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Research Article

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Optimizing the conditions for polysaccharide ultrasonic-assisted extraction in *Cordyceps gunnii* mycelia using genetic algorithm

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ABSTRACT

The study was performed to optimize the polysaccharide ultrasonic-assisted extraction conditions from the mycelium of Cordyceps gunnii by artificial neural network coupled with genetic algorithm. The effects of various experimental parameters in extraction step including ultrasonic time, ultrasonic power and liquid-solid ratio were studied using the method of single factor test design. On the results of single factor test design, a Box-Behnken design was performed to determine the effect of different parameters on the polysaccharide extraction rate. Using response surface methodology and artificial neural network coupled with genetic algorithm to analysis the results of Box-Behnken design. The optimum conditions for polysaccharide extraction obtained by the GA-ANN was ultrasonic time 225 s, ultrasonic power 430 W and liquid-solid ratio 45 mL ·g^{-1}, which was more reliable than the conditions obtained by RSM since better polysaccharide extraction rate was validated.

Key words: Polysaccharide, *Cordyceps gunnii*, Artificial neural network, Genetic algorithm

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INTRODUCTION

Cordyceps gunnii (berk.) Berk, a major entomogenous fungus, belongs to the Ascomycota, Pyrenomycetes, Sphaeriales, Clavicipitaceae and parasites on the larvae of Hepialidae. It is also well known as the Chinese rare caterpillar fungus and has similar pharmacological activities to the famous Chinese traditional medicine Cordyceps sinensis[1-2]. Many important secondary metabolic products were found in *Cordyceps gunnii* mycelia including adenosine, cordycepin, cordycepic acid and anti-ultraviolet (UV) radiation constituents[3-4]. Consequently, it has received special attention for medicinal purpose due to its various physiological constituents.Artificial neural network (ANN) is a non-linear computational model based on biological neural networks[5-7]. It simulates the human brain learning process by mathematically modeling the network structure of interconnected node cells. ANN possesses good predictive capability for complex and non-linear systems. There were several literatures demonstrated that the predictive accuracy of ANN models were superior to RSM model using the same experiment design. However, ANN is known as a black box modeling approach. The relationships between the variables and responses are not described by specification of suitable fitting function in ANN models. The effects of factors on response values and the interaction effects among the factors cannot be studied by ANN model[8-9]. Genetic algorithm (GA) is usually suggested to search for optimum solutions in non-linear systems. It mimics the principles of biological evolution, namely "survival-of-the-fittest" and "random exchange of data during propagation" followed by biologically evolving species[10]. In present study, sequential statistical methods including BBD, ANN and GA were successfully applied to optimize the extraction conditions for maximizing the extraction rate of polysaccharide from *Cordyceps gunnii* mycelium. The predictive capability of ANN model was much more satisfied than BBD model in this complex system.

EXPERIMENTAL SECTON

Microorganism and medium

The strain of *Cordyceps gunnii* Mycelia was given by Jilin Tonghua Yongcang Pharmaceutical Co., Ltd. The *Cordyceps gunnii* Mycelia was maintained in cuvette with 20% glycerol modified PDA medium at -80 ℃. The modified PDA medium composed of (g Ł-1): glucose 20, peptone 20, KH2PO4 5H2O 3.0, MgSO4 7H2O 3.0 and was autoclaved at 121℃ for 30 minutes. The maintained strain was inoculated to 100 mL sterile modified PDA medium in Erlenmeyer flask (250 mL) incubated at 26℃ in rotary shaker at 150 r/min for 4 d. Then, the culture was inoculated to Erlenmeyer flask (250 mL) containing 100 mL sterile modified PDA medium, which was incubated at 26℃ in rotary shaker at 150 r/m for 4 d, and the seed culture had been prepared. After submerged culture of this strain, the mycelium was harvested by centrifugation for 8min at 5000 r/min to separate it from the liquid medium. After repeated washing with distilled water, the mycelial pellets were lyophilized for this experiment.

Sample preparation

The polysaccharide was extracted by water assisted with ultrasound at various ultrasonic time, ultrasonic power and liquid-solid ratio from the powder of lyophilized mycelium. After this extraction, the solution was centrifuged at 8000 rpm for 5 min to obtain the crude polysaccharide solution.

Analysis Method

The total sugar content was measured by anthrone-sulfuric acid colorimetry method[11]. Reducing sugar content was measured by 3,5-dinitrosalicylic acid method[12].

Experimental Design

Single-factor test. In this study, in order to select a suitable ultrasonic power, ultrasonic time and liquid-solid ratio, samples (0.1g) were soaked and extracted using 4mL water at the power of 200W, 240W, 280W, 320W, 360W, 400W, 440W, 480W, 520W, and kept for 210s. The best ultrasonic power was selected according to the values of polysaccharide extraction rate. Then, the influence of the ultrasonic time on the extraction was investigated by considering ten ultrasonic time (60s; 90s; 120s; 150s; 180s; 210s; 240s; 270s; 300s). Finally, based on the results above, the best liquid-solid ratio (20 mL g-1, 30 mL g-1, 40 mL g-1, 50 mL g-1, 60 mL g-1, 70 mL g-1, 80 mL g-1) with the scopes of investigation was selected.

Box-Behnken design. To determine the optimum levels of three variables, the Box-Behnken method was used for experimental design. In this work, a Box-Behnken design was set up to investigate the empirical relationship between the extraction rate of polysaccharide and three controlled factors. The independent factors considered for optimization included ultrasonic power (X1; W), ultrasonic time (X2; s) and liquid-solid ratio (X3; mL g-1). The levels of independent variables were shown in Table 1. The design and results were shown in Table 2. The experiments were carried out in triplicate. The experimental data were analyzed by two different approaches, including RSM and ANN-GA. In the present work, we used ANN that consists of three layers of nodes, which are the basic computing units: input layer, hidden layer, output layer. As the number of hidden nodes greatly affects the capabilities of the ANN model, we employed a criterion named the degree of approximation (Da) to select the suitable number of hidden nodes. This function can be represented as follow:

$$D_a = \frac{c}{\frac{n_c}{n} \times MSE_c + \frac{n_t}{n} \times MSE_t + |MSE_c - MSE_t|}$$

where MSEc and MSEt were the mean-square-errors (MSE) of calibration set and test set, respectively; n was the sum number of calibration set and test set; nc and nt were the number of calibration set and test set; and c was a constant number (in present work c was 0.1). The initial weight played an important role in ANN model optimization. In present work, the ANN model with a fixed topology structure was trained thirty times with the initial weight given randomly and the best ANN model were selected according to their Da. Once the ANN model was developed to the desired level of precision, GA was implemented as a computerized search and optimization procedure that uses the principles of natural genetics and natural selection.

RESULTS AND DISCUSSION

Single-factor test design

On the basis of single variable at a time experiments, suitable ultrasonic power, ultrasonic time and liquid-solid ratio were identified. The results were shown in Fig. 1. It can be seen, the most suitable ultrasonic power, ultrasonic time and liquid-solid ratio were 400W, 210s, 40 mL g-1.

Table 1 NDEPENDENT CARIABLES AND THEIR LEVELS IN BOX-BEHNKEN DESIGN

Independent variables	Coded values		
	-1	0	1
ultrasonic power (X_1)/ W	360	400	440
ultrasonic time (X_2)/ s	180	210	240
liquid-solid ratio (X_3)/ mL·g^{-1}	30	40	50

Box-Behnken Design

The value of X1, X2 and X3 were further optimized using box-behnken design. The results were shown in Table 2. Response surface methodology. The results of Box-Behnken design were analyzed by RSM. A regression analysis was carried out by the software of SAS Version 8.0 and the results were shown in Table 3. A multivariate quadratic regression (MQR) model was developed based on Box-Behnken design data for determining the individual effects and mutual interaction effects of candidate variables. The regression equation for polysaccharide extraction rate, as a function of the three independent variables (X1, X2 and X3) and their linear and quadratic interactions is presented in the following equation：

$$Y=2.1729+0.0663*X_1+0.0559*X_2+0.1069*X_3-0.1244X_1^2-0.0753*X_1X_2-0.0184*X_1X_3-0.1157*X_2^2+0.0184*X_2X_3-0.0713*X_3^2$$

The R2 of the model was 0.9544. These results demonstrated that the fit of the model was satisfied. F-test method was employed to exam the significances of the coefficients in this model. The results were shown in table 3 and the effects of individual variables and the mutual effects between the variables were learnt from these results. As can be seen in table 3, the effects of X1, X2, X3, X12, X1X2, X22, X32 were significant. The other terms were insignificant. SAS software was employed to search for the maximum value in model. The optimum condition was found as follows: ultrasonic time 214s, ultrasonic power 410W and liquid-solid ratio 48mL g-1. Then the highest polysaccharide extraction rate of 2.226% predicted by the model could be obtained. The validation experiments with these optimum conditions were implemented in triplicate and the average extraction rate of adenosine was 2.703%. The relative error between expected value and experimental value was 1.81%. 3D surface plots were drawn to show the effects of independent variables on the response, the results was shown in Fig. 2. They simply described the relationship between the response values and the variables.

Artificial neural network and genetic algorithm. One of the data of the Box-Behnken design experiments were randomly chosen and used as prediction set. Another one was randomly chosen and used as test set. The other experimental data were used as calibration set. A three layers feed back ANN model was established. The ANN model was optimized by selecting the suitable number of hidden nodes. The most suitable number of hidden nodes with the maximum Da was 11, as the Fig.3. The determination coefficient (R2) of the optimized ANN model was 0.9721, indicating that the fitting of the ANN was satisfied. The root mean square error of test set (RMSET) and the root mean square error of prediction set (RMSEP) were 0.0021 and 0.0064. These results indicated that the predictive capability of the ANN model was satisfied. While the ANN model was developed, genetic algorithm (GA) was employed to search for the optimum medium which was as follow: ultrasonic time 225s, ultrasonic power 430W and liquid-solid ratio 45mL g-1. Then, the highest polysaccharide extraction rate of 2.351% predicted by the model could be obtained, which increased 5.61% from that obtained by MQR model. At last, the validation experiments with these optimum conditions were implemented in triplicate and the average extraction rate of polysaccharide was 2.293%. Therefore, the ANN-GA model performed better than RSM model in the optimization studies.

TABLE 2 THE DESIGN MATRIX AND THE RESULTS OF BOX-BEHNKEN DESIGNABLE TYPE STYLES

Runs	X_1	X_2	X_3	Y (%)
1	-1	-1	0	1.68875
2	-1	1	0	2.0175
3	1	-1	0	1.99875
4	1	1	0	2.02625
5	0	-1	-1	1.895
6	0	-1	1	2.03125
7	0	1	-1	1.90375
8	0	1	1	2.11375
9	-1	0	-1	1.77875
10	1	0	-1	1.92125
11	-1	0	1	2.07
12	1	0	1	2.13875
13	0	0	0	2.1825
14	0	0	0	2.16375
15	0	0	0	2.1725

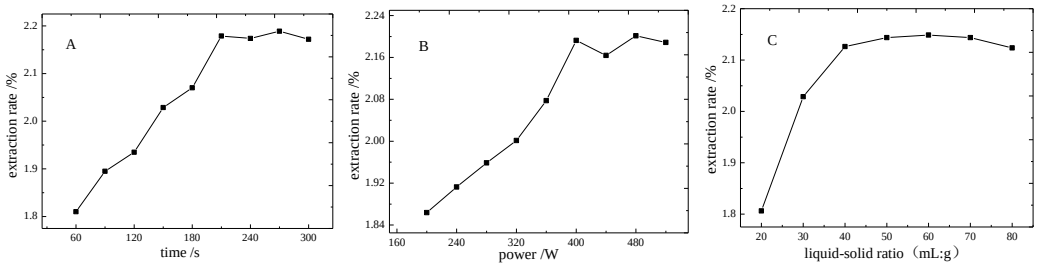

Fig. 1 The results of single-factor test design (A ultrasonic time; B ultrasonic power; C liquid-solid ratio)

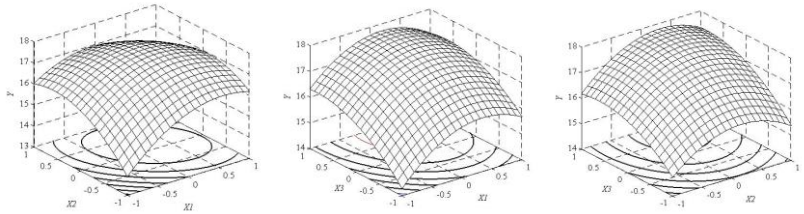

Fig. 2 Response surface plot showing relative effect of three extraction parameters on polysaccharide extraction rate

TABLE 3 THE STATISTICAL RESULTS OF MQR MOEDL

Source	*DF*	*SS*	*MS*	*F*	*Pr > F*
X_1	1	0.0351	0.0351	12.8153	0.0159
X_2	1	0.0250	0.0250	9.1361	0.0293
X_3	1	0.0914	0.0914	33.3509	0.0022
$X_1 \times X_1$	1	0.0572	0.0572	20.8638	0.0061
$X_1 \times X_2$	1	0.0227	0.0227	8.2806	0.0351
$X_1 \times X_3$	1	0.0013	0.0014	0.4963	0.5036
$X_2 \times X_2$	1	0.0494	0.0494	18.0326	0.0083
$X_2 \times X_3$	1	0.0014	0.0014	0.4963	0.5162
$X_3 \times X_3$	1	0.0188	0.0188	6.8512	0.0476
Model	9	0.2869	0.0319	11.6330	0.0074
Lack of fit	3	0.1515	0.0505	18.4341	0.0074
Total	14	0.3005		R^2	0.9544

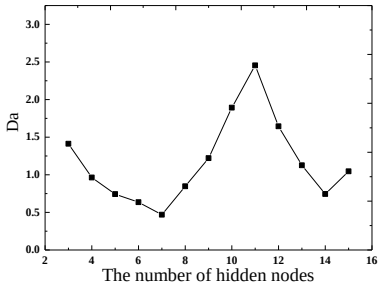

Fig. 3 The effect of the number of hidden nodes on Da

CONCLUSION

In the case exposed here, ANN provided more reliable results than BBD for optimizing the conditions for polysaccharide ultrasonic-assisted extraction in mycelium of *Cordyceps gunnii*. Optimum conditions (ultrasonic time 225s, ultrasonic power 430 W and liquid-solid ratio 45 mL g-1) for extraction procedure were identified at last. The extraction rate of polysaccharide under this condition could reach the highest value of 2.351% with one time extraction. These results demonstrated that the predictive capability of the ANN model was good and this method is feasible. The optimization process in this paper has greatly enhanced the extraction rate of polysaccharide. It should be popular in optimizing any other condition.

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