Amino Acid Profile of Gelatin Extracted From The Skin of Starry Triggerfish (*Abalistes stellaris*) and Determination of its Physical Properties

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Abstract- Gelatin is widely used in the food, pharmaceutical, and cosmetic industries. The demand for gelatin increases every year, gelatin derived from pigs and cows is the main source of gelatin in the market. Gelatin that is sourced from other animals such as from poultry and fish is only about 1%. Gelatin from fish becomes a better prospect to develop. Gelatin raw materials can be obtained from the skin, bones, and fish fins. Fish skin is a waste processing of fishery products. The extraction method, fish type and intensity of the treatment determines the fate of produced gelatin. The purpose of this research determined the amino acid profile of starry triggerfish gelatin which was extracted using 2.5% sulfuric acid. In addition, it also contains the physical properties of gelatin which contains the yield, gel strength and viscosity. The results showed that gelatin products had a composition of essential and non-essential amino acids with the highest percentage of glycine, and then proline, alanine, glutamate acid and arginine. While the lowest was amino acid tyrosine. The average yield of gelatin reached 15.4%, gel strength 15.4 N and viscosity 7,2 cP. The use of sulfuric acid with high concentration can decide amino acids that have been formed so the amino acid chain of the broken gelatin causes the gel strength decreased. While the use acid solution with low concentration can result in small gel strength because collagen converted into gelatin is less. So the type of acid and optimal concentration will affect the acid amino composition and physical characteristics. This is related to the structure of the gelatin Ala-Gly-Pro-Arg-Gly-Glu-Hyd-Gly-Pro which affects the gel strength of the gelatin, where the length of the amino acid chain, the gel strength of gelatin is also getting better. Gel strength will affect viscosity and yield.

Keywords : *amino acid, fish skin, gelatin, physic properties*

I. Introduction

Gelatin is a protein obtained from the partial hydrolysis of collagen from the skin, connective tissue and bones of animals. As the global demand for gelatin is continuously on the rise, many potential sources are being sought for combating this growing need. The global production of gelatin reached 326 thousand tons. Majorly derived from pig skin up to 46%, bovine hides 29,4%, bones 23,1% and others sources only 1,5%. Industrial utilization of gelatin is very wide. Gelatin makes an ideal food ingredient with high digestibility in certain types of diets. As an additive, it improves water holding capacity, texture, elasticity, consistency and stability of foods. Additionally, it has been used as a stabilizer, emulsifier, clarifying agent and as a protective coating material. Desserts, ice cream, jelled meat, confectionary, dairy and bakery foods are few of the main consumption areas for gelatin. Moreover, in pharmaceutics, it is used in manufacturing of capsules, tablet coatings, emulsions, ointments and skincare products.

The main sources of industrial gelatin are limited to those from pigskins and bovine hides and bones. According to Plavan *et al.* (2014), the occurrence of Bovine Spongiform Encephalopathy (BSE) and Foot and Mouth Disease (FMD) along with religious constraints has resulted in an anxiety among users of gelatin from land-based animals. In recent years, increasing attention has been paid to alternative gelatin sources, such as fish skin, which comprise about 30% of the total fish weight available after fish fillet preparation. One of gelatin sources is starry triggerfish (*Abalistes stellaris*). It is estimated that nearly 7.3 million tons of by-product waste are produced annually. Partial use of them, on one hand, leads to the loss of an important protein based product and other purposes, and to the other, to reduce the pollution.

In the conversion process of collagen to gelatin, acid pretreatment hydrolyze the cross-linking bonds between polypeptides and irreversibly results in gelatin. The gelatin is water soluble and forms thermo-reversible gels with the melting temperature near to the body temperature. The amino acids that make up gelatin are bonded to each other through a peptide bond to form gelatin with the Gly-X-Y rearrangement unit, where X is proline and Y is hydroxyproline. The composition and sequence of amino acids gelatin differ from one another depending on the species and type of tissue but always contain high levels of glycine, proline, hydroxyproline. The quality of resultant gelatin is determined by its physicochemical behavior that is further based on the species as well as the process of manufacture. Moreover, the specific amino acids and their respective amounts determine physical and functional behavior of gelatin. The higher the level of proline and hydroxyproline, the higher will be the viscosity and gel strength (Bostaca *et al*., 2103).

So far, there has been no research of the potential of protein in starry triggerfish (*Abalistes stellaris*), especially regarding the profile of amino acids making up proteins of starry triggerfish skin that have been hydrolyzed into gelatin product. The importance of this research, given the potential for hydrolysis of collagen protein to gelatin is a potential product that is very much determined by its constituent amino acids. The aim of this research was to identify amino acid profiles in the gelatin of starry triggerfish skin. Besides that, also to find out the physical characteristics of gelatin extracted from of starry triggerfish skin used acid pretreatment.

II. MATERIALS AND METHODS

2.1 Materials

As an object of our research was used skin of starry triggerfish obtained after filleting of the fish. Typically, this waste are not reused and disposed to the landfills. Wastes were preserved cutting into small pieces and washed with water. The chemical composition of the starry triggerfish skin is presented in Table 1.

Table 1. The chemical composition of the starry triggerfish skin

Parameter	Composition(%)
Protein substances	24,52
Fatty substances	0,13
Water substances	53,49
Mineral substances	17,75

In the pre-treatment gelatin extraction process with acid treatment aims to hydrolyze collagen in fish skin by breaking down intramolecular bonds in collagen triple helix molecules. The results may form three free alpha chains, or the formation of beta chains and gama chains and to dissolve minerals in bone and fish skin which will affect the physical characteristics and amino acid gelatin. The types of acids used are from organic acids such as CH_3COOH, $C_6H_8O_7$ or $C_4H_6O_5$. While the inorganic acids used are H_2SO_4, HCl or H_3PO_4 with different concentrations.

After the preliminary study, the best gelatin extraction method was obtained using H_2SO_4 as acid pre-treatment with concentration of 2.5%. Because the acid solution with very high concentration can decide the amino acid that have been formed so the chain of amino acids will break up and causes the gel strength will decrease. While the low concentration of acid can also cause decreasing the gel strength because collagen converted into gelatin is also less.

This is the most widely adopted processing method where mild to harsh acidic treatment is involved. The fish skin was washed under running tab water for around 1 hour to remove the superfluous materials. Then the cleaned raw material was soaked in 2,5% H_2SO_4 for 20 hours at room temperature by maintaining a ratio of 1:3 skin/solution (w/v). After the given time of treatment, the solution was neutralized. The treated material was blended with distilled water in 1:3 ratio (w/v), and extracted at 70 °C for 5 hours used waterbath. The extracted material was filtered through double-layered cheese cloth. The filtrate was dried at 60 °C in a hot air oven earlier for 48 hours. After that gelatin in the form of sheets will be mashed into powder. All gelatin samples were weighed, calculated for extraction yield, amino acid analyze, gel strength analyze and viscosity analyze.

2.2 Yield Analyze

Gelatin was calculated by the following equation. Yield (%) = $\frac{\text{weight of dry gealtin (g)} x \text{ 100}}{\text{weight of initial dry starry triggerfish (g)}}$

Where the weight of dry starry triggerfish was calculated by subtracting moisture content determined by AOAC (2000) from the initial wet weight.

2.3 Amino Acid Analyze

0.2 g of gelatin sample was prepared in a closed test tube and 5 mL of 6 N. And then HCl was added. The sample was put in an oven with a temperature of 100ºC for 18-24 hours. Next the sample is filtered with Whatman 40 filter paper. The hydrolysis results are piped as much as 10µl and put in a test tube. Then add 30 µl of the drying solution, then dry it with a vacuum pump. The dried sample was added 30 µl of derivate solution and left to dry for 20 minutes. The sample was then diluted with 200 µl of 1M sodium acetate dilution solution. The sample is ready to be analyzed by HPLC.

2.4 Gel Strength Analyze

Gelatin gel was prepared by the method of Kaewdang *et al.* (2015), gelatin was dissolved in distilled water (60 °c) to obtain a final concentration of 6.67% (w/v). The solution was stirred until the gelatin was completely solubilized and then transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. the solution was incubated at the refrigerated temperature (4°C) for 18 h prior to analysis. the gel strength was determined at 8-10°C using a texture analyzer with a load cell of 5 kg and crosshead speed of 1 mm/s. A 1.27 cm diameter flat faced cylindrical teflon plunger was used. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.5 Viscosity Analyze

To determine the viscosity value is a gelatin solution with a concentration of 6.67% prepared with distilled water (7 g gelatin plus 105 ml of distilled water) then the solution was measured for viscosity using a *Brookfield Syncro-Visric Viscometer*. Measurements were carried out at 60ºC with a shear rate of 60 rpm using a spindle. The measurement results are multiplied by the conversion factor. This test uses spindle no.1 with the conversion factor being 1, the viscosity value is expressed in units of centipoise (cP).

III. RESULTS AND DISCUSSION

3.1 Amino Acid Composition

Fish gelatin properties are affected by the presence and concentration of amino acids, molecular weight and particular structural fragments. In general, glycine, proline/hydroxyproline and alanine are the predominant amino acids with respective percentages of 33, 20 and 11%. In a comparative study designed by Mahmood *et al.* (2016), it was observed that proline and hydroxyproline contents in mammalian, warm-water fish and cold-water fish gelatins are 30%, 23% and 17%, respectively. Table 2 presents a comparative list of amino acids that are found in gelatins obtained from different sources.

Table 2. Amino acid composition of different types of gelatins

Amino Acids	Gelatin of Starry Triggerfish Skin(%)	Gelatin of Yellowfin Tuna Skin (%)*
L-Histidin	0,9	0,9
Threonine	3,3	3,7
Prolin	13,0	11,6
Tirosin	0,6	0,5
Leusin	2,4	2,7
	6,5	3,5
Asam aspartat		
Lisin	4,3	3,5
Glisin	26,1	27,7
Arginin	9,5	10,0
Alanin	11,9	8,1
Valin	2,9	2,1
Isoleusin	1,1	1,2
Fenilalanin	2,2	2,6
	11,2	8,2
Asam glutamat		
Serin	4,0	4,1

*) Nurilamala *et al*., 2017

Amino acids composition starry triggerfish gelatin are different from yellow fin tuna gelatin, these are caused by the use of different raw materials. Grouping of amino acids according to Silva *et al.* (2014), can be grouped into 2, namely amino acids that cannot be produced by the body and amino acids that can be produced by the body. Amino acids that cannot be produced by the body are usually called essential amino acids, which can be added to food intake to fulfill them. While the amino acids that can be produced by the body are called non-essential amino acids. Examples of amino acids included in essential amino acids are like lysine, methionine, valine, histidine, phenylalanine, arginine, isoleucine, threonin, leucine, and tryptophan. In non-essential amino acids there are aspartic acid, glutamic acid, alanine, tyrosine, cystine, glycine, serine, proline, hydroxylisine, glutamine, and hydroxyproline.

Starry triggerfish gelatin extracted by acid treatment using 2.5% sulfuric acid has 8 essential amino acids from a total of 10 known essential amino acids. The highest essential amino acid in Starry triggerfish gelatin was 9.5% arginine and 0.9% histidine lowest. While the highest non-essential amino acid composition was glycine 26.1% and the lowest tyrosine was 0.6%. According to Gaidau *et al*. (2013), the structure of gelatin in fish skin is dominated by amino acids which include 14% hydroxyproline, 16% proline and 26% glycine, it depends on the composition of collagen contained in the raw material. Collagen protein is a fibrous protein containing 35% glycine and about 11% alanine and a fairly high proline content. Collagen has a range of strengths, a special structure containing hydroxylisin and hydroxyproline, which are amino acids that are not present in other proteins. The collagen triple helix structure is assembled from the α-specific polypeptide with the position of Glysin-X-Y, where position X is filled with proline amino acids and at position Y is filled with hydroxyproline amino acids. Proline amino acids are amino acids that can create curves in the α-helical structure and hydroxyproline functions in increasing collagen stability.

The triple helix is the basic structural unit of collagen called tropocolagen. The breakdown of the structure of tropocolagen into a random twist uses this acid solution called gelatin. Conversion of collagen to gelatin can cause changes in amino acid composition, this is related to the method used. Extraction of gelatin with an acidic process generally contains more glycine and less tyrosine. The type and concentration of acid solution can affect the characteristics of the gelatin produced.

The use of sulfuric acid with high concentration can decide amino acids that have been formed so the amino acid chain of the broken gelatin causes the gel strength decreased. While the use acid solution with low concentration can result in small gel strength because collagen converted into gelatin is less. So the type of acid and optimal concentration will affect the acid amino composition and physical characteristics. This is related to the structure of the gelatin Ala-Gly-Pro-Arg-Gly-Glu-Hyd-Gly-Pro which affects the gel strength of the gelatin, where the length of the amino acid chain, the gel strength of gelatin is also getting better. Gel strength will affect viscosity and yield.

3.2 Extraction Yield

Different extraction methods result into gelatins with different functionality. In general, fish gelatin holds some characteristic properties that make it different from other sources. Conversion of collagen to gelatin modifies it solubility, making it water soluble. It is readily soluble in hot water, swells in cold water, while insoluble in alcohol or non-polar solvents. It is colorless to yellowish, tasteless, transparent to slightly translucent, powder or flakes or sheets. Mainly, the physicochemical properties are based on the source of collagen and the given treatment of extraction. However, the intensity of treatment and hydrolysis also impart significant variations in the end product. The gelatin yield of starry triggerfish skin used 2,5% sulphuric acid reach 15,4%. According to Karim and Bhat (2009), the gelatin yield is varies from 6-19% on wet weight basis, is less than the mammalian sources.

In the previous study, gelatin yield from stingray fish between 5.79% and 10.90%, gelatin from kuma-kuma skin was 18.27%, gelatin from tilapia skin was 5.4%, gelatin from shark skin was 17 % and yellowfin was 20%. The difference in the yield of gelatin produced from the extraction process of fish skin collagen tissue produces different yields depending on the method used.

3.3 Gel Strenght

One of the most important properties of gelatin is its gel strength, also termed as bloom strength. The molecular weight (of α, β chains) in gelatin defines the strength of bloom. Figure 1 presents the hypothetic model of polypeptide chains in collagen. Bloom strength is also correlated to the viscosity of the gel, and is an important criteria for its application in food. Typically, fish gelatin gel bloom varies from 50 to 300. Nonetheless, factors such as temperature, pH, acids, bases, enzymes and bacteria may alter the strength variably. The thermo-reversibility of gels is one of the most interesting inherent property of gelatin that make its use in jellies possible (Gómez-Guillén *et al*., 2011).

Based on the results of this research, the average gel strength of starry triggerfish gelatin obtained from immersion solution with 2.5% sulfuric acid treatment was 15.7 Newton or 220 bloom. Furthermore shorter the chain of amino acids causes low molecular weight so that gel strength becomes low. Furthermore according to Karim and Batt (2009), gel strength, viscosity, melting point are influenced by many factors, such as molecular weight distribution, concentration of gelatin solution, and salt content. The molecular weight of gelatin is related to the chain length of the amino acid bonds that make up the gelatin. The longer the chain of amino acids, the greater the molecular weight and the higher the value of the strength of the gel

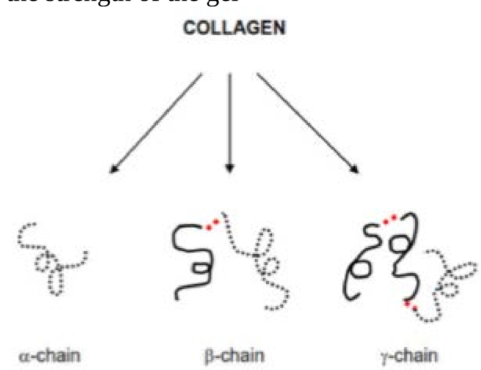

Figure 1. Typical polypeptide strands in fish gelatin

3.4 Viscosity

Another considerable functional property of gelatin is its viscosity, highly related to bloom strength. Various calibrated viscometers or pipettes are used for viscosity measurement, where a standard solution of 6.67% gelatin is used. The viscosity of gelatin from the skin or fish bone is relatively higher compared to gelatin from mammals. This is because fish have a relatively shorter lifespan than mammals, allowing inter and intra-molecular bonds of collagen contained in the skin and fish bones to become less stable in forming triple helical conformation resulting in high viscosity values.

Based on the results of this research, the average viscosity of starry triggerfish gelatin obtained from immersion solution with 2.5% sulfuric acid treatment reach 7,2 cP. Viscosity is influenced by the structure of the protein in gelatin, a longer chain of amino acids will result in higher protein molecular weight, which will increase the viscosity value. Decomposition of collagen's triple helix bond into a single chain by acid causes shorter amino acid chains and smaller molecular weight so that the viscosity value will be smaller.

IV. CONCLUSION

Skin from starry triggerfish (*Abalistes stellaris*) could be an alternative source of gelatin. Gelatin was extracted by 2,5% sulphuric acid (H_2SO_4) as acid pre-treatment had complete amino acid composition, highest extraction yield, highest gel properties and viscosity Being unique to its properties, gelatin is overwhelmingly used in different food recipes. Mainly, mammalian gelatin is leading the market, but concerns for Halal or Kosher applications create a need to find some alternatives. In this regard, fish gelatin is getting commercial interests. Fish gelatin is thoroughly studied for its physicochemical and functional properties that allowed to optimize its use in various applications.

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