COMU J Mar Sci Fish 2023 6(2): 166-177
https://doi.org/10.46384/jmsf.1387324

e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Extraction and Characterization of Acid Soluble Collagen From Golden Grey Mullet (*Chelon auratus*) Scale

Cansu Metin Hacisa*, Taçnur Baygar

Muğla Sıtkı Koçman University, Faculty of Fisheries, Department of Seafood Processing Technology, 48000 Muğla, Türkiye https://orcid.org/0000-0002-2290-1489
Muğla Sıtkı Koçman University, Faculty of Fisheries, Department of Seafood Processing Technology, 48000 Muğla, Türkiye https://orcid.org/0000-0001-8070-0653

Received: 07.11.2023 /Accepted: 21.12.2023 / Published online: 27.12.2023

Key words:

Bioactive substances
Acid soluable collagen
Fish scale
Golden grey mullet
Collagen characterization

Abstract: In the present study, the potential of waste fish scales for obtaining a valuable bioactive material was evaluated. Acid soluble collagen from golden grey mullet (*Chelon auratus*) scales was isolated and characterized successfully. Proximate composition, denaturation temperature, Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE), amino acid composition, Scanning electron microscopy and Energy dispersive X-ray spectroscopy (SEM-EDS), Fourier transform infrared spectrophotometer (FTIR), Thermogravimetric analysis (TGA), antioxidant and antimicrobial activity analyses were performed for the extraction of collagen and extracted collagen was compared with commercial collagen. As a result, an alternative and useful source for mammalian collagen for the industrial applications like food and cosmetics was extracted from the fish scale waste material, which may help mitigate the management of natural wastes or environmental problems.

Anahtar kelimeler:

Biyoaktif madde
Asitte çözünür kollajen
Balık pulu
Altınbaş kefal
Kollajen karakterizasyonu

Altınbaş Kefal (*Chelon auratus*) Pullarından Asitte Çözünür Kollajen Ekstraksiyonu ve Karakterizasyonu

Öz: Bu çalışmada değerli bir biyoaktif materyal elde etmek amacıyla atık balık pullarının potensiyali incelenmiştir. Altınbaş kefal (*Chelon auratus*) pullarından asitte çözünür kollajen izole edilerek karakterize edilmiştir. Ekstrakte edilen kollajenin besin kompozisyonu, denatürasyon sıcaklığı, Sodyum dodesil sülfat jel elektroforezi (SDS-PAGE), amino asit bileşimi, Taramalı elektron mikroskobu ve Enerji dağılımlı X-ışınları spektrometre (SEM-EDS), Fourier dönüşümü kızılötesi spektrofotometre (FTIR), Termogravimetrik analiz (TGA), antioksidan ve antimikrobiyal aktivite analizleri yapılarak ticari kollajenle karşılaştırılmıştır. Sonuç olarak, balık pulu atıklarından, doğal atıkların veya çevre sorunlarının yönetimine katkı sağlayacak, memeli kollajenine alternatif, gıda ve kozmetik gibi endüstriyel alanlarda kullanılabilecek bir kaynak elde edilmiştir.

Introduction

Bioactive substances of fish such as lipids, proteins, vitamins, minerals, and also other fish by-products are considered important functional sources due to their high nutraceutic and cosmeceutic and therapeutic potential (Atef and Ojagh, 2017; Ashraf *et al.,* 2020). These resources have a wide variety of applications i.e in the field of nutrition as a dietary supplement, in pharmacy as antitumor and anti-viral, in agriculture due to their herbicide, insecticidal, and fungicidal activities, and in cosmetics as sunscreen and anti-aging (Zayed, 2018). Today, various fish wastes such as skin, muscle, skeleton, bone and internal organs are used to isolate many bioactive materials (Ashraf *et al.,* 2020). The annual total production of aquatic products exceeded one hundred million tons in the world, and approximately 10 % of this is discarded due to spoilage (Yu and Gu, 2015). These wastes cause danger for the environment because of high biological and chemical oxygen demand, associated pathogens and organic substances, etc. (Knidri *et al.,* 2018). Recently, in numerous scientific studies, the potential of the versatile by-products of fish and crustaceans processing, by converting into high-value bio-compounds such as collagen and gelatin, bioactive peptides, enzymes and specific proteins, chitin, chitosan and pigments, were reported to provide economic and environmental benefits (Hamdi *et al.,* 2017).

Fish scales constitute about 5 % of fish waste and are considered as an important industrial waste (Fehng, 2016). Fish scales are not generally recycled and therefore serious environmental problems are caused by their storage, incineration or disposal to water bodies. Hence, it is very

*Corresponding author: cansumetin@mu.edu.tr

How to cite this article: Hacisa, C. M., & Baygar, T. (2023). Extraction and characterization of acid soluble collagen from golden grey mullet (*Chelon auratus*) scale. COMU J. Mar. Sci. Fish, 6 (2): 166-177. doi:10.46384/jmsf.1387324

important to evaluate these wastes which are a good sources of proteins, minerals, bioactive peptides, fish oil, enzymes and amino acids (Bhuimbar *et al.*, 2019).

Collagen is the most abundant structural protein in the extracellular matrix of the various connective tissues in the body (i.e., skin, bones, ligaments, tendons, and cartilage) (Jafari *et al.*, 2020). It plays an important role in maintaining the structural and biological integrity of the extracellular matrix and provides physiological functions and mechanical strength to tissues. Collagen has multifunctional properties related to their groups of protein side chains such as having high nutritional value, gelling, emulsifying, foaming and film-forming properties, high water absorption capacity, biocompatibility, biodegradability, low toxicity, non-antigenicity, high stability, easy processing, native ability to combine with other materials and large-scale extraction (Shalaby *et al.*, 2020; Subhan *et al.*, 2021; Tang *et al.*, 2022). All these properties make them functional ingredients in various food systems, and also it is used in cosmetics, pharmaceutical and biomedical industry in the forms of injectable solutions, thin substrates, porous sponges, nanofibrous matrices, and micro- and nano-spheres (Shalaby *et al.*, 2020; Subhan *et al.*, 2021, Tang *et al.*, 2022; Rajabimashhadi *et al.*, 2023). Marine collagen obtained from fish parts such as skin, bone, cartilage and scales, as well as from marine vertebrates and invertebrates, are more bioavailable and a good alternative to bovine or porcine collagen (Jafari *et al.*, 2020).

Golden grey mullet has high economic value that is frequently fished in Dalyan,Köyceğiz (Türkiye). According to Dalyan Fisheries Cooperative, 408 tonnes of golden grey mullet were caught in 2018. Therefore, golden grey mullet is an important source of fish scale in this region. Although Turkey has important aquaculture resources, utilization of these resources for valuable bioactive components is very limited. In the present study, and in accordance with the concept of sustainability, a valuable bioactive substance, collagen, was isolated from golden grey mullet fish scales.

Material and Methods

Golden grey mullet (*Chelon auratus*) scales were used as study material. Commercial marine fish skin collagen (MM Ingredients Ltd./UK) in the powder form was used to compare the isolated collagen as control. The golden grey mullet scales were obtained from DALKO Fisheries Cooperative (Köyceğiz/Muğla/Turkey). Scales were transferred to Muğla Sıtkı Koçman University Fisheries Faculty Quality Control Laboratories under cold storage. Scales were washed with tap water then dried for 3 days at room temperature (approximately 24 °C).

Acid soluble collagen (ASC) extraction

Acid soluble collagen (ASC) was extracted from golden grey mullet scales according to the method of Ali et al. (2017) with slight modifications. Non-collagenous protein was removed from fish scales using 0.1 M NaOH for 6 h (1:10 w/v). The solution was changed every 3 hours (2 buffer changes). After 6 hours, the pH was decreased by washing with distilled water. Demineralization of the filtered scales was achieved with 0.5 M $EDTA_2$-Na solution (pH:7.4) (1:10 w/v) using a magnetic stirrer for 48-hour. At this stage, the solution was changed every 12 hours and the removal of non-collagen proteins and demineralization phase was completed. The scales were stored at –80 °C for further procedures. After pretreatment, scales were extracted with 0.5 M acetic acid for 48 hours. After 48 hours, the scales were filtered with a double layer of cloth and the treatment continued with the liquid part. The extract was precipitated with 2.5 M NaCl + 0.05 M Tris (hydroxymethyl) aminomethane at pH 7.0 (1:1 v/v) and centrifuged at +4 °C 14,000 g for 1 h using a high speed refrigerated centrifuge machine (HANIL SUPRA 22K). Precipitated gels were dissolved in 0.5 M acetic acid (w/v, 1:9) and dialyzed. Dialysis was carried out with 0.1 M acetic acid (24 hours/2 buffer changes) and than with distilled water (5 hours). Purified collagens were then dried with freeze-dryer for 72 hours at –50 °C. Entire process of extraction was performed at 4 °C.

Proximate composition analysis

The protein, lipid and ash contents of the ASC from fish scale and commercial collagen samples were determined according to the method of AOAC (2006a), AOAC (2006b) and AOAC (2002), respectively. Moisture content was measured with an automatic moisture analyzer (SARTORIUS MA 35). A conversion factor of 5.95 was used for calculating protein content of fish scale (Chuaychan *et al.*, 2015) and 6.38 for collagen (Chen *et al.*, 2018).

Denaturation temperature

The denaturation temperature was determined by the method of Nagai and Suzuki (2000) with slight modification by measuring its viscosity. Viscosity of 1.5 mg/ml of collagen solution in 0.1 M acetic acid was measured. The thermal denaturation curve was obtained from 10 to 60 ºC by measuring at temperature intervals of 2 ºC. The denaturation temperature, *Td*, was determined as the temperature at which change in viscosity was half completed and calculated by the following equation of Wang *et al.* (2008):

Fractional viscosity = (Measured viscosity – Minimum viscosity) / (Maximum viscosity – Minimum viscosity)

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

SDS-PAGE of collagen extracted from grey mullet fish scale was performed according to the method of Laemmli (1970) with 8% separating gel and 4 % stacking gel. 10.6 µg of protein was loaded in each well. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 and then destained in a solution of 40 % (v/v) methanol and 7 % (v/v) acetic acid to visualize band formation. A wide range molecular-weight marker was used to estimate the molecular weight of collagen samples. Molecular weight was calculated by

densitometric analysis in Image J (1.52a) program (Günlü *et al.*, 2014).

Amino acid analysis

Amino acid analysis of fish scale and commercial collagen was performed according to modified method of Erkan et al. (2010) using a Shimadzu Prominence LC-20A (Kyoto, Japan) high performance liquid chromatograph (HPLC) system by comparison of their retention time with those of authentic standard (Pierce, Amino Acid Standard Hydrolyzate, product no: 20078 20088 20089 1800180 NCI0180, Rockford, IL 61105 USA).

Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS)

Scanning electron microscopy analysis of fish scale and commercial collagen was examined with JSM 7600F Field Emission Scanning Electron Microscope (JEOL, Japan) at an accelerating voltage of 15 kV. A piece of powder was placed on a specimen stub with double-sided adhesive carbon tape. Elemental analysis of collagen samples was also carried out by energy dispersive X-ray spectroscopy (EDS) (Oxford Instruments, UK) combined with SEM.

FTIR spectrum analysis

FTIR spectrums of fish scale collagen and commercial collagen was monitored by FTIR (Thermo Scientific Nicolet iS10-ATR, USA) at a resolution of 4 cm^{-1} in potassium bromide (KBr) pellets and the spectrum were recorded in the wavelength range from 4,000 to 400 nm^{-1}.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis of the fish scale and commercial collagen was executed by using a TGA instrument (Perkin Elmer TGA 4000, Perkin Elmer, Waltham, MA). Samples were heated from 30 to 600 °C at a rate of 10 °C/min under a nitrogen flow rate of 20 ml/min.

Antioxidant and antimicrobial activity

The antioxidant activity (AA %) of fish scale collagen and commercial collagen were carried out by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity according to method of Brand-Williams *et al.* (1995). The scavenging activity percentage (AA %) was calculated according to Mensor *et al.* (2001).

Antimicrobial activity of the fish scale and commercial collagen was carried out using agar well diffusion method (NCCLS, 1993). For this purpose, a Gram positive strain, *Staphylococcus aureus* ATCC (American Type Culture Collection) 25923, a Gram negative strain, *Escherichia coli* ATCC 25922 and a yeast *Candida albicans* (ATCC 10231) provided from Ankara Refik Saydam Hıfzısıhha Institute (Ankara, Turkey), were used. After the convenient incubation period for each microorganism, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms.

Statistical analysis

All experiments were carried out in triplicate and for each parameter, results were reported as mean and standard deviation. Means were compared by the analysis of variance (ANOVA) in SPSS (Version 21, SPSS Inc., Chicago, IL, USA) software. Tukey's multiple range test ($p < 0.05$) was used to detect differences among mean values of all test intervals.

Results and Discussion

Proximate composition

The proximate composition of fish scale, fish scale collagen and commercial collagen was shown in Table 1. The protein, lipid, ash and moisture contents of golden grey mullet fish scales were 39.99 ± 1.54 %, 0.08 ± 0.01 %; 40.07 ± 0.20 % and 21.42 ± 0.09 %, respectively. As a result of the ash analysis after demineralization of the collagen production, the ratio of ash obtained was 0.04 ± 0.02 %. In this context, it has been observed that the removal of minerals was carried out successfully.

Protein, lipid, ash and moisture values were similar to those reported for *Sciaenops ocellatus* scales; 41.1 ± 0.1 %; 0.4 ± 0.1 %; 42.4 ± 0.1 % and 16.1 ± 0.1 % respectively (Chen *et al.*, 2016). These values were 24.32 %; 0.68 %; 47.31 % and 26.72 %; respectively, for the scales of seabass (*Lates calcarifer*) (Chuaychan *et al.*, 2015). Scale composition reported for mullet was 31.12 % protein, 0.20 % lipid, 30.01 % ash and 37.87 % moisture (Cao *et al.*, 2017). Thuy *et al.* (2014) found protein content of mullet scale as 50.4 ± 0.4 % and ash content as 47.9 ± 0.6 %, while Wang *et al.* (2008) found the amount of protein in *Sebastes mentella* scale as 56.9 %; ash amount as 39.4 %. The nutrient contents of golden grey mullet scale examined in the present study are similar to those reported by other researchers. With respect to lipid content, our findings were low as expected.

The values of protein, lipid, ash and moisture of fish scale collagen isolated in the present study were found as 94.52 ± 2.02 %, 0.67 ± 0.11 %, 0.98 ± 0.01 % and 0.17 ± 0.01 %, respectively. Also the protein, lipid, ash and moisture values of the commercial fish skin collagen were determined as 98.89 ± 0.85 %, 0.03 ± 0.01 %, 1.49 ± 0.02 % and 3.09 ± 0.93 %, respectively. Collagen, a connective tissue protein, is expected to have a high content of protein and a fairly low content of lipid, moisture and ash. It was determined that the difference between protein, lipid, ash and moisture values in fish scale collagen and commercial collagen was statistically significant ($p < 0.05$) (Table 1). Nurilmala *et al.* (2019) detected 82.95 % protein, 0.96 % lipid, 3.64 % ash and 12.07 % moisture for skin collagen from yellow fin tuna (*Thunnus albacares*).

Table 1. Proximate composition of fish scale, fish scale collagen and commercial collagen (dw %)

%	Fish scale	Fish scale collagen	Commercial collagen
Protein	39.99 ± 1.54	94.52 ± 2.02[b]	98.89 ± 0.85[a]
Lipid	0.08 ± 0.01	0.67 ± 0.11[a]	0.03 ± 0.01[b]
Moisture	21.42 ± 0.09	0.17 ± 0.01[b]	3.09 ± 0.93[a]
Ash	40.07 ± 0.20	1.49 ± 0.02[a]	0.98 ± 0.01[b]

*Data are expressed as the mean ± SD. Lower case letters in the same line show statistical difference between fish scale collagen and commercial collagen.

Denaturation temperature

Denaturation temperature of fish scale collagen and commercial collagen was determined as 30 ± 0.02 ºC and 28 ± 0.01 ºC, respectively ($p < 0.05$) (Figure 1).

Pati *et al.* (2010) found the denaturation temperature of collagen from scales of *Labeo rohita* and *Catla catla* as 35 °C. They stated that the denaturation temperature is closely related to the temperatures in the natural habitat of fish. El-Rashidy *et al.* (2015) determined the denaturation temperature of the scale collagen from Nile Tilapia (*Oreochromis niloticas*) as 32.09 °C and concluded that scale collagen was close to mammalian collagen. Sionkowska *et al.* (2015) found the denaturation temperature of *Brama australis* skin collagen as 24 °C in their study. The use of collagen from different species in different applications depends on the thermal stability of the obtained species, which depends on the environmental conditions and body temperature. Similarity between the denaturation temperature of the fish collagen and the mammalian collagen is an important advantage in terms of its use in biomedical applications (Pati *et al.*, 2010). Therefore, the denaturation temperature of the collagen obtained from the mullet scale in this study, offers advantages for its use in food and cosmetics industries.

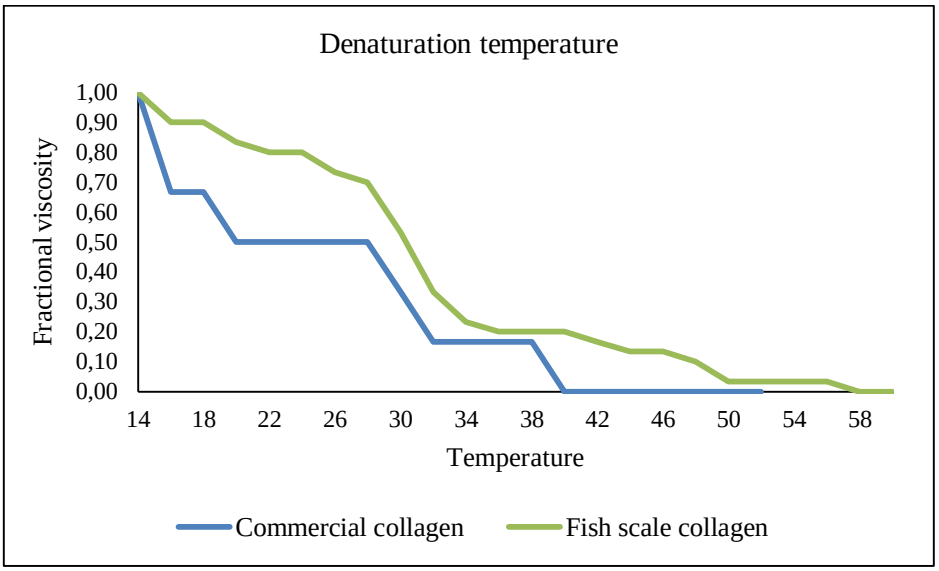

Figure 1. Denaturation temperature of fish scale collagen and commercial collagen

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

Figure 2 shows the electrophoretic pattern and densitometric analysis results of the fish scale collagen. ASC from fish scale was composed of two different α chains, α1 and α2; density wise α1 was far intense than α2 chain, with high molecular weight components β (dimmer) and γ (trimmer). According to subunit composition of SDS-PAGE, ASC was mainly composed of type I collagen. Molecular weights of α1 and α2 chains were found with densitometric analysis as 126 and 116 kDa, β and ɣ chains were 197 and 210 kDa, respectively. Similar results were obtained by Pal *et al.* (2016); the molecular mass of collagen from Mrigal carp (*Cirrhinus cirrhosus*) scale was about 120 kDa for α1 and 110 kDa for α2.

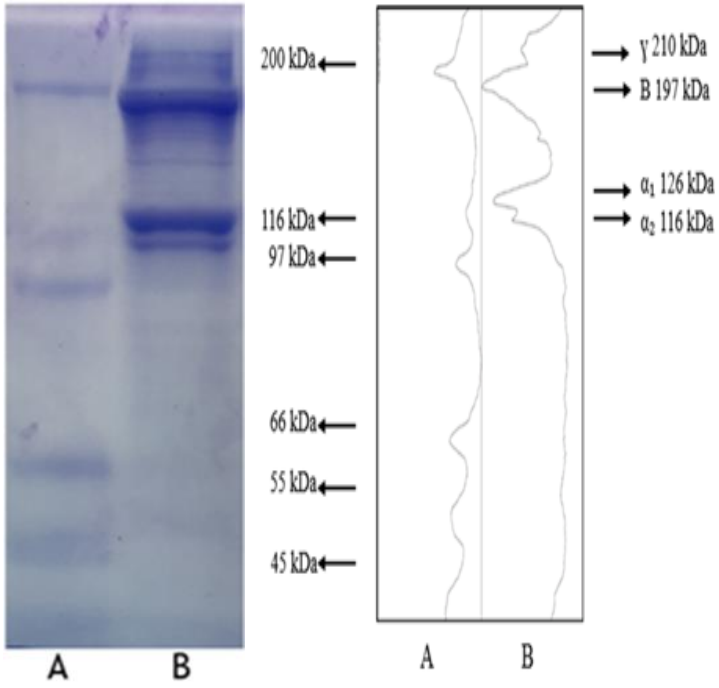

Figure 2. SDS-PAGE pattern and densitometric analysis of protein marker (A) and collagen from fish scale (B)

Chinh *et al.* (2019a) reported that they obtained type I collagen from the scales of tropical freshwater carp, molecular weights of α1 and α2 chains were detected as 139 kDa and 129 kDa, respectively, and the β-chain was also observed. Chen *et al.* (2016) extracted acid-soluble collagen from tilapia (*Oreochromis niloticus*) scales and skin and reported that the electrophoretic positions of the α chains in the scale collagen (α1-132 kDa; α2-121 kDa) was in the higher position compared to skin collagen (α1-130 kDa; α2-120 kDa). In the present study, slightly lower molecular weight was observed compared with those of ASC. Tan and Chang (2018) observed the molecular weights of α1 and α2 chains in the collagen they extracted from the skin of canal catfish (*Ictalurus punctatus*) as 123 kDa and 113 kDa, respectively. The molecular weights of β and γ chains, was found to be 226 kDa and 338 kDa, respectively. They found the molecular weights of β and γ chains in higher positions compared with our study. Different collagen extraction methods applied in fish species can reveal small differences in the structure of collagen. In this context, depending on the species and collagen extraction method, molecular weights may also differ.

Amino acid

The amino acid composition of fish scale and commercial fish skin collagen is presented in Table 2. The extracted collagen was rich in arginine, threonine, glycine and proline. The highest amino acid was arginine + threonine with 39.86 ± 0.93 g/100 g. The peaks of arginine and threonine amino acids were calculated together since they cannot be distinguished clearly. Glycine (13.75 ± 0.34 g/100 g), proline (8.05 ± 0.31 g/100 g) and arginine had higher levels compared to other amino acids. Alanine level was found to be 1.64 ± 0.03 g/100 g. In the commercial fish skin collagen, the highest amino acid was arginine with 13.39 ± 0.09 g/100 g, followed by glycine with 12.56 ± 0.42 g/100 g, and proline with 6.67 ± 0.00 g/100 g. The total amount of essential amino acids of commercial collagen was found to be lower than that of the fish scale collagen ($p < 0.05$). Both collagens had low concentrations of tyrosine, histidine and methionine.

Pal and Suresh (2017) found glycine as the highest amino acids of acid-soluble collagen extracted from two cyprinids species (*Catla catla* and *Labeo rohita*) with 22.43 and 22.63 g/100 g, respectively. Glycine was followed by proline with 15.40 and 15.30 g/100 g, alanine with 12.81 and 12.88 g/100 g, while arginine was found to be 8.52 and 7.24 g/100 g. Similar findings have been reported in our study. The amount of arginine was also lower in the study of Pal and Suresh (2017). In their study, in which collagen was extracted from the tropical frehwater carp scales, threonine was the most dominant amino acid Chinh *et al.* (2019a) with 39.79 % followed by proline with 11.37 %, glutamic acid with 12.84 % and arginine with 9.92 %. Glycine level was found as 3.27 %, alanine as 5.09 %, and hydroxyproline was not detected. Reported glycine content was lower than that determined in the present study. In the amino acid sequence of the collagen, there is one glycine in every three amino acids which explains the higher abundance of glycine.

Table 2. Amino acid composition of the fish scale collagen and commercial collagen (g/100 g)

Amino acids	Fish scale collagen	Commercial collagen
Lysine	2.32 ± 0.11^{a}	2.10 ± 0.01^{a}
Methionine	1.73 ± 0.05^{a}	1.37 ± 0.05^{b}
Arginine + Threonine*	39.86 ± 0.93^{a}	17.02 ± 0.09^{b}
Isoleucine	0.99 ± 0.01^{a}	0.92 ± 0.02^{a}
Leucine	1.78 ± 0.03^{a}	1.44 ± 0.02^{b}
Phenylalanine	1.34 ± 0.02^{a}	1.04 ± 0.01^{b}
Valine	1.77 ± 0.03^{a}	1.53 ± 0.00^{a}
Histidine	0.55 ± 0.01^{a}	0.60 ± 0.00^{a}
Serine	1.08 ± 0.03^{a}	0.89 ± 0.01^{a}
Cysteine	7.00 ± 0.01^{a}	2.37 ± 0.02^{b}
Tyrosine	0.89 ± 0.02^{a}	0.33 ± 0.01^{b}
Alanine	1.64 ± 0.03^{a}	1.61 ± 0.00^{a}
Aspartic acid	2.6 ± 0.02^{a}	2.47 ± 0.03^{a}
Glutamic acid	3.89 ± 0.13^{a}	3.24 ± 0.04^{a}
Glycine	13.75 ± 0.34^{a}	12.56 ± 0.42^{a}
Proline	8.05 ± 0.31^{a}	6.67 ± 0.00^{b}
ΣAA	91.11^{a}	57.02^{b}

Data are expressed as the mean ± SD. Lower case letters in the same line show statistical difference between fish scale collagen and commercial collagen.
*The peaks of arginine and threonine were calculated together since they cannot be distinguished clearly.

Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS)

As shown in SEM images of fish scale and commercial collagen (Figure 3), fibrous structures and a porous, cottony structure were observed in scale collagen. A similar morphology was observed in the commercial collagen and porous structures. More fibrillar structures were found in the scale collagen compared to commercial collagen. In addition, nodular structures were observed in the commercial collagen. Microstructure may be affected by the method of collagen purification and the solvent used. Collagen can form fibrillar structures spontaneously under suitable conditions such as pH, temperature and ionic strength. The pore size of collagen increases as a factor of the amount of water used during collagen production.

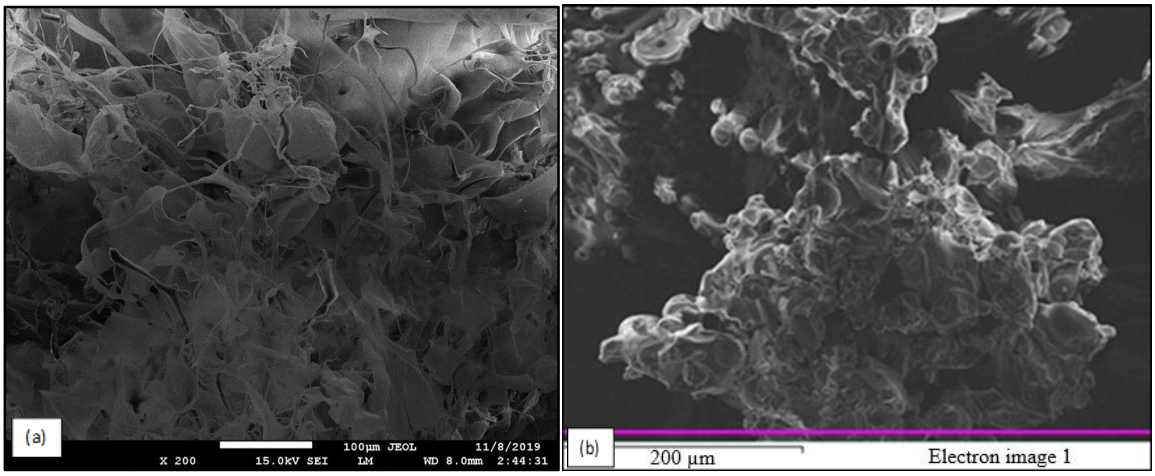

Figure 3. SEM image of fish scale collagen (a) commercial collagen (b)

Shalaby *et al.* (2020) characterized scale collagen from tilapia for wound healing purposes. The SEM image of tilapia collagen is similar to the collagen analyzed in the present study. According to Shalaby *et al.* (2020), the microstructure of lyophilized tilapia collagen shows homogeneous, multi-layered, orderly and highly fibrillated structures which is beneficial to the cell adhesion and proliferation.

Pal and Suresh (2017) examined the physico-chemical properties and fibril structure of ASC and PSC collagen from carp (*Catla catla* and *Labeo rohita*) scales. According to SEM images, porous, three-dimensional collagen fibrils was observed. Nodular structures were seen in PSC. In *Catla catla* scale collagen more porous structures were observed in PSC compared to ASC, but also more branched structures were seen in ASC. ASC from *Labeo rohita* were more porous and fibril structures were less complex than PSC. According to Pal and Suresh (2017) uniform fibril structure is a desired property for collagen.

EDS spectrum of scale and commercial collagen are shown in Figure 4. For scale collagen percent mass weight of C, N and O was found as 46.09 %, 22.05 % and 28.48 %; percent atomic weights as 52.53 %, 21.55 % and 24.37 %, respectively. In the commercial collagen the percent mass weights of the C, N, O elements were determined as; 43.79 %, 23.52 % and 32.69 % and atomic weights as 49.48 %, 22.79 % and 27.73 %, respectively. In scale collagen, unlike commercial collagen, Na and Cl elements were observed corresponding to mass weights of 1.14 % and 2.25 %, atomic weights of 0.68 % and 0.87 %, respectively. It is likely that NaCl, which was used to precipitate collagen proteins dissolved in acetic acid, could not be completely removed during the collagen production process.

Element	Weight%	Atomic%	Element	Weight%	Atomic%
C K	46.09	52.53	C K	43.79	49.48
N K	22.05	21.55	N K	23.52	22.79
O K	28.48	24.37	O K	32.69	27.73
Na K	1.14	0.68	Totals	100.00	
Cl K	2.25	0.87			
Totals	100.00				

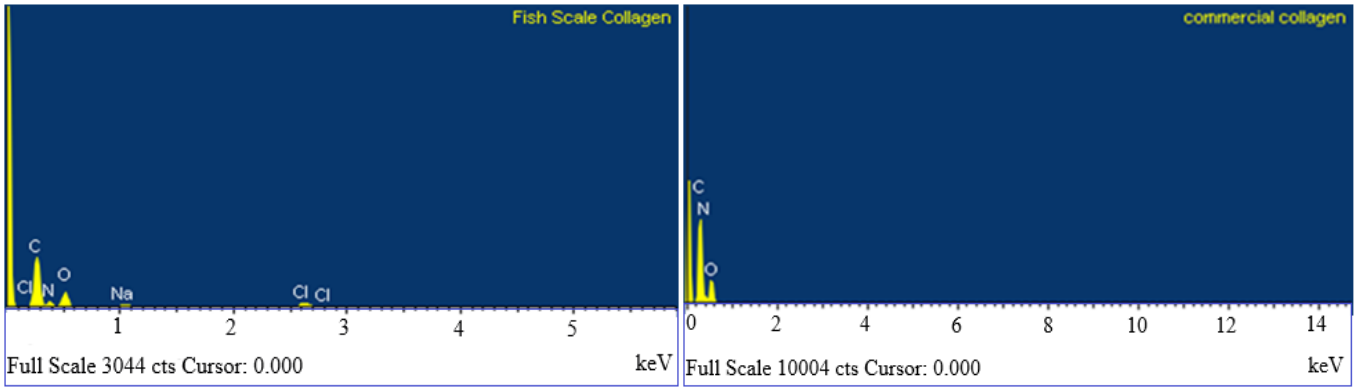

Figure 4. EDS spectrum of fish scale and commercial collagen

Chinh *et al.* (2019b) found the mass weight of Na, Cl, O, C and N in hydroxy apatite and collagen from tilapia fish scale as 33.68 %, 51.06 %, 2.23 %, 11.38 % and 1.65 %, atomic weights as 35.65 %, 35.04 %, 3.40 %, 23.05 %, and 2.86 % according to the EDS analysis. They reported that the higher contents of Na and Cl is due to the NaCl solution which is used for precipitation of the collagen. In future studies, the amount of NaCl solution should be decreased to obtain pure collagen.

FTIR spectrum

Secondary structure of fish scale collagen and commercial collagen was detected by FTIR (Figure 5). FTIR spectrum of fish scale collagen showed main absorption bands of amide A (3262.97 cm^{-1}), amide B (2873.86 cm^{-1}), amide I (1639.38 cm^{-1}), amide II (1532.85 cm^{-1}) and amide III (1234.13 cm^{-1}). In commercial collagen amide A, B, I, II and III bands were observed at 3274.42, 2879.21, 1639.55, 1522.36 and 1237.20 cm^{-1}, respectively. The resulted spectrum was similar for both fish scale and commercial collagen.

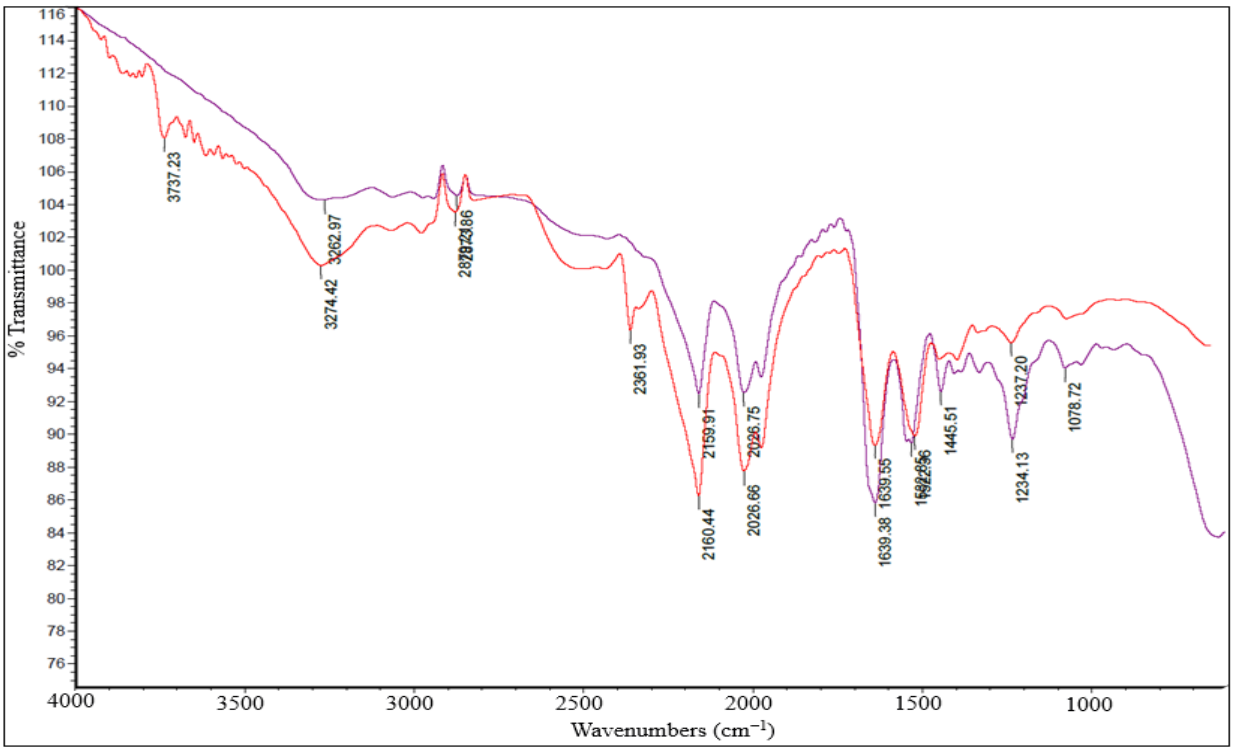

Figure 5. FTIR spectrum of fish scale collagen (purple) and commercial collagen (red)

Amide A consists of N–H stretching vibration. Amide B band consociated with CH_2 asymmetrical stretching (Bhagwat and Dandge, 2016). The range of amide A, I, II, and III is known to have a direct relation to the polypeptide chain (Abinaya and Gayathri, 2019). The amide I wave number ranges from 1600 to 1700 cm^{-1}, which is related to the carbonyl (C=O) stretching vibration of the peptide chain and is the most important factor for determining the secondary structure of proteins. Similarly, the ASC absorption of the regional band was observed at 1639.38 cm^{-1} in the present study. Amide II is related to N–H bond and C-N expansion, whereas amide III is related to CN stretching and N–H bending and participates in the triple helix structure of collagen (Woo *et al.*, 2008). Chen *et al.* (2016) found the main absorption bands in the collagen of red drum (*Sciaenops ocellatus*) scale at 3328, 3080, 1658, 1548 and 1240 cm^{-1}, respectively. Chinh *et al.* (2019b) determined the characteristic peaks at 3451.95, 1635.26, 1400.15, 1065.54 and 462.32 cm^{-1} wavelengths as a result of FTIR measurements made on tilapia fish scale collagen. In this present study, the main bands of the collagen in FTIR spectrum was observed in the range of values specified in the literature, and are quite similar to purified collagen from other fish species.

Thermo gravimetric analysis (TGA)

According to the results of mass changes examined with thermogravimetric analysis, there was no significant weight loss up to 100 °C for both collagens. The first weight loss in scale and commercial collagen occurred at 308 ºC and 286 ºC, respectively. The initial decrease in this temperature is thought to be due to the breakdown of organic compounds. The major lost also occurred in scale and commercial collagen at 350 ºC and 410 ºC. After 600 ºC there was no significant change in weight (Figure 6).

According to TGA results of collagen obtained from the puffer fish (*Lagocephalus inermis*) skin, no significant weight loss was observed before 100 °C (Iswariya *et al*,. 2018). The major weight losses occurred at 315 °C and 520 °C. Pati *et al.* (2010) reported that according to the TGA results, there were no weight losses before 100 ºC in the type I collagen from *Labeo rohita* and *Catla catla* fish scales. The main weight and organic compound losses occurred at 286 ºC and 411 ºC, respectively. Similar to our findings, in both studies, no significant lost or change was observed after 600 °C.

Antioxidant activity

In the present study the DPPH radical scavenging activity of fish scale collagen and commercial collagen was determined as 81.90 ± 1.27 % and 80.54 ± 3.34 %, respectively ($p > 0.05$). Pal and Suresh (2017) found the the DPPH radical scavenging activity of the collagen from *Catla catla* and *Labeo rohita* in the range of 9–24 % for ASC. Collagens can inactivate reactive oxygen species, reduce hydroperoxides, enzymatically eliminates specific oxidants and scavenge free radicals, which may contribute to their antioxidant activities· (Pal and Suresh, 2017). Nakchum and Kim (2016) determined the DPPH radical scavenging activity of squid skin collagen as 57.38 %. Slimane and Sadok (2018) produced films with collagen extracted from the common shark (*Mustelus mustelus*) skin and chitosan, and reported that DPPH radical scavenging activity of films with a pure collagen and mixture of

collagen:chitosan (1:1) was 30.88 ± 0.03 % and 23.91 ± 1.15 %, respectively. These results are considerably lower than the DPPH radical scavenging activity obtained in our study. It was reported that the antioxidant properties can change depending on the peptide sequence and molecular weight (Bu *et al.*, 2017). In our study, it was determined that the DPPH radical scavenging activity of collagen was quite high and similar to that of the commercial collagen. These results further highlight the importance of golden grey mullet scale collagen.

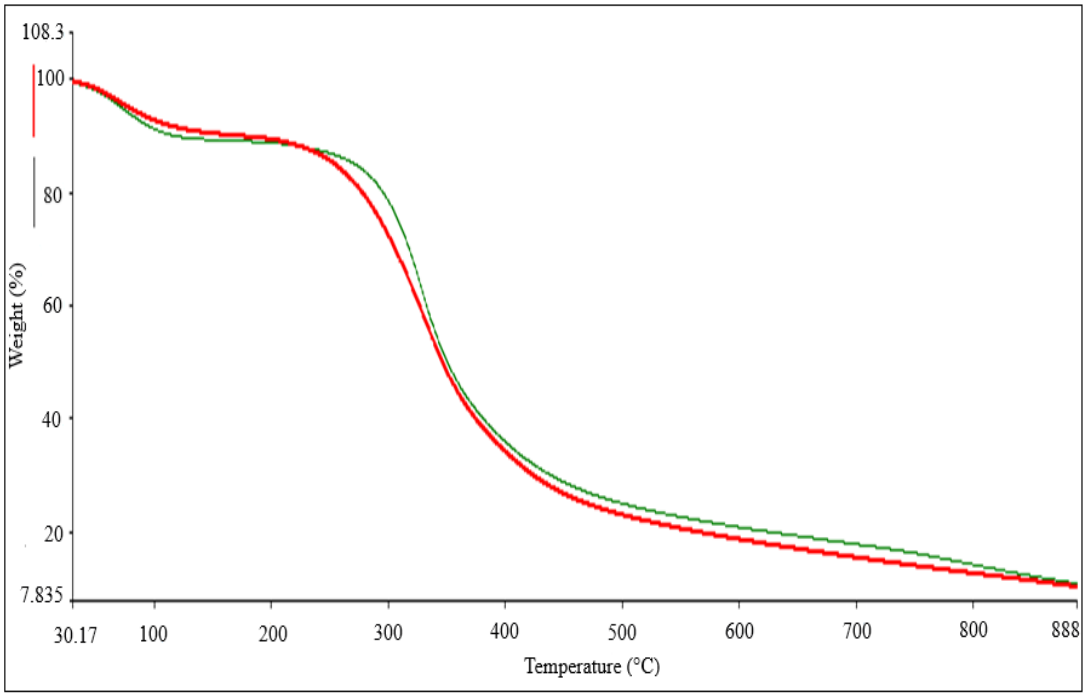

Figure 6. TGA results of the scale collagen (green) and commercial collagen (red)

Antimicrobial activity

The antimicrobial activity of the scale and commercial collagen was evaluated against two common pathogenic bacteria; a Gram negative strain *E. coli* and a Gram positive strain *S. aureus* and a yeast *C. albicans*. There was no inhibition zone against *E. coli*, *S. aureus* and *C. albicans* in both scale and commercial collagen. Venkatesan *et al.* (2017) reported that peptides from *Setipinna taty* and *Scomber scombrus*, produced by enzymatic hydrolysis using different enzymes (pepsin, papain, trypsin) inhibited *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria innocua*. It is thought that due to the small structure of the peptides formed by the hydrolysis of collagen, penetration into microorganisms occurs more easily. Shalaby *et al.* (2020) produced and characterized collagen from fish scales for wound healing purposes, and reported that the mean inhibition circle diameters on *Escherichia coli* and *Staphylococcus aureus* were 0.08 mm and 0.5 mm, respectively, having a very low antibacterial effect againts these bacteria.

Conclusion

Collagen was extracted by using acetic acid from the scale of golden grey mullet. Based on proximate and amino acid composition, denaturation temperature, SDS-PAGE, SEM-EDS, FTIR, TGA, antioxidant and antimicrobial activity, ASC was characterized and compared with commercial fish skin collagen. Extracted collagen was quite similar to commercial collagen, yielding even higher amounts of total amino acids and higher antioxidant properties. ASC from scale of golden grey mullet can be a promising alternative source to mammalian collagen from the fish processing industry. The present study will contribute to the reduction of the environmental pollution as well as having economic and scientific benefits by using unevaluated fish scale wastes for collagen extraction. Isolated collagen is a promising renewable biological source that can be used in various sectors such as food as a functional ingredient and cosmetics, that will contribute to human health.

Acknowledgements

This study is a part of Ph.D. thesis. The authors wish to thank DALKO (Dalyan Fisheries Cooperative/Turkey) for providing the fish scale samples, to Yunus Alparslan for his help and support in all laboratory studies, to Tuba Baygar for SEM-EDS analysis, to Çiğdem Elif Demirci for TGA analysis to Özkan Özden for amino acid analysis, to Özge Tokul Ölmez for FTIR analysis and to Daniela Giannetto for English editing.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Cansu Metin Hacisa and Taçnur Baygar planned and designed the research. Cansu Metin Hacisa carried out laboratory analysis. All authors contributed to the writing of the final manuscript.

Ethics Approval

Ethics committee approval is not required for this study.

References

Abinaya, M., & Gayathri, M. (2019). Biodegradable collagen from *Scomberomorus lineolatus* skin for wound healing dressings and its application on antibiofilm properties. *Journal of Cellular Biochemistry*, *120*, 15572-15584. doi: 10.1002/jcb.28824

Ali, A. M. M., Benjakul, S., & Kishimura, H. (2017). Molecular characteristics of acid and pepsin soluble collagens from the scales of golden carp (*Probarbus jullieni*). *Emirates Journal of Food and Agriculture*, *29*(6), 450-457. doi: 10.9755/ejfa.2016-09-1316

AOAC (2002). Ash content. In Official Methods of Analysis, (17th Ed.) Association of Official Analytical Chemists, Method 920.153, Gaithersburg, Maryland.

AOAC (2006a). Crude protein in meat. In official methods of analysis (17th ed.) 984.13. Gaithersburg, Association of Official Analytical Chemists.

AOAC (2006b). Crude fat determination-soxhlet method. Meat technology information sheet, pp. 1-3.

Ashraf, S. S., Adnan, M., Patel, M., Siddiqui, A. J., Sachidanandan, M., Snoussi, M., & Hadi, S. (2020). Fish-based bioactives as potent nutraceuticals: Exploring the therapeutic perspective of sustainable food from the sea. *Marine Drugs*, *18*(265), 1-20. doi: 10.3390/md18050265

Atef, M., & Ojagh, S. M. (2017). Health benefits and food applications of bioactive compounds from fish byproducts: A review. *Journal of Functional Foods*, *35*, 673-681. doi: 10.1016/j.jff.2017.06.034

Bhagwat, P. K., & Dandge, P. B. (2016). Isolation, characterization and valorizable applications of fish scale collagen in food and agriculture industries. *Biocatalysis and Agricultural Biotechnology*, *7*, 234-240. doi: 10.1016/j.bcab.2016.06.010

Bhuimbar, M. V., Bhagwat, P. K., & Dandge, P. B. (2019). Extraction and characterization of acid soluble collagen from fish waste: Development of collagen-chitosan blend as food packaging film. *Journal of Environmental Chemical Engineering*, *7*, 102983. doi: 10.1016/j.jece.2019.102983

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25-30. doi: 10.1016/S0023-6438(95)80008-5

Bu, Y., Elango, J., Zhang, J., Bao, B., Guo, R., Palaniyandi, K., Robinson, J. S., Geevaretnam, J., Regenstein, J. M., & Wu, W. (2017). Immunological effects of collagen and collagen peptide from blue shark cartilage on 6T-CEM cells. *Process Biochemistry*, *57*, 219-227. doi: 10.1016/j.procbio.2017.04.008

Cao, T. H., Nguyen, T. T. O., Nguyen, T. M. H., Le, N. T., & Razumovskaya, R. G. (2017). Characteristics and physicochemical properties of gelatin extracted from scales of seabass (*Lates calcarifer*) and grey mullet (*Mugil cephalus*) in Vietnam. *Journal of Aquatic Food Product Technology*, *26*(10), 1293-1302. doi: 10.1080/10498850.2017.1390026

Chen, J., Li, L., Yi, R., Xu, N., Gao, R., & Hong, B. (2016). Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (*Oreochromis niloticus*). *LWT - Food Science and Technology*, *66*, 453-459. doi: 10.1016/j.lwt.2015.10.070

Chen, S., Chen, H., Xie, Q., Hong, B., Chen, J., Hua, F., Bai, K., He, J., Yi, R., & Wu, H. (2016). Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenops ocellatus*). *Food Hydrocolloids*, *52*, 468-477. doi: 10.1016/j.foodhyd.2015.07.027

Chen, Y., Liang, C., Wu, H., Pang, H., Chen, C., Wang, G., & Chan, L. (2018). Antioxidant and anti-inflammatory capacities of collagen peptides from milkfish (*Chanos chanos*) scales. *Journal of Food Science and Technology*, *55*, 2310-2317. doi: 10.1007/s13197-018-3148-4

Chinh, N. T., Manh, V. Q., Trung, V. Q., Lam, T. D., Huynh, M. D., Tung, N. Q., Trinh, N. D., & Hoang, T. (2019a). Characterization of collagen derived from tropical freshwater carp fish scale wastes and its amino acid sequence. *Natural Product Communications*, 1-12. doi: 10.1177/1934578X19866288

Chinh, N. T., Manh, V. Q., Trung, V. Q., Trang, T. D. M., & Hoang, T. (2019b). Extraction of hydroxyapatite and collagen from the Vietnamese tilapia scales. *Vietnam Journal of Chemistry*, *57*(2), 225-228. doi: 10.1002/vjch.201900021

Chuaychan, S., Benjakul, S., & Kishimura, H. (2015). Characteristics of acid- and pepsin-soluble collagens from scale of seabass (*Lates calcarifer*). *LWT - Food Science and Technology*, *63*, 71-76. doi: 10.1016/j.lwt.2015.03.002

El-Rashidy, A. A., Gad, A., Abu-Hussein, A. E. G., Habib, S. I., Badr, N. A., & Hashem, A. A. (2015). Chemical and biological evaluation of Egyptian Nile Tilapia (*Oreochromis niloticas*) fish scale collagen. *International Journal of Biological Macromolecules*, *79*, 618-626. doi: 10.1016/j.ijbiomac.2015.05.019

Erkan, N., Selçuk, A., & Özden, Ö. (2010). Amino acid and vitamin composition of raw and cooked horse mackerel. *Food Analytical Methods*, *3*, 269-275. doi: 10.1007/s12161-009-9108-x

Fehng, S. P. C. (2016). Extraction of collagen from fish wastes, optimization and characterization. (M.Sc. Dissertation), Universiti Tunku Abdul Rahman, Kampar.

Günlü, A., Sipahioğlu, S., & Alpas, H. (2014). The effect of high hydrostatic pressure on the muscle proteins of rainbow trout (*Oncorhynchus mykiss* Walbaum) fillets wrapped with chitosan-based edible film during cold storage (4±1 °C). *High Pressure Research*, *34*(1), 122-132. doi: 10.1080/08957959.2014.894510

Hamdi, M., Hammami, A., Hajji, S., Jridi, M., Nasri, M., & Nasri, M. (2017). Chitin extraction from blue crab (*Portunus segnis*) and shrimp (*Penaeus kerathurus*) shells using digestive alkaline proteases from *P. segnis* viscera. *International Journal of Biological Macromolecules*, *101*, 455-463. doi: 10.1016/j.ijbiomac.2017.02.103

Iswariya, S., Velswamy, P., & Uma T. S. (2018). Isolation and characterization of biocompatible collagen from the skin of puffer fish (*Lagocephalus inermis*). *Journal of Polymers and the Environment*, *26*, 2086-2095. doi: 10.1007/s10924-017-1107-1

Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. (2020). Fish Collagen: Extraction, characterization, and applications for biomaterials engineering. *Polymers*, *12*(2230), 1-37. doi: 10.3390/polym12102230

Knidri, H. E., Belaabed, R., Addaou, A., Laajeb, A., & Lahsini, A. (2018). Extraction, chemical modification and characterization of chitin and chitosan. *International Journal of Biological Macromolecules*, *120*, 1181-1189. doi: 10.1016/j.ijbiomac.2018.08.139

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680-685.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*(2), 127-130. doi: 10.1002/ptr.687

Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste material-skin, bone and fins. *Food Chemistry*, *68*, 277-281. doi: 10.1016/S0308-8146(99)00188-0

Nakchum, L., & Kim, S. M. (2016). Preparation of squid skin collagen hydrolysate as an antihyaluronidase, antityrosinase, and antioxidant agent. *Preparative Biochemistry & Biotechnology*, *46*(2), 123-130. doi: 10.1080/10826068.2014.995808

NCCLS (1993). Performance standards for antimicrobial disc suspectibility tests. Approved Standard NCCLS Publication M2-A5, Villanova, PA, USA.

Nurilmala, M., Pertiwi, R. M., Nurhayati, T., Fauzi, S., Batubara, I., & Ochiai, Y. (2019). Characterization of collagen and its hydrolysate from yellowfin tuna *Thunnus albacares* skin and their potencies as antioxidant and antiglycation agents. *Fisheries Science*, *85*, 591-599. doi: 10.1007/s12562-019-01303-5

Pal, G. K., & Suresh, P. V. (2017). Comparative assessment of physico-chemical characteristics and fibril formation capacity of thermostable carp scales collagen. *Materials Science and Engineering: C*, *70*, 32-40. doi: 10.1016/j.msec.2016.08.047

Pal, P., Srivas, P. K., Dadhich, P., Das, B., Maity, P. P., Moulik, D., & Dhara, S. (2016). Accelerating full thickness wound healing using collagen sponge of mrigal fish (*Cirrhinus cirrhosus*) scale origin. *International Journal of Biological Macromolecules*, *93*, 1507-1518. doi: 10.1016/j.ijbiomac.2016.04.032

Pati, F., Adhikari, B., & Dhara, S. (2010). Isolation and characterization of fish scale collagen of higher thermal stability. *Bioresource Technology*, *101*, 3737-3742. doi: 10.1016/j.biortech.2009.12.133

Rajabimashhadi, Z., Gallo, N., Salvatore, L., & Lionetto, F. (2023). Collagen derived from fish industry waste: Progresses and challenges. *Polymers*, *15*(3), 544. doi: 10.3390/polym15030544

Shalaby, M., Agwa, M., Saeed, H., Khedr, S. M., Morsy, O., & El-Demellawy, M. A. (2020). Fish scale collagen preparation, characterization and its application in wound healing. *Journal of Polymers and the Environment*, *28*, 166-178. doi: 10.1007/s10924-019-01594-w

Sionkowska, A., Kozlowska, J., Skorupska, M., & Michalska, M. (2015). Isolation and characterization of collagen from the skin of *Brama australis*. *International Journal of Biological Macromolecules*, *80*, 605-609. doi: 10.1016/j.ijbiomac.2015.07.032

Slimane, E. B., & Sadok, S. (2018). Collagen from cartilaginous fish by-products for a potential application in bioactive film composite. *Marine Drugs*, *16*, 1-19. doi: 10.3390/md16060211

Subhan, F., Hussain, Z., Tauseef, I., Shehzad, A., & Wahid, F. (2021). A review on recent advances and applications of fish collagen. *Critical Reviews in Food Science and Nutrition*, *61*(6), 1024-1037. doi: 10.1080/10408398.2020.1751585

Tan, Y., & Chang, S. K. C. (2018). Isolation and characterization of collagen extracted from channel catfish (*Ictalurus punctatus*) skin. *Food Chemistry*, *242*, 147-155. doi: 10.1016/j.foodchem.2017.09.013

Tang, C., Zhou, K., Zhu, Y., Zhang, W., Xie, Y., Wang, Z., Zhou, H., Yang, T., Zhang, Q., & Xu, B. (2022). Collagen and its derivatives: From structure and properties to their applications in food industry. *Food Hydrocolloids*, *131*, 107748. doi: 10.1016/j.foodhyd.2022.107748

Thuy, L. T. M., Okazaki, E., & Osako, K. (2014). Isolation and characterization of acid-soluble collagen from the scales of marine fishes from Japan and Vietnam. *Food Chemistry*, *149*, 264-270. DOI: 10.1016/j.foodchem.2013.10.094

Venkatesan, J., Anil, S., Kim, S., & Shim, M. S. (2017). Marine fish proteins and peptides for cosmeceuticals: A Review. *Marine Drugs*, *15*, 1-18. doi: 10.3390/md15050143

Wang, L., An, X., Yang, F., Xin, Z., & Zhao, L. (2008). Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*). *Food Chemistry*, *108*, 616-623. doi: 10.1016/j.foodchem.2007.11.017

Woo, J., Yu, S., Cho, S., Lee, Y., & Kim, S. (2008). Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin. *Food Hydrocolloids*, *22*, 879-887. doi: 10.1016/j.foodhyd.2007.04.015

Yu, P., & Gu, H. (2015). Bioactive substances from marine fishes, shrimps, and algae and their functions: Present and future. *Critical Reviews in Food Science and Nutrition*, *55*, 1114-1136. doi: 10.1080/10408398.2012.686933

Zayed, A. E. A. F. (2018). Bioactive compounds from marine sources. (M.Sc. Dissertation), Technischen Universität Kaiserslautern, Kaiserslautern.