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Biochemical and Microstructural Characteristics of Insoluble and Soluble Dietary Fiber Prepared from Mushroom Sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinocerus*, and *Wolfiporia cocos*

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The proximate composition of sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinoceros*, and *Wolfiporia cocos*, together with the yield, purity, monosaccharide profile, and microstructure of their insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) fractions prepared from AOAC enzymatic−gravimetric methods were investigated and compared. All three sclerotia were typical carbohydrate rich sclerotia [ranging from 90.5 to 98.1% dry matter (DM)] with an exceptionally low amount of crude lipid content (ranging from 0.02 to 0.14% DM). Besides, all three sclerotia possessed substantial amounts of IDF (ranging from 77.4 to 94.6% DM) with notably high levels of nonstarch polysaccharides (NSP) (89.9−92.2% DM) in which glucose was the predominant sugar residue (90.6−97.2% of NSP DM). On the contrary, both the yield (only ranging from 1.45 to 2.50% DM) and the amount of NSP (ranging from 22.4 to 29.6% DM) of the three sclerotial SDF fractions were very low. Scanning electron micrographs showed fragments of interwoven hyphae and insoluble materials in the three sclerotial IDF fractions, but only an amorphous structure of soluble materials was observed in the SDF fractions. The potential use of these fiber preparations was discussed.

KEYWORDS: ***P. tuber-regium*; *P. rhinoceros*; *W. cocos*; IDF; SDF; monosaccharide profile; microstructure; mushroom sclerotia**

INTRODUCTION

Recognition of DF as a food substance of significant nutritional value has increased dramatically over the past 40 years as the physical and chemical nature of DF have become more precisely characterized. The latest proposed definition of DF from the American Association of Cereal Chemists (AACC) defines DF as being made up of edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine as well as having beneficial physiological effects such as laxation, blood glucose attenuation, and/or blood cholesterol attenuation (*1*). In general, DF can be divided into soluble (SDF) and insoluble (IDF) fractions based on their solubility in aqueous medium. The viscosity of SDF is responsible for slower digestion and absorption of nutrients, which helps to attenuate blood cholesterol and glucose levels. In contrast, IDF is characterized by its ability to increase fecal bulk (non- or partially fermentable in colonic microflora) and decrease intestinal transit time, thus promoting laxation (*2*). Therefore, consumption of sufficient DF is well-known to confer a protective effect against a number of chronic diseases (such as coronary heart disease, diabetes, colon cancer, constipation, and diverticular diseases) prevalent in the Western world (*3*, *4*). However, according to a current report of the National Academy of Science on dietary reference intakes for macronutrients including DF (*5*), the amount of DF consumed in Western countries such as the United States was only 14−15 g per person per day on average, which was well below the recommended level (about 20−25 g per healthy person per day) reported by the American Dietetic Association (*4*). Except in certain therapeutic situations, DF should be normally obtained through consumption of foods. One way to improve the dietary intake of DF is through utilization of DF isolates as food ingredients, i.e., fiber supplementation. The increased awareness of the potential health benefits of DF among consumers has undoubtedly encouraged food manufacturers to explore new DF sources and developed fiber-enriched or fiber-fortified food products such as snack foods, beverages, cookies, canned meat, etc. (6, *7*). Nowadays, most fiber supplements are obtained from byproducts resulting from the processing (e.g., milling) of cereals, fruits, vegetables, and legumes (*6*). Because of the highly competitive market of fiber-enriched food products, there is an urgent need in the exploration of more alternative source of DF.

Edible mushrooms or fungi have been widely utilized as human foods for centuries and have long been appreciated for

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their texture and flavor as well as some medicinal and tonic attributes (*8*, *9*). However, the awareness of mushrooms as a health food and as an important source of biologically active substances with medicinal value has only recently emerged (*8*). Mushrooms are considered a healthy food because they are low in calories and fat (with a high proportion of unsaturated fatty acids) but rich in proteins, minerals, and DF (*8*, *10*). Edible and/or medicinal mushrooms can be considered as a potential new source of DF, since fungal cell walls are rich in NSPs such as chitin and β-glucans, which are homo- and heteroglucans with β-1,3, β-1,4, and β-1,6 glucosidic linkages (*9*, *11*). Our previous studies have shown that some edible and/or medicinal mushrooms possessed a substantial amount of DF in their different developmental stages including mycelia, sclerotia, and fruiting bodies (*12*−*15*). Among these developmental stages, the amount of DF content prepared from the sclerotia was more than 70% of dry weight (*11*, *13*, *14*, *16*). To explore and develop the sclerotia as a novel alternative DF source, more comprehensive information on the chemical and physical characteristics of sclerotial DF is necessary. However, studies on this area are very limited. In the present study, IDF and SDF fractions were prepared from three edible and medicinal sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinoceros*, and *Wolfiporia cocos* and their characteristics such as yield, purity, monosaccharide profile, and microstructure were compared.

MATERIALS AND METHODS

Sample Preparation. Sclerotia of *P. tuber-regium* (Fr.) Sing., *P. rhinoceros* Cooke, and *W. cocos* (Schw.) Ryv. et Gilbn. were obtained from the Sanming Mycological Institute in the Fujian Province of China. All sclerotia were cleaned with a minimum amount of double-distilled water to remove the dust and soils adhered on their surfaces and then air-dried. The rind (outer covering) of the sclerotia was removed in order to avoid any contaminant that might come from the compost and the plastic wrapping bag during cultivation, and the sclerotia were then pulverized to pass through a screen with an aperture of 0.5 mm by using a cyclotech mill (Tecator, Höganäs, Sweden). The milled mushroom powders were transferred to airtight plastic bags and stored in a desiccator at room temperature (24 °C) prior to proximate analysis as well as DF determination.

Proximate Analysis. The crude protein content was calculated by multiplying the nitrogen (N) content, which was determined by a CHNS/O Analyzer (Perkin-Elmer 2400, Connecticut) with a factor of 4.38 (*17*). The ash contents were estimated by heating the mushroom samples overnight in a temperature-controlled muffle furnace (NEY 1100C, VULCAN A-550, A&P Instrument Co. Ltd.) at 525 °C (*18*). Crude lipids were extracted from the mushroom powders in a Soxhlet extractor (Soxtec System HT6, Tecator) using petroleum ether. The content of crude lipids was then determined gravimetrically after oven drying (100 °C) the extract overnight. Moisture content determined by an infrared moisture analyzer (Mettler LJ 16, Greifensee, Switzerland) at 120 °C was expressed as percentage by weight of sample.

Mushroom IDF and SDF Contents. Before determining the IDF and SDF content, simple sugar was removed from mushroom samples by using a procedure described by Lee and her coinvestigators (*19*). In short, the mushroom sample was desugared by initially extracting it with 47% ethanol under 30 min of sonication at 60 °C (30 mL/g) and further stirring it for 1 h at room temperature after the addition of 95% EtOH (70 mL/g), making the final concentration of EtOH to about 80%. Subsequently, the mixture was filtered via ashless filter paper (Whatman No. 41) and the ethanol insoluble residue was further washed three times each with 80% EtOH, 95% EtOH, and acetone sequentially (50 mL/g). The desugared mushroom sample was oven-dried at 40 °C overnight and milled to powder by using a hammer mill (MFC, IKA, Stanfeni, Br.) equipped with a 0.5 mm screen.

The content of IDF and SDF in the three sclerotia was determined according to the AOAC enzymatic−gravimetric method (*20*) with slight modifications. In brief, 1 g of desugared mushroom sample was added to 40 mL of 0.05 M Mes-Tris buffer (pH 8.2) followed by the addition of 50 μL of heat-stable α-amylases (EC 3.2.1.1 from *Bacillus licheniformis*, catalog no. A33306, Sigma Chemical Co., St. Louis, MO). Then, the mixture was incubated in a boiling water bath (95−100 °C) for 30 min. After it was cooled to 60 °C, 100 μL of bacterial protease (from *B. licheniformis*, catalog no. P3910, Sigma) was added to the mixture and was incubated at 60 °C for 30 min with continuous agitation. Subsequently, 5 mL of hydrochloric acid (0.561 M) was dispensed into the mixture, and the pH of the mixture was adjusted to 4.00−4.70 at 60 °C by using 1 M sodium hydroxide or 1 M hydrochloric acid. Then, 100 μL of fungal amyloglucosidase (EC 3.2.1.3 from *Aspergillus niger*, catalog no. A3513, Sigma) was added into the mixture and incubated at 60 °C for a further 30 min with continuous shaking. The heat stable α-amylase enzymes used to gelatinize and depolymerize the glycogen into dextrins had been tested to be free of β-glucanase, while the protease and amyloglucosidase were used to hydrolyze protein and to further break down dextrins into glucose, respectively. The enzyme-treated mixture containing the buffer solution and nondigestible materials was filtered with the Fibertec System E (1023 Filtration Module, Tecator). The residue recovered (IDF) was washed twice each with 15 mL of 78% ethanol, 95% ethanol, and acetone sequentially and was dried in an air oven at 40 °C overnight. The filtrate was precipitated with four volumes of 95% ethanol overnight. Then, the ethanol insoluble residue (SDF) recovered from centrifugation (4800 rpm, 5 min, 25 °C) was washed with the solvent exchange system and dried in the same way as the IDF. Following the same procedure, a reagent blank was prepared without any sample added. The gravimetric yield of the mushroom SDF and IDF were obtained after corrections for the content of ash, residual protein (see below), and the reagent blank. The moisture content of the desugared mushroom powder and IDF and SDF samples was also determined.

Neutral and Amino Sugar as well as Uronic Acid Contents. The neutral and amino sugar contents of the mushroom IDF and SDF prepared from the aforesaid enzymatic−gravimetric method were determined by gas chromatography (GC) according to the method described by Cheung and Lee (*16*) with slight modifications. In brief, the mushroom IDF and SDF samples first underwent a modified Saeman hydrolysis (*21*) with 12 M sulfuric acid for 1 h at 35 °C followed by a further hydrolysis for 1 h at 100 °C after dilution with distilled water to 2 M concentration. Alditol acetates of the neutral and amino sugars in the acid hydrolysates were prepared according to the methods as described by Blakeney et al. (*22*) with β-D-allose as the internal standard. The alditol acetate derivatives of polysaccharide sugars were quantified by a HP 6890 GC using an Alltech DB-225 capillary column (15 m × 0.25 mm; i.d. 0.25 μm film) and an oven temperature program of initial temperature 180 °C with a hold of 4 min, followed by a temperature rise of 2 °C/min to 220 °C with a final hold of 25 min. Helium was used as the carrier gas, and the sugar derivatives were detected by flame ionization. Individual sugars were corrected for losses during hydrolysis as well as derivatization and for their difference in their responses to GC detector by a molar correction factor determined from the recovery of sugar standards subject to the same treatment as the mushroom fiber samples. The values for the neutral and amino sugars were expressed as polysaccharide residues (anhydrosugars) by multiplying the amount of pentose, deoxy hexose, and hexose with a factor of 0.88, 0.89, and 0.90, respectively. The uronic acid content in the acid hydrolysate was determined separately by a modified colorimetric method using 3,5-dimethylphenol with D-galacturonic acid monohydrate as the standard and expressed as a polysaccharide residue by multiplying by a factor of 0.83 (*23*). Residual protein content in the mushroom IDF and SDF was estimated by multiplying the N, which was determined by a CHNS/O Analyzer (Perkin-Elmer 2400), by a factor of 6.25 after correction for corresponding chitin N (*13*). The total monosaccharide content or NSP of the mushroom IDF and SDF was calculated as the sum of neutral, amino, and uronic acids polysaccharides residues.

Scanning Electron Microscopy. Mushroom SDF and IDF powders were fixed on copper specimen stubs with double-sided, sticky carbon tape followed by coating with gold−palladium in a sputter coater (S150B, Edwards, England). The samples were then examined using a

Table 1. Proximate Composition of the Three Sclerotia (g/100 g Sample DM)[a]

fungal name	moisture	protein	ash	lipid	carbohydrate[b]
P. tuber-regium	12.9 ± 0.10[a]	6.71 ± 0.23[a]	2.78 ± 0.23[a]	0.05 ± 0.01[a]	90.5 ± 0.49[a]
P. rhinoceros	15.3 ± 0.10[b]	2.75 ± 0.02[b]	1.52 ± 0.06[b]	0.02 ± 0.01[b]	95.7 ± 0.09[b]
W. cocos	15.3 ± 0.20[b]	0.67 ± 0.06[c]	1.09 ± 0.13[c]	0.14 ± 0.01[c]	98.1 ± 0.22[c]

[a] Data are mean values of three determinations ± SD. Means in columns with different letters (a–c) are significantly different ($p < 0.05$, one way ANOVA, Tukey-HSD). [b] Carbohydrate = 100 − protein − ash − lipid.

Table 2. Gravimetric Quantification of IDF and SDF Contents (g/100 g Sample DM) Obtained from Three Sclerotia by the AOAC Method[a]

fiber fraction	fungal name	DF rich materials	residual protein[b]	ash	DF[c]
IDF	*P. tuber-regium*	83.4 ± 1.77[a]	4.73 ± 0.05[a]	1.21 ± 0.01[a]	77.4 ± 0.09[a]
	P. rhinoceros	87.1 ± 0.51[b]	1.65 ± 0.16[b]	1.02 ± 0.12[a]	84.4 ± 0.25[b]
	W. cocos	95.7 ± 1.40[c]	0.39 ± 0.01[c]	0.70 ± 0.23[b]	94.6 ± 0.23[c]
SDF	*P. tuber-regium*	3.55 ± 0.11[d]	1.02 ± 0.01[d]	0.03 ± 0.01[c]	2.50 ± 0.02[d]
	P. rhinoceros	2.51 ± 0.04[d]	1.04 ± 0.01[d]	0.02 ± 0.01[c]	1.45 ± 0.01[e]
	W. cocos	2.15 ± 0.31[d]	0.22 ± 0.01[c]	0.02 ± 0.01[c]	1.91 ± 0.01[f]

[a] Data are mean values of three determinations ± SD. Means in columns with different letters (a–f) are significantly different ($p < 0.05$, one way ANOVA, Tukey-HSD). [b] Residual protein = (total N-chitin N) × 6.25. [c] DF = DF rich materials − residual protein − ash.

JEOL scanning electron microscope (JSM-5300, Japan) at an accelerating voltage of 10 kV.

Statistical Analysis. All data were presented as mean values of three determinations ± SD. The results of all mean values were analyzed by one way analysis of variance (ANOVA) and Tukey-HSD at $p < 0.05$ (*24*) to detect significant differences among groups.

RESULTS AND DISCUSSION

Proximate Analysis. The sclerotia produced by the three species had a similar yield of about 50% biomass conversion (i.e., 2 kg of compost material produced about 1 kg of sclerotium). **Table 1** shows the proximate composition of the sclerotia of *P. tuber-regium*, *P. rhinocerus*, and *W. cocos*. In general, the sclerotia exhibited a similar pattern of proximate composition with a substantial amount of carbohydrate (ranging from 90.5 to 98.1% DM) and extremely low lipid content (ranging from 0.02 to 0.14% DM), indicating that all three sclerotia may belong to the type of carbohydrate rich sclerotia previously suggested by Colely-Smith and Cooke (*25*). Similar results on these sclerotia were reported previously (*13*, *26*−*28*). The crude protein (<7.00% DM) and ash contents (<3.00% DM) of the three sclerotia were low and were significantly different from each other (*P. tuber-regium*, the highest; *W. cocos*, the lowest). Although the crude protein content of *P. tuber-regium* was much lower than that (10.8% DM) reported by Ude and his coinvestigators (*28*), these findings would become comparable if the protein conversion factor of 4.38 instead of 6.25 was used in the latter case (7.58% DM). In fact, Basidiomycetes such as *P. tuber-regium* possess an appreciable amount of nonprotein N (mainly coming from chitin/chitosan), which must be corrected before the crude protein content is estimated from the N content and protein conversion factor (*27*, *29*). The ash content of *P. tuber-regium* was much higher than that (0.71% DM) reported previously (*28*); however, similar findings (4.00% DM) had also been reported by Nwokolo (*27*). All air-dried sclerotia possessed a notably high level of moisture; that of *P. tuber-regium* was the significantly lowest. Although the moisture content of the *P. tuber-regium* sclerotium was consistent with that grown in Nigeria (14.9% DW) (*28*), a much higher level of moisture content (23.7% DW) of this air-dried sclerotium had been reported by Nwokolo (*27*). Except for moisture content, all proximate compositions of the *W. cocos* were in agreement with those of our previous study (*13*).

IDF and SDF Content. The gravimetric quantification of IDF and SDF content obtained from the sclerotia is presented in **Table 2**. All three sclerotia possessed a remarkably high level of IDF content (ranging from 77.4 to 94.6% DM, which represents 92.5−98.9% of the IDF rich material) and an exceptionally small amount of SDF content (ranging from 1.45 to 2.50% DM, which represents 57.8−88.8% of the SDF rich material) (**Table 2**). Both the IDF (*W. cocos*, the highest; *P. tuber-regium*, the lowest) and the SDF (*P. tuber-regium*, the highest SDF; *P. rhinocerus*, the lowest) content of the three sclerotia were significantly different from each other. The TDF content in the sclerotia of the *P. tuber-regium*, *P. rhinocerus*, and *W. cocos* was 79.9, 85.9, and 96.5% DM, respectively (the sum of IDF and SDF content). This notably high amount of TDF suggested that all three sclerotia could be considered as an alternative source of DF. In contrast to our previous findings (73.5% DM) (*13*), the extremely high TDF content in *W. cocos* sclerotia might due to the fact that mature *W. cocos* sclerotia (medulla region is light brown in color) instead of young one (medulla region is white in color) was used in the present study. By comparing the crude protein content of the three sclerotia (**Table 1**) with their corresponding residual protein content in both IDF and SDF rich materials, it clearly indicated that the removal of fungal proteins in the three sclerotia by using the bacterial protease normally applied in the AOAC official methods was not efficient and the majority of the sclerotial proteins remained in their insoluble fractions. The coprecipitation of the sclerotial proteins during the ethanol precipitation steps in the AOAC methods may be attributed to using inappropriate protease since enzymatic hydrolysis of protein required very high specificity. It was worthy to find out whether a fungal protease would be an alternative, since fungi commonly secrete proteases to digest and utilize their storage proteins during growth in different developmental stages including the sclerotial stage. However, the best way might be selecting the most appropriate protease according to the amino acid profile of the three sclerotia. Furthermore, inaccessibility of protein by protease in the presence of high fiber content as well as the physically impeccable structure of sclerotia may be the other reasons (*16*).

Monosaccharide Profile. As shown in **Table 3**, all of the three sclerotia shared similar monosaccharide patterns in their IDF (only glucose, glucosamine, and uronic acids were present)

Table 3. Monosaccharides Profile (g/100 g DF Rich Materials DM) in the IDF and SDF Fractions of the Three Sclerotia[a]

fiber fraction	fungal name	glucose	glucosamine	mannose	galactose	uronic acids	NSP[b]
IDF	*P. tuber-regium*	81.5 ± 1.06[a]	6.31 ± 0.55[a]	ND[c]	ND	2.13 ± 0.09[a]	89.9 ± 1.19[a]
	P. rhinoceros	89.6 ± 1.45[b]	1.98 ± 0.04[b]	ND	ND	0.57 ± 0.06[b]	92.2 ± 1.56[a]
	W. cocos	86.8 ± 2.08[b]	1.53 ± 0.09[bd]	ND	ND	1.95 ± 0.07[a]	90.3 ± 2.27[a]
SDF	*P. tuber-regium*	14.8 ± 0.12[c]	1.63 ± 0.26[bd]	4.12 ± 0.04[a]	0.52 ± 0.06[a]	1.28 ± 0.09[c]	22.4 ± 0.39[b]
	P. rhinoceros	18.0 ± 0.89[c]	ND	9.57 ± 0.32[b]	0.92 ± 0.06[b]	1.12 ± 0.16[c]	29.6 ± 0.91[c]
	W. cocos	6.71 ± 0.18[d]	1.01 ± 0.11[c]	13.1 ± 0.20[c]	5.31 ± 0.16[c]	1.27 ± 0.04[c]	27.4 ± 0.39[c]

[a] Data are mean values of three determinations ± SD. Means in columns with different letters (a–d) are significantly different ($p < 0.05$, one way ANOVA, Tukey-HSD). [b] NSP = sum of available individual sugar (amino, neutral, and acidic sugar). [c] ND = not detected.

as well as SDF fractions [monosaccharides present included glucose, glucosamine (except *P. rhinocerus*), mannose, galactose, and uronic acids]. The monosaccharide composition of the IDF and SDF fractions did not only reflect the constituent of fungal polysaccharides in the three sclerotia but also the solubility of these polysaccharides. Because the amount of IDF rich material (83.4−95.7% sample DM) of the three sclerotia was remarkably higher (at least 20 times) than that of the SDF rich material (only 2.15−3.55% sample DM) (**Table 2**), the major composition of the fungal cell wall polysaccharides in these sclerotia could be reflected from the monosaccharide constituents of the IDF fraction. The predominant presence of glucose (81.5−89.6% IDF rich material DM) together with glucosamine content (1.53−6.31% IDF rich material DM) in the IDF fractions suggested that β-glucan and chitin would be the main matrix and fibrillar components of the fungal cell wall polysaccharide in these three sclerotia, respectively. In addition to chitin, the majority of the β-glucan was insoluble.

Other minor sugar residues found in the sclerotial fiber rich materials included mannose, galactose, and uronic acids, and this may indicate the presence of a small amount of mannan, galactan, and polyuronides. The three sclerotia might possess glucuronic acids, since the presence of glucuronic acids in other edible fungi like *Tremella aurantia* and *Tremella fuciformis* has also been reported (*30*, *31*). Previous studies had reported that the two major components of sclerotial hyphae cell wall were β-1,3-glucan with β-1,6-linked branch chains (at different extents) as well as chitin (*32*). Besides the extracellular matrix, which is composed of highly hydrated materials expanding and filling the interhyphal spaces within the sclerotia, it was also found to be composed of mainly β-1,3-glucan with β-1,6-linked side branches. These extracellular polysaccharides may contribute to the soluble β-glucan detected in the SDF fraction while the existence of glucosamine in the SDF fraction may reflect the presence of chitosan (probably derived from the cell wall chitin). Apart from morphogenesis, storage, and supply of water to withstand unfavorable environmental conditions (e.g., drought), one of the most important functions of the extracellular matrix in sclerotia is to provide a large reserve of carbohydrates for energy during germination.

Table 3 also shows that apart from lack of mannose and galactose, the IDF fractions of the three sclerotia exhibited significantly higher glucose (90.6−97.2% of the NSP) but significantly lower uronic acids composition (0.62−2.37% of the NSP) than those of their corresponding SDF fractions. However, no particular trend was observed in the glucosamine content. Besides, glucose was the predominant monosaccharide found in both fractions (60.8−97.2% of the NSP) except the SDF fraction of *W. cocos* (only 24.5% of the NSP) in which mannose was the major monosaccharide present (47.8% of the NSP).

Among the monosaccharide profile of the IDF fractions, *P. rhinocerus* IDF and *W. cocos* IDF were similar except that the uronic acid content of the *P. rhinocerus* IDF was the lowest. *P. tuber-regium* IDF was characterized by its lowest glucose and highest glucosamine content (**Table 3**). On the contrary, the monosaccharide composition of the SDF fractions was significantly different from each other (**Table 3**). *P. tuber-*

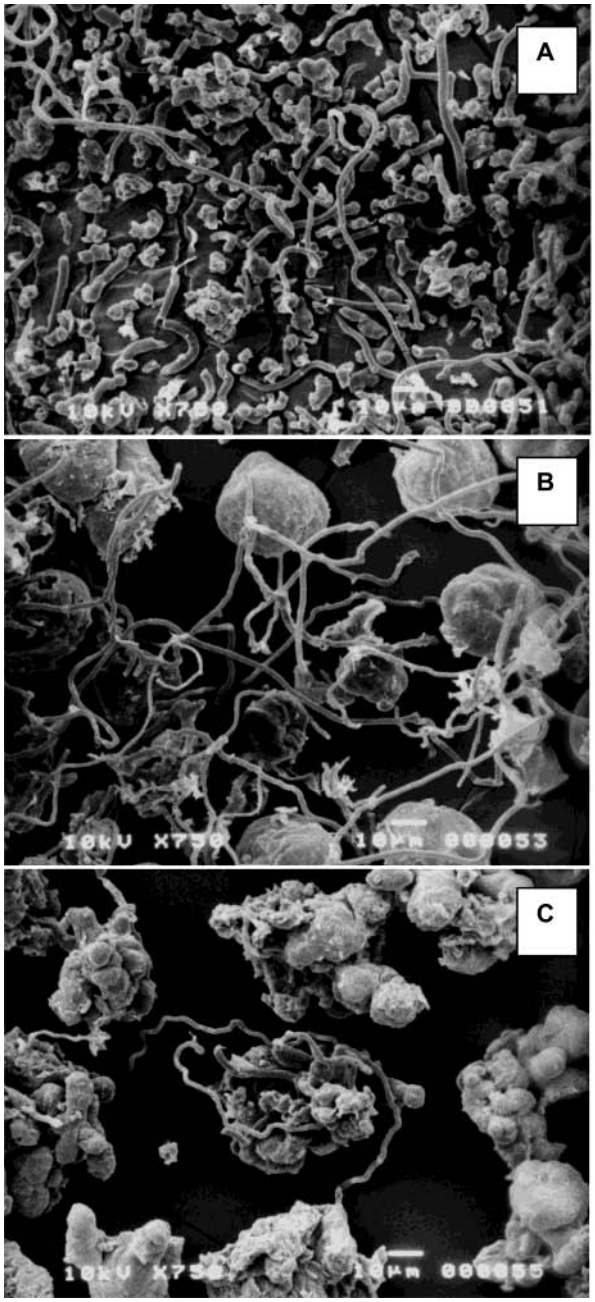

Figure 1. Scanning electron micrographs of IDF fractions from the sclerotia of PTR (**A**), PR (**B**), and WC (**C**); magnification, 750×.

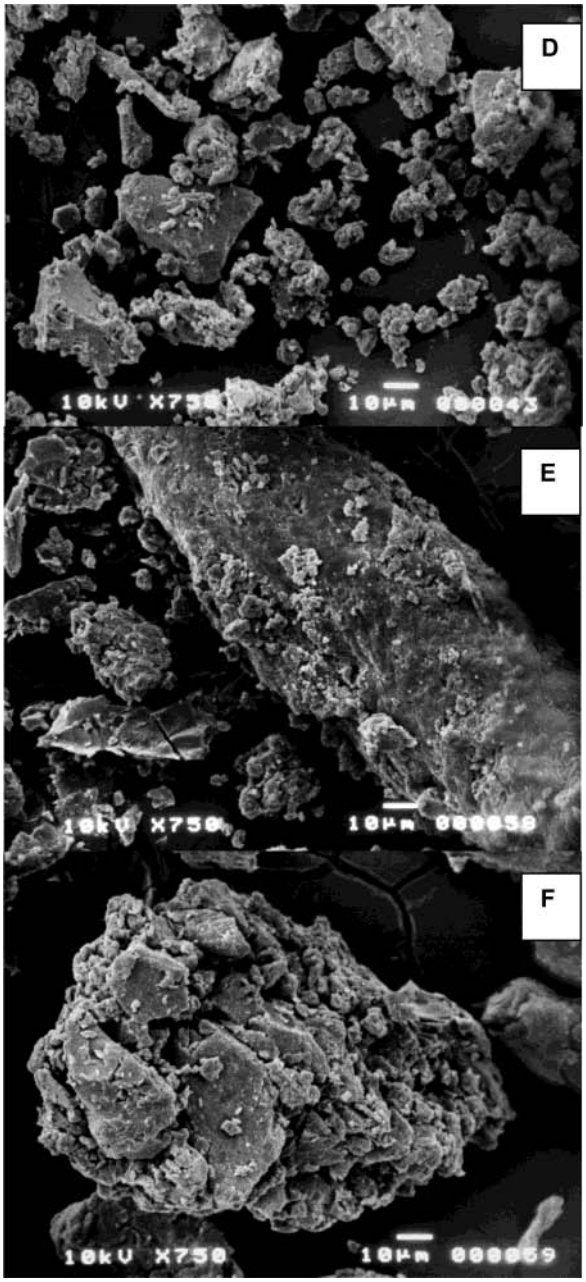

Figure 2. Scanning electron micrographs of SDF fractions from the sclerotia of PTR (**D**), PR (**E**), and WC (**F**); magnification, 750×.

regium SDF exhibited the highest glucose, glucosamine, and uronic acid composition but the lowest mannose and galactose content. Apart from possessing the lowest uronic acid composition, no glucosamine was detected in the *P. rhinoceros* SDF. Although *W. cocos* SDF had the lowest glucose composition, its mannose as well as galactose contents were shown to be the highest.

Scanning Electron Microscopy. Figure 1A−C shows the scanning electron micrographs of the IDF as well as SDF fractions of the three sclerotia. Unlike the SDF fractions, after enzymatic removal of glycogen and protein, all three sclerotial IDF fractions showed fragments of interwoven hyphae, which is one of the main morphological characteristics of sclerotia at the medulla region, together with some insoluble materials. The relative amount of hyphae present in the IDF fractions was in the order of *P. tuber-regium* > *P. rhinoceros* > *W. cocos*. The more hyphae present, the fewer the amount of insoluble materials were observed and vice versa. Regardless of the amount of interwoven hyphae, no significant difference on percent of NSP was obtained among the IDF fractions (**Table 3**). This may suggest that the insoluble materials would likely be fiber rich materials similar to that of hyphae cell wall polysaccharides.

Only amorphous structure was observed in the SDF fractions of the three sclerotia (**Figure 2D−F**). All three SDF fractions possessed a high percent of residual protein (ranging from 10 to 41.5% SDF rich material DM) [residual protein content in SDF fraction (g/100 g sample DM)/amount of SDF rich material (g/100 g sample DM) × 100%; **Table 2**] especially in *P. rhinocerus***,** and their percent of residual proteins was also significantly different from each other. Because only 22.4−29.6% DM of this soluble and irregular materials was NSP (**Table 3**), the sclerotial extracellular matrix (mainly β-1,3-glucan with β-1,6-linked side branches) mentioned above was not the major constituent of the SDF fractions in these three sclerotia. Among the three SDF fractions, the percent of NSP in *P. tuber-regium* was found to be the lowest.

As compared to the SDF fraction, the remarkably high yield and high purity of IDF of the three sclerotia suggested their great potential to act as an alternative source of some common IDF like wheat bran. However, for successful incorporation into formulated food, technological properties such as swelling, water holding capacity, oil holding capacity, color, etc. of the sclerotial IDF should be further investigated (*33*). Besides, the β-glucan rich sclerotial IDF may also be used as a raw material in the popular nutraceutical market nowadays, since mushroom NSPs have been found to possess biopharmacological effects such as antitumor (*34*−*36*) as well as cholesterol-lowering effects (*37*−*38*) in our previous studies.

ABBREVIATIONS USED

NSP, nonstarch polysaccharides; DF, dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; DM, on dry matter basis.

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