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

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Abstract

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

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

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

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INTRODUCTION

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

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

According to the extent of processing, we distinguished primary and intensively processed rice-based products. Primary processed products include brown, white (or polished) and germinated rice and still have the kernel shape. Of these, white rice is the most important consumer product. Although brown rice and germinated rice contain higher levels of nutrients, they are not popular with the public because of their darker colour and
unaccepted sensory properties (Huang, 2004). Intensively processed products include rice noodles, rice crackers
and rice-based infant foods, and mainly originate from further processing of white rice. Whereas several studies
addressed process innovations for improved sensory quality (Lu et al., 2005; Park et al., 2001; Wang et al., 2003;
Zhu, 1990), only Ma included several rice products in a survey of minerals and phytic acid in common Chinese
foods (Ma et al., 2005). In previous studies we reported the natural variation in phytate and mineral levels in
Chinese rice (Liang et al., 2007), and the efficacy of dry fractionation and wet processing in improving mineral
bioavailability (Liang et al., 2008; Liang et al., 2008b).
With the present study, we aim to gain understanding of the effect of standard commercial processing on the
mineral contents and bioavailability of commercial rice products. To this end we collected 30 representative rice-
based products from commercial outlets in China. All samples were analyzed for their contents of minerals and
phytic acid. We also assessed the in vitro solubility of minerals after enzymatic digestion. The objectives were to
1) analyze levels and in vitro solubility calcium, iron and zinc in different rice-based products and their relation
to levels of phytic acid; and 2) evaluate the suitability of rice products as dietary sources of minerals or as
carriers for mineral fortification.
MATERIALS AND METHODS

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

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

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

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

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

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

[Insert Figure 1 here]

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

Group a: low concentrations of both phytic acid and minerals: This group had phytic acid levels lower than 3.7 mg g\(^{-1}\) (wet weight) and calcium, iron and zinc levels below 66, 3.1 and 1.9 mg 100g\(^{-1}\), respectively. Twenty-two samples, including all white rice samples, rice noodles and rice crackers, can be found in this group. Concentrations of calcium were in the range of 2-66 mg 100g\(^{-1}\). They were lower than 20 mg 100g\(^{-1}\) in sixteen samples and ranged from 22-66 mg 100g\(^{-1}\) for the other six samples. Sixteen samples had concentrations of iron 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 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 than 0.6 mg 100g\(^{-1}\).

Group b: low concentrations of phytic acid and high levels of minerals: This group consisted of four infant foods. Samples in this group had similar levels of minerals, which were about 450 mg 100g\(^{-1}\), 10 mg 100g\(^{-1}\) and 4 mg 100g\(^{-1}\) for calcium, iron and zinc, respectively due to minerals fortification, and different levels of phytic acid (0.9-5.9 mg g\(^{-1}\)) resulting from different pre-treated ingredients. Consumption of 50 g food from this group 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, 50-300 mg of phytic acid would intake.

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

Table 2 presents the levels of phytic acid, calcium, iron and zinc and the molar ratios of phytic acid to minerals in the various product categories. Compared with the other categories, infant foods had higher levels of minerals. Among the other five categories, the highest levels of calcium and zinc occurred in germinated rice, and the highest levels of phytic acid and iron in brown rice. White rice had the lowest contents of calcium and iron. Noodles and crackers had similar mineral levels. Phytic acid levels in noodles and crackers were similar to those of white rice, about 10% of brown rice. This indicated that milling brown to white rice significantly reduced
phytic acid levels, but that further processing, involving soaking, fermentation and enzymatic treatment, did not significantly affect phytic acid.

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

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

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

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

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

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

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

Intensively processed rice products are popular with various groups of Chinese consumers, so they also have an impact on the human mineral status. Rice noodles are used as staple foods, especially in Southern China (Lu et al., 2005). The nature of the noodle making process makes noodles an attractive vehicle to enhance intake of minerals. During noodle making, rice is soaked and fermented during periods ranging from several hours to 3 days, prior to noodle making. During these preliminary phases, endogenous rice phytase as well as microbial phytases produced by fermentation microbiota reportedly degrade phytic acid (Umeta et al., 2005), which may explain the low levels of phytic acid observed in rice noodles. Differences in noodle making procedures result in variations of product composition (Marfo et al., 1990). Like phytic acid, also the levels of minerals in rice noodles are determined by processing procedures or the use of ingredients and equipment. For calcium, the lowest value was caused mainly by the loss of calcium by leaching effects, while the highest levels are probably due to the application of calcium-rich additives. Concerning iron, all noodles had higher iron levels than white rice. We suppose this is related to low pH values occurred during noodle processing and the use of cast iron processing machines. Neither equipment nor ingredients affected zinc of which the levels are similar to those in white rice. Crackers are mainly consumed in small quantities as a snack and thus will not greatly affect the
mineral status. Compared to other products, the infant foods are more important, since they constitute an almost exclusive dietary source of macro- and micronutrients, and because they are consumed in relatively small quantities (Lind et al., 2003). Mineral solubility in e.g. infant foods can be improved by various strategies. Enzymatic pre-treatment of white rice e.g. with phytase will significantly improve mineral solubility, as was shown elsewhere with soya bean formulas (Davidsson, 2003).

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

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

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

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REFERENCES


FAO. 2004. The state of food insecurity in the world, rice and food security, Rome, Italy.


Table 1. Commercial consumer rice products investigated

<table>
<thead>
<tr>
<th>Product Category (number of samples)</th>
<th>Processing methods</th>
<th>Moisture contents (g·100g⁻¹)</th>
<th>Main ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Brown rice a (3)</td>
<td>Dehulled only</td>
<td>11.5</td>
<td>10.2 - 12.7</td>
</tr>
<tr>
<td>Germinated rice (1)</td>
<td>Brown rice was germinated till the sprout length was 0.5 - 1.0 mm (Huang, 2004)</td>
<td>26.3</td>
<td>----</td>
</tr>
<tr>
<td>White rice b (8)</td>
<td>Brown rice was milled and polished to remove the outer layer of brown rice and obtain nice appearance and edible quality (Ruan, 2005)</td>
<td>12.9</td>
<td>11.6 - 13.5</td>
</tr>
<tr>
<td></td>
<td>Short-grain (5)</td>
<td></td>
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<tr>
<td></td>
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<td>12.8</td>
<td>11.6 - 13.5</td>
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<tr>
<td></td>
<td>Long-grain (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>----</td>
<td>12.9</td>
<td>12.6 - 13.2</td>
</tr>
<tr>
<td>White rice (8)</td>
<td>Brown rice was milled and polished to remove the outer layer of brown rice and obtain nice appearance and edible quality (Ruan, 2005)</td>
<td>12.9</td>
<td>11.6 - 13.5</td>
</tr>
<tr>
<td>Rice noodles (7)</td>
<td>White rice soaked in water, ground with or without water, steamed, extruded, cooled and dried (Zhu, 1990)</td>
<td>11.9</td>
<td>10.7 - 13.0</td>
</tr>
<tr>
<td>Rice crackers (7)</td>
<td>Rice mixed with water, pulped, moulded, puffed, and baked (Wang et al., 2003)</td>
<td>2.3</td>
<td>1.2 - 3.5</td>
</tr>
<tr>
<td>Infant foods c (4)</td>
<td>White rice ground to powder, roasted, enzyme-treated, drum dried and formulated with other ingredients (Perez-Conesa et al., 2002; Zhao and Liu, 2004)</td>
<td>4.0</td>
<td>3.3 - 4.8</td>
</tr>
</tbody>
</table>

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**Table notes:**

a: all brown rice samples were short-grain
b: white rice samples were divided into two groups according to the length of kernel
c: ingredients of rice noodles are white rice and water, no other cereal materials added
d: other cereal materials were used together with rice
e: all infant foods were fortified with calcium, iron, zinc and some vitamins as stated on product labels
Table 2. Levels of phytic acid and minerals and molar ratios of phytate to minerals in rice products (dry mass basis)

<table>
<thead>
<tr>
<th>Processing extent</th>
<th>Categories of products</th>
<th>Phytic acid (mg g⁻¹)</th>
<th>Ca (mg 100g⁻¹)</th>
<th>[PA]/[Ca]</th>
<th>Fe (mg 100g⁻¹)</th>
<th>[PA]/[Fe]</th>
<th>Zn (mg 100g⁻¹)</th>
<th>[PA]/[Zn]</th>
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<td></td>
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<tr>
<td>Brown Rice</td>
<td></td>
<td>17.5</td>
<td>14.9-19.4</td>
<td>28.6</td>
<td>24-33</td>
<td>3.1-4.6</td>
<td>4.2</td>
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<td>Germinated rice</td>
<td></td>
<td>13.1</td>
<td>---</td>
<td>38.7</td>
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<td>2.1</td>
<td>2.2</td>
<td>---</td>
</tr>
<tr>
<td>White rice</td>
<td>Short grains</td>
<td>1.6</td>
<td>0.6-2.4</td>
<td>6.8</td>
<td>5-11</td>
<td>0.6-2.8</td>
<td>0.8</td>
<td>0.7-1.2</td>
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<tr>
<td></td>
<td>Long grains</td>
<td>2.1</td>
<td>1.8-2.4</td>
<td>7.7</td>
<td>5-11</td>
<td>1.3-2.8</td>
<td>0.9</td>
<td>0.7-1.2</td>
</tr>
<tr>
<td>Primarily processed</td>
<td>Rice noodles</td>
<td>2.1</td>
<td>1.8-2.4</td>
<td>7.7</td>
<td>5-11</td>
<td>1.3-2.8</td>
<td>0.9</td>
<td>0.7-1.2</td>
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<td>Group 1</td>
<td>0.8</td>
<td>0.6-1.2</td>
<td>5.5</td>
<td>5-6</td>
<td>0.6-1.4</td>
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<td>0.7-0.9</td>
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<td>Group 2</td>
<td>1.9</td>
<td>0.2-4.1</td>
<td>27.0</td>
<td>3-46</td>
<td>0.0-3.1</td>
<td>1.6</td>
<td>1.4-1.9</td>
</tr>
<tr>
<td></td>
<td>Rice cracker</td>
<td>1.4</td>
<td>0.8-2.6</td>
<td>26.7</td>
<td>6-59</td>
<td>0.3-0.8</td>
<td>1.9</td>
<td>1.2-3.2</td>
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<tr>
<td>Intensively processed</td>
<td>Infant foods</td>
<td>2.3</td>
<td>0.9-5.9</td>
<td>455.0</td>
<td>408-532</td>
<td>0.0-0.1</td>
<td>10.4</td>
<td>9.9-11.2</td>
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<tr>
<td>Products</td>
<td>Calcium</td>
<td>Iron</td>
<td>Zinc</td>
<td></td>
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<tr>
<td>Brown Rice (3)</td>
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<td>12</td>
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<td>Short-grain (4)</td>
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<td>50</td>
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<tr>
<td>Rice noodles (7)</td>
<td>44</td>
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<td>5</td>
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<td></td>
<td></td>
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<tr>
<td>Group 1 (4)</td>
<td>46</td>
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<td>3</td>
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<tr>
<td>Group 2 (3)</td>
<td>34</td>
<td>21</td>
<td>7</td>
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<td></td>
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<tr>
<td>Rice crackers (7)</td>
<td>29</td>
<td>32</td>
<td>11</td>
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<td>50</td>
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</table>
Figure captions:

Figure 1: Levels of phytic acid and minerals (calcium, iron and zinc) in consumer rice products
Each sample was in one of the three groups as a) white rice based products; b) brown rice based products; c) minerals fortified products
For better comparison, contents of phytic acid and minerals in most of the white rice based products were presented in enlarged figures below.

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

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