AGRICULTURAL AND FOOD CHEMISTRY

Dietary Fibers from Mushroom Sclerotia: 1. Preparation and Physicochemical and Functional Properties

KA-HING WONG AND PETER C. K. CHEUNG*

Food and Nutritional Sciences Program, Department of Biology, The Chinese University of Hong Kong, Shatin, N. T., Hong Kong, China

Preparation of three novel dietary fibers (DFs) from mushroom sclerotia, namely, *Pleurotus tuberregium, Polyporous rhinocerus*, and *Wolfiporia cocos*, by a scale-up modified AOAC procedure using industrial enzymes was investigated. A remarkably high level of total dietary fiber (TDF) ranging from 81.7 to 96.3% sample dry matter (DM), in which a content of nonstarch polysaccharide (NSP) ranging from 86.6 to 94.3% sclerotial TDF DM, was obtained from the three sclerotia. All sclerotial DFs were rich in β -glucan (the glucose residue ranged from 89.7 to 94.5% NSP DM) with a very low level of resistant glycogen (ranged from 3.77 to 3.94% sclerotial TDF DM). All three novel sclerotial DFs also exhibited similar, if not better, physicochemical and functional properties (pH, color, water binding capacity, oil holding capacity, and emulsifying properties) as those of barely DF control and commercial DF-rich ingredients. The potential use of the three mushroom sclerotial DFs as a new β -glucan type DF-rich ingredient in the food industry was discussed.

KEYWORDS: Mushroom sclerotia; total dietary fiber; industrial enzymes; physicochemical and functional properties

INTRODUCTION

It has been demonstrated by extensive research in the past three decades that sufficient dietary fiber (DF) intake has benefits for health maintenance and disease prevention (1). The importance of DF on nutrition and health has led to the development of a large and potential DF market as well as a trend of searching for new DF sources (2, 3). In response to the demand of high DF foods from consumers, numerous fiber-incorporated food products using fiber-rich ingredients have been developed by the food manufacturers (4). In addition to its physiological benefits, DF has desirable functional properties to provide texture, gelling, thickening, emulsification, and stabilization in DF-enriched foods (4, 5). Therefore, DF research has drawn much attention recently, particularly in the growing nutraceutical industry (6, 7). DF of different origins has different structures, chemical compositions, and physicochemical properties that would exhibit different nutritional, technological, and physiological benefits (4, 7-9). Our previous study has found that some mushroom sclerotia, which were dry compact biomass of fungal hyphae, had over 80% dry matter (DM) of total dietary fiber (TDF) [mainly β -glucan type nonstarch polysaccharide (NSP)], that could be used as a potential new source of DF (10). Although there are distinct advantages (e.g., energy saving, environmental friendly, nontoxic, specific, etc.) of using the analytical enzymes listed in the AOAC enzymatic-gravimetric method for scale-up preparation of the sclerotial DF in food industry, the high cost of these enzymes, especially the protease, is a major concern. An enzymatic procedure for preparing DF from three mushroom sclerotia, namely, *Pleurotus tuber-regium* (Fr.) Sing., *Polyporous rhinocerus* Cooke, and *Wolfiporia cocos* (Schw.) Ryv. et Gilbn., using industrial food grade glycolytic and proteolytic enzymes has been recently developed in our laboratory (*11*). Moreover, to explore the application of these three novel sclerotial DFs, the ways of incorporating them into formulated foods should be determined. Therefore, some physicochemical and functional properties [such as color, pH, water binding capacity (WBC), oil holding capacity (OHC), and emulsifying properties] of the three novel sclerotial DFs prepared from the aforesaid enzymatic procedure were investigated and compared with those of commercial DF in the present study in order to evaluate their potential in developing fiber-enriched products with high consumer's acceptance.

MATERIALS AND METHODS

Sample Materials. Sclerotia of *P. tuber-regium, P. rhinocerus*, and *W. cocos* were cultivated by the Sanming Mycological Institute in the Fujian Province of China. The peeled sclerotia were pulverized to pass through a screen with an aperture of 0.5 mm by using a cyclotech mill (Tecator, Höganäs, Sweden), and the sclerotial powders were stored in a desiccator at room temperature (24 °C). Industrial glycolytic (Termamyl Supra and Dextrozyme E) and proteolytic enzymes (Alcalase 2.4 L FG and Protamex) kindly donated by the Novozymes A/S (Bagsvaerd, Denmark) were chosen in this study based on our previous results in the optimal combination of these enzymes (*11*). A commercial barley fiber-rich ingredient (ID 76) kindly provided by ID FOOD (IDIRC, France) was used as the control since it is also rich in β -glucan and possesses a similar insoluble DF to soluble DF ratio (88:12, manufacturer's information) to that of the three sclerotial DFs (*10*).

^{*} To whom correspondence should be addressed. Tel: 852-26096144. Fax: 852-26035646. E-mail: petercheung@cuhk.edu.hk.

Scale-Up Enzymatic Preparation of Sclerotial DF. DF was prepared from individual mushroom sclerotia by a scale-up procedure modified from the AOAC TDF enzymatic-gravimetric method 985.29 (12), as described previously (13). In brief, each gram (a total of 54 g was divided into six portions of 9 g in six separate beakers) of desugared sclerotial powders was added to 50 mL of 0.08 M sodium phosphate buffer (pH 6.0) and gelatinized in a boiling water bath for 30 min. The mixture was then cooled to 75 °C followed by pH adjustment to pH 5.4. Subsequently, 41.6 μ L of Termamyl Supra (heat stable industrial α -amylases) was added to the mixture, which was then incubated for 30 min with continuous shaking. For preparing P. tuber-regium DF, the temperature and pH of the mixture were adjusted to 60 °C and pH 7.5, while in the case of both P. rhinocerus and W. cocos, the temperature and pH of their mixture were adjusted to 50 °C and pH 6.5 instead. Both industrial proteases, Alcalase 2.4 L FG (7.95 μ L) and Protamex (225.2 μ L) were then separately added to the mixture of P. tuber-regium as well as P. rhinocerus and W. cocos, respectively. All mixtures were incubated for a further 30 min with continuous agitation. Consequently, the temperature and pH of all mixtures were adjusted to 60 °C with a pH of 4.3 followed by addition of 136.2µL of Dextrozyme E (industrial amyloglucosidase). All mixtures were then incubated at 60 °C for a further 30 min with continuous shaking. The heat stable α -amylase enzymes were used to depolymerize the sclerotial glycogen into dextrins, which had been tested to be free of β -glucanase, while the protease and amyloglucosidase were used to hydrolyze the sclerotial proteins and to further break down the dextrins into glucose, respectively. The enzyme-treated mixture containing the buffer solution and nondigestible materials was precipitated with four volumes of 95% ethanol overnight. The residue recovered was washed twice each with 78% ethanol, 95% ethanol, and acetone sequentially and was dried in an air oven at 40 °C overnight. The resulting indigestible materials were pooled together to give the sclerotial DF. The gravimetric yield of the three sclerotial TDF was obtained after corrections for their corresponding content of ash, residual protein, and the reagent blank as reported previously (10). The moisture content of the three sclerotial DF samples was also determined.

Carbohydrate Composition. The monosaccharide profile (neutral and amino sugar as well as uronic acid contents) and resistant glycogen (RG) content of the three sclerotial DF were investigated as previously described (*10*, *11*).

pH and Color. The pH was determined potentiometrically with a pH meter using a 10% (w/v) suspension of each sclerotial DF at 25 °C. The color of the three sclerotial DFs and barley DF control powder was individually measured at three different locations by using a full scanning spectrocolorimeter (LabScan XE, Hunter Associates Laboratory, Inc., Reston, VA) after standardization with a white ceramic plate ($L^* = 93.3$, $a^* = -1.01$, and $b^* = 0.63$) at 10° with a D-65 illuminant source. The color was recorded using CIE- $L^* a^* b^*$ uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis. The total color difference between each sclerotial DF sample and the barley DF control was calculated by the following formula: $\Delta E^* = [(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})]^{1/2}$.

Scanning Electron Microscopy (SEM). SEM of the three sclerotial DFs and barley DF control was prepared by fixing the DF powder 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 JEOL scanning electron microscope (JSM-5300, Japan) at an accelerating voltage of 10 kV.

WBC. The WBC of all DF samples was measured by the modified centrifugation method described by Suzuki and his co-investigators (*14*). In brief, 20 mL of ultrapure water was added into a centrifuge tube containing 200 mg of sample. After they were incubated in a 25 °C shaking water bath for 24 h, the tubes were centrifuged at 14000*g* for 30 min at 25 °C (J2 Series, High-Speed Centrifuge, Beckman Instruments, Inc., Palo Alto, California). The supernatant (unbound water) was discarded, and the amount of water held in the hydrated sample was determined by heating the preweighed pellet in an air oven for 2 h at 120 °C. The WBC of each DF sample was expressed as the weight of water held by 1 g of corresponding DF sample.

 Table 1. Gravimetric Determination of the Three Sclerotial Dietary

 Fibers (g/100 g Sample DM) by the Scale-up AOAC Method Using

 Industrial Enzymes^a

sclerotia	TDF	residual protein ^b	ash	corrected TDF ^c
P. tuber-regium P. rhinocerus W. cocos	$\begin{array}{c} 89.3 \pm 2.12 \text{ a} \\ 92.7 \pm 2.88 \text{ a} \\ 98.1 \pm 1.11 \text{ b} \end{array}$	$\begin{array}{c} 6.29 \pm 0.13 \text{ a} \\ 3.33 \pm 0.44 \text{ b} \\ 1.17 \pm 0.18 \text{ c} \end{array}$	$\begin{array}{c} 1.33 \pm 0.01 \text{ a} \\ 1.00 \pm 0.01 \text{ b} \\ 1.21 \pm 0.01 \text{ c} \end{array}$	$\begin{array}{c} 81.7 \pm 2.22 \text{ a} \\ 88.4 \pm 3.13 \text{ b} \\ 96.3 \pm 2.14 \text{ c} \end{array}$

^a Data are mean values of three determinations \pm SD. Means in columns with different letters (a–c) are significantly different (one-way ANOVA using Kruskal–Wallis test, p < 0.05; multiple comparisons using Mann–Whitney U test with Bonferroni correction, p < 0.025). ^b Residual protein = (total N – chitin N) × 6.25. ^c Corrected TDF = TDF – (residual protein + ash).

OHC. The OHC of all sclerotial DF samples and the barley DF control was determined by the method of Caprez et al. (15) with slight modifications. About 2 g of each DF sample was placed in a 50 mL graduated centrifuge tube, followed by the addition of 20 mL of corn oil (Mazola, CPC International Inc., United States). The tube was left for 30 min at room temperature (25 °C) with agitation. Subsequently, the mixture was centrifuged (2500g, 25 °C, 30 min; Beckman GS-15R tabletop centrifuge, Beckman), and supernatant (excess oil) was decanted and weighed. The OHC of the individual DF sample was expressed as the number of grams of oil held by 1 g of corresponding DF sample. The density of the oil was found to be 0.92 g/mL.

Emulsifying Activity (EA) and Emulsion Stability (ES). The EA and ES of the three sclerotial DF samples and barley DF control were determined according to the method described by Yasumatsu et al. (16) with slight modifications. In brief, 1 g of each DF sample was weighed into a 50 mL graduated centrifuge tube, into which 12.5 mL of ultrapure water was added and the mixture was homogenized at 12000 rpm for 30 s with a PT-3000 Homogenizer (Polytron, Kinematica AG, Switzerland). An aliquot (12.5 mL) of corn oil was then added into the slurry, and the mixture was further homogenized at 12000 rpm for 1 min. The emulsion formed was centrifuged (1200 rpm, 25 °C, 5 min), and the EA (%) was calculated from the ratio of the height of the emulsified layer to the height of the total volume of content inside the centrifuge tube. For determining ES, the emulsion formed was further heated in a 80 °C water bath for 30 min and then cooled to room temperature (25 °C) followed by centrifugation under the same conditions. The ES was calculated in the same way as EA and also expressed as percentage of the unheated control.

Statistical Analysis. All data were presented as mean values of three determinations \pm standard deviation (SD) and analyzed by nonparametric one-way analysis of variance (ANOVA) using Kruskal–Wallis test ($p \le 0.05$). When the ANOVA indicated a significant effect, multiple comparisons among the samples were also performed by Mann–Whitney U test with Bonferroni correction ($p \le 0.025$) in order to detect significant differences among groups (17).

RESULTS AND DISCUSSION

Yield and Carbohydrate Composition of the Three Sclerotial DFs. The gravimetric determination of the three sclerotial TDF contents as well as their monosaccharide profiles and RG contents prepared by the scale-up modified AOAC method using industrial enzymes are presented in Tables 1 and 2, respectively. As shown in Table 1, although the residual protein, ash, and TDF contents in all three sclerotia were significantly (p < 0.025) different from each other, their corrected TDF contents were remarkably high (ranged from 81.7 to 96.3% sample DM) and comparable to that of some commercial DF-rich supplements [HUMAMIL (glucomannan), 82.9% DM; FYBOGEL (Ispaghula husk), 88.5% DM; and FIBRAPLAN (soluble fiber from algae, seeds, flours, plants nonspecific), 86.6% DM] (18). This finding suggested that all three sclerotial DFs have great potential to act as an alternative source of high fiber ingredients in the food industry.

Table 2. Monosaccharide Profile (g/100 g Sclerotial TDF DM) of the Three Sclerotial Dietary Fibers Prepared by Industrial Enzymes^a

DF	glucose	glucosamine	mannose	galactose	rhamnose	uronic acids	NSP ^b	RG
P. tuber-regium P. rhinocerus W. cocos	77.7 \pm 2.34 a 84.6 \pm 2.67 b 88.6 \pm 1.13 b	$\begin{array}{c} 5.44 \pm 0.03 \text{ a} \\ 2.17 \pm 0.21 \text{ b} \\ 1.73 \pm 0.01 \text{ b} \end{array}$	0.97 ± 0.03 a 1.11 \pm 0.13 a 1.21 \pm 0.01 b	$\begin{array}{c} 0.05 \pm 0.01 \text{ a} \\ 0.61 \pm 0.02 \text{ b} \\ 0.33 \pm 0.01 \text{ c} \end{array}$	$\begin{array}{c} 0.26 \pm 0.01 \text{ a} \\ 0.53 \pm 0.03 \text{ b} \\ 0.55 \pm 0.03 \text{ b} \end{array}$	2.14 ± 0.01 a 0.51 ± 0.06 b 1.89 ± 0.03 a	86.6 ± 2.55 a 89.5 ± 1.25 ab 94.3 ± 2.50 b	3.91 ± 0.12 a 3.77 ± 0.13 a 3.94 ± 0.27 a

^a Data are mean values of three determinations \pm SD. Means in columns with different letters (a–c) are significantly different (one-way ANOVA using Kruskal–Wallis test, p < 0.05; multiple comparisons using Mann–Whitney U test with Bonferroni correction, p < 0.025). ^b NSP = sum of available individual sugar (amino, neutral, and acidic sugars).

Table 3.	Physicochemical	and Functiona	I Properties of the	Three Sclerotial and	Commercial Barle	y Dietary Fibers
----------	-----------------	---------------	---------------------	----------------------	------------------	------------------

		CIE-Lab ^b				WBC	OHC			
dietary fiber	рН	L*	a*	<i>b</i> *	ΔE^{*c}	(g/g DW ^d)	(g/g DW)	EA (%)	ES (%)	
P. tuber-regium P. rhinocerus W. cocos barley	$\begin{array}{c} 6.11 \pm 0.01 \text{ a} \\ 5.69 \pm 0.01 \text{ b} \\ 5.59 \pm 0.04 \text{ b} \\ 6.64 \pm 0.01 \text{ c} \end{array}$	$\begin{array}{c} 91.5\pm 0.07 \text{ a} \\ 90.4\pm 0.03 \text{ a} \\ 75.4\pm 0.08 \text{ b} \\ 76.6\pm 0.03 \text{ b} \end{array}$	$\begin{array}{c} 0.99 \pm 0.01 \text{ a} \\ 1.43 \pm 0.02 \text{ b} \\ 3.44 \pm 0.13 \text{ c} \\ 3.58 \pm 0.11 \text{ c} \end{array}$	$\begin{array}{c} 9.12 \pm 0.04 \text{ a} \\ 11.6 \pm 0.11 \text{ b} \\ 11.5 \pm 0.03 \text{ b} \\ 21.1 \pm 0.09 \text{ c} \end{array}$	20.9 ± 2.73 a 19.0 \pm 3.25 a 9.84 \pm 0.41 b	$\begin{array}{c} 2.78 \pm 0.11 \text{ a} \\ 2.72 \pm 0.22 \text{ a} \\ 6.66 \pm 0.23 \text{ b} \\ 2.54 \pm 0.21 \text{ a} \end{array}$	$\begin{array}{c} 1.37 \pm 0.04 \text{ a} \\ 1.87 \pm 0.02 \text{ b} \\ 1.36 \pm 0.02 \text{ a} \\ 1.88 \pm 0.01 \text{ b} \end{array}$	$\begin{array}{c} 71.9 \pm 1.07 \text{ a} \\ 69.8 \pm 3.33 \text{ a} \\ 69.2 \pm 3.11 \text{ a} \\ 56.7 \pm 1.17 \text{ b} \end{array}$	$\begin{array}{c} 71.9 \pm 1.07 \text{ a} \\ 69.5 \pm 3.08 \text{ a} \\ 69.2 \pm 3.16 \text{ a} \\ 53.1 \pm 1.43 \text{ b} \end{array}$	

^a Data are mean values of three determinations \pm SD. Means in columns with different letters (a–c) are significantly different (one-way ANOVA using Kruskal–Wallis test, p < 0.05; multiple comparisons using Mann–Whitney U test with Bonferroni correction, p < 0.025). ^b Color was recorded using CIE-Lab uniform color space, where L^* indicates lightness, a^* indicates hue on a green (–) to red (+) axis, and b^* indicates hue on a blue (–) to yellow (+) axis. ^c ΔE^* = total color difference between the three sclerotial DFs and the barley DF control. ^d DW = on dry weight basis.

Table 2 also shows that the three sclerotial DFs had notably high levels of NSP (86.6-94.3% sclerotial TDF DM) in which glucose (89.7-94.5% NSP DM) was the predominant sugar residues. Because only 3.77-3.94% DM of the three sclerotial TDF content was RG, the majority of the glucose residue (95.0-95.6% glucose residue DM) would be the building block of the β -glucan, which is the main component of the fungal cell wall polysaccharide in the three sclerotia (10, 19). Other minor sugar residues found in the three sclerotial DFs also included mannose, galactose, rhamnose, and uronic acids (Table 2). It is worth noting that in the present scale-up preparation, the yield of TDF (Table 1), monosaccharide profile, and RG content (Table 2) of the three sclerotial DFs were in good agreement with those DFs prepared either by a smaller scale (1 g quantity) employing the same industrial enzymes (11) or by the same scale-up procedure using analytical enzymes from Sigma (13). This indicated that the present scale-up DF preparation method using industrial enzymes could obtain sclerotial DF effectively in terms of both quantity (corrected TDF content) and quality (purity and monosaccharide profile) but at a lower cost.

Physicochemical and Functional Properties. Similarly, the physicochemical and functional properties of the three sclerotial DFs obtained in the present study were consistent with our previous results using the same scale-up process but with analytical enzymes from Sigma (*13*). **Table 3** shows that the pH of the suspension of all three sclerotial DFs was slightly acidic (ranged from 5.59 to 6.11), which was consistent with their low levels of uronic acids. Besides, their pH values were significantly (p < 0.025) lower than that of barley DF and higher than that of the DF concentrates prepared from peach (ranged from 3.63 to 3.86) (20) and orange (ranged from 3.85 to 3.93) (21).

As compared with the color of barley DF control, both *P.* tuber-regium and *P. rhinocerus* DFs possessed significantly (p < 0.025) higher values of lightness (L^*) but smaller increments of redness (a^*) and yellowness (b^*) (**Table 3**). Such a high degree of whiteness was a technological advantage of these two sclerotial DFs when added to bakery products such as white pan bread and sugar type cookies, since their incorporation would not likely give off-colors (darker than desired) to these food products (4, 22). The color of *W. cocos*

DF was characterized by exhibiting a similar values of L^* and a^* but a significantly (p < 0.025) lower value of b^* when compared with the color of the barely control. Table 3 also shows that among the three sclerotial DFs, the value of total color difference (ΔE^*) between the *W*. *cocos* DF and the barley DF control was the lowest (9.84; p < 0.025) indicating their relatively higher similarity in color than that of the other two sclerotial DFs. The pinkish brown color of W. cocos DF suggested that its incorporation into a food system might affect the color of the final product. Color is influenced by many factors including species variety, maturity of sample, and processing method (e.g., drying) (21). In this study, because the rind (presence of melanin-like pigment) of all sclerotia was removed during sample preparation, the pinkish brown color of the W. cocos DF would mainly result from the original color of the medulla region of the mature W. cocos sclerotia (23, 24). Although the W. cocos DF prepared from a young W. cocos sclerotia (the medulla region is white in color) exhibited a very high value of lightness ($L^* = 90.8$), its TDF content (73.5%) DM) (25) was remarkably lower than that of the mature one used in the present study.

The WBC of a fiber measures the amount of water retained by the fiber after subject to a stress such as centrifugation (4). This hydration property of DF ingredient is crucial to its successful application in food that will be subjected to physical stress (e.g., extrusion of cereals). Table 3 shows that the W. cocos DF had the highest (p < 0.05) value of WBC (6.26 g/g DW), which was highly comparable to that of some DFs derived from cereal processing byproducts [wheat bran, 6.4-6.6 g/g DW (26, 27); oat bran, 5.5 g/g DW (28)] and fruits [apple DF, 6.3-6.9 g/g DW; pear DF, 6.8 g/g DW (20)], as well as some commercial DF-rich supplements [AGIOLAX (Ispaghula seed and husk, cassia fruit), 6.6 g/g DW (18); FIBREX (sugar beet), 4.56 g/g DW (29)]. The WBC of P. tuber*regium* and *P. rhinocerus* DFs did not differ significantly (p < p0.025) from that of the barley DF, but their WBC levels were also consistent with those of various high fiber ingredients from apple pulp (2.3 g/g DW), wheat bran (2.6 g/g DW), corn bran (2.5 g/g DW), and soy bran (2.4 g/g DW) (5). Besides, the WBC of the barley DF control found was similar to the value (2.0 g/g DW) reported by the manufacturer. The remarkably high



Figure 1. Scanning electron micrographs of dietary fiber prepared from the sclerotia of *P. tuber-regium* (A), *P. rhinocerus* (B), and *W. cocos* (C) and the barley dietary fiber control (D).

WBC of the *W. cocos* DF suggested that this material could be used as a functional ingredient to avoid syneresis (weeping) and to improve the rubbery texture of formulated products such as cheese (4) in addition to reducing calories by total or partial substitution of high-energy ingredients.

Figure 1 presents the scanning electron micrographs of all DF samples, indicating that fragments of interwoven hyphae were only observed in the three sclerotial DFs but not in the case of the barley control. The relative amount of hyphae present in the sclerotial DFs was in the order of P. tuber-regium > P. rhinocerus > W. cocos, which was also consistent with the observation of their IDF fractions reported previously (10). The particle size of the amorphous structural materials in P. tuberregium DF was similar to that of the barley control but obviously smaller than that of the other two sclerotial DFs (Figure 1), even though all three sclerotial DFs were pulverized to powders by the same hammer mill fitted with a 0.5 mm screen. This finding might also explain their differences in WBC among the four DF samples shown in Table 3. Because the smaller the particle size of water insoluble fiber-rich ingredients, the larger surface area for exposure of their hydrophobic regions would result, which might give a low WBC value. Nevertheless, the notably low WBC value of P. rhinocerus DF might even attribute to the presence of higher amount of hyphae (Figure 1). Furthermore, methods of measurement and food system environments (such as pH, ionic strength, concentration, presence of other water binding materials, etc.) are also crucial important factors that affect the WBC of a high fiber ingredient (4, 30, 31).

In the present study, only the OHC of *P. rhinocerus* DF was comparable to that of barley DF (**Table 3**) and wheat DF (2.3 g/g DW) (7). The OHC value of barley DF was also in good agreement with that reported by the manufacturer (1.6 g/g TDF DW). Although the OHC values of DF obtained from *P. tuberregium* and *W.* cocos were significantly (p < 0.025) lower (1.36–1.37 g/g DW), they were comparable to that of orange

DF concentrate (0.86-1.28 g/g) (21) and a commercial DF-rich supplement, FIBREX (1.29 g/g DW) (29).

The ability of a fiber to bind oil is more of a function of the porosity of the fiber structure than the affinity of the fiber molecule for oil (4). Therefore, both presoaking of the high fiber ingredient with water and reduction of particle size would result in a lower OHC value (4, 7), since the number of pores in fiber is reduced or the pores are preoccupied by water. However, in the present study, the SEM of all sclerotial DFs did not show any obvious porous structures (Figure 1). This observation indicated that a more complex mechanism involving other factors such as number of lipophilic sites, overall hydropoboicity, and capillary attraction (32) determined the variations of OHC in the sclerotial DFs. A high fiber ingredient with a high OHC allows the stabilization of high fat content and emulsion by retaining the fat in formulated food products such as comminuted or emulsified meat. In low fat meat applications, the OHC of the high fiber ingredients could also retain the low amount of fat present, which aids the flavor, texture, and juiciness of the final cooked product.

Table 3 also shows that the emulsion formed by all DF samples was generally good, since their EA values (56.7-71.9%) were >50% (16, 33). Besides, the EA of all sclerotial DFs was notably (p < 0.0.5) higher than that of the barley DF control, rice bran DF (14.4%), and the commercial fiber rich supplement, FIBREX (3.46%) (31), suggesting their great potential to act as an emulsifier in formulated food. Furthermore, the emulsions formed from all DF samples were very stable, which was evident by their similar high percentage of ES after incubation at 80 °C for 30 min. It is worth noting that among the three sclerotial DFs, the significantly (p < 0.025)higher level of residual protein in P. tuber-regium DF (Table 1) did not result in better physicochemical and functional properties than the other two sclerotial DFs. This finding indicated that the DF component in the three sclerotial DFs would likely be the main determining factor for their physicochemical and functional properties evaluated in the present study.

The scale-up DF preparation process using industrial enzymes did not only prepare the three sclerotial DFs economically but also maintained their quantity (corrected TDF content), purity (residual protein and ash), and quality (RG and NSP contents and physicochemical and functional properties) when compared with those of the three sclerotial DFs prepared on laboratory scale using the same industrial enzyme combination (11) as well as those using the same scale-up procedure but with the analytical enzymes from Sigma (13). Besides, their physicochemical and functional properties were also comparable to or even better than those of the barley DF control and some commercial fiber rich supplements. Therefore, the three novel sclerotial DFs appeared to be versatile low calorie food ingredients with several technological advantages (natural origin, high DF content, and good physicochemical functional properties) that are of interest to the food ingredient market and could be incorporated into a wide range of formulated foods such as bakery products, noodles, snacks, etc. Further investigations on their physiological performance such as in vitro mineral binding capacity and in vitro fermentability using human faecal microflora are reported in parts 2 and 3 of this series, respectively, in order to evaluate their potential role as a functional food fiber or nutraceutical.

ABBREVIATIONS USED

EA, emulsifying activity; ES, emulsion stability; NSP, nonstarch polysaccharides; OHC, oil holding capacity; RG, resistant glycogen; SEM, scanning electron microscopy; TDF, total dietary fiber; WBC, water binding capacity.

ACKNOWLEDGMENT

We thank Nian-lai Huang of Sanming Mycological Institute, Fujian, China, for the cultivation and identification of the mushroom sclerotia. We also acknowledge the technical assistance of C. C. Li and Freddie Kwok of the Department of Biology, The Chinese University of Hong Kong.

LITERATURE CITED

- ADA Reports. Position of the American Dietetic Association: Health implication of dietary fiber. J. Am. Diet. Assoc. 2002, 102, 993–1000.
- (2) Chau, C. F.; Huang, Y. L. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. J. Agric. Food Chem. 2003, 51, 2615–2618.
- (3) Sloan, A. E. Dietary fiber moves back into mainstream. Food Technol. 2001, 55, 18.
- (4) Nelson, A. L. High-Fiber iIngredients; Eagan Press: Minnesota, 2001.
- (5) Dreher, M. L. Handbook of Dietary Fiber: An Applied Approach; Marcel Dekker: New York, 1987.
- (6) Jalili, T.; Wildman, R. E. C.; Medeiros, D. M. Nutraceutical roles of dietary fiber. J. Nutr. Funct. Med. Foods 2000, 2, 19– 34.
- (7) Thebaudin, J. Y.; Lefebvre, A. C.; Harrington, M.; Bourgeois, C. M. Dietary fibers: Nutritional and technological interest. *Trends Food Sci. Technol.* **1997**, 8, 41–48.
- (8) Blackwood, A. D.; Salter, J.; Dettmar, P. W.; Chaplin, M. F. Dietary fiber, physicochemical properties and their relationship to health. J. R. Soc. Promo. Health 2000, 120, 242– 247.

- (9) Guillon, G.; Champ, M. Structural and physical properties of dietary fibers, and consequences of processing on human physiology. *Food Res. Int.* 2000, *33*, 233–245.
- (10) Wong, K. H.; Cheung, P. C. K.; Wu, J. Z. Biochemical and microstructural characteristics of insoluble and soluble dietary fiber prepared from mushroom sclerotia of *Pleurotus tuberregium*, *Polyporus* rhinocerus and *Wolfiporia cocos. J. Agric. Food Chem.* **2003**, *51*, 7197–7202.
- (11) Wong, K. H.; Cheung, P. C. K. Preparation of nondigestible carbohydrates from mushroom sclerotia using industrial enzymes. *Abstract of Vahouny-ILSI Japan International Symposium on Nondigestible Carbohydrate*; Vahouny Symposia ILSI Japan: Tokyo, Japan, 2004; P-21.
- (12) AOAC. Official Methods of Analysis, 16th edition; Association of Official Analytical Chemists: Washington, DC, 1995.
- (13) Cheung, P. C. K.; Wong, K. H.; Masuyama, R. Dietary fiber from mushroom: an evaluation of their biochemical and physicochemical properties. *Abstract of Seventh International Vahouny Symposium: Dietary Fiber in Health and Disease*; Vahouny Symposium: Edinburgh, United Kingdom, 2002; P-12.
- (14) Suzuki, T.; Ohsugi, Y.; Yoshie, Y.; Shirai, T.; Hirano, T. Dietary fiber content, water holding capacity and binding capacity of seaweeds. *Fish. Sci.* **1996**, *62*, 454–461.
- (15) Caprez, A.; Arrigoni, E.; Amado, R.; Neukom, H. Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran. *J. Cereal Sci.* **1986**, *4*, 233–239.
- (16) Yasutmasu, K.; Sawada, K.; Moritaka, S.; Nfisaki, M.; Toda, J.; Wada, T.; Ishi, K. Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem. Tokyo* **1972**, *36*, 719– 737.
- (17) SPSS Inc. SPSS Base 9.0 Application Guide; SPSS Inc.: Chicago, 1999.
- (18) Goñi, I.; Martin-Carrón, N. *In vitro* fermentation and hydration properties of commercial dietary fiber-rich supplements. *Nutr. Res.* **1998**, *18*, 1077–1089.
- (19) Willetts, H. J.; Bullock, S. Developmental biology of sclerotia. *Mycol. Res.* **1992**, *96*, 801–816.
- (20) Grigelmo-Miguel, N.; Martin-Belloso, O. A dietary fiber supplement from fruit and vegetable processing waste. *1997 Conference* of Food Engineering (CoFE'97); American Institute of Chemical Engineers: Los Angeles, 1997; 73 G.
- (21) Grigelmo-Miguel, N.; Martin-Belloso, O. Characterization of dietary fiber from orange juice extraction. *Food Res. Int.* **1999**, *31*, 355–361.
- (22) Good, H. Measurement of color in cereal products. *Cereal Foods World* 2002, 47, 5–6.
- (23) Bi, Z.; Zheng, G. Y.; Li, T. H. *The Macrofungus Flora of China's Guangdong Province*; The Chinese University Press: Hong Kong, 1993.
- (24) Xu, L.; Wang, W. Chinese Materia Medica—Combination and Applications; China Domica Publishing Ltd.: China, 2002; pp 217–219.
- (25) Cheung, P. C. K. Dietary fiber content and composition of some edible fungi determined by two methods of analysis. J. Sci. Food Agric. 1997, 73, 255–260.
- (26) Adams, R. G.; Evans, A. J.; Oakenfull, D. G.; Sidhu, G. S. Fruit processing wastes as dietary fiber supplements. *Proc. Nutr. Soc. Aust.* **1986**, *11*, 115.
- (27) Ralet, M. C.; Thibault, J. F.; Della Valle, G. Influence of extrusion cooking on the physicochemical properties of wheat bran. J. Cereal Sci. 1990, 11, 249–259.
- (28) Cadden, A. M. Comparative effects of particle size reduction on physical structure and water binding properties of several plant fibers. *J. Food Sci.* **1987**, *52*, 1595–1599.
- (29) Abdul-Hamid, A.; Luan, Y. S. Functional properties of dietary fiber from defatted rice bran. *Food Chem.* 2000, 68, 15– 19.

- (30) Auffret, A.; Ralet, M. C.; Guillon, F.; Barry, J. L.; Thaibault, J. F. Effect of grinding and experimental conditions on the measurement of hydration properties of dietary fibers. *Lebensm.-Wiss. Technol.* **1994**, *27*, 166–172.
- (31) Fleury, N.; Lahaye, M. Chemical and physicochemical characterization of fibers from *Laminaria digitata* (Kombu Breton): A physiological approach. J. Sci. Food Agric. 1991, 55, 389–400.
- (32) Kinsella, J. E. Functional properties of protein in foods: a survey. *J. Food Sci. Nutr.* **1976**, *7*, 219–280.
- (33) Wang, J. C.; Kinsella, J. E. Functional properties of novel protein: Alfafa leaf protein. J. Food Sci. 1976, 41, 286–290.

Received for review May 11, 2005. Revised manuscript received September 12, 2005. Accepted September 26, 2005. This project was financially supported by the Direct Grant allocated from the Research Committee of The Chinese University of Hong Kong.

JF0510788