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Review

Dietary roles of phytate and phytase in human nutrition: A review

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ABSTRACT

Phytate is the primary storage form of both phosphate and inositol in plant seeds. It forms complexes with dietary minerals, especially iron and zinc, and causes mineral-related deficiency in humans. It also negatively impacts protein and lipid utilisation. It is of major concern for individuals who depend mainly on plant derivative foods. Processing techniques, such as soaking, germination, malting and fermentation, reduce phytate content by increasing activity of naturally present phytase. Supplementation of phytase in diets results in increase in mineral absorption. Apart from negative effects, its consumption provides protection against a variety of cancers mediated through antioxidation properties, interruption of cellular signal transduction, cell cycle inhibition and enhancement of natural killer (NK) cells activity. It has therapeutic use against diabetes mellitus, atherosclerosis and coronary heart disease and reduces kidney stone formation, HIV-1 and heavy metal toxicity; however, information on the dosage for humans for eliciting beneficial effects is limited.

Contents

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1. Introduction

Plant-based food products are the main staple food for human beings in many parts of the world. They constitute an important source of carbohydrates, protein, dietary fibre, vitamins and non-nutrients (Katina et al., 2005). Among all the antinutritional components, phytic acid is of prime concern for human nutrition and health management. The chemical description for phytic acid is myoinositol (1,2,3,4,5,6) hexakisphosphoric acid. The unique structure of phytic acid offers it the ability to strongly chelate with cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts. It therefore adversely affects the absorption and digestion of these minerals by animals (Raboy, 2001). Salts of phytic acid are designated as phytates (*myo*-inositol-1,2,3,4,5,6-hexakisphosphates) which are mostly present as salts of the mono- and divalent cations K^+, Mg^{2+} and Ca^{2+}. Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2002). Phosphorus, in this form, is not utilised by human beings, dogs, pigs, birds or agastric animals because they lack the intestinal digestive enzyme phytase (Holm, Kristiansen, & Pedersen, 2002). Phytate works in a broad pH-region as a highly negatively charged ion and therefore its presence in the diet has a negative impact on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Fredlund, Isaksson, Rossander-Hulthén, Almgren, & Sandberg, 2006; Lopez, Leenhardt, Coudray, & Rémésy, 2002; Lönnerdal, 2002). Besides, phytate has also been reported to form complexes with proteins at both low and high pH values. These complex formations alter the protein structure, which may result in decreased protein solubility, enzymatic activity and proteolytic digestibility. Hitherto, massive investigations have been carried out on the negative aspects of phytate that have offered overwhelming evidence that dietary phytate is an antinutrient component. As a solution, the phytate-degrading enzyme, phytase, is in vogue for degradating phytate during food processing and in the gastrointestinal tract. Major efforts have been made to reduce the amount of phytate in foods by different processes and/or the addition of exogenous enzymes. In spite of many negative aspects on human health, the consumption of phytate, however, has been reported to have some favourable effects. The outcome of surveillance of populations consuming vegetarian-type diets has shown lower incidence of cancer, which suggests that phytate has an anticarcinogen effect (Shamsuddin, 2002; Vucenik & Shamsuddin, 2003). The metal binding characteristics of phytate endow it an anti-oxidant function, inhibiting the production of hydroxyl radicals that normalise cell homeostasis (Minihane & Rimbach, 2002) and it also acts as a natural food anti-oxidant (Raboy, 2003). Dietary phytate may have health benefits for diabetes patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying (Thompson, 1993). Likewise, phytate has also been shown to regulate insulin secretion (Barker & Berggren, 1999). It is believed that phytate reduces blood clots, cholesterol and triglycerides and thus prevents heart diseases (Jariwalla, Sabin, Lawson, & Herman, 1990; Onomi, Okazaki, & Katayama, 2004). It is also suggested that it prevents renal stone development (Grases, Prieto, Simonet, & March, 2000a; Grases et al., 2000b; Selvam, 2002). It is used as a complexing agent for removal of traces of heavy metal ions (Wise, 1982). *In vitro* studies have indicated that phytic acid incubated with HIV-1 infected T cells inhibits the replication of HIV-1 (Otake, Mori, & Morimoto, 1999; Otake, Shimonaka, & Kanai, 1989). Hitherto, many literature reviews, primarily focussing on the antinutritional aspects of phytate, have been published but information on the beneficial effect of phytate is still very scarce and scattered. The purpose of this review is to discuss both negative and prophylactic and therapeutic effects of phytate and the mechanisms responsible for these effects.

2. Phytate

Phytic acid is the hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol (Fig. 1). Phytic acid (known as inositol hexakisphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues. Inositol penta- (IP5), tetra- (IP4) and triphosphate (IP3) are also called phytates. Molecular formula: $C_6H_{18}O_{24}P_6$ and molecular mass: 660.04 g mol^{-1}.

Phytate is formed during maturation of the plant seed and in dormant seeds it represents 60–90% of the total phosphate (Loewus, 2002). Phytate is, therefore a common constituent of plant-derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans on vegetarian diets, on an average, is 2000–2600 mg

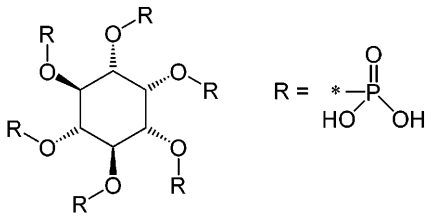

Fig. 1. Chemical structure of phytic acid.

whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150–1400 mg (Reddy, 2002). Usually legume-based food (cooked) items contain higher amounts phytate than do cereal-based food items (Table 1). Few food items, such as sesame seeds (toasted), soy protein concentrate, rice (unpolished and cooked), maize bread (unleavened) and peanuts, containing 39–57, 11–23, 13–22, 12–19 and 10–20 mg/g, respectively, have exceptionally high amounts of phytate. Phytate content in plant-derived human foods is shown in Table 1.

3. Negative aspects of phytate

Table 2 presents an overview of the negative interactions of phytate with nutrients and the mode of actions for the negative effects of phytate.

3.1. Effect on mineral uptake

The presence of phytate in the human diet has a negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Konietzny & Greiner, 2003; Lopez et al., 2002). Among them, bioavailability of Zn^{2+} was reported to be the most adverse effect in humans (Lopez et al., 2002; Lönnerdal, 2002). First reports of Zn^{2+}-deficiency in humans were reported in 1963 among Egyptians, feeding mainly on bread and beans (Prasad, Miale, Farid, Sandstead, & Darbv, 1963). The presence of phytate in their plant-based foods is an important factor in the reduction of Zn^{2+}-absorption and Zn^{2+}-homeostasis, which resulted in dwarfism and hypogonadism (Oberleas, 1983). The order of the ability of the mineral cations to form complexes with phytate *in vitro* has been found to be: $Cu^{2+} > Zn^{2+} > Cd^{2+}$ at pH 3–7 (Persson, Türk, Nyman, & Sandberg, 1998). Dietary phytate forms an insoluble phytate-mineral complex. These complexes are not readily absorbed by the human gastrointestinal tract, which reduces the bioavailability of minerals. Moreover, the small intestine of the human is devoid of phytate-degrading enzyme and also the microbial population in the upper part of the digestive tract is limited (Iqbal, Lewis, & Cooper, 1994). Thereby, the phytate-mineral complex remains partially hydrolysed in the human gut.

Studies on humans also show that phytate has a very strong inhibitory effect on non-haeme iron absorption (Brune, Rossander-Hulthén, Hallberg, Gleerup, & Sandberg, 1992). It has also been demonstrated that phytate reduces Ca^{+2} – absorption but this is less pronounced than Zn^{2+} and $Fe^{+2/+3}$ reduction in humans (Lopez et al., 2002). The bacterial flora residing in the colon is capable of dephosphorylating the phytate and consequently releases Ca^{+2} which gets absorbed from the colon (Sandström, Cederblad, Stenquist, & Andersson, 1990). Studies on the effects of phytate on dietary Cu^{+2}, Mn^{+2} and Mg^{+2} are limited (Lopez et al., 2002; Lönnerdal, 2002). The stability and solubility of the complexes depend on the pH value, the individual cation, the phytate to cation molar ratio and the presence of other compounds in the solution (Oberleas, 1983). The pH is an important factor influencing the solubility of phytate (Cheryan, 1980; Cheryan, Anderson, & Grynspan, 1983), it being more soluble at lower than at higher pH values (Torre, Rodriguez, & Saura-Calixto, 1991). Ca^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+} salts tend to be soluble at pH lower than 4–5, whereas Mg-phytate is soluble at acid pH up to pH 7.5 (Brown, Heit, & Ryan, 1961; Nolan, Duffin, & Mcweeny, 1987). In contrast, ferric phytate is insoluble at pH values in the range 1.0–3.5 at equimolar Fe^{3+} to phytate ratios and solubility increases above pH 4 (Askar, El-Samahy, & Abd El-

Table 1
Phytate content (mg/g on dry matter basis) in plant-derived human food (Greiner & Konietzny, 2006).

Food types	Phytate (mg/g)
Cereals	
Rice (polished, cooked)	1.2–3.7
Rice (unpolished, cooked)	12.7–21.6
Maize bread	4.3–8.2
Unleavened maize bread	12.2–19.3
Wheat bread	3.2–7.3
Unleavened wheat bread	3.2–10.6
Rye bread	1.9–4.3
Sourdough rye bread	0.1–0.3
French bread	0.2–0.4
Flour bread (70% wheat, 30% rye)	0.4–1.1
Flour bread (30% wheat, 70% rye)	0–0.4
Cornflakes	0.4–1.5
Oat flakes	8.4–12.1
Pasta	0.7–9.1
Sorghum	5.9–11.8
Oat porridge	6.9–10.2
Legume-based food	
Green peas (cooked)	1.8–11.5
Soybeans	9.2–16.7
Tofu	8.9–17.8
Lentils (cooked)	2.1–10.1
Peanuts	9.2–19.7
Chickpea (cooked)	2.9–11.7
Cowpea (cooked)	3.9–13.2
Black beans (cooked)	8.5–17.3
White beans (cooked)	9.6–13.9
Kidney beans (cooked	8.3–13.4
Miscellaneous	
Sesame seeds (toasted)	39.3–57.2
Soy protein isolate	2.4–13.1
Soy protein concentrate	11.2–23.4
Buckwheat	9.2–16.2
Amaranth grain	10.6–15.1

Table 2
Negative interactions of phytate and nutrients in food.

Nutrients	Mode of action	Reference
Mineral ions (zinc, iron, calcium, magnesium, manganese and copper)	Formation of insoluble phytate-mineral complexes leads to decrease in mineral availability	Brune et al. (1992), Iqbal et al. (1994), Lopez et al. (2002), Konietzny and Greiner (2003)
Protein	Formation of non-specific phytate-protein complex, not readily hydrolysed by proteolytic enzymes	O'Dell and de Boland (1976), Ravindran et al. (1995)
Carbohydrate	Formation of phytate carbohydrate complexes making carbohydrate less degradable. Inhibition of amylase activity by complexing with Ca^{++} ion and decrease of carbohydrate degradation	Rickard and Thompson (1997), Selle et al. (2000)
Lipid	Formation of 'lipophytin' complexes, may lead to metallic soaps in gut lumen, resulting in lower lipid availability	Matyka et al. (1990), Leeson (1993), Vohra and Satyanarayan (2003)

Fadeel, 1983). However, solubility studies of bran phytate prove that, at gastric pH (approximately pH 2), Ca actually does not bind and this component does not contribute to the solubility of the Ca ion (Siener, Heynck, & Hesse, 2001). Phytate also interacts directly and/or indirectly with various dietary minerals to reduce their bioavailability. In this context the synergistic effect of secondary cations (Ca^{2+}) has been most prominently exhibited (Wise, 1983). Two cations may, when present simultaneously, act jointly to increase the quantity of phytate precipitation. In the presence of phytate and calcium, absorption of other mineral is depressed due to formation of insoluble complexes (Sandberg, Larsen, & Sandstrom, 1993). For example, calcium-bound phytate shows more affinity for Zn and forms co-precipitates, thereby reducing the reabsorption of endogenous Zn as well as affecting availability of dietary Zn (Hardy, 1998). However, the molar ratio of Zn^{2+} and phytate regulates the effect of Ca^{2+} on the amount of Zn^{2+} co-precipitating with phytate. At high Zn^{2+} to 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 (Wise, 1983). Interestingly, Mg^{2+} has also been shown to potentiate the precipitation of Zn^{2+} in the presence of phytate, but it exerted a less pronounced effect on Zn^{2+}-solubility than did Ca^{2+} (Wise, 1983).

3.2. Effect on protein digestibility

Phytate forms a strong complex with some proteins and resists their proteolysis. In general, the interaction of phytate with protein is dependent on pH (Cheryan, 1980). At a pH value lower than the isoelectric point of proteins (Cosgrove, 1966), phosphoric acid groups of phytate bind with the cationic group of basic amino acid, e.g., arginine, histidine, lysine, and form binary protein–phytate complexes. They are insoluble complexes that dissolve only below pH 3.5. Such complex formations may affect the protein structures that can hamper enzymatic activity, protein solubility and protein digestibility. *In vitro* studies show that the extent of phytate–protein interaction is governed by various factors, including pH, the source and solubility of protein and dietary levels of calcium and magnesium (Kemme, Jongbloed, Mroz, Kogut, & Beynen, 1999). For example, protein–phytate complexes have been well documented in wheat (Hill & Tyler, 1954) but are less likely to be present in maize (O'Dell and de Boland, 1976). *In vitro* studies have shown that phytate–protein complexes are less likely to be digested by proteolytic enzymes (Ravindran, Bryden, & Kornegay, 1995) and even digestive enzymes, such as pepsin, trypsin, chymotrypsin (Deshpande & Damodaran, 1989; Inagawa, Kiyosawa, & Nagasawa, 1987; Singh & Krikorian, 1982), lipase (Knuckles, 1988) and amylase (Deshpande & Cheryan, 1984; Knuckles & Betschart, 1987) are inhibited by phytate.

This inhibition may be due to the non-specific nature of phytate–protein interactions and the chelation of calcium ions, which are essential for the activity of trypsin and α-amylase. The reduction in the protease enzyme activity might also be partially responsible for poor protein digestibility. Hitherto, the significance of the phytate–protein complex in human nutrition is still to be elucidated.

3.3. Effect on carbohydrate utilisation

In humans, phytate intake reduces the blood glucose response (glycaemic index) (Lee et al., 2006). This may be because phytate forms complexes with carbohydrates of feedstuffs thereby reducing their solubility and adversely affecting the digestibility and absorbtion of glucose. Phytate may bind with starch either directly, via hydrogen bonds, or indirectly via proteins associated with starch (Rickard & Thompson, 1997). Moreover, the reduction in glucose response, i.e., low glycaemic index, as a result of cereal and legume foods consumption may aid diabetics to control blood glucose (Thompson, Button, & Jenkins, 1987; Yoon, Thompson, & Jenkins, 1983). It was also postulated that phytate, by complexing with Ca^{++} ion, inhibits amylase activity (Selle, Ravindran, Caldwell, & Bryden, 2000). In bean flour, subjected to dephytation, an increment of glycaemic index in humans had been reported by Thompson et al. (1987). Furthermore, *in vitro* studies have shown that incubation of human saliva with either wheat or bean starch incorporated with Na phytate reduced the phytilin-mediated hydrolysis of starch (Thompson et al., 1987; Yoon et al., 1983).

3.4. Effect on lipid utilisation

Phytate forms 'lipophytins' (complexes with lipid and its derivatives), along with other nutrients (Vohra & Satanarayana, 2003). Lipid and Ca phytate may be involved in the formation of metallic soaps in gut lumen of poultry, which is a major restraint for energy utilisation derived from lipid sources (Leeson, 1993). Young chicks, when fed with diets supplemented with fat and phytate, exhibit hampered phytate-P utilisation and a large percentage of fat is excreted as soap fatty acids (Matyka, Korol, & Bogusz, 1990). However, there is a paucity of evidence to support the existence of lipid phytate complexes in the human.

It is evident from the above that the effects of phytic acid are attributed to its ability to form complexes with positively charged food components, such as proteins, carbohydrates, minerals and trace elements.

4. Chemical interaction of phytate in gastrointestinal (GI) tract

The interaction of phytate with minerals and other dietary nutrients is pH-dependent (Reddy, 2002). In the human body, food digesta pass from low pH in the stomach to neutral pH, prevailing in the upper small intestine. During digesta movement, dietary phytate-mineral complexes may dissociate and may form other complexes through the gastrointestinal tract. In the upper part of the small intestine, which is characterised by maximum mineral absorption, the insoluble complexes are highly unlikely to provide absorbable essential elements. Thereby, the chemical interactions of phytate in the upper gastrointestinal tract are of particular concern since the site and degree of phytate degradation can affect the nutritional value of a phytate-rich diet. However, the form in which many minerals occur in foodstuffs and in the gut is largely unknown. Therefore, it is difficult to predict the specific interactions of phytate in the GI tract and the nutritional implications of these interactions. To date, little attention has been paid to the understanding of the *in situ* interaction of phytate with nutrients and minerals in the gastrointestinal tract of human.

5. Degradation of phytate

The dephosphorylation of phytate is a prerequisite for improving nutritional value because removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate. This results in increased bioavailability of essential dietary minerals (Sandberg et al., 1999). Various food processing and preparation techniques, along with the addition of exogenous enzymes, are the major efforts made to reduce the amount of phytate in foods. Hydrolysis of phytate during food processing (and then preparation, for example by germination, soaking, cooking and fermentation) is a result of the phytate-degrading activity of phytase, which is naturally present in plants and microorganisms. Thus, phytases have an important application in human nutrition both for degradation of phytate during food processing and in the gastrointestinal tract. However, the capability to dephosphorylate

phytate differs greatly among different plant and microbial species due to differences in their intrinsic phytate-degrading activities (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002).

During the course of food processing or preparation, phytate is not fully hydrolysed by endogenous phytase. Phytate must be reduced to very low levels to increase mineral bioavailability, especially of iron (Hurrell, 2003). For this purpose addition of exogenous phytase is desired. So far, commercial phytase products have been mainly used as animal feed additives in diets, largely for swine and poultry, and to some extent for fish. But in spite of its immense potential in processing and manufacturing of food for human consumption, no phytase product for human food application has found its way to the market. Conclusively, many researchers have reported a convincing improvement of food products by adding microbial-based phytase during food processing for bread-making (Haros, Rosell, & Benedito, 2001), plant protein isolates (Fredrikson et al., 2001), corn wet milling (Antrim, Mitchinson, & Solheim, 1997) and the fractionation of cereal bran (Kvist, Carlsson, Lawther, & DeCastro, 2005).

5.1. Enzymatic degradation of phytate

Phytases are chemically known as *myo*-inositol (1,2,3,4,5,6) hexa*kis*phosphate phosphohydrolase, and catalyse the sequential release of phosphate from phytate. Phytase sequesters orthophosphate groups from the inositol ring of phytic acid to produce free inorganic phosphorus, along with a chain of intermediate myo-inositol phosphates (inositol pentaphosphate to inositol monophosphate) (Debnath et al., 2005). Phytase not only releases the phosphorus from plant-based diets but also makes available calcium, magnesium, protein and lipid. Thus, by releasing bound phosphorus in feed ingredients of vegetable origin, phytase makes more phosphorus available for bone growth and protects the environment against phosphorus pollution (Baruah et al., 2007).

The first report of phytase activity was from rice bran (Suzuki, Yoshimura, & Takaishi, 1907) and blood of calves (McCollum & Hart, 1908). Later, its presence in plant, bacteria, yeast and fungi was found. Beside these, animals and humans are also a potent source of phytases which are generated endogenously by the small intestinal mucosa and microflora associated with large intestine. In general, endogenous phytase activity of humans and animals is insignificant in contrast to plant and microbial phytase (Weremko et al., 1997).

The first commercial phytase, Natuphos® was produced from *Aspergillus niger* and was released to the market in 1991. Following the prologue of commercial phytase in the market, its acceptance as an animal feed supplement started gaining attention worldwide (Yi, Kornegay, Ravindran, & Denbow, 1996). Moreover, its potential in improving human nutrition (De Silva, Trugo, Terzi, & Couri, 2005) and in other areas, e.g., aquaculture (Yoo et al., 2005) is also being extensively explored.

5.1.1. Classification of phytase

Phytase has been categorised on two bases, depending on the site where the hydrolysis of the phytate molecule is initiated and on the pH of activity. The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB) acknowledged that phytase falls into two categories, depending on the site where the hydrolysis of the phytate molecule is initiated (Selle, Gill, & Scott, 2007). These are 3-phytase (EC 3.1.3.8 or myo-inositol hexakisphosphate 3-phosphohydrolase) and 6-phytase (EC 3.1.3.26 or myo-inositol hexakisphosphate 6-phosphohydrolase). The former liberates the P moiety at position C3, whereas latter releases it from position C6 of the *myo*-inositol hexaphosphate ring (Selle & Ravindran, 2007) In theory, enzymatic hydrolysis of phytate generates a series of lower *myo*-inositol phosphate esters (IP6-IP5-IP4-IP3-IP2-IP1), via a progression of stepwise dephosphorylation reactions and ultimately leads to the production of free myo-inositol, along with six inorganic phosphates (Selle et al., 2007). Recently it was reported that phytase isolated from *A. niger* shows 3-phytase activity whilst *Peniophora lycii* and *E. coli* had 6-phytase (Selle et al., 2007).

Phytases can also be broadly categorised into two major classes based on their optimum pH: the histidine acid phosphatases and alkaline phytases. The former show the optimum activity at pH around 5.0 whilst the latter are more pronounced at pH near to 8.0 (Baruah et al., 2007). With the exception of *Bacillus*, most of the microbial phytate-degrading enzymes, and also plant phytase, belong to the acid type (Selle et al., 2007). Therefore, more focus has been on acidic phytases due to their applicability in human food and broader substrate specificity than alkaline phytases.

5.1.2. Sources of phytase

In general, there are four possible sources: plant phytase, microbial phytase (fungal and bacterial phytase), phytase generated by the small intestinal mucosa and gut-associated microfloral phytase.

5.1.2.1. Plant phytase. Phytase enzymes have been isolated and characterised from a number of plant sources; rice (Hayakawa, Toma, & Igaue, 1989), rape seed (Houde, Alli, & Kermasha, 1990), soybean (Hamada, 1996), maize (Maugenest, Martinez, Godin, Perez, & Lescure, 1999), wheat (Nakano, Joh, Tokumoto, & Hayakawa, 1999) and rye (Weremko et al., 1997). Moreover, some plant ingredients, such as rye and triticale, possess acid phosphatase activity (Viveros, Centeno, Brenes, Canales, & Lozano, 2000). Most plant phytases initiate the hydrolysis of phytate at position C6 of the *myo*-inositol hexaphosphate ring, and hence are reported to be type 6 phytases. However, in raw soybean, the major InsP5 is DL-Ins(1,2,4,5,6)P5 and thus soybean phytase seems to be a 3-phytase (Phillippy & Bland, 1988). Similarly, pea phytase is also found to have a degradation pathway of InsP6 dissimilar to that of cereals (Skoglund, Carlsson, & Sandberg, 1997). In addition, microbial genes encoding phytases with desired properties can be cloned and inserted into plants, yielding increased levels of phytase. In general, the microbial phytase-encoding gene is mainly derived from *Aspergillus niger*, *Bacillus subtilis*, *Aspergillus fumigatus*, *Escherichia coli*, and *Schwanniomyces occidentalis* (Greiner & Konietzny, 2006). The transgenesis has been carried out in rice (Hamada et al., 2005), wheat (Brinch-Pedersen, Olesen, Rasmussen, & Holm, 2000), sugarcane (Santosa, Hendroko, Farouk, & Greiner, 2004), alfalfa (Ullah, Sethumadhavan, Mullaney, Ziegelhoffer, & Austin-Phillips, 2002), arabidopsis (Mudge, Smith, & Richardson, 2003), sesame (Jin et al., 2004), soybean (Li et al., 1997), canola (Ponstein et al., 2002) and potato (Ullah, Sethumadhavan, Mullaney, Ziegelhoffer, & Austin-Phillips, 2003). For human nutrition applications, only one transgenic plant expressing phytase from *Aspergillus fumigatus* gene has been developed (Lucca, Hurrell, & Potrykus, 2001). It is used to improve white or polished rice as a source of iron (Wyss et al., 1999).

5.1.2.2. Microbial phytase. Fungi and bacteria are the most important sources of phytase. The first report on phytase from yeast was in 1984 (Nayini & Markakis, 1984). Later on, several yeast strains were screened for their ability to hydrolyse phytates and, among all, the most extensively used are *Aspergillus niger*, *A. ficuum* and *A. fumigatus* from which the commercially produced phytases originate. Genes have been cloned and research has been undertaken to further improve the *Aspergillus* enzymes by genetic engineering (Wyss et al., 1999). Among yeast phytases, *Saccharomyces cerevisiae* is of particular importance for bread-making. Interestingly, Vohra and Satyanarayana (2001) and Quan, Zhang, Wang, and Ohta (2001) reported the production of a cell-bound

phytase from *Pichia anomala* and *Candida krusei* WZ-001, respectively. Such phytases have potential applications in food processing because they remain stable, even at high temperature and acidity. Moreover, the phytases which are most resistant to high temperature have been isolated from *Pichia anomala* (Vohra & Satyanarayana, 2002), *Schwanniomyces castellii* (Segueilha, Lambrechts, Boze, Moulin, & Galzy, 1992) and *Lactobacillus sanfranciscensis* (de Angelis et al., 2003). Furthermore, the yeast *Pichia pastoris*, was used to significantly increase the thermal stability of *E. coli* phytase by means of recombinant technology (Rodriguez, Wood, Karplus, & Lei, 2000). Recently, there was a report on phytase-producing marine yeasts (Hirimuthugoda, Chi, Li, Wang, & Wu, 2006). These marine yeasts have not yet been fully characterised but are phylogenetically related to *Hanseniaspora uvorum*, *Yarrowia lipolytica*, *Kadamaea ohmeri*, *Candida tropicalis* and *C. carpophila*. They are capable of producing alkaline phytase and thus their applicability in the bioremediation of marine phosphorus pollution can be predicted. Among bacteria, *E. coli*, *Bacillus subtilis*, *Klebsiella terringa*, *Lactobacillus* sp., *Pseudomonas* spp (Greiner, Haller, Konietzny, & Jany, 1997; Greiner, Konietzny, & Jany, 1993; Kerovuo, Lauraeus, Nurminen, Kalkkinen, & Apajalahti, 1998; Richardson & Hadobas, 1997) can degrade phytate during growth through production of extracellular phytases.

5.1.2.3. Mucosal phytase derived from small intestine. Intestinal mucosal phytase activity has been described in pigs by Hu, Wise, and Henderson (1996) with jejunum being the pioneer site for phytase activity. Studies on humans demonstrate that very low phytase activity occurs in small intestine and has limited ability to degrade phytate (Iqbal et al., 1994). Interestingly, many animals, including humans, have the adaptive capacity to increase intestinal phytase and phosphatase activities when offered P-inadequate diets (Zhang, Aggrey, Pesti, Bakalli, & Edwards, 2005).

5.1.2.4. Gut microfloral phytase. This group of phytases had predominantly been reported in pigs. Sandberg et al. (1993) detected phytate hydrolysis by microfloral phytase in the large intestine of pigs. However in humans, large numbers of bacteria present in the colon are able to degrade phytate to some extent but this is inversely related to calcium intake (Walker, Fox, & Irving, 1948). To date, there are few studies, which claim the inclusion of gut microfloral-derived phytase as a food additive.

5.1.3. Commercial prospects of phytase

5.1.3.1. Phytase as food additive. The phytate-hydrolysing enzyme has several applications in food industries. During processing, the endogenous dietary phytase is inactivated and therefore phytate digestion is very poor. This affects mineral absorption in the small intestine. Phytase has a potential for producing low phytin bread (Simell, Turunen, Pironen, & Vaara, 1989). Addition of phytase improves the quality of bread by two ways; first, it improves the nutritional value by reducing the phytate content and, second, it promotes the activation of endogenous α-amylase by making more calcium available (Haros et al., 2001). Likewise, wheat bread rolls treated with fungal phytase doubles non-haeme iron absorption in humans compared to bread untreated with phytase (Sandberg, Hulthen, & Turk, 1996). Soymilk has a high level of phytate (0.56%) and phytase can very well be added for the production of phytate-free soyabean milk (Khare, Jha, & Gupta, 1994). Porridges based on flours from rice, wheat, maize, oat, sorghum and wheat-soy flour blends have been tested on humans for iron absorption. The iron absorption improved when porridges were prepared with water but had no effect with milk (Hurrell, Reddy, Juillerat, & Cook, 2003). Reports also suggest that microbial phytase could accelerate the process of steeping required in the wet milling of corn, thereby improving the properties of corn steep liquor (Caransa, Simell, Lehmussaari, Vaara, & Vaara, 1988). Tarhana, a traditional Turkish fermented and dried cereal food, is a good source of minerals (Ca, Mg and K) with good bioavailabilities. Fermentation with increasing acidity, in addition to use of baker's yeast as a phytase source, resulted in a significant decrease in phytic acid and an increase in total amounts of minerals and proteins (Bilgicli, Elgun, & Turker, 2006). Chapathi (commonly called roti) is a staple food in parts of India and some surrounding countries. It is prepared from whole wheat flour that contains considerable amounts of phytic acid. For the reduction of phytate level, a mutated strain of the yeast *Candida versatilis*, as a source of phytase, has been recommended during the preparation of chapathi dough, which reduces the level of phytate by 10–45% (Bindu & Varadaraj, 2005).

5.1.3.2. Source of myo-inositol phosphates. The inositol phosphates and phospholipids regulate transmembrane signalling and the mobilisation of calcium from intracellular reserves. Phytase plays a crucial role for various inositol phosphate preparations (Billington, 1993). The *myo*-inositol phosphates have various other beneficial health effects, as enzyme stabilizers (Siren, 1986), enzyme substrates, inhibitors of enzymes and thus as potential drug blockers (Laumen & Ghisalba, 1994). The number and position of the phosphate groups on the *myo*-inositol ring is of great significance for their physiological functions.

5.2. Phytate degradation during food processing

Biological processing techniques, such as soaking, germination, malting, cooking, hydrothermal processing and fermentation, result in the phytate dephosphorylation of the food. Dephosphorylation of phytate in food occurs by increasing the activity of naturally present phytate-degrading enzyme in plants and microorganisms. It must be noted that, during food processing or preparation, phytate is, in general, not completely hydrolysed by the endogenous phytases of plants and microorganisms. For the optimisation of the food process for increased mineral bioavailability by phytate degradation, it is crucial to know optimal conditions of the phytases responsible for phytate degradation. Naturally, there are dissimilarities in the capacities of various plant and microbial species to dephosphorylate phytate, due to differences in their intrinsic phytate-degrading activities (Egli et al., 2002) and the properties of the enzymes, such as protein stability and pH, as well as temperature optimum for phytate degradation (Konietzny & Greiner, 2002).

5.2.1. Soaking

Soaking of legume grains and cereal seeds are done as a pretreatment to facilitate processing, which lasts for 15–20 min, or even longer, depending on further processing steps. Generally, cereals and legumes are soaked in water overnight; phytate is water-soluble, so a considerable amount of phytate is removed to the water. In addition, this process also enhances the action of naturally occurring phytase in cereals and legumes. It has been shown that phytate hydrolysis, during soaking, is greatly influenced by temperature and pH (Greiner & Konietzny, 1999). The optimal temperatures for the intrinsic plant phytases during soaking were found out to be 45 and 65 °C and pH values between pH 5.0 and 6.0 (Greiner & Konietzny, 1999).

5.2.2. Germination and malting

During the germination of cereals and legumes, phytate is degraded by intrinsic phytase. Plant seeds utilise phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value. Many researchers (Egli et al., 2002; Viveros et al., 2000) have reported little intrinsic phy-

tate-degrading activity in non-germinated legume grains and cereal seeds, with the exception of rye, wheat, triticale and barley. During germination of cereals and legume, an increase in phytate-degrading activity, with a concomitant decline in phytate content, was observed (Greiner et al., 2001). Moreover, when the malted cereals were ground and soaked under optimal conditions, a complete degradation of phytate was observed (Larsson & Sandberg, 1992), except for oats which, under these conditions, had a low phytase activity (Larsson & Sandberg, 1992).

5.2.3. Cooking

Phytate, being a heat stable component in plant foodstuffs, is not easily degraded whilst cooking. However, the intrinsic plant phytase is thermolabile; prolonged exposure to high temperature may lead to the inactivation of endogenous enzyme. Therefore, to improve phytate dephosphorylation during cooking, plants with heat-stable phytases or addition of exogenous heat-stable phytases has been recommended.

5.2.4. Fermentation

Food fermentation is a microbial and enzymatic method for food processing to achieve prolonged shelf life. Plant products, e.g., cereals, legumes and vegetables, are extensively used in the preparation of a variety of fermented foods. Microorganisms and/or enzymes, during fermentation, may be in microflora found in the raw material that is fermented or exogenous microbial cultures preparations, to activate the process. Lactic fermentation is the preferred method for cereal, legume, maize, soybean and sorghum fermentation. Lactic fermentation leads to lowering of pH as a consequence of bacterial production of lactic and acetic acids, which is favourable for phytase activities, resulting in lowering of phytate (Lopez, Gordon, & Fields, 1983). The acidity of the dough plays an important role in phytate degradation during scalding and sourdough fermentation of bread (Bartnik & Florysiak, 1988). Larsson and Sandberg (1991) found that in oat and rye bran bread, made with 10% sourdough having pH 4.6, a 96–97% reduction of phytate occurred. It has been shown that combined germination and lactic fermentation of white sorghum and maize gruels can yield an almost complete degradation of phytate (Svanberg, Lorri, & Sandberg, 1993). Food products, such as tempeh, miso, koji and soy sauce are produced by fermentation of soybeans with *Rhizopus oligosporus* and *Aspergillus oryzae*. Both moulds have been shown to produce intra- as well as extracellular phytate-degrading activity (Fujita et al., 2003).

5.2.5. Addition of isolated phytase

As an alternative to the optimisation of phytate dephosphorylation by naturally occurring enzymes, addition of a phytase preparation during food processing is suggested. Microbial phytase preparations are now commercially available, making their use in food processing technically feasible. Effectiveness of supplemental phytase in reducing phytate content during food processing was demonstrated for cereal as well as for legume-derived food products (Greiner & Konietzny, 1999). Very effective phytate degradation was obtained by adding *A. niger* phytase to an oat-based nutrient solution fermented by *Lactobacillus plantarum* (Marklinder, Larsson, Fredlund, & Sandberg, 1995). It should be noted that the isolated phytase to be used in food processing should remain active, even at high temperature and over a broad pH range.

Though a large body of research has been focussed on the negative aspects of phytate on human health, consumption of phytate does have many positive effects. The beneficial effects of phytate are evident from the fewer incidences of cancer, diabetes mellitus, renal lithiasis and arteriosclerosis in developing countries where people rely mainly on plant-based diets, which constitute a considerable amount of phytate. However, in western countries, these diseases are common because of the greater dependence on processed food, characterised by a low phytate content. In the next sections we discuss roles of phytate as an anti-oxidant in food products and its therapeutical uses.

6. Phytate as anti-oxidant in food products

Oxidation of food is a destructive process, causing substantial loss of nutritional value. Foods with high contents of unsaturated fatty acid and iron are more prone to undergo oxidation in the presence of oxygen. Even at very low percentages of oxygen (as low as 1%), the oxidation reaction can proceed and produce undesirable flavour changes, discoloration, nutritional losses and microbiological spoilage. The oxidation reaction can be minimised through the addition of anti-oxidant. In this regard, phytate, by chelating free iron, is one such naturally occurring anti-oxidant. Since phytate has the ability to form a unique iron chelate that becomes catalytically inactive (Graf, Empson, & Eaton, 1987; Graf, Mahoney, Bryant, & Eaton, 1984), it inhibits iron-driven hydroxyl radical (–OH$^{\cdot}$) formation and strongly suppresses lipid peroxidation. Iron-mediated hydroxyl radical generation requires availability of at least one coordination site that is open or occupied by a readily dissociable ligand, such as water. The Fe^{3+}-phytate complex does not retain a reactive coordination site, so it consequently fails to support –OH$^{\cdot}$ generation. This property makes the phytate-driven iron complex different from other iron chelates. Out of 22 readily available iron chelators previously studied, only phytate appears to be the most effective and nontoxic food anti-oxidant (Graf et al., 1984). Biochemical studies suggest that phytate provides an exclusive trivalent oxidation (Fe^{3+}) state that blocks the necessary redox cycling of iron required in many oxidation reactions (Graf et al., 1987). This is possibly because phytate shifts the redox potential of iron and maintains it in the ferric form (Fe^{3+}). This effect also affords protection against oxidative damage since the ferrous form (Fe^{2+}) alone has been shown to cause production of oxyradicals and lipid peroxidation, whereas Fe^{3+} was relatively inert (Halliwell & Gutteridge, 1989). These reports signify that phytate represents a rational and economical approach to the preservation of a variety of oxygen-sensitive biological food materials.

Iron-chelating capacity of phytate allows it to inhibit the formation of warmed-over flavour (WOF). During cooking of poultry, meat and fish, myoglobin releases considerable amounts of free iron which binds to phosphatidylethanolamine (PE). This Fe^{3+}-PE complex then catalyses rapid autoxidation of its unsaturated fatty acyl moieties. This results in a large rise in WOF (Graf & Panter, 1991). However, sequestration of the iron greatly reduces the rate of peroxidation of phospholipids. Therefore, the addition of small amounts of phytate inhibits WOF development, both by displacing the iron from PE and by forming catalytically inactive iron chelates. Moreover, the latter function renders phytate more effective than other iron-chelating agents at inhibiting WOF.

7. Therapeutic uses of phytate

Table 3 presents various therapeutic effects of phytate.

7.1. Phytate as an anti-cancer agent

Numerous studies in the medical literature have reported phytate as a broad-spectrum anti-neoplastic agent (Vucenik & Shamsuddin, 2003). Human colon cancer HT-29 cells (Sakamoto, Venkatraman, & Shamsuddin, 1993), human leukaemic hematopoietic cell lines, such as K-562 (Deliliers et al., 2002) and human normal and leukaemic hematopoietic cells (Deliliers et al., 2002)

Table 3
Therapeutic effects of phytate.

Effects	Mode of action	Reference
Protection against colon cancer	Produces butyric acid through fermentation and leads to reduction in gut pH and bile acid metabolism. Inhibits iron-mediated oxidative reactions. Upregulation of the expression of tumour suppressor genes like p53, p21 WAF1/Cip1	Midorikawa et al. (2001), Coradini et al. (2000), Shamsuddin (2002), Saied and Shamsuddin (1998)
Protection against mammary carcinoma	Stimulates the apoptosis of human breast cancer cells. Synergestic effect of phytate and inositol on arresting cell division	Vucenik and Shamsuddin (2003), Vucenik et al. (1993), El-Sherbiny et al. (2001)
Prevention of hepatocellular carcinoma	Enhances tumour suppressor gene activity. Favours the differentiation of malignant cells and conversion of the cancer cells to less aggressive phenotypes	Vucenik et al. (1998a, 1998b)
Prevention of prostate cancer cells (PC3 cells and DU145 prostate cancer cells)	Impairs both receptor-mediated (growth factor receptors EGFR or erbB1) and fluid-phase endocytosis. Inhibits mitogenic signals	Zi et al. (2000)
Prevention of rhabdomyosarcoma (RMS)	Suppresses the cancerous cells and induces the cell differentiation	Vucenik et al. (1998c)
Prevention of pancreatic cancer	Acts as adjunct for pancreatic cancer treatment and increases the sensitivity to conventional therapies	Somasundar et al. (2005)
Prevention of blood and bone marrow cancer	Increases differentiation of carcinoma cells. Increases haemoglobin synthesis	Shamsuddin et al. (1992)
Reduction of the risk of coronary heart disease	Lowers serum cholesterol and triglyceride levels. Decreases serum zinc level and stabilises zinc-copper ratio	Jariwalla et al. (1990), Klevay (1975), Persson et al. (1998)
Reduction of the incidence of fatty liver	Reduces the activity of hepatic enzymes involved in lipogenesis	Katayama (1997)
Reduction of the incidence of Diabetes mellitus	Lowers of blood glucose response. Regulates insulin secretion via its effect on calcium channel activity	Larsson et al. (1997), Barker and Berggren (1999)
Protection against human immunodeficiency virus (HIV)	Acts on HIV and the HIV-specific antigen at an early replicative stage	Otake et al. (1989, 1999)
Protection against teeth decay/ dental caries	Lowers the solubility of calcium, fluoride and phosphate, the major components of enamel. Shows high affinity for hydroxyl-apatite and reduces demineralisation	Kaufman and Kleinberg (1971)
Prevention of renal lithiasis	Stabilizes the rate of crystal (mineral and acid salts) nucleation in urine	Felix et al. (2006)

were inhibited by administration of phytate. Moreover, phytate could also limit the proliferation of breast cancer cells (Shamsuddin, Yang, & Vucenik, 1996), cervical cancer (Ferry, Matsuda, Yoshida, & Hirata, 2002), prostate cancer (Singh, Agarwal, & Agarwal, 2003) and HepG2 hepatoma cell lines (Vucenik et al., 1998a) in humans. The growth of mesenchymal tumours, murine fibrosarcoma (Vucenik, Tomazic, Fabian, & Shamsuddin, 1992), and human rhabdomyosarcoma (Vucenik, Kalebic, Tantivejkul, & Shamsuddin, 1998b) was reduced by the consumption of phytate-rich food. Furthermore, it was also demonstrated that phytate shows different mechanisms of action, depending on the type of cell lines, and that too in a in a dose- and time-dependent manner (Vucenik & Shamsuddin, 2003).

Tran et al. (2003) confirmed that IP6 can inhibit the colony formation of Kaposi's sarcoma (KS) cell lines, KS Y-1 (AIDS-related KS), KS SLK (Iatrogenic KS), and CCRF-CEM (human adult T lymphoma) cells in a dose-dependent manner. The proposed mechanisms of action of phytate against tumour cells are: an increase in natural killer cell activity (Baten, Ullah, Tomazic, & Shamsuddin, 1989), alteration in signal transduction (Dong, Huang, & Ma, 1999), stimulation of genes toward greater cell differentiation (Saied & Shamsuddin, 1998), and anti-oxidant activity (Graf & Eaton, 1990). Recently, it has been shown, for phytate, that it only affects malignant cells and does not influence normal cells and tissues. Deliliers et al. (2002) observed a lethal effect when CD341cells from bone marrow were treated with different doses of phytate. This toxic effect was specific to leukaemic progenitors from chronic myelogenous leukaemia patients but no cytotoxic or cytostatic effect was observed on normal bone marrow progenitor cells under the same conditions. It was further shown that phytate has the potential to induce differentiation and maturation of malignant cells, which results in reversion to the normal phenotype. This was first demonstrated in K-562 hematopoietic cells (Shamsuddin, Baten, & Lalwani, 1992), followed by human colon carcinoma HT-29 cells (Yang & Shamsuddin, 1995), prostate cancer cells (Shamsuddin & Yang, 1995), breast cancer cells (Shamsuddin et al., 1996), and rhabdomyosarcoma cells (Vucenik et al., 1998b).

The first *in vitro* study to verify phytate as an anti-cancer agent was done by cell culture transformation assay, where benzo[α]pyrene was introduced to a rat tracheal cell culture (Arnold, Wilkinson, Sharma, & Steele, 1993). The model BALB/c mouse 3T3 fibroblasts (Babich, Borenfreund, & Stern, 1993) with modest efficacy was used for testing. It was observed that phytate impeded the transformation induced by epidermal growth factor or phorbol ester in mouse epidermal cells (JB6 cells) (Huang, Ma, Hecht, & Dong, 1997). Since this model has been a well-characterised cell system for studying tumour promotion and molecular mechanisms of antitumour agents, the outcome of cell culture transformation assay strongly suggested the potential role of phytate as an antitumour agent (Vucenik & Shamsuddin, 2003).

7.1.1. Colon cancer

Colon cancer, a major neoplastic disease, is one of the main causes of morbidity and mortality in western countries (Landis, Murray, Bolden, & Wingo, 1999). This may be associated with low intakes of dietary fibres. Epidemiological studies and animal research have suggested an inverse relationship between colon cancer and consumption of high-fibre foods. Among the many components of fibre, phytate has been studied extensively for its inhibitory effects against colon carcinogenesis (Shamsuddin & Vucenik, 1999). Animal model assays have demonstrated that the protective effects of dietary fibre on colon cancer development depend on the nature and source of the fibre. In humans, wheat bran seems to suppress the development of cancerous growths in the colon more than corn or oat bran (Reddy et al., 2000). An *in vitro* study by Yang and Shamsuddin (1995) showed that human colon cancer cells, HT-29, were inhibited when subjected to phytate. A down regulation of tumour proliferation marker named PCNA was also seen (Yang & Shamsuddin, 1995). Moreover, the incidence of aberrant crypts was also decreased when phytate was used as an

intermediate biomarker for colon cancer (Challa, Rao, & Reddy, 1997). Several investigations have been focussed on the synergistic effects of phytate and inositol on large intestinal cancers (LIC). A significant reduction in the prevalence of LIC, induced by 1,2-dimethylhydrazine (DMH), was seen in mice (Shamsuddin, Ullah, & Chakravarthy, 1989). Interestingly, the protective effect of phytate was seen even after 5 months of carcinogenic induction with azoxymethane (AOM) (Shamsuddin et al., 1989). It was suggested that phytate and lipid operate together to inhibit carcinogenesis since removal of either of the two components from the human diet had no significant effect on colon tumour incidence (Reddy et al., 2000). Likewise, metabolic epidemiology studies have demonstrated that populations who consume diets high in dietary fibre and/or low in dietary fat, are at low risk for colon cancer, and excrete low levels of putative colon tumour promoters, such as secondary bile acids compared with those at high risk for colon cancer who consume diets with low fibre content and/or high fat content (Reddy et al., 1987).

7.1.2. Breast cancer

Many studies have demonstrated that phytate has an inhibitory effect against mammary carcinoma. Though not much work has been done on human breast cell, a significant and consistent inhibition of mammary cancer by phytate has been shown in 7,12-dimethylbenz[α]- anthracene (DMBA)-induced mammary cancer in rats. Considerable reductions were observed in tumour number, multiplicity (number of tumours per tumour-bearing animal), and tumour burden. It was also noted that phytate protected rats from spontaneous mammary tumours. This study demonstrated that phytate was more effective than a high fibre diet in preventing experimental mammary tumours (Shamsuddin & Vucenik, 1999). Moreover, Vucenik, Sakamoto, Bansal, and Shamsuddin (1993) reported that phytate and inositol showed synergistic effects against mammary carcinogenesis, resulting in 48 percent reduction in tumour multiplicity, as well as slight decreases in tumour size and incidence, when compared with control animals. Based on the above findings of inhibitory effects of phytate on the development and progression of mammary tumours in animal models, similar consequences may be hypothesised for humans. In this context, research on the anti-cancer functions of phytate has revealed that its growth inhibition of human mammary cancer cell lines is independent of the oestrogen receptor status. Two human mammary carcinoma cell lines, of different oestrogen receptor status, exhibited dose-dependent growth inhibition after treatment with phytate (Shamsuddin et al., 1996). Moreover, Gollapudi and Ghoneum (2008) demonstrated that rice bran inhibits the growth and stimulates the apoptosis of human breast cancer cells.

7.1.3. Hepatocellular carcinoma (HCC)

The diagnosis of liver cancer cells in human is minimal and therefore HCC is regarded as a deadly malignant disease. Vucenik et al. (1998a, 1998b) studied the potential role of phytate in the treatment of liver cancer in humans. HepG2, a human liver cancer cell line, was treated *in vitro* with phytate. This resulted in a dose-dependent growth inhibition of HepG2 cells and it also reduced the cells' ability to form colonies.

In addition, a reduction in the cells' production of alpha-fetoprotein (AFP), a HCC tumour marker, was detected. Phytate favoured the differentiation of malignant cells, contributing to conversion of the cancer cells to less aggressive phenotypes (Vucenik et al., 1998b). Consequently, a reduction in the expression of mutant p53 protein and increment in the expression of p21WAF1 protein were observed after treating HepG2 cells with phytate. This suggests that phytate enhances tumour suppressor gene activity (Vucenik et al. (1998a, 1998b)).

7.1.4. Prostate cancer (PCA)

Prostate cancer (PCA) is the most invasive and frequently diagnosed malignancy, and in the USA it is the second leading cause of cancer deaths in males (Godley et al., 1996). A multistage process is entailed in the induction of PCA, involving progression from small, latent carcinomas of low histological grade, to large, metastatic carcinomas of higher grade (Godley et al., 1996). The same authors showed that diet and androgen play a major role in the pathogenesis and promotion of PCA. Among dietary components, phytate has been shown to inhibit the growth and induce differentiation of human prostate carcinoma PC3 cells (Shamsuddin & Yang, 1995).

Zi, Singh, and Agarwal (2000) observed that epidermal growth factor receptor (EGFR or erbB1) endocytosis and associated mitogenic signalling occur in human DU145 prostate cancer cells, suggesting that erbB1 endocytosis might be involved in advanced and androgen-independent PCA growth. In their study, phytate impaired both receptor-mediated and fluid-phase endocytosis, resulting in the inhibition of mitogenic signals associated with growth and proliferation of human prostate carcinoma DU145 cells. The results obtained further suggest a novel molecular pathway to be further explored for the intervention of advanced and androgen-independent human PCA by phytate.

7.1.5. Rhabdomyosarcoma (RMS)

Rhabdomyosarcoma (RMS) is a tumour of mesenchymal origin and is the most common soft tissue sarcoma in children (Vucenik et al., 1998b). Patients with advanced metastatic RMS frequently do not respond to therapies currently available. *In vitro* and *in vivo* studies of phytate effects on the human rhabdomyosarcoma cell line have demonstrated that phytate suppressed the growth *in vitro* in a time- and dose-dependent manner and also induced cell differentiation. However, once phytate was removed from the media, the rhabdomyosarcoma cells were able to recover their logarithmic growth (Vucenik, Zhang, & Shamsuddin, 1998c). Phytate may eventually have a role in the treatment of fibrosarcomas in humans, since intraperitoneal injections of phytate in mice reduced the growth of subcutaneous transplanted murine fibrosarcomas. This prolonged the survival of tumour-bearing mice, and also reduced the number of pulmonary metastases (Vucenik et al., 1992). These findings suggest a potential therapeutic role for phytate in humans, against RMS and possibly for other mesenchymal neoplasms.

7.1.6. Pancreatic cancer

Pancreatic cancer is one of the malignancies most resistant to therapy. The number of mortalities from this cancer was estimated to be 31,270 in 2004 (Somasundar et al., 2005). Pancreatic adenocarcinoma is characterised by its poor prognosis (Vucenik & Shamsuddin, 2003). A resistance to apoptosis contributes to its insensitivity to conventional therapies. It has been reported that *in vitro* administration of corn- and rice-derived phytate on human pancreatic adenocarcinoma cells PANC 1 and MIAPACA, significantly reduced their growth (range 37.1–91.5%) (Somasundar et al., 2005). This suggests that phytate has the potential to become an effective adjunct for pancreatic cancer treatment. Further *in vivo* and human studies are needed to evaluate safety and the clinical utility of this agent in patients with pancreatic cancers.

7.1.7. Blood/bone marrow cancer

The efficacy of phytate in growth reduction of human erythroleukaemia cells K-562 *in vitro* was tested. It was observed that phytate decreased the K-562 cell population by 19–36%, concomitant with an increased differentiation, as evidenced by ultrastructural morphology and increased haemoglobin synthesis (Shamsuddin et al., 1992).

7.2. Mechanism of action against cancer

The mechanism involved in the anti-neoplastic activity of phytate is not fully understood. It was suggested that phytate offers beneficial effects through its chelating ability. However, various theories have been proposed for the anti-cancer activity of inositol compounds. These include anti-oxidant functions, mineral binding ability, pH reduction, interrupting cellular signal transduction, cell cycle inhibition and enhancing natural killer (NK) cells activity.

7.2.1. Anti-oxidant properties

An anti-oxidant property is one of the greatest biological advantages of phytate against cancer cells. The phosphate grouping in positions 1,2,3 (axial–equatorial–axial) is unique to phytate, especially interacting with iron and inhibiting its ability to catalyse hydroxyl radical formation. In another words, it inhibits iron-mediated oxidative reactions and also limits site-specific DNA damage (Midorikawa, Murata, Oikawa, Hiraku, & Kawanishi, 2001). The suppression of hydroxide free radicals and other reactive oxygen radicals by phytate reduces carcinogenesis and cell injury. Moreover, chelation with Fe^{+3} may also reduce the chances of iron-catalysed lipid peroxidation (Phillippy & Graf, 1997). It has also been proposed that antioxidative properties of phytate help in the suppression of colon carcinogenesis (Graf & Eaton, 1985). However, it is yet not certain that a physiological intake of phytate has significant impact on the anti-oxidant status of humans.

7.2.2. Mineral binding ability

The anti-cancer action of phytate is also related to its mineral binding ability with other positively charged compounds. By complexing $Zn^{2+}$and/or Mg^{2+}, phytate can affect thymidine kinase activity, an enzyme critical for DNA synthesis (Shamsuddin, Vucenik, & Cole, 1997; Thompson & Zhang, 1991).

7.2.3. pH reduction

In human gut, undigested starch and fibre reach the colon, where they either contribute to faecal bulk or get fermented to short chain fatty acids, particularly butyric acid. The increased production of butyric acid may play a protective role in colon carcinogenesis, because this organic acid has been shown in several *in vitro* studies to slow down the growth rate of human colorectal cancer cell lines (Coradini, Pellizzaro, Marimpietri, Abolafio, & Daidone, 2000). Reduction in gut pH may influence the metabolic activity of colonic flora (Mallett, Bearne, & Rowland, 1989) and bile acid metabolism (Thornton, 1981) and inhibit ammonia production and absorption (Clinton et al., 1987). Thereby, it offers protection against colon carcinogenesis (Newmark & Lupton, 1990).

7.2.4. Interruption of cellular and nuclear signal transduction pathways

Inositol phopholipids present in plasma membranes have attracted much attention because of their role as intermediaries in transmission of signals elicited by growth factors and mitogens acting at the cell surface. Since inositol occurs ubiquitously in cell membranes, in conjugation with lipids as phosphatidylinositol, it plays a critical role in cell signalling. Additionally, IP6 is the only known dietary source of inositol phospholipids. During cell stimulation, the enzymes PI kinases and phospholipase C convert IP6 to inositol triphosphate (IP3) and diacylglycerol, which act as second messengers inside cells. They also confer a range of cellular functions, including cell proliferation via mobilising intracellular Ca^{2+} (Shears, 1998). These observations led to the postulation that phytate exerts anti-cancer effects by affecting cell signalling mechanisms in mammalian cells (Shamsuddin et al., 1992).

Moreover, anti-neoplastic activity of phytate may also relate to its ability to control export of mRNA from nucleus to cytoplasm. This idea is supported by the fact that the enzyme phospholipase C and two proteins that influence the generation of IP6 are also required for proper and efficient export of mRNA from the nucleus to the cell (Shamsuddin, 2002; York, Odom, Murphy, Ives, & Wente, 1999).

7.2.5. Promotion of DNA repair

DNA is essential for maintaining the stability of the genome. Failure to repair may result in loss of genetic information and, once the cell has gone past the scope of DNA repair, the otherwise normal cell is likely to transform to a malignant (cancer) cell. In this, phytate has been demonstrated to stimulate end joining of DNA, more specifically non-homologous end-joining (NHEJ). NHEJ is thought to be effective at all times in the cell cycle and plays an important role in the repair of double-strand breaks in DNA (Essers et al., 2000; Takata et al., 1998). This process of repair is probably due to the binding of phytate to the DNA-dependent protein kinase (DNA-PKcs) (Hanakahi, Bartlet-Jones, Chappell, Pappin, & West, 2000). Another study reported that it is not DNA-PKcs but the DNA end-binding protein Ku 70 (mol. wt. 70 kDa) and Ku 86 (mol wt. 83 kDa) that binds to phytate (Ma & Lieber, 2002). However, these studies, in spite of differences in their specific findings, clearly show a very important role of phytate in DNA repair mechanism and thus in cancer therapeutics.

7.2.6. Gene alteration

Phytate has been shown to exert an influence at the genetic level. It does this by influencing cell signal transduction pathways, cell cycle regulatory genes, and tumour suppressor genes (Shamsuddin et al., 1997). Reports suggest that phytic acid significantly blocked phosphatidyl inositol-3 kinase (PI-3K), known to influence neoplastic cell transformation activity in a dose-dependent manner (Huang et al., 1997). Subsequently, it can be recommended that PI-3K may ultimately serve as a biomarker for the effectiveness of phytic acid in future clinical studies (Dong et al., 1999). Phytate also upregulates the expression of tumour suppressor genes, e.g., p53, p21 WAF1/Cip1 (Saied & Shamsuddin, 1998). Thus, at gene level, phytate may cause greater differentiation of malignant cells. This perception was supported by several colon cancer studies that have confirmed phytate's ability to influence colon morphology in a constructive way by increasing both cell apoptosis and differentiation (Jenab & Thompson, 2000).

7.2.7. Arrest of cell cycle

Tumours are characterised by uncontrolled growth in cell number and cell size. However, dietary phytate restricts the S phase of mitosis and arrests cells in the G0/G1 phase of the cell cycle. Thereby, it offers an anti-proliferative effect on tumour cells. In this regard, it has been demonstrated that phytate lowers the percentage of cells expressing Ki-67, a proliferative marker in human breast and colon cancer cell lines (El-Sherbiny, Cox, Ismail, Shamsuddin, & Vucenik, 2001).

7.2.8. Augmentation of natural killer (NK) cells

The lower form of phytate, produced by dephosphorylation, performs an integral role in cellular signal transduction and intracellular function. It boosts the intracellular phosphate pool that amplifies NK cell cytotoxicity (Urbano et al., 2000). This enhancement in NK cell activity augments the body's immune response to carcinogenic threat (Reddy, 1999) and also contributes to tumour cell destruction. In an experiment where mice were exposed to a colon carcinogen, dimethylhydrazine, it depressed NK cell activity and, when treated with dietary phytate, an enhancement in NK cell activity and tumour cell suppression were observed (Baten et al., 1989).

7.2.9. Dephosphorylation

Apart from the mentioned mechanisms of phytate, it has also been elucidated that dephosphorylation of phytate to lower forms also accounts for its anti-cancer activity. In fact, lower forms, such as myo-inositol (1,3,4,5,6) pentakisphosphate specifically inhibit the activity of phosphatidylinositol 3-kinase. This enzyme plays a crucial role in angiogenesis, a fundamental step in the transition of tumours from a dormant state to a malignant state (Maffucci & Falasca, 2001). Phosphatidylinositol 3-kinase catalyses the phosphorylation of inositol phospholipids at the D3 position to generate 3′-phosphorylated phosphoinositides (Foster, Traer, Abrahanm, & Fry, 2003), which act by recruiting specific signalling proteins to the plasma membrane (Maffucci & Falasca, 2001).

7.3. Phytate against coronary heart disease

Coronary heart disease is the principal cause of morbidity and mortality in much of the industrialised part of the world. About 30% of all deaths in the United States are due to this disease (Anon, 1969). Etiology of this disease is linked to elevated plasma cholesterol, resulting in hypercholesterolaemia. In developing countries where people consume more cereals, nuts and legume foods, the risk of coronary heart disease is low. This may be correlated with considerable amounts of phytate in their food. *In vitro* studies on animals have demonstrated that dietary phytate supplementation results in significant lowering of serum cholesterol and triglyceride levels (Jariwalla et al., 1990). This effect was accompanied by decrease in serum zinc level and in zinc–copper ratio since it is hypothesised that coronary heart disease is predominantly due to imbalance in zinc and copper metabolism (Klevay, 1975).

Phytate is a naturally occurring chelating agent which preferentially binds with zinc rather than copper; thereby it can be presumed that phytate decreases the absorption of zinc without affecting copper absorption (Persson et al., 1998). Thus in theory, diets higher in fibre, or more specifically in phytate, may have the protective effect of decreasing the ratio of zinc to copper absorbed from the intestinal tract. However, studies on the preventive role of phytate in heart diseases have only been conducted in animals and further investigations on humans are necessary.

7.4. Hypolipidaemic activity

At physiological dosages (0.1–0.5% of diet), IP6 inhibits rise in hepatic total lipids and triglycerides, resulting from administration of sucrose. The mechanism of this hypolipidaemic effect in the liver appears to be related to the inhibition of hepatic enzymes involved in lipogenesis. Although physiological levels of phytate slow the accumulation of lipids, they have little effect on elevated serum lipids. Indeed, these dietary treatments (up to 2.5% IP6) do not produce significant changes in hepatic cholesterol or serum total lipid levels in sucrose-treated animals, consistent with findings of Katayama (1997) showing reduction of serum hyperlipidaemia at higher supplementary levels of dietary phytate.

7.5. Antiplatelet activity of phytate

Platelet adhesion to endothelial cells and their aggregation are key steps in the pathogenesis of thrombosis and atherosclerosis. A study of phytate effect on platelet aggregation was conducted, using whole blood obtained from 10 healthy volunteers.

It was seen that phytate effectively inhibits human platelet aggregation *in vitro*, suggesting its potential in reducing the risk for cardiovascular disease (Vucenik, Podczasy, & Shamsuddin, 1999).

7.6. Phytate against diabetes mellitus

Diabetes mellitus is caused by regular intake of a quickly available glucose diet; thereby it is more prevalent in a western society. This nutritional disease results in abnormally high blood sugar levels (hyperglycaemia). However, phytate-rich foods are of great concern because a negative correlation exists between phytate intake and blood glucose response (Thompson, 1993; Yoon et al., 1983). *In vitro* studies on humans verified that more dependence on phytate-enriched diets results in low blood glucose response. This has great nutritional implication in the prevention and management of diabetes mellitus (Yoon et al., 1983). Moreover, phytate can be a key element in modulating insulin secretion; diminished production of insulin or resistance to its effects results in diabetes. The real mechanism of action is not fully understood but it seems that phytate regulates insulin secretion via its effect on calcium channel activity because it specifically inhibits serine threonine protein phosphatase activity. This, in turn, opens intracellular calcium channels, driving insulin release (Barker & Berggren, 1999; Larsson et al., 1997).

7.7. Phytate against HIV

Phytate was investigated for its antiviral effect on the human immunodeficiency virus (HIV) *in vitro*. In MT-4 cells, phytate completely inhibited the cytopathic effect of HIV and the HIV-specific antigen expression at a concentration of 1.67 mg/ml (Otake et al., 1989). Also, phytate inhibited the replication of HIV-1 in a T cell line, as well as that of a freshly isolated strain in peripheral blood mononuclear cells (Otake et al., 1999). Although the mechanisms of IP6 action remain unclear, it can be speculated that it acts on HIV-1 at an early replicative stage. It is not possible to develop phytate itself as an anti-AIDS drug. Studies of this anti-HIV agent might be expected to provide a basis for eventual production of superior drugs for AIDS treatment.

7.8. Phytate against dental caries

Dental caries, also known as tooth decay, remains one of the most common diseases throughout the world, especially in the western society. Epidemiological studies have shown a significantly elevated incidence of dental caries concomitant with the change in dietary habits in western societies. This increase has been hypothesised to be the result of decreased phytate consumption, substantiated by the increased cariogenicity of flour on refinement. The cariostatic effect of phytate has been ascribed to its ability to lower solubilities of calcium, fluoride and phosphate, the major components of enamel (Kaufman & Kleinberg, 1971). Moreover, phytate has a very high affinity for hydroxyl-apatite. Thus by protecting the teeth from demineralization, phytate may also prevent the formation of cavities, plaque and tartar.

7.9. Phytate against renal lithiasis

Renal lithiases, popularly called kidney stones, are small, hard deposits of mineral and acid salts on the inner surfaces of kidneys. The consequence of alteration of the normal crystallization conditions of urine in the urinary tract lead to this disorder. When normal urine crystallization conditions are disturbed, the rate of crystal nucleation and growth may become such that the crystals cannot easily be eliminated, due to their size. Epidemiological studies have shown that renal stones are more prevalent in developed countries, where populations consume diets based on refined flour compared, than in developing nations, consuming predominantly cereals and legumes, known for their high phytate contents. In human studies, phytate has been demonstrated to be effective

against four types of renal stones (Felix, Costa-Bauza, & Prieto, 2006), namely calcium oxalate monohydrate papillary calculi (characterised by induction of subepithelial calcifications) (Pieras, Costa-Bauza, Ramis, & Grases, 2006), calcium oxalate monohydrate unattached calculi (formed in renal cavities) (Daudon, Bader, & Jungers, 1993), calcium oxalate dihydrate/hydroxyapatite mixed calculi (associated with hypercalciuria and hypocitraturia) (Grases, Costa-Bauza, Ramis, Montesinos, & Conte, 2002) and Brushite calculi (associated with tubular acidosis) (Felix et al., 2006). The mechanism is that phytate can interfere with formation of calculi (crystals) of calcium oxalate and phosphate (Grases et al., 2000a, 2001). This was verified by the evidence that individuals prone to calcium oxalate stone formation were found to have lower urinary phytate excretion than had healthy ones (Grases et al., 2000b).

8. Conclusion

In the past few decades, scientists and entrepreneurs working in the field of human nutrition, human health and environmental protection have been focusing their attention on phytate and phytase. Dietary phytate has received much investigative attention as an antinutrient. We chose not to review this area of research extensively. The interactions of phytate and dietary minerals and beneficial health effects of phytate have been the subject of this review. The interactions of phytate and minerals have both food processing and nutritional implications. The removal of phytate during food processing and/or by adding exogenous phytase results in the improvement of the bioavailability of essential minerals, such as calcium, iron and zinc. This has been ascribed as a potential way to reduce the risk of mineral deficiency among populations, mainly in developing countries, consuming unrefined cereals and/or pulses as a major diet. Moreover, removal of phytate from human food positively influences the purity, yield and economy of the production, as reported for bread-making, production of plant protein isolates and corn wet-milling. A great potential exists for the use of phytases in processing and manufacturing of foods for human consumption but, up to now, no phytase product for a food application has found its way to the market. Apart from being an antinutrient, dietary phytate exhibits beneficial health effects, such as protection against a variety of cancer and heart-related diseases, diabetes mellitus and renal stones. The beneficial health effects of phytate are more significant for populations in developed countries because of the higher incidence of cancer especially colon cancer which is associated with higher fat and lower fibre-rich food intakes. Such populations generally do not suffer from mineral deficiencies. On the one hand, the chelating ability of phytate is considered to be a detriment to one's health whilst, on the other hand, many researchers consider this ability to bind with minerals as its most powerful asset. Such a variant topic signifies that more intensive studies are needed to obtain better insight into the mechanism responsible for the "friend or foe" challenge of phytate. Moreover, regardless of a series of researches on the positive and negative features of phytate, the information on the dosage for humans eliciting positive or negative effects is limited and the optimal dosage for clinical therapies is yet to be determined.

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