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Optimization of glycosaminoglycan extraction on *Patinopecten yessoensis* waste

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Abstract

On the basis of single factor experiment, the pH of enzymatic hydrolysis, enzymolysis temperature, enzymolysis time and solid-liquid ratio as independent variable, extraction rate as response value, extraction technology of glycosaminoglycan from Patinopecten yessoensis waste were optimized using response surface methodology. The order affecting glycosaminoglycan extraction rate was determined: the enzymatic pH > solid-liquid ratio > enzymatic time > enzymatic temperature. The optimal conditions of extraction were: the pH of enzymatic hydrolysis was 8.0, enzymolysis temperature was 40 ℃, enzymolysis time was 3.5 h and solid-liquid ratio was 1:2.
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Keywords: Patinopecten yessoensis waste; Glycosaminoglycan; Optimization of extraction conditions; Response surface methodology

1. Introduction

Glycosaminoglycan, also known as acid mucopolysaccharide, is a kind of heteropolysaccharide[1]. As the sugar chain of animal protein polysaccharide, glycosaminoglycan exists widely in the extracellular matrix of the vertebrate organization. It has many biological activities such as anticoagulant, hypolipidemic, antitumor, antiviral, enhancing immunity of body[2-5]. The studies showed that molluscs contained a variety of glycosaminoglycan[6]. The glycoprotein or glycosaminoglycan have been extracted from Argopecten irradias, Ruditapes philippinarum, Sinonovacula constricta, Scapharca subcrenata, Mactra veneriformis and Bullacta exarata et al, and some activities were studied [7-14].

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Patinopecten yessoensis is a large cold-water shellfish. It was introduced into China in the 1980s, andgradually become the main aquaculture species in the North. About 30% of the waste is produced in P. yessoensis processing, the waste is rich in fatty acids and peptides[15], also contains a certain mass concentration of glycosaminoglycan. Therefore, we conducted the research of extraction conditions optimization of glycosaminoglycan from P. yessoensis waste, in order to provide a theoretical basis for development and utilization of the waste.

2. Materials and methods

2.1. Biological materials and reagents

P.yessoensis were purchased from Tianjin Tanggu farmers markets, trypsin (enzyme activity 250 U/g, optimum temperature 50 ℃, optimum pH 8.0), neutral protease of *Bacillus subtilis* (enzyme activity 60000 U/g, Optimum temperature 40 ℃, Optimum pH 7.5), heparin (150 IU/mg),Alcian Blue 8 GX.

The above materials were purchased from Beijing Solarbio Science & Technology Co.,Ltd.

Ethanol absolute,acetone,hydrogen peroxide and sodium acetate anhydrous were of analytical grade.

2.2. Experimental instruments

Ds-1 high-speed-organization broken machine, SHA-C water bath oscillator, T6 new century UV-Vis spectrophotometer, TGL-16LM high-speed refrigerated centrifuge, 99-1 magnetic stirrer, RE-3000 rotary evaporator, FD-10-50 vacuum lyophilizer.

2.3. Methods

2.3.1. Glycosaminoglycan extraction process

P.yessoensis waste→homogenate→autolysis→enzymolysis→inactivation of enzyme→decoloration →centrifugation→deproteinization→alcohol precipitation→washing by ethoanol and acetone respectively→drying

2.3.2. Determination of glycosaminoglycan content

Glycosaminoglycan content was determined by Alcian Blue colorimetric[16].

2.3.3. The extraction conditions of glycosaminoglycan were optimized by response surface methodology

Enzymatic pH, enzymatic temperature, enzymatic time and solid-liquid ratio were represented by A, B, C and D respectively. Extraction rate of glycosaminoglycan was represented by response value (Y). Experimental factors and levels were shown in the Table 1.

Table 1. The coded values of experimental factors and levels

Factor	Coded values		
	1	0	-1
A	8.00	7.00	6.00

B	30.00	40.00	50.00
C	3.00	4.00	5.00
D	1:3	1:2	1:1

3. **Experimental results and analysis**

3.1. Experimental results and analysis of response surface

Experiment was optimized using the central combination of Box-Behnken, experimental program and results were shown in Table 2.

Table 2. Experimental program and results of response surface

Run	A	B	C	D	Y($\times10^{-4}$)
1	-1	0	-1	0	5.9438
2	1	-1	0	0	5.2162
3	0	0	-1	1	4.5779
4	0	-1	0	1	4.5820
5	0	-1	-1	0	5.9395
6	1	0	-1	0	4.7562
7	0	0	0	0	8.1636
8	0	0	1	1	5.6821
9	0	-1	0	-1	6.6458
10	-1	0	0	1	4.2913
11	1	1	0	0	6.2571
12	1	0	1	0	5.9034
13	0	0	0	0	8.0304
14	0	1	-1	0	4.3905
15	0	1	0	-1	5.3842
16	-1	-1	0	0	5.0881
17	-1	0	1	0	3.7953
18	1	0	0	1	6.0587
19	-1	1	0	0	4.9558
20	1	0	0	-1	5.9840
21	0	1	0	1	6.1448
22	0	0	1	-1	5.1563
23	0	-1	1	0	4.1587
24	0	0	-1	-1	5.8454
25	-1	0	0	-1	5.2990
26	0	1	1	0	5.5824

The above results were analysized using Design expert 7 software. Quadratic regression models were fitted, and the regression analysis and results were shown in Table 3, Table 4 and Table 5.

Table 3. The variance analysis of regression model

Variance source	Sum of square	Degrees of freedom	Mean square	F-value	P-value
Model	26.50	14	1.89	23.23	< 0.0001
A	1.92	1	1.92	23.59	0.0005
B	0.10	1	0.10	1.20	0.2962
C	0.12	1	0.12	1.41	0.2597
D	0.74	1	0.74	9.07	0.0118
AB	0.34	1	0.34	4.22	0.0644
AC	2.72	1	2.72	33.33	0.0001
AD	0.29	1	0.29	3.60	0.0845
BC	2.21	1	2.21	27.12	0.0003
BD	1.99	1	1.99	24.48	0.0004
CD	0.80	1	0.80	9.87	0.0094
A^2	8.84	1	8.84	108.48	< 0.0001
B^2	7.64	1	7.64	93.84	< 0.0001
C^2	11.88	1	11.88	145.87	< 0.0001
D^2	5.88	1	5.88	72.11	< 0.0001

Table 4. Lack of fit test and error analysis of regression model

Variance source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Residuals	0.90	11	0.08		
Lack of fit	0.89	10	0.09	10.00	0.2416
Pure error	0.01	1	0.01		
The total deviation	27.39	25			

Table 5. The credibility analysis of regression model

Item	Value	Item	Value
Standard deviation	0.2854	Multiple correlation coefficient R^2	0.9673
Mean	5.5320	Correcting correlation coefficient R^2	0.9256
Variation coefficient	5.1596	Predicting correlation coefficient R^2	0.8121
PRESS	5.1464	The ratio of signal to noise	20.2742

From the analysis results in Table 3 and Table 4, the model was very significant ($P<0.0001$), and lack of fit was not significant ($P>0.05$). These results suggested that the experimental program was reliable, the selected model fitted to the actual situation.

According to the P values in Table 3, the order affecting extraction rate was: enzymatic pH >solid-liquid ratio > enzymatic hydrolysis time > enzymolysis temperature.

In the Table 5, multiple correlation coefficient was 0.9673, correcting correlation coefficient was 0.9256, and variation coefficient of Y was lower, indicating that the predicted values of the model fitted to experimental values well and the reliability of the experiment was higher. Therefore, the best extraction condition might be determined using the regression model, and the regression equation was determined ultimately:

$$Y=8.10+0.40A+0.090B-0.098C-0.25D+0.29AB+0.82AC+0.27AD+0.74BC+0.71BD+0.45CD-1.42A^2-1.32B^2-1.65C^2-1.16D^2 \quad (1)$$

3.2 The determination of the optimum extraction condition

The response surface analysis charts were plotted by the response surface regression analysis and regression equation (Fig.1-6). The Y values was gradually increased with the increasing in enzymatic pH and hydrolysis temperature, the Y value was the maximum as enzymatic pH and enzymatic temperature reached a certain value, then the Y value gradually decreased with the increasing in enzymatic pH and enzymatic temperature (Fig. 1), a similar law was appeared in Fig. 2-6. Six graphs were opening-downward-convex surface, indicating that the maximum response values existed. The contour center of six graphs was located in –1 to 1, indicating that the optimal extraction condition existed in the levels of designed factors. The optimal conditions of extraction were: enzymatic pH values (A)=7.88, enzymatic temperature(B)=38.55 ℃, enzymatic time(C)=3.68 h, Solid-liquid ratio(D)=1:2.29, predicting extraction rate was 0.084%, the actual extraction rate was 0.081% by three validation experiments. The optimal extraction condition was modified considering the operation convenience: Enzymatic pH values(A)=8.00, enzymatic temperature(B)=40.00℃, enzymatic time(C)=3.5h, Solid-liquid ratio(D)=1:2, the actual extraction rate was 0.082% under this condition, little difference was between the actual extraction rate and the theoretical extraction rate, indicating that the parameters of extraction condition obtained using response surface method were accurate and reliable. The model had a higher value.

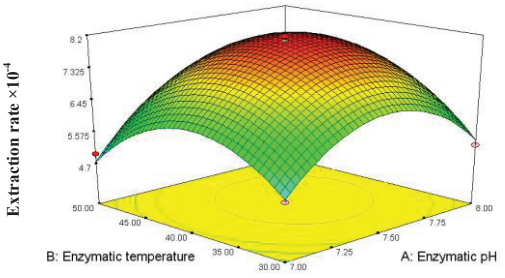

Fig. 1. Effects of enzymatic pH and enzymatic temperature on the extraction rate of glycosaminoglycan

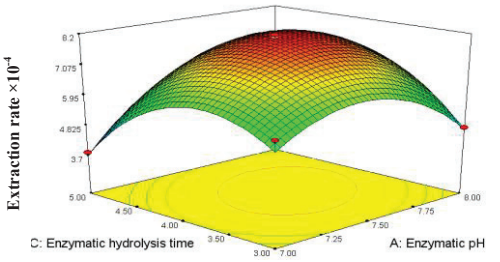

Fig. 2. Effects of enzymatic pH and enzymatic time on the extraction rate of glycosaminoglycan

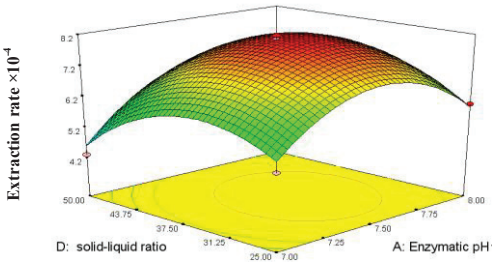

Fig. 3. Effects of enzymatic pH and solid-liquid ratio on the extraction rate of glycosaminoglycan

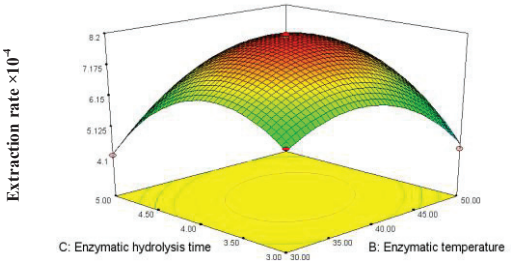

Fig. 4. Effects of enzymatic temperature and enzymatic time on the extraction rate of glycosaminoglycan

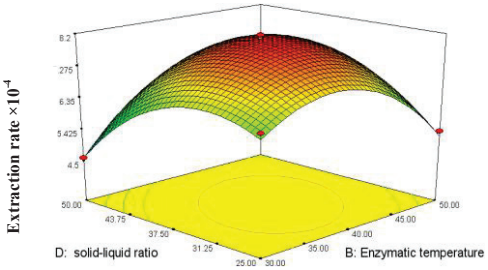

Fig. 5. Effects of enzymatic temperature and the solid-liquid ratio on the the extraction rate of glycosaminoglycan

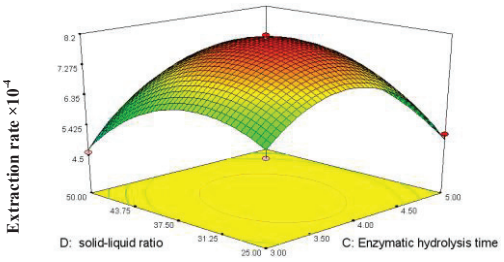

Fig. 6. Effects of enzymatic time and solid-liquid ratio on extraction rate of glycosaminoglycan

4. Conclusions

(1) The order of affecting the extraction rate of glycosaminoglycan from P. yessoensis: enzymatic pH > solid-liquid ratio > enzymatic time > enzymatic temperature

(2) The optimal extraction conditions were determined by response surface methodology: enzymatic pH was 8.0, enzymolysis temperature was 40 ℃, enzymatic hydrolysis time was 3.5 h and solid-liquid ratio was 1:2, glycosaminoglycan extraction rate was 0.265% under the conditions.

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