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Full Length Research Paper

Effect of processing on phytic acid content and nutrient availability in food grains

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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. Zinc and iron are among the important nutrients required for human growth and development. This study was aimed to know the effect of cooking on nutrient availability with respect to relative phytic acid contents. Seeds of five different economically important food crops namely peanut, sunflower, soybean, pigeon pea and rice were used. Reduced phytic acid content was observed in cooked seeds of peanut (47 %), soybean (52 %), sunflower (56.5 %), pigeon pea (49.5 %) and rice (47.9 %). However, soybean and rice zinc contents and peanut, pigeon pea and rice iron contents were increased in cooked seeds. The mean nutrient availability in cooked and non- cooked seeds was statistically non-significant ($p < 0.05$). The cooking treatment significantly ($p < 0.05$) reduced the inherent phytic acid content in seeds besides soaking and germination treatments, although the phytic acid break down did not show increased nutrients content as expected in all food crops.

Key words: Cooking, processing, food grains, nutrients, phytic acid, zinc, iron.

INTRODUCTION

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). 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). 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). 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). A few cases such as iodine, zinc, iron and selenium micronutrient deficiencies can be attributed to particular geological conditions where the soils are low in these minerals (Coelho et al., 2007; Raboy et al., 2001). Fortification and strategies for supplementation of food have proved to be unrealistic in several developing countries for economic reasons, due to poorly developed education and poor communication systems and lack of general infrastructure (Turner et al., 2002). In addition, the presence of endogenous anti-nutritional factors (ANFs) poses a serious threat to bioavailability of essential micronutrients apart from soil fertility. These "antinutritional factors" or antinutrients, include phytate, oxalate, trypsin inhibitors, lectins, glucosinolates and others (Shi et al., 2003), among which phytic acid is seen extensively in seeds of all crops.

Phytic acid (PA; myo-inositol hexaphosphate) is a ubiquitous biomolecule present abundantly in plants wherein PA phosphorus constitutes the major portion of total phosphorus in several seeds and grains (Harland and Overleas 1987). It accounts for 50–80% of the total phosphorus in different cereals (Raboy 2003; Stangoulis et al., 2007; Debjit et al., 2010). However, the presence of certain forms of a particular nutrient can hinder the uptake of other nutrients especially micronutrients. PA forms insoluble complexes with polycations like zinc and

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iron due to reactive phosphorus groups attached to its inositol ring (Pedersen et al., 2007; Sandberg and Svanberg 1991), which in turn renders these essential nutrients unavailable for human intestinal absorption. Phytic acid especially reduces the bioavailability of divalent cations.

Zinc and iron are the essential micronutrients and are key co-factors for many enzymes, involved in growth and developmental processes (Duhan et al., 2002). Zn deficiency is affecting 3 to 4 billion people which accounts for 49% of the world population (Ramakrishna et al., 2006).

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). 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).

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). 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).

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 cooking, germination and soaking treatments for effective removal of phytic acid to improve the micronutrient bioavailability in the seeds of economically important food crops such as peanut, sunflower, soybean, pigeon pea and rice.

MATERIALS AND METHODS

Seed Procurement and Sample Preparation

Seeds of groundnut (variety K-134), sunflower (variety KBSH), soybean (variety MAUS 61), pigeon pea (varietyTTB7) and rice (varietyKRH2) were procured from the National Seed Project, Department of Seed Science and Technology, University of Agricultural Sciences, GKVK campus, Bangalore, Karnataka, India. The seed coat of sunflower and rice husk was mechanically removed from procured seeds and processed as follows: a) one batch of seeds were pressure cooked at 15 1bs per square inch for 10 minutes, b) second batch of seeds were used for germination and, c) third batch of seeds were used for water soaking for 8 hours (water to seed ratio being 1:5). A batch of unsoaked seeds was maintained as control.

Determination of phytic acid and inorganic P (P_i)

Phytic acid and P_i in dry, mature seeds were assayed following Robay et al. (2001). One millilitre of 0.4 N HCl was added to 35 mg each of ground sample in an eppendorff tube and shaken on a gyratory shaker at room temperature for 3.5 hours. The tubes were then centrifuged at 3,900 x g for 15 minutes and the supernatant was transferred into fresh tubes and used for both phytic acid and Pi estimation.

Estimation of Phytic Acid

For phytic acid assay, 35 µLof distilled water and 140 µL of 0.02% (w/v) ammonium iron (III) sulfate-0.2 N HCl were added to 35 µL of each extract in an eppendorf tube and heated on water bath at 99˚C for 30 minutes. The tubes were then cooled to 4˚C for 15 minutes and then incubated at room temperature for 20 minutes. The tubes were then centrifuged at 3,900 x g at 24°C for 30 minutes and85 µL of each supernatant was added to 120 µL of 1% (w/v) 2, 2'-bipyridine-1% (v/v) thioglycolic acid in a 200 µL tube and absorbance was recorded at 519nm using UV-spectrophotometer (Shimadzu UV-1800). Authentic phytic acid (P-7660, Sigma-Aldrich) was used as the standard.

Estimation of Inorganic Phosphorus (Pi)

To determine Pi, 100 µL of 30% (w/v) aqueous trichloroacetic acid was added to 200 µL each of the extract in eppendorf tubes and the tubes were shaken and centrifuged at 3,900 x g for 10 minutes. 50 µL of each supernatant was transferred into a fresh tube and 100 µL of 0.42 % (w/v) ammonium molybdate-1 N H_2SO_4: 10% (w/v) ascorbic acid (7:1) was added. The tubes were incubated at 37°C for 30 minutes and then A_{800} was measured. Potassium phosphate dibasic anhydrous (7758-11-4, Sisco Research Laboratories Pvt. Ltd) was used as a standard and the estimated Pi content was expressed as Pi phosphorus.

Estimation of Micronutrients

Acid digestion: 5 ml of concentrated nitric acid was added to 250 mg of powdered grain sample and incubated overnight for digestion. The next day, 5 ml of diacid mixture (Nitric acid: Perchloric acid in 10:4 ratio) was added and placed on a sand bath till all the white fumes evaporated and thick white residue was left in the flask. It was allowed to cool and the volume was made up to 25 ml using triple distilled water and further dilutions were made wherever necessary. These diluted solutions were used for zinc and iron estimation.

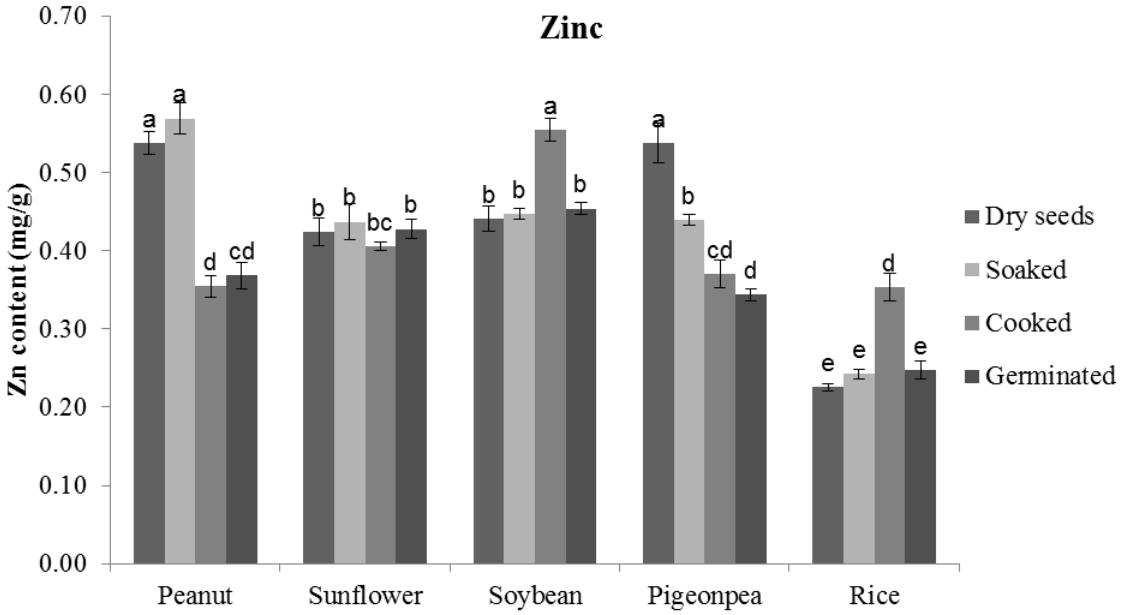

Figure 1. Estimation total zinc (mg g^{-1} of seed) content. The dry, soaked, cooked and geminated seeds of peanut, sunflower, soybean, pigeonpea and rice were used to estimate Zn content. A known amount of seeds were taken in three sets and maintained in three replications. Different treatments like overnight soaking in distilled water, unsoaked seeds, cooking and germination were used. All the experiments were carried in three replications. Statistical significance was calculated ($p<.05$).

Zinc and iron were estimated by using polarized Zeeman Atomic Absorption Spectrophotometer (AAS-2-6100). Standard zinc and iron solutions (Sisco Research Laboratories Pvt. Ltd) ranging from 0- 0.6 ppm and 0.2-6.0 ppm respectively were used in the study. These standards were fed to AAS to get the standard curve to which the sample readings were compared and then computed.

Suitable dilutions were made from the extractant with de-ionized triple distilled water and the absorbance was recorded. The grain zinc and iron content was calculated using the following formula (Liang et al., 2008; Yamunarani et al., 2013):

$$\text{Total Zinc/ Iron (mg/100g seed)} = \frac{\text{Average ppm x Volume of digested sample x Volume made up}}{10^6 \text{ x Weight of the sample x Aliquot taken}}$$

Statistical Analysis

The analysis of variance for different treatments among different crops was carried out to assess the variability in estimated nutrients using MSTATC software. Duncan's multiple range test was performed using MSTATC to assess the significance across the different treatments ($p < 0.05$).

RESULTS

Effect of Soaking, Germination and Cooking on Zinc, Iron, Inorganic Phosphorus and Phytic Acid Content of Seeds

Zinc

Zinc content of peanut seeds decreased significantly upon cooking (0.35mg/g) and germination (0.37mg/g) (Figure 1).

In pigeonpea, all treatments significantly reduced seed zinc content (up to 0.32mg/g). In contrast however, seed processing did not have any effect on seed zinc content in sunflower. In soybean seeds, cooking increased seed zinc content (0.57mg/g) (Figure 1). However, the soaking and germination did not have any effect on seed zinc content in soybean.

Similarly, zinc content of rice seeds increased significantly upon cooking (0.35mg/g). The soaking and germination did not have effect on seed zinc content in rice well (Figure 1). The seed processing treatments did not have constant effect on seed zinc content of peanut, sunflower, soybean and rice.

Iron

Seed iron content increased with soaking, cooking and germination of peanut seeds compared to dry seeds ($p< 0.05$; Figure 2). On the contrary, seed iron content decr-

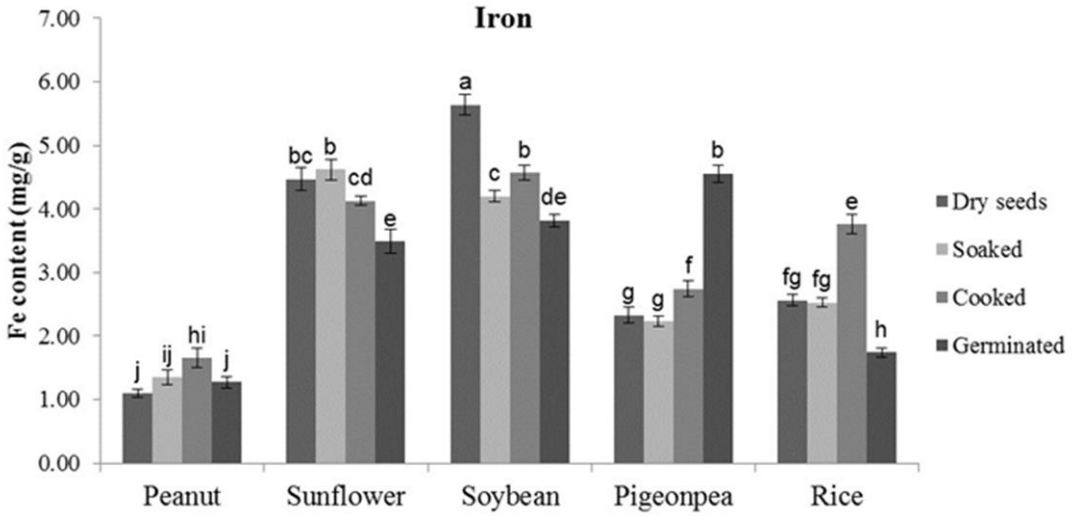

Figure 2. Total iron (mg g-1 of seed) content. Estimated the iron content in dry, soaked, boiled and geminated seeds of peanut, sunflower, soybean, pigeonpea and rice. Three sets of each crop seeds were taken and subjected to overnight soaking in distilled water, cooking and germination. All the experiments were carried out in three replications. Statistical significance was calculated (p<.05)

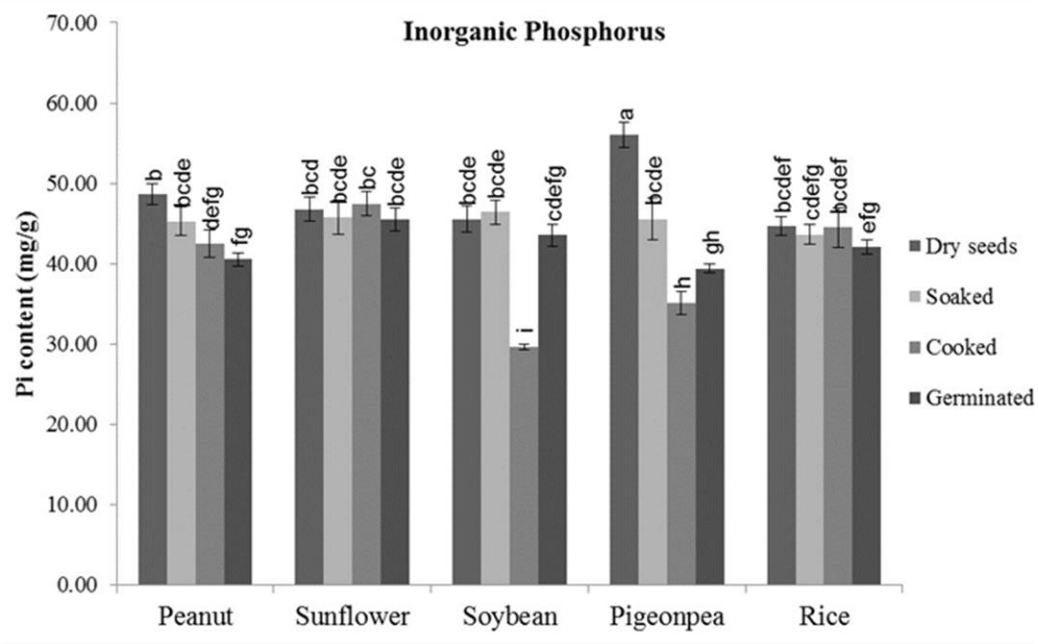

Figure 3. Estimation of inorganic phosphorus (mg g^{-1} of seed) content. The dry, soaked, boiled and geminated seeds of peanut, sunflower, soybean, pigeonpea and rice were used to estimate inorganic phosphorus content. Three sets of each crop seeds were taken and subjected to overnight soaking in distilled water, cooking and germination. All the experiments were carried out in three replications. Statistical significance was calculated (p<.05)

eased significantly in sunflower (4.15mg/g) and soybean (4.6mg/g) upon germination and cooking. Iron content increased in germinated pigeonpea seeds (4.8mg/g). The iron content of pigeon pea seeds was however unaffected by seed treatment. Antagonistically iron content increased with cooking (3.8mg/g) and however decreased significantly upon germination (1.7mg/g) in rice (Figure 2).

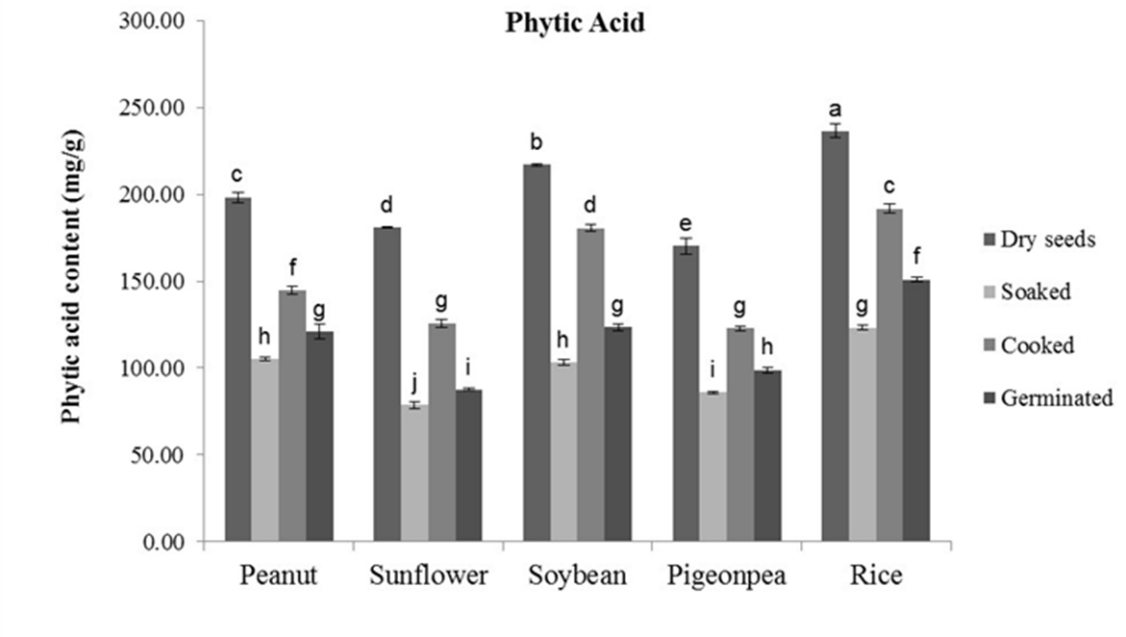

Figure 4. Estimation of phytic acid content (mg g^{-1} of seed) in dry, soaked, boiled and geminated seeds of peanut, sunflower, soybean, pigeonpea and rice. Three sets of each crop seeds were taken and subjected to overnight soaking in distilled water, cooking and germination. All the experiments were carried out in three replications. Statistical significance was calculated (p<.05)

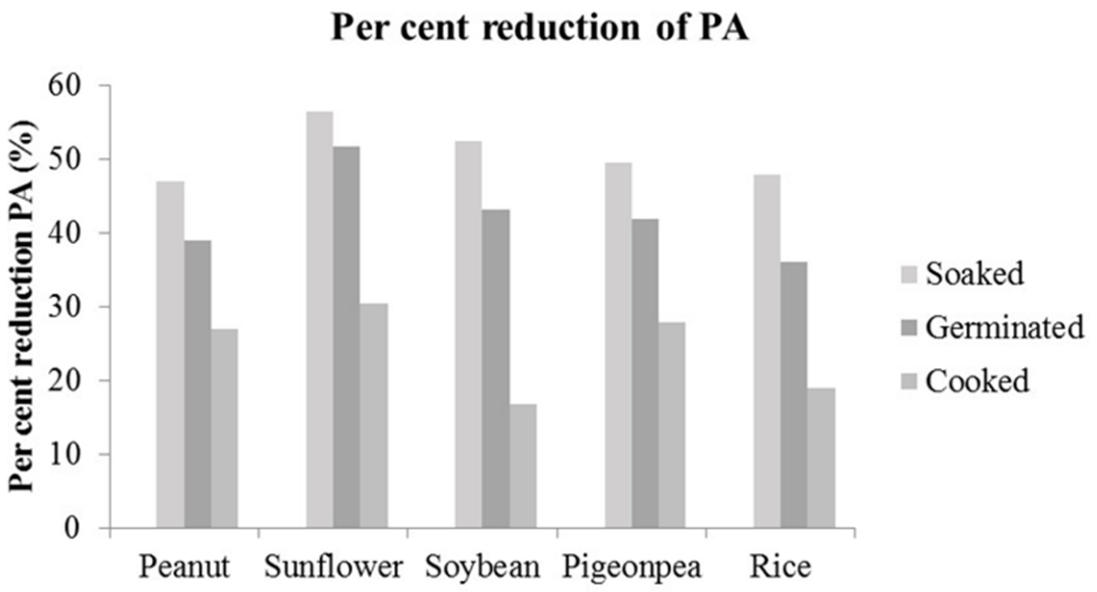

Figure 5. Per cent (%) phytic acid reduction over the dry seeds of different crop seeds. The total phytic acid contents of the soaked, germinated and cooked seeds were used to calculate per cent reduction (%) over dry seeds, wherein dry seed phytic acid was considered as control. Three sets of each crop seeds were taken and subjected to overnight soaking in distilled water, cooking and germination. All the experiments were carried out in three replications.

Inorganic Phosphorus

Inorganic phosphorus content of peanut and pigeonpea seeds decreased significantly in soaked, cooked and germinated seeds when compared to dry seeds (Figure 3). However, in case of rice and sunflower, the total inorganic phosphorus content remained unaffected by seed treatments. However the seed inorganic phosphorus content was reduced significantly upon cooking in soybean (Figure 3).

Phytic Acid

Phytic acid content of peanut, sunflower, soybean, pigeonpea and rice reduced significantly in soaked, cooked and germinated seeds when compared to dry seeds (Figure 4). The seed phytic acid content reduced significantly in peanut (47%), sunflower (56.5%), soybean (52%), pigeonpea (49.5%) and rice (47.9%) upon soaking (Figure 5).The cooking and germination treatments however reduced significantly the seed phytic acid content in peanut, sunflower, soybean, pigeonpea and rice (Figure 4 and 5).

DISCUSSION

In this study we found that soaking, germination and cooking significantly reduces phytic acid of seeds in all species studied.

These results support earlier findings in faba bean (Yuwei et al., 2009), white rice flour (Reddy and Salunkhe, 1980), and oats and corn (Fageer et al., 2004; Larsson and Sandberg, 1995). Among the various seed treatments, soaking appeared to be most effective in decreasing phytic acid content. Soaking small millet, soybean, maize, sorghum and mungbean seeds at room temperature for 24 h decreased phytic acid content by 50% (Lestienne et al., 2005; Lestienne et al., 2005b). Similarly, a 21 % reduction in phytic acid was obtained in sorghum flour soaked at room temperature for 24 h (Mahgoub and Elhag, 1998) and a 51% reduction in case of pounded maize (Hotz et al., 2001). 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).

In contrast to soaking, the cooking treatment did not cause substantial decrease in seed phytic acid, perhaps because of reduction in the longevity of the phytase enzyme due to heating. Pertaining to this circumstance studies in barley and wheat had shown that non-heated treatment maintained higher phytase enzyme and hence decreased phytic acid in seeds (Carlson and Poulsen, 2003). 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). Besides reduction in phytic acid caused by soaking seeds, retention and solubility of zinc and iron were also affected by seed processing (Pedersen et al., 2007; Yuwei et al., 2009; Bishnoi and Yadav, 1994). Retention of zinc and iron was significantly lower in seeds that were soaked and germinated (Figure 1 and 2).

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) and hence inconsistency in seed zinc and iron content upon processing.

The treatment soaking, germination and cooking significantly reduced the seed phytic acid in all the species studied. However this did not result in a proportionate increase in available zinc and iron, probably due to the presence of dietary fibre in the seed.

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