The Characteristic of Gelatin Extracted from The Skin of Adult and Sub-Adult Striped Catfish (Pangasius hypophthalmus) Using Acid-Base Pretreatment: pH and FTIR

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Abstract. The gelatin is the biomaterial that produced by collagen denaturation. The age of animal was reported influences the collagen crosslinks which by that contribute to the gelatin quality. The aims of this study were to determine the characteristics of gelatin that extracted from the skin of adult and sub adult striped catfish (Pangasius hypophthalmus) using acid-base pre-treatment based on pH and FTIR (Fourier Transform Infrared) analysis. The gelatine were extracted from striped catfish skins of adult fish (total body length or TBL= 48.6 ± 1.9 cm) and sub-adult (TBL=38.9 ± 0.5 cm) using acid-base pre-treatment (CH3COOH and NaOH solution). The pre-treated skin was washed with tap water until neutral. Finally, the swollen skin was mixed with sterile water at a ratio 1/10 (w/v) and extracted using water bath at 40-70 °C for 60 minutes. In general, the pre-treated acid-base of adult and sub adult fish skin resulted the neutral pH gelatin. The FTIR spectra analysis of gelatin from adult and sub adult striped catfish skin exhibited the similar of wavenumbers included amide A, B, I, II, and III region. In both striped catfish skin gelatin adult and sub-adult were shown the functional groups of O-H, C-H, N-H, C = O, C = N, C = C, and N = O.

1. Introduction

Gelatin is a mixture of several high molecular polypeptides derived from collagen. The main source of collagen for gelatin is cartilages, bones and animal skin [1][2]. Gelatin is composed of protein (85-92%), mineral salt, and water were obtained by partial hydrolysis of collagen [3]. Partial hydrolysis of collagen breaks the crosslink and some peptide bonds produced water-soluble hydrocolloid product [4]. Worldwide consumption of gelatin reached 450.7 kilotons in 2018. Most commercial gelatin sources is pork skin (46%), cowhide (29.4%), bone (23.1%), and other sources (1.5%) [5][6]. Some of these sources are forbidden by religion reasons, hence several countries to increase demand for gelatin from other source including fish [7]. The food industry of striped catfish (Pangasius hypophthalmus) fillet has been developing in Indonesia which resulted the side products including fish skin, bone, and head. The amount of fish skin is about 30% of total fish processing waste and contains high amount of collagen [8][9]. Several studies were reported that striped catfish skin can be extracted for gelatin production by acid or base extraction [10]. The characteristics of gelatin are influenced by the raw materials sources including species, the selected organ such as skin
or bones and the age of animal that influencing the crosslinking of collagen [11]. Gelatin is widely used for industrial applications, including photography, laboratory materials, pharmacy and food [12]. In industrial applications, gel strength, viscosity and melting point are very important characteristics of gelatin. This property is constructed by several factors, such as the concentration of gelatin solution, temperature of the gel operated, and. The pH and weight values affect the viscosity of gelatin, and an alkaline pH level (above 10) results in a significant decrease in viscosity, while an acidic pH only results in a slight decrease [13].

Amino acids composition determines the gelatin functional group. Gelatin functional group show the molecule reactivity of molecular structure of amino acids in gelatin [14][15]. FTIR (Fourier Transform Infrared) spectra analysis use to determine the functional group of gelatin resulted the Amide B absorption area Fish body size will increase with increasing age. The age of fish can be represented by the size of body fish. The increasing of age influences the amount and stability of crosslink hence more difficult process to break the crosslink and allow collagen denaturation [16][17][18]. Accordingly, we would examined the gelatin characteristics (pH and the functional groups) that extracted from skin of striped catfish at different age (sub adult and adult).

2. Material and Methods

2.1. Raw Materials and extraction of gelatin

The striped catfish skins of adult fish (total body length (TBL) = 48.6 ± 1.9 cm) and sub-adult (TBL=38.9 ± 0.5 cm) were cleaned to removed fat by manual scraped using knife. Skin that has been clean washed with tap water and placed in a water and then brought to refrigerator for preserved at -20 °C. The sample of sub-adult was coded by A and adult was coded by B.

Gelatin was extracted from striped catfish skin according to the method of [19]. The freezed skin were thawed and weighed of 10 grams and soaked with 0,12 mol CH₃COOH solution with ratio of 1:10 (w/v) and stirred for 60 minutes at room temperature. The pretreated skin was washed with tap water until neutral. Then, was soaked in 0,12 mol NaOH solution. The ratio of fish skin and NaCl was 1:10 (w/v) and stirred at room temperature for 60 minutes. The pretreated skin was washed with tap water until neutral. Finally, the swollen skin was mixed with sterile water at a ratio 1/10 (w/v) and extracted using water bath at 40-70 °C for 60 minutes. The pre-treatments and treatments variation of skin fish extraction were summarized at Table 1. The extracted mixture from fish skin were filtered and freezed prior to freeze-dried at -35 °C and vacuum at ±0,3 mbar. Then, the freeze dried gelatin was stored in dry conditions till further analysis was carried out.

Table 1. Summary of Gelatin Extraction: Pre-treatment (acid-base) and temperature treatment of skin of sub-adult striped catfish

| Sample Code | Duration of Acid-Base pre-treatment (minutes) | Temperature (°C) |
|-------------|---------------------------------------------|-----------------|
| Sub adult   | Adult                                      | Acid Base       |                |
| A1          | B1                                         | 60 60           | 70             |
| A2          | B2                                         | 50 50           | 55             |
| A3          | B3                                         | 60 60           | 40             |
| A4          | B4                                         | 50 33           | 55             |
| A5          | B5                                         | 60 40           | 70             |
| A6          | B6                                         | 60 40           | 40             |

2.2 The measurement of pH

The gelatin pH was measured refer to the Wainewright method in Ward and Courts (1977) (20), which performed during the gel form before freeze dried process. The pH measurement conducted at room temperature using a pH meter.

2.3 The Analysis of Fourier Transform Infrared (FTIR) spectra
The Freeze-dried gelatin (± 30-40 mg) was mixed with potassium bromide (± 1.5 mg) and macerated using mortar and pestle to get powder. The powder was placed in 13 mm diameters die and made into a pellet using a hydraulic press with pressure of 5000-10000 psi. The sample was analyzed for presence of functional groups in the regions of 4000 – 400 cm⁻¹ wavenumber. The spectra of FTIR was analyzed quantitatively based on the wavenumber value (cm⁻¹) according to amide absorption regions A, B, I, II, and III to determine the functional groups of gelatin.

3. Result and Discussion

3.1 The Ph OF Gelatin

The value of gelatin pH suspension is very important in chemical properties because it can affect the properties of gelatin to determine the next application [21]. The pH measurement obtained that generally the pretreated acid-base of adult and sub adult fish skin resulted the neutral pH gelatin (Table 2). The neutral or near to the neutral pH of gelatin were possibly occurred due to the pretreatment on the gelatin extraction process that using acid and base chemicals. The gelatin pH reflects the chemical treatment used during the extraction stage (Alfaro et al., 2014). The pH value affects the viscosity of gelatin, and an alkaline pH level (above 10) results in a significant decrease in viscosity, while an acidic pH only results in a slight decrease [13].

| Temperature of treatment | pH of gelatin |
|--------------------------|---------------|
|                          | Sub Adult (A) | Adult (B) |
| 40°C                     | 7.0           | 7.0       |
| 55°C                     | 7.7           | 7.0       |
| 70°C                     | 7.7           | 7.0       |

3.2. The result of FTIR analysis

FTIR spectra of gelatin extracted from sub-adult and adult striped catfish skin are depicted in Figure 1 and Table 3 and 4. FTIR spectra analysis has been used to determining the gelatin based on the functional groups [22]. FTIR spectra used to study functional groups and secondary structure such as α-helix and β-sheet. The characteristic of IR absorption bands of polypeptides and protein were called amides A, B and amides I-VII [23]. Gelatin is obtained from the extraction of proteins derived from animal collagen [3]. The spectrum that shows collagen denaturation is a change in the absorption area of amide A, amide B, amide I, amide II, and amide III [18][17][24].

![Figure 1. FTIR spectra of gelatin extracted from skin of sub-adult (A) and adult (B) striped catfish.](image)

All gelatin samples showed similar spectra and has absorption areas of amide A, amide B, amide I, amide II, and amide III. Skin gelatin Sub-adult striped catfish has amide A band at wavenumber of
3275.91-3284.95 cm\(^{-1}\) and at 3270.32-3294.56 cm\(^{-1}\), respectively. The larger amplitude was shown in the adult catfish skin gelatin. According to [24], showed the presence of short peptide of the NH group indicating degradation of the molecule during the gelatin extraction process. Absorption area of 3290-3280 cm\(^{-1}\) shows a vibration of NH group due to the presence of hydrogen bonds on the amide group and vibration of the OH group [25]. In the amide A region, the lowest wave number was found in adult fish skin gelatin (sample B3), at 3270.32 cm\(^{-1}\). Amide B band on sub-adult and adult striped catfish skin gelatin appeared at 2921.82-3069.13 cm\(^{-1}\) and 2922-3069.13 cm\(^{-1}\), respectively. Amide B shows the asymmetric stretching vibration of group =C–H and –NH\(_3\). The lower wave number at the peak of amide B indicates the interaction between –NH\(_3\) groups on the peptide chain. Meanwhile, the peak in the area of wave number 2920 cm\(^{-1}\) shows asymmetrical strain vibrations of C–H in the C–H2 group [26]. Presence of amide A and B regions indicated that at sub-adult and adult gelatin samples, contained OH, CH, and NH functional groups. Functional groups OH, CH, and NH were found in the absorption region with wave numbers 4000-2500 cm\(^{-1}\) [15].

### Table 3. FTIR spectra and peak position and assignment of gelatin extracted from skin of sub-adult striped catfish (A).

| Absorption Area | A1   | A2   | A3   | A4   | A5   | A6   | Assignment                      |
|-----------------|------|------|------|------|------|------|---------------------------------|
| Amide A         | 3279.77 | 3281.38 | 3280.30 | 3282.27 | 3284.95 | 3275.91 | NH stretching; O-H groups      |
| Amide B         | 2924.80 | 2924.63 | 2924.22 | 3069.13 | 2926.07 | 2924.76 | 2921.82 | CH\(_2\) stretching asymmetrical |
| Amide I         | 1633.49 | 1628.50 | 1631.15 | 1628.70 | 1628.33 | 1633.24 | C=O stretching; O-H coupled with COO |
| Amide II        | 1538.68 | 1535.39 | 1536.47 | 1541.27 | 1540.92 | 1538.76 | CN stretching; NH bending      |
|                 | 1447.18 | 1448.56 | 1450.02 | 1448.86 | 1448.57 | 1454.95 | CH\(_2\) bending                |
|                 | 1398.01 | 1389.26 | 1401.20 | 1399.82 | 1370.56 | 1370.56 | CH\(_3\) wagging of proline     |
| Amide III       | 1237.94 | 1236.05 | 1237.66 | 1236.66 | 1237.02 | 1236.56 | CN stretching; NH bending      |
|                 | 1080.39 | 1079.96 | 1080.79 | 1079.93 | 1080.31 | 1079.93 |                                |

### Table 4. FTIR spectra and peak position and assignment of gelatin extracted from skin of adult striped catfish (B).

| Absorption Area | B1   | B2   | B3   | B4   | B5   | B6   | Assignment                      |
|-----------------|------|------|------|------|------|------|---------------------------------|
| Amide A         | 3275.93 | 3276.78 | 3270.32 | 3282.92 | 3281.07 | 3294.56 | NH stretching; O-H groups      |
| Amide B         | 2924.08 | 2923.45 | 2923.57 | 3062.80 | 3069.13 | 2922.42 | CH\(_2\) stretching asymmetrical |
| Amide I         | 1664.13 | 1627.79 | 1626.58 | 1633.24 | 1627.91 | 1628.78 | C=O stretching; O-H coupled with COO |
|                 | 1636.86 | 1539.64 | 1537.01 | 1538.01 | 1538.57 | 1532.05 | CN stretching; NH bending      |
|                 |       | 1541.36 | 1448.20 | 1448.01 | 1447.28 | 1446.30 | CH\(_2\) bending                |
|                 |       | 1534.46 | 1398.42 | 1391.31 | 1393.63 | 1397.69 | CH\(_2\) wagging of proline     |
|                 |       | 1448.81 | 1395.66 | 1335.63 | 1335.63 | 1335.63 |                                |
| Amide III       | 1236.88 | 1236.61 | 1237.78 | 1237.40 | 1232.76 | 1235.65 | CN stretching; NH bending      |
|                 | 1080.63 | 1079.84 | 1080.73 | 1080.25 | 1079.96 | 1080.30 |                                |

Amide I region at 1600-1700 cm\(^{-1}\) widely used for analysis of secondary structure of the protein [17]. In this study, sub-adult and adult striped catfish skin gelatin has amide I absorption at
wavenumber 1628.33-1633.49 cm⁻¹ and 1626.58-1664.13 cm⁻¹. Amide I absorption area in catfish skin gelatin is in the region of the wavenumber 1628 cm⁻¹, which indicates the presence of a C=O stretching vibration or OH group paired with COO⁻ [11]. Gelatin extracted from sub-adult and adult striped catfish skin gelatin has C=N, C=C, and N=O functional groups at wavenumber of 1650-1550 cm⁻¹ [15]. Amide I shows the secondary structure of proteins such as α-helix, β-sheet, β-turn and random coil. When the collagen has denaturation, the triple helical conformation of collagen changes into a random coil. Pre-treatment process using acetate acid can increase H⁺, so that water can easily enter the collagen fibers and form hydrogen bonds between non-polar collagen groups to break the non-covalent bonds in the collagen fibers and facilitate the collagen breaking process [27][28]. Loss of non-covalent bonds causes the peptide to form a random coil structure. This condition causes the stabilization of hydrogen bonds and folding of the polypeptide backbone to form α-helical, β-sheet, and β-turn structures [29]. The α-helical structure has wavenumber at 1660-1640 cm⁻¹, the β-sheet shows wavenumber 1640-1620 cm⁻¹, 1645-1657 cm⁻¹ shows a random coil structure, as well as wave numbers 1670 cm⁻¹, 1683 cm⁻¹, 1688 cm⁻¹, and 1694 cm⁻¹ shows the β-turn structure [17][23][27].

Gelatin extracted from adult striped catfish skin showed β-sheet structure in all samples and the α-helix structure with wavenumber of 1626.58 to 1664.13 cm⁻¹, while sub-adult area at 1628.33-1633.49 cm⁻¹. The difference in the spectrum of amide I regions in gelatin samples is related to differences in chain conformation [24] and be related to differences in the amino acid composition of fish skin gelatin extracted from different sizes. Research [18] showed that the amino acid composition of glycine and proline tended to increase in gelatin from the snapper extracted from fish skins of different sizes. According to [30], proline and glycine are amino acid residues which have a low tendency to form β-sheet structures. The α-helix structure can form when a single chain of polypeptides entwines and forms a rigid cylindrical structure. Hydrogen bonds are formed between the fourth peptide bond, connecting C=O in one peptide bond with NH in the other peptide bond [29]. Amide II has absorption area at wavenumber 1560-1335 cm⁻¹ [38]. Gelatin extracted from sub adult and adult striped catfish skin has amide II region at 1535,39-1335,24 cm⁻¹ and 1532,05-1335,63 cm⁻¹, respectively. The presence of the amide II region is caused by the deformation of the NH bond in the protein and the vibration of the C=N stretching and associated with deformation of tropocollagen into α-chains [17][22].

Gelatin extracted from adult striped catfish skin at amide I region showed β-sheet structure in sample of A4 (1541.27 cm⁻¹) and A5 (1540.92 cm⁻¹), while in adult one were shown at sample of B1 (1541.36 cm⁻¹) and B6 (1541.38 cm⁻¹). In this research, the amide II regions did not exhibit the β-sheet structure, because the β-sheet structure have wavenumber of 1525-1520 cm⁻¹ [23]. The absorption band of amide II in sub-adult and adult striped catfish skin gelatin was lower than the adult one. According to [24], it shows that the NH group is more involved in binding α chains to gelatin with lower amide II absorption. It similar with the previous research by [17] that reported the amide I and II peaks in collagen extracted from the skin of adult tilapia had a higher frequency than that of collagen from young tilapia skin. The amide III absorption area has a low intensity and is almost invisible. This is related to the loss of the triple helical structure of collagen during denaturation to gelatin [4]. Amide II region found at wavenumber of 1300-1000 cm⁻¹ [32]. In this study, area of amide III were found at 1237.94-1079.93 cm⁻¹ and 1232.76-1079.84 cm⁻¹, respectively, which mostly were in wavenumbers of 1237 cm⁻¹ and 1080 cm⁻¹. It indicates the breakdown of most molecules associated with the transformation from the triple helical structure to the coil structure which takes place during heating, and this change is the result of denaturation of the collagen triple helical structure to gelatin [33][34]. Areas in the wavenumber range of 1083-1031 cm⁻¹ showed a vibrational stretching of the CO group of carbohydrate residues in collagen [35], which were found in all samples of gelatin. Amide III shows a combination of peaks between CN group strain vibrations and NH group deformation of amide linkages resulting from absorption of wagging vibrations from CH₂ groups of glycine backbone and proline side chain [34].
4. Conclusion
Generally the pretreated acid-base of gelatin extraction from the skin of adult and sub-adult fish skin resulted the neutral pH. Moreover, the FTIR spectra analysis of sub adult and adult striped catfish skin gelatin showed the similarity of wavenumbers namely amide A, amide B, amide I, amide II, amide III and functional groups including O-H, C-H, N-H, C = O, C = N, C = C, and N = O.

Acknowledgement
This research was supported by Indonesian Government Research Funding namely KEMENRISTEK/BRIN (Director of Research and Community Service, Deputy for Strengthening Research and Development, Ministry of Research and Technology / National Research and Innovation Agency. We also thank for Research Center of Institut Teknologi Sepuluh Nopember for any help and facilities

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