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The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran

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Abstract

The effect of phytic acid on the solubility of mineral elements in oat bran was studied by digesting phytic acid with phytase enzyme. The combined effect of phytase treatment and the addition of three chelating agents common in food were also tested. Starch and proteins were digested enzymatically. The sample was dialysed, using an equilibrium dialysis method, and the soluble mineral elements were analysed from the dialyzate. The minerals studied were calcium, magnesium, iron, manganese, zinc, potassium and phosphorus. The chelating agents used were citric and malic acids and glucose. The phytase treatment increased the solubility of minerals less than expected. Citric acid was the most efficient chelating agent. The effect of malic acid was small. The results confirmed that the minerals were tightly bound to the dietary fibre of oat bran and were only partially released when the influence of phytic acid was reduced by degradation.

Keywords: Oat bran; Phytic acid; Chelating agents; Mineral elements

1. Introduction

The chyme is an extremely complex matrix, in which the physico-chemical conditions change continuously with diet. The absorption of mineral elements is a very complicated system and several components can form soluble and insoluble complexes with these elements under intestinal conditions. Dietary fibre and fibre-rich food are associated with a decrease in bioavailability of nutritionally significant mineral elements. Fibre components, such as cellulose, hemicellulose, pectins, other polysaccharides and lignin, may form insoluble complexes with mineral elements, and thus reduce their bioavailability. In cereals this negative effect is also attributed to phytic acid (Idouraine, Khan, & Weber, 1996; Persson, Nyman, Liljeberg, Önning, & Frølich, 1991; Rendleman & Grobe, 1982).

Phytic acid (*myo*-inositol-hexaphosphate, IP6) is a constituent of dietary fibre in cereals. It has a high capacity to form insoluble complexes (phytates) with divalent metal ions present in food (Persson, Türk, Nyman, & Sandberg, 1998). Metal complexes of *myo*-inositol-hexaphosphate (IP6) and lower inositol phosphates (IP5–IP1) are poorly soluble at the pH of the gastrointestinal tract and may reduce the bioavailability of minerals such as Fe, Zn, Ca and Cu (Torre, Rodriquez, & Saura-Calixto, 1991). The affinity of IP4 and IP3 for mineral elements is lower than that of IP6, and the solubility of metal complexes formed with IP4 and IP3 is higher than with IP6 (Persson et al., 1998).

Dietary fibre and phytic acid are believed to interact with minerals in the small intestine in vivo. However, in vivo experiments are often complex, time-consuming and expensive. In vitro studies and simplified models may give a tentative explanation of the real mechanism affecting the bioavailability of nutrients. The mineral binding capacity of dietary fibre has been studied *in vitro* using isolated and carefully purified fibre fractions and components (Idouraine et al., 1996; Nair, Asp, Nyman, & Persson, 1987; Persson et al., 1991) or under simulated conditions of the stomach and the small intestine (Miller, Schricker, Rasmussen, & Van Campen, 1981; Sandberg, Carlsson, & Svanberg, 1989).

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The bioavailability of mineral elements can be improved by increasing dietary factors that enhance mineral absorption in the intestine. Natural chelating agents may play a key role in the solubilisation of food minerals and trace elements (Hazell & Johnson, 1987; Miller et al., 1981; Reinhold, Estrada, Garcia, & Garzon, 1986). In our earlier study (Ekholm, Virkki, Ylinen, Johansson, & Varo, 2000) it was shown that it may be possible to increase the availability of minerals when chelating agents, such as citric acid, are added to a fibre-rich diet.

To investigate the binding capacity of fibre constituents, oat bran was chosen as a model to keep the procedure simple. Oat bran represents a commercial milled product; its mineral element concentration is high and it is often used to increase the dietary fibre intake in human diets and animal feeds.

Earlier (Ekholm et al., 2000) we found that oat fibre binds mineral elements tightly. In this study, the aim was to clarify the influence of phytic acid on the solubility of K, Ca, Mg, Fe, Zn, Mn and P in fibre-rich oat bran in a situation where all the fibre components interact. Phytase enzyme was used to degrade phytic acid. Furthermore, the effect of natural chelating agents on the solubility of mineral elements was studied after the phytase treatment.

2. Materials and methods

2.1. Sample matrix and chemicals

The oat bran was a commercial product of Melia Oy (Raisio Group, Nokia, Finland). The sample and the method were the same as in the study of Ekholm et al. (2000). The sample was ground (Cyclotec 1093, Tecator AB, Höganäs, Sweden) to a particle size less than 0.5 mm. The contents of fat, dietary fibre and protein in the bran sample were as specified by the manufacturer (Table 1).

Table 1
The mineral element, dietary fibre, protein and fat contents of the sample

Mineral elements mg/100 g, mean ±S.D.	
Ca	81±0.8
Fe	7.5±1.0
K	630±12.0
Mg	231±8.3
Mn	5.5±1.9
Zn	4.6±0.2
Dietary fiber g/100 g[a]	17
Protein g/100g[a]	17
Fat g/100 g[a]	7.5

[a] Based on manufacturer's specifications.

Milli-Q water (Millipore, Bedford, MA, USA) was used and all chemicals were the purest available. The enzymes were phytase from wheat (EC 3.1.3.26, Sigma, St. Louis, MO, USA), Termamyl 300 L (Novo Nordisk A/S, Bagsvaerd, Denmark), Pepsin (0.7 FIP-U/mg, EC 3.4.23.1, Merck, Darmstadt, Germany) and Pancreatin (8 X U.S.P., Sigma, St.Louis, MO, USA).

2.2. Mineral analysis

The contents of mineral elements of the bran sample were determined by flame atomic absorption spectrometry (AAS; Perkin-Elmer 5100, Norwalk, CT), using an air-acetylene flame. The minerals studied were Ca, Mg, Fe, Mg, Zn and K. The phosphorus content was determined spectrometrically by the method of Olsen and Sommers (1982). For K, Mg, Mn and Zn determinations, wet-digestion with 10 ml concentrated HNO_3 was used, and for Ca, Fe and P, the samples were dry-digested. Lanthane solution (0.09%) was used as an ionisation buffer in Ca, K and Mg determinations.

2.3. Description of the method

The samples were treated according to the method shown in Fig. 1. This method, with some modifications, is according to Asp, Johansson, Hallmer, and Siljeström (1983) for dietary fibre. Starch and protein of the sample were digested enzymatically. To avoid the addition of phosphorus, water was used instead of phosphate buffer throughout the procedure. Furthermore, the amount of Termamyl, an α-amylase, was decreased to minimise mineral contaminations. In this study, phytic acid was also digested enzymatically. The samples were dialysed and the soluble minerals were analysed in the dialyzate. Insoluble minerals were bound by fibre components of the sample and remained in the dialysis residue.

The ground sample (5 g) was placed into a 100-ml flask. Purified water (50 ml) and 65 mg phytase were added. The mixture was incubated for 2 h at 55 °C. The digestion process was according to the manufacturer. After the incubation, the chelating agents (citric and malic acids and glucose) were added. The concentrations were 0, 1.0 and 3.0% of the amount of bran. The mixture of sample and chelating agent was kept in a boiling water bath with horizontal agitation for 15 min to gelatinise the starch. After adding 0.5 ml of Termamyl 300 L, the mixture was further incubated in a boiling water bath for 1 h 45 min. The presence of any unhydrolysed starch was checked with I_2/KI-solution, and an additional 0.15 ml of the enzyme was added if necessary. The pH of the reaction mixture was adjusted to 1.5 with 1 M HCl. After addition of 0.5 g pepsin, the mixture was incubated at 40 °C for 60 min. The pH was adjusted to 6.8 with 1 M NaOH, 0.5 g of pancreatin was

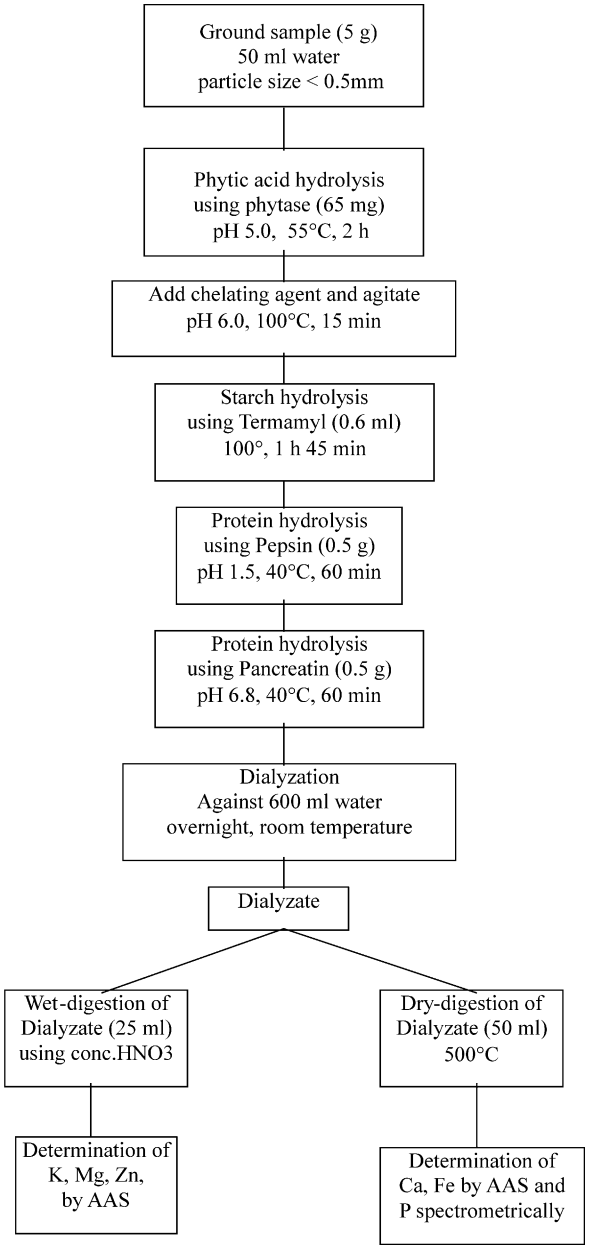

Fig. 1. Main steps in the digestion of samples and the determination of mineral elements.

added and the sample was incubated for an additional 60 min at 40 °C. The sample was dialysed (molecular weight cut-off 3500) against 600 ml of purified water, overnight, at room temperature. The concentrations of mineral elements in the dialyzate were determined by flame atomic absorption spectrometry. Twenty-five millilitres of the dialyzate were digested for K, Mg, Mn and Zn determinations and 50 ml for Ca, Fe and P. Phosphorus was determined spectrometrically using the molybdate–vanadate method (Olsen & Sommers, 1982). All treatments of the samples were made in triplicate and the determinations of mineral elements in duplicate.

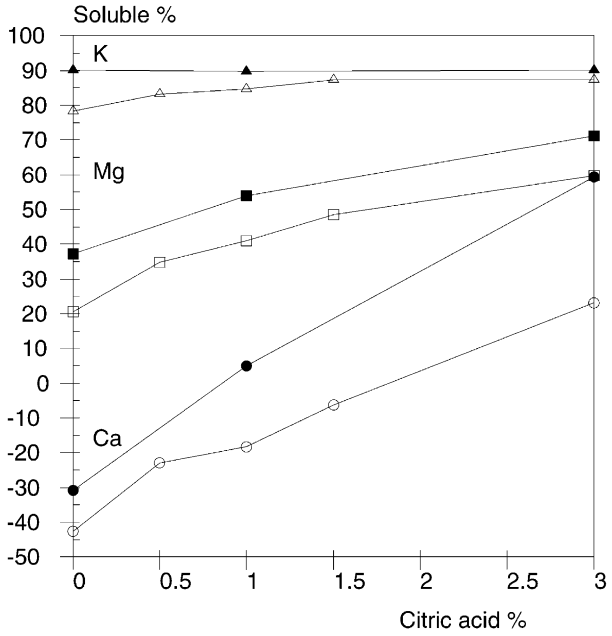

Fig. 2. The effects of phytase and citric acid on the solubility of Ca, Mg and K in oat bran sample. Filled symbols—sample with phytase treatment; open symbols—sample without phytase treatment.

2.4. Analysis of results

The high mineral content of the enzymes used created a problem in the processing of the data. Therefore, the amounts of dialyzable minerals of all enzymes used (i.e. the background level) were determined from the same amount of enzymes and chelating agent as in the determination of the oat bran sample itself but without any substrate. The mineral elements of oat bran were considered to be soluble when their concentration in the dialyzate exceeded the background level. When the mineral element concentration of the dialyzate was lower than the background level, the fibre was considered to have bound these minerals. Thus, the results indicate the solubilities of the minerals native in the samples. The negative values (see Figs. 2–5) indicate the amounts of mineral element released from the enzymes used and then retained by the dietary fibre.

The accuracy and the precision of the analytical method was tested using NBS 1567a Wheat flour reference material (National Bureau of Standards, Gaithersburg, MD) and one unofficial home-made reference solution containing known amounts of K, Mg, Mn and Zn (Table 2). The data were tested statistically using analysis of variance (ANOVA).

3. Results and discussion

3.1. General

In the method used, all other components of the sample, except the dietary fibre are digested. Thus, the

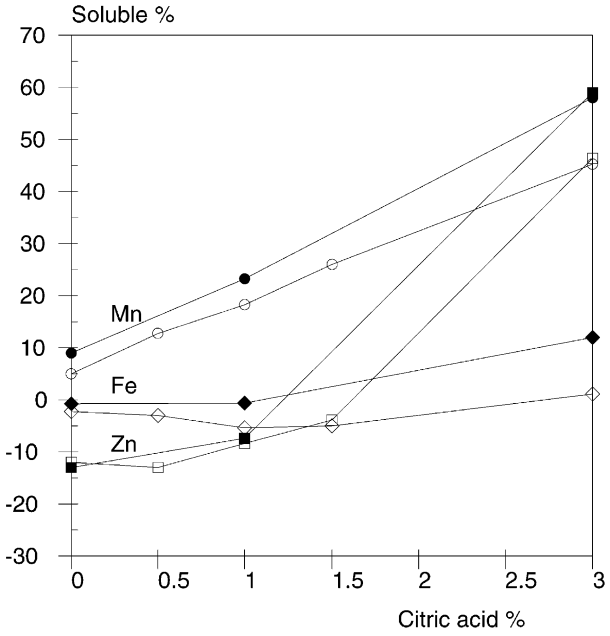

Fig. 3. The effects of phytase and citric acid on the solubility of Zn, Fe and Mn in oat bran sample. Filled symbols—sample with phytase treatment; open symbols—sample without phytase treatment.

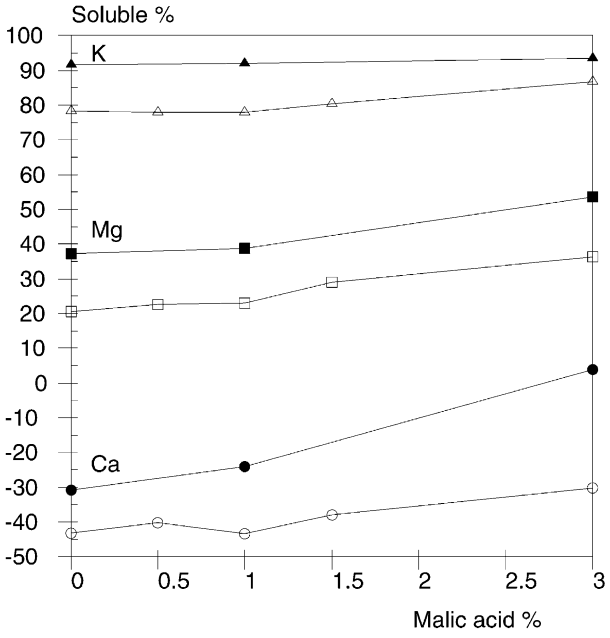

Fig. 4. The effects of phytase and malic acid on the solubility of Ca, Mg and K in oat bran sample. Filled symbols—sample with phytase treatment; open symbols—sample without phytase treatment.

origin of dialyzable minerals is not only fibre and phytates but also proteins, some amino acids and other compounds present in oat bran. These origins were considered to represent the available fraction of mineral elements of the samples. The different components of the dietary fibre can interact with these minerals and decrease their availability. Phytic acid is considered to

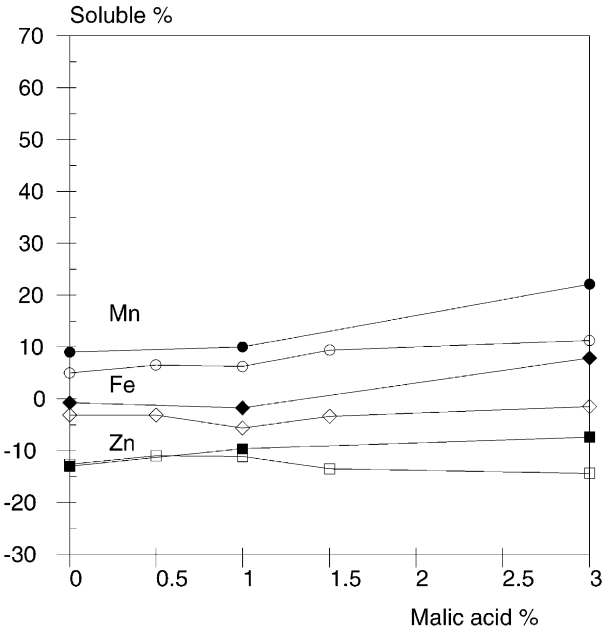

Fig. 5. The effects of phytase and malic acid on the solubility of Zn, Fe and Mn in oat bran sample. Filled symbols—sample with phytase treatment; open symbols—sample without phytase treatment.

be one of the most important dietary fibre components to decrease the availability of minerals in cereal foods.

3.2. The effect of phytase treatment on the solubility of minerals in oat bran

Phytic acid is known to have a high affinity for many divalent mineral elements. It forms stable phytate complexes which can prevent absorption of essential elements from food (Torre et al., 1991). Phytase enzymes can hydrolyse IP6 to free *myo*-inositol and inorganic phosphate via lower inositol phosphate esters (IP5–IP1), and thereby increase the bioavailability of mineral elements (Persson et al., 1998). Earlier studies (Ekholm et al., 2000) found that the solubility of mineral elements from oat bran was very poor and the dietary fibre was able to bind extra Ca and Zn. In this study the digestion of bran was started with the hydrolysis of phytates. According to Persson and co-workers (1991) the phytase treatment, prior to starch and protein hydrolysis, releases phytic acid efficiently. The solubilities of mineral elements with and without phytase enzyme treatment are given in Figs. 2–5 (0% added chelating agent). Results of the mineral solubility without phytase treatment (Figs. 2–5) are from our earlier studies (Ekholm et al., 2000). The solubilities of Ca, Mg and K (Fig. 2) clearly increased when the phytates were hydrolysed. Potassium was the most soluble mineral element in all conditions. The effect of phytase on the solubility of Zn, Mn and Fe was negligible (Fig. 3). Sandberg and Svanberg (1991) have shown that the digestion of cereal samples, with

Table 2
Accuracy and precision of the analytical method

NBS 1567a Wheat flour Element	N	Present study	Certified value
Ca	30	0.0197±0.0021% by weight	0.0191±0.0004% by weight
Fe	33	15.1±2.1 g/kg	14.1±0.5 g/kg
K	5	0.131±0.001% by weight	0.133±0.003% by weight
Mg	5	0.034±0.002 g/kg	0.040±0.002 g/kg
Mn	5	9.1±0.2 μg/kg	9.4±0.9 μg/kg
P	11	0.111±0.014% by weight	0.134±0.006% by weight
Zn	5	11.3±0.4 μg/kg	11.6±0.4 μg/kg
		Noncertified control	
		Analysed	Weighted amount
K	21	39.4±0.9 mg/l	40 mg/l
Mg	20	10.1±0.2 mg/l	10 mg/l
Mn	20	98.8±4.6 μg/l	100 μg/l
Zn	20	203±13 μg/l	200 μg/l

phytase, increased the iron availability significantly. This was not seen in the present study. Phytic acid accounts for 60–90% of the total phosphorus in seeds (Torre et al., 1991). In our studies on oat bran, only 35% of phosphorus was soluble after the phytase treatment when the wheat phytase was used in degradation. According to Sandberg and Svanberg (1991), the use of wheat phytase, under optimal conditions, degraded phytic acid in wheat bran and in wholemeal rye flour almost completely in two hours but, with oatmeal, the reduction of phytates was only 73% in 17 h. The reason for the low phytate degradation in this study might be that the conditions of hydrolysis chosen (pH, temperature, time) were optimal for phytase isolated from wheat. Larsson and Sandberg (1992) have shown that the optimal conditions for phytate degradation in oats are different from those for wheat and the phytate reduction in oats was much lower than those of other cereals.

The ability of phytic acid to bind mineral elements depends on the pH value of the solution and on the number of phosphate groups in the molecule (Persson et al., 1998). Phytic acid forms insoluble chelates with many divalent cations in vitro at pH values between 5 and 7, similar to that in the duodenum (Persson et al., 1998). The other dietary fibre components can also bind mineral ions significantly (Persson, Nair, Frølich, Nyman, & Asp, 1987). In this study the final equilibrium dialysis was carried out at pH 6.8, which is also the intestinal pH. The decreasing order of metal complexation with phytic acid has been reported to be $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ at pH 7.4 (Reddy, Sathe, & Salunkhe, 1982). According to our results (Figs. 2–5, 0% added chelating agent), it seems that the degradation of phytic acid with phytase enzyme alone, without any other chelating agent, releases some K, Ca and Mg in oat bran in the decreasing order Ca > Mg > K but has little if any effect on the binding of Mn, Zn and Fe. Metal complexation with phytic acid depends on pH, and other metals present (Reddy et al., 1982; Torre et al., 1991) which explains the differences in complexation order between our results and those of Reddy et al. (1982).

3.3. Solubility of minerals in oat bran with both phytase treatment and chelating agents

The addition of chelating, agents together with phytase enzyme, increased the solubility of the mineral elements studied, except for potassium. Citric acid was the most efficient chelating agent (Figs. 2 and 3) as also found in our earlier study (Ekholm et al., 2000). The combined effect of phytase and citric acid increased the solubilities of Ca, Mg, Zn, and Mn in the oat bran sample significantly. With the addition of 3% citric acid, the total solubility of Mg and Mn increased from 21 to 70% and from 6 to 54%, respectively. The increase in the solubility of Ca was also significant and exceeded the background level, with a citric acid concentration of 1.0% (Fig. 2). Citric acid treatment, together with phytase, increased the solubility of Zn 71% (Fig.3). The effect of citric acid on the solubility of iron was only moderate in samples treated with phytase (Fig. 3). The low iron solubility and the effect of citric acid on this solubility were also seen in our earlier study (Ekholm et al., 2000). Comparison with the earlier results, obtained when citric acid was used alone, shows that the solubilities of all mineral elements increased markedly as phytase and citric acid (the chelating agent) were used together.

Malic acid, together with phytase, increased the solubility of the mineral elements studied only moderately (Figs. 4 and 5). The increasing effect was highest on the solubility of Ca (49%). The solubility increased

32% for Mg and 11% for Mn. Malic acid treatment did not increase the solubility of Zn and Fe. Glucose had no effect on any mineral element.

Our results show that the mineral element availability in oat bran could be increased by adding both phytase enzyme and a chelating agent. The increase of the solubility for mineral elements differs and depends on the mineral. According to the results of this study, Ca and Mg have a small affinity for phytic acid. It also seems that components of dietary fibre other than phytic acid, are more important in binding Zn, Fe and Mn at physiological pH. Citric acid with three carboxylic acid groups in its structure forms soluble complexes with all mineral elements studied. Thus, we conclude that citric acid, together with phytase enzyme, may increase the availability of mineral elements when high dietary fibre cereal products are eaten.

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