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Effect of soaking and phytase treatment on phytic acid, calcium, iron and zinc in rice fractions

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ABSTRACT

With the aim to maximise phytic acid removal and minimise losses of dry matter and minerals (Ca, Fe, Zn) in rice, three products (whole kernels and flour milled from white and brown rice; and bran, all from the same batch of variety Kenjian 90-31) were soaked in demineralized water at 10 °C (SDW), NaAc buffer of pH 3.5 at 10 °C (SAB), and 500 U L^{-1} phytase of pH 5.5 at 50 °C (SPS). In whole kernels and flour of white rice, phytic acid removal was 100% by all treatments; losses of dry matter, Ca, Fe, and Zn were 2–5%, 12–63%, 9–10%, and apparent gain of 63–72%, respectively. In whole brown rice, SAB removed 75% phytic acid, and SPS 100% from flour; dry matter, Ca, Fe, and Zn losses were 1–16%, 26–56%, 39–45%, and 23–24%. In rice bran, SPS removed 92% phytic acid, and SAB 50%; dry matter, Ca, Zn, and Fe losses were 20%, 48%, 63%, and apparent gain of 5%, respectively.

1. Introduction

In China, rice plays an important role in human nutrition. In 2002 and 2003, the intake of rice and rice products represented 35% of the total energy intake (FAO, 2006; Wang, 2005). In addition to energy, rice also supplies important micronutrients, such as iron, zinc, calcium and some vitamins, especially in rural diets. In 2002, iron consumption in rural areas was estimated at 23.1 mg/capita/day (Wang, 2005), of which about 8.5% was estimated to originate from rice and rice products. Poor mineral bioavailability is a major cause of mineral deficiencies. For example, it was reported that prevalence of iron deficiency related anaemia could be associated with poor iron bioavailability (Ma, 2007) caused by low intake and the occurrence of antinutritional factors such as phytic acid.

In order to increase the bioavailability of minerals in rice and rice products, rice varieties with higher mineral contents and lower levels of antinutritional factors (phytic acid, PA) could be selected for cultivation (Liang, Han, Han, Nout, & Hamer, 2007), rice milling by dry abrasion could be optimised for maximum PA removal with minimum losses of minerals (Liang et al., 2008b), and wet processing of rice could be promoted to remove PA (Liang, Han, Nout, & Hamer, 2008a). Although the first two approaches were shown to improve in vitro solubility of minerals, residual PA levels were still too high to achieve adequate availability of minerals for nutrition. Studies on other cereals indicated that wet processing, such as soaking, germination and fermentation could effectively decrease PA and improve the bioavailability of minerals (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005c).

Soaking is widely applied at both household and industrial scale. It is the most important operation in the process of rice noodle making (Lu, Li, Min, Wang, & Tatsumi, 2005) to soften the kernel prior to pulping. During rice soaking for noodle making, a natural fermentative acidification takes place, and this is regarded as important for noodle quality. Previously, it was reported that soaking of other cereals such as pearl millet with endogenous or exogenous (i.e. added) phytase enzymes at optimum conditions increased the in vitro solubility of iron and zinc by 2–23% (Lestienne, Besançon, Caporiccio, Lullien-Pellerin, & Trèche, 2005a; Lestienne, Caporiccio, Besançon, Rochette, & Trèche, 2005b). In millet, dehulling and milling prior to soaking facilitated phytate degradation by endogenous phytases, whereas in contrast, soya beans had increased phytate levels after dehulling (Lestienne, Mouquet-Rivier, Icard-Vernière, Rochette, & Trèche, 2005d). Whilst it has been observed that soaking has the advantage of decreasing PA levels of e.g., brown rice (Liang et al., 2008a) and legume seeds (Sattar, Durrani, Mahmood, Admad, & Khan, 1989; Vijayakumari, Siddhuraju, Pugalenthi, & Janardhanan, 1998), it also caused undesirable losses of water-soluble nutrients from e.g., soya beans (Bayram, Kaya, & Oner, 2004), common beans (Barampama & Simard, 1995), and other leguminous seeds (El-Adawy, Rahma, El-Bedawy, & Sobihah,

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2000; Sattar et al., 1989). It is therefore important to balance both positive and negative aspects of soaking treatments.

Despite the importance of rice for human nutrition, the opportunities and limitations of wet processing for the improvement of mineral bioavailability in rice have not yet been clarified. Therefore the objectives of the present study were: (1) to explore the potential of soaking, endogenous phytase, exogenous phytase and combined treatments for the removal of PA from whole and ground rice products; and (2) to quantify the negative effects of nutritional relevance, particularly losses of dry matter and minerals.

2. Materials and methods

2.1. Rice products

Brown rice kernels, white rice kernels and rice bran (all of the same batch of variety Kenjian 90-31, harvested in 2005) were collected from Beijing Huateng Model Rice Mill Company, Beijing, China. Brown rice was obtained by de-husking rough rice; white rice was obtained by removing germs, testa and aleurone layers from brown rice; bran consisted of germs, testa and aleurone. Rice flours were prepared in a hammer-mill type grinder (HY-04B, Beijing Xinhuanya, China) and sieved through a 1 mm screen.

2.2. Phytase

Phytase was obtained from DSM (Delft, The Netherlands), with activity of 6000 U g^{-1}, optimum temperature 55 °C, and optimum pH 2.5–5.5. The suggested dose based on application in animal feeds was 500 U kg^{-1} feed dry matter.

2.3. Processing

Raw materials were preheated, and soaked in various soaking media as outlined in Table 1. Preheating was included to allow differentiation between effects of exogenous (added) and endogenous phytase activity. No phytase activity was detected in preheated rice products (China National Standard Analysis Method, 2002). After soaking, kernels were separated from soaking media by decanting, and flours were separated by centrifugation at 5000*g* during 15 min). All treatments were carried out at least in duplicates. All solid residues were freeze-dried and kept at 4 °C prior to analysis.

2.4. Phytic acid (PA)

Contents of PA of all materials were analysed by spectrophotometric detection with ferric chloride ($FeCl_3$) and sulfosalicylic acid after extraction, separation on anion exchange resin according to the China National Standard Analysis Method as described previously (Liang et al., 2007).

2.5. Phosphorus in soaking media

The colorimetric method AOAC 995.11 (Horwitz, 2000) was used to determine phosphorus levels. Acid soluble phosphate forms a blue complex with sodium molybdate in the presence of ascorbic acid as reducing agent. The intensity of blue colour was measured spectrophotometrically at 823 ± 1 nm (7200, Unico, Shanghai, China).

2.6. Calcium, iron and zinc

Minerals in solid residues were analysed using an inductively coupled plasma optical emission spectrometer (ICP-AES) (Optima 2000, Perkin–Elmer) after wet acid digestion with concentrated nitric acid (HNO_3, 65%) and perchloric acid ($HClO_4$, 60%) following the procedure of AOAC official method 975.03 (Horwitz, 2000).

2.7. Data analysis

Data were analysed using the SPSS package (Sony DADC, version 12.0.1). Significance was tested at a 5% level using an unrelated *t*-test.

3. Results

The effects of rice products processing on phosphorus and phytic acid levels are presented in Figs. 1–3. In Table 2, the effects of processing on dry matter loss and Ca, Fe, and Zn levels are presented.

3.1. White rice

For white rice kernels, phytic acid (PA) started at relatively low levels and disappeared from the solids with a concomitant release of soluble phosphorus in the soaking medium (Fig. 1). No difference ($p > 0.05$) between unheated and preheated white rice was observed. Although the differences between treatments were small, phytase application led to significantly higher ($p < 0.05$) levels of phosphorus in the soaking media. For white rice flour, only treatments with phytase decreased PA to below detection level, and led to significantly higher ($p < 0.05$) levels of phosphorous in soaking media.

Acid soaking caused higher dry matter losses than other soaking media (Table 2). Calcium levels showed varying losses, which were bigger than for dry matter. Iron levels followed the same order of magnitude as calcium, with relatively higher losses from kernels

Table 1
Preheating and soaking treatments.

Treatments	Methods	Code	Conditions
Preheating	None	None	No preheating (control)
	Dry preheating	DP	Heat in an ventilated hot air oven at 100 °C, kept for 30 min, then cool to room temperature in sealed glass vessels
Soaking	Soaking medium: Demineralised water (pH 5.95)	SDW	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v), bran with medium at 1:5 ratio (w/v), then soak in a thermostat incubator at 10 °C, during 172 h (kernels) or 24 h (flour and bran)
	Soaking medium: Acidic buffer (HAc-NaAc, 1 M, pH 3.5)	SAB	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v), bran with medium at 1:5 ratio (w/v), then soak in a thermostat incubator at 10 °C, during 172 h (kernels) or 24 h (flour and bran)
	Soaking medium: Phytase solution (500 U L^{-1})	SPS	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v), bran with medium at 1:5 ratio (w/v), adjust to pH 5.5 with HAc and NaAc, then soak in a thermostat water bath at 50 ± 2 °C, for 30 min

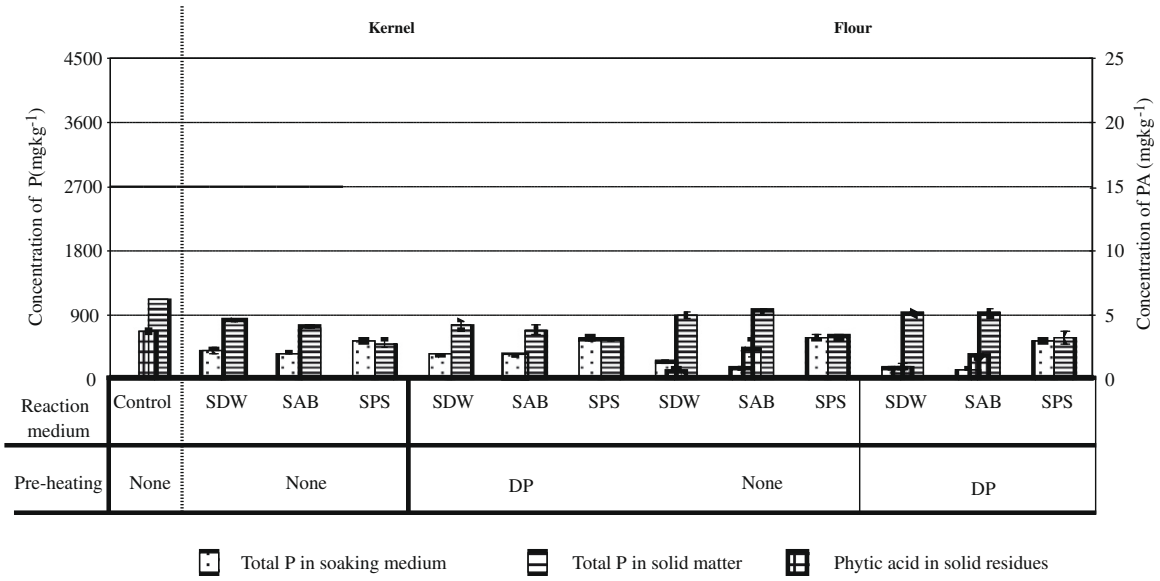

Fig. 1. Effect of preheating and soaking on phosphorus and phytic acid in white rice.

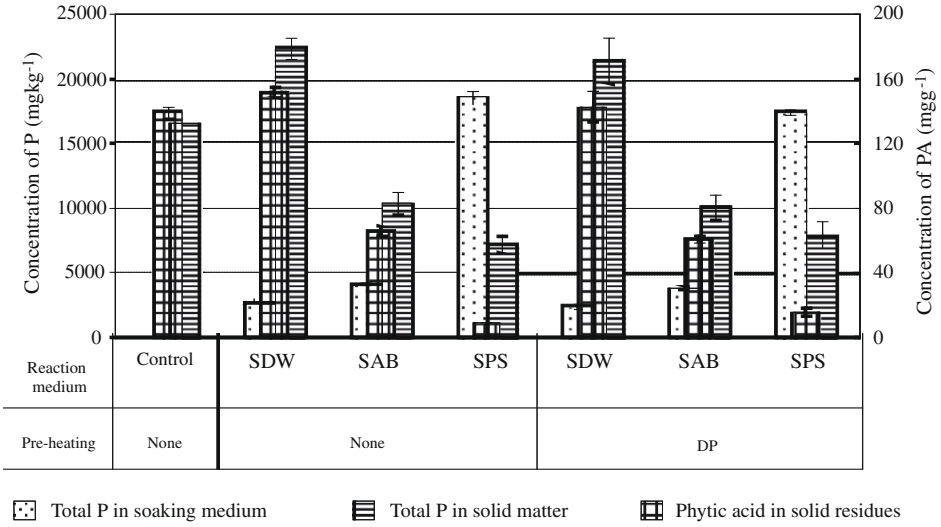

Fig. 2. Effect of preheating and soaking on phosphorus and phytic acid in rice bran.

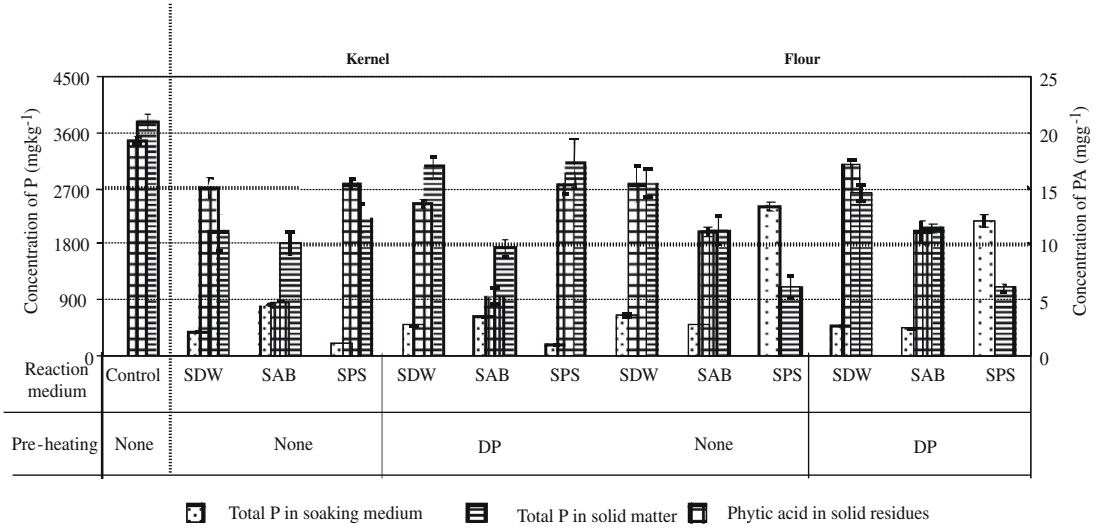

Fig. 3. Effect of preheating and soaking on phosphorus and phytic acid in brown rice.

Table 2
Effect of preheating and soaking on dry matter, calcium, iron and zinc in rice products.

Rice products	Preheating[a]	Soaking[a]	Dry matter loss[b]	Ca[c]	Fe[c]	Zn[c]
White rice						
Control	None	Control	0	173 ± 1	28.7 ± 3.8	24.5 ± 0.3
Kernel	None	SDW	8.6	127 ± 5 (−27)	21.5 ± 4.9 (−25)	24.5 ± 2.6 (−0)
		SAB	15.1	86 ± 17 (−50)	18.6 ± 3.7 (−35)	22.8 ± 3.6 (−7)
		SPS	2.0	153 ± 16 (−12)	25.9 ± 3.4 (−10)	42.1 ± 9.1 (+72)
	DP	SDW	13.0	123 ± 23 (−29)	17.2 ± 3.0 (−40)	23.0 ± 3.6 (−5)
		SAB	19.7	118 ± 10 (−32)	16.5 ± 3.0 (−43)	24.2 ± 3.3 (−1)
		SPS	3.9	73 ± 1 (−58)	20.9 ± 1.8 (−27)	40.8 ± 7.2 (+67)
Flour	None	SDW	6.2	136 ± 24 (−21)	21.0 ± 1.3 (−27)	27.8 ± 12.6 (+13)
		SAB	16.1	106 ± 18 (−39)	29.2 ± 6.2 (+2)	15.3 ± 2.9 (−38)
		SPS	4.9	64 ± 14 (−63)	26.0 ± 3.2 (−9)	40.0 ± 1.6 (+63)
	DP	SDW	6.5	149 ± 22 (−14)	23.3 ± 1.0 (−19)	33.3 ± 7.0 (+36)
		SAB	13.1	114 ± 4 (−34)	21.1 ± 1.3 (−26)	30.2 ± 7.0 (+23)
		SPS	9.6	127 ± 28 (−27)	33.5 ± 0.2 (+17)	28.1 ± 2.2 (+15)
Rice bran						
Control	None	Control	0	617 ± 26	94.6 ± 3.0	57.5 ± 1.1
Bran	None	SDW	7.4	628 ± 44 (+2)	163.9 ± 13.4 (+73)	19.3 ± 1.7 (−66)
		SAB	15.1	493 ± 20 (−20)	99.3 ± 11.4 (+5)	15.4 ± 2.4 (−73)
		SPS	20.2	318 ± 22 (−48)	90.0 ± 8.4 (+5)	21.0 ± 0.4 (−63)
	DP	SDW	28.7	671 ± 50 (+9)	141.3 ± 11.7 (+49)	22.3 ± 3.0 (−61)
		SAB	29.8	524 ± 18 (−15)	125.2 ± 5.5 (+32)	16.1 ± 1.5 (−72)
		SPS	19.4	334 ± 8 (−46)	95.2 ± 12.0 (+1)	21.9 ± 3.1 (−62)
Brown rice						
Control	None	Control	0	284 ± 18	50.7 ± 9.2	34.0 ± 0.4
Kernel	None	SDW	5.0	173 ± 15 (−39)	34.7 ± 9.4 (−32)	26.3 ± 2.6 (−23)
		SAB	16.0	209 ± 4 (−26)	28.0 ± 10.6 (−45)	26.3 ± 1.9 (−23)
		SPS	0.1	222 ± 15 (−22)	26.6 ± 4.1 (−47)	31.4 ± 4.4 (−8)
	DP	SDW	12.6	183 ± 7 (−36)	27.6 ± 4.1 (−46)	29.0 ± 2.3 (−15)
		SAB	18.9	121 ± 10 (−57)	20.2 ± 4.1 (−60)	23.1 ± 2.5 (−32)
		SPS	8.6	127 ± 28 (−55)	33.5 ± 0.2 (−34)	28.1 ± 2.2 (−17)
Flour	None	SDW	31.2	192 ± 16 (−32)	35.0 ± 5.1 (−31)	26.5 ± 8.4 (−22)
		SAB	31.4	108 ± 3 (−62)	31.4 ± 4.5 (−38)	20.8 ± 4.2 (−39)
		SPS	1.5	124 ± 27 (−56)	30.9 ± 3.3 (−39)	26.0 ± 7.7 (−24)
	DP	SDW	6.2	175 ± 18 (−38)	28.2 ± 5.8 (−44)	29.2 ± 0.0 (−14)
		SAB	13.4	153 ± 15 (−46)	32.8 ± 4.4 (−35)	17.1 ± 2.7 (−50)
		SPS	10.3	121 ± 20 (−57)	27.5 ± 2.0 (−46)	21.2 ± 0.1 (−38)

[a] For abbreviations, see Table 1.
[b] Dry matter loss: % of control.
[c] Ca, Fe and Zn: mg kg^{-1}, data in brackets refers to % change compared to control.

than from flour. Zinc followed another trend, with a number of apparent increases. In particular the phytase treatment results in higher zinc levels in the solid matter.

3.2. Rice bran

Levels of PA in rice bran were considerably higher than in white rice. No difference ($p > 0.05$) between unheated and preheated white rice was observed (Fig. 2). Acidic buffer reduced PA and total phosphorus by about 50% with a concomitant release of soluble phosphorus into the soaking medium, whilst soaking in demineralized water had no such effect. In contrast, mild increases of especially total P in soaking medium and in solid matter were observed which are most likely related to leaching into the soaking medium, and apparent increase due to shifts in solid matter composition, respectively. The application of phytase was even more effective; considering the very high initial phytate level a higher phytase activity or longer exposure time might have been required to obtain a complete PA removal (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002).

The dry matter losses (Table 2) were very high, up to almost 30%. The mineral levels in bran, especially of calcium and iron, were more than four times higher than in white rice. Acidic buffer, and more so phytase soaking resulted in strong migration of calcium and zinc into the soaking medium. Zinc levels were about twice higher than in white and brown rice, and suffered more than calcium, from leaching into the soaking media.

3.3. Brown rice

The results obtained with brown rice to a large extent paralleled those of rice bran. An interesting contrast was observed between the kernels and flour (Fig. 3). In kernels, soaking in water and acidic buffer gave similar results as in bran, i.e. water was not effective whereas acidic buffer extracts phytate phosphorus with release of soluble phosphorus into the medium. Phytase had no significant effect ($p > 0.05$). In contrast, in brown rice flour, phytase was highly effective resulting in removal of PA to below detection level.

The dry matter losses (Table 2) from whole kernels were of the same order as from white rice kernels. From brown rice flour, much more dry matter was lost by soaking. Calcium levels in brown rice were higher than in white rice, but the relative losses were similar as a result of soaking. The same can be observed for iron and zinc.

4. Discussion

Preheating and soaking conditions had diverse effects on PA and minerals in the tested rice products. In order to distinguish influences of endogenous and added phytase on PA degradation, endogenous phytase was inactivated by preheating. The effect of dry preheating was however, negligible, indicating that under the experimental conditions, rice endogenous phytase does not have a significant effect ($p > 0.05$) on PA. Phytase addition treatments resulted in significant ($p < 0.05$) PA degradation, with concomitant release of phosphate into the soaking medium; the release of phos-

phate as a result of phytase treatment was also observed in feed (Wu, Ravindran, Pierce, & Hendriks, 2004).

Soaking in water and acidic buffer also resulted in lower PA levels. In principle, two factors could be responsible for the impact of soaking, i.e. endogenous phytase activity and diffusion of PA into the soaking medium. Whilst endogenous phytase was functional in rice-based products (Perlas & Gibson, 2002) and mungbean (Sattar et al., 1989), it was of no relevance in our study as mentioned earlier. Diffusion of PA was reportedly influenced by the nature of the phytate, which may be in the form of salts with different minerals, such as potassium, calcium or magnesium, and the pH of the medium (Mahgoub & Elhag, 1998). Soaking in distilled water was more effective to remove PA from pulses than in solution of sodium bicarbonate (0.02%, w/v) (Vijayakumari et al., 1998). We observed that soaking in acidic buffer was more effective to remove PA from brown rice and rice bran than in demineralised water, presumably because of the higher solubility of phytates in acidic conditions.

We previously reported (Liang et al., 2008a) that levels of minerals in rice products can be ranked in decreasing order as follows: rice bran > brown rice > white rice; this is related to their distribution in the rice kernels and the effect of processing. Investigations elsewhere reported losses of minerals from rice products during soaking to be mainly caused by diffusion into the soaking medium (Barampama & Simard, 1995). Increases of mineral concentration occurred in selected instances, which could be attributed to a proportionately greater loss of dry matter. Quantities of minerals lost are influenced by the soaking conditions (e.g., pH, temperature) and also by complexation of minerals with other components, such as PA, fibre and polyphenols. Only those minerals released from such complexes would be soluble in the soaking medium (Lestienne et al., 2005b). We observed that soaking caused a significant decrease (12–63%) of calcium in almost all rice products. From rice bran, absolute losses of calcium ranging from 46% to 48% (phytase treatment) and from 15% to 20% (acidic buffer soak) were observed whilst calcium concentrations on dry matter basis apparently increased slightly (2–9%); we consider this to be a consequence of the considerable dry matter losses (7–30%) that took place simultaneously. We suggest that phytase-catalysed release of calcium from insoluble complexes, and acidic pH favouring solubility, are the main causes for this mineral extraction. An increase of zinc in white rice dry matter is explained by the loss of water-soluble matter from the surface and retention of zinc which is distributed in the endosperm; an increase of iron in rice bran dry matter is explained by its retention by bonding with phytate and the loss of soluble endosperm remainders which result in a loss of zinc. Further work will be required to establish a material balance for the minerals and other components during soaking of rice components. Regardless of other applications, acidic or phytase soaking of rice bran may be a profitable way to recover minerals for food or feed uses.

The results suggested that acid soaking of white rice was adequate to dephytinize intact kernels. For bran, dephytinizing with phytase caused a majority of minerals to be dissolved in liquid medium, which could be applied profitably as a natural mineral enrichment for food or feed use. The data on whole brown rice vs. milled rice give a very clear illustration of the function of the testa and aleurone layers as a barrier to influx of e.g., phytase, and diffusive loss of matter. It has been reported that the presence of bran, which retards water penetration and therefore the leaching of solids (Bello, Tolaba, & Suarez, 2004), limits the passage of phytate and phytase (Lestienne et al., 2005c). The effect of the bran layer is also the main reason for the lower mass loss of whole brown rice soaked in demineralised water than in acidic buffer; this difference might be caused by a modification of the outermost layer of bran in acidic conditions, so that increased losses of solid mass would occur. A similar situation was observed in dehulled beans (Aminigo & Metzger, 2005; Bayram et al., 2004).

5. Conclusions

White rice, rice bran and brown rice underwent different patterns of losses of dry mass, minerals and phytic acid, when soaked with demineralized water, acidic buffer and phytase solutions after preheating. White rice contained 4 mg g^{-1} phytic acid which was removed by all treatments to below detection level. Dry matter losses due to soaking ranged from 2–20% with the highest losses observed after soaking in acidic buffer. Whereas relatively high losses of calcium (12–63%) and iron (9–43%) were recorded, zinc losses were lower (0–38%). Bran contained 140 mg g^{-1} phytic acid which was removed best (−92%) by phytase treatment, followed by soaking in acidic buffer (−50%). Dry matter losses from bran due to soaking were highest (7–30%) of all rice products. Bran contains the highest levels of calcium (671 mg g^{-1}), iron (95 mg g^{-1}) and zinc (57 mg g^{-1}), and phytase treatment resulted in dissolution of 50–70% in the soaking medium. Brown rice contained 20 mg g^{-1} phytic acid which was removed best from kernels (75% of initial) by soaking in acidic buffer, but in flour by phytase application to below detection levels. Dry matter losses due to soaking ranged from 0.1% to 31% with highest losses observed after soaking in acidic buffer. Brown rice contains about two-fold higher levels of calcium, iron and zinc than white rice. However, losses due to soaking were similar as in white rice, for calcium (22–62%), iron (31–60%), and zinc (8–50%).

Phytase application is an effective method to rapidly remove phytic acid whilst preserving relatively more dry matter and minerals than other soaking approaches in rice products.

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