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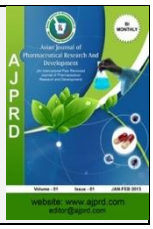

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Review Article

Isolation and Characterization of Collagen and Nanocollagen from Snakehead Fish (*Channa Striata*) Bone

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A B S T R A C T

Objective: This study aims to find out the collagen compounds of the isolation result of the fish bones of the Snakehead have fulfilled the standard of fish collagen and can be formed into nanocollagen.

Methods: All extraction processes are conducted at 4 ^{0}C. Snakehead's bones are soaked with a solution of NaOH 0.1 M with a ratio of 1:10 for 12 hours with occasional stirring, immersion is performed twice every 6 hours (pre-treatment process), then neutralized with bidistilled water up to pH 7. Extracted with a solution CH_3COOH 0.5 M with a ratio of 1:10 for 3 days (check pH), added bidistilled water until pH 4.6. The filtrate is centrifuged at a speed of 4000 rpm for 15 minutes (the residue obtained is extracted back by the same treatment). Acquired Supernatan added 10% NaCl is allowed for 24 hours (collagen precipitate). The precipitate is dried in an oven with a temperature 40 0C for 24 hours, the sample is dry mashed, collagen powder obtained.

Results: Moisture content and an ash content of the collagen's bone isolation is obtained at 0.50%. The protein content of the cored collagen is 85.20%. The existence of fat in the collagen of the Snakehead fishbone is an impurities element that needs to be eliminated through the pretreatment process optimization.

Conclusions: Collagen compounds result from the isolation of the Snakehead fish bones meet the standard of fish collagen, including moisture content, ash content, protein levels, and fat levels and can be formed into nanocollagen.

Keywords: Channa striata, collagen, nanocollagen, isolation, characterization.

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INTRODUCTION

Collagen is the most abundant and exists protein in every animal, which covers about 30% of the total protein. Collagen is primarily present in all connective tissues, including the skin, bones, cartilage, tendons, and veins involved in the formation of fibrillar and microfibrillar tissues of the extracellular matrix and base membrane. Fibrillar protein is a constituent of protein components of bone, cartilage, tendons, skin and other forms of connective tissue.

Bone is one form of waste produced from the fish processing industry [1], such as the bone Snakehead containing collagen. The content of collagen in the Hard Fish Bones (Teleostei) ranges from 15-17%, while the cartilage (elasmobranch) ranges from 22-24% [2]. The fish-derived collagen has smaller denaturation than the collagen derived from mammals. Snakehead's fish bones as waste material can be utilized as an alternative source of collagen because it contains about 16.57% of collagen from the insulation results [3].

Collagen isolation from Snakehead fish bones must meet the standard requirements of fish collagen with a characterization of chemical content in the form of ash content, moisture content, protein levels, and fat content[4]. Testing the morphological particle of raw collagen, results of collagen isolation and nanocollagen results of isolation using Scanning Electron Microscopy (SEM) EVO * MA 10[5], as well as analysis of the functioning of the collagen group of Snakehead fish bones with Spectrophotometer Transform Fourier Infrared (FTIR) [6].

MATERIAL AND METHODS

Material

The materials used in this study were Bidistilled water, boric acid, sulfuric acid, acetic acid, bromocresol green, raw collagen fish, glycerine, solution NaCl 0.9%, sodium chloride 10%, sodium hydroxide pellets, sodium alginate, Snakehead fish bones (*Channa striata*)

Sampel Preparation

The fresh Snakehead is weighed, separated from the inside, washed until clean (no blood) is weighed back then steamed for 30 minutes. Bone-separated from fish meat, collected and weighed throughout the bones.

The Procedure of Collagen Isolation from Snakehead's Fish Bones

Conducted based on previous research [7-8] and modified. All extraction processes are conducted at 4^0C. Snakehead's bones are soaked with a solution of NaOH 0.1 M with a ratio of 1:10 for 12 hours with occasional stirring, immersion is performed twice every 6 hours (pre-treatment process), then neutralized with bidistilled water up to pH 7. Extracted with a solution CH_3COOH 0.5 M with a ratio of 1:10 for 3 days (check pH), added bidistilled water until pH 4.6. The filtrate is centrifuged at a speed of 4000 rpm for 15 minutes (the residue obtained is extracted back by the same treatment). Acquired supernatan added 10% NaCl is allowed for 24 hours (collagen precipitate). The precipitate is dried in an oven with a temperature 40 ^{0}C for 24 hours, the sample is dry mashed, collagen powder obtained.

Nanocollagen Manufacturing Procedure

Using the method of milling, that is using the ball that will be used as a media destroyer inserted into the Jar (Vial HEM), where the first inserted is the ball with a larger diameter, then followed by inserting the balls are smaller in size. Next the sample is inserted into the Jar. The total volume of the balls and samples that can be inserted into the jar does not exceed 2/3 volumes of the jar. The commonly used BPR (Ball to Powder Ratio) is 20:1; 10:1; 8:1. (BPR 20:1 is 20 g ball weight used for 1 g sample to be in milling). The Jar containing the ball and the sample closed tightly. Then Jar is installed on the holder in the HEM. Then HEM is ignited by operating the electronic buttons [9].

Collagen Characteristics of The Snakehead Bonefish

Analysis

The principle of moisture analysis is to know the moisture content of ingredients. The porcelain cup is dried in an oven at 105 ^{0}C for an hour. The dried porcelain cup is inserted in the desiccator for 15 minutes and weighed up to indicate a constant weight (A). A sample of 2 g is inserted into the cup of the dried porcelain is already known to weigh (B). The sample cup is inserted in the oven at 105 ^{0}C for 3 hours, and then the Cup and its contents are cooled in the desiccator for 30 minutes and weighed until the constant weight is obtained (C).

Ash Content Analysis

The principle of ash content analysis is to know the amount of ash contained in an ingredient related to the minerals of the material analyzed. Porcelain cups are dried in an oven with a temperature of about 105 ^{0}C for 1 hour. The oven's dried porcelain cup is inserted in the desiccator for 15 minutes and then weighed up to a constant weight (A). A sample of 3 g (C) weighed and then inserted into the porcelain cup and burned on an electric stove until smokeless and inserted into the furnace with a temperature of 600 ^{0}C for 6 hours. The porcelain cup contains a sample of the result of an attack inserted in the Desiccator for 30 minutes and then weighed until the constant weight was obtained (B).

Analysis of Protein Levels

Thoroughly weighed 2 g samples and then inserted into the 100 mL Kjeldahl flask, added 2 g of selenium mixture added 25 mL H_2SO_4 (p) Heat over the electric bath or burner fire until boiling and the solution becomes clear greenish (about 2 hours). Then allowed to cool, diluted, and put in a calibrate flask 100 ML, suffered until the signing line. Next is the pipetted 5 mL NaOH 40%, 10 mL H_3BO_3 4%, and some droplet indicators cell. Then the distillate was mined with a solution of HCl 0,1N until obtained the discoloration of blue to greenish-blue. Then done setting blank.

Analysis of Fat Levels

The round flask is first drained in the oven with a temperature of 105 ^{0}C for 30 minutes, and then inserted in the desiccator for 15 minutes and weighed until the constant weight (W1). The sample weighed as much as 2 g (W2) and was inserted into a filter paper lined with cotton (fat sleeve) and a paper sleeve plug containing the sample with cotton, then inserted into a soxhlet that has been linked to a fat flask. The extraction process is carried out for 6 hours with a solvent of hexane as much as 150 mL. Hexane mixture and fats are distilled to separate fats from the banishment. The fat flask containing fat extraction result is heated in the oven at 105 ^{o}C for 60 minutes and inserted in the Desiccator for 30 minutes then weighed to a constant weight (W3) [4].

Analysis with Scanning Electron Microscopy (SEM)

Morphology of surface ann of collagen and nanocollagen isolation result of Snakehead fish bones observed using SEM tools with an acceleration voltage of approximately 1.5-20 kV [5].

Analysis with Spectra Fourier- Transform InfraRed (FTIR)

Spectra Fourier-Transform InfraRed is obtained using an FT-IR spectrophotometer. The raw collagen and collagen samples are isolated from the Snakehead bones, blended evenly with the potassium bromide in a comparison of 1:5 weight (Sample: Potassium bromide). The potassium bromide plate is prepared by pressing the powder at a pressure of 5 tons selama5 minutes in a hydraulic suppressor. Then measured transmittance percent on the number of waves 400-4000cm^{-1} [6].

Statistical Analysis

All data were analyzed with regression analysis using SPSS 22.

RESULT AND DISCUSSION

The cleared Snakehead fish then weighed 27 kg, after the fish bones were separated from the meat, cleaned and acquired fish bones 2.26 kg, then carried out the process of isolation obtained filtrate 20 liters and centrifuged obtained 8 liters of the and dried obtained collagen fishbone of Snakehead 374.5 gram. The yield is 16.57%. Collagen isolation of the Snakehead fish is characterized to find out (moisture content, ash, proteins, and fats). Characteristic results can be seen in table 1.

Table: 1 Result characteristics of collagen chemical content from Snakehead fish bones

No.	Parameters	Result (%)	Terms (%)
1.	Moisture content	5,79	≤ 7,0
2.	Ash content	0,60	≤ 2,0
3.	Protein levels	85,2	> 90
4.	Fat content	0,50	-

The results showed that the moisture content and the ash content of the cored collagen results in bone insulation of the Snakehead fish, the level of collagen fat insulating from the bone of the Snakehead obtained by 0.50%. The protein content of the cored collagen is 85.20%, while the collagen protein level is 90%. The percentage of low protein levels is suspected because of the less maximal treatment process. The existence of fat in the collagen of the Snakehead fishbone is an impurities element that needs to be eliminated through the pretreatment process optimization [10].

The Characteristic Result of Collagen Morphology and Nano Collagen of The Snakehead Fishbone with Scanning Electron Microscopy (SEM)

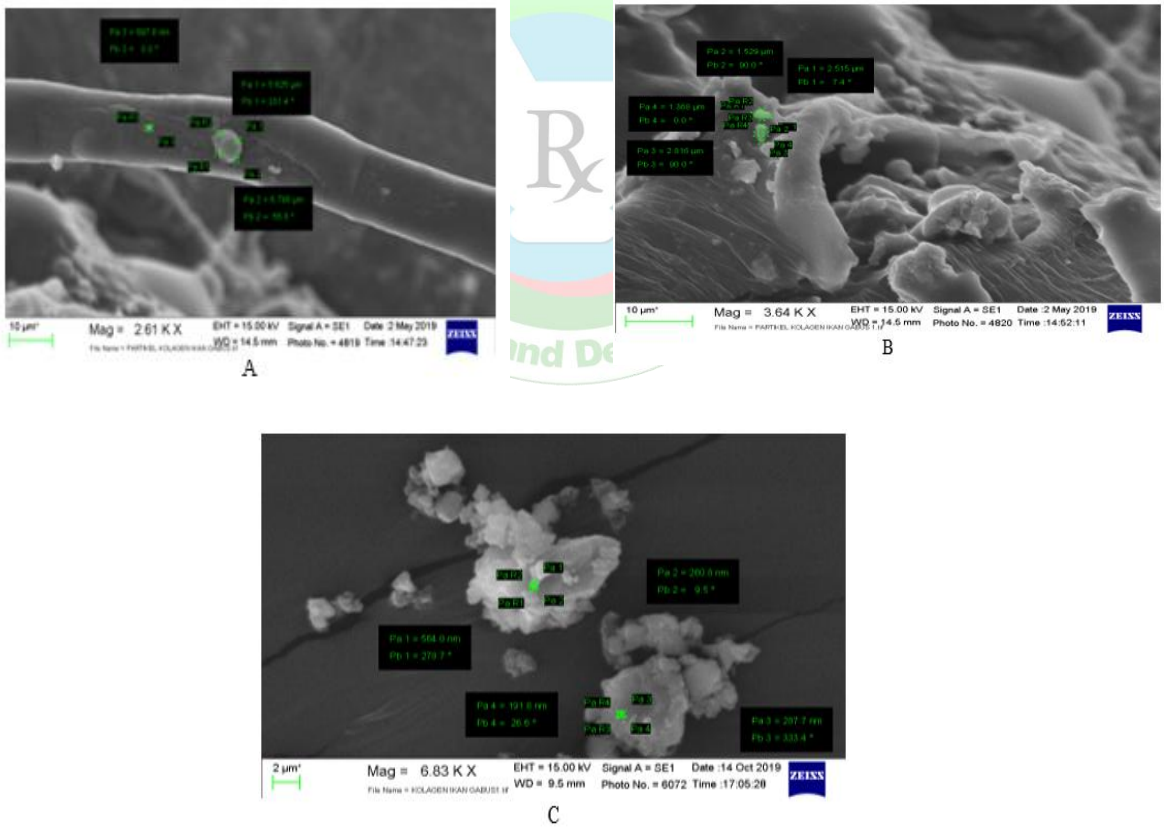

Figure: 1. Morphology of the particle surface with scanning electron microscopy (SEM). A: raw collagen of fish, B: collagen isolation results, C: nanocollagen isolation results.

Testing the morphological particle of raw collagen, results of collagen isolation, and nanocollagen isolation results using Scanning Electron Microscopy (SEM) EVO * MA 10. The observation showed that the surface of the collagen raw particles formed a large aggregate (Fig A). On the surface of the isolated collagen (Fig B) also shows a large aggregate, it has the same particle shape as the fish raw collagen while the isolated nanocollagen particles (Fig C) Form smaller aggregates compared to the raw collagen and the resulting collagen, as shown in Figure 1.

Analysis of the Function Group of Collagen Bone of Snakehead with Spectrophotometer Transform Fourier Infrared (FTIR)

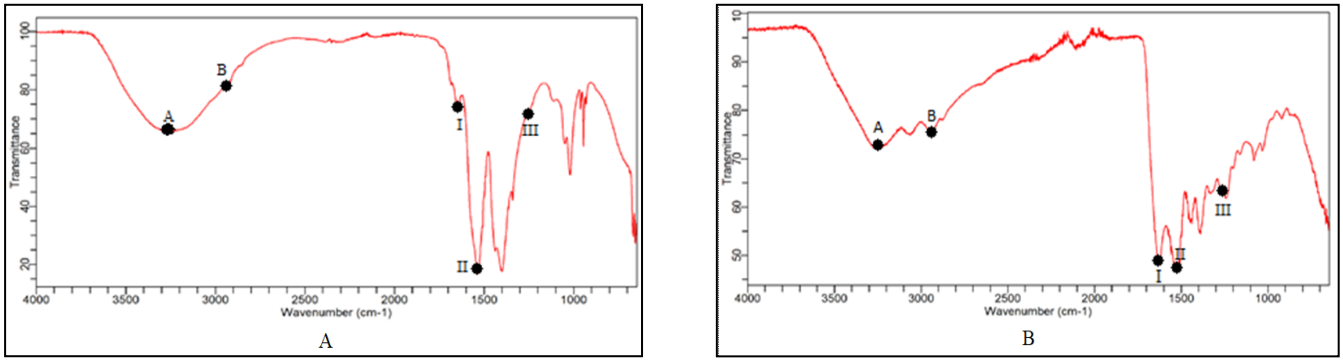

Figure: 2 Characteristics of a function group with Spectrophotometer Transform Fourier Infrared (FTIR). A: collagen bone Snakehead Fish, B: raw collagen Fish

Table: 2 Characteristics of a functioning groups of snakehead fish

Amide Type	Absorption area (cm^{-1})	Absorption peak (cm^{-1})	Description
Amide A	3400-3440	3250	NH
Amide B	2922-2924	2920	CH_2
Amide I	1600-1700	1650	Carbonil (bending C=O)
Amide II	1480-1575	1535	CN stretching, NH bending
Amide III	1229-1301	1240	CN stretching, NH bending

Table: 3. Characteristic of collagen raw function groups

Amide Type	Absorption area (cm^{-1})	Absorption peak (cm^{-1})	Description
Amide A	3400-3440	3280	NH
Amide B	2922-2924	2920	CH_2
Amide I	1600-1700	1630	Carbonil (bending C=O)
Amide II	1480-1575	1530	CN stretching, NH bending
Amide III	1229-1301	1270	CN stretching, NH bending

Results of analysis of the group of collagen function of Snakehead fishbone insulation can be seen in Figure 2 and Table 2, the results of the analysis of collagen group of functions can be seen in Figure 3 and Table 3, the results show the apex of amide A, amide B, amide I, amide II and amide III. The absorption area is in the range of 3400-3440 cm^{-1} indicating the presence of an amide group with the NH stretching is free, but when the NH group is involved in the hydrogen bond then its position will shift to a lower frequency [11].

The absorption of the amide-A collagen of the Snakehead fishbone is 3250 cm^{-1} and the raw collagen 3280 cm^{-1}, this means the collagen of the Snakehead fishbone is an NH group that binds to the hydrogen bond. The absorption area of amide B is in the range of 2922-2924 cm^{-1} indicating the presence of CH2 groups [12]. The culmination of amide B collagen of Snakehead bonefish and the raw collagen is 2920 cm^{-1}, this means there is a CH_2 group on the collagen of the Snakehead fishbone and the raw collagen. The absorption area of the amide I is in the range of 1600-1700 cm^{-1} which corresponds to the vibration of the stretching C- or O-(bond C = O) along the polypeptide chain [13], the peak of the collagen uptake of the Snakehead fishbone is 1650 cm^{-1} and the raw collagen 1630 cm^{-1}.

The peak areas of absorption 1630, 1650 and 1675 cm^{-1} are characteristic of the amino acid residue (β-sheet), random coil and β-turn [14], amide I has 4 components of secondary protein structure namely α-helix, β-sheet, β-turn, and random coil [15], this means the collagen of the Snakehead fishbone and the raw collagen has a β-sheet structure that has not been denatured to α-helix (the characteristic gelatin).

The uptake areas of amide II and III are in the range of 1480-1575 cm^{-1} and 1229-1301 cm^{-1} [16]. The absorption of the amide II and III collagen of the Snakehead bones resides at 1535 cm^{-1} and 1240 cm^{-1} and the collagen raw is at 1530 cm^{-1} and 1270 cm^{-1}, this means the collagen of the Snakehead fishbone and the raw collagen has a II and a Group III. A triple helix structure on the collagen can also be demonstrated based on the intensity ratio between the peak amide III and the peak area of 1450 cm^{-1}. The ratio value between the peaks of the absorption area of amide III and the peak of 1450 cm^{-1} is 1.17, that the ratio value close to 1.0 signifies that the collagen still has a triple helix structure [17].

The characteristic result of the function of the group of Snakehead bone collagen and the raw collagen does not differ considerably and in the collagen bony fish there are groups of amide A, amide B, amide I, amide II, amide III which is a characteristic of collagen, besides, there is also a β-sheet structure and a triple helix structure that is a characteristic of collagen.

CONCLUSION

Collagen compounds result from the isolation of the Snakehead fish bones meet the standard of fish collagen, including moisture content, ash content, protein levels, and fat levels and can be formed into nanocollagen.

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