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Effects of superfine grinding on physicochemical and antioxidant properties of *Lycium barbarum* polysaccharides

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ABSTRACT

The objective of this study was to investigate the effects of superfine grinding on the physicochemical and antioxidant properties of *Lycium barbarum* polysaccharides (LBP). Superfine grinding treatment could decrease the average molecular weight (changed from 834.0, 85.2 and 70.6 to 538.5, 71.2 and 2.1, respectively), the spherical particle's heights (changed from 0.86, 0.59 and 0.51 nm to 0.74, 0.55 and 0.43 nm, respectively) and the spherical particle's diameter (changed from 98.33, 70.67 and 86.33 nm to 88.67, 47.67 and 51.33 nm, respectively) of LBP. After being superfine grinding treated, the ΔH values of LBP changed from −154.6, −136.5 and −105.0 J/g to −220.8, −97.2 and −46.5 J/g, respectively. The IC_{50} of LBP from treated materials on DPPH radical (4.96, 1.98 and 2.97 mg/mL) and ABTS radical (2.25, 0.20 and 0.25 mg/mL) were extremely lower than those of LBP from untreated materials. The results suggested that superfine grinding treatment enhanced LBP's antioxidant activities. Molecular weight and solution behavior were key factors in polymer's antioxidant activities.

1. Introduction

Natural polysaccharides and their conjugates have been used in food and medicine for a long time. The structure and bioactivity mechanism of polysaccharides have been extensively studied, and more and more natural polysaccharides have been applied in therapies (Wang & Fang, 2004). It was demonstrated that some natural polysaccharides were good at preventing oxidative damage in living organism, so could be a potential resource of novel antioxidants (Ge, Duan, Fang, Zhang, & Wang, 2009; Tsiapali et al., 2001; Xu, Liu et al., 2009; Yuan, Zhang, Fan, & Yang, 2008).

Lycium barbarum belongs to the family Solanaceae, which can nourish kidney and liver, brighten eyes, reduce blood glucose and serum lipids, immuno-modulate, reduce senescing, and male fertility-facilitate (Gan & Zhang, 2003; Wang, Wang, Zhang, & Zhang, 2002; Wang, Zhao et al., 2002), has been widely used as a famous traditional Chinese herbal medicine and functional food for more than 2500 years (Shi, Jia, & Dong, 1997; Wen, Chung, Chou, Lin, & Hsieh, 2006). Modern pharmacological studies indicated that *L. barbarum* polysaccharides (LBP), which were the main functional component of *L. barbarum*, possessed a range of bioactivities, including antioxidant properties (Liang, Jin, & Liu, 2011).

Superfine grinding technology is an emerging process technology showing great potential in producing nutraceuticals and functional foods (Chen, Weiss, & Shahidi, 2006). Superfine ground powder refers to small solid particles with the size from 1 nm to 100 μm. The surface of superfine ground powder can undergo some changes, which bring out some outstanding characteristics that crude particles do not possess. Recently, superfine grinding technology drew a great attention in extraction methods of functional components, mainly due to its virtue of saving time, solvent and energy. Furthermore, it considerably enhances the efficiency of the extraction and is friendly to environment. So far, it has been widely employed to extract polysaccharides from different materials with great extraction efficiency and antioxidant property (Hu, Chen, & Ni, 2012; Li, Xia, Wang, & Xie, 2005; Yang, Fang, Wang, & Ma, 2004).

Physicochemical properties of polysaccharides such as chemical composition, molecular weight distribution, thermodynamic property and conformation were significantly influenced by extraction process including microwave-assisted extraction (Zeng, Zhang, Gao, Jia, & Chen, 2012), ultrasonic-assisted extraction (Zhou, Yu, Zhang, He, & Ma, 2012), and superfine grinding technology (Zhao et al., 2010). Usually, antioxidant activity of polysaccharides mainly depends on their structural features including sugar composition, molecular weight, type of glycosidic bond of the main chain, degree of modification of polysaccharides and flexibility configuration of the chains (Lo, Chang, Chiu, Tsay, & Jen, 2011). However, up to now, the effects of superfine grinding treatment on

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the physicochemical properties and antioxidant activities of LBP were unknown yet.

In this study, to explore the influence of superfine grinding treatment on physicochemical properties and antioxidant activities of LBP, several polysaccharide conjugate fractions from superfine grinding treated and untreated *L. barbarum* were obtained by a graded ethanol precipitation. Then their chemical composition, molecular weight distribution, thermodynamic property, conformation and antioxidant activities were determined. And the structure–activity relationships of LBP were also discussed to seek new biological functional principle used in food and pharmaceutical industry.

2. Material and methods

2.1. Materials

Dried fruits of *L. barbarum* were purchased from Shihezi City, XinJiang Province, China. Trifluoroacetic acid (TFA) and the standard monosaccharides (fucose, rhamnose, ribose, arabinose, xylose, mannose, galactose, glucose, sorbose) were purchased from Merck Co. (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO). Dextrans were from Pharmacia Co. (Uppsala, Sweden). 1,1′-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS$^{\bullet+}$) were products of Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were purchased locally and were of analytical grade.

2.2. Superfine grinding treatment of L. barbarum and particle size measurement

The dried *L. barbarum* fruits were milled coarse particles by a disc-mill, which were screened through different sized sieves to separate granulates ($d < 1$ mm); then the uniform coarse particles were ground in an multidimensional swing high-energy nano-ball-milling (CJM-SY-B Qinhuangdao Taiji Ring Nano-Products Co., Ltd., Hebei, China).

Small angle X-ray scattering (SAXS) (Rigaku-3014, Rigaku, Japan) was used for the determination following China National Standard GB/T 13321-2004 (2004). Briefly, sample powders were dispersed in a celloidin–acetone solution and then dried at 20–50 °C to remove acetone completely. Samples were analyzed at 35 kV, 20 mA with Co kα radiation. The scattering angle 2θ was set as 0–3°. A background scattering intensity of multilayer filter was subtracted from the sample intensities for data correction. The mean size (D), median size (d) and distribution spread (B) were calculated using the dividing distribution function (DDF) method according to GB/T 13221:2004.

2.3. Extraction of polysaccharides

Dried fruits of *L. barbarum* were powdered by a beater (conventional grinding method) or a CJM-SY-B type micronizer (superfine grinding method), respectively. Briefly, the dried powder (200 g) were refluxed with 80 g/100 mL ethanol at 80 °C for four times to remove most of the monosaccharides, oligosaccharides, pigments and other low molecular compounds. The organic solvent was volatilized. Then the powder was extracted four times with 1000 mL of distilled water at 85 °C for 95 min. The combined extracts were filtered, vacuum concentrated, precipitated by ethanol, and then centrifuged at 3400 rpm for 15 min. The precipitation was collected, and the proteins in the precipitate were removed using Sevag reagent (Navarini et al., 1999). Then the solution was vacuum freeze-dried and LBP were collected.

2.4. Fractionation of LBP by ethanol-fractional precipitation

One gram of polysaccharides from untreated *L. barbarum* was redissolved in 40 mL of distilled water. Ethanol (95%, v/v) was added slowly to a final ethanol content of 40% (v/v) and kept for 6 h, then the solution were centrifuged at 3400 rpm for 15 min. The precipitation was collected and named as LBP-40. Ethanol was continually added to the supernatant till the ethanol content reached to 50% (v/v) and kept for 6 h. Centrifugation was conducted again, and LBP-50 was obtained. Then LBP-70, LBP-75 and LBP-80 were obtained successively by the same way. Finally, these fractions were obtained and lyophilized (Zha et al., 2009).

Similarly, polysaccharides from superfine grinded *L. barbarum* were then successively sub-fractionated, and LBP-S50, LBP-S60, LBP-S70, LBP-S75, LBP-S80 were obtained. LBP-50, LBP-75, LBP-80, LBP-S50, LBP-S75 and LBP-S80 were used for subsequent experiments because their contents were much higher than other fractions.

2.5. Analysis of chemical components

Neutral sugar content of LBP was analyzed by the modified phenol–sulfuric method using D-glucose as standard (Masuko et al., 2005). The modified carbazole assay was conducted to analyze the uronic acid content with galacturonic acid as standard (Bitter & Muir, 1962). Protein content was analyzed by the coomassie brilliant blue method using bovine serum albumin (BSA) as the standard (Wei, Li, & Tong, 1997).

The composition of neutral monosaccharide in LBP was analyzed as alditol acetates by gas chromatography (Chen, Zhang, Qu, & Xie, 2008). Briefly, 10 mg of polysaccharide samples was hydrolyzed with 1 mL of trifluoroacetic acid (TFA, 2 mol/L) at 120 °C for 4 h, after that, TFA was evaporated to dryness at 60 °C under reduced pressure. Then, 1.5 mL of methanol was added, and the solution was evaporated to dryness again, which were repeated for four times. The hydrolyzed polysaccharide sample (2 mg) with fucose (Fuc), rhamnose (Rha), ribose (Rib), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glu), sorbose (Sorb) as monosaccharide standard, were dissolved in distilled water (0.5 mL), reduced by sodium borohydride ($NaBH_4$, 30 mg) for 1.5 h with oscillation every 30 min, treated with glacial acetic acid (AcOH), and dried. Methanol (1–2 mL) was added to vessel and vacuum dried, and repeated five times to avoid the influence of AcOH, then dried at 105 °C for 15 min in the drying cabinet. Then pyridine and acetic anhydride with the ratio of 1:1 were added and reacted in sealed condition at 100 °C for 1 h. After filtrated with 0.22 μm filter membrane, the derivatives were analyzed by gas chromatography (GC) in an Agilent 6890 system GC (Agilent Technologies, Palo Alto, CA, USA) equipped with flame ionization detector (FID) on OV1701 (30 m × 0.32 mm × 0.5 μm). The samples (3 μL) were injected into GC analyzer. The operation was performed using the following conditions: H_2 40 mL/min, air 450 mL/min, N_2 34 mL/min, injection temperature 250 °C, detector temperature 250 °C, column temperature programmed from 150 °C to 200 °C at 10 °C/min, holding for 10 min at 200 °C, then increasing to 220 °C at 5 °C/min, finally increasing to 240 °C at 1.5 °C/min with a 20 min holding at 240 °C.

2.6. Determination of molecular weight

The molecular weights of *L. barbarum* polysaccharide conjugate fractions were determined by gel permeation chromatography, in combination with a high-performance liquid chromatography (HPLC) instrument (LC-2010A, Shimadzu, Tokyo, Japan) equipped with a refractive index (RI) detector (RID-10A, Shimadzu, Tokyo,