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Sustainable Collagen Extraction from Fish Bones: A Green Solution for Waste Management

Balık Kemiklerinden Sürdürülebilir Kolajen Ekstraksiyonu: Atık Yönetimi için Yeşil Bir Çözüm

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ABSTRACT

The global food industry's exponential growth has made it crucial to assess food waste. In this study, collagen extraction from waste fish bones was carried out in the presence of 0.5 M acetic acid. The physicochemical properties of the successfully obtained collagen were determined by Ultraviolet and Visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), and Differential Scanning Calorimetry (DSC). The results of UV-Vis Spectra of collagen showed maximum absorption at 238 nm. The extracted collagen was found to have a triple helix structure by UV-Vis and FTIR analysis. It was determined that it was semi-crystalline with the XRD diffraction pattern. The thermal denaturation temperature was between 129 ºC and 141 ºC with a flow rate of -4.881 mW (141 ºC) and the enthalpy change (ΔH) was 39.2 mJ/mg. The study has shown that sufficient collagen can be isolated from fish waste simply and inexpensively. Moreover, the present study found that collagen obtained from fish processing waste can be used as a high-value-added material in many areas for various industrial purposes, such as the cosmetics and pharmaceutical industries. Most importantly, processing waste can be utilized, and environmental pollution can be prevented.

Key Words
Collagen, extraction, fish waste, sustainability.

ÖZ

Küresel gıda endüstrisinin hızlı büyümesi, gıda atıklarının değerlendirilmesini zorunlu hale getirmiştir. Bu çalışmada, atık balık kemiklerinden kolajen ekstraksiyonu 0.5 M asetik asit kullanılarak gerçekleştirilmiştir. Elde edilen kolajenin fiziko-kimyasal özellikleri, Ultraviyole ve Görünür Bölge Spektroskopisi (UV-Vis), Fourier Transfer Infrared Spektroskopisi (FTIR), X-ışını Kırınımı (XRD) ve Diferansiyel Tarama Kalorimetrisi (DSC) ile belirlenmiştir. Kolajenin UV-Vis Spektrumu sonuçları, maksimum emilimin 238 nm'de olduğunu göstermiştir. UV-Vis ve FTIR analizleri, ekstrakte edilen kolajenin üçlü sarmal yapıya sahip olduğunu ortaya koymuştur. XRD kırınım desenine göre kolajenin yarı kristalin olduğu belirlenmiştir. Termal denatürasyon sıcaklığı 129 ºC ile 141 ºC arasında olup, akış hızı -4.881 mW (141 ºC) ve entalpi değişimi (ΔH) 39.2 mJ/mg olarak ölçülmüştür. Çalışma, balık atıklarından yeterli miktarda kolajenin basit ve ekonomik bir şekilde izole edilebileceğini göstermiştir. Ayrıca, bu çalışmada balık işleme atıklarından elde edilen kolajenin, kozmetik ve farmasötik endüstriler gibi çeşitli endüstriyel amaçlar için yüksek katma değerli bir malzeme olarak kullanılabileceği belirlenmiştir. En önemlisi, işleme atıkları değerlendirilebilir ve çevre kirliliği önlenebilir.

Anahtar Kelimeler
Kolajen, ekstraksiyon, balık atığı, sürdürülebilirlik.

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INTRODUCTION

Fishing and seafood play a crucial role globally, with nearly 70% of the Earth's surface covered by water, thus providing a significant source of nutrition and livelihood for many communities worldwide [1-2]. Over recent years, the consumption of fish and seafood products has notably increased, largely because these foods are considered essential components of a balanced diet and a healthy lifestyle [3-5]. Seafood is an excellent source of high-quality protein [2], vitamin D [6], iodine [7], and long-chain unsaturated fatty acids such as docosahexaenoic acid (DHA) [8]. These nutrients are critical for human health, highlighting the importance of regularly incorporating seafood into our diets.

Despite the nutritional value of fish, more than half of the fish tissues—including viscera, skin, heads, and fins—are discarded as "waste" by the fish processing industry at various stages [9]. This practice results in the production of vast amounts of fish waste, not only in Turkey but across the globe [10-11]. Such waste poses serious environmental concerns; when disposed of improperly, it damages terrestrial and aquatic ecosystems, leading to a decrease in oxygen levels in marine environments and contributing to the spread of various pathogenic microorganisms [1,12]. Addressing this issue, the European Commission's Blue Growth Plan emphasizes the efficient use of biomass waste by promoting its recycling and repurposing as a valuable input in chemical production, thereby aligning with a circular economy model [13].

Fish biomass presents a valuable source of collagen, the most abundant structural protein in the extracellular matrix of connective tissues, including skin, bones, ligaments, tendons, and cartilage [14,15]. Collagen has extensive applications in biomedical fields due to its versatility as a biopolymer, making it particularly useful in cosmetics and tissue engineering for human health applications [16–18]. Traditionally, collagen has been derived from terrestrial sources such as cattle [19], frog skin [20], chicken, and pig [21]; however, with increasing demand, there is a need for more sustainable, cost-effective sources that reduce reliance on land animals. In biomedical applications, collagen is widely utilized in drug and gene delivery systems, tissue engineering, absorbable surgical sutures, bone regeneration materials, and wound care dressings [3–8]. Its role in wound healing is especially critical, where it functions as a natural scaffold that supports new tissue growth and plays an essential role across all stages of healing, from hemostasis and inflammation to proliferation and tissue remodeling.

In this context, marine biomass has emerged as a promising and increasingly attractive source of collagen [23]. Fish skin, scales, fins, and bones are particularly rich in collagen [24]. Marine-derived collagen is gaining attention for several unique qualities: it has a lower molecular weight, is more readily absorbed by the human body, and possesses high biocompatibility. Additionally, marine collagen carries a lower risk of transmitting animal diseases or pathogens, is more environmentally friendly, and faces fewer religious and ethical restrictions [26]. Nevertheless, marine collagen does have certain limitations. Its relatively low melting and denaturation temperatures limit its range of applications [23-27]. Thus, optimizing the extraction and purification methods for marine collagen is essential for enhancing its physicochemical and biological properties, ultimately expanding its potential uses in various fields.

In this study, collagen extraction from fish waste was targeted using an acetic acid-assisted method, and comprehensive analyses were conducted to characterize the obtained collagen. The extracted collagen's physicochemical properties, including its structural integrity, thermal stability, and purity, were determined using UV-Vis spectroscopy, FTIR, XRD, and DSC techniques. A diagram summarizing the process steps is presented below (Figure 1). This approach aims to provide a sustainable solution for utilizing fish processing waste, potentially reducing environmental impact and promoting the application of collagen in various industrial fields such as cosmetics and pharmaceuticals.

MATERIALS and METHODS

Materials

Fish waste samples were gathered from a local fisherman and the Adem Doruk Trout Facilities located in Etimesgut, Ankara. These samples were transported to the laboratory in a cold chain bag to maintain their integrity and were subsequently stored at -20ºC until use. Chemicals used in the study, including sodium hydroxide (NaOH), acetic acid, and sodium chloride (NaCl), were sourced from Merck KGaA in Darmstadt, Germany. Additionally, butyl alcohol and dialysis tubing made from a cellulose membrane were purchased from Sigma Aldrich Supelco, based in Massachusetts, United States.

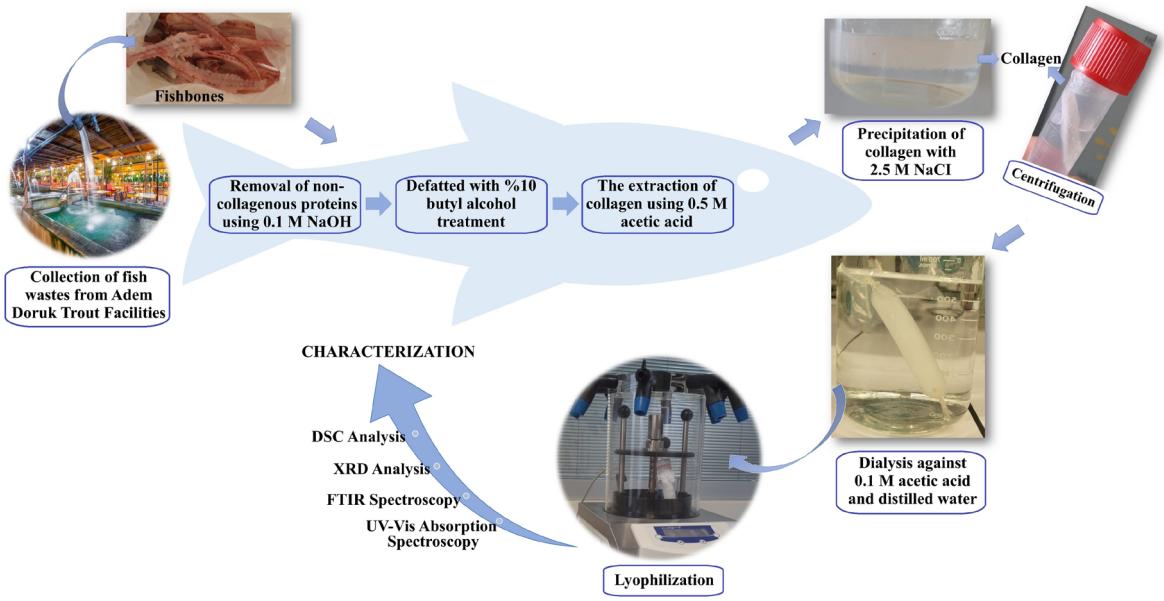

Figure 1. Schematic representation of the processes of collagen extraction.

Pre-Treatment Procedure

The collected bone and skin materials were carefully separated under a cold chain. The bones were finely chopped into small pieces using a blender and washed with pure water. All preparations were meticulously carried out around a low-temperature ice bath to ensure precision. A specific quantity of bone samples was placed in a 0.1 M NaOH solution (1:30 w/v) at +4°C for 3 days to remove non-collagenous proteins, with the solution being renewed every 24 hours to maintain effectiveness. On the final day, the samples were washed with distilled water repeatedly until a neutral pH was achieved. Following this, the samples were submerged in a 10% butyl alcohol solution at a 1:10 ratio (bone/ml solution) for 3 days at +4°C to remove oils, with the solution refreshed daily (Figure 2). After obtaining the oil-free residue, the samples were thoroughly washed with cold distilled water to prepare them for the next processing stage [28-29].

Extraction Procedure

Following the pre-treatment process, the prepared samples were transferred into a 0.5 M acetic acid solution and maintained at 4°C for three days, with the solution replaced every 24 hours to ensure consistency. After this extraction period, the solutions were carefully filtered to remove any solid residues, and each filtrate was collected into a separate container. Once all extracts were combined, 2.5 M NaCl was added to the pooled solution to initiate collagen precipitation. The resulting precipitated collagens were then subjected to centrifugation at 9,000 rpm for one hour at 4°C to separate them from the supernatant. The collagen precipitate obtained was subsequently dissolved in 0.5 M acetic acid. To further purify the collagen, the solution underwent dialysis, initially against 0.1 M acetic acid and then against distilled water, allowing for the gradual removal of residual salts and other small molecules. (Figure 3) Finally, the purified collagen gels were freeze-dried to obtain a stable, dry collagen powder, completing the extraction process [28-29].

Characterization of Collagens

To characterize the extracted collagens, multiple analyses were performed to assess their structural, chemical, and thermal properties:

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy Analysis: UV-Vis absorption spectroscopy was conducted using the Thermo Scientific Genesys 150 UV-Vis Spectrophotometer. A standard collagen solution, prepared in 0.5 M acetic acid at a 0.2 mg/mL concentration, was scanned over a wavelength range of 200–1100 nm. This analysis provided insight into the absorption characteristics of collagen, useful for understanding molecular interactions within the sample.

Fourier-Transform Infrared (FTIR) Spectroscopy: FTIR spectroscopy was employed to investigate functional groups, adsorption peaks, fingerprint regions, and the

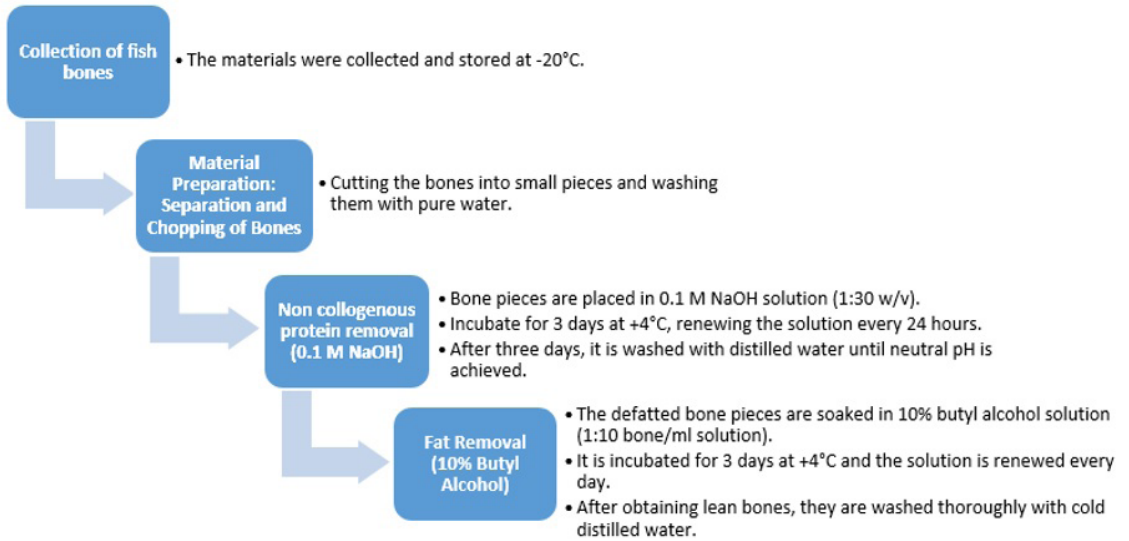

Figure 2. Pre-treatment procedure for collagen extraction from fish bones.

secondary structure of collagen molecules. This analysis used the JASCO FT/IR-6X FTIR spectrophotometer, covering a wavenumber range of 400-4000 cm^{-1} with a resolution of 2 cm^{-1}. By examining the vibrations and movements within the collagen molecule, FTIR provided detailed information about chemical bonding and the overall structure.

X-ray Diffraction (XRD) Analysis: XRD analysis was conducted to examine the crystalline structure of the collagen samples. Using an APD 2000 PRO XRD instrument equipped with a Cu beam tube (Co, voltage: 40 kV, current: 30 mA, wavelength: (CoKa) 1.54 Å), XRD patterns were recorded at a scanning speed of 0.01°/min across a range of 10° to 90°. This technique allowed for the identification of crystalline phases and provided insight into the alignment and packing of collagen fibers within the sample.

Differential Scanning Calorimetry (DSC): To assess the thermal properties of collagen, DSC analysis was performed using a Hitachi DSC 7020 Differential Scanning Calorimeter. The analysis was conducted under a nitrogen atmosphere with a heating rate of 10 °C/min across a temperature range of 50 to 400 °C, with an empty pan serving as the reference. DSC analysis allowed for the observation of thermal transitions, including denaturation temperatures, offering insight into the thermal stability and behavior of the collagen polymer.

These analyses collectively provided comprehensive information on the structural integrity, chemical properties, and thermal stability of the collagen samples.

RESULTS and DISCUSSION

Absorption Spectra

The UV-Vis absorption spectra of collagen isolated from fish wastes, presented in Figure 4, show characteristic absorption peaks at 238 nm and 280 nm wavelengths. Pure type I collagen typically displays a strong absorption peak within the wavelength range of 220–240 nm, which is associated with the $n \rightarrow \pi^*$ transition of the C=O group in peptide bonds, as well as the -COOH and $-CONH_2$ groups in the triple-helical polypeptide chains [30-31]. In this study, a prominent peak was observed at 238 nm, consistent with the findings in prior collagen research.

For example, Bhuimbar et al. [24] reported an absorption peak at 232 nm in collagen extracted from ***Centrolophus niger*** fish waste using an acidic extraction method, a result that aligns well with the absorption range observed here. Similarly, an acidic extraction of collagen from ***Mustelus mustelus*** produced a maximum absorption peak at 235 nm [31]. Research on collagen isolated from bluefin tuna showed an absorption peak at 238 nm [32], matching the main peak observed in this study. These findings are consistent across various studies, supporting the absorption characteristics of the collagen extracted in this research.

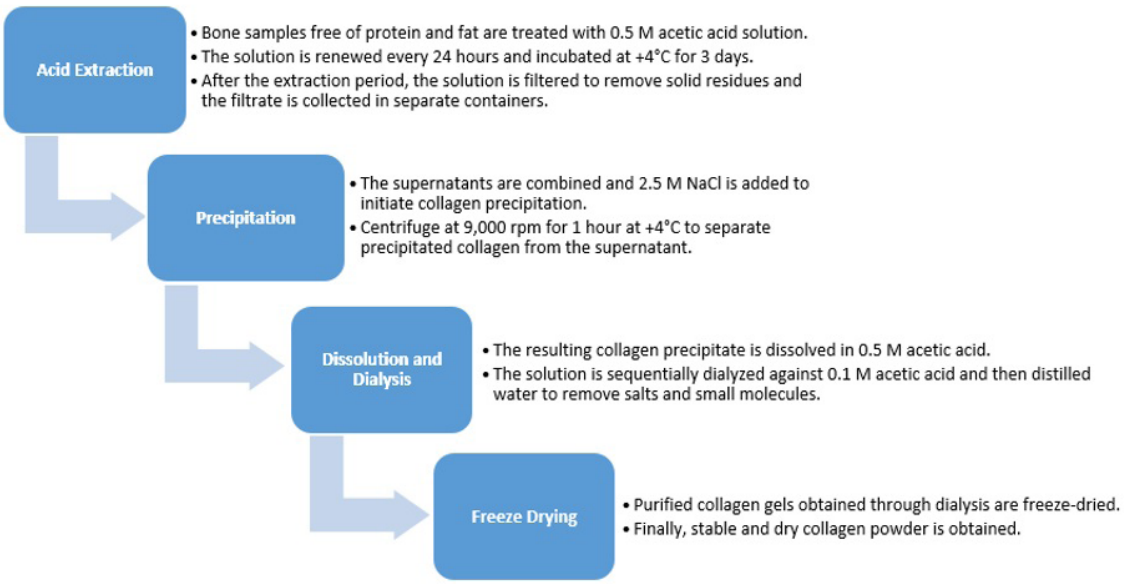

Figure 3. Extraction procedure for collagen isolation from fish bones.

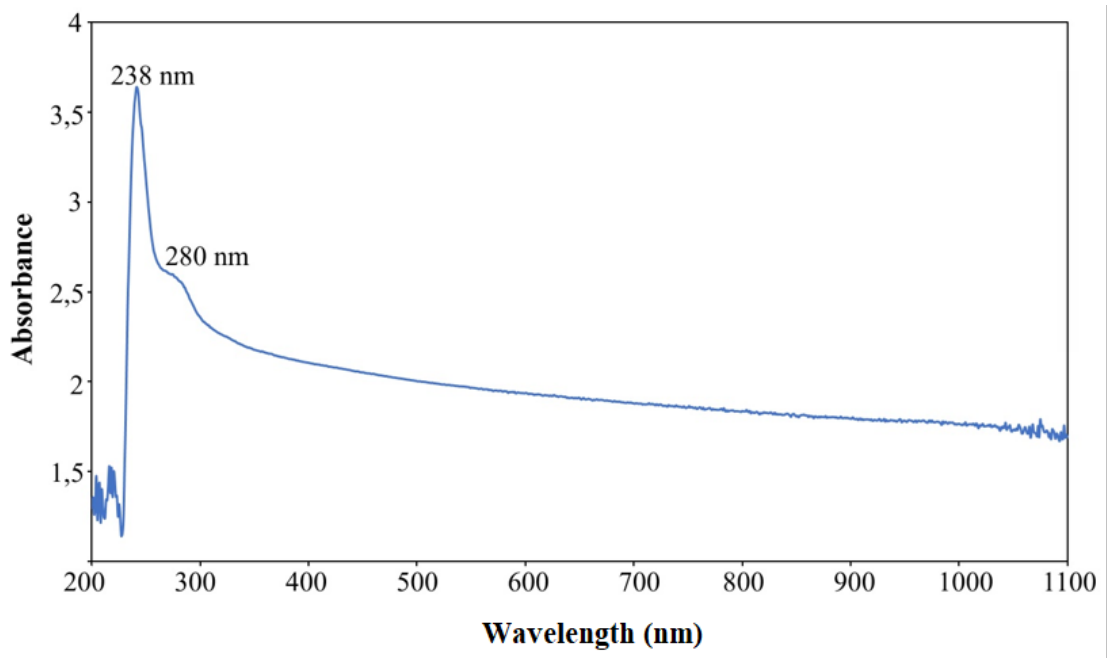

Figure 4. The UV absorption spectrum of collagen from fish wastes.

Collagen's amino acid composition includes small amounts of tyrosine and phenylalanine, with no tryptophan, as previously noted [33, 34]. The low-intensity peak at 280 nm corresponds to the presence of these aromatic amino acids, found in limited quantities within the collagen structure. This lower peak intensity serves as an additional indicator of the collagen extract's purity, as it reflects the minimal presence of aromatic residues. This observation aligns with other studies that associate the 280 nm peak with a low content of aromatic amino acids, further confirming the purity of the extracted collagen [24, 35-38].

FTIR Spectroscopy Analysis

The FTIR spectrum of collagen is illustrated in Figure 5. According to Doyle [39], the amide A band is associated with the N-H stretching frequency. Previous studies by Duan et al. [40], Li et al. [41], and Yan et al. [42] have identified the N-H stretching vibration at 3313.85 cm^{-1}, 3335 cm^{-1}, and 3328.57 cm^{-1}, respectively. Yan et

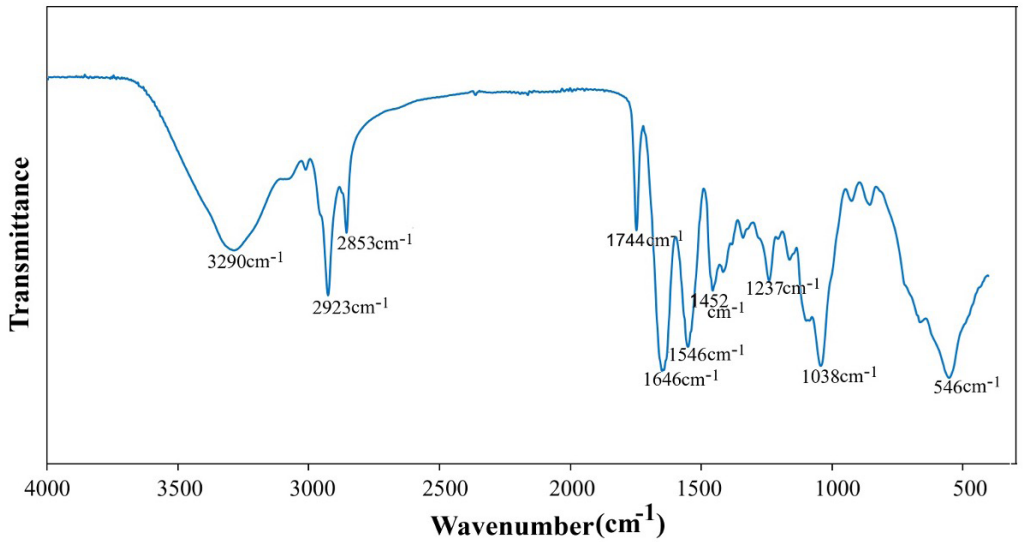

Figure 5. Fourier transform infrared spectrum of fish collagen.

al. noted that when the NH group of a peptide forms a hydrogen bond, the corresponding absorption band shifts to a lower frequency [42]. In our study, the N-H stretching vibrations of collagen were observed to shift to 3290 cm^{-1}, which is likely attributable to the presence of hydrogen bonds in the N-H groups of the collagen structure.

The FTIR spectrum also revealed CH_2 asymmetric and symmetric stretching vibrations at 2923 cm^{-1} and 2853 cm^{-1}, respectively [43-44]. The characteristic lipid C=O stretching was detected at 1744 cm^{-1}, aligning with findings by Wei et al., who reported similar results [45]. Additionally, the distinctive peaks associated with collagen were identified as follows: Amide I (C=O stretching mode) at 1646 cm^{-1}, Amide II (N-H bending mode) at 1546 cm^{-1}, symmetric CH_3 bending mode of the protein at 1452 cm^{-1}, and Amide III (asymmetric C-N stretching mode) at 1237 cm^{-1}, which were also noted in various studies referenced in [1, 41-46].

Furthermore, the absorption peak corresponding to C–OH stretching vibrations of carbohydrate moieties was observed at 1040 cm^{-1}. In their FTIR analysis, Riaz et al. suggested that collagens may contain carbohydrates linked to hydroxylysine residues within the polypeptide chain via O-glycosidic bonds [47]. Lastly, C-S stretching vibrations were noted with slight shifts at a wavelength of 546 cm^{-1} [48].

Overall, these spectral characteristics provide valuable insight into the molecular structure and functional groups present in the collagen extracted from fish waste, confirming the presence of various structural components essential for its biological function.

XRD Analysis

The X-ray diffraction (XRD) pattern serves as a valuable tool for elucidating the fibrillar structure of collagen. The XRD pattern of collagen extracted from fish waste materials is depicted in Figures 6-7. Upon close examination of this figure, it is clear that the pattern exhibits multiple distinct and sharp crystallization peaks characteristic of collagen. Notably, sharp peaks observed at approximately 31.74°, 45.46°, 56.48°, and 75.28° indicate a robust crystalline structure within the collagen matrix.

In addition to the prominent peaks, weaker diffraction peaks were identified at around 27.38°, 53.86°, 66.21°, 73.09°, and 83.98°, which are consistent with findings reported by Wang et al. [49], Usha et al. [50], and Takallu [51]. These weaker peaks suggest additional structural features within the collagen sample, supporting the complexity of its crystalline arrangement.

The second diffraction peak is particularly significant as it provides insight into the intermolecular spacing between collagen fibrils [52-53]. To calculate the minimum distance between the collagen skeletons, the Bragg equation was applied (d (Å) = $\lambda/2\sin\theta$, where λ = 1.54 Å) [54]. The calculated minimum distance value was found

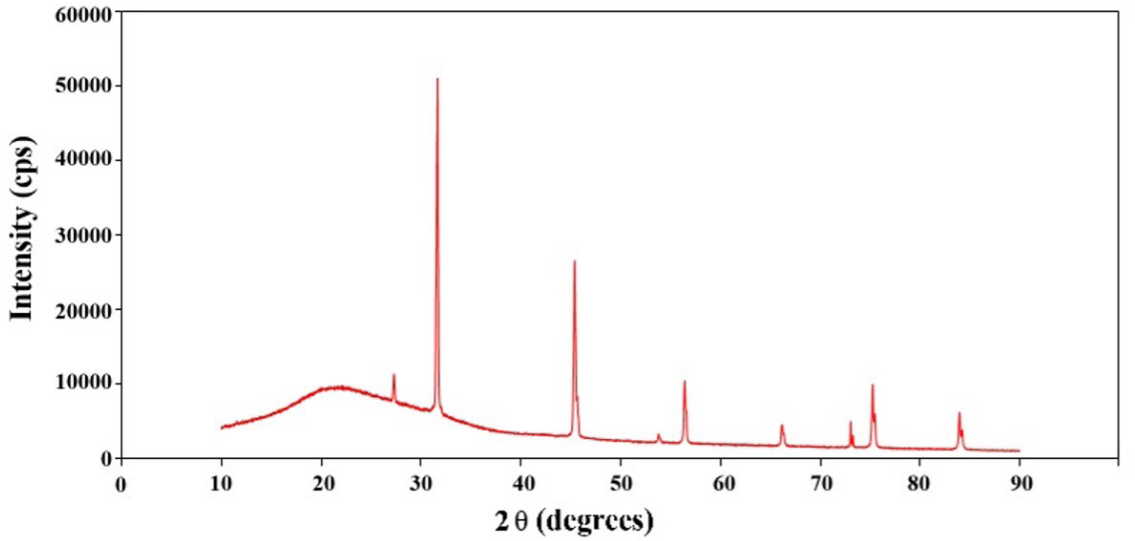

Figure 6. XRD pattern of collagen.

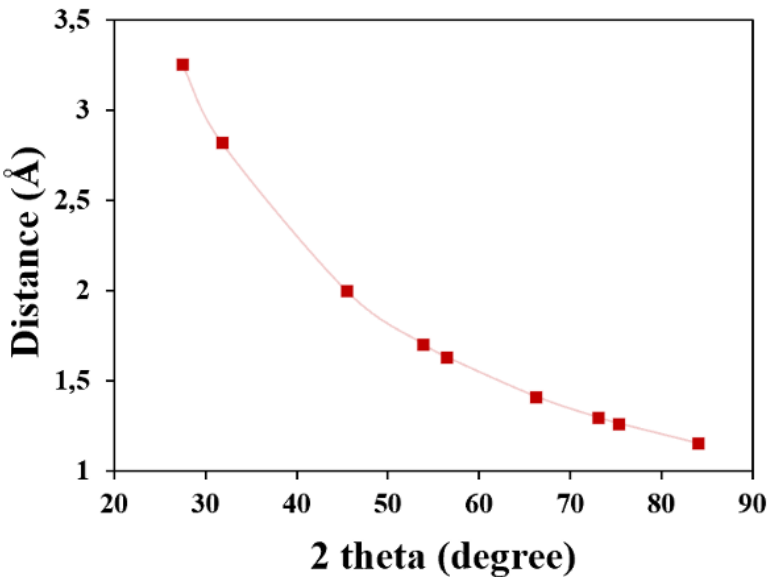

Figure 7. The 2θ and distance (Å) values of collagen.

to be 3.26 Å, which aligns closely with the findings reported by Ampitiya et al. [39].

Additionally, the 2θ and 'd' spacing values of collagen are summarized in Figure 5, further illustrating the crystalline characteristics of the collagen extracted from fish waste. This analysis underscores the structural integrity of the collagen, confirming its potential for various applications in biomedical and industrial fields.

DSC Analysis

The differential scanning calorimetry (DSC) thermograms for collagen extracted from fish waste are presented in Figure 8. The first endothermic peak is observed at approximately 68°C, with a corresponding heat flow of approximately -2.209 mW. This initial endothermic process may indicate moisture loss, as well as the release of free and bound water from the collagen matrix.

The second significant endothermic peak occurs between 129°C and 141°C, with a peak heat flow of -4.881 mW recorded at 141°C. The enthalpy change (ΔH) for this peak is measured at 39.2 mJ/mg. This pronounced endothermic peak is indicative of thermal denaturation processes occurring within the collagen structure. Thermal denaturation (Td) is a critical factor influencing

the thermal stability of proteins, including collagen [55, 56]. During this process, the disruption of the triple helical structure of collagen leads to a subsequent loss of its structural properties [57].

The literature suggests that variations in the Td of collagen sourced from different fish species can be attributed to factors such as habitat, body temperature, and amino acid composition [38, 55]. Elevated heat transfer can significantly alter several physical properties of collagen, including light scattering, optical activity, viscosity, sedimentation, and diffusion. These changes may ultimately cause the breakdown of the collagen's triple helical structure [55, 58].

A third endothermic peak is noted at 239.3°C, with a heat flow of around -2.508 mW. This peak corresponds to the pyrolysis process, indicating the temperature at which chemical decomposition begins, resulting in the irreversible degradation of the amino acids and the protein backbone. In the study conducted by Gauza-Włodarczyk et al., the thermal denaturation temperature of collagen obtained from fish skin was reported to range between 380-420 K, while the thermal decomposition temperature was found to be around 510 K [33].

Overall, the DSC analysis highlights the thermal behavior of collagen extracted from fish waste, providing insights into its stability and structural integrity under varying thermal conditions.

Conclusions

This study aims to contribute to sustainable resource use and waste reduction by targeting collagen extraction from fish waste using an acetic acid-assisted method. Through comprehensive analysis, the extracted collagen was characterized in terms of physicochemical and structural properties, confirming its triple helix structure and semi-crystalline structure. UV-Vis analysis showed strong absorption at 238 nm, consistent with the triple helix polypeptide structure of collagen, and FTIR analysis identified functional groups and bonds important for maintaining the structural integrity of collagen. XRD analysis confirmed the semi-crystalline fibrillary structure, while DSC analysis highlighted the thermal stability of collagen with a denaturation temperature (Td) range of 129–141 °C. This indicates that it has a high resistance to thermal degradation.

The findings indicate that fish waste is applicable as a

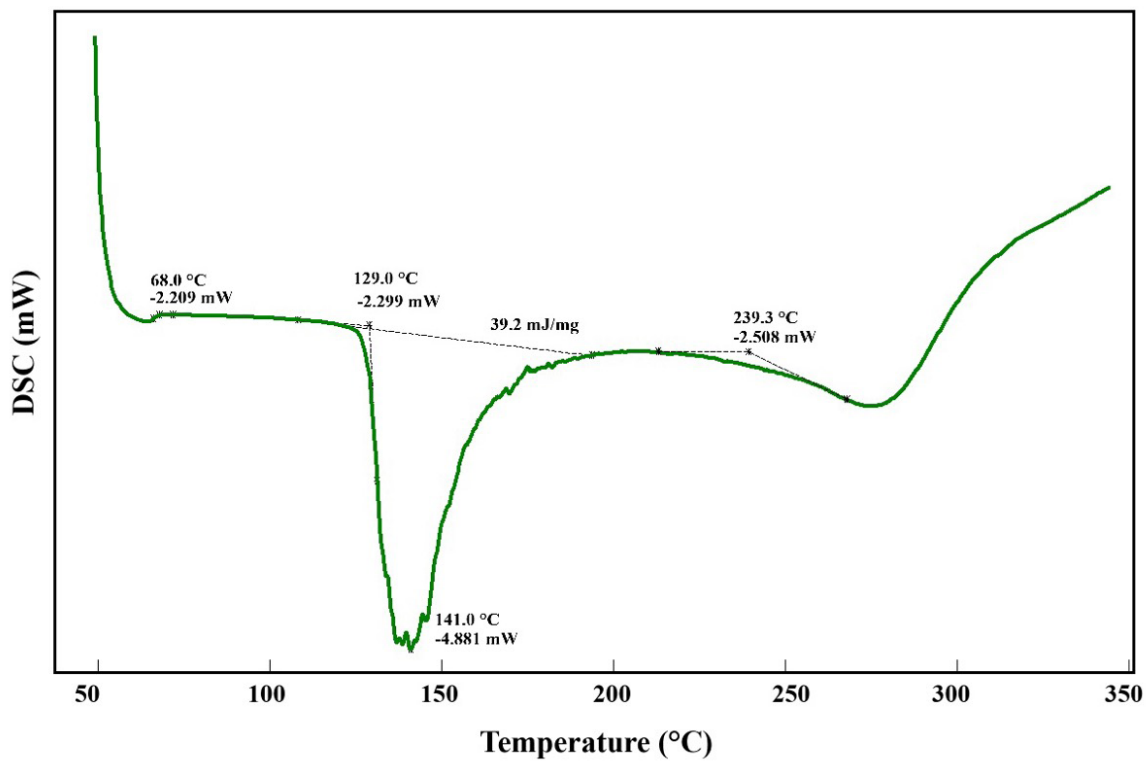

Figure 8. DSC Thermogram of collagen.

valuable source of collagen, with promising applications in various industries, particularly in pharmaceuticals, cosmetics, and tissue engineering. This study is compatible with environmental goals by proposing a sustainable approach to recycle fish processing waste and thus reduce the ecological impact. Future research aims to optimize the extraction efficiency and examine potential modifications to expand the application range of marine-derived collagen in various industrial applications.

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