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Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice

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Abstract

Rice is an important staple food in Asian countries. In rural areas it is also a major source of micronutrients. Unfortunately, the bioavailability of minerals, e.g. zinc from rice, is low because it is present as an insoluble complex with food components such as phytic acid. We investigated the effects of soaking, germination and fermentation with an aim to reduce the content of phytic acid, while maintaining sufficient levels of zinc, in the expectation of increasing its bioavailability. Fermentation treatments were most effective in decreasing phytic acid (56–96% removal), followed by soaking at 10 °C after preheating (42–59%). Steeping of intact kernels for 24 h at 25 °C had the least effect on phytic acid removal (<20%). With increased germination periods at 30 °C, phytic acid removal progressed from 4% to 60%. Most wet processing procedures, except soaking after wet preheating, caused a loss of dry mass and zinc (1–20%). *In vitro* solubility, as a percentage of total zinc in soaked rice, was significantly higher than in untreated brown rice while, in steeped brown rice, it was lower ($p < 0.05$). Fermentation and germination did not have significant effects on the solubility of zinc. The expected improvement due to lower phytic acid levels was not confirmed by increasing levels of *in vitro* soluble zinc. This may result from zinc complexation to other food components.

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Keywords: Soaking; Germination; Fermentation; *In vitro* solubility; Phytic acid; Zinc

1. Introduction

In developing countries, cereals and legumes are the main dietary sources of macronutrients (carbohydrate, protein), as well as micronutrients, such as iron, calcium and zinc (Lestienne, Besançon, Caporiccio, Lullien-Pellerin, & Trèche, 2005; Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005; Lestienne, Mouquet-Rivier, Icard-Vernière, Rochette, & Trèche, 2005). In China, plant foods provide at least 50% of the dietary energy and nutrients (Ma et al., 2005), and rice is the most important staple

food. Unfortunately, rice is not a good source of metabolizable micronutrients, due to the presence of phytic acid. In plants, phytic acid is one of the main inhibitors of the availability of divalent cations such as Fe^{2+} , Ca^{2+} , Mg^{2+} and Zn^{2+} . The phosphate groups of phytic acid (inositol hexakisphosphate) form stable complexes with such cations, thus preventing their bioavailability.

The bioavailability of minerals from foods is defined as the proportion of the minerals that can be absorbed and utilized within the body (Larsson, Minekus, & Havenaar, 1997; Lestienne, Besançon et al., 2005; Lestienne, Icard-Vernière et al., 2005; Lestienne, Mouquet-Rivier et al., 2005). Solubility of minerals, pH of intestinal lumen, dietary factors and residence time at the absorption site influence the bioavailability of minerals (Larsson et al., 1997). The former could be predicted by molar ratios of phytic acid to minerals, HCl (hydrochloric acid)-extractability

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and *in vitro* solubility of minerals. Ma (2007) recently proposed that, when phytate and zinc are present in a molar ratio of 16:1, the zinc is no longer bioavailable. Typical ratios found for rice are: 4–15 and 61–74, for white and brown rice, respectively.

Since the health benefits of improving the bioavailability of minerals in rice are considerable, we have undertaken a systematic study consisting of three parts: (1) a screening of a wide range of rice varieties and growing conditions with respect to phytate and mineral contents, (2) a dry fractionation study aimed at identifying optimum conditions for removing phytate while retaining minerals and (3) a wet processing study with the same aim. The first study was published (Liang, Han, Han, Nout, & Hamer, 2007) and reported a wide variation in both minerals and phytate. The optimum combination (highest mineral concentration and lowest phytate content) did not provide a significant increase in predicted mineral bioavailability. In the second study (Liang et al., accepted), it was found that milling leads to considerable losses of both phytic acid (minerals inhibitor) and minerals. Since, for example, iron and phytate are similarly distributed in the kernel, a dry fractionation would provide only limited opportunities for their separation. On the other hand, zinc is more evenly distributed in the kernel and could be separated from phytic acid. Wet processing provides a third technical option to improve the bioavailability of minerals. Such food processing methods, which can be used both on the industrial scale and in the household, appear to be promising to combat deficiencies of iron and zinc (Gibson et al., 2000). It was reported that wet processing (including soaking, germination and fermentation) leads to a reduction in phytic acid and increase of solubility of minerals in foods, and could thus improve bioavailability of minerals in cereals and legumes.

Soaking of millet, soya bean, maize, sorghum, and mung bean at 30 °C for 24 h decreased the contents of phytic acid by 4–51% (Lestienne, Besançon et al., 2005; Lestienne, Icard-Vernière et al., 2005; Lestienne, Mouquet-Rivier et al., 2005), and soaking of sorghum flour (80% extraction) at room temperature for 24 h reduced phytic acid levels by 16–21% (Mahgoub & Elhag, 1998). Soaking of pounded maize for 1 h at room temperature already led to a reduction of phytic acid by 51% (Hotz, Gibson, & Temple, 2001).

The use of moist conditions to stimulate germination is another technique for reducing phytate levels. Germination of sorghum for 4 d reduced phytic acid by 68–87% (Mahgoub & Elhag, 1998). Badau reported that, with longer germination times, HCl-extractability of calcium, iron and zinc in pearl millet was increased by 2–16%, 15–45% and 12–25%, respectively (Badau, Nkama, & Jideani, 2005).

Sourdough fermentation (30 °C for 4 h), led to a reduction of 60% of phytic acid in whole wheat flour with a >30% increase of iron and zinc during *in vivo* absorption in rats (Lopez et al., 2003; Leenhardt, Levrat Verny, Chantiaud, & Remesy, 2005). After 12 h of accelerated fermentation, 60% of phytic acid in sorghum was degraded (Mahgoub & Elhag, 1998).

In summary, although a complete removal of phytic acid was not reported, wet processing technologies can help to reduce phytic acid so that solubility of minerals in foods could be increased. To our knowledge, there is little information in the literature on wet processing of rice.

Brown rice contains more minerals than does white rice. Compared to white rice, consumption of brown rice would increase the intakes of iron and zinc by factors of 3 and 1.7, respectively (Doesthale, Devara, Rao, & Belavady, 1979; Kennedy, Burlingame, & Nguyen, 2002; Heinemann, Fagundes, Pinto, Pentead, & Lanfer-Marquez, 2005). We therefore selected brown rice for this study. The objectives of the present study were (1) to analyse the effects of wet processing (soaking, germination and accelerated lactic fermentation) on the mass balance and levels of minerals and phytic acid in brown rice and (2) to study the effect of phytic acid contents on the solubility of zinc in wet processed materials.

2. Materials and methods

2.1. Materials

Brown rice (Kenjian 90-31, cultivated in Heilongjiang province and harvested in 2003) was collected from Beijing Huateng Model Rice Mill Company (Beijing, China).

2.2. Soaking

Brown rice was either heated as is, in a drying oven for 30 min at 100 °C (dry-preheated), or mixed with demineral-

Table 1
Preheating and soaking conditions

Treatment code	Preheating		Soaking medium		Soaking time (d)	
	Dry heated ^a	Wet heated ^b	Natural demineralized water (pH 5.9)	Acidic solution ^c	1	7
SDN	×		×			×
SDA	×			×		×
SWN		×	×		×	
SWA		×		×	×	

^a Brown rice was heated in drying oven at 100 °C for 30 min before soaking.

^b Mixture of brown rice and demineralized water (1:1, w/v) was heated in autoclave at 115 °C for 10 min.

^c pH of mixture was adjusted to 3.5 with 5 N HCl before soaking.

ized water (1:1, w/v) and autoclaved for 10 min at 115 °C (wet-preheated). After this step, the rice was soaked at 10 °C with demineralized water (1:5, w/v). The mixture was either left as is, natural (pH 5.9), or adjusted with 5 N HCl to pH 3.5. Rice was soaked for either 1 (wet pre-heated samples) or 7 (dry heated samples) days. After soaking, the total mixture was centrifuged at 5000g (10 min). The pellet was freeze-dried and stored in sealed plastic bags at 4 °C for further analysis. Table 1 summarizes the different soaking conditions used. All soaking treatments were in duplicate.

2.3. Steeping and sprouting

Steeping and sprouting were carried out, following the procedure developed by Capanzana and Buckle (1997). For one separate small sample, approximately 30 g of brown rice were used. At the steeping phase, brown rice was soaked in 150 ml of demineralized water in plastic boxes at 25 °C. Small samples were taken out from the incubator at intervals of 4, 8, 12 and 24 h. After steeping for 24 h, brown rice was separated by decanting and placed in plastic boxes and covered with punctured lids for sprouting for 72 h at 30 °C. Samples were taken at 12, 24, 36, 48 and 72 h. Treatments of steeping and sprouting were in duplicate. After steeping and sprouting, samples were freeze-dried and stored in sealed plastic bags 4 °C for further analysis.

2.4. Fermentation

Mixtures of brown rice and demineralized water (ratio 1:5, w/v) were fermented naturally for 24 h at 30 °C. Starting on the second day and after each consecutive day, a fresh mixture was inoculated with 10% of the water of the previously fermented mixture, in order to obtain an accelerated fermentation by enrichment of acidifying microbiota (Nche, Odamtten, Nout, & Rombouts, 1994).

All treatments were carried out in duplicate. After fermentation, samples were freeze-dried and stored in sealed plastic bags at 4 °C for further analysis.

2.5. Zinc analysis

2.5.1. Total zinc content

Rice samples were digested in microwave oven with hydrofluoric acid (40%, w/w) and concentrated nitric acid (65%, w/w) to allow the analysis of total zinc. Zinc contents were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Elan 6000, Perkin–Elmer, Norwalk, CT). Each sample was digested in duplicate.

2.5.2. *In vitro* soluble zinc content

In vitro soluble zinc was defined as the relative amount of zinc that becomes soluble after enzymatic treatment. Rice samples were digested consecutively with enzymes,

including amylase, pepsin, pancreatin and bile, under certain conditions following the enzymatic degradation procedure described by Kiers, Nout, and Rombouts (2000). Then, mixtures were centrifuged at 5000g for 15 min at 4 °C. The resulting supernatant was filtered (0.45 µm membrane, FP 030/3) and frozen until further analysis. Zinc levels were analyzed with ICP-OES. Each sample was enzymatically extracted in duplicate.

2.6. Phytic acid

A 100 mg amount of sample (previously dried and ground) was placed in a glass tube and mixed with 10 ml of 18.3 g dm⁻³ HCl containing 50 mg dm⁻³ *cis*-aconitate (internal standard). The mixture was boiled at 100 °C for 15 min in a water-bath. Next, samples were transferred into 2 ml Eppendorf tubes and centrifuged at 21,000g for 10 min. A 0.1 ml volume of the supernatant was homogeneously mixed with 0.1 ml of 18.3 g dm⁻³ HCl and 0.8 ml of MilliQ water (Millipore, Billerica, MA, USA) and, from this mixture, 200 µl were immediately transferred to HPLC phials.

Phytic acid was determined by high-performance liquid chromatography (HPLC) (Bentsink, Yuan, Koornneef, & Vreugdenhil, 2003) using a Dionex (Sunnyvale, CA, USA) DX300, ICS2500 system with a suppressed conductivity detector range of 10 µS. An AS11 chromatographic column (Dionex) with a guard column was used. All analyses (20 µl injection volume) were carried out at room temperature. The eluents (flow rate 1 ml min⁻¹) used were as follows: 0–5 min, 0.2 g dm⁻³ NaOH; 5–15 min, linear gradient of 0.2–4 g dm⁻³ NaOH; 15–20 min, 20 g dm⁻³ NaOH; finally 20–35 min, 0.2 g dm⁻³ NaOH.

2.7. Statistical analysis

Data were analyzed with SPSS 10.0 for windows. Significance was tested at a 5% level using an independent-samples *t*-test.

3. Results

3.1. Effect of wet processing on dry matter, phytic acid and zinc

3.1.1. General

The various wet processing treatments were tested for their efficacy on decrease of phytic acid, while retaining micro- and macronutrients. Fig. 1 shows the different results obtained.

3.1.2. Dry Matter

Wet treatments led to a loss of dry matter of 7–16%. Mass loss in accelerated fermented brown rice was 9–12%. Soaking led to a higher mass loss than other treatments, especially soaking after wet preheating, with losses

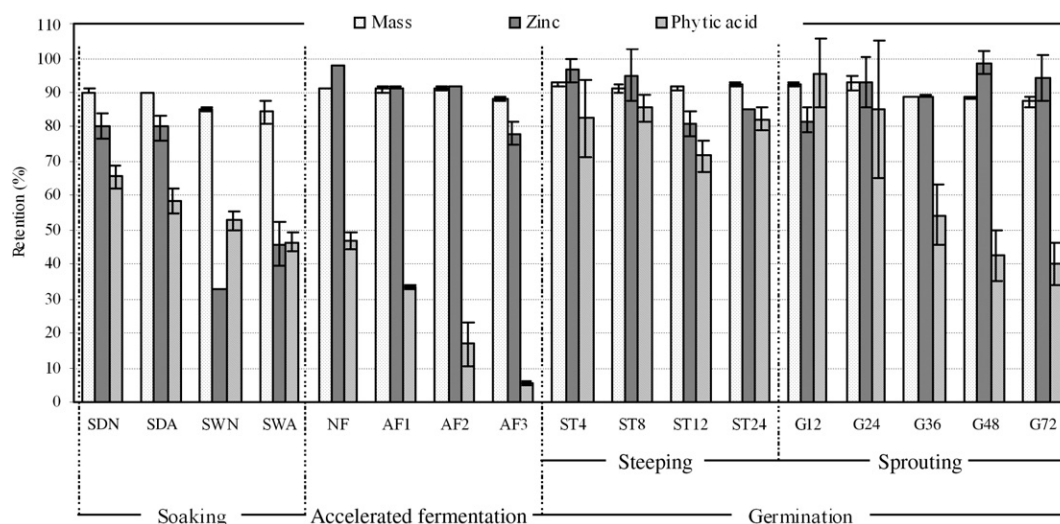


Fig. 1. Retention of mass, zinc and phytic acid in brown rice upon wet processing. Abbreviations: SDN, SDA, SWN, SWA: see Table 1; NF: natural fermentation without starters addition at 30 °C for 24 h; AF1/AF2/AF3: accelerated fermentation with one/two/three cycles of starter enrichment for 24 h and fermented at 30 °C for 24 h; ST4/ST8/ST12/ST24: brown rice was steeped for 4, 8, 12 and 24 h, respectively; G12/G24/G36/G48/G72: brown rice was germinated for 12, 24, 36, 48 and 72 h, respectively; Retention: Percentage of dry mass/zinc/phytic acid in certain quantities of brown rice after or before treatments.

up to 16%. Mass loss during steeping and sprouting ranged from 7% to 13%.

3.1.3. Decrease of phytic acid

Compared to untreated brown rice, wet processes (soaking, steeping and fermentation) significantly decreased phytic acid levels ($p < 0.05$). However, they varied in their ability to reduce phytic acid levels, which can be derived from the retention of phytic acid in samples (5–96%). Fermentation was clearly the most effective: decreasing phytic acid by 53% up to 95%. Steeping of intact grains, as a first step of germination, was not significantly effective: only a reduction of 14–28% was obtained. During fermentation, as well as germination, decrease of phytic acid progressed with time. No significant difference was observed between dry and wet preheating ($p < 0.05$).

3.1.4. Retention of zinc

Results also varied with the different wet processes applied. In general, zinc retention was between 80% and 99%. Only wet preheating led to a considerable loss of zinc (~60%). In most wet treatments of brown rice, the retention of zinc was at similar levels as the retention of dry matter, which was in the range of 80–93%. Rice soaked after wet preheating formed the exception, with dry matter retentions of 33% and 46%, after soaking in demineralized water and acidic solutions, respectively. Retention of zinc in brown rice soaked after dry heating was about 80%, which was much higher than after wet preheating.

3.2. Contents of phytic acid and zinc in treated brown rice

3.2.1. General

The relative losses of phytic acid, zinc and dry matter resulted in changes of absolute concentrations that are of

nutritional relevance. Table 2 presents the concentrations of phytic acid and zinc in treated materials.

3.2.2. Phytic acid

Level of phytic acid in untreated brown rice was 12.5 mg g^{-1} , while it ranged from 1 to 13 mg g^{-1} in treated samples. After soaking, the phytic acid was around 7–9 mg g^{-1} with wet preheated rice having lower levels than

Table 2
Effects of soaking, germination and fermentation on brown rice^{A,E}

Treatments	Methods ^B	Phytic acid ^C (mg g^{-1})	Zinc ^D (mg kg^{-1})
Untreated	None	12.5 ± 0.2	19.6 ± 1.4
Soaking	SDN	$9.1 \pm 0.5\text{a}$	$16.5 (15.7, 17.2)\text{a}$
	SDA	$8.0 \pm 0.5\text{a}$	$16.4 (15.7, 17.1)\text{a}$
	SWN	$7.7 \pm 0.4\text{a}$	$7.1 (7.1, 7.1)\text{b}$
	SWA	$6.9 \pm 0.4\text{a}$	$10.0 (8.6, 11.4)\text{b}$
	NF	$6.4 \pm 0.3\text{a}$	$19.9 (19.9, 19.9)\text{a}$
Natural and accelerated fermentation	AF1	$4.5 \pm 0.1\text{b}$	$18.5 (18.5, 18.6)\text{a}$
	AF2	$2.3 \pm 0.9\text{c}$	$18.6 (18.6, 18.6)\text{a}$
	AF3	$0.7 \pm 0.1\text{c}$	$16.4 (15.7, 17.1)\text{b}$
	ST4	$11.1 \pm 1.5\text{a}$	$19.3 (18.6, 19.9)\text{a}$
Germination: (1) steeping	ST8	$11.7 \pm 0.5\text{a}$	$19.3 (15.7, 22.9)\text{a}$
	ST12	$9.7 \pm 0.6 \text{ a}$	$16.4 (15.7, 17.1)\text{a}$
	ST24	$8.4 \pm 0.5\text{a}$	$17.1 (17.1, 17.1)\text{a}$
	G12	$12.9 \pm 1.3\text{a}$	$16.4 (15.7, 17.1)\text{a}$
Germination: (2) sprouting	G24	$11.4 \pm 2.7\text{a}$	$18.5 (17.1, 20.0)\text{a}$
	G36	$7.6 \pm 1.2\text{b}$	$18.6 (18.5, 18.6)\text{a}$
	G48	$6.0 \pm 1.0\text{b}$	$20.7 (19.9, 21.4)\text{a}$
	G72	$5.7 \pm 0.9\text{b}$	$20.0 (18.5, 21.4)\text{a}$

^A All data expressed on dry matter basis.

^B Abbreviations of treatments are the same as in Fig. 1.

^C Contents of zinc in untreated brown rice and contents of phytic acid in all materials: mean \pm standard deviation ($n = 4$).

^D Contents of zinc: average of results of replicates of treatments (values of replicates).

^E Different characters mean significantly different at $p < 0.05$.

dry preheated. Rice fermented with enrichment starter (AF3) had the lowest phytic acid content (0.7 mg g^{-1}), but natural fermentation (NF) also reduced the initial phytic acid level by 50%. During germination, contents of phytic acid slightly decreased, from 11.7 to 8.4 mg g^{-1} , during the steeping phase, and decreased further from 12.9 to 5.7 mg g^{-1} during sprouting over a period of 12–72 h.

3.2.3. Zinc

The zinc level in initial brown rice was 19.6 mg kg^{-1} . It ranged between 16 and 21 mg kg^{-1} in treated rice, except in brown rice soaked after wet heating, which contained less than 10 mg kg^{-1} .

Further data analysis showed (Table 2) that duration of sprouting and enrichment starters in accelerated fermentation had significant effects on decrease of phytic acid. However, except for wet preheating and AF3, other wet processing did not have a significant effect on contents of zinc in final products ($p < 0.05$).

3.3. Molar ratios of phytic acid to zinc ($[PA]/[Zn]$) and *in vitro* solubility (IVS) of zinc

The molar ratio of phytate to zinc is assumed to provide an indication of the bioavailability of zinc. In untreated brown rice, this ratio was 63 while, after processing, molar ratios of $[PA]/[Zn]$ varied from 4 to 107. The lowest ratio was obtained by fermentation (AF3), and the highest in soaked rice after wet preheating. The amount of IVS-zinc

of treated brown rice is assumed to provide an indication of the amount of zinc available for absorption *in vivo*. The IVS is expected to be more relevant than the phytate to zinc ratio, which is only based on contents. Untreated brown rice had 7.3 mg kg^{-1} of IVS-zinc and, after the various treatments, IVS zinc levels ranged from 4.2 to 8.3 mg kg^{-1} (Table 3).

4. Discussion

Given the important role of rice in the diet as a source of both macro- and micronutrients, an improvement of the bioavailability of zinc and iron is needed. Our studies indicated that selection of rice varieties (Liang et al., 2007), or optimization of dry processing (milling) showed limited promise for improvement of mineral availability. We therefore investigated the potential effect of wet processing. We aimed at identifying wet operations that could be used in combination with optimum growing and dry fractionation procedures. For the same reason, our analysis of the samples was limited to three main factors: phytate, zinc and dry matter. Other micronutrients, such as iron, are equally important but in this study we focussed on zinc.

Our results with wet processes, showing that they could significantly decrease phytic acid in brown rice, agree well with reports about similar treatments on other cereals, such as fermentation of white rice flour (Reddy & Salunkhe, 1980), and steeping and sprouting of oats or corn (Fageer, Babiker, & El Tinay, 2004; Larsson & Sandberg, 1995). We found that soaking, germination and fermentation have different efficacies in reducing the content of phytic acid. Accelerated fermentation, especially fermentation with starters, enriched three times, is the most effective approach for reducing phytic acid in brown rice. In sorghum, soaking and natural fermentation had similar impacts on phytic acid (Mahgoub & Elhag, 1998) which is in line with our results with NF. Decreases caused by fermentation and germination are mainly based on the action of enzymes while, in soaking, a combination of diffusion and enzymatic action is expected (Henderson & Ankrah, 1985; Mahgoub & Elhag, 1998). Other studies have proposed that the activity of endogenous phytase was the main factor leading to a reduction of phytic acid during soaking (Lestienne, Besançon et al., 2005; Lestienne, Icard-Vernière et al., 2005; Lestienne, Mouquet-Rivier et al., 2005). For brown rice, because of the presence of a tough and tight outer layer in the structure, which hinders the diffusion of substances, and low activity of endogenous phytase, which will be activated during steeping, decrease of phytic acid by soaking and steeping was limited. Furthermore, main factor(s) that cause the reduction of phytic acid in soaking and steeping, effect of phytase, or solubilization of phytate, or both, need further study. In a study by Carlson and Poulsen (2003) in which heated and non-heated barley and wheat were soaked, reduction of phytic acid was clearly higher in the non-heated treatment. Unfortunately, the heat treatment was not specified, so we cannot compare

Table 3
In vitro solubility of zinc and molar ratios of phytic acid to zinc in treated materials

Treatments	Methods ^A	<i>In vitro</i> soluble zinc (mg kg^{-1}) ^{B,C}	Solubility (%) ^D	$[PA]/[Zn]$ ^E
Untreated	UR	7.3 ± 0.2	37.5	62.8
Soaking	SDN	$7.7 (7.7, 7.7)\text{a}$	46.9	54.3
	SDA	$6.7 (4.1, 9.3)\text{a}$	40.9	48.0
	SWN	$5.8 (4.6, 7.0)\text{a}$	33.8	106.8
	SWA	$5.7 (5.3, 6.0)\text{b}$	56.8	68.0
Natural and accelerated fermentation	NF	$6.0 (3.4, 8.6)\text{a}$	30.2	31.7
	AF1	$6.8 (6.8, 6.9)\text{a}$	36.8	24.0
	AF2	$7.4 (5.3, 9.6)\text{a}$	40.0	12.2
	AF3	$4.1 (3.2, 4.9)\text{a}$	24.8	4.2
Germination: (1) steeping	ST4	$5.4 (4.9, 6.0)\text{a}$	28.2	56.6
	ST8	$5.5 (4.7, 6.3)\text{a}$	28.6	59.7
	ST12	$4.6 (3.3, 5.9)\text{a}$	28.0	58.3
	ST24	$6.6 (5.0, 8.1)\text{a}$	38.4	48.4
Germination: (2) sprouting	G12	$6.6 (6.3, 6.9)\text{a}$	40.0	77.5
	G24	$4.2 (3.3, 5.1)\text{a}$	22.7	60.7
	G36	$6.2 (5.7, 6.6)\text{a}$	33.1	40.2
	G48	$6.1 (4.7, 7.5)\text{a}$	29.5	28.5
	G72	$8.3 (8.2, 8.3)\text{b}$	41.3	28.1

^A Abbreviations of treatments are the same as in Fig. 1.

^B Contents of zinc: average of results of replicates of treatments (values of replicates).

^C Different characters mean significantly different at $p < 0.05$.

^D Expressed on dry matter basis.

^E Calculated on total contents of phytic acid and zinc in treated materials.

their results with ours. They ascribed the difference in phytate degradation to the inactivation of endogenous phytase. This again indicates the role of endogenous phytase. We did not obtain such differences between our untreated and preheated samples, which may be due to the use of different grains and a different extent of heat treatment; in any case, we found phytase activity in both untreated and preheated rice (data not shown). Our results demonstrate that the reduction of phytic acid increased with germination time, which agrees with previous studies reporting that the activity and/or production of phytase increased during steeping (Henderson & Ankrah, 1985; Larsson & Sandberg, 1995). We observed similar trends with fermentation. Abdalla, El Tinay, Mohamed, and Abdalla (1998) suggested that enzymatic hydrolysis of phytic acid by both endogenous phytase and microbial phytase (lactic acid bacteria can produce significant levels of phytase), and may account for most of the loss of phytic acid during fermentation (Abdalla et al., 1998; Leenhardt et al., 2005; Towo, Matuschek, & Svanberg, 2006). Our observation of low phytic acid contents after fermentation is similar to results obtained with fermented whole wheat flour (Lestienne, Besançon et al., 2005; Lestienne, Icard-Vernière et al., 2005; Lestienne, Mouquet-Rivier et al., 2005). The results indicated that both endogenous phytase and microbial phytases contributed to a reduction of phytic acid in fermented brown rice. During accelerated fermentation, endogenous phytase was activated and accumulated and, at the same time, with growth of micro organisms, microbial phytase also accumulated. From Tables 2 and 4 we observed that longer duration of lower pH values also gave lower levels of phytic acid. From Table 4 we also observed that NF was much less effective for acidification and phytate removal than fermentation with cycle 3 starter (AF3). This could be due to a more rapid pH decrease and/or higher phytase activity, since optimal pH values for plant phytase and microbial phytase were about 5 ± 0.5 and 3 ± 0.5 , respectively (Andriotis & Ross, 2003). In addition to the influence of reaction conditions and ingredients used, factors such as enzymatic degradation, effect of pH on solubility and diffusion, could all be involved in the overall effect of the fermentation. Low starting pH (3.5) was not significantly affected by levels of phytic acid in SWN since the low pH was not maintained throughout the whole soaking period because of the buffering effect of components, such as protein, in brown rice.

Table 4
Initial and final pH values after natural, and accelerated fermentation of brown rice

Treatment ^a	Initial pH ^b	Final pH ^b
NF	7.6 (7.6, 7.6)	5.5 (5.5, 5.6)
AF1	6.6 (6.5, 6.6)	5.3 (5.2, 5.4)
AF2	6.3 (6.2, 6.3)	5.2 (5.0, 5.4)
AF3	5.7 (5.6, 5.9)	4.8 (4.5, 5.1)

^a Abbreviations are the same as in Fig. 1.

^b Average (values of replicates).

The retention and solubility of individual minerals were also affected by process conditions. For zinc, only soaking after wet processing resulted in significantly lower retention compared with the other treatments. This can be explained by the uniform distribution of zinc in the grain and the effect of wet preheating. Whereas dry preheating only results in minor physical damage, such as fissures (or cracks) in the kernels, wet preheating induced swelling of the kernel and gelatinization. The swollen kernel would allow better diffusion and we expect this to be the main reason for the higher loss of zinc and dry matter in soaked brown rice after wet preheating. In fermented brown rice, zinc losses ranged from 1% to 20%.

We expected that, with lower molar ratios of [PA]/[Zn], the *in vitro* solubility of zinc would increase, but this relation was not significant. This discrepancy was also observed by authors who studied other cereals, such as sorghum, millet and millet bran fractions (Lestienne, Besançon et al., 2005; Lestienne, Icard-Vernière et al., 2005; Lestienne, Mouquet-Rivier et al., 2005; Matuschek, Towo, & Svanberg, 2001). Possibly, the molar ratio of [PA]/[Zn] must be very low (possibly <0.2) to achieve increased zinc solubility. Theoretically, one mole of phytic acid could bind four moles of zinc. Under the conditions used, soaking, germination or fermentation are not able to completely eliminate phytic acid, so the residual phytate might still interfere with zinc solubility. Another explanation is that a prediction of solubility of minerals is not feasible by phytic acid to mineral ratios alone. For instance, it was reported that lower inositol phosphates (inositol mono-, bi-, tri- and tetraphosphates), produced during fermentation, increased the capacity of binding minerals of higher-phosphorylated inositol phosphates (IP6 and IP5: *myo*-inositol pentaphosphate), although they were present at a low level (Sandberg et al., 1999). In addition, all forms of zinc phosphate are poorly soluble. Similar to polypeptides in sorghum and millet (Matuschek et al., 2001; Towo et al., 2006), other matrix components that limit *in vitro* solubility, such as dietary fibre, can be present in brown rice. Little is known about the affinity of such components for minerals. Their inhibition might be even more effective than that of phytic acid. Clearly, these hypotheses need further study.

Another aspect that needs further attention is the validity of *in vitro* solubility of zinc as an indicator for *in vivo* bioavailability. Although it has been demonstrated that the addition of phytic acid to, e.g., white bread (Sandberg et al., 1993), results in a strong decrease of *in vivo* human zinc uptake, the situation for rice may be different, and more comprehensive data for solubility and *in vivo* uptake from food matrices are urgently needed.

From this study, we conclude that neither soaking, germination, nor fermentation significantly improve the apparent bioavailability of minerals in rice. It may be of interest to combine wet processing methods, including phytase treatment, with other approaches, such as the application of uptake enhancers, to improve the availability of

minerals in rice. More research will be required to understand the effects of matrix components, and to explore opportunities for combined process approaches.

5. Conclusion

Soaking, germination and fermentation, led to varying decreases of phytic acid in brown rice. The most effective approach (AF3) could reduce 96% of total phytic acid and decrease the molar ratios of phytic acid to zinc below 5. Wet processing (except soaking after wet heating) led to 1–20% of mass loss, and a similar loss of zinc as well.

The sharp decrease in ratio would suggest that fermentation especially would be a successful way to increase zinc bioavailability. However, results from *in vitro* solubility measurement of zinc showed little improvement over untreated brown rice. This could result from the presence of matrix components such as fibre or phosphates, leading to the formation of insoluble zinc complexes. It remains to be investigated to what extent this would affect IVS-solubility and bioavailability.

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