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Harnessing Marine Byproducts and Optimization of Biopolymer Extraction Quality Using Response Surface Methodology and Study of Its Physicochemical Properties

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Abstract

Chitosan, a versatile biopolymer derived from chitin, holds immense potential across various industries owing to its antimicrobial, antioxidant, and biocompatible properties. This study aims to optimize the deacetylation process of chitin, sourced from shrimp shells, using Response Surface Methodology (RSM) to produce high-quality chitosan. The Box-Behnken Design (BBD) was employed to evaluate the effects of temperature, time, and alkali concentration on the degree of deacetylation (DD%), a key determinant of chitosan's functional properties. Seventeen experimental runs were conducted, and the data were analyzed using a quadratic model validated through ANOVA. The results revealed that time and alkali concentration significantly influenced DD%, with a notable interaction between these factors. Optimal conditions for deacetylation yielded chitosan with a DD% above 85%, ensuring enhanced solubility. The solubility ranged from 86% to 96%, strongly correlating with DD%. Additionally, the produced chitosan exhibited moisture content below 10% and ash content under 1%, meeting industrial quality standards. Residual analysis confirmed the model's reliability, and 3D response surface plots illustrated the synergistic effects of the variables. This research highlights the effective use of RSM to optimize chitosan extraction, promoting the sustainable utilization of shrimp shell waste from seafood industries. The high-quality chitosan produced is suitable for applications in biomedicine, agriculture, and water treatment. This study underscores the importance of precise parameter control in enhancing chitosan's properties and sets the groundwork for future exploration of alternative sources and tailored deacetylation processes.

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INTRODUCTION

Chitosan is a linear polysaccharide composed of glucosamine and N-acetylglucosamine units in a partially distributed way [1]. The units are connected by β-(1 → 4) glycosidic linkages. They are derived from chitin by a process called deacetylation. Chitin is the natural polymer composed of repeated units of N-acetyl glucosamine, which are linked by β-(1 → 4) glycosidic bonds [2]. They are predominantly sourced from insects and the exoskeletons of crustaceans, including shrimp, crab, lobster, and krill. Other sustainable sources of chitosan include

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the cell walls of fungi. Due to the abundance of seafood processing industries around the world and to utilize the increasing waste, consumption of marine waste as a source can be a promising option. For their rigid and tough structure, chitin is insoluble in water, restricting them with the fewest applications [3]. Hence, Chitin is modified to chitosan either through chemical method or by enzymatic process. Industrially, chemical processes are carried out for the precise degree of deacetylation and time efficiency. Alkaline hydrolysis is the most common method for the deacetylation of chitin, where the acetyl groups (-$COCH_3$) attached to the N-acetyl glucosamine units of chitin are hydrolysed and replaced with hydrogen atoms, forming amino groups (-NH_2) [4].

The process of deacetylation plays a major role in showcasing the properties of the end product, chitosan. Even a slight modification tends to vary the degree of deacetylation, the acetyl group distribution, the viscosity, and its molecular weight, which in turn influences solubility, antimicrobial property, and other properties. Commercially available chitosan will have a degree of deacetylation ranging from around 70% to 95% and 50 to 2000 kDa of molecular weight [5]. For their abundant properties such as antimicrobial, anti-cancerous, anti-inflammatory, anti-inflammatory, anti-oxidant, anti-cholesterolemic [6], it has been a notable biopolymer in research as well as in various fields such as biomedical and pharmaceuticals, agriculture, and plant protection, in food industries, for water treatment, cosmetics and personal care, textiles, bioplastics, and materials, and also in bioremediation. Even though they are rich in various properties and applications, the quality of chitosan mainly affects the success of its application [7]. The degree of deacetylation and the chitosan's molecular weight are the primary factors influencing this.

Even though there are numerous ways to determine the degree of deacetylation of the chitosan, the simplest, pocket-friendly, and most efficient method is potentiometric titration for degree of deacetylation and Viscometry for molecular weight analysis. Mark-Houwink-Sakurada's empirical equation can be used to determine the viscosity of chitosan by relating its intrinsic viscosity to the polymer's molecular weight [8]. The aim of the current study is to optimize the deacetylation process using response surface methodology for providing a better-quality chitosan for various industrial purposes.

MATERIALS AND METHODS

Raw Material

The raw material used was shrimp wastes, which were obtained from a seafood processing place located in Vanagaram, Chennai, Tamil Nadu (13.0572°N, 80.1483°E). Approximately 1000 grams of shrimp shells were rinsed and fragmented into smaller pieces with a meat tenderizer, thereafter air-dried for 3 hours. Air-dried samples were placed in a hot air oven maintained at 65°C for four consecutive days to get desiccated material. The dry weights of the samples were ascertained prior to the extraction of chitin and preparation of chitosan (Figure 1)

Figure 1. Segregated and processed shrimp shells.

Obtainment of Chitin

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- *Deproteinization:* The dried shrimp shell waste was treated with a 4% (w/v) NaOH solution at 45ºC for 24 hours to eliminate protein. The alkali-insoluble fraction was separated by centrifugation at 4000 RPM for 15 minutes. It was thereafter rinsed multiple times with distilled water until the pH reached neutrality.
- *Demineralization:* To eliminate minerals, deproteinized shells were subjected to a 4% (v/v) HCl solution at 30ºC for 12 hours. Centrifugation at 4000 rpm for 15 minutes to isolate the acid-insoluble fraction. The isolated section was rinsed with distilled water until devoid of acid, then it was subjected to overnight drying at 40°C to produce chitin. Given that the extracted chitin has a little pink hue, the decolorization process was conducted prior to the synthesis of chitosan.
- *Decolourization:* The procedure involved immersing the chitin in 1% potassium permanganate for 30 minutes, followed by treatment with 1% oxalic acid for 30 minutes to 2 hours until complete decolorization occurred. The resulting product can be classified as pure chitin derived from shrimp shells [9].

Extraction of Chitosan

- *Optimization of deacetylation using Response Surface Methodology (RSM):* The deacetylation optimization of chitin was conducted via Response Surface Methodology (RSM). The experimental design and data analysis were performed with Design-Expert software (Stat-Ease Inc., Minneapolis, MN, USA). A three-factor, three-level Box-Behnken design (BBD) was utilized to examine the impacts of temperature (A), time (B), and alkali concentration (C) on the degree of deacetylation (DD%). Seventeen trials were undertaken, comprising five central points to assess experimental error.
- *Chitosan Purification:* Chitosan samples were purified by dissolving in a 1% solution of glacial acetic acid. The solutions were further centrifuged at 6000 RPM for 30 minutes to eliminate insoluble material. The complete precipitation of chitosan can be attained by adding sodium hydroxide till reaching a pH of 12.5, followed by neutralization. The chitosan suspension was centrifuged to separate the supernatant, which was subsequently preserved for future use.
- *Determination of degree of deacetylation:* The extent of deacetylation was assessed using the acid-base titration method, with minor changes to the approach of Domard, A. et al., 1983. In summary, 0.1 g of chitosan was solubilized in 30 ml of a 0.1 mol/l aqueous HCl solution at ambient temperature, with the addition of 5–6 drops of methyl orange. The red chitosan solution was titrated with 0.1 mol/L NaOH solution until it became orange. The degree of deacetylation was determined using the formula:

$$DD\% = \frac{C_1V_1 - C_2V_2}{M \times 0.0994} \times 0.016$$

Where, C1 = concentration of standard HCl aqueous solution (mol/l),
C2 = standard NaOH solution (mol/l),
V1 = volume of the standard HCl aqueous solution used to dissolve chitosan (ml),
V2 = volume of standard NaOH solution consumed during titration (ml), and
M = weight of chitosan (g).

The value 0.016 represents the equivalent weight of the NH_2 group in 1 ml of a typical 1mol/l HCl aqueous solution, while 0.0994 is the weight fraction of the NH_2 group in chitosan.

- *Statistical Analysis:* The experimental data were analyzed using ANOVA to assess the relevance of the model and its component terms. The quadratic model was chosen based on the fit statistics and the results of the lack of fit tests. The model was used to generate response surface plots and to determine the optimal conditions for maximum degree of deacetylation.

Physio-chemical properties:

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- *Solubility:* From the deacetylation process, 100 ml of 1% acetic acid solution was used to dissolve 1g of chitosan and homogenized using a magnetic stirrer. The acidic chitosan solution was then filtered using a vacuum pump. The process was repeated thrice. The percentage of the solubility was determined by [10]:

$$Insoluble\ (g) = final\ weight\ of\ filter\ paper\ (g) - initial\ weight\ of\ filter\ paper\ (g)$$

$$\text{Insoluble (\%)} = \frac{\text{Insoluble(g)}}{\text{Sample weight(g}}$$

$$Solubility\ (\%) = 100 - \%\ insoluble$$

- *Moisture and ash content:* These properties were measured according to the standards. Moisture content was determined by the gravimetric method [11] and ash content by the standard method [12].

RESULTS AND DISCUSSION

The results of this study highlight the successful extraction of chitin from shrimp shells and the optimization of chitosan production. The deproteinization step effectively removed proteins from the shrimp shells, as indicated by the absence of foaming during the NaOH treatment and the consistent pH neutrality after multiple washes. This result shows that the protein removal was complete and that the NaOH solution concentration and treatment time (24 hours) were sufficient for thorough deproteinization. Comparatively, using a 4% NaOH concentration aligns with previous studies [13] but could be optimized further by evaluating shorter treatment times or alternative temperatures, potentially reducing energy input. The success of the demineralization step was evident through the complete dissolution of mineral components, as indicated by the absence of residue after rinsing. This was further confirmed by the acid-neutralized pH of the final product. The use of 4% HCl for 12 hours at 30°C successfully removed minerals such as calcium carbonate, which is essential for enhancing the purity of the chitin. Decolorization was completed using potassium permanganate and oxalic acid, with the pink hue of the chitin effectively removed after treatment. The final product was a visually pure white chitin, indicating successful pigment removal. This step is critical in removing residual pigments that can affect the quality of the subsequent chitosan, particularly in high-purity applications like pharmaceuticals and biomedicine.

Table 1. The 3-Factor BBD Matrix of the Experimental Data.

Sample	Temperature	Time	Alkali concentration
1	1	1	0
2	0	-1	-1
3	0	1	1
4	1	0	-1
5	1	0	1
6	0	0	0
7	0	1	-1
8	0	-1	1
9	0	0	0
10	1	-1	0
11	-1	0	1
12	-1	-1	0
13	-1	1	0
14	-1	0	-1
15	0	0	0
16	0	0	0

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17	0	0	0

Our results have given successful indications for the optimization of chitosan development using Box-Behnken Design (BBD) software. The experimental investigation aimed to optimize the degree of deacetylation (DD%) of chitin using a factorial design approach with temperature (A), time (B), and alkali concentration (C) as influential independent process factors. The study employed a systematic approach to evaluate how variations in these parameters affect the degree of deacetylation, a crucial property influencing the functional properties of chitin in various applications through the BBD approach. The factorial design involved 17 experimental runs (Table 1), systematically varying the levels of temperature, time, and alkali concentration to observe their individual and interactive effects on DD%. The analysis showed that time (B) and alkali concentration (C) had the most significant impact on the DD%, while temperature (A) had a lesser effect. The optimal conditions derived from the RSM model yielded a high DD% of around 85–95%, which is comparable to or exceeds the deacetylation efficiency reported in previous studies. Higher DD% indicates improved solubility and bioactivity of the chitosan.

For analysis, each combination of factors was carefully selected to cover a broad operational range while ensuring sufficient replication to capture variability in the response variable. The primary statistical analysis utilized Analysis of Variance (ANOVA) to assess the significance of each factor and their interactions on the degree of deacetylation. The results from the ANOVA (Table 2) highlighted several key findings: the overall model was found to be significant. The quadratic model demonstrated a highly significant fit ($p < 0.0001$), indicating that it effectively captured the variability in DD% explained by the factors A, B, C and their interactions. Time (B) and alkali concentration (C) showed noteworthy positive effects on DD%. Longer durations (B) and higher concentrations of alkali (C) led to increased deacetylation, suggesting that these factors play crucial roles in enhancing the degree of deacetylation. The interaction between time (B) and alkali concentration (C) (BC) was also significant ($p = 0.0258$), indicating that the combined effect of these factors influences DD% beyond their individual impacts. This interaction phrase indicates a synergistic link, wherein the influence of one component is contingent upon the level of the other. Quadratic terms (A^2, B^2, C^2) were significant ($p < 0.0001$), implying that the relationship between temperature, time, alkali concentration, and DD% is non-linear. This suggests that optimal conditions for maximizing deacetylation may not simply involve maximizing or minimizing individual factors but rather finding a balance that optimizes the quadratic response surface.

Table 2. Analysis Of Variance (ANOVA) of the Response Surface Quadratic Model for Degree of Deacetylation

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	575.25	9	63.92	32.58	< 0.0001
A-Temperature	0.1513	1	0.1513	0.0771	0.7893
B-Time	163.81	1	163.81	83.50	< 0.0001
C-Alkali concentration	70.21	1	70.21	35.79	0.0006
AB	54.02	1	54.02	27.54	0.0012
AC	0.0000	1	0.0000	0.0000	1.0000
BC	15.60	1	15.60	7.95	0.0258
A^2	163.16	1	163.16	83.17	< 0.0001
B^2	3.04	1	3.04	1.55	0.2531
C^2	86.21	1	86.21	43.95	0.0003
Residual	13.73	7	1.96		
Lack of Fit	13.73	3	4.58		
Pure Error	0.0000	4	0.0000		

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Cor Total	588.98	16			

While temperature (A) was not statistically significant in isolation, its quadratic term (A^2, $p < 0.0001$) suggests that temperature could still play an important role under certain conditions. The non-linear effects of temperature indicate that while small changes in temperature may not drastically affect deacetylation, larger deviations from the optimal temperature could have significant consequences, potentially disrupting the balance between time and alkali concentration.

Model Summary and Predictive Power

The model fit statistics further confirm the adequacy of the quadratic model. Adjusted R^2 measure reflects the proportion of variability in the response variable (DD%) explained by the independent variables (A, B, C, AB, AC, BC, A^2, B^2, C^2) adjusted for the number of terms in the model. The adjusted R^2 value of 0.9467 indicates that approximately 94.67% of the variability in the DD% can be explained by the model after accounting for the number of predictors. Predicted R^2 metric estimates how well the model predicts future observations. The predicted R^2 value of 0.6269, although lower than the adjusted R^2, still indicates that the model has a reasonable predictive capability. The disparity between these values suggests that while the model fits the existing data well, its predictive power could be improved, possibly by including more data points or refining the experimental design.

The Adeq precision value of 15.267, which measures the signal-to-noise ratio, is well above the threshold value of 4, indicating a sufficient signal. This reinforces the reliability of the model for navigating the design space and making predictions within the studied range of factors.

Parameter Estimates and Interpretation

Table 3 provides estimates of the coefficients for each factor and their interactions in both coded and actual units. These coefficients quantify the direction and magnitude of each factor's impact on DD%, allowing for a deeper understanding of how changes in temperature, time, and alkali concentration affect the deacetylation process. Temperature (A), although not statistically significant individually, temperature's role in influencing DD% should not be disregarded. It may interact significantly with other factors or play a role outside the tested ranges. Time (B) being the positive coefficient suggests that increasing the duration of the deacetylation process enhances DD%, likely due to more extensive chemical reactions leading to higher deacetylation levels. Similarly, alkali concentration (C), a higher concentration of alkali, contributes positively to DD%, indicating that stronger alkaline conditions facilitate more efficient deacetylation of chitin. The significant interaction terms (AB, AC, BC) indicate that the combined effects of temperature, time, and alkali concentration are not simply additive but rather synergistic or antagonistic, depending on their specific combinations (Table 4).

Table 3. Regression Analysis for the Experimental Data

Std. Dev.	**1.40**	**R^2**	**0.9767**
Mean	80.46	Adjusted R^2	0.9467
C.V. %	1.74	Predicted R^2	0.6269
		Adeq Precision	15.2666

Table 4. Coefficients of the Coded Factor Along With SE

Factor	**Coefficient Estimate**	**df**	**Standard Error**	**95% CI Low**	**95% CI High**	**VIF**
Intercept	75.00	1	0.6264	73.52	76.48	
A-Temperature	-0.1375	1	0.4952	-1.31	1.03	1.0000
B-Time	4.53	1	0.4952	3.35	5.70	1.0000
C-Alkali concentration	2.96	1	0.4952	1.79	4.13	1.0000
AB	-3.68	1	0.7003	-5.33	-2.02	1.0000
AC	0.0000	1	0.7003	-1.66	1.66	1.0000

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BC	1.98	1	0.7003	0.3190	3.63	1.0000
A^2	6.23	1	0.6826	4.61	7.84	1.01
B^2	0.8500	1	0.6826	-0.7641	2.46	1.01
C^2	4.53	1	0.6826	2.91	6.14	1.01

The empirical relationships between tested factor and response are presented by following equation:

Coded Factors

$$Degree\ of\ Deacetylation = 75.00 - 0.1375A + 4.53B + 2.96C - 3.68AB + 0.0000AC + 1.98BC + 6.23A^2 + 0.8500B^2 + 4.53C^2$$

Actual factors

$$Degree\ of\ Deacetylation = 694.52778 - 11.54500(Temperature) + 14.23333(Time) - 2.74611(Alkali\ concentration) - 0.183750(Temperature \times Time) + 2.89293E - 17(Temperature \times Alkali\ concentration) + 0.065833(Time \times Alkali\ concentration) + 0.062250(Temperature^2) + 0.212500(Time^2) + 0.020111(Alkali\ concentration^2)$$

A positive sign in the above equation indicates a synergistic effect of the factors, while a negative sign indicates an antagonistic effect of the factors [14].

Residual Analysis

Examining the residuals helps in validating the model's adequacy. Residuals represent the discrepancies between actual and expected values, and their examination might indicate the fulfilment of model assumptions. The residuals show no obvious pattern, which suggests that the model assumptions of homoscedasticity (constant variance) and normality are satisfied. This signifies that the model's predictions are impartial and dependable within the examined range (Figures 2-3).

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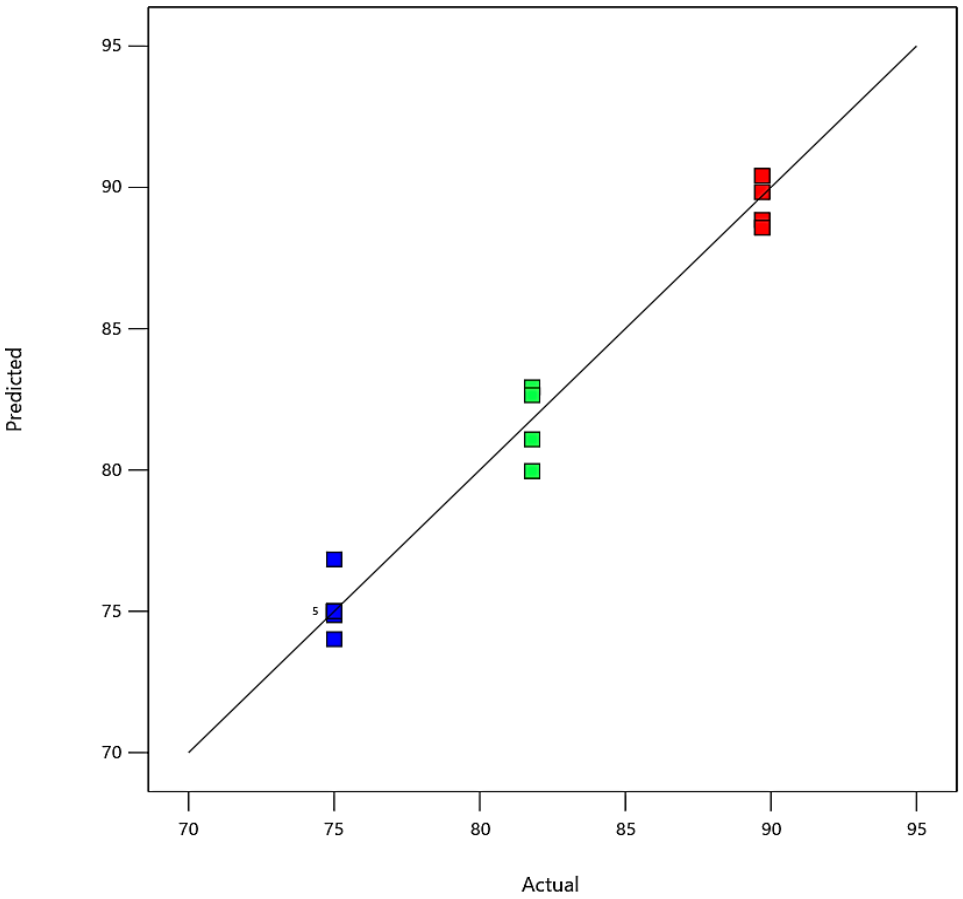

Figure 2. Predicted vs. Actual value of the Experimental Design.

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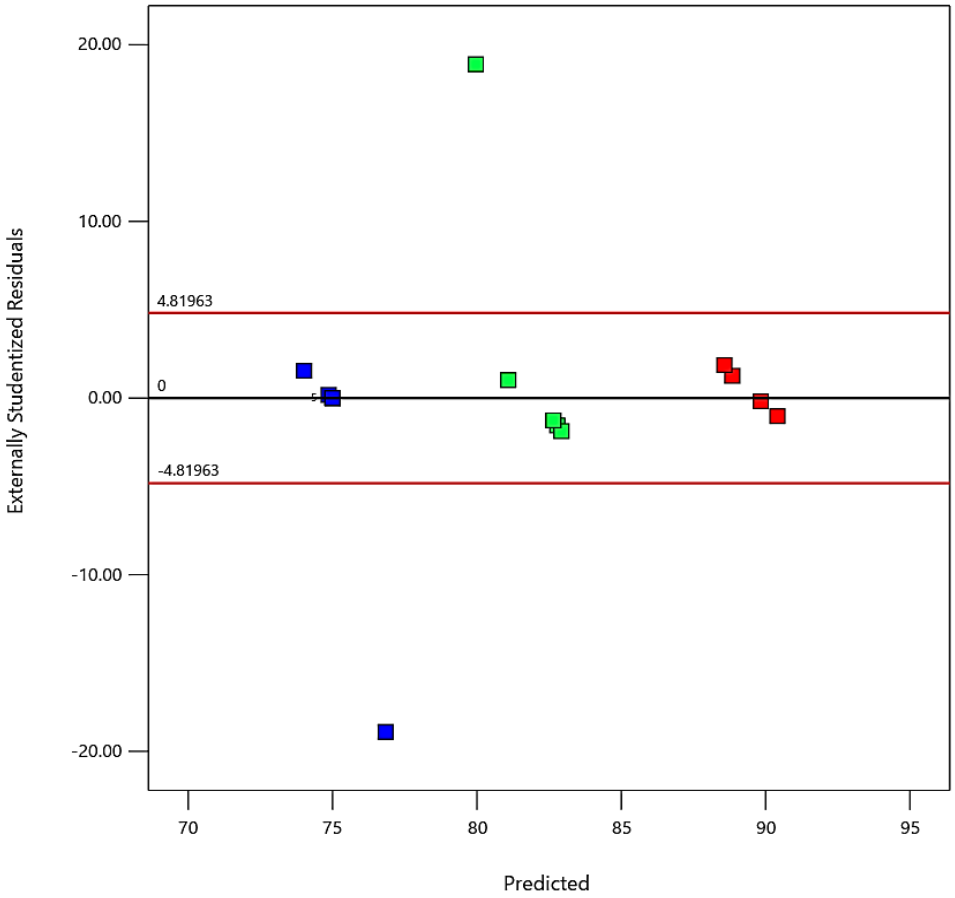

Figure 3. Residual vs. Predicted values of the Experimental Design

The 3D response surface plots for experimental runs with a higher degree of deacetylation presented in Figures 4a to 4d depict the interaction effects of temperature, alkali concentration, and time on the degree of deacetylation (DD%) of chitosan. These interactions are critical in optimizing the efficiency of deacetylation, as various parameters show complex interdependencies. Keeping the alkali concentration constant, the parametric interactions between temperature and time graphs were presented in Figures 4a & 4b for the experimental runs 3 and 5, while temperature is kept as constant, and the interaction between the rest of the two operative parameters like, time and alkali concentration of the runs 11 and 13 is represented in Figures 4c & 4d.

The interaction between temperature and alkali concentration (Figure 4a.1) reveals that increasing temperature significantly enhances DD%, particularly when combined with moderate to high alkali concentrations. This aligns with previous findings, which suggest that higher temperatures accelerate the breakdown of acetyl groups, thus improving deacetylation rates [15]. However, it is observed that beyond a certain alkali concentration, the effect on DD% plateaus. This phenomenon could be due to the saturation of the deacetylation reaction, where excess alkali no longer contributes to further conversion, and could even lead to structural degradation of the chitosan polymer, as noted in earlier studies [16]. Excessive alkali might cause over-degradation or depolymerization, reducing the molecular weight of chitosan [17].

Similarly, Figure 4a.2 highlights the role of time in conjunction with alkali concentration, showing that prolonged reaction times improve DD%, but beyond an optimal point, the benefits diminish. This observation may be linked to the completion of deacetylation, after which prolonged exposure could lead to undesirable side reactions or even degradation of the chitosan chains, as reported in other deacetylation studies [18].

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Figures 4b and 4c further illustrate the combined effects of temperature, time, and alkali concentration. The interaction between temperature and time (Figure 4b.1) demonstrates that while higher temperatures significantly improve DD%, longer reaction times only enhance deacetylation up to a certain extent. Beyond that, extended exposure to high temperatures can negatively impact the molecular integrity of chitosan, causing thermal degradation, which has been corroborated by works on the thermal sensitivity of biopolymers [19]. The synergistic interaction between alkali concentration and temperature (Figure 4b.2) reinforces that while higher alkali concentrations enhance deacetylation at elevated temperatures, an optimal concentration exists, beyond which the effect stabilizes.

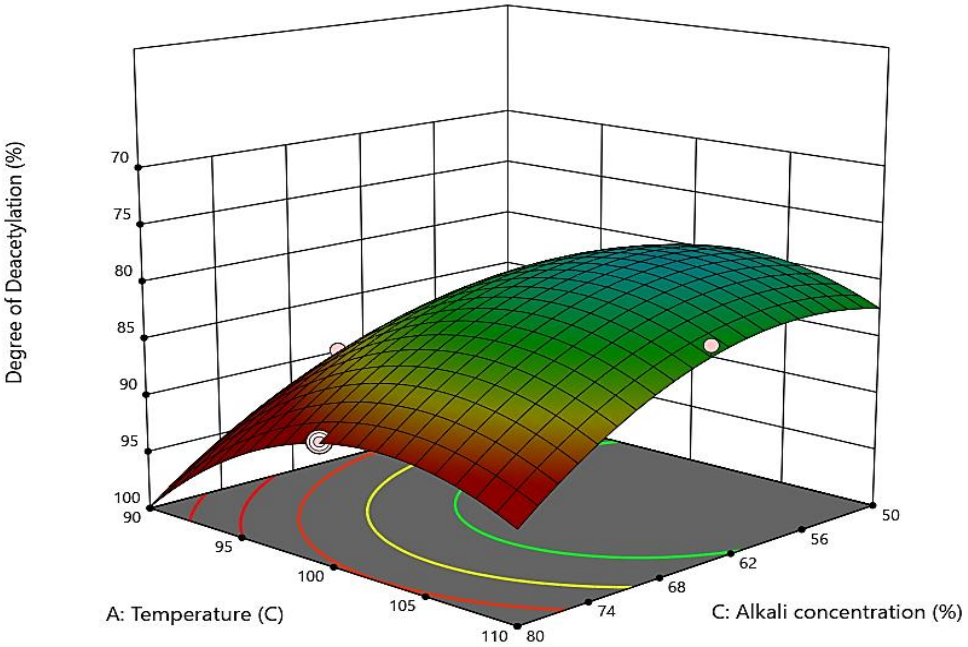

Figure 4a.1. 3D plot of DD% With Interactions Between Temperature and Alkali Concentration.

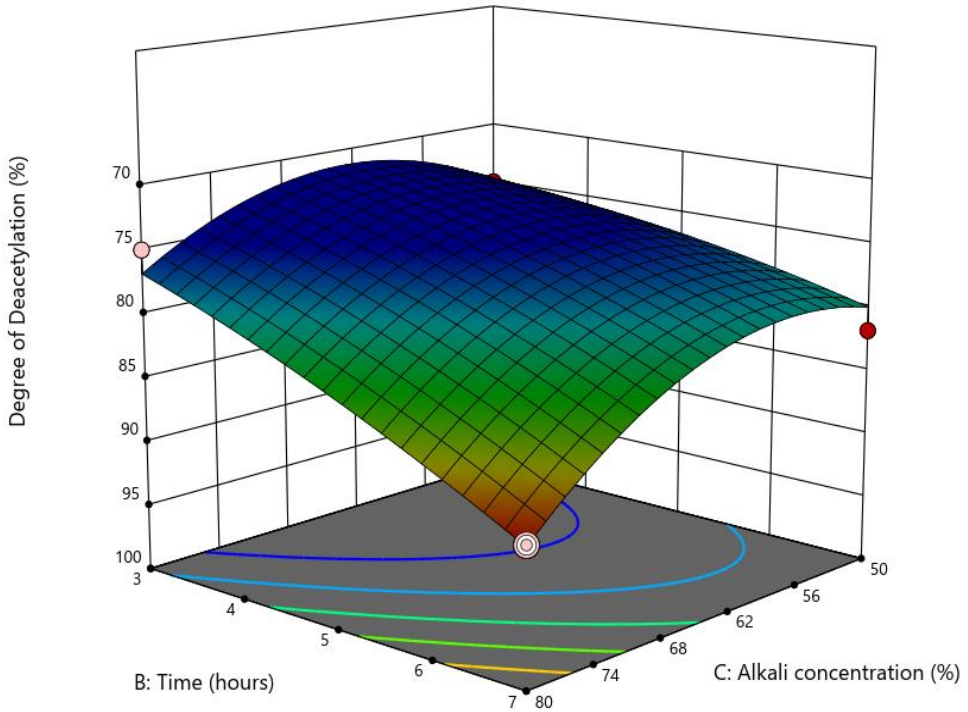

Figure 4a.2. 3D plot of DD% With Interactions Between Time and Alkali Concentration.

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Figures 4d.1 and 4d.2 validate these trends, emphasizing that time and temperature must be balanced to avoid compromising the material's quality. The results show that excessive reaction times or high temperatures can lead to the degradation of the chitosan structure, corroborating earlier studies on the thermal and chemical sensitivity of chitosan under extreme processing conditions [20].

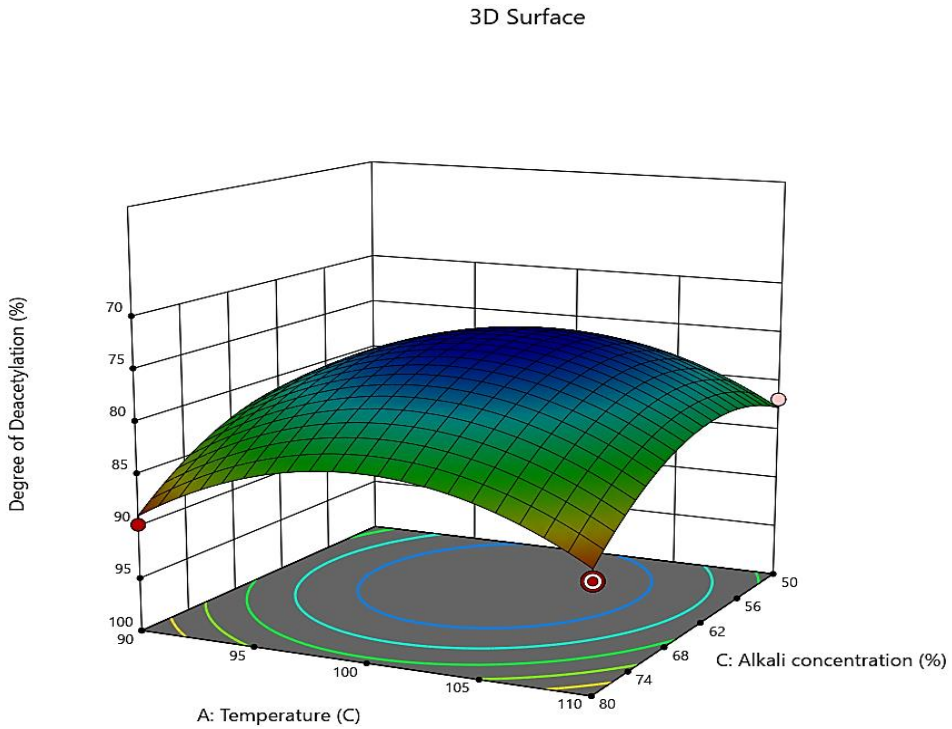

Figure 4b.1. 3D plot of DD% With Interactions Between Temperature and Alkali Concentration.

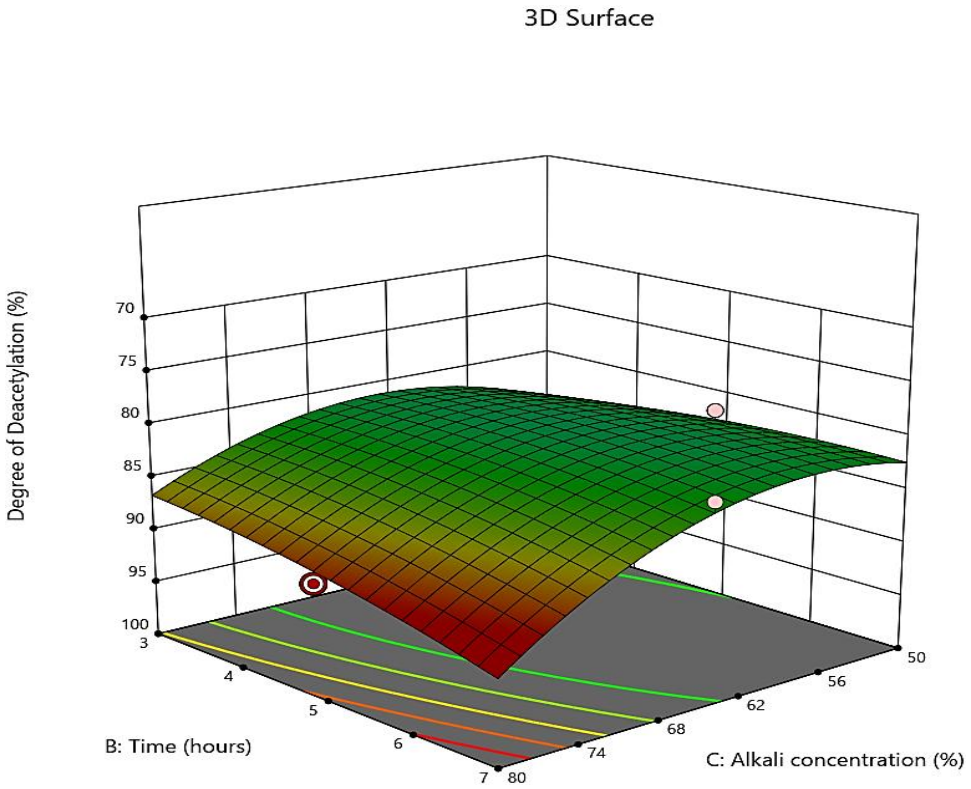

Figure 4b.2. 3D plot of DD% With Interactions Between Time and Alkali Concentration.

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In comparison with previous literature, our findings provide further insight into the optimal conditions for chitosan deacetylation. While it is well established that temperature, time, and alkali concentration are key factors in the deacetylation process [21], this study reveals the precise ranges for these parameters that maximize DD% without compromising the structural integrity of the polymer.

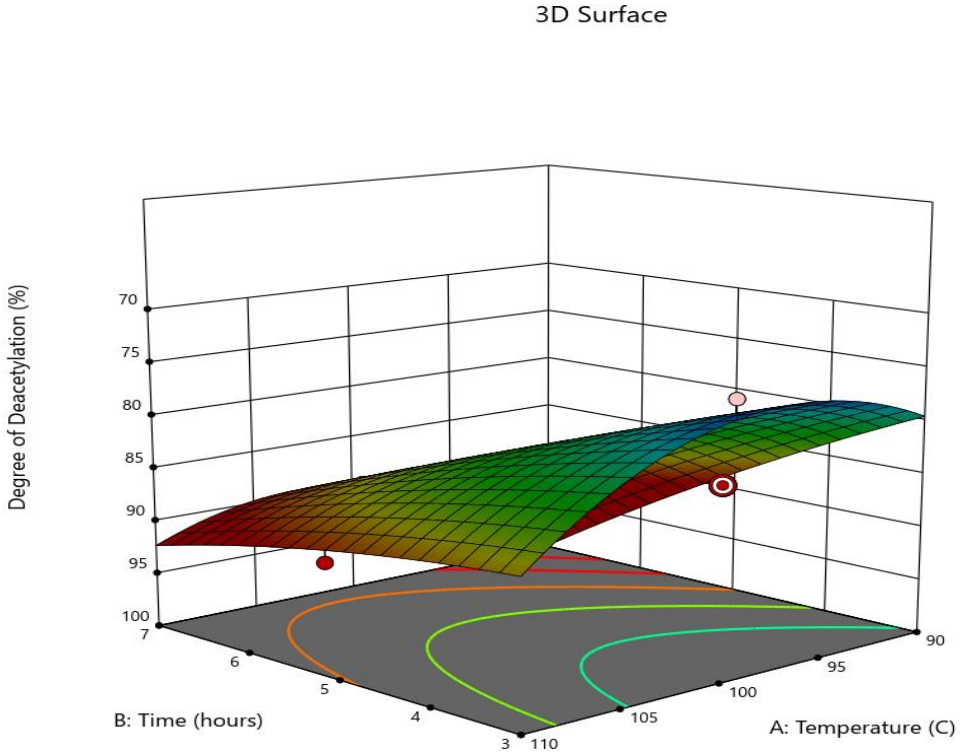

Figure 4c.1. 3D plot of DD% With Interactions Between Temperature and Time.

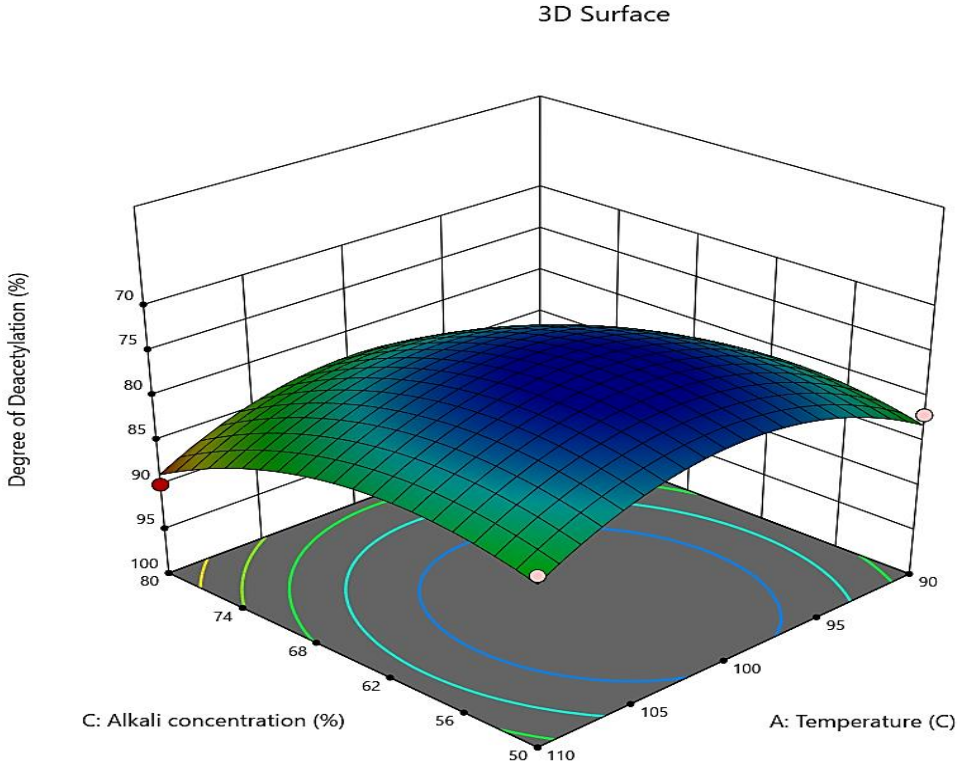

Figure 4c.2. 3D plot of DD% With Interactions Between Temperature and Alkali Concentration.

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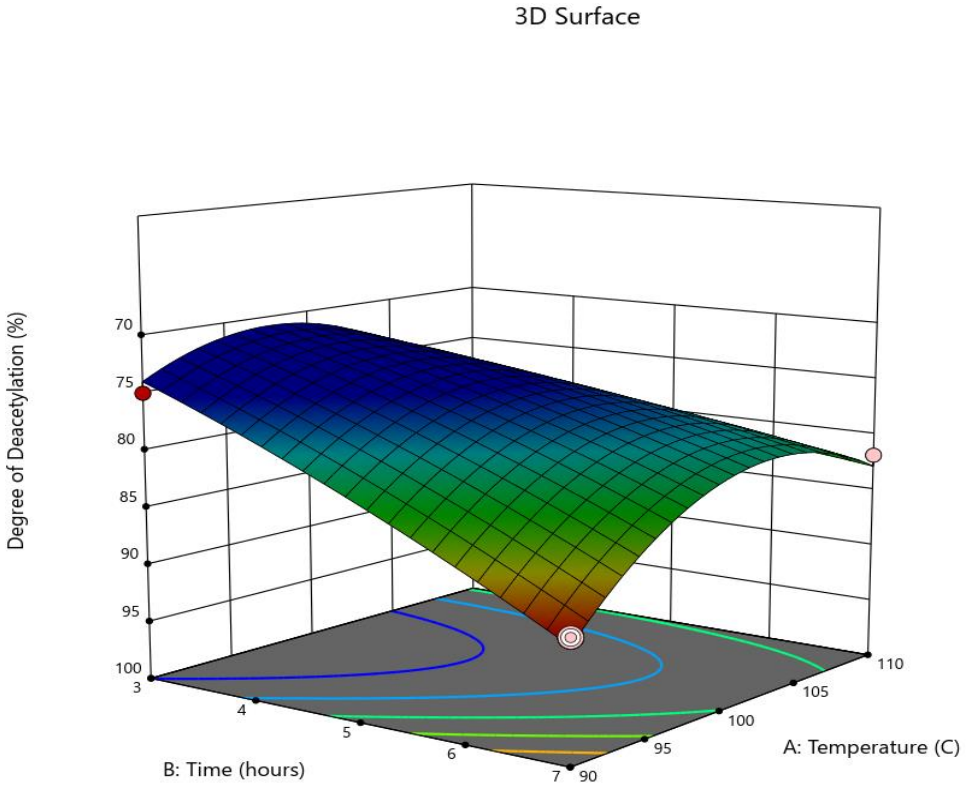

Figure 4d.1. 3D plot of DD% With Interactions Between Temperature and Time.

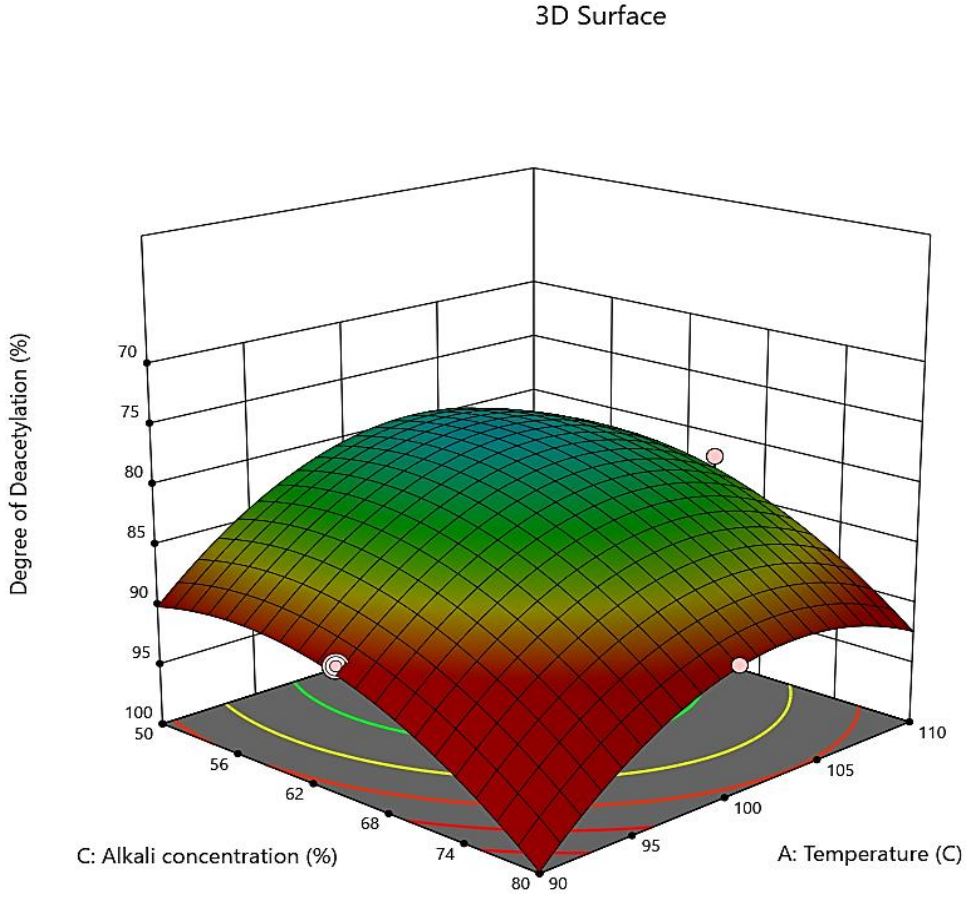

Figure 4d.2. 3D plot of DD% With Interactions Between Temperature and Alkali Concentration.

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Every experiment's yield was computed and graphically displayed (Figure 5). Particles only react when they collide, according to collision theory. Nevertheless, the yield dropped to 43% when the temperature was raised to 110°C. The yields achieved in this study surpassed NO HK's reported yields, which ranged from 43% to 88% [22]. Raising the concentration of NaOH results in an increase in hydroxide ions, which catalyze the processes of not just deacetylation but also depolymerization. When it comes to breaking down the amide link during deacetylation and the glycosidic bond during depolymerization, the hydrolysis reaction is accelerated by the presence of hydrogen ions.

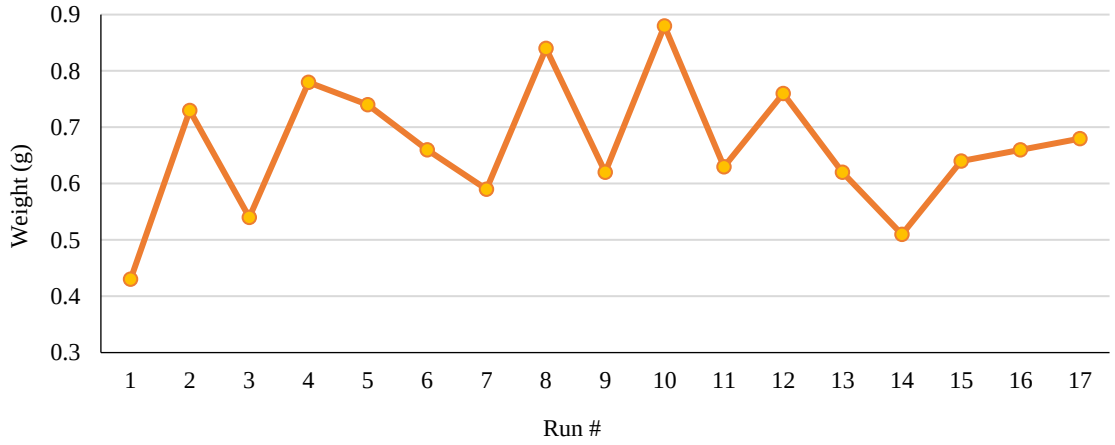

Figure 5. Yield Of Chitosan From 17 Experimental Runs.

One of the key factors affecting chitosan quality is its solubility; a higher solubility will result in a higher-quality chitosan. The temperature and duration of deacetylation, alkali concentration, previous treatments used for chitin isolation, the ratio of chitin to alkali solution, and particle size are all important factors that impact chitosan's solubility. However, the degree of deacetylation mostly determines the solubility; to reach the minimal solubility, the degree of deacetylation must be at least 85% [23]. The results of this investigation showed that the solubility of chitosan varied significantly with respect to NaOH concentration, ranging from 86% to 96% (Figure 6). Lower solubility was seen in deacetylated samples, which is related to a lower DD value. As the degree of deacetylation increased, solubility increased proportionately [24]. determined that the inadequate elimination of protein and acetyl groups is likely the cause of diminished solubility values. The solubility of chitosan is mostly contingent upon the deacetylation of chitin; thus, a lesser degree of deacetylation (DD) may negatively impact the results.

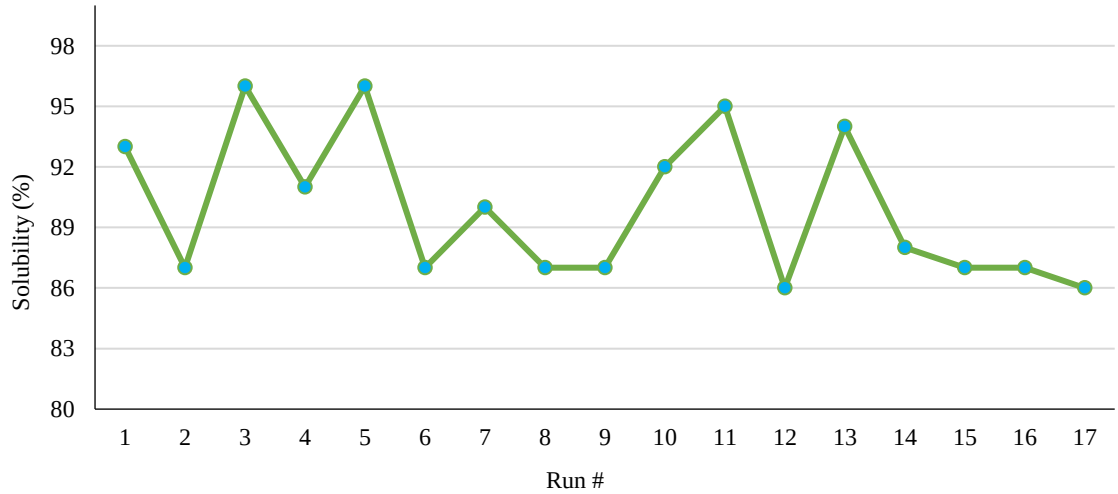

Figure 6. Solubility of Chitosan From 17 Experimental Runs.

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The chitosan should be less than 1% ash-free. This is the right high-quality grade. Some chitosan residue may have an impact on the solubility of the product, which will reduce its viscosity, or it may have an impact on other, more significant features [25]. The shrimp chitosan in the study contained less than 1% ash ranging around 0.3 (±0.1). According to Li, Q. et al., 2020, Commercial chitosan products have a moisture content below 10%. Shrimp shell chitosan samples have a moisture content ranging from 7.5 (±0.02). This is a crucial point since chitosan is hygroscopic by nature [26], which makes it difficult to store for extended periods of time because it absorbs moisture.

CONCLUSION

The findings reveal that both time and alkali concentration significantly influence the deacetylation process, with a notable interaction effect between these parameters. This indicates that precise control over these variables is crucial for producing high-quality chitosan with desirable properties such as enhanced solubility, moisture, and ash. Unlike previous studies that focus on the deacetylation process alone, the novelty of this study ensures the production of chitosan with superior solubility and low ash content, making it more versatile and expanding its potential industrial applications, including biomedicine, agriculture, and water treatment. This research not only contributes to the efficient utilization of marine waste but also enhances the sustainability of the seafood processing industry by providing a valuable biopolymer from otherwise discarded materials. In conclusion, the study successfully demonstrates the potential of using RSM to optimize the deacetylation process, thereby improving chitosan's properties and expanding its industrial applications. Future research could explore the use of alternative sources and further refine the deacetylation parameters to produce chitosan with tailored properties for specific applications. This work sets the stage for broader utilization of chitosan, fostering innovation and sustainability in various fields.

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