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Dedicated to the memory of
Academician Dr. Eng. Emilian BRATU (1904–1991)

OPTIMIZATION OF CHITOSAN EXTRACTION FROM CRUSTACEAN EXOSKELETON WASTES

Cristiana Luminița GÎJIU,[a] Manuela Rossemary APETROAEI,[b] Miruna-Maria APETROAEI,[c] Raluca ISOPESCU,[a,*] Mirela ANTON,[a] Ileana RĂU[a] and Verginica SCHRÖDER[d]

[a] Faculty of Chemical Engineering and Biotechnology, National University of Science and Technology "Politehnica" Bucharest, 1 Ghe. Polizu Str., Bucharest, Roumania
[b] Department of Marine Electric and Electronic Engineering, Faculty of Marine Engineering, Mircea cel Batran Naval Academy, 1 Fulgerului Street, 900218 Constanta, Roumania
[c] Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia Street, 020956 Bucharest, Roumania
[d] Faculty of Pharmacy, "Ovidius" University Constanta, Constanta 900470, Roumania

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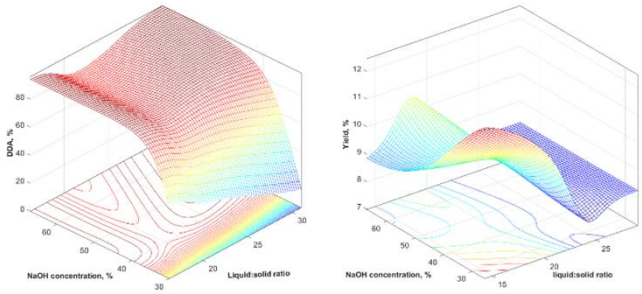
The degree of deacetylation, DDA is the most important characteristic for a specific chitosan sample as it mainly influences its chemical and biological properties. Several natural wastes may be used as sources for chitin as for example crabs' and shrimps' exoskeletons, and the yield of chitosan extraction is generally small and depends on the chitin extraction and the deacetylation step. The simultaneous maximization of yield and deacetylation degree DDA pointed out some optimal operating conditions of the deacetylation procedure. The maximum yield and maximum DDA can be obtained working with higher NaOH concentrations and longer duration of this step.



INTRODUCTION

Chitosan is a natural polysaccharide which due to its low toxicity has many and diverse applications in food biotechnology, cosmetics, pharmaceutics, etc. The main source for chitosan is chitin which by alkaline deacetylation is transformed into chitosan. Several natural wastes may be used as sources for chitin as for example crabs' and shrimps' exoskeleton. In the last years, as shrimp consumption increased, the valorization of their exoskeleton is both interesting for chitosan preparation and as a means of depollution. At present, globally, there is great concern and considerable efforts are being made regarding the seafood industry. Thus, processing crustacean waste from seafood restaurants on the Roumanian coast remains an important step for economic management.[1,2] Crustacean exoskeletons, typically discarded as waste in the seafood industry, are rich in chitin, a natural polymer that by processing

* Corresponding author: raluca.isopescu@upb.ro

produces chitosan. Depending on its main characteristics (DDA and molar mass), this biopolymer has a variety of applications in various industries, including water treatment, biomedicine, agriculture, and food packaging, due to its biocompatibility, biodegradability, and antimicrobial properties.[3,4]

In Roumania, particularly along the Black Sea coast, recognized as an important area for its fishing industry and tourism, valorizing crustacean exoskeletons waste could tackle crucial environmental challenges like waste management and pollution mitigation. Traditionally, this bio-waste is disposed of in landfills or discarded into the sea, contributing to environmental harm and marine pollution. However, transforming these waste exoskeletons into useful chitosan can greatly diminish the amount of organic waste and the problems related to its disposal.

From an economic perspective, the extraction of chitosan from crustacean waste can generate new business prospects and invigorate local economies. The chitosan extraction process serves not only as a method for reducing waste but also as a way to transform a low-value byproduct (chitin) into a high-value commodity (chitosan). This transformation has the potential to open up new markets and enhance the competitiveness of Roumanian seafood enterprises by valorizing their waste flows.

Furthermore, the development of a chitosan industry could increase Romania's scientific and technological capabilities. It would promote research and innovation in green chemistry and sustainable materials science,[5,6] aligning with the worldwide trends towards more eco-friendly industrial methods. It also aligns with the wider goals of the European Union concerning the circular economy and sustainable development, placing Roumania at the forefront of these efforts. Therefore, the processing of crustacean waste into chitosan on the Roumanian coast is a promising initiative that aligns environmental management with economic development and technological progress.

Chitosan offers better properties than the collagen largely used in biological applications.[7,8] Chitosan applications depend on its main properties as degree of deacetylation, mean molar mass, solubility, crystallinity. The degree of deacetylation, DDA is the most important characteristic for a specific chitosan sample as it mainly influences its chemical and biological properties.[9] The degree of deacetylation of chitosan indicates the percentage of β-1,4-D-glucosamine units in the biopolymer. These amine groups result from the conversion of acetamide groups in the polysaccharide's glycosidic ring through a hydrolysis process involving strong alkaline solutions and elevated temperatures. The functional properties of biopolymers, such as solubility, crystallinity, swelling ratio, bioactivity, and biodegradation are imprinted by the degree of deacetylation. Chitosan can be categorized according to its degree of deacetylation (DDA) as follows: high deacetylated chitosan (HDD) with 70–99%, low deacetylated chitosan (LDD) with 55–70%.[10] Some of the classical applications are favored by high deacetylation degree: drug delivery,[11] food preservation,[12] textiles finishing.[13] The process of deacetylation practically means the removal of the acetyl group from the pyranose ring conformation and replacing them with reactive amino groups ($-NH_2$) that ensure the solubility of chitosan by making it able to be protonated in an acidic environment.[13]

As crustacean exoskeletons are mainly composed of chitin, proteins, minerals and pigments,[14] the extraction and purification of chitin from crustacean's wastes is a laborious procedure. The main two steps are the deproteinization by alkaline treatment with NaOH solution and demineralization with HCl solution. The yield of obtaining chitosan from such raw material is generally low. Some alternative solutions using enzymatic hydrolysis, microbial fermentation are suggested by recent research.[15] The optimization of working conditions in chitosan extraction from natural sources aims to obtain good yields and desired properties of chitosan. Process optimization studies for chitosan extraction generally apply statistical modelling based on programmed experiments[16,17] as a multitude of factors is implied in the extraction of chitosan. The present paper is a continuation of some previous studies[10,18–20] referring to the optimization of chitosan extraction from various marine type wastes from the Roumanian Black Sea cost released seasonal on the beaches or after storm conditions. The optimization of the yield and deacetylation degree was carried out based on a complex experiment consisting in chitin extraction from crustacean exoskeleton and its deacetylation to chitosan. The aim of the present study consisted of optimizing the deacetylation stage in producing chitosan with high DDA and high yields from shrimp wastes using artificial neural networks (ANN) and genetic algorithms, having NaOH solutions concentration, solid/liquid ratio, and reaction time as variables.

RESULTS AND DISCUSSION

To obtain chitosan with certain characteristics, it is necessary to carefully manage the amount produced and the deacetylation degree (DDA) by optimizing the process parameters.

The results obtained for chitosan extraction from 20 samples of crustacean's exoskeletons that have been collected from the Black Sea coast are presented in Table 1. The chitin extraction was carried out in similar conditions using 5% HCl solutions for demineralization and 4% NaOH solution for deproteinization. The degree of deacetylation (DDA) is significant in defining the quality of chitosan's physicochemical and biological properties. It varies based on the origin, species, and methods employed in synthesizing chitosan from chitin, as well as the optimization of the involved extraction processes, which include demineralization, deproteinization, discoloration, and deacetylation. Also, specific working conditions such as concentrations, temperature, and time reaction cannot be neglected.[6,10]

The deacetylation step was considered crucial for the main chitosan characteristic, DDA and the working conditions were varied around the generally recommended values[10] (45% NaOH solution, 18 liquid:solid ratio and 120 minutes duration of the process), at constant temperature, of 95°C. The parameters were varied according to a central composite experiment based on a full 2^3 factorial program considering the above-mentioned conditions as the center point of the experiment. Accordingly, the range of parameters variation is given in Table 1.

Table 1

Variables range for experimental measurements

Variable	Minimum value	Maximum value
NaOH solution concentration wt%	28	62
Liquid: solid ratio mL/g chitin	9.5	26.5
Deacetylation duration min	70	170

Table 2

Experimental data

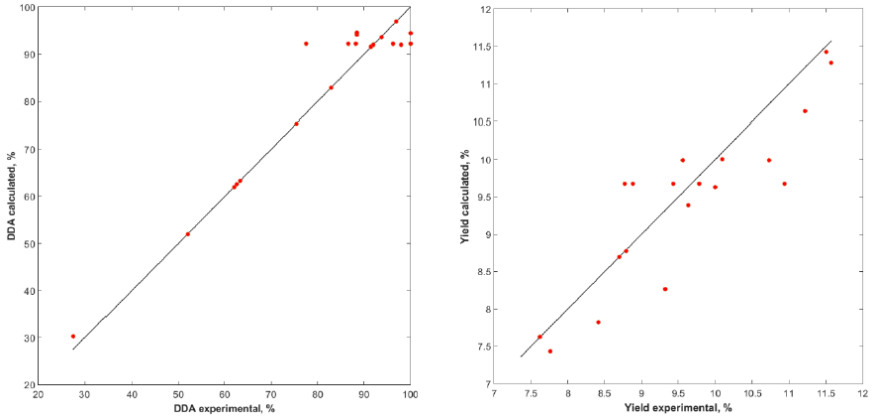
Nr.	Initial crustacean' powder g	Chitin extraction yield	Deacetylation conditions				Chitosan yield referred to crustacean' powder %	DDA %
			NaOH conc. %	Liquid: Solid mL/g	Duration min	T °C		
1	10.0016	12.81	28	18	120	95	11.57	27.5
2	10.001	8.66	35	13	90	95	7.36	62
3	10.0039	11.42	35	13	150	95	9.63	75.5
4	10.007	10.45	35	23	90	95	8.41	52
5	10.0014	11.80	35	23	150	95	10.09	62.5
6	10.0025	13.34	45	9.5	120	95	10	88.5
7	10.0068	11.47	45	18	120	95	8.88	96.27
8	10.0055	11.84	45	18	120	95	8.77	88.25
9	10.0029	12.74	45	18	120	95	9.78	77.47
10	10.0028	12.48	45	18	120	95	9.43	86.65
11	10.0006	13.56	45	18	120	95	10.94	100
12	10.0014	13.65	45	18	70	95	11.22	63.33
13	10.0011	14.18	45	18	170	95	11.5	96.95
14	10.0081	13.44	45	26	120	95	9.32	93.77
15	10.0041	9.85	55	13	90	95	7.76	100
16	10.0047	10.70	55	13	150	95	8.79	88.5
17	10.003	10.82	55	23	90	95	7.62	82.95
18	10.0036	10.62	55	23	150	95	8.7	91.5
19	10.0049	13.25	62	18	120	95	9.56	92
20	10.0005	13.33	62	18	120	95	10.73	97.99

The extraction of chitosan from crustacean exoskeletons typically results in a low yield, which is highly dependent on the deacetylation process parameters such as the concentration of NaOH, the liquid-to-solid ratio, and the duration of the reaction. The study reported yields varying between 7.36% and 11.5%, as can be seen in the experimental values obtained for the 20 powders of crustacean exoskeletons waste samples used (Table 2).

As can be seen, after the demineralization and deproteinization steps chitin is extracted at a low yield varying between 9.85% and 14.18%. In the deacetylation step, according to various values of main parameters (NaOH solution concentration, liquid:solid ratio and duration) chitosan is obtained with various yields resulting in an overall yield between 7.36% and 11.5%. This proves that the deacetylation step is responsible for reaching a given overall yield and of course a DDA which is the main characteristic of final chitosan.

The modelling of the variation of the yield and DDA as function of the deacetylation operating parameter was performed using artificial neural networks (ANN) implemented in Matlab 2023. Two neural models were built, one for the Yield and the other for the DDA, based on feed forward ANNs with backpropagation. The hyperbolic tangent was selected as activation function and Levenberg-Marquard algorithm for training the two networks. The architecture of the two networks was defined with: 3 input nodes (NaOH concentration, liquid:solid ratio and duration), a hidden layer with 4 nodes for the DDA model and 5 nodes for the Yield model, and one node in the output layer standing for the DDA and chitosan yield respectively. 70% of the experimental samples were used for training, 15% for testing and 15% for validation to ensure the capability of the ANN model to correctly capture the process features.

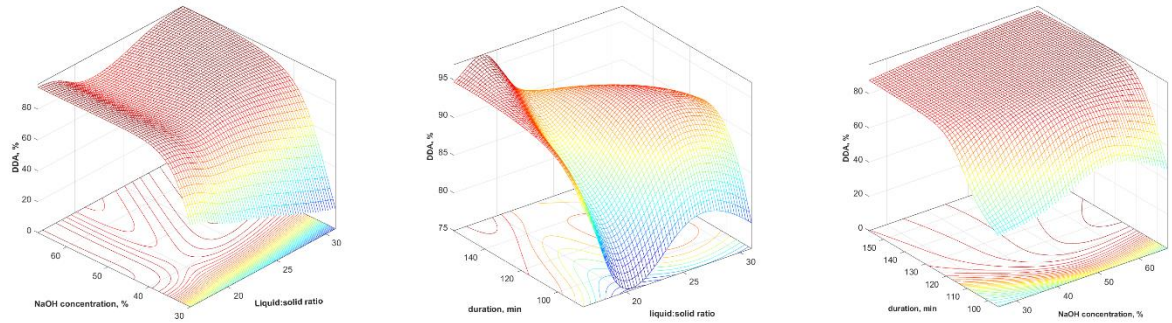
The final neural models were able to reproduce the experimental variation of the Yield and DDA (Fig. 1).



(a) (b)

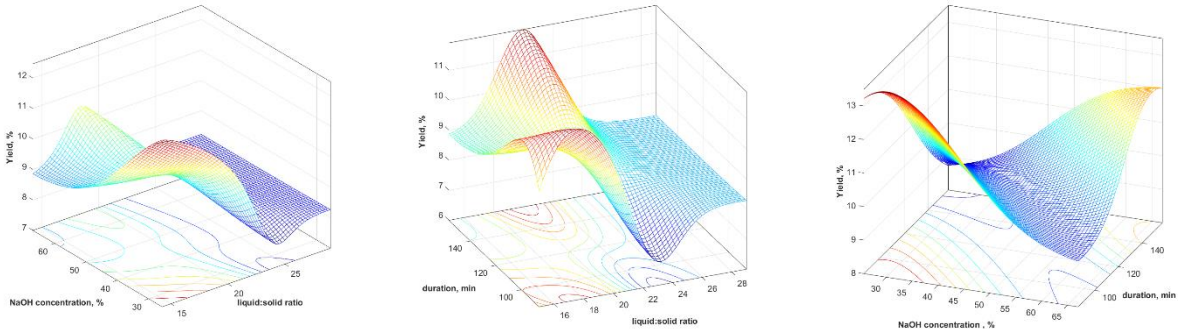
Fig. 1 – Parity plot for the neural models: a) DDA model; b) Yield model.

Using the neuronal models the visualization of the two response surfaces was possible by 3D plots of the Yield and DDA in the space of the variables. Figure 2 shows the variation of DDA with working conditions while Fig. 3 presents the variation of the chitosan yield.



(a) (b) ©

Fig 2 – Response surfaces for DDA: a) process duration 120 min; b) NaOH concentration 45wt%; c) liquid:solid ratio 18 mL/g.



a b c

Fig. 3 – Yield variation with operating parameter: a) for a duration of 120 min; b) for 45% NaOH solution concentration; c) for liquid:solid ratio of 18 mL/g.

Analyzing the response surfaces for both DDA and yield, some combination of operating factors leading to high values for these two characteristics can be found. As Fig 2c shows, higher DDA are possible on quite large domains when NaOH solution concentrations over 45% are used with long durations of the deacetylation process. The liquid:solid ratio influences less the value of DDA, lower or medium values seem to be favorable (Fig. 2 a and b).

The yield variation is more complicated, as it clearly presents a multimodal surface. Some maximum values can be reached at very low NaOH concentrations and small liquid:solid ratios (Fig. 3a), while Fig. 3c reveals a possible maximum value at short duration and low NaOH solution concentration.

The ANN models were used in the process optimization step. The objective of the optimization is to maximize simultaneously the yield and DDA. For this goal the first approach was to define compound objective function, Fob as the weighted sum of the two criteria DDA and YIED represented by the two neural models and maximize it.

$$\text{Max Fob} = 0.1 \cdot \text{DDA} + \text{Yield} \qquad (1)$$

The weight value of 0.1 stands for making the two values of the same order of magnitude, giving thus equivalent importance of the two criteria. The optimization was carried out using genetic algorithms implemented in Matlab 2023. The final solution obtained is: NaOH solution concentration 49.1 wt%, liquid: solid ratio 19.06 mL/g, duration 170 min. This solution corresponds to a DDA of 99.1% and Yield of 12.43%.

A second approach may be the multiobjective optimization using two objective function: f1=max(DDA) and f2=max(Yield) and build up the so called Pareto front that represents a multitude of equivalent optimal solution reflecting a trade-off of the two objective functions. The multiobjective optimization was carried out in the frame of Matlab 2023 using genetic algorithms.

The particular shape of the neural model, mainly the clearly multimodal shape of the yield model, led to difficulty in identifying a Pareto front which has a discontinuous shape (Fig. 4). The break in the front is due to the fact that for different values of operating conditions the yield has almost the same value while the DDA varies. This can be noticed also from some sets of operating points extracted from the Pareto front. It is noticed that a yield of about 12.4% may be reached in various conditions, but with very different DDA values. High DDA is reached if the NaOH solution is about 50% and long durations are used while low DDA is obtained for lower NaOH concentrations and shorter duration of the deacetylation process, with a very small increase in the yield.

Table 3

Selected optimal solutions

NaOH conc. %	Liquid: Solid mL/g	Duration min	DDA %	Yield %
50.48	18.95	169.85	99.01	12.44
49.91	18.99	169.81	99.08	12.44
35.96	17.91	88.67	53.07	12.73
35.95	18.06	87.65	51.57	12.75

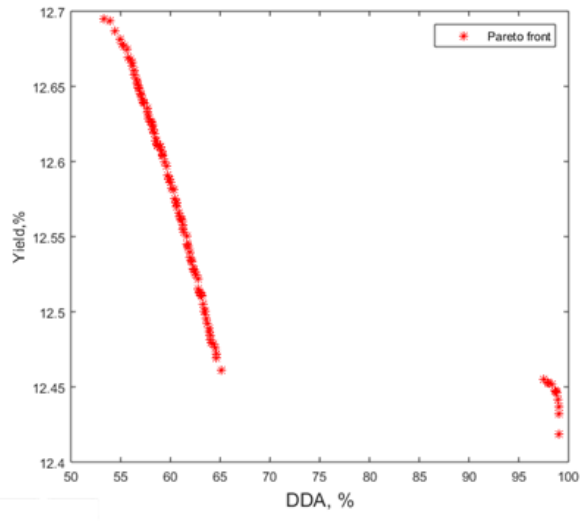


Fig. 4 – Pareto front.

The multiobjective approach confirms the optimum value obtained by using a composite objective function, that is a yield of 12.4 % may be reached together with high DDA (99%) if the deacetylation process is carried out using NaOH solution of 50%, 18–19 liquid: solid ratios and long durations (about 170 min).

EXPERIMENTAL

Materials

This study used crustacean exoskeletons waste, gathered from local seafood restaurants along the Romanian Black Sea coast during summer-autumn 2022. The exoskeletons waste was frozen prior to processing. To extract chitin and chitosan chemically, the initial processing steps involve washing the waste, removing soft tissues, then drying the exoskeletons in an oven at 60 ±1 °C for 3 hours, and finally grinding them into a fine powder. The extraction process involved the use of reagents like 37% hydrochloric acid (HCl) solution obtained from Chemical Company S.A. in Iaşi, Roumania. A 4% solution was prepared from this, and sodium hydroxide (NaOH) pellets purchased from ChimReactiv SRL, Bucharest, Roumania, were necessary for preparing a 5% NaOH solution. Furthermore, ethanol (EtOH) and acetone (p.a.) were used for the discoloration step of the chitin and were purchased from Sigma Aldrich, Taufkirchen, Germany.

The potentiometric pH measurements were used to determine the DDA values of the chitosan samples obtained in this study, according to our previous studies.[10,21] Two inflection points marked the endpoint of the titration; thus the initial inflection point represents the neutralization of the excess protons in the hydrochloric acid, used as a solvent in the chitosan solution tested. The difference between the first and second inflection points indicates the amount of NaOH required to react with the H^+ ions of the amine groups. Measurements were carried out in triplicate per sample. By using Eq (2) and (3), the DDA values were calculated[22]

$$\mathrm{DDA}(\%) = \frac{203 \cdot Q}{1+42 \cdot Q} \quad (2)$$

$$Q = \frac{c_M \cdot \Delta V}{m} \quad (3)$$

where: c_M – molar concentration of NaOH solution, used for titration (mol/L, in the present study: 0.1 mol/L);

ΔV – volume difference of NaOH added corresponding to the two inflection points (L);

m – weight of analyzed chitosan (g).

The yield of chitosan was calculated by using Eq 4

$$\text{Yield } (\%) = \frac{\text{Extracted chitosan (g)}}{\text{Crustacean waste powder (g)}} \cdot 100 \quad (4)$$

Chitin/Chitosan Extraction Procedure

The procedure for chitin extraction involved demineralizing the pre-washed and dried waste, followed by deproteinization and discoloration of the chitin powder obtained. This process was applied to all samples in the following manner:

Demineralization was carried out using a 4% HCl solution (waste powder to HCl solution ratio of 1:13 w/v) at room temperature, with medium stirring, for approximately 50 minutes. The mixture was subsequently rinsed with distilled water until the pH reached approximately 6. Lastly, the powder was dried in an oven at 105 ±1 °C.

Deproteinization was carried out using a 5% NaOH solution (ratio of demineralized powder to NaOH solution = 1:16 w/v) at 65 ±1 °C, with medium stirring for 2 hours. Subsequently, the mixture was rinsed with distilled water until it reached a neutral pH. Ultimately, the chitin powder was oven-dried at 105 ±1 °C.

Discoloration of the chitin sample was carried out using a mixture of acetone and ethyl alcohol (1:1, v/v ratio) in a solid-solvent ratio of 1:1 (w/v), followed by successive washing with distilled water and drying the light yellow-white chitin in an oven at 105±1 °C.

The research on the deacetylation process was conducted using a central composite experimental design, considering NaOH concentration, liquid volume to mass ratio, and the duration of the deacetylation step as independent variables. The parameters values for the experiments were set around a NaOH concentration of 45%, a liquid to solid ratio of 18, and a duration of 120 minutes. These values have been consistently utilized and tested in our prior studies. Following the deacetylation step described above, the wet chitosan pellets were rinsed with distilled water until a neutral pH was reached. Subsequently, the material was oven-dried to constant mass.

CONCLUSIONS

The valorization of crustacean waste can be realized through the extraction of chitosan, which brings environmental benefits by reducing marine pollution and decreasing the challenges of waste disposal. From an economic point of view, this process leads to the introduction of new business oportunities and increased competitiveness in the seafood company sector by converting worthless waste. This paper used genetic algorithms for multi-objective optimization, demonstrating the feasibility of achieving high accuracy in obtaining high DDA and satisfactory yields at the same time. The Pareto front analysis underscored the trade-offs between yield and DDA, directing the choice of operating conditions to achieve a balance between the two objectives.

The deacetylation process can be carried out with high yields in two different regions, with low and high DDA respectively. If low concentration of NaOH solution and short duration are used, yields up to 13 % can be expected, but the DDA is below 60 %. When higher NaOH concentrations and longer duration are used, the DDA is greater than 95 %, and the yields of about 12.5 % may be obtained. By tuning the parameters of the extraction process, this study illustrates that it is possible to enhance the value of the product obtained from crustacean waste, thereby aiding environmental sustainability and economic feasibility. This research aligns with global trends advocating for sustainable and eco-friendly industrial methods, supporting the tenets of the circular economy and the goals of sustainable development.

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