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Effect of Gastrointestinal Conditions on the Mineral-Binding Properties of Dietary Fibers[1, 2]

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Investigations into the relationship between dietary fiber content and mineral-nutrient bioavailability of diets have generally shown an inverse relationship (Munoz, 1986). The inhibitory effect of fiber on mineral absorption, although perhaps more appropriately attributed to phytic acid in some studies, thus seems well established. The process by which fiber exerts its inhibitory effect is less well characterized. Three modes of inhibition have been hypothesized (Kelsay, 1981): 1) fiber greatly increases fecal bulk and motility, thus reducing the time available for absorption or access to transport mechanisms; 2) fiber directly or indirectly alters luminal-to-serosal transport mechanisms in the mucosa (such as the ferritin system); 3) the formation of stable, unabsorbable mineral-fiber complexes reduces the pool of available minerals. The latter hypothesis, utilized by most investigators, as well as in this work, posits that minerals bound to large macromolecules can not be absorbed directly nor transfer to mineral-transporter moieties. Thus, within the context of the subject matter of these proceedings, the present work addresses the issue of "To what extent might a plant-derived macromolecular complex (i.e., plant cell walls) impede mineral absorption?"

Dietary fiber may be simply characterized as being the undigestible fraction of plant cell walls, although more exact definitions are available (Trowell, 1974; Trowell, 1976). Cell walls are comprised of assorted polysaccharides (cellulose, pectins and hemi-celluloses), polyphenols (lignin and tannin), and proteins (Selvendran, 1984). The amount and type of each of these polymers in the cell wall varies considerably with the plant source. Thus, the relationship between mineral bioavailability and dietary fiber may vary substantially depending on the source of the fiber.

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Two disparate sources of dietary fiber, corn bran (pericarp) and soybean hull (seed coat), were examined in this study. The cell walls of corn, a monocot, and soybean, a dicot, differ substantially, particularly in their acidic polysaccharide content (Darvill et al., 1980). This difference should be reflected in the mineral-binding properties of the two tissues. The *in vivo* behavior of corn bran (Dintzis et al., 1985, 1989) and soybean hull (Dintzis et al., 1985; Ward & Reichert, 1986; Lykken et al., 1987; Moore & Kornegay, 1987) have been examined in relationship to mineral bioavailability, and various aspects of their mineral-binding attributes have been explored *in vitro* (Rasper, 1979; Thompson & Weber, 1979; Laszlo, 1987, 1988a,b). Both tissues are employed as ingredients in human and animal diets.

In this work, the mineral-binding properties of corn bran and soybean hull were compared under simulated monogastric gastrointestinal conditions. Although soluble pectins present in such tissues have demonstrable effects on mineral absorption (Monnier et al., 1980), only the insoluble fraction is treated here.

Experimental Procedures

Fiber sources. Dry-milled corn bran (18-30 mesh) was provided by Lauhoff Grain Co. (Danville, IL). Soybean seeds (cultivar Century) were obtained locally. Seeds were passed through a cracking mill. The hulls were collected by hand and ground to a coarse powder with a coffee mill.

Fiber preparation. For experiments in which the endogenous minerals of the fiber sources were examined, the fibers were washed extensively with 70% ethanol and then with acetone. The washed material was collected by filtration on a coarse glass filter, air dried, then stored under vacuum. The ethanol-washed corn bran and soybean hull fibers were suspended in 1.0 mM 4-morpholinepropanesulfonic acid (Mops) buffer, pH 7.2, at a concentration of 4 g fiber/L. The fibers were stirred for 2 h at room temperature. The buffer was decanted and the process repeated. The buffer-washed fibers were washed with acetone, collected by filtration, air dried, and stored under vacuum. For experiments concerned with the binding of added minerals, buffer-washed fibers were treated with 0.05 N HCl (25 g fiber/L) and washed extensively with water. The acid-extracted fibers were collected, dried and stored as above.

Analysis of endogenous-mineral content. Buffer-washed fibers were subjected to solutions of various ionic composition to observe the extent of mineral retention by the fibers. Buffer-washed fiber (0.2 g) was suspended in a 100-mL solution. For experiments in which only the effect of pH was studied, the solution/fiber mixture was pH adjusted with dilute HCl or KOH. For studies of the effect of ionic strength, the KCl concentration of 1.0 mM Mops buffered solutions were varied. In either case, fiber was equilibrated with solution for 2 h while stirring at 25°C. The treated fiber was collected by filtration, washed with acetone, and dried under vacuum overnight. The recovered fiber samples were wet ashed by a microwave digestion procedure. Digestion was performed in a Microwave Digestion System model MDS-81D (CEM Corp., Indian Trail, NC) operated in closed-vessel mode. Samples were contained in 20-mL scintillation vials placed within 100-mL capacity Teflon digestion vessels. Trace-metal-grade nitric acid (6 mL)

was added to the scintillation vials, then the samples were heated in three stages at 5, 15 and 25% power for 10 min at each stage. After cooling, the digestion vials were unsealed. Samples were taken to dryness (open-vessel mode) with a heating regime of 60, 80 and 100% power, 10 min each. The sample ash residue was suspended in 1 mL of 0.2 N HCl containing 0.5 mM ascorbic acid, then filtered through a Centrifree micropartition unit (Amicon Corp.), and stored frozen until analyzed.

Mineral analysis was performed with a Dionex 2010i series ion chromatography system. Transition metals were detected colorimetrically after post-column reaction with 4-(2-pyridylazo)resorcinol. Calcium and magnesium were detected by conductivity. The signal from either detector was fed to a Spectra-Physics 4270 reporting integrator for quantitation.

Analysis of added-mineral binding. The acid-extracted fibers were tested for their mineral-binding affinity and capacity in solutions of varying ionic composition. Acid-extracted fiber (0.1 g) was suspended in 100-mL buffered solutions. Buffered solutions were composed of 2.0 mM 4-morpholineethanesulfonic acid (Mes), and were adjusted to pH 6.0 with KOH. The use of Good's buffers (Mes or Mops) to control solution pH is justified because they don't bind cations (Good et al., 1966), nor should the zwitterionic or anionic forms of these buffers compete with cations for binding to the fibers. Solution ionic strength was adjusted by the addition of KCL. Indicated solution ionic strengths are based solely on the total K concentration, ignoring the contributions of other added ions (i.e., calcium or Mes). Calcium was added to the solutions to give total calcium concentrations of 0.05-2.5 mM. The solutions were stirred for 2 h, except where noted, and then the fiber was collected by filtration and washed sequentially with small aliquots of water and acetone. The collected fiber was dried, weighed, and extracted with 10 mL of 0.2 N HCL. The acid extract was filtered, and analyzed for calcium and potassium content by ion chromatography. Calculation of the solution free-calcium concentrations at equilibrium with the fiber was performed as described previously (Laszlo, 1987).

Results

The endogenous magnesium, calcium, iron and zinc contents of the corn-bran and soybean-hull fiber preparations are given in Table I. Soybean hull contained 10- to 20-fold greater quantities of calcium and iron. Thus, soybean hull represents a potentially richer source of nutritionally important minerals. However, the availability of minerals from these fibers for absorption may be limited by the extent to which the minerals are extracted from the matrix of the fiber under the conditions present in the gastrointestinal tract.

Simulation of stomach pH conditions. To examine the amount of minerals that may be extracted in the stomach, the fibers were subjected to treatment over a range of pH values expected to be encountered. The ionic strengths of these test solutions were not adjusted, thus were quite low. A more realistic simulation of stomach ionic conditions would include a supporting ionic strength of ~0.075 (Alexander, 1962), but this approach was not elected so that the individual effects

Table I. Mineral contents of corn bran and soy hull

Element	Mineral contents (μg/g dry wt)	
	corn bran	soy hull
Mg	400	1,360
Ca	150	3,870
Fe	15	200
Zn	10	20

of pH and ionic strength could be evaluated. The data depicted in Figure 1 demonstrate that little calcium or magnesium was removed from the fibers over the pH range 4.5-6.0. Below pH 4.5, calcium and magnesium were substantially extracted, with little or none remaining below pH 3.0. The behavior of zinc and iron in these tissues is strikingly different (Figure 2). At or above pH 4.0, zinc and iron were not extracted. Over the pH range 3.2-0.7, zinc in both tissues and iron in corn bran were progressively extracted. Much higher acid concentrations were required to remove iron from soybean hull. These results imply that the fibers bind zinc and iron more tightly than calcium or magnesium.

The acid extractability of soybean-hull iron was examined more closely. The cultivar of soybean utilized in this study contained approximately equal proportions of Fe(II) and Fe(III) in the hull (Laszlo, 1988a). Figure 3 shows the extent of extraction of each of these ions at various HCl concentrations. At HCl concentrations approaching the lower achievable physiological stomach pH range (~pH 1.0), Fe(II) was readily extracted, substantially more so than Fe(III). Only 2.0 N HCl was able to quantitatively remove Fe(III) from the soybean hull. More than 90% of the corn-bran iron was found to be in the Fe(II) oxidation state. This suggests that the difference between soybean-hull and corn-bran iron extractability (Figure 2) is due to the presence of the more tightly bound Fe(III) in soybean hull.

Simulation of small intestine ionic conditions. As food passes from the stomach to the intestinal tract, it mixes with secretions from the pancreas resulting in a nearly-neutral pH, high ionic strength solution. The mineral-binding behavior of corn-bran and soybean-hull fibers was examined under simulated small intestine ionic conditions.

It is presumed in these simulations that ingested fibers are in contact with digesta media sufficiently long to allow complete equilibrium of fiber and digesta ionic constituents to be achieved. The adequacy of this assumption was tested. Figure 4 demonstrates the time required for two samples of hydrated, acid-extracted corn bran to equilibrate with a solution containing calcium. Corn-bran fiber bound its maximum amount of calcium (i.e., reached equilibrium) within 30 min. Ninety-five percent of the equilibrium value was reached in about 15 min. This implies that ingested fiber should have little difficulty in equilibrating with digesta ions during transit, at least when fully hydrated and not enveloped in food boluses or fecal matter. Therefore, fiber-solution ion exchanges examined at equilibrium adequately reflect the *in vivo* state.

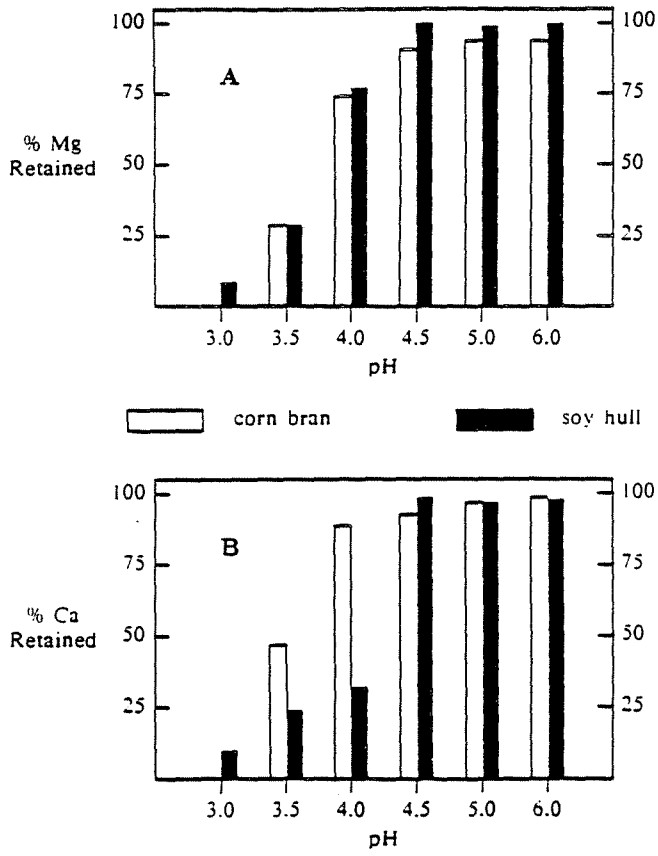

Figure 1. Effect of simulated gastric pH on Ca and Mg contents of fibers. Buffer-washed corn-bran and soybean-hull fibers were treated with solutions at indicated pH values, then examined for amounts of endogenous (A) magnesium and (B) calcium remaining in the fibers. See Table I for mineral contents of untreated samples.

Depending on the acidity of the stomach, the minerals in soybean hull and corn bran may be only partially extracted, as indicated by the results given above, before passing into the small intestine. Figure 5 shows that the calcium and magnesium present in these fibers are progressively more extracted in higher ionic strength media. Thus, the high salt conditions of the intestine should facilitate removal of calcium and magnesium. Note, however, that soybean hull retained a greater proportion of these ions than corn bran at any given ionic strength. This suggests that soybean hull binds calcium and magnesium more tightly. Also, at any given ionic strength, more calcium was retained than magnesium (percentage basis) in either fiber. Solutions with KCl concentrations as high as 150 mM released less than 10% of the iron and zinc from these samples (data not shown). Therefore, while any residual quantities of calcium or magnesium in the fibers leaving the stomach would be extracted in the small intestine, iron and zinc would remain bound.

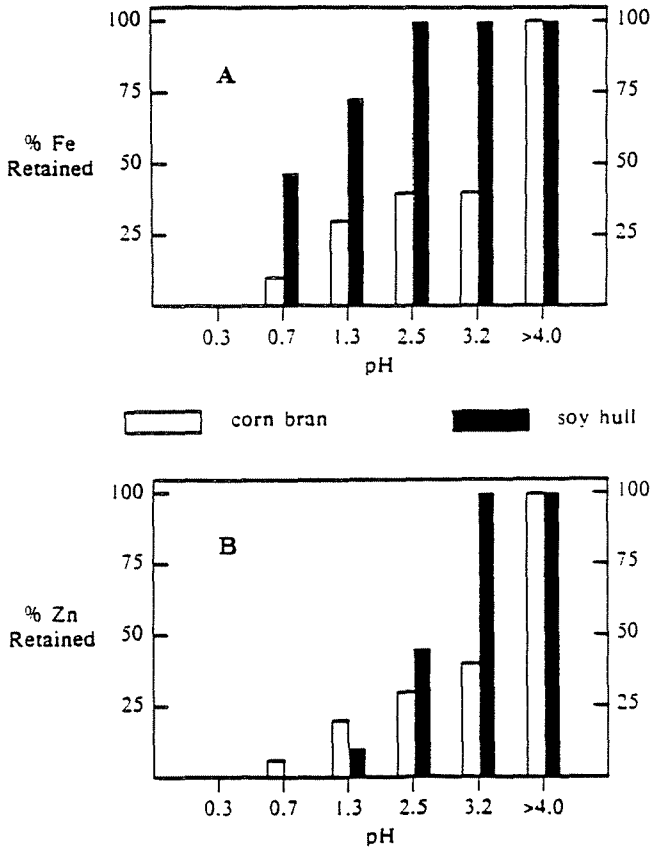

Figure 2. Effect of simulated gastric pH on Fe and Zn contents of fibers. Buffer-washed corn bran and soybean hull were examined for amount of (A) Fe (total) and (B) Zn retained after treatment in various pH solutions. The control (100%) values are given in Table I.

To test whether demineralized fiber emptying from the stomach may re-bind nutritionally important minerals in the small intestine, the mineral-binding capacity and affinity of corn bran and soybean hull were investigated. The total ion-binding capacity of the corn-bran fiber was 195±5 μeq/g dry wt. Figure 6 demonstrates the relationship between solution calcium concentration and the amount of calcium bound by acid-extracted corn bran in various ionic strength media. As expected, the extent of calcium bound was proportional (but not linearly so) to solution free-calcium concentration, and inversely proportional to solution monovalent cation concentration. In 100 mM KCl, calcium filled less than 25% of the corn-bran ion-exchange sites at solution calcium concentrations exceeding the likely physiological level (< 2.5 mM) of free calcium. This is in agreement with the observed effects of ionic strength on endogenous-calcium binding in corn bran (Figure 5).

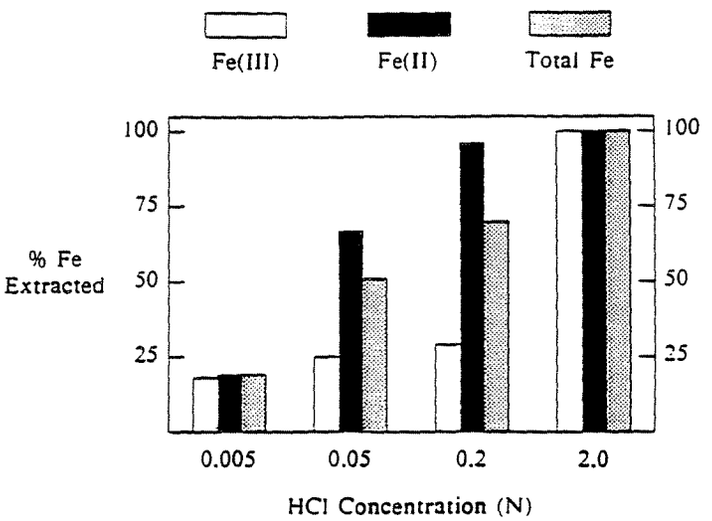

Figure 3. Effect of HCl concentration on iron contents of buffer-washed soybean hulls.

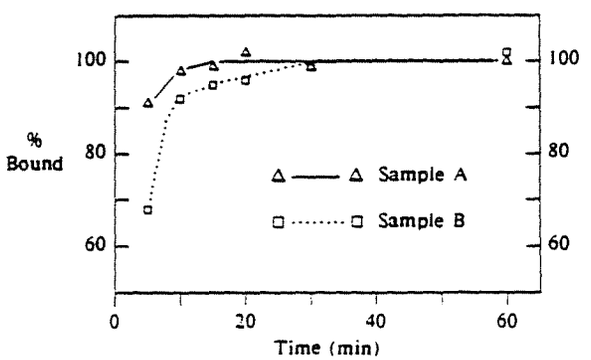

Figure 4. Time required for corn bran to equilibrate with calcium. Two identical samples (A and B) of acid-extracted corn bran (0.2 g) were suspended in 100-mL 2.0 mM Mes buffer, pH 6.0, containing 10 mM potassium. After rehydrating the corn bran for 1 h, Ca was added to a final concentration of 0.25 mM. At time intervals, aliquots (0.75 mL) were sampled to measure free Ca. Bound Ca values were calculated by subtraction of free values from the total (initial) value, and expressed as a percentage of the values found after 24 h.

In similar fashion, the relative affinities of acid-extracted soybean-hull and corn-bran fibers for calcium were compared. At any given solution concentration of calcium and monovalent cations, soybean hull bound a greater total amount of calcium (not shown) and filled a higher percentage of its available binding sites with calcium (Figure 7). This behavior is consistent with the indication of a

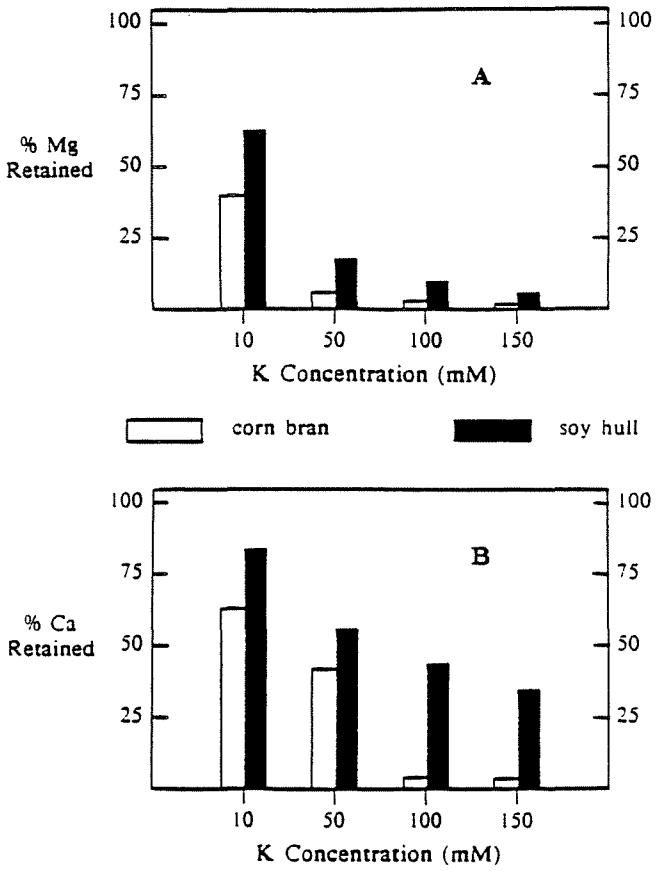

Figure 5. Effect of simulated small intestinal ionic conditions on Mg and Ca in fibers. Buffer-washed corn bran and soybean hulls were treated in 1.0 mM Mops buffer, pH 7.2, with KCl added to adjust the final solution K concentration to the indicated values. (A) Magnesium and (B) calcium contents were measured on the treated fibers and compared to the mineral values given in Table I.

greater binding affinity of soybean hull for calcium suggested by the data in Figure 5. The total ion-exchange capacity of acid-washed soybean hull was approximately twice that of corn bran.

Discussion

The vast majority of ion-exchange (i.e., cation binding) sites in dietary fiber are contributed by acidic polysaccharides present in the plant cell wall. Since dicot cell walls usually contain far more acidic polysaccharides than monocot cell walls, it is not surprising that the soybean hull tissue was found to have a higher cation-binding capacity, and a concomitantly higher level of endogenous minerals

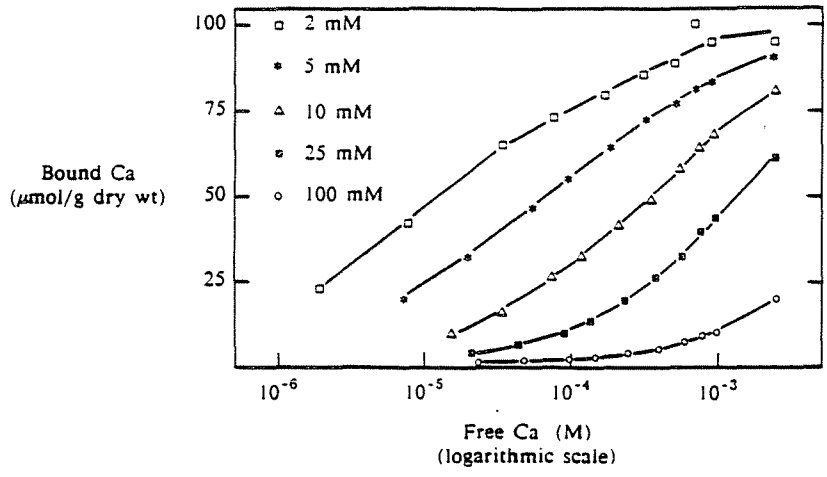

Figure 6. Relationship between calcium concentration, ionic strength, and calcium bound to corn bran. Acid-extracted corn bran was equilibrated with solutions of varying ionic strength (K concentration) and Ca, then examined for Ca content. (See Experimental Procedures for details.)

than corn bran tissue (Table I). In fact, much of the binding capacity of soybean hull is lost by the removal of pectins during the buffer-wash step (Aspinall et al., 1966, 1967; Schweizer & Würsch, 1979; Laszlo, 1987). Rasper (1979) found similar cation-exchange capacities of dietary fibers prepared by several different methods from corn bran to that reported here. Thus, while soybean hull may serve as a greater source of minerals than corn bran, it may potentially act as a greater sink for nutritionally important minerals under physiological conditions.

In addition to defining the total mineral binding capacity of a fiber (James et al., 1978), the uronic acid groups of the constituent acidic polysaccharides determine ion selectivity as well. Typically, plant cell walls display a relative binding affinity for divalent cations of: Cu > Fe, Zn > Ca > Mg (Van Cutsem & Gillet, 1982, 1983; Amory & Dufey, 1984). Previous work on acid-extracted soybean hulls demonstrated they too exhibit this trend (Laszlo, 1987). The extraction behavior of calcium and magnesium in soybean-hull and corn-bran fiber by protons (Figure 1) or salts (Figure 5) was entirely consistent with the notion that these ions are bound to uronic acid groups. However, it is unclear why soybean hull bound calcium more tightly than did corn bran under similar solution ionic conditions (Figures 5 and 7). Two causes can be suggested. The type of uronic acid comprising the acidic polysaccharide fraction of each tissue might be different, with different intrinsic affinities for calcium (and maybe magnesium). Alternatively, the concentration of uronic acid groups may differ, giving rise to different Donnan potentials between the fibers and test solutions (Dainty & Hope, 1961; Sentenac & Grignon, 1981). Resolution of this question will require further compositional and physical studies.

The properties of the endogenous iron and zinc, particularly iron in soybean hull, strongly suggest that these ions are bound to the fibers other than by uronic

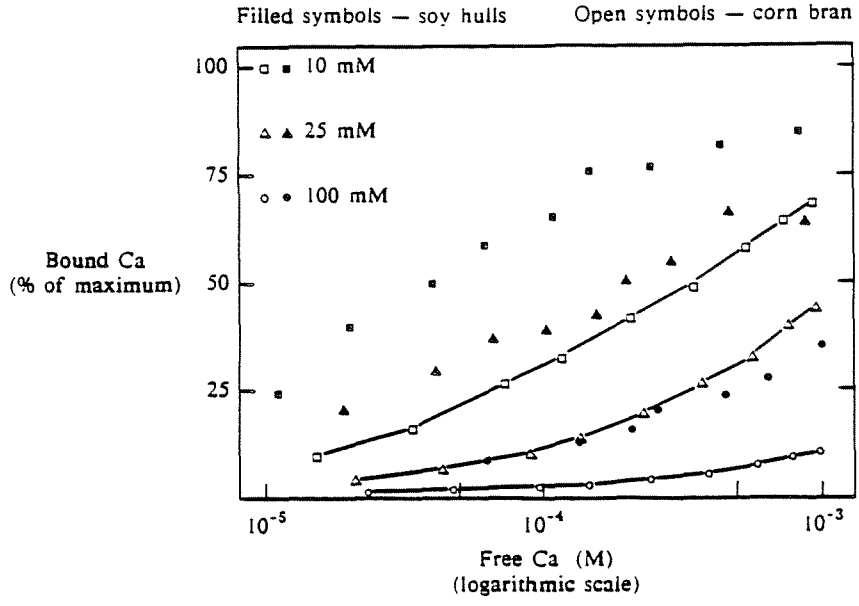

Figure 7. Comparison of calcium binding to corn bran and soybean hull. Acid-extracted corn bran and soybean hull were treated as in Figure 6.

acid groups. Both the very low pH required for extraction (Figures 2 and 3) and ineffectiveness of high salt concentrations are indicative of the presence of other types of mineral-binding sites in these fibers. The inability of low pH to extract iron from the soybean-hull tissue has been noted by others (Thompson & Weber, 1979). The unusual oxidation-reduction properties of the soybean-hull iron also suggests a unique binding site environment (Laszlo, 1988b). Phytic acid, a metal chelator commonly found in dietary fiber preparations, is not present in these tissues (Graf & Dintzis, 1982). Lignin, a potential iron-binding polymer (Fernandez & Phillips, 1982; Platt & Clydesdale, 1987), is present in these tissues at low levels (Rasper, 1979). However, the phenolic groups in lignin have such a high pKa that ferrous iron binding occurs only near neutrality (Reinhold et al., 1981). The tenacity with which soybean hull retains its iron at low pH seems to rule out a role for lignin in the binding of endogenous minerals. In fact, the release of the very tightly held iron at low pH may reflect a pH-induced structural change of the iron-binding site, rather than a simple proton-iron exchange reaction.

The applicability of the results reported herein are dependent on how well the *in vitro* conditions employed mimic *in vivo* states. *In situ* measurements of human stomach acidity suggest that pH 2.0 is an appropriate value to utilize when modeling normal (i.e., healthy) gastric conditions (Ovesen et al., 1986). Ingestion of food causes a transient pH rise, but the original gastric pH value is restored within about an hour (Ovesen et al., 1986). Therefore, it would be expected that all minerals except iron would be completely extracted, or nearly so, from the studied fibers in the human stomach. However, the certainty of such an assertion may be

questioned. Tadesse (1986) has recently demonstrated that the buffering capacity of ingested fiber is sufficient to significantly raise the pH (> 3.0) of the gastric contents. Thus the minerals may be less than completely solubilized.

The poor extractability of soybean-hull iron seems to be at odds with reports of its high bioavailability (Jacob et al., 1980; Lykken et al., 1987). Part of this apparent disparity may be due to the fact that the soybean-hull samples examined in this work were depleted of a soluble fraction representing 10-25% of the hull iron (Laszlo, unpublished observation). This iron may be associated with the solubilized pectins, and may be the source of bioavailable iron. Other components of the gastrointestinal tract (i.e., free amino acids, bile salts, etc), while effective in increasing iron solubility in certain food systems (Slatkavitz & Clydesdale, 1988), are not expected to increase the extractability of soybean-hull iron because strong iron-chelating agents such as EDTA are ineffective (Laszlo, unpublished observation). The one exception, ascorbic acid, has been shown to solubilize a substantial portion of soybean-hull iron (Laszlo, 1988a,b).

The mineral content of corn bran during passage through the swine gastrointestinal tract has been recently examined by Dintzis and co-workers (Dintzis et al., 1989). Their work indicated that the calcium content of corn bran actually increased in the stomach, contradicting the *in vitro* studies described here. One plausible explanation for this apparent discrepancy rests on the observation of Alexander (1962) that the pig stomach pH falls in the range of 4.2-5.1, substantially higher than human gastric pH and more nearly in the region where corn bran can bind significant quantities of calcium. This fact, coupled with the very high mineral supplementation of swine feed and the relatively low amount of calcium initially in corn bran, may produce conditions wherein calcium loading of bran proceeds in accordance with the *in vitro* model.

Although two diverse sources of dietary fiber were examined in this study, several potential mineral-binding components commonly found associated with other dietary fibers were not included. Lignins, tannins, phytic acid and silica all may significantly alter the mineral-binding properties of fiber, changing both its ion-exchange capacity and ion selectivity (or rather, providing multiple ion selectivities).

A critical question left unanswered by this research is whether fiber from corn bran or soybean hull, or dietary fiber in general, diminishes mineral bioavailability. If it does, then the present study suggests that fiber affects mineral absorption by a process other than by virtue of its inherent ion-exchange properties. Future research may well lead us to find that fiber, in-of-itself, has no significant adverse impact on human mineral nutrition. Such a conclusion, as yet unproven, will not negate the value of current investigations into fiber-mineral interactions. Work in this field has broader significance in terms of understanding the mechanisms of mineral absorption in the human gastrointestinal tract.

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