Minerals and phytic acid interactions: is it a real problem for human nutrition?

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Summary Because of its high density of negatively charged phosphate groups, phytic acid (PA) forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake. Indeed, the first step in mineral absorption requires that the mineral remains in the ionic state. As the PA content of the diet increases, the intestinal absorption of zinc, iron and calcium decreases. The inhibitory effects of PA on magnesium or copper are more controversial. Nevertheless, PA does not occur alone in foods and is often consumed with various compounds. Phytates are always present in vegetal matrix composed of fibres, minerals, trace elements and other phytomicronutrients. Thus, in order to evaluate mineral absorption from phytate-rich products, all components of diet and food interactions should be considered and it is hard to predict mineral bioavailability in such products by using only the phytate content.

Keywords Absorption, bioavailability, calcium, fibres, iron, phytate, trace element, zinc.

General considerations about mineral absorption

Some mineral deficiencies are common in developing countries, but mineral subdeficiencies may also occur in developed countries. Minerals are involved in activation of intracellular and extracellular enzymes, in regulation of critical pH levels in body fluids necessary for the control of metabolic reactions and in osmotic balance between the cell and its environment. A deficiency of any one of the essential minerals can result in severe metabolic disorders and compromise the health of the organism.

Intestinal absorption is a key and complex stage for maintaining normal mineral homeostasis. There exists several major mechanisms affecting mineral absorption from the gastrointestinal tract.

Competition of chemically similar ions for the same transport carriers

The gut is an important site for these interactions. The carrier proteins found in duodenum are subject to competition among the ions for their binding and consequential absorption. The competition can be between trace elements, macrominerals or between micro- and macrominerals. For instance, copper is preferentially bound to tranferrin, the protein transport molecule in the mucosa, when competing with iron. Normally this transport mechanism is not completely saturated, so there are adequate binding sites for both iron and copper. Nevertheless, when copper and iron are administered to an excess, iron absorption is inhibited because of the preferential binding of copper to transferrin. Thus, some metals will be bound to the carrier protein before others, and a displacement can occur if a stronger metal requires the binding site held by a weaker metal.

Abbreviations: PA, phytic acid; SCFA, short chain fatty acids

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Changes in the metal component of a digestive enzyme

A metal that activates a specific enzyme can be replaced by another metal that may either block or accelerate that particular enzymatic activity. For example, the carboxypeptide enzyme is activated by zinc. The zinc can be replaced by cobalt, which will cause a decrease in peptidase activity. So, digestive enzyme activity has an impact on mineral absorption. The competition of different minerals for those enzymes therefore influences their overall absorption.

Importance of intestinal pH

The different regions of the small intestine vary in their capacities to absorb the mineral. In general, there is a decreasing absorption rate as the ion goes through the intestine: duodenum → jejunum → ileum → large intestine

The availability of a metal ion for mucosal absorption is generally dependent upon the pH of the intestinal lumen. Certain forms of mineral salts require very low pH to be solubilized and then absorbed. They remain insoluble in an alkaline or slightly acid environment. In an alkaline pH, such as occurs in the intestine below the bile duct, there appears to be little attraction for the carrier protein to be attached to the mineral and carry it across the membrane into the mucosal cells. A very small amount of these minerals may be absorbed into the cells through diffusion or through some natural chelation consequential to pH changes if the ions remain soluble. Many metals have a tendency to form insoluble salts with either anions in the diet or of physiological origin in the higher pH range of the small intestine. In general, the more alkaline the lumen, the lower the rate of absorption of most minerals.

In addition, the transport of a given mineral through the mucosal cell membrane is because of the affinity of the metal for the binding carrier protein or in the cell membrane. The total capacity of the cell membrane to bind or retain the metal is obviously a major consideration in determining the total quantity absorbed.

Interactions between minerals and other nutrients

Vitamins

Not only do minerals interfere with the absorption of other minerals as indicated above, but other nutrients, such as vitamins, can have equally significant positive or negative influence on mineral absorption. Vitamin D is a very interesting example. Its role in the regulation of intestinal absorption of ionic calcium has been conclusively proven. Vitamin D must be altered metabolically to regulate calcium absorption. The change commences in the liver, followed by additional changes in the kidney to produce a hormone, 1,25-dihydroxyvitamin D. This hormone controls the absorption of the calcium ion from the intestine by stimulating the active transport of calcium against an electrochemical potential gradient. The hormone 1,25 dihydroxyvitamin D is also involved in supplying genetic instruction for the production of the carrier protein across the mucosal cell to the serosa. A deficiency of vitamin D in elderly people for instance will interfere with the absorption of ionic calcium (Andon *et al.*, 1993).

Vitamin C from orange juice enhances the absorption of non-haemic iron. The addition of ascorbic acid to aqueous acid solution of the stomach and the upper part of the duodenum causes a displacement of hydrogen ions from the resulting iron ascorbate chelate. This iron chelate has the ability to remain in the solution over a pH range of between 2 and 11. (It should be stressed that the reaction is initiated optimally at an acid pH because ferric chloride as well as ascorbate are both insoluble at an alkaline pH.) Thus, the presence or absence of vitamin C will affect the absorption of this particular form of mineral.

Fat

The absorption of calcium can be depressed in the presence of high levels of fat. Some unsaturated fatty acids also form calcium salts which are poorly absorbed. Thus, the degree to which the fats interfere with intestinal absorption depends on the type of fat in the diet. Presumably, if calcium can be bound by the unsaturated fatty acids, other bivalent metal ions can up also be tied. Nevertheless, it has been reported that even

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with calcium, the amount of insoluble soaps formed is not usually a significant factor in absorption.

Formation of insoluble complexes

The fifth action often involves only one mineral plus a precipitating ligand. The ligand may be inorganic, such as a phosphate, or organic such as a phytate. The complexing with a precipitating ligand can occur in the tissues wherein a mineral can displace another mineral from its position in the tissue. The displaced mineral is then free to enter into a reaction with a precipitating ligand. More appropriate to this discussion are the interactions occurring in either the diet and / or the digestive tract. Again there is competition among the minerals for the precipitating ligand. The competition depends upon the concentrations of the minerals involved, the relative strengths of the association constants of the minerals, and the solubility of the formed product. Precipitating substances derived from the diet are a major factor affecting intestinal absorption of metals. Once a mineral salt is ionized in preparation for membrane transport of the ion, it is by its very physical nature unstable. In the ionic state, it is highly susceptible to sequestering by phytic acid (PA) from cereals and other plant seeds. The PA forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake because the first step in mineral absorption requires that the mineral remain in the ionic state. As the PA content of the diet increases, the intestinal absorption of certain metals, specifically zinc, iron and calcium, decreases. The reduction in mineral PA absorption may be caused by the lack of ionization of the mineral complex in the stable phytate form. If precipitation were to occur, there would be no inducement for carrier proteins located on the intestinal cell membrane to bind themselves to the metal ion prior to membrane transport because the metal is not in ionic form.

In general, hydrolysis of PA increases mineral absorption. Nevertheless, phosphates from PA hydrolysis can react with calcium, magnesium, zinc, iron to form insoluble precipitates. Most phosphates are slightly soluble in water or acid solutions. However, the intestine tends to become alkaline which reduces the solubility of the phosphates when appearing in that environment. When the mineral is trapped within an insoluble compound, there is little likelihood of significant intestinal absorption as the precipitated salt is generally stable, creating no attraction to induce integral carrier proteins in their lipid environment in the membranes of the mucosal cells to bind the ion and move it across the membrane.

Interactions between PA and minerals

PA (or myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the major phosphorus (P) storage compound in plant seeds and can account for up to 80% of seed total P. The remaining P is represented by soluble inorganic phosphate and cellular P (P bound in nucleic acids, phosphorylated proteins, P-lipids, P-sugars). Because of its high density of negatively charged phosphate groups, phytate forms mixed salts with mineral cations which are assumed to play an important role in mineral storage. These salts called phytins contain predominantly K and Mg, whereas other metals such as Ca, Zn, Fe or Cu are found in much smaller amounts. In many plant species, 90% of the phytin is localized in the aleurone layer and only 10% in the embryo. In contrast, in maize, most of the phytin is found in the germ and only a small fraction is found in the aleurone (O'Dell *et al.*, 1972). During germination, phytin is degraded by the action of phytases, which provides the growing seedling with phosphate, mineral cations and myo-inositol. Apart from its storage function, phytate has also been assumed to play an important role in P homeostasis, buffering cellular P levels.

Zinc and phytic acid

Zinc is an essential trace element involved in the immune function, in the activation of many enzymes and in the growth. However Zn deficiency has been recognized in Western countries due to inadequate dietary supply, abnormal blood losses or high physiological requirements for growth, puberty, pregnancy and lactation.

The availability of Zn for intestinal absorption and body utilization is the net effect of absorption inhibiting and promoting components of the diet. The amount of PA, the type and amount of protein and the total Zn content have a major impact on

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the amount of Zn absorbed from foods. Under normal physiological conditions, factors affecting the amount of Zn available for intestinal absorption determine Zn bioavailability. Certain types of diets could alter the reabsorption of endogenous intestinally excreted Zn and thereby affect utilization.

PA is the major determinant of Zn absorption, especially for diets with a low animal protein content. PA strongly binds Zn in the gastrointestinal tract and reduces its availability for absorption and reabsorption (Flanagan, 1984) The inhibitory effects of PA on Zn can be predicted by the molar ratios of PA-to-Zn in the diet, when the dietary Zn intake is close to the requirement. Molar ratios in excess of 15 : 1 progressively inhibit Zn absorption and have been associated with suboptimal Zn status in humans (Gibson *et al.*, 1997). Furthermore, high levels of Ca exacerbate the inhibitory effect of PA on Zn absorption in humans by forming a Ca–Zn–PA complex in the intestine that is even less soluble than phytate complexes formed in either ion alone. Hence, PA × Ca/Zn ratios may be better predictors of Zn bioavailability than PA/Zn molar ratios alone (Fordyce *et al.*, 1987).

In order to increase Zn bioavailability, foods can be improved:

- By increasing the total amount of dietary Zn. However fractional Zn absorption is dependent on the Zn content of the diet. At low Zn content with no potential inhibitory agents, fractional absorption can be as high as >50%. High Zn intakes result in a low absorption per cent, because of the saturation of the active transport mechanisms for Zn.
- By enhancing the bioavailability of Zn consumed. This is possible by promoting the intake of enhancers and reducing the impact of PA on intestinal Zn absorption.

Food interactions are also determinant for Zn absorption (Lönnerdal, 2000). Dietary proteins can potentially facilitate Zn absorption even in the presence of PA. Proteins prevent the precipitation of Zn in the intestinal lumen and amino acids such as cysteine or peptides which facilitate Zn uptake by the mucosal cells (Sandström *et al.*, 1989). Consuming fermented foods leads to enhanced Zn absorption. This effect is attributed to the presence of organic acids (acetic, citric, lactic or malic acids) which form soluble ligands with Zn, thereby preventing the formation of insoluble Zn phytates (Pabon & Lönnerdal, 1992). The solubility of Zn at the site of absorption probably has a major impact on its availability. Zn in foods is relatively easily solubilized at gastric pH, whereas it binds to organic components at higher pH. Thus, small molecular weight ligands, such as organic acids, have the potential to increase solubility and facilitate absorption of Zn. During germination, endogenous phytase activity in cereals and legumes increases as a result of *de novo* synthesis and/or activation of endogenous phytase, the rate of PA hydrolysis varying with the species and variety. This reduction in the PA content of cereals and legumes increased Zn absorption (Gibson *et al.*, 1998).

Iron and phytic acid

The single most prevalent deficiency on a worldwide scale is iron (Fe) deficiency anaemia, affecting an estimated 30% of the world's population. In Europe, Fe deficiency is considered to be one of the main nutritional deficiency disorders (Hercberg *et al.*, 2001). As for Zn, Fe deficiencies are caused by insufficient intakes of Fe, by increased needs of Fe for pregnancies or by blood loss or impaired absorption. For the last point, the source of Fe as well as composition of meal is of great importance because dietary factors play an important role in Fe absorption. There are two kinds of dietary iron: haem Fe and non-haem Fe. If haem Fe is poorly affected by other components in the diets, non-haem Fe, which comprises the main part of the Fe intake, is absorbed in ionic form by receptors on the mucosa cells and its bioavailability varies depending on the Fe status of the subjects and different dietary factors. There is evidence that PA has a very marked inhibitory effect on the absorption of non-haem Fe in man.

As for Zn, PA decreases Fe solubility (Sandberg & Svanberg, 1991) and the inhibition of Fe absorption is closely related to the content of phytate in bread (Brune *et al.*, 1992). The inhibitory effects of PA on Fe can be counteracted by Fe absorption enhancers such as protein (Reddy *et al.*, 1996) or organic acids (Gillooly *et al.*, 1983). Ascorbic acid is the most effective enhancer

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of non-haem Fe absorption. Vitamin C promotes non-haem Fe absorption by reducing ferric iron to the ferrous state which is more soluble at the pH present in the duodenum and small intestine. The enhancing effect of ascorbic acid is strongly dependent on the dose and also varies with the content of PA in the meals (Hallberg *et al.*, 1989). As for ascorbic acid, the precursors of vitamin A in corn partially inhibit the effect of phytate on Fe absorption in humans. Vitamin A or beta-carotene forms a complex with Fe keeping it soluble in the intestinal lumen and preventing the deleterious effect of PA on Fe assimilation (Layrisse *et al.*, 2000).

Nevertheless, high Fe consumption has been proposed to relate to an increase in the risk of colon cancer. Dietary Fe remains largely unabsorbed in the intestine and it is hypothesized to participate in the generation of hydroxyl radical by a Fenton-type reaction in conjunction with colonic microflora. Therefore, inhibition of Fe ion-mediated lipid peroxidation is suggested to be important for the prevention of gastrointestinal diseases. PA and their hydrolysis products can prevent lipid peroxidation, reduce Fe-induced oxidative injury and reverse Fe-dependent augmentation of colorectal tumorigenesis (Miyamoto *et al.*, 2000).

Calcium and phytic acid

The factors controlling calcium bioavailability can be divided into intrinsic and extrinsic factors. Intrinsic factors are age, sex, pregnancy and lactation. Extrinsic factors include a number of dietary variables that may influence Ca absorption, such as amount of calcium, vitamin D, oxalate, phosphopeptides, fat, lactose and PA and a number of dietary variables that may influence urinary Ca excretion such as salt, phosphorus, protein, alcohol and caffeine (Gueguen & Pointillart, 2000).

In humans, PA decreases Ca absorption (Reinhold *et al.*, 1976) and PA breakdown improves Ca availability. Although the literature has frequently reported an inhibitory effect of PA on Ca absorption (Lönnerdal *et al.*, 1989; Rimbach *et al.*, 1995), some researchers failed to observe any negative effect of PA on Ca absorption on rat model (Miyazawa *et al.*, 1996; Nickel *et al.*, 1997).

If Ca assimilation can be reduced by PA, a high Ca/PA molar ratio in food leads to the absence of hydrolysis products in the intestine (Wise *et al.*, 1983). The reason for the decreased PA degradation caused by Ca may be the formation of insoluble Ca phytate complexes which are poor substrates for phytase (Sandberg *et al.*, 1993). Furthermore, the consumption of diets poor in whole products and rich in Ca have a propensity to cause kidney calcifications. The ingestion of PA-rich foods (brans, whole products, etc.) maintains adequate Ca urinary levels to permit effective crystallization inhibition of Ca salts and consequently prevents renal stone development (Grases *et al.*, 2000).

Magnesium and phytic acid

In France, the SU-VI-MAX study showed that 72% of men and 76% of women had magnesium intakes lower than the French recommended dietary allowances (Galan *et al.*, 1997). The main causes of this low Mg intake are the decrease of total energy intake (Mg is ingested together with the macronutrients such as carbohydrate in whole wheat products), the decrease of the nutritional quality of products (high consumption of foods rich in energy and poor in micronutrients such as sugar, sodas, etc.) and the increase of refined products available in the markets (white flour, white sugar, etc.).

Magnesium retention depends on absorption and on homeostatic mechanisms at the level of the kidney and on individual magnesium status. Magnesium absorption is dose dependent but may also vary as a function of the composition of the diet, which contains either enhancers or inhibitors of Mg absorption (Wester, 1987). Mg absorption is mainly influenced by the total quantity of Mg ingested and by the Mg solubility (Coudray *et al.*, 2002a).

It has been reported that diets rich in Ca, marginal in Mg and supplemented with PA decrease Mg bioavailability dose dependently and thereby affect the dietary Mg requirement (Pallauf *et al.*, 1998). In human nutrition, this situation is not common: in general, plants rich in PA (such as whole cereal products, legumes or oilseeds) also contain large amounts of Mg. Under these conditions, unrefined foods remain the major sources of Mg. Less purified products such as whole wheat flour are rich in PA but they provide fivefold more Mg than white wheat

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flour. Thus, in comparison with white wheat flour, the consumption of whole wheat flour can contribute to improve Mg balance in rats (Levrat-Verny *et al.*, 1999; Coudray *et al.*, 2001). Moreover, the importance of the distal part of the digestive tract for Mg absorption is well documented (Karbach & Rummel, 1990). As wholegrain products contain fermentable complex carbohydrate such as resistant starch, pentosans or fructans, Mg absorption can be stimulated (Rayssiguier & Rémésy, 1977) and the inhibitory effects of PA on Mg metabolism can be lessened by fermentable fibres (Lopez *et al.*, 1998). Inspite of the presence of PA, it is difficult to ascribe a negative effect to whole products on Mg absorption. Furthermore, results showing Mg/PA interactions are mostly expressed as absorption percentage of minerals which do not reflect the beneficial effect of mineral-rich products on Mg status.

Copper and phytic acid

Data on the relationship between copper availability and PA are still confusing. For some authors, PA had no effect on Cu absorption in men (Turnlund *et al.*, 1985), whereas others studies showed inhibitory effect of PA on Cu absorption in animal model (Davies & Nightingale, 1975) or a positive effect of PA on Cu absorption in rats (Lee *et al.*, 1988). The effect of PA on Cu absorption seems to be modulated by several factors, especially the Zn level in the diet. PA can indeed enhance Cu absorption because of its ability to bind Zn, thus counteracting its capacity to compete with Ca at the intestinal absorption sites (Champagne & Hinojosa, 1987).

Mineral bioavailability, phytic acid and fibres

Even if phytates are the main cause of the inhibitory effect of wheat bran on Zn or Fe absorption (Hallberg *et al.*, 1987), PA does not occur alone in foods and is often consumed with various compounds. PA always includes in vegetal matrix composed of fibres, minerals, trace elements, and other phytomicronutrients.

Fibre and PA occur together in fibre-rich diets and, thus, it is difficult to separate the effects of fibre and PA in the utilization of most essential metallic ions. Dietary fibres and associated substances, such as PA, are known to have strong *in vitro* mineral binding or complexing capacities (Persson *et al.*, 1991). Moreover, exaggerated amounts of dietary fibres were often used in animal model studies. Thus, animal and human studies have shown that foods or diets rich in dietary fibres may alter mineral metabolism, especially when phytate is present (Andersson *et al.*, 1983; Sandstead, 1992). However, many other studies have indicated that dietary fibres do not appear to affect trace element absorption (Behall *et al.*, 1987; Sunvold *et al.*, 1995). Thus, it is clear that the observed effect of fibres on mineral absorption largely depended on the type (soluble or insoluble, fermentable or non fermentable) and the amount of dietary fibres and the associated components such as PA in the meal.

For instance, fermentable carbohydrates present in seeds, legumes or oilseeds, counteract the inhibitory effects of PA on mineral absorption (Lopez *et al.*, 2000a). These non digestible carbohydrates such as resistant starch, pentosans or fructans have been found to stimulate absorption of several minerals and to improve mineralization of bone. In rat model, the fermentation of such carbohydrates by the flora results in the production of short chain fatty acids (SCFA) in particular acetate, propionate and butyrate. These acids may form complexes with ions with very low charges able to cross the cellular membrane of enterocytes. These acids are also responsible for the acidification (pH decreasing) of caecal content which in turn improves the solubility of minerals and hence their absorption. Finally, the enlargement of the large intestine that occurred in the animals receiving the fermentable complex carbohydrates may also be responsible for better exchange between the colon and the enterocytes which can increase mineral absorption rate. (Scholz-Ahrens *et al.*, 2001). Unfortunately, limited information is still available for humans: indirect evidence that this effect could occur in humans has been reported (Trinidad *et al.*, 1996) and short duration studies using inulin or fructo oligo saccharides showed that calcium absorption was stimulated by fermentable carbohydrate (Coudray *et al.*, 1997). Indeed the colon plays an important role in the absorption of Ca after small intestine resection (Hylander *et al.*, 1990). Recently,

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the data from our laboratory have shown that fermentable carbohydrate can increase significantly intestinal Mg absorption and its balance in young men and in post-menopausal women (Tahiri *et al*., 2001; Coudray *et al*., 2002b).

Finally, the recommendation for increasing dietary fibre in Western communities would not be expected to have any adverse effect on mineral absorption if we increase not only the intake of fibre, but also the dietary intake of other food components such as protein (both vegetable and animal protein) and ascorbic, citric and oxalic acids (in fruits and vegetables). The adequate intake of minerals, fat and simple sugars are maintained with this type of diet. The recommendations should be best interpreted in such a way as to prevent the consumption of excessive amounts of PA, particularly for those whose mineral needs are great (Torre *et al*., 1991).

Effects of breeding, growing conditions and genetic engineering

Using a same vegetal species (wheat), mineral bioavailability from whole wheat flour is different between varieties. The most surprising fact is that varieties with the same PA content does not have the same bioavailability of Mg, Fe or Zn. It could be explained mainly by the variability in vegetal phytase activities and by the mineral/PA ratio. Thus, the total mineral content in whole cereals plays a predominant role in bioavailability. In seeds, PA accumulation is correlated to phosphorus accumulation but not to total mineral content. Thus, if PA content was relatively constant in the wheat tested, varieties with higher mineral bioavailability had greater mineral stores and higher activity of vegetal phytase (Lopez *et al*., 2002a).

Agronomic growing conditions also influence the mineral content of cereals. El-Gindy *et al*. (1957) reported a correlation between fertilizer treatment and total ash of three hard red winter wheat grains grown in thirteen locations utilizing seven different fertilisers. They also concluded that concentrations of minerals depends on the varieties tested. In the same way, Peterson *et al*. (1983) investigating mineral composition of bran originating from twenty-seven varieties of durum wheat, found significant influences of variety and region of cultivation on this composition.

Moreover, organic crops contain significantly more magnesium, iron and phosphorus than conventional crops. These differences could be explained by the presence of a greater population of micro-organisms in organically managed soils. These micro-organisms produce many compounds that help plant, including substances such as citrate or lactate that combine with mineral soil and make them more available to plant roots. For iron, in particular, this is especially important because many soils contain adequate iron but in an unavailable form. Furthermore, potassium fertilizer can reduce the Mg content and indirectly the phosphorus content of some plants (Worthington, 2001). Thus, the mineral bioavailability would be different between organic and conventional crops.

In order to optimize animal growth with reducing mineral accumulation in the soil, nutritional availability of P is most commonly improved by supplying animal fodder with microbial phytases. Other strategies, however, aim at overexpression of phytases in transgenic cereals and on mutational breeding of low-phytate mutants (Hatzack *et al*., 2000). Low phytate crops such as corn or barley, represent a strategy to improve P-utilization and to reduce the environmental impact of phytate-rich manure. It must be noted that other nutritionally important components such as K, Mg, Ca, Zn, protein and starch levels are not severely affected by low-phytate mutations (Mendoza *et al*., 1998; Sugiura *et al*., 1999).

Effects of food processes on mineral/PA interactions

Manufacturing processes (kneading, soaking, fermentation, baking, toasting, extrusion, cooking) play a key role on the PA content of the final cereal product and thus on the mineral bioavailability. Varying losses of PA occur during the manufacturing process. For instance, the whole PA from the ingredients is found in the whole wheat biscuits, because there is no destruction of PA in a whole wheat pastry (Le François, 1988). The PA reduction in extrusion cooking products is still discussed: a 25% decrease was found by Le François (1988) whereas Sandberg *et al*. (1986) observed no change in the PA content in extruded products. Moreover, it must be underlined that

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extrusion cooking may lead to a considerable impairment in the digestion of PA, because of a loss of intrinsic cereal phytase activity (Sandberg *et al.*, 1987). The plant phytase, PA-degrading enzyme that is deactivated during extrusion cooking, is of significance for phytate hydrolysis in the stomach and small intestine. Thus a possible impairment of mineral absorption mediated by the resistance of PA to digestion in the human gut, must be considered (Sandberg, 1997).

Bread is a staple food in the world. Table 1 shows the mineral composition of three cereal products: wheat bran rich in minerals and PA, white bread poor in PA but also poor in minerals and whole bread rich in minerals and the content of PA can strongly fluctuate from 0 to 300 mg 100 g^{-1} of whole bread. As consumption of whole grain breads is increasing in Western countries, a wholewheat bread with low PA level and increased mineral bioavailability would be beneficial and attractive in improving mineral status and consequently in promoting preventive nutrition.

During bread making, the degradation of PA is caused by the action of phytases present in the dough. The activity of baker's yeast seems to have no significant effect in these conditions (Tangkongchitr *et al.*, 1981). Nevertheless, if very little phytate is hydrolysed in unleavened wholemeal breads including bicarbonate of soda breads, PA hydrolysis during all stages of yeast bread making occurs (MacKenzie-Parnell & Davies, 1986). Reduction of PA contents in different bread types may vary between 13 and 100%, with the lowest decrease being in unleavened breads. PA contents of ryebread may be, under optimal conditions, reduced to near zero values (Fretzdorff & Brümmer, 1992). The substantial decrease of PA in wholewheat products can improve mineral availability to humans (Nävert *et al.*, 1985; Brune *et al.*, 1992). Bread has the advantage to conjugate the vegetal (from cereals) and micro-organism (yeast or/and lactic acid bacteria) phytasic activities. Leavening of bread reduces PA and improves Zn absorption. Thus, in Iran and Turkey, Zn deficiencies have been observed when people consume non fermented whole cereal products whereas Zn status has been reported normal in people consuming leavened bread (Reinhold *et al.*, 1976). Bread fermentation improves Zn bioavailability in wholewheat products (Nävert *et al.*, 1985), and that natural leavening process itself (lactic acid fermentation) is more efficient than yeast fermentation to improve mineral bioavailability. Lactic acid fermentation increases Ca, Mg, Fe or Zn solubility estimated *in vitro* (Svanberg *et al.*, 1993). Thus food acidification increases solubility of Mg or Zn independently of phytic acid. (Fig. 1) A lactic acid fermentation of PA-rich foods (bread, etc.) allows to release Mg or Zn from vegetal matrix and leads to a better bioaccessibility. In parallel, it must be underlined that lactic acid bacteria isolated from sourdough have the ability to degrade PA (Shirai *et al.*, 1994) and thus the destruction of PA by micro-organisms occurs during sourdough bread making (Lopez *et al.*, 2000b). Bread making using Baker's yeast contributes to PA degradation. Although a PA decrease was detected during bread making assigned to yeast phytase activity, the main degradation of PA seems to be a result of activity by endogenous plant phytases present in the flour (Türk *et al.*, 1996). It must be noted that PA breakdown was less pronounced in yeast bread than sourdough bread. As the PA content in bread affects mineral assimilation, it appears coherent that Mg, Fe, Zn and Cu were less absorbed from yeast-fermented bread than from sourdough bread. The increased whole bread consumption should raise the intake of essential elements, as high mineral contents are found in wholewheat breads, particularly K, Mg, Zn and Fe (Lopez *et al.*, 2002b).

Various physical and chemical factors affect mineral absorption, including particle size, water content, pH, temperature and fermentation time. Flour granulometry is essential for mineral bioavailability: a wholewheat flour reconstituted

Table 1 Comparison of three wheat products

mg 100 g^{-1}	Wheat whole bread	Wheat white bread	Wheat bran
Potassium	200–300	100–150	1300–1500
Magnesium	50–80	20–25	500–700
Calcium	30–100	20–80	40–80
Zinc	1.5–2.0	0.2–0.8	8–15
Iron	2–4	0.4–1.5	10–20
Phytic acid	0–330	0–20	3000–5000

From: Souci, Fachmann & Kraut, *Food Composition and Nutrition tables*, 6th edition, 2000, CRC Press.

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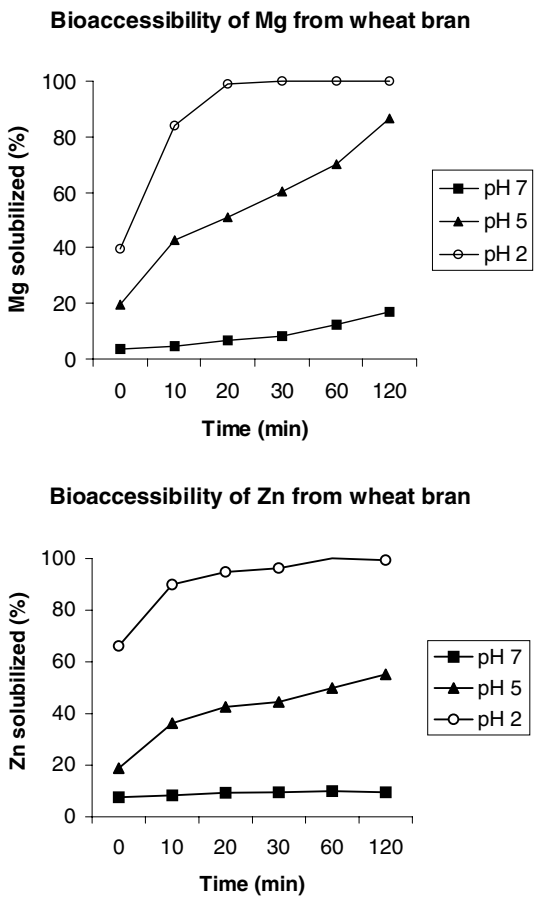

Figure 1 Kinetics of Mg and Zn solubility of wheat bran according to pH modifications. Procedure: wheat bran (500 mg) was mixed with 10 mL of buffer (pH 2, pH 5, pH 7) under agitation during 2 h at room temperature. The soluble and total Mg and Zn were determinated every 30 min by atomic absorption spectrometry. Results were expressed as soluble Mg (or Zn)/total Mg (or Zn) ratio or as percentage of soluble Mg (or Zn). The pH has a major impact on solubility of Mg and Zn from wheat bran, independently of phytic acid.

(white flour + bran) reduces Mg, Fe, Zn and Cu absorption in rats whereas the grain from the same variety that is ground in order to obtain the similar granulometry between white flour and bran did not alter Mg and Cu absorption in rats. Thus, if PA seems to be the main determinant of Fe and Zn absorption in whole products, the particle size of flour may play a role in Mg and Cu assimilation in experimental animals (Lopez *et al.*, 2002b). Furthermore, soaking is important during bread making, because it activates endogenous vegetal phytase. Thus, a high water content of dough increases PA hydrolysis, and phytate reduction in doughs made of coarse meal from wheat and rye is lower than that made of the corresponding flours (Fretzdorff & Brümmer, 1992). To increase PA destruction and mineral bioavailability in whole bread, dough pH has been suggested as the main determining factor (Larsson & Sandberg, 1991). Solubilities of the phytic acid chelates with cations depend on the pH and the amounts and type of cations. The limiting factor for phytate destruction in whole wheat dough above pH 6 is the insolubility of its Mg salt, whereas at pH 5 the limiting factor appears to be the activity of phytase (Tangkongchitr *et al.*, 1982). Moreover, in some circumstances, the calcium ions can play a role in preventing PA disappearance during baking and it has been shown that the addition of milk-derived calcium inhibits phytate hydrolysis (Snider & Liebman, 1992). On the other hand, it has been found that fermented milk did not inhibit phytate degradation to the same extent (Türk & Sandberg, 1992). Possible explanations are that lactic acid present in fermented milk increased the solubility of Ca phytate, or that the lower pH in dough containing fermented milk was close to the optimum pH of phytase than that in the dough made with regular milk. The optimum pH for PA hydrolysis of wheat is 4.5–5.0 and the optimum temperature is 55 °C. Thus, the acidification of the dough by lactic acid bacteria associated with a long fermentation time significantly enhances the phytate hydrolysis. PA breakdown during bread making by rapid processes is indeed less extensive than after a long fermentation process. If bread has a high phytate or a low Fe, Zn, Cu or Mg content, increasing the rising time may result in a considerable improvement in mineral availability (Harland & Harland, 1980). If all these parameters are optimized to increase PA breakdown, increased consumption of whole products may contribute to increased mineral intake, without compromising their bioavailability.

Adaptation of the intestine to PA-rich diets?

Some investigators have suggested that long-term vegetarians can adapt to habitually high intakes of phytate. When diets contain PA, the induction of mucosal phytase exists in rodents and the enhancement of intestinal phytase leads to an

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improved Ca intestinal absorption, demonstrating the adaptation of the small intestine to diets rich in PA and poor in Ca (Lopez *et al.*, 2000c). For other authors (Larsen, 1993; Sandberg *et al.*, 1987), no adaptation to increased small intestinal phytase degradation seems to occur in rats and humans. Brune *et al.* (1989) did not find any reduction in the inhibitory effect of a high-phytate diet on iron absorption in long-term vegetarians. Nevertheless, Moore & Veum (1983) showed that rats fed a marginal phosphorus diet can compensate for the lack of available phosphorus by a greater degradation of PA. Furthermore Yang *et al.* (1991) showed that the intestinal phytate-degrading enzyme was formed by 70K and 90K subunits that are expressed differently. The 70K subunit is detected at birth, whereas the 90K subunit appears at the weaning period, and the induction of the 90K subunit seems to be accelerated by PA intake. A direct extrapolation of the rat results to humans may be premature because the ability of various species of monogastric animals to hydrolyse PA varies. Rats and chicks appear to have high intestinal phytase activity whereas humans and pigs have very much lower activity.

Conclusion

In developed countries, there is clear interest in the health effects of food and increased use of whole grains. Recent epidemiological findings support the protective role of wholegrain foods against several western diseases such as obesity, diabetes or cardiovascular diseases (Kushi *et al.*, 1999). However, whole products are suspected of impairing mineral absorption. PA present in these products is considered to be the major factor causing impaired absorption of Zn, Fe or Ca. The inhibitory effects of PA on Mg or Cu are more controversial. Effective reduction of PA can be obtained via the action of exogenous PA-degrading enzymes (use of microbial or fungal phytases), breeding (selection of low PA varieties or high phytase varieties), agronomic conditions (optimization of fertilization, better knowledge of the benefits of organic crop growing), genetic engineering (over expression of vegetal phytase) or food processes (bread making, lactic acid fermentation).

Nevertheless, PA does not occur alone in foods and is often consumed with various compounds. Phytates always include in vegetal matrix composed of fibres, minerals, trace elements and other phytomicronutrients. Thus, in order to evaluate mineral absorption from phytate-rich products, all components of diet should be considered. Thus, even if PA content remains important, other factors such as vegetal phytasic activity, the phytate-to-mineral ratios or the content of fermentable fibres must be taken into consideration to predict mineral assimilation from whole products. Moreover, food diversity is important in developed countries and therefore, food interactions are also important for determining mineral absorption. Mineral assimilation can be stimulated by the presence of ascorbic acid, beta carotene, fermentable carbohydrates or organic acids whereas excessive Ca in diet reduces PA breakdown and improves mineral losses.

Finally, the impact of the same meal with the same content of PA on mineral bioavailability would vary from case to case. It can be explained by the fact that there are inter individual variations and that whole products consumers seem to have the gastrointestinal tract adapted to high PA diets. The intestinal phytase in such people would be enhanced by the presence of PA in food and the increased PA breakdown would lead to improved mineral absorption. Consequently, it is hard to predict mineral bioavailability in PA-rich products only by using the phytate content.

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