Study of the Optimization of the Ultrasonic Extraction Technology of Gelatin from the Scales of *Aristichthys Nobilis* by Means of Quadratic Regression Orthogonal Optimization

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Abstract: In order to reasonably use of the *aristichthys nobilis* scale resources, gelatin was extracted from the scales of *aristichthys nobilis* with the aid of ultrasonic method. The ultrasonic power, time, temperature and the ratio of material to solvent on extraction of gelatin from *aristichthys nobilis* scales were studied. The ultrasonic extraction conditions of gelatin from *aristichthys nobilis* scales were optimized by quadratic regression orthogonal test based on single factor experiments. The results showed that the optimum conditions were ultrasonic temperature of 72 °C, ultrasonic time of 121 min, ultrasonic power of 300 W, the ratio of solid of solvent 1:11 (g/mL), and the extraction rate of gelatin from *aristichthys nobilis* scale was 48.58% under optimal extraction conditions.

Key words: Quadratic regression orthogonal, scale, gelatin, extraction.

1. Introduction

Scales are the by-products of fishing farming and usually thrown away as the wastes, which wastes resources and pollutes the environment. The results of study indicated that there is a large amount of gelatin. With the wreak havoc of all kinds of infectious diseases such as mad cow disease and foot-and-mouth disease carried by such animals as cows and pigs, foreign countries have banned the production of food and medicines from the raw materials isolated from cows [1]. Extracting gelatin from scales has eased the shortage of gelatin supply in the markets. Gelatin, acting as an important biomacromolecules, is widely used in the fields of food, medicines, light-sensitive materials, chemical engineering and so on because of its of hot reversible feature of sol-gel.

Gelatin acting as the receptor of halogen has the

irreplaceable function by any other mediums in the preparation of emulsion. Such amino acids as tyrosine, histidine, methiodine in gelatin all can react with halogen, making the crystals of silver halides in emulsion equally and stably scatter in the mediums. The trace of active impurities and metal ions can improve the emulsion photographic property by several times, even by tens of times [2]. Using auxiliary ultrasonic method to assist in extracting gelatin from *aristichthys nobilis* scales is both efficient, environmental friendly and saving resources. At the same time, the ultrasonic cavitation and mechanical action are both beneficial to the degradation of collagen in the scales to gelatin to improve the extraction efficiency of scales gelatin.

In this paper, quadric regression orthogonality was adopted to optimize the ultrasonic extracting conditions of *aristichthys nobilis* scales so as to provide theoretical references to the more application

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of the gelatin of *aristichthys nobilis* scales.

2. Experimental Materials and Methods

2.1 Experimental Materials and Instruments

Aristichthys nobilis scales (collected from certain agricultural products markets), hydrochloric acid, sodium hydroxide, citric acid, sodium acetate, paradime thylaminobenzaldehyde, perchloric acid, chloramine-T and magnesium acetate used are analytically pure.

KQ3200 Supersonic Cleaner, made in Kunshan Ultrasonic Instrument Limited Company; LD5-10 Centrifugal Machine, made in Beijing Jingli Centrifugal Machine Limited Company; GR-200 Electronic Scales, made in Japan AND; HH-6 Digital Readout Thermostat Water Bath, Made in Shanghai Jiazhan Instrument and Equipment Limited Company; WXL-5 Intelligent Muffle Furnace, made in Hebi Tianhong Instrument Limited Company; Cary50 Ultraviolet Visible and Visible Spectrophotometer, made in Varian Inc. U.S.A.; Jelly Strength Tester, made in Shanghai Precise Instrument Limited Company.

2.2 Pretreatment of Scales [3]

The scales of *aristichthys nobilis* contain mineral substances, moisture and other impure proteins, which interfere with the extraction of gelatin. So purification was conducted. First of all the scales were cleaned, dried by airing and cut up. Then the samples were put into the solution of 1.0 mol/L HCl (The ratio of solution to the samples was 10:1 ml/g) for 30 min. After that, they were filtered with gauze. This process was repeated twice. Finally, the scales were cleaned by pure water to eliminate the mineral substances from scales and prepare them for use. The purified samples were added with 0.1 mol/L NaOH solution (The ratio of solution to the samples was 10:1 mL/g), stirred and soaked for 6 h, and the solution was changed every 3 h. The treated scales were cleaned by pure water until the solution presented neutral. Then the impure proteins were removed and they were kept

at the constant temperature of below 30 °C to prepare for use.

2.3 Content Determination of Gelatin in Scales

The samples of scales were hydrolyzed in 6 mol/L HCl solution at 105 °C for 6 h. The contents of the extracted hydroxyproline from scales were measured by means of colorimetric method and then the hydroxyproline in the extracted gelatin were measured. Their specific values were used as the extraction ratio of scale gelatin. The compute mode of extraction ratio was given as follows.

Extraction ratio (%) = (the contents of hydroxyproline in the extracted scale gelatin/the contents of hydroxyproline in the scales samples) × 100%.

2.4 Quality Determination of Scale Gelatin [5]

According to the Chinese standard GB 6783-2013, preparing mass fraction 6.67% of gelatin solution sample 360 mL, which was equally divided into 3 shares. After being cooled for 18 h in cryostat at 10 ± 0.1 °C, they were quickly put on resistance dynamometer circular truncated cone. 4 mm was selected on the “depth” of resistance dynamometer, and 0.5 mm/s was selected on “speed” to determine the intensity of congelation. Testing the samples was completed within 2 min. The average values of three tests were taken as the final determination results of congelation intensity, and the expressed unit was Bloom g.

According to the Chinese standard GB 6783-2013, for the extracted gelatin from *aristichthys nobilis* scales, such three indexes as moisture content, ash content and transmittance were tested. The average value of the results of three tests were taken as the final determined result to assess the quality of gelatin.

2.5 Experimental Design

2.5.1 Single Factor Experiment Design

In the experiment, ultrasonic temperature,

ultrasonic wave duration, ultrasonic wave power and the ratio of material to solution were selected as the influence factors in extracting gelatin from aristichthys nobilis scales. According to the principle of control variable method, setting other conditions invariable and the influence level on a certain factor by a single change explored into the influence on the ratio of extracting gelatin, which laid a basis for the quadratic regression optimal experiment.

2.5.2 Quadratic Regression Optimal Experiment Design

On the basis of single factor, ultrasonic wave temperature, ultrasonic wave duration, ultrasonic wave power and ration of material to solution were selected as the investigated objects and the extracting ratio of scale gelatin as the response value. According to the principle of quadratic regression center combination experiment design, the experiment design of 4 factors and 5 levels was selected. Table 1 shows factors and levels of experiment design.

3. Experiment Results and Analysis

3.1 Results of Single Factor Experiment and Analysis

3.1.1 Influence of the Temperature of Ultrasonic Waves on Extracting Gelatin from Aristichthys Nobilis Scales

1.0000 gram prepared aristichthys nobilis scales was precisely weighed up, which had been hydrolyzed for 6 h in 6 mol/L HCl solution at 105 °C and dried up at the normal temperature, the ultrasonic wave duration was set at 120 min, ultrasonic wave power was 200 W and the ratio of materials to solution was 1:10 (g/mL) to conduct ultrasonic extraction. According to the method in step 2.3, it is feasible to

compute the extraction efficiency of gelatin to examine the influence of different ultrasonic wave temperatures on the extracting efficiency of gelatin from aristichthys nobilis scales (Fig. 1).

Through degradation, the collagen in the scales could be extracted in the structure of gelatin. High temperature was beneficial to the degradation of collagen. So, in the range of rising temperature between 50 ~ 80 °C, the extracting efficiency could become higher with the constant rise of the temperature. But with the continuous rise of the temperature, the extracting efficiency would become lower, which was probably because the high temperature had made some gelatin deteriorated and at the same time increased the micromolecular quantity which had been excessively degenerated to make the purity of the extracted gelatin deteriorated. In view of the above, 80 °C was selected as a better temperature for ultrasonic extraction.

3.1.2 Influence of the Duration of Ultrasonic Wave on the Extracting Efficiency from Aristichthys Nobilis Scales

1.0000 gram prepared aristichthys nobilis scales was precisely weighed up, which had been hydrolyzed for 6 h in 6 mol/L HCl solution at 105 °C and dried up at the normal temperature, the ultrasonic wave temperature was set at 70 °C, the ultrasonic wave power at 200 W and the ratio of materials to solution was 1:10 (g/mL) to conduct ultrasonic extraction. According to the method in step 2.3, it is feasible to compute the extracting efficiency of gelatin to examine the influence of different ultrasonic wave durations on the extracting efficiency of gelatin from aristichthys nobilis scales. Fig. 2 shows the results.

Table 1 Factors and levels of the experiment design.

Levels	X ₁ Ultrasonic wave temperature (°C)	X ₂ Ultrasonic wave duration (min)	X ₃ Ultrasonic wave power (W)	X ₄ Ratio of materials to solution (g/mL)
-2	50	60	100	1:6
-1	60	90	200	1:8
0	70	120	300	1:10
1	80	150	400	1:12
2	90	180	500	1:14

Fig. 1 Influence of ultrasonic wave temperatures on extracting efficiency of gelatin from aristichthys nobilis scales.

Fig. 2 Influence of different ultrasonic wave durations on the extracting efficiency of gelatin from aristichthys nobilis scales.

With the prolonging of the extracting duration, the cavitation effects and mechanical functions produced by ultrasonic waves become stronger and stronger. The high temperature and high pressure produced were easy to make the covalent bond and the molecular chain of the fiber break to accelerate the degradation of the fish scale collagen protein and to constantly increase the extracting efficiency of gelatin. Between the extracting duration of 120 ~ 150 min, the increase of extraction efficiency was not obvious, which illustrated that within this range, the extraction of gelatin tended to be complete. While the extraction

duration was longer than 150 min, the extracting efficiency became lower. By overall consideration of extracting cost and energy consumption, selection of 120 min was a better ultrasonic wave duration.

3.1.3 Influence of Ultrasonic Wave Power on the Extraction Efficiency of Gelatin from *Aristichthys Nobilis* Scales

1.0000 gram prepared aristichthys nobilis scales was precisely weighed up, which had been hydrolyzed for 6 h in 6 mol/L HCl solution at 105 °C and dried up at the normal temperature, the ultrasonic wave temperature was set at 70 °C, the ultrasonic wave duration at 120 min and the ratio of materials to solution was 1:10 (g/mL) to conduct ultrasonic extraction. According to the method in step 2.3, it is practical to calculate the extraction efficiency of gelatin to examine the influence of different ultrasonic wave durations on the extracting efficiency of gelatin from aristichthys nobilis scales. Fig. 3 shows Influence of ultrasonic wave power on the extracting efficiency of gelatin from aristichthys nobilis scales.

Ultrasonic wave power directly decided the strength of its cavitation effect and mechanical functions. From Fig. 3, it could be learnt that with the increase of the ultrasonic wave power, the extraction efficiency notably increased within the range of 100 ~ 300 W, which illustrated that within this range, most collagens

Fig. 3 Influence of ultrasonic wave power on the extracting efficiency of gelatin from aristichthys nobilis scales.

were degraded as gelatin with the increase of ultrasonic wave power and small part of collagens continued to degrade with the reinforcement of cavitation effects and mechanical functions. The bigger the ultrasonic waves, the easier to increase attenuation of scattering to form sound barrier to weaken the extraction efficiency. In view of the above, 300 w was selected as a better ultrasonic wave power.

3.1.4 Influence of the Ratio of Materials to Solution on the Extraction Efficiency of Gelatin from *Aristichthys Nobilis* Scales

1.0000 gram prepared *aristichthys nobilis* scales was precisely weighed up, which had been hydrolyzed for 6 h in 6 mol/L HCl solution at 105 °C and dried up at the normal temperature, the ultrasonic wave temperature was set at 70 °C, the ultrasonic wave duration at 120 min and the ultrasonic wave power was at 200 W to conduct ultrasonic extraction. According to the method in step 2.3 it can be realized to calculate the extraction efficiency of gelatin to check the effects of different ratios of materials to solution on the extracting efficiency of gelatin from *aristichthys nobilis* scales (Fig. 4). Influence of the ratios of materials to solution on the extracting efficiency of gelatin from *aristichthys nobilis* scales.

With the increase of the proportion of extractant, the dissolution space of gelatin was enlarged, which was

Fig. 4 Influence of the ratios of materials to solution on the extracting efficiency of gelatin from *aristichthys nobilis* scales.

beneficial to the extraction of gelatin. From Fig. 4, it could be learnt that the extraction efficiency of gelatin from *aristichthys nobilis* scales was between 1:6 ~ 1:12 g/mL. With the increase of the ratios of materials to solution, the range was enlarged. After that, the extraction efficiency basically remained the same. When the ratio of materials to solution was 1:14 g/mL, the extraction efficiency was the biggest. But compared with 1:12 g/mL, the increase of extraction efficiency was not obvious. Through overall consideration of the extraction cost and the purification process in later phase, 1:12 g/mL was a better choice.

3.2 Analysis of the Quadratic Regression Orthogonal Experiment

In order to optimize the ultrasonic wave extracting process of gelatin from *aristichthys nobilis* scales, on the basis of single factor and according to the principle of quadratic regression orthogonal experiment, the experiment designs were optimized. The experiment designs were worked out according to Table 1 and see Table 2 for the results of the experiments. There were 36 experimental points in Table 2, in which 1 ~ 24 were factorial experiments, which a comprehensive analysis of the influence of the intercombination of each factor on the extraction efficiency of gelatin from *aristichthys nobilis* scales and 25 ~ 36 were zero experiment, which examined the reliability of the errors and models of quadratic orthogonal models.

3.3 Analysis of Variance

The RsReg software package of SAS8.01 statistical software was adopted to conduct quaternary non-linear regression analysis (polynomial regression analysis) of the data in 36 groups. Fig. 3 shows the results of analysis of variance and for the regression equation analysis in Fig. 4.

It could be learnt from Fig. 3 that because of the four factors of ultrasonic wave temperature, ultrasonic

Table 2 Results of experiments.

Experiments	X ₁	X ₂	X ₃	X ₄	Y
1	-1	-1	-1	-1	28.97
2	-1	-1	-1	1	33.85
3	-1	-1	1	-1	31.07
4	-1	-1	1	1	31.67
5	-1	1	-1	-1	33.73
6	-1	1	-1	1	37.47
7	-1	1	1	-1	39.13
8	-1	1	1	1	40.61
9	1	-1	-1	-1	38.79
10	1	-1	-1	1	44.88
11	1	-1	1	-1	37.35
12	1	-1	1	1	39.16
13	1	1	-1	-1	35.44
14	1	1	-1	1	37.42
15	1	1	1	-1	39.28
16	1	1	1	1	39.97
17	-2	0	0	0	29.61
18	2	0	0	0	40.79
19	0	-2	0	0	35.94
20	0	2	0	0	39.54
21	0	0	-2	0	35.61
22	0	0	2	0	38.26
23	0	0	0	-2	38.25
24	0	0	0	2	43.82
25	0	0	0	0	48.16
26	0	0	0	0	47.22
27	0	0	0	0	47.36
28	0	0	0	0	47.27
29	0	0	0	0	48.69
30	0	0	0	0	48.89
31	0	0	0	0	47.34
32	0	0	0	0	47.11
33	0	0	0	0	48.07
34	0	0	0	0	47.57
35	0	0	0	0	48.82
36	0	0	0	0	48.37

wave duration, ultrasonic wave power and the ratio of materials to solution, the extraction of gelatin from all achieved a very notably statistical level of 0.01, which had a highly obvious influence on extracting gelatin from aristichthys nobilis scales. What was reflected in Test F is the validity of the regression model. The bigger the value of F was, the more notable the influence of this factor was to the extraction efficiency. As a result, the influence sequence of the four factors were ultrasonic wave temperature > the ratio of

materials to solution > ultrasonic wave duration > ultrasonic wave power. Each quadratic component got to the very notably statistical level of 0.01. The interaction between X₁ and X₃ reached the notably statistical level of 0.05. In the interactions, X₁X₂, X₂X₃ and X₃X₄ got to the very notably statistical level of 0.01. So, in the practical extraction process, each factor should be strictly controlled. When one of the conditions changed, other conditions should be regulated so as to ensure a high extraction efficiency and at the same time to lower the extraction costs.

Pr value in the regression model was less than 0.01, which illustrated that the model was very remarkable. Thus, the regression equation could be used to replace the true points to analyze the results of experiment. From Table 4, it could be learnt that the mean sum of square of the total model reached 98.76%, that is, the changes of all independent variables in the regression equation could be explained as 98.76% changes of dependent variables. There were comparatively more influential factors in the process of extracting gelatin from aristichthys nobilis scales while the equation only included 4 variables and the random errors in the experiment were inevitable. At the same time, the lack-of-fit item value of Pr was 0.0726, bigger than 0.05. So, the lack-of-fit factors did not exist. As a result, this model could be used to anticipate and analyze the experimental results. The fitted equation was given as follows:

$$Y = 47.90583 + 2.422917 X_1 + 1.02125 X_2 + 0.54125 X_3 + 1.350417 X_4 - 3.331146 X_1^2 - 2.090625 X_1X_2 - 0.576875 X_1X_3 - 0.008125 X_1X_4 - 2.696146 X_2^2 + 1.385625 X_2X_3 - 0.343125 X_2X_4 - 2.897396 X_3^2 - 0.756875 X_3X_4 - 1.872396 X_4^2.$$

The Monomial coefficient of the fitted equation was positive while all the quadratic coefficients were negative, which illustrated that these four factors have the positive effects on extracting gelatin from aristichthys nobilis scales and the fixed values made the percent conversion maximum. The anticipated best values of SAS software were X₁ = 0.174916, X₂ = 0.018424, X₃ = 0.010696 and X₄ = 0.176077,

Table 3 Analysis of variance of the experimental results.

Sources of variation	Degree of Freedom	Sum of squares deviations	Mean square deviation	F	Pr > F
X ₁	1	140.8926	140.8926	181.0296	0.0001
X ₂	1	25.03084	25.03084	32.16153	0.0001
X ₃	1	7.030837	7.030837	9.033758	0.006735
X ₄	1	43.767	43.767	56.23519	0.0001
X ₁ ²	1	355.089	355.089	456.2456	0.0001
X ₁ *X ₂	1	69.93141	69.93141	89.85322	0.0001
X ₁ *X ₃	1	5.324556	5.324556	6.841397	0.016154
X ₁ *X ₄	1	0.001056	0.001056	0.001357	0.970961
X ₂ ²	1	232.6145	232.6145	298.8809	0.0001
X ₂ *X ₃	1	30.71931	30.71931	39.47051	0.0001
X ₂ *X ₄	1	1.883756	1.883756	2.420394	0.134708
X ₃ ²	1	268.6369	268.6369	345.1652	0.0001
X ₃ *X ₄	1	9.165756	9.165756	11.77686	0.002504
X ₄ ²	1	112.1877	112.1877	144.1474	0.0001
Models	14	1,302.275	93.01966	119.5188	0.0001
Errors	21	16.34398	0.778285		
Total	35	1,318.619			

Table 4 Analysis of regression equations.

Sources of variation	Degree of Freedom	Sum of squares deviations	Mean square deviation	F	Pr
Once migration	4	216.721283	0.1644	69.62	< 0.0001
Quadratic component	4	968.528118	0.7345	311.11	< 0.0001
Interactive items	6	117.025838	0.0887	25.06	< 0.0001
Total models	14	1,302.2239	0.9876	119.62	< 0.0001
Lack-of-fit Tests	10	11.378892	1.137889	2.25	0.0726
Pure errors	11	4.965092	0.451372		
Total variance	21	16.343983	0.778285		

the maximum value of anticipated extraction was 48.592021% and the maximum value was at stationary point.

The anticipated optimum value of the model was substituted into Table 1 to acquire the such optimal technological conditions as the ultrasonic wave temperature was 71.74916 °C, the ultrasonic wave duration was 120.55272 min, the ultrasonic wave power was 310.696 W and the ratio of materials to solution was 1:10.352154 g/mL. For the convenient practical operation, ultrasonic wave temperature of 72 °C, ultrasonic wave duration of 121 min, ultrasonic wave power of 300 W and the ratio of material to solution of 1:11 g/mL were selected. Under these conditions to conduct confirmatory experiments of three times in parallel, the extraction efficiency were

48.54%, 48.58% and 48.62%, respectively. The average value of 48.58% was taken as the optimal extraction efficiency of gelatin from aristichthys nobilis scales, which had an error of 0.024% compared with the experimental value.

4. Determination of the Properties of the Gelatin Extracted from Aristichthys Nobilis Scales

The intensity of gelatin solution is an important index to evaluate the quality of gelatin and also decides the applications of gelatin. Gelatin has a wide application and different applied ranges have different demands on the intensity of congelation of gelatin. So, determining the properties of gelatin is of great significance. According to the methods in 2.4, the

Table 5 Results of the properties of gelatin of *aristichthys nobilis* scales.

Determination indexes	First share	Second share	Third share	Average value	Chinese standard
Intensity of congelation (Bloom g)	74	70	72	72	≥ 50
Moisture content (%)	11.6	11.9	11.3	11.6	≤ 14
Ash content (%)	0.98	1.05	1.12	1.05	≤ 2
(450 nm, 6.67%)/%	51.6	51.9	51.2	51.5	≥ 30

intensity of congelation, moisture content, ash content and the ratio of transmittance of the gelatin of *aristichthys nobilis* scales extracted through the optimal technology were determined. See Table 5 for the results.

Table 5 showed that the results of determining all the indexes of *aristichthys nobilis* scales gelatin meet the requirements of Chinese standard, among which the intensity of congelation and transmittance were far bigger than those in Chinese standard. In the practical process of extraction, technological parameters could be adjusted to obtain the needed gelatin products on the basis of different operational requirements in combination of the properties of *aristichthys nobilis* scales gelatin.

5. Conclusions

(1) The obtained optimal technological parameters of extracting gelatin from *aristichthys nobilis* scales through quadratic regression orthogonal optimization were that ultrasonic wave temperature was at 72 °C, ultrasonic wave duration was 121 min, ultrasonic wave power was 300 W, and the ratio of materials to solution 1:11 g/mL. Under this condition, the extraction efficiency of the gelatin from *aristichthys nobilis* scales was 48.58%. As compared with the anticipated results of models, the error was only

0.024%, which provided reference for industrial production;

(2) All the determined indexes of the gelatin from *aristichthys nobilis* scales met the requirements of Chinese standards. The technological parameters could be adjusted to obtain the needed gelatin products according to different operational requirements in combination of the properties of *aristichthys nobilis* scales gelatin. This study could provide references for rational development and utilization of the gelatin resources from *aristichthys nobilis* scales.

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