naked barley (c.v. Taiga) were used in Experiment 4. Whole grains of hulled oats (c.v. Sang) were used in Experiment 5. Untreated and hydrothermally treated oats (c.v. Sang) were dehulled with a laboratory dehuller at Svalöf AB, Svalöv, Sweden and used in Experiment 8. The oats were not heat-treated.

Hydrothermal treatments

Experiment 1 was performed on a labscale with plastic pots, which were covered. The whole grains (300 g) were wet-steeped in 2 volumes of water or buffer at 55 °C for wheat, rye and barley and at 37 °C for oats until a weight gain of 40 g/100 g cereal was achieved (15–60 min). The grain was dry-steeped in an oven and fluid lost was replaced through repeated wet-steeping. The grain was incubated for 24 h in total.

Experiment 4 was performed on a labscale. Whole grains (300 g) were wet-steeped in 2 volumes of deionised water or buffer at 55 °C for 20 min. Water, buffer, citric acid or lactic acid solutions were added afterwards, and the grain was incubated at 53–55 °C for a total of 12 h.

Experiments 2–3 and 5–7 were performed in pilot plant equipment, constructed for this purpose at the University of Lund, Institute of Technology, Division of Food Engineering. Batches of whole grains (5–10 kg) were washed for 3–5 min in cold deionised water (except when buffer was used). The grain was wet-steeped in 2·5 volumes of deionised water at 53–57 °C, and, for one batch in Experiment 3, at 70 °C, barley for 30 min and oats for 20 min, in a thermostated tank. The steeping solution was drained off, and the grain was incubated in another covered thermostated tank. Buffer, water, citric acid or lactic acid solutions were added, 3L/10 kg barley and 2L/10 kg oats (Experiments 2, 3, 6, 7). The solutions were recirculated at the beginning, but no new fluid was added during the incubations. In Experiment 5, wet-steeping was repeated after 4 h. The grain was stirred for 1 min every 15 min, and air was automatically blown through the cereals for 1 min every 10 min. Barley was incubated at 50–55 °C for a total of 24 h (Experiments 2, 3), and oats were incubated at room temperature (20–25 °C), 37–40 °C or 50–55 °C for a total of 8 h (Experiments 5–7).

The grain was finally dried in a convection oven at 80–90 °C for 2–3 h (Experiments 1–4) or at 40–50 °C for 10 h, followed by 80 °C for 3 h (Experiments 5–7).

In Experiment 8, the degradation of inositol hexaphosphates after soaking was used as an indirect measurement of phytase activity. This was studied in dehulled oats (c.v. Sang) and naked oats (c.v. Rhiannon), which were either untreated or had been hydrothermally treated in Experiments 5 and 6. The cereals were ground to flour in a laboratory mill (Cyclotec, 1093 Sample Mill; 1·0 mm sieve). Oatflour (57 g) was suspended in deionised water (243 mL) and soaked for 8–17 h at 20 and 37 °C. To stop phytate degradation 0·58 M HCl (168 mL) was added to the incubated samples (32 g) at the end of incubation.

Analytical methods

Moisture determination

Dry matter was determined by drying at 105 °C for 17–20 h. All analytical values were calculated on a dry-matter basis.

pH-determination

pH in the cereals was determined in one or two ways. The equipment used was a GK2713 combined pH electrode and pH-meter PHM82 (Radiometer, Copenhagen). To obtain an estimate of the pH in the outer layers of the cereals, pH was measured by dipping the electrode directly into the wet kernels (pHd). To obtain a mean value of the pH in the cereals, pH was measured after homogenising (Heidolph, type E50) whole grains (5 g) in deionised water (50 mL) for 1 min (pHd).

Phytate determinations

Raw materials and dried samples (0·5 g) were extracted with 0·5 M HCl (20 mL) for 3 h. The inositol phosphates were separated from the crude extract by ion exchange chromatography and determined by ion pair carbon 18 column (C18) reverse phase high-performance liquid chromatography (HPLC) using formic acid/methanol and tetrabutylammoniumhydroxide in the mobile phase. The amounts of inositol tri-, tetra-, penta- and hexaphosphatase were determined by HPLC according to Sandberg and Ahderinne[17] and Sandberg *et al.*[18]. The analyses were performed using a HPLC pump (Waters model 510, Waters Ass. Inc. A) and a C18 Chromasil (5 μm) column (15 cm, 2 mm i.d.). The inositol phosphates were detected by refractive index (ERC-7510 RI-detector, Erma Optical Works Ltd., Japan). Retention times and peak areas were measured with a laboratory datasystem, HP 3350 (Hewlett Packard, Talo Alto, CA). Injections were made with a Poole 20 μL loop. The accuracy of the HPLC method was tested, as reported earlier, and was estimated to be approximately $\pm 3\%$[15].

Bacterial counts

Bacterial counts were determined for naked barley, hulled oats and naked oats (Experiments 4–7). Grain (30 g) was mixed with 0·9% (w/w) NaCl (270 mL), and treated for 2 min in a Stomacher 400 (Colworth, London, UK). The suspensions obtained were diluted successively in test portions (1 mL). The viable counts, expressed as colony forming units (cfu) per g cereal, were determined by the poured plate method. The total counts of viable bacteria were made on Tryptone Glucose Extract Agar (TGEA, Merck). The plates were incubated at 30 °C for 3 days. The counts of *Enterobacteriaceae* were made on Violet Red Bile Agar (VRBG, Biokar). The plates were incubated at 37 °C for 1 day. The counts of lactic acid bacteria were made on Rogosa agar (Rogosa, Biokar). The plates were incubated in an anaerobic jar (GasPak, BBL) at 30 °C for 3 days. The counts of yeasts and moulds were made on Potato Dextrose Agar (PDA, Biokar). The pH was reduced to 3·5 by adding filter sterilised 10% (w/w) tartaric acid (1·4 mL) to the substrate (100 mL). The plates were incubated at 20–25 °C for 5 days. For estimation of spore-forming bacteria 15 mL were pipetted in sterilised media, heated at 80 °C for 10 min and immediately cooled. After dilution, 1 mL portions were inoculated on Trypticase Soy Agar (TSA) and incubated at 30 °C for 5 days.

RESULTS AND DISCUSSION

Phytate reduction, pH and bacterial counts

Wheat, rye, hulled and dehulled barley and hulled and naked oats (Experiment 1)

The phytate (inositol hexaphosphate) reduction for wheat was 46%; for rye, 57%; for hulled barley, 56% and for dehulled barley, 77% after incubation in water at 55 °C for 24 h (Table I). When acetate buffer (pH 4·8) was used, the phytate reduction for wheat was 91%; for rye, 92%; for hulled barley, 89% and for dehulled barley, 99% (Table I).

Phytate reduction for oats, incubated at 37 °C for 24 h, was considerably less – for hulled oats 13%, and for naked oats 7% when water was used and for hulled oats 26% and for naked oats 8% when acetate buffer (pH 4·8) was used (Table I). For dehulled barley, 86% of the phytate was degraded within 2 h (Fig. 1). The pH measured directly (pHd) in the whole wet grains was in the range of 4·8–5·7 when buffer was used and in the range of about 6–7 when water was used.

Mellanby[16] found that phytate in whole grains of wheat was reduced by 84% after wet-steeping in acetate buffer, pH 4·5 at 45 °C for 12 h. In ground wheat incubated under the same conditions, phytate was completely degraded within 1 h. For whole grains of oats, even when ground, phytate was found to be hydrolysed very slowly; after 120 h, a large proportion still remained. It was found recently that phytate was reduced by 99% and 23%, respectively, in whole wheat kernels and dehulled oats kernels after wet-steeping at pH 5·5 at 55 °C for 24 h[19].

Table I Inositol phosphates[a] in whole grains, untreated and hydrothermally treated at 55 °C, except for oats, treated at 37 °C, for 24 h (Experiments 1–3)

Sample	IP_3–IP_6[b] (μmol/g dry matter basis)	IP_6[b] (μmol/g dry matter basis)	Phytate reduction[c] (%)
Wheat[d]	14·59	13·89	
water	7·83	7·48	46
acetate buffer[e]	1·48	1·23	91
Rye[d]	13·14	12·57	
water	5·85	5·42	57
acetate buffer[e]	2·05	1·05	92
Barley, hulled[d]	14·25	13·79	
water	9·44	6·01	56
acetate buffer[e]	4·27	1·57	89
acetate buffer[e,f]	4·63	2·19	84
acetate buffer[f,g]	5·35	1·81	87
citric acid			
0·02 M[f,h,i]	8·09	4·98	64
0·02 M[f,h,i,j]	14·70	7·05	49
0·02 M[f,i]	6·23	3·78	73
0·04 M[f,i]	7·02	4·69	66
Barley, dehulled[d]	10·62	10·35	
water	3·13	2·78	77
acetate buffer[e]	0·26	0·10	99
Oats, hulled[d]	13·55	13·28	
water	11·94	11·54	13
acetate buffer[e]	11·31	9·88	26
Oats, naked[d]	12·49	12·25	
water	12·12	11·37	7
acetate buffer[e]	13·18	11·25	8

[a]Mean values of at least two replicates.
[b]IP_3 to IP_6 = inositol containing three to six phosphates per inositol residue.
[c]Percentage phytate reduction (IP_6) calculated on initial content in untreated samples.
[d]Untreated grains.
[e]pH 4·8.
[f]Performed in pilot plant equipment.
[g]pH 4·5.
[h]Wet-steeped in acid solution.
[i]Acid solution added after wet-steeping.
[j]Wet-steeped at 70 °C.

Hulled barley (Experiments 2 and 3)

The experiment with hulled barley was repeated in the pilot plant equipment (Experiment 2). Phytate was reduced by 84% after incubation in acetate buffer (pH 4·8) and by 87% after incubation in acetate buffer (pH 4·5) at 50–55 °C for 24 h (Table I and Fig. 1). By using acetate buffer (pH 4·5) phytate was reduced by 51% within 2 h and 84% within 12 h (Fig. 1). After 6 h, the pH values measured directly in whole kernels and in homogenised samples were similar, about 5·6 (Fig. 1). The moisture content increased from 12% to 40–42% during the incubation steps.

When hulled barley instead was wet-steeped in water or 0·02 M citric acid and 0·02 or 0·04 M citric acid was added after wet-steeping, phytate reduction was 64–73% (Experiment 3) (Table I). Initial wet-steeping at 70 °C for 30 min seemed to diminish the phytase activity, as judged by the lower extent of phytate reduction during the following incubation at 50–55 °C, compared with initial wet-steeping at 53–57 °C (49% instead of 64%; Table I). The sums of tri-, tetra-, penta- and hexaphosphates in both the samples were 14·70 and 8·09 μmol/g (d.m.), respectively. Samples analysed at different times also showed high remaining

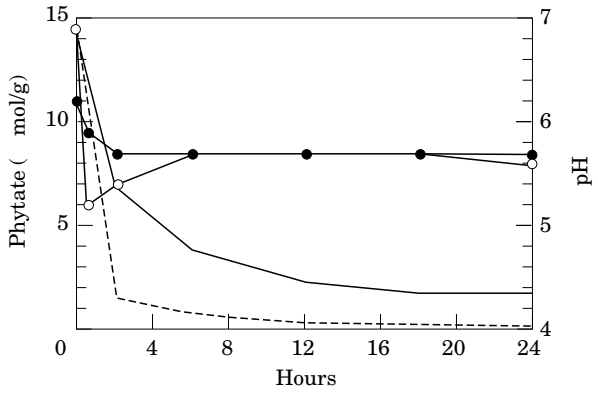

Figure 1 Phytate reduction in dehulled (– – – –) and hulled (——) barley after 0·5, 2, 6, 11–12, 16–18 and 24 h, during incubation in acetate buffer, pH 4·8 and 4·5 respectively at 50–55°C (Experiments 1, 2). pH measured directly (pHd ○) and pH measured after homogenising (pHh ●) in hulled barley during the incubation.

amounts of all inositol phosphates when the wet-steeping temperature was 70 °C (data not presented here). The moisture content increased from 12% to 40–44% during the incubation steps.

In a previous study, phytase in wholemeal of wheat was rapidly inactivated at temperatures over 80 °C and in suspensions with purified phytase at a temperature as low as 60 °C[13]. Phytase in dry cereals, on the other hand, is rather heat resistant[20].

Phytate and phytases are located in the outer layers of the grain. The pH measured directly in the wet kernels with a glass electrode, according to Bella and Labuza[21], differed from the pH in homogenised samples. The acetate buffer lowered the pH in the homogenised grains as the moisture content was increased, which, after some hours, resulted in similar pH values in the homogenised kernels as measured directly in the kernels (about 5·6; Fig. 1). Incubation in citric acid solutions resulted in rather stable pH values, for example, about pH 4 in outer layers and about pH 5 in homogenised samples when 0·04 M citric acid was used. Acetic acid may diffuse into the grains more easily than citric acid, which has the capacity to bind other molecules and metals and form larger complexes.

Naked barley (Experiment 4)

The level of phytate in naked barley was reduced by 41–71% after incubation in water, acetate buffer (pH 4·8), citrate buffer (pH 4·4 and 4·8), 0·02 M citric acid or lactic acid solutions (lactic acid added at 0·23, 0·45 and 0·90% of the weight of the cereals) at 53–55 °C for 12 h (Table II). Incubations in water, citrate or citric acid gave

Table II Inositol phosphates[a] in naked barley, untreated and hydrothermally treated at 53–55 °C for 12 h (Experiment 4)

Sample	IP_3–IP_6[b] (μmol/g dry matter basis)	IP_6[b] (μmol/g dry matter basis)	Phytate reduction[c] (%)
Barley naked[d]	12·61	11·96	
water	9·23	7·02	41
acetate buffer[e]	7·91	4·62	61
citrate buffer[e]	9·33	5·50	54
citrate buffer[f]	8·56	5·75	52
citric acid[g]			
0·02 M	8·89	6·65	44
lactic acid[g]			
0·23%[h]	7·82	4·51	62
0·45%[h]	7·73	3·74	69
0·90%[h]	9·31	3·51	71

[a]Mean values of at least two replicates.
[b]IP_3 to IP_6 = inositol containing three to six phosphates per inositol residue.
[c]Percentage phytate reduction (IP_6) calculated on initial content in untreated samples.
[d]Untreated grains.
[e]pH 4·8.
[f]pH 4·4.
[g]Acid solution added after wet-steeping.
[h]In percentage of the amount of the cereals.

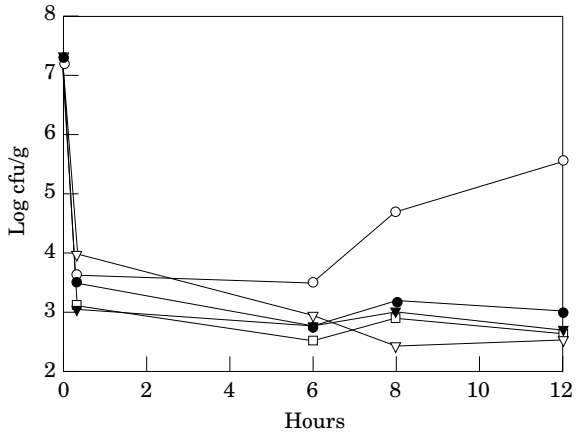

Figure 2 Total counts of bacteria in naked barley after wet-steeping in water for 20 min, and after incubation in water (○), 0·02 M citric acid (●) or lactic acid solutions (0·23%, ▽; 0·45%, ▼; 0·90%, □) incubated for 6, 8 and 12 h at 53–55°C (Experiment 4).

less phytate reduction than in acetate or lactic acid. When citrate or citric acid were used, both pHd and pHh were stable and different during time, whilst, when acetate or lactic acid were used, the pH values became more similar to each other. The moisture content increased from 9 to 37–40%.

At harvest, cereals contain microorganisms, which are mainly located on the surface. Bacterial counts showed that, after wet-steeping of naked barley in water at 53–57 °C for 20 min, the total count of bacteria was reduced by 99·97% (Fig. 2). An increase in the total counts of bacteria was seen in grain incubated in water for more than 6 h, but the bacteria were not identified (Fig. 2). No bacterial growth, whether total counts of bacteria, *Enterobacteriaceae*, lactic acid bacteria or yeast and mould, was observed when the grain was incubated in citric acid or lactic acid for 12 h (Fig. 2). The counts of spore-forming bacteria did not increase during the incubation and the counts were 1·6–2·6 log cfu/g.

Hulled oats (Experiment 5)

No phytate reduction was observed in hulled oats after initial and repeated wet-steeping at 53–57 °C followed of incubation at 37–40 °C, and incubated in total for 8 h (Table III). The grains were dehulled before analysis. The pHd value was 6–6·5, and pHh about 7. The moisture content increased from 10 to 39–40% during the incubation steps. Wet-steeping at 53–57 °C for 20 min reduced the bacterial counts to levels below 1–2·9 log cfu/g, and no bacterial growth was observed during the incubation.

Naked oats (Experiments 6 and 7)

Phytate in raw material from four different batches of naked oats was analysed in duplicate and inositol hexaphosphate (IP_6) varied from 11·55 to 13·57 μmol/g (mean ± SD = 12·81 ± 0·87) (Table III). Phytate in naked oats was reduced by 9–19% after incubation in water or 0·04 M citric acid at 20–25, 37–40 or 50–55 °C for 8 h (Experiment 6) (Table III). When lactic acid solutions (0·45% of the amount of the cereals) were added to naked oats after wet-steeping and incubated at various temperatures for 8 h phytate was reduced by 7–20% (Experiment 7) (Table III). The moisture content increased from 11% to 39% during the incubation steps.

Bacterial counts showed that untreated naked oats had as high counts of *Enterobacteriaceae*, yeast and mould as untreated naked barley. Initial washing and wet-steeping at 53–57 °C for 20 min reduced the counts of bacteria to levels below 1–2·6 log cfu/g. Incubation in water resulted in an increase in the counts of total bacteria after 6 h, and the grain acquired an unpleasant odour. The bacteria were not identified, however. The addition of acid prevented the growth of bacteria.

Ground oats (Experiment 8)

The phytate in ground dehulled oats, whether untreated and hydrothermally treated, was reduced by 72–77% when flour was soaked at 20 °C or 37 °C for 17 h (Table III). The phytate in ground naked oats, whether untreated and hydrothermally treated, was reduced by 88–94% when flour was soaked at the same conditions (Table III). The main part of the reduction (80–82%), occurred during the first 8 h of incubation, and increased to 88% when the pH was adjusted to 5·0.

Phytate was degraded to a greater extent in ground oats than in whole grains, suggesting that grinding allowed better contact between phytate and phytase. Phytase activity was not lost from the hydrothermally treated and dried oat grains as judged by a phytate reduction similar to that for untreated oats. Phytate reduction for untreated flour of dehulled oats was reported earlier to be 61% (pH 5, 55 °C, 17 h)[10] and 65% (37 °C, 22 h)[22].

Table III Inositol phosphates[a] in whole grains of oats, untreated and hydrothermally treated for 8 h (Experiments 5–7) and in ground and soaked oats (Experiment 8)

Sample	Temperature of incubation[d] (°C)	Time of incubation[e] (h)	Temperature of incubation[e] (°C)	IP_3–IP_6[b] (μmol/g dry matter basis)	IP_6[b]	Phytate reduction[c] (%)
Oats, dehulled[f]	—	—	—	15·14	14·70	
	—	17	20	5·09	3·96	73
	—	17	37	5·38	3·34	77
water[g]	37–40	—	—	15·69	14·58	1
water[g]	37–40	17	20	4·65	3·94	73
water[g]	37–40	17	37	5·26	4·06	72
Oats, naked[f]	—	—	—	13·18	12·81	
	—	8	20	4·32	2·51	80
	–	17	20	3·65	1·26	90
	—	17	37	2·25	0·81	94
water[g]	20–25	—	—	12·08	11·59	9
water[g]	20–25	8	20	3·77	2·25	82
water[g]	20–25	8[h]	20	3·72	1·49	88
water[g]	20–25	17	20	3·59	1·18	91
water[g]	37–40	—	—	12·58	11·47	10
water[g]	50–55	—	—	11·16	10·43	19
citric acid[g]						
0·04 M[i]	20–25	—	—	12·49	11·02	14
0·04 M[i]	20–25	17	20	4·04	1·55	88
Oats, naked[f]	—	—	—	14·26	14·08	
lactic acid[g,j]						
0·45%[k]	20–25	—	—	14·49	13·10	7
0·45%[k]	37–40	—	—	13·78	12·32	12
0·45%[k]	50–55	—	—	14·27	11·25	20

[a]Mean values of at least two replicates.
[b]IP_3 to IP_6 = inositol containing three to six phosphates per inositol residue.
[c]Percentage phytate reduction (IP_6) calculated on initial content in untreated samples.
[d]Incubation temperature for whole grains after wet-steeping at 53–57 °C for 20 min.
[e]Incubation of ground cereals.
[f]Untreated grains.
[g]Steeping solution used for whole grains.
[h]pH adjusted to 5·0 with 0·1 M citric acid.
[i]Wet-steeped in acid solution.
[j]Acid solution added after wet-steeping.
[k]In percentage of the amount of the cereals.

This suggests that there is a higher phytase activity in naked oats or that phytate in naked oats is more susceptible to phytase attack.

An increased consumption of dietary fibre from cereals is recommended in the western diet, but this would be accompanied by an increased phytate intake. The significance of phytate on mineral nutrition in western diets has been discussed with special regard to people with higher requirements (e.g. children, young adults, elderly)[23,24].

To achieve a strong increase in iron availability, phytate must be reduced to levels below 0·5 μmol/g or 10 mg inositol phosphate-phosphorus (sum of tri-, tetra-, penta- and hexaphosphates) in a meal if no absorption-promoting factors, such as ascorbic acid or meat, are present[10–12,25]. In 26 diets, zinc absorption *in vivo* was correlated with inositol phosphate-phosphorus (the sum of tri-, tetra-, penta- and hexaphosphates)[25]. Diets containing less than 50 mg resulted in a markedly increased zinc absorption. Whether all inositol phosphates inhibit iron and zinc absorption to the same extent is currently under discussion[11,26–28]. The dose-dependent inhibitory effect of phytate on zinc and calcium absorption is not documented.

Phytate phosphorus is not available for human absorption unless the phosphate groups have been removed from the inositol molecule. Food pro-

cesses such as soaking, germination and fermentation often lead to a degradation of phytate and an increased bioavailability of minerals[25]. It is also possible to hydrolyse inositol hexaphosphates to lower inositol phosphates in the stomach and small intestine of humans when phytase is intact in the meal[29,30].

To avoid adverse effects on mineral nutriture cereal products with reduced phytate and intact phytase should be further developed with hydrothermal treatment of whole grains. Hydrothermal treatment, as shown in the present study, seems to be a suitable processing method with advantages also regarding the microbiological quality.

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