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Response surface optimization of ultrasound-assisted enzymatic extraction polysaccharides from *Lycium barbarum*

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ABSTRACT

In this study, an efficient ultrasound-assisted enzymatic extraction procedure for the water-soluble polysaccharides from the fruit of *Lycium barbarum* was investigated and optimized. Response surface methodology (RSM) based on a three-level four-factor Box Behnken Design (BBD) was employed to optimize the extraction conditions inlcluding extraction time, ultrasonic output power, cellulose concentration and extraction temperature. The experimental data were adequately fitted into a second-order polynomial model. The optimized conditions were as follows: extraction time 20.29 min, ultrasonic output power 78.6 W, cellulose concentration 2.15%, extraction temperature 55.79 °C. Under these conditions, the experimental yield of polysaccharides was 6.31 ± 0.03%, which matched with the predictive yield of 6.32% well.

1. Introduction

Fruit from *Lycium barbarum* in the family of Solanaceae has been used as a kind of traditional Chinese herbal medicine for thousands of years. Nowadays it is widely used as popular functional food (Li, Li, & Zhou, 2007). Modern studies indicated that polysaccharides from *L. barbarum* have a range of biologic activities, including enhancing immunity, protecting hepatic function, reducing blood glucose, reducing myocardial injury, antivirus, anti-tumor and anti-aging (Chan et al., 2007; Wang et al., 2010). In order to realize all its promising applications, there is an increase in demand to supply the market with high-quality *Lycium barbarum* polysaccharides (LBP). Although there were many researches about biological activity of LBP, little attention was paid to the extraction of LBP.

There are many factors that can affect the yield of LBP, such as the different types of *L. barbarum* and the different extraction methods et al. Among these factors, the relation of the types of *L. barbarum* to the polysaccharides content was quite intimate. Inferior raw material always leads to lower polysaccharides content (Ye, 2009). We selected inferior *L. barbarum* in our study due to considering the industrial production of LBP.

Enzyme-assisted extraction, which is considered as a mild, efficient and environmental friendly extraction method, has been used in the extraction of various kinds of compounds recently (Li, Smith, & Hossain, 2006; Moura et al., 2008; Santamaria et al., 2000). The addition of specific enzymes such as celluloses and proteases during the extraction process can promote the release of intracellular contents by breaking the cell wall and lipid bodies (Moura et al., 2008). Compared with conventional extraction procedures, Enzyme-assisted extraction method has some advantages such as mild extraction condition and a usually higher extraction yield.

Compared with the conventional extraction method, ultrasound-assisted extraction (UAE), which combines ultrasound and traditional solvent extraction, has been proved to improve the extraction efficiency by increasing the yield and shortening the extraction time (Chua et al., 2009; Dudiey Thompson, 1955; Romdhane, 2002). Recently, many researches were conducted to indicate the application of ultrasound in diverse extraction processes (Chen et al., 2007; Chua et al., 2009; Rodríguez-González, Femenia, Minjares-Fuentes, & González-Laredo, 2012). Moreover, previous studies have shown that the introduction of ultrasonic energy during enzymatic treatment have resulted in significant improvement (Yachmenev Val, Blanchard Eugene, & Lambert Allan, 1998; Zhang, Fu, & Liang, 2008). So, the combinational usage of enzyme and UAE during the extraction process would be more effective probably. Up to our best knowledge, this method has not been reported in plant materials extraction previously.

Alternatively, RSM is a powerful technique that explores the relationships and interactions among multiple variables by reducing experimental trails. Until now, RSM has been successfully

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Table 1
Box–Behnken design matrix (in coded level of four variables) and response values for the yield of LBP.

Number	X_1 [a]	X_2 [b]	X_3 [c]	X_4 [d]	Polysaccharide yield (%)
1	−1 (10)	−1 (60)	0 (2.0%)	0 (60)	4.63
2	−1	1 (100)	0	0	4.47
3	1 (30)	−1	0	0	4.92
4	1	1	0	0	4.54
5	0 (20)	0 (80)	−1 (1.5%)	−1 (50)	4.72
6	0	0	−1	1 (70)	5.60
7	0	0	1 (2.5%)	−1	6.14
8	0	0	1	1	4.66
9	−1	0	0	−1	5.20
10	−1	0	0	1	4.92
11	1	0	0	−1	5.26
12	1	0	0	1	5.00
13	0	−1	−1	0	4.96
14	0	−1	1	0	5.29
15	0	1	−1	0	4.79
16	0	1	1	0	4.74
17	−1	0	−1	0	4.47
18	−1	0	1	0	4.96
19	1	0	−1	0	4.68
20	1	0	1	0	4.99
21	0	−1	0	−1	5.21
22	0	−1	0	1	5.43
23	0	1	0	−1	5.30
24	0	1	0	1	4.79
25	0	0	0	0	6.27
26	0	0	0	0	6.24
27	0	0	0	0	6.30

[a] Extraction time (min).
[b] Ultrasonic output power (W).
[c] Cellulose concentration (wt.% of *L. barbarum* powder).
[d] Temperature (°C).

applied in the optimization of conditions in food and pharmaceutical research frequently (Baş & Boyacı, 2007; Ghasemlou, Khodaiyan, Jahanbin, Gharibzahedi, & Taheri, 2012).

In the current study, the ultrasound-assisted enzymatic extraction (UAEE) of LBP was firstly investigated. A three-level four-factor BBD was employed combining with RSM to optimize the extraction conditions for obtaining the maximum yield of LBP.

2. Materials and methods

2.1. Materials and chemicals

Fruits of *L. barbarum*, family Solanaceae, were purchased from an herb market in Tianjin, China. Samples were ground and passed through 100 mesh screen.

Cellulose from *Trichoderma viride* (11000 U/mg) was supplied by Sigma Chemical Company. D-glucose was obtained from Tianjin Zhong Ao Tian Yuan Company, and the other chemicals were purchased from Tianjin Qian Cheng Wei Ye Company. All chemicals were reagent grade or better.

2.2. Methods

2.2.1. Instrument

The UAEE was performed in an ultrasound cleaning bath (KQ2200DB, Kunshan ultrasound instrument Co. Ltd., Jiangsu Province, China) working at a frequency of 40 kHz and a ultrasound input power of 40–120 W with a usable capacity of 3 L.

2.2.2. UAEE procedure

The powder of *L. barbarum* (20 g) was double extracted with petroleum ether at 90 °C for 3 h each time to remove lipids, some colored materials, and oligosaccharides under reflux in the Soxhlet set. After being vacuum dried at 60 °C for 12 h, the defatted powder (1 g) was mixed with 30 mL of cellulose solution at the given concentration and fixed pH 4.6 in a 100 ml conical flask (Zhang, Jia, Liu, Wu, & Ran, 2011). This mixture was ultrasonicated at the designed temperature, ultrasonic output power and extraction time. The extracting solution was concentrated, and then precipitated by adding ethanol (12 h, 4 °C) to a final concentration of 80% (v/v). The precipitate was collected and dried to obtain crude polysaccharides. The polysaccharides content was measured by phenol-sulfuric acid method using D-glucose as a standard (Dubios, Gilles, Hamilion, Rebers, & Smith, 1956). The percentage of LBP extraction yield (%) was calculated with the formula of y (%) = $c/w \times 100\%$, where c was the content of polysaccharides, and w represented dried sample weight.

2.3. Box–Behnken design and statistical analysis

On the basis of single-factor experiments, RSM was used to further optimize the ultrasound-assisted enzymatic extraction conditions of LBP. A BBD with four independent variables (X_1, extraction time; X_2, ultrasonic output power; X_3, cellulose concentration; X_4, temperature) at three levels was performed. The range of independent variables and their levels of the independent variables and the results of whole design consisted of 27 experimental points carried out in random order were presented in Table 1, which were based on the results of preliminary experiments. For statistical calculation, each variable was coded at three levels: −1, 0, 1. The variables were coded according to the formula as follows:

$$x_i = \frac{X_i - X_0}{\Delta X}. \quad (1)$$

where x_i was a coded value of the variable; X_i was the actual value of variable; X_0 was the actual value of the X_i on the center point; and ΔX was the step change value.

From Table 1, three replicates (treatment 25–27) at the center of the design were used to allow for estimation the stability and variability (Ye & Huang, 2012). All trials were performed in triplicate.