

# *In-vitro* mineral binding capacity of five fiber sources and their insoluble components for magnesium and calcium<sup>1</sup>

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*In-vitro* binding of calcium (Ca) and magnesium (Mg) by total dietary fiber, hemicellulose A (HCL A), lignocellulose (LCL), cellulose (CL), and lignin (L) fractions isolated from rice bran (RB), wheat bran (WB), oat fiber (OF), apple fiber (AF) and tomato fiber (TF) was evaluated. At pH 6.8, significant amounts of Ca were bound by whole fibers, ranging from 800  $\mu\text{g g}^{-1}$  for RB to 10 097  $\mu\text{g g}^{-1}$  for TF. Mg bound by whole fibers varied from 496  $\mu\text{g g}^{-1}$  for OF to 2177  $\mu\text{g g}^{-1}$  for WB. Re-acid washing (pH < 2.0) released 95–99% of the Ca and Mg bound to the fibers. Fibers with the highest endogenous Ca and Mg concentrations bound significantly ( $P < 0.05$ ) the highest amounts of the minerals studied. The Ca bound by HCL A varied from 9753  $\mu\text{g g}^{-1}$  for RB to 11 337  $\mu\text{g g}^{-1}$  for TF, whereas Mg bound varied from 1151  $\mu\text{g g}^{-1}$  for OF to 5626  $\mu\text{g g}^{-1}$  for TF hemicellulose fractions, respectively. Among the fiber components, Mg binding decreased in the order HCL A > LCL > L > CL, whereas Ca bound was in the order HCL A > LCL > CL > L. A relatively strong correlation was observed between the combined effects of protein content, hemicellulose, and lignin vs total Ca and Mg bound. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Interest in the extent to which selected fibers can bind and influence the bioavailability of divalent minerals has attracted the attention of many workers in the last two decades. Ward and Reichert (1986) reported no significant difference in the absorption of calcium, phosphorus and magnesium by young or adult rats fed wheat, corn, and oat fibers. Similarly, Bagheri and Gueguen (1981) found that addition of wheat bran to rat diets had no adverse effects on calcium, magnesium, phosphorus and zinc absorption. In humans, Moak *et al.* (1987) found that addition of oat and wheat bran to the diet of adult males decreased calcium and magnesium absorption. *In-vitro* binding capacity studies on various fiber sources for minerals showed that binding capacity varies not

only with fiber sources and their fractions but also with their protein content, phytic acid levels, and pH (Claye *et al.*, 1996a; Idouraine *et al.*, 1996a,b).

In studies relating to mineral binding capacity of fiber components such as cellulose, hemicellulose and lignin, there appear to be numerous speculations with little concrete evidence of support, particularly when the fractions studied were extracted from other sources different from fiber-rich foods. Of all the components of fiber, lignin has most consistently been shown to bind minerals with high affinity (Fernandez & Phillips, 1982; Platt & Clydesdale, 1984; Clydesdale *et al.*, 1991). Earlier studies (Branch *et al.*, 1975; James *et al.*, 1978) suggested that non-cellulosic polysaccharides of plant cell wall theoretically may be responsible for cation binding and a significant relationship was found between uronic acid content and calcium binding. McHale and co-workers (1979) reported significant amounts of calcium and magnesium excreted in the urine of adolescent humans fed purified cellulose and hemicellulose supplements in basal diets. Torre *et al.* (1992), in an

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experiment to study interactions of calcium ions with purified lignin, cellulose and pectin, found that lignin exhibited the highest affinity for calcium and the amount bound rose with increasing initial calcium concentration, pH and quantity of fiber. Although high pH levels increased the potential binding capacity of lignin for Ca, cellulose binding was not affected by pH changes. In an experiment comparing the effects of feeding rats diets containing hemicellulose vs cellulose, fecal excretion of most minerals including Ca and Mg was found to be higher in microcrystalline-cellulose supplemented diets than with rice hemicellulose (Mod *et al.*, 1985). In a previous study, the same authors (Mod *et al.*, 1982) showed that significant amounts of calcium and magnesium were bound by alkali-soluble hemicellulose but that these minerals were readily released by low pH and enzyme treatment.

Based on this literature review, there appears to be little agreement regarding which fibers interact with minerals. Moreover, most of the studies investigating the effect of fiber components utilize lignin and wood cellulose. Studies examining which fiber component in dietary sources is the potent binder of calcium and magnesium are still therefore needed. The purpose of this research was to investigate whether or not isolated fiber constituents, namely cellulose, hemicellulose and lignin bind calcium and magnesium and to what extent this binding compares with that of intact fiber found in fiber-rich food sources under conditions simulating those in the gastrointestinal tract.

## MATERIALS AND METHODS

### Fiber sources and sample preparation

Sources of whole fibers were as follows: Hard Red Spring wheat bran (American Association of Cereal Chemists, St. Paul, MN), rice bran (California Natural Products, Lathrop, CA), oat fiber (D. D. Williamson, Louisville, KY), apple fiber (Tree Top, Inc., Selah, WA) and tomato fiber (H. J. Heinz Co., Tracy, CA). The fiber constituents, namely cellulose, hemicellulose, lignocellulose, and lignin were isolated as previously described (Claye *et al.*, 1996a).

Whole fiber samples were treated according to the methods described by Weber *et al.* (1993). Twenty gram samples of various fibers were first defatted and ground to pass through a 60 mesh screen. Separate samples of the defatted fibers were taken for endogenous Ca and Mg analysis in duplicate. The remaining portions were acid-washed by mixing fiber with 1% HCl in a ratio 1:20 w/v, pH < 2.0. The mixture was shaken for 3 h and washed repeatedly with distilled deionized water until a pH of 7 was achieved and the residue air-dried. Defatted whole fibers and acid-washed defatted samples, and cellulose, hemicellulose and lignocellulose fractions were analyzed for protein and ash (AOAC, 1990). The fiber materials were then studied for their Ca and Mg binding

capacity according to the method of Weber *et al.* (1993). Duplicate 10-g samples of the various fibers were mixed with Ca or Mg standard solutions in a ratio 1:100 w/v and shaken overnight at 20°C. The slurry was filtered, repeatedly washed with distilled deionized water (pH 7) and then freeze-dried. Some of the mineral-fiber bound sample was saved for Ca and Mg analysis, while the remaining portion was treated with 1% HCl again. Acid-washed mineral-fiber bound samples were also analyzed for Ca and Mg.

### Ca and Mg analysis

Ca and Mg of bound and acid-washed mineral-fiber bound samples were determined in duplicate using a wet ashing method as reported previously (Claye *et al.*, 1996b). Acid-digested samples were quantitatively made up to a given volume in 10-ml volumetric tubes and appropriate standards were prepared from Fisher Scientific reference standards. Lanthanum chloride to a final concentration of 1% (wt/v) was added to sample solutions and standards to mask interference by phosphorus. After the appropriate dilutions were made, the minerals were determined by flame atomic absorption spectrophotometry (Hitachi Model 180-70) at wavelengths of 285.2 and 422.7 nm for Ca and Mg, respectively. Standards were run after every 10 samples.

### Statistical analysis

Data, expressed as mean  $\pm$  SD, were analyzed by analysis of variance (ANOVA) and *t*-test (Steel & Torrie, 1960) differences being significant when  $P < 0.05$ . The relationship between variables studied was analyzed using Pearson's correlations (Brunner & Kintz, 1987).

## RESULTS AND DISCUSSION

### Endogenous Mg and total binding by whole fibers

Endogenous Mg concentration varied significantly among the five fiber sources studied (Table 1). Values ranged from 519  $\mu\text{mg g}^{-1}$  for apple fiber to 8825  $\mu\text{mg g}^{-1}$  for rice bran. The results obtained in this study are within the range of values reported by Saunders (1990) and Idouraine *et al.* (1995) for oat, tomato, and apple fibers. Significant amounts (99%) of the endogenous Mg were removed during acid washing of the whole fibers. This may suggest that the low pH treatment was effective in releasing Mg, which was loosely bound between the interstices of the fiber constituents. Mod *et al.* (1982) similarly found that a high percentage of Mg was released by low pH treatment.

The affinity of wheat bran, rice bran, oat fiber, apple fiber and tomato fiber to bind Mg ions varied significantly. Mg bound varied from 496  $\mu\text{g g}^{-1}$  for oat fiber to 3249  $\mu\text{g g}^{-1}$  for rice bran. With the exception of apple

**Table 1. Endogenous magnesium content and total binding capacity of five fiber sources\* ( $\mu\text{g g}^{-1}$ )**

Types of fiber	Endogenous	Acid-washed	Total bound	Re-acid washed
Wheat bran	5953 $\pm$ 69 <sup>b,w</sup>	49 $\pm$ 10 <sup>a,y</sup> (99)	2177 $\pm$ 77 <sup>b,x</sup>	39 $\pm$ 5 <sup>a,y</sup> (99)
Rice bran	8825 $\pm$ 183 <sup>a,w</sup>	20 $\pm$ 6 <sup>b,c,z</sup> (99)	3246 $\pm$ 9 <sup>a,x</sup>	41 $\pm$ 7 <sup>a,y</sup> (99)
Oat fiber	771 $\pm$ 31 <sup>d,w</sup>	16 $\pm$ 2 <sup>b,c,y</sup> (99)	496 $\pm$ 28 <sup>e,x</sup>	10 $\pm$ 2 <sup>b,y</sup> (98)
Apple fiber	519 $\pm$ 22 <sup>e,x</sup>	6 $\pm$ 1 <sup>c,y</sup> (99)	1211 $\pm$ 89 <sup>d,w</sup>	17 $\pm$ 4 <sup>b,y</sup> (98)
Tomato fiber	3475 $\pm$ 64 <sup>c,w</sup>	22 $\pm$ 4 <sup>b,y</sup> (99)	1944 $\pm$ 103 <sup>c,x</sup>	32 $\pm$ 5 <sup>a,y</sup> (98)

\*Determined in duplicate fat free dry samples (Mean  $\pm$  SD).

<sup>a-e</sup>Mean values sharing a common superscript within columns are not significantly different ( $P < 0.05$ ).

<sup>w-z</sup>Mean values sharing a common superscript within rows are not significantly different ( $P < 0.05$ ).

Numbers in parentheses indicate percent reduction after acid washing.

fiber, levels of Mg bound dropped by 36% for oat fiber to about 67% for rice bran, compared with endogenous concentrations. Apple fiber, on the contrary, bound 57% more Mg than was originally found in the fiber. About 98–99% of the bound Mg was released when mineral-fiber bound material was re-acid washed. Similar results were obtained by Weber *et al.* (1993), and Idouraine *et al.* (1995, 1996b).

#### Endogenous Ca and total binding by whole fibers

Significantly different ( $P < 0.05$ ) levels of endogenous Ca were observed in the fiber samples (Table 2). The values varied from 701  $\mu\text{g g}^{-1}$  for rice bran to 2812  $\mu\text{g g}^{-1}$  for tomato fiber. Acid-washing released 92–99% of Ca originally found in the fibers. Bound Ca varied from 800  $\mu\text{g g}^{-1}$  for rice bran to 10 097  $\mu\text{g g}^{-1}$  for tomato fiber. These values are within the range of Ca levels reported by Weber *et al.* (1993). Although large amounts of Ca were bound, 91–99% was removed by re-acid washing. These results reaffirm the findings of Laszlo (1987), who found that at about neutral pH conditions divalent cations such as Mg and Ca can be bound to fiber in significant amounts, but may be readily released at low pH  $< 2$ . Some specificity for binding was exhibited by the fibers. While rice bran bound the highest amount of Mg, the same fiber source bound the lowest amount of Ca.

#### Ca and Mg content of fiber components

Varying amounts of Ca and Mg were found in cellulose, lignocellulose and hemicellulose fractions isolated from

the five fiber sources (Table 3). Rice bran and oat fiber sources contained significantly the highest and lowest Ca concentrations, respectively. Isolated lignin fractions showed no significant differences in their Ca content. Generally, the isolated fractions contained Ca levels in the order lignocellulose  $>$  hemicellulose A  $>$  cellulose  $>$  lignin. There were also significant variations in Mg concentrations of the isolated fractions from various fiber sources. Fractions isolated from rice bran again contained the highest Mg levels, whereas wheat bran fractions showed the lowest. Mg concentrations in fiber fractions decreased in the order lignocellulose  $>$  hemicellulose A  $>$  lignin  $>$  cellulose. Overall, the results indicate that hemicellulose fractions of the various fibers contained more Ca and Mg than the rest of the components. Mg content was significantly higher than Ca for all the fiber fractions.

#### Total Ca and Mg bound by fiber fractions

Total binding of Mg and Ca by hemicellulose A, lignocellulose, cellulose and lignin are presented in Table 4. All the fractions isolated from tomato fiber, except lignin, bound the largest amount of Ca. On the other hand, hemicellulose and lignin from rice bran, lignocellulose in apple fiber, and cellulose in oat fiber, bound significantly the smallest amounts of Ca, respectively. The various fractions studied were found to bind Ca in the order, hemicellulose A  $>$  lignocellulose  $>$  cellulose  $>$  lignin. As in Ca, the highest amounts of Mg bound were observed in all the fractions isolated from tomato fiber, except in the lignin component. The lowest Mg levels, however, were observed in all components of oat

**Table 2. Endogenous calcium content and total binding capacity of five fiber sources\* ( $\mu\text{g g}^{-1}$ )**

Types of fiber	Endogenous	Acid-washed	Total bound	Re-acid washed
Wheat bran	1259 $\pm$ 62 <sup>c,w</sup>	83 $\pm$ 17 <sup>b,x</sup> (93)	8153 $\pm$ 45 <sup>b</sup>	98 $\pm$ 6 <sup>a,x</sup> (99)
Rice bran	701 $\pm$ 16 <sup>d,w</sup>	38 $\pm$ 3 <sup>c,x</sup> (95)	800 $\pm$ 8 <sup>e</sup>	69 $\pm$ 13 <sup>b,x</sup> (91)
Oat fiber	1904 $\pm$ 23 <sup>b,w</sup>	21 $\pm$ 8 <sup>c,x</sup> (99)	1990 $\pm$ 26 <sup>d</sup>	7 $\pm$ 3 <sup>c,y</sup> (99)
Apple fiber	726 $\pm$ 412 <sup>d,w</sup>	56 $\pm$ 15 <sup>b,c,x</sup> (92)	4006 $\pm$ 65 <sup>c</sup>	71 $\pm$ 4 <sup>b,x</sup> (96)
Tomato fiber	2812 $\pm$ 12 <sup>a,w</sup>	145 $\pm$ 24 <sup>a,x</sup> (95)	10 097 $\pm$ 29 <sup>a</sup>	114 $\pm$ 6 <sup>a,x</sup> (99)

\*Determined in duplicate fat free dry samples (mean  $\pm$  SD).

<sup>a-e</sup>Mean values sharing a common superscript within columns are not significantly different ( $P < 0.05$ ).

<sup>w-z</sup>Mean values sharing a common superscript within rows are not significantly different ( $P < 0.05$ ).

Numbers in parentheses indicate percent reduction after acid washing.

**Table 3. Calcium (Ca) and magnesium (Mg) contents of insoluble fiber fractions\***

Fiber sources	HCLA	LCL	CL	L	HCLA	LCL	CL	L
	Ca ( $\mu\text{g g}^{-1}$ )				Mg ( $\mu\text{g g}^{-1}$ )			
Wheat bran	30 ± 2 <sup>b,x</sup>	82 ± 9 <sup>b,u</sup>	21 ± 4 <sup>c,x</sup>	61 ± 5 <sup>a,v</sup>	93 ± 10 <sup>c,t,u</sup>	144 ± 2 <sup>d,s</sup>	48 ± 3 <sup>b,w</sup>	96 ± 1 <sup>d,t</sup>
Rice bran	92 ± 6 <sup>a,w</sup>	131 ± 11 <sup>a,v</sup>	77 ± 5 <sup>a,w</sup>	54 ± 6 <sup>a,x</sup>	230 ± 19 <sup>a,t</sup>	279 ± 0 <sup>a,s</sup>	73 ± 6 <sup>a,w,x</sup>	206 ± 6 <sup>a,u</sup>
Oat fiber	25 ± 6 <sup>b,w</sup>	78 ± 17 <sup>b,u</sup>	7 ± 0 <sup>d,w</sup>	71 ± 17 <sup>a,u,v</sup>	158 ± 3 <sup>b,s</sup>	161 ± 3 <sup>c,s</sup>	51 ± 3 <sup>b,v</sup>	110 ± 0 <sup>c,t</sup>
Apple fiber	87 ± 28 <sup>a,u,v</sup>	107 ± 9 <sup>a,b,u</sup>	36 ± 0 <sup>b,w</sup>	76 ± 14 <sup>a,v</sup>	149 ± 15 <sup>b,t</sup>	182 ± 6 <sup>b,s</sup>	69 ± 8 <sup>a,v</sup>	113 ± 2 <sup>c,u</sup>
Tomato fiber	115 ± 18 <sup>a,t</sup>	113 ± 9 <sup>a,t</sup>	38 ± 2 <sup>b,v</sup>	75 ± 7 <sup>a,u</sup>	129 ± 17 <sup>b,t</sup>	192 ± 12 <sup>b,s</sup>	70 ± 9 <sup>a,u</sup>	127 ± 8 <sup>b,s</sup>

\*Determined in duplicate fat free dry sample (mean ± SD,  $n=2$ ).

HCLA, Hemicellulose A; LCL, Lignocellulose — consists of mainly cellulose and lignin;

CL, Cellulose; L, Lignin estimated by calculation (lignocellulose—cellulose).

<sup>a-e</sup>Mean values sharing a common superscript within columns are not significantly different ( $P < 0.05$ ).

<sup>t-z</sup> Mean values sharing a common superscript within rows are not significantly different ( $P < 0.05$ ).

**Table 4. Total binding capacity of insoluble fiber fractions for Ca and Mg\***

Fiber sources	HCLA	LCL	CL	L	HCLA	LCL	CL	L
	Ca ( $\mu\text{g g}^{-1}$ )				Mg ( $\mu\text{g g}^{-1}$ )			
Wheat bran	10 295 ± 142 <sup>b,s</sup>	6502 ± 87 <sup>b,t</sup>	3127 ± 17 <sup>c,v</sup>	3375 ± 99 <sup>b,u</sup>	1576 ± 11 <sup>d,x</sup>	2090 ± 62 <sup>d,w</sup>	1354 ± 56 <sup>b,y</sup>	736 ± 17 <sup>d,z</sup>
Rice bran	9753 ± 115 <sup>c,s</sup>	5352 ± 31 <sup>d,t</sup>	4727 ± 47 <sup>b,u</sup>	625 ± 23 <sup>e,z</sup>	2325 ± 14 <sup>c,v</sup>	2204 ± 10 <sup>c,w</sup>	1034 ± <sup>c,y</sup>	1170 ± 38 <sup>b,x</sup>
Oat fiber	10 161 ± 198 <sup>b,s</sup>	5980 ± 58 <sup>c,t</sup>	1768 ± 27 <sup>d,v</sup>	4212 ± 44 <sup>a,u</sup>	1151 ± 22 <sup>c,w</sup>	1596 ± 31 <sup>e,v</sup>	779 ± 16 <sup>d,x</sup>	816 ± 23 <sup>c,x</sup>
Apple fiber	10 427 ± 170 <sup>b,s</sup>	4774 ± 45 <sup>e,t</sup>	2203 ± 103 <sup>d,x</sup>	2571 ± 82 <sup>c,w</sup>	4087 ± 79 <sup>b,u</sup>	3558 ± 47 <sup>b,v</sup>	1443 ± 79 <sup>b,y</sup>	2416 ± 47 <sup>a,w</sup>
Tomato fiber	11 337 ± 96 <sup>a,s</sup>	8171 ± 33 <sup>a,t</sup>	6524 ± 60 <sup>a,u</sup>	1647 ± 38 <sup>d,v</sup>	5626 ± 102 <sup>a,v</sup>	4293 ± 25 <sup>a,w</sup>	3144 ± 62 <sup>a,x</sup>	1199 ± 18 <sup>b,z</sup>

\*Determined in duplicate fat free dry sample (mean ± SD,  $n=2$ ).

HCLA, Hemicellulose A; LCL, Lignocellulose — consists of mainly cellulose and lignin; CL, Cellulose; L, Lignin estimated by calculation (lignocellulose—cellulose).

<sup>a-e</sup>Mean values sharing a common superscript within columns are not significantly different ( $P < 0.05$ ).

<sup>t-z</sup> Mean values sharing a common superscript within rows are not significantly different ( $P < 0.05$ ).

fiber, except lignin. The general trend of Mg binding exhibited by the fractions was in the decreasing order hemicellulose A > lignocellulose > cellulose > lignin. Limited data were available to compare Ca and Mg binding capacity of fractions from similar fiber sources, except for rice bran. Alkali-soluble hemicellulose A, according to the study by Mod and co-workers (1982), bound Ca levels similar to those in our present study. In that same study, Mg levels bound were lower than Ca in hemicellulose, a finding which supports the results of our present study.

#### Ca and Mg remaining in fiber-bound components

The Mg and Ca bound at pH 6.8 were readily released during acid-washing (pH < 2.0) (Table 5). Significant amounts of Ca and Mg remained in the fiber fractions after acid-washing. All isolated fractions from rice bran and tomato fiber retained the highest amounts of Ca, whereas fractions from oat fiber contained the lowest. The fiber fractions retained Ca in the order lignocellulose > hemicellulose > lignin > cellulose. Some previous researchers (Fernandez & Phillips, 1982a; Platt & Clydesdale, 1984; Clydesdale *et al.*, 1991), have suggested that of all the fiber constituents, lignin is the potent binder of minerals. In this present study, however, the

results indicate that hemicellulose A (the type soluble in alkali) bound significantly higher amounts of Ca and Mg than lignin and cellulose at a physiological pH of 6.8. It can be noted that the predictions of the previous authors were based on either binding studies using commercial wood sources or estimations by difference, and not experiments involving the actual fiber fractions. In comparing the affinity of lignin and cellulose to bind Ca, our findings were not perfectly in agreement to those of Rendelman (1982) and Torre *et al.* (1992), who observed that lignin binds relatively higher Ca and retained more of the mineral than cellulose at low pH. The affinity of hemicellulose or lignin to bind Ca, as postulated by Wieber *et al.* (1988) and Torre *et al.* (1992), may be attributed to the increasing ionization of the functional groups of the polymers with increasing pH. At low pH, mass action may not favor ionization of exposed functional groups and as a result there is no release of H ions. Only those sites on the polymers which were densely charged or were sterically accessible to the metal ions might retain the mineral by adsorption. Cellulose, a linear glucose polymer, is not greatly affected by pH changes and this may explain the minimal binding by the fiber constituent. With respect to the type of fiber-mineral bond, it might be weaker than a hydrogen bond but it is unclear whether the bond is electrostatic or a chelate (Torre *et al.*, 1992).

Table 5. Ca and Mg contents of fiber-bound components after acid washing\*

Fiber sources	HCL A	LCL	CL	L	HCL A	LCL	CL	L
	Ca ( $\mu\text{g g}^{-1}$ )				Mg ( $\mu\text{g g}^{-1}$ )			
Wheat bran	113 ± 9 <sup>a,t,u</sup>	69 ± 7 <sup>c,u</sup>	36 ± 4 <sup>c,v</sup>	33 ± 4 <sup>b,c,v</sup>	123 ± 6 <sup>b,s</sup>	99 ± 3 <sup>d,t</sup>	62 ± 8 <sup>b,u</sup>	37 ± 7 <sup>b,v</sup>
Rice bran	120 ± 8 <sup>a,t</sup>	128 ± 3 <sup>a,t</sup>	74 ± 6 <sup>a,u,v</sup>	54 ± 4 <sup>a,v,w</sup>	178 ± 21 <sup>a,s</sup>	127 ± 10 <sup>c,t</sup>	81 ± 8 <sup>a,u</sup>	46 ± 3 <sup>b,w</sup>
Oat fiber	23 ± 5 <sup>c,v</sup>	36 ± 2 <sup>d,t</sup>	12 ± 2 <sup>d,w</sup>	24 ± 0 <sup>c,u,v</sup>	32 ± 0 <sup>d,t,u</sup>	59 ± 5 <sup>e,s</sup>	20 ± 0 <sup>d,v,w</sup>	39 ± 7 <sup>b,t</sup>
Apple fiber	65 ± 12 <sup>b,u,v</sup>	98 ± 15 <sup>b,u</sup>	33 ± 5 <sup>c,u,v</sup>	65 ± 14 <sup>a,u,v</sup>	100 ± 22 <sup>b,c,u</sup>	216 ± 19 <sup>a,s</sup>	66 ± 0 <sup>b,u,v</sup>	150 ± 27 <sup>a,t</sup>
Tomato fiber	119 ± 2 <sup>a,t</sup>	108 ± 14 <sup>a,b,t</sup>	49 ± 2 <sup>b,v</sup>	49 ± 3 <sup>a,b,v</sup>	84 ± 3 <sup>c,u</sup>	161 ± 9 <sup>b,s</sup>	43 ± 2 <sup>c,v</sup>	118 ± 10 <sup>a,t</sup>

\*Determined in duplicate fat free dry sample (mean ± SD,  $n=2$ ).

HCL A, Hemicellulose A; LCL, Lignocellulose — consists of mainly cellulose and lignin; CL, Cellulose; L, Lignin estimated by calculation (lignocellulose—cellulose).

<sup>a-e</sup> Mean values sharing a common superscript within columns are not significantly different ( $P < 0.05$ ).

<sup>1-2</sup> Mean values sharing a common superscript within rows are not significantly different ( $P < 0.05$ ).

Table 6. Linear regressions ( $y_1, y_2, y_3$ ), Pearson's coefficient correlation ( $r_1, r_2, r_3$ ) and multiple correlation ( $R_{1,2,3}$ ) of protein, lignin, and hemicellulose A vs total binding capacity of Mg and Ca for combined values of five fiber sources

Dependent variables <sup>a</sup> vs Independent variables	Magnesium	Calcium
Protein <sup>b</sup>	$y_1 = 95.16x + 654$ ( $r_1 = 0.896$ )	$y_1 = 153.55x + 3136$ ( $r_1 = 0.376$ )
Lignin	$y_2 = -45.03x + 1896$ ( $r_2 = -0.381$ )	$y_2 = -10.11x + 2632$ ( $r_2 = 0.042$ )
Hemicellulose A	$y_3 = 27.90x + 2310$ ( $r_3 = -0.133$ )	$y_3 = 28.08x + 9748$ ( $r_3 = 0.429$ )
	( $R_{1,2,3} = 0.873$ )	( $R_{1,2,3} = 0.430$ )

<sup>a</sup>Dependent variables (Mg and Ca) vs independent variables (protein content, lignin, hemicellulose).

<sup>b</sup>Protein ( $N \times 6.35$ ) content calculated from values reported previously (Claye *et al.*, 1996b).

### Correlations between some variables and total Ca and Mg binding

Protein, lignin and hemicellulose are among the constituents of fiber that have been speculated to be the potent binders of minerals. Linear regression, Pearson's correlation coefficient and multiple correlation coefficients of these variables vs total Ca and Mg binding capacity are reported in Table 6. Protein had the strongest correlation with total mineral bound over lignin and hemicellulose A. However, combined effects of all three variables gave much higher correlations with total mineral bound, particularly Mg. Factors such as phytic acid, initial mineral concentration, pH, and proteins have been associated with mineral binding (Idouraine *et al.*, 1996a). Overall, individual fiber components bound more Ca and Mg than the whole fibers. This present study suggested that treatments used in the preparation of fiber fractions may have exposed more binding sites in the polymers for Ca and Mg ions. The mechanisms involved, however, require further investigation. Although the initial Ca concentration was lower than for Mg in the fiber components (Table 3), binding affinity for Ca was significantly stronger regardless of fiber type. This finding is not consistent with those reported by Torre *et al.* (1992). These authors reported that the quantity of fiber bound rose as initial Ca concentration was increased. Other additional factors may be involved. Differences in fiber source and methodology employed may explain the inconsistency in results.

### CONCLUSION

Fibers varied in their ability to bind Ca and Mg. This binding ability was largely influenced by pH, lignin, hemicellulose, and protein content of the fiber source. Individual components, particularly hemicellulose A, bound significantly higher Ca and Mg than the whole fibers from the same source. However, exposure of the fiber-bound fractions to low pH levels  $< 2$  readily released the minerals. Overall, hemicellulose and not lignin was found to be the potent binder of Ca and Mg. Cellulose appeared to bind the least amount of Ca and Mg. These results may be useful to compare with studies in which commercial non-food sources have been utilized.

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