Study of the Interactions of Calcium Ions with Lignin, Cellulose, and Pectin

Mercedes Torre,* Adela R. Rodriguez, and Fulgencio Saura-Calixto†

Departamento Quimica Analitica, Facultad de Ciencias, Universidad de Alcala de Henares, 28871 Alcala de Henares, Madrid, Spain

Interactions of calcium ions with insoluble (lignin and cellulose) and soluble (pectin) fiber constituents were systematically investigated in vitro as a function of several physicochemical variables: initial concentration of calcium in solution, pH, and the amount of fiber constituent. The investigation was carried out under experimental conditions which may be achieved in physiological circumstances. Lignin exhibited a high affinity for calcium ions in solution. The amount of bound metal rose with increasing initial calcium concentration, pH, and quantity of fiber. It seemed that proton-ionizable functional groups of lignin were involved in calcium retention by this polymer. The interaction of cellulose and pectin with Ca^{2+} was very weak; calcium could probably be retained by adsorption to the surface of these polysaccharides.

INTRODUCTION

The calcium nutritional status of human populations is actually a matter of major concern since much evidence does suggest that a low calcium intake is one of the risk factors associated with diseases such as osteoporosis and hypertension (Poneros-Schneier and Erdman, 1989).

Several in vivo studies indicate that diets rich in dietary fiber and phytic acid may be a causative factor in calcium deficiency states (Davies, 1978; Kelsay, 1987; Southgate, 1987; Poneros-Schenier and Erdman, 1989; Torre et al., 1991). However, the extent of this effect is not clearly known.

In order to provide explanations for these effects observed in vivo, in vitro studies have been carried out. The ability of numerous natural fiber sources (vegetables, cereals, and fruits) to interact in vitro with calcium has been reported (Camire and Clydesdale, 1981; Rendleman, 1982; Torre et al., 1990). However, only limited studies have been done on interactions of the major isolated constituents that make up the plant cell wall with this metal cation. Although purified components of dietary fiber do not behave the same as fiber in food sources, the data from in vitro mineral binding studies with individual fiber constituents are relevant to the understanding of the physicochemical parameters which determine the interactions between fiber and metallic ions and, also, may be useful to elucidate the relative importance of these components in mineral binding by food sources. Studies on the possible effect of lignin on calcium availability are scarce and poorly representative (Camire and Clydesdale, 1981; Platt and Clydesdale, 1985). Camire and Clydesdale (1981) and Rendleman (1982), in experiments conducted to study the amount of calcium bound to cellulose, found that this polysaccharide exhibited a very low affinity for calcium over a physiological pH range. The results from in vitro studies focused on the effects of soluble fiber constituents are also scarce and, very often, contradictory. Low-methoxylated pectin and guar gum were observed for their ability to bind calcium in aqueous solution (Camire and Clydesdale, 1981). Similarly, Ha et al. (1989) found calcium binding by six industrial hydrocolloids over a wide pH range. A study of Kelly and Potter (1990) showed that when 10% nonfat dry milk was processed with gum arabic, gum karaya, carrageenan, and low-methoxylated pectin, a decrease in dialyzable calcium from the milk was observed. On the contrary, Rendleman (1982) and Schlemmer (1989) found that, under relevant physiological conditions of the intestine, the in vitro interaction of Ca(II) with pectin (Rendleman, 1982; Schlemmer, 1989), alginate, carrageenan, and guar gum (Schlemmer, 1989) was very weak.

More research is needed about the calcium binding capacity of dietary fiber in vitro, as well as about the influence on binding of physicochemical experimental variables such as pH, metal ion concentration, and the amount of fiber in solution, which may help us to provide information on the nature of the interactions between fiber and calcium.

The work presented in this paper has been conducted to systematically investigate whether or to what extent some pure constituents of insoluble (lignin and cellulose) and soluble (pectin) fiber, chosen to be representative of fiber composition in our diet, may interact with calcium ions in aqueous solutions at experimental conditions approximating some of the physiological conditions of the gastrointestinal tract.

MATERIALS AND METHODS

Components of Fiber. Insoluble fiber components were lignin (Therapharm, Ltd., Dowhan Market, Norfolk, U.K.) and cellulose (Sigma Chemical Co., St. Louis, MO). The soluble fiber component was an apple pectin (Sigma Chemical Co., St. Louis, MO) stated as having 76% galacturonic acid content and a methoxy content of 7%.

Reagents and Materials. All reagents were of analytical grade. Anhydrous calcium chloride ($CaCl_2$), fused granular about 0.5–2.0 mm, was obtained from Merck. Solutions were prepared with distilled deionized water.

All glassware was acid washed with HNO_3 (1:1, v/v) and distilled deionized water.

Apparatus. A Perkin-Elmer Model 2380 atomic absorption spectrophotometer with an air–acetylene flame (impact bead and corrosion resistant nebulizer) was used to measure Ca^{2+} at 422.8 nm of wavelength (slit, 0.7 nm; intensity of the monoelemental hollow cathode lamp, 30 mA).

* Author to whom correspondence should be addressed.
† Present address: Instituto de Nutricion (CSIC-UCM), Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain.

0021-8561/92/1440-1762$03.00/0

The apparatus for pH measurements was a MicropH 2000 Crison pH meter, with a combined glass/reference electrode designed for measurements within a wide temperature range.

Calcium Analysis. For calcium determinations, the concentration of this metal was read directly, in duplicate, from the atomic absorption spectrophotometer, using appropriate standards (within the range 0–5 ppm of Ca) prepared from the 1000 ppm of Carlo Erba atomic absorption reference solution of calcium. Lanthanum chloride, to a final concentration of 0.4 % (wt/v), was added to each sample and standard to prevent the formation of calcium phosphate compounds.

Procedure. The method of mixing the sample with calcium solutions and following centrifugation after equilibration for quantitative measurement of soluble calcium in the supernatant was considered the best procedure to investigate mineral binding by insoluble fiber constituents (Rendleman, 1982; Garcia-Lopez and Lee, 1985; Wieber et al., 1988).

In the study of calcium binding properties of pectin, the necessity for the equilibrium dialysis system was evident in view of its solubility in water even at 20 °C (Ha et al., 1989; Schlemmer, 1989; Kelly and Potter, 1990).

Calcium Binding by Lignin and Cellulose. Calcium binding to insoluble fiber components was studied by a binding–centrifugation model system (at room temperature). Duplicate samples of, approximately, 100 mg of lignin or cellulose were weighed into screw-capped centrifuge tubes of 50 mL capacity and 25 mL of calcium solution were added after an approximate adjustment to the desired pH with diluted HCl or NaOH, in order to keep the final ionic strength as low as possible. The tubes were then stoppered and shaken for 3 h, time which was sufficient to achieve equilibrium between fiber and calcium ions in aqueous media. The reaction mixtures were then centrifuged for 15–30 min at 1500*g*, and the resulting supernatants were subsequently checked for pH and analyzed for equilibrium calcium ion concentration by atomic absorption spectrometry (AAS).

Lignin and cellulose (ca. 100 mg) were also evaluated for endogenous calcium solubilization in water as a function of pH, in order to make blank corrections in binding experiments. With this purpose, parallel studies were done in which calcium was omitted and fiber samples were shaken with 25 mL of distilled deionized water.

Binding was studied as a function of initial mineral concentration (calcium concentration in solution was varied from 0 to 5 μequiv of cation mL^{-1}), initial pH of the calcium solution (over the pH range 3.5–7.5) and fiber concentration (from 0 to 12 mg of fiber constituent mL^{-1} of solution).

Calcium Binding by Pectin. A equilibrium dialysis system (at 29.8 ± 0.2 °C) was used to study the interactions between calcium and apple pectin. A mixture of, approximately, 15 mg of pectin and 5 mL of calcium in aqueous solution, contained in a cellulose wet membrane (Spectra/Por 6, Spectrum Medical Industries, Inc., LA) of 100-mm length, 34-mm width, 21.6-mm diameter, and 25 000 MW cutoff, was dialyzed against 100 mL of distilled deionized water. The system was shaken for 24 h at 100 oscillations min^{-1}, to complete dialysis. After binding equilibrium was reached both the internal and external solutions were centrifuged (30 min, 1500*g*), and then the supernatants were checked for pH and analyzed for calcium by atomic absorption spectrometry. In order to correct for endogenous calcium dialyzability, control or blank experiments (dialysis of suspensions of pectin in water and dialysis of solutions containing calcium and no fiber) were also made in parallel.

Calculations. The amount of bound calcium (BC) in an insoluble form by lignin and cellulose was calculated from the calcium equilibrium concentration in the supernatant (EC), the initial metal ion concentration (IC), and the mass of fiber (*m*). The expression was

$$BC = (IC - EC^*)/m \text{ (expressed in } \mu\text{equiv } g^{-1}) \quad (1)$$

or

$$BC = 100 \times (IC - EC^*)/IC \quad (2)$$

where

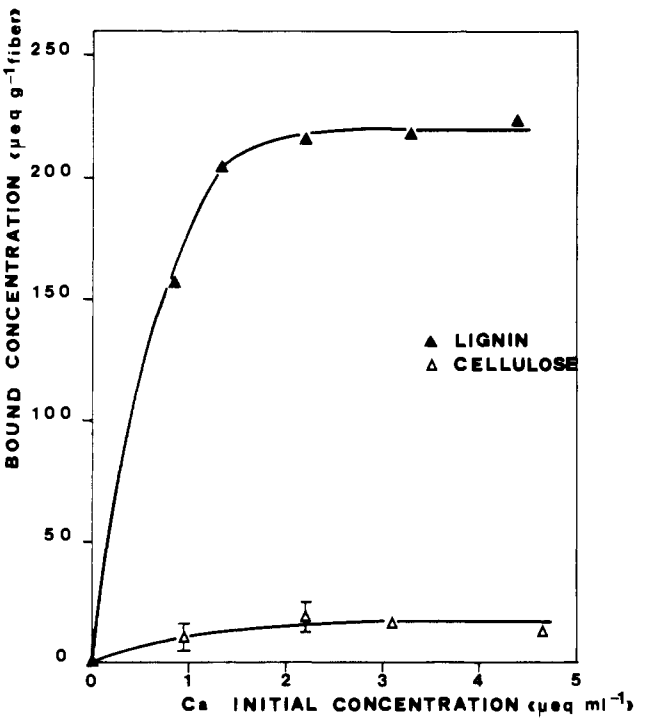

Figure 1. Effect of initial Ca(II) concentration on calcium binding by lignin (4.15 ± 0.12 mg of fiber mL^{-1} of solution) and cellulose (4.11 ± 0.12 mg of fiber mL^{-1} of solution) when pH was constant (5.60 ± 0.000, for lignin, and 5.63 ± 0.06, for cellulose). Results are referred to dry matter of the fiber constituents. The perpendicular bars show ± 1 SD.

$$EC^* = EC - EC_b \text{ (}\mu\text{equiv mL}^{-1}\text{)} \quad (3)$$

was the calcium concentration in the supernatant, corrected by the soluble endogenous calcium concentration of blank or control experiments (EC_b).

Assays were performed at least twice. Results are expressed as the mean value of binding determinations.

Calcium bound by pectin was calculated from the metal content of the internal solutions or retentate (RC), corrected by the calcium content of blank experiments (RC_b):

$$BC = 100 \times [IC - (RC - RC_b)]/IC \quad (4)$$

where

$$RC_b = RC_{bPectin} + RC_{bCalcium} \quad (5)$$

The results of bound concentration are expressed as the mean value of four determinations.

RESULTS AND DISCUSSION

Calcium Binding by Lignin and Cellulose. Results obtained from control experiments, when lignin and cellulose were mixed with water at room temperature, showed that no naturally occurring calcium was released by these fiber components over the pH range used in our investigations. Thus, the residual calcium content of lignin and cellulose did not interfere with the analysis of mineral binding.

Effect of Initial Calcium Concentration on Binding. The influence of calcium concentration in solution on binding by lignin and cellulose when pH and fiber concentration were constant is illustrated in Figure 1. It was apparent from these graphs that lignin exhibited a higher capacity for calcium retention than cellulose.

Binding by lignin increased at higher initial metal ion concentration. The relationship was linear over the concentration range 0–1 μequiv mL^{-1} but a constant value of bound calcium concentration was reached when initial calcium in solution was increased from 1 to 4.5 μequiv

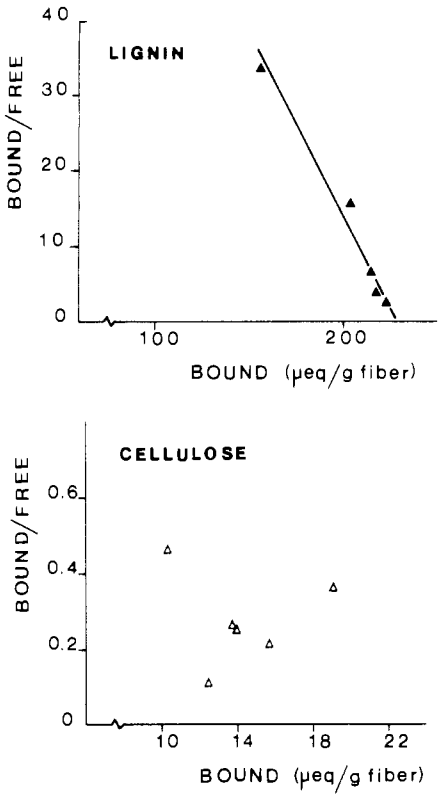

Figure 2. Scatchard plots of the binding of calcium by lignin and cellulose. Estimates of binding are based on the means of measurements whose results are illustrated in Figure 1.

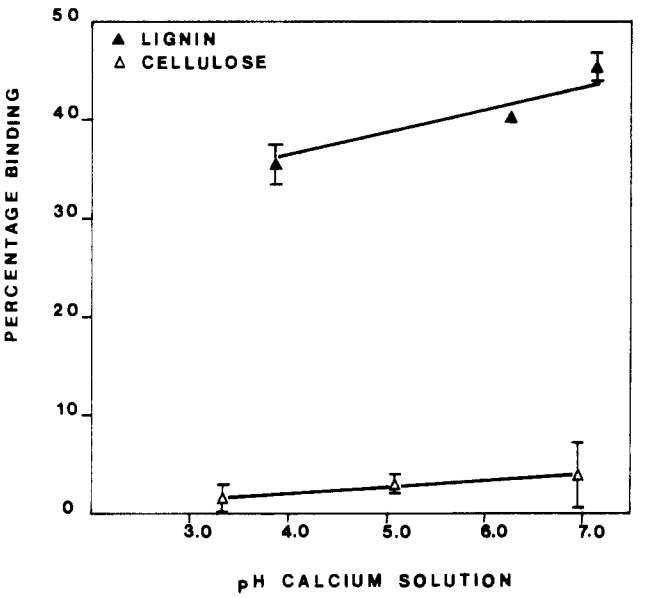

Figure 3. Influence of solution pH on calcium binding to lignin (4.14 ± 0.13 mg of fiber mL^{-1} of solution) and cellulose (4.00 ± 0.04 mg of fiber mL^{-1} of solution) when initial calcium concentration was constant (2.25 ± 0.03 and 2.26 ± 0.02 μequiv mL^{-1}, for lignin and cellulose, respectively). Results are expressed as a percentage of initial calcium concentration in solution. The perpendicular bars show ± 1 SD.

Table I. Change of pH Resulting from Addition of Lignin and Cellulose to Calcium Solutions at Different pH's

lignin	pH_i[a]	3.88	6.28	7.14
	pH_e[b]	5.70 ± 0.09	5.66 ± 0.00	6.31 ± 0.01
cellulose	pH_i	3.32	5.07	6.96
	pH_e	3.35 ± 0.01	4.86 ± 0.06	6.92 ± 0.00

[a] Initial pH of calcium solution. [b] pH of the supernatant solution when binding equilibrium between fiber constituents and calcium was reached. Results are expressed as the mean of two values of pH ± standard deviation.

mL^{-1}. It means that lignin became saturated with calcium at a fiber to metal ion ratio of, approximately, 100:0.5 (milligrams of fiber to milligram of calcium). Maximum calcium binding by lignin was 220 μequiv g^{-1} of fiber (dry matter).

Calcium interactions with cellulose were of little or no importance. Moreover, no relationship was found between bound calcium by this polysaccharide and the metal ion concentration in solution. The maximum amount of calcium bound by cellulose was 20 μequiv g^{-1} of fiber (dry matter).

If the results shown in Figure 1 are replotted according to Scatchard (Rosenthal, 1967; Pennock, 1973), in order to elucidate the possible mechanisms which describe the binding of calcium with the insoluble fiber components (Figure 2), it seems that lignin bound calcium according with the theory of multiple equilibria, with formation of a stoichiometric bond (Kohn, 1987). In effect, when the quotient bound concentration to free concentration was plotted against bound concentration a straight line was found, indicating only one type of binding site for calcium in lignin. The stability constant or the intrinsic association constant (K_{eff}) (Rosenthal, 1967; Platt and Clydesdale, 1987; Ha et al., 1989), calculated from the slope of this straight line, was 2.4×10^4 M^{-1}. Thus, lignin may strongly interact with calcium through formation of stoichiometric bonds involving those easily ionizable functional groups (carboxyl, methoxy, and hydroxyl) of its structure (Selvendran, 1984; Van Soest and Jones, 1988; Torre et al., 1991). With respect to the kind of bond, whether it is electrostatic or a chelate remains an open question. Moreover, the contribution of other mechanisms such as adsorption or ion exchange should not be completely excluded (Torre, 1991).

On the contrary, cellulose did not associate with Ca(II) in accordance with the theory of multiple equilibria since there was no binding line which satisfied the equation of Scatchard. Calcium could probably be retained by adsorption to the surface of this glucose polymer (Torre, 1991), which would explain the weak binding of this cation on cellulose.

Effect of pH on Binding. The study described herein was designed to examine the capacity of lignin and cellulose to bind Ca(II) when pH are brought up from 3.5 to neutrality, approximating in vivo conditions in the gastrointestinal tract.

The influence of pH in the range 3.5–7.5 on the degree of binding by lignin and cellulose is shown in Figure 3.

The data plotted in this figure indicated that lignin bound calcium over a wide range of pH. At higher levels of pH the potential binding capacity of lignin was moderately higher. It could be probably attributed to increased ionization of functional groups of this polymer with increasing pH (Leight and Miller, 1983; McBurney et al., 1986; Wieber et al., 1988). The release of protons from the easily ionizable functional groups of lignin at less acidic conditions of the calcium solution resulted in a small but reproducible decline in pH of the supernatant solutions in which the fiber was suspended (Table I). On the contrary, when pH was initially low (pH 3.88) the mass action law did not favor a change of ionization of the functional groups of lignin, and thus, no release of H^+ ions occurred. At this pH or near, calcium may be retained by adsorption to those binding sites which had a great charge density or were sterically accesible to calcium ions.

From Figure 3 the extremely small interaction between cellulose and Ca(II) in the pH range studied is evident.

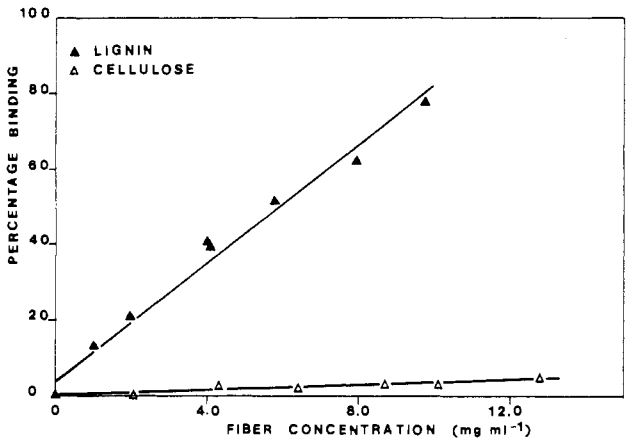

Figure 4. Effect of lignin and cellulose concentration on calcium binding. Results are expressed as a percentage of initial calcium concentration in solution. Initial Ca(II) concentration (μequiv mL^{-1}) and pH was, respectively, 2.26 ± 0.02 and 6.26, for lignin, and 2.21 ± 0.01 and 5.63, for cellulose.

The pH had little or no effect on calcium binding by this polysaccharide, which corroborated that the binding ability of cellulose was not related to the hydroxyl functional groups of its polymeric chains. This is in agreement with the fact that calcium binding by cellulose was not associated with a significant decrease in pH of the supernatant solutions (Table I).

Only limited studies have been done on interactions of calcium with lignin in vitro. However, the binding of calcium by cellulose has been studied by some authors. Our findings agree with those of Rendleman (1982) and Camire and Clydesdale (1981), who found that cellulose had little ability to bind calcium over the physiological pH range 5–8.

Effect of Fiber Concentration on Binding. The relationship between initial lignin and cellulose concentration in aqueous solution (over the range 0–4.5 mg of fiber mL^{-1}) and their affinity for calcium is shown in Figure 4. It is apparent from these results that the extent of calcium binding by lignin in systems of identical pH and metal ion concentration was proportional to fiber concentration in solution. The cause of this pronounced increase in the percentage binding by lignin was the considerable rise in the number of functional groups in solution which may act as ligands of calcium.

On the contrary, addition of increasing levels of cellulose to calcium solutions did not modify the low capacity of this polysaccharide for binding of calcium. This behavior confirmed the fact that the hydroxyl groups of cellulose did not interact with calcium through complex formation. Thus, the contribution of other mechanisms, probably metallic adsorption to the surface of the polymer, which determine the retention capacity of cellulose should be considered (Southgate, 1987; Torre, 1991).

Calcium Retention by Pectin. The results of the study done to measure the effect of pH on the release of endogenous calcium from low-methoxylated pectin when this polysaccharide (14.0 ± 0.1 mg) was dialyzed against distilled deionized water showed that when initial pH of the internal solution was increased from 5.68 to 7.00 the amount of calcium in the retentate, assuming a final state of calcium equilibrium, increased from 9.02 to 10.57 mg Ca g^{-1} of pectin (dry matter), while the concentration of calcium diffused across the dialysis membrane decreased from 9.78 to 5.19 mg of Ca g^{-1} of pectin (dry matter). This is in agreement with the results of Thompson and Weber (1979), who found that most of the residual minerals from different fiber sources were released into solution at the very acidic pHs but were rebound when the pH was raised to near pH 6.8.

At constant amounts of pectin (2.78 mg of pectin mL^{-1} of solution), the addition of Ca(II) ions at constant concentrations (6.58 ± 0.00 μequiv mL^{-1} of solution) exceeding the stoichiometric relation of two carboxyl groups of pectin to one Ca^{2+} ion and different pH values (pH of calcium solutions varied from 5.5 to 7.0) resulted in very little interaction between the metal ion and the soluble polysaccharide. Moreover, change of pH had little effect on binding affinity of pectin. In effect, the percentage of calcium in the retentate varied only from 7.0 to 10.0 when initial pH of calcium solutions added to pectin increased from 5.62 to 7.05. Thus, low-methoxylated pectin did not significantly modify the calcium content of aqueous solution in equilibrium with this fiber constituent. One explanation for the low calcium binding capacity of low-methoxylated pectin may be the high concentration of endogenous calcium of this hydrocolloidal polysaccharide, which complicated the retention process by contributing to the total mineral content of the system. Our findings agree with those of Rendleman (1982).

Previous studies on interactions between calcium and D-galacturonic acid, the main monomeric unit of pectin, in aqueous media (Rendleman, 1982; Laszlo, 1987; Deiana et al., 1989) and those undertaken on the interactions of D-galacturonic acid and other monouronates with Ca(II) ions (Kohn et al., 1968; Malovíková and Kohn, 1983; Kohn and Hirsch, 1986) provided sufficient evidence for the little calcium binding capacity of these molecules. On the basis of these results it seems that in the calcium binding by pectin, the predominant mechanism of interaction was different from complex formation, since the carboxyl group of the D-galacturonic monomeric unit of this polysaccharide did not bind calcium ions. Thus, it is very likely that the calcium adsorption to the surface of pectin should explain the minimal binding of this metallic cation.

It is clear from the in vitro research presented in this article that lignin is a potent binder of calcium ions in aqueous solution and, thus, has a strong capacity to bind this metal ion. The predominant mechanism of binding seemed to be complex formation through the easily ionizable functional groups of this polymer. Cellulose and low-methoxylated pectin are quantitatively less active than lignin. The calcium adsorption to the surface of both cellulose and pectin may explain the minimal binding by these fiber constituents.

ACKNOWLEDGMENT

We acknowledge the financial support for the research from Comision Asesora de Investigacion Cientifica y Tecnica (Project ALI-89-0551).

LITERATURE CITED

Camire, A. L.; Clydesdale, F. M. Effect of the pH and heat treatment on the binding of calcium, magnesium, zinc, and iron to wheat bran and fractions of dietary fiber. *J. Food Sci.* 1981, *46*, 548–551.

Davies, N. T. The effects of dietary fibre on mineral availability. In *Dietary Fibre: Current Developments of Importance to Health*; Heaton, K. W., Ed.; Libbey: London, 1978.

Deiana, S.; Gessa, C.; Solinas, V.; Piu, P.; Seeber, R. Analytical study of the interactions of D-galacturonic acid with iron(III) and iron(II) in solution and with iron(III)-bentonite. *Anal. Chim. Acta* 1989, *226*, 315–322.

Garcia-Lopez, J. S.; Lee, K. Iron binding by fiber is influenced by competing minerals. *J. Food Sci.* 1985, *50*, 424–425; 428.

Ha, Y. W.; Thomas, R. L.; Dyck, L. A.; Kunkel, M. E. Calcium binding of two microalgal polysaccharides and selected industrial hydrocolloids. *J. Food Sci.* **1989**, *54*, 1336–1340.

Kelly, B. J.; Potter, N. N. Dialyzable calcium from milk processed with soluble fiber-containing gums, thickeners, and cocoa. *J. Food Sci.* **1990**, *55*, 1004–1007.

Kelsay, J. L. Effects of fiber, phytic acid, and oxalic acid in the diet on mineral bioavailability. *Am. J. Gastroenterol.* **1987**, *82*, 983–986.

Kohn, R. Binding of divalent cations to oligomeric fragments of pectin. *Carbohydr. Res.* **1987**, *160*, 343–353.

Kohn, R.; Furda, I.; Haug, A.; Smidsrod, O. Binding of calcium and potassium ions to some polyuronides and monouronates. *Acta Chem. Scand.* **1968**, *22*, 3098–3102.

Kohn, R.; Hirsch, J. Binding of calcium, lead, and copper(II) cations to galactaric and 2,5-furandicarboxylic acids and to D-galacturonic acid and its derivatives. *Collect. Czech. Chem. Commun.* **1986**, *51*, 1150–1159.

Laszlo, J. A. Mineral binding properties of soy hull. Modelling mineral interactions with an insoluble dietary fiber source. *J. Agric. Food Chem.* **1987**, *35*, 593–600.

Leight, M. J.; Miller, D. D. Effects of pH and chelating agents on iron binding by dietary fiber: implications for iron availability. *Am. J. Clin. Nutr.* **1983**, *38*, 202–213.

Malovíková, A.; Kohn, R. Binding of zinc cations to pectin and its oligomeric fragments. *Collect. Czech. Chem. Commun.* **1983**, *48*, 3154–3165.

McBurney, M. I.; Allen, M. S.; Van Soest, P. J. Praseodymium and copper cation-exchange capacities of neutral-detergent fibres relative to composition and fermentation kinetics. *J. Sci. Food Agric.* **1986**, *37*, 666–672.

Pennock, B. E. A calculator for finding binding parameters from a Scatchard plot. *Anal. Biochem.* **1973**, *56*, 306–309.

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 neutral detergent fiber under simulated duodenal pH conditions. *J. Food Sci.* **1987**, *52*, 1414–1419.

Poneros-Schneier, A. G.; Erdman, J. W. Bioavailability of calcium from sesame seeds, almond powder, whole wheat bread, spinach and nonfat dry milk in rats. *J. Food Sci.* **1989**, *54*, 150–153.

Rendleman, J. Cereal complexes: binding of calcium by bran and components of bran. *Cereal Chem.* **1982**, *59*, 302–309.

Rosenthal, H. E. A graphic method for the determination and presentation of binding parameters in a complex system. *Anal. Biochem.* **1967**, *20*, 525–532.

Schlemmer, U. Studies of the binding of copper, zinc and calcium to pectin, alginate, carrageenan and guar gum in HCO_3^--CO_2 buffer. *Food Chem.* **1989**, *32*, 223–234.

Selvendran, R. R. The plant cell wall as a source of dietary fiber: chemistry and structure. *Am. J. Clin. Nutr.* **1984**, *30*, 320–327.

Southgate, D. A. T. Minerals, trace elements, and potential hazards. *Am. J. Clin. Nutr.* **1987**, *45*, 1256–1266.

Thompson, S. A.; Weber, C. W. Influence of pH on the binding of copper, zinc and iron in six fiber sources. *J. Food Sci.* **1979**, *44*, 752–754.

Torre, M. Sc.D. thesis, Universidad de Alcala de Henares, Madrid, 1991.

Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Binding of minerals by new sources of high dietary fibre content. In *Dietary Fibre: Chemical and Biological Aspects*; Southgate, D. A. T., Waldron, K., Johnson, I. T., Fenwick, G. R., Eds.; AFRC Institute of Food Research, The Royal Society of Chemistry: Norwich, 1990.

Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Effects of dietary fiber and phytic acid on mineral availability. *CRC Crit. Rev. Food Sci. Nutr.* **1991**, *1*, 1–22.

Van Soest, P. J.; Jones, L. H. P. Analysis and classification of dietary fibre. In *Trace Element Analytical Chemistry in Medicine and Biology*; Bratter, P., Schramel, P., Eds.; de Gruyter: New York, 1988.

Wieber, J.; Kulik, F.; Pethica, B. A.; Zuman, P. Sorptions on lignin, wood and celluloses. III. Copper(II) and zinc(II) ions. *Colloids Surf.* **1988**, *33*, 141–152.

Received for review February 26, 1992. Accepted June 2, 1992.

Registry No. Ca^{2+}, 7440-70-2; lignin, 9005-53-2; cellulose, 9004-34-6; low-methoxyl pectin, 9049-34-7.