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Fabrication of Collagen Foam Sheets from Fish Scale Waste through Controlled Acid Hydrolysis

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Abstract

Marine fish processing generates a significant amount of waste, particularly fish scales, which are often discarded despite being a valuable source of type 1 collagen, a structurally important protein with wide industrial applications. This study focuses on the extraction of collagen from fish scales using a hydro-extraction approach combined with acid hydrolysis. Initially, the scales were pretreated with 0.1 n NaOH to remove non-collagenous impurities and enhance extraction efficiency, as alkaline treatment facilities the breakdown of crosslinked structures. Subsequently, hydrochloric and acetic acid were employed to hydrolyze the collagen, as acids are known to disrupt intermolecular bonds and solubilize collagen effectively. The hydro extraction was carried out at a solid-to-liquid ratio of 1:20, followed by purification and freeze-drying to develop collagen foam sheet. The results revealed that collagen extracted using both acids exhibited comparable structural characteristics, as confirmed by SEM analysis. However, hydrochloric acid-based extraction yielded a greater quantity of collagen sheets. Overall, the study demonstrates that fish scale waste can be efficiently valorized into high-quality collagen using hydro-extraction, supporting sustainable resource utilization and potential applications in biomedical and textile fields.

Key words: Marine Fish scale, Collagen, Acid hydrolysis, Hydro extraction method, Freeze Drying, Collagen foam sheet, SEM Analysis

1. Introduction

Marine fish wastes were discarded into the sea land areas. Fish skin, fin, bone, liver, and scale were discarded to the lands (Wang et al., 2019). Especially, the fish scale wastes were not used for any purposes. Fish scale-extracted collagen is a valuable type-1 structural protein obtained mainly from marine fish scale wastes (Phon et al., 2023). Which is widely found in human skin, bone, and connective tissue. It characterized by a triple-helix structure composed mainly of glycine, proline and hydroxyproline amino acids (Shoulders & Raines, 2009). It is typically extracted through process involving cleaning, demineralization (to remove calcium content) and acid or enzymatic treatment to solubilize collagen (Kozlowska et al., 2015). This collagen exhibits excellent biocompatibility, biodegradability and water binding capacity (Phon et al., 2023), making it suitable for biomedical (Rezvani Ghomi et al., 2021), cometic (Avila Rodríguez et al., 2018) and food applications (Xu et al., 2019) (Bhagwat & Dandge, 2016). Additionally, it is considered a safer and more sustainable alternative to mammalian collagen because it eliminates the risk of zoonotic diseases and utilizes marine waste effectively, making it highly suitable for eco-friendly material development (Matinong et al., 2022).

Hydro-extraction (water-based) of collagen from fish scales offers several important advantages compared to conventional acid or enzymatic methods (Huang et al., 2016). It considered a more efficient and sustainable technique because it enables higher extraction yield (Suparno & Prasetyo, 2019). The process is typically continuous and easy to operate, reducing processing time, labour, energy consumption and cost intensive. In addition, hydro-extraction uses fewer harsh chemicals, making it more environmental-friendly and safer, while

also minimizing chemical residues in the final collagen product. Another benefit is the improved product quality, as the extracted collagen retain essential amino acids such as hydroxyproline and maintain its type-1 structure, which is important for biomedical (Afifah et al., 2019) and textile applications (Paul et al., 2012).

2. Materials and Method:

Fish scales were collected from fish markets in Thoothukudi, a major coastal region known for its abundant marine resources and active fish processing activities. Among the available species, sardine and emperor were selected for collagen extraction due to their high availability and significant scale yield, making them suitable raw materials for efficient collagen preparation. The other materials used in this study included sodium hydroxide (NaOH) pellets, hydrochloric acid (HCl), acetic acid, single distilled water and double distilled water, all of which were utilized during the collagen extraction and purification processes.

The extraction procedure was carried out using a water bath shaker to ensure uniform mixing and effective interaction between the fish scales and the extraction solutions, as continuous agitation enhances collagen solubilization. For the preparation of collagen foam sheets, the extracted collagen solution was subjected to freeze drying, a technique that removes water through submission under controlled conditions, thereby preserving the structural integrity and porous architecture of the material. The microstructural characteristics and elemental composition of the extracted collagen were analysed using a Scanning Electron Microscope (SEM) Instrument, which is widely employed to observe surface morphology. Figure 1 illustrates the overall methodology adopted in this research.

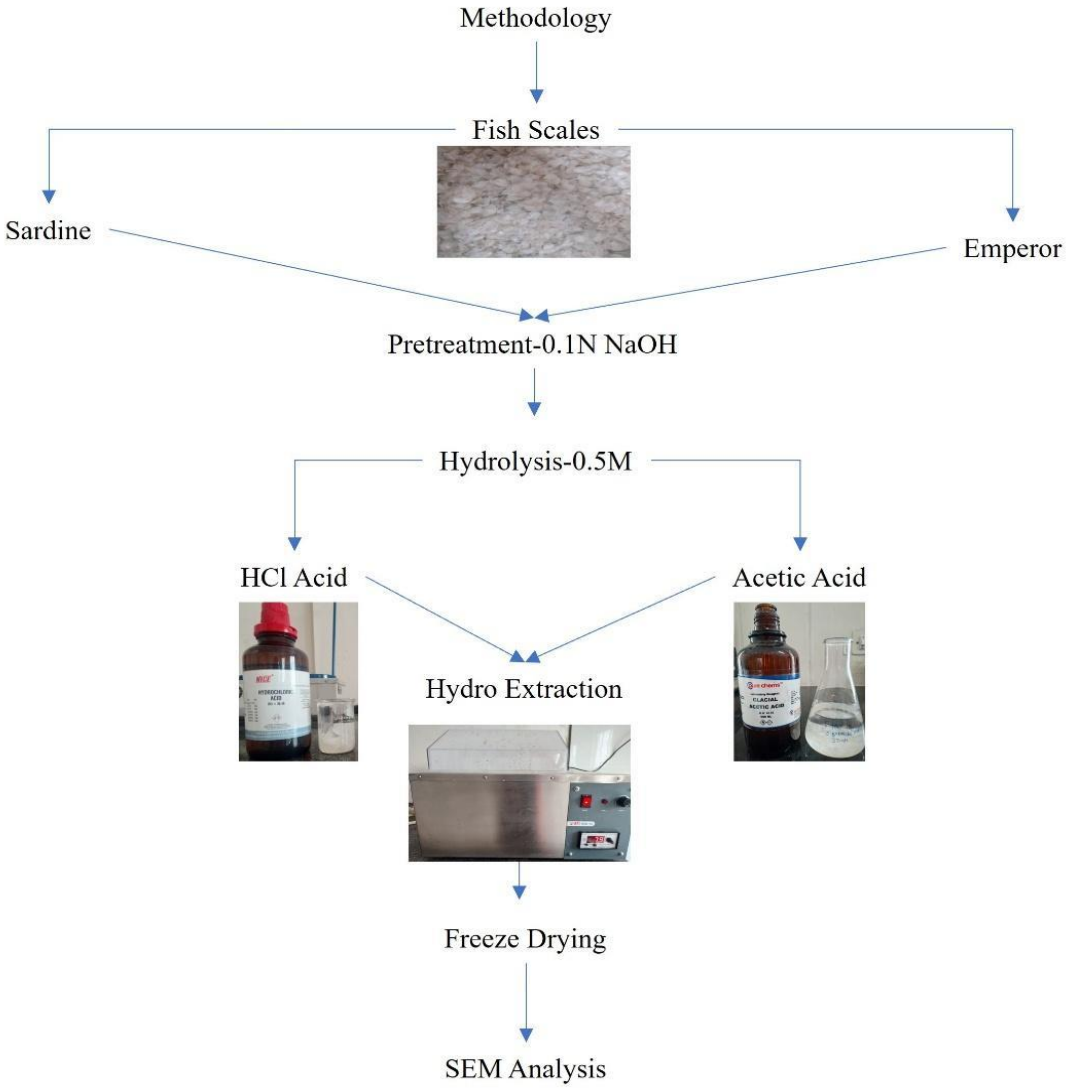

figure: 1 flow chart of collagen extraction and preparation of collagen foam sheet

3. Pretreatment

Fresh fish scales were thoroughly washed with tap water to remove adhering impurities such as dirt, blood, sand and residual salts, as proper cleaning is an essential initial step in collagen extraction to reduce contamination and improve product quality. Subsequently, the scales were rinsed three times with distilled water and allowed to dry to room temperature to eliminate excess moisture. A measured quantity of 5g of dried fish scales was then subjected to pretreatment using 0.1 N sodium hydroxide (NaOH) solution for three days (Zhang et al., 2011). This alkaline treatment plays a crucial role in removing non-collagenous contents, pigments and other unwanted substances, while also facilitating the breakdown of cross-linked structures in the collagen matrix. After the pretreatment process, the scales were again washed three times with distilled water to remove residual alkali and neutralize the sample prior to future extraction steps.

4. Hydrolysis

The pretreated fish scales were subjected to acid hydrolysis using both hydrochloric acid (HCl) and acetic acid under controlled to extract collagen. Each acid was prepared at a concentration of 0.5 M and applied separately for a duration of three days to ensure effective solubilization of collagen (Suparno & Prasetyo, 2019). Acid hydrolysis plays a crucial role in braking down intermolecular cross-links within the collagen structure, thereby enhancing its extraction efficiency and purity. Following the hydrolysis process, the treated scales were thoroughly washed with distilled water to remove residual acids and impurities, ensuring the stability and quality of the extracted collagen for further processing.

5. Extraction

The hydro extraction method was selected in this study due to its cost-effectiveness, minimal chemical usage and environmentally sustainable nature (Huang et al., 2016). The hydrolyzed fish scales were mixed with distilled water at a material-to-liquid ratio of 1:20 to facilitate efficient collagen extraction. The mixture was then subjected to thermal treatment at 80˚C for 2 hours using a water bath shaker, which provides uniform heating and continuous agitation, thereby enhancing the release of collagen into the solution. Following extraction, the solution was filtered through a fine mesh cloth to remove residual solid impurities. The obtained crude collagen solution was subsequently stored at -20˚C to preserve its stability and prevent degradation prior to further analysis or processing.

6. Freeze Drying

Both of the emperor and sardine fish scale extracted-collagen solution were combined in a 1:1 ratio to obtain a uniform collagen mixture for further processing. The collagen solutions obtained from hydrochloric acid (HCl) and acetic acid hydrolysis were then separately subjected to freeze-drying at -80˚C to fabricate collagen foam sheets. Freeze-drying, or lyophilization, removes water through sublimation, resulting in a highly porous and sponge-like structure that preserves the integrity of the collagen matrix.

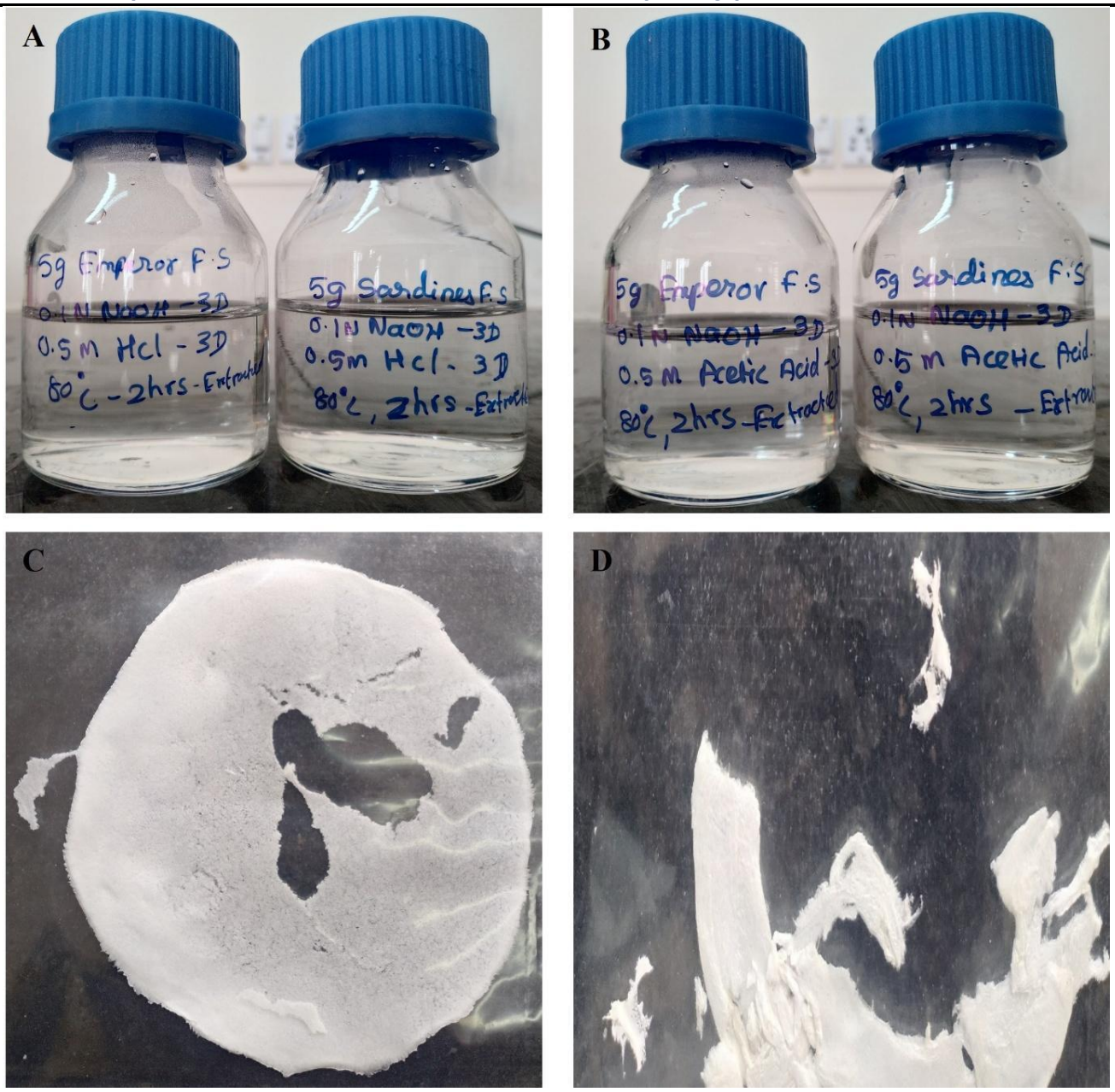

figure: 2 a- raw collagen solution from emperor and sardine fish scales using HCl hydrolysis, b- raw collagen solution from emperor and sardine fish scales using acetic acid hydrolysis, c- collagen foam sheet using HCl-based hydrolysis, d- collagen foam sheet using acetic acid-based hydrolysis

Figure2-B illustrates the formation of collagen sheets using this method. It was observed that the HCl-based collagen exhibited better sheet formation and structural uniformity compared to the acetic acid-based collagen. The resulting sheets displayed a thin, lightweight and sponge-like texture, indicating the successful development of porous collagen materials suitable for further application.

7. Results and Discussion

7.1 SEM Analysis

The scanning electron microscope (SEM) analysis was performed on both hydrochloric and acetic acid hydrolyzed collagen foam sheets to evaluate their surface morphology and elements identifications.

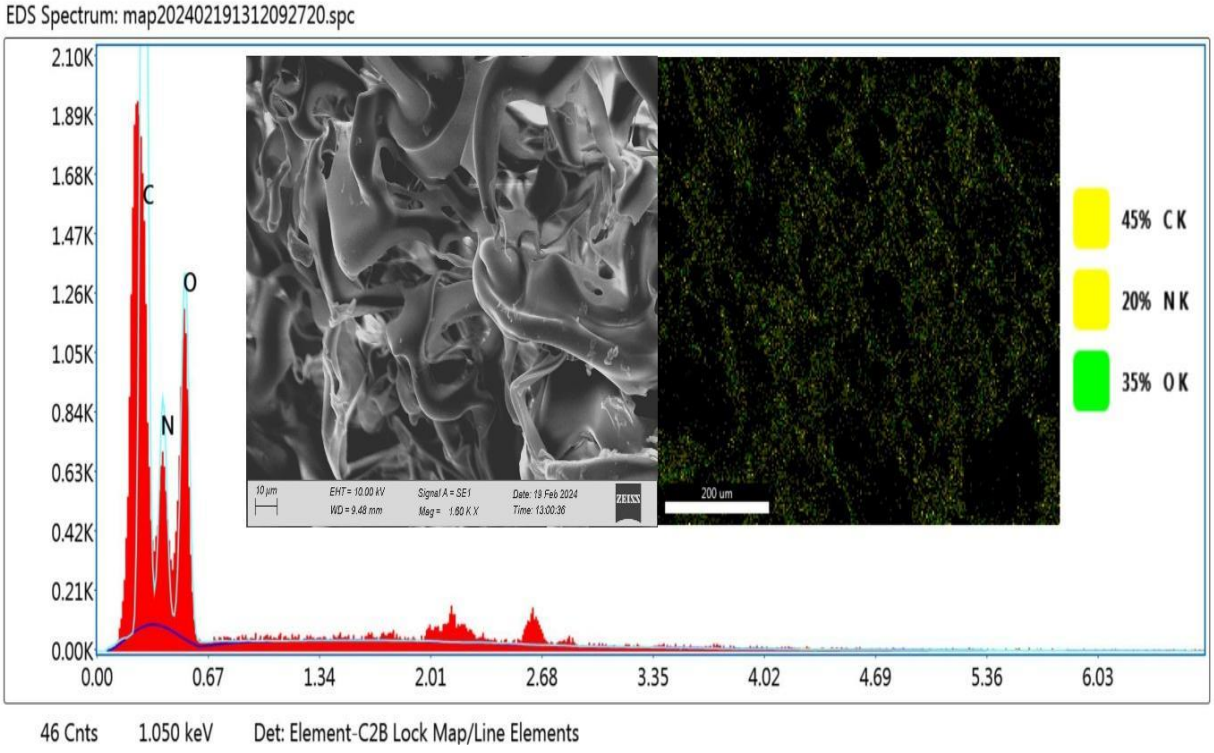

figure: 3 HCl acid hydrolyzed collagen foam sheet

table: 1 SEM analysis for HCl acid-based hydrolyzed collagen foam sheet

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	40.87	46.43	245.23	4.65	0.3357	1.0272	0.7994	1.0000
N K	25.91	25.24	55.41	11.15	0.0806	0.9958	0.3124	1.0000
O K	33.22	28.33	97.69	9.99	0.1113	0.9690	0.3457	1.0000

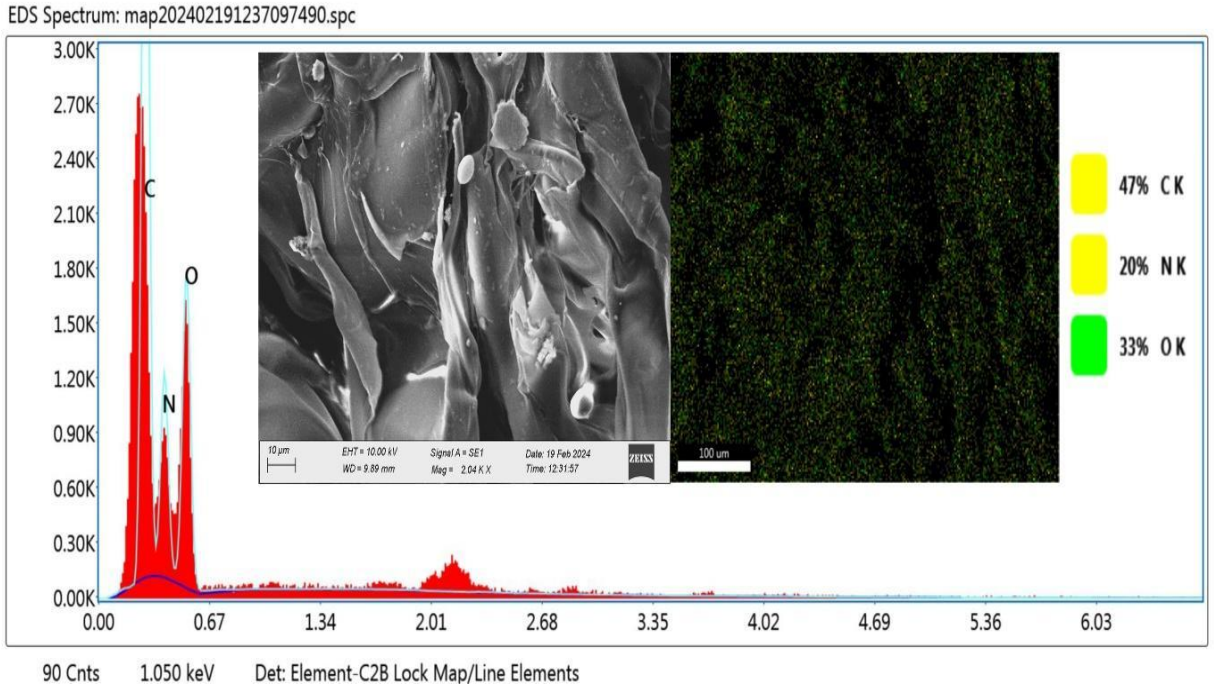

figure: 4 acetic acid hydrolyzed collagen foam sheet

table: 2 SEM analysis for acetic acid-based hydrolyzed collagen foam sheet

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	41.79	47.33	350.53	4.41	0.3449	1.0266	0.8039	1.0000
N K	26.31	25.55	76.84	10.88	0.0804	0.9952	0.3069	1.0000
O K	31.90	27.12	128.36	9.84	0.1051	0.9684	0.3403	1.0000

The SEM micrographs of collagen foam sheets obtained from hydrochloric acid and acetic acid hydrolysis exhibited distinct morphological characteristic while maintaining the general porous nature typical of freeze-dried collagen. Freeze-drying produces a porous structure due to ice crystal sublimation, leading to interconnected pore networks that enhance functional properties such as absorption and permeability. The HCl-hydrolyzed collagen (Figure 3) showed a highly porous, well developed and interconnected sponge-like structure with relatively larger and more open pores. The fibrillar network appeared loosely arranged with clear void spaces, indicating effective hydrolysis and better expansion during freeze-drying. This morphology supports improved surface area and making it suitable for applications requiring high absorbency and bioactivity (Brodsky & Persikov, 2005).

In contrast, the acetic acid-hydrolyzed collagen (Figure 4) exhibited a comparatively denser, layered and more compact structure with reduced pore size and partial aggregation of collagen fibrils. The structure appeared more folded and less open, suggesting relatively milder hydrolysis and lower expansion during freeze-drying. The EDS spectra of both samples confirmed the presence of major elements carbon (C), oxygen (O), and nitrogen (N), which are characteristic of protein-based materials like collagen. The HCl sample showed approximately 45% C, 35% O and 20% N, while the acetic acid sample showed 47% C, 33% O, and 20% N, indicating no significant difference in elemental composition (Sun et al., 2017). The presence of nitrogen confirms the proteinaceous nature of collagen due to amide linkages, while the absence of unwanted elements such as calcium suggests effective demineralization during pretreatment.

8. Conclusion

The present study confirms that fish scales can be effectively utilized as a sustainable and economical source for collagen extraction using the hydro-extraction method. The combined pretreatment and acid hydrolysis processes successfully removed non-collagenous components and enabled efficient collagen recovery. The freeze-drying technique resulted in the formation of highly porous, sponge-like collagen foam sheets, which is consistent with typical collagen scaffold structure formed through ice crystal sublimation. SEM analysis revealed that both HCl and acetic acid hydrolyzed collagen exhibited well-developed porous morphologies, confirming the successful formation of collagen matrices.

However, the HCl-hydrolysed collagen showed a more open, interconnected porous structure, while the acetic acid-based collagen appeared comparatively denser and more compact. These structural variations indicate that the type of acid influences the microstructure of collagen rather than its fundamental composition. EDS analysis further confirmed the presence of carbon, oxygen and nitrogen in both samples, validating the proteinaceous nature and purity of the extracted collagen. Overall, the study demonstrates that hydro-extraction combined with freeze-drying is an effective approach for producing structurally stable collagen materials from fish scale waste, with HCl hydrolysis showing better performance in terms of porosity, sheet formation and yield, making it more suitable for advanced biomedical and textile applications.

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