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Research Article

Application of Box-Behnken Design to the Hot-Water Extraction of Polysaccharide Fraction of *Bletilla striata*

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Abstract: The quantitative effects of extraction temperature, extraction time and ratio of solid to liquid on yield of *Bletilla striata* polysaccharides were investigated using Response Surface Methodology (RSM). The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods. By solving the regression equation and also by analyzing the response surface contour plots, the optimal polysaccharides extraction conditions were determined: Extraction temperature of 92.8°C, extraction time of 2.33 h and liquid to solid ratio 67.2 mL/g. Under these conditions, maximum yield of polysaccharides 31.41% can be achieved.

Keywords: *Bletilla striata*, extraction, polysaccharide, response surface methodology

INTRODUCTION

Bletilla striata is an ornamental orchid which is mainly grown in Eastern Asian countries, which has been widely used to treat alimentary canal mucosal damage, ulcer, bleeding, bruises and burns. *Bletilla striata* is used in Herbal Medicine (baiji). When employed in herbal remedies, the tuber is peeled and dried in the sun, then cut into slices or ground into a powder. *Bletilla striata* is associated with the Lung, Stomach and Liver meridians in traditional Chinese medicine and has a bitter taste and cool properties. Its main functions are to reduce swelling and stop bleeding in the lungs and stomach. It is often used with gelatin, donkey glue and cuttlefish bone as part of a larger herbal formula (Wang *et al*., 2006; Hirano *et al*., 2005; Morita *et al*., 2005).

There is abundant polysaccharide in the tuber of *Bletilla striata*, which is a kind of heteropolysaccharide composed of mannose and glucose. It is novel biodegradable polymers with some pharmacological activities such as anti-ulcer, anti-gastrointestinal bleeding, intestinal adhesion prevention (Wang *et al*., 2006; Diao *et al*., 2008; Dong *et al*., 2009). Whereas, there have been few reports on extraction technology of *Bletilla striata* polysaccharides. Therefore, it's essential and desirable to find an economical and high efficient extraction method of polysaccharides from *Bletilla striata*.

Response Surface Methodology (RSM) is an effective statistical technique for optimizing complex processes. Box-Behnken Design (BBD), one of RSM, only have three levels and need fewer experiments. It's more efficient and easier to arrange and interpret experiments in comparison with others and widely used by many researches (Yin and Dang, 2008).

In this study, the main objective was to optimize the extraction technology conditions of polysaccharides from *Bletilla striata*.

MATERIALS AND METHODS

Experimental materials and chemicals: Dry *Bletilla striata* tuber was bought from Chongqing Tongjunge Big Drugstore Chain Co. (Chongqing, China). All chemicals used in this investigation were analytical grade and purchased from Beijing Chemicals Co. (Beijing, China).

Extraction of polysaccharides from *Bletilla Striata*: Dry *Bletilla striata* tuber was ground and then extracted twice with 80% (v/v) EtOH (1 g/20 mL) at 80°C for 2 h to remove most of the phenolic compounds, monosaccharides and oligosaccharides. The pretreated *Bletilla striata* was then air-dried. The water extraction process of polysaccharides from *Bletilla Striata* was performed at different extraction temperature (X_1: 55-

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Table 1: Variables and experimental design levels for response surface

Independent	Coded symbols	Levels -1	0	1
Extraction temperature (°C)	X_1	55	75	95
Extraction time (h)	X_2	1	2	3
Ratio of liquid to solid (g/ml)	X_3	40	60	80

Table 2: Box-Behnken experimental design with the independent variables

	Coded variable levels			Yield of polysaccharide (%)	
Run	X_1	X_2	X_3	Actual values	Predicted values
1	-1	0	1	16.49	16.60
2	0	1	-1	18.42	18.30
3	0	0	0	24.47	24.72
4	0	-1	1	19.46	19.58
5	1	-1	0	23.67	23.52
6	1	0	-1	24.63	24.52
7	1	0	1	31.09	31.12
8	0	0	0	24.82	24.72
9	0	1	1	23.04	22.78
10	0	0	0	24.73	24.72
11	0	-1	-1	12.51	12.77
12	-1	0	-1	11.94	11.91
13	-1	1	0	14.16	14.31
14	-1	-1	0	12.36	12.13
15	0	0	0	23.91	24.72
16	1	1	0	29.83	30.06
17	0	0	0	25.67	24.72

95°C), extraction time (X_2: 1-3 h) and ratio of liquid to solid (X_3: 40-80 g/mL).

Isolation and determination yield of *Bletilla striata* polysaccharides: After the extraction with water, the extracted slurry was centrifuged at 2504 g for 15 min to collect the supernatant. The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (Senco Technology and Science Inc., Shanghai, China) at 55°C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and precipitation was allowed to take place for 5 h. Then the solution was centrifuged at 2504 g for 15 min, washed three times with dehydrated ethanol and the precipitate was collected as crude extract. The extract was dissolved in water. The polysaccharide content was determined by the phenol-H_2SO_4 method, using Glc as a standard (Dubois *et al*., 1956). The polysaccharide yield (%) was calculated using the following equation:

$$\text{Polysaccharide yield (\%)} = \frac{C \times N \times V}{W \times 1000} \times 100\% \quad (1)$$

where,

C = The concentration of polysaccharide calculated from the calibrated regression equation (mg/mL)
N = The dilution factor
V = The total volume of extraction solution (mL)
W = The weight of raw material (g)

Experimental design: A three level, three variable Box-Behnken factorial Design (BBD) was applied to determine the best combination of extraction variables for the yields of *Bletilla striata* polysaccharides. Three extraction variables considered for this research were X_1 (extraction temperature), X_2 (extraction time) and X_3 (ratio of solid to liquid) and the proper range of three variables were determined on the basis of single-factor experiment for the polysaccharides production. The coded and uncoded (actual) levels of the independent variables are given in Table 1. Table 2 listed the whole design consisted of 17 experimental points, three replicates (treatment 3, 8, 10, 15, 17) at the centre of the design were used to allow for estimation of a pure error sum of squares. The triplicates were performed at all design points in randomized order.

Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{i=0}^{3} \beta_i X_i + \sum_{i=0}^{3} \beta_{ii} X_i^2 + \sum_{i=0}^{3} \sum_{j=0}^{3} \beta_{ij} X_i X_j \quad (2)$$

where,

Y = The measured response associated with each factor lever combination
β_0 = An intercept
β_1 = Regression coefficients computed from the observed experimental values of Y
X_i = The coded levels of independent variables

The terms $X_i X_j$ and X_i^2 represent the interaction and quadratic terms, respectively.

Statistical analyses: Data were expressed as means Standard Errors (SE) of three replicated determinations. The responses obtained from each set of experimental design (Table 2) were subjected to multiple non-linear regressions using Design expert 8.0 software (Stat-Ease, Minneapolis, MN, USA). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the significances of the regression confident were checked by F-test and p-value.

RESULTS AND DISCUSSION

Fitting the model: A regression analysis (Table 3) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the

Table 3: Analysis of variance for extraction variables as linear, quadratic terms and interactions on response variables

Source	SS	DF	MS	F value	Prob>F
Model	577.02	9	64.11	227.24	<0.0001
Residual		5	0.28		
	1.98				
Lack of fit	0.34	3	0.11	0.28	0.8734
Pure error	1.63	4	0.41		
Cor Total	579.00	16			

Table 4: Estimated regression model of relationship between response variables (yield of *Bletilla striata* polysaccharides) and independent variables (X_1, X_2, X_3)

Variables	SS	DF	MS	F-value	p-value
X_1	368.15	1	368.15	5272.73	<0.0001
X_2	38.06	1	38.06	545.17	<0.0001
X_3	63.73	1	63.73	912.83	<0.0001
$X_1 X_1$	4.36	1	4.36	15.45	0.0057
$X_2 X_2$	57.56	1	57.56	204.02	<0.0001
$X_3 X_3$	29.90	1	29.90	105.99	<0.0001
X_1X_2	4.75	1	4.75	16.84	0.0045
X_1X_3	0.91	1	0.91	3.23	0.1152
$X_2 X_3$	1.36	1	1.36	4.81	0.0644

responses studied. Predicted response *Y* for the yield of *Bletilla striata* polysaccharides could be expressed by the following second-order polynomial equation in terms of coded values:

$$Y = 24.72+6.78X_1 + 2.18X_2+2.82X_3+1.09X_1X_2 +0.48X_1X_3-0.58X_2X_3-1.02X_1^2-3.70X_2^2 -2.67X_3^2 \quad (3)$$

where, *Y* is the yield of *Bletilla striata* polysaccharides (g) and X_1, X_2 and X_3 are the coded variables for extraction temperature, extraction time and ratio of solid to liquid, respectively.

The ANOVA of the quadratic regression model showed that the values of the determination coefficient (R^2) and the adjusted determination coefficient (Adj. R^2) were 0.9966 and 0.9922, which were reasonably close to 1 and indicated a high degree of correlation between the observed and predicted values. A very low value of Coefficient of the Variation (C.V.) (2.50) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values (Zhang *et al*., 2011).

The data in the Table 4 indicated that 3 independent variables (X_1, X_2 and X_3), 3 quadratic terms (X_1^2, X_2^2 and X_3^2) and 1 crossproduct term (X_1X_2) significantly (p<0.05) affected the yield of *Bletilla striata* polysaccharides.

Analysis of response surface: The 3-D response surface plots, as presented in Fig. 1a to 1c are very useful to see interaction effects of the factors on the responses. These types of plots show effects of two factors on the response at a time. In all the presented figures, the other two factors were kept at level zero.

Figure 1 shows the 3-D response surface plot and the contour plot at varying extraction temperature (X_1) and extraction time (X_2) at fixed liquid to solid (X_3) (0 level). As expected, a greater increase in polysaccharides yield resulted when the extraction temperature (X_1) was increased in the range from 55 to 95°C, but the temperature curve leveled off at about 93°C, which may indicate that the temperature of 93 is required to achieve maximum increase. However, the yield of polysaccharides extracted first increased and then decreased when the extraction time (X_2) changed in the range from 1 to 3 h. It indicated that the maximum extraction yield of polysaccharides can be achieved when extraction temperature (X_1) and extraction time (X_2) at the threshold level of 93°C and 2.3 h, respectively.

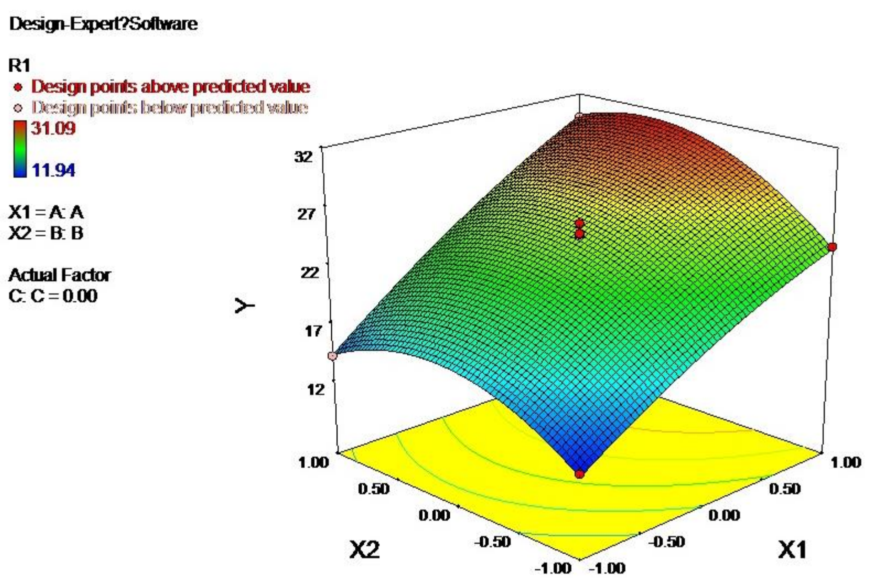

Fig. 1: Response surface showing the effect of extraction temperature (X_1) and extraction time (X_2) on extraction yield (Y) of *Bletilla striata* polysaccharides

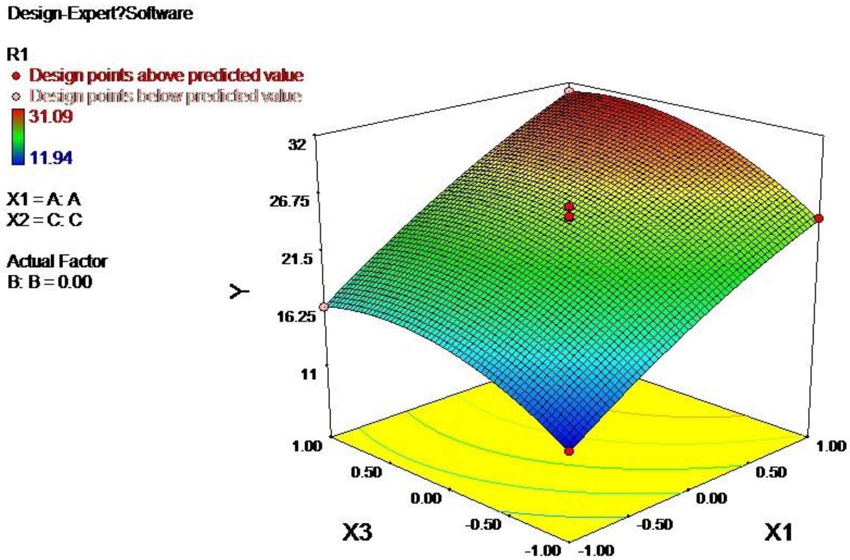

Fig. 2: Response surface showing the effect of extraction temperature (X_1) and ratio of solid to liquid (X_3) on extraction yield (Y) of *Bletilla striata* polysaccharides

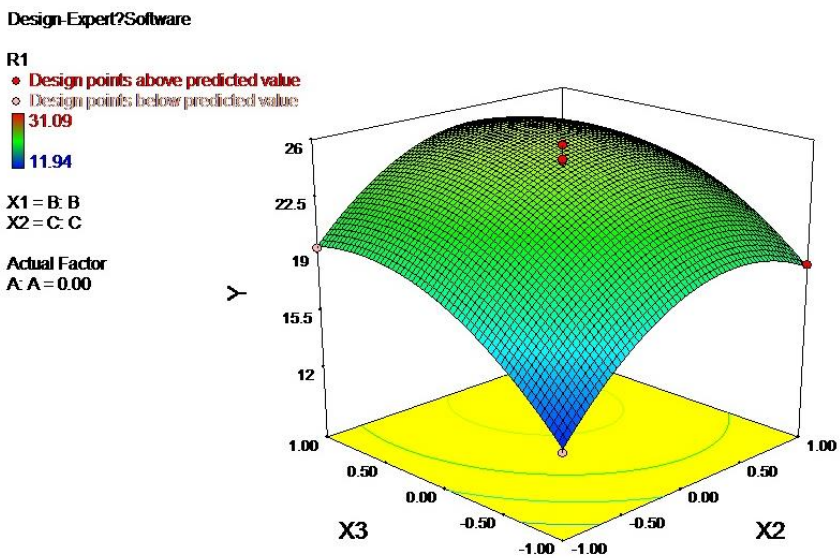

Fig. 3: Response surface showing the effect of extraction time (X_2) and ratio of solid to liquid (X_3) on extraction yield (Y) of *Bletilla striata* polysaccharides

Figure 2 shows the 3-D response surface plot at varying extraction temperature (X_1) and liquid to solid (X_3) at fixed extraction time (X_2) (0 level). The yield of polysaccharides extracted first increased and then decreased when the ratio of liquid to solid (X_3) was increased from 40 to 80 mL/g. The maximum extraction yield of polysaccharides can be achieved when extraction temperature (X_1) and liquid to solid (X_3) at the threshold level of 93°C and 67.2 mL/g, respectively.

Figure 3 shows the 3-D response surface plot at varying extraction time (X_2) and liquid to solid (X_3) at fixed extraction temperature (X_1) (0 level). The yield of polysaccharides extracted first increased and then decreased when both the extraction time (X_2) and the ratio of liquid to solid (X_3) were increased in the experimental range.

Validation of the model: Experiment was carried out under the optimal conditions (within the experimental range): Extraction temperature 92.8°C, extraction time 2.33 h and liquid to solid ratio 67.2 mL/g. Under the optimal conditions, the model predicted a maximum response of 31.33 (%).

To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 31.41±0.34 (%) (N = 3), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization. The results of analysis indicated that the experimental values were in good agreement with the predicted ones and also suggested that the models of Eq. (3) are satisfactory and accurate.

RSM is proved to be useful optimization of technology of polysaccharides extraction. Yin and Dang (2008) investigated the Optimization of extraction technology of the *Lycium barbarum* polysaccharides using RSM. The optimum conditions for *Lycium barbarum* polysaccharide extraction were determined: Extraction time 5.5 h, extraction temperature 100°C, ratio of water to raw material 31.2 and number of extraction 5. Zhang *et al*. (2011) optimized ultrasonic assisted extraction of total flavonoids from Prunella vulgaris L. The results indicated that the highest extraction yield of flavonoids by ultrasonic-assisted extraction could obtain to be 3.62% using ethanol concentration of 41% (v/v) as solvent and liquid to solid ratio of 30:1 (mL/g) for 30.5 min at the temperature of 79°C. RenJie (2008) obtained the optimal condition for Glycyrrhiza glabra polysaccharides yield within the experimental range of the variables studied was at 4.3 h for extraction time, 6 for extraction number and 35 mL/g for ratio of water to raw material. At this condition, the yield of polysaccharides extracted was 3.5%. Wu *et al*. (2007) studied the extraction of polysaccharides from boat-fruited sterculia seeds. Extraction temperature, pH, extraction time and water to seed ratio were found to have a Signiant influence on the yield of the polysaccharides. The optimum conditions were: temperature 60-65°C, time 2.3-3.1 h, pH at 7.0 and water to seed ratio at 75:1. Under these conditions, the experimental yield of polysaccharides was 17.36%. Zhong and Wang (2010) optimized the ultrasound-assisted extraction parameters (ultrasonic power, extraction time and ratio of water to raw material) of longan pulp polysaccharides and concluded that optimum extraction were: Ultrasonic power of 680 W, extraction time of 4.5 min, ratio of water to raw material of 25 mL/g. Under these conditions, the experimental yield of polysaccharides was 4.5%. XuJie and Wei (2008) stated that optimum conditions for polysaccharides extraction from wild edible BaChu mushroom included the following parameters: extraction temperature 94°C, extraction time 10 h, particle size 33 mesh and ratio of water to mushroom 6 mL/g. Ye and Jiang (2011) obtained the optimum conditions of polysaccharides extraction and concluded that optimum extraction were: the extraction time of 3.9 h, extraction temperature of 91°C, number of extraction of 4 and ratio of water to raw material of 24 mL/g. Under these optimized conditions, the optimal polysaccharides extraction yield of 4.37% was obtained.

CONCLUSION

The experiment was carried out under the optimal conditions (within the experimental range): extraction temperature of 92.8°C, extraction time of 2.33 h and liquid to solid ratio 67.2 mL/g. Under these conditions, maximum yield of polysaccharides 31.41% can be achieved.

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