

This is a "Post-Print" accepted manuscript, which has been published in
"International Journal of Food Sciences and Nutrition".

Please cite this publication as follows:

Liang, J., Han, B.Z., Nout, M.J.R., Hamer, R.J. *In vitro* solubility of calcium, iron and zinc in relation to phytic acid levels in rice-based consumer products in China. *International Journal of Food Sciences and Nutrition* 61 (1), 2010, 40-51.

You can download the published version at:

<http://dx.doi.org/10.3109/09637480903229017>

1 **In vitro solubility of calcium, iron and zinc in relation to phytic acid levels in rice-based**
2 **consumer products in China**

3
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7
8 **Abstract**

9 *In vitro* solubility of calcium, iron and zinc in relation to phytic acid (PA) levels in 30 commercial rice-based
10 foods from China were studied. Solubility of minerals and molar ratios of PA to minerals varied with degrees of
11 processing. In primary products, [PA]/[Ca] values were less than 5, [PA]/[Fe] and [PA]/[Zn] were similarly
12 ranged by 5-74 with most values between 20 to 30. [PA]/[mineral] molar ratios in intensively processed products
13 were lower. Solubility of calcium ranged in 0-87%, with the lowest in brown rice (12%) and the highest in infant
14 foods (50%). Iron solubility in two thirds of samples was lower than 30%, and that of zinc narrowly ranged in 6-
15 30%. Solubility of minerals was not significantly affected by [PA]/[mineral]. At present, neither primary nor
16 intensively processed rice-based products are good dietary sources of minerals. Improvements should be
17 attempted by dephytinization, mineral fortification or, or preferably, combination.

18
19 **Key words:** Calcium, iron, zinc; phytic acid, rice-based food

20 **Running title:** Minerals and phytic acid in rice-based foods

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30 INTRODUCTION

31 Rice is a major food cereal in China and about 95% of harvested rice is used for consumer food products either
32 as white rice or as processed foods (e.g. noodles) (FAO, 2006). Rice serves as the major dietary source of energy,
33 protein, thiamine, riboflavin, niacin, iron and calcium (Juliano, 1997; Kennedy et al., 2002). It was reported that
34 in China, during the period 1997-2001, rice products supplied about 30-40% of the dietary energy intake (FAO,
35 2004; Kennedy et al., 2002). In 2002 and 2003, intake of rice and rice products in China was 238 and 215
36 g/capita/day, respectively, which supplied 35% of total energy intake (FAO, 2006; Wang, 2005). This number is
37 even higher in rural areas: estimates from 2002, give an intake of rice and rice products of 246 g/capita/day.

38 In China, iron deficiency related anaemia is quite common: it affects about 30% of pregnant and lactating
39 women. This situation did not improve by changes of the dietary structure (increased consumption of legumes
40 and vegetables) aimed at increasing iron intake (Wang, 2005). The main reason for iron deficiency anaemia and
41 mineral malnutrition in China and other predominantly rice consuming countries is related to the poor
42 bioavailability of iron and other important micronutrients (calcium, zinc). Ma recently reported that, while
43 Chinese rely for 60% of their mineral intake on rice and other plant derived foods, the presence of phytate, a
44 very potent inhibitor of mineral bioavailability, causes a low bioavailability of the minerals concerned (Ma,
45 2007). In order to predict the bioavailability of minerals, molar ratios of phytic acid to minerals have been used
46 as indicator (Adeyeye et al., 2000; Fordyce et al., 1987; Grewal et al., 1999; Hira and Kaur, 1993; Ma et al.,
47 2005; Perlas and Gibson, 2002). Bioavailability levels could also be related to the amount of minerals recovered
48 after in vitro digestion of products with gut enzymes (Glahn et al., 2002; Kiers et al., 2000; Larsson et al., 1997).
49 Ma (2007) proposed that at a molar ratio of $[PA]/[Fe] > 1$, iron uptake was inhibited. In earlier studies we reported
50 average values of $[PA]/[Fe]$ of 50 in brown rice (predicted bioavailability <5%). Perlas and Gibson mentioned
51 that 15 is the critical value of $[PA]/[Zn]$ for zinc bioavailability and gave changes of these ratios after soaking
52 (Perlas and Gibson, 2002). Although increasingly, information on prediction of the bioavailability of trace
53 elements in unprocessed products becomes available, information for in processed products is still scant. In a
54 previous paper, we demonstrated that especially wet processing like fermentation could lead to a substantial
55 reduction in phytate levels (Liang et al., 2007).

56 According to the extent of processing, we distinguished primary and intensively processed rice-based products.
57 Primary processed products include brown, white (or polished) and germinated rice and still have the kernel
58 shape. Of these, white rice is the most important consumer product. Although brown rice and germinated rice
59 contain higher levels of nutrients, they are not popular with the public because of their darker colour and

60 unaccepted sensory properties (Huang, 2004). Intensively processed products include rice noodles, rice crackers
61 and rice-based infant foods, and mainly originate from further processing of white rice. Whereas several studies
62 addressed process innovations for improved sensory quality (Lu et al., 2005; Park et al., 2001; Wang et al., 2003;
63 Zhu, 1990), only Ma included several rice products in a survey of minerals and phytic acid in common Chinese
64 foods (Ma et al., 2005). In previous studies we reported the natural variation in phytate and mineral levels in
65 Chinese rice (Liang et al., 2007), and the efficacy of dry fractionation and wet processing in improving mineral
66 bioavailability (Liang et al., 2008; Liang et al., 2008b).

67 With the present study, we aim to gain understanding of the effect of standard commercial processing on the
68 mineral contents and bioavailability of commercial rice products. To this end we collected 30 representative rice-
69 based products from commercial outlets in China. All samples were analyzed for their contents of minerals and
70 phytic acid. We also assessed the in vitro solubility of minerals after enzymatic digestion. The objectives were to
71 1) analyze levels and in vitro solubility calcium, iron and zinc in different rice-based products and their relation
72 to levels of phytic acid; and 2) evaluate the suitability of rice products as dietary sources of minerals or as
73 carriers for mineral fortification.

74

75 **MATERIALS AND METHODS**

76 **Sample collection**

77 Rice-based solid products with a shelf-life longer than 6 months were used for study. 30 commercial products
78 were selected and purchased at three supermarkets in Beijing. A description of these products is presented in
79 table 1.

80 [Insert Table 1 here]

81 **Contents of total and in vitro soluble minerals**

82 For analysis of total contents of calcium, iron and zinc, 1 g (accuracy 0.0001 g) sample was wet digested with
83 nitric acid (HNO₃, 65 %) and perchloric acid (HClO₄, 60 %) following the procedure of the AOAC 975.03
84 (Horwitz, 2000). In vitro soluble minerals were measured in the supernatant after enzymatic digestion of
85 suspended food samples. Enzymatic digestion followed the procedure of Kiers et al. (2000). After digestion, the
86 reaction mixture was centrifuged at 3600 g at 4 °C for 15 min and the supernatants were filtered through a 0.45
87 µm membrane. Calcium, iron and zinc in acid digests and supernatants of enzymatic digests were determined
88 with an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 2000, Perkin-Elmer)
89 (Bentsink et al., 2003). During analysis, the sample flow rate was 1.5 mL min⁻¹. All samples were digested and
90 analyzed in triplicates.

91 **Contents of phytic acid**

92 Phytic acid contents of the product were analysed in triplicate by spectrophotometric detection with ferric
93 chloride (FeCl₃) and sulfosalicylic acid after extraction, separation on anion exchange resin following the
94 procedures described by Ma et al. (Ma et al., 2005).

95 **Moisture Contents**

96 Moisture contents in collected samples were analysed following AOAC official method 4.1.09A.

97 **Statistical analysis**

98 Data were analyzed with SPSS 10.0 for windows. Significance was tested at a 5% level using an independent-
99 samples *t*-test.

100

101

102 **RESULTS**

103 **Phytic acid, calcium, iron and zinc, and molar ratios of phytic acid to minerals**

104 [Insert Figure 1 here]

105 Phytic acid, calcium, iron and zinc levels of all samples were mapped in figure 1. In this figure, samples could be
106 clustered into three groups a, b and c as follows:

107 **Group a: low concentrations of both phytic acid and minerals:** This group had phytic acid levels lower than 3.7
108 mg g⁻¹ (wet weight) and calcium, iron and zinc levels below 66, 3.1 and 1.9 mg 100g⁻¹, respectively. Twenty-two
109 samples, including all white rice samples, rice noodles and rice crackers, can be found in this group.
110 Concentrations of calcium were in the range of 2-66 mg 100g⁻¹. They were lower than 20 mg 100g⁻¹ in sixteen
111 samples and ranged from 22-66 mg 100g⁻¹ for the other six samples. Sixteen samples had concentrations of iron
112 in the range of 0.6-1.5 mg 100g⁻¹, and the other six contained 1.5-3.1 mg 100g⁻¹. Eighteen samples had
113 concentrations of zinc ranging from 0.8 to 1.6 mg 100g⁻¹, three in the range 1.7-2.0 mg 100g⁻¹, and one lowered
114 than 0.6 mg 100g⁻¹.

115 **Group b: low concentrations of phytic acid and high levels of minerals:** This group consisted of four infant
116 foods. Samples in this group had similar levels of minerals, which were about 450 mg 100g⁻¹, 10 mg 100g⁻¹ and
117 4 mg 100g⁻¹ for calcium, iron and zinc, respectively due to minerals fortification, and different levels of phytic
118 acid (0.9-5.9 mg g⁻¹) resulting from different pre-treated ingredients. Consumption of 50 g food from this group
119 would result in about 200-260 mg of calcium intake; 5 mg of iron and 2-3 mg of zinc; but at the same time, 50-
120 300 mg of phytic acid would intake.

121 **Group c: high concentration of phytic acid and low levels of minerals:** This group represents brown and
122 germinated rice. Levels of calcium, iron and zinc were 20-40 mg 100g⁻¹, 2-6 mg 100g⁻¹ and 2-3 mg 100g⁻¹
123 respectively, and phytic acid levels were 10-17 mg g⁻¹. Consumption of rice products from this group will result
124 in mineral intake, however, a considerable intake of phytic acid will take place at the same time.

125 [Insert Table 2 here]

126 Table 2 presents the levels of phytic acid, calcium, iron and zinc and the molar ratios of phytic acid to minerals
127 in the various product categories. Compared with the other categories, infant foods had higher levels of minerals.
128 Among the other five categories, the highest levels of calcium and zinc occurred in germinated rice, and the
129 highest levels of phytic acid and iron in brown rice. White rice had the lowest contents of calcium and iron.
130 Noodles and crackers had similar mineral levels. Phytic acid levels in noodles and crackers were similar to those
131 of white rice, about 10% of brown rice. This indicated that milling brown to white rice significantly reduced

132 phytic acid levels, but that further processing, involving soaking, fermentation and enzymatic treatment, did not
133 significantly affect phytic acid.

134 As shown in table 2, the molar ratios of phytic acid to minerals were different for the respective types of
135 minerals and rice products. In all product categories, [PA]/[Ca] ratios ranged between 0.0 and 4.6. This was
136 much lower and the range was narrower than for [PA]/[Fe] (0.2-61) and [PA]/[Zn] (0.3-74). Ratios of [PA]/[Ca],
137 [PA]/[Fe] and [PA]/[Zn] in brown rice and germinated rice were much higher than in other products. Infant
138 foods had the lowest ratio of [PA]/[Ca], likely due to the calcium added. In the category of noodles, the largest
139 variation of phytic acid to minerals ratio was observed. These variations result from diverse concentrations of
140 phytic acid and/or minerals.

141 **In vitro solubility of calcium, iron and zinc in rice products**

142 The phytate to mineral ratio provides a crude indicator for mineral bioavailability. The amount of minerals
143 solubilised after in vitro digestion of a sample is closer to the in vivo situation and may therefore be more
144 predictive for true bioavailability.

145 [Insert Table 3 here]

146 As shown in table 3, the in vitro solubility of minerals differed among the product categories. The solubility of
147 calcium ranged between 0-87%, with the lowest average (12%) in brown rice and the highest (50%) in infant
148 foods. Iron and zinc solubility ranged between 0-83% and 0-34%, respectively. Both white and brown rice
149 categories showed the highest average solubility of iron and zinc. In both primary and intensively processed
150 products, the average solubility of calcium and iron was much higher than in brown rice. In contrast, germination
151 as well as intensive processing (noodles and crackers) led to a decreased solubility of zinc.

152 [Insert Figure 2 and Figure 3 here]

153 Figures 2 and 3 show the in vitro solubility of calcium, and iron and zinc, respectively, in relation with the
154 [PA]/[mineral] ratios for each of the samples analyzed. Calcium solubility varied in a wide range of <1%
155 to >80% when the [PA]/[mineral] ratios were lower than 1. Beyond a ratio of 1, most products had a calcium
156 solubility of around 20%. A similar phenomenon was observed in model studies. The iron solubility in about two
157 thirds of the products was lower than 30%, and that of zinc had a narrow range between 6-30%. The solubility of
158 iron and zinc was not significantly affected by molar ratios of phytic acid to minerals. Figures 2 and 3 indicate
159 that mineral solubility is not exclusively determined by the molar ratio [PA]/[Fe], but that other factors also play
160 a role. Such factors may include added fortificants and the presence of food matrix components such as dietary
161 fiber and free phosphate.

162

163 **DISCUSSION**

164 According to Chinese Dietary Reference Intake (DRIs) for adults, the adequate intake (AI) of iron is 10 mg/day
165 and of calcium is 400 mg/day, and the DRI of zinc is 8 mg/day (Chinese Nutrition Society, 2006). When
166 calculated on the basis of the per capita consumption of rice and rice products of 238 g/day (Wang, 2005) and
167 the average contents of minerals found in the present study, rice-based products supply 11% of calcium AI, 38%
168 of iron AI, and 39% DRI of zinc.

169 Primary processed rice products cannot be considered as good sources of minerals for several reasons. First, the
170 solubility (as an index for bioavailability) of calcium and iron was very low in brown rice because of a high level
171 of phytic acid. Milling and polishing achieves 90% removal of phytic acid thus increasing the minerals solubility.
172 However, the levels of calcium and iron are also reduced significantly (70-80%) by primary processing. With
173 respect to calcium and iron, germination is a good way to improve their solubility (bioavailability), since both
174 the levels as well as the solubility of calcium and iron increased after germination. Enzymatic degradation of
175 phytic acid and other components chelated to minerals during steeping and germination of brown rice
176 contributed to the increase of solubility. On the other hand, for all primary products, white rice is the most
177 important staple food, while brown rice - a good source of zinc - and germinated rice are not widely consumed
178 because their sensory properties are not appreciated by most Chinese consumers (Huang, 2004).

179 Intensively processed rice products are popular with various groups of Chinese consumers, so they also have an
180 impact on the human mineral status. Rice noodles are used as staple foods, especially in Southern China (Lu et
181 al., 2005). The nature of the noodle making process makes noodles an attractive vehicle to enhance intake of
182 minerals. During noodle making, rice is soaked and fermented during periods ranging from several hours to 3
183 days, prior to noodle making. During these preliminary phases, endogenous rice phytase as well as microbial
184 phytases produced by fermentation microbiota reportedly degrade phytic acid (Umata et al., 2005), which may
185 explain the low levels of phytic acid observed in rice noodles. Differences in noodle making procedures result in
186 variations of product composition (Marfo et al., 1990). Like phytic acid, also the levels of minerals in rice
187 noodles are determined by processing procedures or/and the use of ingredients and equipment. For calcium, the
188 lowest value was caused mainly by the loss of calcium by leaching effects, while the highest levels are probably
189 due to the application of calcium-rich additives. Concerning iron, all noodles had higher iron levels than white
190 rice. We suppose this is related to low pH values occurred during noodle processing and the use of cast iron
191 processing machines. Neither equipment nor ingredients affected zinc of which the levels are similar to those in
192 white rice. Crackers are mainly consumed in small quantities as a snack and thus will not greatly affect the

193 mineral status. Compared to other products, the infant foods are more important, since they constitute an almost
194 exclusive dietary source of macro- and micronutrients, and because they are consumed in relatively small
195 quantities (Lind et al., 2003). Mineral solubility in e.g. infant foods can be improved by various strategies.
196 Enzymatic pre-treatment of white rice e.g. with phytase will significantly improve mineral solubility, as was
197 shown elsewhere with soya bean formulas (Davidsson, 2003).

198 Data presented here show an inverse relation between phytic acid levels and solubility of calcium and zinc. The
199 inadequate solubility of iron in some infant foods tested, might be due to the use of an ineffective chemical form
200 of iron used for fortification (Engle-Stone et al., 2005). In addition, interaction of minerals also affects the
201 solubility of calcium, iron and zinc (Fordyce et al., 1987). However, some studies mentioned that even the
202 combination of favourable factors such as reduction of phytic acid, sufficient fortification of iron and addition of
203 ascorbic acid (an enhancer of iron absorption) did not clearly improve iron and zinc status in infant foods
204 (Lachat et al., 2006; Lind et al., 2003; Mamiro et al., 2004). This suggests that other inhibitory factors such as
205 dietary fiber and the product matrix, may interfere with mineral uptake. Therefore, the ultimate test remains the
206 *in vivo* measure of uptake.

207 Promising approaches for the enhancement of bioavailability of minerals in rice products are to increase mineral
208 levels by supplementation or fortification, increase bioavailability through added enhancers or by removal of
209 inhibitors (Davidsson, 2003; Gibson et al., 2000) or combinations thereof. Considering the variability of iron and
210 zinc levels in rice varieties from growing regions in China (Graham et al., 1999; Liang et al., 2007), there is a
211 potential in selecting crops with maximum mineral and minimum phytic acid levels. The specific localization of
212 minerals and phytic acid also enable an optimised milling procedure resulting in maximum retention of minerals
213 and removal of phytic acid (Liang et al., 2008b). This would contribute to improved bioavailability of
214 particularly iron, zinc and calcium (Engle-Stone et al., 2005). Fortification with minerals should take into
215 account their interactions, palatability especially for iron compounds, and opportunities for enhancement by
216 ascorbic acid-rich fruit and vegetables (Davidsson, 2003; Engle-Stone et al., 2005; Kennedy et al., 2002).

217

218 **CONCLUSIONS**

219 The diversity of products and processing methods for rice offers opportunities for improvement of mineral
220 bioavailability in rice-consuming regions. From the presented results of contents and solubility of minerals, it
221 was found that primary processed rice products are poor sources of minerals, either because of low
222 bioavailability or because of their low consumer acceptance. Some intensively processed products (except infant
223 foods), e.g. rice noodles, could improve mineral nutrition via the approaches of the use of materials (ingredients)
224 and application of processing methods. Some infant foods need further processing to decrease their levels of PA
225 or other inhibitors, thus increasing mineral bioavailability.

226

227 **ACKNOWLEDGEMENT**

228 Financial support was provided by Wageningen University through the North-South Interdisciplinary Research
229 and Education Fund (INREF). We gratefully acknowledge the assistance of Lin Li and Jin Ying for mineral
230 analysis.

231

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315

316 Table 1. Commercial consumer rice products investigated

Product Category (number of samples)	Processing methods	Moisture contents (g·100g ⁻¹)		Main ingredients
		Average	Range	
Brown Rice ^a (3)	Dehulled only	11.5	10.2 - 12.7	Brown rice
Germinated rice (1)	Brown rice was germinated till the sprout length was 0.5 - 1.0 mm (Huang, 2004)	26.3	----	Germinated rice
White rice ^b (8)	Brown rice was milled and polished to remove the outer layer of brown rice and obtain nice appearance and edible quality (Ruan, 2005)	12.9	11.6 - 13.5	White rice
Short-grain (5)	----	12.8	11.6 - 13.5	White rice
Long-grain (3)	----	12.9	12.6 - 13.2	White rice
Rice noodles (7)	----	11.4	10.2 - 13.0	----
Group 1 ^c (4)	White rice soaked in water, ground with or without water, steamed, extruded, cooled and dried (Zhu, 1990)	11.9	10.7 - 13.0	Rice and water
Group 2 ^d (3)	White rice together with other materials, such as starch or soy protein soaked or not soaked in water, ground, steamed, extruded, cooled and dried	10.6	10.2 - 11.0	Rice, water, starch, amorphophallus rivieri flour and soy protein
Rice crackers (7)	Rice mixed with water, pulped, moulded, puffed, and baked (Wang et al., 2003)	2.3	1.2 - 3.5	Rice, sugar, oil, salt and soy sauce
Infant foods ^e (4)	White rice ground to powder, roasted, enzyme-treated, drum dried and formulated with other ingredients (Perez-Conesa et al., 2002; Zhao and Liu, 2004)	4.0	3.3 - 4.8	Ingredients differed from products for infants' age and manufacturers

- 317 a: all brown rice samples were short-grain
318 b: white rice samples were divided into two groups according to the length of kernel
319 c: ingredients of rice noodles are white rice and water, no other cereal materials added
320 d: other cereal materials were used together with rice
321 e: all infant foods were fortified with calcium, iron, zinc and some vitamins as stated on product labels
322

323

324 Table 2. Levels of phytic acid and minerals and molar ratios of phytic acid to minerals in rice products (dry mass basis)

Processing extent	Categories of products	Phytic acid (mg g ⁻¹)		Ca (mg 100g ⁻¹)		[PA]/[Ca]	Fe (mg 100g ⁻¹)		[PA]/[Fe]	Zn (mg 100g ⁻¹)		[PA]/[Zn]
		Average	Range	Average	Range	Range	Average	Range	Range	Average	Range	Range
Primarily processed	Brown Rice	17.5	14.9-19.4	28.6	24-33	3.1-4.6	4.2	2.6-5.8	28-61	2.6	2.2-3.0	61-74
	Germinated rice	13.1	---	38.7	---	2.1	2.2	---	52	2.8	---	47
	White rice	1.6	0.6-2.4	6.8	5-11	0.6-2.8	0.8	0.7-1.2	7-29	1.8	1.4-2.2	4-15
	Short grains	2.1	1.8-2.4	7.7	5-11	1.3-2.8	0.9	0.7-1.2	14-29	1.8	1.6-2.2	9-15
	Long grains	0.8	0.6-1.2	5.5	5-6	0.6-1.4	0.8	0.7-0.9	7-12	1.8	1.4-2.0	4-6
Intensively processed	Rice noodles	1.2	0.0-4.1	27.8	3-75	0.0-3.1	1.8	1.1-3.0	0.2-21	1.4	1.0-2.0	0.3-27
	Group 1	0.8	0.0-1.5	28.3	3-75	0.0-0.6	1.9	1.2-3.0	0.2-12	1.5	1.0-2.0	0.3-10
	Group 2	1.9	0.2-4.1	27.0	3-46	0.0-3.1	1.6	1.4-1.9	0.9-21	1.3	1.0-1.5	1.3-27
	Rice cracker	1.4	0.8-2.6	26.7	6-59	0.3-0.8	1.9	1.2-3.2	3.5-11.1	0.9	0.7-1.2	8-27
	Infant foods	2.3	0.9-5.9	455.0	408-532	0.0-0.1	10.4	9.9-11.2	0.7-5.1	5.0	4.3-6.5	2.0-12.7

325 Table 3: In vitro solubility (% of total content) of calcium, iron and zinc in rice products

Products (number of samples)	Calcium		Iron		Zinc	
	Average	Range	Average	Range	Average	Range
Brown Rice (3)	12	5.6 - 15.4	16	5.2 - 22.5	20	15.8 - 25.4
Germinated rice (1)	38	----	25	----	12	----
White rice (8)	18	0.0 - 29.7	39	0.0 - 82.8	19	13.5 - 33.5
Short-grain (4)	16	0.0 - 29.7	50	7.0 - 82.8	18	13.5 - 22.7
Long-grain (4)	21	15.8 - 29.2	19	0.0 - 46.7	21	14.2 - 33.5
Rice noodles (7)	44	28.6 - 80.4	23	0.0 - 44.0	5	1.0 - 14.4
Group 1 (4)	46	29.0 - 80.4	25	14.8 - 38.9	3	1.1 - 5.5
Group 2 (3)	34	28.6 - 49.1	21	0.0 - 44.0	7	1.0 - 14.4
Rice crackers (7)	29	3.4 - 49.4	32	0.0 - 69.7	11	0.0 - 26.6
Infant foods (4)	50	15.9 - 86.8	10	1.4 - 19.8	14	6.1 - 30.1

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327

328 **Figure captions:**

329 Figure 1: Levels of phytic acid and minerals (calcium, iron and zinc) in consumer rice products

330 Each sample was in one of the three groups as a) white rice based products; b) brown rice based products; c) minerals fortified products

331 For better comparison, contents of phytic acid and minerals in most of the white rice based products were presented in enlarged figures
332 below.

333

334 Figure 2: *In vitro* solubility of calcium in consumer rice products

335 Figure 3: *In vitro* solubility of iron and zinc in consumer rice product

336





