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Original Research Article

Content of some minerals and their bioavailability in selected popular rice varieties from Bangladesh

Sonia Tamanna[1], Sayma Parvin[1], Sanjay Kumar[1], Alak K Dutta[1], Aysha Ferdoushi[1], M Ali Siddiquee[2], Sunil K Biswas[2] and M Zakir H Howlader[1*]

[1]Department of Biochemistry and Molecular biology, University of Dhaka, Dhaka-1000,
[2]Bangladesh Grain quality and Nutrition Laboratory, Bangladesh Rice Research Institute (BRRI), Gazipur, Dhaka, Bangladesh
******Corresponding author e-mail:* hhzakir@yahoo.com

A B S T R A C T

Rice grain is the staple food for over half of the world's population especially in the developing countries as such, is an important source of essential minerals. Mineral bioavailability in rice could be improved by the selection of varieties with high Fe, Zn, Ca and Se as well as low PA content. Therefore, the study was undertaken to investigate the variations of Fe, Zn, Ca, Se and PA content in some popular high yielding BRRI varieties consumed in Bangladesh as well as to estimate the potentially inhibiting effect of phytate on the bioavailability of iron, zinc, calcium and selenium by measuring their molar ratios. The study also investigated the effect of morphology of rice kernels on these parameters. For this purpose, we elected 8 BRRI (Bangladesh Rice Research Institute) developed varieties (BR-3, BR-11, BR-16, BRRI Dhan-26, BRRI Dhan-28, BRRI Dhan-29, BRRI Dhan-30, BRRI Dhan-40 and two local varieties (Pajam and Chinigura) and categorized as parboiled and unparboiled on the basis of parboiling and thus the effect of parboiling was also studied. The mean value of phytic acid content for parboiled and unparboiled group was found to range between 4.25 to 6.65 mg/g and 4.05 to 6.35 mg/g, respectively. The highest amount of phytic acid content was found in BR-3 (6.65 ± 0.27 mg/g) and BR-11 (6.65 ± 0.05 mg/g) among parboiled samples. The range of iron and zinc content in the parboiled group were 0.017 to 0.044 mg/g, 0.022 to 0.070 mg/g and that in the unparboiled group were 0.020 to 0.044 mg/g, 0.015 to 0.074 mg/g respectively. The zinc content varies greatly from rice to rice. The results of the mineral contents and PA content can be interpreted in terms of expected bio-availability. Despite the variation in mineral contents, in all cases the PA present is expected to render most mineral present unavailable. And thus may impair the bioavailability of the mineral in the body.

Keywords

Phytate;
Iron;
Zinc;
Calcium and Selenium bioavailability;
BRRI varieties;
molar ratios.

Introduction

Rice grain is the staple food for more than half of the world's population and grows in more than 100 countries. China, India, Indonesia, Bangladesh, Thailand, and Vietnam produce about 80 percent of the world rice production. Among the leading rice growing countries of the world, Bangladesh ranked fourth of in both rice area and production. But the position of Bangladesh is more critical due to highest population density in a situation where majority of rice growing areas are rainfed and flood pron. In Bangladesh, rice accounts for 95 percent of the food grain production. Rice production in Bangladesh was less than 10 million tons in 1970 and then exceeded 20 million tons in 1999. In Asia, it serves as the major source of energy, protein, thiamine, riboflavin, niacin, iron (Fe) and calcium (Ca) in the diet (Juliano,1997).Rice is also the major source of intake of micro-nutrients such as zinc, iron, calcium, selenium and vitamins for Bangladeshi people specially those who live in the urban countryside. Unfortunately, rice does not furnish minerals adequately. It only has limited contents of Fe and Zn, and moreover the loss of minerals, particularly of Fe, during rice milling is high (Doesthale *et al.*, 1979). In addition, rice contains phytic acid (PA), the most important anti-nutritional factor impeding availability of divalent minerals (Jianfen *et al*, 2007). It forms complexes with mineral ions, such as Fe, Zn and Ca, and ultimately affects their bio-availability (Gibson RS *et al.*, 2000; Lucca P *et al.*, 2001; Mendoza C, 2002). Phytic acid is also able to form complexes with proteins and thus impairs digestibility and bioavailability of proteins in seeds (Reddy *et al.*, 1982). In most cases, PA to Zn molar ratio in foods is considered a predictor of Zn bioavailability. Ratios above 20 to 30 have been reported to reduce Zn absorption and growth of animal (Oberleas and Harland, 1981; Solomons, 1982). Although rice is one of the most predominant cultivated cereals and has a substantial effect on the nutritional status of the Bangladeshi people, research on rice in Bangladesh has until now been focused mainly on yield, macronutrients such as protein and starch, and sensory quality. In Bangladesh, micronutrient malnutrition, apart from protein energy malnutrition and, is one of the prime concerns along with an increasing trend of diabetes and urban obesity. It is well established that malnutrition is a health concern, but its solution lies more on food based actions rather than on other preventive or curative approaches. Food based approach, though not suitable for treatment purposes, constitute the most desirable and sustainable methods of preventing malnutrition. To achieve food-based actions to prevent malnutrition, nutrient contents of foods must be known.

In Bangladesh, many high yielding variety of rice are produced to achieve higher productivity, however, the nutrient content of these new variety of rice are not yet been explored. There is no doubt that the bioavailability of dietary minerals in diets in many developing countries including Bangladesh is very low, due to an excess of inhibitors of mineral absorption especially phytate and a low intake of absorption promoters. Therefore, the present study is designed to investigate the nutritive value, especially the mineral (Fe, Zn, Ca and Se), protein and phytate contents of these varieties of rice as a partial effort to explore the relationship between the phytate contents of rice and mineral absorption and also to estimate the inhibitory effect of phytate on the bioavailability of iron, zinc, calcium and selenium in some Bangladeshi rice varieties by measuring their molar ratios.

Materials and Methods

Collection and Preparation of Samples

Ten varieties of paddy were collected on many occasions from the Bangladesh Rice Research Institute (BRRI), Gazipur. A portion of collected every variety of paddies was run through a process of parboiling. Thus collected varieties were divided into two groups- parboiled (PB) and unparboiled (UPB), on the basis of parboiling except the Chinigura for which only unparboiled sample was used. The husk of the paddy was removed by using blender and paddy was converted to rice. These rice grains were used as raw sample in different biochemical analysis.

Measurement of kernel dimensions

Length and breadth was measured using a slide calipers and L/B ratio was determined by dividing length with breadth. For every grain, the maximum length and breadth were measured. Ten grains were measured for each variety, and the average was reported.

Estimation of minerals

Extreme caution was taken to avoid the contamination of glassware and reagent used for mineral analysis. All apparatus were soaked overnight in 2% nitric acid followed by washing with deionized water and oven dry. Deionized water was used for mineral analysis throughout the procedure (Minami *et al*., 2004). Five gram of sample was digested using 25 ml acid mixture of H_2SO_4, $HClO_4$ and HNO_3 (0.5:1.0:0.5) by volume. The digestion continued in an electro thermal resistant high neck volumetric flask at approximately 200ºC until the solution was not clear (slightly greenish). This solution was then cooled and transferred to a test tube. The volume of the solution was made 10 ml using deionized water. A reagent blank was run with the sample in the same way. After dilution, the concentration of Fe^{++}, Ca^{++}, Zn^{++} and Se were determined by the use of Chemito atomic absorption spectrophotometer, 201. The wavelength used for Fe^{++}, Ca^{++}, Zn^{++} and Se were 248.3, 422.7, 213.9 and 196 nm respectively. The samples were analyzed against a calibration curve prepared by standard solution of each element.

Estimation of Phytic Acid content of Rice

The Phytic acid and Phytic acid chelates react with ferric chloride and forms ferric phytate. The available ferric ion after reaction is determined by developing blood-red color with potassium thiocyanate. (Wheeler and Ferral, 1971).

Determination of molar ratio of phytate/ mineral

The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/ mol; Ca: 40 g/mol; Se: 79g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals.

Statistical analysis

Results were expressed as mean ± SEM (standard error mean) and as data analyses were carried out using the Statistical package for Social Sciences (SPSS) (version 17.0 for Windows, SPSS Inc., Chicago, USA). The statistical method used was student's *t*-test (two tailed) and analysis of variance (ANOVA) analysis. Differences were considered significant with $p < 0.05$.

Results and Discussion

All ten rice varieties were analyzed for protein, phytate and mineral content as well as their molar ratio. Table 1 presents the protein, phytate and mineral content in all rice varieties.

The mean value of phytic acid content for parboiled and unparboiled group was found to range between 4.25 to 6.65 mg/g and 4.05 to 6.35 mg/g, respectively. The highest amount of phytic acid content was found in BR-3 (6.65 ± 0.27 mg/g) and BR-11 (6.65 ± 0.05 mg/g) among parboiled samples. They also represent the highest amount between parboiled and unparboiled group whereas BR-3 alone showed the highest content (6.35 ± 0.15 mg/g) in the unparboiled group.

The lowest amount of phytic acid content in the parboiled group was found in BRRI Dhan-28 which was 4.25 ± 0.05 mg/g. In the unparboiled group Chinigura and BRRI Dhan-28 had the lowest content (4.05 ± 0.05). They also showed the lowest content between parboiled and unparboiled group. Parboiled rice samples were found to possess higher amount of phytic acid than the corresponding unparboiled one. Iron content varies gently from one variety to another. The range of iron content in the parboiled group was 0.017 to 0.044 mg/g and that in the unparboiled group was 0.020 to 0.044 mg/g. The maximum concentration of iron was found in Pajam among the parboiled sample and in the unparboiled group BR-3 represented the maximum.

The minimum concentration was found in BRRR Dhan-28 among the parboiled samples and BR-16 showed the minimum in the unparboiled group. The zinc content varies greatly from rice to rice. Zinc content of analyzed rice varieties were found to be in the range of 0.022 to 0.070 mg/g for parboiled and 0.015 to 0.074 mg/g for unparboiled group. Pajam showed the highest amount of zinc content among both parboiled and unparboiled group which were 0.070 and 0.074 mg/g respectively.

The lowest amount of zinc content was found in BR-11 (0.022mg/g) among parboiled samples and in BR-26 (0.015 mg/g) among unparboiled samples. Rice is also a good source of calcium. The range of calcium content in the parboiled group was 0.013 to 0.023 mg/g and that in the unparboiled group was 0.017 to 0.032 mg/g. The maximum concentration of calcium was found in BR-3 (0.023 mg/g) among the parboiled sample and, in the unparboiled group, BRRI Dhan-28 (0.032 mg/g) represented the maximum. The minimum concentration was found in BR-11 for both the parboiled (0.013 mg/g) and unparboiled (0.017mg/g) group. An increasing trend of calcium content was shown to be found in the unparboiled group.

The selenium content also varies greatly from rice to rice. Selenium content of analyzed rice varieties were found to be in the range of 0.011 to 0.023 μg/g for parboiled and 0.013 to 0.025 μg/g for unparboiled group. BRRI Dhan-29 showed the highest amount of selenium content (0.023 μg/g) in the parboiled group and chinigura (0.025μg/g) showed the highest in the unparboiled group. The lowest amount of selenium content was found in BR-16 (0.011 μg/g) among parboiled samples and in BRRI Dhan-28 (0.013 μg/g) among unparboiled samples.

Table.1 Phytate, iron, zinc, calcium, selenium and protein content of the rice varieties analyzed.

Rice Varieties	Phytate content (mg/g)	Iron content (mg/g)	Zinc content (mg/g)	Calcium content (mg/g)	Selenium content (µg/g)
BR-3(P.B)	6.65 ± 0.27	0.039	0.022	0.023	0.016
BR-3(U.P.B)	6.35 ± 0.15	0.044	0.037	0.026	0.016
BR-11(P.B)	6.65 ± 0.05	0.031	0.022	0.013	0.022
BR-11(U.P.B)	6.30 ± 0.20	0.026	0.026	0.017	0.015
BR-16(P.B)	5.35 ± 0.15	0.029	0.025	0.019	0.011
BR-16 (U.P.B)	5.35 ± 0.15	0.020	0.025	0.024	0.017
BR-26(P.B)	5.46 ± 0.14	0.029	0.024	0.019	0.022
BR-26 (U.P.B)	5.35 ± 0.05	0.029	0.015	0.025	0.021
BRRI Dhan-28(P.B)	4.25 ± 0.05	0.017	0.024	0.022	0.016
BRRI Dhan-28 (U.P.B)	4.05 ± 0.05	0.026	0.042	0.032	0.013
BRRI Dhan-29(P.B)	5.5 ± 0.30	0.028	0.026	0.016	0.023
BRRI Dhan-29 (U.P.B)	5.25 ± 0.25	0.026	0.030	0.018	0.023
BRRI Dhan-30(P.B)	4.68 ± 0.29	0.030	0.036	0.021	0.019
BRRI Dhan-30(U.P.B)	4.65 ± 0.20	0.033	0.022	0.023	0.013
BRRI Dhan-40(P.B)	4.55 ± 0.25	0.029	0.053	0.019	0.018
BRRIDhan40(U.P.B)	4.25 ± 0.15	0.040	0.061	0.018	0.017
PAJAM(P.B)	5.0±0.20	0.044	0.070	0.019	0.019
PAJAM (U.P.B)	4.95 ± 0.05	0.0212	0.074	0.019	0.021
CHINIGURA(U.P.B)	4.05 ± 0.05	0.029	0.074	0.020	0.025

Data are presented as mean ± SEM (standard error of mean) of two readings in case of Phytate, (P.B) -parboiled and (U.P.B) – Unparboiled.

Molar ratios of phytic acid, iron, zinc, calcium and selenium in different rice varieties

In order to predict the inhibitory effect of phytate on iron, zinc, Calcium and Selenium bioavailability from different rice varieties, phytate/iron, phytate/zinc, phytate/calcium and phytate/selenium molar ratios were calculated (Table 2). Phytate/iron molar ratio >1 is an indication of poor iron bioavailability and phytate/zinc molar ratio >15 is regarded as been associated with reduced zinc absorption and negative zinc balance (Turnlund et al., 1984; Hallberg et al., 1989). The critical molar ratio, above which ion absorption may be impaired, has been determined by several authors at PA:Ca >1.56, PA:Fe >14 and PA:Zn >10 (Saha et al.,1994; Lopez et al.,1998). Molar ratios of phytate to iron, phytate to zinc, phytate to calcium and phytate to selenium of different rice varieties ranged from 8.8 to 21.18, 5.65 to 30.32, 7.68 to 31.98 and 19.77 to 56.27 respectively. All these ratios are above the critical values, suggesting that absorption of iron, zinc, calcium and selenium from different rice varieties in this study might be very poor. Molar ratio of phytate to iron should be ideally decreased to <0.4 to attain adequate iron bioavailability (Hurrell et al., 2004). According to Lestienne et al. (2005b), molar ratio of phytate to iron is not a sufficient indicator of iron availability if the level of antinutritional factors other than phytate is high. According to the model of Wolters, the availability of Fe in Chinese brown rice would be 4.0–6.5% if only taken into account the effect of PA. Since rice also contains other complex forming substances, such as dietary fibre, and does not contain enhancers of Fe availability, the bioavailability of Fe in rice in Bangladesh will probably be even lower. The mean molar ratio of PA to Zn in the different Bangladeshi rice varieties is 17.64, which is higher than the critical value determined by several authors. There is some correlation (r = -0.501, P < 0.05) between the levels of PA and Zn in rice. Davies and Olpin mentioned that regardless of the absolute levels of Zn and PA, the ratios of PA to Zn would be the major determinant of Zn availability. Marginal Zn deficiency in rats appeared at a ratio of PA to Zn of 10–15. These data suggest that molar ratio of PA to Zn in Bangladeshi rice must be reduced significantly to increase bio-availability of Zn.

Effect of the morphology of rice kernels on the content of PA, Fe, Zn, Ca and Se

The distribution of minerals and PA in rice kernels is not homogenous. In general, the bran contains higher levels of Zn, Fe, as well as PA (Doesthale YG etal; 1979).Since the kernel size and shape could also relate to the ratio of volume to surface area, we analysed the possible effect of the morphology of rice kernel on the content of minerals and PA. The morphology of a rice kernel can be described by several parameters, such as length, breadth, Length to breadth ratio. The properties of the collected samples are presented in Table 3.

Uniformity in shape and size is considered as the first quality characteristics of rice. The length and breadth varies differently for different varieties. The length of collected varieties ranged from 3.93 mm to 6.70 mm whereas breadth varied from 1.82 mm to 2.42 mm. The highest length (6.70 mm) was observed in BR-16 followed by BR-26 (6.54 mm). The highest breadth (2.4mm) was observed in

Table.2 The molar ratios of PA to Fe, Zn, Ca and Se

Parameters	**PA/Fe**	**PA/Zn**	**PA/Ca**	**PA/Se**
Average	15.26±0.879	17.64±2.0465	16.032±1.202	35.28±2.224
Range	8.8-21.18	5.65-30.32	7.68-31.98	19.77-56.27
Correlation between the two components (Pearson correlation, r)	0.263	-0.501*	-0.295	0.030

Data are presented as mean ± SEM (standard error of mean)
* Correlation is significant at the 0.05 level (2-tailed) (P < 0.05).

Table.3 Grain quality characteristics of collected rice varieties.

Varieties	Milling Yield (%)	Length (mm)	Breadth (mm)	L/B ratio	Size and Shape
BR-3	70.8	5.88	2.42	2.43	MB
BR-11	72.3	5.43	2.10	2.59	MB
BR-16	71.8	6.70	2.05	3.27	LS
BR-26	70.9	6.54	1.82	3.59	LS
BRRI Dhan-28	71.7	6.19	1.89	3.28	LS
BRRI Dhan-29	71.0	6.06	1.91	3.17	LS
BRRI Dhan-30	72.3	6.01	2.07	2.90	LB
BRRI Dhan-40	71.3	5.32	2.42	2.20	MB
PAJAM	70.0	4.50	2.03	2.22	SB
CHINIGURA	69.0	3.93	1.92	2.05	SB
MB: Medium bold, LS: Long slender, LB: Long bold, SB: short bold.					

BR-3 and BRRI Dhan-40 followed by BR-11 (2.10 mm) (Table 3). The lowest length and breadth were measured in Chinigura (3.93 mm) and BR-26 (1.82 mm), respectively. The list showed that the L/B ratio of tested rice varieties ranged between 2.05 to 3.59. Highest length breadth ratio was measured in BR-26 (3.59). The lowest was Chinigura (2.05). Milling yield is the measure of rough rice performance during milling. Milling yield of the ten rice varieties were satisfactory. Highest milling yields (72.3%) were found in BR-11 and BR-30 and other rice varieties had more or less similar milling yield (69.0-71.8%). Less than 67% milling yield is not acceptable. All the tested varieties gave higher milling yield (Table 3).

Although PA, Fe, Ca and Se levels in different Bangladeshi rice varieties did not show a significant relation with L/B ratio, Zn level had a significant relation with the ratio L/B and length at the 0.01 level. So, these results further confirmed that the shape of the kernels has a significant effect on the levels of Zn in Bangladeshi rice. This could be due to the influence of factors such as soil, climate, and duration of growth period. We also observed a correlation between length, x, in millimeters, of rice kernels and yield factors, *Y*, in %, as follows: Y=0.824x +66.449(R^2= 0. 497)

The relation of PA, Fe, Zn, Ca and Se levels with the kernel length is presented in Table 4 and shows that the mean value of PA content tends to increase with kernel length, although not statistically significant.

Table 4 also demonstrates the wide variation of Fe content in Bangladeshi rice. It appears that the variability in the short-grain rice is slightly lower than in the medium grain rice and slightly higher than long grain rice.

Table.4 Phytic acid, iron, zinc, calcium and selenium in short-, medium- and long-grain rice

Kernel Shape	**Length (mm)**	**L/B ratio**	**PA content (mg/g)**	**Fe content (mg/g)**	**Zn content* (mg/g)**	**Ca Content (mg/g)**	**Se content (µg/g)**
Short - grain	4.22±0.285	2.1350±0. 085	4.67±.309	0.032±.007	0.073±.001	0.019±.000	0.022±.001
Medium - grain	5.5433±0.171	2.4067±0.113	5.79±.446	0.0352±.003	0.037±.007	0.019±.002	0.017±.001
Long-grain	6.30±0. 136	3.2420±0.111	4.99±.169	0.027±.002	0.027±.002	0.022±.001	0.018±.001

Data are presented as mean ± SEM (standard error of mean)
*Based on one –way ANOVA test significant differences were found only for zinc content. (P< 0.01).

The mean value of Zn levels in rice of different kernel lengths (Table 4) reveal average Zn contents of short-, medium- and long-grain rice to be 0.073, 0.037, and 0.027mg/g respectively. The mean levels differ significantly (P < 0. 01). The Zn content is also correlated with kernel length and L/B ratio (P < 0. 01).

From this study it was conclude that the levels of Fe, Zn, Ca and Se in BRRI developed rice varieties are very diverse. This may be both due to varietal and environmental effects. In principle, rice has the potential to furnish an adequate intake of Fe, Zn, Ca and Se. However, the bio-availability of Fe, Zn, Ca and Se is very low because of the presence of phytic acid, even in rice varieties with the lowest PA levels and highest levels of Fe, Zn, Ca and Se. Our study suggests that there is a need to minimize mineral losses during milling and polishing and to maximize the bio- availability of minerals by dephytinisation.

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