A review phytic acid: As antinutrient or nutraceutical

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Abstract
Phytic acid is a substance found in many types of plant foods, such as grains, legumes (including peanuts and soybeans), nuts, and seeds. It is the storage form of phosphorus, an important mineral used in the production of energy as well as the formation of structural elements like cell membranes (Jacela et al., 2010). These foods, are getting a bad reputation due to phytic acid content (Navert, et al., 1985) and its ability to bind to essential minerals such as iron, zinc, calcium, and magnesium in the digestive tract and inhibit their absorption by the body (Weaver & Kannan, 2002). Recent studies indicate despite being somewhat demonized for its ability to reduce mineral absorption, phytic acid actually has some potentially beneficial properties. On the plus side, phytic acid can act as antioxidant, exhibits anti-cancer properties, and may have a positive impact on cholesterol and blood sugar (Omni et al., 2004).

Preparation methods can reduce the phytic acid content in food, as well as adjusting meal times and food choices (Sade., 2009), can help to have better mineral absorption.

Keywords: phytic acid, mineral absorption, beneficial properties, Preparation methods.

Introduction
Phytic acid (PA; myo-inositol [MI] hexaphosphoric acid) is an abundant plant constituent and, based on weight, comprises 1% to 5% of the edible legumes, cereals, oil seeds, and nuts that serve as major sources of human and animal sustenance (Reddy et al., 1982) [43]. The amount of phytate in grains, nuts, legumes and seeds is highly variable; the levels that researchers find when they analyze a specific food probably depends on growing conditions, harvesting techniques, processing methods, testing methods and even the age of the food being tested. Phytic acid will be much higher in foods grown using modern high-phosphate fertilizers than those grown in natural compost. Numerous studies show that PA forms insoluble complexes with polycations, due to the reactive phosphate groups attached to the inositol ring, and thereby renders them unavailable for intestinal absorption in humans and animals (Kumar et al., 2010) [44]. Therefore, PA has been traditionally considered an antinutrient (Reddy et al., 1982) [43]. However, since the late 1980s, several studies have indicated that PA has beneficial effects such as antioxidant, anticarcinogenic, and antidiabetic properties (Omoruyi et al., 2013). It is mainly present as a salt of mono-valent and divalent cations such as K⁺, Mg⁺, and Ca⁺. It is accumulated into the seed during the ripening period. In dormant seeds, phytate represents 60-90% of total phosphate.

The phytic acid being the principal storage form of phosphorus in many seeds is named myo-inositol hexaphosphoric acid, IP6. Its molecular formula is C₆H₁₈O₂₄P₆ and its molecular weight is 660.03. The structure of phytic acid is shown in Fig.1 and then Fig.2 shows the structure of phytic acid with different possibilities to interact with both metal cations as with protein residues. It contains mineral phosphorus tightly bound in snowflake like molecule.
Phytate as an Antinutrient
The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn 2+, Fe 2+ / 3+, Ca 2+, Mg 2+, Mn 2+, and Cu 2+ (Lonerdel et al., 2002), but also a negative effect on the nutritional value of protein by dietary phytate is discussed.

Phytate and Mineral interaction
Phytate forms complexes with numerous divalent and trivalent metal cations. Stability and solubility of the metal cation-phytate complexes depends on the individual cation, the pH-value, the phytate:cation molar ratio, and the presence of other compounds in the solution (Oberaleas et al., 1983) [9]. Phytate has six reactive phosphate groups and meets the criterion of a chelating agent. In fact, a cation can bind to one or more phosphate group of a single phytate molecule or bridge two or more phytate molecules (Reddy et al., 1982) [40]. Most phytates tend to be more soluble at lower compared to higher pH-values (Torre et al., 1991) [10].

Solubility of phytates increase at pH-values lower than 5.5–6.0 with Ca 2+, 7.2–8.0 with Mg 2+ and 4.3–4.5 with Zn 2+ as the counter ion. In contrast, ferric phytate is insoluble at pH values in the range of 1.0 to 3.5 at equimolar Fe 3+: phytate ratios and solubility increases above pH 4 (Askar et al., 1983) [11]. Another important fact is the synergistic effect of secondary cations, among which Ca 2+ has been most prominently mentioned (wise 1983) [12]. Two cations may, when present simultaneously, act jointly to increase the quantity of phytate precipitation. For example, Ca 2+ enhanced the incorporation or adsorption of Zn 2+ into phytate by formation of a calcium-zinc phytate. The effect of Ca 2+ on the amount of Zn 2+ co-precipitating with phytate is dependent on the Zn 2+: phytate molar ratio. For high Zn 2+: phytate molar ratios, Ca 2+ displaces Zn 2+ from phytate binding sites and increases its solubility. The amount of free Zn 2+ is directly proportional to the Ca 2+-concentration. For low Zn 2+: phytate molar ratios, Ca 2+ potentiate the precipitation of Zn 2+ as phytate.

Thus, higher levels of Ca 2+ result in a more extensive precipitation of the mixed phytates. Mg 2+ also has been shown in vitro to potentiate the precipitation of Zn 2+ in the presence of phytate, however, Mg 2+ has been found to exert a less pronounced effect on Zn 2+-solubility than Ca 2+.

The capacity to bind cations was found to be a function of the number of phosphate groups on the myo-inositol ring. The cation-myo-inositol phosphate complexes are more soluble as the number of phosphate groups decreases. There is also some evidence for weaker complexes when phosphate groups are removed from phytate. In addition, the binding affinity of cations to myo-inositol phosphates has been shown to be affected by the distribution of the phosphate residues on the myo-inositol ring.

Effect of Phytate on Mineral Availability
The formation of insoluble metal cation-phytate complexes at physiological pH-values is regarded as the major reason for a poor mineral availability, because these complexes are essentially non-absorbable from the gastrointestinal tract. Most studies have shown an inverse relationship between phytate content and mineral availability, although there are great differences in the behaviour of individual minerals. Zn 2+ was reported to be the essential mineral most adversely affected by phytate (Lopes et al., 2002) [8]. Zn 2+-deficiency in humans was first reported in 1963 in Egyptian boys whose diets consisted mainly of bread and beans (Prasad et al., 1963) [13]. These patients, who were characterised by dwarfism and hypogonadism, showed a response to dietary Zn 2+-supplementation. It became accepted that the presence of phytate in plant-based foods is an important factor in the reduction of Zn 2+-absorption. Phytate affects Zn 2+-absorption in a dose-dependent manner. There is, however, some lack of agreement among studies, particularly with respect to specific foods and their individual components. In addition, phytate was shown not only to depress the availability of dietary Zn 2+, but also to affect Zn 2+-homeostasis negatively (Oberaleas et al., 1983) [9].

Great deal of controversy exists regarding the effect of phytate on the availability of dietary iron (Halberg et al., 1987) [14]. Much of this controversy may be due to the low absorption of iron in general, the presence of different iron-phytates with different solubility, and the existence of two types of food iron, heme and nonheme iron. Heme iron is better absorbed and its absorption is little affected by dietary factors; nonheme iron, however, is less easily absorbed, and its absorption is affected by other dietary factors. Since many human studies indicate that phytate has a very strong inhibitory effect on iron absorption, it is well accepted today, that phytate appears to be the major but not the only contributor to the reduction in iron availability in man (Brune...
et al., 1992) [15]. Human studies also indicated that phytate inhibits Ca 2+-absorption, but the effect of phytate on Ca 2+-availability seems to be less pronounced compared to that on the availability of iron and particularly Zn2+. This may be due to the relatively high Ca 2+-content of plant-based foods, the capability of the bacterial flora in the colon to dephosphorylate phytate and the fact, that Ca 2+ could be absorbed from the colon (Sandstrom et al., 1992).

Relatively few studies have dealt with the effects of phytate on dietary Cu 2+, Mn 2+ and Mg 2+ utilisation. Phytate has been shown to decrease their bioavailability in in vivo studies, but it appears that the effect of phytate on Cu 2+, Mn 2+ and Mg 2+ availability is less marked than those for some other essential elements (Lopez et al., 2002) [18].

Phytate-Protein Interaction
Phytate interactions with proteins are pH-dependent (Cheryan, 1980) [1]. At pH-values below the isoelectric point of the protein, the anionic phosphate groups of phytate bind strongly to the cationic groups of the protein to form insoluble complexes that dissolve only below pH 3.5. The α-NH2 terminal group, the ε-NH2 of lysine, the imidazole group of histidine and guanidyl group of arginine have been implicated as protein binding sites for phytate at low pH-values. These low pH protein-phytate complexes are disrupted by the competitive action of multivalent cations. Above the isoelectric point of the protein, both protein and phytate have a negative charge, but in the presence of multivalent cations, however, soluble protein-cation-phytate complexes occur. The major protein binding site for the ternary complex appears to be the nonprotonated imidazole group of histidine, but the ionized carboxyl group of the protein are also suggested sites. These complexes may be disrupted by high ionic strength, high pH (> 10), and high concentrations of the chelating agents.

Phytate and Protein digestibility
Phytate is known to form complexes with proteins at both acidic and alkaline pH (Cheryan, 1980) [1]. This interaction may effect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. The inhibitory effect increases with the number of phosphate residues per myo-inositol molecule and the myo-inositol phosphate concentration. This inhibition may be due to the non-specific nature of phytateprotein interactions, the chelation of calcium ions which are essential for the activity of trypsin and α-amylose, or the interaction with the substrates of these enzymes. The inhibition of proteases may be partly responsible for the reduced protein digestibility.

Interaction of phytate in GI tract
Regarding mineral availability, solubility of phytate complexes is a critical and perhaps overriding issue. Complexes that are insoluble in the upper small intestine, where maximum mineral abs option normally occurs, are highly unlikely to provide absorbable essential elements. Thus, chemical interactions of phytate in the upper gastrointestinal tract are of particular concern. The form in which many minerals occur in foods is largely unknown, as is also the form in which they occur in the gut. Therefore, predicting the specific interactions of phytate in the gastrointestinal tract and the nutritional implications of these interactions is very difficult. As foods are ingested and the digesta travels through the gastrointestinal tract, phytate may continue to maintain associations developed during ripening or food processing. Because binding of phytate with minerals or proteins depends upon pH-value (Cheryan, 1980) [1], which changes from low pH in the stomach to about neutral in the upper small intestine, dietary phytate complexes may dissociate and phytate may form other chelates during its passage through the gastrointestinal tract.

Beneficial health effects of phytate
In the last years, some novel metabolic effects of phytate or some of its degradation products have been recognised. Dietary phytate was reported to prevent kidney stone formation (Grases et al., 2000) [2], protect against diabetes mellitus (Thompson, 1973), caries (Kaufman et al., 1971) [4], atherosclerosis and coronary heart disease (Jariwalla et al., 1990) [5] as well as against a variety of cancers (Vaucenik et al., 2003) [6].

Phytate and Diabetes Mellitus
Diabetes mellitus is one of the most common nutrition-dependent diseases in Western society. It may be caused by hyper-caloric diets with high percentage of quickly available carbohydrates. Foods that result in low blood glucose response have been shown to have great nutritional significance in the prevention and management of diabetes mellitus. In this regard phytate-rich foods are of interest, since a negative relationship between phytate intake and blood glucose response was reported (Yoon et al., 1983) [20]. For example, phytate-enriched unleavened bread based on white flour reduced the in vitro starch digestibility besides flattening the glycemic response in five healthy volunteers in comparison with bread without phytate addition (Yoon et al., 1983) [20]. The in vitro reduction of starch digestion was positively correlated with the myo-inositol phosphate concentration and negatively with the number of phosphate groups on the myo-inositol ring. It has to be noted, that there are also studies which have not found an inhibition of α-amylase and starch digestion by phytate.

Phytate and Coronary Heart Disease
Heart disease is a leading cause of death in Western countries, yet it is low in Japan and developing countries. Elevated plasma cholesterol or more specifically, elevated LDL-cholesterol concentrations have been shown to be one of the risk factors. It has been proposed that dietary fibre or more specifically phytate, as a component of fibre, may influence the aetiology of heart disease (Potter, 1995) [21]. Animal studies have demonstrated that dietary phytate supplementation resulted in significantly lowered serum cholesterol and triglyceride levels (Jarivalla et al., 1990) [4]. This effect w as accompanied by decrease in serum zinc level and in zinc-copper ratio. Thus, the hypothesis was put forward that coronary heart disease is predominantly a disease of imbalance in regard to zinc and copper metabolism (Kievay, 1975) [22]. The hypothesis is also based on the production of hypercholesterolemia, which is a major factor in the aetiology of coronary heart disease, in rats fed a diet with a high ratio of zinc and copper (Kievay, 1973) [23]. It was thought that excess zinc in the diets resulted in decreased copper uptake from the small intestine, since both minerals compete for common mucosal carrier systems. As phytate preferentially binds zinc rather than copper (Persson et al., 1998) [28], it was presumed that phytate exerts its effect probably by decreasing zinc without affecting copper absorption.

Phytate and Renal Lithiasis
In recent years, research on phytate as a potent inhibitor of renal stone formation has been intensified (Grases et al.,
Phytate and Caries

Anticancer effects of phytate. It was demonstrated that phytate is a broad-spectrum antineoplastic agent, affecting different cells and tissue systems (Vaucenik et al., 2003) [9]. Phytate inhibited the growth of human cell lines such as leukaemic haematopoietic K-562 cell line (Lambertenghi et al., 2002) [27], colon cancer HT-29 cell line (Shamsuddin et al. 1992) [28], breast cancer cell lines (Shamsuddin et al., 1995), cervical cancer cell lines (Ferry et al., 2002), prostate cancer cell lines (ZiX et al., 2000) [33], HepG2 haepatoma cell line (Vacunik et al., 1998) [34, 36, 40], in a dose- and time-dependent manner. However, cells from different origin have different sensitivity to phytate, suggesting that phytate may affect different cell types through different mechanisms of action. It was also demonstrated, that phytate has the potential to induce differentiation and maturation of malignant cells, which often results in reversion to the normal phenotype (Shamsuddin et al. 1992) [28]. Phytate was further shown to increase differentiation of human colon carcinoma HT-29 cells (Yang et al., 1995) [17], prostate cancer cells (Shamsuddin et al., 1995) [28], breast cancer cells, (Shamsuddin et al., 1996) [30], and rhabdomyosarcoma cells (Vacunik et al., 1998) [34, 36, 40]. The effectiveness of phytate as a cancer preventive agent was also shown in colon cancer induced in rats and mice. Phytate was effective in a dose-dependent manner given either before or after carcinogen administration. The phytate-treated animals demonstrated a significantly lower tumour number and size. Studies using other experimental models showed that the antineoplastic properties of phytate were not restricted to the colon. Phytate significantly reduced experimental mammary carcinoma (Hirose et al., 1994) [38], skin papillomas (Ishikawa et al., 1999) [39], tumour size of metastatic fibrosarcoma and experimental lung metastases (Vacunik et al., 1992) [33], growth of rhabdomyosarcoma cells (Vacenic et al., 1998) [34, 36, 40], and regression of pre-existing liver cancers (Vacenik et al., 1998) [34, 36, 40]. In addition synergistic cancer inhibition by phytate when combined with inositol was demonstrated in several cancers in experimental animals (Shamsuddin et al., 1989) The in vivo experiments were performed either by adding phytate to the diet or by giving phytate via drinking water. Comparable of even stronger tumour inhibition was obtained with much lower concentrations of phytate when it was given in drinking water.

Phytate and Caries

The higher incidence of caries in industrialised compared to developing countries was suggested to be nutrition-dependent. Phytate lowers the solubility of calcium, fluoride and phosphate, the major components of enamel (Kaufman et al., 1971) [4]. Thus, teeth are more protected against the leading cause of caries, the attack of acids and bacteria. Furthermore, the very high affinity of phytate for hydroxyl apatite may prevent the formation of plague and tartar.

Phytase

Phytase is the enzyme that neutralizes phytic acid and liberates the phosphorus. This enzyme co-exists in plant foods that contain phytic acid. Ruminant animals such as cows, sheep and goats have no trouble with phytic acid because phytase is produced by rumen microorganisms; monogastric animals also produce phytase, although far less. Mice produce thirty times more phytase than humans (Iqbal., 1994), so they can be quite happy eating a raw whole grain. Data from experiments on phytic acid using mice and other rodents cannot be applied to humans.

In general, humans do not produce enough phytase to safely consume large quantities of high-phytate foods on a regular basis. However, probiotic lactobacilli, and other species of the endogenous digestive microflora can produce phytase (Fumaluro, 2005) Thus, humans who have good intestinal flora will have an easier time with foods containing phytic acid. Increased production of phytase by the gut microflora explains why some volunteers can adjust to a high-phytate diet.

The Phytate Threshold

It appears that once the phytate level has been reduced, such that there is more available phosphorus than phytate in the grain, we have passed a critical point and the food becomes more beneficial than harmful. Retention of phosphorus decreases when phytate in the diet is 30-40 percent or more of the total phosphorus (Gontzea, 1968).

For best health, phytates should be lowered as much as possible, ideally to 25 milligrams or less per 100 grams or to about 03 percent of the phytate-containing food eaten. At this level, micronutrient losses are minimized. RDI changes from country to country, table 2 shows phytate RDI in various country

<table>
<thead>
<tr>
<th>Country</th>
<th>RDI mg/day</th>
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<tbody>
<tr>
<td>Uk &amp; US</td>
<td>631-746</td>
</tr>
<tr>
<td>Finland</td>
<td>370</td>
</tr>
<tr>
<td>Italy</td>
<td>219</td>
</tr>
<tr>
<td>Sweden</td>
<td>180</td>
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Determination of phytate content

Phytate is subsequently estimated either by determining the phosphate, inositol or iron content of the precipitate (direct method), or by measuring the excess iron in the supernatant (Indirect method). These approaches are not specific for phytate due to the co-precipitation of partially phosphorylated myo-inositol phosphates (Xu et al., 1992) [17] and should therefore be limited to the analysis of material which contains negligible amounts of phytate dephosphorylation products. If substantial amounts of partially phosphorylated myo-inositol phosphates are present such as in processed foods, the content of phytate will be overestimated by using phytate determination methods based on iron precipitation.

More recently, high performance liquid chromatography (HPLC) techniques have been introduced into phytate
determination, Amon (Xu et al., 1992) [17] g these ionpair reverse-phase and anion-exchange chromatography are largely used today. These systems allow the simultaneous separation and quantification of myo-inositol tris- to hexakisphosphates (ion-pair reverse-phase chromatography) (sandsberg et al., 1986) [18] or myo-inositol mono- to hexakisphosphates (anion- exchange chromatography) [30]. Furthermore, a number of isomer specific ion-exchange chromatography methods with gradient elution for the separation and quantification of myo-inositol phosphates in the picomolar range have been developed very recently (Chen et al., 2003) [19].

How to curtail phytates
The potential benefits of phytic acid occur in instances with high dietary phytic acid intake. However, a high intake has also been associated with reduced mineral absorption. So, in order for us to get the best of both worlds (if such a thing is possible) it’s important to discover some ways in which we can minimize the negative effects while maximizing the beneficial effects.

Vitamin C incorporation
One way we can do this (specifically in regards to iron) is by incorporating more vitamin C (ascorbic acid) into our diet. These two work well together, with vitamin C placing iron in a chemical state that is more readily absorbed by the body.

Processing
Preparation methods such as milling, soaking, germinating, or fermenting can be very effective in reducing the amount phytic acid present in foods. Some methods are better for different foods. In the case of nuts and legumes, soaking and germinating are most successful, but for grains and cereals, all three are effective.

1. Milling
Milling grains and removing the bran decreases phytic acid. Unfortunately, milling also tends to remove many of the minerals! Removing the bran and then enriching a food with minerals might allow for enhanced nutrient absorption in the body.

2. Germination
Germination enhances native phytase activity in plants and thus decreases phytic acid. Soaking and germinating grains is a good idea, but it does not eliminate phytic acid completely. Significant amounts of phytic acid will remain in most sprouted grain products. For example, malting reduces wheat, barley or green gram phytic acid by 57 percent. However, malting reduces anti-nutrients more than roasting.36 In another experiment, malting millet also resulted in a decrease of 23.9 percent phytic acid after 72 hours and 45.3 percent after 96 hours.

In legumes, sprouting is the most effective way to reduce phytic acid, but this process does not get rid of all of it. Germinating peanuts led to a 25 percent reduction in phytates. After five days of sprouting, chick peas maintained about 60 percent of their phytate content and lentils retained about 50 percent of their original phytic acid content. Sprouting and boiling pigeon pea and bambara groundnut reduced phytic acid by 56 percent. Germinating black eyed beans resulted in 75 percent removal of phytate after five days sprouting (Lestienne et al., 2005) [42].

Germination is more effective at higher temperatures, probably because the heat encourages a fermentation-like condition. For pearled millet, sprouting at 92 degrees F for a minimum of 48 hours removed 92 percent of the phytate. At 82 degrees F, even after 60 hours, only 50 percent of phytic acid was removed. Higher temperatures above 86 degrees F seem less ideal for phytate removal, at least for millet (Reddy, 2001) [49].

Sprouting releases vitamins and makes grains and beans and seeds more digestible. However it is a prefermentation step, not a complete process for neutralizing phytic acid. Consuming grains regularly that are only sprouted will lead to excess intake of phytic acid. Sprouted grains should also be soaked and cooked.

3. Roasting
Roasting wheat, barley or green gram reduces phytic acid by about 40 percent. 40 If you subsequently soak roasted grains, you should do so with a culture that supplies additional phytase, as phytase will be destroyed by the roasting process (Sade, 2009).

4. Fermentation
Sourdough fermentation of grains containing high levels of phytase-such as wheat and rye-is the process that works best for phytate reduction. Sourdough fermentation of whole wheat flour for just four hours at 92 degrees F led to a 60 percent reduction in phytic acid. Phytic acid content of the bran samples was reduced to 44.9 percent after eight hours at 92 degrees F.46 The addition of malted grains and bakers yeast increased this reduction to 92-98 percent. Another study showed almost complete elimination of phytic acid in whole wheat bread after eight hours of sourdough fermentation. A study of phytates in recipes used typically by home bread bakers found that leavening with commercial yeast was much less effective at removing phytates. Yeasted whole wheat breads lost only 22-58 percent of their phytic acid content from the start of the bread making process to the complete loaf (Helland et al., 2004) [53].

Mineral supplementation
Another way to maximize benefit of phytic acid is to simply increase the consumption of foods rich in iron, zinc, magnesium, and calcium that are naturally low in phytic acid. For example, consider eating more animal-based proteins. Understandably, this can be challenging for vegetarians and vegans and may warrant the inclusion of a vitamin and mineral supplement.

Meal timing
Yet another strategy is to focus more on meal timing. In other words, eat foods that contain phytic acid separately from foods that are richer in minerals. From a practical standpoint, one could accomplish this by eating meals of protein and fat separate from meals of carbohydrate and fat.

Seed breeding
Scientists are working on to produce to produce low phytate grain varieties or to breed high phytase grain varieties. There are modern seed hybrids of grain and legume plants that contain less phytic acid.

Conclusion
One of the easiest mistakes to make in the world of nutrition is to assume that any nutrient or substance behaves in just one way, whether it’s good or bad. Truth is, the body is much
more unique and complex than we can truly understand, and stuff we eat tends to have many different functions once it’s inside of us. Every person, though sharing similar nutrient needs, is going to respond differently and accommodate foods, such as those containing phytic acid, more or less easily than others. In healthy people eating balanced diets, phytic acid’s effects on iron, zinc, and manganese status is minimal and it doesn’t seem to cause nutrient deficiencies. To argue that some plant foods are “unhealthy” because of their phytic acid content seems mistaken, especially when phytic acid’s potential negative effects on mineral assimilation may be offset by its health benefits. So we should aim to reduce phytic acid rather than eliminate it.

References