Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes

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Abstract: Iron (Fe) bioavailability in unpolished, polished grain and bran fraction of five rice genotypes with a range of Fe contents was measured by *in vitro* digestion and cultured Caco-2 cells of cooked grain. There was a significant difference in Fe bioavailability among the five rice genotypes tested, in both the unpolished and polished grain. The range of Fe bioavailability variation in polished rice was much wider than that of unpolished, suggesting the importance of using Fe levels and bioavailability in polished rice grain as the basis for selecting high-Fe rice cultivars for both agronomic and breeding purposes. Milling and polishing the grain to produce polished (or white) rice increased Fe bioavailability in all genotypes. Iron bioavailability in polished rice was high in the UBON2 and Nishiki, intermediate in both IR68144 and KDML105, and low in CMU122. All genotypes had low bioavailability of Fe in bran fraction compared to unpolished and polished grain, except in CMU122. CMU122 contained the lowest level of bioavailable Fe in unpolished and polished grain and bran, because of the dark purple pericarp colored grain and associated tannin content. The level of bioavailable Fe was not significantly correlated with grain Fe concentration or grain phytate levels among these five genotypes tested. The negative relationship between Fe bioavailability and the levels of total extractable phenol was only observed in unpolished ($r = -0.83^{**}$) and bran fraction ($r = -0.50^{*}$). The present results suggested that total extractable phenol and tannin contents could also contribute to lowering bioavailability of Fe in rice grain, in addition to phytate.

Keywords: Fe bioavailability; rice; Caco-2 cell; total extractable phenol; tannin; grain color

INTRODUCTION

The World Health Organization has recently reported that there are more than 3 billion Fe-deficient people globally (i.e., 50% of the world population).[1] In human nutrition, Fe-deficiency-induced anemia impairs growth, development and immunity especially in infants and young children.[1,2] In Asia, 60–70% of preschool children and pregnant women are estimated to be affected by Fe-deficiency anemia.[3] On the basis of delivery effectiveness and economic consequence, enhanced Fe intake through staple food is the preferred effective mechanism in the long term,[4,5] as it has been recently confirmed in a study in Mindanao, the Philippines, that almost 50% of the Fe diet of the local population comes from Fe sources contained in cereals.[6] Rice is the staple food for the majority of people in Asia, but, unfortunately, contains the lowest Fe concentration among the cultivated Gramineae species.[6,7]

It is necessary to improve both the net Fe concentration and Fe bioavailability in rice grain (particularly polished rice known as white rice) for improving the Fe intake in populations dependent on rice as a staple food and without access to other Fe-rich food sources. In addition to agronomical management, selecting genotypes with high efficiency of Fe accumulation in the endosperm and high Fe bioavailability from existing germplasm collection may be an efficient and reliable way to deliver Fe nutrition benefits to farmers and local population. A wide variation of Fe concentration in rice grain exists among rice genotypes and high grain Fe concentrations were found in some traditional genotypes.[8]

Iron bioavailability in rice grain can be greatly inhibited by certain polyphenols,[9] flavonoid such as proanthocyanidins known as tannin,[9] and phytate.[9–11] Phytate, the naturally occurring form of phytic acid in seeds, is a salt of *myo*-inositolhexaphosphoric acid[12] associated with a wide range of cations including K,

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Contract/grant sponsor: Thailand Research Fund
Contract/grant sponsor: McKnight Foundation
(*Received 8 June 2005; revised version received 20 August 2005; accepted 6 January 2006*)
Published online 10 May 2006; DOI: 10.1002/jsfa.2471

Mg, Zn, and Fe.[13] Glahn *et al.*[14] reported that the bioavailability of Fe, assessed by using an *in vitro* digestion/Caco-2 cell model, was not related to the total grain Fe content or to grain phytate levels in the genotypes they tested, but was correlated with grain pericarp color. Little work has been done to relate grain Fe bioavailability to the levels of phenolic compounds in rice such as soluble phenols and tannin.

Previous studies have reported the variation in the bioavailability of Fe in rice grain among 15 genotypes by using an *in vitro* digestion/Caco-2 cell model.[14] However, these studies have only used brown rice: unpolished rice grain. There is little information on genotypic variation of Fe bioavailability in polished rice grain of different rice genotypes, in comparison with that in unpolished rice. Polished or white rice is the preferred form of rice grain commonly consumed by people. It is important to identify genotypes with high Fe concentration and high Fe bioavailability in the polished rice (the endosperm). Polishing processes reduce the Fe concentration in the grain by as much as 60%.[15] In the meantime, most of the anti-Fe nutrition compounds such as phytate, polyphenols and flavonoid are located in the polishing or bran fraction, so grain processing also substantially decreases the amount of inhibitors of grain Fe bioavailability. As a result, it is important to know if the polishing process changes the genotypic variation of Fe levels, distribution of anti-Fe nutrition compounds (such as phenolic compounds, proanthocyanidin and phytate) and associated Fe bioavailability in the rice grain. The information will provide the basis for genetic selection to improve nutritional quality of Fe particularly in polished rice grain. The objectives of the present study are to investigate the variation of Fe accumulation and bioavailability in polished rice, in comparison with those in unpolished rice, among five selected rice genotypes representative of a range of total Fe concentrations in rice grain; and to compare Fe bioavailability in different grain form and fractions including unpolished, polished and bran, in relation to the distribution of inhibitors in different forms of grain and grain fractions.

MATERIALS AND METHODS

Sample preparation

Five rice genotypes (*Oryza sativa* L.) were selected to represent two contrasting grain Fe status: three high- and two low-Fe grain lines (Table 1), based on the classification of grain Fe status in different genotypes in Senadhira *et al.*,[6] Prom-u-thai and Rerkasem,[8] Graham *et al.*[16] and Welch *et al.*[17] Rice grains were harvested at maturity from paddy plants grown in the same season on a sandy loam (San Sai soil) at Chiang Mai University, Thailand (18°48′ N; 98°59′ E). The hulls were removed by hand from the paddy rice grain to yield brown rice called 'unpolished grain'. The 'polished grain' samples were obtained by milling a 100 g lot of unpolished rice grain in a sample mill (Ngek Seng Huat company, model K-1) and the 'bran fraction' (the residue remaining after the polishing process) of the grain samples were also collected for the analysis of Fe and anti-nutrient compounds. Polishing for 30 s was found to be optimal for the visual whiteness of the rice grains. Subsamples of unpolished, polished grain and bran fraction were autoclaved for 15 min to simulate the cooking process and to produce corresponding cooked rice samples. The autoclaved grain samples were then homogenized in a Polytron homogenizer. The homogenate was frozen and then lyophilized to dryness. After being digested with concentrated nitric acid at 140 °C, Fe concentrations in rice grain and bran were determined by an inductively coupled plasma atomic emission spectrometry (ICAP-AES model 61E trace analyzer, Thermo Jarell Ash Corp., Franklin, MA).[18] A standard reference material of ground wheat grain was included in each batch of digestion for analytical quality control.

Bioavailability of Fe

Iron bioavailability was determined by *in vitro* digestion of 1 g cooked rice samples (including unpolished, polished rice and bran fraction) and cultured Caco-2 cells were used to simulate Fe absorption in human digestion system (intestine) from cooked rice of unpolished, polished rice grain and bran fraction, and the amount of ferritin formed in Caco-2 cells was used as proxy for Fe absorption by humans.[14] Immunoradiometric assay was used to measure Caco-2 cell ferritin content (FER-Iron II Ferritin Assay, RAMCO

Table 1. Iron concentration in polished, unpolished grain and bran fraction of five rice genotypes

Genotype	Description	Type	Fe concentration ($\mu g\,g^{-1}$)[a]		
			Unpolished	Polished	Bran fraction
Nishiki	Popular Japanese rice	NG[c]	18.70 ± 0.43	10.42 ± 0.93	145.69 ± 2.70
IR68144	Improved IRRI[b] rice variety	NG	21.30 ± 0.67	12.25 ± 0.84	157.75 ± 0.59
CMU122	Traditional Thailand hill-tribe genotype	G[d]	22.85 ± 0.10	11.45 ± 0.13	123.62 ± 0.63
KDML105	Popular Thailand jasmine rice	NG	13.45 ± 0.04	10.72 ± 0.08	82.25 ± 0.16
UBON2	Newly released Thailand genotype	G	14.05 ± 0.07	12.80 ± 0.05	134.13 ± 1.67

[a] Mean ± standard error of the mean ($n = 3$).
[b] IRRI, International Rice Research Institute.
[c] NG, non-glutinous rice.
[d] G, glutinous rice.

J Sci Food Agric **86**:1209–1215 (2006)
DOI: 10.1002/jsfa

Laboratories, Houston, TX). A 10 μL sample of the sonicated Caco-2 cell monolayer, harvested in 2 mL of water, was used for each ferritin measurement.[14]

Total extractable phytate, phenol and tannin content (CT)

Approximately 0.25 g rice sample were extracted with 10 mL of 1.25% H_2SO_4 for 2 h on a rotating shaker. The extraction mixture was centrifuged at $2000 \times g$ for 10 min. An aliquot (1 mL) of the extract solution was subsampled for the analysis of phytate. The extract of bran fraction was diluted 5–10 times before the analysis of phytate. The amount of phytate was measured by high-performance liquid chromatography (HPLC) as described by Lehrfeld[19] using inositol hexakis- (IP6), pentakis- (IP5) and phosphate (PO_4) as reference standards. Phytate concentration in each sample was calculated from the sum of IP6 and IP5.

For phenol analysis, aliquots (2 g) of rice sample were extracted with three changes of 20 mL 50% methanol for 60 min each time. The extraction mixture was centrifuged at $2683 \times g$ for 5 min each time and the supernatants were pooled for analysis of total extractable phenol using the Folin Ciocalteu's method.[20] For the total extractable tannin, 500 mg rice sample were extracted with three changes of 10 mL 70% acetone containing 0.1% $Na_2S_2O_5$. The sample extraction mix was centrifuged and supernatants from the three extractions were pooled for each sample. The residue was dried with N_2 for the extraction of bound CT fraction. Total extractable tannin was quantified using the butanol/HCl colorimetric methods.[21] Standard CT was extracted specifically for quantitative calibration, from the bran fraction part of *Oryza sativa* cv. Dramuda with 70% aqueous acetone containing 200 μg mL^{-1} ascorbic acid. The CT standard was purified by adsorption chromatography on Sephadex LH-20.[21]

Statistical analysis

The analysis of variance was carried out to detect the differences among the genotypes by using Statistic 7. The least significant difference (LSD) at $P < 0.05$ was applied to compare the means for any significant difference. The correlation coefficient was determined among five genotypes separately for each fraction of unpolished, polished and bran fraction.

RESULTS

Among the five genotypes investigated, there was a wide range of variation of Fe concentration in unpolished rice grain from 13.45 to 22.85 μg Fe g^{-1} dry weight. Nishiki, IR68144 and CMU122 were categorized as high-Fe genotypes, with 18.70–22.85 μg Fe g^{-1} dry weight, whereas, KDML105 and UBON2 only contained 13.45–14.05 μg Fe g^{-1} dry weight in the unpolished rice. However, the range of variation of Fe concentration was narrower in the polished rice than in the unpolished rice. The polishing process significantly decreased the Fe concentration in the rice grain to 10.42–12.25 μg Fe g^{-1} for the three high-Fe genotypes and to 10.72–12.80 μg Fe g^{-1} dry weight for the two low-Fe genotypes (Table 1).

In contrast, polishing enhanced the bioavailability of Fe and widened the range of Fe bioavailability variation among the five genotypes tested. Iron bioavailability in unpolished rice ranged from 2.3 to 8.4 mg ferritin (mg protein)$^{-1}$, with a similar value among the four genotypes Nishiki, IR68144, KDML105 and UBON2, and the lowest in CMU122, which was about one-third of the former four genotypes.

The polishing process increased Fe bioavailability in all five genotypes (Fig. 1), with the highest Fe bioavailability in UBON2 (13.6 ng ferritin (mg protein)$^{-1}$) and Nishiki (12.0 ng ferritin (mg protein)$^{-1}$), intermediate in IR68144 (8.5 ng ferritin (mg protein)$^{-1}$) and KDML105 (10.1 ng ferritin (mg protein)$^{-1}$), and the lowest in CMU122 (5.7 ng ferritin (mg protein)$^{-1}$). Iron bioavailability in the rice bran fraction was significantly lower than in unpolished and polished rice in all genotypes, except for CMU122 (Fig. 1). CMU122, which retained its purple color even in the polished rice, had comparable Fe bioavailability between the bran fraction and unpolished rice.

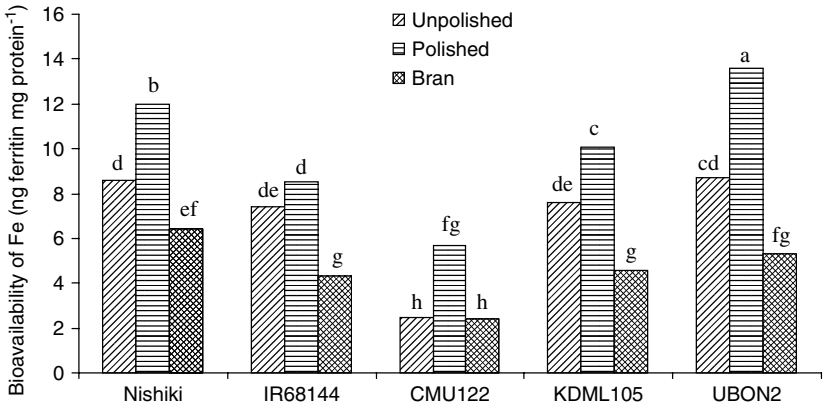

Figure 1. The comparison of iron bioavailability in unpolished, polished grains and bran fraction of five rice genotypes. Ascorbic acid (200 μmol L^{-1}) was added to the digest to promote iron absorption. Different letters are used to compare the significant difference ($P < 0.05$).

The effectiveness of the polishing process in increasing Fe bioavailability varied among the genotypes tested. After polishing, Fe bioavailability (ng ferritin (mg protein)$^{-1}$) in the polished rice increased from 8.7 to 13.6 for UBON 2, from 8.6 to 12.0 for Nishiki, from 7.6 to 10.2 for KDML 105 and from 2.6 to 5.8 for CMU122 (Fig. 1), whereas IR68144 showed no difference in the amount of bioavailable Fe between unpolished and polished grains. Low Fe bioavailability was found to be particularly associated with grain pericarp color of CMU122, which had a dark-purple pericarp.

The levels of phytate in unpolished grain did not differ significantly among the five genotypes (Table 2). In the unpolished rice, phytate concentrations ranged from 10.2 to 13.2 μmol g^{-1} dry weight, with the highest levels in KDML105 and CMU122 and the lowest in Nishiki (Table 2). In contrast, the levels of phytate in the polished rice were substantially decreased by polishing, ranging from about 1.5 μmol g^{-1} dry weight (KDML105) to about 4.3 μmol g^{-1} dry weight (Nishiki and UBON2) (Table 2). There was no direct relationship between phytate concentration and bioavailability of Fe and between phytate concentration and total grain Fe concentration among these five genotypes tested. Most of the phytate in the unpolished rice were distributed in the bran fraction.

However, Fe bioavailability was significantly correlated with the level of total extractable phenol in unpolished rice grain ($r = -0.83^{**}$) and bran fraction ($r = -50^{*}$), but not in polished grain. The levels of total extractable phenol in the unpolished rice ranged from 225 to 883 mg gallic acid equivalent kg^{-1} dry matter, with a declining order of: CMU122 > IR68144 = Nishiki > UBON2 > KDML105. In contrast, the levels of total extractable phenol in polished rice only ranged from 76 to 147 mg gallic acid equivalent kg^{-1} dry matter (Table 2). Similar to the distribution pattern of phytate, high concentrations of total extractable phenol were present in the bran fraction, ranging from 501 to 1485 mg gallic acid equivalent kg^{-1} dry matter.

Total extractable tannin was largely under detectable in four genotypes with white–brown pericarp. The one genotype with dark-purple pericarp, CMU122, was found to have total extractable tannin 0.23% of condense tannin in unpolished rice, 0.01% in polished rice and 1.07% in bran fraction (Table 3). The high tannin contents in unpolished, polished rice grain and bran fraction were consistent with pericarp color as well as bioavailability of Fe in rice grain. Overall, the combined effects of total grain Fe concentration, phytate levels and the levels of total extractable phenols explained the genotypic variation of Fe bioavailability in the unpolished and polished rice grain (Fig. 1).

DISCUSSION

The results demonstrated that polishing altered the magnitude of variation in Fe concentrations and substantially reduced Fe concentrations in the polished rice (white rice), in comparison with those in the unpolished, brown rice. This suggested that

Table 2. Total phytate and extractable phenol content in unpolished, polished and bran fraction of rice grain in five genotypes

		Phytate concentration (μmol g^{-1})			Total phenol (mg gallic acid kg^{-1})[a]		
Genotype	Source	A[b]	B	C	A	B	C
Nishiki	Japan	10.2a[c]	4.3a	45.6d	362.3b	147.0a	1034.3b
IR68144	IRRI	12.2a	2.0b	85.2a	382.2b	129.8b	1044.8b
CMU122	Thailand	13.0a	2.1b	49.3cd	883.7a	105.0c	1485.8a
KDML105	Thailand	13.2a	1.4b	59.4c	225.1d	75.9d	501.4c
UBON2	Thailand	12.0a	4.2a	73.2b	298.1c	102.4c	616.9c

[a] Recovery test = 91%.
[b] A, unpolished; B, polished; C, bran fraction.
[c] Different letters are used for comparing the significant different in each genotype ($P < 0.05$).

Table 3. Total extractable tannin content in soluble and bound form of unpolished, polished and bran fraction of five rice genotypes

		Total extractable tannin content[a] (% CT)					
		Unpolished		Polished		Bran	
Genotype	Source	Soluble	Bound	Soluble	Bound	Soluble	Bound
Nishiki	Japan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IR68144	IRRI	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CMU122	Thailand	0.04	0.19	0.01	n.d.	0.54	0.53
KDML105	Thailand	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
UBON2	Thailand	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Recovery test = 87%.
n.d., undetectable.

J Sci Food Agric **86**:1209–1215 (2006)
DOI: 10.1002/jsfa

care should be exercised when selecting high-Fe rice genotypes on the basis of Fe concentration in the unpolished rice (brown rice). However, Fe bioavailability in the rice grain was significantly enhanced by the polishing process, and the net amount of Fe available for apparent absorption by human intestine cells was higher in the polished than the unpolished, on the basis of the net amount of ferritin formed from digesting the same weight of each rice fraction (unpolished, polished and bran). The variation in Fe bioavailability among the five genotypes tested was explained by the combined effects of anti-nutrients phytate and extractable phenol. The presence of condensed tannin coincided with the dark-purple pericarp color in CMU122, which had the lowest Fe bioavailability, though it had the highest Fe concentration in the unpolished rice among the genotypes tested. The present results did not show any correlation between the grain Fe concentration and bioavailable Fe in unpolished rice grain, which was consistent with earlier findings.[14] A similar phenomenon was also observed in grains of early- and late-maturing tropical maize.[22,23]

Polished rice is the preferred form by most people who consume rice as a staple food.[15] Polishing can reduce grain Fe concentration from 9% to 50% depending on the genotype of rice. Polished grain loses its embryo and aleurone cell layers, which form the bran fraction. Many have reported that most of the Fe in the rice grain is localized in the outer layer of the grain, which includes the embryo and aleurone tissues.[12,15] The amount of bran removed during grain processing may depend on the size and shape of the grain and embryo and the thickness of the aleurone cell rows.[24–26] After polishing, the percent Fe retained in the UBON2 grain was higher than in the other four genotypes studied. This may be a result of morphological differences in the grain between genotypes such as the thickness of aleurone cell row layer and variations in embryo size.

Importantly, the polishing process was shown to increase Fe bioavailability in all the genotypes studied. Polished UBON2 grain had higher levels of bioavailable Fe compared to polished IR68144 grain (Fig. 1), even though polished UBON2 and IR68144 grain contained similar Fe concentrations (Table 1). Although Fe concentrations in the polished rice were lower than those in the unpolished rice (brown rice), the apparent net amount of Fe available for the Caco-2 cells was higher for the polished rice than unpolished and the bran fraction, given that the same amount of each fraction was digested. This enhanced Fe bioavailability further supports the notion that polished rice should be used in assessing and selecting rice genotypes with high Fe concentration and bioavailability.

Although polishing removed Fe from the grain, it also removed inhibitors of Fe absorption (e.g., polyphenols and flavonoid).[5] Many researchers have reported that phytate is a significant inhibitor of Fe bioavailability from cereal crops[10,11] as well as phenolic compound[27] and tannic acid.[9] However, the effect of phytate appears to be somewhat complex as it was not consistently correlated with bioavailability of Fe in rice grain as has been reported in a previous study.[14] Clearly, phytate is one of the important Fe bioavailability inhibitors, as reduction of phytate content in cereals led to a strong increase in bioavailability of Fe.[28–30] However, phytate concentration was not consistently related to the Fe concentrations and bioavailability in unpolished, polished rice grain and bran fraction, probably owing to the limited range of variability in phytate concentrations among the five genotypes tested. The lack of differential impact of phytate on Fe bioavailability in the rice grain may be due to the fact that phytate:Fe molar ratios in excess of 10:1 produce maximal inhibition of Fe uptake *in vitro*,[9] and in this study all of the samples were above this molar ratio. To our knowledge, this specific ratio effect has not been documented in humans, though the body of literature indicates that phytate:Fe levels need to be reduced to below a 10:1 ratio for the phytate effect to be attenuated. Thus, it takes a reduction to about 1:1 of phytate: Fe to eliminate the phytate effect (Hurrell RF, personal communication).

The levels of total extractable phenol seemed to contribute to the variation in Fe bioavailability among the five genotypes. Previous studies suggested that bioavailability of Fe in rice grain was correlated with the intensity or darkness of the pericarp color.[14] Biochemical compounds that impart color to the grain, such as phenol and tannins, may be responsible for the low Fe bioavailability observed in the colored grain.[14] The current results showed that the bioavailability of Fe was correlated with total extractable phenol in unpolished rice grain ($r = 0.83^{**}$) and bran fraction ($r = 0.50^{**}$). The varieties containing high levels of total extractable phenol had low bioavailability of Fe in unpolished and bran fractions. However, most of the extractable phenols were removed in the polished rice grain, which had a small range of total extractable phenol levels. In low-tannin sorghum, degradation of phenolic compounds increased bioavailability of Fe.[31,32] A larger number of genotypes with a wide range of extractable phenol in the endosperm should be investigated in further studies to clarify the relationship between the levels of phenol in the endosperm and Fe bioavailability.

Furthermore, CMU122 with dark-purple pericarp color coincidently had the lowest bioavailability of Fe in the genotypes tested. This is probably related to the high total extractable tannin content in rice grain. But tannin was presented in only one variety in this study and the direct effect of tannin on bioavailability of Fe remains to be investigated. Nevertheless, condensed tannin binds Fe, rendering it unavailable for absorption in the gastrointestinal tract.[29,31–33] However, further studies in humans are required to

investigate the comparative effects of phytate, phenolic acid and tannins on Fe bioavailability in rice grain.

CONCLUSIONS

The present study established that the magnitudes of genotypic variation in Fe concentration and bioavailability of rice grain can be significantly altered by the simple polishing process for yielding white rice – a preferred form of human consumption. The polishing process increases Fe bioavailability in the rice grain, which was estimated by using an *in vitro* digestion/Caco-2 cell model. Polishing removed a substantial proportion of anti-nutrient inhibitors such as extractable phenol and phytate, which underlined the enhanced Fe bioavailability responses. However, Fe concentrations in the polished rice grain were also substantially reduced, due to the loss of Fe in the bran fraction. As a result, rice genotype selection and breeding for high-Fe genotypes should be based on the Fe concentrations and bioavailability in the endosperm, rather than the unpolished rice. Compounds associated with pericarp color, such as total extractable phenol and tannins, appear to be strong inhibitors of Fe bioavailability in the rice grain, which may use an initial parameter in screening rice germplasm collection for high Fe level and bioavailability. Further research is required to identify these anti-nutrient substances associated with bioavailability of Fe in rice grain and to establish the comparative effects of phytate and phenols on Fe bioavailability in human trials.

ACKNOWLEDGEMENTS

We acknowledge financial support from the Thailand Research Fund and McKnight Foundation. Zhiqiang Cheng, Yongpei Chang and Larry Heller at US Plant, Soil and Nutrition Laboratory, Agricultural Research Service, US Department of Agriculture, and Mr Graham Kervin, a senior researcher at the analysis center, University of Queensland, are acknowledged for their technical assistance. Seed for planting was provided by the Thailand Rice Research Institute (KDML105, UBON2), CMUPN*lab* (CMU122) and IRRI (IR68144).

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