# Combined Effects of Various Types of Dietary Fiber and Protein on *in Vitro* Calcium Availability

Sonia Blaney,† John A. Zee,*,† Roger Mongeau,‡ and Johanne Marin†

Groupe de Recherche en Nutrition Humaine, Département des Sciences des Aliments et de Nutrition, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Sainte-Foy, Québec G1K 7P4, Canada, and Nutrition Research Division, Bureau of Nutritional Sciences, Health Canada, Banting Research Centre, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

The effect of the nature of dietary fiber was determined by measuring soluble and ionic calcium using an *in vitro* method. Results show the existence of a chemical interaction between calcium and fiber and/or protein. Lignin, oat bran, and wheat bran, which contain endogenous protein and phytic acid, with or without added protein, reduced calcium availability by 2−20% possibly through the formation of stable and insoluble complexes between fiber/phytic acid and calcium. When combined with fiber (with the exception of pectin), beef and casein had no significant effect on soluble calcium. When compared with the fiber-free control, soy and lignin decreased soluble calcium. As well, soy, wheat bran, lignin, and cellulose decreased ionic calcium, suggesting an interaction effect.

**Keywords:** *Dietary fiber; protein; interaction; calcium availability*

## INTRODUCTION

Inadequate calcium (Ca) intake and poor absorption of Ca are only two of several risk factors for osteoporosis and some other diseases (Osteoporosis Society of Canada, 1994). Indeed, Cumming (1990) suggested that increasing Ca intake positively influences bone health and lowers risk of osteoporosis.

On the other hand, many dietary fibers can bind Ca (Weber, 1993) and may decrease calcium availability *in vitro* (Slavin, 1985; Platt and Clydesdale, 1987) or lead to negative calcium balance *in vivo* (Nnakwe et al., 1989). According to Slavin (1985), insoluble fibers (lignin, hemicellulose, wheat bran) bind more calcium as compared to soluble fibers (pectin, gums). The negative effect of dietary fiber is probably due to the formation of stable complexes with calcium owing to the anionic character of fibers (Platt and Clydesdale, 1987). Another factor in fiber sources which may affect calcium availability is the presence of phytic acid which may form insoluble complexes with calcium (Grynspan and Cheryan, 1989).

According to James et al. (1978), increased fiber consumption may lead to some potential deleterious effects. In vegetarian diets, the intake of dietary fiber (30−40 g/day) is usually higher than in omnivorous diets (20 g/day) (Locong, 1986). The proportion of various fiber constituents (pectin, lignin, and cellulose) and phytic acid in these diets is also different (Calkins, 1986). For these reasons, it has been assumed that the availability of calcium in vegetarian diets is lower than in omnivorous diets.

The protein source may also affect calcium absorption. Casein from dairy products increased calcium solubility *in vivo* due to the presence of phosphoserin groups on the molecule (Berrocal et al., 1989). However, beef protein did not affect calcium balance in humans (Spencer et al., 1988), while soy protein isolate decreased calcium solubility due to its phytic acid content (Champagne, 1988).

Some fibers can decrease *in vitro* protein digestibility by interfering with enzyme activity and proteolysis (Isaksson et al., 1982). It is therefore possible that the effect of dietary fiber on calcium availability is dependent on the nature of protein. The purpose of this study was to evaluate the combined effect of various types of dietary fiber and protein on *in vitro* calcium availability.

In this study, calcium availability was determined by measuring soluble and ionic calcium. According to Miller et al. (1981) and Pantako et al. (1994), soluble calcium as determined after simulated gastrointestinal digestion is a good indicator of available calcium for absorption. Kim and Zemel (1986) also reported that ionic calcium is a better indicator of calcium availability since soluble calcium complexes are not necessarily available for absorption. While both soluble and ionic calcium levels provide a satisfactory qualitative differentiation of calcium bioavailability from milk, ionic calcium is expected to provide a better index of calcium availability from foods which contain substantial amounts of soluble chelating agents.

## MATERIALS AND METHODS

**Materials.** Calcium chloride ($CaCl_2 \cdot 2H_2O$), lanthanum chloride ($LaCl_3 \cdot 7H_2O$), citrus pectin, and cellulose (Alphacel) were purchased from Sigma Chemical Co., St. Louis, MO. The methoxyl content of the pectin was 8.9%. Purified lignin (99%) was obtained from Daishowa Co., Quebec City, Quebec. Wheat bran and oat bran were purchased at a local grocery store. Pepsin (1:60000, No. P 7012) and bile extract (porcine, B 8631) were purchased from Sigma Chemical Co. Pancreatin (1 × NF, No. 102557), soy protein isolate, and casein were purchased from ICN Biochemicals, Cleveland, OH. Lean beef meat (500 g) was purchased at a local grocery store, lyophilized and defatted at room temperature by shaking with petroleum ether for 12 h, and ground (Galibois and Savoie, 1987). The characteristics of the nonpurified and purified protein and fiber sources are shown in Tables 1 and 2. As can be seen, the amounts of calcium found in various sources studied were negligible.

**Procedures.** In this study, protein sources (soy isolate, beef concentrate, and casein), fiber sources (wheat bran and

* Author to whom correspondence should be addressed [phone, (418) 656-2131 ext 7150; fax, (418) 656-3353; e-mail, john.zee@aln.ulaval.ca].

† Université Laval.

‡ Banking Research Centre.

 

**Table 1. Fiber and Phytic Acid Content of Soy Protein and Unpurified Fiber Sources**

| product | cellulose (%) | lignin (%) | hemicellulose (%) | phytic acid (%) |
|---|---|---|---|---|
| soy isolate | 1.4 ± 0.1 | 0.3 ± 0.1 | 0.0 ± 0.0 | 1.9 ± 0.1 |
| wheat bran | 8.8 ± 0.1 | 3.4 ± 0.1 | 28.5 ± 1.1 | 3.6 ± 0.2 |
| oat bran | 0.5 ± 0.1 | 1.0 ± 0.2 | 8.8 ± 0.2 | 1.1 ± 0.1 |

**Table 2. Protein[a] and Calcium Contents in Protein and Fiber Sources**

| product | protein (%) | total calcium (%) |
|---|---|---|
| soy isolate | 87.8 ± 0.1 | 0.04 ± 0.01 |
| beef concentrate | 85.1 ± 0.1 | 0.02 ± 0.01 |
| casein | 86.3 ± 1.4 | 0.05 ± 0.00 |
| wheat bran | 16.46 ± 0.4 | 0.03 ± 0.00 |
| oat bran | 15.25 ± 0.2 | 0.01 ± 0.00 |
| lignin | ND[b] | 0.14 ± 0.05 |
| cellulose | ND | 0.07 ± 0.01 |
| pectin | ND | 0.04 ± 0.00 |

[a] N × 6.25. [b] Not determined.

oat bran), and dietary fibers (lignin, cellulose, and pectin) were used. The amounts of protein and calcium concentrations used were based on those found in omnivorous diets (Calkins, 1986; Locong, 1986). The amounts of fiber sources and dietary fibers were the same as that of pectin in omnivorous diets. (It should be noted that wheat and oat brans contain about 40% and 17% total dietary fiber, respectively.) Although fiber effects on calcium availability may be more of a problem in vegetarian or semivegetarian diets, the omnivorous diet was chosen to observe any effect which may be caused by the nature of fibers. Morever, dietary fibers and fiber sources were used in their original forms without further grinding. Some controls without fiber or protein were also digested and analyzed as described below.

*In Vitro Method.* During the experimentation, all glassware used was acid washed and distilled deionized water rinsed. Calcium availability was evaluated in triplicate by the *in vitro* method of Keane et al. (1988), as modified by Marin and Zee (1992).

To determine the effect of the nature of fibers, each fiber−protein mixture was prepared by mixing 0.20 g of a fiber source and 0.25 g of a protein source in distilled deionized water. Calcium chloride (0.0412 g) was then added to the fiber−protein mixture and the final volume brought to 100 mL with distilled deionized water. Final concentration of each component was 0.2%, 0.25%, and 0.02% for fiber, protein, and calcium, respectively. To facilitate *in vitro* digestion, the test materials in the reaction mixtures were reduced by a factor of 35. The reaction mixture was incubated with 20 mL of pepsin (0.024 g of pepsin/1.2 g of protein suspended in 0.1 N HCl) at 37 °C with shaking for 2 h at pH 1.9. An aliquot (3 mL) of a suspension of pancreatin (0.04 g of pancreatin/1.2 g of protein) and bile (0.0625 g of bile/1.2 g of protein) in 0.1 N $NaHCO_3$ was added to the pepsin digest after adjusting the pH to 6.8 using 1 M $NaHCO_3$ and incubated for another 2 h at 37 °C. The final pH was measured and adjusted in each step of the *in vitro* enzyme reactions. Reaction mixtures were then kept on ice to prevent further reaction.

Further, the digested sample was centrifuged at 5000*g* for 30 min at 5 °C and the supernatant centrifuged at 27000*g* for an additional 45 min. The final supernatant was filtered through Whatman No. 1 paper, and the filtrate was subsequently analyzed for soluble and ionic calcium.

*Determination of Total, Soluble, and Ionic Calcium.* All analyses were performed in duplicate. Total calcium was determined by digesting 10 mL aliquots of a sample in 12 mL of $HNO_3$−$HClO_3$ (3:1) in Kjeldahl flasks for 45 min followed by atomic absorption spectrophotometry (AAS). Lanthanum chloride (0.5% final concentration) was previously added to all samples and standards to avoid the formation of calcium phosphate complexes.

Soluble calcium was determined from the supernatant (10 mL) by AAS, as described above. For ionic calcium, a 10 mL aliquot of sample was brought to 100 mL with distilled deionized water. To this was added 2 mL of 4 M KCl solution. Ionic calcium was determined using a calcium ion-selective electrode (Model 93-21, Orion Research Inc., Cambridge, MA). The precision within runs was 1% and that between runs 2%.

*Determination of Protein and Fiber Contents.* The protein content (% protein = N × 6.25) was measured using an automatic Kjel-Foss apparatus (Model 16200, Foss Co., Denmark). In brans and soy isolate, the proportions of cellulose, hemicellulose, and lignin were measured by the Van Soest method (AOAC, 1975), and phytic acid was measured by the method of Latta and Eskins (1980).

**Statistical Analysis**. A completely randomized factorial design was used for this study. Bartlett's test was used to confirm homogeneity of variances (Steel and Torrie, 1980). Variance analysis (ANOVA) was used to determine the fiber effect, the protein effect, and the fiber × protein interaction effect. Fisher's equation, based on total and partial sums of squares and their degrees of freedom, was used for testing the source of the protein × fiber interaction (Neter et al., 1985). The partial sum of squares is associated with the protein × fiber interaction without consideration of one or several fibers. Tukey's multiple range test was also used to separate significantly different protein−fiber combinations (Kirk, 1982).

## RESULTS AND DISCUSSION

The effects of fiber−protein mixtures on calcium availability are shown in Table 3. Variance analysis also showed an independent protein ($P < 0.001$) and fiber effect ($P < 0.001$) as well as a protein−fiber interaction for soluble and ionic calcium ($P < 0.0001$) (Table 4).

**Soluble Calcium.** With or without the protein sources, lignin reduced ($P < 0.05$) calcium solubility, particularly in the presence of soy protein (Table 3). The hydroxyl and methoxyl groups of lignin may form insoluble complexes with calcium according to Platt and Clydesdale (1987). When compared with fiber-free control (FF), pectin reduced calcium solubility by 4.3% ($P < 0.05$) in the presence of casein but increased it by 6.0% ($P < 0.05$) in the presence of soy protein. This may be due to the nonliberation of phytic acid from soy protein caused by the presence of pectin. The latter may decrease enzyme activity by increasing the viscosity of the reaction medium, resulting in reduced production of phytic acid−calcium complexes. Since a high-methoxy pectin was used in the present work, calcium solubility should not have been affected (Marin et al., 1990). Indeed, the calcium−pectin association depends on the degree of methoxylation of pectin (Inglett and Falkehag, 1979). No effect ($P < 0.05$) of oat and wheat brans was observed with or without added protein source as compared to FF in spite of the phytic acid present (see also Table 2). However, a reduction in calcium solubility has been reported by Rendleman (1982) with a calcium:wheat bran ratio of 0.037 as compared to 0.075 in our study. This may be due to differences in particle size of the bran (and thus the surface area) used in the two studies. Other minerals may also compete with calcium to bind phytate or fiber (Platt and Clydesdale, 1985). Furthermore, proteins in brans (Table 2) may also affect calcium availability (Torre and Rodriguez, 1991).

For each fiber studied, beef and casein had no effect on calcium, except for pectin−casein which decreased ($P < 0.05$) calcium availability compared to protein-free control (PF) (Table 3). When soy protein was added to fiber−calcium mixtures, calcium solubility decreased in the presence of oat bran (7%), lignin (11%) and cellulose (9%), compared to PF ($P < 0.05$). Soluble calcium was

**Table 3. Effects of Protein−Fiber Mixtures on Soluble and Ionic Calcium Availability (%)[a]**

| protein | | fiber source | | | | | |
|---|---|---|---|---|---|---|---|
| | | FF | oat bran | wheat bran | lignin | pectin | cellulose |
| PF | $Ca_s$ | 99.4 ± 0.9[abx] | 94.9 ± 1.1[bx] | 98.2 ± 4.1[abx] | 86.8 ± 1.2[cx] | 100.3 ± 0.8[ax] | 99.8 ± 1.0[abx] |
| | $Ca_i$ | 98.7 ± 2.4[ax] | 89.4 ± 3.4[byz] | 86.8 ± 0.7[by] | 84.4 ± 1.0[bx] | 101.9 ± 1.1[ax] | 99.4 ± 0.7[ax] |
| beef | $Ca_s$ | 99.7 ± 0.9[ax] | 97.8 ± 1.2[ax] | 100.3 ± 3.4[ax] | 89.6 ± 0.1[bx] | 100.3 ± 2.3[ax] | 98.2 ± 2.8[ax] |
| | $Ca_i$ | 102.2 ± 1.3[ax] | 97.4 ± 1.5[ax] | 96.9 ± 2.7[ax] | 87.4 ± 1.2[bx] | 88.3 ± 1.5[bz] | 101.8[ax] ± 6.1 |
| casein | $Ca_s$ | 99.5 ± 0.8[ax] | 96.9 ± 0.2[abx] | 98.4 ± 1.4[abx] | 86.7 ± 2.0[cx] | 95.2 ± 2.0[by] | 98.5[abx] ± 0.4 |
| | $Ca_i$ | 97.9 ± 2.6[abx] | 94.8 ± 2.7[abxy] | 95.7 ± 1.9[abx] | 86.8 ± 1.7[cx] | 92.6 ± 2.4[bcy] | 99.3[ax] ± 1.9 |
| soy | $Ca_s$ | 93.1 ± 1.5[by] | 87.8 ± 1.6[bcy] | 95.6 ± 2.2[abx] | 75.6 ± 1.9[dy] | 99.1 ± 1.2[axy] | 90.6[bcy] ± 2.6 |
| | $Ca_i$ | 89.7 ± 1.3[by] | 86.6 ± .07[bcyz] | 83.3 ± 1.3[cy] | 79.1 ± 1.7[dy] | 98.6 ± 1.1[ax] | 84.9[cy] ± 2.1 |

[a] $Ca_s$, soluble calcium; $Ca_i$, Ionic calcium; FF, Fiber-free (control); PF, protein-free (control). Different letters (abc) in each row denote significant differences ($P < 0.05$). Different letters (xyz) in each column denote significant differences ($P < 0.05$).

**Table 4. Variance Analysis for Protein−Fiber Interactions for Soluble ($Ca_s$) and Ionic ($Ca_i$) Calcium**

| | | α | *P* |
|---|---|---|---|
| protein | $Ca_s$ | 0.05 | 0.0001 |
| | $Ca_i$ | 0.05 | 0.0001 |
| fiber | $Ca_s$ | 0.05 | 0.0001 |
| | $Ca_i$ | 0.05 | 0.0001 |
| protein−fiber | $Ca_s$ | 0.05 | 0.0001 |
| | $Ca_i$ | 0.05 | 0.0001 |

not significantly decreased in the presence of wheat bran. The soy protein effect may be attributed to the presence of phytic acid (Table 1) which binds calcium to form insoluble complexes at pH 4−7 (Champagne, 1988; Torre and Rodriguez, 1991). Grynspan and Cheryan (1983) found similar results with calcium: phytate ratios of 0.50−2.74. It should be noted, however, that inositol phosphate isomers other than phytate may also reduce mineral availability of foods (Lehrfeld and Morris, 1992). These authors suggested that measurement of individual inositol phosphates will more accurately predict the potential negative effect on mineral availability. This may explain why wheat bran containing higher phytic acid had no effect on Ca availability. The fact that the addition of soy to pectin did not modify calcium solubility may again be explained by increased viscosity of the reaction medium.

**Ionic Calcium.** Pectin and cellulose had no effect on ionic calcium in the absence of protein *in vitro* ($P <$ 0.05) (Table 3). However, ionic calcium content in the presence of oat bran, wheat bran, and lignin was reduced by 5−17% due to the presence of fibers such as lignin and hemicellulose. Indeed, Mod et al. (1982) have observed that hemicellulose may bind Ca under gastrointestinal conditions and thus interfere with Ca absorption. Soluble fibers in oat and wheat brans can form soluble complexes with calcium, and they can reduce calcium availability as measured by ionic calcium (Table 3). When beef or casein proteins were present in oat bran/wheat bran/cellulose−calcium mixtures, the amount of ionic calcium (94.8−101.8%) was not affected as compared to FF (Table 3).

Lignin significantly reduced ionic calcium by 15% and 11% in the presence of beef and casein, respectively, as compared to FF ($P < 0.05$) (Table 3). Similarly, pectin reduced the amount of ionic calcium by 13.9% and 5.3% in the presence of beef and casein respectively. This indicates that pectin−beef−Ca or pectin−casein−Ca combinations form soluble complexes which are not necessarily all in an ionic form. Ionic calcium content was significantly lower ($P < 0.05$) in the presence of soy protein and wheat bran (83.3%), lignin (79.1%), or cellulose (84.9%), compared to FF (89.7%). Pectin, however, seemed to have a positive effect on calcium availability in the presence of soy protein as measured by ionic calcium.

Compared to PF for each fiber studied, beef and casein did not affect calcium availability except in the case of pectin and wheat bran ($P < 0.05$). When soy protein was added to lignin or cellulose, ionic calcium was reduced ($P < 0.05$) by 5% and 15%, respectively, compared to PF.

Measurements of soluble and ionic calcium *in vitro* suggest that fiber and protein can affect calcium availability (soluble and ionic forms). The latter was decreased in the presence of lignin with or without protein and by fiber−soy protein mixtures. Diets, however, are not likely to contain sufficient lignin to make this constituent of mature plants to be of importance. Pectin also decreased calcium availability when associated with casein and beef protein. It should also be noted that results obtained in this study may relate only to potential availability for absorption in the small intestine but not in the colon. Indeed, certain fibers such as pectin may further be degraded in the gut (Cummings et al., 1979). Further studies are needed to test the effect of fibers and proteins on calcium availability using concentrations corresponding to those found in vegetarian diets. As well, *in vivo* studies should be carried out to confirm the results presented in this paper.

ACKNOWLEDGMENT

We express our gratitude to Charles Lavigne for the statistical interpretation.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed., Association of Official Analytical Chemists: Washington, DC, 1975; pp 162−163.

Berrocal, R.; Chanton, S.; Juillerat, M. A.; Pavillard, B.; Scherz, J. C.; Jost, R. Tryptic phosphopeptides from whole casein. II. Physicochemical properties related to the solubilization of calcium. *J. Dairy Res.* **1989**, *56*, 335−341.

Calkins, B. M. Consumption of fiber in vegetarians and nonvegetarians. In *Handbook of Dietary Fibers*; CRC Press: Boca Raton, FL, 1986; pp 407−413.

Champagne, E. T. Effects of pH on mineral-phytate, protein-mineral-phytate and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high-fiber foods. *J. Am. Coll. Nutr.* **1988**, *7*, 499−508.

Cummings, J. H.; Southgate, D. A. T.; Branch, W. J.; Wiggins, H. S.; Houston, H.; Jenkins, D. J. A. The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. *Br. J. Nutr.* **1979**, *41*, 477−485.

Cumming, R. G. Calcium intake and bone mass: a quantitative review of evidence. *Calcif. Tissue Int.* **1990**, *47*, 194−201.

Galibois, I.; Savoie, L. Relationship between amino acid intestinal effluent in rat and *in vitro* protein digestion products. *Nutr. Res.* **1987**, *7*, 65−79.

Grynspan, F.; Cheryan, M. Calcium phytate: effect of pH and molar ratio on *in vitro* solubility. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1761−1764.

Grynspan, F.; Cheryan, M. Phytate-calcium interactions with soy protein. *J. Am. Oil. Chem. Soc.* **1989**, *66*, 93−97.

Inglett, G. E.; Falkehag, S. I. *Dietary Fibers; Chemistry and Nutrition;* Academic Press Inc.: New York, 1979; pp 33−42.

Isaksson, G.; Lundquist, I.; Ihse, I. Effect of dietary fiber on pancreatic enzyme activity *in vitro*. *Gastroenterology* **1982**, *82*, 918−924.

James, W. P. T.; Branch, W. J.; Southgate, D. A. T. Calcium binding by dietary fiber. *Lancet* **1978**, *1*, 638−639.

Keane, L. A.; Potter, N. N.; Sherbon, J. W. Estimation of calcium status in selected food systems. *J. Food Sci.* **1988**, *53*, 1111−1112.

Kim, H.; Zemel, M. B. *In vitro* estimation of the potential bioavailability of calcium from sea mustard (*Undaria pinnatifida*), milk and spinach under simulated normal and reduced gastric acid conditions. *J. Food Sci.* **1986**, *51*, 957−959.

Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*, 2nd ed.; Brooks/Cole Publishing Co.: Pacific Grove, CA, 1982; pp 116−118.

Latta, M.; Eskins, M. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* **1980**, *28*, 1313−1315.

Lehrfeld, J.; Morris, E. R. Overestimation of phytic acid in foods by the AOAC anion-exchange method. *J. Agric. Food Chem.* **1992**, *40*, 2208−2210.

Locong, A. Nutritional status and dietary intake of a selected sample of young adults vegetarians. *J. Can. Diet. Assoc.* **1986**, *47*, 101−106.

Marin, J.; Zee, J. A. Influence des hydrocolloïdes sur la biodisponibilité potentielle du calcium dans les produits laitiers. (Effect of hydrocolloids on potential availability of calcium in dairy products.) *Microbiol. Alim Nutr.* **1992**, *10*, 23−28.

Miller, D. D.; Schricker, B. R.; Rasmussen, R. R.; Van Campen, D. An *in vitro* method for estimation of iron availability from meals. *Am. J. Clin. Nutr.* **1981**, *34*, 2248−2256.

Mod, R. R.; Ory, R. I.; Morris, N. M.; Normand, F. I. In vitro interaction of rice hemicellulose with trace minerals and their release by digestives enzymes. *Cereal Chem.* **1982**, *59*, 538−542.

Neter, J.; Wasserman, W.; Kutner, M. H. Multiple regression-II. *Applied Linear Statisticals Models*, 2nd ed.; Richard D. Irwin Inc.: Homewood, IL, 1985; pp 44−46.

Nnakwe, N.; Kies, C.; Fox, H. M. Calcium and phosphorus utilization from wheat bran by lacto-ovo-vegetarians and omnivores. *Nutr. Rep. Int.* **1989**, *39*, 897−906.

Osteoporosis Society of Canada. Consensus on calcium nutrition (1993). *Nutr. Actual.* **1994**, *18*, 62−70.

Pantako, T. O.; Amiot, J. Correlation between *in vitro* solubility and absorption in rat of calcium, magnesium and phosphorus from milk protein diets. *Sci. Aliment* **1994**, *14*, 139−158.

Platt, S. R.; Clydesdale, F. M. Binding of iron by lignin in the presence of various concentrations of calcium, magnesium, and zinc. *J. Food Sci.* **1985**, *50*, 1322−1326.

Platt, S. R.; Clydesdale, F. M. Mineral binding characteristics of lignin, guar gum, cellulose, pectin and NDF under simulated duodenal pH conditions. *J. Food Sci.* **1987**, *52*, 1414−1419.

Rendleman, J. A. Cereal complexes: binding of Ca by bran and components of bran. *Cereal Chem.* **1982**, *59*, 302−309.

Slavin, J. L. The availability of minerals in fibre diets. *The Clinical Role of Fiber*; Medical Education Services: Mississauga, Ontario, Canada, 1985; pp 41−49.

Spencer, H.; Kramer, L.; Osis, D. Do protein and phosphorus cause calcium loss? *J. Nutr.* **1988**, *118*, 657−660.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics: a Biometrical Approach*, 2nd ed.; McGraw-Hill Book: New York, 1980.

Torre, M.; Rodriguez, A. R. Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* **1991**, *1*, 1−22.

Weber, C. W.; Kohlhepp, E. A.; Idouraine, A.; Ochoa, L. J. Binding capacity of 18 fiber sources for calcium. *J. Agric. Food Chem.* **1993**, *41*, 1931−1935.

Received for review January 18, 1996. Revised manuscript received July 16, 1996. Accepted August 12, 1996.[⊗] This study was funded by the Conseil de Recherche en Pêcheries et Agroalimentaire du Québec.

JF960027V

[⊗] Abstract published in *Advance ACS Abstracts*, September 15, 1996.