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Review Article

Phytase: A Boom in Food Industry

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ABSTRACT

Phytate [myo-inositol(1,2,3,4,5,6)hexakisphosphate] have been considered negative in food industry as it is the main storage form of phosphorus (P) in many plants but this phytate-bound P is not available to non – ruminants as they don't have these endogenous enzyme and hence the availability and digestibility of phytate phosphorous is very low in these animals. Phytic acid has antinutrients behavior and has a potential for binding positively charged multivalent cations, proteins and amino acids in foods. Phytase [myo-inositol(1,2,3,4,5,6)hexakisphosphate phosphohydrolases], a phytate-specific phosphates is an enzyme that can break down the undigestible phytic acid and thus release digestible phosphorus, calcium & other nutrients. Research in the field of animal nutrition has put forth the idea of supplementing phytase enzyme, exogenously, so as to make available bound nutrients from phytic acid and, thereby helps in food processing and digestion in the human alimentary tract. This review covers the application of phytase in food industry and emphasizes is given on developing new effective phytase with improved properties.

INTRODUCTION

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisdihydrogenphosphate) & mixed cation salts of phytic acid, designed as phytate, are a group of organic phosphorus (P) compounds found widely in nature especially in legumes, cereals, and oilseed crops which serve as a major source of nutrients for the animals (Table 1). These crops have an important constituent of phytic acid whose salt form, phytate, is an anhydrous storage form of more than 80% of the total phosphorus in cereals and legumes. In terrestrial ecosystems, they are synthesized by plants, accumulate in seeds during the ripening period and are regarded as the primary storage form of myo-inositol (Reddy et al. 1982) and phosphorus in grains (Turner et al. 2002) and in pollen.

The ruminants digest phytic acid with the help of phytase produced by their anaerobic ruminal micro flora. However, simple-stomached animals such as pig, poultry and fish are deficient in gastrointestinal tract phytase. So, in the context of human and animal nutrition, the following two aspects of phytic acid are emanated (Wodzinski et al. 1996) Firstly, Monogastric animals have only low levels of phytate-degrading enzymes in their digestive tracts, and since phytic acid itself is not resorbed, feed for animals is supplemented with inorganic phosphorus to meet phosphorous requirement; and Secondly, phytic acid is an antinutrients constituent in plant-derived food and feed, since it form complexes with proteins, amino acids (Pallauf and Rimbach 1996) and variety of metal ions . It forms complexes with these minerals because it poses a high phosphate content, which results in a high negative charge over a wide pH range. So, it chelates with positively charged divalent cations, rendering a poor absorption of the bound metals in small intestine. This is partially attributed to the wide-spreading human nutritional deficiencies of calcium, iron, zinc in developing countries (Manary et al. 2002) but mineral sub deficiencies may also occur in developed countries (Lopez et al. 2002). They have been studied most intensively in the seeds of plants (Greiner 2002). Phytate are create hurdle in the path of food assimilation (Noureddini and Dang 2008).

Because of these problems, there is considerable interest in phytate degrading enzyme. A diverse class of enzymes, phosphates, catalyzes the cleavage of monophosphoester bonds in various organo-phosphate compounds, but these enzymes are not capable of hydrolyzing the monophosphoester bonds in phytic acid.

As the hydrolysis of phytic acid has great importance, a special class of enzymes hydrolyzing phytic acid has evolved – the phytases (*myo*-inositol hexakisphosphate phosphohydrolases) which hydrolyze phytic acid to less phosphorylated *myo*-inositol derivatives (in some cases to free *myo*-inositol), releasing inorganic phosphate (Fig 1). Therefore, phytase in animal feed reduces the need of extra supplementation of phosphorus. As a result, fecal phosphate excretion by the animal is reduced by up to 50 % and hence, environment is protected from excessive phosphorus runoffs pollution. Phytases have been found in plants, microorganisms, and in some animal tissues (Konietzny and Greiner 2002). The first commercial phytase products were launched into market in1991 (Haefner et al. 2005) and after that many commercial products were launched (Table 2).Phytic acid is Myo-inositol (1,2,3,4,5,6) hexakisphosphate. Inositol phosphates consist of an inositol ring and at least one phosphate group. "myo" refers to the conformation of the hydroxyl groups on the inositol ring. The nine possible configurations of the inositol ring have been annotated in a number of ways, but the adopted nomenclature is according to the set of rules suggested by Posternak (1965) (Fig. 2). Based on the carbon in the myo-inositol ring of phytate at which dephosphorylation is initiated, phytases can be referred as 3-phytases (E.C. 3.1.3.8), 6-phytases (E.C. 3.1.3.26) and 5-phytases (E.C. 3.1.3.72). Depending on their pH optima, phytases have been divided into acid and alkaline phytases and based on the catalytic mechanism, phytase can be referred to as histidine acid phytases, β-propeller phytases, cysteine phytases or purple acid phytases (Mullaney and Ullah 2003).Therefore, phytases are considered to be potential candidate for use as an enzyme that have great value in enhancing the nutritional quality of phytate-rich foods & feeds (Oboh and Elusiyan 2007). In addition, phytase would be an eco-friendly product.

PHYTATE IMPACT ON FOOD

Phytate, salts of phytic acid, is the storage form of both phosphate and inositol in plants product especially in seeds and grains. It is formed during maturation of 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 (Table 1). Depending on the amount of plant derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4500mg (Reddy 2002). On average, daily intake of phytate was estimated to be 2000-2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries and 150- 1400 mg for mixed diets (Reddy 2002).

PHYTATE DEPHOSPHORYLATION DURING PROCESSING OF FOOD

Phytate hydrolysis can occur during food preparation and production, either by phytase from plants or by microorganisms. Foods processing which increase the activity of native enzymes are soaking, cooking, malting, germination, baking and fermentation technology. Fermentation leads to lowering of pH as a result of bacterial production of organic acids especially lactic and acetic acid, which is favorable for cereal phytase activity. Compared to legumes, cereals exhibit mostly higher phytate –degrading activity in the pH range from pH 5 -5.5 (Egli et al. 2002), whereas phytate-degrading activity at pH 8.0 was slightly lower in cereals compared to legumes. Products of phytase action on phytate i.e. myo-inositol pentakis-,tetrakis-, tris-, bis-, and monophosphates are further dephosphorylated by phosphatases as they do not accept phytate as substrate. During food processing, phytate is not fully hydrolyzed by the phytases naturally present in plants and microorganisms. So, it is important to optimize the properties of naturally occurring phytases.

Soaking

Soaking is pretreatment to enhance processing of legume grains and cereal seeds. It may be of short period or for a long period. In household activities cereals and pulses are typically soaked in water at room temperatures overnight. For example, soaking of wheat bran, rye flour at optimal conditions for wheat phytase activity (pH 4.5-5, 55^0C) resulted in complete phytate dephosphorylation (Sandberg 1991). Sandberg (1991) found that the $InsP_6$ and $InsP_5$ content reduced to about 0.5µmol g^{-1} enhancing the physiological condition to increase iron solubility. As phytate is water soluble, a significant phytate reduction can be seen after discarding the soak water. In addition action of endogenous phytases contributes to phytate reduction. Temperature and pH value have significant effect on enzymatic phytate hydrolysis during soaking (Greiner and Konietzny 1999). Soaking temperatures between 45 and 65^0C and pH values between pH 5.0 and 6.0 causes significantly enzymatically phytate reduction (Greiner and Konietzny 1999).

Cooking

Cooking process is not able to hydrolyze phytate, as phytate is heat stable. Therefore, considerable phytate dephosphorylation during cooking only takes place either by discarding the cooking water or by the enzymatic phytate hydrolysis due to the action of the intrinsic plant phytase during the early part of the cooking phase (Greiner and Konietzny 1999). Progressive inactivation of endogenous enzyme occurs if kept for long time in elevated temperatures. Thus, heat-stable phytases or additions of exogenous heat stable phytases are the only possibilities to improve dephosphorylation during cooking of plant products.

Table 1. Phytate contents in plant or plant products (adjusted from Tyagi et al. 1998; National Research Council 1993; Selle and Ravindran 2006)

Food	Phytate p (%)	Food	Phytate p (%)
Cereals/millets		Cowpea (cooked)	3.9-13.2
Wheat	0.21-0.27	Black beans (cooked)	8.5-17.3
Maize	0.25	White beans (cooked)	9.6-13.9
Rice	0.09	Lima beans (cooked)	4.1-12.7
Sorghum	0.22-0.21	Faba beans (cooked)	8.2-14.2
Barley	0.19-0.20	Kidney beans (cooked)	8.3-13.4
Bajra	0.23	Navy beans (cooked)	6.9-12.3
Oat	0.21	Soybeans	9.2-16.7
Rye	0.19	Tempeh	4.5-10.7
Corn grain	0.18	Lentils (cooked)	2.1-10.1
Cereal-based		Green peas (cooked)	1.8-11.5
French bread	0.2-0.4	Peanuts	9.2-19.7
Mixed flour bread (70% wheat, 30% rye)	0.4-1.1	Oilseed meals	
Mixed flour bread (70% rye, 30% wheat)	0-0.4	Groundnut meal	0.46
Sourdough rye bread	0.1-0.3	Soybean meal	0.38-0.56
Whole wheat bread	3.2-7.3	Cotton seed meal	0.77-0.78
Whole rye bread	1.9-4.3	Sunflower meal	0.45
Unleavened wheat bread	12.2-19.3	Canola meal	0.64
Oat bran	7.3-2.1	Corn glutton	0.26
Oat flakes	8.4-12.1	Rapeseed meal	0.63
Oat porridge	6.9-10.2	Miscellaneous	1.41
Pasta	0.7-9.1	Rice bran	0.83
Maize	9.8-21.3	Wheat bran	39.3-57.2
Cornflakes	0.4-1.5	Sesame seeds (toasted)	2.4-13.1
Rice (polished, cooked)	1.2-3.7	Soy protein isolate	2.9-11.7
Wild rice (cooked)	12.7-21.6	Soy protein concentrate	11.2-23.4
Sorghum	5.9-11.8	Buck weed	9.2-16.2
Legume-based		Amaranth grain	10.6-15.1
Chickpea (cooked)	2.9-11.7		

Malting

Malting is a process during which the whole grain is soaked and then germinated. But when the malted cereals were ground and soaked at optimal conditions for wheat phytase only then there was complete degradation of phytate (Larson and Sandberg 1992) otherwise if we use malted grains of wheat, rye and oats as such there was only slightly or not at all reduction of phytate content. Oat germination require 5 days at 11^0C followed by incubation at 37-40^0C, then phytate content of oats was reduced by 98%. Both iron and particularly zinc absorption was significantly improved from the porridge made from malted flour (Larson and Sandberg 1992).

Phytate is hydrolyzed during germination in a step wise manner by phytases or by the combined action of phytate and phosphates which supply nutritional requirement by accumulating less phosphorylated myo-inositol intermediates. But in non-germinated grains and cereals, only little intrinsic phytate-degrading activity is found (Egli et al. 2002). As long time is required to improve mineral bioavailability through germination, this approach is not economical beneficial in industries food processing.

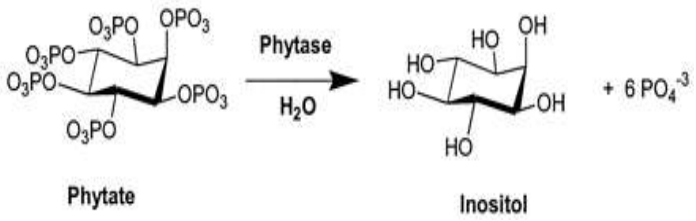

Fig.1 Mechanism of dietary phytate hydrolysis by microbial phytase.

Germination

Germination is a process which is also used to breakdown antinutrients, such as phytate and protease inhibitors, in cereals and legumes to increase their nutritional value. In household cooking we basically followed this process and it helps in significant increase in mineral uptake after 6 to 10 days of germination.

Fermentation and Baker Technology

Food fermentation covers a wide range of microbial and enzymatic processing of food to achieve desirable characteristics such as prolonged shelf life, nutritional enrichment, attractive flavor and elimination of anti-nutrients. Major fermentation microorganisms include lactic acid bacteria, moulds and yeast.

Table 2. Commercial production of microbial phytases.

Company	Country	Phytase source	Production strain	Trademark
Roal	Finland	*A. awamori*	*T. reesei*	Finase
Novozymes	Denmark	*A. oryzae*	*A. oryzae*	Ronozyme®
AB enzymes	Germany	*A. awamori*	*T. reesei*	Finase
BASF	Germany	*A. niger*	*A. niger*	Natuphos
Fermic	Mexico	*A. oryzae*	*A. oryzae*	Phyzyme
Alko Biotechnology	Finland	*A. oryzae*	*A. oryzae*	SP, TP, SF
Genencor International	USA	*P. simplicissimum*	*P. funiculosum*	ROVABIO
Finnfeeds International	Finland	*A. awamori*	*T. reesei*	Avizyme
Alltech	USA	*A. niger*	*A. niger*	Allzyme phytase
BioZymes	USA	*A. oryzae*	*A. oryzae*	AMAFERM
DSM	USA	*P. lycii*	*A. oryzae*	Bio-Feed Phytase

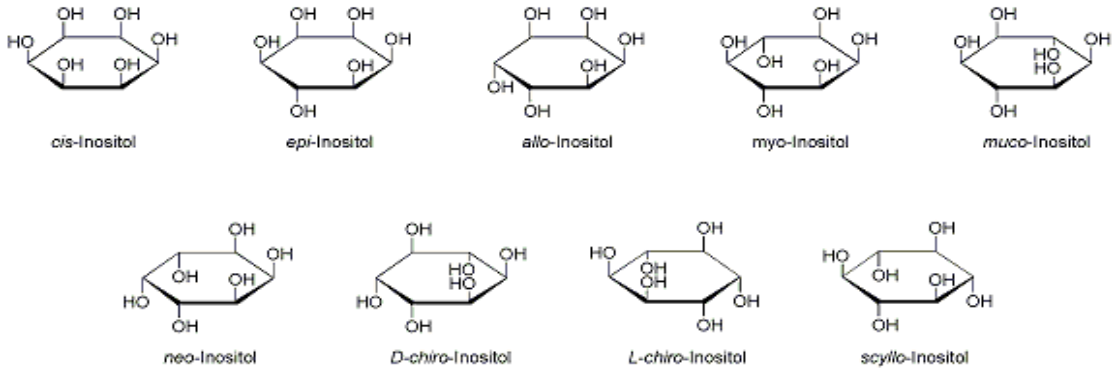

Fig. 2 The nomenclature of the nine stereo isomers of inositol (Haworth projections), Numbering of the carbon atoms can be performed either counterclockwise (D) or clockwise (L). Only the chiro form has specific D and L conformations.

Yeast or lactic acid bacteria are, for example, used to produce bread. Bread is an important source of both iron and the inhibiting phytate. Phytase is best in improving bread making. But lactic-yeast and mixed fermentation are old methods for food processing and preservation. Moreover, conditions during bread fermentation disfavor yeast phytase expression (Turk et al. 1996). Today, defined starter cultures and controlled conditions are generally used in food fermentation. The type of microorganisms, the fermentation used, and the starting amount of phytate present in the raw material significantly affect the extent of phytate removal during the fermentation process. Phytate hydrolysis is affected by the type, extraction rate and freshness of flour, presence and absence of yeast, pH, fermentation time and water content of dough, baking conditions and by various additives. In last two years, bifidobacterial strains, novel phytate-degrading enzymes bacteria, are added in wheat dough as a fermentation starter replacing the common lactic acid bacteria.

Milling process defines the chemical composition of any flour. Flours with higher extraction contain increasing amount of bran. The higher the extraction of the flour, the higher the content of iron and phytate in it as these originates from the bran. Now days, as consumption of whole grain breads are increased so it would be beneficial and attractive to improve the mineral status and consequently level of nutrition increased. Addition of acetic acid or lignoberries to the dough increased the phytate reduction to 96 and 83%, respectively, compared with 55% in control bread without additives (Turk et al. 1996). The pH of the dough's with 96% reduction was between 4.5 and 5. In oriental food fermentation, phytase of the microorganisms used for fermentation contribute significantly to phytate degradation.

Food products such as tempeh, miso, koji and soy sauce are produced by fermentation of soybeans with *Rhizopus oligosporus* and *Aspergillus oryzae*, respectively. Both moulds have been shown to produce intra- as well as extracellular phytate-degrading activity (Fujita et al. 2003).

High levels of phytate degradation in the fermentation medium have been described by using economically competitive expression systems for *Escherichia coli* (Miksch et al. 2002) as well as for yeasts i.e. *Hansenula polymorpha* (Mayer et al. 1999) and *Pichia pastoris* (Yao et al. 1998). If phytate degrading capability of microorganisms used in fermentation such as *Saccharomyces cerevisiae*, *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum* are increased, then there is no need for protein purification. Application of these improved microorganisms in fermentation of plant derived products is expected to result in food products having significant low level of phytate.

Addition of isolated enzymes during food processing

Addition of a phytase preparation during food processing is suggested, as an alternative to the optimization of phytate dephosphorylation by naturally occurring enzymes. The extent of phytate hydrolysis during food processing is affected by the raw material used, the source of phytase, the manufacturing process and the amount of enzyme activity added. 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. Microbial phytase preparations are now commercially available, making their use in food processing technically feasible. Very effective phytate degradation was obtained by adding *A. niger* phytase to an oat-based nutrient solution

fermented by *Lactobacillus plantarum* (Marklinder et al. 1995).

DEPHOSPHORYLATION OF PHYTATE IN THE GASTROINTESTINAL TRACT

A number of factors have to be considered when discussing phytate degradation in the gut, e.g. if there is an effect of dietary phytase secreted from intestinal mucosa or phytase produced by the intestinal micro flora. By changing the intestinal flora or by increasing enzyme secretion, phytate degradation can be enhanced in the gastrointestinal tract. Balance studies in human indicate that an increasing dietary calcium level reduces the degree of phytate degradation in humans (Ellis et al. 1986).

HYDROLYSIS OF ENZYMES IN THE STOMACH, SMALL INTESTINE AND COLON

Phytate can be degraded during food processing in the gastrointestinal tract. Inositol hexaphosphate can be degraded by enzymatic and nonenzymatic hydrolysis. Enzymatic hydrolysis generally occurs during biological processing and preparation of plant related food product such as steeping, malting, fermentation, hydrothermal processing as well as during degradation in the gastrointestinal tract but when food is treated with strong acid or high temperature and pressure than non-enzymatic degradation occurs. The enzymatic degradation is more specific and selective (Larson and Sandberg 1992) and give maximum yield at optimum temperature and pH (Table 3). So, by increasing the amount of exogenous enzyme or by physical separation of phytate-rich parts of plant seeds we can enhance phytate degradation in final food.

NEGATIVE ASPECTS OF PHYTIC ACID ON HUMAN DIET

Phytic acid is the major cause in mineral diminution and deficiency in plant derived foods. Human population mainly depends on whole grain and legume staple foods for their survival and these food items contain large amounts of phytic acid so this lead the risk of mineral deficiency in them (Konietzny and Greiner 2002). Phytate not only cause nutritional deficiencies but also effects yield and quality of food ingredients (Kvist et al. 2005). Formation of insoluble mineral-phytate complexes mainly causes poor mineral bioavailability and these complexes are not absorbed by human intestine. Moreover, monogastric animals do not have endogenous phytate degrading enzymes (Iqbal et al. 1994). So, there is a problem in the digestion of phytate containing food by these animals. At physiological pH, zinc was reported to form the most insoluble salt with phytic acid (Maddaiah et al. 1964) suggesting that zinc is the most deficient mineral in phytate-rich diets and this interaction may change protein structure by decreasing enzyme activity, proteolytic digestibility and protein solubility.

Solubility is prerequisite for absorption of most minerals. Phytic acid structure is indicative of strong chelating potential. It has six strongly dissociated protons (pKs 1.1 to 2.1) and six weakly dissociated protons (pKs 4.6 to 10.0). The effect on minerals is observed through the formation of phytate-mineral (M) or peptide-mineral-phytate complexes. These complexes have stoichiometries of the $M^{+}(n)$- phytate type (n=1-6). Phytate forms a wide variety of insoluble salts with divalent and trivalent cations. Usually, the divalent cations (e.g: Zn^{2+}, Ca^{2+} and Mg^{2+}) form insoluble penta- and hexa- substituted salts. Solubility and stability of myo-inositol phosphate-mineral complexes have been found to decrease as the number of phosphate residues on the myo-inositol ring decreases. Therefore there is reduced impairment of intestinal uptake of essential dietary minerals by the removal of phosphate residues from phytate (Larson and Sandberg 1992).

Only myo-inositol pentakisphosphate suppressed absorption of iron, zinc, calcium in humans in isolated form, while myo-inositol tetrakis- and trisphosphates had no effect. But in the presence of higher phosphorylated myo-inositol phosphates, myo-inositol tetrakis- and trisphosphates were shown to contribute to the negative effect of phytate on iron absorption (Larson and Sandberg 1992). Because a strong negative correlation was found between zinc absorption and the sum of myo-inositol tris-through hexakisphosphate from cereal and legume meals (Sandberg 1991), such a contribution is also true for zinc absorption but solubility at neutral pH has been shown to be less important for calcium absorption. So, to reduce the risks for mineral deficiency in fast growing children, pregnant women, people of developing countries different formulation have been developed by pharmaceutical supplementation (Maberly et al. 1994). But practically, none of supplementations are very successful. So, alternative approaches are adopted for increasing the amount of these micronutrients in diet which enhance their uptake either by plant breeding or by genetic engineering.

Recently, low phytate mutants in maize, barley, rice and soybeans have been isolated (Raboy 2002) and their potential for improving the absorption of divalent cations has been shown. To improve rice as source of iron, three proteins were expressed in the central endosperm of the rice seed: a Phaseolus phytoferritin, an endogenous cysteine-rich metalothionein-like protein, and an *Aspergillus fumigatus* phytase (Lucca 2001). If properly targeted, over expression of phytase during seed development can result in reduced phytate levels in the mature seed (Coello et al. 2001).

Table 3. Comparison of microbial phytases from different sources

Phytase source optimum	Phytase activity (U/mg) (37^0C)	Temperature optimum (^{0}C)	pH
Fungi			
Aspergillus fumigatus 6.0	23-28	60	5.0-6.0
Aspergillus oryzae	11	50	5.5
Aspergillus caespitosus	-	80	5.5
Aspergillus terreus	142-196	70	5.0-5.5
Aspergillus niger 5.5	50-103	55-58	2.2
Thermomyces lanuginosus	110	65	6.0
Emericella nidulans	29-33	-	6.5
Myceliophthora thermophila	42	-	5.5
Penicillum simplicissimum	3	55	4.0
Peniphora lycii	1080	58	5.5
Sporotrichum thermophile	-	45	5.0
Yeast			
Saccharomyces castelii	418 (70^0C)	77	4.4
Pichia anomala	-	60	4.0
Candida krusei	1210	40	4.6
Cladosporium	909	40	3.5
Bacteria			
Escherichia coli	811-1800	55-60	4.5
Klebsiella terrigena	205	58	5.0
Klebsiella pneumonia	224,297	50,60	5.0,5.5
Klebsiella aerogenes	-	68	4.5,5.2
Bacillus subtilis	9-15	55-60	6.5-7.5
Bacillus amyloliquefaciens	20	70	7.0-8.0
Pantoea agglomerans	23	60	4.5
Citrobacter braakii	3457	50	4.0
Lactobacillus sanfranciscensis	-	45	4.0
Pseudomonas syringae	769	40	5.5

Table 4. Phytase expression in genetically engineered plants

Host plant	Tissue	Phytase source	Host plant	Tissue	Phytase source
Maize	seed (embryo)	A. niger phy A2	Potato	leaf	*A. niger*
Tobacco	seed	*A. niger*	Potato	root (secreted)	*consensus*
Tobacco	leaf	*A. niger*	Sugarcane	callus	*E. coli*
Tobacco	leaf	*B. subtilis*	Wheat	seed	*A. niger*
Tobacco	leaf (secreted)	*A. niger*	Soybean	callus (secreted)	*A. niger*
Tobacco	root (secreted)	*A. niger*	Soybean	seed	*A. niger*
Tobacco	root (secreted)	*B. subtilis*	Sesame	root (secreted)	*A. niger*
Rice	seed	*E. coli* + *S. ruminantium*	Arabidopsis	seed	*E. coli*
Rice	seed	*A. fumigatus*	Arabidopsis	root (secreted)	*B. subtilis*
Rice	leaf	*S. occidentalis*	Arabidopsis	root (secreted)	*A. niger*
Alfalfa	leaf	*A. niger*	Canola	seed	*A. niger*

BENEFICIARY EFFECT OF PHYTATE ON HUMAN

Almost researches have been focused on the negative aspects of phytate on human health but consumption of phytate, however, have many positive effects, for example, as an antioxidant and an anticancer agent. Chelation action of phytate with iron provides it antioxidant property (Burgess and Gao 2002). The beneficial effects of phytate are also evident from the diabetes mellitus, renal lithiasis and arteriosclerosis in developing countries where people rely mainly on plant-based diets, which constitute a considerable amount of phytate (Kumar et al. 2010). However, in western countries, these diseases are common because of the greater dependence on processed food, characterized by low phytate content. Evidence also indicates that phytate inhibitory effects on mineral absorption are not seen in diets containing animal protein. These evidences indicate that phytate is beneficial as a dietary antioxidant in an animal protein diet (Cornforth 2002).

PHYTASE APPLICATIONS

Role of probiotics in phytase production

FAO/WHO working group suggest the definition of probiotics as live microorganisms that when administered in adequate amounts confer a health benefit on the host (Vasilijevic and shah 2008). Hirimuthugoda et al (2007) have isolated a novel microbial marine phytase from the gastrointestinal tract of sea cucumbers, *Holothuria scabra*. They found two strains of *Holothuria scabra,* W2B and YF 12C, which are similar to *Yarrowia lipolitica* and *Candida tropicalis* through a confirmation of DNA sequences analysis of phylogeny with those in the National Centre for Biotechnology Information (NCBI) database. The species of *Yarrowia lipolitica* can be used at the commercial level for marine phytase production. Whereas, *Candida tropicalis* is well-known yeast species found all over the world, and is common pathogenic strain on humans. Therefore, industrial application of this species is limited, although extracted phytase can be used as an industrial product.

Phytase as animal feed Supplement

Phytase has been known for several decades and up to now, it is solely used in monogastric animal's feed such as swine, fish (Nwanna et al. 2008), poultry and in human diet also. Phytase is used by these animals as they produce little or no phytase in the intestine. But ruminant animals can enzymatically hydrolyze phytic acid and release inorganic phosphorus as they have micro flora in their gut which hydrolyzes phytic acid. This requirement of phosphorus of monogastric animals is met by supplementing soybean and other meals with inexpensive phosphate. The excess phytin phosphorus is disposed of in the animal's manure (Mullaney et al. 2000) where it is cleaved by soil and water-borne microorganisms. This excess phosphorus is then released in water bodies causing eutrophication (Bali and Satyanarayana 2001). So, the availability of phosphorus can be improved by adding microbial phytase in to their feed.

However, feed industry could not use it economically due to its high production cost. Another limiting factor is its inactivation at the high temperatures required for pelleting feed (above 80^0C) and its activity loss during storage (Greiner and Farouk 2007). The cost of using phytase as an animal feed additive can be reduced if the heat tolerance of the enzyme is increased. These problems can also be overcome if phytase is produced endogenously by monogastric animals. For this a transgenic mouse has been developed that secretes phytase in its saliva (Golovan et al. 2001). Thermo stability of enzymes can also be increased by sorghum liquor wastes supplemented with starch, by the use of salts and polyols (Lamosa et al. 2000) and by the immobilization of *E. coli* phytase (Greiner and Konietzny 1999).

Among the bacterial phytases, the pH optimums for intracellular and extracellular phytases are 4.5-6.0 and 6.0-7.0, respectively (Afinah et al. 2010). There is no report of bacterial phytases which worked at alkaline pH except *Bacillus* species (Kim et al. 1998) but alkaline phytase have been purified from lily pollen (Jog et al. 2005) and the rat intestine (Yang et al. 1991). Compared to many other phytase producing strains which exhibit low enzyme activity at pH values associated with the upper digestive tract, the *Rhizoctonia* sp. and *F. verticilloides* phytase activity is significantly higher. Both of phytases exhibited maximum activity as high as Natuphos and pGP209 phytase (commercial phytase), at 50^0C (Martin et al. 2005). Site-directed mutagenesis confirmed that a replacement of Gly-277 and Tyr-282 of *A. fumigatus* wild-type phytase with the corresponding residues of *A. niger* phytase (Lys and His) gave rise to a phytase with a pH optima at 2.8 to 3.4 (Tomschy et al. 2002). For industries, a phytase with a pH activity profile ideally suited for maximal activity in the digestive tract of monogastric animals is desirable.

Several studies presented that addition of phytase significantly improve digestibility of protein, amino acids (Chen 2000), phosphorus, calcium and zinc utilization (Cao et al. 2007). However, Ai et al (2007) argues that phytase supplementation do not improve protein utilization and subsequently growth of Japanese seabass. The results presented by Cowieson et al. 2008, confirm previous reports that the ingestion of phytic acid by broiler chickens stimulates an increase in endogenous losses of amino acids and N from the gastrointestinal tract. In studies with pigs the doses of microbial phytases (Natuphos^R) in the range of 500 to 2000 phytase units/Kg of feed resulted in generation of 0.8 to 1.0 g digestible P/Kg of feed. The dose-response relationship seems to be dependent on the type of feed and the doses of microbial phytase. In addition, the content of digestible calcium is increased, and amounts on average from 50 to 80% of the increase of digestible P content. In contrast, soaking a phytate-rich diet or addition of lactic or formic acid to a diet with microbial phytase from natuphos^R had a synergistic effect on the apparent digestibility of P and calcium.

Sometimes, workers exposed to microbial enzymes have suffered allergic responses. There are some reports that phytase is an occupational allergen that cause specific IgE immune responses. There are some commercial phytase feed such as Bio-feed^R, which are dust free that offers several advantages over the powdered enzyme (Mullaney et al. 2000).

With the help of recent advances in biotechnology field, now new phytase can be expressed in different microorganisms, for example a new phytase was expressed in yeast, and this, when fed to weanling pigs, improved the bioavailability of phytate phosphorus. This phytase is also as effective as Natuphos at the inclusion level of 700 or 1200 U/Kg of a P-deficient (Stahl et al. 2000).

SOURCES OF PHYTASES

Fungal phytase

Transgenic rice has been developed to over-express genes encoding for phytase *Aspergillus fumigatus*, ferritin from *Phaseolus vulgaris*, and a cysteine –rich metalloprotein- like protein to improve rice iron bioavailability to humans. The plant has been crossed with a recently developed β-carotene producing rice line (Lucca et al. 2001). Expression of the phytoferritin approximately doubled the endosperm iron content and the cysteine-rich peptides have been shown to improve iron absorption in the gut. *Aspergillus fumigatus* phytase was selected because of its reported high thermal stability (Wyss et al. 1999) and the hope of retaining activity during cooking. *Aspergillus niger* phyA gene was over-expressed in maize seeds using a construct driven by the maize embryo-specific globulin 1 promoter. Low-copy-number transgenic lines with

simple integration patterns were identified. Phytase activity in transgenic maize seeds reached approximately 2,200 U/Kg seed, about a 50-fold increase compared to non-transgenic maize seeds (Chen et al. 2007). *A.niger* phyA gene was also expressed in maize endosperm under the control of the rice glutelin-1 promoter (Drakakaki et al. 2005). But there are some hurdle in the use of phytase i.e. production of phytase, stability of enzyme, thermostability of enzyme. Native *Aspergillus* phytase had high regeneration abilities after heat treatment. So, WT *Aspergillus fumigatus* phytase as well as heat stable engineered phytase has been expressed in wheat and barley (Brinch-Pedersen et al. 2003). For the production of phytase "biofarming" is the cost effective approach. The most common approach is to use the Cauliflower Mosaic Virus (CaMV) 35S promoter for the construct, and the results are same as that with fungal phytase (Ullah et al. 2003). Similar results with a heat stable *A. fumigatus* phytase expressed in tobacco root (George et al. 2005), subterranean clover root (George et al. 2005), potato leaves (Ullah et al. 2003), alfalfa leaves (Ullah et al. 2003), and wheat seed (Brinch –Pedersen et al. 2000) have also shown significant results (Table 4).

Bacterial phytase

When *E.coli* appA phytase gene was targeted to vacuoles of transgenic *Arabidopsis* seeds significant result of phytate reduction and P increase in seeds were seen (Coello et al. 2001). Same results were also seen, when phytase was expressed in transgenic soyabean (Chiera et al. 2004) and in sugarcane callus (Santosa et al. 2004). Phytase of *Bacillus subtilis* was also expressed in tobacco root (Lung et al. 2005) and leaves (Yip et al. 2003), arabidopsis root (Lung et al. 2005). Combination of *E.coli* and *S. ruminantium* phytase was also expressed in rice seed (Hong et al. 2004). All these phytases show significant results of phytate reduction and increase in P content in respective part.

Purification is not always the big problem with phytase enzyme as it is produced both extracellularly as well as intracellularly. A transgenic strain of *Bacillus mucilaginosus* express high phytase activity extracellularly and degrade PA in the soil and helpful in promoting tobacco growth, hence, increase its phosphorus content and limiting eutrophication (Li et al. 2007).

Yeast phytase

Experiments with pigs that were fed with rice leaves expressing yeast phytase, indicates that yeast phytase is quite resistant to denaturation when expressed in rice (Hamada et al. 2006).

CONCLUSION

Up to now, phytate blocks the path of mineral bioavailability and protein digestibility in human nutrition but now phytases solve all these problems. They are also recognized in reducing the need to supplement phosphorus in diets and it also minimizes the phosphorus levels in manure. Phytases not only supply ample amount of phosphorus but its use is also eco-friendly. Phytases also solved the problems of developing countries and other vulnerable groups such as baby- boomers, strictly vegetarians; those have unhealthy diet which leads to inadequate nutrient uptakes.But there is no one ideal phytase which can fulfill all requirements. Thus researchers has to become more vigorous in isolating novel and best phytate-hydrolyzing enzymes microorganisms and optimizing their catalytic features and specific activity via genetic engineering with improved thermo stability, pH optimum, and substrate specificity. Thus research aiming at improving food texture and digestibility at a cost effective level should continue.

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