



JOURNAL OF SCIENCE, TECHNOLOGY AND EDUCATION (JSTE)

**A PUBLICATION OF THE
DEPARTMENT OF SCIENCE,
TECHNOLOGY & MATHEMATICS
EDUCATION (STME),
NASARAWA STATE UNIVERSITY, KEFFI**



**VOLUME
9**

ISSN: 2651-5539

ADDRESSING MICRONUTRIENT DEFICIENCY: THE ROLE OF PHYTASE ENZYME IN MITIGATING ANTINUTRITIONAL EFFECTS DUE TO PHYTIC ACID

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Citation: Ohaegbu, C. G., Achi, O. K., Nwachukwu, E., Achi, N. K. (2025). Addressing micronutrient deficiency: The role of phytase enzyme in mitigating antinutritional effects due to phytic acid. *Journal of Science, Technology, and Education (JSTE)*; www.nsukjste.com/ 9(9), 109-122

Abstract

Micronutrient deficiency remains an international health difficulty, particularly in developing areas where plant-primarily based diets predominate. Phytic acid, a commonplace anti-nutritional component in cereals, legumes, and nuts, drastically impairs the bioavailability of important minerals along with iron, zinc, magnesium, and calcium, by forming insoluble complexes. This exacerbates micronutrient deficiencies, mainly among inclined populations. Phytase, an enzyme capable of hydrolyzing phytic acid, is important in enhancing mineral bioavailability. Additionally, Lactic Acid bacteria (LAB) widely used in

fermentation enhance the degradation of phytic acid through the production of natural acid, and in a few instances intrinsic phytase activity. This paper explores biochemical mechanisms, sources, and applications of phytase, highlighting its position in improving dietary mineral absorption. This comprehensive evaluation integrates biochemical insights and realistic packages to deal with phytic acid's challenges and harness phytase's advantages for enhancing worldwide dietary results.

Keywords: Phytic acid, micronutrient, deficiency, phytase, phytate.

Introduction

Micronutrient deficiencies, specifically of vital minerals inclusive of iron, zinc, and calcium, remain a considerable public health concern affecting populations dependent on plant-based foods. A chief contributing factor is phytic acid, the form of phosphate, observed in all plant-primarily based foods (Kumar *et al.*, 2010).

Phosphorus is an important nutrient for animals as it's far found in plant seeds and feeding plants, however, it is not digested by humans or monogastric animals. It interacts with critical molecules including iron, zinc, magnesium, calcium, and protein, to supply a salt known as phytate (Kumar *et al.*, 2010). Von Grebmer *et al.* (2014) identified phytic acid as a negatively

charged Phytate in mono-, di- or trivalent metal salts. This is due to the various affinity of phytic acid protons for dissociation to their pH range inside the stomach and small intestine (Nagar *et al.*, 2021). Phytic acid salts, referred to as phytate or myoinositol hexakisdi-hydrogen phosphate, are the number one storage form for both myoinositol and phosphate in oilseeds, legumes, and cereal grains (Priyodip and Balaji, 2018). Due to their low solubility within the upper gastrointestinal tract's pH range, where most minerals are absorbed, these salts tend to precipitate as pH increases (Rutherford *et al.*, 2014).

The unfavorable consequences of phytate inside the human food plan are normally due to its decreased mineral absorption (Ca^{2+} , Mg^{2+} , Fe^{2+} , Zn^{2+} , Cu^{2+} , and Mn^{2+}). The activation of intracellular and extracellular enzymes, pH regulation in bodily fluids, and maintaining the balance between cells inside their surroundings, all depend on these minerals. Inadequate mineral availability in the human gastrointestinal tract is due increased formation of insoluble mineral-phytate complexes (Konietzny and Greiner, 2003; Kumar *et al.*, 2021).

The inability of monogastric animals like swine and poultry to hydrolyze the substrate through the absorption of phytate phosphorus necessitates the addition of inorganic phosphate to fulfill the dietary requirements. The undigested phytate being excreted in manure leads without delay to pollutants of floor water and contributes to an extensive hassle with nutrient loss.

Non-enzymatic and non-assisted techniques can remove phytate (Greiner and Konietzny, 2006). Soaking, germination, malting, cooking, exogenous phytase supplementation, fermentation, and hydrothermal remedy are among the food processing techniques that could cause fractional degradation of a few or all of the phytate (Vadivel and Biesalski, 2012). However, the remaining portion causes problems associated with malnutrition (Song *et al.*, 2019).

The breakdown of phytate is more efficiently achieved through the use of enzymes than other means (Gupta *et al.*, 2019). Phytases (Myo-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.8/EC 3.1.3.26) can perform enzymatic hydrolysis on phytic acid. Phytate hydrolysis is initiated by way of phytases, which release solubilized varieties of inorganic phosphate, myo-inositol, and lower sorts of inositol phosphate (Askelson *et al.*, 2014; Zeller *et al.*, 2015). This hydrolytic response is important for carbohydrate metabolism, regulation of metabolism, and signal transduction pathways inside the biological system. Depending on the position of the hydrolyzed phosphate, phytases can be categorized into either 3-phytases or 6-phytases (da Silva *et al.*, 2019). Phytases are derived from plants, animals, and microorganisms in nature, but human beings do not possess endogenous phytases or can harbour microorganisms that own phytases (Rodehutschord *et al.*, 2022). These enzymes are used to improve phosphorus nutrition in animal feed, processing and production of human food,

and additives to enhance mineral health and reduce animal waste pollutants.

This article emphasizes the role of phytase enzymes in mitigating the adverse nutritional impacts of phytic acid, highlighting their potential for developing sustainable and nutritionally enhanced food products.

Micronutrient Deficiency Due to Phytic Acid

A significant portion of the global population is impacted by micronutrient deficiencies (MNDs), which are serious public health issues (Manjeru *et al.*, 2019). They are common throughout the world, with the greatest risk being experienced by expectant mothers and their young children (Bailey *et al.*, 2015). Worldwide, there are approximately 800 million undernourished people and 1.5 to 2 billion people with one or more chronic motor neuron diseases (MNDs), with the most common causes being deficiencies in calcium, iodine, iron, zinc, selenium, and vitamins like A and folate (Bailey *et al.*, 2015; Kumssa *et al.*, 2015; Beal *et al.*, 2017; WHO, 2017; Herforth *et al.*, 2020). In 2011, Africa and Asia accounted for 90% of the population at risk of zinc and calcium insufficiency (Kumssa *et al.*, 2015). Micronutrients are critical to human health; MNDs can hinder immune response, stunt growth, and cognitive development, and raise the risk of non-communicable diseases such as metabolic, cardiovascular, and skeletal problems (WHO, 2003; Huang *et al.*, 2022). They raise the risk of morbidity and death, prenatal problems, and intellectual deficits (Bailey *et al.*, 2015). When vitamin and mineral consumption and absorption

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are insufficient to support optimal health and development, micronutrient deficiencies arise (Bouis and Saltzman, 2017).

In underdeveloped nations, plants are the main food source. The reduced bioavailability of iron and zinc in unprocessed cereal and legume meals leads to metabolic disorders associated with these nutrients. Therefore, raising the nutritional content of such foods will raise everyone's nutritional status. The bran contains significantly more minerals, phosphorus, and phytate than the entire grain (Santis Santis *et al.*, 2019). Programs for fortifying food rely on widely available, industrially processed foods that are typically out of reach for the majority of people on the planet (Martorell and de Romaña, 2017). Anemia affects almost one-third of people on the planet, with iron deficiency responsible for half of the cases. Iron deficiency harms pregnancy, work capacity, infection resistance, cognitive development, and productivity. Cellular differentiation and growth are mediated by zinc. Although low to moderate levels of zinc insufficiency are widespread worldwide (Gupta *et al.*, 2015), approximately one-third of the global high-risk population resides in low-income nations. Impaired growth, immunological dysfunction, elevated morbidity and mortality, unfavorable pregnancy outcomes, and aberrant neurobehavioral development are all consequences of zinc deficiency. Depending on the mineral and the kind of food matrix, there were considerable differences in the minerals' in vitro bioaccessibility. Pulses and nuts were generally determined to be the finest sources of bio-

accessible Fe and Zn (Marcinek *et al.*, 2015). There are nutritional issues since grains have limited mineral content and low bioavailability due to phytic acid and other anti-nutritional components that lower bioavailability to 5–15% (Das *et al.*, 2012).

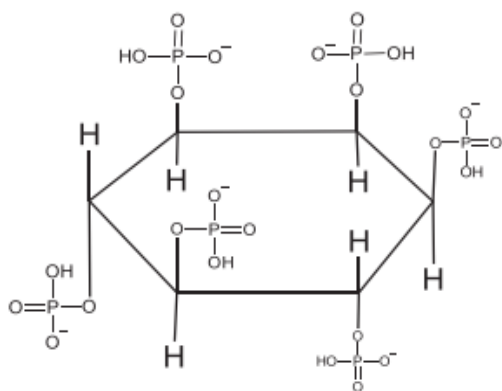


Fig. 1: Structure of phytic acid

Impacts of Phytic Acid on Vital Mineral Elements

According to Lawal *et al.* (2002), phytic acid (PA) impairs the absorption of calcium, iron, zinc, and iron and may lead to mineral shortages. It can bind simultaneously to two or more minerals. PA's capacity to form stable complexes with two or more positively charged elements, particularly minerals (Ca, Zn, and Fe) of nutritional value, is attributed to its six phosphate groups with a negative charge (Nissar *et al.*, 2017). The mineral molar ratio of PA determines whether or not it causes a mineral shortage. According to Marin *et al.* (2009), one helpful tool for estimating mineral

bioavailability is the molar ratio between PA and Ca, Fe, magnesium, and Zn.

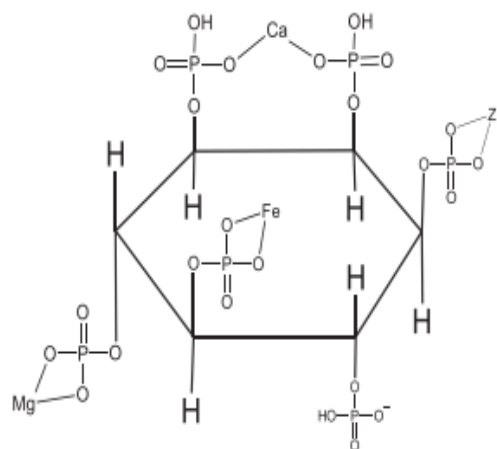


Fig. 2: Structure of phytic acid-metals complex calcium

Calcium

One of the vital minerals impacted by PA is calcium (Ca). Using an atomic absorption spectrophotometer (AAS) and capillary electrophoresis, the capacity of PA to bind with Ca in various food samples was found to be pH-dependent (Dendougui and Schwedt, 2004). Furthermore, their binding feasibility is occasionally defined by the concentration of PA and Ca (PA/Ca ratio). Foods low in PA, for example, might not affect calcium at all, especially when combined with foods high in calcium. Tamim *et al.*'s (2004) study made clear that adding calcium to a diet lacking in calcium reduces the effectiveness of PA binding.

Although PA has a detrimental effect on calcium, there are some health advantages. According to Fuster *et al.* (2017), it prevents the formation of

kidney stones and, consequently, kidney stone illness (urolithiasis). Since PA most likely shows a stronger affinity for calcium than for the antinutrient oxalate, its presence inhibits the crystallization of calcium and oxalate, which has been linked to the formation of kidney stones (D'Alessandro *et al.*, 2019). Higher ratios of PA to Ca are adequate to avoid the Oxalate-Ca complex and, consequently, urolithiasis, according to a study by Israr *et al.* (2013).

Magnesium

One element that is important to biology is magnesium (Mg). Magnesium acts as a co-factor in about three hundred (>300) metabolic processes, particularly those that include macromolecules (metabolism of lipids, proteins, and carbohydrates, among others) (Kostov, 2019). In no way is the detrimental impact of PA on magnesium's bioavailability unusual. Human Mg absorption has been demonstrated to be reduced when PA is added to food (Bohn, 2008), which may have an impact on multiple metabolic processes. While the amount of magnesium determines the impact of PA on the absorption of magnesium, research has shown that the presence of PA influences the marginal content of magnesium in a meal (Pallauf *et al.*, 1998), implying that if the nutrients are not received, PA may exhibit antinutritional characteristics.

Iron

Desferrioxamine and PA both have detrimental effects on iron (Fe) bioavailability. Desferrioxamine is frequently employed to physically eliminate Fe beyond physiologic demand (Hawkins *et al.*, 1993). Fe deficiency anemia, which is considered to be a very serious nutritional challenge globally; may be exacerbated by changes in Fe bioavailability caused by PA. A malfunction in the synthesis of hemoglobin is its defining feature. Anemia affects over two billion individuals worldwide (Ortega-Rodes *et al.*, 2014; Hackl *et al.*, 2019). Fe deficiency is the main cause of anemia, while there are other contributing factors as well (Skolmowska and Glabska, 2019). Plant sources of iron are poorly absorbed, which could become more problematic when allergens, such as PA, are present (Feitosa *et al.*, 2019). Nonetheless, the accessibility and absorption of non-heme iron are improved by eating meals high in vitamin C and bio-fortifying plant-based diets with iron (Magdalena, 2015).

Zinc

Zinc forms the most stable complex with PA among the important minerals chelated by PA. Accordingly, in terms of bioavailability, it is most likely the most impacted mineral (Weaver and Kannan, 2002). Zn insufficiency impacts one-third of the global population, according to a study (Gupta *et al.*, 2015), and PA may play a role in this condition.

Sources of Phytase

The pre-requisite requirement for phytase extraction is the identification of potent sources. There are a variety of them, viz., plants, animals, and microorganisms. Many of the food supplements from plant sources like wheat, peas, corn, maize, rye, soybeans, etc contain phytase, which is derived from them.

Plants

Phytase was first identified in rice bran, and its activity was later revealed in cereals, legumes, and oil seeds (Jatuwong *et al.*, 2020). Wheat, barley, and rye contained the highest levels of phytic acid, while beans, lily pollen, maize, lettuce, spinach, soybeans, oilseeds, and grass had the lowest concentrations (Gupta *et al.*, 2015). Phytase sources derived from plants and their byproducts are tested to feed animals. Plant phytases are more temperature and pH-stable than microbial phytases. The fundamental challenge involved in producing plant phytases is a less labourious, economically viable, and less time-consuming technique for synthesizing enzymes. Gupta *et al.* (2015) stated that industrial or domestic processing techniques such as bio-processing, can be used to enhance the internal phytase activity present in plant-based foods

Bacterial phytase

Fungi produce extracellular phytases, whereas bacteria create largely cell-associated enzymes. *Enterobacter* spp., *Bifidobacterium* sp., *Aerobacter aerogenes*, *Citrobacter braakii*, *Megasphaera elsdenii*, *Pseudomonas* sp., *Bacillus*

sp., *Prevotella* sp., *Mitsuokella multiacidus*, *Klebsiella* sp., and *Escherichia coli* have all been shown to produce phytases. Furthermore, *Lactobacillus* was demonstrated to be the greatest phytase producer among the several lactic acid bacteria isolated from sour doughs (Konietzny and Greiner, 2003). Compared to endogenous phytases, microbial phytases break down 73–80% more phytate. The regulating component of phytase activity is pH, with the highest levels discovered at 5.0–5.5 and 2.5 (Jain *et al.*, 2016). Bacterial phytases have different characteristics such as broad substrate specificity, high catalytic efficiency, proteolysis resistance, and extreme heat stability. Phytase production was observed during bacterial cell growth, suggesting that it may be involved in a metabolic signal-transduction pathway. In gram-negative bacteria, many proteins containing myoinositol phosphates have been discovered, and they are likely involved in signal transduction. For instance, *Salmonella* Dublin secretes a myoinositol polyphosphate 4-phosphatase that enhances virulence by interfering with cellular Myo-inositol phosphate processes. Bacterial phytases are commonly produced by Submerged fermentation (Coban *et al.*, 2017, Hussain *et al.*, 2021).

Fungal phytase

The first commercially available phytases for industrial use were obtained from fungal strains, either through three common mutation processes or via recombinant DNA technology (Puppala *et al.*, 2021). At the commercial level, microbial

phytase is the most common source of enzyme production, with the majority of studies concentrating on microbial phytase derived from filamentous fungi such as *Trichoderma*, *Penicillium*, *Myceliophthora*, *Rhizopus*, *Mucor*, and *Aspergillus* (Dailin *et al.*, 2019). Fungal phytase is preferred over bacterial phytase due to its thermostability and greater chitin effects, while

bacterial phytase exhibits activity across a range of acidic to alkaline pH, but has protease limitations in the gut (Song *et al.*, 2019). Most filamentous fungi, unlike unicellular fungi, are good for both submerged and solid-state fermentations owing to their ability to grow across a large surface area and penetrate solid substrate (yeasts).

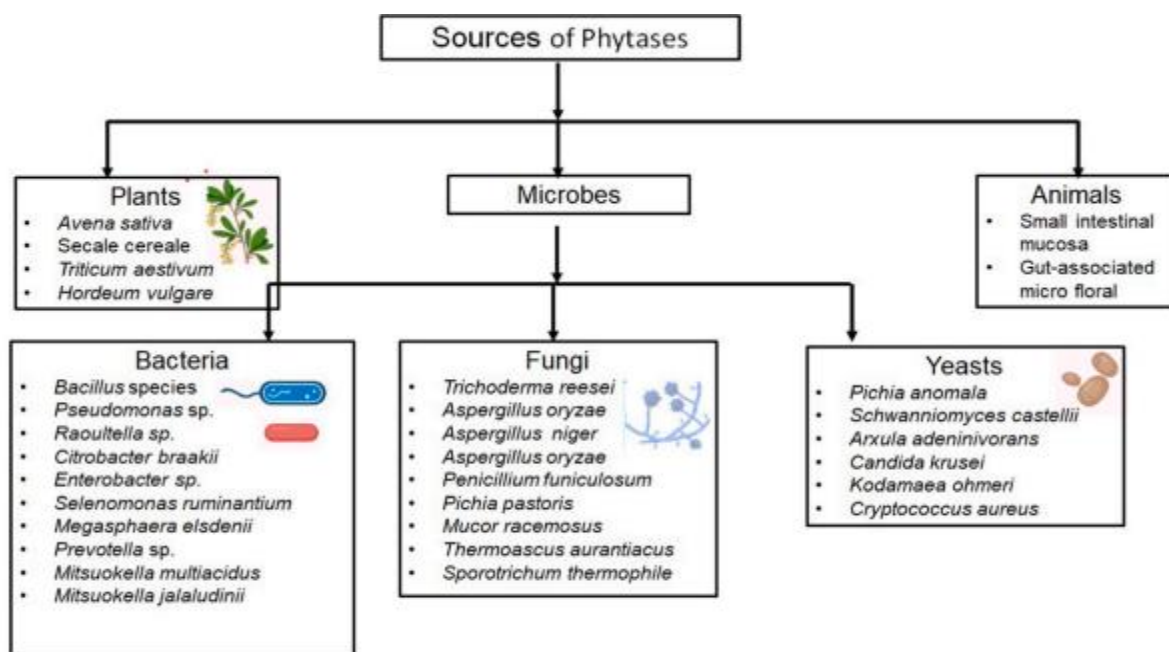


Fig 3: Various sources of phytases (Dailin *et al.*, 2019).

Application of Phytases in Food Processing

Fortification of food products with microbial phytase

The food industry relies on phosphatase enzymes, which can improve the health of non-ruminant individuals. The cost of producing food with phytase during processing and manufacturing is reduced, which also enhances the quality of the final product. In food processing, phytase has been

used to improve the separation of wheat from barley, the production of plant protein isolates, wet

milling of maize, and cereal husks separation (Singh and Satyanarayana, 2015). Phytic acid is present in wheat and whole grain flour, which is used to make various doughs and breads. Phytase's ability to increase bread volume and texture quality is due to its ability to increase fermentation time without altering pH. The alpha-amylase

activity of phytase has been indirectly linked to the improvements in bread quality.

The dephosphorylation of phytate is essential for the nutritional value of food and feed, as it increases the availability of vital minerals by the release of phosphate groups from the inositol ring structure. This has led to a reduction in the mineral binding capacity of phytate (Sandberg *et al.*, 1999). The addition of minerals to the development of whole wheat bread, which had phytic acid losses, resulted in increased nutritional value. Adding commercial fungal phytase from *Aspergillus niger* to the dough ingredients improved bread quality and also softened crumbs. Phytase indirectly enhances the quality of bread through its action on alpha-amylase activity (Greiner and Konietzny, 2006).

Phytase activity has been identified as an excellent candidate for producing whole meal bread with high bioavailable minerals, thanks to the discovery of yeasts with high phytase activity. At pH 5 and 30°C, yeasts like *Saccharomyces cerevisiae*, *Pichia kudriarzevii*, and *Pichia occidentalis* with phytase activity were isolated from sourdough bread.

Role of Phytases in animal feed to improve nutritional value

Phytase is used as an animal feed supplement to increase the availability of nutrients and decrease phosphorus content in the ecosystem. The absorption of trace minerals and phosphorus in phytate is dependent on phytase, which is essential

for monogastric animals due to its limited accessibility (Maqsood, 2013). Phytase is an additive found in about 70% of animal feed (Ranjan and Satyanarayana, 2016). The incorporation of phytase supplements to broiler diets can enhance their growth and development (Humer *et al.*, 2015; Attia *et al.*, 2021; Saleh *et al.*, 2021). Phytases, which are mostly made up of microalgae, have been genetically engineered to handle thermostability and survive in the gastrointestinal tract and are safe for consumption (Erpel *et al.*, 2016).

Phytase inhibits the nutritional impact of phytate and enhances the digestion of starch, phosphorous, calcium, and amino acids. Fungal phytase supplements are utilized in pisciculture, poultry, and pig industries. Natural water contains insufficient amounts of phosphorus to meet the nutritional needs of fish. Most pisciculture fish species require 0.4–0.9% of phosphorus in their diet, as reported by Wang *et al.* (1998). External feed additives added to the water make up for that. Freshwater fishes may absorb phosphorus via their gills, whereas marine ecosystem fishes may consume it through the gut. The effectiveness of biological bioreactors for extracting fungal and microalgae phytase from soy protein substrate was proposed in such cases (Santos *et al.*, 2019). Furthermore, other byproducts generated during different fermentation stages in a bioreactor can have varying effects on the phytase activity and feed derivatives. Marine pollution can be controlled by phytases, which reduce phosphate

levels in water bodies and prevent eutrophication, as demonstrated by Handa *et al.* (2020).

Mechanism of Action of Phytase

The elimination of phosphate from phytic acid or its salt phytate is accomplished by the enzyme phytase (Yu *et al.*, 2012). To eliminate the phosphate group, one can start with IP6 and proceed to penta- (IP5), tetra- (IP4), tris (IP3), di-, and mono-esters of inositol. Phytases hydrolyze the entire phosphorylated phytic acid to produce penta-esters of inositol before hydrolysis of those latter into tetra-esters and so on (Yu *et al.*, 2012). If given sufficient time, complete hydrolysis will produce phosphate and myo-inositol (along with amino acids/minerals/nutrients) from phytic acid. *In vivo*, the hydrolysis reaction is incomplete, which typically results in a mixture of inositol-phosphate esters such as IP5, IP4, and IP3.

According to Yu *et al.* (2012) IP6 and its esters (IP1-5) were found to have a higher protein binding capacity than previously reported. A phytase from a fungal or an *E. coli* was used to hydrolyze IP6 through enzymatic hydrolysis in that study. The testing of the binding of the resulting molecules to soy protein was conducted using *E. coli* phytase. This involved analyzing the different IP5 positional isomers. The inositol ring contains phosphate at different carbon positions. To test for protein binding, a turbidity test was employed. High turbidity values with low solubility and high absorbance indicate higher binding capabilities. IP6 has a greater affinity for soy protein, and the system's absorbance decreased

rapidly with decreasing phosphorylation from IP6 to IP3. IP1 to 4 have low protein binding abilities, whereas IP5 is still active but not as effective in binding soy proteins.

According to the research, *E. coli* 6-phytase intermediate products ie (IP5 (1, 2, 3, 4, 5) were significantly more effective in the aggregation of protein than the 2 IP5 isomers (IP5 (1, 3, 4, 5, 6) and IP5 (1, 2, 4, 5, 6), which are generated by *Aspergillus niger* 3-phytase showing 6.6- vs. 4.6-fold turbidity reduction respectively. The discovery indicated that the location of the initial hydrolysis step can significantly affect protein binding, as it differs from one phytase source to another. From IP6 to IP3, the capacity to bind with Fe³⁺ decreased proportionally in that study. To achieve the maximum reduction of pepsin inhibition, it is necessary to reduce IP6 to IP1-2 (Yu *et al.*, 2012). Phytate binding to calcium is associated with the composition of its ester, and its ability to bind calcium has been significantly reduced. Adeola and Cowieson (2011) reported that IP3's binding affinity is roughly 11% less than that of IP6. Rapid elimination of IP6 and IP5 in the stomach will greatly decrease the amount of calcium binding in small intestines.

The acidic pH of the environment (e.g. the abdominal region) is where IP6's effect on protein binding was reported to be most detrimental (Morales *et al.*, 2011). The authors found that casein, treated with fish acid protease (pH 2) and placed on top of sodium phytate decreased protein solubility by 80%. At pH 2.5, 16°C, and for 180

min, the protein solubility did not decrease throughout intestinal digestion (pH 8.5). Similar results were obtained by using soybean meal (SBM) as a substrate. An *E. coli* phytase was associated with a 60% change in amino acid release during acidic digestion, but during intestinal digestion, it was not. The data demonstrates that early and complete hydrolysis of phytate by phytase in the upper digestive tract is necessary for better digestion of minerals such as Ca, Fe, phosphorus, and protein. The data indicate that to eliminate the negative impacts of phytate, it is necessary to hydrolyze IP6 in the upper part of the digestive tract as much as possible.

Conclusion

Phytic acid, while abundant in many plant-based foods, poses significant nutritional challenges due to its strong chelating properties, which reduce the bioavailability of essential minerals including iron, zinc, and calcium. Addressing the anti-nutritional impacts of phytic acid is critical for combating micronutrient deficiencies, particularly in populations relying heavily on cereals and legumes as dietary staples.

Phytase, a potent enzyme for degrading phytic acid, has proven to be an effective tool in improving mineral bioavailability. Its diverse sources, mode of action, and broad applications in food processing and animal feed underscore its importance.

Acknowledgments

We would like to thank Ukwuru Mike Ukwuru for his valuable input and revision of the manuscript.

Funding

The authors declare no financial support for the research, authorship, or publication of this article.

Author contributions

Conceptualization, O.K.; writing—original draft preparation, C.G.; writing—reviewing and editing, C.G and E. All the authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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