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New source of chitosan from Black Sea marine organisms identification

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ABSTRACT

The Romanian Black Sea environment, due to its biodiversity, contains a lot of organism, which represents a rich natural resource of many biologically active compounds, such as: sterols, proteins, polyunsaturated fatty acid, polysaccharides, pigments and antioxidants.

In their evolution, marine organisms in the Black Sea adapted excellently to the marine environment such as: low temperature, absence of light, extreme pH and pressure, low salinity, low oxygen, the presence of one toxic abiotic chemical (H_2S). Moreover, they produce a wide variety of secondary metabolites (biologically active), which cannot be found in other terrestrial organisms.

Chitosan is a copolymer of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. Usually, it is obtained by deacethylation of the natural chitin, which is extracted from the exoskeleton of marine organisms, mainly crabs and shrimps. Chitosan could have a lot of applications in biomaterials, pharmaceuticals, cosmetics, metal ion sequestration, agriculture and foodstuff treatment (flocculation, clarification) because of its efficient interaction with other polyelectrolytes.

In this paper, we propose new possible source of chitosan, the spawning of *Rapana venosa*, a predatory gastropod, which invaded the Black Sea in earlier 1940s. Preliminary spectral studies on this biopolymer extracted from this biomaterial and another marine source (*Eriphia verucosa*) will be discussed and compared with that of chitosan standard in order to put in evidence the presence of chitosan in the obtained extracts.

KEYWORDS

Chitosan; biopolymer; spawning; *Rapana venosa*

Introduction

The Black Sea is a semi-enclosed sea, located between 40°27′N-46°32′N latitude and 27°27′E-41°42′E longitude, which communicates with the Mediterranean Sea to the South and with the Azov Sea to the North [1]. There are two climate types: submediterranean on the Eastern and Southern coasts, and Crimean and temperate on the Western and Northern coasts.

The Romanian Black Sea environment, due to its biodiversity contains a lot of organisms, which represents a rich natural resource of many biologically active compounds like: sterols,

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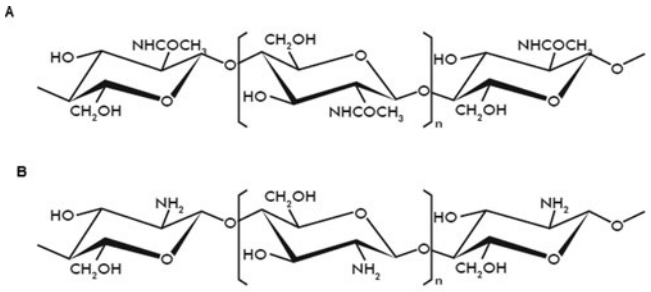

Figure 1. Chemical structure of chitin (A) and chitosan (B).

proteins, polyunsaturated fatty acid, polysaccharides, pigments and antioxidants. In their evolution, marine organisms in the Black Sea adapted to the marine environment variations especially rapid changes in the physicals and chemical gradients such as: temperature, oxygen and salinity. Moreover, they produce a wide variety of secondary metabolites (biologically active), which cannot be found in other terrestrial organisms.

Chitosan is produced from chitin, which is a natural carbohydrate polymer found in the exoskeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton, including coral and jellyfishes [2]. Chitin (fig. 1A) and chitosan (fig. 1B), the naturally abundant and renewable polymers have excellent properties, like biodegradability, biocompatibility, non-toxicity, adsorption, anticoagulant activity [3, 4].

The crustaceans represent an important group of aquatic organisms of Black Sea, their body is covered by an exoskeleton, which consists mainly of chitin impregnated with calcium carbonate.

The objectives of this study were to identify the marine resources (the crustaceans and the spawning of *Rapana venosa* on Romanian Black Sea waters), to isolate the chitin and chitosan from the exoskeleton and spawning of identified species, and to check the chitin and chitosan extracted from the shell of crustaceans decapod with standards of chitin and chitosan through FTIR/ATR and UV-VIS-NIR spectral analyzes.

The large Asian gastropod, *Rapana venosa* (Valenciennes, 1846) is a predatory specie native in the Sea of Japan and it has been unintentionally introduced into marine and brackish water areas of Black Sea due to naval activity.

Materials and methods

Figure 2 presents the places from where the samples were collected. The spawning of *Rapana venosa* (Mollusca, Gastropoda) (fig. 3) was collected from Cape Midia Gulf (44.5°N; 28.6°E) in February 2014, while *Eriphia verrucosa* (Crustacea, Decapoda) (fig. 4), a specie of crustaceans specific to Black Sea waters, was collected from Tuzla (44°N; 028°40′E) during the period April 2014 ÷ November 2014.

Chitosan extraction from samples of *R. venosa* spawning was done in the following steps: pre-treatment, deproteinization, filtration and drying. After collection, all the samples were identified. Then, the samples of *R. venosa* were washed, dried and analyzed, whereas the samples of *Eriphia verrucosa* were identified [3], weighted and body length measured and analyzed at Univesity POLITEHNICA of Bucharest, Faculty of Applied Chemistry and Materials Science. Also, the carapace and tissue were detached.

Figure 2. Map of collecting place.

Figure 3. *Rapana venosa* spawning.

Figure 4. Eriphia verrucosa.

The spawning of *Rapana venosa* sample was washed with distilled water, dried in oven at 60°C, for 3 hours and grounded in small pieces with a scissors. Then, 10 g of the sample were taken for the extraction process, soaked in 4% NaOH solution (1:15 w/v) at 65°C for 2 hours, filtered, and the precipitate was washed with distilled water until a neutral pH was obtained. The resulting chitosan (fig. 5) was then dried to constant weight and prepared for characterization.

The extract process of chitosan from crustacean shell waste consists of three basic steps: demineralization (calcium carbonate and calcium phosphate separation), deproteinization (protein separation) and deacetylation. These three steps represent the standard procedure for chitosan production [6].

The carapaces and the claws of *Eriphia verrucosa* samples (fig. 6) were washed thoroughly with distilled water and dried in oven to constant weight at a temperature of 60°C for 3 hours. The dried sample was size-reduced and soaked in 4% HCl solution (1:15 w/v) at ambient

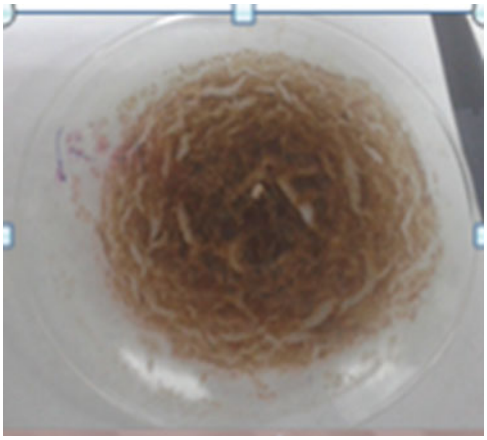

Figure 5. Spawning chitosan.

Figure 6. *Eriphia verrucosa* shells drying.

temperature (approximately 23°C) for 30 minutes and filtered. The resulting precipitate was washed with distilled water untill a neutral pH was obtained and the demineralized sample was dried to constant weight.

The demineralized sample obtained was further soaked in 4% NaOH solution (1:15 w/v) at 65°C, for one hour. The chitin was then dried to constant weight and it was deacetylated in 45% NaOH solution (1:20 w/v) at 100°C, for 15 minutes. After deacetylation, the obtained chitosan (fig. 7) was washed with distilled water untill a neutral pH was obtained, and it was dried to constant weight and prepared for spectral characterization.

The chitosan standard was purchased from LGC Standards, Germany.

Spectral measurements were performed using UV - VIS - NIR spectrophotometer Jasco, V 670 model and FT/IR spectrophotometer equipped with ATR device, Perkin-Elmer spectrum 100. UV – VIS – NIR spectra were registered in 200–2000 nm region, with a step of 0.5 nm, and the IR spectra were recorded in 4000 ÷ 600 cm^{-1} domain as a result of the average of four scans with a resolution of 4 cm^{-1}.

Figure 7. Crustacean chitosan.

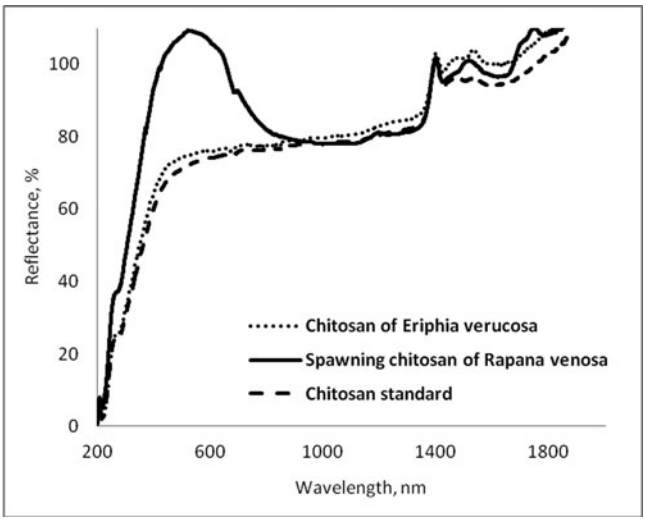

Figure 8. UV-VIS-NIR spectra of the chitosan samples.

Results

It was selected one specie of crustaceans from Romanian Black Sea waters: the spawning of *Rapana Venosa* (Valenciennes 1846) (fig. 3) and *Eriphia verrucosa* (Forskal, 1775) (fig. 4), in order to extract and to identify the chitin and respectively, chitosan [5]. The results obtained by UV-VIS-NIR and FT-IR/ATR spectroscopy are presented in figures 8 and 9.

Chitosan has two UV chromophoric groups namely, N-acetylglucosamine (GlcNAc) and glucosamine (GlcN), because chitosan cannot be completely deacetylated. The UV – VIS – NIR spectra recorded for the chitosan obtained from the two sources were compared with the UV – VIS – NIR spectrum of standard chitosan (fig. 8). The two characteristic peacks absorption at 210 and 260 nm confirm the presence of chitosan.

The FT-IR/ATR spectra (fig. 9) recorded for the crustacean chitosan samples and spawning chitosan sample of *Rapana venosa*, comparative with standard chitosan showed the main absorption bands as following:

– The peaks observed at 3400 ÷ 3200 cm^{-1} are due to different vibrations, like stretching vibration of hydrogen-bonded ($\nu_{O\text{-}H}$), which is overlapped to the stretching vibration of N – H from the free amino group (- NH_2) at C_2 position of glucosamine: at 3356 cm^{-1}

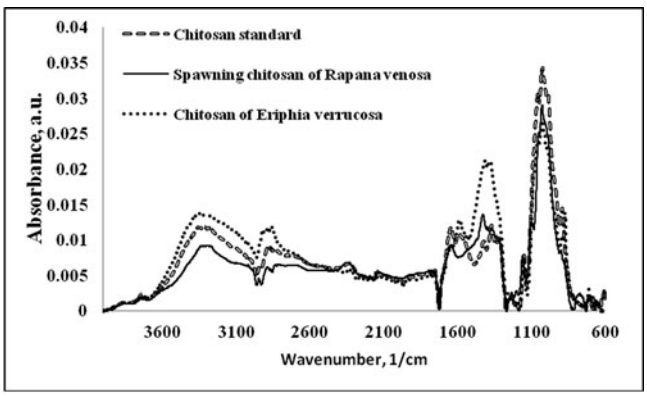

Figure 9. FT-IR spectra of the chitosan samples.

and 3292 cm^{-1} for standard chitosan, at 3336 cm^{-1} and 3287 cm^{-1} for spawning chitosan sample of *Rapana venosa*, at 3362 cm^{-1} and 3292 cm^{-1} for chitosan of *E.verrucosa*;
- At $\sim$ 2935 cm^{-1} and 2884 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;
- The band at 1656 cm^{-1} representing acetylated amino group, which is due to the C = O stretching vibrations ($\nu_{C=O}$) of Amide I is present in all chitosan samples;
- The band at 1590 cm^{-1} is assigned to NH_2 bending vibrations (δ_{N-H}) (N-acetylated residues, amide II band) [7] for chitosan standard. This band is shifted to 1587 cm^{-1} for crustacean chitosan samples and desappeared in the case of spawning chitosan sample of *Rapana venosa*. The absence of this band for spawning chitosan sample of *Rapana venosa* indicates a higher degree of deacetylation than in chitosan standard sample;
- Vibrations of –OH group of the primary alcoholic group are registered at 1421 cm^{-1} for standard chitosan and at 1428 cm^{-1} for spawning chitosan sample of *Rapana venosa* and at 1417 cm^{-1} for chitosan sample of *E.verrucosa*;
- In all chitosan samples vibrations of O–H and C–H in the ring are displayed between 1385 ÷ 1316 cm^{-1}, and the band located near 1150 cm^{-1} is related to –C–O–C in glycosidic linkage [8];
- The primary alcoholic group ($-CH_2-OH$) represented by a strong absorption band at 1025 cm^{-1} for standard chitosan could be seen at 1028 cm^{-1} for spawning chitosan sample of *Rapana venosa* and 1032 cm^{-1} for chitosan sample of *E.verrucosa*.

The results from the spectroscopic analysis indicated the presence of chitosan in all studied samples. The chemical extraction method from spawning *Rapana venosa* leads to a chitosan sample with a higher degree of deacetylation than in chitosan standard sample.

Conclusions

The studies of some Romanian marine resources in Black Sea waters demonstrated that these are a rich source of chitosan, which make them useful in a various fields, ranging from waste management to food processing, medicine and biotechnology.

The chitosan, obtained by chemical extraction of crustacean carapace (exoskeleton) was analyzed and compared by UV – VIS – NIR and FT-IR/ATR spectroscopy with those of chitosan standards and the results confirmed the existence of chitosan in the collected samples. The absorption bands present in the UV – VIS – NIR and IR spectra obtained for the chitosan extracted from the two marine sources were compared with those of standard chitosan showing that spawning of *Rapana venosa* as well as crustacean *E.verrucosa* are good sources of chitosan, source unexploited yet.

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