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ARTICLE

Effects of Sulfation on the Physicochemical and Functional Properties of Psyllium

Wei Liu,[†,‡] Boce Zhang,[‡] Qin Wang,[‡] Zhouhong Xie,[‡] Wenbing Yao,[†] Xiangdong Gao,*[,†] and Liangli (Lucy) Yu*[,‡]

[‡]Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742 and [†]School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, PR China

The sulfation of psyllium was carried out with sulfur trioxide−pyridine in dimethyl formamide. Three sulfated psyllium derivatives, named SP1, SP2, and SP3, were characterized by sulfur content determination, elemental analysis, FT-IR, and surface charge analysis. The sulfated derivatives were also evaluated for their morphological and rheological properties, water uptake capacities, swelling volumes, and in vitro bile acid-binding abilities. The results showed that sulfation reduced the gelling capacity of psyllium and the viscosity of its solution, and significantly increased its bile acid-binding capacity. Sulfation might also increase the water uptake ability of psyllium but might decrease its swelling capacity. The three sulfated psyllium derivatives had in vitro binding capacities against cholic and chenodeoxycholic acids comparable to that of cholestyramine resin on a per same as it is weight basis. The bile acid-binding capacity of SP1 was about 8.4-fold of that observed for the original psyllium preparation under the same assay conditions. The results from this study suggest that sulfation is a possible approach to obtain novel psyllium derivatives with desirable physicochemical, functional, and biological properties for utilization in functional foods or supplemental and pharmaceutical products.

KEYWORDS: Psyllium; sulfation; water uptake; swelling volume; viscosity; bile acid binding

INTRODUCTION

Psyllium, a mucilaginous seed husk preparation from *Plantago* genus, possesses many physiological activities such as hypolipidemic effect, reducing the risk of colon cancer, hyperglycemia and body weight control, and could be used to treat irritable bowel syndrome, constipation, and gastric hypoacidity (*1*, *2*). It could also be used in drug delivery systems and cosmetic products, and for water treatment (*3*−*5*). *Plantago ovata* is a preferred species commercially grown in India, and its mucilage polysaccharide is a highly branched acidic arabinoxylan (*6*). The major challenges to incorporate psyllium in food and beverage formulas or in dietary supplements and other consumer products at the level required for health effects are still its physicochemical properties such as the development of high viscosity and its strong gelling capacity when placed in aqueous systems.

It is well accepted that the physiological and functional properties of psyllium preparations are highly dependent on their physicochemical properties, which are determined by their molecular and chemical structures (*7*). Physical, mechanical, enzymatic, and chemical approaches have been developed to improve the physicochemical properties of psyllium and consequently to promote its utilization in food or other consumer products (*6*−*10*). Both enzymatic and chemical approaches might modify the molecular and chemical structures of psyllium and effectively improve its physicochemical and biological properties (*2*, *6*, *7*, *9*, *10*). The primary limitation for the enzymatic modification of psyllium is the availability of food grade enzymes and the processing cost, although these enzymatic approaches are generally considered green since they do not involve or generate any organic solvents and chemicals (*6*, *7*, *10*). However, chemical modification is less expensive and may produce psyllium derivatives with more diverse chemical and molecular structures, and becomes an important approach to modify the properties of natural biopolymers including psyllium.

Recently, a few psyllium derivatives have been prepared using chemical methods and investigated for their functionalities (*10*−*13*). In 2008, carboxymethylated arabinoxylan was successfully prepared from the arabinoxylan isolated from psyllium (*Plantago ovata*) seed husk by reacting with sodium monochloroacetate under strong alkaline conditions. The carboxymethylation was able to enhance the water solubility of the arabinoxylan from psyllium (*11*). Recently, ethylation of psyllium arabinoxylan was successfully achieved with ethyl iodide and sodium hydroxide in methanol, ethanol, or acetone. Ethylation was shown to alter the intrinsic viscosity of the arabinoxylan derivatives (*12*). In 2009, the treatment with an ethanol solution of hydrochloric acid was shown to improve the functionality of psyllium by reducing its gelling, water-uptake, and swelling

*To whom correspondence should be addressed. (L.Y.) Department of Nutrition and Food Science, 0112 Skinner Building, University of Maryland, College Park, MD 20742, Tel: (301)-405-0761. Fax: (301)-314-3313. E-mail: lyu5@umd.edu. (X.G.) School of Life Science and Technology, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing, PR China 210009. Tel: +86-25-83271298. Fax: +86-25-83302827. E-mail: xiangdong_gao@yahoo.com.

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capacities (*10*). In addition, grafting and cross-linking were able to modify psyllium for application in drug delivery devices (*13*). These research activities have indicated the potential of improving psyllium functionality through chemical modifications.

Sulfation is a widely accepted method to add polyanionic charges to polymers, and sulfonic groups are naturally present in food gums such as carrageenans (*14*, *15*). Introduction of the strong anionic sulfonic groups in the psyllium molecule may inhibit its gelling capacity because of electrostatic repulsion. Sulfation has been shown to be a low cost, rapid, and effective method in improving physicochemical and functional properties of polysaccharides such as starch, dextran, pullulan, cellulose, and chitosan (*14*−*19*). To our knowledge, the effects of sulfation on physicochemical and functional properties of psyllium have not been investigated. The objective of this study was to prepare sulfated psyllium derivatives using sulfur trioxide−pyridine and to characterize the chemical structures of the sulfated psyllium derivatives by using FT-IR, elemental analysis, surface charge determination, and morphology observation. In addition, the effects of sulfation on functional and biological properties of psyllium were evaluated by examining and comparing the sulfated psyllium derivatives with the original psyllium preparation for their rheological properties, water uptake capabilities, swelling behaviors, and their bile acid-binding capacities.

MATERIALS AND METHODS

Chemicals and Reagents. Sulfur trioxide−pyridine complex ($SO_3 \cdot Py$), dimethyl formamide (DMF), pyridine, barium chloride, and dialysis membrane tubing (MWCO12000-14000) were purchased from Thermo Fisher scientific Inc. (NY, USA). Acid treated psyllium preparation (Psy) was obtained from psyllium husks by acid hydrolysis as described previously (*10*). Cholestyramine, individual bile acids (cholic and chenodeoxycholic acid), diphorase, nicotinamide adenine dinucleotide, nitro blue tetrazolium, and 3-α hydroxysterol dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analytical grade and were used without further purification.

Preparation of Sulfated Derivatives. Sulfated derivatives were prepared according to the method described previously with minor modifications (*14*, *20*, *21*). The psyllium sample (Psy, 2 g) was placed in dry DMF (40 mL) and then mixed with the sulfating agent $SO_3 \cdot Py$ (36.73 g) dissolved in 50 mL of DMF. For every mole of $SO_3 \cdot Py$, 1 mL of pyridine was added to the mixture. The reaction was carried out with continuous stirring at 60 °C for different durations (3 and 4 h, respectively). After cooling to room temperature, 250 mL of cold water was added to terminate the reaction, and followed by neutralization with 5 M NaOH solution. The sulfated psyllium was precipitated by adding ethanol to the mixture. The precipitation was resuspended in 200 mL of water, dialyzed for 72 h to remove salt and other low molecule weight chemicals, and concentrated to a final volume of about 250 mL under reduced pressure, followed by precipitation with four volumes of EtOH. The solids were collected, washed with ethanol and acetone three times, and dried under nitrogen to obtain the sulfated psyllium products. Two products, SP1 and SP2, were obtained for the two reaction times of 3 and 4 h. The two supernatants after SP1 and SP2 collection were combined, and another four volumes of EtOH were added to precipitate the carbohydrates. The precipitates were washed and dried to obtain the sulfated psyllium sample SP3. The three samples including SP1, SP2, and SP3 were characterized for their structural, physicochemical, and functional properties.

Structural Characterization. Psy and sulfated derivatives were examined for sulfur content determination, elemental analysis, Fourier transform−infrared spectroscopy (FT-IR), and surface charge (ζ-potential). Sulfur content determination was performed according to a previously described barium chloride−gelatin nephelometery with a few modifications (*22*). A calibration curve was constructed with sodium sulfate as standards. Elemental analysis was carried out using a Perkin-Elmer PE 2400-Series II, CHNS/O analyzer (PerkinElmer, USA) to confirm the sulfur content determined by barium chloride−gelatin nephelometery. The degrees of substitution (DS) which indicated the average number of sulfonic groups attached to a xylose unit was calculated according to the following formula (*23*):

$$DS = \frac{132 \times \frac{S\%}{32}}{100 - \left(\frac{80}{32} \times S\%\right)}$$

FT-IR spectra were recorded on a Thermo Nicolet IR 200 spectrometer (Thermo, USA). The surface charge of particles could be characterized by measuring the ζ-potential. The concentration of each sample solution was 0.1 mg/mL. Briefly, the folded capillary cell was filled with the solution and sealed by two stoppers, and then the cell was mounted to the particle analyzer (Malvern, Zetasizer Nano ZS90). The output data quantitatively indicate the charge that the particles carry.

Scanning Electron Microscope (SEM). The morphological information of Psy and sulfated derivatives were investigated by using a SU-70 Analytical Ultra-High Resolution Scanning Electron Microscope (Hitachi, Tokyo, Japan). All samples were placed directly onto conductive carbon tapes on the specimen stub and coated with a 10 nm layer of gold.

Examination of Rheological Properties. An AR 2000ex rheometer (TA Instrument, Leatherhead, UK) was used to measure the viscoelastic properties of the psyllium samples following a previous procedure (*24*). Samples were dissolved and well mixed in water at a concentration of 5 mg/mL. The rheological properties were characterized immediately after each solution was prepared at 25 ± 1 °C with a 60 mm 59° steel cone. The shear viscosity was measured at a range of shear rates from 0.01 to 100 s^{-1}, and the dynamic oscillatory testing was performed as a function of frequency (0.1 to 1000 rad/s). The mean values of at least two measurements were reported for each measurement.

Water-Uptake Capacity Assay. Water-uptake capacities of Psy and sulfated derivatives were determined and compared according to a previously reported protocol (*9*) with minor modifications. Briefly, the samples were equilibrated in a 15% relative humidity chamber at ambient temperature (22 °C) for 72 h, and their weights were measured. The samples were then transferred to a 90% relative humidity chamber and kept for 30 min, and measured for total weight. The weight change was calculated and reported in milligram of water taken by per gram of the sample per min. Duplicate tests were conducted for each psyllium sample.

Swelling Capacity Assay. Swelling capacities of the samples were determined following the procedure described by Zhou and others (*25*). Briefly, 0.1 g of each sample was suspended in 7 mL of simulated intestinal fluid without enzyme according to U.S. Pharmacopeia (*26*). After keeping the suspensions at 37 °C for 8 h with occasional shaking, the mixture was kept for another 16 h at ambient temperature (22 °C) for the sediment to settle. Then the volumes of the sediments were recorded. The swelling capacity was calculated as mL of sediment/g of each psyllium sample.

Bile Acid-Binding Capacity Assay. The bile acid-binding capacities were determined following previously reported procedures (*10*, *25*). Briefly, 50 mg of the sample was treated with 0.5 mL of 0.01 mol/L HCl, which simulated gastric conditions, and was incubated at 37 °C for 60 min with continuous shaking (50 rpm). The solution was adjusted to pH 7.0 by adding 0.05 mL of 0.1 mol/L NaOH and mixed with 2.5 mL of 400 μmol/L bile acid stock solution prepared in 0.01 mol/L phosphate buffer (pH 7.0), which simulated intestinal conditions. The mixture was incubated for another 60 min at 37 °C and then centrifuged for 10 min at 6000 rpm, and the supernatant was collected for bile acid determination. Quantification of the unbound bile acids was conducted using a commercial kit from Sigma-Aldrich (St. Louis, MO). The final assay mixture was added with 100 μL of supernatant or bile acid standards, 125 μL of 1.22 mmol/L nicotinamide adenine dinucleotide, 125 μL of 5 mmol/L nitro blue tetrazolium salt, 100 μL of 625 units/L 3-α hydroxysterol dehydrogenase, and 100 μL of 625 units/L diphorase. The mixture was incubated for 60 min at ambient temperature. After incubation, 100 μL of 1.33 mol/L phosphoric acid was added to stop the reaction, and the absorbance of each reaction mixture was measured at 530 nm. The phosphate buffer without bile acid was used for a reagent blank and cholestyramine used as a positive control to verify the enzyme. The levels of unbound bile acids were obtained using a standard curve prepared with each of the two pure bile acids, which were cholic and chenodeoxycholic acids. The bile

Table 1. Sulfur Contents and Degree of Sulfation (DS) of Sulfated Psylliums Measured by Two Different Methods[a]

	yield (%)	sulfur content$_{BCG}$ (%)	DS$_{BCG}$	sulfur content$_{EA}$ (%)	DS$_{EA}$
SP1	93.53	7.49 a ± 0.09	0.38	10.37 a ± 0.09	0.58
SP2	66.43	11.40 b ± 0.16	0.66	11.11 b ± 0.03	0.63
SP3	33.61	13.55 c ± 0.16	0.84	13.11 c ± 0.07	0.80

[a] BCG, barium chloride−gelatin method; EA, elemental analysis. Data are expressed as mean ± SD. Values with different letters are significantly different ($P < 0.05$). SP1, SP2, and SP3 represent different sulfated psylliums.

acid-binding capacity (mg/g sample) was calculated against a reagent blank. Duplicate tests were performed for each sample against each bile acid.

Statistical Analysis. Data were reported as mean ± SD for duplicate determinations. ANOVA and Tukey's tests were performed (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to identify differences among means. Statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Structural Characterization. Table 1 shows the sulfur contents and the degree of sulfation (DS) values measured via both barium chloride−gelatin and elemental analysis methods. Two methods were employed since the NMR analysis could not be performed for these samples because of their high viscosity in the available NMR solvent systems. As shown in **Table 1**, SP2 had greater sulfur content and DS value than SP1, suggesting that longer sulfation reaction time may increase the sulfur content and the degree of sulfation of psyllium. SP3 had the greatest sulfur content and the degree of sulfation, which may partially be explained by the way it was prepared. SP3 was collected by adding a greater level of ethanol in the solution, which indicated that SP3 had greater water solubility. The greater water solubility may be associated with the incorporation of more hydrophilic sulfonic groups in the molecule. The sulfur content and DS values obtained by the two methods were in a good agreement except for the low sulfur content for SP1 determined by the barium chloride−gelatin method.

Figure 1 shows the FT-IR spectra of psyllium (Psy), SP1, SP2, and SP3 in the 400−4000 cm^{-1} region. In comparison with Psy, two new characteristic absorption bands appeared in the FT-IR spectra of SP1, SP2, and SP3, one at 1224.85 cm^{-1} describing an asymmetrical S═O stretching vibration and the other at 800 cm^{-1} representing a symmetrical C−O−S vibration associated with a C−O−SO_3 group, indicating the incorporation of the sulfonic groups (*27*). The stronger peaks at 1224.85 and 800 cm^{-1} in SP3 also indicated that it had higher sulfur content than SP1 and SP2, which was in good agreement with the results of sulfur content determination.

To further confirm the introduction of the negatively charged sulfonic groups to psyllium molecules, surface charge (ζ-potential) was examined for SP1, SP2, and SP3 and compared to that of Psy. Although all sample molecules coagulated quickly, it did not affect the measurement of surface charge (ζ-potential). The ζ-potential of Psy, SP1, SP2, and SP3 were −17.3, −30, −39, and −44.7 mv, respectively, showing a remarkable increase of surface negative charge in the sulfated psyllium samples. Furthermore, the negative charge of SP1, SP2, and SP3 was associated with their sulfur contents (**Table 1**). It is understandable that the sulfonic group carries negative charge, while the hydroxyl groups do not carry any charges. The more sulfonic groups in the sample, the more negative charge could be determined. Taking together, these surface charge determination data supported the sulfation of psyllium under the reaction conditions.

Morphological Properties. Scanning electron microscope (SEM) measurements of Psy, SP1, SP2, and SP3 are shown in **Figure 2**. SEM results showed that sulfation caused a dramatic impact on the morphology of psyllium. Psy had filamentous structures (**Figure 2a**), which contained regular strands forming a network-like structure on the surface. Sulfated samples were found to be less fibrillar (**Figures 2b−d**), indicating surface changes in morphology. The more sulfonic groups attached to the polymer, the less filamentous structure existed, suggesting that sulfation might reduce the surface area of psyllium particles. The surfaces of SP1 and SP2 were still filamentous but had been significantly eroded as compared to that of Psy (**Figures 2a−c**). Furthermore, instead of the filamentous surface structure, SEM of SP3 showed the presence of small aggregated clusters or broken particles (**Figure 2d**). It was reported earlier that particle size and specific surface structure might influence the hydration behavior of gel forming polymers and change their intrinsic viscosities (*28*, *29*). Therefore, the SEM results also suggested that sulfation might alter the intrinsic viscosity or rheological properties of psyllium.

Rheological Properties. The rheological properties of polysaccharides are of great interest for basic research and potential application. **Figure 3** shows that during the whole measurement range, the elastic modulus (G') of all samples was higher than their viscous modulus (G''), indicating a typical weak gel-like property, which is consistent with what Guo et al. (*30*) have reported on their psyllium samples. Moreover, as the sulfation degree increases, there were more and more missing points of G' and G'' values in the low frequency range as shown in **Figure 3b−d**. This trend of both deduced moduli maybe caused by the variation of the degree of sulfation substitution in individual psyllium molecules and their aggregates. Hydrolysis might also occur under the sulfation reaction conditions and lead to the breakdown of psyllium molecules (*14*). These broken molecules, which might have a molecular weight larger than the dialysis membrane cutt-off size of 12,000 and remain in the sulfated psyllium samples, might not be homogeneous in their size, shape, hardness, particle density, and surface roughness, which may partially explain the SEM image of SP3 (**Figure 2**) and might also further explain the missing points in **Figure 3b−d** (*31*). The value of gel strength (G') also dropped after sulfation (**Figure 3**). Psy had the strongest gel under the experimental conditions, whereas the weakest gel was observed for the solution of SP3 with the highest sulfur content. These data indicated that sulfation greatly reduced the gelling capacity of psyllium.

Apparent viscosity η^* of Psy and sulfated derivatives decreased with increasing shear rate (**Figure 3**), suggesting that they were all pseudoplastic materials and perform shear thinning behavior. Moreover, sulfated derivatives had lower apparent viscosity than that of Psy at the experimented shear rating range. When plotting the apparent viscosity versus shear rate at the double logarithm scale (**Figure 4**), it can be seen that sulfation derivatives not only had substantially lower apparent viscosities but also had narrow shear thinning regions. Their apparent viscosities decreased linearly at the low shear rate region and changed to the Newtonian behavior region at the high shear rate. This might be explained by the fact that the introduction of anionic sulfonic groups may change the molecular conformation, strengthen electrostatic repulsion, and intra- and intermolecular interactions as evidenced by the SEM images presented in **Figure 2**. These rheological behavior changes of sulfated derivatives suggested that they may have reduced sliminess and improved mouthfeel when used in food products such as breads and crackers (*24*). They may also have superior properties than Psy during food processing and food consumption.

Effects of Sulfation on Water-Uptake Properties. Sulfation significantly increased water uptake capacity of psyllium (**Figure 5**). The psyllium derivative with higher sulfur content had stronger

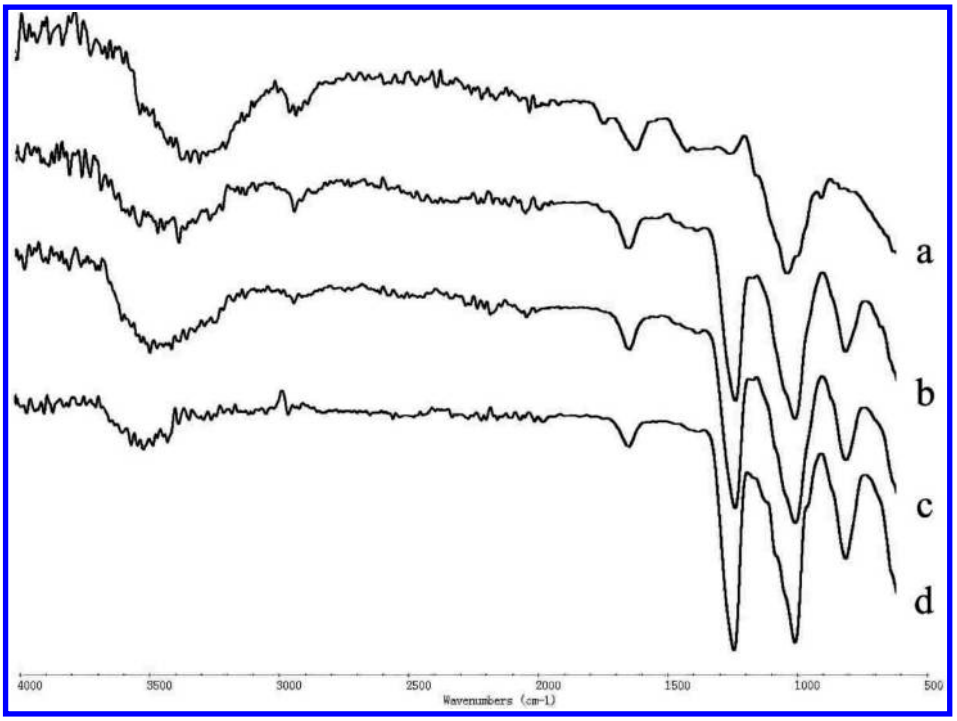

Figure 1. IR spectra of psyllium samples. 'a' represents the starting psyllium preparation, while 'b', 'c', and 'd' represent different sulfated psylliums SP1, SP2, and SP3, respectively.

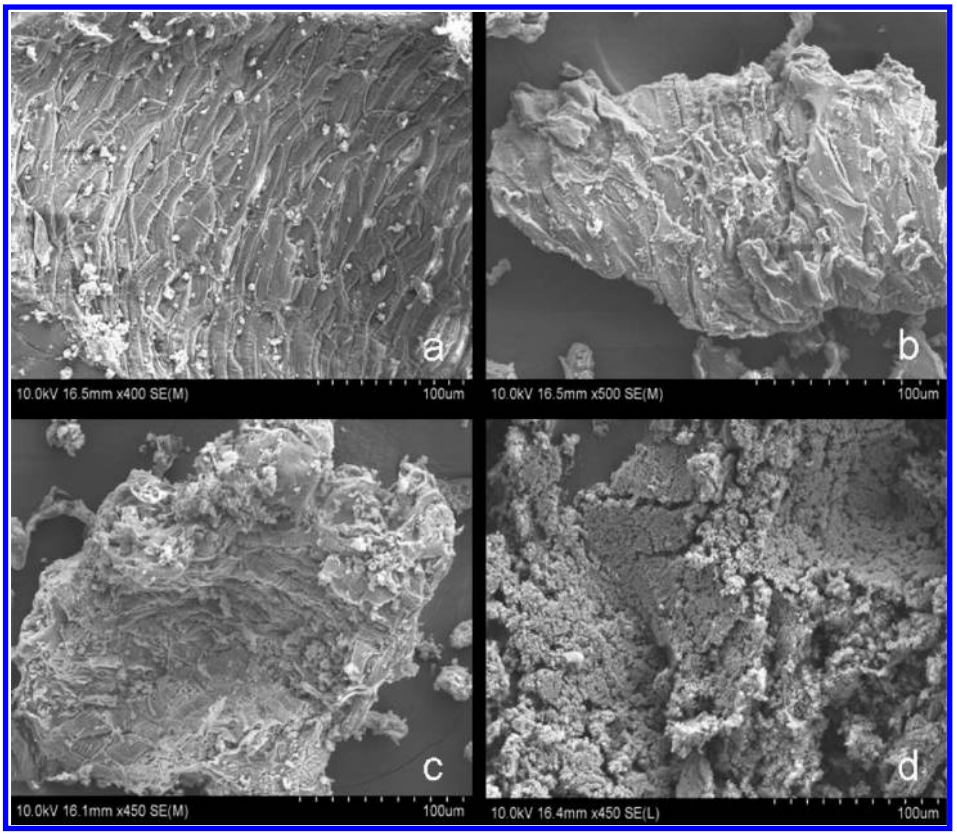

Figure 2. SEM measurement of psyllium samples. (**a**) Starting psyllium preparation; (**b**−**d**) different sulfated psylliums SP1, SP2, and SP3, respectively.

water uptake ability. SP1, SP2, and SP3 could absorb water at an average rate of 2.88 mg/g/min, 3.55 mg/g/min, and 3.81 mg/g/min under the experiment conditions in the first 30 min, which was 1.65-fold, 2.03-fold, and 2.18-fold of that for Psy. Water uptake property depends on the particle surface area and structure. The reduction in surface area evidenced by the SEM determination (**Figure 2**) could not explain the increased water uptake capacity. However, the sulfonic group is a negatively charged hydrophilic group which can be hydrated rapidly in the presence of moisture and contributed to the increased water uptake ability of SP1, SP2, and SP3 possibly through electrostatic interaction and hydrogen bond formation (*32*, *33*).

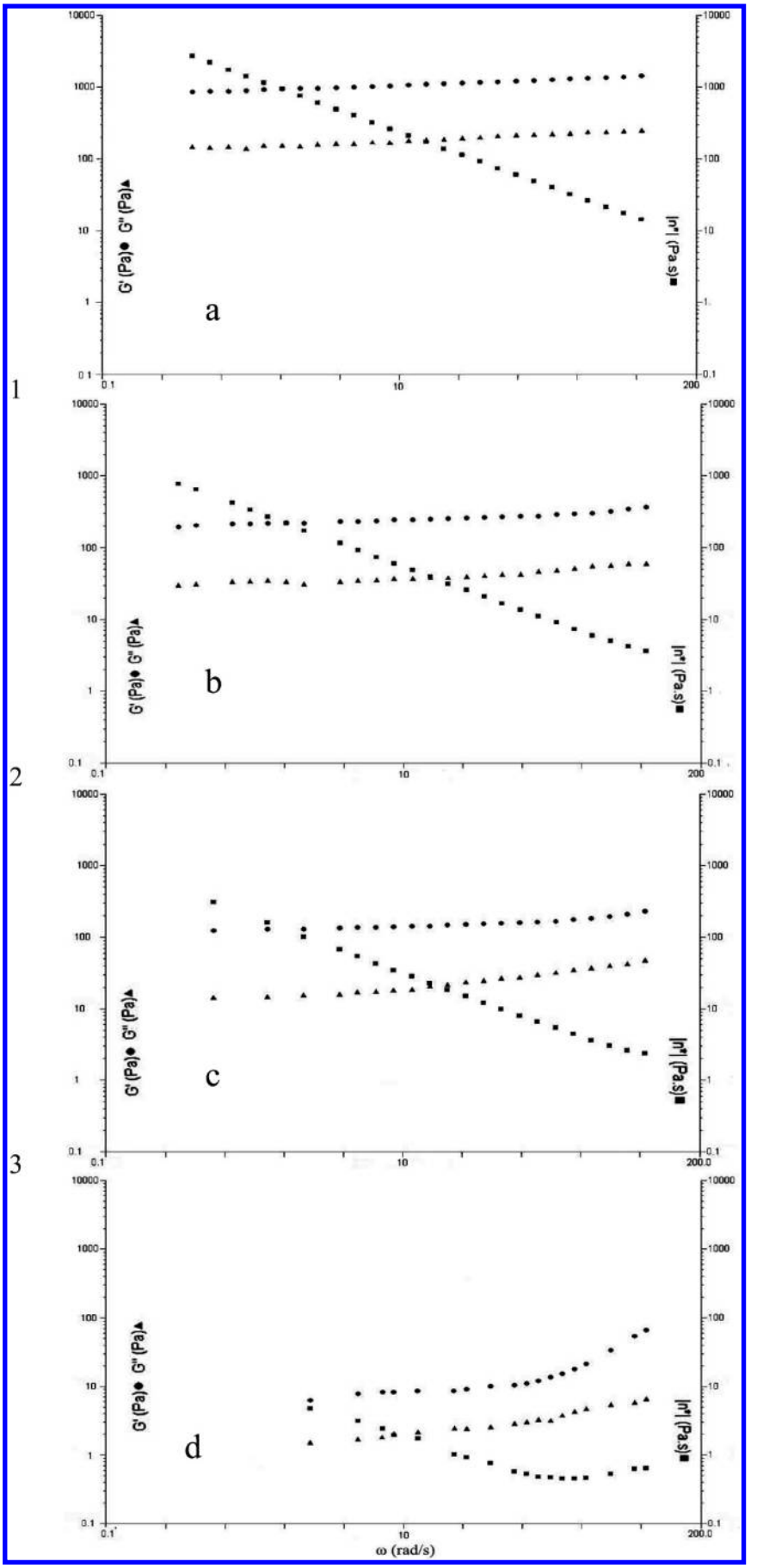

Figure 3. Effects of sulfation on the G', G'', and η^* with frequency (ω) of psyllium samples. (**a**) Starting psyllium preparation; (**b**−**d**) different sulfated psylliums SP1, SP2, and SP3, respectively.

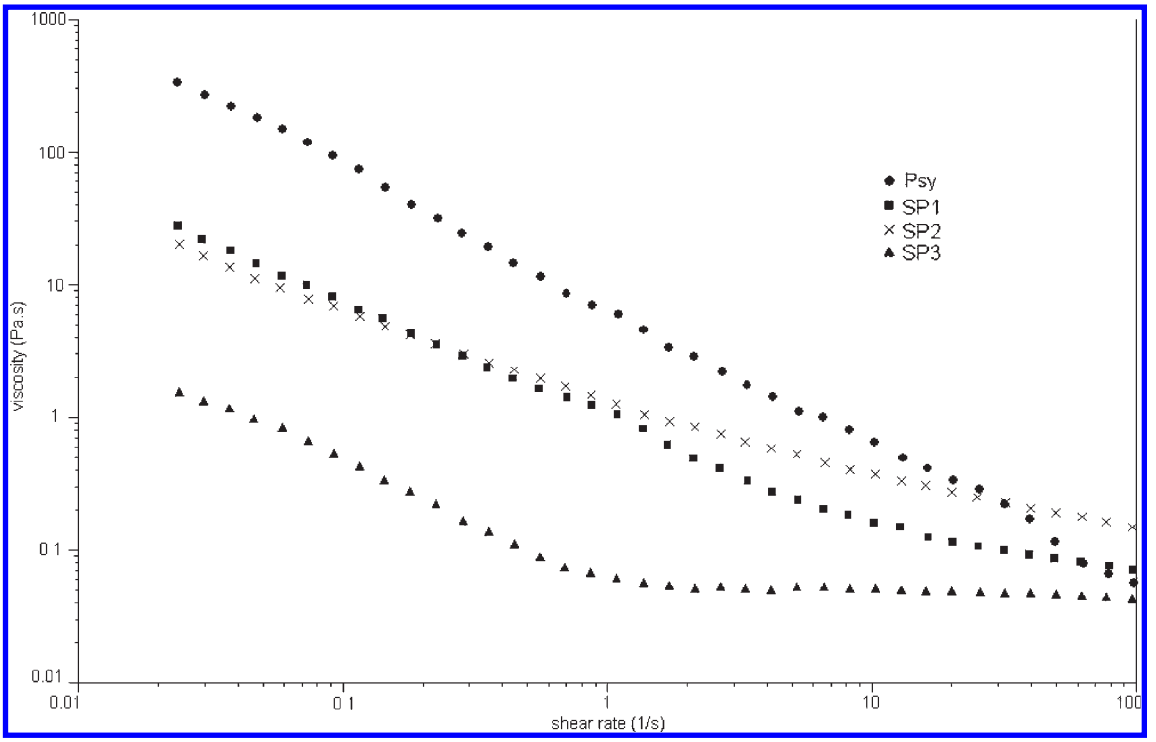

Figure 4. Shear thinning behavior of psyllium samples. Psy represents the starting psyllium preparation, while SP1, SP2, and SP3 represent different sulfated psylliums.

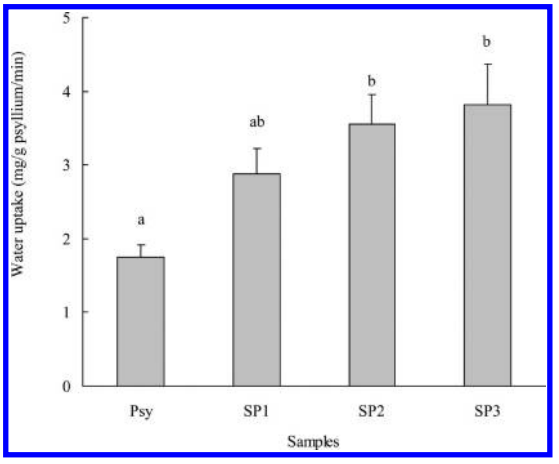

Figure 5. Effects of sulfation on water uptake capacities of psyllium samples. Psy represents the starting psyllium preparation, while SP1, SP2, and SP3 represent different sulfated psylliums. Data are expressed as mean ± SD. Vertical bars represent the SD. Values with the same letters are not significantly different ($P > 0.05$).

Effects of Sulfation on Swelling Properties. Swelling volume measures the water-holding capacity of polymers, and it is also an important parameter for predicting the health beneficial properties of psyllium. In the present study, SP3 did not form sediment or gel under the experimental conditions, but SP1 and SP2 did. Furthermore, the swelling volume of SP1 was significantly lower than that of Psy and SP2, only about 75% of that for Psy; and no difference was observed between the swelling volumes for Psy and SP2 (**Figure 6**). These data suggested that sulfation might alter the swelling volume of psyllium and that the influence might depend on the sulfation degree. These data also indicated the possibility to obtain nongelling psyllium derivatives through introducing a certain degree of sulfation into the molecule.

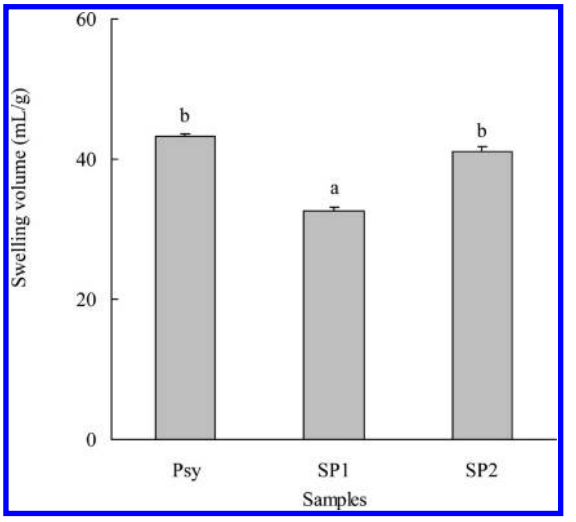

Figure 6. Effects of sulfation on swelling volume of psyllium samples. Psy represents the starting psyllium preparation, while SP1, SP2, and SP3 represent different sulfated psylliums. Data are expressed as mean ± SD. Vertical bars represent the SD. Values with the same letters are not significantly different ($P > 0.05$).

Effects of Sulfation on Bile Acid-Binding Properties. Binding of bile acids to polymers may enhance their elimination and promote the conversion of cholesterol to bile acids, which may reduce the plasma total and LDL cholesterol levels, and the risk of cardiovascular diseases. SP1, SP2, and SP3 were evaluated and compared to Psy for their in vitro binding capacities against the selected bile acids including cholic acid (CA) and chenodeoxycholic acid (CDCA), which are the primary bile acids synthesized in liver. All three sulfated psyllium derivatives had significantly higher bile acid-binding capacities than Psy (**Figure 7**). The binding capacity of SP1, SP2, and SP3 against cholic acid was about 8.4 mg/g, 7.6 mg/g, and 8.1 mg/g, which was 6.5-fold, 5.9-fold,

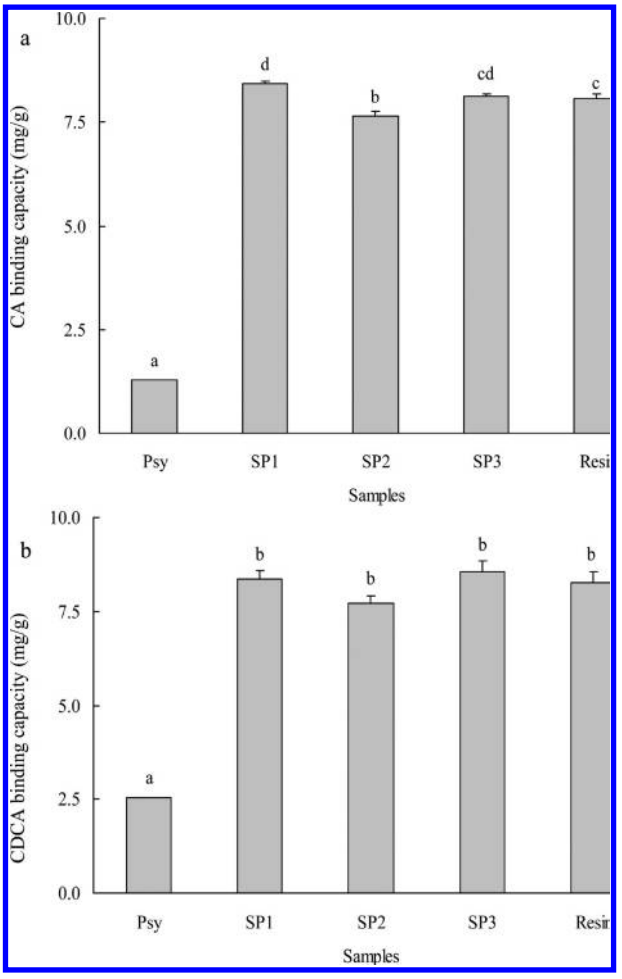

Figure 7. Effects of sulfation on bile acid-binding capacities of psyllium samples. (**a** and **b**) Cholic acid (CA) and chenodeoxycholic acid (CDCA) binding capacity of each sample, respectively. Resin means cholestyramine resin, which is the positive control. Psy represents the starting psyllium preparation, while SP1, SP2, and SP3 represent different sulfated psylliums. Data are expressed as mean ± SD. Vertical bars represent the SD. Values with the same letters are not significantly different ($P > 0.05$).

and 6.3-fold of that for Psy, respectively (**Figure 7a**). The chenodeoxycholic acid-binding capacities of SP1, SP2, and SP3 were also significantly greater than that for Psy (**Figure 7b**). Additionally, SP1 and SP3 had stronger binding capacities against both cholic and chenodeoxycholic acids than that for cholestyramine resin under the experimental conditions. SP1 showed 104.5% and 101.3% binding capacities against cholic and chenodeoxycholic acids, respectively, as compared to cholestyramine resin on a same per as it is weight basis. Cholestyramine resin was included as a positive control in the present study. Cholestyramine resin is a quarternary ammonium anion exchange resin with a polystyrene backbone and is commercially used as an adjunctive therapy for binding bile acids in the intestinal system and reducing plasma cholesterol level (*34*). These data suggested the possible application of sulfated psyllium derivatives as cholesterol-lowering adjutant(s).

Interestingly, a few previous studies indicated that amination of chitosan or oat β-glucan could increase their bile acid-binding capacity possibly due to the introduction of cationic groups into the polysaccharide molecules (*34*). But, introduction of anionic groups such as the sulfonic group could decrease the bile acid-binding capacity of polysaccharides including oat β-glucan (*35*). Results from the present study showed that introduction of negatively charged sulfonic groups to psyllium could increase the bile acid-binding capacity (**Figure 7**). These conflicting observations may be due to the different mechanisms involved in their bile acid-binding actions. A number of factors have been suggested to possibly contribute to the overall bile acid-binding capacity of polymers (*34*−*36*). Ionic interaction, hydroxyl group interaction, and trapping in the polymer matrix may explain the bile acid-binding capacity of cholestyramine, native chitosan, and cationic aminated β-glucan, but could not explain the strong in vitro bile acid-binding capacity of sulfated psyllium observed in the present study (*25*, *34*−*36*). Taken together, the ionic interaction may not be the primary contributor for the in vitro bile-acid-binding capacity of sulfated psyllium.

In summary, sulfated psyllium containing anionic sulfonic groups could be prepared using $SO_3 \cdot Py$ in DMF. Sulfation may improve physicochemical and functional properties and the in vitro bile acid-binding ability of psyllium. Further in vivo bioactivity and toxicological evaluations of sulfated psyllium are recommended to promote its food or pharmaceutical applications.

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