Bioavailability of Zinc in Cookies Fortified with Soy and Zinc[1]

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ABSTRACT

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The bioavailability of zinc in cookies fortified with three soy protein products (flour, concentrate, and isolate) was compared with that in cookies fortified with egg albumin. Protein products were added at the same (26%) protein level. The levels of zinc and with one exception (albumin-based cookies), of phytate phosphorus were also equal in all test cookies. Bioavailability was studied using young rats fed a submarginal (9 ppm) level of zinc provided entirely by the cookies. Growth rate, diet efficiency, and serum zinc levels of rats did not differ significantly between the five test cookies (three soy-based and two albumin-based). More sensitive parameters (femur zinc concentration and apparent zinc absorption), however, showed significant differences. When phytate was added to albumin-based cookies, both the femur zinc concentration and the zinc absorption were significantly reduced and approached values obtained with soy-based cookies. Among soy-based cookies, serum and femur zinc levels and apparent zinc absorption tended to be lower in rats fed cookies made with soy isolate than in rats fed cookies made with soy flour or concentrate. Less than 20% of phytate in cookies was hydrolyzed during baking.

Deficiencies of zinc, iron, and magnesium are associated with nutritional problems in a sizable segment of the American population. Fortification of cereal-based products with these minerals has been proposed (NAS/NRC 1974) to improve the nutritional profiles of these products and thus to correct such deficiencies.

The nutritional profiles of cereal-based products such as bread, cookies, and crackers can also be enhanced through fortification with various plant proteins such as those from soy (McWatters 1978, Ranhotra et al 1974a). However, such a use of soy may adversely affect the bioavailability of the minerals because of possible complexing of minerals with soy protein and/or phytate (Erdman and Forbes 1977, Rackis and Anderson 1977). The purpose of this study was to examine the effect of three major soy protein products (flour, concentrate, and isolate) on the bioavailability of an important trace mineral, zinc, added to a popular food product, the cookie. No attempt was made to assess or improve the quality characteristics of the cookies.

MATERIALS AND METHODS

Cookies were made using cake flour enriched with zinc and with three soy protein products or with egg albumin (Table I). Information on other ingredients in cookie formulas and a brief description of the cookie-making process are also presented in Table I. Protein products were added at the same protein level. The level of zinc was also equal in each cookie, as was the level of phytate (except in cookie A). Sodium phytate, which contained 17.7% phosphorus, was obtained from Sigma Chemical Co.

Bioavailability was studied in male weanling rats (Sprague-Dawley) initially weighing an average of 50 g. All animals were housed individually in mesh-bottom stainless-steel cages under controlled environmental conditions. Diets (Table II) were developed, using air-dried and finely-ground cookies to provide 9 ppm of zinc. Diet and deionized water were offered (eight rats per diet) ad libitum for four weeks. Body weight gain and diet intake were recorded. Fecal collections were made throughout the four-week period.

Bioavailability was based on growth response, diet efficiency, "apparent" zinc absorption (urine was not collected), and tissue (serum and femur) concentration of zinc. Zinc levels in protein products, diets, dried feces, blood serum (blood collected by heart puncture), and femur were determined by atomic absorption spectrophotometry (Ranhotra et al 1977), with an IL (Instrumentation Laboratory, Wilmington, MA) model 251 spectrophotometer. Protein was determined by the standard AOAC method (1975). Phytic acid in ingredients and in baked cookies was determined by Makower's method (1970) as described earlier (Ranhotra et al 1974b). The data were subjected to analysis of variance.

RESULTS AND DISCUSSION

A substantial amount of phosphorus in soy and other plant proteins exists as phytic acid phosphorus (Erdman and Forbes 1977, Rackis and Anderson 1977, Ranhotra et al 1974b). Soy also contains a substantial amount of zinc (Table I). However, the absorption of zinc in soy is poor (Erdman and Forbes 1977, Rackis and Anderson 1977) because of the possible formation of zinc-phytate-protein complexes. Although complexing may involve both the naturally occurring and the added zinc, our study was not designed to distinguish this. Only the effect of three important soy protein products (Table I) on the bioavailability of zinc was studied. Five types of cookies (A–E) were made for this purpose.

The level of protein in cookies A–E and of phytate in cookies B–E was equal (Table I). Cookie A (albumin-based) contained no added phytate, and the amount of phytate naturally occurring in cookie A (from cake flour) was negligible. The level of zinc in cookies A–E was also equal (Table I). This necessitated, especially for albumin-based cookies, addition of zinc at a level higher than the level (2.2 mg/100 g of flour) recommended (NAS/NRC 1974). The equalization of protein and zinc in cookies A–E permitted us to keep these two diet variables constant with a minimal change in the amount of cookies used (Table II). Thus, we studied only the effect of type of protein (soy vs albumin) and of phytate (cookie A vs B–E) on the bioavailability of zinc.

Zinc in the diet (Table II) was provided at a submarginal level (NAS/NRC 1972) to enable us to detect differences in bioavailability. No significant difference in weight gain or in diet efficiency was found among the rats eating cookies A–E (Table III). Although serum zinc levels tended to be low from cookie E (soy-isolate-based), differences again were not significant. This suggests that 9 ppm of zinc was probably adequate for maximum growth and normal circulating zinc levels, as we observed earlier (Ranhotra et al 1977, 1978).

Differences between cookies A–E, however, became apparent when more sensitive measurements such as femur zinc concentration (Momcilovic et al 1976) and apparent zinc absorption were examined (Table III). Although femur weight (moisture-free) did not differ significantly, femur zinc concentration was significantly ($P < 0.05$) higher from cookie A (albumin-based, no phytate) than from cookie B (albumin-based, phytate added) and cookies C–E (soy-based, phytate-containing). This suggests that phytate, rather than protein, is the major determinant

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TABLE I
Cookie Formula

	Cookies				
	A	B	C	D	E
Cake flour (g)	100	100	100	100	100
Other ingredients[a] (g)	58	58	58	58	58
Protein source[b] (g)					
Egg albumin	33	33	...	...	...
Soy flour (defatted)	...	...	50	...	...
Soy concentrate	...	...	...	39.9	...
Soy isolate	...	...	...	...	30.9
Zn[c] (mg)	4.49 (0.07)	4.49 (0.07)	2.20 (2.36)	3.40 (1.16)	2.24 (2.32)
Na-phytate[d] (mg)	0 (0)	1107 (0)	0 (1107)	66 (1041)	448 (659)

[a] Includes: sucrose, 30; dextrose, 10; shortening, 15; salt, 1; and soda, 2. Water added varied (51–100 ml, flour basis). The procedure was as follows: Preblend soy with shortening (7 min), add the remaining ingredients (including albumin for albumin cookies), mix (3 min) with water added simultaneously, scrape the dough, deposit cookies on sheet pan, and bake (13 min, 350°F).

[b] Amount adjusted to same (26.1%) protein ($N \times 6.25$) level. Soy samples obtained from ADM Company (flour-Nutrisoy) and from Central Soy Company (concentrate-Promosoy and isolate-Promine D).

[c] As $ZnCl_2$; naturally occurring Zn in the protein source is shown in parentheses.

[d] Added to cookies B, D, and E to increase the phytate P level to that in the soy flour used in cookie C. The phytate contributed by the protein source (moisture-free basis) is shown in parentheses. The phytate in other ingredients was negligible. Amount of Na-phytate added is expressed on moisture-free basis.

of zinc bioavailability, as has been suggested (Davies et al 1977, Davies and Nightingale 1975). Among the soy-based cookies (cookies C–E), femur zinc concentration tended to be lowest, although not significantly, from the cookies made with isolate, compared with those made with soy flour or concentrate. This suggests, as previously postulated (Erdman and Forbes 1977, Rackis and Anderson 1977), that the type of phytate-protein complexes, rather than phytate alone, probably accounts for the differences, especially with soy isolate. However, the differences may be due in part to the type of phytate (natural vs added) involved (Rotruck and Luhrsen 1979).

The apparent absorption of zinc was also significantly ($P < 0.05$) higher from cookie A (albumin-based, no phytate) than from cookies (B–E) made with albumin or soy but containing phytate (Table III). Like femur and serum zinc levels, the zinc absorption level tended to be lower from cookies made with soy isolate than from those made with soy flour or concentrate. Barring differences due to the type of phytate (natural or added), soy isolate seems to affect the availability of zinc more adversely than do soy flour or concentrate.

Less than 20% of the phytate in phytate-containing cookies (cookies B–E) was hydrolyzed during baking (Table IV). When hydrolysis of phytate in baked products is substantial, such as in yeast-fermented bread (Ranhotra et al 1974b, 1978), bioavailability of zinc increases appreciably (Ranhotra et al 1978, Reinhold et al 1974). Otherwise phytate, when added to the diet as its sodium salt (Davies and Nightingale 1975), reduces the body's retention of zinc, whether or not diet is supplemented with zinc. This is attributed to possible inhibition of absorption of endogenous zinc (Davies and Nightingale 1975).

The addition of soy protein products to cereal-based foods thus makes imperative the enrichment of these foods with zinc, as has been proposed (NAS/NRC 1974). Such enrichment would ensure the adequacy of zinc in a total diet that, for health and economic reasons, has come to include increasing amounts of food products containing plant proteins.

TABLE II
Composition of Test Diets

	Diet[a]				
	A	B	C	D	E
Egg albumin (g/100 g)	20.00	20.00	20.00	20.00	20.00
Other ingredients[b] (g/100 g)	10.54	10.54	10.54	10.54	10.54
$CaCO_3$[c] (g/100 g)	1.24	1.24	1.19	1.19	1.20
$NaH_2PO_4 \cdot H_2O$[c] (g/100 g)	2.11	1.94	1.88	1.88	1.89
Cookies[a] (g/100 g)	35.71	36.02	36.02	36.32	34.84
Cornstarch (g/100 g)	30.40	30.26	30.37	30.07	31.53
Zn (ppm)	9.0	9.0	9.0	9.0	9.0

[a] Diets determined by cookie type. Amounts added furnished 9 ppm Zn.

[b] Includes: vitamin diet fortification mixture from ICN Pharmaceuticals, 2.2 g (extra biotin added, 0.4 mg); alphacel, 2 g; trace minerals (in starch base and containing: Cu, 0.5 mg; Fe, 2.5 mg; Mn, 5 mg; Mg, 40 mg; and I, 0.015 mg), 1 g; corn oil, 4 g; NaCl, 1 g; KCl, 0.3432 g.

[c] Amounts added to attain 500-mg level of Ca and P each.

TABLE III
Tissue Concentration and Absorption of Zinc[a,b] in Four-Week Experiment

	Diet[c]				
	A	B	C	D	E
Tissue Concentration					
Weight gain (g)	163 ± 10 a	160 ± 9 a	165 ± 9 a	166 ± 14 a	165 ± 12 a
Diet/gain ratio[d]	2.22 ± 0.20 a	2.08 ± 0.17 a	2.16 ± 0.16 a	2.15 ± 0.10 a	2.11 ± 0.24 a
Serum Zn (μg/dl)	98.1 ± 13.7 a	89.6 ± 13.5 a	100.0 ± 10.9 a	86.5 ± 15.1 a	86.0 ± 12.8 a
Femur wt (g)	0.349 ± 0.017 a	0.338 ± 0.015 a	0.341 ± 0.019 a	0.334 ± 0.022 a	0.343 ± 0.020 a
Femur Zn (μg)	45.5 ± 2.8 a	33.6 ± 2.9 b	34.2 ± 2.7 b	33.4 ± 3.0 b	32.4 ± 4.1 b
Femur Zn (μg/g)	130.6 ± 6.3 a	99.6 ± 8.3 b	100.3 ± 9.0 b	100.0 ± 7.3 b	94.9 ± 12.6 b
Apparent Absorption[e]					
Zn intake (μg)	3,197 ± 162 a	2,995 ± 296 a	3,188 ± 182 a	3,204 ± 258 a	3,132 ± 296 a
Zn absorbed (%)	89 ± 2 a	85 ± 2 b	83 ± 3 bc	83 ± 3 bc	81 ± 3 c

[a] Values indicate average ± SD.

[b] Within each row, means without a common letter are significantly different ($P < 0.05$).

[c] Diets determined by cookie type; all diets contained 9 ppm Zn.

[d] Diet eaten (g)/weight gain (g).

[e] Feces collected throughout the four-week period.

TABLE IV
Phytic Acid Hydrolysis

	Cookies[a]				
	A	B	C	D	E
Phytic acid P (mg)[b]					
Before baking	9.7	232.3	232.4	232.7	232.6
After baking	···	186.8	215.5	209.8	189.7
Hydrolyzed (%)	100	19.6	7.3	9.8	18.4

[a] Diets determined by cookie type; all diets contained 9 ppm of Zn.
[b] Total in formula.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official Methods of Analysis. (12th ed.). The Association: Washington, DC.

DAVIES, N. T., HRISTIC, V., and FLETT, A. A. 1977. Phytate rather than fiber in bran as the major determinant of zinc availability to rats. Nutr. Rep. Int. 15(2):207.

DAVIES, N. T., and NIGHTINGALE, R. 1975. The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of zinc, copper, iron and manganese in rats. Br. J. Nutr. 34:243.

ERDMAN, J. W., and FORBES, R. M. 1977. Mineral bioavailability from phytate-containing foods. Food Prod. Dev. 11(10):46.

MAKOWER, R. U. 1970. Extraction and determination of phytic acid in beans (Phaseolus Vulgaris). Cereal Chem. 47:288.

McWATTERS, K. H. 1978. Cookie baking properties of defatted peanut, soybean and field pea flours. Cereal Chem. 55:853.

MOMCILOVIC, B., BELONJE, B., GIROUX, A., and SHAH, B. G. 1976. Bioavailability of zinc in milk and soy-protein-based infant formulas. J. Nutr. 27:113.

NAS/NRC. 1972. Nutrient requirements of laboratory animals. No. X. National Academy of Sciences, National Research Council. Publ. 990:68. Washington, DC.

NAS/NRC.1974. Proposed fortification policy for cereal-grain products. National Academy of Sciences, National Research Council: Washington, DC.

RACKIS, J. J., and ANDERSON, R. L. 1977. Mineral availability in soy protein products. Food Prod. Dev. 11(10):38.

RANHOTRA, G. S., LEE, C., and GELROTH, J. A. 1978. Bioavailability of zinc in soy-fortified wheat bread. Nutr. Rep. Int. 18(4):487.

RANHOTRA, G. S., LOEWE, R. J., and LEHMANN, T. A. 1974a. Breadmaking characteristics of wheat flour fortified with various commercial soy protein products. Cereal Chem. 51:629.

RANHOTRA, G. S., LOEWE, R. J., and PUYAT, L. V. 1974b. Phytic acid in soy and its hydrolysis during breadmaking. J. Food Sci. 39:1023.

RANHOTRA, G. S., LOEWE, R. J., and PUYAT, L. V. 1977. Bioavailability and functionality (breadmaking) of zinc in various organic and inorganic sources. Cereal Chem. 54:496.

REINHOLD, J. G., PARSA, A., KARIMIAN, N., HAMMICK, J. W., and ISMAIL-BEIGI, F. 1974. Availability of zinc in leavened and unleavened wholemeal wheaten breads as measured by solubility and uptake by rat intestine "in vitro". J. Nutr. 104:970.

ROTRUCK, J. T., and LUHRSEN, K. R. 1979. A comparative study in rats of iron bioavailability from cooked beef and soy protein. J. Agric. Food Chem. 27:27.

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