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Optimization studies of chitin and chitosan production from *Penaeus notialis* shell waste

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Optimization studies of extraction of chitin and chitosan from pink shrimp (*Penaeus notialis*) shell waste and of the degree of deacetylation (*DDA*) of extracted chitosan were investigated via the Box-Behnken design of experiments using response surface methodology. Robust quadratic models for predicting the extraction yields of chitin and chitosan and *DDA* of chitosan were obtained. These models were verified by determining their eigenvalues and determinants, thereby revealing the nature of the optimum points and Hessian matrices. The respective modelled optimization conditions for the maximum yields of chitin and chitosan and for the highest *DDA* of chitosan were obtained thus: (3.25 M HCl solution, 19.03 h demineralization time, 2.43 M NaOH solution, and 2.03 h deproteinization time), (50% w/w NaOH solution, 87.9°C deacetylation temperature, and 145.26 min deacetylation time) and (50% w/w NaOH solution, 97.2°C deacetylation temperature, and 90 min deacetylation time). Excellent agreements were achieved between the experimental responses (extraction yields of chitin and chitosan, and *DDA* of extracted chitosan) and their predicted values with % error <5 in all cases.

Key words: Chitin, deproteinization, deacetylation, chitosan, optimization, response surface methodology.

INTRODUCTION

Shrimps are one of the most important seafood worldwide. Industrially, about 45-55% of raw shrimp weight is generated as shell waste during shrimp processing, clean-up and packaging (Hossain and Iqbal, 2014; Lertsutthiwong et al., 2002). These biological wastes can be used to produce value-added products (such as chitin and chitosan) instead of causing major environmental concerns such as air and water pollution (Nouri and Khodaiyan, 2014a, b). On a dry basis, shrimp shell waste contains 30 to 40% w/w protein, 30 to 50% w/w calcium (II) trioxocarbonate (IV) ($CaCO_3$) and 10 to 30% w/w chitin (Hajji et al., 2014; Nithya et al., 2014).

The second most abundant natural bio-polymer after cellulose is chitin, β- (1 → 4) N- acetyl-D-glucosamine, and is one of the chief components of the exoskeleton of crustaceans (crabs, shrimps, krill, barnacles, lobsters, etc.), insects and fungal cell walls. On partial deacetylation of chitin, the cationic amino biopolymer obtained is chitosan, β- (1 → 4) D-glucosamine (Ibitoye et al., 2018). Due to the compact structure of solid state of chitin, it remains insoluble in most solvents and dilute acids. This then usually leads to carrying out a chemical deacetylation of chitin to solve the problem of insolubility and produce the most common derivative (chitosan)

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(Hajji et al., 2014; Roberts, 1992). Chitin and its derivative, chitosan, can be distinguished mainly by the amount or percentage of the acetyl-glucosamine group present in the bio-polymer. In a case where the acetyl-glucosamine group is > 50%, the bio-polymer is referred to as chitin but if the percentage is < 50%, the bio-polymer is chitosan (Kamboj et al., 2015; Nouri et al., 2016). The structural formulae of chitin and chitosan show the linear chain of acetyl-glucosamine, the removed acetyl groups (CH_3-CO) and the bond types of both biopolymers (Okoya et al., 2016).

Chitin and chitosan are natural, non-toxic, highly stable, and biodegradable polymers, which are difficult to degrade thermally and chemically. They find extensive applications in industries like textiles (Al-Sagheer et al., 2009; Muzzarelli and Peter, 1997), food processing (Ko et al., 2003; Rhoades and Roller, 2000), medicine (Kaya et al., 2014), agriculture (Hirano et al., 2001), and wastewater treatment (Kaya et al., 2016; Rinaudo, 2006). However, the most significant applications in chitin/chitosan technology have been in the area of environmental studies, which include removal of dyes (Kyzas et al., 2017; Szymczyk et al., 2015), polychlorinated biphenyl (PCB) removal (Ikeda et al., 1999), and chemical waste detoxification (Mohanasrinivasan et al., 2014; Wagner and Nicell, 2002). Chitin and chitosan also find applications in water treatment such as filtration (Al-Manhel et al., 2018; Juang and Chiou, 2001), desalination (Arai and Akiya, 1978; Raeiatbin and Acikel, 2017), and flocculation/coagulation (Eikebrokk and Saltnes, 2002; Pontius, 2016; Sudha et al., 2017).

Over the years, chitin has been produced from various crustaceans, the different sources of which affect the production of chitin and in turn of chitosan. Equally, the origin of the source of the crustacean has an influence on the percentage of chitin present in it (Abdou et al., 2008; Muzzarelli and Peter, 1997). Therefore, several works have been reported on the extraction and characterization of chitin and its derivatives from different origins. Limam et al. (2011) investigated the extraction and characterization of chitin and chitosan from two species of crustacean of Tunisian origin. Also, Nouri et al., (2016) isolated chitosan with high functionality from species of Indian white shrimp, *Penaeus indicus*, shell waste. Recently, Ibitoye et al. (2018) examined the physicochemical characteristics of the extracted chitin and chitosan from house cricket and concluded that they compared favourably with the commercial chitin and chitosan.

Despite all these reported works, literature is scanty on the comprehensive optimization studies of the production of chitin and chitosan from crustacean bio-wastes. Chitin sources are abundantly available along river banks and coastal areas (Amos, 2007). The shells of crustaceans are discarded after processing, without proper method of disposal in Nigeria and perhaps in some developing countries thereby constituting environmental pollution and these can be utilized to produce chitin that can be chemically deacetylated to chitosan.

Response surface methodology (RSM) is a useful statistical technique for designing experiments where the number of experimental trials can be reduced, for building models, and for analysing the influences of numerous design variables on the response being investigated, whereby the significant and insignificant factors can be determined. RSM can equally be employed to optimize treatment conditions and processes (Krishnaiah et al., 2015; Younes et al., 2012). This study aims at determining the optimum conditions for the respective extraction yields of chitin and chitosan, and for the degree of deacetylation (*DDA*) of the extracted chitosan from pink shrimp shell waste using RSM via Box-Behnken Design (BBD) of experiments. The chitin and chitosan were extracted and isolated using the chemical processes of demineralization, deproteinization and deacetylation, respectively with high yield and degree of deacetylation (*DDA*).

MATERIALS AND METHODS

Materials and Reagents

Shrimp (*Penaeus notialis*) shell bio-wastes of Nigeria origin were obtained from a fish market in Lagos State, Nigeria. Loose tissue was removed from the shrimp shell, the shell was then washed, and dried. The dried samples were ground in a 500 W-blender and sieved in a 250 µm sieve. At ambient temperature of 28±2°C, the samples were stored in polyethylene bags for further analysis.

NaOH pellets (97%) and HCl (~37%) were purchased from Fischer Scientific Company, USA, while potassium permanganate (99.0%) and oxalic acid dihydrate (99.5%) were purchased from J. T. Baker Company, USA.

Extraction of chitin from *P. notialis* shell waste

The extraction of chitin for *P. notialis* shell waste involved the processes of demineralization, deproteinization, decolourization, and subsequent deacetylation of the extracted chitin to chitosan.

Demineralization

The process of production of chitin from *P. notialis* involved demineralisation with 2 to 4 M hydrochloric acid for 12 to 24 h at ambient temperature of 28±2°C, constant agitation speed of 100 rpm, and solvent to solid ratio of 10:1 (w/v). Separation of the acid-shell mixture was done by vacuum filtration and distilled water was used to wash thoroughly the demineralized shell until a neutral pH was achieved.

Deproteinization

The demineralized shells were deproteinized with 1.5 to 3.5 M NaOH for 1 to 3 h at a temperature of 70±0.5°C, constant agitation speed of 100 rpm and solvent to solid ratio of 15:1 (w/v). The produced heterogeneous mixture was mixed thoroughly to form insoluble particles (chitin) and separated by vacuum filtration. The

Table 1. Coded and uncoded factors of RSM experimental design for chitin extraction.

Variable	Unit	Symbol code	Coded variables level		
			-1	0	+1
			Experimental value		
HCl concentration	mol/L	X_1	2	3	4
Demineralization time	H	X_2	12	18	24
NaOH concentration	mol/L	X_3	1.5	2.5	3.5
Deproteinization time	H	X_4	1	2	3

precipitate was then washed thoroughly with distilled water to a pH of 7.0.

Decolourization

The extracted crude chitin from the treated shrimp shells was decolourised by treating it with 10 g/L potassium permanganate for 1 h and then reacted with 10 g/L oxalic acid for another 1 h. The decolorized chitin was separated from the resulting mixture via vacuum filtration, after which washing with distilled water was performed until pH=7.0. Drying of the sample was carried out at 80°C for 3 h and the dry weight recorded.

Deacetylation of chitin

The deacetylation of chitin produced was carried out via immersion in 30 to 50% w/w of NaOH solution for 1.5 to 4.5 h at a temperature of (60-100)±0.5°C, constant agitation speed of 100 rpm, and solvent to solid ratio of 10:1 (w/v). Vacuum filtration was used to separate the resulting mixture which was thoroughly washed with distilled water until pH was neutral. The solid matter obtained (that is chitosan) was oven-dried at 80°C for 3 h and the dry weight recorded.

Response surface optimization of chitin and chitosan extraction

The optimum conditions for production of chitin and chitosan were determined by using response surface methodology (RSM) in MINITAB 17.1 environment. Design of experiments (DOE) was performed employing three levels and four variables for the chitin extraction process and three levels and three variables for the chitosan extraction process. The optimized conditions obtained from the chitin extraction process were then used for the chitosan production from the shrimp shell wastes. The parameters employed for both extraction processes are shown in Tables 1 and 2.

Analysis of extraction yield

The respective extraction yields of chitin and chitosan from *P. notialis* shell wastes were analysed using Equation 1:

$$Y_j = \left(W_j / W_s\right) \times 100, \quad j = ch,\ cs \tag{1}$$

where ch and cs represent chitin and chitosan, respectively, Y_j represents extraction yield of *j* in %, W_j represents dried extraction weight of *j* in g, W_s represents weight of shrimp shell bio-wastes in g (= 25 and 45 g for chitin and chitosan extraction, respectively).

Determination of the degree of deacetylation (*DDA*) of chitosan

The degree of deacetylation of the chitosan produced from shrimp shell waste was determined by using acid-base titration method of Zhang et al., (2011) with some modification. 0.125 g of chitosan was dissolved in 30 mL of 0.1 M standard HCl aqueous solution, 5 to 6 drops of methyl orange was added as indicator and then stirred for 30 min until total dissolution was observed at room temperature. The resulting red chitosan solution was titrated with 0.1 M NaOH solution until a colour change to orange was observed. The degree of deacetylation of chitosan, *DDA*, in %, was calculated using Equation 2:

$$DDA = \left(\frac{c_1 V_1 - c_2 V_2}{M \times 0.0994} \times 0.016 \right) \times 100 \tag{2}$$

where c_1 and c_2 represent respective concentration of standard HCl and standard NaOH solutions in mol/L, V_1 represents volume of the standard HCl solution used to dissolve chitosan in mL, V_2 represents volume of standard NaOH solution consumed during titration in mL, and *M* represents weight of chitosan in g. The factor 0.016 in Equation 2 is the equivalent weight of NH_2 group in 1 mL of standard 1 M HCl solution, in g, and 0.0994 is the proportion of NH_2 group by weight in chitosan.

Experimental design and statistical analysis

The conventional technique for the optimization of a multi-variable system is to treat one variable at a time (OVAT). However, this technique is time-consuming, not cost efficient, and does not show the interactive and square effects of the factors. A response surface methodology (RSM) in form of a 3^k-Box-Behnken Design (BBD) (k = number of experimental factors) was chosen to statistically optimize the extraction of chitin and chitosan from shrimp shell wastes using four experimental factors $\left(X_1, X_2, X_3, X_4\right)$ and three experimental factors $\left(X_5, X_6, X_7\right)$, respectively in three factor levels $\left(-1, 0, +1\right)$, as shown in Tables 1 and 2. Hence, the chitin extraction optimization required 27 experimental runs while the chitosan production optimization required 15 experimental runs, as determined using Equation 3:

Table 2. Coded and uncoded factors of RSM experimental design for chitosan extraction.

Variable	Unit	Symbol code	Coded variables level		
			-1	0	+1
			Experimental value		
NaOH concentration	% by weight (w/w)	X_5	30	40	50
Reaction temperature	°C	X_6	60	80	100
Reaction time	Min	X_7	90	180	270

$$N = 2k(k-1) + c_p \quad (3)$$

where *N* represents total experimental runs, *k* represents number of variables (=4 for extraction of chitin, and =3 for extraction of chitosan and *DDA* of chitosan), and c_p represents number of central points (=3). Each experimental run was conducted in triplicates and the average value of the experimental response taken.

The Box-Behnken design is a design for fitting response surfaces called response surface designs or designs for quadratic models (Bezerraa et al., 2008). It reveals three levels in order to fit a model that is indicative of the curvature of the response. The quadratic regression model for predicting the response variables is given in Equation 4, which was used to fit the experimental results:

$$Y = \Phi_0 + \sum_{i=1}^{k} \Phi_i X_i + \sum_{i=1}^{k} \Phi_{i,i} X_i^2 + \sum_{1 \le i \le j}^{k} \Phi_{i,j} X_i X_j + \varepsilon \quad (4)$$

where Φ_0, Φ_i, $\Phi_{i,i}$, and $\Phi_{i,j}$ represent regression coefficients of constant, linear, quadratic, and interactions terms, respectively, X_i, X_j represent independent variables, *k* represents number of variables and *Y* represents predicted response (Montgomery, 2001).

The quality and adequacy of the model were evaluated using coefficient of determination, R^2, adjusted R^2 (*adj.* R^2), and predicted R^2 (*pred.* R^2). Analysis of variance (ANOVA) was conducted to show the efficacy of the fitted mathematical model. Three-dimensional response surface plots were used to examine the influence of independent variables on the responses investigated.

Determination and verification of optimum conditions

The optimized conditions for the production of chitin and chitosan and the *DDA* of chitosan were determined by analysing the response surface plots and the composite desirability function, with the objective of finding maximum yield, $(Y)_{\max}$, of both the chitin and chitosan, in %, and the maximum degree of deacetylation, $(DDA)_{\max}$, of the extracted chitosan, in %. The optimized conditions were verified by running the experiments again using the RSM results on MINTAB 17.1 software. The experimental responses were then compared with the predicted values. The optimized response quadratic models were also verified by equating the first derivatives of the mathematical functions to zero. The Hessian matrices were obtained, determinants of the leading principal minors of the Hessian matrices and eigenvalues were calculated to reveal the nature of the optimized variables and those of the Hessian matrices. The quadratic function obtained for *k* number of variables as described in Equation 4 is used to illustrate the necessary and sufficiency conditions needed to determine the nature of extrema points.

$$\frac{\partial \underline{Y}}{\partial \underline{X}} = \underline{0} \text{ at } \underline{X} = \underline{X}^* \quad (5)$$

$$Q = \sum_{i=1}^{k} \sum_{j=1}^{k} h_i h_j \left. \frac{\partial^2 Y}{\partial X_i \partial X_j} \right|_{\underline{X} = \underline{X}^*} \quad (6)$$

$$H(\underline{X})\big|_{\underline{X} = \underline{X}^*} = \left[\left. \frac{\partial^2 Y}{\partial X_i \partial X_j} \right|_{\underline{X} = \underline{X}^*} \right] \quad (7)$$

where $\underline{X}^*$ represents optimum (extremum) point, Q represents quantity of the quadratic form of the Hessian matrix, and $H(X)\big|_{\underline{X} = \underline{X}^*}$ represents Hessian matrix of the predicted response. Equation 5 shows the necessary condition needed for Equation 4 to calculate optimum of the design variables and Equation 6 is the sufficiency condition needed for the stationary points of Equation 4 to be an extremum (optimum) point.

RESULTS AND DISCUSSION

Development of regression model equations for chitin and chitosan extraction yield and optimization studies

In this investigation, RSM via BBD was employed to determine the optimum combination of parameters for production yield of chitin and chitosan from shrimp shell bio-wastes. The respective observed and predicted yields, $(Y_1)_{\exp}$ in g, and $(Y_1)_{pred}$ in g, for the 27 experimental runs during the chitin extraction are shown in Table 3, where

Table 3. Box-Behnken Design arrangement for the experimental and predicted values for the yield of chitin extraction from shrimp shell waste.

Run order	Symbol code				Yield of chitin		Yield of chitin (%) (g/25 g)	% error, ε_1
	X_1	X_2	X_3	X_4	$(Y_1)_{\exp}$ in g	$(Y_1)_{pred}$ in g		
1	-1	-1	0	0	3.20	3.13000	12.80	2.1875
2	+1	-1	0	0	4.70	4.76833	18.80	1.4539
3	-1	+1	0	0	4.50	4.38500	18.00	2.5556
4	+1	+1	0	0	5.01	5.03333	20.04	0.4657
5	0	0	-1	-1	4.45	4.41000	17.80	0.8989
6	0	0	+1	-1	4.50	4.57667	18.00	1.7037
7	0	0	-1	+1	4.95	4.82667	19.80	2.4916
8	0	0	+1	+1	4.55	4.54333	18.20	0.1465
9	-1	0	0	-1	3.40	3.37792	13.60	0.6495
10	+1	0	0	-1	5.10	4.97125	20.40	2.5245
11	-1	0	0	+1	3.90	4.01958	15.60	3.0662
12	+1	0	0	+1	4.70	4.71292	18.80	0.2748
13	0	-1	-1	0	4.18	4.19958	16.72	0.4685
14	0	+1	-1	0	5.10	5.15458	20.40	1.0703
15	0	-1	+1	0	4.40	4.33625	17.60	1.4489
16	0	+1	+1	0	4.93	4.90125	19.72	0.5832
17	-1	0	-1	0	3.60	3.66042	14.40	1.6782
18	+1	0	-1	0	5.40	5.42875	21.60	0.5324
19	-1	0	+1	0	4.20	4.22708	16.80	0.6448
20	+1	0	+1	0	4.75	4.74542	19.00	0.0965
21	0	-1	0	-1	4.00	4.05208	16.00	1.3021
22	0	+1	0	-1	4.50	4.56208	18.00	1.3796
23	0	-1	0	+1	4.00	3.99375	16.00	0.1563
24	0	+1	0	+1	5.00	5.00375	20.00	0.0750
25	0	0	0	0	6.40	6.39667	25.60	0.0521
26	0	0	0	0	6.42	6.39667	25.68	0.3635
27	0	0	0	0	6.37	6.39667	25.48	0.4186

$$\varepsilon_1 = \frac{(Y_1)_{\exp} - (Y_1)_{pred}}{(Y_1)_{\exp}} \times 100$$

It was observed that the extracted chitin from 25 g of dried shell waste of pink shrimp (*Penaeus notialis*) was in the range of 3.20 to 6.42 g corresponding to yield of 12.80 to 25.68%.

Table 4 shows the results of the 15 experimental runs for the chitosan extraction from the shrimp shell waste, where $(Y_2)_{\exp}$ represents observed extraction yield of chitosan in g, $(Y_{2a})_{pred}$ represents predicted extraction yield of chitosan with both insignificant and significant effects in g, and $(Y_2)_{pred}$ represents predicted extraction yield of chitosan with significant effects only. The percentage yield of chitosan, Y^*, was calculated thus:

$$Y^* = \left(\frac{chitosan\ dry\ weight}{precursor\ dry\ weight} \right) \times 100 \quad (8)$$

In Table 4, it was observed that the extracted chitosan from 45 g of the shrimp shell waste was in the range of 4.27 to 7.52 g corresponding to yield of 9.49 to 16.71%. The resulting quadratic regression equations for estimating the optimal conditions for chitin extraction yield, $(Y_1)_{pred}$ and for chitosan extraction yield, $(Y_2)_{pred}$, from the shrimp shell waste are given in Equations 9 and 10, respectively.

$$\begin{aligned}(Y_1)_{pred} = & -30.6374 + 9.1454X_1 + 1.1535X_2 + 5.3321X_3 + 4.7821X_4 - 1.1X_1^2 - 0.0269X_2^2 \\ & - 0.7813X_3^2 - 1.0262X_4^2 - 0.0413X_1X_2 - 0.3125X_1X_3 - 0.2250X_1X_4 - 0.0162X_2X_3 \\ & + 0.0208X_2X_4 - 0.1125X_3X_4\end{aligned} \quad (9)$$

Table 4. Box-Behnken Design arrangement for the experimental and predicted values for the yield and *DDA* of chitosan extracted from shrimp shell waste.

Run order	X_5	X_6	X_7	$(Y_2)_{\exp}$ in g	$(Y_{2a})_{pred}$ in g	$(Y_2)_{pred}$ in g	Y^* in %	% error, ε_2	$(DDA)_{\exp}$ in %	$(DDA_{is})_{pred}$ in %	$(DDA)_{pred}$ in %	% error, ε_3
1	-1	-1	0	4.27	4.4275	4.4275	9.4889	3.6885	79.25	79.3800	79.3781	0.1616
2	+1	-1	0	5.30	5.2175	5.2175	11.7778	1.5566	84.15	84.1950	84.1931	0.0512
3	-1	+1	0	4.48	4.5625	4.5625	9.9556	1.8415	80.39	80.3450	80.3431	0.0584
4	+1	+1	0	7.39	7.2325	7.2325	16.4222	2.1313	88.11	87.9800	87.9781	0.1497
5	-1	0	-1	5.59	5.4175	5.6375	12.4222	0.8497	81.98	81.8750	81.8769	0.1257
6	+1	0	-1	7.52	7.5875	7.3675	16.7111	2.0279	88.90	88.8800	88.8819	0.0203
7	-1	0	+1	5.16	5.0925	4.8725	11.4667	5.5717	80.79	80.8100	80.8119	0.0271
8	+1	0	+1	6.21	6.3825	6.6025	13.8000	6.3205	86.15	86.2550	86.2569	0.1241
9	0	-1	-1	5.21	5.2250	5.2050	11.5778	0.0960	82.50	82.4750	82.4769	0.0278
10	0	+1	-1	6.17	6.2600	6.2800	13.7111	1.7828	86.05	86.2000	86.2019	0.1766
11	0	-1	+1	4.51	4.4200	4.4400	10.0222	1.5521	82.13	81.9800	81.9819	0.1803
12	0	+1	+1	5.55	5.5350	5.5150	12.3333	0.6306	82.98	83.0050	83.0069	0.0324
13	0	0	0	6.99	7.1200	7.1200	15.5333	1.8598	85.00	84.9033	84.9008	0.1167
14	0	0	0	7.20	7.1200	7.1200	16.0000	1.1111	84.80	84.9033	84.9008	0.1188
15	0	0	0	7.17	7.1200	7.1200	15.9333	0.6974	84.91	84.9033	84.9008	0.0109

$$(Y_2)_{pred} = -20.3650 + 0.2985X_5 + 0.4349X_6 + 0.0180X_7 - 0.0050X_5^2 - 0.0031X_6^2 - 0.0001X_7^2 + 0.023X_5X_6 \quad (10)$$

with % error being

$$\varepsilon_2 = \frac{(Y_2)_{\exp} - (Y_2)_{pred}}{(Y_2)_{\exp}} \times 100.$$

The positive and negative signs in the models, Equations 9 and 10, signify synergetic and antagonistic effects of the factors, $X_i, i = 1-7$, respectively.

The respective extraction yields of chitin and chitosan varied with all the combinations of conditions during the demineralization, deproteinization, and deacetylation stages of the shrimp shells. The conditions of extraction run 26 (3 M, 18 h, 2.5 M, 2 h) and extraction run 6 (50% w/w, 80°C, 90 min) as shown in Tables 3 and 4 corresponded to the maximum chitin and chitosan yield of 6.42 g (25.68%) and 7.52 g (16.71%), respectively. Furthermore, results obtained showed that the optimal conditions (6.52 g, 26.08%) for chitin preparation from shrimp shells were at 3.25 M HCl solution, 19 h demineralization time, 2.43 M NaOH solution, and 2.03 h deproteinization time, while the optimal conditions (7.62 g, 16.93%) for chitosan extraction yield from shrimp shells were achieved at 50% w/w NaOH concentration, 87.8°C reaction (deacetylation) temperature, and a reaction (deacetylation) time of 145.2 min.

The analysis of variance (ANOVA) and the estimated regression coefficients of each term of the regression models, Equations 9 and 10, are illustrated in Tables 5 and 6. Generally, the smaller and the larger the values of *p* (<0.05) and *t,* respectively, the more significant the corresponding coefficient term is. Based on the results shown in Table 5, the extraction yield of chitin from shrimp shells had significant linear effect, quadratic effect and interaction effect on all

Table 5. Estimated regression coefficients and the Analysis of Variance (ANOVA) for the second-order polynomial model for chitin extraction from shrimp shells (uncoded units).

Chitin extraction yield regression model, $(Y_1)_{pred}$								
Factor/source	***Seq SS***	***DF***	***Adj MS***	***Coef***	***SE Coef***	***F*-value**	***t*-value**	***p*-value**
Regression model	17.2201	14	1.23001	-	-	149.13	-	0.000
Constant	-	-	-	-30.6374	1.08467	-	-28.220	0.000
Linear	5.7748	4	2.24015	-	-	271.60	-	0.000
X_1	3.9216	1	7.18185	9.1454	0.30993	870.75	29.508	0.000
X_2	1.7328	1	4.11338	1.1535	0.05165	498.72	22.332	0.000
X_3	0.0102	1	2.76814	5.3321	0.29105	335.62	18.320	0.000
X_4	0.1102	1	2.50041	4.7821	0.27465	303.16	17.411	0.000
Square	10.4559	4	2.61399	-	-	316.93	-	0.000
X_1^2	1.9802	1	6.45333	-1.1000	0.03933	782.42	-27.972	0.000
X_2^2	1.7016	1	4.99230	-0.0269	0.00109	605.28	-24.602	0.000
X_3^2	1.1572	1	3.25521	-0.7813	0.03933	394.67	-19.866	0.000
X_4^2	5.6170	1	5.61701	-1.0262	0.03933	681.02	-26.096	0.000
Interactions	0.9893	6	0.16488	-	-	19.99	-	0.000
X_1X_2	0.2450	1	0.24503	-0.0413	0.00757	29.71	-5.450	0.000
X_1X_3	0.3906	1	0.39062	-.0.3125	0.04541	47.36	-6.882	0.000
X_1X_4	0.2025	1	0.20250	-0.2250	0.04541	24.55	-4.955	0.000
X_2X_3	0.0380	1	0.03802	-0.0162	0.00757	4.61	-2.147	0.053
X_2X_4	0.0625	1	0.06250	0.0208	0.00757	7.58	2.753	0.018
X_3X_4	0.0506	1	0.05062	-0.1125	0.04541	6.14	-2.477	0.029
Residual error	0.0990	12	0.00825	-	-	-	-	-
Lack of fit	0.0977	10	0.00977	-	-	15.43	-	0.062*
Pure error	0.0013	2	0.0063	-	-	-	-	-
Total	17.3191	26	-	-	-	-	-	-

R^2 99.43%, Predicted R^2 96.73%, AdjustedR^2 98.76%. *S*: Standard deviation (= 0.0908180); PRESS: prediction error sum of squares (= 0.56565); *Coef*: coefficient; *SE Coef*: standard error coefficient; *t*: student test; *p*: probability value; *S*: standard deviation; *insignificant *p* > 0.05 at 95% confidence level; *DF*: degree of freedom; *Seq SS*: sequential sum of squares; *Adj SS*: adjusted sum of squares; *Adj MS*: adjusted mean Square; *F*: Fisher's variance ratio.

the variables ($p < 0.05$; that is, significant at 95% confidence level) while the chitosan extraction yield had insignificant interaction effects of X_5X_7 and X_6X_7 since *p* > 0.05 at 95% confidence level, as presented in Table 6. Le Man et al. (2010) reported that for a regression model to be adequate, the correlation coefficient, R^2, value should not be less than 0.75. Large R^2 value does not usually indicate an acceptable regression model; a similarly high *adj*. R^2 value can be used to arrive at the conclusion of acceptable regression model (Koocheki et al., 2009). The values of *adj*. R^2 for the chitin and chitosan extraction yield from shrimp were 0.9876 and 0.9583, respectively while the respective R^2 values were 0.9943 and 0.9791.

Figure 1a and b shows the respective plots of predicted extracted chitin and chitosan yields by the developed models (Equations 9 and 10) against their corresponding experimental yields.

It was observed that the predicted yields were in consonance with the experimentally obtained yields of chitin and chitosan. Hence, an excellent correlation was achieved between the quadratic models prediction and the observed values. The residual plots for the extracted chitin and chitosan yields from shrimp shell waste are as shown in Figure 2a and b, respectively.

The assumption of normality of error terms is checked by the normal probability plot (Montgomery, 2001). The present results showed that most of the points were clustered around the blue line in Figure 2a and b, which is an indication that the error terms are approximately normal. Thus, the assumption of normality is valid in our investigation. The residuals in Figure 2a and b appear to be normally distributed (shown by the Normal probability

Table 6. Estimated regression coefficients and the Analysis of Variance (ANOVA) for the second-order polynomial model for chitosan extraction yield (uncoded units).

Chitosan extraction yield regression model, $(Y_2)_{pred}$				
Term/Factor	***Seq SS***	***Coef***	***F*-value**	***p*-value**
Regression model	17.3395	-	46.9300	0.0000
Constant	-	-20.3650	-	0.0010
Linear	9.4675	-	25.1800	0.0000
X_5	5.9858	0.2985	7.8700	0.0260
X_6	2.3113	0.4369	67.4000	0.0000
X_7	1.1704	0.0180	11.1200	0.0130
Square	6.9884	-	44.1300	0.0000
X_5^2	0.5230	-0.0050	17.4900	0.0040
X_6^2	5.5423	-0.0031	111.0500	0.0000
X_7^2	0.9231	-0.0001	17.4900	0.0040
Interactions	1.0788	-	10.3200	0.0140
X_5X_6	0.8836	0.0023	16.7400	0.0040
X_5X_7	0.1936	-0.0002	5.5500	0.0650*
X_6X_7	0.0016	0.0000	0.0050	0.8390*
Residual error	0.1743	-	-	-
Lack of fit	0.1485	-	3.8400	0.2140*
Pure error	0.0258	-	-	-
Total	17.7090	-	-	-

R^2 =97.91%, Predicted R^2= 87.62%, Adjusted R^2=95.83%. Standard deviation, *S*=0.229751; Prediction error sum of squares, *PRESS*=2.19155; *Seq SS*: sequential sum of squares; *Coef*: coefficient; *F*: Fisher's variance ratio; *p*: probability value; *insignificant ($p > 0.05$) at 95% confidence level.

and Histogram plots) and generally random (shown by the residuals against their fitted values and observation order) for the extraction yields of chitin and chitosan from shrimp shell waste.

Development of regression model equation for degree of deacetylation of chitosan and optimization studies

The degree of deacetylation of chitosan is one of the factors affecting its solubility, chemical reactivity and biodegradability and thus influences its performance in many applications (Abdel-Salam, 2013). The observed and predicted *DDA*s of chitosan produced from shrimp shell waste for the 15 experimental runs are shown in Table 4, where $(DDA)_{\exp}$ represents observed *DDA* of chitosan in %, $(DDA_{is})_{pred}$ represents predicted *DDA* of chitosan in %, with both insignificant and significant effects, and $(DDA)_{pred}$ represents predicted *DDA* of chitosan in %, with significant effects only. These results indicated that the *DDA* of chitosan ranged from 79.25 to 88.90%. The regression model for estimating the extremum conditions for degree of deacetylation, $(DDA)_{pred}$, of chitosan is given by Equation 11:

$$(DDA)_{pred} = 41.4627 + 0.4623X_5 + 0.5794X_6 + 0.0371X_7 - 0.0044X_5^2 - 0.0037X_6^2 + 0.0035X_5X_6 - 0.0004X_5X_7 - 0.0004X_6X_7 \quad (11)$$

with % error being

$$\varepsilon_3 = \frac{(DDA)_{\exp} - (DDA)_{pred}}{(DDA)_{\exp}} \times 100.$$

The optimal conditions for the degree of deacetylation, $(DDA)_{pred}$, of chitosan from the precursor shell waste were achieved at X_5 = 50% w/w, X_6 = 97.17°C, and

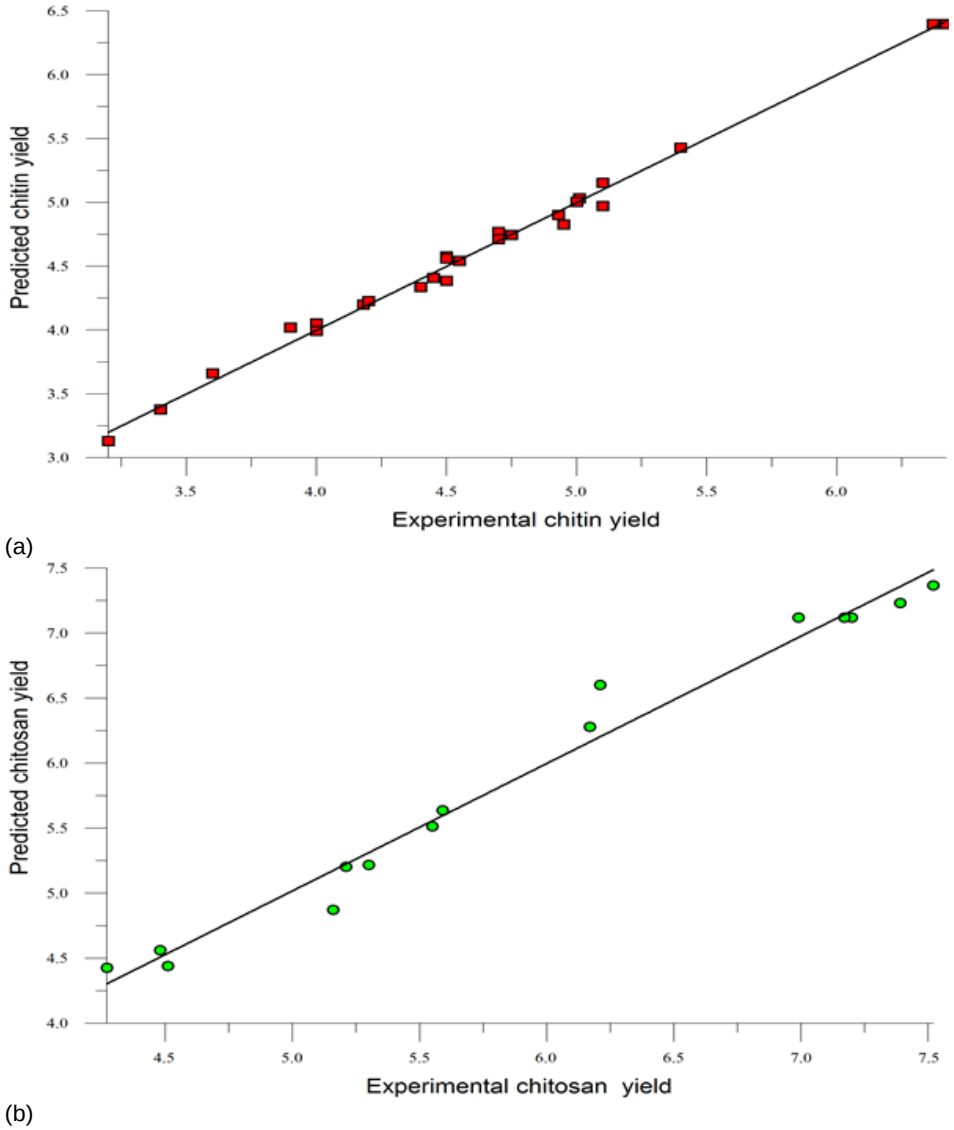
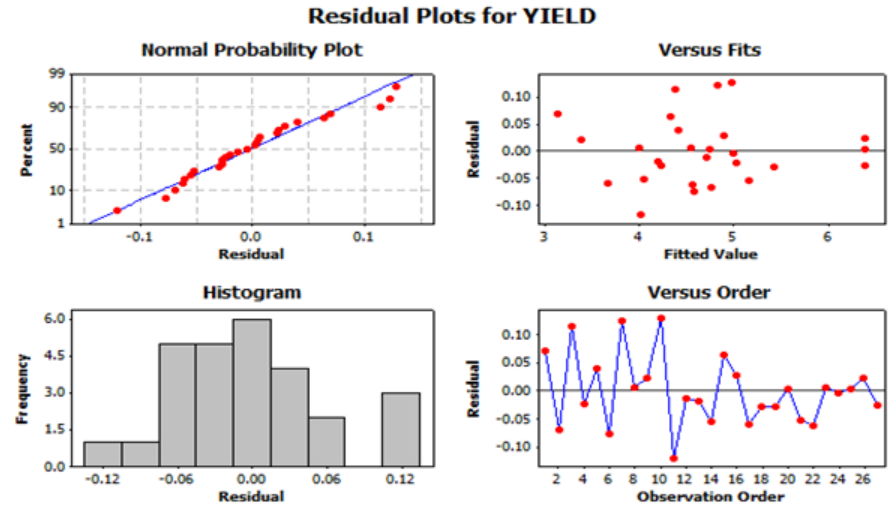


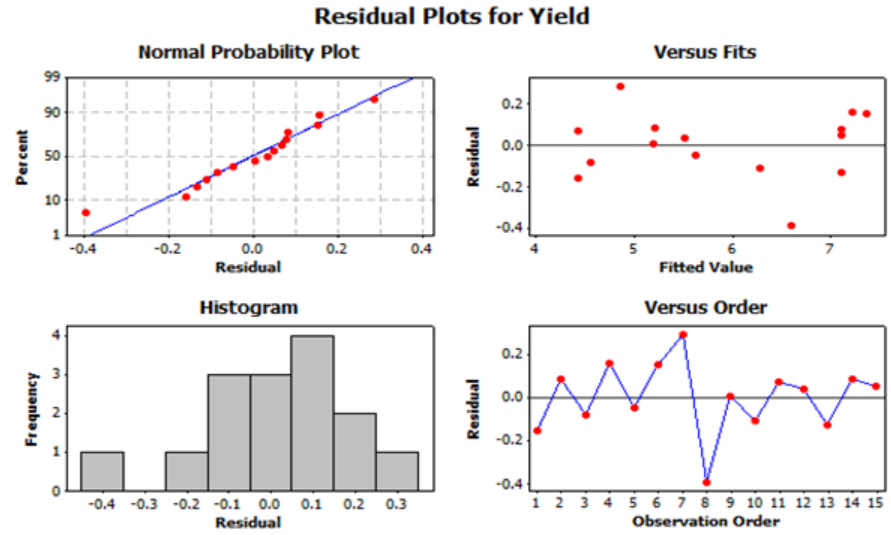
Figure 1. Predicted extraction yields of (a) chitin; (b) chitosan from shrimp shell waste against experimental yields.

X_7 = 90 min using the RSM software of MINITAB 17.1, with $(DDA)_{pred}$ = 89.73%. Figure 3 shows the plot of the predicted *DDA* of chitosan by the developed model (Equation 11) against their corresponding experimental values. Excellent agreement was achieved between the predicted and experimental *DDA* of chitosan. Figure 4 shows the residual plots for the degree of deacetylation of chitosan from shrimp shell waste corroborating the authenticity and robustness of the regression model.

The statistical analyses of the *DDA* of chitosan with significant interaction of design variables are shown in Table 7. Here, the *p*-values and *F*-values were used as tools to check the significance of each of the variables as well as their interactive and quadratic effects. As regards the importance and relationship amongst R^2, *adj*. R^2, and *pred*. R^2, same principle was used for the resulting quadratic regression equation. The values of R^2, *adj*. R^2, and *pred*. R^2 in Table 7 indicated an excellent agreement between $(DDA)_{\exp}$ and $(DDA)_{pred}$ of the chitosan, making the model sufficient for prediction of *DDA* of chitosan in the range of operational/test variables. The significance of the experimental variables for the *DDA* and the summary of analysis of variance (ANOVA) of the regression model of chitosan are shown in Table 7. Here, the ANOVA of the regression model equation revealed that the quadratic model derived from the Box-Behnken Design could adequately be used to predict the response (Table 4) as evident from the high *F*-values and very low *p*-values ($p \leq 0.05$).



(a)



(b)

Figure 2. Residual plots for (a) chitin; (b) chitosan extraction yield from shrimp shells.

Analysis of response surface

Three-dimensional response surface curves and two-dimensional contour plots were depicted to study the interactions between the design variables in the extraction yields of chitin and chitosan, and the degree of deacetylation of chitosan. These plots were used to determine the optimum levels of each factor required to obtain maximum response. The effects of individual factors on the chitin extraction yield from the shrimp shell waste are as shown in Figures 5 and 6. The plots were obtained by holding the third and fourth variables at maximum point (high) values. The surface plots (Figure 5) and contour plots (Figure 6) illustrate the interactive effects of HCl concentration, X_1 in M, time of demineralization, X_2 in h, NaOH concentration, X_3 in M, and time of deproteinization, X_4 in h, on the extraction yield of the chitin. Figures 5i and 6i show the effects of HCl concentration and demineralization time on the extraction chitin yield from shrimp shell waste, maximum yield was likely to occur between 3 to 3.5 M and 18 to 19.5 h; Figures 5ii and 6ii show that maximum response (chitin yield) should be between 3 to 3.5 M and 2 to 2.5 M; while Figures 5iii to vi and 6iii to vi show that maximum extraction chitin yield should be between 3 to 3.5 M and 1.5 to 2.2 h, 18 to 19.5 h and 2 to 2.5 M, 18 to 19.5 h and 1.5 to 2.2 h, and 2 to 2.5 M and 1.5 to 2.2 h, respectively. This is a strong indication of the dependence of the extraction yield of chitin on the HCl concentration, time of demineralization, NaOH concentration and the deproteinization time.

The interactive effects of the NaOH concentration, X_5

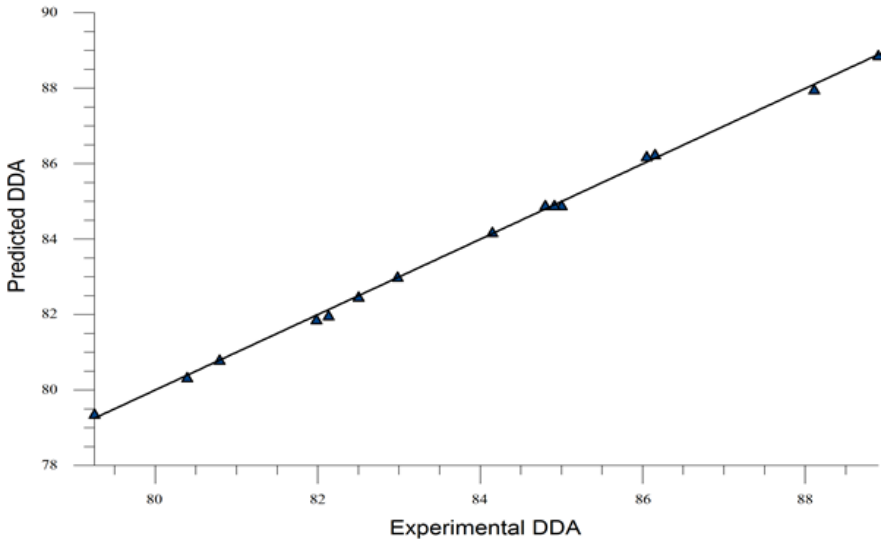


Figure 3. Predicted *DDA* of extracted chitosan from shrimp shell waste against experimental *DDA*.

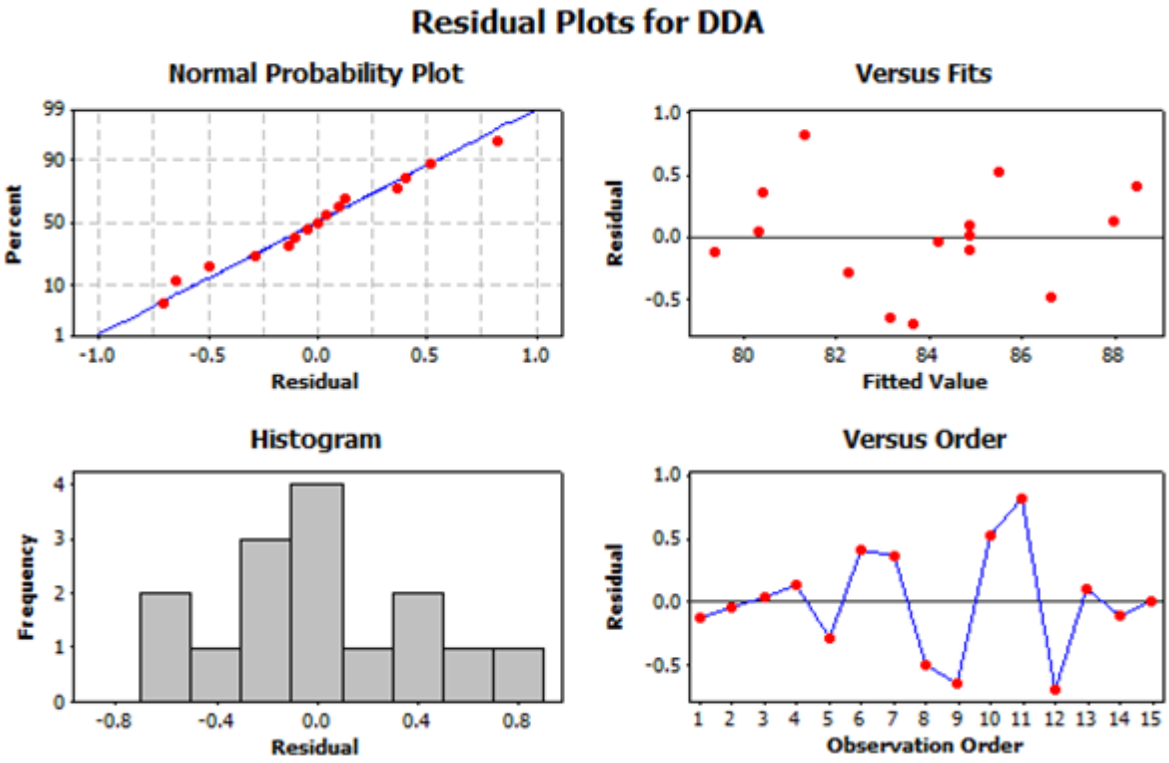


Figure 4. Residual plots for *DDA* of chitosan from shrimp shell waste.

in % w/w, reaction (deacetylation) temperature, X_6 in °C, and reaction (deacetylation) time, X_7 in min, on the extraction yield of chitosan by holding the third variable at mid-point value are as shown in Figures 7 and 8. Figure 7i and ii revealed that as NaOH concentration, deacetylation temperature and deacetylation time increase, the extraction chitosan yield increased to a certain point before evening out. These, therefore, showed positive significant interactions between X_5

Table 7. Estimated regression coefficients and the Analysis of Variance (ANOVA) for the second-order polynomial model for *DDA* of chitosan (uncoded units).

DDA of chitosan regression model, $(DDA)_{pred}$				
Term/factor	***Seq SS***	***Coef***	***F*-value**	***p*-value**
Regression model	108.6140	-	641.0100	0.0000
Constant	-	41.4627	-	0.0000
Linear	95.5910	-	106.6500	0.0000
X_5	77.5010	0.4623	45.1400	0.0010
X_6	11.2810	0.5794	283.6100	0.0000
X_7	6.8080	0.0371	64.7300	0.0000
Square	8.6040	-	203.1200	0.0000
X_5^2	0.4260	-0.0044	34.5500	0.0010
X_6^2	8.1780	-0.0037	386.1200	0.0000
X_7^2	0.0000	0.0000	0.0000	0.9620*
Interactions	4.4190	-	69.5500	0.0000
X_5X_6	1.9880	0.0035	93.8700	0.0000
X_5X_7	0.6080	-0.0004	28.7300	0.0020
X_6X_7	1.8220	-0.0004	86.0500	0.0000
Residual error	0.1270	-	-	-
Lack of fit	0.1070	-	2.6700	2.9100*
Pure error	0.0200	-	-	-
Total	108.7410	-	-	-

R^2 =99.88%, Predicted R^2= 98.93%, Adjusted R^2=99.73%. Standard deviation, *S*=0.145534; Prediction error sum of squares, *PRESS*=1.16407; *Seq SS*: sequential sum of squares; *Coef*: coefficient; *F*: Fisher's variance ratio; *p*: probability value; *insignificant ($p > 0.05$) at 95% confidence level.

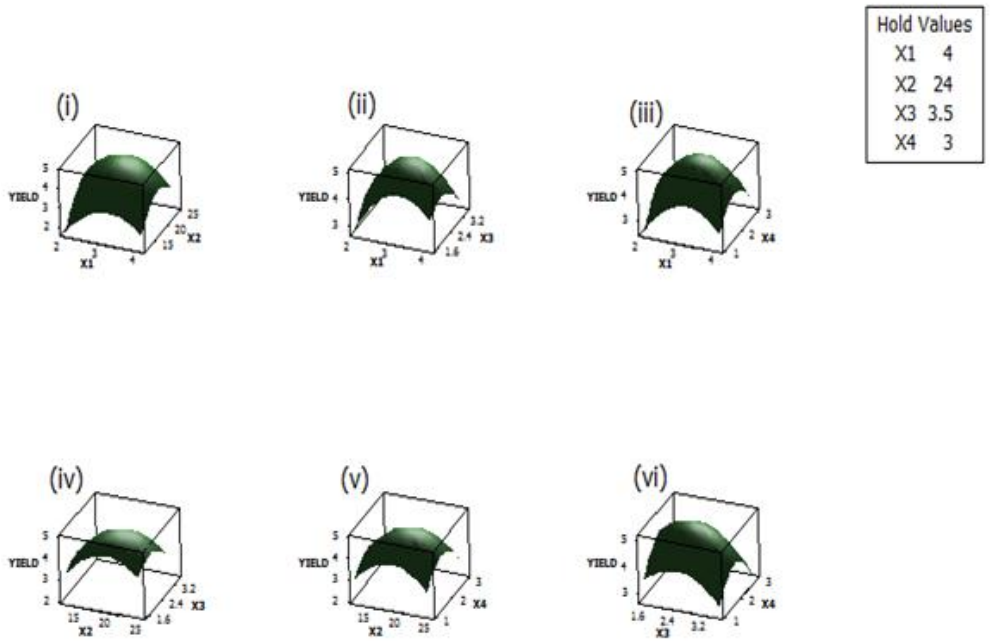


Figure 5. Three-dimensional surface plots of the effects of HCl concentration, X_1 in M, time of demineralization, X_2 in h, NaOH concentration, X_3 in M and time of deproteinization, X_4 in h, on the yield of chitin from shrimp (*Penaeus notialis*) shell waste.

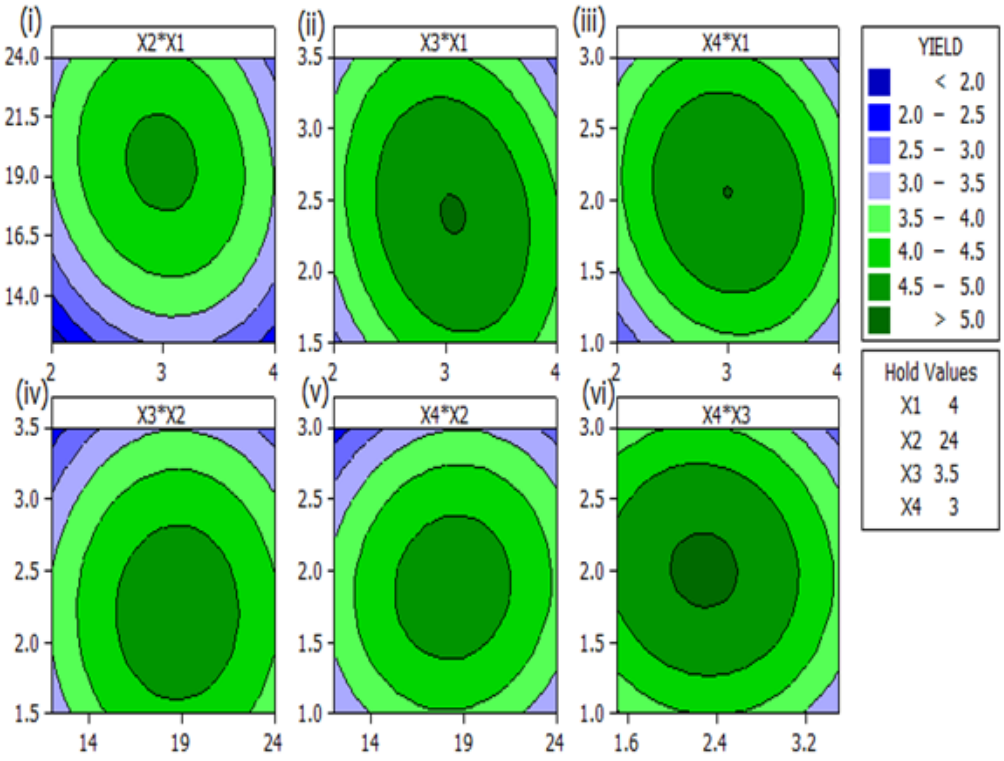


Figure 6. Contour plots of the effects of the HCl concentration, X_1 in M, time of demineralization, X_2 in h, NaOH concentration, X_3 in M, and time of deproteinization, X_4 in h, on the yield of chitin from shrimp (*Penaeus notialis*) shell waste.

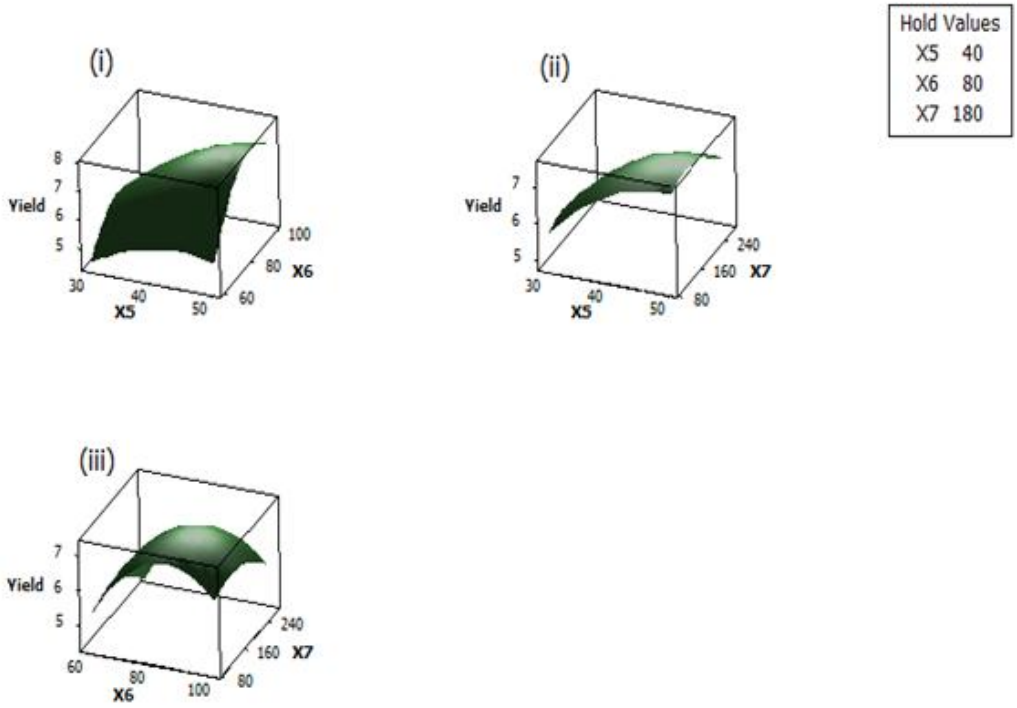


Figure 7. Three-dimensional surface plots of the effects of NaOH concentration, X_5 in % w/w, reaction (deacetylation) temperature, X_6 in °C and reaction (deacetylation) time, X_7 in min, on the yield of chitosan from shrimp (*Penaeus notialis*) shell waste.

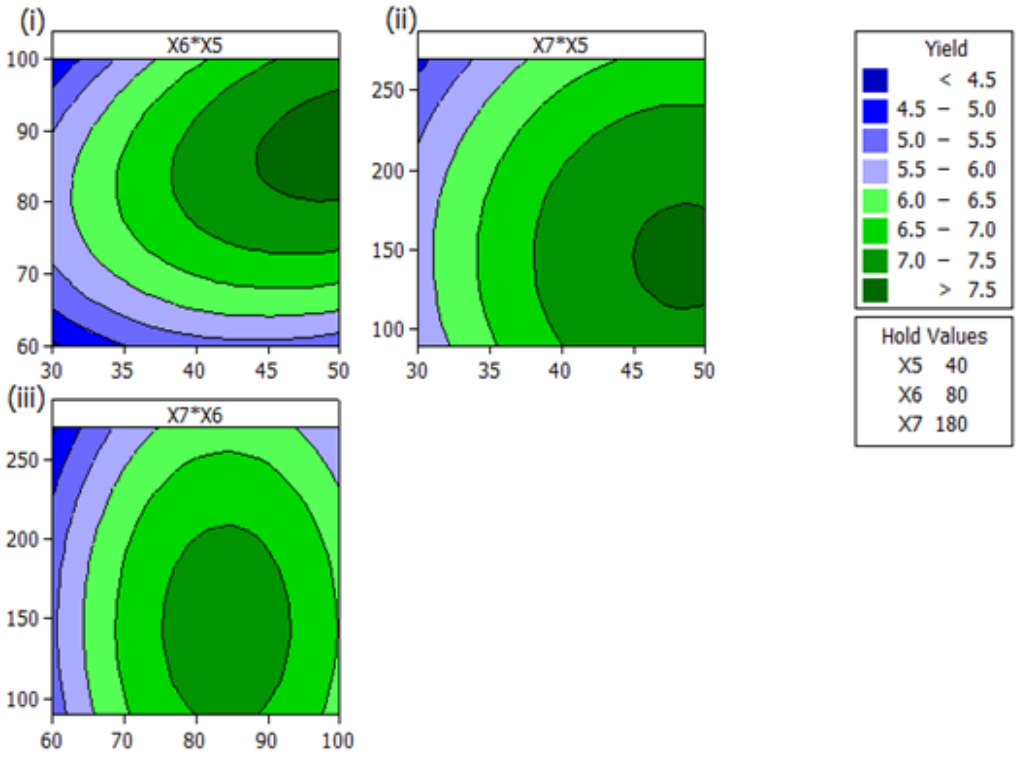


Figure 8. Contour plots of the effects of NaOH concentration, X_5 in % w/w, reaction (deacetylation) temperature, X_6 in °C and reaction (deacetylation) time, X_7 in min, on the yield of chitosan from shrimp (*Penaeus notialis*) shell waste.

and X_6 and $X_5 X_7$. Figure 7iii shows that a mixed and divergent relationship existed between the chitosan yield and input variables (X_6 and X_7); a positive interaction was observed between X_6 and X_7 before experiencing a negative effect after an optimum reaction temperature of approximately 88°C was obtained. Figure 8i to iii shows the effects of NaOH concentration, deacetylation temperature and deacetylation time on the yield of chitosan, indicating that maximum response was likely to occur between 45-50% w/w, 85-90°C and 130-150 min, respectively.

Figures 9 and 10 illustrate the interactive effects of the NaOH concentration, X_5 in % w/w, reaction temperature, X_6 in °C, and reaction time, X_7 in min, on the *DDA* of chitosan produced by holding the third variable at mid-point value. In Figure 9i, the degree of deacetylation was observed to rapidly increase with an increase in the NaOH concentration, X_5, and deacetylation temperature, X_6, while holding the deacetylation time, X_7, constant at a mid-point value of 180 min. This thus confirms the positive significant interaction effect between the NaOH concentration and deacetylation temperature. Additionally, this shows that the degree of deacetylation (*DDA*) of chitosan increases with an increase in NaOH concentration and deacetylation temperature. Figure 9ii and iii shows the effects of the input experimental variables (X_5, X_6 and X_7) on the DDA of chitosan from the *P. notialis* shell waste. The effects indicated a positive interaction between the independent variables (X_5, X_6 and X_7) and the *DDA* of produced chitosan. Figure 10i, ii and iii furthermore shows the effects of the NaOH concentration (X_5), deacetylation temperature (X_6) and deacetylation time (X_7) on the *DDA* of chitosan, illustrating that maximum response should be between 45 to 50% w/w, 80 to 100°C, and 80 to 100 min, respectively.

Verification of optimum conditions and response variables

To obtain the maximum responses, that is, the maximum extraction chitin yield, maximum extraction chitosan yield, and maximum *DDA* of chitosan from the shrimp shell waste, an optimization process was performed using the MINITAB RSM Optimizer® software. The response

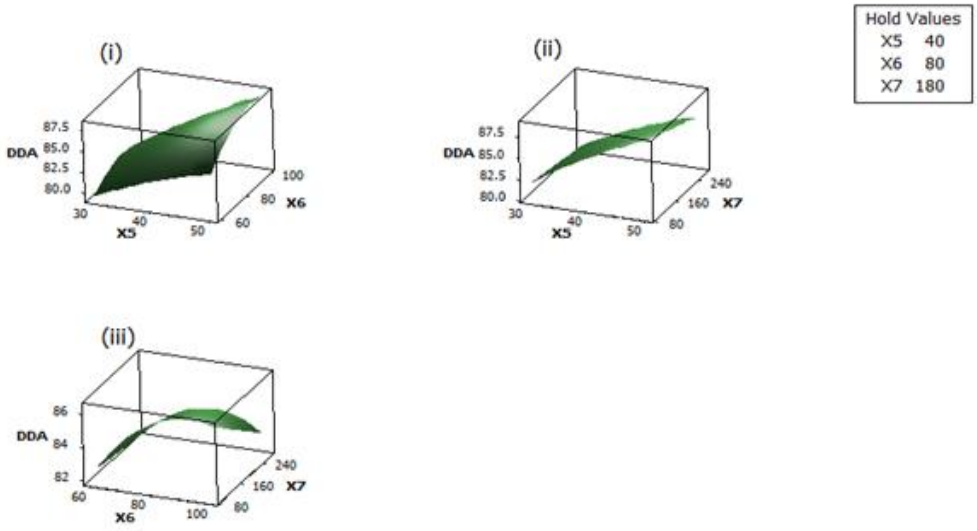


Figure 9. Three-dimensional surface plots of the effects of NaOH concentration, X_5 in % w/w, reaction (deacetylation) temperature, X_6 in °C and reaction (deacetylation) time, X_7 in min, on the *DDA* of chitosan from shrimp (*Penaeus notialis*) shell waste.

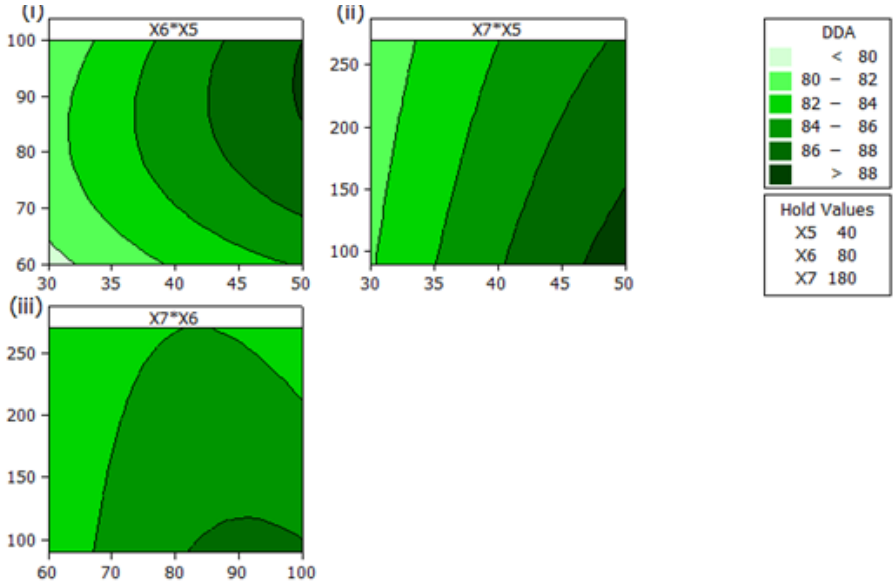


Figure 10. Contour plots of the effects of NaOH concentration, X_5 in % w/w, reaction (deacetylation) temperature, X_6 in °C and reaction (deacetylation) time, X_7 in min, on the *DDA* of chitosan from shrimp (*Penaeus notialis*) shell waste.

optimizer was used to determine the exact optimum values of the design variables (Zainal et al., 2014). The results of the response optimizer at optimum conditions for maximum goals for the extraction chitin yield, extraction chitosan yield, and *DDA* of chitosan were obtained as: (X_1 = 3.25 M, X_2 = 19 h, X_3 = 2.43 M, and X_4 = 2.03 h, $(Y_1)_{pred}$ = 6.5018 g), (X_5 = 50% w/w, X_6 = 87.8°C, and X_7 = 145.2 min, $(Y_2)_{pred}$ = 7.7595 g), and (X_5 = 50% w/w, X_6 = 97.17°C, and X_7 = 90 min, $(DDA)_{pred}$ = 89.9925%), as shown in Figures 11a, 11b and 12, respectively.

Validation experimental runs were conducted using the exact optimum conditions in duplicate and the average values of the responses were obtained as shown in Table 8. It was observed that there was an excellent agreement between the experimental response values and the predicted values based on the regression models.

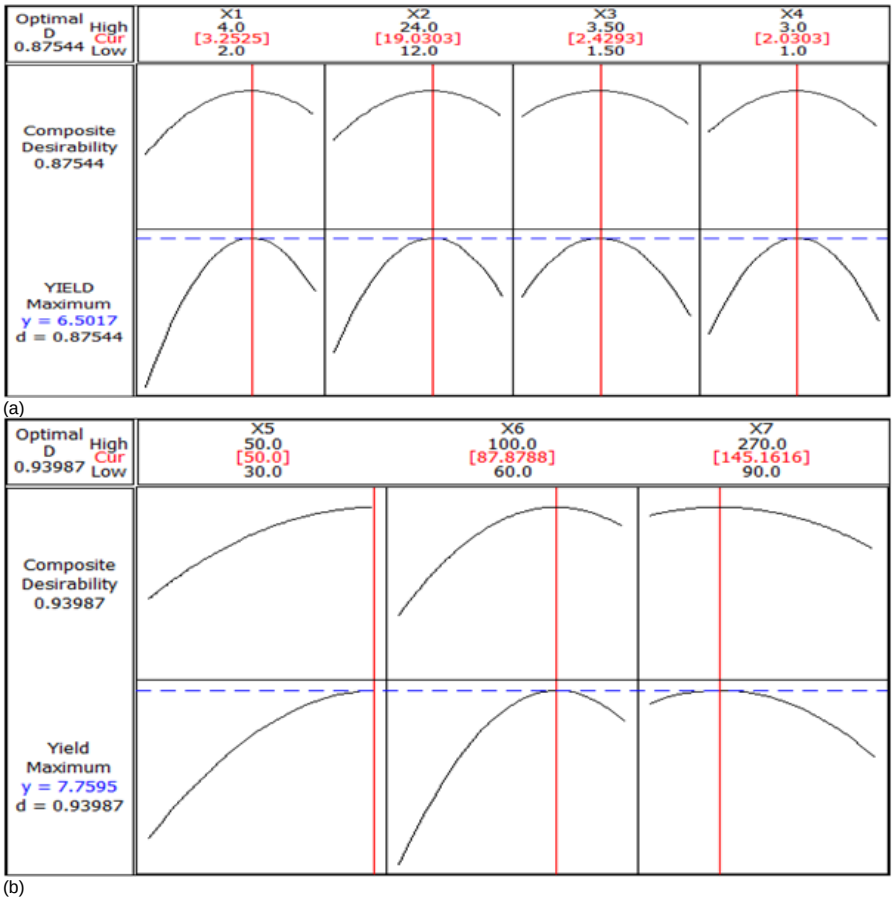


Figure 11. Response optimizer at the optimum conditions for the maximum extraction yields of (a) chitin, and (b) chitosan from shrimp (*Penaeus notialis*) shell waste.

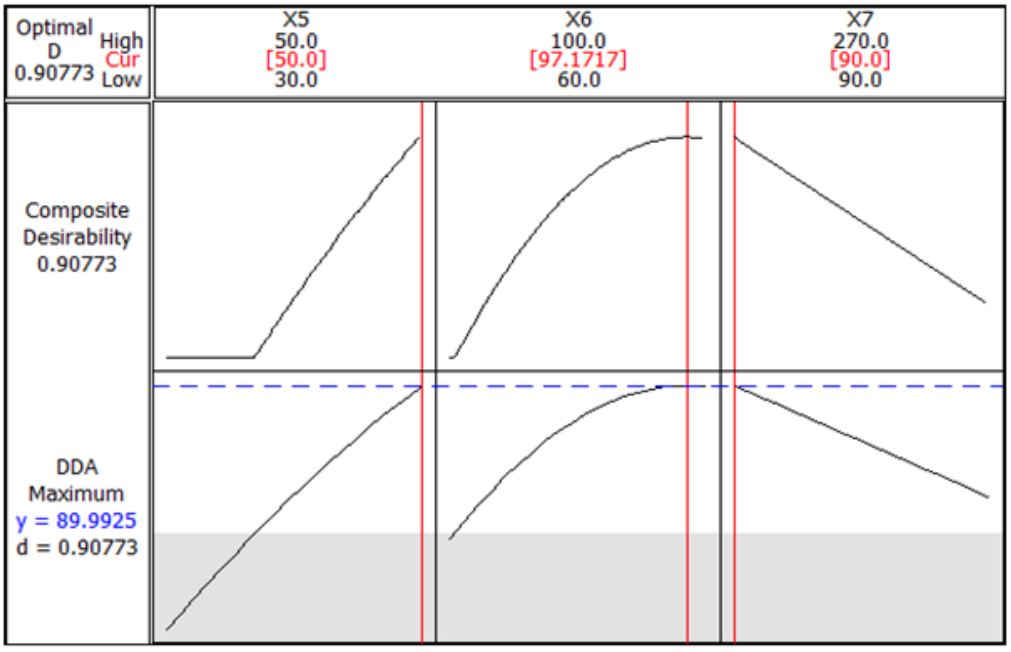


Figure 12. Response optimizer at the optimum conditions for maximum degree of deacetylation (*DDA*) of chitosan from shrimp (*Penaeus notialis*) shell waste.

Table 8. Verification of the optimum conditions of the regression models for the extraction yields of chitin and chitosan, and *DDA* of chitosan from shrimp shell waste.

Equation	Parameter	Response	Calculated optimum point ($\underline{X}^*$)	MINITAB optimum point ($\underline{X}^*$)	Eigenvalues ($\underline{\lambda}$)	Determinant values ($\underline{M}$)	Nature of Hessian matrix $H(\underline{X}^*)$
Equation 9 ($(Y_1)_{pred}$ = g/25 g)	Experimental value (g)	6.52000	X_1^*= 3.24860	X_1^*= 3.2525	λ_1= -2.4765	M_1= -2.2000	Negative definite
	MINITAB value (g)	6.50174	X_2^*= 19.0046	X_2^*= 19.030	λ_2= -1.9054	M_2= +0.1167	
	Calculated value (g)	6.49110	X_3^*= 2.41920	X_3^*= 2.4293	λ_3= -1.4343	M_3= -0.1767	
	-	-	X_4^*= 2.03390	X_4^*= 2.0303	λ_4= -0.0526	M_4= +0.3560	
Equation 10 ($(Y_2)_{pred}$ = g/45 g)	Experimental value (g)	7.62000	X_5^*= 50.0320	X_5^*= 50.000	λ_5= -0.0111	M_5= -0.0100	Negative definite
	MINITAB value (g)	7.75947	X_6^*= 87.5217	X_6^*=87.8788	λ_6= -0.0051	M_6= +5.67×10^{-5}	
	Calculated value (g)	7.74920	X_7^*= 145.981	X_7^*=145.161	λ_7= -0.0002	M_7= -1.134×10^{-8}	
Equation 11	Experimental value (%)	89.7300	X_5^*= 49.9880	X_5^*= 50.000	λ_5= -0.0117	M_5= -0.0088	Negative definite
	MINITAB value (%)	89.9925	X_6^*= 96.8750	X_6^*= 97.172	λ_6= -0.0046	M_6= +5.287×10^{-8}	
	Calculated value (%)	89.9820	X_7^*= 90.0800	X_7^*= 90.000	λ_7= -0.0010	M_7= -3.712×10^{-9}	

The optimum conditions of the regression models, Equations 9 to 11, were also verified by calculating the optimum values of the independent (or design) variables $X_i, i=1-7,$ and then equating the first derivatives of the mathematical functions to zero (necessary conditions), as given in Equation 5. The nature of the test (that is, design) variables and Hessian matrices were also obtained by calculating the eigenvalues ($\underline{\lambda}$) and values of the determinant ($\underline{M}$) of the Hessian matrices (sufficiency conditions), as given in Equation 7. As presented in Table 8, the eigenvalues of $H(\underline{X}^*)$ were all negative, the determinants of the leading principal minors of $H(\underline{X}^*)$ alternated in signs being negative and positive for odd and even values of *i*, that is $M_i\{H(\underline{X}^*)\}<0$ and $M_i\{H(\underline{X}^*)\}>0$, respectively. Hence, the Hessian matrices of the regression models, Equations 9 to 11, were negative definite indicating global (or local) maxima of the optimum points ($\underline{X}^*$). Moreover, the obtained regression models in this study were strictly concave. Table 8 also shows a robust comparison of the calculated optimum points with the optimum points obtained from MINITAB RSM Optimizer® software generated from the regression models and a comparison of the experimental, MINITAB, and calculated responses. Excellent agreements were achieved amongst these responses for extraction yields of chitin and chitosan, and the *DDA* of chitosan.

Conclusion

The extraction of chitin and chitosan from pink shrimp obtained from the coastal area of Lagos State, Nigeria, was investigated. The extraction process was studied via the Box-Behnken Design (BBD) of experiments using response surface methodology. The step-by-step studies showed the input variables that had tremendous influence on the extraction processes. The present study showed that chitin and chitosan could be obtained from the shell waste of pink shrimp (*P. notialis*), with high yield and high degree of deacetylation. It also demonstrated that response surface methodology (RSM) is an advantageous statistical technique for the investigation of the effects of major independent factors on the chitin and chitosan yield, and on the *DDA* of chitosan from

pink shrimp shell waste. Equally, the optimum factors for the extraction yield of the chitin were determined to be 3.25 M HCl, 19.03 h demineralization time, 2.43 M NaOH solution, and 2.03 h deproteinization time with an optimized (maximum) extraction chitin yield of 6.52 g (26.08%). Also, the optimized yield for chitosan extraction was obtained as 7.62 g (16.93%) at optimized conditions of 50% w/w NaOH solution, 87.9°C deacetylation temperature, and 145.26 min deacetylation time while the maximum degree of deacetylation (*DDA*) of chitosan was obtained as 89.73% at optimized conditions of 50% w/w NaOH solution, 97.2°C deacetylation temperature, and 90 min deacetylation time. Analysis of variance (ANOVA) and other statistical tools were used to test the authenticity and robustness of the obtained quadratic regression models, which were found to be very adequate and accurate in predicting the respective responses of the processes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abdel-Salam HA (2013). Evaluation of nutritional quality of commercially cultured Indian white shrimp Penaeus Indicus. International Journal of Nutrition and Food Sciences 2(4):160-166.

Abdou ES, Nagy KSA, Elsabee MZ (2008). Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology 99(5):1359-1367.

Al-Manhel AJ, Al-Hilphy ARS, Niamah AK (2018). Extraction of chitosan, characterisation and its use for water purification. Journal of the Saudi Society of Agricultural Sciences 17(2):186-190.

Al-Sagheer FA, Al-Sughayer MA, Muslim S, Elsabee MZ (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. Carbohydrate Polymers 77(2):410-419.

Amos TT (2007). Production and productivity of crustacean in Nigeria. Journal of Social Sciences 15(3):229-233.

Arai S, Akiya F (1978). Desalination reverse osmotic membranes and their preparation. US Patent 4111810.

Bezerraa MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76:965-977.

Eikebrokk B, Saltnes T (2002). NOM removal from drinking water by chitosan coagulation and filtration through lightweight expanded clay aggregate filters. Journal of Water Supply: Research and Technology – AQUA 51(6):323-332.

Hajji S, Younes I, Ghorbel-Bellaaja O, Hajji R, Rinaudo M, Nasri M, Jellouli K (2014). Structural differences between chitin and chitosan extracted from three different marine sources. International Journal of Biological Macromolecules 65:298-306.

Hirano S, Hayashi M, Okuno S (2001). Soybean seeds surface-coated with depolymerised chitins: chitinase activity as a predictive index for the harvest of beans in field culture. Journal of the Science of Food and Agriculture 81:205-209.

Hossain MS, Iqbal A (2014). Production and characterization of chitosan from shrimp waste. Journal of Bangladesh Agricultural University 12:153-160.

Ibitoye EB, Lokman IH, Hezmee MN, Goh YM, Zuki ABZ, Jimoh AA (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. Biomedical Materials 13(2):1-12.

Ikeda M, Gotanda T, Imamura Y, Hirakawa C (1999). Method for microbially decomposing organic compounds and method for isolating microorganism, US Patent 5919696.

Juang RS, Chiou CH (2001). Feasibility of the use of polymer-assisted membrane filtration for brackish water softening. Journal of Membrane Science 187(1-2):119-127.

Kamboj S, Singh K, Tiwary A, Rana V (2015). Optimization of microwave assisted Maillard reaction to fabricate and evaluate corn fiber gum-chitosan IPN films. Food Hydrocolloids 44:260-276.

Kaya M, Akyuz B, Bulut E, Sargin I, Eroglu F, Tan G (2016). Chitosan nanofiber production from drosophila by electrospinning. International Journal of Biological Macromolecules 92:49-55.

Kaya M, Baran T, Mentes A, Asaroglu M, Sezen G, Tozak KO (2014). Extraction and characterization of α-chitin and chitosan from six different aquatic invertebrates. Food Biophysics 9(2):145-157.

Ko JA, Park HJ, Park YS, Hwang SJ, Park JB (2003). Chitosan microparticle preparation for controlled drug release by response surface methodology. Journal of Microencapsulation 20(6):791-797.

Koocheki A, Taherian AR, Razavi S, Bostan A (2009). Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium Perfoliatum seeds. Food Hydrocolloids 23:2369-2379.

Krishnaiah D, Bono A, Sarbatly R, Nithyanandam R, Anisuzzaman SM (2015). Optimisation of spray drying operating conditions of Morinda Citrifolia L. fruit extract using response surface methodology. Journal of King Saud University – Engineering Sciences 27:26-36.

Kyzas GZ, Bikiaris DN, Mitropoulos AC (2017). Chitosan adsorbents for dye removal: A review. Polymer International 66:1800-1811.

Le Man H, Behera SK, Park HS (2010). Optimization of operational parameters for ethanol production from Korean foodwaste leachate. Internationa Journal of Environmental Science and Technology 7(1):157-164.

Lertsutthiwong P, How NC, Chandrkrachang S, Stevens WF (2002). Effect of chemical treatment on the characteristics of shrimp chitosan. Journal of Metals, Materials and Minerals 12(1):11-18.

Limam Z, Selmi S, Sadok S, El Abed A (2011). Extraction and characterization of chitin and chitosan from crustacean by-products: Biological and physiochemical properties. African Journal of Biotechnology 10(4):640-647.

Mohanasrinivasan V, Mishra M, Paliwal J, Singh S, Selvarajan E, Suganthi V (2014). Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste. 3 Biotechology 4(2):167-175

Montgomery DC (2001). Design and Analysis of Experiments, 5th ed., John Wiley and Sons, New York, NY, USA.

Muzzarelli RAA, Peter MG (1997). Chitin Handbook, 1st ed., European Chitin Society, Atec, Grottammare, Italy.

Nithya A, Jothivenkatachalam K, Prabhu S, Jeganathan K (2014). Chitosan based nanocomposite materials as photocatalyst (A Review). Materials Science Forum 781:79-94.

Nouri M, Khodaiyan F, Razavi HS, Mousavi M (2016). Improvement of chitosan production from Persian Gulf shrimp waste by response surface methodology. Food Hydrocolloids 59:50-58.

Nouri M, Khodaiyan F (2014a). Determination of parameters of chitosan extraction from shrimp shell, in: 1st International Conference on Natural Food Hydrocolloids, Mashhad, Iran.

Nouri M, Khodaiyan F (2014b). Persian Gulf shrimp waste optimization of chitosan extraction condition, in: 1st International Conference on Natural Food Hydrocolloids, Mashhad, Iran.

Okoya AA, Akinyele AB, Amuda OS, Ofoezie IE (2016). Chitosan-grafted carbon for the sequestration of heavy metals in aqueous solution. American Chemical Science Journal 11:1-14.

Pontius FW (2016). Chitosan as a drinking water coagulant. American Journal of Civil Engineering 4(5):205-215.

Raeiatbin P, Acikel YS (2017). Removal of tetracycline ny magnetic chitosan nanoparticles from medical wastewaters. Desalination and Water Treatment 73:380-388.

Rhoades J, Roller S (2000). Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Applied and Environmental Microbiology 66(1):80-86.

Rinaudo M (2006). Chitin and chitosan: properties and applications. Progress in Polymer Science 31:603-632.

Roberts GAF (1992). Chitin Chemistry, 1st ed. Macmillan Press, London, United Kingdom.
Sudha PN, Aisverya S, Gomathi T, Vijayalakshmi K, Saranya M, Sangeetha K, Latha S, Thomas S (2017). Application of chitin/chitosan and its derivatives as adsorbents, coagulants, and flocculants, in: Chitosan. Scrivener Publishing LLC pp. 453-487.
Szymczyk P, Fliipkowska U, Jozwiak T, Kuczajowska-Zadrozna M (2015). The use of chitin and chitosan for the removal of reactive black 5 dye. Progress on Chemistry and Application of Chitin and its Derivatives 22:260-272.
Wagner M, Nicell JA (2002). Detoxification of phenolic solutions with horseradish peroxidase and hydrogen peroxide. Water Research 36(16):4041-4052.
Younes I, Ghorbel-Bellaaj O, Nasri R, Chaabouni M, Rinaudo M, Nasri M (2012). Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization. Process Biochemistry 47(12):2032-2039.
Zainal S, Noorul FK, Ri Hanum YS, Rahmah M (2014). Optimization of chitosan extract from cockle shell using response surface methodology (RSM). Asian Journal of Agriculture and Food Science 2(4):314-323.
Zhang AJ, Qin QL, Zhang H, Wang HT, Li X, Miao L, Wu YJ (2011). Preparation and characterisation of good-grade chitosan from housefly larvae. Czech Journal of Food Sciences 29(6):616-623.