

## THE IMPORTANCE OF CHITIN BIOPOLYMER EXTRACTED FROM THE EXOSKELETONS *LITOPENAEUS VANNAMEI* SHRIMP

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**Abstract.** Chitin is biopolimer  $\beta$ -(1-4)-N-acetil-D-glucosamine is the most abundant found in the nature, after the cellulose. Chitin derived from the Greek word *khitón*, meaning a protective covering of invertebrates and is found in the crustaceans' exoskeletons, in the cellular wall of fungi and other biological materials. The main sources of chitin are the residues of shrimp, crab and lobster. Shrimp has about 5-7% of chitin in its composition and crab has about 15-20%. The shrimp residue contains: 15 to 20% of chitin, 25 to 40% of proteins and 40 to 55% of calcium carbonate. The expansion of the shrimp industry has generated environmental impacts as a result of the disposal of industrial residues in sanitary bankments, seas and rivers. The objective of this study was to develop chitin present in exoskeletons *Litopenaeus vannamei* shrimps, which is being discarded as waste, thus causing pollution and environmental problems and thus get a better utilization of these raw materials. Also, show the process of extraction of chitin. The *Litopenaeus vannamei* shrimp is native from the Pacific Ocean and was introduced in the Brazilian culture since the decade of the eighty. It has had its productivity increased in the northeast for presenting favorable handling characteristics and excellent adaptation to the climate and regional diversities. The State of Rio Grande do Norte is the largest producer of shrimps in Brazil. The chitin is a polysaccharide that has attracted great interest of scientists and technologists as functional polymeric materials with great potential applications in several areas: medicine, food industries, chemical, agriculture, pharmaceuticals, cosmetics and the development of biomaterials, such as gels, films and polymeric membranes. Chitin presents to excellent properties such as: biodegradability, biocompatibility, non toxicity, antibacterial properties, emulsifier and chelate. The process of chitin obtaining follows the stages of demineralization, deproteinization and deodorization. The chitin can be found in three different forms of structural arrangement depending on yours crystalline structures, of the chains disposition and of the presence of water molecules such as: alpha, beta and gamma. The alpha chitin has intermolecular connections of hydrogen strong some with reticular structure more disorganized that other.

**Keywords:** *Litopenaeus vannamei*, chitin, biopolymer.

### 1. INTRODUCTION

The biopolymers are natural polymeric such as collagen, elastin, hialurônico acid, dextran, cellulose and chitin. The chitin in Fig. 1a is the biopolymer  $\beta$ -(1-4)-N-acetil-D-glucosamine most abundant found in the nature, after the cellulose. Its chemical structure is similar to cellulose in Fig. 1b and can be differentiated by the hydroxyl group located at position 2, which were replaced by chitin in acetamine groups. It has been estimated that  $10^0$ - $10^{12}$  tons of chitin are biosynthesized each year (Percot, 2003).

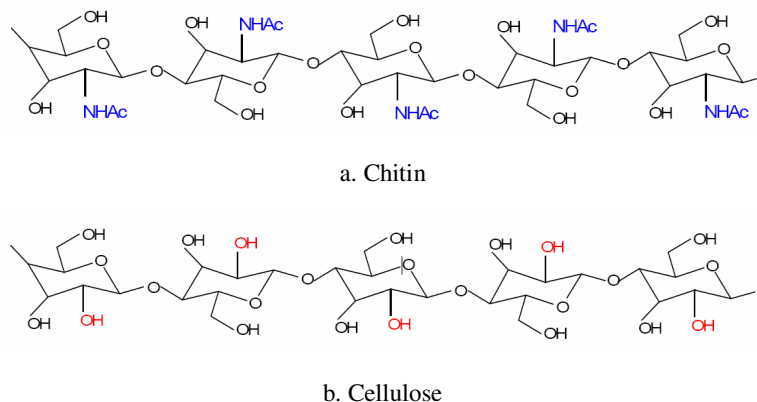


Figure 1. Molecule structure of chitin and cellulose

Chitin is derived from the Greek word *khitón*, meaning a protective covering of invertebrates and is found in the crustaceans' exoskeletons (shrimp, crab and lobster), in the cellular wall of fungi and other biological materials.

Ahead of the concern to the appropriate destiny for the residues of crustaceans of the fishing industry, studies have been accomplished in the sense of finding an appropriate destiny for the *Litopenaeus Vannamei* shrimp so that the aggressions to the environment are more and more reduced. The residue from shrimp is constituted by 15 to 20% of chitin, 25 to 40% of proteins and 40 to 55% of calcium carbonate (Mathur and Narang, 1990). With this can cause great problem of social order for being unpleasant in the smell, for attract insects and moreover, it can to cause damages the human health.

The shrimp is becoming the main sea product of the northeast and of the country. However the *Litopenaeus vannamei* in Fig. 2 is not native of this area, but original of the Pacific Ocean, being created actually in nurseries in coastal farms. It is more cultivated in Brazil answering for more than 95% of the national production, has been introduced in Brazil in the decade of eighty. The cause of it great search was accelerated growth rate in high densities, conversions excellent and big alimentary capacity to adapt to the different climatic conditions.



Figure 2. *Litopenaeus vannamei*

The northeast has been chosen to base projects of that nature, for it possesses extensive coastal strip and of hot waters, ideals for the development of the shrimp. The shrimp mainly peeled, could generate four hundred and sixty ton/year of residues (peel and head). These residues composed of chitin, proteins and minerals are discarded usually in the sea, in embankments used for animal ration or as fertilizers. When played in the sea that residue liberates unpleasant odors, it provides the exaggerating proliferation of algae and with the great generated volume becomes a serious environmental problem. When deposited in embankments, the residues are responsible for the formation of having leached and biogas, advancing pollution, propagation of bad smells and proliferation of vectors of diseases as mice and insects.

The chitin biopolymer was isolated by the French teacher Henri Braconnot in 1811, thirty years before the isolation of cellulose, but the lack of basic knowledge about its properties, including chemical reactivity, has severely limited their industrial applications until the early 1970s. Discovery in mushrooms received the initial denomination of fungina.

The name chitin was given by Odier in 1823 that it isolated this insoluble substance of the armour/shell of the insects (Sandford, 1989). Odier also observed the presence of the chitin in the crab shell and suggested that it would be the basic material in the formation of the exoskeleton of all of the insects and possibly of the arachnids. Only in 1843, Payen discovered that the chitin contained nitrogen in its structure (Craveiro *et al.*, 1999). In 1859, Rouget (Velásquez, 2003) discovered the chitosan.

Although chitin has been discovered two centuries ago, studies and research of applications have been stepped up around 1970, when if realized the great potential of application that presented both the chitin and chitosan amongst themselves. Chitosan has been produced industrially for the first time in 1971 in Japan and in 1986 fifteen industries producing chitin and chitosan, in commercial scale, it existed at that country. Nowadays, these polysaccharides have been researched and their applications are considered high potential for the twenty-first century.

The chitosan in Fig. 3 has one primary amine and two free hydroxyl groups for each monomer with a unit formula of  $C_6H_{11}O_4N$ . This natural biopolymer is a glucosaminoglycan and is composed of two common sugars, glucosamine and N-acetylglucosamine, both of which are constituents of mammalian tissues (Khan, 2001; Snyman *et al.*, 2002).

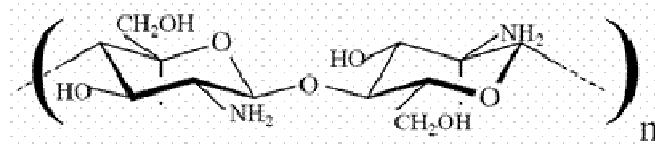


Figure 3. Structures chemistry of chitosan, where “n” is the degree of polymerization

Chitin presents to excellent properties such as: biodegradability, biocompatibility, non toxicity, antibacterial properties, emulsifier and chelate. The chitin can be found in three different forms of structural arrangement depending on yours structures crystalline, of the chains disposition and of the presence from water molecules such as:  $\alpha$ ,  $\beta$ , and  $\gamma$  chitin in Fig. 4. These variations in the crystalline domains attach to the different polymorphisms properties, because the possibilities of hydrogen bonding intra and intermolecular.

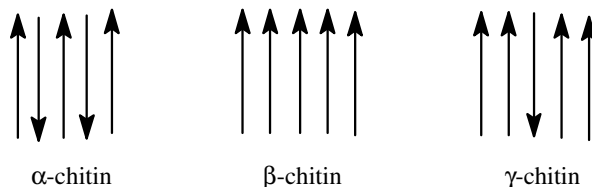


Figure 4. Orientations of the polymeric chains in the different chitin forms

The  $\alpha$ -chitin is the most common and most abundant form of this biopolymer. It's also considered the most stable. The chains are antiparallel, it has the strongest magnetic field, resulting in a dense packaging and it's present in crustaceans as shrimp, insets and fungi. It from which nowadays is obtained the most of commercial chitin (Peesan *et al.*, 2003).

The  $\alpha$ -chitin is found in resistant and rigid structures, such as the cuticle of arthropods (crab and shrimp) and in these cases is strongly associated with proteins, inorganic materials or both. The  $\alpha$ -chitin has intermolecular connections of hydrogen strong.

The  $\beta$ -chitin has a parallel structure resulting in less dense packing than for  $\alpha$ -chitin. It's obtained from squids and microscopic algae. It exhibit greater reactivity, swelling and solubility in water than the  $\alpha$ -chitin.

The form  $\alpha$  chitosan has strong intermolecular hydrogen bond, while  $\beta$  chitosan is characterized by weak intermolecular effects.

The  $\gamma$ -chitin form has not been fully classified but an arrangement of two parallel chains and one antiparallel chain has been suggested. The  $\gamma$ -chitin is obtained from ganglia of squids. Forms  $\beta$ -and  $\gamma$ -chitin occurs in flexible structures but also tough.

The process to obtain the chitin follows the stages of demineralization, deproteinization and deodorization. After those phases the material is dry. The chitosan is obtained starting from the deacetylation of the chitin, as in Fig. 5 although it is found naturally in the cellular wall of some mushrooms (Chatterjee *et al.*, 2005).

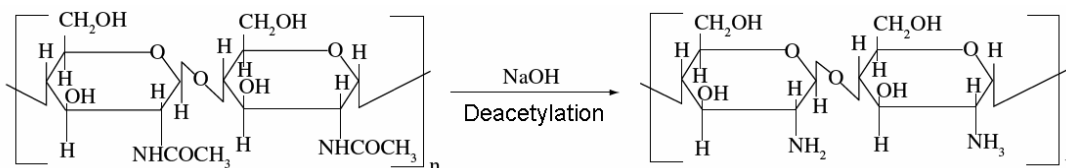


Figure 5. Deacetylation of the chitin

Chitin contains 6–7% of nitrogen and in its deacetylated form (chitosan) contains 7–9.5% of nitrogen. In chitosan between 60 to 80% of the acetyl groups available in chitin are removed (Mathur and Narang, 1990).

## 2. APLICABILITY OF CHITIN AND CHITOSAN

The poor solubility of chitin is the major limiting factor in its utilization and investigation of its properties and structures. This is resulting from the presence of strong hydrogen bonds between the chains polymeric.

The chitin and the chitosan are polysaccharides that have attracted great interest of scientists and researchers as functional polymeric materials with great potential applications in several areas: medicine/pharmaceuticals, food industries, agriculture, cosmetics, development of biomaterials, such as gels, films, photographs, used also in treatment of waters and scaffolds for the regenerations of tissues.

Medicine/pharmaceuticals: The chitosan is a very useful polymer for biomedical applications because of its biocompatibility, biodegradability and low toxicity. Chitin has properties of flexible materials with applications for specific purposes, such as sponges and bandages for the treatment of wounds and sutures lines and is very favorable for the cure of the wound. It can be used in the ophthalmology as protecting ocular, fabrication of films, contact lens, blood cholesterol control and skin burn. In the recovery submitted to oculars of intra of surgeries or in cases of reaching a chronic agreement of the cornea because it doesn't need removal, because it is biodegradable.

**Food industries:** Chitosan offers a wide range of unique applications in the food industry, including preservation of foods from microbial deterioration, formation of biodegradable films and recovery of material from food processing discards. Moreover, it can act as a dietary fibre and as a functional food ingredient.

**Agriculture:** Most of the recent studies display the chitin as a good mechanism for defense in plants, acting as agent antifungal in the control of diseases. It was also evaluated as a fertilizer that can improve global crop incomes. Chitosan is derived of chitin, which is used as a biocontrol elicitor in agriculture and horticulture.

**Cosmetics:** Because of its cationic character the chitosan interacts with negatively charged biological surfaces, such as skin and hair. It is used in the field of cosmetics as products for hair (conditioning, shampoo) products for hands, face and for body creams, lotions of bath and moisturizer.

**Biomaterial:** The great variety of chitosan applications in the field of biomaterial is due to its excellent properties. The same interact with the human body due the: bioactivity, antimicrobial activity, immune stimulation, chemotactic action, enzymatic biodegradability, mucoadhesion and epithelial permeability which supports the adhesion and proliferation of different cell types. This biopolymer has been used in treatment of wounds, ulcers and burns, calling upon its haemostatic properties and its accelerating wound healing effect.

**Film:** Are obtained eatable films that promote better quality and conservation of fresh products, as: frozen meat, vegetables and fruits, besides it is biodegradable and it increases the life of storage of the frozen products. Chitin can substitute the plastics eventually (products of petroleum) because the same has a very long life (more than 300 years) different of the chitin that is biodegradable. Besides the possibility to be employed in construction materials as resistance of extreme pressure.

**Photography:** The chitosan has been used in the development of products for photography and printing. The same has optical characteristics and ability to form films that act as protector on photographic paper, mainly due of its resistance to the wear.

**Treatment of waters:** The chitosan presents a great capacity to fasten molecules as pesticides, proteins and colorations. The amino group of chitosan checks the greatest similarity to form complex with transition metals than other natural polysaccharides such as cellulose.

**Scaffold for the Regeneration of Tissue :** Chitin and its derivatives have been used as scaffolds for bone and other natural tissue regeneration as well as structures by which three-dimensional formation of tissues are supported. There are at least four important factors that should be taken into account: ability to form temporary matrix, ability to form porous structure for tissue to grow, biodegradability and finally non-toxic by products from the digestion.

### 3. MATERIALS AND METHODS

#### 3.1 Materials

The raw material used in this work was the crustacean residues (shrimp - *Litopenaeus Vannamei*) for obtaining of chitin. The samples were acquired of local restaurants of the city of the 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.

Table 1. Reagents

Reagents	Symbologi	Origin
Sodium hydroxide micro pearl P.A	NaOH	Vetec
Cloridric acid P.A	HCl	Quimex
Sodium hydroxide P.A	NaOCl	QM
Acetic acid P.A	CH <sub>3</sub> COOH	Vetec
Propane acetone P.A	(CH <sub>3</sub> ) <sub>2</sub> CO	Vetec

#### 3.2 Methods

The *Litopenaeus Vannamei* shrimp was the source to extract the chitin through of stages of demineralization, deproteinization and deodorization. After was realized the deacetylation of chitin to obtain the chitosan. Starting from the initial mass of the shrimp residues it was weighed and the obtained products was made calculations of percentage of yield process. Morphologics analysis was accomplished with using SEM- Scanning electron microscope and X-Ray diffraction with the aid XRD.

#### 3.3. Preparation 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, as show in Fig. 6. The steps used for the extraction of chitin and chitosan followed the phases of the

pretreatment, demineralization, deproteinization, deodorization and deacetylation. These stages were accomplished at the textile laboratory of UFRN/LABTEX.

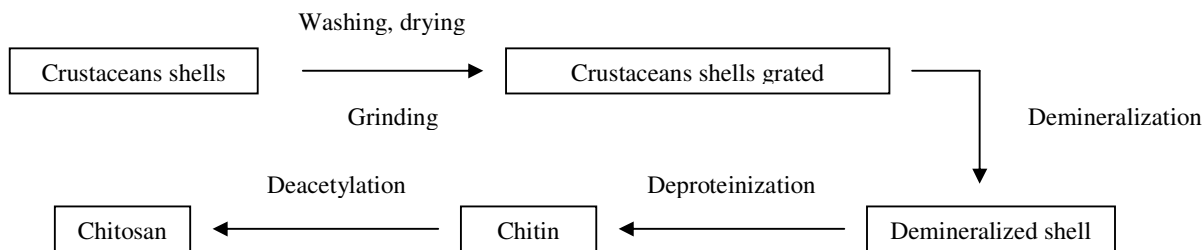


Figure 6. Obtaining chitin and chitosan

The preliminary operations to the chitin obtained had as objective the separation of the rude material, among them vegetable material, fabric portions and other materials that eventually can accompany the residue. In the case of the crustaceans' residues included the pretreatment of grinding to obtain smaller size. The size used was 0,297 mm. The demineralization stage had for objective to reduce the tenor of ashes of the raw material. It was used chloride acid 2,5% v/v, in the case of the shrimp residues and agitation. After washed until neutral pH. The deproteinization stage had the function of reducing the tenor of nitrogen full of protein and consisted of adding of sodium hydroxide solution 5% p/v to the demineralized raw material with agitation. Soon afterwards it was made the wash of this material to neutral pH. In the deodorization stage, the desproteinization raw material was put in agitation in the solution of sodium hypochlorite 0,36% v/v. The objective of operation is to accentuate the reduction from odor the material and the retreat of pigments. It was made the wash with water to remove the sodium hypochlorite, until neutral pH. After the deodorization it was necessary the drying of the obtained product (humid chitin). That drying was accomplished to the temperature of 80°C by four hours.

The process of chitosan production was accomplished starting from the deacetylation of the chitin, in that the chitin reacted with solution of NaOH 45°Bé (42,3 %). The reaction happened with agitation and heating for two hours. At the end of the time of reaction a wash was accomplished with water removing the excess of the reagent, what was verified through the measurement of the pH. After the deacetylation of the chitin it was obtained the chitosan and then gone by a purification process. The chitosan was added in solution of 1% acetic acid, of which was obtained the dissolved chitosan, since this is soluble in diluted organic acids. After it was made the neutralization even with acid pH 7,0 and soon afterwards the drying was made in greenhouse and after having left constant temperature with aid of the desiccator.

## 4. RESULT AND DISCURSION

### 4.1. Calculation of yield

Worked with about of 3 000 g *Litopenaeus vannamei* shrimp, where each 1 000 g corresponded about of 132 units. In Fig. 7a it can be observed the variability with different size and weights of the samples. The samples were weighed on an analytical balance and showed a variation of weights in nature between 4,34 to 10,21 g in the Fig.7b, obtained an average of 6,67 g. Each 1000 g corresponded it about 458 g of meat, 103 g of shells of shrimp, 258 g of residue and the rest of water.



Figure 7 a. Samples of *Litopenaeus vannamei* shrimp used in this work

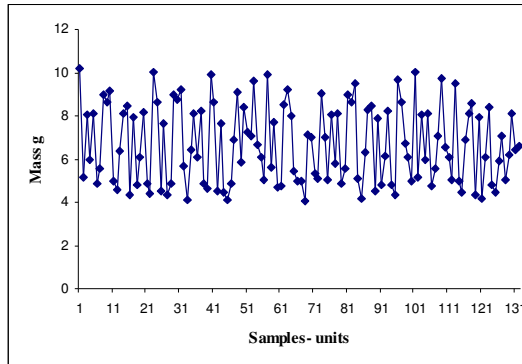


Figure 7 b. Weight of the samples

In Table 2 it presents the income of the obtaining of chitin and production of chitosan in relation to initial mass used in this work after the stages of demineralization, deproteinization and deacetylation.

Table 2. Changes in mass and income of chitin and chitosan.

Steps	Mass (g)	Yield (%)
Raw material (In natura)	3000	
Shells	313,00	-
Chitin	144,00	46,00
Chitosan	67,40	21,53

The yield of chitin presented approximately 46 % of the initial mass concentration of chitin in the shrimp exoskeleton. As the reaction of chitin deacetylation is more aggressive due to high alkalinity and temperature employed, the result of this step equaled 21,53 % of the initial mass used. It was noticed a mass reduction in the chitosan production, which was due the retreat of the acetyl group of chitin.

#### 4.2. Scanning electron microscope (SEM)

The morphological characteristics of *Litopenaeus Vannamei* chitin and chitosan were evaluated by technique of scanning electron microscopy (SEM). Both results showed similar structures with various aspects of geometrically irregular particles, fine and loosely attached, as seen in Fig. 8a and 8c.

The micrographs of the samples in analysis showed morphologies of quite wrinkled and fibrous surfaces, since it was observed in the Fig. (8b, 8d, 8e and 8f). The characteristics presented in the micrographs of the Fig. 8g and 8h showed some pores being able to be due to concentrations accented of NaOH.

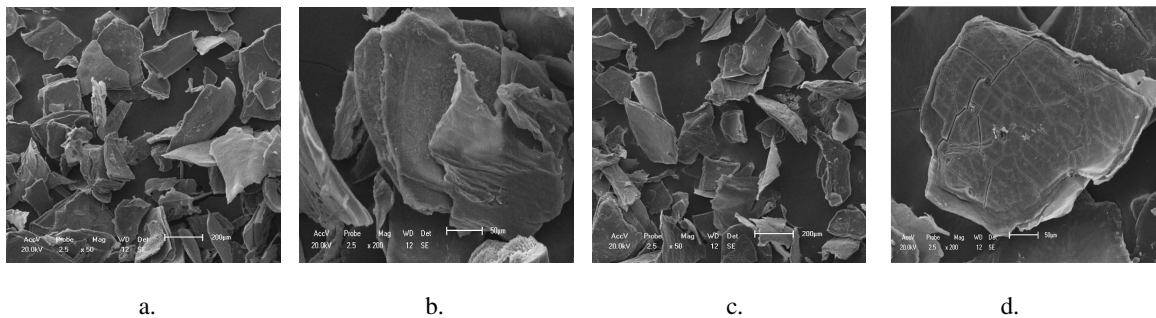


Figure 8 a. chitin 50X b. chitin 200X c. chitosan 50X d. chitosan 200X



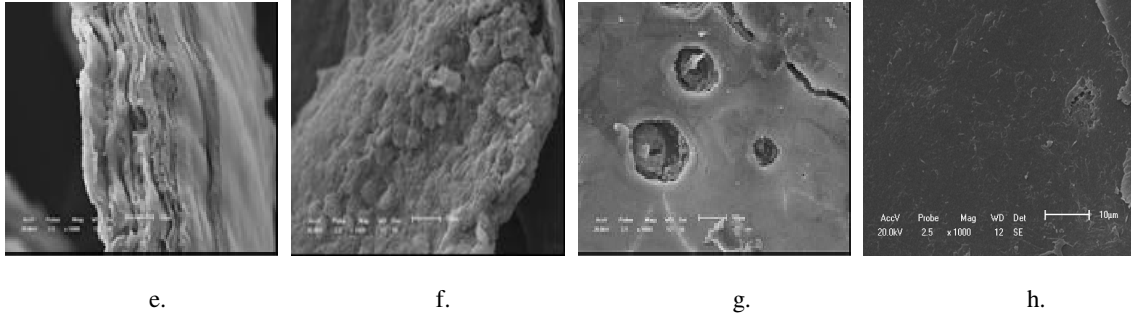


Figure 8 e. Chitin 1000 x f. chitin 1000 x g. chitosan 1000x h. chitosan 1000x

### 4.3. X-ray diffraction

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 80°.

The diffractograms of chitin showed in the Fig. 9a and 9b a lot of similarity. They had in common a strong intensity peak near the angle of 20°, which is of greater intensity. The differentiation of types of chitin can be made using the width at half height of this peak, which is a concept used by the construction of the Scherrer equation that determines the interplanar distance (Focher, *et.al.*, 1990). The width at half height was determined by the program origin, from selection of the largest intensity peak.

In the Figure 9a, the crystalline peak of chitin was found with the great intensities of 2622 cps, correspond to  $2\theta = 19.06^\circ$ , observed that the value of width at half height of this peak it was of (2,612). In Figure 9b the crystalline peak of chitin, with the great intensities of 672 cps correspond to  $2\theta = 19.144^\circ$ , observed that the value of width at half height of this peak it was of (2,655). The parts inserted in the graphs determined the value of the width at half height of the chitin.

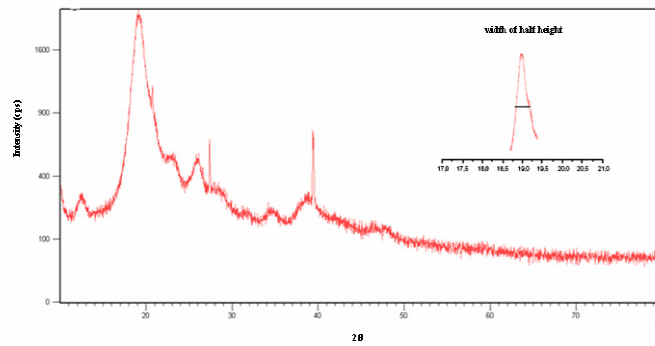


Figure 9 a. X-ray diffractogram of chitin

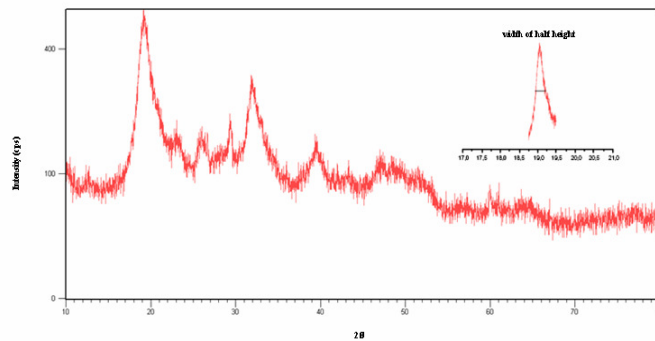


Figure 9 b. X-ray diffractogram of chitin

The picks of the diffractograms in the Fig. 9b were more intense and more crystalline than the Fig. 9a, confirming that so it is possible to realize that some chitins has reticular structure more disorganized than other. The parameters of net, that says of the crystalline characteristics of the polymer, stand back with larger intensity for the chitin of Fig. 9a than for chitin Fig. 9b.

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## 7. RESPONSIBILITY NOTICE

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