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Marine By-Product Valorization: Collagen Extraction from Sardine Scales for Circular Cosmetics and Nutrition †

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Abstract

The increasing consumption of fish products has resulted in significant waste generation, with sardine scales (*Sardina pilchardus*) representing a notable by-product of the canning industry. This work investigates the sustainable valorization of these scales through collagen extraction for applications in cosmetics and nutrition. Collagen was extracted using acid and pepsin-assisted methods and characterized by spectroscopic and imaging techniques (UV-Vis, FTIR, PXRD, SEM, SDS-PAGE). Subsequent enzymatic hydrolysis with papain produced low-molecular-weight peptides. Biological assays revealed enhanced antioxidant activity of the hydrolyzed peptides compared to native collagen, while no antimicrobial effects were detected. Permeation studies in Caco-2 cells indicated moderate intestinal absorption (~6.4% in 6 h). These findings support the potential of sardine-derived collagen as a bioactive ingredient aligned with circular economy principles.

Keywords: collagen; marine by-products; natural products; circular economy

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1. Introduction

Oceans cover over 70% of the planet and sustain global economies through fisheries and aquaculture. However, intensive exploitation of marine resources generates large amounts of underutilized biomass, with scales, viscera, heads, and tails representing the main by-products of fish processing. In Europe, sardines (Sardina pilchardus) are widely consumed, and their industrial processing produces considerable quantities of waste, particularly scales, which are often discarded [1,2].

Marine by-products are an attractive source of bioactive compounds such as polyphenols, peptides, polysaccharides, chitin, and collagen. Collagen, the most abundant

structural protein in connective tissues, has high potential in the food and cosmetic sectors due to its biocompatibility and beneficial effects on skin health, nutrition, and tissue regeneration. Hydrolyzed collagen peptides further expand these applications because of their antioxidant activity, digestibility, and capacity to permeate intestinal barriers [3,4].

Valorization of sardine by-products through collagen extraction not only reduces environmental impact but also supports the development of high-value products aligned with the principles of the circular blue economy. This study investigates the extraction, characterization, and bioactivity of collagen obtained from sardine scales, aiming to contribute to sustainable strategies for marine biomass utilization.

2. Methods

2.1. Fish Scales

Scales from *Sardina pilchardus* were provided by Conservas Pinhais & Cia, Lda. (Matosinhos, Portugal). Upon arrival, the scales were rinsed with tap water to remove residual tissue and stored at 4 °C until further processing.

2.2. Collagen Extraction

Two extraction protocols were employed: acid solubilization (ASC) and pepsin-assisted digestion (PSC) following Santos Filipe et al. (2024) [5].

2.3. Collagen Characterization

Extracts (1 mg/mL) were analyzed using UV-Vis and ATR-FTIR spectroscopy to confirm the presence of collagen-specific absorption bands following Srinivasan et al. (2021) [6]. Powder X-ray diffraction (PXRD) was used to assess crystallinity, while scanning electron microscopy (SEM) provided morphological insights as described in Santos Filipe et al. (2024) [5]. Elemental analysis was performed via energy-dispersive X-ray spectroscopy (EDS) to detect residual salts [5].

2.4. Papain-Mediated Hydrolysis of Acid-Soluble Collagen

Acid-soluble collagen was hydrolyzed using papain under optimized enzymatic conditions (pH 6.3, 55 °C, 4.25 h). The resulting peptides were thermally inactivated, centrifuged, freeze-dried, and stored at 4 °C for subsequent analysis, as described in Santos Filipe et al. (2024) [5].

2.5. SDS-PAGE Analysis

Protein profiles of native collagen and hydrolyzed peptides were evaluated by SDS-PAGE using gradient gels (4–12%). Gels were stained with Coomassie Brilliant Blue and imaged to determine molecular weight distribution, as described in Santos Filipe et al. (2024) [5].

2.6. Biological Activities of Collagen

2.6.1. Antioxidant Capacity

The antioxidant activity of ASC extract and collagen peptides was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, as described by Hernández-Ruiz et al. (2023) [7].

2.6.2. Antimicrobial Activity

The antimicrobial activity of the ACS extract and collagen peptides was evaluated via well diffusion against selected Gram-positive (Staphylococcus aureus ATCC 25923 and ATCC 6538, Staphylococcus epidermidis ATCC 12228), Gram-negative

(Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922), and yeast strains (Saccharomyces cerevisiae ATCC 2601 and Candida albicans ATCC 10231), following the guidelines of the Clinical and Laboratory Standards Institute [8].

2.6.3. Evaluation of Collagen Peptides Cytotoxicity

The cell viability of Caco-2 cells when exposed (2 h) 60 collagen peptides (0.1–1 mg/mL) was measured using the MTT assay as previously described [9].

2.6.4. Caco-2 Cell Culture

The capacity of collagen peptides to permeate the intestinal barrier were assessed with differentiated Caco-2 cells monolayers on Transwell® insert (Corning Inc., Lowell, MA, USA) as described in Santos Filipe et al. (2024) [5].

3. Results and Discussion

3.1. Collagen Extraction

Collagen was successfully extracted from sardine scales using both acid solubilization (ASC) and pepsin-assisted methods (PSC). The PSC methos result in a yield of 0.55% (*w*/*w*), which was approximately three times higher than that obtained via acid extraction (0.18% *w*/*w*). These differences are consistent with previous literature reports for other fish species. Differences in yield can be influenced by extraction methods, tissue composition, and fish size or age [5].

3.2. Structural Characterization of Collagen

UV–Vis spectra confirmed collagen presence with absorption maxima at 233 nm (ASC) and 236 nm (PSC), while FTIR analysis showed typical amide bands (A, I, II, III) indicating preserved triple-helix structure. PXRD patterns revealed a more ordered structure in ASC, suggesting higher molecular cross-linking. SEM imaging displayed uniform fiber structures, and EDS analysis identified NaCl crystals, more abundant in PSC [5].

3.3. Hydrolysis and SDS-PAGE Analysis

Hydrolysis with papain effectively fragmented collagen into peptides (12–25 kDa), confirmed by SDS-PAGE. The ASC extract showed typical type I collagen bands (α1, α2, and β chains), while hydrolyzed peptides lacked high-molecular-weight bands, demonstrating successful enzymatic breakdown [5].

3.4. Biological Activities

Collagen peptides exhibited significantly higher antioxidant activity compared to native ASC (3.6-fold increase, IC50 = 19.79 mg/mL). No antimicrobial activity was detected under the tested conditions, consistent with peptide size-dependent effects and differences in bacterial strains [5].

Table 1. Antioxidant (DPPH, IC50) and antimicrobial activities of ASC and collagen peptides (PEP).

Sample	Antioxidant Activity (DPPH, IC50 mg/mL)	Antimicrobial Activity (10 mg/mL)
Collagen peptides (PEP)	19.79 ± 0.85 (high activity)	N/a
Acid-soluble collagen (ASC)	> 70 (low activity)	N/a

3.5. Intestinal Permeation

To assess the absorption potential of collagen peptides, their permeability was evaluated using differentiated Caco-2 cell monolayers that mimic the human intestinal barrier. Collagen peptides (0.5 mg/mL) exhibited moderate permeability, with an apparent permeability coefficient (Papp) of 5.25×10^{-6} cm/s, compared to 26.54×10^{-6} cm/s for caffeine, a high-permeability reference (Table 2). No cytotoxic effects were observed at the tested concentrations. The moderate permeation of collagen peptides is consistent with their molecular size (10–25 kDa) and agrees with previous studies showing size-dependent transport across Caco-2 monolayers. These results reinforce the feasibility of using sardine-derived collagen peptides in oral formulations, given their moderate permeability and absence of cytotoxic effects [5].

Table 2. Intestinal permeability (Papp) of collagen peptides and caffeine across Caco-2 cell monolayers.

Sample	Papp (cm/s) × 10^{-6}	Permeability Classification
Collagen peptides (0.5 mg/mL)	5.25	Moderate
Caffeine (0.8 mg/mL, control)	26.54	High

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