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IONIZING RADIATION APPLIED TO ONE STEP CONVERSION FROM DIFFERENT SOURCES OF CHITIN

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ABSTRACT

Chitosan is a polyssacharide obtained from chitin's molecule deacetylation which is the main constituent of some fungi species and the exoskeleton of crustaceans, insects and mollusks. Frequently the production of chitosan is from the crab shells and shrimps that are byproducts of the fishing industry, so it is highly dependent on seasonality. Therefore, finding new chitin's sources become important. The amino groups present in chitosan give important biological properties such as biodegradability and biocompatibility, activity/immunological effects and antibacterial healing. The chitosan deacetylation process is an aggressive reaction since it requires the attack of chitineous substrate in hot and high concentrated alkalis solution by 1 to 17 hours. It is possible to reduce reagent concentration and time using high-energy irradiation (gamma rays and electron beam). The advantages of radiation use in high-energy include: the absence of chemical initiators, the process can be performed at room temperature and there is no need for the use of solvents. In this work, crab shell, shrimps, squid glads and blattaria were used in order to compare the quality of chitosan found in each animal source. After pretreatment, which include the steps of demineralization and deproteinization, the samples were irradiated at a dose of 20 kGy (gammacell) in order to reduce the deacetylation time. The chitosan from the used chitin sources was characterized by FTIR analysis and its degree of deacetylation was determined.

1. INTRODUCTION

Chitin is a biopolymer with a linear chain formed by N-acetyl-2dioxy-D-glucopyranose units connected by β (1 → 4) glycosidic bonds (Fig. 1).It is the second most abundant biopolymer in nature, being preceded only by cellulose.

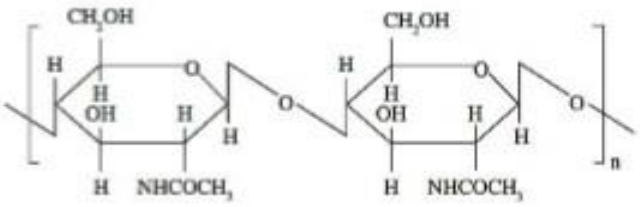

Figure 1: Chitin structure.

The exoskeletons of crustaceans may present 15 - 20 % of chitin, 25 - 40 % of proteins and 40 - 55 % of calcium carbonate [1-2], as well as other impurities. There is no standard process for the isolation of chitin, but the following order usually follows: demineralization followed by deproteinization. For this reason, it is very difficult to obtain chitins with identical characteristics (p. ex., molar mass and crystallinity).

Chitin demineralization is a step on chitin's pretreatment where calcium carbonate and other inorganic salts are dissolved. This step uses many kinds of inorganic acids and the most used is HCl. Already in order to eliminate the proteins, aqueous inorganic alkaline solutions are normally used. Details of chitin's pretreatment depends of its source type, so it can be used different concentrations and reaction time. Strict conditions should be avoided because they may cause degradation of molecular structure and crystallinity of chitin [3].

The proportion of chitin in exoskeletons is different according the type of living being, from species to species and from the region where they are found (Tab. 1) [4].

Table 1. Proportion of chitin in living resources

Living Resources	Chitin (%)
Fungi	2 - 40
Shrimps	5 - 30
Crabs	10 - 70
Squids/Octopus	40
Beetle	5 - 15
Blattaria	10 - 35

Fishery activities are the common source of chitosan and it depends of season demands. An alternative option is the extraction of chitin from fungi that have large amounts of this polymer in their cell wall, highlighting the class Zygomycetos, division Zygomycotina and more precisely the order Mucorales [5]. Chitin from insects, such as blattaria (cockroaches) is another possibility of extraction because these terrestrial organisms exhibit a rapid reproduction, very low cost of creation, besides they do not present the problem of seasonality. Particularly in the case of blattaria, it is not necessary to carry out the demineralization step [6].

Chitin is a colorless amorphous powder, where crystalline structures are present. It is insoluble in water, organic solvents and also in some diluted bases and acids. Chitin is applied in form sutures or membranes that act in the healing process, but its solubility in some high concentrated acids limits its use [7-8].

Its derivatives chitosan is obtained by the deacetylation process – the chitin's acetamido groups ($-NHCOCH_3$) are transformed into amino groups ($-NH_2$) - and has the following structure: 2-amino-2-deoxy-D-glucopyranose and 2-acetamide-2-deoxy-D-glucopyranose, which units are

also linked by β- (1,4-) (Fig. 2). Unlike chitin, it is soluble in practically all organic solvents [9].

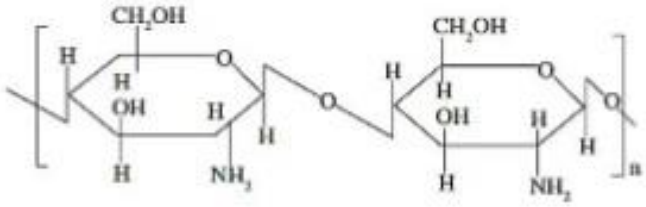

Figure 2: Chitosan structure.

Deacetylation is made in highly alkaline aqueous solutions (sodium or potassium hydroxide) for long time periods and at high temperatures, causing in some cases, depolymerization during the reaction [10]. Thus, complete deacetylation of chitin is rarely achieved.

More recent studies already consider chitosan when the sample shows a degree of deacetylation (DD) from 50 % [9; 10]; the higher degree of deacetylation, higher will be chitosan solubility in aqueous solution [11]. Depending on the application, it is also necessary to carry out the purification process in order to obtain a chitosan impurities free [12-13]. Some applications may highlighted as: removal of heavy metals, application in the cosmetics industry, immobilizing agent in the controlled release of drugs, in the treatment of obesity, artificial skin, contact lenses, curative treatment of wounds and burns, among others [11; 14] and production of a bactericidal agents, acting in the elimination of bacteria and fungi.

The degree of deacetylation, crystallinity and molar mass directly influence chitosan applications. In order to minimize the time of reaction and consequently this effect, the ionizing radiation can be used after the deproteinization step, where part of the chain already breaks [3].

In this work, the chitin from crab shells, shrimp, squid glads and cockroaches was converted to chitosan by classical and irradiation processes methodologies. Its quality, evaluated by the DD, was obtained by FTIR spectral analysis.

2. EXPERIMENTAL METHODS

2.1. Materials

The chitinous material used in this study were crab shells from *Charybdis hellerii*, known as Siri bidu at Brazilian coastal region that is an invasive specie that threatens our native coastal fauna and they were kindly provided by technicians staff from IP-SP (Fishing Institute of São Paulo State). The shrimp shell and the squid glads were waste discarded by local commerce. The cockroaches were obtained through the purchase of breeders. The standard chitosan used was Sigma Aldrich.

2.2. Radiation process

The pulverized samples were irradiated in a gamma cell at 20 kGy. The samples mass were determined before and after irradiation process.

2.3 Chitin pretreatment and chitosan deacetylation

The chitin from animal sources needs a pretreatment cycle; in this work, the pretreatment steps were that setted for crab shells chitin by Ferreira [3], as well the chitosan deacetylation and purification process.

2.3.1. Demineralization process

The demineralization step was carried out by washing chitin with 1 M hydrochloric acid during 60 minutes at room temperature.

2.3.2. Deproteinization process

This step was performed using 1 M sodium hydroxide solution at tempertatures between 60ºC – 80ºC for 60 minutes.

2.3.3. Deacetylation process

The chitin's conversion in chitosan was carried out under the following conditions: dissolution of pretreated chitin in 60 % NaOH under reflux for 1 h (irradiated samples) and 6 h (non-irradiated samples).

2.3.4 Purification process

Chitosans were solubilized in acetic acid (0.1 mol/L) and precipitated with 1 mol/L NaOH. A colloid was formed and it was filtered.

2.4. Characterization

The infrared spectra of the chitosans were carried out in a Perkin Elmer-Spectrum 100 Fourier transform infrared spectrometer (FTIR) from Center of Technology of Radiation (CTR) - IPEN/CNEN-SP. Samples were embeded in KBr pellets and all spectra were obtained in transmission mode at the medium infrared frequency (4000 cm^{-1} at 400 cm^{-1}) after 16 scans.

In this technique the degree of deacetylation is calculated according to equations derived from empirical studies that are related to the carbonyl (-C=O), N-acetyl and hydroxyl (-OH) groups present in chitosan. The proposed equations generally use absorbance values in the bands at

1655 cm^{-1} and 3450 cm^{-1}, respectively associated to carbonyl and hydroxyl groups. The degree of deacetylation (DD) in this work was extracted by Rojas [15] equation (1):

$$DD = 100x[1-1,33x(A_{1655}/A3_{450})] \quad (1)$$

3. RESULTS AND DISCUSSION

Figures 3 and 4 show the spectra of chitosans from commercial standard and animal sources (non irradiated and irradiated).

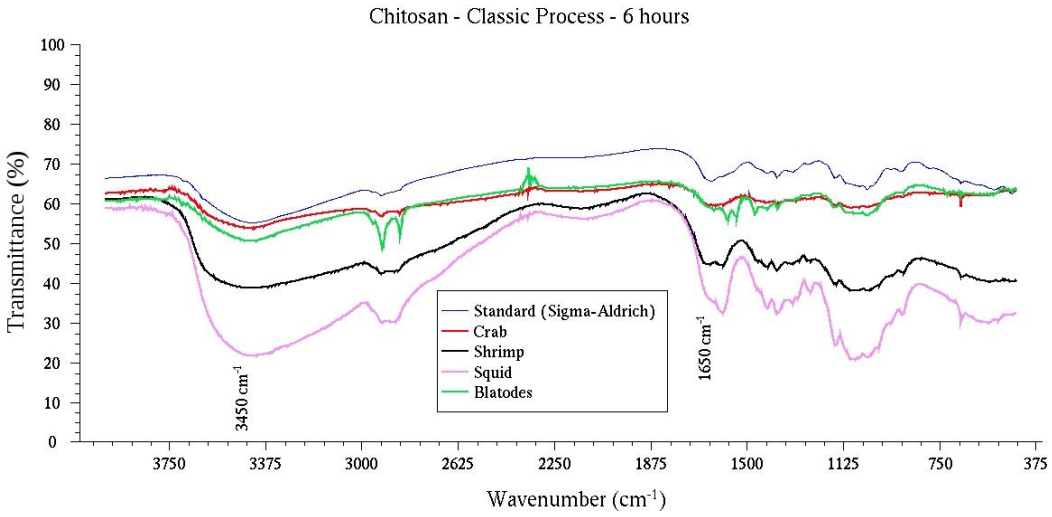

Figure 3: Spectra of non irradiated chitosans compared to standard chitosan.

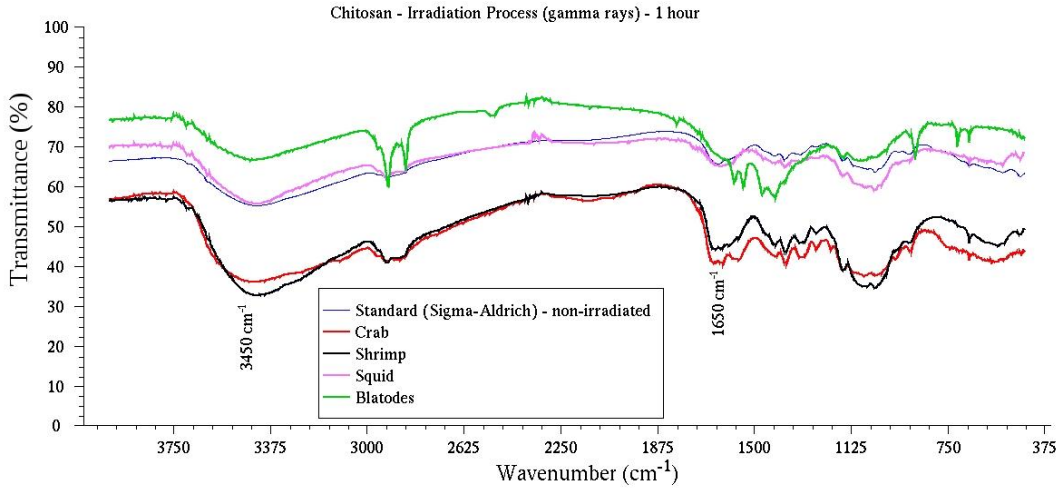

Figure 4: Spectra of irradiated chitosans compared to standard chitosan.

Chitosan presents relevant peaks in the region of 3570-3200 cm^{-1} and 3450 cm^{-1}, related to the stretches of the -OH and -NH groups at approximately and 1650 cm^{-1} (-C=O) and 1380 cm^{-1} (-CH) related to respectively amide I and N-acetyl metyl group [16].

The DD values calculated from equation (1) are shown in Table 2:

Table 2. Degree of deacetylation of non-irradiated (NI) and irradiated (IR) chitosan samples.

Sample	DD (%)
Standard	**78**
Crab-NI	76
Crab-IR	79
Shrimp-NI	48
Shrimp-IR	57
Squid-NI	34
Squid-IR	42
Blattaria-NI	38
Blattaria-IR	83

Standard chitosan showed a degree of deacetylation of 78 %, which is in agreement with the value described on its MSDS (DD = 75-85 %). Crab shell's classical method showed a value near to that presented in standard sample, however the DD increase in this sample when the

irradiation method is applied. These results indicate irradiation method is effective in deacetylation process and the time reaction is minimized, avoiding chitosan's chain damage.

Shrimp's chitosan showed low DD value in classical method and it can be related to effects of pretreatment. The time and concentration of reagents used in demineralization and deproteinization steps were optimized in chitin conversion to chitosan from crab shells studied by Ferreira [3]. It is possible shrimp's chitin pretreatment was not effective and it needs an optimization study of pretreatment parameters.

In the same way, chitosan from squid glads presented the lowest DD value in classical method may be related to the non-optimized pretreatment process. However, despite its complete solubilization in acetic acid, this sample showed a brown coloration, indicating temperature degradation; in this work deacetylation ocurred at 100-120 °C and it may be very high. Locilento indicates that the appropriate temperature for deacetylation squid glads chitin is 80 °C [17].

Also, deacetylation performed by classical method applied to chitin from cockroaches showed a low DD value. It suggest the optimization of pretreatment parameters are necessary in this case too. The temperature in deacetylation process may be not sufficient to complete chitin's conversion, since Bezerra [6] suggested temperature values are between 110-150 °C for chitosan obtaining.

All irradiated samples showed the highest degree of deacetylation, indicating deacetylation may be performed by ionizing radiation and it is an efficient process to minimize the reaction time, since the low time of 1 hour was enough for chitin's coversion to chitosan.

4. CONCLUSIONS

In this work, was observed irradiation process allow chitosan with high DD values, reducing the reaction time necessary for the conversion of chitin to chitosan. Consequently, the quality of chitosan obtained in this method is better than chitosan obtained in classical method, once chitosan chains may be preserved.

Blattaria chitosan was configured as a source of chitosan in addition to crustaceans. To high convertion of cockroaches's chitin it is required a long time and high temperature.

On the other hand, squid glads chitin is more sensitive to the temperature of deacetylation process, occurring degradation of the sample.

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