



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 coats 7.8%, the content of SDF of modified black bean coats 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.

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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 (Choung et al., 2001; Guajardo-Flores, García-Patiño, Serna-Guerrero, Gutiérrez-Urbe, & Serna-Saldívar, 2012; Jiang et al., 2014; Todd & Vodkin, 1993). 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; Feng et al., 2014; Galisteo, Duarte, & Zarzuelo, 2008; Mateos-Aparicio, Mateos-Peinado, & Rupérez, 2010). 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

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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 black 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% H₂O₂ (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. Preparation 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 were 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 μ L 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 μ L 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 μ L) 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

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

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