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Optimization of Process Parameters for the Production of Chitin from the Shell of Flowertail Shrimp (*Metapenaeus dobsoni*)

C. G. Joshy*, A. A. Zynudheen, George Ninan, V. Ronda and Sabeena Muhammed
ICAR-Central Institute of Fisheries Technology, Matsyapuri P. O., Cochin - 682 029, India

Abstract

The objective of present study was to optimize the process parameters for the production of chitin from the shell of *Metapenaeus dobsoni* by deproteinization and demineralization using linear regression. The response variables viz: yield and quality of chitin were optimized in terms of concentration, treatment time and ratio of acid and alkali used in the production of chitin. First order polynomial regression model with interaction coefficients for alkali concentration with treatment time was found as optimal to explain the variability in the response variables in terms of process variables. Based on the fitted model, the coefficients of acid concentration (2.48), acid ratio (0.006) and acid treatment time (0.039) were found significant for chitin yield at 1% level and linear regression coefficients showed a non-significant increasing trend. The regression coefficient for interaction between acid concentration and treatment time showed a significant decreasing trend (-0.025). The optimum combination of process variables to improve the yield and quality of chitin from *M. dobsoni* shell was sodium hydroxide concentration at 3 % in 1:2 ratio and treatment time of 30 minutes and hydrochloric acid concentration at 1.25 N in1:2 ratio and treatment time of 90 minutes.

Keywords: Chitin, *Metapenaeus dobsoni*, Deproteinization, Demineralization, Regression, Optimization

Introduction

Chitin ($C_8H_{13}O_5N$) is a long-chain natural polymer of N-acetyl glucosamine (Muzzarelli, 1970). It is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans and insects, the radulae of molluscs, and the beaks of cephalopods, including squid and octopuses (Al sagheer et al. 2009; Chaussard & Domard, 2004). Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is produced commercially by deacetylation of chitin (Mathur & Narang, 1990). At present, chitin, chitosan and their derivatives have a large range of application in chemistry, medicine, bio-technology, pharmacy, water treatment, cosmetics and food technology (Kumar, 2000). Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%) (Muzzarelli et al, 1990). As most of the present-day polymers are synthetic materials, their biocompatibility and biodegradability are much more limited than those of natural polymers such as cellulose, chitin, chitosan and their derivatives. Chitin is mainly produced from the crab and shrimp shell waste through some chemical process, i.e., deproteinization with dilute alkali and demineralization with dilute acid (Khanafari et al. 2008, Percot et al. 2003a). The resulting chitin is deacetylated with alkali at higher temperature to get chitosan. In India, shell fish processing is a very important industry, which generates 20-50% shell waste of the processed product (Sathiadas & Aswathy, 2004). The shell waste is a rich source of protein, minerals and chitin, which can be recovered through an optimized extraction process.

Approximating dependent variable as a function of independent variables is a useful technique to improve the process and it can be ideally employed to optimize the extraction process of biopolymers like chitin. The major process parameters involved

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* *E-mail: cgjoshy@gmail.com*

in the production of chitin by chemical method are temperature, concentration and treatment time of alkali and acid and ratio of reagents used (Percot et al., 2002; 2003b). The yield and quality attributes of extracted chitin is largely affected on the preciseness of these process variables. The objective of the study was to obtain a suitable mathematical formula to approximate the relationship between yield and process variables and to optimize the process variables to improve the chitin yield and quality from the shell of flower tail shrimp, *Metapenaeus dobsoni*, a commercially important penaeid shrimp which constitutes a major share of seafood export from India.

Materials and Methods

The shells of marine prawn, *M. dobsoni,* were collected from a processing plant in Cochin. The prawn wastes were washed thoroughly and drained in the laboratory. The shell waste was packed in 500g lots and frozen stored until use. Chitin was prepared from the sample as per the method of Madhavan & Nair (1974). In the present study, different levels of six process variables *viz*: sodium hydroxide concentration, treatment time and sample alkali ratio in deproteinization process, hydrochloric acid concentration, treatment time and sample acid ratio in demineralization process were taken into consideration by keeping temperature as constant.

Three levels of sodium hydroxide (NaOH) concentration, alkali ratio and treatment time in minute were considered as processing variables for the deproteinization process (Table 1).

Table 1. Process variables for Deproteinization process

Processing variable	Levels of process variables		
NaOH Treatment time (min)	20	30	40
NaOH concentration	2%	3%	4%
NaOH ratio (sample/alkali)	1:1	1:2	1:3

The stored shell (500 g) was thawed and boiled with three concentration of sodium hydroxide in three different time interval, i.e, 20, 30 and 40 minutes (Table 1). The resulting solution containing protein was drained off and washed with water to remove residue alkali content completely (Madhavan & Nair, 1974).

Once the deproteinization process was over, the resultant material was subjected to demineralization process to obtain the chitin. In this step, three levels of hydrochloric acid (HCl) concentration, acid ratio and treatment time were considered (Table 2).

Table 2. Process variables for Demineralization process

Processing variable	Levels of process variables		
HCl Treatment time (min)	75	90	105
HCl concentration	1 N	1.25 N	1.5 N
HCl ratio (sample /acid)	1:1	1:2	1:3

The samples were treated with different concentrations of HCl in different acid ratio in three time intervals. Excess acid containing minerals was drained off and the residue was repeatedly washed with water to make the sample acid free (Madhavan & Nair, 1974). Residue was collected and dried under the sunlight to get the chitin. The yield of chitin was calculated by taking the difference between initial sample weight and final product. Ash content was estimated by the AOAC method (1975).

In the present study, data on two response variables *viz*: yield and ash were collected from 35 out of total experimental units using fractional factorial experimental set up as per Myers & Montgomery (2002).

The response variables are modeled as a function of process variables using polynomial models. The general form of polynomial equation is given in the formula 1.

$$Y_i = \beta_0 + \beta_i X_i + \beta_{ii} X_i^2 + \beta_{ij} X_i X_i \ , \ i{=}j, \ i<j \ \ldots\ldots\ldots\ldots(1)$$

Where Y_i is the dependent variable

β_i, β_{ii}, β_{ij} 's are regression coefficients

X_i 's are independent or explanatory variable

All statistical analysis was carried out using SAS 9.2. Once the suitable polynomial model is fitted, steepest ascent and descent optimization technique was used in the present study to optimize yield and ash content of chitin sample (Khuri & Cornel. 1996).

Results and Discussion

The observed data on yield and ash content of chitin is given Table 3. The range of chitin yield and ash content were 3.30 – 5.14 and 0.63 – 26.73 % respectively. The lowest chitin yield 3.30% with ash content 0.63% was obtained from the process

Table 3. Effect of process variables on yield and quality of chitin

Sample No.	Alkali			Acid			Response variables	
	Time	Ratio	Concentration	Time	Ratio	Concentration	Yield (%)	Ash (%)
1	20	1:1	2	75	1:1	1	4.00	11.86
2	40	1:1	2	75	1:1	1.5	3.60	9.17
3	20	1:3	2	75	1:1	1.5	3.90	9.18
4	40	1:3	2	75	1:1	1	4.80	15.18
5	20	1:1	4	75	1:1	1.5	4.17	8.97
6	40	1:1	4	75	1:1	1	4.57	21.63
7	20	1:3	4	75	1:1	1	4.98	21.16
8	40	1:3	4	75	1:1	1.5	3.89	10.62
9	20	1:1	2	105	1:1	1.5	3.91	6.14
10	40	1:1	2	105	1:1	1	5.00	21.42
11	20	1:3	2	105	1:1	1	5.14	21.19
12	40	1:3	2	105	1:1	1	3.62	9.02
13	20	1:1	4	105	1:1	1.5	4.97	24.57
14	40	1:1	4	105	1:1	1	3.92	7.33
15	20	1:3	4	105	1:1	1.5	3.73	7.08
16	40	1:3	4	105	1:1	1.5	5.08	26.73
17	20	1;1	2	75	1:3	1.5	3.48	0.99
18	40	1:1	2	75	1:3	1	3.47	2.74
19	20	1:3	2	75	1:3	1	3.94	4.60
20	40	1:3	2	75	1:3	1.5	3.51	3.25
21	20	1:1	4	75	1:3	1	3.89	2.86
22	40	1:1	4	75	1:3	1.5	3.30	0.63
23	20	1:3	4	75	1:3	1.5	3.73	2.60
24	40	1:3	4	75	1:3	1	3.35	4.44
25	20	1:1	2	105	1:3	1	3.78	3.27
26	40	1:1	2	105	1:3	1.5	3.65	1.32
27	20	1:3	2	105	1:3	1.5	4.42	1.55
28	40	1:3	2	105	1:3	1	3.53	2.41
29	20	1:1	4	105	1:3	1.5	3.84	1.30
30	40	1:1	4	105	1:3	1	3.59	3.23
31	20	1:3	4	105	1:3	1	3.58	3.69
32	40	1:3	4	105	1:3	1.5	3.45	1.35
33	30	1:2	3	90	1:2	1.25	4.26	5.36
34	30	1:2	3	90	1:2	1.25	4.03	3.75
35	30	1:2	3	90	1:2	1.25	3.56	3.16

variable combination of alkali concentration of 4 % at 1:1 ratio treated for 40 minutes and acid concentration of 1.5 N at 1:3 ratio treated for 75 minutes. The highest chitin yield of 4.03% with acceptable ash content of 3.75% was obtained from the process variable combination of alkali concentration of 3 % at 1:2 ratio treated in 40 minutes and acid concentration of 1.25 N at 1:2 ratio treated for 90 minutes.

First order polynomial model of interaction coefficients for alkali concentration with treatment time and acid concentration with treatment time were found to be suitable to explain the variability in the dependent variables viz: ash and yield in terms of process variables. The regression coefficients of higher order polynomial were found non-significant. The mathematical expression of the selected model for the dependent variable is

$Y=\beta_1 x_1+ \beta_2 x_2+ \beta_3 x_3+ \beta_4 x_4+ \beta_5 x_5+ \beta_6 x_6+ \beta_7 x_1 x_2+ \beta_8 x_4 x_5+ e$

Where Y is the dependent variable

β_1 is the regression coefficient for alkali concentration (x_1)

β_2 is the regression coefficient for alkali treatment time (x_2)

β_3 is the regression coefficient for alkali ratio (x_3)

β_4 is the regression coefficient for acid concentration (x_4)

β_5 is the regression coefficient for acid treatment time (x_5)

β_6 is the regression coefficient for acid ratio (x_6)

β_7 is the regression coefficient for interaction between alkali concentration (x_1) and alkali treatment time (x_2)

β_8 is the regression coefficient for interaction between acid concentration (x_4) and acid treatment time (x_5)

e is the error term

The coefficient of variation (CV) of original values of ash content was found high, hence it was transformed into new scale using logarithmic transformation. The transformed data was used to fit the model. The resultant model with the estimated regression coefficients for ash and yield are given below along with the goodness of fit of the model.

$$\text{Log (Ash)} = 0.122x_1+0.009x_2+0.012x_3+0.753^{*}x_4 + 0.008x_5 - 0.007^{**}x_6 - 0.003x_1x_2 - 0.007x_4x_5$$

R^2=0.94

$$\text{Yield} = 0.265x_1+0.014x_2+0.014x_3+2.48^{**}x_4 + 0.039^{**}x_5 - 0.006^{**}x_6 - 0.007x_1x_2 - 0.025^{**}x_4x_5$$

R^2=0.98

* - indicates regression coefficient is significant at 5% level of significance ($p < 0.05$)

**- Indicates regression coefficient is significant at 1% level of significance ($p < 0.01$)

The all regression coefficients of alkali treatment were non-significant, whereas the regression coefficients for acid concentration and ash content were found significant with a high R^2 value. However, the sign and magnitude of regression coefficients were taken into consideration for optimizing the ash content. The positive regression coefficients indicated that the ash content increased (0.122 and 0.753%) with increase in concentration of NaOH and HCl. The treatment time of NaOH and HCl also showed negligible effect on ash content. The ash content was decreasing (0.007%) significantly as the acid ratio increases, but the same was increasing as the alkali ratio changes. The interaction coefficient for alkali concentration and treatment time and acid concentration and treatment time showed a non-significant decreasing trend on ash (-0.003 and -0.007% respectively).

Table 4. Computation of steepest ascend method for the chitin yield

point	Process variables					
	Alkali (NaOH)			Acid (HCl)		
	Concentration	Time	Ratio	Concentration	Time	Ratio
Base	3	30	15	1.25	90	104
Δ (increment)	0.107	0.005	0.006	0.250	0.016	0.002
Base+Δ	3.107	30.005	15.006	1.500	90.016	104.162
Base+Δ	3.214	30.011	15.011	1.750	90.032	104.165
Base+Δ	3.320	30.016	15.017	2.000	90.048	104.167
Base+Δ	3.427	30.022	15.022	2.250	90.063	104.169

Table 5. Computation of steepest descend method for the Ash content of chitin

point	Process variables					
	Alkali (NaOH)			Acid (HCl)		
	Concentration	Time	Ratio	Concentration	Time	Ratio
Base	3	30	15	1.25	90	104
Δ (increment)	0.161	0.012	0.016	0.250	0.011	0.010
Base+Δ	3.161	30.012	15.016	1.500	90.011	104.170
Base+Δ	3.161	30.012	15.016	1.750	90.021	104.179
Base+Δ	3.161	30.012	15.016	2.000	90.032	104.189
Base+Δ	3.161	30.012	15.016	2.250	90.043	104.198

The variability in the yield was explained by the model with significant R^2 (0.98) value. The yield was increasing significantly as the acid concentration (2.48 %) and treatment time (0.039 %) increases and the yield was decreasing significantly as the acid ratio increases. The regression coefficients of alkali concentration, treatment time and alkali ratio showed non-significant increasing trend on yield. The regression coefficients for interaction between alkali concentration and treatment time showed a non-significant decreasing trend (-0.007%) and acid concentration and treatment time showed a significant decreasing trend (-0.025%).

The computed value of steepest ascent method for chitin yield and steepest descend method for the ash content is given in table 4 and 5 respectively. The steepest ascend paths of chitin yield and steepest descend paths of ash content showed slightly increasing trend as the levels of process variables increases from the baseline. The alkali concentration, treatment time, alkali ratio, acid treatment time and ratio did not show marked change from the base, whereas the acid concentration showed significant rate of change from the base for chitin yield and quality.

Conclusion

Most of the process variables had positive direct effect on yield and ash content of chitin except the acid and alkali ratio. The interaction of acid and treatment time showed a negative trend on yield and ash content of chitin. The effect of alkali (NaOH) found to be non-significant during chitin extraction process, whereas the acid produced significant effect during demineralization process. The optimum combination of process variables to improve the chitin yield and quality was alkali concentration, treatment time and ratio at 3 %, 30 minutes and ratio 1:2, respectively and acid concentration, treatment time and ratio at 1.25 N, 90 minutes and 1:2, respectively.

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