Influence of Germination on Mineral Bioavailability and Phytic Acid Content in Rice

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Abstract

The effect of germination on phytase activity, phytic acid content, antioxidant activity, and bioavailability of minerals in rice (*Oryza sativa* L.) was investigated. Paddy rice was germinated for 0,1,2,3 and 4 days at room temperature (28±2°C) and the rice husks, rice bran, brown rice, white rice, rootlets, and shoots were individually separated and examined. Phytase activity of all rice fractions increased significantly after germination, and was highest in brown rice followed by rice bran after four days of germination. The phytic acid content of germinated rice and rice bran decreased from 39.24–14.18 mg/g and 63.35–46.34 mg/g, being 63.9% and 26.9% reductions compared to the initial phytic acid content, respectively. Only a small amount of phytic acid was detected in the rootlets and shoots, while it was not detected in the husks. The germination of the rice significantly increased the phosphorus content and the bioavailability of calcium (Ca), iron (Fe), magnesium (Mg), and phosphorus (P). There was no effect on the content of Ca, Fe, Mg (rice bran fraction), and antioxidant activity of germinated rice extract. Results suggest that germination is an effective process to reduce the level of phytic acid and to improve the quality of rice grains for enhanced the bioavailability of minerals.

Keywords: Bioavailability, Germinated rice, Phytic acid, Mineral, Antioxidant

1. Introduction

Phytic acid or phytate is a phosphorus reserve stored in the form of phosphorylate inositol which is synthesized and gradually accumulated in cereal and legume seeds (Miller *et al*., 1980). Six forms of phosphorylate inositol including inositol monophosphate (IP1), inositol biphosphate (IP2), inositol triphosphate (IP3), inositol tetraphosphate (IP4), inositol pentaphosphate (IP5), and inositol hexaphosphate (IP6) are found naturally (García-Estepa *et al*., 1999). IP6 is the most common form found in cereals and seeds (Park *et al*., 2006). However, phytic acid occurs naturally as a mixed-phytate in a complex with a number of mineral cations, such as zinc, calcium, potassium, and iron (Brinch-Pedersen *et al*., 2002). This mixed-phytic form is almost indigestible by monogastric animals and humans, who have low phytase activity to hydrolyze the phytic acid to smaller molecules, resulting in a reduction of the bioavailability of these nutrient elements (Brinch-Pedersen *et al*., 2002; Pointillart and Fontaine, 1986).

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Therefore, phytic acid is regarded as an anti-nutrient (Urbano *et al*., 2000). Alternatively, IP6 may have high potential to reduce the risk of various diseases such as colon cancer, heart disease, and kidney stones (Shamsoddin *et al*., 1992). Epidemiological studies have reported that phytic acid was efficient at inhibiting the development of colonic aberrant crypt foci (ACF) and colonic tumors in rats (Shamsuddin and Ullah, 1989; Thompson and Zang, 1991; Graf and Eaton, 1993).

Rice (*Oryza sativa* L.) is the main energy source in food in several countries. It is also a good source of phytic acid, especially in the bran fraction (5.88%) (Canan *et al*., 2011). Phytic acid is considered a natural anti-nutrient as it has strong ability to chelate multivalent metal ions which can result in poor bioavailability of such minerals. If consumers ingest a marginal diet in essential minerals, the phytic acid might cause nutritional deficiencies. This may be a significant problem as phytic acid weakens the function of the existing essential minerals in rice, especially in brown rice since it accumulates mainly in the bran layer and exists in the brown rice grain. The consumption of rice bran is a good source of dietary fiber. When brown rice is consumed as a functional food, there is a resultant increased in intake of phytic acid. To reduce the phytic acid intake, research work has examined possible activation of the native phytase and the impacts of different storage conditions on phytic acid content in rice. Development changes during the germination process provide a potential mechanism to convert the large phosphorylate inositol molecules to smaller inositol phosphates. This option has been examined by Centeno *et al*. (2001) with rye and barley and by Berrier-Guillot *et al*. (1996) with sorghum showing that germination reduced the phytic acid content. However, little work has been carried out to examine the influence of germination to improve the bioavailability of minerals in rice.

During the germination process, hydrolytic enzymes are activated with the potential to hydrolyze large molecules into smaller molecular substances, resulting in significant biochemical changes. Germination produces a significant increase in the range of chemical components and of bioactive compounds such as crude protein, crude lipid, total sugar, γ-oryzanol, and γ-aminobutyric acid. Simultaneously some substances, including phytic acid, show a decrease in content (Moongngarm and Saetang, 2010; Donkor *et al*., 2012; Kim *et al*., 2012). The germination process can also improve the digestibility of a cereal (Yang *et al*., 2001; Fernandez-Orozco *et al*., 2008; Frias *et al*., 2005). During germination the enzyme phytase is activated with the potential to hydrolyze phytic acid (IP6) to a lower IP value, such as IP5, IP4, and IP3. This may cause the release of minerals and improve bioavailability (García-Estepa *et al*., 1999; Ekholm *et al*., 2003). This study aimed to investigate the changes in phytase activity, phytic acid content, and the bioavailability of minerals in germinated rice.

2. Materials and Methods

2.1 Plant materials

Paddy or rough rice (cultivar RD-6) was obtained from a rice-milling factory in Mahasarakham Province, Thailand.

2.2 Chemical reagents

All chemicals and reagents used in this study were analytical grade and they were purchased from Fluka Chemical (Buchi, Switzerland), including butyratehydroxyanisole (BHA), ascorbic acid, and 2, 2-diphenyl-2-picylhydrazyl (DPPH).

2.3 Preparation of germinated paddy rice

The rice grains were prepared following the method of Moongngarm and Saetung (2010) with small modifications. Rough or paddy rice (4 kg/replicate; 3 replicates) was soaked in tap water at room temperature (28±2°C) for 48 h. The water was changed every 6–7 h throughout this time. The water was drained and the steeped rice seeds were collected and divided into four parts (1 kg/part). The steeped grains were then germinated in a basket following the method of Moongngarm and Saetung (2010). The germinated rice seeds were randomly collected every day for four days and dried in a tray dryer at 50±2°C until final moisture content reached 10% (% wet basis). The shoots and rootlets of the dried seeds were separated and the grains were milled. Four different fractions, namely, husk, rice bran, brown rice, and white rice were obtained. All samples were stored at 4°C in a sealed polyethylene bag until required for analysis.

2.3.1 Determination of phytase activity

Phytase activity was measured according to the method of Eeckhout and Paepe (1994).

2.3.2 Determination of phytic acid content

Phytic acid content was determined following the method of García-Estepa *et al*. (1999) as modified by Febles *et al*. (2001). The phytic acid content was calculated as:

$$\text{Phytic acid (\%)} = 1.32(10–V)/P$$

Where V is ethylenediaminetetraacetic acid (EDTA) solution volume (mL), and P is sample weight (g).

2.3.3 Determination of mineral elements in germinated rice

The mineral elements were analyzed following the AOAC (2000) official method. The sample (5g) was wet-digested with 10 mL conc. HNO_3 prior to determining the mineral composition by flame atomic absorption spectrometry (Model AA 660, Shimadzu, Japan) using an air-acetylene flame. A solution of ash, obtained from wet ashing, was used for Ca, Mg, and Fe analysis. Phosphorus was determined spectrometrically using the molybdate–vanadate method of Olsen and Sommers (1982).

2.3.4 Determination of bioavailability of minerals

Mineral availabilities were evaluated in vitro using the continuous-flow dialysis method of Bosscher *et al*. (2000). The availability of the element was calculated from the amount of element that had passed the dialysis membrane, proportional to the total element content of the grain sample, using the following equation:

$$\text{Availability (\%)} = (D \times 100)/(W \times A)$$

where D is the total content of the element in the dialysate after intralumenal digestion (mg), W is the weight of the grain sample used for the intestinal stage (g), and A is the concentration of the element in the grain sample (mg/g).

2.4 Sample extraction for antioxidant activity

To determine antioxidant activity, the phytic acid extract was prepared following the method described by Centeno *et al.* (2001) with some modifications. Samples (5 g) were added to 100 mL of 0.5 M HCl (pH 1) and held for 4 h in an automatic shaker with medium speed (Model LSI-1005R) at room temperature (27°C). The chloride extract was centrifuged at 17,300 g for 30 min at 15°C, and the supernatants were collected and neutralized with NaOH. The neutralized extract was then dried by freeze-drying and used for analysis. The dried extract was diluted with 20 mL of 80% methanol containing 0.15% HCl. The extracts obtained were used for antioxidant activity including DPPH radical scavenging activity and lipid peroxidation assay.

2.4.1 DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay, was evaluated using the method of Dasgupta and De (2004). The absorbance of the mixture at 517 nm was measured using a UV-visible spectrophotometer, and the percent inhibition activity was calculated as

$$[(A_0 - A_e)/A_0] \times 100$$

where A_0 is the absorbance without extracts, and A_e is the absorbance with extracts. The radical scavenging activity of the extracts was expressed as the concentration of the rice bran required for 50% inhibition of the free radicals (IC_{50} g/mL). In order to calculate the IC_{50} of the extracts, the logarithmic regression curve was established by plotting percentage of inhibition against serial dilutions of sample concentration. This represents sample concentration (mg/mL) required to decrease the radicals by 50% of the extracts.

2.4.2 Lipid peroxidation assay

Lipid peroxidation was determined using the method of Dasgupta and De (2004). The absorbance of the supernatant was measured at 532 nm. Inhibition of lipid peroxidation (%) was calculated as

$$[(1 - Ae)/Ac] \times 100$$

where *Ac* is the absorbance value of the control (without extracts), and *Ae* is the absorbance value of the samples (in the presence of oil extracts). The results were calculated and expressed as 50% inhibition of lipid peroxyl radical (IC_{50} values).

2.5 Statistical analysis

Data were statistically analyzed using the statistical software version SPSS 19.0 (trial version). F-test was used for data analysis of the effect of germinating times and germinated rough rice fractions on phytase activity, contents of phytate and essential minerals, as well as antioxidant activity. The difference between the means was compared using the Duncan Multiple Range Test at 5% probability.

3. Results and Discussion

3.1 Phytase activity

Germination time significantly increased phytase activity but reduced phytic acid content ($p<0.05$). For both brown rice and the rice bran fraction, phytase activity increased with germination time, with the highest phytase activity (1117.0 U/kg) shown by brown rice after four days of germination (Table 1). This may have been due to the high concentrations of phytic acid in these fractions, the main substrate which activates phytase activity. After four days of germination, the phytase activity increased tenfold compared to that in un-germinated rice. In contrast, phytase activity in the rootlets and shoots was not detected in the raw sample but it was found to increase rapidly on the first day of germination in the rootlets and on the second day in shoots. The phytase activity of rootlets and shoots decreased significantly after germination on days three and four (Table 1). Phytase activity in the rootlets and shoots was highest after two days of germination, and then it decreased. This result is in agreement with Centeno *et al*. (2001), Liang *et al*. (2008), who found that the phytic acid content of cereal seeds decreased with germination. An inverse correlation between the levels of both phytase activity and inorganic phosphorus was observed. Phytate breakdown was noted by Kikunaga *et al*. (1991). This reduction in phytic acid could have resulted from phytase and other related enzymes having complementary modes of action for reducing large phosphorylate inositol molecules to smaller molecular units.

Table 1 Phytase activity (U/kg) of germinated seed fractions prepared with different germination times

Germination time (days)	Brown rice	Rice bran	Rootlet	Shoot	Husk
0 (Raw)	152.52±14.14^{c}	166.30±8.62^{c}	ND	ND	4.22±0.28^{b}
1	161.67±6.05^{c}	179.08±8.59^{c}	506.45±22.84^{a}	ND	5.66±0.08^{a}
2	173.00±7.07^{c}	184.93±6.47^{c}	129.79±5.50^{b}	433.50±18.51^{a}	3.50±0.11^{c}
3	889.70±29.70^{b}	216.53±8.52^{b}	106.78±8.31^{b}	352.36±14.01^{b}	2.01±0.02^{d}
4	1,117.00±31.11^{a}	403.68±14.18^{a}	103.60±2.83^{b}	143.59±14.06^{c}	2.00±0.04^{d}

Note: Values are means as mean ± standard deviation (n = 3). ND = not detected.

^{a,b,c} Means within a column with different letters were significantly different (p<0.05).

3.2 Phytic acid content

Phytic acid content in brown rice and rice bran decreased with increasing germination time (Table 2). Phytic acid levels in ungerminated brown rice and rice bran were 39.24 mg/g and 63.35 mg/g, respectively. During germination from one to four days, the phytic acid content continually reduced from 57.73 mg/g to 46.34 mg/g in rice bran, 38.51 mg/g to 14.18 mg/g in brown rice, and 8.25 mg/g to 6.60 mg/g in white rice. In contrast, the phytic acid level in the rootlets and shoots did not change with germination time, and no phytic acid was detected in the husks.

The rice bran showed the highest levels of phytic acid ranging between 46.34 mg/g and 63.35 mg/g, followed by brown rice from 14.18 mg/g to 39.24 mg/g. Phytic acid was not detected in the husk fraction. These results concur with a previous study by García-Estepa *et al*. (1999), who reported that 57.71 mg/g of phytic acid in cereals was distributed in the rice bran layer. The results were also similar to Moongngarm and Saetung (2010), who found that phytic acid in germinated rice reduced significantly by 12.9%, 30.3%, and 25.8% for brown rice, rough rice, and rough rice powder, respectively, when compared with ungerminated grain. Kim *et al*. (2012) noted that germination significantly decreased phytic acid content in rough rice (from 3.57 mg/g to 2.17 mg/g), and brown rice (from 4.34 mg/g to 3.42 mg/g). This decrease in phytic acid may be due to an increase in phytase activity during germination. The phytase enzyme has the potential to hydrolyzephytic acid, changing the phytate to phosphate and myoinositol phosphates (Centino *et al*., 2001). A reduction of phytic acid has also been attributed to leaching during soaking of the grains (Lestienne *et al*., 2005).

Table 2 Total phytic acid content (mg/g) and phytic acid reduction (RD; %) in germinated rice fractions prepared with different germination times

Time (days)	Brown rice		White rice		Rice bran		Rootlet	Shoot	Husk
	Content	RD	Content	RD	Content	RD			
0 (Raw)	39.24±0.18^{a}	0	8.57±0.07^{a}	0	63.35±0.05^{a}	0	ND	ND	ND
1	38.51±0.11^{b}	1.86	8.25±0.46^{a}	3.73	57.73±0.25^{b}	8.87	0.35±0.07	ND	ND
2	20.78±0.04^{c}	47.04	7.28±0.03^{b}	15.05	57.38±0.13^{b}	9.42	0.32±0.05	0.18±0.03	ND
3	16.66±0.08^{d}	57.54	7.22±0.49^{b}	15.75	55.05±0.14^{c}	13.10	ND	0.19±0.04	ND
4	14.18±0.11^{e}	63.86	6.60±0.13^{c}	22.99	46.34±0.06^{d}	26.85	0.34±0.04	0.23±0.04	ND

Note: Time refers to germination time (day); Values are means as mean ± standard deviation (n = 3), ND refers to not detected; ^{a,b,c} Means within a column with different letters were significantly different (p<0.05). RD (Reduction) = % phytic acid reduction compared with initial content.

3.3 Effect of germination on mineral contents

The edible fractions of brown rice and rice bran were selected for further study on the content of minerals and their bioavailability in rice containing high phytic acid content. The concentration of Ca, Fe, and Mg (in rice bran) did not significantly increased following germination, especially with (Table 3). The concentrations of Ca and Fe in the rice bran from the raw sample (0 day) were 0.96 mg/g and 1.56 mg/g, and in brown rice 0.59 mg/g and 0.38 mg/g, respectively. After germination, the results indicated that germination had no effect on the content of Ca and Fe in both the bran fraction and the brown rice kernel whereas germination slightly reduced magnesium content. Phosphorus, the major mineral in the rice bran changed from 285.11 mg/g to 698.97 mg/g over the first three days and in brown rice it changed from 304.90 mg/g to 371.71 mg/g from day one today four.

Table 3 Concentration of minerals in rice bran and brown rice with different germination times

Germination time (day)	Ca (mg/g)	Fe (mg/g)	Mg (mg/g)	P (mg/g)
Rice bran				
0 (Raw)	0.96±0.05	1.56±0.05	33.71±0.30	296.02±18.87^{c}
1	1.04±0.07	1.68±0.10	33.93±0.12	285.11±15.31^{c}
2	1.03±0.01	1.59±0.08	33.78±0.12	336.93±14.30^{b}
3	1.01±0.02	1.51±0.10	30.44±0.15	698.97±40.2^{a}
4	1.00±0.01	1.75±0.18	31.65±0.20	667.95±17.40^{a}
Brown rice				
0 (Raw)	0.59±0.03	0.38±0.00	8.15±0.03^{b}	304.90±8.94^{b}
1	0.59±0.08	0.40±0.04	8.33±0.04^{a}	319.28±4.61^{b}
2	0.60±0.04	0.41±0.02	8.29±0.02^{a}	313.97±4.26^{b}
3	0.61±0.07	0.47±0.01	7.17±0.03^{c}	371.70±5.11^{a}
4	0.60±0.05	0.39±0.02	6.12±0.02^{d}	360.11±5.80^{a}

Note: Values are means as mean ± standard deviation (n = 3).

^{a,b,c} Means within a column with different letters were significantly different (p<0.05).

3.4 Effect of germination on bioavailability of minerals

The availability of minerals was obtained by determining the mineral content in the dialysate and compared with that in the rice fractions without dialysis for each germination time. The availability of Ca, Fe, and P in rice bran increased with increasing germination time (Figure 1A). The highest availability in both rice bran and brown rice was observed after days three and four of germination (Figure 1A and 1B). This maximum availability ranged from 85% to 86% for Ca, 16% to 29% for Fe, and 15% to 17% for P in rice bran, and in brown rice from 86% to 88% for Ca, 23% to 24% Fe, and 21% to 26% for P. Germination had no effect on the availability of Mg in rice bran but significantly increased after day four of germination in brown rice.

The minerals were likely chelated to the phytic acid with a negative effect on bio-availability and hence on the functioning of mineral nutrients which can lead to malnutrition in humans (Jacobsen and Slotfeldt-Ellingsen, 1983; Tang *et al*., 2008). The degradation of phosphorylate inositol from large to smaller molecules would have resulted from phytase activity. Phytase activity, therefore, reduced the mineral binding and consequently released minerals, increasing their availability as observed particularly in the rice bran. Ekholm *et al*.

(2003) reported that phytic acid efficiency was high leading to chelation with divalent metal ions of mineral elements with resultant poor mineral solubility. Oat bran treated with phytase produced higher mineral solubility, especially for Ca, Mg, and potassium (K). The greatest change in solubility was observed with K and zinc (Zn), while manganese (Mn), and Fe only increasing slightly. An uneven distribution of mineral elements has been found across different tissues in germinated rice. For example, Wang *et al*. (2011) found that phytic acid and four mineral elements, including Mg, Ca, Mn, and Fe, were not equally distributed with a high concentrations occurring in the outer layer (0–15%) depending on the degree of milling of the rice kernel. Itani *et al*. (2002) found that mineral elements decreased from the outer bran layers to the endosperm.

Results from this study indicated that most of the mineral elements and phytic acid were present in the rice bran, the mixture of pericarp, aleurone, sub-aleurone, and outer endosperm bran layers. This is in agreement with Lamberts *et al*. (2007), who reported that proteins were mostly distributed in the outer endosperm and the bran fraction contained most of the minerals (61.0%). Wang *et al*. (2011) found that mineral elements and phytic acid were highly concentrated in the fractions of the outermost part of indica rice kernels.

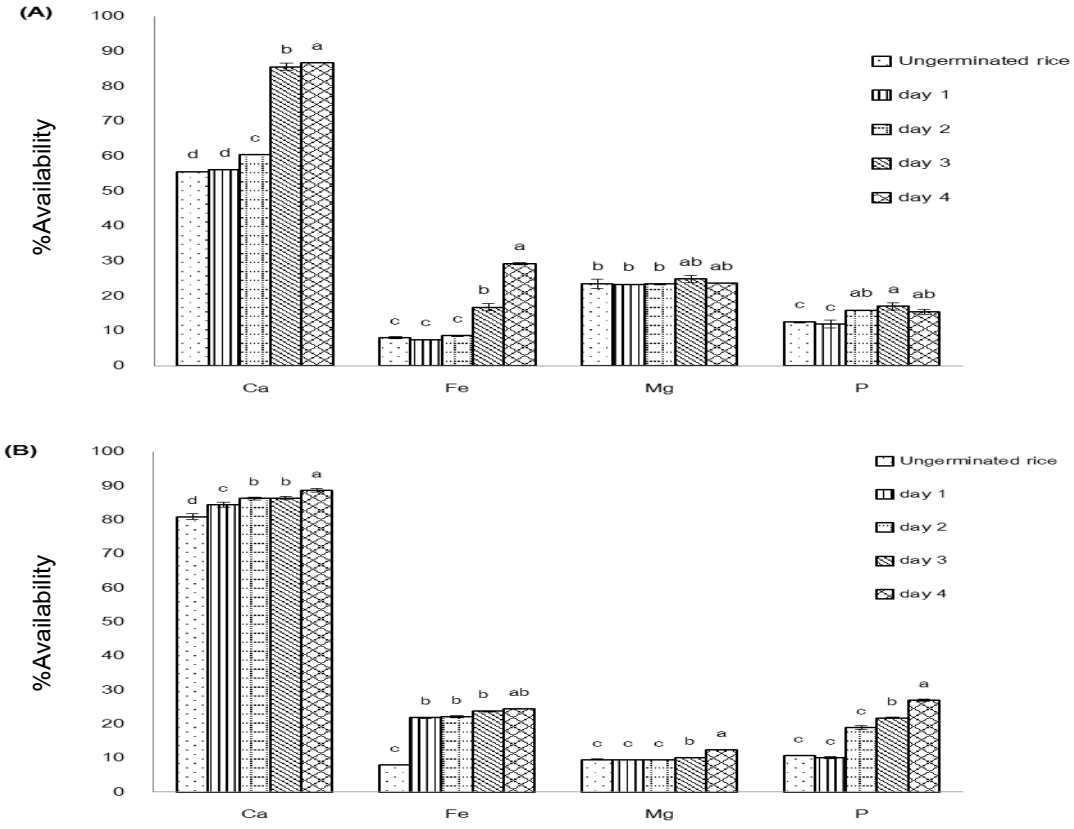

Figure 1 Percent availability of minerals in rice bran (A) and brown rice (B) obtained with different germination times. Each observation is mean ± SD of replicate experiments (n=3). The same letter above columns indicates no significant difference (p<0.05). The vertical bars on each column indicate the standard deviation.

3.5 Antioxidant activity of phytic acid rich rice bran extract

A rice bran fraction sample was extracted to prepare phytic acid rich extract (PRE) and used to evaluate antioxidant activity using two different methods, namely DPPH and lipid peroxyl radical scavenging activity. The antioxidant activity, expressed as an IC_{50} value (concentration of the extract required to inhibit 50% of the free radical), of PRE showed no significant difference due to germination time (Figure 2A). This may be due to several forms of phytic acid in the PRE had similar antioxidant efficiency (Figure 2B). This is in agreement with the studies of Graf *et al*. (1987), Sakaĉ *et al*. (2010) who reported that phytic acid in different forms, obtained from edible legumes, cereals, and seeds, could greatly accelerate Fe^{2+}-mediated oxygen reduction, block iron-driven hydroxyl radical generation, and suppress lipid peroxidation. Canan *et al*. (2012) noted that a maximum inhibitory activity of 40%, associated with its chelating capacity was found in rice bran phytic acid. Norhaizan *et al*. (2011) reported that phytic acid from rice bran, measured by DPPH radical scavenging, was 41.5% lower than butylatedhydroxytoluene (BHT) at 95.2%. Irradiation of phytic acid could increase DPPH radical scavenging which is achieved by transferring an electron or hydrogen atom to the corresponding DPPH radical (Norhaizan *et al*., 2011; Dasgupta and De, 2004). Lipid peroxidation is an undesirable reaction in food processing and storage, which can be retarded by antioxidants (Sakaĉ *et al*., 2010). However, studies on the antioxidant activity and antioxidant capacity of foods depend on several factors including the colloidal properties of substrates, conditions and stages of oxidation, and the localization of antioxidants in different phases (Frankel and Meyer, 2000). Therefore, it is recommended that the evaluation of antioxidant activity should be made under various oxidation conditions using different assays (Zulueta *et al*., 2009). These observations indicated a significant antioxidant function for phytate in germinated rough rice and suggested that phytate may have a use as a natural antioxidant.

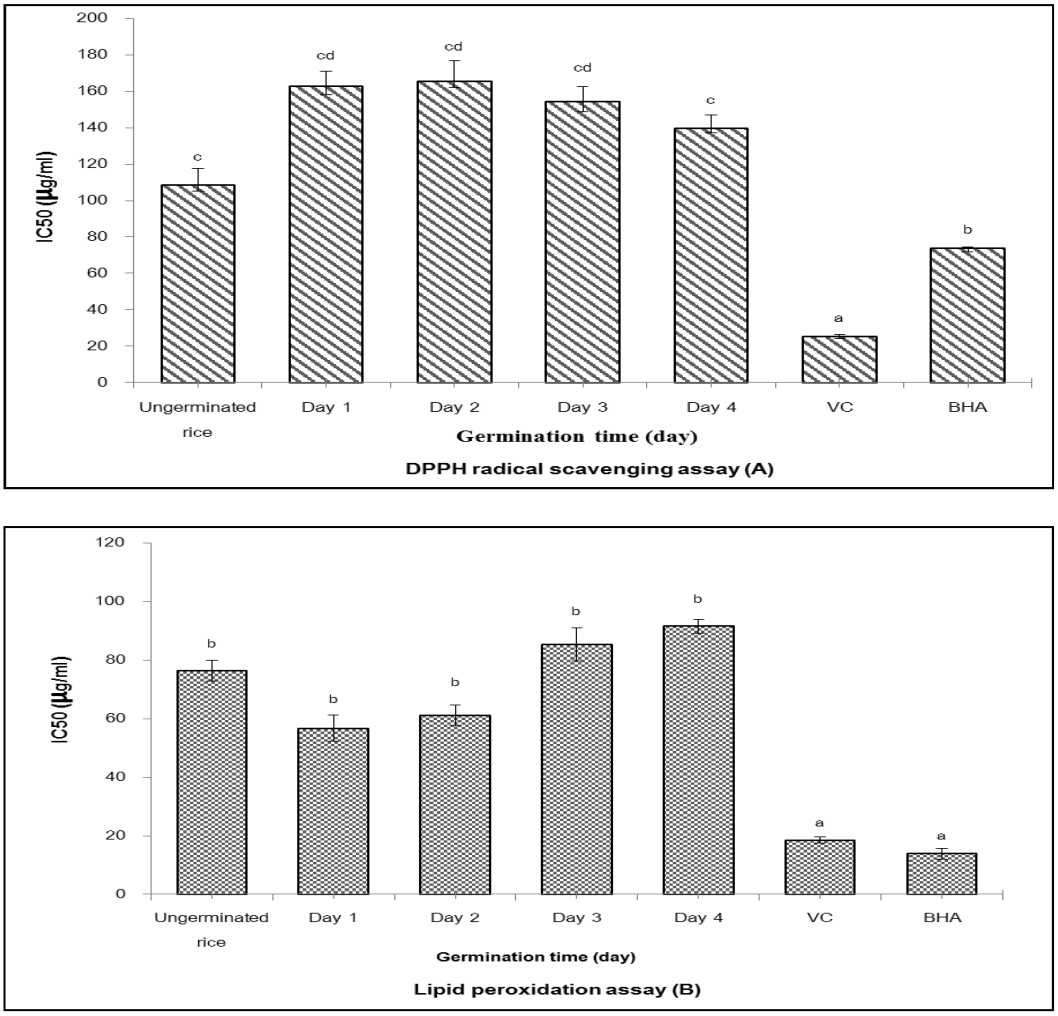

Figure 2 Antioxidant activities of phytic acid rich rice bran extract by DPPH radical scavenging (A) and lipid peroxidation assay (B).

Note: Each observation is mean ± SD of replicate experiments (n=3). The same letter above columns indicates no significant difference (p<0.05). The vertical bars on each column indicate the standard deviation. IC_{50}: Concentration of the extract to inhibit 50% of free radical. VC = ascorbic acid; BHA = Butylhydroxyanisole.

4. Conclusion

This study demonstrated that germination could reduce phytic acid content. The rice bran fraction showed the highest phytic acid content and phytase activity. Germination significantly increased the bioavailability of Ca, Fe, Mg, and P, whereas it did not affect the antioxidant activity of the phytic rich extracts prepared from the rice bran fraction. This suggests that germination of rice seeds is an effective process to lower the level of phytic acid and improve the bioavailability of minerals in rice grains, without any alteration of antioxidant activity of phytic rich extracts.

Acknowledgements

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