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Separation of water-soluble polysaccharides from *Cyclocarya paliurus* by ultrafiltration process

Jian-Hua Xie [1], Ming-Yue Shen [1], Shao-Ping Nie, Qiang Zhao, Chang Li, Ming-Yong Xie *

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

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ABSTRACT

In this study, ultrafiltration membrane process was employed to separate polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*) to simulate industrial production. Meanwhile, the molecular weight distribution of *C. paliurus* polysaccharides was investigated by gel permeation chromatography. Four fractions were obtained and named as CPPS-A, CPPS-B, CPPS-C and CPPS-D, respectively. CPPS-A and CPPS-B contained approximately 69.5% and 12.7% of polysaccharides, whose molecular weight were in the range of 100–300 kDa and 120 kDa, respectively. CPPS-C was comprised of two polysaccharides with average molecular weight of 40 kDa and 15 kDa. Results showed that ultrafiltration resulted in the removal of parts of small molecule weight polysaccharides, the increase of proportion of high molecule weight ones and the obvious improvement of quality of products. Compared with ethanol precipitation and gel permeation chromatography techniques, ultrafiltration showed many advantages, and also provided theoretical support for industrial manufacturing of *C. paliurus* polysaccharides in separation.

1. Introduction

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*), namely sweet tea tree in China, has been used in Chinese medicine for treatment of hyperglycemia, hyperlipidemia and diabetes mellitus for thousands of years (Kurihara, Asami, Shibata, Fukami, & Tanaka, 2003; Xie, Li, Nie, Wang, & Lee, 2006; Xie et al., 2010a), and was also used as beverages (such as herbal tea) in China (Xie et al., 2006). It has been found that *C. paliurus* contains many potentially bioactive substances, including polysaccharides, proteins, minerals, phenolic compounds, vitamins, saponins, sugars, triterpenoids and amino acids (Xie & Xie, 2008; Xie et al., 2013a, 2013b). As the major active constitute in *C. paliurus* leaves, *C. paliurus* polysaccharides have been shown to contain 23.5% uronic acid and 64.8% neutral sugars (Xie et al., 2010b), which consisted of rhamnose, arabinose, galactose, glucose, mannose and xylose in the molar ratio of 1.00:1.85:3.26:3.12:0.85:0.29 (Xie et al., 2013c). We have reported that the polysaccharides in *C. paliurus* possess a variety of bioactivities, such as antioxidant (Xie et al., 2010b), hypoglycemic (Xie et al., 2006), anticancer (Xie et al., 2013d), antimicrobial (Xie et al., 2012) and immunomodulatory activities (Huang, Nie, Xie, Han, & Xie, 2009).

In recent decades, polysaccharides isolated from plants, animals and fungi have attracted a great deal of attention due to their various biological activities (Nie, Cui, Xie, Phillips, & Phillips, 2013). However, most current studies on polysaccharide put an emphasis on its structure or bioactivities. There have been few reports on membrane separation and purification of polysaccharides (Sheng et al., 2007).

Various methods, such as ethanol precipitation, gel permeation chromatography and ion-exchange chromatography have been employed for separation and purification of polysaccharides. However, these methods possess inherent limitations. The traditional ethanol precipitation used to separate polysaccharides is time-consuming and requires large volumes of organic solvents (Du et al., 2010). The gel filtration chromatography is not only complicated but also expensive (Hong & Choi, 2007). Compared to conventional separation techniques, ultrafiltration membrane process has many advantages, such as low cost, energy saving, high efficiency, and being environmental friendly, having the characteristics of continuous operation at room temperature, and no secondary dissolving-out substances in the separation (Mohammad, Ng, Lim, & Ng, 2012). The ideal separation method should be capable of producing high quantities of polysaccharides and be non-destructive with a shorter separation time (Sheng et al., 2007). Therefore, the use of ultrafiltration process for separation of molecular weight-specific polysaccharides with particular biological activities has

* Corresponding author at: State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, Jiangxi, China. Tel.: +86 791 83969009; fax: +86 791 83969009.
E-mail addresses: jhxie@ncu.edu.cn (J.-H. Xie), xmync@163.com, myxie@ncu.edu.cn (M.-Y. Xie).
[1] These authors contributed equally to this work and are considered as co-first authors.

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received increased attention. In recent years, it has been applied in separation and purification of many biomolecules such as proteins (Cheang & Zydney, 2004; Tsou, Kao, Juin Tseng, & Chiang, 2010), antibiotics (Hernández-Campos, Brito-De la Fuente, & Torrestiana-Sánchez, 2010), peptides (Chabeaud et al., 2009; Liu et al., 2013; Segura Campos, Chel Guerrero, & Betancur Ancona, 2010), polyphenols (Nawaz, Shi, Mittal, & Kakudac, 2006; Prodanov et al., 2008) and polysaccharides (Du et al., 2010; Sun, Qi, Xu, Juan, & Zhe, 2011). To our knowledge, the suitability of ultrafiltration membrane for direct separation of *C. paliurus* polysaccharides has not been reported. In this study, ultrafiltration membrane process was successfully employed for the separation of *C. paliurus* polysaccharides and four fractions were obtained.

2. Materials and methods

2.1. Materials and chemicals

The leaves of *C. paliurus* were collected in Xiushui County, Jiangxi Province, China. A voucher specimen was deposited at State Key Laboratory of Food Science and Technology, Nanchang University, China. The leaves were air dried and ground into a fine powder in a mill.

Standard dextrans, namely, T-10, T-40, T-70, T-500 and T-2000 (10, 40, 70, 500 and 2000 kDa) were purchased from Pharmacia Biotech (Uppsala, Sweden). Aqueous solutions were prepared with ultra pure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and solvents used were of analytical reagent grade unless otherwise specified.

2.2. Extraction of crude C. paliurus polysaccharides

Extraction of water-soluble *C. paliurus* polysaccharides was carried out by the procedure reported in our previous study (Xie et al., 2011). Briefly, the dried leaves powder (500 g) were extracted with 5000 mL of 80% ethanol for 24 h at room temperature to remove the interference components such as monosaccharide, disaccharide and polyphenol in the samples. Then the pretreated samples were dried at 45 °C for 8 h. The dried residues were extracted with 5000 mL ultra pure water at 80 °C for 2 h. After filtered, the residues were extracted again with 2500 mL ultra pure water at the same temperature for 2 h. Then the extracts were combined and filtered through glass wool and centrifuged at 8400 × *g* for 15 min to separate the supernatant and the residue. The supernatant was kept in a refrigerator for membrane separation.

2.3. Separation of C. paliurus polysaccharides by ultrafiltration

The *C. paliurus* polysaccharides solution was pretreated through a membrane with glass wool to avoid the fouling of the ultrafiltration membranes. The ultrafiltration process was performed on an ultrafiltration system with different membranes. The polysaccharides solution was pumped to the membrane surface (tangential flow) and the filtrate was collected while the retentate was directed back to the recycle tank. All experiments were carried out with a hollow fiber ultrafiltration module (Suntar Membrane Technology Co., Ltd., Xiamen, China) with different nominal molecular weight cut-offs (300 kDa, 100 kDa and 6 kDa) polysulfone hollow fiber membranes (effective membrane area: 0.15 m^2). The polysaccharide solutions were pumped by a peristaltic pump (PP-05, Chenghe Co., Ltd., Changzhou, China). The schematic of ultrafiltration set-up is shown in Fig. 1.

The *C. paliurus* polysaccharide solution prepared by centrifugation (8400 × *g* for 15 min) was passed through the membrane starting with the largest membrane cartridge (300 kDa). The retentate and permeate were collected separately, and the retentate

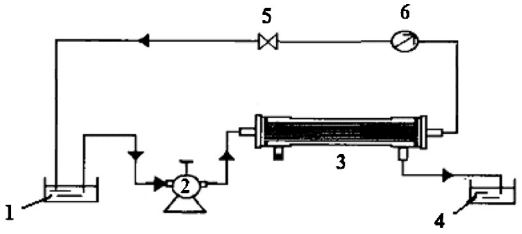

Fig. 1. Experimental set-up of ultrafiltration with hollow fiber. (1) sample liquid pool; (2) peristaltic pump; (3) ultrafiltration module; (4) permeated liquid pool; (5) regulating valve; (6) septum manometer.

was recirculated into the feed until maximum permeate yield was reached, as indicated by a decreased permeate flow rate. Permeate from the 300 kDa membrane was then filtered through the 100 kDa membrane with recirculation until maximum permeate yield was reached, and the 100 kDa permeate was then processed with the 6 kDa membrane as mentioned above (Fig. 2).

Four fractions (CPPS-A, CPPS-B, CPPS-C and CPPS-D) obtained from *C. paliurus* polysaccharide solution were concentrated to small volumes and also precipitated with four volumes of anhydrous ethanol for 12 h at 4 °C. The four precipitates were dissolved in ultra pure water, and freeze-dried respectively.

2.4. Molecular size distribution of C. paliurus polysaccharides

The molecular weight of the polysaccharide fractions was determined by high-performance gel permeation chromatography (HPGPC) with a Waters HPLC apparatus (UK6 injector and 515 HPLC pump, Waters, Milford, MA, USA) equipped with an Ultrahydrogel TM-500 column (300 × 7.8 mm), a Waters 2410 refractive index detector (Waters Corp., USA) and a Waters 2487 UV detector connected in series with a Millennium32 workstation.

The elution was carried out with Milli-Q water at a flow rate of 0.6 mL/min. All sample solutions were filtered through a 0.45 μm membrane filter prior to analysis. The injection volume of standards and samples was 20 μL, and the running time was 30 min. Retention times from HPGPC were recorded and time variations were dependent on the average molecular weight of the dextran standards. Glucose and dextran standards with different molecular weights (10, 40, 70, 500 and 2000 kDa) were prepared using the same solvent as the mobile phase. A standard curve with the retention time (t) plotted against the logarithm of their respective molecular weights (Mw). The equation of the standard curve was: $\log M_w = 8.7928 - 0.3062t$ (where M_w represented the molecular weight, while t represented retention time) with a correlation coefficient of 0.991 (Xie et al., 2010b).

2.5. Determination of polysaccharides content

The polysaccharide content of the four fractions was determined according to the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with some modifications. Briefly, 2 mL of diluted solution containing polysaccharides, 1.0 mL of 5% (w/w) phenol was added in the tubes and mixed up by shaking quickly. Then 5 mL of sulfuric acid was added and mixed, and boiled in the boiling water for 20 min, and the tubes were cooled down to room temperature by tap water, and finally 2 mL ultra pure water was added and mixed. The absorbance of the solution was measured at 488 nm with an ultraviolet-visible spectrophotometer (TU-1900, Pgenenal, Beijing, China) against the same mixture, without adding the sample as a blank. The calibration curve of glucose ($y = 5.441x + 0.025$, where y is the absorbance value of sample, x is the polysaccharide concentration, $R^2 = 0.995$) is shown in Fig. 3.