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Isolation and Characterization of Collagen from the Invasive Sea Urchin (*Diadema setosum* L., 1778) in North-Eastern Mediterranean Sea, Türkiye

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ABSTRACT

In the present study, collagen was obtained from tissues of the invasive sea urchin (*Diadema setosum*) rapidly spread in the Mediterranean Sea. As a result, the yield of collagen isolated from sea urchin was determined to be 23.78±1.33% (dry weight). As a result of SDS-Page analysis, it was determined that it contains (α1)2α2, (Molecular Weight (MA) 124, 114 kDa) and ß chains (MA 245 kDa) similar to calfskin collagen. In the amino acid analysis of collagen, it was determined that the imino acid (proline+hydroxyproline) content was 196.1 residues/1000 residues. The functional bands of amide A (3301 cm^{-1}), B (2924 cm^{-1}), I (1643 cm^{-1}), II (1550 cm^{-1}), and III (1242 cm^{-1}) functional bands were visualized in the FTIR spectrum. These results were like other collagen sources. Invasive sea urchin was used as a source of collagen for the first time in the present study. An alternative source of collagen to mammalian collagen, which is used commercially in many industries such as biomedicine, food and cosmetics, was isolated for the first time from *D. setosum*. It was proposed that marine collagen can be used as an alternative source of collagen and a functional component in areas including food, cosmetics, and pharmaceutical industries.

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Türkiye Kıyılarında Dağılım Gösteren İstilacı Denizkestanesinden (*Diadema setosum* L., 1778) Kolajen Ekstraksiyonu ve Karakterizasyonu

ÖZET

Bu çalışmada, Akdeniz'de hızla yayılan istilacı denizkestanesi (*Diadema setosum*) dokularından kolajen elde edilmiştir. Sonuç olarak, denizkestanesinden elde edilen kolajenin verimi %23.78±1.33 (kuru ağırlık) olarak belirlenmiştir. SDS-Page analizi sonucunda, dana derisi kolajenine benzeyen $(\alpha_1)_2\alpha_2$, (Molekül Ağırlığı (MA) 124, 114 kDa) ve ß zincirleri (MA 245 kDa) içerdiği tespit edilmiştir. Kolajenin amino asit analizinde imino asit (prolin+hidroksiprolin) içeriğinin 196.1 kalıntı/1000 kalıntı olduğu belirlenmiştir. FTIR spektrumuna göre Amid A (3301 cm^{-1}), Amide B (2924 cm^{-1}), Amide I (1643 cm-1), Amide II (1550 cm^{-1}) ve Amide III (1242 cm^{-1}) fonksiyonel bantları görüntülenmiştir. İstilacı denizkestanesinden kolajen ilk defa bu çalışma ile elde edilmiştir. Biyotıp, gıda ve kozmetik gibi birçok endüstride ticari olarak kullanılan memeli kolajenine alternatif olarak istilacı denizkestanesinden (*D. setosum*) kolajen ilk kez elde edilmiştir. Elde edilen bu kolajeninin gıda, kozmetik ve ilaç endüstrileri gibi alanlarda alternatif bir kolajen kaynağı ve fonksiyonel bir bileşen olarak kullanılabileceği önerilmiştir.

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INTRODUCTION

Collagen is found in many tissues including skin, tendons, and connective tissues of vertebrates, and comprise about 30% of the total protein in the body (Muthumari et al. 2016). Approximately 29 different types of collagens have been described with unique amino acid orders and molecular constructions in vertebrate tissues (Ali et al. 2018). It usually has a triple helix (Gly-X-Y) structure wrapped around each other, consisting of Glycine (Gly), proline (X), and hydroxyproline (Y). Collagens are widely used in many fields such as food, cosmetics, tissue engineering, and the pharmaceutical industry due to their properties such as biocompatibility and biodegradability (Muthumari et al., 2016). Collagen is obtained from the skin and bones of terrestrial animals such as cows, pigs, and chickens. However, since communicable diseases such as bird flu, bovine spongiform encephalopathy (BSE), and foot and mouth disease (FMD) have increased in recent years, collagens obtained from terrestrial animals cause disease concerns (Gharagheshlagh et al., 2020). Therefore, marine collagens have become the focus of attention to meet the need for collagen. In addition to their use as a food source, marine species are important resources that can be used in fields such as agriculture, chemical industry, and cosmetics. In recent years, there is a tendency toward marine resources for the production of collagen, which has been rapidly increasing for use, especially in health and cosmetics.

Marine collagen sources are generally vertebrate and invertebrate species such as fish, octopus, cuttlefish, shrimp, sea cucumber, and sea urchin. Skins, tissues or marine animal waste parts of economically important species constitute an important part of marine collagen resources (Ahmad & Benjakul, 2010; Muthumari et al., 2016; Ali et al., 2018; Nurilmala et al., 2019; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020; Li et al., 2020; Sulaiman & Sarbon, 2020). Research on collagen production from species with low or no economic value has increased because of the increase in collagen use and demand (Barzideh et al., 2014; Iswariya et al., 2018). The fact that marine animals that do not have economic value are not used as a resource, there is no hunting pressure on these species, and their rapid spread creates negative effects on the ecosystem and native marine species.

The Mediterranean region is a good example of this case, especially due to the rapidly increasing foreign species inflows in the last decade. The entry of foreign species into the Mediterranean Sea can be through the Suez Canal, the Strait of Gibraltar, or by ship transport. However, the most intense foreign species entry is through the Suez Canal. To date, 80 fish and 123 invertebrates have been introduced from these alien marine species, which are called the lessepsian species (Çınar et al., 2020). Pufferfish are harmful invaders that have spread throughout the Mediterranean Sea within the last 20 years (Kayhan et al., 2021). In a similar way, the lionfish (*Piterois miles*) has been spreading rapidly (Hüseyinoğlu et al., 2021). One of the species that enters the Mediterranean Sea and has a serious invasion potential is the sea urchin *Diadema setosum*.

The invasive sea urchin (*D. setosum*) is a species of Indo-Pacific origin and has a wide distribution in the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea), east coast of Africa, Japan, and Australia (Lessios et al., 2001). It was first reported on the Turkish coasts in 2006 on the Kaş Peninsula of Antalya, and then on the Iskenderun Bay, the Aegean Sea, and the Marmara Sea coasts (Turan et al., 2011; Yapıcı 2018; Artüz & Artüz, 2019). *D. setosum* reaches large sizes due to its unique feeding habits and reproductive behaviors a great threat to endemic species with the increase in population density. Due to the morphological structure of this species (long spines), it damages the fishing gear used in fishing, hindering hunting activities. In addition, its long and black spines cause adverse effects such as painful injury, swelling, and redness when penetrating human skin.

The present study aimed to extract collagen from the invasive sea urchin (*D. setosum*) for the first time and to determine the physical, chemical, and structural properties of this collagen. Thus, the collagen extracted from the sea urchin species can be used in cosmetics, tissue engineering, pharmaceutical industry, etc. This invertebrate, which is not consumed in Türkiye, will be brought to the country's economy as a biomaterial with high economic value.

MATERIAL and METHODS

Materials

In the study, *D. setosum* samples were collected with nets from the coast of Iskenderun Bay in August 2021. (Figure 1). The sea urchin samples collected were brought to the laboratory in sealed plastic boxes. Morphometric measurements of the samples were made with the help of a digital caliper with a precision of 0.01 mm after their weighing using a digital scale with a precision of 0.01 g. The mean total weight, gonad weight, test weight, and test diameter of the individuals were calculated as 84.64±2.77 g, 3.04±0.11 g, 32.72±1.21 g, and 51.8±1.18 mm, respectively (Mean±SD). Then all soft tissues were taken and stored at -80°C until the time of analysis.

Collagen Preparation

The method of Sivakumar & Chandrakasan (1998) was modified for the isolation of collagen from the soft tissues of invasive sea urchin (*D. setosum*). The whole procedure was carried out at 4°C. Soft tissues were cut into small pieces using scissors and kept in 0.2 N NaOH solution for two days (NaOH solution was changed daily). The precipitate was washed three times with distilled water and then lyophilized. The dried precipitate was melted in 1 M acetic acid two days. Then 5% (w/v) pepsin (Sigma p7000), according to the lyophilized weight was added and hydrolyzed for 48 h. The mixture was then centrifuged at 8000xg for 1h at 4 ° C, and the precipitate was collected. The precipitate was salted by adding NaCl to a last concentration of 0.7 M and was followed by precipitation by adding NaCl to a last concentration of 2.3 M in 0.05 M Tris-HCl (pH 7.5). The precipitate was divided by centrifugation at 9000×g for 1 h at 4 °C. The precipitate was then melted in 0.5 M acetic acids, dialyzed in contrast to 0.1 M acetic acid, distilled water, and lyophilized.

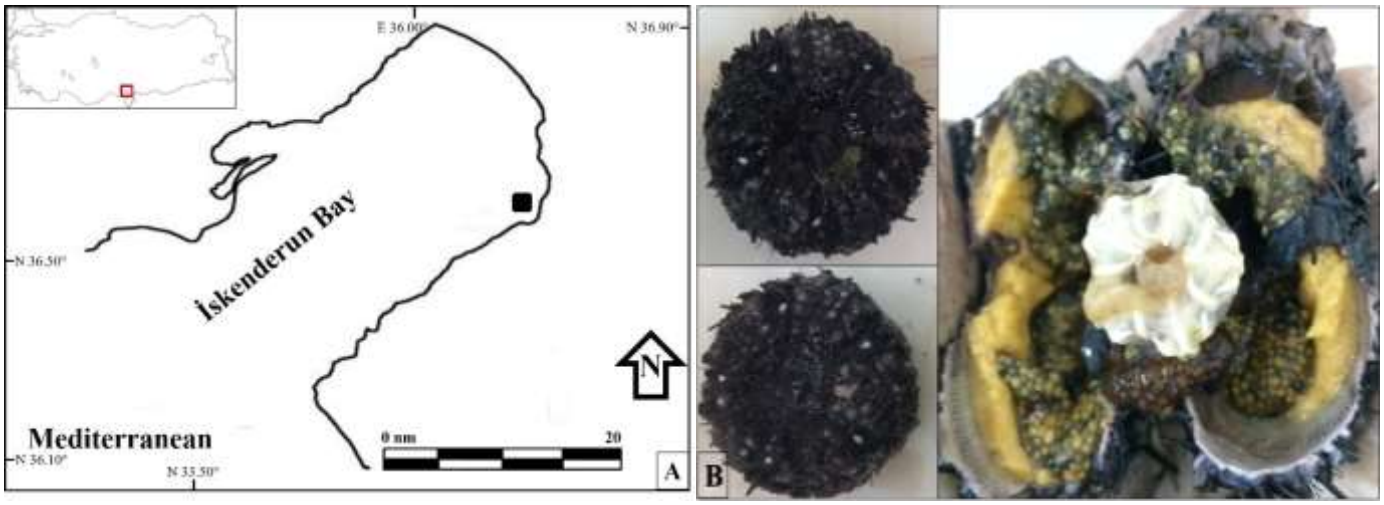

Figure 1 A) Study area, B) Invasive Sea Urchin *D. setosum* species.
Şekil 1 A) Çalışma Alanı, B) İstilacı denizkestanesi *D. setosum* türü

Yield of collagen

The initial weight of the soft tissues of sea urchins was used to calculate the collagen yield and was calculated with the following Eq. 1.

Yield of collagen= (Weight of lyophilized collagen)/(Initial wet weight of tissues)x100 (1)

Proximate analysis

The ash and protein values of the samples taken from the soft tissues of the sea urchin were determined according to the Association of Official Analytical Chemists (AOAC) (2000) method, the lipid analyses according to Bligh & Dyer (1959) and the total crude protein analysis was carried out using the Kjeldahl method (Bligh & Dyer, 1959; AOAC 2000). All analyses were performed in triplicate.

SDS-Page analysis

The collagen was dissolved in 5 mg mL^{-1} 0.1 M acetic acid by agitating at room temperature for four hours. Then it was dialyzed against PBS (phosphate buffered saline) and loaded into the well in 100, 200, and 400 ng wells, respectively, by denaturation using a buffer at 95 °C for 5 minutes. The sample in the first well was 100 ng of BSA (Bovine Serum Albumin). Separation gel was 8% and no stacking gel was used. The sample was marked with Coomassie Brilliant Blue R250 and then destained.

Amino Acid analysis

The amino acid analysis of collagen obtained from sea urchin soft tissues was performed according to the D.05.G106 (UFLC-UV) method (PITC 1999; Dimova 2003). The amino acid content was stated as residues/1000 residues.

FTIR analysis

The FTIR spectra (SHIMADZU F-TIR-IRAffinity-1S) of collagen obtained from soft tissues of sea urchins were performed using the ATR method under dry conditions. All spectra readings were performed between 400 and 4000 cm^{-1} and at a data acquisition rate of 4 cm^{-1}.

SEM analysis

A SEM device was used to examine the surface area and internal structure of sea urchin collagen. Before collagen imaging, the collagen was coated with gold-palladium (Au-Pd) using a POLARON SC7620 sputter coating device. The sample was imagined under SEM (Scanning Electron Microscope) (JEOL JSM-638OLA) using 15 kV.

Statistical analyses

Data were analyzed using Microsoft Office Excel 2016. All samples were analyzed in triplicate. All quantitative results are given as mean±standard deviation.

RESULTS and DISCUSSION

In the last ten years, very few studies have been found on the study of invasive and poisonous *D. setosum* on the Mediterranean coast, such as growth, reproduction, and accumulation of metal. Previous studies have generally been concerned with species registration, availability, distribution in the region, biological properties and extraction of chitin and chitosan (Rahman et al., 2012; Fitriyani et al., 2022; Uğurlu & Duysak, 2022). Furthermore, there are studies on the components of nutrients (protein, lipid, fatty acid), antibacterial effects, cytotoxic activity, and accumulation of metals in tissues consumed such as gonads of this species (Flammang et al., 1997; Marimuthu et al., 2015; Tulandi et al., 2021). Examining studies on the species in Türkiye in recent years, it was seen that they were carried out on the Mediterranean Sea, Aegean Sea and Marmara Sea coasts and showed only the first records (Yokes & Galil, 2006; Turan et al., 2011; Yapıcı 2018; Artüz & Artüz, 219; Bilecenoğlu et al., 2019). There are studies of collagen extraction on different species of sea urchins in the literature. Collagen obtained in these studies had two structures of fibrillar collagen (α_1 and α_2) structures (Trotter & Kobb, 1994; Omura et al., 1996; Cluzel et al., 2000; Nagai & Suzuki, 2000). It has the potential to be an alternative marine collagen source for use in several areas such as food, medicine, and cosmetics (Nagai & Suzuki, 2000). It is a promising biomaterial for tissue and regenerative medicine and is environmentally friendly and economically sustainable (Shimizu et al., 1990). However, no biomaterial production studies related to *D. setosum were found.*

Yield in sea urchin collagen

For the collagen obtained from the soft tissues of sea urchins, 17.5 g collagen was obtained from 75 g of soft tissue (dry weight) (Figure 2). The mean collagen yield was calculated as 23.78±1.33%. It has been reported that the mean collagen yields extracted from the skin and bones of fish vary between 2% and 29% (Ahmad & Benjakul, 2010; Muthumari et al., 2016; Ali et al., 2018; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020). The wide range of differences between collagen yields was associated with the species of fish or tissues used, their ecological environments, and different extraction methods. Nagai & Suzuki (2000) have reported a yield of approximately 35% (dry weight) of the collagen obtained from the shells of purple sea urchin (*Anthocidaris crassispina*). Ferrariro et al. (2020) have reported the mean yield of the collagen obtained from the soft tissue of *Paracentrotus lividus* as 4.93±2.22%. The differences in collagen yields obtained from *D. setosum* were associated with the difference in species or the difference in the tissues from which the collagen is obtained.

Figure 2 The collagen obtained from the *D. setosum* (original images).

Şekil 2 D. setosum'dan elde edilen kolajen (orijinal görüntü)

Proximate analysis

It was determined that the total lipid and ash contents of the collagen extracted from *D. setosum* were lower than the total lipid and ash contents of the raw tissue. This was associated with the process of removing collagen from inorganic substances and fat. In the present study, the protein, lipid and ash content of sea urchin tissues were calculated as 20.99±0.33%, 15.78±0.34%, 18.35±0.56%, respectively, while the protein, lipid and ash content of collagen was calculated as 49.5±0.26 and 2.7±0.25% and 1.72 ±0.12%, respectively.

Nurilmala et al. (2019) have reported the protein, lipid, and ash contents of the collagen obtained from *Thunnus albacares* skin as 36.09%, 1.08%, and 2.25%, respectively. Sulaiman & Sarbon (2020) calculated the protein, oil, and ash values of collagen extracted from *Decapterus macrosoma* fish waste as 22.86%, 0.38%, and 60.9%, respectively. Li et al. (2020) have reported the protein, lipid and ash levels of collagen extracted from the body wall of *H. cinerascens* as 10.3±0.4%, 0.3±0.0%, and 0.9±0.1%, respectively. The difference in protein, lipid, and ash content of collagen obtained from *D. setosum* was associated with the fact that it lived in different habitats or with the difference in the tissues from which collagen was obtained.

SDS-Polyacrylamide Gel Electrophoresis (SDS-Page)

SDS-Page analysis of collagen obtained from sea urchin is presented in Figure 3. The collagen extracted from the sea urchin comprised α_1, α_2, and high molecular weight chains ß and γ chains (Figure 3; Lane

1). The molecular weight of this collagen, the α_1, α_2, and ß chains, was determined to be approximately 124 kDa, 114 kDa and 245 kDa, respectively. In addition, it was determined that the collagen has an $(\alpha_1)_2\alpha_2$ molecular structure (Figure 3).

It has been reported that collagen obtained from the test portion of *Asthenosoma ijimai* has an $(\alpha_1)_2\alpha_2$ heterotrimer structure, like that determined in the present study (Omura et al., 1996). Shimizu et al. (1990) have reported that there are four α chains as α_1 α_2 α_3 α_4 in the *A. ijimai*, while Trotter & Koob (1994) have reported that *Eucidaris tribuloides* cidaroid sea urchin has heterotrimer collagen with an $(\alpha_1)_2\alpha_2$ molecular structure. The sea urchin collagen in the present study was found to have an $(\alpha_1)_2\alpha_2$ molecular structure, similar to the collagen of different species in similar studies (Liu et al., 2012; Zhang et al., 2014; Asaduzzaman et al., 2020). As a result, the α_1 and α_2 chains in the sea urchin collagen confirm that it is like type I collagen.

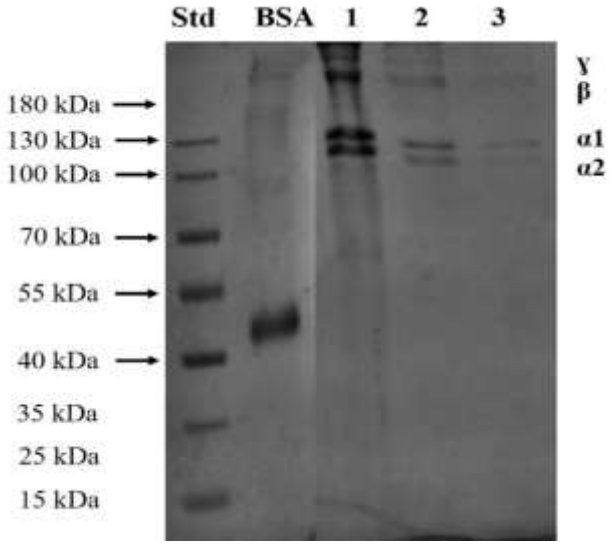

Figure 3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page) of collagen from D. *setosum*. Std: Protein markers; BSA: 100 ng BSA (Bovine Serum Albumin); lane 1: 400 ng collagen; lane 2: 200 ng collagen; lane 3: 100 ng collagen.

Şekil 3 D. setosum kolajenin Sodyum Dodesil Sülfat Poliakrilamid Jel Elektroforezi (SDS-Page). Std: Protein markır; BSA: 100 ng BSA (Sığır Serum Albümin); Şerit 1: 400 ng kolajen; Şerit 2: 200 ng kolajen; Şerit 3: 100 ng kolajen.

Amino Acid Composition of Collagens from the Soft Tissues of *D. setosum*

The amino acid content of the collagen extracted from *D. setosum* is shown in Table 1 as residue/1000 residues. In the present study, proline and hydroxyproline, which are unique in their amino acid content, were determined in collagen extracted from *D. setosum* soft tissues and glycine was determined as the amino acid with the highest amount (Table 1). In general, the amino acid glycine comprises about one-third of the total amount of amino acids. In the present study, 326.94 residues/1000 residues of glycine were found in the collagen extracted from *D. setosum* (Table 1).

Table 1 Amino acid composition of collagen from *D. setosum* (residues/1000 resiudes).

Çizelge 1 D. setosum kolajenin amino asit içerikleri (kalıntı/1000kalıntı)

Amino Acids	*D. setosum*
Alanine (ALA)	101.6
Arginine (ARG)	35.9
Aspartic acid (ASP)	69.3
Cysteine (CYS)	0
Glutamic acid (GLU)	32.4
Glycine (GLY)	326.9
Histidine (HIS)	9.8
Isoleucine (ILE)	11.4
Leucine (LEU)	25.8
Lysine (LYS)	11.2
Methionine (MET)	15.8
Phenylalanine (PHE)	16.2
Proline (PRO)	117.5
Serine (SER)	58.2
Threonine (THR)	56.6
Tyrosine (TYR)	9.9
Valine (VAL)	22.1
Hydroxyproline (HYP)	78.6
Imino acid (HYP + PRO)	196.1

In the literature, the amount of glycine has been reported to be the leading amino acid in collagen samples and comprises approximately 30-35% of the amino acid content in Saurida spp, *Mugil cephalus* and *Cypselurus melanurus* (Kumar et al., 2012; Veeruraj et al., 2013; Kozlowska et al., 2015). It has been reported that the ASC and PSC collagens obtained from the scales of Saurida spp. contained 335 and 338 residues/1000 residues glycine, respectively.

In the collagen extracted from *D. setosum*, alanine, aspartic acid, threonine, and serine amino acids were found to be 101.6, 69.3, 56.6, and 58.2 residues/1000 residues, respectively. No cystine was detected in the *D. setosum* collagen (Figure 4). Similarly to the present study, Senaratne et al. (2006) did not detect cystine in collagens extracted from different marine organisms, and cystine was not found in amino acid analyses of collagen material in other studies (Iswariya et al., 2018) The amino acid content of proline and alanine of collagen obtained from *D. setosum* is expected values for sea urchin, which is a tropical and subtropical species and explains the higher denaturation temperatures compared to cold-climate species.

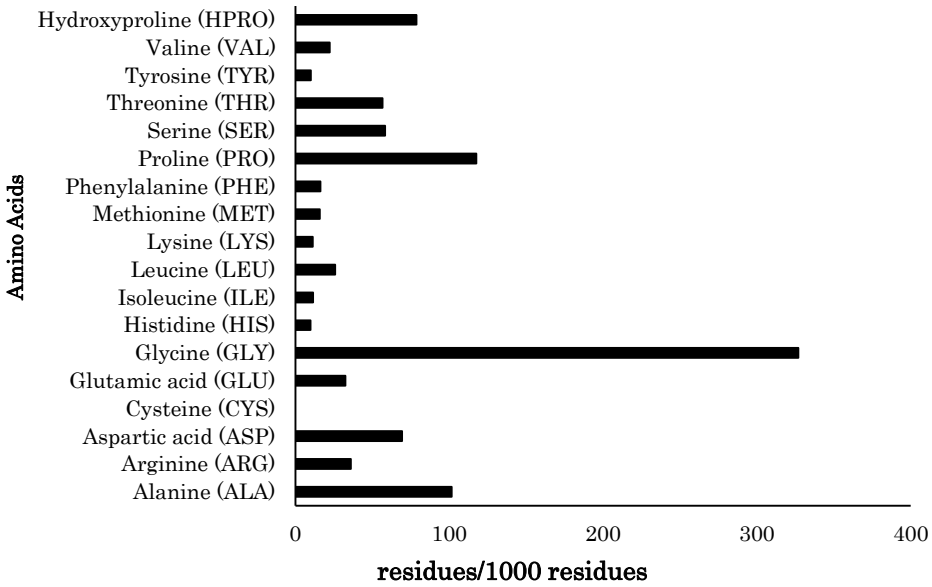

Figure 4 Amino acid composition of sea urchin collagen.
Şekil 4 Denizkestanesi kolajenin amino asit kompoziyonları

In the present study, proline and hydroxyproline contents of the collagen extracted from sea urchin were determined to be 117.5 and 78.6 residues/1000 residues, and the total imino acid content was determined to be 196.1 residues/1000 residues. Sea urchin collagen has higher than those of the Chrysaora sp. (149 residues/1000 residues) (Barzideh et al., 2014), *A. ijimai* (84 residues/1000 residues), *Strongylocentrotus nudus* (86 residues) residues/1000 residues) and *Strongylocentrotus intermedius* (85 residues/1000 residues) sea urchin species (Omura et al., 1996). Imino acid provides structural integrity in collagen. It has been known that the reason for the different amounts of imino acid content is due to the habitat differences in which the animals live and different habitat temperatures.

FTIR spectrum

The FTIR spectrum analysis of *D. setosum* collagen is presented in Figure 5. The positions of characteristic Amide A, Amid B, Amid I, Amid II, and Amid III bands are shown in Table 2. The IR spectrum results of sea urchin collagen showed the typical bands of Amide A, Amide B, Amide I, Amide II and Amide III for Type I collagen. The amide A band is generally associated with the extending vibrations of the N-H group. A free N-H extending vibration occurs in the range of between 3400 and 3440 cm^{-1}. The Amide A band of collagen extracted from *D. setosum* was determined to be 3301 cm^{-1} (Table 2). A shift of the wavenumber to a lower frequency, i.e., 3300 cm^{-1} of the N-H extending vibration, typically indicates that the N-H group has more hydrogen bonds (Gharagheshlagh et al., 2020).

Table 2 The major peak assignments of the FTIR spectra for collagen from *D. setosum.*
Çizelge 2 D. setosum kolajenin FTIR spektrumu için major pikleri.

Region	Peak wavenumber (cm^{-1})	Assignment
Amide A	3301	N-H stretch and H bond
Amide B	2924	CH2 asymmetric stretch
Amide I	1643	C=O stretch/hydrogen bond coupled with COO
Amide II	1550	NH bond coupled with CN stretch
Amide III	1242	NH bond coupled with CN stretch

The Amid B band, which corresponds to the asymmetric extending of the alkanyl C-H group and represents the NH_3^+ group, is observed at a wavelength of between 2850 and 2950 cm^{-1}. In the present study, the Amid B band was observed at 2924 cm^{-1} in the sea urchin collagen. The amide I band has characteristic frequencies of between 1600 and 1700 cm^{-1}, is associated with extending vibrations of the carbonyl group (C=O), and is the most important factor in determining the secondary structure of a protein. The Amide I band was observed at 1643 cm^{-1} in *D. setosum* collagen. The amide II band is associated with the N-H bond due to C-N extending vibrations in the range of between 1550 and 1600 cm^{-1} and its shift to

lower wavelengths indicates a hydrogen bond formation. The Amide II band of collagen obtained from the soft tissues of sea urchins was determined to be 1550 cm^{-1} (Figure 5).

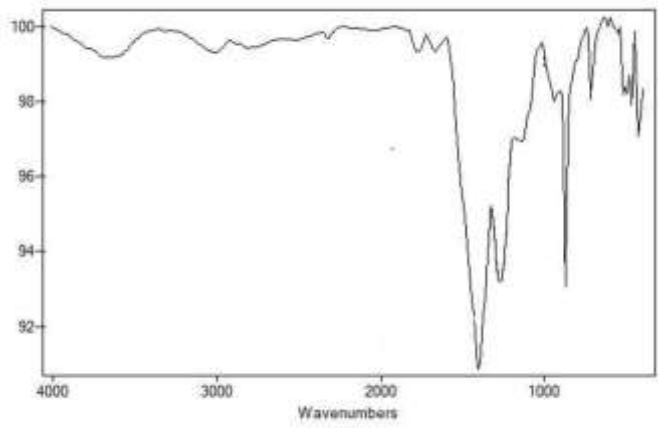

Figure 5 The FTIR spectrum of collagen from *D. setosum*.
Şekil 5 D. setosum kolajenin FTIR spektrumu

Finally, the Amide III band has a characteristic frequency between 1236 cm^{-1} and 1452 cm^{-1}. It shows the combination levels between C-H extending vibrations and N-H deformation of triple helix collagen and is considered a collagen fingerprint. The Amide III band was observed at 1242 cm^{-1} in *D. setosum* collagen. The present study determined that the FTIR results of collagens obtained from different marine organisms were similar to those reported in the literature (Ahmad & Benjakul, 2010; Ali et al., 2018; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020). The FTIR spectrum confirmed that *D. setosum* collagen has a natural triple helix structure.

Scanning Electron Microscope (SEM)

The morphological structure of collagen from *D. setosum* is shown in Figure 6. To the naked eye, collagen is soft, white, and porous. However, examined by SEM, it was determined that it consisted of interconnected, multilayered, scaly, dense, and irregular layers (Figure 6). Due to the excellent properties of collagen, its three-dimensional structure is of great importance. In the present study, the morphological structure of collagen obtained from sea urchin was investigated for the first time and it was concluded that it can be utilized in many fields such as cosmetics, tissue engineering, and biomedicine.

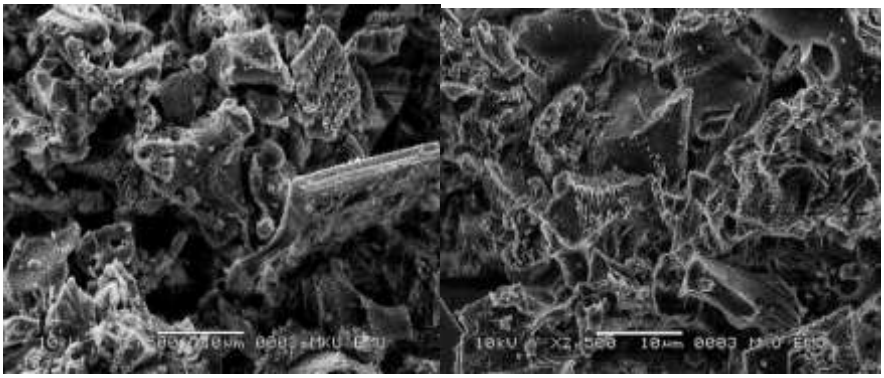

Figure 6. The SEM images of collagen from *D. setosum.*
Şekil 6. D. setosum kolajenin SEM görüntüleri

CONCLUSION

Collagen material was obtained for the first time from *D. setosum* sea urchin. The collagen obtained from *D. setosum* was similar to Type I collagen, which has a wide application area. Invasive sea urchin collagen showed similarities to those of terrestrial vertebrates and marine species, and it was found that it can be used as an alternative source of marine collagen. It can be proposed that alternative marine collagen extracted from *D. setosum* can be used as a biomaterial in fields such as biomedicine, tissue engineering, and cosmeceuticals.

As a result, using the sea urchin, an invasive species, as a collagen material will provide a high added value to Türkiye's economy by meeting the need for collagen biomaterial, which has been a popular research topic recently. On the other hand, in the case of its use in different industrial areas, *D. setosum* will be needed on an industrial scale, and its catch volume will eventually increase. Thus, the sea urchin population will be indirectly controlled. It will be ecologically beneficial and its dangerous situation in terms of tourism will be eliminated.

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Researchers' Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

Authors have declared no conflict of interest.

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