The effect of NaOH addition on the characteristics of tilapia skin collagen

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Abstract. Research on fish collagen is now growing rapidly as the use of collagen in industry increases. Collagen extraction begins with the removal of non-collagen proteins using bases to maximize the extraction process. This research aims to determine the effect of differences in NaOH concentrations on the characteristics of tilapia skin collagen. NaOH in collagen extraction serves to remove alkaline soluble proteins to optimize the collagen extraction process. The bases used were NaOH with the concentration of 0.5, 1.0, and 1.5%. The extraction was carried out using the acid method. Using SEM, observation parameters for crude collagen from the tilapia skin include collagen yield, functional group analysis, lightness, and surface morphology. The results of functional groups analysis showed that the collagen obtained in all treatments had typical collagen characteristics, i.e., amide A, amide B, amide I, amide II, and amide III. The non-collagen deproteination treatment with 0.5% NaOH could produce better collagen than the 1.0 and 1.5% concentrations, as indicated by the highest yield (20.42%) and lightness (93.22). Morphological analysis showed that the collagen extracted has an irregular branched fiber structure.

1. Introduction
Tilapia (Oreochromis niloticus) is one of the leading commodities of freshwater aquaculture in Indonesia. Tilapia production tends to increase every year, with recorded production figures in 2019 of 1,337,382 tons, or an increase of 14.43% from its production in 2018 [1]. Increased production of tilapia cultivation makes Indonesia the second largest tilapia producer in the world after China [2]. The supply-demand projection for this commodity shows a potential that will continue to increase from 2012 to 2030 [3,4]. One of the processed tilapia products with high demand is frozen fillets, and the United States is the largest export market for frozen tilapia fillets [5].

The processing of tilapia fillets produces by-products in the form of fish skin as much as 8.7% of the raw material for fillets [6]. Several publications mention the potential use of tilapia skin for tanned skin [7], medicine, and food to utilize gelatine and collagen fish skin collagen [8,9,10]. Tilapia collagen can be used as a wound dressing. Coppola et al. [11] stated that Tilapia collagen has potential medical use to protect from infection, promote wound healing and skin re-growth. The processed product of tilapia skin that has a high selling price is collagen. The use of fish skin as a raw material for collagen follows the needs of halal products for many consumers, considering that around 60% of the world's collagen uses pork as raw material or as a supporting material for the extraction process [12]. Another reason for using fish skin as a raw material for collagen is that other consumers who
Collagen is a protein found in animals, both vertebrates and invertebrates, with a proportion of about 30% of the total body protein. To date, 27 types of collagen have been identified [15]. The primary type of collagen found in fish is type 1 collagen. This type of collagen is widely applied in industrial activities. Publications state that collagen in fish skin varies from 11 to 63% of the total protein depending on the type of fish, extraction material, and extraction technique [6]. One of the steps in the acid method of collagen extraction is pre-treatment, namely, removing non-collagenous proteins using a strong base [16]. This process will also cause the fat bound to the collagen fibres to undergo saponification and can be separated directly [17]. The concentration of strong bases in the pre-treatment stage was reported to vary between 0.01% to 5%, with a period of 2 hours to 2 days under cold and room temperature conditions [18,19,20]. Blanco et al. [18] stated that of the three independent variables (NaOH concentration, pre-treatment temperature, and processing time), the NaOH concentration factor had a slightly higher influence on the results of the collagen extraction process. For this reason, it is necessary to study the effect of NaOH concentration on the pre-treatment stage of extracting fish skin collagen. NaOH in collagen extraction serves to remove alkaline soluble proteins to optimize the collagen extraction process. Until now, studies on the effect of NaOH concentration in collagen extraction are still limited. Liu et al. [21] reported that the concentration of NaOH affects collagen extraction from the skin of grass carp fish, while the skin of tilapia has not been the object of research.

2. Material and methods

2.1. Materials
The materials used were tilapia fish (Oreochromis niloticus) skin, taken from the tilapia fillet industry in Semarang-Central Java. Other materials used were acetic acid (glacial) 100% (Merck), NaOH (Merck), and NaCl (Merck). The equipment used was beaker glass 5 L (Iwaki), chilling room (capacity 2 tons, -2 to 5°C), centrifuge (Beckman J2-21), and freeze dryer (Labconco Freezone 4.5).

2.2. Methods
Collagen extraction was carried out by the acid method [18,20,22]. The fish skin preparation stage was carried out by cleaning and cutting the skin with an area of 4-6 cm², removing non-collagen proteins with NaOH for 18 hours at 4°C with variations in NaOH concentration 0.5; 1.0; and 1.5%, then extracted using 0.5M acetic acid solution at 4°C for 3 days. The collagen solution was then centrifuged at 10000 rpm, 4°C for 30 min. The filtrate was precipitated with NaCl to a concentration of 0.9M, and then the obtained collagen was filtered and dialyzed with a semipermeable membrane. The collagen was dried using a freeze dryer. Parameters observed were yield of extraction, functional group profile, lightness, and surface morphology.

2.2.1. The yield of collagen [23]. Yield is the ratio between the extract obtained and the initial raw material with the following formula:

\[
% \text{Yield (wet wt basis)} = \frac{\text{dry wt of collagen}}{\text{wet wt of tilapia skin}} \times 100\%
\]  

(1)

2.2.2. Functional groups analysis [6]. The characterization of the collagen functional groups was carried out using Perkin Elmer Spectrum One FTIR (Fourier Transform Infra-Red). A total of 2 mg of Collagen and 200 mg of KBr were ground until homogeneous and placed on a disc printer, then vacuumed to remove air on the disc. The printed disc is inserted into the FTIR device and then measured at a wavelength of 400 cm⁻¹ to 4,000 cm⁻¹, then the IR spectrum will appear.
2.2.3. **Colour Analysis** [24]. Collagen colour was measured using Hunterlab Color Flex (Hunterlab Reston, USA). Dry collagen is placed at the sample port with the side to be measured toward the port. The sample should be flat against the port and completely covers it. The instrument gives three values, i.e L* (lightness), a* (greenness/redness), and b* (yellowness/blueness). White tile standard (L* = 94.20, a* = 1.09, b* = 2.55) used to calibrate the instrument.

2.2.4. **Morphology analysis** [25]. Surface morphology of collagen was observed with the SEM (Scanning Electron Microscopy) tool referring to the JEOL JSM-5310LV type SEM procedure manual. The sample was placed in the specimen holder using carbon double ends with a cross pointing vertically up or the objective lens under a vacuum.

3. **Results and discussion**

3.1. **The yield of collagen**

Extraction of collagen from tilapia skin with different alkaline proportions gave yields ranging from 17.35 to 20.42% (Figure 1). The highest collagen yield (20.42%) was obtained in 0.5% NaOH treatment, while the lowest value (17.35%) was obtained in 1.5% NaOH treatment. This yield value was higher than the yield of tilapia skin collagen reported by Menezes (2020), 13.7 to 19.0%. Figure 1 shows that the higher the concentration of NaOH used, the lower the collagen yield obtained. Liu [21] reported that a high concentration of sodium hydroxide in collagen extraction could remove non-collagenous proteins and cause a significant loss of acid-soluble collagen. The raw material for extracting collagen from tilapia skin cannot use a more than 0.5% sodium hydroxide concentration. As described in other research publications, high concentrations of sodium hydroxide can lead to more significant acid-soluble collagen loss.

![Figure 1](image.png)

**Figure 1.** The yield of tilapia skin collagen.

3.2. **Collagen Functional Groups**

Spectra FTIR for collagen functional groups has some peaks specific. Muyonga et al. [22] state that five major amide bones indicate the characteristic of collagen molecules, including amide A, amide B, amide I, amide II, and amide III bands. Figure 2 shows a spectra IR for collagen molecule from tilapia fish skin with different NaOH concentrations. The increasing of NaOH concentration causes a more substantial peak at wavenumber 3431.20 – 3345.15 cm\(^{-1}\). The Amide bands of collagen molecule from Nile tilapia and channel catfish at wavenumbers of 3315 – 3293 cm\(^{-1}\). The amide A bands associate with the N-stretching band and show the existence of hydrogen bonds. The N-H groups of a peptide are involved in a hydrogen bond, will shift to a lower frequency in wavenumber near 3300 cm\(^{-1}\) [17,22,27].

Amide B for collagen molecule in Figure 2 shows in wavenumber 2937.73 – 2936.82 cm\(^{-1}\). Amide B area indicates a C-H group stretching. Amide I is at wavenumber 1661.67 – 1657.05 cm\(^{-1}\) which indicates C=O stretching, while amide II at wavenumber 1549.10 – 1548.70 cm\(^{-1}\). According to a
previous publication, it indicates the carbonyl group coupled to carboxyl group [19,28]. Amide III at wavenumber 1239.54 – 1239.12 cm\(^{-1}\). It indicates a combination between the stretching vibration of C-N and the bending vibration of N-H [19,28]. IR absorption in this study seems lower than in any previous publication. This could be proved that the amide groups were involved in hydrogen bonds and the triple helix presence. Collagen from tilapia fish skin was extracted with acetic acid 0.5% and pre-treatment by soaking in NaOH 0.1 mol/L solution has strong absorption of IR spectra at wavelengths at Amide I (1600 -1700 cm\(^{-1}\)) [29]. The absorption band of Amide I shows the C=O stretching vibration along the polypeptide backbone in the molecule of collagen. It related the formation of a hydrogen bond between N=H and C=O that main part of the secondary structure molecule of collagen [22,30].

![Figure 2](image_url)  
**Figure 2.** The FTIR spectra peak position of tilapia skin collagen.

The collagen wavelength of FTIR is similar to the profile reported in some publications [28,29]. Combination Amide I and II indicate a collagen molecule helix, which begins to break the triple helix structure bond into a double helix. This can be seen from the strong IR spectra of collagen molecules on the larger wavenumber from a high concentration treatment. NaOH molecule could break the collagen molecule triple helix to a strands helix or double helix which including in five specific area amide mentioned above. IR absorption in wavelengths of amide I was stronger, caused by a partial bond in polypeptide backbone removed by alkaline solution in pre-treatment. The pre-treatment might affect to break structure seconder of the collagen molecule, so the loss of reactive amino acid [22,30].

3.3. Colour analysis
Colour is one of the important factors in the appearance of a product. However, it does not affect the functional properties of collagen. The result of the colour analysis showed that collagen extracted has a relatively high L\(^*\) value with a range of 87.99 to 93.22, indicating a light colour, almost the same as snakehead skin collagen, which uses H\(_2\)O\(_2\) as a bleaching agent (85.27 – 89.49) [31]. This L\(^*\) value was higher than chicken feet collagen (63.21-72.88) [32]. The value of L\(^*\) collagen decreases with the high concentration of NaOH used. Sodium hydroxide can break bonds, due to its ability to promote hydrolysis of amide and ester linkages, functionalities that are commonly found in organic matter. In a hydrolysis reaction, the amide or ester bond is broken down into simpler molecules. The reaction product will have a higher solubility in water than the parent compound because of its smaller size [33]. In industrial use, sodium hydroxide is specifically used to promote darker colour development by promoting oxidation processes and Maillard reaction by controlling the increase of alkalinity [33]. Those can explain the decrease in lightness level on the increase in the proportion of sodium hydroxide added in the collagen extraction process. The a\(^*\) collagen extracted value ranges from 0.01-0.075, lower than chicken feet collagen (1.08-5.57). This positive a\(^*\) value indicates an increasing
redness colour. The $b^*$ value of collagen extracted gave positive result (5.85-6.17), indicating a yellow colour. This $b^*$ value is lower than chicken feet collagen (15.76-19.27). Gaurav et al. [34] report that the colour of collagen depends on the raw materials used and method of extraction.

**Table 1.** The color value of tilapia skin collagen.

| NaOH concentration (%) | L*  | a*  | b*  |
|------------------------|-----|-----|-----|
| 0.5                    | 93.22 | 0.01 | 5.85 |
| 1.0                    | 89.98 | 0.51 | 6.17 |
| 1.5                    | 87.99 | 0.75 | 6.00 |

3.4. Morphology analysis

Morphological analysis was only performed on collagen treated with 0.5% NaOH, which gave the highest yield and lightness (L*). The magnification of the collagen surface image in the SEM analysis carried out is 750 times. The surface shown in Figure 3a indicates that the resulting collagen does not form a porous or hollow texture. The surfaces look like compact fields which are connected to each other to form sheets. When compared with the collagen reported by Li et al. [29] at the same scale size (100 µm), similar surface profiles were obtained. The resulting collagen is in the form of interconnected sheets. It can be seen that the surface appearance shows a complex fibril shape, with irregularly branched filaments, similar to that reported by Zhang et al. [30]. The appearance of an irregular fibril structure indicates that fibrillogenesis occurs in collagen [34]. Collagen is said to have a structural band where this structure is a protein that is cross-linked with other proteins.

![Figure 3a](image1.png)

![Figure 3b](image2.png)

**Figure 3.** The SEM profile of collagen matrix of tilapia skin (a) treated with 0.5% NaOH and collagen matrix of tilapia skin (b) in Li et al. [29].

4. Conclusions

The effect of NaOH addition on the characteristics of tilapia skin collagen was studied. Increasing NaOH concentration could induce a decrease in yield and lightness characteristics. The collagen molecule treated with 0.5% NaOH showed the highest yield (20.42%) and lightness (93.22), it also has a morphological appearance as an irregular branched fiber structure. Specific characterization of collagen molecules showed by spectra FTIR with five peaks in amide A, amide B, amide I, amide II, amide III.

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