



Review Article: Fish Bone Collagen

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Fishbone collagen is an alternative source of bovine and pig collagen. The purpose of this article is to review the types, benefits, extraction methods and characterization of collagen from fish bones, especially freshwater fish. The result of the review shows that the collagen from tilapia bones is shaped like a sheet with a slight porous surface and is of type I. Collagen extraction begins with bone disease from the remaining fat, then degreasing and the next step is extraction. After the extraction process is complete, filtration is carried out to store the filtrate or residue, then the collagen is purified by salting-out with NaCl. Collagen yield and collagen pH value from collagen extraction were different for each type of fish bone. The difference in yield value and protein and amino acid content in the resulting collagen can be caused by differences in the extraction method, the concentration of the solution used, whether acid or alkaline, and the neutralization process and the type of raw material. Fishbone collagen has good benefits in the cosmetic and pharmaceutical fields.

Keywords: Type; benefits; pharmacy; cosmetics; characteristics.

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1. INTRODUCTION

The production of Indonesian freshwater fisheries, especially tilapia, has increased every year. Ministry of Marine Affairs and Fisheries (KKP) data shows that tilapia production reached 1.1 million tons in 2018 and then increased to 1.3 million tons in 2019. Most of the tilapia production is sold in fillet form. One of the wastes produced from the tilapia fillet industry is bone. According to Junianto et al. [1] it is possible to use fish bones to be processed into high value added products, namely extracting the collagen compounds.

Fish collagen is considered to be an alternative to collagen from bovines and pigs which is commonly used in industrial applications. According to Romadhon [2] Muslims and Jews are not allowed to use pig raw materials, while Hindus are not allowed to use bovine raw materials. Muralidharan et al. [3] stated that the main difference between collagen from fish and collagen from other animals is its high biological value, high content of essential amino acids, and low content of hydroxyproline. According to Yunoki et al. [4] stated that collagen from salmon has a smaller denaturation than collagen from mammals. According to Leuenberger [5], fish collagen has a relatively higher viscosity value than mammals. Fish collagen is heat sensitive due to labile cross links as compared to mammals. According to Berillis [6] Amino acids such as proline and hydroxyproline in fish collagen are lower than mammalian collagen, but serine, threonine and methionine in fish collagen are higher than mammalian collagen.

Marine collagen comes from fishery waste such as fish bones which are exploited as an environmentally friendly and inexpensive source of collagen. Marine collagen is recently recognized as a promising biomaterial with great potential in pharmaceutical and biomedical applications. According to Alves et al. [7] collagen extracted from codfish and salmon skin is used as a component of cosmetic formulations, shows the best capacity to retain air and is suitable for application of dermal moisturizers and shows that fish collagen has no irritating or inflammatory effect on the skin. Based on a pilot study Chen et al. [8] obtained collagen oligopeptide-rich hydrolysate from codfish skin by using a collagenolytic protease from the deep-sea bacterium *Pseudoalteromonas* sp., showed that the collagen peptides obtained had antioxidant

activity, reduced free radicals at 10 mg/mL, higher than hyaluronic acid, so that collagen peptides can be an ideal ingredient in cosmetics. Therefore, this article aims to review the types, benefits, methods of extraction and characterization of collagen from fish bones, especially freshwater fish.

2. FISH BONE COLLAGEN

Collagen is the main structural component of connective tissue, covering 30% of the total protein in the tissues of vertebrates and invertebrates [9]. The basic collagen molecule is formed from three polypeptide chains that twist together to form a triple helix structure with a unique amino acid arrangement, namely Gly-X-Y, at position X is proline and position Y is hydroxyproline [10]. Each collagen type has a specific alpha chain with its own domain structure which contributes to the classification by collagen type. Generally, fish collagen consists of two alpha 1 chains and one alpha 2 chain [11]. Collagen from fishbone shows properties of type I collagen consisting of two $\alpha 1$ and one $\alpha 2$ chain [12]. Collagen from fish bones is classified as type I collagen, which contains high levels of the amino acids glycine, alanine and proline.

Collagen which is composed of skin, bone and scales has different morphology. Collagen morphology can be displayed using Scanning Electron Microscopy (SEM). Based on Romadhon's research [2] using SEM with a magnification of 3000x, it can be seen that the collagen from tilapia fish bones is shaped like a sheet with a slight porous surface, while the collagen from the skin and scales of tilapia has the same morphology, which is in the form of small boxes and a smooth surface without pore. Arumugam et al. [13] also conducted morphological observations with SEM at 500x magnification of fish collagen where collagen looks like fiber. The difference in magnification in the collagen morphology observations using SEM affects the observed morphological differences, where at high magnification collagen looks like interconnected sheets. The visible pores in tilapia bone collagen are due to the space between the collagen fibers. According to Arumugam et al. [13] stated that the space between the sheet which is sheathed causes porosity in collagen. Porosity in collagen facilitates the incorporation of any value added chemical like drugs.

3. COLLAGEN EXTRACTION METHODS

Collagen extraction begins with cleaning fishery by-products such as bones, scales and skin from the remains of meat and fat that is still attached. The next stage, namely the degreasing stage, is the stage for eliminating non-collagen proteins as well as impurities by soaking in NaOH. According to Ata et al. [14] the collagen that forms the helix is called the collagen group, where after immersion with NaOH solution a reaction occurs which causes the helix to stretch so that it can bind water. It is characterized by a structure that is initially thin to thick and clear in color. The next stage, namely the extraction stage, in which collagen generally dissolves in acidic solvents. According to Kittiphattanabawon et al. [15] collagen that dissolves in high acid solvents can cause the solubility to be slightly decreased. Therefore, the extraction stage is carried out using a weak acid with a low concentration such as acetic acid (CH₃COOH). According to Nurhayati and Peranginangin [16] Acetic acid has a carboxyl group (-COOH) which can bind to the amine group (-NH₂) of collagen protein, making it easier for the collagen extraction process. Collagen obtained by extraction with acids is called Acid Soluble Collagen (ASC).

Extraction temperature in collagen extraction affects the yield value of the resulting collagen. According to Nurhayati and Peranginangin [16] collagen fibers can experience shrinkage if heated above 60-70°C. The process of collagen shrinkage causes the collagen structure to break down into water-soluble gelatin. Therefore, the collagen extraction process is carried out under the shrinkage temperature. Several studies reported by Nagai et al. [17], Noitup et al. [18] and Senaratne et al. [19] used a temperature of 4°C in collagen extraction. The low extraction temperature used in this extraction process is based on the low denaturation temperature of collagen. After the extraction process is complete, filtration is carried out to separate the filtrate or residue, then the collagen supernatant

or filtrate is purified by salting-out with NaCl salt. The use of high concentrations of salt can cause the water surrounding the protein molecule to be eliminated, so that the protein that settles as a wet collagen residue.

4. COLLAGEN CHARACTERISTICS OF FISH BONES

4.1 Collagen Yield

Based on research by Nagai and Suzuki et al. [20] Ayu fish bones have a high collagen yield compared to skipjack tuna and Japanese sea bass fish bones. Darmanto et al. [9] reported that, tilapia fish bones have a higher collagen yield than milkfish and mackerel fish bones. The difference in yield value in the resulting collagen can be caused by differences in the extraction method, the concentration of the solution to remove non-collagen protein, the type of material, the temperature and the length of time of production [21]. According to Zhou and Regenstein [22], the concentration of acid applied in the collagen extract production process can increase the yield value. Kittiphattanabawon et al. [15] stated that collagen that dissolves in high acid solvents can cause solubility to be slightly decreased. According to Naro [23] the higher the concentration of the acetic acid solution used, the more collagen is produced. This shows that the concentration factor of the acetic acid solution has a significant effect on collagen protein extraction.

4.2 Collagen pH

Based on Romadon's research [2], collagen in tilapia fish bones has a pH value that is not much different from the research of Sembiring et al. [24]. These results are lower than the collagen quality requirements of SNI 8076: 2014, namely 6.5 - 8, but slightly higher than the collagen pH of several collagen brands for cosmetics reported by Peng et al. [25], which ranges from 3.8 to 4.7. According to Zhou and Regenstein [22] the

Table 1. Collagen yield from some types of fish bones

Types of fish bones	Collagen yield (%)	Extraction methods
Skipjack tuna (<i>Katsuwonus pelamis</i>) [20]	42.30	CH ₃ COOH 0.5 M
Japanese sea bass (<i>Lateolabrax japonicus</i>) [20]	40.70	
Ayu (<i>Plecogosus altivelis</i>) [20]	53.60	
Nile tilapia (<i>Oreochromis niloticus</i>) [9]	56.45	HCl 4% 1 N
Milkfish (<i>Chanos chanos</i>) [9]	36.22	
Mackerel (<i>Scomberomorus</i> sp.) [9]	49.80	

Source: Nagai and Suzuki [20], Darmanto et al. [9]

Table 2. Collagen pH from some types of fish bones

Types of fish bones	Extraction methods	pH
Nile tilapia (<i>Oreochromis niloticus</i>) [2]	CH ₃ COOH 0.5 M	5.39
Tuna (<i>Thunnus</i> sp.) [24]	HCl 3%	5.19

Source: Romadhon [2], Sembiring et al. [24]

Table 3. Collagen compositions of fish bones [9]

Proximate Compositions	Nile tilapia	Milkfish	Mackerel
Protein (%)	25.06	32.99	31.92
Fat (%)	0.74	1.32	1.41
Ash (%)	50.75	53.41	54.63
Moisture (%)	7.46	8.48	5.29

Table 4. Amino acid composition of fish bone collagen

Amino Acid	Residues/1,000 total residues		
	Common carp bone collagen (<i>Cyprinus carpio</i>) [12]	Leather jacket bone collagen (<i>Odonus niger</i>) [3]	Bigeye snapper bone collagen (<i>Priacanthus</i> sp.) [15]
Hydroxyproline	87.2	80	20
Aspartic acid	51.5	47	47
Threonine	24.7	25	25
Serine	29.6	33	34
Glutamic acid	35.8	76	74
Proline	103.1	112	95
Glycine	356.8	334	361
Alanine	95.1	122	129
Valine	12.1	19	17
Methionine	11.2	13	8
Isoleucine	11.5	10	5
Leucine	17.2	21	25
Tyrosine	24.1	3	2
Phenylalanine	25.8	13	12
Histidine	10.5	4	6
Lysine	52.8	26	25
Arginine	51	54	46
Hydrolysine	-	8	20
Total	1000	1000	1000

Source: Duan et al. [12], Muralidharan et al. [3], Kittiphattanabawon et al. [15]

difference in the pH value of collagen can be caused by differences in the type and concentration of the solution used, both acidic or alkaline and the neutralization process. The combination of acid and alkaline processes tends to produce a pH close to neutral.

4.3 Collagen Proximate Analysis

Darmanto et al. [9] found that the proximate composition of bone collagen in freshwater fish,

brackish water and sea water is different. The protein content of mackerel fish bone collagen is higher than the bone collagen in milkfish and tilapia, which is 25.06%. However, the protein content in the mackerel fish bones is lower than the dry collagen protein content according to SNI 8076 [26], namely 80-88%. Jamilah et al. [27] stated that differences in protein content could be caused by differences in the extraction method used. The water content of tilapia, milkfish and mackerel bone collagen produced still meets the

collagen quality requirements SNI 8076 [26], namely ≤ 12 .

4.4 Amino Acid Composition

Collagen is composed of several amino acids, especially glycine, proline and hydroxyproline. Glycine is the largest amino acid that makes up collagen. Collagen does not contain tryptophan and cysteine, and is very low in tyrosine and histidine [28]. Fish collagen has a low amount of hydroxyproline and stability compared to terrestrial vertebrate animal collagen [29]. The content of hydroxyproline depends on the type of fish, body temperature, and the temperature of the environment in which the fish live. Collagen produced from fish species that live in cold environments has a lower hydroxyproline content than tropical fresh water fish. This is because hydroxyproline contains hydrogen bonds which stabilize the triple helix structure of the collagen molecule.

Amino acids in collagen in the types of common carp fish bones, leather jacket fish bones and bigeye snapper fish bones are different. Based on a research by Kittiphattanabawon et al. [15] on bigeye snapper fish bones, has a higher glycine content than common carp fish bones [12] and leather jacket fish bones [3]. However, when compared to other compositions such as proline and hydroxyproline, the bigeye snapper fish bones were lower. Collagen produced from fish species that live in cold environments has a lower hydroxyproline content.

5. BENEFITS OF FISH BONE COLLAGEN

Based on a research by Haris et al. [30] the addition of tilapia fish bone collagen with a concentration of 3% in solid bath soap has the best results compared to control concentrations, 1% and 2% through the hedonic test. This is because solid bath soap with the addition of 3% collagen has scrubs that tend to be more than the addition of 1% and 2% collagen. Besides that, from the side of the rough impression and the texture felt by the panelists, it has a rough impression and good texture compared to other treated soaps. The value of water content in soap with the addition of 3% collagen had the lowest water content value, namely 11.84%. This value meets the SNI quality requirements for soap water content, which is a maximum of 15%. This is in accordance with the statement of Nurhayati et al. [31] that the higher the addition

of collagen, the more water is bound so that the water content will decrease.

Based on a research by Sudewi et al. [32] catfish bone collagen has a good effect on the evaluation of anti aging cream preparations. Cream preparations using catfish bone collagen gave homogeneous results, pH 6.0–6.4, stable at 12 weeks of storage and did not irritate the skin of 10 volunteers. According to Nazliniwaty et al. [33] the effectiveness of anti aging creams during the use of preparations for 4 weeks can be measured by calculating the percent increase in water content, decrease in the number of pores, increase in smoothness, reduce the number of blemishes and reduce wrinkles on the skin. The results of the anti aging effectiveness test of catfish bone collagen cream for 4 weeks on the skin of volunteers showed an increase in water content up to 66.03%, a decrease in the number of pores up to 47.43%, an increase in smoothness up to 33.33%, a reduction in blemishes up to 48.23% and reduction of wrinkles 54.83%.

6. CONCLUSION

The results of the review show that the collagen from tilapia bone is shaped like a sheet with a slight porous surface and is of type I. Collagen extraction begins with cleaning the bones from the remaining fat, then degreasing and the next step is extraction. After the extraction process is complete, filtration is carried out to separate the filtrate or residue, then the collagen is purified by salting-out with NaCl. Collagen yield and collagen pH value from collagen extraction were different for each type of fish bone. The difference in yield value and protein and amino acid content in the resulting collagen can be caused by differences in the extraction method, the concentration of the solution used, both acid or base and the neutralization process and the type of raw material. Fishbone collagen has good benefits in the cosmetic and pharmaceutical fields.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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