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Zinc Bioavailability in a Semolina/Soy Protein Mixture Was Not Affected by Extrusion Processing*

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ABSTRACT

Effects of extrusion processing on zinc bioavailability in a product made from 85% semolina and 15% soy protein concentrate were determined using in vitro and in vivo techniques. Soluble and dialyzable zinc contents of the extruded product were 15.3 and 13.0 μg/g, respectively, compared to 15.6 and 15.0 μg/g in the raw (unprocessed) ingredients. Zinc bioavailability in diets in which all of the zinc (14 ppm) was provided either by the extruded product or by its raw ingredients was determined in two groups of male Sprague Dawley rats. No differences were found in concentrations of zinc in plasma, liver and femur between rats fed the two experimental diets. Apparent zinc digestibility was similar for both diets. These results are consistent with the in vitro results showing no effect of extrusion processing on zinc bioavailability.

KEY WORDS: zinc, bioavailability, extrusion.

INTRODUCTION

Extrusion processing, a procedure used in mass production of cereal and legume derivatives, cookies and other preshaped products, has become of major industrial importance.[1] In extrusion processing, some unique conditions are present because the product is subjected to high pressure in combination with severe shear. The degree of mixing and homogenization during extrusion processing also might decrease diffusion barriers or break chemical bonds and thus, increase reactivity in a food system.[2] Extrusion processing has the potential to alter protein structure, solubility, and digestibility by heat, pressure, and shear.[3] Denaturation could, under some circumstances, facilitate digestion.[4] Formation of stable phytate-protein-zinc complexes has been suggested as an explanation for observed differences in zinc absorption with different methods of processing.[5] Interactions among food components such as phytate, protein and zinc during extrusion cooking may have positive, negative or no effects on the bioavailability of zinc. Since extrusion cooking is used increasingly for production of weaning food and breakfast cereals, a thorough knowledge of the effects on nutritional value is essential.

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The purpose of this research was to determine the effect of extrusion processing on zinc bioavailability in a semolina product containing soy protein concentrate using in vitro and in vivo methods.

MATERIALS AND METHODS

1. Product ingredients

Semolina (30 mesh), milled from durum wheat with approximately 65% extraction and enriched with niacin, iron, thiamin and riboflavin, was obtained from the North Dakota Mill & Elevator, Grand Folks, ND. Enriched semolina has been added amounts of 3.13 mg iron, 0.53 mg thiamin, 0.49 mg riboflavin and 2.86 mg niacin per 100 g.[6] Soy protein concentrate (Procon 2000) was purchased from Central Soya (Fort Wayne, IN).

2. Extrusion processing

A mixture of 85% semolina and 15% soy protein concentrate was used to make a product in the shape of small "O's". A corotating twin-screw extruder (Creusot-Loire, Model 45) was used to extrude the semolina/soy product at the temperature of 92 to 96℃. Water was injected into the feed at the rate of 0.12 L/min. The screw was operated at 900 rpm, and the feeder was set to deliver 2.06 kg of the semolina/soy mixture per minute. The final product was dried in a vat dryer/blower to a moisture content of 8 to 9%. The extruded product and an aliquot of the mixed raw ingredients were put in plas-

tic bags and stored at 18℃. The extruded product was ground using a blender prior to analyses.

3. In vitro analyses

Quantitative determination of soluble zinc was done by the assay described by Gifford-Steffen and Clydesdale[7] with the following modifications; zinc concentration of supernatant was analyzed by atomic absorption spectrophotometry (AAS) (Perkin-Elmer Model 2380, Norwalk, CT) after wet ashing with concentrated HNO_3 and 30% H_2O_2. Dialyzable zinc was estimated using the in vitro method developed by Kane and Miller[8] to determine dialyzable iron. An aliquot of the dialysate was wet ashed prior to zinc analysis.

4. In vivo animal experiment

The in vivo bioavailability of zinc in the extruded product and the raw ingredients was determined in weaning Sprague-Dawley male rats (Harland, Indianapolis, IN). Rats were housed individually in suspended stainless steel cages in a temperature- and humidity- regulated room with alternating 12-hour light/dark cycle. Temperature was maintained at 20-22℃, humidity at 68-70%. Food consumption and body weights were measured twice weekly. Rats were housed in metabolic cages during the last week of the experimental diet period for daily collections of feces. Food intake was measured daily during the fecal collection period. Deionized water was supplied ad libitum throughout the experiment using glass water bottles with plastic caps and stainless steel drinking tubes. Care and treatment of rats were approved by the All University Committee on Animal Use and Care at Michigan State University.

After a 8-day adaptation period, all rats were fed a zinc deficient basal diet for 10 days. Six rats were killed to get baseline data. Rats were then assigned to the following three groups: control diet (n = 13) and two experimental diets - extruded diet (n = 13) and raw diet (n = 6). Fewer rats were included in the last group because of a limited supply of the raw ingredient mixture. At the end of week 1 of the experimental diet period, six rats each in the extruded and control groups were killed. During week 3, daily fecal samples for each rat were collected for 7 days. The daily fecal samples were combined and dried in a drying oven to a constant weight. At the end of three weeks, rats were anesthetized with methoxyflurane and killed by drawing blood from the heart using syringes rinsed with sodium heparin. Blood samples were centrifuged (International Centrifuge, Model UV) at 1500 × g for 20 minutes: plasma was removed and frozen. Livers, kidneys, and femurs were removed, weighed and frozen.

5. Diets

The diets were formulated in accordance with the general guidelines prepared by the American Institute of Nutrition[9] with modifications. Composition of the basal zinc deficient diet and three test diets is listed in Table 1. Cupric carbonate and ferric citrate were premixed with Dyetrose (Dyets Inc., Bethlehem, PA) and then incorporated into the basal diet to provide recommended levels of copper and iron. Because of the relatively low phos-

Table 1 Composition of diets[1]

Component	Basal	Extruded	Raw	Control
		g/kg		
Extruded product	-	516	-	-
Product Raw ingredients	-	-	543	-
Egg white[2]	220	107	95	234
Cornstarch	380.5	-	-	366.5
Dyetrose[3]	132	109.5	94.5	132
AIN-93G mineral mix[3] (Zn, Cu and Fe free)	35	35	35	35
		mg/kg		
Zinc carbonate[4]	-	-	-	27.0
Cupric carbonate[5]	9.6	-	-	9.2
Ferric citrate[6]	269.5	-	-	208.7
Potassium phosphate[7]	5461	-	-	5403

1): The following ingredients (g/kg) were added to all diets: sucrose, 100: soybean oil (0.02% TBHQ, Dyets, Inc., Bethlehem, PA), 70: fiber (Cellulose, FCC, Dyets, Inc., Bethlehem, PA), 50: AIN-93 vitamin mix (Dyets Inc., Bethlehem, PA), 10: and choline bitartrate, 2.5.
2): Egg white, spray dried (Dyets, Inc., Bethlehem, PA)
3): Dyets, Inc., Bethlehem, PA
4): Fisher Scientific, Fair Lawn, NJ
5): J.T. Baker Chemical Co. Phillipsburg, NJ
6): $FeC_6H_5O_7 \cdot H_2O$, Matheson Coleman & Bell, Norwood, OH
7): Monobasic, Mallinckrodt, Paris, KE

phorous content of egg white, potassium phosphate was added in order to provide an adequate amount of phosphorous. The control diet was similar to the basal diet except for an increase in the amount of zinc and minor adjustments in the protein and iron to be similar to amounts in the extruded and raw diets. The ground extruded product and the raw product ingredients were used to provide all of the zinc (14 ppm) in the extruded and raw experimental diets, respectively. Approximately 50% of the total protein was supplied by egg white, and the remainder was supplied by the extruded product or raw product ingredients.

6. Analytical methods

1) Phytate

The phytate content of the extruded and raw products was determined using the anion-exchange method of Harland and Oberleas.[10]

2) Protein analysis

Protein content of the diets and dried feces was determined by the AOAC method[11] adapted for the use of the Tecator 1016 digestor (Tecator Co., England), Bchi 323istillation unit (Switzerland) and titrator (716 DMS Titrino, Switzerland). The protein content was calculated on a dry weight basis using a nitrogen conversion factor of 5.70.

3) Zinc analyses

Double deionized water and acid washed glassware were used routinely in all experimental procedures. Bovine and Wheat Flour Standard References (U.S. Department of Commerce, National Institute of Standards, Saithersburg, MD) were used to validate methodology.

Femur samples (one from each rat) were soaked in ether for 24 hours to extract fat and allowed to dry briefly at room temperature. The femur samples were placed in acid-washed ceramic crucibles and dried in a drying oven (Blue M, Blue Island, IL) to a constant weight and then dry ashed in a muffle furnace (Temco, Model No 293C, Barber-Coleman, Rockford, IL) at 600℃ for 40 hours. When the femur samples were cool, 2 ml of 3 N HCl was added. Livers and kidneys were freeze-dried (Unitrap II, Virtis Co., Gardiner, NY) to a constant weight. Liver samples were crushed manually. Duplicate 0.5 g ground liver samples and one kidney (approximate 0.16 g) were soaked overnight in 10 ml concentrated HNO_3 to allow tissues to dissolve in the acid before wet ashing. The dried feces were ground in a blender. Rat diets, the extruded product, raw product ingredients, livers, kidneys and feces were wet ashed using concentrated HNO_3 and 30% H_2O_2; ash was diluted to 10 ml using 0.1 N HCl. Further dilution was made for zinc analysis of diets, product and ingredients(1 : 100), livers and kidneys (1 : 14) and feces (1 : 3). Plasma samples were diluted 1 : 10 with deionized water for analysis of zinc. Standard solutions were made by diluting stock standards with a 5% glycerol solution. The glycerol solution was used to compensate for the viscosity of the plasma. Zinc content of samples was determined by AAS.

4) Statistical analyses

For the in vivo experiment, data obtained at the end of week 1 of the experimental period were analyzed using the Student's t-test. Data at the end of experiment (week 3) were subjected to a one-way analysis of variance at the 95% confidence level to determine the significance of differences between means. Significant differences among the treatments were determined by Fischer's least significant differences (LSD) test when appropriate. All percentage data were log transformed prior to statistical analyses.

RESULTS

1. Analyses of products and diets

The analyzed amounts of zinc and phytate in the extruded product were 27.2 μg/g and 3.42 mg/g, respectively. These values were similar to those in the mixed raw ingredients before extrusion, 25.8 μg/g and 3.73 mg/g for zinc and phytate, respectively. The analyzed amount of zinc was similar in the extruded and raw rat diets (Table 2). However concentration of zinc in the control diet was 14% higher than in the extruded diet and 17% higher than in the raw diet. Concentrations of phytate in the diets containing the extruded and raw product were calculated to be 0.18% and 0.20%, respectively, based on the analyzed amount of phytate in the extruded

Table 2 Zinc, protein and phytate content of diets[1]

Diets	Zinc content (mg/kg diet)	Phytate (mg/g diet)[2]	Protein (%)
Basal (Zn deficient)	3.8 ± 0.3	–	16.9 ± 0.2
Extruded	14.8 ± 0.5	1.76	18.5 ± 0.1
Raw	14.5 ± 0.8	2.02	17.8 ± 0.8
Control	16.9 ± 0.8	–	17.6 ± 0.8

1): Each value represents mean ± SEM of six determinations.
2): Phytate values obtained by calculation from phytate content of extruded and raw (unprocessed) product.

Table 3 Soluble, percent soluble, dialyzable and percent dialyzable zinc in extruded and raw (unprocessed) products[1]

Product	Soluble zn (μg/g)	Soluble zn (%)	Dialyzable zn (μg/g)	Dialyzable zn (%)
Extruded	15.3 ± 3.0	56.5 ± 10.9	13.0 ± 0.5	47.8 ± 1.7
Raw	15.6 ± 1.7	56.6 ± 6.5	15.0 ± 0.2	58.1 ± 0.8

1): Each value represents mean ± SEM of six determinations.

Table 4 Concentrations of zinc in plasma and tissues[1]

Diets	Plasma (μmol/L)	Liver (μg/g dry wt)	Kidney (μg/g dry wt)	Femur (μg/g defatted dry wt)
			Zn deficient	
Basal	10.3 ± 1.40	73.2 ± 4.6	82.7 ± 3.9	109 ± 5
			Week 1[2]	
Extruded	21.7 ± 1.25	80.3 ± 3.8	89.6 ± 0.7	128 ± 3[a]
Control	22.5 ± 1.84	75.3 ± 1.8	94.1 ± 2.6	154 ± 6[b]
			Week 3[3]	
Extruded	23.7 ± 0.93[a]	87.8 ± 2.1	81.9 ± 1.2[a]	157 ± 2[a]
Raw	25.1 ± 1.01[a]	89.9 ± 2.1	85.4 ± 1.7[a]	163 ± 3[a]
Control	27.6 ± 1.04[b]	85.8 ± 2.2	94.0 ± 1.2[b]	216 ± 6[b]

1): Each value represents mean ± SEM (after 1wk, n = 6 in extruded and control; after 3wk, n = 7 in extruded, control and n = 6 in raw)
2): Different letters within a column indicate significant differences at $p < 0.05$ as determined by Student's t-test.
3): Different letters within a column indicate significant differences at $p < 0.05$ as determined by ANOVA and LSD.

Table 5 The weight of feces, fecal concentration of zinc and apparent digestibilty[1][2]

Diets	Fecal weight (g dry wt/d)	Zinc conc. (μg/g dry wt)	Total zinc (μg/d)	Zinc digestibility (%)[3][4]	Protein digestibility (%)[3][5]
Extruded	1.24 ± 0.04[b]	123 ± 12[ab]	152 ± 16[ab]	34.4 ± 7.3	91.2 ± 0.6[b]
Raw	1.52 ± 0.06[c]	117 ± 6[a]	178 ± 11[b]	29.9 ± 4.3	89.3 ± 0.6[a]
Control	0.99 ± 0.06[a]	131 ± 9[b]	131 ± 15[a]	44.6 ± 5.5	92.7 ± 0.4[b]

1): Each value represents mean ± SEM (n = 7 in extruded and control; n = 6 in raw)
2): Different letters within a column indicate significant differences at $p < 0.05$ as determined by ANOVA and LSD.
3): All data were log-transformed prior to statistical analysis
4): Apparent mineral digestibility
= (μg mineral consumed/d-μg mineral in feces/d)/μg mineral consumed/d × 100
5): Apparent protein digestibility = (N consumed/d-N feces/d)/N consumed/d × 100

product or raw ingredients. It was assumed that basal and control diets contained no phytate.

2. In vitro experiments

The analyzed amounts of soluble zinc and percent soluble zinc were similar in extruded and raw products (Table 3). The soluble zinc content of the extruded product was 15.3 μg zinc/g compared to 15.6 μg/g in the raw ingredients, respectively. These amounts represent approximately 56% of the total zinc. The extruded product contained 13.0 g dialyzable zinc/g compared to 15.0 μg/g in the raw ingredients or 47.8 and 58.1% of total zinc, respectively. Results of these in vitro measures to assess zinc bioavailability showed no differences in soluble and dialyzable zinc between the extruded product and its raw ingredients.

3. In vivo animal experiment

At the end of week 3 of the experimental diet period, the mean body weight (211 ± g) of rats in the control group was significantly lower than that of the extruded (236 ± 7 g) and raw (248 ± 3 g) groups. There was no significant difference in the body weight of the extruded and raw groups. The lower total weight gain of rats in the control group may be explained by their lower intake of food. However, because of the higher zinc content of the control diet, total zinc intake was not significantly different for the three groups.

At the end of week 1 of the experimental period, plasma zinc concentration had increased in both control and extruded diet groups and was similar for both groups (Table 4). At the end of week 3, plasma concentration of zinc was significantly higher in rats fed the control diet than in rats fed the extruded and raw diets. There was no significant difference in plasma zinc concentrations between rats fed the extruded and raw diets, suggesting no effect of extrusion processing on zinc bioavailability.

At the end of week 1, femur zinc concentration in the control group was significantly higher than that in rats fed the extruded diet. Similarly at the end of week 3, femur zinc concentration was higher in the control group than groups fed the extruded and raw diets, but no difference was seen between the concentrations for the extruded and raw diet groups (Table 4). After week 3 of

the experimental diet period, the control rats also had higher kidney zinc concentration compared to the rats fed the experimental diets.

Fecal dry weights were significantly different among the groups: raw > extruded > control (Table 5). The fecal concentration of zinc was higher but total fecal zinc excretion was lower in the extruded diet group compared with the raw diet group because of lower total fecal weight. Significant differences in fecal zinc concentration and total zinc were found only between the raw and control diet groups. Rats fed the raw diet had a significantly lower zinc concentration compared to control rats. However, total zinc was significantly higher in rats fed the raw diet compared to control rats.

Although apparent zinc digestibility was 30 to 50% higher in rats fed the control diet compared to the experimental diets, the differences were not significant due to large individual variations. Apparent protein digestibility was lower in rats fed the raw diet compared to rats fed the extruded or control diet (Table 5). Apparent protein digestibility for all three groups was approximately 90%.

DISCUSSION

Similar results for the effect of extrusion processing on zinc bioavailability were found in this study using in vitro and in vivo methods. In vitro methods assume that solubility is a major factor determining bioavailability of minerals, although it is well recognized that other factors may influence absorption in vivo. Other researchers reported contradictory results with respect to the correlation between in vitro and in vivo bioavailability when they investigated the solubility of minerals as a measure of in vivo bioavailability.[12-16] However, positive correlations between in vitro dialyzability and in vivo bioavailability have been reported for iron and zinc.[17][18]

Limited data for the effects of extrusion processing on zinc bioavailability have been reported. In a study reported by Kivisto[19] apparent zinc absorption was determined during two four day periods in ileostomy subjects fed a constant low fiber diet. Supplementation of the diet with an extruded high fiber product resulted in a significant decrease in apparent zinc absorption compared with supplementation with a non-extruded mixture of the product ingredients. Although phytic acid intake was similar in the two periods, the digestion of phytic acid was significantly lower when the extruded product was consumed.[20] The authors suggested that the adverse effect on zinc absorption may have resulted from extrusion causing a deactivation of the phytase naturally present in bran. In a subsequent experiment,[21] zinc absorption was studied in 33 subjects using radioisotopic labeling of single meals and measurement of whole-body retention. When test products were served with a high protein meal containing fish and rice there was no difference in zinc absorption from an extruded high fiber product or corresponding raw ingredients, 36.6 and 31.8%, respectively. In contrast, zinc absorption was only 6.5% when the extruded product was consumed with milk and bread. Reduction of the phytate content of the ingredients prior to extrusion resulted in improved zinc absorption. Fairweather-Tait [22] studied the effect of extrusion cooking of a bran-flour mixture on zinc retention in normal adults. ^{67}Zn was administered with non-extruded or extruded cereal with milk, and isotopic retention measured from fecal excretion. Extrusion cooking had no effect on zinc retention. These results in humans are consistent with our results in rats which showed no differences in apparent zinc digestibility or in plasma, liver and femur zinc concentrations between the extruded- and raw diet-fed groups, thus suggesting that the extrusion conditions used in the present study also did not affect zinc bioavailability.

Conflicting results have been obtained for the effect of extrusion processing on the phytate content of products. A reduction in phytate content in an extruded bran product was reported by Anderson[23] and Fairweather-Tait,[22] whereas Sandberg[20] and Kivisto[21] found little difference. The phytate contents of the extruded and raw rat diets in the present study were 1.76 and 2.02, respectively. The egg white-based control diet was assumed to contain no phytate. Because of the known inhibitory effect of phytate on zinc bioavailability,[24] one might expect improved zinc bioavailability if extrusion processing reduced the phytate content of a product. However, this effect was not observed either in the study by Fairweather-Tait[22] or in our study, although the reductions in phytate in both studies were relatively small. As expected, zinc was more bioavailable in the control egg white diet that did not contain phytate than in the semolina/soy protein diets containing phytate.

The phytate to zinc molar ratio has been suggested as a parameter to predict zinc bioavilaibility. Values greater than 12 have been reported to reduce accumulation of zinc in femurs in rats but not depress growth.[25][26] The estimated phytate to zinc molar ratio of the extruded diet and raw diet were 11.7 and 13.9 respectively. The extent of formation of phytate-protein-zinc complexes is depen-

dent on the presence of other minerals, particularly calcium. Graf and Eaton[27] indicated that a low calcium content in the diet (0.75%) had no effect on zinc bioavailability when the phytate to zinc molar ratio was 12 or less, whereas when the calcium content of the diet was increased to 1.75%, growth of rats was significantly depressed. In the present study, estimated calcium concentrations were lower than the level shown to have a negative effect on zinc bioavailability, and were similar in all diets: 0.55, 0.55 and 0.52% in the extruded, raw and control diets, respectively. Formation of phytic acid-protein-zinc complexes has been suggested as an explanation for observed differences in zinc absorption with different methods for processing soy protein.[5] Although total phytic acid content may not be altered by extrusion processing, interactions among phytic acid, protein and zinc nevertheless may occur. Conflicting results in the various studies may be explained in terms of variations in extrusion conditions together with the use of different raw materials or variations in food components included in the experimental diets or test meals.

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