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Unlocking the Potential of Fishery Waste: Acid-Soluble Ultrasound Extraction of Marine Collagen from Sardine Fish Scales †

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Abstract: Globally, fish consumption generates significant waste from fish markets and processing industries, including fish skin, scales, and bones. If not appropriately managed, this fishery waste can lead to environmental pollution. Collagen, the most abundant protein in animal bodies, has diverse medical, biomedical, and pharmaceutical applications, but its high cost has constrained its usage. Collagen derived from marine sources, particularly from byproducts of fish processing, is seen as an alternative to collagens from land animals. There has been growing interest in utilizing fish scales as a cost-effective source of this valuable collagen-rich protein. Repurposing fish scales could alleviate environmental pressure and create additional commercial value. In a recent study, collagen was isolated from the scales of Moroccan Sardina pilchardus, a fish species renowned for its high collagen content. This marine collagen type I features a triple alpha-helical structure comprising one $\alpha 2$ chain and two $\alpha 1$ chains. The collagen extraction was accomplished using the acid soluble collagen (ASC) method combined with an ultrasound technique after pretreating the fish scales, involving step demineralization to remove a high amount of minerals. The ASC extracted from the sardine scales exhibited high solubility in the highly acidic pH range (pH 2). Various physicochemical techniques such as FTIR, DRX, and SEM confirmed the isolated protein as collagen. Hence, the sardine scale could serve as an alternative source of collagen, and the characteristics of the collagens were minimally affected by the extraction process employed.

Keywords: marine collagen; fish scale; byproducts; ultrasound technology; Morocco Sardina pilchardus

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1. Introduction

Morocco is the leading producer and exporter of sardines and aquaculture among North African countries due to its abundant inland water resources. With a vast coastal area, the fishing sector is vital to Morocco's economy. In 2022, the country achieved a notable feat by recording a national fish production of 1.55 million tons, solidifying its leadership in Africa [1,2].

The fishing industry in Morocco produces a significant amount of fish waste, especially from sardines (Sardina Pilchardus). After processing, parts such as scales, fins, viscera, heads, and skeletons, known as fish waste, make up about 40–60% of the fish's weight [2]. Unfortunately, these by-products are often ignored and can cause environmental problems, including increased pollution, greenhouse gas emissions, and

unpleasant odors due to their high organic matter content and rapid degradation rate. Disposing of such large volumes presents logistical and environmental challenges, requiring prompt removal to prevent the release of atmospheric pollutants in urban areas [3]. Roughly 30% of the total waste by-products consist of scales, bones, and skin, which are rich in high-quality proteins and can be used to produce Collagen [4]. Fish scales, which protect the fish's dermis layer, are made up of valuable materials, including organic components such as Collagen, fat, and lecithin, as well as inorganic components like calcium-deficient hydroxyapatite and trace elements such as magnesium, iron, zinc, and calcium [5–7].

The potential to produce valuable products using fish waste for collagen production can bring economic benefits to the fish processing industry and help address environmental concerns [8]. Fish collagen from fish scales has similar properties to type I Collagen, known as "tropocollagen", characterized by a triple-helical α-domain structure. It consists of three distinct α-chains and is a heterotrimeric molecule comprising two α1-chains and one α-2 chain, with a molecular weight of around 100 kDa [4,8,9]. Fish collagen has higher bioavailability and absorption efficiency than alternatives from bovine and porcine sources, with an absorption efficiency of approximately 1.5 times greater [4]. Additionally, the composition and microstructures of fish scales make them suitable for various applications, including tissue engineering, therapeutic and pharmaceutical use, biological filling, medical treatment, sewage processing, and flexible biomaterial and biocomposite engineering [7].

Implementing a circular economy in the fisheries industry is essential to raise awareness, maintain production and consumption, maximize resource utilization, and minimize waste [2]. Current efforts are focused on reducing biomass waste through innovative methods. For instance, Collagen is extracted from Sardina pilchardus fish scales using the hybrid technique, which involves the acid-soluble extraction (ASC) and ultrasound collagen extraction (UCE) process. The process begins with decalcification pretreatment to remove a significant amount of calcium from fish scales, followed by an acidic extraction process using AcOH with different incubation times and sonication posttreatment to improve collagen peptide extractability. The main goal of acid-ultrasound extraction is to disrupt the cross-links within the collagen helix, resulting in high-quality Collagen. The extracted acid-soluble collagen (ASC) supernatant is then dialyzed and freeze-dried to obtain the desired collagen product. This study specifically focuses on isolating and characterizing type I collagen from the scales of Moroccan Sardine pilchardus and examining their physicochemical characteristics.

2. Methods

2.1. Sample Collection

Moroccan Sardina pilchardus were obtained from the local market, Fish scales were thoroughly washed with tap water, and then distilled. After that, they were washed twice in a 1.0 M NaCl solution and stored at −25 °C until they were ready to be used.

2.2. Extraction of Collagen from Fish Scales

The process of extracting collagen from fish scales involves two main steps. First, the fish scales were demineralized using EDTA. Then, the collagen was isolated through treatment with dilute acetic acid, followed by sonication of the mixture.

2.2.1. Demineralization Process

The demineralization process was carried out according to the method described previously [10] by treating the scales with a high amount of calcium (16–59% mineral content in weight) in fish; decalcification is required to be performed by ethylenediaminetetraacetic acid (EDTA). Sardine scales were treated in a 0.5 M EDTA solution at a pH of 7.4

for 48 h. Once demineralized, the fish scales were washed three times with distilled water before further use.

2.2.2. Isolation of Collagen

The demineralized fish scales were treated with a 3 M acetic acid solution in the highly acidic pH range (pH 2) for either 48 or 96 h. Afterward, the mixture was sonicated for 15 min at 30 °C, and the non-soluble portion of the scales was separated through filtration. Next, acetone was added to the filtrate to cause collagen precipitation, and the solution was left undisturbed for 24 h. The resulting suspension was centrifuged at 3900 rpm for 30 min and then freeze-dried, producing collagen powder.

2.3. Characterization of Collagen

2.3.1. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The Collagen extracted was examined through FTIR spectra using a Perkin-Elmer Model Spectrum RX-1. KBr was mixed with 1 mg of the powdered sample and palletized under pressure. The FTIR Spectrum Software program was used to analyze the data for each sample. Spectra were obtained from the frequency ranges of 4000 cm^{-1} to 450 cm^{-1}.

2.3.2. X-ray Diffraction (XRD) Analysis

The XRD pattern of isolated collagen samples was recorded using an Automated Multipurpose X-ray Diffractometer (XRD) (Rigaku SmartLab) with CuKa radiation (k = 1.5406 Å) worked at 36 kV and 36 mA. The XRD pattern was recorded in a fixed time mode at room temperature in the 30 min which was according to the Bragg eq (X = 2d sin 9).

3. Results and Discussion

3.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were used to analyze the extracted collagens for conformational changes in the polypeptide chains. The analysis revealed a typical collagen profile with distinct absorption bands representing different amides (A, B, I, II, and III) characteristic of the collagen molecule. These absorption bands appeared at wavelengths similar to those found in other studies on marine Collagen [8,11,12].

Figure 1a,b displays the Infrared spectrum of the solid sample obtained from collagen extraction from sardine scales at 48 h and 96 h. The results indicated the presence of an Amide A band at 3340 cm^{-1} and 3418 cm^{-1} for the (N–H) bond (stretching of proteins), as well as an Amide B band at 2890 cm^{-1} and 3027 cm^{-1} for the CH2 asymmetric stretch, signifying the presence of an amide group. The protein conformational arrangements were further studied using amides I–III, with absorption bands of the C = O bond (stretching of proteins) at 1656 cm^{-1} and 1668 cm^{-1} (amide I), the N–H bond (Bending/C–N stretching of proteins) at 1543 cm^{-1} and 1557 cm^{-1} (amide II), and the C–O bond (stretch/NH bond coupled with C–N stretch) at 1240 cm^{-1} and 1242 cm^{-1} (amide III) (Table 1).

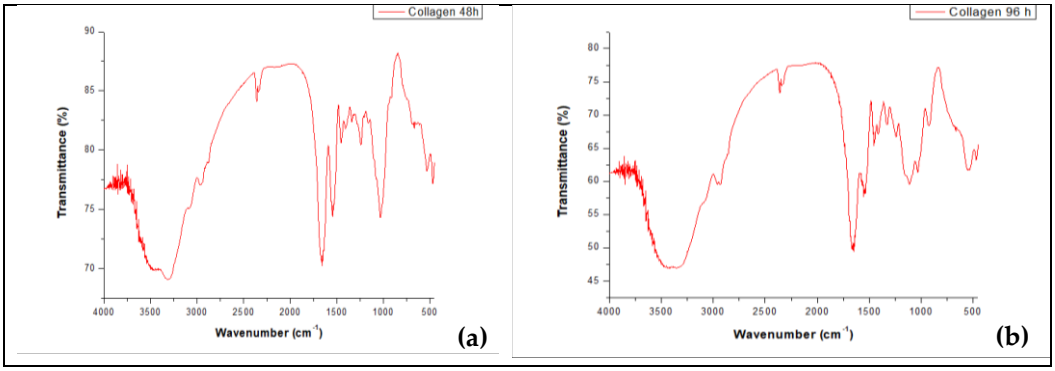

Figure 1. FTIR spectra of extracted collagen 48 h (**a**) and 96 h (**b**) of sardine fish scales.

Table 1. FTIR peak range of extracted collagen from sardine fish scales.

Designation	Approximate Frequency cm^{-1}		Description
	Collagen 48 h	Collagen 96 h	
Amide A	3340	3418	NH bond stretching
Amide B	2890	3027	CH2—asymmetric stretch
Amide I	1656	1668	C = O bond stretching of proteins
Amide II	1543	1557	CN stretching, NH bending
Amide III	1240	1242	CN, CO stretching and NH bending

3.2. X-ray Diffraction (XRD)

The X-ray diffraction (XRD) pattern of sardine scale collagen can be seen in Figure 2a,b with well-defined and sharp diffraction peaks. A comparison of the XRD results of scale collagen with the findings of Belouafa et al. [12] suggests that the crystal structure is different from pure collagen, which is typically amorphous due to its organic nature, likely due to the presence of calcium salt residue. The peaks in Figure 1a corresponding to collagen extracted at 48 h show higher intensity than those in Figure 1b corresponding to collagen extracted at 96 h, indicating that the scales were not adequately decalcified before the extraction process. This implies that the scales needed to be exposed to acid longer to remove calcium salts.

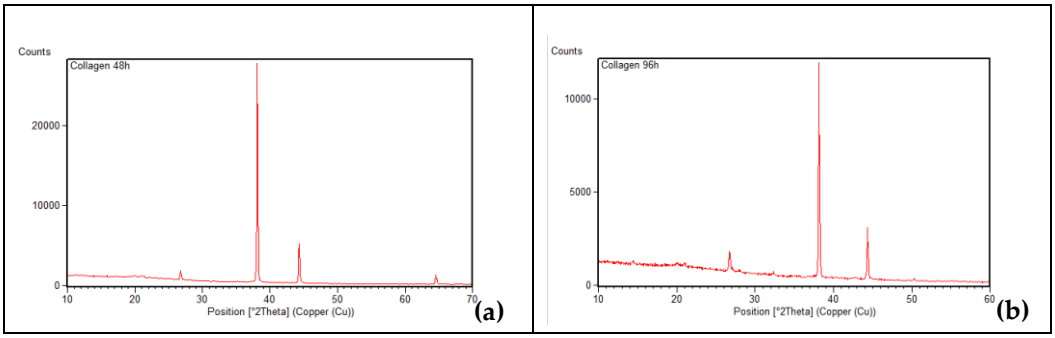

Figure 2. XRD pattern of extracted collagen 48 h (**a**) and 96 h (**b**) of sardine fish scales.

4. Conclusions

After using the acid-soluble Collagen (ASC) method along with ultrasound to extract collagen from the scales of Moroccan Sardines "Sardina Pilchardus", promising results have been observed. The collagen obtained from fish scales shows excellent potential for use in pharmaceuticals, biomedicine, healthy foods, cosmetics, and drug delivery systems. Its safety, absorbability, biocompatibility, degradability, and cost-effectiveness make it a favorable alternative to collagen from land animals. Further research and development in this area could lead to significant advancements in the use of fish-scale collagen, benefiting consumers and various industries.

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