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Arun Kumar
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Deepak Kuma
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

SKZ Rizvi
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Ajit Tiwari
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Akanksha Singh,
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Adesh kumar
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Shambhoo Prasad
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

KN Singh
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Correspondence
Arun Kumar
Department of PMB & GE, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, U.P, India

Effect of processing on phytic acid content and iron availability in selected rice variety

Arun Kumar, Deepak Kuma, SKZ Rizvi, Ajit Tiwari, Akanksha Singh, Adesh Kumar, Shambhoo Prasad and KN Singh

Abstract
Rice (*Oryza sativa* L.) which belongs to the family of grasses, Poaceae, is the main food source for more than two-third of the world's population especially in Southeast Asia. Nutrient paucity due to unavailability of quality food sources is a major impediment to the progress in improvement in the field of human nutrition. Phytic acid is a major anti nutritional factor that binds with cationic nutrients like zinc and iron, and makes them unavailable for human intestinal absorption. Iron is the important nutrients required for human growth and development. This study was aimed to know the effect of soaking on phytic acid contents. Minimum phytic acid measured in the Sarjoo-52 was 5.61 g/kg and maximum phytic acid measured in the Swarna was 9.12 g/kg followed by NDR-359 was 8.05 g/kg. After processing in case of after soaking minimum phytic acid content measured in the Sarjoo-52 was 3.63 g/kg and maximum phytic acid content in parents after soaking measured in Swarna was 6.76 g/kg. Minimum phytic acid content after germination measured in the Sarjoo-52 was 3.08 g/kg and maximum phytic acid content in parents after germination measured in Swarna was 6.05 g/kg. The soaking treatment reduced the inherent phytic acid content in seeds of rice. The highest iron was obtained in sarjoo-52 (24.15 ppm) and lowest obtained in swarna (12.48 ppm).

Keywords: processing, rice, nutrients, phytic acid, iron

Introduction
Rice grain is the staple food for more than half of the world's population and grows in more than 100 countries. The top 10 rice producing countries in the world today are India, China, Indonesia, Bangladesh, Thailand, Vietnam, Burma, Philippines, Cambodia, and Pakistan. China, India, Indonesia, Bangladesh, Thailand, and Vietnam produce about 80 percent of the world rice production. Among the rice producing countries, India ranks second in total production (90.00 million tonnes) next to China (184.25 million tonnes) with an average productivity of 3.09 t/ ha-1 (FAO, 2013) [8]. Nutrient deficiencies have plagued the world's population from ancient days. The number and proportion of undernourished people in the world is estimated to be 870 million and 13% of the population respectively (WHO report, 2014) [47].

In the last few years, food scientists have laid an emphasis on the effects of mineral nutrient deficiencies and it has become increasingly evident that the lack of minerals may have similarly severe negative consequences on human health (Bouis 2000) [5]. These deficiencies have major negative effects on human health, and development; working ability and quality of life (Welch and Graham 2004; Shailen *et al.*, 2005; Anne and Paula 2006; White and Brown, 2010) [43, 38, 1, 46]. Although micronutrient requirement is very small, every one in three humans worldwide is not getting enough quantity, especially the poor, women and children (Gibson 1994; Ramakrishna *et al*., 2006) [32].

Phytic acid (PA) or myo-inositol hexaphosphate is a strong chelator of multivalent metal ions, especially iron, zinc and calcium (Hurrel, 2004) [15]. For many years, phytic acid was considered an antinutritional compound because it reduces the bioavailability of several minerals important for human nutrition (Bohn *et al.*, 2007; Li *et al*., 2008; Schelemmer *et al*., 2009) [4, 37]. However, since the 1990s, phytic acid has been scientifically emphasized for its beneficial effects on human health, particularly in the prevention of diabetes (Lee *et al*., 2006) [21], Renal calculi (Saw *et al.*, 2007) [35], Parkinson's disease (Xu *et al.*, 2008) [48] and cancer (Vucenik and Shamsuddin, 2006). The application of 0.50% PA acid rice bran added to the drinking water after tumor induction, reduced the risk of colon cancer in rats (Norazalina *et al.*, 2009). Its antioxidant effect, described by several researchers (Lee and Hendricks, 1995; Soares *et al*., 2004; Stodolak *et al*., 2007; Harbach *et al.*, 2007) [22, 39, 40, 12], is due to its ability to inhibit the formation of hydroxyl radicals and to form chelates with Fe2+ ions, causing them

to become catalytically inactive (Graf and Eaton, 1990) [11]. In addition, rice contains phytic acid (PA), the most important antinutritional factor impeding availability of divalent minerals (Jianfen *et al*, 2007) [18]. It forms complexes with mineral ions, such as Fe, Zn and Ca, and ultimately affects their bio-availability (Gibson RS *et al*., 2000; Lucca P *et al*., 2001; Mendoza C, 2002) [10, 26, 28]. Phytic acid is also able to form complexes with proteins and thus impairs digestibility and bioavailability of proteins in seeds (Reddy *et al*., 1982) [33]. The importance of Fe in vital metabolic functions is evidenced by Fe being an intrinsic component of haemoglobin, myoglobin and cytochromes (Hurrell *et al*., 2003) [14]. As humans and animals are dependent on plant based foods for their nutrient requirement except Vit B12, the deficiency of any nutrient can lead to malnutrition or under nutrition (White and Broadley, 2009) [44].

It has been shown that wet processing like soaking; germination and fermentation reduced phytic acid content and increased the solubility of nutrients (Cakmak *et al*., 1999; Selle and Ravindran 2008; Bilyeu *et al*., 2008) [6, 36, 3]. Studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and an enhancement of mineral bioavailability (Nunes *et al*., 2005; Paulik *et al*., 2005; Liang *et al*., 2008) [30, 24, 31]. Though the complete removal of phytic acid has not been shown, wet processing techniques can help to reduce phytic acid which in turn increases the availability of minerals in foods.

The present study was aimed at exploring the opportunities of germination and soaking treatments for effective removal of phytic acid to improve the micronutrient bioavailability in the seeds of economically important food crop rice.

Materials and Methods

Phytic acid content in the rice has been analyzed by the method of Wheeler and Ferrel (1971) [45]. The phytate is extracted with tricloroacetic acid and precipitated as ferric salt. The iron content of the precipitate is determined calorimetrically and the phytate phosphorus content was calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate.

Reagents

3% Tricloroacetic acid, 3% Sodium sulphate in 3% TCA, 1.5 N NaOH, 3.2 N $HNO_{3\backslash}$

Procedure

Standard ferric nitrate solutions Fe (NO_3) 433 mg ferric nitrate was weight and dissolved in 100 ml distil water. 2.5 ml of this solution was taken in another flask and make up the volume 50 ml with water. Several test tubes were taken and transferred 2.5, 5, 10, 15 and 20 ml working solution by adding 20 ml 1.5 M potassium thiocynate and volume was made up to 70 ml. The colour intensity was recorded at 480 nm on spectrophotometer against blank solution.

2g finally ground rice seed sample was transferred into conical flask (200 ml capacity). 3% TCA (50 ml) was added in the flask and extracted the material for 30 minutes with mechanical shaking. The whole content was filtered and 10 ml filtrate was collected. 4 ml ferric chloride solution was added and the contents were heated in boiling water bath for 45 minutes. After expiry of the period, the whole content was decanted carefully and the precipitate was washed with 20ml 3% TCA 2-3 times. Finally 3 ml 1.5N NaOH was added in the decanted material and total volume was made 30 ml with water. The whole content was heated in boiling water bath for 30 min. and filtrate by what man filter paper. The residue from the filter paper was dissolved with 40 ml 3.2 N HNO_3 into 100 ml volumetric flask. The flask was cooled at room temperature and 5 ml aliquot was taken in another volumetric flask and diluted 70 ml with water. 1.5 M Potassium thiocynate (20 ml) was also added and colour absorbance was recorded immediately at 480 nm against blank solution. The micro gram iron was calculated from standard curve and phytate phosphorus (ml/100g) in sample was calculated by using following formula.

Phytate (%) = micro gram iron x 15 / weight of sample

Effect of processing treatment on Phytic acid content:

Soaking

The rinsed rice seeds were soaked in distilled water for 12 hours at room temperature. To avoid possible problem that the soaking water may cause germination of seed, the dry seed to soaking water ratio must be selected such that the seeds are completely covered by water. A seed to water ratio of (1:10 w/v) was used. The unimbibed seeds were discarded. The soaked seed were rinsed twice in distilled water and then dry at 55 °C for three to four days in a hot air oven.

Germination

Rice seeds were sterilized by soaking in ethanol for 1 minute. The seeds were kept in water for 12 hours at room temperature. The rinsed and soaked seeds were germinated in sterile Petri dishes with germination paper for 2 days (48 hours). Sprouts were rinsed in distilled water and dried at 55°C for analysis of phytic acid, tannin, protein, total polyphenol, total sugar and reducing sugar content.

Mineral Profiling for iron

The standard for ICP- OES was prepared from stock solution of pb obtained from Perkin Elmer. Working solution was prepared from stock as necessary calibration standards of different concentrations 0.03mg/L, 0.06 mg /L, 0.2mg /L 0.3mg/Lwere prepared from working standard solution. All other reagents and solvent used in this study were of analytical grade obtained from Fisher Scientific. Milli-Q water was used for washing laboratory glasswares and preparation of sample and standard solution.

Sample preparation

0.1 g of finely crushed sample was used for ICP-OES 2ml Nitic acid 1 ml percholoric acid, 1ml hydrogen per oxide 1ml milli-Q water were added and made the final volume 8ml.

Results

Phytic acid (PA) content in selected varieties.

The data pertaining to the phytic acid content in selected rice was given in Table No.1 and graphically was shown in figure– 1. Phytic acid content in rice varieties Sarjoo- 52 was 5.61 g/kg, in Madhukar was 6.39 g/kg, in NDR-359 was 8.05 g/kg, in CSR-13 was 6.72 g/kg, in Swarna Sub-1 was 7.40 g/kg, and in Swarna was 9.12 g/kg. In the highest phytic acid was in Swarna and was 9.12 g/kg and lowest in Sarjoo-52 and was 9.12 g/kg. Highest phytic acid was in Swarna and was 9.12 g/kg and lowest in Sarjoo-52 and was 9.12 g/kg.

Table 1: Phytic acid (PA) content in selected varieties

S. No	Genotypes	Phytic acid content (g/kg)
1	Sarjoo- 52	5.61
2	Madhukar	6.39
3	NDR-359	8.05
4	CSR-13	6.72
5	Swarna Sub-1	7.4
6	Swarna	9.12

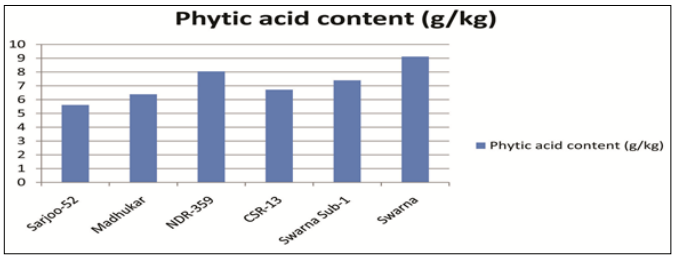

Fig 1: Histogram showing the phytic acid content in rice seed

Phytic acid and its influence on Soaking & germination

Phytic acid content after soaking noticed in Sarjoo-52 was 3.63 g/kg, in Madhukar was 4.44 g/kg, in NDR-359 was 5.74 g/kg, in CSR-13 was 4.89 g/kg, in Swarna Sub-1 was 4.34 g/kg, and in Swarna was 6.76 g/kg. Minimum phytic acid content after soaking measured in the Sarjoo-52 was 3.63 g/kg and maximum phytic acid content after soaking measured in Swarna was 6.76 g/kg.

The data pertaining to the phytic acid content and its influence on soaking and germination in different genotype of rice was given in Table No. 2 and graphically shown in figure- 2. Phytic acid content after germination measured in Sarjoo-52 and was 3.08 g/kg, in Madhukar was 3.89 g/kg, in NDR-359 was 5.49 g/kg, in CSR-13 was 4.31 g/kg, in Swarna Sub-1was 3.73 g/kg, and in Swarna was 6.05 g/kg. Minimum phytic acid content after germination measured in the Sarjoo-52 was 3.08 g/kg and maximum phytic acid content after germination measured in Swarna was 6.05 g/kg. Minimum phytic acid content after germination in Sarjoo-52 was 3.08 g/kg while maximum phytic acid measured in Swarna was 6.05 g/kg. Significant correlation was obtained regarding phytic acid content after germination of seeds in various genotypes in present investigation.

Table 4.3.2: Phytic acid content and its influence on soaking and germination (g/kg)

S. No	Genotypes	Phytic acid content (g/kg)	After Soaking (g/kg)	After Germination (g/kg)
1	Sarjoo- 52	5.61	3.63	3.08
2	Madhukar	6.39	4.44	3.89
3	NDR-359	8.05	5.74	5.49
4	CSR-13	6.72	4.89	4.31
5	Swarna Sub-1	7.4	4.34	3.73
6	Swarna	9.12	6.76	6.05

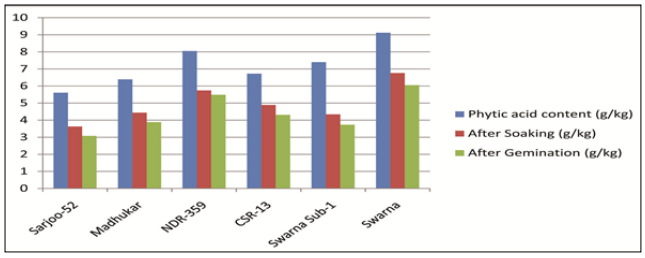

Fig 2: Histogram showing the Phytic acid content and its influence on soaking and germination in rice seed

Iron content

The data pertaining to the iron content presented in Table No. 3 and graphically shown in figure– 3. Iron content in parents Sarjoo-52 was 24.15 ppm, in Madhukar was 17.38 ppm, in NDR-359 was 15.39 ppm, in CSR-13 was 21.13 ppm, in Swarna Sub-1 was 18.20 ppm and in Swarna was 12.48 ppm. The highest iron was obtained in sarjoo-52 (24.15 ppm) and lowest obtained in swarna (12.48 ppm),

Table 3: Iron content in ppm

S. No	Genotypes	Iron content (ppm)
1	Sarjoo- 52	24.15
2	Madhukar	17.38
3	NDR-359	15.39
4	CSR-13	21.13
5	Swarna Sub-1	18.20
6	Swarna	12.48

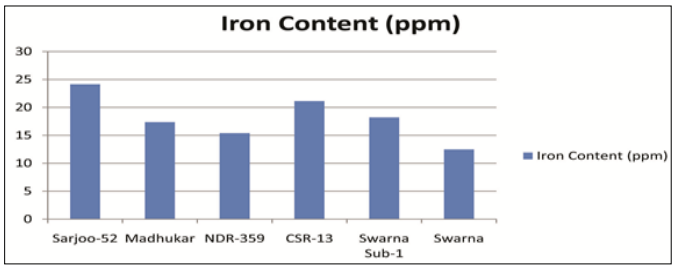

Fig 3: Histogram showing the iron in rice seed

Discussion

In this study we found that soaking, germination significantly reduces phytic acid of seeds in all selected verities of rice studied. These results support earlier findings in white rice flour (Reddy and Salunkhe, 1980) [34]. Among the various seed treatments, soaking appeared to be most effective in decreasing phytic acid content. Soaking and germination of seeds activate the endogenous phytase enzymes that hydrolyse phytic acid to free myo-inositol and inorganic phosphate via lower inositol phosphate esters (IP5-IP1) (Yuwei *et al*., 2009; Honke *et al*., 1998; Beal and Mehta, 1985; Eskin and Wiebe, 1983; Kozlowska *et al*., 1996; Tabekhia and Luh, 1980; Lestienne *et al*., 2005a) [49, 14, 2, 7, 19, 41, 23]. The reduction in phytic acid content during germination was a time-dependent process, confirming previous studies indicating that the activity and/or production of phytase increased during steeping (Henderson and Ankrah, 1985; Larsson *et al*., 1997; Yuwei *et al*., 2009) [49, 13, 20]. The dietary fibres are known to bind to nutritionally significant minerals. Cellulose, hemicellulose, pectins, lignin and other polysaccharides form insoluble complexes with mineral nutrients and thus makes them unavailable. Many reports have shown that the affinity of the dietary fibres for different minerals varies (Idouraine *et al*., 1996; Maha Lakshmi and Sumathi, 1997) [17, 27] and hence inconsistency in seed zinc and iron content upon processing. The treatment soaking, germination significantly reduced the seed phytic acid in all the species studied.

References

1. Anne S, Paula B. Household Dietary Diversity Score (HDDS) for Measurement of Household Food Access: Indicator Guide version 2. Food and Nutrition Technical Assistance Project, Academy for Educational Development, NW, 2006. http://www.fantaproject.org.
2. Beal L, Mehta T. Zinc and phytate distribution in peas: influence of heat treatment, germination, pH, substrate and phosphorus on pea phytate and phytase. J Food Sci. 1985; 50:96-100.
3. Bilyeu KD, Zeng P, Coello P, Zhang ZJ, Krishnan HB, Bailey A, *et al*. Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. Plant Physiol. 2008; 146:468-477.
4. Bohn L, Josefsen L, Meyer AS, Rasmussen S. Quantitative analysis of phytate globoids isolated from wheat bran and characterization of their sequential

dephosphorylation by wheat phytase. Journal of Agricultural and Food Chemistry. 2007; 55**:**7547-7552.
5. Bouis HE. Special issue on improving human nutrition through agriculture. Food Nutr. Bull. 2000; 21:351-576.
6. Cakmak I, Kalayci M, Ekiz H, Braun HJ, Kilinc Y, Yilmaz A. Zinc deficiency as an actual problem in plant and human nutrition in Turkey: a NATO-Science for Stability Project. Field Crops Res. 1999; 60:175-188.
7. Eskin NAM, Wiebe S. Changes in phytase activity and phytate during germination of two faba bean cultivars. J Food Sci. 1983; 48:270-271.
8. FAO. The State of Food and Agriculture, Food System for Better Nutrition, 2013. www.fao.org
9. Gibson RS. Content and bioaccessibility of trace elements in vegetarian diets. *Am. J. Clin. Nutr.* 59: 1223S–1232S.
10. Gibson RS, Hotz L, Temple C, Yeudall F, Mtitimuni B, Ferguson E. Dietary strategies to combat de ciencies of iron, zinc, and vitamin A in developing countries: development, implementation, monitoring, and evaluation. Food. Nutr. Bull. 2000; 21:219 231.
11. Graf E, Eaton JW. Antioxidant functions of phytic acid. Free Radical Biology and Medicine. 1990; 8:61-69.
12. Harbach APR, Costa MCR, Soares AL, Bridi AM, Shimokomaki M, Silva CA, Ida EI. Dietary corn germ containing phytic acid prevents pork meat lipid oxidation while maintaining normal animal growth performance. Food Chemistry, 2007; 100:1630-1633.
13. Henderson HM, Ankrah SA. The relationship of endogenous phytase, phytic acid and moisture uptake with cooking time in *Viciafaba* minor cv. Aladin. Food Chem. 1985; 17:1-11.
14. Honke J, Kozlowska H, Vidal-Valverde C, Frias J, Gorecki R. Changes in quantities of inositol phosphates during maturation and germination of legume seeds. Z Lebensm Unters Forsch A. 1998; 206:279-283.
15. Hurrell RF, Reddy MB, Juillerat MA, cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am. J Clin. Nutr. 2003; 77:1213-1219.
16. Hurrel RF. Phytic acid degradation as a means of improving iron absorption. International Journal for Vitamin and Nutrition Research. 2004; 74:445-452.
17. Idouraine A, Khan MJ, Weber CW. In vitro binding capacity of wheat bran, rice bran, and oat fiber for Ca, Mg, Cu, and Zn alone and in different combinations. J Agric. Food Chem. 1996; 44:2067-2072.
18. Jianfen Liang, Bei-Zhong Han, Longzhi Han MJ, Robert Nout, Robert JH. Iron, zinc and phytic acid c ontent of selected rice varieties from china. J Sci. Food. Agricult. 2007; 87:504-510.
19. Kozlowska H, Honke J, Sadowska J, Frias J, Vidal-Valverde C. Natural fermentation of lentils: Influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. J Sci. Food Agric. 1996; 71:367-375.
20. Larsson M, Sandberg AS. Malting of oats in a pilot-plant process. Effects of heat treatment, storage and soaking conditions on phytate reduction. J Cereal Sci. 1995; 21:87-95.
21. Lee SH, Park HJ, Chun HK, Cho SY, Cho SM, Lillehoj HS. Dietary phytic acid lowers the blood glucose level in diabetic KK mice. Nutrition Research. 2006; 26:474-479.
22. Lee BJ, Hendricks DG. Phytic acid protective effect beef round muscle lipid per oxidation. Journal of Food Science. 1995; 60(2):241-244.
23. Lestienne I, Icard-Verniere C, Mouquet C, Picq C, Treche S. Effects of soaking whole cereal and legume seeds on iron, zinc, and phytate contents. Food Chem. 2005; 89:421-425.
24. Liang J, Han BZ, Nout MJR, Hamer RJ. Effects of soaking, germination and fermentation on phytic acid, total and in vitro soluble zinc in brown rice. Food Chem. 2008; 110:841-848.
25. Li CY, Park DS, Won SR, Hong AK, Ham JK, Choi JK, Rhee HI. Food chemical properties of low-phytate rice cultivar, Sang-gol. Journal of Cereal Science. 2008; 47:262-265.
26. Lucca P, Hurrell R, Potrykus I. Approaches to improving the bioavailability and level of iron in rice seeds. J Sci. Food., Agric. 2001; 81:828-834.
27. Maha Lakshmi R, Sumathi S. Binding of iron, calcium and zinc by fibre of sorghum and ragi. Food Chem. 1997; 60:213-217.
28. Mendoza C. Effect of genetically modi ed low phyticacid plants on mineral absorption. Int. J Food. Sci. Technol. 2002; 37:759-767.
29. Norazalina S, Norhaizan ME, Hairuszah I, Norashareena MS. Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethaneinduced colon carcinogenesis in rats. Experimental and Toxicologic Pathology. 2009; 6(3):259-268.
30. Nunes ACS, Cuneo F, Amaya-Farfán J, Capdeville GD, Rech EL, Aragão FJL. RNAi- mediated silencing of the myo-inositol-1-phosphate syn- thase gene (GmMIPS1) in transgenic soybean inhibited seed development and reduced phytate content. Planta. 2005; 223:45-51.
31. Paulik SJ, Bastidas RJ, Chiou ST, Frye RA, York JD. Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. PNAS. 2005; 102:12612-12617.
32. Ramakrishna VP, Jhansi R, Ramakrishna R. Anti-nutritional factors during germination in Indian bean (*Dolichoslablab* L.) seeds. World Journal of Dairy & Food Sciences. 2006; 1(1):06-11.
33. Reddy NR, Sathe SK, Salunkhe DK. Phytates in Legumes and Cereals. Adv. Food Res. 1982; 28:1-91.
34. Reddy NR, Salunkhe DK. Effect of fermentation on phytate phosphorus and mineral content in black gram and rice. J Food Sci. 1980; 45:1708-1712.
35. Saw NK, Chow K, Rao PN, Kavanagh JP. Effects of inositol hexaphosphate (Phytate) on calcium binding, calcium oxalate crystallization and in vitro stone growth. The Journal of Urology. 2007; 177:2366-2370.
36. Selle PH, Ravindran V. Phytate-degrading enzymes in pig nutrition. Livestock Science. 2008; 113:99-122.
37. Schelemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition & Food Research. 2009; 53:S330-S375.
38. Shailen N, Michelle I, David G, Subramanian SV, George DS. Poverty, child under nutrition and morbidity: new evidence from India. Bull. World Health Organ. 2005; 83(3):210-216.
39. Soares AL, Olivo R, Shimokomaki M, Ida EI. Synergism between dietary vitamin E and exogenous phytic acid in prevention of warmed-over flavour development in chicken breast meat Pectoralis major. M. Brazilian. Archives of Biology and Technology. 2004; 47:57-62.

40. Stodolak B, Starzynska A, Czyszczon M, Zyła K. The effect of phytic acid on antioxidant stability of raw and cooked meat. Food Chemistry. 2007; 101:1041-1045.
41. Tabekhia MM, Luh BS. Effect of germination, cooking and canning on phosphorus and phytate retention in dry beans. J Food Sci. 1980; 45:406-408.
42. Vucenik I, Shamsuddin AM. Protection against cancer by dietary IP6 and inositol. Nutrition and Cancer. 2006; 55:109-125.
43. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp. Bot. 2004; 55:353-364.
44. White PJ, Broadley MR. Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist. 2009; 182:49-84.
45. Wheeler EL, Ferrel RE. A method for acid determination in wheat and wheat fractions. American association of cereal chemists, inc. 1971; 48:314.
46. White PJ, Brown PH. Plant nutrition for sustainable development and global health. Annals of Botany. 2010; 105:1073-1080.
47. WHO Report. Undernourishment around the world in 2012, 2014. http://www.fao.org/docrep/016/i3027e/i3027e02.pdf
48. Xu Q, Kanthasamy AG, Reddy MB. Neuroprotective effect of the natural iron chelator, phytic acid in a cell culture model of Parkinson's disease. Toxicology. 2008; 245:101-108.
49. Yuwei L, Weihua X, Chunyan X, Yan L, Zhenxin G. Impact of soaking and phytase treatments on phytic acid, calcium, iron and zinc in faba bean fractions. Int. J Food Sci. Tech. 2009; 44:2590-2597.