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Extraction and Characterization of Chitin and Chitosan from Invasive Alien Swimming Crab *Charybdis longicollis*

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Abstract

Chitosan is a biocompatible, biodegradable, non-toxic, antibacterial, antioxidant, and antifungal natural polymer. In this study, chitin and chitosan were chemically isolated from the exoskeleton of invasive swimming crab *Charybdis longicollis*. In order to obtain the chitin, demineralization, deproteinization, and decolourization steps were applied to the samples. Chitosan was prepared from the isolated chitin by deacetylation at high temperatures. The chemical composition of chitin from *C. longicollis* was characterized by XRD and FTIR analysis. The yield of chitin extraction from dry crab shells was 25.78 %. The yield of chitosan produced from extracted chitin was 80.23 %. The experimental analyses revealed that the obtained chitin and chitosan could be used as biomaterial.

Keywords:
Charybdis longicollis, swimming crab, chitin, chitosan, scaffold, biomaterial
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Introduction

The invasive swimming crab *Charybdis longicollis* Leene, 1938, is native to the Indian Ocean, the Persian Gulf, and the Red Sea and was first recorded from the Mediterranean Sea in Antalya and Mersin Bays in 1954 (Holthuis, 1961). The population spread to Egypt and Greece in the Mediterranean and dominated to depths of 25 to 80 m (Innocenti & Galil, 2011). After first

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recorded *C. longicollis*, populations dominated the sandy-mud bottoms to a depth of 25 to 80 m. *C. longicollis* population has a consistently high rise in recent decades (Deval, 2020). This high rise is a severe problem for the Mediterranean ecosystem.

C. longicollis prefers infaunal and slow-moving prey, and the most common food items are molluscs, crustaceans, and fish. As the species is highly abundant in the littoral and sublittoral zones of the Mediterranean Sea, it is likely to influence the local biota (Stasolla et al., 2015). Sixty years after its first record in the Mediterranean, the population of *C. longicollis* seems durable, despite the high prevalence of its parasite and its damaging impacts, such as increased host mortality, sterilization, moulting prevention, and reduction of aggressive behaviour (Innocenti et al., 2003). Innocenti & Galil (2011) suggested that the crab's high fecundity and the presence of parasite-free larger individuals, mainly in deeper waters, maintain its population.

Chitin is a natural polymer that has a highly organized crystalline structure that is nitrogenous, white, and hard, having a low chemical reactivity (Pillai et al., 2009). It is the second most abundant polysaccharide in nature, second only to cellulose (Hudson & Smith, 1998). It is insoluble in water and organic solvents, presenting, after purification, as a yellowish powder. Chitin is widely distributed in nature and is the main element of the marine invertebrate exoskeleton; and can be found in the structure of insects, arthropods, and molluscs (Santos et al., 2020).

In this study, chitin was chemically isolated from the exoskeleton of invasive swimming crab *C. longicollis*. This study exhibits the efficiency of this approach and the economic potential of the *C. longicollis* exoskeleton to obtain chitin and chitosan with properties suitable for pharmaceutical and biomedical applications.

Material and Methods

Invasive swimming crab samples were collected from the Gulf of Iskenderun using a commercial trawler. The samples were transferred to the laboratory and stored at -21˚C until starting the extraction procedure.

The first stage of the extraction process involves thermomechanical treatments, where the shells are scraped free of loose tissue and washed individually in lightly saline water. After that, chitin extraction of the swimming crab shell was treated with 1.7 M HCl at an ambient temperature for 6 h and then with 2.5 M NaOH at 75 ◦C for 1 h. Next, the pigments were decomposed by using 1% potassium permanganate. After that, chitosan extraction was performed with 50% NaOH at 121° C for 1h (Figure 1).

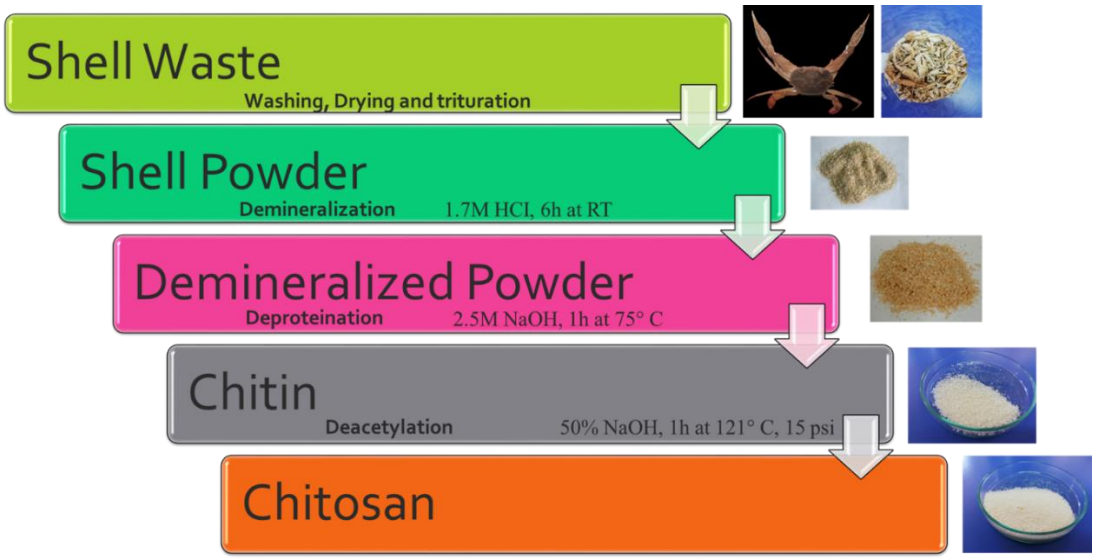

Figure 1. The extraction process of chitin and chitosan

The infrared spectra were registered in a Fourier transform infrared (FTIR) spectrometer (Jasco/FT/IR-6700) connected to a PC with Spectra Manager™ (Jasco) for data processing. The analyses were directly performed on finely powdered *C. longicollis* material. The samples were prepared in KBr pellets at a concentration of 5% concentration. They were placed into the crystal cell, and the cell was clamped into the mount of the FTIR spectrometer. In this work, we used a range of 400–4000 cm^{-1} then the automatic signal gain was collected and rationed against a background spectrum recorded from the clean empty cell.

XRD analysis

X-ray powder diffraction (XRD) was used to identify phase compositions and the crystallinity of the chitin. The XRD patterns were recorded by Rigaku Miniflex 600 Diffractometer with Cu Kα (40 kV, 15 mA, λ=1.54050 Å) radiation. Scanning was conducted between 10 deg<20<70<90 deg (with 0.01-deg and 0.05-deg steps and 1 deg/min rate).

Results

Three repetitive analyses were performed to calculate the quantity of chitin and chitosan extracted from the shells of *C. longicollis*. Dried crab shells in the wet weight state were quantified with a ratio of 25.78 % chitin. The yield of chitosan produced from extracted chitin was 80.23 %.

Fourier transform infrared (FTIR) of chitin from the swimming crab species *C. longicollis* are shown in Figure 2. These spectra presented peaks at 1063 cm^{-1}, which is due to the C–H bonds of the anomeric carbon. This band to characterize the configuration of the anomeric centre from the glucopyranosicyclic residues of chitin: C-H axial at 1063 cm^{-1}. The first wide peak lies between 1229 and 1250 cm^{-1}. This range corresponds to C=O, so this result is also indicative of alpha chitin. The second wide peak is between 1381 and 1405 cm^{-1}. This interval includes the significant amide bands that correspond to the amide II of N–H and the amide III of C–N. The third wide peak is between 2900 and 2987 cm^{-1}. Finally, we found one low peak (3668–3673 cm^{-1}) corresponding to

amide B. The results indicate a system containing amino-polysaccharide alpha chitin alongside proteins.

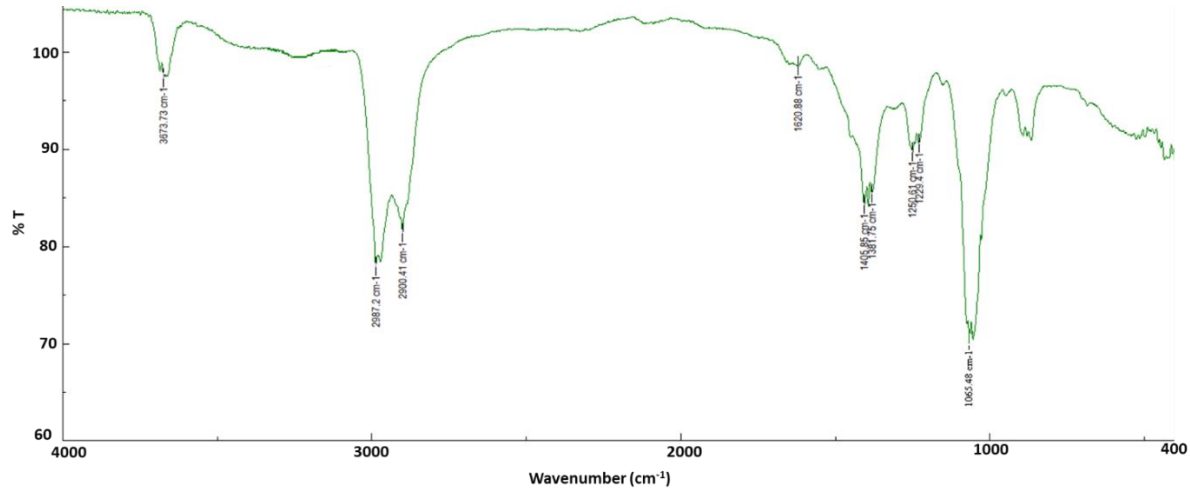

Figure 2. Fourier transform infrared (FTIR) spectra of chitin obtained from *C. longicollis* in the range of 4000–400 cm^{-1}

An analysis of the chitin XRD patterns are given in Figure 3 shows that all intense reflections match with chitin with the chemical formula $C_{32}H_{52}O_{20}N_4$. The reflections of the chitin match perfectly with the data of the alpha chitin presented on ICDD Card No: 96-151-6345 (Sikorski et al., 2009). Its cell parameters were found as follows: a = 4.75, b = 10.3330 ve c = 18.89, which are very close to the ICDD card. The XRD analysis supports the result of the FTIR analysis.

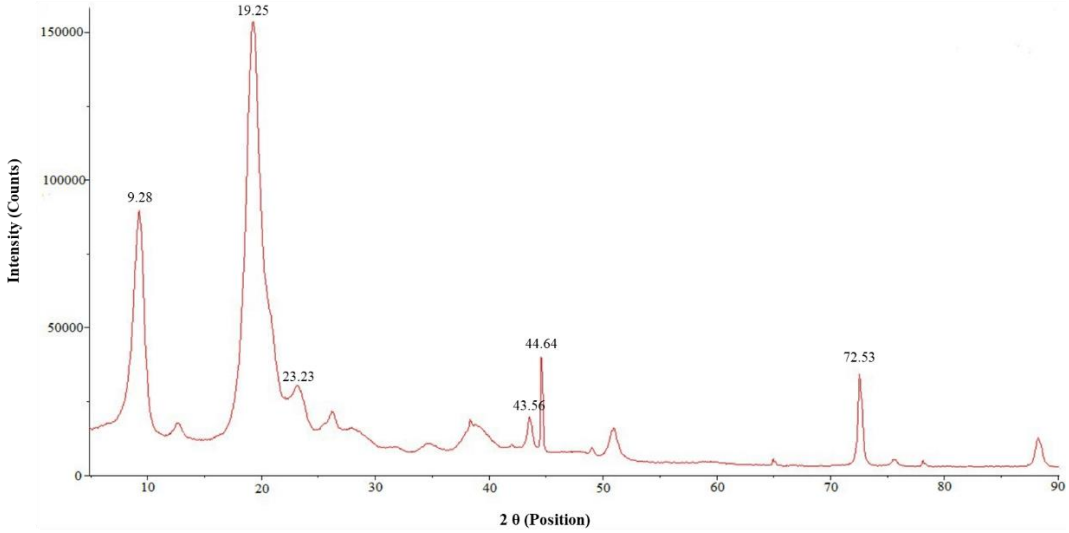

Figure 3. X-ray diffraction patterns of chitin from *C. longicollis*.

Discussion

In the present study, chitin was chemically isolated from the exoskeleton of invasive swimming crab *Charybdis longicollis*. The characterization studies (XRD and IR spectrum) indicated that the obtained material was identified as chitin and the yield of chitin extraction from dry crab shells was found 25.78 %. In the next step, chitosan was produced from the extracted chitin at 80.23 %. This yield is higher than the data reported in the literature (Oduor-Odeto et al., 2005; Kaya et al., 2016; Demir et al., 2016; Varun et al., 2017; Ahyat et al., 2017) and the result proved that the crabs are one of the significant resources of chitin and chitosan among the other crustacean organisms. It's noteworthy that the obtained chitin and chitosan can be used as biomaterial.

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Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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