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Effects of ultrafine grinding and cellulase hydrolysis treatment on physicochemical and rheological properties of oat (*Avena nuda* L.) β-glucans

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ABSTRACT

In order to improve the functionality of oat (*Avena nuda* L.) β-glucans and widen their applications in food industry, the oat bran was processed by ultrafine grinding and enzymatic hydrolysis, and extracted to obtain oat β-glucans. The extracted oat β-glucans were characterized by the molecular weight distribution, particle size, rheological properties, thermal stability, solubility and water holding capacity, and morphological properties. The average molecular weight of raw oat β-glucans (OBG-1) and ultrafine ground oat β-glucans (OBG-2) were 2.79×10^5 Da and 3.26×10^5 Da, respectively; while the average molecular weights of cellulase hydrolyzed oat β-glucans (OBH-1 and OBH-2) significantly decreased to 1.98×10^5 and 1.05×10^5 Da. The particle size, thermal stability, solubility and water holding capacity showed significant correlation with molecular weight distribution. Both OBG-1 and OBG-2 had shear-thinning behavior, whereas OBH-1 and OBH-2 exhibited shear-thickening behavior. Scanning electron microscope observation suggested that cellulase hydrolyzed oat β-glucans had fibrous structures with granular particles in aggregates sticking to the fibrous skeleton. Compared with raw and ultrafine ground oat β-glucans, the partially hydrolyzed oat β-glucans showed different rheological properties, which might be of potential use in the food industry.

1. Introduction

Oat belonging to the genus *Avena* in the family Gramineae is an important grain crop for humans (Duan et al., 2014). Oats are mainly grown in Russia, Canada, the United States, Finland, Australia, and China. There are seventy species of oats around the world, while *Avena sativa* L. (hulled oat) and *Avena nuda* L. (naked oat) are the most cultivated oat species (Jing and Hu, 2012). The hulled oats have been used in the form of rolled oats and steel cut groats in western countries for many years (Welch, 1995). In China, the naked oat has been widely cultivated and was used as a traditional Chinese herb medicine due to its lubricative feature in intestine for hundreds of years (Hu et al., 2014).

Oat contains a high level of nutrients, including protein, fat, minerals and vitamins, and oat bran is a main source of soluble fiber β-glucan. The high content of soluble fiber β-glucan in oats has showed significant positive health effects in lowering cholesterol, modulating glucose, improving gastrointestinal function, and preventing heart diseases (Alfredo et al., 2009; Tapola et al., 2005). Oat β-glucans out of various dietary fibers have been considered as a kind of potential ingredients in health-promoting functional foods not only because of their health benefits, but also because of their ability to bind water molecules and increase apparent viscosity (Lazaridou et al., 2006). Nevertheless, physicochemical properties of oat β-glucans play fundamental roles in their functionality in food systems, which will limit their use as food technological ingredients. Many research efforts have been done to develop various oat β-glucan dietary fiber sources by different processing methods, such as acid or enzymatic hydrolysis, extrusion, superfine grinding and steam heating, for improving their functionality (Gamel et al., 2014). These studies revealed the alterations in the molecular weight and structure of oat β-glucans during processing, as well as their effects on the physicochemical, rheological and functional properties of oat β-glucans (de Moura et al., 2011). Further in-depth studies on the effect of food processing methods on the physicochemical and functional properties of oat β-glucans will widen the applications of oat β-glucans in food industry and open new

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possibilities for designing fiber enriched products and generating new textures in a range of applications (Rosell et al., 2009).

In this study, ultrafine grinding (physical method) and cellulase hydrolysis (enzymatic method) were applied to influence the physicochemical and functional properties of oat (*Avena nuda* L.) β-glucans. The objective of this research was to conduct comparative spectroscopic, chromatographic and rheological studies on the physicochemical properties of ultrafine ground and cellulase hydrolyzed oat β-glucan samples. The solubility and water holding capacity of various oat β-glucan samples were also investigated.

2. Material and methods

2.1. Materials

Naked oat (*Avena nuda* L.) bran was obtained from Yanbei Health-Care Food Co. (Wanquan County, Hebei Province, China). The mixed-linkage β-glucan assay kit was purchased from Megazyme (Co. Wicklow, Ireland). All other chemicals and reagents were purchased locally and were of analytical grade.

2.2. Methods

2.2.1. Sample preparation

Oat bran was ground ultrafine with a LWF-350 μm (Jinan Saixin Machinery Co., Jinan, China). The planetary rubbing mill with power of 0.75 kW was a vertical batch mill. A total of 3 rubbing rings were vertically equipped with potholders on the turntable. The revolution speed of turntable was unfixed at 2500– 2800 r/min. The oat bran was ground ultrafine for 6 h.

Oat β-glucan was extracted from untreated and ultrafine grinding-treated oat bran according to the procedure described in previous literature (Byun et al., 2008; Lazaridou and Biliaderis, 2004) with some modifications. Briefly, 100 g of untreated or ultrafine grinding-treated oat brans were extracted with 400 mL of 75% (v/v) ethanol and refluxed at 80 °C for 10 min to deactivate endogenous β-glucanases. The mixture was filtered and extracted twice with the same solvent to remove most of the polyphenols, flavonoids, lipids and monosaccharides. Then the residues of oat bran were dried in air and extracted with distilled water (1000 mL, 3 times) at 60 °C for 1.5 h. The mixture was then centrifuged at 3000 r/min for 15 min. All supernatants were combined and hydrolyzed with α-amylase (480 U/mL, Beijing Aoboxing Biotechnology Co., Beijing, China) at 90 °C for 10 min (1 U/g oat bran) and β-amylase (4700 U/mL, Beijing Aoboxing Biotechnology Co., Beijing, China) at 60 °C for 10 min (3 U/g oat bran) to remove starch. The pH of the solution was adjusted to 4.5 and the sample was left standing for 4 h. Then it was centrifuged at 3000 r/min for 15 min. The supernatants were concentrated to 10% of the original volume under vacuum pressure and precipitated by adding 4 times of volume of 95% (v/v) ethanol at 4 °C overnight. The mixture was filtered and the protein in oat samples was then removed by the Sevag method. The mixed solution with the volume ratio of chloroform to n-butyl alcohol (4:1) was added in the solution of oat extracts. Then the mixture was oscillated adequately for 5 min. The organic phase containing protein was separated from the water phase, and the extraction solution was vacuum freeze-dried. All the samples were made in triplicate for each extraction. The oat β-glucan samples from untreated and ultrafine grinding-treated oat β-glucan were obtained and named as OBG-1 and OBG-2, respectively.

Cellulase hydrolysis treatment was conducted by hydrolyzing the isolated OBG-1 sample with a commercial cellulase (76 U/g oat β-glucan, Tianjin Noao Sci & Tech Development Co., Ltd, Tianjin, China) at different dosages (5, 10, 20, 40, 60, 80 and 100 U/g) in distilled water (1 mg/mL, 55 °C, pH 5.5) for 20 min under continuous stirring. According to the HPLC chromatogram (Fig. S1), high- and low-molecular weight oat β-glucan hydrolyzate samples for further study were obtained by enzymatic hydrolysis with cellulase dosage of 10 U/g and 60 U/g and named as OBH-1 and OBH-2, respectively. The hydrolyzates were concentrated to about 10% of the original volume under vacuum pressure and vacuum freeze-dried.

The β-glucan content was determined by the method of McCleary and Glennie-Holmes (1985) using the Megazyme® mixed-linkage β-glucan assay kit. The contents of protein and reducing sugars were measured by Coomassie brilliant blue method (Bradford, 1976) and 3,5-dinitrosalicylic acid (DNS) method (Breuil and Saddler, 1985), respectively. The moisture content was determined by drying to constant weight at 105 °C.

2.2.2. Molecular weight distribution determination

Molecular weight (M_w) distributions of oat β-glucan samples were determined using a Shimadzu LC-20AT HPLC system (Shimadzu, Japan) equipped with a RID-10A refractive index detector (Shimadzu Scientific Instruments Inc., Kyoto, Japan) and the chromatographic column was placed in a 30 °C column oven. The molecular weight distributions of OBG-1 and OBG-2 were measured using a Shodex OHpak SB-805HQ column, while OBH-1 and OBH-2 samples were injected into a Shodex OHpak SB-803HQ column. Oat β-glucan samples (1% w/v) were dissolved in ultrapure water and filtered through 0.45 μm filter. The flow rate of the mobile phase (ultrapure water) was set at 0.8 mL/min and sample injection volume was 20 μL. Calibration of the column was performed according to five different M_w standards (Pharmacia International Ltd.) in the range of 1000–4000 kDa for Shodex OHpak SB-805HQ column and 10–100 kDa for Shodex OHpak SB-803HQ column. The M_w of oat β-glucan samples were calculated from the calibration curve.

2.2.3. Particle size determination

The mean diameter and polydispersity index (*PDI*) of oat β-glucan samples were conducted using a BI-200SM dynamic light scattering (DLS) system (Brookhaven Instruments Corporation, New York, USA) equipped with a MGL-III model 100 mV He–Ne laser ($\lambda = 532$ nm), a computer-controlled BI-200SM goniometer, and a BI-9000AT digital correlator. Light scattering was monitored at a 90° angle and the temperature of the sample holder was controlled at 25 °C via a recirculating water bath. Sample solutions (1 mg/mL) were carefully filtered through a 0.45 μm membrane directly into a borosilicate glass tube and measured within 5 min. The average particle size of species in the oat β-glucan solution was obtained by the CONTIN model.

2.2.4. Rheology

Rheological properties of oat β-glucan solution (0.5% and 0.75%) were taken using a rotational rheometer (Brookfield Engineering Laboratories model DV-III + ULTRA, USA) with concentric cylinders spindle ULA. The temperature was controlled at 25 °C. A dependence of apparent viscosity on shear rate was observed in controlled rate mode. The shear rate was linearly increased from 10 to 250 s^{-1}. The experimental data were fitted by a power law constitutive equation (Aziznia et al., 2008)

$$\eta = K\gamma^{n-1} \quad (1)$$

where η is the apparent viscosity (Pa s); K is the consistency coefficient (Pa s^n); γ is shear rate (s^{-1}); n is the flow behavior index (dimensionless) describing the divergence from the Newtonian model, $n < 1$ for a shear-thinning fluid and $n = 1$ for a Newtonian fluid.