Chapter 12
Fungal Phytases: Current Research and Applications in Food Industry

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Contents

12.1 Introduction ... 435
12.2 Phytases of Fungal Origin ... 437
12.3 Phytase Adverse Effects ... 439
12.4 Enzymatic Phytate Dephosphorylation to Increase Mineral Bioavailability During Food Processing ... 441
12.4.1 Soaking ... 442
12.4.2 Cooking ... 443
12.4.3 Germination ... 443
12.4.4 Fermentation ... 443
12.5 Isolated Phytases and Food Processing in Recent Times ... 444
12.6 Isolated Phytase Applications in Food Production ... 446
12.6.1 Bread Making ... 447
12.6.2 Generation of Isolated Plant Proteins ... 447
12.6.3 Wet Corn Milling ... 448
12.6.4 Cereal Branch Split ... 448
12.7 Phytase Degradation in Human Body ... 448
12.8 Production of Metabolically Active Phytate Breakdown Products ... 451
12.9 Conclusion ... 452
References ... 452

12.1 Introduction

Phytases have been one of the most important enzymes in nutrition, environmental protection, and human health over the last two decades. These enzymes sequentially isolate orthophosphate groups from the core of phytate inositol, the principal chemical source of phosphorus in plants (Kumar et al. 2017). Various phytases have been

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isolated from plants or microbes and can be categorized according to their optimal pH and catalytic methods (histidine acid phosphatases, ß-propellate phytases, cysteine, or purple acid phosphatases). The urgent need to boost phytate phosphorus in diets for animals with simple stomachs to reduce the excretion of phosphorus fume into the environment has been motivated by recent phytase activities. However, future phytase applications may be extended to release dietary phytate-bound minerals for human nutrition and to produce specific inositol phosphates for human health (Konietzny and Greiner 2003; Kour et al. 2020).

There is also an excellent opportunity to use phytases in the production and manufacture of food for human consumption, where research focuses on the enhancement of the nutritional value of plant-based food as well as the technical development of food processing. A high diet of phytate contributes to a considerably reduced absorption of dietary mineral products (Sandberg et al. 1999). During food processing, phytate dephosphorylation results in the formation of only partly phosphorylated myo-inositol phosphate esters with a lower capacity to interfere with the dietary intake (Sandström and Sandberg 1992; Han et al. 1999; Shears 1998). Specific myo-inositol phosphate esters have proved to have many essential physiological functions in humans (Greiner et al. 2002). Phytases, therefore, that can be used for food processing to produce functional foods (Haros et al. 2001) should generate these biochemically active phosphate esters of myo-inositol and absorb them into the food tract. This chapter contains technical improvements to the implementation of phytases for bread making (Wang et al. 1999), plant protein isolation production (Fredrikson et al. 2001; Caransa et al. 1988), corn waters (Antrim et al. 1997; Kvist et al. 2005), and cereal bran fractionation (Andlid et al. 2004).

In the gastrointestinal monogastric tract, phytate (Inositol hexaphosphate, IP6), the dominant source of phosphorous and mineral food complexes (calcium, ferrous, magnesium, and zinc), is indigestible (Hellström et al. 2010; Brinch-Pedersen et al. 2007). Phytate is a key antinutritional factor for the bioavailability of dietary minerals that rely exclusively on cereals to dietary consumers (Cao et al. 2007). It involves the global burden of iron deficiency and the resulting complications, particularly among women and children, in low-income countries. Diät mineral bioavailability can be increased with the use of phytase, a catalytic enzyme for phytate sequence hydrolysis (Raghavendra and Halami 2009; Ogunremi et al. 2020; Mullaney and Ullah 2003).

As members of previously recognized phosphatases classes, the basic structural characteristics of several phytate-degrading enzymes were determined (Mullaney and Ullah 2003; Chu et al. 2004). X-ray crystallographic studies, among others, have confirmed their belonging to a class with a new catalytic mechanism (Ha et al. 2000). The description of the molecular 3D structure of the various phytate degrading enzymes strengthened our understanding of the relationship between the molecular structure and the catalytic functioning of the molecular structure. It is now clear that specific phytases have been established to satisfy the unique nutritional requirements of various forms of life in plants, animals, and microbials (Rastegari et al. 2020). A strong connection between the catalytic domain of an enzyme and unique molecular architectural elements also seems to exist. Although certain structural

Table 12.1 Phytase enzyme and their structural and adaptive features

Enzyme family	Unique structural feature	mechanism/adaptation to hydrolyzes phytate	Example	Reference
Histidine Acid Phosphatase	N-terminal RHGXRXP C-terminal HD consensus motif	N-terminal H forms a phosphohistidine intermediate, C-terminal acts as proton donor/ Substrate specificity site residues positively charged	*A. niger* *P. Lycii* *E. coli* Zea mays L.	Wodzinski and Ullah (1996)
Cysteine Phosphatase	P loop structure contains HCXXGXXR(T/S) consensus motif	Protein tyrosine phosphatase mechanism cleaves phosphate groups/ Deeper active site pocket accommodates phytate	*S. ruminatium*	Chu et al. (2004)
Purple Acid Phosphatase	Consensus motif: DXG/GDXXY/GNH (E,D) /VXXH/GHXH	Metalloenzymes, phylogenetically linked to large plant PAP/unknown	Glycine max *M. truncatula*	Oh et al. (2004)

components are important, certain nonessential parts of the molecule may therefore be altered to adapt the catalytic mechanism to the different substrates. In future research, the exact number of catalytic mechanisms formed to hydrolyze the phytate can be determined. It is now recognized that four phosphatase enzyme groups are representative of phytic acid that can be degraded as described in Table 12.1.

12.2 Phytases of Fungal Origin

The majority of phytases isolated from fungi and yeast, typically known as 3-phytases, are histidine acid phosphatases, glycosylated, and active for a wide variety of substrates (Wyss et al. 1999b). *Aspergillus niger* PhyA was the first phytase to be well characterized and marketable. This enzyme, encoded by a 1.4 kb DNA fragment, is a monomer with an estimated molecular weight of 80 kDa, a bi-hump pH profile with two optimal pH at 2.5 and 5.0–5.5, an optimal temperature at 55–60 °C, and a high phytic acid affinity (Han et al. 1999). *Aspergillus fumigatus* phytase has a sequence similarity of 66% to A. PhyA Phytase niger, however, exhibits greater thermo-tolerance (Pasamontes et al. 1997a; Wyss et al. 1998).

The thermo-tolerance was associated with high refolding efficiency after heat denaturation, and the specificity of the buffers used in heat treatment can be modulated (Rodriguez et al. 2000a). The enzyme has a wide range of pH and is highly active against low phosphorylation inositol phosphates (Wyss et al. 1999b; Rodriguez et al. 2000a). Yet its unique phytate activity is small (Tomschy et al. 2000). PhyA phytase *Peniophora lycii* was also sold out. It is a 6-phytase with optimum pH at 4.0–4.5 and optimum temperature at 50–55 °C and has dimeric conformation (Lassen et al. 2001). It seems vulnerable to thermal and protease treatments

(Simon and Igbasan 2002) or low pH (Quan et al. 2004) isolated a low-molecular-weight (32.6 kDa) phytase from *Cladosporium* sp., an airborne fungus. PS-1.

The enzyme is not glycosylated and has an average 3.5 pH and an average 40 °C temperature. It produces tri-phosphate inositol as the product. Phytases isolated from thermophilic fungi *Myceliophtora thermophila* and *Talaromyces thermophilus* (Mitchell et al. 1997; Pasamontes et al. 1997b) show a high degree of homology of sequence to other fungal phytases *A. niger*, *A. Terreus,* and *A. Fumigate*. Berka et al. (1998) isolated a phytase from the *Thermomyces lanuginosus* thermophilic fungus which showed better thermostability and catalytic performance and a higher transition temperature than the A. Phytase to the niger. Chadha et al. (2004) reported that phytase produced by the *Mucor pusillus* thermophilic fungus was active in a wide pH range of 3–7.8. Nakamura et al. (2000) found substantial levels of phytase activity in 35 species from a survey on 738 strains of yeast, with a wide range of optimal pH and temperature. *Arxula adeninivorans* developed well in media containing phytate as the sole source of phosphate and secreted phytase with an optimum pH of 4.5–5.0, and an optimum temperature of around 75 °C (Sano et al. 1999; Quan et al. 2002) also reported substantial phytase development from soil-isolated yeast *Candida Krusei* WZ-001. The isolated phytase produced two different subunits with 116- and 31-kDa molecular masses, had a glycosylation rate of about 35%, and had optimal pH and temperature at 4.6 and 40 °C, respectively. *Pichia anomala* (Vohra and Satyanarayana 2001), *Saccharomyces cerevisiae* (Türk et al. 2000), and *Schwanniomyces castellii* (Segueilha et al. 1992) also showed phytase activity (Loewus 2002). These enzymes were active in the range of acid pH, with the optimum temperature at 60–74 °C.

Phytats Dietary Effects Salts of phytic acid, designated as phytates, are regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. Phytate is formed during the maturation of the plant seed, and in dormant seeds, it represents 60–90% of the total phosphate (Reddy 2002). Phytate is therefore a common constituent of plant-derived foods. 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 4500 mg (Grases et al. 2000). 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. Potential health benefits of phytate-rich diets. Consumption of phytate, however, does not seem to have negative aspects on human health. Dietary phytate was reported to prevent kidney stone formation (Jariwalla et al. 1990) and to protect against atherosclerosis and coronary heart disease (Vucenik and Shamsuddin 2003) as well as against a variety of cancers (Grases et al. 2001).

The levels of phytate and its dephosphorylation products in the urine, plasma, and other biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet. Therefore, the reduction in phytate intake in developed compared to developing countries might be a factor responsible for the increase in diseases typical for Western societies, such as diabetes mellitus, renal lithiasis, cancer, atherosclerosis, and coronary heart diseases. It was suggested that phytate exerts beneficial effects in the gastrointestinal tract and other target tissues through its chelating

ability, but other mechanisms have also been discussed. Because several myo-inositol phosphates, including phytate, are present as intracellular molecules, and because the second messenger D-myo-inositol(1,4,5)trisphosphate is bringing about a range of cellular functions including cell proliferation via mobilizing intracellular Ca^{2+} (16), phytate was proposed to exert its anticancer effect by affecting cell signaling mechanisms in mammalian cells (Grases et al. 2001). An effect of extracellular phytate on the concentration of several intracellular myo-inositol phosphate esters has already been demonstrated in human erythroleukemia cells (Ferry et al. 2002).

Furthermore, it has recently been reported that highly negatively charged myo-inositol polyphosphates can cross the plasma membrane and be internalized by cells. Myo-inositol hexakisphosphate was shown to enter HeLa cells followed by intracellular dephosphorylation to partially phosphorylated myo-inositol phosphates (Maffucci et al. 2005), whereas turnover of myo-inositol(1,3,4,5,6)pentakisphosphate was quite slow after internalization by SKOV-3 cells (Carrington et al. 1993). In addition, individual myo-inositol phosphate esters have been proposed to be metabolically active. D-myo-inositol (1,2,6), for example, has been studied with respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases (Claxson et al. 1990; Konietzny and Greiner 2003), and due to its antiangiogenic and antitumor effects, myo-inositol(1,3,4,5,6)pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies (Carrington et al. 1993).

12.3 Phytase Adverse Effects

Phytate is an antinutrient that functions as a strongly negatively charged ion in a wide pH range and thus has a considerable affinity to positive-charge food components, such as minerals, trace elements, and proteins (Lopez et al. 2002; Sandberg et al. 1999). This relationship not only has nutritional effects but also affects the yield and consistency of food ingredients such as starch, steep corn liquor, or isolates of plant protein (Sandström and Sandberg 1992; Han et al. 1999; Wang et al. 1999; Fredrikson et al. 2001; Caransa et al. 1988). The main issue about phytate's role in the human diet is its detrimental impact on mineral absorption. In this sense, minerals of concern include zinc, iron, calcium, magnesium, manganese, and copper (Antrim et al. 1997). Since such complexes are basically nonabsorbable from the human gastrointestinal tract, the development of insoluble mineral–phytate complexes at physiological pH values is known to be the main explanation for the poor bioavailability. In addition, owing to the lack of endogenous phytate-degrading enzymes and the restricted microbial community in the upper part of the digestive tract, the human small intestine has only a very limited capacity to hydrolyze phytate (Kvist et al. 2005). Myo-inositol phosphate–mineral complexes have been found to decrease in solubility and stability as the amount of phosphate residues on the myo-inositol ring decreases. The removal of phytate phosphate residues,

therefore, results in a decreased impairment of the intestinal absorption of essential dietary minerals (Cheryan 1980; Iqbal et al. 1994; Sandberg 1991).

Only myo-inositol pentakisphosphate in isolated form inhibited human absorption of iron, zinc, and calcium, while myo-inositol tetrakis and trisphosphates had no effect in the concentrations under investigation. Nonetheless, in the presence of higher phosphorylated myo-inositol phosphates, myo-inositol tetrakis- and trisphosphates have been shown to lead to the phytate's negative impact on iron absorption (Cheryan 1980). Since there has been a clear negative association between zinc absorption and the amount of myo-inositol tris- by hexakisphosphate from cereal and legume meals (Sebastian et al. 1998), such a contribution is also possibly true for zinc absorption. Phytate is well known to form protein complexes at both acidic and alkaline pH (Knuckles and Betschart 1987). This interaction can affect changes in protein structure that may reduce enzymatic activity, solubility of proteins, and digestibility of proteolytics. The importance of protein–phytate complexes in feeding is, however, still under scrutiny. There is clear evidence that phytate–protein interactions have an adverse effect on in vitro protein digestibility, and the degree of this effect depends on the source of the protein. Nonetheless, a negative effect of phytate on the protein's nutritional value was not explicitly established in monogastric animal studies (Desphande and Cheryan 1984).

Although some have indicated phytate does not influence the digestibility of proteins, others have found an increase in the supply of amino acids with declining phytate levels. This disparity may be attributed at least in part to the use of various sources of protein. The inhibition by phytate of digestive enzymes such as a-amylase (Knuckles 1988), lipase (Singh and Krikorian 1982), or proteinases (Desphande and Damodaran 1989; Inagawa et al. 1987; Jenab and Thompson 2002), such as pepsin, trypsin, and chymotrypsin, may also be of nutritional significance as shown in in vitro studies. With the amount of phosphate residues per myo-inositol molecule, and the concentration of myo-inositol phosphate, the inhibitory effect increases. This inhibition may be due to the nonspecific nature of phytate–protein interactions, the chelation of calcium ions necessary for the trypsin and a-amylase function, or the interaction with these enzyme substrates. Protease inhibition may be partially responsible for the decreased digestibility of the proteins. Phytate was also considered an in vivo a-amylase inhibitor as shown by the negative relationship between phytate intake and blood glucose response (D'Souza et al. 1987).

Food rich in phytate has therefore been regarded as having significant nutritional significance in the prevention and management of diabetes mellitus, one of Western society's most common nutrition-dependent diseases. The most serious phytate-attributable effects have occurred as a major dietary variable in populations with unrefined cereals and/or pulses. Deficiencies in zinc and iron have been reported due to high intakes of phytate (Lönnerdal 2000; Maberly et al. 1994). Various approaches have been developed to reduce the risk of mineral deficiency in vulnerable groups, such as child-bearing mothers, strictly vegetarians, inhabitants of developing countries, and fast-growing children. Supplementation with nutritional formulations, food fortification, dietary diversification, and disease prevention (Raboy 2002) is the most known methods for reducing micronutrient malnutrition.

None has been very good for different reasons. An alternative solution would be to increase the overall level of micronutrients in the edible parts of staple crops while at the same time increasing the concentration of compounds that encourage their uptake and/or decrease the amount of compounds that inhibit their uptake, either by plant breeding or by genetic engineering. Low phytate mutants have recently been isolated in maize, barley, rice, and soybeans (Mendoza 2002), and their capacity for enhancing the absorption of iron, zinc, and calcium has been shown (Lucca and Hurrell 2001). To boost rice as an iron source, three proteins were expressed in the rice seed's central endosperm: a *Phaseolus phytoferritin*, an endogenous metallothionine-like protein rich in cysteine, and an *Aspergillus fumigatus* phytase (Coello et al. 2001).

If properly managed, phytase overexpression during seed production may result in reduced levels of phytate in the mature seed (Greiner and Konietzny 2006). Increased levels of seed phytase can also lead to an increase in mineral absorption by decreasing phytate levels in plant-based food during human stomach processing and digestion after a meal is eaten. Furthermore, phytate degradation may be improved during food processing by adding exogenous phytases or modifying optimal conditions for the native plant or microbial phytases. In addition to enzymatic degradation, nonenzymatic phytate hydrolysis during food processing or physical separation of phytate-rich sections of plant seed may lead to reduced phytate levels in the final foods. In general, a loss of valuable nutrients that are either extracted along with the phytate-rich sections of the plant or destroyed by the strong acids or high temperatures needed for nonenzymatic phytate dephosphorylation must compensate for the lower phytate rates. However, enzymatic phytate degradation also occurs under moderate conditions and does not affect other components of food (Egli et al. 2002).

12.4 Enzymatic Phytate Dephosphorylation to Increase Mineral Bioavailability During Food Processing

Different methods of food processing and preparation contribute to a reduction of the phytate content of the raw products. As regards enzymatic phytate dephosphorylation during food processing and preparation, it is important to distinguish the modification of optimal conditions during food processing for the native plant or microbial phytases from the introduction of exogenous ones. For example, phytate hydrolysis during germination, soaking, cooking, and fermentation is a consequence of the naturally occurring phytate degrading behavior in plants and microorganisms. Due to variations in their intrinsic phytate-degrading activities (Viveros et al. 2000; Eeckhout and de Paepe 1994; Konietzny and Greiner 2002) and the properties of enzymes such as protein stability and pH, as well as optimum temperature for phytate degradation (Hurrell 2003), the ability to dephosphorylate phytate significantly differs between different plant and microbial organisms. To understand phytate

hydrolysis, it is important to recognize and account not only for phytase activity but also for other phosphatase activities present in the plant material.

All enzymes capable of dephosphorylated phytate are known as phytases per description. However, the products of phytase activity on phytate, myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates can be further dephosphorylated during food processing by phytases as well as phosphatases that do not accept phytate as a substratum. Phytate is generally not completely hydrolyzed during food processing or preparation by the phytases which occur naturally in plants and microorganisms. However, it has been found that phytate must be reduced to very low levels to greatly increase the bioavailability of minerals, particularly iron (Greiner and Konietzny 1999). Knowing the properties of the natural phytases is important to improve food processing and preparation for phytate degradation. Several cereal phytases (Greiner 2002; Greiner and Konietzny 1998; Hayakawa et al. 1989; Konietzny et al. 1995; Laboure et al. 1993; Nakano et al. 1999; Greiner et al. 2001; Greiner 2002;), legumes (Houde et al. 1990; Mandel et al. 1972; Gibson and Ullah 1988; Nayini and Markakis 1984; de Angelis et al. 2003), and microorganisms used for food fermentation (Greiner and Konietzny 1998, 1999) have been isolated in recent years, and their enzymatic properties have been established. However, the properties of a purified enzyme in a food matrix are not necessarily identical to the properties of the same enzyme. For example, the optimum temperature for phytate dephosphorylation was determined by a phytase of black beans (*Phaseolus vulgaris* var. Preto) as 50 °C for the isolated enzyme and 65 °C for the enzyme in the bean matrix (Fredlund et al. 1997) (Table 12.1).

12.4.1 Soaking

Soaking is also used as a pretreatment to promote the production of grains from the legumes and cereals. Soaking can last for a short time, about 15–20 min, or a very long time, about 12–16 h. Cereals and legumes are usually immersed in water overnight at room temperatures in household circumstances. Since phytate is water-soluble, by discarding the soak water, a significant reduction in phytate can be achieved. Additionally, endogenous phytase activity contributes to the reduction of phytate. Temperature and pH values during soaking (Fredlund et al. 1997; Greiner et al. 1997; Vidal-Valverde et al. 1998) have been shown to have a significant effect on enzymatic hydrolysis of the phytate. If the soaking stage is performed at temperatures between 45 and 65 °C and pH values between pH = 5.0 and 6.0, which are similar to the optimum conditions for phytate dephosphorylation by intrinsic plant phytases, a large percentage of phytate (26–100%) has been enzymatically hydrolyzed (Greiner et al. 1997; Vidal-Valverde et al. 1998; Egli et al. 2002).

12.4.2 Cooking

Since phytate is heat stable, phytate is not expected to cause significant heat loss during cooking. Thus, substantial phytate dephosphorylation during cooking occurs only by either discarding the cooking water or by enzymatic phytate hydrolysis due to the action of intrinsic plant phytases during the early part of the cooking process (Fredlund et al. 1997). Prolonged periods at high temperatures lead to a gradual inactivation of the endogenous enzymes. The supply of heat-stable phytases to plants or the introduction of exogenous heat-stable phytases is therefore possible.

12.4.3 Germination

Germination is a process widely used in legumes and cereals, especially through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors, to increase their palatability and nutrient value. A little intrinsic phytate-degrading activity is found in nongerminated legume grains and cereal seeds, with the exception of rye and to some degree wheat, triticale, and barley (85–88), but a marked increase in phytate-degrading activity was observed during germination, with a concomitant decrease in phytate content (Mandel et al. 1972; Türk et al. 1996; Greiner et al. 2003). During germination, phytate is gradually hydrolyzed by phytases or concerted action of phytases and phosphatases that do not accept phytate as a substratum to supply the plant's nutritional needs without the accumulation of less phosphorylated intermediate myo-inositol. After 6–10 days of germination, phytate levels that resulted in a strong increase in mineral uptake could be achieved. Since there is a need for long periods of time to increase mineral bioavailability by germination, this approach is intended to be useful for household applications, but it does not appear to be an economical industrial food processing method.

12.4.4 Fermentation

Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable features such as extended shelf-life, enhanced safety, attractive taste, nutritional enrichment, removal of anti-nutrients, and health promotion. Many cereals, legumes, and vegetables are used extensively to make a variety of fermented foods. Microorganisms used for the fermentation of food may be part of the normal microflora present in the fermented raw material or specially cultivated crops engineered to bring about changes in the fermented content. Established starter cultures and controlled conditions are widely used today in the fermentation of food. During the fermentation process, the form of microorganism, the fermentation conditions used, and the starting amount of phytate present in

the raw material greatly affect the extent of phytate removal. Major microorganisms for fermentation include lactic acid bacteria, molds, and yeast. For starters, in many countries, yeast and/or lactic acid bacteria are used to produce bread, a staple food. Phytate reduction takes place in the different stages of bread making and naturally depends on the type of bread that is being made. Phytase present in the cereal flour is primarily responsible for phytate dephosphorylation during bread fermentation, whereas the contribution of microbial phytate degrading activity from baker's yeast and lactic acid bacteria is very small or even nonexistent (Leenhardt et al. 2005; Lopez et al. 2000; Sutardi and Buckle 1988).

There is still some debate about the ability of the lactic acid bacteria to produce a phytate-degrading enzyme. Some studies appear to demonstrate the ability of lactic acid bacteria to hydrolyze phytate (Greiner and Konietzny 1999; Fujita et al. 2003), while others have failed to identify a phytate-degrading enzyme (Lopez et al. 2000). Lowering the pH value of the dough to a more suitable one for the operation of the endogenous cereal phytases is therefore very likely the contribution of the microorganisms during fermentation to phytate hydrolysis. However, there is convincing evidence in Oriental food fermentation that phytases of the micro-organisms used for fermentation contribute significantly to phytate degradation (Fujita et al. 2003; Konietzny et al. 1995). Food products such as tempeh, miso, koji, and soy sauce are produced by fermenting soybeans with, respectively, *Rhizopus oligosporus* and *Aspergillus oryzae*. Both molds have been shown to develop phytate-degrading activity intra- as well as extracellular (Kerovuo et al. 1998).

12.5 Isolated Phytases and Food Processing in Recent Times

It has been shown that adding a phytase preparation during food processing is an alternative to maximizing phytate dephosphorylation by enzymes already present in the raw material used in food processing. The effectiveness of supplementary phytase in reducing phytate content during food processing was demonstrated for cereals as well as for food products derived from legumes (Greiner et al. 1997; Shimizu 1992), and even full phytate degradation was demonstrated to be feasible. During food processing, the level of phytate hydrolysis is influenced by the raw material used, the manufacturing process, the source of phytase, and the amount of added enzyme activity. There is no suitable phytase for all diet applications. The added phytase must be highly active when processing or preparing food. Since temperature and pH value are the major factors deciding enzyme activity, high phytate degrading capability even at room temperature, appropriate heat resistance, and high activity over a broad pH range are beneficial properties for phytase that should be used in food processing (Kim et al. 1998; Jog et al. 2005; Yang et al. 1991; Wyss et al. 1999a; Greiner et al. 1993; Vohra and Satyanarayana 2002). The activity of the enzymes increases with temperature to the limit. A further rise in temperature results in enzyme denaturation which is heat-induced. The optimum temperature for phytate hydrolysis ranges from 35 to 80 °C, depending on the source of the enzyme.

Plant phytases generally exhibit maximum activity at lower temperatures as opposed to their microbial equivalent. The higher pH and thermal stability as well as the higher specific microbial activity relative to plant phytases make the former more suitable for food processing applications. Specific activity is a crucial factor in the commercial use of an enzyme, as it affects the expected use on the economy. Thus microbial phytases appear to exhibit higher specific activities compared to plant phytases. The stability of most plant phytases decreased significantly at pH values below pH = 4 and above pH = 7.5, while most of the corresponding microbial enzymes even at pH values above pH = 8.0 and below pH = 3.0 are very stable. For example, when exposed to 4 °C for 2 h (Segueilha et al. 1992), a phytase from *Escherichia coli* did not lose any activity at pH = 2.0 and pH = 10.0. Most plant phytases are irreversibly inactivated within minutes at temperatures above 70 °C while most of the corresponding microbial enzymes maintain significant activity even after extended incubation periods. *Pichia anomala* (Doekes et al. 1999), *Schwanniomyces castellii* (O'Conner et al. 2001), and *Lactobacillus sanfranciscensis* (Greiner and Konietzny 1999) have isolated the phytases most resistant to high temperatures reported so far. Incubation of these enzymes at 70 °C for 10 min did not result in significant loss of activity, and it was even confirmed that the phytase of *Pichia anomala* tolerated a 30-h treatment at 70 °C without any loss of activity (Doekes et al. 1999).

It is of practical interest for the technical application of phytases in food processing that a crude enzyme preparation as well as an enzyme present in a food matrix are more pH- and heat-resistant than the corresponding highly purified enzyme. Although microbial phytases are best suited for a food processing application, cereal and legume phytases are thought to be an alternative due to their higher consumer acceptance and their presumed low allergenic potential. Phytases that are found in cereals and legumes are already part of the human diet and none of them have been reported to be an allergen. Conversely, *Aspergillus niger* phytase was thought to be a high-risk factor for occupational asthma and rhinitis. The enzyme has been demonstrated to trigger unique immune responses to IgE among workers exposed to powered phytase preparation (Baur et al. 2002; Rodriguez et al. 2000; Garrett et al. 2004). The phytase preparations of *Aspergillus niger* are commercially available, making their use technically feasible in the food processing industry (Abdel-Azeem et al. 2021; Yadav et al. 2019a, b). Since phytases with the properties needed for food processing applications have so far not been found in nature, phytase engineering is seen as a promising strategy to optimize their catalytic features.

Improving thermal tolerance and rising specific activity are two important issues not only for animal feed but also for phytase applications for food processing. To obtain an enzyme capable of withstanding higher temperatures, various techniques were employed. After the introduction of three glycosylation sites into the amino acid sequence of the *Escherichia coli* phytase by site-directed mutagenesis (Lehmann et al. 2002), a shift in the optimum temperature of the *Escherichia coli* phytase from 55 to 65 °C and a significant increase in its thermal stability at 80 and 90 °C was achieved by expression of the enzyme in the yeast *Pichia pastoris*. A further technique used to improve the efficiency of the *Escherichia coli* phytase

(Tomschy et al. 2000) was the saturation mutagenesis technology of the gene site. A library of clones incorporating all 19 possible changes in amino acids in the 431 residues of the *Escherichia coli* phytase sequence was generated and screened for mutants showing increased thermal tolerance.

When exposed to 62 °C for 1 h and 27% of its initial activity after 10 min at 85 °C, the best mutant displayed no loss of activity, which is a major improvement over parental phytasis. Additionally, there has been a 3.5-fold enhancement in gastric stability. By using a consensus approach based on the comparison of amino acid sequences of homologous proteins and the subsequent calculation of a consensus amino acid sequence using one of the standard programs available, a fully synthetic phytase was generated, showing an increase in intrinsic thermal stability of 21–42 °C compared to the 19 parental fungal phytases used in its design (Tomschy et al. 2000) In addition, a threefold increase in specific activity was achieved by replacing one single amino acid in a fungal phytase sequence with site-directed mutagenesis (Miksch et al. 2002; Mayer et al. 1999). Finally, a phytase will not be efficient if an inexpensive device cannot produce it in high yield and purity. Due to the small amount of phytase obtained from wild-type organisms and their tedious and cost-intensive purification, wild-type organisms are not an appropriate source of enzymes for industrial applications. This has led to the development of highly efficient and cost-effective processes to produce phytase by recombinant microorganisms. The use of economically efficient expression/secretion systems for *Escherichia coli* (Yao et al. 1998) as well as for the yeasts has identified high rates of phytate-degrading activity accumulating in the fermentation media Hansenula (Kerovuo and Tynkkynen 2000) and *Pichia pastoris* (Haraldsson et al. 2005). There is no need for protein purification if phytate-degrading capability is introduced or increased in microorganisms used for food fermentation, such as *Saccharomyces cerevisiae*, *Lactobacillus sanfranciscensis*, or *Lactobacillus plantarum*. Improved use of microorganisms in the fermentation of raw material extracted from plants is expected to result in food products with substantially lower levels of phytate. A genetically modified phytase-secreting strain of *Lactobacillus plantarum* has recently been reported (Haros et al. 2001), but the levels of secretion were far too low for an industrial application. In addition, a *Saccharomyces cerevisiae* strain was constructed which produces high levels of extracellular phytase activity (Sandberg et al. 1996), but its ability to contribute significantly to phytate hydrolysis during fermentation needs to be studied first.

12.6 Isolated Phytase Applications in Food Production

In addition to improving the bioavailability of the mineral and trace elements, phytase addition during food processing has been documented to affect the economy of the production process as well as the yield and quality of the end products. Technical advances have been recorded by the introduction of phytase during food processing for bread making (Pasamontes et al. 1997), plant protein isolate production

(Sandström and Sandberg 1992; Han et al. 1999), maize wet milling (Wang et al. 1999; Fredrikson et al. 2001), and cereal bran fractionation (Caransa et al. 1988).

12.6.1 Bread Making

Phytase proved to be an outstanding improver in bread making (Pasamontes et al. 1997). In addition to reducing phytate content in doughs and fresh breads, phytase addition reduced fermentation time without affecting the pH of the dough. An increase in bread volume and an improvement in crumb texture were also observed. For both formulations, the breadcrumbs' hardness or firmness was popular so that softer crumbs were obtained with the addition of phytase. Certain criteria of texture such as gumminess and chewiness were also reduced. It has been proposed that these changes in bread consistency are consistent with an indirect effect of phytase on a-amylase activities. In the final breads, the introduction of phytase during bread making results in lower levels of phytate. Even so, a complete phytate removal was not attainable. This, in effect, releases calcium ions from calcium–phytate complexes that are important for a-amylase action. No phytase activity could be found in final breads. Therefore, both intrinsic cereal and augmented microbial phytases were inactivated during baking.

12.6.2 Generation of Isolated Plant Proteins

The application of plant protein isolates and concentrates has been finding increasingly important in food production because of their strong nutritional and functional properties. Nonetheless, the relatively high phytate content present in plant seeds and grains and their association with proteins under alkaline conditions, which are typically applied for protein extraction, adversely affect the yield and quality of the protein isolates obtained using standard production processes. The solubility of the proteins decreased by interacting with the phytate resulting in decreased protein content in the final concentrate. Therefore, a considerable amount of phytate ends up in the protein isolate which affects both its nutritional and its functional properties.

However, it was confirmed that the introduction of exogenous phytase into the production process resulted in significantly higher protein yields and almost complete removal of myo-inositolhexakis-, pentakis-, tetrakis-, and trisphosphates from the final plant protein isolate (Sandström and Sandberg 1992; Han et al. 1999). Such phytate-reduced plant protein isolates have been proposed as ideal protein sources for infant formulae due to an increase in mineral bioavailability, their amino acid composition, and their in vitro protein digestibility. In addition, certain phytate-reduced plant protein isolates are addressed in food products as functional additives, due to their strong properties of foaming, emulsifying, and gelling.

12.6.3 Wet Corn Milling

Steeping is a process needed to obtain the valuable corn steep liquor and soften the maize kernel as well as break the maize cell wall in wet maize milling. Starch yield, corn steep liquor consistency, and steeping time are the main issues of corn wet milling. Maize consists of phytate, which in large measure ends up in the steep liquor of corn and constitutes an undesirable portion. Phytate-free corn steep liquor is easier to absorb and is used in the fermentation industry to manufacture compounds such as enzymes, yeast, polysaccharides, antibiotics, and amino acids as well as a high-energy liquid feed product for animals. Through adding phytases to the steep liquor along with plant cell wall degrading enzymes, corn steep liquor was obtained which was completely free of phytate (Wang et al. 1999; Fredrikson et al. 2001). However, the steeping time was substantially decreased which was accomplished by promoting the separation of starch from fiber which gluten, higher starch and gluten yields, and lower energy consumption.

12.6.4 Cereal Branch Split

It is commonly recognized and accepted that the cereal bran, the by-product of flour production, is the most nutritious component of a grain of cereals. Recently, an industrial method was created to isolate economically the main branch fractions to produce high-value protein, soluble non-starch carbohydrates, oil fractions, and insoluble fibers (Antrim et al. 1997). The bran is first subjected to a combination of enzymatic treatment using starch- and phytate-hydrolyzing enzyme group proteins and wet milling, accompanied by concurrent centrifugation and ultrafiltration. The second step is to fractionate the insoluble phase of the above-mentioned first step by enzymatic treatment with xylanase and/or b-glucanase and wet milling, followed again by sequential centrifugation and ultrafiltration. All fractions obtained have much broader business applications and higher values than the branch initial.

12.7 Phytase Degradation in Human Body

Phytate hydrolysis in the human gastrointestinal tract can be achieved by the action of phytate-degrading enzymes from three sources: dietary phytases, small intestine mucosal phytases, and bacterial flora phytases in the colon. Except for calcium, phytate degradation in the colon is not expected to greatly affect mineral absorption, as minerals are mainly absorbed in the upper small intestine. In addition, it has been shown that only very low phytate-degrading activity exists in the small intestine of humans.

The human small intestine, therefore, has a very limited ability to hydrolyze phytate. In comparison, dietary phytases are an essential factor for phytate degradation during digestion because these enzymes are involved in the stomach of humans (Berka et al. 1998).

Generally speaking, the intrinsic phytate-degrading activity in plant-derived foods is not strong enough to hydrolyze the dietary phytate during the passage through the human stomach to such a degree that there is a substantial increase in iron absorption. The production of plants with higher phytase-degrading activities in the edible parts or the application of phytase preparations to the raw materials or the final foods may lead to more extensive phytate degradation in the human stomach. Such phytases should be effective in releasing phytate phosphate into the human stomach, stable to resist inactivation by storage, and may also be suitable to withstand food processing and preparation. Thermal stability is a concern of special significance as food processing and preparation typically require exposure to high temperatures. Until now, phytases with the necessary degree of thermal stability to withstand thermal treatments such as isolated cooking or within a certain food matrix have not been found in nature. It is therefore no surprise that isolation and characterization of thermostable enzymes, as well as engineering phytases, are hot spots of current phytase work (Phillippy 1999; Kim et al. 2003; Lehmann et al. 2002; Tomschy et al. 2000) to boost stability at elevated temperatures and the quest for determinants of thermal stability. Likewise, it is undisputedly desirable to have a phytase that can withstand long-term storage or transport at ambient temperature.

The enzymatic properties of a phytase are determined by its ability to hydrolyze phytate in the digestive tract. Since the stomach is the key functional site of dietary and/or supplementary phytase, it is beneficial to provide an enzyme with optimum acid pH, good stability under acidic pH conditions, and good pepsin resistance. Microbial phytases are thought to have advantages over their plant counterparts regarding their phytate-degrading ability in the human stomach. Microbial phytases demonstrate substantial enzymatic activity over a wide range of pH and are also active below pH = 3.5. Additionally, the stability of certain microbial phytases below pH = 3.0 is noteworthy. Additionally, plant phytases are more susceptible to gastrointestinal enzyme inactivation. It was confirmed that wheat phytase was less resistant to pepsin and pancreatin than *Aspergillus niger* phytase (Simon and Igbasan 2002) and that the phytases of *Escherichia coli* and *Citrobacter braakii* were even more resistant to pepsin and pancreatin than the *Aspergillus niger* phytase (Rodriguez et al. 1999).

Furthermore, *Citrobacter braakii* phytase was stable to trypsin (Rodriguez et al. 1999). The corresponding enzyme from *Bacillus subtilis* demonstrated a comparable vulnerability to pancreatin compared with the *Escherichia coli* phytase but a much higher resistance to pepsin (Haros et al. 2005). As recently recorded for the *Escherichia coli* and *Aspergillus niger* phytase produced in *Pichia pastoris* (Blanquet et al. 2004), it must also be noted that recombinant enzymes can differ in proteolytic resistance compared to their wild-type counterparts.

Quite recently (Sandberg et al. 1996), a radically different approach to enhancing phytate degradation in humans' stomach and upper small intestines was brought up. Healthy for human consumption microorganisms such as baker's yeast (Saccharomyces cerevisiae), lactobacilli, or bifidobacteria have been proposed as carriers of phytate reducing activity throughout the gastrointestinal tract. Tolerance to the conditions in the stomach and small intestine and the ability to produce extracellular phytate-degrading activity under gastrointestinal conditions are therefore characteristics that microorganisms require for such an application. *Saccharomyces cerevisiae* (Shears 1998), as well as some strains of Lactobacillus and Bifidobacterium (Vucenik and Shamsuddin 2003), has also demonstrated the ability to survive the passage across the human gastrointestinal tract.

Neither for *Saccharomyces cerevisiae* nor for any *Lactobacillus* or *Bifidobacterium* strains, however, was a sufficiently high extracellular phytate degrading activity demonstrated. Genetic engineering may be used to solve this restriction for the development of high-recombinant, phytase-producing strains. To be able to hydrolyze the dietary phytate, the microorganisms must secrete this phytase or attach it to its outer cell wall. Two separate strategies for improving the production and secretion of phytase in the target microorganisms were successfully applied. A *Bacillus subtilis* phytase-encoding gene was inserted into a *Lactobacillus plantarum* strain, but the secreted phytate degrading activity was far too small for any application (Haros et al. 2001).

Nevertheless, in yeast, the regulation of phytase synthesis has been changed by removing a gene encoding a negative regulator for the phytase-encoding genes to express themselves. Compared with the corresponding wild-type yeast, the recombinant yeast exhibited multiple-fold higher phytate hydrolysis capacity, both in the presence and in the absence of orthophosphate (Sandberg et al. 1996). However, recombinant yeast was shown to degrade up to 40% of the phytate present in wheat gruel under simulated gastric conditions, while with wild-type yeast no phytate hydrolysis was observed. Studies on in vivo are still lacking, however. Since yeast phytase at pH values above pH = 7 is practically inactive, this phytase will only be active in the human stomach, and no further phytate degradation is expected to occur in the small intestine. Thus, using one or a mixture of food-grade microorganisms with both characteristics, secretion of an optimally active phytase at acidic and another optimally active at alkaline conditions may increase phytate breakdown in the human stomach and upper small intestine during digestion with a concomitant improvement in the bioavailability of mineral products.

12.8 Production of Metabolically Active Phytate Breakdown Products

In recent years, a great deal of scientific evidence has been published connecting diet, foods, or individual food components with preserving human health and preventing chronic diseases such as coronary heart disease, cancer, or osteoporosis. Specific phosphate esters with myo-inositol have been shown to have essential physiological functions in humans (Brinch-Pedersen et al. 2007).

Several of these compounds, in particular D-myo-inositol(1,4,5)trisphosphate and D-myo-inositol(1,3,4,5)tetrakisphosphate, have been shown to play an important role as secondary intracellular messengers (Greiner and Konietzny 1996), and some isomers of myo-inositol phosphates have demonstrated major pharmacological effects, such as complications of diabetes and anti-inflammatory effects as well as an anti-inflammatory effect (Claxson et al. 1990; Konietzny and Greiner 2003). In addition, it has been proposed that dietary myo-inositol phosphates offer benefits for human health, such as improving heart disease conditions by regulating hypercholesterolemia and atherosclerosis (Vucenik and Shamsuddin 2003), preventing the development of renal stone (Jariwalla et al. 1990), and protecting against a variety of cancers, especially colon cancer (Grases et al. 2001).

Phytate can be partially dephosphorylated during food processing and digestion to produce many positional isomers of pentakis, tetrakis, tris, bis, and monophosphates in myo-inositol (Singh et al. 2020). The number and distribution of the residues of phosphates on the myo-inositol ring determine the metabolic effects caused by the individual phosphate isomer of myo-inositol. Different phytases exhibit varied pathways of phytate degradation, resulting in the generation and accumulation of various intermediate myo-inositol phosphate. Nonenzymatically, attempts to produce specified isomers of the various partially phosphorylated myo-inositol phosphates have resulted in mixtures of pentakis, tetrakis, tris, bis, and monophosphate isomers. The purification from the mixture of these isomers is arduous and uneconomical. An alternative approach to making phytate pure breakdown products available in appropriate amounts for physiological studies is the use of a bioreactor based on immobilized enzymes followed by the hydrolysis mixture anion-exchange chromatography. The quantity of the desired product for phytate degradation may be regulated by the number of phytate-containing solution passing through the bioreactor (Sandberg et al. 1987).

When individual phytate degradation products are known to be metabolically active, phytases can be used in food processing to produce foods with enhanced nutritional value, health benefits, and sensory (functional foods) properties. Through attaching phytase to the raw material, phytate is converted during food processing into metabolically active myo-inositol phosphates. To end up with foods with a reduced phytate content and a regulated content and composition of partially phosphorylated myo-inositol phosphate esters with health benefits, phytate dephosphorylation must be closely managed during food processing. An alternative may be

using pure phytate as the source material to produce metabolically active myo-inositol phosphates as food supplements.

Since partially phosphorylated myo-inositol phosphate esters are subject to degradation in the human gastrointestinal tract even if all dietary phosphatases like phytases are inactivated, the desired physiological effects may need to be stimulated by enriching foods with a precursor of the true active myo-inositol phosphate ester. It has already been demonstrated in ileostomy patients (Sandberg and Andersson 1988; Sandberg et al. 1987) that the human intestinal alkaline phosphatase exhibits activity against lower myo-inositol phosphate esters and that the microflora in the human colon is also considered capable of degrading phytate and phytate breakdown items. Myo-inositol phosphates must be consumed in the gastrointestinal tract to achieve their metabolic effects in tissues far away from the food tract. There is some evidence that the human digestive tract consumes myo-inositol phosphates since the levels of phytate and its dephosphorylation products in biological fluids fluctuate with ingestion or phytate deficiency in the human diet (Shamsuddin et al. 1992).

12.9 Conclusion

Phytases, the most significant enzyme in nutrition, human health, and environmental protection, are helpful in the elimination of phytate in the human stomach and upper small intestine during food processing. Surprisingly, in mankind phytase enzyme is reduced. In conclusion, research is required to classify metabolically active isomers and phytases of myo-inositol phosphate or a mixture of phytases and/or phosphatases without phytate-degrading activity able to produce such isomers. There isn't one phytase suitable for all food applications. Thus, scanning nature for phytases with more desirable properties for food applications and engineering phytases to improve their catalytic and stability characteristics are appropriate approaches to make a suitable phytase available for a particular food processing application. The phytases can be used in isolated form or generated at high levels in recombinant microorganisms used for the fermentation of food and/or in recombinant plant edible pieces. If the intrinsic phytases present in the material to be processed are to be used during food processing for phytate dephosphorylation, their catalytic properties must be elucidated to maximize phytate degradation with respect to the intended use of the product.

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