Acta Aquatica Turcica

E-ISSN: 2651-5474

Home Page: https://dergipark.org.tr/actaquatr 20(2): 097-107, 2024 DOI: 10.22392/actaquatr.1301286

Research Article **Araştırma Makalesi**

Evaluation of Gastropods as Biomaterials: *Monodonta turbinata* (Born, 1780)

Gastropodların Biyomalzeme Olarak Değerlendirilmesi: *Monodonta turbinata* (Born, 1780)

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Received: 23.05.2023 **Accepted:** 14.11.2023 **Published:** 01.06.2024

How to Cite: Uğurlu, E. (2024). Evaluation of gastropods as biomaterials: *Monodonta turbinata* (Born, 1780). *Acta Aquatica Turcica, 20*(2), 097-107. https://doi.org/10.22392/actaquatr.1301286

Abstract: *Monodonta turbinata* is a densely populated species along the coasts of İskenderun Bay, Mediterranean Sea. The shells of *M. turbinata* contain a high amount of chitin for the chitosan production. The goal of this research is to produce chitin and chitosan from *M. turbinata* shells and characterize them using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) techniques. In addition, the samples were analyzed for solubility and deacetylation degree. The yields of chitin and chitosan obtained from *M. turbinata* were calculated as 60.26±0.96% and 80±1.29%, respectively. FTIR spectrum analysis results revealed the existence of functional groups in various bands and confirmed that the samples were chitin and chitosan. As a result of the FTIR, the deacetylation degree (DD) value was established to be 84.83%. The crystalline index (CrI) of chitin obtained from shells was computed as 54.92%. SEM analysis results displayed the morphological differences between chitin and chitosan biopolymers. The results indicate that M. turbinata shells, a waste product from İskenderun Bay, hold promise as a chitin and chitosan source for various applications in Türkiye.

Keywords
- Gastropods
- Biomaterial
- Polymer
- Chitin
- Chitosan

Özet: *Monodonta turbinata*, İskenderun Körfezi kıyılarında yoğun olarak bulunan bir türdür. Bu türler, kitosana dönüştürülebilen yüksek miktarda kitin içerir. Bu araştırmanın amacı, *M. turbinata* kabuklarından kitin ve kitosan elde etmek ve X-ışını kırınımı (XRD), Fourier Dönüşümlü Kızılötesi Spektroskopi (FTIR) ve Taramalı Elektron Mikroskobu (SEM) teknikleri kullanarak karakterize etmektir. Ayrıca asitteki çözünürlüğü ve deasetilasyon dereceleri de belirlenmiştir. *M. turbinata* kabuklarından elde edilen kitin ve kitosan verimleri sırasıyla %60,26±0,96 ve %80±1,29 olarak hesaplanmıştır. FTIR spektrum analizi sonuçları farklı bantlarda fonksiyonel grupların varlığını göstermiş ve örneklerin kitin ve kitosan olduğunu doğrulamıştır. FTIR analizi kullanılarak DD değeri %84,83 olarak bulunmuştur. Kabuklardan elde edilen kitinin kristal indeksi (CrI) %54,92 olarak hesaplanmıştır. SEM analiz sonuçları, kitin ve kitosan biyopolimerleri arasındaki morfolojik farklılıklarını ortaya koymuştur. İskenderun Körfezi'nde yoğun bir popülasyona sahip *M. turbinata* kabuk atığının, Türkiye'deki çeşitli uygulamalar için umut verici bir kitin ve kitosan kaynağı olduğunu göstermektedir.

Anahtar kelimeler
- Gastropod
- Biyomateryal
- Polimer
- Kitin
- Kitosan

1. INTRODUCTION

Natural products that play important roles in the regulation of biological systems are known as secondary bioactive metabolites. The marine area is rich in natural products with unrivaled effectiveness. Marine animals are composed of bioactive molecules with extraordinary properties due

to the unique properties of the marine environment (Soltani et al., 2015). In recent years, it is known that the oceans are a rich and sufficient source of natural products (Dhinakaran et al., 2012).

Chitin polymer, which is a structural amino polysaccharide, is the most abundant in the world after cellulose and has a fibrous structure. The sources of chitin are the exoskeleton of crustaceans such as shrimp, crabs and lobsters. Chitin was first described in 1884 and has been reported to have a poly (β-(1→4)-N-acetyl-D-glucosamine) structure (Rinaudo, 2006). The chitin biopolymer has remarkable properties (biodegradability, nontoxicity, biocompatibility, etc.). In addition, this biopolymer is used in pharmaceutical, medical and industrial fields (Yadav et al., 2015). Chitosan is formed as a result of advanced deacetylation of chitin and is the most important derivative of chitin. Chitosan is a weak base and is insoluble in water, but soluble in acidic aqueous solutions. It is mainly evaluated by its molecular weight (MW) and degree of acetylation (DA) (Younes & Rinaudo, 2015). The amine groups in the structure of chitosan have an important advantage in terms of affecting various biological activities.

In recent years, various studies have been carried out on chitin and chitosan. Researchers emphasize obtaining chitin biopolymers from natural sources. There are chemical, biological and thermal methods to obtain chitosan from naturally sourced chitin (Kumari et al., 2015; El Knidri et al., 2018; Kaczmarek et al., 2019; Zainol Abidin et al., 2020). The fact that chitosan biopolymers are biodegradable, biocompatible and non-toxic has attracted a lot of attention in many industries such as cosmetic health, agriculture, food, paper, and wide application areas (Synowiecki & Al-Khateeb, 2003).

Individuals of *Monodonta turbinata (Born, 1780)* (Gastropoda, Prosobranchia, Trochidae) are marine gastropod mollusks. They are distributed in all coastal areas of the Mediterranean and can be easily found all year round. *M. turbinata* lives in the rocky areas of the tidal zone. It can survive out of the water for several hours and tolerate high temperatures. This species eats algae residue scraped from rocks. Monodonta is distributed in the Mediterranean and Western Atlantic, from Portugal to Morocco and the Canary Islands.

Gastropods form the dominant group of molluscs on the coast of İskenderun Bay in the Northeast Mediterranean (Bakır et al., 2012). Most of the studies on *M. turbinata*, which has a dense population on the coasts of İskenderun Bay, were limited to the biology, heavy metal accumulation, nutrition and biomonitoring of this species, and no studies on biomaterial production such as chitin and chitosan were encountered. It is very significant that the species to be used as biomaterials are sustainable, easily available and abundant.

In this study, it was aimed to extract chitin and chitosan from *M. turbinata*, one of the most abundant examples of gastropods in the İskenderun Bay. In addition to the extensive application areas of chitin and chitosan in many industrial areas, this species has not been economically evaluated. In this context, chitin and chitosan were produced from *M. turbinata* shells. Yield, deacetylation degree, solubility, X-Ray Diffraction (XRD), Fourier Transforms Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analyses of chitin and chitosan biopolymers were performed.

2. MATERIALS and METHOD

2.1. Materials

In the present study, *M. turbinata* was randomly collected from the coastal region of İskenderun (36.58968° N, 36.14690° E) at the Northeast Mediterranean coast in April 2023 (Figure 1). Sampling was done manually with a pocket knife, and a total of 50 samples were collected. The *M. turbinata* species were quickly taken to the lab in a box (Figure 1). The soft tissues of *M. turbinata* were removed, washed with plenty of water and the shells were dried in an oven at 60 °C. The dried shells were weighed and then pulverized using a grinder. Approximately 200 g of dry *M. turbinata* shell was used (Alabaraoye et al., 2018).

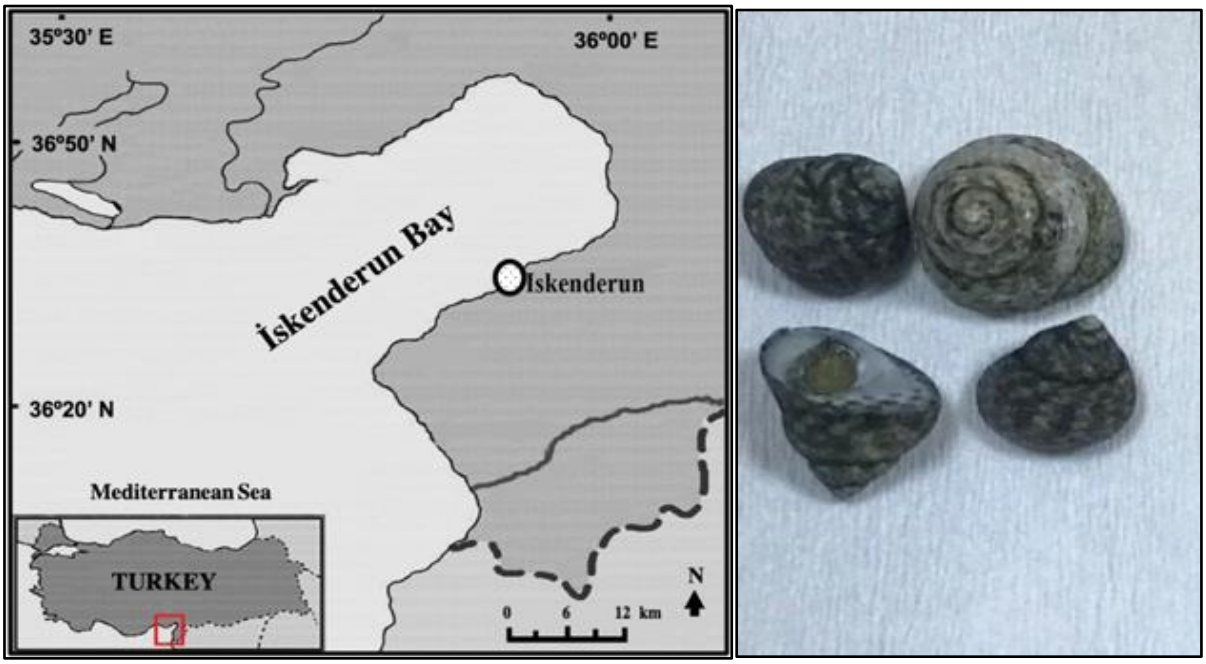

Figure 1. Study area (Anonymous, 2023; GM, 2023) and *M. turbinata*.

2.2. Extraction

The demineralization was stirred at 500 rpm for 6 hours using 1M HCl at a ratio of 1:10 (w/v) at ambient temperature. The obtained shell powder was washed to neutralize under running water. Finally, the shell powder was collected and washed using distilled water and dried at 50 °C for 18 hours. Deproteinization was mixed at 500 rpm by adding 1M NaOH at a ratio of 1:10 (w/v) followed by heating at 70 °C for 18 hours. The shell powder was neutralized by washing under the water. The shell powders were gathered and washed again with distilled water. Finally, the shell powders were dried in an oven for 18 hours , then weighed and placed in polyethylene tubes. The resulting shell powder is chitin. Then, the chitin was deacetylated with 50% NaOH at a ratio of 1:10 (w/v) at 100 °C for 4 hours at 500 rpm (Al Sagheer et al., 2009; Marei et al., 2016). The resulting deacetylated solid was filtered, gathered, and washed using distilled water. Finally, the chitosan was dried in an oven for 18 hours, then weighed and placed in polyethylene tubes.

2.3. Yields

The yields of the chitin and chitosan obtained from the powders were calculated by correlating the weights of the raw bark powders with the weights of chitin and chitosan taken later. The chitin and chitosan yields were calculated as described by Luo et al. (2019).

$$Y_{Chitin} = \frac{W_{chitin}}{W_{Raw\ shell}} x100 \quad (1)$$

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

where, Y: yield, W: weight

2.4. Solubility

To determine the acid solubility of chitin and chitosan powders obtained from *M. turbinata* shells, 1g from each was weighed. It was dissolved in 100 mL of 1% acetic acid solution. The solution was then stirred and kept at ambient temperature for 2 hours. Then, it was filtered through filter paper that was weighed before and the filter paper was dried. The dried paper and samples were weighed again. The percent solubility was analyzed from the weight gain rate of the filter paper x100 (Nessa et al. 2011).

2.5. Fourier Transforms Infrared Spectroscopy (FTIR) Analysis

FTIR analyses of *M. turbinate* chitin and chitosan material were performed with a Jasco/FT/IR-

6700 instrument set with ATR. IR spectra were observed between 4000 and 400 cm^{-1} at a determination of 4 cm^{-1}. The DD of the polymer was computed according to the study used by Brugnerotto et al. (2001) (Eqs. 3 and 4)

$$\%DA = \left[\left(\frac{A_{1320}}{A_{1420}}\right) - 0.3822\right]/0.3133 \quad (3)$$

$$\%DD = 100 - \%DA \quad (4)$$

where, DD = deacetylation degree (%) and DA = acetylation degree (%). A1320 was the peak region of the 1320 cm-1 band and A1420 was the apex area of the 1420 cm-1 band, A1320 is the peak for the amide group and A1420 was the peak for the amine group.

2.6. X-Ray Diffraction (XRD) Analysis

X-Ray diffraction (XRD) studies were performed to determine the crystallinity of the obtained chitin and chitosan biopolymers. Malvern Panalytical EMPYREAN 3rd generation analytical (UK) device was 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 45°. The crystalline index (CrI) method was used by Yuan et al. (2011) Eq (5),

$$Crl_{110} = \left(\frac{I_{110} - I_{am}}{I_{110}}\right) x100 \quad (5)$$

where I_{110} is the highest intensity of the (110) diffraction peak at 2θ = 20° and I_{am} is the amorphous deflection signal at 2θ = 16°.

2.7. Scanning Electron Microscopy (SEM)

The surface areas and structures of *M. turbinate* chitin and chitosan biopolymers 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 (Marei et al., 2016).

3. RESULTS and DISCUSSION

Chitin and chitosan were extracted from *M. turbinata* shell waste as a result of deproteinization, demineralization and deacetylation processes, and the quality of the extracted chitin and chitosan biopolymers was determined (Figure 2).

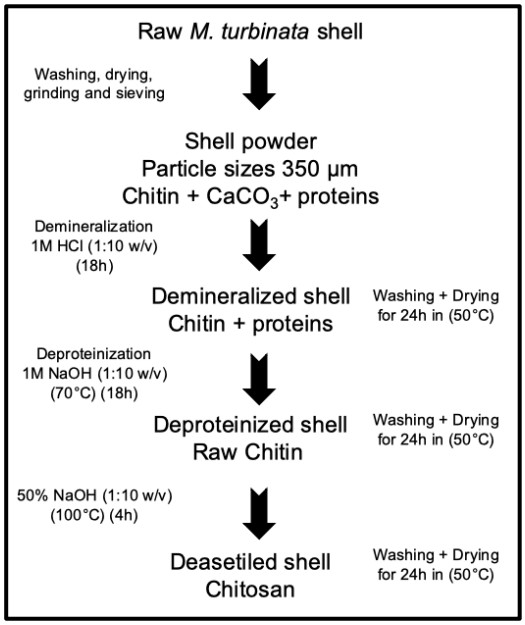

Figure 2. Isolation process of chitin and chitosan from *M. turbinata* shells.

3.1. Yields

Many researchers reported that the yields of the obtained chitin and chitosan biopolymers differ among marine species. The results obtained by Fadlaoui et al. (2019) showed a yield of 8.27% chitin and 5.89% chitosan. Majekodunmi et al. (2017) reported that the yield of chitosan obtained from *Mytilus edulis* shells was 51.8% and the yield of chitosan obtained from *Laevicardium attenuatum* shells was 43.8%. Al Sagheer et al. (2009) reported that the yield of chitin from *Metapenaeus affinis*, *Penaeus semisulcatus*, *Portunus pelagicus* male, *P. pelagicus* female, *Thenus orientalis* and cuttlefish were 19.13%, 16.75%, 20.8%, 20.14%, 21.26%, and 7.4%, respectively. Ahyat et al. (2017) reported that yields of chitin and chitosan from the shells of *Portunus pelagicus* were 20.24% and 13.56%, respectively. Bolat et al. (2010) reported that chitin and chitosan from the shells of *Potamon potamios* were 6.83% and 4.56%, respectively. Kabalak et al. (2020) reported that yields of chitin from *Polyphylla fullo*, *Lucanus. cervus*, *Gryllotalpa gryllotalpa* and *Bradyporus (Callimenus) sureyai* shells were 11.3%, 10.9%, 10.1% and 9.8% respectively. In this study, the dry weight of chitin and chitosan from the shells of *M. turbinata* were 60.26±0.96% and 80±1.29%, respectively (Figure 3). The chitin and chitosan yields are affected not only by the variation of the organisms used for production of the chitin and chitosan, but also by the place where these organisms live in different geographical locations.

Figure 3. Dry weight of chitin and chitosan obtained from *M. turbinata* shells.

3.2. Solubility

According to the results, *M. turbinata* chitin biopolymer had a low solubility value of 59.11±1.48%. It was determined that the chitosan biopolymer obtained from *M. turbinata* shells showed an excellent solubility of 88.69±1.71%. Similarly, Alabaraoye et al. (2018) obtained the chitin content from 6 different shellfish wastes and reported their solubility between 58.33% and 85.71%. Demir et al. (2016) reported that the chitosan solubility obtained from crab shell in acetic acid was 99.29±0.001%. The main purpose of this method is to assure the precision of the readings from acetyl groups and proteins, since the residues or other impurities may adversely affect the results in the samples during the analysis processes. Chitosan biopolymer affects parameters such as the degree of deacetylation and many physicochemical properties including solubility and crystallinity.

3.3. FTIR Analysis

FTIR analysis was achieved to determine the structure of chitin and chitosan obtained from *M. turbinata* shells by identifying characteristic bands of these polymers. Figure 4 shows the FTIR spectra for chitin and chitosan from *M. turbinata* shells.

The FTIR spectrum of *M. turbinata* chitin showed bands at 3395.41 cm^{-1} (stretching and vibrating of aliphatic O-H), 2953.28 cm^{-1} (C-H vibration of -CH_3), 1785.76 cm^{-1} (stretching and vibration of C-O and C=O), 1443.46 cm^{-1} (bending vibration of -NH and stretching vibration of -CN), 1081.87 cm^{-1}

(-C-O-C) and 855.28 cm^{-1} (ß-1,4 glycosidic).

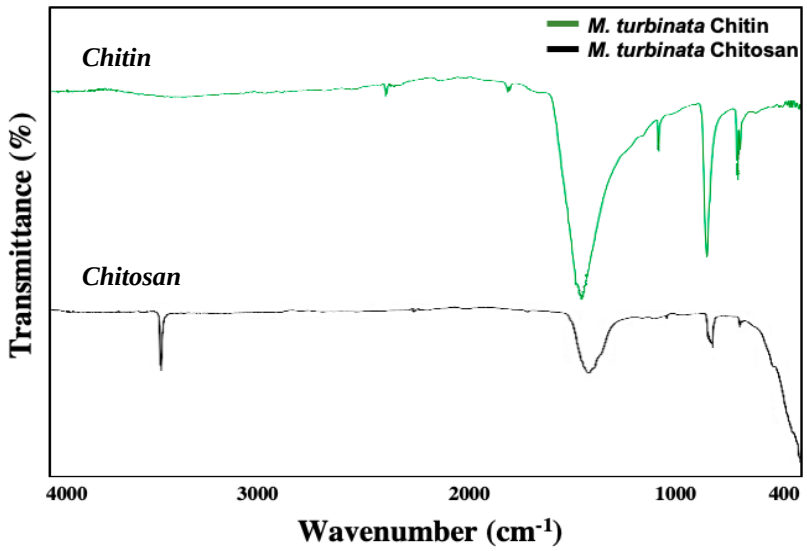

Figure 4. FTIR spectra of chitin and chitosan isolated from *M. turbinata* shells.

FTIR spectra of chitosan obtained from *M. turbinata* chitin observed bands at 3471.62 cm^{-1} (OH stretch overlapped with NH stretch and inter-hydrogen bonds of the polysaccharide), 2948.77 cm^{-1} (C-H stretch), 1627.92 cm^{-1} (amide I band, C=O stretch), 1483.05 cm^{-1} (NH_2 bending), 1407.16 cm^{-1} (C-H bending), 1275.78 cm^{-1} (amide III band, C-N stretch), 1094.32^{-1} (bridge C-O-C stretch), and 983.62 cm^{-1} (C-O-C stretch). Analysis of chitin and chitosan with FTIR spectrum yielded results similar to previous studies (Hajji et al., 2014; Kumari et al., 2015; Demir et al., 2016; Alabaraoye et al., 2018; Kabalak et al., 2020; Varma & Vasudevan, 2020). The absorbance mode of the FTIR spectra was used to calculate DD and DA in *M. turbinata*. The %DA of chitin obtained from *M. turbinata* shells was calculated as 15.17%. In addition, the %DD of chitosan was calculated as 84.83%. Öğretmen at al. (2022) reported that the %DD of chitosan obtained from pink shrimp (*Parapenaeus longirostris*) was 81%. Alabaraoye et al. (2018) reported that the %DA of chitin materials obtained from mussel, oyster, shrimp and crab species were %91, 85.62, 51.61 and 69.4, respectively. It is estimated that the results found in these two studies and the current study are due to the difference in species and experimental process.

3.4. XRD Analysis

To identify the location of the crystal structures and to know the functional properties of the mechanisms in *M. turbinata*, the chitin and chitosan powders were analyzed by XRD. The XRD pattern of the biopolymers of *M. turbinata* shells, chitin and chitosan are presented in Figure 5.

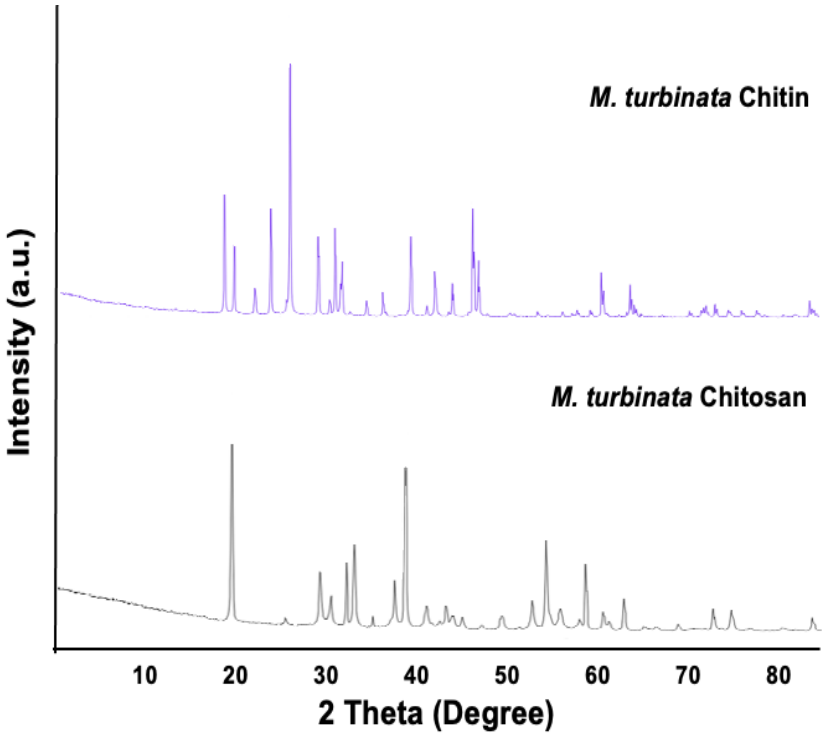

Figure 5. XRD spectra of chitin and chitosan isolated from *M. turbinata* shells.

The XRD analysis in this study shows that α-chitin is extracted. XRD analysis of the *M. turbinata* chitin determined 17 peaks of crystal reflection in the 10-90° range, with the 8 greatest peaks (19.10°, 20.30°, 24.50°, 28.40°, 30.10°, 32.20°, 41.30° and 48.70°) determined (Figure 5). The greatest peak reflection was found to be about 20-30° (1100° count s^{-1}) at 2θ. 18 peaks were defined in the XRD examination of *M. turbinata* chitosan, and the nine greatest peaks (20.15°, 29.60°, 32.90°, 34.10°, 38.20°, 39.60°, 54.65° and 59.40°) were decided. The greatest peak of the chitosan was determined at about 2θ at 20.15° and 39.60° (1200° count s^{-1} and 1080° count s^{-1}) (Figure 5).

The crystalline index (CrI) of chitin obtained from *M. turbinata* shells was calculated as 54.92%. Kaya et al. (2014) reported that CrI value of chitin extracted from *Fomitopsis pinicola* was 52%. Gbenebor et al. (2017) reported that CrI values of chitin from shrimp exoskeleton were between 79.4-87.4%. Phuong et al. (2017) reported that CrI value of chitin from black tiger shrimp was 54.7%. Odili et al. (2020) reported that CrI value of chitin from crab shell was 80%. Uğurlu & Duysak (2023) reported CrI values of chitin obtained from *D. setosum* testa and spines were 68% and 67%, respectively. The CrI value observed in this study was similar to the CrI values of chitins obtained from other organisms.

3.5. SEM analysis

Figure 6 shows the SEM of chitin (Figure A and B) and chitosan (Figure C and D) prepared from shells of *M. turbinata*. The surface structure of the obtained chitin was studied. It was observed that the chitin biopolymers have porous and fibril structures. It also showed that chitin from *M. turbinata* showed regularly arranged dense pores. Chitosan prepared from *M. turbinata* chitin shows a highly porous structure. It is revealed that the chitin biopolymer has a smoother surface area than chitosan.

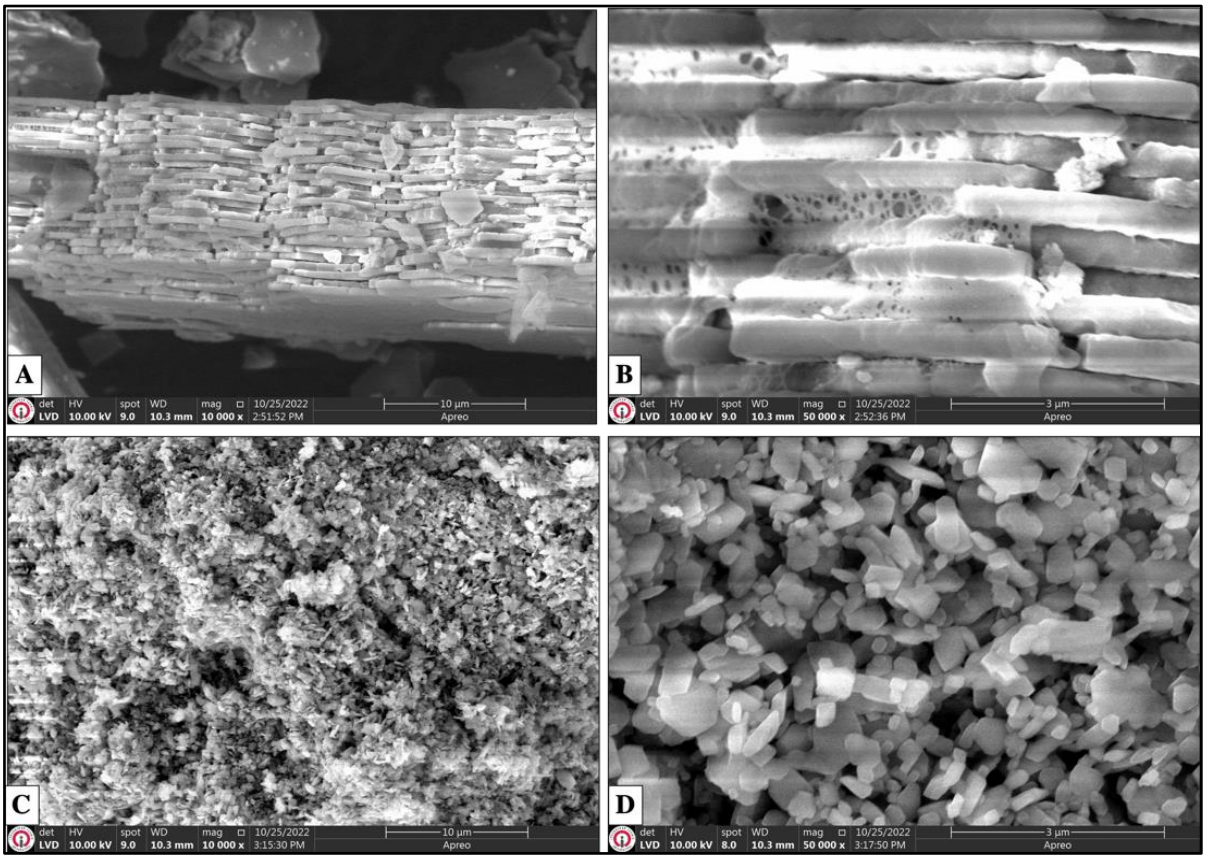

Figure 6. SEM micrographs of chitin (A and B) and chitosan (C and D) extracted from *M. turbinata* at different magnifications.

The presence of pores and fibers in *M. turbinata* chitin and chitosan biopolymers was similar to chitin and chitosan in previous studies in pink shrimp, fishing waste, Nigerian shrimp, and other crustaceans (Uğurlu & Duysak, 2023; Mohan et al., 2021; Varma et al., 2021; Kumari et al., 2015; Isa et al., 2012; Al Sagheer et al., 2009).

4. CONCLUSION

Chitin and chitosan biopolymer were obtained from the gastropod species *Monodonta turbinata*, which has dense populations on the coasts of İskenderun Bay. The chitin and chitosan yields obtained from *M. turbinata* were calculated as 60.26±0.96% and 80±1.29%, respectively. The deacetylation degree from *M. turbinata* was calculated as 84.83% using FTIR analysis. The solubility of chitosan from *M. turbinata* shell was 59.11±1.48%. XRD analysis showed that the *M. turbinata* shells have a crystalline structure. The CrI value of *M. turbinata* shell was found to be 54.92%. XRD FTIR and SEM analysis results of chitin and chitosan biopolymers prepared from *M. turbinata* shells have confirmed that they can be used commercially in many different areas (food, cosmetics, medicine etc.). These results suggest that *M. turbinata* shell is one of the most remarkable and good sources of chitin and chitosan.

ACKNOWLEDGMENT

I would like to thank Cansev Genç for her support in laboratory studies.

FUNDING

No financial support was received for the present study.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests in this paper.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Research data is not shared.

REFERENCES

Ahyat, N. M., Mohamad, F., Ahmad, A., & Azmi, A. A. (2017). Chitin and chitosan extraction from *Portunus pelagicus*. *Malaysian Journal of Analytical Sciences*, *21*(4), 770-777. https://doi.org/10.17576/mjas-2017-2104-02

Alabaraoye, E., Achilonu, M., & Hester, R. (2018). Biopolymer (Chitin) from various marine seashell waste: isolation and characterization. Journal of Polymers and the Environment, 26, 2207–2218. https://doi.org/10.1007/s10924-017-1118-y

Al Sagheer, F. A., Al-Sughayer, M. A., Muslim, S., & Elsabee, M. Z. (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate Polymers*, *77*(2), 410-419. https://doi.org/10.1016/j.carbpol.2009.01.032

Anonymous, (2023). Türkiye'nin Komşuları Dilsiz Haritası. Retrieved on June 11, 2023 from https://www.sosyalciniz.net/turkiyeninkomsulari-dilsiz-haritasi/

Bakır, B. B., Öztürk, B., Doğan, A., & Önen, M. (2012). Mollusc fauna of İskenderun Bay with a checklist of the region. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*(1), 171-184.

Bolat, Y., Bilgin, Ş., Günlü, Izci, L., Koca, D. B., Çetinkaya, S. & Koca, H. U. (2010). Chitin-chitosan yield of freshwater crab (*Potamon potamios*, Olivier 1804) Shell. *Pakistan Veterinary Journal, 30*(4), 227-231.

Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Argüelles-Monal, W., Desbrieres, J., & Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan characterization. *Polymer*, *42*(8), 3569-3580.

Demir, D., Öfkeli, F., Ceylan, S., & Bölgen Karagülle, N. (2016). Extraction and characterization of chitin and chitosan from blue crab and synthesis of chitosan cryogel scaffolds. *Journal of the Turkish Chemical Society Section A: Chemistry*, *3*(3), 131-144. https://doi.org/10.18596/jotcsa.00634

Dhinakaran, D. I., Prasad, D. R. D., Gohila, R., & Lipton, P. (2012). Screening of marine sponge-associated bacteria from *Echinodictyum gorgonoides* and its bioactivity. *African Journal of Biotechnology*, *11*(88), 15469-15476.

El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., & Lahsini, A. (2018). Extraction, chemical modification and characterization of chitin and chitosan. *International Journal of Biological Macromolecules*, 120, 1181-1189.

Fadlaoui, S., El Asri, O., Mohammed, L., Sihame, A., Omari, A., & Melhaoui, M. (2019). Isolation and characterization of chitin from shells of the freshwater crab *Potamon algeriense*. *Progress on Chemistry and Application of Chitin and its Derivatives*, (24), 23-35.

Gbenebor, O. P., Adeosun, S. O., Lawal, G. I,. Jun, S., & Olaleye, S.A. (2017). Acetylation, crystalline and morphological properties of structural polysaccharide from shrimp exoskeleton. *Engineering Science and Technology, an International Journal*, *20*(3), 1155-1165.

GM, [Google Maps]. (2023). Google Maps. Retrieved on June 5, 2023 from https://www.google.com/maps

Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M., & Jellouli, K. (2014). Structural differences between chitin and chitosan extracted from three different marine sources. *International Journal of Biological Macromolecules*, *65*, 298-306.

Isa, M. T., Ameh, A. O., Tijjani, M., & Adama, K. K. (2012). Extraction and characterization of chitin and chitosan from Nigerian shrimps. *International Journal of Biological and Chemical Sciences*, *6*(1), 446-453.

Kabalak, M., Aracagök, D., & Torun, M. (2020). Extraction, characterization and comparison of chitins from large bodied four Coleoptera and Orthoptera species. *International Journal of Biological Macromolecules*, *145*, 402-409. https://doi.org/10.1016/j.ijbiomac.2019.12.194

Kaya, M., Akata, I., Baran, T., & Menteş, A. (2014). Physicochemical Properties of Chitin and Chitosan Produced from Medicinal Fungus (*Fomitopsis pinicola*). *Food Biophysics*, 10, 162–168

Kaczmarek, M. B., Struszczyk-Swita, K., Li, X., Szczęsna-Antczak, M., & Daroch, M. (2019). Enzymatic modifications of chitin, chitosan, and chitooligosaccharides. *Frontiers in Bioengineering and Biotechnology*, *7*, 243.

Kumari, S., Rath, P., Kumar, A. S. H., & Tiwari, T. N. (2015). Extraction and characterization of chitin and chitosan from fishery waste by chemical method. *Environmental Technology & Innovation*, *3*, 77-85.

Luo, Q., Wang, Y., Han, Q., Ji, L., Zhang, H., Fei, Z., & Wang, Y. (2019). Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects. *Carbohydrate Polymers*, 209, 266-275.

Majekodunmi, S. O., Olorunsola, E. O., & Uzoaganobi, C. C. (2017). Comparative physicochemical characterization of chitosan from shells of two bivalved mollusks from two different continents. *American Journal of Polymer Science*, *7*(1), 15-22. https://doi.org/10.5923/j.ajps.20170701.03

Marei, N. H., Abd El-Samie, E., Salah, T., Saad, G. R., & Elwahy, A. H. (2016). Isolation and characterization of chitosan from different local insects in Egypt. *International Journal of Biological Macromolecules*, *82*, 871-877.

Mohan, K., Muralisankar, T., Jayakumar, R., & Rajeevgandhi, C. (2021). A study on structural comparisons of α-chitin extracted from marine crustacean shell waste. *Carbohydrate Polymer Technologies and Applications*, *2*, 100037.

Nessa, F., Masum, S. M., Asaduzzaman, M., Roy, S., Hossain, M., & Jahan, M. (2011). A Process for the Preparation of Chitin and Chitosan from Prawn Shell Waste. *Bangladesh Journal of Scientific and Industrial Research*, 45(4), 323–330. https://doi.org/10.3329/bjsir.v45i4.7330

Odili, C., Sekunowo, O., Gbenebour, P., & Adeosun, O. (2020). Characterization and properties comparison of Nigerian crab-shell extracts. *Uşak University Journal of Engineering Sciences, 3*(1), 1-12.

Öğretmen, Ö. Y., Karsli, B., & Çağlak, E. (2022). Extraction and Physicochemical Characterization of Chitosan from Pink Shrimp (*Parapenaeus longirostris*) Shell Wastes. *Journal of Agricultural Sciences*, 28(3), 490-500 https://doi.org/10.15832/ankutbd.861909

Phuong, P.T.D., Minh, N.C., Cuong, H.N., Minh, N. V., Han, N. T., Hoa, N.V., Yen, H.T.H., & Trung, T. S. (2017). Recovery of protein hydrolysate and chitosan from black tiger shrimp (*Penaeus monodon*) heads: approaching a zero waste process. *Journal of Food Science and Technology*, 54, 1850-1856. https://doi.org/10.1007/s13197-017-2616-6

Rinaudo, M. Chitin and Chitosan. (2006). Properties and applications. *Progress in Polymer Science*, 31, 603-632.

Soltani, M., Parivar, K., Baharara, J., Kerachian, M. A., & Asili, J. (2015). Putative mechanism for apoptosis-inducing properties of crude saponin isolated from sea cucumber (*Holothuria leucospilota*) as an antioxidant compound. *Iranian Journal of Basic Medical Sciences*, *18*(2), 180.

Synowiecki, J., & Al-Khateeb, N. A. (2003). Production, properties, and some new applications of chitin and its derivatives. Critical Reviews in Food Science and Nutrition, 43(2), 145-171. https://doi.org/10.1080/10408690390826473

Uğurlu, E., & Duysak, Ö. (2023). A study on the extraction of chitin and chitosan from the invasive sea urchin *Diadema setosum* from İskenderun Bay in the Northeastern Mediterranean. *Environmental Science and Pollution Research*, 30(8), 21416-21424. https://doi.org/10.1007/s11356-022-23728-9

Varma, R., & Vasudevan, S. (2020). Extraction, characterization, and antimicrobial activity of chitosan from horse mussel *Modiolus modiolus*. *ACS Omega*, *5*(32), 20224-20230.

Varma, R., Vasudevan, S., Chelladurai, S., & Narayanasamy, A. (2021). Synthesis and Physicochemical Characteristics of Chitosan Extracted from *Pinna deltoides*. *Letters in Applied NanoBioScience*, *11*(4), 4061-4070.

Yadav, P., Yadav, H., Shah, V. G., Shah, G., & Dhaka, G. (2015). Biomedical biopolymers, their origin and evolution in biomedical sciences: A systematic review. Journal of clinical and diagnostic research: *JCDR*, 9(9), ZE21.

Younes, I., Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, *13*(3), 1133-74.

Yuan, Y., Chesnutt, B. M., Haggard, W. O., & Bumgardner, J. D. (2011). Deacetylation of chitosan: Material characterization and in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. *Materials*, *4*(8), 1399-1416.

Zainol Abidin, N. A., Kormin, F., Zainol Abidin, N. A., Mohamed Anuar, N. A. F., & Abu Bakar, M. F. (2020). The potential of insects as alternative sources of chitin: An overview on the chemical method of extraction from various sources. *International Journal of Molecular Sciences*, *21*(14), 4978.