

# Simultaneous fortification of iron and zinc in parboiled rice kernel

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**ABSTRACT:** Fortification with Fe or Zn during parboiling process can effectively increase the concentration of each of these nutrients in rice. As Fe and Zn are often both limited in the diet of rice eaters, the present study investigated the feasibility of simultaneous fortification of parboiled rice with Fe (Fe-EDTA) and Zn (ZnSO<sub>4</sub>). The samples were treated with solutions consisting of both elements at different Fe:Zn concentration ratios (as mg/l) in the ranges 50:50, 50:100, 50:150, 50:200, and 100:50, 150:50, 200:50. Parboiling resulted in a nearly 5-folds increase in the concentration of Fe and Zn in rice kernels, depending on the rice cultivars. The added Fe and Zn penetrated across the husk and aleurone layers into the endosperm tissue, with a close relationship between unpolished and polished rice in their Fe ( $r = 0.86$ ,  $p < 0.01$ ) and Zn concentration ( $r = 0.87$ ,  $p < 0.01$ ). All to nearly all of the fortified Fe (80–100%) and Zn (70–100%) were retained after a washing that simulated the common practice of a pre-cooking wash. The fortified Fe and Zn in the polished kernels were highly bio-accessible, which was 45–100% for Fe and 88–97% for Zn. These results show how Fe and Zn concentration in polished parboiled rice can be increased by simultaneous fortification with both nutrients during the parboiling process.

**KEYWORDS:** multi fortification, parboiling process, bio-accessibility, retention, penetration

## INTRODUCTION

Fe and Zn deficiency together account for the most severe nutritional disorder among the world population, particularly in South Asia and Africa, where parboiled rice is the main staple and access to Fe and Zn from rich sources such as animal products is limited<sup>1–3</sup>. Our previous studies have demonstrated that Fe content of polished-parboiled rice kernels can be effectively increased by 5–50 folds by adding the desired amount of Fe during the parboiling process<sup>4,5</sup>. Results were similar with Zn fortification<sup>6</sup> with an increase of 1.5–5 folds. Both Fe and Zn solutions penetrated effectively into the rice endosperm with a retention rate of 25–100% and 64–100%, respectively<sup>4,6</sup>, even after the washing, maintaining a high bio-accessibility without significant adverse impact on cooking quality or sensory attributes<sup>7</sup>. Effective fortification with iodine in parboiled rice has also been reported<sup>7</sup>.

It would be economically advantageous to fortify rice with both elements simultaneously. However, it is unknown whether simultaneous fortification with Fe and Zn in parboiling will achieve the same effective-

ness as separate fortification, in terms of penetration into the endosperm, retention ratio after rinsing, and bio-availability potential.

The present study investigated the feasibility of simultaneous fortification with Fe and Zn in rice parboiling, by examining (1) if there is any competitive interaction between Fe and Zn on the Fe and Zn concentrations present in polished-parboiled rice fortified at various Fe and Zn ratios, and (2) the effectiveness of Fe and Zn fortification, retention ratio, and the bio-accessibility of the retained Fe and Zn. Three different rice cultivars were compared to determine if there is any genotypic difference in the effectiveness of simultaneous Fe and Zn fortification during parboiling.

## MATERIALS AND METHODS

### Fe and Zn fortification in parboiling process

Paddy rice kernels of non-glutinous cv. SPR 1, PSL 1, and CNT 1 (popular cultivars for parboiling in Thailand's export industry) with amylose content of 26, 15, and 29%, respectively, were obtained from Phitsanulok Rice Research Centre in Thailand. All tests

in the present study used food grade Fe-EDTA (Ferrazone, Akzo-Nobel, Co. Ltd., Netherlands) and ZnSO<sub>4</sub> (Merck, Germany), unless described otherwise.

Two hundred gram lots of paddy rice were thoroughly rinsed by 3 changes of filtered-tap water followed by another 3 changes of double de-ionized (DDI) water before applying treatment. The washed paddy rice kernels were soaked in 200 ml of the mixed Fe-EDTA: ZnSO<sub>4</sub> solutions containing 50:50, 50:100, 50:150, 50:200, and 100:50, 150:50, 200:50 mg/l at pH 3.0–3.5 based on results of previous investigations<sup>6,8</sup>. The rinsed paddy rice was soaked at 60 °C for 6 h in solutions containing the designated Fe:Zn ratio described above. The unfortified parboiled rice (control) was produced by soaking the same amount of cleaned paddy rice in 200 ml of triple distilled de-ionized water. The sampled kernels 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<sup>-2</sup> using a pressure cooker (Megafeta, model-supernova, Spain). Each treatment was replicated 3 times. The parboiled paddy kernels were cooled to room temperature and sun-dried (to approximately 11% moisture content) before de-husking and milling.

### De-husking and milling

Sun-dried parboiled paddy rice was de-husked in a testing de-husker (Ngek Seng Huat, model P-1, Thailand) to yield brown rice (unpolished), which was milled to produce white rice (polished) as described previously<sup>4-6</sup>. Each of the 50 g lots of unpolished rice kernels was 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 by Teflon to minimize Fe contamination from the mill. Subsamples of the husk and the unpolished and polished rice kernels were dried at 70 °C in an oven for 72 h, dry-ashed in Muffle furnace at 500 °C<sup>9</sup>, and analysed for total Fe and Zn concentrations using an atomic absorption spectrometer (Hitachi model Z-8230, Japan).

### Fe and Zn retention and solubility

Samples of 1 g Fe:Zn-fortified parboiled polished rice kernels were thoroughly rinsed with 3 changes of 10 ml DDI water at each time<sup>2,10</sup>. The rice kernels were oven dried at 70 °C for 72 h. The kernel weights were then recorded before Fe and Zn analysis.

For Fe and Zn bio-accessibility test, Fe:Zn-fortified parboiled white rice kernels 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 mix, the supernatant was further

filtered through watchman filter paper for the analysis of soluble Fe and Zn as described earlier<sup>5</sup>.

### Physical and chemical properties of unfortified non-parboiled rice kernel

For alkaline spreading test, 100 kernels of white rice were placed in individual glass Petri dishes and covered with 1.7% KOH solution for 23 h. The alkaline spreading value in each Petri dish was recorded<sup>11</sup>. The density of rice kernel was calculated by expressing the ratio of weight/volume. Kernel whiteness and translucency were determined by using milling meter (Satake, model MM1D, Japan). All sampling was made in triplicate.

### Data analysis

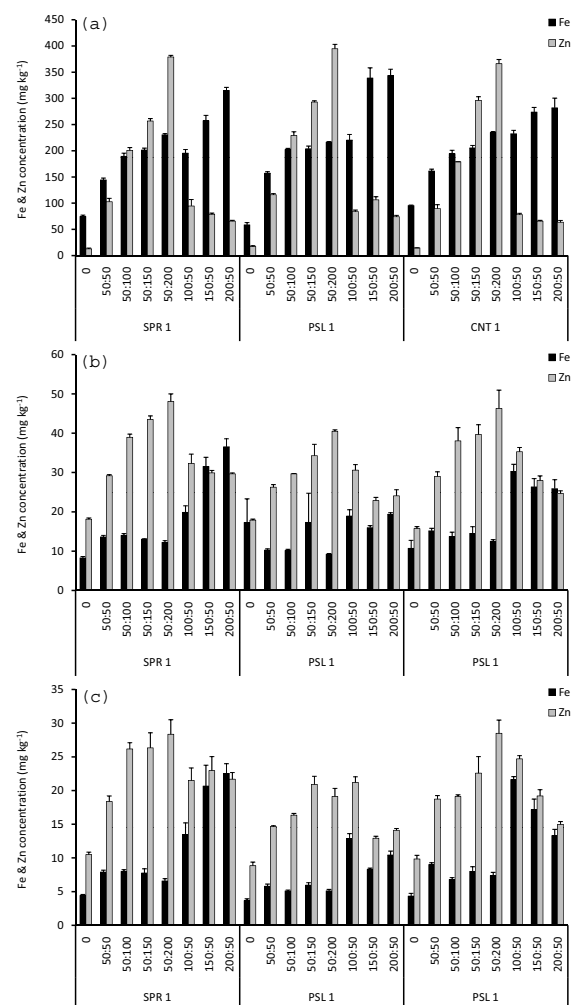
The ANOVA was carried out to detect effects of multi Fe and Zn fortification in parboiling process on Fe and Zn concentration in different kernel tissues by using STATISTIX 8, Analytical Software (Tallahassee, FL, USA). The least significant difference at  $p < 0.05$  was applied to compare the means for significant differences of the treatments. The correlation analysis was used to evaluate the relationships between total Fe and Zn concentration in the husk, unpolished, and polished rice and total Fe and Zn concentration in polished rice and its Fe and Zn retention after rinsing and bio-accessibility of Fe and Zn.

## RESULTS

### Effectiveness of simultaneous fortification for Fe and Zn

The concentration of Fe and Zn in the husk, unpolished and polished rice in Fe and Zn fortified parboiled rice significantly increased with increasing fortification ratios of Fe and Zn, regardless of the rice cultivar ( $p < 0.05$ ) (Fig. 1). In polished rice, Fe concentration in cultivar SPR 1, PSL 1, and CNT 1 increased to 22.6, 12.9, and 21.6 mg/kg with Fe:Zn fortification ratios of 200:50, 150:50, and 100:50 mg/kg paddy rice, respectively, while those in unfortified parboiled rice remained low 3.7–4.4 mg/kg Fe. Zn concentration in the rice kernel of three cultivars increased 21.2–28.5 mg/kg, with Fe:Zn fortification ratio at 50:200, 100:50, and 50:200 mg/kg paddy rice, respectively, while those in unfortified parboiled rice were as low as 8.5–10.5 mg/kg Zn.

Most of the fortified Fe and Zn were retained in the husk of all rice cultivars tested with various fortification ratios (Fig. 1a). Fe concentration was increased when increasing Fe fortification ratio or at a constant fortification ratio but with increasing Zn



**Fig. 1** Fe and Zn concentration in the (a) husk, (b) unpolished rice, and (c) polished rice of parboiled rice fortified with different ratios of Fe and Zn in 3 rice cultivars.

rate. In contrast, Zn concentration in the husks was increased with increasing Zn fortification ratio at a constant Fe rate, but it was decreased by increasing Fe rate at a constant Zn rate.

The increasing and/or decreasing patterns of Fe and Zn concentration varied among rice cultivars when Fe and Zn were fortified at different ratios. For example, Zn concentration in PSL 1 decreased more than in SPR 1 and CNT 1, when increasing Fe fortification ratio at a constant Zn concentration. The effect of Fe:Zn fortification ratio on Fe and Zn concentration was similar in unpolished and polished rice across the cultivars tested (Fig. 1b, c). In all 3 cultivars, Fe concentration varied in a small range, but Zn concentration increased when increasing Zn fortification ratio at a constant Fe rate. In SPR 1 and

PSL 1, Fe concentration increased, while Zn concentration did not change when increasing Fe fortification ratio at a constant Zn rate. In CNT 1, both Fe and Zn concentrations decreased when increasing Fe:Zn ratios from 100:50 to 200:50.

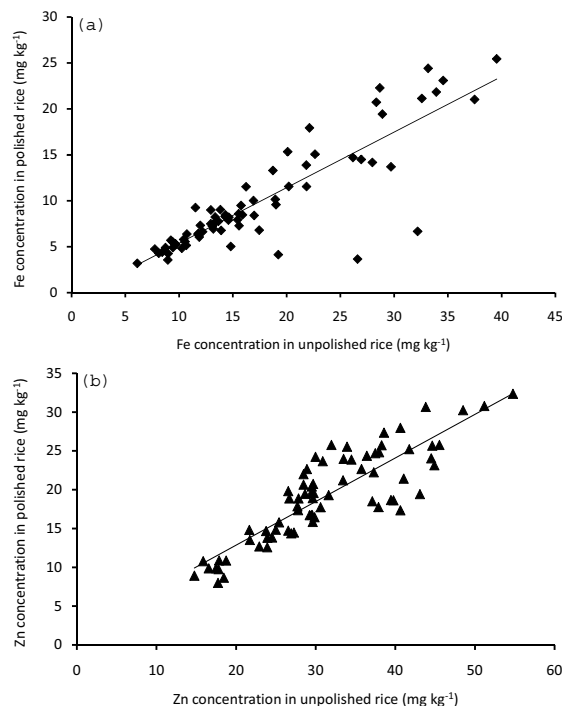
In general, Fe concentration in unpolished and polished parboiled rice was relatively stable at a constant Fe fortification ratio regardless of the Zn rate, but Zn concentration was significantly increased with increasing Zn fortification ratio while the Zn rate was kept constant at 50 mg/kg paddy rice (Fig. 1b, c). In contrast, when increasing Fe fortification ratio at a constant Zn rate, both Fe and Zn concentrations were increased in the unpolished and polished kernels (Fig. 1b, c), which was contrary to the effects on Zn and Fe concentrations in the husk. The increasing and/or decreasing Fe and Zn concentration patterns in polished-parboiled rice, however, did vary among the rice cultivars. Fe concentration in the unpolished and polished kernels of SPR 1 was continually increased with increasing Fe fortification ratio with constant Zn concentration, while Fe and Zn concentration in PSL 1 and CNT 1 kernels tended to decline slightly when Fe fortification ratios increased from 100 to 200 mg/l at 50 mg/l Zn.

There was no relationship between Fe and Zn concentration in the husk and unpolished or polished rice among different Fe:Zn fortification ratios and rice cultivars. But a close correlation was found between unpolished and polished rice in their concentration of Fe ( $r = 0.86$ ,  $p < 0.01$ ) or Zn ( $r = 0.87$ ,  $p < 0.01$ ) (Fig. 2a, b).

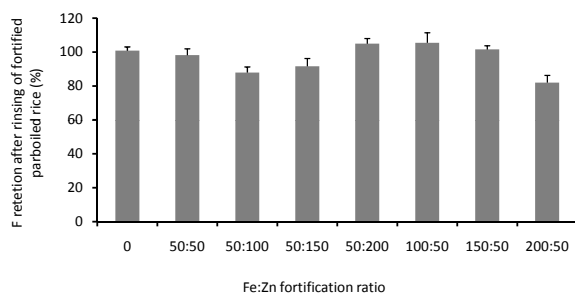
### Fe and Zn retention after rinsing

Nearly all the fortified Fe and Zn in the parboiled polished rice remained after simulated rinsing in all fortification ratios and cultivars (Fig. 3). Fe retention ratio in the rinsed fortified parboiled rice kernels ranged 80–100% of the pre-rinsing concentration of Fe. The retention rate depended on the Fe:Zn fortification ratio, but not on the rice cultivar (Fig. 3). The parboiled rice fortified with Fe:Zn at 50:100, 50:150, and 200:50 mg Fe/kg paddy rice (80–90%) had less Fe retention rate than the others (98–100%). The retention of Zn in the fortified parboiled rice kernels after rinsing ranged 70–100% of the pre-rinsing concentration of Zn, which was independent on either cultivar or fortification ratio (data not shown).

A retention rate of 90% after the pre-cooking wash was indicated by the linear relationship between pre-rinsing concentration and retention after rinsing of both Fe ( $r = 0.99$ ,  $p < 0.01$ ; Fig. 4a) and Zn ( $r = 0.91$ ,  $p < 0.01$ ; Fig. 4b).



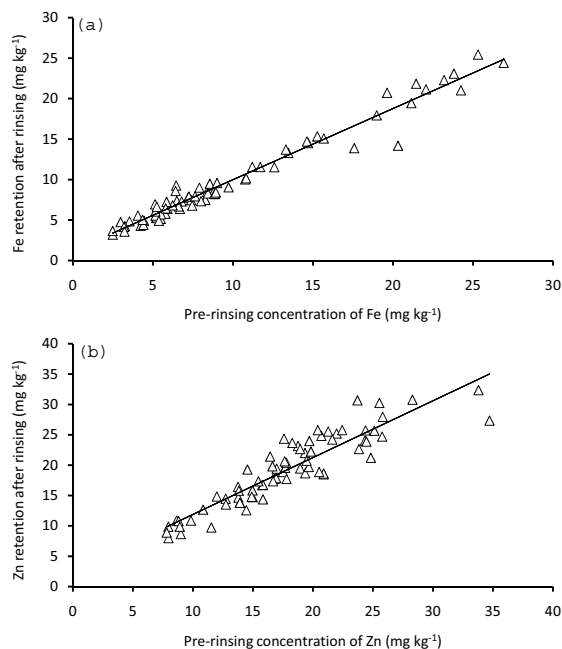
**Fig. 2** The relationship of (a) Fe ( $y = 0.60x - 0.66$ ,  $r = 0.86$ ) and (b) Zn ( $y = 0.56x + 1.62$ ,  $r = 0.87$ ) in polished concentration between unpolished and polished rice in all fortification ratios and rice cultivars ( $n = 72$ ).



**Fig. 3** Fe retention rate (as % of the unrinsed) after rinsing (simulating rice washing) in the Fe:Zn fortified parboiled rice kernels milled for 30 s in three rice cultivars tested.

**Bio-accessibility of Fe and Zn**

The bio-accessibility of Fe (as an assay of bioavailable Fe) in the parboiled rice fortified with Fe:Zn mix significantly increased in all cultivars, but varied among cultivars and with fortification ratios of Fe:Zn ( $p < 0.05$ ) (Fig. 5a). The bio-accessibility of Fe in Fe:Zn fortified parboiled rice ranged 45–100% of the total initial Fe concentration, compared with about 40–45% in unfortified parboiled rice. The bio-



**Fig. 4** The relationship between pre-rinsing concentration and retention after rinsing of (a) Fe ( $y = 0.88x + 1.20$ ,  $r = 0.99$ ) and (b) Zn ( $y = 0.94x + 2.51$ ,  $r = 0.91$ ) in polished unfortified and fortified rice of 3 cultivars ( $n = 72$ ).

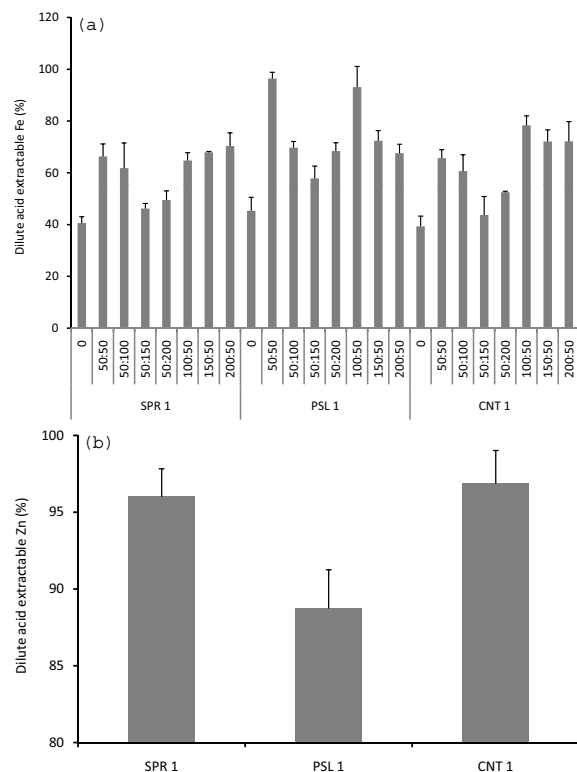
accessibility of Fe increased when increasing both Fe and Zn fortification ratios in all cultivars, even though the increasing of Fe was limited when fortification with high rate of Fe and Zn.

The bio-accessibility of Zn in both unfortified and fortified parboiled rice was high (almost 100% of the total Zn) in all fortification ratios, but varied among the cultivars tested (Fig. 5b). It was higher in cultivars SPR 1 and CNT 1 (96–97%) compared with PSL 1 (88%).

There were linear correlations between the total initial kernel Fe and Zn concentrations and the bio-accessibility of Fe ( $r = 0.96$ ,  $p < 0.01$ ; Fig. 6a) and Zn ( $r = 0.93$ ,  $p < 0.01$ ; Fig. 6b) in the unfortified and fortified parboiled rice of all fortification ratios and rice cultivars.

**DISCUSSION**

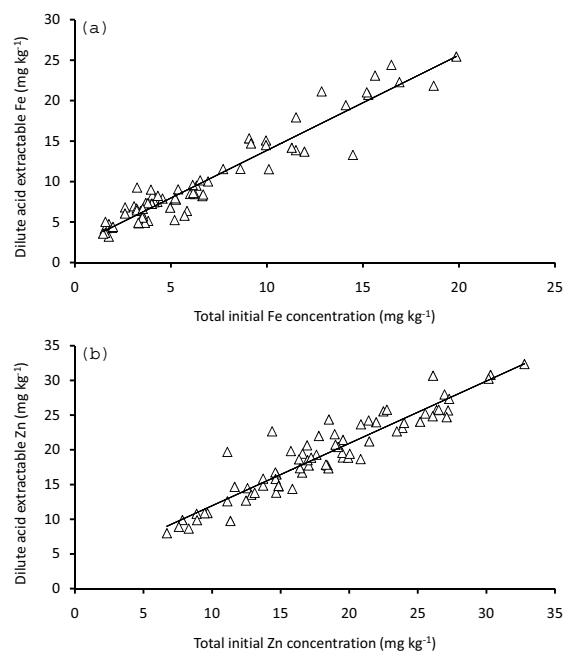
The present study demonstrated the effectiveness of fortification with Fe and Zn in parboiled rice just as previously reported with iodine<sup>7</sup>. We have shown that Fe and Zn can be simultaneously fortified during parboiling process, which effectively raises the Fe and Zn concentration in polished-parboiled rice by up to 5 folds from the very low 4 mg/kg Fe and 9–10 mg/kg Zn of unfortified parboiled rice. The cultivar



**Fig. 5** Dilute acid extractable (a) Fe (% total Fe) and (b) Zn (% total Zn) of unfortified and Fe:Zn fortified parboiled rice kernels milled for 30 s in three cultivars tested.

differences in Fe and Zn concentration of polished-parboiled rice fortified with the Fe:Zn mix was probably due to differences in both physical and chemical properties of rice kernel tissues (e.g., husks, aleurone layer, endosperm cells) in each cultivar<sup>12,13</sup>. Nevertheless, the results confirmed that Fe and Zn can be simultaneously added in the fortification process during rice parboiling. The added Fe and Zn penetrated across the husk and aleurone layer into the endosperm tissue, which was evident by the relationship between unpolished and polished rice in their concentration of Fe and Zn. Moreover, almost all of the fortified Fe and Zn in the polished parboiled rice kernels were retained after a simulated washing process (a common pre-cooking practice among rice consumers). The fortified Fe and Zn in the polished parboiled rice kernels were highly bio-accessible. However, an *in vivo* study on Fe and Zn bioavailability would be required in a further investigation to accurately quantify their bioavailability ratios.

As far as we have known, this is the first study to explore the feasibility of simultaneous fortification of multiple micronutrients in parboiled rice kernels. The



**Fig. 6** The relationship between total initial (a) Fe ( $y = 1.18x + 2.09$ ,  $r = 0.96$ ) and (b) Zn ( $y = 0.89x + 2.85$ ,  $r = 0.93$ ) concentration and dilute acid extractable Fe and Zn concentration ( $n = 72$ ).

potential of enhancing Fe and Zn density in polished-parboiled rice through an individual fortification of Fe and Zn in parboiling process has been previously investigated by our research group<sup>4,6</sup>. Multi-fortification for Fe and Zn can be more economical than individual micronutrient fortification in parboiled rice. However, a previous study reported that multi fortification of Fe with other minerals may cause undesired taste, colour and/or flavour because Fe reacts with several food ingredients and other minerals such as iodine, vitamin A, and other organic compounds<sup>3,14</sup>. However, our previous study reported that parboiled rice fortified with appropriate rates of Fe was acceptable among parboiled rice consumers, because it appeared similar or even better in appearance, cooking quality, and sensory attributes than local parboiled rice<sup>15</sup>. In the present study, doubly-fortified rice was also found to have similar physical properties as those of the singly-fortified rice, but the cooking quality and sensory attributes would need to be tested. Multi-fortification for Fe or Zn and its chelation as Na<sub>2</sub>EDTA in rice flour, wheat, and corn has been reported to be able to improve the bioavailability of both Fe and Zn in human nutrition<sup>2</sup>. However, multi-fortification with several minerals in food products may require an extra care as undesired physical and

chemical properties that influence to consumer's acceptability may occur.

The present study found that multi fortified Fe and Zn effectively penetrated into the inner layer of rice endosperm which was indicated by the correlation of Fe and Zn concentration between unpolished and polished rice and the retention of fortified Fe and Zn in polished-parboiled rice even after simulated washing. This is similar to our previous observations with individual fortification of Fe and Zn<sup>4,6</sup>. The results suggest that simultaneous fortification of Fe and Zn in parboiled rice had no negative impacts on Fe and Zn penetration into the inner layers of rice kernel and its retention after washing. However, the effectiveness of Fe and Zn penetration into the endosperm differed among the cultivars, probably due to their different physical and chemical properties as discussed previously<sup>4,6,15</sup>. It was found in the present study that cultivar SPR 1 and CNT 1 was more efficient in increasing Fe and Zn concentration in polished rice after fortification with Fe and Zn, compared with PSL 1, which was also observed in our previous studies on its individual fortification<sup>6,8</sup>.

The present study has demonstrated the differences of some physical and chemical properties of rice kernel in 3 cultivars (Table 1). The alkaline spread value was higher in PSL 1 (6.6) compared with SPR 1 (2.5) and CNT 1 (3.3), indicating soft texture of the kernel in PSL 1 and hard in SPR 1 and CNT 1, which is also correlated with the previous result in amylose content (26–29% in SPR 1 and CNT 1 and 14.9% in PSL 1)<sup>15</sup>. These properties closely influence the degree of gelatinization of the endosperm which may have influenced Fe and Zn penetration into the endosperm during the parboiling

process<sup>16–18</sup>. Moreover, it was also found that PSL 1 had a lower whiteness and higher translucency than SPR 1 and CNT 1 (Table 1), suggesting differences in starch structure, which may also influence Fe and Zn penetration into the endosperm during parboiling process and retention. However, kernel density may not be correlated with the differences on Fe and Zn penetration as it does not differ among the cultivars (Table 1).

The cultivar variation in Fe and Zn distribution in the husk, unpolished, and polished rice of the parboiled rice kernels may be related to their differences in binding chemicals in different kernel tissues. Previous studies reported that Fe and Zn in rice kernel are commonly bound to organic compounds in rice kernel such as ascorbic acid, phytate, and polyphenolics<sup>12,13,19,20</sup>. The present study found that in the husk of all rice cultivars, Fe concentration was increased when increasing Fe fortification ratio or at a constant fortification ratio but with increasing Zn rate. In contrast, Zn concentration in the husks was increased with increasing Zn fortification ratio at a constant Fe rate, but it was decreased by increasing Fe rate at a constant Zn rate. This may be related to the competition of Fe for the negatively charge sites against Zn in the husk cell walls. Therefore, it is very possible that Fe and Zn share the same specialisation and that their binding and bio-accessibility in the tissues of rice kernel. Further study is required to characterize the chemical properties in relation to Fe and Zn binding in husk, aleurone layer, and endosperm. Interview with operators of parboiling mills revealed that rice cultivars are recognized as an important variable that needs to be adjusted for in the parboiling process, some cultivars are known to be unsuitable for parboiling (C. Prom-u-thai, unpublished). Therefore, it may also be necessary to adjust for cultivar difference in Fe and Zn fortification.

The Fe and Zn in the polished parboiled rice kernels from the simultaneous fortification process showed a similar range of high bio-accessibility as that of the individually fortified rice<sup>5,6</sup>. The bio-accessibility is a rapid method for quantifying level of potentially bio-available Fe and Zn in the Fe and Zn fortified parboiled rice. The bio-accessibility of Fe and Zn in parboiled-polished rice is generally high, ranging 40–60% for Fe and 88–97% for Zn. The higher variation of bio-accessibility of Fe than Zn was probably due to dissociation of Fe from chelates located in the outer layer of Fe-fortified parboiled rice, but Zn bio-accessibility is relatively stable<sup>5,6</sup>. Bio-accessibility of Fe and Zn also varied among the cultivars. The bio-accessibility of Zn in cultivar

**Table 1** The physical and chemical properties in unfortified non parboiled rice in three rice cultivars.

Cultivar	Alkaline spread value <sup>†</sup>	Kernel density <sup>‡</sup> (mg/ml)	Whiteness <sup>§</sup> (%)	Translucency <sup>§</sup> (%)
SPR 1	2.5 <sup>a</sup>	1.59 <sup>a</sup>	50.1 <sup>c</sup>	2.33 <sup>a</sup>
PSL 1	6.6 <sup>b</sup>	1.47 <sup>a</sup>	38.4 <sup>a</sup>	2.60 <sup>b</sup>
CNT 1	3.3 <sup>a</sup>	1.39 <sup>a</sup>	47.3 <sup>b</sup>	2.49 <sup>ab</sup>

<sup>†</sup> The values are average from 100 kernels of each cultivar.

<sup>‡</sup> The density of rice kernel was calculated from weight/volume ( $n = 3$ ).

<sup>§</sup> Kernel whiteness and translucency were determined by using milling meter (Satake, model MM1D, Japan).

The letters indicate significant differences among rice cultivars in each property at  $p < 0.05$ .

PSL 1 was lower than those of SPR 1 and CNT 1. Further studies should be conducted to investigate the different physiological and chemical properties of the endosperm among different rice cultivars that may be the reasons for differences in bio-accessibility of Fe and Zn.

As we can see, parboiled rice fortified with Fe and Zn is a cost effective approach for delivering Fe and Zn nutrition to the mass population as there is an existing infrastructure and marketing networks and consumers in developing countries such as Asia and Africa, without having to invent new process/infrastructure and/or alter the existing consumption behaviour. The bioavailability, cooking, and sensory quality of the multi-fortified parboiled rice are to be further evaluated, before formulating an optimal fortification process for parboiled rice mills.

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