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Exploring collagen diversity in the bone of mesopotamian catfish (*Silurus triostegus*, Heckel, 1843)

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Abstract

Collagen, a pivotal extracellular matrix biomolecule ubiquitous in connective tissues, drew substantial attention due to its widespread presence, notably in skin and bone. This pioneering study delves into the extraction, characterization, and amino acid profiling of acid-soluble collagens (ASC) obtained from the bone (ASC-B) of the Mesopotamian catfish, *Silurus triostegus,* (Heckel, 1843). Notably, this research marks the inaugural exploration of this species as a collagen source.

Fourier transform infrared spectroscopy (FTIR) confirmed the integrated and native nature of both collagens, while X-ray diffraction (XRD) results indicated the preservation of helical structures in both skin and bone collagens. UV-Vis spectra highlighted prominent absorptions at 230 nm. SEM studies revealed the porous and fibrous structure of ACS-B

Collectively considering UV–Vis and FTIR results alongside the amino acid composition, the extracted collagens were characterized as type I collagen. The collagen isolated from the catfish emerges as a potential alternative source of vertebrate collagens with prospective applications in diverse industries, including diet, medical, and pharmaceutical sectors.

Keywords: Catfish; ASC; XRD; FTIR; Type I collagen; Characterization

1. Introduction

According to fish base [1] *Silurus triostegus*, also known as Mesopotamian catfish or Tigris catfish, belongs to the *Siluridae* family. This species is widely distributed in Iran, Iraq, Syria, and Türkiye. It can reach to max 99 cm in length, weigh up to 8.5 kg, and live about 11 years [2]. While the *Siluridae* family members are widespread across Europe and Asia, their greatest diversity is found in Southeast Asia. Beyond this region, their diversity diminishes in temperate East Asia, the Indian subcontinent, Southwest Asia, and Europe. Notably, silurids are largely absent from central Asia. [3]. *S. triostegus* holds significant economic value. In Adiyaman province of Türkiye, it is mostly caught by locals from Atatürk Dam Lake and consumed as a source of food.

Collagen is the predominant protein in connective tissues, serving as a crucial structural component that imparts strength and elasticity [4,5]. This major fibrous glycoprotein is abundantly present in various parts of the body, including the skin, bones, cartilage, tendons, and other connective tissues in both mammals and fish. In mammals, collagen fibers form a vital network that supports tissue integrity and function, while in fish, collagen provides structural resilience and flexibility, especially in the skin and skeletal system. Its role extends beyond mere structural support, influencing

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cellular processes such as wound healing, tissue repair, and overall biological function across different species [6,7]. Collagen constitutes up to 85% of the skin in mammals and accounts for 25-35% of their total body protein. In contrast, fish skin is composed of up to 96% collagen, with the protein making up 19-38% of the total protein content in fish [8]. The market offers a diverse array of collagen products, yet they differ significantly in their sources and suitability for various dietary needs. Many collagen supplements are derived from farm-raised animals such as cattle, pigs, and chickens, while others are sourced from aquatic organisms including fish, shellfish, jellyfish, and crustaceans. Cattle are more commonly used for collagen production than porcine and fish sources, primarily due to their lower cost and the plentiful supply of skin and bones available for processing [9,10]. However, there is increasing concern about the potential transmission of diseases, such as bovine spongiform encephalopathy, to humans through the consumption of collagen derived from bovine products [11].

Collagen is integral to numerous industries, including those producing nutritious foods, cosmetics, tissue engineering materials, and wound care products, owing to its unique properties [12,13]. Its fibrous structure and resistance to stretching confer strength and flexibility to the skin. Additionally, collagen is vital for tissue formation and the fortification of blood vessels, making it indispensable in applications that require robust and resilient biomaterials [14,15]. Given its excellent biocompatibility and low immunogenicity, collagen is a highly desirable biomaterial in biomedical applications. Beyond its industrial uses, collagen's anti-aging properties are increasingly sought after across various medical specialties, including plastic surgery, burn treatment, and weight management [16,17]. This has prompted numerous studies aimed at identifying alternative collagen sources. Notably, collagen derived from aquatic sources has gained popularity due to its advantages over traditional sources. Fish-derived collagen peptides exhibit higher bioavailability, as they are more easily digested, absorbed, and distributed throughout the body, at a rate up to 1.5 times faster than bovine or porcine collagen [18,19]. Several studies have indicated that even fish waste is a potent source of collagen, highlighting its potential for dietary supplementation [20–24]. Consequently, fish-derived collagen, including that extracted from fish waste, has been recognized as a valuable supplement for dietary purposes. Its high bioavailability and efficient absorption make it an attractive option for enhancing overall health and wellness. The inclusion of fish collagen in the diet not only supports skin elasticity and joint health but also promotes the repair of connective tissues and enhances bone density. Additionally, its amino acid profile, rich in glycine, proline, and hydroxyproline, contributes to various physiological functions, further cementing its role as a beneficial dietary supplement. The sustainable use of fish waste for collagen extraction also aligns with environmental conservation efforts by reducing waste and promoting the utilization of natural resources.

In the current study, the catfish (*Silurus triostegus,* Heckel, 1843) was investigated for its collagen resources. In Türkiye, *S. triostegus* found in the Orontes, Euphrates, and Tigris River system. Given that this catfish is one of the least encountered freshwater species in the Adıyaman province, and there is no existing research on collagen extraction from its bones, this study aims to investigate the feasibility of using the catfish bones as an alternative source of collagen. Specifically, the study seeks to evaluate whether the bones of this catfish can provide a viable and effective collagen source for potential applications in various industries.

2. Materials and Methods

2.1. Materials

A catfish (*Silurus triostegus*, Heckel, 1843) was purchased from a local market in Kahta, Adiyaman in April 2019. Its total length was 37 cm and weighed 1.39 kg. Then the fish was brought to the Çukurova University Biotechnology Laboratory and prepared for research after cleaned. The bones were taken apart from the body and frozen at -20 °C until use. The bones of the catfish were thawed in the refrigerator at +4 °C and later was brought to room temperature before performing the extraction procedure.

2.2. Methods

2.2.1. Sample Preparation

The preparation of collagen samples was conducted with meticulous attention to detail, incorporating minor modifications of Nagai and Suzuki's [25] method to suit the specific requirements of the study. Throughout the entire process, strict temperature control was maintained, ensuring that the procedures were conducted at a temperature not exceeding +4 °C. This meticulous approach guaranteed the preservation of sample integrity and minimized the risk of temperature-induced alterations, thereby ensuring the reliability and reproducibility of the experimental outcomes.

2.2.2. Characterization of Collagens

Collagen Yields

The determination of collagen yield was executed meticulously, employing the specified formula and considering the dry weight of the material. This approach ensured accurate quantification and provided a reliable indicator of the extraction efficiency and yield of collagen from the samples under investigation.

$$Collagen\ Yield\ \left(\frac{g}{100g}\right) = \frac{Weight\ of\ lyophilized\ collagen}{Initial\ weight\ of\ lyophilized\ fish\ bone} \times 100$$

Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) analysis of collagen samples adhered to the established methodology outlined by Kittiphattanabawon et al. (2005)[26], ensuring methodological consistency and comparability with prior studies. Lyophilized collagen samples underwent gelation with 0.05 M acetic acid at a solid/liquid ratio of 1:40 (w/v), followed by refrigerated storage at +4 °C for a duration of two days to achieve optimal gel formation. The measurements were conducted using the Mettler Toledo Model DSC 3 instrument, renowned for its precision and reliability (Schwerzenbach, Switzerland). Subsequently, the gelled samples, weighing between 5 to 10 mg, were meticulously weighed in aluminum pans to facilitate accurate thermal analysis. The DSC screening encompassed a 10 °C temperature range, with temperature increments of 1 °C/min, ensuring thorough assessment of thermal transitions. Liquid nitrogen served as the cooling medium, maintaining precise and stable thermal conditions throughout the analysis.

Temperature calibration was accurately conducted using an indium thermogram, with an empty aluminum container employed as a reference. The resulting DSC thermogram facilitated the calculation of crucial parameters, including the maximum transition temperature (Tm) and the total denaturation enthalpy (H), providing valuable insights into the thermal properties and stability of the collagen samples under investigation.

X-Ray Diffraction Analysis

X-ray diffraction (XRD) analysis, employing the PANalytical X'Pert High Score Empyrean with CuKa (=1.54) radiation, was conducted to identify the crystal structures of lyophilized collagen samples. The scanning range spanned from 5 °C to 45 °C, utilizing a scan speed of 0.5 °C/min and a step interval of 0.02 °C.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of collagen were meticulously acquired under controlled conditions, utilizing 2 mg of collagen dispersed in approximately 100 mg of KBr, ensuring a dry environment. The spectral analyses were conducted employing a JASCO ATR Pro One Model 6700 FT/IR spectrometer, manufactured by JASCO International Co. Ltd., Hachioji, Tokyo, Japan. Data acquisition was conducted at a precise rate of 4 cm^{-1}, spanning the wavelength range from 4000 to 600 cm^{-1}, facilitating comprehensive analysis of collagen's molecular structure and composition. The cross-platform program Spectra Manager TM II was used to analyze spectral data.

UV-Vis Measurement

The UV spectra of collagen samples were meticulously acquired and documented utilizing an Agilent Technologies Cary 100 UV-Vis Spectrophotometer. Prior to analysis, the samples were meticulously dissolved in 0.5 M acetic acid at a concentration of 0.2 mg/mL. Subsequently, readings were systematically taken across the wavelength range of 200–900 nm, with the negative control consisting of 0.5 M acetic acid.

Scanning Electron Microscopy (SEM)

The Quanta 650 model, FEI® (Columbus, Ohio, USA), was used for scanning electron microscopy (SEM). The samples' surfaces were made conductive by coating them with Gold-Palladium (Au/Pd) (about 2Å/sec). Samples were observed at 30 kV, and the EDS technique was used to determine the major compounds of the surface regions of the samples.

3. Results

3.1. Collagen Yield

Based on the wet weight, the yields of acid-soluble collagens extracted from Mesopotamian catfish bones (ASC-B) was 10.81%.

3.2. Thermal Stability of Collagen by Differential Scanning Calorimeter (DSC)

The maximum transition temperatures (Tmax) of acid-soluble collagen extracted from template bones dissolved in 0.5 M acetic acid are shown in Figure 1. Two different Tmax and enthalpy (ΔH) values for ASC-B observed, and they were found as 1st 32.59 °C, 0.418 J/g, 2nd 35.08 °C, 0.166 J/g. The amino acid composition of collagen, especially the presence of imino acids, plays a critical role in determining its thermal stability. Proline and hydroxyproline, with their characteristic pyrrolidine rings, contribute to the structural integrity of collagen. Specifically, hydroxyproline enhances collagen's thermal stability by facilitating the formation of inter-chain hydrogen bonds, which reinforce the triple helical structure of collagen [27,28]. Therefore, the Tmax value usually considered to have a positive relationship with the imino acid content [29].

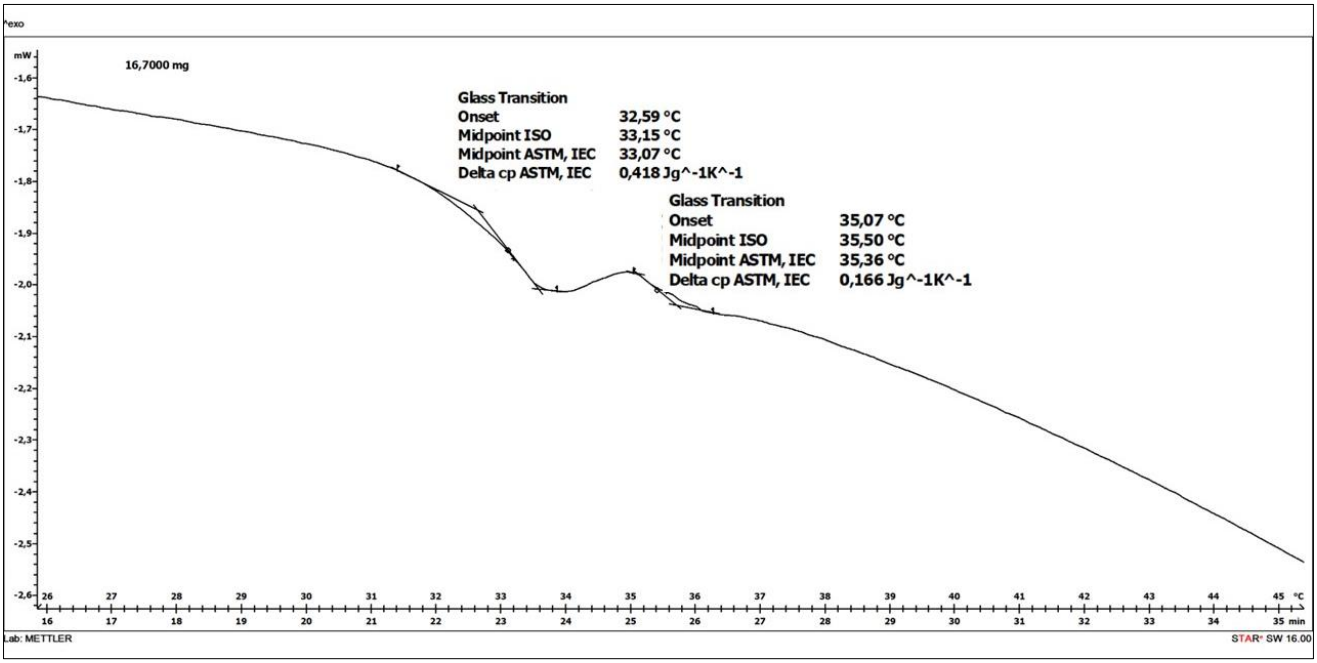

Figure 1 DSC thermogram of ASC-B from the bone of Mesopotamian catfish dispersed in 0.05 M acetic acid

3.3. X-Ray Diffraction (XRD)

As shown in Figure 2, the XRD curve for ASC-B has characteristic two break peaks at diffraction angles (2θ) of approximately 11.84° and 23.98° for ASC-B. The initial prominent peak corresponds to the triple helix configuration inherent to collagen, reflecting its fundamental structural integrity. Meanwhile, the subsequent prominent peak signifies the inter-chain spacing within the collagen molecules. These findings validate the preservation of the triple helix structure in both collagen samples, indicating their resistance to denaturation and reaffirming their structural stability. This suggests that both collagen variants maintain their native conformation, ensuring their suitability for various applications requiring intact collagen structures.

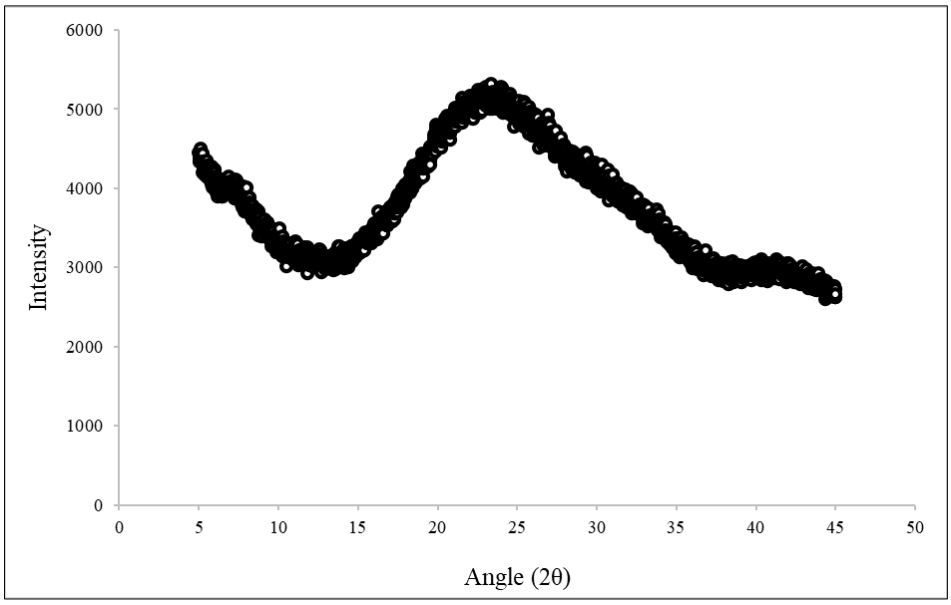

Figure 2 X-ray diffraction spectra of Mesopotamian catfish's ASC-B

3.4. Fourier Transform Infrared (FTIR)

FTIR spectra of collagen extracted from catfish bones is presented in Figure 3. The spectral analysis of collagen revealed consistent characteristics across samples, exhibiting five discernible absorption bands associated with collagen molecules: amide A, amide B, and amide I, II, and III. This pattern indicates a notable abundance of proline and hydroxyproline amino acids within the collagen structure. Such distinct absorption bands are indicative of type I collagen, affirming the identity and composition of the obtained collagen samples. The most common pattern in fish is collagen type I, which consists of bands of -chains (α-1 and α-2) and their dimers (β-components) [30]. Cao et al. characterized both the acid solubilized collagen (ASC) and pepsin solubilized collagen (PSC) extracted from European eel (*Anguilla anguilla*) muscles and found that there was not any apparent difference in the thermal stability of both collagens, probably due to the synergistic effect of molecular weight and hydrogen bonds [31].

According to the obtained results amide A absorption peak of ASC-B was 3298.64 cm^{-1}.

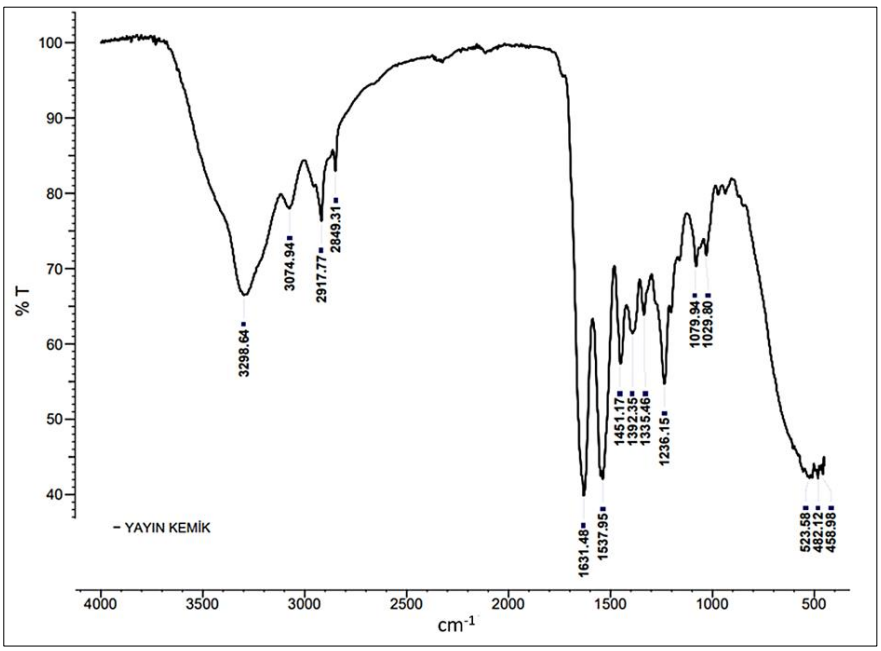

Figure 3 The FTIR spectra of acid-soluble collagens from bone of Mesopotamian catfish

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

UV-Vis spectroscopy serves as a reliable method for assessing the purity of collagen samples [32]. By measuring the absorbance of ultraviolet and visible light across a range of wavelengths, this technique provides valuable insights into the composition and quality of collagen preparations. Through the analysis of absorption patterns, researchers can discern the presence of impurities or contaminants within collagen samples. In the current study, Figure 4 illustrates the UV-Vis measurement results for ASC-B. A solitary absorption peak is observed, with maximum absorbance recorded at 232 nm.

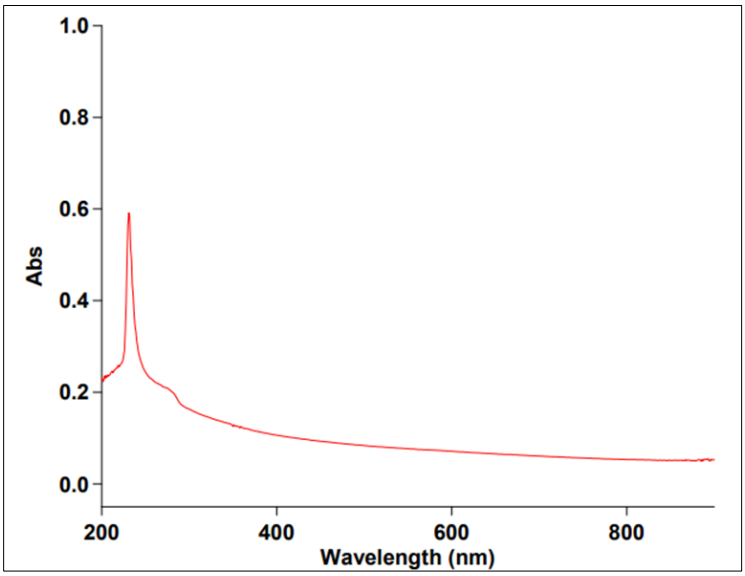

Figure 4 UV- Spectra of acid-soluble collagens from bone of Mesopotamian Catfish

3.6. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was employed to examine the morphological structures of the extracted lyophilized ASC-B at four different magnifications (×200, ×500, ×1000, and ×2,000), as depicted in Figure 5. While the lyophilized collagen samples exhibited a soft, white, and spongy appearance with a porous structure visible to the naked eye, scanning electron microscopy (SEM) revealed a different morphology. The SEM images depicted a dense, irregular surface with partial wrinkling, characterized by randomly arranged filaments. These surface features are believed to result from dehydration during the lyophilization process, a phenomenon consistent with findings reported in previous studies, such as collagen obtained *Amur sturgeon* skin [33], *Mastacembelus mastacembelus* skin and bone [34], *Istiophorus platypterus* skin [35].

In this study, the organization of collagen was observed to be lacking, poorly organized, characterized by intersecting fibers, entangled bundles, and fibrils forming intricate meshes in contact with neighboring structures. Within the porous matrix, fibrils of varying thickness were found to be intertwined. Consequently, the scanning electron microscopy (SEM) images suggest that both collagen samples can be classified as type I collagen, exhibiting a fibrillar structure.

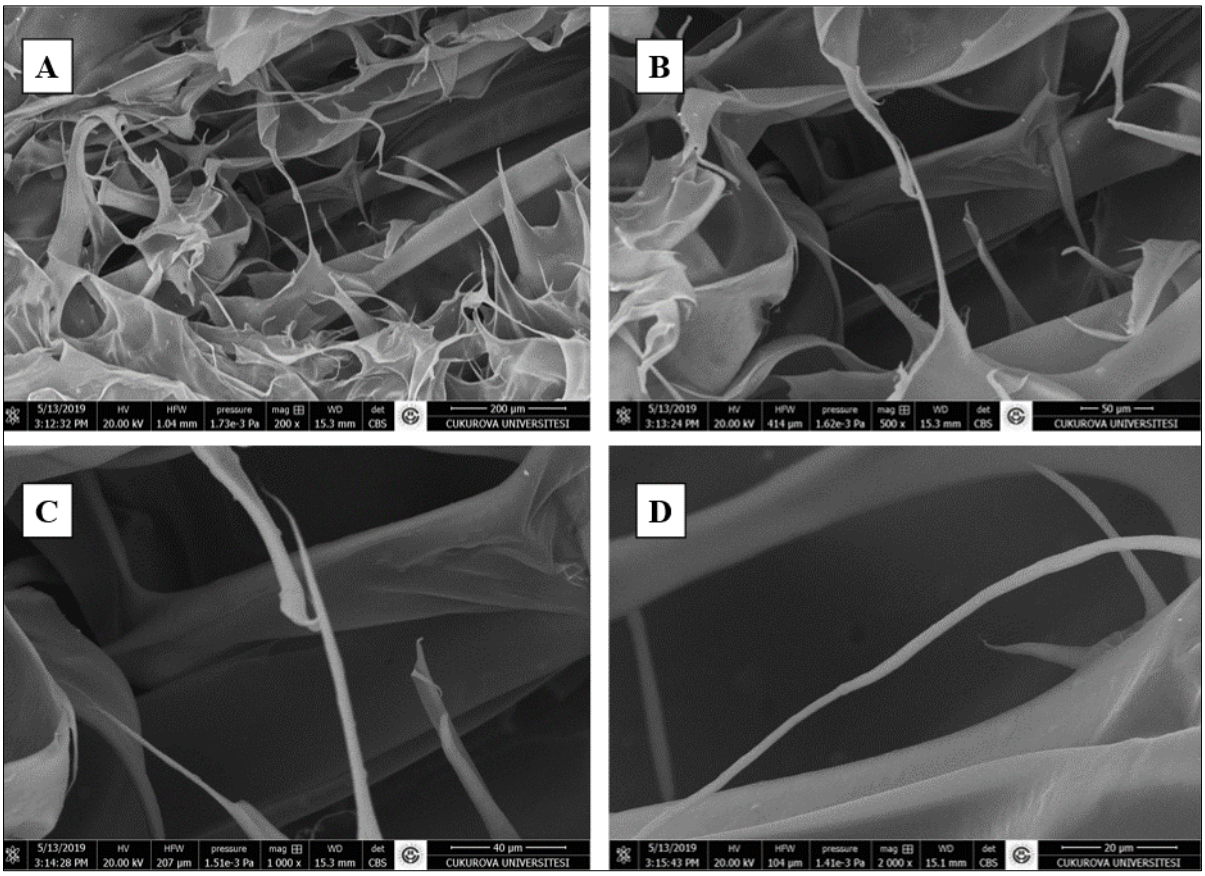

Figure 5 SEM images of acid-soluble collagen from bone of Mesopotamian catfish A: ×200, B: ×500, C: ×1.000, D: ×2.000

4. Discussion

he variability in collagen yield across different extraction sources and methodologies underscores the importance of understanding and optimizing extraction protocols to maximize collagen extraction efficiency. In line with previous studies, our investigation revealed differences in collagen yield between bone and skin extraction from Mesopotamian catfish. Moreover, comparisons with other studies on collagen extraction from various fish species highlight the influence of factors such as species-specific collagen content, tissue source, and extraction methods on yield outcomes. These findings prompt further exploration into refining collagen extraction techniques to enhance yield and ensure the efficient utilization of fish-derived collagen for various applications.

As previously mentioned, Figure 2 depicts the X-ray diffraction (XRD) profile for ASC-B, wherein distinctive twin peaks are evident at specific diffraction angles (2θ). The initial peak, sharp in nature, corresponds to the characteristic triple helix structure inherent to collagen. Subsequently, a more pronounced peak indicates the inter-chain spacing within the collagen molecules. These observations affirm the preservation of the triple helix structure in bone collagens, indicating their resilience against denaturation. Notably, analogous findings have been reported in previous studies, such as the investigation on carp scale collagen conducted by Zhang et al.[40], *Oreochromis niloticus* skin collagen [41], *Gadus macrocephalus* skin collagen (Sun et al. 2017b), Atlantic cod and Atlantic salmon skin collagen [42], and skins and bones of *Arabibarbus grypus* [43], *Luciobarbus esocinus* [44], *Alburnus tarichi* [45] and *Mastacembelus mastacembelus* [34].

FTIR spectra of collagen extracted from Mesopotamian catfish bones showed one Amide A absorption peak of ASC-B was 3298.64 cm^{-1}. According to Sai and Babu (2001), the Amide A band, associated with N-H stretching vibrations, falls within the range of 3400-3440 cm^{-1} [46]. However, Doyle and colleagues (1975) noted that when a hydrogen bond involving the NH group of a peptide is present, the band's position may shift to lower frequencies, around 3300 cm^{-1} [47]. The observed shift in the Amide A band towards lower wavelengths in this study suggests the presence of hydrogen-bonded hydroxyl groups in bone collagen.

This spectroscopy is commonly employed for evaluating the purity of collagen [32]. As depicted in Figure 4, a single absorption peak is evident, with maximum absorbance observed at 232 nm for ASC-B. This specific absorbance range is indicative of type I collagen. Typically, proteins exhibit their highest absorbance around 280 nm. However, our results demonstrate a maximum absorbance at 232 nm, attributed to the absence of tryptophan amino acid and low tyrosine amino acid content in ASC-B.

Upon meticulous examination, intriguing morphological features of the extracted and lyophilized ASC-B became apparent. Initially, it presented as soft, white, and spongy, exhibiting a porous structure discernible to the unaided eye. However, upon closer scrutiny with scanning electron microscopy, a stark contrast emerged. The SEM analysis unveiled a complex morphology, characterized by a dense, irregular surface, interspersed with partial wrinkling, and intertwined with randomly arranged filaments. These observations may be linked to the dehydration process inherent to lyophilization, a phenomenon documented in analogous studies focusing on collagen derived from *Salmo salar* skin [48], *Amur sturgeon* skin [33], *Istiophorus platypterus* skin [35], *Mastacembelus mastacembelus* [34], *Arabibarbus grypus* [43], *Luciobarbus esocinus* [44], and *Alburnus tarichi* [45] skins and bones.

5. Conclusion

In summary, the successful extraction and thorough characterization of collagen from Mesopotamian catfish bone have been accomplished. The extracted collagen was unequivocally identified as type I collagen, exhibiting a typical amino acid composition indicative of its classification. Crucially, the preservation of its triple helical structure post-extraction was confirmed through rigorous FTIR and XRD analyses. Notably, the absence of absorption at 280 nm and the prominent absorption peak at 232 nm for ASC-B further corroborated its type I collagen identity. Additionally, SEM imaging provided valuable insights, revealing interconnected pores and intricate lace-like fibers within the collagen matrix.

In conclusion, the commendable attributes demonstrated by the extracted collagen underscore its significant potential as a viable alternative in diverse applications spanning the realms of diet, medical, and pharmaceutical industries. Its inherent strength and flexibility hold promise for facilitating skin repair and regeneration, while its suitability for incorporation into nutraceutical formulations further broadens its potential utility.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declares no conflicts of interests

Author contributions

Concept: Yasemen YANAR, Mustafa GÖÇER, and Muhsin AYDIN; Sample Collection: Mustafa GÖÇER; Methodology and applications: Yasemen YANAR and Mustafa GÖÇER; Literature review, data collection or processing: Yasemen YANAR, Mustafa GÖÇER, and Muhsin AYDIN; Writing: Mustafa GÖÇER and Muhsin AYDIN.

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