EXTRACTION OF CHITIN AND CHITOSAN FROM THE EXOSKELETON WASTE OF CRUSTACEANS (SHRIMP AND CRAB)

Andrade, S. M. B. de, mestrsan@yahoo.com.br
Ladchumananandasivam, R, sivamrls@hotmail.com
Post-graduate Programme in Mechanical Engineering – PPGEM, Federal University of Rio Grande do Norte – UFRN, Natal, Brazil.

Galvão A. O, alcionegalvao@gmail.com
Belarmino, D. D, debelarmino@yahoo.com.br
Melo, J. P, joãopaulo_candel@hotmail.com
Post-graduate Programme in Mechanical Engineering – PPGEM, Federal University of Rio Grande do Norte – UFRN, Natal, Brazil.

Abstract. *The state of Rio Grande do Norte is the largest producer of shrimps in Brazil. The exoskeletons from these crustaceans are being thrown out as waste and thus resulting in environmental pollution. Chitin is the main substance found in the exoskeletons of these crustaceans and is being used in this study to extract chitosan and then use it to produce nanofibers. The chitosan is a hydrophilic biopolymer obtained from chitin, which is a polysaccharide abundant in nature, second in largest quantity produced in the world after cellulose. The chitin and chitosan have attracted greater interest of the scientists and researchers, as functional polymeric materials with applications in several areas: medicine, food industries, chemical, in agriculture, pharmaceuticals, cosmetics and the development of biomaterials, such as gels, films and polymeric membranes. The choice of chitin is justified due to its excellent properties such as: biodegradability, biocompatibility, non toxicity, antibacterial properties, emulsifier and binders. For the extraction of the chitin demineralization, deproteinization and deodorization process were carried out. After the extraction of chitin, it was submitted to deacetylation process to transform it into chitosan. Chitin and chitosan samples were analysed using X-Ray diffraction to verify the crystalline nature of their structures. It was observed from the analysis that the chitosan has a semi-crystalline structure compared to chitin which is more crystalline than that of chitosan. Thermogravimetric analysis was also carried out to confirm the variation in crystallinity of the two structures. The terminal amino groups in the chitosan structure also contribute to the amorphous structure of chitosan. The hydrogen bonds act as secondary links and contribute to the change in the angle of the bond between the chitosan molecules. Morphology studied of the chitin and chitosan were carried out using SEM, where the surface characteristics were analysed. It was observed that the quality of the chitosan produced depend mostly on the conditions of extraction, chemicals used as well as the time of immersion on the sequence of the treatments.*

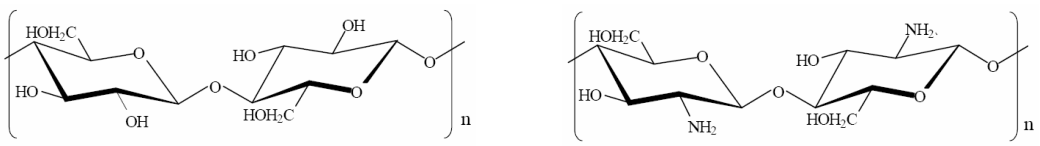
Keywords*: Chitin, chitosan, morphology, crystallinity.*

1. INTRODUCTION

The Northeast, especially the Rio Grande do Norte, offers exceptional conditions for the creation and production of shrimp as: average annual temperature of 27° C, salinity appropriate, high insolation, water rich in foods that come from the growth of mangroves, impervious and plans lands and ventilation, constituting like this the main factors for the carciniculture in the region. The state of Rio Grande do Norte stands out because of its extensive coastal areas available for cultivation of the shrimps. In 2010 the stage produced about 27 000 tonnes of shrimp. Currently the carciniculture of Rio Grande do Norte represent about 40 % of total exports of the state (CB, 2010).

Chitin is the second largest biomass source and the most abundant organic component in the skeletal structure of a lot of classes of invertebrates animals, as the arthropods, annelids, mollusks, coelenterates and is also present in the cell walls of fungi and some algae species (Dinesh and Alok, 2000).

Chitin and chitosan, Fig. 1a and Fig. 1b are polysaccharides that have attracted great interet of scientists and researchers as functional polymeric materials. Are nontoxic polymer, biodegradable, biocompatible and produced by renewable natural source, with applications in several areas: agriculture, food industry, textiles, pharmaceuticals, cosmetics and development of biomaterials, such as gels, films, membranes andpolymer nanofibers (Tonhi and Plephis, 2002).



a. b.

Figure 1. Structures chemistry of the polysaccharides a. chitin and b. chitosan

Chitin is a similar structure the cellulose, in which the hydroxyl groups at carbon-2 are replaced by acetamide residues and therefore resembles in many of its applications. Its structure was first studied around 1930 based on their chemical and enzymatic hydrolysis (Mathur and Narang, 1990).

The Japan, the USA and China are the largest world producers of chitin, but the polymeric 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 choice of chitin is justified due to its excellent properties such as biodegradability, biocompatibility, no toxicity, antibacterial properties, emulsifiers and binders. The present work extracted the chitin through the waste of crustaceans (shrimp-*Litopenaeus vannamei*, *Aristeus antennatus* and crab-*Ucide cordatus*). For the extraction of the chitin used the demineralization, deproteinization and deodorization processes. After extraction of chitin, the same was submitted to deacetylation process to transform in chitosan, which is an abundant hydrophilic biopolymer in the nature, second in largest quantity produced in the world after cellulose.

Chitin and chitosan samples were analyzed in this work using X-ray diffraction for verify the crystalline nature of their structures. It was observed from the analysis that chitosan has a semicrystaline structure compared the chitin which is more crystalline than chitosan. Thermogravimetric analysis was also carried out to confirm the variation in crystallinity of the two structures. The terminal amino groups in the chitosan structure also contributed to the amorphous structure. In accordance with Boschi (2006) the hydrogen bonds act as secondary links and contributed to the change in the angle of the bond between the chitosan molecules.

Morphology studied of the chitin and chitosan were carried out using SEM where the surface characteristics were analyzed. It was observed that the quality of the chitosan produced to depend mostly on the conditions of extraction, chemicals used as well the time of immersion in the succession of treatments.

2. MATERIALS AND METHODS

2.1 Materials

The raw materials used in this work were the waste of crustaceans (shrimp-*Litopenaeus vannamei, Aristeus antennatus* and *cr*ab-*Ucides cordatus*) in Fig. 2a, 2b and 2c. All the waste for chitin obtaining and chitosan production were acquired of local fairs and restaurants in city at Natal/RN, contributing like this to decrease of the pollution in the environment, as well as an educational work among the people there inserted in that activity). All reagents used in this work were of analytical degree P.A, Tab 1.

a. b. c.

Figure 2 a. shrimp - *Litopenaeus vannamei* b. shrimp - *Aristeus antennatus* c. crab - *Ucides cordatus*

Table 1. Reagents used in this work.

REAGENTS	SIMBOL	ORIGIN
Sodium hydroxide micro pearl P.A.	NaOH	Vetec
Cloridric 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

Chitin and chitosan were extracted from crustaceans, (*Litopenaeus vannamei, Aristeus antennatus e Ucides cordatus*). Morphological characteristics of the studied solids (chitin and chitosan) were evaluated by technique of scanning electron microscopy (SEM). X-ray diffraction studies were carried out using a universal X-ray diffractometer, model Shimadzu XRD-6000 with Cu radiation, with power of 30 kV and 30 mA. The samples were scanned with 10 to 80º. The thermogravimetric curves were obtained with a heating rate of 10 ^{o}C min^{-1} under a dynamic atmosphere of air in the temperature range of 25-700 ^{o}C.

2.3. Process of obtaining of chitin and chitosan

The process used for the chitin obtaining starting from shells of crustaceans was similar to the process used by Soares *et.al,* 2003, for shrimps and crabs residues, where it follows the pretreatment stages, demineralization, deproteinization, deodorization and drying. These stages were accomplished at the textile laboratory of UFRN/LABTEX.

One of the preliminary operations to the chitin obtaining had as objective the separation of the rude material, among its vegetable material, fabric portions and other materials that eventually can accompany the residue. In the case of the crustaceans' residues, the pretreatment included the washing, drying and grinding, in order to obtain smaller size. The granulation used in this work was of 48 mesh (0,297 mm), as show the Fig. 3. The demineralization stage had for objective to reduce the tenor of ashes with objective of verifying the elimination of carbonates and phosphates.

Figure 3. Granulation used in this work

For shrimps crustaceans worked up with hydrochloric acid (HCl) 2,5% v/v and 7,0% v/v for crabs crustaceans. Both samples after the washing until neutral pH were filtrated and put in hothouse in temperature 80ºC for 1:00 hour and 25 min and then transferred to a desiccator to attain constant temperature.

A solution of sodium hydroxide 5% w/v was used for the deproteinization step to reduce the nitrogen content of protein, under stirring and finally the samples were repeatedly washed to remove any traces of sodium hydroxide. In the deodorization step 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 the pigments. The samples were filtered, washed many times to remove any traces of the chemical used as well as any soluble impurities in the sample. The filtered samples were then dried in an oven at 80ºC for 4 hours.

The dried deproteinized and deodorized samples of chitin were treated with NaOH 45 °Bé (42.3%) and the solution was heated for 2 hours while stirring. At the end of the reaction time the samples were filtered and then washed until neutral pH. 1% of acetic acid was added to the chitosan to dissolve it and measured pH was 6.0. The solution was precipitated in an alkaline solution of NaOH and the samples were neutralized using acid until the pH of 7.0 was attained. The chitosan was obtained after drying it in an oven at a constant temperature.

3. RESULTS AND DISCUSSION

3.1. X-Ray diffraction (DRX)

The use of X-ray diffraction allowed to clearly distinguish the difference between chitin and its derivative deacetylated chitosan. In fact, the diffractograms of chitin in the Fig. 4a, 4b and 4c showed best resolved signs and in greater numbers than observed in the diffractograms of chitosan in the Fig. 4d, 4e and 4f, which could be attributed to the presence of higher crystalline areas in chitin than chitosan (Roberts, 1992 and Zhang *et al*, 2000).

Looking at Fig. 4a, the spectrum of chitin from shrimp showed several crystalline peaks, the most intense ones were related to the intensity of 514 and 318 cps, corresponding to 2θ = 19 280 ^{o} and 2θ = 31 900 ^{o}, respectively, while in Fig. 4b the crystalline peaks, with the intensities of 954 and 334 cps, corresponded to 2θ = 19 256 ^{o} and 2θ = 32 040 ^{o}. In Figure 4c, the crystalline peaks for the intensities 2396 and 1524 cps corresponded to 2θ = 19 040 ^{o} and 2θ = 26 500 ^{o}.

In Figure 4d the crystalline peak with the intensity of 2050 cps, corresponded to 2θ = 19 100 °. In Figure 4e crystalline peaks with the intensities of 1566 cps corresponded to 2θ = 18 729 °. By analyzing the spectrum of chitosan in the Fig. 4f, the crystalline peak with the intensity of 140 cps corresponded to 2θ = 19 840 °. By analyzing the diffractograms of chitin and chitosan samples, it could be verified that chitosan had a semi-crystalline structure.

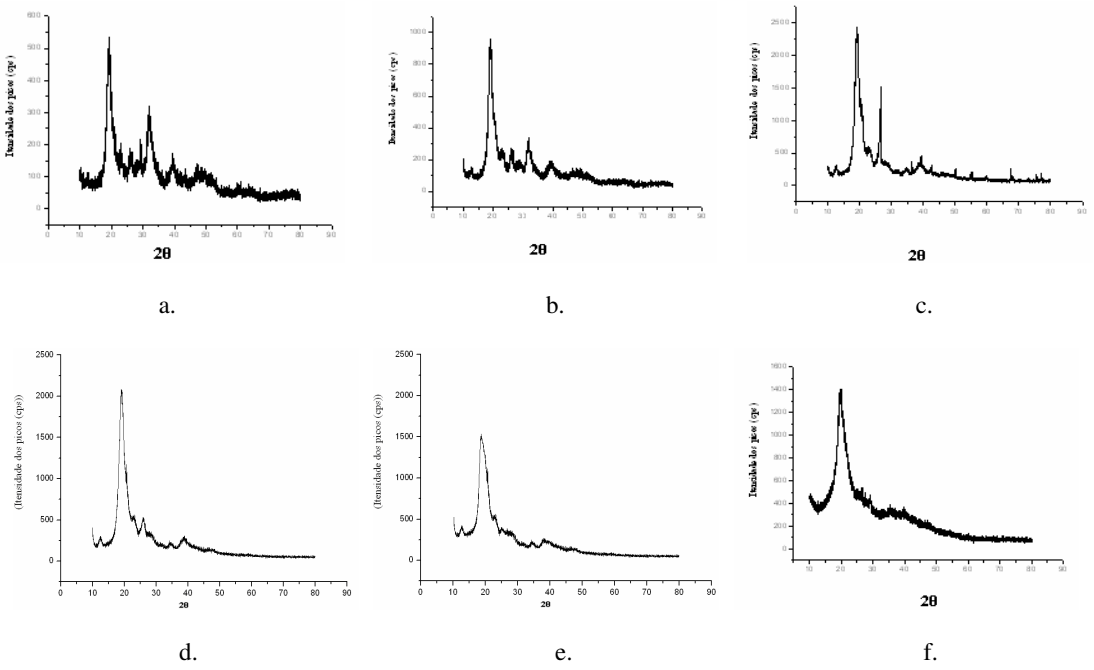


Figure 4. X-ray difratogramas. a. chitin from shrimp-*Litopenaeus vannamei*; b. and c. chitin from shrimp -*Aristeus antennatus*; d. chitosan from shrimp-*Aristeus antennatus*; e. and f. chitosan from shrimp-*Litopenaeus vannamei*

In the analysis of Fig. 5a, it was observed that the spectrum of chitin from crab showed several crystalline peaks, the most intense ones attained an intensity of 1232, 1262, 474, 380 and 228 cps, corresponded to 2θ = 34 140 °, 2θ = 18 040 °, 2θ = 47 160 °, 2θ = 50 820 ° and 28.780 ° respectively, while in Fig. 5b the crystalline peak related to the intensity of 566 cps, corresponding to 2θ = 29 520 °.

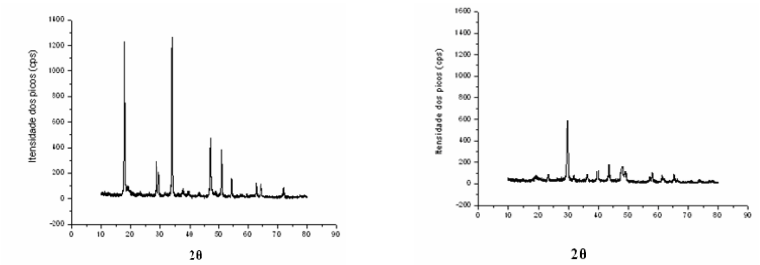


Figure 5. X-ray diffractogram from 10 to 80º (2θ). a. Chitin from crab- *Ucides cordatus* b. Chitosan from crab-*Ucides cordatus*

Chitosan itself has no absolute standard of crystallinity; therefore, from the two peaks of highest intensity of X-ray diffraction of this material, the crystallinity index was determined. The crystallinity index (I_{CR}) could be 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 ($2\theta \approx 20$) and amorphous ($2\theta \approx 10$ or 13 °) respectively. The relationship between the degree of deacetylation (DD) and the relative crystallinity index is inversely proportional. The higher is the crystallinity index; the lower is the degree of deacetylation, which is characteristic to chitins that possess high degree of crystallinity. Data correlating the samples with their crystallinity index and degree of deacetylation is shown in Tab 2.

Table 2. Results for chitin and chitosan samples of shrimp and crab.

SAMPLES	I_A	I_C	% I_{CR}	%GD
Chitin from shrimp - *Litopenaeus vannamei*	116	514	77,4	-
Chitin from shrimp - *Aristeus antennatus*	151	954	84,2	-
Chitin from shrimp - *Aristeus antennatus*	274	2396	88,5	-
Chitosan from shrimp - *Aristeus antennatus*	290	2050	85,8	73,0
Chitosan from shrimp - *Litopenaeus vannamei*	431	1566	72,4	70.2
Chitosan from shrimp - *Litopenaeus vannamei*	474	1404	66,2	69,4
Chitin from crab - *Ucides cordatus*	37	1262	97,0	-
Chitosan from crab - *Ucides cordatus*	72	566	87,0	71,8

The results of the crystallinity index (I_{CR}) showed very close values to chitin under chemical treatment with sodium hydroxide. Chitin has led to low crystallinity of chitosan, as well as and the conditions of the treatments used for chitin and chitosan. 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. Thus, samples of chitin obtained in this study have a diffraction pattern with well defined peaks and higher crystallinity.

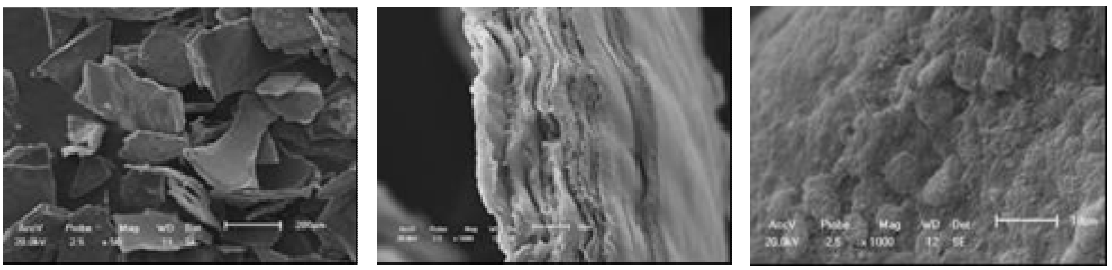
The chitosan extracted from crab carapace had crystallinity index of 87% while that of shrimps varied between 66 and 85.8%. These value were similar to that presented in the literature by Weska, *et.al*., 2007. This proves that the deacetylation process was efficient, due to removed of a portion of the acetyl groups from the chitin structure and, thus, to the greater presence of primary amine groups in the chitosan (Craveiro *et.al*., 1999). Soon the crystallinity was decreased during the deacetylation process.

The crystalline structure of chitosan has been shown to be similar to cellulose in the arrangements of inter and intrachain hydrogen bonding. The terminal amino groups in chitosan structure also contribute to the amorphous structure of chitosan, because the hydrogen bonds act as secondary links and contribute to the change in the angle of the link between the molecules of chitosan (Boschi, 2006). The hydrogen bonds generate zones in amorphous polymer with a capacity greater than the swelling of the crystalline zones, due in part to the high affinity primary amine groups to water. In summarizing, the chitosan has a lower crystallinity of chitin and therefore is more susceptible to hydration and dissolution in aqueous media, especially in low PH values.

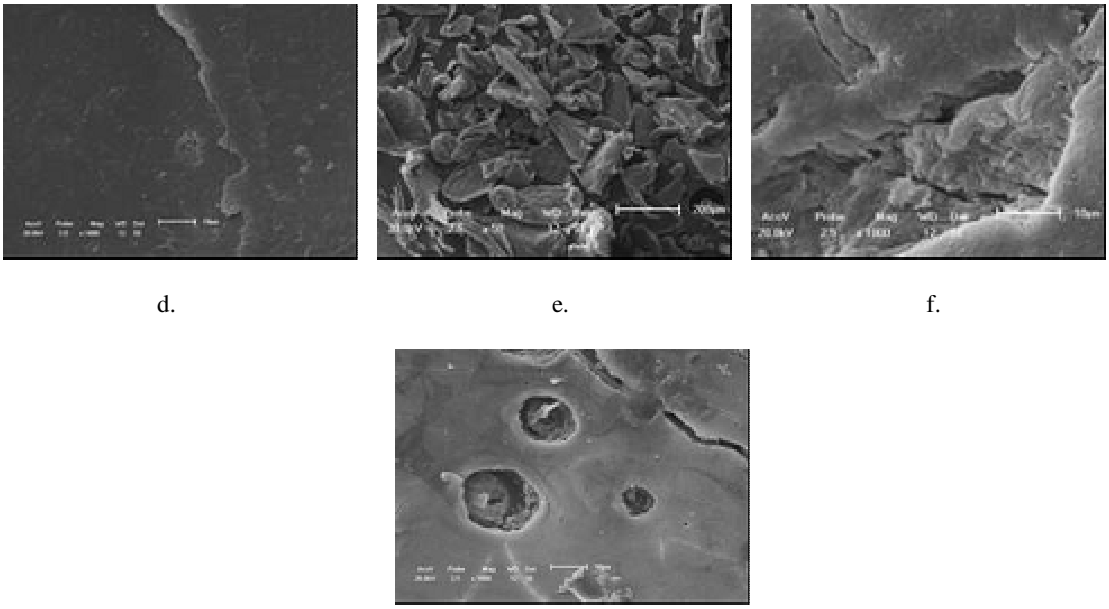
3.2. Scanning electron microscope (SEM)

The results showed that the chitin and chitosan had similar structures presenting various aspects with irregular geometry, fine particles and loosely attached to it, as seen in Fig. 6a and 6e and still presented a heterogeneous surface.

The micrographs of the samples in analysis showed morphologies of surfaces very wrinkled and fibrous, as was observed in the Fig. 6b, 6c, 6d and 6f. The characteristics presented in the micrographs of the Fig. 6g showed some pores, may be due to the accented concentration of NaOH.



a. b. c.



d. e. f.

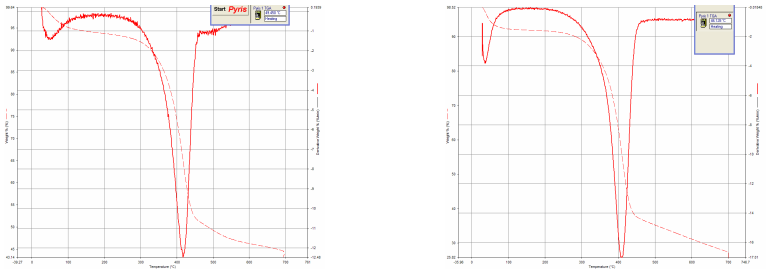
g.

Figure 6. Micrographs of a. Chitin 50 x b. Chitin 1000 x c. Chitin 1000 x d. Chitosan 1000 x e. Chitosan 50 x f. Chitosan 1000 x g. Chitosan 1000 x

3.3. Thermogravimetric analysis (TG/DTG)

In the thermogram of chitin, Fig. 7a can be observed two stages of decomposition. The first occurred in the range of 50-100 ^{o}C and was attributed to evaporation of water molecules. The second stage of decomposition occurred in the range of 400-500 ^{o}C 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 in Fig. 7b, occurred two stages of decomposition similar to that found in chitin, occurring respectively in the ranges of 40-100 ^{o}C and 400-500 ^{o}C.



a. b.

Figure 7 a. Chitin b.Chitosan

With the analyzed results it was observed that thermogravimetric analysis and X-ray diffraction both revealed that the reaction deacetylation decreases the thermal stability and crystallinity of the sample, proving this, in Fig. 7a (endothermic effect to 50 ºC) and in the deacetylated form, Fig.7b (endothermic effect to 40 ºC).

4. CONCLUSION

The study showed a form to use of crustaceans the exoskeletons (*Litopenaeus vannamei, Aristeus antennatus* and *Ucides cordatus*) that are played out as waste, through the extraction of the chitin and chitosan, contributing like this to avoid the environmental pollution. The method of deacetylation of chitin was effective and may be proved by the

deacetylation degree, presented for the degree of chitosan deacetylation higher than the minimum recommended in the literature (GD >50%). The control of time and of temperature is important to the initial stage of treatment of chitin, in order of removed a percentage of proteins and salts. The technique of scanning electron microscopy was essential for particulars format of the chitin and chitosan samples. It was observed from the X-ray diffraction analysis that the chitosan has a semi-crystalline structure compared to chitin which is more crystalline than that of chitosan. 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.

5. ACKNOWLEDGEMENTS

Thank to God, the CAPES, to PPGEM, The CTPETRO-INFRA and FINEP/LIEM. To Dr. R. Anadjiwala, Dr. Rakesh Kumar, Dr. Maya John and Mrs. Valencia Jacobs of CSIR.

6. REFERENCES

Abram, P., 2004. Chitin and Chitosan: Obtención Caracterización y Aplicaciones, Pontificia Universidad Católica-Perú.

Boschi, A. O., 2006. Mechanical Tests of Chitosan Membrane Coated with hydroxyapatite: Effect of Addition of Sodium Hydroxide. UFSCar, SP.

Centro de Biociências. 2010. Carciniculture in Rio Grande do Norte, 12 Dec. 2010. <http://www.cb.ufrn.br/>.

Craveiro, A. A., Craveiro. A. C, and Queiroz, D. C., 1999. Chitosan Fiber of the Future. Park Technological Development. PADATEC, Fortaleza, Ceará.

Dinesh, K.S. and Alok, R.R., 2000. Journal of Macromolecular Science-Polymer Reviews Part *C*; 40**.**

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

Mathur N.K. and Narang CK., 1990. "Chitin and Chitosan: Versatile Polysaccharides from Marine Animals", J. Chem. Educ.67(11), 938.

Roberts, G. A. F., 1992. Chitin Chemistry, Macmillan: London.

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

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.

Tonhi, E. and Plepis, A. M. G., 2002. New Chemistry, pp. 25, 943.

Weska, R. F., Moura, J. M., Batista, L. M., Rizzi, J. and Pinto L. A. A., 2007. Optimization of Deacetylation in the Production of Chitosan from Shrimp Wastes- Use of Response Surface Methodology. Journal of Food Engineering, Vol. 80, pp. 749 - 753.

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

7. RESPONSIBILITY NOTICE

The authors: Andrade, S. M. B. de., Ladchumananandasivam, R., Galvão A. O., Belarmino, D. D., and Melo, J. P. are the only responsible for the written material included in this paper.