

Effect of Acetic Acid Concentration on Acid-Soluble Collagen Extracted from Red Tilapia Skin: A Preliminary Study

Umi Hartina Mohamad Razali ^{1*}; Andi Muhammad Nurfalauddin Binhar ²; Nena Sakinah Matali ² and Hasmadi Mamat ^{3*}

¹Halal Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Malaysia.

²Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Malaysia.

³Innovative Food Processing and Ingredients Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Malaysia.

*umi.hartina@ums.edu.my

ABSTRACT

The present work investigated the effect of acetic acid concentration on the yield and physicochemical properties of acid-soluble collagen extracted from red tilapia skin. Treated fish skin was extracted using three acetic acid concentrations, namely 0.1 M, 0.5 M and 0.9 M, followed by evaluation of collagen yield, hydroxyproline content, moisture, protein, ash, pH, colour and relative viscosity. Collagen extracted using 0.5 M acetic acid produced the highest yield, with values of $5.88 \pm 0.87\%$, $12.42 \pm 1.84\%$ and $12.81 \pm 1.91\%$ on wet, dry and protein bases, respectively. The same treatment also showed the highest hydroxyproline content (55.59 ± 0.11 mg/g) and protein content ($88.73 \pm 2.14\%$), indicating better collagen purity. Moisture content remained low across all treatments, while ash content increased significantly ($p < 0.05$) with acid concentration. The pH decreased from 4.37 to 3.68 as acetic acid concentration increased, reflecting greater residual acidity. Colour analysis showed that 0.1 M produced the lightest collagen powder, whereas 0.5 M provided better compositional quality. Relative viscosity declined with increasing temperature in all samples, but 0.5 M collagen consistently showed the highest viscosity, suggesting better molecular integrity and thermal-related functional stability. Overall, 0.5 M acetic acid was the most suitable concentration for extracting acid-soluble collagen from red tilapia skin. Further studies should evaluate the structural, functional, and application-related properties of the purified collagen to establish its suitability for food, nutraceutical, cosmetic, and biomaterial applications.

Received: 14 July 2026

Accepted: 26 August 2026

Published: 30 September 2026

Doi: <http://10.51200/ijf.v3i2.8013>

Keywords: acetic acid concentration; acid-soluble collagen; red tilapia skin

1. Introduction

Due to its biocompatibility, biodegradability, film-forming ability, water-binding capacity, and functional behaviour in gel, emulsion, and foam systems, collagen is widely used in food, nutraceutical, cosmetic, pharmaceutical, and biomedical applications (Coppola *et al.*, 2020; Gaikwad & Kim, 2024). Nowadays, however, conventional bovine and porcine collagen sources are increasingly challenged by concerns related

to disease transmission, religious restrictions, consumer acceptability, and sustainability. Hence, waste valorisation and circular use of aquaculture resources can be achieved through fish-processing by-products such as skin, scales, bones and fins as valuable sources of collagen.

Tilapia is widely farmed and commercially processed, particularly for fillet production, with fillets accounting for only about 30–33% of the whole fish and approximately 60–70% remaining as processing by-products, including skin, scales, heads, frames and viscera (Peñarubia *et al.*, 2023). Therefore, red tilapia skin represents a potentially abundant industrial by-product that can be valorised as a raw material for collagen recovery and other value-added applications. Tilapia-derived collagen has been demonstrated to be type I collagen and to have potential functional properties for food, biomedical, and biomaterial applications (Song *et al.*, 2021; Huang *et al.*, 2024). In this regard, tilapia by-products, such as skin and scale, have also been found to be acceptable collagen sources (Menezes *et al.*, 2020; Kittiphattanabawon *et al.*, 2019). Acid-soluble collagen extraction remains one of the most practical and widely used methods. Appropriate control of the extraction conditions can maintain the triple-helical structure of the collagen, whereas weak organic acids, such as acetic acid, could swell the tissue and solubilise the collagen (Liu *et al.*, 2015; Mohamad Razali *et al.*, 2023). In addition, recent research highlights the sensitivity of collagen recovery and quality to extraction conditions, including acid type, acid concentration, extraction time, temperature, and the purification process (Gaikwad & Kim, 2024; Hasanuddin *et al.*, 2024).

Although there has been a surge in fish collagen research, few studies have focused on the effects of increasing acetic acid concentration on both collagen yield and basic physicochemical characteristics. This is important as insufficient acid strength can limit collagen solubilisation, while higher acid strength can disrupt the molecules, reduce collagen purity and alter functional properties such as viscosity and colour (Hadfi & Sarbon, 2019; Carattri *et al.*, 2025). The purpose of this research was thus to determine the effect of various concentrations of acetic acid on the yield and physicochemical properties of acid-soluble collagen extracted from red tilapia skin and to find the optimum concentration that could provide not only optimal collagen recovery but also high-quality collagen.

2. Materials and Methods

2.1 Materials

The fish used were red tilapia (500–600 g), obtained from the hatchery centre of Universiti Malaysia Sabah (UMS). On arrival at the laboratory, all the fish were washed thoroughly to remove dirt, blood, and other impurities. The skin was then carefully removed from the fish using a knife. The skin was then cut into small pieces (about 0.5 × 0.5 cm) for further processing. The prepared skin samples were then kept in clean containers in a chest freezer at –20 °C for one week before the collagen extraction process was started. All the chemicals used were of analytical grade.

2.2 Collagen Extraction

Fish skin was washed with tap water to thaw and remove foreign material. Afterward, the skin was homogenized in 0.1 M NaOH at a 1:10 ratio to remove non-protein substances. The mixture was stirred for 6 hours, and the alkali solution was replaced every 2 hours. Following this, the fish skin was washed with tap water until a neutral pH was obtained. Then the skin was soaked and stirred in 1:10 butyl alcohol for 18 hours to remove fat from the skin. Three cycles of exchange of the butyl alcohol solution were performed every 6 hours. During extraction, the homogenized fish skin was treated with acetic acid at concentrations of 0.1 M, 0.5 M, and 0.9 M. The mixture was centrifuged for 30 minutes at 20,000 × g. Collagen was then salted out with 0.8 M sodium chloride. The precipitate was dissolved in 0.5 M acetic acid at a 1:10 ratio and then dialyzed against acetic acid and distilled water. Lastly, the collagen precipitate was frozen prior to drying in a freeze-dryer (Model 701221010, Labconco Corporation, Kansas City, MO, USA) to obtain collagen powder (Jamilah *et al.*, 2013).

2.3 Yield of Collagen

The yield of collagen (%) extracted from red tilapia skin was determined using the method described by Jamilah *et al.* (2013).

$$(i) \text{ Yield (wet wt basis)} = \frac{\text{Dry wt of collagen}}{\text{Wet wt of fish skin}} \times 100\% \quad (1)$$

$$(ii) \text{ Yield (dry wt basis)} = \frac{\text{Dry wt of collagen}}{\text{Wet wt of fish skin} - \text{moisture content}} \times 100\% \quad (2)$$

$$(iii) \text{ Yield (protein basis)} = \frac{\text{Dry wt of collagen}}{\text{Protein content of fish skin}} \times 100\% \quad (3)$$

2.4 Hydroxyproline Content

Hydroxyproline content of the collagen samples was determined according to Kolar (1990) with slight modifications. Hydroxyproline standard was obtained from Merck (Darmstadt, Germany). An amount of 60 mg hydroxyproline was dissolved in distilled water in a 100 mL volumetric flask to obtain a stock solution. A solution of hydroxyproline (6 µg/mL) was prepared by placing 1 mL of the stock hydroxyproline solution in a 100 mL volumetric flask and filling it with 99 mL of distilled water. Working standard solutions (0.6, 1.2, 1.8, 2.4, 3.6, and 4.8 µg/mL) were prepared. The hydrolytic digest of the collagen sample (0.1 g) was prepared by treating it with 0.75 mL of sulphuric acid (3.5 M) for 16 h at 105 °C. The sample solutions were then diluted with 11.5 mL of water. Each sample and standard solution (2 mL) was treated with 1.0 mL of Chloramine-T solution and allowed to stand for 20 minutes at room temperature. Then, 4-dimethylaminobenzaldehyde solution (1 mL) was added and heated at 60 °C for 15 min. A hydroxyproline standard curve was used to measure collagen extract at 558 nm after a 5-min incubation in iced water to stop the reaction.

2.6 Moisture, Protein and Ash Content

Moisture, crude protein ($N \times 5.55$) and ash content of the samples (red tilapia skin and collagen) were determined according to AOAC Official Method 935.46 (AOAC, 2001).

2.7 Determination of pH

The pH value of the extracted collagen was measured using British Standard Method BS 757 (British Standard Institute, 1975). A 1.0% (w/v) collagen solution was prepared using distilled water and allowed to reach room temperature (about 25 °C). The pH was then measured using a glass electrode pH meter (Toledo 320 pH meter, Mettler-Toledo Instrument, Greifensee, Switzerland) after calibration with pH 4.0 and 7.0 buffer solutions. All measurements were performed in triplicate.

2.8 Determination of Colour

Colour determination of the collagen sample was carried out using a ColorFlex colourimeter (HunterLab, USA). The black and white standard tiles were used to verify the accuracy of the readings. Prior to introducing the sample into the sample cup, the sample cup was cleaned with fibreless Kimwipes tissue. This cup was then placed on the sensor and covered. The colour values were measured as lightness (L^*), redness (a^*) and yellowness (b^*). All measurements were performed in triplicate.

2.9 Determination of Viscosity

The viscosity of collagen was determined based on the method described by Kittiphattanabawon *et al.*

(2005). Five hundred milliliters of 0.03% collagen in 0.1 M acetic acid were subjected to viscosity measurement using a Brookfield Synchroelectric viscometer (Model DV II+, Brookfield Engineering Labs Inc., Stoughton, MA, USA). This measurement was made on spindle No. 1 with a spindle speed of 100 rpm. The collagen solution was heated from 4 °C to 50 °C with a heating rate of 4 °C per minute; at each set temperature the solution was held for 30 minutes before measuring the viscosity. Each measurement was performed in triplicate. The viscosity value was determined by dividing the viscosity value by the one measured at 4 °C.

2.10 Statistical Analysis

All analyses were performed in triplicate. The data were expressed as mean $\pm$ standard deviation. All data collected were analysed using Analysis of Variance (ANOVA) and Tukey's Comparison Test. The significance level among the means ($p < 0.05$) was evaluated. The analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) version 2.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Moisture, Protein and Ash Content of Red Tilapia Skin

The chemical composition of red tilapia skin revealed a moisture content of $76.33 \pm 1.00\%$, an ash content of $0.55 \pm 0.01\%$, and a protein content of $22.93 \pm 0.35\%$. The moisture content was relatively high compared with the 62.73% reported for tilapia skin by Liao *et al.* (2018) and 68.5% reported for barramundi skin by Jamilah *et al.* (2013). Nevertheless, it remained comparable to that reported for tilapia skin, which range from approximately 50-70% in most studies and may reach about 76.5% depending on fish age, rearing conditions and source (Gallo *et al.*, 2023). The ash content of 0.55% was considerably lower than the 2.95% reported for barramundi skin (Jamilah *et al.*, 2013) and was within the range reported for tilapia skin (0.12-0.70%) (Gallo *et al.*, 2023). The relatively low ash content indicates a low proportion of mineral matter in the skin, which is favourable for its subsequent use as a collagen extraction material. Although the protein content differed from that previously reported for barramundi skin ($63.3 \pm 1.8\%$) (Jamilah *et al.*, 2013), it remained within the approximately 21-34% range reported for collagen-rich fresh fish skins, supporting its suitability as a raw material for collagen recovery and value-added applications (Liao *et al.*, 2018; Tan & Chang, 2018).

3.2 Yield of Collagen

The collagen from red tilapia skin obtained using different acetic acid concentrations is depicted in Figure 1. It was found that the concentration of acetic acid used affected the collagen yield, with the highest recovery obtained at 0.5 M acetic acid (Table 1). On a wet-weight basis, the yield increased from $4.43 \pm 2.27\%$ at 0.1 M to $5.88 \pm 0.87\%$ at 0.5 M, then decreased to $5.01 \pm 0.48\%$ at 0.9 M. The yield was similar to that of purple-spotted bigeye snapper skin acid-soluble collagen (3.19% w/w) but lower than that of southern catfish skin collagen (8.00 to 8.66% w/w) (Oslan *et al.*, 2022; Zhang *et al.*, 2024). The present dry weight yield was found to be between $9.36 \pm 4.79\%$ and $12.42 \pm 1.84\%$ (dry weight basis), lower than that of southern catfish skin collagen reported by Zhang *et al.* (2024) and Nile tilapia skin collagen reported by Zheng *et al.* (2024).

The 0.1 M acid used to swell the skin matrix may not have been strong enough to disrupt the non-covalent interactions holding collagen within the tissue, leading to incomplete collagen solubilisation (Kiew & Mat, 2013). Increasing the concentration to 0.5 M significantly improved collagen recovery, indicating a more favourable balance between tissue swelling, collagen solubilisation and preservation of the collagen structure. Moderate acetic acid conditions have similarly been reported to support effective collagen extraction while maintaining its physicochemical quality (Carattri *et al.*, 2025; Hasanuddin *et al.*, 2024).

However, further increasing the concentration to 0.9 M reduced collagen recovery, possibly due to excessive acid-induced swelling, partial disruption of collagen molecules, or loss of soluble collagen fractions during subsequent purification. A similar concentration-dependent effect has been reported for silver catfish (*Pangasius* sp.) skin collagen, where 0.5 M acetic acid provided favourable physicochemical and viscosity characteristics compared with higher acid concentrations (Hadfi & Sarbon, 2019). Therefore, among the concentrations evaluated in the present study (0.1–0.9 M), 0.5 M was the optimum acetic acid concentration for red tilapia skin collagen extraction. The increase in recovery from 0.1 to 0.5 M, followed by a decline at 0.9 M, indicates that the experimental range encompassed the optimum region, although further evaluation at narrower concentration intervals around 0.5 M is needed to define the precise optimum concentration.

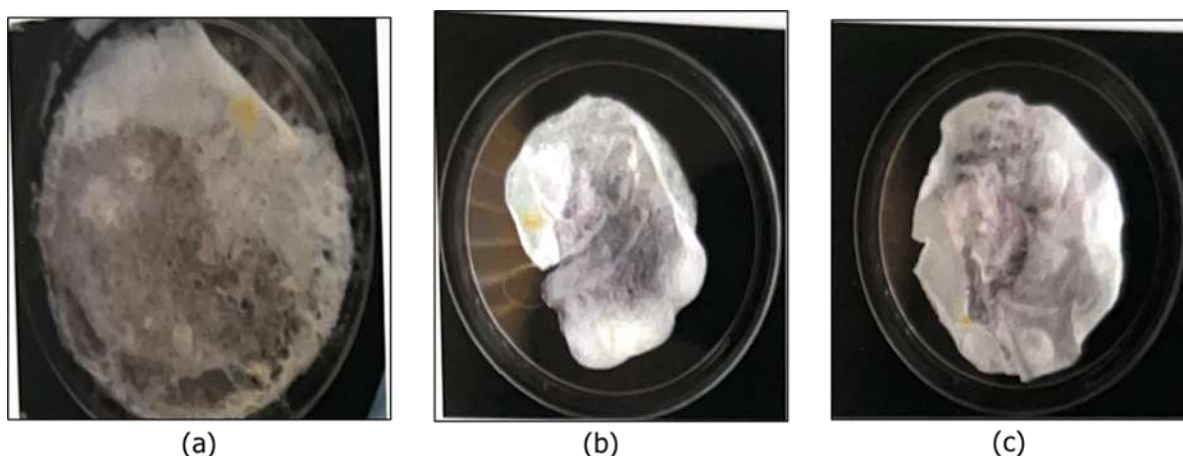


Figure 1. Collagen extracted from red tilapia skin using (a) 0.1 M acetic acid, (b) 0.5 M acetic acid, and (c) 0.9 M acetic acid

Table 1. The yield of collagen extracted from red tilapia skin using different acetic acid concentrations

Yield (%)	Acid Concentration		
	0.1 M	0.5 M	0.9 M
Wet basis	4.43 ± 2.27 ^a	5.88 ± 0.87 ^c	5.01 ± 0.48 ^b
Dry basis	9.36 ± 4.79 ^a	12.42 ± 1.84 ^c	10.58 ± 1.01 ^b
Protein basis	9.07 ± 5.79 ^a	12.81 ± 1.91 ^c	10.90 ± 1.04 ^b

^{a,b,c} Data are given as means ± SD. Means with different superscript letters in the same column are significantly different ($P < 0.05$).

3.3 Hydroxyproline Content

Acetic acid concentration significantly affected hydroxyproline content (Table 2). The highest hydroxyproline content was recorded in collagen extracted using 0.5 M acetic acid (55.59 ± 0.11 mg/g), followed by 0.9 M (38.50 ± 0.33 mg/g) and 0.1 M (27.60 ± 0.56 mg/g). Considering that hydroxyproline is one of the indices of collagen, with a quantitative value strongly correlated with collagen after hydrolysis (Melo *et al.*, 2023), this suggests that 0.5 M acetic acid was the most effective concentration for determining the medium of tilapia skin to recover the collagen-rich material. The hydroxyproline content provides a more robust estimate of the purity of collagen because hydroxyproline is a typical amino acid of collagen molecules, whilst collagen yield is primarily an expression of the amount of recovered material. The highest hydroxyproline values at 0.5 M acetic acid indicate that it produced the most collagen-rich extract, with the least non-collagen proteins or other impurities. Possibly, the lower hydroxyproline content at 0.1 M resulted

from incomplete collagen solubilisation because the lower acid concentration provided insufficient protonation to effectively disrupt ionic and hydrogen-bonding interactions within the skin matrix, thereby limiting tissue swelling and collagen release (Liu *et al.*, 2015). However, the reduction at 0.9 M indicates that a higher acid concentration did not improve collagen purity and may have caused some degradation or loss of collagen fragments during extraction and purification. In addition, the hydroxyproline content of the present 0.5 M sample was found to be higher compared to the previously reported acid-soluble collagen (ASC) from salmon skin, which contained 48.28 and 49.15 mg/g (d.w.) for conventional ASC and ultrasound-defatted ASC (Nilsuwan *et al.*, 2022a). It was, however, lower than some highly purified fish collagens, for example, needlefish skin collagen (79.07-81.69 mg/g) (Ramle *et al.*, 2022) and unicornfish bone collagen (80.17-81.41 mg/g depending on the type of acid used) (Fatiroi *et al.*, 2023).

Table 2. The yield of collagen extracted from red tilapia skin using different acetic acid concentrations

Physicochemical Properties	Acid Concentration		
	0.1 M	0.5 M	0.9 M
Hydroxyproline content (mg/g)	27.60 ± 0.56 ^a	55.59 ± 0.11 ^c	38.50 ± 0.33 ^b
Moisture content (%)	7.99 ± 0.22 ^a	7.32 ± 0.18 ^a	7.22 ± 0.85 ^a
Protein content (%)	77.60 ± 1.79 ^b	88.73 ± 2.14 ^c	65.48 ± 0.66 ^a
Ash content (%)	0.23 ± 0.10 ^a	0.28 ± 0.01 ^b	0.32 ± 0.21 ^c
pH	4.37 ± 0.44 ^c	3.94 ± 0.10 ^b	3.68 ± 0.12 ^a
Hunter colour value			
<i>L</i> *	80.11 ± 1.49 ^b	71.71 ± 6.17 ^a	72.11 ± 1.47 ^a
<i>a</i> *	-0.07 ± 0.03 ^a	-0.25 ± 0.08 ^b	-0.08 ± 0.06 ^a
<i>b</i> *	0.11 ± 0.05 ^b	0.52 ± 0.28 ^c	-0.22 ± 0.09 ^a

^{a,b,c} Data are given as means ± SD. Means with different superscript letters in the same column are significantly different ($P < 0.05$).

3.4 Moisture, Protein and Ash Content of Collagen

As for the moisture content of the extracted collagen, it was found to be between $7.22 \pm 0.85\%$ and $7.99 \pm 0.22\%$, not showing any significant difference among the three acetic acid concentrations. The relatively low moisture content is desirable as lower moisture generally means better powder stability, reduced susceptibility to microbiological spoilage and longer storage keeping quality. This slight reduction from 0.1 M to 0.9 M could be due to the greater efficiency of removing loosely bound water during extraction and drying. These values are similar to those of the materials from a collagenous fish, like tuna tendon collagen, reported to contain approximately 7-8% moisture (Chanmangkang *et al.*, 2024). Protein content showed a clear significant difference among treatments, with the highest value recorded at 0.5 M acetic acid ($88.73 \pm 2.14\%$), followed by 0.1 M ($77.60 \pm 1.79\%$) and 0.9 M ($65.48 \pm 0.66\%$). This is important since protein quantity, together with hydroxyproline content, is an indication of the compositional quality of the collagen extract. This value is higher than the one observed for Nile tilapia skin collagen obtained in an acid extraction medium (68.73-80.58%) (Costa *et al.*, 2024). However, it was lower than the acid-soluble collagen protein content (97.80%) reported for salmon skin by Nilsuwan *et al.* (2022b), suggesting that the present extract may contain a very low amount of other non-collagen components or may benefit from further purification. A recent study on lumpfish collagen-rich hydrolysates also indicated that protein content can vary significantly depending on pretreatment, ranging from 72.18-81.19% in non-pretreated to 96.18-99.26% in chemically treated samples (Scheja *et al.*, 2024). The higher amount of protein at 0.5 M acetic

acid indicated superior collagen purity among the samples, whereas the lower amount of protein at 0.9 M acetic acid may be due to excessive acid exposure, partial degradation of protein, and loss of the soluble protein fraction during washing and/or recovery process.

Ash content increased significantly with acetic acid concentration, from $0.23 \pm 0.10\%$ at 0.1 M to $0.28 \pm 0.01\%$ at 0.5 M and $0.32 \pm 0.21\%$ at 0.9 M, but the overall values remained very low. This implies that the collagen was extracted with minimal inorganic residues, corresponding to high removal of minerals and salts during washing or purification. The obtained values also showed a lower result than collagen isolated from salmon skin (0.75%) and Nile tilapia skin (1.16-1.82%) acid-soluble extract respectively, which validates the lower amount of mineral impurity present in the current samples (Nilsuwan *et al.*, 2022b; Costa *et al.*, 2024). The increase in ash at 0.9 M may result from increased mineral matrix release from the skin matrix, as the difference is slight.

3.4 pH

The pH decreased significantly as acetic acid concentration increased, from 4.37 ± 0.44 at 0.1 M to 3.94 ± 0.10 at 0.5 M and 3.68 ± 0.12 at 0.9 M. This does not necessarily reflect greater purity in the collagen; rather, it suggests that stronger acid treatment left an increased amount of residual acidity in the collagen matrix after extraction and drying. The current pH levels are lower than the pH of Nile tilapia collagen produced by acid extraction (5.52 to 6.85) reported by Costa *et al.* (2024), which suggests that variations in washing, dialysis, neutralisation or acid retention could have a high impact on the final pH of the collagen powder. The lowest pH observed at 0.9 M coincided with the lowest protein and hydroxyproline contents, suggesting that the higher acid concentration may have promoted excessive swelling or partial disruption of collagen molecules, resulting in loss of soluble collagen fractions during extraction and purification.

3.5 Colour properties

The colour results showed that the L^* values of the samples differed significantly among treatments, wherein the 0.1 M acetic acid sample showed the highest L^* value ($L^* = 80.11 \pm 1.49$), indicating the lightest collagen powder, while the 0.5 M ($L^* = 71.71 \pm 6.17$) and 0.9 M ($L^* = 72.11 \pm 1.47$) samples were darker. This indicates that intense acid treatment might have facilitated the release or retention of pigment-related compounds, oxidised residues, or other non-collagenous components, rather than colour being directly translated as an indication of the purity of collagen. The L^* value of 0.1 M was similar to that of barracuda skin collagen ($L^* = 78.54$) extracted with acetic acid (Matarsim *et al.*, 2023), while the value for salmon skin acid-soluble collagen is reported to be higher at $L^* = 85.53-85.37$ (Nilsuwan *et al.*, 2022b). In the present study, the a^* values were near zero and slightly negative, revealing a low red-green colour contribution, while the low values of b^* refer to a weak yellowness-blueness, which was favourable because brighter collagen exhibiting lower colour contribution is generally preferable in the development of food, pharmaceutical and biomedical products (Matarsim *et al.*, 2023). Generally, the 0.1 M sample had the highest visual brightness, but the 0.5 M sample presented the best compositional properties in terms of hydroxyproline and protein concentration, indicating that colour and collagen purity did not follow the same trend.

3.6 Viscosity

All collagen samples showed a decrease in relative viscosity as temperature increased from 10 to 60 °C, indicating the temperature-sensitive property of red tilapia skin collagen. The collagen extracted using 0.5 M acetic acid exhibited the highest relative viscosity within the whole temperature range, with a value close to 100% at 10 to 20 °C and decreased to around 36% at 60 °C. The 0.1 M sample exhibited an intermediate profile and the 0.9 M sample had the lowest viscosity, particularly above 30 °C. This trend indicates that 0.5 M acetic acid produced collagen with the best molecular integrity and highest intermolecular interaction, as reflected in the hydroxyproline and protein contents for the same treatment. The lower viscosity at 0.1 M might be attributed to incomplete collagen solubilisation, while 0.9 M might have caused excessive acid-

induced disruption, partial chain depolymerisation, or weakening of the collagen fibre structure (Kiew & Mat, 2013; Hadfi & Sarbon, 2019). Thus, viscosity confirms the earlier compositional observations; 0.5 M acetic acid yielded not only more collagen-rich material, but also more stable collagen in terms of functionality.

The lower viscosity values at higher temperatures are consistent as heating the sample will increase the mobility of the molecules and the weakening of hydrogen bonding and intermolecular interactions involved in the stabilisation of the collagen triple helix. The more pronounced reduction at higher temperatures (~ 30 °C) is also significant, as it could represent the onset of a collagen structural transition or partial denaturation. This aligns with the findings of Zheng *et al.* (2024) on the denaturation temperature of collagen from Nile tilapia, a warm-water fish, of 31.31 °C, which exhibited more stable rheological behaviour than cold-water chum salmon collagen, owing to both higher molecular weight (HMW) and imino acid (IMA) content. Farooq *et al.* (2024) also mentioned that the thermal stability of fish collagen is closely related to the content of proline and hydroxyproline; generally warm-water fish collagen is more thermally stable than cold-water fish collagen. The current trend is also in line with Hadfi & Sarbon (2019), who found that the relative viscosity of silver catfish skin collagen prepared in 0.5 M acetic acid showed greater value under mild conditions than at higher acid concentrations, and that the value of relative viscosity decreased with increased temperature. The viscosity profile reveals that it is not necessarily the highest acid concentration which is most suitable for enhancing collagen functionality; 0.5 M acid extraction is able to preserve the structure of collagen chains, intermolecular association and heat-dependent viscosity behaviour.

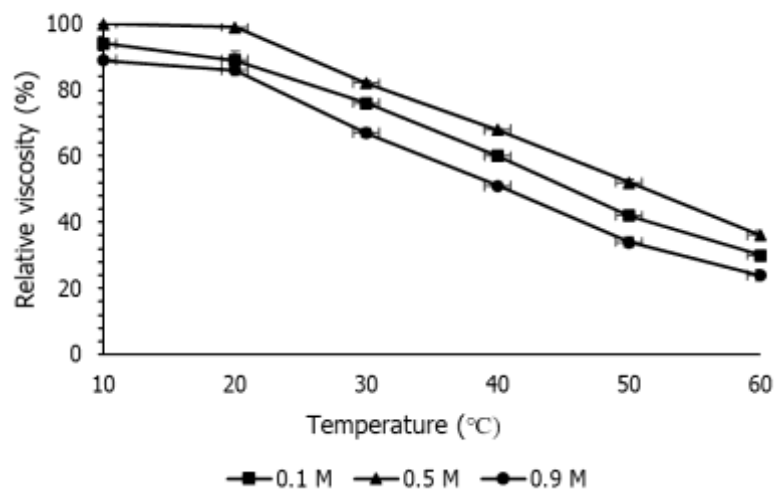


Figure 2. Collagen extracted from red tilapia skin using (a) 0.1 M acetic acid, (b) 0.5 M acetic acid, and (c) 0.9 M acetic acid

4. Conclusion

It is shown that the acetic acid concentration is an important variable in the recovery, composition and functional behaviour of the acid-soluble collagen extract from red tilapia skin obtained in this study. Of the concentrations investigated, 0.5 M acetic acid overall presented the best conditions with the highest collagen yield, hydroxyproline content, protein content and relative viscosity, indicating better recovery, purity and molecular stability of collagen. On the other hand, 0.1 M acetic acid seemed not enough to completely dissolve collagen from the skin matrix, and 0.9 M acetic acid did not improve the extraction but may have caused excessive acid exposure, resulting in decreased protein, hydroxyproline content and

viscosity. The results suggest that 0.5 M acetic acid is a suitable concentration for red tilapia skin collagen extraction; however, only limited physicochemical properties were evaluated in this preliminary study. Further studies are required to structurally characterise the collagen using SDS-PAGE, FTIR, amino acid profiling, and thermal analysis, and also to analyse the purified collagen's suitability in terms of solubility, functional properties, purification efficiency, colour improvement and application-based testing to better determine its suitability for food, nutraceutical, cosmetic or biomaterial development.

Author contributions: Conceptualization, U.H.M.R.; methodology, N.S.M.; validation, H.M.; formal analysis and investigation, N.S.M.; resources, U.H.M.R.; data curation, A.M.N.B.; writing—original draft, A.M.N.B.; writing—review and editing, U.H.M.R.; visualization, H.M.; supervision, U.H.M.R. All authors have read and approved the final manuscript.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflicts of interest.

References

- AOAC. (2001). *Official Methods of Analysis of AOAC International* (17th ed.). AOAC International, Gaithersburg, MD, USA.
- British Standards Institution (BSI). (1975). Methods for sampling and testing gelatine (physical and chemical methods) (BS 757:1975). London: British Standards Institution
- Carattri, M., Shofy Mubarak, A., & Sulmartiwi, L. (2025). Catfish (*Pangasius hypophthalmus*) skin as a nanocollagen source: Impact of extraction time and acetic acid concentration with ultrasonication assistance. *Journal of Marine and Coastal Science*, 14(2), 86-100. <https://doi.org/10.20473/jmcs.v14i2.72265>
- Chanmangkang, S., Maneerote, J., Surayot, U., Panya, A., You, S. G., & Wangtueai, S. (2024). Physicochemical and biological properties of collagens obtained from tuna tendon by using ultrasound-assisted extraction. *Journal of Agriculture and Food Research*, 15, 100984. <https://doi.org/10.1016/j.jafr.2024.100984>
- Coppola, D., Oliviero, M., Vitale, G. A., Lauritano, C., D'Ambra, I., Iannace, S., & de Pascale, D. (2020). Marine collagen from alternative and sustainable sources: Extraction, processing and applications. *Marine Drugs*, 18(4), 214. <https://doi.org/10.3390/md18040214>
- Costa, F. T., Oliveira, T. P., Droval, A. A., Marques, L. L. M., Fuchs, R. H. B., & Cardoso, F. A. R. (2024). Evaluation of physicochemical properties of Nile tilapia skin collagen extracted in acid medium. *Brazilian Journal of Biology*, 84, e255440. <https://doi.org/10.1590/1519-6984.255440>
- Farooq, S., Ahmad, M. I., Zheng, S., Ali, U., Li, Y., Chen, S., & Zhang, H. (2024). A review on marine collagen: Sources, extraction methods, colloids properties, and food applications. *Collagen and Leather*, 6(1). <https://doi.org/10.1186/s42825-024-00152-y>
- Fatiroi, N. S., Jaziri, A. A., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2023). Biochemical and microstructural characteristics of collagen biopolymer from unicornfish (*Naso reticulatus* Randall, 2001) bone prepared with various acid types. *Polymers*, 15(4), 1054. <https://doi.org/10.3390/polym15041054>
- Gaikwad, S., & Kim, M. J. (2024). Fish by-product collagen extraction using different methods and their application. *Marine Drugs*, 22(2), 60. <https://doi.org/10.3390/md22020060>
- Gallo, N., Terzi, A., Sibillano, T., Giannini, C., Masi, A., Sicuro, A., Blasi, F. S., Corallo, A., Pennetta, A., De Benedetto, G. E., Montagna, F., Maffezzoli, A., Sannino, A., & Salvatore, L. (2023). Age-related properties of aquaponics-derived tilapia skin (*Oreochromis niloticus*): A structural and compositional study. *International Journal of Molecular Sciences*, 24(3), 1938. <https://doi.org/10.3390/ijms24031938>
- Hadfi, N. H., & Sarbon, N. M. (2019). Physicochemical properties of silver catfish (*Pangasius* sp.) skin collagen as influenced by acetic acid concentration. *Food Research*, 3(6), 783-790. [https://doi.org/10.26656/fr.2017.3\(6\).130](https://doi.org/10.26656/fr.2017.3(6).130)
- Hasanuddin, H., Jaziri, A. A., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2024). Effect of different acids during collagen extraction from the bone and fins of purple-spotted bigeye (*Priacanthus tayenus* Richardson, 1846) and their

- physicochemical properties. *Food Research*, 8(1), 326-335. [https://doi.org/10.26656/fr.2017.8\(1\).224](https://doi.org/10.26656/fr.2017.8(1).224)
- Huang, J. Y., Wong, T. Y., Tu, T. Y., Tang, M. J., Lin, H. H., & Hsueh, Y. Y. (2024). Assessment of tilapia skin collagen for biomedical research applications in comparison with mammalian collagen. *Molecules*, 29(2), 402. <https://doi.org/10.3390/molecules29020402>
- Jamilah, B., Umi Hartina, M. R., Mat Hashim, D., & Sazili, A. Q. (2013). Properties of collagen from barramundi (*Lates calcarifer*) skin. *International Food Research Journal*, 20(2), 835-842
- Kiew, P. L., & Mat, M. (2013). The influence of acetic acid concentration on the extractability of collagen from the skin of hybrid *Clarias* sp. and its physicochemical properties: A preliminary study. *Focusing on Modern Food Industry*, 2(3), 123-128.
- Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry*, 89(3), 363-372. <https://doi.org/10.1016/j.foodchem.2004.02.042>
- Kittiphattanabawon, P., Sriket, C., Kishimura, H., & Benjakul, S. (2019). Characteristics of acid and pepsin solubilized collagens from Nile tilapia (*Oreochromis niloticus*) scale. *Emirates Journal of Food and Agriculture*, 31(2), 95-101. <https://doi.org/10.9755/ejfa.2019.v31.i2.1911>
- Kolar, K. (1990). Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *Journal of the Association of Official Analytical Chemists*, 73(1), 54-57
- Liao, W., Guanghua, X., Li, Y., Shen, X.R., & Li, C. (2018). Comparison of characteristics and fibril-forming ability of skin collagen from barramundi (*Lates calcarifer*) and tilapia (*Oreochromis niloticus*). *International Journal of Biological Macromolecules*, 107, 549-559. <https://doi.org/10.1016/j.ijbiomac.2017.09.022>
- Liu, D., Wei, G., Li, T., Hu, J., Lu, N., Regenstein, J. M., & Zhou, P. (2015). Effects of alkaline pretreatments and acid extraction conditions on the acid-soluble collagen from grass carp (*Ctenopharyngodon idella*) skin. *Food Chemistry*, 172, 836-843. <https://doi.org/10.1016/j.foodchem.2014.09.147>
- Matarsim, N. N., Jaziri, A. A., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2023). Type I collagen from the skin of barracuda (*Sphyraena* sp.) prepared with different organic acids: Biochemical, microstructural and functional properties. *Journal of Functional Biomaterials*, 14(2), 87. <https://doi.org/10.3390/jfb14020087>
- Melo, M. M. P., Mesquita, R. B. R., Coscueta, E. R., Pintado, M. E., & Rangel, A. O. S. S. (2023). Assessment of collagen content in fish skin - development of a flow analysis method for hydroxyproline determination. *Analytical Methods*, 15(43), 5901-5908. <https://doi.org/10.1039/d3ay01589k>
- Menezes, M. do L. L. R., Ribeiro, H. L., Abreu, F. de O. M. da S., Feitosa, J. P. de A., & Filho, M. de S. M. de S. (2020). Optimization of collagen extraction from Nile tilapia skin (*Oreochromis niloticus*) and its hydrogel with hyaluronic acid. *Colloids and Surfaces B: Biointerfaces*, 189, 110852. <https://doi.org/10.1016/j.colsurfb.2020.110852>
- Mohamad Razali, U. H., Yaakob, H., Sarbon, N. M., Zainan, N. H., Dailin, D. J., & Zaidel, D. N. A. (2023). Improving collagen processing towards a greener approach - current progress. *Journal of Chemical Technology & Biotechnology*, 98, 1063-1082. <https://doi.org/10.1002/jctb.7332>
- Nilsuwan, K., Fusang, K., Pripatnanont, P., & Benjakul, S. (2022a). Properties and characteristics of acid-soluble collagen from salmon skin defatted with the aid of ultrasonication. *Fishes*, 7(1), 51. <https://doi.org/10.3390/fishes7010051>
- Nilsuwan, K., Patil, U., Tu, C., Zhang, B., & Benjakul, S. (2022b). Salmon skin acid-soluble collagen produced by a simplified recovery process: Yield, compositions, and molecular characteristics. *Fishes*, 7(6), 330. <https://doi.org/10.3390/fishes7060330>
- Oslan, S. N. H., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2022). Characterization of acid- and pepsin-soluble collagen extracted from the skin of purple-spotted bigeye snapper. *Gels*, 8(10), 665. <https://doi.org/10.3390/gels8100665>
- Peñarubia, O., Toppe, J., Ahern, M., Ward, A., & Griffin, M. (2023). How value addition by utilization of tilapia processing by-products can improve human nutrition and livelihood. *Reviews in Aquaculture* 2023; 15(Suppl. 1): 32-40. doi:10.1111/raq.12737
- Ramle, S. Z., Oslan, S. N. H., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2022). Biochemical characteristics of acid-soluble collagen from food processing by-products of needlefish skin (*Tylosurus acus melanotus*). *Applied Sciences*, 12(24), 12695. <https://doi.org/10.3390/app122412695>

- Scheja, J. M., Sigurdarson, J. J., Olafsson, H. G., & Einarsson, H. (2024). Maximizing collagen yield from underutilized lumpfish (*Cyclopterus lumpus*) skins by optimizing pre-cleaning and extraction methods. *Marine Drugs*, 22(12), 525. <https://doi.org/10.3390/md22120525>
- Song, Z., Liu, H., Chen, L., Chen, L., Zhou, C., Hong, P., & Deng, C. (2021). Characterization and comparison of collagen extracted from the skin of the Nile tilapia by fermentation and chemical pretreatment. *Food Chemistry*, 340, 128139. <https://doi.org/10.1016/j.foodchem.2020.128139>
- Tan, Y. & Chang, S.K.C. (2018). Isolation and characterization of collagen extracted from channel catfish (*Ictalurus punctatus*) skin. *Food Chemistry*, 242, 147–155. <https://10.1016/j.foodchem.2017.09.013>.
- Zhang, Q., Hou, S., Liu, Y., Du, J., Jia, Y., Yang, Q., Xu, T., Takagi, Y., Li, D., & Zhang, X. (2024). Properties of skin collagen from southern catfish (*Silurus meridionalis*) fed with raw and cooked food. *Foods*, 13(18), 2901. <https://doi.org/10.3390/foods13182901>
- Zheng, Y., Li, Y., Ke, C., Gao, X., Liu, Z., & Chen, J. (2024). Comparison of structural and physicochemical characteristics of skin collagen from chum salmon (cold-water fish) and Nile tilapia (warm-water fish). *Foods*, 13(8), 1213. <https://doi.org/10.3390/foods13081213>