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Short communication

Optimization of enzyme-assisted extraction of the *Lycium barbarum* polysaccharides using response surface methodology

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ABSTRACT

In this study, an efficient enzyme-assisted extraction procedure was developed and optimized for the extraction of polysaccharide from *Lycium barbarum*. The concentrations of papain and cellulose were optimized by an orthogonal experimental design and the optimal results were cellulose concentration 2.0% (wt.% of *L. barbarum* powder) and papain concentration 1.0%. The response surface methodology (RSM) based on Box Behnken Design (BBD) was employed to further optimize the extraction conditions which included extraction time, extraction temperature and pH. The optimal conditions were as follows: extraction time 91 min, extraction temperature 59.7 °C and pH 5.0. Under these conditions, the experimental yield was 6.81 ± 0.10%, which was well matched with the value predicted by the model.

1. Introduction

Lycium barbarum L. in the family Solanaceae has been used as a kind of traditional Chinese herbal medicine for thousands of years (Li, Ma, & Liu, 2007). *L. barbarum* polysaccharide (LBP), the main bioactive component of *L. barbarum*, has a large variety of bioactivities, such as enhancing immunity, anti-tumor, protecting hepatic function, anti-aging, antivirus, reducing neuronal damage, and lowering blood glucose (Chan et al., 2007; Wang et al., 2010). In order to realize all its promising applications, there is a strong need to supply the market with high-quality LBP. However, compared with the numerous studies of LBP biological activity, little attention was devoted to extraction of LBP.

Hot-water technology is the traditional extraction method for polysaccharides, and has been widely investigated (Hou & Chen, 2008; Sun, Liu, & Kennedy, 2010; Wang, Luo, & Ena, 2007). Nonetheless, this technology usually requires long extraction time and high temperatures. Recently, enzyme-assisted extraction, which is considered as a mild, efficient and environmentally friendly method, has been employed to extract different compounds from plants, and has been proved to be effective in improving the yield of the target component (Li, Smith, & Hossain, 2006; Moura et al., 2008; Santamaria et al., 2000).

The objective of this study was to investigate the extraction process of LBP with compound enzymes (cellulase and papain), and further to optimize the extraction conditions for the yield of LBP by response surface methodology (RSM). To the best of our knowledge, there were no reports of systematic studies of enzyme-assisted extraction process of LBP with compound enzymes.

2. Materials and methods

2.1. Materials

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

Cellulase from *trichoderma viride* (11,000 U/mg), and Papain (P3250, 0.5–2 U/mg) were 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. Extraction 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 extracted with 30 ml of compound enzymes solution at the given concentration in a designed temperature, extraction time and pH. The extract was concentrated, and then precipitated by adding ethanol (12 h, 4 °C) to give a final concentration of

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Table 1
Results and analysis of orthogonal experiment.

No.	(A) Cellulose concentration (wt.% of *L. barbarum* powder)	(B) Papain concentration (wt.% of *L. barbarum* powder)	Polysaccharide yield (%)
1	1 (1.5%)	1 (0.5%)	4.24
2	1	2 (1.0%)	5.17
3	1	3 (1.5%)	4.29
4	2 (2.0%)	1	6.03
5	2	2	6.64
6	2	3	6.05
7	3 (2.5%)	1	5.47
8	3	2	5.68
9	3	3	5.38
K_1	13.7	15.74	
K_2	18.72	17.49	
K_3	16.53	15.72	
R	5.02	1.77	

Table 2
Independent variables and their levels used in the response surface design.

Independent variables	Factor levels		
	−1	0	1
X_1: extraction time (min)	60	90	120
X_2: temperature (°C)	50	60	70
X_3: pH	3.6	4.6	5.6

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 (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The percentage LBP extraction yield (%) was calculated with the formula of y (%) = $c/w \times 100\%$, where c was the polysaccharides content of extraction, and w represented dried sample weight (1 g).

2.2.2. Orthogonal test design of compound enzymes concentrations

An orthogonal $L_9(3)^4$ test design was applied to investigate the optimal concentrations of cellulase and papain with the following extraction conditions: pH 4.6, temperature 60 °C, and extraction time 90 min. As seen from Table 1, the extraction experiment was carried out with 2 factors and 3 levels, and the range of each factor was based on the results of preliminary single-factor experiments. The yield (%) of LBP was the dependent variable and was obtained following the method in Section 2.2.1.

2.2.3. Box Behnken design and statistical analysis

On the basis of orthogonal test of compound enzymes concentrations, RSM was used to further optimize the enzyme-assisted extraction conditions of LBP. A BBD with three independent variables (X_1, extraction time; X_2, temperature; X_3, pH) at three levels was performed. The coded and uncoded (actual) levels of the independent variables are presented in Table 2, which were based on the results of preliminary experiments. As seen from Table 3, the whole design consisted of 17 experimental points, and the experiment was carried out in random order. All trials were performed in triplicate. The extraction yield was treated as response. SAS (Version 9.2, USA) software package was used to generate the experimental design, statistical analysis and regression model.

Data from BBD were analyzed by multiple regressions to fit the following quadratic polynomial model:

$$y_k = b_{k0} + \sum_{i=1}^{3} b_{ki}x_i + \sum_{i=1}^{3} b_{kii}x_i^2 + \sum_{i<j=2}^{3} b_{kij}x_ix_j \quad (1)$$

Table 3
Box Behnken design matrix (in coded level of three variables) and response values for the yield of LBP.

Number	x_1	x_2	x_3	Polysaccharide yield (%)
1	−1	−1	0	5.89
2	−1	1	0	5.57
3	1	−1	0	6.03
4	1	1	0	5.37
5	−1	0	−1	4.24
6	−1	0	1	5.97
7	1	0	−1	4.53
8	1	0	1	6.06
9	0	−1	−1	4.56
10	0	−1	1	5.65
11	0	1	−1	3.54
12	0	1	1	6.16
13	0	0	0	6.64
14	0	0	0	6.74
15	0	0	0	6.51
16	0	0	0	6.74
17	0	0	0	6.69

y_k was the response function; b_{k0}, b_{ki}, b_{kii}, and b_{kij} represented the constant regression coefficients of the model, and x_i and x_j were the independent variables.

3. Results and discussion

3.1. Orthogonal analysis of compound enzymes concentrations

In the enzyme-assisted extraction process, various parameters played a role in increasing extraction yield of LBP. In order to learn more about a range of factors which affect the process and reduce number of experimental trials, an orthogonal test design was applied to obtain the optimal compound enzymes concentrations, and then extraction time, temperature and pH were further optimized using RSM.

Orthogonal analysis of results of $L_9(3)^4$ was showed in Table 1. The results indicated the influence (R) of extraction factors on the extraction yield was $R_A > R_B$. The optimal combination parameters were *A*2*B*2, namely, cellulose concentration (2.0%) and papain concentration (1.0%). In these conditions, the maximum extraction yield of 6.64% was achieved.

3.2. Optimization of the procedure by RSM

The design matrix and the corresponding results of RSM experiments were shown in Table 3. The mathematical model describing the extraction yield of LBP (y) as a function of the coded independent variables in the selected ranges was given by the following equation:

$$y = 6.664 + 0.04x_1 - 0.18625x_2 + 0.87125x_3 - 0.36325x_1^2 - 0.085x_1x_2 - 0.58575x_2^2 - 0.05x_1x_3 + 0.3825x_2x_3 - 1.10075x_3^2 \quad (2)$$

After the response surface regression procedure, the value of the determination coefficient ($R^2 = 0.9923$) showed a good fit to the model and indicated that only 0.77% of the total variations were not explained by the model. The value of the adjusted determination coefficient (Adj. $R^2 = 0.9692$) also confirmed that the model was highly significant. A very low value of coefficient of the variation (C.V.) (2.24%) clearly indicated a very high degree of precision and a good deal of reliability for the experimental values.

The significance of each coefficient of Eq. (2) was determined using P-value (Table 4). The smaller was the value of P, the more

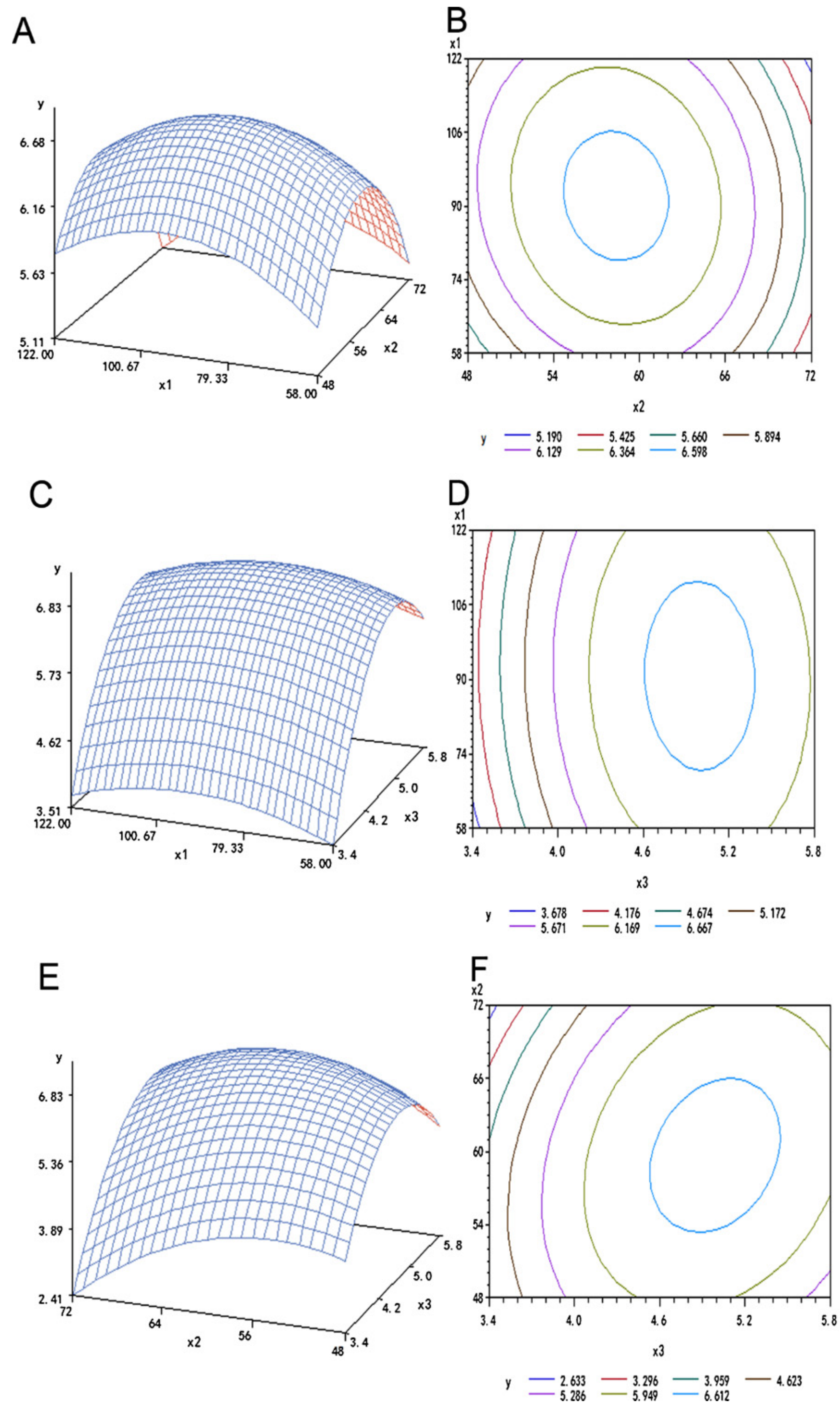

Fig. 1. Response surface plots (A, C, and E) and contour plots (B, D and F) showing the effects of variables (X_1: extraction time, min; X_2: temperature, °C; X_3: pH) on extraction yield of LBP (y).

significant was the corresponding coefficient (Hou & Chen, 2008). It can be seen that the linear coefficients (x_1, x_2, x_3), quadratic term coefficients (x_1^2, x_2^2, x_3^2) and cross product coefficient ($x_2 \times x_3$) were significant, with very small P values ($P < 0.05$). The results indicated that the extraction time, temperature and pH were all significantly correlated with the LBP extraction yield.

The graphical representations of the regression Eq. (2), and the contour plots are presented in Fig. 1. Two variables within the experimental range were depicted in one 3D surface plot while the other variable was kept constant at zero level. Different shapes of the contour plots indicated different interactions between the variables. Elliptical contours were obtained when there was a perfect interaction between the independent variables while circular contour plot indicated otherwise (Guo, Zou, & Sun, 2010). As shown in Fig. 1(A) and (B), the extraction yield of LBP increased with increase of temperature (x_2) from 50 to 59.66 °C, but beyond 59.66 °C, extraction yield of LBP decreased with increasing temperature as higher temperatures reduced the enzyme activity. When temperature was set, extraction yield of LBP was also found to increase with increase of extraction time (x_1) from 60 to 90.97 min, and then

Table 4
Test of significance for regression coefficients.

Effect	Estimate	Stand error	*t*-Ratio	*P*-value
x_1	0.04	0.020353	4.85	0.0019[a]
x_2	−0.18625	0.082443	6.48	0.0003[a]
x_3	0.87125	0.714863	12.38	<0.0001[a]
$x_1 \times x_1$	−0.36325	0.000068995	−5.85	0.0006[a]
$x_1 \times x_2$	−0.085	0.000212	−1.33	0.2239
$x_1 \times x_3$	−0.05	0.002124	−0.78	0.4583
$x_2 \times x_2$	−0.58575	0.000621	−9.43	<0.0001[a]
$x_2 \times x_3$	0.3825	0.006371	6.00	0.0005[a]
$x_3 \times x_3$	−1.10075	0.062095	−17.73	<0.0001[a]

[a] Significant coefficient ($P < 0.05$).

there was a little decrease when the extraction time continued to increase. From Fig. 1(C) and (D), it indicated that extraction yield of LBP increased rapidly when the pH (x_3) increased from 3.6 to 4.99, and then began to decrease. In Fig. 1(E) and (F), maximum extraction yield of LBP (6.83%) was achieved when extraction temperature and pH were 59.66 °C and 4.99, respectively.

It could be concluded that the optimal extraction conditions of LBP were extraction time 90.97 min, extraction temperature 59.66 °C, pH 4.99, and the model predicted a maximum response of 6.84%. Among the three extraction parameters studied, pH was the most significant factor to affect the extraction yield of LBP, followed by temperature and extraction time.

The suitability of the model equation for predicting the optimum response values was tested by confirmation experiments. The experiment yield of LBP was 6.81% (±0.10%), which was not significantly different from the predicted value of 6.84%. These data proved that the model designed in this study was valid.

4. Conclusion

Enzyme-assisted extraction was a green and efficient extraction technique that could be used to improve the extraction yield of LBP. The optimal compound enzymes concentrations (cellulose concentration 2.0%, papain concentration 1.0%) were obtained by an orthogonal test design. Then extraction time, temperature and pH were chosen to further optimize LBP extraction by RSM. These optimal conditions were as the following: extraction time 91 min, extraction temperature 59.7 °C and pH 5.0. Under the optimal conditions, the experiment extraction yield of LBP was 6.81 ± 0.10%, which was agreed closely with the predicted value.

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