

Process and Kinetic Models of Hydrochloric Acid -Extracted Collagen from Bighead Carp Scale

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Abstract. Fish scale contains rich collagen. Collagen is an important material which is widely used in many kind of industries. Extracting collagen from fish scale not only reduces the pollution but also obtains new raw material of commercial collagen. In this paper, single factor test method was firstly used to investigate the effects of liquid/solid ratio, concentration of hydrochloric acid, extraction time and extraction temperature on collagen extraction yield from bighead carp scale and their optimal values were determined. Based on which, the response surface method was used to establish the process and kinetic models for the collagen extraction process. The results show that the two models in the form of multiple regression are basically identical with the actual values and can be used to predict the collagen extraction yield and collagen extraction velocity under different operation conditions, and to adjust the operation parameters for obtaining the expected collagen extraction velocity with high collagen extraction yield in order to realize the ideal utilization efficiency and production intensity of the collagen in fish scale.

Introduction

Fish scale is a kind of waste produced in fish processing. Fish scale contains reach collagen which is an important biological, food and cosmetic material and can be widely used in pharmaceutical, food and cosmetics industries because of its excellent biocompatibility, low antigenicity, immunogenicity, and good function of auxiliary cell regeneration. To extract collagen from fish scale can not only reduce biological pollution but also obtain potential new collagen resources in the world. Recently, fish collagen has attracted many scholars and researchers to engage into this research area. Most of the published articles focused on the isolation and characterizations of collagen from the scales of different kind of fishes[1-2].The process model reflects the relations between collagen extraction yield (as target) and main operation parameters(as influence factors). The kinetic model reflects the relations between collagen extraction velocity (as target) and main operation parameters(as influence factors). These two models can predict and adjust the technical parameters to achieve the expected collagen extraction yield and collagen extraction velocity under different operation conditions, which is conducive to realizing idea utilization of fish scale and collagen production intensity. In this paper, single factor test and response surface test methods were used to establish the process and kinetic models for the collagen extraction from bighead fish scale and the significance of the models and model terms were analyzed and validated with the actual values.

Experimental

Materials and chemicals.

The scale of bighead carp was collected from the local market in Nanchang, Jiangxi, China. L-hydroxyproline (BR) was obtained from Sinopharm Chemical Reagent Co.,Ltd.(Shanghai,China). The other chemicals used in the experiment were chemical pure and made in China.

Decalcification of fish scale.

The scales were leached in a 0.5 mol/L HCl solution with oscillation for 57 min and then soaked for 10 h in a 0.5 mol/L Na₂CO₃ solution with constant stirring. After that the decalcified fish scales were repeatedly rinsed with water.

Extraction of collagen.

a) Single factor test.

a.1 g fish scale, firstly accurately weighed and decalcified, was added 15 distilled water and certain amount of 0.5 mol/L hydrochloric acid solution and the effect of extraction time(6,12, 24, 36,48 h) on collagen extraction yield was investigated at 28°C. b.1 g fish scale, firstly accurately weighed and decalcified, was added 15 mL distilled water and certain amount of hydrochloric acid solution with different concentrations and the effect of hydrochloric acid concentration (0.25, 0.5, 0.75,1.00,1.25 mol/L) on collagen extraction yield was investigated at 28°C. c.1 g fish scale, firstly accurately weighed and decalcified, was soaked in certain amount of 0.5 mol/L hydrochloric acid solution and the effect of the added amount of distilled water (5,10, 15, 20, 25 mL) on collagen extraction yield was investigated at 28°C. d. 1 g fish scale, firstly accurately weighed and decalcified, was added 15 mL distilled water and a certain amount of 0.5 mol/L hydrochloric acidsolution and the effect of temperature(10, 20, 28, 30, 40°C)on collagen extraction yield was investigated. The results show the optimal technical parameters are determined to be: liquid/solid ratio 15 mL/g, hydrochloric acid concentration 0.5 mol/L (the collagen will be greatly lost when hydrochloric acid concentration is over 0.5 mol/L), extraction time 24 h and extraction temperature 28 °C(the collagen will be easily denatured when temperature is higher than 28°C).

b) Response surface test

Based on the results obtained by single factor test, the optimal parameters were taken as the investigation factors and collagen extraction yield and collagen extraction velocity were taken as the double targets. The design of four factors with three levels was listed in Table 1.

Table 1 Design of four factors/three levels for response surface test

Factor	code	level		
		-1.00	0.00	1.00
Ratio of liquid/solid, mL/g	X ₁	10	15	20
Concentration of hydrochloric acid, mol/L	X ₂	0.25	0.50	0.75
Extraction time, h	X ₃	12	24	36
Extraction temperature, °C	X ₄	20	28	36

Measurement of collagen extraction yield and velocity.

The collagen extraction yield (%) is defined as the ratio of hydroxyproline content in extraction solution (w_2 , mg/g) to total hydroxyproline content in raw fish scale (w_1 , mg/g). The standard curve of hydroxyproline was drawn and its regression equation was obtained as $y= 0.10946x-0.00639$ ($R^2=0.9982$), where y is the absorbance and x is the mass concentration of hydroxyproline, µg/mL. The collagen extraction velocity is defined as mass of extracted collagen per unit volume and per unit time, mg/(L·h),

Results and discussions.

According to the design in Table 1,29 sets of test (including 5 sets of central group tests) were separately conducted when the extraction yield and extraction velocity were taken as double targets and the results were listed in Table 2.

Table 2 Results of response surface test

Order No.	X ₁	X ₂	X ₃	X ₄	Extraction Test	yield Model	relative error %	Test	Extraction Model g/(L·h)	velocity relative error %
1	15	0.50	12	20	4.20	4.13	-1.67	0.3022	0.30	-0.73
2	15	0.75	24	36	14.92	14.75	-1.14	1.0734	1.06	-1.25
3	20	0.75	24	28	10.53	10.68	1.42	0.7576	0.77	1.64
4	10	0.25	24	28	7.00	7.01	0.14	0.5036	0.50	-0.71
5	15	0.5	24	28	9.25	9.25	0.00	0.6655	0.6655	0.00
6	15	0.5	36	20	4.73	4.88	3.17	0.3403	0.35	2.85
7	15	0.25	12	28	6.45	6.45	0.00	0.4640	0.46	-0.86
8	15	0.25	36	28	7.26	7.45	2.62	0.5223	0.54	3.39
9	15	0.25	24	36	9.75	9.50	-2.56	0.7014	0.68	-3.05
10	15	0.25	24	20	3.61	3.44	-4.71	0.2597	0.26	0.12*
11	15	0.75	36	28	11.10	11.27	1.53	0.7986	0.81	1.43
12	20	0.5	24	20	4.48	4.67	4.24	0.3223	0.34	5.49*
13	15	0.5	24	28	9.25	9.25	0.00	0.6655	0.6655	0.00
14	15	0.5	24	28	8.58	8.81	2.68	0.6173	0.63	2.06
15	15	0.5	24	28	9.25	9.25	0.00	0.6655	0.6655	0.00
16	15	0.75	24	20	5.52	5.43	-1.63	0.3971	0.39	-1.79
17	20	0.25	24	28	6.88	7.09	3.05	0.4950	0.51	3.03
18	20	0.5	24	36	12.11	12.39	2.31	0.8712	0.88	1.01
19	10	0.5	24	20	4.56	4.55	-0.22	0.3281	0.33	0.58
20	15	0.5	12	36	11.35	11.37	0.18	0.8165	0.82	0.43
21	10	0.75	24	28	10.70	10.65	-0.47	0.7698	0.77	0.03
22	10	0.5	36	28	9.17	8.99	-1.96	0.6597	0.65	-1.47
23	15	0.5	36	36	12.78	13.02	1.88	0.9194	0.94	2.24
24	15	0.75	12	28	9.87	9.86	-0.10	0.7101	0.71	-0.01
25	10	0.5	24	36	12.31	12.30	-0.08	0.8856	0.88	-0.63
26	20	0.5	36	28	10.64	10.07	-5.36	0.7655	0.72	-5.94*
27	15	0.5	24	28	9.25	9.25	0.00	0.6655	0.6655	0.00
28	15	0.5	24	28	9.25	9.25	0.00	0.6655	0.6655	0.00
29	20	0.5	12	28	8.01	7.85	-2.00	0.5763	0.56	-2.83

The results of variance analysis of regression model with collagen extraction yield and extraction or reaction velocity as targets were exhibited in Table 3 and Table 4, respectively.

Table 3 Results of variance analysis of regression model (with extraction yield as target)

Source	Sum of square	Degree of freedom DF	Mean square MS	F value	Prob>F	Significance level
model	228.04	14	16.29	266.59	<0.0001	significant
X ₁	9.075×10 ⁻³	1	9.075×10 ⁻³	0.15	0.7057	
X ₂	39.20	1	39.20	641.65	<0.0001	
X ₃	4.34 ³	1	4.34 ³	71.10	<0.0001	
X ₄	177.25	1	177.25	2901.08	<0.0001	
X ₁ X ₂	6.250×10 ⁻⁴	1	6.250×10 ⁻⁴	0.010	0.9209	
X ₁ X ₃	1.04	1	1.04	17.03	0.0010	
X ₁ X ₄	3.600×10 ⁻³	1	3.600×10 ⁻³	0.059	0.8117	
X ₂ X ₃	0.044	1	0.044	0.72	0.4099	
X ₂ X ₄	2.66	1	2.66	43.48	<0.0001	
X ₃ X ₄	0.20	1	0.20	3.31	0.0901	
X ₁ ²	0.076	1	0.076	1.25	0.2831	
X ₂ ²	0.51	1	0.51	8.37	0.0118	
X ₃ ²	0.29	1	0.29	4.78	0.0464	
X ₄ ²	3.08	1	3.08	50.48	<0.0001	
Residual	0.86	14	0.061			
Lack of Fit	0.86	10	0.086			
Pure error	0.000	4	0.000			
Total variation	228.90	28				
R ²	0.9963					

Table 4 Results of variance analysis of regression model (with extraction velocity as target)

Source	Sum of square	Degree of freedom DF	Mean square MS	F value	Prob>F	Significance level
model	1.18	14	0.084	266.61	<0.0001	significant
X ₁	4.720×10 ⁻⁵	1	4.720×10 ⁻⁵	0.15	0.7050	
X ₂	0.20	1	0.20	641.84	<0.0001	
X ₃	0.022	1	0.022	71.10	<0.0001	
X ₄	0.92	1	0.92	2901.00	<0.0001	
X ₁ X ₂	3.240×10 ⁻⁶	1	3.240×10 ⁻⁶	0.010	0.9208	
X ₁ X ₃	5.388×10 ⁻³	1	5.388×10 ⁻³	17.04	0.0010	
X ₁ X ₄	1.849×10 ⁻⁵	1	1.849×10 ⁻⁵	0.058	0.8124	
X ₂ X ₃	2.280×10 ⁻⁴	1	2.280×10 ⁻⁴	0.72	0.4101	
X ₂ X ₄	0.014	1	0.014	43.51	<0.0001	
X ₃ X ₄	1.050×10 ⁻³	1	1.050×10 ⁻³	3.32	0.0899	
X ₁ ²	3.938×10 ⁻⁴	1	3.938×10 ⁻⁴	1.25	0.2832	
X ₂ ²	2.651×10 ⁻³	1	2.651×10 ⁻³	8.38	0.0117	
X ₃ ²	1.512×10 ⁻³	1	1.512×10 ⁻³	4.78	0.0462	
X ₄ ²	0.016	1	0.016	50.55	<0.0001	
Residual	4.427×10 ⁻³	14	3.162×10 ⁻⁴			
Lack of Fit	4.427×10 ⁻³	10	4.427×10 ⁻⁴			
Pure error	0.000	4	0.000			
Total variation	1.18	28				
R ²	0.9925					

The Design-expert 7.0 software was used to make the multiple regression fitting of the data in Table 2 to obtain the multiple regression models as below. The model (1) reflects the relations between extraction yield and the four factors of ratio of liquid/solid (X₁), hydrochloric acid concentration (X₂), extraction time (X₃) and extraction temperature (X₄). The model (2) reflects the relations between collagen extraction velocity and the four factors.

$$Y = -10.1266 - 0.0425X_1 - 0.3767X_2 - 0.0898X_3 + 0.8351X_4 - 10^{-2}X_1X_2 + 8.5000 \times 10^{-3}X_1X_3 - 7.5.0000 \times 10^{-4}X_1X_4 + 0.0350X_2X_3 + 0.4075X_2X_4 + 2.3438 \times 10^{-3}X_3X_4 - 4.3333 \times 10^{-3}X_1^2 - 4.4933X_2^2 - 1.4728 \times 10^{-3}X_3^2 - 0.01078X_4^2 \quad (1)$$

$$Y = -0.7286 - 3.0683 \times 10^{-3}X_1 - 0.0270X_2 - 6.4625 \times 10^{-3}X_3 + 0.0601X_4 - 7.2000 \times 10^{-4}X_1X_2 + 6.117 \times 10^{-4}X_1X_3 - 5.375 \times 10^{-5}X_1X_4 + 2.5167 \times 10^{-3}X_2X_3 + 0.0293X_2X_4 + 1.6875 \times 10^{-4}X_3X_4 - 3.1167 \times 10^{-4}X_1^2 - 0.3235X_2^2 - 1.0602 \times 10^{-4}X_3^2 - 7.7565 \times 10^{-4}X_4^2 \quad (2)$$

It is known from Table 3 that the model F-value of 266.59 implies the model is significant. There is only a less than 0.01% chance that “a Model F-value” this large could occur due to noise. The values of “Prob>F” less than 0.0001 indicate the model terms are very significant, in this case, X_2 , X_3 , X_4 , X_2X_4 and X_4^2 are very significant model terms. The values of “Prob>F” between 0.0001 and 0.0500 indicate the model terms are significant, in this case, X_1X_3 , X_2^2 , X_3^2 are significant model terms. The values of “Prob>F” between 0.0500 and 0.1000 indicate the model terms are general significant, in this case, X_2X_4 is general significant model term. The values of “Prob>F” greater than 0.1000 indicate the model terms are not significant, in this case, X_1 , X_1X_2 , X_1X_4 , X_2X_3 and X_1^2 are not significant model terms. The correlation coefficient of the model is $R^2=0.9963$, which means that the model can correctly describe the test results and only 0.37% of total variation of response value can not be explained by the model. Furthermore, as shown in the 8th column of “relative error” of Table 2, the model values of collagen extraction yields are close to those of the test and their relative errors are ranged from 0.00% to 5.36% (absolute values) within the test range, indicating that the model is accurate and can be used to predict and adjust the process parameters for obtaining the expected collagen extraction yield. The degrees of the effects of factor X_2 , X_3 and X_4 on the collagen extraction yield are larger than that of factor X_1 according to their “Prob>F” values in Table 3 and the order of their effects on collagen extraction yield are , according to the contour maps shown in Fig.1, determined to be $X_4 (D) > X_2 (B) > X_3 (C)$.

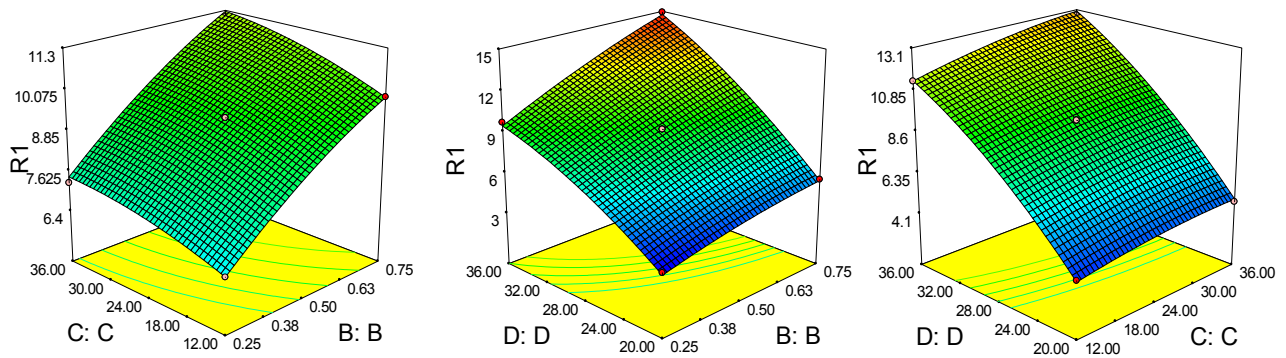


Fig.1 Response surface with extraction yield as target

It is known from Table 4 that the model F-value of 266.61 implies the model is significant. There is only less than 0.01% chance that “a Model F-value” this large could occur due to noise. According to the values of “Prob>F” in Table 4, X_2 , X_3 , X_4 , X_2X_4 , X_4^2 are very significant model terms, X_1X_3 , X_2^2 and X_3^2 are significant model terms, X_3X_4 is general significant model terms, X_1 , X_1X_2 , X_1X_4 , X_2X_3 and X_1^2 are not significant model term. The model “Prob>F” value less than 0.0001 indicate that the relations between the response value (collagen extraction velocity) and the four factors are extremely significant and the test results are reliable. The correlation coefficient of the model is $R^2=0.9925$, which means that the model can well describe the test results and only about 0.75% of total variation of response value can not be explained by the model. Furthermore, as shown in the 11th column of “relative error” of Table 4, the model values of collagen extraction velocity are basically close to those of the test and their relative errors are ranged from 0.00% to 5.94% (absolute values) within the test range, indicating that the model is basically agreed with the

practical values and can be used to predict and adjust the process parameters for obtaining the expected collagen extraction velocity. The degrees of the effects of factor X_2 , X_3 and X_4 on the collagen extraction yield are larger than that of factor X_1 according to their “Prob> F ” values in Table 4 and the order of their effects on collagen extraction yield are , according to the contour maps shown in Fig.1,determined to be $X_4(D) > X_2(B) > X_3(C)$.

The size of the effects of the four factors on the collagen extraction velocity can not be determined according to the values of “Prob> F ” because all their values of “Prob> F ” are <0.0001. However, it can be determined to be $X_4(D) > X_2(B) > X_3 > X_1(C)$ according to the contour maps shown in Fig.2.

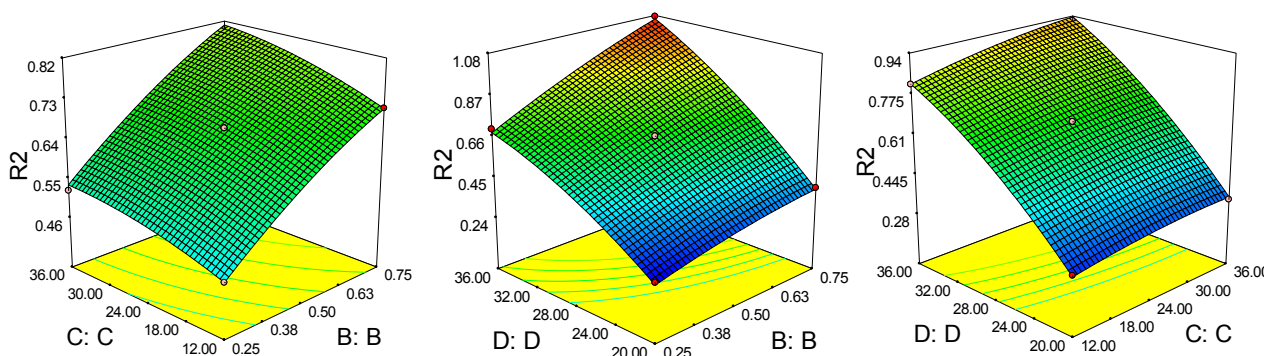


Fig.2 Response surface with extraction velocity as target

Conclusion

The process and kinetic models in the form of multiple regression for collagen extraction from bighead carp scale were established by response surface test. The models are basically in agreement with the test values. They can be applied to predict the collagen extraction yield and collagen extraction velocity under different operation conditions and the collagen extraction velocity corresponding to the collagen extraction yield under same conditions in order to adjust the operation parameters to gain the expected extraction yield and extraction velocity for realizing higher utilization efficiency of raw material and higher collagen production capacity at the same time.

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