

**RESEARCH ARTICLE**

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# Iron and zinc variation along the grain length of different Thai rice varieties

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**ABSTRACT:** This study examined the distribution of iron (Fe) and zinc (Zn) along the grain length of seven rice varieties. The experiment was conducted in a completely randomized design with two factors (variety and grain fraction) and three independent replications. Samples of brown and white rice of six common Thai rice varieties and a high Fe and Zn variety, IR68144, were transversely cut into three fractions per grain (basal, middle, and distal) with approximately the same length in each fraction. The concentration of Fe and Zn was determined by the dry ashing method and quantified using atomic absorption spectrometry. The middle grain fraction of brown rice was found to have the lowest Fe and Zn with greater concentration of Fe and Zn in the basal (embryo end) than the other fractions. The rice varieties differed in the amount of Fe and Zn allocated to different fractions of the endosperm (white rice). The potential for loss of Fe and Zn during milling due to their uneven distribution along the grain length will become more significant when higher nutrient concentrations are involved, such as those achieved by biofortification efforts. Micronutrient distribution needs to be taken into consideration to ensure that rice consumers benefit from Fe and Zn biofortification.

**KEYWORDS:** grain fraction, micronutrient distribution

## INTRODUCTION

Human malnutrition from Fe and Zn are the most prevalent nutritional disorders in developing countries of Asia where the population has limited consumption of foods rich in Fe and Zn such as meat and dairy products, and derive most of their nutritional as well as calorie needs from rice<sup>1</sup>. Previous studies have reported a wide range in Fe and Zn concentrations in brown rice (caryopsis with intact pericarp and embryo) of different genotypes, e.g., 4–24 mg Fe/kg and 14–58 mg Zn/kg in a germplasm collection at the International Rice Research Institute<sup>2</sup> and 6–16 mg Fe/kg and 17–59 mg Zn/kg among Thai rice varieties<sup>3</sup>. Based on such information, breeding, and selection of Fe and Zn rich rice genotypes has been suggested as a means to increase the intake of these nutrients<sup>4,5</sup>.

Rice grains are harvested as rough rice or paddy (husk enclosed caryopsis). Pre-cooking processing includes removal of the husk that produces brown rice, followed by milling by a polishing action leav-

ing just the white endosperm or white rice (de-husked caryopsis without pericarp, the aleurone, some subaleurone cells and embryo), the form most commonly preferred by rice consumers. Iron and Zn have been shown to have particular localization patterns in different parts of the rice seed, being most concentrated in the aleurone, followed by the embryo and least concentrated in the endosperm<sup>3,6</sup>. Zinc has also been reported to be much more concentrated in the dorsal than the ventral section of rice grain<sup>7</sup>. Due to the removal of the aleurone and embryo, the concentrations of Fe and Zn are reduced in white rice, ranging from 2–11 mg Fe/kg and 10–40 mg Zn/kg<sup>3,8–10</sup>. Furthermore, rice varieties can differ significantly in the loss of Fe and Zn by milling<sup>3</sup>.

During the process of rice milling, some grains are broken; those that remain more than three quarters of the full grain length are known in the rice trade as head rice, those with shorter pieces are graded as substandard, broken rice<sup>11</sup>. The more stringent standard in Thailand requires that head

rice must be at least four-fifths of the full grain length<sup>12</sup>. The price of milled rice is determined by the percentage of head rice, with 100% head rice receiving the highest price, and prices decreasing with increasing percentage of broken rice. Our preliminary investigation found higher concentrations of Fe and Zn in broken rice than in whole grain<sup>13</sup>. The rice with lower percentage of head rice, e.g., 20–45% broken, is consumed by people with lower incomes. Thus the intake of Fe and Zn by different groups of rice consumers may depend on the distribution of these nutrients along the grain length and the position of grain breakage. This study therefore evaluates variation in the Fe and Zn content of 6 common Thai rice varieties and a high Fe and Zn genotype IR68144-2B-2-2-3 in 3 transverse fractions along the grain length. Samples of broken rice from the market were also examined for the proportion of the different grain fractions and Fe and Zn concentrations.

## MATERIALS AND METHODS

### Rice sample preparation

Six common paddy Thai rice varieties (KDML105, PTT1, SPR1, PSL1, CNT1, and CNT80) and IR68144 (IR68144-2B-2-2-3) were grown in the field on San-sai series soil under wetland condition at Chiang Mai University (18° 47' N, 98° 57' E) during the rainy season, the main rice season in Thailand. Four-week-old seedlings of each genotype were transplanted into 20 × 40 m plots at 0.25 × 0.25 m spacing. The field was kept flooded under 0.1–0.2 m of water until maturity. Four weeks after transplanting N (25 kg/ha) and P (14 kg/ha) were applied, followed by N 63 kg/ha 2 weeks later. Seeds of all varieties were harvested at maturity. The experiment was conducted in completely randomized design with two factors (variety and grain fraction) and three independent replications. One hundred grams of each variety was de-husked with a laboratory husker (model P-1, Ngek Seng Huat) to produce brown rice. All relevant parts of the husker were Teflon-coated to avoid Fe contamination during the husking process<sup>8</sup>. After husking, 30 g subsamples of the brown rice were milled for 30 s with laboratory mill (model K-1, Ngek Seng Huat) to produce white rice. Subsamples of brown and unbroken white rice were cut transversely into three fractions of approximately the same length with a Teflon knife (Personna, Verona VA, USA), identified as basal (embryo end), middle, and distal (the opposite end to the embryo) fractions.

### Determination of Fe and Zn

Distribution of Fe and Zn along the grain length was evaluated by atomic absorption spectrophotometer (Hitachi Model Z-8230) after dry ashing at 535 °C for 8 h<sup>14</sup>. The distribution of Fe and Zn along the grain length of white rice was determined from the nutrient content in the different grain fractions. The experiment was conducted in 3 replications with completely randomized design.

### Proportion of grain fraction among market broken rice

Two sets of the bulked broken rice samples were collected from the local retail market in Chiang Mai. The first set of samples consisting of 4 samples of whole grain (100% head rice) and 4 samples of broken grain of unknown variety from separated bulk samples, were analysed for Fe and Zn, in triplicate. The second set of samples were 16 samples of non-glutinous aromatic Thai Hom Mali (the most common broken rice on sale in the retail market, produced largely from the variety KDML105). Fe and Zn were determined from the basal, middle and distal grain fractions. For each broken rice sample, the proportion by weight and number of basal (identified by the scar left after removal of the embryo), middle (sharp cross grain break at both ends) and distal (sharp cross grain break at one end and smooth grain tip at the other end) grain fractions were determined from 3 replicates of 10 g subsamples.

### Data analysis

The Fe and Zn concentrations and proportion of broken fractions were subjected to ANOVA. Data on proportion were arcsine transformed before analysis. Significant differences between means were determined by the least significant difference (LSD) at  $p < 0.05$ . Correlation analysis was used to test the significance of each correlation. All statistical analyses were performed using Statistic 8 (analytical software, SXW).

## RESULTS

Based on their brown rice contents, all Thai rice varieties had low Fe (< 13 mg Fe/kg), and Zn (< 30 mg Zn/kg), while IR68144 was high in both Fe (16 mg Fe/kg) and Zn (40 mg Zn/kg). Distribution of both Fe and Zn (Table 1) along the length of brown rice grain varied significantly among the rice varieties, but did not seem to be related to the low or high Fe/Zn status of the varieties. In general, the

**Table 1** Iron and zinc concentrations in 3 transverse fractions of brown rice of seven varieties.

Variety	Fe concentration (mg/kg) <sup>†</sup>						Zn concentration (mg/kg) <sup>†</sup>					
	Basal		Middle		Distal		Basal		Middle		Distal	
KDML105	8.9	bB	5.2	aAB	6.3	aA	30.8	cC	20.9	aA	26.2	bB
PTT1	9.4	cB	4.8	aA	6.5	bA	29.7	cBC	19.5	aA	27.3	bB
SPR1	6.2	aA	6.3	aB	8.2	bB	15.5	aA	20.6	bA	26.3	cB
PSL1	6.3	aA	5.6	aAB	5.9	aA	17.6	aA	18.6	aA	25.1	bB
CNT1	15.4	cD	7.8	aC	9.3	bBC	28.1	cB	19.4	aA	22.5	bA
CNT80	11.7	cC	6.3	aB	9.9	bC	28.3	bB	19.6	aA	26.9	bB
IR68144	17.3	cE	10.1	aD	13.7	bD	40.9	cD	29.4	aB	38	bC
Mean	10.7	c	6.5	a	9	b	27.3	b	21.1	a	27.5	b

<sup>†</sup> Effects by F-test: Variety (V),  $p < 0.001$ ; Grain fraction (F),  $p < 0.001$ ;  $V \times F$ ,  $p < 0.001$ .

Significant difference (by  $LSD_{0.05}$ ) between grain fractions in each variety indicated by different lowercase letters and between varieties for each grain fraction by different uppercase letters.

**Table 2** Iron and zinc concentrations in 3 fractions of white rice of seven varieties.

Variety	Fe concentration (mg/kg) <sup>†</sup>						Zn concentration (mg/kg) <sup>‡</sup>					
	Basal		Middle		Distal		Basal		Middle		Distal	
KDML105	4.5	abB	3.7	aAB	4.9	bC	18.9	aB	19.6	aA	24.3	bB
PTT1	5.9	bC	4.5	aBC	5.3	abC	22.3	aC	22.6	aB	28.6	bC
SPR1	3.5	aA	3.1	aA	3.9	aB	14.9	aA	19.2	bA	24.2	cB
PSL1	4.9	bB	3.0	aA	2.5	aA	19.3	aB	19.3	aA	22.9	bAB
CNT1	4.2	aAB	3.5	aA	3.8	aB	13.6	aA	18.6	bA	21.9	cA
CNT80	6.9	bD	4.8	aC	5.5	aC	18.7	aB	19.4	aC	24.5	bB
IR68144	10.1	bE	8	aD	10.8	bD	31.2	aD	30.4	aD	39.3	bD
Mean	5.7	c	4.4	a	5.4	b	19.8	a	21.3	b	26.5	c

<sup>†</sup> Effects by F-test: Variety (V),  $p < 0.001$ ; Grain fraction (F),  $p < 0.001$ ;  $V \times F$ ,  $p < 0.01$ .

<sup>‡</sup> Effects by F-test: Variety (V),  $p < 0.001$ ; Grain fraction (F),  $p < 0.001$ ;  $V \times F$ ,  $p < 0.001$ .

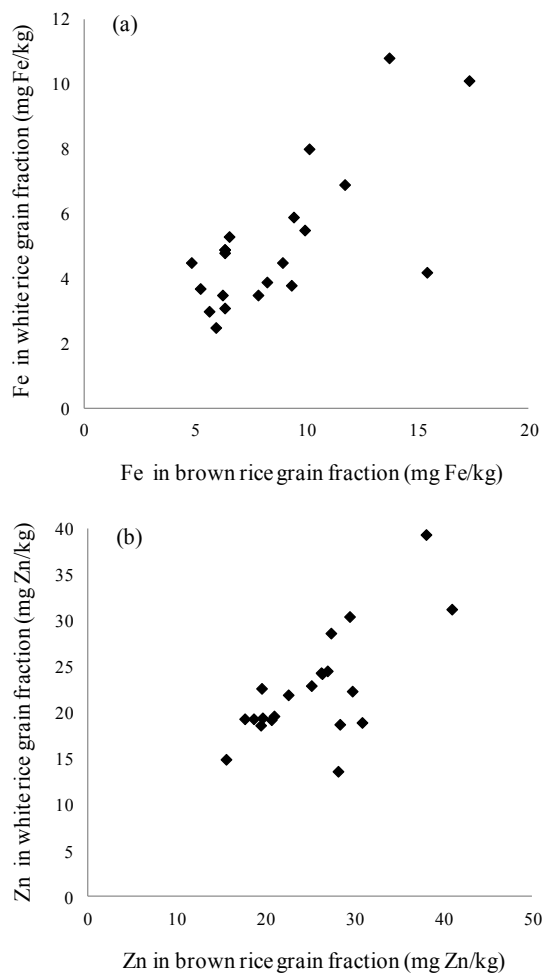
Significant difference (by  $LSD_{0.05}$ ) between grain fractions in each variety indicated by different lowercase letters designates and between varieties for each grain fraction by different uppercase letters.

middle grain fraction of brown rice was lowest in both Fe and Zn. There were greater concentrations of Fe and Zn in the basal (embryo end) than the other fractions of the grain in 5 out of 7 rice varieties. Brown rice of the varieties SPR1 and PSL1 were much more uniform in Fe concentration along the grain length, but their Zn concentrations were highest in the distal fraction.

The concentrations of Fe and Zn in white rice, with the pericarp, the aleurone, some subaleurone cells and embryo removed, were lower than in brown rice in all grain fractions. Milling had different effects on the relative Fe content of the grain fractions (Table 2). The concentration of Fe in white rice was indistinguishable in the 3 grain fractions of all varieties. SPR1 and CNT1 were about the same in basal and distal fractions while the same fractions from KDML105, PTT1, IR68144 were higher than that from the middle fraction. The basal fraction in PSL1 and CNT80 was higher

than both the middle and distal fractions of SPR1 and CNT1 which had similar concentration. The relative Zn concentrations of the grain fractions were affected by milling differently from those of Fe. White rice Zn concentration was highest in the distal fraction in all 7 rice varieties, although there were some differences among the varieties in relative Zn concentration of the 3 grain fractions (Table 2). In SPR1 and CNT1, there was a significant gradient of increasing Zn in white rice from the basal to distal fraction. In the remaining 5 varieties, the white rice Zn concentration of the basal and middle fractions was indistinguishable.

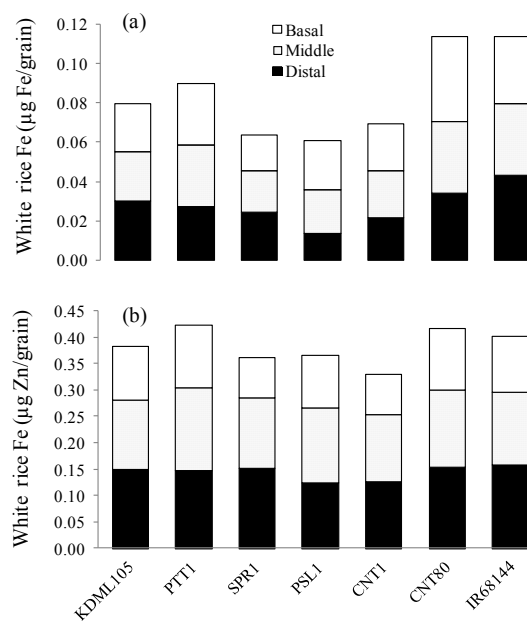
The white rice Fe concentration of the grain fractions was closely correlated with their brown rice Fe ( $R^2 = 0.51$ ,  $p < 0.001$ ), and similarly white rice Zn concentration of the grain fractions was closely correlated with their brown rice Zn ( $R^2 = 0.48$ ,  $p < 0.001$ ) (Fig. 1). With white rice Fe content that ranged from 0.06–0.11  $\mu\text{g}/\text{grain}$  and Zn from



**Fig. 1** Relationship between the concentration in brown and white rice grain fractions of (a) Fe,  $R^2 = 0.51$ ,  $p < 0.001$  and (b) Zn,  $R^2 = 0.48$ ,  $p < 0.001$ , of 7 rice varieties. (Significance of linear regression by ANOVA).

0.36–0.42  $\mu\text{g}/\text{grain}$ , distribution of the nutrients differed significantly among the 3 grain fractions, and differently between Fe and Zn (Fig. 2). About the same amount of Fe was allocated to the 3 grain fractions in 6 of the 7 varieties; the exception was PSL1 which had significantly less Fe in the distal fraction. All 7 rice varieties allocated significantly less Zn to the basal fraction, especially SPR1 and CNT1.

Rice samples from the retail market varied within the same range of Fe concentration for full grain (1.3–2.7 mg/kg) and broken rice (1.9–2.9 mg/kg), and similarly for the range of Zn concentration for full grain (16.2–21.2 mg/kg) and broken rice (17.8–22.2 mg/kg) (Fig. 3). By weight, broken samples of Thai Hom Mali, a long-grain

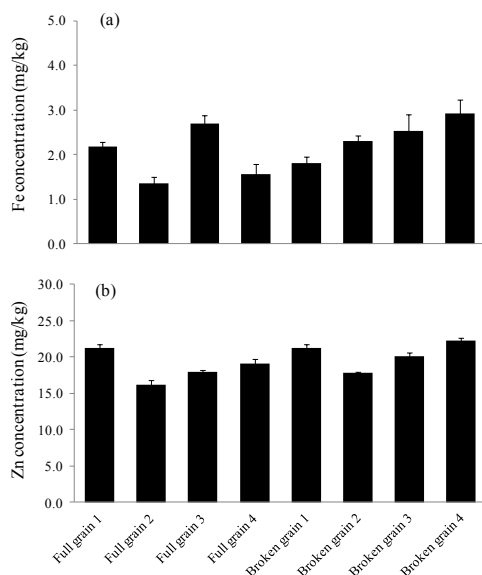


**Fig. 2** Distribution of (a) Fe and (b) Zn content along the grain length of white rice of 7 varieties. For Fe, the variety (V), grain fraction (F), and  $V \times F$  were  $p < 0.001(0.003)$ , not significant  $p < 0.05$ , and  $p < 0.001(0.005)$ , respectively. For Zn, V, G, and  $V \times F$  were  $p < 0.001(0.008)$ ,  $p < 0.001(0.005)$ , and  $p < 0.01(0.014)$ , respectively. Numbers in brackets are LSD at  $p \leq 0.05$ .

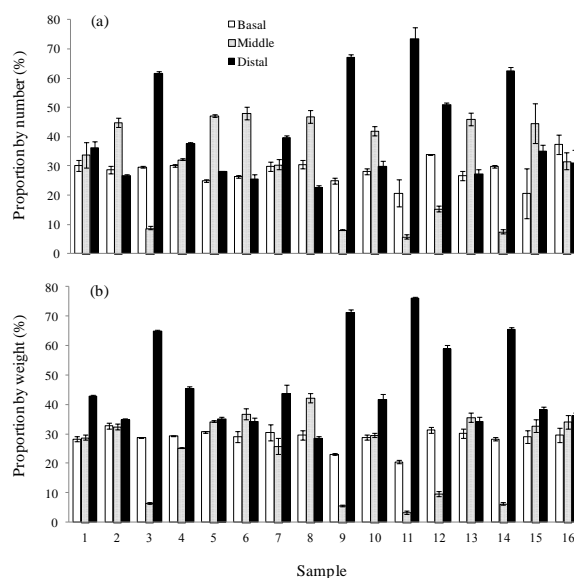
aromatic rice, in this study were made up mostly of the distal grain fraction, although by their number the middle and distal fractions were dominant in about the same number of samples (Fig. 4).

**DISCUSSION**

Significant differences in concentration of the nutrients in white rice grain fractions indicate longitudinal variation in the distribution Fe and Zn in the endosperm of rice. The rice varieties also differed in the amount of Fe and Zn allocated to different fractions of the endosperm. On average, the Fe concentration in the embryo and aleurone is 14 times that in the endosperm; and the embryo Zn is 9 times while the aleurone Zn is twice that of the endosperm<sup>3</sup>. In spite of this, there was a close correlation between the Fe concentration of white and brown rice grain fractions, and similarly between the Zn concentration of white and brown rice grain fractions. The variation in Fe and Zn distribution along the endosperm length would have implications for the concentration of these nutrients in milled rice. The loss of grain mass by milling, also called degree of milling, is influenced by mor-



**Fig. 3** Concentrations of (a) Fe and (b) Zn in full and broken grain of the commercial samples from the local market in Chiang Mai, Thailand. Bars represent standard error of means ( $n = 3$ ).



**Fig. 4** Proportion of different grain fractions in 16 broken Thai Hom Mali rice samples (a) by number and (b) by weight from the retail market in Chiang Mai, Thailand. Bars represent standard error of means ( $n = 3$ ).

phological characters of the rice grain such as its size and shape, which can differ widely among different rice varieties<sup>15–17</sup>. Loss of Fe during milling in rice varieties grouped by their grain length and shape was closely associated with the degree of milling<sup>18</sup>. Among the long-slender grain Thai rice varieties, however, the loss of Fe and Zn during milling is influenced by variation in partitioning of the grain Fe and Zn into the aleurone and embryo as well as the degree of milling<sup>3</sup>. The variation in Fe and Zn distribution along the length of rice grain found in this study would add another variable to the effect of milling on nutrient concentration of milled rice, with the magnitude and direction of the effect dependent on the grain fraction that is broken off and its nutrient concentration relative to the remainder of the grain. The loss of Fe and Zn during milling in the grain tips would contribute towards loss of the nutrients along with the removal of embryo and aleurone. This, on the other hand, may not be a complete loss, as the cheaper broken rice would become enriched with Fe and Zn.

Grain breakage is a well-known feature of rice milling<sup>19,20</sup>. However, it is as yet unknown which end of the grain is more likely to be broken off, or how this may be influenced by genotype and environment. The observation that the distal grain fraction made up the major proportion by weight of

the broken Hom Mali rice samples from the market suggested that this may not happen randomly. As Hom Mali rice is produced largely from the variety KDML105, the loss of distal grain tip with its higher concentrations of Fe and Zn during milling would result in significant loss of the nutrients. The limited samples of full grain and broken rice from the market were not distinguishable, neither in their Fe nor their Zn concentration. With estimated requirement of 8–18 mg/day of Fe (27 mg Fe for a pregnant woman) and 8–12 mg/day of Zn<sup>21</sup> and per capita rice consumption of 0.2–0.3 kg<sup>22,23</sup>, both full grain and broken rice from the market would have furnished only a small fraction of the nutritional needs of consumers. At the low Fe and Zn concentration of common rice varieties<sup>3,24</sup>, reflected in the 1–3 mg/kg Fe and 16–22 mg/kg Zn found in the market rice, and narrow variations in the nutrient concentration along the grain length, loss of Fe and Zn during milling due to grain breakage may be of little consequence. The potential for loss of Fe and Zn during milling due to their uneven distribution along the grain length will, however, become more significant when involving higher concentration of the nutrients, such as achieved by biofortification and/or fortification through parboiling process efforts<sup>13</sup>. For example, milling will affect Fe and Zn concentration of milled rice more noticeably in high

Fe and Zn varieties like IR68144 than in varieties with lower Fe and Zn. Loss of grain tips with higher Fe and Zn concentrations during milling will result in lower concentrations of the nutrients in the remaining grain fraction, while the broken rice will become more enriched.

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