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Phytases for improved iron absorption

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Abstract Phytase enzymes present an alternative to iron supplements, because they have been shown to improve iron absorption by means of catalysing the degradation of a potent iron absorption inhibitor: phytic acid. Phytic acid is a hexaphosphate of inositol and is particularly prevalent in cereal grains, where it serves as a storage molecule for phosphorous. Phytic acid is also associated with minerals. The minerals are bound by chelation to the negatively charged phosphate groups in phytic acid. Phytases catalyse the dephosphorylation of phytic acid, thus releasing bound minerals to make them available for absorption. This article presents research on phytase catalysis in gastric conditions and considers potential benefits and drawbacks for using phytases as a food supplement.

INTRODUCTION

The World Health Organization has recently approved a phytase enzyme from the mould *Aspergillus niger* for use in foods (1). Several studies have demonstrated the efficacy of phytase enzymes for improving iron absorption from phytate-rich meals (Figure 1) (2–12). The objective of this study is to assess whether phytase catalysis in the gastric ventricle post ingestion may be practically possible and nutritionally useful.

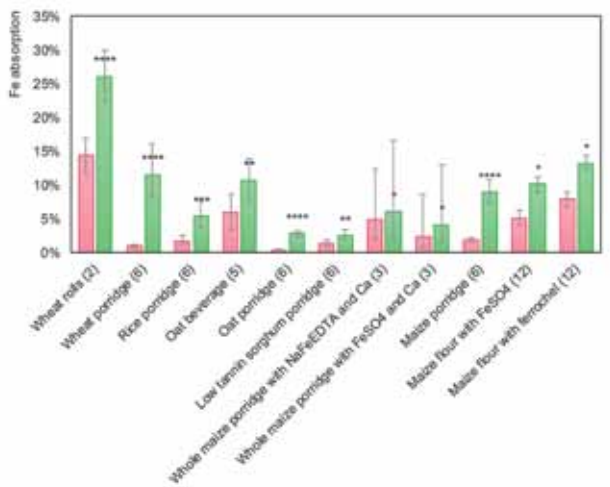

Figure 1. *In vivo* iron absorption from different cereal meals without (red) and with (green) phytase catalysis (i.e. with microbial phytase added), references as indicated in the x-axis labels (2, 3, 5, 6, 12). Error bars correspond to standard errors or 95% confidence intervals as given by the respective authors. Asterisks indicate P values for the difference between iron absorption with and without phytase: * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Phytases (myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphate phosphohydrolase; EC 3.1.3.8) catalyse dephosphorylation of phytic acid (myo-inositol (1, 2, 3, 4, 5, 6)-hexakisphosphoric acid). Phytic acid is the major phosphorous storage compound in seeds and kernels and may constitute up to ~1.5 % of the kernels by weight (13). The phosphate groups of phytic acid are negatively charged at physiological pH values, thus making phytic acid a strong chelator of positively charged ions, notably minerals such as iron (Fe^{3+}) and zinc (Zn^{2+}) (13, 14). This chelation causes low bioavailability of minerals from phytate-rich foods, notably cereals. For iron, the bioavailability in typical cereals is as low as 2-3 % (15). Iron deficiency is the most common nutritional deficiency worldwide and it is estimated that globally around 2 billion people are affected (16). Unfortunately, human biology does not possess an efficient mechanism to naturally release appreciable amounts of the phytate-bound iron during digestion. However, by action of exogenously added microbial phytases, iron can be released from phytic acid by phytase-catalysed dephosphorylation. This dephosphorylation essentially involves removal of phosphate groups from the inositol base of phytic acid. At least four phosphates must be cleaved off from each inositol base molecule to eliminate inhibition of iron absorption (17), i.e. to release chelated minerals, notably iron, from phytate. *In vivo* studies have confirmed that addition of microbial phytases to cereal-based foods can increase iron absorption via this mechanism (18). This phytase catalysis could take place before food consumption as a means of pretreatment or the phytase could be ingested along with the phytate-rich meal, thus acting in the human gastrointestinal tract. In any case, as iron absorption occurs in the upper part of the small intestine, the iron should preferably be released before the food digesta exits the gastric ventricle.

PHYTASE STABILITY AND ACTIVITY IN THE GASTRIC ENVIRONMENT

For phosphate cleavage and iron release from phytic acid to take place efficiently in the gastric ventricle, phytases need to be stable and active in the gastric environment. Recent research has shown that the phytases from *Escherichia coli* and *Aspergillus niger* have sufficient activity under conditions simulating the environment in the stomach ventricle, i.e. at low pH range (~2-6) and with proteolytic pepsin present and moreover that these two enzymes are more stable than the (thermostable) commercial phytases from *Citrobacter braakii* and *Peniophora lycii* (19). In fact, no significant loss of activity due to acidity or presence of the pepsin was observed during two hours of incubation in simulated gastric ventricle conditions (19).

CEREAL PHYTATE DEGRADATION AT GASTRIC VENTRICLE CONDITIONS

An additional requirement for improved iron release from phytate is the ability of the phytases to work on the phytic acid in genuine cereal substrates. In e.g. wheat grains, the phytate is embedded in globoids in the bran layer of the grain (14). It has been shown that phytates to a large extent are soluble under gastric conditions and that ongoing solubilisation will occur once dephosphorylation of phytic acid has been initiated (20). This solubilisation enables the catalysis of dephosphorylation and in turn induces more or less complete removal of phytic acid and inositol phosphates lower with a lower degree of phosphorylation, which can contribute to iron absorption inhibition (18). We recently found that a dosage of 0.4 phytase units of microbial phytase (either from *E. coli* or *A. niger*) per µmol phytic acid in different cereal substrates, wheat bran, rice bran and milled sorghum flour resulted in complete degradation of inositol hexakis- to triphosphates (InsP3-6) within 15-30 min at gastric ventricle conditions (one unit of phytase activity corresponds to the amount of enzyme needed to release 1 µmol inorganic phosphate per min). The degradation occurred at an initial rate of ~0.1-0.2 µmol InsP3-6 removed per phytase activity unit per minute.

IRON RELEASE AT GASTRIC VENTRICLE CONDITIONS

After 2 hours of simulated gastric incubations with or without addition of microbial phytase, we adjusted pH to 6.5 to measure soluble iron by ICP-OES (Table 1). Except for sorghum flour incubated at pH 2 and wheat bran incubated at pH 5, addition of a microbial phytase significantly enhanced *in vitro* iron solubility

	pH 2		pH 5	
	phytase	no phytase	Phytase	no phytase
Wheat bran	1.24 ± 0.21*	0.09 ± 0.03	6.46 ± 1.85	6.09 ± 0.23
Rice bran	1.59 ± 0.26*	0.04 ± 0.02	3.66 ± 1.02*	0.10 ± 0.02
Sorghum flour	0.51 ± 0.04	0.49 ± 0.02	1.23 ± 0.03*	0.80 ± 0.08

Table 1. Mg iron per 100 g dry cereal soluble at pH 6.5 after 120 min gastric incubation at pH 2 or pH 5 with or without *A. niger* phytase addition. Asterisks indicate significant difference ($p < 0.005$) between soluble iron with or without added phytase (within each cereal and pH).

(Table 1). Wheat had endogenous phytase activity that contributed to almost complete removal of InsP3-6 during two hours of incubation, although the rate was only about ~1/6 compared to the level of added microbial phytases. Soluble iron contents should be seen in relation to the total iron contents of the applied cereals, which were ~16, ~17 and 4 mg/100 g dry cereal for wheat bran, rice bran and milled sorghum flour, respectively.

Although iron solubility does not equal iron absorption, the amount of soluble iron present has been shown to correlate well with human iron absorption studies (investigating non-fortified meals) and serves well as a way to understand the influence of different factors on iron absorption (21). *In vivo* data show increases in iron absorption from 1.7 to 11.7 times with the addition of phytases (Figure 1). Assuming the same relative absorption from non-fortified meals as shown in Figure 1, some examples of the quantitative significance of iron absorption improvement can be calculated (Table 2). Total iron absorption from a serving of oatmeal porridge (made from 45 g oats) may increase from ~6 µg to ~53 µg with addition of phytases and for a similar serving of wheat porridge from 15 to 176 µg. Considering two slices of whole wheat bread (relative absorption from wheat rolls are used), iron absorption may potentially increase from 226 µg to 412 µg. Nutrition data from the USDA National Nutrient Database have been used (22). Thus, phytases have the potential of improving iron absorption from these meals from 0.6-23% of the daily physiological iron requirements up to 5-41% of the daily physiological iron requirements (~1-2 mg depending on age and gender) (18). While single meals do not cover the entire amount of iron needed to rectify severe iron deficiency and iron deficiency anaemia, phytase-assisted iron solubilisation is expected to be nutritionally relevant in the long term, notably as a preventive measure against iron deficiency anaemia.

ADVANTAGES OF PHYTASES FOR IMPROVED IRON BIOAVAILABILITY

Current approaches to improve iron status include the administration of iron supplements or fortification of common foods with iron. Supplemental iron may have both acute and chronic side effects. Iron supplements often induce undesirable gastrointestinal side effects, which are thought to be related to the total dose of elemental iron (23). The usual dosage for treatment of iron deficiency anaemia in adults is 60 mg of inorganic iron three to four times a day (23), causing high concentrations of iron in the gastrointestinal tract. These prolonged high concentrations of free iron in the gut produce oxidative stress and reduce fractional iron absorption due to mucosal block of the intestinal cells (24). Also, unabsorbed iron in the colon can have negative effects on the gut microbiota (25). In contrast, improving absorption of native iron present in cereal foods via phytase treatment will diminish any negative side effects related to the high concentrations of free iron in the gut. As a final note on the advantages of phytases, high levels of supplemental iron has a negative sensory effect.

POTENTIAL DISADVANTAGES OF PHYTASE SUPPLEMENTATION

Concerns have been raised about the safety of removing a nutritional chelator, which may aid in decreasing

Meal	Serving size	mg iron per serving (22)	Iron absorption without phytase treatment		Iron absorption with phytase treatment	
			[%]	[µg]	[%]	[µg]
Oat porridge	45 g	1.91	0.33%	6	2.79%	53
Wheat porridge	45 g	1.53	0.99%	15	11.54%	176
Whole wheat bread	2 slices	1.58	14.30%	226	26.10%	412

Table 2. Iron absorption from different cereal-based meals. Serving sizes for the porridges are given on cereal basis. Relative iron absorptions [%] are from Sandberg *et al*. and Hurrell *et al*. (2, 6) and are shown in Figure 1. One slice of bread was calculated as weighing 32 g.

bioavailability of toxic heavy metals. Our recent results show that *in vitro* solubilities of arsenic, cadmium and lead are *not* increased by the addition of microbial phytases. One *in vivo* study in rats found a tendency to increased bone lead concentration correlated to phytic acid degradation, but no change in concentration of cadmium and lead in the liver and kidneys (26). A phytase supplement study in humans showed no change in blood lead levels due to the ingestion of phytase (27). In addition, sufficient iron status and plentiful dietary supply of magnesium, calcium and zinc are known to limit cadmium absorption by means of down-regulation of intestinal mineral transporters as well as competition for the transporters between cadmium and magnesium, calcium and zinc (28).

A final concern related to degradation of phytic acid in food is the potential loss of the possible anticancer activity of phytic acid that has been reported in many studies (reviewed by Vucenik and Shamsuddin (29)). It is reported that the presumed anticancer activity of phytic acid likely depends on rapid dephosphorylation *in vivo* and that inositol as well as inositol hexakisphosphate has antioxidant properties (29). The presence of an active phosphatase in human plasma has recently been published along with the result that no inositol hexakisphosphate could be detected in human plasma (30). Therefore, the anticancer effect of phytic acid may be preserved even after partial dephosphorylation in the gastrointestinal environment.

When comparing the direct ingestion of phytases with different phytase pretreatments of foods (including action of endogenous cereal phytases during e.g. leavening of bread), there are advantages of both approaches. Phytase pretreatment of foods can be carried out centrally in such a way that no actions from the end users are required, thereby potentially improving compliance. On the other hand, many food products, especially in the developing countries, do not undergo central processing, thus not providing the opportunity for adding phytases (or fortification iron). Application of phytase directly at the point of the consumers, e.g. as a sprinkle for application on porridges or similar, requires highly thermostable phytases and clear guidelines, notably because the required "reaction time" in the final product may provide a window for undesirable microbial contamination and growth.

CONCLUSIONS

Phytases used as a pretreatment of phytate-rich cereal foods or ingested along with phytate-rich cereal meals provide a means of improving iron absorption and may aid in decreasing the prevalence of iron deficiency. It is expected that this strategy does not produce the common, undesirable side effects experienced with traditional iron supplements. The recent approval by WHO of the *A. niger* derived phytase for use in foods or supplements opens a door for using phytases in foods and supplements. Although more knowledge is needed on the phytase-mediated iron release and iron absorption *in vivo*, hopefully the recent progress, i.e. both the WHO approval and the new knowledge on phytases, can help provide viable solutions to improve iron bioavailability from cereal foods, and in this way hopefully aid in decreasing the prevalence of iron deficiency anemia.

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