Modified soluble dietary fiber from black bean coats with its rheological and bile acid binding properties

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ABSTRACT

Alkaline hydrogen peroxide (AHP) was investigated to enhance the content and functionality of soluble dietary fiber (SDF) extracted from black bean coats. Compared with the content of SDF of original black bean coat 7.8%, the content of SDF of modified black bean coat was 16.9% after treated by 15% H₂O₂ (v/v) at pH 11 and 1:18 (w/v) as liquid-to-solid ratio for 0.5 h. Monosaccharide composition confirmed that the ratio of galacturonic acid in modified SDF (M-SDF) was higher than that of original SDF (O-SDF), and M-SDF was also with smaller molecular weight (Mw) of 1.24×10^6 Da and lower ζ -potential of -42.3 mV. Other structural characters were determined by FT-IR and TEM. In the range of 2-6% (w/v), both O-SDF and M-SDF showed a shear thinning behavior. The latter showed stronger gelation ability at the presence of Ca²⁺ evaluated by dynamic oscillatory rheometry. Moreover, M-SDF exhibited good abilities of binding bile acids *in vitro*. It could be concluded that M-SDF had a great potential to be applied as a novel kind of functional ingredient or additive in food industry.

Keywords: Black bean coats; Soluble dietary fiber; AHP; Rheology; Bile acid binding

1. Introduction

Black bean has been widely used as nutrient-rich food and described in Compendium of Materia Medica. Its coat was a kind of byproduct during the process of black bean oil production. Black bean coats were rich in many natural compounds, such as polysaccharides, proteins, flavonoids, saponins and polyphenols (Guajardo-Flores, García-Patiño, Serna-Guerrero, Gutiérrez-Urbe , & Serna-Saldívar, 2012; Todd & Vodkin, 1993; Choung et al., 2001; Jiang et al., 2014). It was demonstrated that the black bean coat extract could prevent obesity and diabetes by enhancing energy expenditure and suppressing inflammation (Kanamoto et al., 2011). However, most current researches are focusing on flavonoids, saponins or polyphenols of black beans coats. Other main functional fractions, such as dietary fiber, have been barely reported.

Dietary fiber (DF) has drawn a great attention because of its significant benefits, such as reducing the risk of coronary heart diseases, strokes, hypertension, diabetes, obesity, and certain gastrointestinal diseases (Elleuch et al., 2011; Huang, Ye, Chen, & Xu, 2013). DF could be classified into insoluble (IDF) and soluble dietary fiber (SDF) based on whether it could form a dispersion or not when mixed with water (Jiménez-Escrig & Sánchez-Muniz, 2000). Compared to IDF, SDF had certain better physiological and bioactive properties, such as stronger antioxidant activity, higher capacity to form gels, greater fermentability and viscosity (Esposito et al., 2005; Mateos-Aparicio, Mateos-Peinado, & Rupérez, 2010; Feng et al., 2014; Galisteo, Duarte, & Zarzuelo, 2008). Thus SDF could be readily applied to food products as thickeners, emulsifiers, stabilisers, and fat replacers (Cui, Wu, & Ding, 2013; Sozer, Cicerelli, Heiniö, & Poutanen, 2014).

Our previous study showed that black bean coats had dietary fibers as 69.2%, but only 7.8% were soluble. Thus, the objective of this study is to enhance the content of SDF from black bean coats, as well as develop the application of byproducts in food industry. AHP was chosen as the modification method in this study. The molecular

weight, monosaccharide composition and ζ -potential of M-SDF were determined. Its structure was also analyzed by FT-IR and TEM. In addition, the rheological and bile acid binding capacity were determined to demonstrate the gelling property and potential health benefits of M-SDF.

2. Materials and methods

2.1 Materials

Black bean coats were donated by Shanxi Qingyu Oil Company Limited (Shanxi, China). After carefully removing dusts, beans and grass blade, the coats were ground into powder (40 mesh) using a grinder. Standard monosaccharides including rhamnose, fructose, and xylose were purchased from Tokyo Chemical Industry (Shanghai, China). Glucose, cholestyramine resin, cholic and chenodeoxycholic acids, nicotinamide adenine dinucleotide, diphorase, and nitro blue tetrazolium were purchased from Sigma-Aldrich (St. Louis, USA). Arabinose, fucose, mannose, glucuronic acid, galactose, galacturonic acid and 3- α -hydroxysterol dehydrogenase were purchased from J&K Chemical Technology (Beijing, China). Dextrans with different molecular weights were purchased from the National Institute for Food and Drug Control (Beijing, China). Heat-stable α -amylase, protease, and amyloglucosidase solutions were purchased from Megazyme (Co. Wicklow, Ireland). Other reagents for analysis were of analytical grade and used without further purification.

2.2 Modification of bean coats

Based on our previous orthogonal experiment, AHP modification was conducted under the optimal conditions. Certain weight of black bean coats powder was firstly mixed with 15% H_2O_2 (v/v) solution as 1:18 liquid-to-solid ratio (w/v) under pH 11, and the suspension was then stirred for 0.5 h by magnetic stirring. After reaction, the solid matters of suspension were collected by vacuum filtration followed by distilled

water-washing twice. Finally, the washed solid was transferred to oven overnight at 60 °C to get the modified black bean coats for SDF extraction.

2.3 Preparations of SDF from modified black bean coats

The SDF was extracted by enzymatic-gravimetric procedure according to the AOAC method 985.29 (AOAC, 2001) with slight modifications. Six gram of original or modified black bean coats was dispersed in 240 mL MES-TRIS buffer (pH 8.2) and mixed by magnetic stirring for 1 h. Then the suspension was added with 300 µL of α -amylase solution, and hydrolyzed at 95 °C-100 °C under constant shaking for 35 min. After the temperature of the hydrolysate decreased to 60 °C, 600 µL of protease solution was added for further hydrolysis under constant shaking for 30 min. Next, the pH of mixture was adjusted to 4.5 by acetic acid (3 M). Amyloglucosidase solution (600 µL) was then added for final hydrolysis at 60 °C for 30 min under constant shaking. The ultimate hydrolysate was filtrated and the sediment was washed by distilled water twice. All the filtrate was collected and condensed to about one-tenth in vacuum rotary evaporation system. Afterwards, the concentrated filtrate was mixed with 95% (v/v) ethanol at 60 °C for 1 h followed by centrifugation at 4500 rpm for 10 min. Finally, the precipitated flocculate was subjected to vacuum freeze-drying to get SDF.

2.4 Monosaccharide composition

2.4.1 Hydrolysis of SDF

The SDF sample (2.0 mg) was dissolved with 0.4 mL distilled water and then added 0.4 mL of 4 M trifluoroacetic acid (TFA) in a sealed tube. The sealed tube was kept at 110 °C for 3 h. After cooled to room temperature, methanol reagent was used to remove excessive acid by nitrogen-blow, which was repeated for three times. The dry sample was then dissolved with 1.0 mL distilled water.

2.4.2 Preparation of monosaccharide standard solutions

Stock standard solutions (2.0 mg/mL) were prepared by dissolving each standard monosaccharide in a mixture of water solution. Working monosaccharide standard solutions (0.01 mg/mL) for generating the calibration curve were further obtained by appropriate dilution of the stock standard solutions with distilled water. The sample solutions were filtered through 0.45 μ m syringe filters prior to use. All the solutions were stored at 4 °C until being used.

2.4.3 Ion Chromatography

The monosaccharide compositions of SDF were analyzed by HPAEC after acid hydrolysis. HPAEC was performed on a Dionex ICS-2500 system, equipped with PAD and a Carbo PACTM PA20 column (3 mm $\times$ 150 mm). Hydrolysates (25 μ L) were injected for ion chromatography analyzed. The eluant was a mixture of three solutions: solution A: distilled water, solution B: 50 mM sodium hydroxide solution and solution C: 0.5 M sodium acetate solution. Elution was performed using a gradient (Table 1) that was optimized for efficiency and also provided specific quantitative analysis of neutral and acidic monosaccharides. The flow rate was adjusted to 0.45 mL/min and the column temperature was set at 30 °C. The retention time of each monosaccharide standard in the mixtures was confirmed by the analysis of each monosaccharide.

Calibration was performed with a standard solution of L-rhamnose, L-arabinose, D-glucose, D-xylose, D-fructose, D-mannose, D-galactose, L-fucose, glucuronic acid and galacturonic acid. Data were processed using the Chromeleon software (Chromeleon 7.2, Dionex, USA).

2.5 Molecular weight determination

The molecular weight of SDF was determined using a high-performance size exclusion chromatograph (Agilent, USA) equipped with a gel filtration column (Shodex SUGAR KS-805, 8 mm i.d. $\times$ 300 mm, Showa Denko, Japan) and a refractive index detector according to published method (Gong et al., 2015). In brief,

distilled water was used as the mobile phase and eluted at a flow rate of 1.0 mL/min. The molecular weight of SDF was calculated by the standard curve (different dextrans molecular weight against its retention time) prepared with a series of dextran standards with different molecular weights (180, 2 700, 5 250, 9 750, 13 050, 36 800, 64 650, 135 350, 300 600 and 2 000 000 Da, respectively).

2.6 ζ -potential determination

The surface charges of O-SDF and M-SDF were measured using a particle analyzer (Zetasizer Nano ZS90, Malvern, UK). SDF solution (2.0 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 output data indicated the charge of the SDF quantitatively. The ζ -potential tests were performed by triplicate.

2.7 Infrared spectral analysis

The Fourier transform infrared (FT-IR) spectrum was carried out on a FT-IR spectrophotometer with an ATR accessory (Nicolet 6700, Thermo Fisher, USA) in the range of 4000-400 cm^{-1} .

2.8 Transmission electron microscope (TEM)

To observe the microstructure, 0.01 mg/mL of SDF solution was prepared by distilled water. A droplet of SDF solution was deposited on the carbon film specimen (200 mesh, Beijing Zhongjingkeyi Technology, China). Transmission electron microscopy (Tecnai G2 Spirit BIOTWIN, FEI, USA) with an accelerating voltage of 120 kV was applied to visualize the molecular morphology of the O-SDF and M-SDF samples after they were dried at ambient temperature and humidity.

2.9 Rheological measurements

2.9.1 Instrument

The rheological measurements were carried out by using an ARG2 rheometer (TA Instruments, USA) equipped with a SRS Peltier Circulator (TA Instruments, USA) as the temperature controller which made the temperature of the plate sensor system within ± 0.1 °C controllable. All the rheological studies were performed by using parallel plate (40 mm diameter with a gap of 500 μm).

2.9.2 SDF solution preparation

The SDF aqueous solutions were prepared as follow: 2%, 4%, 6% (w/v) of samples was prepared with distilled water at 55 °C under continuous magnetic stirring for 1 h. After totally dissolved, the solutions were equilibrated at 4 °C overnight to allow the elimination of entrapped air.

2.9.3 Flow behavior

The appear viscosity was recorded by a function of shear rate ranging from 0.1 to 1000 s^{-1} acquired in a logarithmic ramp. All the experiments were performed dublicately at 25 °C with 5 min equilibration before each rheological test.

2.9.4 Preparation of gels

The 1% (w/v) SDF dispersion was prepared with distilled water by magnetic stirring. The dispersion was heated continuously up to 90 °C and the necessary amount of 50 mM or 60 mM CaCl_2 stock solution was added at the end of the heating. Then, the hot solutions were poured onto the preheated fixture of the rheometer (90 °C) immediately after preparation.

2.9.5 Oscillatory dynamic tests

After poured onto the rheometer plate, SDF gels were equilibrated for 2 min before tested. A few drops of water were placed at the cone's edge to avoid the evaporation of the sample during measurements. Gel formation of SDF samples with different concentrations of Ca^{2+} was investigated under a controlled cooling from 90 °C to 25 °C at a rate of 1 °C/min. After that, a gel cure test was run at 1 rad/s and 25 °C during 30 min. Then, a frequency sweep at 25 °C from 0.1 to 100 rad/s was carried

out at a constant strain (1%). Finally, strain sweeps were performed at a constant frequency (1 rad/s) to verify that all the measurements could be completed within the region of linear viscoelasticity. All the oscillatory dynamic tests were performed by duplicate.

2.10 Bile acid binding capacity

According to a previous study (Niu, Xie, Zhang, Sheng, & Yu, 2013), the bile acid binding capacities of M-SDF samples with different concentrations of CaCl_2 were determined. Each sample (25 mg) was mixed with 500 μL different concentrations of CaCl_2 . To simulate gastric conditions, the mixture was treated with 0.25 mL of HCl (0.01 M) followed by incubation at 37 °C with continuous shaking for 1 h. NaOH solution (0.1 M) was used to adjust the pH of mixture to 7.0, and then 1.25 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 4500 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 of reaction solution or bile acid standard (100 μL) was added with 125 μL of nicotinamide adenine dinucleotide (2.5 mM), 125 μL of nitro blue tetrazolium (0.61mM), 100 μL of 3- α 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). Triplication tests were taken for each sample against each bile acid.

2.11 Statistical analysis

All the results were expressed as mean $\pm$ standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) and least significant difference (LSD) using SAS 9.3 TS level 1M2 (SAS Institute Inc., USA). Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1 SDF from black bean coats by AHP modification

Compared with the content of SDF of original black bean coat 7.8%, the content of SDF of modified black bean coat increased to 16.9% (shown in supporting material Fig. S), which indicated the method established in this study is of great efficiency in enhancing the SDF content of black bean coats. It is well known that the physical properties of dietary fibers could be altered dramatically by treatment with alkaline hydrogen peroxide (AHP), because AHP could reduce cellulose crystallinity as a solubilizing part of the lignin through rupture of the hydrogen bonding in the molecular chains, leading to more open internal structure in fibers. Thus, AHP could increase content of SDF in products and growth in the water retention, swelling capacity and sensory characteristics of modified dietary fibers as well (Larrea, Grossmann, Beléia, & Tavares, 1997; Cao et al., 2016). In this study, AHP was selected as the modified method because of its great safety and good applicability in food industry.

3.2 Structural characters of SDF

3.2.1 Monosaccharide composition of SDF

Monosaccharide composition analysis by HPAEC (Table 2) showed that both O-SDF and M-SDF were composed of eight kinds of monosaccharide: galacturonic acid, fucose, glucose, rhamnose, arabinose, xylose, galactose and mannose. Among

these monosaccharide, xylose, mannose, galactose, rhamnose, arabinose and galacturonic acid are the mainly typical compositions of hemicellulose in most plant cell walls. The result indicated that hemicellulose was one of the main components of black bean coats. On the other hand, the amount of galacturonic acid was also high in both O-SDF and M-SDF SDF. As galacturonic acid was one of the major pectic monosaccharides in pectin (Lira-Ortiz et al., 2014), another major component of SDF from black bean coats may be pectin. Compared to the O-SDF, there was a significant decrease in the amount of galactose and mannose, and also an obvious increase in the content of pentose (arabinose and xylose) and galacturonic acid in the M-SDF, which may indicate an oxidizing reaction on polysaccharides induced by AHP.

3.2.2 Molecular weight of SDF

Both of HPLC spectrogram of O-SDF and M-SDF showed one main peak with 84.0% peak area and other three small peaks. The molecular weight was calculated as 1.44×10^6 Da for O-SDF and 1.24×10^6 Da for M-SDF using a dextran standard curve. The decrease in molecular weight could be explained by the oxidation of AHP, which implied that AHP treatment could severely degrade the structure of SDF.

3.2.3 FT-IR and ζ - potential of SDF

FT-IR spectra have been utilized to elucidate the functional groups of polymers, as well as the bonding information of these functional groups. The FT-IR spectrum was shown in Fig. 1. The general spectral profiles of the O-SDF and M-SDF were similar except some characteristic bands. A broad band located at about 3441 cm^{-1} both in M-SDF and O-SDF was attributed to the O-H stretching of hydrogen bound of the hydroxyl groups mainly from cellulose and hemicellulose (Ma & Mu, 2016). In addition, a significant reduction in intensity and width of the peak at 3441 cm^{-1} was observed in M-SDF, indicating the degradation of cellulose or hemicellulose due to AHP treatment. The weak band at 2925 cm^{-1} was originated from C-H stretching, which represented the presence of the typical structure of polysaccharide compounds (Yan, Ye, & Chen, 2015). The typical peak at 1634 cm^{-1} was corresponded to

characteristic bending or stretching of aromatic hydrocarbons of lignin (Zhao et al., 2013) which was another mainly component of dietary fiber. It was noteworthy that carbonyl group stretching of ester group (at 1733 cm^{-1}) (Zhang, Liang, Pei, Gao, & Zhang, 2009) was only observed in O-SDF, implying the oxidant effect of AHP. The signals at 800 cm^{-1} and 1200 cm^{-1} were resulted from the highly coupled C-C-O, C-OH and C-O-C stretching vibrations of the polymer backbone (Mudgil, Barak, & Khatkar, 2012; Shobha, Vishu Kumar, Tharanathan, Koka, & Gaonkar, 2005).

The surface charge (ζ - potential) was examined to further explain the mechanism of gelation of M-SDF. The ζ -potentials of O-SDF and M-SDF were -9.76 and -42.3 mV, respectively. These data showed an over 4-fold increase of surface negative charge of the M-SDF compared to that of the original, indicating the stronger electrostatic forces between M-SDF and Ca^{2+} may be another essential reason for gelation in M-SDF apart from hydrogen bonding.

3.2.4 Transmission Electron Microscopy of SDF

The TEM images of O-SDF and M-SDF were showed in Fig.2. It was obvious that the SDF treated by AHP tended to form a network with more open internal structure, whereas the molecular morphology of O-SDF presented as chains with lots of branches. The different microscopic morphology between O-SDF and M-SDF could explain the improvements of water solubility, the ability of gel formation and the bile acid binding capacity after modification.

3.3 Rheological properties

3.3.1 Flow properties

The flow properties of SDF from black bean coats showed in Fig. 3, which illustrated the effect of shear rate on viscosity of different concentrations of SDF at 25°C . A shear thinning behavior was observed in both O-SDF and M-SDF at all concentrations. As for SDF of 6% (w/v), a sharp and linear decrease of viscosity presented throughout. With the decrease of SDF concentration, the viscosity began to

reduce slowly at high shear rates, especially for M-SDF. A decreasing number of chain entanglements could account for the decrease in viscosity at high shear rates (Qi, Cui, Chen, & Guo, 2005). In addition, the viscosity of both O-SDF and M-SDF decreased as the reducing SDF concentration, and the viscosity of M-SDF decreased sharply when the concentration lowered to 4%. Compared to O-SDF, M-SDF aqueous solution exhibited lower viscosity at each concentration.

3.3.2 Gelling properties

Since gelling properties had little significant difference among O-SDF with different concentrations of Ca^{2+} , Fig 4 only showed the results of oscillatory dynamic test of O-SDF (1%, w/v) in the presence of 7.5 mM CaCl_2 as an example. Fig. 4a showed the variation of storage modulus (G') and loss modulus (G'') during controlled cooling. It was obvious that G' and G'' had similar variation trend and approximate value with strong temperature dependence, indicating gel formation did not occur in this system during cooling. At 25 °C, G' and G'' increased rapidly at beginning and reached a steady value after 500 s with the circular frequency of 1 rad/s, indicating a stable system formed (Fig. 4b). Mechanical spectra of O-SDF- CaCl_2 system showed in Fig. 4c after 30 min at a constant strain (1%). G'' increased linearly as increasing frequency, while there was an abrupt rise after 10 rad/s in G' , implying a sudden structural change in the system. The strain sweeping results (Fig. 4d) confirmed that all the measurements above were within the linear viscoelastic region.

The viscoelastic behavior of M-SDF solution at a concentration of 1% (w/v) was investigated in the presence of 5, 7.5 and 10 mM CaCl_2 . Fig. 5a showed the semi-logarithmic plots of G' and G'' versus temperature which obtained at a constant frequency during controlled cooling. It revealed that a cross-linked network was formed in the presence of CaCl_2 , which was characterized by the temperature independence of G' and G'' on cooling. In fact, no crossover between G' and G'' occurred during cooling from 90 °C to 25 °C. All system showed a typical gel-like behavior ($G' > G''$), indicating that Ca^{2+} might act as a crosslink agent in the M-SDF solution. Also, G' and G'' increased with the enhancement of Ca^{2+} concentration.

The time evolution of both G' and G'' moduli for M-SDF system was showed in Fig. 5b at fixed frequency of 1 rad/s within linear viscoelastic region at 25 °C. Obviously, all systems appeared stable G' and G'' moduli during 30 min and presented a typical gel-like behavior. In the presence of 10 mM CaCl_2 , G' reached almost 1000 Pa which was 200-fold greater than that of 5 mM CaCl_2 .

Fig. 5c showed the mechanical spectra of M-SDF- CaCl_2 system. The applied stress yielded 1% strain which was within the linear viscoelastic region. The M-SDF system with 7.5 mM and 10 mM CaCl_2 denoted a strong gel-like behavior, with G' higher than G'' and both of them with slight frequency dependence. Whereas, as for low concentration of CaCl_2 (5 mM), the storage modulus (G') decreased sharply to negative value after frequency increased to 10 rad/s, suggesting a structural collapse in this system. It was also noteworthy that the formed gels were soft and elastic rather than strong and hard gels.

The linear viscoelastic region of M-SDF gels was exhibited by the strain sweep. As shown in Fig. 5d, the linear viscoelastic region became narrower when calcium concentration increased. G' of gel system with 5 mM CaCl_2 remained constant until strain values reached 12%. However, when strain values only increased to 6%, G' of M-SDF with 10 mM CaCl_2 began to decrease dramatically. The strain at which G' decreased sharply was defined as the critical strain S (Guo, Cui, Wang, Goff, & Smith, 2009). At the critical strain S , gel structure began to collapse. Therefore, critical strain S reflected the deformability of a gel. The decreasing critical strain S value proved that M-SDF with high concentration of calcium obtained a greater deformability which could make it easier to process in food industry. In addition, all of systems had a critical strain S higher than 1%. Thus, the selected strain (1%) in the entire oscillatory dynamic experiments ensured that the gel network was not damaged by the imposed strain during the measurements.

According to the published study, the networks formed by calcium-pectin could be described as the egg-box model which Ca^{2+} was chelated between the carboxyl groups of different pectin chains (Powell, Morris, Gidley, & Rees, 1982). Additionally,

divalent ions gelation for pectin chains depended on the number of non-methylated galacturonic acid residues. (Mierczyńska, Cybulska, Sołowiej, & Zdunek, 2015; Fang et al., 2008). Referring to the former monosaccharide composition, ζ - potential and TEM results, SDF modified by AHP was composed of pectin mainly with more galacturonic acid residues, carried much more negative charge and presented more open internal structure with plenty of porous structure than those of O-SDF. Therefore, the M-SDF showed more significant gel-forming property than the O-SDF. The gelation mechanism might be the contribution of hydroxyl groups via hydrogen bonds as well as ionic interactions between dissociated carboxyl groups via Ca^{2+} -bridges.

3.4 Bile acid binding capacity

The ability of polymers to bind bile acids may enhance the elimination of bile acids and promote the conversion of cholesterol to bile acids in liver. It could reduce the levels of total plasma and LDL cholesterol and lower the risk of cardiovascular diseases as a result (Zhou, Xia, Zhang, & Yu, 2006; Niu, Xie, Zhang, Sheng, & Yu, 2013). As United States Food and Drug Administration claimed, it was allowable to make a cholesterol-lowering health statement for foods containing adequate amount of soluble fiber. As shown in Fig.6, M-SDF had remarkable binding capacities against both cholic (CA) and chenodeoxycholic acids (CDCA) at the present of Ca^{2+} . The capacities enhanced as concentration of Ca^{2+} increased. The capacities at the highest concentration of Ca^{2+} were comparable with those of the resin which was a commercial drug to reduce plasma cholesterol level by the bile acid-binding mechanism. The study also proved that there was little bile acid binding capacity exhibited by O-SDF with or without Ca^{2+} as well as M-SDF without Ca^{2+} (data not shown). Thus, the significant bile acid binding capacity could be the result of the gel formed by SDF with Ca^{2+} . As the concentration of Ca^{2+} increased, the growing amount of gel presented more open intern structure for bile acid binding, which was corresponding to the results of rheological properties discussed above.

4. Conclusion

Alkaline hydrogen peroxide (AHP) was an efficient and safe modification method for improving the SDF content, rheological and functional properties of black beans coats. The content of SDF could be significantly increased from 7.8% to 16.9% under optimal AHP modified conditions. Compared to O-SDF, M-SDF had different ratio of monosaccharide composition, smaller molecular weight and higher surface negative charge. Both O-SDF and M-SDF aqueous solution exhibited a shear thinning behavior. However, the M-SDF showed greater gelation ability at the presence of Ca^{2+} , which made it as a promising thickening and gelling additive in food applications. Moreover, the results of bile acid binding capacity suggested a high potential of modified SDF as cholesterol-lowering functional ingredient.

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References

- AOAC. (2001). Official methods of analysis (17th ed.). MD: Association of Official Analytical Chemists.
- Cao, W. X., Sun, C., Qiu, J. P., Li, X. D., Liu, R. H., & Zhang L. (2016). Pretreatment of sweet sorghum bagasse by alkaline hydrogen peroxide for enhancing ethanol production. *Korean Journal of Chemical Engineering*, 33(3), 873-879.
- Choung, M. G., Baek, I. Y., Kang, S. T., Han, W. Y., Shin, D. C., Moon, H. P., et al. (2001). Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.). *Journal of Agricultural and Food Chemistry*, 49(12), 5848-5851.
- Cui, S.W., Wu, Y., & Ding, H. H. (2013). The range of dietary fibre ingredients and a comparison of their technical functionality. In J. A. Delcour, & K. Poutanen (Eds.), *Fibre-rich and Wholegrain Foods: Improving Quality* (pp. 96-115). FL: Woodhead Publishing.
- Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food chemistry*, 124(2), 411-421.
- Esposito, F., Arlotti, G., Maria Bonifati, A., Napolitano, A., Vitale, D., & Fogliano, V. (2005). Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International*, 38(10), 1167-1173.
- Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., & Williams, P. A. (2008). Binding behaviour of calcium to polyuronates: Comparison of pectin with alginate. *Carbohydrate Polymers*, 72(2), 334-341.
- Feng, T., Su, Q., Zhuang, H., Ye, R., Gu, Z., & Jin, Z. (2014). Ghost structures, pasting, rheological and textural properties between mesona blumes gum and various starches. *Journal of Food Quality*, 37(2), 73-82.
- Galisteo, M., Duarte, J., & Zarzuelo, A. (2008). Effects of dietary fibers on disturbances clustered in the metabolic syndrome. *The Journal of Nutritional*

Biochemistry, 19(2), 71-84.

- Gong, L., Zhang, H., Niu, Y. G., Chen, L., Liu, J., Alaxi, S., et al. (2015). A Novel Alkali Extractable Polysaccharide from *Plantago asiatic* L. Seeds and Its Radical-Scavenging and Bile Acid-Binding Activities. *Journal of Agricultural and Food Chemistry*, 63(2), 569-577.
- Guajardo-Flores, D., García-Patiño, M., Serna-Guerrero, D., Gutiérrez-Urbe, J. A., & Serna-Saldívar, S. O. (2012). Characterization and quantification of saponins and flavonoids in sprouts, seed coats and cotyledons of germinated black beans. *Food Chemistry*, 134(3), 1312-1319.
- Guo, Q., Cui, S. W., Wang, Q., Goff, H. D., & Smith, A. (2009). Microstructure and rheological properties of psyllium polysaccharide gel. *Food Hydrocolloids*, 23(6), 1542-1547.
- Huang, Z., Ye, R., Chen, J., & Xu, F. (2013). An improved method for rapid quantitative analysis of the insoluble dietary fiber in common cereals and some sorts of beans. *Journal of Cereal Science*, 57(3), 270-274.
- Jiang, L. Z., Wang, J., Li, Y., Wang, Z. J., Liang, J., Wang, R., Chen, Y., et al. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Research International* 62, 595-601.
- Jiménez-Escrig, A., & Sánchez-Muniz, F. J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, 20(4), 585-598.
- Kanamoto, Y., Yamashita, Y., Nanba, F., Yoshida, T., Tsuda, T., Fukuda, I., et al. (2011). A black soybean seed coat extract prevents obesity and glucose intolerance by up-regulating uncoupling proteins and down-regulating inflammatory cytokines in high-fat diet-fed mice. *Journal of Agricultural and Food Chemistry*, 59(16), 8985-8993.
- Larrea, M., Grossmann, M., Beléia, A., & Tavares, D. (1997). Changes in water absorption and swollen volume in extruded alkaline peroxide pretreated rice hulls. *Cereal Chemistry*, 74(2), 98-101.

- Lira-Ortiz, A. L., Reséndiz-Vega, F., Ríos-Leal, E., Contreras-Esquivel, J. C., Chavarría-Hernández, N., Vargas-Torres, A., et al. (2014). Pectins from waste of prickly pear fruits (*Opuntia albicarpa* Scheinvar'Reyna'): Chemical and rheological properties. *Food Hydrocolloids*, 37, 93-99.
- Ma, M. M., & Mu, T. H. (2016). Effects of extraction methods and particle size distribution on the structural, physicochemical, and functional properties of dietary fiber from deoiled cumin. *Food Chemistry*, 194, 237-246.
- Mateos-Aparicio, I., Mateos-Peinado, C., & Rupérez, P. (2010). High hydrostatic pressure improves the functionality of dietary fibre in okara by-product from soybean. *Innovative Food Science & Emerging Technologies*, 11(3), 445-450.
- Mierczyńska, J., Cybulska, J., Solowiej, B., & Zdunek, A. (2015). Effect of Ca^{2+} , Fe^{2+} and Mg^{2+} on rheological properties of new food matrix made of modified cell wall polysaccharides from apple. *Carbohydrate Polymers*, 133, 547-555.
- Mudgil, D., Barak, S., & Khatkar, B. S. (2012). X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *International Journal of Biological Macromolecules*, 50(4), 1035-1039.
- Niu, Y. G., Xie, Z. H., Zhang, H., Sheng, Y., & Yu, L. L. (2013). Effects of Structural Modifications on Physicochemical and Bile Acid-Binding Properties of Psyllium. *Journal of Agricultural and Food Chemistry*, 61(3), 596-601.
- Powell, D. A., Morris, E. R., Gidley, M. J., & Rees, D. A. (1982). Conformations and interactions of pectins: II. Influence of residue sequence on chain association in calcium pectate gels. *Journal of Molecular Biology*, 155(4), 517-531.
- Qi, F., Cui, R. Q., Chen, C. P., & Guo, B. N. (2005). Some completely monotonic functions involving polygamma functions and an application. *Journal of Mathematical Analysis and Applications*, 310(1), 303-308.
- Shobha, M. S., Kumar, A. B. V., Tharanathan, R. N., Koka, R., & Gaonkar, A. K. (2005). Modification of guar galactomannan with the aid of *Aspergillus niger* pectinase. *Carbohydrate Polymers*, 62(3), 267-273.
- Sozer, N., Cicerelli, L., Heiniö, R.-L., & Poutanen, K. (2014). Effect of wheat bran

- addition on *in vitro* starch digestibility, physico-mechanical and sensory properties of biscuits. *Journal of Cereal Science*, 60(1), 105-113.
- Todd, J. J., & Vodkin, L. O. (1993). Pigmented soybean (*Glycine max*) seed coats accumulate proanthocyanidins during development. *Plant Physiology*, 102, 663-670.
- Yan, X. G., Ye, R., & Chen, Y. (2015). Blasting extrusion processing: The increase of soluble dietary fiber content and extraction of soluble-fiber polysaccharides from wheat bran. *Food Chemistry*, 180, 106-115.
- Zhang, M., Liang, Y., Pei, Y., Gao, W. W., & Zhang, Z. S. (2009). Effect of Process on Physicochemical Properties of Oat Bran Soluble Dietary Fiber. *Journal of Food Science*, 74(8), C628-C636.
- Zhao, X. Y., Chen, J., Chen, F. L., Wang, X. C., Zhu, Q. J., & Ao, Q. (2013). Surface characterization of corn stalk superfine powder studied by FTIR and XRD. *Colloids and Surfaces B: Biointerfaces*, 104, 207-212.
- Zhou, K. Q., Xia, W. S., Zhang, C., & Yu, L. (2006). *In vitro* binding of bile acids and triglycerides by selected chitosan preparations and their physico-chemical properties. *LWT-Food Science and Technology*, 39(10), 1087-1092.

Table 1

Ion chromatography gradient program for SDF samples analyzed.

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0.0	95.0	5.0	0.0
20.0	95.0	5.0	0.0
20.1	85.0	5.0	10.0
30.0	85.0	5.0	10.0
30.1	0.0	100.0	0.0
35.0	0.0	100.0	0.0

Table 2

Monosaccharide composition of O-SDF and M-SDF.

Area (%)	O-SDF	M-SDF	
Ara	3.42	20.29	
Xyl	1.52	15.10	
Gal A	16.24	29.59	increased
Fuc	0.43	2.95	
Rha	2.54	9.04	
Galac	24.52	12.90	
Man	46.38	6.84	decreased
Glu	4.94	3.29	

Figure captions

Fig. 1. FTIR spectra of M-SDF (a) and O-SDF (b)

Fig. 2. TEM images of SDF in water solution: (a) O-SDF; (b) M-SDF.

Fig. 3. Viscosity curves of aqueous solution of soluble dietary fiber (SDF) at 2% (circles), 4% (triangles) and 6% (squares) (w/v): (a) O-SDF; (b) M-SDF.

Fig. 4. Oscillatory dynamic test results of O-SDF (1%, w/v) in the presence of 7.5 mM CaCl_2 . (a) G' , G'' vs temperature ($\omega=1$ rad/s, strain=1%); (b) G' , G'' vs time ($\omega=1$ rad/s, strain=1%, $T=25$ °C); (c) G' , G'' vs angular frequency (strain=1%, $T=25$ °C); (d) G' vs strain ($\omega=1$ rad/s, $T=25$ °C).

Fig. 5. Oscillatory dynamic test results of M-SDF (1%, w/v) in the presence of 5mM (square), 7.5 mM (circle) and 10 mM (triangle) CaCl_2 . (a) G' , G'' vs temperature ($\omega=1$ rad/s, strain=1%); (b) G' , G'' vs time ($\omega=1$ rad/s, strain=1%, $T=25$ °C); (c) G' , G'' vs angular frequency (strain=1%, $T=25$ °C); (d) G' vs strain ($\omega=1$ rad/s, $T=25$ °C).

Fig. 6. Bile acid binding capacity of M-SDF in the presence of difference concentrations of CaCl_2 . Resin means cholestyramine resin, which is the positive control. Data are expressed as the mean $\pm$ standard deviation (SD). Vertical bars represent the SD value of each data point. Values in the same acid treatment group with different letters were significantly different ($P<0.05$).

Supplemental Material Fig. S Content of SDF from original and modified black bean coats. Data are expressed as the mean $\pm$ standard deviation (SD). Vertical bars represent the SD value of each data point. Values carrying different letters were significantly different ($P<0.05$).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

1 Graphical abstract

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