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Review Article
Phytase: application in food industry

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Abstract: Phytates have been considered as a threat in human diet due to its antinutrients behaviour which known as strong chelators of divalent minerals such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}. Phytic acid has a potential for binding positively charged proteins, amino acids, and/or multivalent cations or minerals in foods. The resulting complexes are insoluble, difficult for humans to hydrolyze during digestion, and thus, typically are nutritionally less available for absorption. The reduction of this phytates can be achieved through both enzymatic and non-enzymatic removal. Enzymatic degradation includes addition of either isolated form of wild-type or recombinant exogenous phytate-degrading enzymes microorganisms in the food matrix. Non-enzymatic hydrolysis of phytate occurred in the final food during food processing or physical separation of phytate-rich parts of the plants seed. The application of phytase with respect to breadmaking process, probiotics, animal feed supplement and transgenic crops are emphasised in this paper.

Keywords: Phytase, phytic acid, food application

Introduction

Phytic acid is a major component of all plant seeds, constituting 1-3% by weight of many cereals and oilseeds and typically accounting for 60-90% of the total phosphorus (Graf, 1983). Phytates serve several physiological functions, especially in seed germination. Historically, phytates have been considered solely as antinutrients because they are known as strong chelators of divalent minerals such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}. Moreover, phytates are also capable of binding with starch and proteins while preventing their assimilation through the digestive system (Noureddini and Dang, 2008).

Conversely, as a strong chelator of iron and zinc, phytate in plant foods actually can serve as an antioxidant to reduce free radical formation mediated by these metals (Lei and Porres, 2003). The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially nonabsorbable from the human gastrointestinal tract (Greiner and Konietzny, 2006).

Minerals are involved in activation of intracellular and extracellular enzymes, in regulation of critical pH levels in body fluids necessary for the control of metabolic reactions and in osmotic balance between the cell and its environment. A deficiency of any one of the essential minerals can result in severe metabolic disorders and compromise the health of the organism. Some minerals deficiencies are common in developing countries, but mineral subdeficiencies may also occur in developed countries (Lopez *et al*., 2002).

However, many populations might ingest inadequate quantities of calcium, iron, and zinc and may be marginally deficient in magnesium. Thus, concern continues about the impact of dietary phytate upon the mineral status of certain vulnerable segments of the population including children, teenagers, pregnant women, and the elderly. For the first three groups, representing periods of prolific growth, the phytate/mineral ratios are critical. In the final group, the baby-boomers, inadequate nutrient intakes coupled with use of over-the-counter medications may further compromise mineral status. Still others

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will strive to increase phytate consumption even as a supplement because of the health benefits. The changing face of the food supply through genetic modification, fortification, creation of functional foods, and increased use of supplements will affect mineral nourishment (Weaver and Kannan, 2002).

Effective reduction of phytic acid (PA) can be obtained via the action of both enzymatic and non-enzymatic degradation (Greiner and Konietzny, 2006). Enzymatic degradation involve of addition either isolated form of wild type or recombinant exogenous PA-degrading enzymes bacteria from various source of fungi and bacteria. Whereas, in nonenzymatic hydrolysis of phytate, reduction levels of phytate occurred in the final food during food processing or physical separation of phytate-rich parts of the plants seed. Engineering of phytases in order to optimise their catalytic features is seen as a promising strategy to efficient reduction of phytate. Enhancement of thermal tolerance and increase in specific activity are two important issues not only for animal feed, but also for food processing applications of phytases. Different strategies have been used to obtain an enzyme capable of withstanding higher temperatures for instance shifting in temperature optimum of the *Escherichia coli* phytase from 55°C to 65°C achieved by expression of the enzyme in the yeast *Pichia pastoris* after introduction of three glycosylation sites into the amino acid sequence of the *Escherichia Coli* phytase by site-directed mutagenesis (Rodriguez *et al*., 2000). Furthermore, breeding which selection of low PA varieties or high phytase varieties is another successful way to reduce level of phytate amount. As in food industry, processed foods like breads, lactic acid fermented products are beneficially healthy as the level of phytate content are lessen at the end product as well as acquirement of good product quality (Lopez *et al*., 2002 and Haros *et al*., 2001).

Phytases are a special class of phosphatases that catalyze the sequential hydrolysis of *myo*-inositol-(1,2,3,4,5,6)-hexakisphosphate or phytic acid ($InsP_6$) to less phosphorylated *myo*-inositol derivatives and inorganic phosphate (Haros *et al*., 2007). Phytase reduces the antinutritional properties of phytic acid and eutrophication, caused by the excretion of undigested phytic acid by monogastrics because of the lack of adequate levels of phytase in their digestive tracts (Greiner and Konietzny 2006). Phytate-degrading activity has been detected in plants, micro-organisms, and in some animal tissues and phytases from several plant and microbial species (Hill *et al*., 2007 and Haros *et al*., 2007) have been purified and characterized. Hence, although currently phytases are used mainly as animal feed additives in diets of monogastric animals, there is a great potential for the use of this class of enzymes in processing and manufacturing of food for human consumption (Jorquera *et al*., 2008).

Nevertheless, there are still limited sources of phytase which suitable to be use in all food application. Thus, screening for ideal phytase with more approving properties and engineering phytases in order to optimize their catalytic and stability features still are enduring research interest. Some of the criteria of ideal phytase is capable of remain highly active during food processing or preparation. Moreover, it is a favourable property of phytase to have high phytate-degrading capability even at room temperature, withstanding of acceptable heat and a high activity over a broad pH range (Greiner and Konietzny, 2006). Various studies have been performed to manipulate phytase in food application.

Phytic acid issues on human nutrition

The major past anxiety over seed-derived dietary phytic acid has been its role in mineral diminution and deficiency. Human populations that manage to survive on whole grain and/or legume staple foods consume large amounts of phytic acid (Table 1), and this may contribute to their risk for mineral depletion and deficiency. However, dietary phytic acid may also have vital positive roles, for example, as an antioxidant and an anticancer agent. The recognized benefits of dietary phytic acid may be a more important consideration in certain populations than concerns over mineral deficiency. The question of seed-derived dietary phytic acid in human nutrition and health is more complicated by the fact in the cereal grain, phytic acid is deposited in the aleurone and germ, which is also the site for the grain's main mineral stores. Removal of these tissues during milling or polishing removes most phytic acid and most of the grain's mineral deposits. The impact of dietary phytic acid in human health must, therefore, be evaluated on a case-by-case basis, considering positive and negative roles in a given population consuming a given diet (Raboy *et al*., 2002).

Because phytate binds essential minerals and can prevent their absorption, most human nutritionists view the compound negatively. However, its unique chelating action with iron provides phytate with antioxidant characteristics (Burgess and Gao, 2002). More recently, studies indicate that phytic acid is a natural antioxidant important for seed viability. Evidence also indicates that the inhibitory effects of

Table 1: Seeds/grains/fruits with seeds that are commonly eaten by humans that contain phytic acid (PA) concentrations of one percent or more on a weight basis. (Adapted from Lott et al., 2002).

Plant	Structure	% Phytic Acid
Sesame	Dry seed	4.71
Pumpkin/squash	Embryo	4.08
Flax (linseed)	Dry seed	3.69
Rapeseed (canola)	Dry seed	2.50
Sunflower	Embryo	2.10
Mustard	Dry seed	2.00
Cashew	Embryo	1.97
Brazil and other tree nuts	Embryo	1.80
Hemp	Dry fruit	1.74
Peanut	Seed in shell	1.70
Tomato	Seed only	1.66
Soybean	Dry seed	1.55
Almond	Dry embryo	1.42
Eggplant	Seed only	1.42
Beans	Dry seed	1.41
Pistachio	Embryo	1.38
Watermelon	Seed only	1.36
Kiwi fruit	Fleshy fruit	1.34
Broad beans	Dry seed	1.11
Cucumber	Immature seed	1.07
Sorghum	Dry grain	1.06
Cocoa beans	Dry seed	1.04
Barley	Dry grain	1.02
Oats	Dry grain	1.02
Wheat	Dry grain	1.02
Peas	Dry seed	1.00

phytate on mineral absorption are not seen in varied diets containing animal protein. Phytate may actually be beneficial as a dietary antioxidant in an animal protein diet. There may be nutritional advantages, or at least no disadvantage, to addition of phytate to meat products (Cornforth, 2002).

Phytic acid has a potential for binding positively charged proteins, amino acids, and/or multivalent cations or minerals in foods. The resulting complexes are insoluble, difficult for humans to hydrolyze during digestion, and thus, typically are nutritionally less available for absorption. Phytate forms chelating conjugates with nutritionally important minerals such as calcium, magnesium, copper, iron (Fe^2 and Fe^{3+}), zinc, cobalt, and manganese. Figure 4 shows the example of a phytate interaction with iron and a protein. Solubility is a prerequisite for absorption of most minerals, although solubility at neutral pH has been shown to be less important for calcium absorption. The chemical structure of phytic acid is indicative of strong chelating potential (Figure 2). Phytic acid 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. The insolubility of these complexes is regarded as the major reason for the reduced bioavailability of minerals due to diets high in phytic acid. Several factors determine the effect of phytate on mineral bioavailability: pH, size and valence of the mineral, mineral and phytate concentrations and ratios, and food matrix that include the presence of enhancers and/or inhibitors

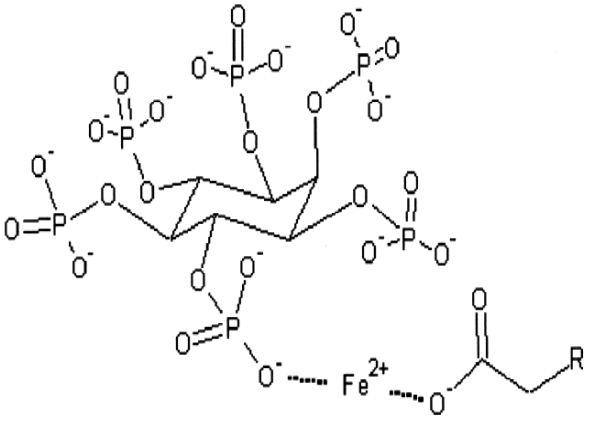

Figure 4: Phytate showing an example of an interaction with iron and protein. (Source from Weaver and Kannan, 2002)

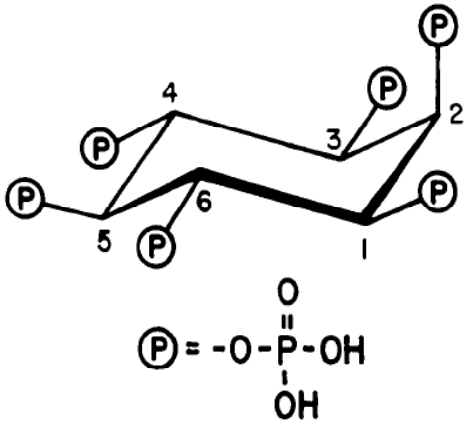

Figure 2: Structure of phytic acid in dilute solution. (Graf, 1983)

(Weaver and Kannan, 2002).

From numerous *in vitro* and *in vivo* studies, it is clear that purified exogenous PA, given either in the drinking water or the diet, as well as endogenous PA, as a phytochemical component of wheat bran, have cancer protective effects on a variety of tissues. It is also apparent that pure PA may play a role in reducing serum cholesterol and lipids and thus reduce the risk of heart disease, while both exogenous and endogenous PA may have hypoglycemic effects and thus be of consequence in diabetes. PA consumption may also be beneficial for those suffering from buildup of renal calculi. Although only a limited number of studies have been conducted on the role of endogenous versus exogenous PA, it appears that endogenous PA maybe, in part, responsible for the cancer protective effects of high-fiber foods, particularly wheat bran, a rich source of PA, while exogenous PA is also very effective as possible mechanisms of phytic acid acid are illustrated on Figure 1. However, the effects of PA observed in animal studies need to be validated in humans, so well-designed, prospective clinical studies are necessary. Several mechanisms have been suggested for the disease protective effects of PA, including its ability to bind starch, proteins, enzymes, and minerals such as the pro-oxidant iron, its potential participation in cellular inositol phosphate pools and its involvement in signal transduction, cell signaling cascades and gene expression. PA is a major phytochemical in many high dietary fiber foods, which may, in part, be responsible for the disease risk reduction effect attributed to many of these foods. However, additional research is necessary to better characterize the effects of PA on various diseases and to further compare the effectiveness of endogenous versus exogenous PA, particularly in human populations (Jenab and Thompson, 2002).

Degradation of phytate (*myo*-inositol hexakisphosphate, $InsP_6$) occurs during food processing and in the gastrointestinal tract. This degradation is of nutritional importance because the mineral binding strength decreases and the solubility increase when phosphate groups are removed from the inositol ring, resulting in an increased bioavailability of essential dietary minerals. $InsP_6$ can be degraded by enzymatic and nonenzymatic hydrolysis. Enzymatic hydrolysis generally occurs during biological processing and preparation of plant food/feed such as steeping, malting, hydrothermal processing, fermentation, and addition of phytase as well as during degradation in the gastrointestinal tract. Nonenzymatic hydrolysis usually takes place when food/feed is treated with strong acid or high temperature and pressure. The enzymatic degradation is more selective and isomer specific (Sandberg, 2002).

Bakery technology

Bread is a staple food in the world and is an important source of both iron and the inhibiting phytate. The chemical composition of flour depends on the ratio of the cereal grain removed by the milling process. Low extraction white flour mainly initiates from the endosperm, whereas flours with higher extraction also contain increasing amounts of bran. The higher the extraction of the flour, the higher the content of iron and phytate that originates from the bran (Brune *et al*., 1992). As consumption of whole grain breads is increasing in Western countries, a whole wheat bread with low phytic acid level and increased mineral bioavailability would be beneficial and attractive in improving mineral status and consequently in supporting preventive nutrition.

Phytase was revealed to be an exceptional

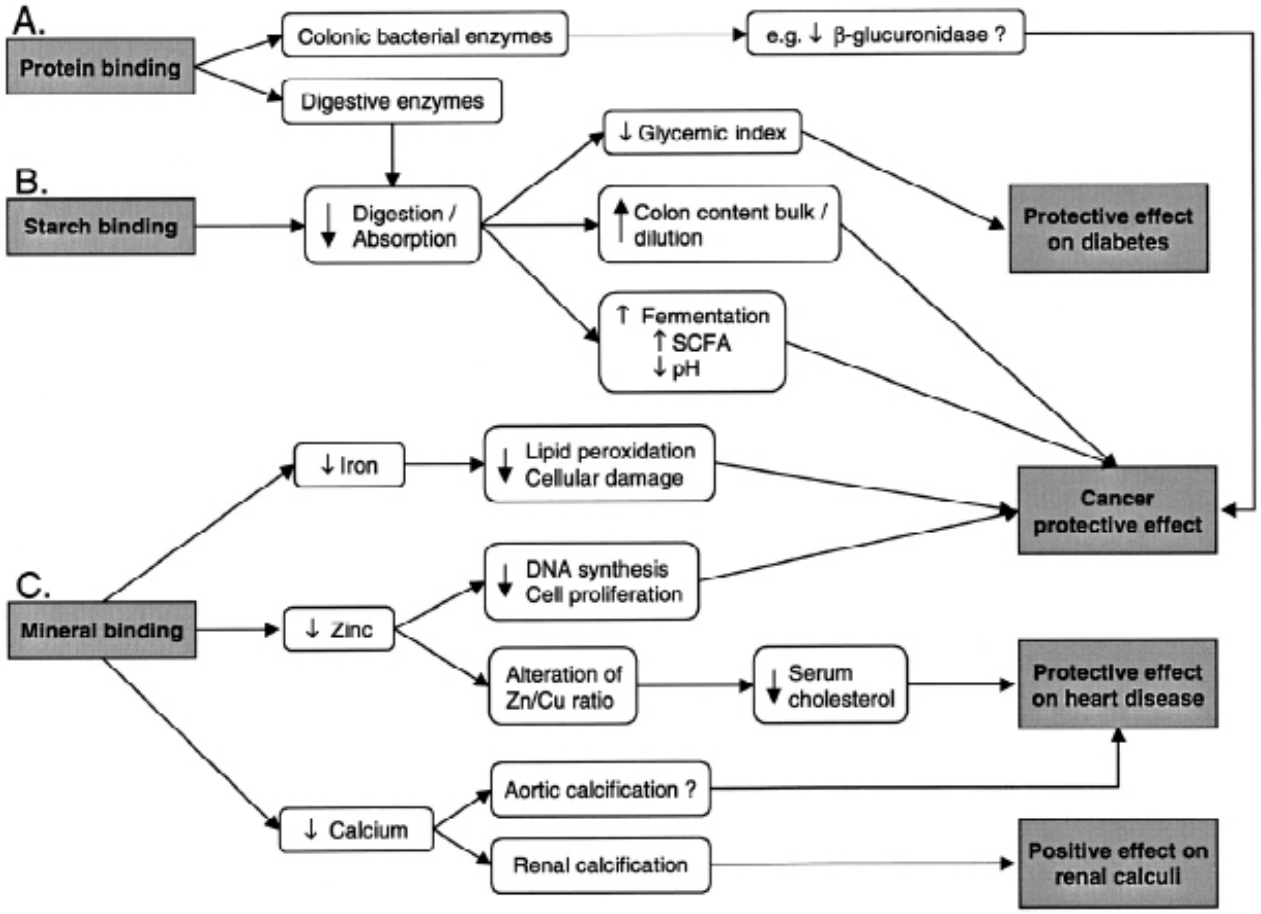

Figure 1: Possible mechanisms of phytic acid action. (Adapted from Jenab and Thompson, 2002)

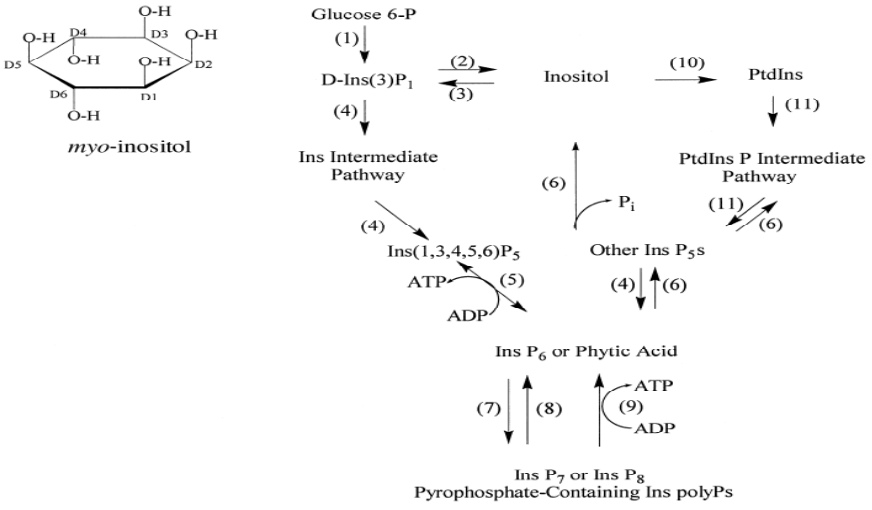

Figure 3: An outline of biochemical pathways involving phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate or Ins P6) in the eukaryotic cell. (a) Structure of myo-inositol. The numbering of the carbon atoms in the ring is given following the D-conversion. (b) Biochemical pathways. Numbers at arrows indicate the following enzymatic activities: (1) D-myo-inositol 3-P1 synthase (MIPS); (2) D-Ins 3-P1 phosphatase, or Ins monophosphatase; (3) D-Ins 3-kinase or Ins kinase; (4) Ins P or polyP kinases; (5) Ins 1,3,4,5,6 P5 2-kinase or phytic acid-ADP phosphotransferase; (6) phytases and phosphatases; (7) Ins P6 or pyrophosphate-forming kinases; (8) pyrophosphate-specific phosphatases; (9) pyrophosphate-containing Ins PolyP-ADP phosphotransferases; (10) phosphatidylinositol (PtdIns) synthase; and (11) PtdIns and PtdIns P kinases followed by PtdIns P-specific phospholopase C (Adapted from Raboy et al., 2002).

breadmaking improver. The supplementation of commercial fungal phytase (3.1.3.8) from *Aspergillus niger* in the dough ingredients containing fiber formulation leads to an acceleration of the proofing, an improvement of the bread shape, a slight increase of the specific volume, and also confers softness to the crumb. These improvements in bread quality were suggested to be associated with an indirect impact of phytase on α-amylase activity (Greiner and Konietzny, 2006). From the nutritional point of view, a further hydrolysis of the phytates which considered as anti-nutritional compounds is reached by adding exogenous phytase, therefore an enhancement in the mineral adsorption can be obtained with the consumption of phytase supplemented bread (Haros *et al*., 2001).

Recently, novel phytate-degrading enzymes bacteria from bifidobacterial strains are incorporated in wheat dough as a fermentation starter replacing the common lactic acid bacteria. However, taking into account the phytate degrading activity besides the pH and the total titrable acidity of the resulting dough, the Bifidobacterium strains from infants could be good starters for being used in breadmaking (Palacios *et al*., 2008).

Sathe and Venkatachalam, (2002) proposes that phytate hydrolysis occurs throughout the different stages of bread making and obviously depends on the type of bread being made. Some of the factors that significantly affect phytate hydrolysis include the following:

(1) *Flour*: type, freshness, extraction rate,

(2) *Yeast*: presence/absence (depending on type of bread), amount,

(3) *Dough*: pH, water content, fermentation time,

(4) Baking conditions: leavening time, temperature,

(5) *Additives*: calcium and magnesium salts, sodium bicarbonate.

Lopez et al., 2002, supports the claims from Sathe and Venkatachalam when it was noted that phytate breakdown was less marked in yeast bread than sourdough bread. As the phytate content in bread affects mineral assimilation, it appears consistent that Mg, Fe, Zn and Cu were less absorbed from yeast-fermented bread than from sourdough bread. It can be suggested that sourdough bread should be replaced with the common choice of yeast-fermented bread consumption due to its remarkable nutritional value.

Probiotics

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 (Vasiljevic 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 yeasts strains of W2B and YF12C are similar to *Yarrowia lipolitica* and *Candida tropicalis* through a confirmation of DNA sequences analysis of phylogeny with those in the National Center for Biotechnology Information (NCBI) database. The species of *Yarromia 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. The role or impact of yeasts in the gastrointestinal tract of sea cucumbers is not clearly known but obviously the significant phytase synthesis is favourable for disgesting phytate phosphorous as well as a probiotic form.

Animal feed supplement

Up to now, phytases have been mainly, if not solely, used as animal feed additive in diets largely for swine and poultry, and to some extent for fish. The first commercial phytase products were launched into market in 1991 (Greiner *et al.*, 2007). The addition of phytase to feed for monogastric animals is commonly used to enhance the digestibility of phytate-associated phosphorus (Pontoppidan *et al*., 2007). The effectiveness and limitations of phytase supplementation may also depend on substrate specificity (Greiner and Farouk 2007). The effect of microbial phytases (EC 3.1.3.8) as feed supplement is well documented in the literature (Noureddini and Dang, 2008).

One constraint of using phytase as a feed supplement is the expenditure associated with production and application of the enzyme. In countries where regulations controlling nutrient management have been implemented, such as the Netherlands, avoiding fines for overload phosphorus output can counterbalance the expense associated with enzyme supplementation. However, in areas where animal waste management remains less heavily regulated, enzyme costs have prevented widespread use. Another factor limiting use has been the incapability of the commercially available phytase supplements to endure the elevated temperatures associated with feed pelleting. To have an impact on nutrient

utilization, an enzyme present in the plant based animal feed must maintain active until it is consumed and can take action to release phytate phosphorus in the animal digestive tract. Improving the thermal and protease stability of phytases is an active area of research (Grabau, 2002).

Phytases used as feed additives should be effective in releasing phytate phosphate in the digestive tract, stable to resist inactivation by heat from feed processing and storage, and cheap to produce (Greiner and Farouk, 2007). Ruminants have the ability to utilize phytate phosphorous (P), partially as a result of the phytase activity of the ruminal microflora. However, fish cannot digest phytate P in plant-based diets because they lack intrinsic gastrointestinal phytase. Therefore, in intensive fish production, large amounts of P are discharged into the environment where they pose serious P pollution problems (Nwanna *et al*., 2007).

Several studies have revealed that phytase supplementation has significantly improve digestibility of protein, phosphorus, calcium and zinc utilization (Liebert and Portz, 2007; Sardar *et al*., 2007; Baruah *et al*., 2007 and Cao *et al*., 2007). However, Ai *et al*., (2007) argues that phytase supplementation do not improve protein utilization, and subsequently growth of Japanese seabass. Despite the availability of large number of phytase producing organisms, there is a direct need to have an isolate which produce stable phytase with high specific activity that efficiently hydrolyze phytic acid without any considerable loss in its enzymatic activity (Vats *et al*., 2009). Among the bacterial phytases, the pH optimums for extracellular and intracellular phytases are 6.0-7.0 and 4.5-6.0, respectively. For industrial application, a phytase with a pH activity profile ideally suited for maximal activity in the digestive tract of monogastric animals is desirable.

Transgenic crops

Phytases have been cloned and expressed transgenically in plants, yeast, fungi, and bacteria. These exercises have exposed valuable information on how differences in posttranslational modification *in vivo* and processing for secretion or storage influence phytase stability (Phillippy, 2002), and phytic acid level (Raboy *et al*., 2002).

The resulting variation in seed or grain total phosphorus (P) is almost completely accounted for by variation in phytic acid P. In normal, nonmutant or "wild-type" seeds or grains, essentially all P over and above a concentration necessary for nominal cellular function accumulates as phytic acid P. The concentration necessary for cellular function, nonphytic acid P, is usually about 15% to 25% of total P in mature grain produced under nominal or standard conditions. If any nonmutagenized population is screened for grain phytic acid P concentration, and lines are identified with "low" or "high" levels, one is most likely selecting for low or high levels of grain total P concentration. A "low phytic acid" line would, therefore, be low total P. In addition, phytic acid P is highly and positively correlated with grain total nitrogen and, to a lesser extent, with several mineral elements. Selecting "low phytic acid" lines would result in "low protein" and, in certain cases, "low minerals." An approach was needed that could avoid these undesirable "correlated responses," most of which are an outcome of the relationship between phytic acid P and total P (Raboy *et al*., 2002).

Another potential route to improved phosphorus availability is the targeted expression of phytase to modify phosphorus reserves in mature seeds. Subcellular localization of a phytase to the site of phytate accumulation during seed development may allow a reduction in seed phytate levels with an accompanying increase in available phosphorus. To achieve proper targeting, a better understanding of the site of phytate biosynthesis and accumulation as well as signals for protein localization will be required. Iron and other mineral deficiencies are common in populations consuming diets rich in seeds and grains due to the chelating effects of phytate. Phytase is among the gene fragment that is being introduced to improve the nutritional value of rice. The expression of a thermostable phytase to lower phytate content in rice and other crops would represent a critical nutritional enhancement (Grabau, 2002).

In order to increase phytate-degrading activity during food processing, incorporation of plants with a high phytase activity into the plant-derived raw material to be processed is seen as an alternative to the addition of exogenous phytases (Greiner and Konietzny, 2006). Hong *et al.,* (2008) have demonstrated in their studies unique features of genetically engineered sweet potato which the expression of secretory phytase in transgenic potato improving phytate utilization and increasing size, number and yield of potato tubers when organic fertilizers containing phytate as a sole phytate source. Moreover, it possesses high activity of the sweet potato sporamin promoter in potato making this promoter an ideal alternative choice for expressing recombinant proteins in transgenic potato tubers. Thus, this high-level expression of phytase with high activity over broad pH ranges making potato tubers

a suitable phytase carrier. In addition, based on its promising results of animal feeding tests suggesting that transgenic potato tubers containing recombinant phytase are an efficient feed additive.

Concluding remarks

The presents of phytic acid in food matrices has become the major concerns due to its negative effect on mineral bioavailability and protein digestibility in human nutrition. Furthermore, the baby-boomers generation and several developed countries people have been applying unhealthy lifestyle with indelicate meal diet eating which may lead to inadequate nutrient intakes. Thus, the inclusion of exogenous phytase in food medium and reduction of phytate level in plant based food via genetic engineering have been seen as promising area. Application of phytase in food business seems to be a gifted approach nutritionally and economically. However, it is not an easy task to simply assign any commercially available phytase to food application as many tests yet to be conducted to approve its effectiveness. Scientist has to become more vigorous in isolating novel and best phytate-hydrolyzing enzymes microorganisms and optimizing their catalytic features, thermal tolerance and specific activity via genetic engineering to generate an idyllic phytase for food application.

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