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Research Article

Characteristics of Water-Soluble Collagen Extracted from Catfish (*Pangasius* sp.) Skin Using Different Acetic Acid (CH3COOH) Concentrations

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Abstract

Collagen extraction from fish skin offers a sustainable approach to valorize fish processing by-products, and fish skin catfish (*Pangasius* sp.) is recognized as a promising collagen source. However, previous studies reported that in low water solubility, limiting its functional application in food, pharmaceutical, and cosmetic industries. To address this challenge, this study investigates the use of varying acetic acid concentrations (0.4, 0.6, and 0.8 M) during the hydrolysis stage prior to hydro-extraction, aiming to enhance the solubility and quality of the extracted collagen. Parameters measured included yield, solubility, proximate composition (protein, fat, air content, ash), organoleptic quality, molecular weight, amino acid profile and functional groups analysis. The best results were achieved with 0.6 M acetic acid, resulting in type I collagen coupled by the amide groups A, B, I, II, III, and molecular weights (65, 95, 130 and 270 kDa). The dominant amino acids identified was glycine. This treatment yielded a collagen extraction rate of 9.04% and solubility of 79.71%. The proximate composition included 67.34% protein, 14.87% fat, 8.48% moisture, and 10.69% ash. Organoleptic scores for appearance, odour, and texture were 7.80, 7.93, and 6.80, respectively. The collagen met the SNI 8076:2020 standard for protein content, moisture, and organoleptic attributes; however, fat and ash contents exceeded the specified limits. In conclusion, the acetic acid concentration significantly affects the physicochemical and sensory properties of collagen. Catfish skin shows strong potential as a raw material for collagen production, which supports its use in the food, pharmaceutical, and cosmetic sectors.

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1. Introduction

Catfish (*Pangasius* sp.) is one of the leading freshwater fish commodities in Indonesia. According to the Ministry of Marine and Fisheries (2022), catfish production in Indonesia reached 332 tons in 2021 and nearly doubled to 635 tons in 2022. This increase aligns with the growth of the catfish industry, particularly in fillet processing (Devi *et al*., 2017). However, the rise in catfish production also resulted in a higher volume of fishery by-products, such as heads, viscera, bones, and skin. Fillet processing generates fish skin by-products, accounting for approximately 5.12–6.14% of the total fish weight, which have not been optimally utilized. Fish skin can be processed into collagen, creating a value-added product (Yanti *et al*., 2022).

Collagen is a fibrous protein composed of three polypeptide chains arranged in a triple-helix structure, playing a crucial role in the formation of connective tissue. Collagen found in the cornea, teeth, tendons, bones, and skin accounts for 25–30% of the total animal protein (Yanti *et al*., 2022). Additionally, more than 50% of the extracellular protein in the skin consists of collagen (Suptijah *et al*., 2018). Due to its structural and functional properties, collagen has significant applications in the food, pharmaceutical, cosmetic, and biomedical industries (Suptijah *et al*., 2018). Catfish skin can serve as an alternative collagen source, replacing commercial collagen derived from pigs and cows. This is particularly important because pork-based collagen is prohibited for Muslims and Jews, while Hindus do not consume beef-based products. Furthermore, bovine collagen carries the risk of contamination with Foot and Mouth Disease (FMD) and Bovine Spongiform Encephalopathy (BSE), both of which have emerged over the past few decades (Nining, 2020). Fish-derived collagen offers several advantages, including a smaller molecular structure that enhances absorption by the body (Astiana *et al*., 2016). Additionally, its amino acid composition and biocompatibility closely resemble those of collagen derived from cows and pigs (Jafari *et al*., 2020).

Over the past decade, research on water-soluble collagen from fish skin, particularly catfish, has advanced significantly, focusing on optimizing extraction methods, characterizing its properties, and exploring its applications in various industries. Optimal pretreatment methods, such as degreasing and bleaching with hydrogen peroxide, have been shown to enhance the purity and yield of collagen extracted from the skin of Southern catfish (*Silurus meridionalis*) (Xu *et al*., 2017). Ultrasonic-assisted extraction (UAE) with ethanol has also been proven effective in removing up to 85.6% of lipid content from catfish skin while preserving the integrity of the extracted collagen (Agustina *et al*., 2023). Additionally, a previous study indicated that a 24-hour extraction period using pepsin on silver catfish (*Pangasius* sp.) skin yields collagen with optimal physicochemical properties (Shaik *et al*., 2023). Collagen extracted from the skin of sucker catfish (*Pterygoplichthys pardalis*) has been confirmed as Type I collagen, characterized by a distinct amino acid composition and valuable for medical and cosmetic applications (Nurubhasha *et al*., 2019). Research has also revealed that collagen derived from African sharptooth (*Clarias gariepinus*) exhibits high biocompatibility with mesenchymal stem cells, making it a promising candidate for tissue engineering and regenerative medicine (Rusinek *et al*., 2023). Furthermore, the application of catfish-derived collagen in the food industry has been explored in fresh cheese products, with sensory evaluations showing no significant impact on taste or texture (Nurubhasha *et al*., 2019). In biomedical applications, collagen hydrolysate from fish skin has demonstrated the ability to accelerate cell proliferation and wound healing in in-vitro studies (Manjushree *et al*., 2023). With these advantages, collagen derived from catfish skin presents a sustainable and promising alternative to mammalian collagen sources. In addition, water-soluble collagen research is essential due to its superior solubility, safety, and bioavailability compared to traditional collagen. Unlike conventional collagen, which has poor water solubility, water-soluble collagen dissolves more efficiently, allowing for broader applications in the food, pharmaceutical, and cosmetic industries (Yang *et al*., 2020). Its smaller molecular structure also enhances absorption and bioavailability, making it highly beneficial for skin and joint health (Choi *et al*., 2019). These advantages make water-soluble collagen a crucial focus for future innovations in industrial applications. However, research on water-soluble collagen from catfish (*Pangasius* sp.) is still limited. Hence, this study is essential to be carried out to meet the growing demand for collagen.

Collagen extraction can be carried out using acid, base, and enzymatic methods. However, the enzymatic extraction method has the drawback of high enzyme costs (Kittiphattanabawon *et al*., 2010). Base extraction is typically used for raw materials with complex cross-links, such as bones and cowhide, while acid extraction is more suitable for materials with fewer cross-links, such as fish and pig skin (Astiana *et al*., 2016). Common acids used in collagen extraction include organic acids like acetic acid, lactic acid, and citric acid, with acetic acid (CH_3COOH) being the most frequently used due to its superior extraction efficiency compared to other solvents (Hadfi

and Sarbon, 2019). Liu *et al.* (2015a) stated that water-soluble collagen extraction from *Pangasius* sp. fish skin should focus on optimizing various aspects of the extraction process to improve yield, solubility, and functional properties. The acid plays a crucial role in breaking down collagen's intermolecular bonds. Acetic acid plays a crucial role in disrupting the non-covalent bonds and loosening the triple-helix structure of collagen, thereby enhancing its solubility in aqueous solutions. By adjusting the concentration of acetic acid during hydrolysis, the extent of structural modification can be controlled, which in turn affects the yield, solubility, and functional quality of the extracted collagen (Liu *et al*., 2012; Nagai and Suzuki, 2000). Hence, the treatment of acetic acid concentration and extraction conditions is an important area for further research.

Hydro-extraction is another collagen isolation method that utilizes temperature and water, with distilled water serving as a heat transfer medium (Kolanus *et al*., 2019). Collagen obtained through hydro-extraction is water-soluble and offers several advantages, including continuous production, high yield, and lower production costs. Additionally, hydro-extracted collagen is biodegradable and considered safer for long-term consumption compared to collagen extracted using acid or base (Huang *et al*., 2016). The characteristics of collagen can also be influenced by the concentration of acetic acid used during extraction (Liu *et al*., 2015b). This study aims to investigate the effect of acetic acid concentration on the solubility and quality characteristics of water-soluble collagen extracted from catfish (*Pangasius* sp.).

2. Materials and Methods

2.1 Materials

2.1.1 The equipment

The equipment's used in this study were analytical balance (PA224, Ohaus), analytical balance (PA2102, Ohaus), beaker glass (CTE33, Iwaki), orbital shaking incubator (LM-450D, Yihder), hotplate stirrer (Thermo Scientific), measuring cylinder (Herma), measuring pipette (Herma), moisture analyzer (BEL i-Thermo 163L, India).

2.1.2 The material

The materials used in this study were catfish (*Pangasius* sp.) skin obtained from a fish fillet company in Surabaya, East Java, Indonesia. Other materials used in this study included distilled water, sodium hydroxide (NaOH) (Merck), acetic acid (CH_3COOH) (Merck), TRIS buffer (Vivatis), sodium chloride (NaCl) (Merck), and materials for analysis.

2.1.3 Ethical Approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

The procedure for producing water-soluble collagen from catfish (*Pangasius* sp.) skin using different concentrations of acetic acid consists of several stages, including raw material preparation, deproteinization using NaOH, hydrolysis with acetic acid, and hydro-extraction. The analyses conducted include proximate analysis (protein, fat, moisture, and ash), yield and solubility testing. Additionally, amino acid profiling, FTIR and SDS-PAGE analysis were carried out on the best-performing treatment, which used 0.6 M acetic acid.

2.2.1 Preparation of catfish (Pangasius sp.) skin

The catfish skin samples were cleaned of dirt, meat, fat, and scales using running tap water. All parts of the skin were cut into 1×1 cm^2 pieces. The remaining skin samples were stored in the freezer until further use.

2.2.2 Deproteinization process

The catfish skin samples were weighed first before entering the deproteination process. The deproteination process was carried out by soaking the skin samples using sodium hydroxide (NaOH) solution which aims to remove non-collagen proteins. The ratio between the skin and NaOH solution was 1:10 (w/v) with a soaking period of 3 hours and a new NaOH solution was replaced every hour. The skin samples and 0.1 M NaOH solution were put into an Erlenmeyer flask, placed on a hot plate, and stirred using a magnetic stirrer. The deproteinized samples were neutralized using distilled water.

2.2.3 Hydrolysis process

The neutralized catfish skin samples were macerated using an acetic acid solution. The collagen hydrolysis process in this study used variations in acetic acid concentrations (0.4 M; 0.6; and 0.8 M) with a soaking time of 72 hours at a chilling temperature. The ratio between the skin sample and the acetic acid solution was 1:10 (w/v). The skin sample was filtered, and the liquid was collected. The volume of the extracted liquid obtained from the extraction was measured using a measuring cup and precipitated by adding NaCl until the solution reaches a concentration of 2.5 M maintained for 30 minute in room tempera-

ture. The sample was then mixed with a TRIS buffer with a concentration of 0.05 M to neutralize the pH for 1 hour in room temperature. The precipitate formed from the salting-out process and the addition of TRIS buffer were filtered and collected separately by using centrifuging at 20°C for 15 minutes at 4600 rpm.

2.2.4 Hydro-extraction process

The neutralized sample was hydro-extracted using an incubator shaker at 40°C at 150 rpm for 2 hours. The ratio between skin and distilled water was 1:1 (w/v). The sample was then centrifuged at 20°C for 15 minutes at 4600 rpm. The hydro-extraction resulted in water-soluble collagen, which was stored in a refrigerator to form a gel. The sample was then dried in an oven for 48 hours at 45°C. The dried *water-soluble* collagen sample was stored at a chilling temperature for further testing.

2.2.5 Yield analysis

Yield is the percentage of *water-soluble* collagen produced from the initial raw material. Yield indicates the portion of the raw material that can be utilized, making it a parameter for determining its economic value (Suptijah et al., 2018). The *water-soluble* collagen yield is obtained by dividing the weight of dried collagen by the weight of the used catfish skin material (Normah and Afiqah, 2018). The yield of water-soluble collagen from catfish skin can be calculated using the following formula.

$$\text{Water soluble collagen yield (\%)} = \frac{\text{Weight of dried collagen (g)}}{\text{wet weight of fish skin (g)}} \text{ x } 100 \quad ..(1)$$

2.2.6 Proximate analysis

The water-soluble collagen from catfish skin was determined by the proximate analysis based on the standard method (AOAC, 2007). Ash content was analysed by placing the sample in a furnace with a temperature of 550°C until the sample turned to ash with a grey colour. Moisture content was determined using a moisture analyser. The lipids content is characterized using Soxhlet methods. Crude protein content was analysed using the Kjeldahl method including destruction, distillation, and titration.

2.2.7 Organoleptic analysis

The organoleptic evaluation of water-soluble collagen was conducted according to SNI 8076:2020 with 30 panelists. The panellists assessed the collagen samples based on appearance, odour, and texture for each treatment.

2.2.8 Functional group analysis using Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted according to Nurhayati and Murniyati (2013) with modifications. A total of 2 mg of water-soluble collagen was accurately weighed and thoroughly mixed with 100 mg of potassium bromide (KBr) in an agate mortar at a ratio of 1:50 (w/w). The mixture was ground until a fine, homogeneous powder was obtained. The homogenized mixture was then transferred into a die set and compressed using a hydraulic press at 10 tons of pressure for 2 minutes to form a transparent pellet (disk). To prevent interference from atmospheric moisture and CO_2, the pellet was vacuum treated to eliminate trapped air. The prepared pellet was then placed in the FTIR sample holder, and spectra were recorded using an FTIR spectrophotometer within a scanning range of 400–6000 cm^{-1}, at a resolution of 4 cm^{-1} with 32 scan repetitions per sample to ensure spectral clarity and accuracy. The resulting FTIR spectrum revealed absorbance peaks at specific wave numbers corresponding to the functional groups present in the collagen sample. Characteristic bands for protein structures, such as Amide A (~3300 cm^{-1}), Amide I (~1650 cm^{-1}), Amide II (~1550 cm^{-1}), and Amide III (~1240 cm^{-1}), were identified and interpreted according to protein infrared absorption patterns as reported by (Safithri *et al*., 2020).

2.2.9 Amino acid analysis using High-performance liquid chromatography (HPLC)

Amino acid analysis of water-soluble collagen was conducted according to the method outlined by AOAC (1995), with slight modifications. Approximately 20 mg of freeze-dried water-soluble collagen sample was weighed and placed into a hydrolysis tube. The sample was then hydrolyzed using 5–10 mL of 6 N hydrochloric acid (HCl), maintaining a solid-to-liquid ratio of approximately 1:400 (w/v) to ensure complete hydrolysis. The sealed tube was incubated in an oven at 110°C for 22 hours. After hydrolysis, the sample was cooled to room temperature, transferred into a 500 mL volumetric flask, and dried under a gentle stream of nitrogen gas to remove residual acid and moisture. Following drying, derivatization reagent was added to the hydrolyzed sample to form stable, detectable amino acid derivatives. Typically, 20–50 µL of derivatization reagent (such as o-phthalaldehyde or phenyl isothiocyanate, depending on the HPLC system) was added per 100 µL of sample. The mixture was allowed to react at room temperature for 15–30 minutes. The resulting solution was diluted to the desired volume (usually up to 1 mL) with 0.1 M sodium acetate buffer or mobile phase, then filtered using a 0.2 µm nylon

membrane syringe filter to remove particulates. A 20 µL aliquot of the filtered sample was injected into the HPLC system equipped with a C18 reverse-phase column and a UV detector (typically set at 254 nm or 338 nm, depending on the derivatization agent used). The mobile phase used was typically a gradient of buffer and acetonitrile or methanol, with a total runtime of 30–60 minutes. The amino acid composition was determined by comparing the retention times and peak areas of the sample with those of a standard amino acid mixture processed under identical conditions.

2.2.10 Molecular weight of water-soluble collagen using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The molecular weight of water-soluble collagen from the catfish skin was tested using SDS-PAGE. The method for determining molecular weight refers to Li *et al.* (2013). The SDS-PAGE sample preparation involved suspending the water-solublle collagen sample in a 5% SDS solution and incubating it at 85˚C for 60 minutes in a water bath (Aminudin *et al*., 2015). The sample was centrifuged at 6000 rpm for 5 minutes at room temperature to remove insoluble particles. The dissolved sample was then analysed by SDS-PAGE using a 7.5% resolving gel and a 4% stacking gel with a sample-to-loading buffer ratio of 20 µL sample in 5 µL loading buffer. The gel composition was as follows: 3.22 mL of distilled water; 2.7 mL of 30% polyacrylamide; 2 mL of Tris buffer (pH 8.8); 80 µL of 10% SDS; 2.7 µL of 15% APS; and 6 µL of TEMED. The sample was then loaded into the wells and electrophoresed using an electrophoresis instrument. The electrophoresis gel was stained with 0.1% (w/v) Coomassie Blue R-250 in 45% (v/v) acetic acid for 12 hours.

2.3 Analysis Data

The research design used in this study was a Completely Randomized Design (CRD) using three treatments and three independent replications. The research data were tested for normality and homogeneity before being tested further. If the research data were normally distributed and homogeneous, an ANOVA test was carried out using Microsoft Excel on catfish skin water soluble collagen's proximate test, yield, and solubility of catfish skin collagen. Furthermore, a Duncan test was conducted to determine if there was a significant difference. Organoleptic testing was carried out using an ANOVA test in SPSS and further analysed with the Mann-Whitney test if there was a significant difference.

3. Results and Discussion

3.1 Results

3.1.1 Analysis of physicochemical and yield of water-Soluble Collagen from Catfish (Pangasius sp.*) skin with different concentration of acetic acid*

The production process of water-soluble collagen from catfish skin consists of several stages, namely raw material preparation, collagen deproteinization, collagen hydrolysis, and collagen hydro-extraction. The main treatment in this study focused on the collagen hydrolysis stage using acetic acid at concentrations of 0.4 M, 0.6 M, and 0.8 M. The resulting water-soluble collagen was then characterized to evaluate its quality. The characterization included proximate analysis (protein, fat, moisture, and ash content), solubility, yield, organoleptic assessment. Additionally, functional group, amino acid and molecular weight analyses were conducted on the best treatment sample, determined based on its protein content.

The protein content analysis aimed to determine the percentage of protein in the extracted water-soluble collagen. The results showed protein contents of 65.22%, 67.34%, and 84.02% for the 0.4 M, 0.6 M, and 0.8 M acetic acid treatments, respectively (Table 1). Among these, the 0.6 M treatment met the collagen quality standard according to SNI 8076:2020, indicating its suitability for further application.

The fat content analysis revealed values of 16.72%, 14.87%, and 17.63% for the 0.4 M, 0.6 M, and 0.8 M treatments, respectively (Table 1). The moisture content, a determinant factor for product stability and shelf life, was recorded at 11.82%, 8.48%, and 1.61%, respectively, across the treatments, with all results falling well within the acceptable threshold of ≤14% as stipulated by SNI 8076:2020.

The ash content, reflecting the mineral residue within the water-soluble collagen, showed a decreasing trend corresponding to increased acetic acid concentration, with values of 16.21%, 10.69%, and 5.60% (Table 1) respectively. This reduction indicates effective removal of inorganic components during processing.

The solubility analysis, which is pivotal for evaluating the functional performance of water-soluble collagen in aqueous solutions. The highest solubility was observed in the 0.8 M acetic acid treatment, reaching 86.98% (Table 1). Suggesting its enhanced applicability in formulations requiring high water solubility.

Yield analysis, which reflects the efficiency of the production process, showed values of 6.35%, 9.04%, and 4.94% for the 0.4 M, 0.6 M, and 0.8 M

treatments, respectively (Table 1). The 0.6 M acetic acid treatment yielded the highest recovery rate.

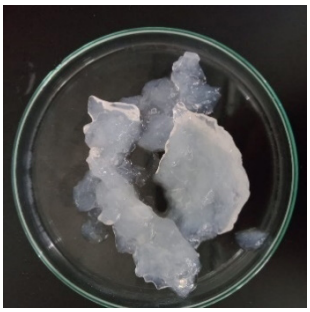
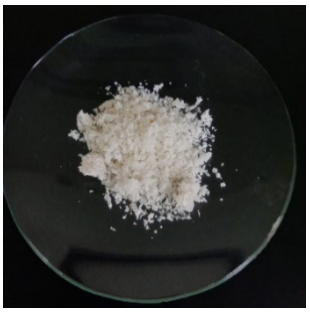
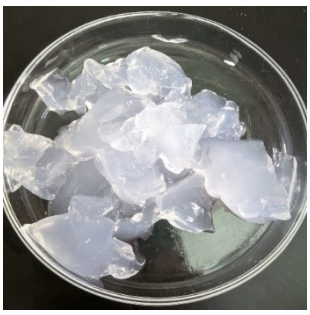
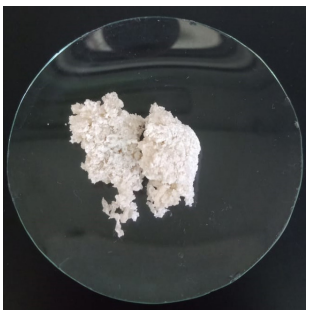
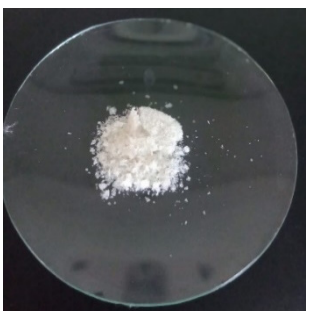
Organoleptic analysis was conducted on non-dried water-soluble collagen samples using a scoring

Table 1. Characteristics of water-soluble collagen from catfish (*Pangasius* sp.) skin with concentration of acetic acid

Parameter	Acetic Acid Concentration			Indonesian National Standard
	0.4 M	0.6 M	0.8 M	
Protein Content (%)	65.22 ± 1.68[a]	67.34 ± 4.64[a]	84.02 ± 2.26[b]	≥75
Lipid Content (%)	16.72 ± 0.17[b]	14.87 ± 0.23[a]	17.63 ± 0.43[c]	-
Water Content (%)	11.82 ± 0.92[c]	8.48 ± 0.63[b]	1.61 ± 0.52[a]	≤14
Ash Content (%)	16.21 ± 1.14[c]	10.69 ± 0.41[b]	5.60 ± 0.57[a]	≤1
Yield (%)	6,35 ± 0,32[b]	9,04 ± 0,09[c]	4,94 ± 0,09[a]	-
Solubility (%)	74,66 ± 1.08[a]	79,71 ± 1.26[b]	86,98 ± 0.71[c]	-

Description: Different superscripts in the same column shows that there are significant differences ($p<0.05$)

Table 2. Wet and dried collagen in different concentrations of acetic acid

Acetic Acid Concentration	Wet Collagen	Dried Collagen
0.4 M		
0.6 M		
0.8 M		

method based on SNI 8076:2020. Evaluations by 30 panelists resulted in average scores above 7 for appearance, odor, and texture parameters (Table 3), indicating favorable sensory attributes across all treatments.

Table 3. Organoleptic value of catfish skin collagen with different concentration of acetic acid

Parameter	Mean Organoleptic Value			Indonesian National Standard
	0.4 M	0.6 M	0.8 M	
Appearance	7.60 ± 1.19	7.80 ± 1.13	7.87 ± 1.36	
Odor	7.87 ± 1.55	7.93 ± 1.26	7.33 ± 1.30	≥7
Texture	7.27 ± 1.64	6.80 ± 1.42	6.80 ± 1.70	

Table 4. The functional groups of catfish skin collagen with 0.6 M of acetic acid

Amide Groups	Absorbance Peaks from the Wavelength Range (cm^{-1})			Chain of Groups
	Absorption Region (Nurjanah *et al*., 2021)	Collagen Sample	Catfish Collagen (Suptijah *et al*., 2018)	
A	3500-3100	3277.06	3407.37	*Stretching* NH
B	2935-2915	2927.94	2933.24	*Asymmetrical stretch of* CH_2
I	1690-1600	1633.71	1640.89	*Stretching* C=0
II	1575-1480	1537.27	1550.28	CN *stretching*, NH *bending*
III	1301-1229	1240.23	1239.46	CN *stretching*, NH *bending*

Based on the characterization results, the water-soluble collagen extracted using 0.6 M acetic acid demonstrated the most optimal overall performance. Although the highest protein content was observed in the 0.8 M treatment, the fat, moisture, and ash contents of the 0.6 M treatment were within the ideal range in accordance with SNI 8076:2020 standards. Moreover, this treatment yielded the highest recovery rate (9.04%), indicating superior extraction efficiency compared to the other concentrations. Considering the balance between chemical quality, production efficiency, and compliance with national quality standards, the water-soluble collagen extracted with 0.6 M acetic acid was selected as the best sample and will be used for further analyses, including functional group characterization, amino acid profiling, and molecular weight determination.

3.1.2 Analysis of functional group of water-Soluble Collagen from Catfish (Pangasius sp.*) skin with 0.6 M of acetic acid*

Fourier Transform Infrared (FTIR) spectroscopy was conducted on the collagen sample extracted using 0.6 M acetic acid, selected as the best-performing treatment based on its high protein yield. Functional group and amino acid analyses were conducted only on the best sample (0.6 M acetic acid treatment), selected based on its high protein content. FTIR analysis confirmed the presence of characteristic collagen functional groups, with absorption peaks at amide A (3277.06 cm^{-1}), amide B (2927.94 cm^{-1}), amide I (1633.71 cm^{-1}), amide II (1537.27 cm^{-1}), and amide III (1240.23 cm^{-1}) (Figure 1). The FTIR spectra revealed the presence of characteristic functional groups associated with collagen, confirming the structural integrity of the molecule. A distinct peak at 3277.06 cm^{-1}, corresponding to amide A, was attributed to N–H stretching vibrations, indicating the presence of hydrogen bonding essential for maintaining the triple

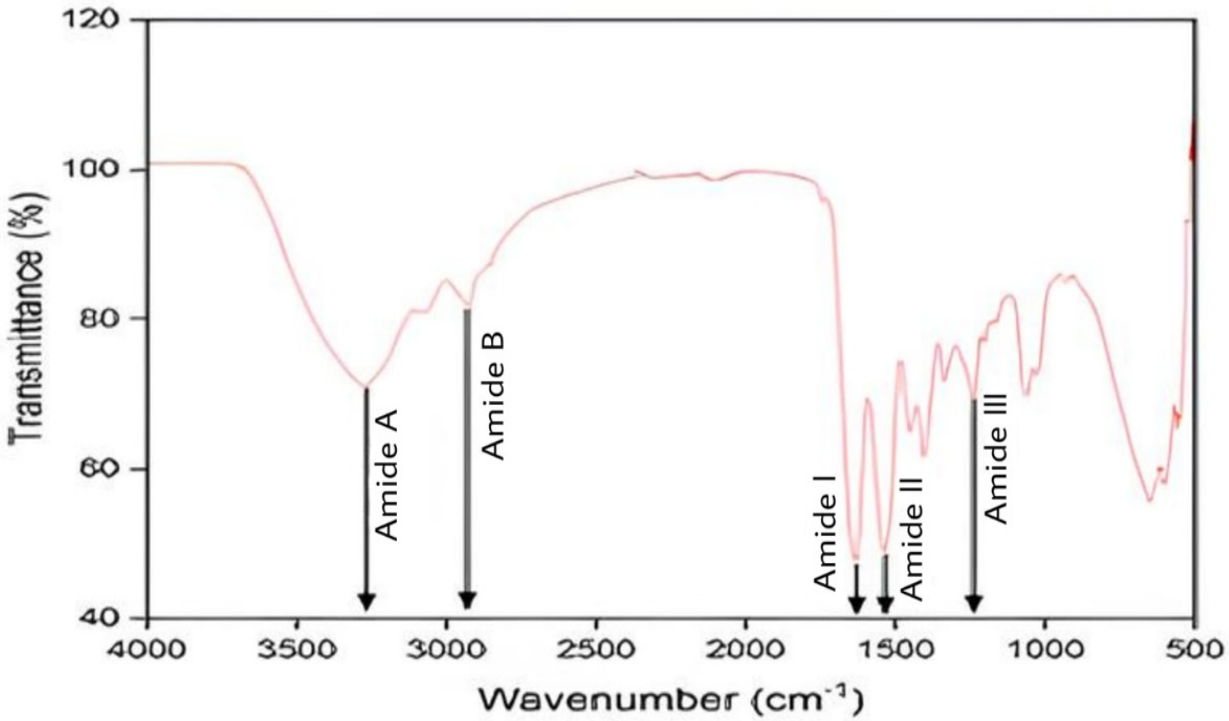


Figure 1. The FTIR spectrum of catfish skin collagen with 0.6 M of acetic acid

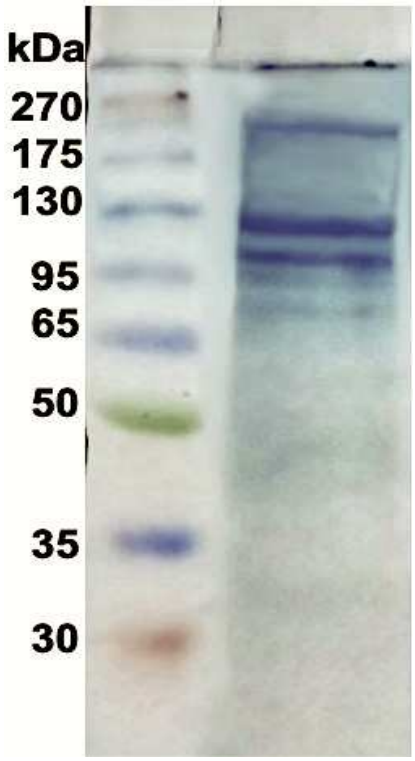


Figure 2. SDS-PAGE of catfish skin collagen with 0.6 M of acetic acid

helix structure. The absorption at 2927.94 cm^{-1}, associated with amide B, reflected asymmetrical stretching of C–H bonds from methyl or methylene groups on amino acid side chains. The amide I band observed at 1633.71 cm^{-1}, primarily linked to C=O stretching of peptide bonds, is a reliable indicator of protein secondary structure and confirmed the presence of α-helices and β-sheets typical of type I collagen. Additionally, the amide II peak at 1537.27 cm^{-1} reflected N–H bending coupled with C–N stretching vibrations, supporting the presence of intact polypeptide chains. The amide III band at 1240.23 cm^{-1}, which represents complex vibrations of C–N and N–H modes, further supported the retention of the collagen's triple-helical conformation (Table 4). Collectively, the well-defined absorption bands across all five characteristic regions strongly suggest that the collagen structure remained intact following the 0.6 M acetic acid extraction, with minimal denaturation or degradation.

3.1.3 Analysis of amino acid of water-Soluble Collagen from Catfish (Pangasius sp.) skin with 0.6 M of acetic acid

The amino acid profile is a critical indicator of collagen quality, as collagen exhibits a unique amino acid composition compared to other proteins. The analysis showed that water-soluble collagen extracted with 0.6 M acetic acid contained glycine as the dominant amino acid, accounting for 20.77% (Table 5).

3.1.4 Analysis of molecular weight of water-Soluble Collagen from Catfish (Pangasius sp.) skin with 0.6 M of acetic acid

SDS-PAGE analysis was performed to determine the molecular weight and type of collagen. The results indicated that catfish skin of water-soluble col-

lagen from all treatments had a molecular weight was 65, 95, 130 and 270 kDa (Figure 2).

Table 5. Amino acid composition of catfish skin with 0.6 M of acetic acid

No	Amino Acid	Average Content (%b/b)
1	Glycine	20.77
2	Alanine	9.199
3	Arginine	6.266
4	Phenylalanine	2.467
5	Valine	2.461
6	Isoleucine	2.284
7	Lysine	2.244
8	Aspartate	1.777
9	Leucine	1.485
10	Cysteine	1.319
11	Histidine	1.182
12	Tyrosine	1.114
13	Serine	1.017
14	Methionine	1.009
15	Threonine	0.740
16	Glutamic Acid	0.483

3.2. Discussion

3.2.1 Characteristic of physicochemical of water-Soluble Collagen from Catfish (Pangasius sp.) skin with different concentration of acetic acid

To evaluate the quality and suitability of water-soluble collagen extracted using different acetic acid concentrations, a comprehensive chemical characterization is necessary. This includes assessing yield, solubility, proximate composition (protein, fat, moisture, and ash content), organoleptic properties, amino acid composition, molecular weight, and functional groups. These parameters provide critical insights into the structural integrity, purity, and functional properties of the extracted collagen, which are essential for its potential applications in various industries.

The protein content results were closely related to the acid treatment applied. The highest protein content in this study was 84.02% (Table 1) when extracted using 0.8M acetic acid, higher than the SNI 8076:2020 standard but still lower than the protein content in Devi *et al.* (2017), which was 87.56%. The higher the acidity of the solution used in the demineralization process, the higher the protein content. This occurs because acetic acid penetrates the skin cells, breaking the hydrogen bonds in the peptide chains, and the H^+ ions from the acid help water penetrate the collagen fibres, causing skin swelling (Nugraheni *et al*., 2021). The composition and condition of the collagen raw material significantly affect the final protein content. The better the quality of the raw material, the higher the protein content (Jaziri *et al*., 2019). Protein content is also influenced by the type of fish used, the body part, age, habitat, and diet (Febriana *et al*., 2021). The soaking time and extraction temperature also affect the protein content. Longer soaking times and higher extraction temperatures can lower the collagen protein content (Pradarameswari *et al*., 2017). Furthermore, variations in acetic acid concentration affect the composition of proximate the extracted collagen. Protein content is directly affected because collagen is the main protein component; efficient extraction ensures higher protein content in the final product (Devi *et al*., 2017).

The lowest lipid content in this study was 14.87% (Table 1) when extracted using 0.6M acetic acid, higher than the lipid content in Devi *et al.* (2017), which was 2.13%. The higher the acetic acid concentration, the lower the lipid content. This is because acetic acid tends to break down protein structure bonds. Hydrolysis using acid can bind lipids, which are then removed during the neutralization process with distilled water, lowering the lipid percentage. The high lipid content in this water-soluble collagen study is suspected to be due to an insufficient NaOH pretreatment process (Jamilah *et al*., 2013). Lipid content is also influenced by acid concentration, as proper extraction conditions facilitate lipid removal while maintaining collagen integrity (Kurniawan, 2016).

The moisture content of water-soluble collagen from catfish skin extracted with different concentrations of acetic acid met the collagen quality standards (SNI 8076:2020), which set a maximum moisture content of 14%. The water content in the 0.8 M treatment in this study was 1.61% (Table 1), which is lower than the 7.76% moisture content found in Devi *et al.* (2017). Moisture content that exceeds the standard can decrease collagen quality, as high moisture makes collagen more susceptible to enzymatic activity that can degrade or damage its structure. High moisture content also increases the risk of microbial contamination, creating favourable conditions for the growth of bacteria and fungi. The higher the moisture

content, the shorter the shelf life of collagen, whereas lower moisture content extends its shelf life (Kusa *et al*., 2022; Nurhidayah *et al*., 2019). The moisture content of each sample varied depending on the sample's humidity. The wetter the sample, the higher its moisture content (Harjo *et al*., 2015). In addition, excessive acid concentrations may change the water and ash content by excessively breaking down non-collagen residues, thereby affecting the final composition (Nurjanah *et al*., 2021).

Ash content can be influenced by the acetic acid concentration used in the hydrolysis or demineralization process during collagen production. The higher the acetic acid concentration, the more minerals are eliminated, resulting in a lower ash content percentage (Suptijah *et al*., 2013). The ash content of a material depends on the mineral content in the raw material. If a material has a high mineral content, its quality is poor, but if it has low ash content, it is safe for use. A low ash content in collagen indicates high purity (Hutomo *et al*., 2015). The ash content observed in this study—16.21% (0.4 M acetic acid), 10.69% (0.6 M), and 5.60% (0.8 M)—was significantly higher than that reported by Devi *et al*. (2017) for catfish skin collagen (0.04%). This elevated ash content is likely attributed to insufficient NaOH pretreatment (Jamilah *et al*. 2013).

The difference in acetic acid concentrations (0.4 M, 0.6 M, and 0.8 M) in water-soluble collagen extraction significantly affected the yield characteristics. The highest collagen yield from catfish skin was obtained at the 0.6 M concentration (Table 1). This finding aligns with previous studies indicating that acetic acid concentration plays a crucial role in collagen extraction efficiency. For example, Makgobole *et al*. (2024) reported that lower concentrations of acetic acid (0.5 M) produced higher collagen yields, whereas increasing the concentration resulted in diminished extraction efficiency (Makgobole *et al*., 2024). Similarly, Davison *et al*. (2009) observed that higher concentrations beyond 0.6 M can degrade collagen, reducing yield (Almirón *et al*., 2014). The water-soluble collagen yield in this study was lower than the yield of tuna skin collagen (13.97%) (Hema *et al*., 2013) and catfish (17.27%) (Devi *et al*., 2017). However, the water-soluble collagen yield in this study was higher than the yield from parang-parang fish skin (2.15%) (Kurniawan, 2016), rohu fish skin (4.13%) (Hema *et al*., 2013), and yellowfin tuna skin collagen (2.13%) (Nurjanah *et al*., 2021).

Different concentrations of acetic acid significantly affect collagen yield due to its role in breaking down non-collagen proteins and affecting the structural integrity of collagen. Acetic acid functions as a weak acid that disrupts cross-linking between collagen and other matrix components, thus facilitating extraction. At optimal concentrations (e.g., 0.5–0.6 M), collagen dissolution is efficient, resulting in higher yields. However, lower concentrations (e.g., 0.4 M) may not sufficiently break down the connective tissue, resulting in lower extraction efficiency, while higher concentrations (e.g., 0.8 M) may damage the triple helix structure of collagen, reducing the yield and compromising its functional properties (Davison *et al*., 2009; Hema *et al*., 2013).

0.8 M acetic acid concentration resulted in a decrease in water-soluble collagen yield. As the concentration of acetic acid increases, collagen solubility also increases. This occurs because acetic acid disrupts collagen fibres and skin tissue. When the skin is soaked in a high concentration of acetic acid, it swells continuously, softening the tissue and making it easier to break apart. However, during the neutralization process, a significant portion of the damaged skin and collagen is lost, leading to a reduction in yield. Wulandari (2016) stated that excessive swelling and increased collagen solubility result in lower collagen yield. Additionally, collagen yield is influenced by factors such as skin type, acetic acid concentration, and the amount of collagen lost during pretreatment and washing processes (Ratnasari *et al*., 2013).

The increase in collagen yield is due to the acetic acid solution penetrating the pores of the fish skin and reaching the collagen inside. Collagen tends to absorb water, leading to an increase in volume. Water absorption by collagen causes the collagen molecule cross-links to open, resulting in volume expansion and skin swelling (Cahyono *et al*., 2022). Therefore, selecting an optimal acetic acid concentration is crucial for maximizing collagen yield while preserving its structural and nutritional properties.

Solubility is the ability of a substance (solute) to dissolve in a solvent. Catfish water-soluble collagen solubility was affected by acetic acid (Table 1), as per previous studies. The higher the acetic acid concentration, the higher the collagen's solubility. Acetic acid has a significant effect on solubility because it can alter the tertiary structure of collagen by breaking ionic bonds, allowing collagen to dissolve (Jaswir *et al*., 2011) and disrupting cross-linking between collagen chains, making non-crosslinked collagen easier to dissolve (Nadjamuddin *et al*., 2014). Collagen is a protein that tends to dissolve in acidic solvents. It dissolves more easily at low pH (Devi *et al*., 2017), requiring a combination of hydro-extraction methods to make collagen easily soluble at neutral pH or wa-

ter-soluble.

The organoleptic characteristics of water-soluble collagen are important factors in assessing quality using the senses. Organoleptic testing is an initial step in evaluating material quality and detecting deviations (Said *et al*., 2011). Wet water-soluble collagen from catfish skin was evaluated for appearance, odor, and texture according to SNI standards. The test was conducted using 30 untrained panelists, each presented with a collagen sample and given a rating form. Panelists provided evaluations based on the criteria and specifications provided in the form. All three treatments in this study met the collagen quality standard specified by SNI 8076:2020, achieving a minimum appearance score of 7. All three treatments in this study met the collagen quality standard specified by SNI 8076:2020, achieving a minimum appearance score of 7.

The odor results based on the organoleptic evaluation of water-soluble collagen for all three treatments in this study met the collagen quality standards of SNI 8076:2020, with a minimum score of 7. The water-soluble collagen had a slight fishy smell. Wijaya *et al.* (2021) suggest that collagen extracted from aquatic animals typically has a fishy odour because fish are known for their distinctive fishy smell. Normah and Suryati (2015) also reported that collagen from fish has the drawback of a fishy odour. The lack of effect of acetic acid on organoleptic properties can be attributed to its volatile nature. During the washing and drying process, the remaining acetic acid evaporates, thus minimizing its impact on the final collagen odor (Jongjareonrak *et al*., 2005). Furthermore, the fishy odor of collagen is mainly caused by the remaining non-collagenous proteins, lipids, and volatile compounds such as trimethylamine (TMA), not the acid used for extraction (Liu *et al*., 2020). Since acetic acid mainly functions to break down collagen from the skin matrix without significantly affecting these odor-causing compounds, its concentration does not play a major role in determining the final organoleptic characteristics.

The texture results based on the organoleptic evaluation of water-soluble collagen with 0.4 M acetic acid treatment in this study met the collagen quality standards of SNI 8076:2020, with a minimum score of 7. The water-soluble collagen texture was flexible. Collagen texture can be influenced by the content of myofibril proteins and moisture content in the raw materials used (Baehaki *et al*., 2019). The absence of acetic acid's influence on texture can be attributed to its role in breaking down non-collagenous proteins and facilitating collagen extraction without significantly altering the collagen's triple-helix structure, which is responsible for its flexibility (Jongjareonrak *et al*., 2005). Additionally, the neutralization and drying processes after extraction help remove excess acetic acid, preventing any direct impact on the final texture of the collagen (Liu *et al*., 2020). The flexibility of collagen is primarily determined by its molecular integrity and hydration level rather than the acid concentration used during extraction. Studies have shown that proper extraction conditions maintain collagen's fibrillar structure, ensuring elasticity and flexibility in the final product (Skierka and Sadowska, 2007).

The acetic acid (0.6 M) was identified as the optimal concentration for extracting water-soluble collagen from catfish skin, the rationale behind its selection requires further clarification. At a lower concentration, such as 0.4 M, the acetic acid may not have been sufficient to effectively solubilize the collagen fibers, leading to incomplete extraction. Acid concentration plays a crucial role in disrupting intermolecular cross-links and promoting collagen dispersion; thus, inadequate acidity may result in lower protein yields and poor solubilization (Kittiphattanabawon *et al*., 2010). Conversely, at a higher concentration like 0.8 M, there is a potential risk of partial denaturation or structural alteration of collagen molecules due to excessive protonation, which can disrupt the native triple-helix structure (Shoulders and Raines, 2009). This could explain why collagen extracted at 0.6 M exhibited the highest protein content while maintaining its structural integrity, as confirmed by FTIR and SDS-PAGE analyses for the next step of experiment. Therefore, 0.6 M strikes a balance between sufficient extraction efficiency and could be preservation of collagen's native structure.

3.2.2 Characteristic of functional group of water-Soluble collagen from catfish (Pangasius sp.*) skin with 0.6 M of acetic acid*

The FTIR analysis performed in this study aimed to confirm that the compound produced was collagen based on its functional groups (Table 4.). The resulting FTIR spectrum revealed absorbance peaks at specific wave numbers corresponding to the functional groups present in the collagen sample. Characteristic bands for protein structures, such as Amide A (~3300 cm^{-1}), Amide I (~1650 cm^{-1}), Amide II (~1550 cm^{-1}), and Amide III (~1240 cm^{-1}), were identified and interpreted according to protein infrared absorption patterns as reported by Safithri *et al.* (2020). These absorption bands are directly associated with the characteristic vibrational modes of peptide bonds in the collagen backbone and provide insight into the preservation of its triple-helical structure.

Water-soluble collagen has characteristic functional groups, including five amide regions with absorption peaks at amide A (3277.06 cm^{-1}), amide B (2927.94 cm^{-1}), amide I (1633.71 cm^{-1}), amide II (1537.27 cm^{-1}), and amide III (1240.23 cm^{-1}) (Figure 1). Based on the spectra obtained (Figure 1.), collagen from catfish skin exhibited the same characteristics as catfish skin's collagen (Suptijah *et al*., 2018) and yellowfin tuna skin (Nurjanah *et al*., 2021). The amide a region shows NH groups and hydrogen bonding. The amide B region results from asymmetric CH_2 stretching. The amide I region shows the C=O stretch vibration, a secondary protein structure. Amide I include four secondary protein structures: α-helix, β-sheet, β-turn, and random coil (Suptijah *et al*., 2018). The amide II region shows NH bonds and the amide III region shows NH bonds indicative of a helix structure. Collagen's secondary structure is a three-dimensional protein structure influenced by non-covalent bonds like hydrogen bonds (Nurhayati and Murniyati, 2013). The intensity of amide III is associated with the triple helix structure and retains its structural integrity of collagen. The triple helix structure in the collagen sample indicates that the collagen has not yet transformed into gelatine (Suptijah *et al*., 2018).

3.2.3 Characteristic of amino acid of water-Soluble Collagen from Catfish (Pangasius sp.*) skin with 0.6 M of acetic acid*

Amino acid composition determines collagen characteristics. Generally, glycine is the most abundant amino acid in collagen. Water-soluble collagen from catfish skin extracted with 0.6 M acetic acid in this study contained amino acids dominated by glycine, indicating that catfish skin collagen is Type I collagen. Glycine affects the formation of the collagen triple helix chain because glycine has a smaller molecular size compared to other amino acids, making it easier for the collagen chain to bend and form a stable triple helix structure (Girsang *et al*., 2020). Beyond structural roles, proteins are also known to function as biotemplating agents in material synthesis, as demonstrated by prefoldin in the biosynthesis of gold nanoparticles (Djohan *et al*., 2019). This evidence supports the potential of fish collagen as a biomaterial with both structural and functional value for biomedical applications.

3.2.4 Molecular weight of water-Soluble Collagen from Catfish (Pangasius sp.*) skin with 0.6 M of acetic acid*

Collagen molecular weight was analyzed using SDS-PAGE, a technique that separates polypeptide chains of proteins based on their molecular weight through the application of an electric current (Jaziri *et al*., 2017). The SDS-PAGE analysis of the extracted water-soluble collagen revealed protein bands at approximately 65, 95, 130, and 270 kDa (Figure 2). Collagen with a molecular weight of 65–270 kDa typically represents a mixture of polypeptide chains derived from type I collagen, the most abundant type in fish skin (Shoulders and Raines, 2009). The bands observed at 95 and 130 kDa are consistent with the molecular weights of α1 and α2 chains of type I collagen, the most abundant collagen type found in fish skin (Liu *et al*., 2012; Kittiphattanabawon *et al*., 2005), and higher weights (200–250 kDa) indicate β-chain dimers formed through natural crosslinking (Liu *et al*., 2015b). However, the appearance of a lower molecular weight band at 65 kDa is not commonly reported in the literature and may indicate partially hydrolyzed α-chains, which could enhance the solubility of collagen in water. This characteristic may explain the specific designation of the extract as water-soluble collagen.

4. Conclusion

The acetic acid concentration significantly influences the characteristics of water-soluble collagen from catfish skin. Among the tested concentrations, 0.6 M acetic acid was found to be optimal, yielding the highest protein content while preserving the native triple-helical structure. Lower concentration (0.4 M) resulted in inefficient solubilization, while higher concentration (0.8 M) risked partial denaturation. However, acetic acid concentration did not affect the organoleptic properties. FTIR analysis confirmed collagen-specific functional groups, and SDS-PAGE revealed subunits around 65–270 kDa, consistent with type I collagen. Glycine was the most dominant amino acid (20.77%), aligning with the typical collagen sequence. These results indicate that 0.6 M acetic acid enables effective extraction of structurally intact type I collagen. Future studies are recommended to explore thermal stability, bioactivity, and peptide composition for broader industrial applications.

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Authors' Contributions

The contribution of each author as follow, Patmawati; supervised the research, devised the main conceptual ideas, article writing, did critical revision of the article. Puput Nuzil Romadhoni; collected the

data, analyzed, and article writing. Devi Puspitaningsih; collected the data, analyzed, and article writing. Laksmi Sulmartiwi; supervised the research and did critical revision of the article. Dwitha Nirmala; supervised the research and did critical revision of the article. Lastiko Endi Rahmantyo; article writing, supervised the research and did critical revision of the article. Oemar Moechtar: supervised the research and did critical revision of the article. Siva Raseetha; supervised the research and did critical revision of the article. Mohamad Akmal Alwi Husein; collected the data, analyzed, and article writing. Khadijah Zai: supervised the research and did critical revision of the article

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) acknowledge the use of ChatGPT for searching of sources articles which related with this topic areas and language refinement in preparing this manuscript. All AI-generated content was rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the author(s). To ensure transparency and support the review process, a comprehensive delineation of the tool's application is furnished in the "Introduction" or "Materials and Methods" section of this manuscript in compliance with the publisher's ethical guidelines.

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