CHAPTER 4

Iron and zinc distribution along the grain length of different Thai rice varieties and implications on grain quality

4.1 Introduction

Iron (Fe) and zinc (Zn) are the most prevalent nutritional disorder worldwide, especially in developing countries where the population has limited consumption of foods rich in Fe and Zn, such as animal products, and who derive most of their nutritional as well as calorie needs from rice (Hotz and Brown, 2004). In brown rice, previous studies have reported a wide variation in Fe and Zn concentrations among varieties, from 4 to 24 mg Fe/kg and 13.5 to 58.4 mg Zn/ kg (Gregorio, 2002). However, Fe and Zn in white rice is generally much lower at 2 to 11 mg Fe/ kg (Prom-u-thai *et al.*, 2007a) and 9.7 to 26.5 mg Zn/kg (Prom-u-thai *et al.*, 2010; Sellappan *et al.*, 2009) due to removal in the milling process.

However, a preliminary investigation established that the Fe density in broken rice of both unfortified and Fe-fortified parboiled rice can be higher than that in full grain (Prom-u-thai *et al.*, 2009a). This provides an economic advantage among low income populations who consume broken rice as the staple food because of its much lower price compared with full grain rice. For example, from 2009 to 2011 the average FOB Bangkok prices per metric ton of Thai Hom Mali jasmine rice were about US$ 500 for broken and US$ 1000 for full grain, and for non-jasmine white rice the prices were US$ 400 for broken and US$ 600 for full grain (Thai Rice Exporters Association, 2012). Importers in developing countries often buy broken rice to supply the low end markets for human consumption and sometimes for feeding animals. Differential Fe and Zn concentrations in broken compared with full grain rice would have significant implications for the health of poorer rice consumers.

The rice grain is harvested as rough or paddy (the husk encloses the caryopsis) rice. Pre-cooking processing includes removal of the husk that produces brown rice, followed by milling to remove the pericarp, seed coat, aleurone and embryo, producing the white endosperm or white rice, the form most commonly preferred by rice consumers. In the process some grains are broken, with those that are more than three quarters to four-fifths of the full grain length (depending on local market condition) considered as head rice (full grain), those with less length graded as sub-standard, broken rice and small pieces are generally screened and included in the bran and germ fractions. Uneven distribution of Fe and Zn along the grain length would contribute to different concentrations of these nutrients in broken and full grain. However, there is currently no available information on the Fe and Zn distribution along the grain length. To address this gap in knowledge, which could contribute to improvement in the Fe and Zn nutrition of rice eaters, the present study aimed to evaluate how Fe and Zn densities of broken rice compare with those of head rice (> 4/5 whole grain length, by Thai standard (Ministry of Commerce 1997) in rice cultivars with differing Fe and Zn concentrations. Distribution of Fe and Zn in the basal, middle and distal segments was assessed. For comparison, commercial samples from the local market were evaluated for Fe and Zn contents in different grain fragments in broken rice samples.

4.2 Materials and methods

4.2.1 Samples and varieties examined

Four commercial samples of full grain white non-glutinous rice and 4 samples of broken grain A1 super grade (length ≤ 0.65 of full grain or not passing thought sieve no.7, Ø 1.75 mm mesh) were purchased from 2 city markets in Chiang Mai, Thailand and used to investigate Fe and Zn in rice grain.

Three commercial samples of broken parboiled rice A1 super grade obtained from three parboiled rice producers in Kampheang Phet province, Thailand were used to investigate the variation in the proportion of broken segments of parboiled rice. Two broken white samples obtained from two city markets in Chiang Mai, Thailand, were used for comparison.

Four samples of white rice of laboratory standard long slender grain variety (SPR1, CNT1, PTT1 and KDML105) obtained from Division of

Agronomy, Chiang Mai University were used to investigate the variation in proportion of broken segments of laboratory milled rice.

Lastly, 14 samples of brown and white rice of 7 varieties; SPR1, PSL1, CNT1 KDML105, PTT1, CNT80 and IR68144-2B-2-2-3 from Division of Agronomy, Chiang Mai University were used to investigate Fe and Zn distribution along the grain length.

4.2.2 Sample preparation

1) Milling process

One hundred gram of paddy rice was dehusked with a laboratory dehusker machine (model P-1, Ngek Seng Huat Company, Thailand) to yield brown rice. The dehusker was Teflon-coated for all containers and handles to avoid contamination during the dehusking process (Prom-u-thai *et al.*, 2007a). After dehusking, 30 g of whole grain of brown rice were subsampled for each variety and milled for 30 s in a laboratory milling machine (model K-1, Ngek Seng Huat Company, Thailand) to yield white rice. The experiment was conducted with 3 replicates for each variety.

2) Proportion of grain fraction

Ten g sub-samples of broken rice were separated manually into three groups; basal (adjacent to the embryo), middle and distal segments and proportions were determined by number and by weight. Each sample was replicated 3 times.

3) Iron and Zn distribution along the grain length

Five g sub-samples of brown and white rice were transversely cut into three fractions per grain (as in 2 above) with approximately the same length in each fraction with a Teflon knife (Personna, Verona VA, USA). Each sample was replicated 3 times.

4) Iron and zinc analyses

Samples were oven dried at 75 °C for 72 h and dry-ashed in a muffle furnace at 535 °C for 8 h. The ash was dissolved in HCl (1:1; HCl:deionized water) and the Fe and Zn were determined using an Hitachi Z-8230 atomic absorption spectrophotometer (AAS) (Allan, 1961). Soybean leaf was used for reference material in all samples to check the quality of Fe and Zn analyses.

5) Statistical analysis

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

4.3 Results

4.3.1 Comparison of Fe and Zn concentrations between full and broken grain of commercial rice

The concentrations of Fe and Zn in full and broken grain were significantly different among samples. The Fe concentration in full grain showed more variation than in broken grain (Table 4.1); it ranged from 1.3 to 2.7 mg/kg in full grain (CV 30.9%) and 1.9 to 2.9 mg/kg in broken grain (CV 23.6%). The Zn concentrations varied much more narrowly, ranging from 16.2 to 21.2 mg/kg in full grain (CV 11.0%) and 17.8 to 22.2 mg/kg in broken grain (CV 9.0%) (Table 4.2).

Table 4.1 The concentration of Fe in full and broken grains of four commercial rice samples from two markets in Chiang Mai, Thailand.

Samples	Fe concentration (mg/kg)		Samples	Fe concentration (mg/kg)
Full grain 1	2.19	A	Broken grain 1	1.81
Full grain 2	1.35	B	Broken grain 2	2.30
Full grain 3	2.70	A	Broken grain 3	2.54
Full grain 4	1.57	B	Broken grain 4	2.93
Mean	18.6		Mean	2.4
F-test	**		F-test	ns
LSD $_{0.05}$	1.72		LSD $_{0.05}$	
CV (%)	11.0		CV (%)	23.6

Uppercase letters are used for comparison between rows. The different letters are significantly different by LSD (P<0.05).

Table 4.2 The concentration of Zn in full and broken grains of four commercial rice samples from two markets in Chiang Mai, Thailand.

Samples	Zn concentration (mg/kg)		Samples	Zn concentration (mg/kg)	
Full grain 1	21.19	A	Broken grain 1	21.26	AB
Full grain 2	16.19	CB	Broken grain 2	17.75	C
Full grain 3	17.90	BC	Broken grain 3	20.13	B
Full grain 4	19.10	B	Broken grain 4	22.18	A
Mean	1.95		Mean	20.33	
F-test	**		F-test	**	
LSD $_{0.05}$	0.53		LSD $_{0.05}$	1.24	
CV (%)	30.9		CV (%)	23.6	

Uppercase letters are used for comparison between rows. The different letters are significantly different by LSD (P<0.05).

4.3.2 The proportion of broken fractions

The middle grain section accounted for the smallest proportion of grain fractions in all 9 broken rice samples representing commercial raw and parboiled rice and 4 specific long slender grain varieties milled in the laboratory (Table 4.3). The actual proportion by weight of the middle grain fraction varied from 14 to 24% in commercial broken rice samples and 2 to 12% in laboratory milled broken rice samples. The biggest proportion of broken grain was generally represented by the distal fraction, ranging from 32 to 52% in the commercial and laboratory milled broken rice samples. The exception was KDML105 from laboratory milling which had more basal (56%) than the distal (32%) segment. The actual proportion by number of the middle grain fraction in broken commercial rice varied more than the proportion by weight (Table 4.4), ranging from 20 to 52% while the laboratory broken rice varied from 2 to10%. The biggest proportion of grain fraction was represented by the distal and basal fractions except commercial KDML105-1 and -2, which had similar grain fraction number.

Table 4.3 Proportion by weight of the basal, middle and distal fractions of 5 commercial and 4 laboratory broken rice samples.

Sample		Grain Fraction		
		Basal	Middle	Distal
Commercial	Parboiled rice 1	26.7 Fb	21.0 ABb	52.3 Aa
	Parboiled rice 2	33.4 DEb	23.3 Ac	43.4 Ca
	Parboiled rice 3	41.9 BCa	14.2 CDb	43.9 Ca
	KDML105 -1	31.9 EFb	24.1 Ac	44.1 BCa
	KDML105 -2	32.7 DEFb	16.4 BCc	51.0 ABa
Laboratory	SPR1	47.1 Ba	2.0 Eb	51.4 ABa
	CNT1	39.2 CDb	10.7 Dc	50.5 ABCa
	PTT1	38.0 CDEb	10.4 Dc	51.7 ABa
	KDML105	55.9 Aa	12.0 Dc	32.2 Db
	Mean	38.5	14.9	46.7
	Variety	Fraction	Variety x Fraction	
F-test	ns	***	***	
LSD $_{0.05}$		0.03	0.08	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.4 Proportion by number of the basal, middle and distal fractions of 5 commercial and 4 laboratory broken rice samples.

Sample		Grain Fraction		
		Basal	Middle	Distal
Commercial	Parboiled rice 1	28.5 Bb	19.8 Bb	51.7 Aa
	Parboiled rice 2	32.0 Bab	26.5 ABb	41.6 ABCa
	Parboiled rice 3	37.5 Ba	19.5 Bb	43.0 ABCa
	KDML105 -1	31.6 Ba	29.6 Aa	38.8 Bca
	KDML105 -2	37.4 ABa	27.9 ABa	34.6 Ca
Laboratory	SPR1	45.8 Aa	2.1 Db	52.2 Aa
	CNT1	37.8 ABb	10.4 Cc	51.8 Aa
	PTT1	40.9 Aa	10.9 Cb	48.2 ABa
	KDML105	46.4 Aa	5.4 CDb	48.2 ABa
	Mean	37.5	16.9	45.6
	Variety	Fraction	Variety x Fraction	
F-test	ns	***	***	
LSD $_{0.05}$		0.27	0.27	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

4.3.3 Distribution of Fe and Zn between grain fractions

In general, the concentrations of Fe and Zn in brown and white rice of seven varieties were lowest in the middle fraction *(P<0.001)* (Table 4.5-4.8). In brown rice in five cultivars, the Fe concentrations in the distal, middle and basal fractions ranged from 6.3 to 13.7, 4.9 to 10.1 and 8.9 to 17.3 mg/kg, respectively. By contrast, SPR1 had lower Fe concentrations in the basal and middle fractions (6.2 mg/kg) and in PSL1 the Fe concentration did not differ among grain fractions and ranged from 5.6 to 6.3 mg/kg (Table 4.5). By comparison, the Fe concentration in brown rice ranged from 5 to 17 mg/kg.

The Fe concentration in white rice was considerably lower than brown rice in all fractions and varieties, ranging from 2 to 11 mg/kg. The lowest Fe concentration generally occurred in the middle fraction, ranging from 3.0 to 8.0 mg/kg. The ranges for the other two fractions were 3.5 to 10.1 mg/kg in the base and 2.5 to10.8 mg/kg in the distal end. An exception was PSL1 and CNT80 where there was no difference in Fe concentration between the middle and distal fractions (Table 4.6).

The zinc concentration in brown rice ranged from 15 to 41 mg/kg. The lowest concentration generally occurred in the middle fraction which ranged from 18.6 to 29.4 mg/kg. By contrast, the Zn concentrations ranged from 28.1 to 40.9 mg/kg in the basal fraction and 22.5 to 38.0 mg/kg in the distal fraction. Exceptions were SPR1 and PSL1 which had the lowest Zn concentration in the basal part of the grain (15.5 and 17.6 mg/kg, respectively) (Table 4.7).

Unlike for brown rice, the lowest Zn concentration in white rice occurred in the basal fraction or in both the basal and middle fractions (range 13.6 to 31.2 mg/kg). The Zn concentrations were significantly higher in the distal fraction, ranging from 21.9 to 39.2 mg/kg (Table 4.8)

Table 4.5 The Fe concentration in three fractions of brown rice of seven varieties

Variety	Fe concentration (mg/kg)			Mean
	Basal	Middle	Distal	
SPR1	6.2 Eb	6.3 Cb	8.2 Ca	6.9
PSL1	6.3 Ea	5.6 Ca	5.9 Da	5.9
CNT1	15.4 Ba	7.8 Bc	9.3 BCb	10.8
KDML105	8.9 Da	5.2 Cb	6.3 Db	6.8
PTT1	9.4 Da	4.8 Dc	6.5 Db	6.9
CNT80	11.7 Ca	6.3 Cc	9.9 Bb	9.3
IR68144-2B-2-2-3	17.3 Aa	10.1 Ac	13.7 Ab	13.7
Mean	10.74	6.49	9.01	
	Variety	Fraction	Variety x Fraction	
F-test	***	***	***	
$LSD_{0.05}$	0.1	0.5	1.4	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.6 The Fe concentration in three fractions in white rice of seven varieties

Variety	Fe concentration (mg/kg)			Mean
	Basal	Middle	Distal	
SPR1	3.5 Ea	3.1 Da	3.9 Ca	3.5
PSL1	4.9 DEa	3.0 Db	2.5 Db	3.5
CNT1	4.2 DEa	3.5 Da	3.8 Ca	3.8
KDML105	4.5 Dab	3.7 CDb	4.9 Ba	4.4
PTT1	5.9 Ca	4.5 BCb	5.3 Bab	5.2
CNT80	6.9 Ba	4.8 Bb	5.5 Bb	5.7
IR68144-2B-2-2-3	10.1 Aa	8.0 Ab	10.8 Aa	9.6
Mean	5.7	4.4	5.4	
	Variety	Fraction	Variety x Fraction	
F-test	***	***	**	
$LSD_{0.05}$	0.6	0.3	0.9	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.7 The Zn concentration in three fractions of brown rice of seven varieties

Variety	Zn concentration (mg/kg)			Mean
	Basal	Middle	Distal	
SPR1	15.5 Ec	20.6 Bb	26.3 Ba	20.8
PSL1	17.6 Bb	18.6 Bb	25.1 Ba	20.4
CNT1	28.1 Ca	19.4 Bc	22.5 Cb	23.3
KDML105	30.8 Ba	20.9 Bc	26.2 Bb	26.0
PTT1	29.7 BCa	19.5 Bc	27.3 Bb	25.5
CNT80	28.3 Ca	19.6 Bb	26.9 Ba	24.9
IR68144-2B-2-2-3	40.9 Aa	29.4 Ac	38.0 Ab	36.1
Mean	27.3	21.1	27.5	
	Variety	Fraction	Variety x Fraction	
F-test	***	***	***	
$LSD_{0.05}$	1.3	0.9	2.3	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.8 The Zn concentration in three fractions of white rice of seven varieties

Variety	Zn concentration (mg/kg)			Mean
	Basal	Middle	Distal	
SPR1	14.9 Dc	19.2 Cb	24.2 CDa	19.4
PSL1	19.3 Cb	19.3 Cb	22.9 CDa	20.5
CNT1	13.6 Dc	18.6 Cb	21.9 Da	18.3
KDML105	18.9 Db	19.6 Cb	24.3 CDa	20.9
PTT1	22.3 Bb	22.6 Bb	28.6 Ba	24.5
CNT80	18.7 Cb	19.4 Cb	24.5 Ca	20.9
IR68144-2B-2-2-3	31.2 Ab	30.4 Ab	39.3 Aa	33.6
Mean	19.8	21.3	26.5	
	Variety	Fraction	Variety x Fraction	
F-test	***	***	**	
$LSD_{0.05}$	1.4	0.9	2.5	

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD ($P<0.05$).

4.3.4 Partitioning of Fe in grain fractions of brown and white rice

In brown rice the basal and distal fractions generally had a greater (*P<0.001*) proportion of the total grain Fe than the middle fraction (Table 4.9). However, there was a strong interaction between variety and fraction. In particular, CNT1, PTT1 and IR68144-2B-2-2-3 had more Fe in the basal fraction (39.9, 41.2 and 37.6% of total grain Fe, respectively) than in the middle and distal fractions. By contrast, in SPR1 and PSL1 the grain Fe was distributed across fractions in the following order: distal (42.2 and 50.1% of total grain Fe, respectively) > middle (32.3 % and 29.9% of total grain Fe) > basal (25.3 % and 20.1% of total grain Fe). Overall, individual fractions accounted for 20.1 to 50.1% of the total grain Fe.

The effect of variety on Fe distribution in fractions of brown rice disappeared in white rice (Table 4.10). However, there was an interaction between variety and fraction in white rice. In particular, three varieties (SPR1, KDML105 and IR68144-2B-2-2-3) had a higher proportion of grain Fe in the distal fraction and this contrasted with PSL1 and CNT80 which had a higher proportion of grain Fe in the basal fraction. The remaining two varieties (CNT1 and PTT1) had higher but equal proportions of grain Fe in the basal and middle fractions. Although these differences were significant, the range in values was relatively small, namely from 26.7 to 40.6.

Table 4.9 Proportion of grain Fe in three grain fractions; basal, middle and distal of brown rice of seven varieties.

Variety	Fe distribution (%)		
	Basal	Middle	Distal
SPR1	25.3 Bc	32.2 Ab	42.4 Ba
PSL1	20.1 Cc	29.9 ABb	50.1 Aa
CNT1	39.9 Aa	28.0 ABb	32.1 CDb
KDML105	38.6 Aa	32.8 Aab	28.5 Db
PTT1	41.2 Aa	25.7 Bc	33.1 CDb
CNT80	38.5 Aa	26.1 Bb	35.4 Ca
IR68144-2B-2-2-3	37.6 Aa	29.1 ABb	33.2 CDab
	34.5	29.1	36.4
	Variety	Fraction	Variety x Fraction
F-test	ns	***	***
LSD0.05		0.03	0.07

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.10 Proportion of grain Fe in three grain fractions; basal, middle and distal of white rice of seven varieties.

Variety	Fe distribution (%)		
	Basal	Middle	Distal
SPR1	28.2 Cc	33.3 ABb	38.5 Aa
PSL1	40.6 Aa	32.6 ABb	26.7 Cc
CNT1	34.4 Ba	34.2 ABa	31.4 Bb
KDML105	30.8 Cb	31.2 Bb	38.1 Aa
PTT1	34.7 Ba	35.2 Aa	30.1 BCb
CNT80	38.0 ABa	31.7 Bb	30.2 BCb
IR68144-2B-2-2-3	30.0 Cb	32.2 Bb	37.8 Aa
Mean	33.8	32.9	33.3
	Variety	Fraction	Variety x Fraction
F-test	ns	ns	***
LSD0.05			0.04

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

4.3.5 Partitioning of Zn in grain fractions of brown and white rice

In brown rice, the proportion of grain Zn was greater in the basal and distal parts than the middle fraction (Table 4.11). However, there was an interaction between variety and fraction. In general, SPR1, PSL1 CNT1 and PTT1 (44.5, 42.2, 35.4 and 37.1% of total grain Zn, respectively) had more Zn in the distal fraction than in the basal and middle fractions and this contrasted with KDML105 which had more Zn in the basal and middle fractions (34.9 and 34.2% of total grain Zn, respectively) than in the distal fraction. The remaining varieties, CNT1 and IR68144-2B-2-2-3 had equal proportion s of grain Zn in the basal and distal fractions which higher than in the middle fraction.

Unlike brown rice, the middle and distal fractions of white rice generally had higher proportions of grain Zn than the basal fraction (Table 4.12). Nevertheless, there was an interaction between variety and fraction.

In SPR1, KDML105, CNT80 and IR68144-2B-2-2-3 (41.9, 38.9, 36.9, and 39.1% of total grain Zn, respectively) there was more grain Zn in the distal than in the middle and basal fractions. In addition, PSL1 and PTT1 had the highest grain Zn in the middle fraction (39.0 and 31.1% of total grain Zn, respectively). The remaining variety, CNT1 had equal proportions of grain Zn in the middle and distal fractions and these were higher than in the basal fraction.

Table 4.11 Proportion of grain Zn in three grain fractions; basal, middle and distal of brown rice of seven varieties.

Variety	Zn distribution (%)		
	Basal	Middle	Distal
SPR1	20.8 Cc	34.7 Ab	44.5 Aa
PSL1	23.4 Bc	34.4 Ab	42.2 Aa
CNT1	32.9 Bb	31.6 BCb	35.4 BCa
KDML105	34.9 Aa	34.2 Aba	30.9 Dc
PTT1	35.1 Ab	27.9 Dc	37.1 Ba
CNT80	34.1 Aa	30.4 CDb	35.5 BCa
IR68144-2B-2-2-3	33.5 Aab	31.9 BCb	34.7 Ca
Mean	30.7	32.2	37.2
	Variety	Fraction	Variety x Fraction
F-test	ns	***	***
LSD0.05		0.01	0.03

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

Table 4.12 Proportion of grain Zn in three grain fractions; basal, middle and distal of white rice of seven varieties.

Variety	Zn distribution (%)		
	Basal	Middle	Distal
SPR1	21.1 Dc	36.9 Bb	41.9 Aa
PSL1	27.2 ABc	39.0 Aa	33.8 Db
CNT1	23.4 Cb	38.3 Aa	38.3 Ba
KDML105	26.8 Bc	34.2 Cb	38.9 Ba
PTT1	28.0 Ac	37.1 Ba	34.9 Db
CNT80	28.2 Ac	34.9 Cb	36.9 Ca
IR68144	26.4 Bc	34.6 Cb	39.1 Ba
Mean	25.9	36.4	37.7
	Variety	Fraction	Variety x Fraction
F-test	ns	***	***
LSD0.05		0.01	0.02

The lowercase and uppercase letters are used for comparison between columns and rows, respectively. The different letters are significantly different by LSD (P<0.05).

4.4 Discussion

In chapter 3, I found foliar Fe, Zn and N fertilizers to rice panicles can improve rice grain nutritional quality but cannot decrease percent broken rice. The previous study reported that broken rice grain had more Fe than those in the whole grain (Prom-u-thai *et al.*, 2009b), however, there is currently no available information on the Fe and Zn distribution along the grain length. In this chapter, I found the available information on the Fe and Zn distribution of different broken rice components that cause broken rice can sometimes be higher Fe and Zn than in whole grain rice.

From the results there was considerable variation in Fe and Zn concentrations among the grain parts of rice in both brown and white rice. The Fe and Zn concentration of most varieties were higher in basal and/or distal fractions than the middle depend on rice varieties, therefore, it might be suggested that rice grain originally have higher Fe and Zn in the tip part. The transportation of Fe and Zn to rice grain was reviews. Many studied reported that the Fe and Zn concentrations in rice grain originally from grain developing stage. Jiang *et al*. (2007) noted that most of Zn in rice grain originated from root uptake after flowering. However, other studies reported that Zn concentration in rice grain could increase by Zn foliar application which might be suggested that Zn could re-translocated in plant in late developing stage that might be important for Zn content in rice grain (Wissuwa *et al.*, 2008). The Zn in the rice grains and partly in the husks might be supplied via the phloem after mobilization from the blades of the flag and upper leaves and also by xylem-to-phloem transfer in the nodes while Fe stored in the flag and upper leaves may be transported to the grains via the phloem (Yoneyama *et al.*, 2010) to rice panicle then rice grain. The developing grain is connected to the maternal plant by a single vascular bundle trace which along the grain from through the pericarp (Krishnan and Dayanandan, 2003). Nutrients loading into rice grain are thought to move in this vascular stand in dorsal side (Oparka and Gates, 1981). Nutrients are distributed to the tissue surrounding the grain and effluxes into apoplast in grain (Krishnan and Dayanandan, 2003). From the results, the higher concentration of Fe and Zn in basal and distal parts than the middle fraction might be caused by the efflux of nutrients are not eventually or the path way of Fe and Zn transport into rice grain may connect at the basal and/or tip of rice grain. The further study requires investigating the pathway of Fe and Zn accumulation in rice grain during assimilation processes in relation with high nutrients accumulation in broken grain.

Milling generally depressed the concentrations of Fe and Zn by removing the embryo and outer layers (including pericarp, teata, nucellus epidermis and aleurone layer) which have high Fe and Zn (Saenchai *et al.*, 2012). In addition, degree of milling that influenced by grain morphology and milling duration are also affected on the depression of these two minerals (Liang *et al.*, 2008; Prom-u-thai *et al.*, 2007a; Saenchai *et al.*, 2012). From the results, milling depressed grain Fe up to 60%, while grain Zn was depressed not over 24% in all grain fractions. This results extent those of previous works, reported that rice grain Fe was depressed by milling up to 85% (Prom-u-thai *et al.*, 2007b; Resurrection *et al.*, 1979; Saenchai *et al.*, 2012; Villareal *et al.*, 1991) and up to 58% for Zn (Ren *et al.*, 2006; Saenchai *et al.*, 2012; Villareal *et al.*, 1991). It is not surprising to find Fe concentration is more depressed than Zn, it has been reported that Fe in non-transgenic rice is most abundant in the embryo and endosperm but not in endosperm (Sellappan *et al.*, 2009; Sivaprakash *et al.*, 2006), while Zn is localized in endosperm of brown rice up to 75% (Jiang *et al.*, 2008; Saenchai *et al.*, 2012). Moreover, the investigation by using Perls Prussian blue for Fe and Diphenylthiocabazone (DTZ) staining found these two minerals allocated in the peripheral layer of brown rice and Zn was also found in endosperm (Krishnan *et al.*, 2003; Prom-u-thai *et al.*, 2010; Sellappan *et al.*, 2009; Sivaprakash *et al.*, 2006)

The proportion of grain Fe and Zn contents in brown rice of all varieties were particular higher in the basal and distal fractions. It can be suggest that the Fe and Zn contents in the basal were make up by highly concentrated in the embryo and outer layer while the distal fraction may make up by more outer layer than the middle fraction. On the other hand, the Fe partitioning in white rice cannot specify the highly Fe fraction, it depend on variety. The lack correlation between grain Fe of brown and white rice has been reported (Prom-u-thai *et al.*, 2007b) supports this result. It does not know if the Fe and Zn are distributed equally around the grain of brown rice or whether it might be more concentrated on the dorsal segment nearest to the vascular tissue. However, Zn in white rice remains higher in the distal that might be understand that Zn is originally high in the distal fraction even it has lower individual grain dry weight than the middle fraction (data not show).

From this study, broken rice had higher proportion of basal and distal fractions than the middle fraction. It is evident that these two fractions are the key to make up Fe and Zn concentrations in broken rice, therefore, it could be suggested that broken rice can sometimes has higher Fe and Zn than whole grain depends on the grain fractions that make up most of the broken rice as well as how the variety differ in the nutrient concentration in its different grain fractions would determine concentration of the nutrients in broken rice. Theses can directly be the benefit for rice consumers who face Fe and Zn deficiency problems and consume broken rice as the staple food. For example, if consuming 300 g broken white rice per day per person (average rice consumption by Thai people) can provide Fe and Zn up to 21% and 78% of Thai Recommended Daily Intake, respectively. Moreover, this is great information for utilize broken rice for food industry. Because of it low price compared with whole grain, broken rice usually ends up as raw material in food industry such as noodle, various snacks, crackers, breakfast cereals (Bond, 2004) and might be used in complementary foods for infants (Chitpan *et al.*, 2005). Therefore, the use of rice flour prepared from broken rice with high Fe and Zn concentration varieties would be to promising ways to reduce the incidence of Fe and Zn deficiency in rice consumers in developing countries.

In conclusion, this study has found the Fe and Zn concentrations in broken rice can sometimes be higher than in whole grain rice but not always. The grain fractions that make up most of the broken rice as well as how the variety differ in the nutrient concentration in its different grain fractions would determine concentration of the nutrients in broken rice.