PRODUCTION OF CHITOSAN NANOFIBERS EXTRACTED FROM THE EXOSKELETONS OF CRUSTACEANS THROUGH ELECTROSPINNING

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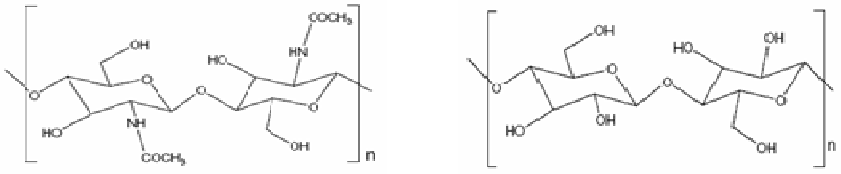
Abstract. *Chitosan is an amino polysaccharide derived from chitin deacetylation process. The chitin is the second polysaccharide more abundant in nature after cellulose and is the main component found in the crustaceans exoskeletons, insects, in the cell walls of fungi and yeasts. Chitin is a biodegradable natural polymer, of low cost, renewable and of great economic and environmental importance. The objective of this study was to develop chitosan from chitin present in the exoskeletons of shrimps and crabs that are being discarded as a waste, thus causing pollution and environmental problems. Beyond of extract the chitin and chitosan, objective was chitosan membranes manufacture to adding economic values the product that was considered as a waste product in the first place. The nonwoven or membrane produced by electrospinning has functional properties and can be used in the medical area, food industry, pharmaceuticals and chemical industries, due to the characteristic properties such as biodegradability, biocompatibility, non toxicity as well as used as antibacterial, emulsifying and chelate agent. Chitin was obtained from cleaned and selected exoskeletons. First it was ground into smaller particles and then submitted through the stages of demineralization, deproteinization and deodorization. In the demineralization process the samples of crustaceans exoskeleton (shrimp and crab) were treated with acid solutions at the ratios of 1:3, 1:6 and 1:10, in order to select the best possible ratio that eliminates the maximum amount of carbonates. Samples extracted from chitin and chitosan were used to study the morphological properties using SEM, DRX and the thermal properties through TG and DTG as well as FTIR analysis. The curves obtained by TG/DTG showed evidences of thermal decomposition in the chitosan samples. Through analysis by x-ray diffraction was observed that the chitosan has a semi-crystalline structure. The low crystallinity of chitosan can be explained from the thermogravimetric analysis, where degradation temperatures of chitosan showed less than the chitin. It was also observed that the deacetylation degree (GD) was inverse to the relative crystallinity index, in other words, the higher the crystallinity index, lower is the degree of deacetylation, as it is the characteristic of chitin having higher crystallinity. Chitosan membranes were produced by electrospinning and analysed the surface characterisstics through the scanning electron microscope (SEM).*

Keywords*: chitosan, crystallinity, nanofibres, electrospinning.*

1. INTRODUCTION

Chitin in Fig.1a is the most abundant biopolymer found in nature after the cellulose, as in Fig. 1b. The chemical structure of chitin is similar to cellulose and can be differentiated by the hydroxyl group located at position 2, that in the chitin were replaced by acetamide groups (Lehninger *et al.,* 1995). It is easily obtained and naturally renewable (Tsigos *et al*., 2000).



a. b.

Figure 1 a. Chitin b. Cellulose

Chitin is a linear polymer with high molecular weight composed of *N*-acetyl-2-amido-2-deoxy-D-glucose with units linked through β (1→4) bonds. A point of difference from other polysaccharides is the presence of nitrogen (Roberts, 1992).

The chitin and the chitosan, in Fig. 2 are amino polysaccharides that are waking up great interest of scientists and researchers as functional polymeric materials. Both are biodegradables, biocompatibles and non toxics and are used as antibacterial, emulsifying and chelating agent. Chitosan is obtained by deacetylation of chitin, of low cost and of great economic and environmental importance (Azevedo *et al*., 2007). Chitin and chitosan are produced by natural renewed source having applications in several areas: agriculture, industry of foods, textile industry, pharmaceutical industry, cosmetics and biomaterials, such as gels, films, polymers membranes and nanofibers (Tonhi and Plepis, 2002).

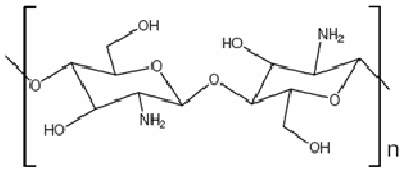


Figure 2. Chitosan

Chitin occurs essentially in crustacean, mollusks, insects, in the cell walls of fungi and yeasts. The exoskeleton is associated the organic substances, mainly proteins and impregnated with inorganic substances, such as calcium salts.

The Japan, the USA and China are the largest world producers of chitin, but the polymer is also produced, although in smaller scale, in India, Norway, Canada, Italy, Poland, Chile and Brazil, among other (Abram, 2004) and ahead of wastefulness for the industries of conserves in some parts of the world, including U.S.A. Oregan, Washington, Virginia, Japan and for several fishing fleets in Antarctica (Rinaudo, 2006). Several studies have been conducted over the past 20 years with aiming to obtain biomaterials that not only improve in quantity, but also the quality of patient's life.

The study present was developed starting of the chitin and chitosan from shrimps and crabs exoskeletons that are discarded as waste, causing pollution and environmental problems. The process to obtaining of the chitin followed the stages of demineralization, deproteinization and deodorization and then carried out the chitin deacetylation to obtain chitosan. After these stages the drying of the material was accomplished.

Electrospinning has won popularity in the last 10 years, due in large part to increased interest of nanoscale properties and technological. Potential applications of electrospinning include: membranes, nanofibers, tissue engineering scaffolds, among others. One attractive feature is the simplicity and inexpensive nature of installing the apparatus. The electrospinning process consists of a capillary, which can be a syringe with needle, a high voltage source and a collector, as in the Fig. 3.

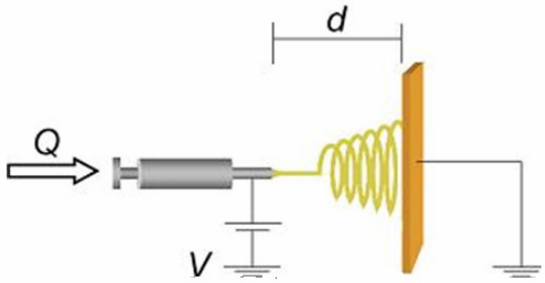


Figure 3. Electrospinning process. *Q*, flow rate; *d*, distance between plate and needle; *V*, applied voltage

Already were produced by electrospinning process, polymeric nanofibers and several biopolymers, what demonstrates the versatility of this process. The nanofibers produced by electrospinning process in the form of nonwoven (TNT) result in materials with large ratio of surface area and volume with morphology similar to natural tissue, making it an excellent candidate for use in the production of support structures for cell growth, grafts, bandages and medicines for application systems (Hirano et al., 1996).

2. MATERIALS AND METHODS

2.1 Materials

The raw materials used in this work were the residues of crustaceans (shrimp - *Litopenaeus vannamei, Aristeus antennatus* and *c*rab -*Ucides cordatus*), as show the Fig. 4a, 4b and 4c. The raw materials were acquired of local fairs and restaurants in city at Natal/RN, for extraction of chitin and chitosan to produce of chitosan membrane. The same contributed to decrease of the pollution in the environment, as well as an educational work among the people there inserted in that activity. For the formation of chitosan membranes was used the parameters: working distance (needle-collector) 10 cm, 7 wt% chitosan solution was dissolved in acetic acid aqueous solution 60%, was used high a voltage power supply to produce voltages of 15 KV.

All reagents used in this work were of analytical degree P.A, to see Tab 1.

a. b. c.

Figure 4 a. shrimp - *Litopenaeus vannamei* b. shrimp - *Aristeus antennatus* c. crab - *Ucides cordatus.*

Table 1. Reagents used.

Reagents	Origin
Sodium hydroxide micro pearl P.A (NaOH)	Vetec
Hydrochloric acid P.A (HCl)	Quimex
Sodium hypochlorite P.A (NaOCl)	QM
Acetic acid P.A (CH_3COOH)	Vetec
Propane acetone P.A $(CH_3)_2CO$	Vetec

2.2. Methods

The morphological analysis was evaluated using of the SEM and DRX. The measures of x-ray diffraction were accomplished in universal x-ray diffractometer; model Shimadzu XRD- 6000 with radiation of Cu, potency of 30 kV and current of 30 mA. The samples were scanned of 10 to 80º. Analysis of the thermal properties (TG/DTG) were obtained with a heating rate of 10ºC min^{-1} under a dynamic of atmosphere air in the temperature range of 25-700ºC. The spectroscopy in the infrared region (FTIR) was one of the techniques also used for characterization of chitin and chitosan.

The structural morphology of chitosan membrane was determined using the scanning electron microscope (SEM).

2.3. Process of obtaining of chitin and chitosan

The procedure for obtaining of chitin from crustaceans exoskeletons (shrimp and crab) was similar to the process used by Soares *et.al*, 2003, which followed the steps of pretreatment, demineralization, deproteinization, deodorization and drying, followed by deacetylation of chitosan. Such steps were performed in the textile laboratory, LABTEX/UFRN, Brazil and some analysis at CSIR - South Africa.

One of the preliminary operations to the obtaining of chitin was the separation of unwanted material in the collected samples. In the case of residues of crustaceans, the pre-processing included the milling to obtain smaller particles. Particle size used in this study was 0,297 mm. The demineralization step was aimed at reducing the ash content found in the samples. In this stage, worked with ratios of 1:3, 1:6 and 1:10, to the exoskeletons of shrimp and crab and were analyzed with the aim to verify which ratio would best to eliminate the carbonates and phosphates. These steps are described in Tab. 2a and 2b.

Table 2a. Demineralization of *Litopenaeus vannamei* shrimp diluted in ratio of HCl 1:3, 1:6 and 1:10.

Process	Weight (g)	Hcl (%)	Temperature (ºC)	Time (h)	Ratio sol/liq
Demineralization	10	2,5	23	1	1:3
Demineralization	10	2,5	23	1	1:6
Demineralization	10	2,5	23	1	1:10

Table 2b. Demineralization of *Ucides cordatus* crab diluted in ratio of HCl 1:3, 1:6 and 1:10.

Process	Weight (g)	Hcl (%)	Temperature (ºC)	Time (h)	Ratio sol/liq
Demineralization	10	7,0	23	1	1:3
Demineralization	10	7,0	23	1	1:6
Demineralization	10	7,0	23	1	1:10

As was showed by Table 2a and 2b used hydrochloride acid (HCl) 2.5% v/v for shrimp and 7.0% v/v for crabs, Fig. 5a and 5b. Both samples were washed repeatedly until the neutral pH. After filtering, the samples were placed in an oven at 80ºC for 1 hour and 25 minutes and then put to a desiccator to attain constant temperature, Fig. 5c.

a. b. c.

Figure 5. Samples in ratio of 1:3, 1:6 and 1:10. a. Shrimp b. Crab. c. Samples dried of shrimp and crab in desiccator.

Solution of sodium hydroxide 5% w/v was used for the deproteinization to reduce the content of protein nitrogen; soon afterwards the samples were repeatedly washed until pH neutral. In the deodorization process the deproteinized material was mixed with a solution of sodium hypochlorite 0.36% v/v under stirring to reduce the odour of the material and to remove any pigments. The samples were filtered, washed to remove any traces of the chemicals used as well as any impurities in the sample. The filtered samples were then dried in an oven at 80°C for 4 hours.

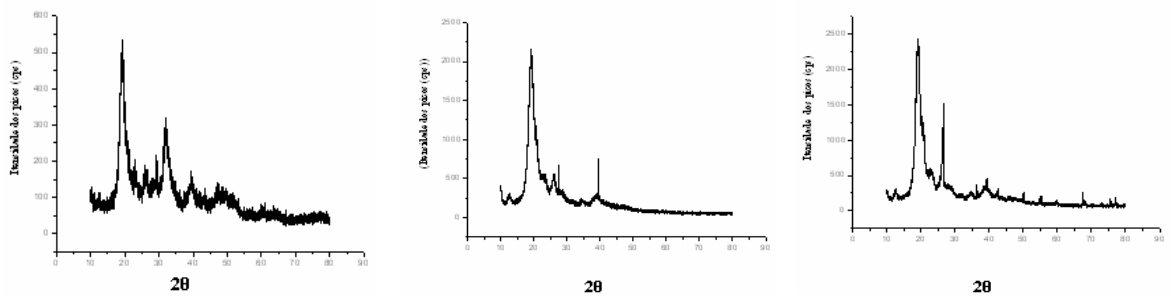
The deacetylation process occurred from the dried deproteinized samples of chitin, where were treated with NaOH 45ºBé (42.3%) and the solution was heated for 2 hours while stirring. The samples were filtered and washed until pH neutral. 1% of acetic acid was added, obtaining the chitosan dissolved. The solution was precipitated in an alkaline solution and neutralized using acid until the pH of 7.0. The purified chitosan was obtained after drying it in an oven at a constant temperature.

For the formation of chitosan membranes worked up with a chitosan solution, where was prepared and stirred continuously for 12 h at room temperature and later poured into a syringe of 5 ml, with distance of needle to collector was 10 cm. Both the syringe and the needle were tilted 45° from a horizontal baseline. The chitosan membrane was collected in aluminum sheet and the electrical potential was fixed at 15 kV. The same were dried at 70°C.

3. RESULTS AND DISCUSSION

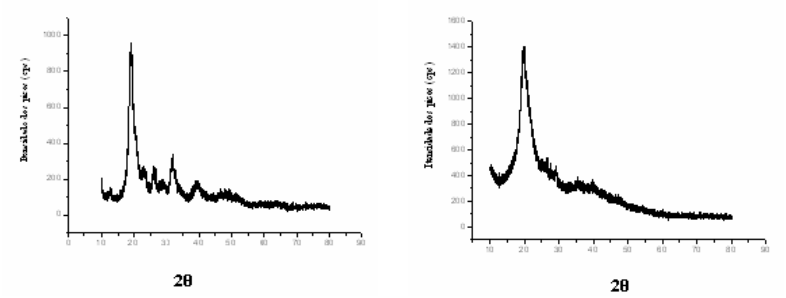
3.1. X-Ray diffraction (DRX)

The use of X-ray diffraction allowed distinguish the chitin clearly of its derived deacetylated. In fact the chitin diffractograms in the Fig. 6a, 6b and 6c presented more resolved signs and in larger number of the than observed in the chitosan diffractograms in Fig. 6d and 6e, what was attributed to the existence of crystalline domains, larger in more number in the case of the chitin (Roberts, 1992 and Zhang *et al.,* 2000).



a. b. c.

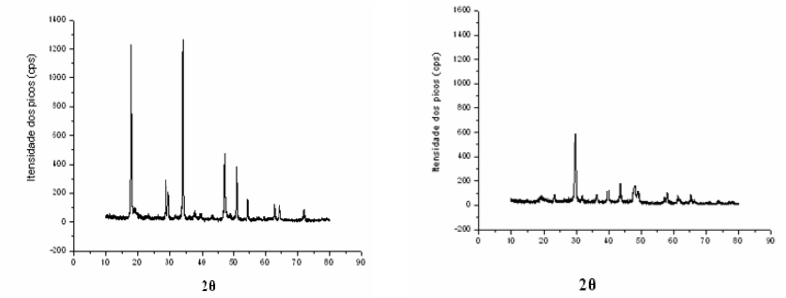
Figure 6. X-ray diffractograms a. shrimp chitin - *Litopenaeus vannamei* b. and c. shrimp chitin - *Aristeus antennatus*



d. e.

Figure 6. X-ray diffractograms d. shrimp chitosan -*Aristeus antennatus* e. shrimp chitosan -*Litopenaeus vannamei*

In the analysis of Fig. 7a and 7b, it could be observed that the spectrum of crab chitin showed several crystalline peaks and the crab chitosan showed aspect semicrystaline.



a. b.

Figure 7. X-ray diffractograms a. crab chitin -*Ucides cordatus* b. crab chitosan -*Ucides cordatus*.

The crystallinity index (I_{CR}) was determined with the use of Equation (1) (LI *et al*, 1998).

$$I_{CR} = \frac{I_C - I_A}{I_C} \times 100 \quad (1)$$

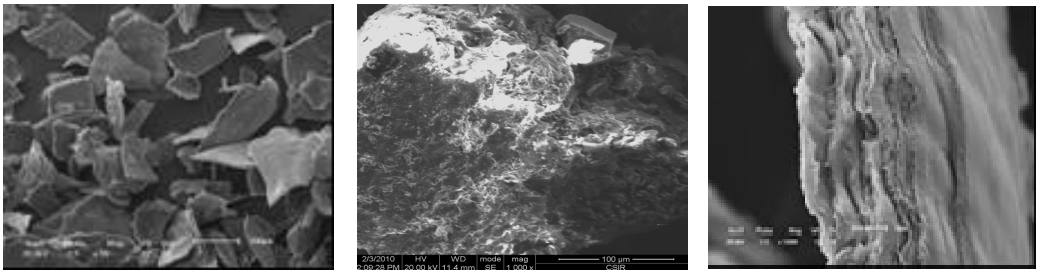
Where: I_C and I_A are the intensities of the signals from the crystalline regions (2θ ≈ 20) and amorphous (2θ ≈ 10 or 13 °), respectively. The chitosan extracted from crab exoskeletons showed crystallinity index of 87 % and (GD) 71,8 %, while that of shrimps varied between 66 % and 85.8 % and (GD) varied between 69,4 % and 73 %. Compared with the recommended deacetylation degree in literature showed that (GD>50%), proving the chitin deacetylation (Suh and Matthew, 2000).

Were also observed that the deacetylation degree (GD) was reverse to the relative crystallinity index, in other words, as higher the crystallinity index, lower the deacetylation degree, as is the characteristic of chitin having higher crystallinity (Antonino, 2007). It should also be noted that the crystallinity of the samples depends on several factors, such as the nature of the organism from which the chitin was extracted and the conditions employed in the extraction of the polymer (Gow and Goodway, 1987).

3.2. Scanning electron microscope (SEM)

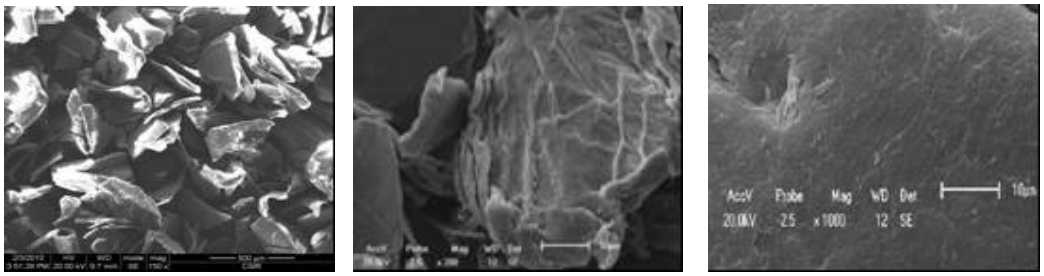
The morphological characteristics of the studied solids (chitin and chitosan) were evaluated by technique of scanning electron microscope (SEM) and the results showed that they had similar structures presenting various aspects of geometrically irregular, fine particles and loosely attached, as seen in Fig. 8a and 8d. Also still present a heterogeneous surface.

The micrographs of the samples in analysis showed morphologies quite wrinkled and fibrous surfaces, since it is observed in the Fig 8b, 8c, 8e and 8f.



a. b. c.

Figure 8 a. Chitin 50X b. Chitin 1000X c. Chitin 1000X



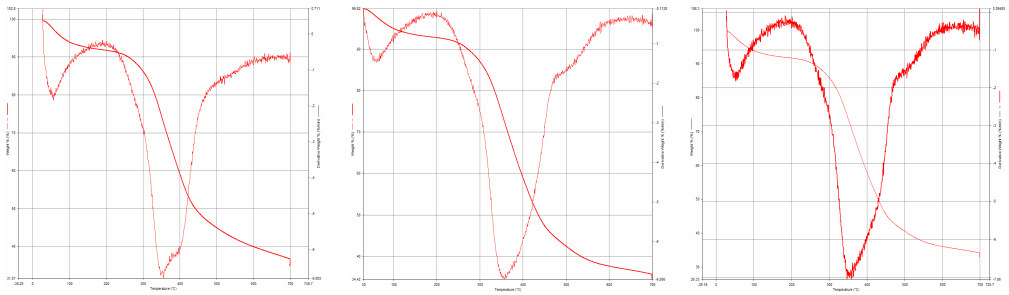
d. e. f.

Figure 8 d. Chitosan 150X e. Chitosan 200X f. Chitosan 1000 X

3.3. Thermogravimetric analysis (TG/DTG)

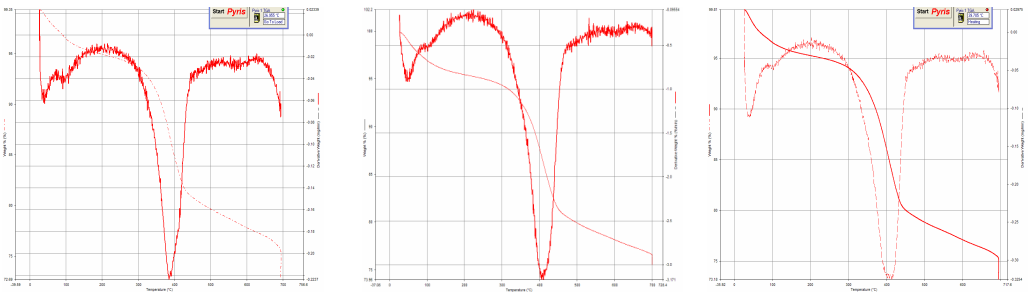
Thermogravimetric curves of the samples were obtained to verify the profile of thermal decomposition and to determine the temperature intervals corresponding to the percentage of hydration, decomposition of organic material and also used DTG curves corresponding to the first derivative of TG. Initially, a study on the effect of HCl concentration in samples from exoskeleton of shrimp and crab were carried out in order to determine the carbonate content.

The demineralization process, Fig. 9 and Fig. 10 was carried out in three ratios: 1:3, 1:6 and 1:10, as can be seen in the curves of weight loss as a function of temperature in shrimps and crabs exoskeletons.



a. Demineralization - ratio 1:3 b. Demineralization - ratio 1:6 c. Demineralization - ratio 1:10

Figure 9. TG/DTG of demineralization *Litopenaeus vannamei* shrimp in HCl ratios of: a. 1:3; b. 1:6 and c. 1:10.



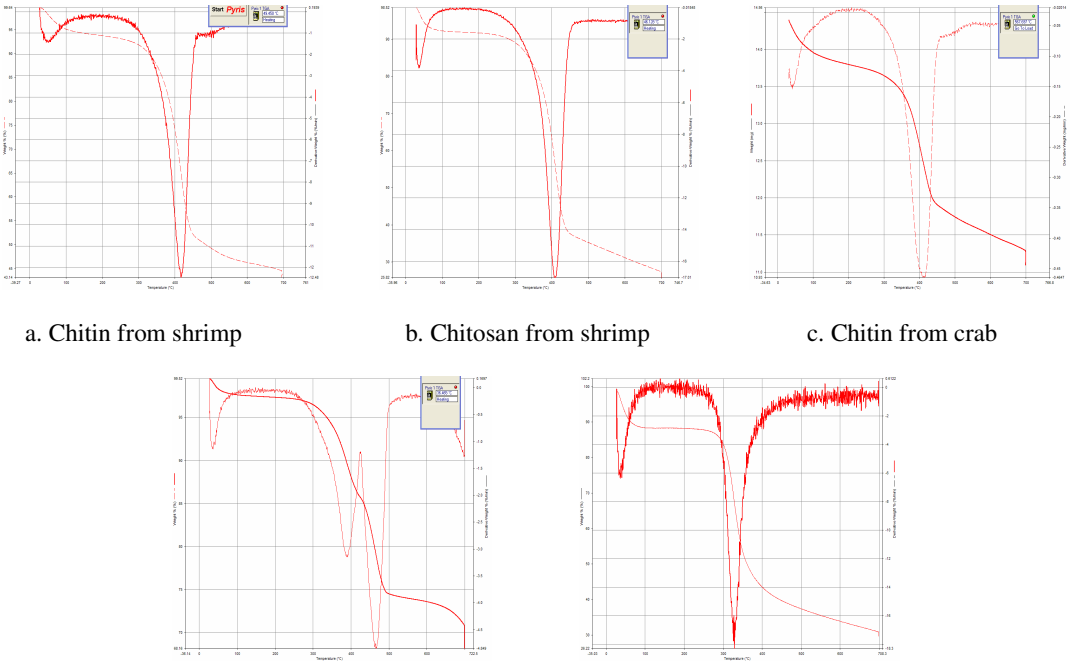
a. Demineralization - ratio 1:3 b. Demineralization - ratio 1:6 c. Demineralization - ratio 1:10

Figure 10. TG/DTG of demineralization *Ucides cordatus* crab in HCl ratios: a. 1:3; b. 1:6 and c. 1:10.

It was observed that the profiles of the thermogravimetric curves (TG/DTG) were similar. In agreement with the obtained results it was preferable to use low concentration in the demineralization process avoiding like this degradation. In Figure 11a, 11b, 11c, 11d and 11e the profile of the thermal decomposition of a TG / DTG of chitin and chitosan samples can be seen. In the thermograms of chitin, Fig. 11a and 11c two stages of decomposition can be observed. The first occurred in the range of 50-100ºC and was attributed to evaporation of water molecules and the second stage of decomposition occurred in the range of 400-500ºC and could be attributed to the degradation of saccharide structure of the molecule, including dehydration of saccharide rings and polymerization and decomposition of the acetylated and deacetylated units of chitin.

In the thermogram of chitosan, Fig. 11b had two stages of decomposition similar to that found in chitin, occurring respectively in the ranges of 40-100ºC and 400-500ºC. In Figure 11d had four stages of decomposition, occurring respectively in the ranges of 40-100ºC, 300-400ºC, 400-500ºC and 600-700ºC. In Figure 11e occurred in two stages, in the range 40-100ºC and in the range of 300-400ºC.

The second stage of chitosan decomposition from crab and shrimp chitosan, Fig. 11d and Fig. 11e, occurred at a temperature lower than that observed for the chitin decomposition. This suggests that chitosan has a lower thermal stability. The peak that appears around 390ºC and 329ºC respectively may be due to the degradation of the part of the molecule that has been deacetylated. Thus, it can be seen that the sample showed a dehydration process followed by decomposition of the biopolymer generating carbonized material.



a. Chitin from shrimp b. Chitosan from shrimp c. Chitin from crab

d. Chitosan from crab e. Chitosan from shrimp

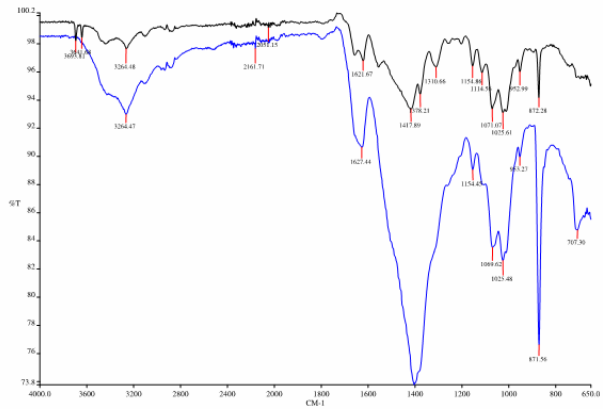
Figure 11. TG/DTG a. and b. chitin and chitosan from shrimp c. and d. chitin and chitosan from crab e. chitosan from shrimp.

3.4 Fourier transform infrared (FTIR) spectroscopy

The spectrographs of chitin and chitosan from *Ucides cordatus* crab are shown in Fig. 12a. The infrared spectrum of chitin showed the band with peak at 3264.47 cm^{-1} due to axial stretching vibrations of OH groups present in chitin, which is superimposed to the stretching band of N-H. The peak at 1627.44 corresponded to the characteristic absorption band of amide I, attributed to axial deformation of the carbonyl C = O present in chitin. The band at 1400 cm^{-1} was attributed to the symmetrical angular deformation of CH_3 group.

Comparing the spectrum of chitosan with the spectrum of chitin, there were significant changes in the region between 1400 cm^{-1}, a reduction in the peak. The band at 1655 cm^{-1} is associated the deformation axial carbonyl (C = O), called amide II.

The peak at 1378.21 cm^{-1} referred the vibration of C-H of CH_3 group, referred the symmetrical angular deformation band. The spectrum still showed absorption of peak at 3264.5 cm^{-1}, associated with the N-H, the hydrogen bond and the stretching of O-H.



– crab chitosan
– crab chitin

Figure 12 a. FTIR spectrogram of the chemical structure of chitin and chitosan from *Ucides cordatus* crab.

Figure 12b showed the characteristic bands of chitosan from *Litopenaeus vannameis* shrimp. It can be observed in the region of 3357 cm^{-1}, the corresponding to the axial OH stretch and at 1646 cm^{-1} is the band of C = O of amide I. The region 1375.72 corresponded to axial deformation of the C-N of amide. At 2875.97, corresponded to the stretching of C-H and the region near 1150 cm^{-1} corresponded to COC (polysaccharide).

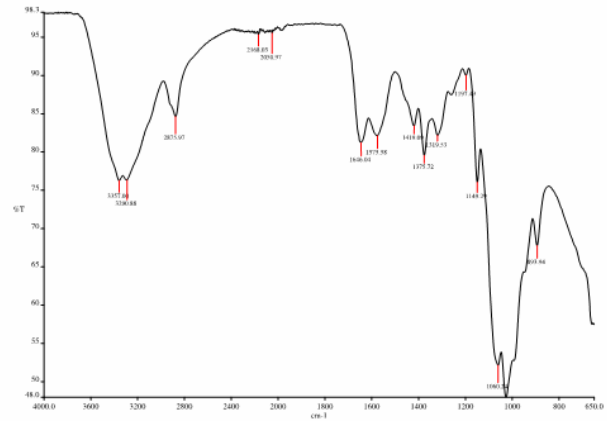
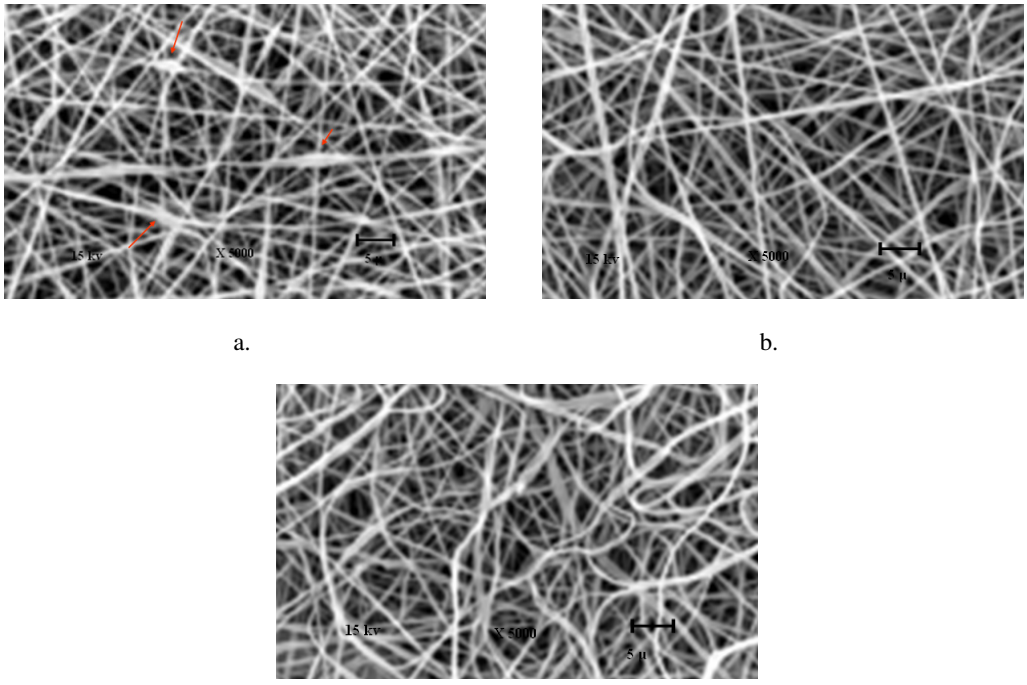


Figure 12 b. FTIR spectrogram of the chemical structure of chitosan from *Litopenaeus vannamei* shrimp.

3.5. Scanning electron microscope in nanofibers

Analysis in the chitosan membranes was performed in the scanning electron microscope (SEM), which are showed the in Fig. 13 a, 13b and 13c. This membrane was produced at a voltage of 15 kV, on 7 wt% chitosan solution was dissolved in acetic acid aqueous solution 60%. The micrographs were an increase of 5000 X and the diameter with (~120 nm).

The formation of beads in the presented nanofibers can be observed in Fig. 13a. The presence of moisture in the sample or the solvent can cause the appearance of beads, beyond electrical loading of the sample during testing of the SEM (Pham *et al.*, 2006).



a. b.

c.

Figure 13 a. b. c. Chitosan nanofibers in 5000 X.

4. CONCLUSION

Through this simple and versatile technique of electrospinning, biopolymers such as chitosan have been obtained into membrane and their production variables and morphology have been established. The polysaccharides (chitin and chitosan) obtained from crustacean exoskeletons from crab and shrimp are biodegradable, of low cost, renewable and of economy and environment important. The method to chitin deacetylation was effective compared with the recommended deacetylation degree in the literature (GD>50%). The technique of scanning electron microscopy showed format of the chitin and chitosan samples and their surfaces. It was observed from the x-ray diffraction analysis that the chitosan had a semicrystalline structure compared to chitin, which is more crystalline than that of chitosan. Also it was observed that the profiles of the thermogravimetric curves (TG/DTG) were similar. The characteristic bands analyzed by FTIR were very similar and showed that the same have basically the same functional groups and that chitosan has a lower thermal stability. This can be showed in the thermograms of chitin with endothermic effect to 50ºC and in the deacetylated form (chitosan) with endothermic effect to 40ºC. In this study, electrospinning was used for the production of chitosan membranes. It was noticed that the quality of chitosan produced to depend mainly on the extraction conditions, of the origin of raw material, of chemicals used and the immersion time in the sequence of treatments. The structures of membrane were controlled by adjusting the applied voltage, polymer concentration and solvent composition.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Abram, P., 2004. Chitin and Chitosan: Obtention, caracterization and applications. Pontifica Universidad Católica-Perú.

Antonino, A. N., 2007. Optimization of the process of obtaining of chitin and chitosan from shrimp exoskeletons derived of the Paraibana fishing industry. João Pessoa, PB, 84p.

Azevedo, V.V.C., Chave, S.A., Bezerra, D.C., Lia F.M.V. and Costa, A.C.F.M. 2007. "Chitin and Chitosan: applications as biomaterials." Journal of Electronic Materials and Processes Vol.2, pp.27-34.

Gow, N. A. R. and Goodway, G. W., 1987. Carbohydr. pp. 165, 105.

Hirano, S., 1996. Chitin Biotechnology Applications. Biotechnology Annual Review, Vol. 2, pp. 237-258.

Lehninger, A. L., Nelson, D. L. and Cox, M. M., 1995. Principles of Biochemistry. 2 ed. São Paulo: Sarvier.

Li, J. K., Wang, N. and Wu, X. S.,1998. J. Control Release, 56, 117 p.

Pham, Q. P., Sharma, U., Mikos, A. G. 2006. Electrospinning of Polymeric Nanofibers for Tissue Enginnering Applications: a review. Tissue Eng. 12. pp 1197-1211.

Rinaudo, M., 2006, Prog. Polym. Sci. 31, pp. 603-632.

Roberts, G. A. F., 1992, Chitin Chemistry, The Macmillan: Press Ltd, Hong Kong.

Soares, N. M., Moura, C. M., Rizzi, J., Vasconcelos, S. R., Santos, V.O.B. and Pinto, L.A.A., 2003. Study of the Chitosan Production Starting from Shrimp Residues in Pilot Scale. XVI - CRICTE_Regional Congress of Scientific and Technological Initiation in Engineering, Ijuí, RS.

Suh, J. K. F. and Matthew H. W. T. 2000. Biomaterials, 2589p.

Tonhi, E. and Plepis, A. M. G., 2002. "Quim. Nova", pp. 25, 943.

Tsigos, I., A. Martinou, D. Kafetzopoulos., and V. Bouriotis., 2000. Chitin Deacetylases: New, Versatile Tools in Biotechnology. Trends Biotechnol.

Zhang, M., Haga, A., Sekiguchi, H. and Hirano, S., 2000., "Int. J. Biol. Macromol". pp. 27.

7. RESPONSIBILITY NOTICE

The authors: Andrade, S. M. B., and Ladchumananandasivam, R., are the responsible for the written material in this paper.