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Effects of Structural Modifications on Physicochemical and Bile Acid-Binding Properties of Psyllium

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Supporting Information

ABSTRACT: The effects of sulfation, hydroxypropylation, and succinylation on gelling, water uptake, swelling, and bile acid-binding capacities of psyllium were examined and compared at the same molar substitution degree. Sulfated, hydroxypropylated, and succinylated psyllium were prepared with substitution levels of 1.02, 0.88, and 0.79, respectively, and their structures were characterized using FT-IR, SEM, and ζ-potential determination. All three derivatization methods reduced the gelling and swelling capacities of psyllium and increased the water uptake and bile acid-binding capacities compared to the original psyllium. Interestingly, it was observed for the first time that introduction of a stronger negatively charged group into the molecule might more effectively enhance the bile acid-binding capacity of psyllium. On the other hand, the steric effect of the substitution groups seemed to be more critical in altering the gelling and swelling properties of psyllium.

KEYWORDS: *psyllium, modification, water uptake, swelling, gelling capacities, bile acid binding*

INTRODUCTION

Polysaccharides have been recognized for their health properties, such as antitumor, anti-inflammatory, antidiabetic, and antihyperlipidemic effects.[1−4] Their health activities are highly dependent on their molecular weight and chemical structure including monosaccharide composition, glycosidic bonds, the structure of main chain and branches, degree of branching, and substitution.[5−8]

Psyllium, a mucilaginous product derived from the seed husk of the *Plantago* genus, is a highly branched arabinoxylan and consists of 1→3 and 1→4 β-D-xylopyranosyl residues as its main chain.[9] It has been utilized in functional foods, folk medicine, and supplemental products for potential cholesterol-lowering, laxative, and insulin sensitivity improvement properties.[10−13] The major challenge in delivering an adequate amount of psyllium in a single serving of a functional food product for a health claim is its extremely strong water-absorbing and gelling capacities. To promote the application of psyllium for human health, several physical, mechanical, enzymatic, and chemical approaches have been taken to improve its physicochemical and functional properties, without decreasing its health and safety properties.[14−17] Our recent studies demonstrated that sulfation, hydroxypropylation, and succinylation could significantly improve the gelling property of psyllium and its bile acid-binding capacities.[18−20] However, it remains unclear which modification is more effective in altering each functionality and bile acid-binding ability of psyllium at the same molar substitution degree.

As a continuation of our effort to enhance the application of psyllium in supplemental and food products, the objective of this study was to determine and compare the effectiveness of sulfation, hydroxypropylation, and succinylation on the physicochemical, functional, and bile acid-binding properties of psyllium at the same molar degree of substitution (DS). Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and surface charge were also examined for the three psyllium derivatives to compare their morphological and structural characteristics. The physicochemical and functional properties of psyllium derivatives were evaluated as their gelling, water uptake, and swelling properties.

MATERIALS AND METHODS

Materials. Sulfur trioxide−pyridine complex (SO_3·Py), *N*,*N*-dimethylformamide (DMF), 4-dimethlaminopyridine, propylene oxide, isopropanol, succinic anhydride, barium chloride, and tributylamine were purchased from Thermo Fisher Scientific Inc. (Monroe, NY, USA). Nitroblue tetrazolium chloride (NBT), cholic and chenodeoxycholic acids, diphorase, nicotinamide adenine dinucleotide, and 3-R hydroxysterol dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification.

Sulfation. Sulfation was carried out according to a previously reported method (Figure 1a).[18] Briefly, psyllium (2.0 g) was placed in 40 mL of DMF and then mixed with the sulfating agent (SO_3·Py, 36.7 g) dissolved in DMF (50 mL). One milliliter of pyridine was added to the mixture per mole of SO_3·Py. The reaction was continuously stirred at 60 °C for 4 h. After the mixture had cooled to ambient temperature, 250 mL of cold water (4 °C) was added to terminate the reaction, followed by neutralization with 5 M sodium hydroxide solution. The sulfated psyllium was dialyzed for 72 h to remove salt and other small molecules and concentrated to a volume of 250 mL under reduced

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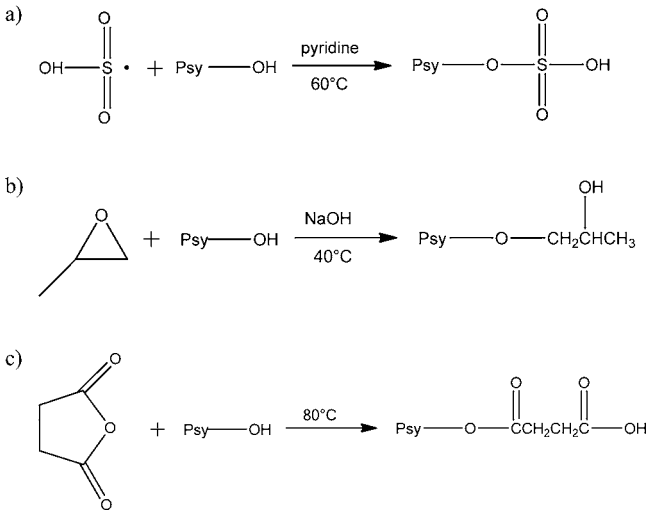

Figure 1. Reactions of different modifications: (a) sulfation; (b) hydroxypropylation; (c) succinylation.

pressure, followed by precipitation with 3-fold volumes of 95% ethyl alcohol. The precipitate was collected by centrifugation, washed with ethanol and acetone, and dried under nitrogen to obtain the sulfated psyllium (SFP).

Hydroxypropylation. According to the method described previously,[19] 5.0 g of psyllium was mixed with isopropanol and 50 mL of sodium hydroxide solution (Figure 1b). After 30 min of stirring, 30 mL of propylene oxide was added to the reaction mixture. The reaction was continuously stirred at 40 °C for 36 h. The reaction product was neutralized using 5 M HCl and dialyzed against distilled water for 96 h. The dialysate was concentrated, and 3-fold volumes of 95% ethyl alcohol were added to precipitate the polysaccharide. The solids were collected, washed with ethanol and acetone, and dried under nitrogen to obtain the hydroxypropylated psyllium (HP).

Succinylation. Succinylated psyllium was prepared according to the method described previously with minor modifications (Figure 1c).[20] Two grams of psyllium was added to 80 mL of DMF and 325 mg of 4-dimethlaminopyridine. After heating to 60 °C, 3 g of succinic anhydride and 8 mL of tributylamine were added to the reaction. Then the mixture was heated at 80 °C for 6 h. After the reaction, the mixture was cooled to 4 °C using ice water, and then a 1000 mL cold saturated ethanolic solution of sodium acetate was added. After centrifugation, the precipitate was dissolved in distilled water and dialyzed against 0.5% $NaHCO_3$ for 48 h, followed by purified water for 96 h. The dialysis solution was mixed with 3-fold volumes of 95% ethyl alcohol. The precipitate was collected, washed with ethanol and acetone, and dried under nitrogen to obtain the succinylated psyllium (SCP).

Structural Characterization. FT-IR spectra of samples were recorded on a JASCO FT-IR-4100 spectrometer with an ATR PRO450-S single-reflection ATR accessory (JASCO, USA). The DS values of the derivatives were determined by weight gain method. The weight gain was determined according to eq 1

$$\mathrm{WPG} = \frac{(m_{\mathrm{dp}} - m_{\mathrm{op}})}{m_{\mathrm{op}}} \times 100\% \quad (1)$$

where WPG is the percentage of weight gain, m_{op} the mass of original psyllium, and m_{dp} the mass of the derived psyllium. The degree of substitution (DS), which indicated the average number of modifying groups attached to a xylose unit, was calculated according to eq 2

$$\mathrm{DS} = \mathrm{WPG} \times \frac{132}{M_{\mathrm{d}}} \quad (2)$$

where M_{d} is the molar mass of the substitution group.

The surface charges of derivatives were measured using a particle analyzer (Malvern, Zetasizer Nano ZS90, Worcestershire, UK). The sample (0.1 mg/mL) was placed in the folded capillary cell and sealed with two stoppers. Then the cell was mounted to determine the ζ-potential of molecules. The data indicated the charge of the sample quantitatively.

Scanning Electron Microscopy (SEM). The morphological information of original psyllium and derivatives was examined using an SU-70 Analytical Ultra-High Resolution scanning electron microscope (Hitachi, Tokyo, Japan). Samples were placed on the conductive carbon tapes of a specimen stub and coated by a 10 nm layer of gold.

Gelling Properties. According to a laboratory protocol,[21] the gelling properties of original psyllium and psyllium derivatives were measured using a TA-XT Plus Texture Analyzer with a 25 mm diameter probe (Texture Technologies Corp., Scarsdale, NY, USA). Gel samples were prepared by mixing 0.50 g of powder with 10 mL of distilled water. After setting for 24 h at room temperature, the gel sample was subjected to a double-compression test. Instrumental parameters were set as follows: pretest speed, 2.0 mm/s; test speed, 5 mm/s; post-test speed, 5 mm/s; and test distance, 6 mm. Gel hardness was the maximum force to break the gel sample, and the force of gel to hold the probe was determined as gel adhesiveness. Duplicate measurements were taken for samples.

Water Uptake Capacities. Water uptake capacities of original and derived psyllium were determined according to the procedure reported by Niu et al.[20] Briefly, the sample (0.1 g) was placed in a chamber of 10% relative humility at room temperature for 48 h. The weights of different status were measured respectively. The samples were then transferred to a chamber of 80% relative humility, kept for 30 min, and then measured for total weight. The weight change of each sample was calculated and compared in grams of water taken per gram of each sample. Duplicate measurements were performed for samples.

Swelling Capacities. Swelling volumes of different psyllium samples were determined and compared following a previous laboratory procedure.[14] Each sample (0.1 g) was suspended in simulated intestinal fluid (10 mL) according to the U.S. Pharmacopia. The suspensions were equilibrated at 37 °C with occasional shaking for 8 h. The sedimentation was then kept at room temperature for 16 h. The volume of the sediment was determined. The swelling capacity of each psyllium sample was calculated as milliliters of sediment per gram of sample.

Bile Acid-Binding Capacities. According to a previous study,[20] the bile acid-binding capacities of derived psyllium samples were determined and compared to that of original psyllium. To simulate gastric conditions, 50 mg of each psyllium sample was treated with 0.5 mL of HCl (0.01 M) and incubated at 37 °C with continuous shaking (50 rpm) for 1 h. The mixture was adjusted to pH 7.0 by NaOH (0.1 M), and then 2.5 mL of bile acid stock solution (400 μM in 0.01 M phosphate buffer, pH 7.0) was added to simulate intestinal conditions. The reaction mixture was incubated for 1 h at 37 °C. The supernatant was collected for bile acid assay after centrifugation for 10 min at 6000 rpm. Bile acid determination was determined by calculating the unbound bile acids quantitatively by a commercial kit from Sigma-Aldrich (St. Louis, MO, USA). The supernatant or bile acid standard (100 μL) was added to 125 μL of nicotinamide adenine dinucleotide (1.22 mM), 125 μL of nitroblue tetrazolium salt (5 mM), 100 μL of 3-R hydroxysterol dehydrogenase (625 units/L), and 100 μL of diphorase (625 units/L) as the final assay mixture. After incubation for 1 h at room temperature, 100 μL of phosphoric acid (1.33 M) was added to stop the reaction. The absorbance of each reaction mixture was measured at 530 nm with cholestyramine resin as a positive control. The levels of unbound bile acids were determined by standard curves of cholic and chenodeoxycholic acids, respectively. The bile acid-binding capacity (mg/g sample) was calculated against 0.01 M phosphate buffer (pH 7.0). Duplicate tests were taken for each psyllium sample against each bile acid.

Statistic Analysis. Data were expressed as the mean ± SD for duplicate determinations. ANOVA and Tukey's tests were conducted (SPSS for Windows, version rel. 10.0.5., 1999, SPSS Inc., Chicago, IL, USA) to identify differences among means. Statistical significance was considered at $P < 0.05$.

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RESULTS AND DISCUSSION

Structural Characterization. The degrees of substitution were 1.02, 0.88, and 0.79 for sulfated psyllium (SFP), hydroxypropylated psyllium (HP), and succinylated psyllium (SCP), respectively. The surface charge (ζ- potential) was examined to further confirm the introduction of the substitution groups with charge to psyllium molecules. The ζ-potentials of psyllium, SFP, HP, and SCP were −23.6, −49, −19.25, and −54.45 mV, respectively. These data showed an over 2-fold increase of surface negative charge in the SFP and SCP compared to that of the original psyllium, confirming the presence of sulfonic and succinyl groups in the derivatives.

Figure 2 presents the FT-IR spectrum of psyllium and derivatives in the 500−4000 cm^{-1} region. In comparison with

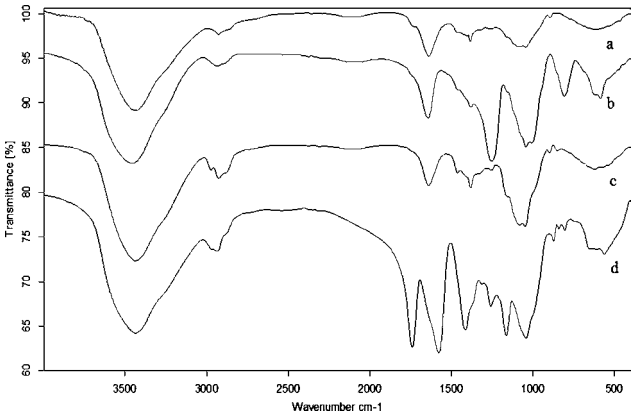

Figure 2. FT- IR spectra of different psyllium samples: (a) psyllium; (b) SFP; (c) HP; (d) SCP. SFP, HP, and SCP stand for sulfated, hydroxypropylated, and succinylated psyllium, respectively.

original psyllium (Figure 2a), new characteristic absorption bands appeared in the IR spectra of SFP (Figure 2b), HP (Figure 2c), and SCP (Figure 2d) due to the introduction of individual derivative groups. In Figure 2b, the characteristic absorption band at 1250 cm^{-1} represents an asymmetrical S═O stretching vibration and the other at 800 cm^{-1} describes a symmetrical C—O—S vibration associated with a C—O—SO_3 group, which confirmed the introduction of the sulfonic groups.[22] Two new absorption bands appear in the IR spectrum of HP (Figure 2c), one at 2970 cm^{-1}, corresponding to the C—H stretching, and the other at 1390 cm^{-1}, for the bending of the CH_3 group in the hydroxypropyl group.[23,24] In Figure 2d, the absorption band at about 1730 cm^{-1} could be attributed to the carbonyl stretching vibration of the ester linkage. The absorption bands at 1560 and 1390 cm^{-1} describe asymmetrical stretching vibration and symmetrical stretching vibration of $-COO^-$ of carboxylate.[25] These results indicate the incorporations of the sulfonic, hydroxypropyl, and succinyl groups into psyllium molecules.

Morphological Properties. Figure 3 represents the SEM of psyllium, SFP, HP, and SCP. SEM results suggested dramatic impacts of the substitutions on the morphology of psyllium. Psyllium (Figure 3a) had a filamentous surface structure, which indicated regular strands forming a network-like folding structure on the surface. Compared to the surface characteristics of psyllium, the surfaces of sulfated (Figure 3b), hydroxypropylated (Figure 3c), and succinylated psyllium derivatives (Figure 3d) were still filamentous but had been significantly eroded and were also found to be less fibrillar, suggesting that sulfation, hydroxypropylation, and succinylation might reduce the surface area of psyllium particles. It was reported previously that specific surface structure and particle size might influence intrinsic viscosities, hydration behavior, and gel-forming capacity of polymers.[26] Thus, the SEM results suggested that these chemical modifications might change the solution behavior and gelling capacities of the modified psyllium preparations.

Gelling Properties. Gel hardness and adhesiveness were determined for the psyllium and derivatives, reflecting the force required for breaking the gel and the ability of the gel matrix to hold the compressing probe of the texture analyzer under the experimental conditions.[14] All three derivatizations were able to reduce the gel hardness and adhesiveness significantly compared to the original psyllium (Figure 4). SFP showed a 35.5% reduction in gel hardness and a 41.9% reduction in gel adhesiveness as compared to the original psyllium. HP formed a weaker gel than SFP with a dramatic reduction of gel hardness for 92.7%, whereas 81.2% reduction was observed in forces to hold the compressing probe. SCP showed a 91.6% reduction in gel hardness and an 86.3% reduction in gel adhesiveness. There was no difference between the gelling properties of HP and SCP (Figure 4). Interestingly, SFT with a molar substitution degree of 1.02 had greater gel hardness and adhesiveness than HP and SCP with molar substitution levels of 0.88 and 0.79, respectively (Figure 4), suggesting that the introduction of a bulky side chain might be more effective than a charge group in reducing the gelling capacities of psyllium. The gelling process is initiated with the formation of junction zones, which grow and join the polysaccharide molecules to form the gel network.[21] These results suggested that introducing a bulky substituent was more effective in reducing the gelling capacity of psyllium due to the steric hindrance. This finding is important for future efforts in structural modification to promote the utilization of psyllium preparations in functional foods for human health.

Water Uptake Capacities. In this assay (the figure is in the Supporting Information), SFP, HP, and SCP could absorb water at average rates of 6.15, 4.93, and 6.16 mg/g/min, respectively, under the experimental conditions, which were 1.33-, 1.07-, and 1.34-fold of that for psyllium. Furthermore, both sulfation and succinylation significantly increased water uptake capacity of psyllium, but hydroxypropylation did not. This observation suggested that introduction of a negative charge or a highly polar hydrophilic group might increase the water uptake capacities of psyllium. On the other hand, introduction of bulky hydroxypropyl substitutions with a polar hydroxyl group did not significantly alter the water uptake ability of psyllium. Taking both the gelling and water uptake properties into account, introduction of additional bulky substitutions including hydroxypropyl group together into psyllium molecules may be a preferred approach to improve its functionalities.

Swelling Capacities. Swelling volume measures the water-holding capacity of a testing material and is an important parameter for predicting the beneficial properties of psyllium.[14] As shown in Figure 5, the swelling volumes of SFP, HP, and SCP were 65.1, 30.2, and 51.1% that of psyllium. The data indicated that hydroxypropylation reduced the swelling volume of psyllium most remarkably, followed by succinylation and sulfation (Figure 5). These results might be partially explained by the reduction of gel network due to the structure modification. The incorporation of bulky substitutions might

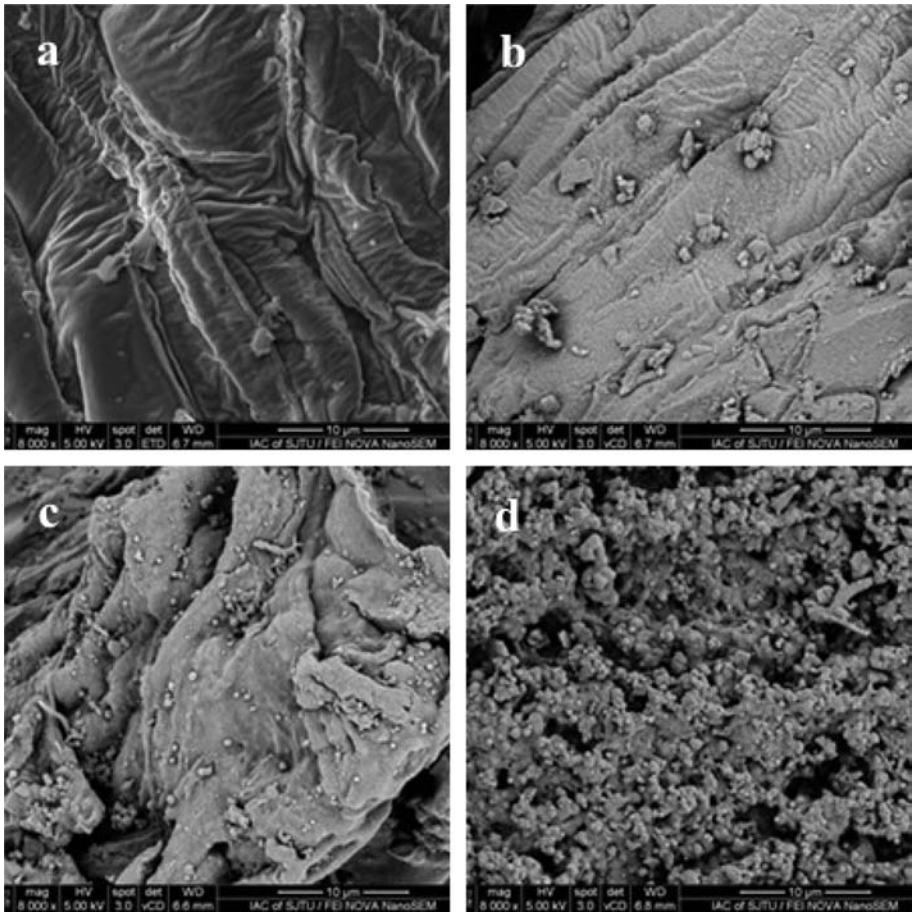

Figure 3. SEM of psyllium samples: (a) psyllium; (b) SFP; (c) HP; (d) SCP. SFP, HP, and SCP stand for sulfated, hydroxypropylated, and succinylated psyllium, respectively.

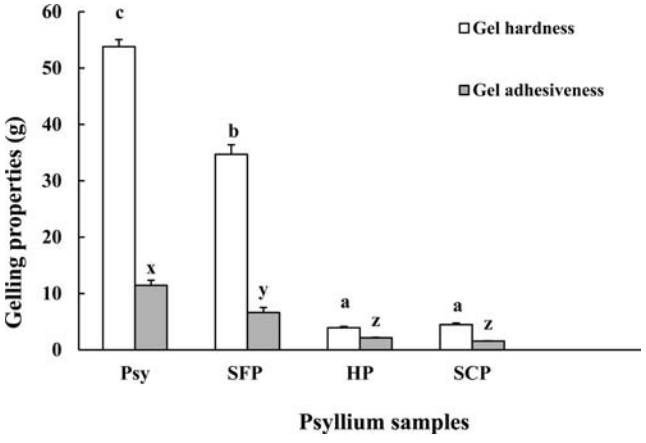

Figure 4. Gelling properties of psyllium samples. Psy stands for original psyllium obtained commercially, whereas SFP, HP, and SCP stand for sulfated, hydroxypropylated, and succinylated psyllium, respectively. Data are expressed as the mean ± standard deviation (SD). Vertical bars represent the SD value of each data point. Values carrying the same letters are not significantly different ($P > 0.05$).

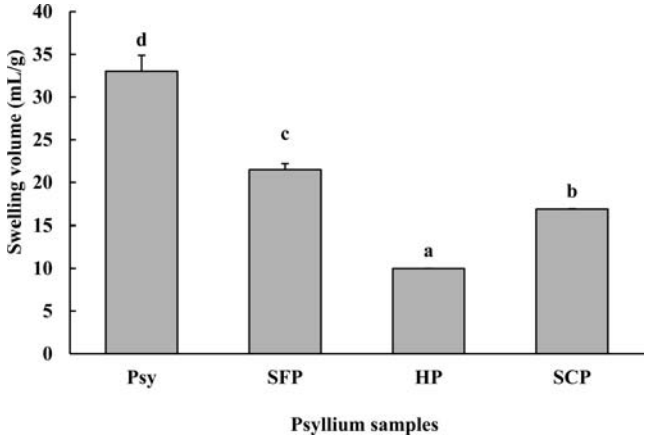

Figure 5. Swelling capacity of psyllium samples. Psy stands for original psyllium obtained commercially, whereas SFP, HP, and SCP stand for sulfated, hydroxypropylated, and succinylated psyllium, respectively. Data are expressed as the mean ± standard deviation (SD). Vertical bars represent the SD value of each data point. Values carrying the same letters are not significantly different ($P > 0.05$).

alter the network-like folding structure more effectively, whereas anionic groups could also reduce the gel formation through electronic repulsion.

Bile Acid-Binding Capacities. Binding of bile acids to polymers may enhance their elimination, which promotes the conversion of cholesterol in liver to bile acids, may reduce the total plasma and LDL cholesterol levels, and finally reduce the risk of cardiovascular diseases.[27] The U.S. Food and Drug Administration allows a cholesterol-lowering health claim for foods containing adequate amounts of dietary fiber including psyllium. The cholesterol-lowering benefit of psyllium might be

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partially explained by its ability to bind bile acids and stimulate cholesterol metabolism.[14,28]

Cholic acid (CA) and chenodeoxycholic acid (CDCA) are two primary kinds of bile acids synthesized in the liver. As shown in Figure 6, SFP had the greatest bile acid-binding capacities among the three derivatives, which was 6.00-fold greater in binding CA and 6.23-fold stronger in binding CDCA compared to those of the original psyllium. The bile acid-binding capacities of SFP were comparable to that of cholestyramine resin, a quarternary ammonium anion exchange resin with a polystyrene backbone. It was commercially used as an adjunctive therapy for reducing the plasma cholesterol level by the bile acid-binding mechanism in the intestinal system.[29] The order of bile acid-binding capacity was SFP > SCP > HP > psyllium (Figure 6). HP had a 1.98-fold stronger CDCA-binding capacity than psyllium, but had the same CA-binding capacity as psyllium. These results might imply that introduction of anionic groups such as sulfonic and carboxylic groups to psyllium could enhance its bile acid-binding capacity. This could be explained by the complexation of hydrogen bonds, which affected the interaction between the gel network and bile acids.

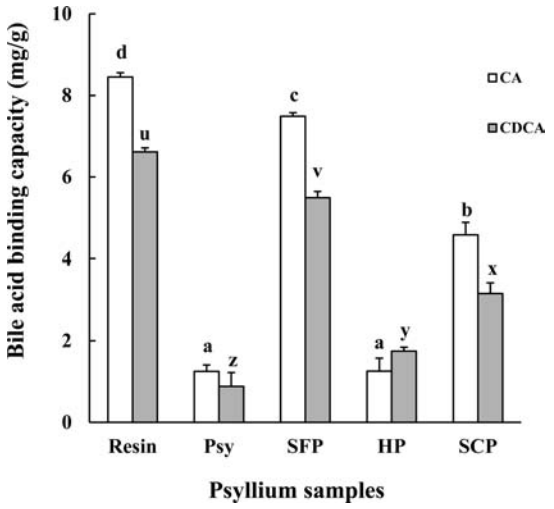

Figure 6. Bile acid-binding capacity of psyllium samples. Resin means cholestyramine resin, which is the positive control. Psy stands for original psyllium obtained commercially, whereas SFP, HP, and SCP stand for sulfated, hydroxypropylated, and succinylated psyllium, respectively. Data are expressed as the mean ± standard deviation (SD). Vertical bars represent the SD value of each data point. Values carrying the same letters are not significantly different ($P > 0.05$).

In summary, results from this study indicate that the negative charge and the size of the substitution group may alter the physicochemical, functional, and bile acid-binding capacities of psyllium differently. Charge from the substitution group has a strong impact on the bile acid-binding capacity and water-uptake behavior, whereas the size of the substitution groups may be more important for the gelling and swelling properties of psyllium. These findings are important for improving the physicochemical, functional, and biological properties of psyllium.

■ ASSOCIATED CONTENT

Supporting Information

Additional figure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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