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Industrial applications of phytases

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Abstract
Phytases have a profound role in animal feed and various food industries due to the non-availability of phosphorus to the animals present in the feed, along with improving the digestion and absorption of the phosphorus and certain other poorly available nutrients. Especially, phytate-degrading enzymes from microorganisms offer technical and economical feasibility for their production and application. Phytates have been considered as a threat in human diet due to its antinutrient behaviour which is known as strong chelator of divalent minerals such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}. Biotechnologically produced phytases may be possible for use tomorrow in food processing as well as plant raw material and starter cultures with inserted phytase genes. Potential uses of phytases in various industries mainly food industry has been emphasized in this paper.

Keywords: Phytase, antinutrients, food processing, mineral absorption, phytic acid.

1. Introduction

Inositol hexakisphosphate (InsP6), commonly known as phytate, is a major component of plant Storage organs such as seeds, roots and tubers, where it serves as a phosphate source for germination and growth (Kuhar *et al*, 2008) [14]. 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 (Dahiya and Singh, 2009) [5]. At the same time, phytate may have beneficial roles as an antioxidant, anticarcinogen and more (Jenab and Thompson, 2002) [13]. Reduction of phytates can be achieved through both enzymatic and nonenzymatic removal. Food processing techniques increasing the activity of the naturally occurring plant phytases are soaking, malting, hydrothermal treatment and fermentation. Because of the interest in the use of microbial phytate-degrading enzymes in feed applications, highly efficient and cost-effective processes for their production by recombinant microorganisms have been developed. The phytase required for commercial feed preparation must meet certain technical specification of being thermo stable with high specific activity (Dahiya and Singh, 2014) [6]. In addition it must be active over a wide ph range, as the ph of the stomach in finishing and weanling pigs, poultry and aquaculture varies from highly acidic to neutral. Microorganisms producing phytases are all mesophiles, with the exception of thermophilic fungi, *Thermomyces lanuginosus, Talaromyces thermophilus and Sporotrichum thermophile.* The optimum temperature for the production of phytases from most of the microorganisms lies in the range 25 to 37 °C. The pH has also a profound effect on the production of the enzyme. For phytase production, the optimum pH of most bacteria and fungi is in the range between 5.0 and 7.0. The phytases must also be resistant to proteolysis and show in vitro broad substrate specificity. The active sites of phytases typically contain ionisable groups (Arg, Lys, His, Glu, Asp) that are involved in substrate or product binding and/or catalysis and that determine the pH activity profile of an enzyme.

2. Application of Phytase enzyme in multidisciplinary fields

1. Animal feed industry

Monogastric animals such as humans, chickens, and pigs produce little or no phytase in the intestine. This requirement of phosphorus is met by supplementing soybean and other meals with relatively inexpensive rock phosphate, which provide the animal with this necessary nutrient. Phytic acid present in the manure of these animals is enzymatically cleaved by soil and water-borne microorganisms. The phosphorus thus released is transported into the water bodies causing eutrophication (Bali and Satyanarayana, 2001) [2]. This results in oxygen depletion due to excessive algal growth. The availability of phosphorus can be improved by adding microbial phytase to the feed or by using phytase-rich cereal diet (Dahiya and Singh, 2014) [6]. The enzyme minimizes the need for supplementation with inorganic phosphorus due to improvement in the utilization of organic phosphorus in poultry, and thus markedly reducing the excretion of phosphorus in manure (Bali and Satyanarayana, 2001) [2]. Phytase hydrolyzes phytate, and the addition of phytase to feed (250 to 1000 U/kg) can fully replace phosphorus supplementation (Golovan *et al*., 2001). To properly formulate diets with phytase and to realize its full potential, the exact value of the nutrients it releases must be investigated.

1. Recently concluded research projects indicate that the nutrient values for phytase for phosphorus, calcium, amino acids and the dietary components that produce energy can be used in diet formulation for broilers fed from hatch to slaughter with no negative effects on growth performance, carcass traits or meat quality.
2. Adding phytase decreases the amount of total and soluble phosphorus in the litter, which has positive effects on the environment when poultry litter is used as fertilizer.
3. Phytase may reduce the cost of the diet by reducing the amount of soybean meal, fat and crystalline amino acids that must be added.
4. Research also has indicated that adding phytase to nutritionally adequate diets for young chicks' increases growth performance. Chicks fed phytase ate more and thus gained more weight regardless of the nutrient content of the diet. Results from recent research have indicated that the increase in daily feed intake may be explained by an increase in feed transit time (time from ingestion to excretion) in chicks fed diets with phytase (Boling *et al*., 2000) [3].
5. Phytase has been shown to increase the availability of some trace minerals, including copper, manganese, iron and zinc. Because of the positive effect of phytase on trace mineral utilization, commercial use may lead to removing trace minerals in diets where phytase is added (Chantasartrasamee *et al*., 2005) [4].
6. Phytase may have positive effects on quality of retail cuts of pork, because adding phytase (more specifically, reducing inorganic phosphorus levels in diets with phytase) decreased the amount of water lost from dripping, thawing and cooking.
7. The effect of phytase on energy and amino acid availability is a much-debated topic. Research results from several experiments have indicated that phytase improves the availability of amino acids, starch and the dietary components that produce energy in diets for growing pigs.

New phytase products are being produced for use in animal agriculture, and one area of research is to evaluate each product to see how they compare with one another. Two of these phytase products are Ronozyme and Natuphos. Research at the LSU AgCenter has shown that adding phytase to swine and poultry diets has positive effects on calcium, phosphorus, amino acid, trace mineral digestibility and the dietary components that provide energy. These positive effects can be achieved without affecting carcass composition or meat quality. Furthermore, by taking advantage of all of the positive aspects of phytase, producers can reduce the negative effects of animal waste on the environment (by reducing the nutrient content of manure used as fertilizer) and reduce diet cost by reducing the levels of some of the high-cost feed ingredients.

2. Food industry

There is a great potential for the use of phytases in processing and manufacturing of food for human consumption. Research in this field focuses on the improvement of the nutritional value of plant-based food as well as on the technical improvement of food processing (Dahiya and Singh, 2009) [5]. A diet rich in phytate leads to a considerably reduced absorption of dietary minerals and the dephosphorylation of phytate during food processing results in the formation of only partially phosphrylated myo-inositol phosphate esters with a lower capability to impair with the intestinal uptake of dietary minerals. Individual myo-inositol phosphate esters have been shown to have several important physiological functions in man (S.B. Shears 1998). Therefore, phytases may find application in food processing to produce functional foods (Greiner and Farouq, 2007) [9], if such bio-chemically active myo-inositol phosphate esters could be generated by phytases and absorbed in the alimentary tract of humans. Technical improvements by adding phytases during food processing have been reported for bread making (Haros *et al*., 2007) [10], production of plant protein isolates (Fredrikson *et al*., 2001) [7], corn wet milling (Antrim *et al*., 1997) [1] and the fractionation of cereal bran (Kvist *et al*., 2005) [15].

2.1 Bread making

Phytase was shown to be an excellent bread making improver (Haros *et al*., 2007) [10].

Some of its advantages are as follows:

1. Reduction of phytate content in dough & fresh breads
2. Shortening of formulation time without any change in pH.
3. Increase in bread volume and an improvement in crumb texture.
4. Softer bread crumbs were obtained.
5. Other texture parameters like gumminess and chewiness were also decreased.

All these improvements in bread quality were suggested to be associated with an indirect import of phytase on α-amylase activity; also, it lowers phytate levels in the final breads, which in turn, releases Ca^{2+} ions, essential for α - amylase activity, from calcium-phytate complexes.

2.2 Production of plant protein isolates

Due to their good nutritional properties, the application of plant protein isolates and concentrates has been found increasingly interesting in food production Phytate under alkaline conditions, interacts with proteins and negatively affects the yield and quality of the protein isolates in the find concentrate. Exogenous phytase application into the production process, however, was reported to result in significantly higher protein yield and an almost complete removal of myo-inositolhexakis-, pentakis-, tetrakis-, and triphosphates from the final plant protein isolate (Fredrikson *et al*., 2001) [7]. Due to an improvement in mineral bioavailability and their *in vitro* protein digestibility, these phytate-reduced protein isolates were suggested as suitable protein sources for infant formulae and also these are discussed as functional additives in food products, because of their good foaming, emulsifying and gelling properties.

2.3 Corn wet milling

Maize comprises phytate, which ends up in the corn seep liquor and constitutes an undesirable product. By adding phytases to the steep liquor, corn steep liquor that was entirely free from phytate was obtained (Antrim *et al*., 1997) [1] and also steeping time was reduced alongwith the separation of starch from fibre and gluten. This phytate free liquor is easier to concentrate and is used in fermentation industry for the production of enzymes, antibiotics, amino acids as well as a high-energy liquid animal feed ingredient.

2.4 Fractionation of cereal bran

Cereal bran, by product of flour production, is the most nutritious part of a cereal grain. It is fractionated sequentially, to obtain fractions having broader market applications and greater value than the original bran. Firstly, the bran is subjected to a mixture of starch- and phytate- hydrolysing enzymes and wet-milling, followed by centrifugation and ultrafiltration. Secondly, the insoluble phase obtained after first step was treated with xylanase and/or ß-glucanase and wet milling, followed by centrifugation and ultra-filtration (Kvist *et al*., 2005) [15].

3. 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) [19]. Hirimuthugoda *et al.,* (2007) [12] have isolated a novel microbial marine phytase from the gastrointestinal tract of sea cucumbers, *Holothuria scabra*. Industrial application of this species is limited, although extracted phytase can be used as an industrial product for digesting phytate phosphorous as well as a probiotics form.

Recently Yuzhi Miao *et al*., (2013) [16] engineer lactic acid bacteria to produce the enzyme phytase from a gene native to *Bacillus subtilis* GYPB04. The phytase gene (*phyC*) of *B. subtilis* GYPB04 was cloned into the plasmid pMG36e for expression in *Lactococcus lactis.* The results of this study may be used in the dairy fermentation industry for the development of functional, healthy yogurts and other fermented dairy foods that provide both active phytase and viable probiotics to the consumer.

3.1 Future trends

Despite considerable economic interest only limited data on the catalytic properties of about a dozen microbial phytases, including bacteria, fungi and yeast is available of these two fungal phytases, one from a deuteromycetes A*spergilus. ficuum* and other from a basidomycete*, Peniophora lycii*, have recently been commercialized. Further active research must, therefore, be directed for identifying new native phytase proteins from diverse micro flora and plant that would form the basis for creating consensus phytase using genetic and protein engineering approaches. Optimization of catalytic properties has been approached in the past mostly on a trial-and-error basis by random mutagenesis. Most recently, technological advances have paved the way for several direct evolution approaches, i.e. rapid, iterative processes of mutation and/or recombination of genes and selection or screening of improved protein variants. Furthermore, by replacing a considerable part of the active site of the generated enzyme with the corresponding residues of the phytase enzyme of *A. niger* NRRL 3135 a shift in catalytic properties was observed, demonstrating that rational transfer of favorable catalytic properties from one phytase enzyme to another is possible by using this approach. It is evident that the inability of plants to utilize phosphorus from soil phytate is associated with a lack of extra cellular phytase activity (Hayes *et al*., 2000) [11]. Thus, an opportunity exists for using gene technology to improve the ability of plants to utilize phytate phosphorus. Extra cellular secretion of the phytate- degrading enzyme from *A. niger* from plant roots was shown to enable the plants to obtain phosphorus from phytate A more effective utilization of phosphorus from soil and fertilizer sources would be particularly beneficial to agriculture throughout the world.

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