EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2024, 25(9), 348–357
https://doi.org/10.12912/27197050/190801
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Received: 2024.06.24
Accepted: 2024.07.10
Published: 2024.08.01

Optimization of Chitin Extraction from Shrimp Shells Using Full Factorial Design Methodology

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ABSTRACT

This study aims to optimize chitin extraction conditions by reducing water and chemical consumption using a two-level factorial design approach. Two variables were studied for the demineralization process: the volume of hydrochloric acid (400 to 500 mL) and the demineralization time (1 to 1.5 hours) at room temperature. For deproteinization, the variables examined were NaOH concentration (0.6 to 1 mol/L) and deproteinization time (1 to 1.5 hours) at 70–80 °C. The ash and protein contents were measured to evaluate the efficiency of the demineralization and deproteinization processes, respectively. Minitab 18 software was used for the experimental design. The results showed that the best conditions for extracting high-quality chitin are a demineralization time of 1 hour, an HCl volume of 500 mL, a NaOH concentration of 1 M, and a deproteinization time of 1 hour. These optimal conditions reduce the amount of water, time, and chemicals required for chitin extraction. Compared to previous studies, we significantly reduced the amount of water and chemicals as well as the extraction duration. These results are innovative as they offer a more efficient and sustainable solution for chitin extraction, thus filling a gap in current research by proposing an optimized method. By providing these new data and demonstrating their effectiveness, our research makes a significant contribution to improving chitin extraction techniques, addressing an unmet need in the industrial field.

Keywords: chitin, extraction, modeling, demineralization, deproteinization, water consumption, chemical consumption, Minitab.

INTRODUCTION

Many international shrimp peeling companies operate in Morocco, processing a wide range of shrimp from around the world. Moroccan shrimp processing industries generate more than 0.15 million tons of shrimp waste each year, which unfortunately generates a large amount of

shrimp waste and causes major pollution issues. This waste emits offensive odors into the atmosphere, resulting in chemical pollution that combines with toxic gases like carbon dioxide CO_2 and methane CH_4, significantly contributing to the risks of climate change [Rissouli et al., 2016] This waste can be used to make chitin, one of the most abundant polymers on Earth. It can be found in a wide range of animal products, including shrimp shell, crab shell, and insects [Pakizeh et al., 2021]. This compound can be converted into chitosan, which has high commercial value due to its biocompatibility, antimicrobial activity, and lack of toxicity. Chitosan finds multiple applications, including agriculture, water treatment, and pharmaceuticals [Rissouli et al., 2017]. Chitin can be extracted using either chemical or enzymatic methods [Islam et al., 2023]. The chemical method is the most widely used in the industry. The extraction experiments with hydrochloric acid and sodium hydroxide were the most successful [Knidri et al., 2018]. Furthermore, the chemical process is inexpensive, quick, and simple. However, there are some drawbacks, such as the need for a large amount of water and energy, and the production of significant amounts of alkaline and acidic wastewater [Tolaimate et al., 2003]. Today, reducing water consumption is a health, environmental, and economic concern. Chitin extraction conditions influence the properties of its main derivative, chitosan. Adeyi el et al. (2017) have optimized the chitosan extraction conditions using the surface response method (SRM) [Adeyi et al., 2017]. A longer demineralization and deproteinization time (49 hours) at 8% HCl and 3.5N NaOH concentrations resulted in a greater extraction yield. A study on Omani shrimp shells used RSM to determine the best conditions for extracting chitin and chitosan. The highest yield was achieved with 3% HCl and 50% NaOH for 4 hours [Amoo et al., 2019; Al Hoqani et al., 2021]. Amoo et al have optimized the extraction of chitin and chitosan using SRM. A high-quality deacetylation chitosan was obtained under the following conditions: HCl (3.25 M), demineralization time (19.03 h), NaOH (2.43 M), and deproteinization time of 2.03 h [Amoo et al., 2019].

Our objective is to improve the process of producing high-quality chitin from Moroccan shrimp shells by adjusting the water volume, NaOH concentration, and deproteinization and demineralization times. This is the first optimization study of chitin production in Morocco. The chitin obtained will be analyzed to determine the efficacy of our methods. The study's findings will help to reduce water and energy consumption, shorten production times, and lower costs. The conditions for demineralization and deproteinization were selected based on previous research findings [Rissouli et al., 2017]. The goal is to produce higher-quality chitin faster and with less water, energy, and chemical consumption. A two-factor design was used to investigate the effects of demineralization and deproteinization factors at each stage. The study was conducted using Minitab version 18.

MATERIAL AND METHODS

Extraction of chitin

Previous research and experience were utilized to determine the optimal conditions for demineralization and deproteinization [Tolaimate et al., 2003; Amoo et al., 2019; Al Hoqani et al., 2021]. Chitin extraction consisted of four steps: pretreatment, demineralization, deproteinization, and bleaching [Firzanah et al., 2024]. The frozen shrimp shell was first washed with hot water (80–90 °C) while constantly stirring to remove free shell residues, lipids, and other materials, then dried in an oven at 60 °C for 24 hours before being ground into small pieces. The shells were demineralized by treating 50 grams of the samples with varying volumes of HCl (400–500 mL) with constant stirring at room temperature for 1 hour, 1.25 hours, and 1.5 hours. The demineralized samples were then filtered, washed several times, and dried at 60 °C for 24 hours. The demineralized samples were deproteinized by treating them with different concentrations of NaOH (0.6 mol/L, 0.8 mol/L, and 1 mol/L) at 80 °C for 1 hour, 1.25 hours, or 1.5 hours while stirring constantly. After stirring, the deproteinized samples were filtered, washed, and dried.

Optimization of chitin extraction from shrimp shells

Ash content

The demineralized shells were subjected to ash analysis to determine the minerals removed and the efficacy of this step. The samples were placed on a petri dish and heated in the furnace at 650 °C for 4 hours. The ash content can be

calculated using the following formula [Puvvada et al., 2012] :

$$C\% = \frac{M_1}{M_2} \times 100 \quad (1)$$

where: $\%C$ – ash content, M_1 – mass of demineralized dried shells before incineration in (g), M_2 – mass demineralized dried shells after incineration in (g).

Protein content

The protein content was calculated using the Biuret method. Bovine serum albumin (BVA) was used as the standard solution, and a stock solution of 2 g/l was prepared. 0; 0.6; 1.2; 1.8; 2.4, and 3ml of solution were placed in tubes and filled to the 3ml mark, followed by 4mL of Biuret solution [Gornall et al., 1948]. The standard solution concentrations were calculated, and a calibration curve was created [Lin et al., 2012]. The protein content of chitin samples was measured by immersing 3 g in 10 mL of 4% NaOH. The mixture was stirred for six hours at 95 °C. The hydrolysate was filtered and placed in a volumetric flask, which was then filled with 100 mL of distilled water. We took 10 mL of this solution and filtered twice. Then we measured 3 mL and added 12 mL of Biuret solution. The solutions were kept at 30 °C for one hour. A spectrophotometer measured absorbance at 540 nm [Yapa et al., 2008].

Optimization of the demineralization

We applied the full two-level factorial design methodology to optimize the demineralization and deproteinization processes [Eddaoukhi et al., 2023; Eddaoukhi et al., 2024]. The variables investigated in the demineralization step were the volume of HCl (X_1) and the demineralization time (X_2). The response variable is ash content (Y_1) (Table 1). By using two levels for each factor, we get $2^2 = 4$, with two points in the center and two repeatability tests for each assay, for a total of ten assays (Table 2). Equation 2 depicts the empirical model for planning 2^2, with a denoting the model's coefficients.

$$Y_1 = a_0 + a_1X_1 + a_2X_2 + a_{12}X_1X_2 \quad (2)$$

Optimization of the deproteinization

The deproteinization step was optimized using demineralized shells. The variables studied during the deproteinization process were the concentration of NaOH and the stirring time. These parameters have a significant influence on the deproteinization reaction [Amoo et al., 2019; Eddaoukhi et al., 2023]. The response variable is protein content (Y_2) (see Table 3). Taking two levels for each factor yields $2^2 = 4$ tests. Each test was repeated, with two essays added in the middle. So there will be ten assays (see Table 4).

This experimental design produced a first-degree polynomial equation.

$$Y_2 = b_0 + b_1Z_1 + b_2Z_2 + b_{12}Z_1Z_2 \quad (3)$$

where: Y_2 is the dependent variable representing ash content, Z_1 and Z_2 are the independent variables corresponding to NaOH concentration and reaction time, respectively. b_0 is a constant, b_1 and b_2 are the coefficients explaining the linear effect of Z_1 and Z_2, and b_{12} is the coefficient that explains the relationship interaction between the two variables.

Statistical analysis

The experiments were duplicated, and the results were presented as averages and standard deviations. The results of the experimental design were analyzed using Minitab®18.1 (©2017 Minitab, Inc.) software using the mean of analysis of variance (ANOVA), with a significance level of p-value < 0.05.

Table 2. Test plan for demineralization optimization (given by Minitab)

N° Essay	V HCl (ml)	Demineralization time (h)
1	400	1
2	500	1
3	400	1.5
4	500	1.5
5	400	1
6	500	1
7	400	1.5

Table 1. Factors and levels used for demineralization optimization

Factors	-1	0	1
V (HCl) ml	400	450	500
Time (min)	1h	1.25h	1.5h

Table 3. Factors and levels used for deproteinization optimization

Factors	-1	0	1
[NaOH] mol/L	0.6	0.8	1
Deproteinization time (h)	1	1.25	1.5

Table 4. Test plan for deproteinization optimization (given by Minitab)

N° Essay	NaOH (mol/L)	Deproteinization time (h)
1	0.6	1
2	1	1
3	0.6	1.5
4	1	1.5
5	0.6	1
6	1	1
7	0.6	1.5
8	1	1.5
9	0.8	1.25
10	0.8	1.25

Table 5. Levels and results of factorial design 2^2 response variables for shrimp shell demineralization

Treatment	X_1- V HCl(ml)	X_2-time(h)	Y_1 Ash content%
1	(-1) 400	(-1)1 h	7.3
2	(+1) 500	(-1)1 h	0.95
3	(-1) 400	(+1)1.5 h	5.2
4	(+1) 500	(+1)1.5 h	1.8
5	(-1) 400	(-1)1 h	8.2
6	(+1) 500	(-1)1 h	0.95
7	(-1) 400	(+1)1.5 h	5.2
8	(+1) 500	(+1)1.5 h	1.35
9	(0) 450	(0) 1.25 h	2.5
10	(0) 450	(0) 1.25 h	2.05
Level -	400 ml	1 h	
Level0	450 ml	1.25 h	
Level+	500 ml	1.25 h	

RESULTS AND DISCUSSION

Optimization of demineralization processes

During the one-hour demineralization process, 500 mL of HCl produced the lowest mineral content value of %C = 0.95 (Table 5). The reaction's efficiency was 99.05%. This value exceeds previous findings [Bajaj et al., 2011]. The highest percentage C = 8.2% was obtained for V = 400 mL and t = 1 h, with a 91.8% efficiency. This implies that the input parameters and levels were appropriate, and that the shells were demineralized using moderate amounts of water and HCl over an average time period.

The results of the experiments were used to perform the analysis of variance. Table 6 displays the estimated coefficients for each factor. The p-value represents the significance of each factor and its interactions. A low p-value indicates that the factor has a statistically significant effect. Table 6 shows p-values of 0 for HCl volume, 0.044 for the interaction of the two factors, and 0.174 for time. In this case, we can conclude that both the individual effect of HCl volume and the interaction effect of the two variables on ash content are statistically significant. Notably, the effect of HCl volume is the greatest. The regression equation for estimating the optimum demineralization conditions is given below:

$$Ash\ content\ \% = 65.1 - 0.1315\ V - 30.5\ t + 0.0635\ V \times t \quad (4)$$

where: V – HCl volume, t – Demineralization duration (h).

The square of the correlation coefficient (R^2) is used to verify the model's precision and accuracy. In this case, the R^2 regression is nearly 100% (R^2 = 92.91%), indicating that the model is valid [Adeyi et al., 2017]. The adjusted R^2 value was 89.37%, which is very similar to the normal R^2. This validates the model's reliability. R^2 (pred) = 86.46 %, indicating that the model can accurately predict ash content values for new experiments at 86.46% (Table 7). Based on these findings, we can conclude that the model was well-suited to the original conditions and can accurately predict

Table 6. Estimated regression coefficients for the chitin mineral content model

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		3.550	0.279	12.72	0.000	
Volume HCl (ml)	-5.213	-2.606	0.312	-8.35	0.000	1.00
Time (h)	-0.962	-0.481	0.312	-1.54	0.174	1.00
Volume HCl (ml)*time (min)	1.587	0.794	0.312	2.54	0.044	1.00

Table 7. Regression statistics for shrimp shell demineralization

S	R-sq	R-sq (adj)	R-sq (pred)
0.882380	92.91%	89.37%	86.46%

the demineralization process under new conditions. This could indicate that our model is reliable and capable of optimizing demineralization processes on a larger scale.

Figure 1 shows the Pareto chart, which displays the absolute values of factor effects from highest to lowest. The diagram also includes a reference line to indicate significant effects. Bars that cross the reference line indicate statistical significance. It is obvious that the volume of hydrochloric acid has the greatest effect on ash content, followed by the interaction of the two factors, and finally by time, which has a minor effect on the demineralization stage.

The main effects graph (Figure 2) shows that as the volume of HCl increases, the ash content decreases gradually from 6.16% to 0.94%. However, the ash content only slightly changes from 4.03% to 3.07% as reaction time increases. The interaction diagram (Figure 3) shows that at time t = 60 min, the average ash content decreases as the HCl volume increases from 400 mL to 500 mL. Similarly, at t = 90 minutes, the average ash content decreases as the HCl volume increases from 400 mL to 500 mL. As a result, we can conclude that 500 mL of HCl and one hour of reaction time are sufficient for effective demineralization.

According to the contour plot in Figure 4, the lowest values of the response variable (% ash content) are obtained for HCl volumes of around 500 ml and demineralization times of 60 to 75 minutes. On the other hand, the highest values are obtained after using 400 mL of HCl for an hour, which makes sense. For volumes ranging from 495 mL to 500 mL, increasing the duration of demineralization increases the ash content. Despite a long period of demineralization, the ash content remains constant in volumes ranging from 465 mL to 495 mL. However, for volumes ranging from 400 mL

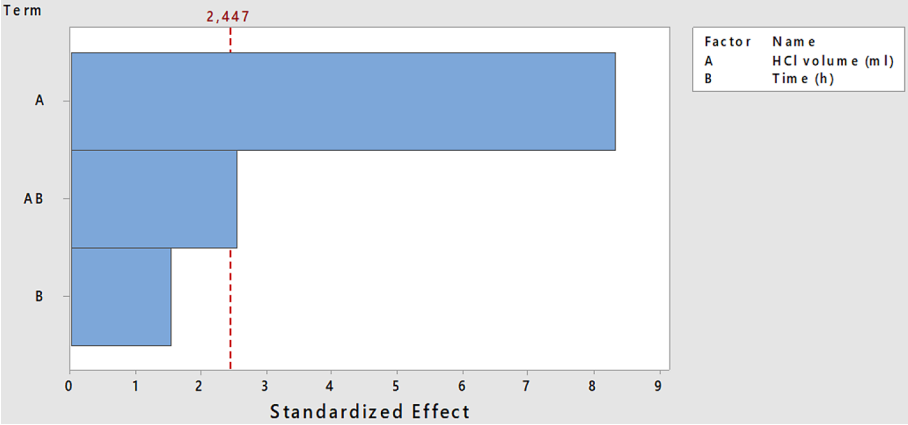

Figure 1. Pareto diagram for the effects of factors and their interaction

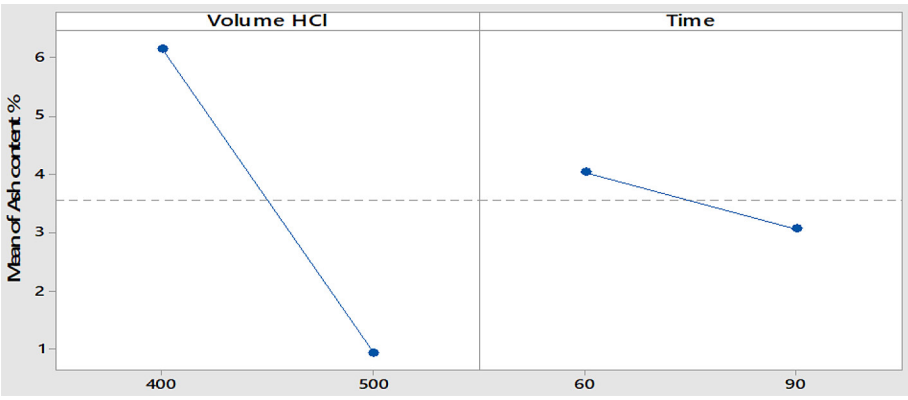

Figure 2. Diagrams of main effects on ash contents

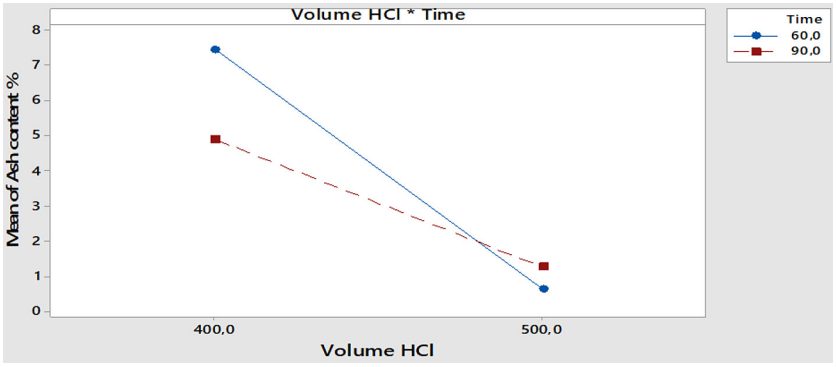

Figure 3. Diagrams of effects interactions on ash contents

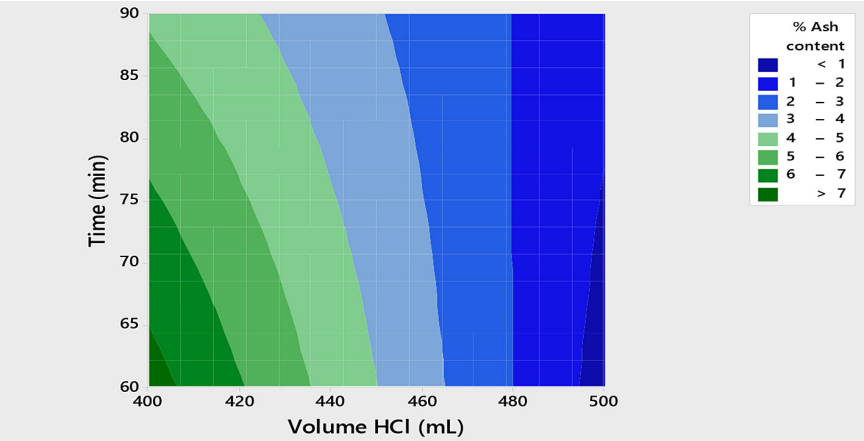

Figure 4. Correlation between % ash and time-volume variation: contour plot

to 465 mL, the ash content decreases as the demineralization time increases. The increase in ash content with increasing demineralization duration could be attributed to the occurrence of certain undesirable chemical reactions that result in the formation of more mineral residue.

Optimization of deproteinization processes

Table 8 depicts the protein content of the chitin samples used in each experiment. As can be seen, the values vary from 1.7 to 2.65%. These values fall within acceptable ranges [Yapa et al., 2009; Benhabiles et al., 2013]. The lower value, Y_2 = 1.7%, was obtained on the deproteinized sample at a concentration of 1mol/L for 1 hour and a (chitin mass) / (volume NaOH) ratio of 1/1, with an efficiency of 98.3%. The regression equation is as follows:

$$Protein\ content\ \% = \\ = 3.73 - 2.70\ C - 0.19\ t + 0.95\ C \times t \qquad (5)$$

where: C – NaOH concentration mol/L, t – Deproteinization duration.

Table 8. Factor levels and results of factorial design 2^2 response variables for shrimp shell deproteinization

Treatment	X_1- C NaOH (mol/l)	X_2-time (h)	Y_2 protein content%
1	(-1) 0.6	(-1) 1	2.61
2	(+1) 1	(-1) 1	1.70
3	(-1) 0.6	(+1) 1.5	2.65
4	(+1) 1	(+1) 1.5	2.08
5	(-1) 0.6	(-1) 1	2.27
6	(+1) 1	(-1) 1	1.78
7	(-1) 0.6	(+1) .5	2.61
8	(+1) 1	(+1) 1.5	2.16
9	(0) 0.8	(0) 1.25	2.58
10	(0) 0.8	(0) 1.25	2.35
Level -	0.6	1	
Level0	0.8	1.25	
Level+	1	1.25	

Since R^2 greater than 0.75, we can accept the model. Based on the p-values (Table 10), we can conclude that the effect of NaOH is more significant (p-value = 0.003) than the effect of time (p = 0.058).

Table 9. Regression statistics for shrimp shell deproteinization

S	R-sq	R-sq(adj)	R-sq(pred)
0.172211	83.68%	75.52%	61.79%

Furthermore, the interaction between the two factors is not significant (p = 0.465). These findings show that the NaOH concentration has a significant impact on demineralization efficiency. The effect of reaction duration is less pronounced, but with a p-value of 0.058, there is a trend toward significance. The interaction effect is weak, indicating that the use of NaOH and the duration of the experiment have no significant effect on the outcome. This means that the effect of each factor (NaOH and time) on the ash content is independent of each other. From the Pareto diagram shown in Figure 5, we can deduce that NaOH concentration has a significant effect on protein content in chitin samples. The effect of time remains weak. The interaction effect of the two factors is very weak. The main effects graph (Figure 6) shows that as NaOH concentrations rise, protein content in chitin samples falls significantly. Protein content in longer-deproteinized chitin samples is higher than in shorter-deproteinized samples. The interaction diagram (Figure 7) shows that protein content decreased with increasing NaOH concentration

Table 10. Estimated regression coefficients for the chitin protein content model

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		2.2790	0.0545	41.85	0.000	
NaOH concentration(mol/L)	-0.6050	-0.3025	0.0609	-4.97	0.003	1.00
Time (min)	0.2850	0.1425	0.0609	2.34	0.058	1.00
NaOH concentration (mol/L) *time (min)	0.0950	0.0475	0.0609	0.78	0.465	1.00

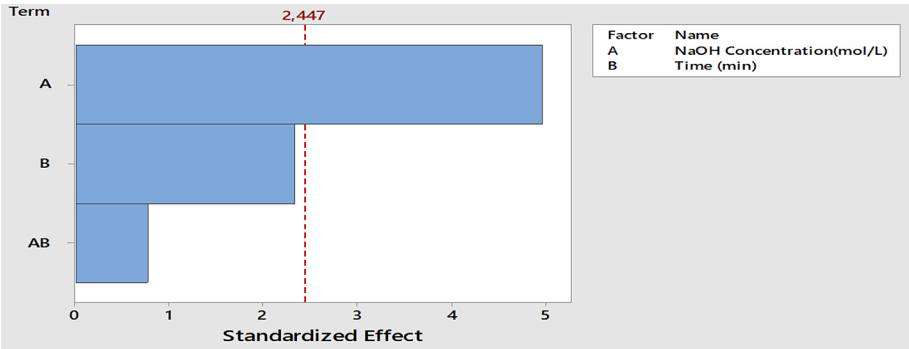

Figure 5. Pareto chart for the effects of factors and their interaction

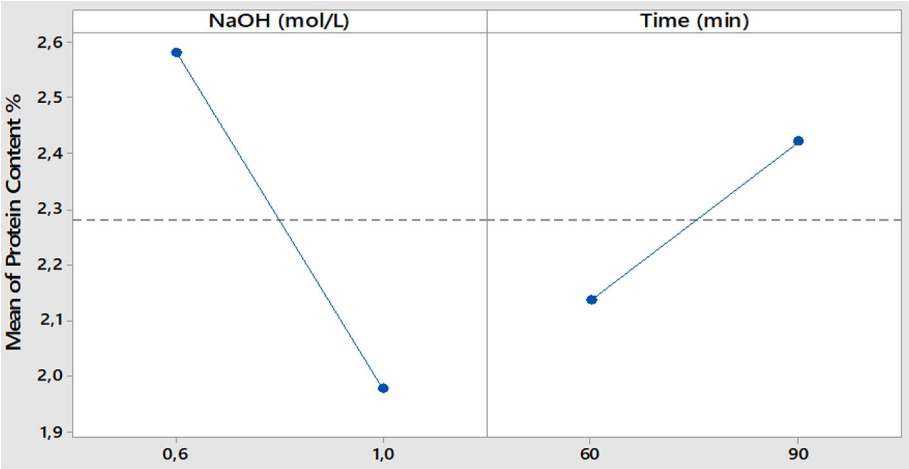

Figure 6. Diagrams of main effects on protein contents

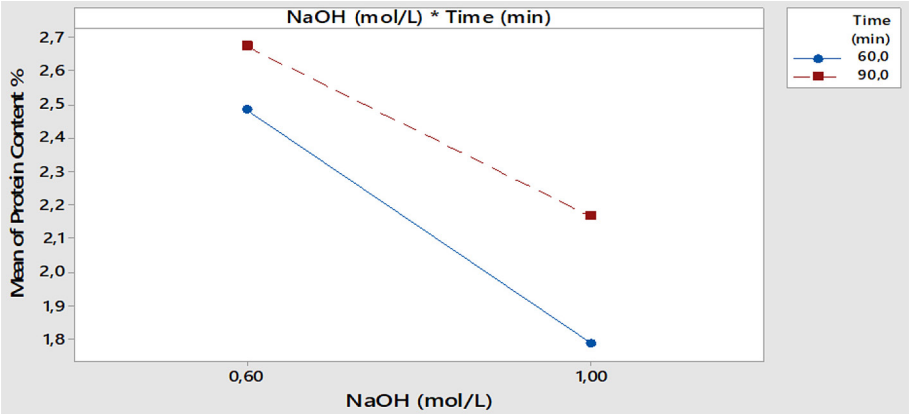

Figure 7. Diagrams of main effects interactions on protein contents

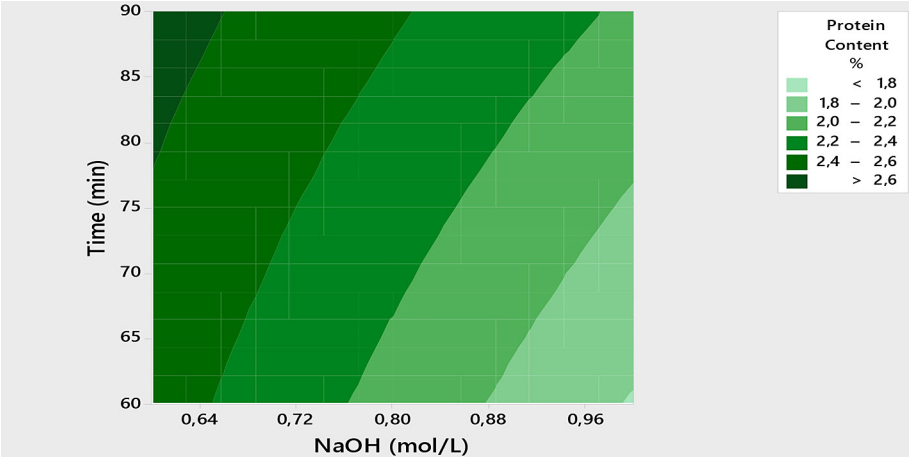

Figure 8. Curve of protein content versus time (min) and NaOH concentration (mol/L)

Table 11. Comparison of optimal chitin extraction conditions according to the literature

Parameter	HCl concentration	Demineralization time	NaOH concentration	Temperature	Deproteinization time	Yied %	Exeriemntal plan	References
Shrimp shell	8%	48 h	3.5 N		1h		Response surface methodology (RSM)	Adeyi et al., 2017
Penaeus notialis	3.25M	19.03 h	2.43 N		2.03h		Response surface methodology	Amoo et al., 2019
Omani shrimp shell	3%	1 h	50%	110	3h	53.31%	RSM	Al Hoqani et al., 2020
Shrimp shell	0.73 mol/l	132.61 min	0.95 mol/l	60.49	75.65 min	10.13%	RSM	Tokatlı et al., 2017

for both deproteinization times (1 h and 1 h 30 min). The slope values of the two lines are nearly identical, indicating that the effect of NaOH concentration on protein removal remains consistent across deproteinization times. Figure 8 shows that higher NaOH concentrations and shorter deproteinization times yielded the lowest protein contents (< 1.8). The highest contents were obtained using lower NaOH concentrations and longer deproteinization times. It can be concluded that a higher NaOH concentration and a moderate deproteinization time result in lower protein concentrations.

CONCLUSIONS

The findings of this study clearly demonstrate the validity of the parameters and levels

we selected. The optimal conditions we established for chitin extraction, which included a one-hour demineralization process with 500 mL HCl followed by a one-hour deproteinization with a 1 mol/L NaOH concentration, produced excellent results. The Ash and Biuret analyses revealed that chitin is produced with a low concentration of minerals and proteins. Furthermore, the regression models developed demonstrated a strong ability to describe and predict the responses associated with chitin removal. As a result, the volume of HCl and the concentration of NaOH can be used as control variables to improve chitin extraction. Other factors that can affect chitin extraction include shell pre-treatment, HCl concentration, NaOH volume, temperature, and so on. More research and studies are thus needed to improve the chitin extraction process. Complementary analyses, such as infrared (IR) spectroscopy, may be useful in determining chitin's chemical structure and quality. However, our method allowed us to save water and chemicals while maintaining the quality of the extracted chitin. In addition, we were able to significantly reduce the time required for these steps, which saved time during the manufacturing process. Comparing our findings to those of previous studies has allowed us to highlight the significant advances we have made. Our optimal extraction conditions have demonstrated significant advantages over existing methods (Table 11) This comparison validates our optimization approach and demonstrates its ability to improve the environmental and economic efficiency of chitin extraction in the industrial sector. To summarize, this method offers a viable pathway for producing high-quality chitin that can be used in a variety of applications, including medicine, fertilizer production, and water treatment.

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