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Characteristics of Physical-Chemical Properties of Collagen Extracted from the Skin of Bonylip Barb Fish (*Osteochilus vittatus*)

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Abstract: Bonylip barb fish is one of the local fish species in Indonesia. This fish has a high egg productivity so that the eggs used as raw materials caviar. One of the waste generated from the production process is the skin of Bonylip barb fish. This study aims to characterize the physical and chemical properties of collagen extracted from the skin of Bonylip barb fish. The extraction method used is soluble collagen enzyme pepsin (Pepsin - soluble collagens). The results showed that the yield of collagen extracted from the skin of Bonylip barb fish was 6.18% (wet weight). The collagen has moisture, protein, fat and ash content of 9.04%, 90.09%, 0.56% and 1.28%, respectively. The most common type of amino acid is glycine (16.56%) and the smallest is histidine (0.28%). Based on the amino acid content and functional groups, the collagen extracted from the skin of this fish includes collagen type I. The specific viscosity and reduced at a temperature of 28°C each of 0.1784 cp and 8.922 Dl/g and the denaturation temperature point is 31.5°C.

Key words: Collagen · Bonylip Barb Fish Skin · Characteristic · Physical · Chemical

INTRODUCTION

Collagen is a product that has a wide range of uses in the food, pharmaceutical, biomedical and cosmetic industries, but its use is limited because of its high price [1]. Collagen is a product generally extracted from the skin and bones of cattle, pigs, or poultry, but its use for food is limited due to religious and health reasons [2]. Another alternative source of collagen that is currently being developed is extracted from the skin, scales, bones and fish swimming bubbles [3]. Collagen from fish is now a concern to be applied to various fields by reason of a relatively cheap and easily accessible protein [4]. Based on the qualitative and quantitative, collagen that obtained from fish is better than mammals collagen [5].

The physical and chemical properties of collagen extracted from the mammalian part will be different from those extracted from the fish part [6]. The extraction of collagen from the fish part has been carried out by previous researchers, namely from the scales of tilapia [7] from Albacore Tuna (*Thunnus alalunga*), Dog Shark (*Scoliodon sorrakowah*) and Rohu (*Labeo rohita*) [8]. To enrich the diversity of collagen extract material made from raw fish, then the research of collagen extraction from the skin of Bonylip barb fish. Extraction collagen from the skin of Bonylip barb fish has never been done by researchers.

Bonylip barb fish is Indonesian local fish (The local name is Nilem fish). This fish is cultivated for its eggs as caviar raw material. One of the waste generated from the processing process is the skin of Bonylip barb fish.

The proportion of skin to the weight of Bonylip barb fish is approximately 10.6%. This value is higher than Common carp (4, 29%), Red snapper (5, 71%) and Milkfish (4.00%) [9]. This study aims to characterize the physical and chemical properties of collagen extracted from the skin of Bonylip barb fish.

MATERIALS AND METHODS

Materials: Bonylip barb fish samples were obtained from cultivators of Bonylip barb fish in Ciajur, West Java-Indonesia. Furthermore, in the circumstances of life brought to the laboratory of Fishery Products Processing Universitas Padjadjaran, Jatinangor-Sumedang, West Java Indonesia. The distance is approximately 60 Km.

Methods

Preparation Fish Skin Samples: The preparation of collagen was based on Kiew and Don modified method [10] is as follows: The Fish turned off by hitting his head. Next in the filet and taken the skin. The skin of the Bonylip barb fish is cleansed from the blood and the

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remains of the meat attached with cold water (<4°C). Next step, the fish cut into pieces with scissors, the size of the skin pieces of fish Bonylip barb is approximately ≤ 0.5 cm^2. Thereafter, the skin of Bonylip barb fish was soaked in 0.1 M NaOH solution with a sample ratio and volume of 1: 20 (w/v) solution for 6 hours. During the immersion, the stirring is done continuously. The NaOH solution is changed every 2 hours. Then after the immersion was completed, the sample of skin of the Bonylip barb fish was washed with cold (4°C) washers until the pH of a washed neutral or slightly alkaline aqualine wash (pH = 7.0 - 8.0). Then the sample is packed in plastic and stored on the frezeer until ready for use in the next stage.

Collagen Extraction Process: Bonylip barb leather fish samples from the thawing frezeer then weighed as much as 100 grams and then inserted into a 1000 ml glass beaker. After that, the enzyme pepsin as much as 1 gram. Then into a glass beaker that has contained fish skin samples and the enzyme inserted a 0.7 M acetic acid solution of 1000 ml. Then stirred until homogeneous and allowed to stand for 24 hours.

After immersion, the mixture was centrifuged at 10, 000 rpm for 20 minutes at 4°C. The viscous liquid phase (Supernatant) is accommodated and the solid phase is discarded. Then the viscous liquid is precipitated by adding NaCl until the final concentration is 0.8M. Precipitation is carried out for 24 hours in the refrigerator at cold temperatures (5 - 8°C). The precipitate is then separated by centrifugation at 10, 000 rpm for 20 min at 4°C.

Then the precipitate was reconstituted with 0.5 M acetic acid until dissolved. The solution was dialyzed with 0.1 acetic acid and aquadest. The process of dialysis for each type of solution is done in 2 stages. Each stage need 3 hours. Next, the solution is filtered with a filter cloth. The filtered collagen solution was then dried using a freeze dryer. The collagen obtained is called soluble pepsin collagen (SPC).

Physical-Chemical Properties Determination: Yields, physical characteristics and chemical characteristics of collagen were studied. Parameters of observed collagen physical characters were viscosity and denaturation temperature [11] method using a Brookfield viscometer). Collagen chemistry character parameters observed were proximate analysis (AOAC 1995 method [12]), functional group metallization [13] (With FTIR = (Fourier Transform Infra-Red) Perkin Elmer Spectrum One) and amino acid composition (HPLC = High Performance Liquid Chromatography; The condition of HPLC during amino acid analysis: Column Temperature: 38°C; Fluent flow rate: 1 mL / min; Pressure: 3000 psi; Mobile phase: Acetonitrile 60% and sodium acetate 1 M 40%; Column type: Pico tag 3.9 x 150 nm column; Program: Gradients; Detector: UV / 254 nm; and Merk: Waters Corporation USA) (AOAC 1995 method [12].

Statistical Analysis: The measurement results of proximate analysis were three replication and presented with mean ± standard deviation. Data on the physical and chemical characteristics of collagen were analyzed descriptively comparative.

RESULTS AND DISCUSSION

Yields: The yield of collagen is the result of the calculation of the collagen weight obtained from the extraction divided by the skin weight of the extracted fish and then multiplied by 100 percent [8]. The yield of collagen of Bonylip barb fish obtained from this research is 6.18% wet weight. The yield of collagen skin collagen is larger than the collagen of Rohu (*Labeo rohita*) fish skin (4.13%) [9], *Pangasianodon hyphothalmus* fish skin, i.e. 5.1% [14] and *Nerita crepidularia* (1, 28%) [15].

If the results of this skin yield of Bonylip barb fish compared with tuna skin collagen, 13.97% and shark skin, 8.96% [9] and big-eye snapper fish skin, 10.94% [11] is smaller. This difference in yield of collagen is due to differences in protein content in fish skin, where the skin of Bonylip barb fish used has lower protein content than shark, tuna and bigeye snapper [9, 11]. Differences of species, habitat, pre treatment and extraction methods are other factors that may cause different yields.

Proximate Analysis: The proximate composition of skin of Bonylip barb and collagen extracts greatly determines the physical and chemical properties of the use of the material. The results of proximate analysis of the skin of Bonylip barb fish and collagen extracted results are informed in Table 1.

Based on the dry weight, the skin protein content of Bonylip barb fish (92.53%) was higher than the protein content of the tilapia (72.47%, Alfaro *et al.* [16]) but lower than the catfish (95.76%, Rawdkuen *et al.* [17]). The protein content of the skin of the fish greatly determines the amount of collagen in the fish skin tissue [16]. Bonylip barb fish skin containing protein, 21.80% (wet weight) so potential as a raw material source of collagen.

Table 1: Proximate analysis of Bonylip barb fish skin

Proximate parameters (%)	Bonylip barb fish skin	Collagen extracted from Bonylip barb fish skin
Moisture	78.64 ± 2.74	9.04 ± 1.86
Crude protein	21.80 ± 1.94	90.09 ± 2.27
Crude fat	1.5 ± 0.80	0.56 ± 0.15
Ash	0.26 ± 0.03	1.28 ± 0.75

Description: The measurement results of three replications

Table 2: Amino acid composition (%) of collagen extracted from the skin of Bonylip barb fish

Amino Acid	Value (%)
Aspartic acid	4.78
Glutamic acid	7.92
Serine	2.88
Histidine	0.28
Glycine	16.56
Theonine	2.16
Arginine	6.83
Alanine	7.58
Tyrosine	0.39
Methionine	1.09
Valine	2.09
Phenylalanine	1.60
I-leucine	0.95
Leucine	3.01

The content of collagen protein extracted from the skin of Bonylip barb fish (97.99% dry weight) was lower than that of shark skin collagen (98.67% dry weight [9]). The low content of this skin collagen protein nilem because the content of the ash is still high, i.e. 1.28% (Wet weight). The differences in collagen content are strongly influenced by the type of fish, the extraction method and the use of the used dryers [18].

Analysis of Amino Acids: The amino acid composition in collagen is influenced by its physical-chemical properties, such as cross-linking and thermal stability. The composition of the amino acid, collagen differs in each species because of differences in molecular structure [19]. The results of the amino acid analysis of collagen extracted from the skin of Bonylip barb fish are presented in Table 2. Amino acid analysis is only performed on 15 amino species only.

Based on Table 2 above, the glycine content is the largest compared to other types of amino acids. In collagen from other fish skin sources such as tilapia, grass carp and silver carp obtained the same results that the glycine content is the largest [20]. Glycine was also the largest part in the amino acid compositions of gelatine extracted from silver carp waste, porcine skin and calf skin [21].

Histidine, tyrosine and I-leucine are the smallest amounts of amino acids in collagen extracted from the skin of nilem fish (Table 2). The results of previous studies also informed that the histide, tyrosine and I-leucine content were low in collagen extracted from tuna leather [9] also on collagen extracted from tilapia fish skin, grass carp and silver carp [20].

The collagen extracted from the skin of this Bonylip barb fish shows collagen type 1 because of its high glycine content and low tyrosine and histidine content. According to Liu *et al.* [22] the high glycine content of approximately 30% of the total amino acid content and the very small histidine and tyrosine content, in collagen, reference to the type I collagen. The collagen is extracted from the skin is generally a type I collagen [23].

Analysis of Functional Groups by Fourier Transform Infrared (FTIR): The typical functional groups present in collagen are amida A, B, I, II and III. The results of infrared spectra of collagen extracted from the skin of Bonylip barb fish are presented in Figure 1 and the characteristics of the Bonylip barb fish collagen skin functional groups are presented in Table 3.

Figure 1 shows that the peak absorption of amida group A from collagen extract from Bonylip barb fish is detected at wave number 3336.44 cm^{-1} with NH streching characteristic. This value is in the range of uptake for collagen type 1 [13] which higher than the value of collagen absorption from Squid leather (*Doryteuthis singhalensis*) is 3307 cm^{-1} [2] and lower than the absorption of collagen from fish skin Nile is 3434 cm1 [13]. The amida group A with free stretching NH is usually detected at the wave number 3440-3400 cm^{-1}, but when the NH group on the peptide is involved in the hydrogen bond, its position will shift to a lower frequency [25].

The amida B collagen group of extracts from the skin of the nilem fish was detected at the wave number 2927.95 cm^{-1}. This value is almost similar to the absorbance value of the amida group B of the clarias sp collagen, 2929 cm^{-1} [10]. The range of the collagen type I permeate region for the amida B group is in the range of 2850-2950 cm^{-1} with the CH asymmetric stretching bond [22] thus the B collagen amida group extracted from the skin of the nilem fish is in this range.--

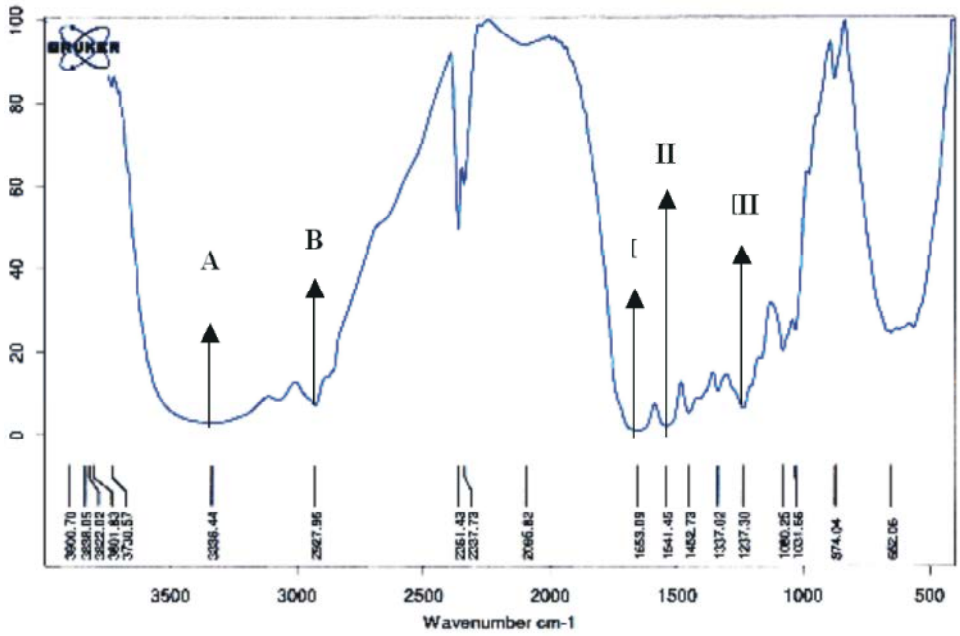

Fig. 1: Infrared spectra of skin collagen of Bonylip barb fish

Table 3: Characteristics of cluster function of skin collagen Bonylip barb fish

The absorption area	Absorption peak wave number (cm^{-1})	Area standards Absorption (cm^{-1})	Explanation
Amida A	3336.44	3440-33000[1]	NH *streching*
Amida B	2927.95	2850-29502	CH Asymmetry streching
Amida I	1653.09	1690-1600[3]	C=O *streching*
Amida II	1541.45	1575-1480[3]	NH *bending*, CN *streching*
Amida III	1237.30	1301-1229[3]	NH *bending*, CN *streching*

Information : [2][13], [1][22], [3][24]

The collagen amida I cluster extracted from the skin of the nilem fish was detected at the wave number 1653.09 cm^{-1}. Kong and Yu [24] state that the amida I group will be detected at the wave number 1690-1600 cm^{-1}. The value of amida group I collagen extracted from the skin of nilem fish is the same as the value indicated by the collagen absorption from the extract of skin of sea eel [2] slightly higher than the collagen extract from the skin of tilapia perch, which is 1650 cm^{-1} [13] and collagen from the extract of all types of goldfish, ie 1650 cm^{-1} [22]. The amida I group is related to the vibrational characteristics of the carbonyl group stretch and is a typical functional group that can differentiate between collagen and gelatin [26]. Generally the amida group I in collagen has a wave number between 1690-1600 cm^{-1} [24].

Collagen II amida collagen group of nilem fish skin extraction was detected at wave number 1541.45 cm^{-1}. The amida II group of collagen extracted from the skin of the fish will be detected in the range of 1575-1480 cm^{-1} [24]. The same cluster value is indicated by the extraction of collagen extracted from sea eels [2] but slightly lower than the skin of nilem fish compared to collagen extracts from the skin of tilapia perch, which is 1542 cm^{-1} [13]. The amida group II is related to the vibrational characteristics of NH bending accompanied by CN stretching vibration [27].

Collagen III amida group of nilem fish skin extraction was detected at wave number 1237.30 cm^{-1}. The amida III group of collagen extracted from the skin of the fish will be detected in the range of wave numbers 1301-1229 cm^{-1} [24]. The value of amida group III in collagen extracted from the skin of nilem fish is higher than collagen extract from the skin of tilapia perch, which is 1235 cm^{-1} [13] but lower than the absorption value of collagen amida group III of extraction of sea eel that is 1243 cm^{-1} [2]. The amida group III having the same characteristic as the amida II group will remain slightly altered from the vibration of NH bending to CN stretching [27]. The amida III group with

Table 4: Collagen viscosity extracted from the skin of Bonylip barb fish at different temperatures

Tempe-rature°C	Flow time (seconds) Collagen solution (t)	Solvent (to)	Specific viscosity (η_{sp}) $\eta_{sp} = \frac{(t - t_0)}{t_0}$	Viscosity Reduced (η_{sp}/c)
28	107.43 ± 0.18	91.6 ± 0.06	0.1784	8.922
30	101.49 ± 0.14	85.16 ± 0.05	0.1779	8.898
32	95.47 ± 0.22	82.92± 0.02	0.1513	7.566
34	90.79 ± 0.15	79.61 ± 0.17	0.1404	7.019
36	87.14 ± 0.04	76.91 ± 0.04	0.1329	6.646
38	84.42 ± 0.08	74.57 ±0.19	0.1321	6.605
40	81.68 ± 0.06	72.29 ± 0.08	0.1298	6.490

Description, C = concentration of collagen sample solution is 0.02 g / dL

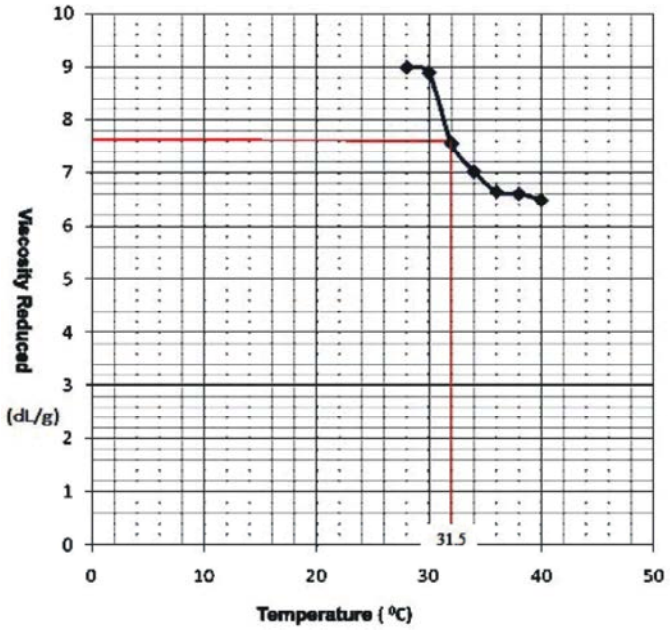

Fig. 2: The collagen denaturation curve extracted from the skin of a Bonylip barb fish

wave numbers 1237-1234 cm^{-1} has a high molecular structure and a dominant triple helix structure with strong bonds [28]. Thus the extraction process undertaken in this study produces collagen with good characteristics.

Viscosity Analysis of Collagen Solution: Viscosity is a measure of viscosity that expresses the magnitude of friction in a fluid. Collagen viscosity is very important to know related to its use. Calculation result of extraction collagen viscosity from skin of nilem fish based on observation of flow time as presented Table 4.

According to Table 4, the higher the temperature, the decreasing the specific viscosity and the reduced viscosity. The highest specific viscosity and highest detoxification were obtained at 28°C i.e. 0.1784 and 8.922 respectively. Conversely the lowest specific and reduced viscosity was found at 40°C i.e. 0.1298 and 6.490 respectively. Decrease in viscosity is caused by a decrease in collagen molecular weight due to polymer chain breakdown. Temperature is one of the factors that affect the chain cutting or protein polymer.

Denaturation Temperature Analysis: The thermal denaturation curve of collagen solution is formed by plotting reduced viscosity against temperature. The denaturation point (Dp), expressed as the mid-temperature between the extrapolated line of the original collagen with the fully denatured collagen. The collagen denaturation point extracted from the skin of the a Bonylip barb fish as presented in Figure 2.

Based on Figure 2, the collagen denaturation temperature of extracted results from the skin of a Bonylip barb fish is 31.5°C. This denaturation temperature is higher when compared with collagen from cod skin source, 15°C, mackerel skin , 26.1°C and skipjack skin, 29.7°C [13] but lower than collagen from goldfish scales,

32.5°C [29]. According to El-Rashidy *et al*. [7], the temperature point of denaturation of collagen shows its stability in heat. The collagen denaturation temperature point derived from fish skin varies depending on the species and their environmental habitats that correlate with the amino acid content. The higher the amino acid content in the collagen the higher the denaturation point, which means the more stable the collagen is to heat.

CONCLUSION

The yield of collagen extracted from the skin of nilem fish was 6.18 % (Wet weight). Collagen has moisture, protein, fat and ash content of 9.04 %, 90.09 %, 0.56 % and 1.28 % respectively. The most common type of amino acid is glycine and the smallest is histidine. Based on its amino acid content and functional group, the collagen extracted from the skin of this fish includes collagen type 1. Specific viscosity and reduced at a temperature of 28°C each of 0.1784 and 8.922 and the denaturation temperature point is 31.5°C.

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