Strategies to Reduce Phytate Content in the Korean Diet

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Abstract : High dietary phytate is a known factor in reducing the bioavailability of minerals such as zinc and calcium which are already chronically low in the Korean diet. This study was conducted to develop methods for reducing dietary phytate through the addition of phytase and/or the substitution of high phytate foods with low phytate foods. Ten units of phytase per 100g of uncooked brown rice were added to brown rice gruel resulted in a 16.2% phytate reduction after a 3-hour incubation period; an 18.2% reduction was produced after a 6-hour incubation period. The addition of ten units of phytase per 100g of soybean curd residue at 45°C, followed by refrigeration for 3 hours, resulted in a 19.1% phytate reduction. The addition of 20 units of phytase under the same conditions reduced phytate content by 24.6%. In this study, two typical Korean meals consisting of legumes and unrefined cereals were prepared as high phytate meals; these were then compared to low phytate meals that had been prepared by treating the foods with phytase and substituting unrefined with refined cereals (i.e., brown rice with white rice, whole wheat bread with white bread). The phytate content of the two high phytate meals was 1878.2mg and 1811.8mg. After the addition of phytase and the food substitution, the phytate content of the low phytate meals was reduced to 788.9mg and 606.0mg. The phytate to zinc molar ratio of high phytate diets was 22.4 and 21.3 and 9.4 and 7.9 for the low phytate meals. These results indicate that the nutritional status of Koreans in terms zinc and other minerals can be improved by phytate reduction. This can be accomplished through the change of milling process for some cereals and/or the enzyme treatment of some high phytate food items.

Key Words : phytate degradation, phytase treatment, phytate content, Korean diet

I. Introduction

High levels of phosphorus are stored as phytate (myo-inositol hexakisphosphate, Ins P6) in legumes, seeds, cereal, grains, roots, tubers, nuts, fruits, and vegetables. In cereal and grains such as rice, sorghum and wheat, phytate is deposited together with protein and minerals in the aleurone layer or, as is the case for maize, in the germ. In legumes and seeds, phytate is deposited in the cotyledons and embryo axes. This dietary phytate has the potential for reducing mineral bioavailability by forming insoluble complexes with minerals that are hard to absorb for humans

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(Reddy and Sathe, 2002). Several studies have reported that the reduction of dietary phytate can improve mineral absorption in animals and humans (Hayashi et al., 2001; Hunt et al., 1998; Manary et al., 2001; Mendoza *et al.*, 2001). Thus, phytate reduction may increase mineral bioavailability, especially zinc. These fingings are significant for Koreans who traditionally consume diets high in phytate and low in zinc; in fact, for Koreans, the major dietary sources of zinc are the cereal and grain products (48.9%) which have a high phytate content (Joung *et al.*, submitted). Thus, dietary patterns of many Koreans may result in their marginal zinc deficiencies.

The effects of the phytate effect on zinc absorption can be estimated from ratios of phytate, zinc, and calcium (Davies and Olpin, 1979). The phytate : zinc molar ratio can be used to estimate the effect of phytate-zinc interaction. However, because calcium exacerbates phytate's effect on zinc absorption, the phytate x calcium:zinc millimolar ratio can be a better predictor of zinc bioavailability (Ellis *et al.*, 1987; Harland *et al.*, 1988).

The degradation of phytate in order to increase the bioavailability of essential dietary minerals can be achieved through physical, chemical, or genetic methods. Food processing methods such as soaking, germination, fermentation, hydrothermal cooking, and exogenous enzyme addition have been used to improve phytate degradation (Lonnerdal, 2000; Reddy and Sathe, 2002; Phillippy and Wyatt, 2001). However, as indicated in our previous study, traditional food preparation methods such as hydrothermal cooking, soaking, and fermentation do not show any notable reduction of phytate in Korean foods (Nam, 2000). The addition of phytase enzyme, however, has been shown to effectively reduce the phytate content of various foods (Manary *et al.*, 2000; Sandberg *et al.*, 1996). Additionally, milling, a physical processing procedure, has been shown to reduce phytate (Hunt *et al.*, 2002). In this study, we have attempted to develop enzymatic and food substitution methods to decrease phytate content of Korean foods in order to increase mineral availability and improve the mineral nutritional status of Koreans.

II. Materials and Method

1. Phytase treatment of food items

Rice and soybeans were selected for phytase treatment because they are high in phytate content and they are staple foods in Korea. Traditional methods for cooking brown rice gruel and soybean curd stew were used. All food items used in this study were purchased from a sole source and refrigerated until use. Phytase (5000U/g, BASF) was diluted in deionized water (2mg/ml) and refrigerated until use.

Phytase treatment experiments were conducted to find optimal conditions for the maximum rate of phytate degradation by regulating pH, the temperature of the enzyme, and length of the time that the food items were refrigerated. Ten units (10U) of phytase per 100g of rice were added to brown rice gruel, which was then cooked at 45°C and refrigerated for 3 or 6 hours. Ten U or 20U of phytase per 100g of soybean curd were added to uncooked soybean curd at 4°C and refrigerated for

3 hours; it was then used to make stew. Phytase treated food was mixed to ensure the homogeneity of the enzyme action. After incubation, foods were heated to >70°C for 3 minutes to inactivate the phytase; portions were then freeze-dried, ground, and stored in airtight containers until phytate analysis. The phytate content was analyzed by the AOAC method (Harland and Oberleas, 1986) as described in Section 4 below. Sensory evaluations of food items before and after the enzyme treatments were conducted as described below.

2. Sensory evaluation of enzyme treated foods

Sensory tests were performed to evaluate differences in sensory characteristics between phytase treated and untreated foods. Tests were conducted by ten female participants, aged 24-30, who had experience profiling food. Twelve descriptive terms (Kim *et al.*, 1993) were used to characterize brown rice gruel (viscosity, mouth feel, stickiness, graininess, color, aftertaste, and overall acceptance) and soybean curd stew (off-flavor, wetness, hardness, beany taste, savory taste, and overall acceptance). Treated and untreated brown rice gruel and soybean curd stew were served at a temperature between 60-65°C. Panel members cleansed their palates with water at room temperature and then rated each food sample according to the given characteristics.

3. Preparation of a Korean diet with high and low in phytate content

Two typical Korean meals were selected for their high phytate content; they were composed of legumes and unrefined cereals (Table 4). The selected dishes were chosen because they were dishes commonly consumed by Koreans. Values for the phytate contents of the milled and unmilled rice/rice products have been previously reported (Nam, 2001; Yoon, 2000). Whole wheat and white bread were purchased from a local bakery where the baker used the same ingredients for both breads. The phytate content of the two meals was reduced by substituting items with grains low in phytate and by the phytase treatment of high phytate food items (Table 4). Because there were some differences between the nutrient contents other than phytate in the four menus, some menus were switched between diet 1 and 2. Meals were prepared, and portions of single food items and a mixture of the meals (to represent a composite one-day menu) were freeze-dried, ground, and stored in airtight containers until analysis.

4. Analysis of phytate content in diet and foods

The phytate content in the food items and composite one-day menus before and after phytase treatment was analyzed using the AOAC anion exchange method developed by Harland and Oberleas (1986). Phytate was extracted from the dried samples using 2.4 % HCl, eluted with 0.7M NaCl solution on an ion exchange column and wet-digested with HNO_3/H_2SO_4 to release P, which was measured colorimetrically at 640nm. The phytate content in the samples was calculated as hexaphosphate equivalents. All measurements were done in triplicate.

5. Evaluation of zinc availability in Korean meals containing high and low phytate

The nutrient compositions of the meals were calculated by using the Korean Food Composition Table (Korean Nutrition Society, 2000). Dietary zinc intakes were calculated using the same table but supplemented with analysis and substitution from another database published in a previous study (Lee, 1998). Phytate content in the meals was analyzed from a composite of one-day diet samples. Zinc bioavailability of the four meals - two with high and two with low phytate content - were estimated based on calculated zinc and calcium content and analyzed phytate content. The phytate : zinc molar ratios were calculated as the millimoles of phytate intake per day divided by the millimoles of zinc intake per day (WHO, 1996). Phytate x calcium : zinc millimolar ratios were calculated as millimoles of phytate intake times calcium intake per day divided by millimoles of zinc and energy intake per day (Ellis *et al.*, 1987; Harland *et al.*, 1988). Amounts of physiologically available zinc were estimated by applying three levels of zinc availability, according to FAO/WHO/IAEA guidelines (WHO, 1996).

6. Statistical analysis

Statistical Analysis System (SAS), version 6.12, was used for the statistical treatment of the data. Differences between each characteristic were tested by paired comparison t-tests.

III. Results and Discussion

In <Table 1>, the phytate content of cereals differs greatly due to different degrees of milling. The phytate contents of highly refined rice, its cooked product, and white bread were decreased by 81.6%, 88.6% and 40.9% as compared to their respective unrefined products. These results paralleled a study by Hunt *et al.* (2002) in which Filipino rice was shown to have similar decreases in phytate after milling and similar increases in phytate : zinc molar ratio and zinc bioavailability. However, minerals are concentrated in the cereal' s aleurone layer that is removed during milling, so increased zinc bioavailability through milling was insufficient to compensate for the decrease in total zinc content of the refined or less refined rice (Hunt *et al.*, 2002). And, since most Koreans consume refined rice, milling may not be an effective strategy for reducing the phytate content of the Korean diet. Furthermore, milling cannot be used to decrease the phytate content of legumes since the phytic acid in legumes is associated with

<Table 1> Effect of milling on phytate contents

Phytate content of cereal	Phytate (mg/dry wt.100g)	% reduction phytate
Brown rice[a]	1118.7±11.8	
Well-milled rice[b]	205.8±43.2	81.6
Cooked brown rice[a]	1080.9±8.2	
Cooked well-milled rice[b]	123.5±70.6	88.6
Whole wheat bread	69.4±3.5	
White bread	41.0±2.1	40.9

[a] Phytate contents from Nam (2000)
[b] Phytate contents are the mean value of the results from Nam (2001) and Yoon (2000)

protein bodies and difficult to remove by physical methods.

<Table 2> shows the phytate reduction rate of brown rice gruel when treated with phytase. Ten units of phytase per 100g of uncooked brown rice were added to brown rice gruel at 55°C. This resulted in a 16.3 % reduction after a 3-hour refrigerated incubation period and in an 18.0 % reduction after 6-hour period. The resulting small differential in phytate reduction between the 3 and 6 hour incubation periods can be attributed to the temperature of the gruel. The temperature of the gruel fell to below 30°C after 3 hours of incubation. According to a study conducted by Greiner and Konietzny (1998), phytase activity decreases when temperatures are below 50°C.

<Table 3> shows the phytate reduction of

<Table 2> Effect of phytase treatment methods on the phytate content of brown rice gruel

Phytase Treatment Methods[a]	Phytate (mg/dry wt.100g)	% reduction phytate
Control	881.3±5.0	
3 hour incubation	738.6±35.3	16.3
6 hour incubation	722.8±16.1	18.0

[a] Addition of 10U phytase per 100g rice at 45°C; incubation under refrigeration

<Table 3> Effect of phytase treatment methods on the phytate content of soybean residue curd stew

Phytase Treatment Methods[a]	Phytate (mg/dry wt.100g)	% reduction phytate
Control	723.0±61.3	
10U phytase addition	585.1±43.2	19.1
20U phytase addition	545.1±18.0	24.6

[a] Addition of phytase per 100g soybean curd residue; incubation at 4°C for 3 hours

soybean curd after phytase treatment. The addition of 10U phytase per 100g-soybean curd resulted in a 19.1% reduction of phytate after a 3-hour incubation period. The addition of 20U phytase under the same conditions reduced phytate by an additional 5%. The maximal reduction of phytate in the soybean curd stew was achieved by the addition of 20U of enzyme at 55°C (47.8%, data not shown); this figure is similar to the optimal temperature reported by Greiner and Konietzny (1998). The most rapid degradation of phytate is achieved by adding peas with endogenous phytase at 45°C (Fredrikson *et al.*, 2001). The addition of more enzymes, however, resulted in a final cooked product with low consistency and was inedible. Although temperature control is the most effective method for increasing phytase activity, it was not given priority in this study as temperature is difficult to control outside the laboratory. Additionally, reproducing the phytase dose and incubation time was thought to be a more practical means of phytate reduction for the average household. Further studies are necessary to develop phytase treatment methods under various conditions on more phytate-rich food items.

In the sensory evaluation tests for cooked food items, there was little difference between phytase treated foods and untreated foods (Tables 5 & 6). Other than a decrease in the viscosity of brown rice gruel after phytase treatment, there were no significant differences in sensory characteristics (Table 5). The sensory characteristics of soybean curd stew were not affected by phytase treatment. Overall acceptance showed little difference with or without phytase treatment (Tables 5 & 6).

<Table 4> summarizes menus for two typical

<Table 4> Selected typical menus for Korean diets

	High phytate diet 1	Phytate reduced diet 1	High phytate diet 2	Phytate reduced diet 2
Breakfast	Brown rice gruel Kimchi Grilled seaweed Grilled yellow croaker Seasoned lettuce Orange juice	Brown rice gruel treated with phytase[a] Kimchi Grilled seaweed Grilled yellow croaker Seasoned lettuce Orange juice	Ham and cheese sandwich Orange juice	Ham and cheese sandwich[b] Orange juice
Lunch	Cooked brown rice Steamed egg Grilled tofu with Seasoning Seasoned bean sprout Kimchi Milk Banana Tomato	Cooked rice[b] Soybean curd residue stew (treated biji) [a] Seasoned cucumber Seasoned squash Yogurt Milk Tomato	Cooked brown rice with soybean Kimchi Soybean paste soup with Chinese cabbage Braised lotus root Pan-fried fish Milk Apple	Cooked rice with soybean[b] Kimchi Soybean paste soup with Chinese cabbage Braised lotus root Pan-fried fish Milk Banana Apple
Dinner	Cooked brown rice Kimchi Sea mustard soup Stir-fried chicken Potato salad Seasoned vegetable Watermelon	Cooked rice[b] Kimchi Sea mustard soup Stir-fried chicken Potato salad Seasoned vegetable Watermelon	Cooked brown rice Soybean curd residue stew (biji) Seasoned cucumber Seasoned squash Melon	Cooked rice[b] Steamed egg Grilled tofu with seasoning Seasoned bean sprout Kimchi Melon

[a] Menu containing phytase treated food
[b] Menu containing refined cereal

Korean daily diets. <Tables 7> and <Tables 8> give mean values for the phytate content and nutrient values of these Korean meals. High phytate diets 1 and 2 contained 1878.2 mg and 1811.8 mg phytate, respectively and phytate-reduced diets 1 and 2 contained 788.9 mg and 606.0 mg phytate, respectively. The substitution of cereals and the utilization of phytase treatment resulted in a 62.2 % reduction of phytate in phytate-reduced meals. Only 6.9 % of the reduction was achieved through phytase treatment; 55.3 % resulted from substitution of cereals. These finding suggest that milling methods would be easier to increase the zinc bioavailability and,

<Table 5> Mean value and paired t-test results of sensory evaluation of brown rice gruel

	Panel mean rating[a]		
	Gruel	Enzyme treated gruel	Paired t-test P
Viscosity	9.92±2.14	9.95±2.47	<0.05
Mouth-Feel	8.34±2.84	8.41±3.28	NS[b]
Granule	6.38±2.77	7.17±2.88	NS[b]
Adhesiveness	10.09±2.01	9.25±2.10	NS[b]
Color	7.39±2.21	7.60±2.35	NS[b]
Aftertaste	6.67±2.76	7.23±2.75	NS[b]
Overall acceptance	9.95±2.47	9.24±3.09	NS[b]

[a] 15cm line scale used; data presented by mean value ± standard deviation
[b] Not significant

<Table 6> Mean value and paired t-test results of sensory evaluation of soybean curd reside stew

	Panel mean rating[a]		
	Stew	Enzyme treated stew	Paired t-test P
Off-flavor	5.86±3.16	5.63±2.86	NS[b]
Wetness	9.32±1.88	9.92±2.09	NS[b]
Hardness	6.61±2.47	6.75±2.98	NS[b]
Beany flavor	7.94±2.38	8.10±1.59	NS[b]
Savory taste	9.90±1.71	9.62±1.55	NS[b]
Overall acceptance	10.98±1.84	10.87±1.65	NS[b]

[a] 15cm line scale used; data presented by mean value ± standard deviation
[b] Not significant

consequently, the zinc nutritional status of Koreans. However, it may not be a practical way as we found because 1) consumption is already higher for refined rather than unrefined cereals in Korea, 2) total zinc content is reduced through the milling process, and 3) high phytate food items such as legumes can not be milled to reduce phytate. Cereal and grain products and legumes and their products supplied 68.57% and 19.27% of total phytate intake, respectively (Joung *et al.*, submitted). Therefore, phytase treatment is an alternative way to reduce phytate in Korean diets, even though a mere 6.9% reduction by phytase is not enough to reduce dietary phytate. To achieve enough phytate degradation, further research on phytate reduction methods with more food items and various treatments is required.

<Table 7> Nutrient contents of selected typical Korean diets before and after phytate treatment

	High phytate diet 1	Phytate reduced diet 1	High phytate diet 2	Phytate reduced diet 2
Energy (kj)	439.6	430.9	442.6	433.8
Protein (g)	85.6	73.2	80.3	76.4
Fat (g)	53.8	49.9	52.7	45.9
Calcium (mg)	810.63	776.2	824.8	832.6
Phosphorous (mg)	1699.9	1639.0	1427.7	1308.7
Iron (mg)	14.2	14.8	13.8	10.7
Potassium (mg)	3532.8	3265.8	3420.5	2740.9
Zinc (g)	8.3	8.3	8.4	7.6
Fiber (g)	7.4	12.7	7.9	5.2

[a] Calculated by Korean food composition table (Korean Nutritional Society, 2000).

<Table 8> Phytate contents of selected typical Korean diets before and after phytate treatment

Diet type		Phytate contents (mg/diet)	% reduction rate
Diet 1	High phytate diet	1878.2±66.1	
	Phytate reduced diet	788.9±43.4	58.0
Diet 2	High phytate diet	1811.8±92.2	
	Phytate reduced diet	606.0±44.7	66.6

As shown in <Table 9>, phytate reduction increased zinc bioavailability. Phytate to zinc molar ratios for high phytate meals were 22.4 and 21.3; these were reduced to 9.4 and 7.9 by phytate reduction methods. Thus, zinc availability in the phytate-reduced meals showed an increase from low to moderate levels of zinc availability. The available zinc content was increased in phytate-reduced meals from 1.25 and 1.26mg to 2.28 and 2.91mg. Consequently, available zinc content for phytate-reduced meals can meet the Korean RDA value for adults (Korean Nutrition Society, 2000). In conclusion, phytate degradation and/or production of phytate - reduced grains are methods that can be used to improve the zinc status of Koreans. In fact, some phytate reduction methods can be used in institutional settings such as schools and care facilities to make sure that more zinc is available to children and seniors.

VI. Conclusion

In this study, methodologies to reduce phytate content were developed for the cereals, grains, and legumes that are staple components of the Korean diet. Utilization of a phytase treatment method and the substitution of unrefined cereals with refined cereals reduced phytate content and phytate to zinc molar ratios, and increased the bioavailability of zinc. Since many Koreans already consume refined cereals, phytase treatment is a better strategy for phytate reduction than the milling process. Our study can be used to help reducing dietary phytate and increasing zinc bioavailability in the Korean diet.

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<Table 9> Zinc availability of selected typical Korean diets before and after phytate treatment.

Diet type		Zn(mg)[a]	Phy: Zn Molar ratio[a]	Ca × phy/Zn ratio (mmol/1000kcal)[a]	Available zinc (mg)[b]
Diet 1	High phytate diet	8.3	22.4	246.7	1.25
	Phytate reduced diet	8.3	9.4	104.7	2.29~2.91
Diet 2	High phytate diet	8.4	21.3	229.7	1.26
	Phytate reduced diet	7.6	7.9	90.5	2.28~2.66

[a] Zinc and calcium content were calculated by Korean food composition table (Korean nutritional society, 2000).
[b] Calculated using classification of diet by zinc availability table by WHO (1996).

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