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Production of Chitosan from Crab Shells Using an Aqueous Extract of Wood Ash for the Deacetylation of Chitin: An Innovative, Eco-friendly, and Low-cost Method

Juliao Monjane[1, *], Dercio Chemane[2], Amandio Zimba[2], Paulo Dimande[2], Amalia Uamusse[1]

[1]Department of Chemistry, Eduardo Mondlane University, Maputo, Mozambique

[2]Rural Development High School, Eduardo Mondlane University, Vilankulo, Mozambique

Email address:

juliao.monjane@uem.mz (J. Monjane), monjane.juliao@gmail.com (J. Monjane)

[*]Corresponding author

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Abstract: The importance of chitosan has grown significantly over the last years due to its medicinal, agricultural, and industrial applications, as well as the biodegradability properties. The present study aimed to describe for the first time, the preparation of chitosan from chitin using wood ash aqueous extract as an alkaline medium. Chitin was obtained from crab shells in a microbial fermentation process using fish viscera for protease. Pilled and milled sweet potatoes and cassava water extracts were used separately in the deproteinization and demineralization steps as sources of carbon. The obtained chitin samples were then converted into the respective chitosan samples (B-C2 and M-B2) by an innovative, low-cost, and eco-friendly method using an aqueous extract (pH=12.4) prepared from wood ash, instead of the chemical process using sodium hydroxide solution in the deacetylation of chitin. The yield of the obtained chitosan was found to be 11.5% for B-C2 and 9.4% for M-C2. The presence of chitosan was determined by the analysis of the FTIR spectrum of both samples. The degree of acetylation was found to be 77.3% and 83.2% for B-C2 and M-B2, respectively, based on the IR analysis of absorption bands at 3456 and 1636 cm^{-1}, and 75.4% and 84.1% based on the potentiometric titration. The average molecular weight (192.1 kDa for B-C2 and 194.3 kDa for M-B2) was determined using the viscometer method. The solubility of B-C2 and M-B2 in 1% acetic acid was found to be 77.8% and 63.9%, respectively. The analysis of SEM photographs of B-C2 showed an amorphous morphology while M-B2 showed a crystalline morphology. The TGA and DSC curves obtained in a dynamic air atmosphere showed three degradation stages. In the main event (the second stage), an exothermic peak was observed at 355°C for B-C2 coupled with the weigh-loss of 7%, while for M-C2 was observed at 365°C coupled with weight-loss of 10%. Due to their high degree of deacetylation, low molecular weight, and viscosity, both chitosan samples could be utilized unambiguously in the agri-food field as a biofertilizer or as a bio-adsorbent in the wastewater treatment.

Keywords: Chitosan, Crab Shells, Enzymatic Treatment, Aqueous Extract Of wood Ashes, Sources of Carbon

1. Introduction

Crustaceans, such as crabs, shrimps, prawns, and lobsters are among the most important fisheries products worldwide. In Mozambique, the fishery industry, mainly the restaurants are the most producers of the bio-waste derived from the exoskeleton of the crustaceans. However, the real numbers of this bio-waste are unknown. Worldwide, nearly 45% of processed seafood is disposed on landfill, consequently leads to environmental issues. Fishery by-products such as chitin and its derivative chitosan have economical value and are well-recognized for having various applications in many fields, including agriculture, medicine, and the cosmetic industry [1].

Chitin is considered the second most abundant biopolymer found in nature after cellulose. This biopolymer has been produced by an enormous number of living organisms and is found in the exoskeleton shells of crustaceans and arthropods

or the cell walls of fungi and yeast [1–3]. Chitosan usually refers to a group of polymers that are obtained after deacetylation of chitin by changing the number of degrees of deacetylation, which reflects the balance between the two types (Figure 1) and differentiates chitin from chitosan [4].

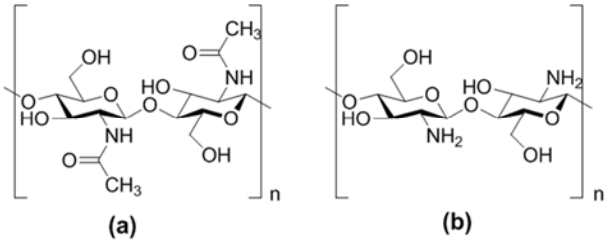


Figure 1. *Structures of chitin (a) and chitosan (b).*

The extraction of chitin from crude materials requires the removal of the two significant constituents: proteins by deproteinization and inorganic calcium carbonate by demineralization, along with small amounts of pigments and lipids that are in general eliminated during these two steps. Both deproteinization and demineralization steps could be carried out utilizing chemical and enzymatic or biological treatments. The order of the two above-mentioned steps might be reversed with some advantages, particularly when enzymatic or biological treatment is used. Microbial fermentations are also employed, in this situation, the deproteinization and demineralization steps are handled at same time [1, 3].

The extraction by chemical treatments has numerous disadvantages to the environment [2]. The improvement of green extraction techniques based on the concept of ‘‘Green Chemistry’’ is acquiring more attention, favoring the use of enzymes and microorganisms for chitin extraction [1]. The biological or enzymatic extraction of chitin offers high reproducibility in a short period, simple to control, smaller solvent consumption, and low energy input. However, this method is yet restricted to laboratory-scale researches [1, 3]. In the biological process, chitin extraction requires the utilization of proteases. Crude proteases are mainly derived from bacteria and fish viscera. In several of the major fish-producing countries, such as Mozambique, these by-products might represent about 45% of the seafood harvest. These materials are to a great extend underutilized and disposed of as waste, harming consequently the environment. Hence, the utilization of these crude enzymes in the chitin extraction could be interesting in decreasing the cost of this process [5].

Alternative methods to obtain chitosan from chitin without utilization of environmentally unsafe reagents, for example, the highly concentrated sodium hydroxide have been investigated. However, in spite of the numerous investigations of the issue, it is still far from being solved [5].

The study aimed to enhance the use of crab shells waste and help to minimize environmental pollution, in which the main objective was to extract chitin and prepare chitosan from crab shells using an eco-friendly method. Here, we wish to report the first example describing the deacetylation of chitin using a strongly basic aqueous extract prepared from wood ash as an alternative to strong sodium hydroxide solution, in an eco-friendly, low-cost, and innovative method by applying ‘‘Green Chemistry’’ approach.

2. Experimental Part

2.1. General Methods

The crabs were purchased in the Municipal Market of Vilankulo village, Vilankulo District, Inhambane Province, located in the Southern part of Mozambique. After purchase, the crabs were cooked and the shells were separated. Then, the crab shells were dried in an oven at 90°C for 2 h. After removal from the oven, they were cooled at room temperature, milled into a fine powder, stored in a cool and dried place at Rural Development High School, in Vilankulo District until use. The sweet potatoes (0.5 Kg), cassava (0.5 Kg), fish (source of fish viscera, 1.0 Kg); vinegar (as the source of acetic acid, 1.0 L), and sugar (100 g) were purchased in the local markets in the same district. The wood ash (1.0 Kg) was obtained from the local households. The Brucker FT-ATR instrument was used to record the infrared spectra and a Scanning Electronic Microscopic Jeol JSM-IT100 was used to obtain the photographs of the morphological structure of the obtained chitosan. For Thermogravimetric Analysis and Differential Scanning Calorimetric, an SDT Q600 V20.9 Build 20 instrument was used. The average molar mass determination was performed using a micro-Ostwald viscometer (SI Analytics GmbH^{TM}, type No. 285404405, model 51810, with a capillary inner diameter of 0.43 mm. The measurements were done at 25±1°C). For potentiometric titrations, a pH meter was used.

2.2. Extraction of Chitin by Biological Method

143.8 g of dried and milled crab shells were mixed with an aqueous extract prepared from sweet potatoes (0.5 Kg of pilled and milled were extracted with 0.5 L of water tap) in a 5 L mud pot, and then 100 g of fish viscera were added. The mixture was left at room temperature for 48 h for fermentation (aerobic at daytime and anaerobic at night time), with occasional stirring. During this period, the pH of the reaction mixture was kept under control (3.5 to 4.2). After fermentation, 50 g of sugar were added to complete the deproteinization and demineralization processes (all of these steps are recommended to be done outside of the laboratory due to the strong odour). 48 h later, the demineralized and deproteinazed chitin was washed with water tap and dried for 2 h at 90°C in an oven to obtain 34.1 g of a sample named C. The same procedure was applied to obtain the sample M (33.2 g) from 143.0 g crab shells treated with cassava aqueous extract.

2.3. Chitosan Preparation Using an Aqueous Extract of Wood Ash

An aqueous extract of wood ash (200 g in 800 mL of water tap, measured pH=12.4) was boiled for 40 minutes with

occasional stirring. After filtration, 200 ml of the extracted solution were added to 25 g of previously prepared chitin in an Erlenmeyer and then was shacked for 10 minutes. The mixture was transferred into an opened bottle and subjected to a water bath for 4 h with occasional stirring. The reaction mixture was then cooled at room temperature, filtrated, washed using a water tap, and dried in an oven for 2h at 90°C to obtain (19.1g) of chitosan (B-C2). For purification, the obtained chitosan was dissolved in 500 mL of vinegar at 5%, heated at 120°C for 4 h with occasional stirring. Then, the mixture was cooled at room temperature and filtrated. The purified chitosan was precipitated by adding 660 mL of vinegar. The precipitate was filtrated and dried in an oven at 110°C for 4 h. The pure chitosan (16.5 g) was obtained as a white amorphous solid. The same procedure was applied for chitosan M from pilled and milled cassava aqueous extract to obtain sample M-B2 (13.5 g) as a crystalline white solid. Due to the shortage of time, the sample M-B2 was not further purified.

2.4. Potentiometric Titration of Chitosan Samples

The dried chitosan sample (50 mg, B-C2) was dissolved in a 250 mL flask with 20 mL of 0.1 M hydrochloric acid, and then 25 mL of deionized water was added. The mixture was stirred continuously for 30 minutes. After, another portion of 25 mL of deionized water was added and continued stirring for 30 minutes. When chitosan was completely dissolved, the final volume of the solution was adjusted for 100 mL using deionized water. The solution was titrated with 0.1 M of sodium hydroxide. The respective titration curves of pH vs NaOH titration volume were generated (Figure 2). The potentiometric titration was made in triplicate. The DD of chitosan was calculated using Eq. 1:

$$DD[\%] = 2.03x\frac{V_2 - V_1}{m + 0.0042(V_2 - V_1)} \quad (1)$$

where: m is the weight of the chitosan sample, V_1 and V_2 are volumes of 0.1 M NaOH solution corresponding to the deflection points (the first and the second equivalent points). 2.03 is the coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 is the coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units [6].

2.5. Determination of the Average Molecular Weight

The molecular weight of the chitosan was calculated based on its intrinsic velocity ($[\eta]$) by applying the Eq. 2 of Mark, Haouwink, and Sakurada, where K and a are constants and M_v is the molecular weight in Daltons. The values of 0.72 for a and 4.74×10^{-5} dL/g for K were adopted, which are constants for a given solvent-solute system. We used 1% of acetic acid solution at a temperature of 30°C.

$$[\eta] = KM_v^a \quad (2)$$

The intrinsic viscosity of chitosan was determined by capillary viscometer using an Ostwald viscometer. The method is based on the measurement of the flow time (t_0) of the volume solvent (consisting of 1% of acetic acid) or chitosan solution (t) contained between two lines marked on the viscometer using a chronometer. Eq. 3 defines the specific viscosity (η_{sp}) [4, 7, 8].

$$\eta_{sp} = \frac{t - t_0}{t_0} \quad (3)$$

Dividing this value by concentration of the chitosan solution (C_i) in g/dL gives the reduced viscosity (η_{red}) defined by Eq. 4.

$$\eta_{red} = \frac{\eta_{sp}}{c_i} \quad (4)$$

Thus, for different concentrations of chitosan (0.010, 0.025, 0.050, 0.075, and 0.100 g/dL) different reduced viscosity values are obtained. The plotting of the reduced viscosities as a function of the concentrations gives rise to a line whose ordinate at the origin is equal to the intrinsic viscosity (Figure 3). The intrinsic viscosity can be determined otherwise through the inherent viscosity (η_{inh}), obtained from the relative viscosity (η_{rel}), and defined by Eq. 5 [4, 7, 8]:

$$\eta_{inh} = \frac{\ln(\eta_{red})}{c_i} \quad (5)$$

2.6. Solubility Test

Chitosan powder (0.1 g in triplicate) was placed into a centrifuge tube (known weight). Then was dissolved with 10 mL of 1% acetic acid and was shaken for 30 minutes using an incubator shaker operating at 40 rpm and 25°C. The solution was then immersed in a boiling water bath for 10 minutes, cooled to room temperature, and centrifuged at 10,000 rpm for 10 minutes. The supernatant was decanted. The undissolved particles were washed in distilled water (25 mL) and then centrifuged at 10,000 rpm. The supernatant was removed, the undissolved pellets were weighed, and the percentage of the chitosan solubility was determined. The solubility of chitosan was calculated using Eq. 6 [10].

$$S(\%) = \frac{m_i - m_f}{m_i} x100 \quad (6)$$

where: m_i is the initial mass of chitosan and m_f represents the final mass of chitosan.

3. Results and Discussions

3.1. Demineralization and Deproteinization of Chitin

The mass analysis showed that the crab shells used consisted of 34.1 g (23.7%) and 33.2 g (23.2%) of dry weight chitin for B-C2 and M-B2, respectively. Crabs are considered a good source of chitin (20-30%). The values obtained were in agreement with those reported in the literature using fish viscera as a source of enzymes for deproteinization and demineralization of chitin [11]. The demineralization process is carried out by lactic acid produced by bacteria derived from fish viscera, through the conversion of the carbohydrates present on cassava (or sweet potatoes) aqueous extracts as a

source of carbon. The deproteinization is carried out by proteases secreted by bacteria into the fermentation medium. The lactic acid is formed from the breakdown of glucose creating at low pH (measured, 3 to 4) and reacts with calcium carbonate present in the chitin, leading to the formation of calcium lactate, which precipitates and could be removed by washing with water [12]. The amylose content in cassava is higher than in sweet potatoes. These differences in amylose content affect the time of the deproteinization and demineralization processes since it was observed that the cassava extract reacts more quickly than the sweet potatoes extract. However, it did not affect directly the yields of chitin and chitosan obtained, meaning that both cassava and sweet potato aqueous extracts could be used in the same way as sources of carbon during the extraction of chitin.

3.2. Deacetylation of Chitin Using an Aqueous Extract of Wood Ash

Wood ash consists mainly of inorganic minerals and organic compounds remaining in the ash due to incomplete combustion. Besides these chemicals, all the major macronutrients are also found in the ash. The significant extent of the minerals in the ash is combination of oxides, hydroxides, silicates, and carbonates of the base-forming cations. A portion of the oxides and hydroxides in the ash dissolve easily in water and produce a strongly alkaline solution. From the literature, the pH of the aqueous extract of wood ash is found between 12.1–12.6 [9]. The measured pH value of the solution was found to be 12.4. This pH value is in some way very close to a pH of a solution of NaOH 50% w/v (pH 13.7) normally used for the chemical preparation of chitosan from chitin.

The yield of chitosan was obtained by comparing the weight of the raw material and the weighed chitosan, which was obtained after the treatment. The yields of the purified sample B-C2 and unpurified M-B2 were 16.5 g (11.5%) and 13.5 g (9.4%), respectively. The yield of chitosan extracted from crab shells has been reported to be in the range of 30 – 36%. The differences in the yield could be due to the reaction time, which has a positive effect on the yield [13]. The yield of the obtained chitosan samples could also be affected by the loss of sample mass from excessive removal of the acetyl groups from the polymer during deacetylation, i.e., the conversion of chitin to chitosan [13].

3.3. Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy is a technique that is used to determine the vibrational functional groups of samples. The FT-IR spectra of B-C2 and M-B2 samples were examined and are presented in Table 1. Both samples possessed similar functional groups. In the sample B-C2, the –OH stretching band was depicted at 3495 cm^{-1}, which showed the alcohol group in the chitosan, while for M-B2 was at 3380 cm^{-1}. According to the literature, the vibration identified as the alcohol group (-OH band) is found between 3650-3200 cm^{-1}. The stretching band for C-O was found at 1076 cm^{-1} for B-C2, while for M-B2 chitosan stretching band for C-O was at 1130 cm^{-1}. Also, the stretching band for NH for B-C2 was observed at 3440 cm^{-1}, while for M-B2 appeared at 3369 cm^{-1}. As mentioned in the literature, the band identified as amine group (N-H stretching bands) absorbs infrared from 3500 to 3100 cm^{-1}. Moreover, the bending band pf $N\text{-}H_2$ in the chitosan B-C2 was 1622 cm^{-1}, while for M-B2 this band was depicted at 1655 cm^{-1}. Both samples showed slightly different absorption N-H and O-H peaks in the spectrum. The appearance of these bands depends on the DD.

Table 1. *IR absorptions frequencies of the extracted chitosan samples.*

Functional group	Type of vibration	Characteristic absorption (cm^{-1})	
		B-C2	M-B2
OH, alcohol group	stretch	3495	3381
NH_2, amine group	stretch	3440	3369
C-O-C, glc group	stretch	1160	1128
CH, aliphatic	stretch	2924	2921
CH, aliphatic	bending	1412	1430

3.4. Determination of Degree of Deacetylation (DD)

The DD influences the physical, chemical, and biological properties of chitosan, such as acid-base characteristics, biodegradability, self-aggregation, as well as the ability to chelate metal ions. In addition, the DD determines the content of free anime groups in the polysaccharide that can be used to differentiate chitin and chitosan. Several methods are used for determining the DD, which include the FT-IR and potentiometric titration methods.

3.4.1. FT-IR Spectroscopy

DD may range from 30-90% depending on the available source and procedure. It is calculated by using the Eq. 7 and FT-IR analysis of the prepared chitosan:

$$DD = 100 - \left[\frac{A_{1655}}{A_{3450}} x \frac{100}{1.33}\right] \quad (7)$$

where A_{1655} and A_{3450} are the absorbances at 1655 cm^{-1} of the amide-I band as a measure of the *N*-acetyl group content and 3450 cm^{-1} of the hydroxyl group band as an internal standard to correct film thickness or for differences in chitosan concentration powder form. The factor 1.33 denotes the value of the ratio between A_{1655}/A_{3450} for full *N*-acetylated chitosan. It was assumed that the value of this ratio was zero for full-deacetylated chitosan and that there was a rectilinear relationship between the *N*-acetyl group content and the absorbance of the amide-*I* band [3].

From the equation above the DD was found to be 77.3 and 83.2% for M-B2 and B-C2, respectively. From these results, it was observed that both chitosan prepared have DD≥ 50%, which according to the literature are soluble in diluted organic acids [14]. Another main characteristic of both samples relating to DD, they could be considered to be used for medicinal purposes (DD=70-90%).

3.4.2. Potentiometric Titration

The titrant was the solution of standard NaOH containing 0.1 M KCl. The standard titrant solution was added to the

chitosan solution gradually. Both the volume of NaOH added and pH values of the solution were recorded using a digital pH-meter. The differential and integral titration curves were drawn between pH and the volume of titrant added, which produced an integral curve with two equivalent points. Around pH=5.7, chitosan begins to precipitate. In the present work, the precipitation of chitosan oligomer at the first equivalence point is low since the samples have relatively low molecular weights and dilute solutions of HCl and NaOH were used [15]. The variation of pH for chitosan solution as a function of NaOH added volume was plotted. Figure 2 shows the titration curve. From the figure, two equivalent points could be distinguished. The first point corresponds to the neutralization of the excess of the HCl. The second point could be ascribed to the protonation of amine groups of GlcN residues of chitosan. In acidic pH, primary amino groups are protonated, and as the NaOH solution is added during titration, they are neutralized and their concentration could be quantified by plotting the graphs presented in Figure 2. The DD of both chitosan samples was found to be 75% for B-C2 and 85% for M-B2. The results of DD obtained by FT-IR and potentiometric titration showed a good agreement of both methods. Even being the fastest method when compared with the potentiometric titration, the FT-IR spectra quantitative reproducibility and somewhat operator-dependent procedures for setting baselines for reading absorbances are the weak sides of this technique.

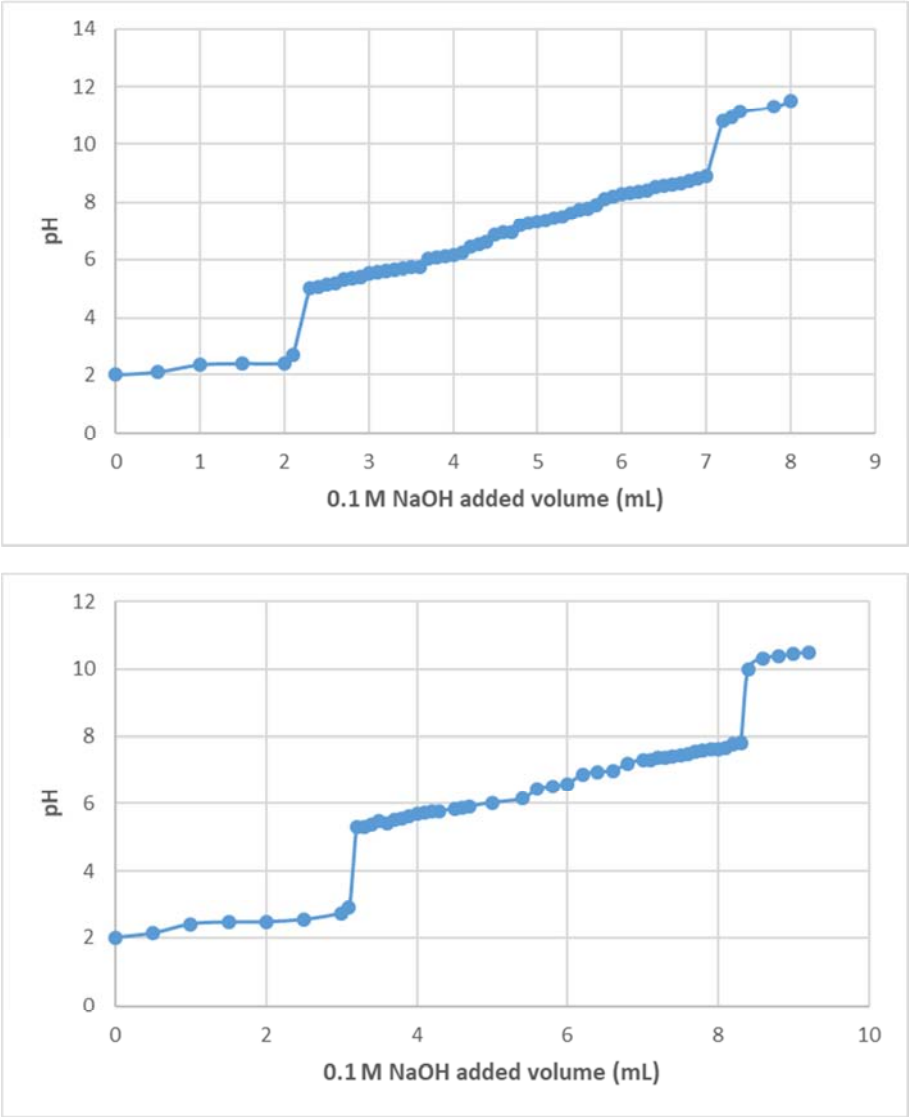


Figure 2. *Chitosan potentiometric titration versus 0.1 M NaOH solution volume: B-C2 (above) and M-B2 (below).*

3.5. Determination of the Average Molecular Weight (M_w)

The evolution of the reduced and inherent viscosity of chitosan samples as a function of concentration is shown in Figure 3. The results of the linear fit of reduced and inherent viscosity (428 and 432 mL/g for B-C2 and M-B2, respectively) are presented in Table 2. By replacing the value of the intrinsic viscosity (the intercept value) in Eq. (3), the viscosimetric average molecular weight could be obtained and was found to be 192.1 and 194.3 kDa for M-B2 and B-C2, respectively. The value of R^2 close to 1 shows that the linear fit or mathematical model obtained is in adequation with the experimental data

observed. The values of the molecular weight of chitosan depend closely on the extraction procedures because they can induce depolymerisation of the macromolecular chains and/or degradation during the extraction. The macromolecular chain of chitosan generally has a molecular weight of 100 to 1500 kDa. The obtained chitosan samples had low viscosity and molecular weight. These results confirmed that both samples are highly *N*-deacetylated derivatives of chitin. Low molecular weight chitosan samples are interestingly used due to their good solubility and good functional properties, such as antibacterial, antifungal, and antioxidant activities.

3.6. Scanning Electronic Microscopic Analysis

Figure 2 shows the SEM photographs examined at 50X magnification of chitosan prepared from a crab shell. The samples exhibited different morphological photographs. The B-C2 showed a morphological amorphous structure while the M-B2 was a crystalline. Since sample C-B2 was submitted for further purification, it is reasonable to state that the purity of chitosan could affect the changes in its morphological structure. Crystalline and amorphous structures of chitosan have been reported in the literature.

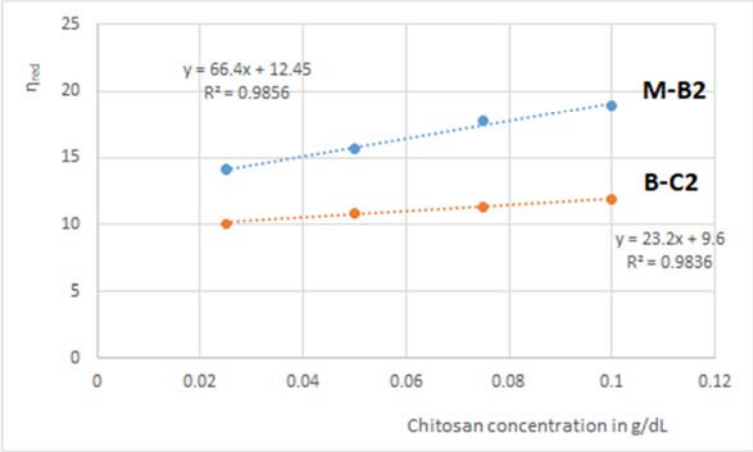
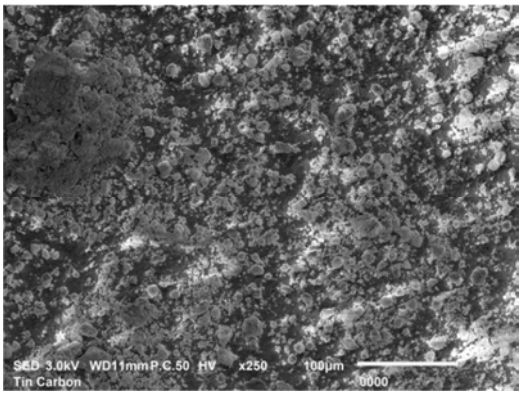
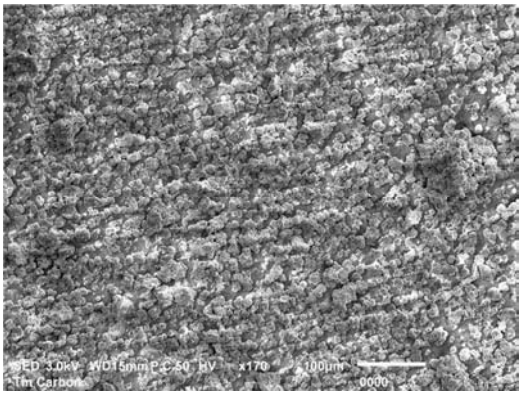


Figure 3. *The reduced viscosities as a function of the chitosan concentrations.*



(a)



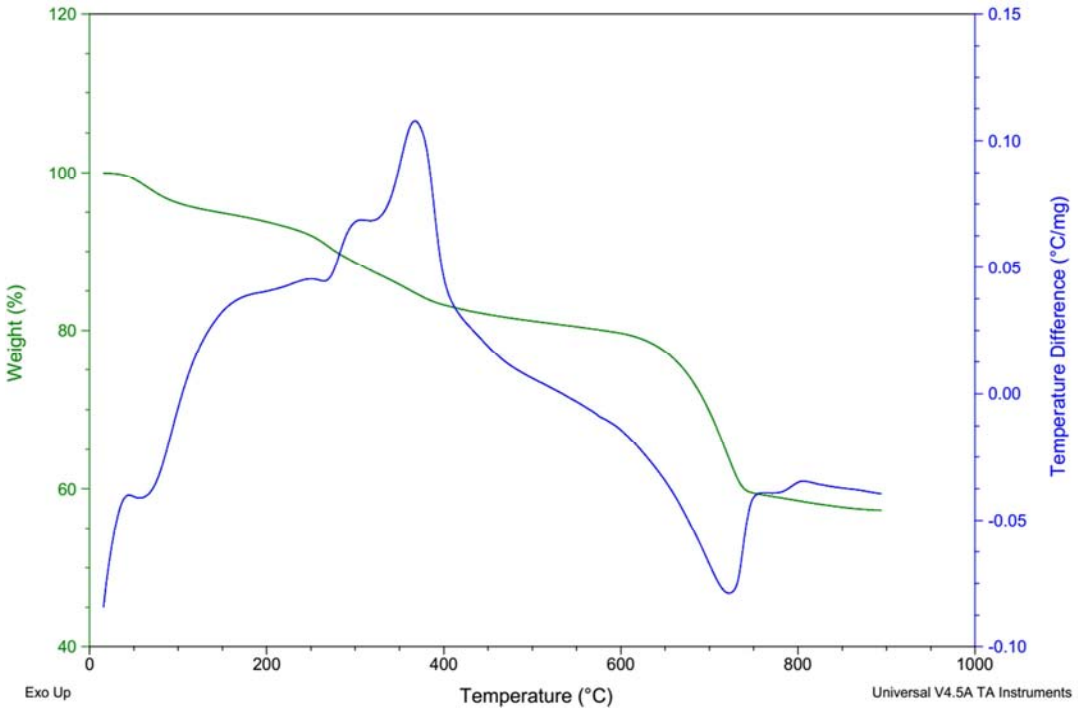
(b)

Figure 4. *SEM photographs of chitosan prepared: (a) B-C2 – amorphous structure; (b) M-B2 – crystalline structure.*

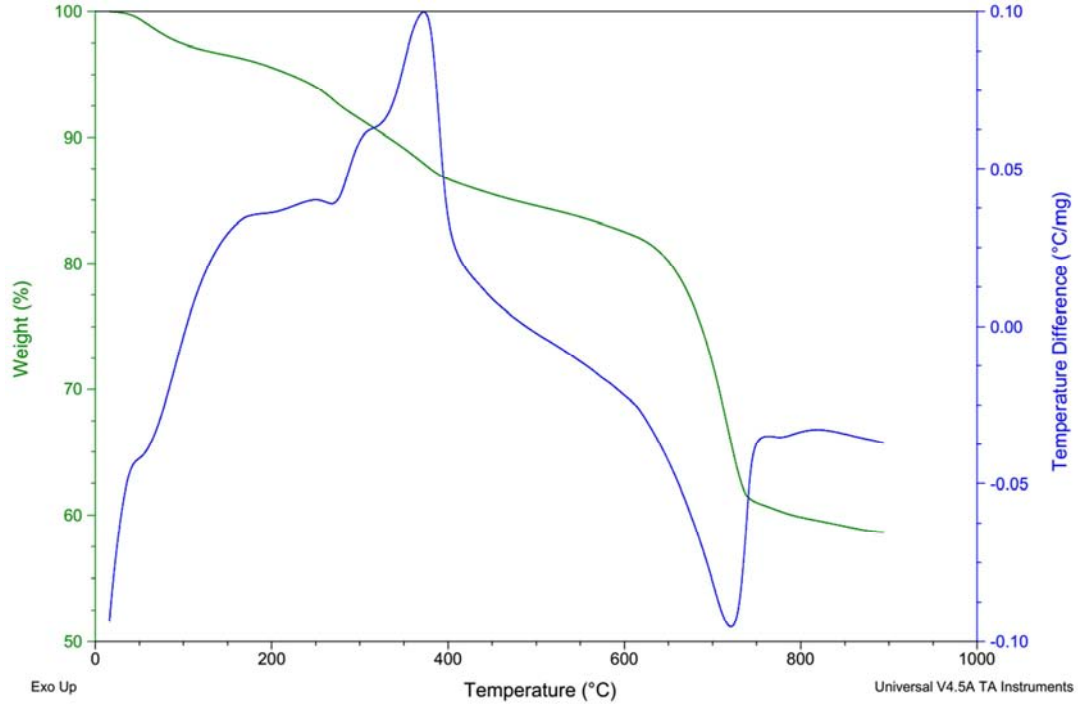
3.7. Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetric (DSC)

To investigate the pyrolysis behaviour, the chitosan samples (M-B2 and B-C2) were heated from room temperature to 900°C in a dynamic air atmosphere, and the corresponding TGA and DSC curves of both samples are shown in Figure 3. The degradation of chitosan in the air atmosphere occurs in three stages [16]. In the first thermal event, both samples showed an endothermic peak in the range of 40-90°C with a weight-loss of 3% for M-C2 and 9% for B-C2, respectively. This event is associated with the release of absorbed water, which is obvious because of the chitosan hydrophilicity and removal of moisture. The second thermal, also called the main event was characterized by the appearance of an exothermic peak from 250-400°C for M-C2 and 220-390°C for B-C2 coupled with the weight-loss of 10 and 7%, respectively. The event suggests that chitosan started to decompose and produce volatile compounds, and the exothermic effect might result from the cross-linking reactions during thermal degradation. In fact, in this range, it was observed two exothermic overlapped peaks for both samples: M-C2 (300 and 365°C) and B-C2 (300 and 355°C). The peak at 300°C could be properly attributed to the decomposition of the content of amine groups, while the later peak appeared due to the decomposition of the acetyl units that predominate in the chitosan structure, indicating that the amine residues are thermally less stable than the acetyl groups [17]. The third degradation stage was characterized by the appearance of an endothermic peak at 610-740°C for M-C2 and 610-720°C for

B-C2 with a weight-loss of 20% for both samples. The appearance of this effect at a higher temperature might result from the thermal degradation of the new cross-linked material that has been formed in the second degradation process, in which the scission abstraction of the side groups and ring-opening reactions occur [18]. In many cases after thermal analysis, there are residues derived from raw material. The carbonaceous residues and mineral impurities in the air atmosphere were found to be 57% for M-C2 and 59% for B-C2, which show the creation of permanent, non-volatile structures that are not burned at higher temperatures. The above-mentioned results suggested that both chitosan samples have similar DD.



(a)



(b)

Figure 5. *TGA and DSC curves (a) M-C2 (b) B-C2, respectively.*

The extracted chitosan samples showed to enhance the grain yield on pigeon pea (0.7 $t.ha^{-1}$) and maize crops (6.0 $t.ha^{-1}$), when compared with Manconzeb (0.5 and 4.5 $t.ha^{-1}$, respectively) and also showed antifungal properties against *Cochliobolus heterostrophus* on maize and *Colletotrichum lindemuthianum* on pigeon pea crops [19].

Table 2. *Summary of characteristics of extracted chitosan samples from crab shells.*

Characteristic	Chitosan sample	
	B-C2	**M-B2**
Carbon source	Sweet potato	Cassava
Yield of chitin (%)	23.7	23.2
Yield of chitosan (%)	11.5	9.4
Intrinsic viscosity (mL/g)	428.5	432.1
DD (%)		
FT-IR:	77.3	83.2
Potentiometric	75.4	84.1
Solubility (%)	77.8 (7.78 g/L)	63.9 (6.39 g/L)
Average molecular weight (kDA)	192.1	194.3
SEM analysis	Amorphous	Crystalline

4. Conclusions

Crab shells waste produced by the seafood industry is one of the important problems contributing to significant environmental and health hazards. Biopolymers like chitin and chitosan are important due to their biological and physiological properties. These properties offer many potential applications in various fields like environmental protection, agriculture, medicine, pharmaceutics, and biotechnology. The current study demonstrated an effective method for the extraction of high purity chitosan from crab shells. The produced chitosan samples had low viscosity, low molecular weight, and high DD. In our previous study, both samples showed antifungal properties and enhance the grain yields on maize and pigeon pea crops. Currently, the optimization and validation processes of the method are underway in our laboratory.

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