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Dietary Fibers from Mushroom Sclerotia: 3. In Vitro Fermentability Using Human Fecal Microflora

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The in vitro fermentability of three novel dietary fibers (DFs) prepared from mushroom sclerotia, namely, *Pleurotus tuber-regium*, *Polyporous rhinocerus*, and *Wolfiporia cocos*, was investigated and compared with that of the cellulose control. All DF samples (0.5 g each) were fermented in vitro with a human fecal homogenate (10 mL) in a batch system (total volume, 50 mL) under strictly anaerobic conditions (using oxygen reducing enzyme and under argon atmosphere) at 37 °C for 24 h. All three novel sclerotial DFs exhibited notably higher dry matter disappearance (*P. tuber-regium*, 8.56%; *P. rhinocerus*, 13.5%; and *W. cocos*, 53.4%) and organic matter disappearance (*P. tuber-regium*, 9.82%; *P. rhinocerus*, 14.6%; and *W. cocos*, 57.4%) when compared with those of the cellulose control. Nevertheless, only the *W. cocos* DF was remarkably degraded to produce considerable amounts of total short chain fatty acids (SCFAs) (5.23 mmol/g DF on organic matter basis, with a relatively higher molar ratio of propionate) that lowered the pH of its nonfermented residue to a slightly acidic level (5.89). Variations on the in vitro fermentability among the three sclerotial DFs might mainly be attributed to their different amounts of interwoven hyphae present (different amounts of enzyme inaccessible cell wall components) as well as the possible different structural arrangement (linkage and degree of branching) of their β-glucans.

KEYWORDS: In vitro fermentability; human fecal microflora; mushroom dietary fiber

INTRODUCTION

Dietary fiber (DF) escapes digestion and absorption in the human small intestine and constitutes the main substrate for colonic fermentation (*1*). Fermentative breakdown of DF in the human colon by anaerobic saccharolytic microflora leads to the production of certain gases (CO_2, CH_4, and H_2), microbial biomass, and short chain fatty acids (SCFAs), which considerably influence the physiological functions of humans (*2*, *3*). According to Hill (*4*), colonic degradation of DF proceeds stepwise with the breakdown of polysaccharides by the extracellular enzymes into oligosaccharides, which are further hydrolyzed with intracellular or cell-bound enzymes of the colonic microflora into simple sugars. These sugars are then consumed and metabolized by the colonic bacteria to produce the SCFAs (*1*, *2*). Because a wide spectrum of enzymes is required to digest the heterogeneous and highly organized DF polysaccharides, a complex bacterial system harboring a wide range of cooperating and competing species is usually involved during the colonic fermentation (*5*).

The rate and extent of fiber fermentation depend on two main categories of factors: (i) host specific factors such as activities and composition of the colonic microflora as well as gastrointestinal tract transit time and (ii) substrate specific factors including physicochemical properties (e.g., particle size, solubility, and cell wall architecture) of the fiber source as well as chemical composition (monosaccharide profile) and structural arrangement (degree of branching and linkages between monosaccharide) of fiber constituents (*6*−*8*). The SCFAs produced during fermentation are rapidly absorbed by the colonic mucosa, stimulating water and sodium absorption as well as peristalsis, which, in turn, aids in the bowel function (*9*, *10*). An appreciable amount of SCFAs resulted from highly fermentable DFs would also lower the colonic pH, modulating the composition of colonic microflora by inhibiting the growth of pathogenic bacteria (nonacid tolerant) but stimulating that of a beneficial one like *Lactobacillus* (*11*, *12*).

For investigating the fermentability of DF, sampling of SCFAs in humans is time consuming, expensive, and difficult; thus, numerous in vitro batch systems utilizing human fecal bacteria have been developed for determining DF degradation and SCFAs (total and individual) production as well as predicting the potential physiological effects of various DF sources during their fermentation in the human colon (*13*, *14*). In vitro fermentation systems are rapid and inexpensive, allowing for screening of new fiber source that is not yet permitted for human consumption and the results of which are also well-correlated with those of human studies (*15*, *16*). In

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the present study, the fermentability of three novel DFs prepared from sclerotia, namely, *Pleurotus tuber-regium* (Fr.) Sing., *Polyporous rhinocerus* Cooke, and *Wolfiporia cocos* (Schw.) Ryv. et Gilbn., was evaluated in vitro by using human fecal microflora in order to predict their fate in the human colon after consumption as a food ingredient.

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 (*17*). The DFs of the three sclerotia were prepared by a modified AOAC procedure using industrial enzymes as reported in our previous study (*18*). Cellulose, which has been widely used in similar studies (*2, 19*), was applied as the negative control.

In Vitro Fermentation Using Human Fecal Microflora. For assessing the in vitro fermentability, the three sclerotial DFs and the cellulose control were fermented in vitro with a human fecal homogenate in a batch system under strictly anaerobic conditions for 24 h as described by Edwards et al. (*20*) as well as Velázquez et al. (*21*) with some modifications. Fresh human feces were separately collected from three healthy adult volunteers, who had consumed a nonspecific Western diet, taken no antibiotics for at least 3 months, and had no history of gastrointestinal diseases prior to the study. In brief, each individual fresh fecal sample was immediately collected into a sterilized and preweighed 100 mL reagent bottle containing 50 mL of 100 mM reduced sodium phosphate buffer (pH 6.5) and 1 mL of oxygen reducing enzyme supplement (Oxyrase For Broth, Mansfield, OH). As soon as possible, the three fecal samples obtained were aseptically pooled together and homogenized for 30 s in a Waring blender at low speed under anaerobic condition. The resulting human fecal homogenate was then further diluted with the reduced sodium phosphate buffer (containing Oxyrase For Broth, 50:1 v/v) to a final ratio of 1:6 (w/v) and passed through three layers of sterilized filtering bags. The filtrate of this diluted human fecal homogenate was immediately used as the fermentation inoculum.

Duplicate samples (500 mg) of the cellulose control and the three sclerotial DFs were separately and aseptically added into sterilized 100 mL reagent bottles containing 40 mL of the reduced sodium phosphate buffer. The bottles were then tightly closed, sealed with Parafilm, and stored at 4 °C for 24 h in order to allow complete hydration of the substrates and to limit the possibility of microbial growth before initiating fermentation. Two hours before inoculation, 0.8 mL of the oxygen reducing enzyme supplement was aseptically applied into the substrate mixture and the headspace of the bottle was flushed with ultrapure argon gas for 1 min. The bottles were then tightly closed, sealed with Parafilm, and preincubated at 37 °C. To begin fermentation, each substrate-containing bottle was aseptically inoculated with 10 mL of the aforesaid human fecal homogenate and the headspace of the bottle was flushed with the ultrapure argon gas for 1 min. All bottles were then tightly closed, sealed with Parafilm, and incubated for 24 h with mild agitation (50 rpm) on a vibration shaker placed in a 37 °C incubation room. At the end of the experiment, the nonfermented residues of the control and the three sclerotial DFs were individually well-mixed by vigorous shaking. After the pH measurement was taken, aliquots (2 mL) of the nonfermented residue were transferred to a 15 mL centrifuged tube and frozen at −20 °C prior to further SCFAs analysis. Following the same procedure, triplicates of reagent bottles containing no substrate (blank) were also fermented with the human fecal homogenate in order to allow corrections for any dry matter, organic matter, and SCFAs production not arising from the substrates.

Dry matter and organic matter contents of the nonfermented residues of all DF samples were determined according to the procedure described by Bourquin et al. (*14*). In brief, individual nonfermented residue in the remaining fermentation broth (48 mL) was precipitated with four volumes of 95% EtOH overnight. After it was filtered through a tared ashless filter paper (Whatman 541), the residue recovered was sequentially washed with 78% EtOH, 95% EtOH, and acetone followed by oven drying at 105 °C for 24 h. The weight of the oven-dried residue was used to determine dry matter recovery. Together with the ashless filter paper, each oven-dried residue was then quantitatively transferred to a preweighed and acid-washed porcelain crucible and dry ashed in a muffled furnace at 600 °C overnight. The ash content of the nonfermented residue was used for computing its organic matter recovery. Following the same procedure, the dry and organic matter of all DF samples (starting materials for the in vitro fermentation) were also analyzed. The dry matter disappearance (DMD) after 24 h of fermentation was calculated as follows:

$$\mathrm{DMD}\ (\%) = \frac{[\mathrm{DM}_{\mathrm{original}} - (\mathrm{DM}_{24} - \mathrm{B}_{24})]}{\mathrm{DM}_{\mathrm{original}}} \times 100\ \% \qquad (1)$$

where $DM_{original}$ is the weight of dry matter of the original DF sample, DM_{24} is the weight of dry matter of the nonfermented residue after 24 h of fermentation, and B_{24} is the average weight of dry matter in the blank after 24 h of fermentation.

The organic matter disappearance (OMD) was calculated in a similar manner as the DMD:

$$\mathrm{OMD}\ (\%) = \frac{[\mathrm{OM}_{\mathrm{original}} - (\mathrm{OM}_{24} - \mathrm{B}_{24})]}{\mathrm{OM}_{\mathrm{original}}} \times 100\ \% \qquad (2)$$

where $OM_{original}$ is the weight of organic matter of the original DF sample, OM_{24} is the weight of organic matter of the nonfermented residue after 24 h of fermentation, and

B_{24} is the average weight of organic matter in the blank after 24 h of fermentation.

Action of Different β-Glucanases on the Three Sclerotial DFs. Our previous study has shown that the major component of the three novel sclerotial DFs was β-glucan (glucose residue, 89.7−94.5% NSP DM) (*22*). Besides, the structures of their fractionated β-glucans especially for the *P. tuber-regium* and *W. cocos* sclerotia were also found to contain β-1,3- and β-1,6- or even β-1,4-linkages (*23*−*25*). Hence, the possible β-glucanases that might cause endo/exo depolymerization of the three sclerotial DFs would likely include *exo*-1,3-β-glucanase (EC 3.2.1.39), *endo*-1,3-β-glucanase (laminarinase, EC 3.2.1.58), mixed linked *endo*-1,3(4)-β-glucanase (lichenase, EC 3.2.1.73), and *endo*-1,4-β-glucanase (cellulase, EC 3.2.1.4). To investigate the action of these β-glucanases on the three sclerotial DFs, 100 mg of individual sclerotial DF in 5 mL of corresponding buffer [*endo*- and *exo*-1,3-β-glucanase as well as cellulase, 100 mM sodium citrate buffer (pH 4.5); lichenase, 25 mM Na phosphate buffer (pH 6.5)] was separately incubated with the four aforesaid β-glucanases [*exo*-1,3-β-glucanase (8.0 U; E-EXBGL, Megazyme); *endo*-1,3-β-glucanase (1.15 U; E-LAMSE, Megazyme); lichenase (40 U; E-LICHN, Megazyme), and cellulase (2.5 U; E-CELTR; Megazyme)] under their corresponding optimum temperatures (*exo*- and *endo*-1,3-β-glucanase, 40 °C; lichenase and cellulase, 60 °C) for a total of 30 min with continuous agitation. The enzyme concentration of the four β-glucanases used had been found to be in excess when the reaction was performed under the same substrate concentration, temperature, pH, and duration (data not shown). The enzymatic reaction was terminated by heating the digesta at 90 °C for 10 min followed by adding 5 mL of 200 mM sodium acetate buffer (pH 4.0). After they were vigorously vortex-mixed, the diluted digesta were centrifuged (1000*g*, 25 °C, 10 min), and 100 μL of the supernatant was quantitatively transferred to a new test tube. Except for the *exo*-1,3-β-glucanase, the resulting β-linked gluco-oligosaccharides in the supernatant of the three *endo*-β-glucanases (laminarinase, lichenase, and cellulose) were further hydrolyzed to glucose by incubation with 100 μL of β-glucosidase (0.8 U, EC 3.2.1.21, originated from *Aspergillus niger*; E-BGLUC, Megazyme) at 50 °C for 10 min. Consequently, the glucose liberated directly and indirectly from both *exo*- and *endo*-β-glucanases was determined with a glucose assay kit (K-GLUC, Megazyme, Ireland). The performance of the tested β-glucanases was evaluated according to the amount of glucose released from the three sclerotial DFs on dry matter basis.

Gas Chromatographic (GC) Determination of SCFAs. The method to measure SCFAs concentration was modified from Erwin and his coinvestigators (*26*). Briefly, a frozen aliquot (2 mL) of each

Table 1. DMD and OMD of the Three Sclerotial DFs and the Cellulose Control and the pH of Their Nonfermented Residues after 24 h of In Vitro Fermentation Using Human Fecal Microflora[a]

DF	DMD (%)	organic matter disappearance (%)	pH
P. tuber-regium	8.56 ± 0.55	9.82 ± 0.80	6.75 ± 0.23
P. rhinocerus	13.5 ± 1.41	14.6 ± 2.47	6.61 ± 0.21
W. cocos	53.4 ± 0.13	57.4 ± 0.69	5.89 ± 0.21
cellulose	3.34 ± 0.17	3.84 ± 0.63	6.79 ± 0.13

[a] Data are average values of two determinations.

nonfermented residue was thawed and centrifuged (4800 rpm, 4 °C, 30 min). Seven hundred microliters of the supernatant was then transferred into a new 15 mL centrifuge tube and acidified by adding 175 μL of 25% *meta*-phosphoric acid together with 125 μL of an internal standard, 4-methyl pentanoic acid (4 mg/mL of 25% *meta*-phosphoric acid). After they were incubated at room temperature for 30 min, the SCFAs in the mixture were extracted with 1 mL of diethyl ether three times and pooled together followed by dehydration with anhydrous sodium sulfate. The dehydrated SCFAs sample was then transferred into a sample vial through a 0.45 μm filtering disk and stored at −20 °C prior to further GC analysis.

The SCFAs contents of the cellulose control and the three sclerotial DFs produced after the 24 h of in vitro fermentation were quantified by a HP 6890 GC system equipped with an Quadrex 007-FFAP capillary column (30 m × 0.25 mm; i.d. 0.25 μm film). An oven temperature program of an initial temperature of 100 °C with a hold of 5 min, followed by a temperature rise of 3 °C/min to 160 °C with a final hold of 5 min, was used. Both injector and detector temperatures were set at 220 °C. Helium was used as a carrier gas with a constant flow rate of 1.5 mL/min. Five microliters of sample was injected with a split ratio of 20:1, and their SCFAs contents were detected by flame ionization. Individual SCFAs were corrected for losses during solvent extraction, and their different responses to the FID by a molar correction factor was determined from the recovery of SCFAs standards subjected to the same sample treatment. A mixture of individual SCFA standards including ethanoic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid (SCFA standards kit, catalog no. 18600, Alltech, PA), and 4 methyl-pentanoic acid (internal standard, catalog no. 6220601, Alltech) was prepared in 25% *meta*-phosphoric acid at a final concentration of 0.5 mg/mL for both quantitation and identification. The amount of the SCFAs produced from the cellulose control and the three sclerotial DFs after 24 h of in vitro fermentation was expressed as millimoles per gram of substrate on organic matter basis.

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 $p < 0.05$ (*27*).

RESULTS AND DISCUSSION

DMD and OMD. The DMD and OMD of the three novel sclerotial DFs and the cellulose control after 24 h of in vitro fermentation using human fecal microflora are presented in **Table 1**. Cellulose was served as the negative control in this study since its highly ordered crystalline structure and insolubility in water were found to severely limit its degradation by human colonic bacteria (*19*, *28*). Although all three sclerotial DFs possessed notably higher DMDs and OMDs when compared with those of the cellulose control, both the *P. tuber regium* and the *P. rhinocerus* DFs were comparatively less fermented [with lower values of DMD (8.56 and 13.5%, respectively) and OMD (9.82 and 14.6%, respectively)] than the *W. cocos* DF (DMD, 53.4%; OMD, 57.3%).

Detailed studies concerning the fermentation of sclerotial DF by human fecal microflora have not been reported. However, levels of DMD and OMD of some commercial fiber fractions prepared from cereals [oat (DMD, 9.10−12.9%; OMD, 7.30−12.2%); wheat bran (DMD, 15.0%); corn bran (OMD, 11.2%)], apple (DMD, 58.0%), and soy (DMD, 60.3%; OMD, 56.4−57.7%) reported previously were comparable to those of the three sclerotial DFs (*14*, *29*−*31*). In agreement with the observation reported by Bourquin and his coinvestigators (*29*), the OMD values of all sclerotial DF samples were generally higher than their corresponding DMD values (OM = DM ash content, eq 2). Even though the OMD of the three sclerotial DFs was corrected for that originating from the blank, their true OMD might be slightly underestimated since microbial cells produced during fermentation might be recovered as ethanol insoluble material especially for the readily fermented substrates (*14*).

Relationship between the β-Glucan Structure of the Three Sclerotial DFs and Their Fermentability. Numerous studies had reported that the chemical composition (monosaccharide profile) and structural arrangement (linkage and degree of branching) of the fiber constituents in a substrate did not only control the rate and extent of its degradation but also the nature of the SCFAs produced (*8*, *32*). Because the major component (glucose residue, 89.7−94.5% NSP DM) of the three novel sclerotial DFs was β-glucan (*22*), the structural arrangement (linkage and degree of branching) of β-glucan (originated from hyphal cell wall and extracellular matrix) would likely be one of the main factors determining their fermentability. As shown in **Table 2**, the degree of endo/exo depolymerizaion (as reflected by the amount of glucose released) of the three sclerotial DFs was generally low and varied greatly. In the case of *exo*-β-1,3-glucanase and *endo*-β-1,3-glucanase, 2.22−8.41 and 0.38−11.4 g of glucose was released from 100 g of sclerotial DF DM, respectively. For lichenase and cellulase, 0.09−0.50 and 0.04−5.12 g of glucose was released from 100 g of sclerotial DF DM, respectively. The enzyme resistant characteristics of the three sclerotial DFs might be attributed to their impeccable cell wall architectures, which act as a physical barrier hindering the attack of the tested β-glucanases as suggested previously (*17*). **Table 2** also shows that the action of the tested β-glucanases on the three sclerotial DFs was species-dependent, implying the structural (especially for linkage and branching) differences in their β-glucan type DF components. *P. tuber-regium* DF was susceptible to be hydrolyzed by all tested β-glucanases except for the *endo*-β-1,3-glucanase (**Table 2**). This finding suggested that the *P. tuber-regium* DF might possess a complicated structure of β-glucan with linkages including β-1,3 and β-1,4 of different proportions. Different structures of β-glucan isolated from the *P. tuber-regium* sclerotia had also been reported previously (*23*, *24*). It is interesting to note that only *P. tuber-regium* DF could be digested by the cellulase and lichenase (**Table 2**), implying the involvement of β-1,4-linkage in its β-glucan type DF component, which was not commonly found in mushroom sclerotia. Our previous linkage analysis on the *P. tuber-regium* polysaccharides (an alkaline soluble fraction) also gave a similar result, suggesting the presence of β-1,4-linkage apart from β-1,3- and β-1,6-linkages (*23*). In the case of *P. rhinocerus* and *W. cocos* DFs, the glucose residue was notably detected in the digesta resulting from both *exo*- and *endo*-β-1,3 glucanases but not from lichenase and cellulase (**Table 2**). This finding indicated that the structure of their β-glucan type DF component would likely be linked by β-1,3-glucosidic bond without the involvement of the β-1,4-linkage [typical β-glucan structure of most mushroom sclerotia (*33*)]. When compared with *P. rhinocerus* DF, the exo and endo depolymerization of

Table 2. Amount of Glucose Released (g/100 g of Sclerotial DF DM) from the Three Sclerotial DFs after Incubation with Different Levels of β-Glucanases[a]

sclerotial DF	*exo*-1,3-β-glucanase	*endo*-1,3-β-glucanase	lichenase	cellulase
P. tuber-regium	2.22 ± 0.36	0.38 ± 0.15	0.50 ± 0.02	5.12 ± 0.27
P. rhinocerus	3.49 ± 0.32	4.68 ± 0.31	0.09 ± 0.02	0.04 ± 0.02
W. cocos	8.41 ± 0.43	11.4 ± 0.37	0.09 ± 0.01	0.04 ± 0.01

[a] Data are average values of two determinations.

Table 3. Production of Total SCFAs, Acetate, Propionate, and Butyrate by the Three Sclerotial DFs and the Cellulose Control after 24 h of In Vitro Fermentation Using Human Fecal Microflora[a]

	mmol/g DF OM[c]			
DF	total SCFAs[b]	acetate	propionate	butyrate
P. tuber-regium	0.45 ± 0.05	0.21 ± 0.02 (2.10)[d]	0.10 ± 0.01 (1.00)	0.14 ± 0.01 (1.40)
P. rhinocerus	0.64 ± 0.01	0.38 ± 0.04 (4.75)	0.18 ± 0.03 (2.25)	0.08 ± 0.01 (1.00)
W. cocos	5.23 ± 0.13	2.79 ± 0.05 (2.82)	1.45 ± 0.11 (1.46)	0.99 ± 0.12 (1.00)
cellulose	0.28 ± 0.03	0.13 ± 0.07 (1.86)	0.07 ± 0.04 (1.00)	0.08 ± 0.02 (1.14)

[a] Data are average values of two determinations. [b] Total SCFAs = total SCFA (acetate + propionate + butyrate). [c] OM = on organic matter basis. [d] Values in parentheses are relative molar ratio of individual SCFA.

the β-glucan type DF component in the *W. cocos* DF was more extensive (8.41 and 11.4 g of glucose released from 100 g of sclerotial DF DM, **Table 2**). Regardless of the differences in their intact cell wall architecture, a different degree of branching between the β-glucan of the *P. rhinocerus* and *W. cocos* DF might be one of the possible reasons. Previous studies had reported that β-glucan isolated from *W. cocos* sclerotia was an almost linear homoglucan with only a β-1,3-linkage (*25*). During enzyme digestion, the steric effect of this nonbranching β-glucan would be very weak and facilitate the depolymerization by both the *exo*- and the *endo*-β-1,3-glucanases. Therefore, the relatively lower degree of exo and endo depolymerization of *P. rhinocerus* DF implied that its β-glucan structure might possess a certain degree of branching, which only allowed partial degradation by the *endo*-β-1,3-glucanase.

Unlike cellulose (straight chain of β-1,4-glucan; DMD, 3.84%; **Table 1**), numerous β-glucans such as mixed linked β-glucan [straight chain of β-1,(3)4-glucans] from cereals (*34*), laminarian (β-1,3-linked backbone with very few β-1,6-branching, about 7:1) from brown seaweeds (*35*), and curdlan (straight chain of β-1,3-glucan) from bacteria *Alkaligenes faecalis* (*36*) had been reported to be highly fermented (DMD, >90.0%) by both human fecal and rat cecal microflora. In the present study, even though the three sclerotial DFs were β-glucan type polysaccharides with similar linkages to these readily fermented β-glucans, their fermentabilities were relatively low, particularly the *P. tuber-regium* and *P. rhinocerus* DFs (DMD, <14%; OMD, <15%).

Among the three sclerotial DFs, a difference in structural arrangement (linkage and degree of branching) of their β-glucans was insufficient to explain such a large discrepancy of their fermentabilities (in terms of DMD and OMD) especially for the case between the *P. rhinocerus* and *W. cocos* DF (about four times; **Table 1**). The main difference of their β-glucan structure would likely be the degree of branching, which should not be very large, since both of their β-glucans could be partially hydrolyzed by the *endo*-1,3-β-glucanase (**Table 2**). Various amounts of interwoven hyphae present in the three sclerotial DFs in the order of *P. tuber-regium* > *P. rhinocerus* > *W. cocos* were found in our previous study (*22*), and this finding might be a possible reason for their different degree of fermentability. As observed in the β-glucanase digestion shown in **Table 2**, the cell wall architecture of the hyphae present in all three sclerotial DFs might serve as a physical barrier, hindering the access and digestion of extracelluar enzymes secreted by the fecal saccharolytic bacteria and thus lowering the susceptibility of the fiber components to bacterial degradation. Previous findings had also suggested that apart from the chemical composition and structural arrangement (linkage and branching), the accessibility of DF component by extarcellular enzymes of colonic saccharolytic bacteria was also a key factor governing the fermentabiliy as well as SCFAs production of a DF (*6*, *37*). The poor water solubility of the three sclerotial DFs might also play an important role in their low degradation by the human fecal bacteria (*38*, *39*) when compared to other β-glucans. As a result, the variation of fermentability among the three sclerotial DFs would likely be attributed to their difference in the amount of interwoven hyphae present (possessing enzyme resistant cell wall architecture) in addition to the possible structural differences (linkage and degree of branching) of their β-glucan components.

Except for the *W. cocos* DF, the considerably low fermentability of the *P. tuber-regium* and *P. rhinocerus* DF would remain nonfermented in the human colon, which, in turn, contributes to the fecal bulking capacity together with the bacterial biomass. Apart from diluting the carcinogenic and toxic substances by providing a more bulky stool, the nonfermented *P. tuber-regium* and *P. rhinocerus* DF could also decrease the transit time of the stool along the colon and lower the chance of exposure to carcinogens in a similar way as other cereal polysaccharides (*31*).

Total and Individual SCFAs Contents. Apart from fermentability, different DF sources (such as oat, wheat, soy, pectin, guar gum, etc.) were found to produce different amounts of total and individual SCFAs with unique SCFA profiles during a 24 h in vitro fermentation using human fecal microflora (*14*, *19*). In the present study, only negligible amounts (<1%, data not shown) of pentaonic, hexanoic, heptanoic, and octanoic acids were detected while the acetic, propionic, and butyric acids were found to be the major SCFAs obtained (**Table 3**). These results were also consistent with the previous finding of other β-glucan-enriched DFs originating from oat and barley (*34*). **Table 3** also shows that during the 24 h in vitro fermentation, the total SCFAs (the sum of the acetate, propionate, and butyrate) production in all DF samples greatly varied with that of *W. cocos* DF being the highest and cellulose control being the lowest. The two

poorly fermented *P. tuber-regium* and *P. rhinocerus* DFs only produced low levels of total SCFAs (0.45 and 0.64 mmol/g DM, respectively), which was still comparable to that of two commercial oat fibers (0.42−0.87 mmol/g DM) (*29*) but was considerably lower than that of mixed linked β-glucan (10.5 mmol/g) in oats (*34*) and the laminarian (8.76 mmol/g) (*35*). In contrast, the highly fermented *W. cocos* DF produced a considerable amount of total SCFAs (5.23 mmol/g OM; **Table 1**), which also remarkably lowered the pH of its nonfermented residue to a slightly acidic value (5.89; **Table 1**; $r = 0.98$, $n = 8$, $p < 0.05$). This was consistent with previous findings on other readily fermented fiber fractions such as apple fiber (pH 6.14) (*30*) and oat fiber (79.7% β-glucan, pH 6.25) (*34*). Furthermore, a very high correlation between total SCFA production and OMD was also obtained ($r = 0.99$, $n = 8$, $p < 0.05$), indicating that total SCFA production originated from the organic matter of the substrate. This observation on other DF sources was also reported previously (*14*, *29*).

Table 3 also shows that the levels of individual SCFA produced from the three sclerotial DFs were relatively higher than those of the cellulose control except for the acetate and propionate of *P. tuber-regium* DF as well as butyrate from *P. rhinocerus* DF. Among the three sclerotial DFs, *W. cocos* DF was characterized by producing the highest levels of acetate (2.79 mmol/g OM), propionate (1.45 mmol/g OM), and butyrate (0.99 mmol/g OM).

Special attention should also be paid to the relative molar ratios of SCFAs, since they could determine some physiological benefits of a DF in humans (*40*). As shown in **Table 3**, the relative amount of acetate was the highest among all DF samples, which was consistent with previous studies (*19*, *32*). Interestingly, fermentation of both β-1,4-glucan-rich samples, *P. tuber-regium* DF, and cellulose control exhibited similar SCFA profiles with a relatively higher molar ratio of butyrate (acetate:propionate:butyrate in *P. tuber-regium* DF = 2.1:1:1.4; cellulose = 1.86:1:1.14; **Table 3**). This finding was also comparable to that of β-1,4-linkage-rich glucan from cereals with a relative molar ratio of 2.09:1:1.84 (*41*). In the case of *P. rhinocerus* and *W. cocos* DFs that were rich in β-1,3-linkages, they had a relatively higher molar ratio of propionate (*P. rhinocerus* DF = 4.75:2.25:1; *W. cocos* DF = 2.82:1.46:1; **Table 3**) after the 24 h in vitro fermentation and their SCFA profiles were also similar. The production of a relatively higher molar ratio of propionate was also reported in the fermentation of curdlan (3.72:1.41:1) (*36*) and laminarian (1.7:1.25:1) (*35*).

From a physiological point of view, the relatively higher level of propionate produced by the readily fermented *W. cocos* DF implied that it might possess hypoglycemic (probably by increasing hepatic glucose utilization or maximizing the insulin response) and/or hypocholesterolemic effect(s) on humans (probably by suppressing the synthesis of hepatic cholesterol or redistribution of cholesterol from plasma to liver) as proposed from previous human (*42*) and animal studies (*43*).

In the present study, apart from the different extent of enzyme inaccessibility of cell wall components (different amount of interwoven hyphae present in the three sclerotial DFs), the possible structural variations (linkage and branching) in the β-glucans component of the three sclerotial DFs (from both hyphal cell wall and extracellular matrix) might result in their differences in fermentability as well as the amount and profile of SCFAs produced (*8*, *30*). Obviously, a structure−function relationship between the three sclerotial DFs and their in vitro fermentability was present. In this study, the in vitro fermentability of the *W. cocos* DF was found to be the highest accompanied by production of a substantial amount of total SCFAs, which also reduced the pH of its nonfermented residue to a slightly acidic level. These findings suggested that during fermentation of the *W. cocos* DF by the human colonic bacteria, its high fermentability might result in a sufficient amount of SCFAs that acidified the colonic pH, which might in turn promote the ionization of the unabsorbed minerals and enhance their passive absorption in the colon like other highly fermented DF reported previously in both humans (*44*, *45*) and rats (*46*, *47*). Therefore, further investigation on the possible enhancing effect of the *W. cocos* DF on passive mineral absorption in the large intestine by using animal model is underway.

ABBREVIATIONS USED

DMD, dry matter disappearance; OMD, organic matter disappearance; SCFAs, short chain fatty acids; DF, dietary fiber.

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