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Iron fortification in parboiled rice – a rapid and effective tool for delivering Fe nutrition to rice consumers

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Abstract

Parboiled rice production accounts for nearly half of the world's rice production. Its markets and consumer base are firmly established in South Asia and Africa where Fe-deficient populations are mostly concentrated. Our research group has pioneered the technology of Fe-fortification in parboiled rice and demonstrated its feasibility in significantly increasing Fe concentration in the endosperm (white rice) and its bioavailability in rice based diet. Fortification with Fe-EDTA during parboiling resulted in 10 to 50 folds increase in grain Fe concentration, depending on the grain properties among different rice varieties. However, the broken rice of Fe-fortified parboiled rice contained 5 times the Fe concentration of the full grain, which is often bought and consumed by people in low income category. The bioavailability of the fortified Fe is closely correlated with increasing Fe concentration in white rice ($r = 0.90$, $p < 0.01$). The retention rates of the fortified Fe in the white rice range from > 50% to almost 100%, despite repeated rinsing before cooking depending on rice varieties. Perls' Prussian blue staining and prolonged polishing showed that the in vitro Fe penetrated into the interior of the endosperm. Fortification at the rate up to 250 mg Fe kg-1 paddy rice has no deleterious effects on appearance, color and sensory quality and overall acceptance by parboiled rice consumers. It increased Fe concentration up to 27 mg Fe kg-1 of in white rice, compared with 5 mg Fe kg-1 in unfortified parboiled and raw white rice. As a result, we can conclude that parboiled rice is a ready and effective tool for improving Fe nutrition of rice consumers in these regions.

Introduction

Fortifying Fe in food has been suggested as a means to improve Fe level in food products and human diets (Moretti et al. 2005; Moretti et al. 2006). However, Fe fortifying in raw rice grain has not been successful due to consumers' poor acceptance of the different appearance of the fortified grain mixed with normal grain and removal of the Fe fortified grains before cooking (Cook et al. 1997). Surface coating also tends to be washed away during rinsing, a common practice in rice cooking. In addition, fortification by surface coating also requires not only re-educated consumers, but new industrial infrastructure on a large scale, which are often economically unfeasible.

The success and cost-effectiveness of in vitro Fe-fortification will depend on several key aspects (1) industrial feasibility and associated investment costs; (2) biological effectiveness of Fe fortified; and (3) consumers' acceptance. Parboiled rice production overcomes these constraints and offers an ideal vehicle for Fe fortification.

Parboiling, a ready made industrial process for iron fortification

Parboiled rice production already accounts for about half of the world rice crop and the infrastructure for production and market distribution have been well established without major additional investment. Parboiled rice is the form of rice preferred by the majority of rice consumers in the countries of South Asia (Choudhury 1991; Pillaiyar 1981) and Africa, coincidently, where most of the Fe-deficient populations are distributed and concentrated. Thailand, where practically no parboiled rice is consumed, exports 2-3 million tons of milled parboiled rice each year, to countries in Middle East and Africa (Rerkasem 2007). Processing facilities are also common in countries where parboiled rice is consumed such as India and Bangladesh. Our survey in Thailand found mills of varying degrees of sophistication, of which many are modern mills fitted with electronic eyes that screen out blacken or discoloured grains. Briefly, the process of rice parboiling involves soaking the unhusked paddy rice, steaming and drying before milling (Bhattacharya 2004). The soaking time varies from 6 to 24 hours, when Fe fortification can be implemented by spiking suitable form and concentration of Fe in the soaking water under the optimal soaking conditions. As a result, this does not require major changes to the existing parboiling rice production process and infrastructure.

Effectiveness of Iron fortification by parboiling

Adding Fe to the paddy rice in the parboiling process significantly increases both total Fe concentration in the white rice grain and the bioavailable Fe fraction (Prom-u-thai et al. 2008; Prom-u-thai et al. 2009). Under laboratory simulated parboiling conditions, Fe fortification increased Fe concentrations in milled rice by 10 to 50 folds, compared to the background level of 4-7 mg Fe kg^{-1} dry weight, depending on varieties and milling time (Fig. 1). For example, after 60 seconds milling, the cultivars YRF 2 and Opus had the largest increment, with Fe concentration 20 times of those in unfortified raw rice. At 120s milling time, the cultivar Opus, had as much as 50 times of the Fe concentration in the unfortified. Current findings indicate that the optimum rate of Fe fortification in parboiling is about 250 mg Fe kg^{-1} paddy rice, which can increase Fe concentration from 5 mg Fe kg^{-1} in unfortified parboiled and raw white rice, to about 27 mg Fe kg^{-1} white rice, without adverse impact on rice cooking qualities such as color, flavor and textures. The most significant finding is that the fortified Fe remained highly bioavailable and its bioavailability exhibited a close correlation within the total Fe concentration ($r = 0.90$, $p < 0.01$) in the parboiled grains (Prom-u-thai et al. 2009). Parboiled rice can be fortified to contain more bioavailable Fe than white bean, the standard for high Fe legume grain (Fig. 2) (Prom-u-thai et al. 2009).

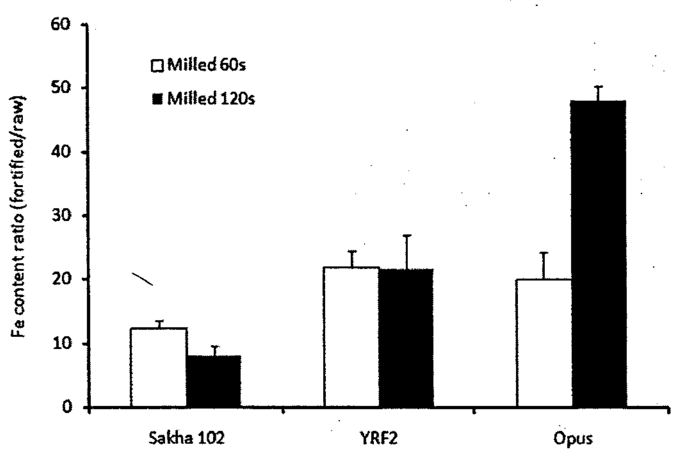

Fig. 1 The ratio of Fe content in fortified rice grain to that of unfortified rice grain. The parboiled and raw grains were milled for 60 and 120s, respectively. The bars represent standard errors of corresponding means from 3 replicates (Prom-u-thai et al. 2008).

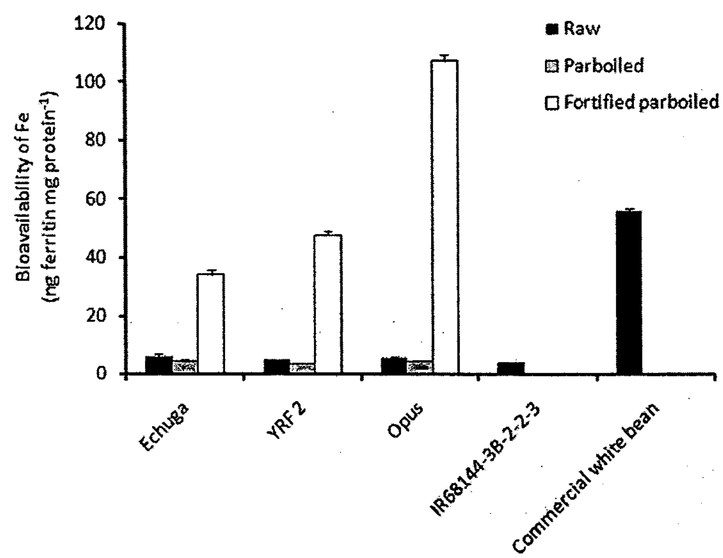

Fig. 2. Bioavailability of Fe from digests of rice samples in 3 cultivars after milling for 120s, IR68144-2B-3-2-2, and a commercial US white bean were included. Values are mean ± SEM (n=3) (Prom-u-thai et al. 2009).

Fe Penetration and retention in the endosperm

The fortified Fe effectively penetrated into the interior of the endosperm, which was clearly demonstrated by Fe localization staining of with Perls' Prussian blue (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. 3B1 and B2) (Prom-u-thai et al. 2008). The results indicated that the distribution of the staining tended to diffuse through the dorsal region of the grain and gradually towards the opposite pole of the grain. From visual observation, the parboiling process achieved a significant penetration through the inner layers of the endosperm of the parboiled rice grains. The advantage of Fe penetration into the inner layer of the endosperm after fortification process ensure the retention of adequate Fe after polishing for optimum cooking qualities of rice grain, in contrast to the problem of significant Fe loss from milling of raw rice grains. This advantage also helps high Fe retention after rinsing prior to cooking, a common practice of rice consumers. The degree of Fe loss from rinsing in the Fe-fortified and parboiled rice grains varied among cultivars and milling time (Fig. 4). For example, 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 after rinsing.

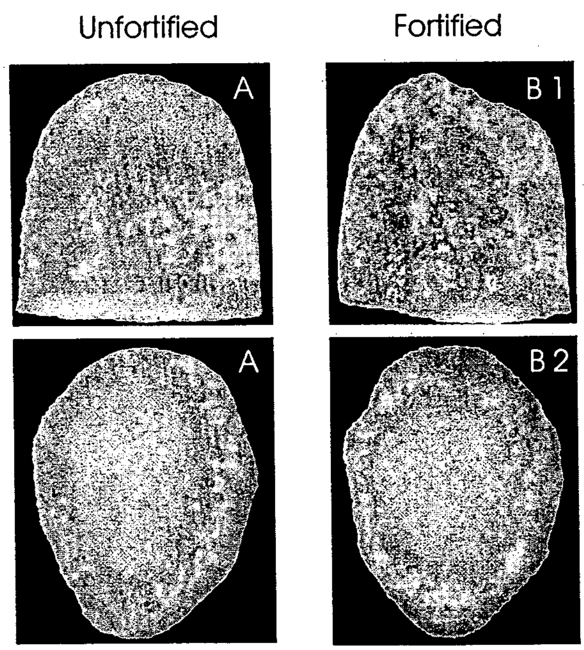

Fig. 3 Stereo-micrographs of unfortified (A1, A2) and fortified (B1, B2) rice grains (cv. Opus), which were milled rice for 60s. The grains were cut transversely across the middle plane of the grain and were stained with Perls Prussian blue (side views - A1, B1; and top view - A2, B2). All scale bar =1 mm. The intensity of staining represented the relative density of Fe in the grains (Prom-u-thai et al. 2008).

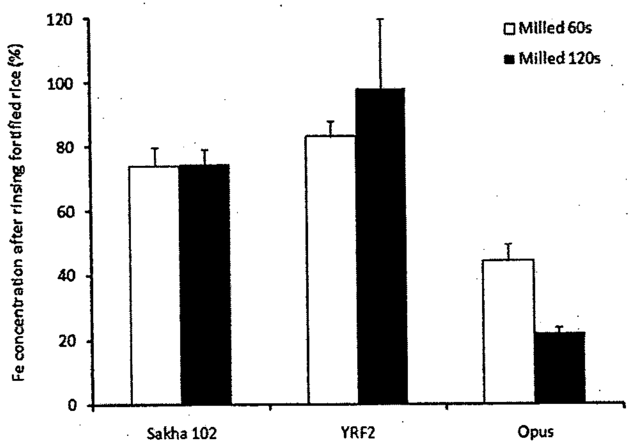

Fig. 4 Iron retention rate (as % of the un-rinsed) after rinsing (simulating rice washing) in the Fe-fortified parboiled rice grains milled for 60 and 120s, respectively, in the 3 rice cultivars tested (Prom-u-thai et al. 2008).

Fe richness in broken parboiled rice fortified with Fe

Broken rice is an undesirable in rice milling which as it lower the quality and price. The percentage of broken grain (<3/4 to grain length) has negative impact on the price. For example, during the first half of March 2009 the price per ton of 100% parboiled rice (no broken) was quoted at US$ 664, dropping to US$ 479 with just 5% broken grain and US$ 336 per ton for rice sold as broken (Thai Rice Exporters Association 2009). Broken rice costs only 25-50% of the 100% full grain rice and is preferred by lower income consumers for cost-saving. Because of its very low price compared with rice that is mostly or all whole grain, broken rice usually ends up as raw material in processing industry from rice flour to noodle and various snacks. Broken rice is also the main staple for consumers with low income in many developing countries. Importers in Africa commonly buy broken parboiled rice from Thailand for the low end market. So we examined the Fe fortification effectiveness and density in broken rice for this neediest sector of the population who happens to have the highest risk of Fe-deficiency anemia. The Fe content of broken rice in unfortified and Fe fortified parboiled rice (with 250 and 450 mg Fe kg^{-1} paddy rice) were compared with whole grain. The broken rice of unfortified and Fe-fortified parboiled rice at 250 and 450 mg Fe kg^{-1} paddy rice, contained Fe concentrations ranging from 16-96 mg kg^{-1} white rice, while it was 7-18 mg kg^{-1} in the full grain (Fig.5). Unfortified, the Fe concentration was 7 mg kg^{-1} in whole grain compared with 16 mg kg^{-1} in the broken. For Fe fortified parboiled rice, Fe concentration in broken rice was 4-5 times of those in whole grain. This difference in Fe concentration between whole and broken grain of parboiled rice is likely to be composed of grain sections containing higher Fe. The fortified Fe tends to distributed in the tips of the grain that tend to break off during milling (Fig. 6). In parboiled rice fortified with Fe, differential rates of Fe diffusion may occur in different sections of the rice grain during fortification. Fortification of Fe during parboiling therefore is a very promising and equitable approach for overcoming Fe-deficiency risks in economically advantaged and disadvantaged populations.

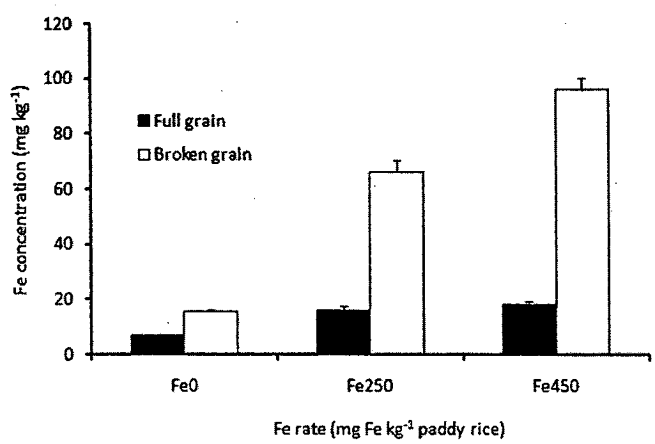

Fig. 5. Fe concentrations in full and broken grains in cultivar CNT 1 of unfortified (Fe0) and fortified parboiled rice at 250 (Fe250) and 450 (Fe450) mg Fe kg^{-1} paddy rice.

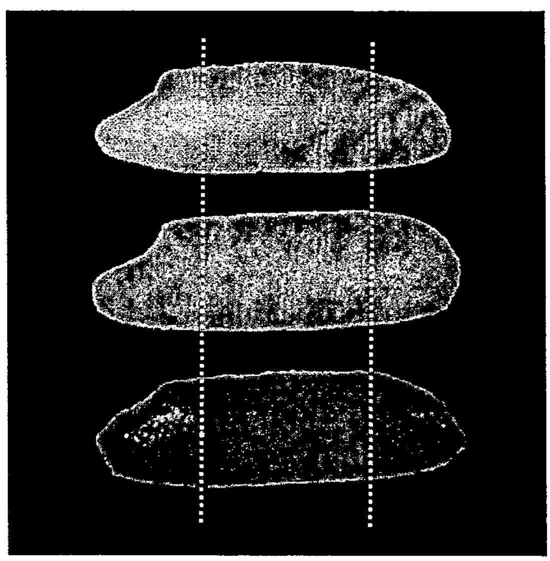

Fig. 6. Stereo-micrographs showing position of rice grain that are likely to break off during milling process at both ends of rice grain (dash lines) in unfortified parboiled (above) and parboiled rice fortified with Fe at 250 (middle) and 450 (below) mg Fe kg^{-1} paddy rice (cv CNT 1), which were milled for 30s. The grains were stained with Perls Prussian blue. The intensity of staining represented the relative density of Fe in the grains.

Consumer acceptance and remaining issues

The changes of pre-cooking quality, cooking quality and consumers' poor acceptance are critical reasons that have contributed to the lack of success of Fe fortification of raw rice (Cook et al. 1997). These fundamental problems have not been found with Fe fortified parboiled rice at optimal Fe density. Two sensory panels, one in parboiled rice eating Bangladesh and one in Thailand where parboiled rice is hardly ever consumed, found the cooked parboiled rice fortified with appropriate rate of Fe to be indistinguishable from commercially available parboiled rice. The sensory test among 10 farmer panelists in Bangladesh gave an overall acceptability of 100% to the parboiled rice fortified with 250 mg Fe kg^{-1} paddy rice. These initial results provide a great confidence of marketability and consumer acceptance of the Fe-fortified parboiled rice.

Further research needs to focus on appropriate cultivar-specific or grain-property-specific protocols of Fe-fortification during parboiling, such as soaking time, temperature, steaming and drying conditions. Physical and chemical properties of rice grain before fortification process may affect Fe entry and retention rate in the endosperm. A social survey on consumer's preference and opinion is also a significant topic to be investigated, particularly among the countries with high parboiled rice consumption.

Conclusion

With an established industry infrastructure and half of the world's rice production already parboiled, Fe-fortified parboiled rice offers a ready tool for significantly improving Fe nutrition in economically disadvantaged populations in south Asia and Africa, where Fe-deficiency anemia poses a great threat to human health and productivity. Fe-fortified parboiled rice can easily and rapidly reach rice consumers in these countries without the need to alter consumption habits of local populations and establishing new market network and access. The cheaper broken rice contains much more Fe, which are mostly consumed by low income consumers. In summary, Fe-fortified parboiled rice offers highly cost-effective tool to reduce incidences of Fe deficiency in developing countries within an immediate future if it is adopted by the current parboiled rice industry.

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Iron fortification and parboiled rice quality: appearance, cooking quality and sensory attributes

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Abstract

BACKGROUND: Iron (Fe) fortification of parboiled rice increases both Fe concentration and bioavailability in milled grains (i.e. white rice). The aim of the present study was to evaluate parboiled rice fortified with 250 and 450 mg Fe kg^{-1} paddy rice for its pre-cooking appearance, cooking quality, basic sensory attributes and overall acceptance in comparison with unfortified parboiled rice in Thailand and local parboiled rice in Bangladesh.

RESULTS: Fe fortification at 250 mg Fe kg^{-1} paddy rice significantly elevated Fe concentration in white rice to as high as 19.1 mg Fe kg^{-1} white rice, compared with 6.2 mg Fe kg^{-1} white rice for unfortified parboiled rice, without any adverse impact on consumer acceptance based on the current preliminary assessment. The added Fe was well retained in the cooked rice, with significant residual value for human intake. Panellists in Thailand and Bangladesh did not detect significant differences in the acceptability of parboiled rice fortified at 250 mg Fe kg^{-1} paddy rice compared with unfortified and local parboiled rice respectively. However, Fe fortification of parboiled rice at the higher level of 450 mg Fe kg^{-1} paddy rice significantly intensified the yellow colour of the grain and changed the off-flavour, chewiness and flakiness of the cooked Fe-fortified parboiled rice. This resulted in a low acceptability ranking of parboiled rice fortified at 450 mg Fe kg^{-1} paddy rice by panellists in both Thailand and Bangladesh.

CONCLUSION: Fe fortification of parboiled rice at an appropriate level (e.g. 250 mg Fe kg^{-1} paddy rice) is dosage-effective and acceptable to rice consumers. Consumer acceptability of Fe-fortified parboiled rice is closely related to pre-cooking appearance, cooking quality and sensory attributes.

Keywords: rice; *Oryza sativa*; iron fortification; parboiled rice; sensory evaluation; cooking quality

INTRODUCTION

Iron (Fe) fortification of parboiled rice significantly increases total Fe concentration and its potential bioavailability in milled grains (i.e. white rice) to as high as 140 mg Fe kg^{-1} dry weight and 110 ng ferritin mg^{-1} protein in white rice respectively.[1,2] In comparison, white rice of the high-Fe rice variety IR68144-3B-2-2-3 from conventional breeding has a total Fe concentration of only 3.5 mg Fe kg^{-1} dry weight and a potential bioavailability of only 5 ng ferritin mg^{-1} protein. Fortifying paddy rice grains with food-grade iron-ethylene diamine tetraacetic acid (Fe-EDTA) during the parboiling process takes advantage of the existing industrial process in parboiled rice mills without the need to make any significant alterations to this process. The evidence of high bioavailability potential and high Fe density in white rice has demonstrated the feasibility of Fe fortification of parboiled rice to significantly increase Fe intake in human diets based on rice.[1,2] As a result, this approach may provide a cost-effective option to combat Fe deficiency-induced anaemia within the immediate future in southern Asia and Africa, where parboiled rice is mostly produced and/or consumed and Fe deficiency is widespread. However, this novel approach requires further optimisation and evaluation before its adoption by the parboiling rice industry in Asian countries such as Bangladesh, India and Thailand. One of the important aspects of Fe-fortified parboiled rice is consumer acceptance, which can be greatly influenced by its visual appearance (e.g. colour and morphology) and sensory attributes compared with unfortified rice.

Earlier attempts to fortify rice grain with Fe were not successful owing to adverse changes induced by the added Fe in important quality attributes affecting consumer acceptance. These include pre-cooking appearance, cooking quality and sensory attributes,

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which were significantly different from those of unfortified rice, causing negative reactions from rice consumers.[3,4] Multi-fortification of rice products with several minerals and nutrients (e.g. iodine, iron, zinc and vitamin A) also caused undesirable appearance and off-flavour.[4–6] Fortification of rice flour or grain with Fe in inorganic form ($FeSO_4$) resulted in unacceptable colour for consumers as a result of lipid oxidation.[7–9] Therefore consumers are likely to accept Fe-fortified parboiled rice only if the Fe fortification does not induce any significant negative changes in quality and sensory attributes of the parboiled rice.

The aim of the present study was to investigate key quality aspects contributing to consumer acceptability of Fe-fortified parboiled rice, namely visual appearance, cooking quality and sensory attributes. The evaluation of sensory attributes was conducted by panellists from Thailand and Bangladesh in comparison with unfortified and local parboiled rice respectively. Texture analysis of cooked rice was also performed to compare unfortified and Fe-fortified parboiled rice.

MATERIALS AND METHODS

Preparation of Fe-fortified parboiled rice

Paddy rice samples of cv. CNT 1 (considered one of the best rice varieties for parboiling for Thailand's export market) were provided by a milling operator in central Thailand (Kasetsomboontanyakit Co. Ltd, Chainat, Thailand) and used to produce Fe-fortified parboiled rice. The experimental Fe fortification process has been described previously.[1,2] Briefly, 1 kg lots of paddy rice were rinsed thoroughly in three changes of filtered tap water followed by three changes of distilled deionised (DDI) water. The washed paddy rice grains were subjected to two levels of Fe fortification treatment by soaking them in Fe solutions (pH 3–3.5) containing 250 and 450 mg Fe L^{-1} in the form of Fe-EDTA (Ferrazone®, Akzo Nobel Co. Ltd, Amsterdam, Netherlands) (equivalent to 250 and 450 mg Fe kg^{-1} paddy rice at 130 g kg^{-1} moisture content respectively) at 60 °C for 6 h. In addition, 1 kg lots of rinsed paddy rice were soaked in 1 L of distilled triply deionised water (TDI) at 60 °C for 6 h to produce unfortified parboiled rice (control). The treated paddy grains were completely drained until there was no free water and then steamed at 119 °C for 10 min under a pressure of 0.8 kg cm^{-2} in a pressure cooker (model Supernova, Magefeta, Derio, Spain). The parboiled paddy grains were cooled to room temperature and sun dried to approximately 110 g kg^{-1} moisture content before being husked and milled.

Husking and milling

The sun-dried parboiled paddy rice was husked in a testing husker (model P-1, Ngek Seng Huat, Bangkok, Thailand) and the resulting brown rice was milled to produce white rice as described by Prom-u-thai *et al.*[1,2] Briefly, 50 g lots of brown rice grains were milled for 30 s in a laboratory milling machine (model K-1, Ngek Seng Huat). Prior to milling, metal parts of the husker and milling machine were cleaned and coated with Teflon to minimise Fe contamination from them. The milled rice grains were subsampled representatively for the measurement of grain size using digital callipers and the assessment of colour using a chroma meter (model CR-300, Minolta, Osaka, Japan). In the assessment of colour the L value represents the degree of lightness or darkness of the sample ($L = 100$, white; $L = 0$, black), the a value represents the tendency from green (−) to red (+) and the b value represents the tendency from blue (−) to yellow (+).

Subsamples of brown and white rice grains were oven dried at 70 °C for 72 h and analysed for total Fe concentration using an atomic absorption spectrometer (model Z-8230, Hitachi, Tokyo, Japan) after dry-ashing in a muffle furnace at 500 °C.

Cooking parboiled rice

The milled parboiled rice grains (white rice) were washed in three changes of distilled water at 1 : 1 (v/v) ratio and drained until there was no free water. Approximately 300 g lots of the grains were cooked in water at 1 : 2 (v/v) ratio in a 2 L household electric rice cooker (model MI-T80, Imarflex, Bangkok, Thailand) for sensory evaluation. When the cooker had automatically switched off after the cooking phase, it was kept at a warm setting for an additional 10 min. The top 1 cm layer of cooked rice was skimmed off and discarded. Test samples were taken from the centre of the cooking bowl, keeping 1 cm away from the edge and bottom of the bowl to minimise edge effects. Each cooked rice sample was loosened gently with a fork and transferred into a glass container covered with a plastic lid for sensory evaluation.

Sensory evaluation

Sensory evaluation of the unfortified and Fe-fortified parboiled rice was conducted in two countries: Bangladesh (a parboiled rice producer and consumer) and Thailand (a parboiled rice exporter). In Thailand, 32 untrained panellists (14 men and 18 women) who did not normally eat parboiled rice were recruited from Chiang Mai University. After an explanation of the test the panellists were instructed to apply the standards and process of descriptive sensory evaluation before conducting each test (Table 1). Cooked rice samples were prepared and tested by the panellists on the same day commencing at 10:30. Sensory attributes of the cooked unfortified and Fe-fortified rice were rated on a nine-point structured scale (1 = extremely low intensity, 9 = extremely high intensity), including aroma, colour, cohesiveness, glossiness, hardness, stickiness and chewiness. Approximately 15 g of cooked rice per treatment was served warm in a plastic cup to each panellist in random order on a tray, with a glass of drinking water, at each testing session. The cups were coded with three-digit random numbers only. The panellists were instructed to clean their palates with water prior to testing each sample. In addition to individual attributes the panellists were also asked to rate the acceptability of Fe-fortified parboiled rice samples in comparison with unfortified parboiled rice on a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) without undue bias towards other sensory attributes.

In Bangladesh, two groups of trained panellists, nine scientists in one and ten farmers in the other, participated in the sensory evaluation. An explanation of the ranking standards and process of descriptive sensory evaluation was given to the panellists before conducting each test (Table 1). They rated the cooked rice on the same set of attributes as those used in Thailand, in comparison with local parboiled rice consumed by them daily, but on a five-point structured scale (1 = significantly poorer than local, 2 = poorer than local, 3 = same as local, 4 = better than local, 5 = significantly better than local). They also rated the acceptability of Fe-fortified parboiled rice samples without undue bias towards other sensory attributes. The sensory evaluation was performed in duplicate with panellists in both Thailand and Bangladesh.

Texture analysis

Texture profile analysis of cooked unfortified and Fe-fortified rice samples was performed in triplicate with a texture analyser

Table 1. Description of pre-cooking appearance, cooking quality and sensory attributes used to evaluate cooked Fe-fortified parboiled rice in comparison with unfortified parboiled rice

Attribute	Definition
Pre-cooking appearance	
Colour	Degree of grain colour before cooking, measured using chroma meter
Size	Grain size before cooking, measured in three dimensions (length, width, thickness) using digital callipers
Cooking quality	
Colour	Degree of yellowish colour
Glossiness	Amount of shine on surface of cooked rice
Volume expansion	Amount of volume expansion of cooked rice
Attractiveness	Force required to remove rice sample adhering to mouth surface
Sensory attributes	
Aroma	Fundamental aroma sensation of cooked rice
Cohesiveness	Degree to which rice sample deforms before rupturing
Hardness	Force required to compress cooked rice using molar teeth
Stickiness	Degree of force required to separate individual grains of cooked rice using tongue
Chewiness	Number of chews required to masticate cooked rice until suitable for swallowing
Flakiness	Difficulty of making small pieces while chewing in mouth
Softness	Degree of softness while chewing cooked rice using molar teeth
Off-flavour	Fundamental off-flavour sensation compared with local parboiled rice
Inner hardness	Amount of force required to compress inner kernels upon chewing

(model TA-XTplus, Stable Micro System Co. Ltd, London, UK). Hardness, stickiness, adhesiveness and cohesiveness of individual grains were determined by compressing 20 grains with a 2 kg load. A 3.5 mm diameter cylindrical aluminium probe (Stable Micro System Co. Ltd, London, UK) was employed. The following conditions were used in the study: test speed, 1 mm s^{-1}; post-test speed, 5 mm s^{-1}; strain, 90%; test time, 13.3 s; points per second, 200; number of points, 2660; trigger force, 10 g.

Data analysis

Analysis of variance (ANOVA) was carried out using Statistic 8 (SXW, Tallahassee, FL, USA) to detect the influence of panellists' variation in parameter ranking in sensory evaluation, texture analysis profile and effects of Fe fortification on the quality attributes of parboiled rice in comparison with unfortified and/or local parboiled rice.

RESULTS

Fe concentration in parboiled rice and grain appearance

For uncooked rice, Fe concentrations in parboiled brown and white rice were increased by Fe fortification (Table 2). Milling decreased the Fe concentration in parboiled brown rice grain regardless of Fe treatment. Fortification at 250 mg Fe kg^{-1} during parboiling increased the Fe concentration 3.5-fold in brown rice and threefold in white rice, while fortification at 450 mg Fe kg^{-1} increased the Fe concentration ten- and ninefold in brown and white rice respectively. Moreover, the concentration of Fe in cooked rice after normal cooking did not decline significantly in either unfortified or Fe-fortified parboiled rice, even with three rinses before cooking, in comparison with uncooked rice.

Concerning grain colour, the measured L values showed no difference between parboiled rice fortified at 250 mg Fe kg^{-1} and unfortified parboiled rice, while parboiled rice fortified at 450 mg Fe kg^{-1} appeared slightly darker in colour than unfortified parboiled rice (Table 3). According to the measured a values, parboiled rice fortified at 250 mg Fe kg^{-1} and unfortified parboiled rice were similar in colour, slightly green, while parboiled rice fortified at 450 mg Fe kg^{-1} had a slightly red tinge. Comparing the measured b values, parboiled rice from all treatments was slightly yellow in colour, but that fortified at 450 mg Fe kg^{-1} was darker yellow than the others.

No difference in grain size was observed between unfortified and Fe-fortified parboiled rice.

Cooking quality

From the sensory evaluation conducted in Thailand, no differences in colour and glossiness were detected by the panellists between Fe-fortified and unfortified parboiled rice, but the 32 panellists showed significant differences in their preferential ranking of these two cooking quality attributes (Table 4, Fig. 1).

In Bangladesh, Fe-fortified parboiled rice was ranked by scientist and farmer panellists in comparison with unfortified and local parboiled rice. The scientist panellists agreed that Fe-fortified and unfortified parboiled rice did not differ from local parboiled rice in all cooking qualities evaluated, but there was a significantly different ranking of volume expansion among them (Table 5, Fig. 2). On the other hand, the farmer panellists' ranking was not different for all cooking qualities, but they detected significant differences in colour and attractiveness between Fe-fortified and unfortified parboiled rice: Fe-fortified parboiled rice was ranked the same as or better than local parboiled rice for colour and attractiveness, while unfortified parboiled rice was ranked poorer than local parboiled rice (Table 5, Fig. 3).

Sensory attributes

In Thailand, no differences were detected between Fe-fortified and unfortified parboiled rice in all sensory attributes (Table 4, Fig. 1). However, the panellists showed a significantly different ranking preference for all sensory attributes except aroma. The scientist panellists' ranking in Bangladesh showed that all sensory attributes except off-flavour were not significantly different between Fe-fortified and unfortified parboiled rice (Table 5). Unfortified parboiled rice and parboiled rice fortified at 250 mg Fe kg^{-1} were ranked similar to or even better than local parboiled rice (Table 5, Fig. 2), but parboiled rice fortified at 450 mg Fe kg^{-1} was ranked inferior to local parboiled rice for off-flavour. The scientist panellists gave a consistent ranking for all attributes. In contrast, the farmer panellists gave a consistent ranking for all sensory attributes but found that Fe-fortified and unfortified parboiled rice were different in chewiness, flakiness, off-flavour and inner hardness (Table 5, Fig. 3). Unfortified parboiled rice and parboiled rice fortified at 250 mg Fe kg^{-1} were ranked better than local parboiled rice for off-flavour, but parboiled rice fortified at 450 mg Fe kg^{-1} was poorer than local parboiled rice.

Table 2. Fe concentrations in brown and white rice after milling for 30 s of Fe-fortified parboiled rice with 250 and 450 mg Fe kg^{-1} paddy rice and unfortified raw and parboiled rice of cv. CNT 1

		Fe concentration (mg Fe kg^{-1})		
		Uncooked rice		Cooked rice
Sample	Fe fortification rate (mg Fe kg^{-1} paddy rice)	Brown rice	White rice	White rice
Unfortified parboiled rice	0	8.32 ± 0.11	6.20 ± 0.12	6.15 ± 0.32
Fe-fortified parboiled rice	250	28.25 ± 0.12	19.14 ± 0.11	22.07 ± 1.35
	450	79.62 ± 0.15	54.15 ± 0.24	55.64 ± 0.42

Values are mean ± standard error ($n = 3$).

Table 3. Grain colour of white rice in unfortified and Fe-fortified parboiled rice at two rates of Fe fortification

		Grain colour		
Sample	Fe fortification rate (mg Fe kg^{-1} paddy rice)	*L*	*a*	*b*
Unfortified parboiled rice	0	57.21 ± 0.9	−0.60 ± 0.2	21.03 ± 0.2
Fe-fortified parboiled rice	250	55.70 ± 1.5	−0.26 ± 0.2	21.17 ± 0.3
	450	54.20 ± 2.0	0.24 ± 0.1	22.19 ± 0.2

Values are mean ± standard error ($n = 3$).

Table 4. Influence of differential preference among 32 panellists in Thailand on attribute ranking and effects of Fe treatment (unfortified parboiled rice, Fe-fortified parboiled rice with 250 and 450 mg Fe kg^{-1} paddy rice) on cooking quality and sensory attributes of parboiled rice

Attribute	Panellists	Fe treatment
Cooking quality		
Colour	3.60***	2.15NS
Glossiness	4.61***	0.57NS
Sensory attributes		
Aroma	1.55NS	0.35NS
Cohesiveness	2.68***	0.15NS
Hardness	2.36**	0.39NS
Stickiness	11.10***	0.07NS
Chewiness	4.32***	0.50NS
Acceptability	2.24**	2.50NS

Values are *F* values from ANOVA, with following levels of significance: ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

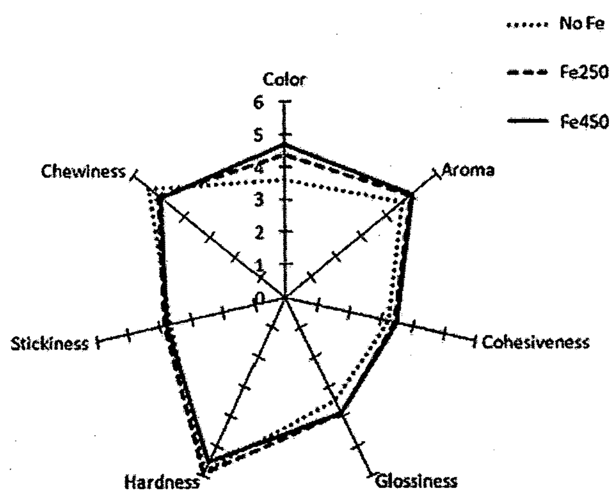

Figure 1. Sensory profiles of cooked Fe-fortified parboiled rice (Fe250 and Fe450) compared with unfortified parboiled rice (No Fe) according to 32 panellists in Thailand. Scale: 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

Acceptability

In the sensory evaluation in Thailand the acceptability did not differ between Fe-fortified and unfortified parboiled rice, but its ranking preference among the 32 panellists was not consistent (Table 4). On the other hand, the acceptability ranking by the nine scientist panellists in Bangladesh was similar between unfortified parboiled rice and parboiled rice fortified at 250 Fe kg^{-1}, with an acceptance frequency of eight and nine respectively. In contrast, the acceptability of parboiled rice fortified at 450 mg Fe kg^{-1} was less than 50% (four out of nine panellists). In comparison, the ten farmer panellists gave an acceptability of 100, 100 and 80% to unfortified and 250 and 450 mg kg^{-1} fortified parboiled rice respectively (data not shown).

Instrumental texture analysis

There were no differences in hardness and cohesiveness between unfortified and Fe-fortified parboiled rice, while stickiness was not detected at all (Table 6). However, adhesiveness was found to be lower in unfortified parboiled rice compared with parboiled rice fortified at both rates of Fe (Table 6).

DISCUSSION

The present study has demonstrated that Fe fortification at an appropriate rate (250 mg Fe kg^{-1} paddy rice) had no adverse impact on consumer acceptance, as it did not affect most of the important quality attributes evaluated, including appearance,

Table 5. Influence of differential preference among 19 panellists (nine scientists, ten farmers) in Bangladesh on attribute ranking and effects of Fe treatment (unfortified parboiled rice, Fe-fortified parboiled rice with 250 and 450 mg Fe kg^{-1} paddy rice) on cooking quality and sensory attributes of parboiled rice

	Scientists		Farmers	
Attribute	Panellists	Fe treatment	Panellists	Fe treatment
Cooking quality				
Colour	1.51NS	2.84NS	0.36NS	3.89**
Glossiness	1.32NS	0.04NS	1.14NS	1.55NS
Volume expansion	2.90*	0.05NS	1.45NS	1.20NS
Attractiveness	2.57NS	2.45NS	0.36NS	9.76**
Sensory attributes				
Aroma	2.14NS	3.26NS	1.09NS	0.25NS
Chewiness	0.60NS	0.19NS	0.30NS	14.00***
Flakiness	2.05NS	0.85NS	0.29NS	5.31*
Off-flavour	1.07NS	6.00**	0.54NS	10.00***
Inner hardness	1.22NS	2.49NS	0.38NS	12.6**
Softness	1.77NS	0.45NS	0.54NS	2.64NS
Stickiness	1.64NS	0.79NS	1.42NS	0.04NS

Values are *F* values from ANOVA, with following levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

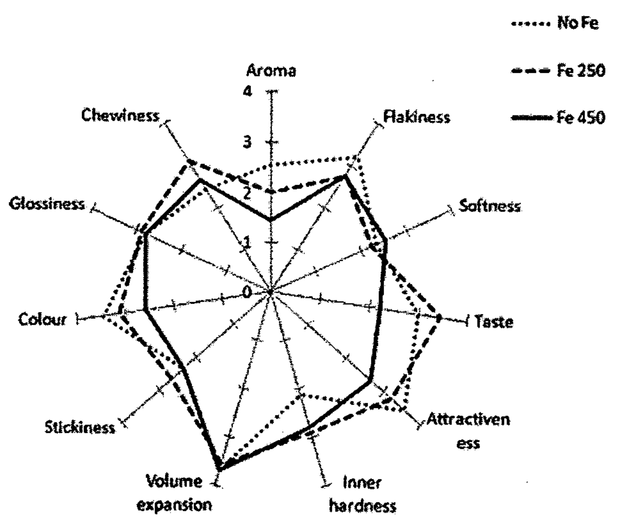

Figure 2. Sensory profiles of cooked Fe-fortified parboiled rice (Fe250 and Fe450) and unfortified parboiled rice (No Fe) compared with local parboiled rice according to nine scientist panellists in Bangladesh. Scale: 1 = significantly poorer than local, 2 = poorer than local, 3 = same as local, 4 = better than local, 5 = significantly better than local.

cooking quality and sensory attributes. Considering also our previous studies,[1,2] the approach of Fe fortification during parboiling can consistently increase the Fe density in parboiled rice grain, which is controlled by the rate of Fe loading per unit weight of paddy rice grain in the parboiling process. Treating paddy rice grain with 250 mg Fe kg^{-1} increased the Fe concentration in white rice to as high as 22.1 mg Fe kg^{-1} white rice, more than threefold that in parboiled rice without Fe fortification. In a preliminary evaluation, panellists in Thailand and Bangladesh did not detect significant differences in the acceptability of parboiled rice fortified at 250 mg Fe kg^{-1}. This fortified parboiled rice was

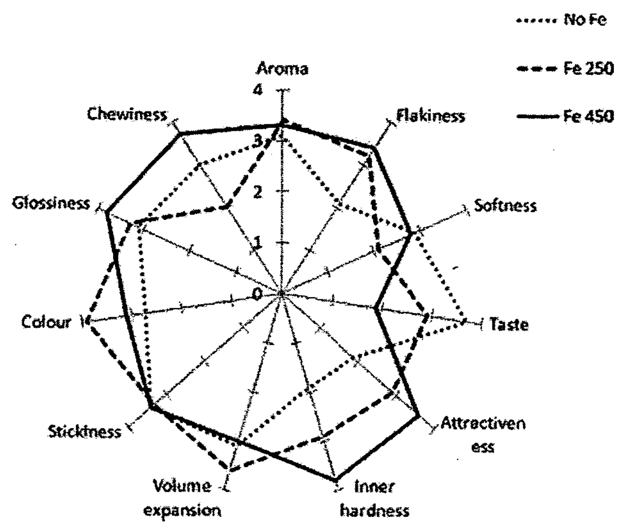

Figure 3. Sensory profiles of cooked Fe-fortified parboiled rice (Fe250 and Fe450) and unfortified parboiled rice (No Fe) compared with local parboiled rice according to ten farmer panellists in Bangladesh. Scale: 1 = significantly poorer than local, 2 = poorer than local, 3 = same as local, 4 = better than local, 5 = significantly better than local.

ranked no different from or even better than a local commercial parboiled rice by the panellists in Bangladesh, which is one of the major producers and consumers of parboiled rice in southern Asia. However, Fe fortification of parboiled rice at 450 mg Fe kg^{-1} significantly intensified the yellow colour of the grain and changed the off-flavour, chewiness and flakiness of the cooked Fe-fortified parboiled rice. This may have led to the lower ranking of parboiled rice fortified at 450 mg kg^{-1} by the panellists in Thailand and Bangladesh in terms of its acceptability.

The colour and size of white rice grain are critical characteristics of pre-cooking quality affecting consumer acceptance of the final product in the market. Fe fortification of raw rice grain has not yet been successful, as consumers can easily recognise fortified grains owing to changes in colour and shape, even when mixed with normal grains.[4] Raw rice grains fortified with several minerals and vitamins appeared yellow to brownish in colour, which had negative effects on consumer acceptance of the rice.[3,5,6] The chemical form of Fe used in the fortification process can also influence the colour of white rice. Fortification of rice grains with $FeSO_4$ resulted in unacceptable grain colour.[7,8]

As a result, the pre-cooking appearance (particularly colour) of Fe-fortified parboiled white rice is the first important aspect critical to the development and application of this technology. It will greatly influence consumer perception and acceptance of Fe-fortified white rice[10,11] and thus its marketability in the existing parboiled rice markets. The colour of the grain surface is directly influenced by the level of Fe deposited on the surface and/or in the top layer of cells of the endosperm. The surface Fe concentration is in turn related to the rate of Fe addition in the parboiling process and/or the efficiency of Fe diffusion into the endosperm. Our previous study showed that Fe-EDTA added to parboiled paddy rice penetrated the deeper layers of the endosperm.[1] However, Fe diffusion into the endosperm may not be adequate when the Fe fortification rate is too high, leaving a significant concentration of Fe on the surface and causing a detectable change in colour. Parboiled rice fortified with 250 mg Fe kg^{-1} did not differ in surface colour from unfortified parboiled rice, but fortification at 450 mg

Table 6. Texture analysis profiles of unfortified and Fe-fortified parboiled rice at two rates of Fe fortification

Sample	Fe fortification rate (mg kg^{-1} paddy rice)	Texture analysis profile			
		Hardness (kg)	Stickiness (kg)	Adhesiveness (g)	Cohesiveness
Unfortified parboiled rice	0	5.95a	ND	−4.59a	0.46a
Fe-fortified parboiled rice	250	6.12a	ND	−12.31b	0.46a
	450	6.41a	ND	−13.53b	0.48a

Different letters within a column indicate significant differences between Fe treatments at $P < 0.05$. ND, not detectable.

Fe kg^{-1} led to a visibly higher intensity of yellow colour compared with the unfortified control, based on colorimeter measurements. Therefore the rate of Fe addition to paddy rice in the parboiling process should be optimised not only to achieve a nutritionally meaningful level of Fe retention in the white rice but also to avoid significant changes in colour that may negatively affect its attractiveness to and acceptance by consumers. Fe fortification itself did not cause any changes in grain size.

Cooking qualities such as colour, glossiness and volume expansion are also critical characteristics of cooked Fe-fortified parboiled rice.[12,13] In a previous study it was found that consumers accepted the cooking quality (e.g. aroma, chewiness) and off-flavour of gluten-free bread fortified with ferric pyrophosphate and emulsifiers.[14] In the present study, post-cooking colour, glossiness, volume expansion and attractiveness were used to evaluate cooking quality differences between Fe-fortified and unfortified parboiled rice. Only the farmer panellists in Bangladesh distinguished differences in colour and attractiveness between Fe-fortified and unfortified parboiled rice. However, no differences between Fe treatments were detected by the panellists in Thailand and the scientist panellists in Bangladesh. The farmer panellists in Bangladesh ranked the cooking quality attributes of Fe-fortified parboiled rice similar to or even better than those of local parboiled rice, while unfortified parboiled rice was ranked poorer than local parboiled rice.

Experienced parboiled rice consumers may be more sensitive than non-consumers to differences in cooking quality between Fe-fortified and unfortified parboiled rice. As a result, further evaluation by experienced parboiled rice consumers in different parboiled rice regions should be carried out to ensure the wide acceptance of this product in parboiled rice markets. In contrast, inexperienced parboiled rice consumers in Thailand exhibited inconsistent preference when ranking the quality attributes. This may be related to the fact that the panellists did not routinely cook and consume parboiled rice, even though Thailand is the world's major exporter of parboiled rice. Bangladesh is not only a producer of parboiled rice but also a consumer. Its farmers cook and consume parboiled rice daily, so their evaluation may be more relevant.

Sensory attributes can also significantly affect consumer acceptance of Fe-fortified parboiled rice. Although a wide-scale sensory evaluation of Fe-fortified parboiled rice will be necessary among parboiled rice consumers, the present study is the first to evaluate sensory attributes of parboiled rice, and its results can at least serve as a preliminary confirmation of the feasibility of Fe fortification of parboiled rice. Apart from the appearance characteristic, sensory attributes of Fe-fortified parboiled rice such as aroma, hardness and stickiness are critical to consumer attitudes towards the product and its acceptance.[11,13] The texture analysis carried out in this study also indicated that there were no differences between unfortified and Fe-fortified parboiled rice in terms of hardness and cohesiveness. Even though adhesiveness was higher in Fe-fortified parboiled rice than in unfortified parboiled rice, the difference was probably not enough to be detected by consumers. Stickiness was not detected in either unfortified or Fe-fortified parboiled rice, probably owing to the low degree of stickiness of parboiled rice and the limitations of the texture analyser. From the evaluation results we can at least confirm that the sensory attributes including aroma and off-flavour were not different between parboiled rice fortified at 250 mg Fe kg^{-1} and unfortified parboiled rice, while the evaluation on parboiled rice fortified at 450 mg Fe kg^{-1} was less consistent.

Similarly, the panellists in Bangladesh were consistent in their preference ranking of the sensory attributes tested, but the ranking of the same attributes by the panellists in Thailand was more variable. It is recognised that the panellists in Bangladesh consume parboiled rice as a staple food and thus were able to reliably discriminate between Fe-fortified and unfortified parboiled rice. The scientist panellists in Bangladesh established that parboiled rice fortified at 250 mg kg^{-1} did not differ in off-flavour compared with unfortified parboiled rice. The farmer panellists in Bangladesh also confirmed the similarity in sensory attributes between Fe-fortified and unfortified parboiled rice and even ranked parboiled rice fortified at 250 mg Fe kg^{-1} above a local product commonly sold in the market. However, the higher rate of fortification (450 mg Fe kg^{-1}) led to a poorer off-flavour. The farmer panellists were more experienced and gave a different ranking from the scientist panellists in terms of chewiness, flakiness and inner hardness.

The acceptability ranking was consistent between the scientist and farmer panellists in Bangladesh. The acceptability by the scientist panellists was 89–100% for parboiled rice fortified at 250 mg Fe kg^{-1} but only 44% for parboiled rice fortified at 450 mg Fe kg^{-1}. In comparison, the acceptability by the farmer panellists was 100% for parboiled rice fortified at 250 mg Fe kg^{-1} and 80% for parboiled rice fortified at 450 mg Fe kg^{-1}. As a result, we can conservatively confirm that a fortification rate of 250 mg Fe kg^{-1} paddy rice will not reduce consumer acceptability, though sensory attributes of other rice varieties used for parboiled rice production need to be evaluated under fortification at similar Fe rates.

The present results confirmed that parboiled rice fortified with Fe at an appropriate rate (e.g. 250 mg Fe kg^{-1} paddy rice) is acceptable to rice consumers. Pre-cooking appearance, cooking quality and sensory attributes are closely related to consumer acceptability. Parboiled rice fortified with 250 mg Fe kg^{-1} in the form Fe-EDTA appeared no different or even better in appearance, cooking quality and sensory attributes than local parboiled rice.

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ey factors affecting Fe density in Fe-fortified-parboiled rice: Parboiling ɔnditions, storage duration, external Fe-loading rate and genotypic differences

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ABSTRACT

The present study evaluated the key factors affecting the efficiency of iron (Fe) penetration into the endosperm in parboiled rice of different varieties. It also investigated effects of storage time on Fe bio-accessibility, rice colour and Fe retention after rinsing. Rice grains of three varieties were fortified with an increasing range of Fe-fortification rates during the parboiling process, under two typical parboiling conditions, which are ambient soaking temperature for 24 h and 60 °C soaking temperature for 6 h at neutral (6.0–6.5) and acidic pH (3.0–3.5). Soaking of paddy rice, at 60 °C in acidic water for 6 h before steaming, was found to be better for maximising the Fe concentration in white-parboiled rice than the former ambient soaking. Under this parboiling condition, adding 250 mg Fe kg^{-1} of paddy rice, at soaking, produced the most desirable Fe concentration in white rice, ranging from 17.5 to 25.4 mg kg^{-1} among the rice varieties tested. The concentrations of Fe in parboiled white rice exhibited an exponential increase with increasing concentrations of Fe in the soaking water in all varieties, which were linearly related to Fe concentration of brown rice ($r = 0.96^{**}$, $p < 0.01$). The colour of the parboiled rice fortified with Fe was initially light yellow, with variation among rice varieties, but it did become slightly darker after 16 weeks of storage, probably because of Fe oxidisation. This may be related to decreasing bio-accessibility after 20 weeks of storage. Storage, however, did not affect the total Fe retention after rinsing, though the retention rate was variety-dependent. Information about parboiling will provide the basis for formulating an optimal industry protocol for producing Fe-fortified-parboiled rice, which can be further refined in pilot studies on the industrial scale.

Introduction

Iron-fortification in parboiled rice is a novel technique that can ovide a readily deployable solution to the Fe-deficiency anaemia oblem of rice diet-based populations in Asia and Africa (Prom-u-ai, Fukai, Godwin, Rerkasem, & Huang, 2008; Prom-u-thai, Glahn, al., 2009). It can be easily integrated into the existing parboiling ocess on an industry scale, in many regions of the world, from uth Asia to South Africa (Bhattacharya, 2004; Choudhury, 91; Pillaiyar, 1981). During the fortification process, added Fe ectively penetrates into the inner tissue layers of the rice grain d is well retained in the washing process prior to cooking rom-u-thai, Fukai, et al., 2008; Prom-u-thai, Glahn, et al., 09). The Fe loaded into the parboiled rice has a high Fe bioavailility potential, based on a simulated bioavailability test using the co-2 cell culture model and dilutes acid-extractable Fe, compared to unfortified raw and parboiled rice (Prom-u-thai, Glahn, et al., 2009).

One major cost of Fe fortification is the high cost of food grade Fe-EDTA used in fortification trials, which may influence the profit margin of parboiled rice and thus adoption by industry. To maximise the cost-effectiveness of the Fe fortification in parboiled rice, it is necessary to achieve the highest rate of Fe penetration into the endosperm at the lowest Fe addition rate under optimised soaking and parboiling conditions. Although our previous investigation found that fortifying Fe in parboiled rice at 250 mg Fe kg^{-1} paddy rice can be well accepted by parboiled rice consumers, in terms of pre-cooking appearance, cooking qualities and other sensory attributes (Prom-u-thai, Rerkasem, Fukai, & Huang, 2009), systematic evaluation of fortifying and parboiling conditions on Fe density in the parboiled rice has not been fully carried out, hindering progress towards commercial testing and scaling-up of this promising novel technique.

Key factors contributing to the efficiency of Fe penetration into the endosperm and the final Fe density in the grain may include, (1) parboiling conditions: soaking temperature, time and solution

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pH, (2) the Fe-loading rate in the soaking solution, (3) the storage stability of Fe-fortified-parboiled rice in relation to Fe retention after rinsing, bio-accessibility of Fe and colour deterioration. The information will provide the basis for formulating optimal industry protocol for producing Fe-fortified-parboiled rice, which can be further refined in pilot studies on the industrial scale. The present study also evaluates the influence of rice variety on the effectiveness of Fe fortification, by comparing three popular varieties used to produce parboiled rice in Thailand. Two common parboiling processes of the parboiled rice industry in Thailand were compared, namely (1) soaking of paddy rice at room temperature for 24 h and (2) soaking at 60 °C for 6 h, before steaming at low pressure and sun-drying.

2. Materials and methods

2.1. Fe-fortified through a parboiling process

Paddy rice grains of cv. SPR1, PSL1 and CNT1 (considered popular varieties for parboiling in Thailand's export market) were obtained from the commercial parboiled rice producers in central Thailand. Cultivar SPR1 was selected to determine effects of parboiling condition on Fe concentration in Fe-fortified-parboiled rice and all cultivars were used to determine the relationship between Fe-fortification rate and Fe concentration in Fe-fortified-parboiled rice. All tests in the present study used food grade Fe-EDTA (Ferrazone® Akzo-Nobel Co. Ltd., Netherland), unless described otherwise.

Approximately 200 g lots of paddy rice were sub-sampled and rinsed thoroughly in three changes of filtered water and then three changes of distilled water before applying treatments. For parboiling condition treatments, rinsed paddy rice was soaked in 200 ml of Fe solution containing150 mg Fe kg^{-1} of paddy rice, at two pH values – neutral (6.0–6.5) and acidic (3.0–3.5). One group at each pH was soaked at room temperature for 24 h and the other at 60 °C for 6 h. For the control treatment, unfortified-parboiled rice, rinsed paddy rice, was soaked with 200 ml of distilled water.

The above soaked paddy rice grains were steamed under the low pressure (0.8 kg cm^{-2}) at 119 °C for 10 min in a pressure steamer (Megafeta, model-supernova, Spain). The steamed paddy rice was cooled and sun-dried to the moisture level of 10–11%. For evaluating the relationship between Fe-loading rate and Fe concentration in the parboiled rice grains, rinsed paddy rice was soaked at 60 °C for 6 h before steaming in 200 ml lots of Fe solutions (pH 3.0–3.5) containing 150, 250, 350 and 450 mg Fe kg^{-1} of paddy rice, respectively. Each treatment was replicated three times.

2.2. Husking and milling

After drying, the above-treated parboiled rice was separated into brown rice (unmilled) and husk (palea and lemma) with a testing husker (Ngek Seng Huat, model P-1, Thailand). Fifty gramme lots of the brown rice were milled for 30 s to yield white rice (milled rice) by using a laboratory milling machine (Ngek Seng Huat, model K-1, Thailand). Metal parts of the husker and the polishing machine were cleaned by Teflon to minimise Fe contamination in rice samples. The milled rice grains were oven-dried at 70 °C for 72 h. Total Fe concentrations in the grain samples were quantified by means of AAS (Hitachi model Z-8230, Japan) after being dry-ashed in a Muffle Furnace at 500 °C (Zarcinas, Cartwright, & Spouncer, 1987).

2.3. Storage stability of Fe-fortified-parboiled rice

Samples of Fe-fortified-parboiled rice, selected from the experiment above, were stored for 24 weeks in plastic zipped bags at ambient temperature, to examine the stability of Fe-fortified-parboiled rice during storage, in comparison with unfortified-parboiled rice. The storage stability was assessed on the basis of grain colour, Fe retention after rinsing and Fe bio-accessibility, as dilute acid-extractable Fe, over 24 weeks of storage.

2.4. Iron retention and Fe bio-accessibility

For assessing Fe retention after rinsing, about 1 g of milled, fortified Fe parboiled rice grains was thoroughly rinsed in three changes of 10 ml of distilled water and then oven-dried at 70 °C for 72 h. The sample weights were then recorded before analysis of the Fe retention after the rinsing treatment. For Fe bio-accessibility analysis, about 1 g of the milled, Fe-fortified and parboiled rice grains was weighed into a 50 ml centrifuge tube, and extracted with 10 ml of 0.1 M HCl at 37 °C for 30 m. The supernatant was centrifuged at 3500 rpm and filtered. Iron concentrations in the extract samples were quantified by means of AAS (Hitachi model Z-8230, Japan) (Zarcinas et al., 1987). Each treatment was replicated three times.

2.5. Grain colour changes

The colour of the Fe-fortified-parboiled rice grains in storage was measured by using a chroma metre (Minolta, CR-300 series, Japan). The value of L represents the degree of lightness or darkness of the samples; $L = 100$ denotes white, $L = 0$ denotes black; b value stands for the tendency from blue (−) to yellow (+), and 0 is neutral. Approximately 10 g of rice grain sample were placed in the container at the constant depth of 2 cm. Each treatment was replicated three times.

2.6. Perls Prussian blue staining

Ten white rice grains of unfortified-parboiled and Fe-fortified-parboiled rice of cultivar CNT 1 were placed in a Petri dish and sub-merged 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 blue colour representing the relative density of Fe in the grains, was assessed under an optical microscope (Nikon SMZ1500, Japan). Prussian blue is an insoluble compound resulting from the reaction between potassium ferrocyanide and ferric Fe released from protein attachments in dilute hydrochloric acid (Doucet & Viel, 2002).

2.7. Statistical analysis

Analysis of variance was carried out to detect the significant differences of Fe concentration, grain colour values, Fe retention after rinsing and Fe bio-accessibility by using Statistic 8, analytical software, SXW (Tallahassee, FL, USA). The least significant difference (LSD) at $p < 0.05$ was applied to compare the means for significant differences of the treatments. Correlation analysis was used to evaluate the relationship among Fe concentrations in brown and white rice and Fe-fortification rate.

3. Results

3.1. Effects of parboiling condition

Parboiling condition significantly affected Fe concentration in brown and white-parboiled rices in response to the Fe-fortified treatments ($p < 0.05$) (Table 1). Iron concentration ranged from

able 1
on concentration in brown and white unfortified raw and parboiled rice and Fe-fortified-parboiled rice (150 mg Fe kg^{-1} of paddy rice) under two parboiling conditions.

Rice type	Soaking temp/time	pH[c]	Fe treatment	Fe concentration (mg kg^{-1})[d]	
				Unmilled	Milled
Raw	–	–	–	11.6 ± 0.4	6.83 ± 0.1
Parboiled	Room temp/ 24 h[a]	Neutral	Fe0	10.6 ± 0.2	5.11 ± 0.2
		Neutral	Fe150	19.5 ± 1.0	10.1 ± 0.5
		Acid	Fe150	19.0 ± 1.5	10.2 ± 1.0
Parboiled	60 °C/6 h[b]	Neutral	Fe0	9.97 ± 0.2	8.09 ± 0
		Neutral	Fe150	15.1 ± 0.5	13.5 ± 1.0
		Acid	Fe150	27.0 ± 0.2	16.9 ± 0.7

[a] Soaking paddy rice at room temperature for 24 h before steaming and drying.
[b] Soaking paddy rice at 60 °C for 6 h before steaming and drying.
[c] Neutral pH means 6.0–6.5, acid pH means 3.0–3.5.
[d] Mean ± SE, $n = 3$.

10.0 to 27.0 mg kg^{-1} in brown rice and from 5.1 to 16.9 mg kg^{-1} in white rice, under the two types of parboiling conditions. Under the parboiling condition of soaking at room temperature for 24 h, Fe concentrations in the Fe-fortified-parboiled rice were not significantly different between acidic (18.9 and 10.2 mg kg^{-1}) and neutral pH (19.5 and 10.1 mg kg^{-1}) in both brown and white rice. However, under the condition of soaking at 60 °C for 6 h, Fe concentrations in both brown and white rice were higher at acidic pH (27.0 and 16.9 mg kg^{-1}) than at neutral pH (15.1 and 13.5 mg kg^{-1}). Parboiling condition with soaking at 60 °C for 6 h was more effective in increasing Fe concentration in parboiled white rice fortified with Fe than was parboiling with soaking at room temperature for 24 h.

Since the parboiling condition of soaking at 60 °C for 6 h is more effective in increasing Fe concentration in the parboiled rice grains, this was selected as the preferred condition for subsequent tests to assess the optimal rate of Fe loading, storage stability of Fe-fortified, parboiled rice in terms of colour change, Fe retention and bio-accessibility of the absorbed Fe in white rice.

3.2. Relationship between Fe-fortification rate and Fe concentration in the rice grain

Iron concentrations in brown and white rice were closely correlated with Fe-fortification rate, but this relationship varied among the three varieties tested ($p < 0.05$) (Fig. 1). For the parboiled rice subject to Fe-fortification treatments, Fe concentration in brown and white rice increased exponentially with increasing Fe-fortification rate in all varieties ($p < 0.01$) (Fig. 1). In unfortified white rice, Fe concentration was not influenced by the parboiling process alone, except for PSL1, in which parboiling appeared to have lowered the Fe concentration of white rice (Fig. 1). Iron concentration, in white rice of the Fe-fortified, parboiled types linearly increased with that in the brown rice fraction ($r = 0.96$, $p < 0.01$) (Fig. 2).

Distribution of the fortified Fe in white rice was visually observed by staining with Perl's Prussian blue and comparing the staining of unfortified raw with those of parboiled rice grains (Fig. 3). The staining was especially intense at the tips of the rice grain. The intensity of blue colour which represented the relative density of Fe in the grains, increased with increasing Fe-loading rates. The unfortified raw and parboiled rice had only low intensity of Fe in the rice grain.

3.3. Grain colour and storage stability

The values of L, which represents the degree of lightness or darkness of the samples ($L = 100$ denotes white; $L = 0$ denotes black) and b, which stands for the tendency from blue (−) to yellow (+), and 0 is neutral were presented in this study as the two colour values are the major ones in parboiled rice. The white-parboiled rice fortified with 250 mg Fe mg^{-1} of paddy rice (which is considered to optimal) was selected to investigate the effect of storage duration on colour deterioration, Fe retention and bio-accessibility of Fe among the three rice varieties during 24 weeks of storage.

Before storage, the samples of Fe-fortified-parboiled rice grains appeared to be light yellow, with some variation among the three varieties (Fig. 4). There was no change of the yellowness in any varieties over 24 weeks of storage. The white colour of Fe-fortified-parboiled rice, of all the rice varieties, slightly declined up to the 16th week, but became stabilised afterwards, compared to that of the initial appearance. The whiteness values of all rice varieties were not different over the 24-week storage, except for the 12th and 16th weeks. At the 12th week, CNT 1 was whiter than were SPR1 and PSL 1, while PSL 1 was darker than SPR 1 and CNT 1 at the 16th week.

3.4. Fe retention and bio-accessibility of Fe

The retention of Fe in white fortified-parboiled rice, after rinsing, correlated exponentially with Fe-fortification rate in all three varieties ($r = 0.98^{**}$, $p < 0.01$). The retention of fortified Fe in the parboiled rice did not change over 24 weeks of storage, but the retention rate varied among the three varieties (Fig. 5). The varieties SPR 1 and PSL 1 retained 80% of the fortified Fe while CNT 1 retained only 60% of the initial Fe from the fortification.

3.5. Bio-accessibility of Fe

The bio-accessibility of the fortified Fe in the freshly produced parboiled rice ranged from 100% of the total Fe in variety PSL 1 to 70% in SPR 1 and 80% in CNT 1 (Fig. 6). The bio-accessibility of Fe decreased over storage time by about 20%, 45% and 33% in varieties SPR 1, PSL 1 and CNT 1, respectively, with the bio-accessibility in PSL 1 remaining higher than that in the other two varieties for most of the storage period of 24 weeks.

4. Discussion

The present study has established that the parboiling condition of soaking paddy rice in acidic Fe solution at 60 °C for 6 h before steaming is more effective to maximise the concentration of Fe in white-parboiled rice, more Fe density in white rice can be achieved for the same rate of Fe loading. Under this parboiling condition, adding 250 mg Fe kg^{-1} of paddy rice during soaking achieved the most desirable Fe concentration of 17.5–25.4 mg kg^{-1} in white rice (R.M. Welch, personal communication), with variation among rice varieties. The concentration of Fe in white and brown rice, exponentially increased with increasing rate of Fe for-

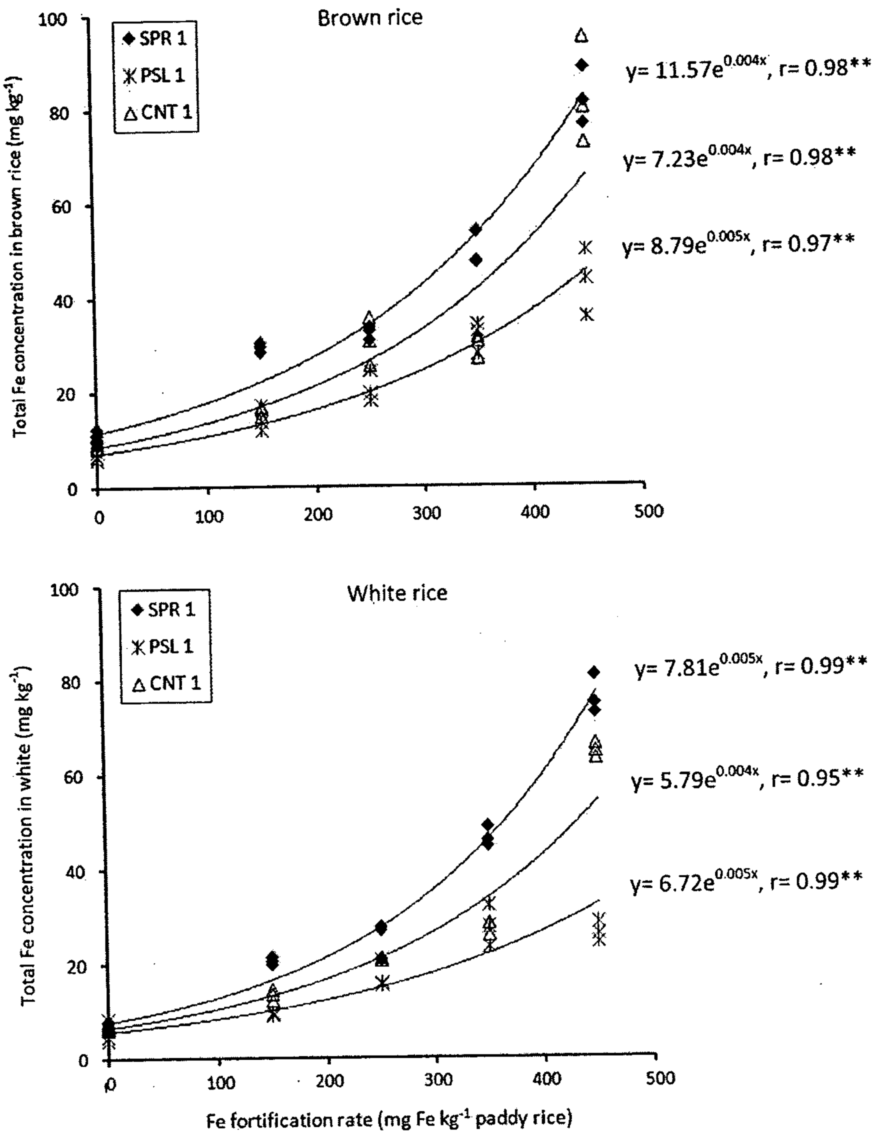


Fig. 1. Relationship between Fe-fortification rates and total Fe concentrations in brown and white Fe-fortified-parboiled rices and unfortified and raw/parboiled rices of three rice varieties pooled ($n = 18$).

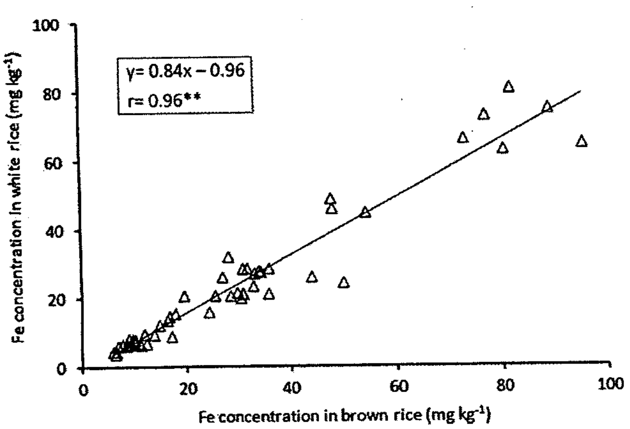
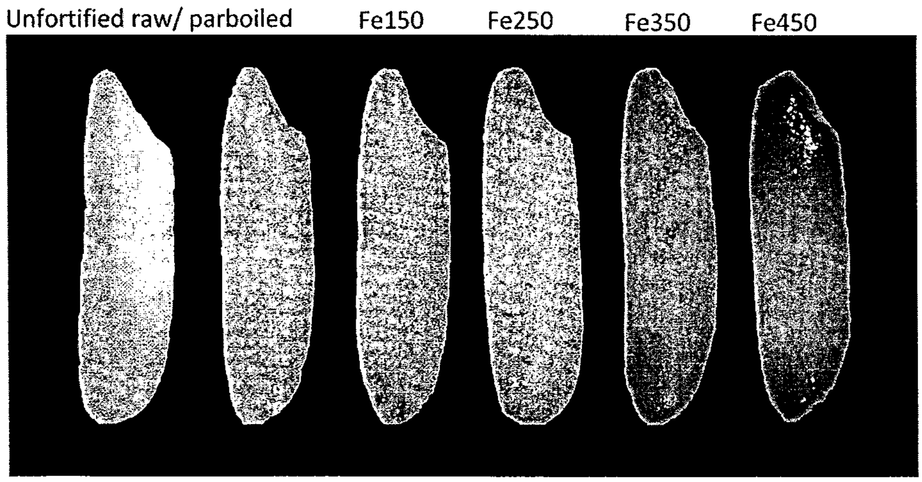


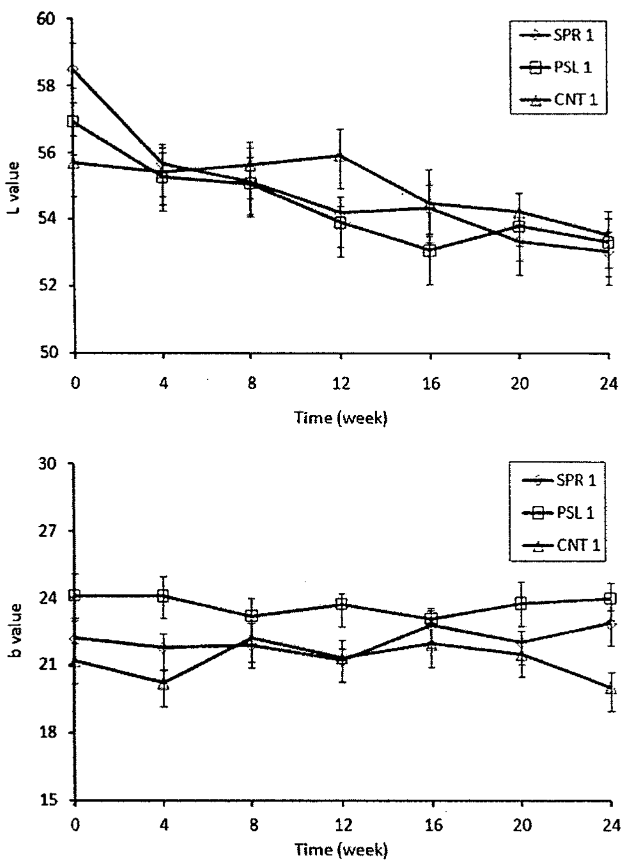
Fig. 2. Relationships between Fe concentrations in brown and white rice unfortified raw/parboiled rice and Fe-fortified-parboiled rice with different rates of Fe loading among three rice varieties.

tification in all three varieties and there was a linear correlation between Fe concentrations in white and brown rice ($r = 0.96^{**}$, $p < 0.01$). The fortified Fe effectively penetrated into the internal part of the endosperm. The intensity of the Perl's Prussian blue staining in the white rice confirmed the efficacy of Fe fortification by parboiling in rice. No available information on the specific physical and chemical properties may explain this genotypic variation among the three varieties. The internal structure and composition of protein bodies in the rice grain differ with differing rice varieties and grain N status (Leesawatwong, Jamjod, Kuo, Dell, & Rerkasem, 2005). Protein bodies in the endosperm are important sinks for Fe (Prom-u-thai, Huang, et al., 2008; Wada & Lott, 1997). However, further research is required to rank the effectiveness of Fe fortification among all popular varieties used in parboiling rice, in order to optimise Fe-loading rate, based on key rice property parameters.

Optimisation of parboiling conditions for Fe fortification is important for maximising the effectiveness of Fe penetration into the grain and thus for the economical use of the food grade EDTA. In the present study, it is confirmed that Fe concentration in white-



ig. 3. Stereo-micrographs of unfortified raw and parboiled rice and parboiled rice fortified with Fe at 150, 250, 350 and 450 mg Fe kg^{-1} of paddy rice (cv. CNT 1), which were ıilled for 30 s. The grains were stained with Perls Prussian blue. The intensity of staining represented the relative density of Fe in the grains.



'ig. 4. Grain colour formulation of white-parboiled rice fortified with !50 mg Fe kg^{-1} of paddy rice in three rice varieties. The value of L represents the legree of lightness or darkness of the samples; L = 100 denotes white; L = 0 denotes ılack; b value stands for the tendency from blue (−) to yellow (+), and 0 is neutral, ı = 3.

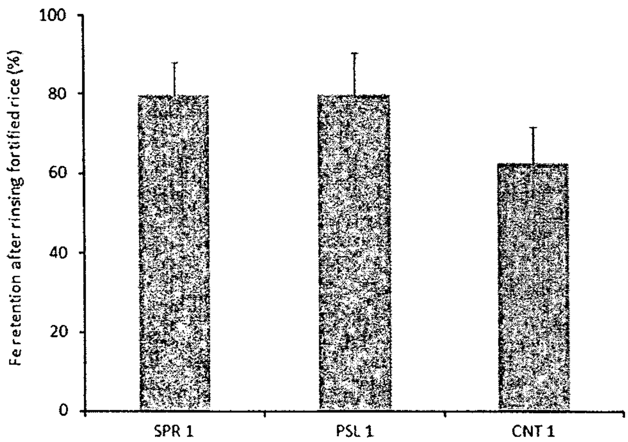


Fig. 5. Fe retention rate (as % of the total Fe concentration) of parboiled rice fortified with 250 mg Fe kg^{-1} of paddy rice after rinsing (simulating rice washing) with three changes of water in three rice varieties, n = 3.

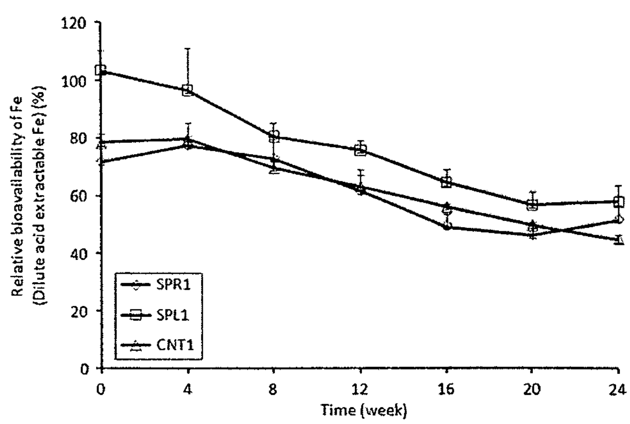


Fig. 6. Relative bioavailability of Fe (dilute acid-extractable Fe) (as % of the total Fe) in white-parboiled rice fortified with 250 mg Fe kg^{-1} during 24 weeks of storage in three rice varieties, n = 3.

ɔarboiled rice can be significantly increased when soaking paddy 'ice in acid conditions, at 60 °C for 6 h before the steaming process, :ompared with Fe concentration in neutral conditions at the same ;oaking temperature and time and soaking at room temperature 'or 24 h with both acid and neutral conditions. However, there is ıo difference of Fe concentration in white-parboiled rice between ıcid and neutral conditions when paddy rice is soaked in room temperature for 24 h. It is possible that ion exchange in grain tissues may have been stimulated during the parboiling process, especially when soaking paddy rice at high temperature in acid conditions, resulting in increases of some necessary minerals for human diets in white-parboiled rice (Ali & Bhattacharya, 1980; Doesthale, Devara, Rao, & Belavady, 1979). The penetration of added Fe into white-parboiled rice in this study was enhanced by soaking at elevated temperature (60 °C), probably due to decreased resistance of water penetration across cell layers of the endosperm and/or enhanced ion exchange, even in a shorter time. Moreover, soaking of paddy rice at room temperature over a long period also carries the risk of deterioration of flavour from fermentation (Islam, Shimizu, & Kimura, 2002; Miah, Haque, Douglass, & Clarke, 2002).

Fortified Fe effectively penetrated into the inner layers of rice endosperm (white rice), which was exponentially correlated with Fe concentration in white-parboiled rice, with some variation among rice varieties. The penetration pathways in the grains of different rice varieties may be effectively different, due to their different physical and chemical properties. For example, the hardness (or physical density), water absorption capacity and protein body density may all contribute to differences in Fe penetration into the endosperm and Fe retention. It was found, in the present study, that PSL 1 had a lower rate of Fe penetration into the endosperm than had SPR 1 and CNT 1. SPR 1 and CNT 1 are non-photosensitive varieties, while PSL 1 is a photosensitive one. The physical and/or chemical properties of these two types may be influenced by photoperiod duration, e.g. starch pattern, which can result in differences in Fe penetration during the fortification process (Ong & Blanshard, 1995; Yang, Peng, Dionisio-Sese, Laza, & Visperas, 2008). On the other hand, amylose content also differs between the two types of rice, 26–29% in SPR 1 and CNT 1 and 14.9% in PSL 1. These properties closely influence the degree of gelatinisation and water absorption of the endosperm (white rice), which may have influenced the rate of Fe penetration associated with water absorption in the white rice grain during the parboiling process (Alary, Laignelet, & Feillet, 1977; Derycke, Vandeputte, et al., 2005; Derycke, Veraverbeke, et al., 2005; González, Livore, & Pons, 2004). However, further study is required to investigate the relationship between typical key indicators of physical and chemical properties of rice grains of different rice varieties and water sorption and cation-exchange capacity, in order to achieve the maximum rate of Fe penetration in the fortification process of parboiled rice.

The retention rate of fortified Fe in white-parboiled rice, after simulated washing, is generally high, ranging from 60% to 80% of the initial Fe concentration. This is because of the inward movement of Fe from the surface layers of rice grain into the endosperm. The Fe retention capacity of the parboiled rice grains was not affected by the 24 weeks of storage. There are some variations of Fe retention rate among the three rice varieties tested. This may also be related to the acute water absorption capacity, dynamic rate of water flux through the endosperm, and/or exchange rate of cations. The fortified Fe chelates with organic compounds (such as phenolic compounds and sugars, and this has been reported to be high in rice grain (Ramalingam & Anthoni Raj, 1996). However, it remains to be found whether the parboiling could reduce the water sorption capacity and water flux rate of the endosperm during the brief washing process, in comparison with raw rice under soaking conditions before steaming.

The storage conditions may have some negative effects on the quality of the Fe-fortified-parboiled rice. After 24 weeks of storage at room temperature, the Fe-fortified-parboiled white rice appeared a bit darker compared with the initial colour. This suggests that oxidation of Fe deposited in the outer cell layer of the Fe-fortified white rice may have occurred. The oxidisation-induced brownish colour of fortified-parboiled rice has been reported when carrying out multi-fortification of Fe and vitamin A, resulting in poor acceptance by rice consumers (Li, Diosady, & Jankowski, 2008). Adding of antioxidant reagents, during the fortification process, was suggested to reduce any oxidising reaction during storage of the fortified products (Iqbal, Bhanger, & Anwar, 2005; Li et al., 2008), but this will increase the complexity and costs of the technology. Further survey should be conducted to assess the influence of the slight colour change, after storage, on the acceptance of the Fe-fortified-parboiled rice by consumers in the near future.

Decreased bio-accessibility of Fe with increasing storage time may also be related to Fe oxidisation, as discussed above. Although the bio-accessibility of Fe was decreased after 24 weeks of storage, it remained about 13 times higher than that in unfortified raw and parboiled rice by the end of the storage period. However, bioavailability tests with humans are necessary to confirm that this loss of bio-accessible Fe during storage is significant for Fe intake in human.

5. Conclusion

The present study presents important information about parboiling conditions (during fortification with Fe) on Fe concentration in the parboiled rice, on which industry adoption of this technology can be based. The parboiling condition of 60 °C for 6 h at acidic pH, before steaming, is much more effective in enhancing Fe concentration in white-parboiled rice, than is 24 h soaking at ambient temperature. The exponential relationships between Fe concentration in white-parboiled rice and rate of Fe fortification can be used to calculate the amount of Fe-EDTA required for producing the parboiled rice with a desirable Fe concentration in white rice, after being standardised for specific varieties. The current results suggested that 250 mg Fe kg^{-1} of paddy rice seems to be the optimal rate of Fe addition to produce the most desirable Fe concentration of 17.5–25.4 mg kg^{-1} in white rice. Storage conditions of the Fe-fortified-parboiled rice may influence the colour and its effects on consumer acceptance require further investigation.

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Zinc fortification of whole rice grain through parboiling process

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ABSTRACT

The present study evaluated the effectiveness of zinc (Zn) fortification in a parboiling process for improving Zn density in parboiled-polished rice and its potential bioavailability in the human diet. Fortification of Zn in whole paddy rice grain with 50–400 mg Zn/kg paddy rice, during parboiling, increased Zn concentrations in polished-parboiled rice from 1.3 to 4.5 times those in unfortified parboiled rice. The added Zn rapidly penetrated into parboiled rice grains in the initial soaking process before saturation. There was an exponential correlation between Zn concentrations in unpolished ($r = 0.63$) ($p < 0.01$) and polished rice ($r = 0.30$) ($p < 0.05$) and soaking time. Zinc concentrations in unpolished rice were linearly correlated with Zn concentration in the polished rice ($r = 0.60$) ($p < 0.01$). Moreover, more than half of the added Zn is retained after a simulated washing process before cooking, ranging from 64–100%. In the Zn-fortified parboiled rice, 57–100% of Zn in polished rice grain was soluble in dilute acid, which was indicative of a high potential Zn bioavailability for human intake. The results suggest that parboiled rice has great potential for Zn fortification.

1. Introduction

Zinc (Zn) deficiency, has been estimated to affect 95.4% of the population in South Asia (Hettiarachchi, Hilmers, Liyanage, & Abrams, 2004), where people largely consume parboiled rice as a staple food. Polished raw and parboiled rice contain very low levels of Zn (Choudhury, 1991; Pillaiyar, 1981; Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008a). Zinc deficiency severely affects the immune system, increasing susceptibility to infections; it restricts growth in young children and impairs taste, smell, memory and spermatogenesis in adults (Hotz & Brown, 2004; Rosado, 2003). Recent studies indicate that Zn deficiency is a critical micronutrient deficiency globally, especially in South Asia (Black, Lindsay, Bhutta, Caulfield, & de Onnis, 2008).

To alleviate Zn deficiency in human populations who have limited access to food sources (e.g., meat) rich in Zn, several strategies have been suggested, including supplementation, dietary modification and food fortification (Brown, Peerson, Rivera, & Allen, 2002), as well as genetic and agronomic biofortification of stable food crops (Cakmak, 2008; Pfeiffer & McClafferty, 2007). A supplementation schedule is not cost-effective in the long term and its efficacy also depends on re-educating consumers. Dietary modification may promote increased consumption of Zn, from food sources, which are produced through cropping high Zn cultivars and improved Zn fertiliser management (Cakmak, 2008). This strategy is useful, but requires nutrient management expertise and its effectiveness in boosting Zn density in grains can vary a great deal, with changing seasonal conditions and other agronomic practices. Fortification of Zn in the flour of wheat, corn and rice has been successfully established (Hettiarachchi et al., 2004; Rosado, 2003). However, rice consumers mostly cook whole grains as staple food. As a result, Zn fortification in whole rice grains warrants a systematic investigation. Since parboiled rice represents the major staple food in South Asia, it would be an effective vehicle for delivering Zn nutrition, especially among low income populations in remote areas, if its Zn density can be substantially improved in a cost-effective manner.

Our previous investigations, using parboiled rice grain, have successfully developed a technique to improve iron density, which is highly bioavailable for human intake (Prom-u-thai et al., 2008a; Prom-u-thai et al., 2009a). Iron fortification of parboiled rice significantly increases total iron concentration and contributes greatly to a potentially high iron bioavailability in polished rice (white rice grains), as high as 140 mg Fe/kg dry weight and 110 ng ferritin/mg protein. This iron-enrichment process did not result in any adverse impact on cooking quality and sensory attributes (Prom-u-thai, Rerkasem, Fukai, & Huang, 2009b).

The present study is to investigate the feasibility of Zn fortification in parboiled rice as a rapid and cost-effective solution to Zn deficiency, particularly for those living in rural areas of developing countries. In such areas of the developing world, the contribution

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of animal-based proteins to the daily calorie intake is very limited and inadequate. The present research focuses on examining; (i) the effectiveness of Zn fortification in increasing Zn concentration in whole grain, (ii) retention and solubility of added Zn in the grain, and (iii) distribution of Zn within the grain (e.g., Zn concentrations in the bran and endosperm fractions, etc.), as a result of fortification treatments.

2. Materials and methods

2.1. Zn fortification in the parboiling process

Paddy rice samples of cv. SPR 1 and CNT 1 (two of the best rice varieties for parboiling in Thailand's export market) were grown and provided by Phitsanuloke Rice Research Center in central Thailand for producing Zn-fortified parboiled rice. Samples of paddy rice (~200 g) were cleaned by rinsing thoroughly in three changes of filtered tap water, followed by another three changes of distilled deionised water (DDI). The washed paddy rice grains were soaked in 200 ml of Zn solutions (pH adjusted to 3.0–3.5 before soaking) containing 50, 100, 150, 200, 250, 300, 350 and 400 mg Zn/l, respectively, as $ZnSO_4$ or ZnO. Zinc sulfate and oxide are commonly used in food fortification due to their chemical stability and cost effectiveness (Hotz & Brown, 2004; Rosado, 2003). The rinsed paddy rice was soaked at 60 °C for 6 h in the Zn solutions, to produce Zn-fortified parboiled rice. Unfortified parboiled rice (control) was produced by soaking the cleaned paddy rice in 200 ml of distilled triple-deionised water (TDI).

Zinc absorption by the paddy rice grains during the soaking period was investigated by using cultivar SPR 1 fortified with 200 mg Zn/kg paddy rice. The soaked grains were sampled every 30 min over a 6-h period. The sampled grains were completely drained until there was no free water and steamed at 119 °C for 10 min under a pressure of 0.8 kg cm^2 using a pressure cooker. The parboiled paddy grains were cooled to room temperature and sun dried (to approximately 11% moisture content) before dehusking and milling.

2.2. Husking and milling

The sun-dried parboiled paddy rice was dehusked in a testing husker (Ngek Seng Huat, Model P-1, Bangkok, Thailand) to yield brown rice (unpolished) and husk. The unpolished rice was milled to produce white rice (polished) as described in Prom-u-thai et al. (2008a) and Prom-u-thai et al. (2009a) Fifty-gram samples of the unpolished rice grains were milled for 30 s using a laboratory milling machine (Ngek Seng Huat, Model K-1, Thailand). Metal parts of the husker and the milling machine were cleaned and coated with Teflon to minimise Fe contamination from the mill. Subsamples of the husk and the unpolished and polished rice grains were oven dried at 70 °C for 72 h and analysed for total Zn concentrations using atomic absorption spectrometry (AAS) (Hitachi Model Z-8230, Japan) after being dry-ashed in a muffle furnace at 500 °C.

2.3. Diphenylthiocarbazone (DTZ) staining

Ten grains of polished (milled for 30 s) unfortified and Zn-fortified parboiled rice of cultivar CNT 1 were submerged in freshly prepared DTZ solution, by dissolving 1,5-diphenyl thiocarbazone (Merck) (500 mg/l) in methanol (AR grade) for 30 min, as described previously (Ozturk et al., 2006). Samples were rinsed thoroughly in DDI water and blotted dry using tissue paper. The intensity of staining (red colour), representing the relative density of Zn in the grains, was assessed under an optical microscope (Nikon SMZ1500, Japan).

2.4. Zn retention and solubility

For evaluating Zn retention in the parboiled rice grain after washing, about 1 g Zn-fortified parboiled white rice grains was thoroughly rinsed three times, with 10 ml DDI water each time (Hettiarachchi et al., 2004; Tulyathan, Laokuldilok, & Jongkaewwattana, 2007; Tulyathan, Mekjarutkul, & Jongkaewwattana, 2005). The rice samples were oven dried at 70 °C for 72 h. The grain weights were then recorded before Zn analysis.

For Zn solubility test, Zn-fortified parboiled white rice grains were finely ground and 1 g lots of the samples were extracted with 10 ml of 0.1 M HCl at 37 °C for 30 min. After centrifuging, the supernatant was filtered through Whatman No. 5 filter paper for the analysis of soluble Zn.

2.5. Data analysis

Analysis of variance was carried out to detect effects of Zn fortification treatments and Zn chemical forms, on Zn concentrations in different grain tissues by using Statistic 8, analytical software (SXW, Tallahassee, FL). The least significant difference (LSD) at $p < 0.05$ was applied to compare the means of the treatments. Correlation analysis was used to evaluate the relationships between parameters of Zn concentration in unpolished and polished rice, and Zn concentration in unpolished and polished rice and soaking times.

3. Results

3.1. Effectiveness of Zn fortification

Fortification of Zn in the parboiling process significantly increased Zn concentrations in both unpolished and polished rice grains, compared with those in unfortified parboiled rice, regardless of cultivars and Zn chemical forms ($p < 0.05$, Figs. 1 and 2). In unpolished rice, there was no difference of Zn concentration between unfortified raw and parboiled rice (Fig. 1A). Increasing Zn fortification rates significantly enhanced Zn concentration in unpolished rice, regardless of the cultivar and Zn form used (Fig. 1A). The concentration of Zn in unpolished rice fortified with Zn ranged from 23.5 to 78.8 mg/kg compared with those in raw and unfortified parboiled rice (16.1–17.1 mg/kg), with decreasing order of: Zn400 > Zn300 > Zn350 > Zn250 = Zn200 > Zn150 = Zn100 > Zn50. Concentration of Zn in unpolished rice was higher in the cultivar SPR 1 (58.5 mg/kg) than the cultivar CNT 1 (49.2 mg/kg) (Fig. 1B). Fortification with soluble Zn as $ZnSO_4$ resulted in higher Zn concentrations in unpolished rice (57.8 mg/kg), compared with the ZnO form (49.9 mg/kg) (Fig. 1C). In polished rice, increasing Zn fortification rates significantly enhanced Zn concentration in polished rice, regardless of the cultivar and Zn form (Fig. 2). The concentration of Zn in polished rice fortified with Zn ranged from 13.2 to 44.1 mg/kg compared with those in raw and unfortified parboiled rice (9.7–12.5 mg/kg). There was a positive correlation between Zn concentration in unpolished and polished rice, with no differences between cultivars and Zn chemical forms ($r = 0.60$; $p < 0.01$; Fig. 3).

3.2. Zn penetration in fortified parboiled rice

The penetration of Zn in rice grain increased with increasing Zn fortification rate (Fig. 4). By examining Zn concentrations in husk, unpolished and polished rice grains after fortification, it was shown that the added Zn penetrated across the rice grain within 30 min after soaking (Fig. 5). There was a high rate of Zn retention in the husk, which contained up to 257 mg Zn/kg dry matter. In

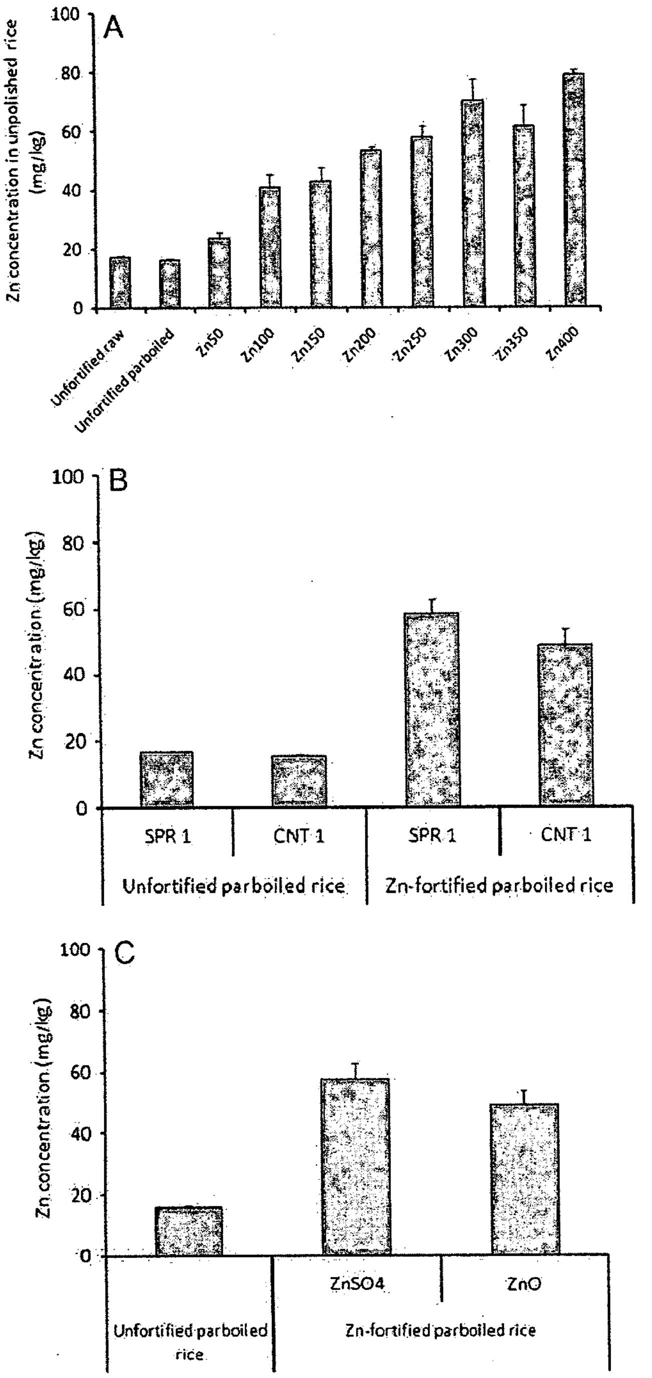

Fig. 1. Zn concentration in unpolished rice of unfortified raw and parboiled rice and parboiled rice fortified with Zn: effects of Zn rate (A), rice cultivar (B) and Zn chemical form (C).

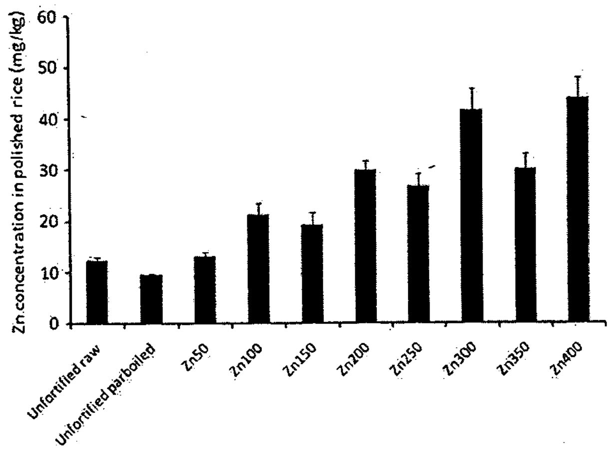

Fig. 2. Zinc concentration in polished rice treated with different rates of Zn fortification. The Zn values were pooled together from two forms of Zn treatments.

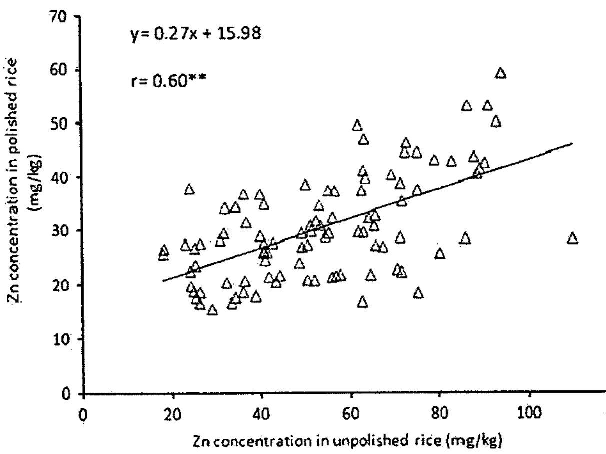

Fig. 3. Relationship between Zn concentration in unpolished and polished-parboiled rice fortified with different concentrations of Zn of two Zn forms in two rice cultivars.

comparison, Zn concentrations in unpolished rice increased from 17 to 28 and in polished rice from 13 to 25 mg/kg, after 30 min of soaking. The concentration of Zn in parboiled rice was correlated exponentially with soaking time, in both unpolished ($r = 0.63$; $p < 0.01$) and polished rice ($r = 0.30$; $p < 0.05$) (Fig. 6).

3.3. Zn retention after rinsing and relative Zn bioavailability

The fortified Zn in the parboiled rice was retained well after rinsing. The retention of Zn fortified in the form of zinc oxide was almost 100%, probably due to its low solubility (Fig. 7). In contrast, the retention rate of the Zn fortified in the form of Zn sulphate was variable, ranging from 70 to 100% for cultivar SPR1 and 60–100% for cultivar CNT1 (Fig. 8). In general, the amount of Zn retained in the rice grains after rinsing was linearly correlated with total Zn concentrations in the fortified and parboiled white rice grains (Fig. 9). There was also a linear correlation between the dilute acid-extractable (as an indirect index of bioavailable Zn pool) Zn and total Zn concentrations in the parboiled rice (Fig. 10).

4. Discussion

The present study demonstrated that Zn fortification in the parboiling process increased Zn concentration in parboiled-polished rice grains by 1.3–4.5 times that of the unfortified rice. The Zn added in the fortification process effectively penetrated across grain layers of the rice endosperm within 30 min of soaking in Zn solution, and was retained after milling (Figs. 2 and 5). The penetration of Zn into grain was enhanced by soaking. The added Zn rapidly penetrated in the initial soaking, but the effect of soaking time declined over time, as indicated by the exponential correlation between Zn concentration in unpolished ($r = 0.63$, $p < 0.01$) and polished rice ($r = 0.30$, $p < 0.05$) and soaking time in the

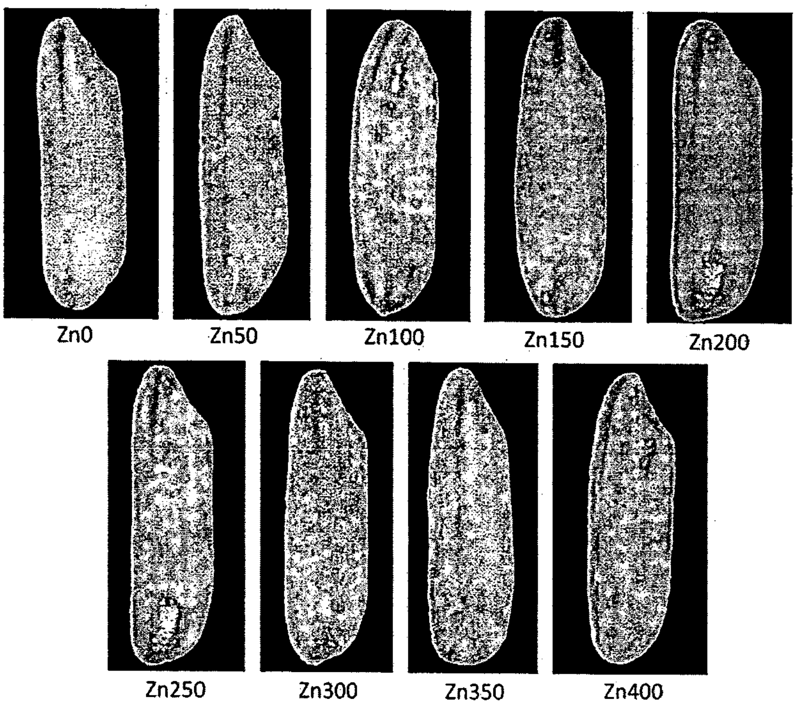

Fig. 4. Stereomicrographs of polished, unfortified or fortified rice grains (cv. CNT 1). Whole polished-parboiled grains were stained with DTZ. The intensity of staining represented the relative density of Zn in the grains.

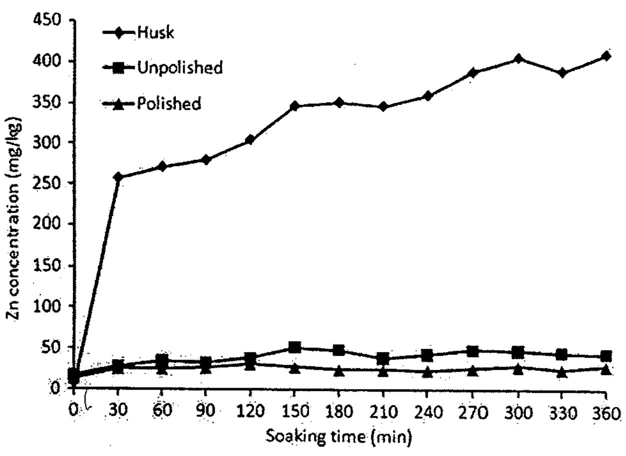

Fig. 5. Zinc concentrations in husk, unpolished and polished rice treated with 200 mg/kg paddy rice over different soaking times during the fortification process.

parboiling process. Zinc concentration in polished rice was correlated with that in unpolished rice ($r = 0.60$, $p < 0.01$). No such correlation was found between Zn concentration in the husk of the fortified parboiled paddy rice and in unpolished and polished rice, possibly due to the completely different tissue structure of rice grain. However, the correlation of Zn concentration between unpolished and polished rice indicated the penetration of fortified Zn into the inner layer of the rice grain.

Moreover, more than half of the Zn was retained after rinsing before cooking. The parboiled fortified Zn that remained after rinsing ranged from 64–100% of total Zn after fortification. In the Zn-fortified and parboiled rice, 57–100% of Zn in polished rice grains was dilute acid-soluble, indicating the high bioavailability potential of the fortified Zn, which is yet to be confirmed in human studies. The results nevertheless suggest that parboiled rice is a promising medium for Zn fortification, to improve the density of Zn in parboiled rice grain and nutritional value in human diet, as well as for Fe fortification (Prom-u-thai et al., 2008a; Prom-u-thai et al., 2009a).

Fortification of Zn in food has been recommended as a potential strategy to increase the level of Zn intake among the Zn-deficient population in developing countries (Hettiarachchi et al., 2004). Zinc fortification in cereal flour, such as wheat, corn and rice, contributes greatly to increasing both Zn concentration in flour and intake of consumers (Hettiarachchi et al., 2004; Rosado, 2003). However, since flour is not a common form of rice consumption in South Asia (Moretti et al., 2006), where Zn deficiency is widespread, parboiled rice grain as a staple food in this region may be considered as a cost-effective tool to enhance Zn intake, as the parboiled rice industry is well-established, removing the need to re-educate existing consumers. However, further study will be required to conduct the effects of Zn toxicity, in case technical problems arise during processing of Zn-fortified parboiled rice in the industrial unit.

The penetration of fortified Zn occurred in a short time during the soaking period, about 30 min. It was found that a large amount of fortified Zn penetrated into the husk during soaking, of which only a proportion was translocated into the grain. This was probably due to the difficulty of Zn transport from the husk into the inner grain part as described previously (Prom-u-thai, Fukai, Godwin, & Huang, 2007; Prom-u-thai et al., 2008a). Most probably the husk is rich in compounds which bind or complex Zn, such as phytate and/or protein (Prom-u-thai et al., 2008b; Wada & Lott, 1997), which may prevent further mobility of Zn into the endosperm. However, the fortified Zn penetrated into the inner grain layers and significantly increased Zn concentration in polished rice, compared with unfortified parboiled rice, from 10 up to 45 mg/kg. The penetration of fortified Zn across the grain layers, particularly in polished rice was not dependent on Zn fortification form and rice cultivar, but on Zn solution concentration.

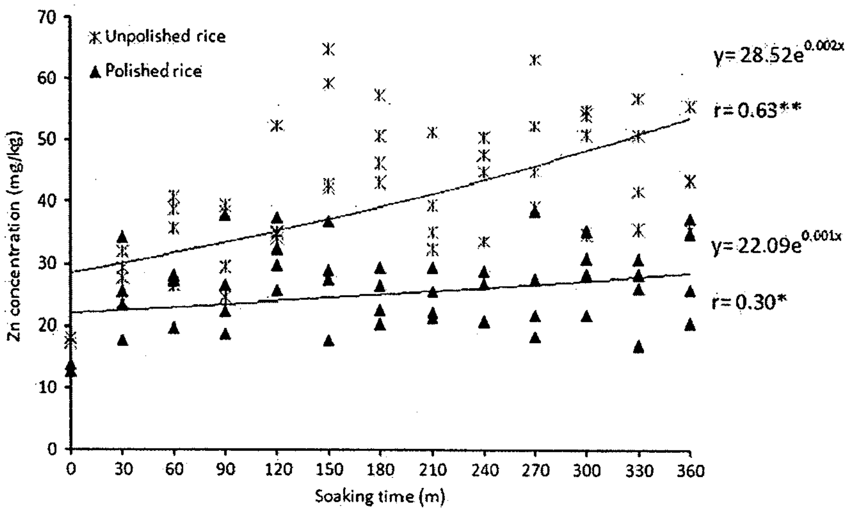

Fig. 6. Zinc concentrations in unpolished and polished (cv. SPR 1) parboiled rice fortified with Zn (200 mg/kg paddy rice) with different soaking times ($n = 51$).

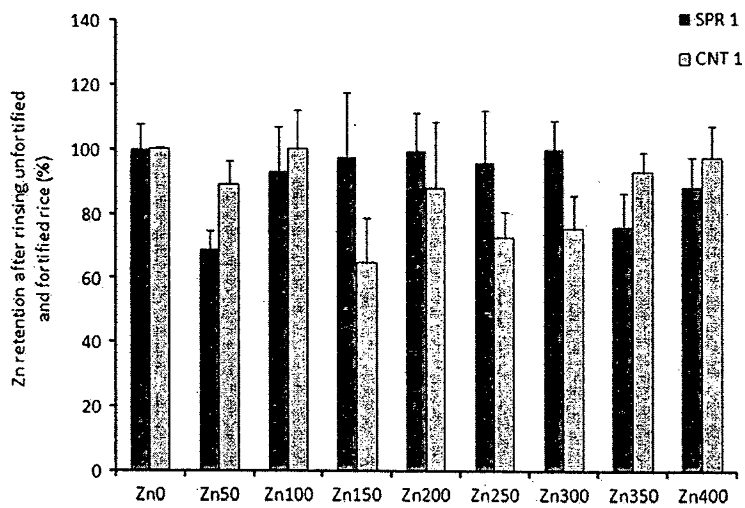

Fig. 7. Zinc retention rate (the amount of Zn remaining after rising treatments/the total amount of Zn before rinsing) in parboiled rice, fortified with different rates of $ZnSO_4$, after rinsing with three changes of water.

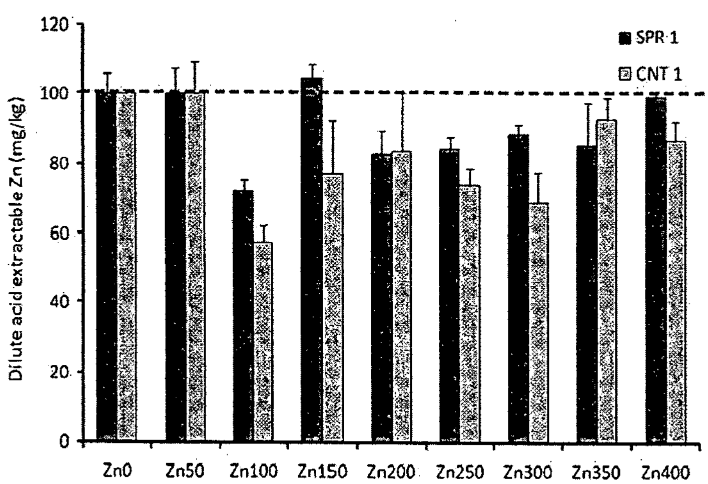

Fig. 8. The concentration of dilute acid-extractable Zn (the potential bioavailable pool of Zn in polished rice of parboiled rice fortified with different concentrations of $ZnSO_4$.

The effectiveness of Zn fortification in parboiled rice was also demonstrated in the high retention rate of the fortified Zn after repeated rinsing – a common habit for rice consumers – before cooking. The percentage of Zn retention varied with different Zn

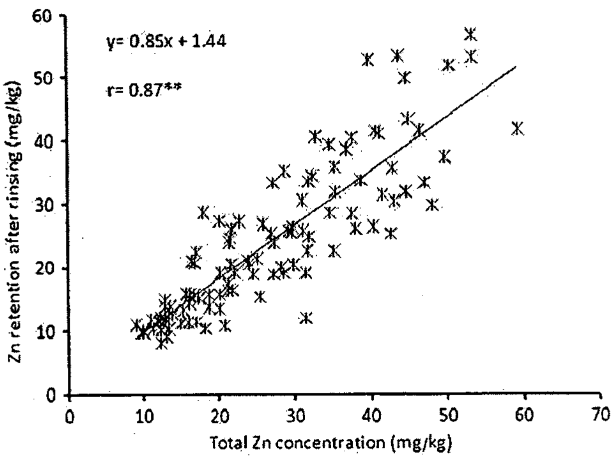

Fig. 9. The relationship between total Zn concentration and Zn retained after rinsing in parboiled rice grains subject to Zn fortification treatments. The data in the graph were pooled from those of the two cultivars subject to fortification by two Zn forms.

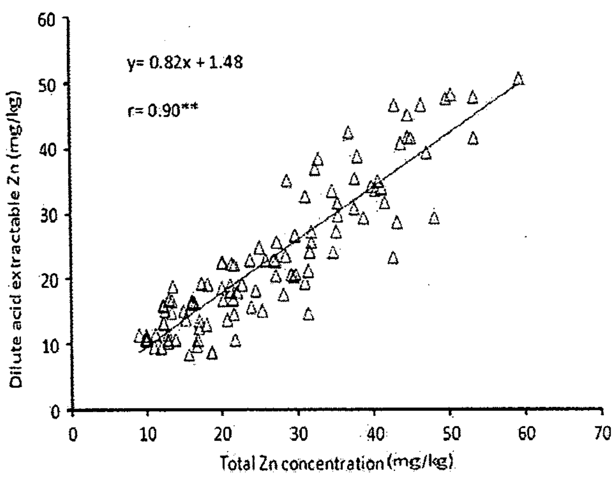

Fig. 10. The relationship between total Zn and dilute acid-extractable Zn concentration in parboiled rice treated with increasing concentrations of Zn. The data were pooled from the two cultivar tests.

fortification rates and chemical forms of Zn, in the range of 64–100% in Zn-fortified parboiled rice. This indicates that Zn in the rice grain may be strongly bound and is not easily washed off during rinsing with several changes of water. Even though there was a small amount of fortified Zn loss during rinsing process, there was still a high amount of Zn retained in fortified parboiled rice grain. For example, 75% of the fortified Zn was retained after rinsing cultivar CNT 1 when fortified with 300 mg Zn/mg paddy rice. The concentration of Zn in this variety after rinsing remained as high as 27.2 mg/kg which is about 2.8 times higher than that in unfortified parboiled rice. Therefore, the present study confirmed that the fortified Zn can be efficiently retained in cooked rice, even after rinsing with several changes of water before cooking.

It was shown that the added Zn should be bioavailable in human diets, as most fortified Zn in polished-parboiled rice grains was removed by dilute acid extraction (Prom-u-thai et al., 2008a; Prom-u-thai et al., 2009a). Previous studies have shown that fortification of ZnEDTA and ZnO in rice and corn flour, respectively, increased the absorption of Zn in blood of a Zn-deficient population (Hettiarachchi et al., 2004; Rosado, 2003). Further animal and human trials will be carried to examine the bioavailability of Zn in polished-parboiled rice after fortification of parboiled rice.

5. Conclusion

Parboiled rice is commonly consumed in South Asia where Zn deficiency in human populations is widespread. The effectiveness of Zn fortification in a parboiling process suggests that consuming Zn-fortified parboiled rice may be a rapid and cost-effective means to improve the amount of Zn intake. The level of Zn fortified in the grains is linearly correlated with Zn fortification rate, regardless of Zn forms and rice cultivars. Parboiled rice fortified with Zn is not only improving the nutritional value of human diets but it also helps to sustain the use of existing food resources. Further research is required to optimise the fortification process and evaluate Zn addition rates on rice quality and Zn bioavailability in human diets.

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อรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ 2522
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เลขที่ 239 ถนนห้วยแก้ว ตำบลสุเทพ อำเภอเมือง
จังหวัดเชียงใหม่ 50200 ประเทศไทย

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3.4 อีเมล์

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หาวิทยาลัยเชียงใหม่
9 ถนนห้วยแก้ว ตำบลสุเทพ อำเภอเมือง จังหวัดเชียงใหม่ 50200

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ผู้ประดิษฐ์ /~~ผู้ออกแบบผลิตภัณฑ์~~/ และที่อยู่ (เลขที่ ถนน ประเทศ)
1. ศ. ดร. เบญจวรรณ ฤกษ์เกษม ที่อยู่ 186 /1 ถนนสุเทพ ซอย 5 ต. สุเทพ อ. เมือง จ. เชียงใหม่ 50200 ประเทศไทย
2. ดร. ชนากานต์ พรมอุทัย ที่อยู่ เลขที่ 209/52 ม. บ้านรุ่งอรุณ 3 หมู่ 9 ต. หางดง อ. หางดง จ. เชียงใหม่ 50230 ประเทศไทย

คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม
ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร
ขที่ - วันยื่น - เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ
☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

.การยื่นคำขอนอกราชอาณาจักร

วันยื่นคำขอ	เลขที่คำขอ	ประเทศ	สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	สถานะคำขอ
.1				
.2				
.3				

.4 ☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอสิทธิให้ถือว่าได้ยื่นคำขอนี้ในวันที่ได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรในต่างประเทศเป็นครั้งแรกโดย

☐ ได้ยื่นเอกสารหลักฐานพร้อมคำขอนี้ ☐ ขอยื่นเอกสารหลักฐานหลังจากวันยื่นคำขอนี้

.การแสดงการประดิษฐ์ หรือการออกแบบผลิตภัณฑ์ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรได้แสดงการประดิษฐ์ที่หน่วยงานของรัฐเป็นผู้จัด

วันแสดง วันเปิดงานแสดง ผู้จัด

0.การประดิษฐ์เกี่ยวกับจุลชีพ

0.1 เลขทะเบียนฝากเก็บ	10.2 วันที่ฝากเก็บ	10.3 สถาบันฝากเก็บ/ประเทศ

1.ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอยื่นเอกสารภาษาต่างประเทศก่อนในวันยื่นคำขอนี้ และจะจัดยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ที่จัดทำป็นภาษาไทยภายใน 90 วัน นับจากวันยื่นคำขอนี้ โดยขอยื่นเป็นภาษา

☐ อังกฤษ ☐ ฝรั่งเศส ☐ เยอรมัน ☐ ญี่ปุ่น ☐ อื่นๆ

2.ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้อธิบดีประกาศโฆษณาคำขอรับสิทธิบัตร หรือรับจดทะเบียน และประกาศโฆษณาอนุสิทธิบัตรนี้ ลังจากวันที่ เดือน พ.ศ

☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอให้ใช้รูปเขียนหมายเลข ในการประกาศโฆษณา

3.คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ประกอบด้วย

ก. แบบพิมพ์คำขอ 2 หน้า

ข. รายละเอียดการประดิษฐ์
หรือคำพรรณนาแบบผลิตภัณฑ์ 10 หน้า

ค. ข้อถือสิทธิ 1 หน้า

ง. รูปเขียน 2 รูป 2 หน้า

จ. ภาพแสดงแบบผลิตภัณฑ์

☐ รูปเขียน รูป - หน้า

☐ ภาพถ่าย รูป - หน้า

ฉ. บทสรุปการประดิษฐ์ 1 หน้า

14.เอกสารประกอบคำขอ

☑ เอกสารแสดงสิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ หนังสือรับรองการแสดงการประดิษฐ์/การออกแบบ ผลิตภัณฑ์

☑ หนังสือมอบอำนาจ

☐ เอกสารรายละเอียดเกี่ยวกับจุลชีพ

☐ เอกสารการขอนับวันยื่นคำขอในต่างประเทศเป็นวันยื่นคำขอในประเทศไทย

☐ เอกสารขอเปลี่ยนแปลงประเภทของสิทธิ

☐ เอกสารอื่น ๆ

5. ข้าพเจ้าขอรับรองว่า

☑ การประดิษฐ์นี้ไม่เคยยื่นขอรับสิทธิบัตร/ อนุสิทธิบัตรมาก่อน

☐ การประดิษฐ์นี้ได้พัฒนาปรับปรุงมาจาก...

6.ลายมือชื่อ (☐ ผู้ขอรับสิทธิบัตร / อนุสิทธิบัตร; ☑ ตัวแทน)

(นางสาวกนกวรรณ ศรีอุทธา)

ตัวแทนผู้รับมอบอำนาจ