Optimization and Models of Decalcification from Bighead Carp Scale with Citric Acid by Response Surface Method

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Abstract. Fish scale is a newly developed alternative resource for extracting collagen,which is one of the important biological materials. To extract collagen from fish scale has a great significance to prevent pollution and receive good economic benefits. In this paper, based on the experimental results of single factor test, the response surface method was used to further optimize the technical parameters for the decalcification of bighead fish scale and establish the process model and kinetic model. The results showed that the maximum calcium removal rate reached 93.40% under the optimal conditions of liquid/solid ratio 23.50, citric acid concentration 11.0%, decalcification time 66.3 min and magnetic steering speed 989r/min and the maximum decalcification velocity reached 241.86mg/(L min) under the optimal conditions of liquid/solid ratio 10.10, citric acid concentration 12.60%, decalcification time 31.6 min and magnetic steering speed 865r/min. The two models in the form of multiple regression were proved to basically agree with the test values and can be used to predict the calcium removal rate and decalcification velocity under different operation parameters and the decalcification velocity corresponding to the calcium removal rate under same parameters, to adjust the operation parameters for obtaining the expected calcium removal rate and decalcification velocity, and to control the production capacity of fish scale treatment and the collagen quality prepared by following collagen extraction from the decalcified fish scale.

Introduction

The scale of fish accounts for 3% -5% of the fish's total weight. The protein in fish scale is about 41% - 84% of total mass of scale. The collagen is the main ingredient in the protein.Therefore, the extraction of collagen from fish scales can not only reduce the pollution by the scales, but also obtain the cheap material in the manufacture of the collagen[1-4]. Collagen is a kind of biological macromolecule material. It plays an important organizational role in animal cells and so is one of the key raw material for the biotechnology industry and also has a huge demand in biomedical materials market. Collagen can be widely used in biomedical materials, cosmetics, food industry, research purposes, etc. In fish scale, collagen fibers are closely adhered with hydroxyapatite each other and is not easy to separate. So before extracting of collagen, the fish scale must be pretreated to remove hydroxyapatite (calcium). In recent years, many researchers have done the studies on fish scale decalcification with organic and inorganic acid as extracting agents. Most of them focused on the optimization of the process conditions. Decalcification of fish scale is a reaction between acid and salt. The process model reflects the relations between calcium removal rate (as target) and operation parameters(as influence factors). The kinetic model reflects the relations between decalcification velocity (as target) and operation parameters(as influence factors). These two models can predict and adjust the technical parameters to achieve the expected calcium removal rate and decalcification velocity under different operation conditions, which is conductive to the control

of the production capacity of fish scale treatment and collagen quality. In this paper, based on the results of single factor test in our previous research work[5]. Response surface method with citric acid as extracting agent was used to further optimize the operation parameters and establish the process and kinetic models of decalcification from bighead fish scale and validate the models with the test values.

Experiment

Raw Materials

Bighead carp scales: collected from local market. The scales were cleaned and dried for use .
Chemicals: calcium carbonate, EDTA, citricacid, chrome black T, hydroxylamine hydrochloride, ethanol, ammonium chloride and ammonia. All the above are analytically pure.
Standard regression equation of calcium concentration
2.7622 g anhydrous calcium chloride, dried to constant weight at 110℃, was accurately weighed and added into a 500 mL volumetric flask to be diluted to scale.for preparing the standard calcium solution with concentration of 0.105 mol/L. Then 0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mL of standard calcium solutions above were respectively added into a 150 conical flasks and diluted to 20.0 mL with distilled water. After that, 15 mL ammonia buffer (pH = 10) and 2 drops of chrome black T indicator were added into the flasks. The solution was measured by titration with 0.01007 mol/L EDTA standard solution until the color of the solution was changed from purple to blue.The consumed volume of EDTA was recorded. This measurement was conducted for two times in parallel. Then, the calcium standard curve was drawn with consumed volume of EDTA as the abscissa and calcium ion concentration as the ordinate[6]. The standard curve equation was then obtained as follows.

$$y = -0.00976 + 0.02143\ x \tag{1}$$

Measurement of calcium content
See literature[5].
Decalcification process of fish scale
See literature [5].
Measurement of calcium removal rate
See literature [5].
Measurement of decalcification velocity
The decalcification velocity of fish scale is defined as mass of decalcified calcium per unit volume and per unit time, g/(L min), which is calculated by following equation:

$$v = \frac{x \times m_1}{V \times \tau}\quad (g/(L\ min)) \tag{2}$$

Where V is the volume of the citric acid solution, mL; τ is the time of decalcification, min; x is the mass fraction in fish scale, 10,5%.

Establishment of Models

The suitable operation parameters for decalcification of bighead fish scale were determined by single factor test[5]: liquid(citric acid solution)/solid(fish scales) ratio 20 mL/g, citric acid concentration 10%, decalcification time 60 min and magnetic stirring speed 900 r/min. Under the suitable conditions, the calcium removal rate reached 92.57%. Based on the results of single factor test, the response surface test was carried out in this experiment. The parameters concerned above were taken as investigation factors and calcium removal rate and decalcification velocity were taken as the targets. The design of four factors with three levels was listed in Tab.1.

Tab.1 Test design of three factors/three levels for response surface method

factor	code	levels -1.00	levels 0.00	levels 1.00
Ratio of liquid/solid/mL/g	X_1	15	20	25
Citric acid concentration/%(mass)	X_2	7.5	10	12.5
Decalcification time /min	X_3	40	60	80
Steering speed/r/min	X_4	800	900	1000

Tab.2 Results of response surface method

Order No.	X_1	X_2	X_3	X_4	calcium removal rate Test	calcium removal rate Model	calcium removal rate relative error %	decalcification velocity Test g/(L·min)	decalcification velocity Model	decalcification velocity relative error %
1	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
2	20	15	60	900	92.58	93.14	0.06	81.00	76.27	-5.84
3	25	7.5	80	800	89.79	90.64	0.95	47.14	44.13	-0.023
4	15	12.5	80	1000	90.67	91.23	0.62	79.34	77.89	-1.83
5	25	12.5	40	1000	90.67	90.91	0.26	95.29	101.61	6.63
6	15	7.5	80	1000	88.81	89.50	0.78	77.71	76.33	-1.78
7	20	10	60	700	91.67	90.33	-1.46[*]	80.21	74.78	-6.77[*]
8	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
9	25	7.5	40	1000	88.82	89.03	0.24	93.26	99.70	6.91
10	15	7.5	40	1000	86.99	87.35	0.41	152.23	160.70	5.56
11	25	12.5	80	1000	92.58	91.80	-0.84	48.60	44.98	-7.45
12	15	12.5	40	800	87.95	88.31	0.40	153.91	162.24	5.41
13	15	12.5	80	800	89.79	90.48	0.77	78.57	77.21	-1.73
14	20	5	60	900	89.72	88.37	-1.50[*]	78.51	72.94	-7.09[*]
15	25	12.5	40	800	89.80	90.17	0.41	94.29	100.75	6.85
16	25	7.5	40	800	87.96	88.47	0.58	92.36	98.88	7.06
17	25	7.5	80	1000	90.66	91.37	0.78	47.60	44.34	-6.85
18	25	12.5	80	800	91.68	92.39	0.77	48.13	44.73	-7.06
19	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
20	15	7.5	40	800	86.15	86.65	0.58	150.76	159.46	5.77
21	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
22	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
23	20	10	60	900	92.57	92.57	0.00	81.00	81.00	0.00
24	10	10	60	900	88.77	87.73	-1.17[*]	155.35	146.34	-5.80[*]
25	20	10	60	1100	92.58	90.84	-1.88[*]	81.00	76.39	-5.69[*]
26	20	10	20	900	86.87	86.48	-0.45[*]	228.03	203.44	-10.78[*]
27	20	10	100	900	92.58	91.79	-0.85[*]	48.60	63.04	29.71[*]
28	15	7.5	80	800	87.94	88.77	0.94	76.95	75.70	-1.62
29	30	10	60	900	92.56	91.47	-1.07[*]	54.00	52.85	-2.13[*]
30	15	12.5	40	1000	88.82	89.19	0.42	155.44	163.53	5.20

According to the design above, 30 sets of test (including 5 sets of central group tests) were separately conducted when the calcium removal rate and decalcification velocity were taken as targets and the results were listed in Tab.2.

The results of variance analysis of regression model with calcium removal rate and decalcification velocity as targets were exhibited in Tab.3 and Tab.4, respectively.

Tab.3 Result of variance analysis of regression model (with calcium removal rate as target)

Source	Sum of square	Degree of freedom DF	Mean square MS	F value	Prob$>F$	Significance level
model	111.83	14	7.99	7.75	0.0002	significant
X_1	20.94	1	20.94	20.31	0.0004	
X_2	17.61	1	17.61	17.08	0.0009	
X_3	28.56	1	28.56	27.70	<0.0001	
X_4	3.21	1	3.21	3.12	0.0979	
X_1X_2	1.600×10^{-3}	1	1.600×10^{-3}	1.552×10^{-3}	0.9691	
X_1X_3	1.600×10^{-3}	1	1.600×10^{-3}	1.552×10^{-3}	0.9691	
X_1X_4	1.000×10^{-4}	1	1.000×10^{-4}	9.699×10^{-5}	0.9923	
X_2X_3	2.500×10^{-3}	1	2.500×10^{-3}	2.452×10^{-3}	0.9614	
X_2X_4	4.000×10^{-4}	1	4.000×10^{-4}	3.880×10^{-4}	0.9845	
X^3X_4	4.000×10^{-4}	1	4.000×10^{-4}	3.880×10^{-4}	0.9845	
X_1^2	15.13	1	15.13	14.68	0.0016	
X_2^2	10.59	1	10.59	10.27	0.0059	
X_3^2	26.22	1	26.22	25.43	0.0001	
X_4^2	3.91	1	3.91	3.80	0.0704	
Residual	15.46	15	1.03			
Lack of Fit	15.46	10	1.55			Not significant
Pure error	0.000	5	0.000			
Total variation	127.30	29				
R^2	0.8785					

The Design-expert 7.0 software was used to make the multiple regression fitting of the data in Tab.2 to obtain the multiple regression models as below. The model (3)with calcium removal rate as target reflects the relations between calcium removal rate and factors of ratio of liquid/solid(X_1), citric acid concentration(X_2), decalcification time(X_3) and steering speed (X_4). The model (4)with decalcification velocity as target reflects the relations between decalcification velocity and factors of ratio of liquid/solid(X_1), citric acid concentration(X_2), decalcification time(X_3) and steering speed (X_4).

$$Y=18.4563+1.3567X_1+2.2863X_2 + 0.3411X_3 + 0.0712X_4 + 8.0000\times10^{-4}X_1X_2 + 1.0000\times10^{-4}X_1X_3+5.0000\times10^{-6}X_1X_4+2.5000\times10^{-4}X_2X_3+2.0000\times10^{-5}X_2X_4+2.5000\times10^{-6}X_3X_4-0.0297X_1^2-0.0994X_2^2 - 2.4443\times10^{-3}X_3^2 -3.7771\times10^{-5}X_4^2 \tag{3}$$

$$Y=400.8988 - 16.0914X_1+6.1055X_2 - 6.9912X_3 + 0.2583X_4 - 0.01838X_1X_2 + 0.0725X_1X_3 - 2.150\times10^{-4}X_1X_4 -6.350\times10^{-3}X_2X_3 + 4.500\times10^{-5}X_2X_4 - 7.6252\times10^{-5}X_3X_4 + 0.1860X_1^2 - 0.2528X_2^2 + 0.0326X_3^2 - 1.3677\times10^{-4}X_4^2 \tag{4}$$

Tab.4 Results of variance analysis of regression model (with decalcification velocity as target)

Source	Sum of square	Degree of freedom DF	Mean square MS	F value	Prob>F	Significance level
model	49133.68	14	3509.55	35.11	<0.0001	significant
X_1	13110.57	1	13110.57	131.15	<0.0001	
X_2	17.58	1	17.58	0.18	0.6809	
X_3	29565.43	1	29565.43	295.75	<0.0001	
X_4	3.33	1	3.33	0.033	0.8576	
X_1X_2	0.84	1	0.84^3	8.375×10^{-3}	0.9283	
X_1X_3	841.58	1	841.58	8.42	0.0110	
X_1X_4	0.18	1	0.18	1.807×10^{-3}	0.9667	
X_2X_3	1.61	1	1.61	0.016	0.9006	
X_2X_4	2.025×10^{-3}	1	2.025×10^{-3}	2.026×10^{-5}	0.9965	
X_3X_4	0.37	1	0.37	3.722×10^{-3}	0.9522	
X_1^2	593.02	1	593.02	5.93	0.0278	
X_2^2	68.49	1	68.49	0.69	0.4208	
X_3^2	4678.17	1	4678.17	46.80	<0.0001	
X_4^2	51.31	1	51.31	0.51	0.4847	
Residual	1499.52	15	99.97			
Lack of Fit	1499.52	10	149.95			Not significant
Pure error	0.000	5	0.000			
Total variation	50633.20	29				
R^2	09704					

It is known from Table 3 that the model F-value of 7.751implies the model is significant. There is only a 0.02% chance that "a Model F-value"this large could occur due to noise.The values of " Prob＞F" less than 0.0500 indicate the model terms are significant, in this case, X_1,X_2, X_3, X_1^2, X_2^2 and X_3^2 are significant model terms. The values of " Prob＞F" greater than 0.1000 indicate the model terms are not significant, in this case, X_4, X_1X_2, X_1X_3, X_1X_4,X_2X_3,X_2X_4,X_3X_4 and X_4^2 X_2X_3 are not significant model terms. The correlation coefficient of the model is R^2=0.8785, which means that the model can basically describe the test results and only 12.15% of total variation of response value cannot be explained by the model. Furthermore, as shown in the 8th column of "relative error" of Table 2, the actual test values of calcium removal rate are close to those of the model and their relative errors are ranged from 0.00% to 0.95% (absolute values)within the value range which was taken in the response surface experiment except the values marked with "*" , indicating that the model is accurate and can be used to predict and adjust the process parameters for obtaining the expected calcium removal rate. According to the "Prob＞F" values of X_1, X_2, X_3 and X_4 in Table 3, the significance of the effects of the four factors on the calcium removal rate can be determined to be $X_3>X_1>X_2>X_4$. The technical parameters were further optimized to be liquid/solid ratio 23.50, citric acid concentration 11.0%, decalcification time 66.3 min and steering speed 989r/min by the solution of the model (3) with the software. Under the optimal conditions, the maximum calcium removal rate was 93.40%.

It is known from Table 4 that the model F-value of 35.11 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. The values of " Prob＞F" less than 0.0500 indicate the model terms are significant, in this case, X_1, X_3, X_1X_3, X_1^2 and X_3^2 are significant model terms. The values of " Prob＞F" greater than 0.1000

indicate the model terms are not significant, in this case, X_2, X_4, X_1X_2, X_1X_4, X_2X_3, X_2X_4, X_3X_4, X_2^2, and X_4^2 are not significant model terms. The model " Prob＞F" value less than 0.0001 indicate that the relations between the response value (decalcification velocity) and the four factors are extremely significant and the test results are reliable..The correlation coefficient of the model is $R^2=0.9704$, which means that the model can well describe the test results and only about 3.0% of total variation of response value cannot be explained by the model. Furthermore,as shown in the 11th column of "relative error" of Table 2, the actual test values of decalcification velocity are basically close to those of the model and their relative errors are ranged from 0.00% to 7.457% (absolute values) within the value range which was taken in the response surface experiment except the values marked with "*" , 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 decalcification velocity.According to the "Prob＞F" values of X_1, X_2, X_3 and X_4 in Table 4, the significance of the effects of the four factors on the decalcification velocity can be determined to be $X_3> X_1>X_2>X_4$, which is same as those for the target of calcium removal rate. The technical parameters were further optimized to be liquid/solid ratio 10.10, citric acid concentration 12.60%, decalcification time 31.6 min and steering speed 865r/min by the solution of the model (4) with the software. Under the optimal conditions, the maximum decalcification velocity was 241.86 mg/(L min).

Conclusions

The technical parameters were further optimized by response surface method.The optimal parameters were determined to be liquid/solid ratio 23.50, citric acid concentration 11.0%, decalcification time 66.3 min and steering speed 989r/min under which the maximum calcium removal rate was 93.40% and liquid/solid ratio 10.10, citric acid concentration 12.60%, decalcification time 31.6 min and steering speed 865r/min, under which the maximum decalcification velocity reached 0.4932g/(L min).The models in the form of multiple regression were basically in agreement with the test values. They can be applied to predict the calcium removal rate and decalcification velocity under different operation conditions and the decalcification velocity corresponding to the calcium removal rate under same conditions in order to adjust the operation parameters for gaining the expected calcium removal rate and decalcification velocity and to control the production capacity of fish scale treatment and the collagen quality and collagen production output in the following collagen extraction from the decalcified fish scale.

Acknowledgements

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