Optimization of Extraction Process of Exopolysaccharide from Lentinus edodes by Response Surface Methodology

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Abstract: In this paper, response surface methodology was used to study the optimal extraction parameters of extracellular polysaccharide from Lentinus edodes on the basis of single factor analysis. The factors of the extraction process of lentinan extracellular polysaccharide were optimized and analyzed, and the optimal extraction process of lentinan extracellular polysaccharide was finally determined. That is, the ethanol concentration was 78.0%, the alcohol precipitation time was 15.8 h, and the pH was 7.8. Under the above optimized conditions, the actual extraction amount of extracellular polysaccharides of Lentinus edodes can be increased to 1562.3mg/L, and the stability and reliability are high. The experimental values are roughly consistent with the predicted values of the theoretical linear regression model constructed in the previous period.

1. Introduction

Lentinus extracellular polysaccharide (LNT) mainly refers to an effective active molecule extracted from the high quality mushroom fruiting body. It mainly comprises two modules: branches β-(1-3)-D-glucan, β-(1-6)-D-glucan. The extraction process of lentinan extracellular polysaccharide can not only effectively treat recurrent bladder cancer, liver cancer, gastric cancer and the like which cannot be cured by surgery, but also LNT can effectively improve the immune function of patients. However, due to the low extraction efficiency of the existing LNT extraction process, the effective application of LNT is seriously affected. Therefore, it is necessary to optimize the LNT extraction process.

2. Optimization of Experimental Materials and Instruments for Extraction of Exopolysaccharide from Lentinus edodes by Response Surface Methodology

The strain used in this LNT extraction test is mainly Lentinus edodes 66# strain, which is provided by an engineering technology research center for edible and medicinal bacteria. The optimized test medium is mainly slant medium. It consists of 0.30% potassium dihydrogen phosphate, 2.0% glucose, 20.0% potato, 0.15% magnesium sulfate heptahydrate and 0.001% vitamin B1. The fermentation medium was mainly composed of 0.10% magnesium sulfate heptahydrate, 0.30% yeast extract, 2.00% corn flour, 1.00% glucose, 2.00% wheat bran, and 0.10% potassium dihydrogen phosphate. The pH of the medium used in this test was natural [1].

In this LNT extraction optimization experiment, PL205 electronic precision balance, aseptic operation Table, DNP-9085 electrothermal incubator, THZ-C-1 constant low temperature gas bath shaker were used. PHS-3CpH acidimeter; MLS-3700 autoclave; HH-S water bath pot; TDZ5-WS centrifuge.

3. Optimization of Extraction of Exopolysaccharide from Lentinus edodes by Response Surface Methodology

After the above materials have been prepared, Lentinus edodes strain 66# is inoculated into slant culture medium and cultured at 28.0℃. After the strain is filled in the tube, two pieces of 0.50cm2 bacteria pieces are taken, and at the same time, 300.0mL fermentation medium is put into a

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500.0mL triangular flask, and the fermentation medium is inoculated. After standing still for 24.0h, put it into a shaking Table, adjust the speed of the shaking Table to 180r per minute, and perform shaking fermentation at 28.0℃ for 240h[2]. After the fermentation, the mycelium was removed from the fermentation broth and the volume of the fermentation broth was 100.0 mL. Fermentation filtrate was 20.0 mL after constant volume, and 80.0 mL absolute ethanol was added. Stand for 24 hours at 5.0 C. In TDZ5-WS centrifuge, the supernatant was absorbed after centrifugation, and the centrifugal precipitation was washed repeatedly with acetone, ether and anhydrous ethanol. Finally, excess water was evaporated in a DNP-9085 electrothermal incubator to concentrate to constant weight. After concentration, store in a 4.0℃refrigerator.

4. Response Surface Methodology to Optimize Extraction of Exopolysaccharide from Lentinus edodes

4.1 Research on Single Factor Test Results

The LNT single factor test mainly relies on the ethanol volume fraction, the fermentation filtrate pH, and the ethanol precipitation time to extract LNT. After repeating the above experiment three times, the average amount of LNT was calculated. First, an ethanol concentration experiment was carried out, and the ethanol concentration was adjusted to 70.0%, 80.0%, and 90.0%, respectively, according to the above experimental operation. Subsequently, the experiment was repeated three times, and the highest concentration of LNT was selected as the optimal ethanol concentration. According to the test results, with the increase of ethanol concentration, the output of LNT also increased. When the concentration of ethanol increased to 78.0%, the output of LNT reached the highest value of 1562.3 mg/L. Therefore, the optimum concentration of ethanol is 78.0%[3].

Secondly, PH test was carried out to keep other experimental conditions unchanged, and the pH of fermentation broth of Lentinus edodes was adjusted to 7.0, 8.0 and 9.0. Choose the best pH. According to the experimental results, as the pH increases, the LNT production increases first, then decreases, and the slope changes. At a pH of 7.8, the LNT yield reached a maximum of 1562.3 mg/L, indicating an optimal extraction pH of 7.8.

Finally, the ethanol precipitation time, i.e. ethanol precipitation experiment, was carried out, i.e. 5.0 mL of concentrated LNT was added with ethanol to promote the ethanol concentration to 78.00%. Subsequently, the PH was adjusted to 7.8 using 1.0mol/L dilute hydrochloric acid and 1.0mol/L sodium hydroxide. Adjust the alcohol precipitation time to 12h, 16.0h and 20.0h respectively, centrifuge for 750s for precipitation, then use acetone, diethyl ether and absolute ethyl alcohol to carry out repeated circulation flushing, and dry to constant weight in an electric heating constant temperature incubator at 60.0℃. Repeat three times and select the highest yield for the best alcohol precipitation time. By analyzing the final test results, it is concluded that as the alcohol precipitation time increases, the LNT yield also increases. When the alcohol precipitation time reached 15.8h, the LNT yield reached the highest value of 1456.5mg/L. From this, the optimum alcohol precipitation time was 15.8h.

4.2 Optimization of LNT Extraction Conditions by Response Surface Methodology

First of all, the response surface method is used to design the experiment. Taking the mass concentration of polysaccharide (mg/L) in LNT extract as response value, a three-factor and three-level response surface design was carried out. At the same time, in the SAS software, the results of the three-factor three-level response surface experiment design were analyzed. Specifically as Table 1:

Table 1 LNT response surface design data and results (partial)

Experiment number	Code value			response value
	X_1(Ethanol concentration/%)	X_2(Alcohol precipitation time/h)	X_3(pH)	Y(Extraction quantity/mg^{-L})
01	70	12	8.0	1456.5
02	70	20	8.0	1452.3
05	80	12	7.0	1522.1
06	80	20	9.0	1520.1
09	90	16	9.0	1452.2
10	90	20	8.0	1325.0
14	80	16	8.0	1562.3
15	80	20	8.0	1232.5

Secondly, the experimental results of the quadratic regression model fitting exploration, through SAS software analysis of the above data, can be obtained regression coefficient coefficient estimates, standard error, F value and probability partial regression coefficient (P value) [4]. For example, the degree of freedom of coefficient X_1 is 1, the estimated value of coefficient is -12.914562, the standard error is 1.832564, the F value is 30.23, and the P value is 0.0089. The degree of freedom of the coefficient X_2X_1 is 1, the estimated value of the coefficient is -3.102560, the standard error is 2.602563, the f value is 26.32, the probability partial regression coefficient (p value) is 0.0058, and the rest is shown in Table 2:

Table 2 LNT extraction test linear regression analysis results (partial)

Source of variance	Coefficient estimation	Root mean square error (standard error)	Freedom	F value	Prop＞F	Significance
X_2	-11.235620	1.985623	1	27.56	0.0111	·
X_3	-10.235610	2.305624	1	26.52	0.0021	··
X_1X_3	-2.365281	0.985623	1	36.52	0.0035	···
Misstated item	-0.023562	0.025441	1	16.14	0.2256	·
X_2X_3	-1.256382	1.235614	1	31.00	0.0065	··
$X3^2$	-0.289562	2.356412	1	33.21	0.0050	··
$X2^2$	-0.456289	1.236521	1	28.92	0.0062	·

Through the analysis of the above coefficients, it can be concluded that the source of variance of the test results is X_1 (ethanol concentration), X_2 (ethanol precipitation time), X_3 (pH), first term, interaction term and second term. The total degree of freedom is 9, the sum of squares and mean sum is 3190.956231, 0.9498, and the F value is 26.8956. Substituting the above data into the quadratic regression model, the regression equation can be obtained as: $Y=267.98-12.89X_1+5.45X_2+11.98X3-7.62X_1^2-3.11X_1X_2-5.16X_2^2+0.70X1X_3-2.06X_2X_3+3.79X_3^2$.

Since the coefficient of the quadratic term in the above formula is less than 0, the quadratic regression equation characterizes that the parabolic surface opening is downward, and there is a maximum value. That is, the LNT extraction process has optimal parameters. At the same time, by analyzing the coefficient R2 of the quadratic regression equation, it can be concluded that the regression equation fits well, and the F-test is more significant. The selected variable is the main source of response value change. It is indicated that the quadratic regression model better describes the actual relationship between each factor and the response value (coefficient of variation is 2.0014). According to this, the quadratic regression equation can be used to determine the optimal LNT extraction parameters. Then, the significance degree of the first term F- test, the second term F- test and the interactive term F- test of the regression equation was analyzed, and it was found that they were extremely significant, insignificant and insignificant respectively, indicating that there was a significant principal effect among the three factors X_1, X_2 and X_3 selected in this experiment [5]. Finally, the effect of various factors on the response value is further analyzed. It is concluded that X_1 has the most significant effect on the response value, X_2 has no significant effect on the

response value, and X_3 has an effect on the response value>X_2, but <X_1.

Finally, the response surface method is explored. In the SAS software, the regression model is studied and discussed. It is concluded that in the process of finding the optimal response area, the key values of X_1, X_2 and X3 are -0.8785, 0.5124 and 1.3256, respectively, and the estimated value is 1562.3. The sTable point type is Maximum value. Through the analysis of the above data, it can be concluded that there is a sTable point in the linear regression model. Combining X_1, X_2 and X_3 coding formulas, the optimum level values of main factors X_1, X_2 and X_3 are calculated. The coding formula of X_1 (ethanol concentration/%) is as follows: $(\xi_1 - 24)/20$

The coding formula of X_2 (alcohol precipitation time/h) is as follows: $(\xi_2 - 4)/2$.

The X3 (pH) coding formula is: $(\xi_3 - 3)/1$.

ξ is the key value in the above formula. By substituting the above values into the formula, it can be concluded that X_1, X_2 and X_3 are 78 ℃, 15.8h and 7.8 respectively. The maximum LNT concentration predicted by the final model was 1562.3 mg/L[6]. Then repetitive validation tests are carried out on the predicted value. That is, 5.0 ml of LNT fermentation broth is taken, and LNT is extracted when the pH is 7.8 and the ethanol concentration is 78.0%. The alcohol precipitation time is 15.8h, and the average LNT mass concentration is 1562.3mg/L, which is generally consistent with the predicted value of the model. This shows that the linear regression equation is appropriate and reasonable, and can be used to provide guidance for the optimization of LNT extraction experiments.

5. Conclusion

To sum up, LNT belongs to polar macromolecular compounds, and its structure has characteristics and is closely related to immune activity. In the process of LNT extraction, ethanol concentration has the greatest influence on the extraction rate of LNT, followed by pH and alcohol precipitation time. Therefore, in the process of LNT extraction, relevant personnel should adjust the concentration of LNT ethanol to 78.0%, pH to 7.8, and alcohol precipitation time to 15.8h to ensure the extraction efficiency of LNT.

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