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Enzyme-assisted extraction of polysaccharides from *Dendrobium chrysotoxum* and its functional properties and immunomodulatory activity☆

Li-Hua Pan [a, b], Jian Wang [a], Xing-Qian Ye [b, **], Xue-Qiang Zha [a], Jian-Ping Luo [a, *]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China
[b] School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

Dendrobium chrysotoxum has long been used as a health-promoting and therapeutic tonic in China. The polysaccharides from *D. chrysotoxum* (DCP) are considered to play an important role in its medical effects. The present study aimed at optimizing the technology for enzyme-assisted extraction (EAE) of DCP and at investigating the physicochemical characteristics and functional properties of DCP-E obtained by EAE and DCP-H obtained by hot water extraction (HWE). Based on single factor tests and orthogonal experiments of extraction pH, extraction temperature, cellulase amount and extraction time, the optimum conditions for CP-E were extraction pH value of 5.5, extraction temperature of 40 °C, cellulase amount of 10 g/L, extraction time of 3.0 h and solid/liquid ratio of 1:25. Under these conditions, the yield of DCP-E was 8.41 g/100 g dry weight, which was 1.25 times of that of DCP-H. As compared to DCP-H, DCP-E displayed an increased purity, a decreased molecular weight and relative viscosity as well as a changed monosaccharide compositions. Foam stabilization and splenic cell proliferation experiments showed that DCP-E had higher cell proliferation rate while DCP-H had better foam stabilization activity. These results indicate that different extraction methods would influence the physicochemical characteristics and subsequently functional properties of polysaccharides, suggesting that the suitable extraction method should be selected according to the application intention of polysaccharides.

1. Introduction

Polysaccharides from edible resources not only have been widely used as emulsifiers, stabilizers and thickeners in the food industry (Harris & Smith, 2006), but also have gained considerable attentions as immunomodulators, antioxidants and hypoglycemic agents in functional food and medicine fields because the growing body of scientific evidence suggests the health benefits of these natural biomacromolecules.

To obtain polysaccharides from edible resources for application, different extraction technologies including hot water extraction (HWE) (Bendjeddou, Lalaoui, & Satta, 2003), enzyme-assisted extraction (EAE) (Yin, You, & Jiang, 2011), ultrasonic-assisted extraction (Chen et al., 2012) and microwave-assisted extraction (Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011) have been used. In general, HWE is a traditional technology that is widely used for polysaccharide extraction although it is associated with low yield, long extraction time and high temperature (Yin et al., 2011). In contrary, EAE has been employed as a powerful method for the acceleration of extraction process and the improvement of extraction yield due to the advantages of environmental compatibility, high efficiency and simple operation processes (Puri, Sharma, & Barrow, 2012). With EAE, the enzyme degradation of cell walls is of major importance for facilitating the polysaccharides inside cells to overcome cell wall limitation into the solvent. Although EAE has been used for the extraction of polysaccharides from different plants, to the best of our knowledge, there are very few reports on the extraction of polysaccharides from *Dendrobium chrysotoxum* using EAE technology.

Dendrobium chrysotoxum Lindl, which is widely distributed in Yunnan, Guangxi and Sichuan provinces of China (Ng et al., 2012), has long been used in traditional medicines for the treatment of asthma and the increasing secretion of saliva (Qu, Liu, & Shang, 2011), in addition to being used as a health-promoting food.

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* Corresponding author. Tel.: +86 551 62901539.
** Corresponding author. Tel.: +86 571 88982155.
E-mail addresses: psu@zju.edu.cn (X.-Q. Ye), jianpingluo@hfut.edu.cn (J.-P. Luo).

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Recent research showed that crude polysaccharides isolated from this plant using HWE method had antioxidative and anti-hyperglycemic activities (Li, Qing, Fang, Liu, & Chen, 2009; Pan et al., 2014; Sun, Wang, & Ye, 2013; Zhao, Son, Kim, Jiang, & Lee, 2007). However, it is unclear whether the polysaccharides from *D. chrysotoxum* (DCP) express the potential of immunological activity. At the same time, it is also unclear whether different extraction methods change the physicochemical characteristics and functional properties of DCP.

Because the effectiveness of enzyme-assisted extraction varies greatly from one material to others (Zuorro, Fidaleo, & Lavecchia, 2011), we investigated the effect of EAE on *D. chrysotoxum* polysaccharides in order to obtain the optimum EAE conditions for the preparation of this polysaccharides. To compare scientifically the physicochemical characteristics and functional properties of DCP obtained by EAE and HWE, the chemical compositions, rheological properties, foam stabilization and immunological activity of polysaccharides obtained by EAE under the optimal extraction conditions were studied in comparison with those obtained by HWE in this study.

2. Materials and methods

2.1. Materials and chemicals

D. chrysotoxum was collected from Xishuangbanna (Yunnan, China) in April, 2011. Male BALB/C mice (18 ± 2 g) were obtained from the Experimental Animal Center, Anhui Medical University of China (Hefei, China). All solvents were analytical grade and from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (MO, USA). Sugar and Dextran T series standards were from Sigma–Aldrich (MO, USA). Cellulase (15,000 U/g) was purchased from Imperial Jade Bio-products Co. Ltd. (Beijing, China).

2.2. Polysaccharide extraction

Fresh stems (25 g) of *D. chrysotoxum* were air-dried at 30 °C for 7 days, crushed into powder and passed through a sieve with 100 um pores (5 g), pretreated with acetone in a Soxhlet system for 24 h to remove most of the pigments and fats. The pretreated powder was extracted by EAE or HWE in a thermostat water bath (Jintan Jingda Instrument Factory, Jintan, China) at 70 °C for 4.0 h with the solid/liquid ratio of 1:20 using HWE method or under the parameters specified in Section 2.3 using EAE method. Extracts were filtered through gauze and Whatman grade 54 filter paper (Whatman, UK) and centrifuged at 8135 g for 10 min by a CT15RT model high speed refrigeration centrifuge (Shanghai Tianmei Biochemical Equipment Engineering Co. Ltd., Shanghai, China). The obtained supernatant was concentrated by a rotary evaporator (Heidolph, Germany) under vacuum pressure at 60 °C and precipitated by adding 4 times volumes of anhydrous ethanol. The precipitate was collected by centrifugation at 8135 g for 10 min, dissolved in distilled water, deproteinized five times using Sevag method (Staub, 1965), dialyzed against distilled water for 48 h and lyophilized to give DCP. The products of DCP obtained by EAE and HWE were named as DCP-E and DCP-H, respectively.

The polysaccharide content was measured by the phenol–sulphuric acid method using D-glucose as a standard at 490 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). In briefly, 2 mL of DCP solution was pipetted into a colorimetric tube, and 0.05 mL of 80mL/100mL phenol was added and mixed. Then 5 mL of concentrated sulfuric acid was added rapidly, directly against the liquid surface. After 10 min, the tubes were shaken and kept for 10–20 min in a water bath at 25 °C, and the absorbance was measured at 490 nm. Blanks were prepared using distilled water. The amount of polysaccharides was determined according to the standard curve of D-glucose and the polysaccharides yields were calculated by the following equation: extraction yield (g/100 g dry weight) = weight of lyophilized DCP (g) ÷ weight of dried materials (g) × 100.

2.3. Parameter optimization of EAE

Extraction process and yield of polysaccharides were described in Section 2.2. Firstly, the effects of four single factors, including pH values (3.0–6.0), temperature (30–70 °C), cellulase amount (5–35 g/L) and time (1–4 h), on the extraction yield of DCP-E were investigated. Then, an orthogonal $L_9(3^4)$ test design was applied to optimize extraction parameters for EAE (Table 1).

2.4. Analysis of total carbohydrates, total proteins and uronic acids

The contents of total carbohydrates (TC), total proteins (TP) and uronic acids (UA) in DCP-E and DCP-H were determined by phenol–sulphuric acid method (Dubois et al., 1956), Kjeldahl nitrogen determination method with an M0825 model auto-analyzer (Behr, Germany) (Kjeldahl, 1883) and m-hydroxylbiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), respectively.

2.5. Determination of molecular weight

Molecular weight (Mw) of DCP-E and DCP-H was determined by HPGPC system with TSK G4000 PWxl column (7.8 × 300 mm) and TSK G5000 PWxl (7.8 × 300 mm) column in tandem (Waters Co. Ltd., USA). The performance conditions were column temperature of 30 °C, injection volume of 20 μL (10 g/L, w/v) and flow rate of 0.5 mL/min with double-distilled water. The molecular weight was calculated by reference to the standard curve of Dextran T series (8000–1,300,000) (Zha, Luo, Luo, & Jiang, 2007).

2.6. Analysis of monosaccharide compositions

GC was used for the analysis of monosaccharide compositions according to the method of Duan, Zheng, Dong, and Fang (2004).

Table 1
Orthogonal tests of enzyme-assisted extraction for DCP-E ($n = 3$).

No.	A/pH	B/temperature	C/cellulase	D/time	Extraction yield/(g/100 g DW)
1	1 (4.5)	1 (30 °C)	1 (10 g/L)	1 (2.5 h)	7.57 ± 0.38
2	1	2 (40 °C)	2 (15 g/L)	2 (3.0 h)	7.17 ± 0.36
3	1	3 (50 °C)	3 (20 g/L)	3 (3.5 h)	5.80 ± 0.31
4	2 (5.0)	1	2	3	7.14 ± 0.36
5	2	2	3	1	6.36 ± 0.32
6	2	3	1	2	8.03 ± 0.41
7	3 (5.5)	1	3	2	6.27 ± 0.32
8	3	2	1	3	8.32 ± 0.43
9	3	3	2	1	7.35 ± 0.37
K_1	20.54	20.98	23.92	21.28	
K_2	21.53	21.85	21.66	21.47	$T = 64.01 \pm 0.36$
K_3	21.94	21.18	18.43	21.26	$x = 7.11 \pm 0.12$
R	1.40	0.87	5.49	0.21	
k_1	6.85	6.99	7.97	7.09	
k_2	7.18	7.28	7.22	7.16	
k_3	7.31	7.06	6.14	7.09	
r	0.47	0.29	1.83	0.07	

DCP-E, the polysaccharides from *D. chrysotoxum* extracted by the method of enzyme-assisted extraction.
T, total extraction yield of 9 tests from No.1 to No.9.
x, average of the total extraction yield of 9 tests from No.1 to No.9, $x = T \div 9$.
K_i, total extraction yield of the same factor and level in the experiments, $i = 1, 2,$ or 3.
R, range analysis value.
k_i, average of the total extraction yield of the same experiment level, $i = 1, 2,$ or 3; r, range analysis average value.