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Optimization and Modeling of Chitin Synthesis from Fish Scale using Response Surface Methodology (RSM)

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ABSTRACT

This paper investigates the effect of process parameters (reaction time, temperature and concentration of acid/base) in the optimization of demineralization and deproteinization processes for chitin synthesis from Oreochromis niloticus waste scale. The processes conditions, the source and pretreatment of raw material significantly affect chitin quality and yield. The effect of these parameters on the quality and yield of the chitin was carried out using statistical design of experiments via central composite design (CCD) of response surface methodology (RSM). The experimental results indicated that the predicted models results could adequately describe the quality and yield of chitin. The results suggested that the optimal condition for chitin synthesis in demineralization; treatment temperature 50 ^{o}C, reaction time of 3 h, and concentration of lactic acid 9.76 % with percentage demineralization of 96.91 % and 96.89 % for actual and predicted respectively with yield of 90.3 %. Similarly, deproteinization; reaction time of 3.34 h, concentration of NaOH 6 % and treatment temperature of 70 ^{o}C with percentage deproteinization of 95.01 % and 94.21 % for actual and predicted respectively with yield of 92.2 %. The quality of the synthesized chitin have the values of 4.2 %, 0.35 %, 0.96 % and 6.25 g/Kg for Moisture content, Ash content, Crude Protein and Mineral content (g/Kg) respectively, which were within the standard value. These analyses revealed that the quality and yield of the chitin obtained from the Oreochromis niloticus waste scale were due to the effective interaction between input parameters and the responses.

Keywords: chitin, chitin synthesis, fish scale waste, response surface methodology.

1 INTRODUCTION

Effective utilization of waste materials obtained from aquatic resources is vital to environmental waste management (Adebayo-Tayo *et al.*, 2012). The conversion of these wastes into valuable chemical materials such as chitin for various applications is one of the foremost goals of this research (Maram *et al.*, 2013; Mohammed *et al.,* 2014).

Chitin is a linear polymer consisting mainly of β-(1→4) - linked 2-acetamido-2-deoxy-β-D-glucopyranose units with two major processes of synthesis (demineralization and deproteinization). It is insoluble in water and common organic solvents (Kurita, 2001; Rinaudo, 2006; Isa *et al.*, 2014; Islem and Marguerite 2015). Chitin the second most abundant polysaccharide is synthesized by a huge amount of living organisms including aquatic animals, fungi and insects (Islem & Marguerite, 2015). Mehdi *et al* (2014) reported that these biopolymers have found numerous applications in different areas such as: wastewater engineering for removal of organic, inorganic metal ion and pigments; food industry in preservative, anti-cholesterol and food additive; agriculture in seed and fertilizer coating; cosmetics and toiletries in body creams, moisturizer and lotions; biomedical in tissue engineering, drug delivery and wound dressing. The majority of these resourceful applications are due to its non-toxicity, biocompatibility and biodegradability (Divya *et al.*, 2014; Hossain and Iqbal, 2014).

Chitin can be synthesized through different approaches but the most common ones are chemically, enzymatic, microbial fermentation, microwave and ultrasonic process, using raw materials from aquatic waste such as fish scale, shrimp and crab shell, through demineralization and deproteinization as part of processing model (Bhaskar *et al.,* 2007; Ghorbel-Bellaaj *et al.,* 2010; Kamboj *et al.,* 2015).

This technique involves an approach where one processing parameter (factor) at a time was considered during the experimental procedure. The approach has been considered to be time consuming, thus making it very expensive (Nessa *et al.,* 2010; Badwaik *et al.,* 2012). However, the need for better options such as statistical design of experiments where combined effect of factors such as reaction time, temperature and concentration of acid for demineralization and base for deproteinization can be easy studied and analyzed (Anayet *et al.,* 2011; Yi *et al.,* 2011; Jimoh *et al.,* 2013).

Several researchers have worked on chitin production using some of these methods but much has not been reported as regards study on the statistical design of experiments via central composite design (CCD) of response surface methodology (RSM) and effect of the process parameters (temperature, time, and concentration of organic acid/base) on the quality and yield of the chitin final product (Mehdi *et al.,* 2014; Faria *et al.,* 2015). The

major advantages of RSM over others approaches may include: low cost of materials, high effectiveness, serve energy and time (Bhaskar *et al.,* 2007; Gerente *et al.*, 2007; Nidheesh *et al.,* 2014).

The application of RSM in the synthesis of chitin may mark a major progress toward it quality and yield (Faria *et al.,* 2015). RSM is a very important tool for design of experiment (DOE), wherein the relationship between response(s) of a process with its primary (input) decision variables is mapped to achieve the objective of maximization or minimization of response properties (Peričin *et al.,* 2008; Melvin *et al.,* 2011; Badwaik *et al.,* 2012). This paper therefore will focus more on the use of RSM to predict the optimal condition for demineralization and deproteinization with more emphasis on the combined effect of operating parameters on the quality and yield of synthesis chitin.

2 METHODOLOGY

2.1 MATERIAL

The fresh samples of Africa Arowana, *Oreochromis niloticus* waste scale were obtained from Fish market in Mobil area of Minna, Niger State, Nigeria.

2.2 CHEMICAL AND REAGENTS

The major chemical and reagents used for the synthesis of chitin 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.

2.3 METHODS

The wet fish scale waste (FSW) was thoroughly washed and oven dried at 60 ^oC for 12 h, then milled and sieved through 300 µm BS sieve. The clean polyethylene bags was used to store the sample at ambient temperature of about 30 ^oC and transferred in to a desiccator to avoid moisture intake. The prepared sample was further used in demineralization, deproteinization and optimization of chitin with aid of design of experiments via central composite design (CCD) of RSM.

2.4 Thermogravimetric Analysis (TGA)

Thermal analyzer (TGA 4000 Perkin-Elmer) was employed to automatically measure the weight loss of the sample as a function of temperature and time.

2.5 DEMINERALIZATION OF FISH SCALE WASTE (FSW)

Demineralization was carried out with aid of design experiments using Erlenmeyer flask (250 ml capacity) each having 10 g of samples. Three factors [concentration of lactic acid (A, %, v/v), reaction time (B, h) and treatment temperature (C, ^oC)], which were expected to have an effect on demineralization of FSW on chitin synthesis using a 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. The experimental plans with respect to their values in actual form are presented in Table 1. After treatment, the demineralized FSW were washed to neutrality with deionised water, centrifuged and filtered. The residues were oven dried at 60 ^oC for 6 h, milled and labelled for further analysis. The percentage demineralization was evaluated by "(1)". And in order to appraise the degree of demineralization (%, w/w), ash content in the dried materials was carried out.

TABLE 1: DESIGN MATRIX OF DEMINERALIZATION PROCESS

Process Variable	-α	Low(-1)	Centre Point(0)	High(+1)	+ α
A: Conc. of lactic acid (%)	4.95	7	10	13	15.05
B: Time (h)	0.32	1	2	3	3.68
C: Temperature (^oC)	36.59	40	45	50	53.41

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

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)

2.6 DEPROTEINIZATION OF DEMINERALIZED FISH SCALE WASTE (FSW)

NaOH solution was used to remove protein from demineralized FSW. For all design experiments, reactions were carried out in Erlenmeyer flask (250 ml capacity), each having 10 g of demineralized FSW. Three factors [concentration of NaOH solution (A, %, w/v), reaction time (B, h) and treatment temperature (C, °C)], which were expected to have an effect on deproteinization of demineralized FSW and identified the most significant variables on chitin synthesis using a 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 (Melvin *et al.,* 2011). The experimental plans with respect to their values in actual form are presented in Table 2. After treatment, deproteinized FSW were washed to neutrality with deionised water, centrifuged and filtered. The residues were oven dried at 60 ^oC for 6 h, milled and labelled for further analysis. The percentage deproteinization was evaluated by "(2)". And in order to evaluate the extent of deproteinization (%, w/w), the protein content of the dried residues was determined.

TABLE 2: DESIGN MATRIX OF PROTEINATION PROCESS

Process Variable	-α	Low(-1)	Centre Point(0)	High(+1)	+ α
A: Conc. of NaOH (%)	0.64	2	4	6	7.36
B: Time (h)	1.32	2	3	4	4.68
C: Temperature (°C)	43.18	50	60	70	76.82

$$D_P = \frac{(P_0 O - P_r R) * 100}{P_0 O} \quad (2)$$

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)

2.7 ANALYSIS OF RSM DESIGN AND MODEL DEVELOPMENT

In this study, important process operating parameters (i.e. reaction time, process temperature and concentration of acid/base) that is expecting to have impact on chitin synthesis through demineralization and deproteination (Hossain and Iqbal 2014; Divya*et al.,* 2014) were examined using RSM. To avoid unnecessary repetition of experiments, central composite design (CCD) of RSM was applied to produce 20 runs of experimental conditions for the three operating parameters on chitin synthesis.

Based on the identification of variables (A, B, and C), the CCD was developed to determine the mutual interactions among the identified variables and their corresponding optimum levels. The analyses were all conducted with aid of CCD matrix and the results of RSM experiments were analyzed using the design expert (software 7.0). The data obtained were subjected to the Analysis of Variance (ANOVA) and optimization. The responses obtained in CCD were subjected to non-linear regression analysis for obtaining empirical models that relate the responses to the independent factors (Nouri & Khodaiyan, 2014). The results of CCD were used to derive second order polynomial model "(3)".

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

Where Y is the predicted response (% demineralization or % deproteinization); β_o is the intercept (regression coefficient); β_a, β_b and β_c are the linear coefficient; β_{aa}, β_{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 and Sanjoy, 2014)

3 RESULTS AND DISCUSSION

3.1 SYNTHESIS OF CHITIN FROM FISH SCALE WASTE (FSW)

To study the combined effect of these variables, experiments were performed using different combinations as shown in Table 3 and 4. Which summarize the experimental design along with the experimental and predicted responses from each individual experiment, with aid of multiple regression analysis on the experimental data,

The chitin from FSW was deminerazed followed by deproteinization, In this study, from the response surface methodology showed that the optimum conditions of demineralization was 96.91 % at treatment temperature 50 °C, concentration of lactic acid solution 9.7 % (v/v) and reaction time 3 h. Similarly, the optimum conditions of deproteinization was 95.01 % at treatment temperature 70 °C, concentration of NaOH solution 6 % (w/v), reaction time 3.34 h. The high values of percentage demineralization and deproteinization may be due to interaction of the process parameters (temperature, time and concentration of acid/base) lead to high quality of chitin yield (Nidheesh *et al.,* 2014).

TABLE 3: FACTORS AND RESPONSE FOR DEMINERALIZATION

	Factors			Responses		
Run	A-Conc. of lactic acid (%)	B-Time (h)	C-Temp. (°C)	Actual Value	Predicted Value	Residual
1	13(1)	1(-1)	40(-1)	79.39	74.61	4.78
2	10(0)	3.68(1.68)	45(0)	93.34	92.79	0.55
3	10(0)	0.32(-1.68)	45(0)	84.33	83.83	0.49
4	13(1)	3(1)	40(-1)	99.39	99.26	0.14
5	4.95(-1.68)	2(0)	45(0)	80.08	78.39	1.68
6	7(-1)	1(-1)	50(1)	98.79	97.47	1.33
7	10(0)	2(0)	53.41(1.68)	87.02	85.75	1.27
8	13(1)	3(1)	50(1)	99.10	102.07	-2.96
9	10(0)	2(0)	45(0)	94.87	98.89	-4.02
10	10(0)	2(0)	45(0)	99.34	97.90	1.44
11	15.05(1.68)	2(0)	45(0)	81.09	85.17	-4.08
12	10(0)	2(0)	36.59(-1.68)	98.31	96.80	1.51
13	10(0)	2(0)	45(0)	88.76	91.43	-2.67

14	10(0)	2(0)	45(0)	97.07	96.98	0.09
15	7(-1)	1(-1)	40(-1)	93.83	93.76	0.07
16	10(0)	2(0)	45(0)	93.62	93.76	-0.14
17	7(-1)	3(1)	40(-1)	94.33	93.76	0.57
18	13(1)	1(-1)	50(1)	93.83	93.76	0.07
19	10(0)	2(0)	45(0)	93.13	93.76	-0.63
20	7(-1)	3(1)	50(1)	94.25	93.76	0.49

TABLE 4: FACTORS AND RESPONSE FOR DEPROTEINIZATION

	Factors			Responses		
Run	**A-Conc. of NaOH (%)**	**B-Time (h)**	**C-Temp. (^{o}C)**	**Actual Value**	**Predicted Value**	**Residual**
1	4(0)	3(0)	60(0)	67.16	60.69	6.47
2	4(0)	1.32(-1.68)	60(0)	78.53	76.43	2.10
3	6(1)	4(1)	70(1)	67.67	66.90	0.77
4	4(0)	3(0)	76.82(1.68)	91.24	96.35	-5.10
5	6(1)	2(-1)	70(1)	78.11	70.07	8.04
6	4(0)	3(0)	60(0)	79.94	77.78	2.16
7	4(0)	3(0)	60(0)	77.97	77.13	0.83
8	6(1)	2(-1)	50(-1)	94.03	98.55	-4.52
9	4(0)	3(0)	60(0)	98.46	96.62	1.16
10	4(0)	3(0)	60(0)	91.88	87.86	4.02
11	2(-1)	4(1)	50(-1)	55.98	65.72	-9.74
12	4(0)	3(0)	60(0)	94.00	88.40	5.60
13	7.36(1.68)	3(0)	60(0)	79.92	81.02	-1.10
14	2(-1)	4(1)	70(1)	87.71	90.75	-3.04
15	2(-1)	2(-1)	50(-1)	87.63	85.14	2.49
16	4(0)	4.68(1.68)	60(0)	82.61	85.14	-2.53
17	4(0)	3(0)	43.18(-1.68)	83.11	85.14	-2.03
18	2(-1)	2(-1)	70(1)	87.99	85.14	2.85
19	6(1)	4(1)	50(-1)	87.36	85.14	2.22
20	0.64(-1.68)	3(0)	60(0)	82.84	85.14	-2.30

In the present study, central composite design of RSM was applied in order to show an optimum combination of parameters for chitin synthesis. The results indicated that the chitin synthesis from 10 g of dried FSW at different conditions in the range of 7 – 13 % lactic acid, 2 – 6 sodium hydroxide, 1 – 4 h and 40 – 70 ^{o}C (Table 1 and 2). The statistical analysis carried out to determine the significant variables of chitin synthesis and it interaction between input and the response. These values showed a good agreement between the actual and predicted values (Table 3 – 4).

In demineralization treatment shown in Table 3, the response was significantly affected by the concentration of lactic acid (13 %), time (3 h) and temperature of 40^{o}C increases percentage demineralization to 99.39 %, means that the factors play role in the removal of mineral from FSW. Same scenario in deproteinization treatment shown in Table 4, the response was significantly affected by the concentration of sodium hydroxide, time and temperature of 50 ^{o}C which increase percentage deproteination to 94.03 %. The same mold was observed for the prolonged reaction time of 2 h, with concentration of 6 % it means that the factors contribute in the protein removal (Hossain and Iqbal 2014).

From the results given in Table 4 and 5, the predicted values of the responses for % demineralization (Y_{dm}) and % deproteinization (Y) were calculated using "(3)", based on the respective coefficients provided in "(4–7)". The predicted values at each experimental runs were close to experimental values which aid in obtaining optimal conditions of the treatments. The predicted values of Y_{dm} and Y were further validated and results suggested that the optimal condition for chitin synthesis in demineralization; treatment temperature 50 ^{o}C, reaction time of 3 h, and concentration of lactic acid 9.76 % with percentage demineralization of 96.91 % and 96.89 % for actual and predicted respectively. Similarly, deproteinization; reaction time of 3.34 h, concentration of NaOH 6 % and treatment temperature of 70 ^{o}C with percentage deproteinization 95.01 % and 94.21 % for actual and predicted respectively (Richa *et al.,* 2014). The percentage yield of demineralization and deproteinization are 90.3 % and 92.2 % respectively.

3.2 REGRESSION MODEL FOR DEMINERAZATIONAND DEPROTEINATION PROCESSES

The coefficients of the quadratic polynomial regression model were applied to fit of the responses. The percentage deminrazation and deprotienation of chitin synthesis were Model by design of experiment software(7.0). The quadratic regression models "(4 – 7)", were obtained for predicting the optimal points of chitin synthesis. The models show that the input variables have significant effect on the quality and yield of chitin during demineralization and deproteinization treatments.

Equation in Terms of Coded Factors:

$$Y_{dm} = +93.76 + 8.62A + 3.46B + 1.65C - 0.69AB + 0.22AC - 0.47BC - 3.67A^2 - 0.98B^2 + 0.16C^2 \quad (4)$$

Equation in Terms of Actual Factors:

$$Y_{dm} = +5.04852 + 10.80809A + 13.88312B - 0.19838C - 0.22945AB + 0.014888AC - 0.093487BC - 0.40722A^2 - 0.98111B^2 + 6.29207e^{-003}C^2 \quad (5)$$

Equation in Terms of Coded Factors:

$$Y = +85.14 + 9.29A + 6.74B + 2.89C + 3.43AB - 2.01AC + 0.21BC - 4.56A^2 - 2.85B^2 + 0.27C^2 \quad (6)$$

Equation in Terms of Actual Factors:

$$Y = -5.10376 + 14.64870A + 15.73034B + 0.30867C + 1.71360AB - 0.10044AC + 0.021392BC - 1.13986A^2 - 2.85409B^2 + 2.65300e^{-003}C^2 \quad (7)$$

The detail of the experimental outcome in Table 5 and 6 shows that the analysis of the variance (ANOVA) for the variables indicated that the quadratic model derived from CCD could adequately be used to describe the factors for demineralization and deproteinization of FSW.

Table 5: ANOVA FOR PERCENTAGE DEMINERALIZATION

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	1426.013	9	158.4459	18.92193	< 0.0001
A-Conc of Acid	1015.698	1	1015.698	121.2968	< 0.0001
B-Time	163.2334	1	163.2334	19.49366	0.0013
C-Temperature	37.13692	1	37.13692	4.434966	0.0615
AB	3.79067	1	3.79067	0.45269	0.5163
AC	0.398953	1	0.398953	0.047644	0.8316
BC	1.747956	1	1.747956	0.208744	0.6575
A^2	193.5771	1	193.5771	23.11737	0.0007
B^2	13.8721	1	13.8721	1.656634	0.2271
C^2	0.356591	1	0.356591	0.042585	0.8406
Residual	83.73664	10	8.373664	-	-

From the result given in Table 5, it can be seen that the F-value of 18.92 implies the model is significant. The model terms are significant base on p-value of 0.0001. In this case A, B, A^2 are significant model terms. And it can be deduce that the models were valid due to Standard Deviattion of 2.89, Root Squared 0.9445 which is 94%, Mean 90.69, Adjusted R-Squared 0.8946, Correlation of Variance 3.19 % and Adeq Precision 16.215, "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable and ratio of 16.215 indicates an adequate signal. This model can be used to navigate the design space.

Table 6: ANOVA FOR PERCENTAGE DEPROTEINIZATION

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	2436.573	9	270.7303	6.605219	0.0034
A-Conc of Acid	1178.12	1	1178.12	28.74351	0.0003
B-Time	621.0859	1	621.0859	15.15312	0.0030
C-Temperature	114.4033	1	114.4033	2.791186	0.1257
AB	93.96566	1	93.96566	2.292554	0.1609
AC	32.285	1	32.285	0.787682	0.3956
BC	0.366109	1	0.366109	0.008932	0.9266
A^2	299.5888	1	299.5888	7.309302	0.0222
B^2	117.3921	1	117.3921	2.864107	0.1214
C^2	1.014329	1	1.014329	0.024747	0.8781
Residual	409.8733	10	40.98733	-	-

From the result given in Table 6, it can be seen that the F-value of 6.61 implies the model is significant. The model terms are significant base on p-value of 0.0034. In this case A, B, A^2 are significant model terms. And it can be deduce that the models were valid due to Standard Deviattion of 6.40, Root Squared 0.8560 which is 86%, Mean 80.25, Adjusted R-Squared 0.7264, Correlation of Variance 7.98 % , Predictected R-Squared -0.0181 and Adeq Precision 9.261. A ratio greater than 4 is desirable and ratio of 9.261 indicates an adequate signal. This model can be used to pilot the design space.

3.3 MODEL VALIDATION FOR DEMINERAZATION AND DEPROTEINATION PROCESSES

In model validation an opportunity exists to carry out additional experiments to improve the prediction and the reliability of the prediction. The validation assessment can be an important component toward maximizing the quality and yield of chitin. The chitin synthesized in this work was compared with standard as shown in Table 7.

TABLE7: COMPARATIVE OF SYNTHESIZED AND STANDARD CHITIN

Parameter	FSW	Chitin synthesized	Standard Chitin
Moisture content (%)	17.5	4.2	<10
Ash content (%)	40.37	0.35	<1
Mineral content (g/Kg)	24.03	6.25	-
Crude Protein (%)	32.71	0.96	<1

According to Table 7, it can deduced that parameters of synthesized chitin have the values of 4.2 %, 0.35 %, 0.96 % and 6.25 g/Kg for Moisture content, Ash content, Crude Protein and Mineral content (g/Kg) respectively, which were within the standard value reported by Maryam & Mahmood (2007). These analyses revealed that the quality and yield of the chitin obtained from the FSW were due to the effective interaction of input parameters and the responses.

3.4 Characterization FSW and chitin

The characteristics of Fish Scale waste (FSW) obtained and chitin produced was carried out using proximate and mineral composition, with the results presented in Table 8. The Analysis showed that the FSW had 17.50 % moisture, 40.37 % ash, 4.88 % lipid, 32.71 % crude protein and 24.03 g/Kg mineral. While chitin proximate and mineral composition were 4.20 %, 0.35 %, 1.54 %, 0.96 %, 6.25 g/Kg, for moisture, ash, lipid, crude protein, and mineral respectively. The demineralization conditions used in this study reduce the mineral content. This is in agreement with the findings reported by Adejonwo, 2010 and Ghorbel-Bellaaj *et al.,* 2010.

TABLE 8: RESULT OF PROXIMATE AND MINERAL ANALYSIS OF CSW AND CHITIN

Sample	**Moisture (%)**	**Ash (%)**	**Lipid (%)**	**Crude Protein (%)**	**$CaCO_3$ (g/Kg)**
FSW	17.50	40.37	4.88	32.71	24.03
Chitin	4.20	0.35	1.54	0.96	6.25

From the results given in Table 8, it can be deduce that the process parameters used in this study reduces proximate and mineral content to permissible limits and pave ways in estimating the quality of the chitin as 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.

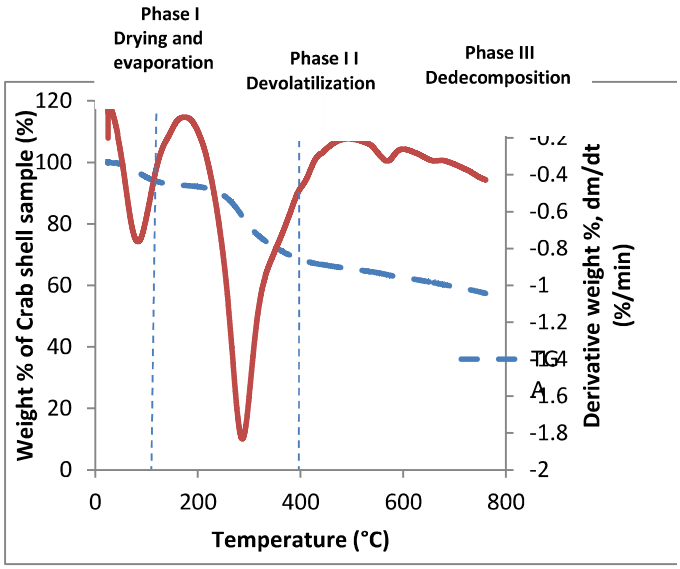

Figure 1: TGA and DTG of Fish scale

4 CONCLUSION

In this paper, Chitin is synthesized from the abundant aquatic wastes by organic acid demineralization and alkali deproteinization treatments. The various input conditions as well as the source and pretreatment of raw material significantly affect its quality. Chitin and its major derivatives chitosan are used in many fields, and their applications are expanding annually. The effect of process variable on chitin preparation from FSW was carried out with aid of design expert (7.0) via CCD of RSM in order to illuminate and optimize the factors which may maximize the quality and yield of chitin.

The quadratic Central composite design (CCD) model used indicates that the operating parameters significantly influence on the quality and yield of synthesized chitin. The optimum conditions, viz. reaction temperature 50 ^{o}C, concentration of lactic acid solution 9.7 % (v/v) and reaction time 3 h which revealed 96.91 % demineralization was achieved. Similarly, the optimum processing conditions, viz. treatment temperature 70 ^{o}C, concentration of NaOH solution 6 % (w/v), reaction time 3.34 h which revealed 95.01 % deproteinization was achieved.

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