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EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM LOCAL MARINE RESOURCES

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***Abstract**: This paper presents the chemical extraction of two marine polysaccharides (chitin and chitosan) from one of the crustacean species of Romanian Black Sea waters. The characterization of these polysaccharides were studied through optical microscopy and FT-IR/ATR spectroscopy. The spectra samples were compared with those of chitin and chitosan standards and results showed the presence of chitin and chitosan in the studied samples.*

***Keywords**: chitin, chitosan, crustacean species, chemical extraction, FT-IR/ATR*

INTRODUCTION

Marine polysaccharides are one of the major components of all living organisms. In nature, marine polysaccharides are derived from various resources such as marine algae, crustaceans, and microorganisms [1].

Marine polysaccharides are easily processed into nanoparticles, nanofibers, microparticles, scaffolds, membranes, gels, beads, and sponge forms, and these forms have been used for various biomedical applications in cancer therapy, drug delivery, tissue engineering, biosensors, wound dressing, and water treatment in the area of nanobiotechnology [2].

Chitin and chitosan are the most acquaintance marine polysaccharides; over the years they have attracted a great deal of attention in food, pharmaceutical and industrial applications due to their distinctive biological and physicochemical characteristics [3, 4]. Besides, the last two decades have demonstrated a great amount of work on these biopolymers, which highlighted wide potential uses [5]. The current trend towards the use of natural products has strongly contributed to the observed increase in demand, which has turned chitin and chitosan production processes into highly profitable ones.[6]

Chitin is a hard, inelastic, nitrogenous polysaccharide, available from variety of sources which include, exoskeleton of crustaceans, cell wall of certain fungi, mushrooms, worms, diatoms, arthropods, nematodes and insects, with shellfish waste such as shrimps, crabs and crawfish being the principal sources [7].

Worldwide, chitin is the second most abundant and most important natural polysaccharide after cellulose. It is composed by mainly repeating unit of β-(1-4) linked N - acetyl – D – glucosamine (fig.1a) [8] and it is used almost exclusively as raw material for production of chitosan.

Chitosan is the most important derivative of chitin, it is a linear polymer of β-(1–4)-2-amino-2 deoxy-D-glucosamine (fig.1 b).

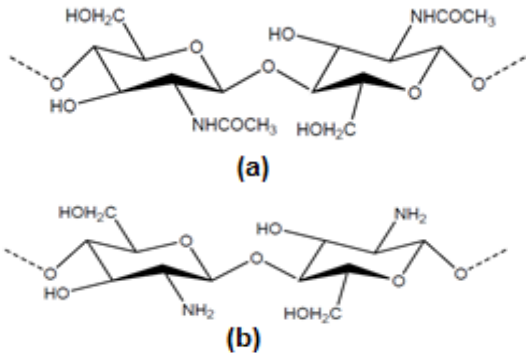

Fig. 1 Chemical structures of chitin (a) and chitosan (b)

The objective of this paper is to demonstrate how our natural marine resources could be used by the extraction of chitin and chitosan, two promising biopolymers with applications in many fields ranging from waste treatment management or ballast water management to biotechnology and medicine.

Moreover, chitin and chitosan extracted from the carapace of collected shrimps were compared

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with standards of chitin and chitosan by using FT-IR/ATR and optical microscopy

MATERIALS & METHODS

Chitin and chitosan were extracted from *P.elegans* (Ratke 1837) (fig.3) one of crustaceans species, collected from Cap Midia Gulf (44,3ºN; 28,6ºE), (fig. 2) at the north of Constanta town, in May-June 2015. The collected samples for analysis were identified [9] and measured. The standards (chitin and chitosan) are purchased from LGC Standards, Germany.

P.elegans (Ratke 1837) is the ordinary shrimp, being one of euryhalinic and eurybiontic species, found on the sandy bottoms covered with algae, [9, 10, 11].

The selection of specimens of *P. elegans* was done providing the carapace consistency of whom dimensions ranged from 3 cm to 4.5 cm and weight body ranged from 0.5 g to 1.1 g.

The selected samples were frozen and transported for analysis at University POLITEHNICA of Bucharest, Faculty of Applied Chemistry and Materials Science. The shrimps were washed with a plenty of distilled water, then they were soacked in hot water (t=50ºC) for detaching easily the carapace and the claws.

The extract process of chitosan from shrimps exoskeleton samples consists of three basic phases: demineralization (inorganic salts separation), deproteinization (protein separation) and deacetylation, according to the standard procedure for chitosan production [12, 13, 14].

In the first phase of the method, the dried samples of *P.elegans* exoskeletons were size – reduced and soaked in small concentration of HCl solution at room temperature and centrifuged. The resulted precipitate was washed with distilled water until a neutral pH, and dried to constant weight.

In the second phase of the method, the demineralized sample was treated with NaOH weakly solution at 75°C. The obtained chitin was centrifuged and washed with distilled water until a neutral pH, followed by drying to constant weight.

Fig. 2 Midia Gulf- the place of collected species

Fig. 3 *Palaemon elegans*

The third phase, deacetylation of the obtained chitin was carried out by soaked it in strong NaOH solution at higher temperature (90°C). The obtained chitosan (fig. 4) was treated with ethanol in order to remove the impurities, washed with distilled water until a neutral pH is obtained, and dried to constant weight.

The standard chitosan (from shrimps shells, CAS no.9012-76-4) and standard chitin (CAS No. 1398-61-4) were purchased from LGC Standards, Germany.

The FT-IR spectrophotometer used to record spectra was a Perkin-Elmer spectrum 100 FT/IR equipped with ATR device and belongs to Faculty of Applied Chemistry and Materials Science, from University POLITEHNICA of Bucharest. In all solid samples, IR spectra were recorded between 4000 cm^{-1} and 600 cm^{-1} by accumulation of 32 scans, with a resolution of 4 cm^{-1}. The optical microscopy analyzes were performed with Optika B 350 microscope, at Ovidius University from Constanta.

Fig. 4 Chitosan powder of *P. elegans*

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RESULTS

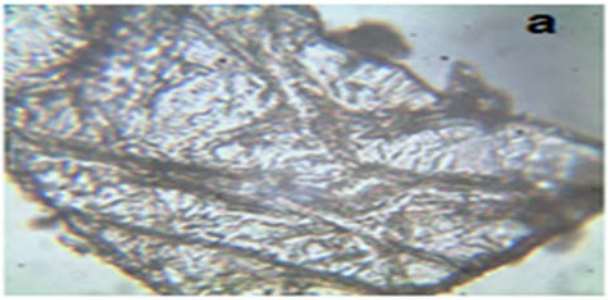
The obtained results of collected shrimps showed that their exoskeleton is composed of inorganic salts (25%), proteins (64% and chitin (11%).

The chitin obtained (fig. 5 b) was analyzed and compared with standard chitin through optical microscopy. The results showed that the chitin extracted from shrimps was arranged distinctly in a microfibrillar crystalline structure, same as standard chitin (fig. 5 a).

The FT-IR/ATR spectra recorded for the *P.elegans* chitosan samples comparative with standard chitosan, showed the main absorption bands as following (fig. 6):

- The peaks observed at 3400 ÷ 3200 cm^{-1} are due to different vibrations, like stretching vibration of hydrogen-bonded (ν_{O-H}), which is overlapped to the stretching vibration of N – H from the free amino group (- NH_2) at C_2 position of glucosamine: at 3357.46 cm^{-1} for standard chitosan, and 3287 cm^{-1} for chitosan of P. elegans;
- At ~ 2876 cm^{-1} and 2361 cm^{-1} the bands are assigned to stretching vibration of C – H bond (ν_{C-H}) in – CH_2 and in – CH_3 groups, respectively [15];
- The primary alcoholic group (– CH_2 – OH) represented by a strong absorption band at 1019 cm^{-1} for standard chitosan could be seen at 1012.45 cm^{-1} for chitosan of analyzed samples.

The results from the spectroscopic analysis indicated the presence of chitosan in *P.elegans* studied samples, a local source unexploited yet.



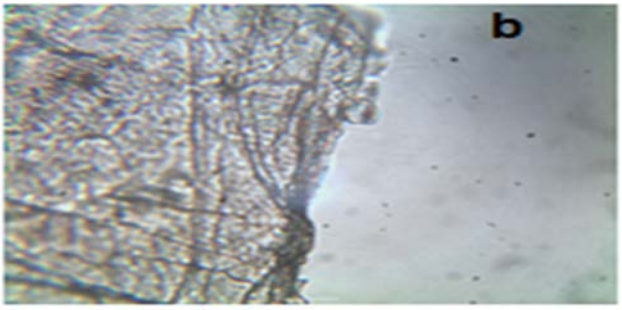


Fig 5 Optical microscope observation of the chitin standard **(a)** and chitin of *P. elegans* **(b)**

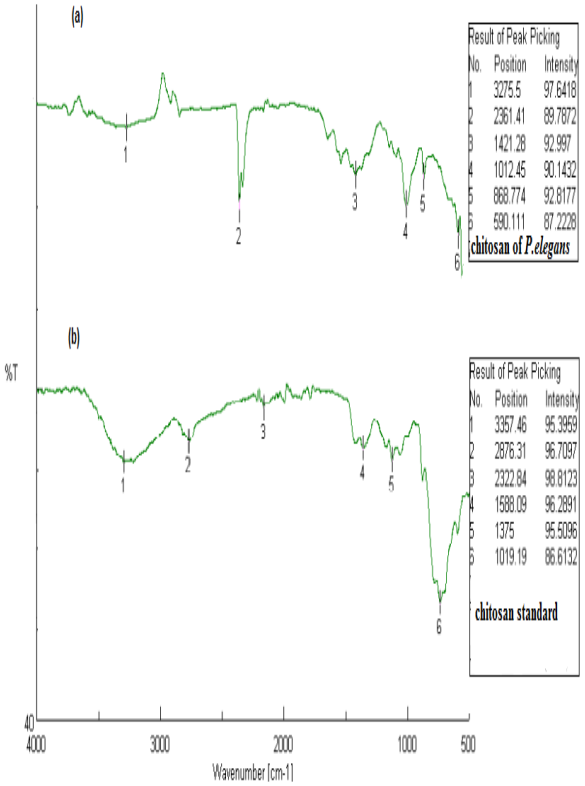


Fig. 6 FT-IR spectra of shrimps chitosan (a) and chitosan standard

CONCLUSIONS

The chitin, obtained by chemical extraction of *P.elegans* exoskeleton was analyzed and compared through optical microscopy with that of chitin standard. The results confirmed that the structure of chitin extracted from shrimps is similar to the structure of standard chitin.

The deacetylation of obtained chitin carried out through chemical extraction, using strong NaOH solutions and higher temperatures.

The absorption bands present in the IR spectra obtained for the chitosan, extracted from the local marine sources were compared with those of standard chitosan showing that *P. elegans* is a good source of chitosan, source unexploited yet.

The extraction of chitosan from crustaceans processing wates (exoskeletons) could minimize the environmental pollutants.

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The experimental study of this species of Romanian marine crustaceans showed that, this is a rich source of chitosan, exhibiting characteristics which make it useful in many fields, ranging from water management or antifouling agent to food processing, medicine and biotechnology.

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