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Determination of Amino Acid Profile and Some Characteristics of Collagen Extracted from Skin and Bone of Mangar (*Luciobarbus esocinus* Heckel, 1843)

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ABSTRACT

Acid soluble collagens from bone (ASC-B) and skin (ASC-S) of mangar (*Luciobarbus esocinus* Heckel, 1843) were extracted, characterized, and their amino acid profiles were determined. To best of our knowledge, the current study is the first research that used this species as a source of collagen. Both ASC-S and ASC-B from mangar skin and bone contained glycine as the major amino acid and high amounts of proline, hydroxyproline, alanine, and glutamic acid. On the basis of dry weight, yields of ASC-S and ASC-B were 9.38 and 3.71%, respectively. Furthermore, fourier transform infrared spectroscopy proved that both collagens were integrated and native. Additionally, the results of X-Ray Diffraction (XRD) demonstrated that both of the collagens reserved their helical structures. The screened collagens had prominent absorptions at 230 nm by UV-Vis spectra. Additionally, the SEM studies have shown that both ACS-S and ASC-B have porous and fibrous nature. According to the UV–Vis and Fourier Transform Infrared Spectrophotometer (FTIR) results, extracted collagens were characterized as type I collagen based on their amino acid composition. According to the obtained results, the collagen isolated from mangar can potentially be an alternative source of vertebrate collagens for use in the diet and other industries such as medical and pharmaceutical industries.

Keywords: Mangar, ASC, FTIR, SEM, type I collagen

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Ferkenin (*Luciobarbus esocinus* Heckel, 1843) Deri ve Kemiklerinden Ekstrakte Edilen Kollajenin Amino Asit Profili ve Bazı Özelliklerinin Belirlenmesi

Öz: Ferkenin (*Luciobarbus esocinus* Heckel, 1843) kemiğinden ve derisinden asitte çözünür kolajenler (ASC) ekstrakte edildi, karakterize edildi ve amino asit profilleri belirlendi. Bildiğimiz kadarıyla, yapılan bu çalışma bahsi geçen türü kolajen kaynağı olarak kullanan ilk araştırma olma niteliği taşımaktadır. Ferkenin derisi ve kemiğinden elde edilen ASC'lerin içerisinde ana amino asit olarak glisin ve yüksek miktarlarda prolin, hidroksiprolin, alanin ve glutamik asit içerdiği belirlendi. Kuru ağırlık, ASC-deri ve ASC-kemik verimleri sırasıyla %9,38 ve %3,71 olarak belirlendi. Ayrıca, fourier dönüşümü kızılötesi spektroskopisi (FTIR) ile her iki kolajenin de entegre ve doğal olduğu kanıtlandı. Ek olarak, X-Işını Kırınımı (XRD) sonuçları şunu gösterdi: her iki kolajen sarmal yapılarını korumuştur. Araştırılan kolajenler UV-Vis spektrumları tarafından 230 nm'de belirgin absorpsiyonlara sahiptir. Ayrıca, SEM çalışmaları hem ACS-deri hem de ASC-kemiğin gözenekli ve lifli yapıya sahip olduğunu göstermiştir. UV–Vis ve FTIR sonuçlarına ve ekstrakte edilen kolajenler, amino asit bileşimlerine göre tip I kolajen olarak karakterize edildi. Bu çalışma ile, ferkeden izole edilen kolajenin, diyet, tıp ve ilaç endüstrileri gibi diğer endüstrilerde kullanım için omurgalı kolajenine göre potansiyel olarak alternatif bir kaynak olabileceği belirlenmiştir.

Anahtar kelimeler: Ferke, ASC, FTIR, SEM, tip I kolajen

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Introduction

The *Luciobarbus esocinus* (mangar, ferke in Turkish) is a species of ray-finned fish in the genus *Luciobarbus*, native to the Tigris–Euphrates River system in the middle eastern countries that include Iran, Iraq, Syria, and Turkey (Freyhof 2014). This species was described as a vulnerable species by The International Union for Conservation of Nature's (IUCN) red list of threatened species. It is among the most important endemic species found in the Euphrates and Tigris rivers (Ozgur 2016). Mangar is usually hunted by locals as a source of food. It can reach about 2.3 m and weigh up to 140 kg (Fricke et al. 2007) and among the most preferred freshwater fish by local people.

As the main component of connective tissue, collagen is the major fibrous glycoprotein found mainly in the skin, bone, cartilage, tendon, and connective tissues of mammals and fish (Huo and Zhao 2009; Aberoumand 2012; Salvatore et al. 2021). Collagen makes up from 25% to 35% of the whole-body protein content and up to 85% of the skin of mammals. However, it makes up from 19% to 38% of the whole-body protein content and up to 96% of the skin of fish (Dave et al. 2019). A wide range of collagen products are available commercially, but not all collagens are from the same source, nor are they suitable for certain types of dietary requirements (Noorzai and Verbeek 2020). Many of these collagens are sourced from livestock production in different farms that include cattle, pig, and chicken, while others come from aquatic sources including fish, shellfish, jellyfish, and crustaceans. Cattle are more widely used in collagen production in compare to porcine and fish sources because of its lower price and the abundance of its skin and bones. However, there are increased concerns about the transmission of diseases such as bovine spongiform encephalopathy (BSE) to humans due to consumption of bovine-based products (Nathanson et al. 1997; EFSA Panel on Biological Hazards (BIOHAZ) et al. 2020).

Due to its resistance to stretching and its fibrous structure, collagen provides strength and elasticity of the skin as well as playing an important role in strengthening blood vessels and tissue development (Sherratt 2009). Collagen's low immunogenicity and high biocompatibility make it a preferred biomaterial in biomedical applications. Beside its industrial uses, there is a great interest in collagen's anti-aging effects in many medical fields such as plastic surgery, burn surgery, and even weight management (Borumand and Sibilla 2014; Sionkowska et al. 2020). For this reason, many studies have been done to find alternative sources of collagen. Recently there has been an increase in the trend towards collagen derived from aquatic sources. Fish-derived collagen has a higher level of bioavailability since fish-derived collagen peptides have been proven to be easily digested absorbed and distributed throughout the body as much as 1.5 time in compare to collagen derived from bovine or porcine sources (Sadowska et al. 2003). In many studies, it has been reported that even fish waste constitutes a high potential source of collagen (Kiew and Mat Don 2012; Wang et al. 2013; Mahboob et al. 2015). Therefore, the isolated collagen has been known that it can be used as a supplement to the diet.

In the current study, mangar (*Luciobarbus esocinus* (Heckel, 1843)) was investigated for its collagen resources and their amino acid profiles. *L. esocinus* (Heckel, 1843) is found in some Middle Eastern countries such as Iran, Turkey, Syria, and Iraq. It is one the most important endemic species found in the Euphrates and Tigris River system, which is located within the Middle East (Ozgur 2016). Since mangar is one of the most consumed fresh water fish in the region and to best of our knowledge there has been no previous study involving collagen extraction from its skin and bones, with this study we determined that whether the skin and bones of mangar can be used as an alternative source of collagen.

Materials and Methods

Materials

Two mangar fish (*L. esocinus* Heckel,1843), each weighed about 6 kg and approximate length was 88 cm, were purchased from the fish market in May 2019, then brought to the laboratory and cleaned, its skin and bones were separated from the body and they were frozen at -20 °C until reuse. Fish skin and bones were thawed in the refrigerator at +4 °C and later was brought to room temperature before application of the extraction procedure.

Methods

Sample preparation

The preparation of the collagen samples was performed with minor modifications of the method that was previously described by Nagai and Suzuki (2000) (Nagai and Suzuki 2000).

All sample preparation procedures were carried out at not exceeding +4 °C. Each sample was studied as duplicate in all examinations.

Characterization of collagens

Collagen yields

Collagen yield was calculated using the dry weight of the material as specified in the following formula.

$$\text{Collagen yield (g/100g)} = \frac{\text{Weight of lyophilised Collogen}}{\text{Initial weight of lyophilised fish skin}} \times 100$$

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis of collagen samples (one repeat) was performed as previously described by Kittiphattanabawon and colleagues (Kittiphattanabawon et al. 2005). Lyophilized collagen samples were gelled with 0.05 M acetic acid at a solid/liquid ratio of 1:40 (w/v) and then stored at +4 °C for two days. Measurements were done by using Mettler Toledo, Model DSC 3, (Schwerzenbach, Switzerland). In an aluminum pan, the gelled samples (5-10 mg) were weighed. The screening was carried out at a temperature of 10 °C, increasing at a rate of 1 °C/minute. As a cooling medium, liquid nitrogen was used. An indium thermogram was used to calibrate the temperature against an empty aluminum container. The DSC thermogram was used to calculate the maximum transition temperature (Tm) and total denaturation enthalpy (ΔH).

X-Ray diffraction analysis

Crystal structures of lyophilized collagen samples were determined using an X-ray diffraction (XRD; PANalytical X'Pert High Score Empyrean, 45kV, 40mA) with CuKa (λ =1.54) radiation in the scanning range of 5 °C to 45 °C at 0.5 °C/min scan speed and 0.02 °C step interval.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of collagen (one repeat was performed) were obtained from 2 mg collagen in about 100 mg of potassium bromide (KBr) under dry conditions. All spectra were performed using a JASCO ATR Pro One Model 6700 FT/IR spectrometer (JASCO International Co. Ltd., Hachioji, Tokyo, Japan) at a data acquisition rate of 4 cm^{-1} from 4000 to 600 cm^{-1}. Analysis of spectral data was performed using Spectra Manager TM II cross-platform software program (JASCO).

UV-Vis measurement

A Cary 100 UV-Vis Spectrophotometer was used to obtain UV spectra of collagen (one repeat) samples (Agilent Technologies). The samples were dissolved in 0.5 M acetic acid at 0.2 mg mL^{-1} concentration. Readings were taken in the 200-400 nm wavelength range against 0.5 M acetic acid (negative control).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed (one repeat) using the Quanta 650 model, FEI®, (Columbus, Ohio, USA). The surface of the samples was made conductive by coating them with Gold-Palladium (Au/Pd) (approximately 2Å/second). Samples were observed using 30 kV, and EDS technique was used to determine the major compounds of the samples surface regions.

Amino acid composition

Collagen samples were hydrolyzed in 6 N HCl for 24 hours at 110 °C under vacuum. HPLC was used to analyze amino acids (Shimadzu model Nexera-X2 device). Two microliters of the derivatized sample were injected into the Shimadzu XR-ODS II shim-pack column. The temperature of the column oven was set to 40 °C. KH_2PO_4 solution (A) (1 mM K_2HPO_4 in water) and (B) Acetonitrile/Methanol/Water (45/40/15) were used as mobile phases. The retention times and peak areas of the standards were used to define and calculate amino acids. All of this method was performed as duplicates from the initial stage.

Statistical Analysis

In the current study, the results are expressed as mean ± standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences SPSS (Pallant 2020). One Way ANOVA was used to determine the differences between groups. The T-test was also used for pair comparisons. Any *p* value below 0.05 ($P<0.05$) were considered as statistically significant.

Results

Collagen Yield

Based on the wet weight, the yields of acid-soluble collagens extracted from mangar skin (ASC-S) and bones (ASC-B) were 9.38% and 3.71%, respectively. Collagen yield obtained from the mangar skin was found to be higher than its bone ($P<0.05$).

Thermal Stability of Collagen by Differential Scanning Calorimeter (DSC)

The maximum transition temperatures (Tmax) of acid-soluble collagen extracted from template skin and bones dissolved in 0.5 M acetic acid are shown in Figure 1. Tmax and enthalpy (ΔH) value of ASC-S was found as 32.62 °C, 0.230 J/g. However, there were three different Tmax and enthalpy (ΔH) values for ASC-B and they were found as 1st 32.66 °C, 0.253 J/g, 2nd 38.12 °C, 0.452 J/g, and 3rd 41.37 °C, 0.320 J/g. The amino acid composition of collagen, particularly the imino acid composition, influences its thermal stability. While proline and hydroxyproline provide the spatial structure of collagen with pyrrolidine rings, hydroxyproline improves collagen's thermal stability by forming inter-chain hydrogen bonds that stabilize the triple helical structure of collagen (Gelse et al. 2003). Therefore, the Tmax value has a positive relationship with the imino acid content. In fact, the reason why the Tmax value of cattle skin collagen is higher than the value we obtained at 40.8 °C is the higher amount of imino acid it contains (Komsa-Penkova et al. 2000).

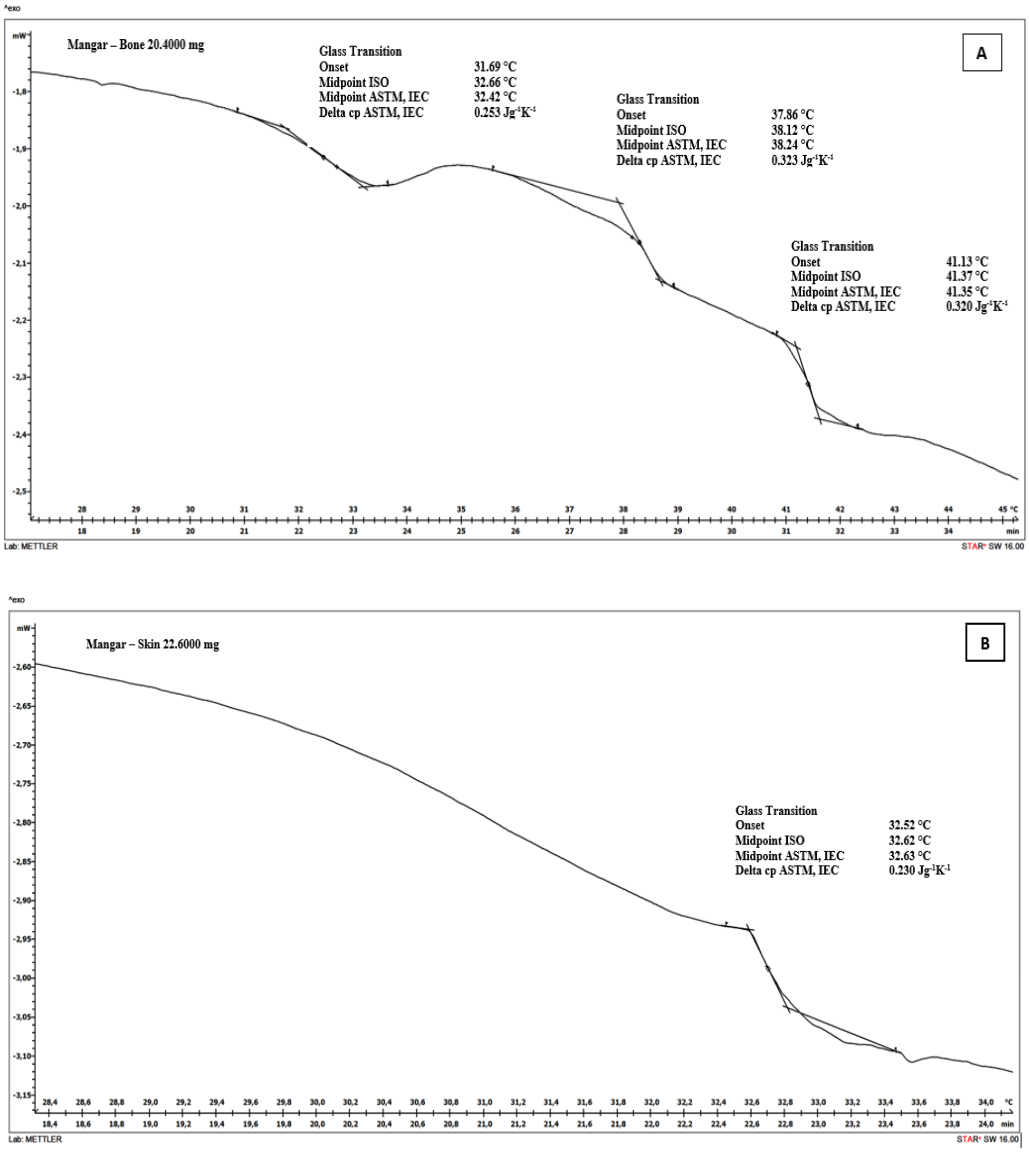

Figure 1. DSC thermogram of ASC-B (a) and ASC-S (b) from the skin and bone of mangar dispersed in 0.05 M acetic acid.

X-Ray Diffraction (XRD)

XRD is frequently used to investigate the crystal structure of polymers. Refraction occurs when an X-ray encounters crystalline particles, and the position and density of the diffraction peak reflect the structural properties of the crystals (Bigi et al. 2001). The XRD curves for both ASC-S and ASC-B have characteristic two break peaks at

diffraction angles (2θ) of approximately 6.93º and 22.78º for ACS-S and 7.68º and 22.17º for ASC-B, as shown in Figure 2. The first sharp peak corresponds to the collagen's triple helix structure, while the second large peak represents the distance between the chains. These findings confirm that both collagens retain their triple helix structure and are not denatured.

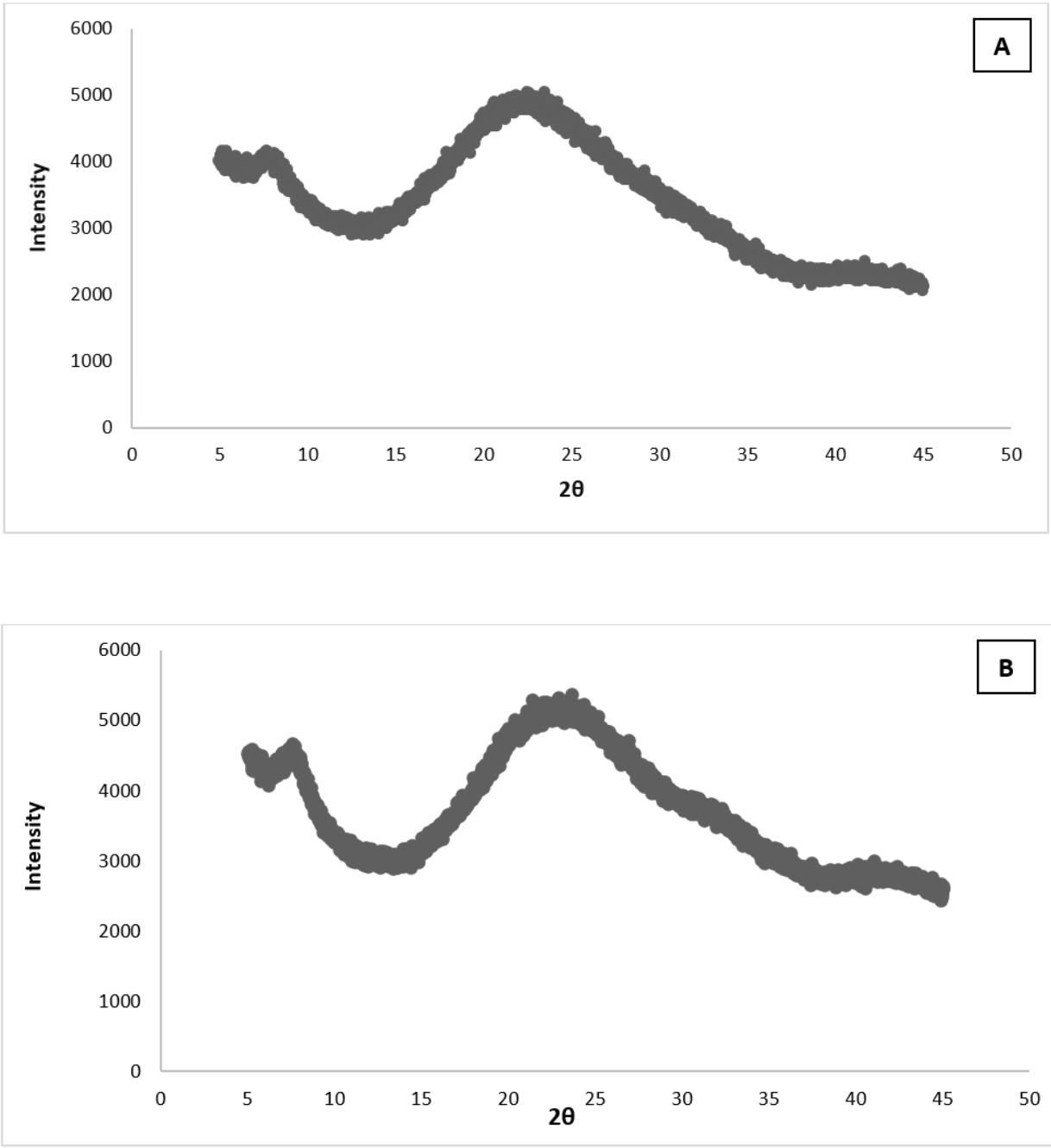

Figure 2. X-ray diffraction spectra of mangar's ASC-S (a) and ASC-B (b).

Fourier Transform Infrared (FTIR)

FTIR spectra of collagen extracted from mangar skin and bones are shown in Figure 3. Collagen exhibited similar spectral properties with five distinct collagen absorption bands (amide A, amide B, and amide I, II, and III), indicating the presence of high proline and hydroxyproline aminoacids in the collagen molecule; these are typical collagen bands and indicate that the collagen obtained was determined to be type I collagen. Amide A absorption peaks of ASC-S and ASC-B were found to be 3219.58 and 3290.93 cm^{-1}, respectively.

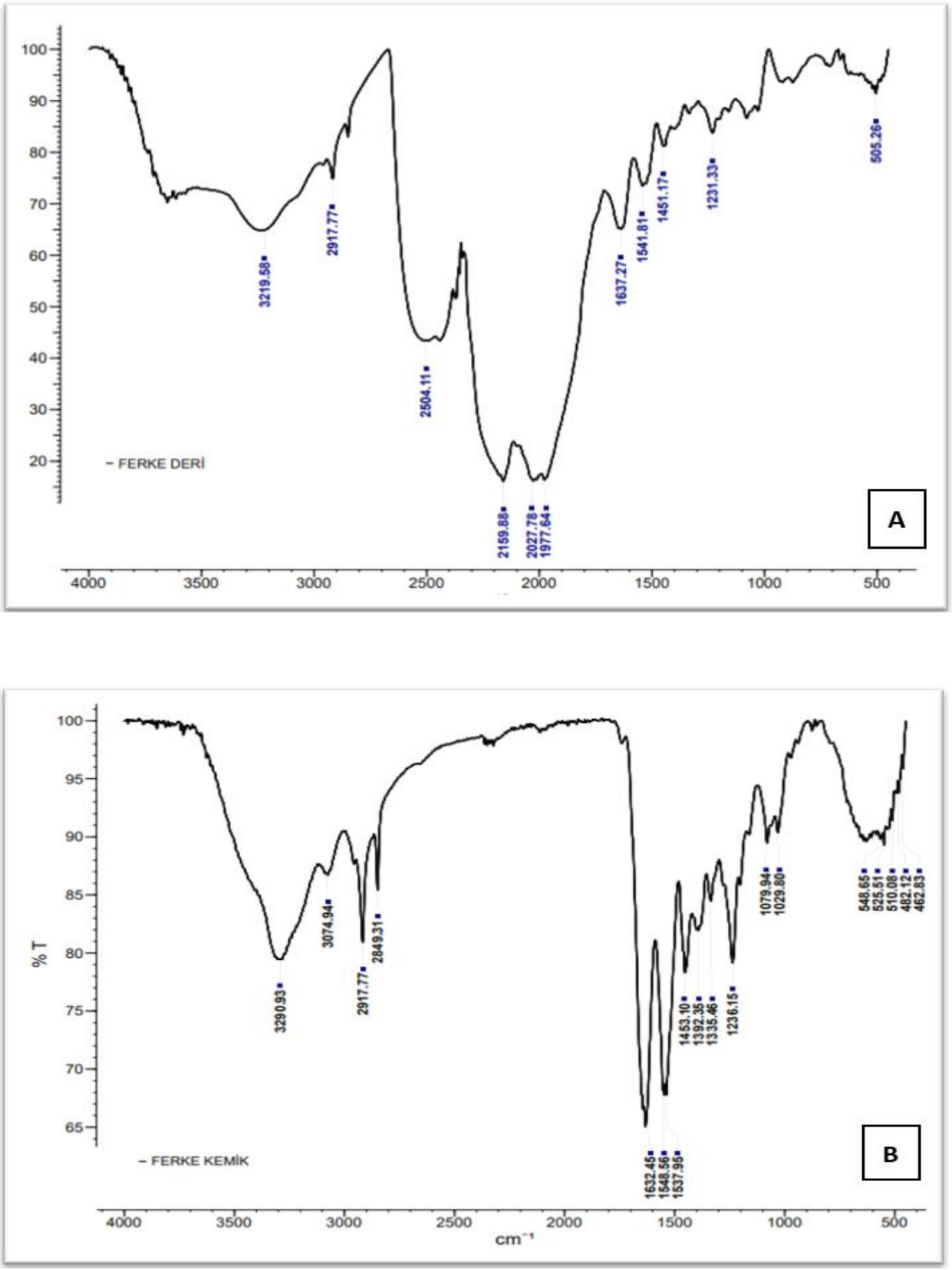

Figure 3. The FTIR spectra of acid-soluble collagens from skin (a) and bone (b) of mangar.

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy Analysis

UV-Vis spectroscopy was used to assess the purity of collagen. ASC-S and ASC-B UV-Vis measurement results are shown in Figure 4. There is a single absorption peak and showed maximum absorbance at 230 and 232 nm, respectively.

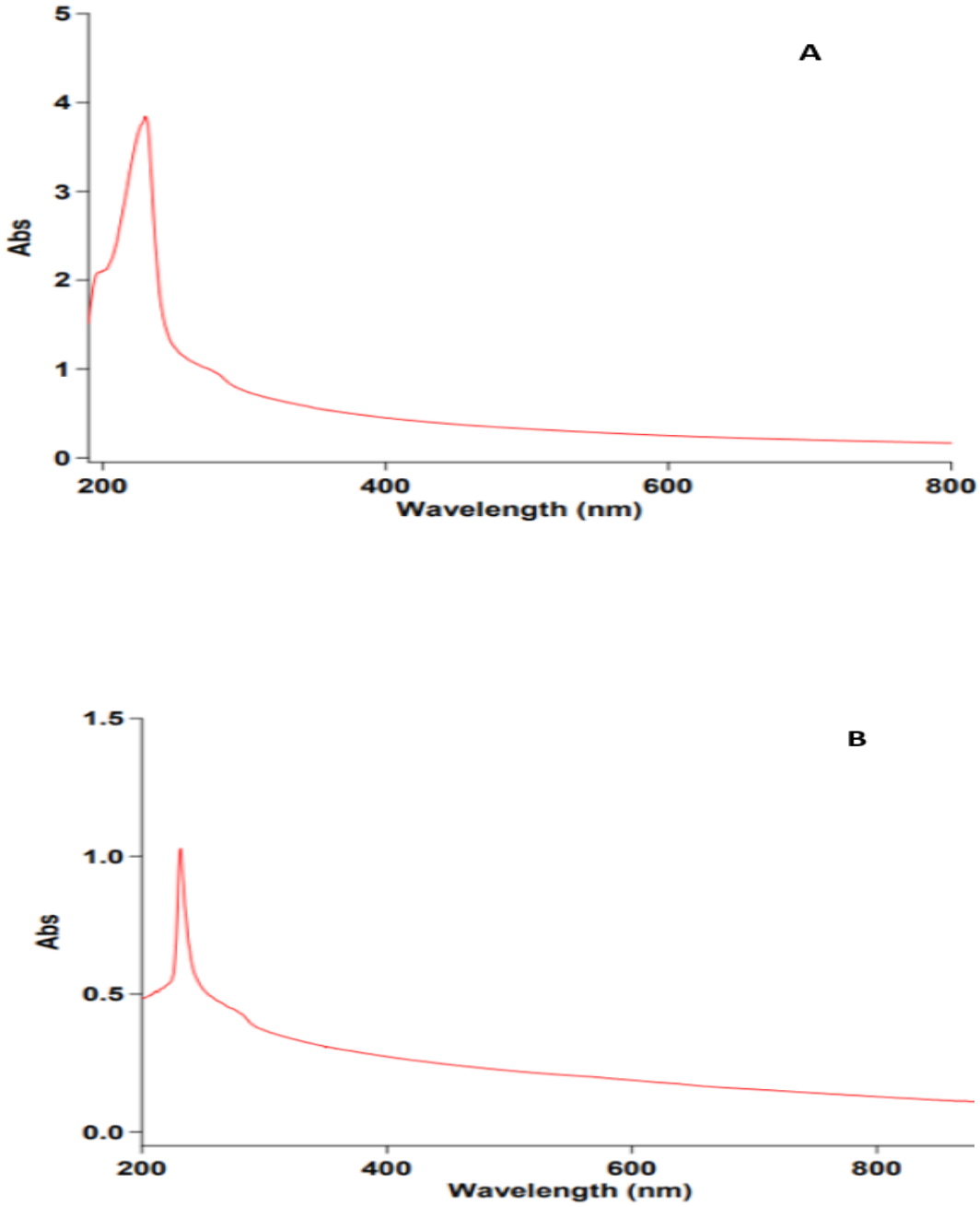

Figure 4. UV- Spectra of acid-soluble collagens from skin (a) and bone (b) of mangar

Scanning Electron Microscopy (SEM)

The morphological structures of the extracted lyophilized (freeze dried) collagens (ASC-S and ASC-B) were visualized by scanning electron microscopy (SEM) under three different magnifications ×200, ×500, ×1000 and ×2,000 (Figure 5 and Figure 6). Both of the lyophilized collagens were seen as soft, white, and spongy with a porous structure when naked eye observations were made. However, SEM analysis revealed that both collagens had a dense, irregular, and partially wrinkled surface image bound by randomly wrapped filaments. This is probably due to dehydration during lyophilization. Likewise, some similar results have been reported by various research such as the observation of collagen obtained from *Arabibarbus gripus* skin and bone (Göçer 2022), *Amur sturgeon* skin (Wang et al. 2014) and *Istiophorus platypterus* skin (Tamilmozhi et al. 2013).

In this study, both of the studied collagens were alike in many ways. They are characterized by poor organization, intersecting fibers, entangled bundles, and some fibrils have intricated meshes in contact with others. Both collagens' fibrils of varying thickness were intertwined throughout the porous matrix. As a result, the collagen SEM images

show that they are type I collagen with a fibrillar structure.

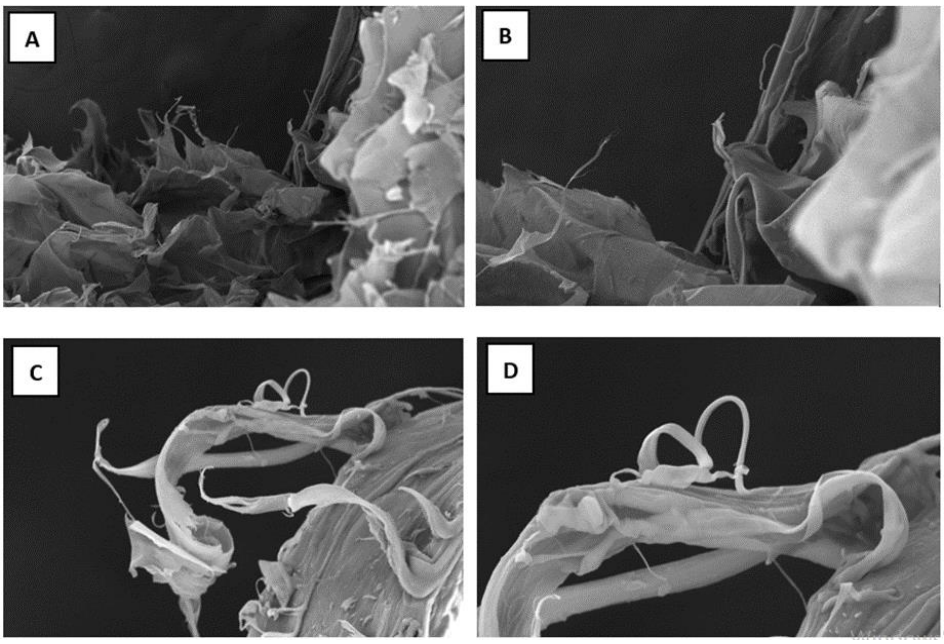

Figure 5. SEM images of acid-soluble collagen from skin of mangar A: ×200, B: ×500, C: ×1.000, D: ×2.000

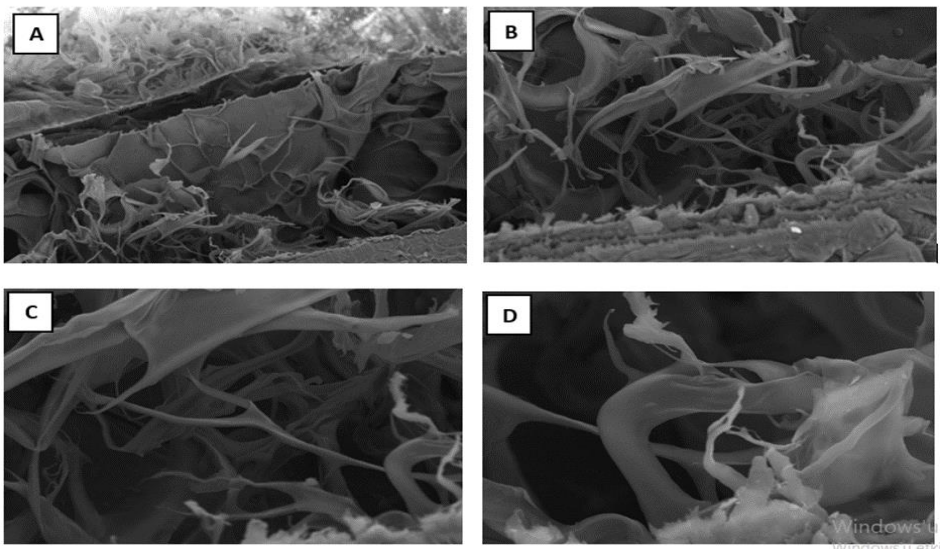

Figure 6. SEM images of acid-soluble collagen from bone of mangar A: ×200, B: ×500, C: ×1.000, D: ×2.000

Amino Acid Composition

Table 1 shows the amino acid compositions of acid-soluble collagen extracted from mangar skin (ASC-S) and bones (ASC-B). Both collagens have amino acid compositions that are similar. Due to the characteristic (Gly-Pro-Hyp) triple-helix repeats of all collagens, the ASC-S and ASC-B samples were high in glycine (Gly), proline (Pro), and hydroxyproline (Hyp). Tryptophan and cystine were not detected. Proline and hydroxyproline found in both ASC-S and ASC-B, which are important imino acids that ensure the structural integrity of collagen. The total amount of imino acid (Pro+Hyp) is 19.32% and 19.17% for ASC-S and ASC-B, respectively, and is statistically similar ($P>0.05$).

Table 1. Amino acid profiles (g/100g protein) of skin (ASC-S) and bone (ASC-B) collagens of mangar

Amino Acid	ASC-S	ASC-B
Aspartic acid	4.91±0.07[b]	5.58±0.06[a]
Glutamic acid	9.46±0.45	9.31±0.17
Serine	3.84±0.07[b]	4.17±0.04[a]
Glycine	29.46±0.56	30.23±0.09
Threonine	3.58±0.33	3.51±0.07
Arginine	7.51±0.26	7.18±0.10
Alanine	9.61±0.18	9.26±0.05
Tyrosine	0.38±0.01	0.36±0.01
Cysteine	0	0
Valine	1.2±0.09	1.06±0.08
Methionine	1.04±0.12	0.97±0.06
Tryptophan	0	0
Phenylalanine	2.17±0.21	1.98±0.03
Leucine	2.39±0.04	2.33±0.08
Lysine	3.25±0.03[a]	2.86±0.11[b]
Hydroxyproline	9.63±0.10	9.48±0.05
Proline	9.69±0.27	9.69±0.22
Total imino acid (Hydroxyproline + proline)	19.33±0.38	19.18±0.24

±, represents standard deviations. The superscript letters [a] and [b] indicate to the statistical differences ($p<0.05$, n=6) between groups within the same line. ND, not determined.

Discussion

Collagen Yield

Similar to the current study, it was found that the yield of collagen obtained from *Priacanthus tayenus* bone (1.6%) to be lower than the yield of collagen obtained from its skin (10.9%) (Kittiphattanabawon et al. 2005). The authors also suggest that their ASC-B yields were lower than the current study, whereas their ASC-S yields were higher. Additionally, the obtained results of this study suggested that the collagen yield of this study is higher than the yield of collagen extracted from carp bone (1.06%) (Duan et al. 2009) by over than 3.5 folds.Unlikely, Doğdu et al. (2019) extracted collagen from silver cheeked pufferfish *Lagocephalus sceleratus* skin and the collagen yield was found to be 50.9%, which is much higher than the current study (Doğdu et al. 2019). A very recent study was accomplished by Jaziri et al. (Jaziri et al. 2022) on skin of lizardfish (*Saurida tumbil* Bloch, 1795) the yields of acetic acid-extracted collagen (AESkC), lactic acid-extracted collagen (LESkC), and citric acid-extracted collagen (CESkC) were 11.73 ± 1.14%, 11.63 ± 1.10%, and 11.39 ± 1.05% (based on wet weight), respectively. That suggests that even if different acids were used in order to extract collagen from skin of *S. tumbil* the collagen yield was around 11%, which is very close to our study results. Reátegui-Pinedo and gis colleagues (Reátegui-Pinedo et al. 2022) isolated collagen from tilapia (*Oreochromis niloticus*) skin the yields were for acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC), 19% and 21%, respectively. In another study, Benjakul et al. (2010) was found the collagen yield (7.7% and 7.1%) extracted from *P. tayenus* and *Priacanthus macracanthus* skin, both of these results represented very close collagen yield to the current study (Benjakul et al. 2010). However, Wei et al. (2019) isolated and characterized collagen from sturgeon fish and reported that the collagen yield was 5.73% (Wei et al. 2019). The current study's results represent about twice as much collagen yield than Wei et al. (2019)'s study. In the current study, the same method which was adopted without any modification or with minor modification of Nagai and Suzuki (2000)

was used as the above-mentioned previous studies. Since all reviewed studies results vary from each other in terms of the collagen yield percentages, all of these studies suggest that the collagen yield could be species specific.

Thermal Stability of Collagen by Differential Scanning Calorimeter (DSC)

In a study conducted by Göçer (2022) Tmax and enthalpy values of ASC-S and ASC-B isolated from *Arabibarbus grypus* were found as 31.59 °C, 0.358 J/g and 32.25 °C, 0.452 J/g, respectively. Whereas, as shown in Figure 1 in this study, Tmax and enthalpy value of ASC-S of was found as 32.62 °C, 0.230 J/g and there were three different Tmax and enthalpy values for ASC-B and they were found as 1st 32.66 °C, 0.253 J/g, 2nd 38.12 °C, 0.452 J/g, and 3rd 41.37 °C, 0.320 J/g. These differences could be due to use of different species for the isolation of ASC-S and ASC-B. The cattle's imino acid content in ASC-S was 19.26%, while the ASC-B was 19.89%. This value was found to be lower than the collagen obtained from many cold climate fish (Ciarlo et al. 1997). This explains why collagen isolated from subtropical and tropical fish have better thermal stability (Hsieh et al. 2016).

X-Ray Diffraction (XRD)

As it mentioned above, Figure 2 shows the XRD curve for both ASC-S and ASC-B has characteristic two break peaks at diffraction angles (2θ) of approximately 6.93º and 22.78º for ACS-S and 7.68º and 22.17º for ASC-B. The first sharp peak is related to the triple helix structure of the collagen, while the second large peak indicates the distance between the chains. These results confirm that both of the collagens preserve the triple helix structure and is not denatured. Similar results have been obtained by several studies include carp scale collagen study by Zhang et al.(Zhang et al. 2007), *O. niloticus* skin collagen (Sun et al. 2017), *Gadus macrocephalus* skin collagen (Sun et al. 2017), Atlantic cod and Atlantic salmon skin collagen (Alves et al. 2017), and *A. grypus* skin and bone (Göçer 2022).

Fourier Transform Infrared (FTIR)

FTIR spectra of collagen extracted from mangar skin and bones showed that Amide A absorption peaks of ASC-S and ASC-B were 3219.58 and 3290.93 cm^{-1}, respectively. According to Sai and Babu (2001), Amide A band generally originates from N-H stress vibration and occurs in the wavelength range of 3400-3440 cm^{-1} (Sai and Babu 2001). However, Doyle et al (1975) mentioned that when the NH group of a peptide is involved in the hydrogen bond, the position can shift to a low frequency, usually around 3300 cm^{-1} (Doyle et al. 1975). Therefore, the shift of amide A towards lower wavelengths, as observed in this study, indicates that hydrogen bonded hydroxyl groups are present in both skin and bone collagens. Amide I band of ASC-S and ASC-B were 1637.27 and 1632.45 cm^{-1}, respectively. These results are consistent with the 1625-1690 cm^{-1} range that is the position of the general amide I bands of collagen. Similar results were acquired by Shalaby and colleagues (Shalaby et al. 2020). Amide II band was found to be 1541.81 cm^{-1} for ASC-S and 1548.56 cm^{-1} for ASC-B, amide II band is generally seen at wavelengths of 1550-1600 cm^{-1} (Krimm and Bandekar 1986), its shift to lower wavelengths represents the formation of hydrogen bond. The triple helix structure of collagen can also be presented by the ratio of the density between the absorption peak of amide III and the absorption peak of 1450 cm^{-1}. In our study, the Amide III absorption peaks of ASC-S and ASC-B were 1231.33 and 1236.15 cm^{-1}, respectively. The ratio of the density between the absorption peak of Amide III and the absorption peak of 1450 cm^{-1} was 1.18 (ASC-S/ASC-B=1.17).

Göçer (2022) found that amide A absorption peaks of ASC-S and ASC-B of *A. grypus* were found to be 3265.86 and 3292.86 cm-1, respectively. The researcher indicated that collagen presents similar spectral properties with five characteristic collagen absorption bands including amide A, B, I, II, and III, indicating the presence of high proline and hydroxyproline amino acids in the collagen molecule, which are considered as typical bands for collagen and they mean that the obtained collagen is type I collagen the same as determined in the current study. Matmaroh et al. (2011) stated that a value approaching 1.0 indicates that collagen still has a triple helix structure.

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy Analysis

This spectroscopy is usually used in order to assess the purity of collagen (Kumar and Rani 2017). As presented in Figure 4, there is a single absorption peak and showed maximum absorbance at 230 and 232 nm, respectively. This range is the distinctive absorbance of type I collagen. Generally, the highest protein absorbance is observed at 280 nm; however, our results have

shown maximum absorbance at 230-232 nm due to the absence of tryptophan amino acid and low tyrosine amino acid content in both ASC-S and ASC-B. Similar study was done by Jaziri and his research group (Jaziri et al. 2022) on skin of lizardfish (*Saurida tumbil* Bloch, 1795) and they found that the max absorbances were for AESkC, LESkC, and CESkC at 230.5 nm, 230.0 nm, and 231.5 nm, respectively. In another study, UV absorption spectrum of *O. niloticus* "tilapia" skin collagen samples were between 232 and 234 (Reátegui-Pinedo et al. 2022).

Scanning Electron Microscopy (SEM)

The morphological structures of the extracted and lyophilized ASC-S and ASC-B were observed as soft, white, and spongy with a porous structure when no magnification applied. However, when SEM results were examined, both of the collagens were found to have a dense, irregular, and partially wrinkled surface image bound by randomly wrapped filaments. This could be due to dehydration during lyophilization. Some similar results have been reported by various research groups, the observation results of collagen obtained from *Salmo salar* L. (Mørkøre et al. 2020), *Amur sturgeon* skin (Wang et al. 2014) and *Istiophorus platypterus* skin (Tamilmozhi et al. 2013).

Amino Acid Composition

The amino acid compositions of ASC-S and ASC-B collagen of mangar presented results as expected. As with other collagens, tryptophan and cystine were not detected (Yata et al. 2001; Muyonga et al. 2004; Jongjareonrak et al. 2005). Proline and hydroxyproline found in both ASC-S and ASC-B are important imino acids that ensure the structural integrity of collagen. The total amount of imino acid (Pro + Hyp) is 19.32% and 19.17% for ASC-S and ASC-B, respectively, and is statistically similar ($P>0.05$). This value is similar to the values reported for *O. niloticus* (19.8 - 19.4%) (Potaros et al. 2009) and Carp (19.4%) (Zhang et al. 2011); higher than the values reported for tilapia, (17.75%), grass carp (17.90%) and silver carp (17.78%) (Tang et al. 2015); lower than the value reported for tilapia (25.4%) (Grossman and Bergman 1992). The difference in imino acid content between different species is due to the different habitats of different species, especially to the difference in temperature (Singh et al. 2011).

In conclusion, collagens were successfully extracted and characterized from mangar skin and bone. Both extracted collagens were type I collagen, with a typical amino acid composition. The FTIR and XRD analyses revealed that their triple helical structure was preserved after the extraction processes. Both extracted collagens demonstrated maximum absorption at 230-232 nm and no absorption at 280 nm. Both collagens' SEM images revealed interconnected pores with lace-like fibers. To conclude, the positive characteristics exhibited by the extracted collagens in this study, there is a high potential for use as a valuable collagen alternative in diet, medical and pharmaceutical (can be used extensively in various medical applications). For example, its strength and flexibility may help in the repair and regeneration of skin and nutraceuticals industries.

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