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Potential of Patin (Pangasius Sp.) Swimming Bubbles as Raw Materials of Anti-Aging Collagen Results For Acid Extraction

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ABSTRACT
Increased production of catfish causes an increase in by-products of catfish consisting of skin, head, bones, and swim bladder. The products produced during fish processing range from 20-60% of the total raw materials. Fish swimming bubbles have a proportion of less than 2% in one fish with a high collagen protein content of 93.39% bk. (3) This study aims to determine whether catfish swimming bubbles have the potential to produce collagen as a cosmetic raw material. chemistry of swimming bubbles of catfish, Pegasus sp. and the characteristics of collagen obtained by extraction using 0.2 M acetic acid. The results showed that the swimming bubbles of catfish contained 23.8% protein, which was dominated by the amino acid glycine 130.32 mg/g, L-arginine 53.92 mg/g and L-alanine 53.85 mg/g. Characteristics of functional groups and molecular weights indicate that the obtained collagen is classified as Type I collagen, and the resulting collagen has high thermal stability so that it can be applied to the cosmetic industry.
KEYWORDS: acetic acid, swim bladder, collagen, catfish

I. INTRODUCTION

Collagen is the main structural protein of connective tissue that accounts for 30% of the total protein in the body and is a constituent component of bones, teeth, muscles, and skin. This compound has a triple helix composed of three polypeptide chains and is a fibrous protein. (1) Collagen in its development has become an important biomaterial in the industrial, cosmetic, food, biomedical, and pharmaceutical fields. The main source of commercial collagen generally comes from terrestrial animals such as cattle and pigs, but the use of cattle has begun to cause concern among producers and consumers because of the spread of prion bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD) as well as religious issues that do not allow it. use of pork and beef as a source of collagen. (2) This condition opens up opportunities for the use of collagen sources other than terrestrial animals, namely aquatic animals, one of which is fish. Fish can be used as an alternative source of collagen because it is unlikely to be associated with prion disease, FMD or halal. Collagen sourced from fish has advantages over terrestrial animals in terms of shorter protein fibers (Muyonga et al. 2004a; Liu et al. 2012) and a simpler molecular structure that makes it easy to absorb (Kumar et al. 2011).

Almost all types of fish can be used as a source of collagen, but it will be more effective to utilize the abundant by-products of the catfish industry.(2)The swim bladder contains an important chemical component which is dominated by the collagen protein(Kaewdang, Benjakul, Kaewmanee&Kishimura, 2014). According to Katili (2009), collagen is dominated by the amino acids glycine, proline, hydroxyproline and alanine. Lehninger (2000) stated that the protein content of collagen is 21% proline and hydroxyproline, 11% alanine and about 35% glycine. Collagen can be applied in the pharmaceutical, biomedical, cosmetic and food industries.

According to Santos et al. (2013), many parts of the bones and skins of land animals such as poultry, pigs and cattle are produced into collagen, but now exploration of aquatic animals in order to find alternative collagen sources has been widely carried out such as skin, bones and fish swimming bubbles (Jamilah, Hartina, Hashim & Sazili, 2013; Kaewdang et al., 2014; Kittiphattanabawon & Benjakul, 2005; Nagai, Suzuki & Nagashima, 2008), as well as from sea cucumber meat (Alhana, Suptijah & Tarman, 2015). As a source of collagen, catfish swimming bubbles have several advantages, including having a fairly high protein content and abundant availability because they have not been utilized. The protein content of fish swim bladders that have been studied, such as the swim bladder of yellow fin tuna (Thunnus albacores) is 12.09% (Kaewdang et al., 2014), 20.27% (Idrus, Hadinoto

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& Kolanus, 2018) and cinch fish. (Muarenesox talabon) by 33.67% (Gadi, Trilaksani & Nurhayati, 2017).

Research on the use of fish swimming bubbles has been widely carried out, both at home and abroad. In Indonesia, research on the use of fish swimming bubbles has been carried out since 2006, including the manufacture of isinglass from catfish swimming bubbles (Trilaksani, Nurjanah & Utama, 2006), collagen extraction from cinchfish and tuna swimming bubbles (Djailani, Trilaksani & Nurhayati, 2016 ; Kartika & Trilaksani, 2016; Gadi et al., 2017; Idrus et al., 2018). However, until now the optimal treatment has not been obtained to obtain the characteristics of collagen that meet the collagen quality requirements in accordance with SNI 8076: 2014 (BSN, 2014). In addition, the Acid Soluble Collagen (ASC) method to extract collagen from the swimming bubbles of catfish has not been widely used. Kaewdang et al. (2014) reported that the yield of tuna swim bladder collagen extracted using 0.5 M acetic acid was 1.07%. Idrus et al. (2018) reported that the use of 0.5 M and 0.75 M acetic acid produced collagen with a glycine content of 1175.05 mg/g and 733.99 mg/g; arginine 848.75 mg/g and 0 mg/g and alanine 338.66 mg/g and 215.35 mg/g. According to Wang et al. (2008), the extraction conditions and methods used affect the yield and amino acid composition of collagen. This study aims to determine whether the swimming bubbles of catfish have potential as raw materials for collagen for cosmetics.The raw material preparation process to get dry collagen as shown below:

			
Freezeraw material Personal Collection (1)	Raw material in water Personal Collection (2)	Rawmaterian in NaOH Personal Collection (3)	Rawmaterian in CH3COOH Personal Collection (4)
			
Wet Collagen Personal Collection (5)	Dry Colagen Personal Collection (6)	Powder Collagen Personal Collection (7)	Serum Google image (8)

Figure 1. The raw material preparation process to get dry collagen

II. MATERIALS AND METHODS

MATERIALS AND EQUIPMENT

The main material used in this study was the swim bladder of catfish (Swim Bladder of Catfish) (Pangasius sp.) which was obtained from PT. Kurnia Mitra Makmur. The chemicals used in the pre-extraction process (pretreatment) and collagen extraction of catfish swimming bubbles are NaOH (Merck), CH 3 COOH (Merck), NaCl (E. Merck), DMSO (Merck), NaH 2 PO 4 (Merck), Na 2 HPO 4 (Merck), kojic acid (sigma aldrich), tyrosine enzyme (333 U/mL in phosphate buffer)

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(sigma Aldrich), and a substrate (L-DOPA 2 mM) (sigma Aldrich). Andaquadest.

The equipment used is a spectrophotometer (SPECTRO UV-VIS), centrifugation (HIMAC 21G), freeze dryer (Eyela FDU-1200 Japan), FTIR (Bruker Tensor 37 Germany), Differential Scanning Calorimetry (Shimadzu Germany), Ultra Performance LiquidChromatography (Water - Coorporation USA), oven (Memmert UNB 400 Germany) and pH meter (ORION 3 STAR Thermo Scientific Germany), Barfield Viscometer, Extensometer.

Collagen extraction from catfish swim bladder begins Pre-treatment of fresh catfish swim bladder according to Alana et al. (2015) with modifications. Pre-treatment was carried out by immersing the swim bladders using 0.1 M Noah solution to remove non-collagenous proteins, fats, minerals, and pigments present in the catfish swim bladders. Immersion was carried out in 0.1 M NaOH solution with a swimming bubble ratio and 1:10 (w/v) NaOH solution. The soaking solution was changed every 6 hours and the remaining soaking solution was tested for protein content by the Bradford method. The pre-treated swimming bubbles were washed with distilled water until they reached pH 7. Hydrolysis of acid-soluble collagen (Modification of Erizal et al. 2012). Extraction of collagen from the swim bladder was carried out by the acid soluble method. Coarse collagen fibers obtained from pre-treatment were soaked with 0.5 M CH3COOH at 4°C for 48 hours. The next process was filtered with a calico cloth, then the filtrate was dried using a freeze dryer to obtain dry collagen.

METHODS

Analysis of collagen

Yield collagen yield value was obtained from the comparison of the dry weight of the collagen produced with the weight of the swimming bubble material of catfish (AOAC 2005). The yield value is obtained by the formula:

Collagen yield (%) = Dry weight of collagen × 100%
Raw material weight

Analysis of protein

Analysis of the protein content (non-collagenous) in the NaOH residue of the catfish swimming bubble immersion was carried out using the Bradford method (1976) with bovine serum albumin (BSA) as the standard. The sample was put into a 50 L test tube, then 2.5 mL of Bradford's reagent was added. The next process was incubated for 5 minutes, then the absorbance was measured using a spectrophotometer at a wavelength of 610 nm. The standard concentration of BSA used is 0; 400; 500; 600; 800; 1,000 and 1,200 ppm.

Functional group analysis

Functional group analysis performed by FTIR was carried out to determine the typical functional groups present in the collagen produced. Collagen as much as 0.02 g was pulverized with 100 mg of Potassium Bromide in a mortar until homogeneous, then compacted into a pellet mold and vacuumed in a pellet molding machine. Measurement of the test sample was carried out at a wave number of 4000-500 cm -1 . The resulting FTIR spectra show the wavenumber absorption peaks of the test sample. The functional groups of the test sample were determined based on the absorption peak of the detected wave number with the absorption region for the protein functional group.

pH analysis

Collagen pH refers to Alhana et al. (2015). 1 g of collagen was dissolved in 20 mL of distilled water, added 50 mL of distilled water, and homogenized. The pH meter is turned on and left to stabilize. The electrode is immersed in the sample for a while until a stable pH value is obtained. Measurements were carried out 3 times.

Amino acid Analysis

Amino acid analysis Amino acid analysis was performed using High performance Liquid Chromatography (HPLC) (AOAC 2005). This method begins with hydrolysis of the sample through tethering of 6 N HCl and heating, then the hydrolyzate is dried and derivative with phenylisothiocyanate (PITC) or Edman's Reagent solution and acetonitrile is added as the mobile phase. Samples were injected into HPLC after being filtered using 0.45 Millipore filter paper.

The concentration of amino acids is calculated by the formula:

Amino acid concentration = sample area×C ×FP×BM ×1OO
The area ofberth×bobot sample (g)
C :Amino acid concentration (ug/mL)
FP :Dilution factor
BM :The molecular weight of each amino acid (g/mol)

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III. RESULTS AND DISCUSSION

Yield

Yield value shows how efficiently raw materials can be converted into products. The yield of collagen hydrolyze obtained is influenced by various factors including the type and quality of the raw materials. Zelechowska (2010) stated that frozen storage time affects the amount of collagen that can be extracted. The longer the raw material is stored the less the amount of collagen that can be extracted, with a decrease of up to 1.2% per week. The yield of collagen hydrolyzed obtained is to the raw material of catfish swimming bubbles. The results of Hartina et al. (2019) showed that the yield of hydrolyzed milkfish skin collagen hydrolyzed using alkalis and bromelain enzymes for 60 minutes was 6.75±2.04% and 16.89±0.12%, respectively. The results of research by Zhao et al. (2018) showed the yield value of collagen hydrolyzed from the skin of Rana chensinensis which was hydrolyzed using the pepsin enzyme of 15.1%. Differences in the yield of collagen hydrolyzed in various species according to Zhang et al. (2007) can be influenced by the hydrolysis process, the specificity of the protease enzyme used, and the characteristics of the crosslinking bonds in collagen and its hydro lysates. Collagen crosslinking can undergo polycondensation

Protein Level

The remaining NaOH immersion in catfish swimming bubbles was analyzed for protein to determine the remaining non-collagen protein content using the Bradford method (1976) with bovine serum albumin (BSA) as the standard. The sample was put into a 50 L test tube, then 2.5 mL of Bradford's reagent was added. The next process was incubated for 5 minutes, then the absorbance was measured using a spectrophotometer at a wavelength of 610 nm. The standard concentration of BSA used is 0; 400; 500; 600; 800; 1000 and 1,200 ppm. The absorbance results showed the levels of non-collagen protein obtained .

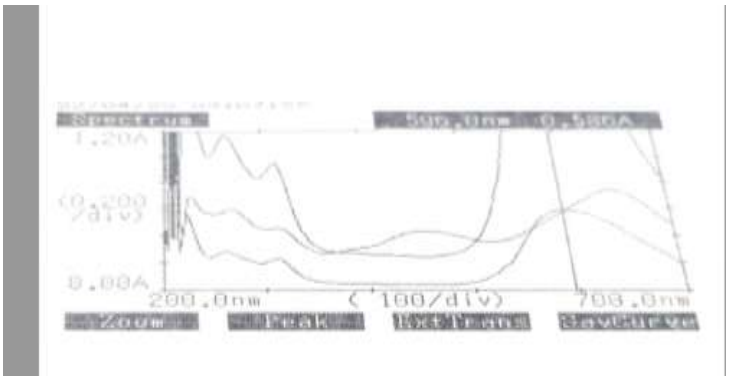

Figure 2. Spectrum BSA

Functional group analysis

Functional group analysis was analyzed using FTIR to determine the specific functional groups present in the collagen produced. Collagen as much as 0.02 g was pulverized with 100 mg of Potassium Bromide in a mortar until homogeneous, then compacted into a pellet mold and vacuumed in a pellet molding machine. The measurement of the test sample was carried out at a wave number of 4000-500 cm -1 . The resulting FTIR spectra show the wavenumber absorption peaks of the test sample. The functional groups of the test sample were determined based on the absorption peak of the detected wave number with the absorption region for the protein functional group. The results were as follows:

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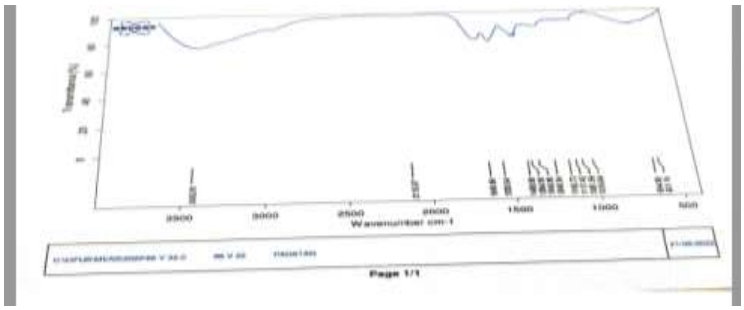

Figure 3. FTIR Spectrum

pH Test

To find out whether the extracted collagen from fish swimming bubbles can function as a cosmetic raw material, it is necessary to test the pH of the extracted collagen by referring to Alhana et al. (2015). 1 g of collagen was dissolved in 20 mL of distilled water, added 50 mL of distilled water, and homogenized. measured with universal pH paper. Measurements were carried out 3 times. The following results were obtained: (Table pH test)

Figure 4. Measurements pH

Amino AcidAnalysis

Being filtered using 0.45 Millipore filter paper. The concentration of amino acids obtained is as follows: Amino acid analysis Amino acid analysis was performed using High performance Liquid Chromatography (HPLC) (AOAC 2005). This method begins with hydrolysis of the sample through tethering of 6 N HCl and heating, then the hydrolyzate is dried and derivative with phenylisothiocyanate (PITC) or Edman's Reagent solution and acetonitrile is added as the mobile phase. Samples were injected into HPLC after

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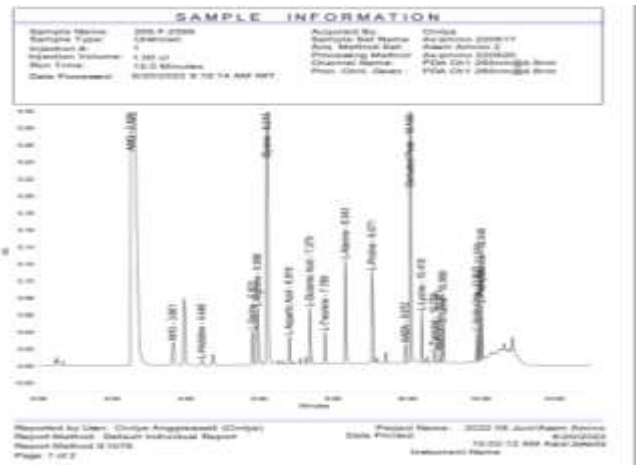

Figure 5. Amino AcidAnalysis

	Peak Name	RT	Area	% Area	Height	Amount
1	AMQ	2.529	4158339.09	54.74	546075	
2	NH3	3.661	89795.28	1.18	28082	
3	L-Histidine	4.448	20141.14	0.27	6750	41.077
4	L-Serine	5.823	96609.32	1.27	40066	
5	L-Arginine	5.956	162703.38	2.14	67081	100.592
6	Glycine	6.213	906724.04	11.94	302585	1513.518
7	L-Aspartic Acid	6.818	72320.34	0.95	32731	109.741
8	L-Glutamic Acid	7.379	133330.82	1.76	65887	
9	L-Treonine	7.789	74002.46	0.97	37262	77.706
10	L-Alanine	8.343	245422.39	3.23	121760	263.020
11	L-Proline	9.071	237671.24	3.13	109104	147.115
12	AABA	9.972	40304.08	0.53	22522	
13	Derivated Peak	10.100	1032477.35	13.59	613590	
14	L-Cystine	10.330				
15	L-Lysine	10.418	76799.18	1.01	60123	54.615
16	L-Tyrosine	10.759	14871.23	0.20	11887	5.876
17	L-Methionine	10.901	9967.07	0.13	7079	
18	L-Valine	10.999	61155.52	0.81	47205	
19	L-Isoleucine	11.910	39967.81	0.53	36932	
20	L-Leucine	11.973	66889.54	0.88	71167	
21	L-Phenylalanine	12.048	56676.68	0.75	65544	4.316
Sum			7596167.97			

Table 1. Amount Amino Acid

IV. CONCLUSION

The swimming bubbles of catfish have a high protein content of 50.18% (wk.) and have a high content of amino acids that characterize collagen, namely amino acids glycine 130.32 mg/g, L-arginine 53.92 mg/g and L-alanine. 53.85 mg/g. The pretreatment process with selected KOH solution was at a concentration of 0.05 M with a soaking time of 8 hours and the selected treatment in the extraction process using acetic acid was at a concentration of 0.5 M for 48 hours with a yield of 16.047%. Acid soluble collagen with the best treatment had amide functional group spectra of A, B, I, II, and III and had a Tg of **88 ^{o}C.**

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