Extraction of Acid Soluble Collagen from Tilapia Fish Scales using Acetic Acid by Simple Method

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**Abstract** Tilapia fish scales have the potential to be a source of collagen of about 6-10% for biomedical, cosmetic, and food applications. In the biomedical industry, collagen can be used for wound healing, organ tissue engineering and drug delivery. This research aims to extract collagen with a simple method using acetic acid so that it can be applied on an industrial scale. The extraction of collagen from tilapia fish scales consists of two stages that are carried out at low temperatures. The pre-treatment stage uses NaOH 0.1M for 12 hours to remove non-collagen proteins. The second stage is the extraction process using acetic acid as a solvent with concentration variations of 0.1M, 0.5M; 1.0M; and 1.5M to determine the effect of acid concentration on the functional groups of amino acids that make up collagen. The highest percent collagen yield of 9.47% was produced at a concentration of 2M acetic acid. Collagen functional groups of various concentrations show amide groups A, B, I, II, and III which are typical of collagen.

**Keywords**: *Collagen, Tilapia fish, Pharmaceutical, Extraction, Wound healing*

**INTRODUCTION**

Collagen is the most abundant structural protein in all animals [1] especially in marine organisms, such as fish (pisces), jellyfish (Cnidaria), sponges (porifera), mollusks (octopus, mussels, cuttlefish, and squid), and certain echinodermata species [2]. Collagen is often applied in various fields such as healthcare, cosmetics, tissue engineering, drug delivery, wound healing, bone disease, dental applications, 3D printing, food and beverages [3]. The usage of marine-derived collagen is becoming compatible with religious beliefs because it avoids the use of pork and beef which are not consumed by Hindus and Muslims. In industrial fish processing, as much as 30% of the total waste by-products consist of scales, bones, and skin that can be used to produce collagen [4]. Tilapia fish is one of the fish that has its potential as a source of collagen [5]. Huang et.al's 2016 research showed that 6-10% of collagen can be extracted from tilapia scales by extrusion–hydro-extraction process [6]. Collagen extraction methods from fish can use acid soluble collagen extraction, supercritical fluid extraction, deep eutectic solvent extraction, ultrasonic extraction, and enzyme extraction [2], [7]. However, these methods are quite difficult to apply in industry due to the use of centrifuge [8], dialysis [9], and supercritical fluid reactor [2]. Based on these methods, a simple method that is easy to apply is acid soluble collagen extraction. The acid soluble collagen extraction method uses acids such as acetic acid, tartaric acid, lactic acid, formic acid, and citric acid [10].

Based on previous research, this research introduces a simplified method for extracting collagen from tilapia fish scales using acetic acid, eliminating the need for centrifugation and dialysis. This enhancement makes the process more practical for industrial applications. The proposed method is a cost-effective, time-efficient, and scalable alternative compared to earlier studies that utilized complex instruments or multi-step purification processes. The extraction of collagen from tilapia fish scales consists of two stages that are carried out at low temperatures. The pre-treatment stage uses NaOH 0.1M for 12 hours to remove non-collagen proteins [11]. The second stage is the extraction process using acetic acid as a solvent with concentration variations of 0.5M, 1.0M, 1.5M, and 2.0M to determine the effect of acid concentration on the functional groups of amino acids that make up collagen [12].

**EXPERIMENTAL SECTION**

**Materials**

The materials that used in this study were tilapia fish scales, sodium hydroxide, acetic acid 100% (Merck, p.a), distilled water, and sodium chloride (Merck, p.a).

**Pre-treatment Tilapia Fish Scales Using Sodium Hydroxide**

Tilapia fish scales was added 0.1M NaOH solution with ratio 1:10 w/v and stirred using magnetic stirrer at the hotplate at room temperature for 24 hours. It is then filtered using a cheesecloth and the residue is neutralized.

**Extraction Acid Soluble Collagen Using Acetic Acid**

The residue was added 0.5M acetic acid at a ratio of 1:10 w/v and continuously stirrer for 7 hours with temperatures below 10°C. Then filtered using a cheesecloth. The filtrate obtained was added NaCl up to 0.9M and stirred for 15 minutes. Then it was left to settle overnight in temperatures below 10°C. The precipitate and filtrate were separated using filter paper. The precipitate was washed with distilled water until the pH was neutral and dried. The yield of collagen was calculated as follows [10]:

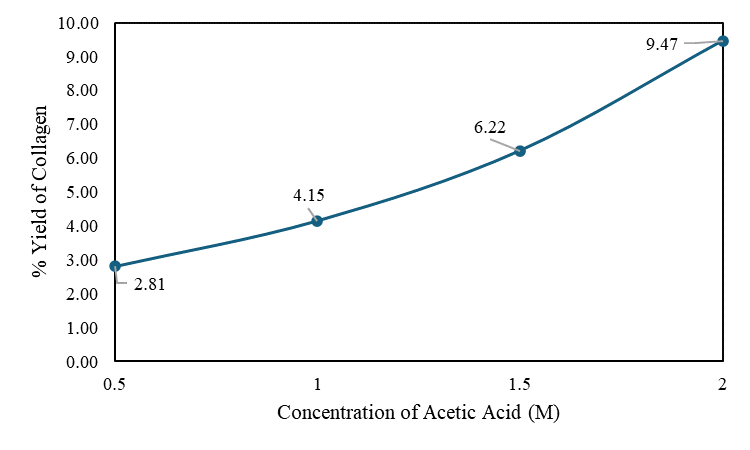
**Materials Characterization**

The crystal phase of the prepared samples was characterized by X-ray diffraction (XRD) PANalytical X’Pert PRO. The XRD analysis was performed using XRD PANalytical under Cu Kα irradiation (λ = 1.5406 Å), with accelerating voltage and current of 40 kV and 40 mA, respectively [13]. The analysis was carried out at a diffraction angle of 2θ of 10 – 100°. The functional groups of the materials were analysed using Fourier Transform Infrared Shimadzu using conventional KBr pellets [14]. The measurements were performed at a wavenumber of 400 - 4000 cm−1. The absorbance of the collagen solution was monitored using Thermo Scientific Genesis 10S UV–Vis spectrophotometer at a wavelength of 200 – 800nm [7].

**RESULT AND DISCUSSION**

**Acid Soluble Collagen from Tilapia Fish Scales**

In this study, collagen from tilapia fish scales was successfully extracted using sodium hydroxide and acetic acid. Sodium hydroxide is used to remove other proteins besides collagen (deproteinase) [15] and acetic acid is used to dissolve collagen [12]. In the pre-treatment of tilapia fish scales using NaOH, the solution becomes brown in colour. The discoloration of the solution is due to the deproteinase process, which is the rupture of telopeptides from collagen molecules [16]. According to Liu et. al [17], NaOH concentration of 0.1M is effective in dissolving non-collagenous proteins without causing collagen loss, while concentrations above 0.1M can significantly cause collagen protein loss. After NaOH pre-treatment, there was a 26% decrease in scale weight due to the removal of non-collagenous proteins. The residue was then extracted using acetic acid. The collagen extraction process was carried out at temperatures below 10°C with various concentrations of acetic acid 0.5M, 1.0M, 1.5M, and 2.0M for 7 hours, resulting in a colourless solution then precipitated with NaCl to form a precipitate which is Acid Soluble Collagen (ASC). The difference in acetic acid concentrations of 0.5M, 1.0M, 1.5M, and 2.0M affects the percent yield value of collagen obtained by 2.81%, 4.15%, 6.22%, and 9.47% ASC, respectively (Figure 1). The yield value obtained (Figure 2) is the same as the collagen yield produced by the research of Huang et.al [6] who used extrusion-hydro-extraction process. The use of simple extraction methods in this study is effective in collagen extraction due to the uncomplicated process used, time efficiency, and solvent cost efficiency so that it can be applied on an industrial scale.



**FIGURE 1.** Yield of Collagen



**FIGURE 2**. (a) Tilapia Fish Scales and (b) Collagen (ASC) from Tilapia fish scales

**Characterization of Acid Soluble Collagen Tilapia Fish Scales**

The infrared spectrum of collagen is shown in Figure 3, based on the FTIR spectra of collagen from fish scales showing spectra in the amide A, amide B, amide I, amide II, and amide III regions which are typical spectra of collagen. The characterization of functional groups of collagens from FTIR analysis is shown in Table 1. Amide A of tilapia fish scale collagen was detected at wave number around 3479-3541cm-1. The amide A spectrum is an N-H stretching vibration in intermolecular hydrogen bonds and is generally observed in the range of 3000-3500 cm-1 [18]. The collagen FTIR spectrum of amide B was at 2920-2944 cm-1 showing asymmetric and symmetric stretching vibrations of CH2 groups [19]. Amide B from ASC was detected at wave numbers 2916-2933 cm-1.

A graph of different colored lines

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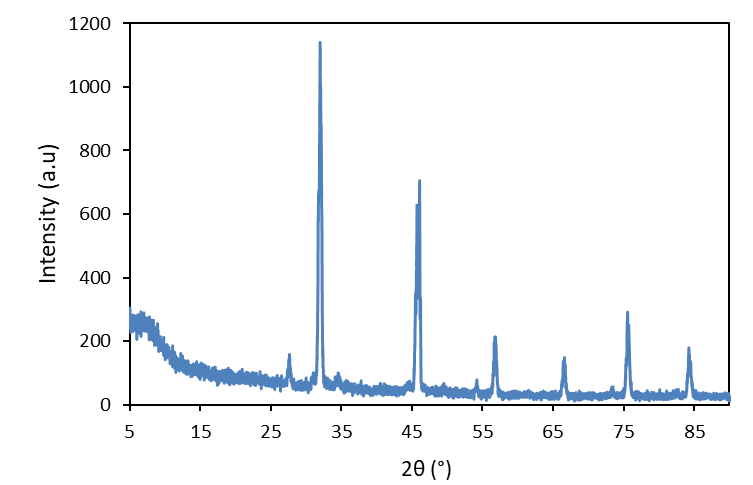
**FIGURE 3.** FTIR spectra of ASC with different concentrations of acetic acid

At wave numbers 1643-1654 cm-1, stretching of the C=O group of the protein secondary structure which is amide I was detected. Amide I absorption is generally found in the range of 1600-1700 cm-1 [20]. The absorption spectra for amide II and amide III of all collagens were similar around 1040-1550 cm-1, showing N-H bending vibrations coupled with C-N stretching vibration and C-H stretching, respectively [21]. The results of FTIR spectra of amide II and amide III from tilapia fish scales are 1055-1548 cm-1. Based on the results of FTIR analysis, increasing the concentration of acetic acid, there was no change in the absorption of amide A, amide B, amide I, amide II, and amide III, but there was a shift in the relevant range. This shows that the concentration of acetic acid affects the yield value and does not affect the purity of collagen.

**TABLE 1.** Characteristics of the collagen functional group from FTIR analysis

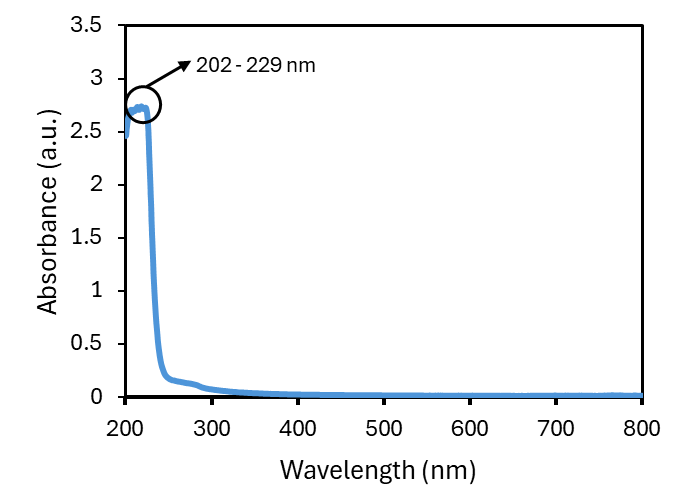
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| --- | --- | --- | --- | --- |
| Amide | Wavenumber standard (cm-1) | Wavenumber obtained (cm-1) | | Characteristic of amide |
| Amide A | 3000-3500 | 3479-3541 | N-H stretching vibration | |
| Amide B | 2920-2944 | 2916-2933 | Asymmetrical or symmetrical stretch CH2 | |
| Amide I | 1600-1700 | 1643-1654 | C=O stretch | |
| Amide II | 1040-1550 | 1055-1548 | C-N stretching and N-H bending | |
| Amide III | 1040-1550 | 1055-1548 | N-H bending and C-H stretching | |

X-ray diffraction patterns were obtained to confirm that the extracted collagen was suitable. Figure 4 shows the X-ray spectrum indicating that the collagen peak is 31.99°. The sharp peak is associated with the triple helix structure of collagen. This diffraction peak represents diffuse scattering caused by the layered structure of collagen. The results are similar to those observed in cow bones [22].



**FIGURE 4**. XRD pattern of collagen extract (ASC)

The UV-Vis spectrum (Figure 5) of collagen in the analyzed sample exhibited an absorption peak at 202-229 nm, due to peptide bonds, alongside additional absorption peaks linked to aromatic amino acids. This result corresponds to closely with the research findings of Chinh et al. (2019), which indicated that the UV-Vis spectrum of pure collagen dissolved in 0.5 M acetic acid exhibited a pronounced absorption peak at 192 nm, indicative of type I collagen [23]. Furthermore, the research carried out by Coscueta et al. (2023), indicated that ASC and type I collagen was observed at 213 and 229 nm, respectively [24].



**FIGURE 5.** UV-Vis spectra of collagen extract (ASC)

**CONCLUSION**

Extraction of collagen from tilapia fish scales using a simple method with acetic acid variations of 0.5, 1.0, 1.5, and 2.0M has been successfully carried out. The concentration of acetic acid affects the ASC yield, with the highest yield obtained at a concentration of 2M, which is 9.47%. The functional groups of collagens from various concentrations of acetic acid show the characteristic collagen spectrum, namely amide A, amide B, amide I, amide II, and amide III, without other contaminants. The UV-Vis spectrum shows peaks at 202-229 nm due to peptide bonds, along with additional absorption peaks linked to aromatic amino acids. The diffractogram XRD indicates that the collagen peak is at 31.99°. The sharp peak is associated with the triple helix structure of collagen.

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