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Research Article

Optimization for Ultrasound-assisted Calcium Hydroxide Extraction of Protein from Shrimp Waste using Response Surface Methodology

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Abstract: Each year a considerable number of shrimp wastes were discarded. It caused a waste of resources, but also led to environmental pollution. In order to make a reasonable use of shrimp waste, protein was extracted from Vannamei waste by calcium hydroxide and ultrasonic-assisted, then used response surface methodology to optimize experiments to find the optimum extraction method. The optimal condition, predicted protein extraction rate of 37%, was obtained under the optimum conditions of the extraction time of 81 min, the extraction temperature of 60.00°C and the extraction concentration of 0.18 g/g dry matter.

Keywords: Calcium hydroxide, extraction, protein, response surface methodology, vannamei waste

INTRODUCTION

According to the Global Outlook for Aquaculture Leadership (GOAL) Statistics in recent years, the global annual production is nearly 4 million tons of shrimp; Of course it would produce so many shrimp wastes. However, only a minor part were used to extract chitin, (Olfa *et al*., 2013) chitosan (Lopez *et al*., 2010; Cahú *et al*., 2012), or astaxanthin (Armenta-López *et al*., 2002; Tao and Kyung, 2013). And in many commercial, medical, or cosmetic applications, chitin and chitosan have been studied in depth (Patomporn *et al*., 2013), each year a considerable number of shrimp processing by-products were discarded. There were also some shrimp processing wastes processed as feed additives (Ravi Kumar, 2000; Shu *et al*., 2009). Generally, shrimp wastes protein hydrolyzate-highly digestible feed were produced by immersed into boiling aqueous sodium hydroxide in the industrial production (Xia, 2003). Here in order to get a protein hydrolyzate feed method, calcium hydroxide with ultrasonic-assisted instead of sodium hydroxide was used in processing. The advantages of calcium hydroxide contain reducing costs and calcium ions could be removed by carbonation precipitated calcium carbonate, or just remained in the protein hydrolyzate as animal feed additives. Ultrasound-assisted alkaline for protein extraction is an emerging method, main role of ultrasonic is to damage physically cell wall and cell membrane, increasing the movement frequency and speed of molecular, to accelerate lye spread, accelerating protein dissolution rate in alkali liquid and to improve the liquid protein extraction rate (Zhao and Li, 2008). The efficiency of protein extraction may be affected by several factors (temperature, pH, concentration, etc.). Therefore, Response Surface Methodology (RSM) becomes a useful tool to optimize the extraction parameters, to evaluate the multiple parameters and to identify their significant interactions with the reduced number of experimental trials (Wani *et al*., 2008). For instance, optimized protein extraction of red pepper seed by response surface methodology (Ebru and Ozgul, 2010). Optimization of protein extraction from Brewer's Spent was done (Tang *et al*., 2010). Optimization of ultrasound-assisted extraction process of perilla seed meal proteins has also been done (Zhu and Fu, 2012).

MATERIALS AND METHODS

Materials: Vannamei was purchased in Agricultural technology market (Baoding, China); calcium hydroxide was purchased from Tianjin Rgent Chemical Reagent Co. Ltd. (Tianjin, China). Coomassie Brilliant blue G-250 was obtained from Shanghai Lanji Technology Development Co. Ltd. (Shanghai, China). Bovine serum albumin was purchased from Beijing Biotopped Science and Technology Co. Ltd. (Beijing, China). Phosphoric acid was obtained from Tianjin Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China). Ethanol was obtained from Tianjin Jinfeng Chemical

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Reagent Co. Ltd. (Tianjin, China). All chemicals were of reagent grade.

Pre-treatment: Shrimp meats were removed and the residual wastes (heads, shrimp-shell, tails, etc.) were blended for 10 min in an organization mixer, measured the moisture with a moisture analyzer, model Ohaus MB35 (Shanghai, China), collected in plastic bottles and finally frozen at -4°C for later use.

Determination of crude protein in shrimp processing waste: Use Kjeldahl method to determine the crude protein in shrimp processing waste. Carried out three times and averaged (AOAC, 1990).

Determination of soluble protein: Because solubility of calcium hydroxide is low and the higher the temperature, the lower the solubility, so the water-bath oscillator, model Jieruier THZ-82 (Changzhou, China) was chosen and the mixture was processed by an ultrasonic cleaning instrument, model Kudos SK5200H (Shanghai, China) for good contact between the suspended solids and the liquid before extraction experiment.

Took shrimp waste (3 g dry matter of 60 mL water) into 250 mL conical flask, added Ca $(OH)_2$ (0.15-0.25 g/g dry matter) and 60 mL water, first mixed the liquid by ultrasound (59 Hz) for 15 min, then placed in a water-bath oscillator (200 r/min), selected three time points (30-90 min), three temperature points (60-80°C), then centrifuged, last, protein was measured with Bradford method. Bradford (1976) 1 mL of the diluted sample was placed in a test-tube. Five milliliter of coomassie brilliant blue solution was added and the resulting mixture was stirred. Bovine Serum Albumin (BSA) as the standard, the absorbances of the mixed samples were measured at 595 nm on an UV-vis spectrophotometer, model Unico UV-2800 (Shanghai, China):

Protein extraction rate = (supernatant protein content/total protein content) ×100%

Box-Behnken design: According to the results of single factor experiments, select three factors of the extraction temperature, extraction time, extraction concentration, use Box-Behnken design scheme of response surface of three factors and three levels to analysis. And +1, 0, -1 encoded factors represent variables (Ni and Zeng, 2010); The independent variables X_i were coded as x_i, which were defined as dimensionless, according to the Eq. (1):

$$x_i = (X_i X_0) / \Delta X_i \quad (1)$$

where, x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the centre point and X_i is the step change value. The independent variables and their levels were presented in Table 1. Five replicates at the centre of design were used to estimate a pure error sum of squares.

Table 1: Box-Behnken central composite experimental design factors and levels of encoded values

	Symbols		Levels		
Factors	Coded	Un-coded	-1	0	+1
Time (min)	x_1	X_1	30	60	90
Temperature (°C)	x_2	X_2	60	70	80
Ca $(OH)_2$ (g/g)	x_3	X_3	0.15	0.2	0.25

Table 2: Box-Behnken design and experimental results

Number	x_1	x_2	x_3	Extraction rate
1	0	-1	+1	0.1708
2	0	+1	-1	0.0376
3	0	+1	+1	0.1997
4	-1	+1	0	0.2534
5	0	0	0	0.3277
6	-1	0	-1	0.1450
7	-1	0	+1	0.2249
8	0	0	0	0.2924
9	-1	-1	0	0.2114
10	0	0	0	0.2936
11	1	0	-1	0.1396
12	0	0	0	0.3353
13	+1	-1	0	0.3517
14	+1	+1	0	0.0680
15	0	-1	-1	0.3024
16	0	0	0	0.3125
17	+1	0	+1	0.1605

Verification of the model: From the RSM generated model, the optimum conditions of protein extraction of temperature, time and concentration were obtained. For verification of the model, protein was extracted under optimal conditions and the extraction rate of protein extracted was determined.

RESULTS AND DISCUSSION

Fitting the model: It can be seen from Table 2 that all of the extraction rate values were not so high. There may be some reasons as follows, firstly, since the solubility of calcium hydroxide itself is low and decreases with increasing temperature, so protein extraction rate was low. Secondly, shrimp contains some scleroproteins including collagen, elastin, keratin, etc., which is to perform the function of protecting the body protein. They do not dissolve in the lye, which may also cause the measured value decreased. Thirdly, the higher PH may cause some protein denatured, resulting in the decreased measured value (Han and Xie, 2008).

The application of RSM extraction rate fits the regression equation, which was an empirical relationship between protein extraction rate and the test variable in coded units, as the following Eq. (2):

$$Y = 0.31 - 0.014x_1 - 0.060x_2 + 0.016x_3 - 0.051x_1^2 - 0.041x_2^2 - 0.094x_3^2 - 0.081x_1x_2 - 0.015x_1x_3 + 0.073x_2x_3 \quad (2)$$

Table 3: Analysis of Variance (ANOVA) for response surface quadratic model for the extracted protein

Source	S.S.	DF	M.S.	F-value	Prob.>F	
Model	0.14000	9	0.01600	49.85	<0.0001	Significant
X_1	0.00160	1	0.00160	5.23	0.0561	
X_2	0.02900	1	0.02900	90.31	<0.0001	
X_3	0.00220	1	0.00220	6.83	0.0348	
X_1^2	0.01100	1	0.01100	34.21	0.0006	
X_2^2	0.00690	1	0.00690	21.90	0.0023	
X_3^2	0.03700	1	0.03700	118.21	<0.0001	
X_1X_2	0.02700	1	0.02700	84.00	<0.0001	
X_1X_3	0.00087	1	0.00087	2.76	0.1408	
X_2X_3	0.02200	1	0.02200	68.30	<0.0001	
Residual	0.00220	7	0.00032			
Lack of fit	0.00070	3	0.00023	0.62	0.6402	Not significant
Pure error	0.00150	4	0.00038			
Cor total	0.14000	16				

R^2: 0.9846; Adj R^2: 0.9649; Pred. R^2: 0.9059; S.S.: Sum of square; M.S.: Mean square

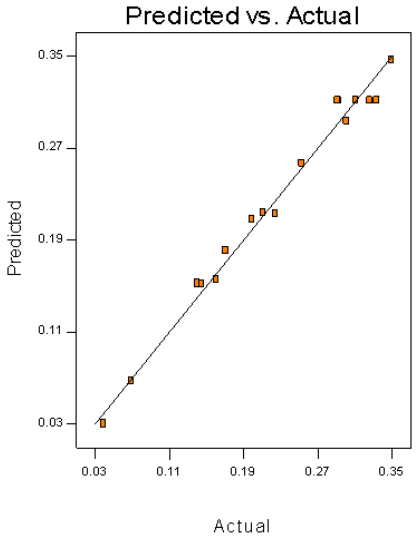


Fig. 1: Comparison between predicted and actual values of protein extraction rate

Analysis of Variance (ANOVA) presents the validity of the model and could explain whether this model adequately fits the variation observed in protein extracted at the designed level. If $p<0.01$, the F-test for the model is extremely significant, if $0.01<p<0.05$, the F-test for the model is significant and if $p>0.05$, the F-test for the model is not significant.

It can be seen from Table 3 that The Model F-value of 49.85 implied the model was significant. Value of "Prob>F" was <0.0500, indicating model terms were significant. Influence of temperature on the extraction rate was extremely significant ($p<0.01$), the impact of the concentration on the extraction rate was significant ($0.01<p<0.05$), but the impact of time was not significant ($p>0.05$). In this case x_2, x_3, x_1^2, x_2^2, x_3^2, x_1x_2 and x_2x_3 were significant model terms. The most significant effect was the temperature (x_2), followed by concentration (x_3), last, time (x_1).

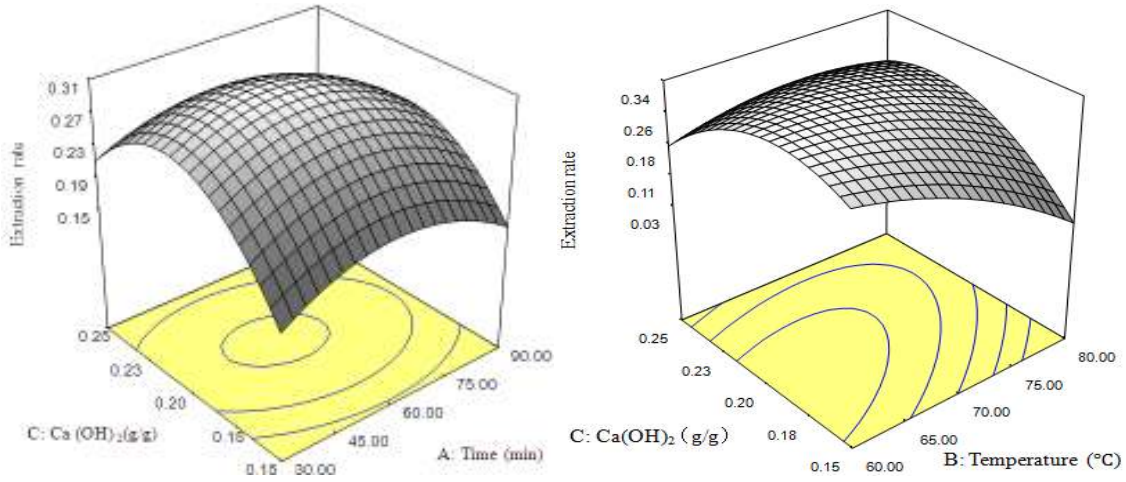
Lack of fit is used to assess the reliability of an equation, if equation modeling shows significant, it needs to be adjusted, if not, it can be good for date analysis in future. The "Lack of Fit F-value" of 0.62 implied the Lack of Fit was not significant relative to the pure error ($p>0.05$). Non-significant lack of fit was good.

For a fit model, R^2 should be at least 0.80 (Joglekar and May, 1987). R-Squared was 0.9846, indicating a reasonable fit of the model to the experimental data. The "Pred R-Squared" of 0.9059 was in reasonable agreement with the "Adj R-Squared" of 0.9648. Therefore, the model adequately represented the real relationship between the parameters chosen.

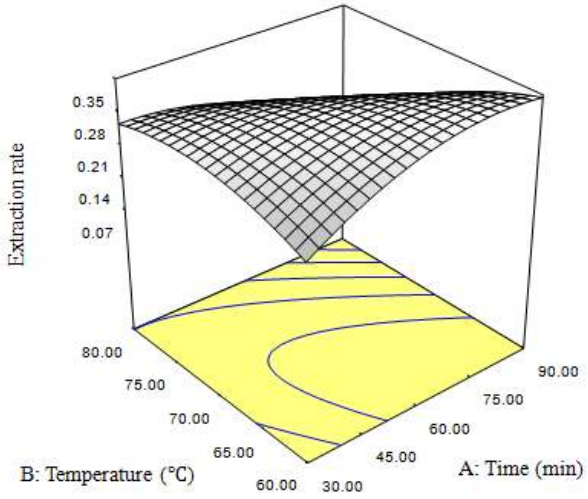
Figure 1 shows that the regression model was in good agreement with the experimental results. The observed values were compared with the predicted value calculated from the model. The result suggested that the models used in the research were able to identify operating conditions for the protein extraction.

In order to visualize, the response surfaces of Ca $(OH)_2$ treatment conditions were shown in Fig. 2 shows the interaction of every two factors was significant. Figure 2a shows the effects of the time and the concentration of Ca $(OH)_2$ (g/g dry matter) on protein production. A quadratic effect of the time on the response was observed, as well as the concentration of Ca $(OH)_2$ (g/g dry matter). Figure 2b shows the effect of the temperature and the concentration of Ca $(OH)_2$ (g/g dry matter) on protein production. A quadratic effect of the temperature, as well as the concentration of Ca $(OH)_2$ (g/g dry matter), on the response was observed. Figure 2c shows the effect of the time and the temperature on protein production. Quadratic effects were observed on both of the impacts of time and the temperature on protein production.

Validation of the model: (36.56%) of protein was obtained in a control experiment carried out under the optimized operating condition (extraction time of 81 min, extraction temperature of 60.00°C, extraction concentration of 0.18 g/g dry matter). The experimental



(a) (b)



(c)

Fig. 2: Surface plots for protein extraction of shrimp waste. (a) Figure plot to show the combination of time and the concentration of Ca $(OH)_2$ (g/g dry matter), (b) figure plot to show the combination of temperature and the concentration of Ca $(OH)_2$ (g/g dry matter), (c) figure plot to show the combination of time and temperature

extraction rate of protein was in agreement with the predicted value.

CONCLUSION

Proteins extraction by ultrasound-assisted calcium hydroxide can be used to shrimp waste recycling, which can greatly reduce the costs of protein extraction. Response surface methodology was successfully used to optimize the extraction parameters for the protein extraction from shrimp waste. Three parameters (extraction time, extraction temperature and extraction concentration) were measured by Box-Benhnken. The optimal predicted protein extraction rate of 37% was obtained under the optimum conditions of the extraction time of 81 min, extraction temperature of 60.00°C, extraction concentration of 0.18 g/g dry matter. The experimental extraction rate agreed closely with the predicted extraction rate under optimized conditions. Response surface methodology to optimize the extraction protein process of shrimp waste is not only scientific and reasonable, but also reliable and fast feasible, which means that this method has certain significance.

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