ISOLATION AND CHARACTERIZATION OF CHITIN FROM SHELLS OF THE FRESHWATER CRAB POTAMON ALGERIENSE

Soufiane Fadlaoui1*, Ouahid El Asri2, Lakrat Mohammed3, Addou Sihame4, Abdelouadoud Omari1, Mohammed Melhaoui1

1Laboratory of Water, Environment, and Sustainable Development, Mohamed First University, Mohammed V avenue, P.O.Box 724, Oujda 60000 Morocco
E-mail: soufiane.fadlaoui@gmail.com

2Biochemistry and Biotechnology Laboratory, Mohamed First University, Oujda, Morocco

3Laboratory of mineral solid and analytical chemistry, Mohamed First University, Oujda, Morocco

4Laboratory of Physics of Matter and Radiation, Mohamed First University, Oujda, Morocco

Abstract
This work presents, for the first time, the extraction and characterization of chitin from the shell of the freshwater crab species Potamon algeriense with a standardized and revised chemical method. Chitin and chitosan were isolated following demineralization, deproteinization, decolouration (raw chitin), and deacetylation (chitosan). After boiling, drying, and grinding, 62.12% of the ground shell was obtained. A yield of 40.92% was obtained after demineralization of ground crab shell, while after the deproteinization process 8.74% was obtained. After decolourization, 8.27% of raw chitin was obtained, and the final amount of chitosan extracted from the crab shells was approximately 5.89%. We also characterized the isolated chitin by determining its physicochemical properties using X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA).

Keywords: Chitin, chitosan, deacetylation, freshwater crab, Potamon algeriense

Received: 15.03.2019
Accepted: 02.05.2019
1. Introduction

Chitin is the second most abundant biopolymer in nature, after cellulose. It is usually isolated from the exoskeletons of invertebrates, insects, marine diatoms, sponges, mollusks, coralline algae, cell walls of certain fungi, and crustaceans like crabs, shrimps, and lobsters by chemical processes using strong acids and bases [1–6]. The colour of chitin is light yellow to brown, and the appearance of chitin is as flocculence or a filiform solid. In addition, chitin is not soluble in water [7]. Chitin does not have a single chemical structure, but many. It includes several polysaccharides composed of N-acetyl-β-D-glucosamine units (from 50 to 100%) and D-glucosamine units (from 0 to 50%).

Structurally, chitin is a straight-chain polymer composed of β-1,4-N-acetylg glucosamine, and it is classified into three different natural polymorphs, α-, β-, and γ-chitin [8,9], with α-chitin being the most common in nature and having a structure of antiparallel chains, usually isolated from the exoskeleton of crustaceans and more particularly from shrimps, crabs, and lobsters [5]. β-chitin can be obtained from squid pens. It has intra-sheet hydrogen bonding by parallel chains [10,11]. Meanwhile, γ-chitin, found in yeast and the cell walls of certain fungi, has not been completely identified. It has been proposed that it is a mixture of two parallel chains and one antiparallel chain. [11,12] have suggested that γ-chitin can be a combination of α and β structures rather than a different polymorph.

Because chitin has a compact structure, it is insoluble in most solvents [13,14]. Therefore, chemical modifications of chitin are performed [15]. The most common derivative is chitosan, a straight-chain polymer of glucosamine and N-acetylg glucosamine, hydrophilic, natural, cationic, nontoxic biopolymer derived from partial N-deacetylation of chitin [16–18].

The last three decades have seen active research into potential usual applications of chitin and its derivatives, mainly chitosan. Because of its biodegradability, biocompatibility, and non-toxicity, chitosan has a wide range of applications in different fields, e.g., cosmetics, agriculture, food, pharmacy, biomedicine, the paper industry, paint and textile industries, wastewater treatment, wound healing, and drug delivery systems [17–43]. Chitosan and chitosan oligomers are also known for their biological activities, such as their antimicrobial [41–55], antitumor [39,40], and hypocholesterolemic functions [41].

The carapace waste of crustaceans is constituted mainly of 30–50% calcium carbonate, 30–40% protein, and 20–30% chitin. Nevertheless, these constituents are changeable, depending on the species and seasons [49]. To quote some examples, the shell waste of the snow crab *Chionoecetes opilio* and the northern prawn *Pandalus borealis* contains approximately 17–32.2% chitin [50–59]. It has been estimated that the chitin content of the blue crab was 14%. It was also determined that the grey shrimp *Crangon crangon* contains 17.8% chitin [1], while the speckled shrimp *Metapenaeus Monoceros* contains 4.5–7% chitin [52].

The freshwater crab, *Potamon algeriense* [53], belongs to the family of the Potamidae, which is the largest of all freshwater crab families and comprises 95 genera and over 505 species [54,55]. *P. algeriense* can be found in North Africa, exclusively in three countries, Morocco, Algeria, and Tunisia [54]. In Morocco, the species has been reported from the north in the watershed of the Oued Laou near Chefchaouen, from the Northeast in the watershed of Moulouya, and from the Middle Atlas in the Oued Oum Rbia watershed near Khenifra. Despite their wide distribution, the population of *P. algeriense* has not been commercially evaluated. In fact, freshwater crabs are also an important source of chitin, like the other crustaceans.

The aim of this study was to extract and determine the yield of chitin and chitosan from the carapace of *P. algeriense*, known as the freshwater crab of Maghreb, and it has not
been economically evaluated, besides its wide range of applications in numerous industrial areas.

2. Materials and Methods

2.1. Chitin and Chitosan Extraction

Specimens of the freshwater crab *Potamon algeriense* [53] from Oued Zegzel northeast of Morocco were used in this study (Fig. 1 and Fig. 2). For extracting chitin and chitosan, crabs were boiled for 15 min in order to take them out from their carapaces. The shells were scraped free of soft tissue, cleaned, rinsed, and dried at 60°C for 24 hours. The shells were ground with a Retsch mill (model Brinkmann Rmo) to obtain a coarse powder and were then sieved to a 350-µm diameter. The extraction of chitin and chitosan from the crab carapace was performed with three repetitive analyses. Chitosan was extracted from the shells by means of mineralization, deproteinization, decolouration, and deacetylation.

![Figure 1. The freshwater crab *Potamon algeriense.*](image)

The ground shells were then soaked very slowly (to avoid overflow of the sample due to the massive emission of CO₂ gas) in a 1 N HCl solution for six hours at room temperature to remove calcium salts (demineralization), with a solid/solvent ratio of 1:15 (w/v) [56]. The decalcified shell was collected on Whatman paper filter in a Buchner funnel. The resulting solid was washed with deionized water until it was neutralized. Then, the demineralized samples were dried and weighed.

Chitin deproteinization was carried out under standard autoclaving conditions (15 psi/121°C). The demineralized shell was treated with aqueous sodium hydroxide solution (3%) for 20 min at 15 psi/121°C, and the solid/solvent ratio was 1:10 (w/v) [49]. The absence of proteins was indicated by the absence of colour of the medium at the last treatment. The resulting solution was then washed to neutrality, filtered, dried, and weighed as mentioned above (Fig. 3).

For the purpose of decolouration, the obtained chitin residue was treated further with 0.32% sodium hypochlorite solution for 10 min, with a solid/solvent ratio of 1:10 (w/v) [56]. Following decolouration, the discoloured chitin was collected and washed with...
deionized water until it was neutralized. It was then dried at 60°C for 24 h to obtain purified discoloured crab chitin.

![Figure 2](image)

*Figure 2. Area of the study in Oued Zegzel, Morocco.*

For deacetylation, purified crab chitin was treated under the conditions of 15 psi/121°C with 50% NaOH for 30 min, and a solid/solvent ratio of 1:15 (w/v) was used [57]. The resulting chitosan was filtered, washed, dried, and weighed. The yield of chitosan extracted from crabs was then calculated. All data collected from this work were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) [58].

### 2.2. Characterization of Chitin

#### 2.2.1. Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra were registered in a Fourier transform infrared (FTIR) spectrometer (Nicolet Magna, Nicolet Analytical Instruments, Madison, WI) connected to a PC with Omnic software (Thermo Electron Corp) for data processing. The analyses were directly performed on finely powdered *Potamon algeriense* material. The samples were prepared in KBr pellets at a concentration of 5% (w/w) [59]. 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 500–4500 cm\(^{-1}\) then the automatic signal gain was collected and rationed against a background spectrum recorded from the clean empty cell.

#### 2.2.2. X-Ray Diffraction (XRD)

The degree of crystallinity and the size of the crystallites were determined by means of the X-ray diffraction (XRD) method. The same finely ground powder samples as used for FTIR were used for this analysis. The polymorphism of the crystals in the samples was determined by an X-ray diffractometer (Phillips) with 30 kV and 40 mA Cu K\(\alpha\) radiations. Sample analyses were carried out in the 20–60° range of the 2\(\theta\) angle, with step sizes of 0.020° and a point measurement time of 2 s.
2.2.3. Thermogravimetric (TG) Analysis
Thermogravimetric (TG) analysis of this material was carried out with a Perkin Elmer TGA 7 apparatus with a platinum sample holder using Pyris software for data handling. Measurements were performed in a nitrogen atmosphere at a heating rate of 15°C min⁻¹. The samples were heated up to at least 700°C, starting from 50°C.

3. Results and Discussion

3.1. Chitin and Chitosan Extraction
Three repetitive analyses were performed for the purpose of calculating the quantity of chitosan extracted from the shells of *P. algeriense*. Dried and ground crab shells in the wet weight state were quantified with a ratio of 62.12%. The results are shown in Fig. 3 and Table 1.

![Figure 3. Isolation process of chitin and preparation of chitosan from crab shells.](image-url)
About 22 crab species from 4500 species over the globe have been commercially evaluated [54,60]. In ecological investigations, crabs are considered biological indicators [61]. Numerous studies have concentrated on the biological, chemical, and ecological aspects of freshwater crabs [61–65], but rare studies have been carried out on the extraction of chitin and chitosan [66].

The quantity of chitosan is estimated to be approximately 1.560 million tons throughout the world [67]. The shells of crustaceans currently present the main industrial source for extraction of this biopolymer. One hundred billion tons of chitin are generated from arthropods, mollusks, and the cell walls of certain fungi every year. Cauchie et al. (1997) estimated that the annual production of this polysaccharide in freshwater ecosystems is only approximately 600 million tons [67]. Knoor (1984) stated that chitin is the most under-exploited polysaccharide around the globe. There is currently a total of 238 genera and 1.476 species of known freshwater crabs from 14 families around the world [53] that could be exploited as sources of chitosan.

The chemical process of isolating chitin and chitosan from crab shells after drying and grinding results in a yield of purified chitin of 8.27% and a yield of chitosan of 5.89%. The results are presented in Fig. 3 and Table 1.

**Table 1.** The yield (%) of chitosan isolated from crab shells.

| Isolation process               | % Yield |
|--------------------------------|---------|
| Wet weight                     | 100.00  |
| Weight of boiled shell          | 64.85   |
| Weight of dried shell           | 62.32   |
| Weight of ground shell          | 62.12   |
| Dry weight                     |         |
| Ground shells                   | 100.00  |
| Demineralized shells            | 40.92   |
| Deproteinized shells            | 8.74    |
| Decoloured shells (Raw chitin)  | 8.27    |
| Deassembled shells (Chitosan)   | 5.89    |

In the present manuscript, the yield after boiling the specimens was quantified as 64.85%, which was statistically significant (P>0.05) according to the yield of dried and ground freshwater crab shell. These results were in accordance with those acquired with the sand crab *Portunus pelagicus* [67, 68]. Approximately 8.27% of chitin was found in the ground shells of *P. algeriense*. The results obtained by Mol [69] showed a yield of around 3–6% chitin. Chakrabarti (2002) reported that the brown shrimp *Metapenaeus monoceros* contains about 4.5–7% chitin in shell waste [62], while Hertrampf and Piedal-Pascual (2000) declared that the content of chitin extracted from the snow crab *Chionoecetes opilio* was nearly 10.6% [70]. The quantity of chitin was 26.6% in *Chionoecetes opilio* [71] and 17.8% in the grey shrimp *Crangon crangon* [72]. Tharanathan (2003) reported that 14% of chitin was found in blue crab, while in *Chionoecetes opilio* and *Pandalus borealis* chitin content ranged from 17 to 32.2% [50,51]. Cho (1998) declared that the difference in the potential amount of chitin and chitosan between various crabs depends on the species and the seasons [49].

Chitosan is a biopolymer resulting from alkaline deasettilation of chitin. It is found mainly in the exoskeleton of arthropods and is quite easy to isolate methodologically. In this paper, 5.89% of chitosan was extracted from the freshwater crab *P. algeriense*. Due
to the easier and inexpensive method of collecting and capturing freshwater crabs compared to that of marine species, freshwater crabs could be raised as a source of chitin and they may also present a new alternative fishing material in freshwater ecosystems. For all of these reasons, freshwater crustaceans, especially crabs, could be commercially evaluated by producing chitin and chitosan because of the ease of processing for chitin extraction.

3.2. Characterization of Chitin and Chitosan

3.2.1. Fourier Transform Infrared Spectroscopy

The infrared (IR) spectra of chitin from the crab species *potamon algeriense* are shown in Fig. 4. These spectra presented peaks at 890 cm⁻¹, which is due to the C–H bonds of the anomeric carbon. Several authors used this band to characterize the configuration of the anomeric centre from the glucopyranosicyclic residues of chitin: C–H axial at 891±7 cm⁻¹ and C–H equatorials at 844±8 cm⁻¹ atoms [72]. Therefore, the chitin of *potamon algeriense* is characterized by the β-configuration in the anomeric centre (C₁) of this polysaccharide.

On the other hand, these spectra were characterized by two wide peaks. The first wide peak lies between 913 and 1108 cm⁻¹. This range corresponds to C=O, so this result is also indicative of chitin [73]. The second wide peak is between 1242 and 1570 cm⁻¹. This interval includes the significant amide bands that correspond to the amide ΙΙ of N–H and the amide ΙΙΙ of C–N.

Finally, we found two low peaks (2801–2889 cm⁻¹) corresponding to amide B (2800–2990 cm⁻¹) [75,76]. The results indicate a system containing amino-polysaccharide chitin alongside proteins.

![Fourier transform infrared (FTIR) spectra of chitin obtained from *potamon algeriense* in the range of 4500–400 cm⁻¹.](image)

**Figure 4.** Fourier transform infrared (FTIR) spectra of chitin obtained from *potamon algeriense* in the range of 4500–400 cm⁻¹.
3.2.2. X-Ray Diffraction

To detect the orientation of the crystal structures and to understand the functional properties of the organic matrix components in *potamon algeriense*, the finely ground shell powder samples were analysed by XRD. The XRD pattern of *potamon algeriense* shell is shown in Fig. 5. The XRD analysis revealed that the biggest diffraction face intensity is at $2\theta=29.4$. This intensity corresponds to the rhombohedral calcite. Rahman et al. (2014) confirmed that this diffraction angle ($2\theta=29.4$) in the calcite (104) indicates the presence of Mg–calcite [74].

The comparison of our XRD diffraction results with different types of calcite (012, 140, 110, 113, 202, 018, 116, 122, 211) shows that is similar to the strongest faces of calcite. Therefore, we showed different crystal surfaces in our case. We found that the crystalline components in the shell of *potamon algeriense* exhibited the characteristics of chitin and collagen calcite planes.

![Image of X-ray diffraction pattern](image)

**Figure 5.** X-ray diffraction (XRD) analysis of chitin obtained from *potamon algeriense*. The diffraction scan identifies the mineral form of calcium carbonate with calcitic crystal planes, which were nucleated by chitin and collagen matrices.

3.2.3. Thermogravimetric Analysis

Concerning the chitin obtained from *potamon algeriense*, the mass loss was observed in four stages (Fig. 6). In the first stage, there was a mass loss of 5%. This loss was due to water evaporation within the structure. In the second stage, mass loss amounted to 15.3%. The mass loss observed at this stage was due to the beginning of the decomposition of the chitin molecules. In the third stage, mass loss was 22.8%, corresponding to the continued decomposition of chitin. During the fourth step, we observed the biggest mass loss rate (24.8%). The mass loss observed at this stage was due to the decomposition of calcite in the collagen calcite. These results show the good thermal stability of the extracted chitin.

---

*S. Fadlaoui, O. El Asri, L. Mohammed, A. Sihame, A. Omari, M. Melhaoui*
4. References

[1] Synowiecki J, Al-Khateeb NA; (2003) Production, properties, and some new applications of chitin and its derivatives. Critical Reviews in Food Science and Nutrition, 43, 145–171.

[2] Klinger C, Żółtowska-Aksamitowska S, Wysokowski M, Tsurkan M V, Galli R, Petrenko I, et al; (2019). Express Method for Isolation of Ready-to-Use 3D Chitin Scaffolds from Aplysinaarcheri (Aplysineidae: Verongiida) Demosponge. Marine drugs, 17, 131. DOI: doi.org/10.3390/md17020131.

[3] Wysokowski M, Bazhenov V V, Tsurkan M V, Galli R, Stelling AL, Stöcker H, et al; (2013). Isolation and identification of chitin in three-dimensional skeleton of Aplysinafistularis marine sponge. International Journal of Biological Macromolecules, 62, 94–100. DOI: 10.1016/j.ijbiomac.2013.08.039.

[4] Shaala L A, Asfour HZ, Youssef DT, Żółtowska-Aksamitowska S, Wysokowski M, Tsurkan M, et al; (2019). New source of 3D chitin scaffolds: the Red Sea demosponge Pseudoceratinaarabica (Pseudoceratinidae, Verongiida). Marine drugs, 17, 92. DOI: 10.3390/md17020092.

[5] Ehrlich H, Shaala LA, Youssef DT, Żółtowska-Aksamitowska S, Tsurkan M, Galli R, et al; (2018). Discovery of chitin in skeletons of non-verongiid Red Sea demosponges. PloS one, 13, e0195803. DOI: 10.1371/journal.pone.0195803.

[6] Żółtowska-Aksamitowska S, Tsurkan MV, Lim SC, Meissner H, Tabachnick K, Shaala LA, et al; (2018). The demosponge Pseudoceratinapurpurea as a new source of fibrous chitin. International journal of biological macromolecules, 112, 1021-1028. DOI: 10.1016/j.ijbiomac.2018.02.071.

[7] Tanigawa T, Tanaka Y, Sashiwa H, Saimoto H, Shigemasa Y; (1992) Various biological effects of chitin derivatives. In C. J. Brine, P. A. Sandford, & J. P.Zirkakis (Eds.), Advances in chitin and chitosan (pp. 206–215). London: Elsevier Science Publisher.

[8] Cabib E; (1981) Chitin: structure, metabolism, and regulation of biosynthesis. In Plant Carbohydrates II (pp. 395-415). Springer, Berlin, Heidelberg.
[9] Cabib E, Bowers B, Sburlati A, Silverman SJ; (1988) Fungal cell wall synthesis: The construction of a biological structure. Microbiological Science, 5, 370–375.
[10] Minke R, Blackwell J; (1978) The structure of α-chitin. Journal of Molecular Biology, 120, 167–172.
[11] Jang MK, Kong BG, Jeong YI, Lee CH, Nah JW; (2004) Physicochemical characterization of α-chitin, β-chitin and γ-chitin separated from natural resources. Journal of Polymer Science, Part A: Polymer Chemistry, 42, 3423–3432.
[12] Rudall KM; (1963) The chitin/protein complexes of insect cuticles. Advances in Insect Physiology, 1, 257–313.
[13] Silva SS, Mano JF, Reis RL; (2017). Ionic liquids in the processing and chemical modification of chitin and chitosan for biomedical applications. Green Chemistry, 19, 1208-1220. DOI: 10.1039/C6GC02827F.
[14] Jaworska MM, Stepniak I, Galiński M, Kasprzak D, Biniaś D, and Górak A; (2018). Modification of chitin structure with tailored ionic liquids. Carbohydrate polymers, 202, 397-403. DOI: 10.1016/j.carbpol.2018.09.012.
[15] Peter MG; (1995) Applications and environmental aspects of chitin and chitosan. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, 32(4), 629–640.
[16] Roberts GAF; (1992) Chitin chemistry (1st ed.). London: Macmillan.
[17] Muzzarelli RAA; (1977) Chitin. Pergamon, Oxford, UK.
[18] Muzzarelli RAA, Rocchetti R, Stanic V, Weckx M; (1997) Methods for the determination of the degree of acetylation of chitin and chitosan. Chitin handbook, 109-119.
[19] Masri MS, Reuter FW, Friedman M; (1974) Binding of metal cations by natural substances. Journal of Applied Polymer Science, 18, 675–81.
[20] Elson CM, Davies DH, Hayes ER; (1980) Removal of arsenic from contaminated drinking water by a chitosan/chitin mixture. Water Research, 14(9), 1307–1311.
[21] Knoor D; (1984) Use of chitinous polymers in food – A challenge for food research & development. Food Technology, 38, 85–97.
[22] Jha IN, Iyengar L, Rao AVSP; (1988) Removal of cadmium using chitosan. Journal of Environmental Engineering, 114, 962–974.
[23] Mckay G, Blair HS, Findon A; (1989) Equilibrium studies for the sorption of metal ions onto chitosan. Indian Journal of Chemistry Section a-Inorganic Bio-Inorganic Physical Theoretical & Analytical Chemistry, 28(5), 356–360.
[24] Coughlin RW, Deshaies MR, Davis EM; (1990) Chitosan in crab shell wastes purifies electroplating wastewater. Environmental Progress, 9: 35–9.
[25] Udaybhaskar P, Iyengar L, Rao AVSP; (1990) Hexavalent chromium interaction with chitosan. Journal of Applied Polymer Science, 39, 739–47.
[26] Rorrer GL, Hsien TY, Way JD; (1993) Synthesis of porous magnetic chitosan beads for removal of cadmium ions from wastewater. Industrial & Engineering Chemistry Research, 32, 2170–2178.
[27] Simpson BK, Gagne N, Simpson MV; (1994) Bioprocessing of chitin and chitosan. In A. M. Martin (Ed.), Fisheries processing: Biotechnological applications (pp. 155–173). London: Chapman & Hall.
[28] Hsien TY, Rorrer GL; (1995) Effects of acetylation and crosslinking on the material properties and cadmium ion adsorption capacity of porous chitosan beads. Separation Science and Technology, 30(2), 455–75.
[29] Jansson-Charrier M, Guibal E, Roussy J, Delanghe B, Le Cloirec P; (1996) Vanadium (IV) sorption by chitosan: kinetics and equilibirum. Water Research, 30, 465–75.
[30] Guibal E, Milot C, Tobin JM; (1998) Metal-anion sorption by chitosan beads: equilibrium and kinetic studies. Industrial & Engineering Chemistry Research, 37, 1454–1463.
[31] Guibal E, Milot C, Roussy J; (1999) Molybdate sorption by cross-linked chitosan-beads: dynamic studies. Water Environment Research, 71, 10–7.
[32] Felse PA, Panda T; (1999) Studies on applications of chitin and its derivatives. Bioprocess Engineering, 20, 505–512.
[33] Ngah WSW, Liang KH; (1999) Adsorption of gold (III) ions onto chitosan and N-carboxymethylchitosan: equilibrium studies. Industrial & Engineering Chemistry Research, 38, 1411–1414.
[34] Shahidi F, Arachchi JKV, Jeon YJ; (1999) Food applications of chitin and chitosan. Trends in Food Science and Technology, 10, 37–51.
[35] Kumar MNVR; (2000) A review of chitin and chitosan applications. Reactive and Functional Polymers, 46, 1–27.
[36] Dutta PK, Dutta J, Tripathi VS; (2004) Chitin and chitosan: chemistry, properties and applications. Journal of Scientific and Industrial Research, 63, 20–31.
[37] Rashidova SSH, Milusheva R Yu, Voropaeva NL, Pultonova SR, Nikonovich GV, Ruban IN; (2004) Isolation of chitin from a variety of raw materials, modification of the material, and interaction its derivatives with metal ions. Chromatographia, 59, 783–786.
[38] Wysokowski M, Motylenko M, Rafaja D, Koltsov I, Stöcker H, Szalaty TJ, et al; (2017). Extreme biomimetic approach for synthesis of nanocrystalline chitin-(Ti, Zr) O2 multiphase composites. Materials Chemistry and Physics, 188, 115-124. DOI: 10.1016/j.matchemphys.2016.12.038.
[39] Petrenko I, Bazhenov VV, Galli R, Wysokowski M, Fromont J, Schupp PJ, et al; (2017). Chitin of poriferan origin and the bioelectrometallurgy of copper/copper oxide. International journal of biological macromolecules, 104, 1626-1632. DOI: 10.1016/j.ijbiomac.2017.01.084.
[40] Bazhenov VV, Wysokowski M, Petrenko I, Stawski D, Sapozhnikov P, Born R, et al; (2015). Preparation of monolithic silica–chitin composite under extreme biomimetic conditions. International journal of biological macromolecules, 76, 33-38. DOI: 10.1016/j.ijbiomac.2015.02.012.
[41] Kendra DF, Hadwiger LA; (1984) Characterization of the smallest chitosan oligomer that is maximally antifungal to Fusarium solani and elicits pisatin formation in Pisumsativum. Experimental Mycology, 8, 276–281.
[42] Sudarshan NR, Hoover DG, Knorr D; (1992) Antibacterial action of chitosan. Food Biotechnology, 6, 257–272.
[43] Sekiguchi S, Miura Y, Kaneko H, Nishimura SI, Nishi N, et al; (1994) Molecular weight dependency of antimicrobial activity by chitosan oligomers. In: Nishinari, K., Doi, E. (1994). Food Hydrocolloids: Structures, Properties, and Functions. Plenum, New York, pp. 71–76.
[44] Abdou ES, Elkholy SS, Elsabee MZ, Mohamed E; (2008) Improved antimicrobial activity of polypropylene and cotton nonwoven fabrics by surface treatment and modification with chitosan. Journal of Applied Polymer Science, 108, 2290–2296.
[45] Elsabee MZ, Abdou ES, Nagy KSA, Eweis M; (2008) Surface modification of polypropylene films by chitosan and chitosan/pectin multilayer. Carbohydrate Polymers, 71, 187–195.
[46] Suzuki K, Mikami T, Okawa Y, Tokoro A, Suzuki S, et al; (1986) Antitumor effect of hexa-N-acetylchitoheaxoase and chitoheaxoase. Carbohydrate Research, 151, 403–408.
[47] Tokoro A, Tatewaki N, Suzuki K, Mikami T, Suzuki S, et al; (1988) Growth-inhibitory effect of hexa-N-acetylchitohexaose and chitohexaose against Meth-A solid tumor. Chemical & Pharmaceutical Bulletin, 36, 784–790.

[48] Sugano M, Yoshida K, Hashimoto M, Enomoto K, Hirano S, et al; (1992) Hypocholesterolemic activity of partially hydrolyzed chitosan in rats. In: Brine, C.J., Sandford, P.A., Zikakis, J.P. (Eds.), Advances in Chitin and Chitosan. Elsevier, London, pp. 472–478.

[49] Cho HR, Chang DS, Lee WD, Jeong ET, Lee EW; (1998) Utilization of chitosan hydrolysate as a natural food preservative for fish meat paste products. Korean Journal of Food Science and Technology, 30, 817–822.

[50] Shahidi F, Synowiecki J; (1991) Isolation and characterization of nutrients and value-added products from snow crab (Chionoecetes opilio) and shrimp (Pandalus borealis) processing discards. Journal of Agricultural and Food Chemistry, 39, 1527–1532.

[51] Tharanathan RN, Kittur FS; (2003) Chitin-the undisputed biomolecule of great potential Critical Reviews in Food Science and Nutrition, 43, 61–87.

[52] Chakrabarti R; (2002) Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. Food biotechnology, 16, 81–90.

[53] Bott R; (1967) Potamidae (Crustacea. Decapoda) aus Afghanistan. Westasien und dem Mittelmeeranam (Eine Revision der Untergattung Potamo R s str.). Videnskabeligemmeddelelser fra den Naturhistoriske Forening, 130, 7–43.

[54] Cumberlidge N, Daniels SR; (2008) A conservation assessment of the freshwater crabs of western Africa (Brachyura: Potamonautidae). African Journal of Ecology, 46, 74–79.

[55] Yeo DCJ, Ng PKL, Cumberlidge N, Magalhaes C, Daniels SR, et al; (2007) Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. In: Freshwater Animal Diversity Assessment. Hydrobiologia, 595, 275–286.

[56] No HK, Lee MY; (1995) Isolation of chitin from crab shell waste. Korean Journal of Food Science and Technology, 24, 105–113.

[57] Philips DJH; (1977) The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments-A review. Environemental Pollution, 13, 281–317.

[58] Siddiquie PJA, Akbar Z, Quasim R; (1987) Biochemical composition and calorific values of the three edible species of portunid crabs from Karachi. Pakistan Journal of Scientific and Industrial Research, 30, 119–122.

[59] Bahuguna SN, Rana AR, Singh S; (2016) Diet composition of freshwater crab, Potamonkoolooense Rathbun, 1904 from hillstream of Uttarakhand. Journal of Applied and Natural Science, 8(1), 301–304.

[60] Kobayashi S; (2012) Dietary preference of the potamid crab Geothelphusa dehaani in a mountain stream in Fukuoka, northern Kyushu, Japan. Plankton and Benthos Research, 7(4), 159–166.
ISOLATION AND CHARACTERIZATION OF CHITIN FROM SHELLS OF THE FRESHWATER CRAB POTAMON ALGERIENSE

[64] Cumberlidge N; (1999) The freshwater crabs of West Africa. Family Potamonautidae. Faune et Flore Tropicales 35, Institut de recherche pour le développement (IRD, ex-ORSTOM), Paris, 382 pp.

[65] Gherardi F, Micheli F; (1989) Relative growth and population structure of the freshwater crab, Potamonpotamios palestiniensis, in the Dead Sea area (Israel). Israel Journal of Zoology, 136, 133–145.

[66] Bolat Y, Bilgin Ş, Günli A, Izci L, Koca SB, et al; (2010) Chitin-chitosan yield of freshwater crab (Potamonpotamios, Olivier 1804) shell. Pakistan Veterinary Journal, 30, 227–31.

[67] Cauchie HM; (1997) An attempt to estimate crustacean chitin production in the hydrosphere. In: Advances in Chitin Science: Vol 2: (Domard A, GAF Roberts, KM Varum, eds): Jacques Andre Publisher, Lyon, France, pp: 32.

[68] Türeli C, M Çelik, ErdemÜ; (2000) Comparison of Meat Composition and Yield of Blue Crab (Callinectessapidus RATHBUN, 1896) and Sand Crab (Portunuspelagicus LINNE, 1758) Caught in Iskenderun Bay, North-East Mediterranean. Turkish Journal of Veterinary and Animal Sciences, 24, 195–203.

[69] Mol S; (2004) Alternative Product Technologies in Fisheries. In: Fish Processing Technology. No: 4465: (Varlık C, ed.): İstanbul Univ. Fisheries Faculty, İstanbul, Turkey, pp: 441–476.

[70] Hertrampf JW, Piedal-PascualF; (2000) Handbook on ingredients for aquaculture feeds. Kluwer Academics Publishers, Dortrecht, The Netherlands, pp: 109-113.

[71] Hong KN, MunYL; (1995) Isolation of chitin from crab shell waste. Korean Journal of Food Science and Technology, 24, 105–113.

[72] Synowiecki J, Al-KhateebNA; (2000) The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangoncrangon processing discards. Food Chemistry, 68, 147–152.

[73] Zhbankov RG, Andrianov VM, Marchewka M K; (1997). Fourier transform IR and Raman spectroscopy and structure of carbohydrates. Journal of Molecular Structure, 436, 637-654.

[74] Rahman MA and Halfar J; (2014) First evidence of chitin in calcified coralline algae: new insights into the calcification process of Clathromorphum compactum. Scientific reports, 4, 6162.

[75] Weiss I M, Schönitzer V; (2006). The distribution of chitin in larval shells of the bivalve mollusk Mytilusgalloprovincialis. Journal of structural biology, 153, 264-277.

[76] Lewandowska K., Furtos G. F; (2017) Characterisation of thin chitosan films for guided tissue regeneration purposes. Progress on Chemistry and Application of Chitin and its Derivatives, 22, 118-124.