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# Effect of phytase application on micronutrient status of plant-based foods

Japheth Abuor Anuro[1], Fredrick Onyango Ogutu[1], Trizer Nelly Okumu[1]
[1]*Food Technology Division, Kenya Industrial Research and Development Institute (KIRDI), P.O. Box 30650, GPO, Nairobi, Kenya.*
*Correspondence should be addressed to Japheth A. Anuro; Japheth.anuro@kirdi.go.ke*

***Abstract***
*Plant-based foods constitute an important source of carbohydrates, protein, dietary fiber and vitamins. They are also associated with anti-nutrients, whose presence result in low bioavailabity of several micronutrients causing metabolic disorders related to the nutritional factors. Of prime concern for human nutrition and health management is phytic acid. In this review the effect of phytase application on micronutrient content and bioavailability of plant-based foods was critically analyzed. PubMed and Google scholar databases were searched for articles using phytase, phytase application in cereals, plant-based foods, micronutrients and deficiency as keywords. A total of 105 articles were obtained out of which 39 were included in the review. Results indicate that application of exogenous phytase to plant-based foods increases micronutrient content and bioavailability alongside improvements in baking and brewing.*

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## I. Introduction

More than half of the world population are affected by malnutrition and together with hunger-related diseases, they account for over 60% of deaths(Felistus et al., 2017; Popova & Mihaylova, 2019).Of greatest concern are iron, zinc and vitamin A deficiencies, while developing countries whose main staple foods are derived from plants are particularly affected(Platel & Srinivasan, 2016).

Plant-based foods are an important source of carbohydrates, protein, dietary fiber and vitamins, but are also associated with anti-nutrients, the highest concentrations being in grains, beans, legumes and nuts, but can also be found in fruits, roots and leaves resulting in low bioavailabity of several micronutrients (Hendek Ertop & Bektaş, 2018; Popova & Mihaylova, 2019).The major anti-nutrients are phytates, tannins, lectins and oxalates, of which phytic acid is of prime concern for human nutrition and health management, affecting approximately two to three billion people worldwide(Kumar et al., 2010).

Chemically, phytic acid is myo-inositol (1, 2, 3, 4, 5, 6) hexa-kisphosphate and structurally consist of hydroxylated inositol ring with at least a phosphate group sterically stable at pH range 0.5~10.5(Singh et al., 2018). Also known as phytin ($C_6H_{18}O_{24}P_6$), phytic acid has a molecular weight of 660 $gmol^{-1}$ and is considered the main source of stored phosphorus in seeds, grains, and vegetables (Cangussu et al., 2018). It is considered anti-nutritional since it occurs as a mineral complex that is insoluble at the physiological pH of the intestine, resulting in reduced uptake of essential dietary minerals in the human intestine (Mamiro et al., 2016).Depending on the grade of food processing and the amount of plant-derived foods in the diet, average daily intake of 2000–2600 mg reported for vegetarian diets and diets of inhabitants of rural areas of developing countries, though can be as high as 4500 mg (Kumar et al., 2010). Generally, cultivars, climatic conditions, location besides moisture content, type of soil, year and fertilizer application affect the levels in food (Humer et al., 2015).

Several efforts have been tried to reduce micronutrient deficiencies globally including supplementation with pharmaceutical preparations, food fortification, as well as dietary diversification and disease reduction. Other strategies include increasing the total level of micronutrients in the edible parts of staple crops while increasing the concentration of compounds promoting their uptake or decreasing the amount of compounds that inhibit their absorption such as phytic acid (Perera et al., 2018). However, choice of an intervention or strategy depends on the cause and severity, as well as scope of the micronutrient deficiency, and the intervention picked should focus on eliminating the root cause, taking into consideration cultural preferences, sustainability and feasibility, besides ensuring continued access (Bailey et al., 2015).

Widespread success has been achieved with vitamin A supplementation, however this strategy requires feasible provisions of supplements alongside adequate educational programs to ensure compliance while it does not address the root cause of the deficiency (Bailey et al., 2015). According to the authors, dietary diversification is also limited by food availability within certain regions while legislative approvals are required

for strategies that increase concentration of micronutrients through genetic modification, leaving fortification and reduction of micronutrient inhibitors as the viable options.

Taking advantage of phytase catalytic activity, phytate degradation and enhanced micronutrient accessibility can be achieved through addition of phytase during food processing (Chen et al., 2018).This is supported by the increased use of enzymes in juice manufacturing, flour milling, baking and brewing as well as winemaking, starch and meat processing, dairy and manufacture of pre-digested foods(Sharma et al., 2019). Most of these are hydrolytic enzymes, used for the degradation of natural substances, including those involved in utilization of phytate-bound phosphorus(Ozatay, 2020).

Phytases have been used mainly as animal feed additives in pigs and poultry diets, and to some extent for fish, however with great potential in processing and manufacturing of human food, an area where phytate reduction has been demonstrated for cereal and legume-derived products(Kumar et al., 2010).Vijayaraghavan et al.(2013) reported an improvement in the nutritional value of plant-based foods through addition of phytases during food processing. Despite such reports, no phytase-treated food product is available in the market currently. This review aims at analyzing published articles covering phytase application and the effects on micronutrient content and bioavailability of plant-based foods with a view to fabricating phytase-treated food products for human consumption.

## II. Methodology

A systematic review of published literature on phytase application in plant-based foods was conducted using PubMed and Google scholar databases. A total of 105 articles were obtained using search terms "phytase, phytase application in cereals, plant-based foods, micronutrients and deficiency." From this number a total of 39 articles that contained relevant content were selected and analyzed for the review.

## III. Results and Discussion

Phytase, also known as myo-inositol hexa-kisphosphate phosphohydrolase (EC 3.1.3.26 and EC 3.1.3.8) is a phosphatase hydrolyzing phytate (phytic acid) into inositol phosphates, phosphorus, inositol as well as other essential nutrients (Cangussu et al., 2018). The nature of raw material used, manufacturing process and source of phytase besides the amount of enzyme activity added affect the extent of hydrolysis (Greiner & Konietzny, 2006). This hydrolysis occurs when phytate is in solution and its solubility is affected by the pH of the system, being more soluble at lower pH values, and since phytase is a protein, it is sensitive to high temperature, causing denaturation at excessive heat (Humer et al., 2015). An ideal phytase has a maximum enzyme activity and stability at high temperatures as well as proteolysis resistance and the ability to work at acidic pH levels(Dokuzparmak et al., 2017).

Nutrient bioavailability in food can be improved by application of phytase hence their supplementation during food processing has the potential to enhance nutrient uptake, consequently contributing to the fight against malnutrition (Gupta et al., 2013; Herrmann et al., 2019). For instance, in vivo studies have confirmed increased iron absorption through phytase-catalyzed dephosphorylation of cereal-based foods (Nielsen & Meyer, 2016).In Malawi, a study of children with rickets recorded a two-fold increase in zinc bioavailability through phytase application while reduced phyticacid:zinc molar ratio from 30:1 to 7:1 is reported for phytase treatment of a corn soy porridge, increasing fractional zinc absorption from 24% to 41% (Moretti et al., 2014).

Effectiveness of phytase application has been illustrated in baking as well as in dephytinization of infant formulas, infant cereals, and complementary foods, besides successful attempts in brewing (Lei et al., 2013).

Sources of phytase

Plants (wheat, barley, peas, soybeans, corn, rice, and spinach) animals, and microorganisms are the conventional sources phytase (Cangussu et al., 2018). However, microbial phytases have high pH and thermal stability compared to plant phytases, making them more investigated for industrial purposes(Gupta et al., 2013). Microbial phytases are also of great interest to industry due to their high level of production and extracellular activity (Cangussu et al., 2018).

Of the microorganisms, fungi, bacteria and yeast are effective in phytase production, with fungi giving better results (Patel et al., 2017;Parhamfar et al., 2015) while over 200 fungal isolates, mainly Aspergillus, Mucor, Penicillium and Rhizopus have been tested. Among these *Aspergillus niger* phytase exhibited activity over a broad pH range, including that of the stomach and is stable during the expected residence time (Gupta et al., 2013; Troesch et al., 2013). It also produces heat stable commercial phytases, a pre-requisite in food processing involving heat treatment (Sharma Vivek, 2017). Complete degradation of phytic acid has been realized through addition of commercial phytase from *Aspergillus niger* to infant formulas based on soy and pea protein isolates (Serafina & Ulf, 2021) while the World Health Organization has found 3-phytase from *Aspergillus niger* safe for use in human food (Nielsen et al., 2013).

The potential of phytase to improve iron bioavailability has been demonstrated in wheat rolls, where fungal phytase increased iron absorption from 14.3% to 26.1% (Perera et al., 2018). In another study, *A. niger* phytase showed an increase of up to 75% in cereal porridge when added with ascorbic acid (Nielsen et al., 2013). The authors also reported degradation of phytate with *A. niger* phytase of up to 97% in bread, increasing the cellular iron uptake by Caco-2 cells of ~150%.

**Factors affecting phytase hydrolysis of phytic acid**

West(2014) reports on the use of *A. niger* phytase at low concentrations on corn-soybean meal used for animal feed to release inorganic phosphorus. The enzyme concentration ranged from 0.15 to 0.45 units/g of meal with the later being the most effective. Increased InsP6 and P disappearance by 17.7 and 5.4, and 8.7 and 4.5 percentage points, up to the duodenum–jejunum and ileum respectively, is also reported in broiler feed when phytase was increased from 1,500 to 3,000 FTU/kg (Ajuwon et al., 2020). According to the authors complete breakdown of anti-nutritive factors is possible at high phytase concentrations.

Effect of pH on phytase action

Enzymes have maximum pH above which their structure deteriorate while their activity decreases (Demir et al., 2017). For instance, many phytases show maximal phytate-degrading activity in the acid pH range with pH 5 to 5.5 reported for plant grains and seeds (Greiner & Konietzny, 2006). While alkaline phytases have an optimum pH around pH 8.0, acid phytases have pH optimum around pH 5.0 (Dechavez et al., 2011).

Most Aspergillus phytases are active between pH 2.5 and 6.0 with the highest activity exhibited at pH 5.5(Igbasan et al., 2000). For instance, *A. niger* PhyA and PhyB exhibited pH optima at pH 2.5and pH 5.0 for the former, and pH 2.5 for the later (Zhang et al., 2013). Dokuzparmak et al.(2017) also reported maximum activities at pH values of 2.2–5.0, 5.5, 6.5, and 5.5, for *A. niger, Aspergillus oryzae, Emericella nidulans*, and *Penicillium lycii* respectively.

Effect of temperature on phytase action

Optimum temperatures for most phytases have been found to vary between 33 and 37°C (Demir et al., 2017). However, depending on the source of enzyme, this may vary from 35 to 80 °C while further increase in temperature my result in a heat-induced denaturation (Greiner & Konietzny, 2006).

Studies have shown that microbial phytases work on a wider temperature range of 35 to 63°C while plant phytases have high activity at temperatures between 45 and 60°C (Singh et al., 2018). For instance Aspergillus phytases are capable of maintaining high stability at processing temperatures of 70°C(Igbasan et al., 2000). In a study by Naves (2012) *A. oryzae* and *A. niger* phytases also showed increasing activity up to 40°C and 45°C, respectively. The two were however inactivated at 50°C and 60°C, respectively.

Optimum temperatures of 60°C, 70°C, 45°C, 50°C, 70°C, 55°C, 40°C, 55°C, 70°C, 50°C, 50°C and 50–60°C have been reported by Dokuzparmak et al. (2017) for *B. subtilis* N-77, Bacillus sp. DS11, *L. sanfranciscensis*, *G. stearothermophilus* DM12, *Bacillus amyloliquefaciens*, *E. coli*, *C. krusei*, *A. niger*, *A. terreus*, *A.oryzae*, *P. lycii* and Enterobacter sp. 4 respectively. According to the authors *A. niger* ATCC 9142 phytase also retained 22% of residual activity after 3 min incubation at 80°C compared to 18 and 6% for two commercial phytases.

**Technological considerations in application of Phytase**

Addition of phytase during food processing has been fronted as a potential solution to phytate dephosphorylation with application of *A. niger* phytase to an oat-based nutrient solution reported (Mittal et al., 2013). While centralized food pretreatment using phytase has been suggested to improve compliance, many food products, particularly in developing countries, do not undergo central processing, hence direct application of phytase at the point of use is possible (Nielsen & Meyer, 2016). According to the authors, this latter method will require thermostable phytases.

Kaleda et al.(2020) heated suspended pea-oat powder in tap water (15% dry matter) to 40 ◦C under continuous stirring followed by addition of 1.5% of phytase (Phyzyme® XP). The mixture was incubated for 4-h at 40 ◦C under minimum agitator speed after which the suspension was collected, frozen at −40 ◦C and then lyophilized. Sanz-Penella et al.(2012) also added α-amylase together with phytase to 60 g of suspended roasted flour in 124 mL of deionized water. The pH was adjusted to 5.5, and the mixture incubated at 55 °C with gentle agitation for 20 min. The reaction was stopped by heating the slurry at 98 °C for 10 min before drying at 120 °C and milling. Chen et al.(2018) however immobilized the enzyme by incubating 50 mg of phytase dissolved in acetate buffer (pH 5.5) on activated carriers for 16 hr at 4 °C.

## IV. Conclusions and future perspectives

Increasing micronutrient intake in food through food based approaches is a sustainable method of prevention of micronutrient malnutrition which remains a widespread global health problem, particularly in developing countries(Gupta et al., 2013). Foods consumed in such countries are often poorly available despite containing significant amounts of minerals, including iron and zinc, due to the presence of high levels of

phytate(Troesch et al., 2013) hence reducing the phytate content of these foods may be a more feasible alternative to enhancing mineral bioavailability (Gibson et al., 2010).

Commercial dephytinization can be achieved through addition of exogenous phytase either during preparation or just before consumption (Gibson et al., 2010; Troesch et al., 2013). This has been illustrated in dephytinization of infant formulas, infant cereals, and complementary foods, besides brewing and baking where it has resulted in improved alcohol production, proofing time, width/height ratio of bread slices, specific volume, and crumb firmness (Lei et al., 2013). Research is however needed to establish the optimum conditions for phytate hydrolysis in different foods using phytase from different sources.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Japheth Abuor Anuro, et. al. Effect of phytase application on micronutrient status of plant-based foods." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 16(03), (2022): pp 29-33.