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Optimization of Deacetylation Parameters during the Extraction of Chitosan from Shrimp Shells Waste

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Abstract

The objective of this work was to valorize shrimp shell waste to produce chitosan by using an experimental design for the extraction. The centered composite plan was used to optimize the extraction of chitosan. The influent factors were the caustic soda concentration, the temperature and the time. Results showed that the values of answers would adapt to a second-degree polynomial model. For statistical analysis, the R-square value obtained was greater than 98.80%; the Absolute Average (AAD) was equal to zero and the Biais factor was equal to the unit, validating the model. Optimal values of caustic concentration were 67.06% while those of the temperature and the time were 100˚C and 35 min respectively. Among these factors, the concentration of caustic soda showed the greatest influence on the degree of deacetylation which varies between 74.39% and 96.02%. The optimal yield of chitosan extraction was 72.16%. Fourier Transformed Infrared (FTIR) spectrum showed absorption bands at 1650 cm^{-1} and 1315 cm^{-1} characteristic of chitosan and confirmed by morphology studies. Concentration has the greatest influence on deacetylation than temperature and time; otherwise, the viscosity increases with the pH. Shrimp shell waste converted into chitosan reduces pollution and improves their value.

Subject Areas

Chemistry & Materials Science, Environmental Chemistry

Keywords

Chitosan, Optimization, Deacetylation, Extraction, Shrimp Shells Waste

1. Introduction

Chitin and chitosan are obtained by successive transformations of the exoskeletons (shells or scales) of crustaceans from waste for food industry. However, the proportion of chitin in this waste can vary from 15% to 30% in terms of dry mass for certain crab carcasses and from 30% to 40 % for brown shrimps [1]-[3]. It is the most abundant polysaccharide on earth after cellulose. Its hydrolysis in a strongly alkaline medium leads to the production of its main derivative which is chitosan; the latter is a substance with little response in nature, which explains why there are no exploitable primary sources. The essence of chitin transformation lies mainly in the production of chitosan which is economically more beneficial.

The use of the latter has affected several areas of daily life, namely fields of pharmacy, agrifood, environment, agriculture, textiles, stationery, not to mention cosmetics. Chitosan is a non-toxic natural, renewable and biodegradable resource. It is also a biocompatible substance since its chemical reactivity is very interesting; it can play an important role in several areas given its film-forming, biological, antifungal and water retention properties [2] [4]-[6]. The physicochemical and biological properties of chitosan are mainly related to the structural characteristics of its degree of deacetylation (DDA) and its molar mass. Optimal extraction conditions can be defined through experimental plans which makes it possible to better organize tests required by research scientific companies. More information can be obtained with minimum experiments from experiment plans. It is then necessary to follow mathematical rules and adopt a rigorous approach [7].

2. Methodologie

2.1. Methodology

The shrimp sampling was collected from Wouri river in Douala, Cameroon; this species (*Litopenaeus vannamei*) is in high asked and liked in local restaurants and fisheries. Our marine waste or co-products have been collected in markets, restaurants and from fishermen; these shells were dried, crushed and stored in hermetically sealed glass bottles away from light for further processing according to the methodology of **Figure 1**.

2.2. Characterization of the Raw Material

This involves determining the water, ash, soluble matter, protein and chitin content of the sampled shrimp shells according to [1].

2.2.1. Water Content (%WC)

A mass of 30 g was introduced into the beaker and placed in an oven at a temperature of 105 ± 2˚C for 24 hours; the water content is calculated according to the following formula:

$$\mathrm{WC} = \frac{m_i'}{m_i} \times 100\% \quad (1)$$

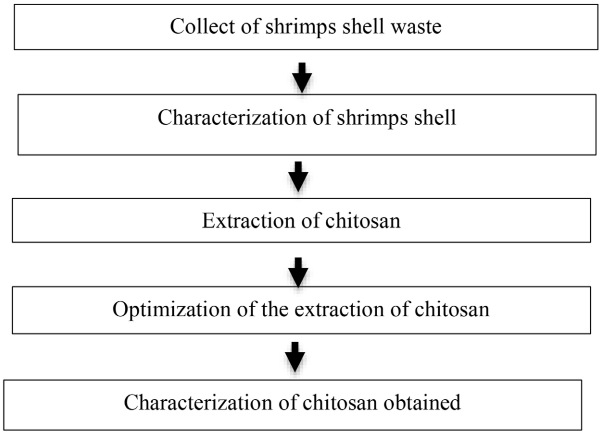

Figure 1. General methodology of work.

where m_i' and m_i are masses obtained after and before incubation respectively.

2.2.2. Ash Content (%AC)

It was based on the calcination of shrimp shells at a temperature of 550˚C for 24 hours in order to obtain ashes, followed by drying in an oven at 105˚C for four hours. The ash content was calculated according to the following formula:

$$\mathrm{AC} = \frac{m_i'}{m_i} \times 100\% \tag{2}$$

where, m_i' and m_i are masses after and before calcination respectively.

2.2.3. Soluble Content

Shell masses were weighed, then macerated in 100 mL of solvent (CH_2Cl_2) for 24 hours. The supernatant was collected and evaporated. The rate of soluble matter was determined using the relationship

$$\mathrm{SC} = \frac{m_s}{m_0} \times 100\% \tag{3}$$

where m_s and m_0 are masses of the residue obtained after evaporation of the solvent and the initially weighed sample respectively.

2.2.4. Total Protein Content

The ninhydrin test is used to have the total protein content by a process that consists of deproteinization several times using a dilute alkaline solution (1.5 M NaOH). The mixture was heated for 30 minutes and the supernatant was extracted and the alkaline solution was then added for the second time. The mixture was stirred for 2 hours, filtered and washed until neutral pH. After washing, the effective deproteinization was discovered using a ninhydrin test (2% in water) staining in characteristic blue-violet in the presence of residual proteins. In their absence, ninhydrin remains colorless. The following formula was used to determine the protein content (PC):

$$\mathrm{PC} = \frac{m_i - m_i'}{m_0} \times 100\% \quad (4)$$

where m_i, m_i' and m_0 were masses of the sample after steaming, remaining after deproteination several times and of the sample initially weighed respectively.

2.3. Extraction of Chitosan

2.3.1. Demineralization (Decalcification)

Shells, once pretreated (washing, crushing and drying), were thoroughly mixed in aqueous solutions of hydrochloric acid (HCl) to dissolve the minerals present in shells [8] [9]. Samples were mixed in a range of HCl concentrations ranging from (1 M to 3.5 M) under magnetic stirring between 1 hour and 2.5 hours at 25˚C. For each test, a mass m_0 of shrimp shell is placed in a jar and treated with a volume of 100 mL of dilute hydrochloric acid solution at the desired concentration. After reaching the recommended demineralization time, the mixture undergoes filtration and washing with demineralized water until neutralization (pH = 7). The efficiency of demineralization was evaluated using the calculation of the demineralization rate (DM) with the given formula.

$$\mathrm{DM} = \frac{A_0 M_0 - A_r M_r}{A_0 M_0} \times 100\% \quad (5)$$

where A_0 and A_r are percentages of ashes in the initial and the hydrolyzed products respectively.

2.3.2. Deproteination

Deproteination consists of solubilizing the proteins present in chitin in an aqueous solution. Demineralized shells, after washing with water to neutral pH, were mixed in caustic soda solutions (NaOH) in a proportion of 1:10 w/v (dry shell weight/NaOH volume). Experiments of this stage were carried out with NaOH concentrations of 0.25 M, 0.5 M, 1 M and 1.5 M at times varying between 1 hour, 2 hours and 3 hours at 65˚C respectively [9]. The effectiveness of deproteination was monitored using ninhydrin tests. These tests can only detect the presence of these proteins in chitin. The reagent used must be freshly prepared by dissolving 0.25 g of ninhydrin in 100 mL of distilled water. The reaction of proteins with ninhydrin is a general reaction of all proteins and free amino acids except proline and oxyproline. The appearance of a blue-violet color makes it possible to detect the presence of proteins.

2.3.3. Deacetylation: (Experiment Plan)

This step consists of changing from chitin to chitosan using a basic treatment (NaOH).

Factors that can most influence the degree of deacetylation (DDA) and the yield (Rd) of obtaining chitosan according to the literature are the concentration of NaOH (%), the temperature (˚C) and the time (min). The chitin obtained will be basic with a basic concentration ranging from 20% to 60% with a temperature variation of between 60 and 140˚C for 10 to 60 minutes. A composite experi-

mental design centered on these three factors was used for this purpose. The total number of tests N is calculated using the formula:

$$N = N_f + N_\alpha + N_0 = 2^{k-r} + 2k + n_0 \quad (6)$$

where 2^k is the number of trials for a full factorial design, r is a function of the number of trial reductions for a fractional factorial design. For example, $r = 1$ for halving the number of trials. In general, $r = 0$ for $k \leq 4$

- N_f is the number of experimental points of a factorial plan at two levels, complete or fractional.
- N_α is the number of points located on the axes of each of the factors studied at a distance α from the center of the experimental domain.
- N_0 is the number of points in the center of the domain (x3)

The number of tests at center n_0 and the parameter α is chosen in accordance with the properties desired for the plan.

The parameter α makes it possible to define tests in addition to the factorial plan. When using coded values, α defines the position at the axial point relative to the center. Ultimately, each factor will take the following coded values: −1 for the low level, +1 for the high level, 0 in the center, $-\alpha$ and $+\alpha$.

With 3 factors and 3 tests at the center, our experience matrix will be made up of 17 experiences. The multiplicity of tests at the center makes it possible to determine the experimental error and to consider that it is the same everywhere in the field.

When applying the principle of rotation, the value of α for three factors is 1.682. Value sought at point α = value at center ± step. α (Table 1)

Answers are deacetylation degree (DDA) and Yield (Rd) for chitosan obtain.

The axial points are defined by the regression equation which shows the contribution of the different factors. This equation is presented as follows:

$$\begin{aligned} Y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \\ & + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \end{aligned} \quad (7)$$

where b_0 is the center value, b_i is the linear factor, b_{ii} is the quadratic factor and b_{ij} is the interaction factor.

To validate the empirical model obtained, the experimental responses obtained are compared to those calculated from the mathematical equation of the model; in addition to the coefficient of determination (R^2),

Table 1. Implicit domain for deacetylation.

Factors	Units	Factors	Variation/Level				
			$-\alpha$	Lower limit (−1)	Center (0)	Upper limit (1)	$+\alpha$
Concentration (*C*)	(%)	X_1	12.937	20	40	60	67.062
Temperature (*T*)	(˚C)	X_2	45.875	60	100	140	154.12
Time (*t*)	(mn)	X_3	1.1718	10	35	60	68.828

The Absolute Average Deviation Analysis (AADM) which provides information on the average error of the manipulations is given by the following expression [10] [11]:

$$\text{AADM} = \frac{\sum_{i=1}^{p}\left|\frac{Y_{iexp} - Y_{ical}}{Y_{iexp}}\right|}{p} \tag{8}$$

With: Y_{iexp} the experimental response and Y_{ical} the response calculated from the model for an experiment i; p being the total number of experiments.

The model is validated if $0 \leq \text{AADM} \leq 0.2$.

- The bias factors

$$Bf = 10^{B} \tag{9}$$

The bias factor is given by the expression

With B the bias

$$B = \frac{1}{n}\sum \log\left(\frac{Y_{theo}}{Y_{obs}}\right) \tag{10}$$

The model is validated if $Bf \leq 1.20$

- Factors of accuracy

The accuracy factors are given by the following expressions:

$$Af_1 = 10^{A_1}, \quad Af_2 = 10^{A_2} \tag{11}$$

With the accuracy

$$A_1 = \frac{1}{n}\sum_{i=1}^{n}\left|\log\left(\frac{Y_{theo}}{Y_{obs}}\right)\right|, \quad A_2 = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\log\left(\frac{Y_{theo}}{Y_{obs}}\right)\right)^2} \tag{12}$$

The model is validated if $1 \leq Af_{1,2}$

2.4. Characteristic of Chitosan

2.4.1. Solubility Test

A mass (m_0) of extracted chitosan is introduced into 100 mL of 2% acetic acid and stirred for 24 hours until the solid is dissolved; the mixture obtained is then filtered and the solid residue is dried in an oven, weighed to obtain a mass m'. The solubility is obtained according to the following formula:

$$S = \frac{m_0 - m'}{m_0} \tag{13}$$

2.4.2. The Degree of Deacetylation (DDA)

The DDA is evaluated by the pH-metric assay method according to the following steps [12]:

- Solubilization of 0.1 g of chitosan in 10 mL HCl 0.1 mol/L with distilled water (10 mL) for 30 min then 12 mL of distilled water is added with stirring for 30 minutes;
- Titration of the solution, with stirring, with a 0.1 mol/L NaOH solution; A solution containing chitosan dissolved in hydrochloric acid is neutralized with sodium hydroxide. The volume of sodium hydroxide solution used cor-

responds both to the volume necessary to neutralize the excess HCl acid used for dissolving the chitosan and to neutralize the ammonium functions of the chitosan.

$$\mathrm{DDA} = \frac{2.03(V_2 - V_1)}{m + 0.0042(V_2 - V_1)} \tag{14}$$

2.4.3. Determination of Viscosity

To determine of viscosity of chitosan, 1.0 g of chitosan was dissolved in 100 mL of 0.1 M acetic acid. The viscosity of the chitosan was measured in a viscometer viscostar, fungilab at 22.3˚C, 100 RPM.

2.4.4. Analysis of Chitosan Phases Groupment and Morphology

The nature of the phase groupings studied is analyzed using the Perkin Elmer Spectrum 100 Fourier Transform Infrared spectrometer (FTIR) equipped with a high-sensitivity broadband (400 - 4,000 cm^{-1}) detector, with a mercury-cadmium-tellurium window. (MCT) and cools to 77K. Morphology of samples was inspected using a Beckman Coulter SA 3100 Scanning Electronic Microscope (SEM) equipped with an energy dispersive spectroscopy (EDS) detector.

3. Results and Discussion

Different transformations undergone by shrimp waste until chitosan is obtained are summarized in Figure 2 below:

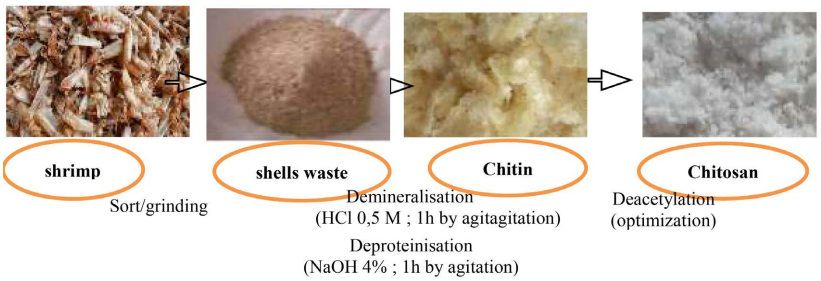

Figure 2. Steps for extraction of chitin and chitosan from shrimp shells waste.

3.1. Characterization of Shrimp Shells Waste

Some prefatory analysis done on the raw material before transformation are shown in Table 2.

Table 2. Characterization of shrimp shells waste.

	Characteristics	Average
1	Water	30.37% ± 0.8%
2	Ash	30.76% ± 2.9%
3	Protein	12.61% ± 0.55%
4	Soluble	19.48% ± 1.1%
5	Chitin	26.26%

All these values are similar to these obtain by [13].

3.2. Production of Chitosan

Different combinations done for the three concentrations of HCl (1, 2 and 3.5 M) and three times (1, 2 and 2.5 hours) permitted to do nine experiences the results are shown in Table 3.

Analyzes relating to the demineralization and deproteination taking into account three concentrations of hydrochloric acid and three values of time show that the yield increases with an increase in acid concentration, which is in agreement with the results of the work of [14].

According to the kinetic study of the reaction, the demineralization is characteristic of the pseudo-second-order. Order 2 is explained by the fact that the structure of crustacean exoskeletons has several layers. The best demineralization (99.99%) is obtained for a concentration of 3.5 M for 2 h 30 mn. It can be observed that higher the concentration of the acid, the faster layers are attacked by the absorption of the acid. On the other hand, a long enough time favors the demineralization process because the shells are also softened over time.

3.3. Optimization of Deacetylation Using the Centered Composite Experimental Design

The main experimental responses of the experimental design are presented in Table 4 below

From the table above, it can be seen that the deacetylation rate varies from 74.39% to 96.02% and the yield varies from 30.81% to 72.16%. These results make it possible to highlight the influence of the parameters which are the concentration, the temperature and the time on the rate of grouping of free amine and the yield of obtaining chitosan. It is clearly observed that a maximum concentration allows better deacetylation.

Table 3. Demineralisation and deproteination results at 65˚C.

N˚	[HCl] (M)	Time (h)	Initial weight (g)	Weight before calcination (g)	Weight after calcination (g)	Ash content (%)	Demineralisation content (%)	Deproteination content (%)
1	1	1	10.01	1.54	0.01	0.65	99.98	48.33
2	1	2	10.09	1.99	0.17	8.54	97.46	54.13
3	1	2.5	10.04	1.20	0.04	3.33	99.61	52.22
4	2	1	10.01	3.00	0.11	3.67	99.53	54.93
5	2	2	10.08	1.65	0.14	3.48	99.5	56.55
6	2	2.5	10.07	2.63	0.15	5.70	99.87	47.06
7	3.5	1	10.06	2.90	0.03	1.03	99.96	55.45
8	3.5	2	10.07	1.65	0.18	10.91	95.86	52.55
9	3.5	2.5	10.04	2.00	0.01	0.5	99.99	50.49

Table 4. Mixture design for optimization of DDA and Rd.

N°	Coded factors			Actual factors			Responses	
	X_1	X_2	X_3	C(%)	T(˚C)	Time (min)	DDA	Y
1	−1	−1	−1	20.0	60.0	10.0	78.81	72.16
2	1	−1	−1	60.0	60.0	10.0	90.61	44.27
3	−1	1	−1	20.0	140.0	10.0	76.57	67.82
4	1	1	−1	60.0	140.0	10.0	81.23	39.14
5	−1	−1	1	20.0	60.0	60.0	80.23	63.38
6	1	−1	1	60.0	60.0	60.0	86.09	40.95
7	−1	1	1	20.0	140.0	60.0	75.3	62.09
8	1	1	1	60.0	140.0	60.0	74.39	45.38
9	−1.682	0	0	12.94	100.0	35.0	85.27	65.74
10	1.682	0	0	67.06	100.0	35.0	96.02	30.81
11	0	−1.682	0	40.0	45.87	35.0	86.58	59.71
12	0	1.682	0	40.0	154.12	35.0	80.32	62.1
13	0	0	−1.682	40.0	100.0	1.17	79.75	66.3
14	0	0	1.682	40.0	100.0	68.83	77.09	59.4
15	0	0	0	40.0	100.0	35.0	88.0	64.48
16	0	0	0	40.0	100.0	35.0	88.0	62.11
17	0	0	0	40.0	100.0	35.0	89.23	65.5

3.3.1. Regression Coefficient and Model Proposal for Deacetylation (DDA)

Analyses of the results deacetylation model are given by the equation below:

$$\begin{aligned} \text{DDA} = {} & 49.6985 + 0.307644 * C + 0.418486 * T + 0.782576 * t \\ & + 0.00205717 * C * C - 0.00217344 * C * T - 0.0028775 * C * t \\ & - 0.00194174 * T * T - 0.00062625 * T * t - 0.00936637 * t * t \end{aligned} \quad (15)$$

Linear factors, concentration, temperature and time positively influence the deacetylation. For the quadratic factors, the concentration only influences but weakly; interactions have no effect. On the other hand, it appears that in absolute value, the effect of time is 2.5 times; the effect of NaOH concentration, 1.9 times the effect of temperature. Main effects of the different factors are shown in **Table 5** and illustrated in **Figure 3**.

The R-squared statistic indicates that the fitted model explains 97.434 % of the yield in obtaining chitosan. Bias factors and Accuracy factors are equal to unity and the AADM is equal to zero so the model is considered valid.

The ANOVA table breaks down the deacetylation rate variability into separate rows for each of the effects. It then tests the statistical significance of each of the effects by comparing the root mean square to an estimate of the experimental error. In this case, 7 effects have a probability less than 0.05, indicating that it is significantly different from zero at the 95.001% confidence level. The R-squared

Table 5. Significance of different effects and indication for validation of the model for DDA.

Source	Amount of square	DDL	Quadratic average	Rapport F	Proba.
$X_1:C$	110.86	1	110.86	70.52	0.0001
$X_2:T$	115.625	1	115.625	73.56	0.0001
$X_3:t$	18.8061	1	18.806	11.96	0.0106
X_1X_1	4.53984	1	4.540	2.89	0.1330
X_1X_2	24.186	1	24.186	15.39	0.0057
X_1X_3	16.56	1	16.56	10.53	0.0141
X_2X_2	64.7154	1	64.715	41.17	0.0004
X_2X_3	3.13751	1	3.137	2.00	0.2006
X_3X_3	229.767	1	229.767	146.17	0.0000
Total slip	11.0036	7	1.572		
Total (corr.)	599.2	16			
R square				**98.877%**	
R square ajusted				97.434%	
AADM				0.004	
Biais factor				1.005	
Exactitude factor				1.028 1.031	

statistic indicates that the fitted model explains 93.243 % of the variability in the degree of deacetylation. The following figure presents the level of significance of each factor.

In this process, the concentration has a great influence on the deacetylation of chitin; this results in the fact that the DDA increases with the concentration. Results show that the DDA of chitin from shrimp shells increases with temperature or with concentration [15].

So, although the concentration is one of the main factors responsible for the multiplication or the release of free amines from chitosan, however, temperature also has a somewhat greater influence on the process of deacetylation of chitin.

A very high temperature of the reaction medium can lead to an attack on the carbon chain, thus leading to a rupture of C-C bonds and consequently to obtaining a chitosan of not very good quality. However, factors of x temperature of concentration and time are in order of increasing influence. It is clear that the impact of DDA processing time is less important than the effect of NaOH concentration; it can also deduce that the deacetylation process is endothermic because the DDA increases with the increase in temperature from 60 to 80˚C (**Figure 3**).

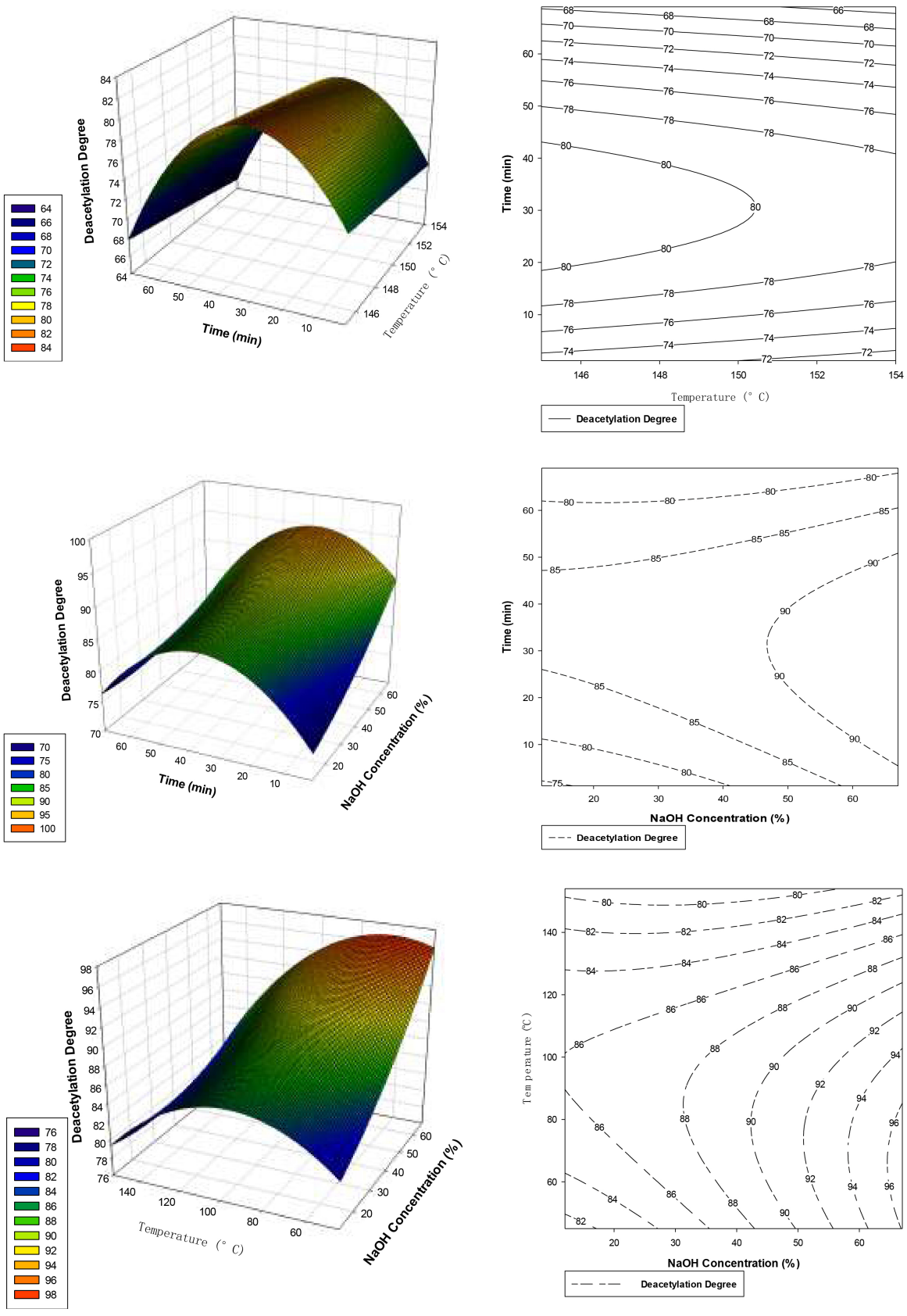

Figure 3. Surface of response and contours graphic for DDA.

3.3.2. Regression Coefficient and Model Proposal for Yield (Y)

Analyses of the results of the yield model is given by the equation

$$\begin{aligned} Rd = {} & 65.7373 + 0.779151 * C + 0.0586817 * T - 0.387114 * t \\ & - 0.0202712 * C * C + 0.000770312 * C * T + 0.0043575 * C * t \\ & - 0.0007565 * T * T + 0.00157625 * T * t - 0.000236975 * t * t \end{aligned} \quad (16)$$

From this equation, it emerges that in absolute value with regard to the main effects, the effect of concentration is 13 times the effect of temperature and 2 times the effect of time. It can notice that the concentration and the temperature have significant effects on the yield of obtaining chitosan. However, the effect of time has a negative impact and, in addition, a high concentration and long reaction time leads to a low yield of chitosan.

The interaction between factors has positive effects on performance; the influence of the concentration-time interaction has a fairly positive impact followed by that of the temperature-time and finally the concentration-temperature interaction, although less on the yield of obtaining chitosan. In the other hand, the quadratic effect of factors has a negative impact on the performance, especially the quadratic effect of the concentration (Table 6).

Table 5 shows combinations of factor levels that maximize Rd in the indicated region. Use the options dialog for analysis to specify the region in which the optimization should be performed. You can set one or more factors to constant levels by setting the low and high limits to these values and illustrated in Figure 4.

The R-squared statistic indicates that the fitted model explains 98.877% of the variability in Rd. The adjusted R-squared statistic, which is better for comparing models with different numbers of explanatory variables, is 97.434%. The probability value is greater than 5.001%; this does not indicate serial autocorrelation of the residuals at the 5.001% significance level (Figure 4).

Table 6. Analysis of variance for yield.

Source	Amount of square	DDL	Quadratic average	Rapport F	Proba.
X_1:C	1752.87	1	1752.87	469.25	0.0000
X_2:T	0.822	1	0.822	0.22	0.6533
X_3:tps	37.552	1	37.552	10.05	0.0157
X_1X_1	440.819	1	440.819	118.01	0.0000
X_1X_2	3.0381	1	3.038	0.81	0.3971
X_1X_3	37.976	1	37.976	10.17	0.0153
X_2X_2	9.823	1	9.823	2.63	0.1489
X_2X_3	19.876	1	19.877	5.32	0.0544
X_3X_3	0.147	1	0.147	0.04	0.8484
X_1:C	0.147	1	0.147	0.04	0.8484
Total slip	26.148	7	3.735		
Total (corr.)	2329.07	16			

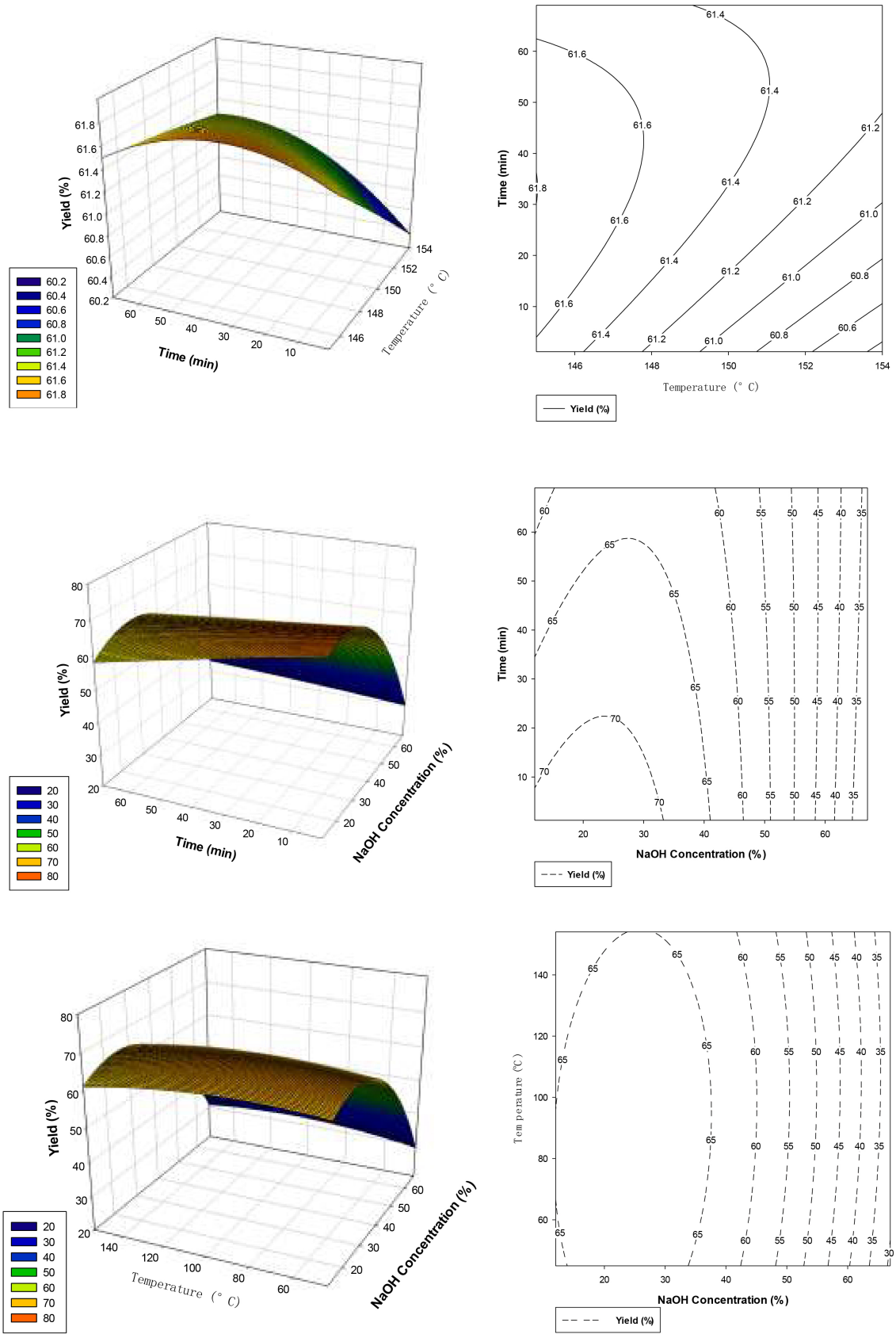

Figure 4. Surface of response and contours graphic for yield.

3.4. Characterization of the Chitosan Obtained

3.4.1. Degree of Deacetylation

The degree of deacetylation obtained by evolution of pH with viscosity of chitosan obtained from shrimp shells waste is represented in **Figure 5**.

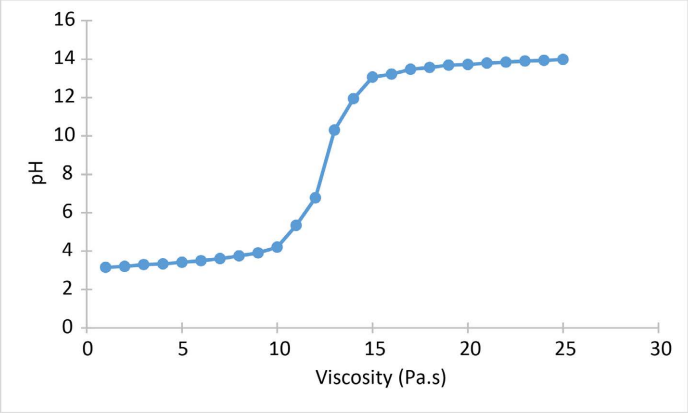

Figure 5. pH-metric dosage curve of chitosan obtained.

The interpretation of the curve shows that the viscosity evolves proportionally to the pH, with formation of the hysteresis between 10 Pa.s and 15 Pa.s where one passes from the very acid pH of to the very basic pH of 13; this is characterized by a sharp change in the curve in this range.

The viscosity of chitosan depends on the DDA of this polymer. The more it is deacetylated, the more free amine groups there are, the more the chitosan is soluble and consequently its viscosity is greater. Viscosity also depends on polymer concentration (it increases with concentration), temperature (it drops as temperature increases), molecular weight (intrinsic viscosity increases with increasing molecular weight) and finally on PH (the lower it is, the higher the viscosity) [16].

The chitosan obtained (%DDA equal to 88%) is of good quality because we speak of chitosan from 50% NacGlu according to [17], and in general chitosan is defined as being the deacetylated form at more than 60% - 70%. The commercial one has a DDA between 66 and 95%. Our result is higher than that obtained by Kumari *et al*., 2015 [18] this due to species diversity and the environment.

3.4.2. Fourier Transformed Infrared (FTIR)

The Fourier Transformed Infrared (FTIR) spectrum of chitosan obtained from shrimp shells under optimal conditions shows many characteristic peaks (**Figure 6**); at 896 cm^{-1}, it can noted an asymmetric C-O-C elongation of the bond glycosidic; between 1028 cm^{-1} and 1159 cm^{-1} of peaks characteristic of the pyranose cycle; elongation of the OH groups and extended vibration of NH at 3335 cm^{-1}; between 2850 and 2960 cm^{-1}, a C-H elongation of the symmetrical or asymmetrical CH_2 group of the pyranose cycle; a CO-NH (amide III) deformation at 1315 cm^{-1}; CH_2 deformation at 1423 cm^{-1}; a band characteristic of O=C of the

amide group I at 1650 cm^{-1} and between 1320 cm^{-1} and 1415 cm^{-1}, the NH_2 of the amine group. Successful deacetylation is assessed on the intensity of the band at 1650 cm^{-1} and 1315 cm^{-1} according to [1] [8] [9] [19].

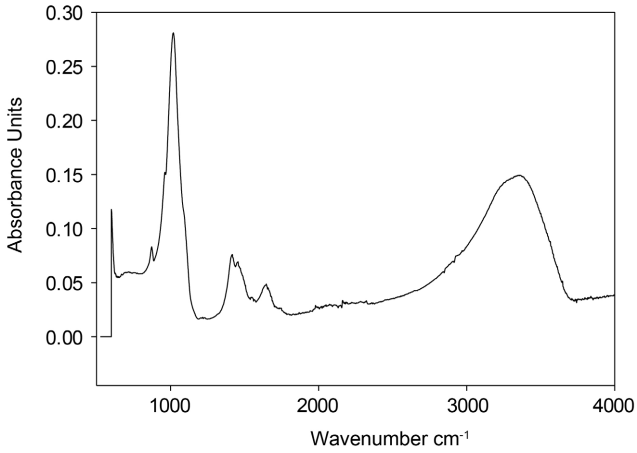

Figure 6. IRTF Spectrum of Chitosan extracted from shrimp shells waste.

3.4.3. Morphology of Chitosan

The morphology of the obtained chitosan was revealed by Scanning Electronic Microscope (SEM) as shown in **Figure 7**. The electron micrograph (5 µM) indicated that the outer of chitosan was rough and had some little parts on the chitosan skin. Energy Dispersive Spectroscopy (EDS) shows that the most abundant element is Carbone (61.2%) followed by Oxygen (34.8%) and Calcium (3.5%); similar to [19]; other elements such as Silicious, Sodium, Sulfur and Aluminum are only in traces.

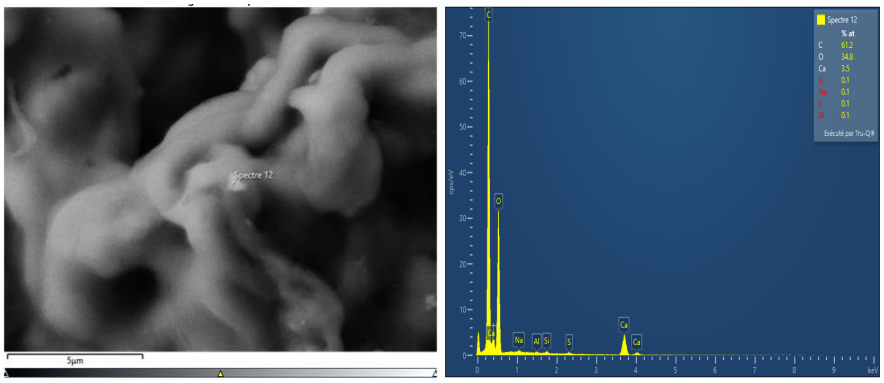

Figure 7. Morphology of chitosan (5 µm) coupled EDS.

4. Conclusion

This work aims to extract chitosan from shrimp shell waste by using the mixture design experiment for deacetylation Among the three independent variables which are sodium concentration, temperature and time, it is the concentration that has the greatest influence on the degree of deacetylation (maximum con-

centration of 67.062%). The temperature should not be very high and not low either. The viscosity increases with the pH with a considerable shift between 10 Pa.s and 15 Pa.s. The IRTF spectrum of chitosan obtained from shrimp shells waste under optimal conditions shows numerous characteristic peaks and successful deacetylation, assessed on the intensity of the band at 1650 cm^{-1} and 1315 cm^{-1}. The morphology of the obtained chitosan indicated the outer of chitosan was rough and had some little parts on the chitosan skin. The transformation of waste like shrimp shells improves their values and reduces pollution. Chitosan can be used in many applications such as cosmetics.

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Conflicts of Interest

The authors declare no conflicts of interest.

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