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Dietary Fibers from Mushroom Sclerotia: 2. In Vitro Mineral Binding Capacity under Sequential Simulated Physiological Conditions of the Human Gastrointestinal Tract

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The in vitro mineral binding capacity of three novel dietary fibers (DFs) prepared from mushroom sclerotia, namely, *Pleurotus tuber-regium*, *Polyporous rhinocerus*, and *Wolfiporia cocos*, to Ca, Mg, Cu, Fe, and Zn under sequential simulated physiological conditions of the human stomach, small intestine, and colon was investigated and compared. Apart from releasing most of their endogenous Ca (ranged from 96.9 to 97.9% removal) and Mg (ranged from 95.9 to 96.7% removal), simulated physiological conditions of the stomach also attenuated the possible adverse binding effect of the three sclerotial DFs to the exogenous minerals by lowering their cation-exchange capacity (ranged from 20.8 to 32.3%) and removing a substantial amount of their potential mineral chelators including protein (ranged from 16.2 to 37.8%) and phytate (ranged from 58.5 to 64.2%). The in vitro mineral binding capacity of the three sclerotial DF under simulated physiological conditions of small intestine was found to be low, especially for Ca (ranged from 4.79 to 5.91% binding) and Mg (ranged from 3.16 to 4.18% binding), and was highly correlated ($r > 0.97$) with their residual protein contents. Under simulated physiological conditions of the colon with slightly acidic pH (5.80), only bound Ca was readily released (ranged from 34.2 to 72.3% releasing) from the three sclerotial DFs, and their potential enhancing effect on passive Ca absorption in the human large intestine was also discussed.

KEYWORDS: Mushroom sclerotia; dietary fiber; in vitro mineral binding capacity; simulated gastrointestinal conditions

INTRODUCTION

Recommendation for the increase of dietary fiber (DF) intake has raised questions about their possible negative effects on mineral bioavailability, particularly in high-risk population groups such as the elderly, infants, and pregnant women (*1*, *2*). Although this issue has raised interest among many scientists for the last two decades, conflicting results from numerous in vitro (*2*−*4*) and in vivo (*5*−*8*) studies have made it difficult to draw a clear conclusion on this controversial subject.

By reviewing the adverse effects of DF on mineral bioavailability in previous literatures (*9*−*11*), three postulations on how DF might limit mineral bioavailability have been suggested. They are (i) shortening the transit time of nutrients when DF moves along the small intestine and thus reducing the time required for mineral absorption, (ii) directly or indirectly impairing the transportation of minerals when DF moves across the intestinal mucosa cells, and (iii) electrostatistic binding and/or trapping of minerals within DF particles, leading to the formation of stable, unabsorbable mineral−fiber complexes and thus reducing the pool of ionized minerals for absorption.

Because the last postulation is believed to be the main factor determining the undesirable effect of DF on the mineral bioavailability, in vitro mineral binding capacity of DF would be a crucial parameter for predicting its effect on mineral bioavailability in humans (*12*). When DF is ingested, it generally passes through three major gastrointestinal sections including the stomach, small intestine, and colon (*13*). These three gastrointestinal sections possess their unique physiological conditions (such as regional pH, ionic strength, and average transit time), which had been found to significantly affect the in vitro mineral binding capacity of DF in numerous previous studies (*12*, *14*−*16*). Other factors such as source, type, and physicochemical properties [e.g., cation-exchange capacity (CEC)] of the fiber (*14*) as well as the presence or absence of potent mineral chelators (e.g., proteins and phytic acid) had also been reported to have a correlation with the mineral binding capacity of DF (*18*−*20*). Nevertheless, in vitro methods for studying the mineral binding capacity of DF under sequential simulated physiological conditions of the human stomach, small intestine, and colon have been well-developed (*15*, *16*, *21*), as the physiological conditions of these three gastrointestinal sections in humans have been well-characterized in the past two decades (*12*, *13*, *22*, *23*).

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Interestingly, our preliminary studies showed that mushroom DF, especially the one prepared from sclerotium of *Pleurotus tuber-regium* using the typical AOAC enzymatic−gravimetric procedure, had a remarkably low in vitro mineral binding capacity at a pH similar to that of the small intestine (pH 7), and the bound minerals, especially calcium (Ca), were also readily released under a strong acidic medium (pH 1) (*24*). Therefore, in this study, three novel DFs prepared from mushroom sclerotia, namely, *P. tuber-regium* (Fr.) Sing., *Polyporous rhinocerus* Cooke, and *Wolfiporia cocos* (Schw.) Ryv. et Gilbn., were evaluated for their in vitro binding capacity on five nutritionally important divalent minerals including Ca, magnesium (Mg), copper (Cu), iron (Fe), and zinc (Zn) under sequential simulated physiological conditions of the human stomach, small intestine, and colon in order to predict their possible effects on mineral bioavailability in humans when they move along the gastrointestinal tract.

In the present study, the regional pH of the simulated colonic condition was adjusted to slightly acidic (5.80) instead of the nearly neutral physiological pH of the human large intestine (pH 6.5) (*25*), with a view to assessing whether the bound minerals would be released from the three sclerotial DFs in the colon, if their fermentability was high enough to produce a sufficient amount of short chain fatty acids that give this colonic pH value similar to that from other highly fermentable DF fractions (such as curdlan, apple, and oat fiber) reported previously (*26*−*28*). Furthermore, the potential enhancing effect of the three novel sclerotial DFs on passive Ca absorption in humans was also discussed.

MATERIALS AND METHODS

Sclerotial DF Preparation. Sclerotia of *P. tuber-regium*, *P. rhinocerus*, and *W. cocos* were obtained from the Sanming Mycological Institute in the Fujian Province of China. All sclerotia were cleaned, dried, and pulverized as previously described (*29*). The DFs of the three sclerotia was prepared by a modified AOAC procedure using industrial enzymes as reported in our previous study (*30*).

In Vitro Mineral Binding Capacity. The in vitro mineral binding capacity of the three sclerotial DFs on Ca, Mg, Cu, Fe, and Zn was investigated under sequential simulated physiological conditions of the human gastrointestinal tract according to the method of Idouraine and his coinvestigators (*21*) with some modifications. As briefly described in **Figure 1**, 25 g of each original sclerotial DF sample was gently shaken in a 1% HCl solution (pH 1.5; ionic strength = 75 mM KCl; 1:20 w/v) in a 600 mL beaker for a period of 3 h at 37 °C in order to assess its mineral binding capacity in the stomach. The slurry was then filtered through a Whatman 541 ashless filter paper, and the residue was washed several times with ultrapure water until the filtrate tested neutral (pH 7) (gastric conditions treated sclerotial DF samples). All gastric conditions treated sclerotial DFs were then freeze-dried, portions (4.5 g) of which were kept for analysis of minerals, CEC, phytate, and residual protein contents. For determining the mineral binding capacity under simulated physiological conditions of the small intestine, a portion (4 g) of each remaining gastric conditions treated sclerotial DF was separately mixed with 40 mL of Ca, Mg, Cu, Fe, and Zn standard solution (1000 mg/L each) (Ca: product no., 141362H; Mg: product no., 141433F; Cu: product no., 141392N; Fe: product no., 141402V; and Zn: product no.: 141503C; Spectrosol, BDH Laboratory Supplies, Poole, England) in a 500 mL Erlenmeyer flask. The volume of the mixture was then made up to 400 mL by adding the 2.0 mM MES buffer solution (pH 6.8; ionic strength = 100 mM KCl) and incubated at 37 °C for 3 h with moderate agitation. The slurry was then centrifuged at 2500*g* for 15 min under room temperature. The supernatant was discarded, and the residue was washed several times with ultrapure water (small intestinal conditions treated sclerotial DF sample). Subsequently, each small intestinal conditions treated sclerotial DF was then freeze-dried, portions (1.5 g) of which were kept for the minerals analysis. Finally, to assess the mineral binding capacity under a mimicked physiological conditions of the colon with slightly acidic pH, portions of the mineral-bound DF (2 g) treated with simulated small intestinal physiological conditions were further incubated with the 2.0 mM MES buffer solution (pH 5.8; ionic strength = 100 mM KCl; 1:100, w/v) in a 250 mL Erlenmeyer flask for 24 h at 37 °C. The resulting mixture was centrifuged at room temperature (2500*g*, 15 min), and the residue was washed several times with ultrapure water until the pH value was about 7 (colonic conditions treated sclerotial DF samples). All colonic conditions treated sclerotial DFs were then freeze-dried, portions (1.5 g) of which were kept for the mineral analysis.

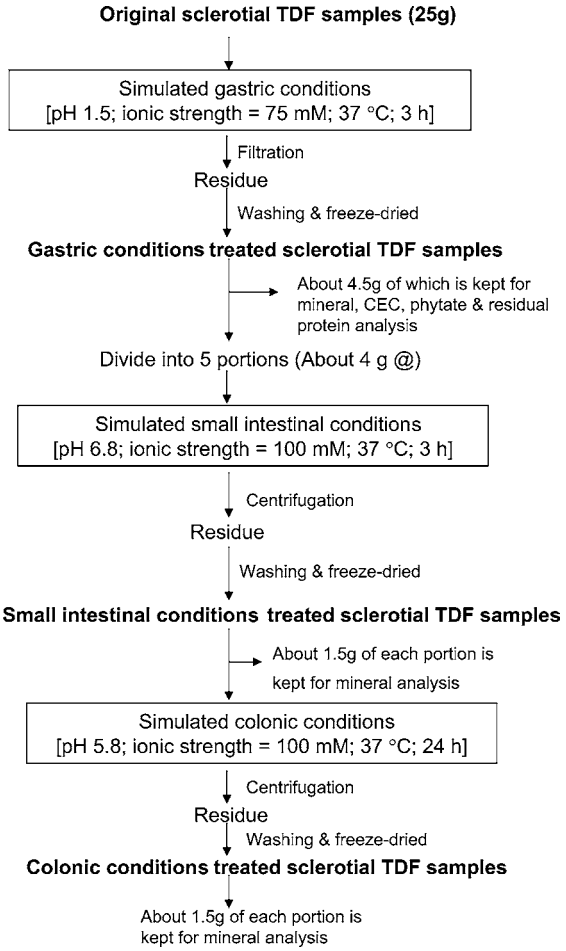

Figure 1. Flow diagram illustrating the major steps of determining in vitro mineral binding capacity of the three sclerotial TDFs under sequential simulated gastrointestinal conditions.

Duplicate samples (0.5 g) of the original (endogenous), gastric conditions treated, small intestinal conditions treated, and colonic conditions treated sclerotial DF samples (**Figure 1**) were wet ashed sequentially with nitric acid (65%) and hydrogen peroxide (30%) followed by appropriate dilution with 1 M HCl. The Ca, Mg, Cu, Fe, and Zn concentrations in the ashed solution were then analyzed using a flame atomic absorption spectrophotometer (Hitachi Z-18100, polarized Zeeman AAS, Japan) as described by Idouraine and his coinvestigators (*2*). Ca and Mg were determined in the presence of 1% lanthanum oxide in order to avoid the interference by P when air−C_2H_2 flame is used (*31*). All glassware was preacid washed in 10% HCl and rinsed three times with ultrapure water to prevent any mineral contamination.

Phytate Content. Duplicate samples of the original and gastric conditions treated sclerotial DF samples were analyzed for phytate using AOAC method 986.11 (*31*) and were calculated as hexaphosphate equivalents.

CEC. The CEC of the three original and gastric conditions treated sclerotial DFs was determined according to the procedure reported by Moorman and his coinvestigators (*32*) with slight modifications. In brief,

Table 1. Phytate, CEC, and Residual Protein Content of the Three Original and Gastric Conditions Treated Sclerotial DFs[a]

	phytate content (mg/g)		CEC (mequiv/kg)		residual protein content (g/100 g)[b]	
DF	original	gastric conditions treated	original	gastric conditions treated	original	gastric conditions treated
P. tuber-regium	0.81 ± 0.11	0.29 ± 0.10	23.7 ± 1.04	16.2 ± 1.21	6.29 ± 0.13	5.27 ± 0.17
P. rhinocerus	0.77 ± 0.20	0.31 ± 0.14	16.7 ± 1.12	11.3 ± 2.16	3.33 ± 0.44	2.07 ± 0.24
W. cocos	0.65 ± 0.14	0.27 ± 0.08	21.6 ± 1.02	17.1 ± 1.78	1.17 ± 0.18	0.88 ± 0.11

[a] Data are average values of two determinations. [b] Residual protein = (total N − chitin N) × 6.25.

the cationic functional groups in each sclerotial DF sample (500 mg) were first converted into their acidic form by stirring in 25 mL of 2 M HCl overnight. After centrifugation (15 min, 2500*g*, 25 °C), the residue was washed extensively with ultrapure water until the total dissolved solute (TDS) of the washing water was similar to that of the ultrapure water (<5.0 mg/L). Subsequently, the acidic residue was suspended in 25 mL of 0.3 M NaCl and centrifuged under the same condition mentioned above. The supernatant was then titrated with 0.01 M KOH, and the CEC was expressed as milliequivalents per kg of sclerotial DF.

Residual Protein Content. The residual protein content of the three original and gastric conditions treated sclerotial DFs was estimated by multiplying the nitrogen content determined by a CHNS/O Analyzer (Perkin-Elmer 2400, CT) by a factor of 6.25 after correction for their corresponding chitin N as previously described (*33*).

Statistical Analysis. All data were presented as mean values of two determinations and analyzed by nonparametric one-way analysis of variance using Kruskal−Wallis test ($p < 0.05$). Bivariate correlation between variables was also determined nonparametrically using Spearman's test at $p < 0.05$ (*34*).

RESULTS AND DISCUSSION

CEC, Phytate, and Residual Protein Content. Results in **Table 1** indicate that all three original sclerotial DFs exhibited similar and remarkably low levels of phytate (ranged from 0.65 to 0.81 mg/g of sclerotial DF DM), which was comparable to that of psyllium (0.58 mg/g) and a commercial fiber, Citrucel (0.92 mg/g), reported by Luccia and Kunkel (*3*). Besides, these values were also markedly lower than those of some fibers prepared from apple (1.35 mg/g), barley (4.53 mg/g), and tomato (8.53 mg/g) (*35*). Polyphosphate granules are one of the major cytoplasmic reserves in the sclerotia, and their quantity increases during the growth of sclerotia, particularly in the outer medulla and cortex (the main storage region at maturity) (*36*). In the present study, the exceptionally low phytate level in all three original sclerotial DFs might partly be attributed to the removal of the rind and/or cortex during the sample preparation (*28*). Besides, the enzymatic treatment during DF preparation would also cause a loss of phytate as suggested by Elhardallou and Walker (*15*). There are several possible roles of polyphosphates in sclerotia including energy storage, regulation of soluble phosphate levels, and storage of phosphorus (*37*, *38*).

Results in **Table 1** also indicate that the CEC of all three original sclerotial DFs was considerably low (only ranged from 16.7 to 23.7 mequiv/kg of sclerotial DF) but was comparable to that of cellulose [22.7−23.0 mequiv/kg (*38*, *39*); 19.0 mequiv/kg (*14*)]. However, the values were markedly lower than those of some fibers prepared from cereals [wheat bran, 95.0 mequiv/kg; oat hulls, 36.0 mequiv/kg (*17*)], legumes [soy bran, 184 mequiv/kg (*17*)], and fruits [orange, 454−997 mequiv/kg (*39*); passion fruit seed, 50.3 mequiv/kg (*40*)] reported previously. The notably low CEC of the three original sclerotial DFs might be due to the exceptionally low level of uronic acids present [only ranged from 0.51 to 2.14 g/100 g sclerotial TDF DM (*41*)], which are the main DF components responsible for the CEC (*42*, *43*). A very good correlation between CEC and uronic acid content of the three sclerotial DFs ($r = 0.98$, $p < 0.05$, $n = 6$) was also obtained.

Although a significant difference ($p < 0.05$) was detected among the residual protein content of the three original sclerotial DFs (*P. tuber-regium*, the highest; *W. cocos*, the lowest), the findings were in agreement with the DF prepared from the same sclerotia by the analytical enzymes used in the AOAC procedure (*29*).

Because the gastric conditions treated sclerotial DFs were used to measure exogenous mineral binding instead of the original ones, potential parameters that would affect the mineral binding capacity including CEC, phytate, and residual protein contents of the gastric conditions treated sclerotial DFs were also assessed. As shown in **Table 1**, acid washing had a remarkable effect on further reduction of the CEC (ranged from 20.8 to 32.3%), phytate (58.5−64.2%), and residual protein contents (16.2−37.8%) of all three gastric conditions treated sclerotial DFs, which were consistent with the previous findings (*2*, *17*). These results implied that when the three sclerotial DFs reached the stomach, the gastric acid might attenuate their adverse binding effects on dietary minerals to a certain extent by removing appreciable amounts of their protein and phytate (potent mineral chelators) in addition to lowering their CECs. Although lignin was also found to be a potent chelator for minerals (*16*), only a trace amount of Klason lignin was detected in both the original and the gastric conditions treated sclerotial DF samples (data not shown).

In Vitro Mineral Binding Capacity. The endogenous Ca, Mg, Cu, Fe, and Zn contents of the three sclerotial DFs are presented in **Table 2**. All three sclerotial DFs exhibited a very low level (<30.0 μg/g of sclerotial DF) of the five tested minerals. This finding indicated that the three raw sclerotia might originally contain very small amounts of these minerals, or considerable amounts of these minerals were lost during the enzymatic preparation of their DFs. A reduction of the endogenous minerals in DF fraction prepared from cereals (wheat bran and oat hull) and legumes (butter bean, broad bean, lentils, and soy bran) using the enzymatic procedure had been reported previously (*14*, *17*). Because their endogenous mineral levels were remarkably lower than those of the fiber prepared from common DF sources such as cereals (wheat bran, rice bran, and oat; Ca, ranged from 701 to 1904 μg/g; Mg, ranged from 771 to 8825 μg/g), fruits (apple and orange; Mg, ranged from 519 to 879 μg/g; Zn, ranged from 9 to 16 μg/g), legumes (butter bean, broad bean, and lentils; Ca, ranged from 977 to 1730 μg/g; Mg, ranged from 286 to 424 μg/g; Fe, ranged from 180 to 390 μg/g; Cu, ranged from 11.1 to 30 μg/g; and Zn, ranged from 29 to 62 μg/g), vegetables (tomato and sugar beet fibers; Mg, ranged from 1530 to 3475 μg/g; Zn, ranged from 13 to 41 μg/g) (*2*, *15*, *43*), and some commercial DF supplements (Citrucel, Fiber One, All-Bran, Metamucil, etc.; Ca, ranged from

Table 2. Endogenous Mineral Content and In Vitro Mineral Binding Capacity of the Three Sclerotial DFs under Sequential Simulated Physiological Conditions of the Human Stomach (% Removal), Small Intestine (% Binding), and Colon (% Releasing)[a]

mineral	DF	endogenous (μg/g)	% removal[b]	% binding[c]	% releasing[d]
Ca	*P. tuber-regium*	28.5 ± 0.02	97.9 ± 0.03	5.91 ± 0.13	37.0 ± 0.35
	P. rhinocerus	25.1 ± 0.03	97.5 ± 0.04	5.32 ± 0.14	34.2 ± 0.73
	W. cocos	21.2 ± 0.03	96.9 ± 0.18	4.79 ± 0.16	72.3 ± 2.03
Mg	*P. tuber-regium*	4.56 ± 0.14	95.9 ± 1.19	4.18 ± 0.13	7.44 ± 1.18
	P. rhinocerus	4.17 ± 0.12	95.9 ± 1.08	3.26 ± 0.12	9.54 ± 1.18
	W. cocos	3.31 ± 0.08	96.7 ± 1.13	3.16 ± 0.10	10.5 ± 0.97
Cu	*P. tuber-regium*	4.17 ± 0.04	56.4 ± 2.94	14.0 ± 0.23	4.15 ± 0.16
	P. rhinocerus	3.23 ± 0.11	46.8 ± 1.59	11.8 ± 0.22	2.70 ± 0.02
	W. cocos	4.51 ± 0.03	62.1 ± 3.07	11.8 ± 0.25	2.28 ± 0.09
Fe	*P. tuber-regium*	27.5 ± 0.14	51.7 ± 4.60	24.3 ± 0.34	4.23 ± 0.33
	P. rhinocerus	23.6 ± 0.18	46.1 ± 2.40	21.3 ± 0.34	0.47 ± 0.04
	W. cocos	21.9 ± 0.23	44.8 ± 0.20	21.2 ± 0.29	0.19 ± 0.07
Zn	*P. tuber-regium*	0.47 ± 0.04	59.5 ± 1.32	9.48 ± 0.21	5.47 ± 0.32
	P. rhinocerus	0.33 ± 0.07	35.3 ± 7.85	8.45 ± 0.21	6.06 ± 0.06
	W. cocos	0.41 ± 0.02	56.2 ± 2.74	8.42 ± 0.36	6.87 ± 1.41

[a] Data are average values of two determinations. [b] % Removal = (endogenous mineral bound − gastric mineral bound) × 100%/endogenous mineral bound. [c] % Binding = (small intestinal mineral bound − gastric mineral bound) × 100%/total exogenous mineral added (10000 μg). [d] % Releasing = (small intestinal mineral bound − colonic mineral bound) × 100%/small intestinal mineral bound.

333 to 6340 μg/g) (*3*), the three sclerotial DFs would not likely be a good dietary source of these five minerals. Although each endogenous mineral content greatly varied among the three sclerotial DFs, similar levels of individual endogenous minerals were observed, with Ca (21.2−28.5 μg/g) and Fe (21.9−27.5 μg/g) being the highest followed by Cu (3.23−4.51 μg/g) and Mg (3.31−4.56 μg/g), while Zn (0.33−0.47 μg/g) was the lowest. Little data had been reported in the literature on the endogenous Ca, Mg, Cu, Zn, and Fe contents of sclerotial DF. However, the present results of the *P. tuber-regium* DF were consistent with those obtained from the same sclerotial DF using analytical enzymes from Sigma (*24*).

Under the simulated physiological conditions of the stomach, the strong acidic environment removed most of the endogenous Ca and Mg from all original sclerotial DFs (Ca, 96.9−97.9% removal; Mg, 95.9−96.7% removal) with the exception of Cu (only 46.8−62.1% removal), Fe (only 44.8−51.7% removal), and Zn (only 35.3−59.5% removal) (**Table 2**). This finding suggested that when the three sclerotial DFs reached the stomach, most of their endogenous Ca and Mg would be readily released while about half of their endogenous Cu, Fe, and Zn still remained bound. Similar phenomenon on fibers prepared from cereals (wheat bran, corn bran, rice bran, and oat) and legumes (soybean hull) had been observed previously (*2*, *12*). In the present study, the more efficient removal of the endogenous Ca and Mg under acidic medium might be due to their weaker bonding energy than that of the Fe, Cu, and Zn, which were more tightly bound to the interstices of the fiber constituents in the three sclerotial DFs (*44*). Besides, this variation also indicated that the binding mechanism for Ca and Mg might be different from that of the others (*35*). According to Laszlo (*12*) as well as Thompson and Weber (*45*), the ineffective release of endogenous Fe, Cu, and Zn under strong acidic medium (gastric condition) might suggest that the binding mechanism of these minerals might only be partially via electrostatistic interactions. Luccia and Kunkel (*3*) proposed that under acidic pH, most cationic functional groups of the fiber would act as cation exchangers, which were responsible for the release of the bound minerals. Besides, an environment possessing a high density of hydrogen ions is also not favorable for the ionization of the exposed cationic functional groups, causing a very weak electrostatic interaction with the divalent cations (*46*, *47*). As a result, only those sites on the fiber, which are densely charged or are sterically accessible to the cations, might retain some minerals by adsorption (*47*). As recently reported by Sangnark and Noomhorm (*48*), the smaller the particle size of the fiber, the weaker the Ca and Mg binding to it, affecting the % removal of endogenous Ca and Mg during acid washing.

The mineral binding behavior of the three gastric conditions treated DFs under the simulated physiological conditions of the small intestine is also shown in **Table 2**. In general, the % mineral binding of the three gastric conditions treated sclerotial DFs to the Ca, Mg, Cu, Fe, and Zn were notably low (Ca ranged from 4.79 to 5.91%; Mg ranged from 3.16 to 4.18%; Cu ranged from 11.8 to 14.0%; Fe ranged from 21.2 to 24.3%; and Zn ranged from 8.42 to 9.48%), suggesting that the three partially demineralized sclerotial DFs from the stomach could only rebind a limited amount of the five nutritionally important minerals in the small intestine and might not possess detrimental effects on mineral bioavailability as compared with other fibers (*9*−*11*, *49*). The exceptionally low mineral binding capacity of the three sclerotial DFs might ascribe to the fact that their major constituent [ranged from 73.9 to 85.2% sclerotial TDF DM (*41*)] was β-glucan, a neutral and almost water insoluble polysaccharide. Similar to cellulose, its relatively stable hydroxyl groups might only provide limited contribution to the overall mineral binding capacity, which, in turn, would likely be determined by their minor components such as protein and phytic and uronic acids (*47*). Similarly, a very weak interaction between other hemicellulosic fiber (e.g., psyllium) and minerals under the stimulated pH of the small intestine had also been reported previously (*3*). Furthermore, **Table 2** also shows that the three gastric conditions treated sclerotial DFs shared a very similar pattern of mineral binding, although the % binding for individual minerals varied remarkably in a descending order of Fe > Cu > Zn > Ca > Mg. Among the three gastric conditions treated sclerotial DFs, *P. tuber-regium* DF exhibited the highest % binding for all of the minerals studied while the *W. cocos* DF possessed similar % mineral binding to that of the *P. rhinocerus* DF except Ca, which was found to be the lowest. These results implied that *W. cocos* DF might contain the lowest number of specific sites for Ca or it might have the same number of sites but with weaker affinity for Ca than that of the other two sclerotial DFs as suggested by Idouraine et al. (*2*).

Uronic acids, protein, and phytate were the minor constituents of the three gastric conditions treated sclerotial DFs that have been speculated to be potent chelators of minerals (*18*, *19*, *44*). In this study, a very good correlation between all % mineral binding and the residual protein content of the three gastric conditions treated sclerotial DFs was observed (r ranged from 0.97 to 0.98, $p < 0.05$, $n = 6$). The data did not only suggest that the residual protein content of the three gastric conditions treated sclerotial DFs would be one of the main factors determining their mineral binding capacity but also explained that the highest % binding for all minerals as in the case of *P. tuber-regium* DF would probably be attributed to its highest protein level (**Table 1**). Such correlation had also been obtained from oat fiber, apple fiber, and tomato fiber (*2*, *44*). Nevertheless, neither the phytate content nor the CEC (indicator of the amount of uronic acids) of the three gastric conditions treated sclerotial DFs was correlated with their % mineral binding (r only ranged from 0.03 to 0.47 and from −0.11 to 0.35, respectively, $p < 0.05$, $n = 6$), suggesting that further reduction of phytate content and CEC by the gastric acid could result in notably low levels of phytate and CEC (**Table 1**), causing very little or no binding effect on the five tested minerals in the small intestine (*8*). Furthermore, other factors such as particle size, in combination with other minerals and water and oil holding capacity, had also been reported to have correlation with the mineral binding capacity of fibers (*3*, *15*, *48*).

In this study, the mineral binding capacity of the three small intestinal conditions treated sclerotial DFs under simulated physiological conditions of colon with "beneficial" pH (5.80) was also investigated. As shown in **Table 2**, only bound Ca was remarkably released (34.2−72.3% releasing, **Table 2**) from the three small intestinal conditions treated sclerotial DFs at pH 5.80 and their % releasing of Ca was also greatly different from each other (with *P. tuber-regium* DF being the highest and *W. cocos* DF being the lowest). This observation indicated that as compared to Mg, Fe, Cu, and Zn, the binding of Ca to the three small intestinal conditions treated sclerotial DFs would be weaker especially for the *W. cocos* DF (*35*). This finding also implied a potential physiological benefit of the three sclerotial DFs on Ca bioavailability. On reaching the human colon, part of the small intestinal conditions treated sclerotial DFs would be fermented by the anaerobic microflora in the large intestine, releasing some of their bound minerals. If the fermentability of the three small intestinal conditions treated sclerotial DFs was high enough to create an acidic colonic environment (pH ≤ 5.80), this would not only release appreciable amounts (34.2−72.3%) of bound Ca from the three nonfermented sclerotial DFs but might also promote their ionization together with the already released and unabsorbed minerals. As a result, passive mineral absorption especially Ca in the large intestine might be enhanced and the overall Ca bioavailability might then be improved. The additional absorption of Ca in the colon is important especially in elderly people and postmenopausal women who have insufficient Ca intake or insufficient active Ca absorption from the small intestine. Nevertheless, the main criterion that determines the potential enhancing effect of the three sclerotial DFs on passive Ca absorption in the human large intestine is their fermentability. Investigation on in vitro fermentability of the three novel sclerotial DFs using human fecal microflora is reported in part 3 of this series.

ABBREVIATIONS USED

CEC, cation-exchange capacity; DF, dietary fiber; TDS, total dissolved solute.

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