OPTIMIZING DEPROTEINIZATION OF CHITIN USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT
The physicochemical parameter which influences the application of chitin in various fields is strongly linked to the source and the conditions of the chitin production. The condition of chitin can be controlled by manipulating solution conditions (temperature, concentration, time) and optimization of deproteinization processes. The *Callinectes pallidus* waste was pre-treated, bleached, decolourized and demineralised which significantly affect chitin quality and yield. The design of experiments (DOE) via central composite design (CCD) of response surface methodology (RSM) was used to optimize the process parameters of deproteinization. The experimental results indicates that the CSW/chitin had 5.24/4 % moisture, 59.31/0.7 % ash, 6.75/0.95 % lipid, 15.75/2.05 % crude protein, 44.17/8.98 g/Kg mineral, 374 m^2/g specific surface area and 0.25 cm^3/g pecific pore volume respectively. The predicted values of the response was further validated and suggested the optimal conditions for deproteinization of chitin were 70 ºC, 4 h, and 4.47 % of NaOH with percentage deproteinization of 92.97 % and 91.08 % for actual and predicted value respectively.

Keywords: chitin synthesis, deproteinization, crab shell waste, response surface methodology.

1 INTRODUCTION

Wastes generated from the production and processing of aquatic resources such as fish, lobsters, crabs and shrimps are enormous which constituting source of land and water pollutions since most of them are littered on river banks thereby restricting tourist activities (Jang *et al.,* 2004; Mejia-Saules *et al.,* 2006; Adebayo-Tayo *et al.*, 2012). The conversion of these wastes into useful chemical materials such as chitin for industrial application is one of the major objectives of this research (Jang *et al.,* 2004; Gerente *et al.*, 2007: Sivakami *et al.,* 2013; Maram *et al.*, 2013; Mohammed *et al.,* 2014).

Chitin is a unique functional material for versatile applications with high commercial interest due to its percentage nitrogen contents compared to artificially replaced cellulose (Kumar, 2000; Yadav *et al.,* 2004; Zaku *et al.,* 2011). It can be found in an array of species in both the animal and plant kingdoms. The tradition source of chitin is the seafood waste, mostly the fish scale, crabs, prawn, lobster and shrimps shells. Chitin is the second most abundant biopolymer in nature after cellulose. It is estimated that its annual synthesis reaches 100 billion tons (Rinaudo, 2006).

Many techniques have been planned and used over the years to achieve pure chitin; nevertheless, no standard technique has been adopted. The usual processes of chitin synthesis consist of the use of strong acids and bases under high temperatures, may cause pollution and significantly lower quality and yield of chitin (No *et al.,* 2002; Rødde *et al.,* 2007). An alternative way to solve these problems is to use organic acid during demineralization. Furthermore, the efficiency can be achieved via optimization of deproteinization treatment (Shirai *et al.,* 2001; Cira *et al.,* 2002). However, Response surface methodology (RSM) is useful software that can reduce the number of experimental trials, determine the significant reaction factors, and can be employed to optimize the treatment conditions (Younes *et al.,* 2012). Hence, the aim of this work was to search for modified ways of getting high quality chitin from the crab shell (*Callinectes pallidus*) for industrial used.

2.0 Meterial and Methods

2.1 Material and Chemical/Reagents

The fresh samples of Gladiator Swim crab shells, *Callinectes pallidus* were obtained from Oyingbo, Lagos, Nigeria. For all experiments, reactions were carried out in Erlenmeyer flask (250 ml capacity). The major chemical/reagents used are include lactic acid ($CH_3CH(OH)CO_2H$), sodium hydroxide (NaOH), Sodium hypochloride (NaOCl) and Acetone($(CH_3)_2CO$ all these chemical and reagents are analytically graded and were obtained from Sigma Aldrich and Analar BDH.

2.2 Methods

The wet crab shell waste (CSW) was thoroughly washed and oven dried at 60ºC for 10 h, then milled and sieved through 300 µm BS sieve. 10 g of the prepared CSW were decolorized with acetone in the ratio 1:1 weight of solid to solvent (W/V) for 10 min and dried for 1 hr at ambient

temperature. The bleached with 0.35 % NaOCl solution for 10 min at ambient temperature. Samples were washed with distilled water and oven dried for about 4 h at 60 ºC, and milled (Nessa *et al.,* 2010; Abdulwadud *et al.,* 2013). The surface area, proximate and thermal gravimetric analyses of CSW and chitin were carried out in other to determine its quality and yield.

2.3 Demineralization of crab shell waste (CSW)

Demineralization was carried out using modified procedures reported by Hossain and Iqbal (2014) and Divya *et al.* (2014). The CSW samples mixed with 10 % concentration of lactic acid (v/v), 2 h reaction time and treatment temperature of 45 ºC. The demineralized CSW were washed to neutrality with deionised water, oven dried at 60 ºC for 4 h, milled and labelled for further analysis (Jimoh*et al.,* 2013). The percentage demineralization was evaluated by Equation 1.0 (Ghorbel-Bellaaj *et al.,* 2011; Khorrami*et al.,* 2012).

$$D_m = \frac{(A_0\mathrm{O} - A_r\mathrm{R}) * 100}{A_0} \qquad (1)$$

Where:

D_m =Percentage demineralization (%); A_0 = Ash content before demineralization (%)

A_r = Ash content after demineralization (%); O= Weight of Sample before demineralization (g); R= Weight of Sample after demineralization (g)

2.4 Deproteinization of demineralized crab shell waste (CSW)

Three parameters which were expected to have an effect on deproteinization of demineralized FSW were identified; concentration of NaOH solution (A, %, w/v), reaction time (B, h) and treatment temperature (C, °C). The treatment were carried out with aid of central composite design (CCD) of RSM. The CCD matrix consisting 20 runs with three input variables were considered at low (-1), medium (0) and high (+1) level with axial point (α) added at a distance of 1.68 (Myers *et al.,* 2009; Melvin *et al.,* 2011). The percentage deproteinization was evaluated by Equation 2.0 (Ghorbel-Bellaaj *et al.,* 2011; Khorrami *et al.,* 2012).

$$D_P = \frac{(P_0\mathrm{O} - P_r\mathrm{R}) * 100}{P_0} \qquad 2.0$$

Where

D_P = Percentage deproteination (%);P_0 = Protein content before deproteination (%)

P_r = Protein content after deproteination (%); O= Weight of Sample before deproteination (g)

R = Weight of Sample after deproteination (g)

The data obtained were subjected to the Analysis of Variance (ANOVA) and optimization. The results of CCD were used to derive second order polynomial model (Equation 3).

$$\mathrm{Y} = \beta\mathrm{o} + \beta_a\mathrm{A} + \beta_b\mathrm{B} + \beta_c\mathrm{C} + \beta_{ab}\mathrm{AB} + \beta_{ac}\mathrm{AC} + \beta_{bc}\mathrm{BC} + \beta_{aa}\mathrm{A}^2 + \beta_{bb}\mathrm{B}^2 + \beta_{cc}\mathrm{C}^2 + \ldots \quad (3)$$

Where Y is the predicted response (% deproteinization); β_o is the intercept (regression coefficient); β_a, $\beta_b$and β_c are the linear coefficient; β_{aa},$\beta_{bb}$and β_{cc} are the quadratic coefficient; β_{ab},β_{ac} and β_{bc} are the interaction coefficient. A, B and C are the independent variables (Manase *et al.,* 2012; Richa *et al.,* 2014)

3 RESULTS AND DISCUSSION

3.1 Characterization CSW and chitin

The result of the surface chemistry, proximate and mineral composition of Crab shell waste (CSW) and chitin were presented in Table 1. The Analysis showed that the CSW had 6.95 % moisture, 59.31 % ash, 6.75 % lipid, 15.75 % crude protein and mineral 44.17 g/Kg. While chitin proximate and mineral composition were 4 %, 0.7 %, 0.95 %, 2,05 %, 8.98 g/Kg, 374 m^2/g and 0.25 cm^3/g for moisture, ash, lipid, crude protein, mineral, specific surface area and specific pore volume respectively. This is in agreement with the findings reported by Fawole, *et al.,* 2007 and Adejonwo, 2010.

Table 1: Result of proximate, mineral and surface chemistry analysis of CSW and Chitin

Sample	Moisture (%)	Ash (%)	Lipid (%)	Crude Protein (%)	$CaCO_3$ (g/Kg)	Surface Area (m^2/g)	Pore Volume (cm^3/g)
CSW	5.24	59.31	6.75	15.75	44.17	229.60	0.15
Chitin	4.00	0.70	0.95	2.05	8.98	374.00	0.25

From the results given in Table 1, it can be deduce that the surface area analysis, proximate and mineral composition of crab shells are essential in estimating the quality of the raw material utilization for different technological processes (Abdul and Sarojanlini, 2012).

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal analysis in which changes in physical and chemical properties of materials were measured as a function of increasing temperature as in Figure 1.

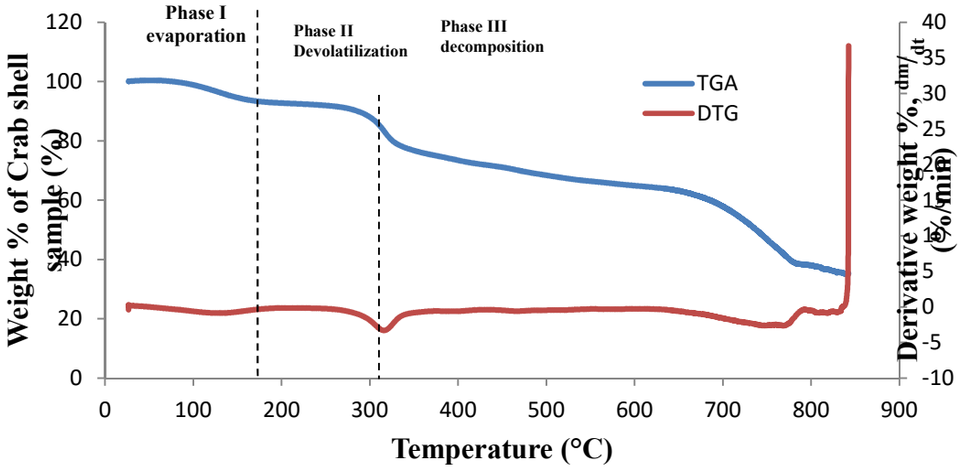

Figure 1: TGA and DTG of Crab shell

The TGA details of crab shell in Figure 1 shows mainly two major weight losses of 190 °C and the second before 310 °C respectively. The first one is due to the elimination of water molecules with respective moisture content of 5.24 % and the second one is due to the depolymerisation and decomposition of volatile products (Vijayalakshmi *et al.,* 2016)

3.2 SYNTHESIS OF CHITIN FROM CRAB SHELL WASTE (CSW)

The percentage deminerization of CSW was 96.41 % obtained from 10 % concentration of lactic acid (v/v), 2 h reaction time and treatment temperature of 45 ºC. The value of percentage deminerization reported in this work is higher than value reported by Neith *et al.,* 2011, which may be due to differences in the approach used. In Table 2 the deminerazed CSW was followed by optimization of deproteinization parameters (Concentration of NaOH, reaction time and treatment temperature). From the response surface methodology showed that the optimum conditions of deproteinization was 92.97 % at treatment temperature 70 ºC, concentration of NaOH 4.47 % (v/v) and reaction time 4 h. The high values of percentage deproteinization may be due to interaction of the process parameters (temperature, time and concentration of base) lead to high quality of chitin yield (Nidheesh *et al.,* 2014).

Table 2: Actual-Predicted Values and Residual for Y (Chitin)

	Factors			Responses		
Run	**A-Conc. of NaOH (%)**	**B-Time (h)**	**C-Temp. (ºC)**	**Actual Value**	**Predicted Value**	**Residual**
1	0	0	0	68.03	61.56	6.47
2	0	-1.68	0	71.11	70.81	0.30
3	1	1	1	91.40	91.86	-0.46
4	0	0	1.68	90.67	96.64	-5.97
5	1	-1	1	78.83	71.26	7.57
6	0	0	0	72.43	70.37	2.05
7	0	0	0	75.50	74.20	1.30
8	1	-1	-1	87.97	88.84	-0.87
9	0	0	0	75.50	74.20	1.30
10	0	0	0	75.50	74.20	1.30
11	1	1	-1	62.94	61.92	1.02
12	0	0	0	87.83	86.11	1.72
13	1.68	0	0	79.53	78.96	0.56
14	1	1	1	76.16	78.98	-2.82
15	1	-1	-1	73.82	70.60	3.22
16	0	1.68	0	80.56	80.60	-0.05
17	0	0	-1.68	79.31	80.60	-1.30
18	1	-1	1	74.53	73.60	0.93
19	1	1	-1	76.31	80.60	-4.30
20	-1.68	0	0	47.60	55.68	-8.08

Central composite design (CCD) of RSM was applied for deproteinization parameters toward achieving quality chitin. From the results given in Table 2, the predicted values of the responses

for % deproteinization (Y) were calculated using Equation 3 based on the respective coefficients provided in Equation 4–5. The predicted values of Y were further validated and results suggested that the optimal condition for chitin synthesis in deproteinization; treatment temperature 70 ºC, reaction time of 4 h, and concentration of NaOH 4.47 % with percentage deproteinization of 92.97 % and 91.08 % for actual and predicted respectivel (Richa *et al.,* 2014)

3.3 REGRESSION MODEL

The percentage deprotienation of chitin synthesis were Model by design of experiment software(7.0). The quadratic regression models (Equations 4 – 5) were obtained for predicting the optimal points of chitin synthesis. The model shows that the input variables have significant effect on the quality and yield of chitin during deproteinization treatments.

Equation in Terms of Coded Factors:

$$Y = +80.60 +8.47*A +7.19*B +2.98*C +6.38*A*B -2.54*A*C +0.66*B*C -3.77*A^2 -2.33*B^2 +1.19*C^2 \quad (4)$$

Equation in Terms of Actual Factors:

$$Y= +50.84402 +9.81415*A +4.42602*B -0.82343*C +3.19132*AB -0.12677*A*C +0.066042*B*C -0.94317*A^2 -2.32706*B^2 +0.011918*C^2 \quad (5)$$

4 CONCLUSION

This research work successfully synthesized high quality chitin from the abundant crab shell wastes by organic acid demineralization and optimization of deproteinization process parameters. The effect of process variable on the chitin preparation and optimizing deproteinization parameters via Central composite design (CCD) of response surface methodology (RSM) maximize the quality and yield of chitin. These findings suggest that the predicted values of the response was further validated and results suggested that the optimal conditions for deproteinization of chitin were 70 ºC, 4 hr, and 4.47 % of NaOH with percentage deproteinization of 92.97 % and 91.08 % for actual and predicted value respectively. This technique makes it attractive for the high quality chitin and avoiding the toxicity of chemical during treatment.

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