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Optimization of Microwave technique conditions for Shrimp chitin deacetylation by response surface methodology

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ABSTRACT

The objective of this study was to optimize chitosan production conditions from shrimp waste using the response surface methodology corresponding to the degree of deacetylation. The influence of alkaline concentration, microwave power, time, and chitin concentration on the N-deacetylation of shrimp wastes chitin by microwave irradiation was investigated. Experimental conditions varied from 30 to 50% NaOH, 1125 to 2450 MHz, 6 to 18 min, and 4.76 to 8.30% chitin concentration. The degree of deacetylation increased significantly with increasing time, NaOH conc. microwave power, and decreasing chitin conc. response surface analysis indicated the maximal degree of deacetylation to occur at 50% NaOH, 2400 MHz, 4.76% chitin conc. and 13 min to obtain chitosan with DDA 90.2%.

Keywords: Chitin, Chitosan, Microwave irradiation, Degree of deacetylation, Optimization, RSM.

INTRODUCTION

The shrimp represent 45 % of the processed seafood, which produced exoskeletons and heads as a waste **(Sila *et al.*, 2014)**. The shrimp as raw materials can be introducing 50-70 % as a waste **(De Holanda and Netto, 2006)**. This waste is a good source of biomolecules, including chitin **(Rødde *et al.*, 2007 and FAO, 2016).** Also, it contains proteins, carotenoids, astaxanthin, nutritive components, and enzymes **(Kurita, 2006; Handayani *et al.*, 2008; and Kaur & Dhillon, 2013).** According to **Nouri *et al.*, (2016) and Ning & Xi (2015),** the shrimp waste contains protein (30-40%), calcium carbonate (30-50%), and chitin (20-30%). Therefore, it is important to exert efforts to convert these wastes into useful and applicable products like chitosan **(Assunção & Pena, 2007 and Rødde *et al.*, 2008).**

Chitin is a natural biopolymer that is found in the exoskeleton of crustaceans **(Yang *et al.*, 2005 and Khanafari *et al.*, 2008),** some mushrooms envelopes, green algae cell walls, fungi, and yeasts **(Croisier and Jérôme, 2013).** It is structure contains mainly poly *β*-(1–4)-2-acetamido-D-glucose **(Ji *et al.*, 2012 and Arbia *et al.*, 2013).** The structure is identical to cellulose except that a secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group **(Yang *et al.*, 2005 and Mejia-Saules *et al.*, 2006)**. Chitosan is a polycationic polysaccharide derived from chitin by deacetylation in alkaline media **(Abdou *et al.*, 2007 and Tokatli & Demirdöven, 2018)**. Actually, chitosan is a copolymer consisting of *β*-(1–4)-2-acetamido-D-glucose and *β*-(1–4)-2-amino-D-glucose units with the latter usually exceeding 60% **(Muzzarelli, 1997 and Dutta *et al.*, 2009)**.

During the last several decades, chitosan had the researcher's attention because of its importance to commercial applications in the food, biomedical, and chemical industries **(Nagasawa *et al.*, 2000; Rinaudo, 2006 and Khorrami *et al.*, 2012).** Chitosan characteristics are biocompatibility, biodegradability, environmentally friendly, non-toxicity, and non-antigenicity **(Kang *et al.*, 2007 and Santos *et al.*, 2016)**. Chitosan plays a good role in a biological system as antimicrobial **(Sudarshan *et al.*, 1992; No *et al.*, 2002 and Tahtat *et al.*, 2011)**, antitumor **(Tokoro *et al.*, 1988)**, hypocholesterolemic functions **(Sugano *et al.*, 1992)**. Chitosan can be used as an emulsifying, thickening, and stabilizing agent in the food industry **(Shahidi *et al.*, 1999; Dong *et al.*, 2000 and García *et al.*, 2008)**. Also, exhibits good film-forming properties and high mechanical strength and adhesion **(Wang *et al*, 2000 and Mucha *et al.*, 2007*).***

Degrees of deacetylation (DDA) express the content of free amino groups that can be employed to differentiate between chitin and chitosan. DDA is the percent of D-glucosamine units to the total D-glucosamine and N-acetyl-D-glucosamine units. Chitosan formed with the DDA value reached to 60% **(Kasaai, 2009)**. According to the DDA value, the usage of chitosan in many food applications is identified **(Elsabee & Abdou, 2013; Taşkın *et al.*, 2014)**.

In the traditional method, chitosan produced by deacetylation of chitin using a 40-50 % sodium hydroxide solution (w/v) at high temperature and pressure **(Zakaria *et al.*, 1998)**. This process takes several hours (10-12hr) to produce chitosan with significant DDA value. Therefore, there is a strong need to find a new technique that can save a massive amount of energy and time. In this regard, microwave irradiation provides an unconventional method as well as a more effective means of energy transfer-promoting deacetylation with a short time period **(Galema 1997 and Rani *et al.*, 2013)**. Microwave heating is a process of electro-heat technique that utilizes specific parts of the electromagnetic spectrum and may be used as an alternative heating method **(Galema, 1997)**. This new technique replaced traditional heating using the three-dimensional heating of the reaction mass **(Safari *et al.*, 2014)** and can make chemical reactions in minutes, rather than hours or days **(Hayes, 2004)**.

Limited works of literature exist on the microwave-induced deacetylation of chitin **(Al Sagheer *et al.*, 2009; Mahdy Samar *et al.*, 2013** and **El Knidri *et al.*, 2016*).*** Also, quaternized chitosan was successfully synthesized in only 50 min under microwave irradiation as reported by **Luo *et al.*, (2010).** All of

these studies reported different conditions for chitin and chitosan extraction. Therefore, it is important to optimize the process conditions for the production of chitosan using the microwave technique.

Response surface methodology (RSM) is a set of statistical and mathematical techniques effective to develop, improve and optimize processes involving a response of interest that is affected by many independent variables **(Myers *et al.*, 2009).** Few studies optimized conventional method conditions to chitosan extraction using RSM **(Ben Seghir & Benhamza, 2017; Tokatli & demirdöven, 2018; and El Knidri *et al., 2016)**, but there is no studies have been discussed the optimization of chitin deacetylation conditions and its effect on chitosan production from shrimp wastes using microwave technique.

The objective of this study has discussed the effect of NaOH concentrations, Microwave power, chitin concentrations, and different times as independent variables on the chitosan DDA as the dependent variable. Three dimension response surface method was applied to predict the optimized independent variables that gave the highest chitosan DDA.

MATERIALS AND METHODS

Materials:

Shrimp waste (heads and exoskeletons) collected from the El-Obour market, Kalubia governorate, Egypt. The waste was packed in plastic bags and stored at – 18°C until use. All used chemicals were of analytical grade and obtained from El-Gomhoria Company for Drugs and Chemicals, Cairo, Egypt.

Methods:

Preparation of shrimp wastes for extraction:

Shrimp wastes washed, dried at 50 ºC overnight, and ground. Then it sieved to obtain coarse powder at particle size 40 mesh. Ground shrimp waste packed into a plastic bag and stored in a dry condition until extraction.

Chitin extraction:

Chitin extraction from shrimp wastes was done according to **Synowiecki & Al-Khateeb (2003),** involved demineralization with 2% (v/v) HCl solution (10:1 v/w, 30ºC, 12h) to remove minerals, separation of an insoluble fraction by centrifugation (4000 rpm, 15min.), washing twice with distilled water then, deproteinization with 4% (w/v) sodium hydroxide solution (10:1 v/w, 90ºC, 12h) to remove proteins, separation of an alkali-insoluble fraction (AIF) by centrifugation (4000 rpm, 15min.), washing of (AIF) with distilled water until neutralization. After drying at 40 ºC overnight the product obtained was designated as purified shrimp waste chitin**.**

Preparation of chitosan by microwave technique:

According to **Sahu *et al.*, (2009)**, with minor modification, chitin was deacetylated using microwave oven with the composite design of four factors, (i) NaOH concentration at 30, 40 & 50%, (ii) microwave power at 1225, 1715 & 2450 MHz,(iii) deacetylation time from 6 to 18 min, and (iv)chitin concentration at 8.33, 6.66, 5.55 & 4.76 (w/v%); were selected as major independent variables factors influencing in the degree of deacetylation of produced chitosan. The resulted mixtures were filtered and the residues were washed with distilled water until neutralization, then dried in a hot air oven at 40 ºC until constant dry weight and stored until further analysis.

Determination of degree of deacetylation (DDA):

The acid-base titration method was used to determine the DDA from the amino group content in chitosan **(Domszy *et al.*, 1985)**. Dry chitosan (0.3 g) was dissolved in 30 mL of HCl standard solution (0.1

mol/L). Methyl orange and aniline blue mixing indicators were added. A standard solution of NaOH was used for titration until the solution became blue-green. The following formulas were used to calculate the DDA of the produced chitosan **(Luo *et al.*, 2000).**

$$(-NH_2)\% = \frac{0.016(C_1V_1 - C_2V_2)*100}{W} \quad \text{[Eq. 1]}$$

$$DD\% = \frac{203(-NH_2\%)*100}{16+42(-NH_2\%)} \quad \text{[Eq. 2]}$$

where*C*1, *V*1, C2, and V2 are the concentrations and a volume for the HCL standard solution and NaOH standard solution, respectively, and W is the weight of the sample.

Statistical Analysis:

For predicting the optimal points, a second-order polynomial function was fitted to correlate the relationship between each independent variables (NaOH conc. (%), Microwave power, chitin conc. and deacetylation time) and the response (DDA%) using the following model:

$$Y = a + bx + cx^2 \quad \textbf{[Eq. 3]}$$

Where Y is the predicted response, a is a model constant; b is a linear coefficient; c is a quadratic coefficient and x and x^2 are independent variables.

A three-dimension contour plot was used as a method to study the response surface of DDA as the dependent variable with NaOH concentration (%), Microwave power, chitin concentration (%), and deacetylation time as independent variables. The response surface method was applied using SigmaPlot **(Sigmaplot, 2000)**54 to locate the optimum conditions to prepare chitosan with a high degree of deacetylation. The Lorentzian model was used for a response of DDA in chitosan against previous independent variables as follows:

$$Z = \frac{a}{[1+\frac{(x+x_O)^2}{b}][1+\frac{(y+y_O)^2}{c}]} \quad \text{[Eq. 4]}$$

The software SigmaPlot **(Sigmaplot, 2000)** was used for experimental design, data analysis. Regression analysis (using PROC REG procedure) was carried out by the Statistical Analysis System **(SAS, 1996).**

RESULTS AND DISCUSSION

1. Polynomial quadratic regression

1.1. NaOH concentration

DDA (%) of produced chitosan was increased gradually by increasing NaOH concentration reaching maximum DDA 73.35% at 50% NaOH concentration. The NaOH concentration had a significant ($p<0.05$) effect on DDA values at microwave power 1715 MHz, chitin conc. 6.66% and deacetylation time 12min. as presented in Figure, 1A. Polynomial quadratic regression (Eq. 5) was used to predict the optimal NaOH concentration of the chitin deacetylation process using a microwave.

$Y = 214.45 - 12.7x + 0.197x^2$ [Eq. 5]

The heterogeneous N-deacetylation of chitin was a typical solid-liquid phase reaction. Deacetylation reaction firstly occurred in the chitin particle's surface and shallow surface. The DDA is slowly at the beginning. The reaction rate becomes faster with increasing the NaOH concentration because more NaOH contacted with the acetyl groups. Thereafter, the surface acetyl groups decreased and the diffusion of NaOH into the chitin particles was farther more difficult. In a sense, it was not an effective method to improve the deacetylation rate by only increasing the concentration of NaOH **(Liu *et al.*, 2009)**.

1.2. Microwave power

The effect of microwave power on the DDA by looking at three different power values (1225, 1715,

and 2450 MHz) was examined. At the same time keping the other independent variables at 40 % NaOH, 6.66% chitin conc., and deacetylation time12 min. For each experiment, the relationship of DDA as a function of microwave power was investigated and the results are shown in Figure, 1B. According to the polynomial quadratic regression, (Eq. 6) the predicted DDA increased from 20.6 to 75.02 % with increasing the microwave power from 1225 to 2426 MHz. Then the DDA was gradually decreased to 74.98 % with increasing the microwave power to 2430 MHz with the correlation coefficient (R^2= 1.000). The split of glycoside bonds and chain length of polysaccharide were downsized by microwave radiation, which led to the dismissal of acetyl groups thus increasing the number of NH_2 groups in fragment structure, theses caused an increase in DDA% (**El-Nesr *et al.*, 2013; Ocloo *et al.*, 2011; Sauperl & Volmajer-Valh, 2013**).

$Y= -166.32 + 0.206x – 4.42x^2$ [Eq. 6]

Microwave irradiation increases the rate of mass transport in the reaction system, which in turn increases the contact between the NaOH solution and the reacting functional groups on the chitin **(Ge and Luo, 2005).** Microwave heating is the use of constantly rotating molecular dipole moment, heating the materials internal and external simultaneously. It is more effective than traditional heating methods, which rely on thermal conduction and radiation model heating the materials from the surface to internal **(Liu *et al.*, 2009).**

1.3. Chitin concentration

Chitin concentrations (4.7-8.3 %) appeared to a high correlation with DDA values at identified condition NaOH concentration 40%, microwave power 1715 MHz, and deacetylation time12 min. The polynomial trend in Figure 1C appeared to inverse relationship between chitin concentration and DDA. Polynomial quadratic equation 3 that achieve the results presented as follows:

$Y= - 2.85 + 23.25x – 2.68x^2$ [Eq. 7]

The highest predicted DDA (46.76 %) was observed at a chitin concentration of 4.76 %. These results in agreement with **Chang *et al.*, (1997)** who reported that the solution-to-chitin ratio shows the minimal effect on the degree of N-deacetylation. On the other hand, **Methacanon *et al.*, (2003)** found that the effect of chitin to alkali solution ratio was insignificant ($p<0.05$).

1.4. Deacetylation time

Effect of deacetylation time on DDA values of produced chitosan at independent variables 40% NaOH, 1715 MHz microwave power, and 6.66% chitin was studied. Polynomial trend presented in Figure 1D. According to the polynomial quadratic equation (Eq. 8), the predicted DDA value increased significantly ($p<0.05$) with increasing the deacetylation time from 6 to 18 min with a correlation coefficient (R^2= 0.8786). The DDA value increased from 2.49 to 56.29 %, respectively. According to **Prashanth *et al.* (2002)**, acetyl groups of chitin cannot be removed in the presence of alkali without deterioration of polysaccharide chains, leading to depolymerization, due to the high temperature of the reagent and reaction times required for complete deacetylation.

***$Y= - 30.7 + 5.88 x – 0.058x^2$* [Eq. 8]**

According to the obtained results the DDA of chitin affected by all independent variables. On the other hand, the polynomial quadratic regression does not clearances the relationship between DDA as the dependent variable and everyone from four

independent variables. Therefore, it could study the interaction relationship between DDA and two independent variables. The obtained results closed with **Weska *et al.*, (2007)**, they showed the nonlinearity of time with the DDA because it is related to higher deacetylation degrees (**Chen and Hwa, 1996**) and depolymerization.

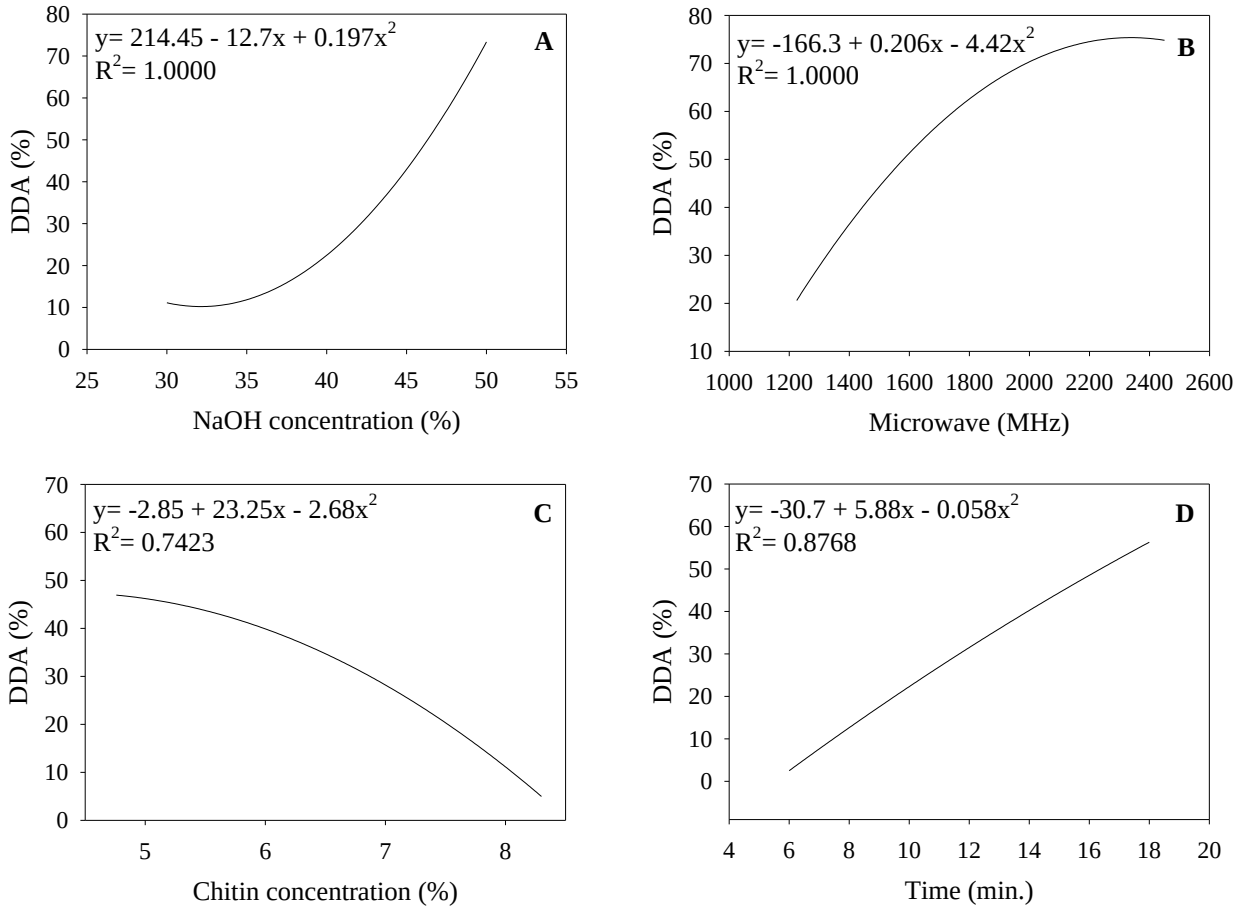

Figure 1. Polynomial trend of NaOH concentration (A), Microwaves power (B), Chitin concentration (C) and time (D) on the degree of deacytelation (DDA) of chitosan.

2. Three dimension response surface study

2.1. Effect of deacetylation time and NaOH Concentration on DDA%:

The three-dimension response surface plot in **Figure,2** is explaining the relationships between the DDA% of produced chitosan and both NaOH concentrations and deacetylation times at 6.66% chitin concentration and 1715 MHz microwave power. DDA was increased with increasing both NaOH concentration from 30 to 50 % and the reaction time from 6 to 18 min. Response surface analysis showed significant (p<0.05) relationships between both NaOH concentration and deacetylation time as independent variables and DDA as a response variable. The predicted Lorentzian model (Eq. 9) had a correlation coefficient (R^2=0.7293).

$$DDA = \frac{272.11}{[1+\frac{(x+72.67)^2}{15.39}]*[1+\frac{(y+16.17)^2}{7.95}]} \quad \text{[Eq. 9]}$$

From the three-dimension plot, we can notice that increasing NaOH concentration had a higher effect on DDA values compared to deacetylation time. The DDA had the same trend with increasing deacetylation time from 6 to 18 min. On contrary, the DDA decreased by increasing the time more than 16 min. The optimal predicted NaOH concentration and time were 50 % and 15.7 min., respectively. The highest DDA value at the optimal studied conditions was 85.6 %. **Sahu *et al*., (2009)** synthesized chitosan by deacetylating chitin under microwave irradiation. They reported that the degree of deacetylation increased with increasing irradiation time. A DDA of 85.3% was achieved after irradiating chitin with a 45% NaOH solution in a microwave for 5.5 min at 900-watt power.

The DDA of chitosan increased with an increase of NaOH concentration, which is related to adding alkaline concentration from 30 to 50%, reduced the content of the free amino group of the chitosan biopolymer but below 50% of NaOH concentration redound inhibiting the deacetylation reaction. Deacetylation of chitosan did not occur at low time and temperature because the acetyl groups cannot be separated at ambient condition, the reaction needs high temperature and time to achieve suitable deacetylation, but if these factors overcome a certain level, these will have a negative effect on DD of chitosan.

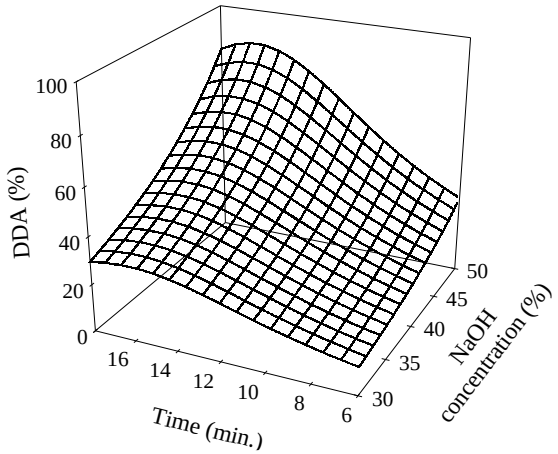

Figure 2: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against time (min.), NaOH concentration (%) as independent variables.

2.2. Effect of deacetylation time and microwave power on DDA%:

The relationship between microwave power and deacetylation time on the DDA of produced chitosan at NaOH and chitin concentrations 40 and 6.66%, respectively was studied. The output data from the response surface method analysis presented in Figure, 3. The DDA was increased with increasing both microwave power and deacetylation time. Output data of response surface study showed significant (p<0.05) relationships between both independent variables. On the contrary, at the time more than 16 min. the DDA values were decreased with increasing the time treatment at all used microwave power values. At the same time, microwave power not had a positive effect on the DDA. At more than 2143.7 MHz the

microwave power had a negative effect on the DDA. The predictive equation (Eq. 10) shows the effect of time (x) and microwave power (y) on DDA with a correlation coefficient of 0.8257.

$$DDA = \frac{88.17}{[1+\frac{(x+15.60)^2}{3.41}]*[1+\frac{(y+2176.8)^2}{756.03}]} \quad \text{[Eq. 10]}$$

The highest observed predictive DDA was 87.94 % at 15.7 min. 2143.7 MHz as deacetylation time and microwave power, respectively. **Alishahi *et al*., (2011) and Al-Sagheer *et al*., (2009)** enhanced chitosan extraction from shrimp wastes by using microwave irradiation at different times and microwave power compared to autoclave treatment and they reported that longer heating times lead usually to a higher percentage of DDA.

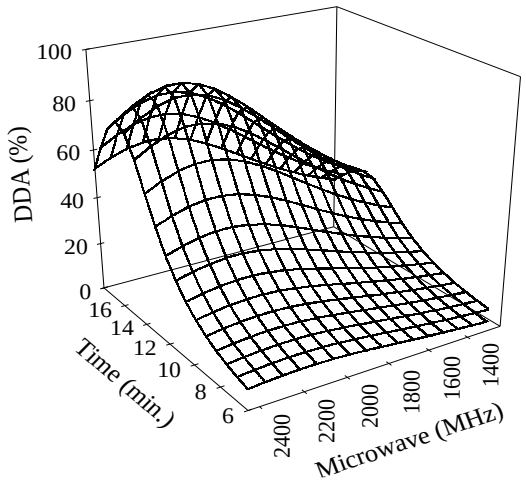

Figure 3: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against time (min.) microwave power (MHz) as independent variables.

2.3. Effect of deacetylation time and chitin concentration on DDA%:

Three dimension response surface for the predicted degree of deacetylation (%) as the dependent variable against time (min.) and chitin concentration as independent variables presented in Figure 4. The reaction time and concentrations of chitin ranged between 6-18 min. and 4.76-8.3 %, respectively. The DDA increased with increasing reaction time. In contrarily, increased chitin concentration lead to obstruction of the acetylation process. According to equation 11, the predicted highest DDA was 59.8 %.

$$DDA = \frac{59.81}{[1+\frac{(x+6.04)^2}{2.29}]*[1+\frac{(y+14.92)^2}{3.94}]} \quad \text{[Eq. 11]}$$

It can be obtained this degree at reaction time 15 min. and chitin concentration 6.08 %.

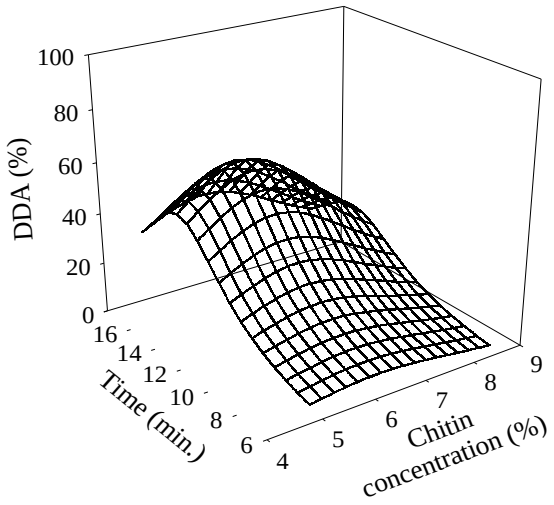

Figure 4: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against time (min.) chitin concentration (%) as independent variables.

2.4. Effect of chitin and NaOH concentrations on DDA%:

The plot in Figure 5 shows the response surface of DDA as observed in the effects of chitin and NaOH at different concentrations. The predicted model (Eq.12) had a suitable determination coefficient (R^2=0.7602). From output data, it could be noticed that the best predicted DDA for that equation was 76.63%.

$$DDA = \frac{77.12}{[1+\frac{(x+4.03)^2}{12.09}]*[1+\frac{(y+9.54)^2}{8.50}]} \quad \text{[Eq. 12]}$$

The response surface showed high determination ($P<0.05$) with combined between the chitin and NaOH at different concentrations. The maximum predicted DDA was obtained at 4.76 % chitin and 50 % NaOH.

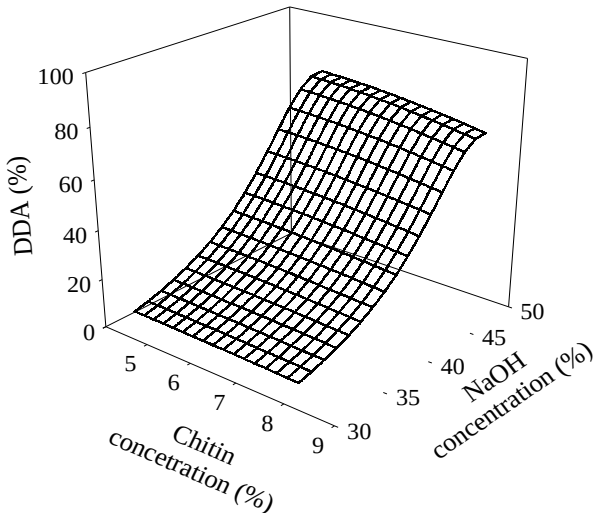

Figure 5: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against chitin concentration (%) and NaOH concentration (%) as independent variables.

2.5. Effect of chitin concentrations and microwave power on DDA%:

The maximum DDA was estimated from a three-dimension response surface plot of different chitin concentrations versus microwave power as shown in figure 6. The maximum DDA was 99.18 % at predicted chitin concentration 5.42 % and microwave power 2220.3 MH. Equation 13 was used to plot the DDA data as dependent variable agents chitin concentrations microwave power as independent variables with R^2= 0.9636 as follows:

$$DDA = \frac{99.48}{[1+\frac{(x+5.40)^2}{1.31}]*[1+\frac{(y+2192.88)^2}{513.77}]}$$ [Eq. 13]

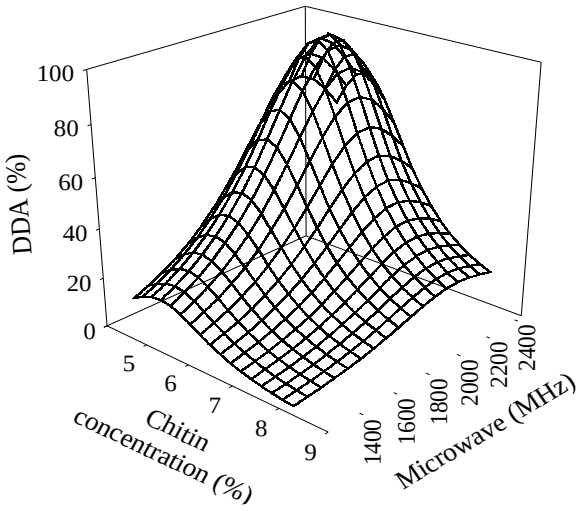

Figure 6: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against chitin concentration (%) and microwave power (MHz) as independent variables.

2.6. Effect of microwave power and NaOH concentrations on DDA%:

The best estimated DDA value for that combination between different microwave power and NaOH concentrations was 98.99 %. The quaternary model (Eq. 14) showed the best correlation between microwave power and NaOH concentration to obtain the high DDA with R^2=0.8106 as follows:

$$DDA = \frac{819.4}{[1+\frac{(x+64.94)^2}{6.17}]*[1+\frac{(y+3672.11)^2}{2843.2}]}$$ [Eq. 14]

Figures 2 to 7 helped to obtain the optimum of each two factors from NaOH concentration, Microwave power, chitin concentration, and deacetylation time with the highest DDA of chitin. These data were extracted from the output data that responded by three-dimension response surface study. The DDA of chitosan increased by simultaneously increasing NaOH concentration and power of microwave **Nouri *et al.*, (2016)**.

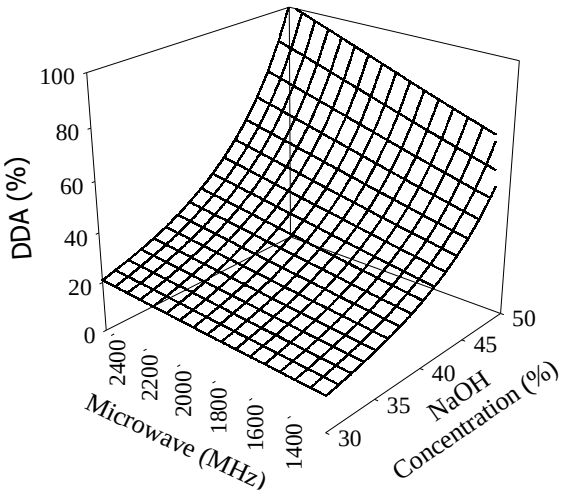

Figure 7: Three dimension response surface for predicted degree of deacetylation (%) as dependent variable against microwave power (MHz) and NaOH concentration (%)as independent variables.

3. Regression coefficients of the predicted cubic polynomial model for a response of the DDA

The previous three-dimension response surface studies for all independent variables (NaOH concentration, Microwave power, chitin concentration, and deacetylation time) appeared significant (P≤0.05) effects on the DDA as the dependent variable with high regression coefficients. It must be noticed that practically the combination between independent variables is necessary for enhancing the DDA. It needs more study on the effect of the interaction between the four independent variables on the DDA. Therefore, the regression coefficient response of different variables on the DDA parameter must be established. Multiple regression coefficients were presented in (Table 1) to predict a cubic polynomial model to optimizing the different independent variables. The model was tested for adequacy by analysis of variance. The regression model for data was highly significant (P≤0.05) with r^2=0.8089. The predicted model for the percentage of DDA (Y) was reported as follows:

$$DDA(\%) = 819.08 - 44.32T - 0.00437P - 208.36C - 15.62N + 2.36T2 + 0.000001P^2 + 32.56C^2 + 0.207N^2 - 0.052T^3 - 1.718C^3 + 0.0037TP + 0.9516TC + 0.365TN - 0.000038PCN - 3.015 * 10^{-8}T^2P^2 - O.OO17T^2C^2 - 0.0002T^2N^2$$

[Eq. 15]

Table 1: Coefficients of predicted Lorentzian model for response of degree deacytelation in chitosan.

Independent variables	Coefficients					R^2
	x_o	y_o	*a*	*b*	*c*	
x= NaOH concentration y=time	72.67	16.17	272.1	15.39	7.95	0.7273
x= time y= Microwave (MHz)	15.60	2176.8	88.17	3.41	756.0	0.8257
x= chitin concentration y=time	6.04	14.92	59.81	2.29	3.94	0.6731
x= chitin concentration y= Microwave (MHz)	5.40	2192.8	99.48	1.31	513.7	0.9636
x= NaOH concentration y= Microwave (MHz)	64.94	3672.1	819.4	6.17	2843.2	0.8106
x= chitin concentration y=NaOH concentration	4.03	49.54	77.12	12.09	8.50	0.7602

R^2 = correlation coefficient

Consequently, the obtained predicted model is possible to identify the optimum conditions required to produce a high percent of DDA from chitin. It could be concluded that the highest predicted DDA was 90.2 % at estimated different independent variables were 50 % NaOH, 2400 MHz, 4.76% chitin, and 13 min. The obtained verified DDA value was 92.8 % with an application of the previous model parameters to produce chitosan.

In many instances, controlled microwave heating under sealed-vessel conditions has been shown to dramatically reduce reaction times, increase product yields and enhance product purities by reducing unwanted side reactions compared to the conventional synthetic method (**Kappe 2004 and DelaHoz *et al*., 2005**). **El Knidri *et al*., (2016)**46 reported that chitosan with a degree of deacetylation of 82.73% was successfully prepared in 24 min via microwave irradiation method, while a much longer time of 6-7 hr was needed for preparing chitosan with the same DDA (81.5%) using a conventional heating method. Microwave radiation (2450 MHz) does not activate specific bonds in molecules and consequently, this form of heating will not lead to any kinetic differences compared to other forms of heating (**Caddick, 1995**).

CONCLUSION

Chitosan was prepared by deacetylation of chitin extracted from shrimp wastes using alkali aqueous solution under microwave radiation. The optimum reaction conditions were as follows: NaOH conc. 50%; microwave power 2400 MHz; chitin conc.

4.76% and deacetylation time, 13 min. Chitosan was obtained from the deacetylation of shrimp wastes chitin under alkaline conditions by using a microwave technique in only 1/50 of the treatment time of the conventional method. The degree of substitution of deacetylation exceeded 90% and thus this is a valuable preparative method.

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