Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 110 (2008) 390–398
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Iron-fortified parboiled rice – A novel solution to high iron density in rice-based diets

Chanakan Prom-u-thai [a,*], Shu Fukai [a], Ian D. Godwin [a], Benjavan Rerkasem [b], Longbin Huang [c]

[a] School of Land, Crop and Food Sciences, University of Queensland, St. Lucia, Qld 4072, Australia
[b] Agronomy Department, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[c] Center for Mined Land Rehabilitation, University of Queensland, St. Lucia, Qld 4072, Australia

Received 26 October 2007; received in revised form 12 November 2007; accepted 8 February 2008

Abstract

The present study pioneered an investigation of a novel and cost-effective approach to fortify Fe in rice and to greatly improve Fe nutrition in rice-based diets through parboiling, though it remains at its preliminary phase. Rice grains of seven cultivars were parboiled in deionised water containing different levels of Fe chelate made by mixing different proportions of Fe sulfate ($FeSO_4$) with ethylenediaminetetra-acetic acid disodium salt (Na_2EDTA). Adding Fe to the parboiling water resulted in an increased Fe concentration in the most grain, effectively where $FeSO_4$ and Na_2EDTA were mixed at 2:1 molar ratio (11.16 g Fe per 100 g raw paddy grain). This treatment resulted in Fe concentrations in white rice milled for 60 s and 120 s, which were 20–50 times higher than those in the unfortified milled raw rice grains. The Fe concentrations in milled rice grains were 50–150 mg Fe kg^{-1} in 60 s milled grains with a slight reduction in 120 s milled grains. Perls Prussian blue staining of the cross section of Fe-fortified parboiled rice grains suggested inward movement of added Fe into the endosperm through the apoplastic pathway in the dorsal region of the rice grain. The retention rates of fortified Fe varied among the different cultivars, possibly due to different physical–chemical properties of the grains. The percentages of soluble fraction of the total Fe were higher than 50% in all cultivars tested, indicating its high bioavailability potential, though it remains to be evaluated. The present findings provided a preliminary basis for further investigation of this innovative technique, before its adoption by parboiled rice industry, such as optimising the levels of Fe addition and industrial process and Fe bioavailability in Fe-fortified-parboiled rice.

Keywords: *Oryza sativa*; Parboiled rice; Parboiling process; Iron; Fortification

1. Introduction

There are on-going international efforts to enhance Fe density in rice (*Oryza sativa* L.) grains for alleviating the risk of Fe-deficiency induced anemia in human populations consuming rice as the staple food. This includes projects coordinated by World Health Organization, Harvest Plus and International Rice Research Institute (Gregorio, 2002; Welch & Graham, 2004). Increasing Fe concentration in rice grain (in white rice) is expected to promote Fe intake by rice consumers and decrease incidences of Fe deficiency anemia in remote communities of developing countries where people have very limited access to Fe-rich food such as animal products (Juliano, 1993). In general, the emphasis of this effort has been on agronomic management and plant breeding (conventional breeding and transgenic selection) (Fidler, Davidsson, Walczyk, & Hurrell, 2003; Graham, Senadhira, Beebe, Iglesias, & Monasterio, 1999; Hettiarachchi, Hilmers, Liyanage, & Abrams, 2004; Takahashi, Nakanishi, Kawasaki, Nishizawa, & Mori, 2001; Vasconcelos et al., 2003). However, crop breeding for high Fe rice is considered as time consuming and expensive (Bouis, 1996), and may not be feasible for economically disadvantaged developing countries. In addition,

* Corresponding author. Tel.: +61 7 33652526; fax: +61 7 33651177.
E-mail address: chanakan@uq.edu.au (C. Prom-u-thai).

0308-8146/$ - see front matter Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.043

genetically engineered crops may face the opposition of consumers and governments, due to their concerns about genetically modified (GM) food. In the mean time, an alternative cost-effective way to deliver Fe nutrition through the staple food system is required for urgently addressing this Fe-deficiency problem, which has to be cost-effective and closely linked to the existing rice products and their market distribution network.

Iron fortification in food has been practiced in areas (Hurrell & Cook, 1990; Mertz, 1997; Tulyathan, Mekjarutkul, & Jongkaewwattana, 2005), requiring industrial infrastructure and changed cooking habits. Iron fortification in rice flour has been promoted in Sri Lanka and the Philippines (Hettiarachchi et al., 2004), but it is not common in most developing countries. Iron fortification in rice by surface coating has not been successful as the consumers can commonly detect the altered colour and remove the fortified grains mixed together with normal grains (Hurrell & Cook, 1990). Thus, these food-fortification methods have proven unsuitable as they are not cost-effective for mass delivery of high Fe rice and require significant changes in cooking culture and habits.

However, fortification of Fe in rice through the existing industrial process of parboiling rice has not yet been exploited. It would be a cost-effective approach for delivering Fe nutrition to the mass population as there is an existing infrastructure and marketing networks and consumers in developing countries such as Asia and Africa, without having to invent new process/infrastructure and/or alter the existing consumption behaviour. Parboiled rice is a potentially powerful vehicle for Fe fortification because approximately 50% of the world rice production is in parboiled form and it is consumed by a high proportion of the population in many countries, especially in South Asia (Choudhury, 1991; Pillaiyar, 1981). Parboiling process consists of soaking, steaming and drying paddy rice grains before milling (Bhattacharya, 2004).

Our study is the first to explore the feasibility of Fe fortification in parboiled rice as a rapid and cost-effective solution to Fe-deficiency anemia in economically disadvantaged populations with rice as the major staple food and poor access to animal proteins. It focused initially on the feasibility of this innovative approach, by examining the effectiveness of Fe fortification and retention, the solubility of Fe in the grain in response to fortification treatments and the likely pathway of Fe movement into the endosperm. The objectives of the present study were to investigate the distribution and movement of fortified Fe in rice grain in the parboiling process with varying degree of Fe chelation by mixing different molar ratios of $FeSO_4$ with Na_2EDTA among different rice cultivars representing a range of grain Fe concentrations and morphology (Table 1) (Prom-u-thai, Fukai, Godwin, & Huang, 2007; Prom-u-thai, Sanchai, et al., 2007). NaFeEDTA has been recently approved as an ingredient to be used in supervised food-fortification programs (Hurrell, 1998, 2003; Thuy et al., 2003), and is the most promising Fe fortification compound for food additives and is used intensively to prevent oxidation and colour changes in food and promote its bioavailability in the human diet (Hurrell, 1998; Macphail, Patel, Bothwell, & Lamparelli, 1994). We also evaluated Fe retention in the grain by measuring rinsing-loss of Fe from the fortified rice grain and Fe solubility the fortified rice grains by extraction in dilute acid, which is used as an indirect estimation of potential Fe bioavailability in the human diet.

Table 1
Fe concentration in raw rice of unmilled and milled grains at 60 and 120 s of seven cultivars and its grain shape

Cultivar	Grain shape[a]	Fe concentration (mg kg^{-1})		
		Unmilled	Milled 60 s	Milled 120 s
Goolarah	Long-slender	6.56 ± 0.2	4.45 ± 0.5	1.72 ± 0.2
Norin PL 11	Short-bold	6.36 ± 0.3	4.92 ± 0.2	1.96 ± 0.7
Sakha 102	Medium-bold	6.92 ± 0.2	4.29 ± 0.4	3.13 ± 0.6
YRF 2	Long-slender	7.19 ± 0.3	3.72 ± 0.8	2.00 ± 0.5
YRM 64	Medium-medium	8.82 ± 0.2	4.39 ± 0.6	3.48 ± 0.7
Echuga	Medium-bold	9.39 ± 0.2	5.18 ± 0.6	2.58 ± 0.6
Opus	Short-bold	9.66 ± 0.1	7.18 ± 0.3	2.23 ± 0.1

The Fe concentrations were means ± S.E. of three individual sub-samples of each cultivar.

[a] Grain shape category was classified using grain length and length/width ratio as explained previously in Prom-u-thai, Sanchai, et al. (2007).

2. Materials and methods

2.1. Fe fortification through parboiling process

Seven rice cultivars were grown in field plots with the same soil type, nutrient management under flooded condition at Yanco Agriculture Institute, NSW, Australia, in October 2005–March 2006. The experiment was laid out in completely randomized block design with three replicates per cultivar. Rice grains were harvested at maturity, with a great care to minimise the chance of Fe contamination in the field. Approximately 150 g of paddy rice were sub-sampled and rinsed thoroughly in three changes of distilled deionised water (DDI) before applying treatments.

For Fe fortification treatments, rinsed paddy rice were soaked with 150 ml of Fe solutions consisting of ferrous sulfate ($FeSO_4$) and ethylenediaminetetra-acetic acid disodium salt (Na_2EDTA) mixed in molar ratios of 1:1, 2:1, 3:1 and 4:1, respectively. The Fe application rates were 5.58, 11.16, 16.74 and 22.32 g Fe per 100 g paddy rice (~13% moisture content), respectively. For the control-unfortified parboiled rice, rinsed paddy rice was soaked with 150 ml of distilled triple deionised water (TDI) for 24 h at room temperature. Soaked grains were steamed with the pressure 2.29 kg cm^{-1} at 110 °C for 10 min. The grains were cooled and sun-dried to approximately 11% moisture content.

2.2. Husking and milling

The dried grains (approximately 11% moisture content) of parboiled rice were separated into brown rice (unmilled caryopsis) and husk (palea and lemma) with a testing husker (Satake model THU-35A, Japan). For each sample, 40 g of the brown rice were milled for 60 or 120 s to yield white rice by using a laboratory milling machine (Satake model TM 05, Japan). Metal parts of the husker and polishing machine were cleaned by sand-blasting and coated with Teflon to minimise Fe contamination in rice samples (Prom-u-thai, Fukai, et al., 2007; Prom-u-thai, Sanchai, et al., 2007). Weights of milled grains were recorded for calculating the percentage of head rice yield and degree of milling (Prom-u-thai, Sanchai, et al., 2007). The samples were oven dried at 70 °C for 72 h. For total Fe analysis, approximately 0.8 g of unmilled grains, 0.5 g of milled grains and 0.3 g of husk were digested in nitric–perchloric acid (5:1) at 120–180 °C until completion, which was quantified by ICP-AES (Zarcinas, Cartwright, & Spouncer, 1987).

2.3. Perls Prussian blue staining

Ten grains of unfortified raw rice and Fe-fortified parboiled rice of cultivar Opus milled for 60 and 120 s, respectively, were cut transversely across the middle plane of the grain with a Teflon knife (Personna, Verona VA, USA) in a petri dish. The specimens were submerged in freshly prepared Perls Prussian blue solution (2% hydrochloric acid mixed with 2% potassium ferrocyanide) for 10 min as described previously (Pintasen, Prom-u-thai, Jamjod, Yimyam, & Rerkasem, 2007; Prom-u-thai, Dell, Thomson, & Rerkasem, 2003). The intensity of staining representing the relative density of Fe in the grains (blue color) was assessed under an optical microscope (Olympus BX61, Australia). Ferric Fe released from any attachments to protein by treatment with dilute hydrochloric acid, reacts with a dilute solution of potassium ferrocyanide to produce an insoluble compound, ferric ferro cyanide (Prussian blue) (Pintasen et al., 2007; Prom-u-thai et al., 2003).

2.4. Fe retention

Approximately 0.5 g milled rice of fortified Fe parboiled rice grains was placed into 50 ml flask. The fortified grain samples were thoroughly rinsed three times with 10 ml TDI water at each time (Hettiarachchi et al., 2004; Tulyathan et al., 2005). Samples were oven dried at 70 °C for 72 h. Weights were then recorded before analysis of the Fe retention after rinsing treatment.

2.5. Fe solubility

Approximately 0.5 g milled Fe-fortified parboiled rice grains were weighed into a 50 ml flask. The sample was extracted with 10 ml of 0.1 M HCl at 60 °C for 30 min. The supernatant was discarded and the total Fe remained in the grain was quantified as described previously. Soluble Fe in the fortified Fe rice was calculated as the difference between total grain Fe concentrations before and after acid extraction.

2.6. Data analysis

The analysis of variance was carried out to detect the differences of Fe concentration in raw and parboiled rice of unfortified and fortified Fe grains among different grain tissues and cultivars by using Statistic 7, analytical software, SXW (Tallahassee, FL, USA). The least significant difference (LSD) at $p < 0.05$ was applied to compare the means for significant differences between raw and parboiled rice of unfortified and fortified Fe grains, in terms of Fe concentrations in different grain tissues and cultivars tested. The correlation analysis was used to test the significance of each correlation.

3. Results

3.1. Effectiveness of fortification

There was variation in Fe concentration between raw and parboiled rice with or without fortified Fe of rice grain among different ratios of $FeSO_4$:Na_2EDTA in the husk, unmilled and milled rice after milling for 60 and 120 s of cultivar Opus ($p < 0.05$) (Table 2). In unfortified rice, Fe concentration in the husk decreased after parboiling process, while there was no difference between unmilled and milled rice at both 60 and 120 s milling time. In Fe-fortified and parboiled rice, Fe concentration significantly increased in all grain tissues investigated, compared to that in raw and parboiled rice without fortified Fe. Iron fortification using the mix of $FeSO_4$ and

Table 2
Iron concentration in raw and parboiled rice with unfortified and fortified Fe in different molar ratios (1:1, 2:1, 3:1, 4:1) of $FeSO_4$:Na_2EDTA (cv. Opus)

Fe treatment/ rice form	Fe concentration (mg kg^{-1})			
	Husk	Unmilled	Milled 60 s	Milled 120 s
Unfortified Fe/raw	64.12 a[A]	9.66 a	7.16 a	2.23 a
Unfortified Fe/parboiled	33.10 b	11.09 a	8.37 a	3.55 a
Fortified Fe/parboiled[B]				
1:1	16.14×10^3 c	493.0 b	79.15 b	29.14 bc
2:1	36.77×10^3 d	2069 d	144.4 c	110.1 d
3:1	49.29×10^3 e	3246 d	80.20 b	36.16 c
4:1	50.69×10^3 e	1947 c	72.70 b	24.89 b

[A] Different letters are used for comparing the significant difference of Fe concentration between unfortified Fe in raw and parboiled rice and fortified Fe in parboiled rice with different ratios of $FeSO_4$:Na_2EDTA in the same column ($p < 0.05$).

[B] Fortified Fe through parboiling process with varying ratio of Fe concentration between $FeSO_4$:Na_2EDTA.

Na_2EDTA (2:1 molar ratio) in rice through parboiling resulted in the highest Fe concentration in milled rice grains regardless of the milling time, containing 144 and 110 mg Fe kg^{-1}, respectively.

There was a significant variation of Fe concentration among seven rice cultivars after fortified Fe with 2:1 molar ratio of $FeSO_4$:Na_2EDTA through parboiling process ($p < 0.05$), regardless of the milling treatments (Fig. 1). The concentration of Fe in milled rice declined substantially, compared to that in the unmilled rice in all cultivars tested. In unmilled rice, Fe concentration ranged from 1121 to 2069 mg Fe kg^{-1}, from 50 to 144 mg Fe kg^{-1} in rice grains milled for 60 s and, from 23 to 110 mg Fe kg^{-1} in rice grains milled for 120 s. Cultivar Opus had the highest Fe concentration, while Sakha 102 had the lowest Fe concentration in all grain tissue layers.

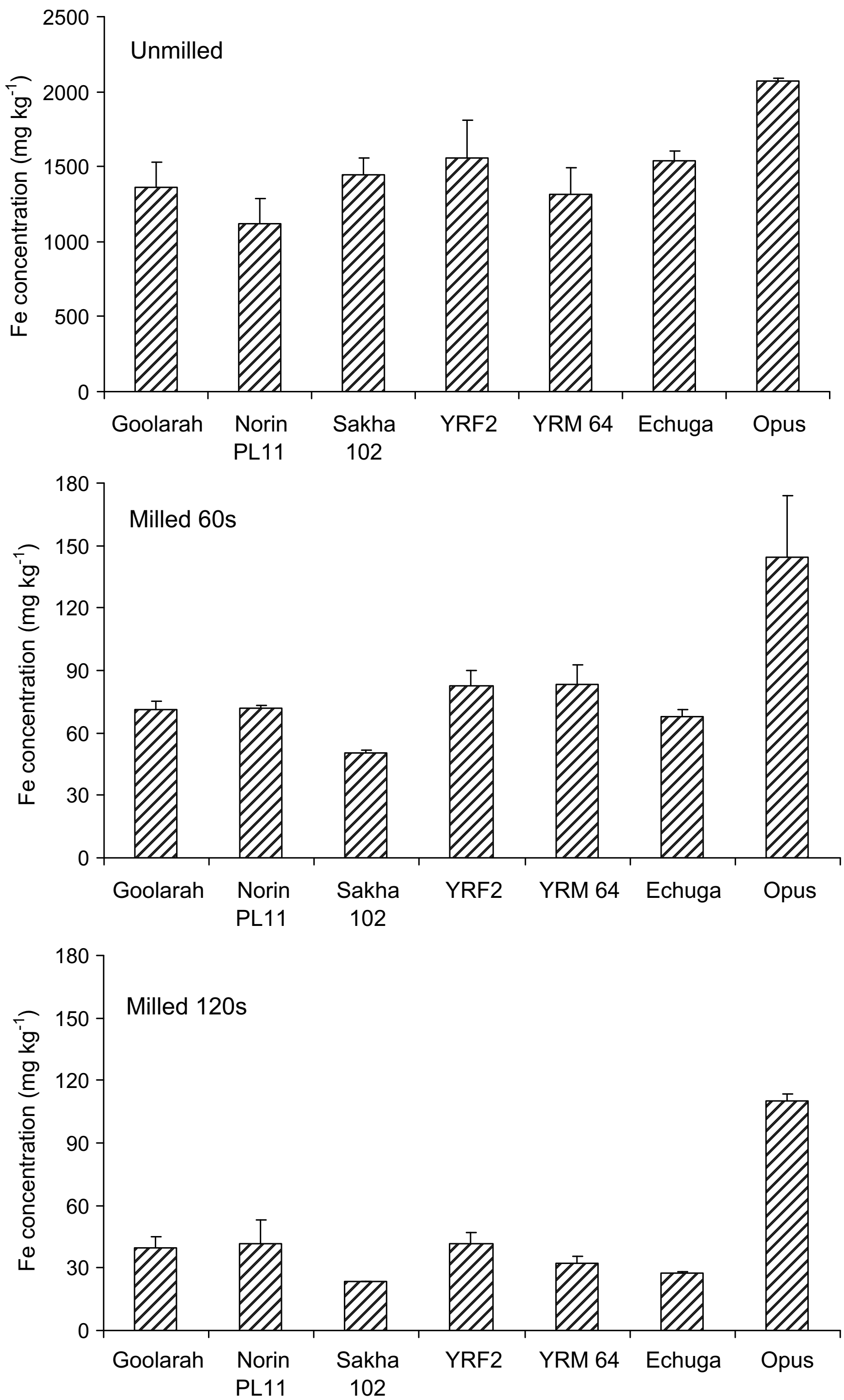

Fig. 1. Total Fe concentrations in fortified rice grains treated with a solution mix of $FeSO_4$ and Na_2EDTA (2:1 molar ratio) during the parboiling process in the seven cultivars tested: Unmilled, milled 60 s and 120 s.

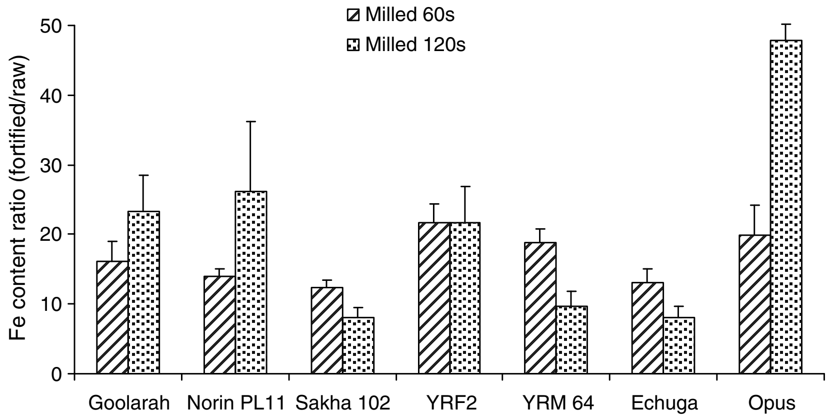

Fig. 2. The ratio of Fe content in fortified rice grain to that of unfortified rice grain. The parboiled and raw grains were milled for 60 and 120 s, respectively. The bars represent standard errors of corresponding means from three replicates.

Fortification of Fe through parboiling produced 10–50-fold increases in Fe content, compared to that in raw rice among cultivars tested (Fig. 2). However, the magnitude of increment varied with milling time and rice cultivars. At 60 s milling, the cultivars YRF 2 and Opus had the largest increment in Fe concentration achieving 20-folds increase compared to the raw rice. At 120 s milling time, the cultivar Opus, had as much as 50-fold increase in Fe concentration, compared to that in the unfortified raw rice.

3.2. Distribution of fortified Fe in the grain

The distribution of fortified Fe across the grain structure was shown in the cultivar Opus, by using Perls Prussian blue staining of Fe-fortified and parboiled rice grains which was compared with the staining of unfortified raw rice grains (Fig. 3). In the grains milled for 60 s, unfortified raw rice grains only had a very low intensity of staining in the surface layer of the endosperm (Fig. 3A1 and A2), while in Fe-fortified and parboiled grain, a high intensity of staining was found in the outer layers (20–30% of the cross-section distance) of the endosperm of the fortified grains (Fig. 3C1 and C2). At 120 s milling, there was little staining reaction in unfortified raw rice (Fig. 3B1 and B2) but, on the other hand, fortified rice grains still had a significant level of staining intensity (Fig. 3D1 and D2). Particularly, the distribution of the staining tended to distribute around the dorsal region of the grain and gradually disperse towards the opposite pole of the grain (Fig. 3D2). From the visual observation, the parboiling process achieved a significant penetration through the outer layers of the endosperm of the parboiled rice grains (Fig. 3C1 and C2).

3.3. Fe retention and solubility

The magnitude of Fe loss caused by washing/rinsing the Fe-fortified and parboiled rice grains varied among cultivars and milling time ($p < 0.05$) (Fig. 4). After rinsing, the milled Fe-fortified rice retained 45–96% Fe in the grains milled for 60 s and 20–98% in the grain milled for 120 s. In grains milled for 60 s, Fe retention rate was the highest in cultivars Goolarah (92.3%) and Echuga (95.9%), and lowest in Opus (44.4%). In grains milled for 120 s, Fe retention rate was the highest in cultivar YRF 2 (97.9%) and lowest in Opus (20.1%). However, there was no significant relationship between the total Fe concentration in fortified rice and the percentage of Fe retention after rinsing in the milled rice regardless of milling time ($r = 0.33$, ns).

The variation of Fe solubility among the seven cultivars at both milling time seemed to be much less than other parameters investigated. The milled grains except for Echuga and Opus, had around 70% solubility for grains milled for 60 s and 75–90% solubility in grains milled for 120 s, except for Echuga (Fig. 5). The percentage of Fe solubility in the grains milled for 120 s was higher than in those milled for 60 s in most cultivars, except for Echuga and Opus.

3.4. Other observations

No significant changes in colour and flavour of milled grain surface was observed in the milled, Fe-fortified and parboiled rice, compared with raw rice grain. The degree of milling (DOM) was not different between parboiled and raw rice as though it varied among rice cultivars, ranging from 12% to 24% at 60 s milling time and from 16% to 31% at 120 s milling time among seven rice cultivars. Head rice yield (HRY) was different among rice cultivars but it was not different between raw and parboiled rice for the same cultivars. The highest HRY was found in Goolarah (89%), YRF 2 (83%) and Opus (80%), lowest in Norin PL 11 (48%) and Sakha 102 (59%) and YRF 2 (75%) and Echuga (78%) were in the middle (data not shown).

4. Discussion

From the evidence presented in the present pilot study, Fe-fortification of parboiled rice is a very promising approach for delivering a cost-effective solution to the

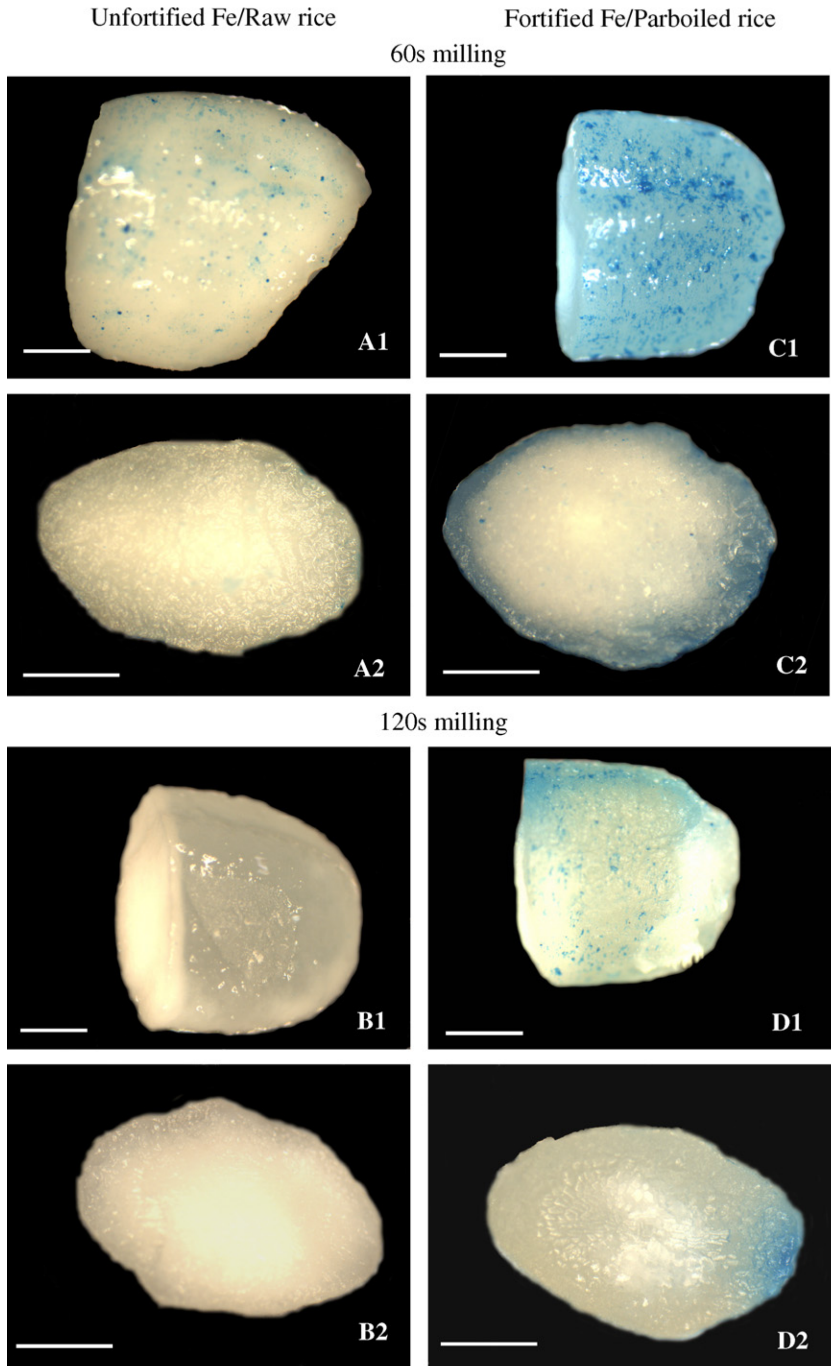

Fig. 3. Stereo-micrographs of unfortified (A1, A2, B1, B2) and fortified (C1, C2, D1, D2) rice grains (cv. Opus), which were milled rice for 60 (A1, A2, C1, C2) and 120 s (B1, B2, D1, D2), respectively. The grains were cut transversely across the middle plane of the grain and were stained with Perls Prussian blue (side views – A1, B1, C1, D1; and top view – A2, B2, C2, D2). All scale bar = 1 mm. The intensity of staining represented the relative density of Fe in the grains.

improvement of Fe nutrition in rice-based diets, at least in regions (mostly developing countries such as Bangladesh and India) already consuming parboiled rice. Iron fortification in the parboiling process dramatically increased Fe concentration in the brown and milled rice grains, with 70–144 and 30–110 mg Fe kg^{-1} in 60 and 120 s milled grains, respectively, compared to 37 mg Fe kg^{-1} in transgenic IR68144-2B-2-2-3 rice variety (Vasconcelos et al., 2003). Iron concentrations in the Fe-fortified parboiled rice grains were 20–50 times higher than those in their corresponding raw white rice (Figs. 1 and 2). More than half of the fortified Fe in the grains was retained in the milled grains even after repeated rinsing, with retention rates ranging from 60% to 95%, except for Opus which had 50–70% of Fe lost to the washing water (Fig. 4). In the Fe-fortified and milled rice grains, 60–95% of the grain

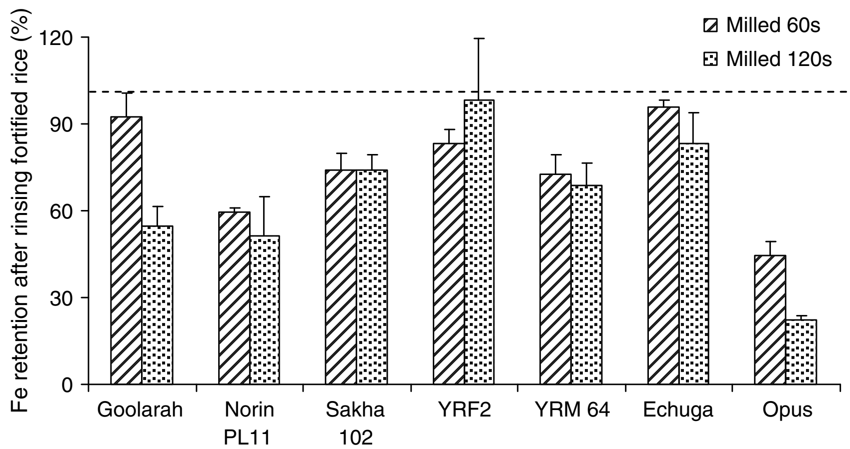

Fig. 4. Iron retention rate (as % of the un-rinsed) after rinsing (simulating rice washing) in the Fe-fortified parboiled rice grains milled for 60 and 120 s, respectively, in the seven rice cultivars tested.

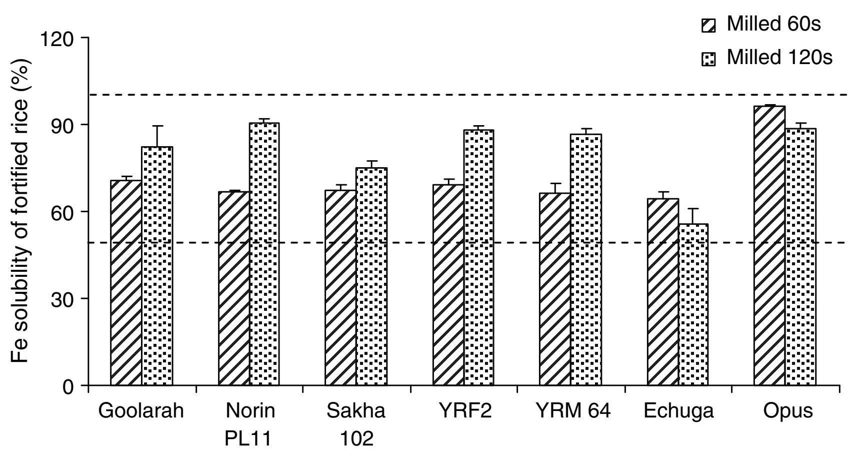

Fig. 5. The Fe solubility (% of the total Fe) of in the Fe-fortified parboiled rice grains milled for at 60 and 120 s, respectively, in the seven cultivars tested.

Fe were acid-soluble, indicating the potentially high Fe bioavailability to human uptake (free Fe form) (Fig. 5). Further research is under way to investigate the bioavailability of Fe in Fe-fortified-parboiled rice and optimal protocols for industrial application of this technology.

The effectiveness of enhancing Fe density in white rice through the Fe-fortified parboiling process is far greater than that achieved from conventional and transgenic rice breeding. For example, Fe concentration in milled rice of IR68144-2B-2-2-3, an improved rice cultivar by conventional breeding is 7–13 mg Fe kg^{-1} (Graham et al., 1999; Prom-u-thai et al., 2006), and 37 mg Fe kg^{-1} in rice containing transferred soybean *ferritin* gene (Vasconcelos et al., 2003). In contrast, Fe concentration in the milled rice grains Fe-fortified in the parboiling had 70–144 and 30–110 mg Fe kg^{-1} in 60 and 120 s milled grains, respectively.

The effectiveness of Fe-fortified parboiled rice is also demonstrated in the high retention rate of the fortified Fe in the endosperm and relatively high potential bioavailability of the Fe. The percentage of Fe retention after rinsing varied among rice cultivars and milling times. YRF 2 and Echuga had highest Fe retention after rinsing after milling both for 60 and 120 s. Loss of Fe in fortified rice after rinsing was not correlated with the total Fe concentration. Apart from the cultivar Opus, on average, 60–98% of the fortified Fe in the milled rice grains was retained in the rice washing process, which is comparable to the retention rates achieved by special and expensive rice surface coating technique, 71.2–96.8% in flour gel coated rice grain (Tulyathan et al., 2005) and 100% in cellulose polymer (Peil, Barrett, Rha, & Langer, 1981). Comparatively, a substantial loss of Fe sprayed on raw rice surface occurs if the rice is rinsed before cooking (Hettiarachchi et al., 2004; Tulyathan et al., 2005). Although a polymer coating technique (Tulyathan et al., 2005) has been suggested for painting Fe on the rice grain surface to minimise the Fe loss from washing and/or cooking, these techniques are expensive and not practical in rice mills of developing countries. In comparison, parboiled rice would be a rapid and cost-effective vehicle to deliver Fe nutrition benefits through the already established parboiling infrastructure, market network and consumers' acceptance in Asia (particularly in the subcontinent) and Africa, where the high risk of Fe malnutrition-induced anemia is present (Bouis, 1996; Graham et al., 1999; Graham, Humphries, & Kitchen, 2000).

Most of the fortified Fe in the milled rice grains remained in the dilute acid-soluble pool, which is considered as potentially bioavailable in the human diet in all cultivars (Swain, Newman, & Hunt, 2003). Previous studies suggested that fortification of FeEDTA into the meal such as rice flour (Hettiarachchi et al., 2004), rice grain (Haas et al., 2005; Macphail et al., 1994), corn (Walter, Pizarro, & Olivares, 2003) and fish/soy sauces (Fidler et al., 2003; Thuy et al., 2003) can help improve Fe absorption in human diet. However, further animal or human trials will be carried to examine the bioavailability of Fe in milled rice after Fe fortification through the parboiling process.

One of the significant advantages of parboiled rice is that parboiling resulted in a significant inward movement of the fortified Fe into the endosperm, countering milling-induced Fe loss in raw rice grains due to the restricted distribution of Fe in the aleurone and embryo (the bran fraction) of brown rice. Parboiling itself may cause the inward migration of some mineral nutrients present in the surface layers of rice grain, resulting in a higher retention rate of these nutrients when being milled to produce white rice (Ali & Bhattacharya, 1980; Doesthale, Devara, Rao, & Belavady, 1979; Palipane & Swarnasiri, 1985), such as P, Ca, Fe, Mn, Mo and Cr in milled parboiled rice (Doesthale et al., 1979). However, the present study did not show any effect of parboiling on the endogenous Fe movement, compared to the raw rice in both unmilled and milled grains. Nevertheless, optimisation of the milling time and conditions may have bearings on Fe retention in both raw and parboiled brown rice, as the degree of milling (DOM) may cause the difference in Fe concentration in milled raw and parboiled rice (Doesthale et al., 1979; Prom-u-thai, Sanchai, et al., 2007). A previous study investigated that Fe concentration in milled rice after parboiling was significantly higher than that in the raw rice only when it was 5% DOM, while there was no difference after 10% DOM (Doesthale et al., 1979), suggesting some weak movement of Fe into the endosperm by parboiling itself. In the present study, DOM were 12–24% and 16–31% at 60 s and 120 s milling time, respectively, which is comparable to the DOM in commercial rice mills to produce white rice. This overcomes the significant loss of Fe present in the aleurone layer and embryos of the brown rice, such as in the case of the high Fe rice variety IR86144-2B-2-2-3 (Prom-u-thai et al., 2006; Prom-u-thai, Sanchai, et al., 2007).

Parboiling resulted in the inward movement of fortified Fe in the endosperm (Figs. 1 and 3). The inward movement of fortified Fe into the inner layers of rice grain declined substantially from the surface layer into the endosperm. Iron distribution in the dorsal region of the endosperm of Fe-fortified rice grains (Fig. 3) suggested that the fortified Fe entered the grain and endosperm via the dorsal vascular bundle present in the kernel. At grain filling stage, assimilates enter the caryopsis by a single vascular bundle which runs through the dorsal of the pericarp region (Oparka & Gate, 1981). Assimilate transport into the caryopsis is dependent on phloem transport and/or apoplasmic diffusion (Welch, 1986). Parboiling process may help to force Fe through the apoplastic pathway in the dorsal part of the grain. This Fe movement has significantly encountered the impact of milling on the retention of Fe in the white rice. However, the amount of Fe moved into the endosperm varied with cultivars, with the highest in Opus and the lowest in Sakha 102, which nevertheless, is still far above the highest Fe concentration in raw white rice reported in the literature.

The efficiency of Fe movement into the endosperm is an important basis of Fe fortification in parboiled rice, which may be affected not only by the degree of Fe chelation, but physical–chemical properties of grains of different cultivars. The responses of Fe concentrations in different grain components to decreasing chelation levels appeared different: with a linear increase of Fe concentrations in the husk, but "*n*"-shaped curvature in the kernel and milled rice grains. By using Opus as an example, the maximum Fe concentration in the milled grains was achieved when the added Fe had a 2:1 molar ratio of $FeSO_4$ and Na_2EDTA, with highest 144 and 110 mg Fe kg^{-1} in 60 and 120 s milled rice. The similar ratio of $FeSO_4$ and Na_2EDTA fortified in rice-based meal was also found to have maximum bioavailability of Fe among anemic people (Macphail et al., 1994). It is possible that at low chelation level, Fe^{2+} may be rapidly oxidised and precipitate on husk surface, thus reducing the opportunity of the Fe moving across the husk. Further optimisation of Fe concentration and Fe chelation ratios will be carried out in further experiments for standardisation of the fortification dosage and protocols.

The different pattern of grain Fe concentration variation in the unmilled parboiled rice (brown rice equivalent to raw rice) across the seven cultivars may be related to the differential sink strength of the surface layer cells of the grain due to different intensity of Fe-chelation compounds in aleurone layer (Prom-u-thai, Fukai, et al., 2007). Interestingly, Fe penetration into the endosperm of Opus was the most efficient, but Fe loss from washing was also the highest in this cultivar, compared to other varieties. The underpinning factors are yet to be identified. Further research will investigate grain physical (e.g., density, apoplastic pathways) and chemical properties (e.g., lipid contents, crystallinity of starch) which may affect Fe entry and retention in the endosperm for optimising Fe fortification for typical rice types in the market.

5. Conclusion

Parboiled rice has already been consumed by more than half of the rice consumers in the world, including Bangladesh, India, and Africa (Choudhury, 1991; Pillaiyar, 1981). This means that parboiled rice with fortified Fe can reach rice consumers in remote areas through established market channels and parboiling is a process that has already existed in many regions of these countries. The partitioning of fortified Fe from the surface layers into the endosperm tissues by apoplastic pathway in the dorsal part of rice grain plays a key role in achieving high Fe concentration in milled rice through parboiling process. On the basis of these preliminary findings and literature review, Fe-fortified parboiled rice may offer the most cost-effective strategy within the short-term, to reduce incidences of Fe deficiency in developing countries within an immediate future, by comparing with the other two major approaches: Agronomic practices and rice breeding.

Acknowledgements

We would like to thank Dr Russell Reinke and all assistants at Yanco Agriculture Institute, NSW, Australia for their help in the field experiment, Geoff Lambert for lab technical support and Eunice Grinan, Center for Microscopy and Microanalysis for microscopy assistant.

References

Ali, S. Z., & Bhattacharya, K. R. (1980). Pasting behaviour of parboiled rice. *Journal of Texture Studies, 11*, 239–245.

Bhattacharya, K. R. (2004). Parboiling of rice. In T. C. Elaine (Ed.), *Rice chemistry and technology* (3rd ed., pp. 329–404). St. Paul, MN: American Association of Cereal Chemists, Inc.

Bouis, H. E. (1996). Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. *Nutrition Reviews, 54*, 131–137.

Choudhury, N. H. (1991). *Parboiling and consumer demand of parboiled rice in south Asia* (pp. 47–54). Manila: International Rice Research Institute.

Doesthale, G. Y., Devara, S., Rao, S., & Belavady, B. (1979). Effect of milling on mineral and trace element composition of raw and parboiled rice. *Journal of the Science of Food and Agriculture, 30*, 40–46.

Fidler, M. C., Davidsson, L., Walczyk, T., & Hurrell, R. F. (2003). Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA. *American Journal of Clinical Nutrition, 78*, 274–278.

Graham, R., Senadhira, D., Beebe, S., Iglesias, C., & Monasterio, I. (1999). Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crops Research, 60*, 57–80.

Graham, R. D., Humphries, J. M., & Kitchen, J. L. (2000). Nutritionally enhanced cereals: A sustainable foundation for a balanced diet. *Asia Pacific Journal of Clinical Nutrition, 9*, S91–S96.

Gregorio, G. B. (2002). Progress in breeding for trace minerals in staple crops. *Journal of Nutrition, 132*, 500s–502s.

Haas, J. D., Beard, J. L., Murray-Kolb, L. E., del Mundo, A. M., Felix, A., & Gregorio, G. B. (2005). Iron-biofortified rice improves the iron stores of non anemic Filipino women. *Journal of Nutrition, 135*, 2823–2830.

Hettiarachchi, M., Hilmers, D. C., Liyanage, C., & Abrams, S. A. (2004). Na(2)EDTA enhances the absorption of iron and zinc from fortified rice flour in Sri Lankan children. *Journal of Nutrition, 134*, 3031–3036.

Hurrell, R. F. (1998). Improvement of trace element status through food fortification: Technological, biological and health aspects. *Bibliotheca Nutritio et Dieta, 54*, 40–57.

Hurrell, R. F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *Journal of Nutrition, 133*, 2973s–2977s.

Hurrell, R. F., & Cook, J. D. (1990). Strategies for iron fortification of foods. *Trends in Food Science & Technology, 1*, 56–61.

Juliano, B. O. (1993). *Rice in human nutrition* (Vol. 26, pp. 162). Prepared in collaboration with FAO. Rome: Food and Agriculture Organization of the United Nations, Rome.

Macphail, A. P., Patel, R. C., Bothwell, T. H., & Lamparelli, R. D. (1994). EDTA and the absorption of iron from food. *American Journal of Clinical Nutrition, 59*, 644–648.

Mertz, W. (1997). Food fortification in the United States. *Nutrition Reviews, 55*, 44–49.

Oparka, K. J., & Gate, P. (1981). Transport of assimilates in the developing caryopsis of rice (*Oryza sativa* L.). *Planta, 151*, 561–573.

Palipane, K. B., & Swarnasiri, C. D. P. (1985). Composition of raw and parboiled rice bran from common Sri Lankan varieties and from different types of rice mills. *Journal of Agricultural and Food Chemistry, 33*, 732–734.

Peil, A., Barrett, F., Rha, C., & Langer, R. (1981). Retention of micronutrients by polymer coatings used to fortify rice. *Journal of Food Science, 47*, 260–262.

Pillaiyar, P. (1981). Household parboiling of parboiled rice. *Kishan World, 8*, 20–21.

Pintasen, S., Prom-u-thai, C., Jamjod, S., Yimyam, N., & Rerkasem, B. (2007). Variation of grain iron content in a local upland rice germplasm from the village of Huai Tee Cha in northern Thailand. *Euphytica, 158*, 27–34.

Prom-u-thai, C., Dell, B., Thomson, G., & Rerkasem, B. (2003). *Easy and rapid detection of iron in rice grain*. Science.

Prom-u-thai, C., Fukai, S., Godwin, D. I., & Huang, L. (2007). Genotypic variation of iron partitioning in rice grain. *Journal of the Science of Food and Agriculture, 87*, 2049–2054.

Prom-u-thai, C., Glahn, R. P., Welch, R. M., Fukai, S., Rerkasem, B., & Huang, L. (2006). Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes. *Journal of the Science of Food and Agriculture, 86*, 1209–1215.

Prom-u-thai, C., Sanchai, C., Rerkasem, B., Jamjod, S., Fukai, S., Godwin, D. I., et al. (2007). The effect of grain morphology on degree of milling and iron loss in rice. *Cereal Chemistry, 84*, 384–388.

Swain, J. H., Newman, S. M., & Hunt, J. R. (2003). Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. *Journal of Nutrition, 133*, 3546–3552.

Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N. K., & Mori, S. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nature Biotechnology, 19*, 466–469.

Thuy, P. V., Berger, J., Davidsson, L., Khan, N. C., Lam, N. T., Cook, J. D., et al. (2003). Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. *American Journal of Clinical Nutrition, 78*, 284–290.

Tulyathan, V., Mekjarutkul, T., & Jongkaewwattana, S. (2005). Iron retention on flour gel-coated rice grains and its storage stability. *Foodservice Research International, 15*, 147–156.

Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torrizo, L., Krishnan, S., et al. (2003). Enhanced iron and zinc accumulation in transgenic rice with the *ferritin* gene. *Plant Science, 164*, 371–378.

Walter, T., Pizarro, F., & Olivares, M. (2003). Iron bioavailability in cornmasa tortillas is improved by the addition of disodium EDTA. *Journal of Nutrition, 133*, 3158–3161.

Welch, R. M. (1986). Effects of nutrient deficiencies on seed production and quality. *Advance in Plant Nutrition, 2*, 205–247.

Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany, 55*, 353–364.

Zarcinas, B. A., Cartwright, B., & Spouncer, L. R. (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communication of Soil Science and Plant Analysis, 18*, 131–146.