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Original Research Article

Characterization and Formulation of Anti-Aging Cream Using Collagen Derived from Sardine (*Sardinella spp*.) Fish Scales

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ABSTRACT

Skin aging is a natural biological process marked by a reduction in collagen production, leading to decreased skin elasticity and the formation of wrinkles. To address this problem, collagen derived from biological sources has attracted considerable attention as a promising active ingredient in cosmetic formulations. This study aimed to extract and characterize collagen from sardine (*Sardinella spp*.) fish scales and to formulate an anti-aging cream containing this collagen. Collagen extraction was performed using the acid-pepsin method. The extracted collagen was characterized using Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups, Scanning Electron Microscopy (SEM) to observe fibrillar collagen structures, and Liquid Chromatography-Mass Spectrometry (LC-MS) to analyze its peptide profiles.The results confirmed that the extracted collagen belonged to type I collagen, as evidenced by the presence of amide groups A, B, I, II, and III. The formulated anti-aging cream, containing collagen at concentrations of 5%, 10%, and 15%, demonstrated good physical stability during 12 weeks of storage, maintaining a stable pH (4.6–4.8) and optimal viscosity (21,200 cP). The antioxidant activity assessed by the DPPH radical scavenging assay revealed that hydrolyzed collagen exhibited a very strong antioxidant activity with an IC_{50} value of 34.97 ppm. Furthermore, hydrolyzed collagen showed maximum solubility (100%) at pH 4, indicating its suitability for cosmetic applications. The present work confirms that collagen derived from sardine fish scales demonstrates significant promise as a natural, efficacious, and safe bioactive component for anti-aging skincare formulations. However, comprehensive in vivo studies and controlled clinical trials remain essential to fully establish its therapeutic efficacy and safety profile for human cosmetic applications.

Keywords: Collagen, Sardine Fish Scales, Anti-Aging Cream, FTIR, SEM, LC-MS .

Introduction

Skin aging is a natural biological process that involves a progressive decline in collagen and elastin production, resulting in a loss of elasticity and the appearance of wrinkles[1]. To address the visible signs of aging, various strategies have been developed, particularly using active ingredients in skincare products that aim to restore moisture, improve elasticity, and reduce oxidative stress. Among the natural sources considered promising, fish-derived collagen has gained considerable attention due to its structural similarity to human collagen and its higher bioavailability compared to mammalian collagen [2] Recent study shows that fish collagen has higher solubility in acidic solutions and possesses antioxidant activity that can protect the, skin From damage caused by free radicals. [3] Unlike bovine or porcine collagen, fish scale-derived collagen is more compatible with cosmetic products due to its lower molecular weight, which allows better skin absorption [4,5]. While prior studies have explored collagen from deep-sea fish for cosmetic applications [5–7], comprehensive research on *Sardinella* spp. fish scale, particularly its characterization as an active anti-aging ingredient, physical stability, and antioxidant efficacy remains limited. Critical gaps persist in its physicochemical profiling and stability performance in topical formulations [6,8].Therefore, this study aims to comprehensively evaluate the characteristics of collagen extracted from sardine fish scales through FTIR spectroscopy, SEM analysis, LC-MS peptide profiling, pH solubility testing, and antioxidant activity using the DPPH method. Furthermore, the extracted collagen was formulated into anti-aging creams containing various collagen concentrations (5%, 10%, and 15%). The physical stability of these creams was evaluated over a 12-week storage period, along with tests on their effectiveness in enhancing skin hydration. The novelty of this study lies in its holistic approach to the characterization and formulation of sardine scales collagen, which has not been extensively reported in previous studies [7–9] .

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Materials and Methods

Sample Preparation

Sardine fish scales used in this study were collected from the coastal waters of Gorontalo, Indonesia, in early January 2025. The samples were submitted to the Laboratory of Fisheries, Faculty of Fisheries,

Gorontalo State University, for taxonomic verification. The specimen was identified and confirmed as Sardinella spp. belonging to the family Clupeidae by a certified ichthyologist, as documented in the official letter No. 2488/IPH.1.01/IL.07/I/2025 dated January 5, 2025. The extraction of collagen from sardine fish scales was conducted following a modified acid-pepsin method. Initially, 400 g of cleaned sardine fish scales were soaked in 0.1 M NaOH solution for 24 hours at 4°C with continuous stirring to remove non-collagenous proteins. The scales were subsequently rinsed thoroughly with distilled water until neutral pH was achieved. Subsequently, the deproteinized scales were immersed in 1.5 M acetic acid for 7 hours at 4°C to facilitate collagen swelling and solubilization. Pepsin enzyme (1:20 w/w) was then added and incubated for an additional 24 hours to extract pepsin-soluble collagen (PSC). The collagen solution obtained was salted out by adding 2.5 M NaCl, followed by centrifugation at 8000 rpm for 20 minutes. The precipitated collagen was washed, filtered through Whatman filter paper, and dried at 40 °C to yield collagen in powdered form. [10,11].

Antiaging Cream Formulation

The anti-aging cream was formulated using sardine fish scales collagen at three concentration levels: 5%, 10%, and 15% w/w, as presented in Table 1. All other components were calculated based on its weight/weight percentage. Polyvinyl alcohol (PVA) was added as a film-forming agent to enhance the physical stability of the emulsion.

Table 1: Composition of Sardine Fish Scales Collagen as Anti-Aging Cream Formulations (w/w%)

Ingredient	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Function
Sardine fish scale collagen	5	10	15	Active ingredient
Polyvinyl alcohol (PVA)	10	10	5	Active ingredient
Hydroxypropyl methylcellulose (HPMC)	4	2	4	Viscosity enhancer
Propylene glycol	10	10	10	Humectant (moisturizer)
Methylparaben	0.2	0.2	0.2	Antimicrobial preservative
Propylparaben	0.5	0.5	0.5	Antimicrobial preservative
Cucumber extract	qs	qs	qs	Fragrance
Ethanol 96%	15	15	15	Solvent

Note: qs = quantum satis (as needed)

Hydroxypropyl methylcellulose (HPMC) was used as a viscosity modifier to ensure consistent texture, while propylene glycol served as a humectant to retain moisture[21]. Methylparaben and propylparaben were included as antimicrobial preservatives to prevent microbial growth and improve shelf life [6]. Menthol was added as a fragrance agent in a quantum satis (qs) amount, and ethanol 96% was used as the primary solvent in the formulation [10]. To prepare the cream, all oil-phase and water-phase ingredients were weighed accurately. The oil-soluble components (parabens and menthol) were first dissolved in ethanol. In a separate beaker, water-soluble components, including collagen, PVA, HPMC, and propylene glycol, were mixed and heated gently at 40–45 °C with continuous stirring. The oil phase was then added gradually to the aqueous phase under homogenization at 2000 rpm for 15 minutes using a high-speed homogenizer. The mixture was stirred slowly for an additional 10 minutes until a homogeneous and stable emulsion was obtained. The final cream was filled into sterilized containers and stored at room temperature for further testing. It is important to note that the anti-aging claim of the formulation was not verified during the formulation stage. Instead, the functional validation was carried out in subsequent phases of the study through antioxidant activity assays of the extracted collagen and by evaluating the physical stability and hydration performance of the cream.

Physical Stability Test

The physical stability test of the anti-aging cream formulations was conducted by evaluating several parameters during a 12-week storage period at room temperature (25 ± 2°C). The parameters measured included organoleptic observation, homogeneity, pH, viscosity, spreadability, and irritation test, following standard cosmetic evaluation methods [11,12]. Organoleptic observations were performed visually to assess changes in color, odor, and texture of the cream during the storage period by spreading 1 g of cream on a clean glass slide and then observing the uniformity of the spread and absence of phase separation. pH measurements were carried out by dispersing 1 g of cream in 10 mL of distilled water and measured using a digital pH meter (Hanna Instruments). Viscosity was measured using a Brookfield viscometer (model DV-E) with spindle no. 4 at 10 rpm at 25°C. The spreadability was determined by placing 1 g of cream between two glass plates and applying a standard weight (500 g) for 1 minute, and then measuring the diameter of the spread area. An irritation test was conducted on 10 healthy human volunteers by applying 0.5 g of cream on the inner forearm area. The skin reactions, including redness, itching, and irritation, were observed and recorded after 24 hours of application.

Morphology Analysis with SEM

The surface morphology of the extracted collagen was examined using SEM. Prior to observation, the collagen samples were freeze-dried and then coated with a thin layer of gold–platinum to enhance conductivity. The prepared samples were observed under SEM (Hitachi SU3500) at magnifications ranging from 100× to 1000×. This analysis was conducted to evaluate the fibrillar structure and surface characteristics of the collagen fibers, particularly the presence of ordered and interconnected collagen bundles as indicators of structural integrity and purity of the extracted material [14].

FTIR Spectroscopy Analysis

FTIR spectroscopy was conducted to identify the functional groups present in the extracted collagen. The collagen samples were finely ground and mixed with potassium bromide (KBr) at a ratio of 1:100 (w/w), then compressed into transparent pellets using a hydraulic press. The spectra were recorded using an FTIR spectrometer (Shimadzu IRTracer-100) in the range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1}. The characteristic peaks corresponding to amide A, amide B, amide I, amide II, and amide III were used to confirm the presence and integrity of collagen structure in the sample [11].

LC-MS analysis

The peptide profile of the extracted collagen was analyzed using LC-MS equipped with Waters QTOF. Prior to analysis, the collagen samples were hydrolyzed and then injected in positive ionization mode. The mobile phase consisted of acetonitrile and water with 0.1% formic acid as the modifier. Mass spectra were acquired using the MassLynx software and analyzed to identify the peptide composition of the collagen extract[15].

pH Solubility Test of Hydrolyzed Collagen

The pH solubility of hydrolyzed collagen was tested by dissolving 50 mg of collagen in 10 mL of phosphate buffer solution at pH 4, 7, and 10. The solutions were stirred at 25 °C for 30 minutes and then filtered through a 0.45 µm membrane filter. The absorbance of each solution was measured at 280 nm using a UV-Vis spectrophotometer. The percentage of solubility was calculated based on absorbance values, and

statistical significance between pH groups was determined using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$) [4,16].

Antioxidant Activity Testing of Pepsin-Soluble Collagen and Hydrolyzed Collagen

The antioxidant activity of both pepsin-soluble collagen and hydrolyzed collagen was evaluated using the DPPH radical scavenging method. A 0.1 mM DPPH solution in methanol was prepared and mixed with collagen solutions at concentrations of 5, 10, 20, 50, and 100 ppm. The mixtures were incubated in the dark for 30 minutes at 37 °C, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition was calculated, and the LC_{50} values were determined from the linear regression curve. The antioxidant activity was categorized as very strong (<50 ppm), strong (50–100 ppm), moderate (100–190 ppm), or weak (>190 ppm). Statistical analysis was performed using one-way ANOVA and Tukey HSD test ($p < 0.05$) to assess significant differences between sample groups [4,15].

Results and Discussion

Collagen Extraction

Collagen extraction from 400 g of sardine fish scales successfully yielded 21.5 g of dried collagen powder. The identification of collagen was conducted using the ninhydrin test to detect the presence of free amino acid residues, particularly proline and hydroxyproline, which are the main components in the triple helix structure of collagen. The test results showed a color change from white to blue, indicating the presence of collagen in the extracted sample. The ninhydrin test is commonly used to determine the success of collagen isolation due to its sensitivity in detecting free amino acids [4,12,16–20]. The extraction method applied in this study, namely the acid-pepsin method, plays a critical role in maintaining the structural integrity and functional properties of the extracted collagen. The use of acid combined with pepsin enzymes was effective in producing collagen with high purity and minimizing the degradation of its triple helix structure [21–23]. Moreover, pepsin-assisted extraction has been reported to efficiently remove non-collagenous proteins without damaging the primary structure of collage [25,26]. Successful collagen extraction is essential because it determines not only the purity but also the stability of collagen, which is crucial for its application in cosmetics and pharmaceuticals [27,28] .

Formulation and Physical Stability of the Anti-Aging Cream

The anti-aging cream formulated with collagen extracted from sardine fish scales was evaluated to determine its physical stability and effectiveness in cosmetic applications. The cream was designed with collagen concentrations of 5%, 10%, and 15% (w/w) to optimize its texture, viscosity, and spreadability for topical use. The formulation process used an oil-in-water (O/W) emulsion system with polyvinyl alcohol (PVA) as a film-forming agent and hydroxypropyl methylcellulose (HPMC) as a thickening agent to maintain product stability [11,12]. Increasing the collagen concentration was found to influence both viscosity and spreadability, where higher collagen content resulted in a thicker texture but still allowed for easy application on the skin surface [28,29].

Stability Test of Anti-Aging Cream During Storage

The physical stability test was conducted over a 12-week storage period at room temperature. Parameters observed included organoleptic characteristics (color, odor, texture), homogeneity, pH stability, and spreadability. The results showed that all cream formulations remained stable during storage without significant changes in color, odor, or consistency ($p > 0.05$). No phase separation or microbial contamination was observed, indicating that the addition of propylene glycol and parabens effectively maintained the product's quality [30,31]. Organoleptic observations revealed that the cream color remained white without turning yellow or brown, indicating the absence of oxidation or degradation reactions. The aroma of the cream also remained pleasant and did not indicate any sign of rancidity or instability, as seen in Figure 1. These findings indicate that the chemical composition of the cream was stable and that the addition of stabilizing agents was effective [11,12,28,29,32].

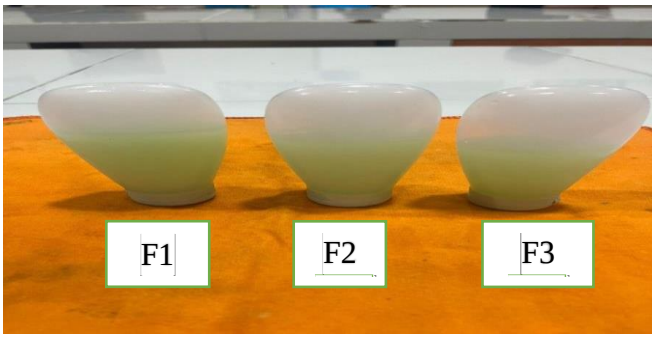


Figure 1: Appearance of Anti-Aging Cream Containing Sardine Fish Scale Collagen and Cucumber Extract (*Cucumis sativus* L.)

The physical stability of a cosmetic formulation is a critical factor determining product integrity during storage and use. Physical stability evaluation includes observations of organoleptic properties, pH, viscosity, and spreadability [28,29]. The anti-aging cream formulated with the sardine fish scales collagen demonstrated good physical stability over 12 weeks of storage, with only minor changes in physicochemical parameters. Organoleptic testing revealed a stable white cream color with no significant alterations during storage [28,29]. The cream's aroma remained unchanged, showing no signs of oxidative degradation or active ingredient decomposition [30]. Texture evaluation confirmed the absence of phase separation or granulation, indicating excellent emulsion stability in the formulation [12,29].

pH, Spreadability, and Viscosity Evaluation of the Anti-Aging Cream

The pH stability results showed that the anti-aging cream formulated with sardine fish scale collagen exhibited an initial pH of 4.7–4.8, which remained within the safe range for topical cosmetic application. After 12 weeks of storage, a slight decrease to 4.6–4.7 was observed, but the pH remained acceptable for maintaining skin integrity and product stability. These results demonstrate that the formulation successfully maintained the chemical stability of active materials and excipients, with no significant degradation observed, as seen in Figure 2 [28,32]. Compared with formulations using collagen from other sources, sardine fish scale collagen cream demonstrated superior pH stability.[6] reported that creams formulated with tilapia collagen exhibited more pronounced pH declines during storage. In contrast, [12] found that cod collagen-based creams maintained stability in the range of 4.5–5.0 [12,29]. Ensuring pH stability is critical to guarantee the safety and effectiveness of long-term topical applications, as excessively acidic or alkaline pH values can lead to skin irritation and reduced efficacy of active compounds [13,28,32]. Spreading ability is a critical parameter for determining a product's effectiveness during topical application. The spreadability test evaluates how uniformly the cream can be distributed across the skin surface under controlled pressure. Key factors influencing spreadability include the formulation's viscosity and rheological properties. Results demonstrate that spreadability increases as viscosity decreases, indicating that lower-viscosity formulations achieve wider distribution on the skin surface (Figure 2). These findings align with [31] who emphasized that optimal spreadability is essential for both user comfort and active ingredient penetration [31,32].

In addition, optimal spreadability enhances the efficient absorption of active substances through the skin. The results demonstrated that formulations with higher collagen concentrations exhibited reduced spreadability due to increased viscosity. Thus, a balanced formulation is essential to maintain both user comfort and optimal spreadability without compromising the efficacy of active ingredients. [32] further emphasized that cream spreadability critically influences the uniform distribution of active compounds on the skin. Consequently, an ideal formulation must achieve equilibrium between spreadability and skin adhesion properties [31,32].

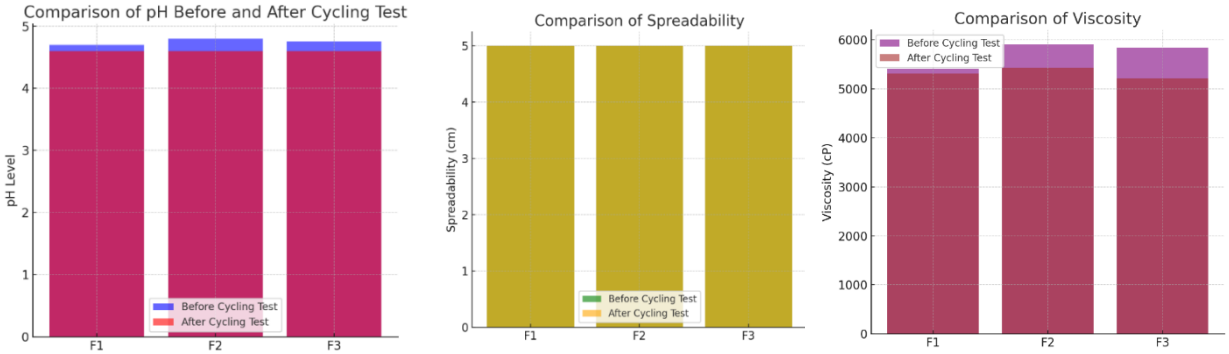


Figure 2: Evaluation results of PH Test, Spreadability, and Formula Viscosity

Viscosity testing revealed that the anti-aging cream formulations containing sardine fish scale collagen had initial viscosities of 18,500 cP (5% collagen), 21,200 cP (10%), and 24,700 cP (15%), as shown in Figure 2. After 12 weeks of storage, all formulations exhibited minor viscosity reductions, with final measurements of 18,100 cP (5%), 20,900 cP (10%), and 24,300 cP (15%). These slight decreases remained within acceptable limits, confirming the emulsion's structural stability throughout storage [29,30].

Compared to collagen creams from other sources, these results demonstrate superior viscosity stability. A study by [6] reported that tilapia collagen-based creams experienced up to 10% viscosity reduction after 12 weeks of storage, while [12] found that codfish collagen creams maintained better viscosity stability with less than 2% decrease [21.31]. Viscosity stability is critically important as it determines both product application performance and user comfort. If viscosity becomes too low, the cream turns watery and difficult to apply, whereas excessive viscosity leads to poor skin distribution [29,30].

The pH test results demonstrated that the cream maintained a stable pH range of 4.6–4.8, which complies with established cosmetic safety standards [29.34]. When compared to other collagen-based formulations such as those derived from tilapia and codfish, the sardine fish scale collagen cream exhibited superior pH stability [21.30]. Spreadability testing revealed optimal performance in the 10% collagen formulation (5.8 cm spread diameter), with the 5% formulation showing 6.2 cm spread and the 15% formulation achieving 5.4 cm [36.37]. These findings correlate with existing research indicating that optimal spreadability enhances active ingredient penetration into the skin [28,32]. Initial viscosity measurements showed values of 18,500 cP (5% collagen), 21,200 cP (10%), and 24,700 cP (15%). After 12 weeks of storage, only minimal viscosity changes were observed, confirming excellent emulsion structural stability [12,29].

Irritation Test

The irritation test was conducted to evaluate the safety of the anti-aging cream formulated with sardine fish scale collagen when applied to human skin. This test aimed to ensure that the cream did not cause adverse skin reactions such as redness, itching, or burning sensations. Based on the results presented in Table 2, none of the volunteers experienced irritation symptoms, including redness, itching, or burning sensations, after using the cream formulations containing 5%, 10%, and 15% collagen concentrations. This indicates that all cream formulations are safe for topical application and do not cause allergic or irritant reactions on human skin. This finding is supported by previous studies which stated that collagen derived from marine sources, particularly fish scales, has a lower potential to cause allergic reactions compared to collagen extracted from bovine or porcine sources [4]. The safety profile of fish collagen-based anti-aging creams enhances their suitability for cosmetic applications, especially for consumers with sensitive skin. The irritation test results demonstrated that the cream formulation caused no skin irritation in volunteers, with no observed signs of erythema, pruritus, or burning sensations after 24 hours of application [4,16]. Comparative studies with collagen formulations from other animal sources revealed that fish-derived collagen exhibits superior safety profiles and lower allergenic potential [21.31]. Collectively, these findings indicate that the sardine fish scale collagen-based cream formulation demonstrates stable physical stability and safety for use as an anti-aging cosmetic product.

Table 2: Irritation Test Results

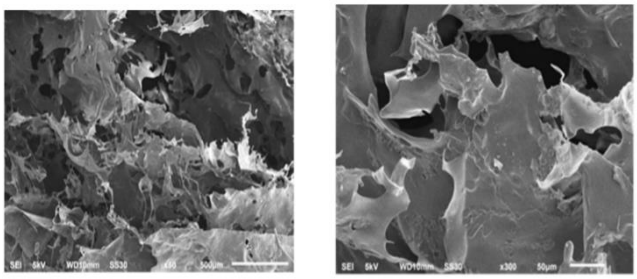
Observation	Formula	Volunteers									
		1	2	3	4	5	6	7	8	9	10
Availability in the market	Redness	-	-	-	-	-	-	-	-	-	-
	Itchy	-	-	-	-	-	-	-	-	-	-
	Perik/hot	-	-	-	-	-	-	-	-	-	-
Formula 5%	Redness	-	-	-	-	-	-	-	-	-	-
	Itchy	-	-	-	-	-	-	-	-	-	-
	Perik/hot	-	-	-	-	-	-	-	-	-	-
Formula 10%	Redness	-	-	-	-	-	-	-	-	-	-
	Itchy	-	-	-	-	-	-	-	-	-	-
	Perik/hot	-	-	-	-	-	-	-	-	-	-
Formula 15%	Redness	-	-	-	-	-	-	-	-	-	-
	Itchy	-	-	-	-	-	-	-	-	-	-
	Perik/hot	-	-	-	-	-	-	-	-	-	-

- indicates no irritation reaction was observed in all volunteers

SEM Analysis

The SEM analysis revealed that the extracted collagen from sardine fish scales exhibited a characteristic fibrillar structure with well-organized morphology, as shown in Figure 3. Observations at 500× and 1000× magnification confirmed the presence of layered and interlocked collagen fibers, demonstrating preservation of the triple-helix structure post-extraction. Furthermore, no significant structural damage or degradation was observed, indicating minimal alteration to protein conformation during the extraction process. These findings confirm that the applied extraction method effectively maintained the structural integrity of collagen [26,29]. Compared to collagen extracted from other sources, such as bovine or tilapia skin, sardine fish scales collagen displayed a more compact and densely packed fibrillar structure. Previous studies reported that collagen from tilapia exhibited a looser structure with more scattered fibers, while bovine collagen fibers tended to be thicker but less densely packed than those from fish collagen. This structural difference results in reduced uniformity and stability in cosmetic formulations[12,28]. The dense and interwoven fibrillar structure observed in sardine fish scale collagen contributes to superior mechanical stability and enhances its adhesive power when used in

cosmetic and and pharmaceutical formulations [29,30]. These characteristics support the potential application of sardine fish scale collagen as an effective active ingredient in anti-aging cosmetic products due to its ability to maintain product integrity and improve skin adherence compared to collagen derived from other sources.



(a) **(b)**

Figure 3: Scanning electron micrographs of sardine fish scale collagen at magnification (a) ×1000 and (b) ×5000

SEM analysis confirmed the preservation of fibrillar collagen structure post-extraction, demonstrating well-organized, layered fiber patterns. These structural features indicate that the extraction method successfully maintained the integrity of the triple-helix collagen network - a critical factor for cosmetic and pharmaceutical applications [28]. Comparative studies by [29] revealed similar fibrous patterns in codfish collagen, though with notably larger fibril diameters than sardine-derived collagen [32]. The denser fibrillar architecture observed in sardine collagen may enhance both mechanical stability and adhesive properties in cosmetic formulations [31].

FTIR Spectroscopy Analysis

FTIR spectroscopy was conducted to identify the functional groups present in the collagen extracted from sardine fish scales. The FTIR spectrum revealed characteristic absorption bands associated with collagen, as shown in Figure 4. The spectral peaks correspond to specific amide groups, confirming the integrity of the protein's triple helix structure. A broad absorption peak was observed at approximately 3316 cm^{-1}, which corresponds to N–H stretching, a typical characteristic of amide A. Another significant absorption was detected at 1636 cm^{-1}, attributed to C=O stretching vibrations in amide I, indicating the presence of the polypeptide backbone. Peaks observed at 1412 cm^{-1} and 1278 cm^{-1} were associated with C–N stretching and N–H bending vibrations in amide II, while a band at 1027 cm^{-1} was linked to amide III, reflecting C–N stretching and N–H bending in the triple helix structure of collagen[26,29]. The detailed wavenumber and functional group assignments of these peaks are summarized in Table 3.

Table 3: Peak Assignments of FTIR Spectra of Extracted Pepsin-Soluble Collagen from Sardine Fish Scales

Peak	Wavenumber (cm^{-1})	Reference (cm^{-1})	Group
Amide a	3316.42	3440-33a00	NH stretching
Amide b	1636.09	3080-2889	CH2 asymmetrical stretch
Amide I	1412.90	1700-1600	C=0 Stretching
Amide II	1278.54	1580-1500	NH bending
Amide III	1032.76	1350-1200	NH bending and CN stretching

The observed spectrum is consistent with the FTIR profiles of collagen reported from other sources (bovine and tilapia skin). However, the intensity of the absorption peaks in sardine collagen appeared higher, suggesting a purer sample with a more preserved triple-helix structure. Fish collagen typically exhibits sharper amide I and II peaks compared to mammalian collagen[12]. Previous studies have also shown that fish-derived collagen demonstrates greater stability and fewer contaminant proteins[28].

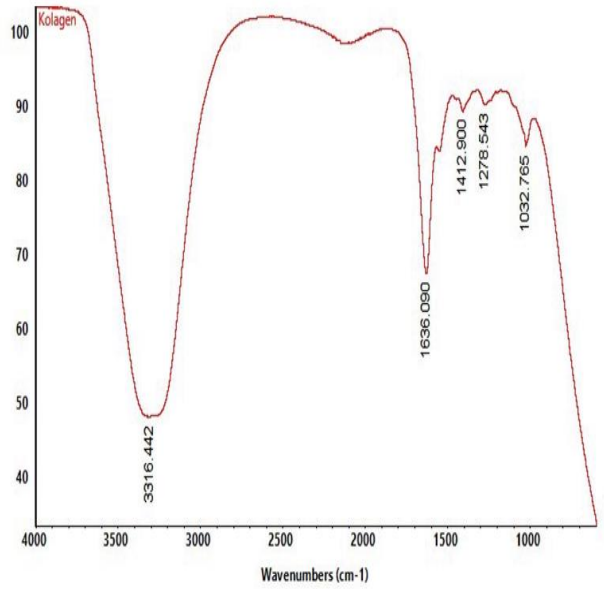
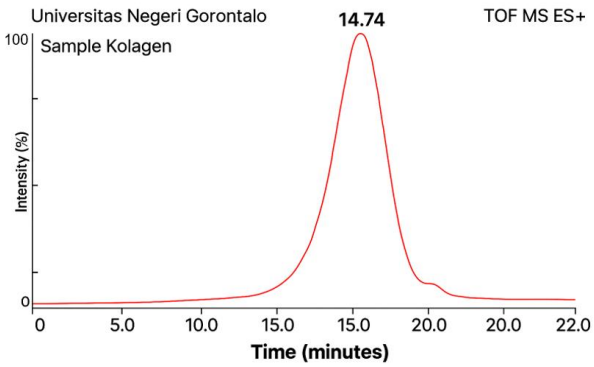


Figure 4: FTIR Spectrum of Extracted Pepsin-Soluble Collagen from Sardine Fish Scales

These findings confirm that the extraction process preserved the primary molecular structure of collagen, validating its purity and structural integrity. The presence of strong amide bands, especially amide I and II, supports the use of this collagen as an active ingredient in cosmetic applications where high molecular integrity is essential[31,32].

LC-MS Peptide Profile Analysis

LC-MS analysis was performed to determine the peptide profile of collagen extracted from sardine fish scales. The LC-MS chromatogram showed a major peak at a retention time of 14.74 minutes, indicating the presence of a dominant peptide fraction, as shown in Figure 5



This major peak suggests that the extracted collagen contains peptides with relatively small molecular sizes, which are typically more easily absorbed and suitable for cosmetic and pharmaceutical applications [7,9]. In addition to the main peak, several minor peaks were observed in the chromatogram, suggesting the presence of diverse peptide fragments with uniform distribution and even intensity. This pattern reflects a complex peptide composition with multiple fragmentations of the collagen protein. The mass spectrum analysis revealed that the major peptide fragment appeared at m/z 760, which indicates peptides rich in proline and hydroxyproline, amino acids characteristic of collagen structure. Furthermore, additional fragments were detected at m/z 820,

900, and 1050, confirming the presence of smaller peptide components in the extracted collagen.

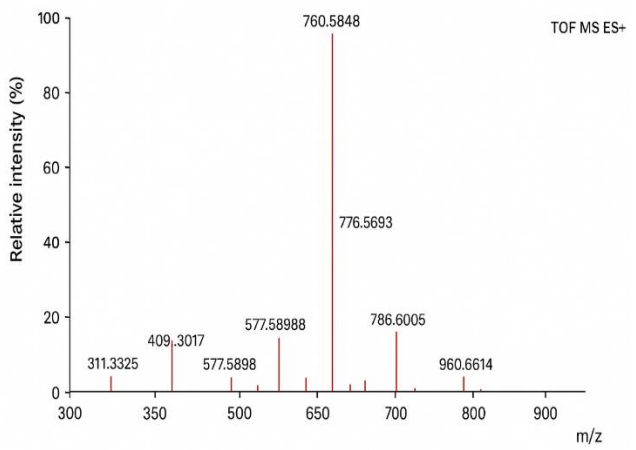


Figure 5: Results of LC-MS Analysis of Sardine Fish Scales Pepsin-Soluble Collagen: Chromatogram (top) and Mass Spectrum (bottom)

Collagen derived from tilapia has been reported to show more fragmented mass spectra with a wider distribution of peptide sizes, while bovine skin collagen exhibited dominant peptide fragments at m/z values above 1000, indicating larger peptide sizes[12,28]. Compared to these other sources, the LC-MS profile of sardine fish scale collagen demonstrated a predominance of smaller peptides, which is advantageous for enhancing bioavailability and absorption in topical cosmetic and pharmaceutical formulations[31,32].

pH Solubility Test of Hydrolyzed Collagen

The solubility profile of hydrolyzed collagen was evaluated at different pH conditions (pH 4, 7, and 10) by dissolving 50 mg of collagen in 10 mL of phosphate buffer solution. The results showed that the maximum solubility was achieved at pH 4, reaching 100%. At pH 7, the solubility decreased to 87.33%, while at pH 10, it further decreased to 86.29%. The solubility results are illustrated in Figure 6. Statistical analysis using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$) confirmed that there were significant differences in collagen solubility at different pH levels. Statistical analysis using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$) confirmed that there were significant differences in solubility at different pH levels. The solubility of hydrolyzed collagen at pH 4 (100.00 ± 0.00%) was significantly higher ($p < 0.05$) compared to pH 7 (87.33 ± 1.53%) and pH 10 (86.29 ± 2.11%).

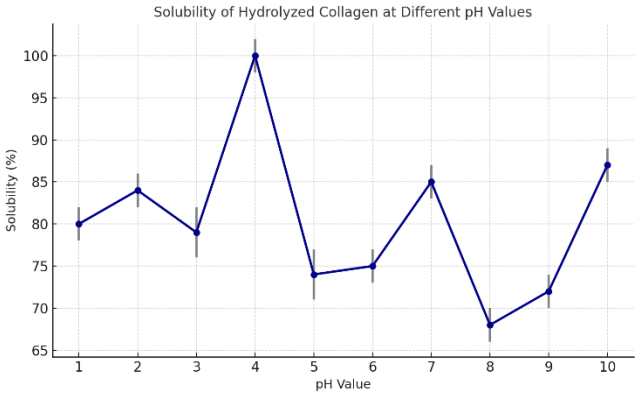


Figure 6: Effect of pH on Solubility of Hydrolyzed Collagen

These findings indicated that hydrolyzed collagen was more soluble in acidic conditions and tended to decrease at neutral and alkaline pH [28,29]. This is in line with previous research, which also reported that collagen from marine sources exhibited greater solubility under acidic conditions compared to neutral or alkaline environments [12,28]. Compared to collagen from cod or bovine skin, sardine fish scales collagen demonstrated a superior solubility profile, particularly at acidic pH, which is essential for stability in various cosmetic formulations [17,31].

The pH solubility test demonstrated that hydrolyzed collagen exhibited maximum solubility (100%) at pH 4. Compared to pepsin-soluble collagen, which showed lower solubility under extreme pH conditions, the hydrolyzed form displayed greater stability and easier dissolution in acidic environments. These solubility characteristics align with findings by [6], who reported that tilapia collagen achieved peak solubility at pH 4-5 but showed reduced solubility at neutral pH relative to sardine scale collagen [29].

Antioxidant Activity of Pepsin-Soluble Collagen and Hydrolyzed Collagen

The antioxidant activity of hydrolyzed and pepsin-soluble collagen extracted from sardine fish scales was assessed using the DPPH radical scavenging method at concentrations of 5, 10, 20, 50, and 100 ppm. The hydrolyzed collagen exhibited a strong antioxidant potential with an LC_{50} value of 34.97 ppm, categorized as very strong ($IC_{50} < 50$ ppm), while pepsin-soluble collagen showed a moderate antioxidant effect with an LC_{50} value of 148.55 ppm ($IC_{50} = 100–190$ ppm), as shown in Figure 7. Statistical analysis using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$) confirmed that the antioxidant activities of the two collagen types were significantly different [12,29].

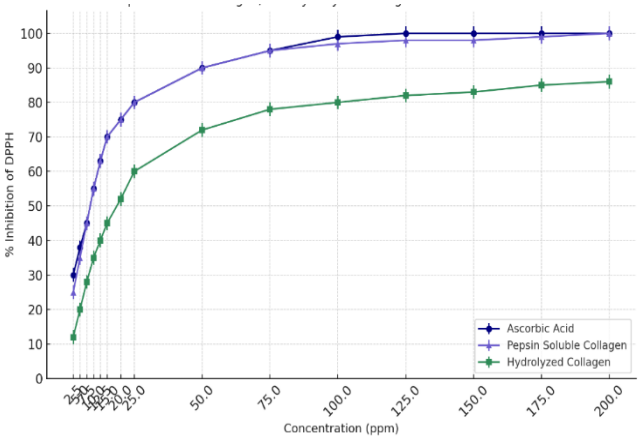


Figure 7: Percentage Inhibition of DPPH Radicals by Ascorbic Acid, Pepsin-Soluble Collagen, and Hydrolyzed Collagen from Sardine Fish Scales

These findings are consistent with previous studies. [9] reported that collagen from tilapia exhibited an LC_{50} value of 56.4 ppm, while [7]. found that bovine skin collagen had an LC_{50} value of 165.3 ppm [7,9]. The lower LC_{50} of hydrolyzed sardine collagen observed in this study indicates a superior radical scavenging capacity, supporting its potential application as an effective bioactive ingredient in cosmetic and pharmaceutical formulations [31,32]. Hydrolyzed collagen derived from sardine scales exhibited significantly enhanced antioxidant activity compared to both pepsin-soluble collagen and collagen from alternative sources (tilapia and bovine skin). These findings strongly support the potential application of sardine-derived collagen as a bioactive ingredient in anti-aging cosmetic formulations. However, additional in vivo studies and clinical trials remain necessary to comprehensively evaluate its long-term safety profile and therapeutic efficacy, particularly under extreme storage conditions[17,31]

Conclusion

This study demonstrated that collagen extracted from *Sardinella spp.* fish scales possesses favorable structural, biochemical, and functional properties for cosmetic application. The extracted type I collagen

maintains its triple helix structure and exhibits strong antioxidant activity and high solubility in acidic conditions, supporting its potential as a natural bioactive ingredient. The formulated anti-aging cream shows good physical stability and is safe for topical use. These findings highlight the promising role of marine-derived collagen in developing safe and effective anti-aging skincare products. Future research should explore its performance under extreme environmental conditions and evaluate its long-term safety through *in vivo* studies.

Conflict of interest
The author declare no conflicts of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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