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Functional Groups of Scale Collagen from Farmed Snakehead Fish (*Channa striata*) and Wild Sources

Gugus Fungsi pada Kolagen Sisik Ikan Gabus (Channa striata) Hasil Budidaya dan Tangkapan di Alam

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Abstract

The study aimed to analyze the functional groups of scale collagen from farmed snakehead fish and wild sources. Scale collagen from farmed snakehead fish was higher than the wild sources (18.82 > 14.27%) with the protein content of both successively (60.24 > 59.78%). The functional groups in scale collagen from farmed snakehead fish were Amide A (3668,77 cm^{-1}), Amide B (2920,35 cm^{-1}) Amide I (2302,14 cm^{-1}), Amide II (1446,67 cm^{-1}), and Amide III (1245,10 cm^{-1}). Meanwhile, scale collagen from wild sources snakehead fish has an Amide A group (3853.94 cm^{-1}), Amide B (2905.89 cm^{-1}), and Amide I (1869.10 cm^{-1}) Amide II (1457.28 cm^{-1}), Amide III (1243.18 cm^{-1}). Scale Collagen from farmed snakehead fish and wild sources is classified as type 1 collagen.

Keywords: Collagen, Scale, Snakehead Fish

Abstrak

Penelitian bertujuan untuk menganalisis gugus fungsi pada kolagen sisik ikan gabus hasil budidaya dan tangkapan di alam. Kolagen sisik ikan gabus hasil budidaya lebih tinggi daripada tangkapan di alam (18,82 > 14,27%) dengan kandungan protein keduanya berturut turut (60,24 > 59,78%). Gugus fungsi pada kolagen sisik ikan gabus hasil budidaya adalah Amida A (3668,77 cm^{-1}), Amida B (2920,35 cm^{-1}), Amida I (2302,14 cm^{-1}), Amida II (1446,67 cm^{-1}), Amida III (1245,10 cm^{-1}). Sedangkan pada kolagen sisik ikan gabus tangkapan di alam memiliki gugus Amida A (3853,94 cm^{-1}), Amida B (2905,89 cm^{-1}), Amida I (1869,10 cm^{-1}), Amida II (1457,28 cm-1), Amida III (1243,18 cm^{-1}). Kolagen sisik ikan gabus hasil budidaya dan tangkapan di alam tergolong kolagen tipe 1.

Kata kunci : Kolagen, Sisik, Ikan Gabus

1. Introduction

The supply of snakehead usually comes from natural catches (rivers, lakes, and swamps) found in the Riau area. Aquaculture in ponds and cages has also been developed, although the seed supply still comes from the wild. However, with the increasing demand for this fish, production in the wild will decline due to overfishing. Therefore, the supply of snakeheads in the future will be highly dependent on aquaculture. The part of snakehead that is commonly consumed is the meat, while some other parts such as scales have not been optimally utilized. Fish scales consist of 3-4% of the total weight of fish and protein is the largest component in it, where 40-90% of the protein is collagen protein (Budirahardjo, 2010). There is still very limited information about collagen content, especially in freshwater fish scales, especially cultured fish.

This study aims to analyze the functional groups of collagen from the scales of snakehead (*Channa striata*) cultivated and caught in nature by an acid method.

2. Material and Method

2.1. Time and Place of Research

The research was conducted from February to May 2022 at the Fishery Products Technology Laboratory, Fishery Products Chemistry Laboratory, Universitas Riau. Industrial Services Standardization and Services Center, Pekanbaru. Material Science Laboratory FMIPA Universitas Riau.

2.2. Methods

The method used in this study was a survey method (case study), namely cultured and wild-caught snakehead in Dayun District, Riau. Data on the proximate composition of scales and collagen used an Independent sample t-test to evaluate the difference.

2.3. Materials

The main materials of this study were the scales of cultured and wild-caught snakeheads. Farmed snakehead was obtained from fish farmers in Kampung Sawit Permai, Dayun Subdistrict, Siak Regency, Riau, and wild-caught snakehead was obtained from fishermen of Danau Zamrud, Dayun Subdistrict, Siak Regency, Riau.

2.4. Procedure

2.4.1. Fish Scale Preparation

The scales were washed thoroughly under running water, and then aerated for 60 minutes. A total of ±350 g of fish scales were taken for analysis of moisture, ash, protein, and fat content; and the rest were soaked in 0.1M NaOH solution at a ratio of 2:3 (b/v) for 9 hours (every 3 hours the NaOH solution was replaced with a new solution). Soaking using NaOH aims to remove non-collagen proteins in the sample. The fish scales were neutralized with distilled water and then dried in a drying oven at 50°C for 8 hours. The dried scales were ground using a blender and sieved on a 20-mesh sieve. The resulting flour was then weighed for yield calculation.

2.4.2. Collagen Isolation

Snakehead scale flour was extracted using 0.5M HCl for 24 hours with a ratio of 1:10 (b/v) at 10oC, and then filtered with the calico cloth. The filtrate containing collagen was precipitated with 212.94 g of NaCl salt for 24 hours and then centrifuged at 8,000 rpm for 30 minutes. The precipitate or pellet obtained was dissolved in 0.5M acetic acid at a ratio of 1:10 (b/v) and put into dialysis bags. The analyzed bag containing the collagen was immersed in 0.1M acetate buffer for 24 hours, where every 6 hours the acetate buffer was replaced, and the last 6 hours the acetate buffer was replaced with distilled water until pH >5. During the analyzed process, salt molecules are diffused out through the dialysis bag so that the collagen obtained is more pure. The wet collagen from analyzed was then lyophilized or freeze-dried using a freeze dryer, resulting in dried collagen in powder form. Furthermore, the yield of the collagen produced was determined and analyzed for functional groups using FTIR.

2.5. Data Analysis

Data analysis at the yield calculation stage was descriptive by calculating the average value. Data analysis on the proximate test of snakehead scales and collagen used an independent T-test which processed the data using Statistical Package for Social Science (SPSS) 28.0 software using a 95% confidence interval (p=0.05) (Mattjik & Made, 2006).

3. Result and Discussion

3.1. Collagen Characteristics of Farmed and Wild-Caught Snakehead Scales

Collagen from the scales of farmed snakeheads is brown and lighter than collagen from the scales of wild-caught snakeheads. This was influenced by the color of the collagen raw material, namely the scales of farmed snakeheads which are more pale brown than the natural catch which is black and partly white. The dried collagen produced from the study can be seen directly and is in the form of granules (Table 1).

Table 1. Collagen yield of farmed and wild-caught snakehead scales

snakehead scales	Collagen weight (g/350 g scales)	Yield (%)
Cultivation results	55,37	15,82
Capture in nature	49,95	14,27

The yield value of collagen from cultured snakehead scales is significantly different and higher than that of collagen from wild-caught snakehead scales. This is because the collagen content is directly proportional to the protein content. It is known that the protein content in the collagen of cultured snakehead scales is higher than the catch in nature. This statement is following the opinion of Tang *et al.* (2011) that cultured fish contain high collagen than wild or natural fish. Most of the protein in these scales is collagen. The statement of Budirahardjo (2010) is that 40-90% of the protein is collagen.

The yield of cultured snakehead scale collagen is higher than the yield value of natural snakehead scale collagen by Widagdo (2016) which is 14.07%. The size of the yield obtained is influenced by the effectiveness of the extraction process which is one of the collagen isolation processes. According to Febrina (2015), the factors that affect extraction results are time, temperature, and solvent. In addition to the type of solvent, sample size also affects the amount of yield.

3.2. Chemical Composition of Collagen of Farmed and Wild-Caught Snakehead Scales

The chemical composition of collagen from farmed and wild-caught snakehead scales is presented in Table 2. Collagen of farmed snakehead scales contains higher moisture, ash, and protein content than wild-caught snakehead, but higher fat and carbohydrate content in wild-caught than farmed fish.

Table 2. Chemical composition of collagen from snakehead scales

Content	Cultivation yield (%)	Caught in nature (%)
Water (bb)	$8{,}37 \pm 0{,}05^{b}$	$6{,}38 \pm 0{,}06^{a}$
Ash (wt)	$28{,}09 \pm 0{,}08^{b}$	$26{,}01 \pm 0{,}23^{a}$
Protein (wt)	$60{,}24 \pm 0{,}02^{b}$	$59{,}78 \pm 0{,}11^{a}$
Fat (wt)	$2{,}57 \pm 0{,}20^{a}$	$3{,}67 \pm 0{,}29^{b}$
Carbohydrate* (wt)	9,10	10,54

*by *difference* ± standard deviation

Notes: Same letter in the same row indicates not significantly different ($p>0.05$); Different letters in the same row indicate significantly different values ($p<0.05$).

The water content in the collagen of cultured snakehead scales was higher than the natural catch ($p<0.05$). This is due to the ability of cultured snakehead scales' collagen to absorb water higher than in nature. In addition, the use of NaOH in the collagen isolation process also affects the moisture content of collagen. However, both values are following the quality requirements of collagen (SNI 8076:2014) which is ≤12%. According to Sinthusarman *et al*. (2013), the removal process of no collagenous protein can be used with alkaline solutions such as NaOH. NaOH solution can minimize the solubility of collagen protein and can cause *swelling in* the sample to allow the entry of water that can break down most of the telopeptide regions in the collagen matrix.

The ash content of collagen in the scales of farmed snakehead was higher than that of wild-caught ($p<0.05$). The variation in the amount of minerals that contribute to ash content is influenced by several factors including season, differences in age, size, species, gonadal maturity, and environmental conditions (Abimbola, 2016). In this study, the samples used came from different environmental conditions, where the cultured snakehead with media in the form of tarpaulin ponds while the snakehead caught in nature with media in the form of lakes. This difference makes a difference to the ash content and the high value of ash content in cultured products is due to the higher residue from the ash process and is a mineral that cannot burn into volatile substances.

The protein content of collagen in the scales of farmed snakehead was higher than that of wild-caught fish ($p<0.05$). The high content of water, ash, and protein in the collagen of cultured snakehead scales is closely related to external, internal, and dietary factors of the fish. The high content or level of protein in collagen can certainly make these snakehead scales a commercial raw material for collagen. Protein is one of the food substances and an important factor for body function. In body tissues, the largest component after water is protein. In liver tissue and meat, there is approximately 50% of the dry weight of cells in the form of protein.

Collagen from the scales of snakeheads also contains fat, while the fat content in collagen from the scales of cultured snakeheads is lower than the natural catch ($p<0.05$). The presence of fat in collagen is an impurity element that needs to be removed through optimization of the sample soaking process using NaOH before extraction. This is following the statement of Tang *et al.* (2011) that farmed fish have low fat than wild or natural fish.

3.3. Functional Groups of Snakehead Scale Collagen

The results of the analysis of functional groups in collagen from cultivated and natural snakehead scales using FTIR can be seen in Table 3.

Table 3. Functional groups in snakehead scale collagen

Function Group	Standard Absorption Area (cm^{-1})	Cultivation Results (cm^{-1})	Conversations in Nature (cm^{-1})	Description
Amide A	3500-3300[1]	3668,77	3853,94	Free NH
Amide B	2935-2915[2]	2920,35	2905,89	CH_2 stretching asymmetry
Amide I	1700-1600[2]	2302,14	1869,10	C=O stretching
Amide II	1575-1480[2]	1446,67	1457,28	NH Bending, C-N stretching
Amide III	1301-1229[2]	1245,10	1243,18	NH Bending, C-N stretching

Note: 1) Muyonga *et al.* (2004), 2) Kong & Yu (2007)

Amide A in the collagen of cultured and wild-caught snakehead scales was found at the absorption peaks of 3668.77-3853.94 cm^{-1}. The absorption peaks of these two collagens were higher than the standard collagen absorption region. This is due to the presence of NH stretch bonds from amide groups associated with hydrogen bonds. The absorption peak of amide B owned by cultured and wild-caught fish scale collagen is in accordance with the standard collagen absorption region which indicates the presence of typical collagen groups. Mayasari (2016) states that the wave number indicating the absorption of amide B is formed from the CH $group_2$ asymmetric stretching, namely asymmetric stretching of the CH $group_2$.

Amide absorption peaks I, II, and III are known to be directly related to the shape of the polypeptide. These peaks indicate the presence of C=O stretching vibrations along the polypeptide chain as well as a marker of the secondary structure of the peptide. This amide I absorption region indicates the presence of C=O stretching and OH groups paired with carboxyl groups. The absorption region in the collagen of cultured and caught snake head scales in nature is much higher than the standard. This is due to the joining or overlap between the absorption peaks of amide I and amide II.

Amide II is related to CN stretching and NH bending, where CN stretching is the regular movement of atoms along the bond axis between C atoms and N atoms so that the distance between atoms can increase or decrease, while NH bending is the movement of atoms that causes changes in the bond angle between two bonds or the movement of a group of atoms towards other atoms. The absorption peak of amide II in collagen scales of cultured and caught snakeheads in nature is in the absorption region of 1446,67 cm^{-1} and 1457,28 cm^{-1}, respectively.

Amide III in the collagen of snakehead scales from cultivation and natural catch was detected at the absorption peak of 1245.10 cm^{-1} and 1243.18 cm^{-1}, this indicates that the collagen of snakehead scales has not been denatured because there is still a triple helix structure. In addition to the amide group found in snakehead scale collagen, there is also a C-H bending alkene group at a peak of 668,36 cm^{-1} for cultivated and 863,18 cm^{-1} for natural catch. The appearance of peaks at wavelengths of 600 - 900 cm^{-1} indicates the presence of aromatic ring C-H functional groups.

4. Conclusions

The yield of collagen from cultured and wild-caught snakehead scales is 15,82% and 14,27% respectively in the dry state with high protein content, namely collagen from cultured snakehead scales 60,24% (wt) and wild-caught 59.78% (wt). The functional groups in the collagen of cultured snakehead scales are Amide A (3668,77 cm^{-1}) Amide B (2920.35 cm^{-1}) Amide I (2302.14 cm^{-1}) Amide II (1446.67 cm^{-1}) Amide III (1245,10 cm^{-1}). Meanwhile, the collagen of snakehead scales caught in nature has Amide A (3853,94 cm^{-1}) Amide B (2905,89 cm^{-1}) Amide I (1869.10 cm^{-1}) Amide II (1457,28 cm^{-1}) Amide III (1243.18 cm^{-1}). Collagen of cultured and wild-caught snakehead scales is classified as type 1 collagen.

5. Suggestion

Suggestions from the author so that further research can be carried out on the collagen of cultured snakehead scales, by adding several tests such as in terms of microbiology and compared with SNI.

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