EVALUATION OF PHYSICAL, CHEMICHAL AND IRRADIATION PARAMETERS ON CRAB SHELL'S CHITOSAN OBTENTION PROCESS

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ABSTRACT

Chitin it is found in exoskeletons of crustaceans and in the cellular wall of fungi. Chitosan is obtained through the stages of deproteinization, demineralization and deacetylation. Specially, crab shells present in their composition 15-20% of chitin, 25-40% of proteins and 40-55% calcium carbonate. The demineralization step aims to reduce the inorganic ions content and is realized under hydrochloric acid dissolution. The deproteinization has the function of reducing the proteins and aminoacids by sodium hydroxide solution added to the raw material. In this work, the experimental design used to determine the best steps conditions for the production of final product – chitosan from chitin of crab shells - had been time (10, 30, 60 and 120 minutes), concentration, relation reagent solution/raw material quantity and irradiation parameters (radiation font, dose and dose rate). The results are discussed in terms of total inorganic materials and proteins quantification and of thermal analysis.

1. INTRODUCTION

Chitin is the second most important natural polymer in the world and it is the most abundant polymer after cellulose, considering the amount of chitinous material discarded annually by exploitation of crustaceans like shrimp and crabs. In spite of your abundance and importance, chitin was only isolated in 1884 [1].

Chitin presents low solubility in aqueous systems, although it may be dissolved in strong acids such as hydrochloric, phosphoric and sulfuric; the dissolution is very slow and causes severe degradation of the polymer [2]. Also, solubility is restricted to a few kinds of organic solvents.

This naturally abundant, low-cost and biodegradable polysaccharide [3] has important properties like non-toxicity, physiological inertness, antibacterial properties, hydrophilicity, gel-forming properties and affinity for proteins that it can found applications in many areas [4]:

- medical and pharmaceutical: used as wound-dressing material and controlled drug release since your biocompatibility with human body; material for bone-filling as a hydroxyapatite–chitin–chitosan composites, which forms a self-hardening paste for guided tissue regeneration in treatment of periodontal bony defects.
- food industry and cosmetics: enzyme and whole cells immobilization; used also to prepare affinity chromatography column to isolate lectins and determine their structure.

- environmental engineering: adsorvent of silver complexes at industrial pollutants treatment plant.

Although chitosan is naturally found in some fungi species, this derivative of chitin is mainly obtained by deacetylation process where it is carrying out under both enzymatic hydrolysis in the presence of a chitin deacetylase [5] and dissolution in high concentration of alkali and temperature conditions. The amine groups on chitosan allow its solubility in acid solutions (acetic and formic acids are the most used), although it is still insoluble in water and alkali.

Chitosan studies have been intensified in the 1990's because of their biological properties such as biodegradation and biocompatibility with human body, immunological, antibacterial and wound-healing activity [5]. Then, chitosan presents similar chitin's area applications, though its chemical interaction is distinctive [6-8]:

- the chelating action allow the removal of heavy metals in industrial waste treatment plants;
- the moisturizing action is profited in the cosmetics industry where it is added to shampoos, sunscreens and creams, it is also used on acne treatments;
- the fats and proteins affinity grants its utilization as immobilizing agent for the controlled drug release, in the obesity treatment, in the production of kidney membranes, artificial skin, contact lenses and other products;
- the hemostatic action is incoming in bandages for treatment of wounds and burns and to induce fast healing;

Some research groups perform chitin deacetylation process by ionizing irradiation that improves the traditional method in terms of reduction of time processing and reagents concentration [9]. The ionizing radiation may undergo several changes in physical and chemical material's structure induced by its interaction; the ionizing radiation choice is therefore a non-polluting technique that may be carried out in room temperature and there is no need to use catalysts [10].

The aims of this work is to explore the effects of some parameters in the pretreatment stages when it is used an *in natura* source of chitin to produces a good quality chitosan, once in the literature some research groups study chitin-chitosan conversion but the pretratment is not used in many times [12, 13 khan e weska] because the starting material is a standard chitin.

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) and Dr. Renata Bazante Yamaguishi (volunteer researcher from IPEN and assistant professor at UNIP).

Forty-one crabs were captured at the coastal pier in Santos city (São Paulo State), shown in Fig. 1, which geographic coordinates are: 23^{o} 99,1' S (latitude) and 46^{o} 30,4' W (longitude), using commercial crab traps (Fig. 2) provided with lure (pieces of chicken carcass); the traps

were immersed from water to sedimentary bed. All captured crabs had been placed in appropriated boxes with sea water to be shipped to the final location where they were freeze.

Only crab shells were used in this work. They were pulverized at a manual grinding and the obtained powder was sieved and separated into nine different grain sizes (mesh) . It was selected three different particle size to develop this study: 32 mesh, 100 mesh and greater than 250 mesh .

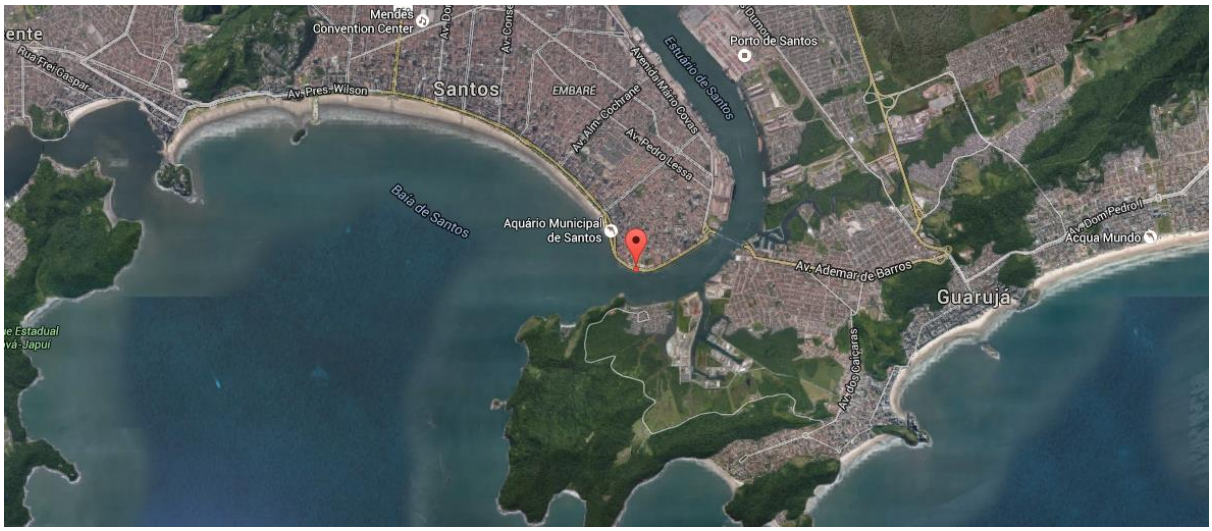

Figure 1: Localization of crabs capture.

Figure 2: Crabs Trap.

2.2. Radiation process

The pulverized crab shells samples were irradiated in gamma radiator at 20 kGy. The samples mass were determined before and after irradiation process.

2.3. Deproteinization

In industrial processing, chitin is extracted from crustaceans by acid treatment to dissolve calcium carbonate followed by alkaline extraction to solubilize proteins. It is known that severe alkali treatment results in degradation of chitin polymer chains and also reduces the quality of the protein extracts. Deproteinization was performed using 1 M sodium hydroxide solution at 70ºC in the following times: 10, 30, 60 and 120 minutes.

2.4. Demineralization

The demineralization step aims to reduce the inorganic ions content and is realized under hydrochloric acid dissolution. The treatment may be performed in different conditions of temperature and time which can vary from 0º to 100ºC and 10 minutes to 48 hours, respectively. However, strict conditions should be avoided because they may cause degradation of molecular properties of chitin [11]. The demineralization in this work was carried out by washing chitin with 1 M hydrochloric acid during 30 minutes at room temperature.

2.5 Material characterization

2.5.1 Protein analysis

The test used for checking the extracted protein content was the Bradford assay (Coomassie Brilliant Blue). The sample absorbances at 580 nm were measured in a UV-vis spectrophotometer Genesys 20 – Thermo.

2.5.2 FTIR analysis

In this work, the infrared was obtained in two ways. *In natura* chitn samples with grains ≤ 32 mesh (irradiated and non-irradiated), spectra in ATR mode were performed in a spectrometer from Perkin Elmer, model Spectrum 100. Spectra of deproteinized samples (1:10, 1:40 and 1:50), were obtained in transmission mode from KBr pellets and performed in a spectrometer Nexus 670, from Nicolet.

2.5.3 Thermogravimetric analysis

The evaluation of thermal decomposition of materials was performed in a TGA model TAA - 100, under synthetic air atmosphere (50 ml/min), in a range from 25 to 800 ° C and heating rate of 20 °C/min.

3. RESULTS AND DISCUSSION

3.1. Protein content

It was observed (Fig. 3) that the particle size of ≤ 32 mesh, the irradiated sample has a peak at 60 minutes and the non-irradiated delivered a larger amount of proteins but at the same point (60 minutes). Chitin particle size of ≤ 100 mesh showed a similar behavior also reaching a point of 60 minutes. However, the irradiated sample has delivered a greater amount of protein than the sample not irradiated. The particle size of 250 mesh was the one that proved to be constant for both the irradiated sample and for the non-irradiated (0,8 and 0,6 mg, respectively). However, the delivery of the protein occurred in a shorter time (30 minutes), which was to be expected since the particles were in a smaller size.

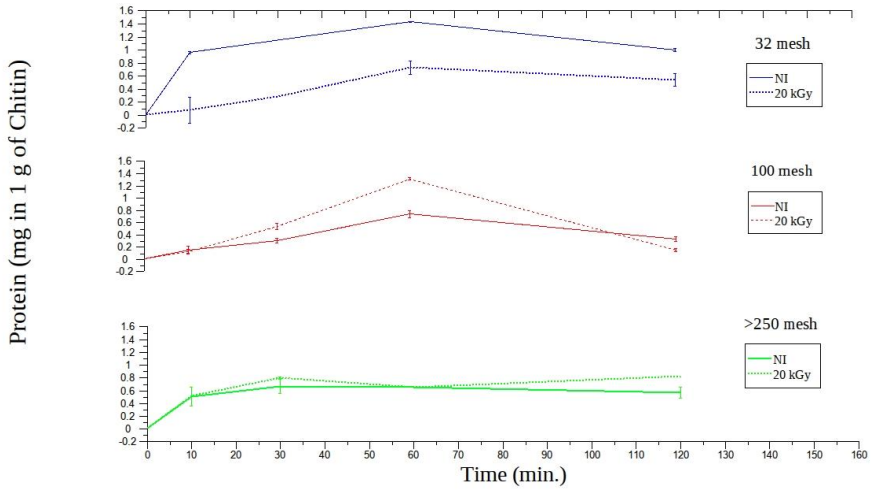

Figure 3: Protein content.

3.2. Infrared spectroscopy

In Fig. 4 is shown the spectrum of chitin in a particle size of $\leq$ 32 mesh. The irradiated chitin has a band in 3470 related to the amine function.

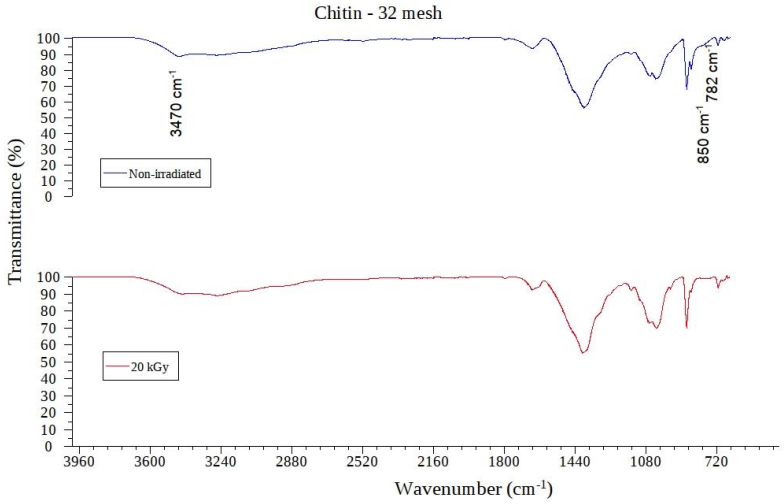

Figure 4: Spectrum of 32 mesh chitin.

The particle size was not a major factor in the removal of the protein content of the sample. The three grain sizes have similar behavior in the analyzed time. The higher protein content delivered is in the particle size of $\leq$ 32 mesh, this suggests that the proteins are adhered to the surface of the shell of the crab. Perhaps the grinding material (to obtain a smaller particle size) occurs to cause a dispersion of the most sensitive proteins. In Fig. 5, is observed sample deproteinization is fast and occurs in 10 minutes; this behaviour indicates instantaneous reaction. Fig 6 shows a large band at 3470 cm^{-1}, that suggests -NH_2 protein group removal and high band intensities, that suggests chitin backbone protein removal.

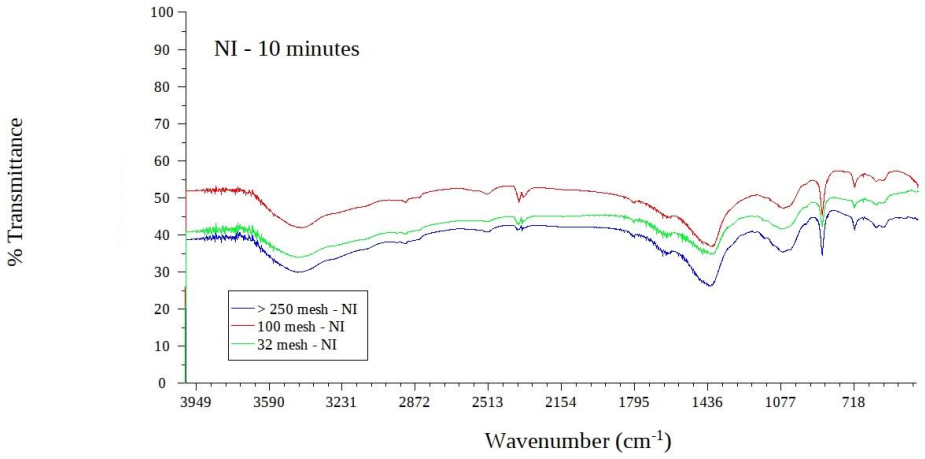

Figure 5: Spectra of chitin demineralized at the time of 10 minutes.

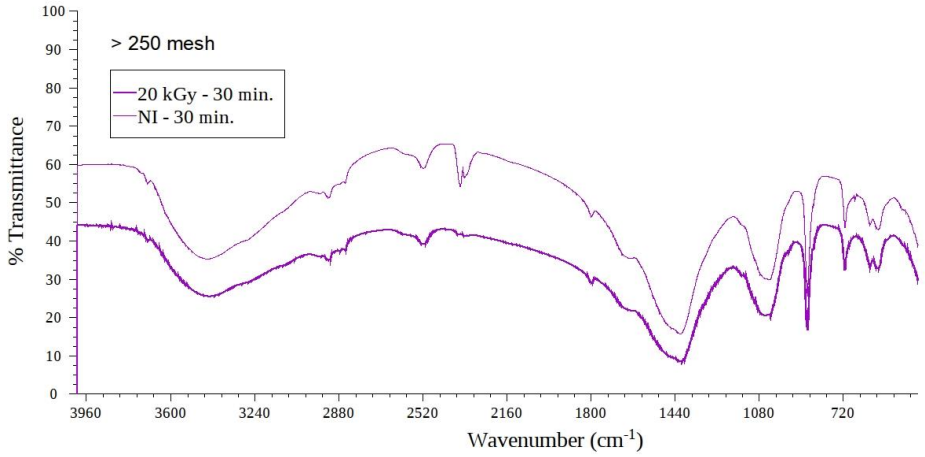

Figure 6: Spectrum of 250 mesh chitin deproteinized.

3.3. Inorganic ions content

The inorganic ion content was calculated by neutralization with 1 M sodium hydroxide solution. It was neutralized 10 ml of sample. The results are shown in table 1. Through the neutralization it was observed that approximately 50 % of the sample is composed of these inorganic ions, which is consistent with the literature that says that the crab shell has 40-55% of inorganic ions in its composition. The proportions used were: 1:40 and 1:50. None significant difference was observed related to these distinct methods.

Table 1: Percentage of inorganic matter of chitin

Sample	Inorganic matter (%)
1:40 chitin : HCl (NI)	26
1:40 chitin : HCl (20 kGy)	58
1:50 chitin : HCl (NI)	32
1:50 chitin : HCl(20 kGy)	57

3.4. Thermal analysis (TG) result

The TG of the samples and the commercial chitin from Sigma were analyzed. In the proportion of 1:50 was possible to observe the presence of a certain quantity of inorganic content, which may indicate that the demineralization time (30 minutes) used has not been enough.

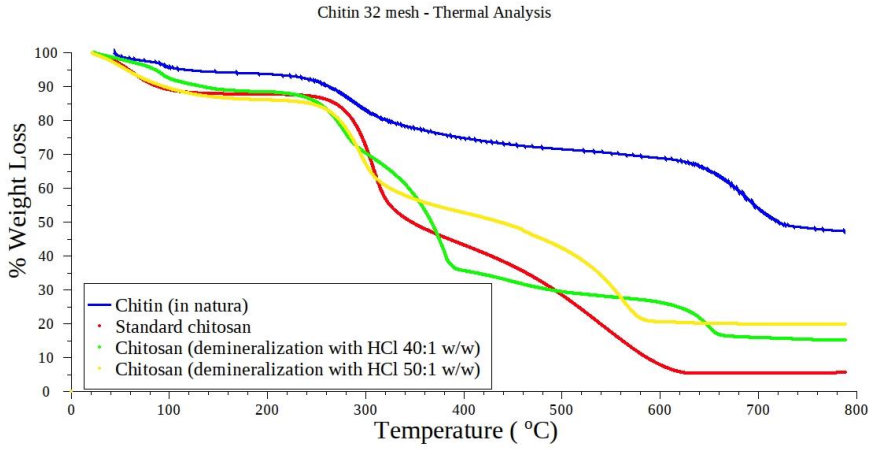

Figure 7: Thermal analysis of chitin.

4. CONCLUSIONS

The objective of the present work was to investigate some parameters of a pretreatment of chitosan as time, concentration and relation reagent solution/raw material quantity. The results showed that the deproteinization step depends on the particle size. It is believed that the proteins found in the surface of the crab shells are labile. The time of 30 minutes was not relatively sufficient for demineralization to produce a good quality chitosan. Some effervescence was observed at the time when chitin is added to the hydrochloric acid which indicate the reaction is instantaneous but the total demineralization should be performed in great times.

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