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Original research

Using Patella caerulea as a biomaterial: Chitin and Chitosan

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Abstract:

Chitin is the most significant polysaccharide that can be obtained from the shells of crustaceans. In addition, the appearance of new application areas of chitin and chitosan is increasing, and the claim for new sources of chitin is increasing. For these reasons, this study was the first time for the new chitin chitosan sources from The Mediterranean Limpet (*Patella caerulea*) species. The physicochemical properties of chitin and chitosan obtained from the Mediterranean Limpet were determined. In addition, Fourier Transforms Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analyzes were performed. The chitin and chitosan yields of the Mediterranean Limpet were 43.42±0.78% and 72.93±1.02%, respectively. Chitin and chitosan from *P. caerulea* shells were found to exhibit some similarities with those from other shellfish. The %DA and %DD of chitin and chitosan obtained from *P. caerulea* shells were calculated as 14.32 and 85.68%, respectively. Chitin and chitosan obtained from the shells of *P. caerulea* can replace other chitin and chitosan in regards to use and applications. For this reason, the shells of *P. caerulea* are a talented alternative foundation of chitin and chitosan.

Keywords: Limpet, *Patella caerulea*, Chitin, Cellulose, İskenderun Bay.

1- Introduction

Limpets are crustaceans that are abundant in rocky areas around the world and are among the best marine herbivores (Jenkins et al., 2005). Diatoms, spores, macro algae and other invertebrates (cyanobacteria and microalgae) feed on microbial biofilms (Coleman et al., 2006; Jenkins et al., 2005). Patella, known as the Chinese hat or stone mussel in our country, is abundant in rocky areas on the seaside or just above sea level (Öztürk and Ergen, 1999). It is distributed in the tidal zones of the marine areas, on the stones on the sea coast, in the supralittoral zone, the mediolittoral zone and the superinfralittoral zone. Patellidae family is represented by 34 species in the world, and the genus Patella is represented by 9 species (Nakano and Ozawa, 2004). It has been reported that it is represented by 6 species in the Mediterranean and three species in our country (Güngör, 2011).

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Limpets of the genus Patella are browsing gastropods that are common populations of firm substrate groups in the central and upper-lower coastal regions of the East Atlantic and Mediterranean Seas in temperate zones (Vafidis et al., 2020). The Mediterranean Limpet *Patella caerulea* Linnaeus, 1758, is among the most common rocky coastal species in the midlittoral and infralittoral, mainly the Mediterranean (Küçükdermenci et al., 2017). *P. caerulea* is thought to be indigenous to the Mediterranean (Christiaens, 1973). Patella species are consumed as human food in the world (Gözler et al., 2003). However, it is neither consumed in our country, nor evaluated in any field. Additionally, no biomaterial studies related to *P. caerulea* (such as chitin and chitosan), which is common in our country, have been found. Only this species has studies on heavy metal accumulation (Duysak and Azdural, 2017; Yücel and Kılıç, 2023; Yüzereroğlu et al., 2010)

It is the second biopolymer of chitin used in the world after cellulose (Özbek, 2010). Chitin biopolymers are a long and linear polysaccharide found in many animals, such as molluscs, arthropods and fungi (Geçer et al., 2004). Chitin is commercially obtained from the exoskeletons of marine animals. Chitin consists of two different forms, α-chitin and β-chitin. Shellfish exoskeletons contain 15-40% of α-chitin (Taşar, 2015). Chitin is colorless, firm and inflexible (Kumar, 2000). There are many derivatives of chitin, the most essential of which is chitosan. Chitosan is extracted from chitin by a deacetylated method (Oyar, 2015). Chitosan, which has many advantages over chitin, is widely used in many different fields, especially in food, cosmetics, agriculture, medicine, paper and textile (Varlık et al., 2004). Moreover, chitin and chitosan can be partially absorbed by human enzymes and are useful in the human body, as they are not poisonous and form saccharide macromolecules that can be converted to glucose when broken down (Islam et al. 2020). When used on injured tissue, it becomes active on the wound and does not show allergic or undesirable reactions (Özdemir, 2006).

P. caerulea should be collected and its shells should be integrated in different areas and used as a source of chitin due to many reasons, such as being abundant in rocks in coastal regions and easily accessible. In this direction, the aim of this study was to examine the physicochemical parameters of chitin and chitosan in order to collect *P. caerulea* and integrate its shells into different areas. In this context, yield, deacetylation degree, solubility, Fourier Transforms Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analyzes of chitin and chitosan materials were performed.

2. Material and Methods

2.1. Materials

In the study, *P. caerulea* were randomly gathered from the coastal region of Iskenderun (36,59572° N, 36.16244° E) of the North-eastern Mediterranean in January 2023 (Figure 1A) (Anonymous, 2023; GM, 2023). Sampling was done manually with a pocket knife. The shell samples were transported to the laboratory by placing them in drums filled with seawater. The samples were quickly taken to the laboratory in a box. The shells were washed with plenty of water and dried in an oven at 60 °C. The shells were weighed and then pulverized using a mixer mill (Figure 1B).

2.2. Extraction of Chitin and Chitosan

The powdered shells were mixed in a solution containing 1M NaOH (g/10mL) at 500 rpm at 70°C for 18 hours for the deproteinization step. Then, the powdered shells were washed, filtered and dried overnight at 50 °C. The dried materials were mixed in a solution containing 1M HCl (g/10mL) for demineralization in a stirrer at 500 rpm at RT for 6 hours (Al Sagheer et al., 2009;

Marei et al., 2016). After demineralization, the powder materials were washed, filtered and dried at 50 °C overnight. The resulting powder material is chitin. The production of chitosan from the obtained chitin is accomplished chitosan by separating the acetyl groups in the chitin structure. Chitin powders were deacetylated in 50% NaOH solution (g/10mL) by stirring at 500 rpm for 4 hours in a magnetic stirrer at 100 °C. The mixture was then washed, filtered and dried overnight at 50°C. The obtained chitin and chitosan materials were stored at +4°C until analysis. Bidistilled water was used for washing at all stages.

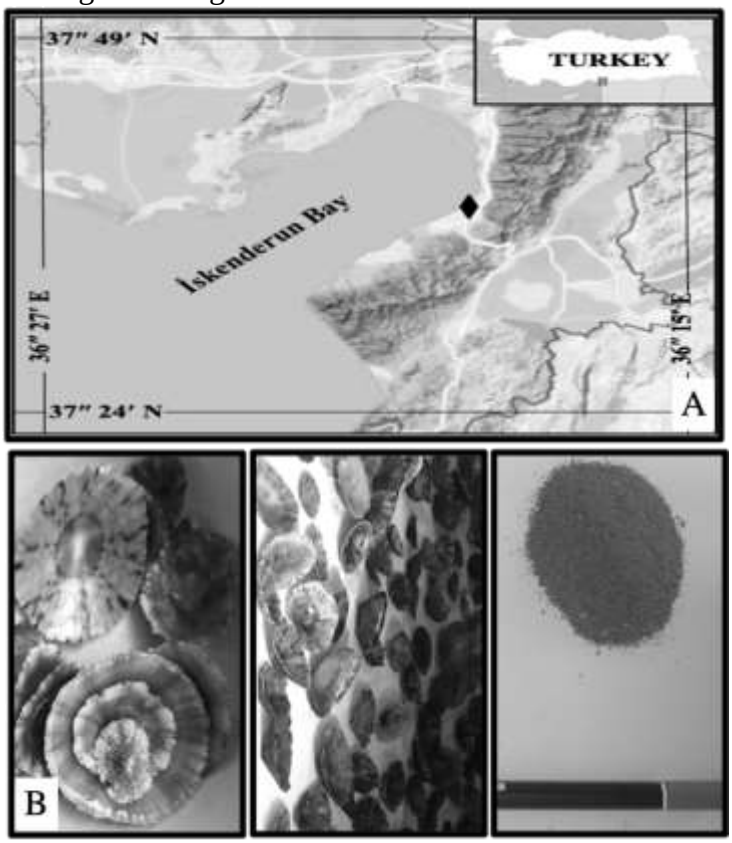

Fig. 1. A) Study area (Anonymous, 2023; GM, 2023) and B) *Patella caerulea* shells.

2.3. Chitin and Chitosan Yields

Yields were computed by relating the weights of the raw shell powders with the weights of chitin and chitosan taken afterward processing. The chitin and chitosan yields were calculated as described by Luo et al. (2019).

$$\%Y_{Chitin} = \frac{W_{chitin}}{W_{raw\ material}} x100 \quad (1)$$

$$\%Y_{Chitosan} = \frac{W_{Chitosan}}{W_{Chitin}} x100 \quad (2)$$

where, Y: yield, W: weight

2.4. Solubility of Chitosan

To determine the solubility of *P. caerulea* chitosan in acid, 0.1 g of chitosan was added into 1% acetic acid (100 mL). It was mixed with the help of a magnetic stirrer. Then it was filtered with filter paper. The remaining powders were washed with water. The washed powders were dried at 50°C for one day. These processes were repeated 3 times. The dried powders were weighed

(Nessa et al. 2011). The resolution was estimated with the help of the following Eqs (3), (4) and (5).

$$Insoluble\ (g) = Final\ weight\ of\ filter\ paper\ (g) - initial\ weight\ of\ filter\ paper(g) \quad (3)$$

$$Insoluble\ (\%) = \frac{Insoluble\ (g)}{Sample\ weight\ (g)} x100 \quad (4)$$

$$Soluble\ (\%) = 100 - insoluble\ (\%) \quad (5)$$

2.5. Fourier Transforms Infrared Spectroscopy (FTIR) Analysis

FTIR analyzes of *P. caerulea* chitin and chitosan material were performed with a Jasco/FT/IR-6700 instrument accoutred with ATR. IR spectra were observed between 4000 and 400 cm^{-1} at a determination of 4 cm^{-1}. The DD of the materials was calculated according to the study used by Brugnerotto et al. (2001) (Eqs. 6 and 7) and measurements were taken in the absorbance mode. A_{1320} was the peak region of the 1320 cm^{-1} band and A_{1420} was the apex area of the 1420 cm^{-1} band, A_{1320} was the peak for the amide group and A_{1420} was the peak for the amine group.

$$\%DA = \left[\left(\frac{A_{1320}}{A_{1420}}\right) - 0.3822\right]/0.3133 \quad (6)$$

$$\%DD = 100 - \%DA \quad (7)$$

where, DD= degree of deacetylation (%) and DA = degree of acetylation (%).

2.6. X-Ray Diffraction (XRD) Analysis

X-Ray diffraction (XRD) study was performed to determine the crystallinity of the obtained chitin and chitosan materials. Malvern Panalytical EMPYREAN 3rd generation analytical (UK) device worked with Cu Ka radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Data were gathered at a scan amount of 1°/min with a scan position of 5 to 60°. The crystal index (CrI) method was used by Yuan et al. (2011) Eq (8).

$$Crl_{110} = \left(\frac{I_{110} - I_{am}}{I_{110}}\right) x100 \quad (8)$$

where I110 is the greatest intensity of the (110) diffraction peak at 2θ = 20° and Iam is the amorphous deflection signal at 2θ = 16°.

2.7. Scanning Electron Microscopy (SEM)

The surface areas and structures of *P. caerulea* chitin and chitosan were visualized by SEM. Before imaging the chitin and chitosan materials, gold-palladium coating was performed with the POLARON SC7620 device. The distribution of coated chitin and chitosan biopolymers was shown with the SEM device (JEOL JSM-638OLA) using 15 kV.

3. Results and Discussion

3.1. Yields

In our study, chitin and chitosan were successfully extracted from *P. caerulea* shells taken from Iskenderun Bay. Yields were estimated for the obtained chitin and produced chitosan. The chitin and chitosan yields obtained from *P. caerulea* shells were 43.42 ±0.78% and 72.93±1.02%, respectively (Eqs. 1 and 2). Some researchers reported that the chitosan yield of the waste shells was 4.65% in *Potamon potamios* (Bolat et al., 2010), 19.2-22.9% and 16.4-18.8% in crayfish (Fernandez-Kim, 2004). Nouri et al. (2015) also indicated that the percentage of extracted

chitosan yield varied between 5.6-13.5%. Vargese (2002) reported an average chitin yield of 2-3.9 g (12-19%) in the exoskeleton of the *Harpiosquilla melanoura* stomatopod. Oduor-Odeto and Peter (2005) reported that the chitin yield was 23.0% for *Scylla serrata* (crab), 15.7% for *Panulirus ornatus* (lobster) and 28.0% for *Penaeus indicus* (shrimp). Abdou et al. (2008) reported that chitin yields were approximately 21.53% and 23.72% from *Penaus aztecus and Penaeus durarum* shrimps, 16.73% from crab shells, 20.60% from shells of *Procambarus clarkia*, 5.40% from cuttlefish and 49% from squid.

3.2. Solubility of Chitosan

The acid solubility of chitosan biopolymers of 85% and above indicates that the degree of deacetylation is good (Kafshgari et al., 2011). The solubility of chitosan obtained from the shells in a 1% acetic acid solution was 85.58±3.22% and showed good solubility (Eq. 3). The good solubility of the obtained chitosan in acetic acid is due to the deacetylation conditions. The temperature was treated at 100 °C in 50% NaOH solution for 4 hours. This study showed that similarity with the DD% of chitosan obtained from different marine animals (Abdelmalek et al., 2017; Birolli et al., 2015; Marei et al., 2016; Sedaghat et al., 2017; Teli and Sheikh, 2012).

Chitosan has N-acetyl-D-glucosamine and D-glucosamine units formed as a result of deacetylation under alkaline conditions (El Knidri et al., 2018). The existence of multiple effective groups such as hydroxyl and amino groups attached to the polysaccharide chain in chitosan offers flexibility for the preparation of molecularly engraved polymers and structural alterations (Wang et al., 2014). It also exhibits chelating ligand properties that hold many metal ions (Al-Manhel et al., 2018; Shajahan et al., 2017).

3.3. XRD Analysis

X-ray diffraction patterns of the extracted chitin and chitosan are presented (Figure 2). XRD of the chitin from the *P. caerulea* shell shown in Figure 2 reveals the presence of $CaCO_3$ (calcite) and chitin. The XRD study in this study shows that α-chitin is extracted. XRD analysis of the patella chitin showed 8 peaks of crystal reflection in the 5-60° range, with the five greatest peaks (12.30°, 26.40°, 40.80°, 49.20°, and 57.60°) observed (Figure 2). The greatest peak reflection was found to be about 20-30° (1060° count s^{-1}) at 2θ. Nineteen peaks were detected in the XRD investigation of *P. caerulea* chitosan, and the nine greatest peaks (16.70°, 20.55°, 26.60°, 30.90°, 32.40°, 35.20°, 39.10°, 43.15° and 43.80°) were determined. The greatest peak of the chitosan was defined at about 2θ at 20-30° (1090° count s^{-1}) (Figure 2). Altered studies also confirmed the existence of two same peaks at about 10° and 20° for altered shell powders with altered degrees of deacetylation (Kumari et al., 2015; Trung et al., 2006). The reason for this was due to differences in species or regional differences.

The crystal index (CrI) of chitin extracted from *P. caerulea* shells was calculated as 61.74%. Ugurlu and Duysak (2022) reported Crl values of chitin obtained from *D. setosum* testa and spines of 68% and 67%, respectively. Cárdenas et al. (2004) found that the Crl value of chitin obtained from shrimp shells of 76.2%. Kaya et al. (2014) found that the Crl values of chitins obtained from 6 different invertebrate species were between 66-74%. Ibitoye et al. (2018) found that the Crl value of chitin obtained from house cricket (*Brachytrupes portentosus*) was 88.02%. Such a wide range of Crl values may be due to the altered species, extraction methods and the purity of the materials used.

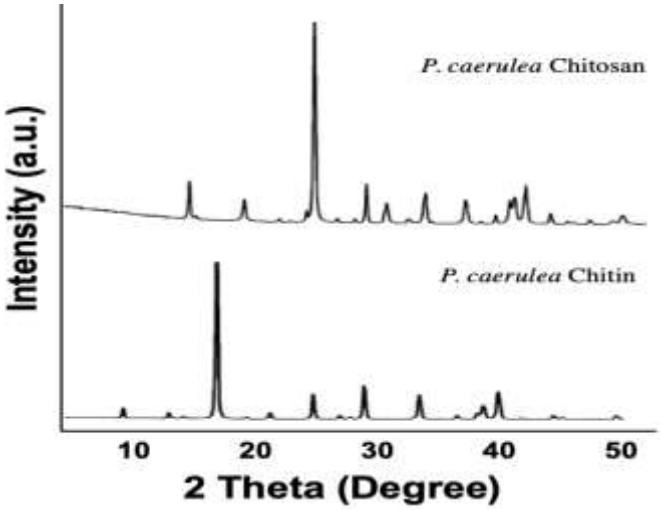

Fig. 2. XRD of chitin and chitosan from the shell of *P. caerulea*.

3.4. FTIR Analysis

IR characterization of chitin and chitosan biopolymers was applied with a Thermo Nicolet Nexus 670 spectrometer in the 4000-400 cm^{-1} frequency range. It can be shown in Figure 3 that the result of IR characterizations was similar to those in the previous studies, indicating that great quality chitin and chitosan biopolymers were gained. The characteristic bands of chitin from spectral data are summarized in Table 1. The FTIR peaks for chitins were observed at 3368.51 cm^{-1} (aliphatic O-H stretching vibrations), 2911.42 cm^{-1} (C-H vibration of $-CH_3$) in the polymer chain), 1693.72 cm^{-1} (Amide I vibration modes), 1532.96 cm^{-1} (N-H straining vibrations of NH_2 groups), 1425.72 cm^{-1} (CH_2 deformation vibrations), 1309.28 cm^{-1} (Amide III vibration modes), 1082.83 cm^{-1} (C-O-C bridge), 1008.59 cm^{-1} (C-O stretching vibration of alcohol groups). Absorption peaks for shell chitin at 1028.83 cm^{-1} is the stretching vibrations for –C–O–C of the glucosamine ring, while peak at 871.67 cm^{-1} is the ring stretching characterization band for β-1,4 glycosidic bonds (Table 1) (Pearson et al., 1960; Focher et al., 1992; Kurita et al., 1993; Al Sagheer et al., 2009).

Table 1. The FTIR bands (cm^{-1}) of chitin isolated from the shell of *P. caerulea.*

Functional group and vibration modes	Classification	Chitin
O–H stretching	-	3368.51
N–H stretching	-	3114.83
CH_3 symmetrical stretch and CH_2 asymmetric stretch	Aliphatic compound	2911.42
C–O secondary amide stretch	Amide I	1693.72
N–H bend. C–N stretch	Amide II	1532.96
CH_2 ending and CH_3 deformation	-	1425.72
CH bends CH_3 symmetrical deformation	-	1337.58
CH_2 wagging	Amide III. components of protein	1309.28
Asymmetric bridge oxygen stretching	-	1209.71
Asymmetric in-phase ring stretching mode	-	1132.33
C–O–C asymmetric stretch in phase ring	Saccharide rings	1082.83
C–O asymmetric stretch in phase ring	-	1008.59
CH_3 wagging	Along chain	938.25
CH ring stretching	Saccharide rings	871.67

The FTIR analysis of the chitosan presented twelve main peaks at the about of 871.67 cm^{-1}, 1021.65 cm^{-1}, 1057.33 cm^{-1}, 1146.18 cm^{-1}, 1322.07 cm^{-1}, 1386.41 cm^{-1}, 1418.87 cm^{-1}, 1583.05 cm^{-1}, 1648.70 cm^{-1}, 2876.49 cm^{-1}, 2919.67 cm^{-1} and 3341.91 cm^{-1} (Table 2 and Figure 3). The use of chitosan as an accelerator in wound healing materials and its usefulness in defending the area from bacteria by preventing bacterial proliferation have been determined (Yadav and Bhise, 2004). The results of the FTIR analysis of chitin and chitosan attained from different marine organisms were also supported by other studies (Al Sagheer et al., 2009; Hassainia et al., 2018; Ibitoye et al., 2018; Islam et al., 2023; Kaya et al., 2014; Kumari et al., 2015; Marei et al., 2016; Sixto-Berrocal et al., 2023; Uğurlu and Duysak, 2022; Yuan et al., 2011).

Table 2. The FTIR bands (cm^{-1}) of chitosan isolated from the shell of *P. caerulea*.

Functional group and vibration modes	Chitosan
O-H stretch overlapped with N-H stretch and inter-hydrogen bonds of the polysaccharide	3341.91
C-H stretch	2919.67
υ (C–H) in pyranose ring	2876.49
υ (C=O) in $NHCOCH_3$ group (Amide I band)	1648.70
δ(CH_2) in CH_2OH group	1583.05
δs(CH_3) in $NHCOCH_3$ group	1418.87
δ (C–H) in pyranose ring	1386.41
Complex vibrations of NHCO group (Amide III band)	1322.07
υs(C–O–C) (glycosidic linkage)	1146.18
υas(C–O–C) (glycosidic linkage)	1057.33
υ (C–O) in secondary OH group	1021.65
Pyranose ring skeletal vibrations	871.67

FTIR analysis were used to compute the degree of acetylation. The absorbance method of the FTIR analysis was used to compute DD and DA in *P. caerulea* shells. The %DA and %DD of chitin and chitosan obtained from *P. caerulea* shells was calculated as 14.32 and 85.68%, respectively.

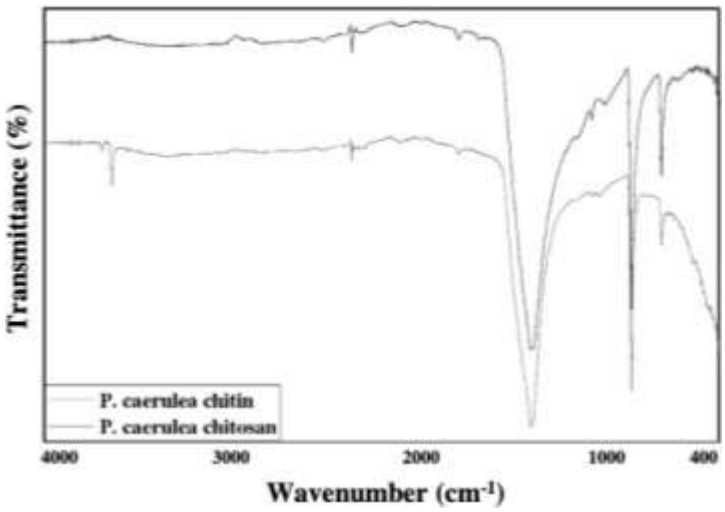

Fig. 3. FTIR of chitin and chitosan from the shell of *P. caerulea*.

3.5. Scanning Electron Microscopy (SEM)

Chitin (Figures 4A, B) and chitosan (Figures 4C, D), extracted from the shells of *P. caerulea*, showed rough, porous, fibrillar and nano-fibrous surface structures under SEM. In addition, chitosan showed similar microfibrillar structure with the accumulation of crystal particles on the fibers. Generally, chitin and chitosan biopolymers have different surface morphologies. These; It has porous and microfibrillar, non-porous, and only microfibrillar structure.

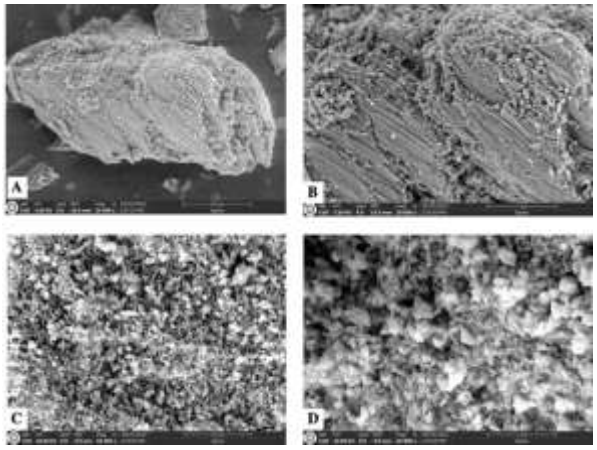

Fig. 4. SEM images, A-B) *P. caerulea* chitin and C-D) *P. caerulea* Chitosan

3.6. Conclusion

This is the first study to show the yield of chitin and chitosan, chitosan solubility and physicochemical things of *P. caerulea* (Linnaeus, 1758) by XRD, FTIR and SEM analyses. According to the results of analysis, it was defined that the chitin biopolymer obtained from the patella shells was in α form. Moreover, chitin and chitosan were defined to have a rough, porous, fibrillar, and nanofibrous surface structure. Chitin and chitosan were obtained from shell of the Mediterranean Limpet *P. caerulea*. The deacetylation of the acquired chitin was shown using a chemical method. The degree of deacetylation was created to be 14.32% of chitin, while the degree of deacetylation of chitosan was 85.68%. Studies show that chitin and chitosan biopolymers are effective materials in applications such as drug release, tissue engineering, cosmetics, and nanomedicine. Modifications of chitin and chitosan biopolymers will extend their application fields far beyond science. Therefore, especially invasive marine organisms or aquaculture wastes should be evaluated.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

Declaration of Competing Interest

The author(s) declare no competing interests.

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