Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 51, No. 1, March 2022
pages (100-114)

sciendo

ISSN 1730-413X
eISSN 1897-3191

Effects of morphometric and biochemical parameters on collagen and pepsin-solubilized collagen yields of *Holothuria tubulosa* (Gmelin, 1790) and *Holothuria* (*Roweothuria*) *poli* (Delle Chiaje, 1823)

by

İbrahim Ender KÜNİLİ*

DOI: **https://doi.org/10.26881/oahs-2022.1.09**
Category: **Original research paper**
Received: **April 1, 2021**
Accepted: **August 24, 2021**

Department of Fishing and Processing Technology, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

* email: enderkunili@yahoo.com

Abstract

Due to their unique biochemical composition, sea cucumbers are highly prized marine echinoderm species. One of their most important properties is that they contain a high amount of collagen in their body wall. In this study, the relationship between collagen and pepsin-solubilized collagen yields from *Holothuria tubulosa* and *Holothuria poli* and morphometric and biochemical parameters were investigated.

Collagen yields were in the range of 10.63–16.04% for *H. tubulosa* and 7.12–13.10% for *H. poli*. It was determined that they may be related to length, body wall weight, and biochemical composition at different length frequencies. Moreover, maturity may have a direct effect on the yield, as mature specimens were found to have lower content of collagen, whereas immature small specimens contained a higher percentage of collagen. It was found that with increasing pepsin concentration, the PSC yield increased to 1.83–1.89% in *H. tubulosa* and *H. poli*, respectively. It was determined that collagen from smaller individuals, which contained more moisture and ash, was likely more susceptible to pepsin hydrolyzation.

This is the first published study demonstrating that collagen yield of sea cucumbers can vary with length, weight, maturity, and biochemical composition, in addition to species-specific differences.

Key words: *Holothuria tubulosa*, *Holothuria poli*, collagen yield, pepsin-soluble collagen, length–weight relationship, maturity, sea cucumber

1. Introduction

Sea cucumber is a benthic marine echinoderm species that occurs in most seas of the world. There are about 1500 sea cucumber species, and about 60 of them are used commercially (Purcell 2010). While some of these economic species such as *Stichopus japonicus* are cultivated in Asian countries, most of them are intensively exploited from natural sources due to high market demand and commercial value, which ranges from USD 15 to USD 385 (Purcell et al. 2013; Purcell 2014). The main reason for the high market demand for sea cucumbers is related to their unique biochemical composition, which includes high amounts of collagen, glycosides, chondroitin sulfate, and a low fat to high protein ratio, making them a perfect choice as a valuable functional food for consumers (Saito et al. 2002; Bordbar et al. 2011; Shi et al. 2016; Senadheera et al. 2020). The sea cucumber is mainly known in its commercial form as "beche-de-mer", which is prepared basically through processes of salting, boiling, and drying (Çaklı et al. 2004; Ferdouse 1999, 2004). Due to the long shelf life of the end product and production greater than the existing end-product stocks, overexploitation in most regions, including the Mediterranean, reaches maximum levels in each season.

Global production of sea cucumber ranged from 227 to 274 gross tons in 2015–2018 (FAO 2020). *Apostichopus japonicus* is the most produced species, but the production volume of this species decreased from 86.37% to 78.54%, following the increase in the production of other Holothurians from 13.58% to 21.40% in global sea cucumber production between 2015 and 2018 (FAO 2020). This may also lead to increased production of species from the Mediterranean such as *H. tubulosa*, *H. poli*, *H. forskali*, *H. mammata*, *H arguinensis*, *H. sanctori*, and *Parastichopus regalis* (Ramon et al. 2010; Antoniadou & Vafidis 2011; Borrero-Perez et al. 2011; Mezali & Thandar 2014; Gonzalez-Wangüemert et al. 2016). Of these species, *Holothuria tubulosa* and *Holothuria poli* are Mediterranean endemic species that occur in higher densities down to a depth of 30 m on a soft benthic substrate consisting of sand, mud, and sea meadows (Coulon & Jangoux 1993; Kazanidis et al. 2010; Gonzelez-Wangüemert et al. 2016; Tolon & Engin 2019). Their colors range from light brown (*H. tubulosa*) to blackish brown with white podia (*H. poli*; Aydın & Erkan 2015). The length of these species can reach up to 35 cm, however, well-represented sizes range from 8–10 cm to 23–26 cm, and commercial interest starts mainly above 10–12 cm (Gonzalez-Wangüemert et al. 2018; Aydın 2019). Both of these holothurians constitute an important proportion of sea cucumber production in Mediterranean countries, mainly Italy, Spain, Greece and Turkey (Mezali et al. 2006; Antoniadou and Vafidis 2011; Valente et al. 2015; Gonzalez-Wangüemert et al. 2016; Künili & Colakoglu 2018; Aydın 2019). The average total production of these species in Turkey was reported as 419 tons for the last seven years and 855 tons for 2017 (Aydın 2020). The marketing of these economic species was generally in the form of fresh frozen and beche-de-mer, as is usual in these countries (Aydın 2008; Antoniadou & Vafidis 2011; Aydın et al. 2011; Sicuro & Levine 2011; Gonzalez-Wangüemert et al. 2016; Aydın 2017; Künili & Colakoglu 2019).

Collagen is an important fibrous protein found in almost all living organisms, as it is the main component of structural tissues (Regenstein & Zhou 2007). In marine animals, collagen is also an important contributor to total proteins in the whole body as it is a major constituent in scales, bones, skin, and fins (Gómez-Guillén et al. 2011; Coppola et al. 2020). However, collagen and collagenous tissues are of particular importance in echinoderms, such as sea urchins, starfishes, and sea cucumbers, because they protect their coelom and body shape, while also playing a key role in motion and defense systems by being the major constituent of their skin, podia, and spines (Santos et al. 2005; Wilkie 2005; Senadheera et al. 2020).

Collagen and its properties in sea cucumber species have been extensively studied in the last decade, however, there is a significant lack of knowledge about collagen and its properties in both *H. tubulosa* and *H. poli*. Although these are economically important sea cucumber species, with collagen being one of the main reasons for their importance, its relationship with morphometric and biochemical characteristics is still unclear. Moreover, the yield of collagen as a function of morphometric and biochemical parameters has not been investigated in any of the sea cucumber species, and this is an important issue for the optimum utilization of sea cucumbers. For this reason, the present study was designed to investigate the collagen yields from *H. tubulosa* and *H. poli* as economic species. In addition, the objective was to determine the yield of collagen hydrolysates, which are indicative of the physiological utility of collagen in the body and in many industries due to their increased solubility and purity (Coppola et al. 2020). By determining the amount of collagen and hydrolysates obtained from both species, we hoped to determine the optimum utilization sizes for both species, while filling the gap in the literature by comparing changes in collagen yields as a function of morphometric and biochemical parameters.

2. Materials and methods

Research materials of *Holothuria tubulosa* and *Holothuria poli* were collected by hand during SCUBA diving from depths ranging from 1 to 15 m along the coasts of the southern Marmara Sea and the northern Aegean Sea. A total of 200 specimens were collected for each species between March 2018 and July 2020 at different time intervals from four locations indicated in Fig. 1. Live specimens individually packed in polyethylene bags were transported to the laboratory using ice-cooled insulated boxes within 4 h. Upon arrival at the laboratory after each sampling, the specimens were divided into five length frequencies according to the distribution of their minimum and maximum lengths. After morphometric measurements were performed, they were stored at –20°C until the number of specimens was sufficient for all analyses in each relevant length frequency. All chemicals used in this study were of analytical grade.

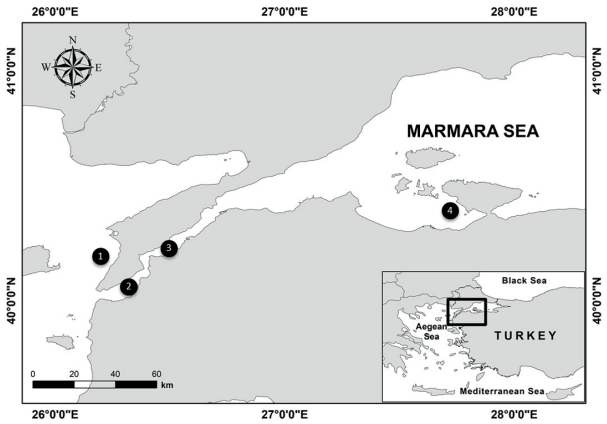

Figure 1

Sampling locations: 1 – Kemikli (40°16'39"N; 26°14'10"E); 2 – Dardanos (40°4'26"N; 26°21'16"E); 3 – Yapıldak (40°12'26"N; 26°28'36"E); 4 – Ocaklar (40°12'26"N; 27°44'46"E).

2.1. Morphometric measurements and preparation of samples

Total weight was measured using a two-digit precision scale at sampling sites when sea cucumbers were alive. Other measurements of specimens, such as length, viscera, gonads, and gutted weight (body wall weight), were performed in the laboratory. Upon arrival at the laboratory, the specimens were weighed again and dissected from the bottom using a sharp knife. The individual bag contents, viscera and gonads, if present, were weighed separately. The length and weight of the remaining body wall were determined and prepared for collagen isolation according to body length frequencies. A total of five length frequencies were determined according to minimum and maximum lengths, and collagen isolation was conducted on these groups separately. Collagen was also isolated and compared from mature and immature specimens according to their length groups. The gonadosomatic index (GSI) of mature specimens was calculated using the ratio of gutted length to gonad weight according to the method described by Conand (1981, 1993).

2.2. Biochemical Composition Analysis

The moisture content was determined for approximately 5 g of minced body wall by oven drying at 105 ± 3°C until constant weight (AOAC 2000). The percentage protein content (Kjeldahl N × 6.25) was determined by the method of AOAC (2000). Extraction of lipids from samples was carried out with a mixture of chloroform, methanol, and water (Bligh & Dyer 1959). Ash content was determined in an oven at 550 ± 5°C for 24 h (AOAC 2000). The total carbohydrate level was calculated using the method of FAO (2003).

2.3. Collagen isolation

Collagen isolation was performed according to the method previously described by Saito et al. (2002) with minor modifications. All steps were performed at 4°C unless a different temperature is specified. A total of 100 g of small pieces of cleaned body wall samples were mixed with a disaggregation solution containing 5 M NaCl, 50 mM EDTA, 0.2 M β-mercaptoethanol, and 0.1 M Tris-HCl (pH 8), after being washed several times and homogenized using Ultra-Turrax (Ika, Yellow Line) with distilled water. The mixture was then filtered through a double-layer cheesecloth and the filtrate was centrifuged at 10,000 x g for 30 min after gentle stirring for 72 h. The precipitate was collected and mixed with 1000 ml 0.1 M NaOH and stirred for 48 h. Collagen fibrils were collected after centrifugation at 7500 x g for 30 min. The collected fibrils were then washed several times with cold distilled water and centrifuged for the last time at 3000 x g for 15 min to extract excess water from the collagen. The remaining collagen fibrils were then used to calculate the wet yield before lyophilization.

2.4. Isolation of pepsin-solubilized collagen (PSC)

Isolation of pepsin-solubilized collagen (hydrolyzation of collagen with pepsin) was performed

according to the methods described by Saito et al. (2002) and Zhu et al. (2012) with minor modifications. All steps were performed at 4°C. An amount of 1 g of lyophilized collagen fibrils was suspended with 500 ml 0.5 M acetic acid containing 1% to 6% pepsin (enzyme:collagen, w/w; Gastric Pepsin from Porcine, Sigma) and stirred at low rpm for two days. The hydrolysis process was followed by centrifugation of the suspension at 7500 x g for 1 h. After centrifugation, the supernatant was mixed with 4.0 M NaCl until the concentration reached 0.8 M NaCl in the total volume. Following the precipitation, collagen hydrolysate was mixed with 0.5 M acetic acid and dialyzed using a 13 kDa molecular cut-off membrane (Thermo, Snake Skin) at 4°C against 0.02 M Na_2HPO_4 (pH 8). After dialysis, samples were centrifuged at 10,000 x g for 45 min and the precipitate was collected. Precipitated samples were then suspended with 0.5 M acetic acid and re-dialyzed against 0.1 M acetic acid for 48 h. Dialyzed samples were directly re-dialyzed against distilled water for 24 h. The dialysate was then collected by centrifugation at 10,000 x g for 15 min. The purified and precipitated samples were then lyophilized and used for calculation of pepsin-solubilized collagen (collagen hydrolysate) yield and SDS-PAGE analysis.

2.5. Determination of the degree of hydrolysis

The hydrolyzation degree (HD) was determined using the o-phthaldialdehyde (OPA) method described by Nielsen et al. (2006). A volume of 3 ml of the prepared OPA reagent (Sigma) was mixed with 400 µl of PSC samples. The mixture was allowed to settle for 2 min after vortexing for 5 sec. Then, the absorbance of the mixture was measured at 340 nm to calculate the content of free amino groups equivalent to that obtained on a standard curve prepared using L-serine (Sigma). The total amount of free amino groups was obtained following acid hydrolysis with 6 M HCl at 110°C for 24 h.

2.6. Calculation of collagen and hydrolysate yields

Collagen yields of *H. tubulosa* and *H. poli* were calculated using the ratio of total weights used in collagen isolation and the wet weight of isolated collagen in g. The calculation was also validated by using the ratio of lyophilized raw body wall weights to lyophilized collagen. The yield of pepsin-solubilized collagen was calculated using the ratio of the initial amount of lyophilized collagen used in pepsin hydrolyzation and the weight of lyophilized pepsin-soluble collagen. The degree of hydrolyzation was used to confirm the yield of collagen hydrolysate.

2.7. Determination of the distribution of molecular weights

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to determine the molecular weights and distribution of proteins. It was also used to isolate proteins and determine the purity and efficiency of isolation and hydrolyzation protocols. To visualize the efficiency of the isolation procedures, the molecular weight distribution of collagen hydrolysates was determined using SDS-PAGE analysis performed according to the method described by Laemmli (1970). A mixture consisting of 0.06 M Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, and 0.1% bromophenol blue was used as a sample buffer and boiled for 5 min before loading a gel consisting of 4% stacking and 7.5% resolving gels. Gels were stained with 0.05% Coomassie Brilliant Blue R-250. An unstained wide range molecular marker (Intron, GangNam-Stain) was used to scale the molecular weight distribution of collagen hydrolysates. Electrophoresis was conducted using a Tris-HCl-Glycine buffer system at 115 V for 3 h.

2.8. Statistical analysis

The data included length, total weight, gutted weight, maturity, and biochemical composition, as well as amounts of protein, collagen, and collagen hydrolysate along with their yields for both sea cucumber species. They were subjected to one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. The suitability of data for ANOVA was tested using the Anderson–Darling test for normality and Levene's test for equal variances (homogeneity). Pearson correlation between variables was conducted after determining the equation of the major determinants affecting the collagen yield in the samples. The software used was Minitab 17 (Minitab, LLC, USA). All experiments were carried out in triplicate and data were calculated as average ± standard deviation. The significance of differences was defined at $p < 0.05$ (Zar 1996).

3. Results

Morphometric data for both sea cucumber species used in this study are summarized in Table 1 for *H. tubulosa* and in Table 2 for *H. poli*.

The minimum and maximum lengths of *H. tubulosa* and *H. poli* were measured as 10.60–25.00 and 8.90–22.40 cm, respectively. The median lengths of *H. tubulosa* and *H. poli* populations were determined as

15.88 ± 1.02 cm and 14.58 ± 0.82 cm, respectively. The minimum and maximum weight and gutted weight were in the range of 26.02–118.08 g and 22.56–59.14 g for *H. tubulosa*, and 26.42–95.70 g and 21.12–55.43 g for *H. poli*, respectively. A total of 68 specimens (34%) of *H. tubulosa* and 44 specimens (22%) of *H. poli* were identified as being in the reproductive period.

3.1. Relationship between collagen yield and length and biochemical composition

In this study, the relationship between the yield of collagen, as the major component of the edible part (body wall) of sea cucumbers, and the length and biochemical composition was investigated. Due to limited sex differentiation in both species, biochemical composition was determined from 100 g randomly subsampled fresh body wall for each sex. The mean content of moisture, proteins, ash, fat, carbohydrates, and the yield of collagen by species in relation to length frequencies are summarized in Fig. 2 and Fig. 3.

For all length frequencies, mean percentage levels of biochemical composition in *H. tubulosa* and *H. poli* ranged from 83.88 ± 1.03 to 84.47 ± 1.09 and from 82.66 ± 0.29 to 83.00 ± 0.28 for moisture, from 8.44 ± 0.99 to 8.66 ± 1.01 and from 8.32 ± 0.24 to 8.54 ± 0.25 for proteins, from 4.66 ± 0.45 to 4.76 ± 0.47 and from 6.59 ± 0.78 to 6.68 ± 0.88 for ash, from 1.75 ± 0.35 to 1.80 ± 0.44 and from 1.51 ± 0.09 to 1.55 ± 0.11 for fat, and from 0.53 ± 0.12 to 1.09 ± 0.66 and from 0.40 ± 0.11 to 0.68 ± 0.15 for carbohydrates, respectively.

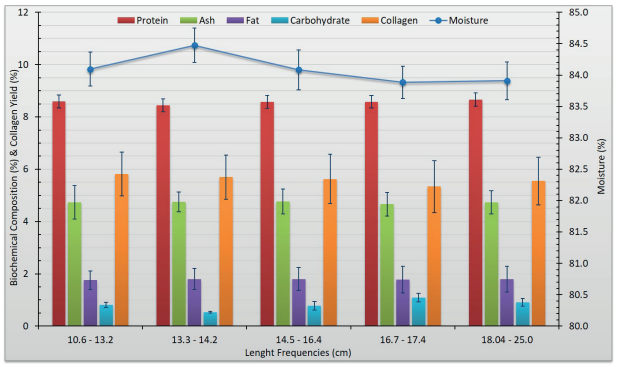

Figure 2

Relationship between *H. tubulosa* collagen yield and length and biochemical composition (mean ± SE).

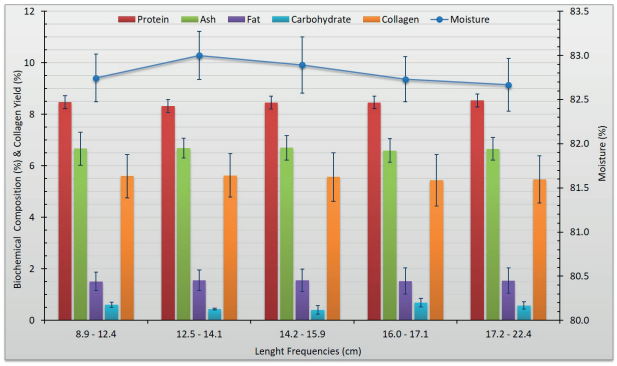

Figure 3

Relationship between *H. poli* collagen yield and length and biochemical composition (mean ± SE).

Table 1

Morphometric data of *H. tubulosa* according to length frequencies (N: 200).

Length Groups (cm)	Length Fq. (%)	Av. Length (cm)	Av. Weight (g)	Av. Gutted Weight (g)	Sex & Ratio (%)		Mean GSI (%)	
					F	M	F	M
10.60 - 13.29	20.00	12.04 ± 1.01^{a}	50.91±5.28^{a}	33.65 ± 0.91^{a}	4	-	3.95 ± 0.09^{a}	-
13.30 - 14.29	20.00	13.83 ± 0.89^{b}	54.73±4.47^{ab}	36.63 ± 0.47^{ab}	6	-	5.52 ± 0.08^{b}	-
14.30 - 16.69	22.00	15.46 ± 1.26^{b}	64.72±5.60^{b}	39.34 ± 0.46^{b}	6	2	5.26 ± 0.01^{b}	2.67 ± 0.02^{a}
16.70 - 18.39	18.00	17.08 ± 1.57^{bc}	80.23±9.28^{c}	45.12 ± 0.67^{c}	4	-	2.77 ± 0.05^{c}	-
18.40 - 25.00	20.00	21.17 ± 2.59^{c}	75.81±7.46^{cb}	45.64 ± 0.01^{c}	10	2	6.55 ± 0.34^{d}	12.84 ± 0.12^{b}

Av: Average, Fq: Frequency, F: female, M: male, GSI: Gonadosomatic Index. Data with different superscripts in the columns indicate significant differences (mean±SE) ($p < 0.05$).

Table 2

Morphometric data of *H. polii* according to length frequencies (N: 200).

Length Groups (cm)	Length Fq. (%)	Av. Length (cm)	Av. Weight (g)	Av. Gutted Weight (g)	Sex & Ratio (%)		Mean GSI (%)	
					F	M	F	M
8.9-12.4	17.00	10.95 ± 0.83^{a}	32.59 ± 5.87^{a}	23.14 ± 0.17^{a}	4	-	5.92 ± 0.05^{a}	-
12.5-14.1	25.00	13.52 ± 0.97^{b}	44.73 ± 4.30^{ab}	29.63 ± 0.25^{b}	2	2	6.91 ± 0.04^{b}	4.89 ± 0.11^{a}
14.2-15.9	30.00	15.10 ± 1.06^{bc}	52.32 ± 6.55^{b}	38.74 ± 0.88^{c}	2	-	6.22 ± 0.03^{ab}	-
16.0-17.1	18.00	16.43 ± 1.22^{cd}	76.44 ± 8.49^{c}	49.52 ± 0.31^{d}	-	2	-	3.47 ± 0.04^{b}
17.2-22.4	10.00	19.12 ± 2.37^{d}	79.65 ± 7.94^{c}	52.78 ± 3.34^{d}	8	2	4.64 ± 0.15^{c}	-

Av: Average, Fq: Frequency, F: female, M: male, GSI: Gonadosomatic Index. Data with different superscripts in the columns indicate significant differences (mean±SE) ($p < 0.05$).

In *H. tubulosa* samples, mean percentage levels of collagen yield for all length frequencies ranged from 5.34 ± 0.98 to 5.81 ± 0.84% of wet weight. The minimum yield was determined from samples belonging to 16.7–17.4 cm length frequency, whereas the maximum yield was obtained from samples belonging to 10.6–13.2 cm length frequency (Fig. 2).

In *H. poli* samples, mean percentage levels of collagen yield for all length frequencies ranged from 5.44 ± 0.15 to 5.63 ± 0.17% of wet weight. The minimum yield was determined from samples belonging to 16.0–17.1 cm length frequency, whereas the maximum yield was obtained from samples belonging to 12.5–14.1 cm length frequency (Fig. 3).

Pearson correlation was applied to the data to compare changes in collagen yield as a function of the major determinants (Fig. 4). To determine the correlation, morphometric data were also used with length and biochemical composition. In both sea cucumber species used in the biochemical composition analysis, a negative non-significant correlation was found between length and collagen yield (r −0.437, r −0.321; $p > 0.05$), whereas the correlation between gutted weight and yield was significantly positive (r 0.069, r 0.239; $p < 0.05$). The correlation between the collagen yield and biochemical constituents was not significant for both sea cucumber species (r −0.18, r 0.76, $p > 0.05$; Fig. 4).

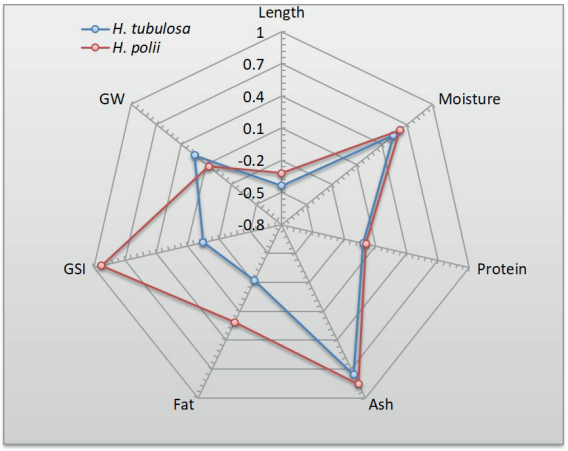

Figure 4

Correlation between collagen yield and major determinants in both sea cucumber species. GW – gutted weight, GSI – gonadosomatic index.

3.2. *Relationship between length, weight, gutted weight, and maturity and collagen yields*

In this study, collagen from mature specimens was isolated separately. Immature specimens with the same length and weight as mature specimens were used as a control group for collagen isolation. The results of collagen yields as per length frequencies along with the comparison of total weight, gutted weight and sexual maturity are summarized for *H. tubulosa* in Fig. 5 and *H. poli* in Fig. 6. Sexual maturity was observed at a minimum length of 12.10–12.30 cm and minimum gutted weight of 41.55 ± 2.59 and 33.16 ± 1.75 g for *H. tubulosa* and *H. poli*, respectively.

Mature *H. tubulosa* specimens with GSIs ranging from 2.77% to 12.4% (M/F ratio of 1/7) were used for collagen isolation. On the basis of length frequencies of mature specimens, the minimum yield was determined as 5.25 ± 0.31 g (10.63% based on gutted weight) in the highest length frequencies. The highest collagen yield, on the other hand, was determined as 6.10 ± 0.18 g (14.68% based on gutted weight) in smaller specimens with the lowest length frequency. Collagen yields of immature *H. tubulosa* specimens of the same length and weight ranged from 6.60 ± 0.27g (16.04%) to 5.69 ± 0.22 g (10.96%), respectively (Fig. 5).

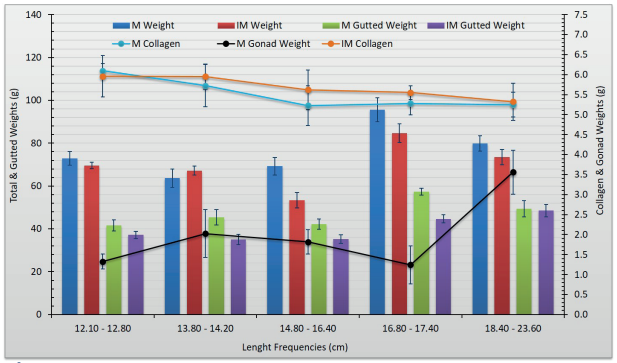

Figure 5

Changes in collagen yield in mature (M) and immature (IM) *H. tubulosa* individuals compared with morphometric parameters (mean weight ± SE).

Mature *H. poli* specimens with GSIs ranging from 4.61% to 6.91% (M/F ratio of 1/4) were used for the collagen isolation. On the basis of length frequencies of mature specimens, the minimum and maximum collagen yields ranged from 5.31 ± 0.04 g (11.90% on the basis of gutted weight) to 5.03 ± 0.11 g (7.12% based on gutted weight) from the smallest to the largest sizes. The collagen yields of immature specimens of the same size ranged from 5.50 ± 0.04 g (13.10%) to 5.09 ± 0.08 g (10.66%), respectively (Fig. 6).

The sex ratio of specimens used for collagen isolation was determined, but differences in the yield between males and females were ignored due to the limited number of male specimens in both species (1/7 M/F of 14 specimens for *H. tubulosa* and 1/4 M/F

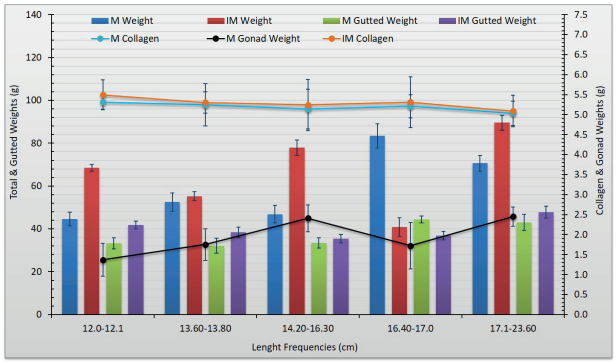

Figure 6

Changes in collagen yield in mature (M) and immature (IM) *H. poli* individuals compared with morphometric parameters (mean weight ± SE).

of 12 specimens for *H. poli*) resulting from the inability to identify the sex until breeding. When considering the GSIs of both species, maturity was found to be an insignificant factor affecting the collagen yield in *H. tubulosa* (r −0.048, $p > 0.05$), while it was an important determinant for *H. poli* (r 0.926, $p < 0.05$). Parallel to this finding, it was found that regardless of the gutted weight, an increase in the number of gonads did not significantly affect the collagen yield of mature specimens ($p > 0.05$). Among mature specimens of both species, *H. tubulosa* produced more collagen than *H. poli* ($p < 0.05$). In both species, however, the collagen yield among immature specimens with the same length and weight was not significantly different ($p > 0.05$).

3.3. Relationship between PSC yield and morphometric and biochemical parameters

The hydrolyzation degrees (HD) of collagens isolated from both species ranged from 14% to 32% (Fig. 7a). The maximum HD, which also gave the highest PSC yield, was determined at 5 and 6% enzyme concentrations. The comparison of PSC yield as a function of length and enzyme concentration was limited in the results to 5% pepsin concentration as no significant increase in the HD was observed after 5% concentration ($p > 0.05$).

It was found that maturity of *H. tubulosa* had a significant positive effect on the PSC yield, compared to immature specimens (r 0576, $p < 0.05$; Fig. 7b). On the other hand, maturity had a negative effect on the PSC yield in *H. poli* (r −0.518, $p > 0.05$). Among biochemical composition variables, protein proved to be a major determinant for the PSC yield in both species (r from 0.768 to 0.904, $p < 0.05$), contrary to the collagen yield (r from −0.02 to 0.0, $p > 0.05$; Fig. 7b).

PSC yields from body walls of *H. tubulosa* and *H. poli* as a function of length frequencies and enzyme concentrations were summarized in Fig. 8. In the lowest length frequency of *H. tubulosa*, the PSC yield ranged from 0.83 ± 0.05 to 1.68 ± 0.11%. In the highest length frequency, the PSC yield ranged from 0.88 ± 0.08 to 1.83 ± 0.12% (Fig. 8a).

In the lowest length frequency of *H. poli*, the PSC yield ranged from 1.02 ± 0.06 to 1.76 ± 0.11%. For the highest length frequency, the PSC yields ranged from 1.1 ± 0.06 to 1.89 ± 0.12% among these length frequencies (Fig. 8b).

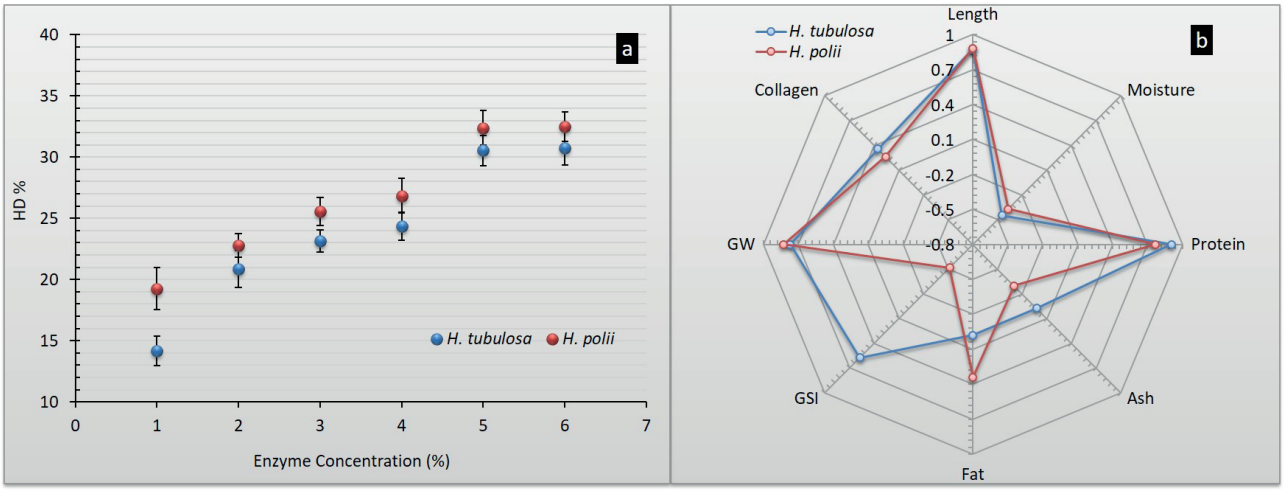

Figure 7

a) Hydrolyzation degrees (HD) of collagens in relation to different enzyme concentrations in *H. tubulosa* and *H. poli* (mean ± SE); b) Pearson correlations between pepsin-solubilized collagen (PSC), morphometric and biochemical parameters as major determinants. GW – gutted weight; GSI – gonadosomatic index.

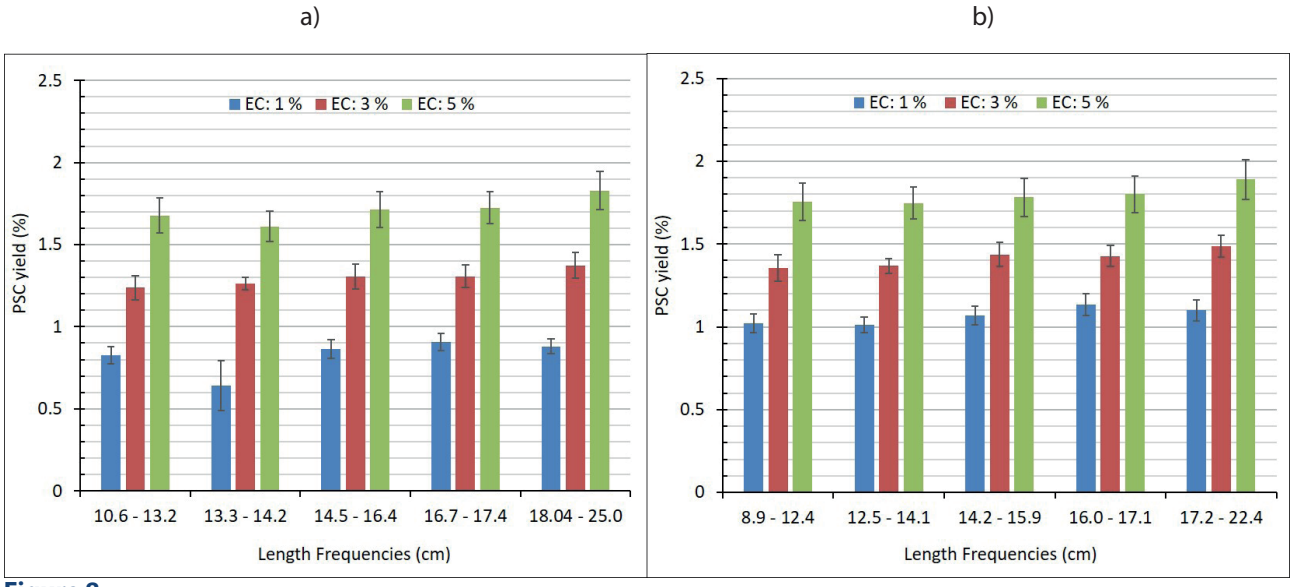

Figure 8

Pepsin-solubilized collagen (PSC) yields in *H. tubulosa* (a) and *H. poli* (b) in relation to different enzyme concentrations (EC) and length frequencies (mean percentage in wet weight of body wall; % ± SD).

3.4. Molecular weight distribution of collagen hydrolysates

SDS-PAGE was applied to verify whether the isolation and hydrolyzation of collagen in both species was successful. The gel image indicating molecular weight distribution of pepsin-solubilized collagen is presented in Fig. 9.

Clear and characteristic bands of sea cucumber pepsin-solubilized collagen, including β, α-1, and α-2, were visualized from the SDS-PAGE gel. Most of the unbroken polypeptides belonging to pepsin-solubilized collagen fibrils were observed above the β chain (≈ 180 kDa) and γ chain residues (> 205 kDa), while characteristic sea cucumber PSC bands of α-1 and α-2 polypeptide chains were determined at ≈ 100–130 kDa. In both species, the density and distribution of protein bands were similar. This can be considered as an indication that the same procedure for both species provided optimum conditions for collagen isolation and hydrolyzation.

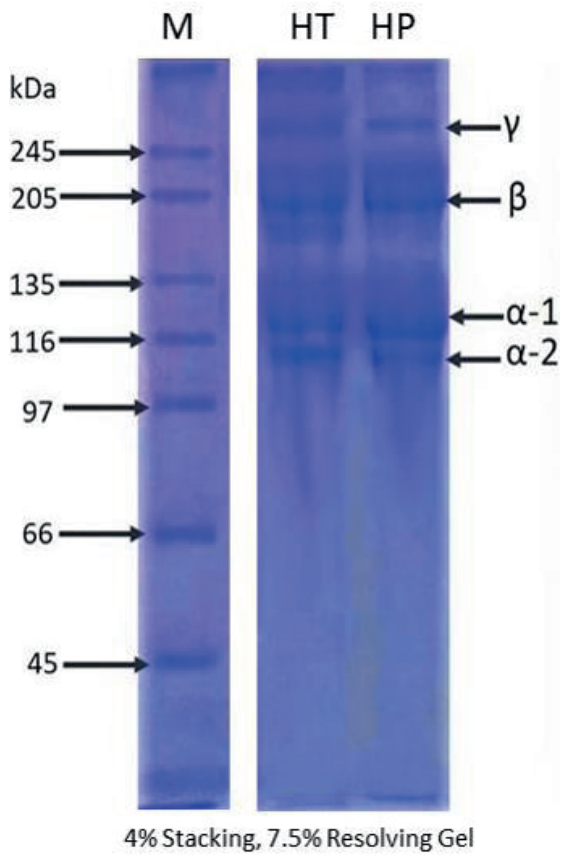

Figure 9

SDS-PAGE pattern of pepsin-solubilized collagen in *H. tubulosa* (HT) and *H. poli* (HP). M – protein marker.

4. Discussion

In this study, collagen and collagen hydrolysate yields – one of the main factors affecting the exploitation of sea cucumber species – were determined in two economic sea cucumber species as a function of their length frequencies. As the first study that reports the relationship between morphometric

parameters and collagen yield of two sea cucumber species, the effects of maturity and biochemical composition on collagen and collagen hydrolysate yields were also investigated. The relationship between length and weight measurements in sea cucumbers was mainly examined after specimens were gutted because of a possible evacuation of their coelomic fluid and internal organs under stress. For this reason, gutted weight (body wall) of sea cucumber is used for a more accurate determination of morphometric data correlations (Conand 1981; Kazanidis et al. 2010; Gonzalez-Wangüemert et al. 2014). In this study, the correlation between the total weight and length was not clear, however, the gutted weight and total length showed a strong positive correlation for both species (r 0.924 and r 0.966, $p < 0.05$). The minimum maturity sizes of both species were 10.6 and 8.9 cm for *H. tubulosa* and *H. poli*, respectively. Non-significant correlations were found between length and maturity for both species (r 0.677 for *H. tubulosa* and r –0.609 for *H. poli*, $p > 0.05$). Previous studies from Turkey reported length distribution in the range of 4.0–30.3 cm for *H. tubulosa* and 3.40–18.0 cm for *H. poli* (Dereli et al. 2016; Gonzalez-Wangüemert et al. 2015; Aydın 2019). The first maturity length of *H. tubulosa* and *H. poli* was reported as 8.1–8.4 and < 13.5 cm, respectively (Dereli et al. 2016; Tamacha et al. 2019). In this study, the median lengths of mature specimens were 15.6 and 13.5 for both species, which are considerably lower than the mean lengths of the total sampled population. Along with the first maturity length, gonadosomatic indices for both species were also at lower levels. However, these lengths and GSIs were still in the same ranges compared to the studies mentioned above.

Biochemical composition, defined as changes in the percentage of moisture, protein, ash, fat, and total carbohydrates in marine organisms, depends and is affected by species differences, feeding regime, geographical location, sex and maturity, seasons, and morphometric characteristics (Colakoglu et al. 2011; Pereira et al. 2014; Khotimchenko 2015; Künili & Colakoglu 2019). In immature specimens of both species, the determined protein amounts and collagen yields were not significantly different across the length frequencies, but moisture and ash showed a strong positive correlation with collagen yields ($p < 0.05$). In general, the content of moisture, protein, and ash was similar to that reported in the range of 81.2–86.0%, 7.41–8.82%, and 5.13–7.04%, respectively, however, the fat content was at higher levels than values reported for different sea cucumber species (Aydın et al. 2011; Omran 2013; Haider et al. 2015; Roggatz et al. 2016). It is believed that the variation in fat levels may be mainly due to regional changes, which may directly affect the fat content and properties of the species (Zhang et al. 2017).

In this study, the length frequency was linked only with biochemical composition, as the sex of both sea cucumber species cannot be identified until they are in the reproduction cycle. The reproduction cycle can vary in holothurians, even within the same species or in individuals of the same length and weight, because the major regulators of maturity are temperature, food availability, and photoperiod (Harriott 1985; Conand 1993; Asha & Muthiah 2008). Nonetheless, reproduction in both species occurs mainly in warmer months, from May to September (Despalotovic et al. 2004; Aydın 2008; Aydın & Erkan 2015; Dereli et al. 2016). In this study, water temperature of sampling areas can reach 25°C during the reproduction period (TSMS 2020), and this may accelerate changes in biochemical and physiological characteristics of the species as a result of increasing food availability and maturity of sea cucumbers (Coulon & Jangoux 1993; Kazanidis 2010; Günay et al. 2015; Künili & Colakoglu 2019). Therefore, the effect of reproduction on collagen yields in both species was determined by performing sampling until sufficient numbers of both immature and mature specimens in different length frequencies were obtained, and biochemical changes along with collagen yields could be compared regardless of temperature (Figs 5, 6, 7b). Although, the amount of proteins in sea cucumbers may not vary significantly between seasons, except in summer (Künili & Colakoglu 2019), the percentage level of predominant and collagen precursor amino acids in the body wall comprised alanine, glycine, glutamic acid, proline, and hydroxyproline at varying levels (Ciu et al. 2007; Zhong et al. 2007; Gao et al. 2011; Liu et al. 2010; Liu et al. 2011; Bechtel et al. 2012; Sicuro et al. 2012; Omran 2013; Wang et al. 2013; Haider et al. 2015; Zhong et al. 2015; Widianingsih et al. 2016). This may also change during reproduction due to the possible demand of the organism for these amino acids. Thus, the change in the ratio of amino acids to total protein may also lead to changes in collagen synthesis and collagen yield.

In this study, we found that maturity was not a non-significant factor affecting collagen yield in *H. tubulosa* (r –0.048, $p > 0.05$), whereas it was an important determinant for *H. poli* (r 0.926, $p < 0.05$). Comparing the yields among mature specimens in both species, *H. tubulosa* had higher collagen yield than *H. poli* ($p < 0.05$). The main reasons for the differences in collagen yield between the two species during the maturation period may be related to

chemical and physiological changes depending on species-specific characteristics, as many results from previous studies indicate that significant changes may occur in the reproduction cycle of different species of echinoderms (Olive 1995; Singh et al. 2001; Muthiga 2006; Mercier & Hamel 2009; Doyle et al. 2012; Kazanidis et al. 2014). In sea cucumbers, the percentage of collagen can be more than 70% of the total proteins in the body wall (Saito et al. 2002; Chen 2003; Li et al. 2020). Therefore, in addition to maturity, the early stage of the life cycle and the feeding regime may also be the main factors that affect the amount and structural properties of the collagen in the body of sea cucumbers (Miller et al. 1983; Silva et al. 2014).

Hydrolyzation of collagen by pepsin, also known as pepsin-solubilized collagen (PSC), shows the potential of collagen to be used to some extent in the body and in industries such as food, cosmetics, and pharmacology. For this reason, as the maximum utilization can vary depending on length as well as amounts of protein and collagen, PSC yield was determined in both species according to these parameters. In addition, the effect of enzyme concentration (EC), which is also an important determinant that directly affects the yield of collagen hydrolysates, was also determined for all length frequencies. The EC may be effective until the hydrolysis reaction is saturated, which means the degree of hydrolyzation may not change significantly after the optimum enzyme concentration is reached. The ECs chosen in the study (1–3–5%) were determined from pilot experiments that indicated the hydrolysis process did not change significantly after reaching a 5% (maximum concentration specified in this study) concentration (Fig. 7a). The pepsin-solubilized collagen has been the subject of many studies due to its potential use based on increased solubility and elevated purity of collagen without losing bioactivity and functionality (Liu et al. 2010; Park et al. 2012; Lin et al. 2015). Sea cucumbers have also been the subject of research in the last decade and observations have been made about the superior properties of collagen hydrolysates (Liu et al. 2011; Park et al. 2012; Zhou et al. 2012; Jin et al. 2019; Li et al. 2020). To the best of our knowledge and the available literature, a number of sea cucumber species have been studied to determine characteristics of collagens and hydrolysates. However, collagen and hydrolysates from *H. tubulosa* and *H. poli* along with the comparison by length, weight, maturity, and biochemical composition have not been the subject of existing studies. In this study, the maximum PSC yield was determined at 5% EC in both species. The PSC yields for *H. tubulosa* were determined as 21.09% of the total protein content (dry weight), 32.95% of the total collagen content (dry weight), and 1.83% of the total body wall (wet weight). The PSC yields for *H. poli* were determined as 34.52% of the total collagen content (dry weight), 22.12% of the total protein content (dry weight), and 1.89% of the total body wall (wet weight). If we consider that moisture levels in both species reach up to 84% (Figs 2 & 3), PSC yields were found to be similar to those reported as 20.8–24.3% (per dry weight of body wall) for *Parastichopus californicus* (Liu et al. 2010) and 26.6% (dry weight of body wall) for *Stichopus japonicus* (Park et al. 2012). However, the PSC yields of both species in this study were significantly lower than the yield reported by Lin et al. (2015) for *Acaudina leucoproocta* as 8.35% (wet weight of body wall). The results obtained in this study for PSC yields in both species were within the range of reported values for some other marine invertebrates. PSC yields on a dry weight basis were reported in the range of 12.3–66% for squids and jellyfishes (Kołodziejska et al. 1999; Barzideh et al. 2013; Khong et al. 2016) and 7–35% for sea urchins (Nagai & Suzuki 2000; Benedetto et al. 2014). However, the determined PSC yields were considerably higher than the levels reported for starfishes as 5.8% for *Asterias amurensis* (Lee et al. 2009) and 6.1% for *Asterina pectinifera* (Qi et al. 2017). Differences in collagen and PSC yields in sea cucumber species as well as other invertebrates are attributed primarily to species differences and protocols or handling procedures used during isolation and hydrolyzation of collagen and its hydrolysates. Although these factors can significantly affect the yield, the characteristics of molecular weight patterns of PSCs remain mostly similar. For this reason, as a final product of collagen hydrolyzation, pepsin-solubilized collagen from both species was subjected to SDS-Page analysis to control the isolation and hydrolysis procedures. In this study, PSC bands comprising β, α-1, and α-2 were visualized from SDS-Page gel. The bands of both sea cucumber species were similar to those reported in previous studies (Saito et al. 2002; Cui et al. 2007; Liu et al. 2010; Liu et al. 2011; Adibzadeh et al. 2014; Liu et al. 2019; Zhong et al. 2015; Li et al. 2020).

5. Conclusion

In this study, the yield of collagen, one of the most important biochemical components of sea cucumbers, was found to be affected by changes in length, weight, and maturity. Length was found to be the most important factor affecting the collagen yields in both species. Moreover, the degree of hydrolyzation in both species, which indicates considerable utilization of

collagen, was also found to be affected by length and maturity. These relationships were also found to be significantly affected by species differences.

In conclusion, collagen and hydrolysate yields from economically important and exploited sea cucumber species in Turkey and the Mediterranean showed significant differences depending on maturity, length, weight, and biochemical composition. It is believed that these results can guide future research and sectors to ensure optimum utilization of these species.

Acknowledgements

This work was supported by the Scientific Research Fund of Canakkale Onsekiz Mart University under Grant Project No. FBA-2019-2977.

References

Adibzadeh, N., Aminzadeh, S., Jamili, S., Karkhane, A. A., & Farrokhi, N. (2014). Purification and characterization of pepsin-solubilized collagen from skin of sea cucumber *Holothuria parva*. *Applied Biochemistry and Biotechnology, 173*, 143–154. https://doi.org/10.1007/s12010-014-0823-4 PMID:24676570

Antoniadou, C., & Vafidis, D. (2011). Population structure of the traditionally exploited holothurian *Holothuria tubulosa* in the south Aegean Sea. *Cahiers de Biologie Marine, 52*, 171–175.

AOAC. (2000). Official Methods of Analysis of the AOAC International (17th). Association of Official Analytical Chemists.

Asha, P. S., & Muthiah, P. (2008). Reproductive biology of the commercial sea cucumber *Holothuria spinifera* (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. *Aquaculture International, 16*, 231–242. https://doi.org/10.1007/s10499-007-9140-z

Aydın, M. (2008). The commercial sea cucumbers fishery in Turkey. *SPC Beche-de-mer Information Bulletin, 28*, 40–43.

Aydın, M., Sevgili, H., Tufan, B., Emre, Y., & Köse, S. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science & Technology, 46*, 500–508. https://doi.org/10.1111/j.1365-2621.2010.02512.x

Aydın, M., & Erkan, S. (2015). Identification and some biological characteristics of commercial sea cucumber in the Turkey coast waters. *International Journal of Fisheries and Aquatic Studies*, 3, 260–265.

Aydın, M. (2019). Biometry, density and the biomass of the commercial sea cucumber population of the Aegean Sea. *Turkish Journal of Fisheries and Aquatic Sciences, 19*(6), 463–474. https://doi.org/10.4194/1303-2712-v19_6_02

Aydın, M. (2020). Length-Weight Relationships and Condition Factor of Four Different Sea Cucumber Species in the Aegean Sea. *Journal of Anatolian Environmental and Animal Sciences, 5*, 80–85. https://doi.org/10.35229/jaes.677940

Barzideh, Z., Latiff, A. A., Gan, C.-Y., Benjakul, S., & Karim, A. A. (2014). Isolation and characterisation of collagen from the ribbon jellyfish (*Chrysaora* sp.). *International Journal of Food Science & Technology, 49*, 1490–1499. https://doi.org/10.1111/ijfs.12464

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917. https://doi.org/10.1139/y59-099 PMID:13671378

Bechtel, P. J., Oliveira, A. C. M., Demir, N., & Smiley, S. (2013). Chemical composition of the giant red sea cucumber, *Parastichopus californicus*, commercially harvested in Alaska. *Food Science & Nutrition, 1*, 63–73. https://doi.org/10.1002/fsn3.12 PMID:24804015

Benedetto, C. D., Barbaglio, A., Martinello, T., Alongi, V., Fassini, D., Cullorà, E., Patruno, M., Bonasoro, F., Barbosa, M. A., Carnevali, M. D., & Sugni, M. (2014). Production, characterization and biocompatibility of marine collagen matrices from an alternative and sustainable source: The sea urchin *Paracentrotus lividus*. *Marine Drugs, 12*(9), 4912–4933. https://doi.org/10.3390/md12094912 PMID:25255130

Bordbar, S., Anwar, F., & Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods—A review. *Marine Drugs, 9*, 1761–1805. https://doi.org/10.3390/md9101761 PMID:22072996

Borrero-Pérez, G. H., González-Wangüemert, M., Marcos, C., & Pérez-Ruzafa, A. (2011). Phylogeography of the Atlanto-Mediterranean sea cucumber Holothuria (Holothuria) mammata: The combined effects of historical processes and current oceanographical pattern. *Molecular Ecology, 20*, 1964–1975. https://doi.org/10.1111/j.1365-294X.2011.05068.x PMID:21463375

Chen, J. (2003). Overview of sea cucumber farming and sea ranching practices in China. *SPC Bech de mer Information Bulletin*. 18:24-33.

Arik Colakoglu, F., Ormanci, H. B., Berik, N., Künili, I. E., & Colakoglu, S. (2011). Proximate and elemental composition of *Chamelea gallina* from the southern coast of the Marmara Sea (Turkey). *Biological Trace Element Research, 143*, 983–991. https://doi.org/10.1007/s12011-010-8943-3 PMID:21225478

Conand, C. (1981). Sexual cycle of three commercially important Holothurian species (Echinodermata) from the lagoon of New Caledonia. *Bulletin of Marine Science, 31*, 523–543.

Conand, C. (1993). Reproductive biology of the holothurians from the major communities of the New Caledonian Lagoon. *Marine Biology, 116*, 439–450. https://doi.

org/10.1007/BF00350061

Coppola, D., Oliviero, M., Vitale, G. A., Lauritano, C., D'Ambra, I., Iannace, S., & de Pascale, D. (2020). Marine Collagen from Alternative and Sustainable Sources: Extraction, Processing and Applications. *Marine Drugs, 18*, 214. https://doi.org/10.3390/md18040214 PMID:32326635

Coulon, P., & Jangoux, M. (1993). Feeding rate and sediment reworking by the holothuroid *Holothuria tubulosa* (Echinodermata) in a Mediterranean seagrass bed off Ischia Island, Italy. *Marine Ecology Progress Series, 92*, 201–204. https://doi.org/10.3354/meps092201

Cui, F., Xue, C., Li, Z., Zhang, Y., Dong, P., Fu, X., & Gao, X. (2007). Characterization and subunit composition of collagen from the body wall of sea cucumber *Stichopus japonicus. Food Chemistry, 100*, 1120–1125. https://doi.org/10.1016/j.foodchem.2005.11.019

Çakli, S., Cadun, A., Kisla, D., & Dincer, T. (2004). Determination of quality characteristics of *Holothuria tubulosa,* (Gmelin, 1788) in Turkish sea (Aegean Region) depending on sun drying process step used in Turkey. *Journal of Aquatic Food Product Technology, 13*, 69–78. https://doi.org/10.1300/J030v13n03_07

Dereli, H., Türk Çulha, S. T., Çulha, M., Özalp, B. H., & Tekinay, A. (2016). Reproduction and population structure of the sea cucumber *Holothuria tubulosa* in the Dardanelles Strait, Turkey. *Mediterranean Marine Science, 17*, 47–55. https://doi.org/10.12681/mms.1360

Despalatović, M., Grubelić, I., šimunović, A., Antolić, B., & Žuljević, A (2004). Reproductive biology of the holothurian *Holothuria tubulosa* (Echinodermata) in the Adriatic Sea. *J. Mar. Biolog. Assoc.UK*. 84:409–414. https://doi.org/10.1017/S0025315404009361h.

Doyle, G. M., Hamel, J. F., & Mercier, A. (2012). A new quantitative analysis of ovarian development in echinoderms: The maturity stage index. *Marine Biology, 159*, 455–465. https://doi.org/10.1007/s00227-011-1823-3

FAO. (2003). Analytical Methods for Carbohydrates (Chapter 2.3) in Food energy – methods of analysis and conversion factors. FAO Food and Nutrition Paper 77, Report of a Technical Workshop, Rome, Italy.

FAO. (2020). *Food and Agriculture Organization of the United Nations, Fisheries & Aquaculture Statistics, Global Production Statistics (online query)*. Retrieved, December 15, 2020, from http://www.fao.org/fishery/statistics/global-production/query/en

Ferdouse, F. (1999). Beche-de-mer markets and utilization. *Beche-de-mer Information Bulletin, 11*, 3–8.

Ferdouse, F. (2004). World markets and trade flows of sea cucumber/beche-de-mer. In: A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J.F. Hamel & A. Mercier (Eds.), Advances in Sea Cucumber Aquaculture and Management (pp. 101–118). Rome, Italy: FAO.

Gao, F., Xu, Q., & Yang, H. (2011). Seasonal biochemical changes in composition of body Wall tissues of sea cucumber *Apostichopus japonicus. Chinese Journal of Oceanology and Limnology, 29*, 252–260. https://doi.org/10.1007/s00343-011-0041-7

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids, 25*(8), 1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

González-Wangüemert, M., Aydin, M., & Conand, C. (2014). Assessment of sea cucumber populations from the Aegean Sea (Turkey): First insights to sustainable management of new fisheries. *Ocean and Coastal Management, 92*, 87–94. https://doi.org/10.1016/j.ocecoaman.2014.02.014

González-Wangüemert, M., Valente, S., & Aydin, M. (2015). Effects of fishery protection on biometry and genetic structure of two target sea cucumber species from the Mediterranean Sea. *Hydrobiologia, 743*, 65–74. https://doi.org/10.1007/s10750-014-2006-2

González-Wangüemert, M., Valente, S., Henriques, F., Domínguez-Godino, J. A., & Serrão, E. A. (2016). Setting preliminary biometric baselines for new target sea cucumbers species of the NE Atlantic and Mediterranean fisheries. *Fisheries Research, 179*, 57–66. https://doi.org/10.1016/j.fishres.2016.02.008

González-Wangüemert, M., Domínguez-Godino, J. A., & Cánovas, F. (2018). The fast development of sea cucumber fisheries in the Mediterranean and NE Atlantic waters: From a new marine resource to its over-exploitation. *Ocean and Coastal Management, 151*, 165–177. https://doi.org/10.1016/j.ocecoaman.2017.10.002

Günay, D., Emiroğlu, D., Tolon, T., Özden, O., & Saygi, H. (2015). Growth and survival rate of juvenile sea cucumbers (*Holothuria tubulosa*, Gmelin, 1788) at Various Temperatures. *Turkish Journal of Fisheries and Aquatic Sciences, 15*, 533–541. https://doi.org/10.4194/1303-2712-v15_2_41

Haider, M. S., Sultana, R., Jamil, K., Zehra, L., Tarar, O. M., Shirin, K., & Afzal, W. (2015). A study on proximate composition, amino acid profile, fatty acid profile and some mineral contents in two species of sea cucumber. *The Journal of Animal and Plant Sciences, 25*, 168–169.

Harriott, V. J. (1985). Reproductive biology of three congeneric sea cucumber species, *Holothuria atra, Holothuria impatiens* and *Holothuria edulis,* at Heron Reef, Great Barrier Reef. *Marine and Freshwater Research, 36*, 51–57. https://doi.org/10.1071/MF9850051

Jin, H. X., Xu, H. P., Li, Y., Zhang, Q. W., & Xie, H. (2019). Preparation and evaluation of peptides with potential antioxidant activity by microwave assisted enzymatic hydrolysis of collagen from sea cucumber *Acaudina molpadioides* obtained from Zhejiang province in China. *Marine Drugs, 17*, 169. https://doi.org/10.3390/md17030169 PMID:30875949

Kazanidis, G., Antoniadou, C., Lolas, A.P., Neofitou, N., Vafidis,

D., Chintiroglou, C.C. & Neofitou, C. (2010). Population dynamics and reproduction of *Holothuria tubulosa* (Holothuroidea: Echinodermata) in the Aegean Sea. *J. Mar. Biolog. Assoc.UK*. 90: 895-901. https://doi.org/10.1017/S0025315410000251

Kazanidis, G., Lolas, A., & Vafidis, D. (2014). Reproductive cycle of the traditionally exploited sea cucumber *Holothuria tubulosa* (Holothuroidea: Aspidochirotida) in Pagasitikos Gulf, western Aegean Sea, Greece. *Turkish Journal of Zoology, 38*, 306–315. https://doi.org/10.3906/zoo-1302-31

Künili, I. E., & Colakoglu, F. A. (2018). Antioxidant and Antimicrobial Activity of Sea Cucumber (*Holothuria tubulosa*, Gmelin 1791) Extracts. Canakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries, *1*(2), 66–71.

Künili, I. E., & Colakoglu, F. A. (2019). Chemical and Nutritional Characteristics of *Holothuria tubulosa* (Gmelin, 1788); A Seasonally Comparative Study. *Journal of Aquatic Food Product Technology, 28*, 716–728. https://doi.org/10.1080/10498850.2019.1637383

Khong, N. M. H., Yusoff, F. M., Jamilah, B., Basri, M., Maznah, I., Chan, K. W., & Nishikawa, J. (2016). Nutritional composition and total collagen content of three commercially important edible jellyfish. *Food Chemistry, 196*, 953–960. https://doi.org/10.1016/j.foodchem.2015.09.094 PMID:26593577

Khotimchenko, Y. S. (2015). The nutritional value of Holothurians. *Russian Journal of Marine Biology, 41*, 409–423. https://doi.org/10.1134/S1063074015060061

Kołodziejska, I., Sikorski, Z. E., & Niecikowska, C. (1999). Parameters affecting the isolation of collagen from squid (*Illex argentinus*) skins. *Food Chemistry, 66*(2), 153–157. https://doi.org/10.1016/S0308-8146(98)00251-9

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685. https://doi.org/10.1038/227680a0 PMID:5432063

Lee, K. J., Park, H. Y., Kim, Y. K., Park, J. I., & Yoon, H. D. (2009). Biochemical Characterization of Collagen from the Starfish *Asterias amurensis*. *Journal of the Korean Society for Applied Biological Chemistry, 52*(3), 221–226. https://doi.org/10.3839/jksabc.2009.040

Li, P.-H., Lu, W.-C., Chan, Y.-J., Ko, W.-C., Jung, C.-C., Le Huynh, D. T., & Ji, Y.-X. (2020). Extraction and characterization of collagen from sea cucumber (*Holothuria cinarascens*) and its potential application in moisturizing cosmetics. *Aquaculture (Amsterdam, Netherlands), 515*, 734590. https://doi.org/10.1016/j.aquaculture.2019.734590

Lin, S., Xue, Y. P., San, E., Keong, T. C., Chen, L., & Zheng, Y. G. (2017). Extraction and Characterization of Pepsin Soluble Collagen from the Body Wall of Sea Cucumber *Acaudina leucopocta*. *Journal of Aquatic Food Product Technology, 26*, 502–515. https://doi.org/10.1080/10498850.2016.1222560

Liu, Z., Oliveira, A. C., & Su, Y. C. (2010). Purification and characterization of pepsin-solubilized collagen from skin and connective tissue of giant red sea cucumber (*Parastichopus californicus*). *Journal of Agricultural and Food Chemistry, 58*, 1270–1274. https://doi.org/10.1021/jf9032415 PMID:20085374

Liu, Z., Su, Y., & Zeng, M. (2011). Amino Acid Composition and Functional Properties of Giant Red Sea Cucumber (*Parastichopus californicus*) Collagen Hydrolysates. *Journal of Ocean University of China, 10*, 80–84. https://doi.org/10.1007/s11802-011-1787-4

Liu, Y. X., Liu, Z. Q., Song, L., Ma, Q. R., Zhou, D. Y., Zhu, B. W., & Shahidi, F. (2019). Effects of collagenase type I on the structural features of collagen fibres from sea cucumber (*Stichopus japonicus*) body wall. *Food Chemistry, 301*, 125302. https://doi.org/10.1016/j.foodchem.2019.125302 PMID:31387034

Mercier, A., & Hamel, J. F. (2009). Endogenous and exogenous control of gametogenesis and spawning in echinoderms. *Advances in Marine Biology, 55*, xi–xii, 1–291. https://doi.org/10.1016/S0065-2881(09)55008-0 PMID:19595321

Mezali, K., Zupo, V., & Francour, P. (2006). Population dynamics of *Holothuria (Holothuria) tubulosa* and *Holothuria (Lessonothuria) polii* of an Algerian *Posidonia oceanica* meadow. *Biologia Marina Mediterranea, 13*, 158–161.

Mezali, K., & Thandar, A. S. (2014). First record of *Holothuria (Roweothuria) arguinensis* (Echinodermata: Holothuroidea: Aspidochirotida: Holothuriidae) from the Algerian coastal waters. *Marine Biodiversity Records, 7*, E40(1-4). https://doi.org/10.1017/S1755267214000438

Miller, A. T., Karmas, E., & Lu, M. F. (1983). Age-related-changes in the collagen of bovine corium studies on extractability, solubility and molecular-size distribution. *Journal of Food Science, 48*, 681–685. https://doi.org/10.1111/j.1365-2621.1983.tb14875.x

Muthiga, N. A. (2006). The reproductive biology of a new species of sea cucumber, *Holothuria* (*Mertensiothuria*) *arenacava* in a Kenyan marine protected area: The possible role of light and temperature on gametogenesis and spawning. *Marine Biology, 149*, 585–593. https://doi.org/10.1007/s00227-005-0224-x

Nagai, T., & Suzuki, N. (2000). Partial characterization of collagen from purple sea urchin (*Anthocidaris crassispina*) test. *International Journal of Food Science & Technology, 35*, 497–501. https://doi.org/10.1046/j.1365-2621.2000.00406.x

Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved Method for Determining Food Protein Degree of Hydrolysis. *Journal of Food Science, 66*, 642–646. https://doi.org/10.1111/j.1365-2621.2001.tb04614.x

Olive, P. J. W. (1995). Annual breeding cycles in marine invertebrates and environmental temperature: Probing the proximate and ultimate causes of reproductive synchrony. *Journal of Thermal Biology, 20*, 79–90. https://doi.org/10.1016/0306-4565(94)00030-M

Nahla, E. S. E. S. O. (2013). Nutritional value of some Egyptian sea cucumbers. *African Journal of Biotechnology, 12*, 5466–5472. https://doi.org/10.5897/AJB2013.13020

Park, S. Y., Lim, H. K., Lee, S., Hwang, H. C., Cho, S. K., & Cho, M. (2012). Pepsin-solubilised collagen (PSC) from Red Sea cucumber (*Stichopus japonicus*) regulates cell cycle and the fibronectin synthesis in HaCaT cell migration. *Food Chemistry, 132*, 487–492. https://doi.org/10.1016/j.foodchem.2011.11.032 PMID:26434320

Pereira, D. M., Andrade, P. B., Pires, R. A., & Reis, L. E. (2014). Chemical ecology of echinoderms: impact of environment and diet in metabolomic profile. In E. Whitmore (Ed.), Echinoderms (pp. 57–76). Nova Publishers.

Purcell, S. W. (2010). Managing sea cucumber fisheries with an ecosystem approach. A. Lovatelli, M. Vasconcellos, & Y. Yimin (Eds), FAO Fisheries and Aquaculture Technical Paper No: 520. Rome: FAO.

Purcell, S. W. (2014). Value, market preferences and trade of Beche-de-mer from Pacific Island sea cucumbers. *PLoS One, 9*(4), e95075. https://doi.org/10.1371/journal.pone.0095075 PMID:24736374

Purcell, S. W., Mercier, A., Conand, C., Hamel, J. F., Toral-Granda, M. V., Lovatelli, A., & Uthicke, S. (2013). Sea cucumber fisheries: Global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries, 14*, 34–59. https://doi.org/10.1111/j.1467-2979.2011.00443.x

Qi, H., Li, N., Zhao, X., Xu, Z., & Qi, L. (2017). Physicochemical Properties and Radical Scavenging Capacities of Pepsin-Solubilized Collagen from the Body Wall of Starfish (*Asterina pectinifera*). *Journal of Aquatic Food Product Technology, 26*(4), 376–389. https://doi.org/10.1080/10498850.2016.1186768

Ramón, M., Lleonart, J., & Massutí, E. (2010). Royal cucumber (*Stichopus regalis*) in the north western Mediterranean: Distribution pattern and fishery. *Fisheries Research, 105*, 21–27. https://doi.org/10.1016/j.fishres.2010.02.006

Regenstein, J. M., & Zhou, P. (2007). Collagen and gelatin from marine by-products. In F. Shahidi (Ed.), Maximising the Value of Marine By-Products (pp. 279–303). Woodhead Publishing. https://doi.org/10.1533/9781845692087.2.279

Roggatz, C. C., González-Wangüemert, M., Pereira, H., Rodrigues, M. J., da Silva, M. M., Barreira, L., Varela, J., & Custódio, L. (2016). First report of the nutritional profile and antioxidant potential of *Holothuria arguinensis*, a new resource for aquaculture in Europe. *Natural Product Research, 30*, 2034–2040. https://doi.org/10.1080/14786419.2015.1107555 PMID:26551335

Santos, R., Haesaerts, D., Jangoux, M., & Flammang, P. (2005). The tube feet of sea urchins and sea stars contain functionally different mutable collagenous tissues. *The Journal of Experimental Biology, 208*, 2277–2288. https://doi.org/10.1242/jeb.01641 PMID:15939770

Saito, M., Kunisaki, N., Urano, N., & Kimura, S. (2002). Collagen as the major edible component of sea cucumber (*Stichopus japonicus*). *Journal of Food Science, 67*, 1319–1322. https://doi.org/10.1111/j.1365-2621.2002.tb10281.x

Senadheera, T. R. L., Dave, D., & Shahidi, F. (2020). Sea Cucumber Derived Type I Collagen: A Comprehensive Review. *Marine Drugs, 18*, 471. https://doi.org/10.3390/md18090471 PMID:32961970

Shi, S., Feng, W., Hu, S., Liang, S., An, N., & Mao, Y. (2016). Bioactive compounds of sea cucumbers and their therapeutic effects. *Chinese Journal of Oceanology and Limnology, 34*, 549–558. https://doi.org/10.1007/s00343-016-4334-8

Sicuro, B., & Levine, J. (2011). Sea Cucumber in the Mediterranean: A Potential Species for Aquaculture in the Mediterranean. *Reviews in Fisheries Science, 19*, 299–304. https://doi.org/10.1080/10641262.2011.598249

Sicuro, B., Piccinno, M., Gai, F., Abete, M. C., Danieli, A., Dapra, F., Mioletti, S., & Vilella, S. (2012). Food quality and safety of Mediterranean sea cucumbers *Holothuria tubulosa* and *Holothuria polii* in Southern Adriatic Sea. *Asian Journal of Animal and Veterinary Advances, 7*, 851–859. https://doi.org/10.3923/ajava.2012.851.859

Silva, T. H., Moreira-Silva, J., Marques, A. L. P., Domingues, A., Bayon, Y., & Reis, R. L. (2014). Marine origin collagens and its potential applications. *Marine Drugs, 12*, 5881–5901. https://doi.org/10.3390/md12125881 PMID:25490254

Singh, R., MacDonald, B. A., Lawton, P., & Thomas, M. L. H. (2001). The reproductive biology of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) using new quantitative methods. *Invertebrate Reproduction & Development, 40*, 125–141. https://doi.org/10.1080/07924259.2001.9652713

Tamacha, F. S., Soualili, D. L., & Mezali, K. (2019). Reproductive biology of *Holothuria (Roweothuria) poli* (Holothuroidea: Echinodermata) from Oran Bay, Algeria. *SPC Beche-de-mer Information Bulletin, 39*, 47–53.

Tolon, T. M., & Engin, S. (2019). Gonadal development of the holothurian Holothuria polii (Delle Chiaje, 1823) in spawning period at the Aegean Sea (Mediterranean Sea). *Su Ürünleri Dergisi, 36*(4), 379–385. https://doi.org/10.12714/egejfas.36.4.09

TSMS. (2020). *Turkish State Meteorological Service, Seawater Temperature Statistics of the Marmara Sea (1970-2020)*. Retrieved February 10, 2021, from https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=K

Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J., & Wu, G. (2013). Glycine metabolism in animals and humans: Implications for nutrition and health. *Amino Acids, 45*, 463–477. https://doi.org/10.1007/s00726-013-1493-1 PMID:23615880

Widianingsih, Z., Zaenuri, M., Anggoro, S., & Kusumaningrum, H. P. S. (2016). Nutritional value of sea cucumber [*Paracaudina australis* (Semper, 1868)]. *Aquatic Procedia, 7*, 271–276. https://doi.org/10.1016/j.aqpro.2016.07.038

Wilkie, I. (2005). Mutable Collagenous Tissue: Overview and Biotechnological Perspective. In V. Matranga (Ed.),

Echinodermata (pp. 221–250). Springer. https://doi.org/10.1007/3-540-27683-1_10

Valente, S., Serrão, E. A., & González-Wangüemert, M. (2015). West versus East Mediterranean Sea: Origin and genetic differentiation of the sea cucumber *Holothuria polii*. *Marine Ecology (Berlin), 36*, 485–495. https://doi.org/10.1111/maec.12156

Zar, J. H. (1996). Biostatistical Analysis. Prentice Hall.

Zhang, X., Liu, Y., Li, Y., & Zhao, X. (2017). Identification of the geographical origins of sea cucumber (*Apostichopus japonicus*) in northern China by using stable isotope ratios and fatty acid profiles. *Food Chemistry, 218*, 269–276. https://doi.org/10.1016/j.foodchem.2016.08.083 PMID:27719909

Zhong, Y., Khan, M. A., & Shahidi, F. (2007). Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (*Cucumaria frondosa*). *Journal of Agricultural and Food Chemistry, 55*, 1188–1192. https://doi.org/10.1021/jf063085h PMID:17243707

Zhong, M., Chen, T., Hu, C., & Ren, C. (2015). Isolation and characterization of collagen from the body wall of sea cucumber *Stichopus monotuberculatus*. *Journal of Food Science, 80*, C671–C679. https://doi.org/10.1111/1750-3841.12826 PMID:25810305

Zhou, X. Q., Wang, C. H., & Jiang, A. L. (2012). In vitro antitumor activities of low molecular sea cucumber Stichopus japonicus peptides sequentially hydrolyzed by proteases. *Advanced Materials Research, 393-395*, 1259–1262.

Zhu, B. W., Dong, X. P., Zhou, D. Y., Gao, Y., Yang, J. F., Li, D. M., Zhao, X. K., Ren, T. T., Ye, W. X., Tan, H., Wu, H. T., & Yu, C. (2012). Physicochemical properties and radical scavenging capacities of pepsin-solubilized collagen from sea cucumber *Stichopus japonicus*. *Food Hydrocolloids, 28*, 182–188. https://doi.org/10.1016/j.foodhyd.2011.12.010