Optimization of Chitoasn Extract from Cockle Shell using Response Surface Methodology (RSM)

Zainal S[1]*, Noorul Fhadila K[1], Ri Hanum Y S[2] and Rahmah M[1]

1. School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.

2. School of Physics and Material Science, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.

**Corresponding author email: zainal147 {at} salam.uitm.edu.my*

ABSTRACT--- ***This study was conducted to determine the optimum condition for the extraction of chitosan by using response surface methodology (RSM). The chitosan was extracted from the cockle shell (Anadara granosa). The extraction was optimized using five levels and five variables such as the concentrations of HCl (%), and its immersion time (hr), the concentration of NaOH (N), the deacetylation temperature (^oC) and its deacetylation time (hr). The extraction process was involved three main steps which were, dimineralization, deproteination and deacetylation. A full factorial of central composite design by MINITAB software version 15 was employed to optimize the yield of chitosan. The optimum condition as predicted by the software was the concentration of HCl at 8% with 16.5 hours time of immersion, concentration of NaOH 2.6N, deacetylation temperature at 61^oC, and 1.5 hours for deacetylation time. The determination of coefficient R^2= 95.4 also was high, which means the experimental data was acceptable.***

Keywords--- cockle shell; chitosan; response surface methodology (RSM)

1. INTRODUCTION

Chitosan as dietary fiber can exhibits the hypolipidemic activity by reducing the triglycerides and cholesterol levels in blood plasma and liver of rats [1]. Chitosan is a copolymer of glucosamine and *N*-acetyglucosamine units linked by 1-4 glucosidic bonds [2]. It is the most abundant biopolymers in nature just after cellulose [3]. It also the most important naturally occurring polymeric materials since they different from synthetic polymers due to the presence of the (acetyl) amino group in the chitosan structure and as classified as cationis polysaccharides making them widely applicable in many field [1-4]. Chitosan is the cationic polysaccharides which obtained from the deacetylation of chitin [2]. According to [1], chitosan has received a great attention because of its higher polymer potential than cellulose in many fields due to their biofunctional polymers. Chitosan can be classified as non- toxic compound and biodegradable. Chitin, the precursor of chitosan is widely available in nature which can be found in the mollusca, onychophora, arthropoda, chaetognatha, pogonophora, tunicat, arthropod shell fungi, algae, protozoa. cnidaria, aschelminthes, echiurida, and annelid [1].

A large amount of the cockle shell waste is produced as result of the rapidly growing cockle processing industry. Normally, the ratio of flesh and cockle shell is 1 to 3, for example 100 kg of the cockle, only 30% is their flesh and other 70% is the shell. The shell is then discarded either at landfill or being dump near the bank of the river or the coastal area. This can caused major pollution to the air and water environment due to their bad odor and waste product.

The production of chitosan from the crustacean shells as food industry waste is economically feasible. The shells contain considerable quantities of astaxantin, a caratenoid that marketed as a fish food additive, antioxidant,preservatives [5].

The purpose of this study is to determine the optimum condition for the extraction of chitosan from cockle shell and being evaluated in full factorial design process by response surface methodology.

2. MATERIALS AND METHODS

2.1 Materials

The fresh cockle shell waste was obtained form cockle processing industry (Arma food Sdn.Bhd.) at Sekinchan, Selangor, Malaysia. Food or pharmaceutical chemical grade used for the this study was obtained form Merck Sdn. Bhd, Malaysia.

2.2 Methods

2.2.1 Preparation of the sample and the extraction of chitosan

The cockle shell was washed to remove all the dirt before dried using oven. Cleaned shell was dried in cabinet dryer until constant weight achieved. The method of experiment was carried out based on method of [6] with slightly modification. Dried shell was crushed by using a hammer mill. Cockle shell powder was undergone demineralization process by using hydrochloric acid (HCl), deproteination process by using sodium hydroxide (NaOH), and followed by deacetylation process by using strong NaOH. Then the chitosan powder was further purified by dissolving in 0.01M HCl solution before drying in oven for overnight.

2.2.2 Optimization of chitosan extract

2.2.2.1 Dimineralization

25g of cockle shell powder was immersed in 120ml of HCl (6- 14%) for (6-48 hours), then treated with 50ml of 2% NaOH for 1 hour. The remaining powder was washed with the distilled water.

2.2.2.2 Deproteination

The shell form the previous process was then immersed in NaOH solution (1.5 - 3.5 N), followed by boiling in water bath for 1hour. The mixture was then cooled at room temperature for 30 minutes. The mixture was filtered then washed until neutral with distilled water.

2.2.2.3 Deacetylation

The shell was immersed in 120ml of 50% NaOH solution, which was then boiled at (40-80 ^{o}C) for (1-3 hour). The sample was placed in fumed hood for 30 minutes. Then the sample was washed continuously with 50% NaOH solution and then filtered in order to obtain the solid matter. The chitosan obtained was placed in 250ml beaker then dried in oven for overnight.

2.2.2.4 Purification of chitosan

Chitosan powder was treated with 120ml of 0.01M HCl solution. Then this mixture was centrifuged until two compound was completely separated (1370 x g for 15min). Then supernatant, was collected in the beaker and dried in the oven for overnight. The purified chitosan obtained was weighed and recorded.

2.2.3 Optimization of experimental design

Response surface methodology (RSM) MINITAB software, was applied to determine the optimum conditions for the chitosan extraction process. The experiment was optimized using five levels and five variables as shown in Table 1. The concentrations of HCl (%) and its immersion time (hr), the concentration of NaOH (N), the deacetylation temperature (^{o}C) and deacetylation time (hr) were the five variables that were varied. Table 2 shows the run of experiment as suggested by the RSM of MINITAB software based on Table 1 by using full factorial central composite design (CCD). In the full factorial designs, experiments were carried out at all levels of every variable. This method gives the effects of all the test variables on response along with their interactive effects [7].

The experimental run was randomized in order to minimize the errors or the unexpected variablity of the response that may occur during the experiment [8]. Those five variables were classified as the independent variables or test varaibles with the yield extract or response as the dependent variable (chitosan). The response surface regreassion, R^2 was employed to obtain the second –order polynomial equation.

Table 1: Coded and uncoded factors for the design of experiment.

	Range and levels				
Test variables	**-α(-2)**	**-1**	**0**	**1**	**α(+2)**
(X_1) Concentration of HCl (%)	6	8	10.	12	14
(X_2) Time of demineralization (hr)	6	16.5	27	37.5	48
(X_3) Concentration of NaOH (N)	1.5	2	2.5	3	3.5
(X_4) Temperature of deacetylation (^{Oo}C)	40	50	60	70	80
(X_5) Time of deacetylation (hr)	1	1.5	2	2.5	3

Where: X_1 = concentration HCl (%), X_2 = time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^{O}C), X_5 = acetylation time (hr)

Table 2. Experimental design recommended by MINITAB Software.

Run No.	X_1	X_2	X_3	X_4	X_5
1	8	16.5	2.0	50	2.5
2	12	16.5	2.0	50	1.5
3	8	37.5	2.0	50	1.5
4	12	37.5	2.0	50	2.5
5	8	16.5	3.0	50	1.5
6	12	16.5	3.0	50	2.5
7	8	37.5	3.0	50	2.5
8	12	37.5	3.0	50	1.5
9	8	16.5	2.0	70	1.5
10	12	16.5	2.0	70	2.5
11	8	37.5	2.0	70	2.5
12	12	37.5	2.0	70	1.5
13	8	16.5	3.0	70	2.5
14	12	16.5	3.0	70	1.5
15	8	37.5	3.0	70	1.5
16	12	37.5	3.0	70	2.5
17	8	27.0	2.5	60	2.0
18	12	27.0	2.5	60	2.0
19	10	16.5	2.5	60	2.0
20	10	37.5	2.5	60	2.0
21	10	27.0	2.0	60	2.0
22	10	27.0	3.0	60	2.0
23	10	27.0	2.5	50	2.0
24	10	27.0	2.5	70	2.0
25	10	27.0	2.5	60	1.5
26	10	27.0	2.5	60	2.5
27	10	27.0	2.5	60	2.0
28	10	27.0	2.5	60	2.0
29	10	27.0	2.5	60	2.0
30	10	27.0	2.5	60	2.0
31	10	27.0	2.5	60	2.0
32	10	27.0	2.5	60	2.0

Where: X_1 = concentration HCl (%), X_2 = time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^{0}C), X_5 = deacetylation time (hr).

The actual values (experimental value yield of chitosan) and the corresponding values (predicted value yield of chitosan) of five variables are shown in Table 3. Based to the Table 3, noticed that the highest actual and predicted responses are 5.3337 and 5.4176, respectively with the condition of 8% of the concentration of HCl, 37.5 hours of the dimineralization time, 3.0N of NaOH concentration, 50^{0}C of deacetylation temperature, and 2.5 hours of dacetylation time. The lowest actual and predicted responses were 0.5422 and 0.43535, respectively with the condition of 12% of the concentration of HCl, 16.5 hours of the dimineralization time, 2.0N of NaOH concentration, 50^{0}C of deacetylation temperature, and 1.5 hours of dacetylation time.

The complete design consist of 32 run with six replicated at the axial point. The data were analyzed to fit the following polynomial equation Y (the yield of the chitosan etract).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_1 X_1 + \beta_7 X_2 X_2 + \ldots\ldots\ldots\ldots \beta_{31} X_3 X_5 + \beta 32 X_4 X_5 \quad (1)$$

Where theY is the predicted response which is dependent variable, i.e. yield of the chitosan extracted, β is the values of constant regression coefficient and X_1, X_2, X_3 is the values of independent variables or test variables (Choorit et al., 2008, Naveena et al., 2005). MINITAB software version 15 was used to analysis of regression coefficients and analysis of variances (ANOVA).

3. RESULTS AND DISCUSSION

Table 3. Factors and comparison between actual (Y) and predicted (FITS) responses.

Run No.	Factors					Responses	
	X_1	X_2	X_3	X_4	X_5	Yield (%)	FITS1 (%)
1	8	16.5	2.0	50	2.5	3.7152	3.72345
2	12	16.5	2.0	50	1.5	0.5422	0.43535
3	8	37.5	2.0	50	1.5	1.9087	1.84063
4	12	37.5	2.0	50	2.5	1.3064	1.27001
5	8	16.5	3.0	50	1.5	2.3321	2.34555
6	12	16.5	3.0	50	2.5	1.1921	1.23723
7	8	37.5	3.0	50	2.5	5.3337	5.41761
8	12	37.5	3.0	50	1.5	2.1001	2.06891
9	8	16.5	2.0	70	1.5	2.6980	2.61796
10	12	16.5	2.0	70	2.5	2.6159	2.56754
11	8	37.5	2.0	70	2.5	2.9000	2.89043
12	12	37.5	2.0	70	1.5	2.4060	2.28132
13	8	16.5	3.0	70	2.5	2.3937	2.46564
14	12	16.5	3.0	70	1.5	2.3937	2.35054
15	8	37.5	3.0	70	1.5	2.8790	2.87462
16	12	37.5	3.0	70	2.5	0.7408	0.76810
17	8	27.0	2.5	60	2.0	3.2433	3.22781
18	12	27.0	2.5	60	2.0	1.5100	1.82820
19	10	16.5	2.5	60	2.0	2.6220	2.76163
20	10	37.5	2.5	60	2.0	2.8071	2.97017
21	10	27.0	2.0	60	2.0	0.8117	1.27741
22	10	27.0	3.0	60	2.0	1.6781	1.51510
23	10	27.0	2.5	50	2.0	0.9476	1.03936
24	10	27.0	2.5	70	2.0	0.8881	1.09904
25	10	27.0	2.5	60	1.5	2.1899	2.63483
26	10	27.0	2.5	60	2.5	3.2177	3.07547
27	10	27.0	2.5	60	2.0	2.0123	2.09808
28	10	27.0	2.5	60	2.0	2.3400	2.09808
29	10	27.0	2.5	60	2.0	2.3320	2.09808
30	10	27.0	2.5	60	2.0	2.0088	2.09808
31	10	27.0	2.5	60	2.0	2.7694	2.09808
32	10	27.0	2.5	60	2.0	2.3368	2.09808

Where: X_1 = concentration HCl (%), X_2 = time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^oC), X_5 = deacetylation time (hr), FITS = prediction value

All the coefficient of linear, quadratic and interaction of test variables were calculated for significant with t statistics and P values, also the estimated coefficient of the model are presented in Table 4. The analysis was done by using Fisher's 'F ' test and student 't' test. The significant of the regression coefficient was determined by the student 't' test and the P value are the tools used to check the significant of each interactions among the test variables [9-10]. The smaller the P-value the more significant the correlation with the corresponding coefficient [10-11].

Table 4 shows the estimated regression coefficients equation for the second order polynomial model for the optimization process. Based on the table 4, the significant of regression equation at the 5% level or the fit model of regression equation for the yield of chitosan extraction is derived as shown in equation 2:

$$Y= -50.9886 + 20.1929\ X_3 + 1.4706\ X_4 + 0.0070\ X_2X_2 - 2.8073\ X_3X_3 - 0.0103\ X_4X_4 + 3.0283\ X_5X_5 + 0.0170\ X_1X_4 - 0.3820\ X_1X_5 + 0.0451\ X_2X_3 - 0.0024\ X_2X_4 - 0.0712\ X_3X_4 - 0.0799\ X_4X_5 \quad (2)$$

Where: Y = response or the yield of the chitosan extracted while X_1 =concentration HCl (%), X_2 =time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^{0}C), X_5= acetylation time (h). In order to develop the fitted responsed surface model equations, all the insignificant terms (P>0,005) were eliminated [8].

Table 4. Estimated regression coefficients of second-order polynomial model for cockle shell optimization

	Coef	SE Coef	t	Term
Constant	-50.9886	8.60965	-5.922	0.000
X_1	-2.2504	1.19057	-1.890	0.085
X_2	-0.2500	0.13871	-1.802	0.099
X_3	20.1929	4.76228	4.240	0.001
X_4	1.4706	0.27915	5.268	0.000
X_5	-0.8580	3.96631	-0.216	0.833
X_1*X_1	0.1075	0.05600	1.919	0.081
X_2*X_2	0.0070	0.00203	3.428	0.006
X_3*X_3	-2.8073	0.89601	-3.133	0.010
X_4*X_4	-0.0103	0.00224	-4.593	0.001
X_5*X_5	3.0283	0.89601	3.380	0.006
X_1*X_2	-0.0062	0.00418	-1.475	0.168
X_1*X_3	-0.1350	0.08785	-1.537	0.153
X_1*X_4	0.0170	0.00439	3.867	0.003
X_1*X_5	-0.3820	0.08785	-4.348	0.001
X_2*X_3	0.0451	0.01673	2.698	0.021
X_2*X_4	-0.0024	0.00084	-2.876	0.015
X_2*X_5	-0.0115	0.01673	-0.686	0.507
X_3*X_4	-0.0712	0.01757	-4.054	0.002
X_3*X_5	-0.7568	0.35139	-2.154	0.054
X_4*X_5	-0.0799	0.01757	-4.547	0.001
		R-Sq = 95.4%		R-Sq(adj) 87.0%

Where: X_1 = concentration HCl (%), X_2 = time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^{O}C), X_5= acetylation time (hr), SE = standard error, t = student test, p = probability, R^2 = R –squared, R^2 (adj) = adjusted R – squared.

Based on the results shown in table 4, the extraction of chitosan from cockle shell has significant linear effect (p< 0.05) on X_3 and X_4 ,but X_1 X_2 and X_3 are not significant at 95% confident level. For the quadratic effect, X_2 X_2, X_3 X_3, X_4 X_4 and X_5 X_5, are significant (p<0.05) but X_1 X_1 are not significant (p>0.05) . For interaction, X_1X_4, X_1 X_5, X_2 X_3, X_2 X_4, X_3X_4, and X_4 X_5 are significant (p<0.05) but X_1X_2, X_1 X_3 and X_2X_5 are not significant (p>0.05).

Table 5. ANOVA for the optimization of cockle shell extraction.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	20	28.1209	28.1209	1.40604	11.39	0.000
Linear	5	10.1548	7.2432	1.44864	11.73	0.000
Square	5	6.0924	6.0924	1.21849	9.87	0.001
Interaction	10	11.8736	11.8736	1.18736	9.62	)0.000
Residual Error	11	1.3582	1.3582	0.12347		
Lack-of-Fit	6	0.9663	0.9663	0.16106	2.05	0.223
Pure Error	5	0.3919	0.3919	0.07838		
Total	31	29.4791				

Where: DF = degree of freedom, Seq SS = sequential sum of square, Adj SS = adjusted sum of square, Adj MS = adjusted mean square, F = fischer, P = probability.

The goodness of model can be checked by determination of coefficient R^2 which provides a measure of how much variability in the observed. Besides, R^2 can be used to determine the adequacy of the model [12]. The higher the R^2 the better the model[13]. In this case the value of determination coefficient (R^2 = 95.4%), indicate that only 4.6% of the total

value of variation are not explained by the model due to other factors which are not included in the model [14]. Adjusted R^2 is the corredted value for R^2 after the elimination of the unnecessary model terms [15]. The value of adjusted R^2 = 87.0% was high and closed to R^2=95.7%, means there is not many non significant terms are included in the model and have high correlation between experimental and test variable.

Analysis of variance (ANOVA) was performed to test the significant of regression,linear,square,and interaction of the model. ANOVA is a statistical technique, which subdivides the total variation of a set of data into component parts such as linear,square,and interaction for the purpose of testing a hypothesis on the parameters of a model [13-16].

The large the value of F indicated most of the variation in the test variables can be explained by the regression model equation [9-16]. Based on Table 5,the F values for regression ,linear,square,and interaction were high compared to P value which means the second order polynomial equation (2) is highly significant and adequate to represent the actual relationship between the response and the test variables.

Based on the Table 5, it was found that the P value for the regression, linear, square and interaction are significant ($p<0.05$). In addition, the lack of fits is insignificant at the 5% level which indicated it is a good model and fitted well with the experimental data [13-17-18].

The response optimiser was formed by using MINITAB software in order to determine the exact optimum condition of the independent variable or test variables which leads to response goals. Response optimizer was used as it to identify the combination of the test variable that jointly optimize a variables [12]. The results of the response optimizer at optimum condition for target, maximum and minimum goals are shown in Figures 1, 2 and 3, respectively.

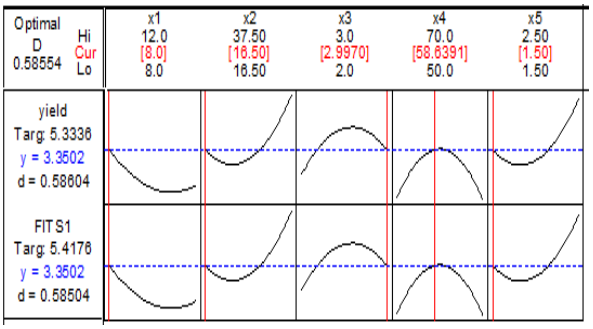

Figure 1. Response optimiser at te optimum condition for the target goal.

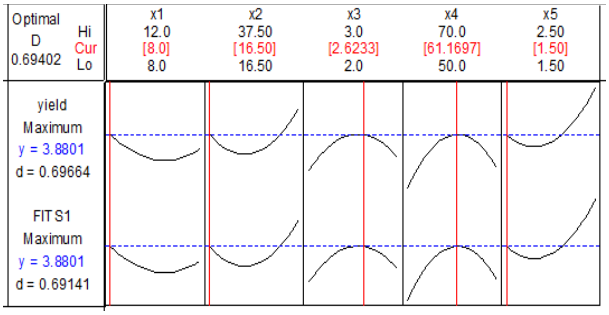

Figure 2. Response optimiser at the optimum condition for the maximum goal

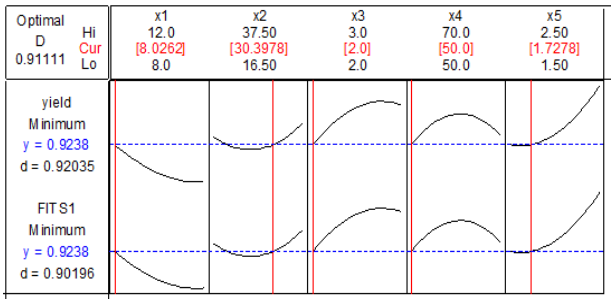

Figure 3. Response optimiser at the optimum condition for the minimum goal

The feasibility of the target, maximum, and minimum goals could be determined from the overlaid contour plot. The results are shown in figures 4, 5 and 6, respectively.

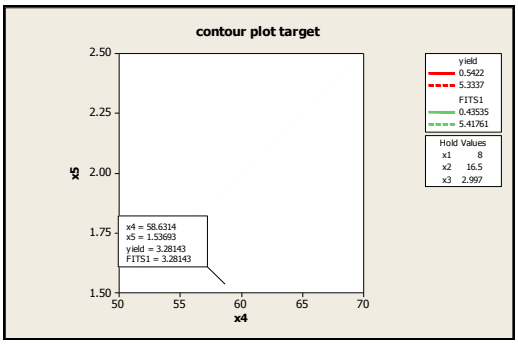

Figure 4. Overlaid contour plot at the optimum condition for target goal: concentration HCl 8%, 16.5hrs time for dimeralization, 3N NaOH concentration, deacetylation temperature 59^{O}C, and 1.5 hrs for deactylation time.

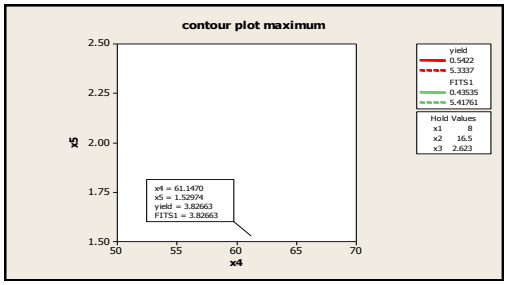

Figure 5. Overlaid contour plot at the optimum condition for max goal: concentration HCl 8%, 16.5hrs time for dimeralization, 2.6N NaOH concentration, deacetylation temperature 61^{O}C, and 1.5hrs for deactylation time.

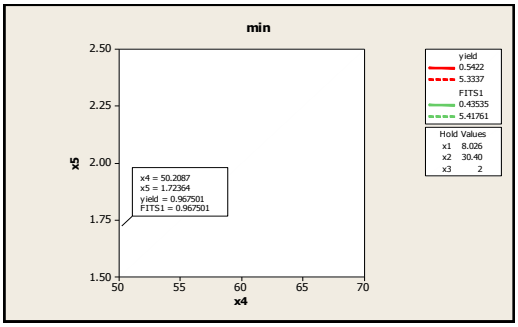

Figure 6. Overlaid contour plot at the optimum condition for min goal: concentration HCl 8%, 30hrs time for

dimeralization, 2N NaOH concentration, deacetylation temperature 50^{O}C, and 1.8 hrs for deactylation time.

The results of the feasibility for three different goals which obtained from the RSM software are shown in table 6.

Table 6. Comparison values of target, maximum, and minimum responses for optimum condition and the feasibility of the experiment.

Goal		lower	target	Upper
Target	Yield	0.5422	5.3336	5.3337
	FITS	0.4353	5.4176	5.4176
Max	Yield	0.5422	5.3337	5.3337
	FITS	0.4353	5.4176	5.4176
Min	Yield	0.5422	0.5422	5.3337
	FITS	0.4353	0.4353	5.4176

Goal	Optimum condition					FITS (%)	F/NF
	X1	X2	X3	X4	X5		
Target	8	16.5	2.997	58.639	1.50	3.350	F
Max	8	16.5	2.623	61.169	1.50	3.880	F
Min	8	30.39	2.0	50.0	1.728	0.924	F

Where: X_1 = concentration HCl (%), X_2 = time of dimineralization (hr), X_3 = concentration NaOH (N), X_4 = deacetylation temperature (^{O}C), X_5= acetylation time (hr), FITS = predicted responses (%), F = feasible, NF = non feasible.

As shown in table 6, the optimum condition for the target goal is the concentration of HCl 8% with 16.5hours for demineralization time, concentration of NaOH 3N, deacetylation temperature 59^{o}C, and 1.5 hours deacetylation time. The optimum condition of maximum goal is the concentration of HCl 8% with 16.5hours for demineralization, concentration of NaOH 2.6N, deacetylation temperature 61^{o}C, and 1.5 hours deacetylation time. The optimum condition of minimum goal is the concentration of HCl 8% with 30hours for demineralization, concentration of NaOH 2N, deacetylation temperature 50^{o}C, and 1.7 hours deacetylation time. All the goals are feasible because they were located at the white region of the overlaid contour plot as shown in figures 4, 5 and 6. However the optimum condition for the maximum goal is chosen because of its target and FITS values was closer to each other compared to target and minimum goals.

Normally, for the graphical optimization procedure, the model of two –dimensional (2D) response contour plot or three-dimensional (3D) response surface plot was of the test variables effect on the chitosan extraction. The 3D plots were drawn by holding three variables constant the centre point and varying the other two test variables within the experimental range in order to show on how each goal response related to two continuous test variables [12].

The contour and surface plots were used to explain the relationship between the response and test variables [19]. The shaped of the contour plot (circular or elliptical) indicate the significant of the test variables interactions. The circular contour plot occur when the corresponding test variables are negligible, and the elliptical countour plot were obtained if the interactions between the corresponding test variables are significant (perfect interaction between test variables and response) [18-20-21].

The contour and surface plots for the cockle shell chitosan at the feasible optimum condition are shown in figures 7 and 8, respectively. Figure 7 is contour plot in elliptical shape whereby, at the lower concentration of the HCl (X_1), the yield was high compared to the high concentration of HCl. As the concentration of HCl was increased the yield of chitosan extract was decreased. The figure 7 is also show that at deacetylation temperature(X_4) of 55ºC to 68ºC the response or chitosan yield of chitosan extract was high which is more than 3.5% could be achieved. The figure 7 illustrates the plot of concentration hydrochloric acid (%)(X_1) and the temperature of the deacetylation (^{o}C) (X_4) while holding another three test variables at fixed point, time of demineralization (X_2), concentration of NaOH (X_3), and the time of deacetylation (X_5) (16.5hours, 2.623N, and 1.5hours respectively).

From the surface plot for maximum goal (figure 8), it was observed that the yield of the chitosan extracted was higher at the lower concentration of the HCl. The yield was decreasing as the concentration of the HCl is increasing. For the temperature of deacetylation it was found that the plot is in quadratic shape, which is when the temperature is increased the yield of chitosan extracted also increased and when the optimum condition of temperature (61^{o}C) is reached the yield was starting to decrease.

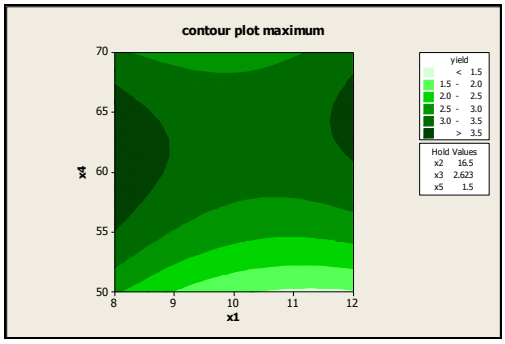

Figure 7.Contour plot of chitosan extract from the cockle shell at feasible optimum condition (goal:maximum): concentration of HCl 8% with 16.5hrs for dimeralization, concentration of NaOH 2.6N, deacetylation temperature 61^{O}C, and 1.5 hrs for deactylation time

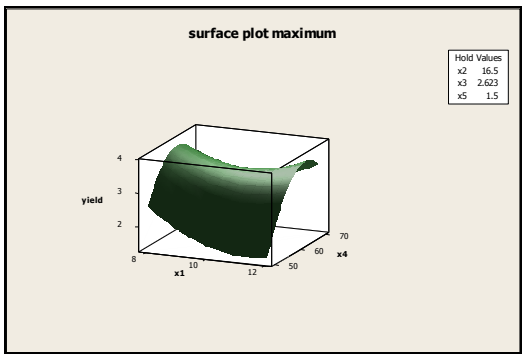

Figure 8. Surface plot of chitosan extract from the cockle shell at feasible optimum condition (goal: maximum): concentration of HCl 8% with 16.5hrs for dimeralization, concentration of NaOH 2.6N, deacetylation temperature 61^{O}C, and 1.5 hrs for deactylation time.

3.1 Verification of predicted values

The verification process was done by running again the experiment using the optimum condition as suggested by the RSM (concentration of HCl at 8% with 16.5hrs time of immersion, concentration of NaOH 2.6N, deacetylation temperature 61^{o}C, and 1.5 hrs for deacetylation time) and then compared this yield of chitoan extract to the predicted values (Table 7). Actual value for the chitosan extract from cockle shell was 3.750 whereas the predicted value was 3.8801. It was found the difference in less than 5% means that the optimum condition for chitoan extract for cockle shell as suggested by MINITAB software version 15 could be accepted.

Table 7. Experimental and predicted results of verification under optimized conditions.

Dependent variable	Predicted value	Experimental value
Yield chitosan	3.880	3.750

Optimized conditons: Concentration of HCl at 8% with 16.5hrs time of immersion, concentration of NaOH 2.6N, deacetylation temperature 61^{O}C, and 1.5 hrs for deactylation time

4. CONCLUSION

The optimum condition for the extration of chitosan from cokle shell by using MINITAB software version 15, was determined. It can concluded that the optimum yield of chitosan (3.880) could be obtained by using the concentration of HCl at 8% with 16.5hours time of immersion, concentration of NaOH 2.6N, deacetylation temperature 61^{o}C, and 1.5 hours for deactylation time. The determination of coefficient R^2= 95.4 also was high, which means the experimental data were acceptable.

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